

Extraction of Ethyl Glucuronide (EtG) from urine using EVOLUTE[®] AX and RapidTrace⁺ Workstation

Introduction

This application note describes for the extraction of Ethyl Glucuronide (EtG) from urine using EVOLUTE AX and RapidTrace⁺ Workstation.

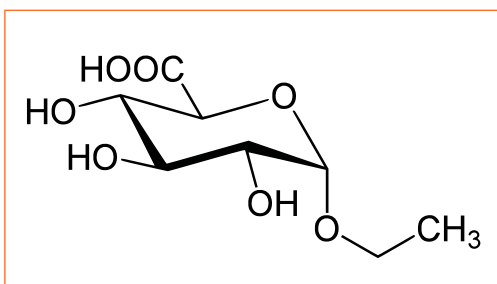


Figure 1. Structure of Ethyl Glucuronide

Ethyl glucuronide (EtG, **Figure 1.**) is an ethanol metabolite formed by the glucuronidation of the parent molecule. The presence of EtG in urine and plasma is a useful and sensitive biomarker of alcohol intake in forensic toxicology. EtG can be detected at very low levels and is useful in distinguishing between ingested ethanol and that which is adsorbed through the skin from ethanol.

This application note has been optimized on the RapidTrace⁺; an updated version of the current RapidTrace software which allows the system to process smaller bed heights than were previously possible. The application note shows analyte recoveries all above 97% with RSDs >10%.

EVOLUTE AX is a resin-based mixed-mode sorbent with an optimized combination of non-polar (hydrophobic), polar (hydrophilic) and weak anion exchange interactions ideally constructed to extract acidic structures like that of Ethyl glucuronide.

Sample pre-treatment

Dilute urine (100 μ L) in acetonitrile (1900 μ L).

RapidTrace⁺ procedure

EVOLUTE AX 100 mg/3 mL 50 μ m part number, 613-0010-B.

	Step	Source	Destination	Volume (mL)	Flow (mL/min)
1	Condition	Methanol	Organic Waste	3.0	3.00
2	Condition	Water	Aqueous Waste	3.0	3.00
3	Condition	ACN	Organic Waste	3.0	3.00
4	Load	Sample	Biological Waste	2.0	1.00
5	Rinse	Water	Aqueous Waste	3.0	2.00
6	Rinse	ACN	Organic Waste	3.0	2.00
7	Collect	ACN:H ₂ O:Formic acid (95:4:1)	Fraction 1	3.0	2.00
8	Collect	Vent	Fraction 1	2.0	2.00
9	Purge-Cannula	ACN	Organic Waste	5.0	30.00
8	Purge-Cannula	Methanol	Cannula Waste	5.0	30.00
9	Purge-Cannula	Water	Cannula Waste	5.0	30.00

Evaporate to dryness and reconstitute in water:methanol (80/20, v/v) (200 μ L). Biotage recommends TurboVap Workstations post elution to evaporate your samples.

HPLC Conditions

Instrument: Waters Acquity UPLC (Waters Assoc., Milford, MA, USA).
Column: Acquity UPLC BEH C18 column (1.7µm, 50 x 2.1 mm id) (Waters Assoc., Milford, MA, USA).
Mobile Phase: Isocratic, 0.1% (v/v) formic acid aq/Methanol (80:20, v/v) at a flow rate of 0.5 mL/min.

Mass Spectrographic Conditions

Instrument: Premier XE triple quadrupole mass spectrometer (Waters Assoc., Manchester, UK) equipped with an electrospray interface for mass analysis. Negative ions were acquired in the multiple reaction monitoring mode
Desolvation Temperature: 450 °C
Ion Source Temperature: 150 °C
Collision Gas Pressure: 3.46 x 10⁻³ mbar
MRM Transitions: 221.0 > 84.9 (quantifier), 221.0 > 74.9 (qualifier).

Results

As shown in figure 2 average analyte recoveries were all above 97% (n=3) based upon six injections per run with RSDs <10% for all recoveries.

