Ultrafast Forensic Screen for Amphetamines in Urine Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Forensic Toxicology

Authors

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Abstract

An ultrafast method for the forensic screening of urine against a five amphetamine panel was developed using an Agilent RapidFire High-throughput Mass Spectrometry System. Amphetamine, methamphetamine, 3,4-methylenedioxyamphetamine (MDA), 3,4-methylenedioxymethamphetamine (MDMA), and 3,4-methylenedioxyethylamphetamine (MDEA) were accurately and precisely measured within a linear range of 25-5,000 ng/mL. Specificity was confirmed by evaluating samples in the presence of commonly interfering substances including phentermine. All five analytes (Figure 1) and their respective internal standards were simultaneously measured in less than 15 seconds per sample, providing a throughput of greater than 240 samples per hour.

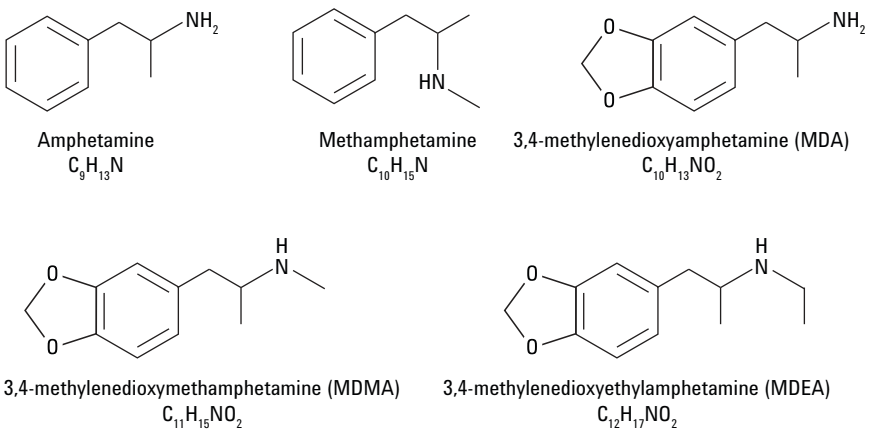

Figure 1. Structures of the five amphetamines in the panel.

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Introduction

Forensic toxicology traditionally relies on immunoassay screening followed by GC/MS, and more recently LC/MS, for quantitative analysis. Although immunoassays provide a high-throughput solution for the forensic drug screening of amphetamines, there is a risk of cross reactivity with common over-the-counter and prescription compounds including ephedrine, pseudoephedrine, phentermine, and phenylpropanolamine.[1] The need for greater throughput, faster turn-around times, and increased specificity have placed increased demands on these traditional technologies. The RapidFire High-throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times under 15 seconds. In the present study, a method was developed for screening urine for a five drug panel (Figure 1) consisting of amphetamine, methamphetamine, MDA, MDMA, and MDEA by ultrafast SPE/MS/MS.

Experimental

RapidFire triple quadrupole conditions

The RapidFire/MS/MS system consisted of the following modules: an Agilent RapidFire 360, an Agilent 6460 Triple Quadrupole Mass Spectrometer, MassHunter Qualitative Analysis (B.05.00), and MassHunter Quantitative Analysis (B.05.00). Samples were analyzed at a rate of 14.5 seconds per sample using the conditions shown in Table 1.

Analyte and internal standard ions were monitored simultaneously in all experiments for all five amphetamine drugs (Table 2).

Table 1. RapidFire/MS/MS conditions.

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 C (reversed-phase C18 chemistry, G9205A)
RF State 1	Sip sensor
RF State 2	2,500 ms
RF State 3	8,000 ms
RF State 4	1,000 ms
Triple quadrupole conditions	
Gas temperature	350 °C
Gas flow	11 L/min
Nebulizer	30 psi
Sheath gas temperatue	400 °C
Sheath gas flow	12 L/min
Nozzle voltage	0 V
Capillary voltage	2,500 V

Table 2. RapidFire/MS/MS conditions.

