



## • Ultrafast confirmation and quantitation of alcohol metabolite markers using the EVOQ Elite TQ coupled to the Elute UHPLC

Quantitation of ethyl glucuronide and ethyl sulfate in urine with the EVOQ TQ MS System.

### Abstract

This study demonstrates a fast and reliable method for the quantitation of the alcohol markers ethyl glucuronide and ethyl sulfate

in urine using the EVOQ Elite™ triple quadrupole mass spectrometer coupled to the new Bruker Elute™ UHPLC. The workflow followed a straightforward sample preparation using protein precipi-

tation with methanol followed by dilution. The method showed excellent precision and linearity and was successfully applied to real samples.

*Keywords:*  
Ethyl glucuronide,  
ethyl sulfate,  
urine



Fig. 1: EVOQ triple quadrupole MS with Elute UHPLC

## Introduction

Ethyl glucuronide (EtG) and ethyl sulfate (EtS) are direct metabolites of ethanol. Although in the human body only a small part of the ethanol consumed is transformed into these two compounds they are used as biomarkers for alcohol consumption or abstinence, e.g. fitness to drive test.

As their detection window is about 3 – 5 days in urine, they can still be found even if the ethanol concentration is no longer measurable. Common cut-off values for EtG are around 100 ng/mL.

As EtG false-negative results can occur due to bacterial degradation e.g. in case of a urinary tract infection, false-positive results may occur after the use of ethanol-based mouthwash or using hand-sanitizers. For these rea-

sons it is important to include EtS in the assay as well.

High Performance Liquid chromatography (HPLC) coupled to a triple quadrupole mass spectrometer provides a specific detection technique for both EtG and EtS.

## Experimental

### Samples

Urine calibrators at six different concentrations (Table 2) as well as a quality control sample were obtained from Recipe (Munich). Two samples of a round robin test organized by the "Society of Toxicological and Forensic Chemistry" (GTFCh) were analyzed. In addition, urine samples of two individuals (both social drinkers; individual 1 female, 30 y, 61 kg; individual 2 male, 37 y, 64 kg) were taken 3 and 24 hours

after the consumption of one glass of wine (200 mL) and one glass of spirits (20 mL) each.

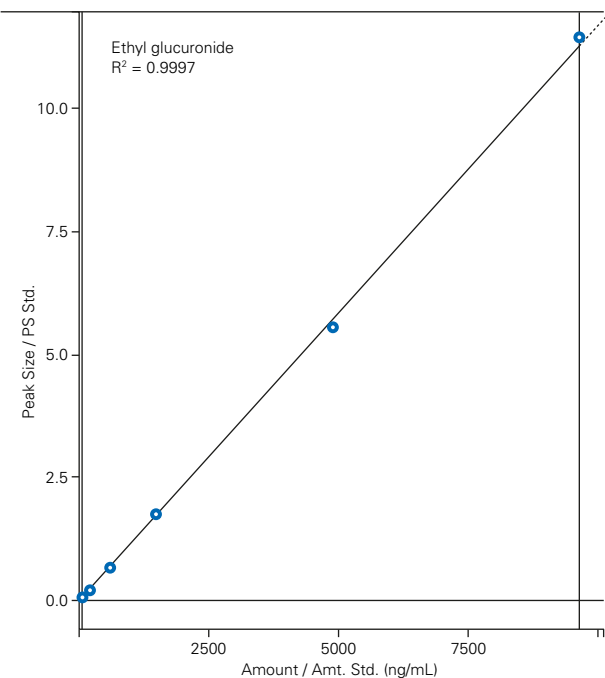
### Sample Preparation

Urine samples were centrifuged at 4000 rpm for 10 min. 100  $\mu$ L urine, 20  $\mu$ L internal standard solutions (5  $\mu$ g/mL EtG-d5 and EtS-d5) and 250  $\mu$ L methanol were added to an Eppendorf tube. After vortexing, the tubes were centrifuged and 270  $\mu$ L supernatant were transferred into an HPLC vial. The supernatant was evaporated in a vacuum concentrator at 45°C and the residue reconstituted in 600  $\mu$ L 0.1 % formic acid.

