Rapid Analysis of Drug Analytes for Forensic Toxicology in Urine Using Ultra-Fast Online SPE/MS/MS

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Introduction

Forensic drug testing has traditionally utilized GC/MS as the analytical method of detection. In the present study, we evaluated the ability of an ultra-fast SPE/MS/MS system to analyze metabolites of some of the drugs of abuse including benzoylecgonine (BE), 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 spectromter with an Agilent JetStream source. Data analysis was performed using RapidFire Integrator software. This methodology is capable of throughputs >240 samples per hour.

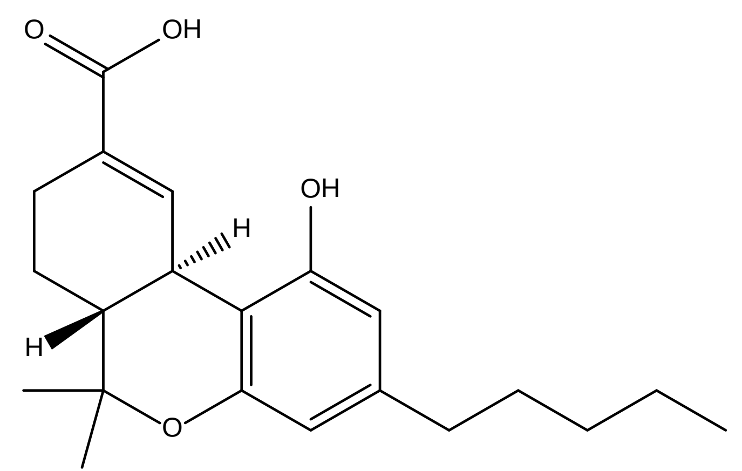
Agilent 6460 Settings

Source Parameters

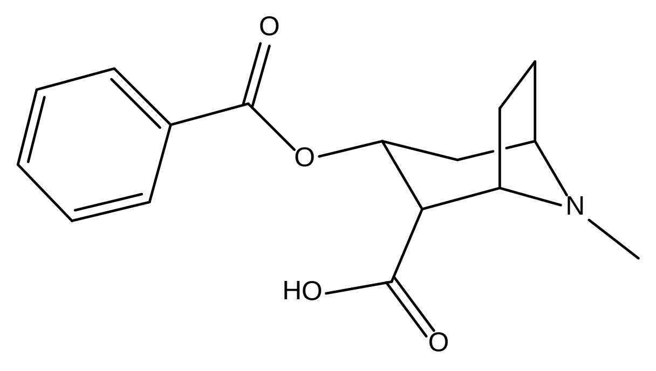
Ionization mode	ESI + Agilent Jet Stream	
Drying gas temp.	350 °C	
Drying gas flow	8 L/min	
Sheath gas temp.	400 °C	
Sheath gas flow	11 L/min	
Nebulizer pressure	45 psi	
Nozzle voltage	2000 V (THC-COOH)	300 V (BE)
Capillary voltage	3500 V	

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.

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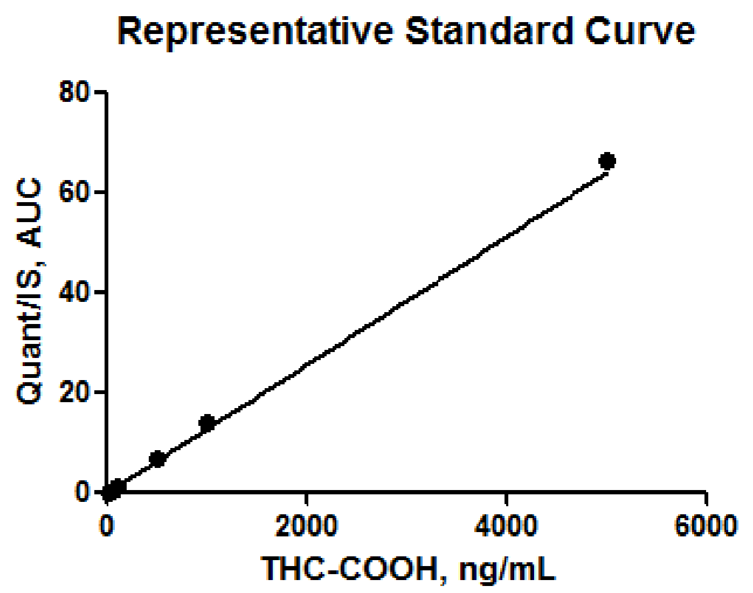

