

HPLC Application

ID No.: 23842

EtG / EtS from Urine on Luna Omega 5u Polar C18, 50x4.6mm

Column: Luna Omega 5u Polar C18 100A, LC Column 50 x 4.6 mm, Ea

Dimensions: 50 x 4.6 mm ID

Order No: 00B-4754-E0

Elution Type: Gradient

Eluent A: 0.1% Formic Acid in H₂O

Eluent B: 0.1% Formic Acid in MeOH

Gradient Profile:	Step No.	Time (min)	Pct A	Pct B
	1	0	98	2
	2	2	40	60
	3	2.01	10	90
	4	3	10	90
	5	3.01	98	2
	6	4	98	2

Flow Rate: 1 mL/min

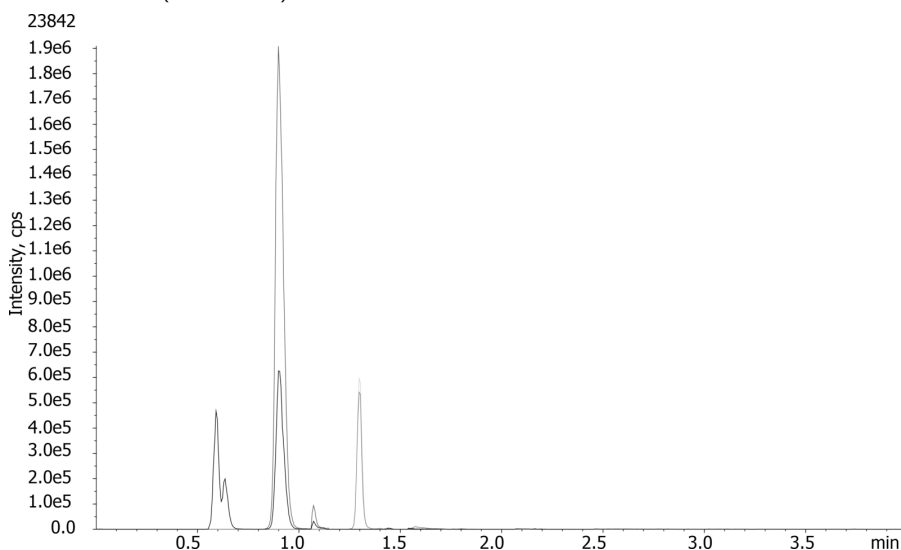
Col. Temp.: 25 °C

Detection: Tandem Mass Spec (MS-MS) @ (550 °C)

Detector Info: <a target="_blank"

Analyst Note: MMA (117.0-->72.9; 117.0-->54.9)

MMA-D3 (120.1-->75.9)



ANALYTES:

- 1 Interference**
Retention Time: 0.593 min
- 2 Ethyl Sulfate (EtS)**
Retention Time: 0.902 min
- 3 Ethyl Glucuronide (EtG)**
Retention Time: 1.299 min



Products used in this application:



Sample Preparation Details

for HPLC Application ID No.: 23842

EtG / EtS from Urine on Luna Omega 5u Polar C18, 50x4.6mm

PRODUCT DESCRIPTION:

Strata[™]-X-AW 33 µm Polymeric Weak Anion, 30 mg / 1 mL, Tubes , 100/Pk

Order No.: 8B-S038-TAK

SOLID PHASE EXTRACTION (SPE) PROCEDURE:

Note: The solvent volumes shown below are for a 30 mg bed mass.

The solvent volumes will need to be adjusted for a smaller or larger bed mass.

Condition:

Load:

1. Into individually labeled 1.5 mL conical micro-centrifuge tubes, combine 0.5 mL of 0.1% acetic acid, 50 µL IS, and 100 µL blank, standard, or sample.
2. Activate the SPE cartridge (Strata-X-AW, 30 mg/1mL) with 1 mL pure methanol.
3. Equilibrate the SPE cartridge with 1 mL of 0.1% acetic acid.
4. Load sample and proceed with elution.
5. Wash the SPE cartridge with 0.5 mL 50% methanol.
6. Dry the SPE bed under high vacuum for 5-10 min.
7. Elute the analyte with 2 x 0.600 mL 2% ammonium hydroxide in methanol.
8. Evaporate the tubes in a concentrator at 45-50°C.
9. Remove the tubes and resuspend the residue in 200 µL Mobile Phase A (0.1% formic acid).

Wash:

Dry:

5-10 min under high vacuum

Elute:

Final Prep and Analysis:

Inject: 10 µL on HPLC Tandem Mass Spec (MS-MS) @ (550°C)

ANALYTES:	Spiked Conc. (ng/mL)	Log P	pKa	% Rec	%RSC (n=0)
1 Interference	0				
2 Ethyl Sulfate (EtS)	0				
3 Ethyl Glucuronide (EtG)	0				

Note: This method is designed as a convenient starting point for further investigation and can be tailored to meet your extraction goals. Call your local Phenomenex Representative for assistance in method development and optimization techniques.

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