Quantitation of Bath Salts/Cathinones in Urine by LC-MS/MS

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Key Words

Cathinone, bath salts, designer drugs, MDPV, methylone, mephedrone, ethylone, butylone, naphyrone, methedrone, TSQ Quantum Ultra, liquid/liquid extraction, forensic toxicology

Goal

To develop an LC-MS/MS method for the analysis of the three Schedule I cathinones (MDPV, methylone and mephedrone), as well as other substituted cathinones (methedrone, ethylone, butylone and naphyrone) in urine with LOQs of 1 ng/mL for forensic toxicology.

Introduction

Substituted cathinones, sometimes know as "bath salts," have become the latest abused designer drugs. Based on cathinone, a substance found in the African *Catha edulis* (khat) plant, substituted cathinones are stimulants with amphetamine- and cocaine-like effects. As with many designer drug classes, variations on base structure abound (Figure 1). On October 21, 2011 the United States Drug Enforcement Agency (US DEA) listed three of the most common substituted cathinones: methylenedioxy-pyrovalerone (MDPV), methylone, and mephedrone, as Schedule I drugs, thereby making them illegal. As these drugs are not detected by current ELISA drug screening tests, new methods are needed to detect and quantify them.

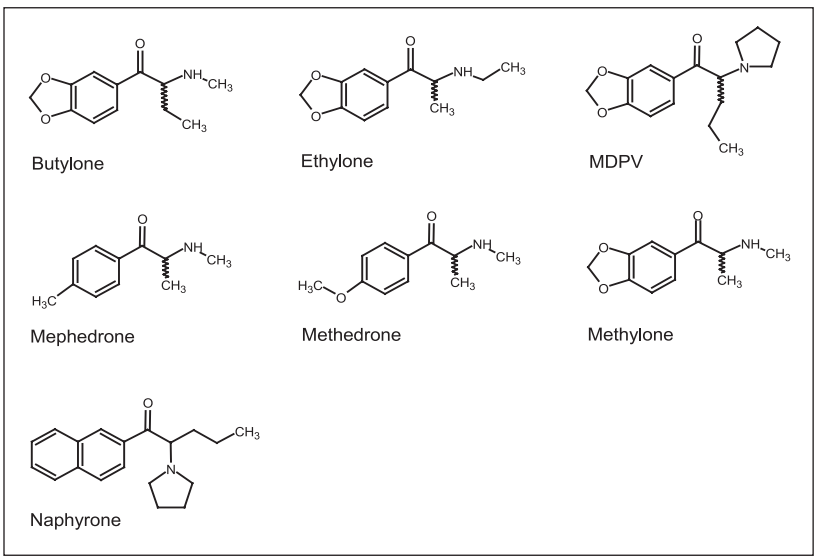

Figure 1. Structures of substituted cathinones (bath salts)

Experimental

Sample Preparation

Deuterated internal standards were available for all compounds except methedrone and naphyrone. Butylone-d_3 was used as internal standard for methedrone and MDPV-d_8 was used for naphyrone.

Sample preparation was a liquid-liquid extraction (LLE). First, 200 µL of urine and 10 µL of internal standard mix solution (2 µg/mL of each deuterated IS) were basified with 100 µL of 1 N NaOH. Extraction was performed by adding 1 mL of ethylacetate/hexane (1:1), mixing, and centrifuging. Then, an 800 µL aliquot of the resulting supernatant was transferred to a clean test tube containing 20 µL of DMSO to prevent complete evaporation of solvent. Analytes have low molecular weight, are slightly volatile, and will evaporate if left too long in the evaporator. The supernatant was evaporated at 37 °C under nitrogen for 15 minutes. Samples were diluted with 200 µL of 5% methanol and transferred to an HPLC vial equipped with a limited-volume insert. Finally, 20 µL was injected into the LC-MS system.

Liquid Chromatography

Chromatographic separations were performed under gradient conditions using a Thermo Scientific™ Accela™ 1250 pump and Accela Open autosampler. The analytical column was a Thermo Scientific™ Hypersil GOLD™ column (50 x 2.1 mm,1.9 µm particle size). The column was maintained at room temperature. The injection volume was 20 µL. Mobile phases A and B consisted of 10 mM ammonium formate with 0.1% formic acid in water and methanol, respectively. Mobile phase C was acetonitrile/1-propanol/acetone (45:45:10). All mobile phases were Fisher Chemical™ brand solvents. A shallow gradient at a flow rate of 500 µL/min was used to separate isomeric ethylone and butylone. The total run time was 5 minutes.

