Ultrafast Analysis of Z-Drugs in Urine Using the Agilent RapidFire High-Throughput Mass Spectrometry System

Application Note

Authors

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Abstract

An ultrafast RapidFire/MS/MS method was developed for the simultaneous determination of the sleep aids (Z-drugs): zopiclone, zolpidem, and zaleplon in human urine. The need for greater analytical capacity and throughput for the analysis of sleep aid medicines (Z-drugs) in forensic toxicology laboratories has placed demands on traditional analytical technologies, GC/MS and LC/MS/MS. We developed a method using the Agilent RapidFire/MS/MS system to analyze Z-drugs in urine with much faster sample cycle times and similar analytical results compared to LC/MS/MS assays. A simple dilute and shoot methodology followed by analysis by RapidFire/MS/MS allowed for the accurate and precise measurement of these analytes in urine over a linear range of 5 to 500 ng/mL. Samples were analyzed on the RapidFire/MS/MS system at 15 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

Z-drug sleep aids, such as zopiclone, zolpidem, and zaleplon (Figure1), have similar pharmacological properties to benzodiazepines. They are often prescribed as hypnotics, and have the risks of reducing the efficiency of driving a car, or working at machines, as well as addiction or severe intoxication.[1] Because of these risks, many forensic toxicology laboratories test for the presence of these drugs in urine.

Steady increases in the need for greater analytical capacity and throughput have placed demands on traditional analytical technologies. The Agilent RapidFire High-throughput Mass Spectrometry (MS) System is an ultrafast SPE/MS/MS system capable of analyzing samples with cycle times of less than 15 seconds. In this study, we developed a method to analyze Z-drugs in urine using a simple dilute and shoot methodology on the RapidFire/MS/MS system, which has much faster sample cycle times and similar analytical results to traditional GC/MS or LC/MS/MS assays. This method allows for the rapid, accurate, and precise measurement of Z-drugs in urine over a linear range of 5 to 500 ng/mL. This methodology provides comparable results to LC/MS/MS, but at > 10x the speed and efficiency of traditional LC/MS/MS methods.

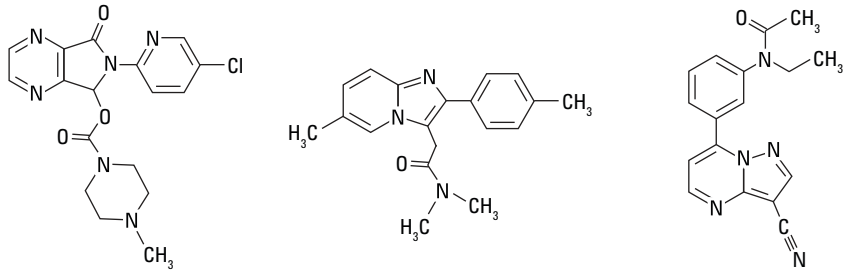

Figure 1. Structures of z-drugs: zopiclone, zolpidem, and zaleplon.

Experimental

RapidFire triple quadrupole conditions

The Agilent RapidFire/MS/MS system consisted of the following modules: Agilent 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 15 seconds per sample using the conditions shown in Table 1. Quantitative and qualitative ions for zopiclone, zolpidem, zaleplon, and internal standards were monitored simultaneously in all experiments (Table 1). Agilent MassHunter Quantitative software automatically calculated qualifier ion ratios.

Table 1. RapidFire MS/MS conditions.

RapidFire conditions						
Buffer A	5 mM ammonium formate in LC/MS grade water; 1.5 mL/min flow rate					
Buffer B	10 % acetonitrile in LC/MS grade water; 1.25 mL/min flow rate					
Buffer C	80 % LC/MS grade methanol:20 % LC/MS grade ethyl acetate; 1.25 mL/min flow rate					
Injection volume	10 µL					
Aqueous wash	HPLC grade water					
Organic wash	HPLC grade acetonitrile					
SPE cartridge	Agilent RapidFire cartridge A (reversed-phase C4 G9203A)					
RF state 1	Sip sensor					
RF state 2	2,500 ms					
RF state 3	2,500 ms					
RF state 4	7,000 ms					
RF state 5	1,000 ms					
Triple quadrupole conditions						
Gas temperature	350 °C					
Gas flow	8 L/min					
Sheath gas flow	11 L/min					
Capillary voltage	3,500					
Sheath gas temperature	400 °C					
Nebulizer	50 psi					
Nozzle voltage	300 V					
MRM transitions						
Analyte	Q1	Q3	Dwell	Fragmentor	CE	CAV
Zopiclone-d4	393.1	245	30	85	13	2
Zopiclone Quant	389.1	244.9	30	85	13	2
Zopiclone Qual	389.1	217.1	30	95	29	2
Zolpidem-d6	314.2	236.1	30	225	29	2
Zolpidem Quant	308.2	235.1	30	145	37	2
Zolpidem Qual	308.2	263.1	30	145	25	2
Zaleplon Quant	306.1	264.3	30	145	20	2
Zaleplon Qual	306.1	236	30	145	25	2

Chemicals and reagents

The analytes zopiclone and zaleplon, and the stable-labeled isotopes internal standards zopiclone-d4 and zolpidem-d6 were purchased from Cerilliant, Round Rock, TX. The 10 and 35 ng/mL quality controls were purchased from Utak Laboratories, Valencia, CA. The 200 ng/mL control was made in-house from a different lot number of material than the calibrators. Zolpidem was purchased from Grace Davison, Deerfield, IL. All other solvents and reagents were purchased from VWR and Fisher Scientific.

