High-Throughput Analysis of Drugs in Urine Using Ultra-fast SPE/MS/MS for Forensic Toxicology

Michelle V. Romm[1], Kari Schlicht[1], Lawrence J. Andrade[2], Matthew Woodcock[2], Vaughn P. Miller[1] and William A. LaMarr[1] 1. Agilent Technologies Inc., Wakefield, MA 2. Dominion Diagnostics, LLC, North Kingston, RI

Agilent Technologies

Introduction

Forensic drug testing has traditionally utilized GC/MS and more recently LC/MS as the analytical method of detection. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. In the present study, we evaluated the ability of an ultra-fast SPE/MS/MS system to analyze metabolites for some of the drugs of abuse including benzoylecgonine, the major metabolite of cocaine, and 11-nor-9-delta-9-tetrahydrocannabinol (THCCOOH), the major metabolite of marijuana, in urine with much faster sample cycle times (under 15 seconds per sample) and similar analytical results compared to GC/MS or LC/MS assays.

Experimental

Mass spectrometry and SPE methods were optimized separately for benzoylecgonine, THCCOOH, and their respective deuterated internal standards on an Agilent High-throughput RapidFire Mass Spectrometry System. Drug-free urine was spiked with analyte, diluted with internal standard, and injected for analysis. Sample analysis was performed at a rate of <15 seconds per sample using a RapidFire 300 system coupled to an Agilent 6460 triple quadrupole mass spectrometer with an Agilent JetStream source. Data analysis was performed using RapidFire Integrator software. This methodology is capable of throughputs >240 samples per hour.

Agilent 6460 Settings

Source Parameters

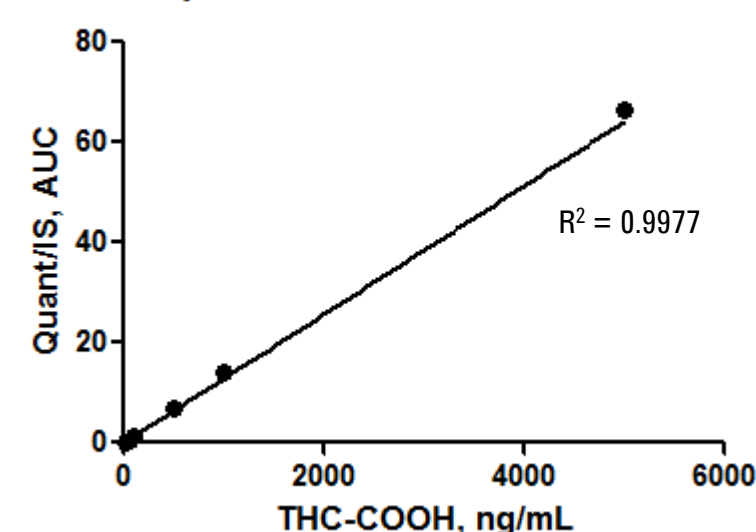| Ionization mode	ESI + Agilent Jet Stream
Drying gas temp.	350 °C
Drying gas flow	8 L/min
Sheath gas temp.	400 °C
Sheath gas flow	11 L/min
Nebulizer pressure	45 psi
Nozzle voltage	2000 V (THC-COOH) 300 V (BE)
Capillary voltage	3500 V

Acquisition Parameters (Positive Mode)

Transition	Precursor Ion	Product Ion	Dwell (ms)	Frag. (V)	CE (V)	CAV
IS						
BE	298.2	171.1	50	120	18	3
THC-COOH	348.2	302	50	110	20	2
Quantifier						
BE	290.1	168	50	125	17	3
THC-COOH	345.2	299	50	110	20	2
Qualifier						
BE	290.1	150.1	50	120	25	3
THC-COOH	345.2	193	50	110	25	2

THCCOOH

Benzoylecgonine
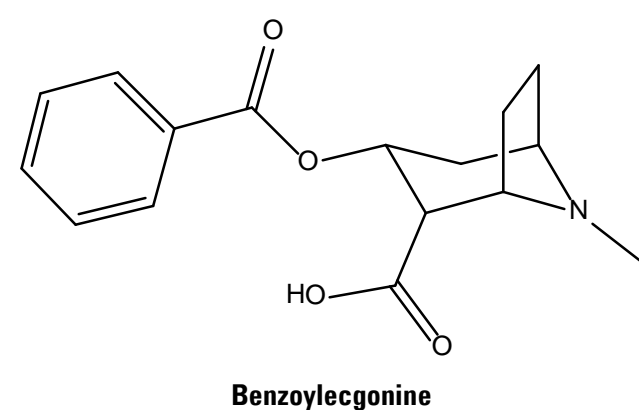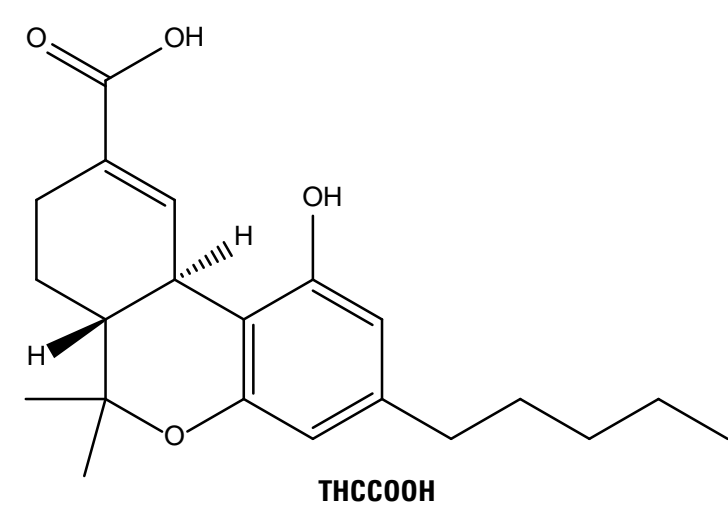
Results and Discussion