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Mass Spectrometry

MS analysis was carried out using a Thermo Scientific™ TSQ Quantum Ultra™ triple-stage quadrupole mass spectrometer equipped with a heated electrospray ionization (HESI-II) probe. Two selected-reaction monitoring (SRM) transitions were monitored for each analyte and each deuterated internal standard to provide ion ratio confirmations (IRC). Data acquisition and processing were performed using Thermo Scientific™ TraceFinder™ software.

Validation

Standard calibration curves were prepared by fortifying pooled blank human urine with analytes. Quality control (QC) samples were prepared in a similar manner at low (LQC), middle (MQC), and high (HQC) concentrations. Intrarun variability and robustness were determined by processing six replicates of each QC level along with a calibration curve, as outlined in the Sample Preparation section, on three different days. Matrix effects were investigated by comparing peak areas of analyte at 10 ng/mL and internal standard prepared in twelve different lots of urine to those of a sample prepared in water.

Results and Discussion

MDPV, methylone, mephedrone, methedrone, ethylone, and butylone were all linear from 1–1000 ng/mL. Figure 2 shows representative calibration curves for all compounds tested. Figure 3 shows representative chromatograms at 1 ng/mL for all compounds. Interassay quality control statistics shown in Table 1 demonstrate the method to be reproducible across the calibration range for the above compounds. Limited matrix effects were seen for the above compounds. These effects were largely mediated by deuterated internal standards. The absolute recoveries of all cathinones tested in various lots of urine, compared to a sample prepared in water, ranged from 85% to 132%. Relative recoveries ranged from 107% to 124%. Precision across all lots also improved when deuterated internal standards were used. Table 2 shows average statistics for all lots showing improvement in both precision and accuracy when internal standards were used.

