Ultrafast Analysis of Methadone and Metabolite EDDP in Urine by the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Forensic Toxicology

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Abstract

The need for greater analytical capacity and throughput for the analysis of methadone and its metabolite, EDDP, in urine laboratories for forensic toxicology has placed demands on traditional analytical technologies. This application note describes a method that uses the Agilent RapidFire/MS/MS system to analyze methadone and EDDP in urine with much faster sample cycle times and comparable analytical results to typical LC/MS/MS assays. A simple dilute and shoot methodology followed by RapidFire/MS/MS analysis allows for the accurate and precise measurement of these analytes in urine over a linear range of 10 to 5,000 ng/mL. Samples were analyzed on the RapidFire/MS/MS system in 11 seconds per sample, providing a much higher throughput method of analysis compared to traditional LC/MS/MS protocols. This new ultrafast method has the speed and accuracy necessary for an efficient screen/confirm (qualitative and quantitative) workflow.

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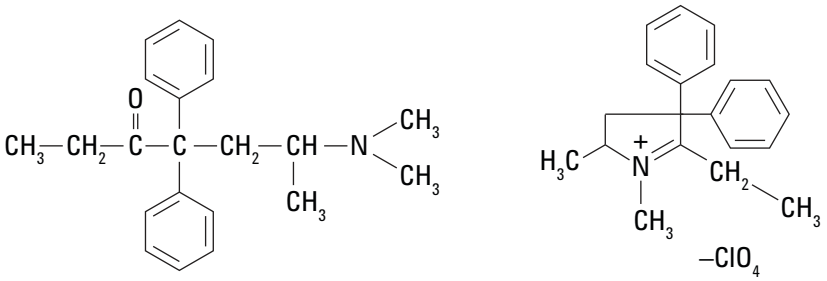
Introduction

Methadone is metabolized by demethylation (cytochrome P450 2D6 [CYP2D6]) to 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP) and to 2-ethyl-5-methyl-3,3-diphenylpyrrolidine (EMDP). Methadone is an unusual drug in that its primary urinary metabolites (EDDP and EMDP) are cyclic in structure (Figure 1), making them very difficult to detect using immunoassays targeted to the parent compound methadone.[1]

Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional analytical technologies. The RapidFire High-throughput Mass Spectrometry System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times of less than 15 seconds. In this application note, we developed a method to analyze methadone and its major metabolite (EDDP), in urine using a simple dilute and shoot methodology[2] and the RapidFire/MS/MS system with much faster sample cycle times and similar analytical results compared to GC/MS or LC/MS/MS assays. This new method, using RapidFire/MS/MS, allows for the rapid, accurate and precise measurement of methadone and EDDP in urine over a linear range of 10 to 5,000 ng/mL. This methodology provides comparable results to LC/MS/MS, but at > 10x the speed and efficiency of traditional LC/MS/MS methods.



Methadone EDDP

Figure 1. Structures of methadone and its metabolite 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (EDDP).

Experimental

RapidFire triple quadrupole conditions

The Agilent RapidFire/MS/MS system consisted of the following modules: RapidFire 360, Agilent 6460 Triple Quadrupole Mass Spectrometer using MassHunter Triple Quadrupole Acquisition Software (B.04.01) with Qualitative Analysis (B.04.00), Quantitative Analysis (B.04.00), and RapidFire Acquisition Software. Samples were analyzed at a rate of 11 seconds per sample using the conditions shown in Table 1.

Quantitative and qualitative ions for methadone, EDDP and internal standards were monitored simultaneously in all experiments (Table 2). Agilent MassHunter Quantitative software automatically calculated qualifier ion ratios.

Chemicals and reagents

The analytes methadone, EDDP, and their stable-labeled internal standards methadone-d3 and EDDP-d3 were purchased from Cerilliant, Round Rock, Texas. All other solvents and reagents were purchased from VWR.