Sample preparation

Urine samples were diluted 1:50 in water. Specifically, 20 µL of each urine sample, blank, calibrators (5, 25, 100, 250, 500 ng/mL) or QC (10, 35, 200 ng/mL) were combined with 1 mL of 100 % ultrapure water containing internal standards (zolpidem-d6 and zopiclone-d4 at 5 ng/mL) in a 2.2 mL 96-well, deep-well plate. The plate was then sealed with an Agilent PlateLoc Thermal Microplate Sealer, mixed for 20 seconds and centrifuged prior to RapidFire/MS/MS analysis.

Data analysis

System control and data acquisition were performed using MassHunter software.

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

Results and Discussion

Samples were prepared by spiking Z-drugs into drug-free human urine and then diluting samples 50-fold with water containing the internal standard. Samples were then analyzed through SPE/MS/MS using the RapidFire/MS/MS system and a C4 cartridge at 15 seconds per sample (Figure 2).

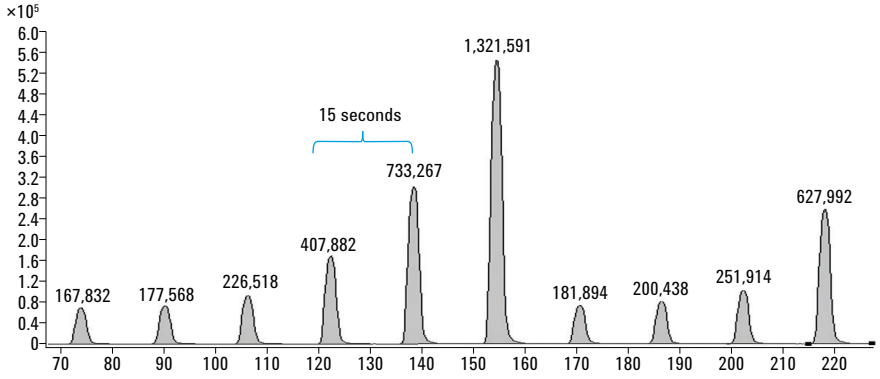

Figure 2. Representative TIC of a Z-drug standard curve and QC injections showing 15 second injection to injection interval.

This RapidFire/MS/MS methodology is capable of throughputs greater than 240 samples per hour providing a high-throughput and very efficient mode of analysis. Zopiclone, zolpidem, and zaleplon standard curves in urine had excellent linearity within the measured range (5–500 ng/mL) with an R^2 value greater than 0.995 (Figure 3). The QC standards were analyzed to obtain inter and intraday precision and accuracy values. Accuracies determined were within 10 %, and coefficient of variation values were all less than 10 % for the concentrations within the measured range (Table 2).

Carryover was assessed by analyzing the AUC of the blank calculated as a % of the mean peak area of the 5 ng/mL samples. No significant carryover (0 %) was determined for all of the Z-drugs (Figure 4). When measuring higher concentrations of Z-drugs (> 500 ng/mL), we recommended using one blank injection between wells by injecting a strong organic solution. Matrix effects were also investigated for both analytes by comparing the AUCs of standard curves prepared in 100 % 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 screening and confirming (quantitating) Z-drugs in urine compared to traditional GC/MS or LC/MS methods.[1,2]