Analyte	Q1	Q3	Dwell	Fragmentor	CE	CAV
MDA	180.1	163.1	10	75	5	7
MDA-d5	185.1	168.1	10	75	8	7
MDEA	208.1	163.1	10	80	8	7
MDEA-d5	213.1	163.1	10	80	8	7
MDMA	194.1	163.1	10	75	8	7
MDMA-d5	199.1	165.1	10	75	8	7
Amphetamine	136.1	91	10	70	15	7
Amphetamine-d11	147.1	98.1	10	70	15	7
Methamphetamine	150.3	119.1	10	80	8	7
Methamphetamine-d14	164.2	98.1	10	80	24	7

Chemicals and reagents

(±)-MDA, (±)-MDA-d5, (±)-MDEA, (±)-MDEA-d5, (±)-MDMA, (±)-MDMA-d5, S(+)-methamphetamine, S(+)-amphetamine, 1S,2R(+)-ephedrine, phentermine, R,R(-)-pseudoephedrine, (±)-phenylpropanolamine (1 mg/mL in methanol), and (±)-amphetamine-d11, (±)-methamphetamine-d14 (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 5,000 ng/mL of MDA, MDEA, MDMA, amphetamine, and methamphetamine. Serial dilutions were used to achieve the remaining standard calibrator concentrations. Quality control (QC) samples were also prepared at 4,000, 800, and 80 ng/mL. A set of standard calibrators containing all five analytes within a concentration range of 125-1,000 ng/mL, as well as a negative matrix control were also spiked with 100,000 ng/mL of the SAMHSA interferent drugs, phentermine, ephedrine, pseudoephedrine, and phenylpropylamine.[2] All samples were diluted 1/50 using water containing all five internal standards (20 ng/mL final concentration for each internal standard). 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 weighting factor was applied during linear regression of the calibration curves. The quantitation using Mass Hunter Quantitative software was performed by spectral peak area ratio to a known concentration of the internal standards.

Results

Samples were prepared by spiking a panel of amphetamines into drug-free human urine and then diluting samples 50-fold with water containing their isotopically labeled internal standards. A representative standard curve for MDMA, showing injection cycle times of 14.5 seconds, is shown in Figure 2. Prepared calibration standards were run in triplicate over a series of days to establish both intra and inter-day precision and accuracy. Amphetamine, methamphetamine, MDA, MDEA, and MDMA had intra and interday accuracies within 15 % and coefficient of variation (CV) values less than 10 % for all concentrations within the linear range (Tables 3-7). This method had excellent linearity within the measured range of 25-5,000 ng/mL with an R^2 value greater than 0.998 (Figures 3-7) for each analyte. The limit of quantification (LOQ) defined as the AUC reproducibility for three injections having a CV of 15 % or less was determined to be 50 ng/mL. The limit of detection (LOD) was determined to be 25 ng/mL for all five analytes. Signal-to-noise ratios were calculated by looking at peak-to-peak height, and were found to be greater than 10:1 at the LOQ concentration for all analytes.

