



Agilent Poroshell 120 色谱柱应用文集

用于 HPLC 和 UHPLC 的 Agilent Poroshell 120 色谱柱

The Measure of Confidence



Agilent Technologies



Agilent Poroshell 120 色谱柱

使实验室中的每一台 LC 和 LC/MS 工作更出色

“我们因为它的耐用性能而选择了 Poroshell 120 色谱柱”

“Poroshell 120 色谱柱拥有可靠的卓越性能——它已经成为我们实验室的新‘标准’”

“对于我经常遇到的最复杂的样品，Poroshell 120 色谱柱为我节省了很多时间”

“Poroshell 120 就是我要找的色谱柱”

来自 Poroshell 120 使用者的评论

Poroshell 120 色谱柱为普通的 HPLC 提供非凡的分析效率，并且显著提升 600 bar 和 1200 bar UHPLC 的分析性能。

该色谱柱在我们早期推出的 Poroshell 300 色谱柱的技术基础上更进一步，在对范围更广的小分子和肽进行分析时，您可以获得比以往更高的通量和更好的分离度。它们的卓越性能如下：

- **卓越的批次间重现性** —— Poroshell 120 色谱柱是由专利的一步多孔壳层工艺制造而成，这极大地减少了色谱柱之间和批次之间的细微差异
- **与亚二微米色谱柱相媲美的分析速度和分离度**，反压却较之降低了 50% —— 使 HPLC 和 UHPLC 的灵活性和效率达到新的水平
- **优异的峰形** —— 尤其是在 pH 6-7 间，可获得更快、更准确的结果
- **较长的柱寿命** —— Poroshell 120 色谱柱使用标准的 2 μm 滤芯，可防止脏样品堵塞
- **多种键合相** —— C18 柱、C8 柱、苯基-己基柱等众多种类！不断增加的键合相系列实现了方法开发的灵活性；查阅我们的网站获取最新信息
- **简单的方法转换及可扩展性** —— 可扩展至 ZORBAX 键合相，实现世界各地实验室间的高效率方法转换
- **UHPLC 保护柱**通过延长 Poroshell 120 色谱柱寿命减少您的运行费用



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前言

在当今液相色谱分析中，实现更快更有效的分析是一大热点也是大势所趋。Agilent Poroshell 120 填料采用安捷伦公司专利的制造工艺，在实心核外包上表面多孔层，能显著提高被分析物在固定相和流动相之间的传质，使其在较低反压下实现快速高效的分离。Poroshell 120 填料平均总粒径为 2.7 μm ，由 1.7 μm 直径的实心核和 0.5 μm 厚的多孔外层组成。使用 Poroshell 120 填料可帮助您获得与亚二微米全多孔色谱柱相媲美的性能和比亚二微米填料低 40~50% 的反压。低的反压可以帮助您在常规液相上实现快速高效分离提供很大程度的可行性。上一版的 Poroshell 120 文集中部份应用充分展示了这一特点：即只需稍加改动，即可将常规 5 μm 柱上的方法转换到 Agilent Poroshell 120 上，为您节省大量实验时间和成本。

作为上一版 Poroshell 120 应用文集的延伸，本版文集整理收录了 2011-2013 年安捷伦公司及相关不同行业的用户使用 Poroshell 120 色谱柱的应用文章。行业应用包括：食品安全、环境分析、药物分析、化工分析和生物分析等热门领域。期间安捷伦公司一直致力于开发更多的 Poroshell 120 固定相以满足日益增长的不同类型物质的分析需求。您将在本版文集中看到更多类型固定相的应用，如用 Poroshell 120 分离水果中的有机酸、用 Poroshell 120 EC-CN 分析炸药残留、Poroshell 120 Bonus RP, Phenyl-Hexyl 在抗氧化剂分析中的应用，以及 Poroshell 120 Hilic 的性能研究报告等最新的应用。本版文集收录的应用的另外一个特色是，随着 Poroshell 120 的性能优势得到了越来越广泛的认可，Poroshell 120 色谱柱在复杂体系中痕量残留

分析得到了越来越广泛的应用，与安捷伦的 Bond Elut 系列样品前处理产品一起成为安捷伦整体解决方案中的亮点，用于体液、食品等复杂基质中药物和兽药残留的分析。希望本文集的出版，能帮助大家更好地了解 Poroshell 120 表面多孔层色谱柱的特点，并在日后工作中能充分利用以实现快速高效的实验室分析工作。

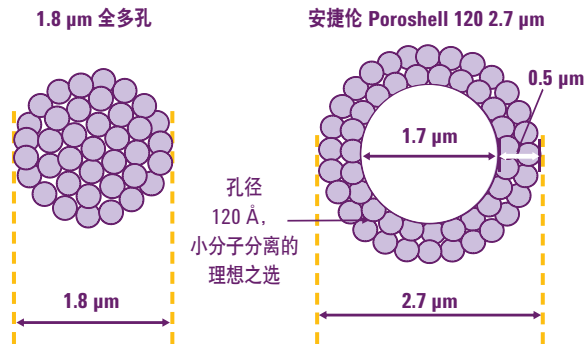


低压条件下即可获得高柱效 经过实际验证的柱间一致性 这就是 Poroshell 120 色谱柱与众不同之处



安捷伦 Poroshell 120 色谱柱的关键特性是表面多孔微粒色谱柱填料。

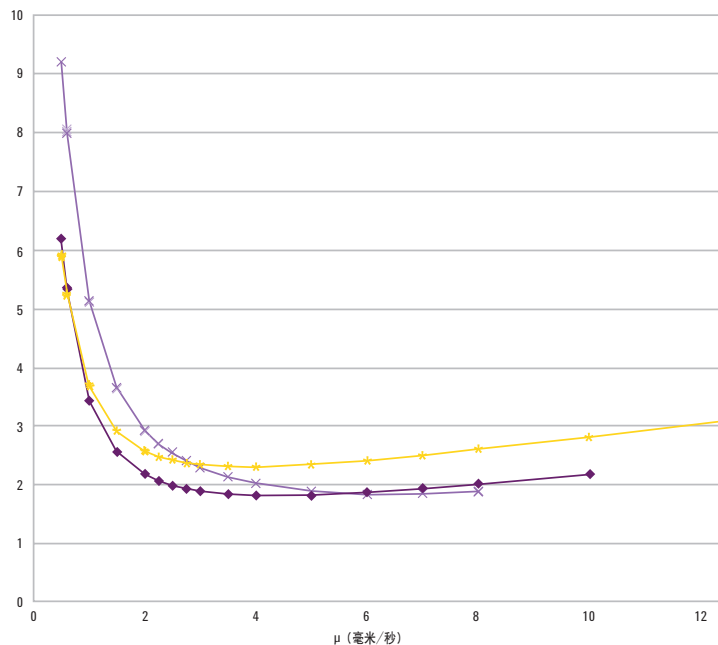
Poroshell 120 填料由 1.7 μm 直径的实心硅胶核和 0.5 μm 厚的多孔外层组成。这种独特的配置能够使色谱柱在亚 3 μm 微粒背压下实现亚 2 μm 微粒的所有性能优点。



表面多孔层填料与亚二微米填料具有相似的性能

图中范德姆特曲线表明表面多孔层、2.7 μm 填料粒径的 Poroshell 120 色谱柱的理论塔板高度可以降低到与 1.8 μm 色谱柱相当，从而获得相似的柱效。

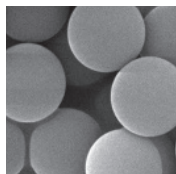
- ◆ Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μm , (USCFX01009) 部件号 695975-302
- ✕ Agilent ZORBAX Eclipse Plus C18, 3.0 x 100 mm, 1.8 μm , (USUYB01455) 部件号 959964-302
- ★ Agilent ZORBAX Eclipse Plus C18, 3.0 x 100 mm, 3.5 μm , (USUXV01435) 部件号 959961-302



Poroshell 120 填料如何制造

为了制造出最适合小分子分离的色谱柱，我们彻底改进了表面多孔层填料技术。具体来说，我们尽量减少生产过程中所需的步骤，以确保填料和色谱性能达到最佳的重现性。

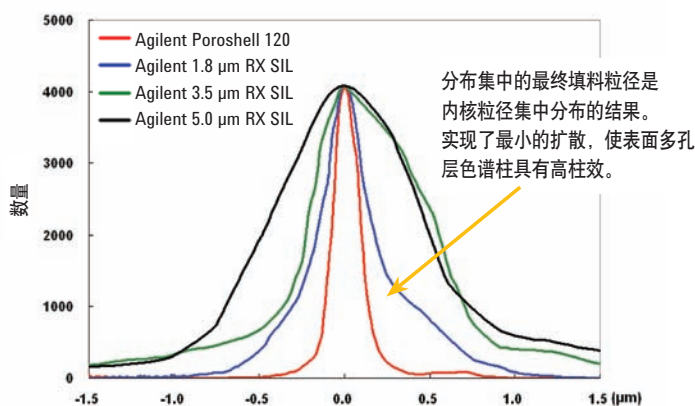
第一步 制造内部实心核



Poroshell 120 色谱柱的填料内核具有非常光滑的表面和均一的 1.7 μm 粒径，整体粒径分布集中。因此，可以获得更紧实填充的柱床，且能获得比全多孔填料更高的效率。

全多孔填料和 Poroshell 120 填料的粒径分布对比

这张图表明 Agilent Poroshell 120 色谱柱最终填料粒径的分布最集中——这是内核填料粒径集中分布的直接结果。



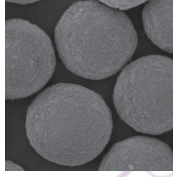
填料粒径分布的标准是粒径 90/10 的比值应低于 1.5

由上述表中可见，ZORBAX 全多孔填料（粒径为 1.8 μm ，3.5 μm 和 5.0 μm ）都具有可接受的粒径分布。然而，Poroshell 120 填料具有比其他三种粒径高 25% 的集中粒径分布，极大地改善了柱效。

	Agilent Poroshell 120 (2.7 μm) LN B10006	Agilent ZORBAX (1.8 μm)	Agilent ZORBAX (3.5 μm)	Agilent ZORBAX (5.0 μm)
10%	2.40 μm	1.67 μm	3.07 μm	4.59 μm
90%	2.85 μm	2.45 μm	4.44 μm	6.21 μm
90%/10% 比率	1.16	1.47	1.45	1.35

如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

第二步 包覆多孔层



一些生产商通过包覆一层又一层的颗粒制造多孔层。然而在安捷伦，我们只采用一步法，即采用与制造传统的 ZORBAX 色谱柱相似的凝聚技术制造多孔层。这种独特的一步法工艺提高了产量，使柱间重现性更好。

第三步 应用键合相

安捷伦 Poroshell 120 固定相的类型已经扩展至能够匹配 ZORBAX 系列固定相，在方法开发方面具备了更强的灵活性和可扩展性。参见下页中所有 Poroshell 120 固定相的详细信息。

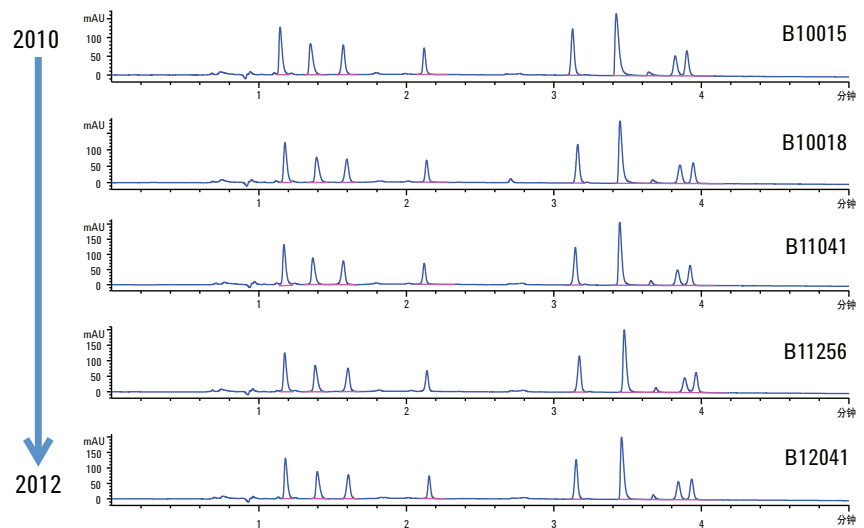
批次之间、年复一年的可重现分析性能

Poroshell 120 颗粒使用安捷伦发明的专利多孔颗粒制作工艺制成。不同于传统的多层化包覆，Poroshell 120 色谱柱使用一步式共凝聚工艺制造，保证最终获得的颗粒更加均匀、色谱结果更加可靠。

制造工艺越简单，色谱柱一致性越好

从对五个批次之间的比较来看，一步壳层工艺得到了高重现性的色谱柱。

Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μ m
部件号 695975-902 —— 来自 5 个不同的批次



各种键合相提供灵活的选择性



Poroshell 120 色谱柱与安捷伦业界领先的 ZORBAX 色谱柱系列来自同一个生产工厂。Poroshell 120 色谱柱与所有 ZORBAX 色谱柱所使用的键合填料相呼应，能够实现世界各地实验室间更简单的方法转换并提供确定的可扩展性优势。

提供各种固定相，完善您的分离：

- **Poroshell 120 EC-C18 和 Poroshell 120 EC-C8**（封端保证最佳峰形）：此类键合相应是您大多数分离的首选，包括使用液质兼容流动相的肽谱分析。我们推荐您首先选择 C18 固定相，然后再使用对各种样品保留较弱的 C8 固定相
- **Poroshell 120 SB-C18 和 Poroshell 120 SB-C8**（不封端用于更大的替代选择性）：这些键合相在低 pH（pH 1-2）条件下能够表现出最佳性能和最长柱寿命。需要注意的是，许多现有使用 ZORBAX SB-C8 色谱柱的 USP 方法都能够转移到 C8 固定相上
- **Poroshell 120 Phenyl-Hexyl**（封端）当加强 π - π 相互作用时，这种固定相可以提供对芳香族化合物的特殊选择性
- **Poroshell 120 SB-Aq**（不封端）这种专利固定相是极性化合物分离的理想选择，并可用于高水性条件
- **Poroshell 120 Bonus-RP**（封端）这种烷基酰胺固定相对大量分离能产生正交结果，并有助于分离难分离化合物
- **Poroshell 120 亲水相互作用色谱（HILIC）色谱柱**：它们保留并分离小极性分析物的能力使得这些色谱柱迅速普及。同时它们也提供了液质应用的高质谱灵敏度
- **Poroshell 120 EC-CN 色谱柱**：这种氰基固定相与我们的 ZORBAX Eclipse XDB-CN 固定相非常相似，为极性化合物正相和反相的 1 维和 2 维分析提供了替代的选择性
- **以及更多**：查阅我们的网站获得键合相的最新列表

如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

Agilent Poroshell 120 EC-C18 和 Poroshell 120 SB-C18 为优化分离提供不同的选择性

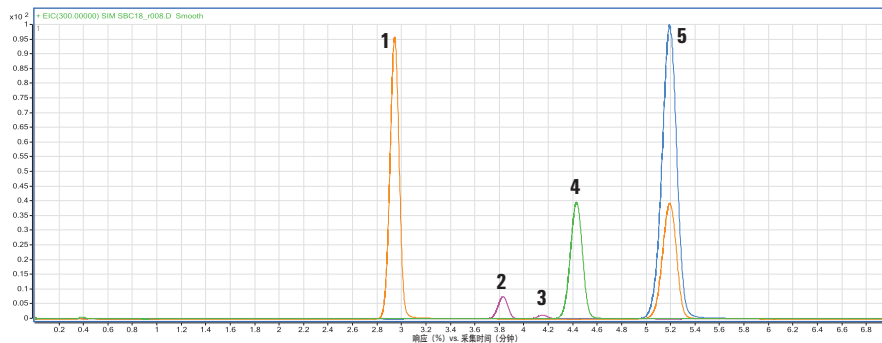
流动相: 35% 水, 65% 乙腈
 流速: 1 mL/ 分钟
 温度: 30 °C
 质谱采集: 动态 MRM

化合物	母离子	碎裂电压
花生四烯乙醇胺	(AEA) 348	135
棕榈酸乙醇胺	(PEA) 300	135
2-花生四烯酸甘油酯	(2-AG) 379	135
油酰乙醇胺	(OEA) 326	135

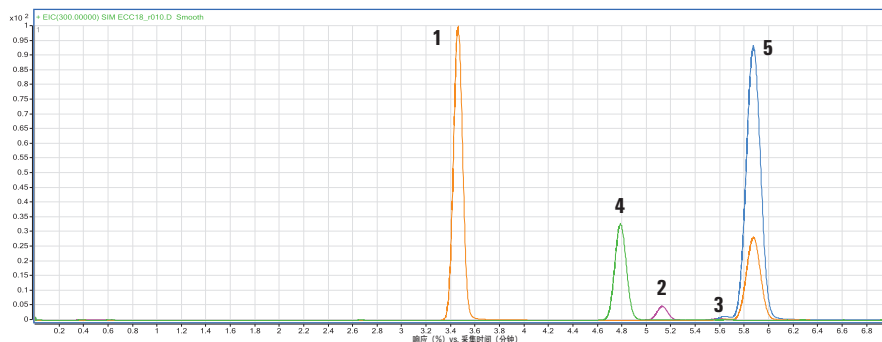
质谱离子源:
 气体温度: 350 °C
 气体流量: 12 升 / 分钟
 雾化器: 40 psi
 毛细管电压: 4000 V

- 分析:
1. 花生四烯乙醇胺 (AEA)
 2. 2-花生四烯酸甘油酯
 3. 杂质
 4. 棕榈酸乙醇胺 (PEA)
 5. 油酰乙醇胺 (OEA)

Agilent Poroshell 120 SB-C18, 3.0 x 100 mm, 2.7 μm
 部件号 685975-302



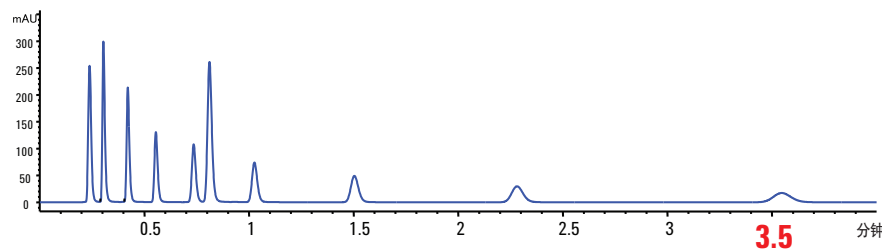
Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μm
 部件号 695975-302



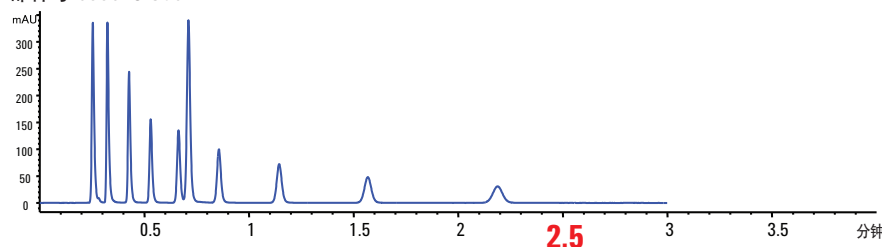
Poroshell 120 EC-C8 色谱柱对非极性化合物的快速分析保留较弱

流动相: 60% 乙腈, 40% 水
 流速: 0.85 mL/ 分钟
 温度: 26 °C
 检测波长: 254 纳米
 样品: 2 μL RRLC 校验样品 (部件号 5188-6529), 烷基苯酮

Agilent Poroshell 120 EC-C18, 3 x 50 mm, 2.7 μm
 部件号 699975-302



Agilent Poroshell 120 EC-C8, 3 x 50 mm, 2.7 μm
 部件号 699975-306



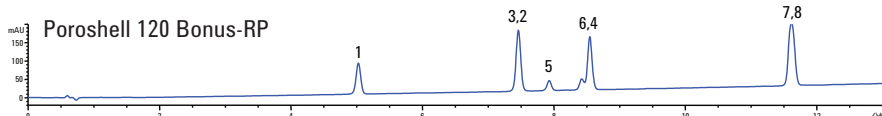
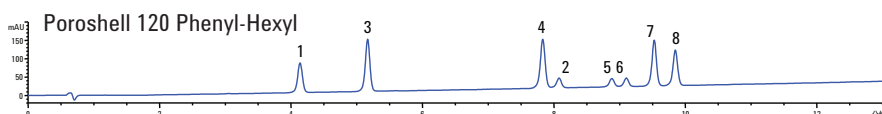
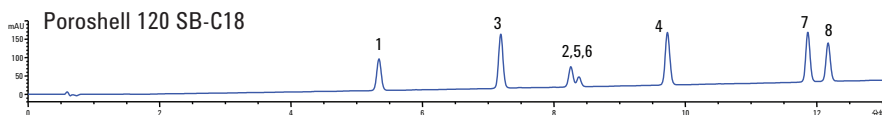
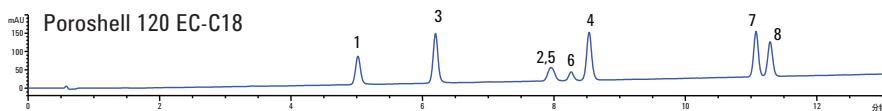
多种可选固定相让您掌控选择性

4 种不同 Poroshell 120 固定相的选择性变化

苯基-己基固定相完成了 8 种类固醇的最佳分离，提供了 C18 柱及 C8 柱的替代选择性，并为这 8 种化合物提供更快速、更干净的基线分离。

色谱柱: Poroshell 120, 2.1 x 100 mm, 2.7 μ m
 流动相: A: 0.1% 甲酸
 B: 甲醇+0.1% 甲酸
 流速: 0.4 mL/min
 温度: 25 $^{\circ}$ C
 检测波长: 260 nm
 样品: 8 种类固醇
 梯度: 40-80% 甲醇 /14 min

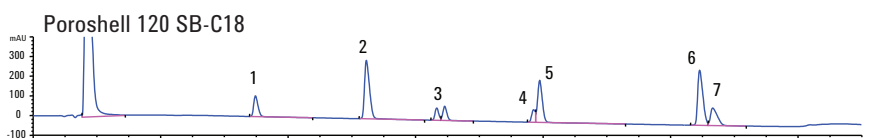
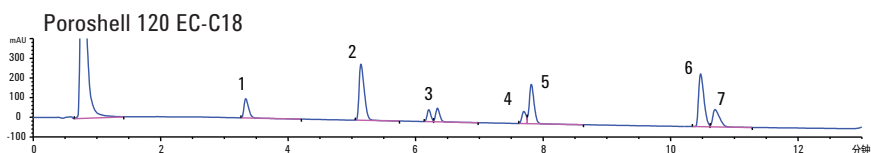
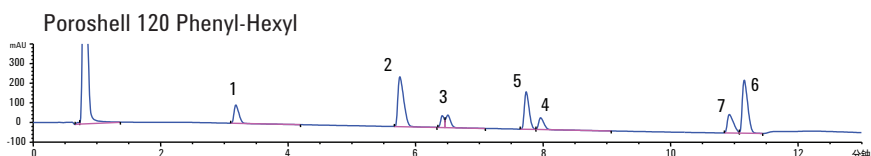
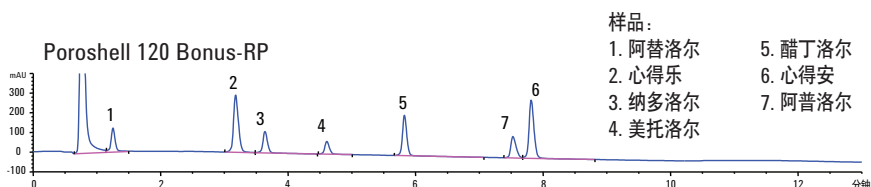
样品:
 1. 氢化可的松
 2. B 雌二醇
 3. 雄甾二烯-3,17-二酮
 4. 睾酮
 5. 乙烯雌二醇
 6. 雌酮
 7. 醋酸诺酮
 8. 孕酮



β -受体阻滞剂的分析: Poroshell 120 固定相的比较

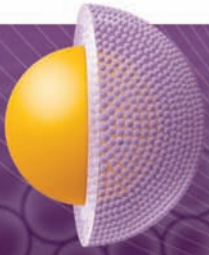
对 β -受体阻滞剂挑战性的分析证明了不同的选择性可以产生不同的结果。总的来说，Bonus-RP 固定相提供了最佳峰形和分离度；这对纳多洛尔尤其适用，它在 C18 和苯基-己基固定相中呈现分裂峰。

色谱柱: Poroshell 120, 2.1 x 100 mm, 2.7 μ m
 流动相: A: 10 mM NH_4CO_2 , pH 3.8
 B: 甲醇
 流速: 0.4 mL/min
 温度: 40 $^{\circ}$ C
 检测波长: 260 nm
 样品: β -受体阻滞剂
 梯度: 10% B 到 30% B/12 min



样品:
 1. 阿替洛尔
 2. 心得乐
 3. 纳多洛尔
 4. 美托洛尔
 5. 醋丁洛尔
 6. 心得安
 7. 阿普洛尔

如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn



标准的高效液相色谱是否能实现快速液相色谱 / 超高压液相色谱的性能？ 安捷伦 Poroshell 120 色谱柱能够实现



通过 Poroshell 120 色谱柱，能够使您在高效液相色谱的压力（低于 400 bar）下实现亚 2 μm 快速液相色谱 / 超高压液相色谱柱效的 80% - 90% 或更多。

这种在低压下快速分离的能力可以极大程度地提高效率，使用您实验室中现有的 HPLC 系统，使您用较少的运行时间分析更多的样品，举例如下。而且，您可以随时将方法无缝对接轻松转换到所选择的安捷伦 1200 Infinity 系列液相色谱仪器上，甚至获得更高的效率。

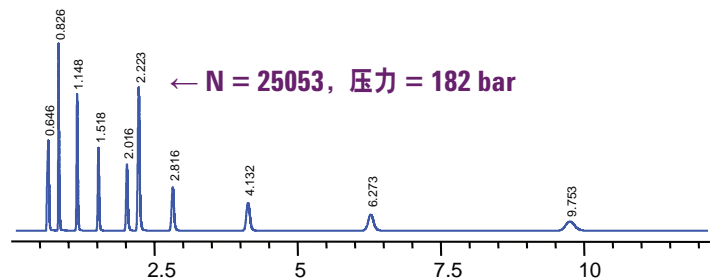
较小压力下的超高压液相色谱效率

对于中性烷基苯酮样品，Poroshell 120 色谱柱可以提供相当于 1.8 μm 色谱柱 90% 以上的效率。也请注意，Poroshell 120 色谱柱上的压力仅为 1.8 μm 色谱柱压力的 50%。

流动相： 60% 乙腈：40% 水
流速： 0.58 mL/分钟
进样量： 4 μL
温度： 26 $^{\circ}\text{C}$
检测器： 二极管阵列检测
波长 = 254, 4 纳米
参比波长 = 360, 100 纳米

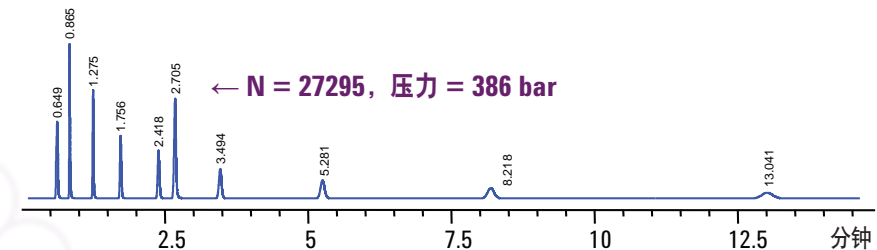
样品： RRLC 校验样品
(部件号 5188-6529)
加标 50 μL 2 mg/mL
硫酸于水 / 乙腈 (65:35) 溶液

Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μm
部件号 695975-302



> 1.8 μm 色谱柱效率的 90%

Agilent Eclipse Plus C18, 3.0 x 100 mm, 1.8 μm
部件号 959964-302



选择 Agilent Poroshell 120 使 HPLC 的效率提高

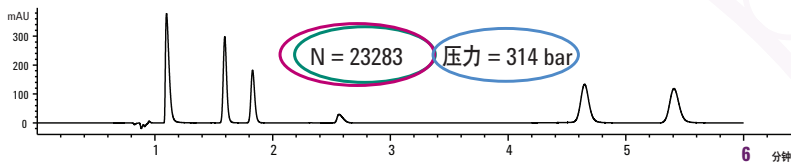
分析此软饮料成分，Poroshell 120 色谱柱达到：

- ▶ 亚 2 μm 色谱柱柱效的 90%
- ▶ 3.5 μm 色谱柱柱效的两倍
- ▶ Poroshell 120 色谱柱压力低于 400 bar，而亚二微米色谱柱压力高于 400 bar

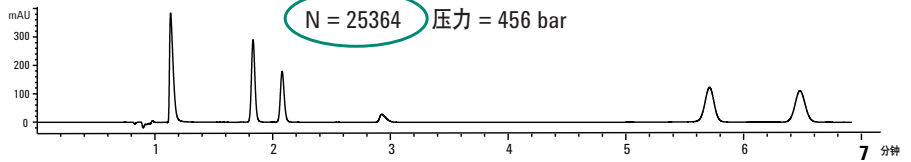
使用甲醇流动相时达到低反压是非常有意义的，因为甲醇比乙腈更容易产生压力。

色谱柱： 3.0 x 100 mm, 2.7 μm
流动相： 65% A: 0.2% 甲酸
 35% B: 甲醇等度
流速： 0.5 mL/min
进样量： 1 μL
温度： 26 $^{\circ}\text{C}$
检测： UV, 220 nm

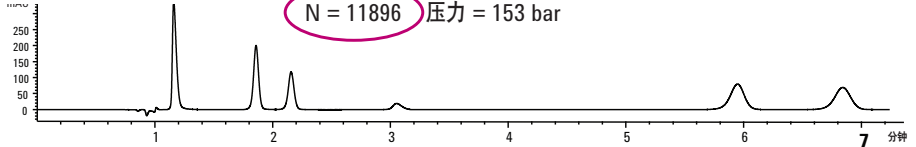
Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μm
部件号 695975-302



Agilent RRHT Eclipse Plus C18, 3.0 x 100 mm, 1.8 μm
部件号 959964-302



Agilent Rapid Resolution Eclipse Plus C18, 3.0 x 100 mm, 3.5 μm
部件号 959961-302



样品：

- | | |
|------------|---------|
| 1. 糖精 | 4. 阿斯巴甜 |
| 2. 咖啡因 | 5. 脱氢乙酸 |
| 3. P-羟基苯甲酸 | 6. 苯甲酸 |

如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

为什么使用保护柱？

安装简便，保护柱能延长分析柱寿命，节约成本。

安装一个较便宜的保护柱——特别是当分析较脏样品时——可防止颗粒物和强吸附物质对色谱柱的损害。作为一种保护，当塔板数、压力或分离度变化超过 10% 时，您需要更换保护柱。

然而，您需要针对您的应用进行一次精确的测定。这里所示为使用 300:1 的水 / Similac（含 0.1 mg 磺胺氯吡嗪和磺胺甲基异恶唑）进行累积寿命测试。

流动相： A: 0.1% 甲酸的水溶液
B: 乙腈

流速： 0.65 mL/min

梯度： 10% B 保持 2 min，
2 min 内提高到 45% B

进样量： 10 μ L

温度： 23 $^{\circ}$ C

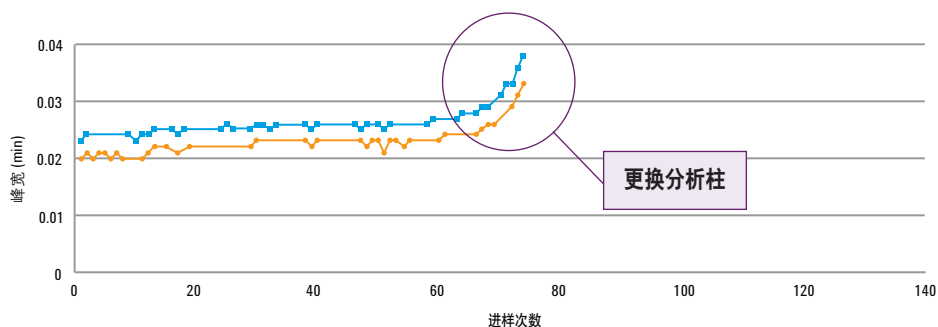
检测： Sig = 254, 4 nm; Ref = Off

仪器： Agilent 1200 系列 RRLC

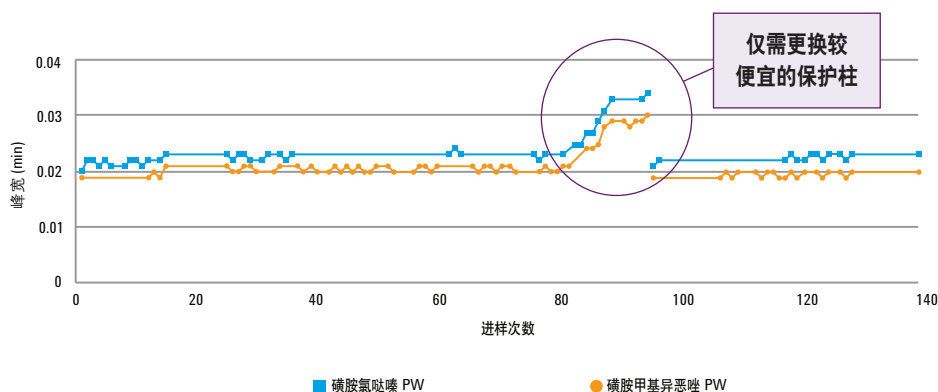
样品制备： 100 mL 水 + 0.333 mL
Similac + 1 mL 0.1 mg/mL
磺胺氯吡嗪和磺胺甲基异恶唑



没有保护住：
分析柱在 80 次进样后失效



有保护住：
80 次进样后保护柱失效 - 保护了分析柱



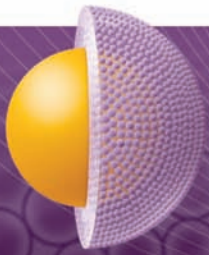
安捷伦快速保护柱使快速液相色谱应用保持超高速分析

用于超高压液相色谱的安捷伦快速保护柱在高压下坚固耐用并且可靠，同时与安捷伦快速液相色谱及超高压液相色谱柱兼容。无需特殊工具就可以安装。

观看在线视频学习如何轻松安装安捷伦快速保护柱：请查看

www.agilent.com/chem/poroshell120.cn





高分离度液质联用系统和液相色谱-串联质谱系统的耐用选择



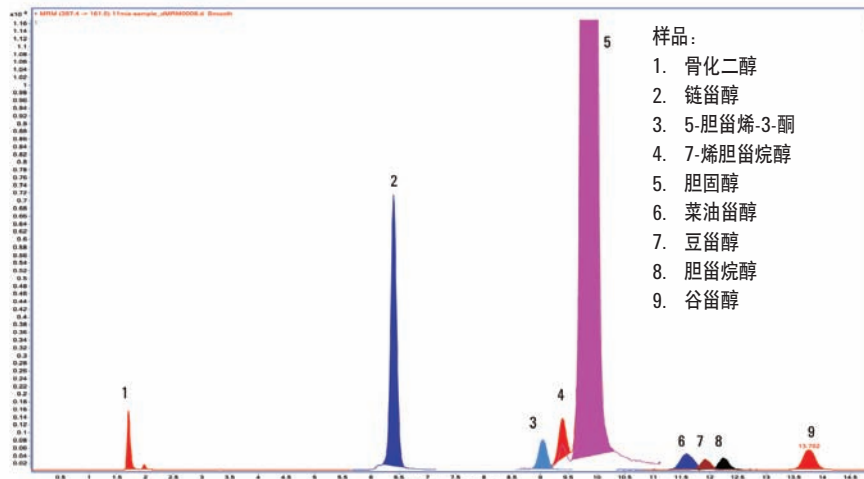
安捷伦 Poroshell 120 色谱柱使您的液质联用系统和液相色谱-串联质谱系统工作更加出色。它们的多孔层和实心核限制了扩散距离、提高了分离速度，而窄粒径分布提高了柱效和分离度。其它优势包括：

- 快速和有效的分离重要的同重化合物
- 能够更好的分离邻近洗脱峰
- 一次分析分离更多化合物
- 液质分析准确度和确定性都得到改善
- 标准的 2 μm 滤芯防止脏样品堵塞

使用 Poroshell 120 EC-C18 液相色谱-串联质谱柱分离胆固醇和其它甾醇

值得注意的是，即使胆固醇与 7-烯胆固醇的比例在 2000:1，也能够获得足够的分离，这对于有效定量很关键，因为这两种化合物具有相同的分子量。

色谱柱: Poroshell 120 EC-C18,
3.0 x 100 mm, 2.7 μm
流动相: 80% 乙腈 / 20% 甲醇
流速: 0.6 mL/min
进样量: 2 μL
温度: 20 $^{\circ}\text{C}$
检测: APCI, 阳离子

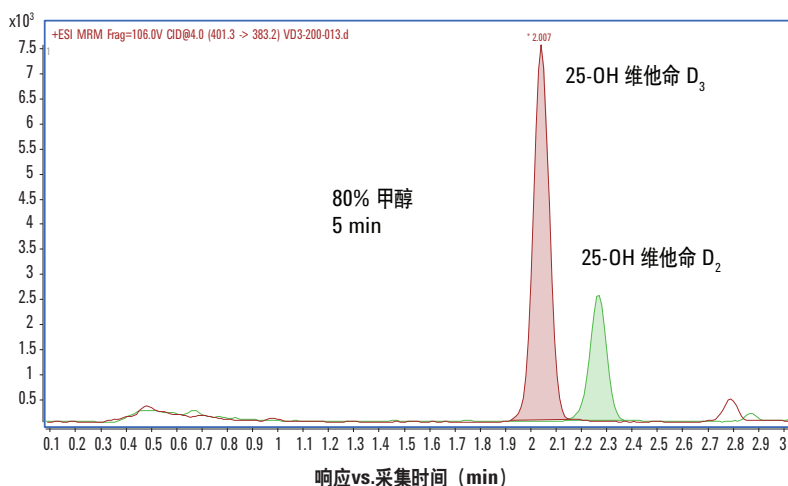
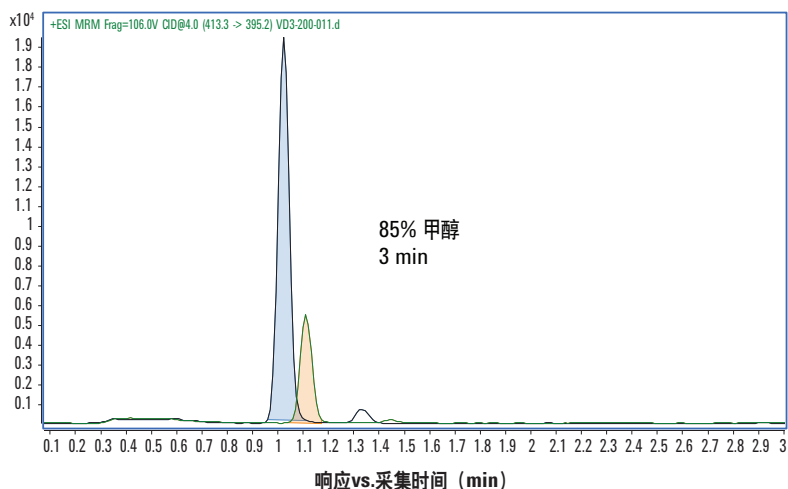


如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

安捷伦 Poroshell 120 EC-C18 色谱柱对维他命 D2/D3 的 1 维分离

Poroshell 120 实现了对血浆中维他命 D2/D3 非常快速的液相色谱-串联质谱分析。在色谱分离的前提下比较了不同等度条件下的分离速度。

色谱柱: Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 μm
 流动相: A: 水 + 0.1% 甲酸
 B: 甲醇 + 0.1% 甲酸
 流速: 0.5 mL/min
 进样体积: 10 μL
 温度: 50 °C
 自动进样器温度: 5 °C
 针头清洗: 冲洗端 (50:25:25, 异丙醇: 甲醇: 水) 5 s
 等度分析: A: 20% B: 80%
 分析时间: 5.0 min



耐用性能 - 即使进样 3000 次以上

这个测试证明了 Poroshell 120 色谱柱卓越的长效性, 在 3000 次进样后仅有较小的性能损失。稳定性由保留时间的一致性来表示 (%RSD)。

分析物	%RSD (RT)	分析物	%RSD (RT)	分析物	%RSD (RT)
吗啡	0.7	哌替啶	0.4	三唑仑	0
可待因	0.4	唑吡坦	0.3	纳曲酮	0.1
氢可酮	0.4	芬太尼	0.1	氯氮	0.1
MDMA	0.3	EDDP	0.1	去甲地西洋	0.1
诺芬太尼	0.2	硝西泮	0.1	丁丙诺啡	0.3
海洛因	0.2	丙氧芬	0.1	古柯乙烯	0.2
哌甲酯	0.2	丁丙诺啡	0.3	11-去-9-羧基-delta9-thc	0

安捷伦 Poroshell 120 色谱柱帮助您提高 超高压液相色谱方法的灵活性

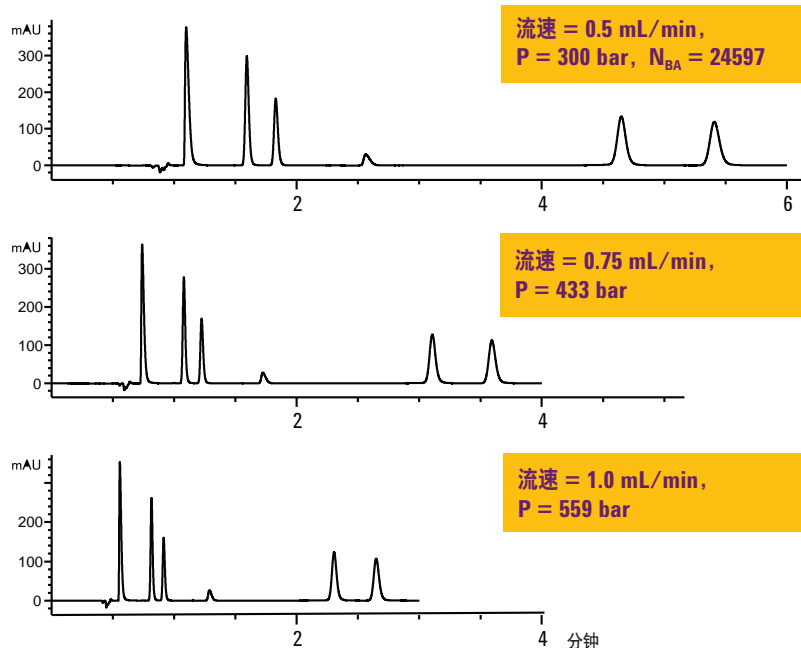


因为 Agilent Poroshell 120 色谱柱可以耐受 600 bar 的压力，您可以将它成功用于您的 UHPLC 方法中——可以使用很长的色谱柱、使用更高的流速，以及粘性溶剂。

Agilent Poroshell 120 EC-C18 色谱柱用于在 UHPLC 上的快速分离

本示例展示了使用产生较高压力流动相的快速分离。在上面色谱图中，使用 3.0 mm 内径色谱柱，流速为 0.5 mL/min，压力低于 400 bar——这是一个典型的液相色谱分离。

虽然上图的分离速度已经很快（仅不到 6 分钟），但是在中图和下图显示您仍可以通过增加流速使运行时间缩短到 3 分钟以下。这些更快的分析需要 400-560 bar 的压力；采用安捷伦 1200 Infinity 系列的灵活升级选件能够帮助您充分发挥 UHPLC 的优势。



粘性更大的溶剂，例如甲醇，可用在 HPLC 或 UHPLC 的工作压力下

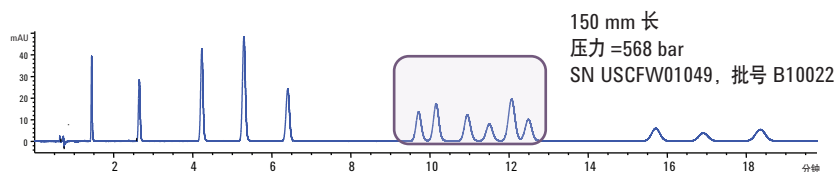
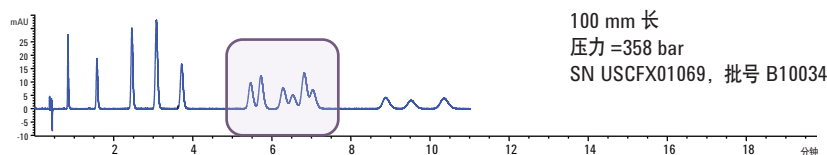
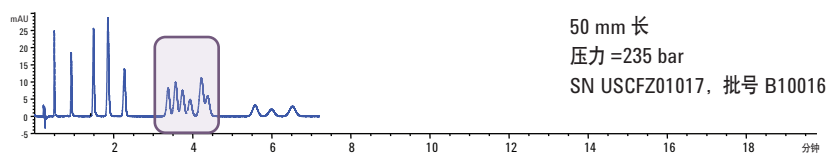
色谱柱:	Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μ m 部件号 695975-302	样品:	1. 糖精 2. 咖啡因 3. P-羟基苯甲酸 4. 阿斯巴甜 5. 脱氢乙酸 6. 苯甲酸
流动相:	65% A: 0.2% 甲酸 35% B: 甲醇 等度洗脱		
流速:	采用不同流速		
进样量:	1 μ L		
柱温箱:	26 $^{\circ}$ C		
检测器:	检测波长: 220, 4 纳米; 参比 = 关		

如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

在 HPLC 或 UHPLC 上使用安捷伦 Poroshell 120 短柱和长柱采用 EPA 8330 方法分离的对比

您可以灵活选择较长的 Poroshell 120 色谱柱实现高分离度分离。从示例中可以看出随着色谱柱逐渐加长，分离度得到改善，压力逐渐增加（最长的色谱柱可达到 UHPLC 的工作压力）。

请注意分离度是受柱长的影响，而不是柱填料批次的影响，进一步证明了 Poroshell 120 色谱柱卓越的重现性。



对于任何分离，柱长、分离度和分析时间的平衡都是至关重要的

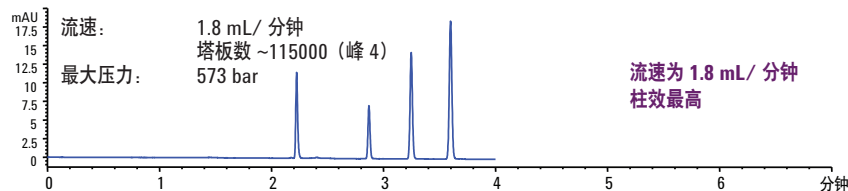
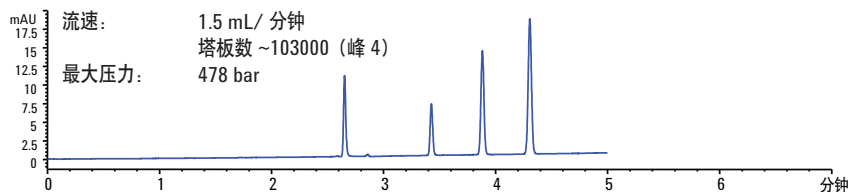
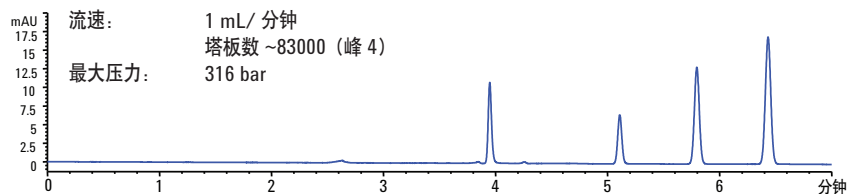
色谱柱: Poroshell 120 EC-C18, 2.7 μm
流动相: 25% 甲醇, 75% 水
流速: 1 mL/分钟
温度: 44 $^{\circ}\text{C}$

串接 Agilent Poroshell 120 系列色谱柱在 HPLC 和 UHPLC 上的工作压力下都可提供最高柱效

因为 Poroshell 120 的优势之一是它的低压，您可以串接色谱柱以达到单位时间内的最佳分离效果。这种方法使复杂的样品得到更好的分离。

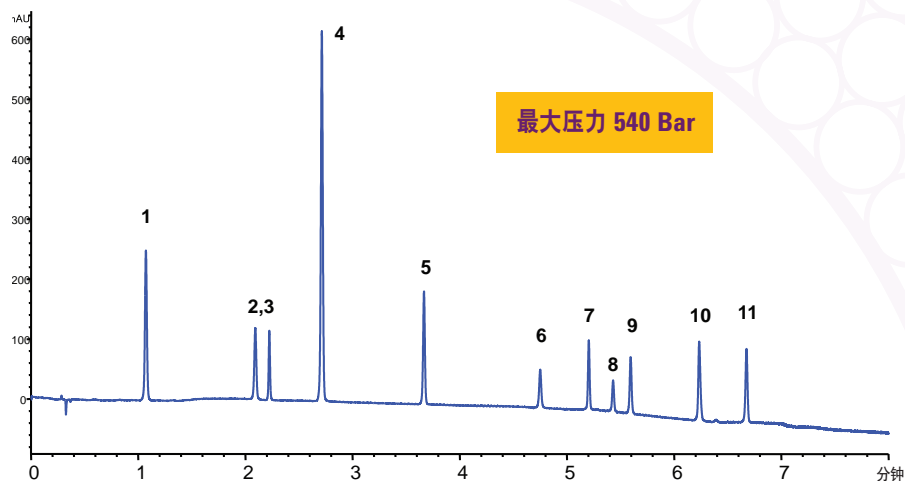
峰号	峰号化合物	塔板数	k'
2	苯乙酮	114120	0.29
3	苯	109931	0.46
4	甲苯	114800	0.65

三个安捷伦 Poroshell 120 EC-C18, 4.6 x 150 mm, 2.7 μm 色谱柱串接实现超高效分离 —— 部件号 693975-902



止痛药中 11 种常见化合物在安捷伦 Poroshell 120 EC-C18 色谱柱上的快速分离

在本例中使用 Poroshell 120 色谱柱，通过提高流速加快 11 种常见镇痛化合物的分离。



色谱柱: Poroshell 120 EC-C18, 4.6 x 100 mm, 2.7 μ m
部件号 695975-902

流动相: A: 水 +0.1% 甲酸
B: 乙腈

流速: 3.5 mL/ 分钟

进样量: 5 μ L

温度: 40 $^{\circ}$ C

检测器: 二极管阵列检测器, 波长: 254 纳米

样品:

1. 对乙酰氨基酚
2. 咖啡因
3. 2-乙酰氨基酚
4. 乙酰胺
5. 对乙酰氨基苯乙醚
6. 舒林酸
7. 吡啶苯噻酰胺
8. 托美汀
9. 酮洛芬
10. 二氟苯水杨酸
11. 扶他林

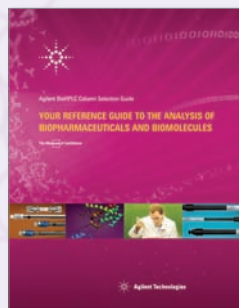
这些信息丰富的选择指南是您实验室的宝贵资源



安捷伦高效液相色谱柱选择指南已经更新并扩展了内容，为安捷伦色谱柱系列中所有小分子色谱柱提供了简介和订购细节。也包括快速液相色谱 / 超高压液相色谱和方法开发的技巧指导。

现在下载，请访问

www.agilent.com/chem/getLCGuide



安捷伦 BioHPLC 色谱柱选择指南包括生物分子分离的方法开发、溶剂选择、流动相改性和优化的建议和技巧及丰富的示例。

下载请访问

www.agilent.com/chem/getLCGuide

如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

方法转换化繁为简



许多在较长的 5 μm C18 色谱柱上开发的方法能够快速轻松地转移到较短的 Poroshell 120 色谱柱上应用。USP 规定的新变化使得从传统方法向较新技术（如安捷伦 Poroshell 120 色谱柱）转变更为容易。它能明显增加色谱通量并降低费用。

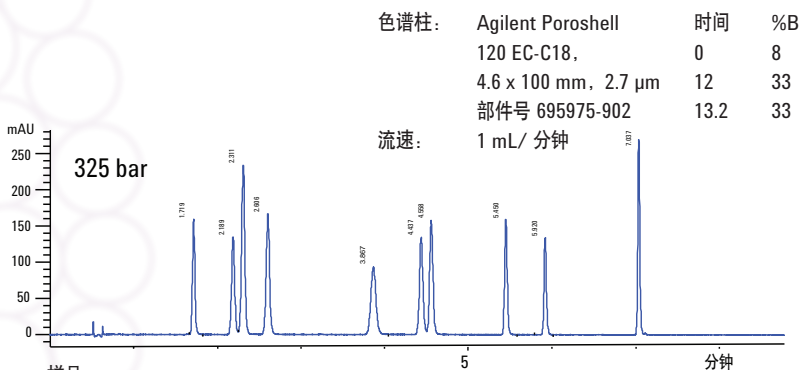
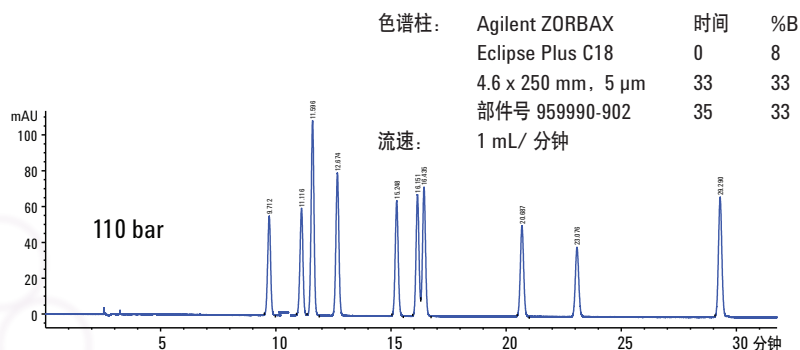
在下面几页中，我们将向您展示包括 USP 方法的五种分离在 Poroshell 120 色谱柱上是如何重现的，以及对相同的分离如何实现比 5 μm 色谱柱快 3-5 倍的分离速度。

安捷伦 Poroshell 120 和 ZORBAX 色谱柱间转换方法以节约时间或增强扩展性

在这个例子中，一个复杂的方法从 ZORBAX Eclipse Plus C18 250 mm, 5 μm 色谱柱被转移到 100 mm 长的 Poroshell 120 EC-C18 色谱柱上。除了需要调整较短色谱柱上的梯度洗脱时间，所有条件保持不变。

如图中所见，分离是完全相同的，而时间却从 30 分钟锐减到 7 分钟，效率大幅提升。

请注意两次分离均使用老款的 Agilent 1100 系列仪器，说明即使是梯度方法也可以轻松转换，同时保持压力低于 400 bar。



样品:

1. 磺胺嘧啶
2. 磺胺噻唑
3. 磺胺吡啶
4. 磺胺甲噻唑
5. 磺胺二甲噻唑
6. 磺胺二甲恶唑
7. 磺胺甲氧基吡嗪
8. 磺胺氯达嗪
9. 磺胺甲基异恶唑
10. 磺胺二甲氧嗪

流动相:

- A: 水 + 0.1% 甲酸
B: 乙腈 + 0.1% 甲酸

分析萘普生片的 USP 方法 —— 在 HPLC 的工作压力下，使用 Agilent Poroshell 120 色谱柱的分析速度提高 4.5 倍

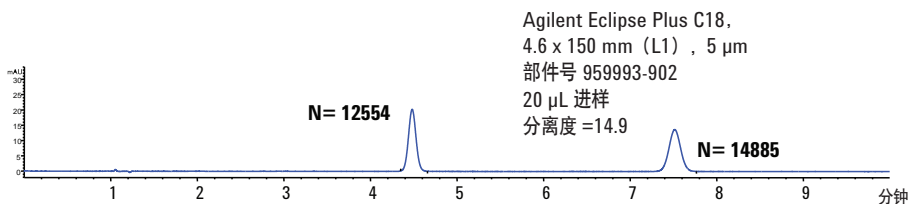
对萘普生的分离证明了使用 Poroshell 120 色谱柱不需要改变流速和流动相就可以轻松实现方法转换。

上图所示为安捷伦 Eclipse Plus C18 色谱柱上的 USP 分析，实现了尖峰、3 倍所需效率以及 15 的分离度。

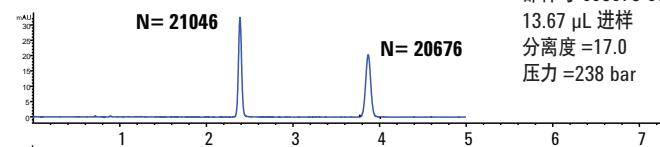
中图，使用安捷伦 Poroshell 120 EC-C18 色谱柱（100 mm 长），获得了更好的柱效和分离度，速度比原始方法快了两倍。并且压力仅为 238 bar，这种等度方法是 HPLC 的极好选择。

下图，使用安捷伦 Poroshell 120 EC-C18 色谱柱（50 mm 长），仍然满足效率和分离度的需要，但分析速度比使用 5 μm 色谱柱快 4.5 倍，而且，压力仅为 133 bar，与 HPLC 方法完全兼容。

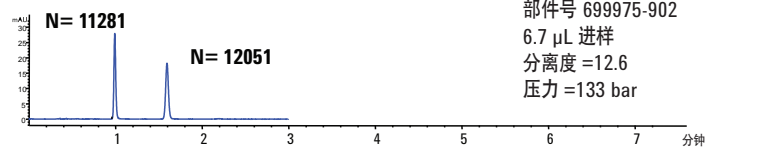
方法要求 $N > 4000$ ， R_s 大于 11.5



2 倍的速度



4.5 倍速度



Agilent Poroshell 120 色谱柱是在 HPLC 工作压力下快速分析的卓越之选

流动相: 50:49:1
乙腈:水:乙酸
流速: 1.2 mL/min
样品:
1. 萘普生
2. 苯丁酮

参见我们的视频教程，学习如何实现萘普生方法向 Poroshell 120 色谱柱的方法转换，优化您的 LC 系统，获得最佳结果。

请访问如下网址：

www.agilent.com/chem/poroshell120video:cn

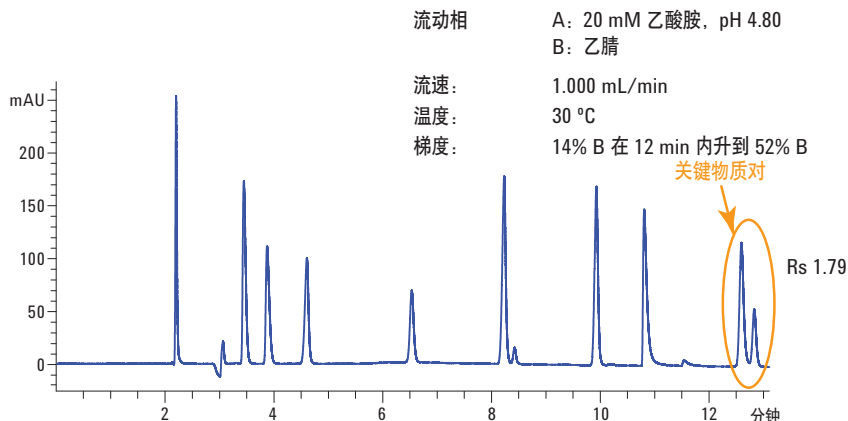


如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

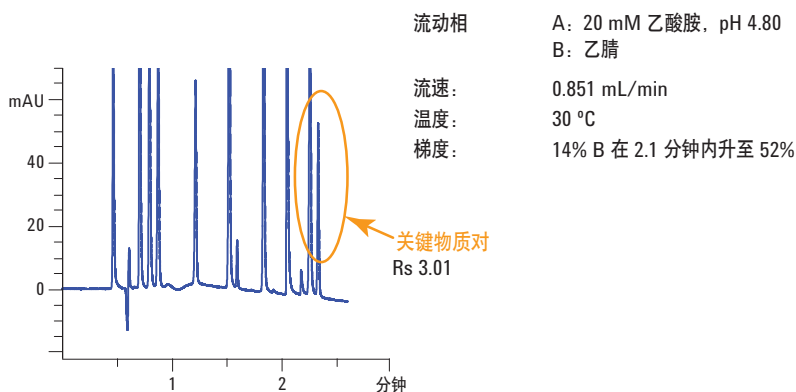
快速低压分析

这里，分析了 11 种非营养食品及饮料添加剂，使用安捷伦 Poroshell 120 EC-C18 色谱柱替换 5 μm ZORBAX Eclipse Plus C18 色谱柱，方法转换的结果是分析时间从 13 min 以上减小到 3 min 以下。溶剂消耗减少 80% 以上，关键组分的分离度从 1.8 提高到 3.0。

5 μm Agilent ZORBAX Eclipse Plus, $P_{\text{max}} = 120 \text{ bar}$



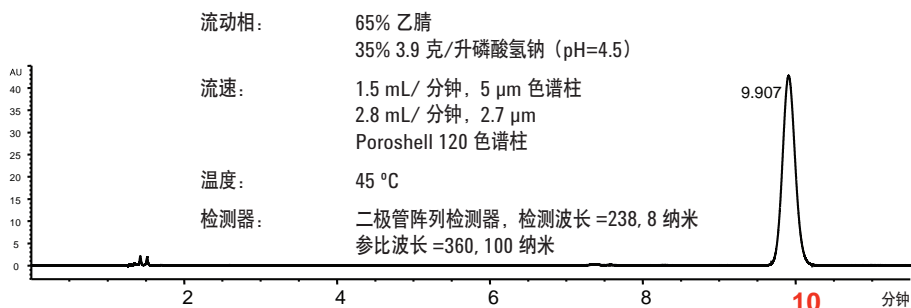
Agilent Poroshell 120, $P_{\text{max}} = 356 \text{ bar}$



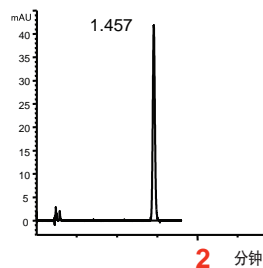
Poroshell 120 对辛伐他汀的快速分析

分析辛伐他汀片的 USP 方法很容易转换为使用 Poroshell 120 色谱柱的方法，前者的分析时间需要 10 分钟，而使用 Poroshell 120 色谱柱比之快 5 倍。请注意，我们使用了 75 mm 的 Poroshell 120 EC-C18 色谱柱代替 250 mm 的长色谱柱，柱长缩短了 70%，这仍可以看作一种方法调整。Poroshell 120 EC-C18 固定相与其他 USP L1 固定相相似，因此得到的结果近似，但速度更快。

Agilent ZORBAX Eclipse Plus C18, 4.6 x 250 mm, 5 μm – 部件号 959990-902



Agilent Poroshell 120 EC-C18, 4.6 x 75 mm, 2.7 μm – 部件号 697975-902



	USP 要求	5 μm (1.5 mL/min)	2.7 μm (2.8 mL/min)
T_R	n/a	9.907	1.457
k'	> 3.0	5.962	5.122
N	> 4500	16939	14439
T_f	< 2.0	1.09	1.10

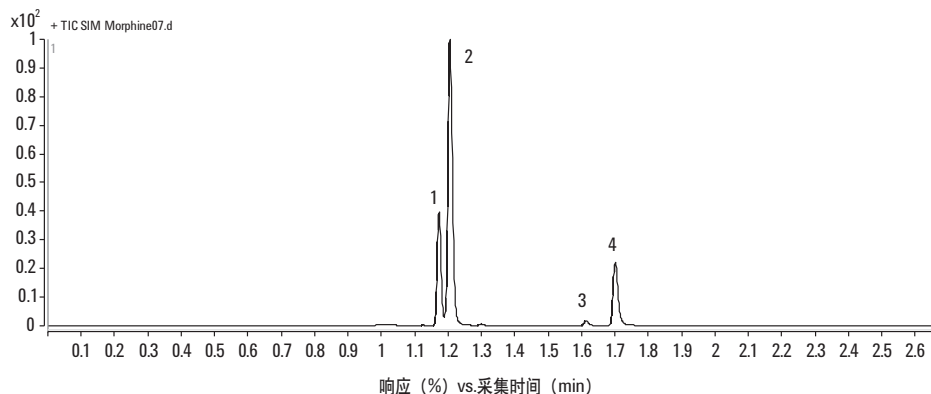
使用 Poroshell 120 HILIC 色谱柱对吗啡及其代谢物的分离

越来越多的实验室开始在药物发现和开发的早期使用 HILIC，原因如下：

- 获得质谱兼容性
- 提高极性化合物及其极性更大的降解产物的保留性
- 提高液质分析的灵敏度

吗啡及其代谢物的分离是快速、有效的 HILIC 液质联用方法的一个示例。这里可以看到，作为一种保留性能较差的高水性反相方法，Poroshell 120 HILIC 色谱柱使这些极性化合物在 2 min 以内得到完全分离，并且具有完美的峰形和效率。

Agilent Poroshell 120 HILIC, 2.1 x 100 mm, 2.7 μ m

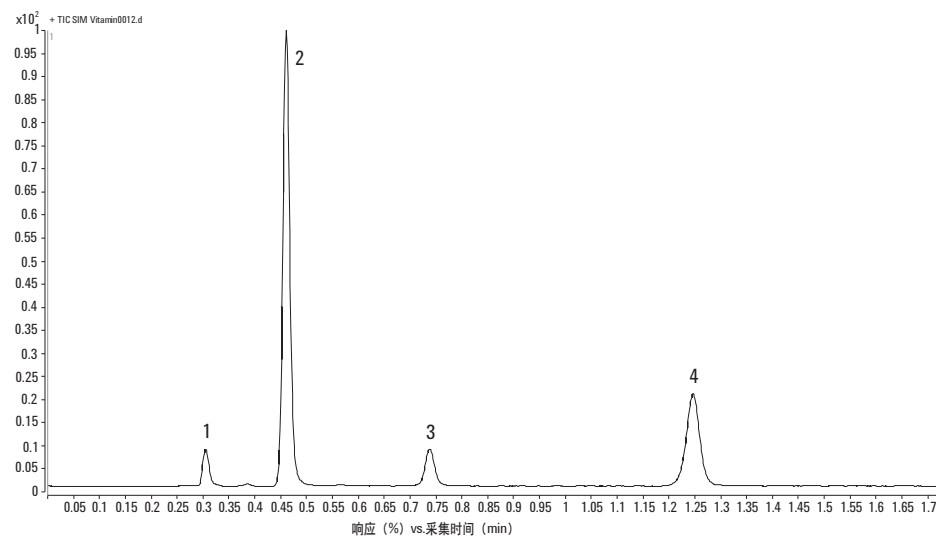


1. 去甲吗啡	流动相:	A: 100 mM NH ₄ HCO ₂ pH 3.2	时间	%B
2. 吗啡		B: 乙腈, 100 mM NH ₄ HCO ₂ pH 3.2 (9:1)	0	100
3. M6G	流速:	0.8 mL/min	0.44	100
4. M3G	温度:	25 °C	1.93	55
	压力:	70 到 505 Bar		
	系统:	Agilent 1290 和 6140 QQQ		

使用 2.1 x 100 mm, 2.7 μ m Poroshell 120 HILIC 色谱柱分析维生素 B 及其相关化合物

HILIC 避免了使用己烷磺酸等离子对试剂，这种试剂被普遍用于分离维生素 B 所用的流动相中。HILIC 也提高了液质分析的兼容性和保留性。

Agilent Poroshell 120 HILIC, 2.1 x 100 mm, 2.7 μ m



1. 4-氨基苯甲酸	流动相:	乙腈 100 mM NH ₄ HCO ₂ pH 3.2 (9:1)
2. 烟酰胺	流速:	0.7 mL/min
3. 核黄素	温度:	25 °C
4. 烟酸	压力:	240 Bar
	系统:	Agilent 1290 和 6140 QQQ

如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn



安捷伦 Poroshell 120 色谱柱： 日常问题强有力的解决之道

2 μm 色谱柱入口筛板可耐受最脏的样品

亚二微米填料提供分析速度和分离度的极大优势，但是由于色谱柱入口使用 0.5 μm 的筛板很容易被脏样品堵塞。Poroshell 120 色谱柱使用了标准的 2 μm 筛板解决了这个问题，有效地防止了脏样品（包括未过滤的血浆）的堵塞。

Poroshell 120 色谱柱的碱性化合物样品负载能力可以与亚二微米色谱柱相媲美

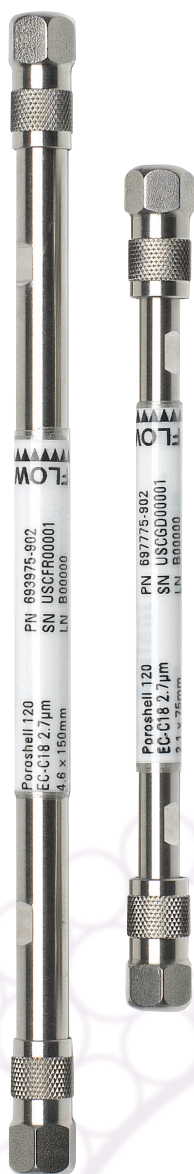
非多孔小粒径填料能为样品提供相互作用的比表面积很小，限制了载样量。而 Poroshell 120 色谱柱采用较大的比表面积的设计，极大地提高了样品负载能力。实际上，即使对于最难分离的碱性化合物，Poroshell 120 色谱柱的负载能力也可以与 1.8 μm 色谱柱相媲美。

理想峰形确保最精确的分析结果

与其他表面多孔层色谱柱相比，Poroshell 120 色谱柱能提供优异的峰形，尤其在 pH 为 6-7 时。

Agilent 1100 和 1200 系列液相色谱系统可轻松优化为适用 Poroshell 120 色谱柱

Poroshell 120 色谱柱的内在性质，使其成为大多数 HPLC 和 UHPLC 仪器的理想色谱柱，包括新的 1200 Infinity 系列液相色谱仪。对 1100 和 1200 系列液相色谱系统，仅需对配置稍微调整（如流速、连接管线长度及内径、流通池体积、检测器峰宽设置等）即可得到优异结果，而压力更低，效率更高。



Agilent Poroshell 120 色谱柱的 2 μm 筛板，有效防止堵塞

即使检测“脏”样品，如未过滤的血浆，Poroshell 120 色谱柱也能有效防止堵塞。本示例中，我们沉析出蛋白质，但是没有离心和过滤样品。即使在这样的条件下进样 2500 次之后，压力也没有任何上升。

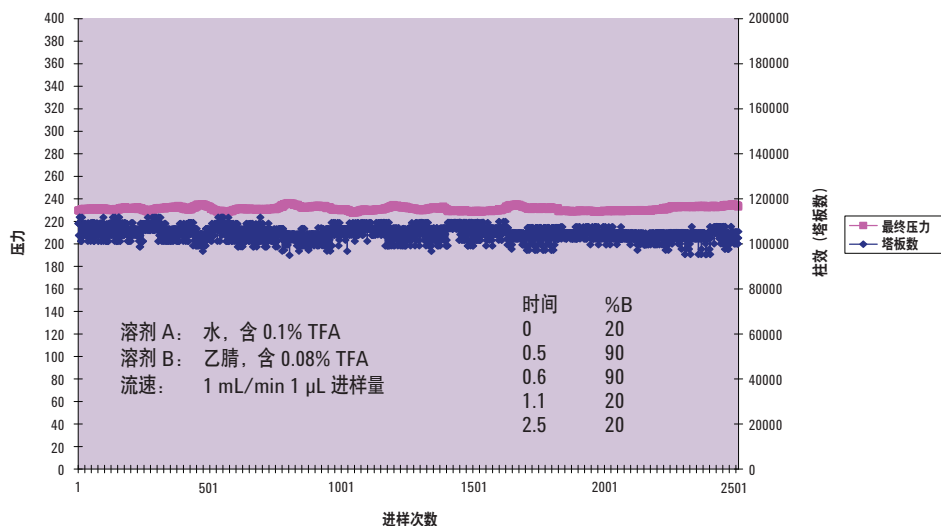
色谱柱: Agilent Poroshell 120 EC-C18,
3.0 x 50 mm, 2.7 μm
部件号 699975-302

进样量: 1 μL

样品: 沉淀血浆
血浆: 20/80 水-乙腈 (含有 0.1% 甲酸):
50/50 水-乙腈 (含二氟苯水杨酸
10 μg/mL) =2:7:1 (二氟苯水杨酸最终
浓度为 1 μg/mL), 摇匀, 静置 10 分钟
不离心, 不过滤

仪器: Agilent 1200 RRLC (SL)

血浆中的二氟苯水杨酸

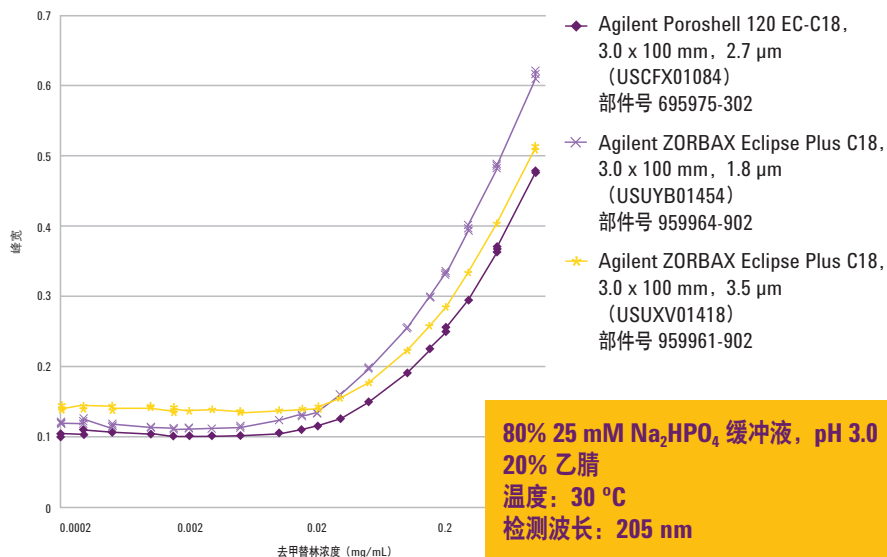


实现与全多孔填料相媲美的载样量

在本示例中，我们在几种安捷伦色谱柱和竞争厂商的色谱柱上进样去甲替林（一种碱性化合物）。可以看到，Agilent Poroshell 120, 2.7 μm 色谱柱与 1.8 μm 色谱柱具有相同的载样量，而 3.5 μm 色谱柱有一个较大的初始峰宽，从而影响分离度。

在这些色谱柱上的载样量非常典型，证明了 Poroshell 120 色谱柱可以被放心地用于碱性化合物的分离。

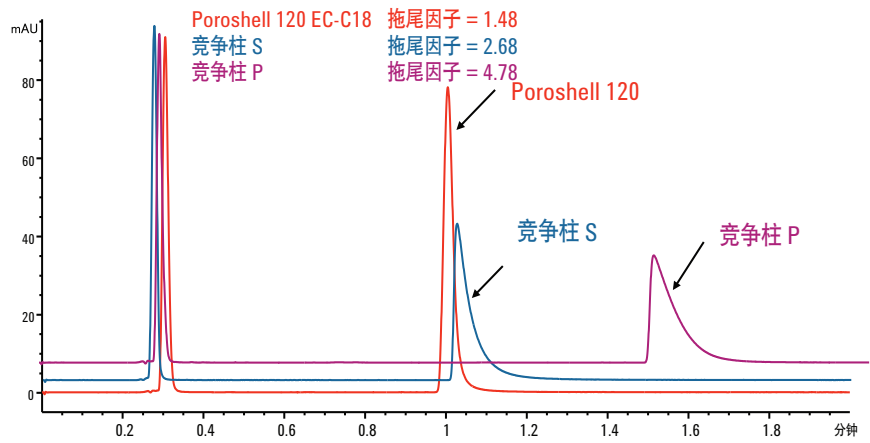
去甲替林碱载样量



如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

安捷伦 Poroshell 120 色谱柱对碱性化合物的分析具有更好的峰形和结果

本示例是对另一种碱性化合物的分离，证明了 Poroshell 120 色谱柱如何在分析挑战性化合物中更胜一筹。



色谱柱: 4.6 x 50 mm
部件号 699975-902

流动相: 20 毫摩尔 40% 磷酸氢二钠, pH 为 7.0
60% 乙腈

流速: 1.5 mL/分

温度: 24 °C

检测器: 二极管阵列检测器, 254 纳米, 2 μ L 流通池

样品: 2 μ L 进样, 250 μ g/mL 阿米替林,
含 50 μ g/mL 腺啶啉的水 / 乙腈 (9:1) 溶液



蛋白质和多肽的快速、可靠分离

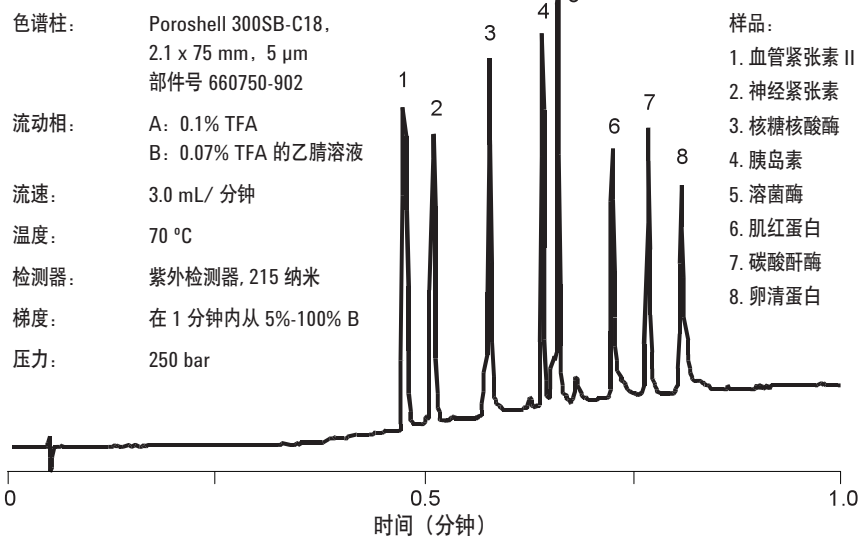


安捷伦 Poroshell 色谱柱是用于分离和表征包括完整和消化蛋白质等复杂生物分子在内的理想选择，安捷伦 Poroshell 300 色谱柱是用于快速分析完整蛋白质的理想选择。安捷伦 Poroshell 120 色谱柱极其适用于肽谱分析，因为它们在较短的分析时间内能够获得比传统的 5 μm 色谱柱更高的分离度。

高流速 2.1 mm 内径的 Poroshell 300 色谱柱用于蛋白质的高分离度分析及快速分离

Poroshell 300 色谱柱，具有较大孔径和较薄的外层，是快速分离完整蛋白质的可靠选择。本示例表明不到 1 分钟就实现了全部分离。

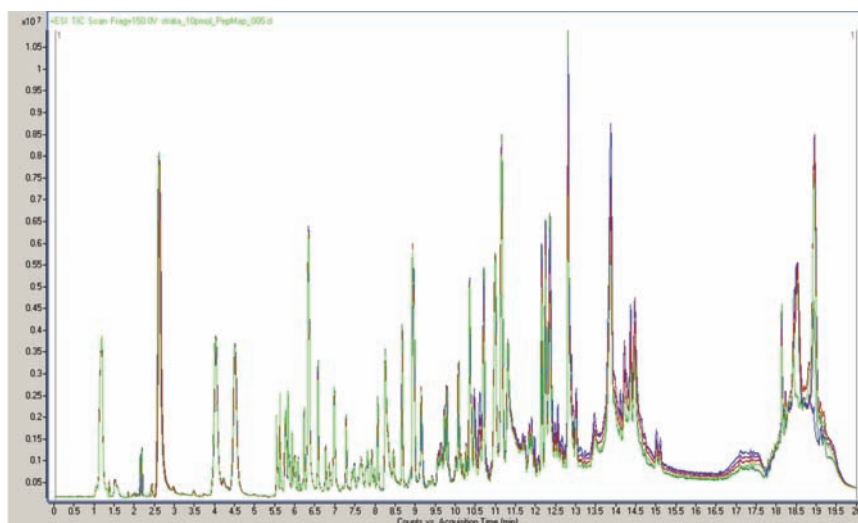
凭借表面多孔颗粒的快速传质能力，Poroshell 300 色谱柱可用于较高流速下蛋白质的极速高效分离。



如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

在安捷伦 Poroshell 120 色谱柱上对单克隆抗体胰蛋白酶肽谱进行 5 次重复分析

此示例是使用 Poroshell 120 色谱柱分离酶解单克隆抗体的肽谱，得到了高分离度和高重现性结果。



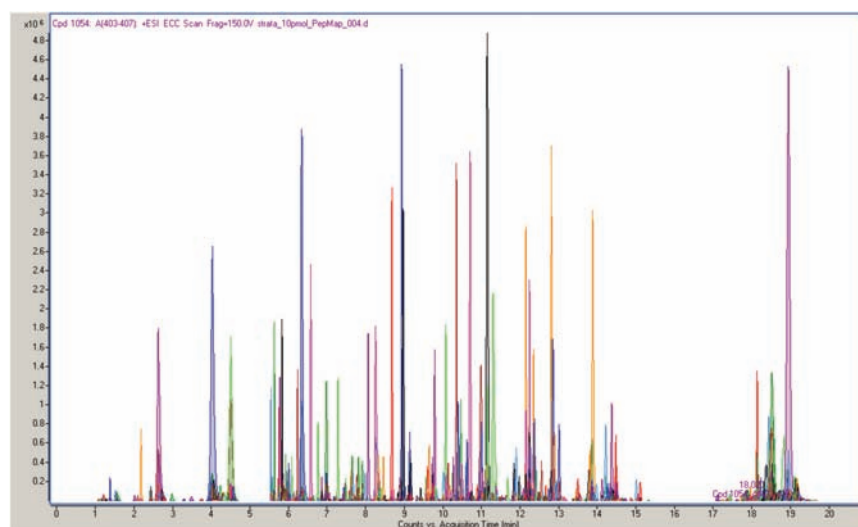
单克隆抗体胰蛋白酶肽谱的 BioConfirm 分子特征提取

通过使用 BioConfirm 分子特征提取软件，能够证明相同的单克隆抗体的轻链和重链上是 100% 的序列覆盖。

四极杆 - 飞行时间质谱参数

离子源：电喷雾正离子模式

气体温度：325 °C
 干燥气流速：10 L/min
 雾化器：40 psi
 毛细管电压：4000 V
 碎裂电压：150 V
 锥孔体电压：65 V
 八极杆 1 射频电压：750 V
 MS：4 Hz
 扫描范围：200-3200 m/z
 参比质量：922.009798
 采集模式：扩展动态范围模式 (2GHz)



色谱柱:	Poroshell 120 SB-C18, 3.0 x 150 mm, 2.7 μm 部件号 683975-302	时间	%B	时间	%B
流动相:	A: 水, 0.1% 甲酸 B: 乙腈, 0.1% 甲酸	0	2	15.1	90
流速:	0.3 mL/分钟	3	2	17	90
柱温:	40 °C	13	45	18	2
检测器:	四极杆 - 飞行时间质谱, 电喷雾正离子模式	15	65		
梯度:	见表				

使用 Poroshell 120 色谱柱分离更多的峰：蛋白裂解液分析

120Å 孔径柱尤其适合于蛋白质裂解物中小分子亲水多肽和肽段的高分离度快速分析。

流动相: A: 水 (0.1% TFA)
B: 乙腈 (0.08% TFA)

流速: 0.30 mL/min

进样量: 10 μL

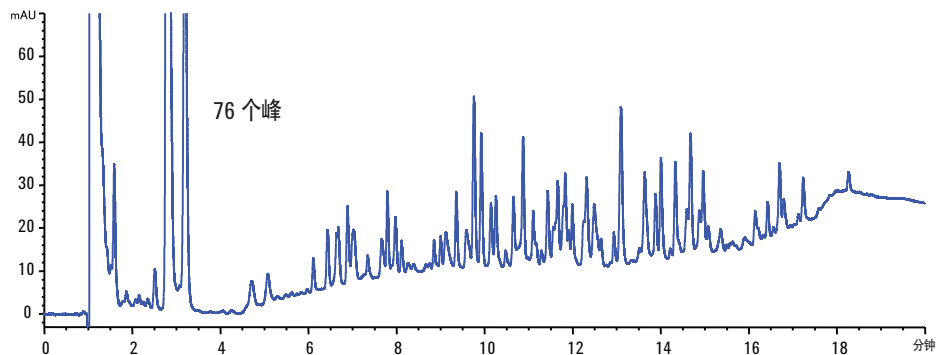
温度: 40 °C

样品: BSA 胰蛋白酶消化液 (供应商)

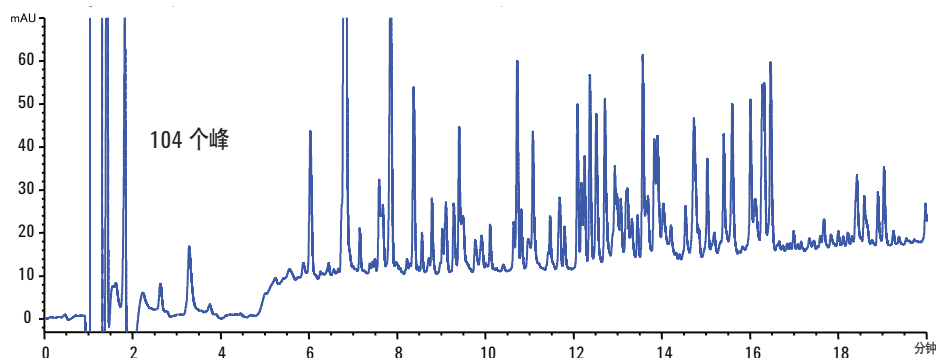
梯度: 保持 3% B, 3 min
35-65% B; 30min
4 min 后运行时间

DAD: 215 nm

Phenomenex Aeris Widepore, XB-C18, 2.1 x 150 mm, 3.6 μm



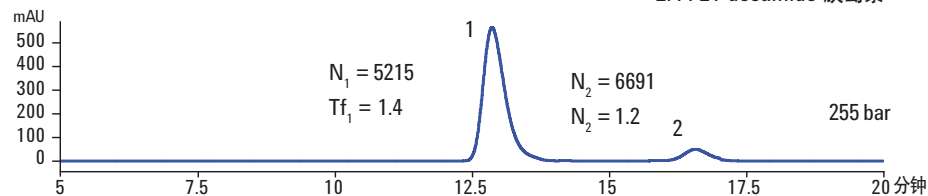
Poroshell 120 EC-C18, 2.1 x 150 mm, 2.7 μm



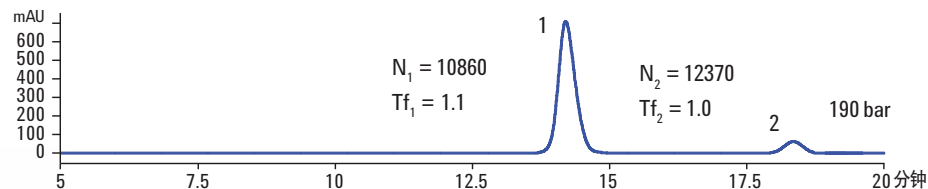
胰岛素分析：把 1.8 μm ZORBAX StableBond 色谱柱转换为 Poroshell 120 色谱柱以提高效率

由于具有更大的孔径及在 120Å 孔中更快速的扩散，Poroshell 120 SB-C18 色谱柱的柱效是 ZORBAX RRHD SB-C18 80Å 色谱柱的 2 倍。Poroshell 120 色谱柱是用于小蛋白质胰岛素或其它多肽分析的理想选择，提供较低压力下的较高柱效。

Agilent ZORBAX SB-C18, 4.6 x 100 mm, 1.8 μm



Agilent Poroshell 120 SB C18, 4.6 x 100 mm, 2.7 μm — P/N 693775-902



如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

哪种快速液相色谱柱最适合您？

安捷伦提供最广泛的快速液相色谱柱，包括 Poroshell 120、ZORBAX 快速分离高分辨（RRHD）、1.8 μm （稳定到 1200 bar）色谱柱及 ZORBAX 快速分离高通量（RRHT）、1.8 μm （稳定到 600 bar）色谱柱。所有这些色谱柱都具有相似的固定相以确保它们的可扩展性。拥有这些选择，您可以灵活的创建方法优化您现有的分析仪器。

您实验室的现有仪器	安捷伦推荐	原理
UHPLC (1000+ bar) 和 HPLC 仪器 (如安捷伦 1290 Infinity 液相色谱和 1260 Infinity 液相色谱 – 600 bar)	1. Poroshell 120 色谱柱 2. ZORBAX RRHD 1.8 μm	Poroshell 120 是在两种仪器类型上都能使用的易用色谱柱。ZORBAX RRHD 能帮助您优化 1290 Infinity 液相色谱的性能, 进行 UHPLC 分离。
仅 400-600 bar HPLC 系列 —— Agilent 1200 系列、Agilent 1100 系列 (400 bar) 以及 1220 Infinity LC 或 1260 Infinity LC (600 bar)	1. Poroshell 120 色谱柱 2. ZORBAX Eclipse Plus 3.5 μm 和 5 μm	利用 Poroshell 120, 您可以提高旧型号 400 bar 仪器的性能, 并且使新型 600 bar UHPLC 仪器获得更佳的性能。对于不能转移的已建立方法, ZORBAX Eclipse Plus 色谱柱能提供出色的峰形和性能。
UHPLC 仪器 (安捷伦 1290 Infinity LC, 其他 1000 bar 以上仪器) 和某些 HPLC 仪器 (例如 1200 LC) 的组合	1. ZORBAX RRHD 1.8 μm 2. Poroshell 120 色谱柱	ZORBAX RRHD 可以在所有这些仪器上获得最佳性能。Poroshell 120 可以在 600 bar 仪器上使用, 以优化其性能。

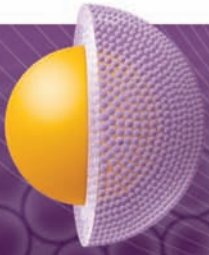
专为色谱工作者设计的简单、可靠的 pH 测试



安捷伦现提供全系列的 pH 计和电极。专为色谱工作者设计, 这些 pH 计为您的实验室提供直观的用户界面设计及出色的耐用性。

了解更多, 请访问

www.agilent.com/chem/AgilentpH



使您的 UHPLC 展现极致性能 更可靠地运行常规分析方法



无论您是需要一台耐用的液相色谱系统运行常规分析，还是需要功能更强的高分离度液相色谱/质谱系统，Agilent 1200 Infinity 系列都能满足您的需要。

1200 Infinity 系列液相色谱使用 Poroshell 120 色谱柱可以提供最高的分离度和灵敏度，同时提高每次分析的分离能力。它们也确保系统间方法轻松转换，而无需方法的再开发或再确认。

了解更多安捷伦 1200 Infinity 系列
无限卓越的性能，请访问：
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无限
超值



1220
Infinity LC

无限
信心



1260
Infinity LC

无限
强大



1290
Infinity LC

如需了解更多安捷伦 Poroshell 120 色谱柱的信息，请访问以下网址：
www.agilent.com/chem/poroshell120:cn

规格和订购信息

安捷伦 Poroshell 120 订购信息 (2.7 μm)

规格 (mm)	EC-C18	EC-C8	苯基 - 己基柱	SB-C18	SB-C8	SB-Aq	Bonus-RP	HILIC	EC-CN
4.6 x 150	693975-902	693975-906	693975-912	683975-902	683975-906	683975-914	693968-901	693975-901	693975-905
4.6 x 100	695975-902	695975-906	695975-912	685975-902	685975-906	685975-914	695968-901	695975-901	695975-905
4.6 x 75	697975-902	697975-906		687975-902					
4.6 x 50	699975-902	699975-906	699975-912	689975-902	689975-906	689975-914	699968-901	699975-901	699975-905
4.6 x 30	691975-902	691975-906		681975-902					
3.0 x 150	693975-302	693975-306	693975-312	683975-302	683975-306	683975-314	693968-301	693975-301	693975-305
3.0 x 100	695975-302	695975-306	695975-312	685975-302	685975-306	685975-314	695968-301	695975-301	695975-305
3.0 x 75	697975-302	697975-306		687975-302					
3.0 x 50	699975-302	699975-306	699975-312	689975-302	689975-306	689975-314	699968-301	699975-301	699975-305
3.0 x 30	691975-302	691975-306		681975-302					
2.1 x 150	693775-902	693775-906	693775-912	683775-902	683775-906	683775-914	693768-901	693775-901	693775-905
2.1 x 100	695775-902	695775-906	695775-912	685775-902	685775-906	685775-914	695768-901	695775-901	695775-905
2.1 x 75	697775-902	697775-906		687775-902					
2.1 x 50	699775-902	699775-906	699775-912	689775-902	689775-906	689775-914	699768-901	699775-901	699775-905
2.1 x 30	691775-902	691775-906		681775-902					

注意: Poroshell 120 色谱柱的压力限值是 600 bar/9000 psi

用于 UHPLC 的安捷伦 Poroshell 120 快速保护柱

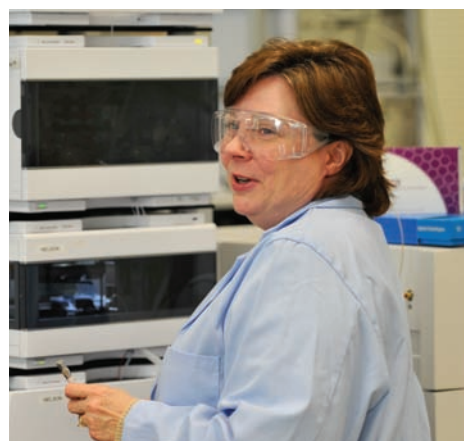
规格 (mm)	EC-C18	EC-C8	SB-C18	苯基 - 己基柱
4.6 x 5	820750-911	820750-913	820750-912	820750-914
3.0 x 5	823750-911	823750-913	823750-912	823750-914
2.1 x 5	821725-911	821725-913	821725-912	821725-914



安捷伦 Poroshell 120 键合相性能指标

键合相	孔径	温度限	pH 范围	封端	碳载量	表面积
EC-C18	120Å	60 °C	2.0-8.0	是	8%	130 m ² /g
EC-C8	120Å	60 °C	2.0-8.0	是	5%	130 m ² /g
Phenyl-Hexyl	120Å	60 °C	2.0-8.0	是	8%	130 m ² /g
SB-C18	120Å	90 °C	1.0-8.0	无	7.5%	130 m ² /g
SB-C8	120Å	80 °C	1.0-8.0	无	4.5%	130 m ² /g
SB-Aq	120Å	80 °C	1.0-8.0	无	专利	130 m ² /g
Bonus-RP	120Å	60 °C	2.0-9.0	三封端	5%	130 m ² /g
HILIC	120Å	60 °C	0.0-8.0	无	N/A	130 m ² /g
EC-CN	120Å	60 °C	2.0-8.0	是	3.5%	130 m ² /g

仅列出典型性能指标



安捷伦 Poroshell 300 订购信息 (5 μm)

产品名称	规格 (mm)	Agilent Poroshell 300SB-C18	Agilent Poroshell 300SB-C8	Agilent Poroshell 300SB-C3	Agilent Poroshell 300Extend-C18
窄径柱	2.1 x 75	660750-902	660750-906	660750-909	970750-902
微径柱	1.0 x 75	661750-902	661750-906	661750-909	971750-902
毛细管	0.5 x 75		5065-4468		
保护柱芯, 4/包	2.1 x 12.5	821075-920	821075-918	821075-924	
保护卡套		820888-901	820888-901	820888-901	
微径保护柱芯, 3/包	1.0 x 17	5185-5968	5185-5968	5185-5968	5185-5968

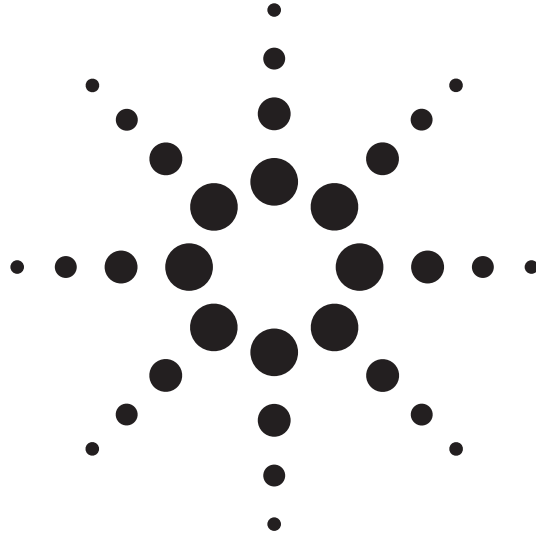
注意: Poroshell 300 色谱柱的工作压力限值 是 400 bar/6000 psi

Agilent Poroshell 300 键合相性能指标

键合相	孔径	温度上限	pH 范围	是否封端
Poroshell 300SB-C18, C8, C3	300Å	90 °C	1.0-8.0	未封端
Poroshell 300Extend	300Å	pH 8 以上 40 °C pH 8 以下 60 °C	2.0-11.0	封端

仅列出典型性能指标

如需了解更多安捷伦 Poroshell 120 色谱柱的信息, 请访问以下网址:
www.agilent.com/chem/poroshell120:cn



食品安全

采用 Poroshell 120 色谱柱快速分析运动饮料中的水溶性维生素

关键词

HPLC Poroshell 120

维生素C 维生素B1

维生素B3 维生素B6

作者

黄桂颖 陈悦娇 马应丹

仲恺农业工程学院

摘要

维生素 C、维生素 B1、维生素 B3、维生素 B6 是运动饮料中常用的食品添加剂，该类添加剂添加过量会对人体健康造成潜在危害，添加不足则达不到运动饮料描述的效果。国家标准对维生素有明确的限量 [1]，但因为不同维生素的国家标准检测方法不一样，检测多种维生素比较耗时费力 [2,3]，采用高效液相色谱法同时检测多种维生素是提高检测效率的有效途径 [4]。由于维生素 C 具有较强的还原性，加热或在溶液中易氧化分解，检测速度越快越准确，超高效液相色谱仪（UHPLC）和亚 2 微米快速液相分析柱有利于维生素检测精密度的提高，使得分析时间和试剂耗量大大降低。但目前基层实验室普遍使用的是常规液相色谱仪，与亚 2 微米快速液相分析柱不兼容，使得快速分析检测很难实现。安捷伦公司最新推出的 Poroshell 120 系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用 Poroshell 120 色谱柱，并采用常规液相色谱仪，对维生素 C、维生素 B1、维生素 B3 和维生素 B6 的同时检测方法加以改进，分析时间和试剂耗量均降低了 1/3，一次分析仅需 3min。现将实验结果报告如下，以供参考。

试验材料与方法

1.1 仪器与试剂

1100 高效液相色谱仪 (Agilent 公司) ; 甲醇 (HPLC 级, 德国默克公司); 水由双蒸水系统制得; 维生素 C (抗坏血酸)、维生素 B1 (盐酸硫胺)、维生素 B3 (烟酰胺)、维生素 B6 (盐酸吡哆辛) 标准品 (分析纯)。

1.2 色谱条件

色谱仪条件: Poroshell 120 SB-C18 柱 (4.6 mm i. d. ×100 mm, 2.7 μ m); 柱温 27 $^{\circ}$ C; 流速 1.0 mL /min; 流动相为磷酸二氢钾溶液 (0.05 mol/L)- 甲醇 =94:6; 紫外检测器: 波长 265nm; 进样体积 3 μ L。

1.3 试验样品

运动饮料, 为市场采购。

1.4 试验方法

1.4.1 标准储备液的配制

用磷酸二氢钾溶液分别和混合配置标准品浓度均为 1000 μ g/mL 的标准储备液, 4 $^{\circ}$ C 避光条件下保存。

1.4.2 混合标准工作液

分别吸取不同体积的储备液, 用磷酸二氢钾溶液配制成浓度分别为 100 μ g/mL、300 μ g/mL、500 μ g/mL、700 μ g/mL、900 μ g/mL 的混合标准工作液。

1.4.3 样品处理

运动饮料用 0.45 μ m 的滤膜过滤后待测。

1.4.4 测定

按 1.2 操作进行, 以保留时间定性, 外标法定量。

结果与讨论

2.1 色谱条件的选择

以 Poroshell 120 柱 (4.6 mm i. d. ×100 mm, 2.7 μ m) 和 Zorbax eclipse XDB C18 (150mm×4.6mm, 5 μ m) 作为分析柱, 以磷酸二氢钾溶液 (0.05 mol/L)- 甲醇的不同配比为流动相进行 HPLC 条件的优化。实验发现, 用 Zorbax eclipse XDB C18 柱, 磷酸二氢钾溶液 (0.05 mol/L)- 甲醇为 95:5 时分离效果最好。用 Poroshell 120 柱, 运动饮料样品在磷酸二氢钾溶液 (0.05 mol/L)- 甲醇为 94:6 分离效果最佳。通过使用 Poroshell120 色谱柱, 每个样品从原来的分析时间 5 分钟缩短至 3min, 提高了工作效率和结果的精密度。标准工作溶液和运动饮料的色谱图分别见图 1 和图 2。

2.2 方法的线性范围和检出限

混合标准工作液的测定按照 1.4 的操作步骤, 以添加剂的峰面积为纵坐标, 含量为横坐标, 绘制标准曲线。本方法中维生素 C、维生素 B1、维生素 B3、维生素 B6 的回归方程和最低检出限 (信噪比为 3: 1) 结果见表 1。

2.3 方法的精密度

对混合标准溶液和样品液重复进样 5 次, 计算样品精密度。

表 1 4 种维生素的回归方程、精密度和检出限

维生素名称	回归方程	相关系数	精密度 (RSD%)	最低检出限 (mg/L)
维生素 C	$\text{Area}=3557.85973 \cdot \text{Amt}+48.998285$	0.9998	0.19	0.001
维生素 B1	$\text{Area}=1467.78391 \cdot \text{Amt}+12.232137$	0.9999	0.29	0.002
维生素 B6	$\text{Area}=334.891305 \cdot \text{Amt}+3.0152544$	0.9999	1.79	0.008
维生素 B3	$\text{Area}=1158.40585 \cdot \text{Amt}+11.031413$	0.9999	1.00	0.002

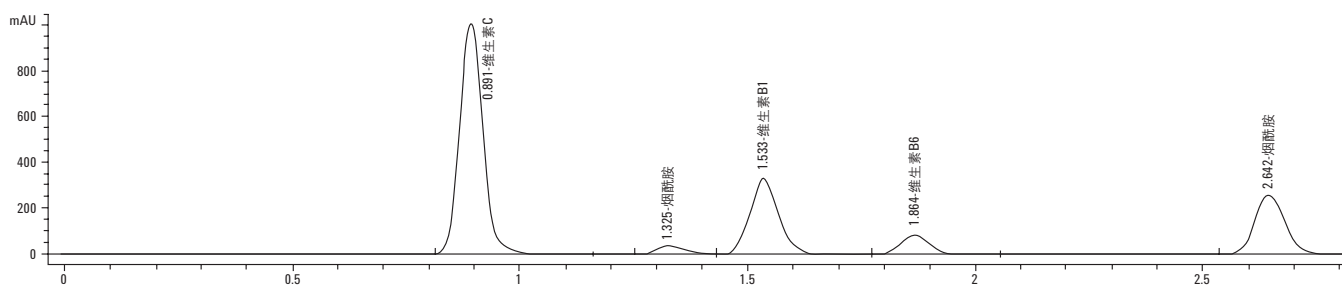


图 1 4 种维生素标准色谱图

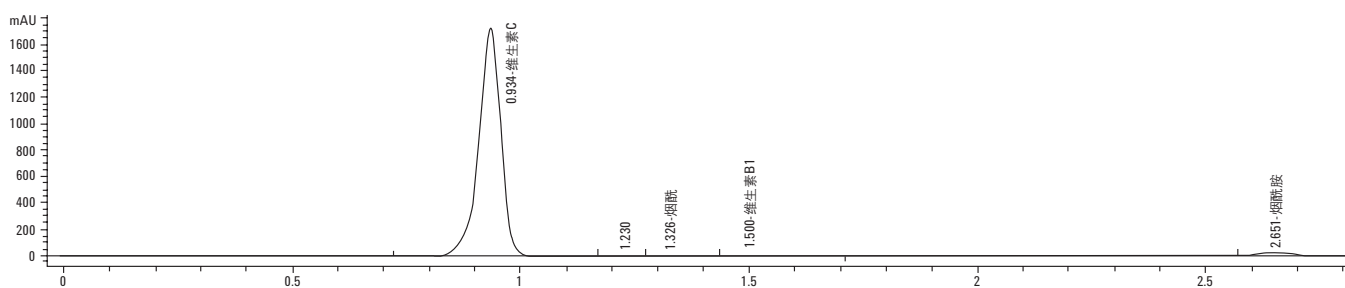


图 2 运动饮料色谱图

2.4 使用传统色谱柱的结果 (图3)

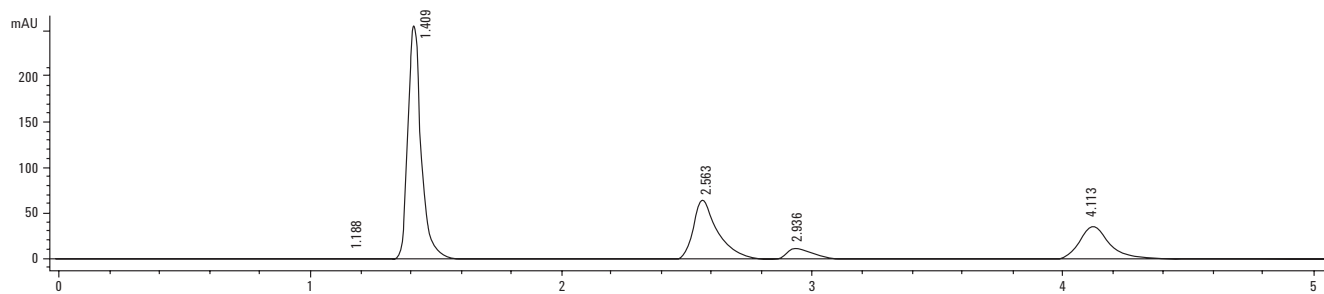


图 3 4 种维生素标准色谱图

色谱条件

1100 液相, DAD 检测器, 标准流通池

色谱柱: Zorbax eclipse XDB C18 (150mm×4.6mm, 5 μ m)

流动相: 5% 甲醇 /95% 磷酸二氢钾 (0.05mol/L)

流速: 1mL/min

柱温: 27 $^{\circ}$ C

进样量: 3 μ L

结论

本法采用 Poroshell120, 在基层普遍使用的常规液相色谱仪上测定维生素 C、维生素 B1、维生素 B3、维生素 B6 共 4 种水溶性维生素。该方法的分析速度迅捷、试剂消耗少、最低检出限和重复性均符合批量样品的检测要求, 结果令人满意, 很适合于基层检测工作的开展。

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采用 Poroshell 120 色谱柱快速分析鸡肉中的磺胺类药物残留

关键词

HPLC

Poroshell 120

磺胺

作者

王凌云

佛山市农产品质量安全监督检测中心

摘要

磺胺类药物 (Sulfonamides, SAs) 具有抗菌谱广、抗菌力强、吸收迅速完全和价廉易得等特点, 广泛应用于畜牧业^[1]。由于 SAs 在动物体内的作用时间和代谢时间相对较长, 往往造成可食性组织中有 SAs 残留, 人们食用了该类药物残留的畜禽产品后会出现急性或慢性中毒, 影响机体的泌尿、免疫系统, 破坏肌肉、肾脏和甲状腺等组织, 如诱发人的甲状腺癌等, 而且还会导致人体内很多细菌对其产生耐药性^[2]。为了保障人类的健康, 各国对 SAs 残留限制越来越严格, 农业部 235 号公告《动物性食品中兽药最高残留限量》中规定 SAs 总量不得超过 100ng/mL, 并规定了 SAs 的液相色谱和液相色谱-质谱联用检测方法^[3]。液质联用法因其价格昂贵, 技术要求高等特点, 不易推广使用。常规液相色谱仪和液相色谱柱普遍应用于基层检测机构, 能够满足几种磺胺类药物残留的测定, 但多组分测定时 (10 种以上) 存在分离难、耗时长等劣势, 使得快速分析检测很难实现, 尤其在应急检测中较难发挥作用。安捷伦公司最新推出的 Poroshell 120 系列表面多孔层色谱柱, 由于其具有低压, 高柱效的特点, 从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用 Poroshell 120 色谱柱, 并采用常规液相色谱仪, 对鸡肉中 12 种磺胺的同时检测方法加以改进, 12 种磺胺得到了很好的分离, 分析时间降低了 1/2, 一次分析仅需 15min。现将实验结果报告如下, 以供参考。

材料与amp;方法

1.1 仪器与试剂

1100 高效液相色谱仪 (Agilent 公司)；甲醇、乙腈 (HPLC 级, 美国默克公司)；水由 Milli-Q -A10 超纯水仪制的；磺胺二甲嘧啶 (SM₂)、磺胺二甲氧嘧啶 (SDM)、磺胺甲噁唑 (SMZ)、磺胺甲氧嘧啶 (SMP)、磺胺嘧啶 (SD)、磺胺喹恶啉 (SQX)、磺胺间甲氧嘧啶 (SMM)、磺胺异恶唑 (SIZ)、磺胺甲基嘧啶 (SMR)、磺胺二甲异嘧啶 (SIM₂)、磺胺对甲氧嘧啶 (SMD)、磺胺吡啶 (SP) 纯度均大于 99%，均购自德国 Dr.Ehrenstorfer GmbH 公司。

1.2 色谱条件

Agilent Poroshell 120 EC-C18 (4.6×75mm 粒径 2.7μm)；柱温 35℃；流速 1.0 mL/min；紫外检测器：波长 270nm；进样体积 20μL；流动相 A：0.1% 乙酸 - 水溶液；流动相 B：乙腈；流动相 C：甲醇。流动相梯度比例见表 1：

表 1 梯度洗脱程序

时间 (min)	流动相 A%	流动相 B%	流动相 C%
0.00	90.5	6.5	3
15.00	63	14	23
16.00	90.5	6.5	3

1.3 试验样品

鸡肉。

1.4 试验方法

1.4.1 标准储备液的配制

精密称取各种 SAs 药物各 10±0.01mg，置于 10mL 棕色容量瓶中，用乙腈溶解并定容，分别配制成 1000μg/mL 的标准储备液，避光保存。

1.4.2 混合标准工作液

分别量取各种 SAs 储备液 (1000μg/mL) 1mL，置于 100mL 棕色容量瓶中，用乙腈定容，配成 10μg/mL 的 12 混合磺胺标准工作液。

1.4.3 样品处理

准确称取 5±0.01g 试样，置于 50mL 聚丙烯离心管中，加入乙腈 25mL，无水硫酸钠少许，涡旋后，以 6000r/min 的速度离心 5min。分离后的残渣再用 25mL 乙腈处理，重复提取一次，合并两次的上清液，45℃ 下旋转蒸发仪减压干燥，残留物用 0.1mol/L HCl 5mL 溶解 (若待测样品含脂肪较多，溶解液加 5 mL 正己烷，涡旋后离心，弃掉正己烷层)，待过柱。

1.4.4 净化

复合阳离子交换柱的平衡：依次用 5mL 甲醇、5mL 去离子水、5mL 0.03mol/L HCl 平衡；

上样：将样品溶液以 5-8s/d 速度流过小柱；

洗涤：依次用 5mL 0.03mol/L HCl、5mL 去离子水、5mL 甲醇洗涤小柱；

洗脱：用 5mL 5% 氯化甲醇洗脱；

收集洗脱液：室温氮气吹干后，用 5mL 流动相溶解，过 0.2μm 有机滤膜，供 HPLC 分析。

结果与amp;讨论

2.1 色谱条件的选择

2.1.1 色谱柱的选择

高效液相色谱法测定磺胺类药物的方法一般采用 C₁₈ 填料的色谱柱，但不同型号的填料对磺胺类药物的色谱行为为有一定的影响，为使 12 种磺胺类药物在色谱柱上得到有效分离，实验选用 Agilent ZORBAX SB-C18 (5μm, 4.6×250mm) 和 Agilent Poroshell 120 EC-C18 (2.7μm, 4.6×75mm) 的色谱柱进行测试。实验发现，流动相采用乙酸水 - 乙腈 - 甲醇体系进行梯度洗脱时，均能对 12 种磺胺类药物达到分离要求，但 SB-C18 的运行时间较长，需要 28min 且基线漂移明显 (图 1)，而 Agilent Poroshell 120 EC-C18 的整个运行时间只需 15min，基线稳定 (图 2)。综合考虑，最后选择使用 Agilent Poroshell 120 EC-C₁₈ (2.7μm, 4.6×75mm) 色谱柱。

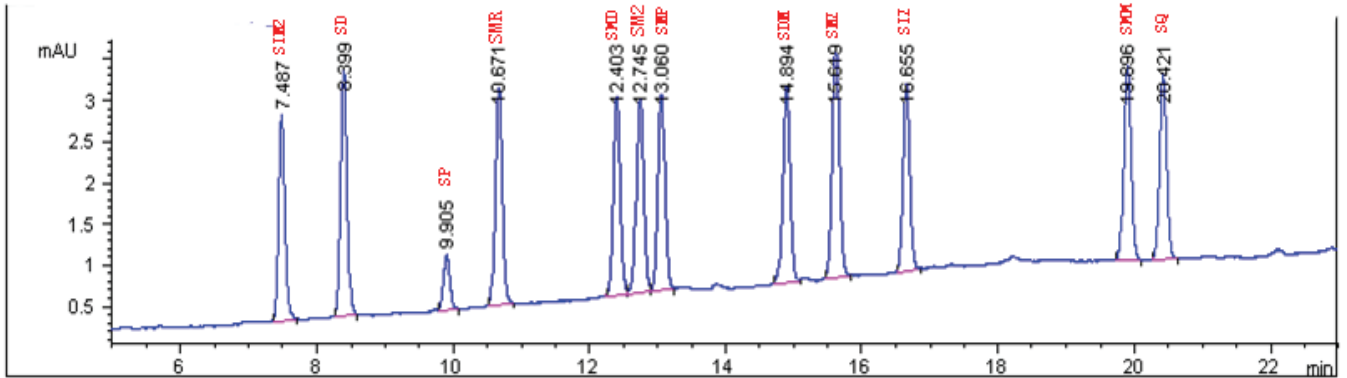


图 1 12 种磺胺类药物的标准色谱图

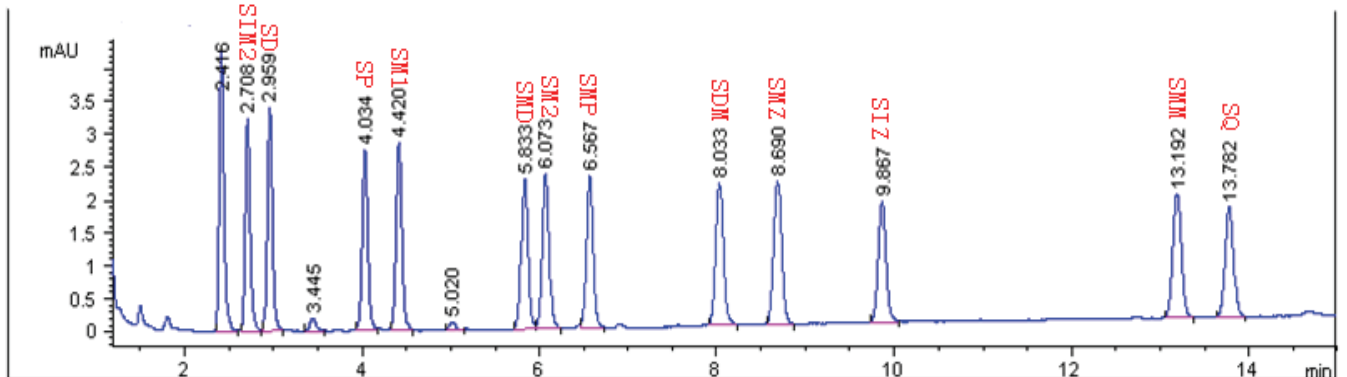


图 2 12 种磺胺类药物的标准色谱图

2.1.2 流动相的选择

磺胺类药物残留分析流动相一般采用缓冲液与甲醇或乙腈按一定比例混合。实验比较了不同浓度的磷酸、乙酸与乙腈、甲醇配比梯度洗脱，结果表明乙酸水-乙腈-甲醇作为流动相，采用梯度洗脱，12 种磺胺类药物能达到基线分离，且峰型尖锐，峰对称性好。12 种磺胺类药物的标准色谱图见图 2。

2.2 旋转蒸发温度对磺胺类药物的影响

实验设立了 30℃、40℃、50℃、55℃、60℃、65℃ 六个温度水平进行试验，结果表明当温度高于 55℃ 时，12 种磺胺类药物的回收率均有不同程度的降低，特别是磺胺异恶唑 (SIZ)，其回收率随温度升高降低相对明显 (回收率约降低 10%)。综合考虑旋蒸时间及温度对药物的影响，确定最佳旋蒸温度为 45℃。

2.3 方法的线性范围

分别取所配制的混合标准工作液 25μL、50μL、100μL、250μL、500μL、1000μL、2500μL 于玻璃管中，室温下氮气吹干后用 5mL 的流动相复溶后，供 HPLC 测定。以峰面积 (Y) 为纵坐标，浓度 (X, μg/mL) 为横坐标，绘制标准曲线。在浓度为 0.05~2μg/mL 范围内，色谱峰峰面积与浓度呈线性相关，线性关系和相关系数见表 2。