Sample preparation

Urine samples were diluted 1:100 in methanol:water (1:1). Specifically, 10 µL of each urine sample, blank, calibrators (10, 100, 500, 1,000, 2,500, 5,000 ng/mL) or QC (150, 750, 2,000 ng/mL) were combined with 1 mL of 50 % ultrapure water: 50 % LC/MS grade methanol containing internal standards (methadone-d3 and EDDP-d3 at 100 ng/mL) in a 2.2 mL 96 deep-well plate. The plate was then sealed with an Agilent PlateLoc Thermal Microplate Sealer and mixed for 20 seconds prior to RapidFire/MS/MS analysis.

Data analysis

System control and data acquisition were performed by MassHunter Triple Quadrupole Data Acquisition Software.

Calibration curves were constructed using linear least squares regression with $1/X^2$ weighting for the multiple reactions monitoring (MRM). The quantitation using MassHunter Quantitative Software was performed by spectral peak area ratio to a known concentration of the internal standards.

Table 1. Experimental conditions.

RapidFire/MS/MS conditions	
Buffer A	Water with 0.1 % formic acid; 1.5 mL/min flow rate
Buffer B and C	50 % methanol: 25 % IPA: 25 % acetonitrile, 0.1 % formic acid, 0.01 % trifluroacetic acid 1.25 mL/min flow rate
Injection volume	10 µL
SPE cartridge	Agilent RapidFire cartridge E (reversed-phase C18 G9205A)
RF State 1	Sip sensor
RF State 2	2,000 ms
RF State 3	0 ms
RF State 4	4,000 ms
RF State 5	800 ms
Triple quadrupole conditions	
Gas temperature	300 °C
Gas flow	10 L/min
Nebulizer	40 psi
Sheath gas temperature	350 °C
Sheath gas flow	12 L/min
Nozzle voltage	0 V
Capillary voltage	2,800 V

Table 2. MRM transitions.

	Q1	Q3	Dwell	Fragmentor	CE	CAV
Methadone-d3	313.2	268.3	35	85	13	5
Methadone Quant	310.2	265.0	35	95	13	5
Methadone Qual	310.2	105.1	35	95	29	5
EDDP-d3	281.2	234.1	35	100	33	5
EDDP Quant	278.2	234.1	35	150	29	5
EDDP Qual	278.2	249.1	35	150	25	5

Results and Discussion

Samples were prepared by spiking methadone and its metabolite EDDP into drug-free human urine and then diluting samples 100-fold with methanol:water (1:1). Samples were then analyzed via SPE/MS/MS using the RapidFire/MS/MS system and a hydrophobic C18 cartridge in 11 seconds per sample (Figure 2). This RapidFire/MS/MS methodology is capable of analyzing more than 300 samples per hour, providing a high-throughput and very efficient mode of analysis. Methadone and EDDP standard curves in urine had excellent linearity within the measured range (10-5,000 ng/mL) with an R^2 value greater than 0.995 (Figure 3). The standard curves were analyzed to obtain intra and interday precision and accuracy values. Intra and interday accuracies determined were within 10 % and coefficient of variation (CV) values were all less than 5 % for concentrations within the measured range (Table 3).