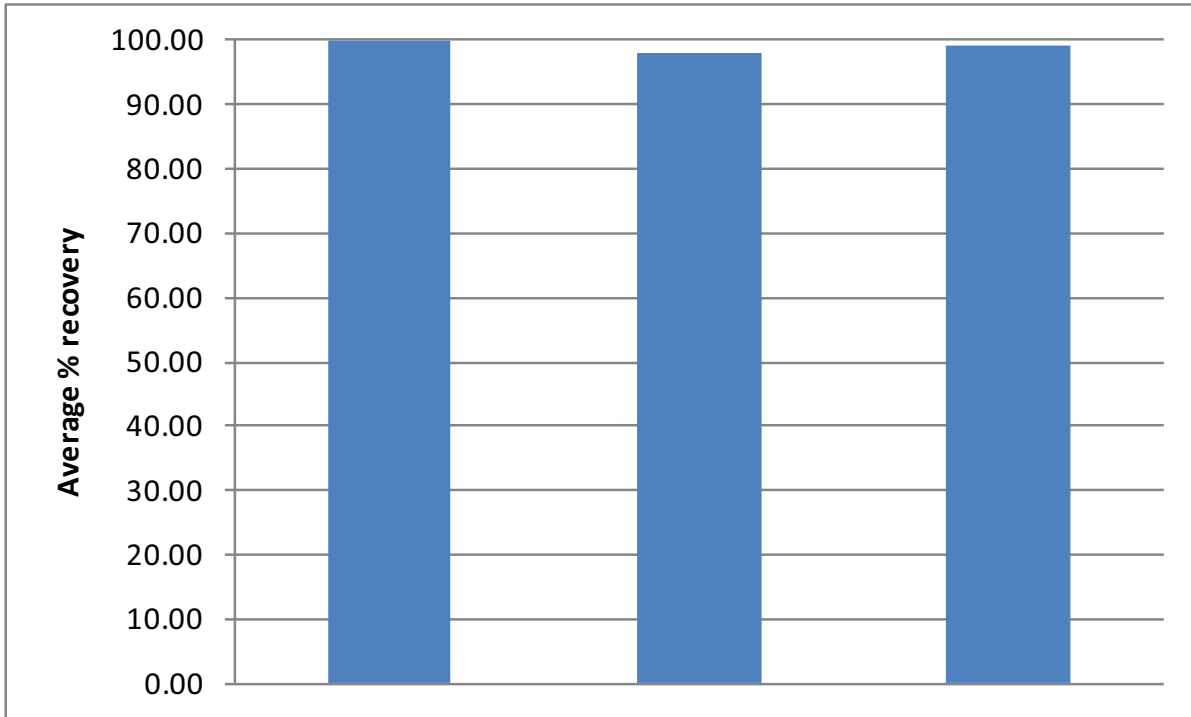


Figure 2. Average analyte recoveries of EtG from urine (n=3)

Reagent lines

Line 1: Water (HPLC grade)

Line 2: Methanol (HPLC grade)

Line 3: Acetonitrile (HPLC grade)

Line 4: ACN:H₂O:formic acid (v/v, 95:4:1)

Ordering information

Part number	Description	Quantity
613-0010-B	EVOLUTE AX 100 mg/ 3mL 50 µm	50
C50000	RapidTrace ⁺ 3 mL Workstation	1
C133966	RapidTrace ⁺ Software Upgrade 2.1	1
C50974	Tube Rack (13 x 100 mm Sample Tubes & 12 x 75 Fraction Tubes)	1
C40707	Test Tubes-13 x 100 mm, Uncap	1000
C44651	Test Tubes-12 x 75 mm, Uncap	1000
C52006	RapidTrace Start-Up Kit	1
C52689	RapidTrace Notebook Controller	1

RapidTrace Overview



The RapidTrace has been designed to eliminate sample preparation bottlenecks and it is a modular, highly scalable, automated platform designed for high throughput. Units are available to accommodate 1 mL, 3 mL and 6 mL SPE cartridges. Up to 10 modular units can be connected together and controlled through a simple, easy-to-use software package. The systems are widely used within analytical industry and are ideal both for industrial settings and for efficient SPE method development.

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