Application Note: 551

Demonstrating High-Performance Quantitative Analysis of Benzodiazepines using Multiplexed SIM with High-Resolution, Accurate Mass Detection on the Q Exactive LC/MS

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Key Words

- **Q Exactive**
- **Accela UHPLC**
- **Selected Ion Monitoring**
- **Drug Quantitation**

Introduction

In today's modern forensic toxicology laboratories, there is a growing demand to have a mass spectrometer with the power and flexibility to perform experiments both for the identification of unknown compounds and for trace-level quantification of target analytes. Additionally, this platform must execute these analyses with minimal sample preparation, provide consistent results and be easily assimilated into the laboratory workflows. With the introduction of the Thermo Scientific Q Exactive high-performance benchtop quadrupole-Orbitrap mass spectrometer, the most stringent qualitative and quantitative objectives can be met. By using high-resolution, accurate mass (HRAM) detection with quadrupole selected ion monitoring (SIM), targeted quantification of benzodiazepines in urine can be accomplished with sensitivity that rivals triple stage quadrupole instruments in selected reaction monitoring (SRM) mode.

Goal

To demonstrate the feasibility of high sensitivity liquid chromatography-mass spectrometry (LC/MS) quantification of benzodiazepines in urine by combining multiplexed SIM with high-resolution, accurate mass detection on the Q Exactive™ high-performance benchtop quadrupole-Orbitrap mass spectrometer.

Experimental

Sample Preparation

Eight benzodiazepines were spiked into blank human urine containing acetonitrile at 10% (v/v) from 0.0125 to 250 ng/mL prior to LC/MS.

UHPLC

Ultra high performance LC (UHPLC) analyses were performed using a Thermo Scientific Accela 1250 liquid chromatography system with an Open Accela™ autosampler. Gradient elution with a Thermo Scientific Hypersil GOLD PFP column (50 x 2.1 mm; 1.9 µm particle size) was used at a flow rate of 500 µL/min. The injection volume was 5 µL.

Mass Spectrometry

MS measurements were accomplished on a Q Exactive mass spectrometer with a heated electrospray ionization (HESI) source in positive ion mode. Quadrupole isolation was set to 1.5 *m/z* with subsequent detection at a mass resolution of 140,000 FWHM via external mass calibration.

Results and Discussion

SIM is a well-established technique for targeted LC/MS quantitation using single quadrupole mass spectrometers. However, its utility is limited owing to the low specificity of unit mass resolution on single quads. The Q Exactive mass spectrometer, which employs Orbitrap-based high-resolution, accurate mass detection, overcomes this limitation. Additionally, the duty cycle on the Q Exactive MS is enhanced by measuring multiple SIM ions simultaneously in the Orbitrap mass analyzer. The process of multiplexed SIM is illustrated in Figure 1. Four different ions are selected by the quadrupole and stored in the C-trap while the Orbitrap analyzer measures the ions from the previous cycle. This process is repeated by passing the four SIM ions from the C-trap to the Orbitrap analyzer for the next mass measurement. The Q Exactive mass spectrometer has the capability to multiplex between two and ten SIM ions.

Table 1 lists the eight benzodiazepines quantified by HRAM LC/MS with their multiplexed SIM time windows, the measured mass errors using external mass calibration, and the lower limits of quantitation (LLOQs) in urine on the Q Exactive mass spectrometer. Two key points to highlight in Table 1 are that (1) mass errors on the Q Exactive system are significantly less than 5 ppm without the need of an internal calibration mass, and (2) the LLOQs of the eight benzodiazepines analyzed in urine are in the pg/mL range.

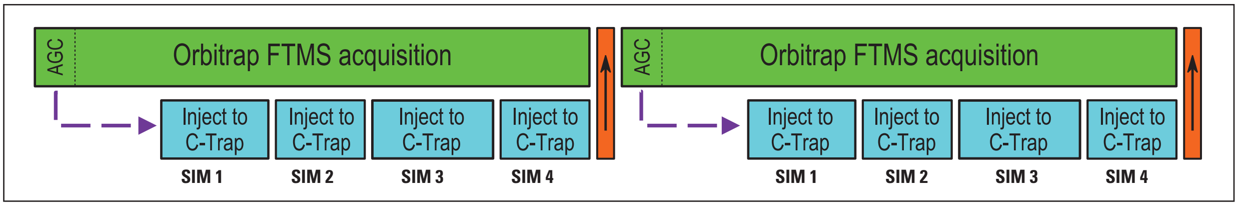

Figure 1. Schematic of multiplexed SIM on the Q Exactive mass spectrometer

Table 1. List of benzodiazepines quantified by HRAM LC/MS on the Q Exactive mass spectrometer

