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Ultra-Sensitive Forensic Analysis Workflow of Cocaine and Its Metabolites in Hair Samples by LC-MS/MS using Strata[®]-X-C Solid Phase Extraction (SPE) and a Kinetex[®] Core-Shell Biphenyl LC Column

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Abstract

A quantitative ultra-sensitive SPE-LC-MS/MS analysis workflow for simultaneous determination of cocaine and metabolites at picogram levels was developed and evaluated in a hair matrix. The method was demonstrated to be sensitive, precise, and accurate. Utilization of the SCIEX QTRAP[®] 6500+ LC-MS/MS system coupled with a SPE extraction using Strata-X-C and separation via LC using a Kinetex core-shell Biphenyl column provided unique advantages in the ability to maximize selectivity when confirming and quantifying low level metabolites in hair.

Introduction

Utilization of the hair matrix in drug analysis has recently grown.^{1-3 and ref. therein} Compared to other biological matrices, hair offers several advantages: its extraction is painless; there are no special requirements for the sample storage; drugs in hair are stable and are not metabolized; considering the rate of hair growth to be on average 1 cm per month, multi-sectional hair analysis enables monitoring the history of drug use.¹

Forensic analysis of cocaine in hair requires a sensitive and reliable analytical workflow. There are two major challenges for the detection of this compound and its metabolites in hair samples: low concentration and matrix interferences. In this technical note, an analysis workflow combining the use of triple quadrupole linear ion trap mass spectrometry with solid phase extraction (SPE) for picogram per mg of hair detection of cocaine and its metabolites in hair is described.

Materials and Methods

Sample Preparation:

Hair samples were washed, dried, and cut into ~ 1 mm segments. The hair was digested with 0.1 N HCl, an aliquot of the extract was mixed with the internal standards and then underwent a SPE procedure with Strata-X-C, a polymeric strong cation-exchange sorbent, as detailed below.

Decontamination:	Wash with dichloromethane and water, air dry
Digest:	Incubate 20mg hair with 1 mL 0.1 N HCl overnight at 45°C
Pre-treatment:	Add 10 µL of IS to 800 µL of hair extract solution, vortex

SPE Protocol

Cartridge:	Strata-X-C, 30 mg/3 mL
Part No.:	8B-S029-TBJ
Condition 1:	1 mL EtOAc/MeOH/28-30 % NH ₄ OH (70:20:10)
Condition 2:	1 mL Methanol
Equilibrate:	1 mL Water
Load:	Pre-treated sample
Wash 1:	1 mL 0.1 N HCl
Wash 2:	1 mL Methanol
Dry:	10 min at high vacuum (~10" of Hg)
Elute:	2x 500 µL EtOAc/MeOH/28-30 % NH ₄ OH (70:20:10)
Add:	50 µL of 0.1 N HCl to eluate
Dry Down:	Evaporate to dryness under nitrogen at 40-45 °C
Elute:	2x 600 µL Ethyl acetate/Hexane (3:1)
Dry:	Sample under slow stream of Nitrogen at 30 °C
Reconstitute:	200 µL of initial mobile phase

HPLC Conditions

Column:	Kinetex 2.6µm Biphenyl	
Dimensions:	100 x 3.0 mm	
Part No.:	00D-4622-Y0	
Mobile Phase:	A: 0.1 % Formic acid in Water B: Methanol	
Gradient:	Time (min)	% B
	0	2
	0.75	2
	1.25	40
	7	90
	9	90
	9.1	2
	13	2
Flow Rate:	600 µL/min	
Temperature:	30 °C	
Detector:	MS/MS (SCIEX QTRAP 6500+)	
Instrument:	ExionLC™ (SCIEX)	
Injection:	10 µL	

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MS/MS Conditions

A SCIEX QTRAP[®] 6500+ LC-MS/MS system with IonDrive[™] Turbo V[™] source and Electrospray Ionization (ESI) probe was used. Cocaine and its 10 metabolites were detected using two MRM transitions per compound to allow quantitation and identification based on the ratio of quantifier to qualifier MRM transitions. MRM transitions utilized in the method are presented in **Table 1**.

