Ultrafast Screen for Bath Salts in Urine Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Forensic Toxicology

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Abstract

The need for greater analytical capacity and higher throughput for the analysis of bath salts in urine for forensic toxicology has placed demands on traditional analytical technologies. An ultrafast method for screening a panel of six bath salts in urine samples was developed using an Agilent RapidFire High-throughput Mass Spectrometry(MS) System. Mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone, and methylenedioxypyrovalerone (MDPV) were accurately and precisely measured within a linear range of 31–1,000 ng/mL. Specificity was confirmed by evaluating samples in the presence of amphetamines and other interfering substances, including ephedrine and pseudoephedrine. All six analytes and a common internal standard, methylone-d3, were simultaneously measured in less than 15 seconds per sample, providing a throughput of greater than 240 samples per hour.

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Introduction

Bath salts, a new family of designer drugs, are synthetic cathinones with effects similar to amphetamines or cocaine. Bath salts can be modified structurally and synthesized easily, preventing them from being detected in regular forensic drug screening. Traditional forensic drug testing relies on immunoassay screening followed by GC/MS and more recently LC/MS for quantitative analysis. The need for greater throughput, faster turn-around times, and increased specificity have placed increased demands on these traditional technologies. The RapidFire High-throughput MS System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times under 15 seconds. In the present study, a method to screen urine for a six-drug panel (Figure 1) consisting of mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone and MDPV by ultrafast SPE/MS/MS was developed.

Experimental

Analytes

The RapidFire/MS/MS system consisted of the following modules: an Agilent RapidFire 360, an Agilent 6490 Triple Quadrupole Mass Spectrometer, MassHunter Qualitative Analysis B.05.00, and MassHunter Quantitative Analysis B.05.00.

RapidFire/MS/MS conditions

Samples were analyzed at a rate of 14 seconds per sample using the conditions shown in Table 1.

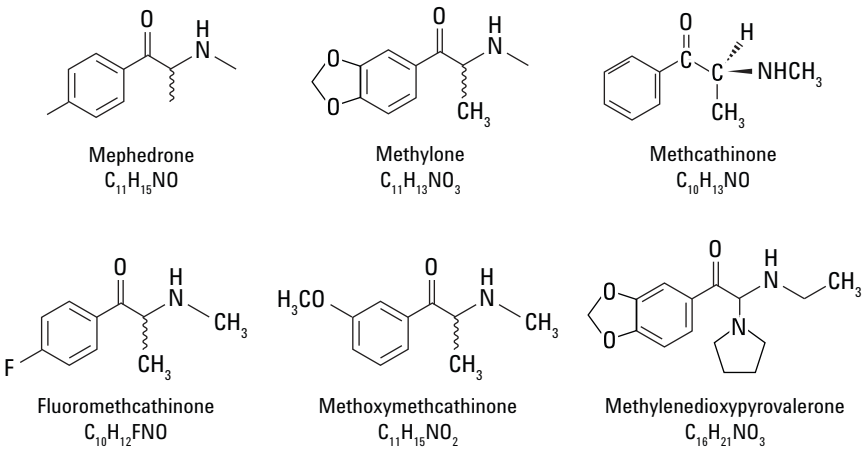

Figure 1. Structures of the six bath salts in the panel.

Table 1. RapidFire conditions.

