

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

## Forensic Toxicology Use Only

Kent Johnson, Fortes Laboratories, Portland, OR; Marta Kozak, Thermo Fisher Scientific, San Jose, CA

### 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  $\mu$ m). A diluted sample of 20  $\mu$ L 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 $\mu$ L	100 $\mu$ L
Analysis Time	6 minutes	6 minutes

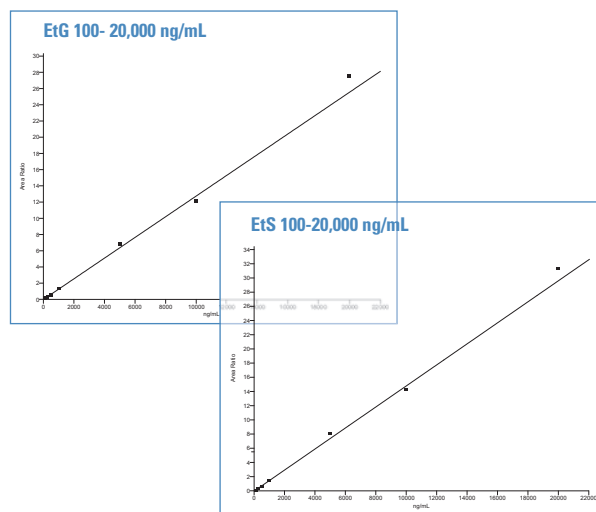


Figure 1: Linear calibration curves for EtG (100-20,000 ng/mL) and EtS (100-20,000 ng/mL).

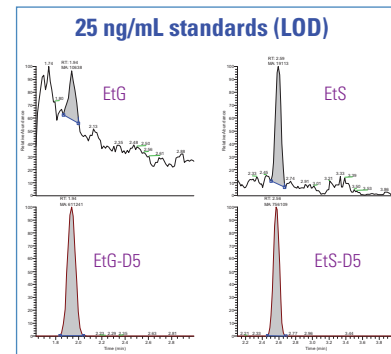


Figure 2: LOD chromatograms of EtG and EtS at 25 ng/mL with deuterated internal standards.

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