The source parameters were held as follow:

Curtain Gas=30; Collision Gas = Medium; Ion Spray Voltage was 4500 V, the temperature of the source was 600 °C, Ion Source Gas 1= 60, Ion Source Gas 2 = 60.

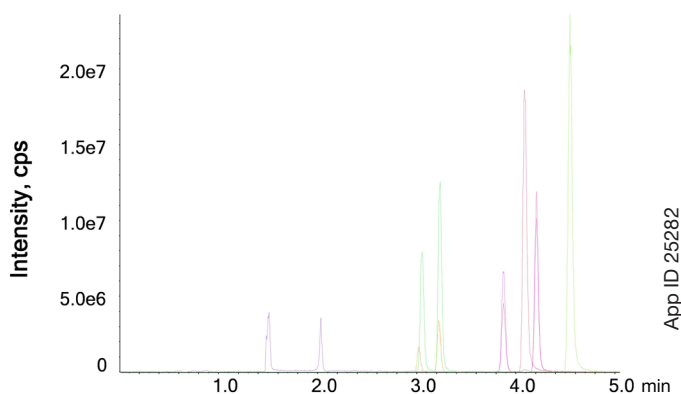
Table 1. MRM transitions and source parameters for cocaine and its 10 metabolites

Q1	Q3	Analyte	DP (volts)	EP (volts)	CE (volts)	CXP (volts)
290.2	168.1	BZE 1	65	10	25	10
290.2	105	BZE 2	65	10	36	10
290.2	168	Norcocaine 1	50	10	21	10
290.2	136.1	Norcocaine 2	50	10	30	10
304.2	182.2	Cocaine 1	70	10	26	10
304.2	82.1	Cocaine 2	70	10	35	10
304.2	105	Cocaine 3	70	10	37	10
186.2	168.1	Ecgonine 1	60	10	23	10
186.2	100.3	Ecgonine 2	60	10	31	10
200.201	182.1	EME 1	45	10	23	10
200.201	82.1	EME 2	45	10	32	10
306.1	168.1	p-OH-BZE 1	70	10	26	10
306.1	186.1	p-OH-BZE 2	70	10	27	10
306.102	168.1	m-OH-BZE 1	80	10	27	10
306.102	121.1	m-OH-BZE 2	80	10	35	10
318.2	196.1	Cocaethylene 1	50	10	26	10
318.2	82.1	Cocaethylene 2	50	10	37	10
320.1	182.1	m-OH-COC 1	70	10	27	10
320.1	82.1	m-OH-COC 2	70	10	42	10
320.101	200.1	o-OH-COC 1	50	10	27	10
320.101	182.1	o-OH-COC 2	50	10	37	10
320.102	182.2	p-OH-COC 1	80	10	26	10
320.102	82.1	p-OH-COC 2	80	10	44	10
293.1	171.1	BZE-D3	68	10	26	10
293.2	171.1	Norcocaine-D3	55	10	22	10
307.1	185.1	COC-D3	65	10	26	10
321.2	199.3	Cocaethylene-D3	51	10	27	10
323.1	185.2	m-OH-COC-D3	60	10	27	10

Results

A method for quantitation and identification of cocaine and its 10 metabolites: benzoylecgonine (BZE), norcocaine, cocaine (COC), ecgonine, ecgonine methyl ester (EME), para-hydroxy-benzoylecgonine (p-OH-BZE), meta-hydroxy-benzoylecgonine (m-OH-BZE), cocaethylene, meta-hydroxy-cocaine (m-OH-COC), ortho-hydroxy-cocaine (o-OH-COC), para-hydroxy-cocaine was developed (**Figure 1**).

