Evaluation of Novel Solid Phase Micro Extraction Sample Preparation Method for LC-MS Analysis of Drugs of Abuse in Urine and Plasma Samples for Forensics

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Poster Note 64702

Overview

Purpose: The novel sample preparation method using solid phase micro extraction (SPME) coupled to LC-MS analysis was evaluated for analysis of 20 chemically diverse drugs of abuse in urine and plasma samples for forensic toxicology.

Methods: After initial, simple sample preparation steps, urine and plasma samples were extracted using SPME procedure. Processed samples were analyzed with 20 min LC-MS method using Thermo Scientific™ Q Exactive™ high resolution mass spectrometer for compound detection and identification.

Results: Obtained data showed suitability of SPME technique for sensitive analysis of drugs of abuse in urine and plasma samples. Limits of quantifications were in range 0.1-5 ng/mL in both matrixes.

Introduction

Solid Phase Extraction (SPE) is the most commonly used sample preparation technique for analysis of drugs of abuse in biological matrixes. Developing SPE method for wide range of chemically diverse compounds is very challenging and requires advanced analytical skills. The developed SPE methods are complex, requiring collection of multiple fractions and usually evaporation and reconstitution steps to obtain low limits of quantitation.

Here we evaluated a new, simple and economical approach using SPME for analysis of drugs of abuse in urine and plasma.

Table 1 presents compounds and corresponding internal standards which were used in method performance evaluation experiments.

TABLE 1. List of analyzed compounds and internal standards.

No	Analyte	M+H	Internal Standard
1	Amphetamine	136.1121	Salbutamol-d3
2	Bisoprolol	326.2326	Salbutamol-d3
3	Buprenorphine	468.3108	Cocaine-d3
4	Cocaine	304.1543	Cocaine-d3
5	Codeine	300.1594	Codeine-d3
6	Diazepam	285.0789	Diazepam-d5
7	GW501516	454.0757	THCCOOH-d3
8	Lorazepam	321.0192	Diazepam-d5
9	Methadone	310.2169	THCCOOH-d3
10	Methamphetamine	150.1280	Salbutamol-d3
11	Metoprolol	268.1911	Salbutamol-d3
12	Morphine	286.1441	Salbutamol-d3
13	Nordazepam	271.0633	Diazepam-d5
14	Oxazepam	287.0582	Diazepam-d5
15	Oxycodone	316.1547	Oxycodone-d3
16	Propanolol	260.1649	Salbutamol-d3
17	Salbutamol	240.1598	Salbutamol-d3
18	Stanozolol	329.2591	Testosterone-d3
19	THCCOOH	345.2063	THCCOOH-d3
20	Trenbolone	271.1696	Testosterone-d3

Calibration standards and QC samples

Calibrations standards at concentrations of 0.1, 0.2, 0.5, 1.0, 5.0, 20, 50 and 100 ng/mL and QC samples at concentrations of 1, 10 and 80 ng/mL were prepared in pooled donor urine and pooled plasma.

Methods

Sample preparation:

Initial sample preparation for urine:

Mix: 1000 µL of urine + 200 µL of phosphate buffer pH 6.6 + 10 µL of internal standard spiking solution.

Initial sample preparation for plasma:

Mix: 250 µL of plasma + 750 µL of water + 10 µL of internal standard spiking solution.

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SPME extraction (Figure 1)

- Blades: single coated HLB (Hydrophylic Lipophilic Balance), 60 μm particles.
- Condition blades in 1.5 mL of 50% methanol.
- Extract prepared samples.
- Wash blades in 1.5 mL of water.
- Desorb from blades in 1 mL of methanol/acetonitrile/water (60/20/20) (v/v/v.)
- All steps performed at room temperature.
- Sample preparation was automated with a Concept 96™ workstation (PAS Technology™)(Figure 2).
- 96-well format for high throughput
- Processing time: 1.3 min/sample

Figure 1. Workflow of SPME method

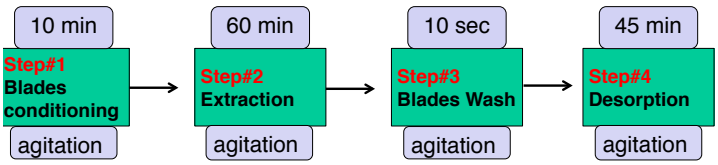
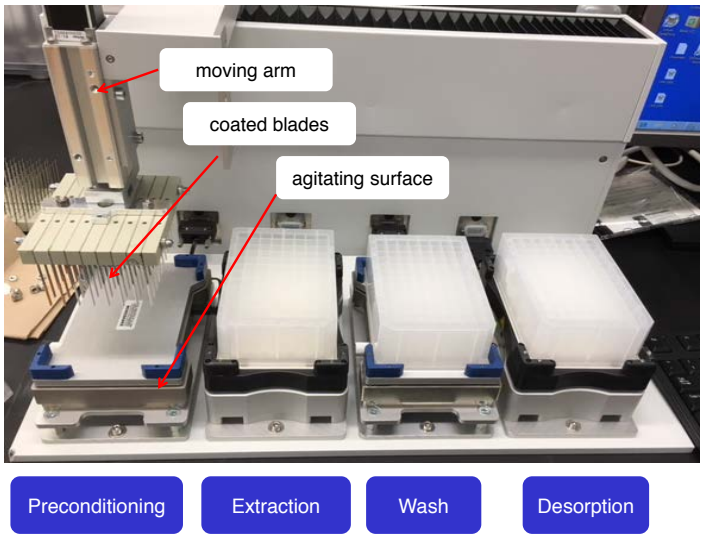


