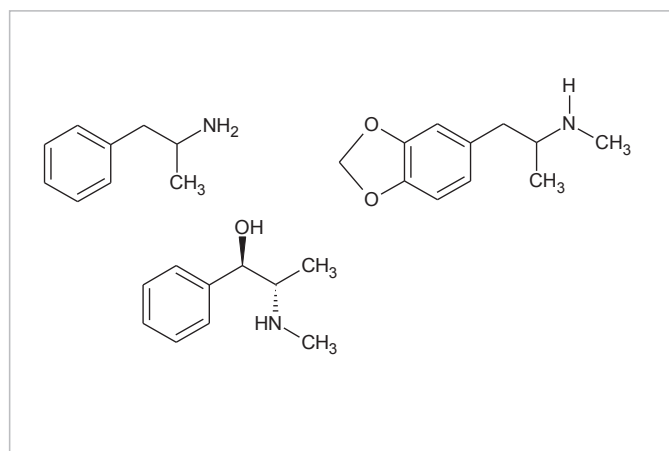


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# Extraction of Amphetamines and Metabolites from Urine (including Elimination of Sympathomimetic Amine Interferences) Using ISOLUTE® SLE+ Prior to GC/MS Analysis



**Figure 1.** Structures of Amphetamine, MDMA and Ephedrine

## Introduction

This application note describes the extraction of a range of amphetamines and metabolites from urine using supported liquid extraction and subsequent analysis by GC/MS. Prior to extraction, a simple oxidation step is performed to eliminate sympathomimetic compounds such as ephedrine and pseudoephedrine so they do not interfere with quantitation of methamphetamine.

ISOLUTE® SLE+ Supported Liquid Extraction plates and columns offer an efficient alternative to traditional liquid-liquid extraction (LLE) for bioanalytical sample preparation, providing high analyte recoveries, no emulsion formation, and significantly reduced sample preparation.

## Analytes

Amphetamine –D5, Amphetamine, Methamphetamine, MDMA, MDA, MDEA, Ephedrine, Pseudoephedrine

## Sample Preparation Procedure

- Sample pre-treatment:** To urine (2 mL), add phosphate buffer (pH 6, 0.8M, 1 mL) and vortex. Add sodium periodate (0.3M, 1 mL). Heat for 15 minutes at 60 °C. Allow to cool, add concentrated ammonium hydroxide (85 µL) and vortex.
- Format:** **ISOLUTE® SLE+ 1 mL Sample Volume columns, part number 820-0140-C**
- Sample Loading:** Load 1 mL of the pre-treated urine mixture onto the column and apply a pulse of vacuum or positive pressure (3–5 seconds) to initiate flow. Allow the sample to absorb for 5 minutes.
- Analyte Extraction:** Apply dichloromethane/isopropanol, (95/5, v/v, 2.5 mL) and allow to flow under gravity for 5 minutes into tubes. Apply a further aliquot of DCM/IPA, (95/5, v/v, 2.5 mL) and allow to flow for another 5 minutes under gravity. Apply vacuum or positive pressure (5–10 seconds) to pull through any remaining extraction solvent.
- Post Elution, Derivatization and Reconstitution:** Remove tubes from the elution rack and add 1% HCl in methanol (100 µL) to each tube. This devolatilizes the analytes and helps to prevent losses on evaporation.
- Dry the extract in a stream of air or nitrogen at ambient temperature using a SPE Dry (20 to 40 L/min) or TurboVap (1.0 bar) for 30 mins.
- Add HFBA (100 µL) and ethyl acetate (100 µL) and vortex for 10 seconds. Transfer to a high recovery vial, cap and incubate at 75 °C for 15 minutes. Cool and then evaporate the HFBA at room temperature. On dryness, reconstitute each vial with ethyl acetate (100 µL). Cap and vortex for 10 seconds.

## GC Conditions

|                       |  |
|-----------------------|--|
| <b>Instrument:</b>    | Agilent 7890A with QuickSwap   |
| <b>Column:</b>        | Agilent J&W DB-5 30 m x 0.25 mm ID x 0.25 µm                               |
| <b>Carrier</b>        | Helium 1.2 mL/min  |
| <b>Inlet:</b>         | 175 °C, Splitless, purge flow: 50 mL/min at 1.0 min                        |
| <b>Injection:</b>     | 1 µL   |
| <b>Wash solvents:</b> | Ethyl acetate  |
| <b>Oven:</b>          | Initial temperature 50 °C, hold for 1.0 minute<br>Ramp 20 °C/min to 275 °C |
| <b>Transfer Line:</b> | 280 °C   |

## MS Conditions

|                    |               |
|--------------------|---------------|
| <b>Instrument:</b> | Agilent 5975C |
| <b>Source:</b>     | 230 °C        |
| <b>Quadrupole:</b> | 150 °C        |
| <b>MSD mode:</b>   | SIM           |