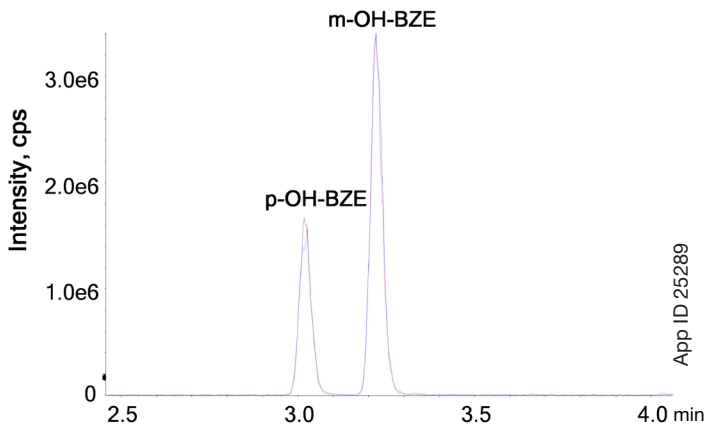
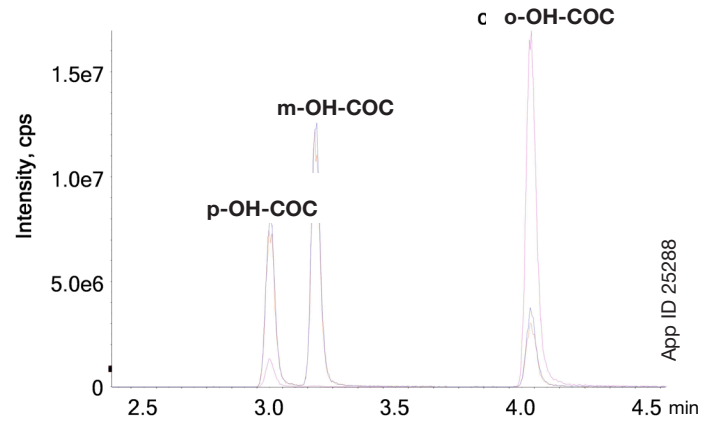
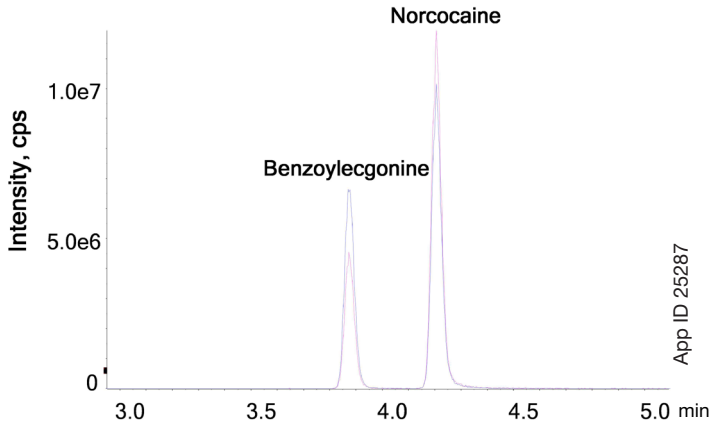
Figure 1. Detection of cocaine and its 10 metabolites in hair at 0.05 ng per mg of hair level



To ensure correct quantitation of cocaine and the possibility of hair sample contamination with cocaine, which is easily available as powder when inhaled, detection and quantification of metabolites is a mandatory part of forensic analysis. Following administration of the drug, the main metabolites that are formed are benzoylecgonine and ecgonine methyl ester while the minor ones are norcocaine and meta- and para-hydroxycocaine.^{2,3} The method discussed here demonstrated a good separation of isomeric compounds present in the mixture under analysis (**Figure 2**).

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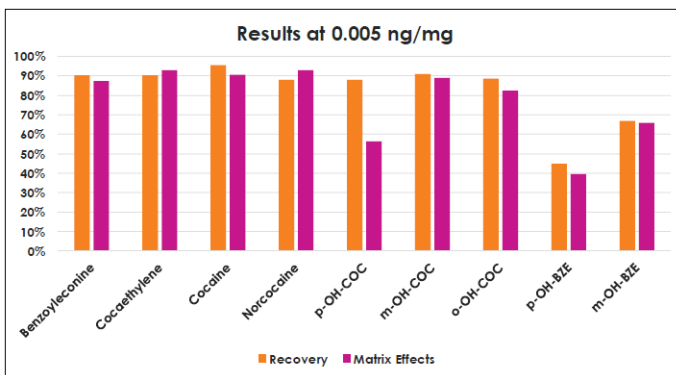
Figure 2.
Separation of isomeric metabolites



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Hair is a very complex matrix, which may represent a problem when detecting analytes at low concentration levels. Robust and reliable extraction procedures are of a great importance in achieving the desired reproducibility, good linear responses, and limits of quantitation. The two-step SPE conditioning process reduced background noise in the final extract. Using methanol rather than isopropanol in the elution solvent produced improved analyte recovery. Our final extraction procedure demonstrated excellent recoveries of the analytes of interest.