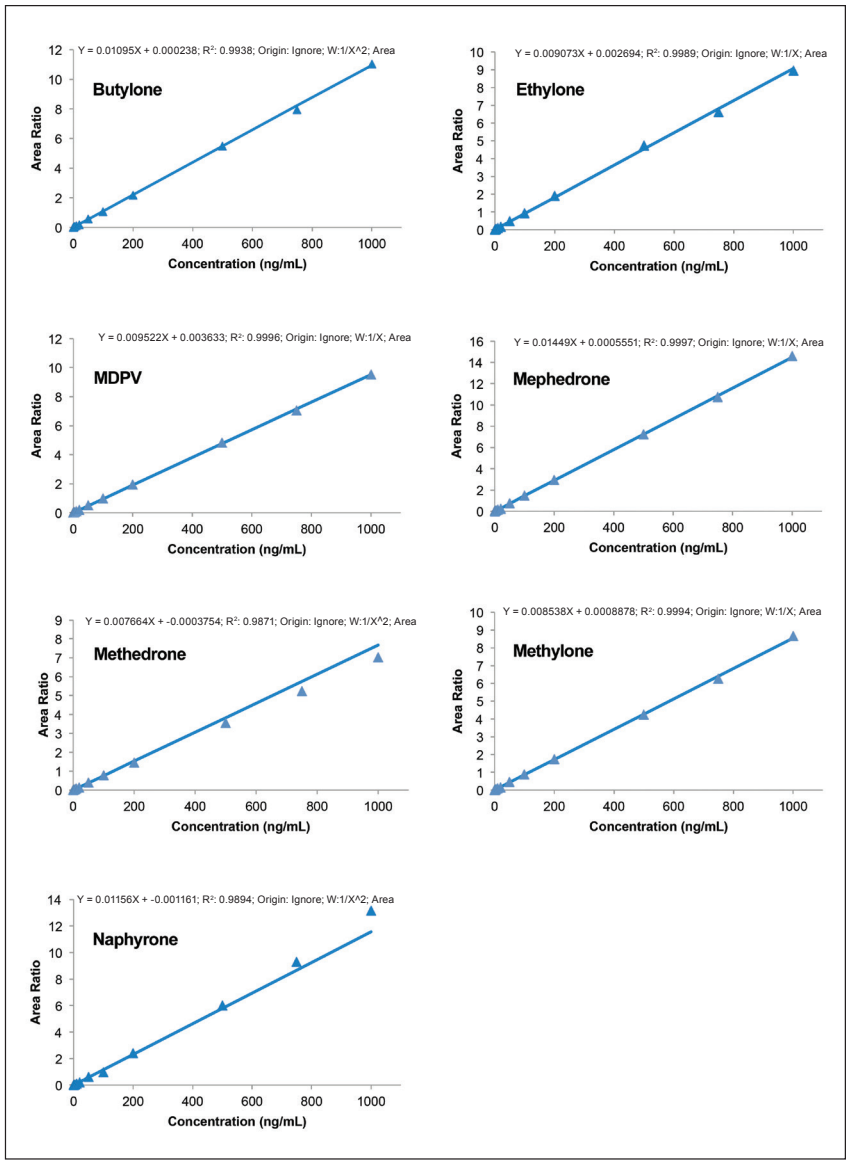

Figure 2. Representative calibrations curves for cathinones in urine

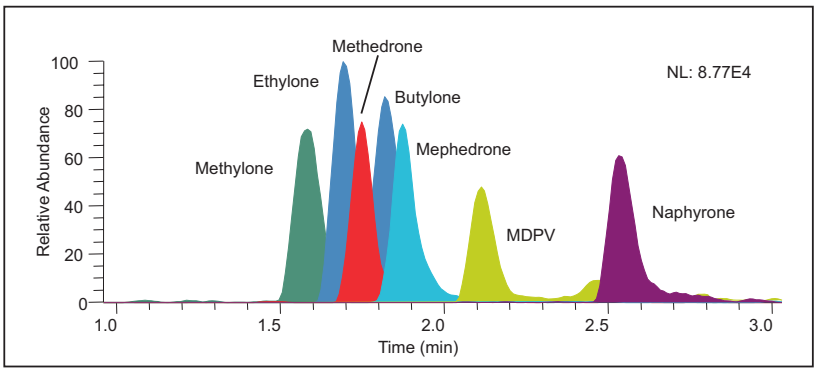

Figure 3. Representative chromatogram of cathinones at 1 ng/mL

Table 1. Interassay QC results

	LQC		MQC		HQC	
n = 18	%Bias	%CV	%Bias	%CV	%Bias	%CV
butylone	-5.80%	5.13%	1.40%	4.81%	-2.86%	3.76%
ethylone	-7.36%	5.93%	6.21%	1.72%	0.777%	1.71%
MDPV	-8.32%	5.48%	5.48%	2.89%	-0.907%	2.92%
mephedrone	-3.23%	2.79%	7.89%	2.64%	0.978%	2.00%
methedrone	-0.0565%	6.21%	8.09%	2.85%	-2.44%	1.79%
methylone	-3.95%	4.86%	6.06%	2.55%	0.394%	2.07%
naphyrone	-40.2%	59.7%	-18.2%	10.9%	-10.3%	8.68%

Table 2. Average imprecision and bias across all lots of urine

	Absolute Recovery		Relative Recovery	
	Imprecision	Bias	Imprecision	Bias
butylone	4.2%	12%	2.5%	13%
butylone-d_3	4.0%	-1.2%	na	na
ethylone	4.8%	16%	1.9%	18%
ethylone-d_5	4.9%	-1.5%	na	na
MDPV	3.0%	25%	1.6%	17%
MDPV-d_8	2.9%	7.1%	na	na
mephedrone	4.7%	21%	2.2%	16%
mephedrone-d_3	5.4%	4.3%	na	na
methedrone[1]	5.4%	18%	2.7%	19%
methylone	6.3%	15%	1.4%	17%
methylone-d_3	6.0%	-1.9%	na	na
naphyrone[2]	17%	49%	16%	39%

[1] Butylone-d_3 used as IS
[2] MDPV-d_8 used as IS

Although naphyrone was detected at 1 ng/mL, it showed more variability than the other compounds and a greater matrix effect from lot to lot. Absolute recoveries for naphyrone ranged from 113% to 207% while relative recoveries using MDPV-d_8 as internal standard ranged from 111% to 191%. All available internal standards were tried, and MDPV-d_8 showed the best results. A lack of a deuterated analog for naphyrone does not allow for matrix effect corrections and negatively affects method precision. In this assay, naphyrone should be considered qualitative.

Conclusion

We achieved our goal of a 1 ng/mL LOQ for the three DEA-regulated cathinones, MDPV, mephedrone, and methylone, as well as methylone, ethylone, and butylone in urine for forensic toxicology. Naphyrone, which shows greater variability, can be detected down to 1 ng/mL in a qualitative manner. Deuterated internal standards are essential for rigorous quantitation of these compounds.

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