表 2: 12 种磺胺药的线性范围、标准曲线回归方程及相关系数

药物		线性范围 (µg/mL)	回归方程	相关系数 R
磺胺二甲异嘧啶	SIM2	0.05-2	$Y=75.40049X-0.382142$	0.99905
磺胺嘧啶	SD	0.05-2	$Y=80.94965X-0.120573$	0.99942
磺胺吡啶	SP	0.05-2	$Y=71.11926X-0.357069$	0.99895
磺胺甲基嘧啶	SMR	0.05-2	$Y=77.12318X-0.370266$	0.99884
磺胺对甲氧嘧啶	SMD	0.05-2	$Y=67.58168X-0.221168$	0.99851
磺胺二甲嘧啶	SM2	0.05-2	$Y=71.50203X-7.01384e^{-2}$	0.99952
磺胺甲氧哒嗪	SMP	0.05-2	$Y=71.58532X-8.71602e^{-2}$	0.99942
磺胺二甲氧嘧啶	SDM	0.05-2	$Y=74.77147X-9.38047e^{-2}$	0.99955
磺胺甲 唑	SMZ	0.05-2	$Y=81.11966X-2.23526e^{-2}$	0.99965
磺胺异恶唑	SIZ	0.05-2	$Y=73.07626X-0.545306$	0.99928
磺胺间甲氧嘧啶	SMM	0.05-2	$Y=74.63985X-8.80582e^{-2}$	0.99945
磺胺喹恶啉	SQ	0.05-2	$Y=75.14487X-0.103483$	0.99932

2.4 加标回收率和精密度的测定

准确称取 5 ± 0.01 g 试样, 添加一定体积的混合标准工作液, 设定 $50 \mu\text{g}/\text{kg}$ 、 $100 \mu\text{g}/\text{kg}$ 、 $200 \mu\text{g}/\text{kg}$ 三个添加水平, 每个水平设 6 个平行, 涡旋 0.5min, 静置 30min 后, 按 1.4.3 和 1.4.4 样品前处理方法处理后进行 HPLC 分析, 方法的加标回收率和精密度见表 3, 鸡肉空白样品以及添加样品色谱图见图 3 和图 4。

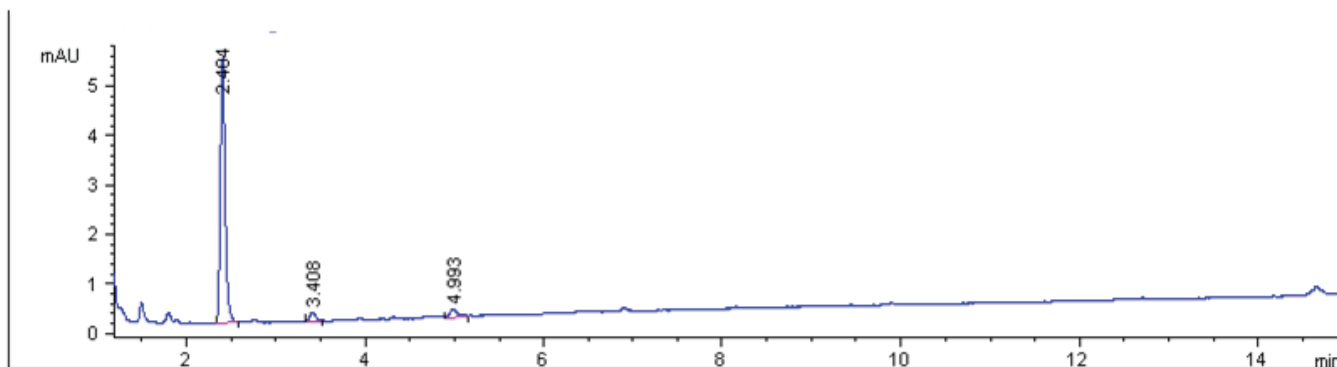


图 3 鸡肉空白样品色谱图

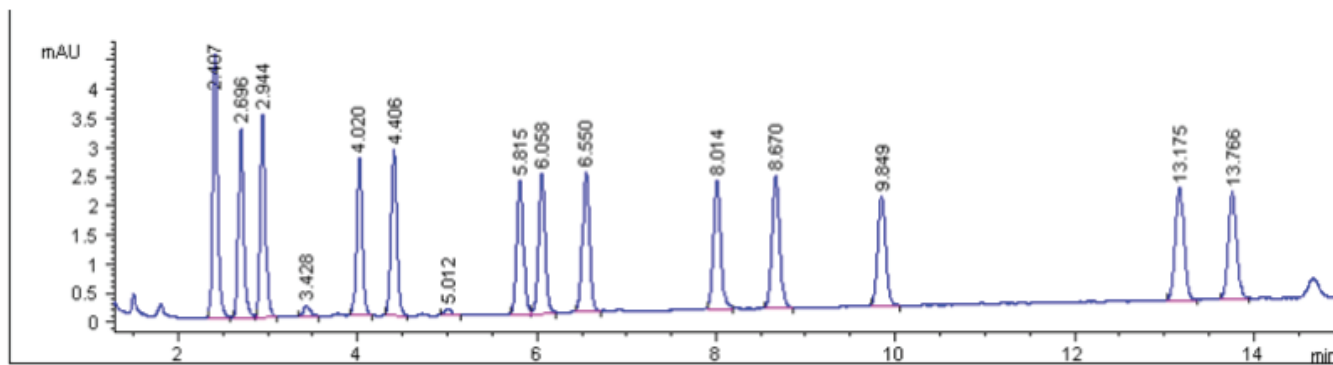


图 4 鸡肉空白样品添加色谱图

表 3: 鸡肉组织中磺胺类药物的添加平均回收率和 RSD (n=6)

药物		添加浓度 (µg/kg)			平均回收率 (%)			RSD (%)		检测限 (µg/kg)	
磺胺二甲异噁啉	SIM2	50	100	200	82.8	84.2	89.7	4.4	3.5	5.0	9
磺胺噻啉	SD	50	100	200	81.6	82.2	89.0	5.2	4.5	4.6	8
磺胺吡啉	SP	50	100	200	80.8	83.5	86.1	6.3	4.8	5.7	11
磺胺甲基噻啉	SMR	50	100	200	84.3	85.0	89.9	3.8	4.7	6.4	11
磺胺对甲氧噻啉	SMD	50	100	200	83.5	84.3	91.0	5.3	5.3	4.3	13
磺胺二甲噻啉	SM2	50	100	200	82.6	85.4	85.8	4.0	4.4	3.5	13
磺胺甲氧吡嗪	SMP	50	100	200	83.3	84.4	85.5	5.1	5.2	5.0	13
磺胺二甲氧噻啉	SDM	50	100	200	82.5	83.0	84.2	4.8	4.1	4.6	14
磺胺甲 唑	SMZ	50	100	200	82.1	82.1	84.0	4.7	4.0	3.7	14
磺胺异恶唑	SIZ	50	100	200	81.3	82.6	83.5	3.4	2.7	3.9	16
磺胺间甲氧噻啉	SMM	50	100	200	80.5	81.6	83.0	5.0	3.9	4.2	16
磺胺喹恶啉	SQ	50	100	200	74.5	76.6	80.0	4.7	4.2	5.1	17

2.5 样品分析

利用本方法测定了来源于农贸市场的 40 个鸡肉样品中的磺胺类药物残留量, 均未检出上述 12 种磺胺类药物。

结论

本文建立了高效液相色谱法测定鸡肉中 12 种磺胺类药物的分析方法, 与以往报道的磺胺类药物 HPLC-UV 检测方法比较可以发现, 本方法前处理操作简单, 耗时少, 从提取至 SPE 柱洗脱结束, 全程不超过 4 小时, 且化学试剂使用量较少。Agilent Poroshell 120 色谱柱的使用实现了在常规液相色谱仪上快速分析的效果, 可满足基层检测机构对检测时效性的要求。

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采用 Poroshell 120 色谱柱研究邓老凉茶颗粒的指纹图谱

关键词

邓老凉茶颗粒 指纹图谱

poroshell 120 质量控制

异绿原酸B HPLC

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摘要

广东凉茶是岭南人民根据本地的气候和水土特性，在长期预防疾病与保健过程中以中医养生理论为指导，以中药为基础，研制总结出来的具有清热解毒、生津止渴等功效的一类植物饮料 [1-2]。邓老凉茶是著名的广东凉茶之一，由金银花、菊花、白茅根、桑叶、蒲公英和甘草 6 味药材组成，具有清热解毒、凉血排毒等功效。随着凉茶走向国际化市场，建立统一的质量标准势在必行。凉茶大多以大组方的形式组成，要确保其统一的质量标准，必须借助于现代分析技术。指纹图谱是目前中药或中药制剂质量控制的有效手段 [3-9]，高效液相色谱法 (HPLC) 是目前最为成熟的指纹图谱技术。本实验对采用 Poroshell 120 色谱柱邓老凉茶颗粒的指纹图谱进行了研究，建立了邓老凉茶颗粒甲醇提取液的 HPLC 指纹图谱，为广东凉茶质量标准的建立提供了技术依据。

材料与amp;方法

1.1 仪器与试剂

Agilent 1100 高效液相色谱系统，配备自动进样器、柱温箱、二极管阵列检测器、ChemStation 色谱工作站。甲醇和乙腈均为色谱纯（美国 Burdick & Jackson 公司），实验用水为超纯水，由超纯水设备（美国 Milipore 公司）制备；磷酸（分析纯，广州化学试剂厂）；绿原酸（ps08072303）、异绿原酸 B（ps08103102）对照品（成都普思生物科技有限公司，质量分数 > 98%）；邓老凉茶颗粒购自广州养和医药科技有限公司。

1.2 色谱条件

色谱柱为 Agilent Poroshell 120 SB-C18 柱（100mm×2.1 mm, 2.7 μm）；流动相为 0.5% 磷酸水溶液（A）- 乙腈（B），梯度洗脱：0 ~ 3 min, 5% B；3 ~ 9 min, 5% ~ 20% B；9 ~ 13 min, 20% B；13 ~ 20 min, 20% ~ 95% B；体积流量 0.5 mL/min；进样量 1.0 μL；柱温 25 °C；检测波长 254 nm。

1.3 对照品溶液的制备

1.3.1 绿原酸对照品溶液的制备 精密称取绿原酸对照品 5.06 mg，甲醇溶解后转移至 25 mL 棕色量瓶中，甲醇定容，过 0.45 μm 滤膜，即得。

1.3.2 异绿原酸 B 对照品溶液的制备 精密称取异绿原酸 B 对照品 5.28 mg，甲醇溶解后转移至 25 mL 棕色量瓶中，甲醇定容，过 0.45 μm 滤膜，即得。

1.3.3 混合对照品溶液的制备 分别吸取 1.00 mL 绿原酸和异绿原酸 B 对照品溶液，组成混合对照品溶液。

1.4 供试品溶液的制备

称取邓老凉茶颗粒 5.00 g，用滤纸包好，置索氏提取器中，加入甲醇 150 mL，索氏提取 1.5 h，提取液旋转蒸馏浓缩至 10 mL，转移至 25 mL 量瓶中，甲醇定容，过 0.45 μm 滤膜，作为供试品溶液。

结果与amp;讨论

2.1 色谱柱以及色谱条件的选择

选用粒径为 2.7 μm 表面多孔壳核填料高效分离色谱柱，分离时间仅为常规 5 μm 粒径色谱柱的 25%，并且具有更高的灵敏度，能够使复杂组分实现快速有效的分离，在指纹图谱研究方面比传统的常规分析柱更具优势。流动相的选择选用不同比例的甲醇-0.5% 磷酸水溶液、乙腈-0.5% 磷酸水溶液、甲醇-0.5% 甲酸水溶液和乙腈-0.5% 甲酸水溶液等流动相体系进行试验对比，以确定最佳流动相的组成及洗脱梯度程序。结果表明，使用乙腈-0.5% 磷酸水溶液系统时，各组分均达到基线分离，流动相中的磷酸明显降低绿原酸的拖尾现象，故选择乙腈-水-磷酸系统为流动相。

2.2 提取方式的选择

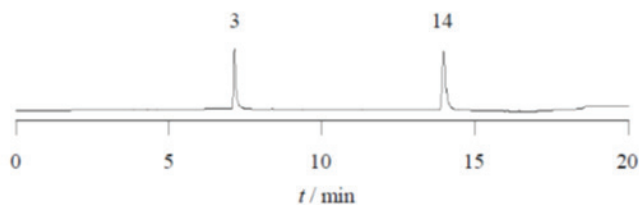
比较水及 50%、70%、90%、100% 甲醇提取效果，结果表明甲醇比其他几种溶剂提取的成分多，故选用甲醇作为提取溶剂。另外，比较了超声提取与索氏回流提取，发现索氏回流提取出的峰较多，提取率较高，因此，样品采用回流提取。

2.3 方法学考察

2.3.1 系统适应性试验 取供试品溶液，连续进样 5 次，考察各共有峰的相对保留时间及相对峰面积的一致性，结果显示各共有峰相对保留时间的 RSD 值在 0.03%~0.79%，相对峰面积的 RSD 值在 0.61%~2.67%，均小于 5%，表明仪器分析精密度良好，分析结果有保障。

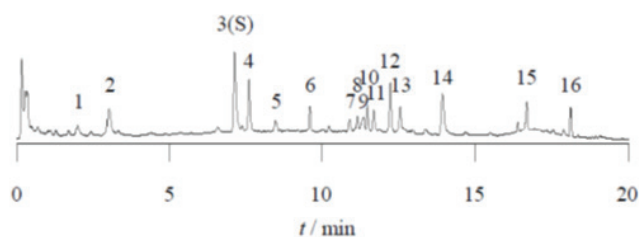
2.3.2 重现性试验 取同一批号样品（S10）6 份，分别配制供试品溶液进行测定，平行 6 份，考察各共有峰的相对保留时间及相对峰面积的重现性，结果显示相对保留时间的 RSD 值在 0.03%~0.40%，相对峰面积的 RSD 值在 1.95%~4.24%，二者均小于 5%，表明该方法的重现性良好。

2.3.3 稳定性试验 取同一批号样品（S10）的供试品溶液，分别在 0、3、6、9、12、24 h 进样，考察各共有峰的相对保留时间及相对峰面积的一致性，结果显示相对保留时间的 RSD 值在 0.06%~0.96%，相对峰面积的 RSD 值在 1.27%~3.74%，二者均小于 5%，表明供试品溶液在 24 h 内稳定。



3- 绿原酸 14- 异绿原酸 B

图 1 对照品的 HPLC 图



S- 绿原酸

图 2 邓老凉茶颗粒的 HPLC 指纹图谱

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2.4 HPLC 指纹图谱分析

取不同批次的 11 批样品分别制备供试品溶液, 测定, 确定 16 个主要色谱峰为共有峰。用《中药色谱图的指纹图谱评价系统》软件(2004 A)进行色谱峰的匹配, 对照品色谱图见图 1, 以绿原酸(S)为参照物的标准指纹图谱见图 2。与对照指纹图谱比较, 11 批邓老凉茶颗粒的相似度分别为 0.978、0.979、0.992、0.988、0.981、0.986、0.990、0.971、0.971、0.986、0.991。相似度评价结果显示, 11 批邓老凉茶颗粒指纹图谱相似度均大于 0.95, 说明工艺稳定, 用该方法对邓老凉茶颗粒进行质量控制较可靠。

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蜂蜜中呋喃唑酮代谢物的液质联用技术确证方法优化 — 应用新型液相色谱柱去除基质干扰

关键词

HPLC-MS/MS

Agilent Poroshell 120

呋喃唑酮代谢物

蜂蜜

作者

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摘要

硝基呋喃类药物为广谱抗生素，对革兰氏阳性及阴性菌均有一定抗菌作用。由于呋喃价格低廉且治疗效果好，所以广泛应用于家禽、家畜、水产品的痢疾、肠炎、球虫病的预防和治疗消化道疾病^[1,2]。但研究证明硝基呋喃类药物及其代谢物具有相当的毒性，有致畸胎作用，且能诱发癌症，这引起了人们高度重视。出于安全考虑，欧盟和美国分别在1995年和2002年全面禁止将硝基呋喃类药物用于食源性动物。2002年3月，我国农业部第193号公告也将硝基呋喃类抗菌剂列入《食品动物禁用的兽药及其他化合物清单》^[3]。蜂蜜是我国重要的出口农产品之一，也一直是药物残留检测的重点产品。但在检测中发现蜂蜜样品在各种检测基质中是最不稳定的，除其本身基质的复杂性，还由于蜜蜂所采的花粉分布难以掌握，每批蜂蜜所包含的蜂蜜种类难以统一以及农户对蜂蜜的采集和调配具有不确定性。近些年来，高效液相色谱-质谱联用技术（LC-MS）越来越多被用于测定动物组织中硝基呋喃类代谢物的残留^[4,5]，并且在日常检测呋喃代谢物时，为了节省时间、提高效率，通常使用短色谱柱进行分离^[3]。但是，此常规方法针对蜂蜜基质的检测有时会出现假阳性现象，因为基质的杂峰会与标准物质峰重叠，而其离子比又与基质添加样品的离子比极为相近^[6]。本实验中采用了安捷伦公司的Poroshell 120系列表面多孔层色谱柱，由于其具有柱子长、低压、高柱效的特点，从而实现了在高效液相色谱串联质谱仪上对假阳性蜂蜜的基质杂峰与标准物质峰的分离。

材料与方法

1.1 仪器与试剂

高效液相色谱串联质谱仪；甲醇 (HPLC 级)、甲酸 (HPLC 级)、乙酸乙酯 (HPLC 级)；水由 Milli-Q Academic 净化系统制得；呋喃唑酮代谢物 AOZ 标准品 (纯度均 >99%, Dr.Ehrenstorfer)、呋喃唑酮同位素内标 AOZ-D4 (纯度均 >99%, WITEGA)。市售蜂蜜。

1.2 标准溶液的配制

称取适量呋喃唑酮内标标准物质用甲醇稀释成 1.0 mg/mL 标准储备液，吸取适量的标准储备液用甲醇稀释成为 10 µg/mL 的标准中间液，吸取适量的标准中间液用甲醇稀释成为 0.2 µg/mL 的标准工作液，-18 °C 避光条件下保存。

移取适量标准工作液，按照样品操作步骤同步操作，使最终样液中呋喃唑酮标准物质的浓度分别为 0 ng/mL, 2.5 ng/mL, 5 ng/mL, 10 ng/mL, 20 ng/mL, 30 ng/mL, 50 ng/mL。

1.3 色谱-质谱条件

采用 Agilent Poroshell 120 SB-C18 柱 (4.6 mm i. d. × 100 mm, 2.7 µm) 色谱柱；流动相 A 为 5 mmol 乙酸铵溶液 + 0.1% 甲酸，流动相 B 为甲醇。梯度洗脱程序：0 ~ 5.0 min, 60% ~ 90% B; 5.0 ~ 6.0 min, 90% B; 6.0 ~ 6.5 min, 90% ~ 60% B; 6.5 ~ 7.5 min, 60% B; 流速 0.4 mL/min; 柱温 40 °C; 进样体积：10 µL。

采用某公司的 50 × 2.1 mm C18 短色谱柱；流动相 A 为 5 mmol 乙酸铵溶液 + 0.1% 甲酸，流动相 B 为甲醇。梯度洗脱程序：0 ~ 0.5 min, 80% A; 0.5 ~ 3.0 min, 80% ~ 20% A; 3.0 ~ 4.2 min, 20% A; 4.2 ~ 5.1 min, 20% ~ 80% A; 流速 0.3 mL/min; 柱温 40 °C; 进样体积：10 µL。

电离方式：电喷雾；正离子扫描；离子传输毛细管温度：350 °C；碰撞气体为氦气；鞘气压力：30 psi；辅助气压力：10 arb；反应监测模式。其它质谱参数见表 1。

样品中待测物质与同时检测的标准具有相同的保留时间，并且所选择的离子相对丰度比相一致，则可判定样品中存在该残留。内标法定量。

表 1 呋喃唑酮代谢物和呋喃唑酮同位素内标质谱参数

化合物 Compounds	保留时间 Retention time (min)	母离子 Parent ion (m/z)	子离子 Daughter ion (m/z)	碰撞能量 Collision energy (eV)	去簇电压 Declustering potential (V)
呋喃唑酮代谢物 (Furazolidone)	1.99	236.0	104.0 134.0*	21 12	122 122
呋喃唑酮代谢物同位素内标 (Furazolidone -D4)	1.95	240.0	134.0	12	122

* 用于定量的子离子。

材料与方法

1.4 样品前处理[7]

准确称取 2.0 g 蜂蜜样品于 50 mL 的离心管，加入适量的内标标准工作液及 0.1 mol/L 2-硝基苯甲醛衍生液，再加入 20 mL 0.12 mol/L 的盐酸溶液，混匀，置于 37 °C 的恒温箱避光反应 16h。按文献 [7] 方法过小柱净化。

结果与讨论

采用 50 × 2.1 mm C18 短色谱柱时，蜂蜜样品的检测结果为呋喃唑酮代谢物 (AOZ) 疑似阳性。疑似样品出峰时间为 2.22 min (如图 1)，与其内标出峰时间一致；离子比为 9.78，其样品添加的出峰时间为 2.22 min (如图 2)，离子比为 10.98，基本一致。

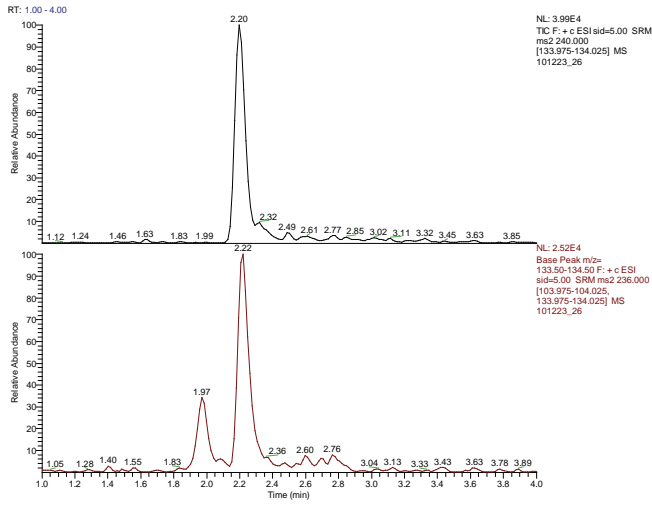


图1 疑似阳性蜂蜜通过 50 × 2.1mm 短柱分离的内标和定量离子的质谱图

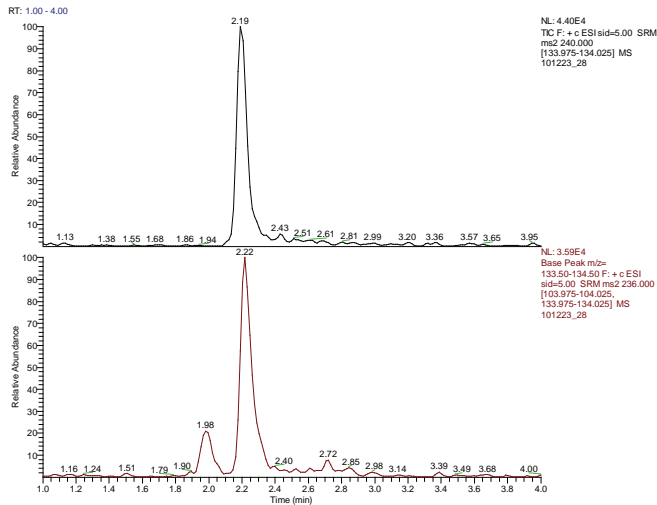


图2 疑似阳性蜂蜜添加通过 50 × 2.1mm C18 短柱分离的内标和定量离子的质谱图

更换 Agilent Poroshell 120 SB-C18 柱 (4.6 mm i. d. × 100 mm, 2.7 μm) 色谱柱、调整流动相后, 疑似样品与内标出峰时间不符, 为杂质峰 (如图 3)。痕量杂质峰与标准物质峰得到良好的分离 (如图 4)。

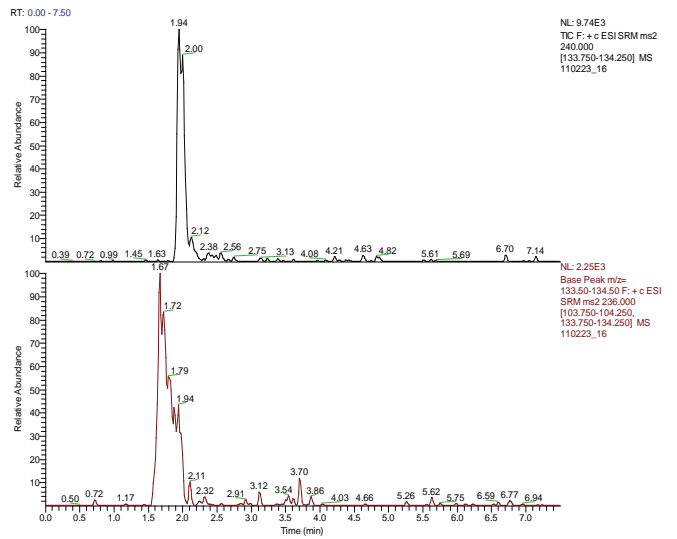


图3 疑似阳性蜂蜜通过 Poroshell 120 SB-C18 柱 (4.6 mm i. d. × 100 mm, 2.7 μm) 长柱分离的内标和定量离子的质谱图

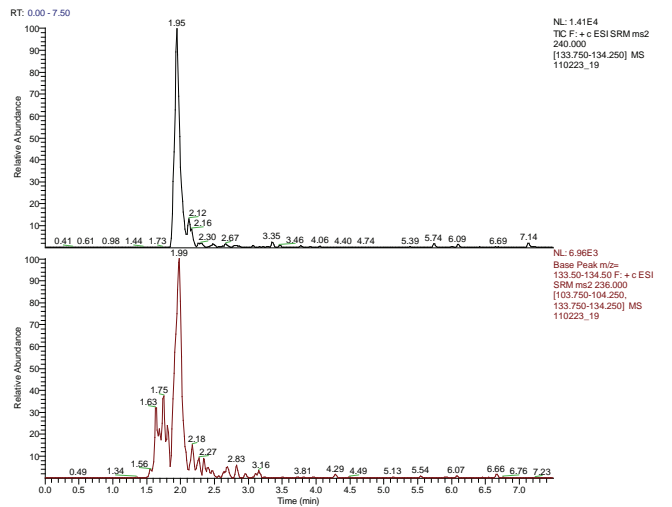


图4 疑似阳性蜂蜜添加通过 Poroshell 120 SB-C18 柱 (4.6 mm i. d. × 100 mm, 2.7 μm) 长柱分离的内标和定量离子的质谱图

以上实验表明, 新型液相色谱柱 Agilent Poroshell 120 SB-C18 的分离流速一般设为 1.0 mL/min, 而在这个实验中, 将流速设为 0.4 mL/min, 在保证分离效果的同时, 峰形也良好。对于种类繁多、基质复杂的蜂蜜, 传统带有 0.5 μm 筛板的色谱柱很容易被样品堵塞, 而 Poroshell 120 色谱柱入口使用了标准的 2 μm 筛板, 有效地防止了样品的堵塞。

总之, 长色谱柱的分离效果明显优于短色谱柱, 但短色谱柱所需时间较短, 适合大批量的样品检测。如果在实验过程中遇到疑似阳性样品的时候, 其有可能为假阳性, 应慎重对待, 建议更换长色谱柱确认, 进行色谱峰的分离, 排除假阳性的干扰, 提高实验的准确性。

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使用 Poroshell 120 色谱柱同时测定食品中 苯甲酸、山梨酸、脱氢乙酸、糖精钠的方法研究

关键词

HPLC

Poroshell 120

防腐剂

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摘要

近年来，由于消费者对食品的安全性日益关注，特别是防腐剂苯甲酸 (BA)、山梨酸 (SA)、脱氢乙酸 (DHA) 和人工合成甜味剂糖精钠 (SS) 的使用越来越受到人们的重视 [1,2]。由于防腐剂、甜味剂都有一定的毒性，过量摄入会对人体带来伤害，还可引发癌症 [3]。在我国食品添加剂使用卫生标准 (GB2760 - 2007) 中对这些添加剂的使用范围和最大使用限量均有明确规定。由于这几种防腐剂和甜味剂允许同时添加在许多食品中，因此同时快速检测成为必要，而现行国标方法 GB/T23495 - 2009 和 GB/T23377 - 2009 分别测定 BA、SA、SS 和 DHA，如果沿用国家标准分开检测，不但增加了工作成本，而且工作效率低。本文方法在文献 [1 - 6] 的基础上加以改进，探讨用高效液相色谱法同时对 BA、SA、DHA 和 SS 进行检测，比较 Poroshell 120 色谱柱和普通色谱柱的区别，结果表明采用 Poroshell 120 色谱柱同时检测食品中的 BA、SA、DHA 和 SS 更快速，更高效。

材料与方法

1.1 仪器与试剂

高效液相色谱仪, AL204 电子天平(瑞士), PT-310 电子天平, 超声波清洗器, 低速台式离心机, Anke TGL-16B 高速离心机。

Poroshell 120 EC-C18(4.6 × 100 mm 2.7 μ m) 色谱柱(美国 Agilent 公司)。苯甲酸标准溶液、山梨酸标准溶液、糖精钠标准溶液均为 1.00 mg/mL(国家标准物质中国计量科学研究院); 脱氢乙酸:(98%, 美国 ACROS 公司); 甲醇(色谱纯); 乙酸铵、硫酸锌、氢氧化钠、石油醚 30~60(分析纯); 水为超纯水。

1.2 样本来源

酱腌菜制品 60 件, 糕点 90 件, 样品均为市场采购。

1.3 测定方法

1.3.1 色谱条件

色谱柱: ZORBAX Poroshell 120 EC-C18(4.6 × 100 mm 2.7 μ m); 流动相: MeOH : 0.02 mol/L NH₄Ac(10 : 90); 柱温: 40 $^{\circ}$ C; 流速: 1.0 mL/min; 检测波长 230nm; 进样量: 10 μ L。根据保留时间定性, 外标法定量。

1.3.2 标准储备液的配制

1.00 mg/mL DHA 标准溶液: 准确称取 0.1 g DHA 标准品, 用 10 mL(20 mg/mL) 氢氧化钠溶液溶解, 置于 100 mL 容量瓶中, 用水定容至刻度(4 $^{\circ}$ C 保存 6 个月)。200 μ g/mL BA、SS、SA 和 DHA 混合标准溶液: 分别准确吸取 10.00 mL 浓度均为 1.00 mg/mL BA、SS、SA 和 DHA 标准溶液置于 50mL 容量瓶中, 用水定容至刻度(4 $^{\circ}$ C 保存 3 个月)。

1.3.3 标准溶液系列的配制

将 200 μ g/mL BA、SS、SA 和 DHA 混合标准溶液逐级配制浓度分别为 1.00、5.00、10.00、20.00、50.00、80.00 μ g/mL 的标准溶液系列(临用现配)。

1.3.4 样品处理

参照国标方法 GB/T23495-2009 和 GB/T23377-2009 以及文献[1-6], 含有二氧化碳的液体样品需超声脱气; 蛋白质含量高的样品需沉淀蛋白质(用氢氧化钠溶液和硫酸锌溶液或者用亚铁

氰化钾溶液和乙酸锌溶液); 脂肪含量高的样品需脱脂(用石油醚或者正己烷)弃去有机层。将样品粉碎混匀, 称取 5g(精确至 0.01g) 样品于 50mL 的比色管中, 加入约 30mL 水, 用氢氧化钠溶液调 pH 值至 7~8, 加水定容至刻度, 超声提取 30min, 3000r/min 离心 10 min, 取上清液经 0.45 μ m 的滤膜过滤后待测。

1.3.5 测定结果

标准系列溶液与样品制备液按照 1.3.1 的色谱条件进行检测, BA、SS、SA 和 DHA 与样品中其它共存物质分离良好, 测定不受干扰。

结果与讨论

2.1 BA、SS、SA 和 DHA 的保留时间分别为 2.4、2.8、3.4、3.7 min, 各组份均能基线分离, 与共存的其他物质分离良好, 其混合标准溶液、阳性样品制备液及样品加标制备液色谱图见图 1~4。

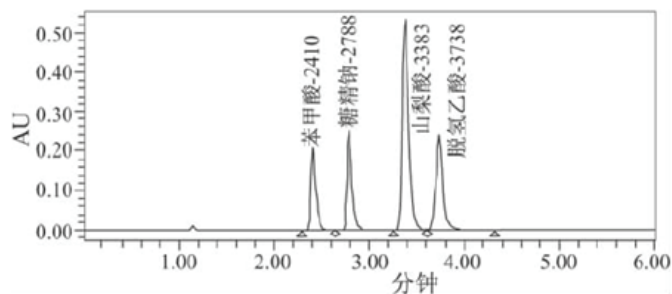


图 1 20 μ g/mL BA、SS、SA 和 DHA 混合标准溶液色谱图(色谱柱: Poroshell 120 EC-C18)

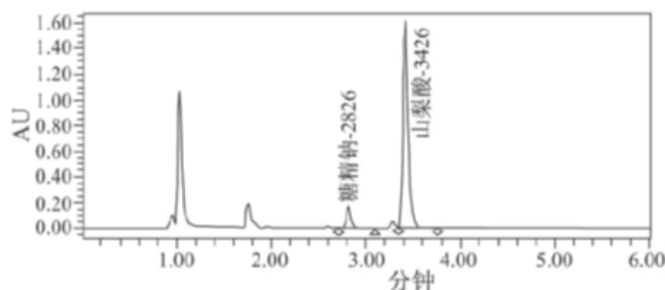


图 2 BA、SS、SA 和 DHA 样品本底制备液色谱图

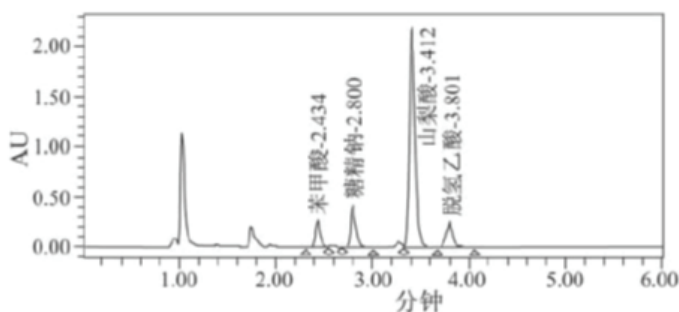


图3 BA、SS、SA 和 DHA 样品加标制备液色谱图

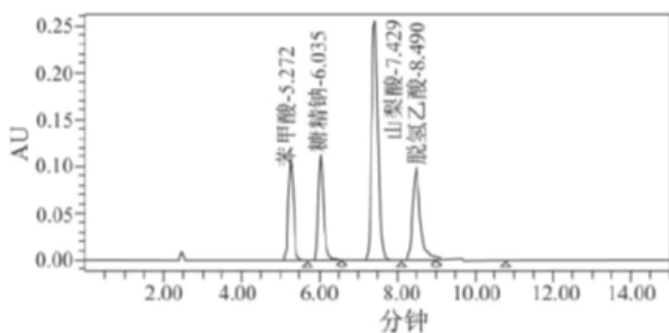


图4 20µg/mL BA、SS、SA 和 DHA 混标溶液液相色谱图(色谱柱:Eclipse XDB-C18)

2.2 工作曲线与检测限

BA、SS、SA 和 DHA 的回归方程在 1 ~ 80µg/mL 浓度范围内与峰面积呈良好的线性关系, 将混合标准溶液逐级稀释至 0.05、0.1、0.2µg/mL 进样量为 10µl 时, BA、SA(0.05µg/mL)、SS(0.1µg/mL)、DHA(0.2µg/mL), 峰高大于 3 倍噪音信号, 即 BA、SS、SA 和 DHA 仪器最低检出量为: 0.5、1.0、0.5、2.0 ng, 该方法 BA、SS、SA 和 DHA 最低检出限为 0.5、1.0、0.5、2.0mg/kg。

2.3 加标回收率与精密度

分别称取 5.00g 同一混合均匀的样品共 8 份, 其中两份本底, 6 份分别准确加入 5.00mL200µg/mLBA、SS、SA 和 DHA 混合标准储备液做加标回收。按照 1.3 的方法操作, 分别定容至 50mL, 制备液相当于各组份均加标 20 µg /mL, 测定样品本底、加标样品制备液浓度, 计算加标回收率。样品本底 BA 和 DNA 均未检出, 山梨酸为 50.27µg/mL, SS 为 11.13µg/mL, BA、SS、SA 和 DHA 的

加标平均回收率依次为 100.3%、102.0%、99.6%、88.8%, 相对标准偏差依次为 0.52%、0.93%、1.74%、0.99%(n=6), 保留时间的相对标准偏差依次为 0.50%、0.54%、0.41%、0.63%(n=6), 用 BA、SS、SA 和 DHA 标准溶液作外标定量, 结果准确可靠, 重现性良好。

2.4 样品中 BA、SS、SA 和 DHA 的检测结果我国食品添加剂使用卫生标准 (GB2760 - 2007) 规定, 酱腌菜中 BA、SS、SA 和 DHA 最大使用量分别为 0.5g/kg、0.15g/kg、0.5g/kg、0.3g/kg; 月饼和糕点中 BA、SS、SA 和 DHA 最大使用量分别为不得检出、0.15g/kg、1.0g/kg、0.5g/kg。按照 1.3 的方法测定了 60 件酱腌菜、90 件糕点中的 BA、SS、SA 和 DHA, 其中酱腌菜中的 BA 检出 15 件, 结果为 0.091 ~ 6.2g/kg, SS 检出 5 件, 结果为 0.076 ~ 0.26g/kg, SA 检出 7 件, 结果为 0.013 ~ 3.2g/kg, DHA 检出 8 件, 结果为 0.027 ~ 0.48g/kg; 糕点中的 BA 和 SS 均未检出, SA 检出 7 件, 结果为 0.024 ~ 0.62g/kg, DHA 检出 17 件, 结果为 0.026 ~ 0.58g/kg。其它样品中的 BA、SS、SA 和 DHA 均为未检出。

2.5 现行国标方法 GB/T23495 - 2009 和 GB/T23377 - 2009 分别测定 BA、SA、SS 和 DHA, 使用该方法可同时测定 BA、SS、SA 和 DHA, 比国标方法大大缩短了分析时间, 提高了灵敏度和工作效率, 节约实验耗材。

2.6 柱温提高到 40°C, 有利于提高灵敏度, 缩短分析时间。

2.7 BA、SS、SA 和 DHA 各组份的最大吸收波长分别为 223、210、254、230 nm, 其中 DHA 还有个次吸收峰波长为 292 nm, 选择 230 nm 测定各组份可得到满意结果, 适合于基层单位具有普通液相色谱仪检测工作的开展。

2.8 色谱柱 1: Poroshell 120 EC-C18(4.6×100mm, 2.7µm), 色谱柱 2: Eclipse XDB-C18(4.6×250mm, 5µm), 色谱柱 3: ZORBAX SB-Aq(4.6×250mm, 5µm), 以上色谱柱均为美国 Agilent 公司提供, 在 1.3 条件下, 使用色谱柱 1、2, BA、SS、SA 和 DHA 各组份均能基线分离, 色谱柱 3 DHA 色谱峰拖尾较严重, 由图 1 和图 4 可以看出, 色谱柱 1 比色谱柱 2 灵敏度更高。其它色谱条件相同, 色谱柱 1 比色谱柱 2 保留时间明显缩短, 6min 即可完成分析。因此, 色谱柱 1 不仅有较高的灵敏度, 而且还提高了工作效率, 节约试剂。

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Identification and determination of the major constituents in Deng's herbal teagranules by rapid resolution liquid chromatography coupled with mass spectrometry

关键词

Deng's herbal tea

Poroshell 120

LC-MS

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摘要

Deng's herbal tea (DHT), a famous traditional Chinese herbal tea consisting of six traditional Chinese medicines (Honeysuckle, Chrysanthemum, Rhizoma imperatae, Folium mori, dandelion and liquorice), is widely used in China for its health benefits. In this paper, a rapid resolution liquid chromatography coupled with mass spectrometry (RRLC-MS) method was developed for the identification and determination of the major constituents in DHT granules. A good RRLC separation was achieved using an Agilent Poroshell 120 SB-C18 column and gradient elution (0.5% formic acid in water/acetonitrile) within 30 min. Twenty-eight compounds were identified or tentatively characterized based on their exact molecular weights and fragmentation patterns. Fifteen major bioactive constituents of those 28 compounds were chosen as the benchmark substances. Their quantitative analyses were performed by a triple quadrupole tandem mass spectrometer (MS/MS) operating in multiple-reaction monitoring mode, and a full quantitative analysis of the 15 major constituents was performed by our developed RRLC-MS/MS method in only 10 min. Of the 16 DHT granule samples tested, the quality of the results was stable, which confirms that the developed method was efficient and robust for the quality control of DHT granules.

材料与amp;方法

1.1 仪器与试剂

Chromatographic separations were performed on an Agilent 1200 Series RRLC system (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump, microvacuum degasser, high-performance autosampler, column compartment and diode array detector (DAD). For the identification of the major constituents in the DHT granules, the RRLC system was coupled to an Agilent 6210 TOF-MS (Agilent Technologies, USA) and an Agilent Trap XCT IT-MS (Agilent Technologies, USA), respectively. However, for quantitative analysis of the 15 major constituents in the DHT granules, the RRLC was coupled to an Agilent 6410 B triple quadrupole mass spectrometer (Agilent Technologies, USA).

Acetonitrile and methanol of HPLC grade were purchased from Burdick & Jackson (Muskegon, MI, USA). Water for RRLC analysis was purified by a Milli-Q water-purification system (Milford, MA, USA). Formic acid was analytical grade and purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China).

1.2 色谱条件

The samples were separated on an Agilent Poroshell 120 SB-C18 column (100 mm × 2.1 mm i.d., 2.7 μm) at a temperature of 25 °C and using a water–formic acid (100:0.5, v/v) (solvent A) and acetonitrile (solvent B) mobile phases at a flow rate of 0.5 mL/min. To identify the major constituents in the DHT granules, a linear solvent gradient was used: 0–1 min, 5% B; 1–15 min, 5–20% B; 15–25 min, 20–45% B; 25–30 min, 45–95% B; and 3-min post-run, 5% B. Next, to determine the contents of the 15 major constituents in the DHT granules, a separate linear solvent gradient was used: 0–1 min, 10% B; 1–8 min, 10–45% B; 8–10 min, 45–95% B; and 3-min post-run, 5% B. For each system used, the DAD was set to monitor absorbance at 254 nm, and each online spectrum was recorded in the range of 190–400 nm.

1.3 试验样品

Five grams of a DHT granule sample was extracted with 50 mL of methanol–water (9:1, v/v) in a flask and sonicated for 15 min followed by filtration. The extraction procedure was repeated twice more, and the extracts were combined. The combined extracts were evaporated under reduced pressure at 50 °C to approximately 10 mL, transferred to a 25-mL volumetric flask, brought up to volume with methanol, and filtered through a nylon membrane filter (0.2 μm, Phenomenex, USA) for RRLC–IT–MS and RRLC–TOF–MS analysis. For RRLC–MS/MS analysis, 1.0 mL of the obtained extracts was diluted to 10.0 mL with methanol.

结果与amp;讨论

2.1 色谱条件的选择

A full separation by RRLC is necessary for the identification of as many of the constituents in the DHT granules as possible. DHT granules consist of six TCMs, and each TCM contains many constituents of similar polarities, which makes their separations quite difficult. Therefore, different elution methods using different elution solvent systems, including water–methanol, water–acetonitrile, water (containing 0.5% formic acid)–methanol and water (containing 0.5% formic acid)–acetonitrile, were investigated. The results show that a linear gradient elution with water (containing 0.5% formic acid)–acetonitrile gave the best resolution and that most of the constituents could be efficiently separated within 30 min (Fig. 1A B and C). Acetonitrile remarkably improved the separation of many of the constituents compared to methanol. Additionally the addition of formic acid had a substantial effect by increasing the retention time, depressing peak tailing of the organic acids, and strikingly improving separation efficiencies and sensitivities.

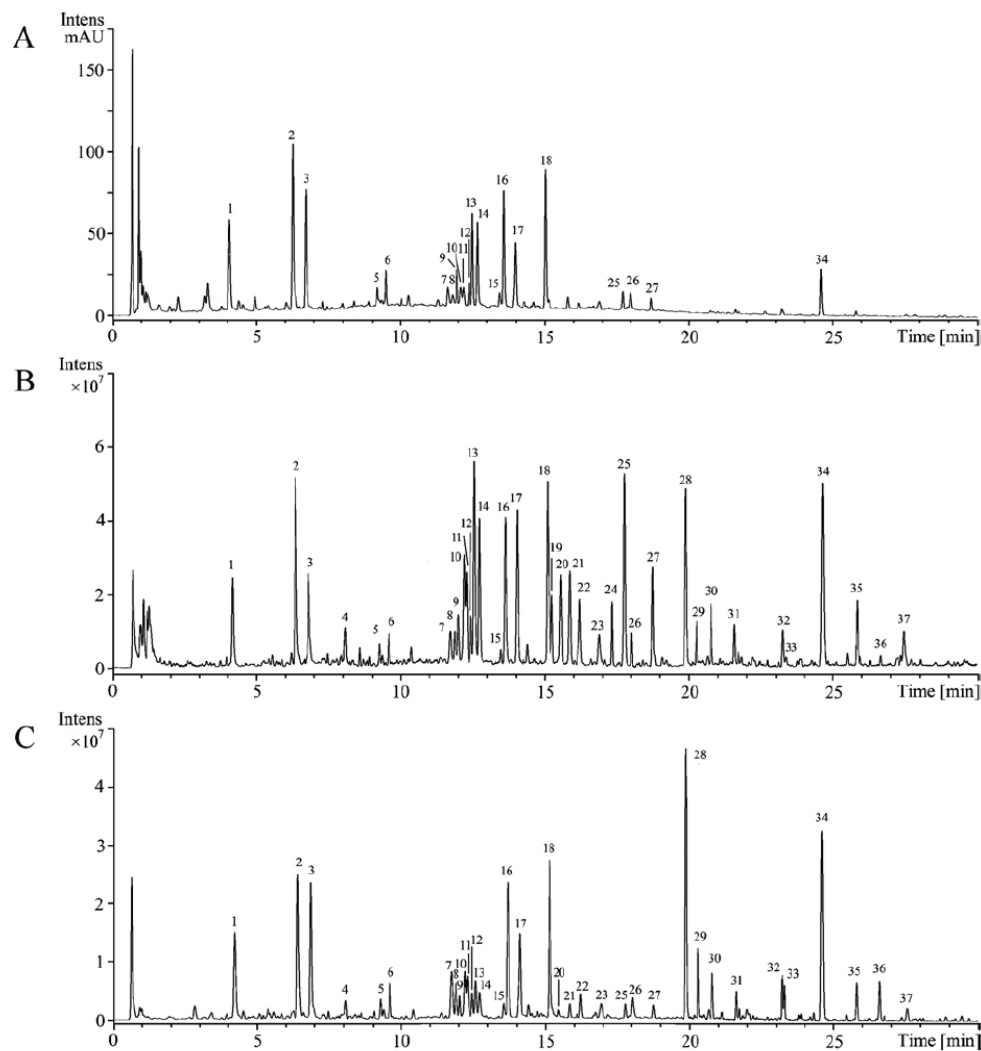


Figure 1 Representative chromatograms of the DHT granule extract. (A) DAD at 254 nm. (B) BPC in positive-ion ESI-IT-MS. (C) BPC in negative-ion ESI-IT-MS

2.2 方法的线性范围以及回收率

The linearities, ranges, regressions, LODs, LOQs, precisions, accuracies and recoveries of the method are listed in Table 3, and the high-correlation coefficient values ($r^2 > 0.9940$) obtained indicated that there were good linear correlations between the concentrations of the investigated compound and their peak areas within the test concentrations. The LODs and LOQs ranged from 1.7 to 146.5 $\mu\text{g/L}$ and from 5.6 to 488.3 $\mu\text{g/L}$, respectively, while the intra and inter-day precisions for

each compound were less than 4.1 and 5.3%, respectively. Furthermore, the intra- and inter-day accuracies were in the range of 96.1–102.8% and 95.3–102.6%, respectively. The developed method showed good accuracy, with mean recoveries ranging from 94.3 to 104.8%. The results also indicated that the developed method was efficient, accurate and sensitive for quantitative determination of the major constituents in the DHT granules.

小结

In this study, a method based on RRLC-MS was developed for the identification and determination of the major constituents in DHT granules. The separation speed and efficiency of RRLC was high, and a full separation of the 37 major constituents and a quantitative determination of the 15 major constituents in the DHT granules were completed within 30 min and 10 min, respectively.

使用配备 Agilent Poroshell 120 EC-C18 色谱柱的 Agilent 1100 系列液相色谱快速分析磺胺类药物

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摘要

本实验将原来使用 4.6 × 250 mm, 5 μm 色谱柱分离 10 种磺胺类药物的方法转移到配备 Agilent Poroshell 120 EC-C18 色谱柱的 Agilent 1100 系列液相色谱上。并提供了方法转换的简单指导原则。新的分离方法将分析时间从 30 min 缩短到 8 min, 由于两根色谱柱均使用了 2 μm 筛板, 因此样品制备方法无需更改。尽管柱压与原方法相比有所增加, 但仍低于 400 bar, 因此该方法可以很容易地转换到几乎所有的 HPLC 系统上。本实验采用未经过滤的蛋白变性牛奶样品 (磺胺二甲氧嘧啶做内标物), 结果显示色谱柱使用寿命超过 1000 次进样。

前言

磺胺类药物是第一种系统地用于预防和治疗人类细菌感染的化学物质。磺胺类药物属于抑菌药；通过抑制细菌的生长和繁殖而非杀死它们作用。目前，磺胺类药物主要用于治疗人泌尿道感染。其它新型药物用来抗感染，如 β -内酰胺类抗生素。当今，磺胺类药物常作为抗生素被兽医使用，主要用来饲养动物以预防感染。此外，它也可用于蜜蜂疾病的治疗。蜂蜜样品中往往可以发现这些抗生素的残留，其毒性或过敏反应引起世界各地消费者的关注。[1,2]

Agilent Poroshell 120 EC-C18, 2.7 μm 色谱柱填料与亚 2 μm 的全多孔填料有相似的性能。由于使用的 2 μm 色谱柱筛板与 5 μm 填料粒径色谱柱的筛板相似，因此无需额外的样品制备就可实现完美的方法转移。

在本实验中，将梯度洗脱方法从 4.6 \times 250 mm, 5 μm 色谱柱转移到 4.6 \times 100 mm Agilent Poroshell 120 EC-C18 色谱柱上并进行了优化。梯度洗脱时间从 30 min 缩短到 8 min。如果应用 4.6 \times 50 mm 色谱柱，虽然分离度有所降低，但洗脱时间可进一步缩短到 4 min 以内。

实验部分

本实验采用快速分离高分离度液相色谱组件对 Agilent 1100 系列 HPLC 仪进行了改进。该系统包括了 G1312A 二元泵，最高输液压力 400 bar；一个 G1316A 柱温箱(TCC)；一台 G1376A 高效自动进样器；一台 G1315B 二极管阵列检测器，配备 3 mm 光程的半微量流通池。使用 Poroshell 120 色谱柱时，所有的管线均改用内径最短的 0.12 mm 管线，改用内径 0.12 mm 的针座；依据先前文献和报告中的优化建议，检测器数据采集速率提至最高(40 Hz)。[3, 4]使用 Agilent ChemStation (A.10.02 版) 采集数据。本实验使用的色谱柱包括 Agilent ZORBAX Eclipse Plus C18 4.6 \times 250 mm, 5 μm 色谱柱 (部件号 959990-902) 和 Agilent Poroshell 120 EC-C18 4.6 \times 100 mm 色谱柱(695975-902)。

以下磺胺类药物购自 Sigma Aldrich 公司：磺胺嘧啶、磺胺噻唑、磺胺吡啶、磺胺甲噁啶、磺胺二甲噁啶、磺胺二甲噻唑、磺胺甲氧嘧啶、磺胺氯吡啶、磺胺甲基异噻唑、磺胺二甲氧嘧啶。各化合物用乙腈:水(50/50, v/v)分别配制成浓度 1 mg/mL 的溶液，然后等量混合配制成每种化合物浓度均为 0.1 mg/mL 的混合溶液。

所用牛奶为 Similac。Similac 是一种类似母乳的婴幼儿配方奶粉商品。制造商严格控制其原料组成，因此与某些牛奶(Abbott Laboratories Columbus Ohio)不同，其蛋白质成分是均匀的。最常见的婴幼儿配方奶粉包含作为蛋白源的纯化牛奶乳清和酪蛋白，作为脂肪源的混合植物油，作为糖源的乳糖，维生素-矿物质组合及其它成分。

将加入磺胺二甲氧嘧啶的 Similac 牛奶 2 mL 加入 18 mL 水:乙腈:甲酸(200:800:1)混合溶液中，剧烈振摇 1 min，静置 3 min 后，吸取 2 mL 上清液到离心管中，5000 rpm 转速下离心 5 min。上清液无需进一步过滤直接转移到自动进样器小瓶中。

讨论

小颗粒填料色谱柱用于快速分析已经有几年的时间了。然而，与 5 或 3.5 μm 填料色谱柱不同，这种色谱柱需要较小孔径的筛板保持色谱柱中的填料。2 μm 筛板用于保持柱中 5 或 3.5 μm 的填料颗粒。对于粒径 3 μm 或更小的颗粒，则需选用 1 或 0.5 μm 孔径的筛板。全多孔颗粒的粒径分布比表面多孔颗粒的大 25%。使用 2 μm 筛板可使窄粒径分布填料如 Poroshell 120 保持在色谱柱中。若新色谱柱的筛板孔径比旧色谱柱的小，方法转化时须格外注意样品的制备以防脏样品堵塞筛板。[5]方法从 5 或 3.5 μm 色谱柱转移到 Poroshell 120 色谱柱时，如果仍使用较大孔径的色谱柱筛板，就不需要额外进行样品净化了。

如图 1 所示，原方法使用了 4.6×250 mm，5 μm 色谱柱，在 30 min 内梯度洗脱分离了 10 种化合物。多数化合物达到基线分离，相邻两峰之间最小的分离度为 1.7。由于实验过于漫长，该分离条件很难再进一步优化。原方法可通过以下几个简单的步骤转移到 2.7 μm 表面多孔填料色谱柱上：

1. 梯度程序的转移（根据柱长按比例缩短原方法的梯度程序，以保持原分离度，保持 k'）。由于原方法分析时间为 30 min，柱长 250 mm，相同流速下使用 100 mm 色谱柱时，梯度洗脱时间缩短为原来的 100/250 即 0.4，进样量按相同比例减少。[4.6.7]如图 2 所示。

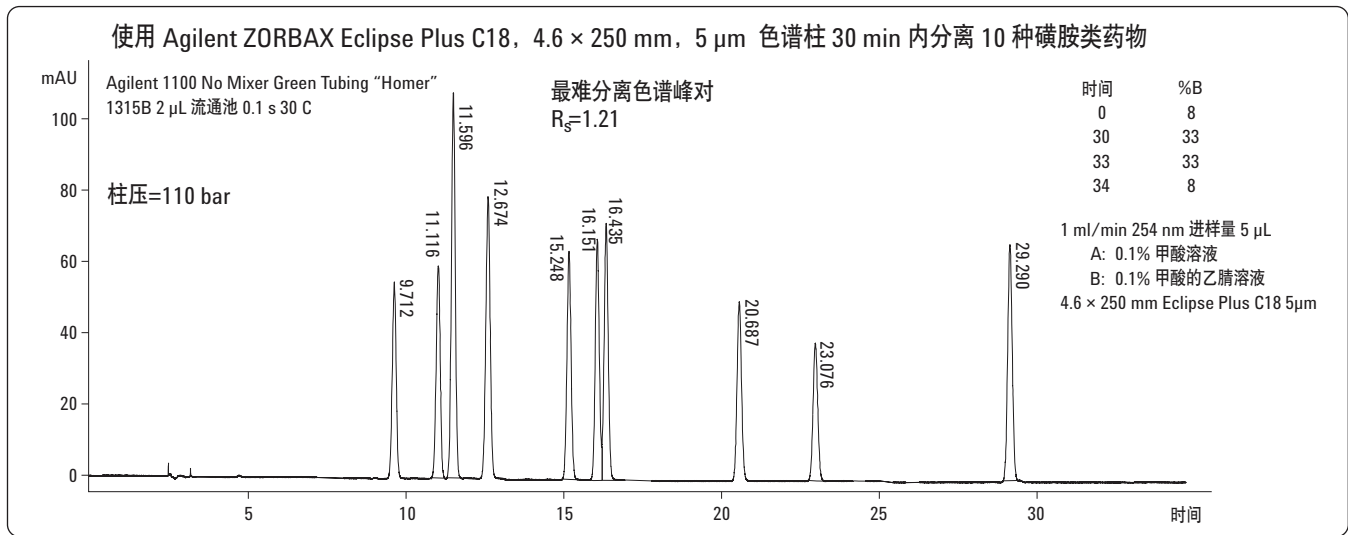


图 1. 采用 Agilent ZORBAX Eclipse Plus C18 4.6 × 250 mm, 5 μm 色谱柱在甲酸/乙腈梯度程序下 30 min 分离 10 种磺胺类药物

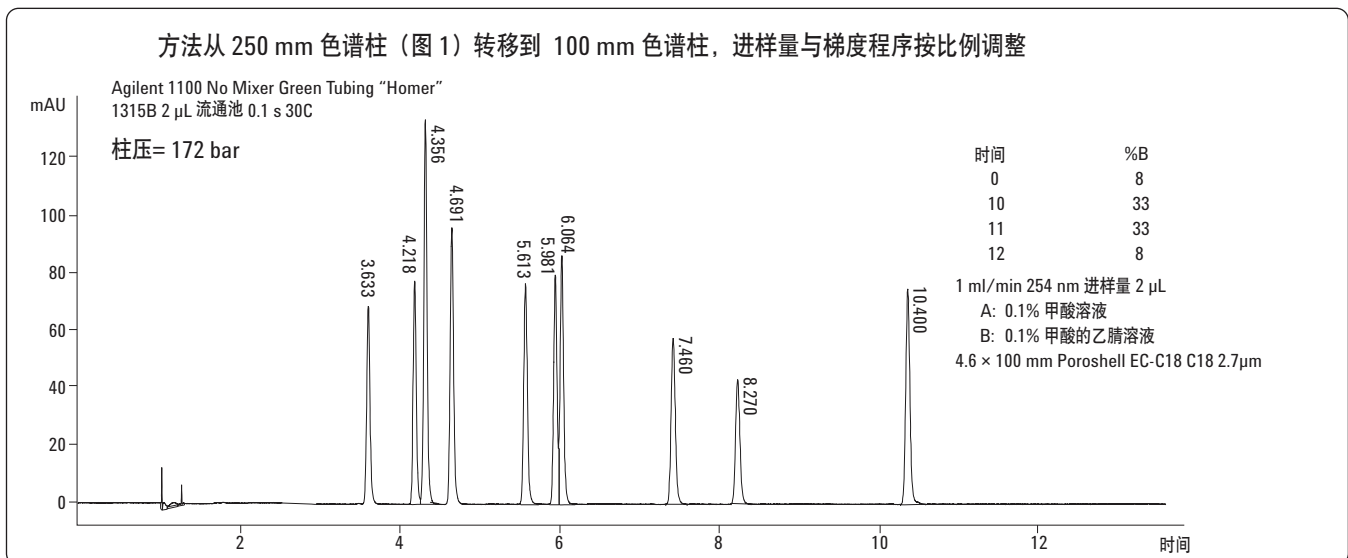


图 2. 采用 Agilent Poroshell 120 EC-C18 4.6 × 100 mm, 2.7 μm 色谱柱在甲酸/乙腈梯度程序下 12 min 分离 10 种磺胺类药物

2. 如图 3 所示, 保持分离中柱体积不变, 增加流速。在分离中采用相似色谱柱通过保持梯度斜率(G_s)不变从而保持 k' 。利用公式计算, 见公式 1

公式 1. $G_s = (V_m/F)(\%B/t_G)$ [7],

该公式可变形为:

公式 2. $(F)(t_G) = ((\%B)(V_m) / G_s)$

其中

F: 流速

t_G : 梯度洗脱时间

V_m : 柱体积

%B: 溶剂强度的改变

我们发现可以通过增加流速缩短梯度洗脱时间。因此, 按照以下公式, 分离条件由原来的 8% 到 33% B 1 mL/min 运行 10 min, 转变成 8% 到 33% B 1.5 mL/min 运行 6.7 min, 最后转变成 8% 至 33% B 2 mL/min 运行 5 min。(1 mL/min \times 10 min = 1.5 mL/min \times 6.7 min = 2 mL/min \times 5 min):

公式 3. $t_2 = t_1 \times \frac{F_1}{F_2}$

其中

t_2 : 新梯度洗脱时间

t_1 : 原梯度洗脱时间

F_2 : 新流速

F_1 : 原流速

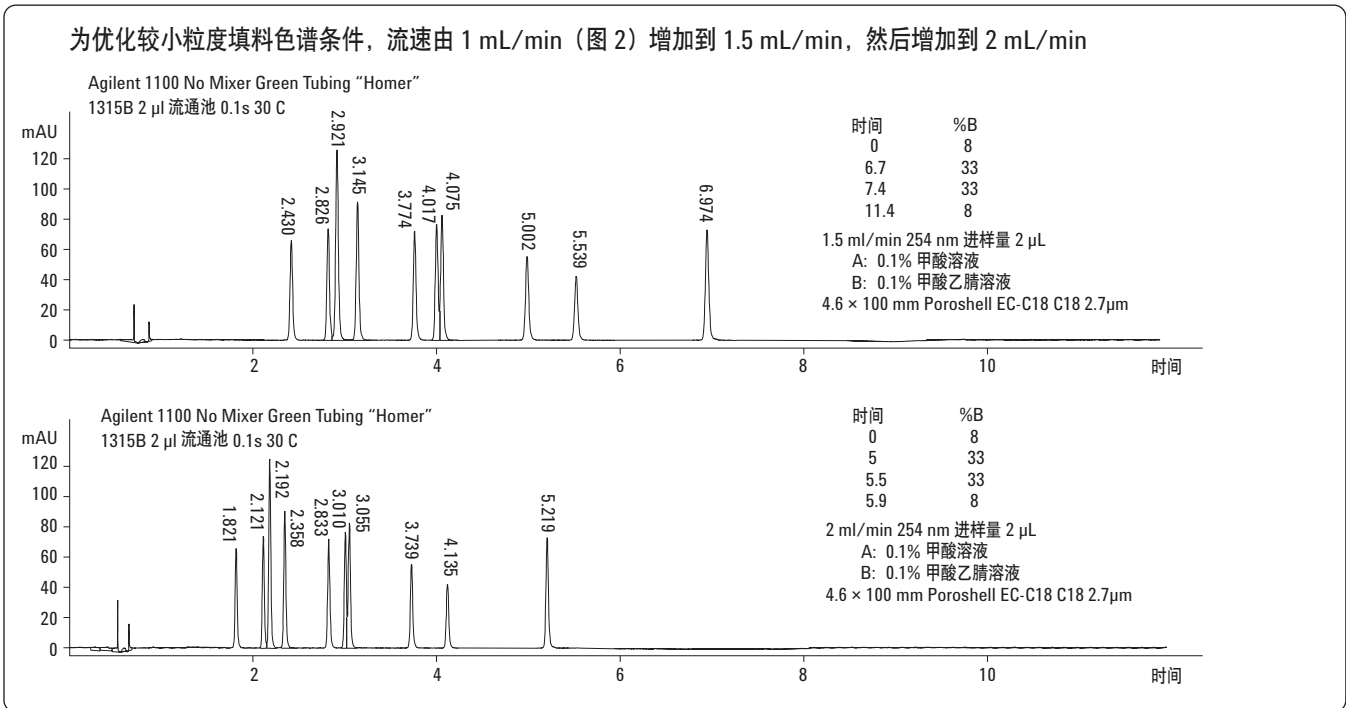


图 3. 采用 Agilent Poroshell 120 EC-C18, 4.6 \times 100 mm, 2.7 μ m 色谱柱在甲酸/乙腈梯度程序下 6 min 分离 10 种磺胺类药物, 流速和梯度程序从 1 mL/min (图 2) 到 1.5 mL/min, 最后到 2 mL/min 按比例调整

3. 梯度程序优化。由于实验可在原实验所需时间的一小部分里运行，因此几个灵活的梯度变化可实现更快的分离或更好的分离度。该优化可在 1 h 内完成。一些工作可在更长的色谱柱上进行，但由于分析时间过长，实验人员需要花费 4 到 8 h 完成这一步骤。图 4 显示了采用 Poroshell 120 色谱柱优化分离选择性采取的各步骤

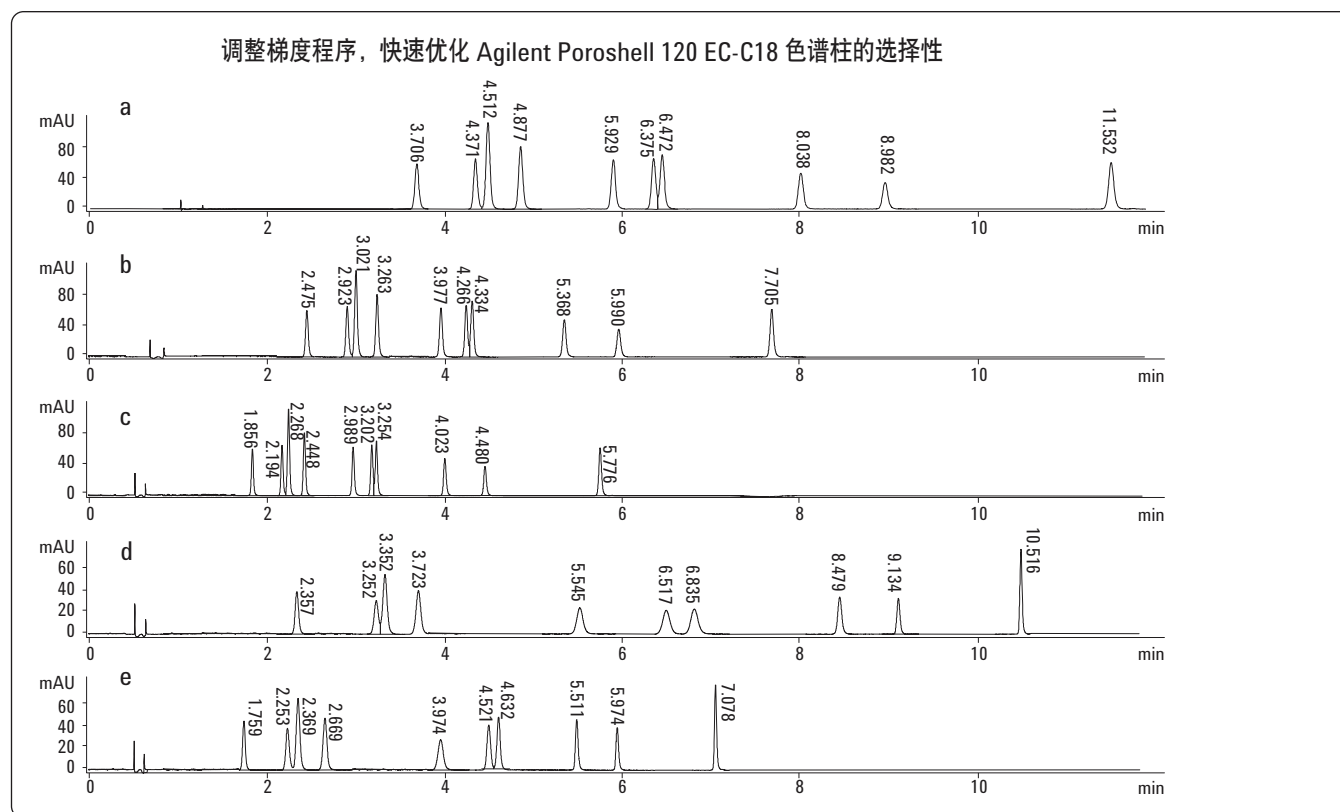


图 4. 调整甲酸/乙腈梯度程序，使用 2.7 μm Agilent Poroshell 120 EC-C18 色谱柱优化分离 10 种磷酸类药物。(a) 采用 250 mm 色谱柱在 1 mL/min 流速下的原梯度洗脱色谱图 (同图 2 所示)。(b) 在 1.5 mL/min 较快的流速下，分析时间缩短 33% 的色谱图 (同图 3 所示)。(c) 在 2 mL/min 较快的流速下，分析时间缩短 50% 的色谱图。(d) 调整梯度程序，改善相邻色谱峰 6、7 的分离度。(e) 调整梯度程序，改善相邻色谱峰 2、3 的分离度

4. Agilent 1100 LC 系统的完整优化。使用小规格色谱柱, 重视仪器配置可获得额外的性能优势。最重要的参数为检测器采集速率。这种情况下, 检测器设置为最大采集速率(40 Hz)。其它重要的因素包括: 尽量缩短管路、减小流通池体积以减少峰展宽、使用小体积的进样针阀座。图 5 为原 250 mm 方法与新 100 mm 方法的叠加色谱图。这些色谱图显示新的更快的方法中的最后一个峰在原 5 μm 方法的第一个峰之前出峰, 显著节省了分析时间

最后, 添加了磺胺二甲氧嘧啶的 Similac 牛奶样品, 通过酸性乙腈沉淀蛋白后提取, 振荡, 静置 5 min 后倒出。由散点图可看出(图 6), 整个实验过程中柱压无变化。此外, 柱效几乎保持不变。未过滤的蛋白变性牛奶样品的直接分析证明了 Agilent Poroshell 120 EC-C18 色谱柱的耐用性。

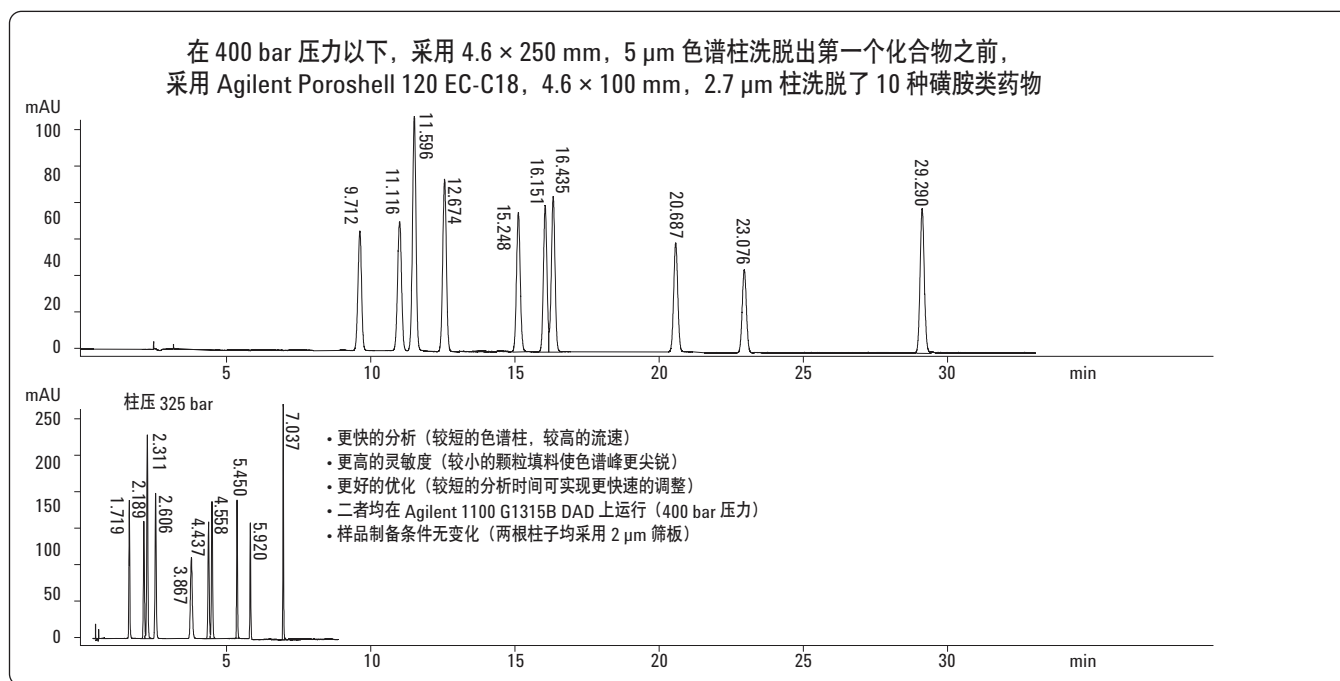


图 5. 从 Agilent ZORBAX Eclipse Plus C18, $4.6 \times 250 \text{ mm}$, $5 \mu\text{m}$ 色谱柱到 Agilent Poroshell 120 EC-C18, $4.6 \times 100 \text{ mm}$, $2.7 \mu\text{m}$ 色谱柱, 在甲酸/乙腈梯度程序下分离 10 种磺胺类药物, 分析时间从 30 min 减少到 8 min

结论

本实验表明，采用 4.6×250 mm, 5 μm C18 色谱柱的现有方法可以很容易地转换为采用 Agilent Poroshell 120 EC-C18 4.6×100 mm 色谱柱的更快但类似的方法。由于运行最大压力远低于 400 bar，故此法还可用于传统 HPLC 系统。此外，由于两种色谱柱均使用 2 μm 筛板，样品制备方法可保持不变。这种方法转移节省了分析时间和溶剂用量，并允许分析人员进一步优化方法参数，时间较长的分析并不理想。

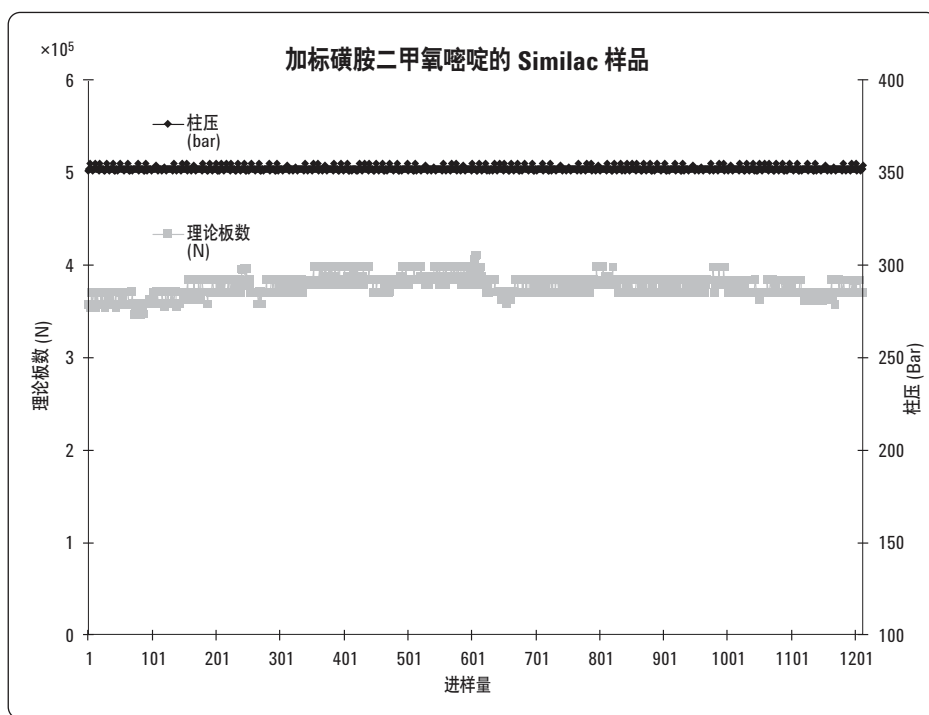


图 6. 使用 Agilent Poroshell 120 100 mm 色谱柱分析的散点图 (磺胺二甲氧嘧啶做内标物的带沉淀的 Similac 样品超过 1000 次连续进样，记录磺胺类药物的柱效，并检测柱压力有无增加)

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采用配备 UV/Vis 二极管阵列和荧光检测器的 Agilent 1290 Infinity LC 系统和 Agilent ZORBAX Poroshell 120 2.7 μm 色谱柱分析辛辣食物和食品中的辣椒碱类物质

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摘要

Agilent 1290 Infinity 液相色谱系统在高效液相色谱和超高压液相色谱中的应用中具有重要地位。与市场上其它系统相比，它的能力范围更广（如压力和流速不同组合的能力），并且可以使用具有不同规格和填料粒径的色谱柱。二极管阵列检测器先进的光学设计可提供宽动态范围和高灵敏度，这在监测天然和合成的食品调味料时非常重要。

这些优点可通过分析干辣椒、酱汁和香料中的主要辣椒碱类成分得进行说明。辣椒科中红辣椒的主成分辣椒素被认为是红辣椒的“辛辣”成分。辣椒碱类成分包括 8 种或更多种化合物，它们可使人感到调味料中的辛辣味道。辣椒素或合成辣椒素（诺香草胺）也已用于外用膏剂，用来缓解关节炎、瘙痒、神经性及其它疾病。核心结构是酚，其主要差异在疏水烷基链上。

该系统具有高耐压性，可使用甲醇、乙腈作为流动相，以比较两种溶剂的选择性。本文评价包括全多孔的 1.8 μm 填料和表面多孔的 2.7 μm 填料在内的不同色谱柱。辣椒素的结构见图 1。

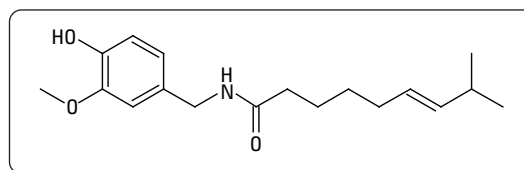


图 1
辣椒素（反式）-N-（4-羟基-3-甲氧基苄基）-8-甲基-6-壬烯酰胺结构

可以使用二极管阵列检测器对辣椒碱色谱峰区的辣椒素特征谱带进行鉴别,以确定是否为活性成分。229 nm/280 nm 的紫外吸光值比一般为 2.4 (图 2)。

使用标准混合物优化分析条件后,对大量样品提取物进行色谱分析(图 3)。

辣椒、其它纯辣椒香料及新鲜的辣椒可采用 UV 检测进行简单分析。但低含量水平样品如美国红辣椒,以及复杂混合物如红辣椒和咖喱混合粉就很可能有干扰。可使用 Agilent 1200 系列荧光检测器(G1321A)解决这一问题,见图 4。FLD 卓越的选择性和高灵敏度可最大程度地减小或消除非辣椒碱类组分对目标化合物色谱峰的干扰。

配置

- 集成真空脱气机的 Agilent 1290 Infinity 二元泵(G4220A)
- Agilent 1290 Infinity 自动进样器(G4226A)
- Agilent 1290 Infinity 柱温箱(G1316C)
- Agilent 1200 系统二极管阵列检测器(G1315C)

结论

该系统具有灵活的溶剂和色谱柱选择性,并能耐高压,可以使用高柱效色谱柱实现高分辨率的快速分离,并且与 4.6 mm 内径的色谱柱相比节省溶剂。荧光检测器选择性更高,可给以前必须通过重新开发分离方法来去除干扰的解决方案提供帮助。

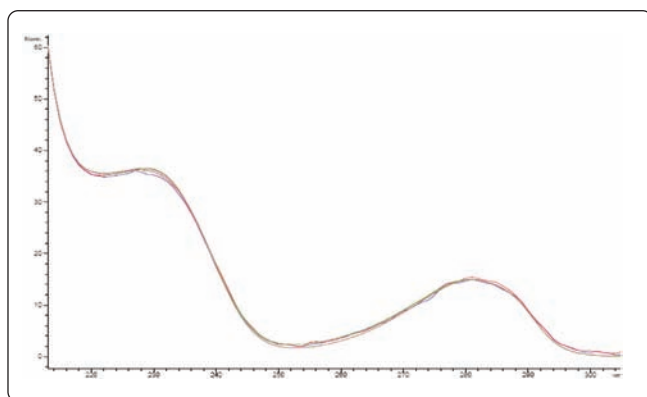


图 2
红辣椒中提取的辣椒碱类化合物的 UV 光谱叠加图

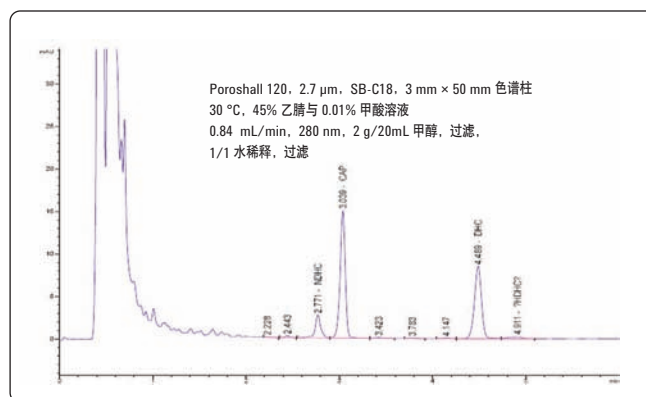


图 3
干辣椒粉的分离 (CAP-辣椒素、NDHC-降二氢辣椒素、DHC-二氢辣椒素)

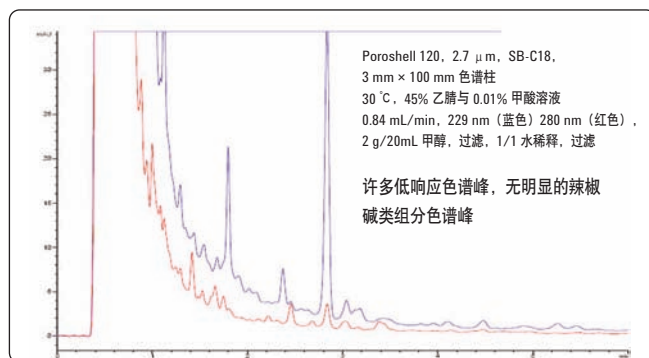
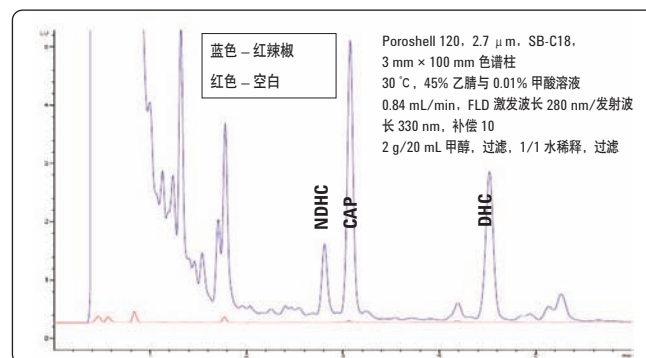


图 4
美国红辣椒提取物的 UV 和荧光信号对比图 (两幅)



www.agilent.com/chem/1290

Poroshell 120 SB-C18 管柱搭配 LC/MS，快速分析茶飲

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摘要

以安捷倫 ZORBAX SB-C18 管柱及安捷倫 Poroshell 120 SB-C18 管柱，分析綠茶中常見的 10 種成分（9 種兒茶素 + 1 種咖啡因），結果顯示兩種管柱的選擇性幾乎相同。以 Poroshell 120 管柱經梯度沖提分析 1.4 分鐘後，再經液相層析儀串聯質譜儀 (LC/MS) 檢測 10 種化合物均能產生直線校正曲線。文中將分析定量幾種罐裝與沖泡的綠茶樣品，並加以比較。經由注入未過濾、未稀釋的沖泡綠茶樣品測試管柱使用壽命，結果證實能在 Poroshell 120 管柱內高壓注入不乾淨的樣品 1500 次以上。

緒論

多酚類化合物可降低罹患心臟疾病的風險、預防癌症，並對抗其他疾病，而新鮮茶葉與綠茶是常見的多酚來源，兒茶素的含量很高。兒茶素會影響茶的顏色與味道，茶的特殊苦味即是兒茶素所致 [1]。茶中的兒茶素，以表沒食子兒茶素沒食子酸酯 (epigallocatechin gallate) 最值得注意，因為是茶的萃取物中，含量最高的多酚類化合物 [2]。

雖然各種類別的茶，均來自相同品種的茶樹 *Camellia sinensis*，但不同的加工處理方式，就會產生不同類別的茶。茶的類別，是由茶葉採收後的發酵（氧化）時間所決定。茶葉採收後，若未儘快乾燥，很快就會枯萎及氧化。此時茶葉的葉綠素會分解，釋出鞣質，導致茶葉變黑。而加熱茶葉會停止氧化，並將分解葉綠素的酵素去除活性，此過程會持續特定一段時間。紅茶為完全發酵茶，烏龍茶為半發酵茶，而綠茶為不發酵茶 [3,4]。由於發酵會降低兒茶素的含量，因此每份綠茶的兒茶素抗氧化劑含量最多，而紅茶最少。

本應用範圍備註中，Yoshida 等人 [5] 利用安捷倫 ZORBAX SB-C18 管柱開發用於檢測茶中兒茶素的 HPLC 分析法，移轉至相同尺寸的安捷倫 Poroshell 120 SB-C18 管柱後，選擇性幾乎不變。本分析法經過了改善，確能適用於 LC/MS，並製作校正曲線，分析罐裝茶及沖泡茶的樣品，以進行比較。另外並實施使用壽命研究，以未稀釋、未過濾的沖泡綠茶樣品，證實裝有大型 2 μm 濾片的 Poroshell 管柱具備優勢，能直接注入不乾淨樣品。

以 HPLC 分析茶飲並非創舉，但此分析法足以證實 Poroshell 120 管柱分析其他天然產品樣品的效用。本分析證實 Poroshell 120 管柱可分析一組 10 種結構非常相似的化合物，包含 4 對差向異構物。這 4 對差向異構物採用價格平庸，又容易取得的樣品做為代表。

實驗

本分析採用安捷倫 1200 系列快速分離 LC (RRLC) 系統，搭配安捷倫 6410 三重四極桿質譜儀 (QQQ)：

表 1. 分析兒茶素的各種分析法參數

	安捷倫 ZORBAX SB-C18 管柱，4.6 × 150 mm、5 μm (p/n 883975-902)	安捷倫 ZORBAX RRHT SB-C18 管柱，4.6 × 50 mm、1.8 μm (p/n 827975-902)	安捷倫 Poroshell 120 SB-C18 管柱，4.6 × 50 mm、2.7 μm (p/n 689975-902)	安捷倫 ZORBAX RRHT SB-C18 管柱，2.1 × 50 mm、1.8 μm (p/n 827700-902)	安捷倫 Poroshell 120 SB-C18 管柱，2.1 × 50 mm、2.7 μm (p/n 689775-902)	安捷倫 Poroshell 120 SB-C18 管柱，2.1 × 50 mm、2.7 μm (p/n 689775-902)	安捷倫 Poroshell 120 SB-C18 管柱，2.1 × 50 mm、2.7 μm (p/n 689775-902)	安捷倫 Poroshell 120 SB-C18 管柱，2.1 × 100 mm、2.7 μm (p/n 685775-902)
流速 (mL/分鐘)	1.00	1.00	1.00	1.00	1.00	1.25	1.50	0.83
移動相 A	0.1% H ₃ PO ₄ 水溶液	0.1% H ₃ PO ₄ 水溶液	0.1% H ₃ PO ₄ 水溶液	0.1% H ₃ PO ₄ 水溶液	各種添加劑的水溶液	0.2% CH ₃ COOH 水溶液	0.2% CH ₃ COOH 水溶液	0.2% HCOOH 水溶液
移動相 B	CH ₃ CN	CH ₃ CN	CH ₃ CN	CH ₃ CN	CH ₃ CN	CH ₃ CN	CH ₃ CN	0.2% HCOOH 溶於 CH ₃ CN
10% B	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15% B	7.50	2.50	2.50	0.50	0.50	0.42	0.36	1.25
27% B	15.00	5.00	5.00	1.00	1.00	0.83	0.71	2.50
沖提停止時間 (分鐘)	15.00	5.00	5.00	1.40	1.40	1.20	0.95	4.00 (含再平衡時間)
沖提後的時間 (分鐘)	10.00	3.00	3.00	1.00	1.00	0.80	0.60	n/a
總循環時間 (分鐘)	25.00	8.00	8.00	2.40	2.40	2.00	1.55	4.00
TCC 溫度 (°C)	40	40	40	40	40	40	40	40
注入量 (μL)	15.0	5.0	5.0	1.0	1.0 (LC/UV), 1.5 (LC/MS)	1.5	1.5	2.0
樣品濃度 (mg/mL)	0.03	0.03	0.03	0.03	0.03 (LC/UV), 0.003 (LC/MS)	0.003	0.003	n/a
系統壓力 (bar)	84	169	117	575	380 (LC/UV), 425 (LC/MS)	505	585	540

- G1312B 二元幫浦 SL 推送移動相 A：H₂O 含多種修飾劑 (0.1% H₃PO₄、0.2% HCOOH、0.2% CH₃COOH、0.02% CF₃COOH、PH 3.6-5.6 的 10 mM CH₃COONH₄、PH 3-4.5 的 10 mM HCOONH₄)，移動相 B：CH₃CN。沖提梯度為 t₀ 時使用 10% B，逐漸升高為 15% B，再逐漸升高為 27% B；梯度沖提時間依管柱的尺寸與流速而變，見表 1
- G1367C 自動液體注射器 (ALS) SL，注入量視特定分析法參數而定，見表 1
- G1316B 恆溫管柱室 (TCC) SL，溫度固定為 40 °C
- G6410A QQQ 質譜儀，質譜離子源：電灑法 AP-ESI，乾燥氣體溫度及流速：350 °C、10 L/分鐘，霧化器氣體壓力：50 Psi，毛細管電壓：選擇離子偵測 (SIM) 模式為 ± 3500 V，m/z 值列於圖 1。兒茶素於負離子模式中監測，咖啡因於正離子模式中監測
- 使用 MassHunter B.02.01 版擷取資料，定性分析則使用 B.02.00 版，定量分析使用 B.03.01 版

綠茶中的兒茶素

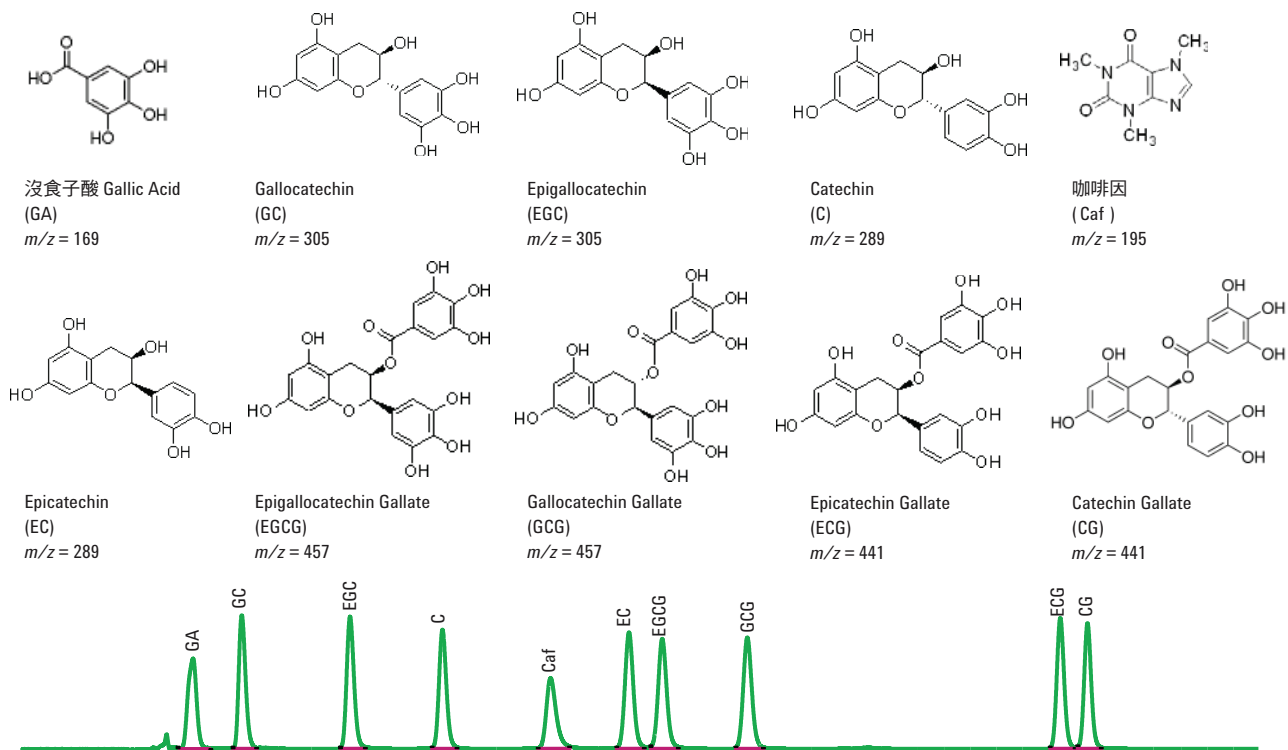


圖 1. 待檢出化合物，自安捷倫 ZORBAX SB-C18 管柱沖提出來的順序；移動相為 H_3PO_4 。（註：後續層析圖顯示的選擇性可能有些微不同，但沖提順序不變。）

本分析使用 6 種安捷倫管柱：

- 安捷倫 ZORBAX SB-C18 管柱， $4.6 \times 150 \text{ mm}$ 、 $5 \mu\text{m}$ p/n 883975-902
- 安捷倫 ZORBAX RRHT SB-C18 管柱， $4.6 \times 50 \text{ mm}$ 、 $1.8 \mu\text{m}$ p/n 827975-902
- 安捷倫 Poroshell 120 SB-C18 管柱， $4.6 \times 50 \text{ mm}$ 、 $2.7 \mu\text{m}$ p/n 689975-902
- 安捷倫 ZORBAX RRHT SB-C18 管柱， $2.1 \times 50 \text{ mm}$ 、 $1.8 \mu\text{m}$ p/n 827700-902
- 安捷倫 Poroshell 120 SB-C18 管柱， $2.1 \times 50 \text{ mm}$ 、 $2.7 \mu\text{m}$ p/n 689775-902
- 安捷倫 Poroshell 120 SB-C18 管柱， $2.1 \times 100 \text{ mm}$ 、 $2.7 \mu\text{m}$ p/n 685775-902

待檢出化合物及其沖提順序的層析圖，顯示於圖 1。所有分析物以乾粉型態均購自 Sigma Aldrich 公司（賓州貝利方特市 (Bellefont) 廠房）。沒食子酸 (gallic acid)、ePigallocatechin、兒茶素 (catechin)、咖啡因 (caffeine)、ePigallocatechin gallate 等標準品，均個別溶於水中，濃度製備成 1 mg/mL ；Galocatechin、ePicatechin、gallocatechin gallate、ePicatechin gallate、catechin gallate 等標準品，則個別溶於 CH_3CN/H_2O 中 (1:1)，濃度製備成 0.5 mg/mL 。上述 1 mg/mL 的標準品溶液，各取 1 單位， 0.5 mg/mL 的標準品溶液，則取 2 單位，混合配製成各種分析物的濃度，均為 0.03 mg/mL 的複合樣品。必要時，可加水稀釋此複合樣品。除了罐裝樣品 A，由日本的同仁寄送至本實驗室之外，其餘茶樣品均購自當地。定量用的罐裝茶及沖泡茶樣品，均以 1:10 的比例，加水稀釋後注入。使用壽命研究用的沖泡綠茶樣品，未經稀釋也未經過濾，直接注入管柱。此外，乙腈、甲酸、醋酸、三氟乙酸 (trifluoroacetic acid)、醋酸銨、甲酸銨等，均購自 Sigma Aldrich 公司（賓州貝利方特市廠房）；水則採用 $18 \text{ M-}\Omega \text{ Milli-Q}$ 水（麻州 Bedford 市）。

結果及討論

先前 T. Yoshida 等人的分析 [5] 顯示，以 $4.6 \times 150\text{-mm}$ 、 $5\text{-}\mu\text{m}$ 的安捷倫 ZORBAX SB-C18 管柱，分析兒茶素的分析時間為 15 分鐘，而使用 $4.6 \times 50\text{-mm}$ 、 $1.8\text{-}\mu\text{m}$ 的安捷倫 ZORBAX 快速分離高處理量 SB-C18 管柱，則分析時間縮短為 5 分鐘。此分析另外使用安捷倫 Poroshell 120 SB-C18 管柱，以進行比較。圖 2 顯示了使用較小顆粒充填的較短型 HPLC 管柱，在保持相同的解析度時，所節省的分析時間。

以 $2.1 \times 50\text{ mm}$ 的管柱執行本分析法，分析時間可再縮短為 1 分鐘。管柱的內徑變小，便能以較低的流速執行相同的分析，而較低流速較適合以 MS 檢測。ZORBAX SB-C18 管柱與 Poroshell 120 SB-C18 管柱，選擇性幾乎相同，因此較容易移轉分析法，如圖 3 所示，但 $1.8\text{-}\mu\text{m}$ 的 ZORBAX 管柱與 $2.7\text{-}\mu\text{m}$ 的 Poroshell 管柱，系統背壓則有明顯差異。ZORBAX 管柱內充填的全多孔性顆粒，直徑較小，而 Poroshell 管柱內充填的表面多孔性顆粒，直徑較大，可大幅降低背壓。Poroshell 管柱的充填顆粒，其多孔性外殼的質量傳導距離短，且顆粒大小的分佈範圍

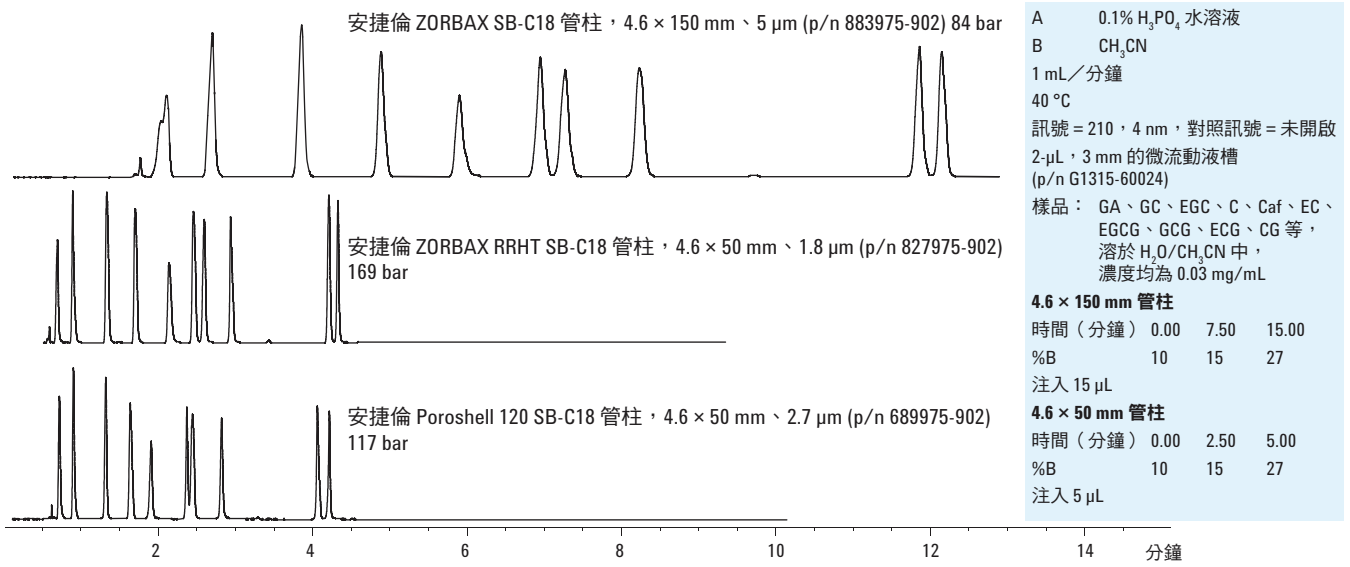


圖 2. 原本以 150 mm 、 $5\text{ }\mu\text{m}$ 管柱，分析兒茶素的方法，調整後套用至 50 mm 、 $1.8\text{ }\mu\text{m}$ 的安捷倫 ZORBAX SB-C18 管柱，及 50 mm 、 $2.7\text{ }\mu\text{m}$ 、以表面多孔性顆粒充填的安捷倫 Poroshell 120 SB-C18 管柱

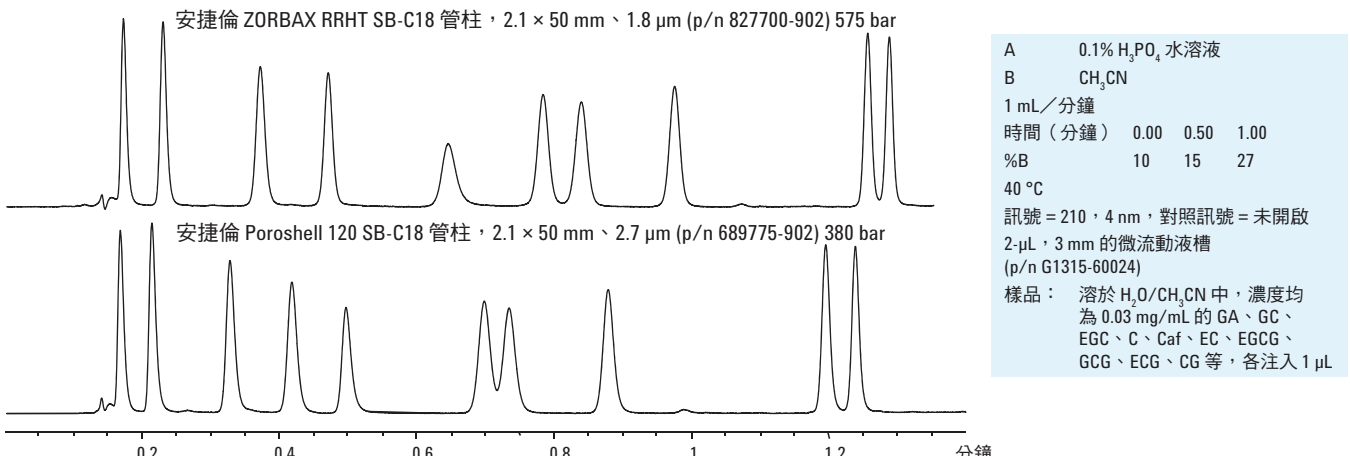


圖 3. 兒茶素分析法移轉至內徑 2.1 mm 的管柱，以搭配 LC/MS 使用，並將流速固定為原先 $4.6 \times 150\text{ mm}$ 管柱分析法的 $1\text{ mL}/\text{分鐘}$ ，依管柱容積調整梯度沖提時間，以進一步縮短分析時間

明顯窄於次 2 μm 的全多孔性顆粒，因此有相似的效能。本分析例顯示，背壓的差異相當大，足以決定該使用 400 bar 或 600 bar 的層析儀。

內徑 2.1 mm 的管柱，採用的流速較低，適合搭配 LC/MS 使用，但原本使用的磷酸移動相，則不能使用於 LC/MS 系統。經篩選後，與 MS 相容，用於本兒茶素分析的數種的移動相，列於圖 4。除了圖 4 呈現的結果之外，本分析也在 PH 3-4.5 時，篩選出 10 mM 的醋酸銨緩衝液，其沖提後的層析圖，幾乎與醋酸銨的數

據相同。整體而言，整個篩選過程中，選擇性保持不變，因此是根據分析物的訊號強度，挑選出最合適的移動相。圖 4 以相同的比例，顯示各個層析圖。銨鹽配製成的緩衝液，及三氟乙酸移動相，均出現顯著的離子抑制現象。2 種最佳的候選溶液，為甲酸與醋酸，其中使用醋酸時，各種化合物的訊號稍微較強。應注意的是，正離子掃描的結果，較不受離子抑制作用影響，因此圖 4 中的負離子掃描的結果，可做為層析圖的代表，不過咖啡因的正離子掃描結果，仍遵循相同的模式。

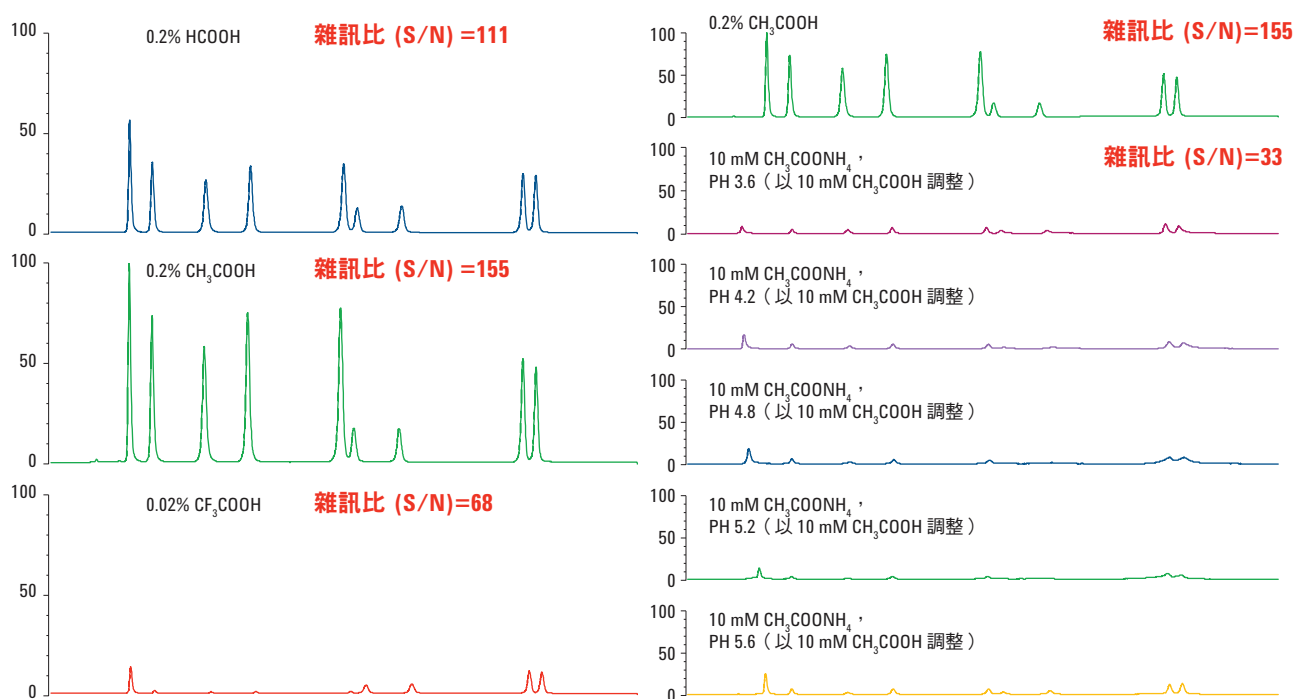


圖 4. 篩選各種與 MS 相容的移動相，尋找可替代原本 LC/UV 法使用的 H_3PO_4 的移動相（註：相較於兒茶素類化合物，咖啡因顯著較不受離子抑制作用影響，因此並未列出其正離子 SIM 層析圖。）

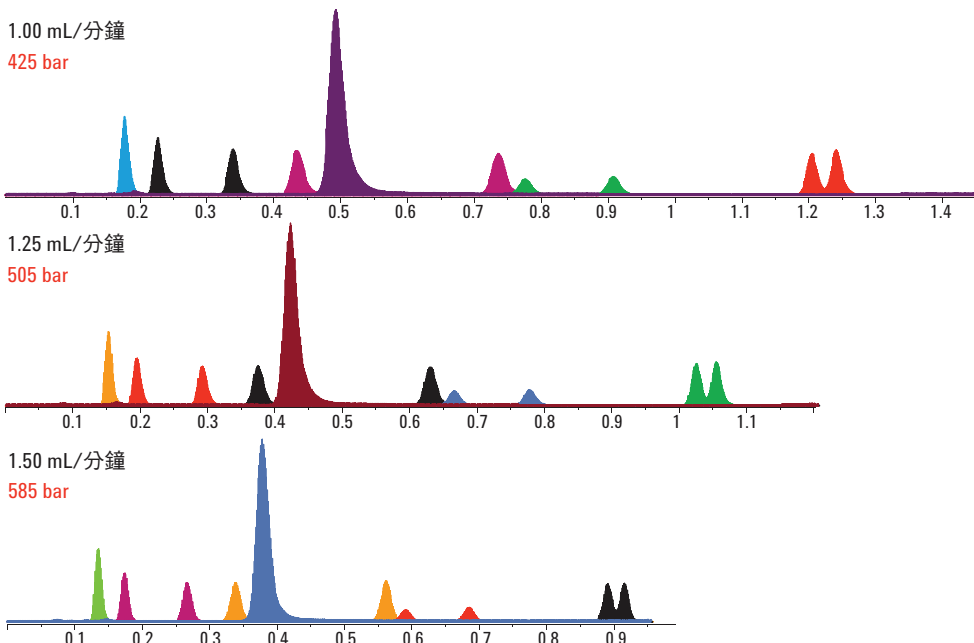
圖 5 並排顯示萃取離子層析圖 (EIC)，可見安捷倫 Poroshell 管柱產生的背壓較低，故能更快完成分析，產生的背壓卻不會高於 600 bar。使用 150 mm 管柱的 15 分鐘分析法，改用 50 mm 的 Poroshell 120 管柱後，分析時間可縮短至少於 1 分鐘，又能保持原本分析法的選擇性。比較圖 3 與圖 5，可見以同樣的管柱 (安捷倫 Poroshell 120 SB-C18 管柱，2.1 × 50-mm、2.7- μ m) 執行相同的分析法 (1 mL/分鐘)，卻產生明顯不同的背壓。產生背壓不同的主要因為連接 HPLC 與 MS 的 0.12 mm 小內徑長型輸送管線。內徑較大的輸送管線不適用於此類分析，因為可能使波帶變寬，降低此精細調整與解析度。

待檢出的 10 種化合物，其各自的校正曲線，至少由 6 個點構成 (至多 10 個)，而每種標準品均分析 3 次。全部 10 種分析物的線性迴歸與相關係數的數據，列於表 2。所有曲線的線性程度均高，各分析物在管柱 (Poroshell 120 SB-C18 管柱，2.1 × 50 mm) 中的最大分析量為 10 ng。所有茶樣品均以 1:10 的比例，加水稀

釋後注入管柱，避免濃度高於校正標準品的最高濃度。唯獨沖泡茶樣品中的 EGCG 化合物，以 1:10 的比例加水稀釋後，仍超出最高濃度，於是利用表 2 的線性迴歸方程式，以外插法計算出 EGCG 的濃度。

表 2. 兒茶素與咖啡因的校正資料；所有標準品均分析 3 次，校正曲線至少由 6 點構成

	線性迴歸線	相關係數，R ²
沒食子酸 (Gallic acid)	$y = 0.466 x$	0.995
Gallocatechin	$y = 0.407 x$	0.996
Epigallocatechin	$y = 0.355 x$	0.996
Catechin	$y = 0.601 x$	0.996
咖啡因	$y = 3.439 x$	0.995
Epicatechin	$y = 0.638 x$	0.995
Epigallocatechin gallate	$y = 0.153 x$	0.998
Gallocatechin gallate	$y = 0.183 x$	0.996
Epicatechin gallate	$y = 0.396 x$	0.998
Catechin gallate	$y = 0.371 x$	0.996



A	0.2% CH ₃ COOH 水溶液	
B	CH ₃ CN	
管柱	安捷倫 Poroshell 120 SB-C18 2.1 x 50 mm, 2.7 μ m	
來源	350 °C, 10 L/分鐘, 50 Psi, +/-3500 V	
擷取	SIM- (169、305、289、457、441), SIM+ (195)	
樣品	溶於 H ₂ O/CH ₃ CN 中，濃度均 為 0.003 mg/mL 的 GA、GC、 EGC、C、Caf、EC、EGCG、 GCG、ECG、CG 等， 各注入 1.5 μ L	
1.00 mL/分鐘		
時間 (分鐘)	0.00	0.50
%B	10	15
1.25 mL/分鐘		
時間 (分鐘)	0.00	0.42
%B	10	15
1.50 mL/分鐘		
時間 (分鐘)	0.00	0.36
%B	10	15

圖 5. 兒茶素分析在提高流速，並依管柱容積調整沖提梯度後，分析速度又再加快

挑選的罐裝茶已進行分析，各種茶樣品的成分及產地，列於表 3，定量結果則顯示於圖 6。茶樣品 A 為日本茶，未開封，已於室溫下儲存約 3 年。樣品 A 只檢出沒食子酸 (gallic acid) 與咖啡因，原本可能有其他兒茶素，但已隨時間分解。罐裝茶樣品 B 及 D，為不同品牌的日本綠茶，兩者標示於標籤上的成份相同，經本分析法分析後，各種兒茶素的含量也約略相同。茶樣品 C 也是綠茶，標示成分也與樣品 B、D 相同，不過是台灣茶。相較於日本綠茶樣品 B、D，樣品 C 的 ePicatechin gallate 與 catechin gallate 含量較高，但其他 8 種分析物的含量則較低。罐裝茶樣品 E 與 F 為日本茶的混合茶，兩者的標籤均標示含有部分綠茶。樣品 E 的主成分為大麥，因此咖啡因與兒茶素的含量，均明顯低於其他茶樣品。茶樣品 F 為烏龍茶的混合茶，成分與其他綠茶有些微差異。相較於純日本茶的樣品 B 及 D，烏龍混合茶的食子酸 (gallic acid) 與咖啡因的含量較高，epicatechin gallate 與 catechin gallate 的含量相同，其餘的兒茶素含量則較低。

表 3. 罐裝茶樣品的成分及產地

罐裝茶樣品	產地	成分
A	日本	(未知)
B	日本	純水、綠茶、維生素 C
C	台灣	礦泉水、綠茶、維生素 C、天然調味料
D	日本	水、綠茶、維生素 C
E	日本	薏仁、糙米、胚芽米、綠茶、大麥、魚腥草、菊苣 (chickory)、藜麥 (quinoa)、明日葉、維生素 C
F	日本	烏龍茶、普洱茶、綠茶、紅茶、菊苣、大豆、芝麻、維生素 C

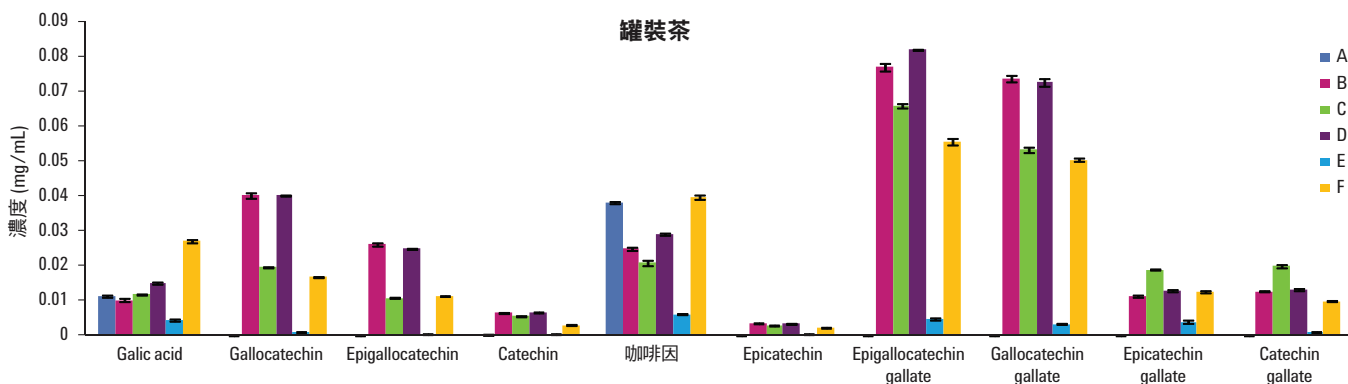


圖 6. 分析的 6 種罐裝茶樣品：3 種綠茶 (2 種日本綠茶 [B、D]、1 種台灣綠茶 [C])、1 種大麥混合茶 (E)、1 種烏龍混合茶 (F)、1 種未知 (A)

圖 6 為罐裝茶樣品的結果比較，圖 7 為現泡的綠茶樣品，圖 8 為現泡的紅茶樣品。兩種沖泡的茶樣品，在茶包沖泡 6-10 分鐘後，大部分化合物均出現最大濃度。過了這段最佳的沖泡時間之後，兩種茶樣品的化合物均開始分解。最值得注意的是 ePigallocatechin gallate，此成分在 60 分鐘內，就分解了最大濃度的 50% 以上。同樣令人感興趣的是，沖泡綠茶樣品與罐裝綠茶樣品，兩者的 epigallocatechin gallate 含量差異為何。