THCCOOH: 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 triplicate over a four day period. Standard curves in urine had excellent linearity within the measured range (10-5000 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. There was no carryover detected.

Representative Standard Curve

$R^2 = 0.9977$

THCCOOH Conc (ng/mL)	Accuracy* (%) Intraday (n=3)	Precision* (%) Intraday (n=3)	Accuracy* (%) Interday (n=4)	Precision* (%) Interday (n=4)
10	101.8	0.2	102.4	0.8
50	93.9	1.3	90.1	5.0
100	92.6	1.4	94.1	2.5
500	103.9	0.7	103.8	1.4
1000	106.3	1.1	107.4	2.2
5000	101.4	0.4	102.2	3.2

*1/x^2 weighing factor

Blinded Human Samples: THCCOOH

Blinded human samples were processed and run at Dominion, while identical samples were shipped to Agilent. Samples were run in the following order: Standards (5-500 ng/ml), QC's, P1-P67. The two analytical methods had a very good correlation. Any samples below 5 ng/ml were considered below the quantitation limit (BQL) and anything above 500 ng/ml were considered above the quantitation limit (AQL) for this experiment.
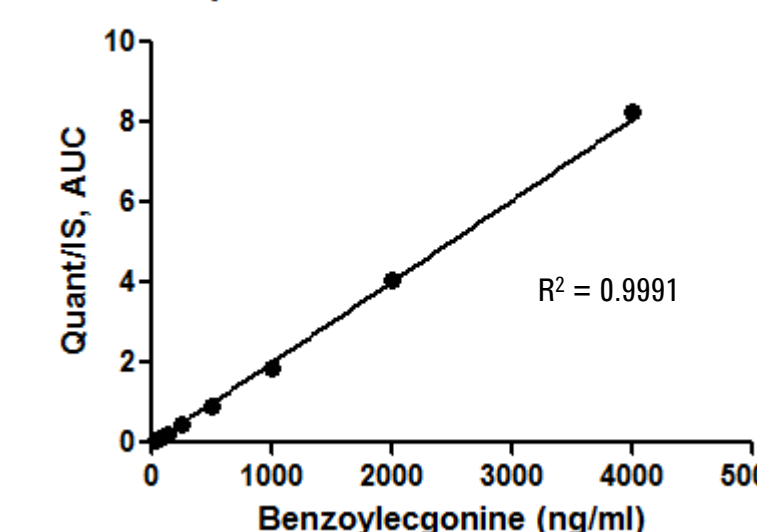
$y = 1.0481x - 0.321$
$R^2 = 0.9841$
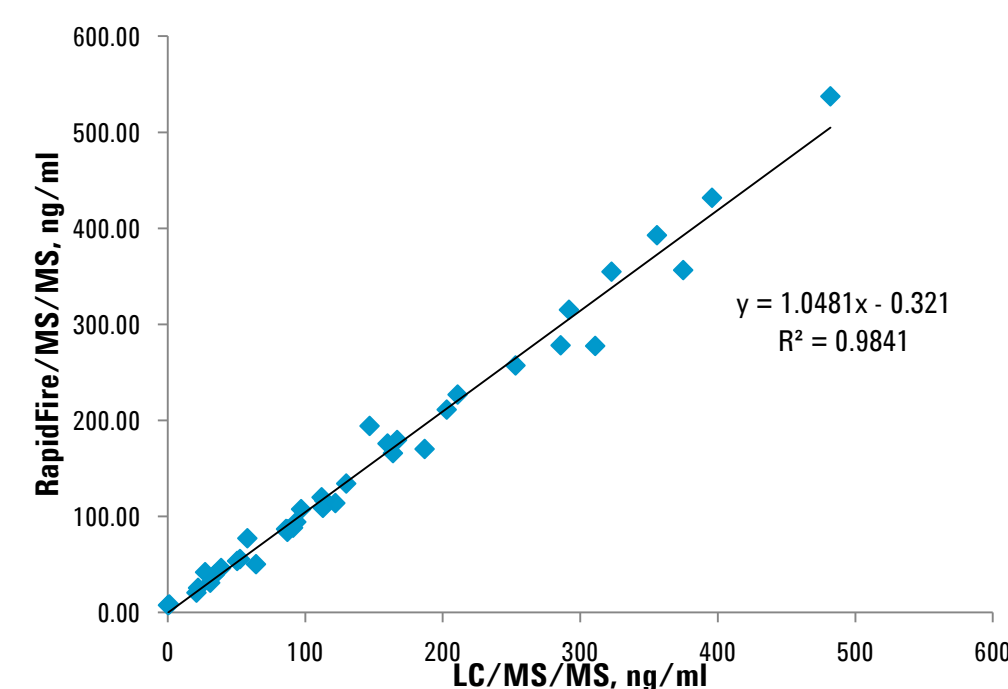
67 Blinded Human Samples
• Base Hydrolysis + offline SPE
• No mis-identified samples
• 15 (BQL) <5 ng/ml
• 13 (AQL) >500 ng/ml