Compound	**SIM Time Window (min)**	**Exact *m/z***	**Measured *m/z***	**Error (ppm)**	**LLOQ (ng /mL)**
Oxazepam	0.00-3.45	287.05818	287.05829	+0.4	0.0625
Lorazepam	0.00-3.65	321.01921	321.01926	+0.2	0.1250
Nitrazepam	0.00-3.65	282.08732	282.08746	+0.5	0.0625
Clonazepam	0.00-3.85	316.04835	316.04828	-0.2	0.0625
Temazepam	3.45-6.00	301.07383	301.07410	+0.9	0.0250
Flunitrazepam	3.65-6.00	314.09355	314.09296	-1.9	0.0625
Alprazolam	3.65-6.00	309.09015	309.09024	+0.3	0.0125
Diazepam	3.85-6.00	285.07892	285.07901	+0.3	0.0125

Figure 2 presents an example LC/MS analysis of benzodiazepines at 0.125 ng/mL in urine using multiplexed SIM on the Q Exactive mass spectrometer. By acquiring these data at a mass resolution of 140,000 FWHM, little or no chemical noise is observed for the ± 5 ppm extracted ion chromatograms of the benzodiazepines in urine. The selectivity afforded by the Q Exactive mass spectrometer at a resolution of 140,000 FWHM is illustrated in the SIM spectrum for oxazepam (Figure 3). In addition to the oxazepam ion at *m/z* 287.05829, there are at least 12 other ions observed within a 0.25 *m/z* range. Yet, the oxazepam ion is easily separated from the other chemical interference ions with the high resolving power of the Q Exactive mass spectrometer.

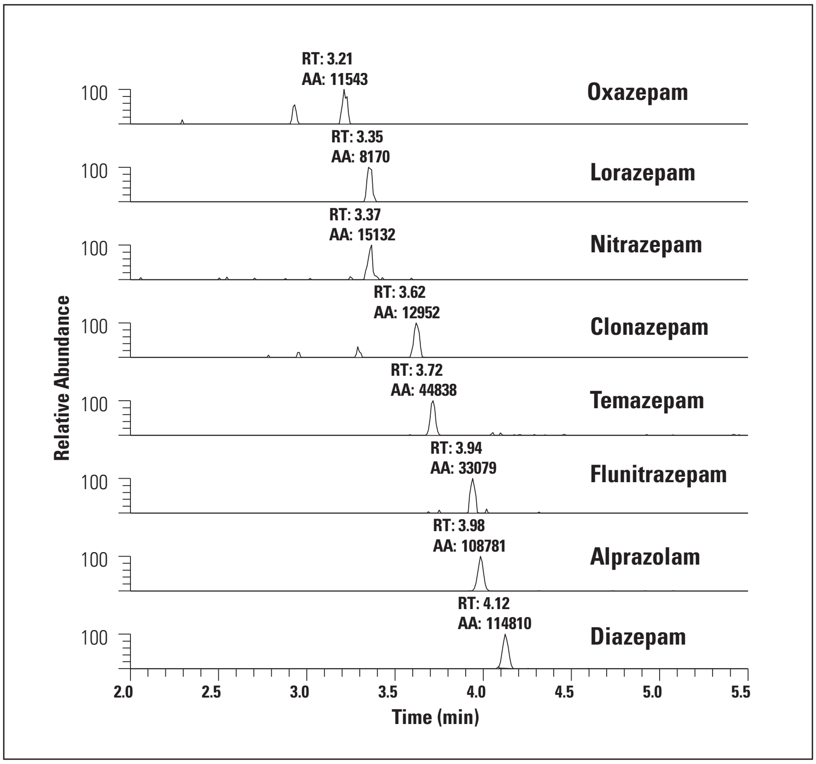

Figure 2. Extracted ion chromatograms (5 ppm) for 0.125 ng/mL benzodiazepines in urine

