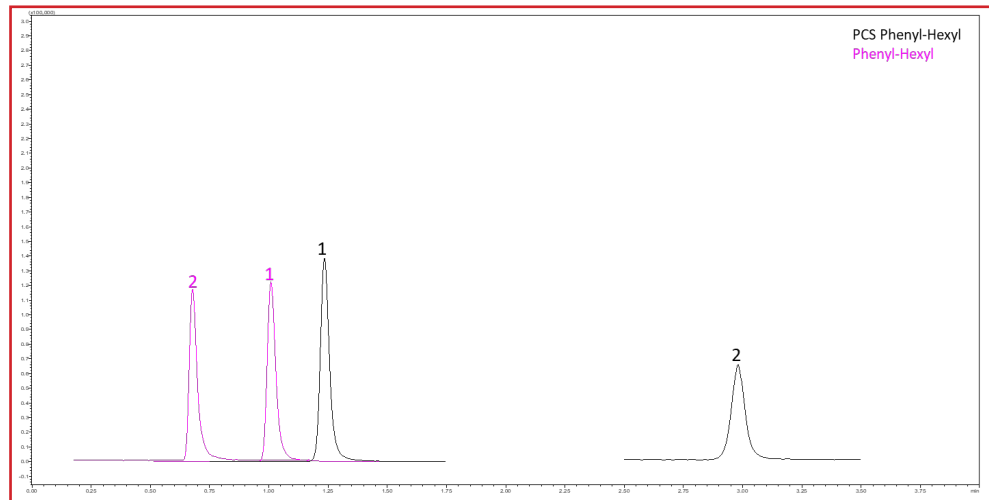




## Boosting EtS Retention: A Charged Approach to Ethanol Metabolite Separation

404



### PEAK IDENTITIES

1. EtG
2. EtS

| Name | Collision energy (eV) | Precursor m/z | Product m/z |
|------|-----------------------|---------------|-------------|
| EtS  | 20                    | 125.1         | 97          |
|      | 40                    |               | 80          |
| EtG  | 16                    | 221.1         | 75          |
|      | 20                    |               | 85          |

### TEST CONDITIONS:

Column: HALO 90 Å PCS Phenyl-Hexyl, 2.7  $\mu$ m, 2.1 x 100 mm

Part Number: 92812-618

Column: HALO 90 Å Phenyl-Hexyl, 2.7  $\mu$ m, 2.1 x 100 mm

Part Number: 92812-606

Mobile Phase A: 0.1% Formic Acid in Water, pH- 2.8

Mobile Phase B: 0.1% Formic Acid in Methanol

|            |      |    |
|------------|------|----|
| Isocratic: | Time | %B |
|            | 0.0  | 3  |
|            | 4.0  | 3  |

Flow Rate: 0.4 mL/min

Back Pressure: 228 bar

Temperature: 30 °C

Injection: 1  $\mu$ L (125ng/mL EtS, 2.5 $\mu$ g/mL EtG)

Sample Solvent: H<sub>2</sub>O

LC System: Shimadzu Nexera X2

MS System: Shimadzu 8060nx Triple Quad

### MS Conditions:

Polarity: Negative mode

Nebulizing Flow: 3 L/min

Heating Gas Flow: 15 L/min

Interface Temperature: 400 °C

Desolvation Temperature: 650 °C

Drying Gas Flow: 3 L/min

DL Temperature: 250 °C

Heat Block Temperature: 400 °C

This application note presents a comparative study on the isocratic separation of ethanol metabolites, ethyl glucuronide (EtG) and ethyl sulphate (EtS), using mass spectrometry detection under formic acid conditions.

The separation is performed on a positively charged (PCS) phenyl-hexyl stationary phase, which demonstrates enhanced retention for EtS compared to a non-charged phenyl-hexyl phase.

The PCS phase significantly improves the retention of EtS, distancing it from the column's void volume. This increased retention is critical for minimizing matrix effects, which are more pronounced when analytes elute near the void volume. In contrast, the non-charged phase shows a substantial drop in EtS retention, leading to potential co-elution with matrix components and reduced analytical sensitivity.

This application highlights how the choice of stationary phase can make a big difference in separating EtS and EtG effectively, especially in complex samples. The HALO® PCS Phenyl-Hexyl phase offers a practical advantage by improving retention and helping reduce matrix interference—making it a solid option for reliable metabolite analysis.