Buffer A	Water with 0.09 % formic acid, 0.01 % trifluoroacetic acid					
Buffer B	50 % methanol, 50 % isopropanol, 0.09 % formic acid, 0.01 % trifluoroacetic acid					
Injection volume	10 µL					
SPE cartridge	Agilent RapidFire cartridge A2 (reversed-phase C4, G9210A)					
RF State 1	sip sensor					
RF State 2	3,000 ms					
RF State 3	7,500 ms					
RF State 4	500 ms					
Triple quadrupole conditions						
Gas temperature	300 °C					
Gas flow	12 L/min					
Nebulizer	45 psi					
Sheath gas temperature	350 °C					
Sheath gas flow	11 L/min					
Nozzle voltage	0 V					
Capillary voltage	4,000 V					
MRM transitions						
Analyte	**Q1**	**Q3**	**Dwell**	**Fragmentor**	**CE**	**CAV**
3,4–MDPV	276.1	205	20	380	15	3
Methylone – d3	211.1	163	20	380	15	3
Methylone	208.1	160	20	380	15	3
3-Methoxymethcathinone	194.1	161.1	20	380	20	3
3-Fluoromethcathinone	182.1	149.1	20	380	20	3
Mephedrone	178.1	145.1	20	380	20	3
S(-) Methcathinone	164.1	131.1	20	380	20	3

Figure 2 is a standard curve demonstrating injection cycle times of 14 seconds. Analyte and internal standard ions were monitored simultaneously in all experiments for all six bath salt drugs.

Chemicals and reagents

Mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone, and MDPV (All at 1 mg/mL in methanol) and mephedrone-d3 (100 µg/mL in methanol), were purchased from Cerilliant, Round Rock, TX. All other LC/MS grade solvents and reagents were purchased from Sigma-Aldrich, St. Louis, MO.

Sample preparation

Standard calibrators were prepared by spiking drug-free human urine with 1,000 ng/mL of mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone, and MDPV. Two-fold serial dilutions were used to achieve the remaining standard calibrator concentrations. A set of standard calibrators containing all six analytes within a concentration range of 31–1,000 ng/mL, as well as a negative matrix control were also spiked with 100,000 ng/mL of the same class of interfering drugs: MDA, MDMA, MDEA, amphetamine, methamphetamine, ephedrine, and pseudoephedrine.

All samples were diluted 1:10 using water containing methylone-d3 as a common internal standard (100 ng/mL final concentration). Samples were transferred to 96-well plates, centrifuged, and injected onto the Agilent RapidFire/MS/MS system.

Data analysis

MassHunter Qualitative Analysis (B.05.00) and Quantitative Analysis (B.05.00) were used for data analysis. A $1/x^2$ weighting factor was applied during linear regression of the calibration curves. The quantitation using MassHunter Quantitative software was performed by spectral peak area ratio to a known concentration of the internal standards.

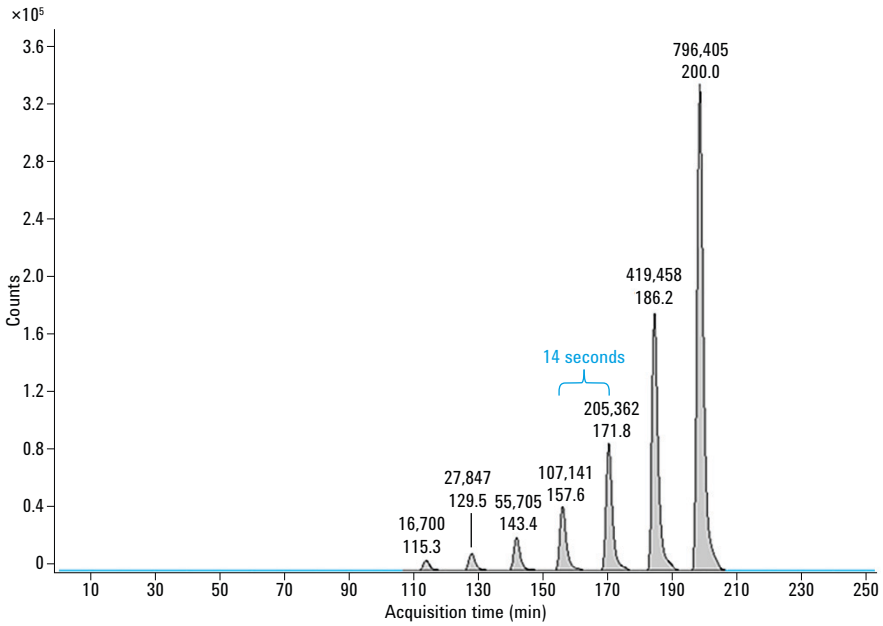

Figure 2. Standard curve showing injection cycle times of 14 seconds.