## Instrumentation

UHPLC:	Bruker Elute UHPLC	Mass spectrometer:	EVOQ Elite triple quadrupole mass spectrometer
Column:	Restek, Ultra Biphenyl, 3 $\mu$ m, 2.1 x 100 mm with guard column 2.1 x 10 mm	Ion source:	VIP H-ESI negative, 4000 V
Mobile phase A:	Water, 2 mM ammonium formate, 0.1 % formic acid	Probe gas:	50 units at 300°C
Mobile phase B:	Methanol, 2 mM ammonium formate, 0.1 % formic acid	Cone gas:	25 units at 350°C
Gradient:	0.0 min 2 % B 1.0 min 80 % B 2.0 min 80 % B 2.01 min 2 % B 3.0 min 2 % B	Nebulizing gas:	50 units
Flow rate:	400 $\mu$ L/min	Active exhaust:	on
Injection volume:	10 $\mu$ L	Collision gas:	Argon, 1.5 mTorr
Column oven:	40°C	MRM transitions:	see Table 1

### Ethylglucuronide



### Ethylsulfate

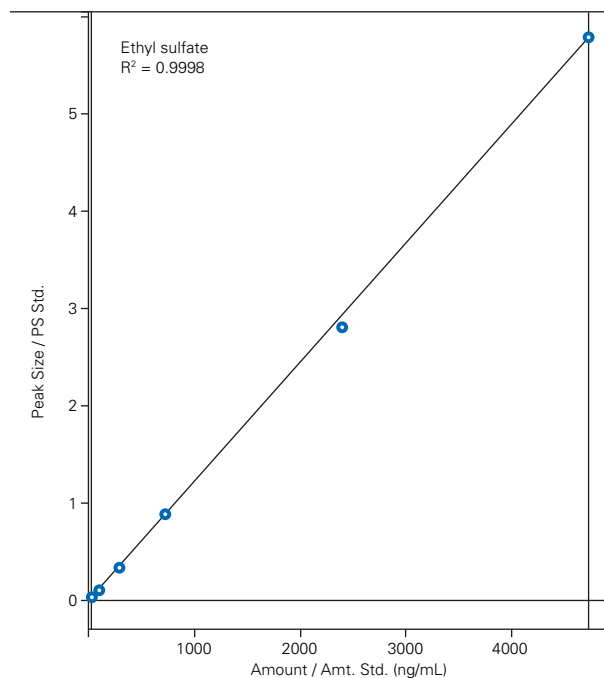


Fig. 2: Calibration curves of EtG (left) and EtS (right) over the range 80 - 9621 ng/mL (EtG) and 27 - 4732 ng/mL (EtS)

## Results and Discussion

Calibration curves showed excellent linearity ( $r^2 \geq 0.999$ ) and a very good accuracy with bias  $< \pm 8\%$  for both EtG and EtS (Figure 2, Table 2). The quality control sample also had a good accuracy with a deviation of 9.4 % for both analytes. Replicate injections ( $n=5$ )

of the two round robin test samples demonstrated excellent precision with  $RSD \leq 2.8\%$  for both compounds. The quantitative results were all within the accepted range (Table 3).

Both EtG and EtS could be detected in the two real samples. In the samples taken 3 h after the consumption

of alcoholic drinks the concentrations even exceeded the calibration range. Both analytes were still detectable in the two samples 24 h after consumption (Table 4). The new Elute UHPLC system provided excellent chromatographic peak shape and a FWHM (Full width half maximum) of only 3-4 sec. as shown in Fig. 3.

