Extraction of Ethyl Glucuronide (EtG) from Urine Using EVOLUTE AX 96-Well Plates and Columns

Application Note

Introduction

This application note describes the extraction of ethyl glucuronide (EtG) from urine using EVOLUTE AX plates or columns.

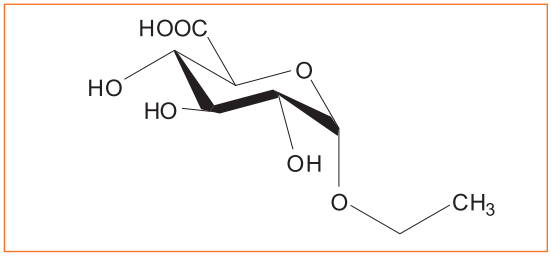

Figure 1. Ethyl glucuronide

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 bio-marker of alcohol intake in forensic toxicology. EtG can be detected at very low levels and being a metabolite it is useful in distinguishing between ingested ethanol and that which is adsorbed through the skin from ethanol containing products such as alcohol based hand washes. Rapid and reliable methods with robust extraction protocols are essential for analysis and quantitation of EtG from various matrices.

EVOLUTE AX is a resin-based mixed-mode sorbent with an optimised combination of non-polar (hydrophobic), polar (hydrophilic) and strong anion exchange interactions ideally constructed to extract acidic compounds like ethyl glucuronide.

Sample Preparation Procedure

Analytes

Ethyl glucuronide (EtG)

EVOLUTE AX Configuration

25 mg 96-fixed-well plate (part number 603-0025-P01)

This application note outlines the procedure using the 25mg 96-well plate format. Other formats are available and optimized methodology is detailed in Table 1.

EVOLUTE AX Procedure

Sample:	Urine (50 µL) spiked at 500 ng/mL with EtG
Sample Pre-treatment:	Urine (50 µL) in acetonitrile (950 µL)
Column Conditioning:	Methanol (1 mL)
Column Equilibration 1:	Water (1 mL)
Column Equilibration 2:	Acetonitrile (1 mL)
Sample Loading:	Pre-treated sample (1 mL)
Interference Elution 1:	Water (1 mL)
Interference Elution 2:	Acetonitrile (1 mL)
Analyte Elution:	Acetonitrile:water:formic acid, (1 mL, 95:4:1, v/v)
Post Extraction:	Evaporate to dryness and reconstitute with water/methanol (200 µL, 80:20, v/v) for LC-MS/MS analysis.

This method is suitable to be applied across the range of EVOLUTE AX formats, dilution factors have been optimized to maximize recoveries and minimize ion suppression.

EVOLUTE AX procedure	**Solution**	25 mg 96-Well-Plate **Part No.** 603-0025-P01	50 mg/3 mL Column **Part No.** 613-0005-B	100 mg/3 mL Column **Part No.** 613-0010-B	200 mg /3 mL Column **Part No.** 613-0020-B	500 mg/6 mL Column **Part No.** 613-0050-C
Sample	Urine	50 µL	200 µL	200 µL	400 µL	1 mL
Sample pre-treatment	Acetonitrile	950 µL	1.8 mL	1.8 mL	3.6 mL	4 mL
Column Conditioning	Methanol	1 mL	2 mL	3 mL	6 mL	6 mL
Column equilibration 1	Water	1 mL	2 mL	3 mL	6 mL	6 mL
Column equilibration 2	Acetonitrile	1 mL	2 mL	3 mL	6mL	6 mL
Sample loading	Pre-treated sample	1 mL	2 mL	2 mL	4 mL	5 mL
Interference elution 1	Water	1 mL	2 mL	3 mL	6 mL	6 mL
Interference elution 2	Acetonitrile	1 mL	2 mL	3 mL	6 mL	6 mL
Analyte elution	Acetonitrile:Water: Formic acid, (95:4:1, v/v)	1 mL	2 mL	3 mL	6 mL	6 mL
Reconstitution	Water/Methanol (80:20, v/v)	200 µL	200 µL	200 µL	200 µL	200 µL

Table 1. Optimized methodology for all EVOLUTE AX formats.

