Ultrafast Online SPE/MS/MS Screening Analysis of Bath Salts in Urine for Forensic Toxicology.

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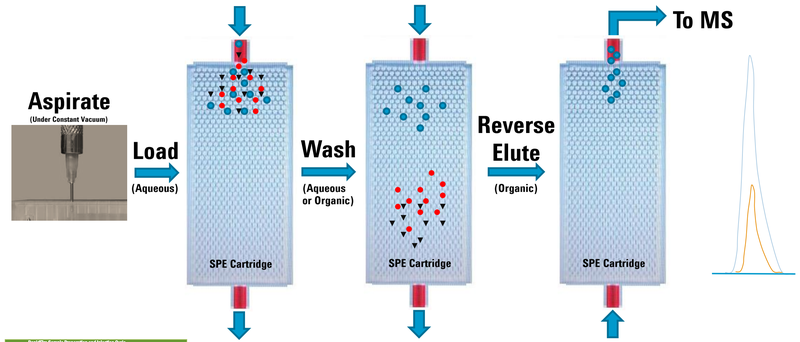
Introduction

Traditionally the screening for drugs of abuse involved analysis by immunoassays followed by a confirmatory test by GC/MS detection and more recently by LC/MS assays. The steady increase in the number of samples requiring analysis has created a bottleneck in screening for these drugs across different classes and longer turnaround times for confirmatory tests. 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 traditional forensic drug screening. Therefore, high-throughput MS-based technologies are best suited for monitoring this class of drugs of abuse. The Agilent RapidFire High-throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times of less than 15 seconds. In the present study, we evaluated the ability of this system to analyze a panel of bath salts in human urine.



RapidFire Sample Preparation and Injection Cycle
1. Sip (withdraw) sample
2. Load (inject) sample on RF Cartridge
3. Wash Cartridge (desalt)
4. Elute sample and inject into MS
5. Re-equilibrate cartridge
6. Repeat

Fig.1: Flowpath for RapidFire

Experimental

Mass spectrometry and SPE methods were optimized on an Agilent High-throughput RapidFire Mass Spectrometry System (RapidFire 300 interfaced to an Agilent 6490 QQQ in ESI Mode). Standard calibrators were prepared by spiking drug-free urine with each drug individually and as a panel (Fig.2) consisting of methylone (208.1→160), methcathinone (164.1→131.1), fluoromethcathinone (182.1→149.1), methoxymethcathinone (194.1→161.1), mephedrone (178.1→145.1) and methylenedioxypyrovalerone (MDPV) (276.1→205), which were then diluted, and injected for analysis.

Experimental

Samples were loaded onto the SPE cartridge using water with 0.09% formic acid and 0.01% trifluoroacetic acid and eluted off the cartridge using 50% methanol and 50% isopropanol with 0.09% formic acid and 0.01% trifluoroacetic acid. Sample cycle times for all compounds were under 15 seconds per sample (Fig. 4). Data analysis was performed using MassHunter Quantitative Analysis B.05.00 software.

RapidFire conditions:

Buffer A = Water with 0.09% FA, 0.01% TFA
Buffer B = 50% Methanol, 50% Isopropanol, with 0.09% FA, 0.01% TFA

Injection volume: 10 µL
SPE Cartridge: Agilent RapidFire Cartridge A2

RF State 1: sip sensor
RF State 2: 3000 ms
RF State 3: 7500 ms
RF State 4: 500 ms.

Analyte	Q1	Q3	Dwell	Frag.	CE	CAV
3,4 – MDPV	276.1	205	20	135	15	3
Methylone – d3	211.1	163	20	135	15	3
Methylone	208.1	160	20	135	15	3
3-Methoxymethcathinone	194.1	161.1	20	135	20	3
3-Fluoromethcathinone	182.1	149.1	20	135	20	3
Mephedrone	178.1	145.1	20	135	20	3
S(-) Methcathinone	164.1	131.1	20	135	20	3

Triple quadruple conditions:

Gas Temp: 300° C
Gas Flow: 12 L/min
Nebulizer: 45 psi
Sheath Gas Temp: 350° C
Sheath Gas Flow: 11 l/min
Nozzle Voltage: 0 V
Capillary Voltage: 4000 V

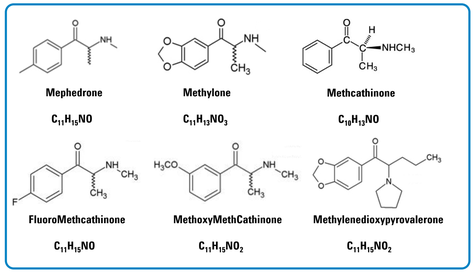
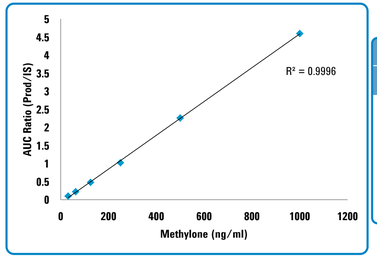


Fig.2. Structures of six bath salts in the panel.

Results and Discussion

Standard curves were prepared in a wide dynamic range 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. Each curve had excellent linearity within its measured range from 31-1000 ng/ml with R^2 values greater than 0.99 (Fig.3). Intraday accuracies were within 15% and intraday coefficient of variation values were all less than 10% for concentrations within the measured range. Ion suppression from urine was accounted for by the use of isotopically labeled internal standards. Accurate and efficient screening of bath salts samples by SPE/MS/MS could be obtained prior to confirmation analysis of this panel by LC/MS/MS. Repeatability and cartridge capacity evaluation was performed by sequentially injecting methylone at one concentration for 2000 times (Fig. 5) . The accuracy and precision were within 3% with no change in cartridge pressure indicating the reuse capabilities of a single RapidFire cartridge.

Results and Discussion



Methylone	Intraday* %		Interday* %	
Conc (ng/mL)	Accuracy (n=4)	Precision (n=4)	Accuracy (n=4)	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
1000	103.0	2.0	103.0	2.7

*1/x^2 weighing factor

Fig.3. Methylone standard curve in urine.

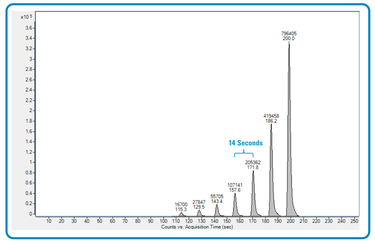


Fig.4. Injection times from peak to peak.

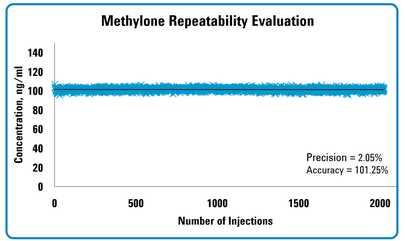


Fig.5. Repeatability and cartridge capacity evaluation.

Conclusions

- A panel of six bath salts including mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone and MDPV was quickly, accurately and precisely quantitated in urine using a simple dilute and shoot method with a potential to add more analytes to this panel on the Agilent RapidFire/MS system.

- Low ng/ml sensitivities across the drug panel were achieved without the loss of linearity and R^2 value greater than 0.99.

- Significant increases in screening efficiency at <15 seconds per sample can be achieved, with an analysis capacity of >240 samples per hour as compared to traditional screening methods.