

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 derivatization 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 (AutoSPE® DOA, FMS Inc.) can greatly assist production labs generate large volumes of samples in relative short periods of time.

Instrumentation

- FMS, Inc. AutoSPE® DOA 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 AutoSPE® DOA: the system can run up to 6 samples simultaneously.

Consumables

- 150mg 6cc DOA Mixed Mode SPE cartridges
- Acetonitrile, LC/MS grade or equivalent
- Methanol, LC/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. Cartridges washed with 5 mL Water.
5. Cartridges 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



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 C₁₈, 2.1 x 100 mm, 1.7 μ m

Column temperature: 30 °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 < 15% for all analytes with most <10%.

With the easy operation, fast extraction and excellent performance the FMS, Inc AutoSPE® DOA demonstrates to be an ideal fit for DOA screening of human urine.

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