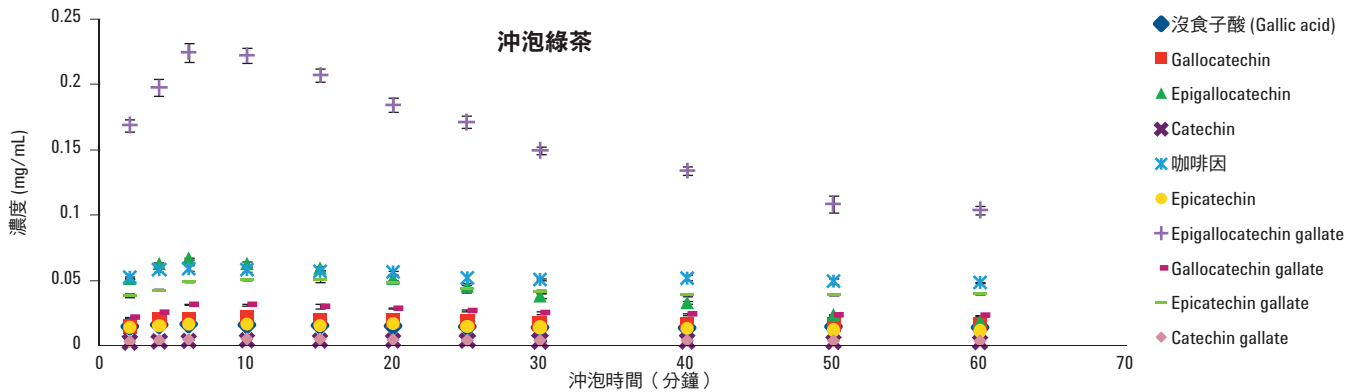


圖 7. 現泡綠茶樣品：1 種市面上的茶包，泡於 6 oz 沸水中，一段時間後取出

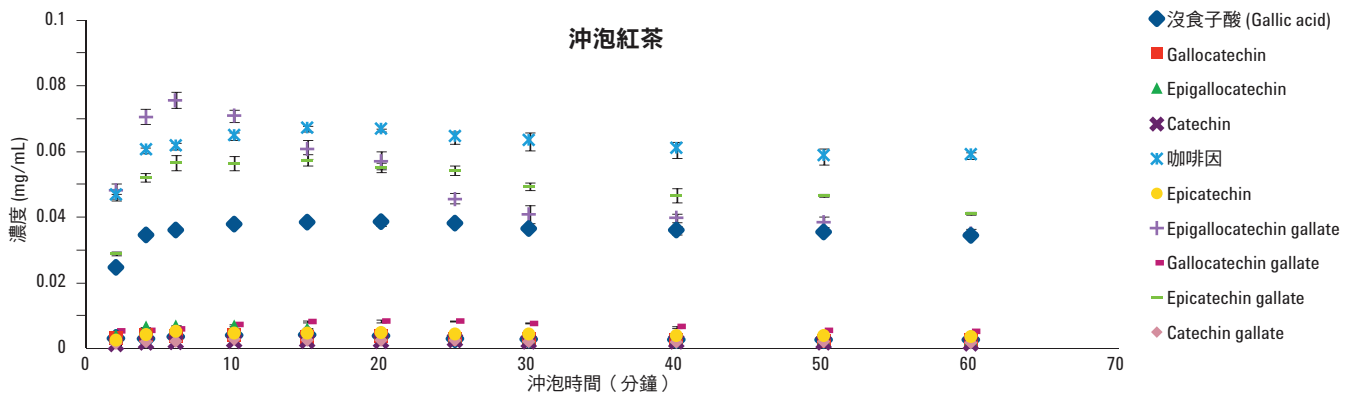


圖 8. 現泡紅茶樣品：1 種市面上的茶包，泡於 6 oz 沸水中，一段時間後取出

圖 9 顯示使用壽命研究的結果，該研究高壓 (550 bar) 注入超過 1500 次不乾淨的樣品，結果壓力並未上升，波峰寬度也未擴大。綠茶樣品為將市面上的茶包，置於 6 oz 的沸水中，沖泡 6 分鐘而成，之後未經過濾也未經稀釋，便直接注入 HPLC 中。兒茶素會大量分解（咖啡因較穩定），因此樣品需每天更換 2 次。安捷倫 Poroshell 120 SB-C18 (2.1 × 100 mm、2.7 μm) 管柱中的 2 μm 濾片，非常適用於不乾淨的樣品，防止堵塞的功能也強於次 2 μm 管柱的 0.5 μm 濾片。

結論

分析綠茶中兒茶素的現有 HPLC 分析法，已自全多孔性顆粒充填的 1.8 μm 安捷倫 ZORBAX SB-C18 管柱，成功移轉至表面多孔性顆粒充填的 2.7 μm 安捷倫 Poroshell 120 SB-C18 管柱。兩種管柱的選擇性幾乎相同，不需調整分析法，分離 10 種化合物的結果便能相同。所有成分的校正曲線，線性程度均相當高，因此各種罐裝茶與現泡茶，均能測定其兒茶素含量，並加以比較。本分析亦使用 Poroshell 120 管柱中較大型的 2 μm 濾片，進行使用壽命試驗，結果顯示，高壓注入不乾淨的樣品 1500 次以上後，不影響層析結果。

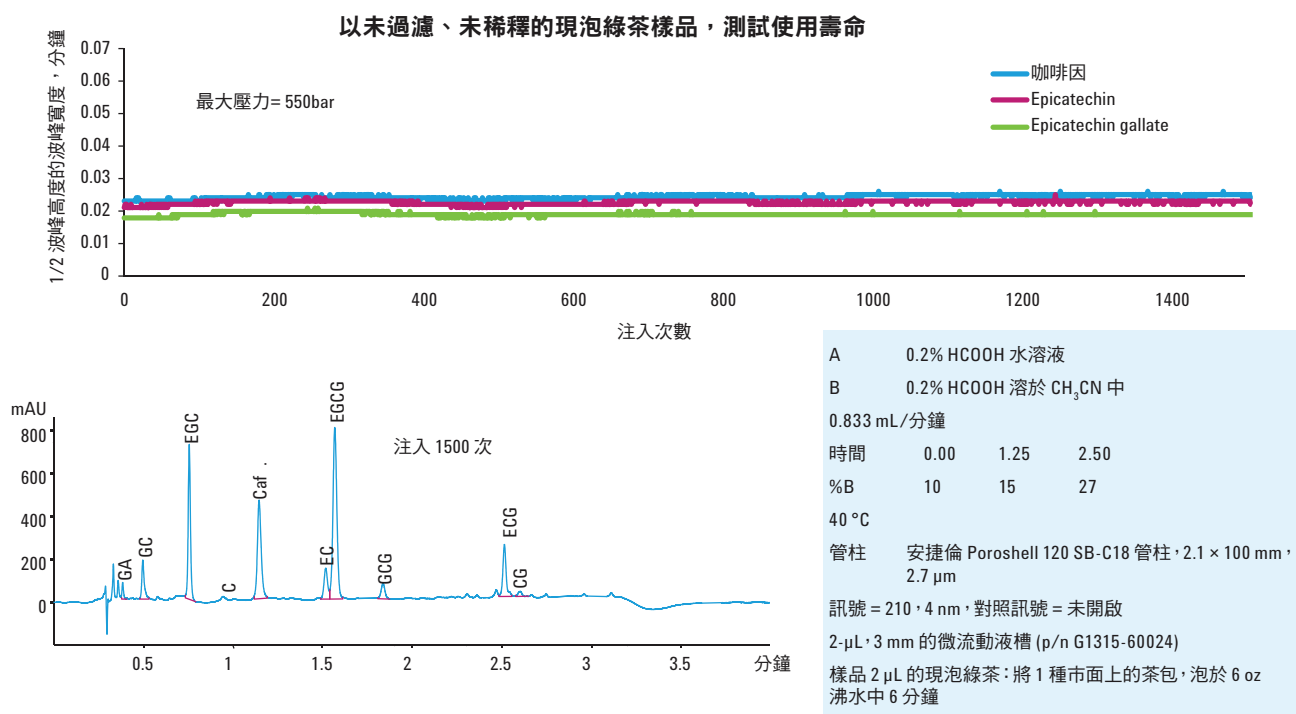


圖 9. 使用壽命研究，以未過濾、未稀釋的現泡綠茶樣品，注入 1500 次以上後，波峰寬度並未增大，壓力也未上升

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采用安捷伦 Bond Elut Plexa 萃取柱和 Poroshell 120 色谱柱结合 LC/MS/MS 分析苹果汁中的杀菌剂及其代谢物

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摘要

本应用报告介绍了一种简单的样品制备方法结合 LC/MS/MS 对苹果汁中的杀菌剂残留进行分析。建立了一种能够同时检测 3 种重要内吸性杀菌剂——多菌灵、噻菌灵、抑霉唑及其代谢物的分析方法，且此方法可以扩展应用到范围更广的农药中去。这是一种无需任何预浓缩即可得到干净提取液的简便快速方法，且该方法检出限可达 2 ppb 以下。采用磷酸三苯酯作为内标进行定量分析。每个被测物在 3 个浓度水平（10 ppb、50 ppb 和 250 ppb）下均有极好的准确度（回收率%）和精度（变异系数 CV %）。

引言

杀菌剂是一类用于抑制真菌生长的农药。由于食品中的杀菌剂残留引起人们对健康问题的广泛关注，因此欧洲、美国和其他许多国家都对其残留进行监管。多菌灵和噻菌灵属于苯并咪唑类杀菌剂，而抑霉唑属于咪唑类农药。鉴于抑霉唑很容易代谢成 α -（2,4-二氯苯基）-1H-咪唑-1-乙醇，在抑霉唑的监管上，美国要求监测其母体及其代谢物的总和。

苯并咪唑类杀菌剂广泛应用于农业中的植物疾病控制和水果的采后处理。在美国，多菌灵（MBC）并没有登记使用，但在很多出口水果和果汁到美国的国家中多菌灵的使用却是合法的。另外，多菌灵是甲基硫菌灵（TM）的代谢物或降解物，后者被允许用于美国的许多农作物和草坪，这增加了人们接触到多菌灵残留的潜在风险。噻菌灵和抑霉唑最常用于包括苹果在内的水果的采后处理如储藏和运输，且这两种化合物在美国是允许使用的。

本文建立的简单提取程序可为目标被测物提供可重现的高回收率，这归功于安捷伦 Bond Elut Plexa 的优越性能，这是一种为提高分析性能和易用性而设计的聚合物吸附剂。该吸附剂对于很多分析物均具有优越的保留性能，此外，由于小粒径的 Bond Elut Plexa 填料不易引起堵塞，所以它能为小柱提供快速而可重现的流速。Bond Elut Plexa 提取物的洁净度降低了离子抑制效应，延长了 HPLC 色谱柱的寿命并使质谱仪器免受污染。

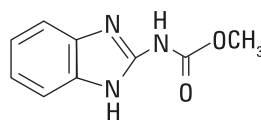
填充 2.7 μm 表面微粒多孔填料的安捷伦 Poroshell 120 EC-C18 色谱柱与亚 2 微米 UHPLC 色谱柱具有相似的柱效，但背压降低了 40%。液相色谱系统分析时的实际压力不会超过 400 bar。

高灵敏度的 Agilent 6460 三重四极杆液质联用系统搭配 AJST 电喷雾离子源能为 4 个目标被测物提供低至 2 ppb 的优异检测性能。由于它大大低于任何现有的测试要求，我们的提取方法可以用灵敏度较低的老型号质谱仪检测，如 Agilent 6410 三重四极杆液质联用系统。使用灵敏度比 Agilent 6460 三重四极杆液质联用系统低的质谱检测器时，可以考虑在萃取过程中复溶这一步对样品进行预浓缩。

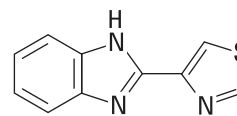
之前安捷伦已经建立了苹果汁中多菌灵与噻菌灵的检测方法 [1]，该方法使用 Agilent Bond Elut SCX SPE 小柱净化，采用 Agilent ZORBAX Eclipse Plus C18, 3.5 μm 色谱柱结合带有可变波长检测器的 Agilent 1200 系列液相色谱系统进行检测。

实验部分

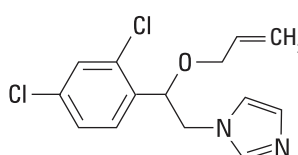
被测物



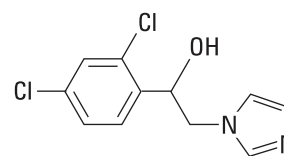
多菌灵 $\text{C}_9\text{H}_9\text{N}_3\text{O}_2$
m.w. 191.19
pKa 4.5 LogP 1.5



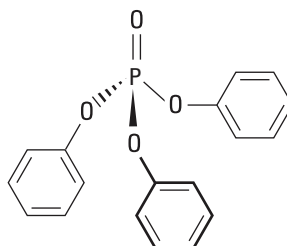
噻菌灵 $\text{C}_{10}\text{H}_7\text{N}_3\text{S}$
m.w. 201.25
pKa 4.7 LogP 2.4



抑霉唑 $\text{C}_{14}\text{H}_{14}\text{Cl}_2\text{N}_2\text{O}$
m.w. 297.18
pKa 6.5 LogP 3.8



抑霉唑代谢物 $\text{C}_{11}\text{H}_{10}\text{Cl}_2\text{N}_2\text{O}$
m.w. 257.12
LogP 2.6



磷酸三苯酯 $\text{C}_{18}\text{H}_{15}\text{O}_4\text{P}$
m.w. 326.28
LogP 4.7

图 1. 被测物的结构和基本信息

农药标准品购自 Sigma-Aldrich 公司 (St. Louis, MO, USA)。内标 (磷酸三苯酯) 由安捷伦科技公司 (Wilmington, DE, USA) 提供。用甲醇配制成储备液 (多菌灵的浓度为 0.25 mg/mL, 其他 3 个被测物和内标的浓度为 1 mg/mL), 并保存于 $-20\text{ }^{\circ}\text{C}$ 。

材料与仪器

SPE: 安捷伦 Bond Elut Plexa 小柱 30 mg, 3 mL
(部件号 12109303)

安捷伦 VacElut 20 固相萃取装置 (部件号 12234100)

安捷伦活塞阀 (部件号 12234520)

安捷伦硅烷化自动进样小瓶, 2 mL (部件号 5183-2072)

安捷伦自动进样小瓶的螺纹口盖 (部件号 5182-0717)

LC: 安捷伦 Poroshell 120 EC-C18, 2.1 x 50 mm, 2.7 μ m 色谱柱 (部件号 699775-902)

Agilent 1260 Infinity LC 系统 (G1379B 微脱气机, 低延迟体积配置的 1312B 二元泵, G1367E 自动进样器, G1330B 恒温器)

MS: 配置 AJST 电喷雾离子源的 Agilent 6460A 三重四极杆液质联用仪

样品制备

前处理

向 0.5 mL 的苹果汁中添加 TPP (内标) 使浓度为 50 ppb, 建议使用 12 x 75 mm 的玻璃试管。用色谱纯的水按 1:3 稀释, 涡旋, 如浑浊需离心。澄清的苹果汁无需离心。

提取

1. 用 0.5 mL 甲醇对 Bond Elut Plexa 小柱进行活化 (先浸透再滴放)
2. 直接上样或者上清液上样
3. 淋洗 1:1 mL 水
4. 淋洗 2:1 mL 30:70 的甲醇水溶液
5. 真空干燥 5~10 min (10~15 in Hg)
6. 用 1 mL 乙酸乙酯/异丙醇 (80:20) 进行洗脱。先浸泡, 再将洗脱液滴至收集瓶中。待洗脱液完全流出后, 于柱上加低真空 (2~3 in Hg)
7. 洗脱液在 55 °C 条件下用氮气流吹干
8. 用 0.5 mL 初始流动相复溶 (10% 甲醇、90% 水、0.1% 甲酸)

LC/MS/MS

LC 条件

流动相 A:	0.1% 甲酸水溶液	
流动相 B:	0.1% 甲酸甲醇溶液	
进样量:	5 μ L	
流速:	0.5 mL/min	
梯度洗脱	时间 (min)	% B
	0.0	10
	0.5	10
	2.0	50
	3.0	95
	7.5	95
	7.1	10

停针时间: 7.2 min

平衡时间: 2.5 min

最大泵压力: 400 bar

洗针: 甲醇/水 (75:25) 洗针时间 10 s

禁止重叠进样

无自动降低延迟体积功能

MS 条件

ES 离子源参数

离子模式:	正离子
毛细管电压:	2800 V
干燥气流速:	12 L/min
干燥气温度:	350 °C
雾化气压力:	40 psi
鞘气流速:	12 L/min
鞘气温度:	300 °C
喷嘴电压:	0 V

MS 参数

扫描模式:	动态 MRM
预运行脚本:	SCP_MS DiverterValveToWaste() {MH_Acq_Scripts.exe}
时间段#1:	1.5 min 后切入 MS
电子倍增管电压:	(+) 400 V

结果与讨论

4 种目标杀菌剂和内标 (TPP) 在 Bond Elut Plexa 小柱上均能得到有效的保留。Bond Elut Plexa 小柱中的吸附材料是极性增强的中性聚苯乙烯-二乙烯苯。这种吸附剂广泛适用于非离子型酸性、中性与碱性物质的提取。我们建立了一种非常简单的萃取方法, 其中唯一的前处理是用水稀释苹果汁。如果苹果汁中有大量的果肉还需要进行离心, 但大多数的苹果汁无需这一步骤。上样和淋洗步骤靠重力流动完成。只需在洗脱前加真空干燥 SPE 柱, 洗脱后加真空使洗脱步骤回收率最大化。

本文建议将第二次淋洗时的甲醇浓度由 5% (通用方法) 增加到 30%, 会显著减少基质干扰。这使较早流出的多菌灵和噻菌灵的 LC/MS/MS 灵敏度提高 30%, 抑霉唑代谢物灵敏度提高 20%。抑霉唑峰面积保持不变。与 100% 甲醇相比, 组合洗脱液 (乙酸乙酯/异丙醇 (80:20)) 对 4 个目标化合物的回收率略微有所提高, 而疏水性强的内标 (TPP) 的回收率大约提高了 2 倍。

表 1 列出了 4 种杀菌剂和内标的动态 MRM 转换参数, 依次为定量转变通道和定性转变通道。

Agilent Poroshell 120 EC-C18, 2.1 × 50 mm, 2.7 μm 色谱柱在 5 分钟内实现对 4 个杀菌剂和内标的分离。图 2 是苹果汁中添加 4 种杀菌剂 (2 ppb) 和内标 (50 ppb) 的 MRM 谱图。所有峰的信噪比均大于 100:1。显然, 每个被测物的检测限均能达到 ppt 水平。每个样品前 1.5 分钟的流出液切入废液以使离子源的污染最小。

表 1. 动态 MRM transitions

化合物名称	是否内标?	母离子	MS1 分辨率	子离子	MS2 分辨率	裂解电压 (V)	碰撞能量 (V)	保留时间	保留时间窗口	极性
多菌灵	<input type="checkbox"/>	192.1	Unit	160.1	Wide	95	15	2.2	2	正
多菌灵	<input type="checkbox"/>	192.1	Unit	132.1	Wide	95	35	2.2	2	正
噻菌灵	<input type="checkbox"/>	202	Unit	175	Wide	110	27	2.5	2	正
噻菌灵	<input type="checkbox"/>	202	Unit	131.1	Wide	110	35	2.5	2	正
抑霉唑代谢物	<input type="checkbox"/>	257	Unit	125	Wide	110	32	2.9	2	正
抑霉唑代谢物	<input type="checkbox"/>	257	Unit	69	Wide	110	22	2.9	2	正
抑霉唑	<input type="checkbox"/>	297.1	Unit	159	Wide	130	23	3.6	2	正
抑霉唑	<input type="checkbox"/>	297.1	Unit	69	Wide	130	22	3.6	2	正
磷酸三苯酯	<input checked="" type="checkbox"/>	327.1	Unit	77	Wide	170	40	4	2	正
磷酸三苯酯	<input checked="" type="checkbox"/>	327.1	Unit	152	Wide	170	45	4	2	正

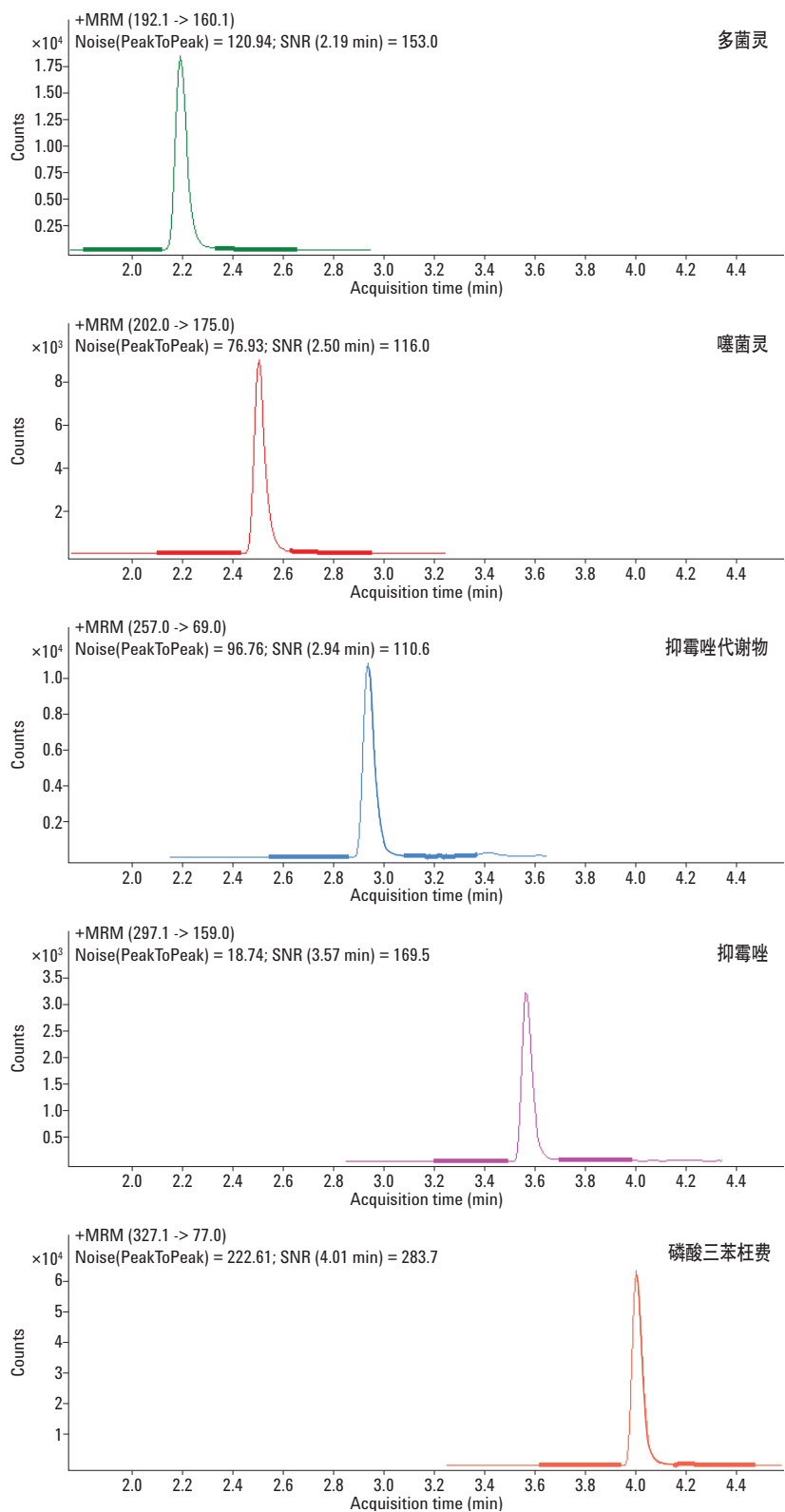


图 2. 苹果汁萃取液中 4 种杀菌剂 (2 ppb) 和 TPP (50 ppb) 的 MRM 提取离子色谱图
Agilent Poroshell 120 EC-C18, 2.1 × 50 mm 2.7 μm 色谱柱。粗线所示为噪音区域

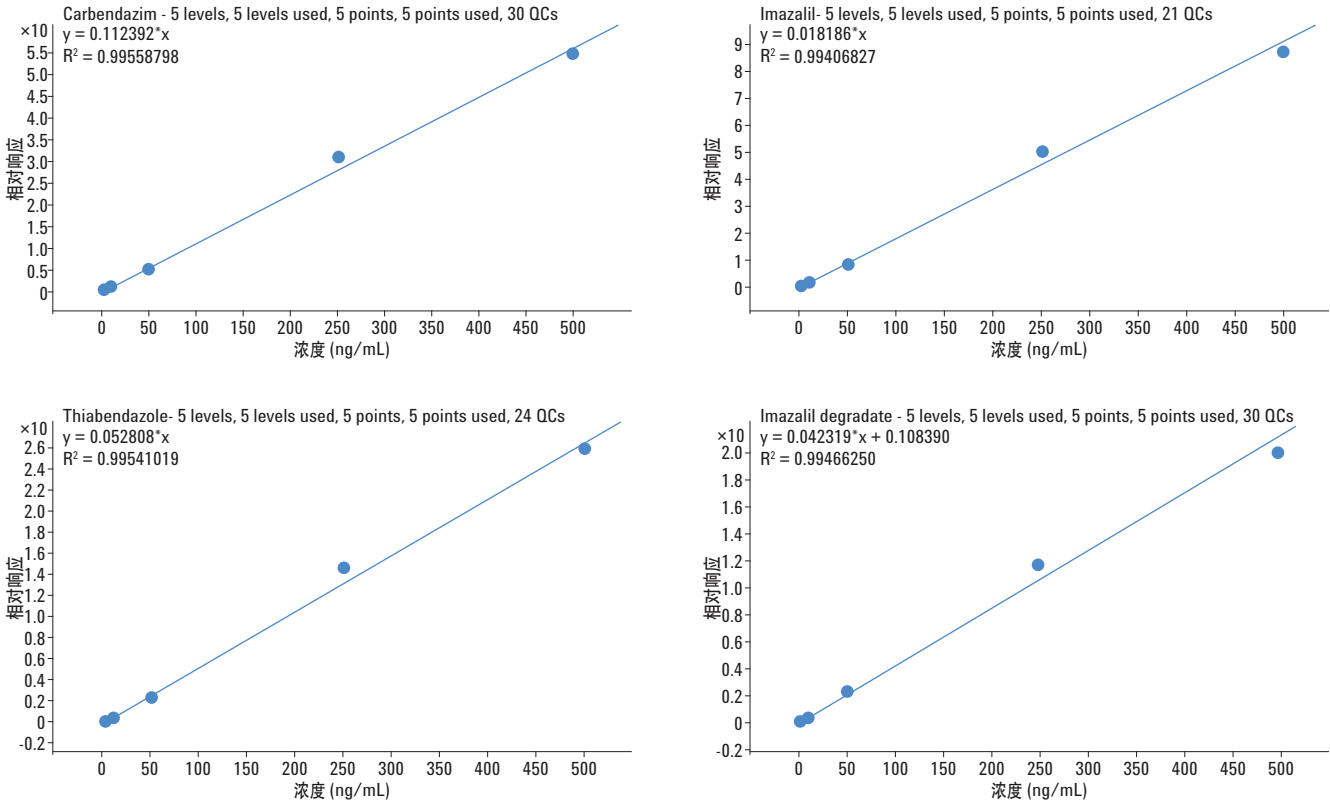


图 3. 苹果汁萃取液中 4 种杀菌剂的校正曲线。线性范围为 2.0~500 ppb。所有的拟合方程线性良好， $R^2 \geq 0.994$

表 2. 苹果汁中杀菌剂测定方法的验证， $n = 5$

化合物名称	R^2	回收率 (%)	10 ppb		50 ppb		250 ppb	
			变异系数 (%)	回收率 (%)	CV (%)	回收率 (%)	变异系数 (%)	
多菌灵	0.996	95.7	1.1	94.1	0.4	107.3	1.2	
噻菌灵	0.995	94.7	1.7	93.6	1	104.8	0.9	
抑霉唑	0.994	95.7	0.4	98.5	1.3	108	0.9	
抑霉唑降解物	0.995	94.1	2.1	100.2	0.8	106.7	1.4	

结论

本文采用固相萃取的前处理方法，结合 LC/MS/MS 检测技术，建立了一种准确性高，重现性好的方法，该方法简单有效。Bond Elut Plexa 吸附剂的独特性质使得该方法可以扩展应用于农药多残留检测。该方法中目标被测物 5 分钟内可以完全分离，且在大多数安捷伦液相系统上都可以实现。

该方法建立的 Agilent 6460 三重四极杆质谱优化参数可以被简单的修改后用于其他的安捷伦三重四极杆液质联用系统。当采用灵敏度较低的检测器时可以在萃取方法中的复溶步骤进行预浓缩。

参考文献

1. C. Zhai, Y. Zou, C. Ball. *Bond Elut Polymer SCX 固相萃取结合高效液相色谱测定苹果汁中苯并咪唑抗真菌剂*。应用简报，安捷伦科技有限公司，出版号 5990-3235CHCN (2008)
2. E. M. Thurman, I. Ferrer, M. Woodman, J. Zweigenbaum. *用液相色谱/飞行时间质谱和液相色谱/离子阱质谱分析柑橘和橙汁中采后杀菌剂及其代谢物*。应用简报，安捷伦科技有限公司，出版号 5989-2728CHCN (2005)
3. Zhai. *应用安捷伦 Bond Elut QuEChERS AOAC 试剂盒和 LC-MS/MS 进行大米农药残留分析*。应用简报，安捷伦科技有限公司，出版号 5990-8034CHCN (2011)
4. Pesticide MRL Database
<http://www.mrlatabase.com/?selectvetdrug=0>

采用安捷伦 Bond Elut PCX 固相萃取柱和 Poroshell 120 色谱柱对鱼肉中的孔雀石绿和结晶紫进行 LC/MS/MS 分析

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摘要

本文建立并验证了一种同时测定鱼肉中孔雀石绿和结晶紫及其代谢产物隐色孔雀石绿和隐色结晶紫的方法。采用固相萃取法对分析物进行提取，并采用液相色谱-正离子多反应监测模式的电喷雾离子化串联质谱法对其进行定量。该方法对鱼肉中所有化合物的定量限均为 0.5 ng/g。这些化合物的动态校正范围为 0.5~100 ng/g。回收率介于 96%~109% 之间，RSD 值介于 1.7%~4.5%。

前言

孔雀石绿 (MG) 和结晶紫 (CV) 是主要用于染色的人工合成色素。此外，由于它们具有抗菌性，所以被用来治疗水生生物的传染性疾病，特别是鱼和鳗鱼。然而，MG 是一种疑似致癌物质，目前在水产养殖中被禁止使用 [1]。

本文采用安捷伦 Bond Elut Plexa PCX 固相萃取 (SPE) 柱对鱼肉中的孔雀石绿 (MG)、结晶紫 (CV) 及其代谢物隐色孔雀石绿 (LMG) 和隐色结晶紫 (LCV) 进行提取, 并对其进行 LC/MS/MS 分析。表 1 列出了这 4 种化合物的名称和化学结构。

实验部分

试剂及化学品

所有试剂均为 MS、HPLC 或者分析级。甲醇、乙腈和水购自 Honeywell 公司。标准品和化学品购自北京百灵威科技有限公司。同位素标记的孔雀石绿 (MG-d5) 和同位素标记的隐色孔雀石绿 (LMG-d6) 作为内标购自 Dr. Ehrenstorfer GmbH 公司。鱼 (鲫鱼) 从当地的超市购买。

分别用甲醇配制各标准溶液, 其浓度均为 1.0 mg/mL, 于 -20 °C 冰箱中储存。用乙腈/水 (10:90) 配制混合工作溶液 (10 µg/mL), 并于 4 °C 储存。加标溶液每日以混合工作溶液用水稀释制得。

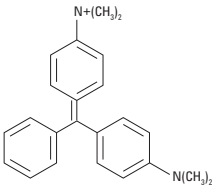
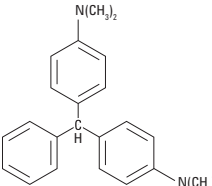
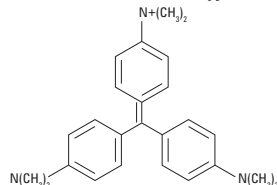
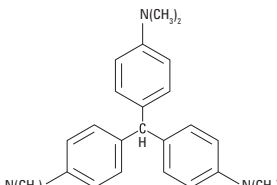
分别用甲醇配制各内标溶液, 并于 -20 °C 冰箱中储存。使用前将各内标溶液混合并用甲醇稀释浓度至 100 ng/mL。

TMPD 溶液: 用甲醇溶解 50 mg N,N,N,N-四甲基-1,4-对苯二胺二盐酸盐 (TMPD) 并定容至 50 mL。

Mcllvaine 缓冲液: 取 445.5 mL 0.1 mol/L 的柠檬酸溶液与 54.5 mL 0.2 mol/L 的磷酸氢二钠溶液, 混合即得。

洗脱缓冲液: 取 50 mL 5 mol/L 的乙酸铵溶液 (调节 pH 值至 7.0)、100 mL 乙酸乙酯和 350 mL 甲醇, 混合即得。

表 1. 本文所用化合物

化合物	CAS 号	结构
孔雀石绿	569-64-2	
隐色孔雀石绿	129-73-7	
结晶紫	548-62-9	
隐色结晶紫	603-48-5	

设备与材料

Agilent 1200 HPLC 系统

Agilent 6460 三重四极杆 LC/MS/MS 系统

Agilent Bond Elut Plexa PCX 固相萃取柱, 60 mg, 3 mL (部件号 12108603)

Agilent Poroshell 120 EC-C18 色谱柱, 窄径, 2.1 × 50 mm, 2.7 μm (部件号 699775-902)

Agilent Vac Elut 20 真空多管装置 (部件号 12234101)

样品制备

样品前处理

将鱼肉切成小块, 用匀浆器匀化。称取 1 g 匀浆样品, 置于离心管中。加入 50 μL TMPD 溶液和 10 mL McIlvaine 缓冲液/乙腈 (1:1, v/v) 混合溶液。涡旋 1 min, 4500 rpm 离心 5 min, 将上清液转移至干净的试管中。在离心管中继续加入 5 mL McIlvaine 缓冲液/乙腈 (1:1, v/v) 混合溶液, 涡旋 1 min, 4500 rpm 离心 5 min, 取上清液。合并两次上清液, 即得用于 SPE 的样品溶液。

固相萃取

SPE 操作过程如图 1 所示。先用 2 mL 甲醇活化安捷伦 Bond Elut Plexa PCX 固相萃取柱, 再用 2 mL 2% 甲酸 (FA) 水溶液平衡萃取柱。将样品溶液加载到萃取柱上, 并在重力的作用下以大约 1 mL/min 的流速通过萃取柱。萃取柱依次用 2 mL 2% FA 水溶液、2 mL 甲醇和 2 mL 正己烷进行淋洗。真空干燥萃取柱 3 min 后树脂吸附剂将干燥完全。用 4 mL 洗脱缓冲液以 1 mL/min 的流速洗脱化合物。将洗脱液加水至 5 mL, 涡旋 10 秒。将样品转移至 2 mL 色谱小样品瓶中用于分析。

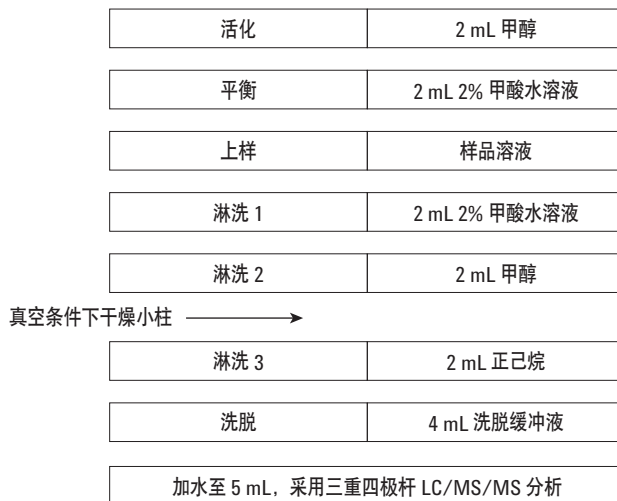


图 1. 提取鱼肉样品中抗菌剂的固相萃取过程

仪器条件

HPLC 条件

色谱柱: 安捷伦 Poroshell 120 EC-C18,
2.1 × 50 mm, 2.7 μm
(部件号 699775-902)

流动相: A: 水 (5 mM 乙酸铵溶液)
B: 乙腈 (0.1% FA 溶液)

进样量: 5 μL

流速: 0.4 mL/min

柱温: 室温

梯度程序:	时间 (min)	%B
	0	30
	5	80
	6	80
	6.5	30
	7	30

MS 条件

采用正离子模式监测标准品及内标化合物。离子源条件详见表 2, MRM transitions 见表 3。

表 2. MS 离子源参数

干燥气温度:	300 °C
干燥气流量:	5 L/min
雾化器压力:	45 psi
鞘气温度:	400 °C
鞘气流量:	11 L/min
喷嘴电压:	正极: 0 V 负极: 0 V
毛细管电压:	正极: 3500 V 负极: 3500 V

表 3. 多反应监测模式监测的离子对

被分析物	MRM 转换通道 (m/z)	碎裂电压 (V)	碰撞能量 (V)
MG	1) 329.3>313.3	175	38
	2) 329.3>208.3		38
CV	1) 372.3>356.2	175	42
	2) 372.3>251.1		36
LMG	1) 331.3>316.2	175	26
	2) 331.3>238.2		16
LCV	1) 374.3>358.3	175	30
	2) 374.3>238.2		26
MG-d5	334.3>318.3	175	38
LMG-d6	337.3>240.2	175	30

结果与讨论

线性和检测限

所有被测农药校准曲线的线性范围为 0.5~100 ng/g。分别向空白基质中添加标准品使浓度为 0.5、1、5、10 和 100 ng/g, 得校准曲线线性范围; 内标浓度为 10 ng/g。经过了整个样品制备过程处理的鱼肉作为空白基质。校准曲线由分析物的相对响应值 (分析物与内标的峰面积比) 与相对浓度 (被分析物与内标的浓度比) 绘制而成。针对所有化合物建立的定量限 LOQ (0.5 ng/g), 低于水产品中这些物质的 MRL。表 4 展示了线性回归方程和相关系数 (R^2) [2]。

表 4. 抗菌剂的线性

化合物	内标	回归方程	R^2
MG	MG-d5	$Y=0.0961x+0.0049$	1
CV	MG-d5	$Y=0.2808x-0.0524$	0.999
LMG	LMG-d6	$Y=0.0649x+0.0033$	1
LCV	LMG-d6	$Y=0.05461x-0.0011$	1

回收率和重现性

在三个浓度水平下进行了回收率和重现性实验；向鱼肉中添加标准品使浓度为 1、10 和 50 ng/g。每个水平重复进样 6 次。回收率和重现性数据详见表 5。图 2 为加标浓度为 10 ng/g 的鱼肉提取物的色谱图。

表 5. 鱼肉中抗菌剂的回收率和重现性

化合物	加标浓度 (ng/g)	回收率 (%)	RSD (n=6)
MG	1	102.1	3.5
	10	102.8	1.8
	50	99.2	1.9
CV	1	96.9	2.1
	10	102.8	2.6
	50	97.4	1.7
LMG	1	103.5	2.6
	10	108.9	3.4
	50	96.7	3.7
LCV	1	99.4	3.8
	10	106.1	3.8
	50	102.7	4.5

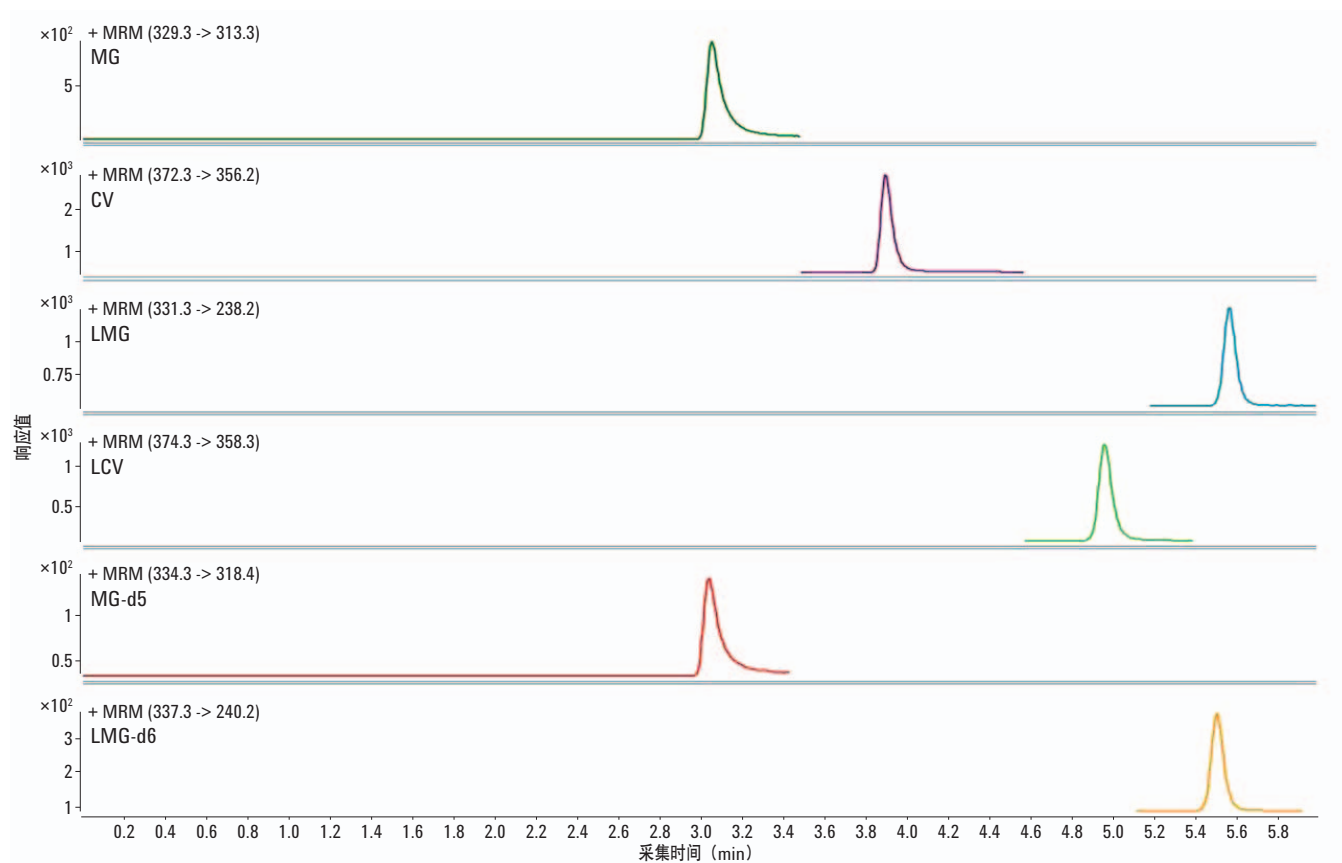


图 2. 采用 Agilent Poroshell 120 EC-C18 色谱柱分析鱼肉中抗菌剂加标浓度为 10 ng/g 时的色谱图

结论

本文建立了同时测定孔雀石绿、结晶紫及其代谢物隐色孔雀石绿和隐色结晶紫的方法。研究表明安捷伦 Bond Elut Plexa PCX 固相萃取柱可以有效地纯化和富集水产品如鱼中的染料。基于空白加标测定所得的回收率和重现性能够满足鱼中染料残留检测相关标准的要求。本方法将杂质与基质效应最小化，对目标化合物的定量分析未产生干扰。

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Fast Separation of Synthetic/Artificial Food Colors on Agilent Poroshell 120 EC-C18

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Ltd

Abstract

Many synthetic or artificial colorants (tartrazine, red 33, sunset yellow, and so forth) are used in food and beverages to improve product appearance. These compounds can be readily separated by reversed-phase liquid chromatography. A new Agilent Poroshell 120 EC-C18 column was used to separate 11 food colorants using a gradient method with an acetate buffer/methanol mobile phase. This method allows a rapid separation of the colorants in 9 minutes. It is suitable for many samples and was applied here to the analysis of these colorants in beverages and cakes.

Introduction

Most synthetic or artificial food colorings are water soluble, making them ideal for analysis by HPLC with reversed-phase columns. These compounds are generally safe, but there are some possible harmful effects, such as allergic reactions and hyperactivity in children; so their use is restricted in some countries. Because of these restrictions, the quantity of these

compounds in food quality control is becoming more important, as is the need to prove that foods using the colorants meet international quality control standards. These compounds are used in food to give them a colorful, attractive appearance. The structures of 11 colorants, mostly azo dyes, are shown in Table 1.

No.	Name	CAS	Structure	No.	Name	CAS	Structure
1	Tartrazine	1934-21-0		7	Red 33	3567-66-6	
2	New red	220658-76-4		8	Allura red	25956-17-6	
3	Amaranth	915-67-3		9	Erioglaucine	3844-45-9	
4	Indigo carmine	860-22-0		10	Azophloxine	3734-67-6	
5	Ponceau 4R	2611-82-7		11	Erythrosine	490C6-10-3	

Table 1. Details of food colorants in this study.

A previous application note described a method developed using Agilent TC-C18(2) and HC-C18(2) columns. The method applied a mobile phase comprised of phosphate buffer at pH 7.0 with methanol [1]. In this work, we focus on developing a method for rapidly separating more colorants in beverages and cakes using Agilent Poroshell 120 EC-C18.

Experimental

The HPLC analysis was performed with the Agilent 1290 Infinity LC system, including an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity Autosampler (G4226A), an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C), and an Agilent Infinity Diode Array Detector (G4212A).

Conditions

Column: Agilent Poroshell 120 EC-C18,
3.0 × 100 mm, 2.7 μm (p/n 695975-302)

Mobile phase: A, 10 mM acetate
B, methanol

Injection volume: 2 μL

Flow rate: 1.0 mL/min

Gradient:

Min	% B
0	5
1.5	5
8	75
12	75

Stop time: 12 minutes, post run 2 minutes

Temperature: 25 °C

Wavelength: 254 nm

The samples were purchased in a local market. Mango juice was diluted 1/10 with water and filtered with 0.2 μm regenerated cellulose membrane filters (p/n 5064-8222) before injection. Cake samples were prepared by weighing 5 g and adding to 40 mL water with a drop of ammonium hydroxide. The samples were then ultrasonicated at 60 - 80 °C for 30 minutes. The samples were increased to 50 mL with methanol and then filtered using 0.2 μm regenerated cellulose membrane filters (p/n 5064-8222) before injection.

Results and Discussion

The colorant compounds shown in Table 1 are polar, water-soluble compounds containing sulfonic acid groups, increasing their solubility in water. In fact, many of these compounds are used in the salt form in food. In a previous work, 7 of these colorants were well separated using a traditional 5 μm TC-C18(2) column with a phosphate buffer and a gradient starting with 10% methanol. Resolution and peak shape are best at mid pH with a phosphate buffer. However, more and more users apply LC/MS to analyze these colorants in very complex food samples. To be compatible with MS detectors, a 10 mM acetate buffer was used. With a highly efficient, superficially porous Poroshell 120 EC-C18,

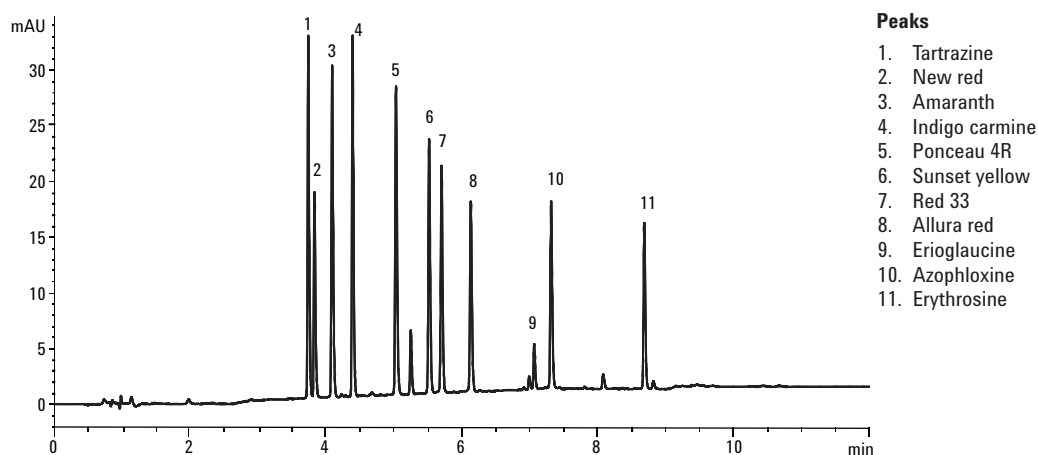


Figure 1. Separation of 11 colorant standards using an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 μm column.

3.0 × 100 mm, 2.7 μm column, 11 synthetic colorants were well separated in only 9 minutes (Figure 1).

Synthetic or artificial food colorants exist in many common beverages and foods, such as fruit-flavored drinks and sodas, cakes, and candy. Colorants in a mango juice and two different types of cake were analyzed. The chromatogram of mango juice is shown in Figure 2. Based on the standard, the first known peak, tartrazine, could be resolved completely from the unknown peak before it, and the amount of tartrazine could be measured.

Cake samples were more complex than mango juice because many polar compounds were extracted with water. To separate colorants from these polar compounds, a gradient starting with low percentage of organic phase was used. Figures 3 and 4 show separations of cake samples, with many peaks in both. The compounds could be identified through retention times of standards. However, for complex samples such as these, other compounds co-eluted with the colorants, which made it difficult to identify the exact colorants. This problem was resolved with an Agilent Infinity Diode Array Detector (G4212A), where the spectra of the peaks in the

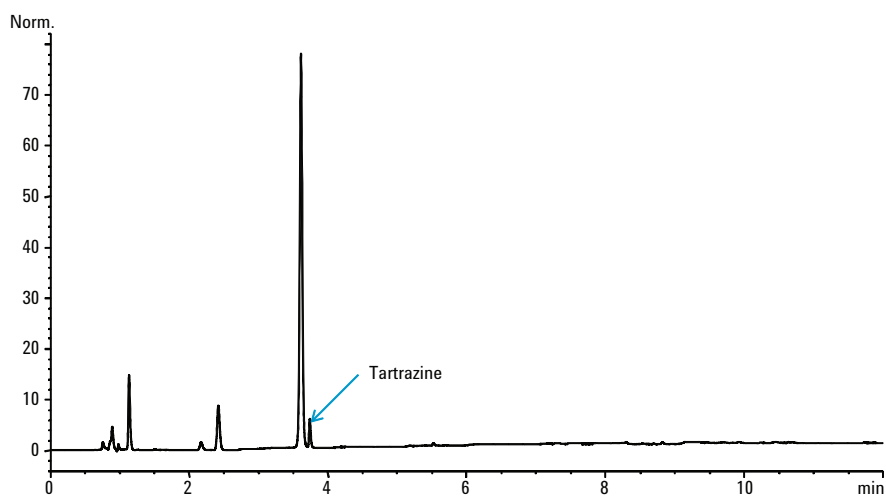


Figure 2. Separation of mango juice with an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 μm column.

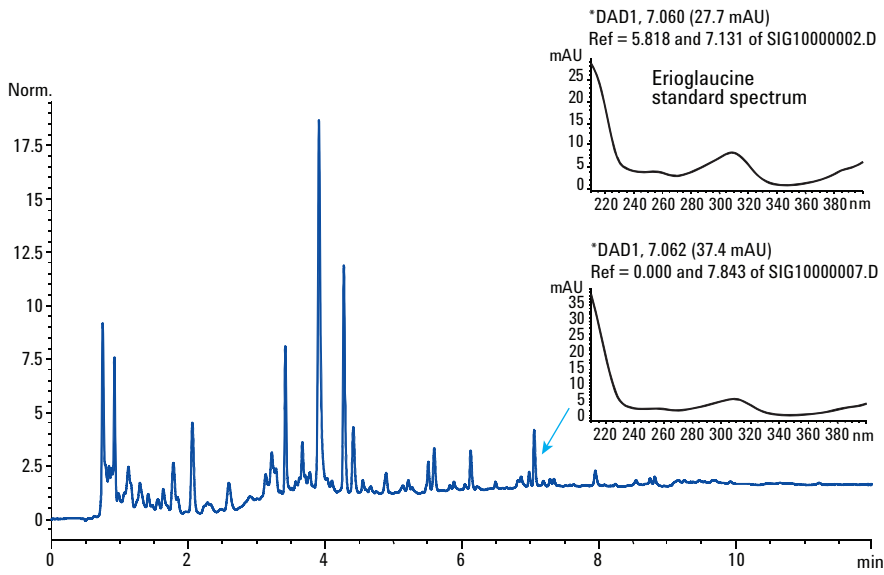


Figure 3. Separation of food colorants in cake sample 1 on an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 μm column.

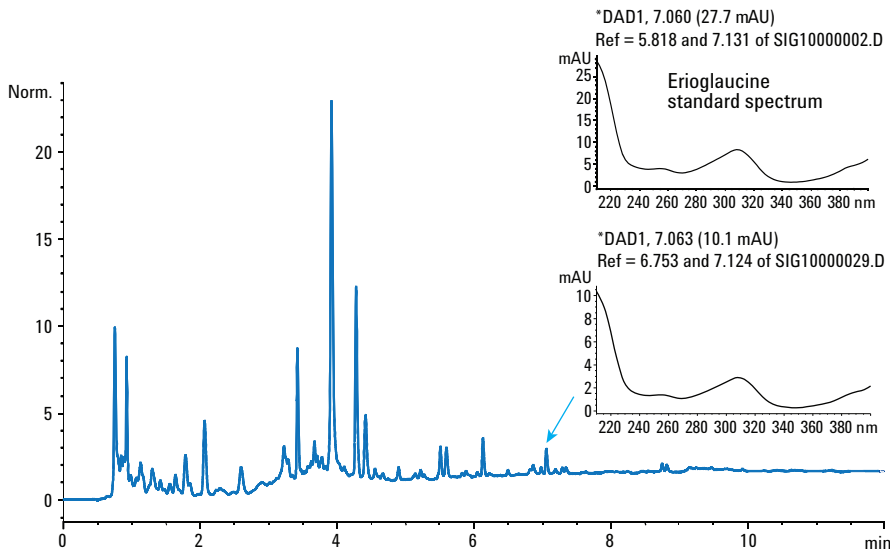


Figure 4. Separation of food colorants in cake sample 2 on an Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 μm column.

sample were matched with those of standards. In both cake samples, the colorant erioglaucine was identified based on the standard retention time coupled with its spectrum.

Conclusions

Using a simple gradient method, many common synthetic colorants can be separated on the Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 μ m column. This method allows rapid separation and screening for many artificial food colorants. In addition, the mass-spectrum-friendly acetate buffer allows the method to be easily transferred to an HPLC/MS method.

Reference

1. Rongjie Fu. "The Separation of Seven Synthetic/Artificial Food Colors on Agilent HC(2)/TC(2) Reversed Phase columns." Agilent Technologies, Inc., Publication 5989-8307EN (2008).

Aminoglycosides in Bovine Muscle Using Agilent Bond Elut Plexa SPE, an Agilent Poroshell 120 Column, and LC/Tandem MS

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Abstract

A method for the simultaneous determination of aminoglycoside residues of spectinomycin, hygromycin B, streptomycin, dihydrostreptomycin, amikacin, kanamycin, apramycin, tobramycin, gentamicin, and neomycin in bovine muscle was developed and validated. The analytes are extracted and cleaned with solid phase extraction and quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry operating in the positive ion multiple-reaction-monitoring mode. The method provides the ng/g level of limit of detection for all aminoglycoside residues in bovine muscle. The dynamic calibration ranges for these compounds were obtained from 10 to 500 ng/g. The overall recoveries ranged from 71 to 98%, with RSD values between 1.4 and 11.2%.

Introduction

Aminoglycosides (AGs) are a class of broad-spectrum antibiotics that have bacterial activity against some aerobic gram-positive and gram-negative organisms. AGs have been extensively employed in animal husbandry for the treatment of bacterial infections or growth promotion. Due to their toxicity and possible antibiotic resistance, considerable attention has been paid to the potential human health risk. The European Union (EU), China, USA, Japan, and other countries have issued strict maximum residue levels (MRLs) for AGs in various animal-origin foods [1, 2].

The objective of this application note was to develop a multiresidue method that would be simple and fast for routine regulatory analysis of aminoglycoside residues in animal tissue. The method relies on a simple SPE step using a polymer sorbent (Agilent Bond Elut Plexa). Table 1 shows details of the β -agonists.

Experimental

Reagents and chemicals

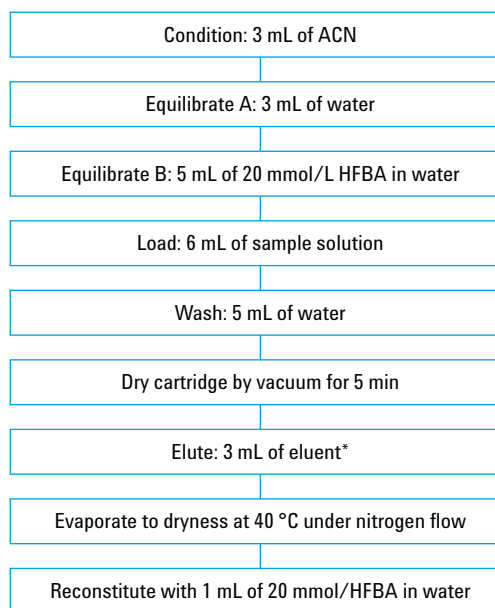
All reagents were MS, HPLC, or analytical grade. Acetonitrile and water were from Honeywell. The standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Bovine muscle was purchased from a local supermarket. Standard solutions (1.0 mg/mL) were made in water individually and stored in a freezer at 4 °C. A combined working solution (10 µg/mL) was made in acetonitrile:water (10:90) and also stored at 4 °C. The spiked solutions were then made weaker by appropriately diluting of the combined working solution in water.

Sample preparation

Bovine muscle (500 g) was minced using a kitchen homogenizer and stored at -20 °C. A 5 g aliquot of homogenized meat was weighed into a 50 mL capped polypropylene tube, and 10 mL of 5% trichloroacetic acid (TCA) in water was added. The mixture was homogenized thoroughly for 1 minute with an Ultra-Turrax T-18 homogenizer (IKA-Labortechnik, Staufen, Germany) and then centrifuged at 4,000 rpm for 5 minutes. The supernatant was transferred to another tube. The same extraction procedure was repeated with 10 mL of 5% TCA in water, and the supernatant was combined into the same tube. A 5 mL volume of 0.2 mol/L heptafluorobutyric acid (HFBA) in water was added to the extracts. After vortex mixing for 1 minute and centrifugation at 4,000 rpm for 5 minutes, the supernatant was adjusted to pH 4.0 with 5% ammonia water. The sample extraction solution was made up to 30 mL with water to be ready for the SPE procedure.

Solid phase extraction

Figure 1 shows the SPE procedure. Bond Elut Plexa cartridges were preconditioned with 3 mL of acetonitrile (ACN) and then equilibrated with 3 mL of water and 5 mL of 20 mmol/L HFBA in water. The 6 mL sample solution was then loaded onto a cartridge and passed through by gravity (approximately 1 mL/min). The cartridges were washed with 5 mL of water. A full vacuum was applied to the cartridge for 5 minutes to completely dry the resin. The compounds were eluted with 3 mL ACN:0.2 mol/L HFBA in water at a rate of 1 mL/min. The eluent was dried under nitrogen at 40 °C. The residue was reconstituted in 1 mL of 20 mmol/L HFBA in water. The sample was then vortex mixed and ultrasonicated to completely dissolve the residue and filtered through a 0.22-µm membrane. Then the sample was finally transferred to a 2 mL chromatography vial for analysis.



* Eluent ACN:0.2 mol/L HFBA in water (8:2).

Figure 1. Bovine muscle clean-up and enrichment – SPE procedure.

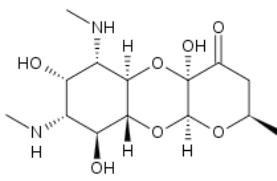
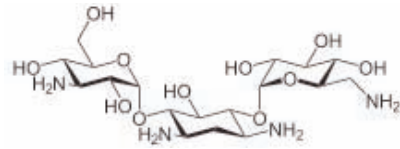
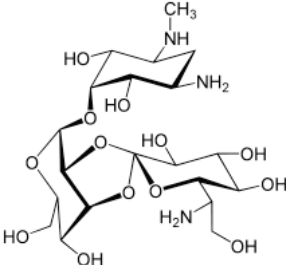
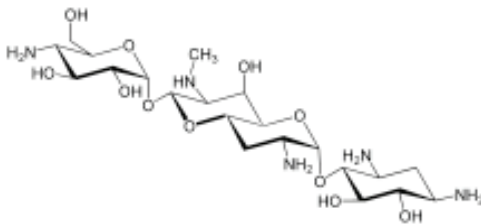
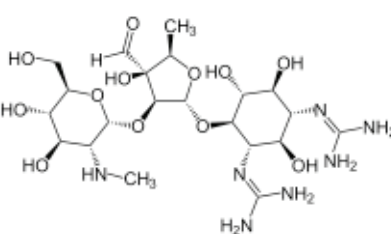
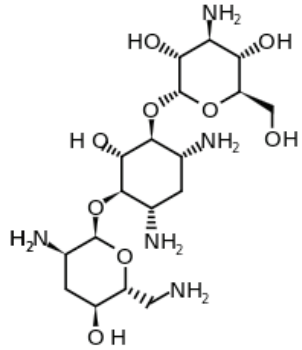
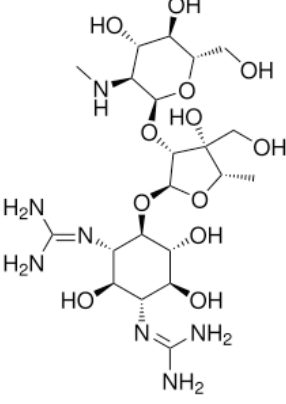
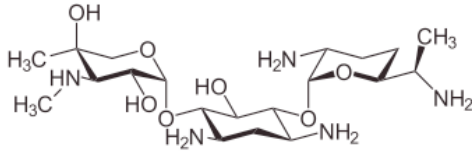
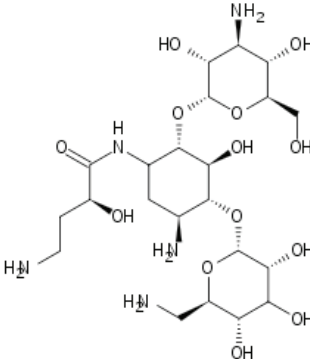
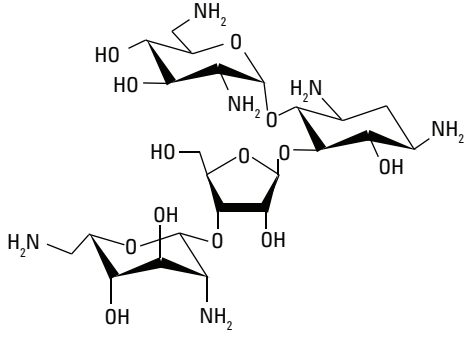
Compound	CAS No.	Log P	Structure	Compound	CAS No.	Log P	Structure
Spectinomycin	1695-77-8	-2.3		Kanamycin	59-01-8	-6.3	
Hygromycin B	31282-04-9	NA		Apramycin	37321-09-8	-6.5	
Streptomycin	57-92-1	-6.4		Tobramycin	32986-56-4	-5.8	
Dihydrostreptomycin	128-46-1	NA		Gentamicin	1403-66-3	NA	
Amikacin	37517-28-5	-7.4		Neomycin	1404-04-2	-7.8	

Table 1. Aminoglycoside compounds used in this study.

Conditions

Column: Agilent Poroshell 120 SB-C18, 2.1 × 100 mm, 2.7 μm (p/n 685775-902)

Sample prep: Agilent Bond Elut Plexa cartridges, 500 mg, 6 mL (p/n 12259506)

Mobile phase: A: Water:acetonitrile (950:50, 20 mmol/L HFBA),
B: acetonitrile:water (800:200, 20 mmol/L HFBA)

Injection volume: 20 μL

Flow rate: 0.3 mL/min

Gradient:

Time (min)	%A	%B
0	85	15
3	85	15
9.5	25	75
9.55	85	15
10	85	15

Temperature: Ambient

Manifold: Agilent Vac Elut 20 Manifold (p/n 12234101)

Instrument: Agilent 1200 Infinity Series LC System
Agilent 6460 Triple Quadrupole LC/MS/MS System

MS conditions

The AGs were monitored in positive mode. Table 2 shows the multiple-reaction-monitoring details.

MS source parameters

Gas temp: 350 °C

Gas flow: 5 L/min

Nebulizer: 45 psi

Sheath gas temp: 400 °C

Sheath gas flow: 11 L/min

Nozzle voltage: Positive, 0 V

Capillary: Positive, 3,500 V

Compound	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)	Retention time (min)
Spectinomycin	351.2	333.2	170	15	4.64
		207.1	170	18	
Hygromycin B	528.3	177.1	170	25	6.77
		352	170	20	
Streptomycin	582.4	263.2	180	30	6.98
		245.8	180	35	
Dihydrostreptomycin	584.4	263.3	180	30	7.06
		246.2	180	40	
Amikacin	586.4	163.1	170	30	7.68
		425.2	170	15	
Kanamycin	485.3	163.1	150	20	7.8
		324.2	150	10	
Apramycin	540.3	217.1	140	25	8.32
		378.2	140	12	
Tobramycin	468.3	163.2	125	20	8.42
		324.2	125	8	
Gentamicin	478.3	322.3	125	8	8.64
		157.2	125	15	
Neomycin	615.3	161.1	175	30	8.74
		293.1	175	20	

Table 2. Masses monitored by multiple-reaction monitoring.

Results and Discussion

Linearity and limit of detection

Solutions used to create external calibration curves were prepared by using a combined working solution to spike matrix blank (10, 20, 50, 100, and 500 ng/g). Matrix blanks were created by taking bovine muscle through the entire procedure, including pretreatment and SPE procedures. The limits of detection (LOD) were chosen as the concentration of each compound that gave a signal-to-noise (S/N) ratio greater than 3:1. The results for the calibration curves and LODs are shown in Table 3.

Compound	Regression equation	R ²	LOD in muscle (ng/g)
Spectinomycin	Y=293.4698x-325.2314	0.9998	2
Hygromycin B	Y=270.2367x-424.6557	0.9999	0.5
Streptomycin	Y=28.7892x+10.1849	0.9999	5
Dihydrostreptomycin	Y=458.6225x-1320.7826	0.9999	0.1
Amikacin	Y=572.3138x-923.7852	0.9999	0.2
Kanamycin	Y=508.1929x-905.1314	0.9998	0.5
Apramycin	Y=239.2452x-646.2071	0.9999	0.5
Tobramycin	Y=696.8031x-1922.6636	0.9999	0.1
Gentamicin	Y=1076.2438x-3690.8511	0.9996	0.1
Neomycin	Y=196.7006x-534.5063	0.9997	2

Table 3. Linearity and LODs of aminoglycosides in bovine muscle.

Recovery and reproducibility

The recovery and repeatability for the method were determined at 3 levels; beef spiked to concentrations of 20, 100, and 500 ng/g. The analysis was performed with 6 replicates at each level. Table 4 shows the recovery and reproducibility data. Figure 2 shows the chromatograms of spiked bovine muscle extracts (20 ng/g).

Compound	Spiked level (ng/g)	Recovery (%)	RSD (n=6, %)
Spectinomycin	20	87.7	2.1
	100	79.7	2.4
	500	91.2	3.2
Hydromycin B	20	75.9	3.9
	100	82.1	3.4
	500	85.6	4.0
Streptomycin	20	71.5	11.2
	100	80.0	9.4
	500	74.5	8.0
Dihydrostreptomycin	20	89.1	4.5
	100	91.2	2.3
	500	93.3	3.6
Amikacin	20	85.9	1.8
	100	90.1	2.4
	500	96.5	3.8
Kanamycin	20	86.7	1.4
	100	90.0	2.2
	500	97.6	2.8
Apramycin	20	84.6	4.9
	100	87.6	3.1
	500	95.4	5.4
Tobramycin	20	89.3	4.4
	100	88.1	3.4
	500	97.7	5.8
Gentamicin	20	82.4	3.5
	100	81.2	4.6
	500	95.8	6.8
Neomycin	20	72.1	2.6
	100	82.8	5.6
	500	90.3	5.5

Table 4. Recoveries and reproducibility of aminoglycosides in bovine muscle.

Conclusions

LC/MS/MS was a reliable and powerful technique for the simultaneous quantification and confirmation of aminoglycosides in bovine muscle. Moreover, the result of this study shows that Agilent Bond Elut Plexa can be used as an effective method for purification and enrichment of multiple aminoglycosides in a complex matrix, such as bovine muscle. The recovery and reproducibility results based on matrix spiked standards were acceptable for aminoglycoside residue determination in bovine muscle under international regulations. The impurities and matrix effect were minimal and did not interfere with the quantification of any target compound. The limits of quantitation were significantly lower than the MRLs [3].

References

1. W. Zhu, J. Yang, W. Wei, Y. L. S. Zhang. *J. Chromatography A*, 1207, 29 (2008).
2. A. Kaufmann, P. Butcher, K. Maden. *Anal. Chim. Acta* 711, 46 (2012).
3. Anon. GB/T 21323-2007 Determination of aminoglycosides residues in animal tissues - HPLC-MS/MS method. China Standard. General Administration of Quality Supervision, Inspection and Quarantine, Beijing, China.

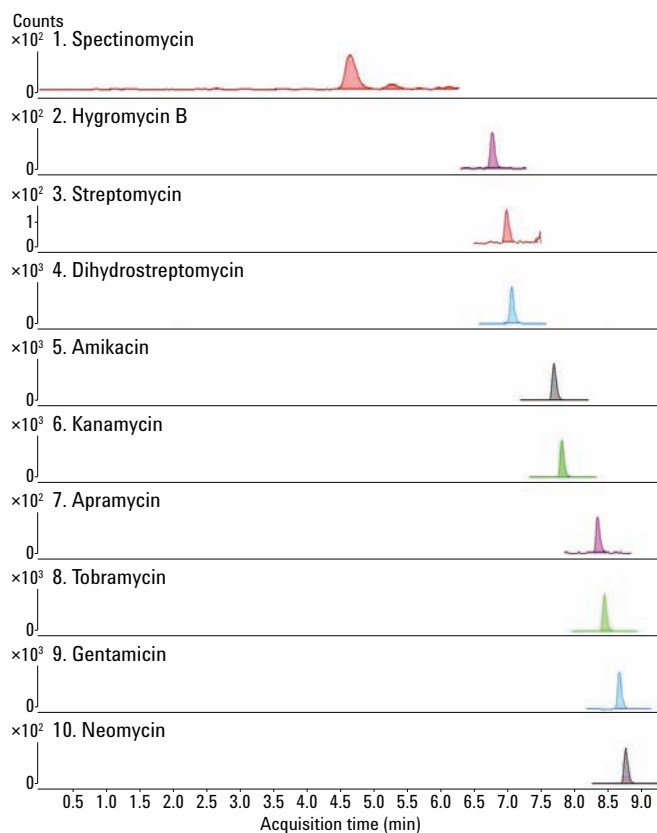


Figure 2. Chromatograms of 20 ng/g spiked bovine muscle sample extract.

Aminoglycosides in Milk Using Agilent Bond Elut Plexa SPE, Agilent Poroshell 120, and LC/Tandem MS

Author

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Abstract

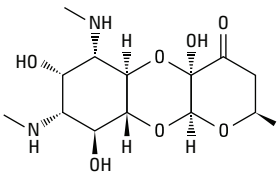
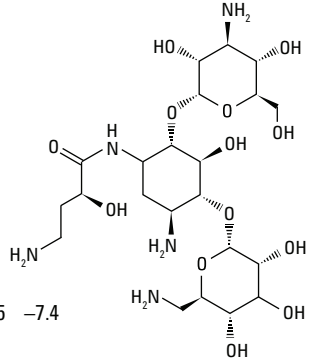
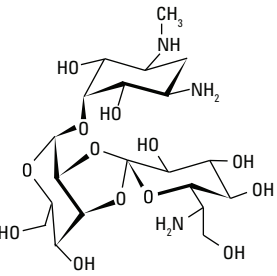
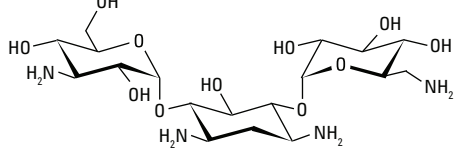
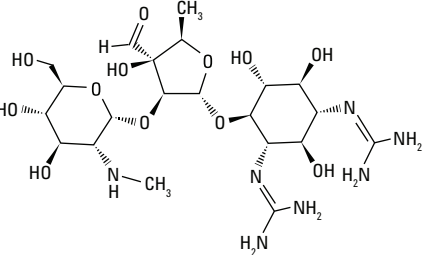
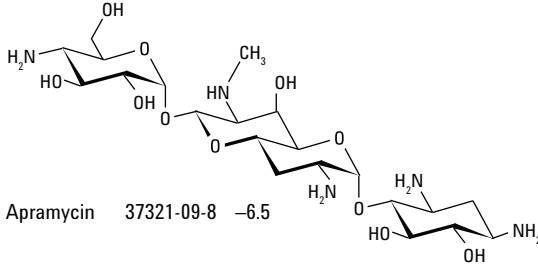
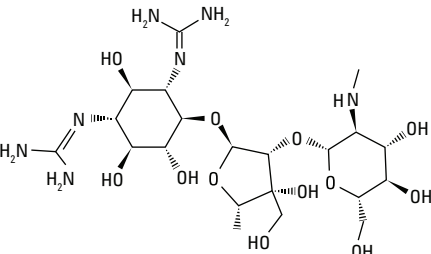
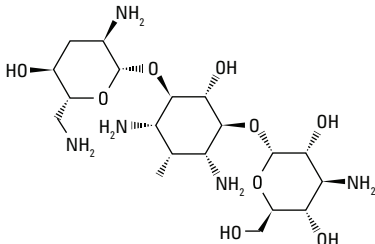
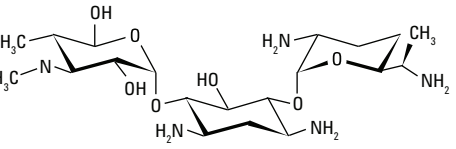
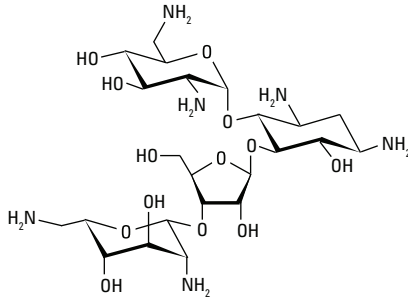
A method for the simultaneous determination of aminoglycoside residues of spectinomycin, hygromycin B, streptomycin, dihydrostreptomycin, amikacin, kanamycin, apramycin, tobramycin, gentamicin, and neomycin in milk was developed and validated. The analytes were extracted and cleaned with Agilent Bond Elut Plexa solid phase extraction (SPE) and quantified by liquid chromatography coupled to electrospray ionization tandem mass spectrometry (LC/Tandem MS) operating in the positive ion multiple-reaction-monitoring mode. The method provided ng/g level of limit of detection for all aminoglycoside residues in milk. The dynamic calibration ranges for these compounds were obtained from 10 to 500 ng/g. The overall recoveries ranged from 67 to 107%, with RSD values between 1.7 and 10.1%.

Introduction

Aminoglycosides (AGs) are a class of broad-spectrum antibiotics that have bacterial activity against some aerobic gram-positive and gram-negative organisms. AGs have been extensively employed in animal husbandry for the treatment of bacterial infections or growth promotion. Due to their toxicity, and possible antibiotic resistance, considerable attention has been paid to the potential human health risk. The European Union (EU), China, USA, Japan, and other countries have issued strict maximum residue levels (MRLs) for AGs in various animal-origin foods [1, 2].

The objective of this work was to develop a multiresidue method that would be simple and fast for routine regulatory analysis of aminoglycoside residues in milk. The method relies on a simple SPE step using a polymer sorbent (Bond Elut Plexa). Table 1 shows details of the aminoglycosides.

Table 1. Aminoglycoside compounds used in this study.

Compound	CAS no.	LogP	Compound	CAS no.	LogP
	1695-77-8	-2.3		37517-28-5	-7.4
Spectinomycin	1695-77-8	-2.3	Amikacin	37517-28-5	-7.4
	31282-04-9	NA		59-01-8	-6.3
Hygromycin B	31282-04-9	NA	Kanamycin	59-01-8	-6.3
	57-92-1	-6.4		37321-09-8	-6.5
Streptomycin	57-92-1	-6.4	Apramycin	37321-09-8	-6.5
	128-46-1	NA		32986-56-4	-5.8
Dihydrostreptomycin	128-46-1	NA	Tobramycin	32986-56-4	-5.8
	1403-66-3	NA		1404-04-2	-7.8
Gentamicin	1403-66-3	NA	Neomycin	1404-04-2	-7.8

Materials and Methods

Reagents and chemicals

All reagents were MS, HPLC, or analytical grade. Acetonitrile and water were from Honeywell International, Inc. The standards were purchased from Dr. Ehrenstorfer (Augsburg, Germany). Bovine milk was purchased from a local supermarket. Standard solutions (1.0 mg/mL) were made in water individually and stored in a freezer at 4 °C. A combined working solution (10 µg/mL) was made in acetonitrile:water (10:90) and also stored at 4 °C. The spiked solutions were then made weekly by appropriately diluting the combined working solution in water.

Sample preparation

Bovine milk (5 g) was weighed into a polypropylene centrifuge tube. Ten mL of extracting solution (5% trichloroacetic acid, 0.6 mmol/L Na₂EDTA, and 15 mmol/L KH₂PO₄) was added to the tube. The mixture was shaken thoroughly for 5 minutes and then centrifuged at 4,000 rpm for 5 minutes at 4 °C. The supernatant was transferred to another tube. The same extraction procedure was repeated with 5 mL of extracting solution, and the supernatant was combined into the same tube. A 5 mL volume of 0.2 mol/L heptafluorobutyric acid (HFBA) in water was added to the extracts. After vortex-mixing for 1 minute and centrifuging at 4,000 rpm for 5 minutes, the supernatant was adjusted to pH 4.0 ± 0.5 with 5 mol/L NaOH in water. The sample extracting solution was then ready for the SPE procedure.

Solid phase extraction

Figure 1 shows the SPE procedure. Bond Elut Plexa cartridges were preconditioned with 3 mL of acetonitrile (ACN) and then equilibrated with 3 mL of water and 5 mL of 20 mmol/L HFBA in water. The sample extracting solution was then loaded onto a cartridge and passed through under gravity (approximately 1 mL/min). The cartridges were washed with 5 mL of water. A full vacuum was applied to the cartridge for 5 minutes to completely dry the resin. The compounds were eluted with 3 mL ACN:0.2 mol/L HFBA in water at a rate of 1 mL/min. The eluent was dried under nitrogen at 40 °C. The residue was reconstituted in 1 mL of 20 mmol/L HFBA in water. The sample was then vortex mixed and ultrasonicated to completely dissolve the residue and filtered through a 0.22 µm membrane. Then the sample was finally transferred to a 2 mL chromatography vial for analysis.

Condition: 3 mL of ACN
Equilibrate A: 3 mL of water
Equilibrate B: 5 mL of 20 mmol/L HFBA in water
Load: sample extracting solution
Wash: 5 mL of water
Dry cartridge by vacuum for 5 minutes
Elute: 3 mL of eluent*
Evaporate to dryness at 40 °C under nitrogen flow
Reconstitute with 1 mL of 20 mmol/L HFBA in water

*Eluent: ACN:0.2 mol/L HFBA in water (8:2).

Figure 1. Milk clean-up and enrichment – Agilent Bond Elut Plexa SPE procedure.

Conditions

Column:	Agilent Poroshell 120 SB-C18, 2.1 × 100 mm, 2.7 µm (p/n 685775-902)		
Sample prep:	Agilent Bond Elut Plexa cartridges, 500 mg, 6 mL (p/n 12259506)		
Mobile phase:	A: Water:acetonitrile (950:50, 20 mmol/L HFBA), B: acetonitrile:water (800:200, 20 mmol/L HFBA)		
Injection volume:	20 µL		
Flow rate:	0.3 mL/min		
Gradient:	Time (min)	%A	%B
	0	85	15
	3	85	15
	9.5	25	75
	9.55	85	15
	10	85	15
Temperature:	Ambient		
Manifold:	Agilent Vac Elut 20 Manifold (p/n 12234101)		
Instrument:	Agilent 1200 Infinity Series Agilent 6460 Triple Quadrupole LC/MS/MS system		

MS conditions

The AGs were monitored in positive mode. Table 2 shows the multiple-reaction-monitoring details.

MS source parameters

Gas temperature:	350 °C
Gas flow:	5 L/min
Nebulizer:	45 psi
Sheath gas temperature:	400 °C
Sheath gas flow:	11 L/min
Nozzle voltage:	Positive, 0 V
Capillary:	Positive, 3,500 V

Table 2. Masses monitored by multiple-reaction monitoring.

Compound	Precursor ion	Product ion	Fragmentor (V)	Collision energy (V)
Spectinomycin	351.2	333.2	170	15
		207.1	170	18
Hygromycin B	528.3	177.1	170	25
		352	170	20
Streptomycin	582.4	263.2	180	30
		245.8	180	35
Dihydrostreptomycin	584.4	263.3	180	30
		246.2	180	40
Amikacin	586.4	163.1	170	30
		425.2	170	15
Kanamycin	485.3	163.1	150	20
		324.2	150	10
Apramycin	540.3	217.1	140	25
		378.2	140	12
Tobramycin	468.3	163.2	125	20
		324.2	125	8
Gentamicin	478.3	322.3	125	8
		157.2	125	15
Neomycin	615.3	161.1	175	30
		293.1	175	20

Results and Discussion

Linearity and limit of detection

Solutions used to create external calibration curves were prepared by using a combined working solution to spike matrix blanks (0.01, 0.02, 0.05, 0.1, and 0.5 mg/kg). Matrix blanks were created by taking milk through the entire procedure, including pretreatment and SPE procedures. The limits of detection (LODs) were chosen as the concentration of each compound that gave a signal-to-noise (S/N) ratio greater than 3:1. The results for the calibration curves and LODs are shown in Table 3.

Table 3. Linearity and LODs of aminoglycosides in milk.

Compound	Regression equation	R ²	LOD in milk (ng/g)
Spectinomycin	$Y = 13790.2955x + 39429.2957$	0.996	0.1
Hygromycin B	$Y = 891.7225x - 1190.5976$	0.998	0.2
Streptomycin	$Y = 1197.4506x - 2934.9645$	0.994	2
Dihydrostreptomycin	$Y = 2240.1902x - 1236.0908$	0.998	0.2
Amikacin	$Y = 1393.7561x - 1742.7928$	0.999	0.2
Kanamycin	$Y = 1107.6982x - 1720.5534$	0.998	0.2
Apramycin	$Y = 288.9123x - 207.5481$	0.999	0.5
Tobramycin	$Y = 804.6063x - 1295.9858$	0.999	0.2
Gentamicin	$Y = 1494.7223x - 894.2355$	0.999	0.2
Neomycin	$Y = 183.7889x - 240.7272$	0.994	0.5

Recovery and reproducibility

The recovery and repeatability for the method were determined at three levels; milk spiked to concentrations of 0.01, 0.02, and 0.05 mg/kg. The analysis was performed with six replicates at each level. Table 4 shows the recovery and reproducibility data. Figure 2 shows the chromatograms of spiked bovine milk extracts (0.02 mg/kg).

Table 4. Recoveries and reproducibility of aminoglycosides in milk.

Compound	Spiked level (mg/kg)	Recovery (%)	RSD (n = 6, %)
Spectinomycin	0.01	78.7	3.8
	0.02	82.5	5.6
	0.1	87.3	4.1
Hydromycin B	0.01	73.1	8.7
	0.02	69.7	6.3
	0.1	77.3	5.9
Streptomycin	0.01	78.1	7.7
	0.02	66.5	10.1
	0.1	71.8	7.1
Dihydrostreptomycin	0.01	84.2	2.1
	0.02	88.2	3.1
	0.1	91.5	5.4
Amikacin	0.01	102.3	2.4
	0.02	97.2	2.7
	0.1	99.4	3.6
Kanamycin	0.01	98.7	4.5
	0.02	92.1	3.9
	0.1	93.6	6.8
Apramycin	0.01	97.1	4.8
	0.02	101.9	6.6
	0.1	89.6	7.1
Tobramycin	0.01	92.5	2.9
	0.02	98.5	4.9
	0.1	94.8	1.7
Gentamicin	0.01	107.3	3.9
	0.02	101.4	3.1
	0.1	105.8	4.5
Neomycin	0.01	88.2	6.7
	0.02	97.4	7.2
	0.1	87.6	5.4

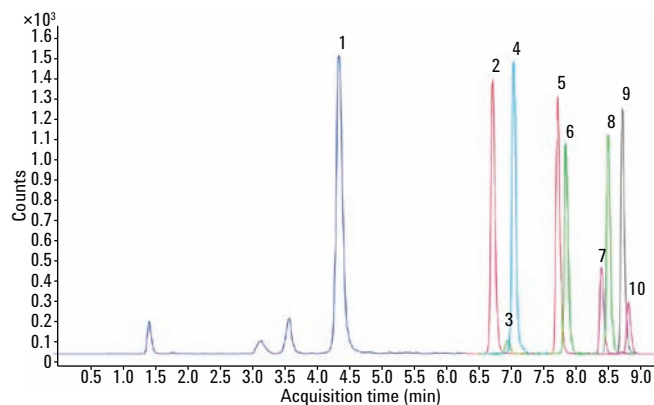


Figure 2. Chromatogram of 0.02 mg/kg spiked milk sample extract. 1. spectinomycin, 2. hygromycin B, 3. streptomycin, 4. dihydrostreptomycin, 5. amikacin, 6. kanamycin, 7. apramycin, 8. tobramycin, 9. gentamicin, and 10. neomycin.

Conclusions

LC/MS/MS is a reliable and powerful technique for the simultaneous quantification and confirmation of aminoglycosides in milk. The results of this application note show that Agilent Bond Elut Plexa can be used as an effective method for purification and enrichment of multiple aminoglycosides in a complex matrix such as milk. The recovery and reproducibility results based on matrix spiked standards are acceptable for aminoglycoside residue determination in milk under international regulations. The impurities and matrix effects are minimal and do not interfere with the quantification of any target compound. The limits of quantitation are significantly lower than the MRLs [3].

References

1. W. Zhu, J. Yang, W. Wei, Y. L. S. Zhang. *J. Chromatography A*, 1207, 29 (2008).
2. A. Kaufmann, P. Butcher, K. Maden. *Anal. Chim. Acta*, 711, 46 (2012).
3. Anon. GB/T 21323-2007 Determination of aminoglycosides residues in animal tissues - HPLC-MS/MS method. China Standard. General Administration of Quality Supervision, Inspection and Quarantine, Beijing, China.

Selectivity Comparison of Agilent Poroshell 120 Phases in the Separation of Butter Antioxidants

Author

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Abstract

The selectivity of various phases of Agilent Poroshell 120, including EC-C18, SB-C18, SB-Aq, Bonus-RP, and Phenyl-Hexyl, were compared in the separation of nine artificial antioxidants. The method was then optimized on an Agilent Poroshell 120 SB-Aq column for these compounds in butter samples with a water (with acetic acid modifier) and acetonitrile mobile phase. The method separated the antioxidants very well and was suitable for quantitative analysis.

Introduction

Foods, such as oils and fats, containing unsaturated fatty acids can easily cause lipid oxidation leading to rancidity, odor problems, and a decrease of their nutritional value. Synthetic ascorbyl palmitate and phenolic antioxidants are often added to foods to prevent oxidation of these unsaturated fatty acids. Single or combinations of antioxidants are permitted to enhance the antioxidative effect in food, but excessive consumption can cause some health problems in humans. For example, 2,4,5-trihydroxybutyrophenone has mutagenic effects, butylated hydroxyanisole and 2,6-di-tert-butyl-4-methylphenol may be carcinogenic, and propyl gallate can cause kidney damage [1].

Regulatory agencies from both Europe and the US have imposed maximum levels for some antioxidants while others have been forbidden [2]. Therefore, the determination of antioxidants in foods and food components is important. Table 1 lists nine antioxidants that are most commonly added to foods along with their structures and abbreviations.

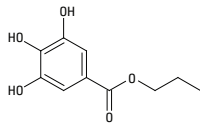
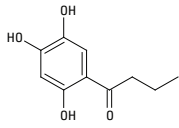
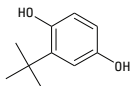
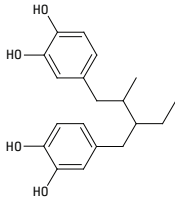
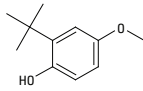
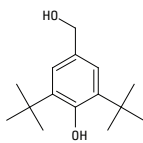
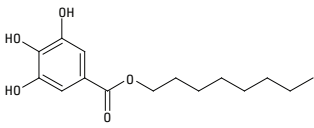
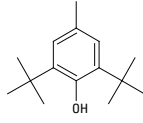
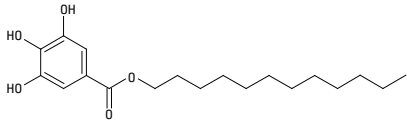
Currently, a regulatory HPLC method in China is used for the determination of antioxidants in fats. This method uses columns 15 to 25 cm long with an internal

diameter of 4.6 mm, packed with 5 μm C18 bonded silica particles, and a mobile phase composed of acetic acid (eluent A) and methanol (eluent B). Unfortunately, this method is quite slow with an analysis time over 30 minutes [3]. QC laboratories in fat-processing industries are looking for a way to shorten analysis time, because some of the antioxidants have been found to suffer significant degradation in solvents at room temperature. Thus, a fast, accurate, and

rugged method is desirable for economical and practical reasons.

This application note describes the analysis of nine antioxidants in butters using the Agilent 1290 Infinity LC with Poroshell 120 columns. Selectivity of various phases was compared for separation. The Poroshell 120 SB-Aq column was chosen for method development for its better selectivity and retention time versus other phases, and then the method was optimized to fit the analysis of various butter samples.

Table 1. Artificial antioxidants used in this study.

Peak No.	Name	CAS	Structure
1	Propyl gallate (PG)	121-79-9	
2	2,4,5-Trihydroxybutyrophenone (THBP)	1421-63-2	
3	2-Tert-butylhydroquinone (TBHQ)	1948-33-0	
4	Nordihydroguaiaretic acid (NDGA)	500-38-9	
5	Butylated hydroxyanisole (BHA)	25013-16-5	
6	Ionox-100	88-26-6	
7	Octyl gallate (OG)	1034-01-1	
8	2,6-Di-tert-butyl-4-methylphenol (BHT)	128-37-0	
9	Dodecyl gallate (DG)	1166-52-5	

Materials and Methods

HPLC analysis was performed with the Agilent 1290 Infinity LC, including an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity Autosampler (G4226A), an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C), and an Agilent 1290 Infinity Diode Array Detector (G4212A).

Columns

Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 μm (p/n 695975-302)

Agilent Poroshell 120 SB-C18, 3.0 × 100 mm, 2.7 μm (p/n 685975-302)

Agilent Poroshell 120 SB-Aq, 3.0 × 100 mm, 2.7 μm (p/n 685975-314)

Agilent Poroshell 120 Bonus-RP, 3.0 × 100 mm, 2.7 μm (p/n 695968-301)

Agilent Poroshell 120 Phenyl-Hexyl, 3.0 × 100 mm, 2.7 μm (p/n 695975-312)

The stock solution of standard's mixture was prepared in methanol at 0.1 mg/mL individually. The standard solutions for linearity were diluted from the stock solution in a series of concentrations including 0.2, 0.5, 1, 2, 5, and 10 ppm in acetonitrile:isopropanol (50:50 v:v).

The butters were purchased in the supermarket, and originated from the US, Switzerland, Australia, and New Zealand. Sample solutions were prepared according to the Chinese regulatory method [3]. For the spiked samples, a stock solution of the antioxidants in the solvent was added prior to extraction. The extraction was carried out by weighing 1.0 g of butter and adding 5.0 mL of *n*-hexane saturated with acetonitrile. This mixture was vortexed to dissolve the butter, was added to 10 mL of acetonitrile saturated with *n*-hexane, was vortexed again for 1 minute, and was allowed to stand until a separate layer developed. The acetonitrile layer was collected and extracted once more with acetonitrile saturated with *n*-hexane. The collected acetonitrile portions were mixed together and concentrated with a flow of nitrogen to a volume of 1 mL, and then made up to 2 mL with isopropanol. These solutions were filtered using Agilent 0.2 μm regenerated cellulose membrane filters (p/n 5064-8222) before transfer into autosampler vials for injection.

Results and Discussion

Selectivity comparison

Poroshell 120 columns were packed with superficially porous particles, which provided performance similar to the sub-2 μm particles but with a 40 to 50% lower pressure than columns with sub-2 μm particles. The recent introduction of new stationary phases available on Poroshell 120 columns made them useful for method development by changing selectivity.

Using a variety of bonded phases to try sequentially for method development demonstrated the different selectivity easily gained from the columns. Figure 1 and Figure 2 are overlays of five different reversed phases with acetic acid:methanol and acetic acid:acetonitrile mobile phases. All gave a symmetrical peak shape, with the exception of the Poroshell 120 Bonus-RP with methanol organic phase. This might have been caused by the amide group embedded in the bonded phase of Poroshell Bonus-RP, which has a strong H-bonding attraction with acidic analytes when methanol is present in the mobile phase.

The differences in selectivity between the five columns were due to the differences in bonding chemistry, such as the type of bonding, the end capping, and the amount and type of silanols on the silica surface. Other factors that influence selectivity, including mobile phase composition, temperature, and pH were identical during the investigation. These five bonded phases were all based on 2.7-μm Poroshell 120 superficially porous silica. They included an EC-C18 column, highly end capped that gave the best overall peak shape; SB-C18 and SB-Aq non-end capped columns, which had interaction with silanol groups and provided alternative selectivity to C18 phases; Bonus-RP, a polar embedded amine column that gave unique selectivity; and a Phenyl-Hexyl bonded column, which had improved selectivity for aromatic compounds.

Under a mobile phase of acetonitrile/1.5% acetic acid, the Phenyl-Hexyl and EC-C18 did not resolve peaks 6 and 7 well. SB-C18 and SB-Aq both resolved all nine peaks, but SB-C18 gave longer retention and less resolution between peaks 6 and 7 than SB-Aq. When changing the mobile phase to methanol:1.5% acetic acid, the elution order changed on all columns with poor separation for several peaks, but SB-Aq still separated all nine compounds well. In a careful comparison between the two mobile phases on the SB-Aq column, acetonitrile:1.5% acetic acid gave even better resolution and higher performance for all the compounds. Therefore, the Poroshell 120 SB-Aq was chosen for further method development with the mobile phase of acetonitrile:1.5% acetic acid.

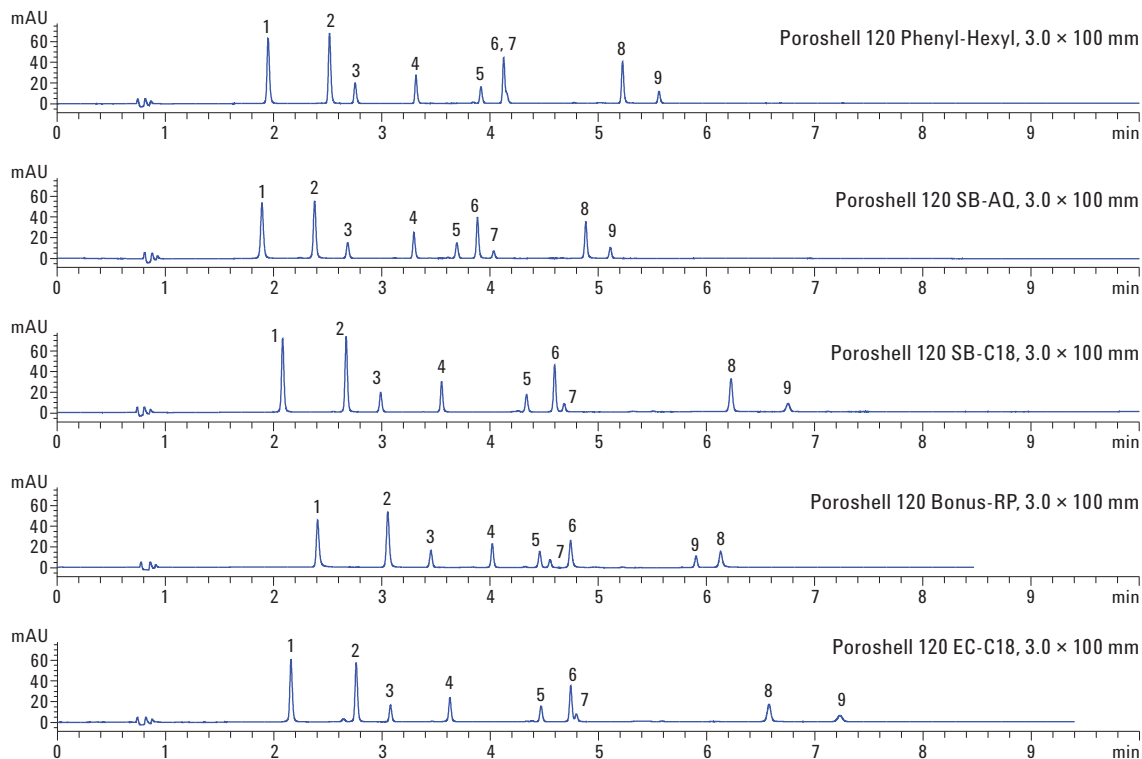


Figure 1. Chromatograms of nine antioxidants in acetonitrile:1.5% acetic acid on various Agilent Poroshell 120 phases showing different selectivity.

Conditions, Figure 1

Eluent: A 1.5% acetic acid, B ACN
 Injection volume: 2 μ L of 10 ppm mixture in 10% methanol
 Flow rate: 0.6 mL/min
 Gradient:

Time (min)	% B
0	25
0.5	25
5	80
8	80

Temperature: 40 $^{\circ}$ C
 Detector: UV, 280 nm

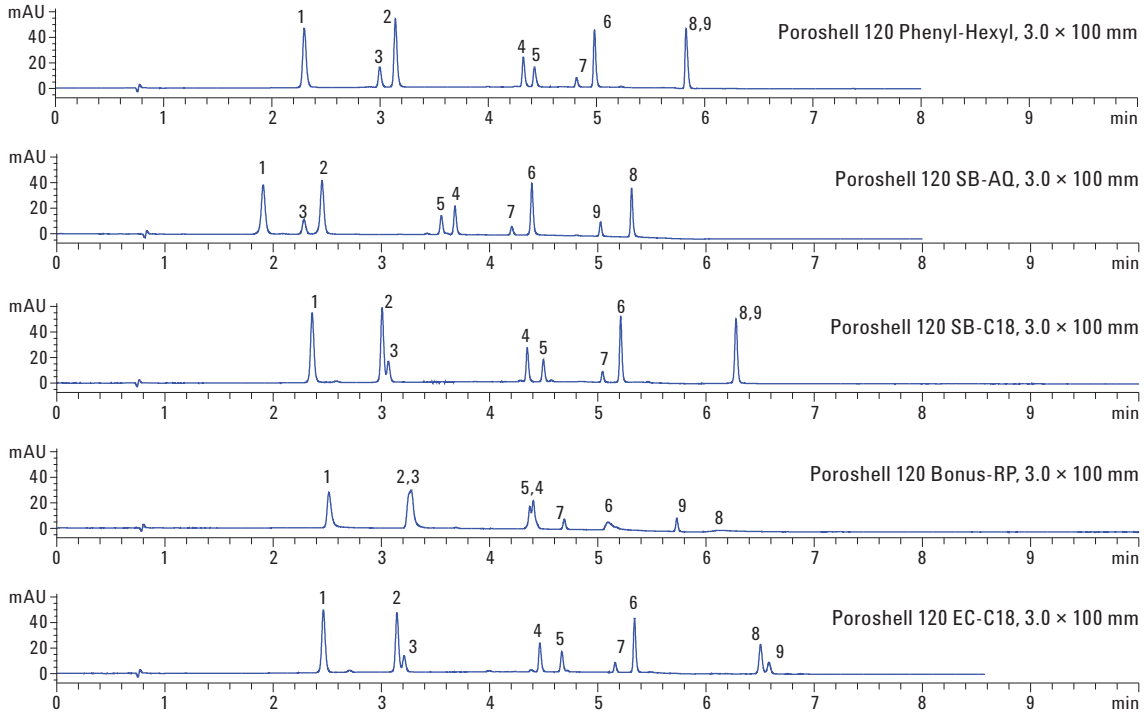


Figure 2. Chromatograms of nine antioxidants in methanol:1.5% acetic acid on various Agilent Poroshell 120 phases showing different selectivity.

Conditions, Figure 2

Eluent: A 1.5% acetic acid, B methanol
 Injection volume: 2 μ L of 10 ppm mixture in 10% methanol
 Flow rate: 0.6 mL/min
 Gradient:

Time (min)	% B
0	40
1	40
5	80
8	80

Temperature: 40 $^{\circ}$ C
 Detector: UV, 280 nm

Method development on Poroshell 120 SB-Aq

The method should be modified according to the above description on Poroshell 120 SB-Aq with acetonitrile:1.5% acetic acid mobile phase, because the real samples (butter) are more complex than the standards. For good separation when dealing with interference components in butter, the gradient was adjusted to get ideal resolutions of target

antioxidants. Figure 3 shows the chromatogram of the nine antioxidants separated under the modified gradient at 10 ppm. All the compounds had a symmetrical peak shape, and the eluted time of the last peak was extended to 8.5 minutes from 5.2 minutes under the original gradient. It was necessary for the real sample separation to use a slightly longer gradient to get ideal separations.

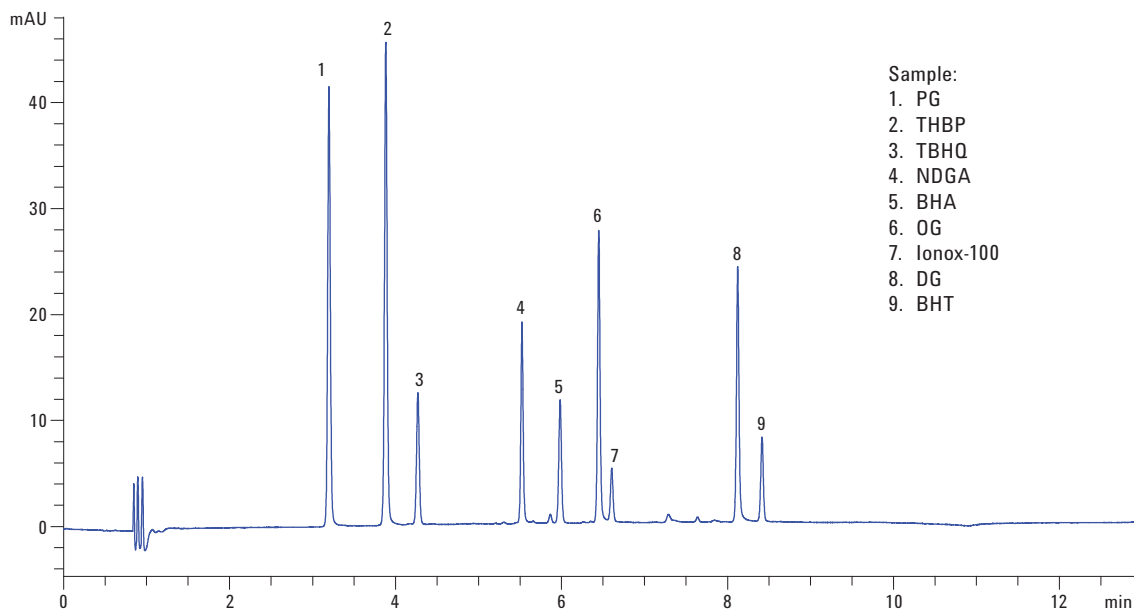


Figure 3. Chromatogram of 10 ppm standards' separation with an Agilent Poroshell 120 SB-Aq column.

Conditions, Figure 3

Eluent: A 1.5% acetic acid, B ACN
 Injection volume: 2 μ L of 10 ppm mixture in 50% ACN/50% IPA
 Flow rate: 0.6 mL/min
 Gradient:

Time (min)	% B
0	10
9	75
10	95

Stop time: 15 minutes
 Temperature: 40 $^{\circ}$ C
 Detector: UV, 280 nm

The data of correlation of linearity and limit of detection (LOD) by UV at 280 nm is shown in Table 2. The coefficients of linearity were excellent for all nine compounds. The LODs were calculated with a signal-to-noise ratio of 3. The LOD was equal to or below 0.1 ppm for all the antioxidants. This corresponded to approximately 0.2 mg/kg or below in a butter sample.

Stable retention times are important for correct identification of analytes in complex food matrixes. Figure 4 shows overlay chromatograms of eight consecutive injections of 1 ppm standard mixture. The retention time of all the peaks was well reproducible and all the relative standard deviations of peak retention time from eight replicate injections on Poroshell 120 SB-Aq column were less than 0.1%.

Table 2. Correlation of linearity and theoretical LOD of nine antioxidants.

No.	Name	Calibration curve	Range (ppm)	Correlation	LOD (s/n=3) (ppm)
1	PG	$Y = 9.144X + 0.936$	0.2 ~ 10	0.9997	0.046
2	THBP	$Y = 10.075X + 1.028$	0.2 ~ 10	0.9998	0.039
3	TBHQ	$Y = 2.815X + 0.309$	0.2 ~ 10	0.9997	0.074
4	NDGA	$Y = 3.659X + 0.306$	0.2 ~ 10	0.9997	0.111
5	BHA	$Y = 2.631X + 0.330$	0.2 ~ 10	0.9997	0.082
6	OG	$Y = 5.796X + 0.550$	0.2 ~ 10	0.9997	0.037
7	Ionox-100	$Y = 1.132X + 0.130$	0.2 ~ 10	0.9994	0.200
8	DG	$Y = 5.208X + 0.112$	0.2 ~ 10	0.9995	0.090
9	BHT	$Y = 1.709X + 0.463$	0.2 ~ 10	0.9992	0.118

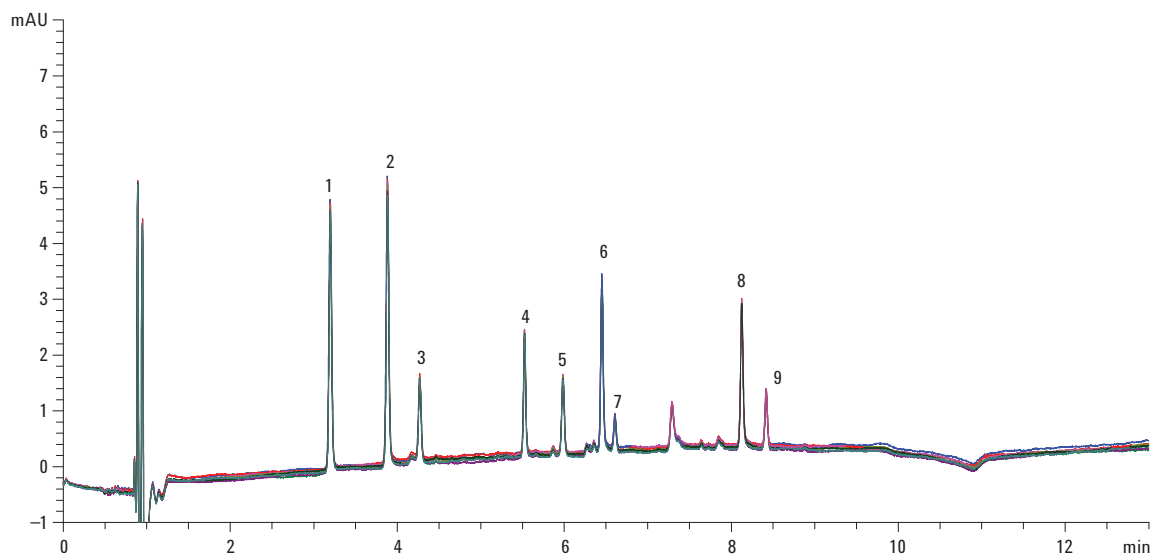


Figure 4. Overlay of chromatograms of eight injections of 1 ppm standards with an Agilent Poroshell 120 SB-Aq column. Conditions same as for Figure 3.

Extracts of five different kinds of butters (Figure 5) and 1 ppm spiked butters (Figure 6) were analyzed to investigate system suitability. Comparing the chromatograms of samples and 1 ppm spiked samples, additional peaks originating from the butter matrix were visible, but there were only a few interferences with the standard peaks. For example, peak 3 was difficult to differentiate from unspiked sample 1 and

sample 5, while peak 9 was difficult to differentiate from unspiked sample 2. Though some interferences were found, we can still measure the amounts of antioxidants from the butter at the ppm level using the HPLC method. If a lower level of LOD is needed, a triple-quadrupole mass spectrometer would be a better choice of detector.

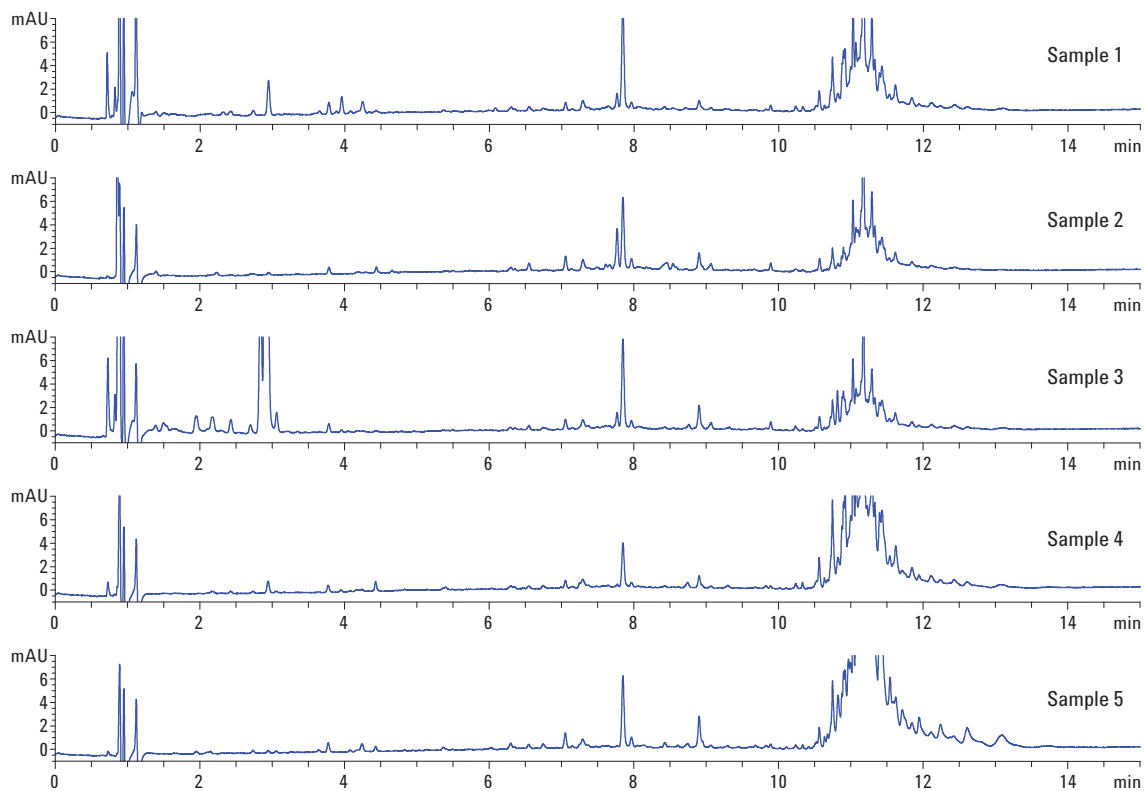


Figure 5. Separation of five different kinds of butter with an Agilent Poroshell 120 SB-Aq column. Conditions same as for Figure 3.

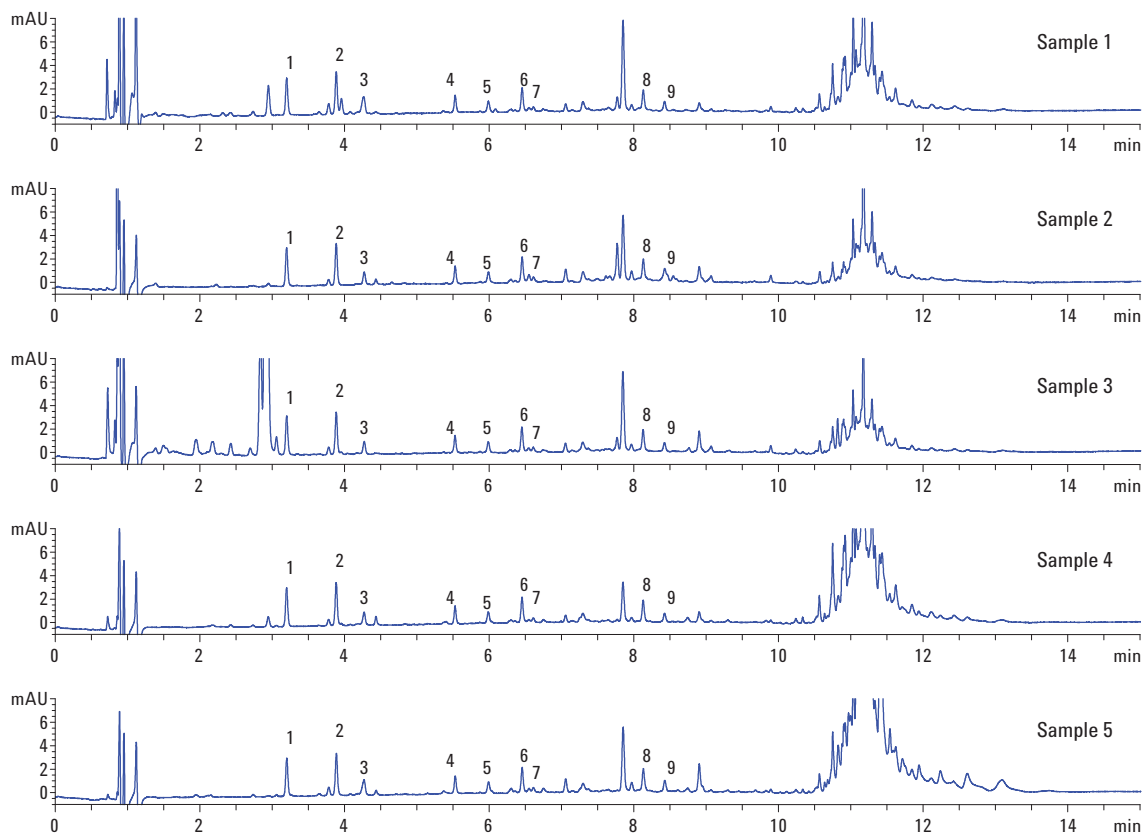


Figure 6. Separation of five different kinds of butter spiked with 1 ppm standards mixture using an Agilent Poroshell 120 SB-Aq column. Conditions same as for Figure 3.

Conclusions

Agilent Poroshell 120 columns are packed with superficially porous particles, which provide high performance similar to the sub-2 μm particles but with a 40 to 50% lower pressure than columns with sub-2 μm particles. The recent introduction of new bonded phases on Poroshell 120 makes them useful in method development by offering unique and differing selectivities across the various chemistries.

Using Poroshell 120 SB-Aq with the Agilent 1290 Infinity LC, a quick analytical method was developed for the determination of antioxidants in butters. The analysis time could be reduced to 15 minutes, one-third of the analysis time with a traditional 5 μm column. Butter samples and spiked butter samples were extracted and the system suitability was investigated. Satisfactory results were achieved at a ppm level. The developed method was suitable for QC laboratories in the food industry for the antioxidant analysis of butter.

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1. Li Tao *et al.* *Food Res. Dev.* (Japan), 24, 23 (2003).
2. Gerd Vanhoenacker, Frank David, Pat Sandra. "Ultrafast analysis of synthetic antioxidants in vegetable oils using the Agilent 1290 Infinity LC system." Application Note, Agilent Technologies, Inc., Publication Number 5990-4378EN (2009).
3. Anon. Method for the determination of antioxidants in oils and fats for import and export - liquid chromatography. SN/T 1050-2002. China Standard (2002).

Fast Separation of Food Preservatives Using an Agilent Poroshell 120 EC-C18 Column

Author

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Abstract

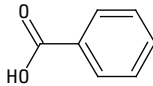
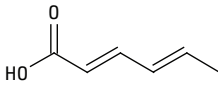
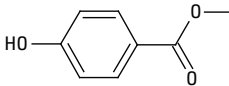
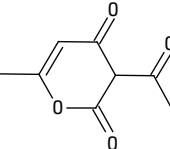
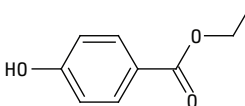
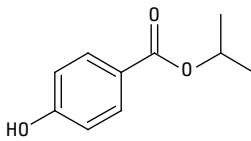
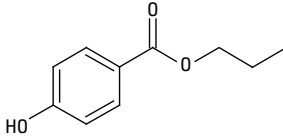
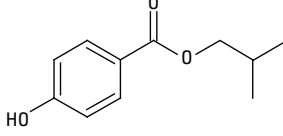
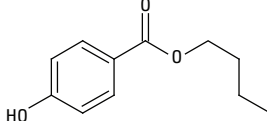
A UHPLC method was developed for the simultaneous determination of the nine preservatives most often used in food and beverages. An Agilent Poroshell 120 EC-C18 column was used for the separation with a gradient method and acetate ammonium buffer: acetonitrile mobile phase. This method gave a rapid separation of the preservatives in 9 minutes. It is suitable for many food and beverage samples and was applied here to the analysis of these preservatives in a cake sample.

Introduction

Preservatives are very popular in the food and cosmetics industries because they prevent these products from degrading within the warranty time. However, preservatives are strictly regulated because their overuse can cause some health problems in humans. For example, some preservatives can accumulate in the human body and negatively influence the metabolism process. Today's trends in food and cosmetics increasingly emphasize the concepts of health and green issues. This means use of safer raw materials as well as fewer preservatives and control of preservatives within a safe limit. Many regulations made by the US Food and Drug Administration (FDA), the European Union (EU), and others set concentration limits on the preservatives in food.

Table 1 lists preservatives commonly used in food. The HPLC method has been the most popular way to analyze the preservatives in food samples. A previous application note described a method developed using a traditional Agilent J&W HC-C18 (2), 4.6 × 250 mm, 5 μm column (p/n 588905-902) that simultaneously separated nine preservatives in 30 minutes [1]. This application note focused on developing a method for rapidly separating the nine compounds and a food sample using a Poroshell 120 EC-C18 column.

Table 1. Preservatives used in this study.

Peak No.	Name	CAS	Structure
1	Benzoic acid	65-85-0	
2	Sorbic acid	110-44-1	
3	Methylparaben	99-76-3	
4	Dehydroacetic acid (DHA)	520-45-6	
5	Ethylparaben	120-47-8	
6	Isopropylparaben	4191-73-5	
7	<i>n</i> -Propylparaben	194-43-3	
8	Isobutylparaben	4247-02-3	
9	<i>n</i> -Butylparaben	94-26-8	

Materials and Methods

HPLC analysis was performed with the Agilent 1290 Infinity LC System, including an Agilent 1290 Infinity Binary Pump (G4220A), an Agilent 1290 Infinity Autosampler (G4226A), an Agilent 1290 Infinity Thermostatted Column Compartment (G1316C), and an Agilent 1290 Infinity Diode Array Detector (G4212A).