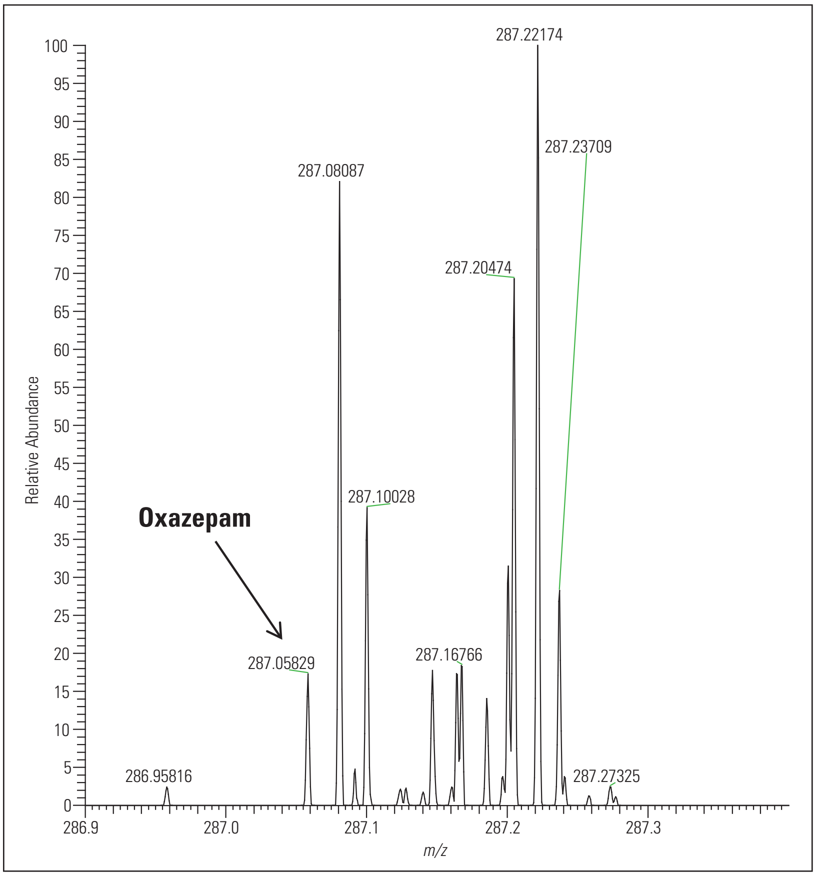

Figure 3. SIM spectrum of oxazepam in urine at mass resolution of 140,000 FWHM

Figure 4 and Table 2 demonstrate the overall quantitative performance of the Q Exactive mass spectrometer for diazepam in urine. The calibration curve for diazepam in Figure 4 shows a linear dynamic range of over four decades (0.0125 – 250 ng/mL), including the inset from 0.0125 to 0.25 ng/mL, with

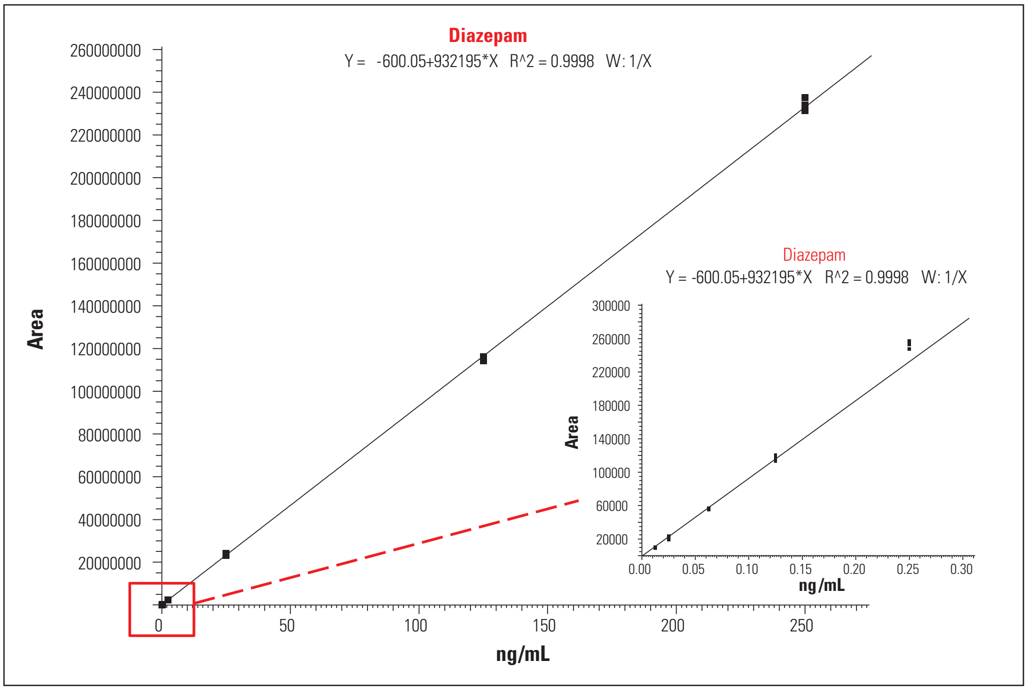

Figure 4. Calibration curve for diazepam in urine from 0.0125 – 250 ng/mL

an R^2 regression value of 0.9998 using 1/x weighting. Table 2 presents the statistical results for the HRAM quantification of diazepam. The quantitative accuracy and precision values obtained by the Q Exactive mass spectrometer using multiplexed SIM are comparable to those observed on triple stage quadrupole mass spectrometers in SRM mode.

Table 2. Statistical results for HRAM LC/MS quantitation of diazepam in urine

Specified Amount (ng /mL)	Mean Calculated Amt. (ng/mL)	%Accuracy	%CV
0.0125	0.0113	90.1	6.0
0.0250	0.0236	94.3	7.9
0.0625	0.0610	97.6	1.4
0.1250	0.127	101.9	2.9
0.250	0.273	109.1	1.7
2.50	2.65	105.9	1.0
25.0	25.5	102.0	1.7
125.0	123.5	98.8	0.8
250.0	250.8	100.3	1.2

Conclusion

The Q Exactive HRAM LC/MS system is a powerful and flexible instrument that can provide both sample identification and quantitative information for forensic toxicology with a single sample analysis. By using the method of multiplexed SIM, eight benzodiazepines in urine were quantified with LLOQs at the pg/mL level and with linear dynamic ranges of 3 to 4 orders of magnitude.

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