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Ultrafast, Ultra-Selective High-Throughput Forensic Drug Screening in Urine

Vaughn P. Miller, Michelle Romm, Kari E. Schlicht, Nikunj Parikh, Mohamed Youssef, Maxcy Stroman, William A. LaMarr and Can Ozbal; Agilent Technologies Inc., Wakefield, MA

Introduction

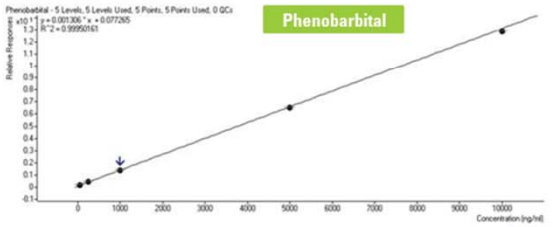
Forensic drug screening has traditionally relied on immunoassays followed by analytical confirmation with a quantitative method such as GC/MS or LC/MS. Immunoassays provide a high-throughput solution for forensic drug screening but introduce the risk of cross reactivity with common over-the-counter and prescription drugs leading to false positives. Mass spectrometry provides a highly selective and highly sensitive methodology, but often lacks the throughput and speed that is required for screening purposes. The ability to develop an ultrafast, but selective, forensic drug screens using an online SPE-MS/MS system was investigated.

Experimental

Methods were developed and optimized for screening panels of barbiturates, amphetamines, and bath salts (synthetic cathinones). The barbiturate panel consisted of (Butalbital, Phenobarbital, Secobarbital, Amobarbital, and Pentobarbital) and was screened in negative ESI mode. Amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), 3,4-methylenedioxyethylamphetamine (MDEA), bath salts (Mephedrone, methylone, methcathinone, fluoromethcathinone, methoxymethcathinone and methylenedioxypyrovalerone), were screened in positive ESI mode. Drug-free urine was spiked with known concentrations of test analytes followed by serial dilutions to create calibrators. Calibrator and quality control samples were simply diluted prior to injecting for analysis. An Agilent High-throughput RapidFire Mass Spectrometry System (RapidFire 300 interfaced to an Agilent QQQ) was used to analyze all samples.

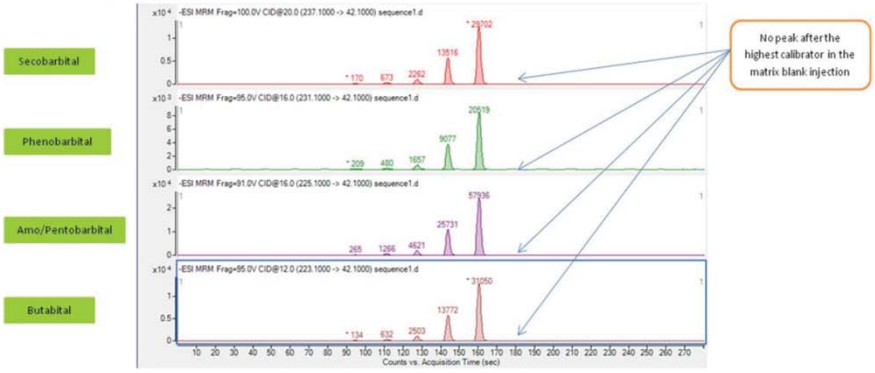
Results

Barbituates: Standard curves were analyzed to obtain intra- and interday precision and accuracy values on the Agilent RapidFire High-throughput Mass Spectrometry System. The samples were analyzed in multiplicate over a four day period. Standard curves in urine had excellent linearity within the measured range (50-10,000 ng/mL) with an R^2 value greater than 0.995. Intra- and interday accuracies determined were within 15% and coefficient of variation values were all less than 5% for concentrations within the measured range.



