Application Note: 489b

Quantitation of 12 Benzodiazepines and Metabolites in Urine Using Ultrahigh Resolution LC-MS for Forensic Toxicology Use

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Key Words

- Exactive
- Accela HPLC
- Pain Management
- Forensic Toxicology

Goal

To demonstrate the quantitation of 12 benzodiazepines in urine using a liquid chromatography-mass spectrometry (LC-MS) method and ultrahigh resolution with the Thermo Scientific Exactive benchtop mass spectrometer for forensic analysis.

Experimental

Standards and Samples Preparation

Calibration standards were prepared by spiking blank urine with 12 benzodiazepines (lorazepam, nordiazepam, oxazepam, temazepam, hydroxytriazolam, 7-aminoclonazepam, 7-aminonitrazepam, hydroxyalprazolam, 7-aminoflunitrazepam, desalkylflurazepam, diazepam, and 2-hydroxyethylflurazepam) to final concentrations ranging from 10 ng/mL to 2,000 ng/mL.

Calibration standards and urine samples were spiked with internal standards (10 deuterated benzodiazepines), hydrolyzed and processed using a solid phase extraction (SPE) procedure.

Third party QC samples containing 6 benzodiazepines were processed and analyzed to obtain method accuracy and precision.

HPLC

HPLC analysis was performed using a Thermo Scientific Accela liquid chromatography system with a Thermo Scientific Hypersil GOLD PFP column (50 x 2.1 mm; 5 µm). A processed sample of 5 µL was analyzed with a 6-minute gradient method.

Mass Spectrometry

MS analysis was carried out on an Exactive™ benchtop LC-MS instrument with an electrospray ionization (ESI) source. Full scan data with resolution of 100,000 (FWHM) was acquired.

Results and Discussion

Figure 1 displays 6 of the 12 selected benzodiazepines at 10 ng/mL and internal standards. Chromatograms for compound detection and quantitation are reconstructed with a mass tolerance of 5 ppm.

Figure 2 shows the calibration curve for this set. Data results for the other six benzodiazepines are available upon request.

Conclusion

The Exactive benchtop LC-MS instrument provides excellent quantitative analysis of 12 benzodiazepines, from 10 ng/mL to 5000 ng/mL in urine, using ultrahigh resolution full scan data acquisition in a 6-minute method. The accuracy, precision, LOQ, and linearity range of the method meet the demands of today's forensic toxicology laboratories.

Method Performance Summary

Target Analytes	Benzodiazepines
Matrix	Urine
Limit of Quantitation (LOQ)	10 ng/mL
Recovery	> 85%
Assay Linearity	10 ng/mL – 5000 ng/mL
Precision (%CV)	< 4%
Carryover at Lower Limit of Quantitation (LLOQ)	< 1%
Sample Volume	2 mL
Analysis Time	6 minutes

Analyte	Mean Conc.(ng/mL)	% Recovery	%RSD
Oxazepam	248	99.3	1
Nordiazepam	234	93.5	1.4
Temazepam	218	87.1	4
Desalkylflurazepam	214	85.7	4
Lorazepam	227	90.8	0.4
Hydroxyalprazolam	255	102	0.4

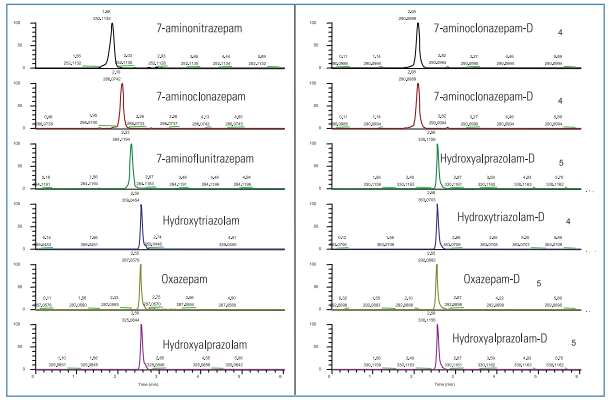

Figure 1: Chromatograms of 6 of the 12 selected benzodiazepines at 10 ng/mL and internal standards.

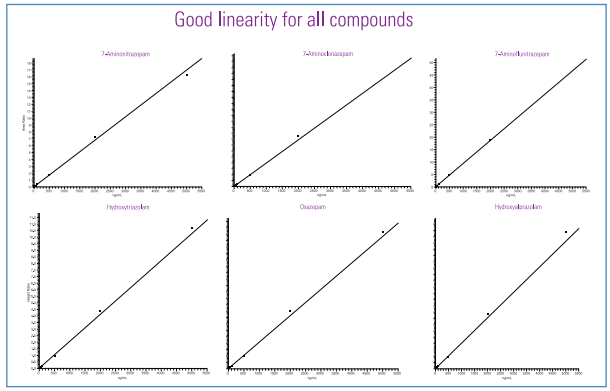

Figure 2: Calibration curves (10-5000 ng/mL) for all analytes

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