HPLC Conditions

Instrument:	Waters Acquity UPLC (Waters Assoc., Milford, MA, USA).
Column:	Acquity UPLC BEH C18 column (1.7 µm, 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.
Injection Volume:	10 µL
Column Temperature:	40 °C

Mass Spectrometry 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. (MRM); 221.0 > 84.9 (quantifier), 221.0 > 74.9 (qualifier).
Desolvation Temperature:	450 °C
Ion Source Temperature:	150 °C
Collision Gas Pressure:	3.46 x 10^{-3} mbar

Results

All results show recoveries greater than 80% with RSD's below 10%. The method shows that optimization is scalable for sample volumes ranging from 50 µL up to 1 mL.

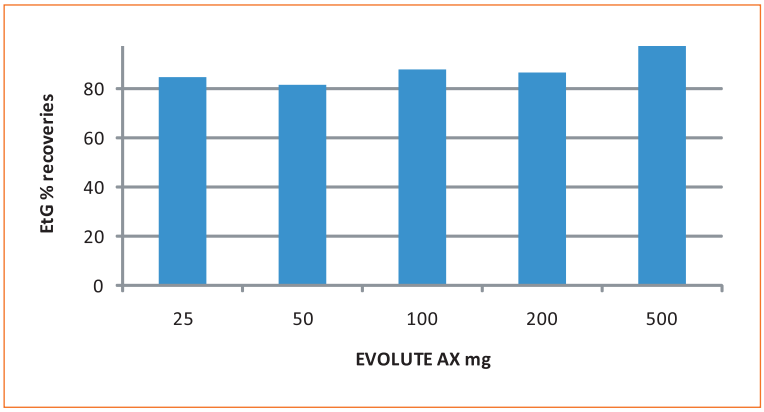

Figure 2. EtG extraction recoveries from urine using EVOLUTE AX

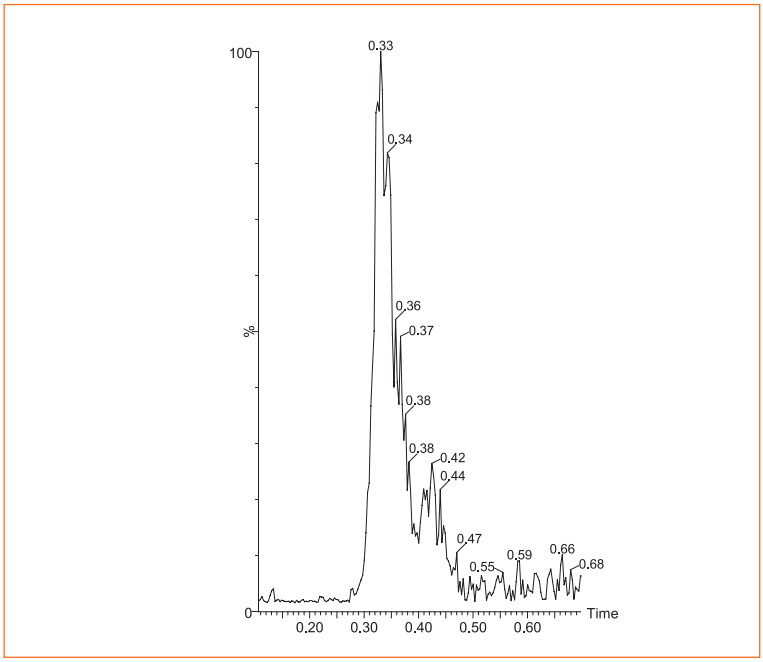

Figure 3. Chromatogram showing extracted EtG (500 ng/mL) using EVOLUTE AX 25 mg 96 well-plate

References

This application note is based on the poster 'Extraction of Ethyl glucuronide (EtG) using a New Resin-Based Mixed-Mode Strong Anion Exchange SPE Sorbent Prior to LC-MS/MS Analysis', L Williams et al, presented at TIAFT, Bonn, August 29 - September 2, 2010.

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