Results and Discussion

Samples were prepared by spiking a panel of six bath salts into drug-free human urine and then diluting samples 10-fold with water containing a common isotopically labeled internal standard. Prepared calibration standards were run four times a day, over a series of four days to establish both intra and interday precision and accuracy. Mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone, and MDPV had intra and interday accuracies within 15 % and coefficient of variation values less than 10 % for all concentrations within the linear range (Tables 3–8). This method had excellent linearity within the measured range of 31–1,000 ng/mL with an R^2 value greater than 0.998 (Figures 3–8) for each analyte. The limit of quantitation (LOQ) was determined to be 31 ng/mL for all six analytes.

Table 2. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of mephedrone in urine.

Mephedrone (ng/mL)	Intraday % accuracy (n=4)	Intraday % precision (n=4)	Interday % accuracy (n=4)	Interday % precision (n=4)
31.25	106.1	1.1	106.7	0.7
62.5	95.1	2.7	94.3	1.6
125	89.7	2.1	87.5	1.2
250	89.8	3.1	92.5	2.5
500	95.9	2.1	96.6	2.1
1,000	103.7	1.7	104.3	1.1

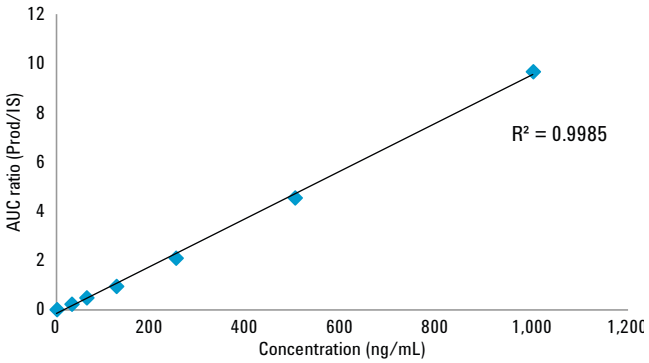

Figure 3. Representative standard curve for mephedrone.

Table 3. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of methylone in urine.

Methylone (ng/mL)	Intraday % accuracy (n=4)	Intraday % precision (n=4)	Interday % accuracy (n=4)	Interday % precision (n=4)
31.25	106.2	1.8	107.4	2.0
62.5	92.7	3.9	90.2	3.4
125	92.6	3.7	91.5	4.9
250	93.3	3.4	95.5	2.5
500	97.4	2.1	98.1	2.4
1,000	103.0	2.0	103.0	2.7

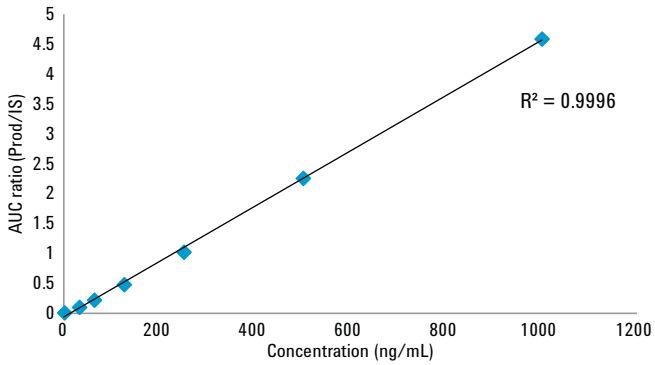

Figure 4. Representative standard curve for methylone.