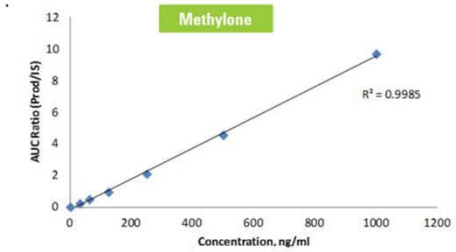
Phenobarbital (ng/mL)	Interday % Accuracy	Interday % Precision	Intraday % Accuracy	Intraday % Precision
50	100.4	5.0	101.6	2.3
250	99.0	5.0	97.9	4.5
1000	99.4	2.6	101.8	3.1
5000	99.9	5.8	101.1	5.1
10000	101.4	2.3	102.6	5.7

Standard curves of the panel of bath salts. Sample analysis from injection to injection is 15 seconds. No significant carryover was detected.



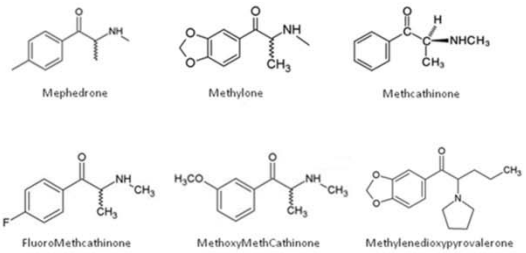
Results

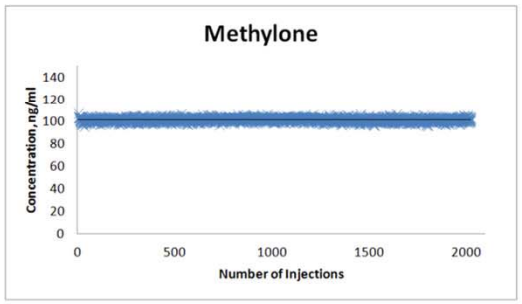
Bath Salts: Standard curves were analyzed to obtain intra- and interday precision and accuracy values on the Agilent RapidFire High-throughput Mass Spectrometry System. The samples were analyzed in multiplicate over a four day period. Standard curves in urine had excellent linearity within the measured range (31-2000 ng/mL) with an R^2 value greater than 0.995. Intra- and interday accuracies determined were within 15% and coefficient of variation values were all less than 5% for concentrations within the measured range.



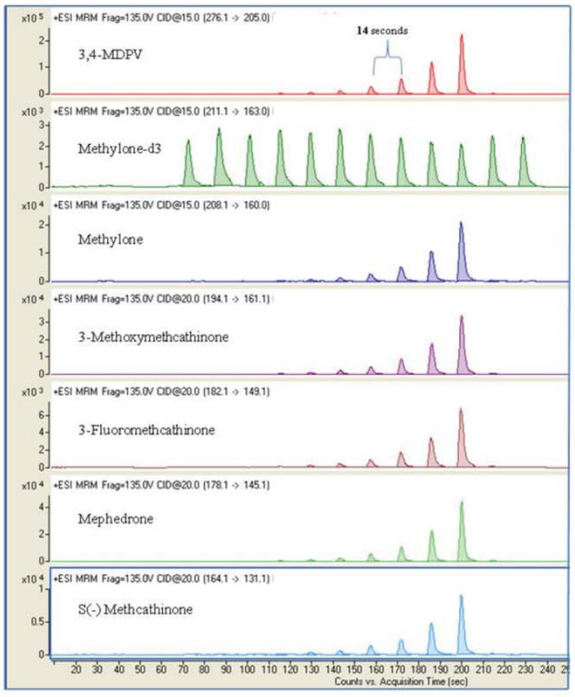
Methylone (ng/ml)	Intraday % Accuracy (n=4)	Intraday % Precision (n=4)	Interday % Accuracy (n=4)	Interday % Precision (n=4)
31.25	106.2	1.8	107.4	2.0
62.5	92.7	3.9	90.2	3.4
125	92.6	3.7	91.5	4.9
250	93.3	3.4	95.5	2.5
500	97.4	2.1	98.1	2.4
1000	103.0	2.0	103.0	2.7
2000	114.8	2.5	114.3	1.8

Structures of the six bath salts in the panel





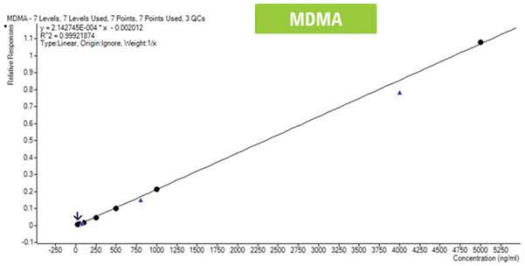
The reproducibility of the method was tested by measuring 2000 sequential injections of all six analytes spiked into urine at 100 ng/mL. The same SPE cartridge was used for all 2000 injections without deviation in pump pressures or peak shape. The instrument response was stable for all six analytes with coefficient of variation less than 5%.