## SIM Parameters

**Table 1.** Analyte ions acquired in the Selected Ion Monitoring (SIM) mode

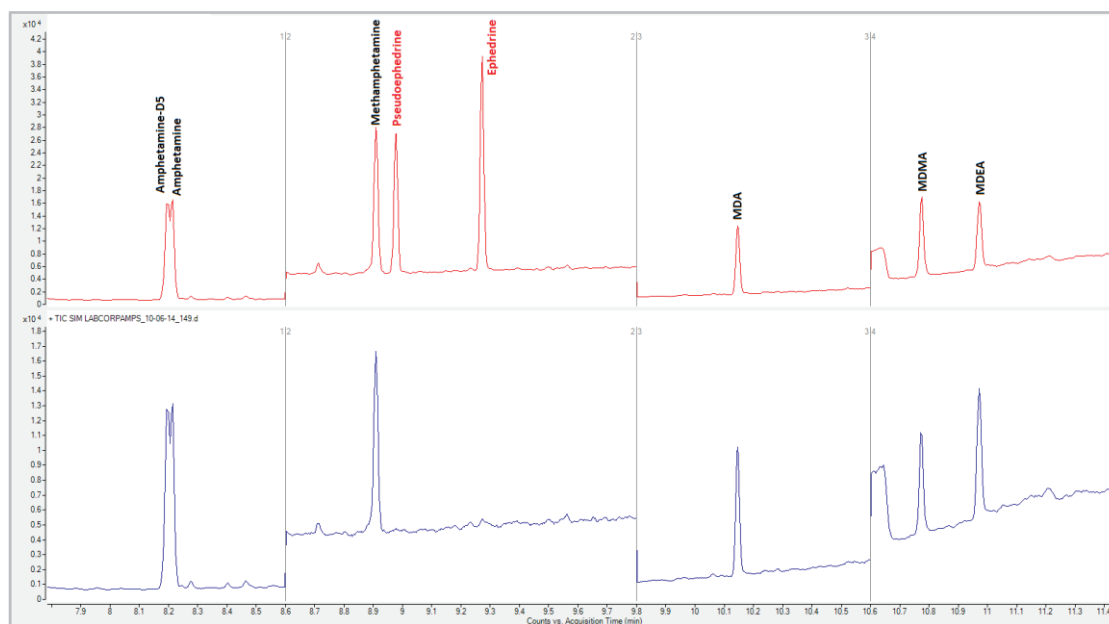
| SIM Group | Analyte         | Quantifier Ion | Quantifier Ion |
|-----------|-----------------|----------------|----------------|
| 1         | Amphetamine-D5  | 244            | 123            |
| 1         | Amphetamine     | 240            | 118            |
| 2         | Methamphetamine | 254            | 210            |
| 3         | MDA             | 162            | 135            |
| 4         | MDMA            | 162            | 210            |
| 4         | MDEA            | 268            | 240            |

and the two compounds that potentially cause interference to methamphetamine

|   |                 |     |     |
|---|-----------------|-----|-----|
| 2 | Pseudoephedrine | 254 | 210 |
| 2 | Ephedrine       | 254 | 210 |

## Results

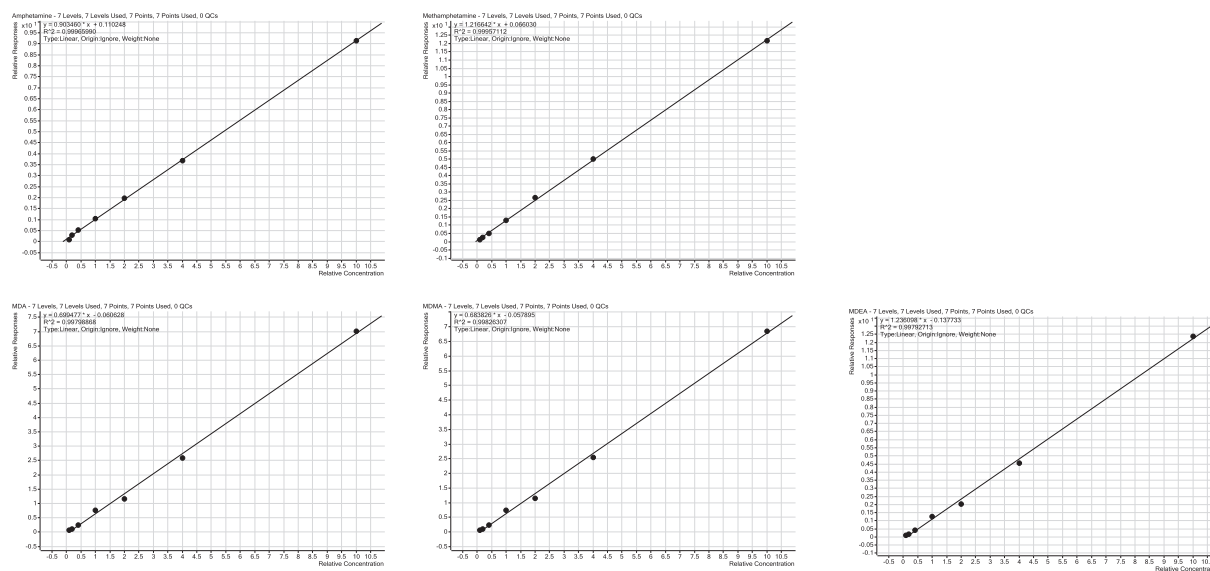
The oxidation protocols prior to the SLE procedure demonstrated complete removal of the ephedrine and pseudoephedrine from urine. This is shown on page 3 in **Figure 2**. The top chromatogram is a urine specimen spiked with the interferents **after** oxidation (demonstrating retention times), the bottom chromatogram illustrates urine spiked with the interferents **before** oxidation (and subsequently removed in the oxidation process). In SIM window 2 it can be seen that ephedrine and pseudoephedrine are completely removed from the sample. (Concentration of all analytes is 100 ng/mL.)