No suppression was observed for any of the six analytes when 100,000 ng/mL of MDA, MDMA, MDEA, amphetamine, methamphetamine, ephedrine, and pseudoephedrine were present in the sample. Mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone, and MDPV were all accurately measured, even in the lower end of the linear range, despite the presence of high concentrations of these drugs. The negative matrix control was also tested and determined to be void of any interference from these drugs.

Table 4. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of methcathinone in urine.

Methcathinone (ng/mL)	Intraday % accuracy (n=4)	Intraday % precision (n=4)	Interday % accuracy (n=4)	Interday % precision (n=4)
31.25	102.7	2.2	103.2	2.9
62.5	101.1	4.9	100.1	6.0
125	89.3	4.6	87.4	2.0
250	93.7	2.8	96.8	3.8
500	98.9	2.8	99.4	3.7
1,000	103.9	3.2	104.9	2.9

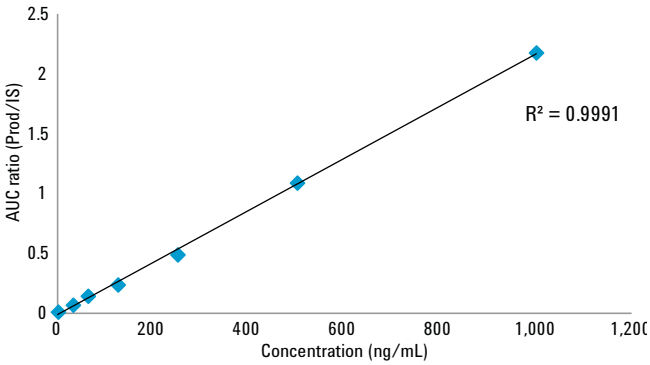

Figure 5. Representative standard curve for methcathinone.

Table 5. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of fluoromethcathinone in urine.

Fluoromethcathinone (ng/mL)	Intraday % accuracy (n=4)	Intraday % precision (n=4)	Interday % accuracy (n=4)	Interday % precision (n=4)
31.25	104.1	4.7	101.7	4.0
62.5	97.4	9.4	100.9	8.4
125	93.0	4.3	92.3	2.0
250	95.3	3.0	98.5	2.9
500	97.9	3.1	97.9	2.1
1,000	103.5	2.8	101.6	3.4

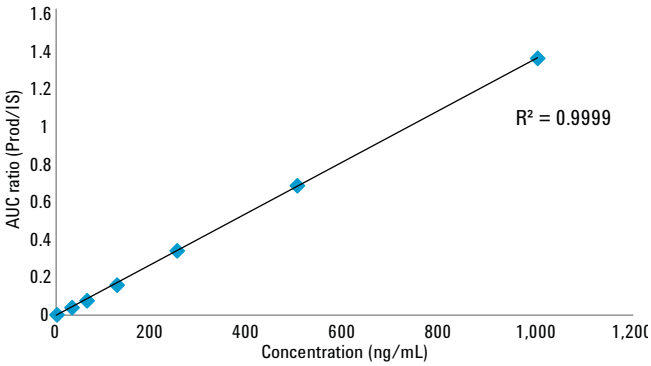

Figure 6. Representative standard curve for fluoromethcathinone.

Table 6. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of methoxymethcathinone in urine.

Methoxymethcathinone (ng/mL)	Intraday % accuracy (n=4)	Intraday % precision (n=4)	Interday % accuracy (n=4)	Interday % precision (n=4)
31.25	103.0	1.6	102.3	1.2
62.5	101.3	3.6	103.5	2.4
125	89.9	2.5	87.3	1.8
250	89.6	3.2	91.4	4.0
500	97.0	2.3	98.0	2.2
1,000	104.3	2.0	104.3	2.0