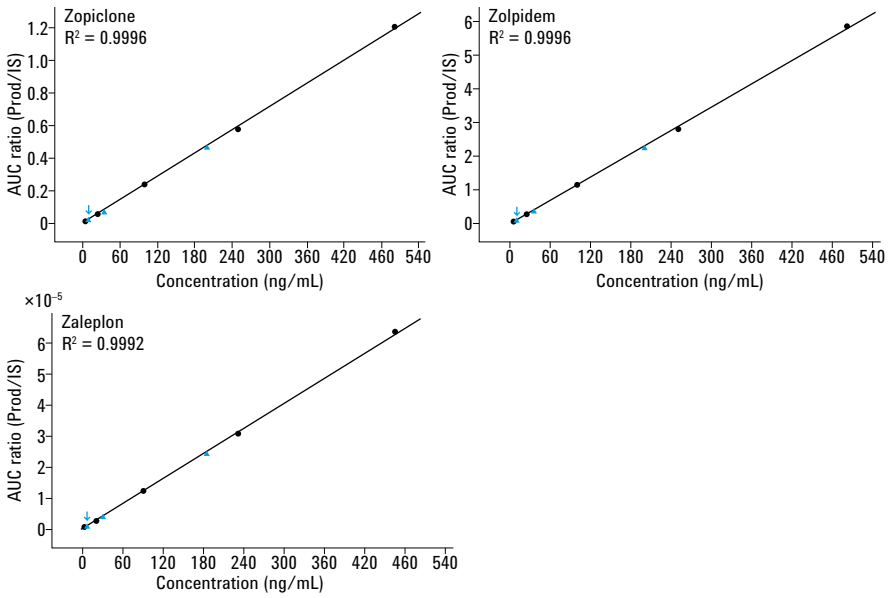

Figure 3. Representative standard curves for Z-drugs in urine. Dark circles are calibrators and blue triangles are QCs.

Table. 2. Interday and intraday accuracy and precision (n=6) for the RapidFire/MS/MS analysis of z-drugs in urine.

Zopiclone (ng/mL)	Intraday % accuracy	Intraday % precision	Interday % accuracy	Interday % precision
10	101.8	8.1	96.7	5.4
35	91.9	3.0	93.0	2.1
200	100.3	2.4	102.5	2.0
Zolpidem (ng/mL)	**Intraday % accuracy**	**Intraday % precision**	**Interday % accuracy**	**Interday % precision**
10	97.5	7.4	91.4	1.1
35	92.6	2.0	91.4	1.4
200	99.3	2.0	100.8	1.0
Zaleplon (ng/mL)	**Intraday % accuracy**	**Intraday % precision**	**Interday % accuracy**	**Interday % precision**
10	100.3	8.2	93.8	5.3
35	97.2	3.6	95.4	3.4
200	100.0	2.9	98.7	2.1

Conclusions

The sleep aid Z-drugs zopiclone, zolpidem, and zaleplon were rapidly, accurately, and precisely quantitated in urine, using a simple dilute and shoot method and the Agilent RapidFire/MS/MS System. Samples were analyzed with injection to injection cycle times of 15 seconds, providing a high-throughput method capable of analyzing more than 240 samples per hour. This methodology provided comparable results to traditional LC/MS/MS, but at > 10x the speed and efficiency for screening and confirming (quantitating) zopiclone, zolpidem, and zaleplone in urine samples.

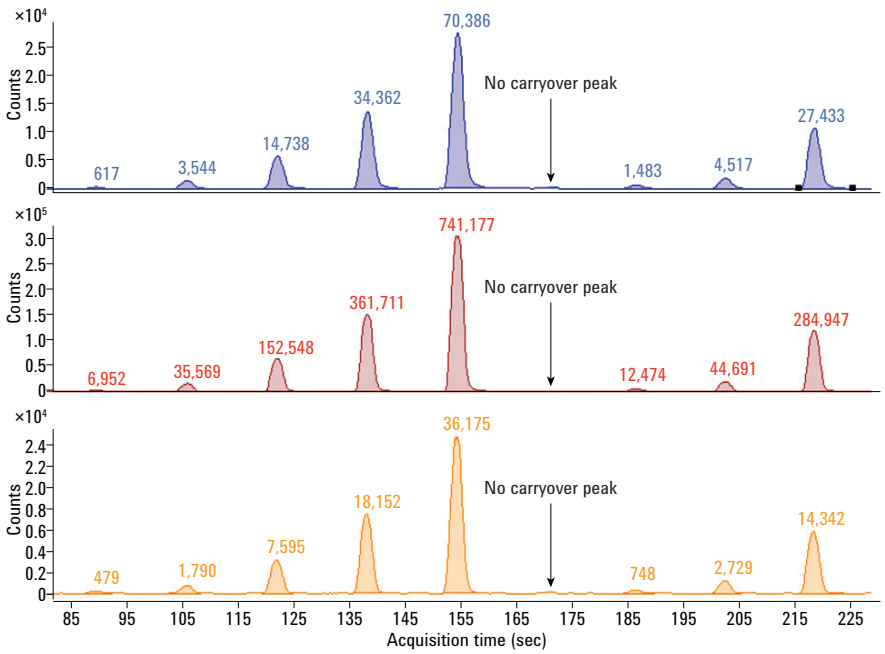

Figure 4. The blank injection after the highest calibrator shows no significant carryover was observed for these analytes.

References

1. Rust, K. Y., *et al*., "Detection and Validated Quantification of 21 benzodiazepines and 3 'Z-drugs' in human hair by LC-MS/MS", *Journal of Forensic Science International*, 2012, 215:64-72.

2. Tonon M. A., Bonato, P. S., "Methods for the analysis of nonbenzodiazepine hypnotic drugs in biological matrices", *Bioanalysis*, 2012, 4(3): 291-304.

www.agilent.com/lifesciences/rapidfire

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