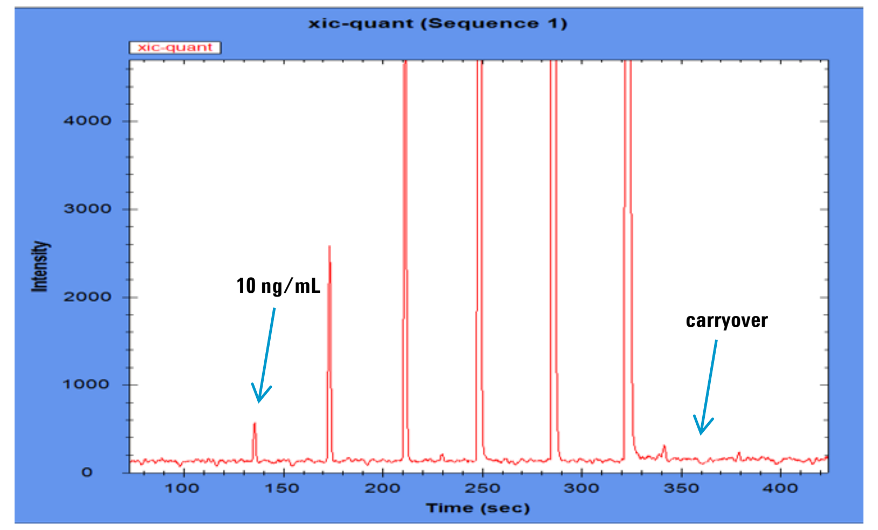

THCCOOH Conc (ng/mL)	Accuracy* (%) Intraday (n=3)	Precision* (%) Intraday (n=3)	Accuracy* (%) Interday (n=4)	Precision* (%) Interday (n=4)
10	101.8	0.2	102.4	0.8
50	93.9	1.3	90.1	5.0
100	92.6	1.4	94.1	2.5
500	103.9	0.7	103.8	1.4
1000	106.3	1.1	107.4	2.2
5000	101.4	0.4	102.2	3.2

*1/x^2 weighing factor

S/N	% Carryover	Linearity
~15	~0	0.9977

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.

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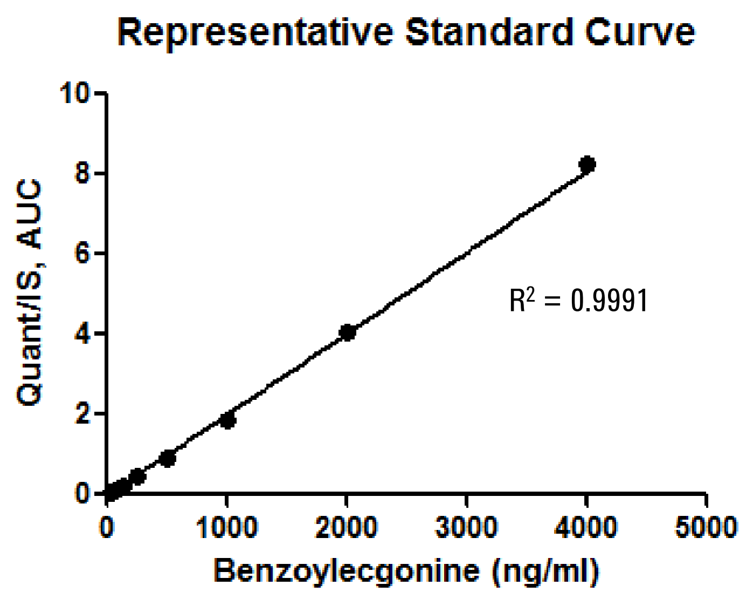
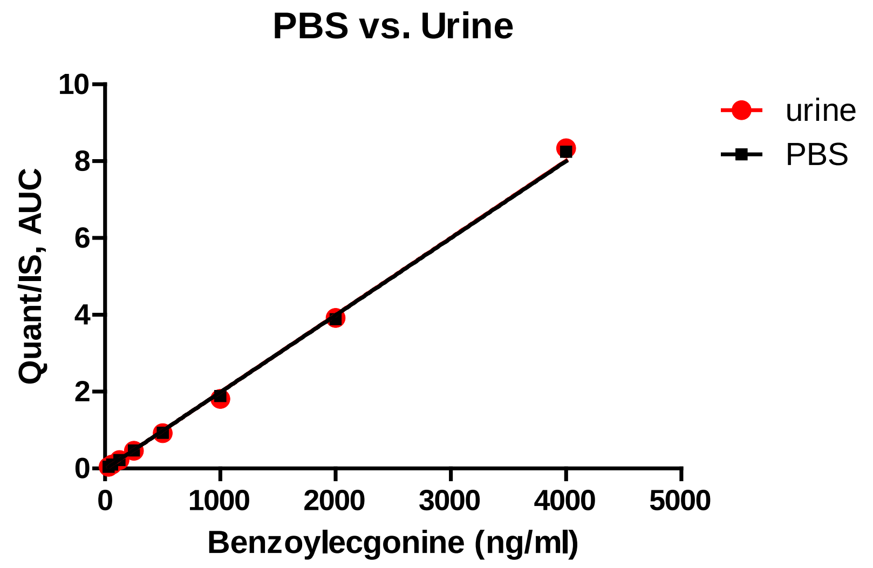