Figure 2. Automated system for SPME extraction



LC method

- Column: Thermo Scientific™ Accucore ™ PFP 100x2.1mm
- Gradient: 20 min total time
- MFA: 0.1% FA, MFB: 0.1% FA in MeOH/ACN=1/1 (v/v)
- Injection volume: 10 μL

MS method

- HESI source in positive ionization mode
- Full scan in range 120-500 m/z
- Resolution: 60K (FWHM at 200 m/z)

Methods performance evaluation

Limit of quantitation: The lowest calibration standard prepared in pooled donor urine and pooled plasma analyzed with accuracy and precision ≥20%RSD.

Matrix effects: % Recovery in nine donor urine samples and five plasma samples analyzed in duplicates and spiked to concentration of 10 ng/mL with all analytes after SPME procedure calculated against the same concentration in water.

Results

Obtained results indicate that SPME is a sample preparation technique giving sensitive and precise LC-MS analysis of drugs c abuse in urine and plasma (Table 2). Matrix effects were observe in urine samples (Table 3) and were corrected by internal standards for those compounds for which deuterated analogs we analyzed (shown by accuracy of QC samples). Negligible matri effects were observed in plasma samples. The data indicates tha deuterated internal standards for each analyte are required in urine analysis but not required in plasma analysis.

TABLE 2. Limits of quantitation and intra-assay precision obtained for Low QC at 1 ng/mL and *10 ng/mL .

No	Analyte	LOQ urine	LOQ plasma	%RSD urine	%RSD plasma
1	Amphetamine	0.2	0.2	<12.0	<3.9
2	Bisoprolol	0.2	1	<5.3	<3.8
3	Buprenorphine	0.1	0.2	<4.5	<8.9
4	Cocaine	0.1	0.2	<4.7	<8.4
5	Codeine	0.1	0.1	<5.1	<3.1
6	Diazepam	0.1	0.1	<2.4	<4.4
7	GW501516	0.1	0.2	<3.2	<12.6
8	Lorazepam	0.1	0.1	<2.1	<4.4
9	Methadone	0.1	0.2	<6.5	<7.7
10	Methamphetamine	0.2	0.1	<5.4	<3.4
11	Metoprolol	5	0.2	<3.0*	<3.3
12	Morphine	0.5	0.1	<3.5	<1.3
13	Nordazepam	0.1	0.1	<3.3	<3.6
14	Oxazepam	0.1	0.1	<1.4	<2.2
15	Oxycodone	5	0.1	<3.5*	<4.0
16	Propanolol	5	0.2	<3.1*	<2.7
17	Salbutamol	1	0.1	<1.0*	<1.8
18	Stanozolol	0.1	0.1	<2.6	<6.3
19	THCCOOH	0.2	20	<4.0	<2.8
20	Trenbolone	0.2	0.1	<3.8	<2.5

TABLE 3. Matrix effects and accuracy of Low QC samples (1ng/mL, *10 ng/mL and **80 ng/ml).

No	Analyte	%Recovery urine	%Recovery plasma	Accuracy Low QC urine	Accuracy Low QC plasma
1	Amphetamine	44.9-65.9	81.7-86.6	<14.9	<8.5
2	Bisoprolol	69.4-100	86.6-91.1	<7.1	<10.4
3	Buprenorphine	30.3-81.3	87.3-93.8	<26.7*	<-19.7
4	Cocaine	79.3-96.5	84.7-92.0	<11.5	<-10,9
5	Codeine	52.2-71.8	79.0-86.4	<8.0	<-6.9
6	Diazepam	51.1-77.5	93.3-98.9	<6.2	<7.3
7	GW501516	78.6-92.4	90.6-96.8	<24.7*	<-19.1
8	Lorazepam	62.7-101	91.4-97.8	<-4.9	<11.7
9	Methadone	63.1-96.1	80.6-86.1	<26*	<17.0
10	Methamphetamine	51.6-87.9	80.3-86.5	<-9.0	<9.4
11	Metoprolol	73.6-92.5	81.1-88.8	<10.1	<-7.1
12	Morphine	34.6-52.6	81.7-85.6	<13.9	<-3.5
13	Nordazepam	51.2-73.0	82.5-94.0	<4.4	<13.5
14	Oxazepam	61.2-77.0	91.3-98.4	<-5.5	<4.8
15	Oxycodone	52.4-76.6	78.5-85.6	<10.9	<-7.4
16	Propanolol	27.1-73.9	78.6-84.0	<20.8*	<13.3
17	Salbutamol	68.7-91.3	90.5-93.9	<1.3	<-5.8
18	Stanozolol	70.3-87.9	90.0-97.2	<-3.7	<-18.8
19	THCCOOH	85.3-97.2	92.3-119	<9.3	<-5.3**
20	Trenbolone	40.9-68.1	92.2-100	<17.6	<5.8

Conclusion

Obtained data show that SPME is a sample preparation techniqu that can yield sensitive, accurate, economical and high throughpu LC-MS analysis of chemically diverse range of drugs of abuse in plasma and urine samples for forensic toxicology.

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