**Figure 2.** Chromatography of all analytes at 100 ng/mL. Analytes spiked after oxidation (top) and spiked before oxidation (bottom).

This experiment was repeated at a pseudoephedrine / ephedrine concentration of 2.5 µg/mL with the remainder of the analytes at 100 ng/mL in urine. Similar results were demonstrated with total removal of the interferences without any adverse effects on the amphetamines.

**Figure 3** shows the linearity of the optimized method on ISOLUTE® SLE+ columns. The coefficient of determination was determined to be greater than 0.997 for all analytes across concentration values, 5, 10, 20, 50, 100, 200 and 500 ng/mL. **Table 2** shows the lower limit of quantitation for each analyte using this protocol on 1 mL capacity columns.



**Figure 3.** Calibration curves for extracted levels of spiked urine, using ISOLUTE SLE+ protocol (1 mL capacity column format).

**Table 2.** Lower limits of quantitation (LLOQ) for each amphetamine or metabolite using this optimized approach.

| Analyte         | LLOQ with 500 µL urine (ng/mL) |
|-----------------|--------------------------------|
| Amphetamine     | 5                              |
| Methamphetamine | 5                              |
| MDA             | 5                              |
| MDMA            | 5                              |
| MDEA            | 5                              |

## Additional Information

1% HCl in methanol was prepared by adding 400 µL concentrated HCL (commercially available 37%) to 39.6 mL HPLC grade methanol.

0.8M potassium phosphate buffer was prepared by weighing 54.436g monopotassium phosphate and adding to 500 mL HPLC grade water. The pH was adjusted to 6 with ammonium hydroxide.

0.3M sodium periodate was prepared by weighing 6.4167g sodium periodate and adding to 100 mL HPLC grade water.

## Ordering Information

| Part Number    | Description   | Quantity |
|----------------|---|----------|
| 820-0140-C     | ISOLUTE® SLE+ 1 mL Sample Volume columns              | 30       |
| PPM-48         | Biotage® PRESSURE+ 48 Positive Pressure Manifold      | 1        |
| SD-9600-DHS-EU | Biotage® SPE Dry Sample Concentrator System 220/240 V | 1        |
| SD-9600-DHS-NA | Biotage® SPE Dry Sample Concentrator System 100/120 V | 1        |
| C103198        | TurboVap® LV, 100/120V                                | 1        |
| C103199        | TurboVap® LV, 220/240V                                | 1        |

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### EUROPE

Main Office: +46 18 565900  
Toll Free: +800 18 565710  
Fax: +46 18 591922  
Order Tel: +46 18 565710  
Order Fax: +46 18 565705  
order@biotage.com  
Support Tel: +46 18 56 59 11  
Support Fax: +46 18 56 57 11  
eu-1-pointsupport@biotage.com

### NORTH & LATIN AMERICA

Main Office: +1 704 654 4900  
Toll Free: +1 800 446 4752  
Fax: +1 704 654 4917  
Order Tel: +1 704 654 4900  
Order Fax: +1 434 296 8217  
ordermailbox@biotage.com  
Support Tel: +1 800 446 4752  
Outside US: +1 704 654 4900  
us-1-pointsupport@biotage.com

### JAPAN

Tel: +81 3 5627 3123  
Fax: +81 3 5627 3121  
jp\_order@biotage.com  
jp-1-pointsupport@biotage.com

### CHINA

Tel: +86 21 2898 6655  
Fax: +86 21 2898 6153  
cn\_order@biotage.com  
cn-1-pointsupport@biotage.com

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