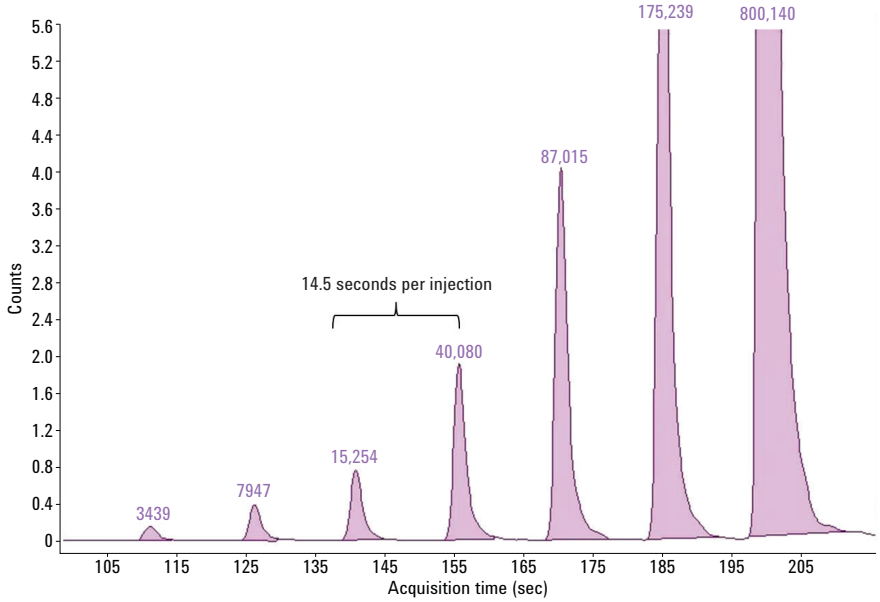

Figure 2. Representative standard curve for MDMA showing injection cycle times of 14.5 seconds.

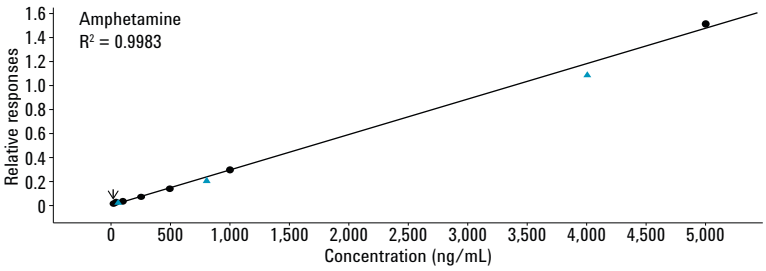

Figure 3. Representative standard curve for amphetamine. Black circles are calibrators and blue triangles are QCs.

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

Amphetamine	Intraday (n = 3)		Interday (n = 4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
50	8.1	104.6	6.3	104.9
100	7.7	91.4	4.3	91.9
250	3.4	91.4	3.2	92.5
500	2.1	92.2	2.2	93.8
1,000	2.4	99.0	2.4	99.6
5,000	1.3	101.4	1.9	102.1
Low QC (80)	7.7	93.8	8.1	91.3
Mid QC (800)	1.5	87.7	1.5	88.9
High QC (4,000)	0.6	92.0	1.8	93.6

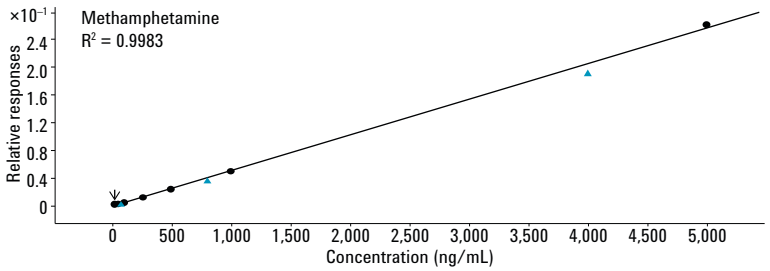

Figure 4. Representative standard curve for methamphetamine. Black circles are calibrators and blue triangles are QCs.

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

Methamphetamine	Intraday (n = 3)		Interday (n = 4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
50	3.6	103.1	4.8	106.3
100	5.8	94.8	6.1	93.2
250	4.8	91.1	3.4	90.9
500	0.8	92.3	2.7	93.2
1,000	3.3	97.6	2.0	98.0
5,000	1.2	102.4	1.5	102.2
Low QC (80)	7.7	95.7	8.3	98.6
Mid QC (800)	1.8	90.5	2.1	90.8
High QC (4,000)	1.6	94.3	1.6	95.0

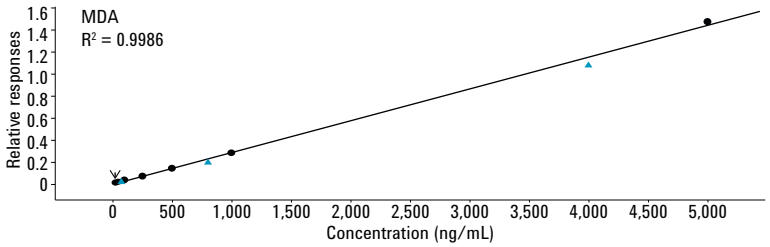

Figure 5. Representative standard curve for MDA. Black circles are calibrators and blue triangles are QCs.