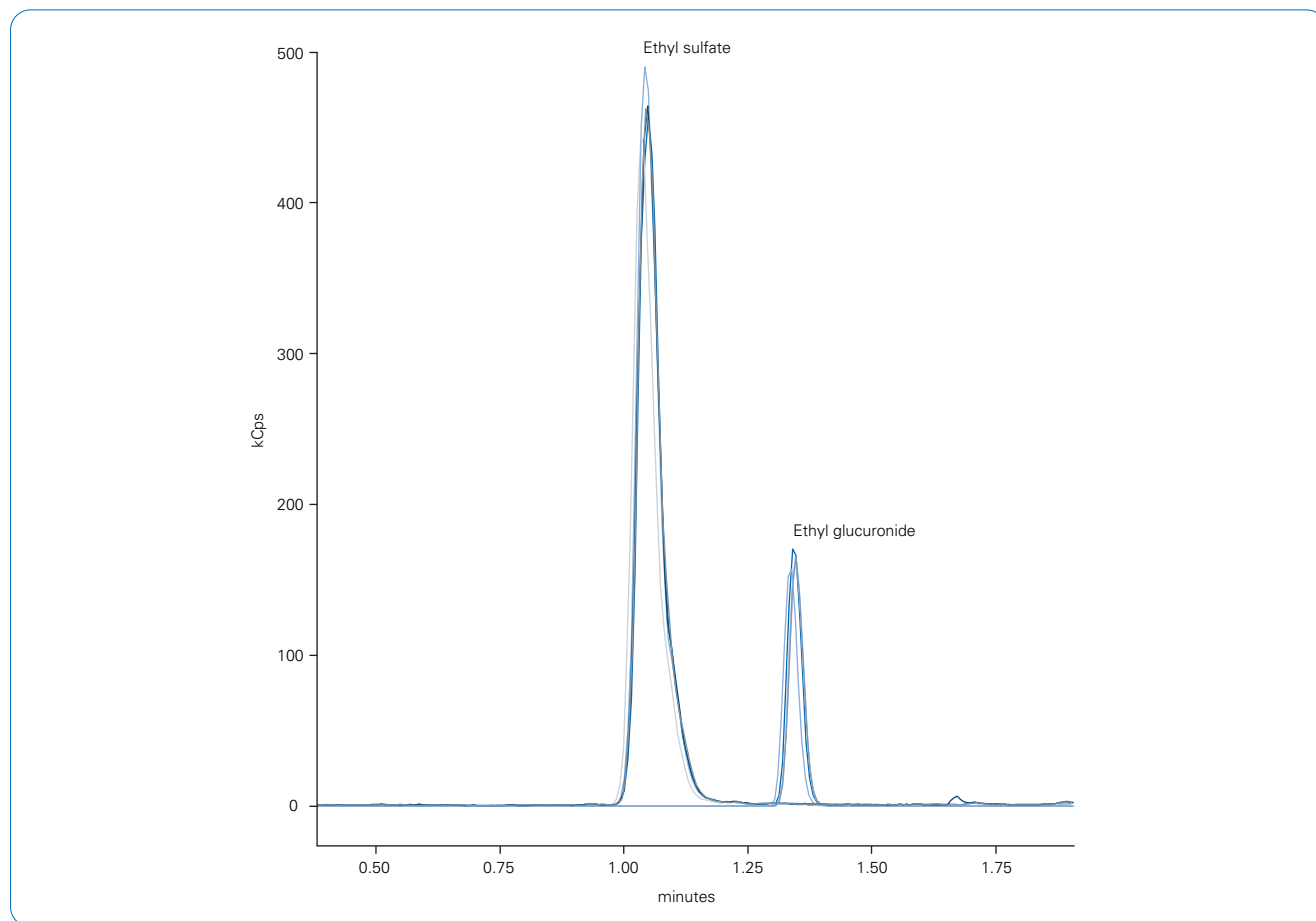


Fig. 3: Overlaid chromatograms of the five replicates of round robin test sample 1; left peak EtS, right peak EtG

Table 1: MRM transitions

Compound	Rt (min)	Precursor Ion	Product Ion 1	CID 1 (V)	Product Ion 2	CID 2 (V)
Ethyl glucuronide	1.34	221.0	75.2	11	85.2	14
Ethyl glucuronide-d5	1.33	226.1	75.0	11		
Ethyl sulfate	1.08	125.0	97.1	12	80.1	26
Ethyl sulfate-d5	1.07	130.0	98.0	12		

Table 2: Calibration and QC of EtG and EtS

Sample	Ethyl glucuronide			Ethyl sulfate		
	Target concentration [ng/mL]	Calculated concentration [ng/mL]	Bias [%]	Target concentration [ng/mL]	Calculated concentration [ng/mL]	Bias [%]
Calibrator 1	80.1	80	-0.1	27.8	27	-2.5
Calibrator 2	191	192	0.6	75.9	82	7.6
Calibrator 3	582	570	-2.1	244	236	-3.2
Calibrator 4	1464	1505	2.8	580	594	2.4
Calibrator 5	4873	4754	-2.4	1933	1860	-3.8
Calibrator 6	9621	9736	1.2	4732	4705	-0.6
Quality control	111	121	9.4	48	53	9.4

Table 3: Results of round robin test

Sample	Analyte	Target concentration [ng/mL]	Accepted Range [ng/mL]	Calculated concentration [ng/mL] (mean, n = 5)	Accuracy Bias [%]	Precision RSD [%] (n = 5)
Round robin test sample 1	EtG	609	399 – 819	613	0.7	2.3
	EtS	886	596 – 1176	856	-3.4	2.4
Round robin test sample 2	EtG	1240	840 – 1640	1558	25.6	2.8
	EtS	790	528 – 1052	807	2.2	2.6

Table 4: Results of real samples; \* concentration above calibration range

Sample	Concentration EtG [ng/mL]	Concentration EtS [ng/mL]
Individual 1: 3 h	approx. 19,000*	approx. 9,000*
Individual 1: 24 h	93	96
Individual 2: 3 h	approx. 19,000*	approx. 8,600*
Individual 2: 24 h	179	124

## Conclusion

A fast three minute method to confirm and quantify EtG and EtS in urine with the new Bruker Elute UHPLC coupled to the EVOQ Elite triple quadrupole MS was presented. The method features excellent linearity and very good precision. EtG and EtS were detected in the two real samples.



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