

Analysis of Ethylglucuronide and Ethylsulfate in Urine, Plasma and Serum by LCMS-8050 using RECIPE ClinMass® LC-MS/MS Complete Kit MS8000

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■ Introduction

In many societies around the world alcohol abuse represents a serious social and economic problem. For alcohol misuse several parameters of laboratory diagnostics are available. They are used for the assessment of acute drinking, chronic abuse (alcoholics), abstinence control and relapse diagnosis.

Acute alcohol abuse, which dates back several hours, is mainly determined by ethanol in the respiratory air (alcohol breath test) and by the blood alcohol level (short-term marker). For the investigation of a long-term, chronic abuse the determination of CDT (Carbohydrate Deficient Transferrin) has been established for routine analysis.

Ethylglucuronide (EtG) and Ethylsulfate (EtS) are formed in the ethanol metabolism and therefore serve in addition to the short-term marker ethanol and the longterm marker CDT for the verification of alcohol abuse. They can be determined in urine in a time range up to 80 h after excessive consume of alcohol. Even in case of low to mid uptake of alcohol, Ethylglucuronide and Ethylsulfate can be detected up to 24 h respectively 48 h.

■ Materials and methods

The LCMS-8050 triple quadrupole mass spectrometer was coupled to a Nexera X2 UHPLC system. EtG and EtS were measured using a commercially available test kit ClinMass® Complete Kit for Ethylglucuronide and Ethylsulfate in Urine, Plasma and Serum, MS8000, MS8100 (RECIPE Chemicals + Instruments GmbH, Dessauerstraße 3, 80992

München, Germany). Calibrators, control samples, analytical column and mobile phase solvents were provided by the kit. 50 µL of urine sample was added to 1000 µL of internal standard solution and mixed for 5 sec. 10 µL of the sample was analysed. For analysis the [M-H]⁻ ion was measured and used as the precursor ion (negative electrospray ionization).

■ Analytical conditions

<i>UHPLC:</i>	Nexera X2 UHPLC
	0.2 mL/min (0.0 - 2.5 min)
	0.5 mL/min (2.6 - 4.8 min)
	0.2 mL/min (4.9 - 5.0 min)
<i>Column temperature:</i>	40 °C
<i>Injection volume:</i>	10 µL
<i>Mass spectrometer:</i>	LCMS-8050
<i>Source conditions:</i>	
<i>Nebulizer Gas:</i>	3 L/min
<i>Heating Gas:</i>	10 L/min
<i>Drying Gas:</i>	5 L/min
<i>Interface temperature:</i>	200 °C
<i>Desolvation Line:</i>	200 °C
<i>Heat Block temperature:</i>	200 °C
<i>Interface voltage:</i>	-2.5 kV
<i>Dwell time:</i>	50 msec
<i>Pause time:</i>	3 msec
<i>Ionization:</i>	Electrospray ionization (ESI) negative mode
<i>Scan Type:</i>	MRM

Table 1 EtG/EtS MRM transitions, retention time (RT). T/I = target or internal standard

Compound	Formula	MRM1	MRM2	RT
EtG	T C ₈ H ₁₄ O ₇	221>75	221>85	2.08
D5-EtG	I C ₈ H ₉ D ₅ O ₇	226>75	226>85	2.07
EtS	T C ₂ H ₆ O ₄ S	125>97	125>80	3.95
D5-EtS	I C ₂ HD ₅ O ₇ S	130>98	130>80	3.92

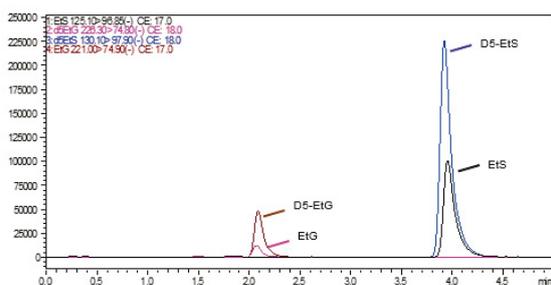


Figure 1 LC-MS separation of EtG / EtS and deuterated standard in five minutes by isocratic chromatography.

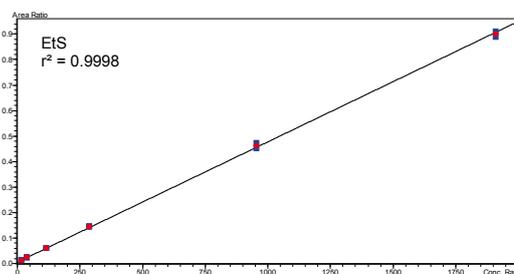
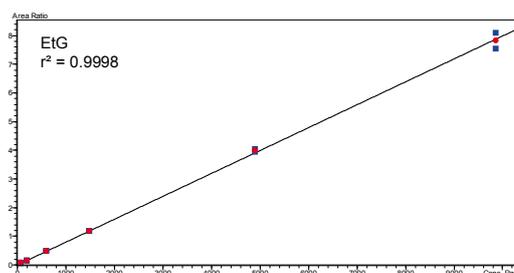


Figure 2 Calibration curves for EtG and EtS.

EtG	Control Level I (111 µg/L)	Control Level II (518 µg/L)	Control Level III (2052 µg/L)
	Conc.	Conc.	Conc.
Control 1	108.055	523.773	2124.918
Control 2	94.895	529.889	2005.968
Control 3	118.378	509.452	2137.082
Control 4	104.611	503.697	2071.541
Mean	106.485	516.703	2084.877
SD	9.691	12.187	59.814
%RSD	9.10	2.36	2.87

Table 2 Reproducibility for EtG

EtS	Control Level I (48 µg/L)	Control Level II (201 µg/L)	Control Level III (799 µg/L)
	Conc.	Conc.	Conc.
Control 1	49.174	209.118	781.053
Control 2	50.413	199.111	772.997
Control 3	46.561	212.100	790.709
Control 4	44.721	200.284	775.448
Mean	47.717	205.153	780.052
SD	2.563	6.434	7.864
%RSD	5.37	3.14	1.01

Table 3 Reproducibility for EtS

■ Results

The rapid elution of EtG and EtS by isocratic chromatography produced excellent peak shape and accuracy with elution in five minutes (Fig. 3).

The calibration curve determined in duplicate showed good linearity over a clinically relevant range of 78.6-9860 µg/L for EtG and 15.3-1910 µg/L for EtS (Fig. 2)

Three control samples at high, mid and low concentration were analyzed in fourfold to measure analytical reproducibility. The percentage relative standard deviation was typically lower than 10% from these measurements.

■ Conclusion

The application of the clinical ClinMass® Complete Kit, for Ethylglucuronide and Ethylsulfate in Urine, Plasma and Serum proved easy to implement and showed good sensitivity and linearity in a clinically relevant concentration range.