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

MDA	Intraday (n = 3)		Interday (n = 4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
50	3.5	109.1	5.5	111.5
100	3.5	109.4	7.1	103.4
250	2.8	92.2	5.2	92.0
500	2.1	91.0	3.4	91.7
1,000	2.6	97.9	3.0	97.1
5,000	1.1	103.0	2.7	102.1
Low QC (80)	5.9	99.8	6.4	100.1
Mid QC (800)	2.4	87.4	3.3	86.3
High QC (4,000)	1.4	94.9	2.4	92.4

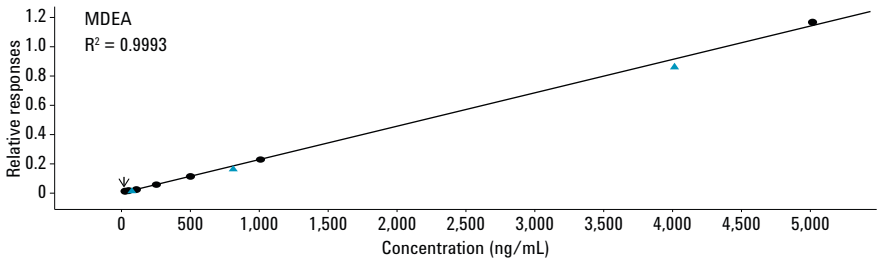

Figure 6. Representative standard curve for MDEA. Black circles are calibrators and blue triangles are QCs.

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

MDEA	Intraday (n = 3)		Interday (n = 4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
50	4.6	99.4	2.7	100.0
100	0.5	93.4	1.8	93.1
250	0.7	92.3	1.3	93.0
500	0.9	95.1	1.6	95.1
1,000	0.8	99.6	1.1	100.3
5,000	0.7	101.6	1.1	101.5
Low QC (80)	1.1	91.2	4.6	93.2
Mid QC (800)	0.7	92.9	1.4	92.5
High QC (4,000)	1.7	94.1	1.3	94.8

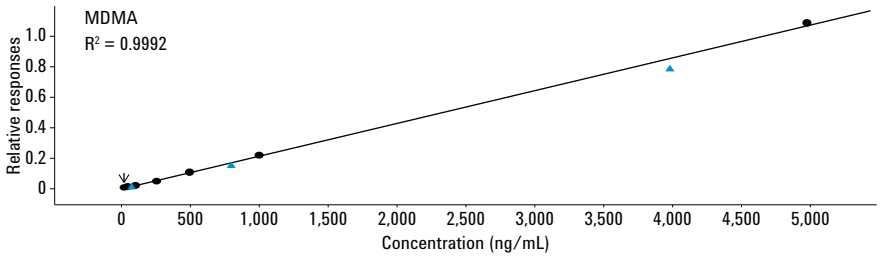

Figure 7. Representative standard curve for MDMA. Black circles are calibrators and blue triangles are QCs.

