LC/MS Application Note

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Rapid Screening of Amphetamine Drugs in Urine by Positive Ion Electrospray LC/MS/MS

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Introduction

Amphetamine drugs are often abused and misused in sports, in the work place, and by recreational users. Forensic, clinical, and doping laboratories are frequently asked to analyze for the presence of amphetamines in urine. Urine samples are most common because large sample volumes can be collected non-invasively. These drugs generally remain detectable in urine for two to three days longer than in blood. For most clinical and forensic applications, initial screening is done by immunoassay with presumptive positive samples confirmed by a second, more specific method such as gas chromatography/mass spectrometry (GC/MS).

A simple and sensitive LC/MS/MS method is described below for high throughput identification and quantitation amphetamine drugs in urine. A rapid and effective solid-phase extraction (SPE) procedure using Focus™ was used to extract amphetamines from urine samples.

Instrumentation

- Varian ProStar 410 AutoSampler
- Varian ProStar 210 Isocratic Solvent Delivery Modules
- Varian 1200L LC/MS equipped with ESI source

Materials and Reagents

- Standard solutions: 1.0 mg/mL ((±)-Amphetamine, (±)-Methamphetamine, 1S,2R(+)-Ephedrine, (±)-MDMA, (±)-MDA and (±)-MDEA), from Cerilliant Corp., Texas, USA.
- Internal standard (IS) solutions: 0.1 mg/mL ((±)-Amphetamine-D5, (±)-Methamphetamine-D5, 1S,2R(+)-Ephedrine-D3 HCl, (±)-MDA-D5, (±)-MDMA-D5 and (±)-MDEA-D5), from Cerilliant Corp., Texas, USA.
- All other chemicals are reagent grade or HPLC grade.
- Focus™ Solid Phase Extraction Cartridges (Varian Part No. A5306021).
- In-house vacuum or vacuum pump (Varian Part No. WL2012B01).
- Vac Elut 20 Manifold with the standard Glass Basin (Varian Part No. 12234505) and Collection Rack for 13 x 75 mm test tubes (Varian Part No. 12234507).

Sample Preparation

A 100 µL aliquot of a 500 ng/mL deuterated internal standards solution was transferred into individually labeled tubes (double blank tube was urine only). To each tube, a 1 mL of urine sample followed by 0.1 mL of 0.1 N KOH solution was added and mixed by vortex.

The mixture was loaded onto the sorbent bed of an activated 3 mL Focus cartridge pretreated with 1 mL of methanol followed by a 1 mL deionized water wash under gentle vacuum of1 to 2 in. Hg. Next, the sorbent bed was washed with 2 x 1 mL acetonitrile/water (10:90, v/v) under gentle vacuum.

The analyte was collected in a 2 mL autosampler vial by eluting with 2 x 100 µL elution solvent (acetonitrile/methanol/water/formic acid (22:68:9:1, v/v) under gentle vacuum. The sorbent bed was then flushed with 600 µL of water under vacuum to wash off the elution solvent and dilute the sample for injection. A 10 µL aliquot was injected directly for analysis.

HPLC Conditions

Column	MonoChrom MS 5 µm, 50 x 2 mm (Varian Part No. A2080050X020)			
Mixer	250 µL static mixer			
Solvent A	0.2% formic acid:10mM NH_4OAc in water (v/v)			
Solvent B	acetonitrile/methanol (1:1, v/v)			
LC Program	Time (min:sec)	%A	%B	Flow (mL/min)
	0:00	75	25	0.25
	6:00	75	25	0.25
Injection Volume	10 µL			
Injection Solvent	acetonitrile/methanol/water/formic acid (5.5:17:77.25:0.25, v/v)			

MS Parameters

Ionization Mode	ESI positive
Collision Gas	2.0 mTorr Argon
API Drying Gas	30 psi at 380 °C
API Nebulizing Gas	59 psi
Scan Time	1.8 sec
SIM Width	0.7 amu
Needle	5000V
Shield	600V
Capillary	30V
Detector	1800V

Scan Parameters

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (V)
(±)-Amphetamine	136	91	14.0
	136	119	6.5
(±)-Amphetamine-D5	141	96	12.5
(±)-Methamphetamine	150	91	17.0
	150	119	9.0
(±)-Methamphetamine-D5	155	92	16.5
1S,2R(+)-Ephedrine	166	117	17.0
	166	148	10.0
1S,2R(+)-Ephedrine-D3	169	151	9.5
(±)-MDA	180	105	20.5
	180	163	9.0
(±)-MDA-D5	185	168	9.0
(±)-MDMA	194	135	19.0
	194	163	10.0
(±)-MDMA-D5	199	165	10.5
(±)-MDEA	208	135	18.0
	208	163	11.5
(±)-MDEA-D5	213	163	12.0