Conditions

Column: Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 μm (p/n 695975-302)
 Eluent: A, 10 mM acetate; B, methanol
 Injection volume: 1 μL
 Flow rate: 0.6 mL/min
 Gradient:

Time (min)	% B
0	20
10	50

 Stop time: 10 min, post run 2 min
 Temperature: 30 °C
 Detector: UV, 230 and 260 nm

The standard solution was prepared in 20% methanol:80% water at 0.1 mg/mL individually. The cake sample was prepared as follows. Weigh 5 g of sample, add 40 mL water and a drop of ammonium hydroxide, and extract in sonicator at 60 to 80 °C for 30 minutes. Add methanol to 50 mL, and filter using Agilent 0.2 µm regenerated cellulose membrane filters (p/n 5064-8222) before injection.

Results and Discussion

The previous method was developed on an HC-C18 (2), 4.6 × 250 mm, 5 µm column that separated nine preservatives compounds to baseline in 30 minutes. The original method was transferred to a Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm column.

Poroshell 120 columns are packed with superficially porous materials with a particle size of 2.7 µm. Compared to traditional totally porous particle columns, the superficially porous particles produce a fast separation with no compromise in performance due to fast diffusion in the particles. The Poroshell 120, 2.7 µm particles have a

Van-Deemter curve similar to columns with sub-2.7 µm totally porous materials. The performance of the Poroshell 120 column does not decrease at high flow rate.

A 3.0 mm id column was used for this separation at a flow rate of 0.6 mL/min, which is approximately 1.5 times of normal flow rate of 0.4 to 0.5 mL/min typically used with this column id. The gradient was optimized according to the column configurations, and nine components were separated completely in 10 minutes on the Poroshell 120 EC-C18 column (Figure 1). The analysis time was shortened from 30 minutes to 10 minutes. Both 230 nm and 260 nm UV wavelength were applied for the detection, because benzoic acid and dehydroacetic acid have a maximum absorption of 230 nm while others have absorptions at 260 nm.

These preservatives are commonly used in many beverages and food, such as fruit-flavored drinks, cakes, candy, and so on. A sample of cake was analyzed, and Figure 2 shows the chromatogram of the sample. Based on the standard, only the dehydroacetic acid was found in the sample at a UV wavelength of 230 nm. The amount could be measured given the standard's concentration.

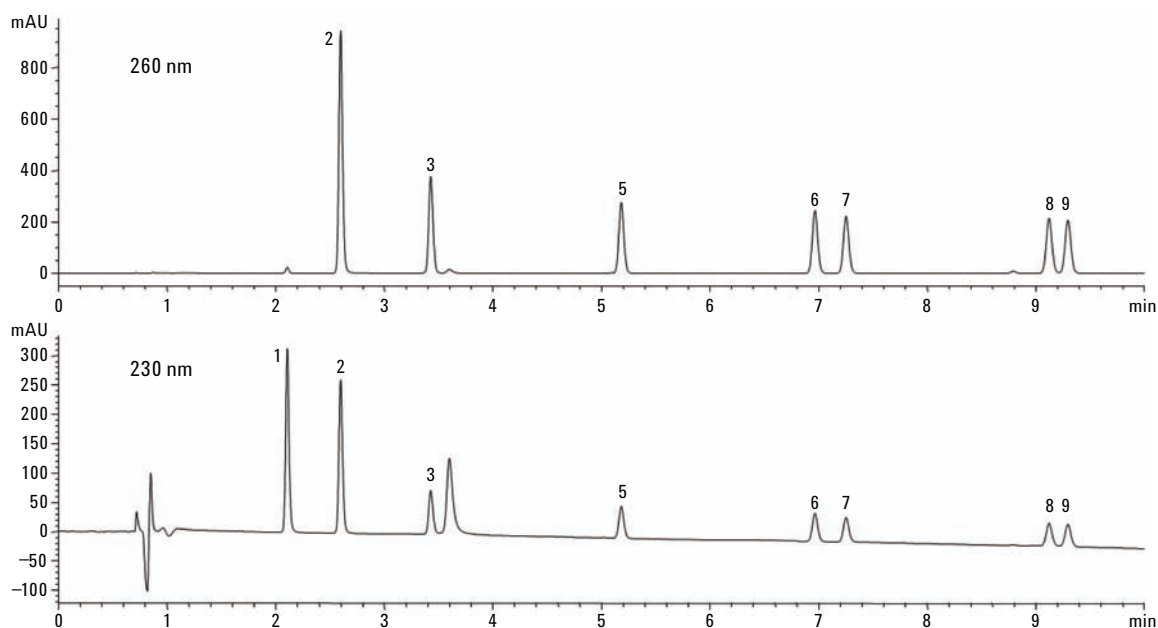


Figure 1. Separation of nine preservative standards using an Agilent Poroshell 120 EC-C18 with UV detection at 230 nm and 260 nm.

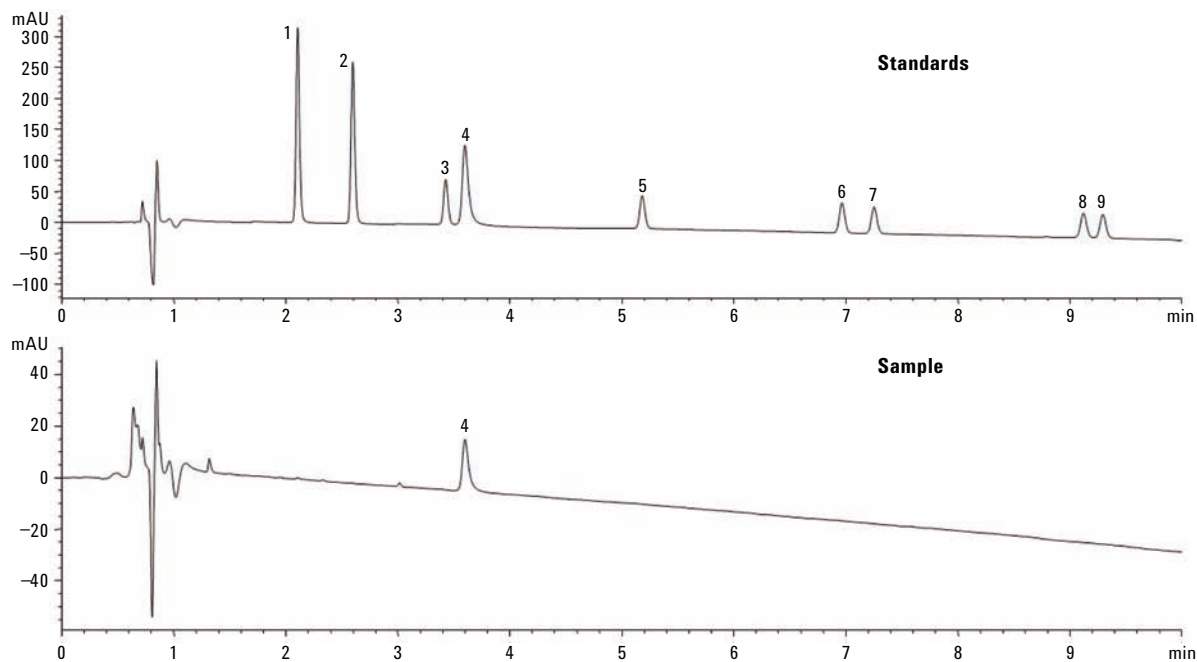


Figure 2. Separation of preservatives in cake using an Agilent Poroshell 120 EC-C18 column with UV detection at 230 nm.

Conclusions

Using a gradient method, preservatives can be rapidly separated on the Agilent Poroshell 120 column. This method allows rapid separation and screening for preservatives in food and cosmetics that can be a routine analysis for product quality control.

Reference

1. Rongjie Fu, Zhixiu Xu. "Analysis of preservatives in food and cosmetics with the Agilent 1120 Compact LC system". Application Note, Agilent Technologies, Inc., Publication Number 5989-8960EN (2010).

Fast Analysis of Fruit Juice Acids with an Agilent Poroshell 120 SB-Aq Column

Author

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Abstract

Fruit juice producers face the problem of contamination of their product by fermentation-causing bacteria or contamination of juice products with cheaper adulterants. The acid profile can be used to identify a juice or verify its purity. A reversed-phase HPLC method for separation of organic acids (tartaric, quinic, malic, citric, and fumaric acids) in fruit juices is demonstrated using an Agilent Poroshell 120 SB-Aq column. The chromatographic separation was performed with an Agilent 1200 Infinity Series using a potassium dihydrogen orthophosphate buffer (pH 2.5) as mobile phase and diode array detection at $\lambda = 226$ nm. Organic acid profiles of several juices are shown.

Introduction

The identification of major organic acids in fruits is considered very important for food and beverage technology and quality evaluation [1]. Organic acids are a useful index of authenticity in fruit products, because they have lower susceptibility to change during processing and storage than other components of fruits [2].

Grapes are known for being acidic, but just how acidic depends on the region in which they are grown. Those grown in cooler climates are more acidic than grapes from warmer areas. Grapes have very low levels of citric acid but have high levels of tartaric acid, which is what makes them sour [3]. Malic acid, from the Latin word malum, or apple, gives green apples their sour quality. It is present in grapes and in most wines. Malic acid confers a tart taste to wine, although the amount decreases with increasing fruit ripeness. Cranberries contain a few different types of acid, including quinic acid, which breaks down the calcium deposits that collect to form kidney stones. Cranberries also contain citric and malic acids. As suggested by the

name, citrus fruits contain a high amount of citric acid, with a general guide that sweeter fruits contain less acid. Limes, lemons, oranges, tangerines, and grapefruits are some acidic citrus fruits. However, berries, such as red currants, raspberries, and blackberries, also have a high amount of citric acid. Figure 1 shows the structures of these common acids.

Acids are generally difficult to separate using reversed-phase liquid chromatography. Bases can be more easily retained at higher pH, where the compounds are not charged. However, in some cases, other compounds in the sample will not be separable at high pH. A possible solution is to use an ion-pair reagent to increase retention. Acidic compounds are also noted as difficult samples to separate or even retain. In general, it is necessary to work below the pKa of the compound, where it will be fully protonated (not charged), and decrease the organic content of the mobile phase [4]. A problem that can occur with many alkyl columns, such as C8 or C18 phases, is poor retention or reproducibility of retention in low organic mobile phase. One of the unique properties of Agilent Poroshell 120 SB-Aq columns is their resistance to dewetting, or what is sometimes referred to as phase collapse [5]. This application note shows separation of some acidic compounds using 100% aqueous conditions.

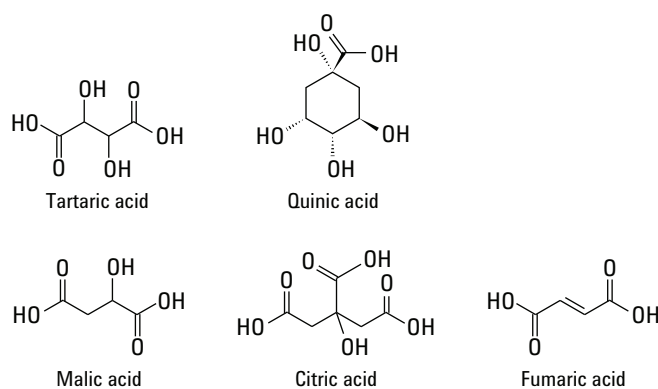


Figure 1. Structures of some fruit acids.

Materials and Methods

HPLC analysis was performed with an Agilent 1200 Infinity Series, comprising:

- Agilent 1200 Series Binary Pump SL (G1312B)
- Agilent 1200 Series Automatic Liquid Sampler (ALS) SL (G1376C)
- Agilent 1200 Series Thermostated Column Compartment (TCC) SL (G1316B)
- Agilent 1200 Series Diode Array Detector (G1315C)

Tartaric, quinic, malic, citric, fumaric acids, potassium phosphate mono basic, and phosphoric acid were purchased from Sigma-Aldrich Corp. Water used in all sample and mobile phase preparation was 18 Mohm.cm Milli-Q (Millipore) produced on site. Juice and cocktail samples were obtained at a local grocery store.

Conditions

Column:	Agilent Poroshell 120 SB-Aq, 3 × 100 mm, 2.7 μm (p/n 685975-314)
Eluent:	100 mM Potassium phosphate buffer, pH 2.5
Injection volume:	5 μL
Flow rate:	0.5 mL/min
Temperature:	50 °C
Detector:	DAD, at 226 nm

Results and Discussion

All samples were freshly prepared and used within the same day. Some of the acids were found to decay when evaluated over more than 1 day. Storing samples in foil wrapping to reduce light or freezing can be used to extend the life of the standards. However, it is best to analyze samples as quickly as possible to achieve the most accurate results. As shown in this method, the entire analysis including sample preparation was accomplished in under 10 minutes. All samples were diluted to 1/3 of their original concentrations, filtered if pulp was present, and then injected. All samples contained tartaric acid and citric acid. Figure 2 shows a chromatogram of the standards and several sample juices.

A useful feature of the Poroshell 120 SB-Aq column is that it can be used in 100% aqueous mobile phase without phase collapse. Use of the high-water-content mobile phase can lead to a dramatic decrease in retention over time. The loss in retention time observed after moving from an organic-aqueous mobile phase into an aqueous mobile phase can be accelerated by turning off the flow for a period of time. The loss in analyte retention over time (or instantaneously with flow stoppage) using an RP column in a high aqueous mobile phase has commonly, but perhaps incorrectly, been referred to as phase collapse.

Using a similar methodology to a published method [5], a 75% methanol and 25% buffer was run on the column for 20 minutes, followed by a 5 minute equilibration to 100% buffer. A sample was injected after the column equilibrated. After this run, the pump was shut down overnight for 18 hours. In the morning, the pump was started, and a new injection was made. Figure 3 compares the chromatograms with identical retention times for all analytes.

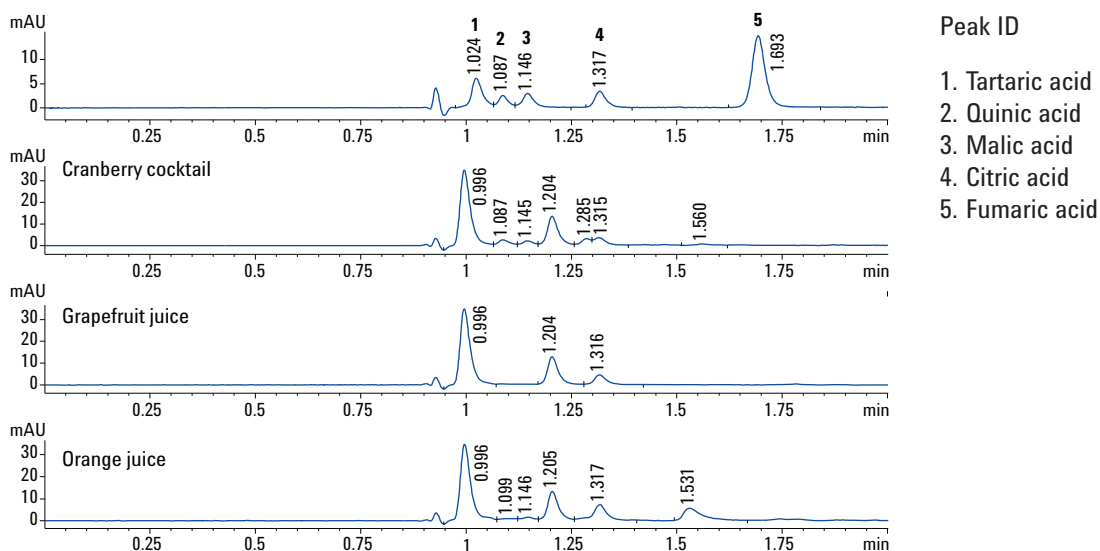


Figure 2. Chromatograms of food acids produced on an Agilent Poroshell 120 SB-Aq column.

Conclusion

This application note is a contribution to the development of a rapid and precise HPLC procedure for quantitative determination of organic acids in fruit juices under reversed-phase conditions. Tartaric, quinic, malic, citric, and fumaric acids were determined simultaneously and eluted from the column within 2 minutes. The method could be used to quantify organic acids in fruit juices. The column was also shown to resist phase dewetting and be effective in a 100% aqueous mobile phase.

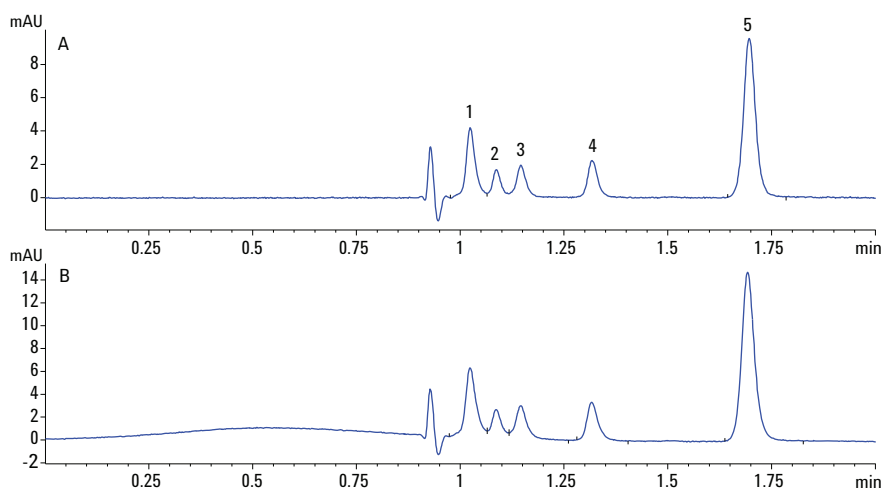
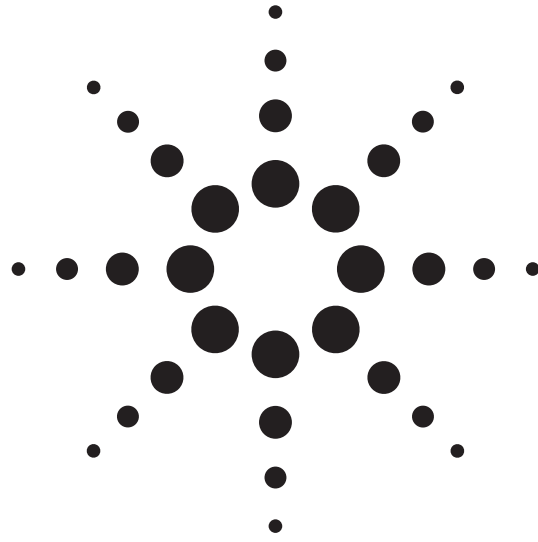


Figure 3. Before (A) and after (B) comparison of two injections of fruit acids after the pump was stopped for 18 hours (injection volume 3 μ L, other conditions as before).

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药物分析

采用安捷伦 Poroshell 120 色谱柱快速分析大鼠血浆中蟾蜍二烯内酯及其代谢物

关键词

HPLC

Poroshell 120

蟾蜍二烯内酯及其代谢物

血浆

作者

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摘要

蟾酥为蟾蜍科动物中华大蟾蜍 (*Bufo gargarizans* Cantor) 或黑眶蟾蜍 (*Bufo melanostictus* Schneider) 等的耳后腺及皮肤腺分泌的白色浆液, 经加工干燥而成 [1]。其性味辛温, 有毒, 具有解毒、消肿、醒神、开窍、强心和止痛等作用。蟾酥的主要化学成分有蟾蜍内酯类、吲哚碱类、甾醇类及多肽类等 [1]。其中已分离鉴定的蟾蜍二烯内酯类化合物逾 40 种 [2-6], 被认为是蟾酥的主要药效成分。蟾蜍内酯类成分在体内的吸收和代谢研究对阐明蟾酥的药理作用以及临床用药安全具有重要的指导意义。然而, 蟾酥的成分复杂, 给药后经动物肠道菌群及体内代谢后成分更为复杂, 而且含量低, 对仪器及分析技术提出了更高的要求。近年来, 液相色谱-质谱联用技术被广泛应用于中药及其代谢物分析, 其优点在于能够实现复杂成分的在线检测和鉴定。质量高分辨和多级串联功能的质谱仪与液相色谱联用, 为成分鉴定的准确性提供了更有力的保障。本研究利用高效液相色谱 (使用 Poroshell 120 快速高效色谱柱) - 高分辨质谱联用仪分析鉴定了蟾蜍内酯类化合物口服给药后在大鼠血浆中的成分及其代谢物, 发现血浆中含有原药中 24 种蟾蜍二烯内酯类成分, 本研究结果为蟾酥活性成分的体内药理毒理作用研究提供了理论依据。

材料与方法

1.1 仪器与试剂

液相色谱仪, 高分辨质谱仪; Poroshell SB-C18 快速分离型高效液相色谱柱(100 mm×2.1 mm, 2.7 mm, 美国 Agilent 公司);TG18M 高速离心机(长沙平凡仪器仪表有限公司);MS2 型涡旋振荡器(广州 IKA 公司)日蟾毒它灵(Gamabufotalin)、沙蟾毒精(Arenobufagin)、蟾毒灵(Bufalin)、脂蟾毒配基(Resibufogenin)和华蟾毒配基(Cinobufagin)对照品(暨南大学中药及天然药物研究所分离并鉴定,HPLC 分析纯度大于 98%);蟾酥饼(山东临沂医药有限公司);甲醇和乙腈(色谱纯,美国 Burdick & Jackson 公司);甲酸(分析纯,广州化学试剂厂);实验用水为超纯水设备(美国 Milli-Q 公司)制备的超纯水。实验动物为雄性 SD 大鼠(体重 200~220 g,广东省医学实验动物中心)。

1.2 色谱条件

Agilent Poroshell SB-C18 快速分离型高效液相色谱柱(100 mm×2.1 mm, 2.7 mm);流动相 A 为水(含 0.5% 甲酸),B 为乙腈;0 min,80% A+10% B;0-7 min,流动相 B 从 0% 升至 30%;7-35 min,流动相 B 从 30% 升至 45%;35-40 min,流动相 B 从 45% 升至 95%;40-45 min,95% B;45-46 min,流动相 B 从 95% 降至 20%;46-55 min,20%B。流速 0.3 mL/min;进样量 1 μ L。离子化方式:电喷雾(ESI)正离子模式;喷雾电压:4.5 kV;管状透镜电压:80 V;鞘气压力:214 kPa;辅助气压力:55 kPa;扫描质量范围:m/z 100-700。

1.3 试验样品

雄性 SD 大鼠 8 只,禁食 12 h 后,其中 6 只 50 mg/kg 剂量蟾酥总内酯灌胃给药,另 2 只灌胃等体积的丙二醇,给药或丙二醇后分别在 10, 20, 35, 60 和 120 min 颈静脉采血,每次取血约 2 mL,转入预先用 0.3% 肝素钠溶液湿润过的 EP 管,置于冰水中。

1.4 试验方法

1.4.1 样品处理

血样静置约 5 min 后,以 3000 r/min 离心 8 min,吸取上清血浆,

加入约 2 倍量的乙腈,漩涡 5 min,超声 10 min,12000 r/min 离心 10 min,吸取上层清液,合并同一大鼠不同时间点血浆经处理后上清液,氮气吹干,残留物加 200 mL 甲醇溶解,过 0.45 μ m 滤膜,待分析

1.4.2 测定

按 1.2 操作进行。

结果与讨论

图 1A 为蟾酥总内酯样品分析的总离子流质量色谱图,通过分析每个色谱峰保留时间、准分子离子和碎片离子的精确质荷比(m/z),并与文献[3,4]比较,推测出蟾酥总内酯样品中共含有 24 个蟾蜍二烯内酯类化合物。每个化合物通过一级高分辨质谱的精确分子量信息推测出其可能分子式,二级高分辨质谱碎片精确 m/z 信息推测出离子碎片化学式,并结合其在 C18 色谱柱的保留时间,与文献[9,10]比较,对 24 个蟾蜍二烯内酯类化合物进行了鉴定,化合物结构见图 2。

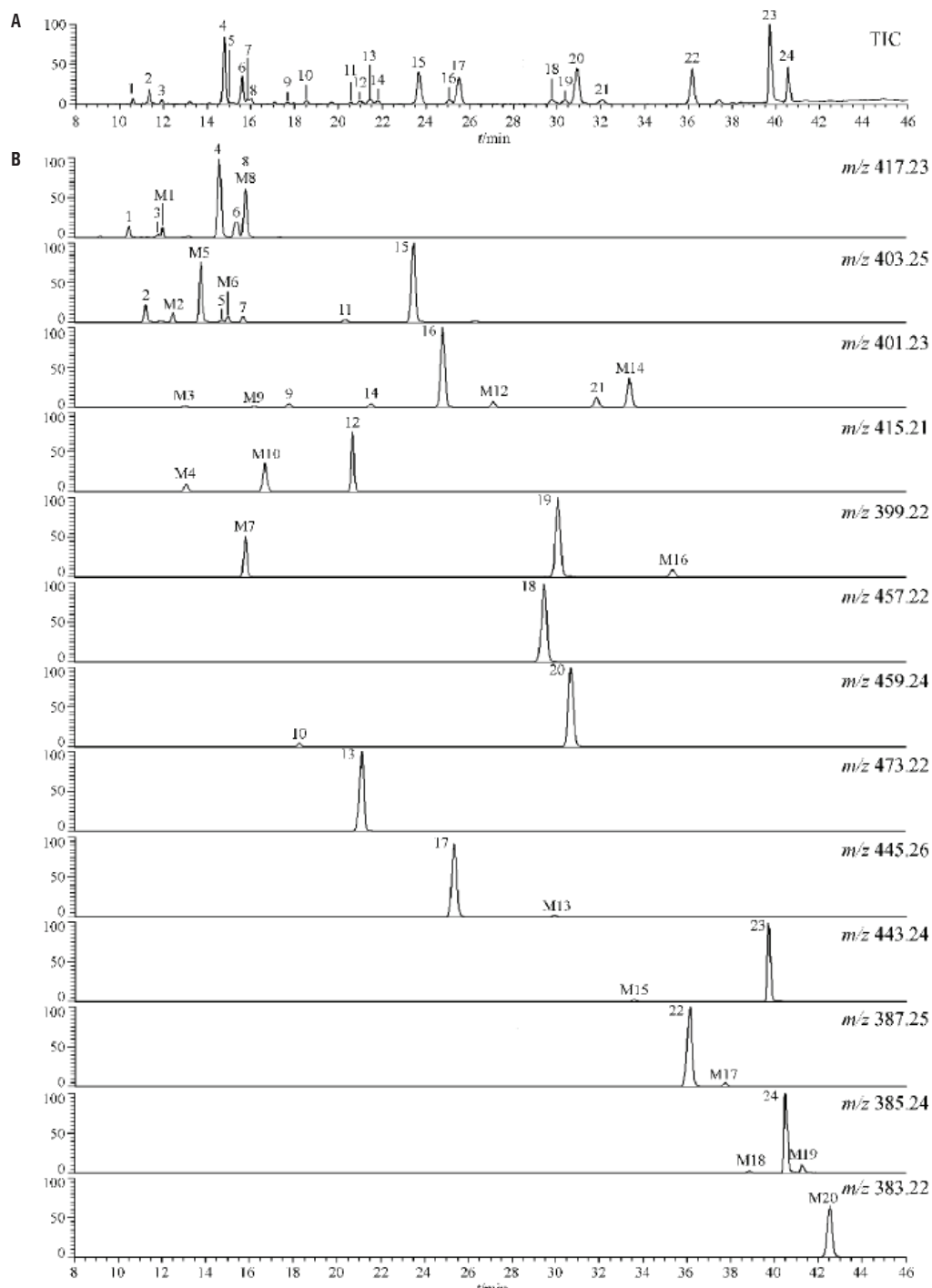


图1 蟾酥内酯提取物的总离子流质量色谱图 (A) 和大鼠给药后血浆样品的提取离子流质量色谱图 (B)

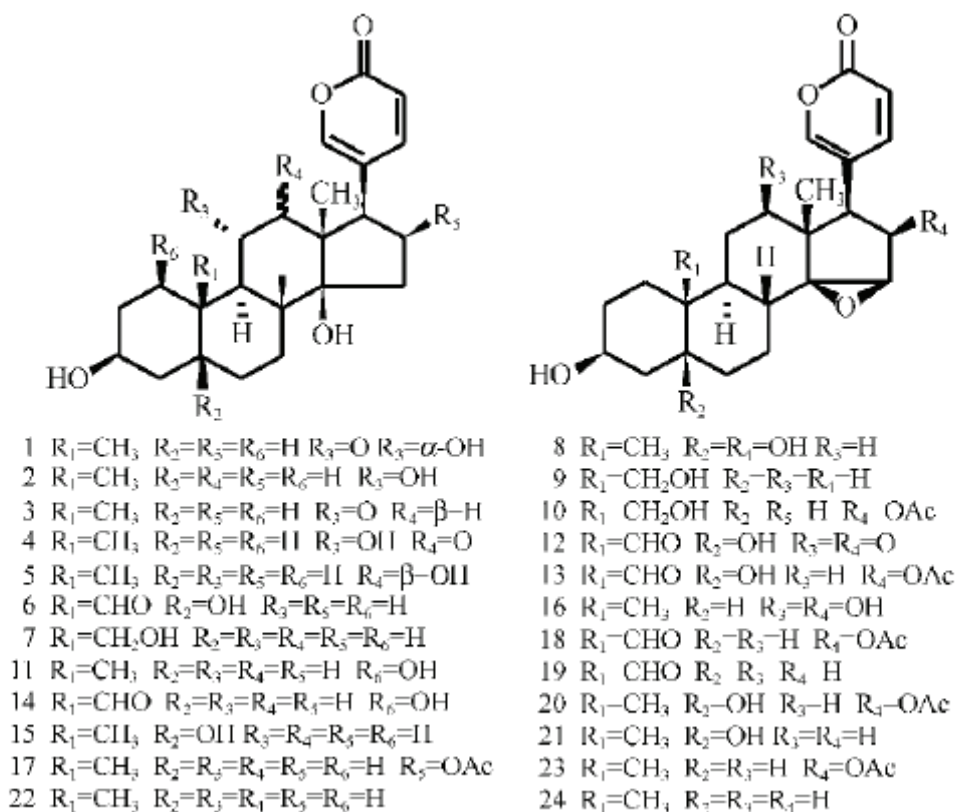


图 2 HPLC-MS 鉴定的蟾酥中蟾蜍二烯内酯类化合物结构

小结

建立了高效液相色谱-质谱鉴定大鼠口服蟾酥总内酯提取物后血浆中蟾蜍二烯内酯类化合物及其主要代谢物的分析方法。大鼠单剂量灌胃 (50 mg/kg) 蟾酥总内酯提取物后收集血浆样品, 乙腈沉淀蛋白, 浓缩定容, 采用 HPLC-MS 分析。利用色谱保留时间、高分辨质谱提供的精确分子量和碎片离子精确质量的信息, 鉴定了 24 种蟾蜍二烯内酯类化合物。本方法快速、灵敏, 适用于鉴定血浆中的微量蟾蜍二烯内酯类化合物及其体内代谢物。

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采用安捷伦 Poroshell 120 EC-C18 色谱柱分析蜂蜜中的阿莫西林残留量

关键词

LC-MS-MS

Poroshell 120

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摘要

阿莫西林是一种广谱半合成青霉素，杀菌作用强，不仅是医学上的重要抗感染药物，也是目前畜禽养殖业中动物疾病预防和治疗的常用药物。阿莫西林使用后会在生物体内残留，人长期食用含青霉素残留的制品，相当于长期低剂量的吸收青霉素，从而使人体产生耐药性。因此对动物性食品中青霉素类抗生素残留进行有效监测已经成为保障食品安全的一个重要环节。安捷伦公司最新推出的 Poroshell 120 系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用 Poroshell 120 色谱柱，并用高效液相色谱-串联质谱，对蜂蜜中的阿莫西林残留量进行检测，该方法获得了较好的回收率，检测限量较低，满足日常检测工作的需要。

材料与方法

1.1 仪器与试剂

液相色谱 - 串联四极杆质谱联用仪, 配备电喷雾离子 (ESI) 源和大气压化学电离 (APCI) 源。氮吹仪 (美国 Organomation 公司); 超纯水器 (Millipore 公司)。

阿莫西林标准品 (纯度 98.0%, Dr. Ehrenstorfer)。甲醇 (色谱纯, 德国 Merck 公司), 甲酸 (色谱纯), 磷酸氢二钾、氢氧化钠和甲醇 (分析纯, 南京化学试剂厂)。

1.2 色谱条件

色谱条件: Poroshell 120 EC-C18 色谱柱 (100 mm × 4.6 mm, 2.7 μm)。流动相: A, 0.1% (v/v) 甲酸水溶液; B, 甲醇。梯度洗脱程序: 0 min, 10%B; 3.0~4.0 min, 30%B; 6.0~8.0 min, 95%B; 8.5~9.5 min, 10%B。流速: 0.4 mL/min。柱温为室温。进样体积为 25 μL。

质谱条件: 采用 ESI 源, 正离子方式检测; 喷射电压: 2500 V; 鞘气 55 bar, 辅助气) 10 bar; 毛细管温度: 350 °C。

阿莫西林的质谱条件为: 母离子: 336 m/z; 子离子: 定性离子为 114 m/z, 碰撞能量 25 eV, 定量离子为 349 m/z, 碰撞能量为 12 eV。

1.3 试验样品

蜂蜜样品来自于市售蜂蜜以及养蜂场的原料蜂蜜。

1.4 试验方法

1.4.1 标准储备液的配制

称取阿莫西林标准品适量, 用乙腈 - 水 (4:6, v/v) 溶解并定容至 10 mL, 配成 1.0 g/L 标准储备液, 于冰箱中冷冻保存。

1.4.2 混合标准工作液

准确吸取一定量的 1.0 g/L 的标准储备液, 用乙腈 - 水 (4:6, v/v) 逐级稀释成 1.0 mg/L。按照检测需求, 用流动相稀释标准储备液, 配制标准工作溶液, 质量浓度范围为 2.0~100.0 μg/L。。

1.4.3 样品处理

称取蜂蜜样品 (5.00 ± 0.05) g 于 50 mL 玻璃离心管内, 加入 0.2 mol/L 磷酸氢二钾溶液 (pH 8.0) 15 mL, 涡旋混匀, 并用中速滤纸过滤到干净的玻璃离心管内, 加入预先用 3 mL 甲醇和 3 mL 水处理的强阴离子交换固相萃取小柱中, 上样流速控制在 1~2 d/s, 用 3 mL 水洗小柱, 弃去全部流出液, 减压抽干, 过程为 10 min, 最后用 5 mL 含 0.2% (v/v, 下同) 甲酸的甲醇溶液洗脱, 收集洗脱液于 10 mL 试管中, 于 45 °C 用氮吹仪吹干, 用甲醇 - 水 (3:7,v/v) 定容至 1.0 mL, 过 0.45 μm 滤膜到进样瓶中, 供液相色谱 - 质谱仪测定。

1.4.5 测定

按 1.2 操作进行, 以保留时间定性, 外标法定量。

结果与讨论

2.1 色谱条件的选择

考察了 Agilent Polaris 5 C18-A (100 mm × 2.0 mm)、Agilent Eclipse Plus C18 (150 mm × 2.1 mm, 5 μm)、Agilent Poroshell 120 EC-C18 (100 mm × 4.6 mm, 2.7 μm) 3 种 C18 柱, 比较后发现, 阿莫西林在前两种 C18 柱上保留时间为 2~3 min, 由于流动相线速度相对较低在 Poroshell 120 柱上的保留时间为 5~6 min, 相对其它两种柱子, 保留时间延长, 峰形有所改善, 而且灵敏度显著提高, 因此选用 Poroshell 120 为色谱柱。阿莫西林标准溶液以及蜂蜜样品和蜂蜜加标样品的色谱图及质谱图见图 1。

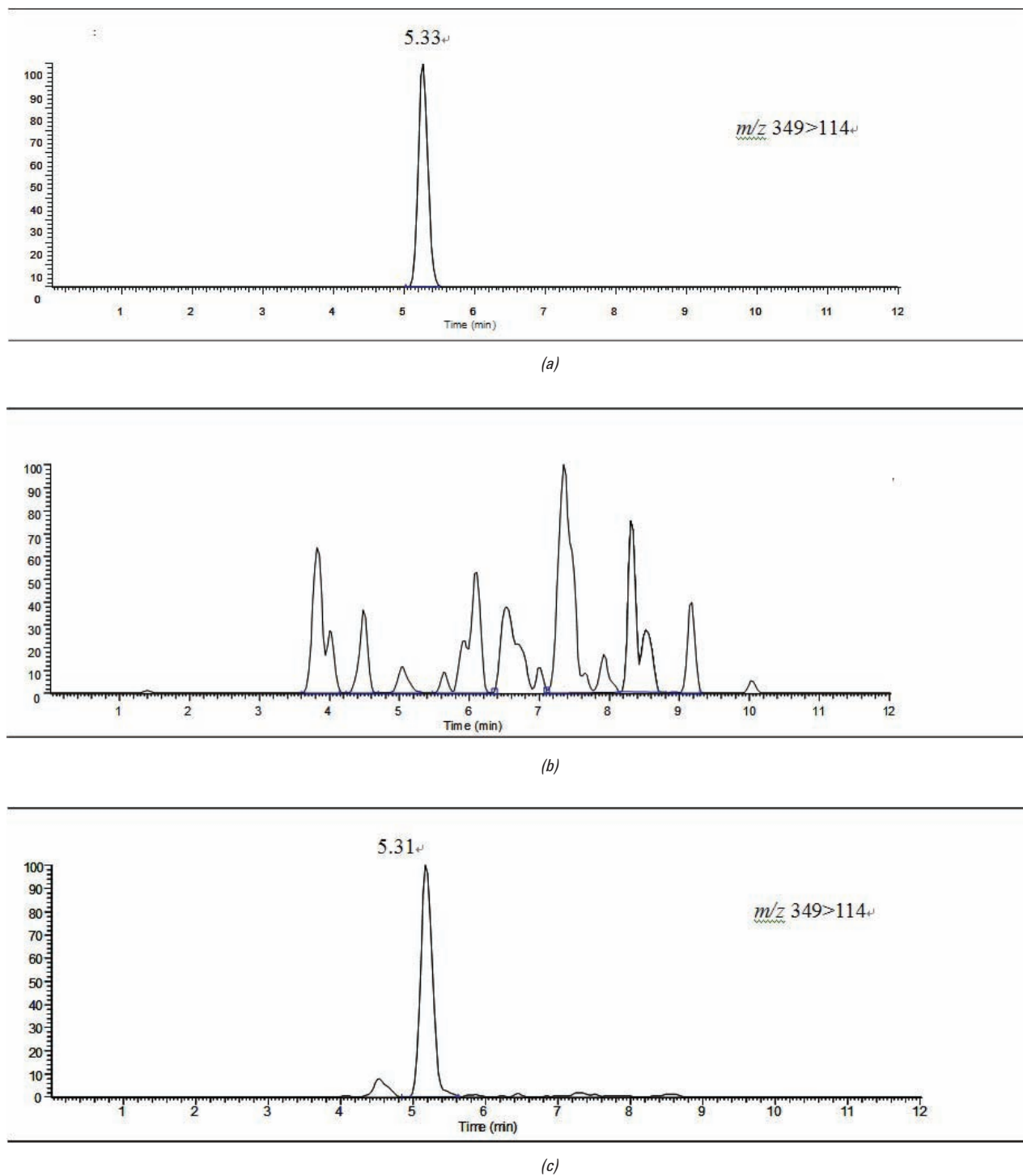


图 1 (a) 阿莫西林标准溶液、(b) 阴性蜂蜜样品、(c) 蜂蜜加标样品 (添加水平为 $5.0 \mu\text{g}/\text{kg}$) 的色谱图

2.2 方法的线性范围和检出限

配制质量浓度为 2.0~100.0 $\mu\text{g/L}$ 的系列标准溶液, 在选定的色谱条件和质谱条件下进行测定, 以待测物的峰面积为纵坐标, 待测物的质量浓度为横坐标进行线性回归, 方程为 $Y = -7167.59 + 319399X$ (X 表示阿莫西林的浓度 (g/L), Y 表示阿莫西林的峰面积), 其线性相关系数 (r^2) > 0.99 。在蜂蜜样品中添加阿莫西林标准溶液, 提取净化后进行测定, 分别以 3 倍信噪比 ($S/N=3$) 和 10 倍信噪比 ($S/N=10$) 确定方法的检出限和定量限, 分别为 2.0 $\mu\text{g/kg}$ 和 5.0 $\mu\text{g/kg}$ 。

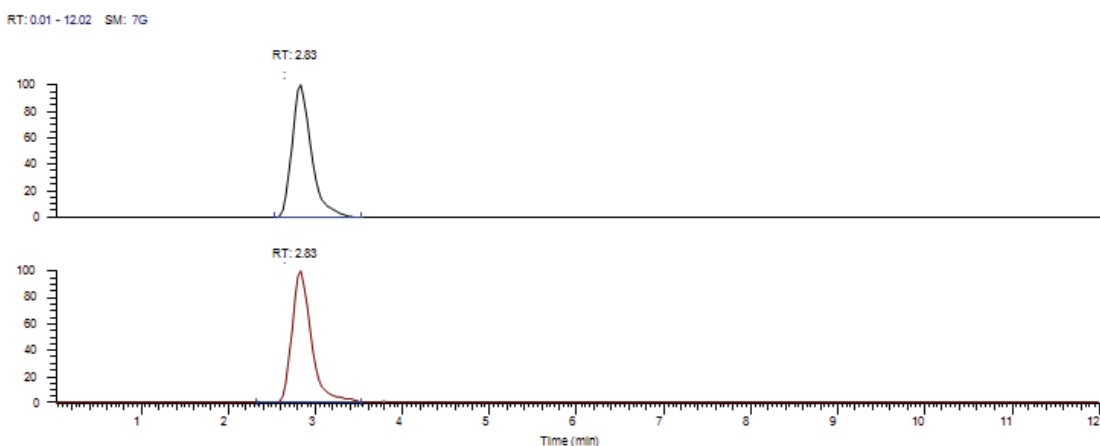
2.3 方法的加标回收率和精密度

选取阴性蜂蜜样品, 添加阿莫西林标准品, 添加水平分别为 2.5、5.0 和 10.0 $\mu\text{g/kg}$, 每个水平做 6 个平行, 连续测定 3 天, 结果见表 4。

表 1 回收率和精密度试验结果
Table 1 Results of recovery and precision tests

Added/ ($\mu\text{g/kg}$)	Found/ ($\mu\text{g/kg}$)	Recovery/%	Intra-day precision (RSD)/% (n=6)			Inter-day precision (RSD)/% (n=3)
			1d	2d	3d	
2.5	2.04	81.7	5.5	6.7	7.3	11.3
5	4.01	80.2	4.7	7.8	5.0	10.4
10	7.42	74.2	6.7	2.8	5.5	9.1

2.4 使用传统色谱柱的结果



小结

建立了高效液相色谱-串联质谱测定蜂蜜中阿莫西林残留的方法, 强阴离子交换固相萃取柱较好地保留了目标物质同时去除了大部分杂质干扰, Poroshell 120 能显著提高灵敏度, 改善峰形。该方法有较好的重现性, 符合实际检测工作的需要。

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采用安捷伦 Poroshell 120 色谱柱快速分析氟替卡松丙酸酯

关键词

HPLC

Poroshell 120

氟替卡松丙酸酯

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摘要

氟替卡松丙酸酯化学名称 S-6 α ,9 α -二氟-11 β -羟基-17 α -丙酰氧基-16 α -甲基-3-氧代雄甾-1,4-二烯-17 β -硫代羟酸氟甲基酯,为化学合成的三氟化糖皮质激素类药物,是由德国葛兰素威康 (Glaxo Wellcome) 有限公司开发,并于 1993 年首次上市,具有较强的抗炎作用,临床上用于治疗哮喘。该产品在 USP 上有记载 [1], 但规定的方法中使用的常规液相色谱仪和液相分析柱,分析时间长,且有机试剂耗用量大,因此,超高效液相色谱仪 (UHPLC) 和快速液相分析柱应运而生,使得分析时间和试剂耗量大大降低。但目前基层实验室普遍使用的常规液相色谱仪与快速液相分析柱的不兼容性,使得快速分析检测很难实现。安捷伦公司最新推出的 Poroshell 120 系列表面多孔层色谱柱,由于其具有低反压,高柱效的特点,从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用 Poroshell 120 色谱柱,并采用常规液相色谱仪,对氟替卡松丙酸酯检测方法加以改进,分析时间和试剂耗量均降低了 2/3,一次分析仅需 26min。现将实验结果报告如下,以供参考。

材料与amp;方法

1.1 仪器与试剂

1260 高效液相色谱仪 (Agilent 公司) ; 乙腈 (HPLC 级, 美国天地公司); 磷酸 (AR 级, 南京试剂有限公司) 水由 Milli-Q Academic 净化系统制得, 氟替卡松丙酸酯由美药星 (南京) 制药有限公司提供。

1.2 色谱条件

溶液 A—0.5 毫升磷酸加入到 1000 毫升水中。

溶液 B—0.5 毫升磷酸加入到 1000 毫升甲醇中。

溶液 C—0.5 毫升磷酸加入到 1000 毫升乙腈中。

流动相: 用溶液 A、溶液 B 和溶液 C 变量混合。必要时做适度调整。

仪器: AGILENT 1260 HPLC, 检测波长: 239nm, 色谱柱: Agilent Poroshell 120 SB-C18 4.6*100mm 2.7um。流速为 1.0mL/min。柱温 40°C。梯度见下表

流动相梯度表

时间	A%	B%	C%
0	50	3	47
10	50	3	47
15	32	3	65
24	32	3	65
26	50	3	47

1.3 试验样品

空白溶液样品、供试品溶液样品、线性溶液样品。

1.4 试验方法

1.4.1 溶液配制

空白溶液: 取溶液 A 与溶液 C 等比例混合, 0.45um 滤膜滤过。

供试品溶液: 取 2 mg 的样品用 5mL 的溶液 A 超声溶解。加 5mL 的溶液 C, 混合, 0.45um 滤膜滤过。

线性溶液: 取 5mg 样品用 5mL 的溶液 A 超声溶解。加 5mL 的溶液 C, 混合作为母液。分别取 1、3、5、7、9mL 母液用溶液 A 与溶液 C 等比例混合溶液分别稀释至 10mL 作为线性溶液。

1.4.2 测定

1.4.2.1 专属性

1.4.2.1.1 空白试验

取空白溶液 10ul 注入液相色谱仪, 按照上述色谱条件测定。观察图谱与样品图谱在相应位置是否有对应峰出现。

结果: 空白溶液在于标准溶液图谱对应位置无任何峰出现, 表明空白对于样品没有干扰 (图 1)。

1.4.2.2 分离度试验

取供试品溶液 10ul 注入液相色谱仪, 按照上述色谱条件测定, 重复进样 3 次, 计算主产物 FP-5 于相邻物质之间的分离度 (图 2)。

编号	主峰与前峰分离度	主峰与后峰分离度
1	12.74	13.97
2	12.36	13.93
3	12.60	13.89

1.4.2.3 精密度

1.4.2.3.1 精密度-重复性

供试品溶液。取 10ul, 注入液相色谱仪, 重复进样 6 次。记录峰面积并计算 RSD。

编号	主峰峰面积
1	5235.18652
2	5241.42041
3	5247.87256
4	5247.17871
5	5254.92920
6	5254.98145
平均	5246.92814
RSD	0.15%

1.4.2.3.2 精密度 - 中间精密度

配制三份供试品溶液按照上述色谱条件分别在 3 天分别进样; 另由实验室另一位分析人员配制三份供试品溶液, 更换仪器, 按照上述色谱条件分别在 3 天分别进样, 记录各检测结果的主峰峰面积百分比并计算 RSD。

编号	浓度 mg/mL	主峰面积百分比
1	0.229	99.3577
2	0.210	99.6944
3	0.233	99.5753
4	0.220	99.2301
5	0.230	99.5443
6	0.249	99.4073
平均	N/A	99.4682
RSD	N/A	0.17%

结果与讨论

2.1 色谱条件的选择

以 Poroshell 120 柱 (4.6 mm i. d. × 100 mm, 2.7 μ m) 作为分析柱, 不改变 USP 标准中的流动相进行 HPLC 条件的优化。实验发现, 在不改变整体梯度只改变梯度变化的时间的情况下 20min 内完成检测有部分峰 (杂质) 分离效果不理想; 30min 完成检测效果良好。故继续进行优化得到最终 26min 完成检测。这样, 每个样品从原来的分析时间 75 分钟 (图 3) 缩短至 26min, 不仅提高了工作效率, 而且节省了有机溶剂。

2.2 方法的线性范围

2.2.1 取线性溶液分别进样记录各浓度样品的主峰总峰面积, 以浓度为横坐标峰面积为纵坐标进行线性回归要求相关系数 R^2 不小于 0.99。

浓度 (mg/mL)	峰面积
0.0506	1134.05945
0.1518	3439.47827
0.2530	5796.76221
0.3542	8011.57074
0.4554	10216.1000

综上作标准曲线得回归方程: $Y=22514.67168X+19.47836$
 $R^2=0.99993$

结果: 经过试验样品在浓度范围 0.0506mg/mL~0.4554mg/mL 范围内呈良好线性关系。可以采用该方法对利用外标法对产品进行定量检测。

2.3 方法的准确度和精密度

取线性溶液分别进样, 各浓度进样 1 次, 各记录各浓度样品的主峰峰面积, 根据回归方程反推各浓度的回收率, 要求回收率在 95%-105% 之间, 并计算 RSD 要求 RSD 不大于 5.0%。

序号	计算浓度 mg/mL	理论浓度 mg/mL	回收率 (%)
1	0.0504	0.0506	99.63%
2	0.1548	0.1518	102.00%
3	0.2584	0.2530	102.14%
4	0.3587	0.3542	101.28%
5	0.4568	0.4554	100.30%
回收率范围	99.63%-102.14%		
回收率 RSD	1.07%		

由于没有产品的标准品, 无法采用外加法进行回收率试验, 因此采用将线性溶液进样后进行反推计算得到回收率, 结果表明该方法的回收率在 95%-10% 之间, 且 RSD 符合要求。

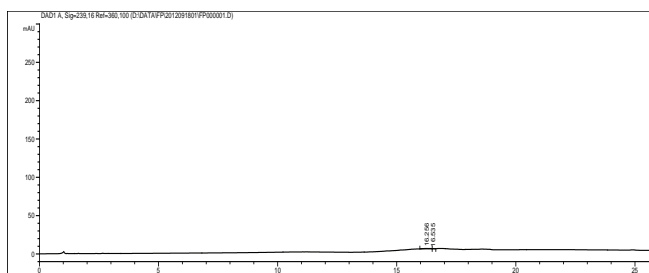


图 1 空白

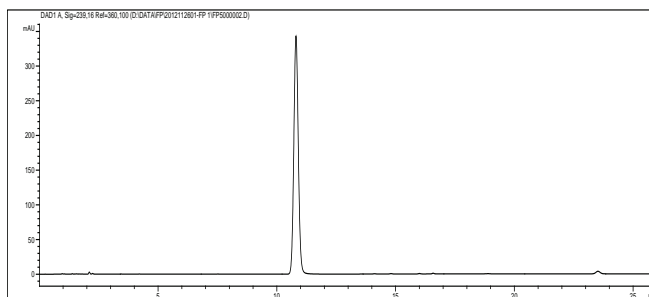


图 2 样品图谱

2.4 使用传统色谱柱的结果

色谱条件

- A 1000mL 水中加 0.5mL 磷酸, 0.45um 滤膜过滤。
 B 1000mL 甲醇中加 0.5mL 磷酸, 0.45um 滤膜过滤。
 C 1000mL 乙腈中加 0.5mL 磷酸, 0.45um 滤膜过滤。

仪器: HPLC, 检测波长: 239nm, 色谱柱: Agilent 4.6mm × 25cm, 5μm L1 的色谱柱。流速为 1.0mL/min。柱温 40°C。梯度见下表:

时间	A%	B%	C%
0	55	3	42
40	55	3	42
60	44	3	53
70	10	3	87
75	55	3	42

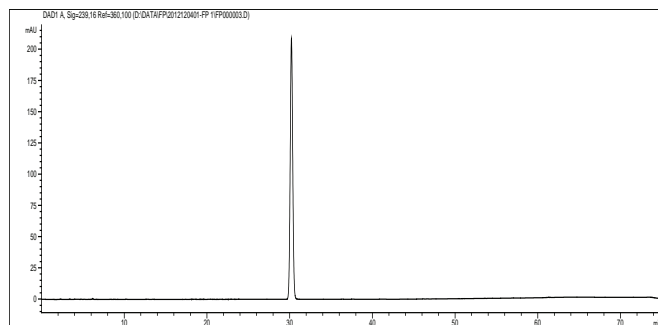


图3 原 USP 方法检测图谱

小结

本法采用 Poroshell120, 在基层普遍使用的常规液相色谱仪上测定氟替卡松丙酸酯。该方法的分析速度迅捷、试剂消耗少、回收率和重复性均符合批量样品的检测要求, 结果令人满意, 很适合于快速高效检测工作的开展。

参考文献

1. Fluticasone Propionate-usp34

采用 Agilent 1290 Infinity 液相色谱系统和 Agilent ZORBAX Poroshell 120 2.7 μm 色谱柱分析装饰或观赏鱼中的药物

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摘要

Agilent 1290 Infinity 液相色谱系统在高效液相色谱和超高效液相色谱中的应用中具有重要地位。与市场上其它系统相比，它的能力范围更广（如压力和流速不同组合的能力），并且可以使用具有不同规格和填料粒径的色谱柱。二极管阵列检测器先进的光学设计，使其具有宽的动态范围和高灵敏度，这在检测精细化学品中的微量杂质时非常重要。

这些优点可通过分析包括磺胺类药物、硝基咪唑类药物和孔雀石绿等鱼类用药的主成分及其相关杂质进行说明。很多药物都可用于治疗装饰或宠物鱼疾病，但其中许多药物都是禁用或受限制的。即使在食用鱼中有残留，其含量也应很低或者不能被高效液相色谱的紫外检测器检测到。本文展示了其中一些药物并详述了一种与 MS 兼容的快速分析分离方法。许多鱼类用药广告中宣传的“药品质量”成份，可能只是意味药品生产及其质量控制的步骤。当对这些产品进行分析时，我们期望产品中的相关杂质含量很低，符合药品质量生产的目标。

该系统具有高耐压性，可以使用甲醇、乙腈作为流动相，以比较两种溶剂的选择性。本文采用 3.0 mm \times 50 mm Poroshell 120 色谱柱，在 1 mL/min 流速下运行 3 min 的简单梯度，包括最后洗脱出人为添加的邻苯二甲酸酯的时间，共用不到 5 min 的时间即可完成所有的分离检测。图 1 为用乙腈和甲醇分离一种药粉中主成分的色谱图，图 2 则是分离含药饲料提取物中成分的色谱图。

通过优化溶剂种类、梯度程序和温度，使用 100 mm Poroshell 120 色谱柱成功分离了磺胺类药物标准混合物，进一步说明了采用该系统分析的快速、高分辨率和灵活性（见图 3）。

提高柱温，采用甲醇进一步优化分离磺胺类药物标准混合物的色谱条件，所有样品都采用最终的甲醇优化色谱条件进行分析，如图 4 所示。

配置

- 集成真空脱气机的 Agilent 1290 Infinity 二元泵 (G4220A)
- Agilent 1290 Infinity 自动进样器 (G4226A)
- Agilent 1290 Infinity 柱温箱 (G1316C)
- Agilent 1200 二极管阵列检测器 (G1315C)

结论

该系统具有灵活的溶剂和色谱柱选择性，并能耐高压，可以使用高柱效色谱柱实现高分辨率的快速分离，并且与 4.6 mm 内径的色谱柱相比节省溶剂。

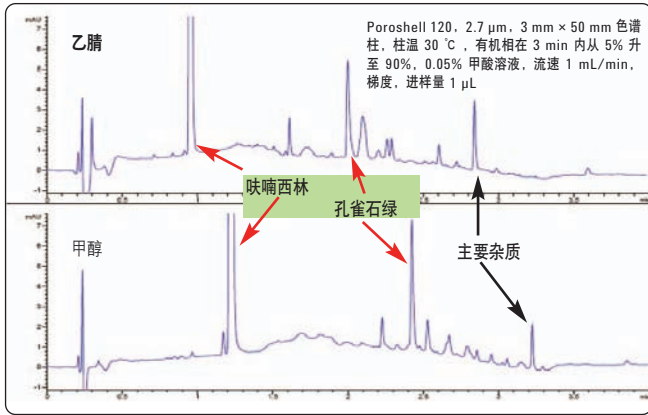


图 1
“Super Ick” medicated powder.

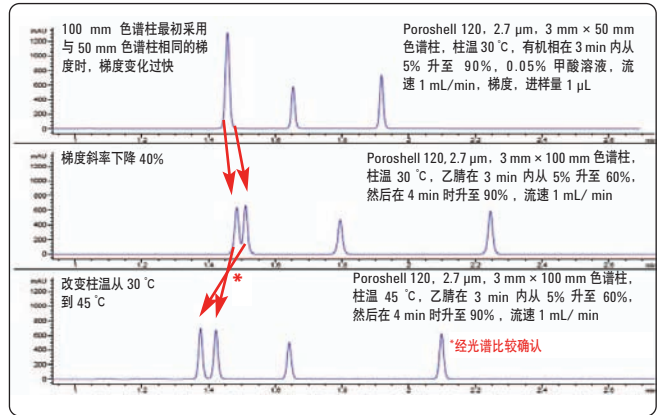


图 3
磺胺类药物标准混合物（安捷伦部件号 59987-20033）

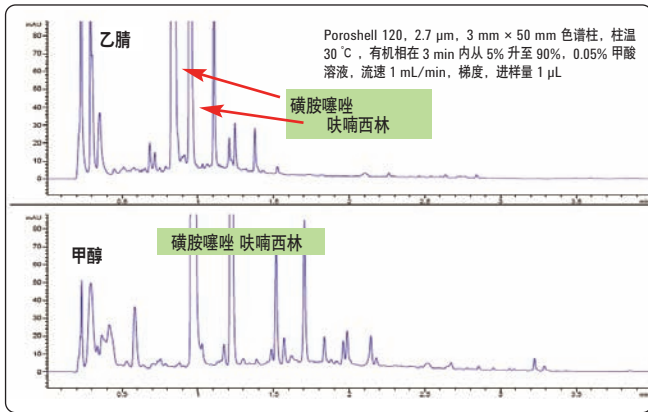


图 2
分离用甲醇/水/甲酸提取的鱼食 “Jungle” 中的药物

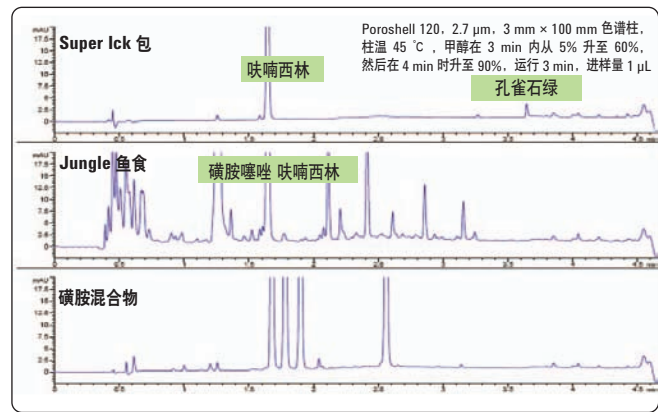


图 4
最终优化方法

使用 Agilent Poroshell 120 色谱柱快速分析现金上的违禁药物残留

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摘要

我们经常可以在美金上发现诸如可卡因等的违禁药物。虽然使用钞票吸毒的想法是可笑的，但真相却非常现实。通常认为贩毒是毒品残留于钞票的源头，且由于残留的毒品为细粉状，所以很容易从一张钞票的表面转移到另一张。由于钞票会经过点钞机和自动柜员机(ATM)处理，因此少量的毒品很容易在钞票间转移。在此之前安捷伦的一份应用报告（安捷伦出版物编号 5990-4254EN）详细阐述了使用配备 Agilent 6410 Triple Quadrupole LC/MS 检测器的 Agilent 1200 Series 液相色谱系统，筛查法医和毒理学分析中关注的 25 个化合物的方法。本实验使用 Agilent Poroshell 120 EC-C18 色谱柱对安捷伦 LC/MS 毒理学测试混合物(安捷伦部件号 5190-0470)中的 25 个化合物进行了分析。实验中采用更高流速，并相应调整甲酸铵/乙腈梯度程序进行洗脱，从而缩短分析时间，并充分利用了这种表面多孔颗粒色谱柱的低反压优势。本文对 25 个化合物分别绘制了校正曲线，并对 \$1 钞票甲醇提取物进行了定性和定量分析。

前言

由于表面多孔填料颗粒性能优越，因而我们尝试着将方法从较大的 5 μm 全多孔填料颗粒色谱柱和亚 2 μm 全多孔填料颗粒色谱柱向表面多孔填料颗粒色谱柱进行转移。由于表面多孔填料颗粒传质距离短、粒径分布较窄，故与亚 2 μm 全多孔颗粒色谱柱具有相似的高柱效。

从较大填料颗粒色谱柱向表面多孔填料颗粒色谱柱进行方法转移的优点之一是显著缩短了分析时间，这是由于短的表面多孔填料颗粒色谱柱能以较高流速（通常两倍）良好运行，并达到与较大填料颗粒长色谱柱相似的分辨率[1-2]。由于分析人员将方法从较大填料颗粒全多孔色谱柱转移到表面多孔填料颗粒色谱柱时，很可能会改变色谱柱柱长和流速，因此必须通过计算按比例调整梯度程序，同时保持选择性（公式 1）。

公式 1

$$t_2 = \frac{t_1 \cdot d_2^2 \cdot L_2 \cdot F_1}{d_1^2 \cdot L_1 \cdot F_2}$$

其中：

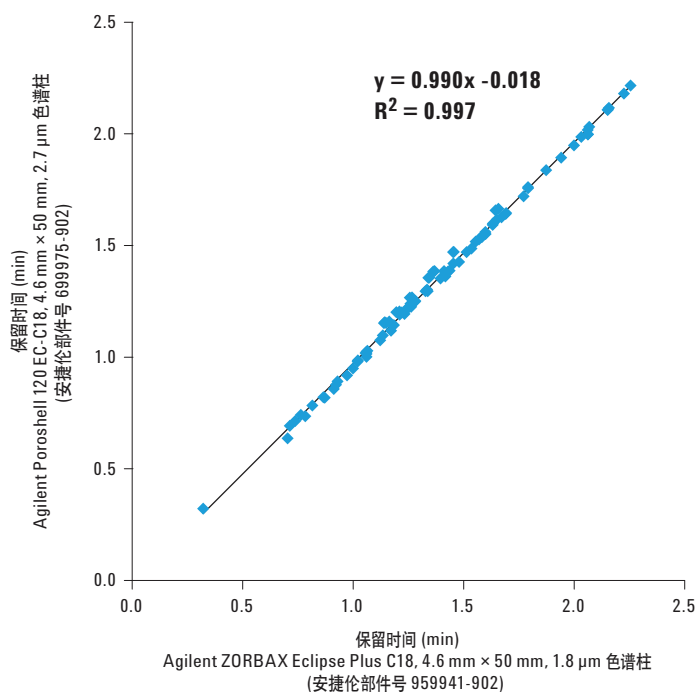
- t_1 和 t_2 分别是原来和调整后的梯度时间 (min)
- d_1 和 d_2 分别是原来和调整后的柱内径 (mm)
- L_1 和 L_2 分别是原来和调整后的柱长 (mm)
- F_1 和 F_2 分别是原来和调整后的流速 (mL/min)

在某些情况下，表面多孔填料颗粒色谱柱的更低反压会非常有用。根据不同的操作条件，表面多孔填料颗粒色谱柱的反压最多可减少 50%。分析人员可以充分利用这一优势增加流速提高分析通量，或在不超过系统压力上限的情况下增加柱长提高分辨率。调整流速和/或柱长需相应地调整梯度洗脱程序（公式 1）。

如果色谱柱填装了性能相似的化学键合相，并采用类似保留机理进行分析时，那么色谱柱间的方法转移将会非常简单，如表面多孔填料颗粒 Agilent Poroshell 120 EC-C18 色谱柱和全多孔填料颗粒 Agilent ZORBAX Eclipse Plus C18 色谱柱。图 1 显示了使用常规梯度分析不同类型的 90 个化合物时，Poroshell 120 EC-C18 色谱柱和 Eclipse Plus C18 色谱柱显示出相似的保留行为。高相关系数(R^2)表明在分离过程中，两种安捷伦 C18 色谱柱与组分间的相互作用机制极为类似，斜率约为 1 表明两种色谱柱与组分相互作用的强度类似[3-4]。当然，虽然使用这两种色谱柱进行分析时，很多组分显示出了相似的选择性，但这并不保证在每个应用中，两种 C18 色谱柱间均能够无需调整直接进行方法转移。

本应用报告显示了使用 Poroshell 120 色谱柱是如何进行复杂样品分析的，该分析之前所用色谱柱为 1.8 μm 色谱柱。在以前的安捷伦应用报告（出版物编号 5990-4254EN）中曾使用 Eclipse Plus 色谱柱对该样品进行了成功的分离[5]。使用含 25 个 LC/MS 毒理学组分测试混合物（安捷伦部件号 5190-0470）说明两种色谱柱的可互换性。使用 Poroshell 120 色谱柱对 25 个化合物分别绘制了校准曲线。是用甲醇对 S1 钞票进行提取，表明钞票表面显著残留了可卡因，以及大量的羟考酮、甲基安非他明、PCP 和 THC。几种痕量违禁和处方药也被检测出来。假设贩毒是毒品出现在美元现金上的源头，那么 ATM 和点钞机将会导致货币残留药物的传播[6]。此外，梯度分析方法被转移到 Poroshell 120 SB-C18 色谱柱上时，一些组分出现了选择性差异，然而 Poroshell 120 SB-C18 色谱柱可在较高温度下运行，从而可以使用更高的流速和更短的分析时间。Agilent Poroshell 120 色谱柱提供两种不同的 C18 固定相，在改变选择性的同时还能保持 C18 色谱柱的性能。升高 Agilent Poroshell 120 色谱柱的流速使压力升至 400 和 600 bar，以用于充分展示传统 HPLC 和新型 UHPLC 的性能。

Agilent Poroshell 120 EC-C18 色谱柱具有与 Agilent ZORBAX Eclipse Plus C18 色谱柱非常类似的选择性



流动相: A: 10 mM 甲酸铵溶液, pH 3
B: 乙腈

梯度程序: t_0 时 B 为 5%, 在 2 min 内升至 95%,
后保持 1 min

流速: 2 mL/min

样品: 1 μL 浓度 1 mg/mL 标准品水溶液

咪喃唑酮	联苯	乙酰苯胺	DL-苯丙氨酸	盐酸奥昔布宁 1
氯霉素	萘	非诺洛芬	盐酸多塞平	苯海拉明
乙胺嘧啶	甲氧基萘	邻苯二酚	盐酸麻黄碱	二氟尼柳
磺胺噻唑啉	苯甲醚	苯酚	洛哌丁胺	尼索地平
磺胺间甲氧嘧啶	二甲氧基苯	间苯二酚	盐酸普鲁卡因	双氯芬酸钠盐
尼莫地平	皮质酮	对苯二酚	非诺洛芬钙盐水合物	氢化可的松
磺胺二甲氧嘧啶	α -羟孕酮	4-硝基苯酚	红霉素	4-羟基苯甲酸
磺胺甲噁唑	黄体酮	邻甲基苯酚	硝酸益康唑	盐酸普鲁卡因胺
磺胺氯哒嗪	α -羟孕酮	对甲基苯酚	吉非罗齐	利多卡因
磺胺甲氧嗪	泼尼松龙	3,4-二甲基苯酚	β -雌二醇	特非那定
磺胺甲噻二唑	美雌醇	2,3-二甲基苯酚	美托洛尔	特非那定
磺胺二甲嘧啶	去氧皮质酮	2-硝基苯酚	泼尼松	盐酸金霉素
磺胺甲嘧啶	黄体酮	2,4-二甲基苯酚	普罗替林	马来酸氯苯那敏
磺胺噻唑	马来酸氯苯吡胺	2,5-二甲基苯酚	2-羟基马尿酸	氯霉素
磺胺嘧啶	小檗碱	1-萘酚	5-羟基间苯二甲酸	盐酸丁螺环酮
苯甲醛	英普咪定	盐酸丙米嗪	氟灭酸	苯佐卡因
碘苯	炔诺酮	d-蛋氨酸	盐酸普莫卡因	安替比林
菲	非那西丁	3,4-二羟基-1-苯丙氨酸	萘普生	乙酰水杨酸

图 1. 使用 Agilent Poroshell 120 EC-C18 色谱柱与 Agilent Eclipse Plus C18 色谱柱分离 90 种化合物的保留时间散点图

实验部分

本文采用配备 Agilent 6410 Triple Quadrupole LC/MS 的 Agilent 1200 Series 快速分离高分离度液相色谱(RRLC)系统:

- G1312B SL 型二元泵, 流动相 A: 含有 0.01% 甲酸的 5 mM 甲酸铵溶液, 流动相 B: 含有 0.01% 甲酸的乙腈。梯度设置: t_0 时 B 浓度为 10%, 后升至 15%, 接着升至 50%, 最后再升至 95% 并保持。梯度程序视色谱柱规格和流速而定 (表 1)
- G1367C SL 型自动液体进样器(ALS), 进样量 1.0 μ L
- G1316B SL 型柱温箱(TCC), 温度设置为 60 °C 或 90 °C (仅限于 Poroshell 120 SB-C18 色谱柱)
- G6410A Triple Quadrupole LC/MS: 电喷雾 AP-ESI, 干燥气温度和流速: 350 °C, 12 L/min, 雾化气压力: 30 psi, 毛细管电压: 2000 V, dMRM 模式, 离子转换如表 2 所示
- MassHunter 软件版本 B.02.01、B.02.00 和 B.03.01 分别用于数据采集、定性分析和定量分析

本文使用了三种安捷伦色谱柱:

- Agilent Poroshell 120 EC-C18, 2.1 mm \times 100 mm, 2.7 μ m 色谱柱 (部件号 695775-902)
- Agilent Poroshell 120 SB-C18, 2.1 mm \times 100 mm, 2.7 μ m 色谱柱 (部件号 685775-902)
- Agilent ZORBAX RRHT Eclipse Plus C18, 2.1 mm \times 100 mm, 1.8 μ m 色谱柱 (部件号 p/n 959764-902)

表 2 展示了采用 Poroshell 120 EC-C18 色谱柱、0.5 mL/min 流速分析目标化合物的保留时间和定性、定量 MRM 离子传输数据。标准溶液为 1 μ g/mL 的标准品甲醇溶液, 购于安捷伦科技公司 (LC/MS 毒理学测试混合物, 安捷伦部件号 5190-0470)。用甲醇和标准溶液稀释制备系列校准溶液。将 \$1 钞票样品浸于 7 mL 甲醇中, 超声 30 min 以制备样品溶液。另外, 乙腈、甲酸和甲酸铵购自 Sigma Aldrich 公司 (Bellefont, PA), 甲醇购自 Honeywell、Burdick and Jackson 公司 (Muskegon, MI)。实验用水为 18 M- Ω Milli-Q 水 (Bedford, MA)。

表 1. 不同色谱柱和实验条件下的高效液相色谱方法参数

梯度和方法参数	2.1 \times 100 mm 1.8- μ m Agilent ZORBAX Eclipse Plus C18	2.1 \times 100 mm 2.7- μ m Agilent Poroshell 120 EC-C18	2.1 \times 100 mm 2.7- μ m Agilent Poroshell 120 EC-C18	2.1 \times 100 mm 2.7- μ m Agilent Poroshell 120 EC-C18	2.1 \times 100 mm 2.7- μ m Agilent Poroshell 120 SB-C18	2.1 \times 100 mm 2.7- μ m Agilent Poroshell 120 SB-C18	2.1 \times 100 mm 2.7- μ m Agilent Poroshell 120 SB-C18
流速 (mL/min)	0.5	0.5	0.7	1.0	0.5	0.9	1.4
10% B (min)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
15% B (min)	0.50	0.50	0.36	0.25	0.50	0.28	0.18
50% B (min)	3.00	3.00	2.14	1.50	3.00	1.67	1.07
95% B (min)	4.00	4.00	2.86	2.00	4.00	2.22	1.43
95% B (min)	6.00	6.00	4.29	3.00	6.00	3.33	2.14
停止时间 (min)	6.00	6.00	4.29	3.00	6.00	3.33	2.14
后运行时间 (min)	2.00	2.00	1.43	1.00	2.00	1.11	0.71
总循环时间 (min)	8.00	8.00	5.71	4.00	8.00	4.44	2.86
TCC 温度 (°C)	60	60	60	60	90	90	90
进样量 (μ L)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
系统压力 (bar)	375	280	385	550	195	370	595

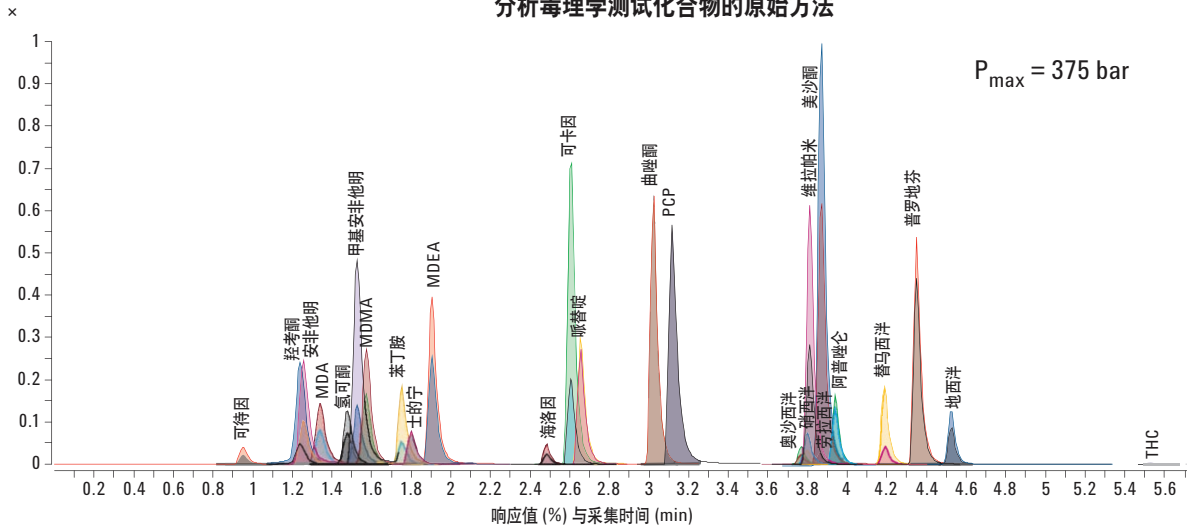
表 2. 25 个毒理学测试混合物组分的 MRM transition

化合物名称	母离子	碎裂电压	子离子 1	碰撞能量 1	子离子 2	碰撞能量 2	保留时间 (min)	Δ保留时间
可待因	300.2	158	165.1	45	58.1	29	0.89	0.4
羟考酮	316.2	143	298.1	17	256.1	25	1.14	0.4
安非他明	136.1	66	119.1	5	91	17	1.19	0.4
MDA	180.1	61	163	5	105	21	1.25	0.4
氢可酮	300.2	159	199	29	128	65	1.34	0.4
甲基安非他明	150.1	92	119	5	91	17	1.43	0.4
MDMA	194.1	97	163	9	105	25	1.46	0.4
士的宁	335.2	195	184	41	156	53	1.66	0.4
苯丁胺	150	66	133	5	91	25	1.66	0.4
MDEA	208.1	107	163	9	105	25	1.8	0.4
海洛因	370.2	149	268.1	37	165	61	2.4	0.4
可卡因	304.2	138	182.1	17	77	61	2.52	0.4
哌替啶	248.2	128	220.1	21	174.1	17	2.59	0.4
曲唑酮	372.2	159	176	25	148	37	2.95	0.4
PCP	244.2	86	91	41	86.1	9	3.05	0.4
奥沙西洋	287	150	269	12	241	20	3.66	0.4
硝西洋	282.1	148	236.1	25	180	41	3.66	0.4
维拉帕米	455.3	158	165	37	150	45	3.75	0.4
劳拉西洋	321	102	275	21	194	49	3.75	0.4
美沙酮	310.2	112	265.1	9	105	29	3.83	0.4
阿普唑仑	309.1	179	281	25	205	49	3.84	0.4
替马西洋	301.1	117	255.1	29	177	45	4.05	0.4
普罗地芬	354.2	153	167	29	91.1	45	4.33	0.4
地西洋	285.1	169	193	45	154	25	4.41	0.4
THC	315.2	150	193.2	20	123.3	30	5.4	0.4

结果与讨论

图 2 展示了 P. Stone 开发的采用 Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 μm 色谱柱的初始分析方法, 该分析在 6 min 内完成, 后运行 2 min, 系统压力 375 bar。图 3 展示了采用 Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 μm 色谱柱进行同一分析时, 具有与 Eclipse Plus 色谱柱相同的分析时间和后运行时间, 而反压却降至 280 bar。虽然图 2 和图 3 中两种色谱柱的洗脱模式存在轻微差别, 但总体选择性如图 1 所预测的那样, 极为相似。

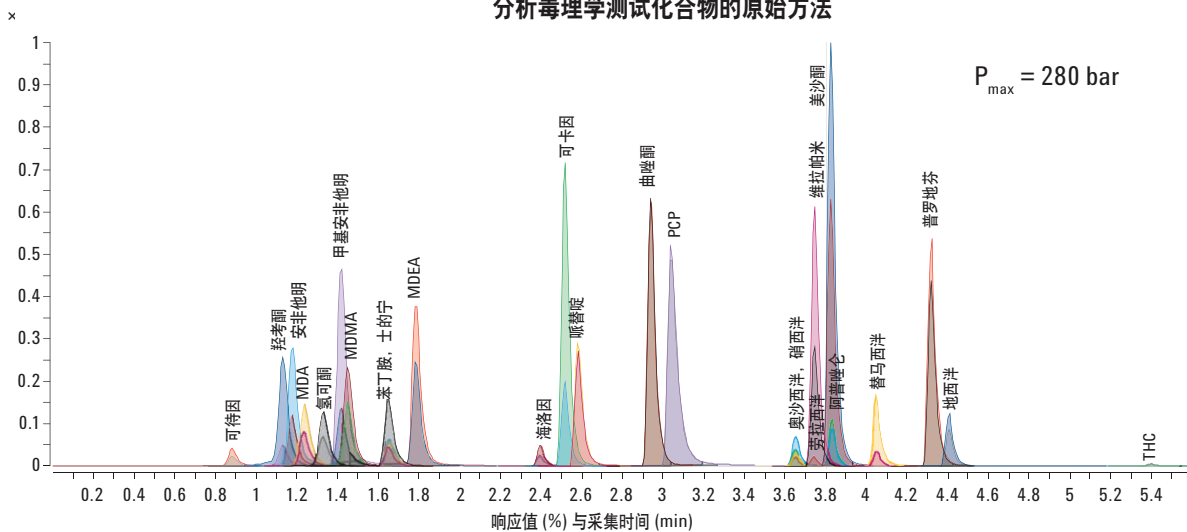
采用 Agilent ZORBAX Eclipse Plus C18, 2.1 mm × 100 mm, 1.8 μm 色谱柱 (安捷伦部件号 959764-902)
分析毒理学测试化合物的原始方法



A: 含有 0.01% 甲酸的 5 mM 甲酸铵溶液 (1 L 水 + 0.3153 g 甲酸铵 + 0.1 mL 甲酸)
 B: 含有 0.01% 甲酸的乙腈溶液 (1 L 乙腈 + 0.1 mL 甲酸); 0.5 mL/min; t_0 时 B 为 10%, 后在 0.5 min 内升至 15%, 接着在 2.5 min 内升至 50%, 然后在 1 min 内升至 95%, 最后保持 2 min; 第 6 min 时停止运行, 后运行 2 min;
 样品: 进样程序: 抽取 5 μL 水, 抽取 1 μL LC/MS 毒理学测试混合物 (部件号 5190-0470) 标准品溶液; 进样: TCC=60 °C;
 MS 离子源: 电喷雾 AP-ESI, 干燥气温度和流速: 350 °C, 12 L/min, 雾化器气压: 30 psi, 毛细管电压: 2000V;
 MS 采集: 动态 MRM (MRM 离子传输通道如表 2 所示), 正电子模式。

图 2. 采用 Agilent ZORBAX Eclipse Plus C18 色谱柱, 在配备 Agilent 6410 Triple Quadrupole LC/MS 的 Agilent 1200 Series 液相色谱系统上分析安捷伦 LC/MS 毒理学测试混合物谱图 (安捷伦部件号 5190-0470)

采用 Agilent Poroshell 120 EC-C18 2.1 mm × 100 mm, 2.7 μm 色谱柱 (安捷伦部件号 695775-902)
分析毒理学测试化合物的原始方法



A: 含有 0.01% 甲酸的 5 mM 甲酸铵溶液 (1 L 水 + 0.3153 g 甲酸铵 + 0.1 mL 甲酸)
 B: 含有 0.01% 甲酸的乙腈溶液 (1 L 乙腈 + 0.1 mL 甲酸); 0.5 mL/min; t_0 时 B 为 10%, 后在 0.5 min 内升至 15%, 接着在 2.5 min 内升至 50%, 然后在 1 min 内升至 95%, 最后保持 2 min; 第 6 min 时停止运行, 后运行 2 min;
 样品: 进样程序: 抽取 5 μL 水, 抽取 1 μL LC/MS 毒理学测试混合物 (部件号 5190-0470) 标准品溶液; 进样: TCC=60 °C;
 MS 离子源: 电喷雾 AP-ESI, 干燥气温度和流速: 350 °C, 12 L/min, 雾化器气压: 30 psi, 毛细管电压: 2000V;
 MS 采集: 动态 MRM (MRM 离子传输通道如表 2 所示), 正电子模式。

图 3. 采用 Agilent Poroshell 120 EC-C18 色谱柱, 在配备 Agilent 6410 Triple Quadrupole LC/MS 的 Agilent 1200 Series LC 系统上分析安捷伦 LC/MS 毒理学测试混合物 (安捷伦部件号 5190-0470)

表 3 展示了采用 Poroshell 120 色谱柱分析安捷伦 LC/MS 毒理学测试混合物中 25 个化合物的校准数据。所有化合物均显示出良好的线性相关性, $R^2 > 0.9979$, 校准数据用于定量测定美元 \$1 钞票的甲醇提取物。色谱分析和定量测定结果如图 4 所示, 在美元钞票

上检测出相当数量的可卡因。羟考酮、甲基安非他明、PCP 和 THC 也被检测出来。此外, 钞票上也检测到少量的安非他明、氢可酮、MDMA、海洛因、美沙酮和地西洋。美元现金上检测出物质的量与之前的测定结果一致[6-8]。

表 3. 使用 Poroshell 120 色谱柱测定 25 个毒理学测试化合物的校准数据

化合物名称	线性校准曲线	相关系数, R^2
可待因	$y = 25.4023x + 3.1628$	0.99990276
羟考酮	$y = 138.9535x - 0.6269$	0.99938632
安非他明	$y = 196.3425x + 50.1606$	0.99987385
MDA	$y = 121.2945x + 180.2165$	0.99945701
氢可酮	$y = 72.1351x - 8.1010$	0.99964622
甲基安非他明	$y = 286.7936x + 429.4970$	0.99789141
MDMA	$y = 121.4217x - 55.0435$	0.99874569
苯丁胺	$y = 110.8083x - 65.1028$	0.99914972
土的宁	$y = 39.3465x - 9.5339$	0.99964358
MDEA	$y = 200.4804x - 14.2886$	0.99980092
海洛因	$y = 18.2969x + 0.4442$	0.99987634
可卡因	$y = 295.8654x - 5.6261$	0.99963342
哌替啶	$y = 145.0367x + 17.2273$	0.99986118
曲唑酮	$y = 286.1986x - 12.4408$	0.99969366
PCP	$y = 287.4395x - 24.8090$	0.99989199
奥沙西洋	$y = 14.7883x - 0.4919$	0.99900677
硝西洋	$y = 49.1750x + 69.2747$	0.99876656
维拉帕米	$y = 273.3001x + 17.3890$	0.99986678
劳拉西洋	$y = 11.2911x + 6.0687$	0.99896851
美沙酮	$y = 439.7238x - 6.7890$	0.9997511
阿普唑仑	$y = 80.2721x + 18.5435$	0.99969734
替马西洋	$y = 70.9899x + 15.5246$	0.99976598
普罗地芬	$y = 243.9474x - 13.0696$	0.99990655
地西洋	$y = 68.9622x + 26.0608$	0.99948978
THC	$y = 3.1838x - 2.7072$	0.99801611

对从一张美元 \$1 钞票中提取出的羟考酮、安非他明、氢可酮、甲基安非他明、MDMA、海洛因、可卡因、PCP、美沙酮、地西洋和 THC 进行了定量测定

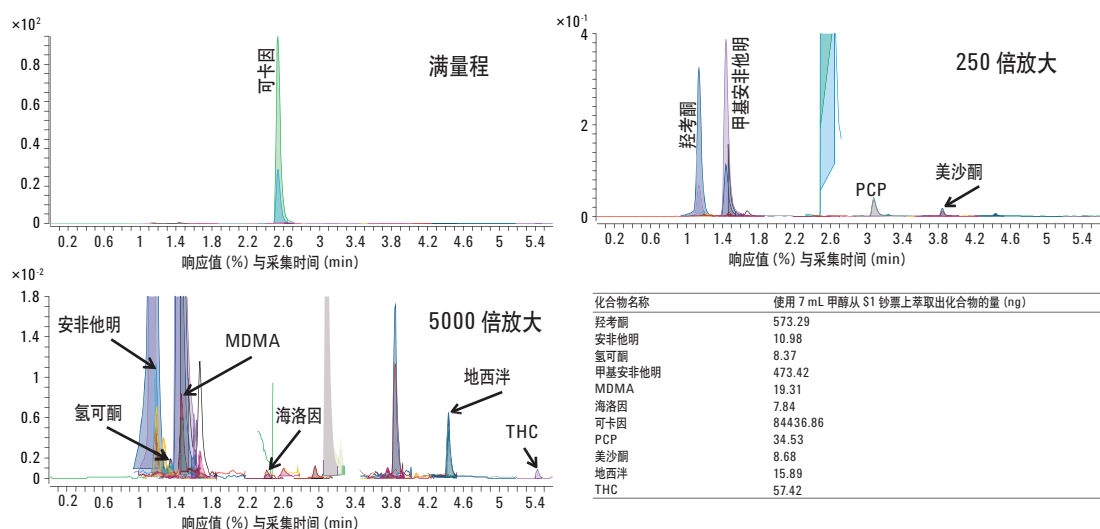


图 4. 使用 7 mL 甲醇、超声 30 min 提取一张随机抽取的美元 \$1 钞票样品得到的色谱分析和定量测定结果

如图 5 所示，基于 Poroshell 120 色谱柱的低反压特性，在不超过 400 bar 的标准高校液相色谱系统压力条件下，流速可从 0.5 mL/min 增至 0.7 mL/min，或者在不超过 600 bar 的超高液相色谱系统压力条件下，流速可提升至 1 mL/min。当十分需要高通量分析，并且具备超高液相色谱分析条件时，流速增加将非常有利。在系统压力低于 400 bar 时，总运行时间能减少 2.3 min，或者在系统压力低于 600 bar 时，总运行时间可减少 4 min（缩短 50% 运行时间）。当然，流速的增加将使得分辨率有所降低，但使用 MS 检测器能够弥补分辨率上的损失。

采用 Agilent Poroshell 120 EC-C18 色谱柱，提高流速，无论系统压力限制是 400 还是 600 bar，都能显著节省分析时间

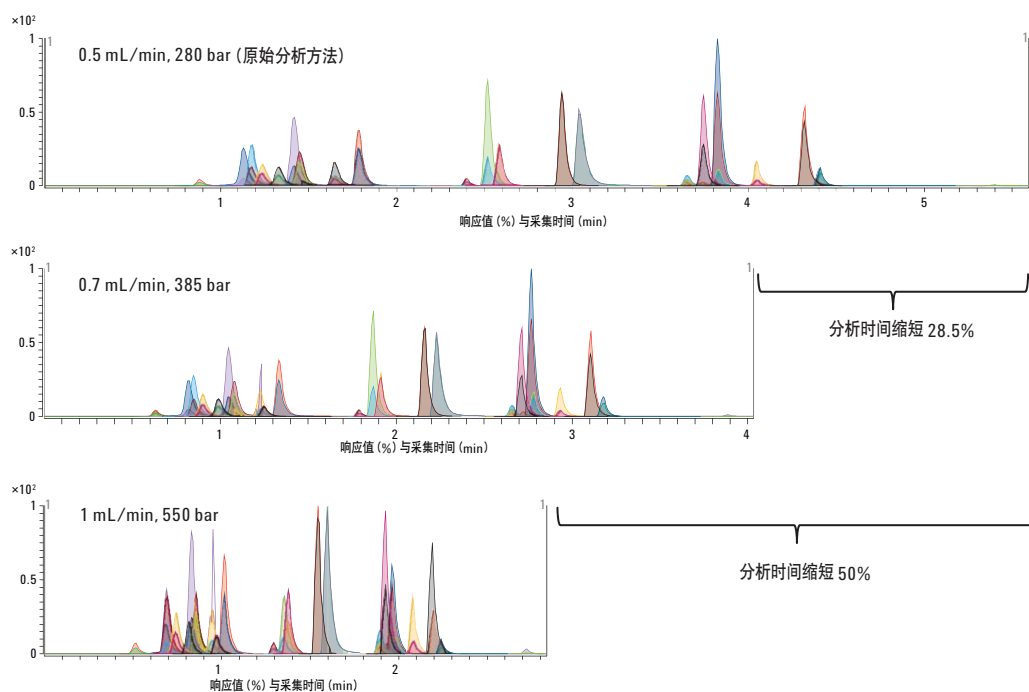


图 5. 采用 Agilent Poroshell 120 EC-C18 色谱柱，提高流速使压力增至 400 或 600 bar 的系统耐受压，缩短了分析毒理学测试化合物的时间

提高温度可以降低流动相的黏度，可使流速进一步增加。原始分析方法的运行温度为 60 °C，是 Eclipse Plus C18 色谱柱和 Poroshell 120 EC-C18 色谱柱的最高运行温度。为了使该分析在更高的温度条件下运行，必须改用 Poroshell 120 SB-C18 色谱柱，其最高运行温度为 90 °C。图 6 展示了采用 Poroshell 120 SB-C18 色谱柱的快速色谱分析。采用 600 bar 的系统耐压运行，可缩短 64.3% 的分析时间，然而这会降低分辨率。对于毒理学测试化合

物等复杂样品分析而言，分辨率损失和化合物严重共洗脱将使得分析总峰数据点减少，从而降低了分析结果的质量。一种简单的解决方案就是增加柱长，从 100 mm 到 150 mm 柱长的轻增加将提高所有组分的分辨率。虽然较长色谱柱不能在很高流速下运行，但分析人员还是可以在不超过系统耐受压力的前提下，运行色谱柱的最高流速缩短分析时间。

使用 Agilent Poroshell 120 EC-C18 色谱柱，提高温度和增加流速使压力增至 400 或 600 bar 的液相色谱系统压力上限，显著缩短了分析时间

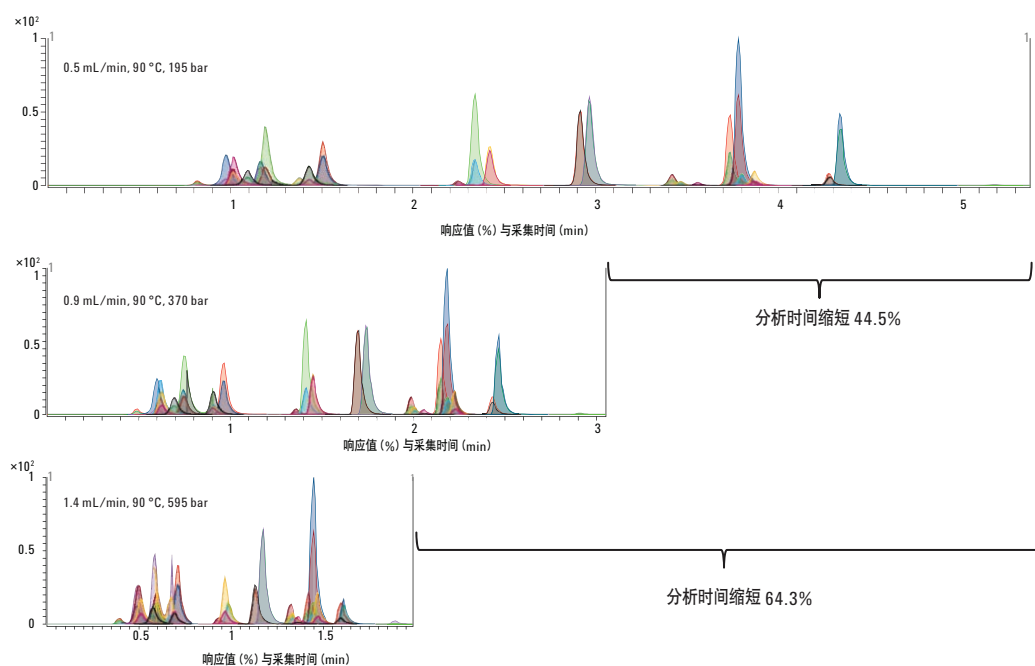


图 6. 采用 Agilent Poroshell 120 EC-C18 色谱柱，通过提高温度和增加流速使压力增至 400 或 600 bar 的系统压力上限，缩短了分析毒理学测试化合物的分析时间

结论

可轻松地将使用 Agilent ZORBAX Eclipse Plus C18 色谱柱分析 25 个毒理学化合物的复杂分析方法转移至表面多孔颗粒填料的 Agilent Poroshell 120 EC-C18 色谱柱, 获得高质量的分析结果, 且能节省大量分析时间。由于 1.8 μm Eclipse Plus C18 色谱柱和 Poroshell 120 EC-C18 色谱柱具有相似的选择性和柱效, 因此, 对于其它复杂样品分析, 无需方法调整即可实现从 1.8 μm Eclipse Plus C18 色谱柱向相同规格 Poroshell 120 EC-C18 色谱柱的转移。Poroshell 120, 2.7 μm 颗粒色谱柱的低反压特性可提高实验效率, 它可在不超过高效液相色谱 400 bar 或超高压液相色谱更高压力上限的条件下, 使用更高流速以缩短分析时间。本分析方法可用于检测和定量测定 \$1 钞票上残留的几种滥用药物, 其中包括可卡因、羟考酮、甲基安非他明、PCP 和 THC。

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将传统方法转移成使用安捷伦 Poroshell 120 色谱柱的快速方法进行人参分析

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摘要

本文实验将使用 4.6×150 mm, $5 \mu\text{m}$ 色谱柱进行人参分析的传统方法转移到了 Agilent Poroshell 120 EC-C18, $3.0 \text{ mm} \times 100 \text{ mm}$, $2.7 \mu\text{m}$ 色谱柱上。在线速度相同的情况下, 所需梯度时间从 100 多分钟降至大约 70 分钟, 如果提高流速, 还可以将分析时间进一步降至 25 分钟以下。在本示例中, 高流速条件下压力超过 400 bar, 因此需要使用 600 bar 的仪器。如果采用 Agilent Poroshell 120 EC-C18 或 Poroshell 120 SB-C18 $3.0 \text{ mm} \times 50 \text{ mm}$ 色谱柱, 梯度时间还可进一步缩短。最短的时间为 11 分钟, 对关键化合物 (人参皂苷 Rg1 和 Re) 的分离度为 1.57。另外, 由于色谱柱使用 $2 \mu\text{m}$ 滤头, 因此, 在样品制备过程中新方法不需要更改。压力仍低于 400 bar, 因此该方法可以在任何 HPLC 仪器上运行。

前言

亚洲人参原产于中国和韩国，数个世纪以来应用于医学的不同系统中。关于人参的治疗报道不计其数，这种药材用于保健和增强免疫系统。人参的传统方法和现代应用包括：增强大病初愈者的健康，增加健康以及耐力，改善精神和身体机能，治疗勃起障碍，丙型肝炎，以及妇女绝经综合症；降低血糖，并且控制血压。亚洲人参的根含有的活性化学物质名为人参皂苷，是人参药用价值的所在。[1]

在中国药典中，采用高效液相色谱方法测定人参中三种主要人参皂苷 Rg1, Re 和 Rb1 的含量 [2]。最初的方法使用传统的 4.6 mm × 150 mm, 5 μm 色谱柱，使用长达 100 分钟的梯度洗脱。因此，有必要建立一种快速的分析方法以增加分析通量并降低实验成本。

在本研究中，将传统方法转移到安捷伦 Poroshell 120 EC-C18 和 Poroshell 120 SB-C18 色谱柱上。安捷伦 Poroshell 120 2.7 μm 色谱柱使用表面多孔材料填充，使其能够实现快速分离，且性能类似于亚 2 μm 全多孔材料。这种方法根据色谱柱的长度、内径以及样品大小进行比例转换。在较短的色谱柱上，仍然能保持原来的线速度。

实验部分

1200 系列 SL 型液相色谱系统包括：二元泵，柱温箱 (TCC)，高性能自动进样器以及二级管阵列检测器 (DAD)。

本文所用色谱柱包括：

- 安捷伦 ZORBAX StableBond C18, 4.6 mm × 150 mm, 5 μm 色谱柱 (部件号: 883975-902)
- 安捷伦 ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 μm 色谱柱 (部件号: 959993-902)

- 安捷伦 Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 μm 色谱柱 (部件号: 695975-302)
- 安捷伦 Poroshell 120 EC-C18, 3.0 mm × 50 mm, 2.7 μm 色谱柱 (部件号: 699975-302)
- 安捷伦 Poroshell 120 SB-C18, 3.0 mm × 50 mm, 2.7 μm 色谱柱 (部件号: 689975-302)

人参样品采用下列样品制备步骤：

1. 称量 1.0 g 干粉。
2. 用 50 mL 乙酸乙酯在索氏提取器中脱脂 3 小时。
3. 冷却，过滤，弃掉液相，之后，将残留物干燥。
4. 用滤纸将残留物移入 25 mL 烧瓶。加入 20 mL 水饱和的正丁醇，超声萃取 30 分钟。
5. 过滤萃取液，收集 10 mL 正丁醇液。
6. 水浴蒸发正丁醇，残留物加入 2 mL 甲醇溶解。
7. 用 0.45 μm 再生纤维素滤膜 (部件号: 5064-8221) 过滤，之后进行高效液相色谱分析。

结果和讨论

将分离人参皂苷 Rg1, Re 和 Rb1 的传统方法在 Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 μm 色谱柱和 Agilent ZORBAX SB-C18, 4.6 mm × 150 mm, 5 μm 色谱柱上进行重复。人参皂苷 Rg1 在这两根色谱柱上的理论塔板数都大于 9000，分离度在 2.0 左右，足够分离三种人参皂苷了。单次运行时间超过 100 分钟，见图 1 所示。

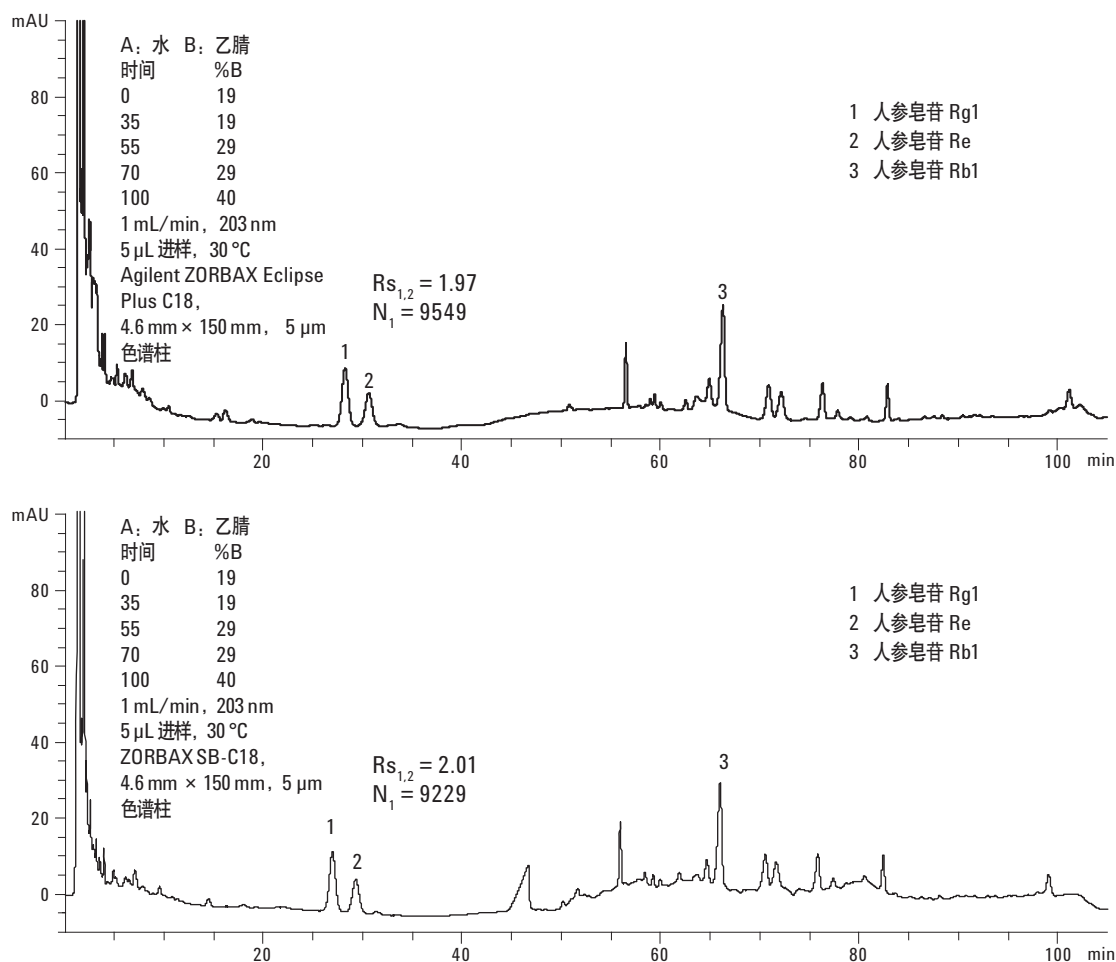


图 1. 在安捷伦 ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 μm 色谱柱和安捷伦 ZORBAX SB-C18, 4.6 mm × 150 mm, 5 μm 色谱柱上运行人参的传统分析方法

随后, 将传统方法转移到安捷伦 Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 μm 色谱柱上。根据下面的方程调整流速以保持相同的线速度。

$$\text{方程 1: } F_1/(r_1)^2 = F_2/(r_2)^2$$

其中

F_1 是传统色谱柱的流速

F_2 是新色谱柱的流速

r_1 是传统色谱柱的半径

r_2 是新色谱柱的半径

如需保持分离度不变, 梯度洗脱时间与色谱柱长度需成比例变化。对比传统的 4.6 mm × 150 mm 色谱柱, 在相同的线速度下使用 3.0 mm × 100 mm 色谱柱, 梯度洗脱时间将会比原来减少三分之一。进样体积适当减少避免样品量过载。所以分析时间由原来的 100 分钟减少到 66.7 分钟, 如图 2 所示。174 bar 的反压对于一台 400 bar 的高效液相色谱仪来说是可以接受的。该色谱图中显示的分度数和理论塔板数比传统方法更高。

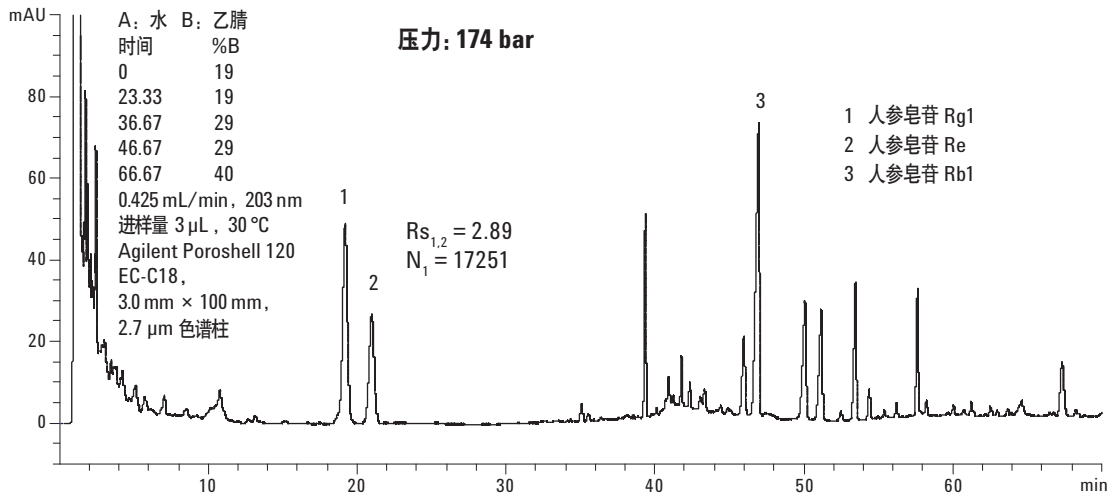


图 2. 采用安捷伦 Poroshell 120 EC-C18, 3.0 mm \times 100 mm, 2.7 μ m 色谱柱在常规流速下的色谱图

新方法比传统方法节省了三分之一的分析时间，且性能有了大幅改进。通过增加流速并减少梯度洗脱时间可以进一步缩短分析时间。使用同一根色谱柱时，梯度洗脱时间是和流速是成正比的。因此，当新方法中使用的流速是原来的二倍时（从 0.425 mL/min 增加到 0.85 mL/min），分析时间就会减少一半。流速是原来的三倍时（图 3）分析周期将会进一步缩短，但是分离度和理论塔板数会有所损失，但即使是这样分离度和理论塔板数仍然比传统色谱柱要好。这可能是由于装填表面多孔 2.7 μ m 填料颗粒

Poroshell 120 色谱柱的范德华曲线类似于装填 1.8 μ m 颗粒色谱柱的原因。不同于装填 1.8 μ m 填料粒径的色谱柱，Poroshell 色谱柱的柱效在高流速下并不会降低。流速是原来流速 3 倍的时候，压力也是原来压力 174 bar 的大概 3 倍，就是大约 485 bar。这个压力对于耐受 600 bar 压力的色谱柱是可以接受的，但是这就需要耐压至少 600 bar 的高效液相色谱仪或超高液相色谱仪了，比如安捷伦 1260 Infinity 液相色谱系统。

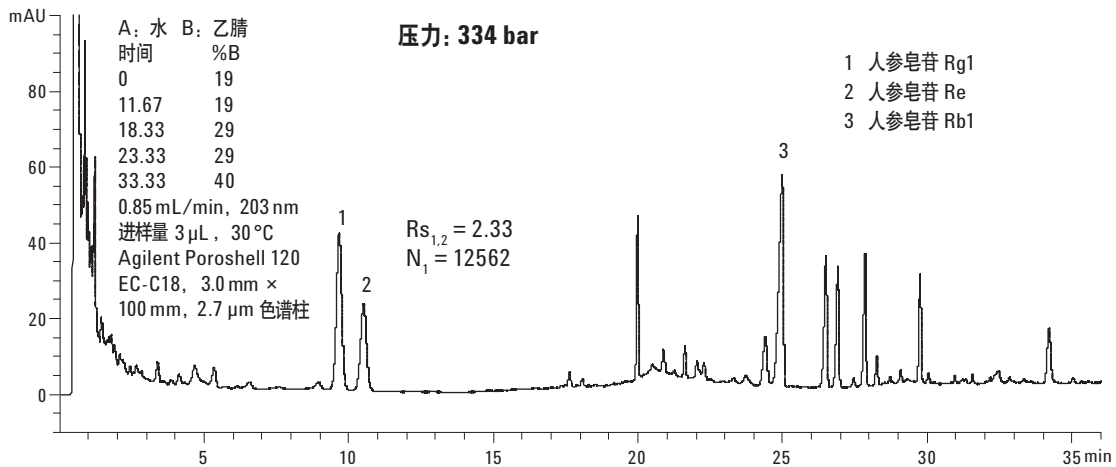


图 3. 采用 Agilent Poroshell 120 EC-C18, 3.0 mm \times 100 mm, 2.7 μ m 色谱柱在较高流速下的色谱图 (接转下页)

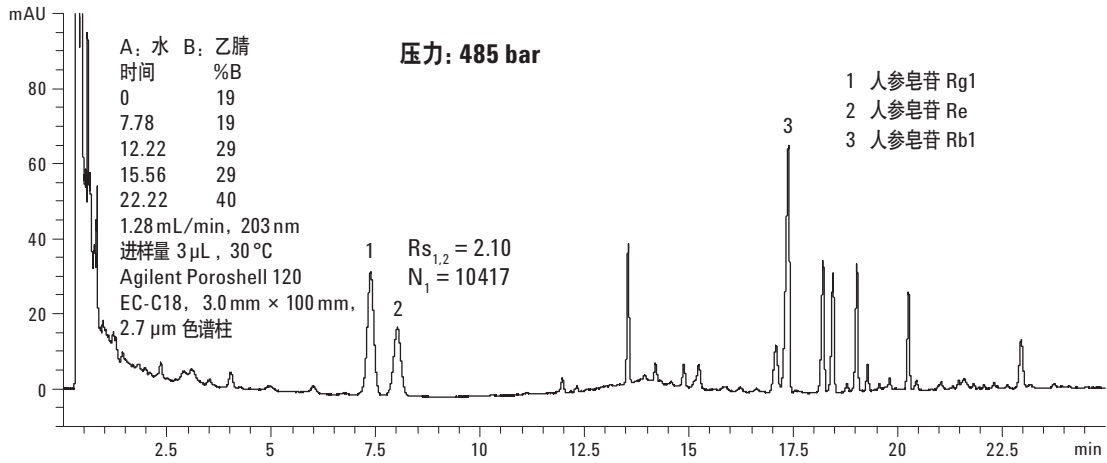


图 3. 采用 Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 μm 色谱柱在更高流速下分析的色谱图

从不同的流速下的重叠色谱图 (图 4) 中可以看出, 采用 Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 μm 色谱柱可以大大节省分析时间。

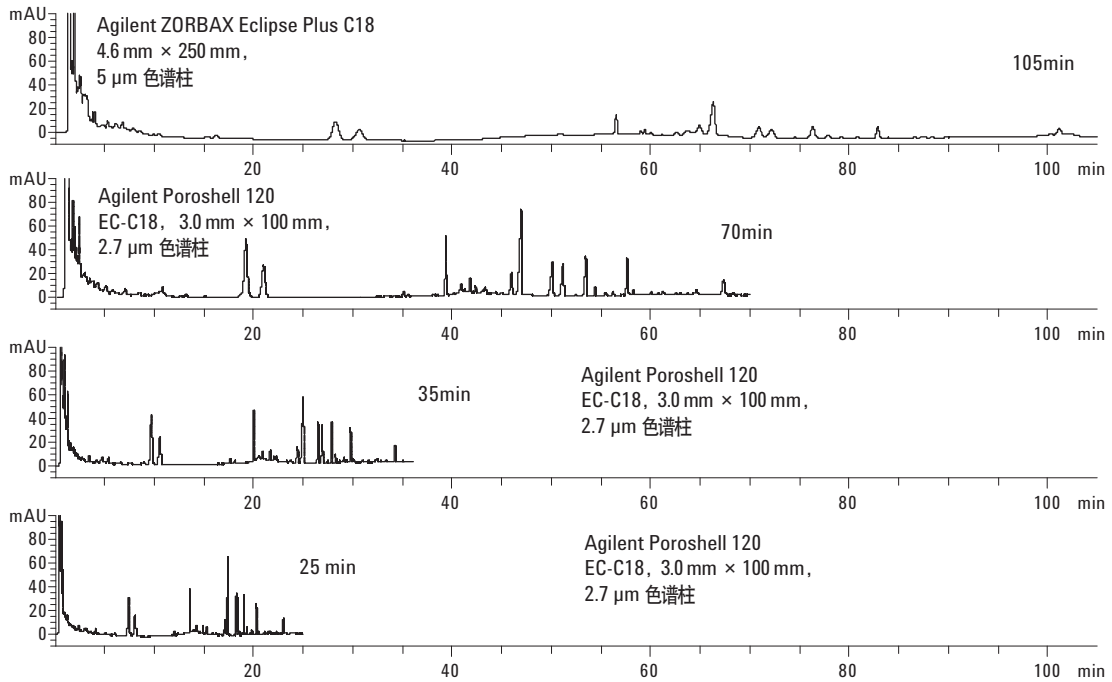


图 4. 采用 Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 μm 色谱柱和 Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 μm 色谱柱在不同流速下的重叠色谱图

基于 Agilent Poroshell 120, 3.0 mm × 100 mm 色谱柱开发的新方法比原方法节约了四分之三 (75%) 的分析时间, 如果采用较短的色谱柱分离时间会进一步缩短。随后, 将这个方法转移到安捷伦 Poroshell 120, 3.0 mm × 50 mm 色谱柱上。分离需要 23 分钟, 人参皂苷 Rs 与 人参皂苷 Rg1, Re 的分离度接近 2.0。当流速加倍, 完成分离仅需 11 分钟 (是原来时间的十分之一), 虽然分离度和性能会有所降低但还是都还可以用于定量分析的。

重要的是, 此时压力只有大约 200 bar, 这远远低于 400 bar 仪器的压力上限。理论上来说, 可以使用 400 bar 仪器运行这一快速分离方法, 但是, 由于延迟体积和柱后体积, 应该优化仪器, 调整高效液相色谱条件, 以获得最理想的分离效果。

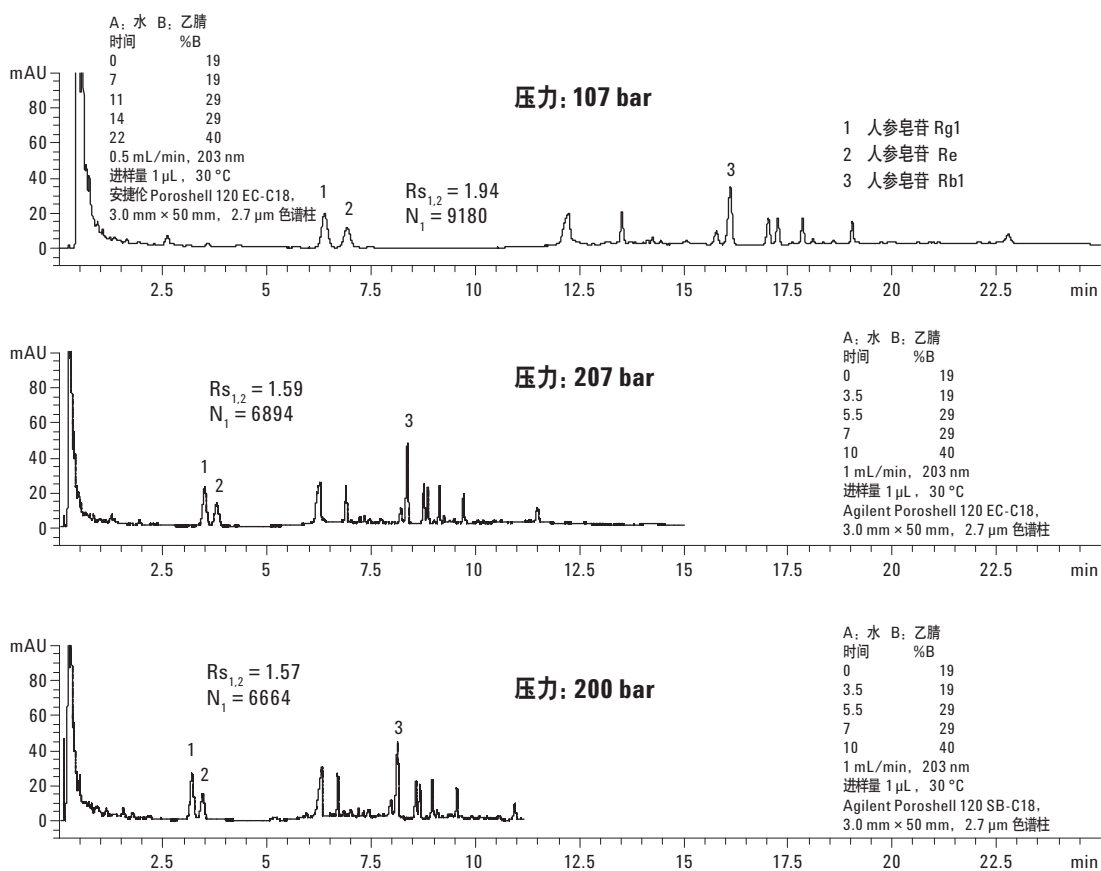


图 5. 采用 Agilent Poroshell 120 EC-C18, 3.0 mm × 50 mm, 2.7 μ m 色谱柱和 Agilent Poroshell 120 SB-C18, 3.0 mm × 50 mm, 2.7 μ m 色谱柱分析人参的色谱图

结论

将人参中人参皂苷的分析方法成功的从传统的 4.6×150 mm, $5 \mu\text{m}$ 色谱柱转移到 Poroshell 120 色谱柱上, 大大节省了分析时间, 同时也没有损失分离度。根据所需要的分析时间以及可用的高效液相色谱仪器选择最好的分析方法。相比于全多孔的亚 $2 \mu\text{m}$ 填料填充的色谱柱, 表面多孔的 $2.7 \mu\text{m}$ 颗粒填充的色谱柱显示出相似的性能, 但是压力较低。由于压力较低, 该方法可以在 400 bar 的仪器上运行。较高的流速在 超高液相色谱仪上可以实现较快的分离, 压力最高可达色谱柱的压力上限 600 bar。