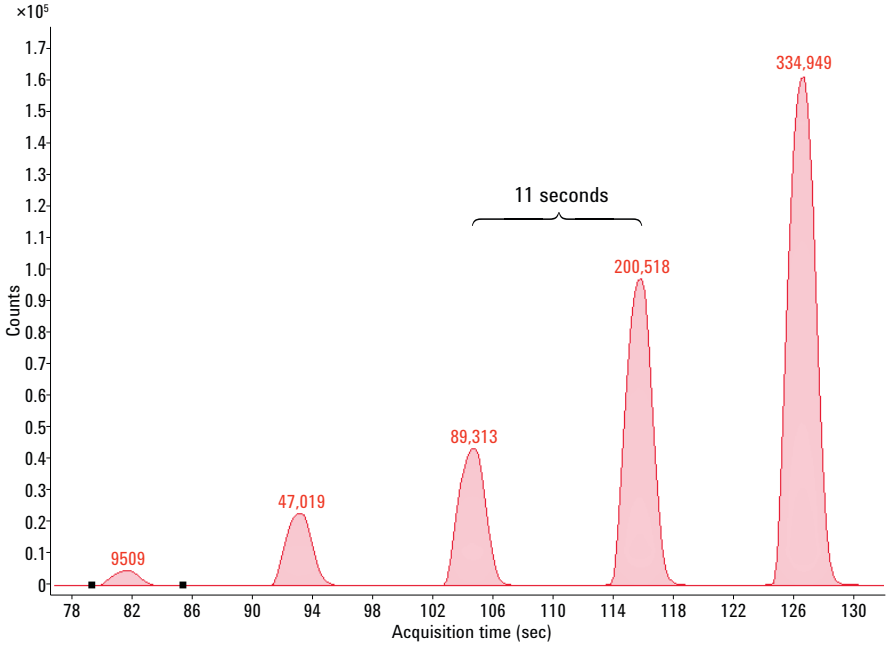


Figure 2. Representative standard curve data for EDDP showing 11 second injection to injection interval.

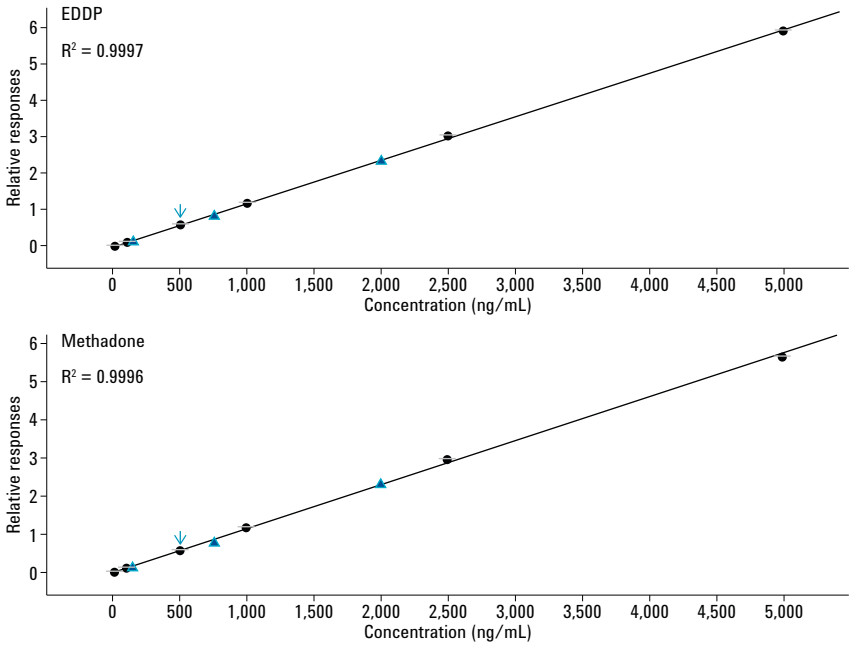


Figure 3. Representative standard curves for EDDP and methadone in urine. Dark circles are calibrators and blue triangles are QC standards.

Carryover was assessed by analyzing the AUC of the blank calculated as a % of the mean peak area of the 10 ng/mL samples. No significant carryover (0 %) was determined for methadone and EDDP (Figure 4). When measuring higher concentrations of both methadone and EDDP (> 5,000 ng/mL), we recommend using one blank injection between wells of a strong organic solution. Matrix effects were also investigated for both analytes by comparing the AUCs of standard curves prepared in 50:50 methanol:water to those in drug-free human urine. No significant matrix effect was observed (<10 %).

This dilute and shoot sample preparation followed by quick analysis on RapidFire/MS provides a very efficient method of forensic screening and confirmation (quantitating) methodone and EDDP in urine compared to traditional immunoassay, GC/MS, or LC/MS methods.[3]

Table 3. Intraday and interday precision and accuracy for methadone and EDDP.