MRM Chromatograms of Amphetamines

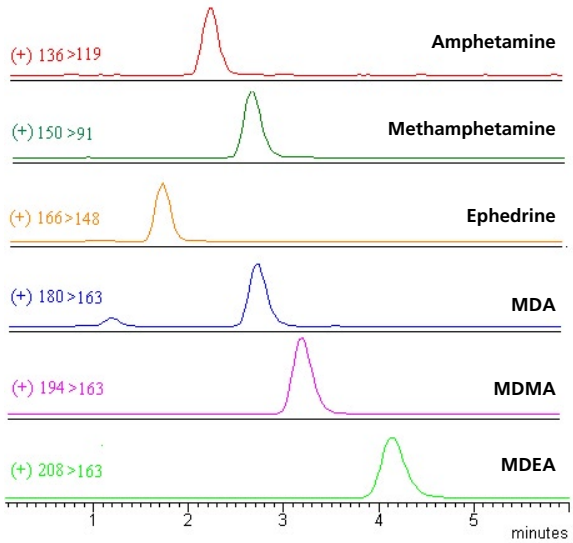

Figure 1. Good separations with short run time and no matrix interferences. Sample: spiked 50 ng/mL in urine.

Example of a Tox Report for Methamphetamine

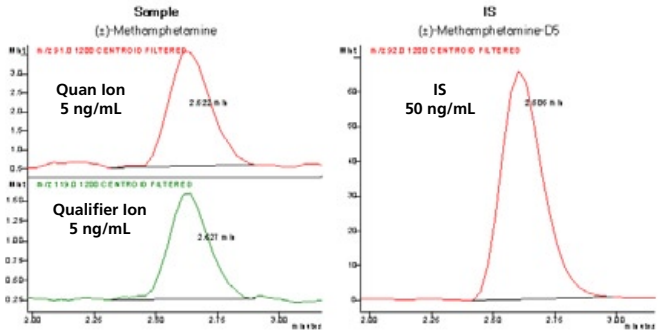

Figure 2. The positive identification was confirmed by retention time matching of the Quan ion with the confirmatory qualifier ion. The IS was used to measure and calculate recovery. Also, the IS was used to provide additional confirmation by retention time as a reference marker.

Example of a Standard Calibration Curve for Methamphetamine

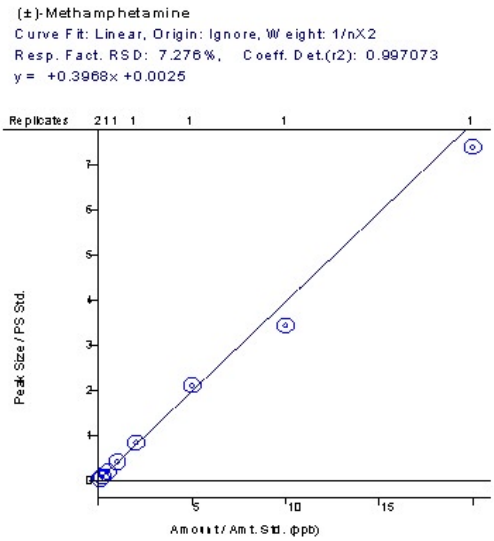

Figure 3. Eight calibration levels (5, 10, 25, 50, 100, 250, 500, and 1000 ng/mL) standard with 50 ng/mL internal standard.

Example of Breakdown Curve for Methamphetamine

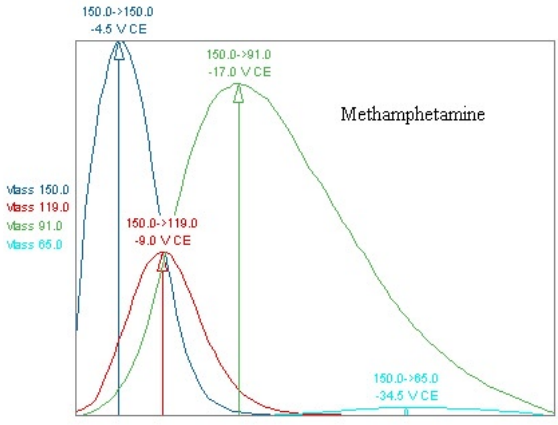

Figure 4. In this typical MS breakdown curve, methamphetamine gives two intense product ions, 150>91 and 150>119.