参考文献

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使用 Poroshell 120 EC-C18 管柱分析 Naproxen： 化繁為簡的調整分析法

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摘要

美國藥典 (USP) 記載的 Naproxen 分析法，需使用 Eclipse Plus C18 管柱與 Poroshell 120 EC-C18 管柱。使用 4.6 x 50 mm 的 Poroshell 120 EC-C18 管柱時，若採用改良型分析法，則能將原本分析法的分析時間縮短 22%，且不須再確效。

緒論

檢測藥物製劑的成本相當可觀，因此許多實驗室的負責人，無不尋求降低成本的解決之道，期待能減少溶劑用量、提升產能，並能繼續使用原有的液相層析儀 (LC)。

美國藥典 (USP) 刊載的分析法，普遍用於檢驗藥物製劑及原料，不過 USP 裡的各種分析法，並非全數使用新技術，因此耗費的時間可能比期望中更長。因此可遵循 USP 第 621 章的建議，可依照需求，適時的更新、調整分析方法。本分析法調整的部分如下：管柱長度、管柱材質、充填顆粒大小、注入量等。雖然其他參數則可按照 USP 載明的方式調整，但改善本分析法的處理量時，不需調整其他參數。若調整前述部分以外的參數，則會改變分析法，需再次確效。

Naproxen 屬非類固醇抗發炎藥物，簡稱 NSAID，市面上已有其學名藥錠劑。USP 明列出一種分析 Naproxen 錠劑的方法，該法採用 L1 (C18)、5 μm 的管柱。Naproxen 的結構如圖 1 所示。

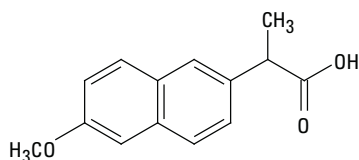


圖 1. Naproxen 的化學結構

安捷倫 Poroshell 120 管柱為能提升效能的 LC 管柱，此類管柱填充 2.7 μm 的表面多孔性顆粒，管柱較短，能加快分析速度、提高解析度，僅需搭配現有的 LC 儀，便能在較短的時間內，檢驗更多的樣品。管柱以 C18 為鍵結相，C18 為一般的 L1 材質。圖 1 顯示，本分析法一開始採用 4.6 mm \times 150 mm、5 μm 的 L1 (C18) 管柱，而後固定分析條件，再以 4.6 mm \times 100 mm、2.7 μm 的安捷倫 Poroshell 120 EC-C18 管柱，及 4.6 mm \times 50 mm、2.7 μm 的安捷倫 Poroshell 管柱分別進行分析，比較其結果。

實驗

- 安捷倫 1200 系列二元幫浦 SL，移動相 A：乙腈：水：冰醋酸 = 500:490:10，流速為 1.2 mL/分鐘，部分分析將流速提高至 2.2 mL/分鐘 (G1312B)
- 安捷倫 1200 系列自動液體注射器 SL (ALS)，150 mm 管柱的注入量為 20 μL ；100 mm 管柱為 13.34 μL ；50 mm 管柱為 6.67 μL (G1376C)
- 安捷倫 1200 系列恆溫管柱室 SL(TCC)，溫度設定為 25 $^{\circ}\text{C}$ (G1316B)
- 安捷倫 1200 系列雙極行列檢出器 SL (DAD)，使用的波長為 254、4 nm，搭配 G1315-60024 微流動液槽（光徑 5 mm，容積 6 μL ）(G1316C)

安捷倫 ZORBAX 管柱：

- 安捷倫 Eclipse Plus C18 管柱，4.6 mm \times 150 mm、5 μm ，p/n 959993-902
- 安捷倫 Eclipse Plus C18 管柱，4.6 mm \times 100 mm、3.5 μm ，p/n 959961-902
- 安捷倫 Poroshell 120 EC-C18 管柱，4.6 mm \times 100 mm、2.7 μm ，p/n 695975-902
- 安捷倫 Poroshell 120 EC-C18 管柱，4.6 mm \times 50 mm、2.7 μm ，p/n 699975-902

乙腈購自 Honeywell 公司，已通過 Burdick & Jackson 試藥級 (ACS) HPLC 溶劑的認證；冰醋酸使用 USP/ACS 級溶劑，購自 VWR 公司；水則採用以密理博 Millipore Milli-Q 系統現場製造，以 18 M Ω 過濾至 0.2 μm 的水。USP 級的 Naproxen，購自美國藥典編輯委員會；Butyrophenone 購自 Sigma-Aldrich 公司。樣品及移動相，均依照 USP 規定的方法配製。[1]

移動相製備

混合乙腈、水及冰醋酸 (500 mL:490 mL:10 mL)，製備移動相。
[1]

樣品製備

樣品及內標準品溶於乙腈與水 (90:10) 的混合液中。取 5 mL 的 butyrophenone，以乙腈稀釋成 100 mL 的內標準品。再取 1 mL 的上述混合液，以乙腈稀釋成 100 mL 的溶液，則每 ml (毫升) 溶液中，含有 0.5 μ L 的 butyrophenone。

精確秤取 USP 級分離標準品：USP 級 Naproxen 分離標準品 (RS)，溶於溶劑混合液，製備成濃度 2.5 mg/mL 的溶液。再取 1.0 mL 的上述溶液，及 2.0 mL 的內標準品溶液，倒入 1 個 100 mL 的容量瓶，並以移動相稀釋至標線後混合。每毫升的此溶液中，含有 25 μ g 的 USP 級 Naproxen RS。[1]

本分析法的層析及效能要求，請見 USP 分析法，摘要如下：[1]

- 4.6 mm \times 150 mm 管柱，L1 管柱 (C18)
- 分析物的理論板數 (N) 不得少於 4000 個
- 分析物波峰與內標準品波峰的解析度解析度，不得低於 11.5

USP 分析法的效率及其他層析效能要求，很容易便能達到，如圖 2 所示。使用 150 mm、5- μ m 的管柱，進行本文所述的 USP 分析法時，每 9 分鐘便能完成 1 次分析，產能為每小時完成 6-7 次分析，若以 9 分鐘注射一次計算，一天約注射 160 次，一週注射 1120 次。多數分析能有如此產能便已足夠，但此分析法稍做調整後，更能提高產能。USP 修訂後的第 621 章，對於分析法的「調整」範圍，提出建議：[2]

- 管柱長度 \pm 70%
- 管柱內徑 \pm 25%
- 充填顆粒顆粒大小：至多縮小 50%，不得增大
- 流速 \pm 50%
- 只要符合系統適合性測試 (SST) 的標準，便能改變注入量

- 管柱溫度 \pm 10%
- 移動相的 pH 值 \pm 0.2
- 紫外光 (UV) 波長：不得改變成製造商規格以外的數據
- 緩衝液的鹽類濃度 \pm 10%

超出上述範圍的調整，將「改變」分析法，需再行確效。若選用較短的管柱，如圖 2 所示的 4.6 mm \times 100 mm、3.5- μ m 管柱，同樣的分析可在原先的 67% 時間內完成，即每項樣品約 6 分鐘完成分析。此分析法可輕易套用至 4.6 mm \times 100 mm，及 4.6 mm \times 50 mm 的 2.7 μ m 安捷倫 Poroshell EC-C18 管柱。50 mm 管柱仍在容許的調整範圍內，且可輕易將分析時間，縮短為原先分析時間的 33%。

為了發揮 Poroshell 120 或其他小容量管柱的最佳效能，必須提高偵測器的速度，並減少多餘的管柱容積。資料收集速率一般設定為 40 Hz。[3] 管柱容量減少時，注入量應進行等比例調整，以防管柱過載，例如 150 mm 管柱的注入量為 20 μ L，則 100 mm 管柱的注入量，應調整為 $20 \times (100/150) = 13.67 \mu\text{L}$ ；50 mm 的管柱應調整為 $20 \times (50/150) = 6.67 \mu\text{L}$ 。

將 5 μ m 的 L1 管柱，換成以 2.7 μ m 表面多孔性顆粒填充的 C18 管柱時，效能將優於本分析法的效能要求。100 mm 長的管柱，分析速度為原先分析法的 2 倍快；50 mm 長的管柱，則為原先分析法的 4.5 倍快，而兩種管柱都能提升處理量。

其中可容許的調整為將流速調整在 \pm 50% 的範圍內，因此本分析法可將流速調高至 1.8 mL/分鐘。而讓管柱的直線速度與顆粒保持恆定，並將流速提高將近 100% (為 2.4 mL/分鐘)，是否需再行確效即在接下來的文章中進行討論。[4] 調整前述項目後，產能將能進一步提升。

圖 3 顯示，4.6 mm \times 100 mm 的安捷倫 Poroshell 120 EC-C18 管柱，提高流速後的變化。此例將 1.2 mL/分鐘的流速，提高為 2 mL/分鐘後，效能及解析度解析度均優於要求的範圍，但背壓仍保持在 400 bar 以內。再將流速提高為 2.2 mL/分鐘時，效能及解析度解析度仍符合要求，但背壓卻微微高於 400 bar 的門檻。

分析法要求：N > 4000，解析度解析度 (Rs) 高於 11.5

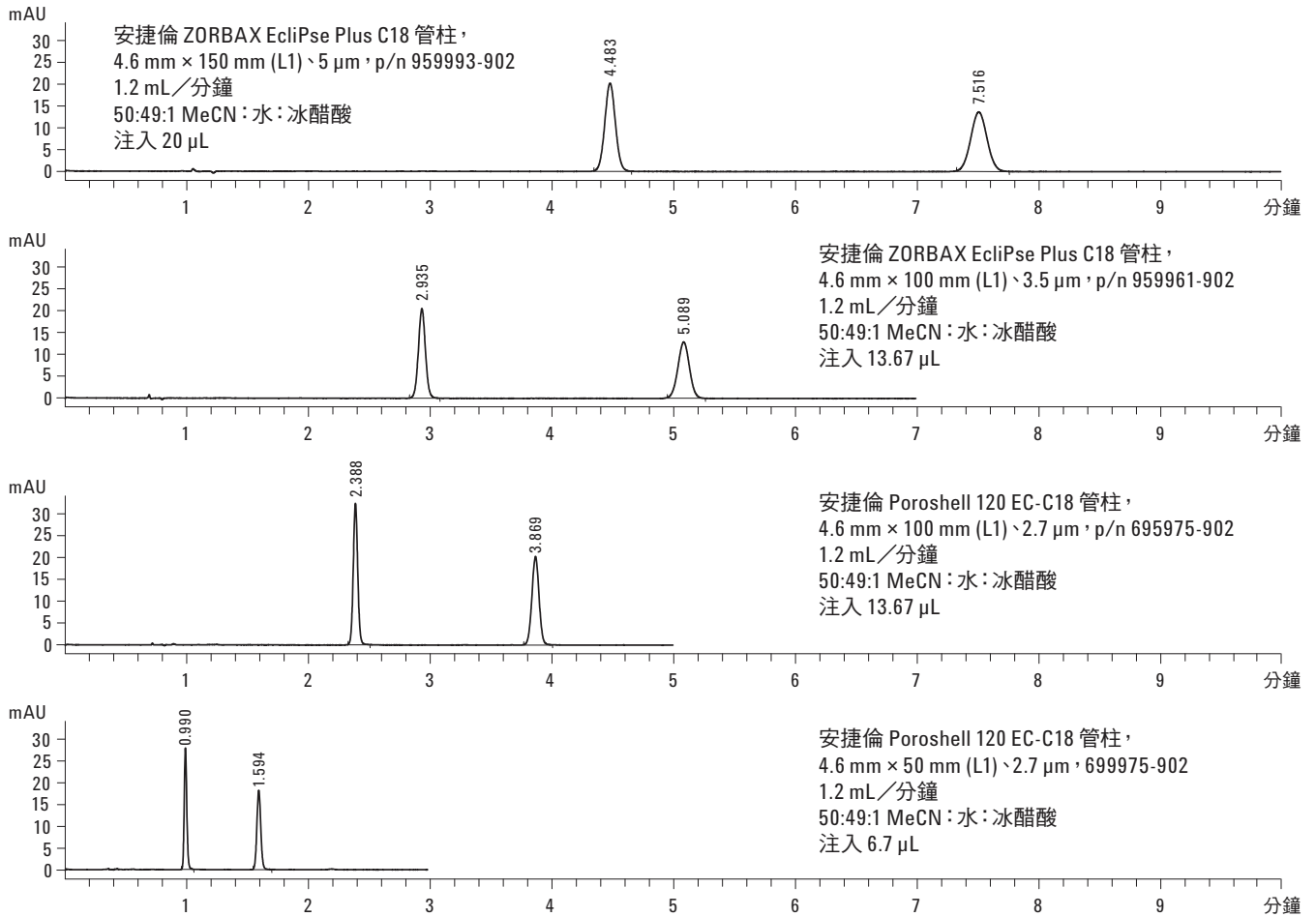


圖 2. 以各種全多孔性顆粒充填管柱，及表面多孔性顆粒充填管柱，執行 USP Naproxen 分析法的結果

分析法要求：N > 4000，解析度解析度 (Rs) 高於 11.5

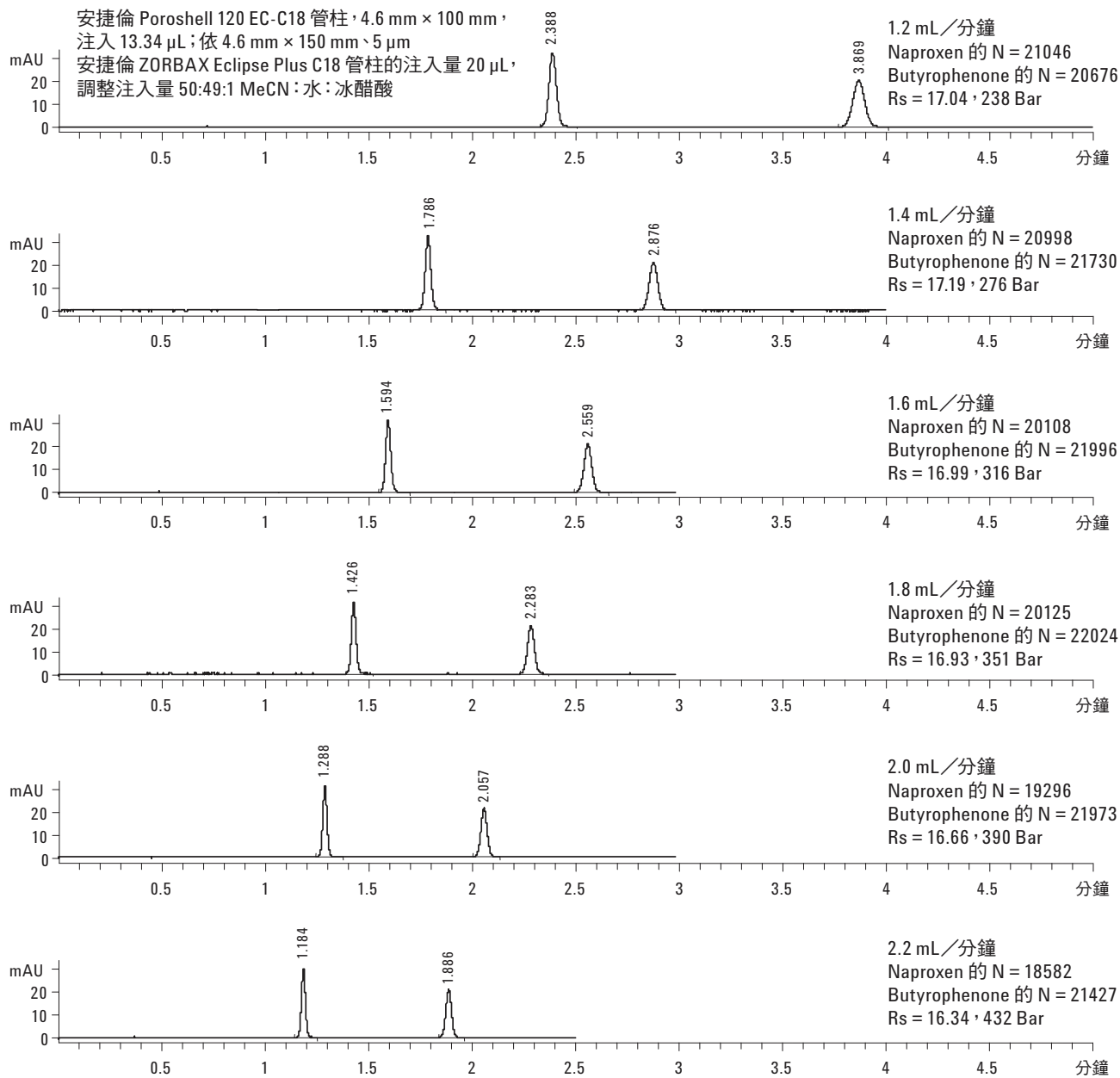


圖 3. 4.6 mm × 100 mm 的安捷倫 Poroshell 120 EC-C18 管柱，以各種流速執行 USP Naproxen 分析法的結果

圖 4 顯示，4.6 mm × 50 mm 的安捷倫 Poroshell 120 EC-C18 管柱，提高流速後的變化。此例將 1.2 mL/分鐘的流速，提高為 2.4 mL/分鐘後，效能及解析度均優於要求的範圍，但回壓仍保持在 300 bar 以內。

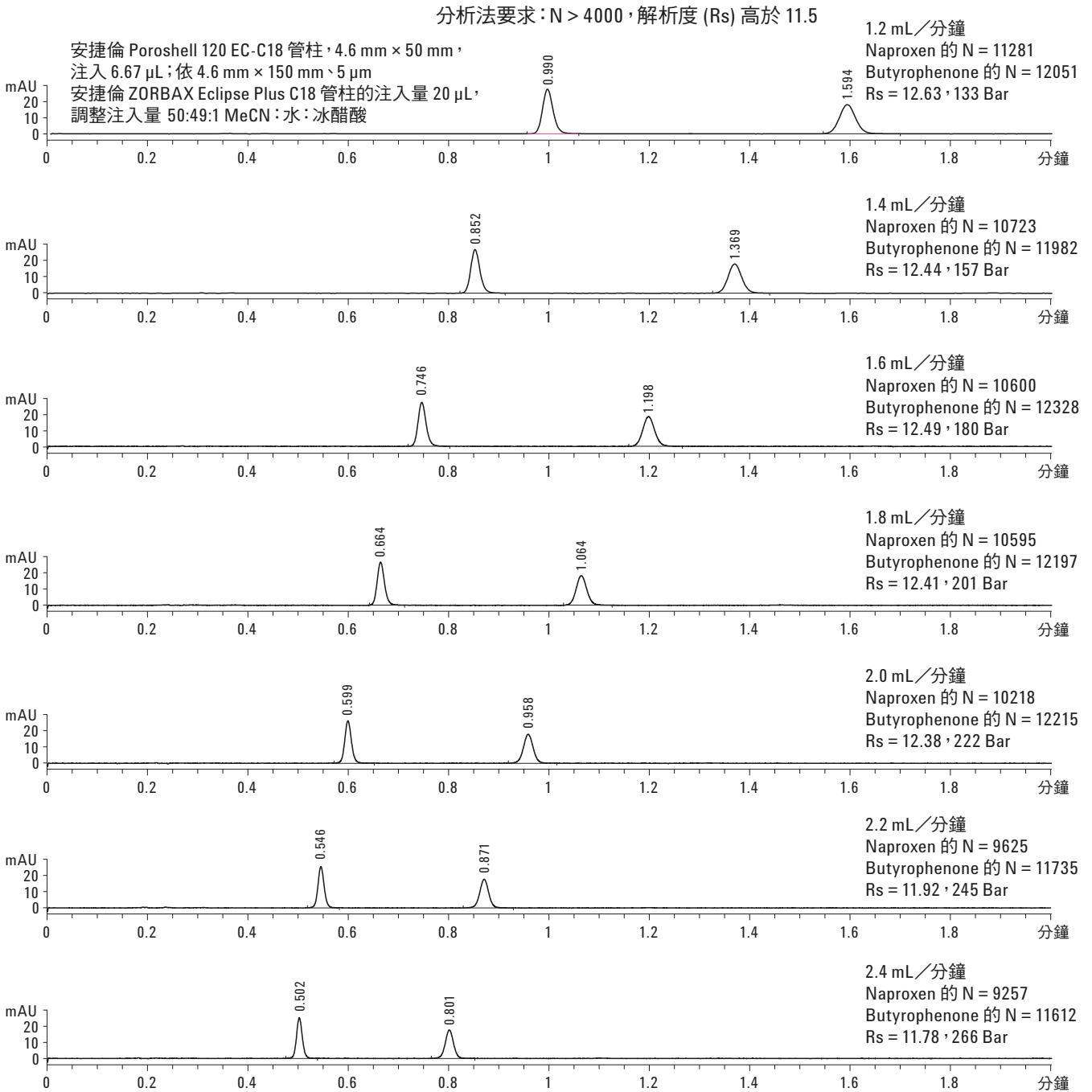


圖 4. 4.6 mm × 50 mm 的安捷倫 Poroshell 120 EC-C18 管柱，以各種流速執行 USP Naproxen 分析法的結果

結論

使用全多孔性顆粒充填的 LC 管柱，執行藥典分析法的實驗室人員，可改用表面多孔性顆粒充填的安捷倫 Poroshell 120 管柱，完全不需更換現有的儀器，便能擁有分析速度、解析度及靈敏度均提升的好處。使用安捷倫 Poroshell 120 管柱，可加快分析速度，提高處理量及產能。適度調整各種藥典分析法，改用較短的管柱，及以較小的 2.7 μm 顆粒充填，其結果便有大幅改善。

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应用 Agilent Poroshell 120 SB-C18 色谱柱分析常见食品中的花青素

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摘要

将最初使用 Agilent StableBond SB-C18 4.6 × 250 mm, 5 μm 或 4.6 mm × 150 mm, 3.5 μm 色谱柱对花青素类化合物进行分析的方法转移到使用 Agilent Poroshell 120 SB-C18, 4.6 mm × 75 mm, 2.7 μm 色谱柱的 Agilent 1260 快速高分离度液相色谱上。按比例调整梯度、进样量及流速, 并保持每一根已评价色谱柱的保留指数不变。如果从 Agilent StableBond SB-C18 4.6 × 250 mm, 5 μm 柱进行转移的话, 时间可从 100 min 减少到 20 min, 并可减少 70% 的溶剂消耗。如果从 Agilent StableBond SB-C18, 4.6 mm × 150 mm, 3.5 μm 柱进行转移的话, 运行时间可从 67 min 减少到 40 min, 并可减少 40% 的溶剂消耗。

前言

花青素是水溶性植物色素，它使水果、花朵和植物呈现红色、蓝色和紫色。花青素的 HPLC 和 HPLC-MS 分析可用于不同种类水果的指纹鉴定，并可辅助鉴别果汁的真伪。

由于花青素作为食品抗氧化剂可能具有保健作用，因此人们对其研究的兴趣越来越浓。自然界中已发现 300 多种不同结构的花青素。花青素是一类黄酮类化合物，属于广泛分布的植物多酚类物质。黄酮醇类、黄烷-3-醇类、黄酮类、黄烷酮类和黄烷酮醇类是另外的黄酮类化合物，它们与花青素的氧化态形态不同。

花青素的定性和定量分析，可用于鉴别不同的蓝莓品种，并鉴定它们的质量。因此农业和酿酒业对花青素色谱分析分离的重视日益增加。最近对花青素作为抗氧化剂/抗癌剂医用价值的研究，也已引起了人们对其色谱分离的兴趣 [1]。

传统上，在这些分离中使用的低 pH 流动相（含甲酸）会引起色谱柱的降解和分离中的变化 [2]。Agilent ZORBAX StableBond SB-C18 色谱柱能够给色谱工作者提供在反相色谱分析中极低 pH 条件下的长期稳定性。许多报道的方法都采用了 50 mL/L (5%) 甲酸或 30 mL/L (3%) 磷酸做流动相。在文中，使用 Agilent Poroshell 120 SB-C18 色谱柱，并按比例调整甲酸和磷酸的用量。应用这种新方法对一些水果和果汁样品进行了分析，包括蓝莓、黑莓、小红莓、草莓和石榴汁等。

实验部分

- G1312B SL 型二元泵，流动相 A：3% 磷酸或 5% 甲酸的水溶液，B：甲醇。
- G1367E SL 型自动液体进样器 (ALS)。
- G1316B SL 型柱温箱 (TCC)，温度设定为 30°C。
- G4212C SL 型二极管阵列检测器 (DAD)，波长设置为 525, 16 nm，未使用参比波长，使用 G4212-60008 微量流通池（光程 10 mm，容积 1 μ L）。
- B.04.02 版化学工作站，可用于控制 HPLC 系统并进行数据处理。
- Agilent Poroshell 120 SB-C18 色谱柱，4.6 mm \times 75 mm，2.7 μ m（部件号 689775-902）。

- Agilent ZORBAX SB-C18 色谱柱，4.6 mm \times 250 mm，5 μ m（部件号 880975-902）。
- Agilent ZORBAX SB-C18 色谱柱，4.6 mm \times 150 mm，3.5 μ m（部件号 863953-902）。

甲酸、磷酸购自 Sigma Aldrich 公司 (Bellefonte, PA)；甲醇购自 Honeywell, Burdick 和 Jackson High Purity 公司 (Muskegon, MI)；18 M-W Milli-Q 超纯水 (Millipore 公司, Bedford, MA)；新鲜的黑莓、蓝莓、草莓、小红莓和石榴汁购自当地商店。

水果提取物的制备方法

首先进行混合：10 g 蓝莓（或其它水果），加入 10 mL 溶剂（70:28:2 的甲醇:水:甲酸），在干冰上混合 10 min，使冰升华。采用玻璃棉，用 10 mL 注射器对样品进行过滤。静置滤液 1 h 后，通过 0.2 μ m 滤膜过滤，取 50 μ L 滤液立即注入 HPLC 进行分析（4.6 mm \times 250 mm 色谱柱）[3,4]，然后将制备得到的澄清溶液转移到标有 Agilent MS Analyzed 的样品瓶中（部件号 5190-2278）。

$$\text{公式 1: } k^* = (t_G F) / (d/2)^2 L (\Delta\%B)$$

式中：

t_G 梯度运行时间

F 流速

L 柱长

d 柱内径

$\Delta\%B$ 梯度运行过程中有机溶剂含量的变化

图 1 显示了使用全多孔的 5 μ m、3.5 μ m SB-C18 色谱柱和较短的、表面多孔的 Agilent Poroshell 120 SB-C18 2.7 μ m 色谱柱对蓝莓提取物进行分析的色谱图。进样量与柱体积成正比。由于它们具有相同的键合相，因此两种色谱柱的选择性应极为相似 [5,6,7]。Agilent ZORBAX StableBond SB-C18 色谱柱尤为适用于极低 pH 流动相的分析条件。在之前的报道中曾说明过，Poroshell 120 色谱柱在大约一半的压力下，可达到 1.8 μ m 全多孔色谱柱峰容量的 90% 左右。此外，Poroshell 120 色谱柱的柱效是 3.5 μ m 色谱柱的两倍 [6,7]。在文中，展示了从 5 μ m 填料的长色谱柱到较短的 3.5 μ m 填料色谱柱再到更短 Poroshell 120 色谱柱的应用渐变过程。根据色谱柱长度（内径保持不变）按公式 1 计算的比例调整洗脱梯度，使保留指数 (k') 保持不变。

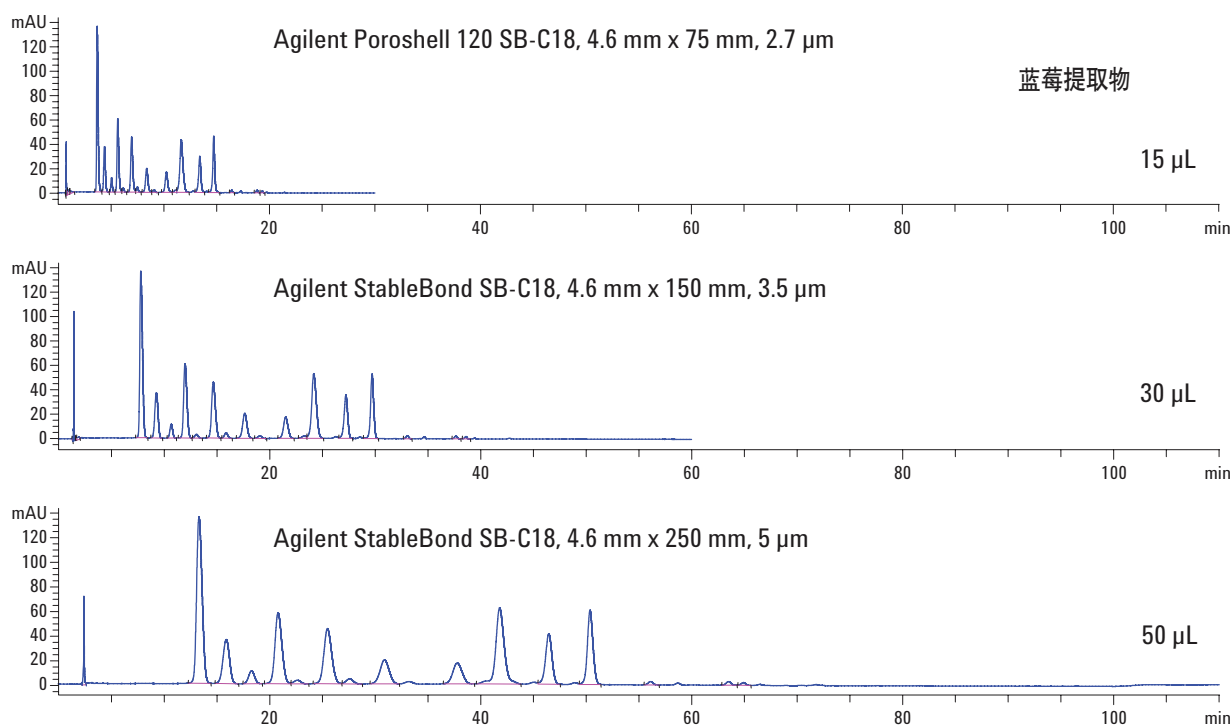


图 1. 使用全多孔和表面多孔 stableBond C18 色谱柱对蓝莓中花青素的分析。柱规格分别为 250 mm 5 μm 、150 mm 3.5 μm 和 75 mm 2.7 μm 以 1 mL/min 的流速对青色素进行分析的叠加色谱图

即使对于蓝莓提取物这种复杂的样品基质，所有峰都能用相同的分析方法被分开（尽管速度更快），这既节约了时间，又减少了溶剂消耗。表 1 列出了所用梯度的参数，时间从 97 min 缩短至 29 min。由于色谱柱使用了 2 μm 柱滤膜，所以不需要额外的样品制备。这些滤膜比全多孔柱上使用的 3 μm 滤膜更耐堵塞 [8]。

表 1. 图 1、2 和 3a 中使用参考文献 3 中的色谱条件，按比例调整的磷酸梯度

柱长	4.6 mm × 250 mm	4.6 mm × 150 mm	4.6 mm × 75 mm	4.6 mm × 75 mm	4.6 mm × 75 mm
粒径	5	3.5	2.7	2.7	2.7
部件号	880975-902	863953-902	689775-902	689775-902	689775-902
流速	1	1	1	1.5	2
最高耐压	183 bar	236 bar	236 bar	349 bar	448 bar
进样量	50	30	15	15	15
% B	时间	时间	时间	时间	时间
23	0	0	0	0	0
26	35	21	10.5	7.5	5.25
60	97	58	29.1	20	10

图 2 中流速或线速度在逐渐增加。在之前的报道中已经说明，对于 Agilent Poroshell 120 EC-C18 4.6 mm 色谱柱，流速在 1.5-2.5 mL/min 范围内时，线速度越大，峰容量越大 [9]。应用公式 1 并保持 k' 值不变，则可使分离保持一致。正如色谱图中所示，在这种情况下，50% 甲醇和 2 mL/min 的流速使压力达到最大值 448 bar。然而流速在 1.5 mL/min 时，压力最大值在 400 bar 以下。实验中所用的梯度如表 1 所示。

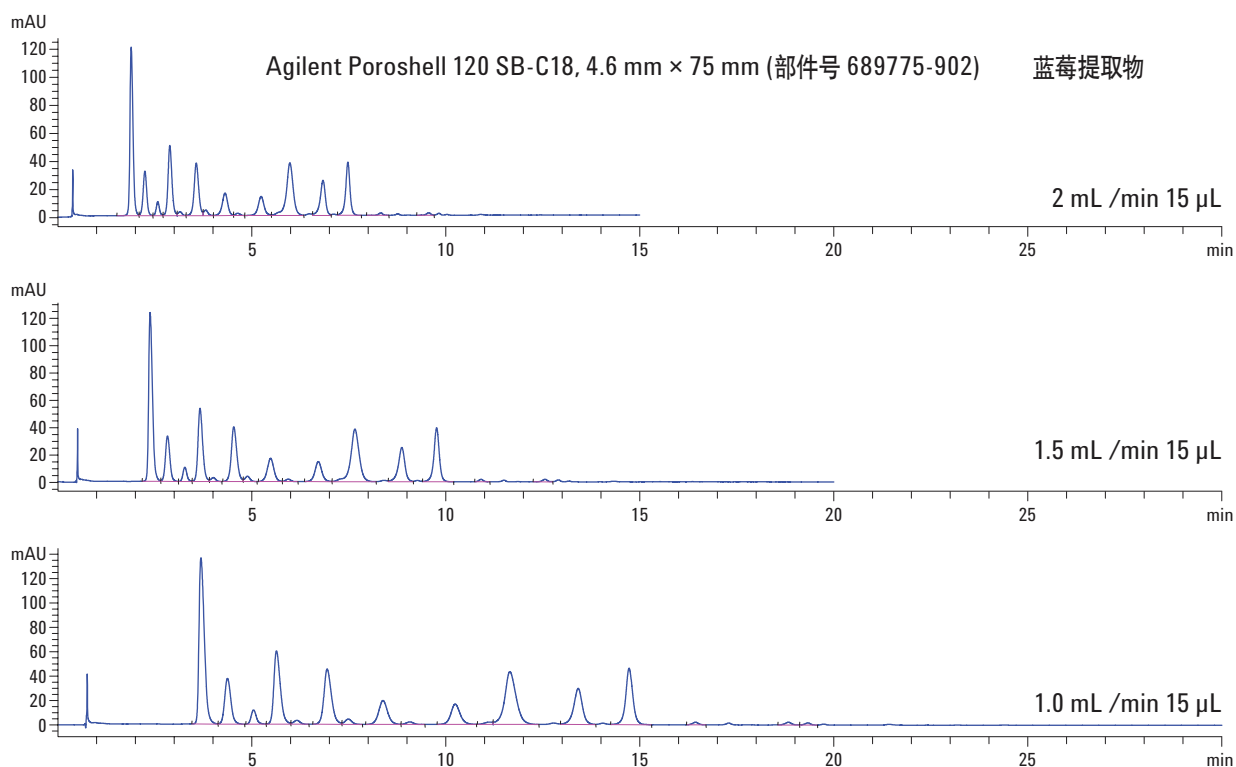


图 2. 使用 Agilent Poroshell 120 SB-C18 4.6 mm × 75 mm 2.7 µm 色谱柱以不同的流速分析蓝莓提取物中花青素的叠加色谱图

图 3a 为使用表 1 中流速为 1.5 mL/min 方法分析实际样品的色谱图。用酸化甲醇制备蓝莓、黑莓、小红莓和草莓样品 [3, 4]。石榴汁无需进一步处理, 可直接进样。如图 1 所示, 复杂的蓝莓样品色谱图大约有 20 个色谱峰 (16 个主峰, 4 个小峰)。Kalt 的研究表明越是趋向野生和生长条件恶劣的蓝莓品种, 花青素含量越多 [4]。黑莓与之前报道的一样, 种植品种只有很少的色谱峰 [10]。小红莓具有一些独特的成分 (cyd-3-gal, cyd-3-glu, cyd-3-arab, pnd-3-gal,

pnd-3-glu, pnd-3-arab), 在参考文献 10、11、12 和 13 中也有相关描述。草莓的测定结果也与先前报道数据一致。图 3b 显示使用 5% 甲酸时, 对梯度洗脱程序稍作调整即可得到相似结果。原始梯度流速为 1.5 mL/min, 且没有增加流动相的线速度。原始和按比例调整的梯度洗脱程序见表 2。用甲酸代替磷酸可实现与质谱联用进行组分鉴定, 但不论采用哪种流动相改性剂, 得到的指纹图谱结果是相似的。

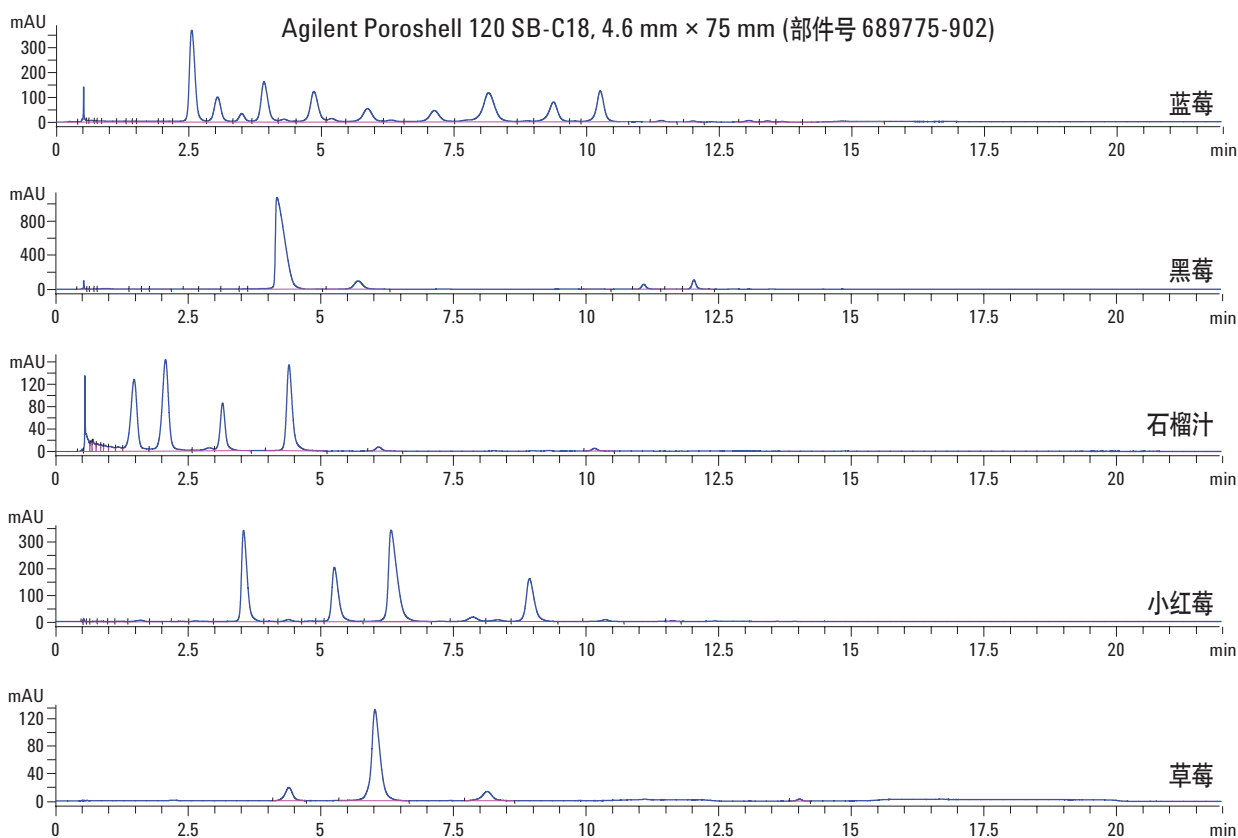


图 3a. 使用 Agilent Poroshell 120 SB-C18 色谱柱和 H_3PO_4 改性剂对不同样品中花青素进行梯度洗脱的叠加色谱图

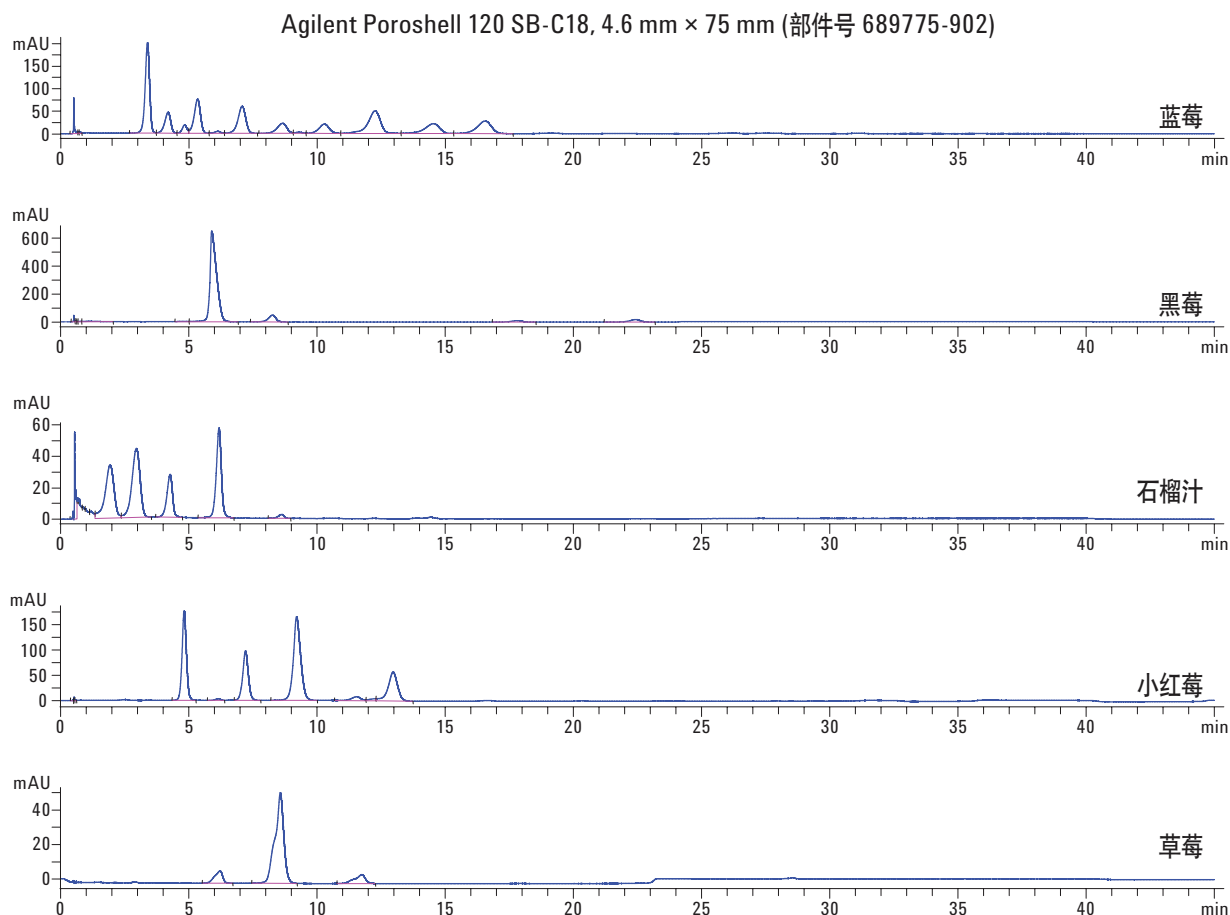


图 3b. 使用 Agilent Poroshell 120 SB-C18 色谱柱和 HCO_2H 改性剂对不同样品花青素进行梯度洗脱的叠加色谱图

表 2. 图 3b 使用参考文献 4 中的色谱条件, 按比例调整的甲酸梯度

柱长	4.6 x 250	4.6 x 75
粒径	5	
部件号	880975-902	68775-902
流速	1.5	1.5
最高耐压	274 bar	349 bar
进样量	50	15
%B	时间	时间
14	0	0
17	10	6
23	35	21
47	65	39
14	67	40.2

结论

与常规的全多孔填料色谱柱相比, 表面多孔填料 HPLC 色谱柱具有众多优势。表面多孔的 Agilent Poroshell 120 SB-C18, 粒径 $2.7\ \mu\text{m}$ 色谱柱拥有与 Agilent ZORBAX StableBond SB-C18 色谱柱相似的选择性。使用 Poroshell 120 SB-C18 色谱柱对花青素进行分析, 结果表明在减少每个样品溶剂消耗的同时可进行更快速分析。将这一分析应用到区分植物品种 [4] 或分析成百个每个样品需要超过 1 小时运行时间的各种食物样品 [10, 12] 时, 我们可以很容易地感受到分析更快速及更少溶剂消耗的重要性。这样可以节省大量时间或允许同等时间内鉴别更多植物品种。用甲酸作为流动相添加剂将可以联用质谱进行组分的进一步鉴定。

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应用 Agilent Bond Elut Plexa PCX 与 Agilent Poroshell 120 对尿液中的 6-乙酰吗啡进行 SAMHSA 标准的 LC/MS/MS 分析

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摘要

由美国药物滥用和精神健康服务管理局 (SAMHSA) 颁布, 于 2010 年 10 月起生效的新准则, 允许在政府认证的药物检测实验室使用 LC/MS/MS 法对初步药物检测结果进行确认。由于 LC/MS/MS 法不需要衍生步骤, 因此比先前使用的 GC/MS 法简便得多。我们提出了一种满足最新 SAMHSA 准则要求的 6-乙酰吗啡的分析方法, 并对其线性、检测限 (LOD)、准确度和精密度进行论证, 还对该方法的基质效应、萃取回收率和总处理效率进行了考察。这是涵盖所有 SAMHSA 监控药物类别的一系列六种简便检测方法之一, 该方法主要使用安捷伦产品进行分析, 包括 Agilent Bond Elut Plexa PCX 混合模式聚合物 SPE 吸附剂、Agilent Poroshell 120 EC-C18 2.7 μm 表面多孔 LC 色谱柱、Agilent 1200 Infinity LC 系统以及应用安捷伦喷射流技术 (AJST) 增强电喷雾离子源的 Agilent 6460 Triple Quadrupole LC/MS 系统。

前言

6-乙酰吗啡或 6-单乙酰吗啡 (6-AM) 是海洛因特有的一种代谢物。海洛因 (或二乙酰吗啡) 是一种由吗啡合成的成瘾性鸦片类药物。海洛因在体内通过脱乙酰化作用迅速代谢生成 6-AM, 然后再缓慢地代谢为吗啡。6-乙酰吗啡被认为是服用海洛因后, 导致所有急性反应的主要成分。6-AM 可在摄入海洛因 24h 内的尿液中检出。在室温下储存的尿样中, 6-AM 可以在 7 天内几乎完全水解为吗啡。在冷冻贮藏的尿液中, 6-AM 被认为是相对稳定的。SAMHSA 最新规定 6-AM 的最高限量浓度为 10 ng/mL, 其 10% 为检测限, 即 1ng/mL。

由于 Agilent Bond Elut Plexa 独特的性质, 本文所描述的简便提取方法实现了 6-AM 可重现的高回收率的提取。与其它聚合物吸附剂不同, Plexa 颗粒具有无氨基的羟基化表面, 避免了与蛋白质的结合。从而获得了最小的离子抑制作用和最高的检测灵敏度。高流速和良好重现性的实现, 主要得益于色谱柱填料的窄粒径分布, 并且不含容易导致堵塞的细颗粒。

选用 Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱是由于其具有高容量和良好的分离性能。与亚 2 μm UHPLC 色谱柱相比, 填充了表面多孔 2.7 μm 填料的 Poroshell 120 色谱柱可提供与其相似的柱效, 但其系统背压却可降低约 40%。因此它甚至允许用户在耐压 400 bar 的常规 LC 系统上采用更高的流速来提高分辨率, 并同时缩短分析时间和再平衡时间。

本方法在较小的样品进样体积 (10 μL) 和无预富集的条件下, 具有极高的信噪比 (样品浓度 1 ng/mL 时 > 190:1, 该浓度为 SAMHSA 规定限量浓度的 10%)。这得益于应用了 AJST 技术的 Agilent 6460 Triple Quadrupole LC/MS 系统电喷雾离子源增强了检测灵敏度。

安捷伦以前的分析方法 (由 Moorman 和 Hughes 开发, 2010) 使用的是 Agilent 6410 Triple Quadrupole LC/MS 系统和其它的 SPE/LC 产品和操作步骤。

实验部分

分析物

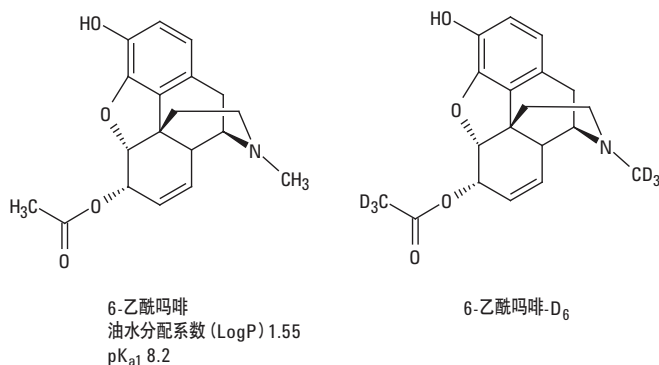


图 1. 6-乙酰吗啡分析物及其结构

药物标准品购自 Cerilliant 公司, 为 1 mg/mL (6-乙酰吗啡) 和 100 μg/mL (6-乙酰吗啡-D₆) 的乙腈溶液。

材料与仪器

SPE

- Agilent Bond Elut Plexa PCX 小柱 30 mg, 3 mL (部件号: 12108303)
- Agilent VacElut 20 多管真空装置 (部件号: 12234100)
- Agilent 截止阀 (部件号: 12234520)
- Agilent 2 mL 自动进样器样品瓶 (部件号: 5182-0716)
- Agilent AS 样品瓶螺纹口瓶盖 (部件号: 5182-0717)

LC

- Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱 (部件号: 699975-302)
- Agilent 1260 Infinity LC 系统 (G1379B 微型脱气机, 低延迟体积配置的 1312B 二元泵, G1367E 自动进样器, G1330B 柱温箱)

MS

- 配有应用安捷伦 AJST 技术电喷雾离子源的 Agilent 6460A Triple Quadrupole LC/MS 系统

样品制备

预处理

往 1 mL 尿液中加入内标，使其浓度为 20 ng/mL，推荐使用 12×75 mm 玻璃试管。加入 2%甲酸 1 mL，涡旋，若溶液浑浊则离心。

萃取

1. 用 0.5 mL 甲醇活化 Bond Elut Plexa PCX 小柱，浸润，然后让其自然滴出
2. 上样/加载上清液
3. 淋洗 1: 1 mL 2% 甲酸溶液
4. 淋洗 2: 1 mL 甲醇
5. 在真空（10–15 英寸汞柱）条件下，抽干 5-10 分钟
6. 用 1 mL 新鲜配制的甲醇：氢氧化铵（100:10）溶液洗脱，先使洗脱液自然滴落至收集小瓶中，然后用低真空（2-3 英寸汞柱）抽滤
7. 氮气吹干
8. 用 1 mL 初始流动相（10%甲醇、90%水、0.1%甲酸）复溶。

LC/MS/MS

LC 条件

流动相 A	0.1% 甲酸水溶液	
流动相 B	0.1% 甲酸甲醇溶液	
流速	0.8 mL/min	
梯度程序	时间 (分钟)	% B
	0.0	10
	1.5	25
	2.0	60
	2.1	90
	5.0	90
	5.1	10
停止时间	5.2 min	
后运行时间	2 min	
最高泵压	400 bar	
进样体积	10 μ L	
进样并洗针		
洗针	用 75: 25 甲醇：水冲洗进样口 10 秒	
禁止重叠进样		
禁止自动减小延迟体积		

MS 条件

ES 离子源参数

离子检测模式	正离子
毛细管电压	2,800 V
干燥气流速	13 L/min
干燥气温度	350 °C
雾化气压力	35 psi
鞘气流速	12 L/min
鞘气温度	400 °C
喷嘴电压	0 V

MS 参数

扫描模式	MRM
预运行脚本	SCP_MS DiverterValveToWaste() {MH_Acq_Scripts.exe}
时间段	#1: 1.2 min-切换阀到 MS
Delta EMV (+)	400 V

结果与讨论

在酸性 pH 条件下，6-乙酰吗啡的叔胺基团质子化，从而分析物可以通过疏水保留和强阳离子交换作用被高效的保留在 Plexa PCX 聚合物吸附剂上。

100%甲醇冲洗 SPE 小柱，可去除大部分基质干扰而无 6-AM 的流失。为破坏分析物和强阳离子交换吸附剂之间的离子相互作用，将一种强碱加入到有机洗脱液中。在样品洗脱前，将 10% NH_4OH 溶液加入甲醇中，以提高 6-AM 的回收率。

Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μ m 色谱柱可实现尿液提取物中 6-AM 的快速分离，并可获得良好的峰形（如图 2 所示）。液相分离开始时采用低有机溶剂比例（10%）的流动相，从而在样品运行开始时使尿液中的盐类和其它极性成分先洗脱下来。每次样品运行开始时先将液流转换使其流至废液（0 到 1.2 分钟），以尽量减少离子源污染。在第 1.2 分钟切换阀将液流转至质谱检测器后立即开始数据采集。0.8 mL/min 的流速可实现较短的保留时间和再平衡时间。

SAMHSA 准则要求分析目标化合物和内标物时必须使用一个定量离子和至少一个定性离子。目标分析物的第三个转换离子（见表 1）信息可提供更高的定性能力。Agilent MassHunter Quantitative 软件可自动计算定性离子比率，并可以标出那些超出可接受范围的离子。

表 1. MRM 转换离子

化合物	母离子	子离子	裂解电压	碰撞能量
6-AM	328.2	165.1	140	40
6-AM	328.2	211.1	140	25
6-AM	328.2	193.1	140	25
6-AM-D ₆	334.2	165.1	140	40
6-AM-D ₆	334.2	211.1	140	25

本方法采用 MRM 扫描模式，设置为常态而非动态检测，因为动态 MRM 模式对单一化合物的检测没有优势。

1 ng/mL 时色谱峰信噪比超过 190:1（如图 2，上图所示），显示了 Agilent 6460 Triple Quadrupole LC/MS 系统优异的性能，能够非常可靠地检测远低于 SAMHSA 限量浓度的 6-AM（10%）。

图 3 给出了 5 个浓度水平的尿液提取标准品 6-乙酰吗啡的校准曲线。向不含有 6-乙酰吗啡的尿液中加入 6-乙酰吗啡标准品，配制浓度分别为 1.0、10、50、200 和 400 ng/mL 的校准标准溶液。添加氘代内标 6-AM-D₆ 的浓度为 20 ng/mL。线性关系极为优异， $R^2 > 0.999$ ，表明该方法在一个较宽的动态浓度范围内呈线性，符合 SAMHSA 准则的要求。

方法评价

表 2 中方法的各项性能指标系根据 Matuszewski 等（2003）提出的原则计算得到的，其作为行业标准用于评价 LC/MS/MS 分析方法已被广泛接受。LC/MS/MS 测试的样品及其萃取步骤如下：第一组，将限量浓度水平的 6-AM 预加到阴性尿液中，然后进行萃取，平行五份，进样分析。第二组，将阴性尿液利用同样方法进行萃取，然后萃取物用初始流动相溶液复溶，之后加入 6-AM 标准品到 10 ng/mL，平行五份，进样分析。第三组是直接加标至初始流动相（即复溶溶剂），使其浓度为 10 ng/mL 的限量浓度（流动相加标）。

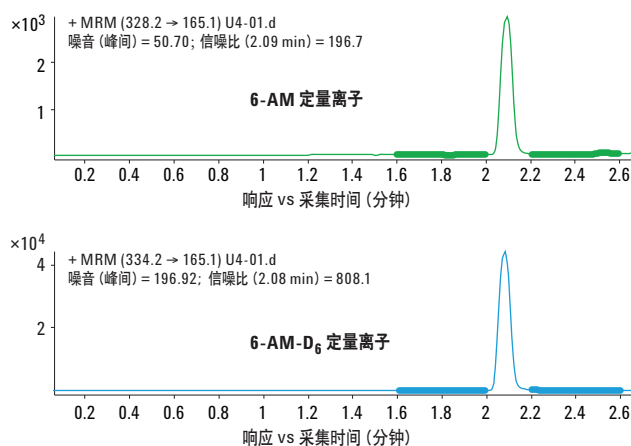


图 2. 尿液提取物中 6-AM（1 ng/mL）和 6-AM-D₆（20 ng/mL）的 MRM 提取离子色谱图。采用 Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱。噪声区域以加粗的形式显示

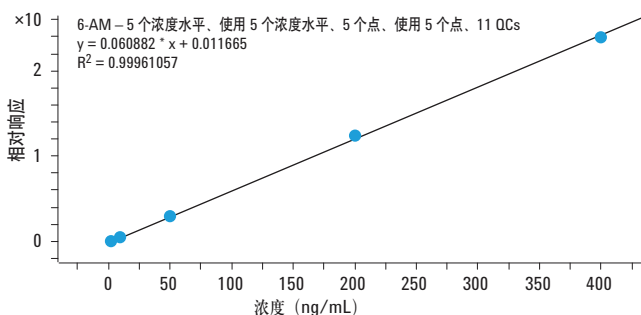


图 3. 尿液提取物中 6-AM 的校准曲线示例。校准范围为 1.0 到 400 ng/mL。线性相关系数 $R^2 > 0.999$

表 2. 6-乙酰吗啡分析方法评价， $n = 5$

	%
处理效率*	83
萃取回收率*	83
基质效应*	100
准确度**	106
精密度 (CV)**	0.6

* 限量浓度条件下测定 ** 限量浓度的 40% 条件下测定

处理效率（即绝对回收率）是 SPE 萃取加标的阴性尿液后进样分析，获得的目标分析物的峰面积与其无基质的流动相加标后进样分析获得的相应峰面积的比值。萃取回收率是 SPE 萃取加标的阴性尿液后进样分析，获得的目标分析物的峰面积与经过相同方法萃取阴性尿液后的提取物加标样品进样分析获得的相应峰面积的比值。基质效应是分析 SPE 萃取阴性尿液后的提取物加标样品，获得的目标分析物的峰面积与其流动相加标后分析获得的相应峰面积的比值。准确度是通过校准曲线计算的分析物检测浓度与添加了已知量目标分析物样品的预期浓度的比值。精密度或变异系数（CV）用来衡量重复性，系采用五个测量值的平均值计算其百分标准偏差获得的。

表 2 显示了 6-乙酰吗啡较高的提取回收率（83%）和良好的准确度（106%）以及精密度（0.6%）。100%的基质效应表明无基质干扰导致的信号增强或抑制，因此可确定经 Plexa 处理的提取物非常纯净。

结论

这里描述的固相萃取样品制备和 LC/MS/MS 检测相结合的分析方法完全遵循 SAMHSA 要求，为认证实验室的药物测试或其它有类似要求的分析环境的测试提供高精密度、准确度和重现性的有法律效力的数据结果。其硬件配置与 2011SAMHSA 方法中的相同均来自安捷伦。这些方法特别为所有安捷伦 1100 和安捷伦 1200 LC 系列的用户开发，这些 LC 系统的背压均不超过 400bar。采用其它型号的安捷伦三重四极杆 LC/MS 系统应用本方法时，离子源参数可以很容易地修改。安捷伦科技可提供电子版本的 LC/MS/MS 数据采集和定量分析方法指导。

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应用 Agilent Bond Elut Plexa PCX 与 Agilent Poroshell 120 对尿液中的阿片类药物（吗啡和可待因）进行 SAMHSA 标准的 LC/MS/MS 分析

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摘要

由美国药物滥用与精神健康服务管理局（SAMHSA）颁布，于 2010 年 10 月生效的新准则，允许在政府认证的检测实验室采用 LC/MS/MS 法确认初步药物测试结果。由于 LC/MS/MS 法不需要衍生步骤，因此它远比以前应用的 GC/MS 法要简便的多。我们提出了一种满足最新 SAMHSA 准则要求的阿片类药物的分析方法，并对其线性、检测限（LOD）、准确度和精密度进行论证，还对该方法的基质效应、萃取回收率和总处理效率进行了考察。这是涵盖所有 SAMHSA 监控药物类别的一系列六种简便检测方法之一，该方法主要使用安捷伦产品进行分析，包括 Agilent Bond Elut Plexa PCX 混合模式聚合物 SPE 柱，Agilent Poroshell 120 EC-C18, 2.7 μm 表面多孔 LC 色谱柱，Agilent 1200 Infinity LC 系统以及应用安捷伦喷射流技术（AJST）增强电喷雾离子源的 Agilent 6460 Triple Quadrupole LC/MS 系统。

前言

阿片类药物（吗啡和可待因）是在罂粟树脂中发现的天然生物碱。可待因是目前世界上使用最广泛的阿片类药物。除了吗啡和可待因的检测，SAMHSA 准则还要求该分析方法应具备使这些药物与其结构类似化合物分离的能力，这些结构类似化合物包括半合成的阿片类药物：氢吗啡酮、羟吗啡酮、氢可酮、羟考酮和可待因的代谢产物去甲可待因。所有这些药物均可与人类的阿片受体结合发挥作用。除了作为止痛处方药使用外，它们还用于娱乐用途，并且可导致阿片类药物依赖，高剂量的阿片类药物/鸦片可导致呼吸衰竭而致人死亡。

吗啡和可待因在体内均可广泛代谢。吗啡主要代谢为吗啡-3-葡萄糖苷酸和吗啡-6-葡萄糖苷酸。可待因的主要代谢产物是吗啡、可待因-6-葡萄糖苷酸和去甲可待因。由于在尿液中发现的吗啡和可待因均主要以葡萄糖苷酸缀合物的形式存在，因此 SAMHSA 要求测定每一种化合物的总浓度。分析前必须将葡萄糖苷酸缀合物完全转换为母体药物。确保游离的阿片类药物完全转化的最可靠的转换方法是酸水解。过去常用的酶解法已被证实不能使母体化合物转换完全，这可能会导致假阴性的结果（Wang 等，2006）。

SAMHSA 规定吗啡和可待因的最高限量浓度为 2000 ng/mL。由于预计在一些尿样中可能出现高浓度的阿片类药物（还有安非他明），我们选择使用更高容量的 3mm id Agilent Poroshell 120 色谱柱代替所有以前安捷伦 SAMHSA 方法中的 2mm id 色谱柱。与亚 2 μm 的 UHPLC 色谱柱相比，填充了 2.7 μm 表面多孔颗粒的 Poroshell 120 色谱柱具有与其相似的柱效，但其背压却减少了 40% 左右。因此，它甚至允许用户在耐压 400 bar 的常规 LC 系统上通过使用更高的流速来提高分辨率，同时缩短分析时间和再平衡时间。

由于 Agilent Bond Elut Plexa 的独特性质，本文所描述的提取方法获得了吗啡和可待因极高的回收率，且重复性好。不像其它聚合物吸附剂，Plexa 颗粒具有无氨基的羟基化表面，避免了蛋白质的结合。从而获得了最小的离子抑制作用和最高的检测灵敏度。高流速和良好重复性的实现，主要得益于色谱柱填料的窄粒径分布，并且不含易引起堵塞的细颗粒。

在较小的样品进样体积（2 μL ）和无预富集的条件下，该方法对吗啡和可待因均获得了极佳的信噪比（样品浓度 200 ng/mL 时 >150:1，该浓度为 SAMHSA 规定限量浓度的 10%），这得益于应用了 AJST 技术的 Agilent 6460 Triple Quadrupole LC/MS 系统电喷雾离子源增强了检测灵敏度。

安捷伦以前的分析方法（由 Moorman 和 Hughes 开发，2010）采用了 Agilent 6410 Triple Quadrupole LC/MS 系统和其它的 SPE/LC 产品及操作步骤。

实验部分

分析物

药物标准品购自 Cerilliant 公司, 为 1 mg/mL (吗啡、可待因、氢吗啡酮、去甲可待因、氢可酮、羟考酮、羟吗啡酮和吗啡 3-葡萄糖苷) 和 100 $\mu\text{g/mL}$ (吗啡- D_6 和可待因- D_6) 的甲醇溶液。

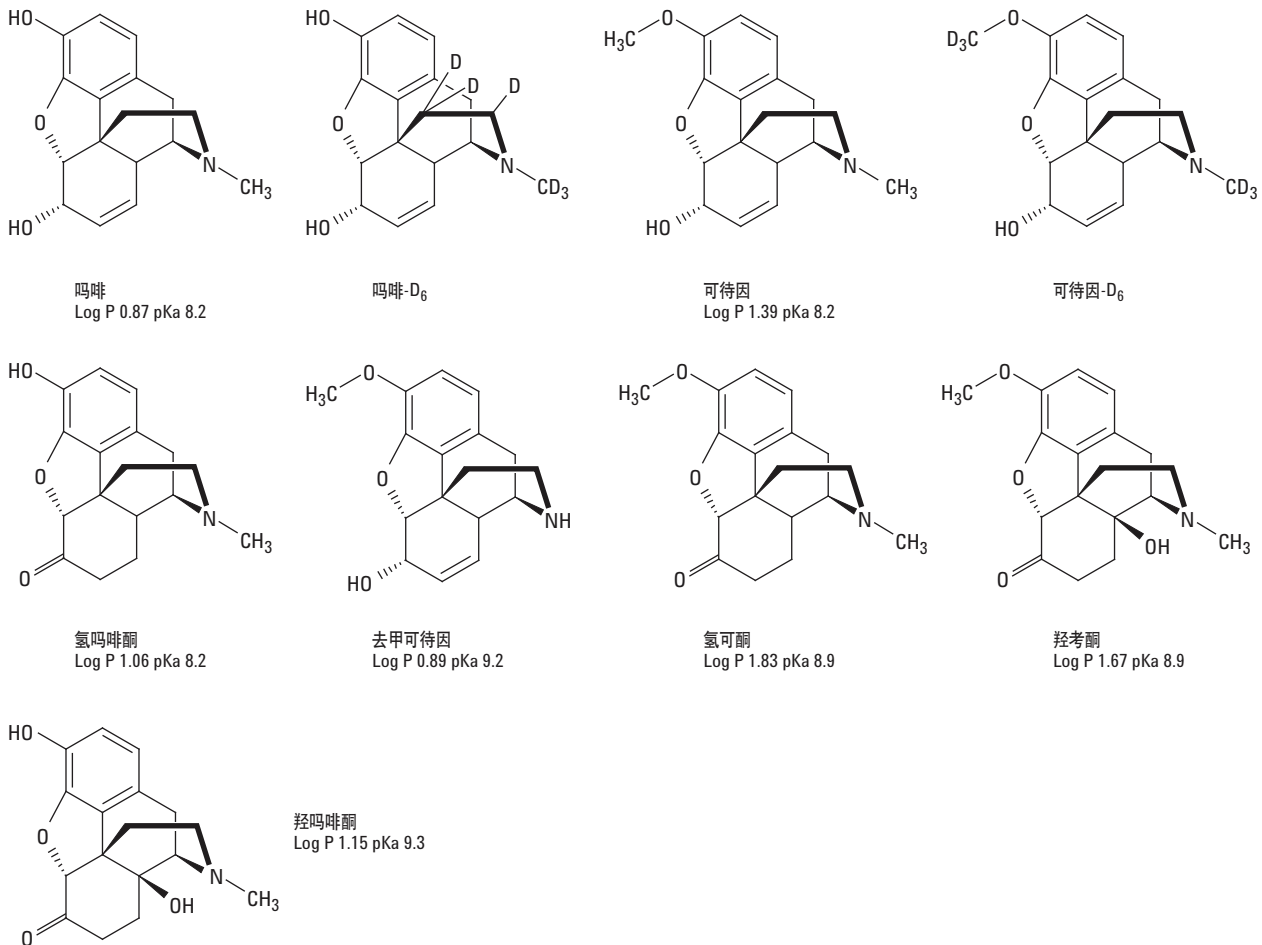


图 1. 阿片类分析物及其结构

材料与仪器

SPE

- Agilent Bond Elut Plexa PCX 小柱 30 mg, 3 mL (部件号 12108303)
- Agilent VacElut 20 多管真空装置 (部件号 12234100)
- Agilent 截止阀 (部件号 12234520)
- Agilent 2 mL 自动进样器样品瓶 (部件号 5182-0716)
- Agilent AS 样品瓶螺纹口瓶盖 (部件号 5182-0717)

LC

- Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱 (部件号 699975-302)
- Agilent 1260 Infinity LC 系统 (G1379B 微型脱气机、低延迟体积配置的 1312B 二元泵、G1367E 自动进样器、G1330B 柱温箱)

MS

- 配有应用 AJST 技术电喷雾离子源的 Agilent 6460A Triple Quadrupole LC/MS 系统

样品制备

水解与样品预处理

1. 往 0.5 mL 尿液中加入内标, 使其浓度为 1000 ng/mL; 推荐使用 12 × 75mm 玻璃试管
2. 加入 125 μL 浓盐酸
3. 在加热装置中于 95 ± 5 °C 水解 90 min
4. 冷却。然后加入 2 mL 0.1 M 醋酸钠缓冲液 (pH 4.5)
5. 用 250 μL 7 M 的 KOH 溶液中和, 涡旋, 测定其 pH 值, 应小于 6
6. 以 6000 rpm 的速度离心 20min

萃取

1. 用 0.5 mL 甲醇活化 Agilent Bond Elut Plexa PCX 小柱, 浸润, 然后使其自然滴出
2. 上样或加载上清液
3. 淋洗 1: 1 mL 2% 甲酸溶液
4. 淋洗 2: 1 mL 甲醇
5. 在真空 (10-15 英寸汞柱) 条件下, 抽干 5-10 分钟

6. 用 2 mL 新鲜配制的甲醇: 氢氧化铵 (100: 20) 溶液洗脱。先使洗脱液自然滴落至收集小瓶中, 然后用低真空 (2-3 英寸汞柱) 抽滤
7. 在 40 °C 下蒸干
8. 用 0.5 mL 初始流动相 (5% 甲醇, 95% 水, 0.1% 甲酸) 复溶

LC/MS/MS

LC 条件

流动相 A	0.1% 甲酸水溶液	
流动相 B	0.1% 甲酸甲醇溶液	
流速	0.8 mL/min	
梯度程序	时间 (分钟)	% B
	0.0	5
	0.5	5
	1.5	25
	2.5	55
	2.6	90
	5.6	90
	5.7	5

停止时间	5.8 min
后运行时间	2 min
最高泵压	400 bar
进样体积	2 μL
进样并洗针	
洗针	用甲醇: 水 (75: 25) 冲洗进样口 10 秒

- 禁止重叠进样
- 禁止自动减小延迟体积

MS 条件

ES 离子源参数

离子检测模式	正离子
毛细管电压	3,000 V
干燥气流速	10 L/min
干燥气温度	350 °C
雾化气压力	35 psi
鞘气流速	12 L/min
鞘气温度	400 °C
喷嘴电压	0 V

MS 参数

扫描类型	动态 MRM
预运行脚本	SCP_MS Diverter Valve To Waste() {MH_Acq_Scripts.exe}
时间段	#1: 1.0min-切换阀到 MS
Delta EMV (+)	0 V

结果与讨论

在酸性 pH 条件下，吗啡、可待因及其衍生物的叔胺基团质子化，从而分析物可以通过疏水作用和强阳离子交换作用被高效的保留在 Plexa PCX 聚合物吸附剂上。

用 100% 甲醇淋洗 SPE 小柱可消除大部分的基质干扰而不会造成阿片类药物损失。在有机洗脱液中添加强碱，以破坏分析物与强阳离子交换吸附剂之间的离子相互作用。在进行样品洗脱之前，将适量 20% 氢氧化铵溶液添加到甲醇中，以提高阿片类药物的回收率。

采用 Agilent Poroshell 120 EC-C18 3×50mm, 2.7 μm 色谱柱能实现阿片类药物及其潜在干扰化合物的良好分离，并获得了极佳的峰形，同时分析可在 2.5min 内完成（如图 2 所示）。液相色谱分离首先采用低有机溶剂比例（5%）的流动相，使尿液中的盐类和其它极性成分在样品运行开始时先被洗脱。每次样品运行开始时先将液流转换使其流至废液（0 到 1 min），以尽量减少离子源污染。1.0 min 时切换阀将液流转至质谱检测器后即刻开始数据采集。0.8 mL/min 的流速可获得较短的分析时间和再平衡时间。

在图 2 所示色谱图中，只有可待因和去甲可待因这一对色谱峰（峰 4 和 5）部分分离，但由于这些化合物具有不同的母离子和转换离子，因此可待因定量时去甲可待因信号所产生的任何可能的干扰都可以排除。

在另一项实验中，测试了 Plexa PCX 吸附剂是否存在使去甲可待因甲基化并转换为可待因的可能性。试验结果均为阴性；在添加了去甲可待因标准品的阴性尿样中并未检测到可待因，因此本文可采用上述提取方法。

在进行干扰物测试时，推荐使用利用一个特定转换离子，通过保留时间和 ΔRT （时间窗）调整的动态 MRM 方法。当然，一旦确定干扰组分已经分离出去，吗啡、可待因及其内标物的数据采集即可通过常规的 MRM 进行。

SAMHSA 准则要求分析目标化合物和内标物时必须使用一个定量离子和至少一个定性离子。目标分析物的第三个转换离子（见表 1）信息可提供更高的定性能力。Agilent MassHunter Quantitative 软件可自动计算定性离子比率，并可以标出那些超出可接受范围的离子。

表 1. MRM 模式数据采集参数

化合物	母离子	子离子	裂解电压	碰撞能量
可待因	300.2	215.1	130	23
可待因	300.2	165.1	130	46
可待因	300.2	153.1	130	50
可待因-D ₆	306.2	165.1	130	44
可待因-D ₆	306.2	218.1	130	23
吗啡	286.1	201.1	130	23
吗啡	286.1	181.1	130	40
吗啡	286.1	165.1	130	43
吗啡-D ₆	292.1	181.1	130	40
吗啡-D ₆	292.1	165.1	130	42
吗啡-3-葡萄糖苷酸	462.2	286.1	162	45
羟考酮	316.2	298.1	130	15
羟吗啡酮	302.2	284.1	130	17
氢可酮	300.2	199.1	130	30
去甲可待因	286.1	225.1	130	20
氢吗啡酮	286.1	185.1	130	28

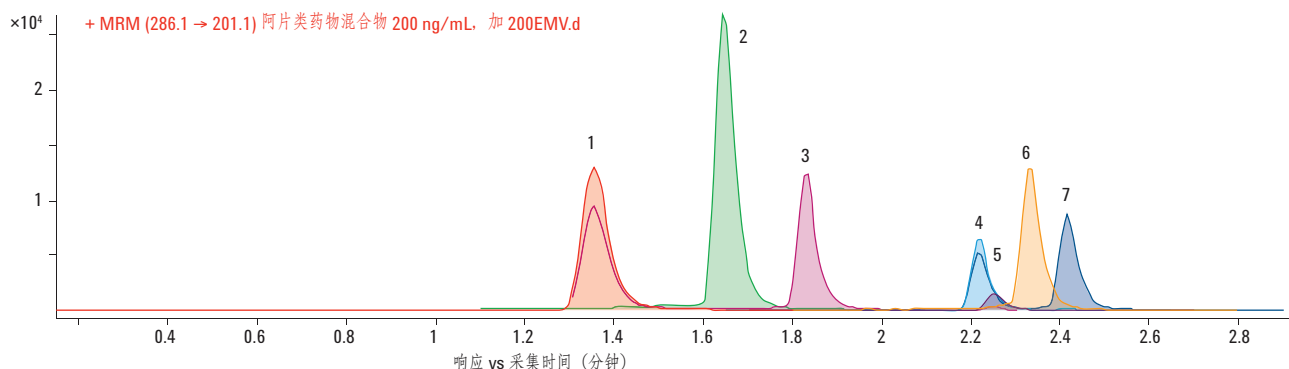


图 2. 阿片类药物与其潜在干扰物在 Agilent Poroshell 120 EC-C18 3×50mm, 2.7 μm 色谱柱上分离的叠加 MRM 提取离子色谱图。各分析物在尿液中的浓度均为 200 ng/mL。色谱峰按洗脱顺序分别为：1、吗啡；2、羟吗啡酮；3、氢吗啡酮；4、可待因；5、去甲可待因（紫红色小峰）；6、羟考酮；7、氢可酮

按照上述步骤处理样品时,尿液中加标浓度为 10,000 ng/mL 的吗啡-β-3-葡萄糖苷酸标准品有 97-99.2% 转换为吗啡。吗啡-β-3-葡萄糖苷酸分析所用的 MS 参数列于表 1 中, 供有兴趣检测水解效率的分析人员参考。

吗啡和可待因的定量峰在浓度为 200 ng/mL 时获得的信噪比超过 150: 1 (如图 3, 上面的 1 和 2 图所示)。这说明 Agilent 6460 Triple Quadrupole LC/MS 系统具有优异的性能, 能够非常可靠地检测远低于 SAMHSA 限量浓度的阿片类药物。

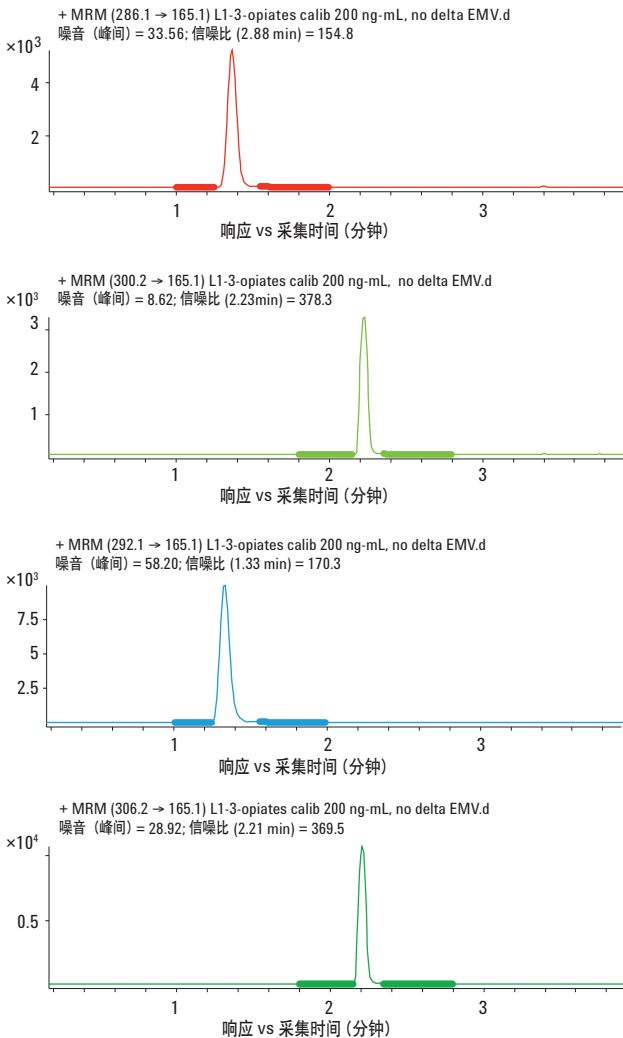


图 3. 尿液提取物中吗啡与可待因 (200 ng/mL) 和吗啡-D6 与可待因-D6 (1000 ng/mL) 的 MRM 提取离子色谱图。采用 Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱。噪声区域以加粗的形式显示

图 4 给出了 5 个浓度水平的尿液提取物中标准品的校准曲线。通过分别向阴性尿液中加入吗啡和可待因标准品, 制备两者浓度分别为 200、1000、2000、10000 和 20000 ng/mL 的一系列校准标准溶液。添加氘代内标吗啡-D6 和可待因-D6 的浓度均为 1000 ng/mL。各曲线良好的线性关系 ($R^2 \geq 0.998$) 表明该方法在很宽的动态浓度范围内呈线性, 符合 SAMHSA 准则的要求。

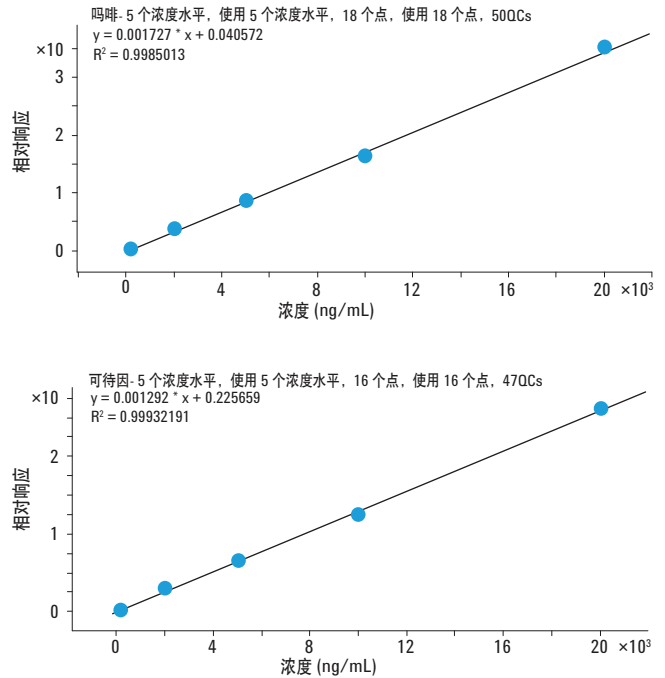


图 4. 从尿液中提取的吗啡 (上图) 和可待因 (下图) 的校准曲线示例。浓度范围为 200 至 20 000 ng/mL。线性相关系数 $R^2 \geq 0.998$

方法评价

表 2 中方法的各项性能指标系根据 Matuszewski 等 (2003) 提出的原则计算得到的, 其作为行业标准用于评价 LC/MS/MS 分析方法已被广泛接受。LC/MS/MS 测试的样品及其萃取步骤如下: 第一组, 将限量浓度水平的吗啡和可待因预加到阴性尿液中, 然后进行 SPE 萃取, 平行五份, 进样分析。第二组, 将阴性尿液利用相同的方法进行萃取, 然后萃取物用初始流动相溶液复溶, 之后加入吗啡和可待因标准品到 2000 ng/mL (固相萃取后加标), 平行五份, 进样分析。第三组是直接加标至初始流动相 (即复溶溶剂), 使其浓度为 2000 ng/mL 的限量浓度 (流动相加标)。

表 2. 在限量浓度条件下阿片类药物的分析方法评价, $n=5$

参数	吗啡	可待因
处理效率 (%)	83	85
萃取回收率 (%)	85	86
基质效应 (%)	98	99
准确度 (%)	108	108
精密密度 (CV) (%)	0.6	0.7

处理效率 (即绝对回收率) 是 SPE 萃取加标的阴性尿液后进样分析, 获得的目标分析物的峰面积与其无基质的流动相加标后进样分析获得的相应峰面积的比值。萃取回收率是 SPE 萃取加标的阴性尿液后进样分析, 获得的目标分析物的峰面积与经过相同方法萃取阴性尿液后的提取物加标样品进样分析获得的相应峰面积的比值。基质效应是分析 SPE 萃取阴性尿液后的提取物加标样品, 获得的目标分析物的峰面积与其流动相加标后分析获得的相应峰面积的比值。准确度是通过校准曲线计算的分析物检测浓度与添加了已知量目标分析物样品的预期浓度的比值。精密密度或变异系数 (CV) 用来衡量重复性, 系采用五个测量值的平均值计算其百分标准偏差获得的。

表 2 显示了啡和可待因的高提取回收率和高处理效率 (约 85%)。高基质效应 (98-99%) 表明只有 1-2% 的信号由于离子抑制作用而损失, 从而证明了经 Plexa 吸附剂处理的提取物具有优异的纯净度。同时该方法具有高准确度 (目标物浓度的 $\pm 10\%$ 之内) 和极好的精密密度 (CV < 1%)。

结论

本文所描述的固相萃取样品制备与 LC/MS/MS 检测相结合的分析方法完全遵循 SAMHSA 要求, 为认证实验室的药物测试或其它有类似要求的分析环境的测试提供高重现性的有法律效力的数据结果。其硬件配置与 2011 版 SAMHSA 方法中的相同均来自安捷伦。这些方法特别为安捷伦 1100 和安捷伦 1200 LC 系列的所有用户开发, 这些 LC 系统的背压不超过 400 bar。采用其它型号的安捷伦三重四极杆 LC/MS 系统应用本方法时, 离子源参数可以很容易地进行修改。安捷伦科技可提供电子版的 LC/MS/MS 数据采集和定量分析方法。

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应用 Agilent Bond Elut Plexa PCX 和 Agilent Poroshell 120 对尿液中的苯环利啉进行 SAMHSA 标准的 LC/MS/MS 分析

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摘要

由美国药物滥用和精神健康服务管理局 (SAMHSA) 颁布, 于 2010 年 10 月生效的新准则, 允许在政府认证的实验室采用 LC/MS/MS 法确认初步药物测试结果。由于 LC/MS/MS 法不需要衍生操作, 因此它远比以前使用的 GC/MS 法要简便得多。我们提出了一种满足最新 SAMHSA 准则要求的苯环利啉的分析方法, 并对其线性、检测限 (LOD)、准确度和精密度进行论证, 还对该方法的基质效应、萃取回收率和总处理效率进行了考察。这是涵盖所有 SAMHSA 监控药物类别的一系列六种简便检测方法之一, 该方法主要使用安捷伦产品进行分析, 包括 Agilent Bond Elut Plexa PCX 混合模式聚合物 SPE 吸附剂, Agilent Poroshell 120 EC-C18 2.7 μm 表面多孔 LC 色谱柱, Agilent 1200 Infinity LC 系统以及应用安捷伦喷射流技术 (AJST) 增强电喷雾离子源的 Agilent 6460 Triple Quadrupole LC/MS 系统。

前言

苯环利啉 (PCP) 是一种合成药物, 为解离型麻醉药的一种。其致幻性和神经毒性作用为人所熟知, 并且在高剂量时, 它可对人体造成永久性损害。5-20% 的 PCP 以原型药物形式从尿液中排出 (Baselt, 2008)。因此, 它以药物原型的形式即可被检测到, 无需水解和测量其代谢物。摄入药物后的数天内均可在尿液中检测到 PCP。在生物样品中它的稳定性良好。含有 PCP 的冷冻尿样可以贮存达一年, 而且仅需 4 °C 冷藏即可满足短期贮存要求。

苯环利啉有一个 3 环结构, 包括一个苯环、一个环己烷环和一个哌啶环 (如图 1 所示)。它是一种弱有机碱, 几乎呈非极性, 并有较高的 logP 值, 为 4.69 (Drug Bank)。SAMHSA 最新规定苯环利啉的最高限量浓度为 25 ng/mL, 其 10% 为检测限, 即 2.5 ng/mL。

由于 Agilent Bond Elut Plexa 吸附剂的独特性质, 本文所描述的简单提取方法可获得 PCP 的高回收率提取, 且重复性好。不像其它聚合物吸附剂, Plexa 颗粒具有无氨基的羟基化表面, 避免了蛋白质的结合。从而获得了最小的离子抑制作用和最高的检测灵敏度。高流速和良好重复性的实现, 主要得益于色谱柱填料的窄粒径分布, 并且不含易引起堵塞的细颗粒。

我们选择了具有高容量和极佳分离性能的 Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱。与亚 2 μm UHPLC 色谱柱相比, 填充了表面多孔 2.7 μm 颗粒的 Poroshell 120 色谱柱与其具有相似的柱效, 但背压却减少了 40% 左右, 因此, 它甚至允许用户在耐压 400 bar 的常规 LC 系统上采用更高的流速来提高分辨率, 并同时缩短分析时间和再平衡时间。

本方法在较小的样品进样体积 (2 μL) 和无预富集的条件下, 获得了极佳的信噪比 (样品浓度 2.5 ng/mL 时 >200:1, 该浓度只有 SAMHSA 规定限量浓度的 10%), 这得益于应用了 AJST 技术的 Agilent 6460 Triple Quadrupole LC/MS 系统电喷雾离子源增强了检测灵敏度。

安捷伦以前的分析方法 (由 Moorman 和 Hughes 开发, 2010) 采用了 Agilent 6410 Triple Quadrupole LC/MS 系统和其它的 SPE/LC 产品及操作步骤。

实验部分

分析物

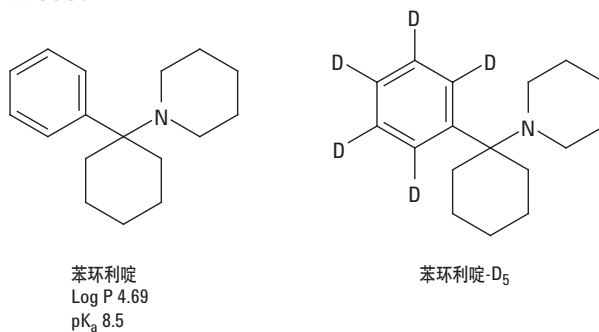


图 1. 苯环利啉分析物及其化学结构

药物标准品购自 Cerilliant 公司, 为 1 mg/mL (苯环利啉) 和 100 μg/mL (苯环利啉-D₅) 的甲醇溶液。

材料与仪器

SPE

- Agilent Bond Elut Plexa PCX 小柱 30 mg, 3 mL (部件号 12108303)
- Agilent VacElut 20 多管真空装置 (部件号 12234100)
- Agilent 截止阀 (部件号 12234520)
- Agilent 2 mL 自动进样器样品瓶 (部件号 5182-0716) 或硅烷化样品小瓶 (部件号 5183-2072)
- Agilent AS 样品瓶螺纹口瓶盖 (部件号 5182-0717)

LC

- Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱 (部件号 699975-302)
- Agilent 1260 Infinity LC 系统 (G1379B 微型脱气机、配置低延迟体积的 1312B 二元泵、G1367E 自动进样器、G1330B 柱温箱)

MS

- 配有应用 AJST 技术电喷雾离子源的 Agilent 6460A Triple Quadrupole LC/MS 系统

样品制备

预处理

往 1 mL 尿液中加入内标, 使其浓度为 50 ng/mL; 推荐使用 12 × 75 mm 玻璃试管。加入 1 mL 2% 甲酸溶液, 涡旋; 如果溶液浑浊则离心。

萃取

1. 用 0.5 mL 甲醇活化 Bond Elut Plexa PCX 小柱，浸润，然后使其自然滴出
2. 上样/加载上清液
3. 淋洗 1: 1 mL 2%甲酸溶液
4. 淋洗 2: 1 mL 甲醇
5. 在真空 (10-15 英寸汞柱) 条件下，抽干 5-10 分钟
6. 用 1 mL 新鲜配制的乙酸乙酯: 甲醇: 氢氧化铵 (80: 20: 5) 溶液进行洗脱。先使洗脱液自然滴落至收集小瓶中，然后用低真空 (2-3 英寸汞柱) 抽滤
7. 氮气吹干
8. 用 1 mL 初始流动相 (10%甲醇、90%水、0.1%甲酸) 复溶

LC/MS/MS

LC 条件

流动相 A	0.1%甲酸水溶液	
流动相 B	0.1%甲酸甲醇溶液	
流速	0.8 mL/min	
梯度程序	时间 (分钟)	% B
	0.0	10
	0.5	10
	2.5	70
	2.51	90
	5.5	90
	5.51	10
停止时间	5.6 min	
后运行时间	2 min	
最高泵压	400 bar	
进样体积	2 µL	
进样并洗针		
洗针	用 75: 25 的甲醇: 水冲洗进样口 10 秒	
禁止重叠进样		
禁止自动减小延迟体积		

MS 条件

ES 离子源参数

离子检测模式	正离子
毛细管电压	3,000 V
干燥气流速	10 L/min
干燥气温度	350 °C
雾化气压力	35 psi
鞘气流速	12 L/min
鞘气温度	400 °C
喷嘴电压	0 V

MS 参数

扫描类型	MRM
预运行脚本	SCP_MS DiverterValveToWaste() {MH_Acq_Scripts.exe}
时间段	#1: 1.2 min -切换阀到 MS
Delta EMV (+)	200 V

结果与讨论

在酸性条件下，苯环利啉的叔胺基团质子化，从而分析物可以通过疏水作用与强阳离子交换作用被高效的保留在 Agilent Bond Elut Plexa PCX 聚合物吸附剂上。

用 100%甲醇洗脱 SPE 小柱可以去除大部分的基质干扰而无 PCP 的流失。向有机洗脱液中添加强碱，以破坏分析物与强阳离子交换吸附剂之间的离子相互作用。在样品洗脱之前，将 5% 氢氧化铵溶液添加到乙酸乙酯-甲醇 (80:20) 中，以提高 PCP 的回收率

采用 Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 µm 色谱柱可实现尿液萃取物中苯环利啉的快速分离，并可获得良好的峰形 (如图 2 所示)。液相色谱分离首先采用低有机溶剂比例 (10%) 的流动相，从而使尿液中的盐类和其它极性成分在样品运行开始时先被洗脱。每次样品运行开始时先将液流转换使其流至废液，以尽量减少离子源污染。在第 1.2 分钟时切换阀将液流转至质谱检测器并即刻开始数据采集。0.8 mL/min 的流速可获得较短的分析时间和再平衡时间。

浓度为 2.5 ng/mL 样品色谱峰 (如图 2, 上图所示) 的信噪比 >200:1, 该结果表明 Agilent 6460 Triple Quadrupole LC/MS 系统具有优异的性能，可用于检测远低于 SAMHSA 规定限量浓度的 PCP (10%)。苯环利啉的疏水性极强，容易吸附在任意活性表面位点上。为避免交叉污染，我们推荐使用高效自动进样器的外部进样针端口冲洗功能，当样品浓度较高时可在进样后运行空白流动相。如果需要，洗针时间可以从 10 秒增加至 20 秒。

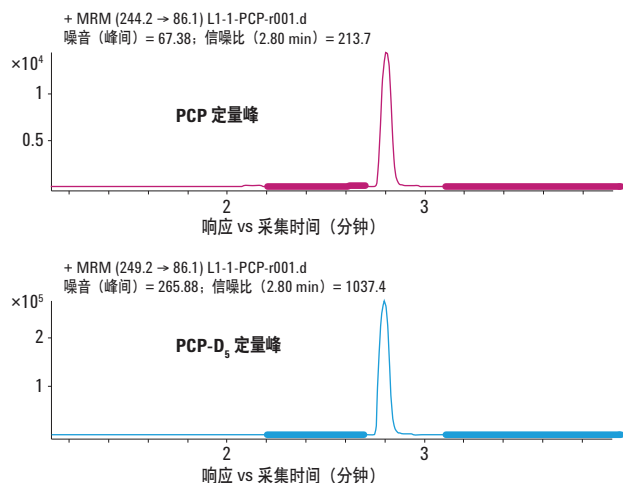


图 2. 尿液萃取物中 PCP (2.5 ng/mL) 和 PCP-D₅ (50 ng/mL) 的 MRM 提取离子色谱图。采用 Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱。噪音区域以加粗的形式显示

SAMHSA 准则要求分析目标化合物和内标物时必须使用一个定量离子和至少一个定性离子。目标分析物的第三个转换离子 (见表 1) 信息可提供更高的定性能力。Agilent MassHunter Quantitative 软件可自动计算定性离子比率, 并可以标出那些超出可接受范围的离子。

图 3 为 5 个浓度水平苯环利啉的尿液萃取标准品的校准曲线。通过向阴性尿液中加入 PCP 标准品, 制备浓度分别为 2.5、25、100、250 和 1000 ng/mL 的一系列校准标准溶液。添加氘代内标 PCP-D₅ 的浓度为 50 ng/mL。R² > 0.999, 线性关系良好, 表明该方法在很宽的动态浓度范围内呈线性, 符合 SAMHSA 准则的要求。

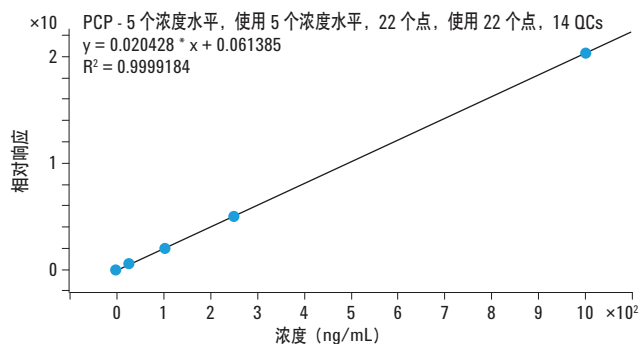


图 3. 尿液萃取物中苯环利啉的校准曲线示例。校准范围为 2.5-1000 ng/mL。线性关系良好, R²>0.999

表 1. MRM 模式数据采集参数

化合物	母离子	子离子	裂解电压	碰撞能量
PCP	244.2	86.1	80	7
PCP	244.2	159.1	80	7
PCP	244.2	91.1	80	34
PCP-D ₅	249.2	164.1	80	7
PCP-D ₅	249.2	86.1	80	7

本方法采用 MRM 扫描模式, 设置为常态而非动态检测, 因为动态 MRM 模式对单一化合物的检测没有优势。

方法评价

表 2 中方法的各项性能指标系根据 Matuszewski 等 (2003) 提出的原则计算得到的, 其作为行业标准用于评价 LC/MS/MS 分析方法已被广泛接受。LC/MS/MS 测试的样品及其萃取步骤如下: 第一组, 将限量浓度水平的 PCP 预加到阴性尿液中, 然后进行萃取, 平行五份, 进样分析。第二组, 将阴性尿液利用同样方法进行萃取, 然后萃取物用初始流动相溶液复溶, 之后加入 PCP 标准品到 25 ng/mL, 平行五份, 进样分析。第三组是直接加标至初始流动相 (复溶溶剂), 使其浓度为 25 ng/mL 的限量浓度 (流动相加标)。

处理效率 (即绝对回收率) 是 SPE 萃取加标的阴性尿液后进样分析, 获得的目标分析物的峰面积与其无基质的流动相加标后进样分析获得的相应峰面积的比值。萃取回收率是 SPE 萃取加标的阴性尿液后进样分析, 获得的目标分析物的峰面积与经过相同方法萃取阴性尿液后的萃取物加标样品进样分析获得的相应峰面积的比值。基质效应是分析 SPE 萃取阴性尿液后的提取物加标样品, 获得的目标分析物的峰面积与其流动相加标后分析获得的相应峰面积的比值。准确度是通过校准曲线计算的分析物检测浓度与添加了已知量目标分析物样品的预期浓度的比值。精密度或变异系数 (CV) 用来衡量重复性, 系采用五个测量值的平均值计算其百分标准偏差获得的。

表 2 显示了苯环利啉较高的萃取回收率 (85%)、良好的准确度 (93%) 和精密度 (0.5%)。98% 的基质效应表明只有 2% 的信号由于基质干扰导致的离子抑制作用而损失 (2%)，从而证明了经 Plexa 吸附剂处理的萃取物拥有优异的纯净度。

表 2. 苯环利啉的分析方法评价, $n=5$

	%
处理效率	83
萃取回收率	85
基质效应	98
准确度	93
精密度 (CV)	0.5

结论

本文所描述的固相萃取样品制备与 LC/MS/MS 检测相结合的分析方法完全遵循 SAMHSA 要求，为认证实验室的药物测试或其它有类似要求的分析环境的测试提供高准确度、精密度和重现性的有法律效力的数据结果。其硬件配置与 2011 版 SAMHSA 方法中的相同均来自安捷伦。这些方法特别为 Agilent 1100 和 Agilent 1200 LC 系列的所有用户开发，这些 LC 系统的背压耐受不超过 400 bar。采用其它型号的安捷伦三重四极杆 LC/MS 系统应用本方法时，我们可以很容易地修改离子源参数。安捷伦科技可提供电子版的 LC/MS/MS 数据采集和定量分析方法。

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应用 Agilent Bond Elut Plexa 和 Agilent Poroshell 120 对尿液中的 11-nor-9-羧基- Δ^9 -四氢大麻酚进行 SAMHSA 标准的 LC/MS/MS 分析

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摘要

由美国药物滥用和精神健康服务管理局 (SAMHSA) 颁布, 于 2010 年 10 月开始生效的新准则, 允许在政府认证的检测实验室采用 LC/MS/MS 法确认初步药物测试结果。由于 LC/MS/MS 法不需要衍生步骤, 因此它远比以前应用的 GC/MS 法要简便的多。我们提出了一种满足最新 SAMHSA 准则要求的 11-nor-9-羧基- Δ^9 -四氢大麻酚的分析方法, 并对其线性、检测限 (LOD)、准确度和精密度进行论证, 还对该方法的基质效应、萃取回收率和总处理效率进行了考察。这是涵盖所有 SAMHSA 监控药物类别的一系列六个简便检测方法之一, 该方法主要应用安捷伦产品进行分析, 例如 Agilent Bond Elut Plexa PCX 混合模式聚合物 SPE 吸附剂、Agilent Poroshell 120 EC-C18 2.7 μ m 表面多孔 LC 色谱柱、Agilent 1200 Infinity LC 系统和应用安捷伦喷射流技术 (AJST) 增强电喷雾离子源的 Agilent 6460 Triple Quadrupole LC/MS 系统。

前言

11-nor-9-羧基- Δ^9 -四氢大麻酚 (THCA、“THC-酸”、THC-COOH) 是四氢大麻酚 (Δ^9 -THC) 的一个代谢产物，而四氢大麻酚是大麻中活性最高的成分。THCA 以其葡萄糖苷酸缀合物的形式，通过尿液排出，并且其排泄时间可达几周。SAMHSA 新规定的 THCA 最高限量浓度是 15 ng/mL，检测限为 10% 的限量浓度，即 1.5 ng/mL。

分析 11-nor-9-羧基- Δ^9 -THC 时的样品制备需要碱水解尿样，以将葡萄糖苷酸缀合物转化为 THCA 来测定该代谢物的总量。尽管 THCA 是一种羧酸，但是为了建立一个可适用于所有 SAMHSA 监测药物预处理的单一的分析方法，我们在安捷伦吸附剂中选择了 Agilent Bond Elut Plexa PCX，一种混合型阳离子交换吸附剂来用于样品萃取。它可通过疏水作用有效地保留 THCA。

由于 Plexa 吸附剂的独特性质，本萃取方法可以获得 THCA 的高回收率萃取，且重复性好。不像其它的聚合物吸附剂，Plexa 颗粒具有无氨基的羟基化表面，避免了蛋白质的结合。从而获得了最小的离子抑制作用和最高的检测灵敏度。高流速和良好重复性的实现，主要得益于色谱柱填料的窄粒径分布，并且不含易引起堵塞的细颗粒。

选择 Agilent Poroshell 120 EC-C18 3×50 mm, 2.7 μ m 色谱柱是由于它具有高容量和极佳的分性能。Poroshell 120 色谱柱填充了表面多孔 2.7 μ m 颗粒，能提供与亚 2 μ m UHPLC 色谱柱相似的柱效，但背压会减少大约 40%。因此，它甚至允许用户在耐压 400 bar 的常规 LC 系统上采用更高的流速来提高分辨率，同时缩短分析时间和再平衡时间。

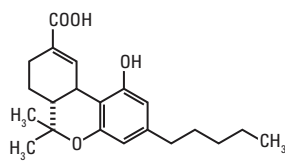
由于基本上是非极性 ($\log P > 6$) 的化合物，大麻素类化合物并不适合使用电喷雾电离，而通常应用 APCI 电离进行分析。然而，由于羧基的存在，在负离子模式下，THCA 比 Δ^9 -THC 和 11-羟基- Δ^9 -THC 更易电离。选择电喷雾离子源用于 THCA 检测也保证了使用单一质谱配置即可方便的用于所有 SAMHSA 监管药物的检测。

本方法在较小的样品进样体积 (10 μ L) 和无预富集的条件下，限量浓度和 10% 限量浓度的样品均获得了极好的信噪比 (分别大约为 100:1 和 10:1)，这得益于应用了安捷伦喷射流技术的 Agilent 6460 Triple Quadrupole LC/MS 系统电喷雾离子源增强了检测灵敏度。

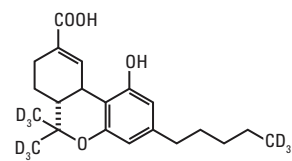
安捷伦以前的分析方法 (由 Moorman 和 Hughes 开发, 2010) 采用了 Agilent 6410 Triple Quadrupole LC/MS 系统和其它的 SPE/LC 产品及操作步骤。

实验部分

分析物



11-nor-9-羧基- Δ^9 -THC
Log P (预测值) 6.06-6.36, pKa 4.5



11-nor-9-羧基- Δ^9 -THC-D₉

图 1. 11-nor-9-羧基- Δ^9 -四氢大麻酚分析物及其结构

药物标准品购于 Cerilliant 公司，为 1 mg/mL (11-nor-9-羧基- Δ^9 -THC) 和 100 μ g/mL (11-nor-9-羧基- Δ^9 -THC-D₉ 和 11-nor-9-羧基- Δ^9 -THC-葡萄糖苷酸) 的甲醇溶液。

材料与仪器

SPE

- Agilent Bond Elut Plexa PCX 小柱 30 mg, 3 mL (部件号 12108303)
- Agilent VacElut 20 多管真空装置 (部件号 12234100)
- Agilent 截止阀 (部件号 12234520)
- Agilent 硅烷化的 2 mL 自动进样器样品瓶 (部件号 5183-2072)
- Agilent AS 样品瓶螺纹口瓶盖 (部件号 5182-0717)

LC

- Agilent Poroshell 120 EC-C18 3×50mm, 2.7 μ m 色谱柱 (部件号 699975-302)
- Agilent 1260 Infinity LC 系统 (G1379B 微型脱气机、低延迟体积配置的 1312B 二元泵、G1367E 自动进样器、G1330B 柱温箱)

MS

- 配有应用 AJST 技术电喷雾离子源的 Agilent 6460A Triple Quadrupole LC/MS 系统

样品制备

水解和样品预处理

1. 往 0.5 mL 尿液中加入内标,使其浓度为 50 ng/mL; 推荐使用甲醇冲洗并干燥过的 12×75 mm 玻璃试管
2. 加入 100 μL 7 M KOH 溶液, 涡旋
3. 在加热装置中于 60 ± 5 °C 水解 30 分钟
4. 冷却。加入 125 μL 甲醇, 涡旋
5. 加入 1.5 mL 0.2 M 醋酸钠缓冲液 (pH 4)
6. 用 100 μL 冰醋酸中和样品溶液, 涡旋
7. 如果溶液浑浊则离心

萃取

1. 用 0.5 mL 甲醇活化 Agilent Bond Elut Plexa PCX 小柱, 浸润, 然后使其自然滴出
2. 上样/加载上清液
3. 淋洗 1: 2mL 10: 90 的乙腈: 2% 乙酸溶液淋洗 2 次
4. 淋洗 2: 2mL 30: 70 的乙腈: 2% 乙酸溶液
5. 在真空 (10-15 英寸汞柱) 条件下, 抽干 5-10 分钟
6. 用 200 μL 正己烷淋洗, 在低真空 (2-3 英寸汞柱) 条件下抽干
7. 在高真空下干燥 3-4 分钟
8. 用 0.5mL 80: 20 的乙酸乙酯: 异丙醇混合液洗脱。浸润, 先使洗脱液自然滴落至收集小瓶中, 然后于低真空 (2-3 英寸汞柱) 抽滤
9. 再向吸附柱中加入 1 mL 相同的洗脱剂, 重复浸润-洗脱过程
10. 在 40 °C 下蒸干
11. 使用 0.5 mL 初始流动相 (30% 甲醇、70% 5 mM 甲酸铵溶液) 复溶

LC/MS/MS

LC 条件

流动相 A	5 mM 甲酸铵水溶液	
流动相 B	甲醇	
流速	0.8 mL/min	
梯度程序	时间 (分钟)	% B
	0.0	30
	1	95
	5	95
	5.1	30
停止时间	5.2 min	
后运行时间	2 min	
最高泵压	400 bar	
进样体积	10 μL	
进样并洗针	用 75: 25 的甲醇: 水溶液冲洗进样口 10 秒	
禁止重叠进样		
禁止自动减小延迟体积		

MS 条件

ES 离子源参数

离子检测模式	负离子
毛细管电压	4000 V
干燥气流速	11 L/min
干燥气温度	320 °C
雾化气压力	18 psi
鞘气流速	12 L/min
鞘气温度	320 °C
喷嘴电压	0 V

MS 参数

扫描类型	MRM
预运行脚本	SCP_MS DiverterValveToWaste(){MH_Acq_Scripts.exe}
时间段	#1: 1.4min 切换阀到 MS
Delta EMV (-)	800 V

结果与讨论

大麻素类化合物易吸附在玻璃和塑料容器上是众所周知的。为使样品损失最小并且保证方法的重现性, 我们强烈推荐只使用新鲜配制的样品贮备溶液和校准溶液, 并且采用硅烷化或者完全用甲醇冲洗并干燥过的玻璃器皿, 最终提取物复溶后应立即进行分析。

THCA 通过疏水作用保留在混合型阳离子交换吸附剂 Plexa PCX 上。100% 甲醇这种常用于淋洗离子交换 SPE 柱的溶剂, 并不适用于淋洗 THCA 萃取物, 因为高浓度的有机溶剂会洗脱掉吸附剂

上的分析物。为使基质干扰最小化，在淋洗步骤 1 和 2 中分别使用了 10% 和 30% 的乙腈，用正己烷冲洗的目的也是如此。当单独使用并且量很小时 (200 μ L)，正己烷可以洗脱掉大多数脂类，同时不会导致分析物的解吸附，因为 THCA 的疏水性很强 ($\log P > 6$)，它可以强烈地保留在 Plexa 颗粒的疏水中心。在洗脱步骤中推荐延长浸润过程来强化溶剂和分析物之间的相互作用，从而提高分析物的回收率。

采用 Agilent Poroshell 120 EC-C 18 3 \times 50 mm, 2.7 μ m 色谱柱可以快速分离尿样提取物中的 THCA，并且可获得极佳的峰形 (如图 2 所示)。LC 分离首先采用较低有机溶剂比例 (30%) 的流动相，从而使尿液中的盐类和其它极性成分在样品运行开始时先被洗脱。由于流动相梯度较大，剩余的疏水干扰物质大部分先于分析物被洗脱出来，因此在目标峰洗脱出来的时候 (1.96 min) 就减小了基质效应。0.8 mL/min 的流速可以获得较短的分析时间和再平衡时间。每次样品运行开始时先将液流转换使其流至废液 (0 到 1.4 min)，以尽量减小离子源污染。在第 1.4 分钟时切换阀将液流转至质谱检测器并立刻开始数据采集。

SAMHSA 准则要求分析目标化合物和内标物时必须使用一个定量离子和至少一个定性离子。目标分析物的第三个转换离子 (见表 1) 信息可提供更高的定性能力。Agilent MassHunter Quantitative 软件可自动计算定性离子比率，并可以标出那些超出可接受范围的离子。

表 1. MRM 模式数据采集参数

化合物	母离子	子离子	裂解电压	碰撞能量
11-nor-9-羧基- Δ^9 -THC	343.2	299.2	135	18
	343.2	245.1	135	30
	343.2	191.1	135	33
11-nor-9-羧基- Δ^9 -THC-D ₉	352.2	308.2	145	18
	352.2	254.2	145	30
11-nor-9-羧基- Δ^9 -THC 葡萄糖苷酸	519.2	343.2	160	22
	519.2	299.2	160	36

根据上述步骤对尿样处理，在阴性尿液中加入 11-nor-9-羧基- Δ^9 -THC 葡萄糖苷酸至浓度为 1000 ng/mL，该样品在处理以后以负离子模式检测。我们发现只出现了一个非常大的 THCA 峰，远远超过了校准最高浓度 600 ng/mL 样品的色谱峰。这表明葡萄糖苷酸通过碱水解已经完全转化为 THCA。检测 11-nor-9-羧基- Δ^9 -THC 葡萄糖苷酸所用的 MS 参数列于表 1 中，供有兴趣检测水解效率的分析人员参考。

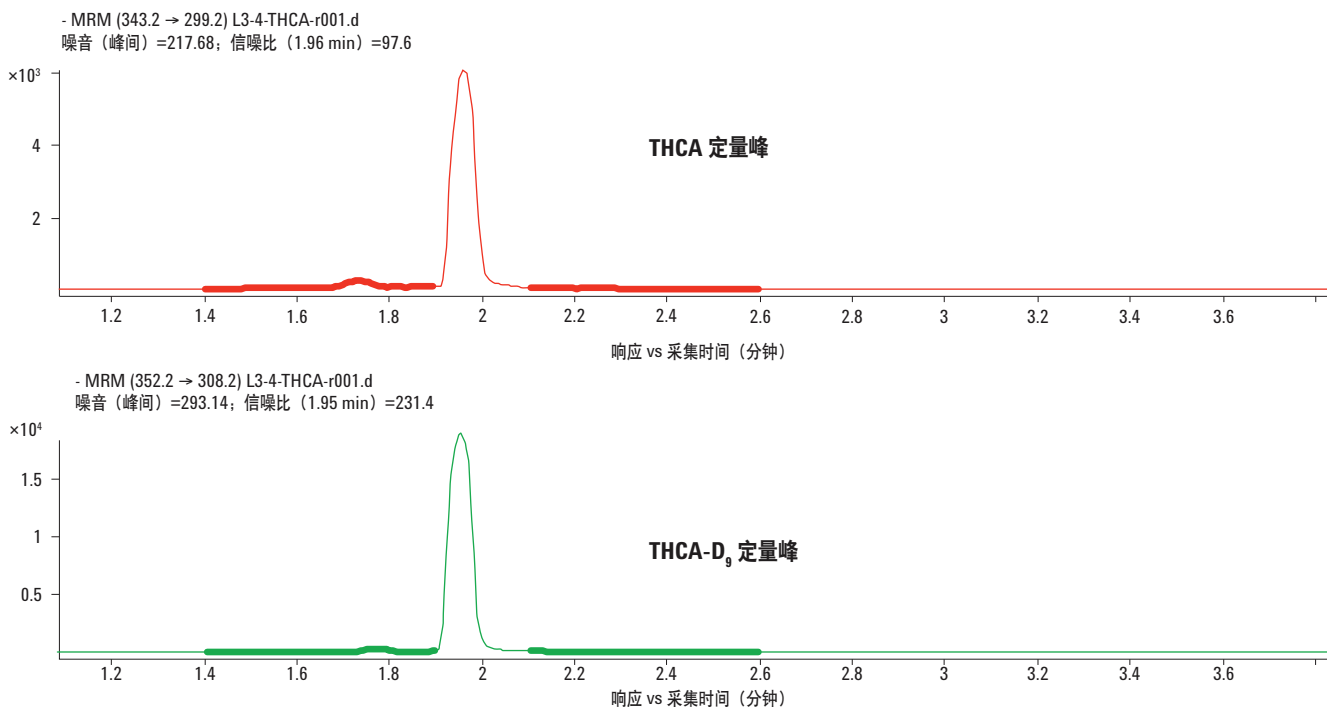


图 2. 尿液萃取物中 THCA (15 ng/mL) 和 THCA-D₉ (50 ng/mL) 的 MRM 提取离子色谱图。采用 Agilent Poroshell 120 EC-C18 3 \times 50 mm, 2.7 μ m 色谱柱。噪音区域以加粗的形式显示

本方法采用 MRM 扫描模式，设置为常态而非动态检测，因为动态 MRM 模式对单一化合物的检测没有优势。

由于具有强烈的疏水性，THCA 不仅可以附着在玻璃器皿上，而且还能附着在进样器部件和液相流路管线上。为了避免交叉污染，我们建议在运行了高浓度样品后运行一个空白流动相，并且使用自动进样器 Injector Flush Pump 选项。如果需要，冲洗针的时间可以从 10 秒提高到 20 秒。

在限量浓度为 15 ng/mL 条件下 THCA 的信噪比大约为 100:1 (如图 2, 上图所示), 表明了 Agilent 6460 Triple Quadrupole LC/MS 系统具有优异的性能, 并且对于远小于 SAMHSA 规定限量浓度的样品 (10%), 也能可靠地进行检测

图 3 展示了五个浓度水平萃取尿液标准品的校准曲线。通过向阴性尿液中加入 THCA 标准品, 配制浓度分别为 1.5、15、75、300 和 600 ng/mL 的一系列校准标准溶液。添加氘代内标 THCA-D9 的浓度是 50 ng/mL。R² > 0.999, 线性关系良好, 表明该方法在很宽的动态浓度范围内呈线性, 符合 SAMHSA 准则的要求。

方法评价

表 2 中方法的各项性能指标系根据 Matuszewski 等 (2003) 提出的原则计算得到的, 其作为行业标准用于评价 LC/MS/MS 分析方法已被广泛接受。LC/MS/MS 测试的样品及其萃取步骤如下: 第一组, 将限量浓度水平的 THCA 预加到阴性尿液中, 然后进行萃取, 平行五份, 进样分析。第二组, 将阴性尿液利用同样方法进行萃取, 然后萃取物用初始流动相溶液复溶, 之后加入 THCA 标准品到 15 ng/mL, 平行五份, 进样分析。第三组是直接加标至初始流动相 (复溶溶剂), 使其浓度为 15 ng/mL 的限量浓度 (流动相加标)。

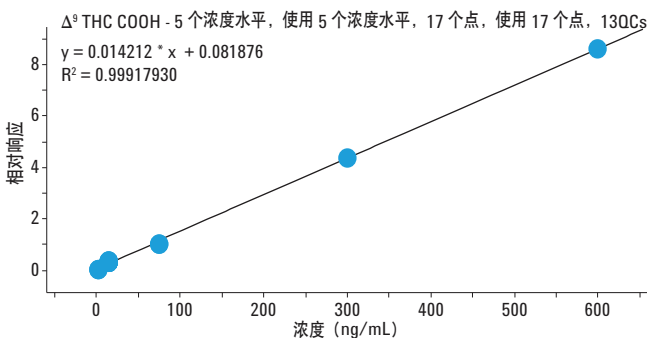


图 3. 尿液提取物中 THCA 的校准曲线示例。校准范围为 1.5 到 600 ng/mL。线性相关系数 R²>0.999

处理效率 (即绝对回收率) 是 SPE 萃取加标的阴性尿液后进样分析, 获得的目标分析物的峰面积与其无基质的流动相加标后进样分析获得的相应峰面积的比值。萃取回收率是 SPE 萃取加标的阴性尿液后进样分析, 获得的目标分析物的峰面积与经过相同方法萃取阴性尿液后的提取物加标样品进样分析获得的相应峰面积的比值。基质效应是分析 SPE 萃取阴性尿液后的提取物加标样品, 获得的目标分析物的峰面积与其流动相加标后分析获得的相应峰面积的比值。