Figure 3.
Recovery and matrix effects



Our measurements also demonstrated excellent linearity of the generated regression curves covering a linear dynamic range from 3 to 4 orders of magnitude; coefficients of variation (CVs) within 10 % and good accuracies. Signal-to-Noise ratios at LLOQ were found to vary from 10 to 50. LLOQs are presented in **Table 2**.

Figure 4.
Calibration curves and statistics information for cocaine, m-OH-cocaine, o-OH-cocaine, benzoylecgonine, and norcocaine

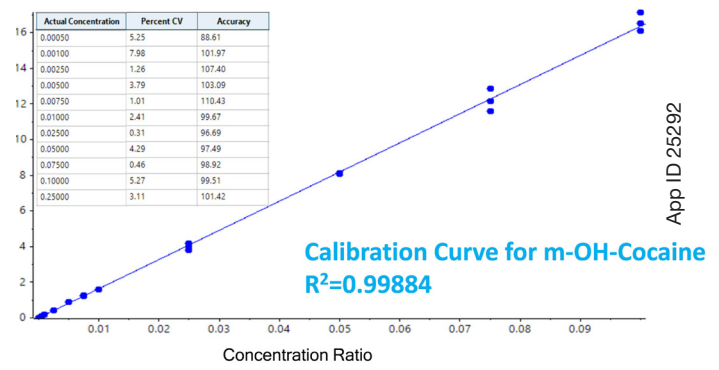
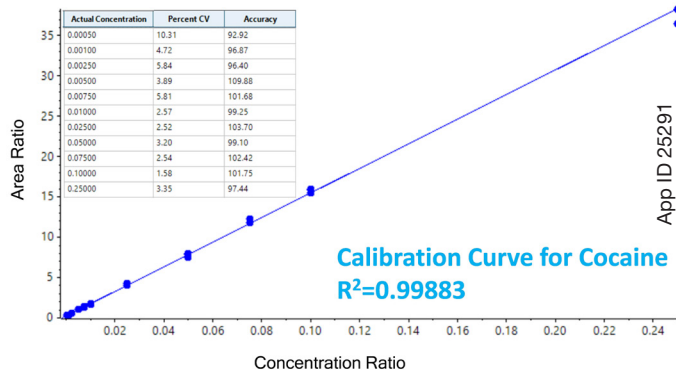


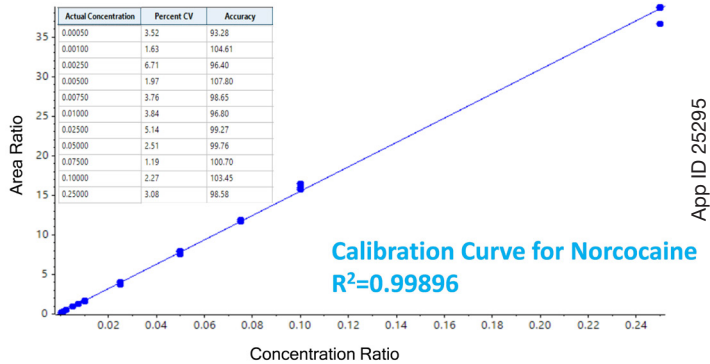
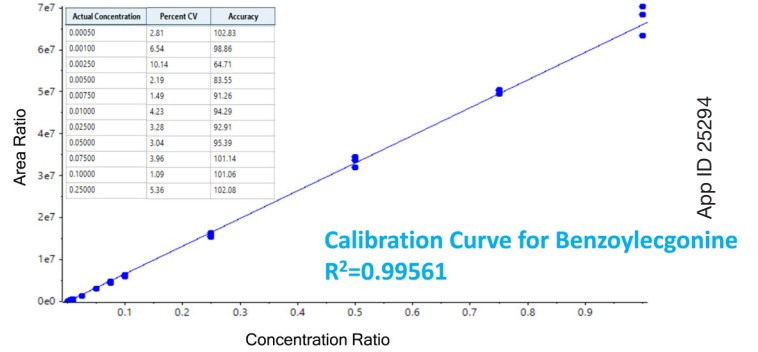
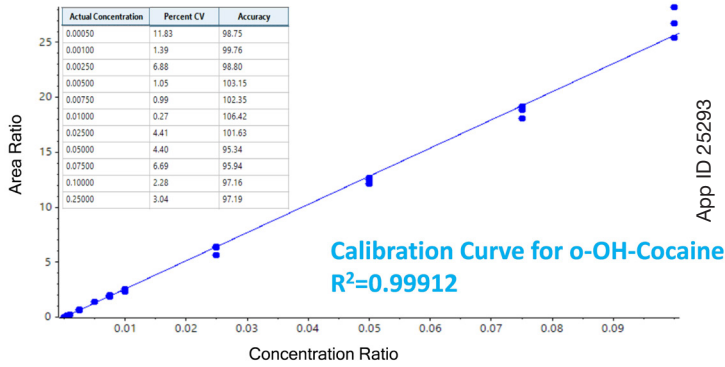
Table 2.
Lower Limits of Quantitation (LLOQ) for cocaine and metabolites panel