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

MDMA	Intraday (n = 3)		Interday (n = 4)	
ng/mL	% Precision	% Accuracy	% Precision	% Accuracy
50	3.9	102.0	4.9	100.0
100	0.3	93.4	2.5	93.1
250	0.9	91.4	3.2	93.0
500	2.1	95.2	2.1	95.1
1,000	0.9	98.7	1.7	100.3
5,000	0.4	100.6	1.9	101.5
Low QC (80)	7.1	90.4	7.3	93.2
Mid QC (800)	2.4	90.0	2.8	92.5
High QC (4,000)	1.0	92.1	1.8	94.8

No interference was observed for any of the five analytes when 100,000 ng/mL of ephedrine, pseudoephedrine, phentermine, or phenylpropanolamine was present in the sample. Methamphetamine, for example, is an isobar of phentermine but maintained accuracy even in the lower end of the linear range despite the presence of 100,000 ng/mL of phentermine and the other interferent compounds (Figure 8). Amphetamine, methamphetamine, MDA, MDEA, and MDMA were all accurately measured in the presence of high concentrations of these common interferent drugs. The negative matrix control was also tested and determined to be void of any interference.

The reproducibility of the method was tested by measuring 2,000 sequential injections of all five amphetamine analytes spiked into urine at 150 ng/mL. The same C18 cartridge was used for all 2,000 injections without deviation in pump pressures or peak shape. The instrument response was stable for all five analytes with coefficient of variation ranging from 2-7 %. MDMA for example had a coefficient of variation of 3.3 % and accuracy within 4 % (Figure 9).

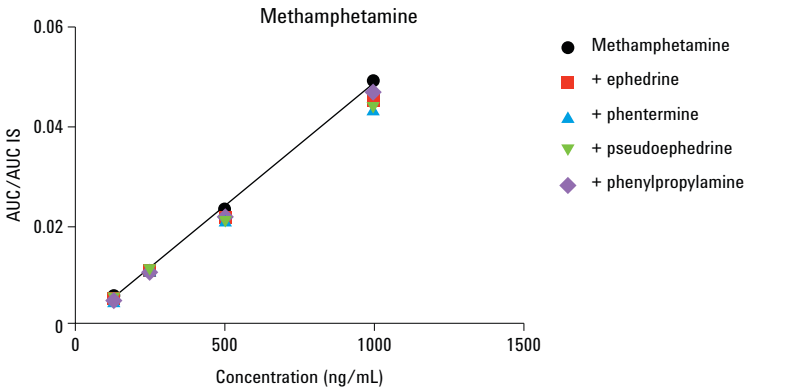

Figure 8. Methamphetamine in the presence of common interferents spiked in at 100,000 ng/mL.

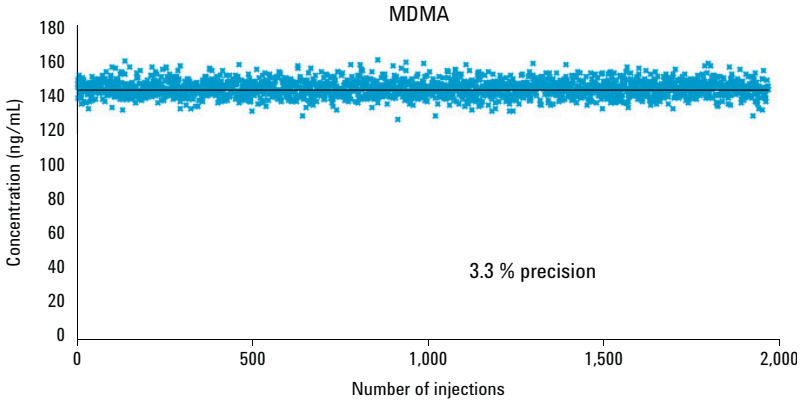

Figure 9. Repeatability evaluation using sequential injections of MDMA.

Conclusions

A panel of five amphetamines including amphetamine, methamphetamine, MDA, MDEA, and MDMA was quickly, accurately, and precisely measured in urine using the Agilent RapidFire/MS system. This forensic screening 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.

References

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2. Manual for Urine Laboratories, National Laboratory Certification Program, SAMHSA, U.S. Department of Health and Human Services, effective October **2010**.

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www.agilent.com/lifesciences/rapidfire

Published in the USA, May 20, 2014
5991-1571EN

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