准确度是通过校准曲线计算的分析物检测浓度与添加了已知量目标分析物样品的预期浓度的比值。精密度或变异系数 (CV) 用来衡量重复性, 系采用五个测量值的平均值计算其百分标准偏差获得的。

本法的特点是拥有较高回收率以及结果数据很高的准确度 (98%) 和精密度 (2.2%) (如表 2 所示)。超过 100% 的基质效应表明了离子增强作用, 而非离子抑制作用。仅 13% 的信号增强证明了 Plexa PCX 萃取物具有比较好的纯净度。鉴于大麻素类化合物分析的挑战性较大, 73% 的总处理效率可以说是非常高的。

表 2. 11-nor-羧基-Δ⁹-四氢大麻酚在限量浓度水平时的分析方法评价, n=5

	%
处理效率	73
萃取回收率	65
基质效应	113
准确度	98.2
精密度 (CV)	2.2

结论

本文所描述的固相萃取样品制备与 LC/MS/MS 检测相结合的分析方法完全遵循 SAMHSA 要求，为认证实验室的药物测试或其它有类似要求的分析环境的测试提供高重现性的有法律效力的数据结果。THCA 分析方法的硬件配置与其它安捷伦 SAMHSA 方法相同。这些方法可以在所有型号的 Agilent 1100 和 Agilent 1200 LC 系列仪器上运行，并且这些 LC 系统的背压不超过 400 bar。采用其它型号的安捷伦三重四极杆 LC/MS 系统应用本方法时，我们可以很容易地修改离子源参数。安捷伦科技可提供电子版的 LC/MS/MS 数据采集和定量分析方法。

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采用 Agilent Bond Elut Plexa PCX 和 Agilent Poroshell 120 色谱柱对全血中的丁丙诺啡和去甲丁丙诺啡进行 LC/MS/MS 分析

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摘要

法医和疼痛科实验室进行全血中丁丙诺啡和去甲丁丙诺啡的检测时，需要一种能在浓度低于 1 ng/mL 的水平内可靠检测这些化合物的分析方法。一种简单的样品净化步骤，结合使用离子对 468.2→55.1 和 414.2→83.1 的 LC/MS/MS 方法，可实现两种分析物的检测限（LOD）低于 0.1 ng/mL。每种分析物校正曲线的线性范围为 0.2 到 20 ng/mL，其 R^2 等于或大于 0.999。采用安捷伦新一代产品分析可获得更高的检测灵敏度。这些产品包括 Agilent Bond Elut Plexa PCX 混合模式聚合物 SPE 吸附剂、Agilent Poroshell 120 EC-C18 2.7 μm 表面多孔填料 LC 柱、Agilent 1200 Infinity LC 系统、以及配备了安捷伦喷射流技术（AJST）增强型电喷雾离子源的 Agilent 6460 三重四极杆 LC/MS 系统。

引言

丁丙诺啡是一种半合成的鸦片样药物，用于治疗慢性疼痛，以及作为美沙酮的替代品治疗阿片成瘾。作为镇痛剂，丁丙诺啡的效果大约是吗啡的 25 到 40 倍。丁丙诺啡的结构与吗啡相似，但疏水性更强（见图 1）。丁丙诺啡在肝脏中存在 N-脱烷基化反应，产生其主要活性代谢物——去甲丁丙诺啡。丁丙诺啡和去甲丁丙诺啡在血液中的浓度非常相近，50% 以上病例中的浓度低于 1 ng/mL (Selden 等, 2011)，给分析人员的检验带来了极大的挑战。此外，由于这些分析物分子结构稳定性强，碰撞诱导裂解产生的碎片量少，因此对这些化合物进行 MS/MS 分析很困难。为了实现检测灵敏度低于 1 ng/mL，检测这些化合物的分析方法不仅要有出色的 MS 性能，还要有一个回收率高、离子抑制低的有效样品净化程序。我们使用 SPE 处理的全血提取物洁净度高，较容易地实现了检测限低于 0.1 ng/mL。与其它聚合物吸附剂不同，所有 Agilent Bond Elut Plexa 系列产品的颗粒均具有不含氨基的羟基化表面，消除了对蛋白质的吸附，从而获得了最低的离子抑制和最高的检测灵敏度。又由于填料粒径分布窄且没有粉末造成堵塞，还实现了高流速和良好的重现性。

利用该方法可实现分析物的良好分离和极佳的峰形，这是采用 Agilent Poroshell 120 系列色谱柱进行分析的特色。采用表面多孔的 2.7 μm 填料，这些色谱柱具有与亚 2 μm UHPLC 色谱柱相似的柱效，但背压却降低了约 40%。这就允许用户甚至可以使用 400 bar LC 系统来提高分离度，并且通过使用更高的流速来缩短分析和再平衡时间。

本文定量分析使用的经鉴定具有最高丰度的新离子对是 468.2 > 55.1 (丁丙诺啡) 和 414.2 > 83.1 (去甲丁丙诺啡)。该方法仅需 0.5 mL 血样，采用 10 μL 较低的进样体积和提取时 5 倍预浓缩，即可在 0.2 ng/mL 浓度时实现良好的信噪比：丁丙诺啡为 84 : 1，去甲丁丙诺啡为 20 : 1 (见图 2)。

实验部分

分析物

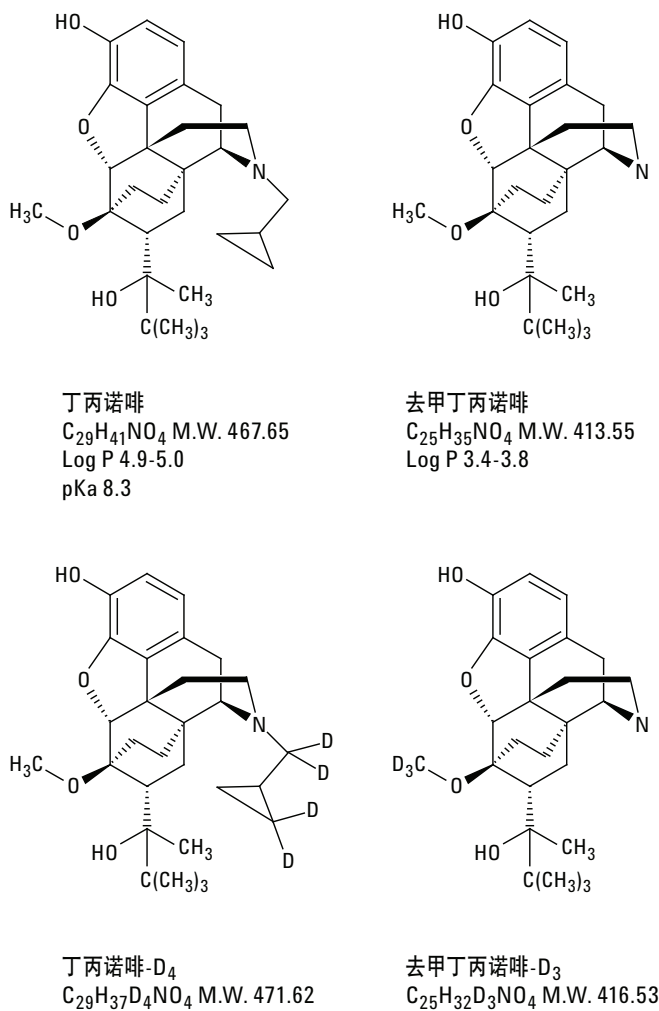


图 1. 分析物丁丙诺啡和去甲丁丙诺啡及其结构。log P 和 pKa 值来自 SRC 和 PubChem

药物标准品为 1 mg/mL (丁丙诺啡、去甲丁丙诺啡) 和 100 $\mu\text{g/mL}$ (丁丙诺啡-D₄ 和去甲丁丙诺啡-D₃) 的甲醇溶液，购自 Cerilliant 公司。

材料和仪器

SPE

- Agilent Bond Elut Plexa PCX 小柱, 30 mg, 3 mL (部件号 12108303)
- 安捷伦真空多管装置 VacElut 20 (部件号 12234100)
- 安捷伦活塞阀 (部件号 12234520)
- 安捷伦硅烷化自动进样瓶, 2 mL (部件号 5183-2072)
- 安捷伦样品瓶内插管, 250 μ L, 脱活玻璃, 聚合物底座 (部件号 5181-8872)
- 安捷伦螺旋盖, 用于 AS 样品瓶 (部件号 5182-0717)

LC

- Agilent Poroshell 120 EC-C18, 3 \times 50 mm, 2.7 μ m 色谱柱 (部件号 699975-302)
- Agilent 1260 Infinity LC 系统 (G1379B 微量脱气机、1312B 低延迟体积二元泵、G1367E 自动进样器、G1330B 柱温箱)

MS

- Agilent 6460A 三重四极杆 LC/MS 系统, 配备 AJST 电喷雾离子源

样品制备

预处理

1. 将适量 ISTD 加入 0.5 mL 血样中, 使浓度为 10 ng/mL, 或采用 0.1 M 磷酸盐缓冲液 (pH 6.0) 配制 10 ng/mL 的 ISTD 溶液, 并在每份血样中加入 0.5 mL 此内标缓冲液。推荐使用甲醇清洗并晾干的 12 \times 75 mm 玻璃管
2. 加入 ISTD 后, 加入 2 至 2.5 mL 磷酸盐缓冲液 (血样至少被稀释了 5 倍)
3. 涡旋后离心, 使沉淀完全

萃取

1. 采用 0.5 mL 甲醇活化 Bond Elut Plexa PCX 小柱浸泡, 然后使其自然滴落
2. 上样/使用 Pasteur 玻璃吸量管吸取上清液
3. 淋洗 1: 2 \times 2 mL 2% 甲酸溶液
4. 淋洗 2: 3 mL 甲醇/2% 甲酸溶液 (70 : 30)
5. 真空干燥 5-10 min (10-15 in Hg)

6. 用 1.5 mL 乙酸乙酯/异丙醇/氨水 (80/20/5) 混合液洗脱。在洗脱即将开始前加入氨水。将洗脱液分成两等份, 依次浸泡吸附剂。关闭活塞阀浸泡约 0.5 min 后, 打开活塞阀让洗脱液在重力作用下滴入收集瓶中。当洗脱液不再滴落时, 使用低真空抽取吸附剂小孔中的洗脱液
7. 45 $^{\circ}$ C 下氮气吹干
8. 用 0.1 mL 初始流动相 (15% 甲醇、85% 水、0.1% 甲酸) 复溶残渣, 涡旋后转移至带聚合物底座的样品瓶内插管中

LC/MS/MS

LC 条件

流动相 A	0.1% 甲酸水溶液	
流动相 B	0.1% 甲酸甲醇溶液	
流速	0.8 mL/min	
梯度程序	时间 (min)	% B
	0.0	15
	2.0	70
	2.1	95
	5.5	95
	5.51	15

停止时间	5.6 min
后运行时间	2 min
最大泵压	400 bar
进样体积	10 μ L
带针头清洗的进样	
针头清洗	用甲醇-水 (95 : 5) 冲洗针座 10 s
禁止重叠进样模式	
无自动减小延迟体积	

MS 条件

ES 离子源参数

离子化模式	正离子
毛细管电压	2800 V
干燥气流量	10 L/min
干燥气温度	350 $^{\circ}$ C
雾化气压力	35 psi
鞘气流量	12 L/min
鞘气温度	350 $^{\circ}$ C
喷嘴电压	0 V

MS 参数

扫描方式	MRM
预运行程序	SCP_MS Diverter Valve To Waste() {MH_Acq_Scripts.exe}
时间段	#1: 1.8 min - 转换阀至 MS
EMV (+) 增益	400 V

表 1 展示了目标化合物及其同位素内标物 MRM transitions 的定量离子和定性离子。

表 1. MRM transitions

化合物名称	母离子	MS1 的分辨率	子离子	MS2 的分辨率	碎裂电压	碰撞能量
丁丙诺啡	468.3	Unit	55.1	Wide	200	62
丁丙诺啡	468.3	Unit	396.2	Wide	200	45
丁丙诺啡-D ₄	472.3	Unit	59.1	Wide	200	62
丁丙诺啡-D ₄	472.3	Unit	400.2	Wide	200	45
去甲丁丙诺啡	414.3	Unit	83.1	Wide	188	60
去甲丁丙诺啡	414.3	Unit	57.1	Wide	188	50
去甲丁丙诺啡-D ₃	417.3	Unit	83.1	Wide	188	60
去甲丁丙诺啡-D ₃	417.3	Unit	57.1	Wide	188	50

结果与讨论

在低 pH 值条件下, 丁丙诺啡和去甲丁丙诺啡的叔胺官能团被质子化, 通过疏水作用和强阳离子交换作用, 它们在 Agilent Bond Elut Plexa PCX 聚合物吸附剂上获得较强的保留。

使用 100% 甲醇淋洗会从 SPE 柱上冲下部分分析物而造成损失。有效去除基质干扰物而又不损失样品的最佳淋洗条件为甲醇-2% 甲酸溶液 (70 : 30)。在有机洗脱液中加入强碱以破坏分析物和强阳离子交换吸附剂之间的离子相互作用。在样品洗脱开始前添加 5% 氨水到乙酸乙酯: 异丙醇混合洗脱液 (80 : 20) 中, 丁丙诺啡和去甲丁丙诺啡可以得到最佳的回收率。推荐使用两步洗脱法并结合浸泡步骤, 以增强溶剂-分析物的相互作用并提高样品回收率。

由于疏水性强, 丁丙诺啡和去甲丁丙诺啡可能粘附到玻璃器皿、LC 管路和进样器部件中, 这也是为什么我们推荐在 LC 方法中使用 95% 甲醇冲洗色谱柱和使用甲醇-水 (95 : 5) 冲洗针座的原因。采用去活处理的样品瓶/内插管和甲醇清洗并晾干的玻璃器皿 (STD 和 ISTD 稀释液用的管和瓶子) 也确保了结果的良好重现性。

LC 分离开始时特意以相对较低比例的有机相 (15%) 进行洗脱, 以使血样中的盐和其它极性化合物在样品运行初期即被洗脱下来。0.8 mL/min 的流速使分析时间和再平衡时间都较短。每次样品运行均将开始部分的洗脱液 (0 到 1.8 min) 导入废液中, 以最大程度地降低对离子源的污染。1.8 min 时开始数据采集, 即转换阀切换后立即开始。

图 2 为丁丙诺啡和去甲丁丙诺啡在 0.2 ng/mL LOQ 时及其氘代内标物在 10 ng/mL 时的色谱图。

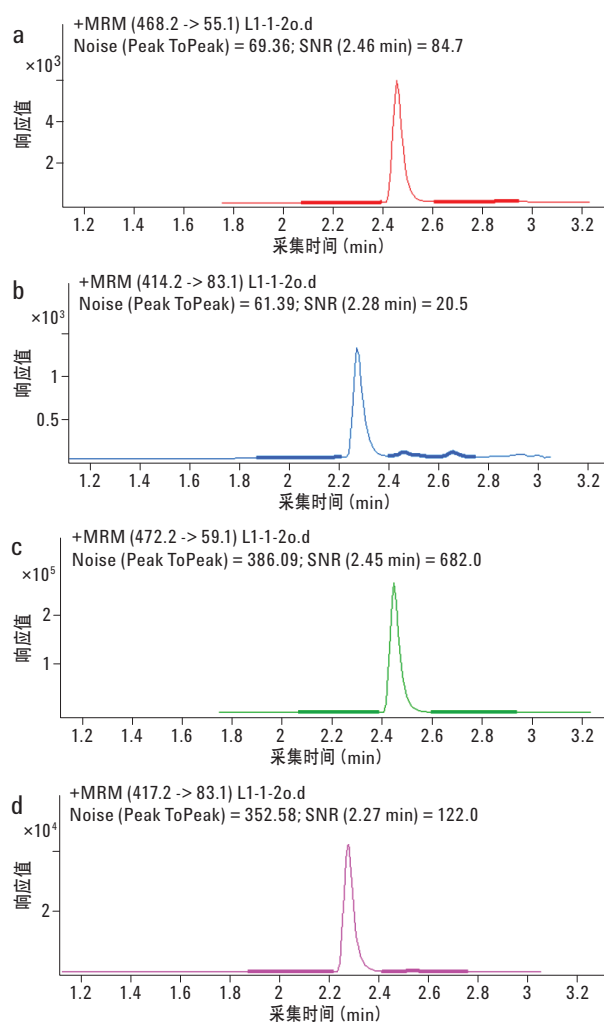


图 2. MRM 萃取离子色谱图: a – 丁丙诺啡, b – 去甲丁丙诺啡 (均为 0.2 ng/mL), c – 丁丙诺啡-D₄, d – 去甲丁丙诺啡-D₃ (均为 10 ng/mL)。全血提取物经 Agilent Bond Elut Plexa PCX 处理, 采用 Agilent Poroshell 120 EC-C18 3 × 50 mm, 2.7 μm 色谱柱分析。噪音区域加粗显示

丁丙诺啡和去甲丁丙诺啡这两种化合物分子离子的高稳定性给 MS/MS 检测造成了困难, 这导致许多研究人员采用 SIM 模式对它们进行定量 (Scislowski et al, 2005; Concheiro et al, 2009), 或者在 SRM 模式下采用较高的碰撞能量 (无碎片) 监测分子离子的转换离子 (Kronstrand et al, 2003; Selden et al, 2011)。与选择性更强的母离子-子离子对定量相比, 这种方法可靠性差。并且, 这种方法有较低的信噪比, 从而具有较高的 LLOQ。利用 Agilent 6460 三重四极杆 LC/MS 系统 (表 1) 在高碰撞能量水平下获得了一种新的稳定碎片类型, 两种分析物在 LLOQ 为 0.2 ng/mL 时均可实现可靠定量。丁丙诺啡丰度最高的碎片是 m/z 55.1 的甲基环丙基 (C_4H_7) 基团。它通过丁丙诺啡- D_4 的 m/z 59.1 碎片得以鉴定确认。去甲丁丙诺啡丰度最高的碎片离子 (m/z 83.1) 可能来自母离子的侧支链, 包括叔丁基基团 ($(CH_3)_3C$)。与最佳碰撞能量下获得的最常用碎片离子相比, 丁丙诺啡的 m/z 55.1 离子的丰度比 m/z 396.2 高 8 倍, 而去甲丁丙诺啡 m/z 83.1 离子的丰度比 m/z 101.1 高 2 倍。

表 1 中列出的 MRM transitions 是两种目标化合物及其氘代 ISTD 的一个定量和一个定性离子。Agilent MassHunter 软件自动计算定性离子的比率, 突出显示那些超出可接受范围的离子。

常态或动态 MRM 采集模式在这种方法下均可使用。

LLOQ 水平为 0.2 ng/mL 时的信噪比分别为丁丙诺啡 84 : 1, 去甲丁丙诺啡 20 : 1 (图 2, a 和 b)。这证明了本样品净化程序的高效性和 6460 三重四极杆质谱出色的检测灵敏度, 从而能够实现现在低于 0.1 ng/mL 的 LOD 下检测这些分析物。

图 3 展示了 5 种浓度全血标样中提取的丁丙诺啡和去甲丁丙诺啡的典型的校正曲线。校正标样由加标分析物至全血中制得, 使浓度分别为 0.2、1、5、10 和 20 ng/mL。添加氘代内标物的浓度为 10 ng/mL。每条曲线出色的线性拟合 ($R^2 > 0.999$) 证明了本方法线性良好。未采用加权处理, 曲线拟合中包括了起点数据。

表 2 展示了五个全血样品的回收率 (准确度) 和精密度 (CV, 或 RSD) 数据, 其每个分析物的加标浓度均为 1 ng/mL。通过对比加标混合标样获得的校正曲线进行定量分析 (图 3)。

表 2 每个分析物在浓度为 1 ng/mL 时进行的方法验证, $n = 5$

	准确度 (%)	CV %
丁丙诺啡	94.2	0.6
去甲丁丙诺啡	103	0.2

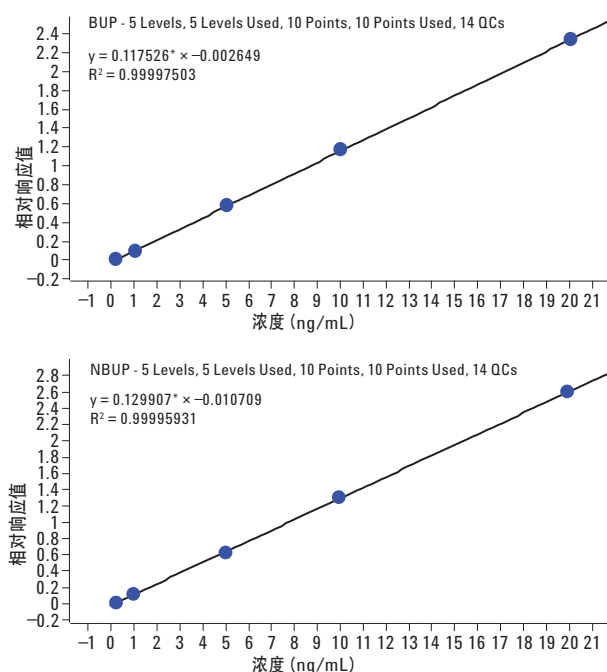


图 3 全血提取物中丁丙诺啡和去甲丁丙诺啡的典型校正曲线。浓度范围 0.2 至 20 ng/mL。线性拟合 $R^2 > 0.999$

结论

本文介绍了一种简单的、固相萃取结合 LC/MS/MS 检测的方法, 实现了全血中浓度低于 0.2 ng/mL 的丁丙诺啡和去甲丁丙诺啡的定量分析。本方法专为安捷伦 1100 和 1200 LC 系列仪器的用户开发, 因为其 LC 系统的背压不能超过 400 bar。如采用其它型号的安捷伦三重四极杆 LC/MS 系统仪器运行本方法, 离子源参数可以很容易修改。由于样品提取物的净化度高, 同时使用经高丰度碎片离子重新鉴定的离子转换通道的耐用的 MS 检测, 使本方法获得了较低检测限。

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使用 Agilent Poroshell 120 色谱柱快速筛选 β -受体阻滞剂的液相色谱分析方法

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引言

β -受体阻滞剂，即 β -肾上腺素受体阻断剂，是用于治疗高血压和由心脏病引起的心率失常的一类药物。 β -肾上腺素受体拮抗剂能减少肾上腺素与其他应激激素的作用。1958 年，礼来实验室合成了第一个 β -受体阻滞剂。而到 1962 年，第一代 β -受体阻滞剂—普萘洛尔和萘心定研制成功并应用于治疗心绞痛，这在临床上意义重大。

β -受体阻滞剂能抑制肾上腺素和去甲肾上腺素的作用，特别是 β -肾上腺素受体（交感神经系统的一部分，用于介导应急反应）。 β -肾上腺素受体包括三种类型，即 β_1 受体、 β_2 受体和 β_3 受体。 β_1 受体主要分布于心肌及肾脏。 β_2 受体主要存在于肺、胃肠道、肝脏、子宫、血管平滑肌和骨骼肌。 β_3 受体主要存在于脂肪细胞 [1、2]。

β -受体阻滞剂可减弱心肌收缩、降低心率及减少冠状动脉血流量，易被滥用于射击等活动量少的运动中。因此，它们被国际奥委会列为禁用药物 [3]。

选择性是优化 HPLC 色谱分离的最有效工具。这个参数随着键合相（包括 C18、C8、极性包埋、苯基键合相）和流动相的改变而改变。本研究中采用 Agilent Poroshell 120 色谱柱与 Agilent 1260 Infinity 方法开发解决方案来快速评估 β -受体阻滞剂分析方法的开发与选择。短柱长和高柱效使得分析时间和平衡时间都大大缩短，从而加速了对选择性的研究 [4]。

实验部分

采用 Agilent 1200 Infinity 系列 LC 多方法解决方案。该系统包括：

- 1260 Infinity 二元泵 (G1312B)
- 1290 Infinity 柱温箱 (G1316C)
- 1260 Infinity 高性能自动进样器 (G1367E)
- 1290 Infinity 二极管阵列检测器 (G4212A)，配备 10mm 最大光强卡套式流通池
- G6140 单四级杆质谱仪

Agilent 1200 Infinity 系列 LC 多方法解决方案是一个高度灵活的系统，最多可容纳 4 种色谱柱 (100 mm)。此外，Agilent ChemStation Method Scouting Wizard (方法筛选向导) 可自动配置方法和序列，以筛选可用的色谱柱组合、溶剂、预设梯度和温度。本实验中采用 4 种 Agilent Poroshell 120 色谱柱：

- Agilent Poroshell 120 StableBond SB-C18, 2.1 × 100 mm, 2.7 μm (部件号: 685775-902)
- Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 μm (部件号: 695775-902)
- Agilent Poroshell 120 Bonus-RP, 2.1 × 100 mm, 2.7 μm (部件号: 685775-901)
- Agilent Poroshell 120 Phenyl-Hexyl, 2.1 × 100 mm, 2.7 μm (部件号: 95775-912)

柱温箱 (TCC) 配备了一个 6 位/4 孔切换阀。这是一种新型的快速切换阀，安装于滑动式轨道上，使得连接与维修更方便。孔 1 与 StableBond C18 色谱柱相连，孔 2 与 EC-C18 色谱柱相连。孔 3 与 Bonus-RP 色谱柱相连，孔 4 与 Phenyl-Hexyl 色谱柱相连，孔 6 则连与短路的连接毛细管相连。

通过每个色谱柱的溶剂经过四个低扩散热交换器中的一个进行独立加热。一个额外的 12 溶剂选择阀连接于 1260 Infinity 二元泵的 A1 阀位。加上 1260 Infinity 二元泵内部的溶剂选择阀，该系统可同时筛选 15 种溶剂。流动相为甲醇和 10 mM 的甲酸铵 (用甲酸调 pH 值至 3.8)。水做为最后的弱溶剂，冲洗色谱柱中的改性剂，以保持合适的色谱柱存储体积。甲酸铵与甲酸购自 Sigma

公司。采用 Milli-Q 18 MΩ 的水。甲醇在整个过程中作为强溶剂使用，购自 Honeywell 公司。温度控制在 25 °C，流速设置为 0.4 mL/min。采用 B.04.02 版 Agilent ChemStation 进行仪器控制 and 数据处理。

本研究测定的化合物包含纳多洛尔、阿替洛尔、阿普洛尔、醋丁洛尔、吲哚洛尔、普萘洛尔、美托洛尔和拉贝洛尔，均购自 Sigma Aldrich 公司。各化合物的结构如图 1 所示。这些基本化合物的 pKa 值范围在 8.8~9.7。每个化合物均配制成 10 mg/mL 的 DMSO 溶液，再用水稀释至 0.1 mg/mL。

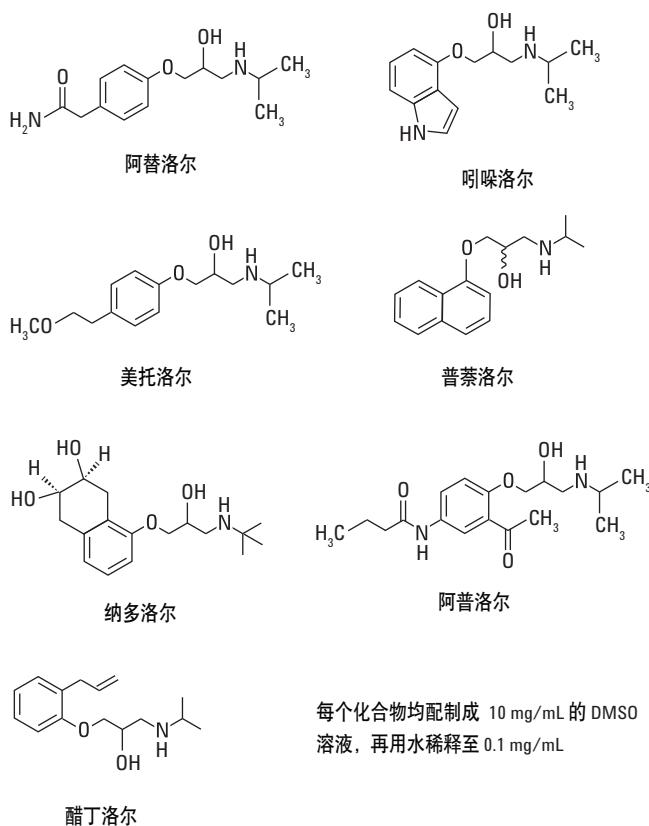


图 1. 几种 β-受体阻滞剂的化学结构

选择合适的色谱柱以提高选择性

选择色谱柱以提高分离选择性。这些色谱柱包括：Poroshell 120 EC-C18，高度封端，是方法开发的首选；Poroshell 120 StableBond SB-C18，无封端，可与硅醇基相互作用，是使用中等 pH 到低 pH 流动相时可选的另一种 C18 固定相；Poroshell 120 Bonus-RP（极性氨基柱）和 Poroshell 120 Phenyl-Hexyl（苯基-己基柱）。众所周知，苯基键合相能够提高对芳香族化合物的选择性。

在疏水性的 C14 烷基链中插入极性包埋基团能够使得 Bonus-RP 固定相完全键合于多孔 Poroshell 120 上，从而使极性样品与硅醇基之间的作用最小化，为各种应用提供对称的色谱峰。这种固定相在中等 pH 条件下效果尤其明显，由于此时胺类与电离的硅醇基之间存在强烈的相互作用。嵌入极性基团还有助于湿润疏水链以防止水相比例过高造成固定相塌陷。

Poroshell 120 Bonus-RP 与普通的 C18 柱适用范围基本一致，并克服了普通 C18 柱的一些缺陷，如不适用于高水相。此外，它对能够发生疏水作用和能够与酰胺基发生氢键作用的分子具有更好的保留性。与单一的烷基固定相相比，Bonus-RP 中的极性基团（氢键受体）与氢键供体（如酚类和酸）可产生很强的氢键作用，从而提高对酚类、有机酸及其他溶质的保留性和选择性。Bonus-RP 的保留性较 C18 柱略小，无需改变流动相条件即可对两种色谱柱进行比较。Bonus-RP 色谱柱为极性化合物提供了不同于 C18 烷基键合固定相的选择性。它还可在 100% 水相条件下使用。

苯基-己基色谱柱具有独特的反相选择性，尤其是对于极性芳香族和杂环化合物。这是由于其键合相的芳香环及其离域电子可与分析物相互作用。Poroshell 120 苯基-己基柱提供与 C18 和 Bonus-RP 色谱柱不同的选择性。对于带有吸电子基团如氟或硝基的芳香类溶质，会得到更好的保留性和选择性 [5、6]。

Poroshell 120 苯基-己基柱为含有芳香族基团的化合物提供独特的选择性和优越的分离度。Poroshell 120 苯基-己基柱也能为中等极性的化合物提供最佳的分离效果，而典型的烷基键合固定相（C18 和 C8）对这类化合物的分离效果不佳。乙腈可减小芳香族、极性化合物和苯基-己基固定相之间的 $\pi-\pi$ 作用力，而甲醇却能增强它们之间的作用力，提高保留性和选择性 [7]。这并不意味着乙腈不能用于苯基键合固定相，或者说它不能提供一个令人满意的分离效果，而是甲醇会使苯基柱的选择性更好。

结果与讨论

由图 2 可见, 7 种化合物在使用的色谱柱中均得到了分离。醋丁洛尔与普萘洛尔在 Poroshell 120 EC-C18 色谱柱上洗脱时间很接近, 而纳多洛尔出现了相同分子离子的双峰。这是由于纳多洛尔存在非对映异构体。Poroshell 120 SB-C18 色谱柱与 Poroshell 120 EC-C18 的分离效果几乎相同。这种现象在 Poroshell 120 Bonus-RP 色谱柱上并未出现, 因为它将次级相

互作用降到最低。与 C18 柱相比, Poroshell 120 Bonus-RP 色谱柱使峰 6 与峰 7 (普萘洛尔和阿普洛尔) 的流出顺序颠倒。Poroshell 120 苯基-己基柱除了使峰 6 和峰 7 顺序颠倒外, 峰 4 和峰 5 (美托洛尔和醋丁洛尔) 的顺序也发生了变化。其他的 C18 色谱柱分离效果与图示相似但保留不同。

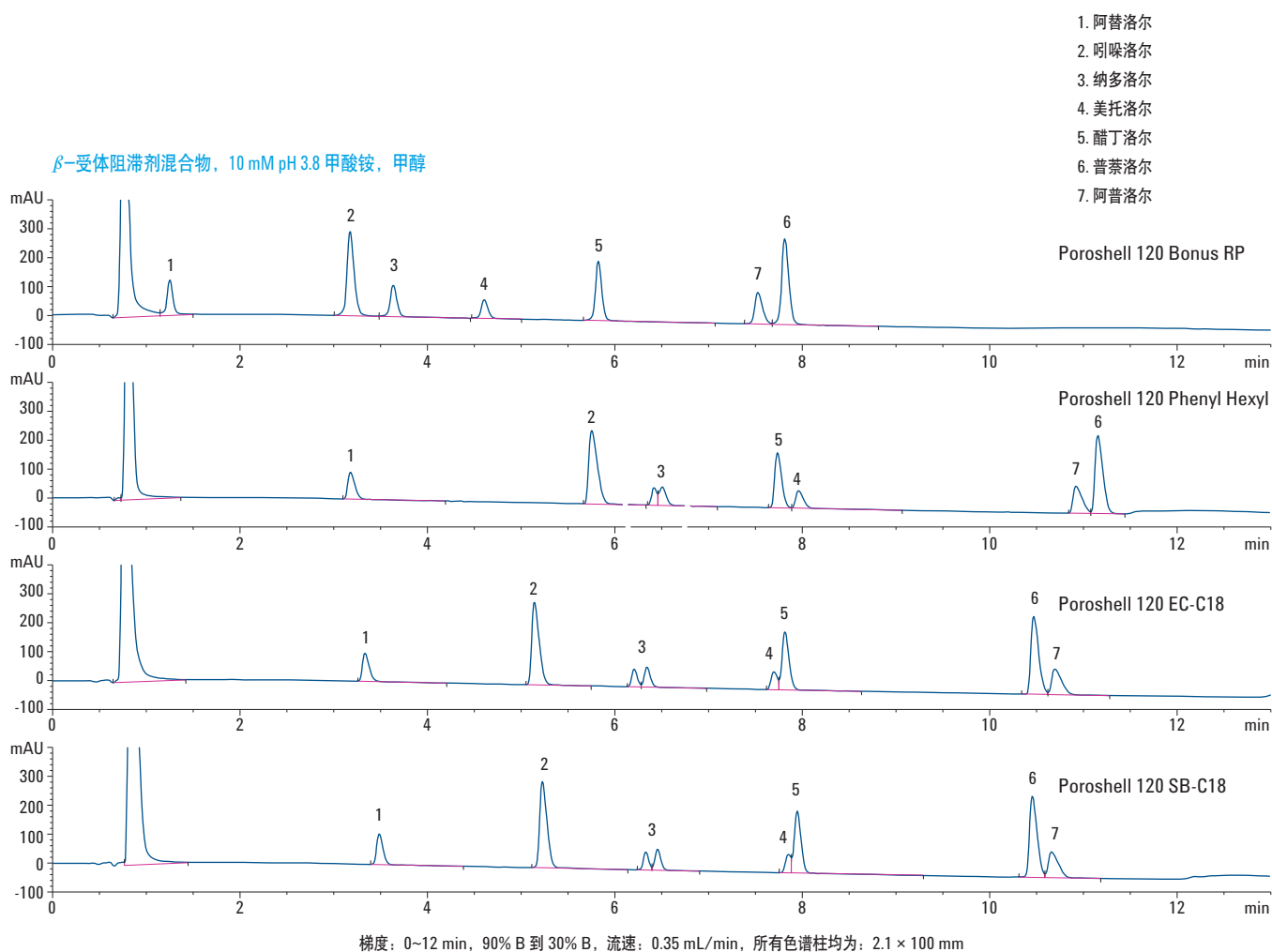


图 2. 采用 Agilent Poroshell 120 色谱柱分离 β -受体阻滞剂

结论

采用本应用研究中的逐步分析方法开发方案，分析问题可以很快得到解决。本研究以 β -受体阻滞剂为例，说明可以使用具有不同选择性的不同固定相来优化分离。虽然 Poroshell 120 EC-C18 和 SB-C18 色谱柱能够提供足够的分离度，但是采用另一种选择性不同的色谱柱，如 Poroshell 120 Bonus-RP 色谱柱，却能得到更好的结果而且还可用于数千个样品。使用 Agilent 1200 Infinity 系列 LC 多方法解决方案能够自动进行 Poroshell 120 色谱柱的方法和序列设置。

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Analysis of Water Soluble Vitamins in Multivitamin Tablets Using Poroshell 120 EC-C18

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Abstract

A method separating nine multivitamin compounds originally developed on Agilent ZORBAX Eclipse Plus C18 columns of various sizes is transferred to an Agilent Poroshell 120 EC-C18 4.6 mm × 75 mm, 2.7 μm column using an Agilent 1260 Rapid Resolution LC. The gradient and flow rate are scaled, maintaining retention index with the objective of determining the optimal flow rate for each column evaluated. The new separation keeps the analysis time constant at 5 minutes and allows the use of a longer column with a higher flow rate and still maintains the pressure under 400 bar. Sample preparation used is dissolution of a tablet in water followed by either filtration through a 0.45 micron syringe filter or centrifugation. Over 800 injections were made without substantially increasing pressure.

Introduction

A compound is called a vitamin when it cannot be synthesized in sufficient quantities by an organism, and must be obtained through diet. Vitamins have diverse biochemical functions. Many vitamins function as precursors for enzyme cofactors, that help enzymes in their work as catalysts in metabolism. Vitamins may also be less tightly bound to enzyme catalysts as coenzymes, detachable molecules that function to carry chemical groups or electrons between molecules.

The two types of vitamins are classified by the materials in which they will dissolve. Fat-soluble vitamins, such as vitamins D, or E, dissolve in fat before they are absorbed in the blood stream to carry out their functions. Excesses of these vitamins are stored in the liver. Because they are stored, they are not needed every day in the diet.

By contrast, water-soluble vitamins dissolve in water and are not stored; they are eliminated in urine. We need a continuous supply of them in our diets. The water-soluble vitamins are the B-complex group and vitamin C.

The B-complex group: thiamin, riboflavin, niacin, pyridoxyl phosphate, folic acid, cobalamin, pantothenic acid and, in addition, ascorbic acid, or vitamin C, are widely distributed in foods. Riboflavin, for example, can be obtained from liver, milk, dark green vegetables, whole and enriched grain products, eggs. Their influence is felt in many parts of the body. They function as coenzymes that help the body obtain energy from food. They also are important for normal appetite, good vision, healthy skin, healthy nervous system and red blood cell formation.

Until the mid-1930s, when the first commercial yeast-extract and semi-synthetic vitamin C supplement tablets were sold, vitamins were obtained solely through food intake, and changes in diet (following a bad harvest) can alter the types and amounts of vitamins ingested. Vitamins have been produced as commodity chemicals and made widely available as inexpensive synthetic-source multivitamin dietary supplements, since the middle of the 20th century [1].

In addition to containing vitamins of interest, other excipient materials such as cellulose, maltodextrin, dextrin, gelatin, dextrose, soy lecithin are formulated into the tablets. Given the large size of the tablet and relatively small quantities of many of the vitamins, clogging problems have plagued this analysis when it has been attempted on small particle size columns.

Reverse Phase HPLC is well suited for vitamin analysis. Qualitative and Quantitative analysis of vitamins is important for clinical, food and pharmaceutical applications. Using a previously described method developed on an Agilent ZORBAX Eclipse Plus C18, 1.8 μm column, a fast separation method is converted to be used on an Agilent Poroshell 120 EC-C18 column [2]. The new method remains fast, but is less prone to clogging by excipients found in multivitamins.

In this work, a method using an Agilent Eclipse Plus C18 4.6 mm \times 50 mm, 1.8 μm column is converted to use an Agilent Poroshell 120 EC-C18 4.6 mm \times 75 mm column. Injection volume is increased and the risk of clogging is reduced.

Experimental

An Agilent 1260 Rapid Resolution LC (RRLC) system was used for this work:

- G1312B Binary Pump SL with mobile phase A: 25 mM Sodium Phosphate pH 2.5 in Water and B: Methanol. The gradient started at 1% B, held at that concentration, then ramped to 12% B and finally 30% B, held at that concentration, and then re-equilibrated to the initial condition. The system is configured with the pulse damper and standard mixer installed.
- G1367E Automatic Liquid Sampler (ALS) SL. Injection volume of 5 μL was used.
- G1316B Thermostatted Column Compartment (TCC) SL with temperature set to 35 $^{\circ}\text{C}$.
- G4212C Diode Array Detector (DAD) SL with the signal set to 230, 4 nm and reference not used, using a G4212-60008 micro flow cell (10-mm path, 1- μL variance).
- ChemStation version B.04.02 was used to control the HPLC and process the data.
- Agilent Poroshell 120 EC-C18, 4.6 mm \times 75 mm, 2.7 μm , p/n 697975-902

The compounds of interest are shown in Reference 2, with their respective structures. Compounds were dissolved in water at 1 mg/mL and used for identification. The following compounds were purchased from Sigma Aldrich: thiamin (vitamin B₁), riboflavin (vitamin B₂), niacin, vitamin B₆, folic acid, vitamin B₁₂, biotin, pantothenic acid, and ascorbic acid. Additionally purchased from Sigma Aldrich (Bellefonte, PA) was the Sodium Monophosphate and Phosphoric Acid. Methanol was purchased from Honeywell, Burdick and Jackson High Purity, (Muskegon, MI). Water used was 18 M Ω WMilli-Q water (Bedford, MA). A multivitamin tablet was purchased at a local pharmacy. (One a Day Women's Active Metabolism, Bayer HealthCare, Morristown NJ.).

Tablets were dissolved by grinding them individually using a mortar and pestle and transferring the entire amount (about 1.6766 g/tablet) with 100 mL water to a 150 mL plastic coated bottle. The bottle is then sealed and shaken vigorously for 5 min. A cloudy solution is produced which is clarified by either filtration (using a 0.45 μm , 30 mm regenerated cellulose filter (p/n 5061-3364) and a 10 mL syringe or by centrifugation in a polypropylene tube (6000 rpm for 5 min). The resulting clear solution is then transferred to an Agilent MS Analyzed Write-On Vial (p/n 5190-2278).

Results and Discussion

In transferring this method from the original method, a longer column was chosen and used at a proportionally higher flow rate. Previous work has shown that higher peak capacity for an Agilent Poroshell 120 EC-C18 (75 mm instead of the previously used 50 mm column) can be obtained at higher linear velocity (1.5 mL/min instead of 1 mL/min) [3]. In addition, a larger injection volume, which should be proportional to column volume, would be possible on the larger Poroshell column. The selectivities of an Agilent ZORBAX Eclipse Plus C18 (75 mm instead of the previously used 50 mm column) and Poroshell 120 EC-C18, have been shown to be very similar in previous work [4,5]. In addition, a larger column would be less affected by extra column effects such as additional tubing [6] required if a cooled autosampler was used, as recommended in a previous note on analysis of water soluble vitamins by Huber [7]. In this work, a slight change in elution order is noted at the beginning of the gradient. This could be the result of the change in column porosity (from fully porous to superficially porous), the

dwell volume of the instrument, or the equilibration time between runs. Figure 1 shows a representative sample chromatogram, scaled to the largest peak. In the upper left corner, an expanded chromatogram is shown that reveals the lower intensity peaks. The chromatographic conditions, as well as the list of components in their elution order, are found on the right.

Over 800 injections of a vitamin tablet extract were made on this column without an increase in pressure. This is due in part to the 2 μm column inlet frit, which is less likely to clog than the smaller porosity frits used on sub 2-micron and totally porous 3 μm columns [8]. At least 100 injections were made using the centrifuge sample clarification method without any change in system pressure. In this case, filtration was found to be a faster method, requiring fewer steps in producing a final sample. The 0.45 μm regenerated cellulose filter presents less resistance than a 0.2 μm filter required for use with a sub 2-micron column. A plot describing pressure changes per injection is shown in Figure 2. An increase in pressure from 177 to 179 bar is noted over 3 days and 6 L of phosphate buffer.

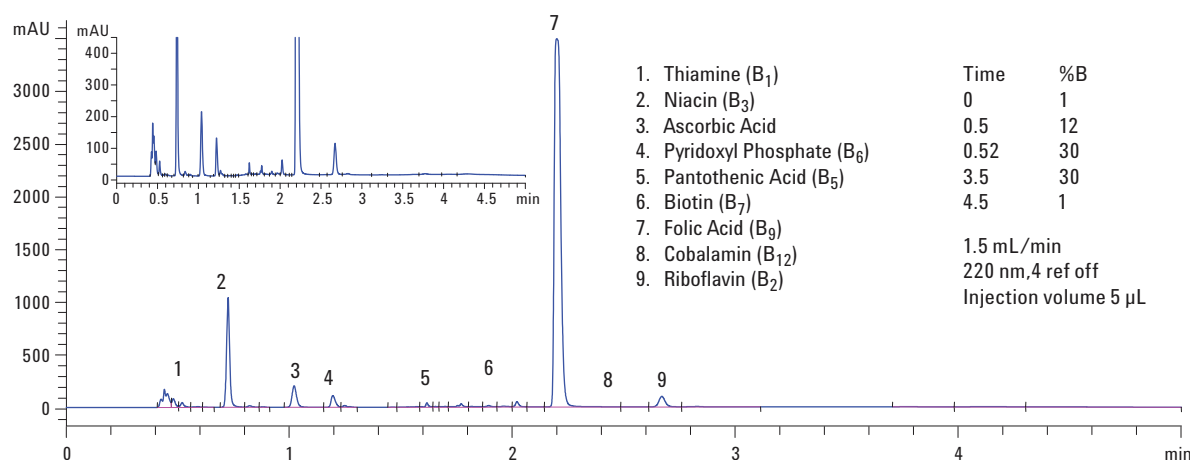


Figure 1. Elution order water soluble vitamins.

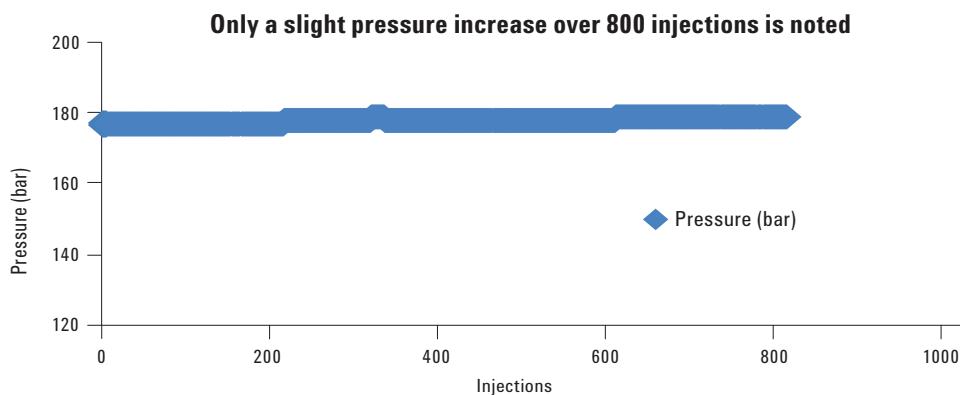


Figure 2. Agilent Poroshell 120 EC-C18 pressure remains nearly constant after 800 injections water soluble vitamin tablet.

Other wavelengths could also be used for this analysis, as several of these compounds form yellow or even red solutions in water, indicating absorbance in the visible region of the spectra [9]. Care should be taken when using a reference wavelength as an improper choice could lead to higher reference absorbance than in the analytical wavelength and to negative peaks.

Conclusion

HPLC columns packed with superficially porous particles offer many advantages over columns packed with conventional, fully porous particles. The superficially porous 2.7- μm Agilent Poroshell 120 EC-C18 offers similar efficiency and selectivity to the 1.8 μm Agilent ZORBAX Eclipse Plus C18 column, without the high back pressure. The 2 μm frit has demonstrated a resistance to clogging through the analysis of over 800 samples.

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Pharmaceuticals in Whole Blood Analyzed on Agilent Poroshell 120, with Modified Mini-Extraction for Sample Preparation

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Abstract

A convenient analytical method for determination of pharmaceuticals in various therapeutic categories in whole blood involves the addition of acetonitrile and salts to a small amount of blood. The mixture is shaken and centrifuged for extraction/partitioning, which removes water and proteins from the sample. An aliquot of the organic layer is cleaned by dispersive solid-phase extraction (SPE) employing SPE sorbent and salts, to remove endogenous matrix components. Analytes are then isolated from spiked samples with recoveries above 80% on average, and RSDs typically below 10% for a wide range of substances. This mini-extraction approach in whole blood delivers successful separation for a variety of pharmaceuticals, with limits of detection below 10 ng/mL. The method is quick, easy, inexpensive, and effective for therapeutic drug monitoring and forensic chemistry.

Introduction

Determination of pharmaceuticals in biological matrices is commonly employed in ADME (DMPK), clinical and forensic analysis. The main techniques used for drug monitoring and analysis are immunoassays, LC, and GC methods. Mass spectral chromatographic methods are the first choice for many applications based on their flexibility, selectivity, sensitivity, qualitative and quantitative capabilities. Analysis of pharmaceuticals in biological samples requires sample preparation that can range from simple protein precipitation (PPT) to more complex solid-phase extraction (SPE). There is a need in classic sample preparation for a method to determine multi-classes of pharmaceuticals in biological samples. Polymeric or mixed-mode SPE sorbents that can isolate acidic, neutral and basic drugs by hydrophobic and, or ion-exchange interactions address this need but there is always room for sample preparation techniques that are rapid and inexpensive to implement.

Previously reported methods provide analysis of multi-residue pesticides in foods. They are known as QuEChERS, (a quick, easy, cheap, effective, rugged, and safe sample preparation approach) [1]. The authors reported outstanding recoveries for a wide range of pesticide classes. Since its inception there have been many reported articles employing QuEChERS for

the analysis of a wide range of compounds including, but not specific to, antibiotics [2], toxins [3], contaminants [4], and pharmaceuticals [5].

In this note we describe an extension of the work presented by Plössl et al. in 2006, for the determination of pharmaceuticals in whole blood employing a modified mini-extraction procedure with LC-MS/MS analysis. The experiments presented in this application note used human whole blood containing either EDTA or citrate as an anticoagulant and were evaluated with both non-buffered and buffered extraction salts used in the QuEChERS methodology, namely non-buffered, AOAC 2007.01 and EN 15662. Modifications to the acetonitrile (extraction solvent) used in the first step (extraction/partitioning) were also evaluated. The experiments were performed using nine different pharmaceuticals (lidocaine, tramadol, amitriptyline, biperidene, oxazepam, lorazepam, chlorpromazine, diltiazem, and naloxone), with a broad range of hydrophobicity and dissociation constants (Table 1). Agilent Poroshell 120 is a good column for this analysis, in part because it has standard 2- μ m frits and is more forgiving for more complex samples relative to a sub-2- μ m column. Poroshell 120 has mass transfer such that it acts very much like a sub-2- μ m particle LC column, without the high back pressure associated with a sub-2- μ m column. The efficient mass transfer equates with faster analysis time and higher throughput with optimum resolution.

Table 1. Characteristics of Pharmaceuticals Under Investigation

Compound	CAS number	Log P	pKa	Therapeutic use
Lidocaine	137-58-6	2.4	8.01	Local anesthetic, antiarrhythmic
Tramadol	27203-92-5	2.5	9.41	Analgesic
Amitriptyline	50-48-6	4.92	9.4	Antidepressant
Biperidene	514-65-8	4.0	10.8	Anticholinergic
Oxazepam	604-75-1	2.23	1.7, 11.3	Antianxiety
Lorazepam	846-49-1	2.47	1.3, 11.5	Antidepressant
Chlorpromazine	50-53-3	5.18	9.3	Antipsychotic
Diltiazem	42399-41-7	3.63	7.7	Calcium channel blocker
Naloxone	465-65-6	1.45	7.9	Opioid receptor antagonist
Nortriptyline (IS)	72-69-5	5.65	9.7	

Experimental

All reagents and solvents were HPLC analytical grade. The compounds were purchased from Sigma-Aldrich (St. Louis, MO, USA).

A stock solution of 1 M ammonium acetate (NH_4OAc) pH 5 was made by dissolving 19.27 g of NH_4OAc powder in 250 mL Milli-Q water. The pH was adjusted to 5 with acetic acid monitored with a pH meter. The solution was stored at 4 °C. MeOH:H₂O (20:80) containing 5 mM NH_4OAc pH 5 was made by combining 200 mL MeOH and 800 mL Milli-Q water, adding 5 mL of 1 M NH_4OAc , pH 5 stock solution. The 5 mM NH_4OAc in ACN was prepared by adding 5 mL of 1 M NH_4OAc , pH 5 stock solution to 1L ACN, sonicating well.

Standard and internal standard solutions (2.0 mg/mL) were made in MeOH and stored at -20 °C. A QC spiking solution of 5.0 µg/mL was made fresh daily in 1:1 ACN:H₂O (0.1% FA). A 0.5 and 5.0 µg/mL standard solution in 1:1 ACN:H₂O (0.1% FA) was made for the preparation of calibration curves in the matrix blank extract with appropriate dilution. A 5 µg/mL of nortriptyline in 1:1 ACN:H₂O (0.1% FA) was used as the internal standard (IS).

Equipment

- Agilent 1260 HPLC with Diode Array
- Agilent 6460 Triple Quadrupole LC/MS system with Electrospray Ionization
- Agilent Bond Elut QuEChERS AOAC Extraction kit (p/n 5982-6755)
- Bond Elut QuEChERS EN Extraction kit (p/n 5982-6650)
- Bond Elut QuEChERS Non-Buffered Extraction kit (p/n 5982-6550)
- Bond Elut QuEChERS AOAC Dispersive SPE kit for General Fruits and Vegetables (p/n 5982-5022)
- Bond Elut QuEChERS EN Dispersive SPE kit for General Fruits and Vegetables (p/n 5982-5021)
- Bond Elut Ceramic Homogenizers (p/n 5982-9312)
- Sorvall ST 16R Centrifuge (Thermo IEC, MA, USA)

- Micro centrifuge 5415D Eppendorf (Brinkman Instruments, Westbury, NY, USA)
- Geno Grinder 2010 (SPEX CertiPrep, Inc., Metuchen, NJ, USA)
- DVX 2500 Multi-Tube Vortexer (VWR International, West Chester, PA, USA)

HPLC conditions

Column	Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 µm (p/n 695775-902)
Flow rate	0.4 mL/min
Column temperature	30 °C
Injection	10 µL
Mobile phase	A. 5 mM Ammonium acetate, pH 5 in 20:80 MeOH:water B. 5 mM Ammonium acetate, pH 5 in ACN
Needle wash	1:1:1:1 ACN:MeOH:IPA:H ₂ O (0.2% FA)
Gradient	20 to 75% B over 5.5 min

MS conditions

ESI	Positive mode
GT	300 °C
GF	7 L/min
Nebulizer	40 psi
SGT	400 °C
SFG	12 L/min
Capillary	3500 V
NV	500 V

Other MS conditions relating to the analytes are listed in Table 2.

Table 2. Instrument Acquisition Data Used for the Analysis of Nine Drugs by LC-MS/MS

Compound	MRM channels (m/z)	Fragmentor (V)	CE (V)	RT (min)	Delta RT
Lidocaine	1) 235.18 > 86.1	97	11	1.37	0.4
	2) 235.18 > 58.1		35		
Tramadol	1) 264.2 > 58.1	97	15	1.20	0.4
	2) 264.2 > 246.1		3		
Amitriptyline	1) 278.2 > 117	112	19	4.25	0.4
	2) 278.2 > 105		19		
Biperidene	1) 312.23 > 98.1	123	19	4.23	0.7
	2) 312.23 > 55.1		60		
Oxazepam	1) 287.06 > 240.9	112	19	3.99	0.4
	2) 287.06 > 268.9		7		
Lorazepam	1) 321.02 > 274.9	113	15	4.09	0.4
	2) 321.02 > 302.9		7		
Chlorpromazine	1) 319.11 > 86.1	112	15	4.63	0.4
	2) 319.11 > 58.1		43		
Diltiazem	1) 415.17 > 177.9	128	19	3.73	0.4
	2) 415.17 > 149.9		43		
Naloxone	1) 328.16 > 310	123	15	0.82	0.4
	2) 328.16 > 212		39		
Nortriptyline (IS)	1) 264.18 > 233	97	7	4.17	0.4
	2) 264.18 > 91		19		

General procedure

1. Add 1 mL of whole blood to a centrifuge tube.
2. Spike with appropriate volume from a concentrated stock mixture to yield 25, 50, and 100 ng/mL of the component mix.
3. Add 20 μ L of IS stock solution, yield 100 ng/mL (Nortriptyline), and two ceramic homogenizers.
4. Vortex.
5. Add 2 mL of an acetonitrile solution (with or without acid), see Table 3.
6. Vortex.
7. Add a premixed amount (see Table 3) of the extraction salts and vigorously shake.
8. Centrifuge at 5,000 rpm for 5 minutes.
9. Transfer 1 mL of the extract into a d-SPE tube (2 mL centrifuge tube) containing 50 mg of PSA and 150 mg of $MgSO_4$ for matrix clean-up.
10. Vortex for 1 minute.
11. Centrifuge at 18,000 rpm for 3 minutes.
12. Transfer a 200 μ L aliquot of the extract into a LC vial containing 800 μ L of water.
13. Vortex and analyze.

The entire series of experiments that were evaluated can be found in Table 3. A matrix matched calibration curve from 10-250 ng/mL was employed to determine recovery.

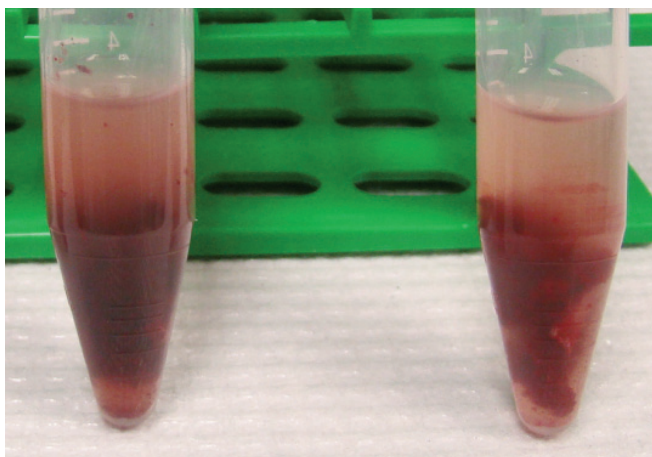
Table 3. Series of Experimental Conditions Investigated

Sample (1 mL)	Extraction solvent	Extraction salts (mg)	d-SPE	Observation
WB	ACN	none	none	Sample: solid mass
WB	ACN, 1% AA	none	none	Sample: solid mass
WB	ACN, 0.4% FA	none	none	Sample: loose particles
WB	ACN, 0.4% FA	Non-buffered, 500	none	Dark extract
WB	ACN, 0.4% FA	AOAC, 500	none	Clear extract
WB	ACN, 0.4% FA	EN, 650	none	Dark extract
WB	ACN, 0.4% FA	Non-buffered, 500	50 mg PSA, 150 mg MgSO ₄	Clear extract
WB	ACN, 0.4% FA	AOAC, 500	50 mg PSA, 150 mg MgSO ₄	Clear extract
WB	ACN, 0.4% FA	EN, 650	25 mg PSA, 150 mg MgSO ₄	Clear extract
WB	ACN, 0.4% FA	EN, 650	50 mg PSA, 150 mg MgSO ₄	Clear extract

WB = whole blood; ACN = Acetonitrile, AA = acetic acid; FA = formic acid, PSA = Primary secondary amine, AOAC = MgSO₄ & NaAcetate, EN= MgSO₄ & Citrate buffers, Non-buffered = MgSO₄ & Sodium Chloride

Results and Discussion

The experiments showed that the use of ACN (0.4% FA) as the extraction solvent offered a better lysed sample versus the other extraction solvents where the sample became a solid mass (see Figures 1 and 2). The AOAC buffered salts yielded the cleanest extract, visually (Figure 3) and was chosen for use with the d-SPE containing 50 mg PSA, 150 mg MgSO₄ for the extraction of the pharmaceuticals in whole blood (Figure 4). It is worth noting that the d-SPE step does in fact offer substantial clean-up for all the extracted samples, especially from the EN and non-buffered salt extracts, which initially showed a significant amount of red blood cells remaining in the extract.



A B

Figure 1. Addition of ACN (A) or ACN (1% AA) (B) to the whole blood, common solvents used in the QuEChERS method.

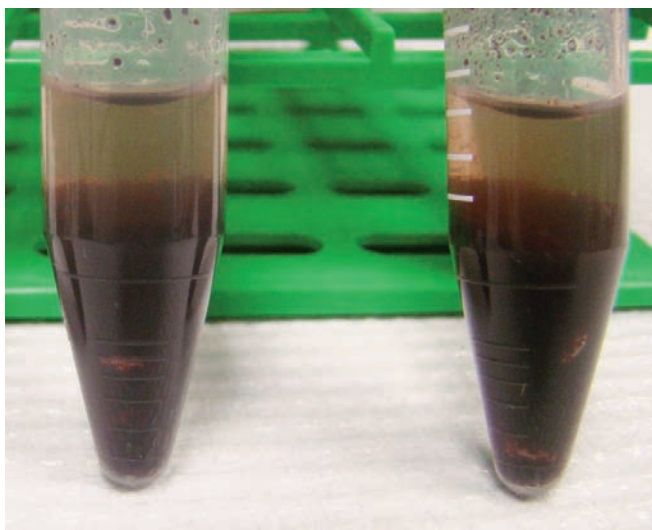
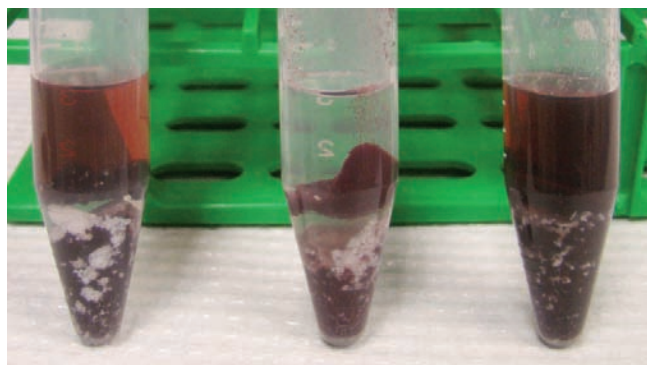


Figure 2. Addition of ACN (0.4% FA) to the whole blood.



A B C

Figure 3. After the addition of ACN (0.4% FA), QuEChERS salts, shake and vortex.

- A EN method citrate salts,
- B AOAC method acetate salts
- C non-buffered method chloride salts



A B C D

Figure 4. Extract after the addition of d-SPE clean-up containing 150 mg $MgSO_4$ and varying amounts of PSA.

- A EN citrate salts and EN d-SPE 25 mg PSA
- B EN citrate salts and AOAC d-SPE 50 mg PSA
- C AOAC acetate salts and AOAC d-SPE 50 mg PSA
- D Non-buffered chloride salts and AOAC d-SPE 50 mg PSA

The mini-extraction procedure is based on the principles behind the QuEChERS methodology. Its purpose is an alternative to more complicated techniques, offering a “just enough” sample preparation technique for complex matrices such as whole blood. This type of sample preparation technique is extremely complimentary to the powerful selectivity of LC-MS/MS multiple reaction monitoring (MRM) mode. The whole blood extract appeared to be clean and free of impurities, indicating that the blank whole blood extract did not contribute any interferences with target compounds. Figure 5 shows the chromatogram of a 10 ng/mL spiked whole blood sample after the mini-extraction procedure.

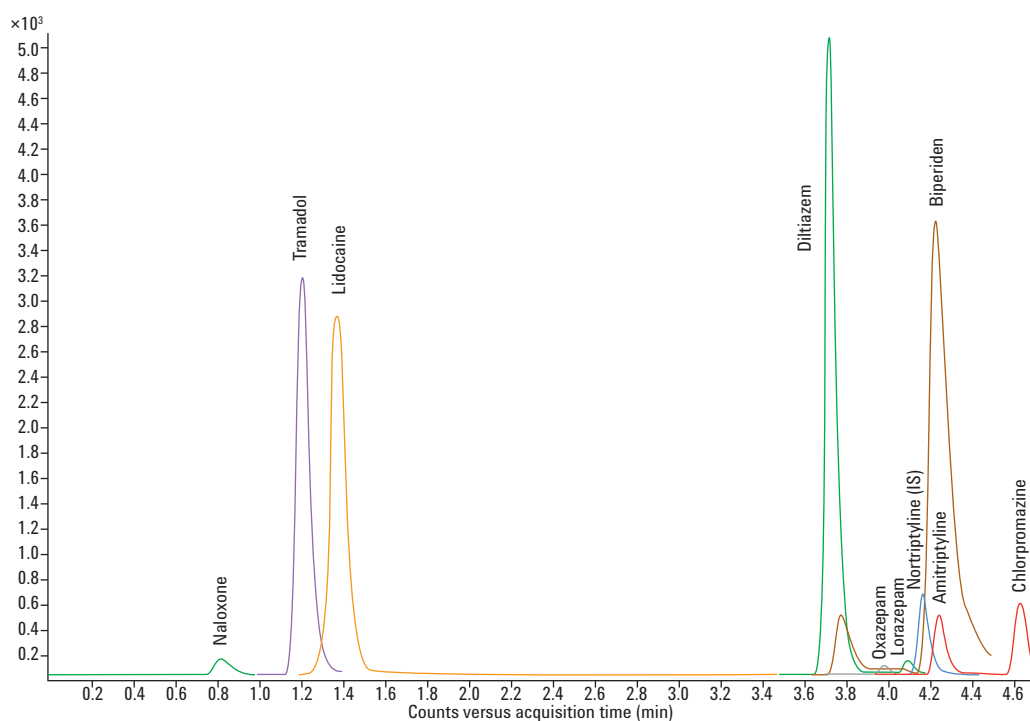


Figure 5. LC-MS/MS chromatograms of 10 ng/mL spiked whole blood sample after mini-extraction; AOAC acetate salts and AOAC d-SPE with 50 mg PSA and 150 mg MgSO₄.

Linearity and limit of quantification (LOQ)

The linear calibration range evaluated for all the pharmaceuticals was 10-250 ng/mL. Matrix blank extracts were prepared for the calibration curves. Calibration curves, spiked in the matrix blank extracts, were made at levels of 10, 25, 50, 100, and 250 ng/mL. The Nortriptyline (IS) was used at 100 ng/mL. The calibration curves were generated by plotting the relative responses of analytes (peak area of analyte/peak area of IS) to the relative concentration of analytes (concentration of analyte/concentration of IS). Figure 6 is an example of the regression equation and correlation coefficient (R^2) observed for the nine pharmaceuticals from whole blood.

Recovery and reproducibility

The recovery and reproducibility were evaluated by spiking standards in the whole blood sample at levels at 25, 50, and 100 ng/mL. These QC samples were quantitated against the matrix spiked calibration curve. The analysis was performed in six replicates at each level. The recovery and reproducibility (RSD) data are shown in Table 4.

It can be seen from the results that all the pharmaceuticals give acceptable recoveries (average > 90%) and precision (average of 7% RSD). We have observed a small degree of matrix interference at low levels of concentration, < 25 ng/mL, with the pharmaceuticals investigated.

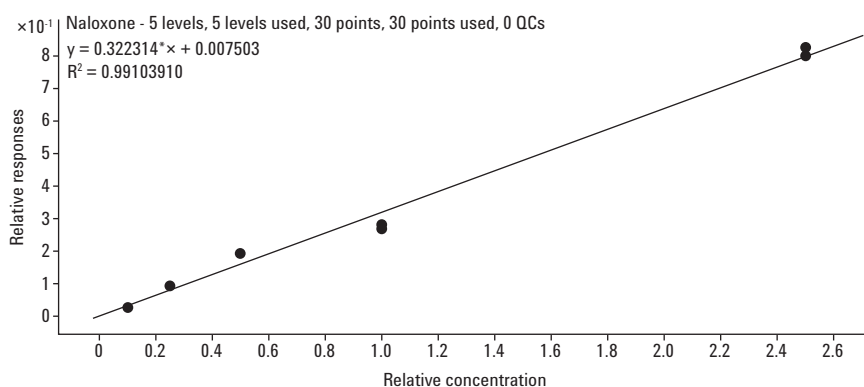


Figure 6. Example of the results from the mini-extraction, standard linear curve for naloxone from 10-250 ng/mL, $R^2 = 0.991$.

Table 4. Recovery and RSD for the Extracted Drug Compounds

Compound	25 ng/mL Spiked		50 ng/mL Spiked		100 ng/mL Spiked	
	Recovery	RSD	Recovery	RSD	Recovery	RSD
Lidocaine	81.6	35.3	98.7	15.7	100	11.8
Tramadol	97.2	18.6	105	3.0	104	8.2
Amitriptyline	85	13.6	104	2.1	104	8.2
Biperidene	75.5	14.8	97	4.5	99	8.2
Oxazepam	60.4	17.3	77.0	9.2	78	8.6
Lorazepam	68.4	17.0	81.9	6.8	81.8	8.6
Chlorpromazine	75	14.1	110	10.3	105	6.3
Diltiazem	63.7	15.8	88.1	2.7	91.7	8.3
Naloxone	68	12.1	80.6	9.0	75.5	7.7

Conclusion

Mini-extraction sample preparation is a simple, easy, and cost effective approach, requiring minimal sample preparation expertise, solvent, or equipment. The mini-extraction approach for the extraction of pharmaceuticals from whole blood offers an alternative sample preparation technique that can be easily implemented by laboratories. Although matrix interference was observed at low level concentrations for some of the pharmaceuticals, improvements in the method will include a dispersive SPE that contains additional solid phase extraction materials to facilitate matrix removal. The Poroshell 120 EC-C18 column offers different selectivity and exceptional peak shape across the wide range of pharmaceuticals used in this study.

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Fast Analysis of Zanthoxylum nitidum Using Agilent Poroshell 120 EC-18 Columns

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Abstract

The Chinese Pharmacopoeia method for analyzing Zanthoxylum nitidum was reproduced successfully on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm column. The good separation was transferred easily to an Agilent Poroshell 120 EC-C18 column; excellent peak shape of nitidine chloride ensured accurate quantitation. Gradient time decreased from 35 minutes to 17.5 minutes on a 75 cm Poroshell 120 column, and to 12 minutes on a 50 cm Poroshell 120 column. Analysis time decreased further, to below 4 minutes by increasing the flow rate; backpressure was still below 400 bar and so the analysis could be run on a traditional HPLC instrument. The Poroshell 120 columns shortened run time while maintaining high resolution of complex traditional Chinese medicine (TCM) components, thereby increasing sample throughput and reducing costs.

Introduction

Zanthoxylum nitidum (Roxb.) DC., also known as liangmi-anzhen, is a plant of the Rutaceae family. It is widely distributed throughout the southeastern part of China, and could also be found in India, northern Queensland and Australia [1]. *Zanthoxylum nitidum* is a commonly used Chinese herbal medicine, which can promote blood circulation, dissipate blood stasis, detumescence, and relieve pain. *Zanthoxylum nitidum* contains many alkaloids, of which nitidine chloride is the major active component that exhibits the main pharmacological and biological activity [2]. Nitidine chloride is best known for its anti-cancer activities [3]. The Chinese Pharmacopoeia [4] specifies that the content of nitidine chloride in *Z. nitidum* as determined by HPLC shall not be less than 0.13% to control the quality of the medicinal material.

Agilent Poroshell 120 EC-C18, 2.7 μm columns are packed with superficially porous particles, which deliver fast separation and achieve performance similar to sub 2 μm totally porous materials. However, since they use 2- μm column frits like those found on 5 μm particle size columns, they require no additional sample preparation. This allows a seamless method transfer to the Poroshell 120 columns from established methods using 5- μm columns. This makes the Poroshell 120 columns very suitable for the analysis of herbal medicines because these samples are often very complex. The Poroshell 120 columns can be used to reduce the analysis time that is normally quite long on a 5- μm column, while providing the high resolution needed for these samples.

In this work, a gradient method was successfully transferred from a traditional 4.6 \times 150 mm, 5 μm column to two different 2.7 μm Poroshell 120 EC-18 columns to increase the speed of the analysis and achieve good resolution and high efficiency, while exceeding the requirements of the Chinese Pharmacopoeia.

Experimental

Reagents and chemicals

Nitidine chloride was obtained from the National Institute for the Control of Pharmaceuticals and Biological Products of China (NICPBP, Beijing, China). Acetonitrile (ACN) and methanol were obtained from Sinopharm Chemical Reagent (Shanghai, China). Water was prepared with a Milli-Q pure water system.

A standard solution of nitidine chloride was prepared by weighing the appropriate standard and dissolving in 70% methanol aqueous solution at 50 $\mu\text{g}/\text{mL}$.

Sample preparation

The raw TCMs of *Z. nitidum* were purchased from a local TCM store. A powdered sample (1 g) was extracted with 20 mL of 70% methanol aqueous solution in an ultrasonic water bath for 30 minutes. Extraction was repeated twice. The extracted solutions were combined and diluted to 50 mL with 70% methanol aqueous solution. This solution was then filtered with a 0.45- μm regenerated cellulose membrane filter (p/n 5064-8221) and injected directly into the HPLC system.

Instrumentation

Analyses were performed on an Agilent 1290 Infinity LC System with a binary pump (G4220A), a thermostatted column compartment (TCC, G1316C), an autosampler (G4226A) and a diode array detector (DAD, G4212A). Table 1 shows method parameters for various column dimensions.

The columns used were:

Agilent ZORBAX Eclipse Plus C18, 4.6 \times 150 mm, 5 μm (p/n 959993-902)

Agilent Poroshell 120 EC-C18, 4.6 \times 75 mm, 2.7 μm (p/n 697975-902)

Agilent Poroshell 120 EC-C18, 4.6 \times 50 mm, 2.7 μm (p/n 699975-902)

Table 1. Method Parameters for the Columns

	Agilent Zorbax Eclipse Plus C18 4.6 \times 150 mm, 5 μm	Agilent Poroshell 120 EC-C18 4.6 \times 75 mm, 2.7 μm	Agilent Poroshell 120 EC-C18 4.6 \times 50 mm, 2.7 μm	
Mobile phase	A: 0.1% formic acid-triethylamine (pH = 4.5), (0.1% (v/v) formic acid aqueous solution adjusted with triethylamine to a pH of 4.5) B: acetonitrile			
Gradient	0 min 20% B 30 min 50% B 35 min 100% B	0 min 20% B 15 min 50% B 17.5 min 100% B	0 min 20% B 10 min 50% B 12 min 100% B	0 min 20% B 2.7 min 27% B 3 min 100% B
Flow rate	1.5 mL/min	1.5 mL/min	1.5 mL/min	3 mL/min
Injection volume	10 μL	5 μL	4 μL	4 μL
TCC temperature	30 $^{\circ}\text{C}$	30 $^{\circ}\text{C}$	30 $^{\circ}\text{C}$	30 $^{\circ}\text{C}$
Detector	UV 273 nm	UV 273 nm	UV 273 nm	UV 273 nm

Results and Discussion

The original separation method of *Z. nitidum* published in the 2010 Chinese Pharmacopoeia was repeated on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm column. Figure 1 shows the chromatograms of nitidine chloride standard solution and *Z. nitidum* extract. Due to the complicated matrix, this type of separation typically requires more than 35 minutes. Nitidine chloride can be baseline separated with excellent peak shape. Performance of the column was shown in Figure 2A.

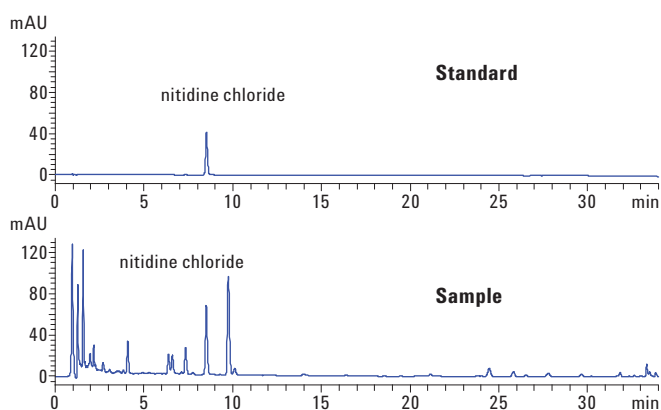


Figure 1. Original method for analyzing *Zanthoxylum nitidum* on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm column.

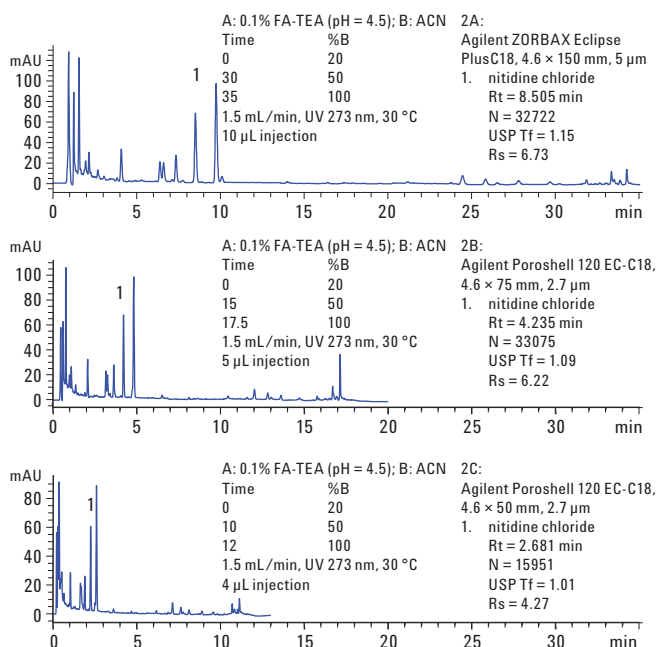


Figure 2. Overlaid chromatograms of *Zanthoxylum nitidum* using Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm; Agilent Poroshell 20 EC-C18, 4.6 × 75 mm, 2.7 μm and Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 μm column.

The original method was scaled from a 4.6×150mm, 5 μm column to an Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 μm column. The flow rate should not be changed, according to Equation 1, to maintain the same linear velocity.

Eq 1

$$F_1/(r_1)^2 = F_2/(r_2)^2$$

where

F_1 is the flow rate of original column

F_2 is the flow rate of new column

r_1 is the radius of original column

r_2 is the radius of new column

The gradient time is proportional to the column length while maintaining the original separation. Since the original column is 4.6 × 150 mm, using a 4.6 × 75 mm column at the same linear velocity shortens the gradient time by 75/150 or a factor of 2, so the injection volume is decreased by half to avoid sample overload. The analysis time also decreased by a factor of 2, from 35 minutes to 17.5 minutes, as shown in Figure 2A and 2B. However, compared to the original method, the chromatogram showed equivalent or slightly better peak shape, theoretical plates and resolution with the Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 μm column. The backpressure of this Poroshell column analysis is less than 250 bar making it possible to use this faster method on any HPLC or UHPLC.

Similarly, the analysis time could be further shortened by using a shorter column. As shown in Figure 2C, the new method saved two-thirds of the original analysis time and the backpressure of 176 bar was quite acceptable for a 400-bar HPLC when using a Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 μm column. The value for theoretical plates of nitidine chloride greatly exceeded the requirement of the 2010 Chinese Pharmacopoeia ($N > 2500$).

This method could be further optimized based on the performance of the Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 μm column. Firstly, the cycle time could be further reduced by using twice the flow rate, because the Van Deemter curve of the superficially porous Poroshell 2.7 μm particles is similar to columns with 1.8 μm particles. Therefore, the efficiency of the Poroshell 120 column does not decrease significantly at higher flow rates. Nitidine chloride is the target compound in this method to control the quality of *Z. nitidum*. After nitidine chloride elutes out of the detector, the proportion of ACN in the mobile phase could be increased to wash the contaminants in *Z. nitidum* out of the column quickly.

As illustrated in Figure 3, having optimized the flow rate and gradient slope for the best possible balance between resolution and analysis time, the analysis time fell to approximately one-tenth of the original analysis time with some loss in resolution and theoretical plates, which were much better than the regulation requirements. The backpressure was less than 370 bar. This pressure was fine for the column, which could be used up to 600 bar. Although a traditional 400-bar HPLC instrument could be used for this method, it is better to use an HPLC or UHPLC with a pressure limit of at least 600 bar.

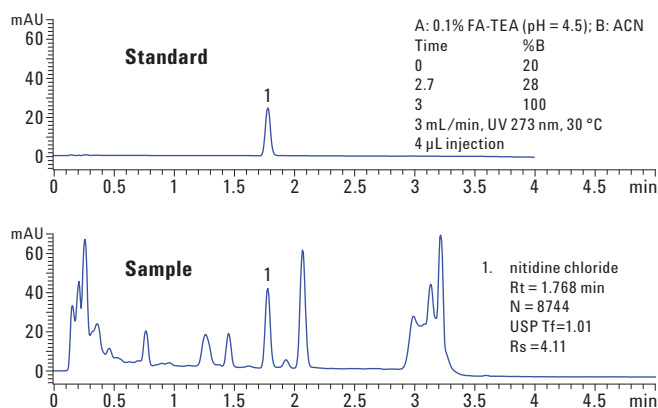


Figure 3. Optimized method for analyzing *Zanthoxylum nitidum* on an Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 μm column.

Conclusions

A method for the analysis of nitidine chloride in *Z. nitidum* was successfully transferred from a traditional 4.6 × 150 mm, 5 μm column to an Agilent Poroshell 120 column. The Poroshell 120 2.7 μm particle columns provide high efficiency and high resolution allowing for faster methods. This is similar to the performance that could be achieved with sub 2 μm columns. But the Poroshell 120 column operates at a lower pressure such that a 400-bar instrument can run the Poroshell 120 column method for routine analysis. A higher flow rate reduces analysis time to less than 4 minutes. The transferred methods are fast with high efficiency, providing good separation and peak shape, and therefore provide substantial time and cost savings.

References

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SAMHSA-Compliant LC/MS/MS Analysis of Amphetamines in Urine with Agilent Bond Elut Plexa PCX and Agilent Poroshell 120

Authors

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Abstract

New guidelines from the US Substance Abuse and Mental Health Services Administration (SAMHSA), effective October 2010, allowed LC/MS/MS methods to be used for confirmation of initial drug tests [1]. LC/MS/MS methods are often less complicated than previously employed GC/MS methods because they do not typically require a derivatization step. We present a method for analysis of five amphetamines that meets the most recent SAMHSA guidelines to demonstrate linearity, limit of detection (LOD), accuracy, and precision, as well as measurement of matrix effects, extraction recovery, and overall process efficiency. This is one of a suite of six simplified methods covering all classes of SAMHSA-regulated drugs and using premier Agilent products, including Agilent Bond Elut Plexa PCX mixed-mode polymeric SPE sorbent, Agilent Poroshell 120 EC-C18 2.7 μm superficially porous LC column, Agilent 1200 Infinity LC system, and Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source.

Introduction

Amphetamines are psychostimulant drugs included in a group of sympathomimetic amines that mimic the effects of the endogenous neurotransmitters, such as epinephrine (adrenaline), norepinephrine (noradrenaline), and dopamine. Amphetamines are found in the leaves of *Ephedra sinica* (for example ephedrine) and were first produced synthetically at the end of the 19th century. Their chemical structure features a phenethylamine backbone with a methyl group attached to the alpha carbon, along with other substitutions (Figure 1). A significant portion of amphetamines is excreted intact in urine. By demethylation, more complicated amphetamine derivatives are metabolized into simpler structures, for example methamphetamine to amphetamine, and MDMA to MDA [2]. The 2011 SAMHSA guidelines require screening for and confirmation of five amphetamines – amphetamine, methamphetamine, MDA, MDMA, and MDEA. The confirmation method should demonstrate the ability to distinguish these drugs from structurally similar compounds that are potential interferences, including ephedrine, pseudoephedrine, phentermine, and phenylpropanolamine (PPA, or norephedrine).

In GC/MS methods traditionally employed for detection of amphetamines, it was common to apply periodate pretreatment to oxidize the hydroxyphenethylamines ephedrine and pseudoephedrine and, thus, exclude a chance of interference by these compounds. We eliminated this step, offering instead a reliable chromatographic separation of all analytes of interest required by the latest SAMHSA guidelines.

The new SAMHSA confirmation cutoff concentration for all amphetamines is 250 ng/mL and a limit of detection at 10% of the cutoff concentration is 25 ng/mL [1]. Because high concentrations of amphetamines can be expected in some urine samples, we chose to use a higher capacity 3 mm id Agilent Poroshell 120 column instead of a 2 mm id column for all Agilent SAMHSA methods. With superficially porous 2.7 μm particles, Poroshell 120 provides similar efficiency to sub-2 μm UHPLC columns but with about 40% less back pressure. Therefore, it allows users of even 400 bar LC systems to increase resolution and to shorten both analysis and re-equilibration times by applying a higher flow rate.

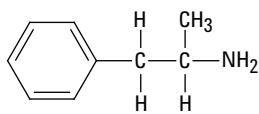
The simple extraction method described here provides reproducible high recoveries of amphetamines due to the unique properties of Agilent Bond Elut Plexa. Unlike other polymeric sorbents, Plexa possesses amide-free hydroxylated particle surface that excludes protein binding. This results in minimized ion suppression and maximum sensitivity. Fast flow and reproducible performance are due to the narrow particle size distribution with no fines to cause blockages.

With a low sample injection volume of 2 μL and no sample preconcentration, the presented method demonstrates excellent signal-to-noise (S/N) ratios (> 400:1 at 25 ng/mL, 10% of the SAMHSA confirmation cutoff) due to the enhanced sensitivity of the Agilent 6460 Triple Quadrupole LC/MS system with the AJST electrospray source.

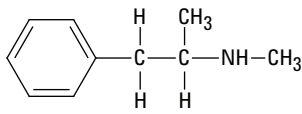
Previous methods from Agilent used the Agilent 6410 Triple Quadrupole LC/MS system and other SPE/LC products and procedures [3,4].

Experimental

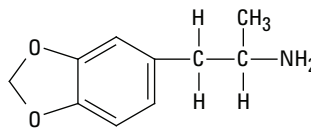
Analytes



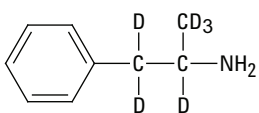
Amphetamine
Log P 1.79 pKa 9.8



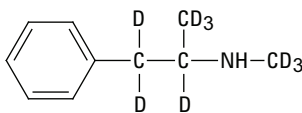
Methamphetamine
Log P 1.94 pKa 9.5



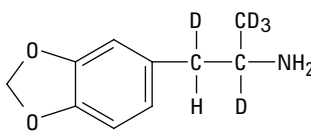
MDA
Log P 1.67 pKa 9.7



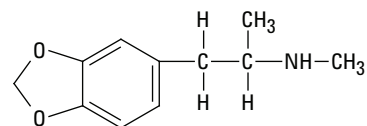
Amphetamine-D₆



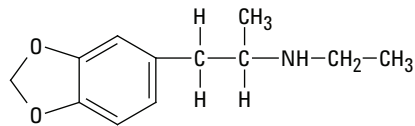
Methamphetamine-D₉



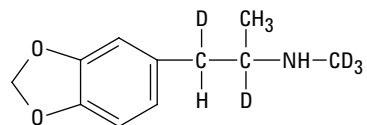
MDA-D₅



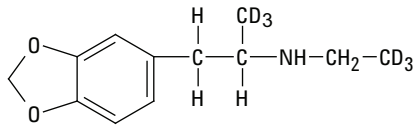
MDMA
Log P 2.05 pKa 9.9



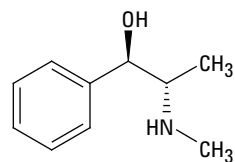
MDEA
Log P 2.34 pKa 9.9



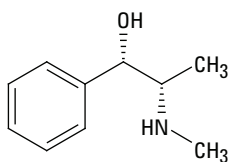
MDMA-D₅



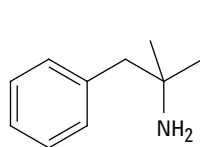
MDEA-D₆



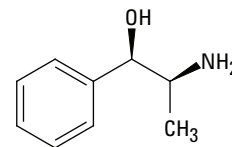
Ephedrine
Log P 1.13 pKa 9.6



Pseudoephedrine
Log P 0.89/1.1 pKa 9.9



Phentermine
Log P 2.16 pKa 10.1



Phenylpropanolamine
Log P 0.81 pKa 9.4

Figure 1. Amphetamines and interferences - analytes and their structures.

Drug standards were purchased from Cerilliant Corporation as 1 mg/mL (amphetamine, methamphetamine, MDA, MDMA, MDEA, ephedrine, pseudoephedrine, phentermine, and phenylpropanolamine) and 100 µg/mL (amphetamine-D₆, methamphetamine-D₉, MDA-D₅, MDMA-D₅, and MDEA-D₆) solutions in methanol.

Materials and instrumentation

SPE

- Agilent Bond Elut Plexa PCX cartridges, 30 mg, 3 mL (p/n 12108303)
- Agilent vacuum manifold VacElut 20 (p/n 12234100)
- Agilent stopcock valves (p/n 12234520)
- Agilent 2 mL autosampler vials (p/n 5182-0716)
- Agilent screw caps for autosampler vials (p/n 5182-0717)

LC

- Agilent Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm (p/n 699975-302)
- Agilent 1260 Infinity LC (G1379B microdegasser, 1312B binary pump in low delay volume configuration, G1367E autosampler, and G1330B thermostat)

MS

- Agilent 6460A Triple Quadrupole LC/MS system with AJST electrospray ionization source.

Sample preparation

Pretreatment

Spike 0.5 mL of urine with ISTDs at 500 ng/mL each; use of 12 × 75 mm glass tubes is recommended. Add 1 mL of 2% formic acid, vortex; centrifuge if cloudy.

Extraction

1. Condition Bond Elut Plexa PCX column with 0.5 mL methanol – soak, then let drip.
2. Load sample/supernatants.
3. Wash 1: 1 mL 2% formic acid.
4. Wash 2: 1 mL of methanol.
5. Dry 5–10 minutes under vacuum (10–15 in Hg).
6. Elute with 1 mL ethyl acetate: methanol: ammonium hydroxide (50:50:20), freshly prepared. Let eluate drip into collection vials, then apply low vacuum (2–3 in Hg).
7. Evaporate under stream of nitrogen to 0.2 mL at ≤ 37 °C.

8. Add 100 µL of 0.025 N hydrochloric acid in methanol, vortex.
9. Evaporate to dryness.
10. Reconstitute in 0.5 mL initial mobile phase (15% methanol, 85% water, 0.1% formic acid).

LC/MS/MS

LC conditions

Mobile phase A	0.1% formic acid in water	
Mobile phase B	0.1% formic acid in methanol	
Flow rate	0.8 mL/min	
Gradient	Time (min)	% B
	0.0	15
	1.5	15
	3.5	30
	3.6	90
	6.6	90
	6.7	15
Stop time	6.8 min	
Post time	2 min	
Max pump pressure	400 bar	
Injection volume	2 µL	
Injection with needle wash		
Needle wash	Flush port 75:25 methanol:water for 10 s	
Disable overlapped injection		
No automatic delay volume reduction		

MS conditions

ES Source Parameters

Ionization mode	Positive
Capillary voltage	4,000 V
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Nebulizer gas	35 psi
Sheath gas flow	12 L/min
Sheath gas temperature	400 °C
Nozzle voltage	0 V

MS parameters

Scan type	MRM
Pre-run script	SCP_MS Diverter Valve To Waste() {MH_Acq_Scripts.exe}
Time segments	#1: 0.6 min (for interferences separation) or 1.2 min (for five amphetamines only) - diverter valve to MS
Delta EMV (+)	200 V

Results and Discussion

At acidic pH, the amine group of amphetamines was protonated, and the analytes were efficiently retained on Bond Elut Plexa PCX polymeric sorbent by a combination of hydrophobic interaction and a strong cation exchange.

A 100% methanol wash eliminated most matrix interferences without the loss of analytes from the sorbent. A strong base was added to organic eluent to break ionic interaction between the amphetamines and strong cation exchange sorbent. The recovery was optimized with two-component organic eluent consisting of 50% ethyl acetate and 50% methanol, with 20% NH_4OH added shortly before sample elution.

Amphetamines are rather volatile and could evaporate at the solvent evaporation step of sample preparation unless precipitated as salts by addition of the hydrochloric acid. It is best to add HCl toward the end of evaporation to avoid the formation of ammonium chloride salts which will cause ion suppression.

Figure 2 shows excellent separation of five amphetamines and potential interferences specified by SAMHSA on the Poroshell 120 EC-C18, 3×50 mm, $2.7 \mu\text{m}$ column, which was completed within 3.2 minutes. LC separation started with a low fraction of organic solvent (15%) to allow salts and other polar components of urine to elute at the beginning of the sample run. Each sample run started with diverting the first portion of flow to waste to minimize source contamination. Data collection started immediately after the diverter valve switch. A flow rate of 0.8 mL/min allowed short separation and re-equilibration times.

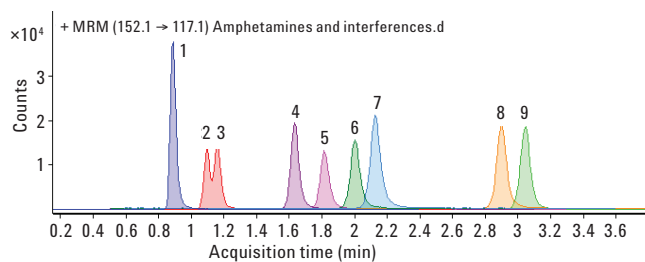


Figure 2. Separation of amphetamines and potential interferences on Agilent Poroshell 120 EC-C18, 3×50 mm, $2.7 \mu\text{m}$ column – overlaid MRM extracted ion chromatograms. Concentration of each analyte corresponds to 50 ng/mL. Peaks in order of their elution are: 1. phenylpropanolamine, 2. ephedrine, 3. pseudoephedrine, 4. amphetamine, 5. methamphetamine, 6. MDA, 7. MDMA, 8. MDEA, 9. phentermine.

A dynamic MRM method using retention time and delta RT (time window) for a certain transition is recommended for the analysis of several compounds. When good separation from interferences is ensured, and data collection is focused on five amphetamines only, the valve can be switched from waste to mass spectrometer at 1.2 minutes instead of 0.6 minutes (time segment no. 1 in the MS method).

SAMHSA guidelines require the use of one quantifier and at least one qualifier ion for both target compound and ISTD. A third transition for target analytes (Table 1) was provided where possible for additional confidence. Agilent MassHunter Quantitative software calculated qualifier ion ratios, automatically highlighting those out of acceptable range.

Table 1. MRM transitions.

Compound name	Precursor	Product	Fragmentor	Collision energy
Amphetamine	136.1	119.1	64	4
Amphetamine	136.1	91.1	64	14
Amphetamine-D ₆	142.1	125.1	66	5
Amphetamine-D ₆	142.1	93.1	66	13
MDA	180.1	163.1	92	5
MDA	180.1	105.1	92	17
MDA-D ₅	185.1	168.1	68	5
MDA-D ₅	185.1	110.1	68	21
MDEA	208.1	163.1	88	8
MDEA	208.1	133.1	88	17
MDEA	208.1	105.1	88	21
MDEA-D ₆	214.2	166.1	90	8
MDEA-D ₆	214.2	108.1	90	25
MDMA	194.1	163.1	84	5
MDMA	194.1	135.1	84	17
MDMA	194.1	105.1	84	21
MDMA-D ₅	199.1	165.1	82	4
MDMA-D ₅	199.1	107.1	82	25
Methamphetamine	150.1	119.1	80	4
Methamphetamine	150.1	91.1	80	16
Methamphetamine-D ₉	159.2	125.2	77	5
Methamphetamine-D ₉	159.2	93.1	77	13
Ephedrine-pseudoephedrine	166.1	133.1	80	21
Phentermine	150.1	133.1	80	6
Phenylpropanolamine	152.1	117.1	80	20

S/N ratios exceeding 400:1 were obtained for quantifier peaks of all five amphetamines at 25 ng/mL (Figure 3, upper panel: S/N is shown for the MDEA quantifier peak). This illustrated the state-of-the-art performance of the Agilent 6460 Triple Quadrupole LC/MS/MS capable of reliably detecting all five amphetamines at a small fraction of the SAMHSA cutoff.

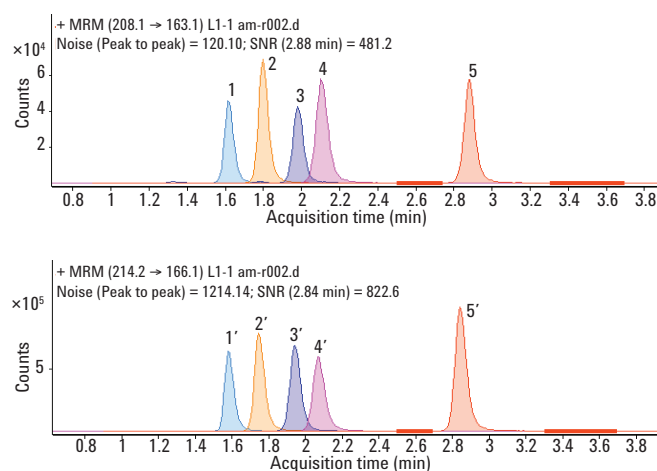


Figure 3. Overlaid MRM extracted ion chromatograms for amphetamines quantifiers (25 ng/mL) and ISTDs quantifiers (500 ng/mL) in urine extract on an Agilent Poroshell 120 EC-C18, 3 × 50 mm, 2.7 μm column. Peaks in order of their elution are: upper panel - 1. amphetamine, 2. methamphetamine, 3. MDA, 4. MDMA, 5. MDEA, lower panel - 1'. amphetamine-D₆, 2'. methamphetamine-D₉, 3'. MDA-D₅, 4'. MDMA-D₅, 5'. MDEA-D₆. Noise regions are shown in bold.

Figure 4 gives examples of calibration curves for extracted urine standards at five concentration levels. Calibration standards were prepared by spiking negative urine at 25, 250, 1,000, 5,000, and 10,000 ng/mL with each of the five members of the amphetamines class. Deuterated internal standards for each analyte were added at 500 ng/mL. The excellent linear fits to all curves with $R^2 > 0.999$ demonstrated linearity of the method across a broad dynamic range of concentrations, as required by SAMHSA guidelines.

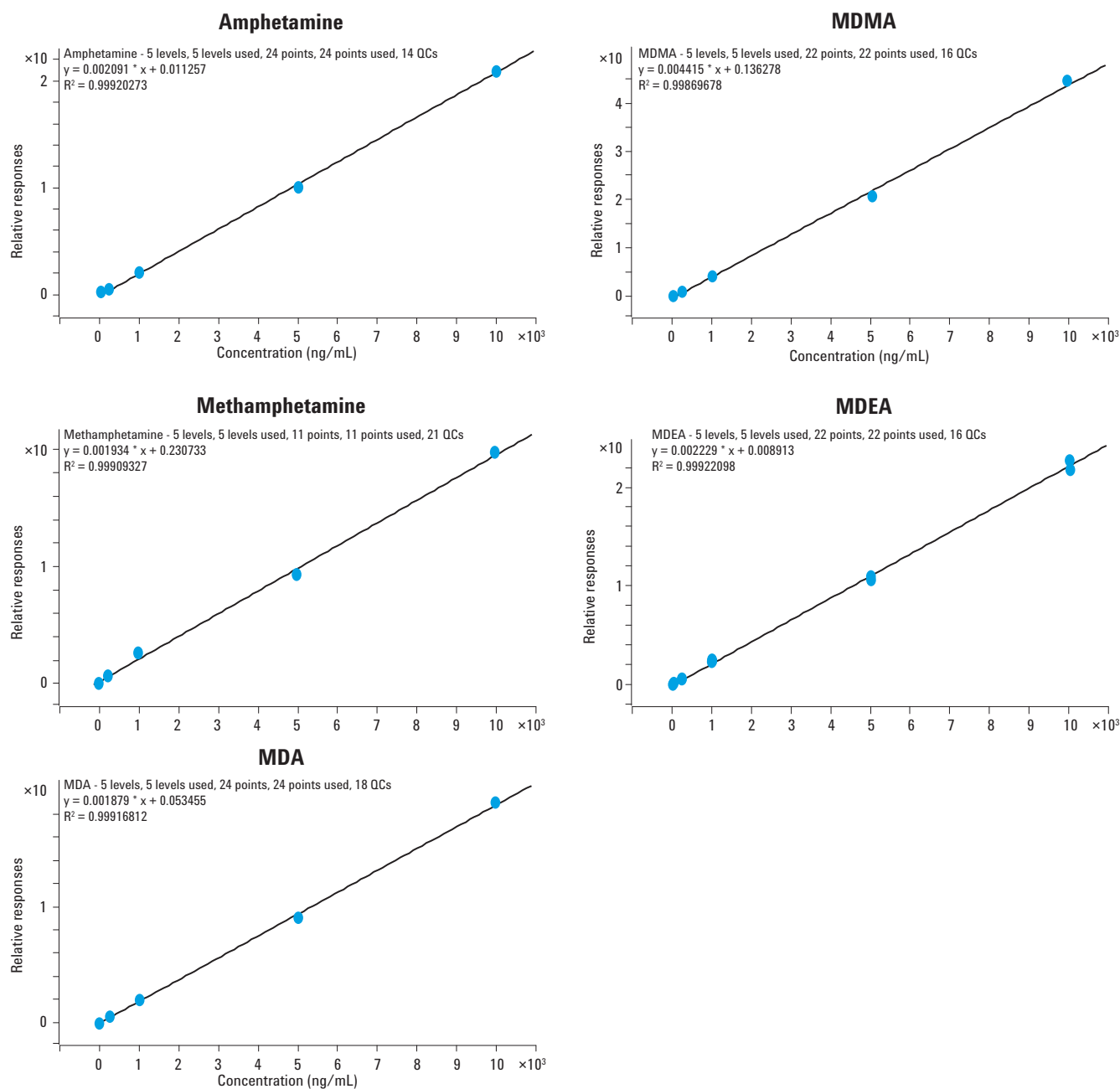


Figure 4. Example calibration curves for five amphetamines in urine extracts. Calibration range 25 to 10,000 ng/mL. All fits are linear, with $R^2 > 0.999$.

Method evaluation

Method performance metrics in Table 2 were calculated according to the principles laid out in Matuszewski *et al* and widely accepted as an industry standard approach for LC/MS/MS methods [5]. The extraction procedure and LC/MS/MS measurement were performed for five replicates of negative urine spiked pre-extraction with each of the five members of the amphetamines class at the cutoff level, and five replicates of negative urine extract reconstituted in initial mobile phase, and then fortified at 250 ng/mL (spiked post-SPE). The third measurement was of initial mobile phase (the reconstitution solvent) fortified to correspond to the cutoff concentration of 250 ng/mL in urine (spiked mobile phase).

Process efficiency (absolute recovery) is a ratio of a peak area of target analyte in urine sample spiked pre-SPE to its peak area in matrix-free spiked mobile phase. Extraction recovery is a ratio of a peak area of target analyte in urine extract spiked pre-SPE to its peak area in an extracted negative urine sample spiked post-SPE. Matrix effect is a ratio of a peak area of target analyte in urine spiked post-SPE to its peak area in spiked mobile phase. Accuracy is a ratio of a measured concentration calculated using the calibration curve to the expected concentration in a sample spiked with a known amount of target analyte. Precision or coefficient of variation (CV) is a measure of reproducibility and is calculated as a percent standard deviation over the mean of the five measurements.

Table 2 shows that the extraction recovery for all five amphetamines was $\geq 94\%$, with overall process efficiency higher than 90% in four out of five analytes; for amphetamine, process efficiency was 86%. The matrix effect of 91 to 99% means only a 1 to 9% signal reduction due to ion suppression, thus, confirming the exceptional cleanliness of Plexa PCX-processed extracts. High accuracy (within 10% of the target) and excellent precision (CV < 1.1%) is typical for this method.

Conclusions

The solid phase extraction procedure coupled with the LC/MS/MS detection method described here is SAMHSA-compliant and provides accurate, precise, and reproducible results for forensic toxicology or other analytical environments with similar requirements for legally defensible data. A hardware setup is the same as in other 2011 SAMHSA methods from Agilent. These methods are intended for all users of Agilent 1100 and Agilent 1200 Series LC because the back pressure in the LC system does not exceed 400 bar. Source parameters can be easily modified to use this method with other models of Agilent Triple Quadrupole LC/MS systems. Electronic copies of the LC/MS/MS acquisition and quantitation methods are available from Agilent Technologies.

Table 2. Method evaluations, n = 5.

Parameter	Amphetamine	Methamphetamine	MDA	MDMA	MDEA
Process efficiency* (%)	86	93	91	93	95
Extraction recovery* (%)	94	94	95	97	96
Matrix effect* (%)	91	99	95	96	98
Accuracy** (%)	107	105	92	101	106
Precision (CV)**(%)	0.6	0.5	1.1	0.5	0.3

*determined at cutoff level

**determined at 40% cutoff level for amphetamine, MDA, MDMA, MDEA, and at the cutoff level for methamphetamine

References

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SAMHSA-Compliant LC/MS/MS Analysis of Benzoyllecgonine in Urine with Agilent Bond Elut Plexa PCX and Agilent Poroshell 120

Authors

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Abstract

New guidelines from the US Substance Abuse and Mental Health Services Administration (SAMHSA), effective October 2010, allowed LC/MS/MS methods to be used for confirmation of initial drug tests [1]. LC/MS/MS methods are often less complicated than previously employed GC/MS methods because they do not typically require a derivatization step. We present a method for analysis of benzoyllecgonine that meets the most recent SAMHSA guidelines to demonstrate linearity, limit of detection (LOD), accuracy, and precision, as well as measurement of matrix effects, extraction recovery, and overall process efficiency. This is one of a suite of six simplified methods covering all classes of SAMHSA-regulated drugs and using premier Agilent products, including Agilent Bond Elut Plexa PCX mixed-mode polymeric SPE sorbent, Agilent Poroshell 120 EC-C18 2.7 μm superficially porous LC column, Agilent 1200 Infinity LC system, and Agilent 6460 Triple Quadrupole LC/MS system with Agilent Jet Stream Technology (AJST) enhanced electrospray source.

Introduction

Benzoyllecgonine (BE) is a major urinary metabolite of cocaine. Cocaine hydrolysis to benzoyllecgonine occurs enzymatically (in the liver), as well as without catalysts at alkaline pH [2]. The SAMHSA-established confirmation cutoff concentration for benzoyllecgonine is 100 ng/mL, and a LOD at 10% of the cutoff would be 10 ng/mL [1].

The extraction method described in this application note provides reproducible high recoveries of benzoyllecgonine due to unique properties of the Agilent Bond Elut Plexa polymer. Unlike other polymeric sorbents, Plexa possesses an amide-free hydroxylated particle surface that excludes protein binding. This results in minimized ion suppression and maximum sensitivity. Fast flow and reproducible performance are due to the narrow particle size distribution with no fines to cause blockages.

A Poroshell 120 EC-C18, 3 × 50 mm, 2.7 μm column was chosen due to its high capacity and excellent separation properties. With superficially porous 2.7 μm particles, Poroshell 120 provides similar efficiency to sub-2 μm UHPLC columns but with about 40% less back pressure, thereby allowing users of even 400 bar LC systems to increase resolution and to shorten both analysis and re-equilibration times by applying a higher flow rate.

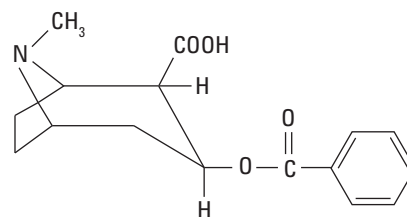
With a low sample injection volume of 2 μL and no sample preconcentration, the presented method demonstrates excellent signal-to-noise (S/N) ratios (> 400:1 at 10 ng/mL, 10% of the SAMHSA confirmation cutoff) due to the enhanced sensitivity of the Agilent 6460 Triple Quadrupole LC/MS system with the AJST electrospray source.

Previous methods from Agilent used the Agilent 6410 Triple Quadrupole LC/MS system and other SPE/LC products and procedures [3,4].

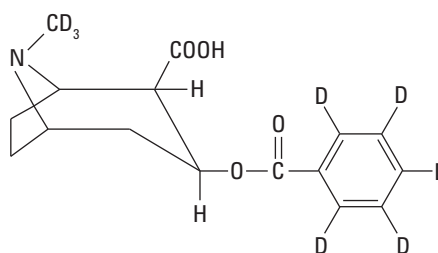
Experimental

Analytes

Drug standards were purchased from Cerilliant Corporation as 1 mg/mL (benzoyllecgonine) and 100 μg/mL (benzoyllecgonine-D₈) solutions in methanol.



Benzoyllecgonine
Log P (predicted) -0.73–2.26
pK_{a1} 10.1



Benzoyllecgonine-D₈

Figure 1. Benzoyllecgonine analytes and their structures. Predicted log P values from DrugBank, ChemSpider, PubChem.

Materials and instrumentation

SPE

- Agilent Bond Elut Plexa PCX cartridges, 30 mg, 3 mL (p/n 12108303)
- Agilent vacuum manifold VacElut 20 (p/n 12234100)
- Agilent stopcock valves (p/n 12234520)
- Agilent 2-mL autosampler vials (p/n 5182-0716)
- Agilent screw caps for autosampler vials (p/n 5182-0717)

LC

- Agilent Poroshell 120 EC-C18, 3 × 50 mm, 2.7 μm column (p/n 699975-302)
- Agilent 1260 Infinity LC (G1379B microdegasser, 1312B binary pump in low delay volume configuration, G1367E autosampler, and G1330B thermostat)

MS

- Agilent 6460A Triple Quadrupole LC/MS system with AJST electrospray ionization source

Sample preparation

Pretreatment

Spike 1 mL of urine with ISTD at 200 ng/mL; use of 12 × 75 mm glass tubes is recommended. Add 1 mL of 2% formic acid, vortex; centrifuge if cloudy.

Extraction

1. Condition Bond Elut Plexa PCX column with 0.5 mL methanol – soak, then let drip.
2. Load sample/supernatants.
3. Wash 1: 1 mL 2% formic acid.
4. Wash 2: 1 mL of methanol.
5. Dry 5–10 minutes under vacuum (10–15 in Hg).
6. Elute with 1 mL methanol: ammonium hydroxide (100:20), freshly prepared. Let eluate drip into collection vials, then apply low vacuum (2–3 in Hg).
7. Evaporate under a stream of nitrogen to dryness.
8. Reconstitute in 1 mL initial mobile phase (10% methanol, 90% water, 0.1% formic acid).

LC/MS/MS

LC conditions

Mobile phase A	0.1% formic acid in water	
Mobile phase B	0.1% formic acid in methanol	
Flow rate	0.8 mL/min	
Gradient	Time (min)	% B
	0.0	10
	0.5	10
	2.5	70
	2.51	90
	5.5	90
	5.51	10
Stop time	5.6 min	
Post time	2 min	
Max pump pressure	400 bar	
Injection volume	2 µL	
Injection with needle wash		
Needle wash	Flush port 75:25 methanol:water for 10 s	
Disable overlapped injection		
No automatic delay volume reduction		

MS conditions

ES source parameters

Ionization mode	Positive
Capillary voltage	3,000 V
Drying gas flow	10 L/min
Drying gas temperature	350 °C
Nebulizer gas	35 psi
Sheath gas flow	12 L/min
Sheath gas temperature	400 °C
Nozzle voltage	0 V

MS parameters

Scan type	MRM
Pre-run script	SCP_MS DiverterValveToWaste() {MH_Acq_Scripts.exe}
Time segments	#1: 1.2 min - diverter valve to MS
Delta EMV(+)	200 V

Results and Discussion

At acidic pH, the tertiary amine of benzoylecgonine was protonated, and the analyte was efficiently retained on Plexa PCX polymeric sorbent by a combination of hydrophobic interaction and a strong cation exchange.

A 100% methanol wash eliminated most matrix interferences without BE loss from the SPE column. A strong base was added to the organic eluent to break the ionic interaction between the analyte and the strong cation exchange sorbent. Benzoylecgonine recovery was optimized with 20% NH₄OH added to methanol shortly before sample elution.

The Poroshell 120 EC-C18, 3 × 50 mm, 2.7 μm column provided fast separation of benzoylecgonine in urine extract and good peak shape (Figure 2). The LC separation started with a low fraction of the organic solvent (10%) to allow salts and other polar components of urine to elute at the beginning of the sample run. Each sample run started with diverting a first portion of flow (0 to 1.2 minutes) to waste to minimize source contamination. Data collection started at 1.2 minutes, immediately after the diverter valve switch. A flow rate of 0.8 mL/min allowed for short analysis and re-equilibration times.

A S/N ratio >400:1 for the 10 ng/mL peak (Figure 2, upper panel) illustrates a state-of-the-art performance of the Agilent 6460 Triple Quadrupole capable of reliably detecting benzoylecgonine at a small fraction (10%) of the SAMHSA cutoff concentration.

SAMHSA guidelines require one quantifier and at least one qualifier ion for both target compound and ISTD. A third transition for target analyte (Table 1) was provided for additional confidence. The Agilent MassHunter Quantitative software automatically calculated qualifier ion ratios, highlighting those out of acceptable range.

Table 1. MRM transitions.

Compound	Precursor	Product	Fragmentor	Collision energy
BE	290.1	168.1	90	15
BE	290.1	105.1	90	30
BE	290.1	82.1	90	32
BE-D ₅	298.2	171.1	90	15
BE-D ₅	298.2	110.1	90	30

Figure 3 is an example calibration curve for extracted urine standards at five concentration levels of benzoylecgonine. Calibration standards were prepared by spiking negative urine at 10, 100, 500, 1,000, and 4,000 ng/mL. Deuterated internal standard BE-D₈ was added at 200 ng/mL. The excellent linear fit with $R^2 = 0.998$ demonstrated linearity of the method across a broad dynamic range of concentrations, as required by SAMHSA guidelines.

Normal, rather than dynamic, MRM scan type can be used with this method, because dynamic MRM has no advantages for detection of a single compound.

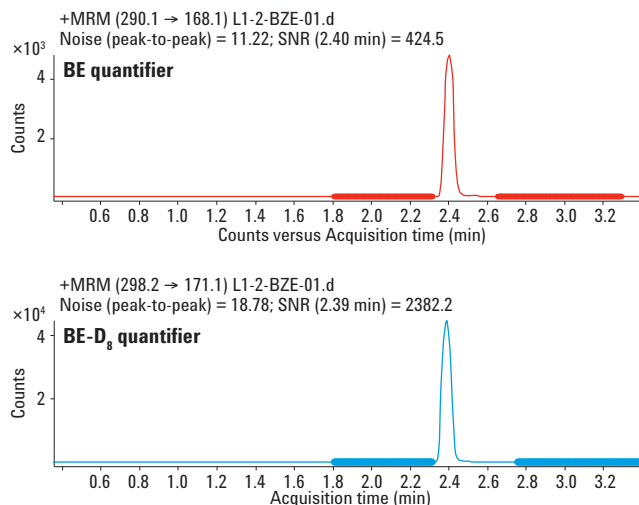


Figure 2. MRM extracted ion chromatograms for BE (10 ng/mL) and BE-D₈ (200 ng/mL) in urine extract. Agilent Poroshell 120 EC-C18, 3 × 50 mm, 2.7 μm column. Noise regions are shown in bold.

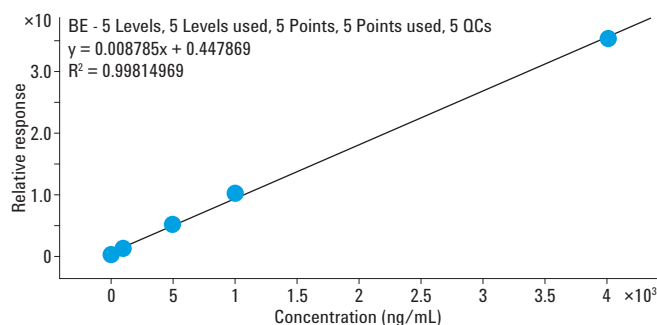


Figure 3. Example calibration curve for benzoylecgonine in urine extract. Calibration range 10 to 4,000 ng/mL. Linear fit, $R^2 = 0.998$.

Method evaluation

Method performance metrics in Table 2 were calculated according to the principles laid out in Matuszewski *et al* and widely accepted as an industry standard approach for LC/MS/MS methods [5]. The extraction procedure and the LC/MS/MS measurement were performed for five replicates of negative urine spiked pre-extraction at the cutoff level, and five replicates of negative urine extract reconstituted in initial mobile phase and then fortified at 100 ng/mL with benzoylecgonine (spiked post-SPE). The third measurement was of initial mobile phase (the reconstitution solvent) fortified to correspond to the cutoff concentration of 100 ng/mL in urine (spiked mobile phase).

Process efficiency (absolute recovery) is a ratio of a peak area of target analyte in urine sample spiked pre-SPE to its peak area in matrix-free spiked mobile phase. Extraction recovery is a ratio of a peak area of target analyte in urine extract spiked pre-SPE to its peak area in an extracted negative urine sample spiked post-SPE. Matrix effect is a ratio of a peak area of target analyte in urine spiked post-SPE to its peak area in spiked mobile phase.

Accuracy is a ratio of a measured concentration calculated using the calibration curve to the expected concentration in a sample spiked with a known amount of target analyte. Precision or coefficient of variation (CV) is a measure of reproducibility and is calculated as a percent standard deviation over the mean of the five measurements.