Results and Discussion

Benzoylecgonine: Standard curves and control samples 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 triplicate over a three day period. Standard curves in urine had excellent linearity within the measured range (30-4000 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. There was less than 1% carryover detected.

Representative Standard Curve

$R^2 = 0.9991$

Benzoylecgonine Conc (ng/mL)	Accuracy* (%) Intraday (n=3)	Precision* (%) Intraday (n=3)	Accuracy* (%) Interday (n=3)	Precision* (%) Interday (n=3)
31.25	103.2	0.9	103.0	0.2
62.5	95.1	2.1	95.3	0.7
125	94.1	0.9	94.5	0.5
250	95.1	0.9	94.8	0.7
500	92.6	0.9	92.6	0.5
1000	92.3	1.4	94.4	1.9
2000	99.7	1.4	98.7	0.8
4000	103.5	1.0	103.5	0.1

*1/x weighing factor

Blinded Human Samples: Benzoylecgonine

Blinded human samples were processed and run at Dominion, while identical samples were shipped to Agilent. Samples were run in the following order: Standards (30-300 ng/ml), QC's, P1-P67. The two analytical methods had a very good correlation. Any samples below 30 ng/ml were considered below the quantitation limit (BQL) and anything above 300 ng/ml were considered above the quantitation limit (AQL) for this experiment.

$y = 0.9666x + 1.5672$
$R^2 = 0.9912$
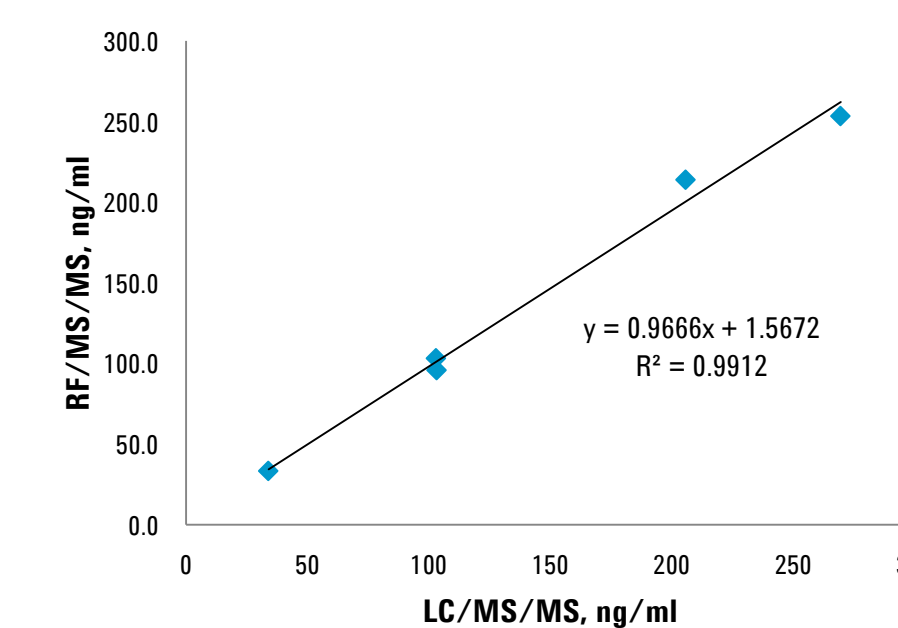
67 Blinded Human Samples
• 1:50 dilution
• No mis-identified samples
• 48 BQL (<30 ng/mL) confirmed
• 14 AQL (>300 ng/mL) confirmed

Conclusions

Drug metabolites such as benzoylecgonine and THCCOOH were accurately and precisely measured using an ultra-fast SPE/MS/MS system. While the analytical results were comparable to LC/MS/MS, the analysis time was much faster at less than 15 seconds per sample. This methodology is capable of throughputs greater than 240 samples/hour. This SPE/MS/MS system may be useful for fast and efficient detection of similar small molecule analytes in urine for forensic toxicology.