Methadone (ng/mL)	Intraday % accuracy (n=6)	Intraday % precision (n=6)	Interday % accuracy (n=6)	Interday % precision (n=6)
150	96.7	3.8	96.3	0.95
750	101.6	2.9	100.2	0.56
2,000	103.6	1.4	103.7	0.94
EDDP (ng/mL)	**Intraday % accuracy (n=6)**	**Intraday % precision (n=6)**	**Interday % accuracy (n=6)**	**Interday % precision (n=6)**
150	99.4	3.3	96.1	1.4
750	100.3	2.3	97.8	1.2
2,000	103.5	2.5	101.7	1.2

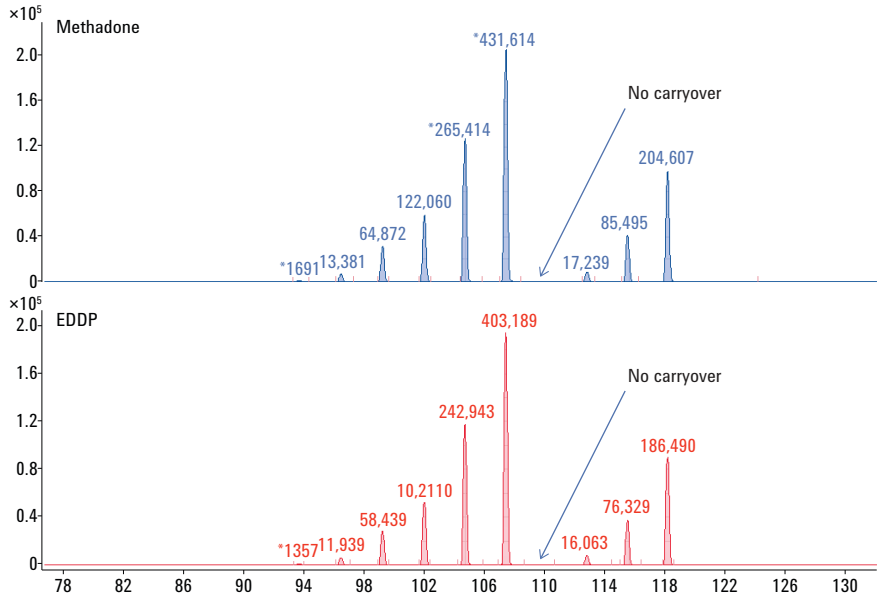


Figure 4. Carryover assessment using a blank injection after the highest calibrator shows no significant carryover was observed for either analyte.

Conclusions

Methadone and its metabolite (EDDP) spiked into urine were rapidly, accurately and precisely quantitated using a simple dilute and shoot forensic method and the Agilent RapidFire/MS/MS System. Samples were analyzed with injection to injection cycle times of 11 seconds, providing a method of analysis with throughputs greater than 300 samples per hour. This methodology provides comparable results to LC/MS/MS, but at > 10x the speed and efficiency of typical LC/MS/MS methods. Therefore, this method provides a very efficient mode for forensic screening and confirmation (quantitating) methodone and EDDP in urine compared to traditional analytical methods. The Agilent RapidFire/MS/MS system may also be useful for the fast and efficient measurement of similar small molecule analytes in urine.

References

1. Feng, J. *et al*. Simultaneous Determination of Multiple Drugs of Abuse and Relevant Metabolites in Urine by LC-MS-MS. *Journal of Analytical Toxicology*, **2007**, 31:369-368.

2. Vlase, L., *et al.* Bioanalysis of Methadone in Human Plasma and Urine by LC/MS/MS. Revue Roumaine de Chimie, **2008**, 53(12):1157-1164.

3. Galloway, E.R., and Bellet, N.F. Methadone Conversion to EDDP during GC-MS Analysis of Urine Samples. *Journal of Analytical Toxicology*, **1999**, 23:615-619.

www.agilent.com/lifesciences/rapidfire

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