Table 2 shows high extraction recovery for benzoylecgonine (86%) together with excellent accuracy (102%) and precision (0.7%). Matrix effect of 99% indicates minor ion suppression of a signal due to matrix interferences (1%), thus, confirming an exceptional cleanliness of Plexa PCX-processed extracts.

Table 2. Method evaluation at the cutoff level, n = 5.

	%
Process efficiency*	85
Extraction recovery*	86
Matrix effect*	99
Accuracy**	102
Precision** (CV)	0.7

*determined at cutoff level

**determined at 40% cutoff

Conclusions

The solid phase extraction procedure coupled with LC/MS/MS detection method described in this application note is SAMHSA-compliant and provides accurate, precise, and reproducible results for forensic toxicology or other analytical environments with similar requirements for legally defensible data. The hardware setup is the same as in the other 2011 SAMHSA methods from Agilent. These methods are intended for all users of Agilent 1100 and Agilent 1200 Series LCs because the back pressure in the LC system does not exceed 400 bar. Source parameters can be easily modified to use this method with other models of Agilent Triple Quadrupole LC/MS/MS instruments. Electronic copies of the LC/MS/MS acquisition and quantitation methods are available from Agilent Technologies.

References

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Faster LC Analysis of Notoginseng Total Saponins Using an Agilent Poroshell 120 EC-18

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Abstract

The traditional method for analyzing notoginseng total saponins was transferred from an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 μm column to an Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 μm column. Gradient time decreased from 60 minutes to 18 minutes. The transferred method was fast, with higher efficiency and a 2.11 resolution for the critical pair of compounds, ginsenosides Rg1 and Re. In addition, backpressure was below 270 bar and so the analysis could be run on a traditional HPLC instrument.

Introduction

Panax notoginseng, also known as San Qi, is a plant of the Araliaceae family. It is a traditional Chinese herb well-known for its therapeutic abilities to stop hemorrhage [1], to influence blood circulation, and to act as a tonic. *P. notoginseng* contains about 8–12% by weight of saponins. Total saponins of *P. notoginseng*, the major bioactive components, are used to treat coronary heart disease, cardiac angina, apoplexy, and atherosclerosis [2, 3]. However, notoginseng total saponins contain several kinds of active components such as notoginsenoside R1, ginsenoside Rg1, Rb1, Re, and Rd. The analysis of saponins is important for evaluating the quality of notoginseng and its Chinese medicine preparations.

Traditionally, the HPLC run time is greater than 60 minutes for the analysis of notoginseng total saponins with the China Pharmacopeia method using a conventional LC column [4]. Agilent Poroshell 120 EC-C18, 2.7 μm columns are packed with superficially porous materials, which deliver fast separation and achieve performance similar to sub-2 μm totally porous materials, but with lower pressure. This application note describes a fast quality control method for the analysis of notoginsenoside R1 and Ginsenosides Rg1, Re, Rb1, and Rd using the Agilent 1290 Infinity LC System and a Poroshell 120 EC-C18 column. Compared to conventional methods, the rapid method is much faster, with better performance, and quality of separation. In addition, solvent consumption is dramatically reduced.

Experimental

Analyses were performed on an Agilent 1290 Infinity LC System consisting of a binary pump (G4220A), a thermostated column compartment (TCC, G1316C), an autosampler (G4226A), and a diode array detector (DAD, G4212A).

Columns

Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 μm (p/n 959990-902)

Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 μm (p/n 697975-902)

Compounds

Compounds of interest are shown in Figure 1, with their respective structures. They were dissolved in 70% methanol aqueous solution at 2.5 mg/mL. Notoginseng total saponins were purchased from a local TCM store. Twenty five mg of the sample powder were transferred to a 10 mL volumetric flask and a 70% methanol aqueous solution was added to dissolve and dilute to volume. This solution was then filtered through a 0.45 μm regenerated cellulose membrane filter (p/n 5064-8221) and injected directly into the HPLC system.

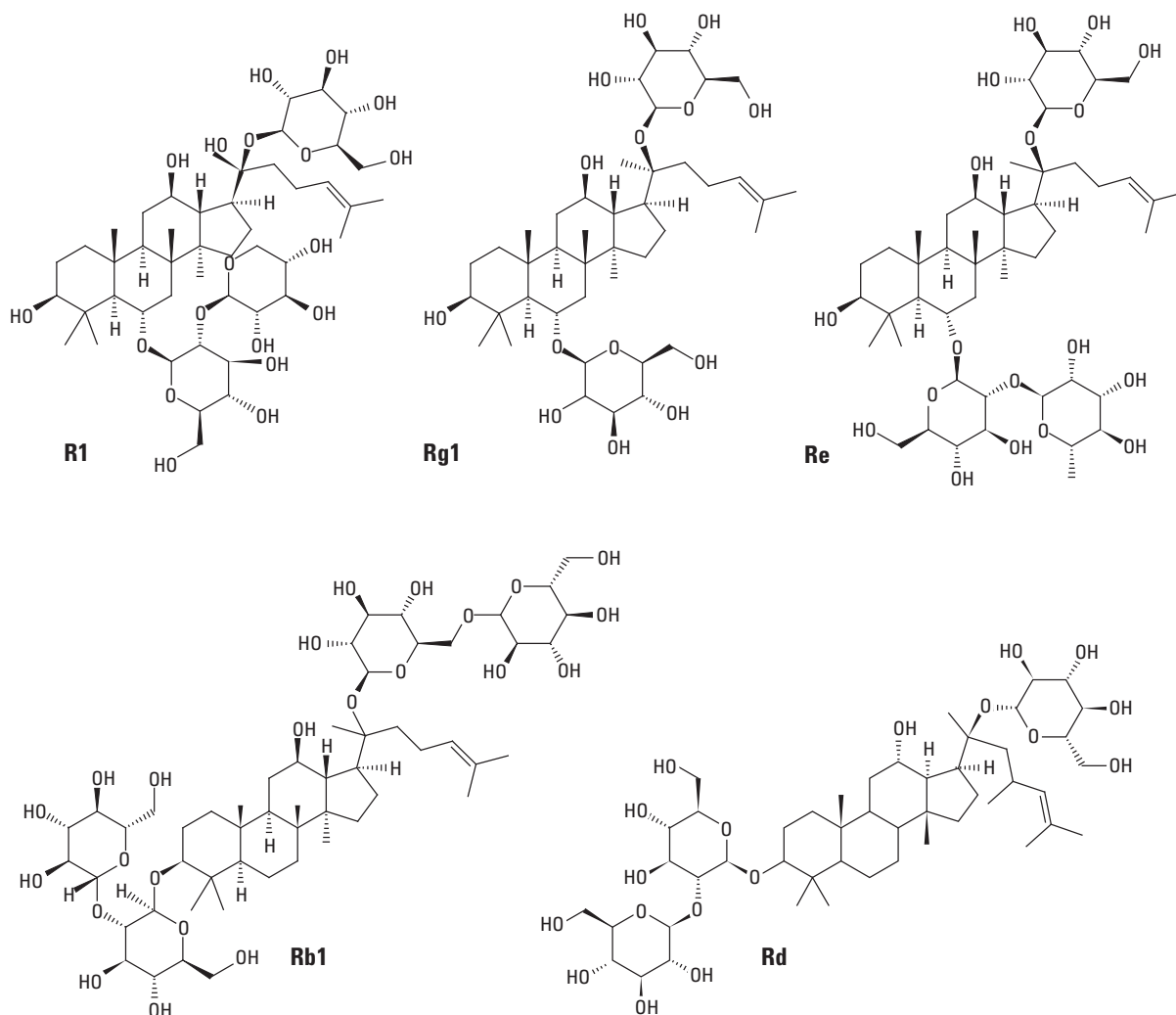


Figure 1. Structure of notoginsenoside R1 and ginsenosides Rg1, Re, Rb1 and Rd.

Results and Discussion

The original separation method of notoginseng total saponins published in the 2010 Chinese Pharmacopoeia was repeated on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 μm column. It took approximately 60 minutes to separate notoginsenoside R1 and ginsenosides Rg1, Re, Rb1, and Rd. Compounds of interest were baseline separated with excellent peak shape. The Agilent Poroshell 2.7 μm particle columns provided similar performance to that of totally porous sub-2 μm columns, but with lower pressure. By using an

Agilent Poroshell 120 EC-C18 4.6 × 75 mm, 2.7 μm column, method transfer and optimization were completed quickly. As shown in Figure 2, the analysis time decreased from 60 minutes to 18 minutes, while achieving better resolution for the critical pair Rg1 and Re, and better theoretical plates for Rg1, which exceeded the requirement of the 2010 Chinese Pharmacopoeia ($N > 6000$). The pressure was less than 270 bar, which is quite acceptable for a 400-bar HPLC when using an Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 μm column. In addition, solvent consumption can be significantly decreased, thereby lowering costs.

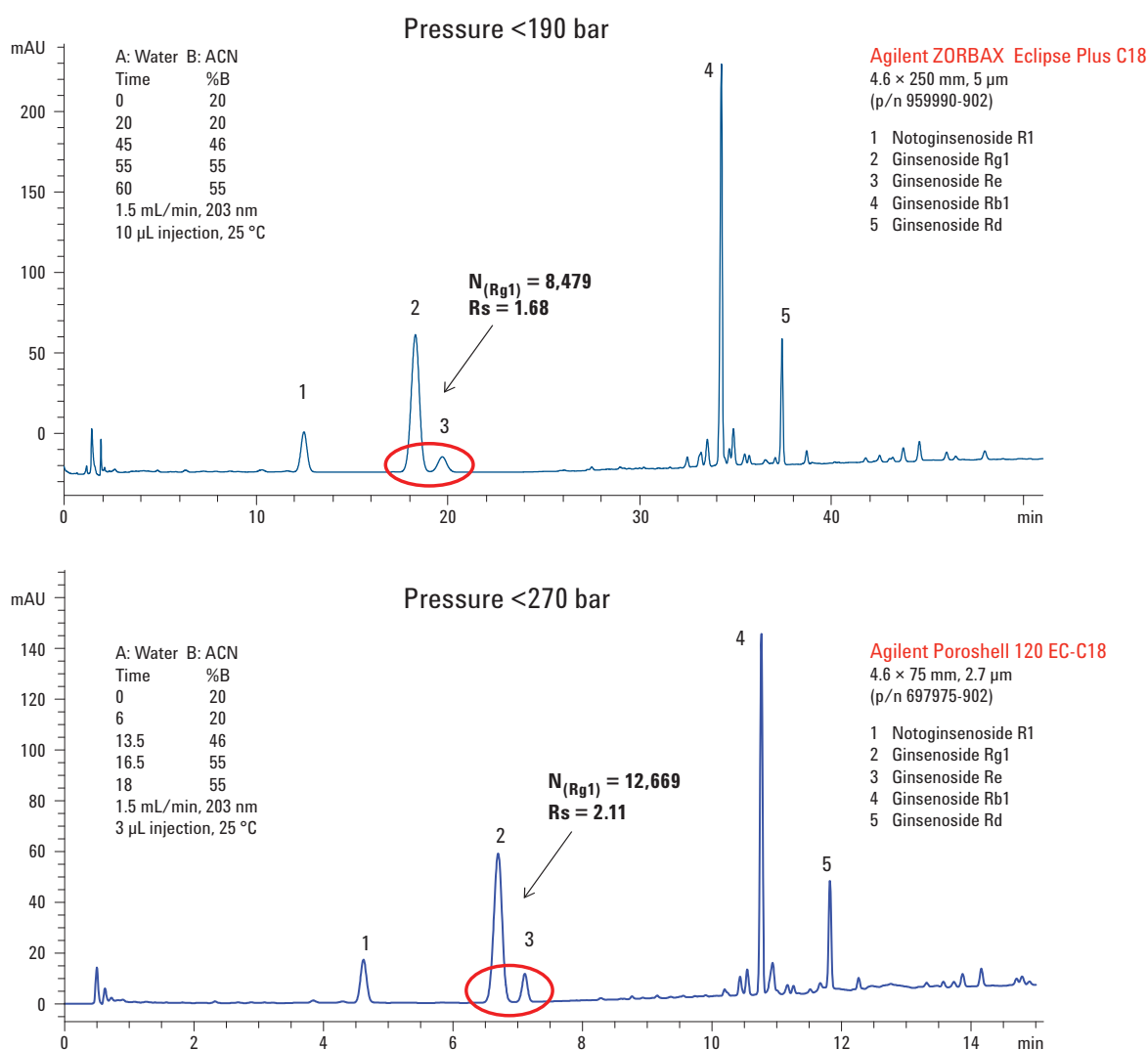


Figure 2. Overlaid chromatograms of notoginseng total saponins using an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 μm and an Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 μm.

Conclusions

The traditional method for analyzing notoginseng total saponins was reproduced successfully on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 μm column. The shorter Agilent Poroshell 120 EC-C18 column can greatly reduce the analysis time and provide better separation and peak shape, and thereby substantial time and cost savings. The Poroshell 120 column can exceed the requirements of the 2010 Chinese Pharmacopoeia for notoginseng total saponins analysis. It is well suited for evaluating the quality of notoginseng and its Chinese medicine preparations.

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Analysis of Echinacoside and Verbascoside in *Cistanche deserticola* Chinese Medicine Using an Agilent Poroshell 120 EC-C18

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Introduction

Due to the complex components in traditional Chinese medicines (TCM), a gradient method with a long time period is often required to separate the target compounds from the complex matrix. HPLC has been an effective method for quality control of TCM applied in the China Pharmacopoeia (CHP).

Cistanche deserticola Y. C. Ma (1960), a commonly used TCM included in the CHP, is prescribed to reinforce the vital function of the kidney and to influence fertility, leukorrhea, and metrorrhagia in women. Phenylethanoid glycosides in *C. deserticola* are the most important active compounds, which have functions of antioxidation, protecting liver and nerves [1]. In the CHP, the amounts of the two main phenylethanoid glycosides of echinacoside and verbascoside (Figure 1) in *C. deserticola* extracts are regulated using HPLC for quality control.

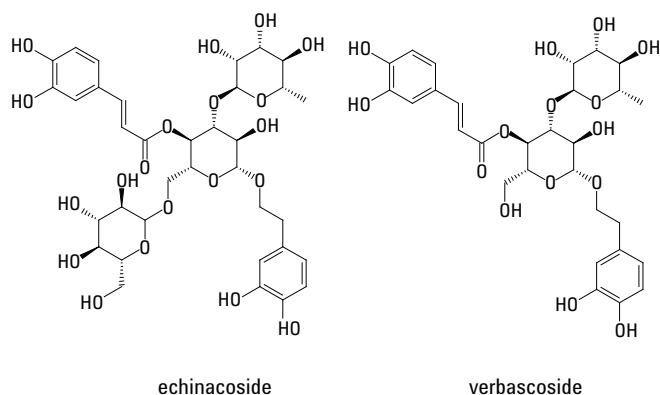


Figure 1. Structures of echinacoside and verbascoside

Traditionally, it takes about 35 minutes to analyze the two compounds with the CHP method using a conventional 5 μm particle column. This application note describes a fast quality control method for the analysis of echinacoside and verbascoside using the Agilent 1290 Infinity LC System and an Agilent Poroshell 120 EC-C18, 2.7 μm column. Compared to conventional methods, the rapid method is much faster, and maintains the same performance and quality of separation. In addition, solvent consumption is dramatically reduced.

HPLC conditions

The analysis was performed with the 1290 Infinity LC System including a G4220A Infinity binary pump, G4226A Infinity sampler (ALS), G1316C Infinity Thermostatted Column Compartment (TCC), and G4212A Diode Array Detector SL (DAD).

Conditions

Sample	Extract of <i>Cistanche deserticola</i>
Mobile phase	A, 0.1% (v/v) formic acid; B, methanol
UV	330 nm
TCC temp	30 $^{\circ}\text{C}$

Conditions for Figure 2

Column	Agilent ZORBAX Eclipse Plus C18 4.6 \times 150 mm, 5 μm (p/n 959993-902)	
Gradient	time (min)	%B
	0	26.5
	17	26.5
	20	29.5
	32	29.5
	33	80
Stop time	35 min	
Flow rate	1 mL/min	
Injection volume	10 μL	

Conditions for Figures 3 and 4

Column	Agilent Poroshell 120, EC-C18, 3.0 \times 50 mm, 2.7 μm (p/n 699975-902)	
Gradient	time (min)	%B
	0	26.5
	5.67	26.5
	6.67	29.5
	10.67	29.5
	11	80
Stop time	11.67 min	
Flow rate (Figure 3)	0.425 mL/min	
Flow rate (Figure 4)	0.425, 0.85, and 1.7 mL/min	
Injection volume	1.4 μL	

Results and discussion

The original LC method for the analysis of *C. deserticola* used an Agilent ZORBAX Eclipse Plus C18, 4.6 \times 150 mm, 5 μm column. Analysis took approximately 35 minutes to separate echinacoside and verbascoside and recondition the column to initial gradient conditions (Figure 2).

By using the 1290 Infinity LC system and a Poroshell 120 EC-C18, 3.0 \times 50 mm column, method transfer and optimization were completed quickly and easily. Analysis was accomplished in 12 minutes, while maintaining the same or even better performance for the two target compounds. Since both Agilent columns have similar chemistry, the separation achieved almost the same selectivity (Figure 3).

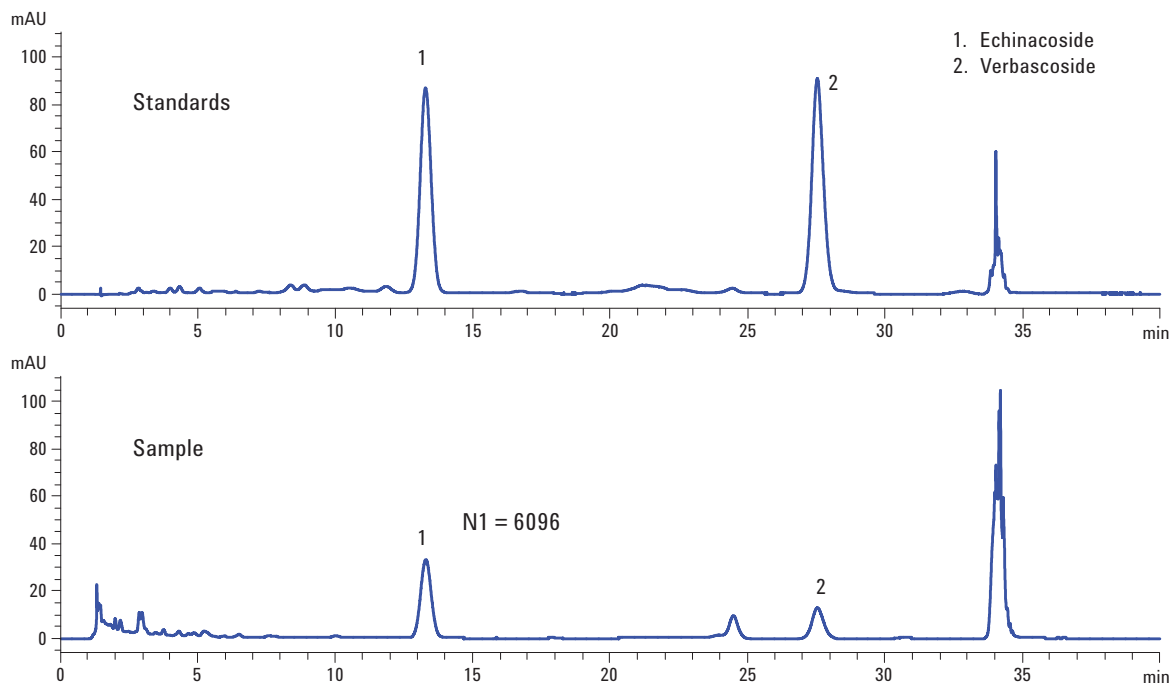


Figure 2. Echinacoside and verbascoside standards, and extract from *Cistanche deserticola*, analyzed on an Agilent ZORBAX Eclipse Plus C18 4.6 × 150 mm, 5 μm column.

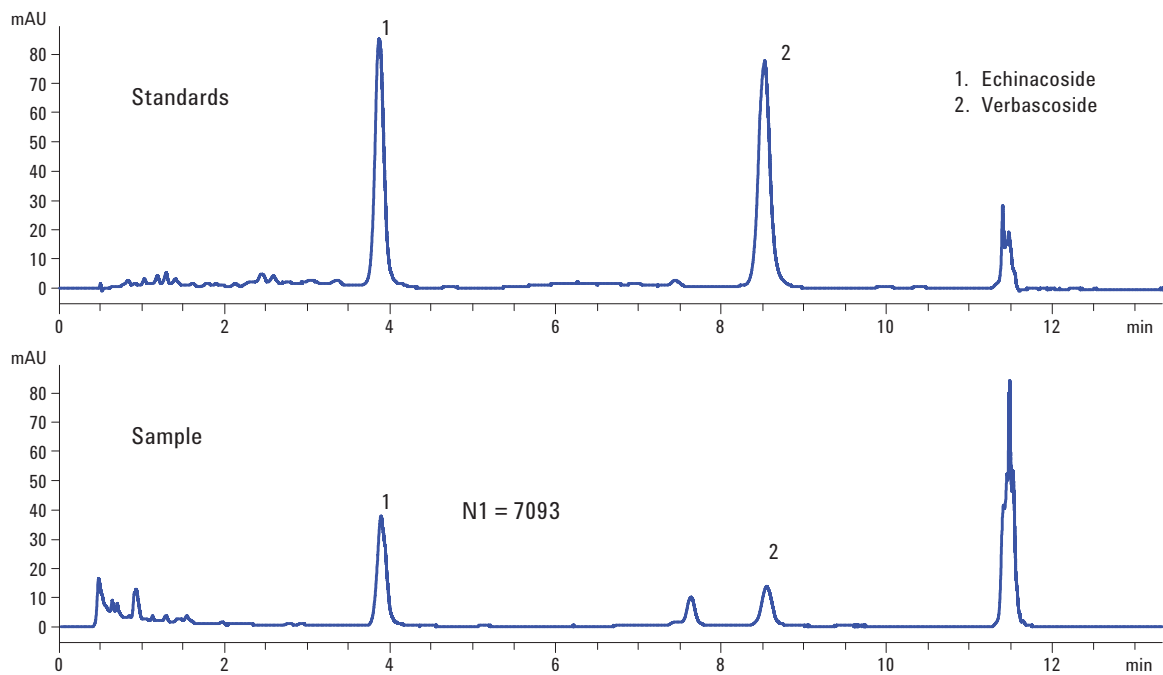


Figure 3. Echinacoside and verbascoside standards, and extract from *Cistanche deserticola*, analyzed on an Agilent Poroshell 120, EC-C18, 3.0 × 50 mm, 2.7 μm column.

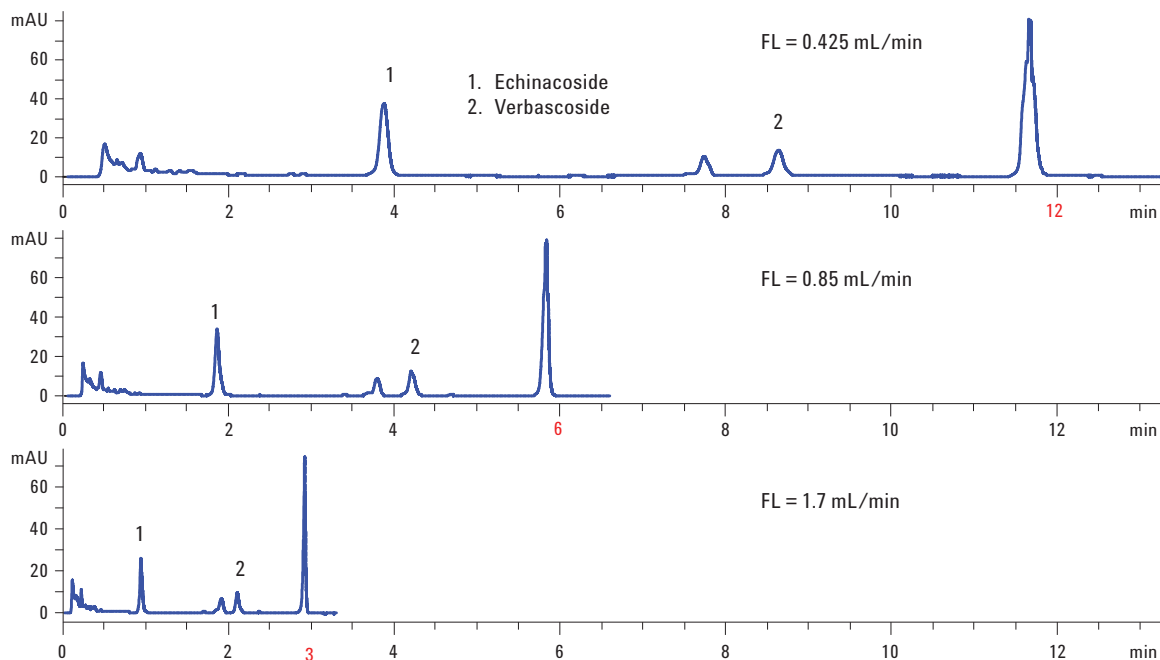


Figure 4. Echinacoside and verbasicoside standards, and extract from *Cistanche deserticola*, analyzed on an Agilent Poroshell 120, EC-C18, 3.0 x 50 mm, 2.7 μm column at different flow rates

In addition, solvent consumption was reduced from 35 mL to 5 mL. To take full advantage of the small-particle column, a higher flow rate could be used to further increase the speed of analysis, as shown in Figure 4.

Conclusion

The shorter Poroshell 120 column with 2.7 μm superficially porous particles dramatically reduce the separation time of *C. deserticola* extracts while maintaining a separation similar to that obtained with conventional 5 μm columns. Therefore, quality control of this traditional Chinese medicine is easy and fast when using Agilent Poroshell 120 columns, and time and solvent could be saved for complex analysis of other such medicines.

Reference

1. Chenghua LI, *et al.* RP - HPLC simultaneous determination of four phenylethanoid glycosides in *Cistanche tubulosa* (Schrenk) Wight.

Fast Screening Methods for Steroids by HPLC with Agilent Poroshell 120 Columns

Author

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Introduction

Steroids are a type of lipid derived from cholesterol. The main feature of steroids is the ring system of 3 cyclohexanes and 1 cyclopentane in a fused ring system, as shown in Figure 1. There are a variety of functional groups that may be attached. The main feature, as in all lipids, is the large number of carbon-hydrogens, which makes steroids non-polar [1].

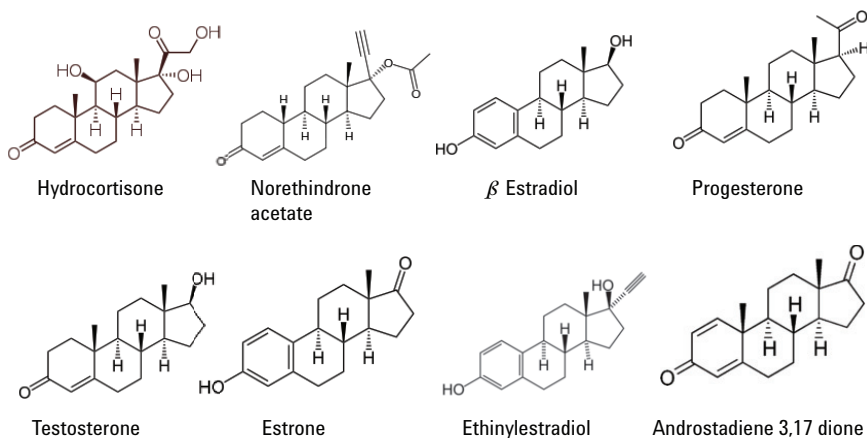


Figure 1. Structures of selected steroids.

Using Selectivity to Enhance Separation of Steroids

When considering the best way to increase chromatographic resolution, it can be useful to consider the resolution equation, which relates efficiency, selectivity, and retention factor.

$$R = \frac{\sqrt{N}}{4} \left(\frac{\alpha - 1}{\alpha} \right) \left(\frac{1 + k'_B}{k'_B} \right)$$

To obtain high resolution, the 3 terms must be maximized. An increase in N , the number of theoretical plates, by lengthening the column, leads to an increase in retention time and increased band broadening. This may not be desirable. Instead, to increase the number of plates, the height equivalent to a theoretical plate can be reduced by reducing the particle size of the stationary phase particles. Superficially porous particles, such as Agilent Poroshell 120, achieve 90% of the efficiency of 1.8 μm materials with considerably lower pressure.

The selectivity factor, α , can also be manipulated to improve separations. Changing selectivity is the variable that can have the largest impact on any separation. Selectivity can be increased by:

- Changing mobile phase composition
- Changing column temperature
- Changing composition of stationary phase

Selectivity is the most powerful tool to optimize separations in HPLC. This parameter is changed by using different bonded phases, including C18, C8, polar embedded, and phenyl bonded phases, or by changing the mobile phase. In this work, Poroshell 120 columns and the Agilent 1200 SL Method Development Solution were used to quickly evaluate method development choices for the analysis of steroids. The short column length and high efficiency provided short analysis times and rapid equilibration leading to fast investigations of selectivity.

Experimental

The Agilent 1260 Infinity Series LC Multi-Method Solution was used. This system consisted of:

- 1260 Infinity Binary Pump (G1312B)
- 1290 Infinity Thermostatted Column Compartment (G1316C)
- 1260 Infinity High Performance Autosampler (G1367E)
- 1290 Infinity Diode-Array Detector (G4212A), equipped with 10 mm MaxiLight cartridge flow cell
- G6140 Single Quadrupole Mass Spectrometer.

The Agilent 1260 Infinity Series LC Multi-Method Solution is a highly flexible system that can be used for up to 4 (100 mm) columns. In addition, the Agilent ChemStation Method Scouting Wizard automates the setup of methods and sequences to screen the available combinations of columns, solvents, predefined gradients, and temperatures. In this work, 4 Agilent Poroshell 120 columns were used:

- Agilent Poroshell 120 StableBond SB-C18, 2.1 \times 100 mm, 2.7 μm (p/n 685775-902)
- Agilent Poroshell 120 EC-C18, 2.1 \times 100 mm, 2.7 μm (p/n 695775-902)
- Agilent Poroshell 120 Bonus-RP, 2.1 \times 100 mm, 2.7 μm (p/n 685775-901)
- Agilent Poroshell 120 Phenyl-Hexyl, 2.1 \times 100 mm, 2.7 μm (p/n 695775-912)

The TCC was fitted with a 6 position/6 port selection valve. This is a new Quick Change Valve mounted on a slide-out rail to make plumbing and maintenance more convenient. Port 1 was connected to a StableBond C18 column, and port 2 was connected to an EC-C18 column. Port 3 was connected to a Bonus-RP column, port 4 to a Phenyl-Hexyl column, and port 6 to a bypass connecting capillary.

The solvent passing into each column was heated using 1 of 4 individual low-dispersion heat exchangers. A G1160 12 solvent selection valve was connected to valve position A1 on the G1312B. Together with the internal solvent selection valve of the Binary SL Pump, up to 15 solvents could be screened using this system. The mobile phase was methanol or acetonitrile with 0.1% formic acid and water with 0.1% formic acid. An acetonitrile/water mixture (50%/50% v/v) was used to rinse the modifiers from the columns and allow proper column storage. Agilent ChemStation version B.04.02 was used to control the instrument and process the data.

The compounds examined included hydrocortisone, norethindrone acetate, estradiol, progesterone, testosterone, estrone, ethinylestradiol, and boldione, which were all purchased from Sigma Aldrich. Structures and details are shown in Figure 1 and Table 1. All samples were prepared at 10 mg/mL in acetonitrile and were diluted in water to a final concentration of 0.1 mg/mL.

Column choice to enhance selectivity

The columns were chosen to improve selectivity in the separation. They included a highly end capped column recommended as a first choice in method development (Poroshell 120 EC- C18), and a non end capped C18 (Poroshell 120 StableBond SB-C18) that could have interaction with silanol groups to provide an alternative C18 selectivity using neutral to low pH mobile phases. A polar-embedded amine column (Poroshell 120 Bonus-RP) and a phenyl-hexyl column (Poroshell 120 Phenyl-Hexyl) were also used. Phenyl bonded phases are known for their improved selectivity for aromatic compounds.

A polar-embedded group inserted into the hydrophobic C14 alkyl chain allows the Bonus-RP phase on totally porous Poroshell 120 to minimize interaction of polar samples with silanols, providing symmetrical peaks for a wide variety of applications. This phase is especially useful at neutral pH where amines can interact strongly with ionized silanols. The polar-embedded group also helps to wet the hydrophobic chains and prevents phase collapse in highly aqueous mobile phases.

Poroshell 120 Bonus-RP can be used for many of the same separations as a C18 column while avoiding some of the disadvantages of C18, such as poor wettability in high aqueous mobile phases. In addition, it is much more retentive for those molecules that can interact by hydrophobic interactions and also by H-bonding with the amide group. Compared to alkyl only phases, Bonus-RP has enhanced retention and selectivity for phenols, organic acids, and other polar solutes due to strong H-bonding between polar group (H-bond acceptor) and H-bond donors, like phenols and acids. Bonus-RP gives retention slightly less than a C18 allows, for easy column comparison without the need to change mobile phase conditions. The Bonus-RP phase gives different selectivity than C18 for polar compounds. It is also compatible with 100% water.

The Phenyl-Hexyl phase has unique reversed-phase selectivity, especially for polar aromatics and heterocyclic compounds, derived from analyte interaction with the aromatic ring of the bonded phase and its delocalized electrons. Poroshell 120 Phenyl-Hexyl can be orthogonal to both C18 and Bonus-RP phases. More retention and selectivity will often be observed for solutes with aromatic electron-withdrawing groups such as fluorine or nitro groups [2,3,4].

Table 1. Steroid nomenclature and molecular characteristics.

Common name	IUPC name	Molecular formula	Molecular weight
Hydrocortisone	Cortisol	C ₂₁ H ₃₀ O ₅	362.460
Norethindrone acetate	(17 α)-17-ethynyl-3-oxoestr-4-en-17-yl acetate	C ₂₂ H ₂₈ O ₃	340.456
β Estradiol	(17 β)-estra-1,3,5(10)-triene-3,17-diol	C ₁₈ H ₂₄ O ₂	272.38
Progesterone	Pregn-4-ene-3,20-dione	C ₂₁ H ₃₀ O ₂	314.46
Testosterone	(8R,9S,10R,13S,14S,17S)-17-hydroxy-10,13-dimethyl-1,2,6,7,8,9,11,12,14,15,16,17-dodecahydrocyclopenta[a]phenanthren-3-one	C ₁₉ H ₂₈ O ₂	288.42
Ethinylestradiol	19-Nor-17 α -pregna-1,3,5(10)-trien-20-yne-3,17-diol	C ₂₀ H ₂₄ O ₂	296.403
Androstadiene 3,17 dione (boldione)	(8R,9S,10R,13S,14S)-10,13-dimethyl-7,8,9,11,12,14,15,16-octahydro-6H-cyclopenta[a]phenanthrene-3,17-dione	C ₁₉ H ₂₄ O ₂	284.39
Estrone	3-hydroxy-13-methyl-6,7,8,9,11,12,13,14,15,16-decahydrocyclopenta[a]phenanthren-17-one	C ₁₈ H ₂₂ O ₂	270.366

Poroshell 120 Phenyl-Hexyl columns deliver unique selectivity for compounds with aromatic groups, providing superior resolution for these samples. Poroshell 120 Phenyl-Hexyl can also provide optimum separations of moderately polar compounds where typical alkyl phases (C18 and C8) do not provide adequate resolution. Acetonitrile tends to decrease the π - π interactions between aromatic and polarizable analytes and the phenyl-hexyl stationary phases, but methanol enhances those same interactions, giving both increased retention and changes in selectivity [5]. This does not mean that acetonitrile should not be used with a phenyl bonded phase or that it might not provide an acceptable separation, but methanol is more likely to deliver the additional selectivity that is desired from a phenyl phase.

Results and Discussion

As can be seen in Figure 2, the separation of all 8 compounds was attempted on all columns surveyed. The Poroshell 120 EC-C18 and Poroshell 120 Phenyl-Hexyl columns showed very similar profiles, although the elution on the Phenyl Hexyl column was faster. This could indicate that the π - π interactions on the Phenyl-Hexyl column were being reduced by the acetonitrile. The overlap of estradiol and androstadiene was less severe on the Phenyl-Hexyl column. The Poroshell 120 SB-C18 column delivered a very different separation, resolving estradiol but losing resolution on ethinylestradiol and estrone. This could be due to the exposed silanols on the SB-C18 phase or to some additional shape selectivity derived

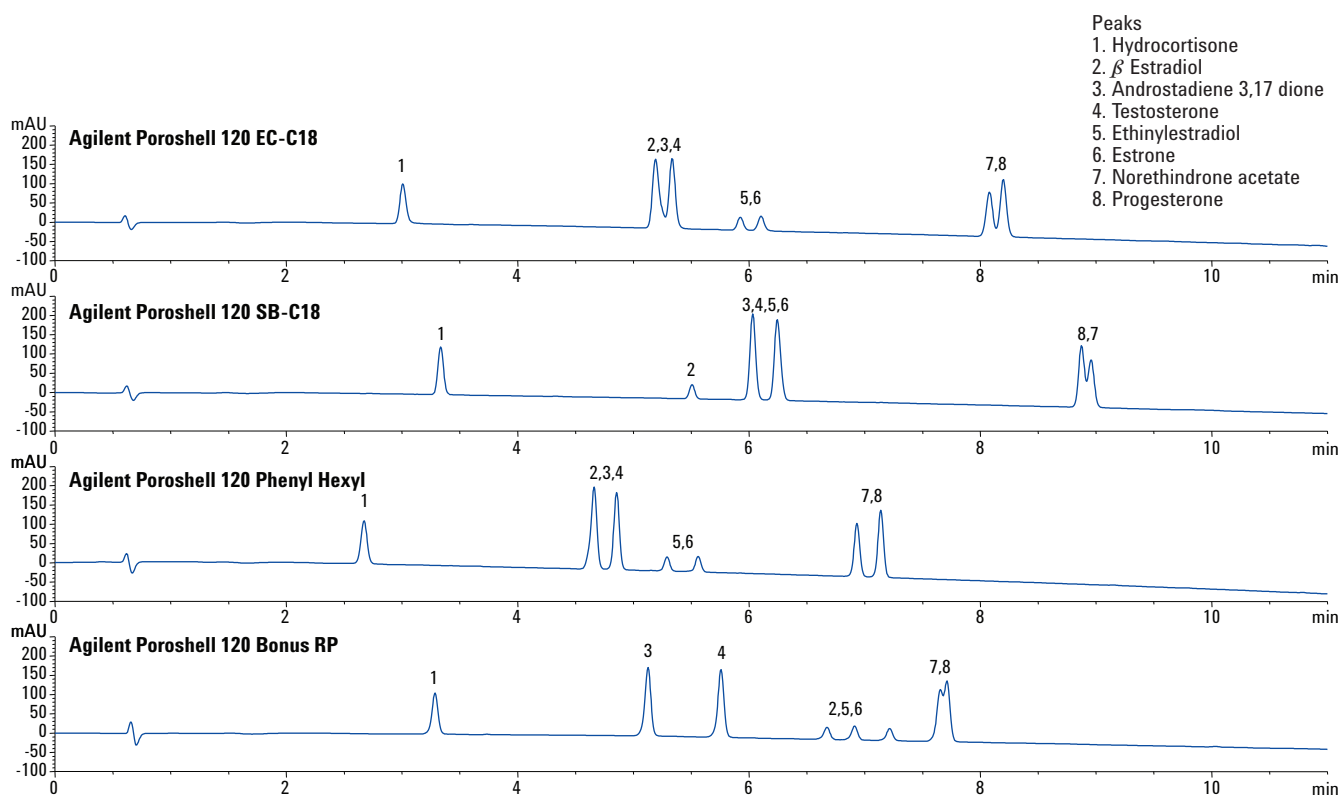


Figure 2. Separation of steroids using Agilent Poroshell 120 columns with acetonitrile.

Conditions

Columns: Agilent Poroshell 120, 2.1 × 100 mm
 Flow rate: 0.4 mL/min
 Gradient: 25-80% MeCN/10 min (0.1% formic acid in water and MeCN)
 Temperature: 25 °C
 Detection: DAD 260,80 ref = off

from the di-isobutyl side chains on the SB-C18 phase. Some additional work is needed to determine this. The Poroshell 120 Bonus-RP phase almost separates all 8 compounds, and when using acetonitrile, it would provide the best method development option for further development.

In Figure 3, the separation was carried out using methanol at slightly elevated temperature (40 °C). In this case, the 2 C18

phases (Poroshell 120 EC-C18 and Poroshell 120 SB-C18) yielded nearly identical chromatographic profiles. Some additional retention was seen on the SB-C18 phase due to some silanol interaction. The Poroshell 120 Bonus-RP chromatogram had 3 overlapping peak pairs, which would likely make further method development difficult in methanol. However, the Poroshell 120 Phenyl-Hexyl phase resolved 8 compounds at better than baseline resolution.

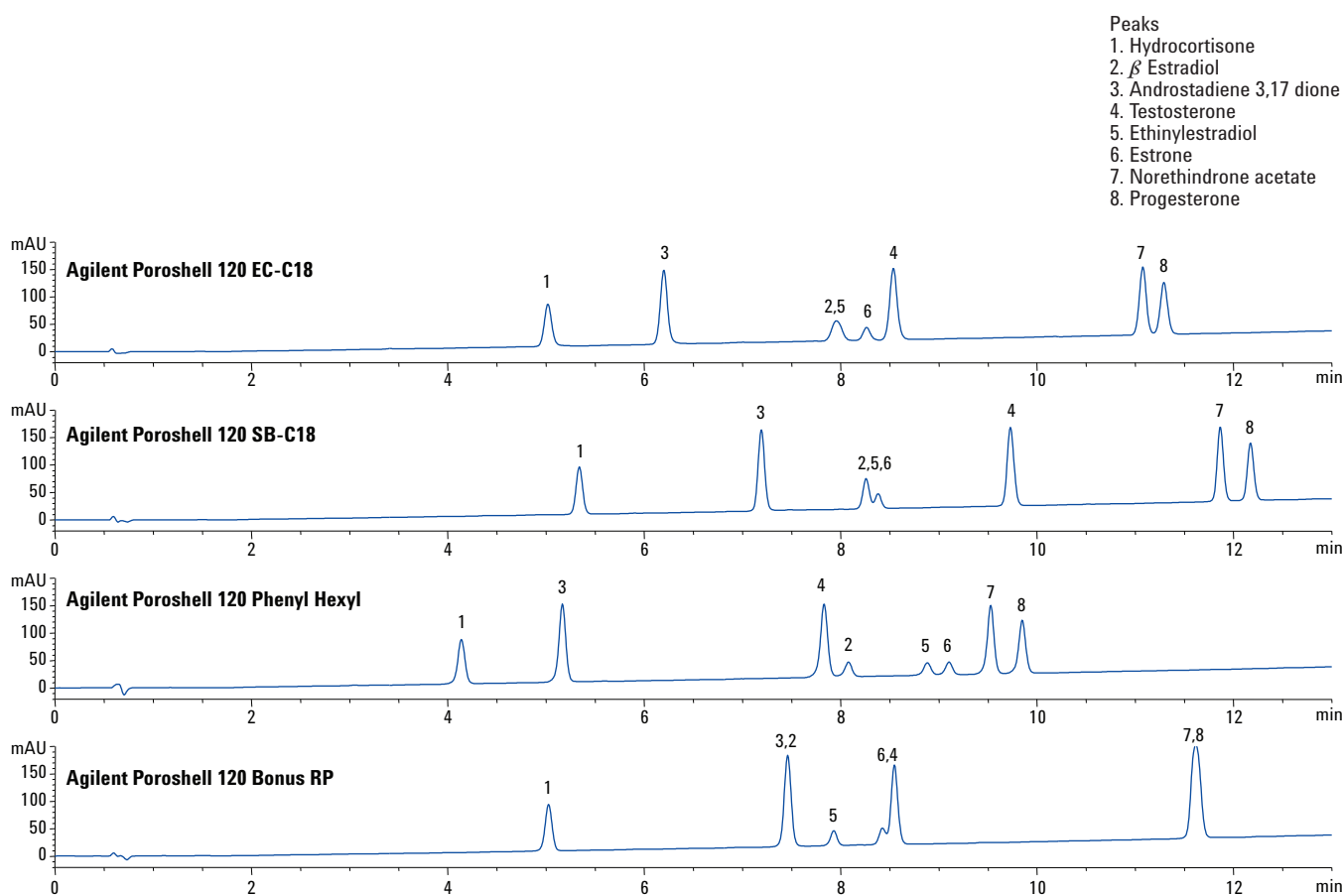


Figure 3. Separation of steroids using Agilent Poroshell 120 columns with methanol.

Conditions

Columns: Agilent Poroshell 120, 2.1 × 100 mm
 Flow rate: 0.4 mL/min
 Gradient: 40-80% MeOH/14 min (0.1% formic acid in water and MeOH)
 Temperature: 40 °C
 Detection: DAD 260, 80 ref = off

Conclusions

Analysis problems can be quickly resolved by including survey methods with generic gradients as part of the method development scheme. This work used steroids as an example, and showed how phases and organic modifiers, such as acetonitrile and methanol, could develop different selectivity that could be used to optimize the separation. In this case, the widely used C18 phases, as found on Poroshell 120 EC-C18 and SB-C18 columns, did not provide adequate separation. Using an alternative selectivity column such as Poroshell 120 Bonus-RP in acetonitrile or Poroshell 120 Phenyl Hexyl yielded better results, and could be used for several thousand samples.

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LC/ELSD and LC/MS/MS of Cholesterol and Related Sterols on a Poroshell 120 Column

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Introduction

Cholesterol and its metabolites, as well as several phytosterols, are separated using Agilent Poroshell 120 columns with LC/ELSD and LC/MS/MS. Two phases of Poroshell 120, EC-C18 and SB-C18, are compared for the sterol separation. Poroshell 120 EC-C18 has better selectivity for these sterols and gives better resolution. LC/MS/MS separates closely eluting compounds, such as lathosterol and cholesterol.

Introduction

The analysis of cholesterol and related compounds can be done by GC and LC. LC methods have several advantages in that they do not require derivatization of sterols prior to analysis, which simplifies the procedure by reducing time spent preparing samples. In addition, LC columns can better withstand the “dirty samples” in which cholesterol is naturally found, such as blood plasma.

Agilent Poroshell 120 columns were selected for this analysis because they can deliver UHPLC efficiencies and throughput with a 2.7 μm particle, resulting in relatively low backpressure. In addition, the 2 μm frit on the Poroshell 120 column is less likely to plug when compared to the 0.5 μm frit typically used on sub 2- μm UHPLC columns, making the Poroshell 120 columns a more rugged column choice for dirty samples.

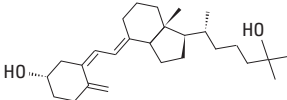
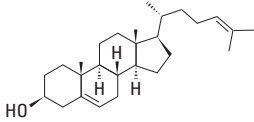
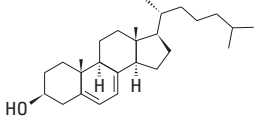
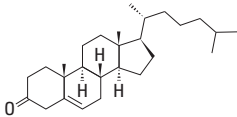
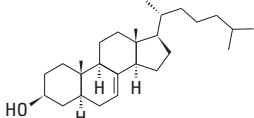
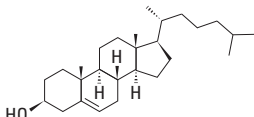
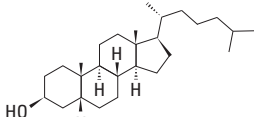
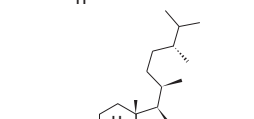
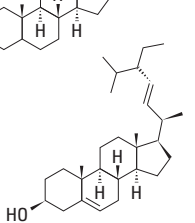
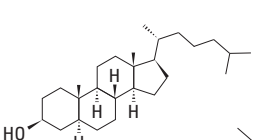
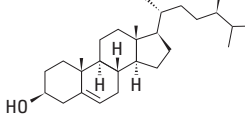
Detection of sterols using LC can be by MS, UV, or ELSD (evaporative light scattering detection). The choice of detector is influenced by the sensitivity needed in the separation, with LC/MS/MS providing very high sensitivity. We chose to develop separations using both ELSD and MS with an Agilent 6460A Triple Quadrupole LC/MS using APCI in positive ion mode [1]. Lathosterol and cholesterol are the most challenging compounds in this separation for 2 reasons. They are difficult to resolve completely, and they need to be resolved for MS/MS detection as they are isobaric. In addition, in a plasma sample, cholesterol is present at a much higher concentration than lathosterol. We selected atmospheric pressure chemical ionization (APCI) in positive ion mode for detection due to the nonpolar nature of sterol compounds. APCI provides good sensitivity for the compounds without derivatization.

Experimental

An Agilent 1200 Infinity Series LC system was used, comprising a binary pump (G1312B), a thermostatted column compartment SL (G1316B), a high performance autosampler (G1367D), and an Agilent LT-ELSD or Agilent 6460A Triple Quadrupole as the detector.

The columns used in the application were:

- Agilent Poroshell 120 EC-C18, 3.0 \times 100 mm, 2.7 μm (p/n 695975-302)
- Agilent Poroshell 120 EC-C18, 4.6 \times 75 mm, 2.7 μm (p/n 697975-902)
- Agilent Poroshell 120 SB-C18, 4.6 \times 75 mm, 2.7 μm (p/n 687975-902)

No.	Compound	CAS No.	Structure
1	Calcifediol	63283-36-3	
2	Desmosterol	313-04-2	
3	Provitamin D3	434-16-2	
4	5-Cholesten-3-one	601-54-7	
5	Lathosterol	80-99-9	
6	Cholesterol	57-88-5	
7	Coprostanol	360-68-9	
8	Campesterol	474-62-4	
9	Stigmasterol	83-48-7	
10	Cholestanol	80-97-7	
11	Sitosterol	64997-52-0	

Results and Discussion

In total, 11 sterols were separated on Poroshell 120 EC-C18 and SB-C18 columns using the ELSD. The ELSD is an excellent detector if sensitivity is not a critical issue and is ideal for many compounds when a UV detector is not appropriate due to the lack of chromophores in the analytes. The separation was done using a simple isocratic mobile phase at a controlled temperature of 20 °C for better resolution. Chromatograms in Figure 1 show better resolution on the Poroshell 120 EC-C18 than on the Poroshell 120 SB-C18.

It is noticeable that the challenging compounds lathosterol and cholesterol are completely resolved on the Poroshell 120 EC-C18 but not on the SB-C18. The well end-capped EC-C18 provides more retention for the nonpolar sterols than the SB-C18, which is not end-capped. These 2 different phases provide slightly different selectivity, which may be very significant for the separation.

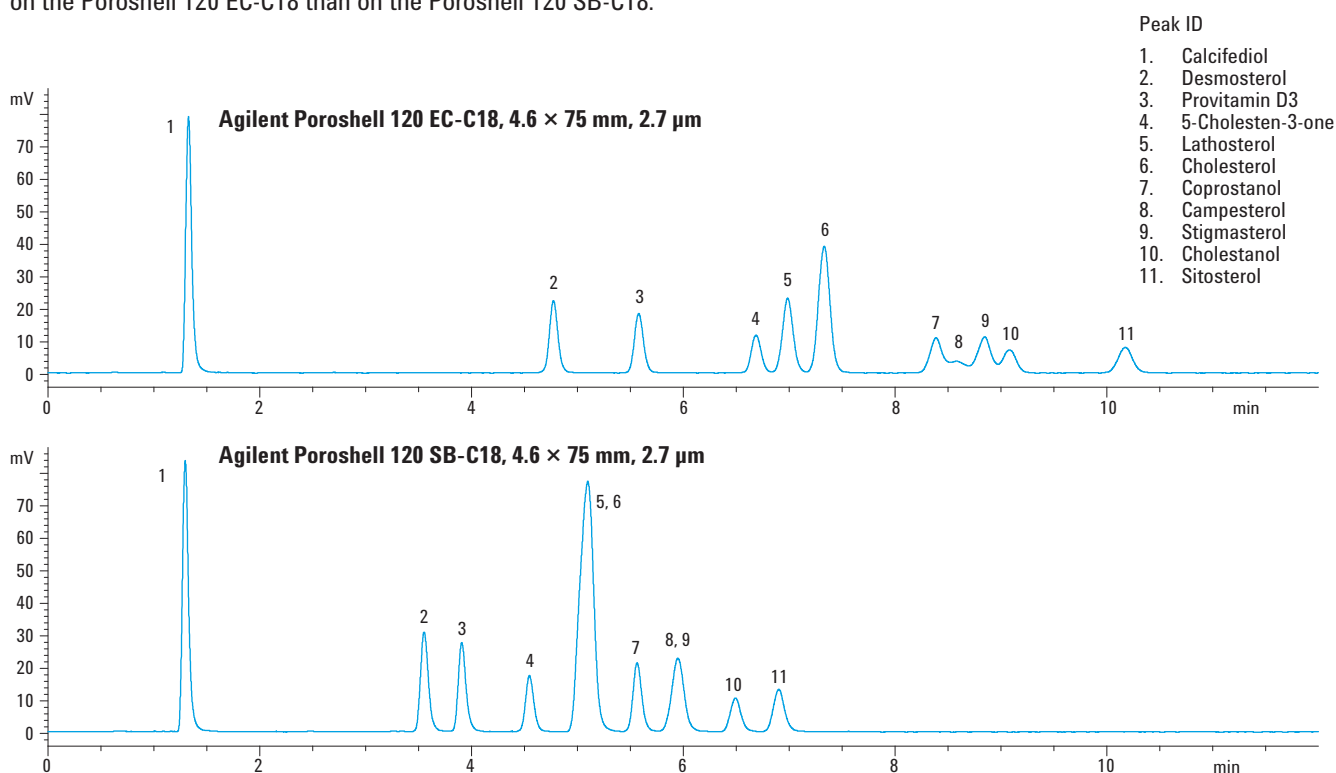


Figure 1. Comparison of Agilent Poroshell 120 bonded phases for the separation of cholesterol and other sterols with evaporative light scattering detection.

Conditions

Sample: 5 μ L injection of 11 sterols in methanol
 Mobile phase: 80% Acetonitrile:20% methanol
 Flow rate: 1.5 mL/min
 Temperature: 20 °C
 Detector: ELSD, gain = 8, filter = 3 s,
 evaporation temperature 60 °C

In a real plasma sample, cholesterol is present at a much higher concentration than other sterols. The ratio of cholesterol to lathosterol is around 2000:1 in a plasma sample, and so it is very difficult for lathosterol to separate from a high concentration of cholesterol. Figure 2 shows a chromatogram of 11 sterols at 2000:1 for cholesterol:other sterols. To achieve sufficient sensitivity for all the sterols with ELSD, 2 measures were adopted. First, a concentration of 10 ppb for low level sterols was needed with ELSD. Second, we chose a 3.0 mm id column that gave higher sensitivity. Though these measures helped to increase sensitivity of low level sterols and thus decrease the concentration of the mixture, cholesterol still had a very high concentration of 20 ppm. With such a high concentration of cholesterol, the

Poroshell EC-C18 was overloaded and gave lower resolution with lathosterol.

LC/MS/MS is the better detector choice if additional sensitivity is needed. However, because lathosterol and cholesterol are isobaric, resolution was still required and so the separation was done on the same Poroshell 120 EC-C18, 3.0×10 mm, $2.7 \mu\text{m}$ column using a 6460A Triple Quadrupole LC/MS for the detector. The ratio of cholesterol to lathosterol was still 2000:1 to evaluate the separation of the sterols as they might occur in a plasma sample. However, the mixture was diluted by 1/10 with methanol to give 2 ppm cholesterol and 1 ppb of other sterols. A small injection volume of $2 \mu\text{L}$ was sufficient for triple quadrupole detection.

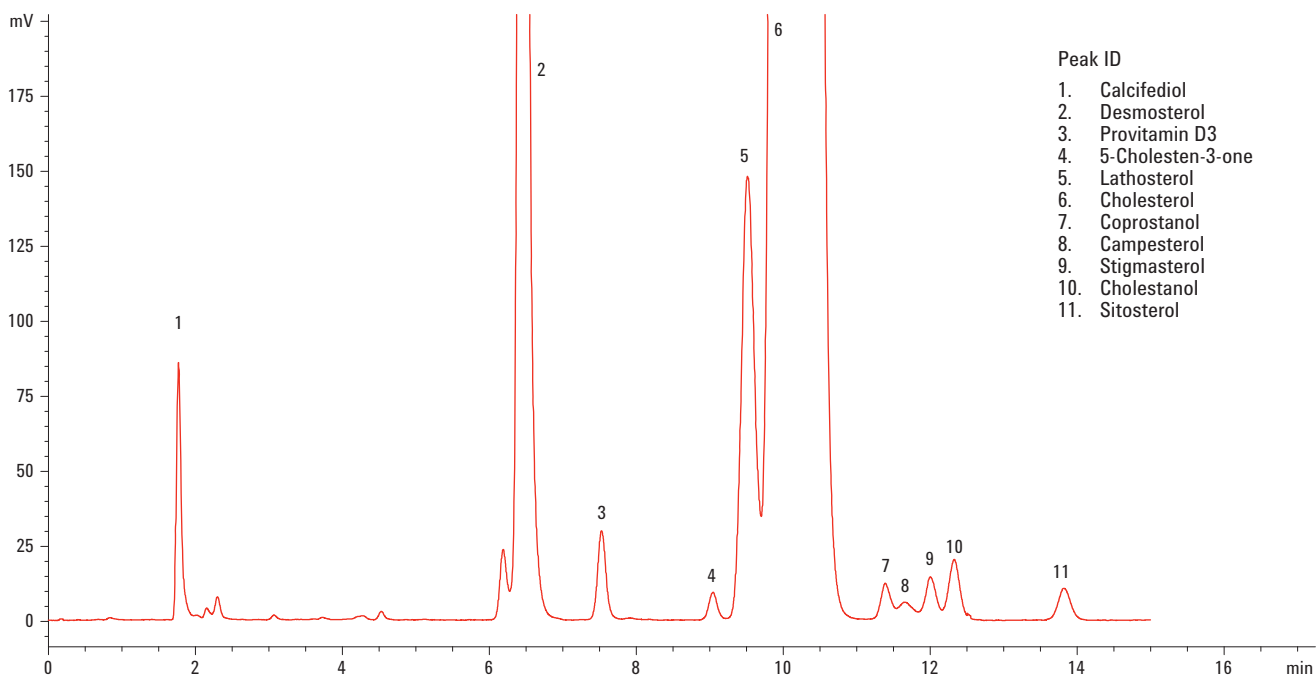


Figure 2. Separation of the sterol mixture (the ratio of cholesterol to other sterols was 2000:1) on an Agilent Poroshell 120 EC-C18, 3×100 mm, $2.7 \mu\text{m}$ column with evaporative light scattering detection.

Conditions

Sample: 5 μL injection of 11 sterols in IPA
(20 ppm for cholesterol, 0.01 ppm for other sterols)

Mobile phase: 80% Acetonitrile:20% methanol

Flow rate: 0.6 mL/min

Temperature: 20 °C

Detector: ELSD, gain = 10, filter = 3 s,
evaporation temperature 60 °C

Figure 3 shows that adequate resolution was obtained in this separation using the Poroshell 120 EC-C18 column, even at 2000:1 for cholesterol:lathosterol, and the separation of all compounds was achieved in less than 15 minutes. Because

this was an isocratic separation, no additional time was spent on column re-equilibration. This was an efficient and effective method for the separation of these 9 sterols.

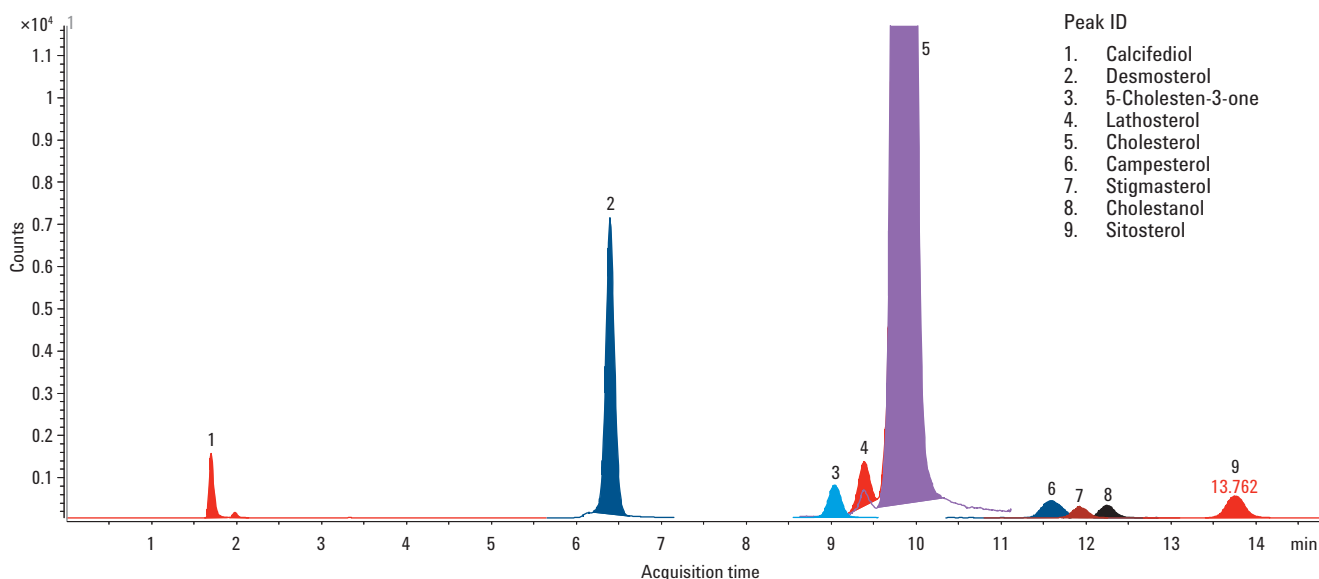


Figure 3. Separation of 9 sterols (the ratio of cholesterol to other sterols was 2000:1) on an Agilent Poroshell 120 EC-C18, 3 × 100 mm, 2.7 μm with LC/MS/MS.

Conditions

Sample: 2 μL injection of 11 sterols in methanol (2 ppm for cholesterol, 1 ppb for other sterols)
 Mobile phase: 80% Acetonitrile:20% methanol
 Flow rate: 0.6 mL/min
 Temperature: 20 °C

MS source parameters

Gas temp: 325 °C
 Vaporizer: 350 °C
 Gas flow: 4 L/min
 Nebulizer: 60 psi
 Positive capillary: 4000 V
 Corona current: 4 μA

APCI acquisition parameters and transitions

Compound name	Precursor ion	Product ion	Fragmentor	Collision energy (eV)	Ret time (min)
Calcifediol	383.3	211.2	144	25	1.7
Calcifediol	383.3	107.1	144	25	1.7
Desmosterol	367.3	161.1	100	17	6.4
Desmosterol	367.3	95	100	22	6.4
5-Cholesten-3-one	385.4	109.1	128	40	9.05
5-Cholesten-3-one	385.4	97	128	21	9.05
Lathosterol	369.4	95.1	112	29	9.4
Lathosterol	369.4	81.1	112	40	9.4
Cholesterol	369.4	161.2	166	10	9.87
Cholesterol	369.4	95.2	166	38	9.87
Campesterol	383.4	161.2	142	16	11.6
Campesterol	383.4	95	142	30	11.6
Stigmasterol	395.4	83.1	148	17	11.95
Stigmasterol	395.4	81.1	148	37	11.95
Cholestanol	371.4	149	150	15	12.27
Cholestanol	371.4	95	150	30	12.27
Sitosterol	397.4	161	125	18	13.78
Sitosterol	397.4	135.2	125	12	13.78

Conclusions

The separation of cholesterol, some of its metabolites, and other phytosterols was most effectively performed with an Agilent Poroshell 120 EC-C18 column with APCI detection in the positive ion mode on an Agilent 6460A Triple Quadrupole LC/MS detector. This column provided good resolution between cholesterol and lathosterol, an indicator of cholesterol synthesis in the body, even at a ratio of 2000:1. This was critical as the 2 compounds had the same molecular weight and resolution was needed to effectively quantitate these 2 analytes.

Reference

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USP Analysis of Warfarin Sodium Tablets Using Agilent Poroshell 120 EC-CN and SB-C8 Columns

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Abstract

Warfarin, warfarin related compound A, and propylparaben were analyzed according to the United States Pharmacopeia (USP) analysis for warfarin sodium tablets. The chromatographic purity and assay analyses were improved by using superficially porous Agilent Poroshell 120 columns as compared to the USP-suggested 5 μm columns. Each method was adjusted within the guidelines in USP Chapter 621 to allow for time and solvent savings with the Poroshell 120 columns. All chromatographic system requirements were met with the improved superficially porous column analyses.

Introduction

There is significant interest in transferring LC methods to superficially porous particles from larger 5 μm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2 μm totally porous particles. This is attributed primarily to a shorter mass transfer distance and a narrower particle size distribution. Furthermore, the larger particle size results in lower backpressure, allowing for these columns to be implemented in methods on virtually any LC system. The benefits of transferring from larger particle columns are very significant time and cost savings, because superficially porous particles are optimally run at faster flow rates and achieve similar resolution with a much shorter column length [1,2].

This application note describes two methods from the USP for the analysis of warfarin sodium tablets that were transferred from suggested 5 μm columns to shorter 2.7 μm superficially porous Agilent Poroshell 120 columns. Each analysis was compared against the USP chromatographic system requirements to ensure column suitability for the analysis. All method modifications are allowable within USP Chapter 621.

Materials and Methods

The instrument setup was optimized for lowest possible extra column volume with short 0.075 mm id capillaries found in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [3]. Analytical method parameters are the same as those specified by the USP.

Warfarin and propylparaben were purchased from Sigma-Aldrich Corp., and warfarin-related Compound A came from the USP. Acetonitrile was purchased from Honeywell International Inc. Water was 18 Mohm.cm Milli-Q.

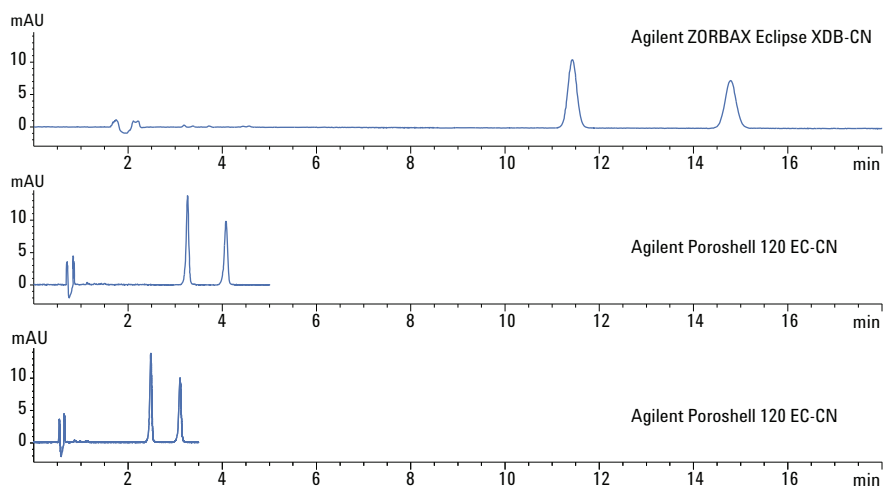
Results and Discussion

USP chromatographic purity analysis of warfarin sodium tablets

Figure 1 shows the USP chromatographic purity analysis for warfarin sodium tablets. The top chromatogram shows the analysis performed as specified by the USP with a 4.6 × 250 mm, 5 μm column with L10 packing, which in this case was a ZORBAX Eclipse XDB-CN column. The two compounds were easily separated in approximately 16 minutes.

Elution order

1. Warfarin (6 μg/mL)
2. Warfarin-related compound A (6 μg/mL)



Conditions

Columns: Agilent Poroshell 120 EC-CN, 3.0 × 100 mm, 2.7 μm (p/n 695975-305)
 Agilent ZORBAX Eclipse XDB-CN, 4.6 × 250 mm, 5 μm (p/n 990967-905)

Sample: Warfarin (6 μg/mL), warfarin related compound A (6 μg/mL)

Eluent: A = 0.1% CH₃COOH in H₂O
 B = CH₃OH

Injection volume: 20 μL for 4.6 × 250 mm column,
 3.5 μL for 3.0 × 100 mm column

Flow rate: 1.5 mL/min for 4.6 × 250 mm column,
 0.64 mL/min or 0.85 mL/min for 3.0 × 100 mm column

Isocratic: 32% B

Temperature: 25 °C

Detector: 260 nm

Instrument: Agilent 1290 Infinity LC

Figure 1. USP chromatographic purity analysis of warfarin sodium tablets using Agilent ZORBAX Eclipse XDB-CN and Agilent Poroshell 120 EC-CN columns. The bottom chromatogram shows the Agilent Poroshell 120 EC-CN column used at its optimal flow rate.

According to the guidelines in USP Chapter 621, the 4.6 × 250 mm, 5 μm analysis can be transferred to a 3.0 × 100 mm, 2.7 μm Agilent Poroshell 120 EC-CN column, shown in the middle chromatogram of Figure 1. The bottom chromatogram shows the same Poroshell 120 EC-CN column used at its optimal flow rate, which accomplishes the desired separation in 3 minutes.

Table 1 lists the USP chromatographic system requirements and the measured values for each of the three chromatograms found in Figure 1. Not only was the 5 μm column suitable for this analysis, but so was the 2.7 μm Poroshell 120 column. Additionally, the Poroshell 120 column, when used at its optimal flow rate, saved significant time and solvent compared to the original analysis, allowing for increased productivity as well as substantial cost savings.

Table 1. USP chromatographic system requirements and measurements for the chromatographic purity analysis of warfarin sodium tablets (W denotes measured values for warfarin, and A for warfarin-related compound A).

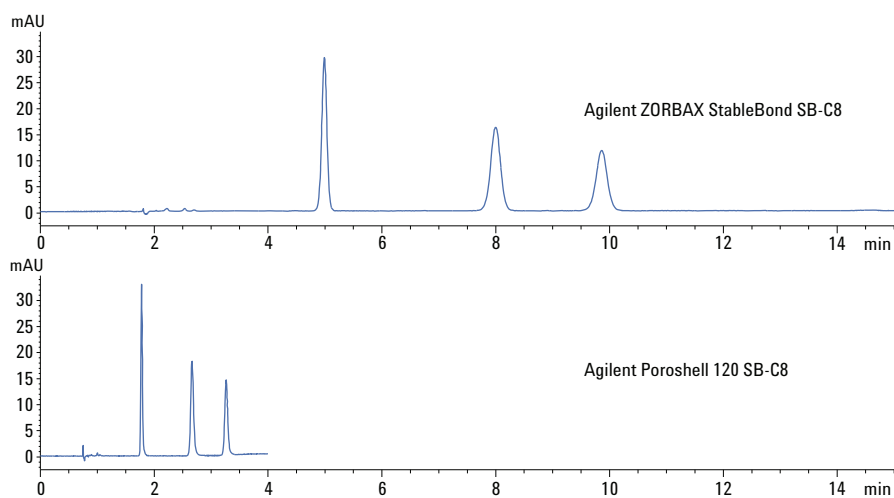
USP chromatographic system requirements	5 μm (1.5 mL/min)	2.7 μm (0.64 mL/min)	2.7 μm (0.85 mL/min)
Resolution between warfarin and warfarin-related compound A is not less than 3	Rs (W,A): 8.3	Rs (W,A): 7.1	Rs (W,A): 7.1
Relative standard deviation for replicate injections is not greater than 5.0%	W: 0.10% A: 0.10%	W: 0.10% A: 0.10%	W: 0.94% A: 0.10%
The relative retention times of warfarin and warfarin-related compound A are 1.0 and about 1.2	W: 1.0 A: 1.3	W: 1.0 A: 1.3	W: 1.0 A: 1.3

USP assay analysis of warfarin sodium tablets

Figure 2 shows the USP assay analysis for warfarin sodium tablets, with propylparaben an internal standard. The top chromatogram is generated using the USP specified 4.6×250 mm, $5 \mu\text{m}$ L7 (ZORBAX StableBond SB-C8) column. The bottom chromatogram is a direct transfer to a 4.6×100 mm, $2.7 \mu\text{m}$ Poroshell 120 SB-C8 column. For this analysis, the Poroshell 120 column could not be used at its optimal flow rate due to pressure limitations. However, regardless of this constraint, the analysis time for these three compounds was still reduced from 10 minutes to less than 3.5 minutes with the Poroshell 120 column.

Elution order

1. Propylparaben ($10 \mu\text{g/mL}$)
2. Warfarin ($5 \mu\text{g/mL}$)
3. Warfarin-related compound A ($5 \mu\text{g/mL}$)



Conditions

Columns: Agilent Poroshell 120 SB-C8, 4.6×100 mm, $2.7 \mu\text{m}$ (p/n 685975-906)
 Agilent ZORBAX StableBond SB-C8, 4.6×250 mm, $5 \mu\text{m}$ (p/n 880975-906)

Sample: Warfarin ($5 \mu\text{g/mL}$), warfarin related compound A ($5 \mu\text{g/mL}$), propylparaben ($10 \mu\text{g/mL}$)

Eluent: A = $0.1\% \text{CH}_3\text{COOH}$ in H_2O
 B = CH_3OH

Injection volume: $15 \mu\text{L}$ for 4.6×250 mm column,
 $5 \mu\text{L}$ for 4.6×100 mm column

Flow rate: 1.4 mL/min

Isocratic: $64\% \text{ B}$

Temperature: $30 \text{ }^\circ\text{C}$

Detector: 280 nm

Instrument: Agilent 1290 Infinity LC

Figure 2. USP assay analysis of warfarin sodium tablets using Agilent ZORBAX StableBond SB-C8 and Agilent Poroshell 120 SB-C8 columns.

All chromatograms met the USP chromatographic system requirements, as shown in Table 2. The superficially porous Poroshell 120 column could easily be substituted for the traditional 5 μm column to save costs and improve productivity.

Table 2. USP chromatographic system requirements and measurements for the assay analysis for warfarin sodium tablets (P denotes measured values for propylparaben, W for warfarin, and A for warfarin-related compound A).

USP chromatographic system requirements	5 μm (1.4 mL/min)	2.7 μm (1.4 mL/min)
The relative retention times of propylparaben and warfarin are about 0.75 and 1.0	P: 0.63 W: 1.0	P: 0.67 W: 1.0
Resolution of propylparaben and warfarin is not less than 2.0	Rs (P,W): 12.0	Rs (P,W): 12.5
Relative standard deviation for replicate injections is not greater than 2.0%	P: 0.26% W: 0.26% A: 0.16%	P: 0.10% W: 0.73% A: 0.97%

Conclusions

Superficially porous Agilent Poroshell 120 columns were successfully substituted for traditional 5 μm columns for the USP chromatographic purity and assay analyses for warfarin sodium tablets. Smaller dimension Poroshell 120 columns could be used to improve productivity and to save time and money over larger 5 μm columns, while meeting all USP requirements for the chromatographic system.

References

1. A. Gratzfeld-Hüsgen, E. Naegele. "Maximizing efficiency using Agilent Poroshell 120 columns". Application Note, Agilent Technologies, Inc. Publication Number 5990-5602EN (2010).
2. V. R. Meyer. *Practical High Performance Liquid Chromatography*, Fourth Ed., p. 34. Wiley (2004).
3. A. Mack. "Optimizing Performance of an Agilent ZORBAX RRHD Eclipse Plus C18 Column by Enhancing an Agilent 1290 Infinity LC System for Ultra-Low Dispersion". Application Note, Agilent Technologies, Inc. Publication Number 5990-9502EN (2011).

USP Analysis of Diphenhydramine and Pseudoephedrine Using an Agilent Poroshell 120 EC-CN Column

Author

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Abstract

Diphenhydramine and pseudoephedrine were analyzed according to the United States Pharmacopeia (USP) assay analysis for diphenhydramine and pseudoephedrine capsules. The assay was improved by using a superficially porous Agilent Poroshell 120 column as compared to the USP-suggested 5 μm column. The method was adjusted within the guidelines in USP Chapter 621 to allow for time and solvent savings with the Poroshell 120 column. All chromatographic system requirements were met with the improved superficially porous column.

Introduction

There is significant interest in transferring LC methods to superficially porous particles from larger 5 μm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2 μm totally porous particles. This is attributed primarily to a shorter mass transfer distance and a narrower particle size distribution. Furthermore, the larger particle size results in lower backpressure, allowing for these columns to be implemented in methods on virtually any LC system. The benefits of transferring from larger particle columns are very significant time and cost savings, because superficially porous particles are optimally run at faster flow rates and achieve similar resolution with a much shorter column length [1,2].

This application note describes a method from the USP for the analysis of diphenhydramine and pseudoephedrine capsules that was transferred from the suggested 5 μm column to a shorter 2.7 μm superficially porous Poroshell 120 column. The analysis was compared against the USP chromatographic system requirements to ensure column suitability. All method modifications are allowable within USP Chapter 621.

Materials and Methods

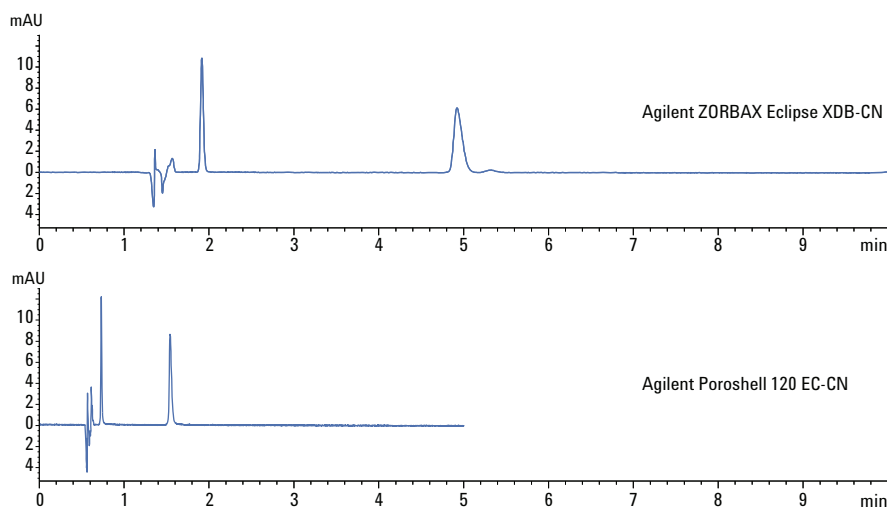
The instrument setup was optimized for lowest possible extra column volume with short 0.075 mm id capillaries found in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [3].

Diphenhydramine, pseudoephedrine, 1-heptanesulfonate, glacial acetic acid, and triethylamine were purchased from Sigma-Aldrich Corp. Acetonitrile and methanol were purchased from Honeywell International Inc. Water was 18 Mohm.cm Milli-Q.

Results and Discussion

Figure 1 shows the USP assay analysis for diphenhydramine and pseudoephedrine capsules. The top chromatogram shows the analysis performed as specified by the USP with a 4.6×250 mm, $5 \mu\text{m}$ column with L10 packing, which in this case was a ZORBAX Eclipse XDB-CN column. The two compounds were easily separated in about 5.5 minutes.

According to the guidelines in USP Chapter 621, the 4.6×250 mm, $5 \mu\text{m}$ analysis can be transferred to a 3.0×100 mm, $2.7 \mu\text{m}$ Poroshell 120 EC-CN column, shown in the bottom chromatogram of Figure 1, which accomplished the desired separation in approximately 1.5 minutes.



Elution order

1. Pseudoephedrine (50 $\mu\text{g}/\text{mL}$)
2. Diphenhydramine (50 $\mu\text{g}/\text{mL}$)

Conditions

Columns: Agilent Poroshell 120 EC-CN, 3.0×100 mm, $2.7 \mu\text{m}$ (p/n 695975-305)
 Agilent ZORBAX Eclipse XDB-CN, 4.6×250 mm, $5 \mu\text{m}$ (p/n 990967-905)

Sample: Diphenhydramine, pseudoephedrine

Eluent: A = 10 mM $\text{C}_7\text{H}_{15}\text{NaO}_3\text{S}$, 13 mM $\text{C}_6\text{H}_{15}\text{N}$, pH 3.3
 B = $\text{CH}_3\text{CN}:\text{CH}_3\text{OH}$ (26:10)

Injection volume: 20 μL for 4.6×250 mm column, 3.5 μL for 3.0×100 mm column

Flow rate: 2 mL/min for 4.6×250 mm column,
 0.85 mL/min for 3.0×100 mm column

Isocratic: 36% B

Temperature: 25 $^\circ\text{C}$

Detector: 254 nm

Instrument: Agilent 1290 Infinity LC

Figure 1. USP assay analysis of diphenhydramine and pseudoephedrine using Agilent ZORBAX Eclipse XDB-CN and Agilent Poroshell 120 EC-CN columns.

Table 1. USP chromatographic system requirements and measurements for the assay analysis of diphenhydramine and pseudoephedrine capsules (P denotes measured values for pseudoephedrine, and D for diphenhydramine).

USP chromatographic system requirements	5 μm (2 mL/min)	2.7 μm (0.85 mL/min)
Resolution between pseudoephedrine and diphenhydramine peaks is not less than 3.0	Rs (P,D): 24.3	Rs (P,D): 21.2
For each analyte peak the tailing factor is not greater than 2.0	P: 1.2 D: 1.4	P: 1.3 D: 1.5
Relative standard deviation for replicate injections is not greater than 2.0%	P: 0.10% D: 0.82%	P: 0.10% D: 1.9%
The relative retention times are about 1.0 for pseudoephedrine and 3.0 for diphenhydramine	P: 1.0 D: 2.6	P: 1.0 D: 2.1

Table 1 lists the USP chromatographic system requirements and the measured values for each of the two chromatograms in Figure 1. Not only is the 5 μm column suitable for this analysis, but so is the 2.7 μm Poroshell 120 column. Additionally, the Poroshell 120 column saved significant time and solvent compared to the original analysis, allowing for increased productivity as well as substantial cost savings.

Conclusions

A superficially porous Agilent Poroshell 120 column was successfully substituted for a traditional 5 μm column for the USP assay analysis for diphenhydramine and pseudoephedrine capsules. The smaller dimension Poroshell 120 column could be used to improve productivity and save time and money over the larger 5 μm column, while meeting all USP requirements for the chromatographic system.

References

1. A. Gratzfeld-Hüsgen, E. Naegele. "Maximizing efficiency using Agilent Poroshell 120 columns". Application Note, Agilent Technologies, Inc. Publication Number 5990-5602EN (2010).
2. V. R. Meyer. *Practical High Performance Liquid Chromatography*, Fourth Ed., p. 34. Wiley (2004).
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USP Analysis of Norethindrone and Mestranol Tablets with Agilent Poroshell 120 EC-CN and EC-C8 Columns

Author

Anne Mack
Agilent Technologies, Inc.

Abstract

Norethindrone, mestranol, and progesterone were analyzed according to the United States Pharmacopeia (USP) analysis for norethindrone and mestranol tablets. The dissolution and assay analyses were improved by using superficially porous Agilent Poroshell 120 columns as compared to the USP-suggested 5 μm columns. Each method was adjusted within the guidelines in USP Chapter 621 to allow for time and solvent savings with the Poroshell 120 columns. All chromatographic system requirements were met with the improved superficially porous column.

Introduction

There is significant interest in transferring LC methods to superficially porous particles from larger 5 μm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2 μm totally porous particles. This is attributed primarily to a shorter mass transfer distance and a narrower particle size distribution. Furthermore, the larger particle size results in lower backpressure, allowing for these columns to be implemented in methods on virtually any LC system. The benefits of transferring from larger particle columns are very significant time and cost savings, because superficially porous particles are optimally run at faster flow rates and achieve similar resolution with a much shorter column length [1,2].

This application note describes two methods from the USP for the analysis of norethindrone and mestranol tablets that were transferred from suggested 5 μm columns to shorter 2.7 μm superficially porous Agilent Poroshell 120 columns. Each analysis was compared against the USP chromatographic system requirements to ensure column suitability for the analysis. All method modifications are allowable within USP Chapter 621.

Materials and Methods

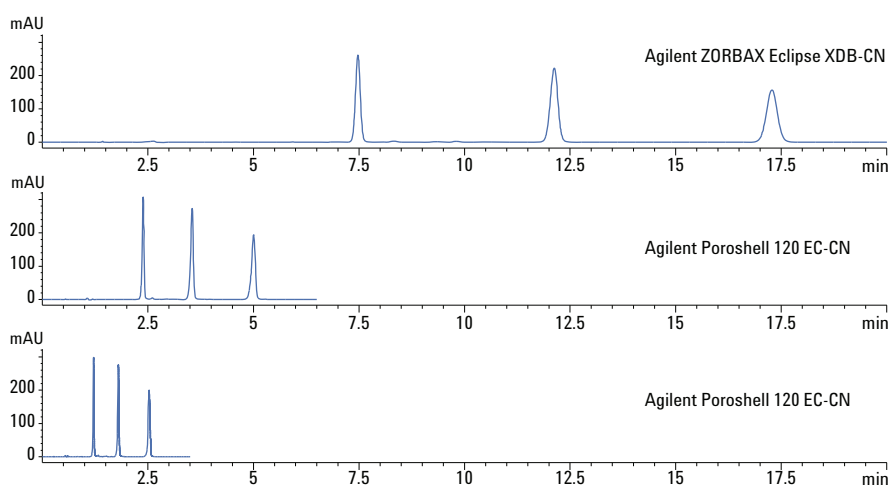
The instrument setup was optimized for lowest possible extra column volume with short 0.075 mm id capillaries found in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [3].

Norethindrone, mestranol, and progesterone were purchased from Sigma-Aldrich Corp. Acetonitrile was purchased from Honeywell International Inc. Water was 18 Mohm.cm Milli-Q.

Results and Discussion

USP dissolution analysis of norethindrone and mestranol tablets

Figure 1 shows the USP dissolution analysis for norethindrone and mestranol tablets, with progesterone as an internal standard. The top chromatogram shows the analysis performed as specified by the USP with a 4.6×250 mm, $5 \mu\text{m}$ column with L10 packing, which in this case was an Agilent ZORBAX Eclipse XDB-CN column. The three compounds were easily separated in approximately 18 minutes.



Elution order

1. Norethindrone (0.5 mg/mL)
2. Progesterone (0.5 mg/mL)
3. Mestranol (0.05 mg/mL)

Conditions

Columns: Agilent Poroshell 120 EC-CN, 3.0×100 mm, $2.7 \mu\text{m}$ (p/n 695975-305)
Agilent ZORBAX Eclipse XDB-CN, 4.6×250 mm, $5 \mu\text{m}$ (p/n 990967-905)

Samples: Norethindrone, mestranol, progesterone

Eluent: A = H_2O
B = CH_3CN

Injection volume: $5 \mu\text{L}$ for 4.6×250 mm column,
 $1 \mu\text{L}$ for 3.0×100 mm column

Flow rate: 1 mL/min for 4.6×250 mm column,
 0.43 mL/min or 0.85 mL/min for 3.0×100 mm column

Isocratic: 40% B

Temperature: $25 \text{ }^\circ\text{C}$

Detector: 205 nm

Instrument: Agilent 1290 Infinity LC

Figure 1. USP dissolution analysis of norethindrone and mestranol tablets using Agilent ZORBAX Eclipse XDB-CN and Agilent Poroshell 120 EC-CN columns. The bottom chromatogram shows the Poroshell 120 EC-CN column used at its optimal flow rate.

According to the guidelines in USP Chapter 621, the 4.6 × 250 mm, 5 µm analysis can be transferred to a 3.0 × 100 mm, 2.7 µm Poroshell 120 EC-CN column, shown in the middle chromatogram of Figure 1. The bottom chromatogram shows the same Poroshell 120 EC-CN column used at its optimal flow rate, which accomplished the desired separation in 2.5 minutes.

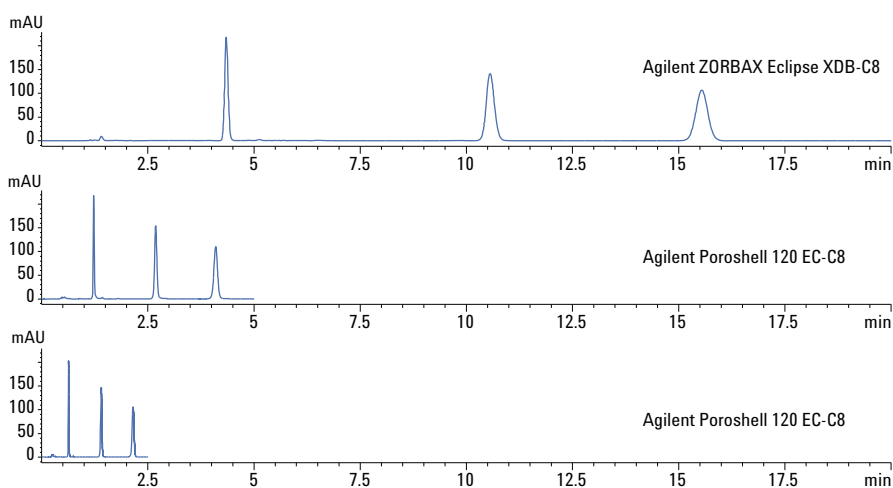
Table 1 lists the USP chromatographic system requirements and the measured values for each of the three chromatograms found in Figure 1. Not only was the 5 µm column suitable for this analysis, but so was the 2.7 µm Poroshell 120 column. Additionally, the Poroshell 120 column, when used at its optimal flow rate, saved significant time and solvent compared to the original analysis, allowing for increased productivity as well as substantial cost savings.

Table 1. USP chromatographic system requirements and measurements for the dissolution analysis for norethindrone and mestranol tablets (N denotes measured values for norethindrone, P for progesterone, and M for mestranol)

USP chromatographic system requirements	5 µm (2 mL/min)	2.7 µm (0.43 mL/min)	2.7 µm (0.85 mL/min)
Relative standard deviation for replicate injections is not greater than 3.0%	N: 0.26% P: 0.24% M: 0.23%	N: 0.92% P: 0.56% M: 0.39%	N: 0.51% P: 0.56% M: 0.39%
Minimum number of theoretical plates for mestranol is 4,000	M: 20304	M: 14790	M: 16297
Tailing factors for norethindrone and mestranol peaks do not exceed 1.5	N: 1.01 M: 1.00	N: 0.87 M: 0.83	N: 0.92 M: 0.86
The relative retention times are approximately 0.4 for norethindrone and 1.0 for mestranol	N: 0.43 M: 1.0	N: 0.48 M: 1.0	N: 0.48 M: 1.0

USP assay analysis of norethindrone and mestranol tablets

Figure 2 shows the USP assay analysis for norethindrone and mestranol tablets, with progesterone as an internal standard (elution order as before). The top chromatogram was generated using the USP-specified 4.6×150 mm, $5 \mu\text{m}$ L7 (ZORBAX Eclipse XDB-C8) column. The middle chromatogram is a direct transfer to a 4.6×50 mm, $2.7 \mu\text{m}$ Poroshell 120 EC-C8 column. The bottom chromatogram shows the Poroshell 120 column used at its optimal flow rate. Analysis time for these three compounds was reduced from 16 minutes to less than 2.5 minutes with the Poroshell 120 column.



Conditions

Columns: Agilent Poroshell 120 EC-C8, 4.6×50 mm, $2.7 \mu\text{m}$ (p/n 699975-906)
 Agilent ZORBAX Eclipse XDB-C8, 4.6×150 mm, $5 \mu\text{m}$ (p/n 993967-906)

Samples: Norethindrone, mestranol, progesterone

Eluent: A = H_2O
 B = CH_3CN

Injection volume: $3 \mu\text{L}$ for 4.6×150 mm column,
 $1 \mu\text{L}$ for 4.6×50 mm column

Flow rate: 1 mL/min for 4.6×150 mm column,
 1 mL/min or 2 mL/min for 4.6×50 mm column

Isocratic: 50% B

Temperature: $25 \text{ }^\circ\text{C}$

Detector: 200 nm

Instrument: Agilent 1290 Infinity LC

Figure 2. USP assay analysis of norethindrone and mestranol tablets using Agilent ZORBAX Eclipse XDB-C8 and Agilent Poroshell 120 EC-C8 columns. The bottom chromatogram shows the Agilent Poroshell 120 EC-CN column used at its optimal flow rate.

Table 2 shows that all chromatograms met the USP chromatographic system requirements. The superficially porous Poroshell 120 column can easily be substituted for the traditional 5 μm column to save costs and improve productivity.

Table 2. USP chromatographic system requirements and measurements for the assay analysis of norethindrone and mestranol tablets (N denotes measured values for norethindrone, P for progesterone, and M for mestranol)

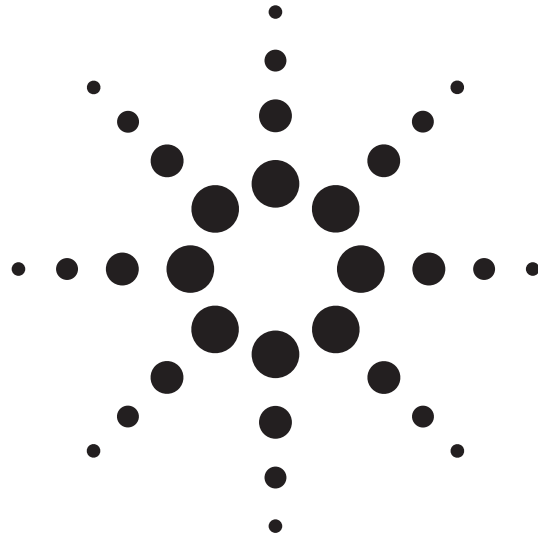
USP chromatographic system requirements	5 μm (1 mL/min)	2.7 μm (1 mL/min)	2.7 μm (2 mL/min)
Column efficiency determined from the mestranol peak is not less than 6,000 theoretical plates	M: 13571	M: 10718	M: 10480
Resolution between progesterone and mestranol is not less than 5.0	Rs (P,M): 11.1	Rs (P,M): 10.9	Rs (P,M): 10.8
Relative standard deviation for replicate injections is not greater than 2.0%	N: 0.26% P: 0.29% M: 0.28%	N: 0.91% P: 0.78% M: 0.59%	N: 1.4% P: 0.95% M: 0.71%
The relative retention times are about 2.5 for mestranol and 1.0 for norethindrone	M: 3.6 N: 1.0	M: 3.3 N: 1.0	M: 3.4 N: 1.0

Conclusions

Superficially porous Agilent Poroshell 120 columns were successfully substituted for traditional 5 μm columns for the USP dissolution and assay analyses of norethindrone and mestranol tablets. Smaller dimension Poroshell 120 columns could be used to improve productivity and to save time and money over larger 5 μm columns, while meeting all USP requirements for the chromatographic system.

References

1. A. Gratzfeld-Hüsgen, E. Naegele. "Maximizing efficiency using Agilent Poroshell 120 columns". Application Note, Agilent Technologies, Inc. Publication Number 5990-5602EN (2010).
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化工分析

采用 Poroshell 120 色谱柱快速分析育发类化妆品中的8种违禁药物

关键词

HPLC

Poroshell 120

违禁药物

化妆品

作者

熊小婷

广州市质量监督检测研究院

摘要

随着生活水平的不断提高，人们对美发也有了更高的要求，各种标识具有防脱、生发功效的育发类化妆品应运而生、飞速发展。然而，部分化妆品企业盲目追求产品的有效性，于该类产品中添加《化妆品卫生规范》(2007年版)明确规定禁止添加的米诺地尔、螺内酯、雌性激素等成分。检测各种育发类化妆品中是否含有上述几种违禁药物，对于控制化妆品的产品质量，保障人民身体健康具有重要的意义。化妆品中米诺地尔、雌性激素、螺内酯等药物成分的检测方法主要有紫外分光光度法^[1]、高效液相色谱法^[2-4]、液相色谱-质谱联用法^[5-7]、气相色谱-质谱联用法^[8]以及毛细管电动色谱^[9-10]等。但是，到目前为止，仍没有全面、系统的对育发类化妆品中不同种类违禁药物进行同时检测的方法报道。本研究采用高效液相色谱-二极管阵列检测法(HPLC-DAD)，使用 poroshell 120 Bonus-RP 快速高效柱，能使8种不同违禁药达到完全分离。方法简单、快速、准确，能满足检测要求，并已成功用于实际样品的检测。

材料与amp;方法

1.1 仪器与试剂

高效液相色谱仪 (Agilent 1200, 配二极管阵列检测器, 美国 Agilent 公司); 超纯水系统 (Milli-Q, 美国 Millipore 公司); Agilent Poroshell 120 Bonus-RP 色谱柱 (4.6×50 mm, 2.7μm); 常规 C18 柱 (4.6×250 mm, 5μm); 涡旋混匀器 (IKAR MS3 digital, 德国 IKA 公司); 超声清洗器 (SK8200HP, 上海科导超声仪器有限公司); 高速冷冻离心机 (Allegra 64R, 美国 Beckman Coulter 公司); 固相萃取小柱 (Bond Elute Plexa, 60mg, 3mL, 美国 Agilent 公司); 甲醇 (HPLC 级, Spectrum 公司); 乙酸铵 (分析纯, 广州化学试剂厂); 水 (超纯水)。

米诺地尔、雌三醇、雌二醇、雌酮、黄体酮标准品购自 Dr. Ehrenstorfer 公司, 螺内酯、坎利酮、己烯雌酚购自 TRC 公司, 含量为 97.0~99.5%。

1.2 色谱条件

色谱柱: Agilent Poroshell 120 Bonus-RP (4.6×50 mm, 2.7μm); 柱温: 35℃; 进样体积: 10μL; 流速: 0.8 mL/min; 流动相 A 为 10mmol/L 乙酸铵, 流动相 B 为甲醇, 梯度洗脱程序: 0-1min 15-40%B, 1-4min 40%-60%B, 4-10min 60%-65%B, 10-10.1min 65%-15%B。色谱柱: 常规 C18 (4.6×250 mm, 5μm); 进样体积: 10μL; 流速: 1.0 mL/min; 梯度洗脱程序: 0-1min 15-58%B, 1-10min 58-62%B, 10-12min 62-95%B, 12-13min 95-15%B。二极管阵列检测器 (DAD) 检测波长: 雌三醇、雌酮、雌二醇、己烯雌酚为 204nm; 米诺地尔为 230nm; 螺内酯、黄体酮为 240nm, 坎利酮为 280nm。

1.3 试验样品

标识具有防脱、育发功效的化妆品, 样品均为市场采购。

1.4 试验方法

1.4.1 标准储备液的配制

分别准确称量 8 种化合物各 10 mg (精确至 0.1 mg) 于 10 mL 容量瓶中, 加甲醇溶解并定容, 分别配成 1g/L 的标准储备液。

1.4.2 混合标准工作液

分别吸取不同体积的储备液, 用甲醇配制成浓度分别为 0.5mg/L、1.0mg/L、5.0mg/L、10.0mg/L、20.0mg/L、50.0mg/L、100.0mg/L 的混合标准工作液。

1.4.3 样品处理

准确称取 1.0g (精确至 0.001g) 样品, 置于 10 mL 比色管中, 甲醇定容至 10mL, 于涡旋混匀器上充分均质, 超声 15min, 10000r/min 20℃ 下高速离心 10min, 取全部上清液, 40℃ 水浴下氮吹至 1mL, 纯水定容至 10 mL, 充分混匀, 10000r/min 20℃ 下高速离心 10min, 上 Bond Elute Plexa 固相萃取小柱。柱子依次用 5mL 甲醇、5mL 水活化平衡, 样品全部上样后, 依次用 3mL 水、3mL 甲醇-水 (10/90, V/V) 淋洗, 5mL 甲醇-水 (90/10, V/V) 洗脱。洗脱液在 40℃ 水浴下氮吹至近干, 甲醇复溶, 定容至 1 mL, 涡旋混匀 1min, 超声 1min, 过 0.22μm 滤膜, 待测。

1.4.5 测定

按 1.2 操作进行, 以保留时间定性, 外标法定量。

结果与amp;讨论

2.1 色谱条件的选择

考察了 Agilent Poroshell 120 Bonus-RP (4.6×50 mm, 2.7μm) 和常规 C18 液相色谱柱 (4.6×250 mm, 5μm) 对 8 种药物的分离效果, 发现用常规液相柱时, 雌二醇与雌酮、坎利酮与己烯雌酚不能很好地分离, 而 Agilent Poroshell 120 Bonus-RP 能得到理想的分离效果。色谱分离图如图 1、图 2 所示。

流动相 A 考察了水、1% 乙酸水溶液和 10mmol/L 乙酸铵水溶液, 流动相 B 考察了甲醇、乙腈的分离效果, 结果显示, 流动相 A 选用水和 1% 乙酸水溶液时, 米诺地尔的保留不强, 出峰太快, 保留时间不易控制, 峰形也有轻微拖尾现象, 而采 10mmol/L 乙酸铵时, 保留相对较强, 能控制保留时间以实现与杂质的分离。流动相 B 选用甲醇和乙腈的分离效果相差不大, 考虑到乙腈的成本与毒性, 选择用甲醇。

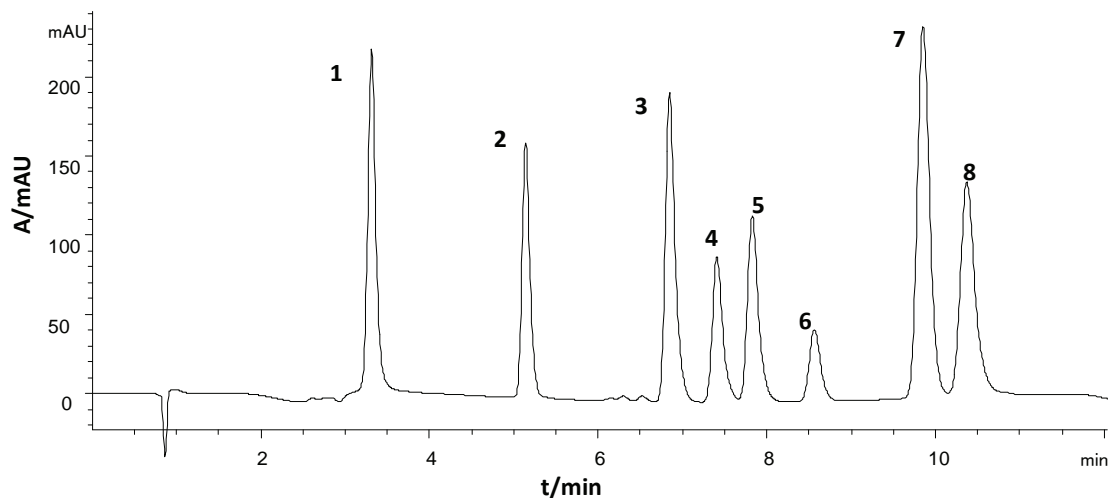


图1 使用 Poroshell 120 Bonus-RP 柱分离的标准色谱图 (浓度为 20mg/L)
1 米诺地尔; 2 雌三醇; 3 螺内酯; 4 坎利酮; 5 雌酮; 6 雌二醇; 7 己烯雌酚; 8 黄体酮

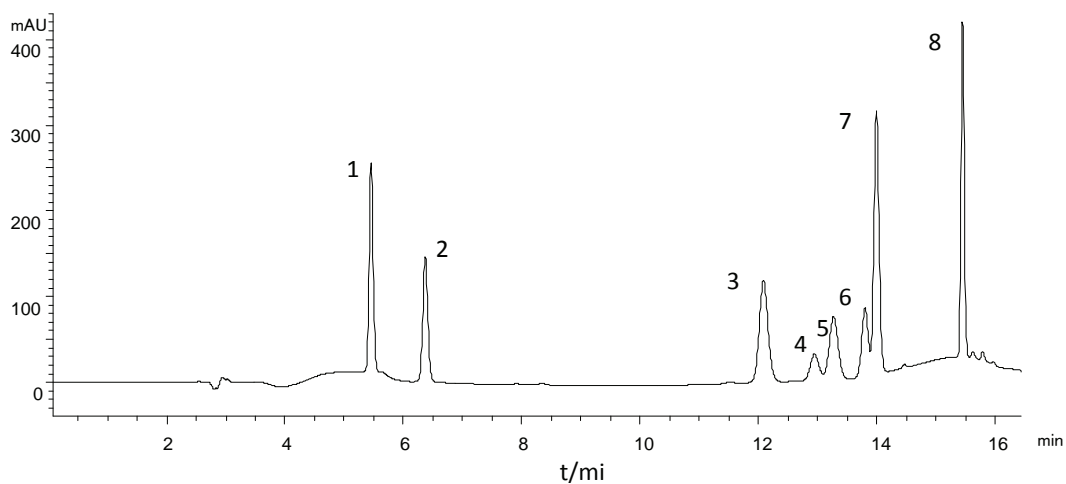


图2 使用常规 C18 液相柱分离的标准色谱图 (浓度为 20mg/L)
1 米诺地尔; 2 雌三醇; 3 螺内酯; 4 雌酮; 5 雌二醇; 6 坎利酮; 7 己烯雌酚; 8 黄体酮

2.2 方法的线性范围和检出限

混合标准工作液的测定按照 1.4 的操作步骤, 以添加剂的峰面积为纵坐标, 含量为横坐标, 绘制标准曲线。本方法中 8 种违禁药物在 0.5 ~ 100 mg/L 范围内均具有良好的线性关系, r 均在 0.999 以上, 螺内酯和黄体酮的方法定量限 ($S/N = 10$) 为 2.0 mg/kg, 其余 6 种药物的方法定量限均为 5.0mg/kg。

2.3 方法的加标回收率和精密度

取上述空白样品 1.0g, 分别添加混合标准储备液, 使得样品中 8 种添加剂的浓度为 50.0g/kg, 按样品前处理操作, 方法的加标回收率和精密度结果见表 1

表 1 8 种违禁药物的回收率、精密度 (n=3)

违禁药物	添加浓度 (mg/kg)	平均回收率 (%)	精密度 (RSD%)
米诺地尔	50.0	74.8	4.2
雌三醇	50.0	91.5	2.7
螺内酯	50.0	91.9	2.8
坎利酮	50.0	91.6	3.5
雌酮	50.0	92.7	2.5
雌二醇	50.0	93.8	3.6
己烯雌酚	50.0	83.8	2.7
黄体酮	50.0	100.4	2.1

小结

本法采用 Poroshell120 Bonus-RP 建立了高效液相色谱 - 二极管阵列法检测育发类化妆品中米诺地尔、雌三醇、螺内酯、坎利酮、雌酮、雌二醇、己烯雌酚、黄体酮 8 种违禁药物的分析方法。该方法的分析速度迅捷、试剂消耗少、检出限低, 结果令人满意, 适合于检测工作的开展。

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采用 Poroshell 120 色谱柱快速分析T项目的中间体

关键词

HPLC

Poroshell 120

ATS02

作者

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摘要

T项目是东阳光的在研项目，为确保项目顺利进行制定了相应的液相检测方法，但方法中使用的常规液相色谱仪和液相分析柱，分析时间长，且有机试剂耗用量大。超高效液相色谱仪（UHPLC）和 1.8 μm 填料快速液相分析柱可以使得分析时间和试剂耗量大大降低。但目前基层实验室普遍使用的常规液相色谱仪与快速液相分析柱的不兼容性，使得快速分析检测很难实现。安捷伦公司最新推出的 Poroshell 120 系列表面多孔层色谱柱，由于其具有低反压，高柱效的特点，从而真正实现在常规液相色谱仪上进行快速分析的可操作性。本文使用 Poroshell 120 色谱柱，并采用常规液相色谱仪，对工艺中的中间体的检测方法加以改进。现将实验结果报告如下，以供参考。

材料与方法

1.1 仪器与试剂

仪器: Agilent 1260 高效液相色谱仪

试剂: 乙腈 (HPLC 级, Honeywell); 超纯水

1.2 色谱条件

色谱仪条件: Poroshell 120 EC-C18 柱 (4.6 mm × 100 mm, 2.7 μm); 柱温 35°C; 流速 1.0 mL/min; 流动相 A: 超纯水; 流动相 B: 乙腈; 紫外检测器: 波长 245 nm; 进样体积 2.5 μL。梯度条件如下

梯度		
Time (min)	A(%V/V)	B(%V/V)
0	55	45
3	55	45
7	40	60
10	13	87
11.2	13	87
16	55	45
20	55	45

1.3 试验样品

工艺中不同阶段的反应液

1.4 试验方法

1.4.1 样品配制

取反应液 2 滴, 并用甲醇稀释至 4 mL, 用 0.2 μm 滤膜过滤即得。

1.4.5 测定

按 1.2 色谱条件下操作。

结果与讨论

2.1 方法转换过程的讨论

在以前分析方法的基础上通过改变梯度运行时间、减少进样量、改进梯度变化速度, 以 Poroshell 120 EC-C18 柱 (4.6 mm × 100 mm, 2.7 μm) 为色谱柱的实验结果显示, 杂质与杂质之间能实现分离, 而主峰与主峰前后峰的分离度在 1.5 以上 (见图 1), 且峰形良好, 柱压低, 色谱柱的柱效能达到分析的要求。

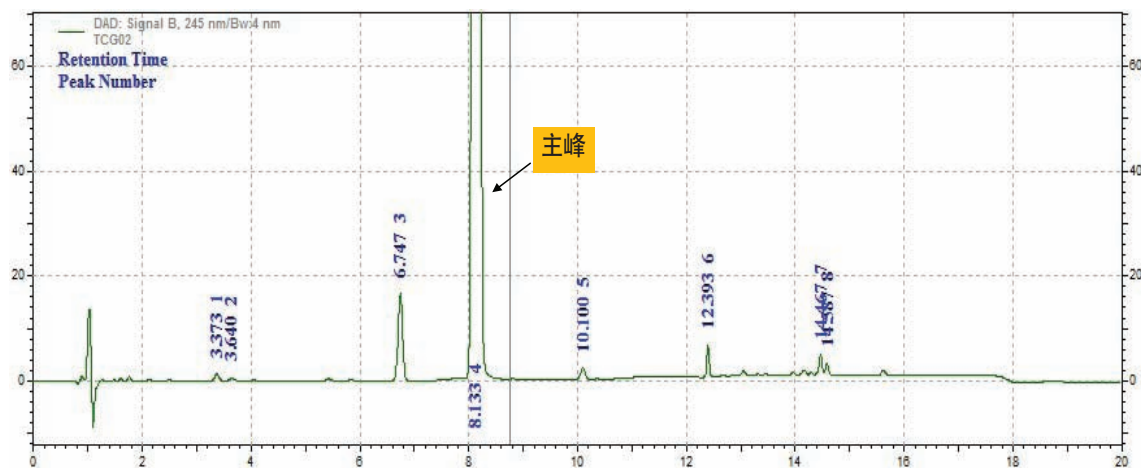
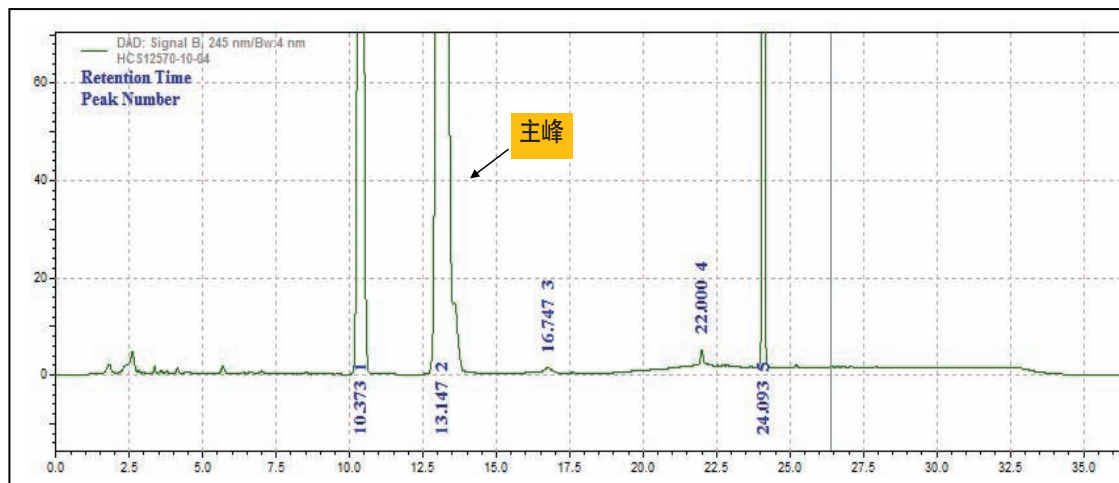


图 1 ATS02 反应液色谱图

2.4 使用传统色谱柱的结果 (图2)



(图2 样品与图1 样品为不同工艺条件下的样品, 主峰上的肩峰是由于在液相条件下形成的互变异构体)

图2 ATS02 反应液色谱图

色谱条件:

色谱柱: zorbax SB -C18 (4.6×250,5μm)

柱温: 35℃

流速: 1.0mL/min

检测波长: 245nm

流动相: A: H₂O B: 乙腈

梯度

Time (min)	A(%V/V)	B(%V/V)
0	55	45
25	55	45
28	10	90
37	10	90

Posttime : 7min

样品配制: ATS02 用甲醇溶解, 进样 5 μl

小结

本法采用 Poroshell120, 在普遍使用的常规液相色谱仪上测定 T 项目工艺中的中间体。该方法的分析时间缩短至原来分析时间的 0.4 倍, 同样的, 流动相的消耗量体积缩小至原来的 0.4 倍, 且峰形良好, 柱压低, 与常规液相分析柱相比有一定的优势。

应用配备 Agilent Poroshell 色谱柱的 Agilent 1290 Infinity LC 在 2 min 内同时测定八种有机紫外线吸收剂

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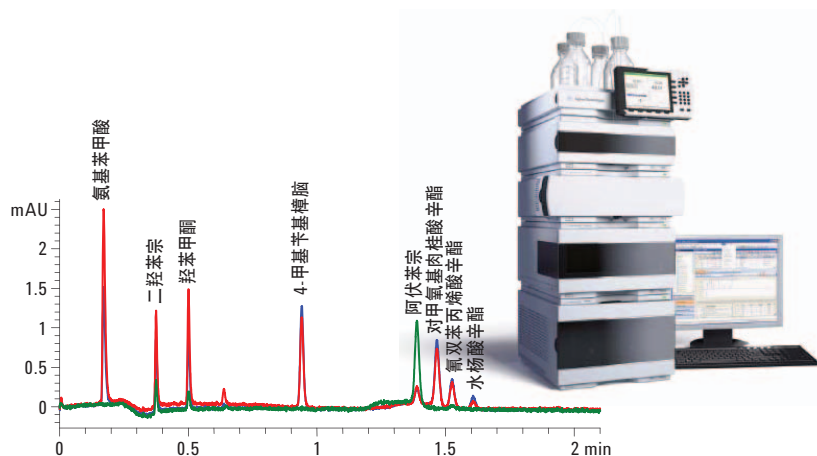
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摘要

FDA 与欧洲药典 (EP) 中都规定了个人护理产品中紫外线吸收剂的含量水平。液相色谱法 (LC) 是用于紫外线吸收剂定性和定量分析的被广泛认可的分析技术。大多数此类分析的传统 LC 方法一次运行需约 20-25 min。在此应用报告中, 采用配有 Agilent Poroshell 色谱柱的 Agilent 1290 Infinity LC, 开发了一种快速、灵敏、耐用和具有良好分辨率的分析方法, 可在 99 s 内分离 8 种 FDA/EP 收录的紫外线吸收剂成分。重复进样保留时间的标准偏差 (SD) 及相对标准偏差 (RSD) 值证实了 Agilent 1290 Infinity 二元泵具有的优良性能。极小的峰面积 RSD 值及有机紫外线吸收剂标准品 0.25 ng-200 ng 载样量的宽线性范围展现了 Agilent 1290 Infinity 二极管阵列检测器的卓越性能。此外, 该方法可有效鉴别 6 种国际个人护理产品中的活性紫外线吸收剂。

引言

紫外线波长介于 X 射线和可见光之间 (~ 10-400 nm)¹。黑色素是可引起皮肤变黑的一种色素，皮肤产生黑色素是人体天然防御紫外线辐射的措施。黑色素吸收紫外线辐射，并将其能量转化为无害的热量散失掉，当然，肤色及其它遗传因素决定了人体对紫外线辐射的反应及黑色素沉着程度^{2, 3}。不考虑肤色和皮肤产生黑色素的能力，与晒伤有关的主要因素是紫外线辐射强度和暴晒时间。紫外光的主要分类见表 1⁴。

保护皮肤免受晒伤的防晒霜中包含以下一个或多个类型的活性成分：

- 吸收紫外线的有机化合物
- 可反射、散射及吸收紫外线的无机微粒
- 具有上述所有功能的有机微粒

有机紫外吸收剂通常是羰基共轭的芳香族化合物。美国 FDA 已批准 7 种 UV-A 吸收剂化合物和 9 种 UV-B 吸收剂化合物可作为防晒霜的成分，同时欧洲药典委员会已批准了另外的 10 种紫外线吸收剂可在欧洲国家使用⁵。

尽管通过认证的几种紫外线吸收剂已经可在市场上见到，但是这些紫外线吸收剂的广泛应用也引起一些关注。一些防晒霜成分已证明具有致癌作用。另外，旧时的和

编号	组成	波长 (nm)	过度暴晒对皮肤的影响
1	UV-A I	340-400	可引起晒黑但极少产生红斑。可引起长期损害。深层渗透。通过间接损伤 DNA 导致皮肤癌。
2	UV-A II	320-340	产生轻度红斑。
3	UV-B	290-320	引起晒伤，是导致皮肤癌的主要原因。
4	UV-C	100-290	很强的辐射。可被臭氧层吸收。可直接损伤 DNA。

表 1
紫外辐射的主要分类

当前广泛应用的防晒化学品不能像黑色素一样有效地消散激发态的能量，因而防晒成分渗透到皮肤底层可增加自由基和活性氧的数量⁶。因此需要对防晒霜进行广泛测试，揭示其中成分的作用效果。此应用报告详述了一个快速液相分析方法，可在 99 s 内分离 8 种当前广泛应用的紫外线吸收剂。在产品开发、法规管理及质量控制中，化妆品生产商可采用这种方法简化防晒霜原材料以及成品的分析，从而提高分析效率。