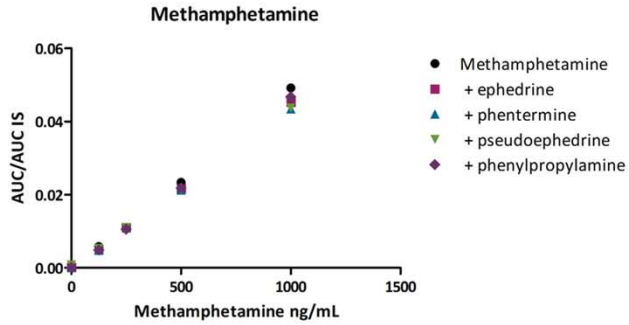
Standard curves of the panel of bath salts. Total sample analysis time from injection to injection is 14 seconds. No significant carryover was detected.

Results

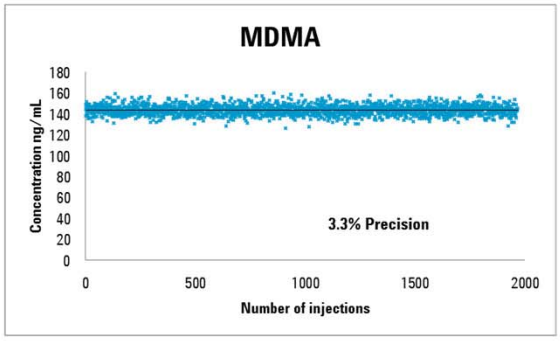
Amphetamines: Standard curves were analyzed to obtain intra- and interday precision and accuracy values on the Agilent RapidFire High-throughput Mass Spectrometry System. The samples were analyzed in multiplicate over a four day period. Standard curves in urine had excellent linearity within the measured range (50-5000 ng/mL) with an R^2 value greater than 0.995. Intra- and interday accuracies determined were within 15% and coefficient of variation values were all less than 5% for concentrations within the measured range.



MDMA (ng/mL)	Interday % Accuracy	Interday % Precision	Intraday % Accuracy	Intraday % Precision
50	102.0	3.9	100.0	4.9
100	93.4	0.3	93.1	2.5
250	91.4	0.9	93.0	3.2
500	95.2	2.1	95.1	2.1
1000	98.7	0.9	100.3	1.7
5000	100.6	0.4	101.5	1.9
Low QC (80)	90.4	7.1	93.2	7.3
Mid QC (800)	90.0	2.4	92.5	2.8
High QC (4000)	92.1	1.0	94.8	1.8



No interference was observed for any of the five analytes when 100,000 ng/mL of ephedrine, pseudoephedrine, phentermine, and phenylpropanolamine were present in the sample. Methamphetamine, for example, is an isobar of phentermine, but the unique SRM transition used in this method maintained accuracy even in the lower end of the linear range despite the presence of 100,000 ng/mL of phentermine and the other interfering compounds. Amphetamine, methamphetamine, MDA, MDEA, and MDMA were all accurately measured in the presence of high concentrations of these common interfering drugs.



The reproducibility of the method was tested by measuring 2000 sequential injections of all analytes spiked into urine at 150 ng/mL. The same SPE cartridge was used for all 2000 injections without deviation in pump pressures or peak shape. The instrument response was stable for all six analytes with coefficient of variation less than 5%.

Conclusions

Fast, specific, and sensitive screens for multiple drug classes were successfully demonstrated using a SPE-MS/MS system. All three panels had sample-to-sample rates of analysis of 16 seconds or less, providing throughputs of greater than 225 samples per hour. These results demonstrate the ability to screen urine for panels of forensic drug analytes without comprising sensitivity, selectivity, or speed of analysis.