Quantitative Analysis of Hydrazine and Acetylhydrazine Using Chemical Derivatization and HPLC-MS/MS

Derek Laine[1], Chad Christianson[1], Kevin Harbol[2], Shane Needham[1]
1. Alturas Analytics, Inc., Moscow Idaho 2. Omeros Corp., Seattle Wa.

Overview

- **Purpose** – To demonstrate the utility of HPLC/MS/MS toward the analysis of low molecular weight molecules Hydrazine and Acetylhydrazine.
- **Methods** – Molecular mass enhancement is achieved by derivatization with 4-Bromobenzaldehyde to produce the corresponding Azine and Hydrazone products that are more suitable to HPLC/MS/MS analysis.
- **Results** – A concentration range from 1.00-1000 ng/mL with a % bias less than 20% for each of the derived analytes was observed. The response was linear with $1/x^2$ weighted regression.

Introduction

Hydrazine and Hydrazine derivatives are used in many applications, including as therapeutic agents. Isoniazid, for example, is a Hydrazine derivative used as an anti-tubercular drug that can potentially metabolize to Hydrazine or Acetylhydrazine. As such, there is demand for a quantitative analysis method of these compounds in a pharmaceutical setting where HPLC/MS/MS is often employed. HPLC/MS has not been widely studied for the detection of Hydrazine, in part due to the molecule's polarity and low molecular weight. In this work, we report the quantitative analysis of Hydrazine and Acetylhydrazine by HPLC/MS/MS using chemical derivatization with 4-Bromobenzaldehyde to produce the corresponding Azine and Hydrazone products. The products are easily extracted and reconstituted in an Acetonitrile:H_2O solvent suitable for HPLC/MS/MS detection.

Methods

Extraction

- Hydrazine and Acetylhydrazine are extracted from rat plasma
- 50 µL aliquot method using Dexamethasone as the internal standard
- Derivation solutions: Acetic Acid, 4-Bromobenzaldehyde and Ammonium Acetate.
- Azine and Hydrazone products are extracted with MTBE
- The MTBE layer is transferred, evaporated, and reconstituted with a Acetonitrile:H_2O solution that is suitable for HPLC.

HPLC

- Gradient HPLC using acetonitrile and water mobile phases
- Flow rate: 0.7 mL/minute
- HPLC column: HSC18 2.1x50 mm (Supelco)
- Column temperature: 50°C

Mass Spectrometry

- Sciex API5500 operating in MRM mode
- ESI
- Positive ion mode
- MRM transitions:
  p-Bromobenzaldehyde Acetylhydrazone: 241.2 → 182.1
  p-Bromobenzaldehyde Azine: 365.4 → 155.4

Conclusions

- The method presented can be utilized to analyze the small and weakly polar Hydrazine and N-Acetylhydrazine, and similar molecules by HPLC/MS/MS.
- The method is straightforward with a short reaction time of approximately 5 minutes.

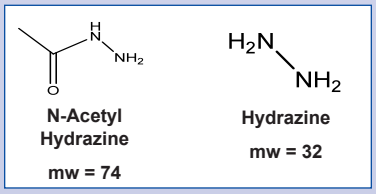

Figure 1: Analysis of Hydrazine and N-Acetyl Hydrazine by LC/MS/MS

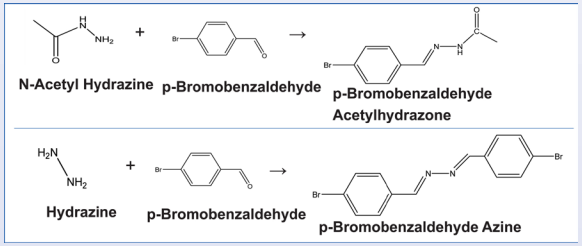

Figure 2: Method for the Derivatization of Hydrazine and N-Acetyl Hydrazine

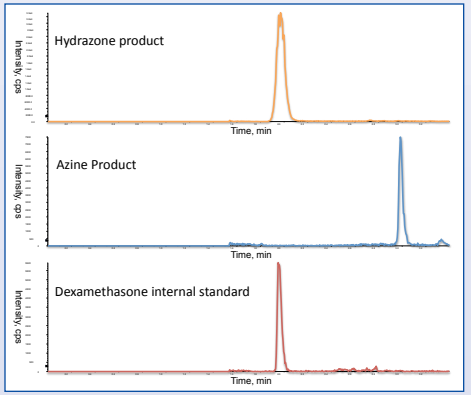

Figure 3: HPLC/MS/MS Chromatogram from a 1000 ng/mL Plasma Sample

Table 1: Bioanalytical Results for N-Acetyl Hydrazine in Rat K_2EDTA Plasma

Sample Name	Analyte Peak Area (counts)	Analyte Concentration (ng/mL)	Area Ratio	IS Peak Area (counts)	Calculated Concentration (ng/mL)	Accuracy (%)
DB	1.26E+03	0	4.73E+00	2.66E+02	<LLOQ	N/A
B	5.71E+02	0	3.16E-03	1.81E+05	<LLOQ	N/A
1	2.75E+04	1	1.07E-01	2.56E+05	1.04	104
1	1.58E+04	1	5.83E-02	2.70E+05	< 0	N/A
5	6.99E+04	5	2.65E-01	2.64E+05	4.51	90.2
5	6.68E+04	5	2.59E-01	2.58E+05	4.37	87.4
30	3.70E+05	30	1.43E+00	2.59E+05	30.0	100
30	3.92E+05	30	1.48E+00	2.65E+05	31.2	104
150	1.76E+06	150	7.35E+00	2.39E+05	160	107
150	1.63E+06	150	6.88E+00	2.36E+05	150	100
1000	1.09E+07	1000	4.75E+01	2.29E+05	1040	104
1000	1.08E+07	1000	4.69E+01	2.31E+05	1030	103

Table 2: Bioanalytical Results for Hydrazine in Rat K_2EDTA Plasma

Sample Name	Analyte Peak Area (counts)	Analyte Concentration (ng/mL)	Area Ratio	IS Peak Area (counts)	Calculated Concentration (ng/mL)	Accuracy (%)
DB	5.66E+02	0	2.13E+00	2.66E+02	<LLOQ	N/A
B	3.21E+02	0	1.78E-03	1.81E+05	<LLOQ	N/A
1	4.56E+03	1	1.78E-02	2.56E+05	2.63	N/A
1	2.15E+03	1	7.96E-03	2.70E+05	1.02	102
5	7.92E+03	5	3.00E-02	2.64E+05	4.64	92.8
5	6.53E+03	5	2.53E-02	2.58E+05	3.86	77.2
30	4.23E+04	30	1.63E-01	2.59E+05	26.5	88.2
30	4.30E+04	30	1.62E-01	2.65E+05	26.3	87.6
150	2.22E+05	150	9.27E-01	2.39E+05	152	101
150	2.36E+05	150	1.00E+00	2.36E+05	163	109
1000	1.54E+06	1000	6.71E+00	2.29E+05	1100	110
1000	1.54E+06	1000	6.68E+00	2.31E+05	1090	109