*Application Note*

# Extraction of Drugs of Abuse in Urine using Solid Phase Extraction

## Introduction

The term Drugs of Abuse (DOA) can be applied to a wide variety of natural and synthetic compounds prevalent in human culture. Many rapidly pass through the human body, while others can persist for extended periods. Screening for traces of these has become common place by analyzing human urine, either by LC/MS or by GC/MS through derivitization of the parent compounds.

Solid Phase Extraction (SPE) provides an ideal extraction media for DOA compounds, as it can be performed with small sample amounts with rapid turnaround. The utilization of automated SPE platforms (EconoTrace SPE, FMS Inc.) can greatly assist production labs generate large volumes of samples in relative short periods of time.

## Instrumentation

- FMS, Inc. EconoTrace® SPE system
- FMS, Inc. SuperVap® 12 Concentrator
- FMS 50 mls evaporator tubes with direct to GC vial termination
- SuperVap® Vial Evaporator
- Waters Accuity LC with Xevo MSD
- Thermo TriPlus Autosampler

FMS EconoTrace SPE with SuperVap concentrator, the system can run up to 8 samples simultaneously

## Consumables

- 150mg 6cc DOA Mixed Mode SPE cartridges
- Acetonitrile, LC/MS grade or equivalent
- Methanol, LS/MS grade or equivalent
- Toluene, pesticide grade or equivalent
- HPLC grade water
- 6N HCL
- Ammonium Hydroxide
- Native and Labeled DOA standards acquired from Cambridge Isotopes Laboratories.

## Sample/Reagent Prep

1. 2 ml Urine samples diluted to 5 mls DI water, pH adjusted to 2 w/HCL.

## SPE Procedure

1. Condition cartridges with 5 mls Methanol at 2ml/min
2. Condition cartridges with 5 mls DI Water at 2ml/min
3. Sample loaded across cartridge at 2.5 ml/min
4. Cartridge washed with 5 ml Water.
5. Cartridge washed with 5 ml Methanol.
6. Cartridges dried with Nitrogen for 20 minutes
7. Cartridges eluted with 4 mls 3:2 methanol – acetonitrile with 5% ammonium hydroxide
8. Cartridges allowed to soak for 3 minutes
9. Cartridges eluted with 3 mls 3:2 methanol – acetonitrile with 5% ammonium hydroxide
10. Remaining elution solvent N2 purged to collection vials.

## SuperVap

1. Preheat temperature: 35 °C
2. Evap mode: 8 PSI Nitrogen with sensor
3. Extracts reduced to 1ml volume

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**SuperVap Vial Evaporator**
At 1ml, GC vials removed from SuperVap tubes and transferred to Vial Evaporator.

Extracts taken to dryness at 2 PSI, ambient temperature.

Extracts reconstituted with recovery standard in initial mobile phase.

**Analytical Conditions**

Waters Acquity H-Class UPLC
Column: Waters BEH $C_{18}$, 2.1 x 100 mm, 1.7 um
Column temperature: 30 $^{O}$C
Solvent A: 0.1% formic acid in MilliQ water
Solvent B: 0.1% formic acid in acetonitrile
Gradient

| Time (min) | Flow (ml/min) | %A | %B | Curve |
|---|---|---|---|---|
| 0 | 0.4 | 98 | 2 | |
| 6 | 0.4 | 52.8 | 47.2 | 6 |
| 7 | 0.4 | 52.8 | 47.2 | 6 |
| 7.5 | 0.4 | 98 | 2 | 6 |
| 8.5 | 0.4 | 98 | 2 | 6 |

Xevo TQD mass spectrometer conditions
Ionization mode: ESI+ and ESI-
Acquisition mode: MRM
Capillary voltage: 1.5 kV
Collision energy (eV): optimized for individual compounds
Cone voltage (V): optimized for individual compounds
Data: acquisition and analysis using MassLynx v.4.1 software

**Results**

| Compound | Percent recovery | RSD |
|---|---|---|
| Amphetamine | 111 | 10.2 |
| Buprenorphine | 114 | 8 |
| Cocaine | 103 | 6.2 |
| Codeine | 100 | 13.6 |
| Fentanyl | 89 | 11 |
| Hydrocodone | 114 | 14.2 |
| Hydromorphone | 92 | 11.2 |
| MDMA | 97 | 9.8 |
| Merperidine | 97 | 7.2 |
| Methadone | 90 | 8.6 |
| Methamphetamine | 100 | 7.2 |
| Naloxone | 106 | 6.1 |
| Naltrexone | 97 | 6.9 |
| Oxycodone | 94 | 6.8 |
| Oxymorphone | 96 | 0.5 |
| PCP | 102 | 4.8 |
| Tramadol | 94 | 2.8 |

**Conclusions**
Extraction efficiencies for DOA compounds in human urine matrix showed excellent precision using the mixed mode cartridge. Between replicates, RPDs were below 15% for all analytes with most falling below 10%. With the easy operation, limited sample prep and excellent performance the FMS, Inc EconoTrace SPE demonstrates to be an ideal fit for DOA screening of human urine.

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