Benzoylecgonine Conc (ng/mL)	Accuracy* (%) Intraday (n=3)	Precision* (%) Intraday (n=3)	Accuracy* (%) Interday (n=3)	Precision* (%) Interday (n=3)
31.25	103.2	0.9	103.0	0.2
62.5	95.1	2.1	95.3	0.7
125	94.1	0.9	94.5	0.5
250	95.1	0.9	94.8	0.7
500	92.6	0.9	92.6	0.5
1000	92.3	1.4	94.4	1.9
2000	99.7	1.4	98.7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

***1/x weighing factor**

Results and Discussion

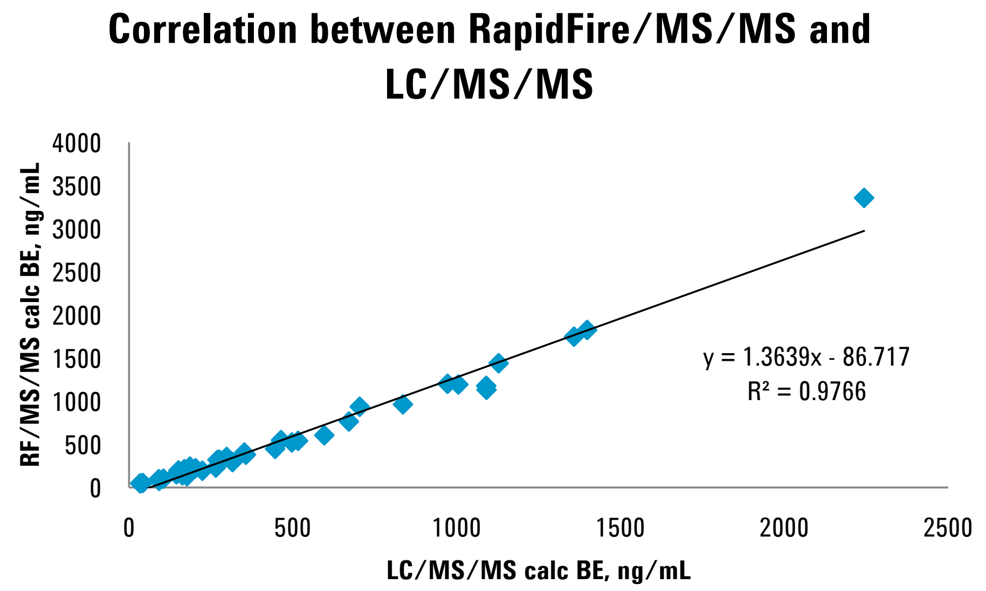


Matrix Effect

Standard curves were prepared in urine or PBS buffer and run on the Agilent High-throughput Mass Spectrometry System. The standard curves were identical demonstrating no interference from a matrix effect for this compound.

Blinded Human Urine Samples

Blinded human urine samples were processed and run at Dominion Diagnostics using LC/MS/MS, while identical unprocessed samples were shipped to Agilent. At Agilent, a new set of standards were prepared and the samples were then processed and run using RapidFire/MS/MS. Samples were run in the following order: Standards, P1-P50, Standards. The methods had a good correlation with an R^2 value of 0.977.



Conclusions

Drug of abuse 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.