实验部分

仪器配置

由化学工作站 (B.04.02版) 控制的 Agilent 1290 Infinity LC 系统，配备了集成真空脱气机的二元泵、自动进样器、柱温箱及用于采集数据的二极管阵列检测器。

进样量 1 μ L，并用乙腈洗针 3 s。样品恒温箱设为 5 $^{\circ}$ C，而色谱柱设定在 55 $^{\circ}$ C 下工作。二元泵流速设置为 1 mL/min。检测器程序设置了 3 个不同的波长 (288、304 和 358 nm)，样品采集速率为 80 Hz (响应时间 0.062 秒，>0.003 min)。采用 Agilent Poroshell 120 EC-C18 色谱柱 (75 mm \times 2.1 mm, 2.7 μ m) 用于色谱分离。

化学品和标准品

8 种紫外线吸收剂标准品和己酸 (流动相改性剂) 均购自 Aldrich 公司 (印度)。超梯度级乙腈 (ACN) 购自 Lab-Scan 公司 (泰国曼谷)。HPLC 级水为 Milli-Q 超纯水净化系统新鲜制备。6 个不同的国际品牌防晒霜购自当地。本实验中所用有机紫外线吸收剂标准品详见表 2。

化合物编号	化合物	结构	UV 光谱
1	名称: 氨基苯甲酸 CAS 号: 150-13-0 分子式: $C_7H_7NO_2$ 分子量: 137.14 检测波长: 288 nm		
2	名称: 二羟苯宗 CAS 号: 131-53-3 分子式: $C_{14}H_{12}O_4$ 分子量: 244.24 检测波长: 288 nm		
3	名称: 羟苯甲酮 CAS 号: 131-57-7 分子式: $C_{14}H_{12}O_3$ 分子量: 228.24 检测波长: 288 nm		
4	名称: 4-甲基苄基樟脑 CAS 号: 36861-47-9 分子式: $C_{18}H_{22}O$ 分子量: 254.37 检测波长: 304 nm		
5	名称: 阿伏苯宗 CAS 号: 70356-09-1 分子式: $C_{20}H_{22}O_3$ 分子量: 310.39 检测波长: 358 nm		
6	名称: 对甲氧基肉桂酸辛酯 CAS 号: 5466-77-3 分子式: $C_{18}H_{26}O_3$ 分子量: 290.397 检测波长: 304 nm		
7	名称: 氰双苯丙烯酸辛酯 CAS 号: 6197-30-4 分子式: $C_{24}H_{27}NO_2$ 分子量: 361.48 检测波长: 304 nm		
8	名称: 水杨酸辛酯 CAS 号: 118-60-5 分子式: $C_{15}H_{22}O_3$ 分子量: 250.33 检测波长: 304 nm		

表 2

本实验中所用的有机紫外线吸收剂标准品详情列表

LC 分析参数

预先混合 0.1% 乙酸溶液/乙腈比例为 90:10 (A), 10:90 (B) 的溶液作为流动相。本实验所用梯度程序见表 3。色谱柱重新平衡的后运行时间设置为 1 min。

时间 (min)	B (%)
0	50
0.1	70
2	85

表 3
实验中所用梯度程序

标准品混合

以乙腈与 0.1% 乙酸溶液按比例为 50:50 的混合液作为稀释剂。分别配制各标准品的母液, 浓度均为 1000 ppm (1000 ng/ μ L)。混合对氨基苯甲酸、二羟苯宗、羟苯甲酮、4-甲基苄基樟脑、阿伏苯宗、对甲氧基肉桂酸辛酯、氰双苯丙烯酸辛酯以及水杨酸辛酯的各标准母液, 并用稀释剂稀释制成每个化合物浓度均为 100 ppm (ng/ μ L) 的标准品混合液。然后采用逐级稀释法稀释较高浓度的标准品混合液, 制成 7 个分析物浓度分别为 50、25、10、5、1、0.5 和 0.25 ng/ μ L 的标准品混合液, 以进行检测器的线性分析。

从配方样品中提取紫外线吸收剂

通过一个简单的样品提取步骤, 使用乙腈从 6 个不同的当地现有国际品牌防晒霜产品中提取紫外线吸收剂。用 5 mL 乙腈对 250 mg 样品进行提取, 超声并离心。上清液用 0.2 μ m 的安捷伦注射式过滤器 (部件号 5061-3361) 过滤。滤液用稀释剂稀释 5 倍, 得到提取样品的贮备液。采用等量的稀释剂进一步稀释样品贮备液, 作为进样样品, 进样量为 1 μ L。加标的萃取样品用 100 ppm 的标准品混合液和提取的样品贮备液等体积混合制备。通过加标样品中组分的保留时间和紫外光谱来鉴定提取样品中的峰。

实验步骤

为考察分离中的色谱干扰, 所有实验均进行空白进样分析。同时进样标准品混合液、线性水平测定溶液、稀释的萃取样品溶液以及加标的稀释萃取样品溶液进行分析。并通过单个标准品进样分析来确定每个标准品的保留时间。

结果与讨论

标准品混合液的液相色谱图

结果表明 8 个防晒霜活性成分可达到良好的基线分离且无色谱空白干扰。标准品混

合液的最后一个峰 (水杨酸辛酯) 在 1.62 min 被洗脱出来。标准品混合液的色谱图如图 1 所示。大约在 0.65 min 处检测到一个未知峰, 这是阿伏苯宗标准品中存在的杂质。通过 200-400 nm 光谱扫描进行峰纯度检测, 结果表明 8 个化合物峰均不包含任何可检测到的杂质共洗脱峰。由于各成分最大吸收值不同因而选定了三个不同的波长进行检测。使用 Agilent Poroshell 120 EC-C18 色谱柱分析, 半峰宽、峰对称性、USP 拖尾因子及分离度数据表明, 所有标准分析物在 99 秒内均达到了基线分离 (表 4)。

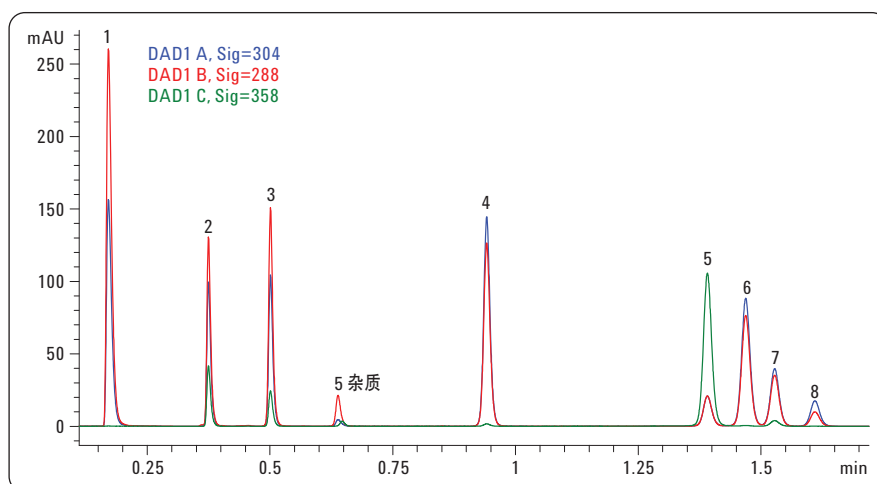


图 1
使用配有 Agilent Poroshell 120 EC-C18 色谱柱的 Agilent 1290 Infinity LC, 8 种有机紫外线吸收剂在 99 秒内的洗脱色谱图 (覆盖了 3 个不同波长)

化合物编号	化合物名称	半峰宽	对称性	USP 拖尾因子	分离度
1	氨基苯甲酸	0.011	0.556	1.438	NA
2	二羟苯宗	0.008	0.648	1.449	12.662
3	羟苯甲酮	0.009	0.720	1.306	8.794
4	4-甲基苄基樟脑	0.014	0.862	1.112	22.988
5	阿伏苯宗	0.018	0.896	1.090	16.456
6	对甲氧基肉桂酸辛酯	0.019	0.920	1.070	2.453
7	氰双苯丙烯酸辛酯	0.019	0.912	1.061	1.805
8	水杨酸辛酯	0.020	0.910	1.061	2.447

表 4
304 nm 波长下, 进样一个标准品混合液 (50 ng), 其中 8 种防晒霜成分标准品的半峰宽、峰对称性、USP 拖尾因子及分离度数据。进样量为 1 μ L

6 次重复进样浓度为 10 ng/μL 样品的色谱叠加图，表明数据重现性良好（图 2）。尽管化学结构相似，各成分在 99 秒内仍得到很好分离。在此水平下，得到的保留时间（RT）的标准偏差值（SD）< 0.0005，相对标准偏差（RSD）< 0.32%，并且峰面积的 RSD < 1.43%。

信噪比

图 3 为分析标准品混合液柱内浓度为 0.25 ng 时所有分析物的色谱图。以各组分可检测到峰的峰高为信号，无化合物峰区域基线的绝对噪声作为噪声，计算各标准品的信噪比值（S/N）。S/N 值、SD 以及 RSD 值列于表 5。在此浓度下，前 6 个化合物的 S/N 值 > 20，其中检测到的最小 S/N 值是水杨酸辛酯（化合物 8），S/N 值是 5。

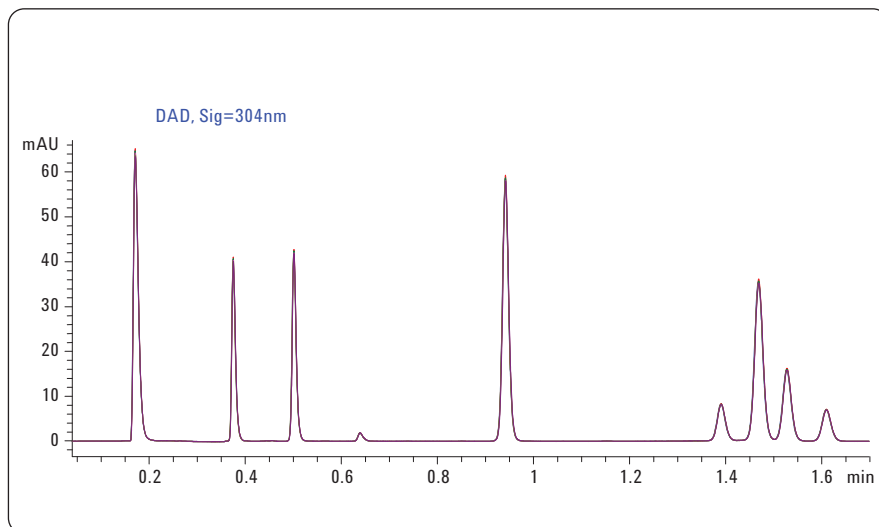


图 2
10 ng/μL 浓度水平下标准品混合液 6 次重复进样的叠加色谱图

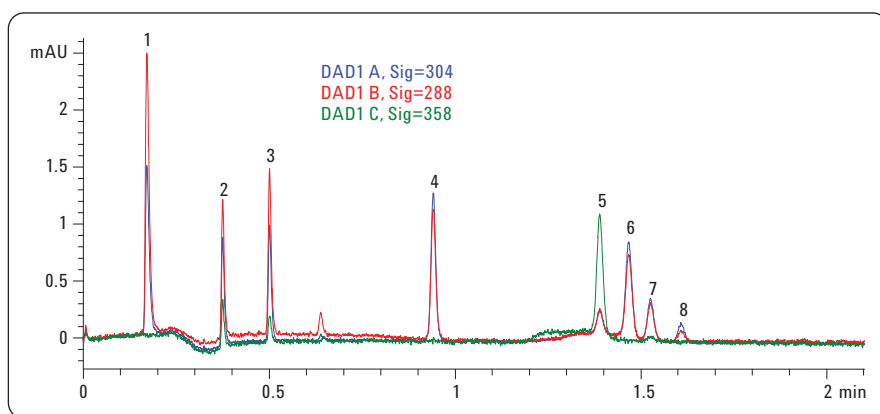


图 3
所有分析物柱内浓度为 0.25 ng 时，标准品混合液的色谱图（覆盖 3 个不同波长）

	化合物 1	化合物 2	化合物 3	化合物 4	化合物 5	化合物 6	化合物 7	化合物 8
进样	288 nm		304 nm	358 nm	304 nm			
3	51.5	26.5	31	36.1	21.1	22.9	10.4	4.6
5	50.3	25.5	28.8	34.7	20.2	22	9.4	4.4
6	56.5	27.3	31.8	34.1	20.8	22	10	4.6
7	50.2	25.6	30	35.1	19.8	21.9	9.5	4.5
8	52.8	26.1	31.4	35.7	19.3	22.2	9.7	4.4
9	50.2	25.3	29.8	35.3	18.6	21.8	9.8	4.5
平均值	51.9	26.1	30.5	35.2	20.0	22.1	9.8	4.5
SD	2.47	0.75	1.13	0.71	0.94	0.40	0.36	0.09
RSD (%)	4.75	2.89	3.71	2.02	4.68	1.80	3.71	1.99

表 5
柱内浓度为 0.25 ng 时，各标准品的信噪比值

线性

在柱内浓度为 0.25 ng-200 ng 的浓度范围进行线性研究 (9 浓度水平, 重复进样 5 次)。浓度水平分别为 200 ng、100 ng、50 ng、25 ng、10 ng、5 ng、1 ng、0.5 ng 和 0.25 ng。通过计算各浓度水平 5 次重复进样峰面积和保留时间的 SD 值和 RSD 值展现其精密度。图 4 所示为保留时间的 RSD。由图可知 RSD 值在可接受的 1.0% 范围内, 表明保留时间的精密度良好。

结果表明除水杨酸辛酯 (化合物 8) 外, 其余所有组分在 0.5 ng 以上的柱内浓度时, 峰面积重现性良好。对于化合物 8, 在 1 ng 及以上柱内浓度时, 其峰面积的 RSD 值也在允许的 2.0% 范围以内。与其它标准品相比, 较小的峰面积表明水杨酸辛酯的紫外响应较弱, 这是其 RSD 值较高的原因。所有线性浓度水平的化合物峰面积的 RSD 值列于表 6。

以标称浓度 (0.25 ng、0.5 ng、1 ng、5 ng、10 ng、25 ng、50 ng、100 ng、200 ng) 为横坐标, 各标准品峰面积为纵坐标制备校准曲线, 建立峰面积与浓度的线性关系, 相关系数 (R^2) > 0.9997。所有标准品的线性曲线叠加如图 5 所示。各组分的 R^2 值列于表 7。

提取样品的分析

创建所有标准品的图谱库用来确认色谱峰成分, 并且提供光谱数据以检测峰纯度或判断是否存在共洗脱峰。对 6 个不同国际品牌防晒霜中的紫外线吸收剂进行提取和分析。所有样品叠加的洗脱色谱图如图 6 所示。样品 5 中可观察到在 1.71 min 处有一未知峰。结果显示, 在已检测的个人防晒护理产品中, 阿伏苯宗和氧双苯丙烯酸辛酯是使用最广泛的紫外线吸收剂成分。从提取样品的色谱图中可明显看出, 样品 b 和 d 提供了抵御 UVA、UVB 和 UVC 射线的广泛保护。

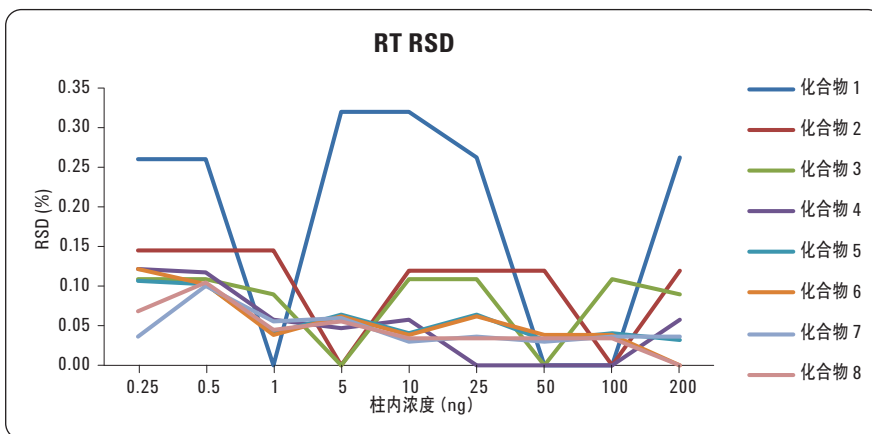


图 4
保留时间 RSD 值

柱内浓度 (ng)	峰面积 RSD 值 (%)							
	化合物 1	化合物 2	化合物 3	化合物 4	化合物 5	化合物 6	化合物 7	化合物 8
0.25	1.70	4.40	3.57	1.53	1.95	2.80	5.93	9.67
0.5	1.48	1.46	1.48	1.45	0.98	1.46	1.80	4.24
1	0.47	0.55	0.73	0.87	1.00	1.34	1.20	1.94
5	0.22	1.37	0.06	0.09	0.31	0.21	0.20	0.50
10	0.95	1.02	0.96	1.10	1.16	1.29	1.43	1.21
25	0.27	0.45	0.50	0.46	0.55	0.54	0.61	0.53
50	1.11	1.16	1.02	0.98	0.80	0.80	0.70	0.79
100	0.11	0.16	0.20	0.26	0.25	0.29	0.27	0.33
200	0.16	0.24	0.19	0.16	0.19	0.18	0.22	0.11

表 6
所有化合物在各线性浓度水平下峰面积的 RSD 值 (n=5)。其中 > 2.0 的 RSD 值标红

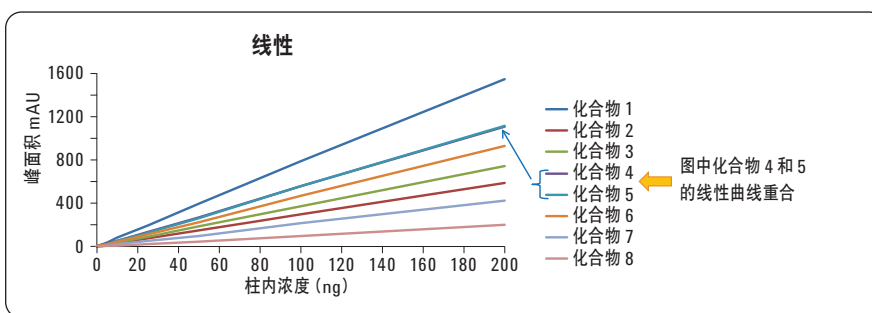


图 5
所有标准品的线性曲线叠加图

化合物	名称	R^2
1	氨基苯甲酸	0.9999
2	二羟苯宗	1.0000
3	羟苯甲酮	0.9999
4	4-甲基苄基樟脑	0.9999
5	阿伏苯宗	0.9997
6	对甲氧基肉桂酸辛酯	0.9998
7	氧双苯丙烯酸辛酯	0.9997
8	水杨酸辛酯	0.9998

表 7
各组分的 R^2 值

具有较高 SPF 值的防晒产品一般含有较大量的防晒成分，因此这些产品中包含相当数量杂质的几率也很大。Agilent 1290 Infinity LC 可在最短的时间内提供个人护理产品中杂质分布的整体概貌，如图 7 所示为样品 e 的色谱图。

结论

本应用报告介绍了使用配有 Agilent Poroshell 120 EC- C18 色谱柱的 Agilent 1290 Infinity LC，在 99 s 内实现了 8 种 FDA/EP 收录的防晒化合物的基线分离。标准品混合溶液色谱图中各组分间最小的分离度 > 1.8。并且在 0.25 ng 水平（柱内浓度）下对各组分的信噪比值进行了考察。所有化合物的线性在柱内浓度 0.25 ng-200 ng 的范围内得到了验证。校准曲线中最差的 R^2 为 0.9997（9 浓度水平，5 次重复进样）。在各线性浓度水平上，得到其保留时间最大的标准偏差值是 0.0018，最大的 RSD 值是 0.32%（n=5）。本法可有效地用于防晒霜和个人护理化妆品中紫外线吸收剂及其杂质的色谱分析。

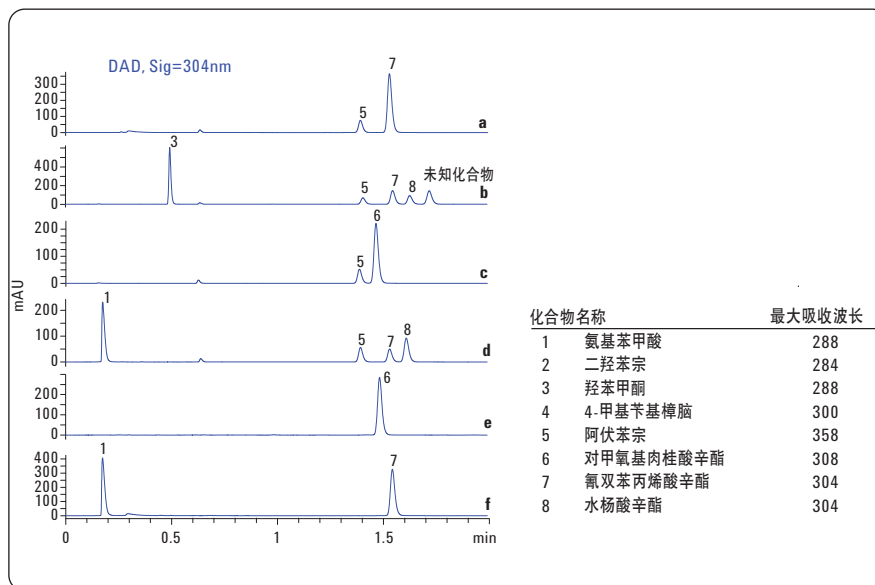


图 6
6 个提取样品的叠加色谱图

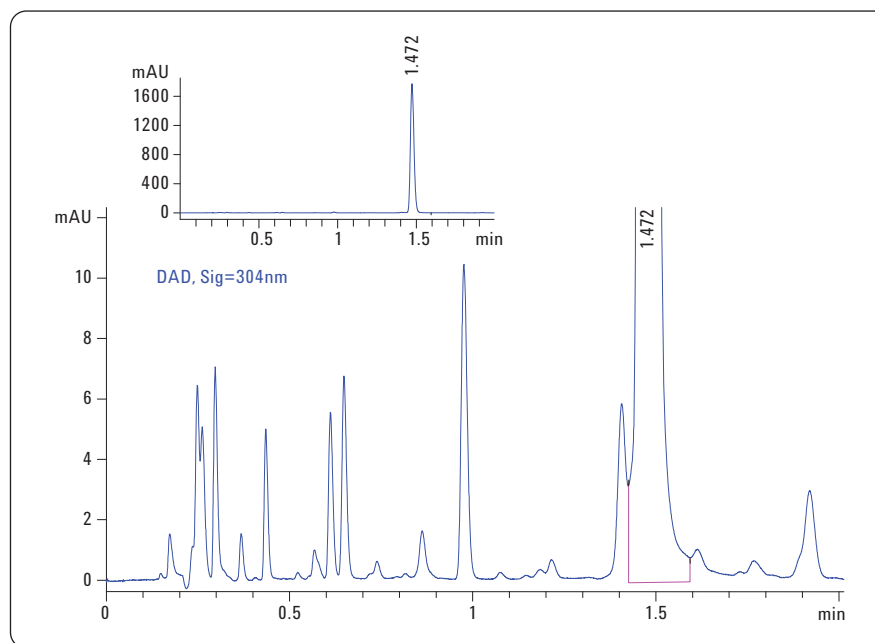


图 7
样品“e”的满刻度放大色谱图，表明杂质峰分离度良好。这些较小的未经确认的峰可能是对羟基苯甲酸酯类或配方中其它相关成分的杂质

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Fast Analysis of Hair Dyes Using an Agilent Poroshell 120 Bonus-RP Column by UHPLC and LC/MS/MS

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Abstract

UHPLC and LC/MS/MS methods were developed for separating components that are banned or restricted for use in hair dyes on an Agilent Poroshell 120 Bonus-RP column. Compared to alkyl-only phases, the Poroshell Bonus-RP column has enhanced retention and selectivity for acidic and polar compounds due to strong H-bonding and can be used in 100% aqueous mobile phase. The UHPLC method using an Agilent 1290 Infinity LC System separated 24 compounds, while 5 more compounds could be analyzed using an Agilent 6460 Triple Quadrupole LC/MS System. All the compounds were separated within 15 minutes. Two commercial samples of hair dyes were analyzed to test the UHPLC method with complex samples.

Introduction

Hair dyes are used by people all over the world. Commonly used hair dyes are composed of aromatic compounds containing modified aniline and phenolic compounds, which may cause allergic reactions and potentially even cause cancer. Due to these potentially harmful effects, the amounts of these compounds are banned or restricted in many countries according to their own regulations. Methods for the quantitative measurement of the compounds in hair dyes include GC, GC/MS, LC, LC/MS [1]. HPLC methods are popular for the quantitative analysis because the compounds analyzed are not thermally stable, and most are strongly polar with low volatility.

A Poroshell 120 Bonus-RP, 2.7 μm column packed with superficially porous materials was used in the application. Poroshell 120 superficially porous columns have nearly the same efficiency as sub-2 μm totally porous columns and can, therefore, be used to provide similar fast and high resolution analyses. The Bonus-RP phases have a polar-embedded group inserted into the hydrophobic C14 alkyl chain that allows minimal interaction of polar samples with silanols, providing symmetrical peak shape for many analytes. The polar embedded group also helps to wet the hydrophobic chains and prevents phase collapse in highly aqueous mobile phase. Compared to alkyl-only phases, Bonus-RP has enhanced retention and selectivity for acidic and polar compounds due to strong H-bonding [2].

This application note describes a UHPLC method that was developed for separating 24 compounds in only 15 minutes. The LC/MS/MS method was used to identify more compounds because the compounds that could not be separated via HPLC, could be resolved by LC/MS/MS. A total of 29 compounds were analyzed using a 6460 Triple Quadrupole LC/MS System. Both methods were developed on a Poroshell 120 Bonus-RP, 3.0 \times 100 mm, 2.7 μm column. Two commercially available samples were purchased and analyzed, and some dye compounds were detected and separated from other components in the hair dyes.

Experimental

The 1290 Infinity LC System includes a binary pump, a Thermostatic Column Compartment (TCC), a high performance autosampler, and a Diode Array Detector (DAD). The LC/MS/MS system includes a 1290 Infinity LC system and an Agilent 6460 Triple Quadrupole LC/MS System. The column used in the application is a Poroshell 120 Bonus-RP, 3.0 \times 100 mm, 2.7 μm .

The stock solutions were prepared individually in methanol at 10 mg/mL and then mixed together and diluted down to an appropriate concentration of standard solution with 2 g/L sodium hydrogen sulfite solution. The standard solutions were then filtered through an Agilent 0.2 μm regenerated cellulose filter (part number 5064-8221). The filtered solutions were transferred to the autosampler vials for HPLC and LC/MS/MS analysis.

The 2 hair dye samples were purchased locally. Each sample (0.5 g) was extracted using 10 mL acetonitrile and then placed in an ultrasonic bath for 10 minutes. The solution was then filtered through a 0.2 μm regenerated cellulose filter, and the filtered solutions were transferred to the autosampler vials for HPLC analysis.

Table 1. The 29 hair dye compounds analyzed in this application.

No.	Name	CAS
1	p-Phenylenediamine	106-50-3
2	2-Amino-3-hydroxypyridine	16867-03-1
3	m-Phenylenediamine	108-45-2
4	p-Aminophenol	123-30-8
5	2,6-Diaminopyridine	141-86-6
6	2,5-Diaminotoluene sulfate	615-50-9
7	o-Phenylenediamine	95-54-5
8	m-Aminophenol	591-27-5
9	o-Aminophenol	95-55-6
10	2-Chloro-1,4-phenylenediamine sulfate	61702-44-1
11	p-Methylaminophenol sulfate	1936-57-8
12	Hydroquinone	123-31-9
13	Resorcinol	108-46-3
14	N,N-Diethyl-1,4-benzenediamine sulfate	6065-27-6
15	3,4-Diaminotoluene	496-72-0
16	1,4-Diamino-2-nitrobenzene	5307-14-2
17	5-Amino-o-cresol	2835-95-2
18	4-(N,N-Diethyl)-2-methyl-p-phenylenediamine monohydrochloride	2051-79-8
19	2-Methylresorcinol	608-25-3
20	6-Amino-m-cresol	2835-98-5
21	4-Nitro-o-phenylenediamine	99-56-9
22	5-Methyl-2-phenyl-1,2-dihydropyrazol-3-one	89-25-8
23	4-Amino-3-nitrophenol	610-81-1
24	6-Hydroxyindole	2380-86-1
25	4-Chlororesorcinol	95-88-5
26	2,7-Dihydroxynaphthalene	582-17-2
27	1,5-Dihydroxy naphthalene	83-56-7
28	4-Aminodiphenylamine	101-54-2
29	1-Naphthol	90-15-3

Results and Discussion

The superficially porous particles have nearly identical efficiency as sub-2 μm totally porous materials and can, therefore, be used to provide similar fast and high resolution analyses at lower pressure. The Bonus-RP phases have a polar-embedded group inserted into the hydrophobic C14 alkyl chain that allows minimum interaction of polar samples with silanols, providing symmetrical peak shape. Compared to alkyl-only phases, Bonus-RP has enhanced retention and selectivity for acidic and polar compounds due to strong H-bonding and can be used in 100% aqueous mobile phase. A previous application note developed a method using a Poroshell 120 EC-C18 separating 17 hair dye compounds [3]. However, to increase retention and separate more compounds, especially polar compounds, a gradient method starting from 100% aqueous phase on the Poroshell 120 Bonus-RP column was used for this application.

Figure 1 shows the separation of 24 potential hair dye standard components on 2 Poroshell 120 Bonus-RP columns in 15 minutes. This separation can be easily reproduced from column to column from different batches. Reasonable resolution is achieved between all the standard components. Due to the complexity of the compounds analyzed, it is difficult to optimize the pH of the mobile phase for all the compounds. In the application a mid pH mobile phase with 10 mM ammonium acetate was used to enhance retention of the compounds on the column because most compounds have amino groups that present neutral in a mid or high pH value mobile phase. These conditions were not optimal for all the components, but they were best for the majority of the components of the sample.

Figure 2 shows overlay chromatograms of sample 1, sample 2, and standards. Compounds vary in different samples. Compounds 2,5-diaminotoluene sulfate, 3-aminophenol, resorcinol, and 5-amino-o-cresol were found in sample 1 and compounds p-phenylenediamine, 3-aminophenol, resorcinol, and 2,7-dihydroxynaphthalene were detected in sample 2. The amounts of the detected compounds can be measured given the concentration of the standards.

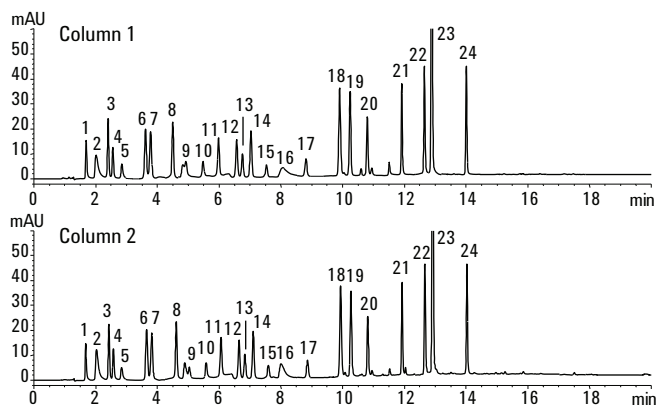


Figure 1. Standards chromatograms on 2 batches of Agilent Poroshell 120 Bonus-RP, 2.7- μm columns on an Agilent 1290 Infinity LC System.

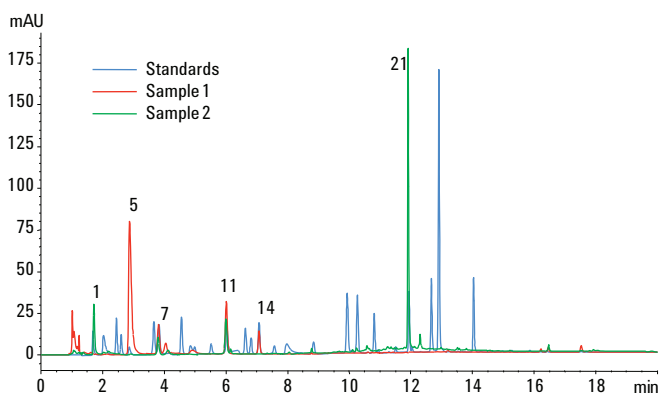


Figure 2. Overlay chromatograms of samples and standard components using an Agilent Poroshell 120 Bonus-RP, 2.7- μm column.

LC conditions

Instrument: Agilent 1290 Infinity LC System (installed with 1290 inline filter after injector valve, p/n 5067-4638)

Column: Agilent Poroshell 120 Bonus-RP, 3.0 × 100 mm, 2.7 μm (p/n: 695968-301)

Mobile phase: A, 10 mM acetate; B, ACN

Gradient: 0 min 0% B
2 min 0% B
8 min 20% B
15 min 70% B
18 min 80% B

Stop time: 20 min, post run 3 min

Flow rate: 0.5 mL/min

Injection: 1 μL

Detector: UV 280 nm

Standards: 1. p-Phenylenediamine	13. 1,4-Diamino-2-nitrobenzene
2. 2-Amino-3-hydroxypyridine	14. 5-Amino-o-cresol
3. m-Phenylenediamine	15. 2-Methylresorcinol
4. 4-Aminophenol	16. 6-Amino-m-cresol
5. 2,5-Diaminotoluene sulfate	17. 4-Nitro-o-phenylenediamine
6. o-Phenylenediamine	18. 4-Amino-3-nitrophenol
7. 3-Aminophenol	19. 6-Hydroxyindole
8. Hydroquinone	20. 4-Chlororesorcinol
9. 2-Chloro-1,4-phenylenediamine sulfate	21. 2,7-Dihydroxynaphthalene
10. 4-Methylaminophenol sulfate	22. 1,5-Dihydroxy naphthalene
11. Resorcinol	23. 4-Aminodiphenylamine
12. 3,4-Diaminotoluene	24. 1-Naphthol

The above LC method could be easily transferred to an LC/MS/MS method because the mobile phase using ammonium acetate is MS friendly. While isomers, such as m-Aminophenol and o-Aminophenol, could not be resolved with LC, these could be identified by LC/MS/MS due to the different product ions. The same for the isomer pairs of hydroquinone and resorcinol. Multiple Reaction Monitor (MRM) chromatograms of respective 29 hair dye components are shown in Figure 3, and the optimized conditions for MRM are listed in Table 2.

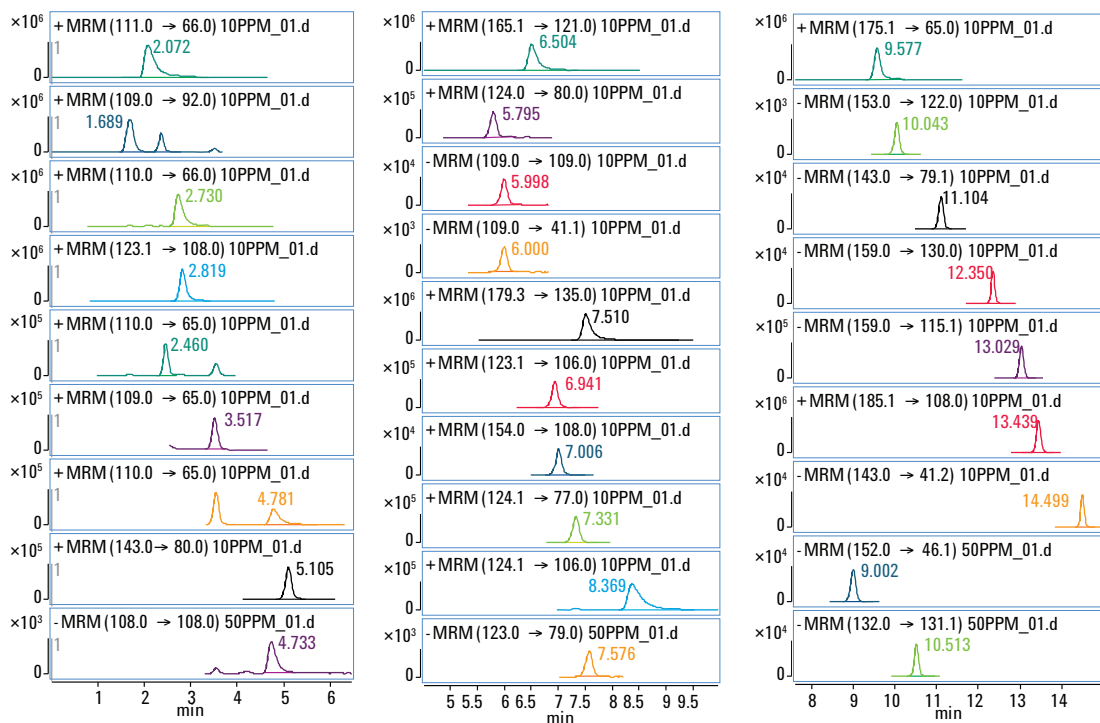


Figure 3. MRM chromatograms of respective 29 hair dye components using an Agilent Poroshell 120 Bonus-RP, 2.7-μm column.

Table 2. MRM conditions for the 29 components analyzed.

No.	Name	Product Ions	Retention time (min)	Polarity
1	p-Phenylenediamine	109 → 92 (12 V); 109 → 65 (22 V)	1.706	Positive
2	2-Amino-3-hydroxypyridine	111 → 66 (25 V); 111 → 94 (25 V)	2.069	Positive
3	m-Phenylenediamine	109 → 92 (12 V); 109 → 65 (22 V)	2.362	Positive
4	p-Aminophenol	110 → 65 (20 V); 110 → 93 (13 V)	2.465	Positive
5	2,6-Diaminopyridine	110 → 93 (21 V); 110 → 66 (23 V)	2.748	Positive
6	2,5-Diaminotoluene sulfate	123.1 → 108 (15 V); 123.1 → 77 (22 V)	2.813	Positive
7	o-Phenylenediamine	109 → 92 (12 V); 109 → 65 (23 V)	3.511	Positive
8	m-Aminophenol	110 → 65 (20 V); 110 → 93 (13 V)	4.731	Positive
9	o-Aminophenol	108 → 108 (0 V); 108 → 107 (22 V)	4.733	Negative
10	2-Chloro-1,4-phenylenediamine sulfate	143 → 108 (16 V); 143 → 80 (25 V)	5.107	Positive
11	p-Methylaminophenol sulfate	124 → 109 (14 V); 124 → 80 (35 V)	5.792	Positive
12	Hydroquinone	109 → 109 (0 V); 109 → 108 (15 V)	6.008	Negative
13	Resorcine	109 → 65 (17 V); 109 → 41.1 (23 V)	6.010	Negative
14	N,N-Diethyl-1,4-benzenediamine sulfate	165.1 → 136 (11 V); 165.1 → 121 (20 V)	6.456	Positive
15	3,4-Diaminotoluene	123.1 → 106 (12 V); 123.1 → 79 (20 V)	6.947	Positive
16	1,4-Diamino-2-nitrobenzene	154 → 108 (16 V); 154 → 91 (20 V)	7.012	Positive
17	5-Amino-o-cresol	124.1 → 77 (19 V); 124.1 → 109 (16 V)	7.329	Positive
18	4-(N,N-Diethyl)-2-methyl-p-phenylenediamine monohydrochloride	179.3 → 150 (11 V); 179.3 → 135 (22 V)	7.477	Positive
19	2-Methylresorcinol	123 → 79 (12 V); 123 → 55 (18 V)	7.573	Negative
20	6-Amino-m-cresol	124.1 → 106 (12 V); 124.1 → 79 (16 V)	8.271	Positive
21	4-Nitro-o-phenylenediamine	152 → 46.1 (23 V); 152 → 105 (14 V)	9.008	Negative
22	5-Methyl-2-phenyl-1,2-dihydropyrazol-3-one	175.1 → 65 (25 V); 175.1 → 133 (20 V)	9.581	Positive
23	4-Amino-3-nitrophenol	153 → 122 (11 V); 153 → 123.1 (19 V)	10.042	Negative
24	6-Hydroxyindole	132 → 131.1 (20 V); 132 → 104.1 (15 V)	10.513	Negative
25	4-Chlororesorcinol	143 → 107 (12 V); 143 → 79.1 (17 V)	11.105	Negative
26	2,7-Dihydroxynaphthalene	159 → 130 (30 V); 159 → 102 (30 V)	12.359	Negative
27	1,5-Dihydroxy naphthalene	159 → 115.1 (22 V); 159 → 131.1 (22 V)	13.025	Negative
28	4-Aminodiphenylamine	185.1 → 108 (18 V); 185.1 → 93 (22 V)	13.425	Positive
29	1-Naphthol	143 → 115.1 (45 V); 143 → 41.2 (45 V)	14.499	Negative

LC conditions

Instrument:	Agilent 1290 Infinity LC System (installed with 1290 inline filter after injector valve, p/n: 5067-4638)	
Column:	Agilent Poroshell 120 Bonus-RP, 3.0 × 100 mm, 2.7 μm (p/n 695968-301)	
Mobile phase:	A, 10 mM acetate; B, ACN	
Gradient:	0 min	0% B
	2 min	0% B
	8 min	20% B
	15 min	70% B
	18 min	80% B
Stop time:	20 min, post run 3 min	
Flow rate:	0.5 mL/min	
Injection:	1 μL	

MS conditions

Gas temp:	325 °C
Gas flow:	10 L/min
Nebulizer:	50 psi
Sheath gas temp:	350 °C
Sheath gas flow:	11 L/min
Capillary:	positive 4,000 v; negative 3,500 v

Conclusion

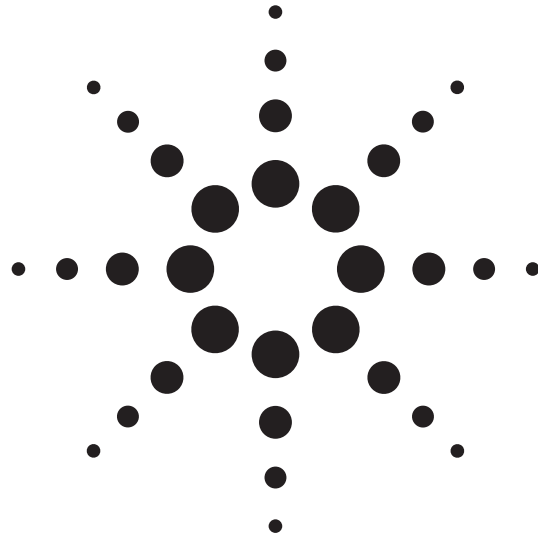
The method was developed for the separation of hair dye components using the Poroshell 120 Bonus-RP column. The column gives a good selectivity for these compounds and provides good resolutions. The method developed on 1290 Infinity LC System is suitable for fast screening and quantitative analysis of these compounds. The LC/MS/MS method enables co-eluted compounds be identified and quantified using MRM and is suitable for low level concentration analysis in complex sample matrices.

Reference

1. "Determination of 22 components in hair dyes by high performance liquid chromatography", *Chinese Journal of Chromatography*, 26(5): 554-558.
2. Anne E. Mack, "Fast screening methods for steroids by HPLC with Agilent Poroshell 120 columns", Application Note, Agilent Technologies, Inc, Publication Number 5991-0245EN.
3. Rongjie Fu, "Fast Analysis of Hair Dyes Using an Agilent Poroshell 120 EC-C18 Column", Application Note, Agilent Technologies, Inc, Publication Number 5990-6501EN.

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环境分析

EPA 8330A Analysis of Explosives Using Agilent Poroshell 120 EC-CN and EC-C18 Columns

Author

Anne Mack
Agilent Technologies, Inc.

Abstract

A group of 14 nitroaromatics and nitramines was analyzed according to EPA Method 8330A for the trace analysis of explosive residues by HPLC/UV. The analysis was improved by using superficially porous Agilent Poroshell 120 columns as compared to the traditional 5 μm columns suggested by the United States Environmental Protection Agency (EPA). Two complimentary phases were used; Poroshell 120 EC-CN and Poroshell 120 EC-C18. As recommended by the EPA, the Poroshell 120 EC-CN column was a good confirmation column when paired with a Poroshell 120 EC-C18 column due to its orthogonal selectivity.

Introduction

There is significant interest in transferring LC methods to superficially porous particles from larger 5 μm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2 μm totally porous particles. This is attributed primarily to a shorter mass transfer distance and a narrower particle size distribution. Furthermore, the larger particle size results in lower backpressure, allowing for these columns to be implemented in methods on nearly any LC system. The benefits of transferring from larger particle columns are very significant time and cost savings, because superficially porous particles are optimally run at faster flow rates and achieve similar resolution with a much shorter column length [1,2].

In this application note, a method from the EPA for the trace analysis of explosive residues by HPLC/UV was transferred from 5 μm columns to shorter 2.7 μm superficially porous Poroshell 120 columns.

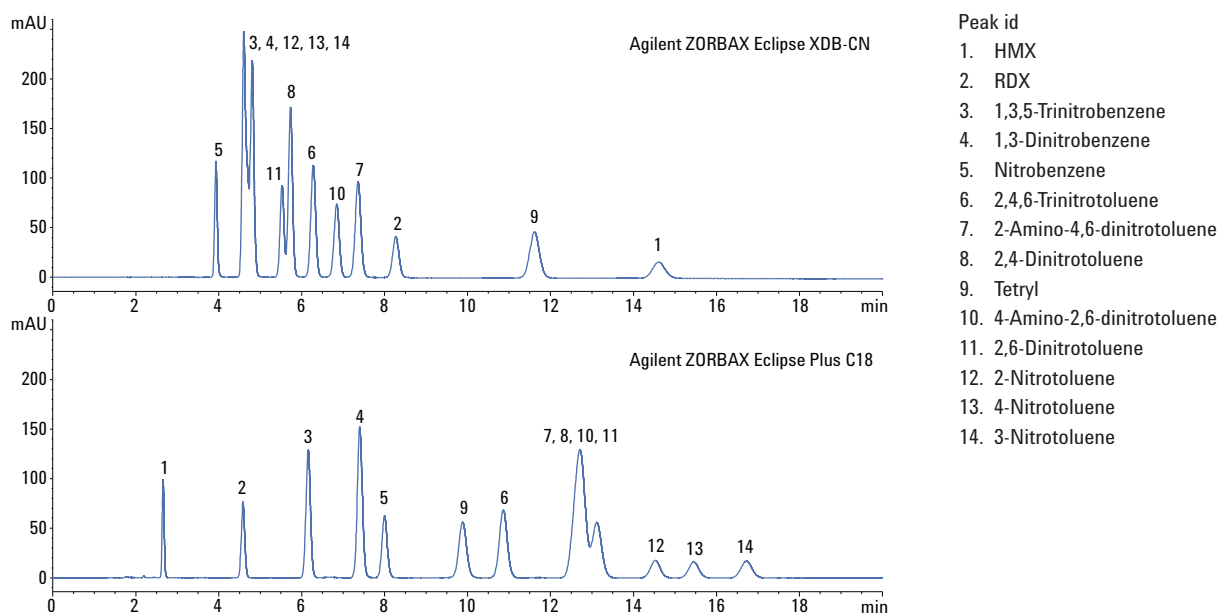
Materials and Methods

The instrument setup was optimized for lowest possible extra-column volume with short 0.075 mm id capillaries found in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [3].

The EPA 8330A stock standard was purchased from Cerilliant Corp. The 14 explosive residues were obtained in an acetonitrile solution at a concentration of 200 µg/mL each. Methanol was purchased from Honeywell International Inc. Water was 18 Mohm.cm Milli-Q.

Results and Discussion

Figure 1 shows EPA Method 8330A for the trace analysis of explosive residues by HPLC/UV, performed as specified by the EPA with 4.6 × 250 mm, 5 µm columns. Two phases were used, Agilent ZORBAX Eclipse XDB-CN, and Agilent ZORBAX Eclipse Plus C18 for confirmatory analysis. Figure 1 shows the two phases had orthogonal selectivity. The nitrotoluene isomers coeluted on the CN column and were separated on the C18 column, while the dinitrotoluene compounds coeluted on the C18 and were separated on the CN column.



Conditions

Columns: Agilent ZORBAX Eclipse XDB-CN, 4.6 × 250 mm, 5 µm (p/n 990967-905)
 Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902)

Samples: Explosive residues

Sample concentration: 20 µg/mL of each compound

Eluent: A, H₂O; B, CH₃OH

Injection volume: 20 µL

Flow rate: 1 mL/min

Isocratic: 50% B

Temperature: 25 °C

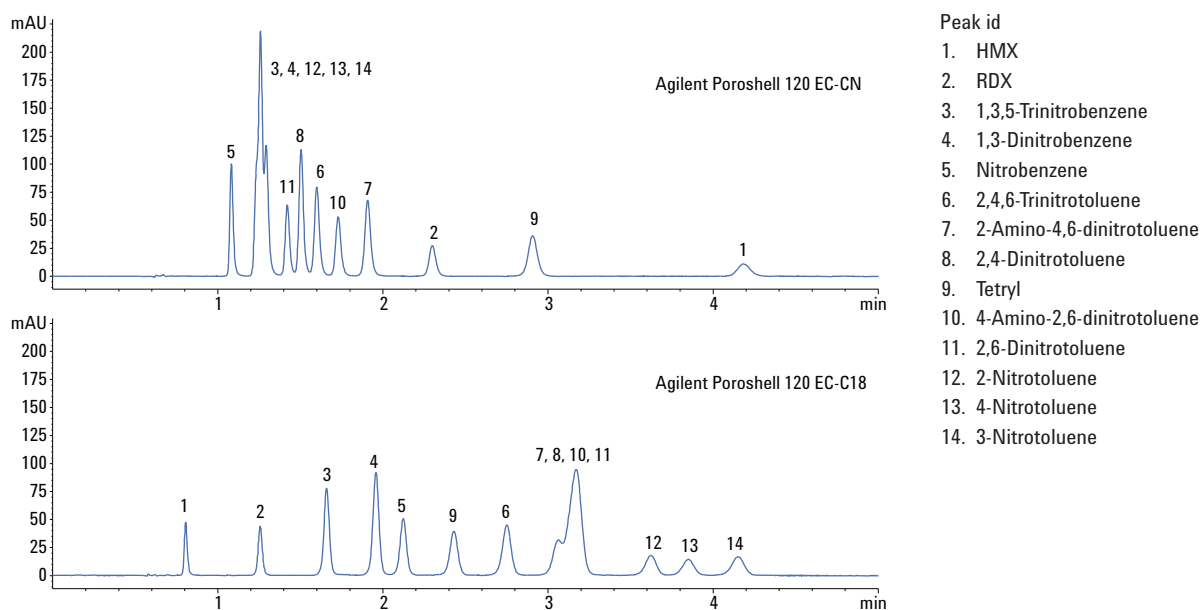
Detector: 254 nm

Instrument: Agilent 1290 Infinity LC

Figure 1. EPA 8330A analysis of explosives using EPA method parameters (isocratic) and Agilent ZORBAX Eclipse columns (5 µm).

Figure 2 shows that the 4.6×250 mm, $5 \mu\text{m}$ analyses can be transferred to 2.1×100 mm, $2.7 \mu\text{m}$ Poroshell 120 columns. Poroshell 120 EC-CN columns have very similar selectivity to ZORBAX Eclipse XDB-CN columns, while Poroshell 120 EC-C18 columns have similar selectivity to ZORBAX Eclipse Plus C18 columns. This allows for simplified method transfer. The flow rate (0.4 mL/min) and injection volume ($1 \mu\text{L}$) were

scaled proportionally to the difference in column volume, while all other method parameters remained the same. The result was nearly the same but accomplished in a fraction of the time, saving significant time and solvent compared to the original analysis. This provided increased productivity as well as substantial cost savings.



Conditions

Columns: Agilent Poroshell 120 EC-CN, 2.1×100 mm, $2.7 \mu\text{m}$ (p/n 695775-905)
 Agilent Poroshell 120 EC-C18, 2.1×100 mm, $2.7 \mu\text{m}$ (p/n 695775-902)

Samples: Explosive residues

Sample concentration: $20 \mu\text{g/mL}$ of each compound

Eluent: A, H_2O ; B, CH_3OH

Injection volume: $1 \mu\text{L}$

Flow rate: 0.4 mL/min

Isocratic: 50% B

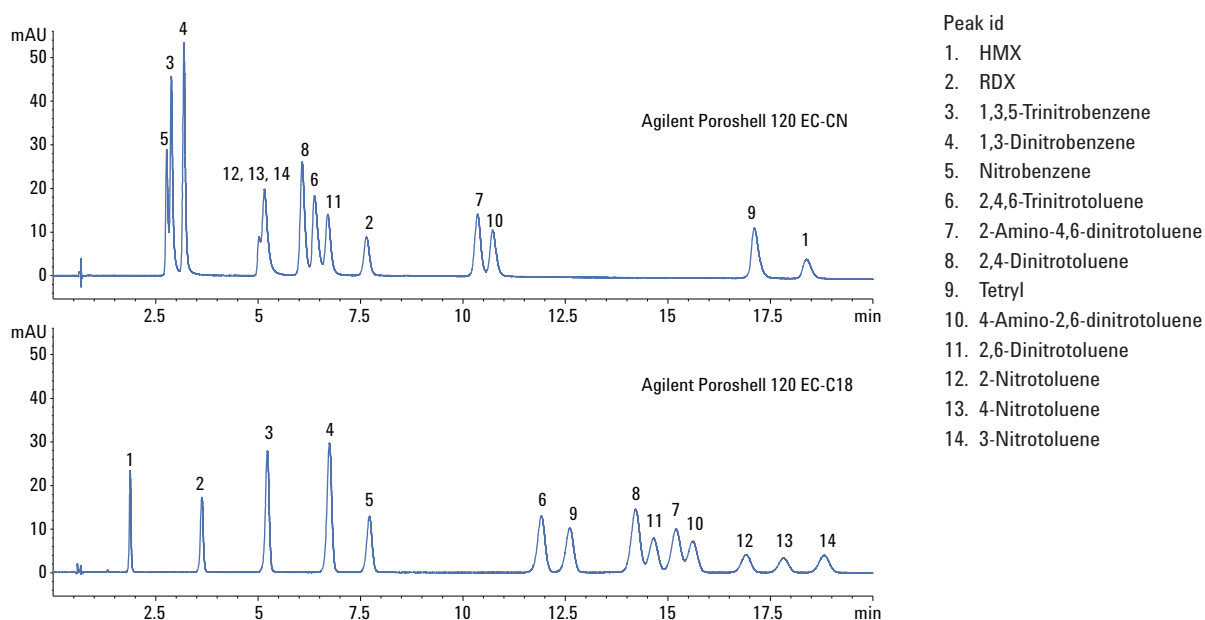
Temperature: $25 \text{ }^\circ\text{C}$

Detector: 254 nm

Instrument: Agilent 1290 Infinity LC

Figure 2. EPA 8330A analysis of explosives using EPA method parameters (isocratic) and Agilent Poroshell 120 superficially porous columns.

Figure 3 shows the two Poroshell 120 columns with independently optimized gradient analyses; Poroshell 120 EC-CN (7% B for 7 minutes, 7-35% B in 13 minutes) and Poroshell 120 EC-C18 (25-36% B in 20 minutes). As can be seen, full resolution of the 14 nitroaromatics and nitramines was difficult, even with an optimized separation. This supports the need for orthogonal columns for confirmatory analyses.



Conditions

Columns: Agilent Poroshell 120 EC-CN, 2.1 × 100 mm, 2.7 μm (p/n 695775-905)
 Agilent Poroshell 120 EC-C18, 2.1 × 100 mm, 2.7 μm (p/n 695775-902)

Samples: Explosive residues

Sample concentration: 20 μg/mL of each compound

Eluent: A, H₂O; B, CH₃OH

Injection volume: 1 μL (ADVR on)

Flow rate: 0.4 mL/min

Gradient: Poroshell 120 EC-CN, 7% B for 7 min, 7 to 35% B in 13 min; Poroshell 120 EC-C18, 25 to 36% B in 20 min

Temperature: Agilent Poroshell 120 EC-CN, 20 °C; Agilent Poroshell 120 EC-C18, 25 °C

Detector: 254 nm

Instrument: Agilent 1290 Infinity LC

Figure 3. EPA 8330A analysis of explosives using gradient elution with Agilent Poroshell 120 superficially porous columns; injection performed with Automatic Delay Volume Reduction.

Conclusions

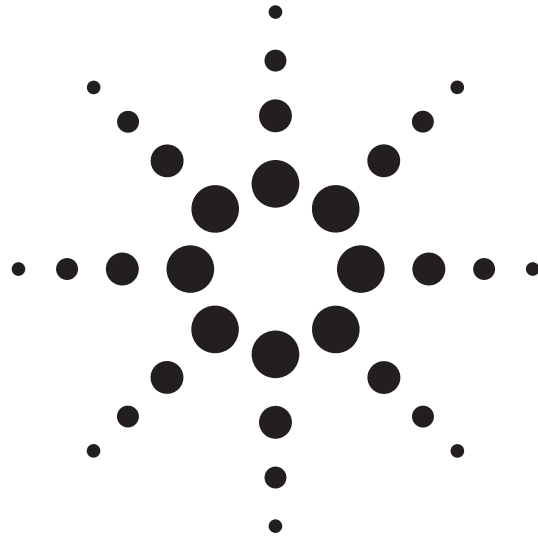
Superficially porous Agilent Poroshell 120 columns were successfully substituted for traditional 5 μm columns for EPA Method 8330A for the trace analysis of explosive residues by HPLC/UV. The smaller dimension Poroshell 120 columns could be used to improve productivity and to save time and money over larger 5 μm columns. Additionally, it is found that the two Poroshell 120 phases (EC-CN and EC-C18) provided orthogonal selectivity, allowing for easy confirmatory analyses.

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生物分析

将中国药典测定胰岛素的方法转换为使用 Agilent Poroshell 120 色谱柱的方法

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摘要

中国药典(Ch. P)规定的检验胰岛素的方法需使用传统的 4.6×150 mm 或 4.6×250 mm, $5 \mu\text{m}$ 的 C18 液相色谱柱。这两种柱长的传统 $5 \mu\text{m}$ 填料色谱柱的胰岛素峰柱效都很低。本应用报告采用 Agilent Poroshell 120 EC-C18 色谱柱或 Agilent Poroshell 120 SB-C18 色谱柱取代传统的色谱柱进行胰岛素分析。采用 Poroshell 120 色谱柱后调整的方法显著提高了柱效和分离度，并且仍满足 Ch. P 或美国药典(USP)规定方法的要求。

引言

胰岛素是由 51 个氨基酸组成的一种肽类激素，分子量为 5808 道尔顿。它产生于胰腺中的朗格汉斯岛（胰岛）。不同动物之间胰岛素的结构略有差异。由于碳水化合物代谢控制作用不同，导致动物胰岛素的“效力”与人类胰岛素不同。牛胰岛素与人胰岛素只有三个氨基酸残基不同，猪胰岛素与人胰岛素最接近。胰岛素已广泛用于 1 型和某些 2 型糖尿病的治疗。Ch. P [1]和 USP [2]规定了长耗时的等度洗脱方法进行胰岛素的含量测定及长耗时的梯度洗脱方法进行胰岛素的有关物质检查。这两种方法的含量测定采用了相同的 HPLC 条件，但用于有关物质分析的梯度洗脱方法略有不同。

本应用报告首先按照 Ch. P 规定的 HPLC 方法条件，采用 Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 μm 色谱柱或 Agilent ZORBAX Eclipse Plus C18, 4.6 × 150, 5 μm 色谱柱测定胰岛素的含量和有关物质。然后将方法转移至表面多孔颗粒的 Agilent Poroshell 120 色谱柱上，该色谱柱可达到与用于快速分离的亚 2 μm 色谱柱相似的柱效。

材料与方法

Ch. P 中测定猪胰岛素有关物质和含量的 HPLC 条件：

色谱柱	十八烷基硅烷键合硅胶 (C18)
流速	1.0 mL/min
进样量	20 μL
柱温	40 °C
检测波长	214 nm

用于有关物质检查的流动相：

流动相 A	0.2 mol/L 硫酸盐缓冲液（将 28.4 g 无水硫酸钠溶于 1000 mL 水中，加入 2.7 mL 磷酸，并用乙醇胺调 pH 至 2.3，混匀）-乙腈 (82:18)
流动相 B	乙腈：水(50:50)

参考以下梯度洗脱程序，调整流动相的组成和等度洗脱区间，以得到大约为 25 min 的胰岛素保留时间，A-21 脱酰胺胰岛素则刚好在梯度洗脱开始之前出峰。

时间 (min)	%B
0	22
35	22
61	67
67	67

用于含量测定的流动相：

流动相 A	0.2 mol/L 硫酸盐缓冲液（将 28.4 g 无水硫酸钠溶于 1000 mL 水中，加入 2.7 mL 磷酸，并用乙醇胺调节 pH 至 2.3，混匀）
流动相 B	乙腈：水(74:26)

本文所用材料

样品	猪胰岛素（由中国食品药品检定研究院提供）
色谱柱	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 μm (部件号 883975-902)
	Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm (部件号 959993-902)
	Agilent Poroshell 120 SB-C18, 4.6 × 100 mm, 2.7 μm (部件号 685975-902)
	Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 μm (部件号 695975-302)
色谱系统	Agilent 1200 SL LC 系统，配备二元泵、柱温箱(TCC)、高效自动进样器和二极管阵列检测器(DAD)

结果与讨论

传统的色谱柱

Ch. P 规定的色谱柱填料为十八烷基硅烷键合硅胶(C18)，该填料也属于 USP L1 填料。Ch. P 方法通常使用传统的 5 μm 填料色谱柱，但在结果满足要求的情况下，也可使用更小粒径填料的色谱柱。因此，首先采用 Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 μm 色谱柱运行该方法，根据 Ch. P 方法的要求，调整流动相的组成以使胰岛素的保留时间大约为 25 min。有关物质和含量测定的色谱图分别见图 1 和图 2。

胰岛素分析的系统适用性实验要求胰岛素峰和 A-21 脱酰胺胰岛素峰的分度度不小于 1.8，且胰岛素峰的拖尾因子不大于 1.8。如图 1 和图 2 所示，这两种传统的色谱柱均满足 Ch. P 中系统适用性实验的要求。与 SB-C18 色谱柱相比，Agilent ZORBAX Eclipse Plus C18 色谱柱展现了更为对称的色谱峰及更高的柱效，这可能是由于两种色谱柱之间的两点差异造成的。第一个差异是 Eclipse Plus C18 色谱柱完全封端，而 SB-C18 色谱柱未封端。封端的色谱柱通常使碱性化合物获得更好的峰形。第二个差异是孔径不同（Eclipse Plus C18 颗粒孔径为 95 Å，SB-C18 颗粒孔径为 80 Å）。较大孔径的 Eclipse Plus C18 色谱柱有利于扩散，进而提高柱效并改善峰形。

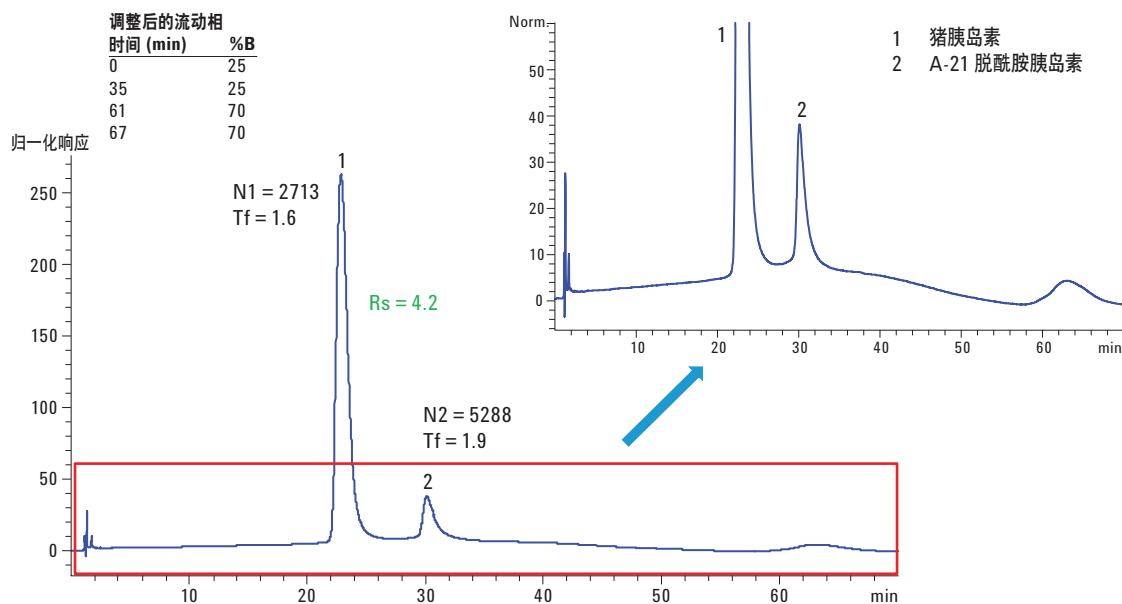


图 1. 采用传统的 Agilent ZORBAX SB-C18 色谱柱 (4.6 × 150 mm, 5 μm) 进行有关物质分析的色谱图

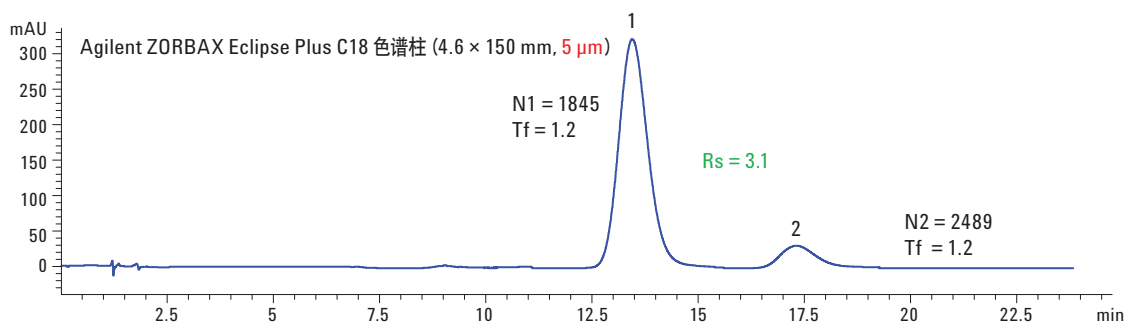
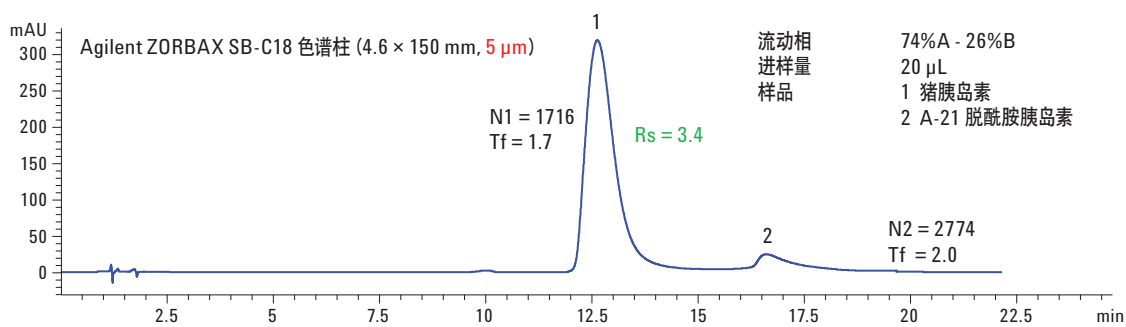


图 2. 采用传统的 Agilent ZORBAX SB-C18 色谱柱 (4.6 × 150 mm, 5 μm) 和 Agilent ZORBAX Eclipse Plus C18 色谱柱 (4.6 × 150 mm, 5 μm) 进行含量测定的色谱图

Agilent Poroshell 120 色谱柱

本研究通过采用 Agilent Poroshell 120 SB-C18 色谱柱 (4.6 × 100 mm, 2.7 μm) 来实现胰岛素中有关物质分析方法的转换。对流动相组成稍作调整以满足 Ch. P 的相关要求。当将原方法调整成为一个新方法时必须重新计算相关的梯度洗脱时间和进样量。

为保证分离度和整体分离效果, 应保持方程 1 中组分的保留指数 K^* 恒定。

$$\text{方程 1: } K^* = (t_r F) / (S \Delta\Phi V_m)$$

其中:

t_r 表示洗脱时间

F 表示流速

S 是一个常数

V_m 表示柱体积 ($V_m = \pi (d/2)^2 L (0.6)$, L 表示柱长, d 表示柱内径)

$\Delta\Phi$ $\Delta\Phi$ 表示梯度程序中有机相改变的百分比

根据方程 1, 在柱内径和柱长改变时需相应改变流速和梯度洗脱时间。

为保证色谱峰响应与原方法一致, 进样量需按比例随柱体积调整 (方程 2)。

$$\text{方程 2: } (d_1/2)^2 L_1 = (d_2/2)^2 L_2$$

其中:

d_1, d_2 分别代表柱 1、柱 2 的柱内径

L_1, L_2 分别代表柱 1、柱 2 的柱长

采用 Agilent Poroshell 120 SB-C18 色谱柱 (4.6 × 100 mm, 2.7 μm) 运行一个新的梯度洗脱方法, 用于分离分析胰岛素中的有关物质。色谱图及相关数据见图 3。采用 Poroshell 120 SB-C18 色谱柱 (4.6 × 100 mm, 2.7 μm) 和 Poroshell 120 EC-C18 色谱柱 (4.6 × 100 mm, 2.7 μm) 运行含量测定方法。采用 Poroshell 120 色谱柱获得的色谱峰与传统的 SB-C18 色谱柱获得的色谱峰 (图 1) 相比, 在许多方面得到了改善, 诸如峰型、柱效和分离度。Poroshell 120 色谱柱的柱效比传统 5 μm 色谱柱高 4 到 6 倍。采用 Poroshell 120 色谱柱分析检测到一些微小的杂质峰 (图 3), 这是由于采用了表面多孔的色谱柱增加了柱效、提高了分析灵敏度、改善了峰型和分离度。当然, 这些杂质可能也存在于用传统 5 μm 色谱柱分离的过程中, 但其低柱效的宽峰降低了分离度, 以至于很难检测到这些杂质。

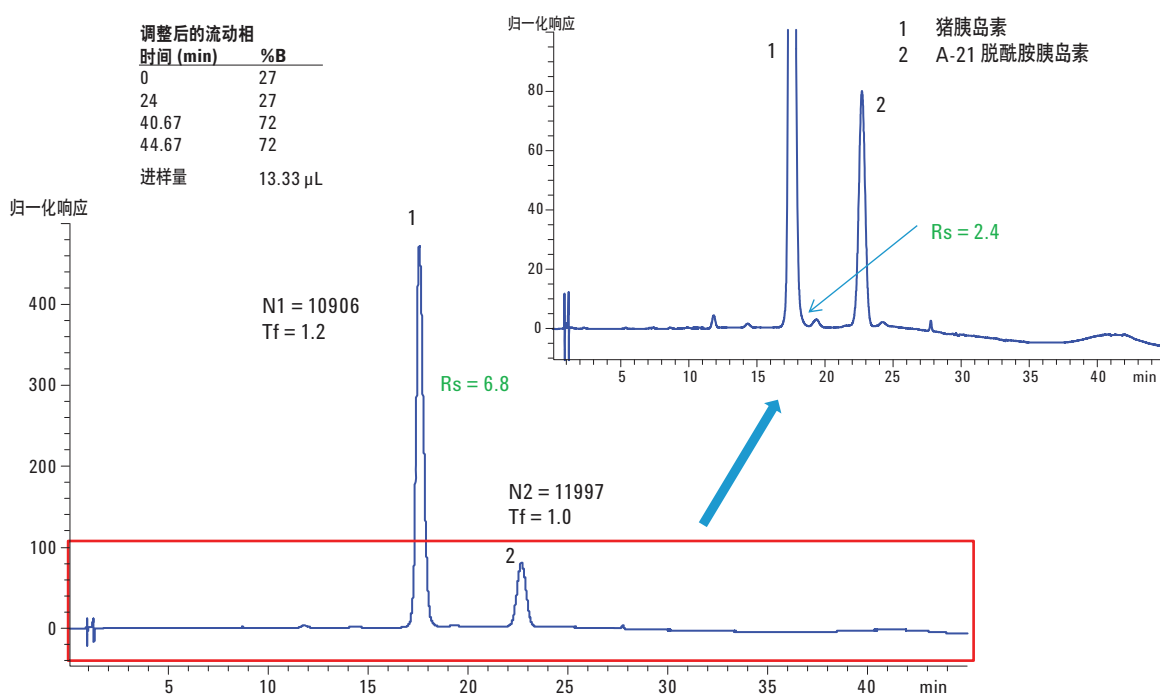


图 3. 采用 Agilent Poroshell 120 SB-C18 色谱柱 (4.6 × 100 mm, 2.7 μm) 分析胰岛素中有关物质的色谱图

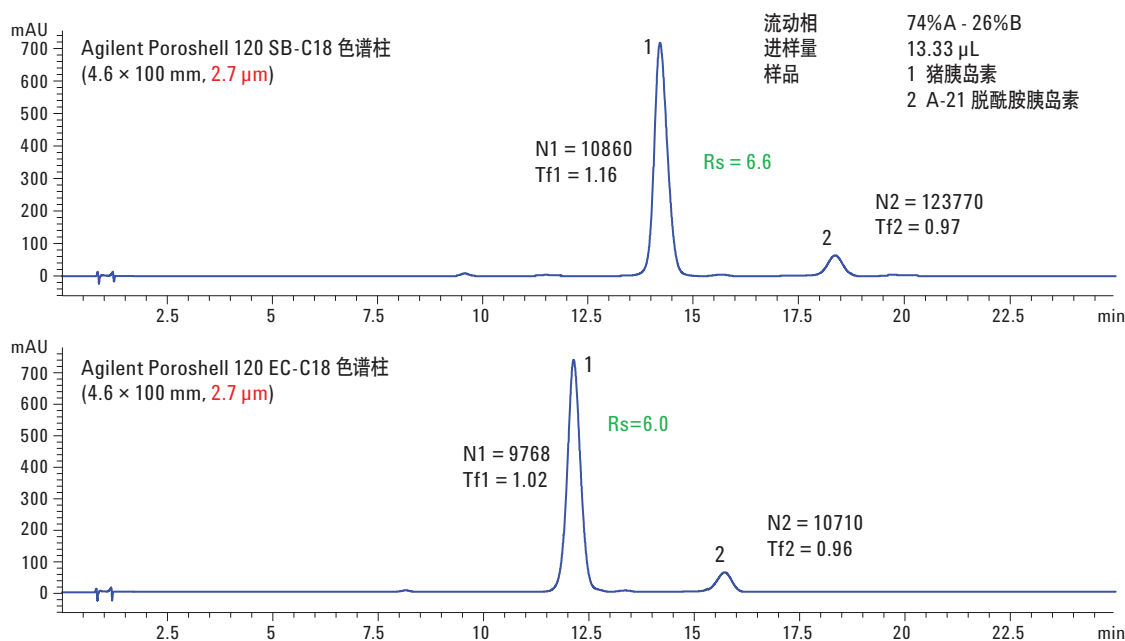


图 4. 采用 Agilent Poroshell 120 SB-C18 色谱柱 (4.6 × 100 mm, 2.7 µm) 和 Poroshell EC-C18 色谱柱 (4.6 × 100 mm, 2.7 µm) 分析胰岛素的色谱图

采用 Agilent Poroshell 120 SB-C18 和 EC-C18 色谱柱分析胰岛素能获得良好的分离性能 (图 4)。它们可轻易满足系统适用性实验的要求。表面多孔的 Poroshell 120 色谱柱所具有的小粒径 (2.7 µm) 和大孔径 (120 Å) 的特点, 使其分离性能得到极大提升。关于 Poroshell 120 系列色谱柱填料孔径、粒径和分子量之间关系的更多信息, 见应用报告 5990-9028CHCN。

进样的重现性对于可靠的分析结果十分重要。Ch. P 要求连续 5 次重复进样的相对标准偏差 (RSD) 小于 2%。这是大多数 LC 方法的一个典型要求。采用 Poroshell 120 色谱柱 (图 5) 分析猪胰岛素和 A-21 脱酰胺胰岛素, 连续重复进样 5 次, 其峰面积的 RSD 值分别为 0.2% 和 0.4%, 轻松满足该项要求。

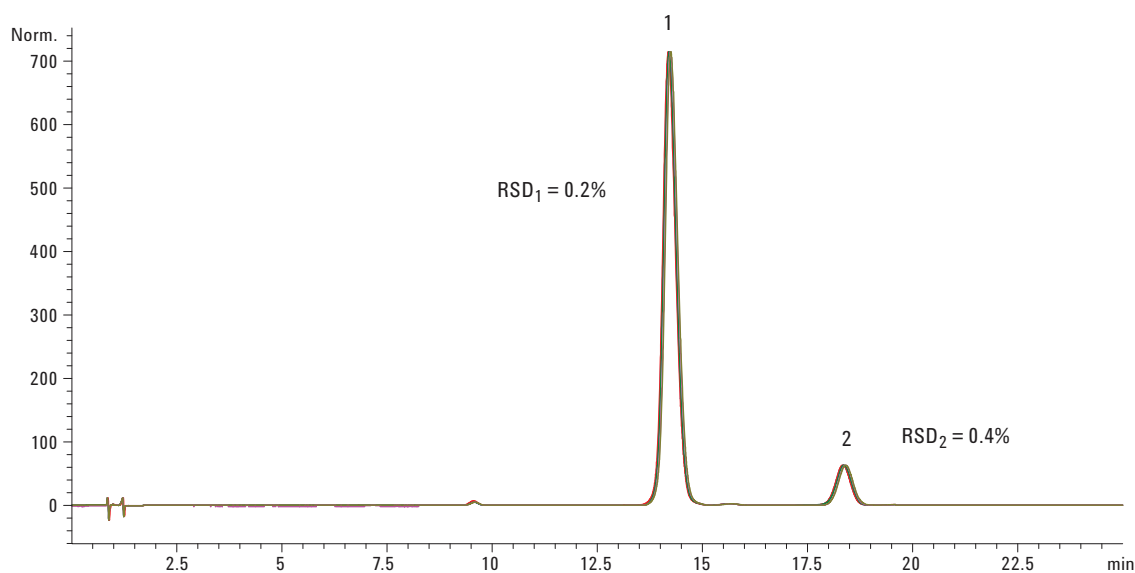


图 5. 采用 Agilent Poroshell 120 SB-C18 色谱柱 (4.6 × 100 mm, 2.7 µm) 连续进样 5 次获得的叠加色谱图

结论

胰岛素分析方法从传统的 5 μm 色谱柱上成功转换到表面多孔的 Agilent Poroshell 120 色谱柱上, 分析性能得到显著提升。粒径为 2.7 μm , 孔径为 120 \AA 的 Poroshell 填料色谱柱能高效地分析小分子蛋白, 如胰岛素, 且容易满足 Ch. P 胰岛素分析的系统适用性实验要求。采用 Poroshell 120 色谱柱的新方法非常适合用于胰岛素的质量控制分析。

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2. 美国药典 USP 31 (第 2 卷), 胰岛素, 2403–2404。

使用 Agilent Poroshell 120 色谱柱最大限度地提高柱效

应用串联色谱柱技术，5 min 内理论板数可达 100000

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摘要

基于表面多孔技术的色谱柱可替代亚 2 μm 颗粒色谱柱。配备这些色谱柱的 Agilent 1290 Infinity LC 系统可实现高效分离。Agilent Poroshell 120 色谱柱可提供：

- 较低的反压
- 最高的柱效
- 可观的柱容量

前言

近年来，亚 2 μm 填料颗粒色谱柱由于其柱效高而备受关注。它可在比范第姆特方程估算的更高流速下运行。与最佳流速下的柱效相比，较高的流速引起的柱效损失可以忽略。亚 2 μm 填料颗粒色谱柱减少了运行及循环时间，可更快地获得分析结果。

这类色谱柱的缺点是由于粒径小，会显著地引起较高反压。在许多情况下，尤其对于亚 2 μm 长柱，液相色谱仪必须能够承受 400 bar 以上的反压。

表面多孔填料颗粒技术制备的色谱柱可显著降低反压，从而提供了另一种高分辨率分析方法 1。与亚 2 μm 颗粒填料色谱柱柱效相比，这些色谱柱柱效略低。但由于反压低，可通过串联色谱柱方式获得极高的塔板数。

本应用报告表明三根长的 Agilent Poroshell 120 色谱柱串联, 可获得极高的柱效。除特殊液相仪器外, 反压可以保持在 400 bar 以下。这种情况下, 较高的流速可节省分析及平衡时间。最后, 对 2.7 μm 多孔外壳色谱柱与亚 2 μm 颗粒色谱柱进行了比较。

实验部分

仪器

本实验选用 Agilent 1290 Infinity 液相色谱系统, 该系统配备二元泵、自动进样器、柱温箱及带有 10 mm 光程流通池的二极管阵列检测器。

使用 Agilent ZORBAX 快速高分离度高通量 4.6 mm \times 150 mm, 1.8 μm 色谱柱和 Agilent Poroshell 120, 4.6 mm \times 150 mm, 2.7 μm 色谱柱。耐压最高均可达到 600 bar。

使用 B.04.02 版 ChemStation 工作站。

结果和讨论

表面多孔填料色谱柱的潜在优势

表面多孔填料色谱柱技术是以填料颗粒的实心内核和表面多孔外壳为基础的。这种填料包括 1.7 μm 实心内核和 0.5 μm 多孔硅胶外壳。整个粒径大约为 2.7 μm 。2.7 μm 表面多孔填料可使背压降低 40–50%, 并提供亚 2 μm 全多孔填料柱效的 80–90%。与全多孔填料相比, 表面多孔填料有更窄的粒径分布, 可获得装填更均一的色谱柱, 从而减少组分在色谱柱中的扩散。与此同时, 小颗粒和多孔外壳的传质阻力更低, 从而在较高流速运行时无柱效损失^{1,2}。

系统配置

以下实验评估了 Agilent Poroshell 120 色谱柱的性能。所有色谱柱内径均为 4.6 mm, 柱长 150 mm。

- 计算单根色谱柱在 1.5 mL/min 运行时的塔板数
- 计算三根色谱柱串联在 1.5 mL/min 运行时的塔板数
- 计算三根色谱柱串联在更高流速运行时的塔板数

- 等度和梯度洗脱条件下保留时间的精确度
- 表面多孔色谱柱与亚 2 μm 颗粒色谱柱的比较

柱效 (塔板数) 通常在等度洗脱条件下测定。对于对称色谱峰运用下列公式计算理论塔板数(N):

$$N = 5.54 (RT/W)^2$$

其中:

RT: 保留时间

W: 半峰宽

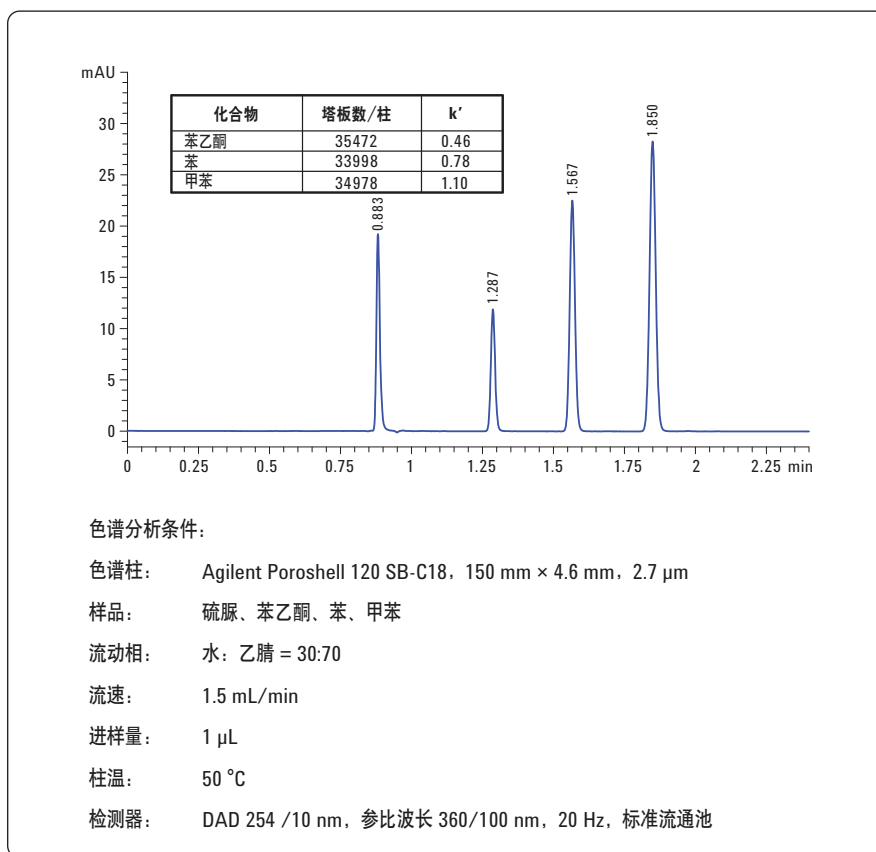


图 1
计算 Agilent Poroshell 120, 150 mm \times 4.6 mm 色谱柱塔板数的色谱图

计算单根色谱柱的塔板数

选用以下化合物评价单根色谱柱的塔板数：尿嘧啶、苯乙酮、苯和甲苯。

结果色谱图及计算得到的塔板数如图 1 所示。

特定色谱条件下甲苯的塔板数/柱约为 35000。

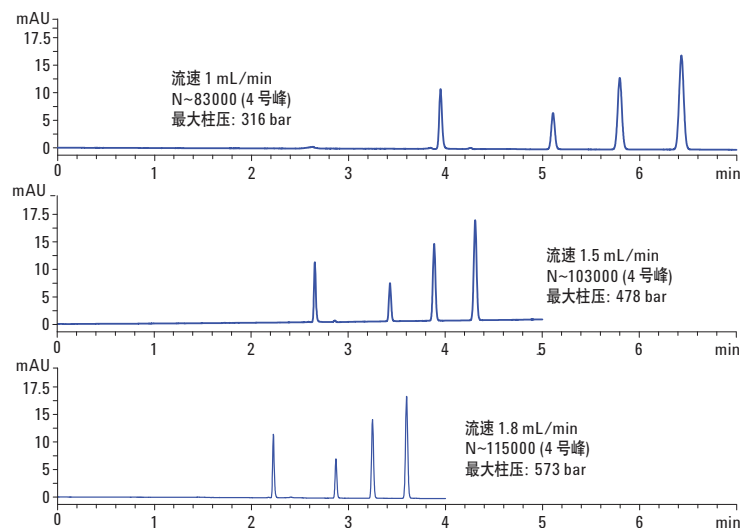
计算三根色谱柱串联的塔板数

单根色谱柱的塔板数约为 35000。三根色谱柱串联，塔板数预计可达 105000。采用 90 mm × 0.12 mm 不锈钢管串联色谱柱。计算了不同流速下的理论塔板数。

色谱图如图 2 所示。如果采用 400 bar 液相色谱系统，在 1 mL/min 流速下运行时，理论塔板数约达 80000。当然，该液相色谱系统耐压高达 1200 bar，因此采用该系统可实现更高的流速和柱效。

1.5 mL/min 流速下，理论塔板数约达 103000，接近预期值。

甲苯获得的最佳结果是 1.8 mL/min 流速下，理论塔板数约为 115000，保留时间 < 5 min。（表 1）。



色谱分析条件：

样品： 尿嘧啶、苯乙酮、苯、甲苯

色谱柱： 三根串联的 Agilent Poroshell 120 SB-C18, 150 mm × 4.6 mm, 2.7 μm 色谱柱

流动相： 水：乙腈 = 20:80

流速： 1、1.5、1.8 mL/min

进样量： 1 μL

柱温： 60 °C

检测器： DAD 254 /10 nm, 参比波长 360/100 nm, 20 Hz, 标准流通池

图 2
计算三根串联的 Agilent Poroshell 120, 150 mm × 4.6 mm 色谱柱在不同流速下塔板数的三张色谱图

化合物	塔板数	k'
苯乙酮	114120	0.29
苯	109931	0.46
甲苯	114800	0.62

表 1
1.8 mL/min 流速下的理论塔板数

在较高的 k' 下，三根色谱柱串联可获得良好的分析结果。流速为 1.2 mL/min。(图 3)

等度条件下保留时间的精密度

图 4 是等度条件 1.5 mL/min 流速下,连续六次进样评估精密度的叠加图,除尿嘧啶外,保留时间精密度 RSD < 0.034%,峰面积精密度 RSD < 0.66%。

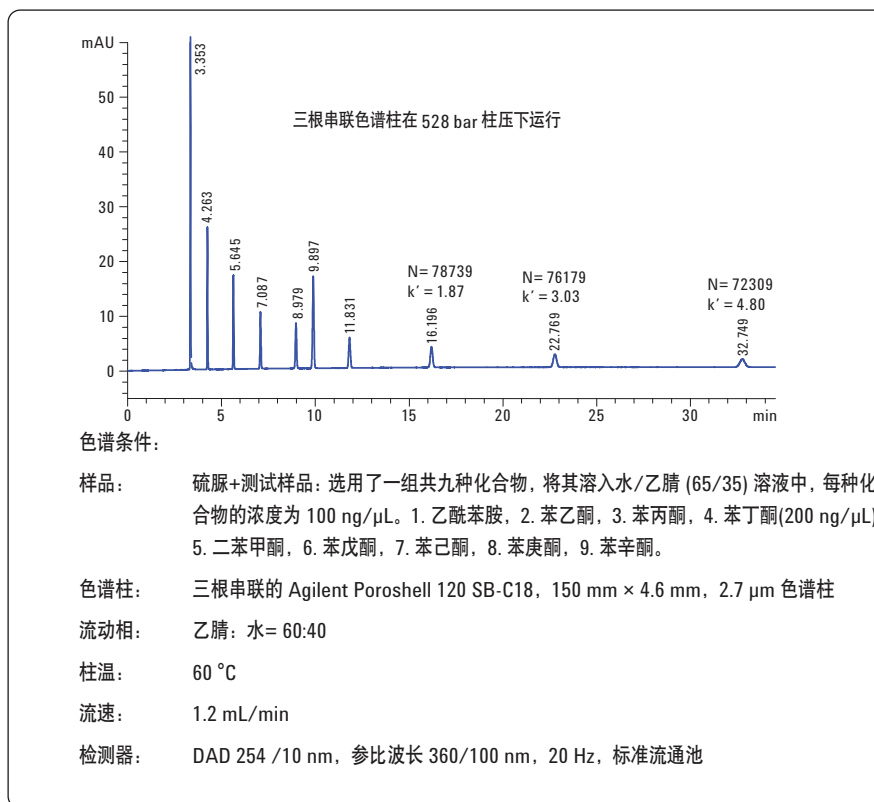


图 3

三根串联色谱柱在柱压 528 bar, 流速 1.2 mL/min, 高 k' 值下的理论塔板数

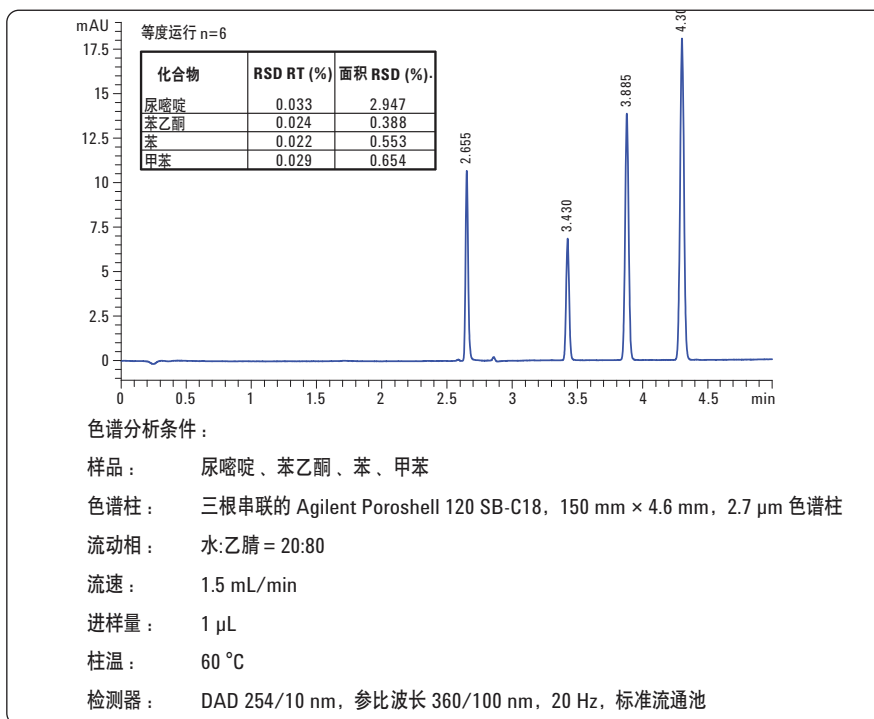


图 4

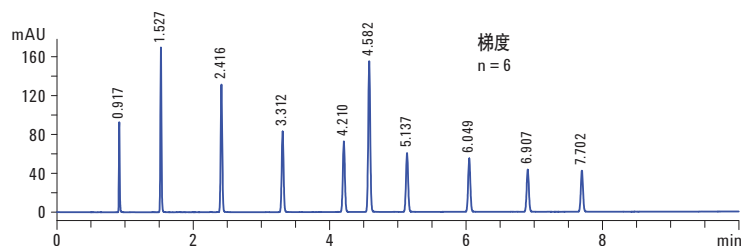
等度条件下连续六次进样组分保留时间和峰面积精密度的叠加色谱图

梯度条件下保留时间和峰面积的精密度

对在 10 min 内 35% 到 95% 梯度条件下分析的精密度进行评价。图 5 是连续 6 次进样评价精密度的叠加色谱图。

除硫脲外，所有化合物保留时间精密度良好(RSD < 0.04%) (图 5)。

所有化合物进样量 1 μ L，峰面积 RSD < 0.38%。



峰	保留时间 (%)	面积 RSD (%)
硫脲	0.092	0.372
1	0.020	0.238
2	0.038	0.255
3	0.033	0.211
4	0.029	0.186
5	0.027	0.227
6	0.023	0.194
7	0.018	0.183
8	0.017	0.251
9	0.017	0.167

样品： 硫脲+测试样品：选取了九种化合物，将其溶于水/乙腈(65/35)溶液中，每种化合物的浓度为 100 ng/ μ L。1. 乙酰苯胺，2. 苯乙酮，3. 苯丙酮，4. 苯丁酮 (200 ng/ μ L)，5. 二苯甲酮，6. 苯戊酮，7. 苯己酮，8. 苯庚酮，9. 苯辛酮。

色谱柱： Agilent Poroshell 120 SB-C18, 150 mm \times 4.6 mm, 2.7 μ m

流动相： 水和乙腈

梯度程序： 0 min 35% 乙腈，10 min 内升到 95% 乙腈

流速： 1.5 mL/min

进样量： 1 μ L

柱温： 60 $^{\circ}$ C

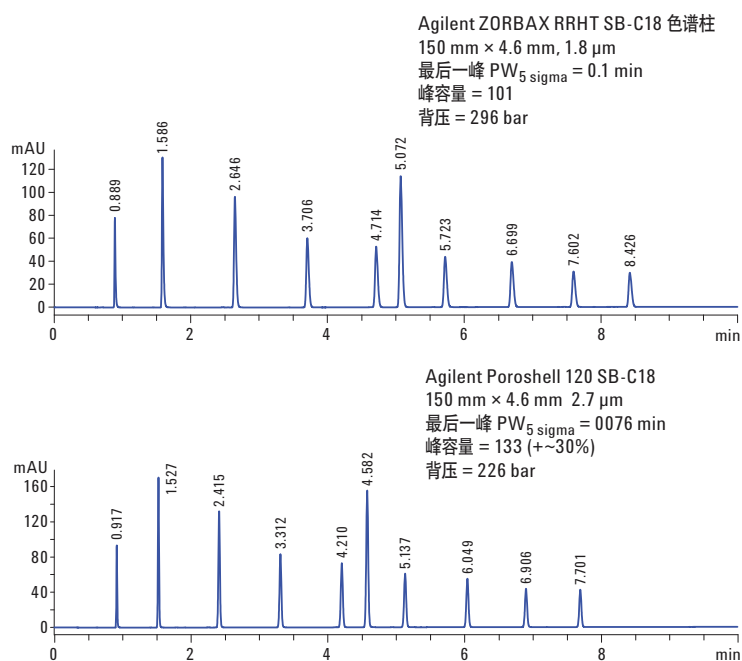
检测器： DAD 245 /10 nm，参比波长 400/100 nm，20 Hz，标准流通池

图 5. 连续 10 个梯度运行的保留时间和峰面积精密度的叠加色谱图

表面多孔色谱柱与亚 2 μm 填料颗粒色谱柱峰容量对比

为了说明表面多孔色谱柱与亚 2 μm 填料颗粒色谱柱的不同, 使用 150 mm \times 4.6 mm 内径的两种色谱柱对比分析了 10 种化合物的混合物 (图 6)。

Agilent Poroshell 120 色谱柱显示出更短的洗脱时间和更窄的峰宽, 因此多孔外壳色谱柱可获得更高的峰容量。Agilent Poroshell 120 色谱柱峰容量为 133 个峰, 亚 2 μm 色谱柱只有 101 个峰, 前者比后者峰容量高。与亚 2 μm 色谱柱相比, 相同条件下, Agilent Poroshell 120 色谱柱柱效高出 30%。



色谱分析条件:

样品: 硫脲+待测样品: 选用了九种化合物, 将其溶于水/乙腈(65/35)溶液中, 每种化合物的浓度为 100 ng/ μL 。1. 乙酰苯胺, 2. 苯乙酮, 3. 苯丙酮, 4. 苯丁酮 (200 ng/ μL), 5. 二苯甲酮, 6. 苯戊酮, 7. 苯己酮, 8. 苯庚酮, 9. 苯辛酮

色谱柱: Agilent ZORBAX RRHT SB-C18, 150 mm \times 4.6 mm, 1.8 μm ;
Agilent Poroshell 120 SB-C18, 150 mm \times 4.6 mm, 2.7 μm

流动相: 水和乙腈

梯度程序: 0 min 35% 乙腈, 10 min 内升到 95%

流速: 1.5 mL/min

进样量: 1 μL

柱温: 60 $^{\circ}\text{C}$

检测器: DAD 245 / 10 nm, 参比波长 400/100 nm, 20 Hz, 标准流通池

图 6

应用表面多孔色谱柱与亚 2 μm 颗粒色谱柱分析“苯酮类”混合物的色谱图

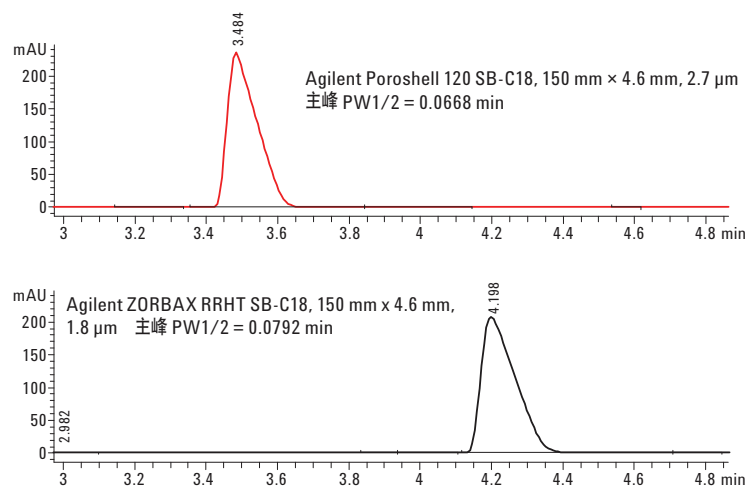
色谱柱体积容量比较

为了考察表面多孔色谱柱与 1.8 μm 填料颗粒色谱柱的体积容量是相同还是前者比后者低，采用了高浓度样品进样。进样量 10 μL ，含量大约为 20 μg (图 7)。

在选定条件下，主峰无显著差异。由于采用 Poroshell 120 色谱柱较早洗脱出峰，因此峰宽较小。

信噪比对比

采用分析药物中的杂质来评估信噪比。杂质含量在 0.02%–0.03% 范围内。色谱条件见图 7。



色谱分析条件

测试样品:	含杂质的 2.022 mg/mL 曲马多
色谱柱:	Agilent Poroshell 120 SB-C18, 150 mm x 4.6 mm, 2.7 μm Agilent ZORBAX RRHT SB-C18, 150 mm x 4.6 mm, 1.8 μm
泵:	
流动相 A:	水 + 0.2% TFA, 流动相 B: ACN + 0.16% TFA
梯度程序:	5 min 内 B 由 17% 升至 45%, 运行时间 7 min, 后运行时间 3 min
流速:	1.5 mL/min
自动进样器	
进样量:	10 μL
洗针时间:	10 s
柱温箱	
温度:	30 $^{\circ}\text{C}$
二极管阵列检测器:	1290 270/10 nm, 参比波长 360/100 nm, 20 Hz, 光程 10 mm 的标准流通池

图 7
表面多孔色谱柱与亚 2 μm 色谱柱性能对比；进样量 10 μL =20 μg

图 8 是局部色谱图的叠加图。红色曲线代表 Poroshell 120 色谱柱得到的色谱图, 黑色曲线代表亚 2 μm 色谱柱得到的色谱图。

表 2 列出了两个色谱柱信噪比的计算结果。使用 Poroshell 120 色谱柱和亚 2 μm 色谱柱分析杂质 1 和杂质 2。

结论

表面多孔色谱柱可完全替代亚 2 μm 色谱柱。较低的背压允许在 4.6 mm \times 150 mm, 2.7 μm 色谱柱上使用 1 mL/min 的流速而不超过 400 bar 压力限制。在这种情况下, 理论塔板数可达 35000 或高于 235000 /m。

三根串联 4.6 mm \times 150 mm 色谱柱理论塔板数可达 100000, 在 5 min 内柱压不超过 600 bar。

Agilent Poroshell 120 色谱柱在等度和梯度洗脱分析中均获得极好的精密度。

相同色谱条件下, 可以预期采用 Agilent Poroshell 120 色谱柱通常比采用具有类似键合相的亚 2 μm 色谱柱洗脱时间短。较短的洗脱时间可获得更窄的峰和更高的峰容量。

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2. F. Griiti, A. Cavazzini, N. Marchetti, G. Guiochon, "Comparison between the efficiencies of columns packed with fully and partially porous C18-bonded silica materials", *Journal of Chromatography A*, 1157, 289-303, 2007

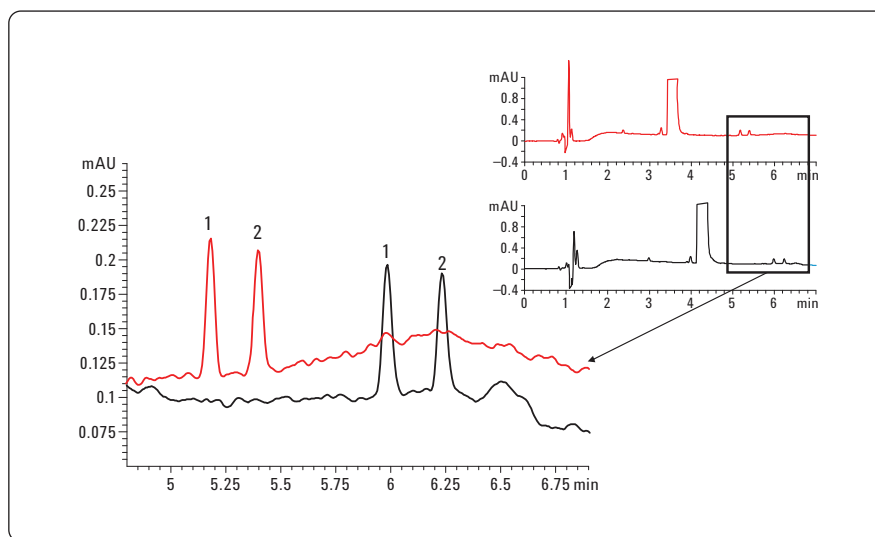


图 8 信噪比对比, 红色曲线代表多孔外壳色谱柱, 黑色曲线代表 1.8 μm 颗粒色谱柱。改性剂选用 TFA

峰	Poroshell 120 S/N	1.8 μm S/N
1	14	13.6
2	12.8	12

表 2 多孔外壳色谱柱与 1.8 μm 颗粒色谱柱的信噪比对比

Hydrophilic Interaction Chromatography (HILIC) Using Agilent Poroshell 120 HILIC

Authors

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Agilent Technologies, Inc.

Introduction

Hydrophilic interaction chromatography (HILIC) is gaining popularity in liquid chromatography particularly for its ability to retain and separate small polar analytes - an area where common reversed-phase liquid chromatography (RPLC) methods often fail. This novel mode of chromatography results in unique retention mechanisms, because water is used as the strong eluting solvent and can have distinct advantages over traditional RPLC in both sample preparation and LC/MS sensitivity due to the use of highly organic mobile phases. The highly organic mobile phases do not require samples in organic solvents to be dried prior to injection, and their higher volatility than traditional RPLC mobile phases makes this technique well suited for applications with mass spectrometers [1].

HILIC retention on a silica-based column is believed to involve a combination of mechanisms. First, a water layer must be adsorbed onto the polar silica surface creating a liquid/liquid extraction system. The polar analytes can then partition into and out of this adsorbed water layer with more polar analytes having a stronger interaction with this immobilized water layer. Charged polar analytes can also undergo ion exchange with the charged silica. Elution is typically from least to most polar, the opposite of RPLC. For HILIC method development, it is important to remember that the solvent strengths are different than in RPLC. For HILIC mode, solvent strength is tetrahydrofuran < acetone < acetonitrile < isopropanol < ethanol < methanol < water, with water being the strongest solvent [2,3].

The development of superficially porous particles has led to the possibilities of method transfer from larger 5- and 3.5- μm particles and from smaller 1.8- μm particles. HILIC mobile phases have much lower viscosities and so generate lower backpressure. The columns possess the advantage of robustness in sample preparation because they have the same 2- μm frit used with larger columns. HILIC columns deliver nearly the same efficiency as the sub-2 μm columns with better robustness. In this work, several applications of HILIC are transferred from totally porous columns (Agilent ZORBAX Rx-Sil, 5 μm , or RRHD HILIC Plus, 1.8 μm) to superficially porous Agilent Poroshell 120 HILIC columns [4].

Experimental

Two systems were used in this work, an Agilent 1200 SL with a G1315C diode array detector and an Agilent 1290 Infinity LC System with an Agilent 6410A Triple Quadrupole Mass Spectrometer. The setup on the mass-spectrometer-equipped system was optimized for the lowest possible extra-column volume with short 0.075 mm id capillaries in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [5]. The 1200 SL system was configured with the shortest possible 0.012 mm id tubing; delay volume was reduced by removing the pulse damper and mixing column and by using the automatic delay volume reduction (ADVR) for gradient analyses [5].

Columns

- Agilent ZORBAX Rx-Sil, 2.1 \times 150 mm, 5 μm (p/n 883700-901)
- Agilent ZORBAX RRHD HILIC Plus, 2.1 \times 100 mm, 1.8 μm (p/n 959758-901)
- Agilent Poroshell 120 HILIC, 2.1 \times 100 mm, 2.7 μm (p/n 695775-901)
- Agilent Poroshell 120 HILIC, 3 \times 150 mm, 2.7 μm (p/n 693975-301)
- Agilent Poroshell 120 EC-C18, 3 \times 150 mm, 2.7 μm (p/n 693975-302)

Napthalene, uracil, epinephrine, dopamine, and norepinephrine were purchased as powders from Sigma Aldrich and prepared to desired concentrations in dimethyl sulfoxide (DMSO) and acetonitrile (MeCN). Morphine, normorphine, morphine-3- β -D-glucuronide (M3G), and morphine-6- β -D-glucuronide (M6G) were purchased as solutions from Cerrilant and diluted in mobile phase. Acetonitrile, methanol, and DMSO were purchased from Honeywell. Ammonium formate and formic acid were purchased from Sigma Aldrich. Water used was 18 M Ω Milli-Q water and was produced in house.

Results and Discussion

In Figure 1, a sample containing a polar analyte, uracil, and a nonpolar analyte, naphthalene, were analyzed on Poroshell 120 EC-C18 and Poroshell 120 HILIC columns. Figure 1 shows the elution order was reversed. In the case of the HILIC column, the uracil, a difficult to retain compound that is analyzed in many metabolomic studies, was strongly retained.

Conditions

Mobile phase: 90/10 MeCN/100 mM $\text{NH}_4\text{HCO}_2\text{H}$ pH 3.2
 Flow rate: 0.84 mL/min
 Pressure: 220 bar Poroshell 120 HILIC;
 224 bar Poroshell 120 EC-C18

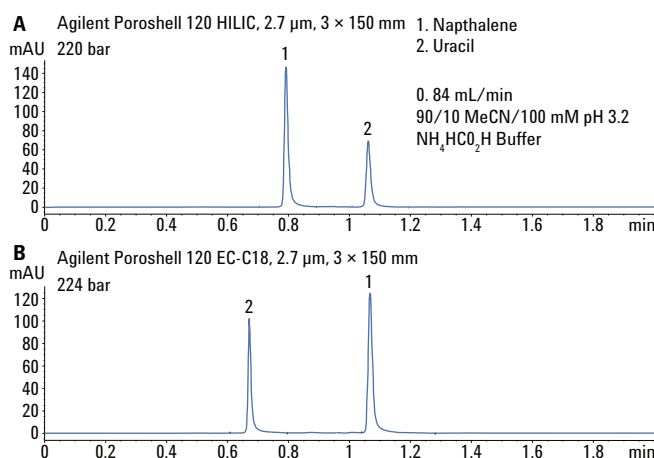


Figure 1. Comparison of the Agilent Poroshell 120 HILIC, 3 \times 150 mm, 2.7 μm column (A) and the Agilent Poroshell 120 EC-C18, 3 \times 150 mm, 2.7 μm reversed phase column (B).

Catecholamines are often analyzed by RPLC [6]. In Figure 2, a gradient separation of 3 catecholamines was carried out using a 5- μm ZORBAX Rx-Sil column and a shorter 2.7- μm Poroshell 120 HILIC column. Several quick observations can be made. Because the Poroshell 120 HILIC column was shorter than the original ZORBAX Rx-Sil column, 66% of the length, each segment of the gradient was reduced proportionally. Because both columns were of the same diameter, scaling was done by changing the gradient according to the length of the column. The injection volume was changed according to the volume of the column. In the case of the smaller Poroshell 120 HILIC column, the injection volume was 66% of the ZORBAX Rx-Sil column (0.5 μL versus 0.75 μL). Also evident is the higher pressure generated by the 2.7 μm Poroshell 120 HILIC particles as compared to the 5 μm ZORBAX Rx-Sil particles. This increase in pressure was due to the smaller particle size of the superficially porous particles. The shorter length of the Poroshell 120 HILIC column helped reduce the pressure. The separation achieved was similar on the 2 columns. However, the epinephrine/norepinephrine pair were slightly better separated on the Poroshell 120 HILIC column. Overall, the peaks were sharper on the Poroshell 120 HILIC column, and the last peak eluted at 3 minutes versus more than 6 minutes on the ZORBAX Rx-Sil column.

Conditions

Samples: Prepared individually in DMSO at 5 mg/mL, mixed in equal parts. ZORBAX Rx-Sil injected neat; Poroshell 120 HILIC diluted with acetonitrile to 2/3 neat injection volume.

Mobile phase: A: 100 mM $\text{NH}_4\text{HCO}_2\text{H}$
B: acetonitrile

Gradient
Agilent Poroshell
120 HILIC:

Time	%B
Initial	97
4	85
4.67	85
5	97
6.67	97

Gradient
Agilent ZORBAX
RX-Sil:

Time	%B
Initial	97
6	85
7	85
7.5	97
10	97

Injection volume: 0.5 μL

Pressure: 168 bar Agilent Poroshell 120 HILIC
68 bar Agilent ZORBAX Rx-Sil

Flow rate: 0.6 mL/min

Instrument: Agilent 1200 SL AVR "ON" no mixer, no pulse damper

Flow cell: 6 mm, 5 μL (p/n G1315-60025) G1315C

Binary pump: Agilent G1312B

Autosampler: Agilent G1367C Automatic Liquid Sampler

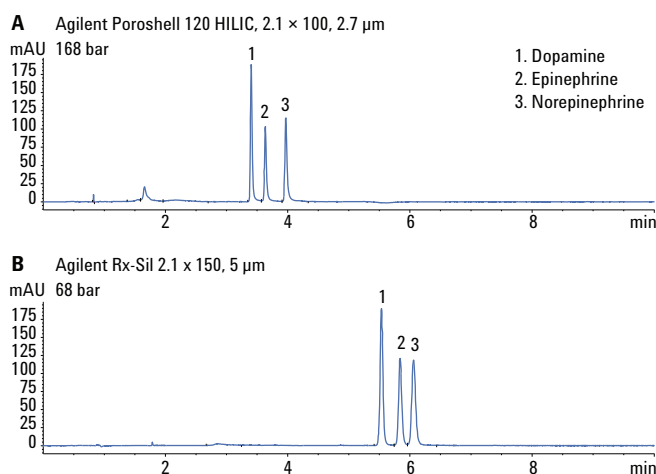


Figure 2. Overlay of HILIC separation of catecholamines on a superficially porous Agilent Poroshell 120 HILIC, 2.1 × 100, 2.7 μm column (A) and a totally porous Agilent ZORBAX Rx-Sil, 2.1 × 150, 5 μm column (B).

Figure 3 shows a gradient separation of morphine and its metabolites on a Poroshell 120 HILIC column and a ZORBAX RRHD HILIC Plus column [7]. While this separation can also be carried out using reversed phase, several advantages have been shown in using the HILIC separation with mass spectrometry detection. A fourfold increase in sensitivity with HILIC mode was produced due to more efficient spraying and desolvation in the ESI-MS source as compared to RPLC. This generated less baseline noise and a more intense MS signal. Figure 3 shows the elution order and selectivity of the compounds were the same on the superficially porous and totally porous columns. The pressure generated by the ZORBAX RRHD HILIC Plus 1.8 μm column was approximately 100 bar higher than the pressure on the Poroshell 120, 2.7 μm column. At this flow rate, the method can be run on a wide variety of instruments. The 0.075 mm id tubing from the low dispersion kit allows easy connection to the mass spectrometer with minimal peak dispersion. In reverse phase mode with its high aqueous content, this may be more of a problem due to high pressure caused by highly viscous solvents, limiting the analysis to only UHPLC systems.

Conditions

Sample:	2 μL injection of 1 $\mu\text{g}/\text{mL}$ each of morphine, normorphine, morphine-3- β -D-glucuronide, and morphine-6- β -D-glucuronide. HILIC sample was prepared in CH_3CN ; RPLC sample was prepared in H_2O
Mobile phase:	A: 100 mM NH_4HCO_2 pH 3.2 B: acetonitrile:100 mM NH_4HCO_2 pH 3.2 (9:1)
Gradient:	Time %B 0 100 0.88 100 3.85 55
Flow rate:	0.4 mL/min
Temp:	25 $^\circ\text{C}$
MS source:	Positive ESI, capillary, 4000 V, drying gas temperature, flow rate and nebulizer pressure vary with mobile phase flow rate
MS acquisition:	Selected ion mode (SIM), delta EMV 200 V, MS dwell time varies with mobile phase flow rate
Software:	Agilent MassHunter version B.03.01 for data acquisition and qualitative analysis.

When coupled to reversed phase SPE or other sample preparation methods that would present the sample in acetonitrile, less work needs to be carried out because a HILIC method could be injected with little or no filtration.

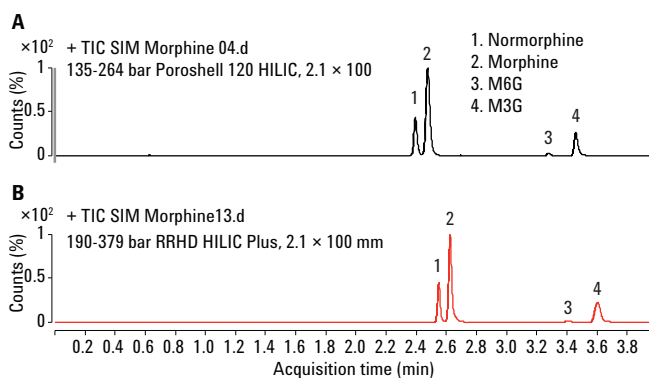


Figure 3. Morphine and metabolite separation on an Agilent Poroshell 120 HILIC 2.7 μm column (A) and Agilent ZORBAX RRHD HILIC Plus 1.8 μm (B).


Conclusions

Elution of polar and nonpolar compounds was reversed on the Poroshell 120 HILIC column as compared to the Poroshell 120 EC-C18 column. The Poroshell 120 HILIC column was similar to the ZORBAX Rx-Sil column for many applications. Peaks were narrower and pressure was higher on the Poroshell 120 HILIC column as compared to the ZORBAX Rx-Sil column, but not sufficient to cause a problem due to the low viscosity solvents used in HILIC. The Poroshell 120 HILIC column was also similar to the ZORBAX RRHD HILIC Plus column for many applications.

The Poroshell 120 HILIC column is ideal for mass spectrometry applications especially when low dispersion tubing is used. The extra pressure generated by the tubing may be offset by the lower pressure of the Poroshell 120, 2.7 μm particles as compared to sub-2 μm particles. The Poroshell 120 HILIC column yields similar efficiencies to the sub-2 μm totally porous ZORBAX RRHD HILIC Plus column, and it may prove to be more robust in situations with moderately dirty samples due to a larger frit at the inlet of the Poroshell 120 column.

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