Analyte	LLOQ (ng/mg)
Ecgonine	0.05
Ecgonine Methyl Ester	0.0025
Benzoylecgonine	0.001
Norcocaine	0.0005
Cocaine	0.0005
p-OH-Benzoylecgonine	0.01
m-OH-Benzoylecgonine	0.01
Cocaethylene	0.0001
m-OH-Cocaine	0.00005
o-OH-Cocaine	0.00005
p-OH-Cocaine	0.001

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Figure 4. (cont'd)

Calibration curves and statistics information for cocaine, m-OH-cocaine, o-OH-cocaine, benzoylecgonine, and norcocaine

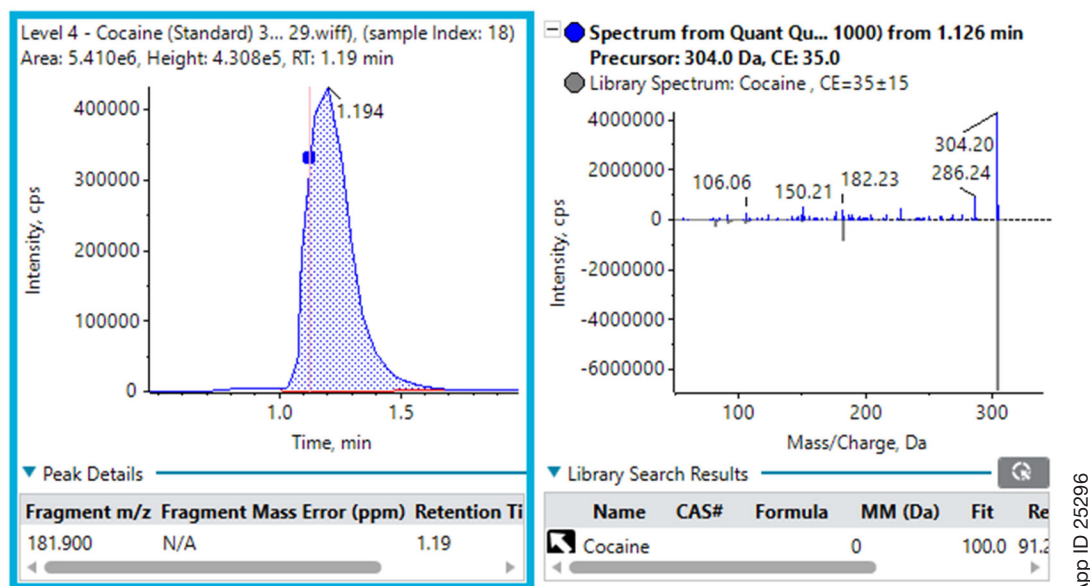


Utilization of a SCIEX QTRAP[®] 6500+ LC-MS/MS system, a hybrid linear ion trap, enables generation of enhanced product ion spectra that contain information of the complete molecular fingerprint of cocaine and metabolites that were searched against relevant spectral libraries. This approach to compound confirmation significantly reduces the risk of false positives in the unknown samples.