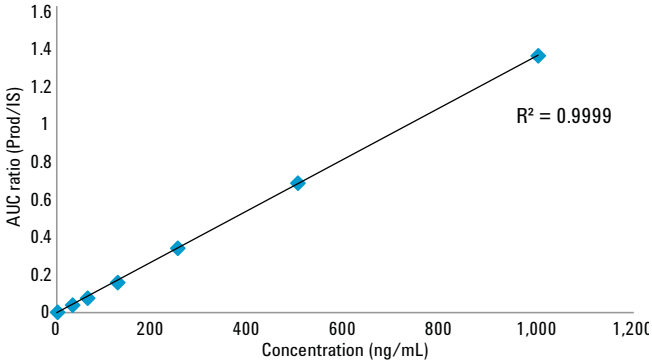

Figure 7. Representative standard curve for methoxymethcathinone

Table 7. Intraday and interday precision and accuracy for RapidFire/MS/MS analysis of MDPV in urine.

MDPV (ng/mL)	Intraday % accuracy (n=4)	Intraday % precision (n=4)	Interday % accuracy (n=4)	Interday % precision (n=4)
31.25	106.9	3.0	106.0	0.7
62.5	94.5	2.5	94.5	1.8
125	93.7	3.6	90.3	1.0
250	88.3	2.3	91.7	2.6
500	96.1	2.8	97.5	1.9
1,000	103.5	1.7	102.8	0.7

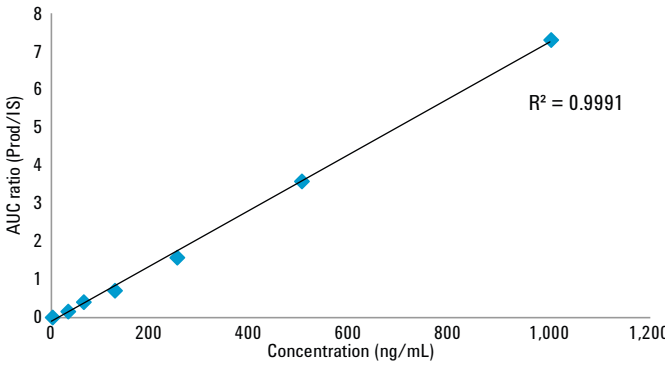

Figure 8. Representative standard curve for MDPV.

The reproducibility of the method was tested by measuring 2,000 sequential injections of all six analytes spiked into urine at 100 ng/mL. The same cartridge was used for all 2,000 injections without deviation in pump pressures or peak shape. The instrument response was stable for all six analytes. For example, methylone had a coefficient of variation of 2.05 % and accuracy within 2 % (Figure 9).

Conclusions

A panel of six bath salts that included mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone, and MDPV was quickly, accurately, and precisely quantitated in urine using a simple dilute and shoot method. With peak-to-peak injection cycle times of 14 seconds, this analytical method is capable of throughputs greater than 240 samples per hour. Using this SPE/MS/MS methodology, increased sensitivity and specificity were achieved compared to traditional screening methods without compromising throughput and speed. In addition, there is potential to add more analytes to this panel on the Agilent RapidFire/MS system.

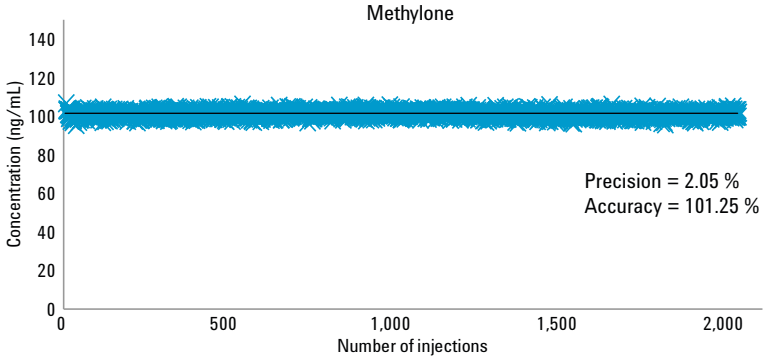

Figure 9. Repeatability evaluation using sequential injections of methylone.

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