Results and Discussion

The LC method used a six-minute run cycle time with the first peak at 1.65 minutes and the last peak at 4.12 minutes (Figure 1). The two product ions for each analyte can be quantitatively analyzed at the level of 5 ng/mL in urine (Figure 2, about 50 pg on-column). This level is 50 times below the proposed drug cutoff levels published by the Substance Abuse and Mental Health Services Administration (SAMHSA).[1] Eight concentration levels were used to generate the calibration curves for the standard. The linearity of the detector response and the response factor-Relative Standard Deviation (rf-RSD) are excellent (Table 1, Figure 3).

The recovery of the drugs from urine was > 85%. The eluent from the Focus cartridge can be injected directly into LC/MS system without derivatization, evaporation, and reconstitute steps. The 96-well format Focus can be used for automation and high-throughput screening.

Only two product ions were used for this analysis because amphetamine and methamphetamine only give two intense product ions (Figure 4, Table 2) while ephedrine, MDA, MDMA, and MDEA produce multiple intense product ions (Table 2). Run-to-run retention time is very reproducible with a <1.4% RSD. Two product ions with a retention time match can be strong evidence for positive identification of amphetamine drugs (Figure 2). Both the urine double blank and the blank with IS show no interference of the analysis at low quantitation level (LQL). For the standard calibration curve, the LQL is 5 ng/mL and upper quantitation level (UQL) is 1000 ng/mL. This LC/MS/MS method is very sensitive and can be possibly adapted to other body fluid analysis for amphetamines, such as sweat and oral fluid which have confirmatory drug cutoff levels of 25 ng/mL and 50 ng/mL, respectively.

Conclusion

The LC/MS/MS method described in this application note is simple and sensitive. This method can quantitatively analyze amphetamine drugs at 50 times below the drug cutoff levels in urine. The Varian SPE and 1200L LC/MS/MS system demonstrated excellent performance for the urinalysis of amphetamines. The system can be a useful tool for forensics, clinical, and doping laboratories.

Reference

1. http://workplace.samhsa.gov/ResourceCenter/DT/FA/GuidelinesDraft4.htm

*ng/mL=ppb

Combined Results of LC/MS Study of Amphetamines

	Retention Time		Curve Parameters				Drug Cutoff Levels in Urine	
Drug Name	min	RSD (%)	R^2	rf-RSD (%)	LQL (ng/mL)	S/N (5 ng/mL)	Initial (ng/mL)	Confirmatory (ng/mL)
Amphetamine	2.16	0.91	0.995	7.96	5	163	500	250
Methamphetamine	2.57	0.94	0.997	7.29	5	1242	500	250
Ephedrine	1.65	0.94	0.999	3.79	5	1031	500	250
MDA	2.67	1.22	0.999	9.39	5	521	500	250
MDMA	3.14	1.24	0.999	1.86	5	729	500	250
MDEA	4.12	1.40	0.999	4.84	5	440	500	250

Table 1. Run-to-run retention time over 13 injections was very reproducible. The linearity of the detector response and the response factor-RSD are excellent. The LQL of this method is 50 times below the proposed drug cutoff levels as published by the SAMHSA.

Summary of Breakdown Data by Ion Transition, Ion Intensity, and Collision Energy (V)

Amphetamine	Methamphetamine	Ephedrine	MDA	MDMA	MDEA
136>91, 100%, -14.5	150>150, 100%, -4.5	166>148, 100%, -10.0	180>163, 100%, -9.0	194>163, 100%, -10.0	208>163, 100%, -11.5
136>136, 64.26%, -4.5	150>91, 88.54%, -17.0	166>166, 75.85%, -4.0	188>188, 50.46%, -4.0	194>194, 95.26%, -4.5	208>208, 89.31%, -4.0
136>119, 63.06%, -7.5	150>119, 43.65%, -9.0	166>117, 15.39%, 17.0	180>105, 32.34% -20.5	194>105, 33.3%, -22.0	208>105, 31.77%, -23.0
136>65, 3.99%, -32.5	150>65, 2.26%, -34.5	166>115, 14.5%, -24.0	180>133, 29.25%, -16.0	194>135, 29.93%, -19.0	208>135, 29.97%, -18.0
		166>133, 13.68%, -18.5	180>135, 29.5%, -16.0	194>133, 29.74%, -18.5	208>133, 29.11%, -18.0
		166>91, 8.82%, -29.0	180>77, 6.83%, -32.0	194>77, 6.34%, -35.5	208>103, 8.03%, -33.0

Table 2. Amphetamine and methamphetamine only give two intense product ions while ephedrine, MDA, MDMA, and MDEA produce multiple intense product ions.

These data represent typical results.
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