To demonstrate these capabilities of the 6500+ system we have acquired the samples in MRM-IDA-2EPI experimental set-up. **Figure 5** illustrates typical results of MS/MS library searching.

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Figure 5.
MS/MS library searching results for cocaine in a standard solution prepared by spiking in blank hair extract



Conclusion

The data presented here demonstrate a complete method for analysis of cocaine and metabolites from hair, including sample extraction, chromatography, and MS detection across a wide analytical range. Analyte extraction recoveries were demonstrated to be greater than 80 %, enabling the analytical workflow to obtain a sub pg/mg LLOQ in hair matrix. The workflow showed excellent accuracy (>95 %) and precision (<15 %), with excellent linearity resulting in R2 values of 0.9990 for all analytes.

Specifically, hydroxycocaine isomers demonstrated acceptable accuracy and precision down to 0.00005 ng/mg. Overall, the LLOQ for cocaine and metabolites was shown to be in low pg per mg of hair sample ranges.

Linear dynamic ranges of the panel under analysis were found to be of 3 to 4 orders of magnitude. It is necessary to note that hydroxybenzoylecgonines suffered from sample stability issues during transport, having previously produced 92 % (m-OH-BZE) and 82 % (p-OH-BZE) recoveries. Recovery for ecgonine was 87 % at 0.1 ng/mg. Ecgonine methyl ester (EME) suffered from high background and produced recoveries greater than 100 %.

In addition to quantitation, the SCIEX QTRAP[®] 6500+ LC-MS/MS system enabled simultaneous identification and confirmation of illicit drugs and their metabolites through utilization of Enhanced Product Ion Scan (EPI) by acquiring full scan MS/MS data. Forensic drug identification and confirmation was achieved using automated MS/MS library searching.



References

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3. K.B. Scheidweiler, M.A. Huestis. Simultaneous quantification of opiates, cocaine and metabolites in hair by LC-APCI-MS/MS. *Anal. Chem.* (2004), 76, 4358-4363

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Ordering Information

Strata®-X-C Solid Phase Extraction (SPE)

Format	Sorbent Mass	Part Number	Unit
Microelution 96-Well Plate			
	2 mg	8M-S029-4GA	1 Plate/Box
96-Well Plate			
	10 mg	8E-S029-AGB	2 Plates/Box
	30 mg	8E-S029-TGB	2 Plates/Box
	60 mg	8E-S029-UGB	2 Plates/Box
Tube			
	30 mg	8B-S029-TAK*	1 mL(100/box)
	30 mg	8B-S029-TBJ	3 mL (50/box)
	60 mg	8B-S029-UBJ*	3 mL (50/box)
	100 mg	8B-S029-EBJ	3 mL (50/box)
	100 mg	8B-S029-ECH	6 mL (30/box)
	200 mg	8B-S029-FBJ	3 mL (50/box)
	200 mg	8B-S029-FCH	6 mL (30/box)
	500 mg	8B-S029-HBJ	3 mL (50/box)
	500 mg	8B-S029-HCH	6 mL (30/box)

* tab-less tubes available

Kinetex® Core-Shell LC Columns

2.6 µm Minibore Columns (mm)					SecurityGuard™ ULTRA Cartridges†
Phases	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	3/pk
Biphenyl	00A-4622-AN	00B-4622-AN	00D-4622-AN	00F-4622-AN	AJ0-9209 for 2.1 mm ID

2.6 µm MidBore™ Columns (mm)				SecurityGuard ULTRA Cartridges†
Phases	50 x 3.0	100 x 3.0	150 x 3.0	3/pk
Biphenyl	00B-4622-Y0	00D-4622-Y0	00F-4622-Y0	AJ0-9208 for 3.0 mm ID

2.6 µm Analytical Columns (mm)				SecurityGuard ULTRA Cartridges†
Phases	50 x 4.6	100 x 4.6	150 x 4.6	3/pk
Biphenyl	00B-4622-E0	00D-4622-E0	00F-4622-E0	AJ0-9207 for 4.6 mm ID

† SecurityGuard ULTRA Cartridges require holder, Part No.: AJ0-9000

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