Ultra Fast Analysis of the Cocaine Metabolite (BZE) in Urine Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Authors

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Introduction

Forensic drug testing has traditionally relied on GC/MS as the analytical detection method of choice. Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional technologies. The Agilent RapidFire High-throughput Mass Spectrometry System is an ultra fast 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 the Agilent RapidFire/MS/MS system to analyze benzoylecgonine (BZE), the major metabolite of cocaine, in urine. Results were achieved with much faster sample cycle times and similar analytical output compared to GC/MS or LC/MS assays.

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Experimental

The Agilent RapidFire/MS/MS system consisted of the following modules: an Agilent RapidFire 360, an Agilent 6460 Triple Quadrupole Mass Spectrometer using MassHunter Triple Quadrupole Acquisition Software (B.04.01) with Qualitative Analysis (B.04.00), and RapidFire Integrator Software.

RapidFire triple quadrupole conditions

Samples were analyzed at a rate of less than 15 seconds per sample. Quantitative and qualitative ions for BZE and an internal standard were monitored simultaneously in all experiments (Table 1).

Chemicals and reagents

BZE (1.0 mg/mL in methanol) and BZE-[D_8] (100 µg/mL in methanol) were purchased from Cerilliant, Round Rock, TX. All other solvents and reagents were purchased from Sigma-Aldrich, St. Louis, MO.

Sample preparation

Standard calibrators were prepared by spiking blank urine or phosphate buffered saline (PBS) with 4,000 ng/mL of BZE. Serial dilutions were used to achieve the remaining standard calibration concentrations. Standard samples were diluted 1:50 using 1 % acetic acid in water containing the internal standard BZE-[D8]. Samples were transferred to 96-well plates, centrifuged, and injected onto the Agilent RapidFire/MS/MS system.

Data analysis

RapidFire Integrator software was used for peak integration. The quantifier ion area under curve (AUC) of each analyte was normalized using the AUC of the internal standard. BZE data was subjected to linear regression with 1/x weighting.

Table 1. RapidFire/MS/MS conditions.

RapidFire conditions	
Buffer A	Water with 0.09 % formic acid, 0.01 % triflouroacetic acid; 1.5 mL/min flow rate
Buffer B	50 % isopropanol, 50 % methanol with 0.09 % formic acid, 0.01 % triflouroacetic acid; 1.25 mL/min flow rate
Injection volume	10 µL
SPE cartridge	Agilent RapidFire cartridge C (reversed-phase C_{18} chemistry, p/n: G9205A)
RF state 1	sip sensor
RF state 2	3,000 ms
RF state 3	3,000 ms
RF State 4	500 ms
Triple Quadrupole conditions	
Gas temp	350 °C
Gas flow	8 L/min
Nebulizer	45 psi
Sheath gas temp	400 °C
Sheath gas flow	11 L/min
Nozzle voltage	300 V
Capillary voltage	3,500 V

	Q1	Q3	Dwell	Fragmentor	CE	CAV
IS	298.2	171.1	50	120	18	3
Quantifier	290.1	168	50	125	17	3
Qualifier	290.1	150.1	50	120	25	3

Results and Discussion

Samples were prepared by spiking BZE into drug-free human urine and then diluting samples 50-fold prior to analysis on the Agilent RapidFire/MS/MS system. Standard curves in urine were analyzed separately to obtain intra and interday precision and accuracy values. Intra and interday accuracies determined for BZE were within 8 % and coefficient of variation values were all less than 3 % for concentrations within the measured range (Table 2). The standard curves had excellent linearity within the measured ranges, with R^2 values greater than 0.995 (Figure 1).

BZE was quantified between 31–4,000 ng/mL and was determined to have a limit of detection (LOD) of less than 2 ng/mL. Carryover was assessed by analyzing the AUC of the blank calculated as the % of the mean peak area of the 31 ng/mL samples. No significant carryover (< 1 %) was seen using this method. Matrix effects were also investigated by comparing standard curves prepared in PBS to those prepared in urine. No significant differences in the standard curve results were observed. Signal to noise ratios were calculated by measuring peak to peak height and found to be greater than 300 at 31 ng/mL.

Table 2. Intraday and interday precision and accuracy for BZE.

Benzoylecongine (ng/mL)	Intraday % accuracy (n=3)	Intraday % precision (n=3)	Interday % accuracy (n=3)	Interday % precision (n=3)
31.25	103.20	0.88	103.01	0.22
62.5	95.12	2.05	95.31	0.74
125	94.14	0.94	94.50	0.54
250	95.11	0.98	94.76	0.68
500	92.62	0.97	92.61	0.50
1,000	92.27	1.41	94.35	1.92
2,000	99.65	1.35	98.70	0.83
4,000	103.48	1.00	103.45	0.07

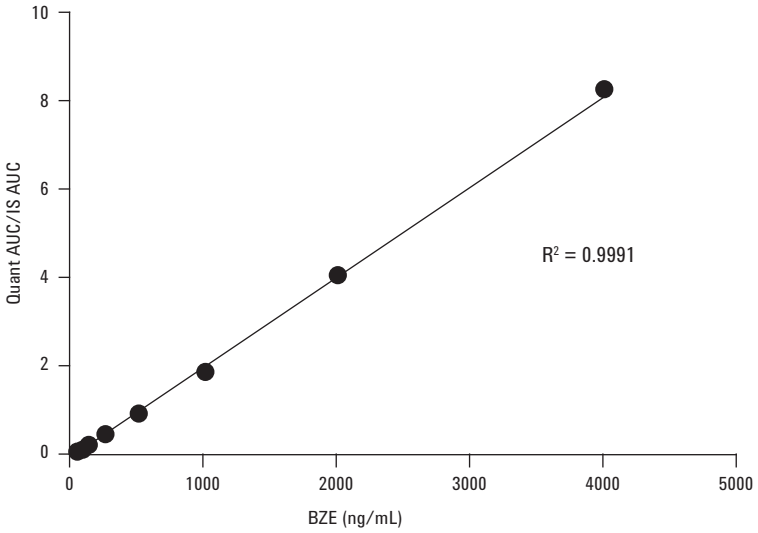

Figure 1. Representative standard curve for BZE in urine.

Conclusions

The drug of abuse metabolite benzoylecgonine (BZE) was accurately and precisely measured using a simple dilute and shoot method on the Agilent RapidFire/MS/MS System. Samples were analyzed in less than 15 seconds per sample, providing a high-throughput method of detection of this analyte. This methodology is capable of throughputs greater than 240 samples per hour, making the Agilent RapidFire/MS/MS system useful for fast and efficient detection of similar small molecule analytes in urine.

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