Application Note: 488b

Quantitation of Urinary Ethyl Glucuronide and Ethyl Sulfate Using Ultrahigh Resolution LC-MS

Forensic Toxicology Use Only

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Key Words

- Exactive
- Accela HPLC
- EtG / EtS
- Pain Management
- Forensic Toxicology

Introduction

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are sensitive and specific urinary biomarkers of recent alcohol intake that are of great interest in today's forensic toxicology laboratories.

Goal

To demonstrate the quantitation of EtG and EtS in urine using a liquid chromatography-mass spectrometry (LC-MS) method with ultrahigh resolution on the Thermo Scientific Exactive benchtop mass spectrometer.

Experimental

Calibration Standards and Sample Preparation

Calibration standards were prepared by spiking blank urine with EtG and EtS to final concentrations ranging from 25 ng/mL to 20,000 ng/mL.

Calibration standards and urine samples were spiked with internal standards (EtG-d5 and EtS-d5) and diluted 10 times with an LC mobile phase prior to injection onto the analytical column.

Commercial QC samples were used 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 C18 column (50 x 2.1 mm; 5 µm). A diluted sample of 20 µL was analyzed with a 6-minute gradient method.

Mass Spectrometry

MS analysis was carried out on the Exactive™ benchtop LC-MS instrument equipped with an electrospray ionization (ESI) source. Full scan data with resolution of 100,000 FWHM at *m/z* 200 was acquired.

Results and Discussion

Figure 1 shows the linear calibration curves for EtG (100-20,000 ng/mL) and EtS (100-20,000 ng/mL).

Figure 2 shows chromatograms of EtG and EtS at 25 ng/mL and the respective deuterated internal standards. Chromatograms for compound detection and quantitation are reconstructed with a mass tolerance of 5 ppm.

Conclusion

The Exactive benchtop LC-MS instrument provides excellent quantitative analysis of EtG and EtS in a 6-minute method. When applied to real samples, the method meets the demands of today's forensic toxicology laboratories with exceptional performance.

Method Performance Summary

Target Analytes	Ethyl glucuronide	Ethyl sulfate
Matrix	Urine	Urine
LOD	25 ng/mL	25 ng/mL or less
LOQ	100 ng/mL	100 ng/mL
Recovery	> 85%	> 85%
Precision	< 15%	< 15%
Assay Linearity	100 – 20,000 ng/mL	100 – 20,000 ng/mL
Carryover at LLOQ	< 1%	< 1%
Sample Volume	100 µL	100 µL
Analysis Time	6 minutes	6 minutes

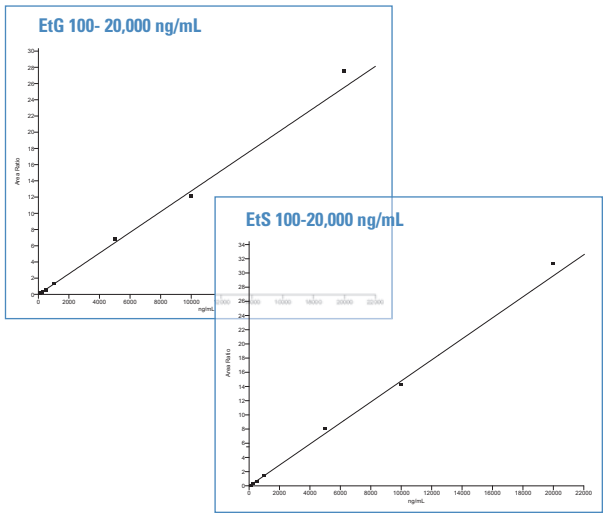

Figure 1: Linear calibration curves for EtG (100-20,000 ng/mL) and EtS (100-20,000 ng/mL).

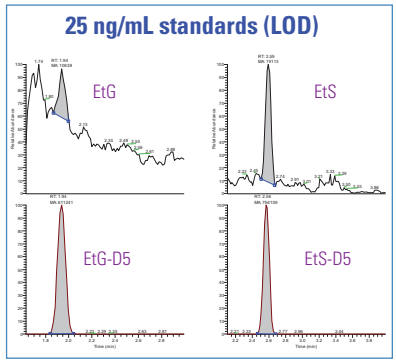

Figure 2: LOD chromatograms of EtG and EtS at 25 ng/mL with deuterated internal standards.

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