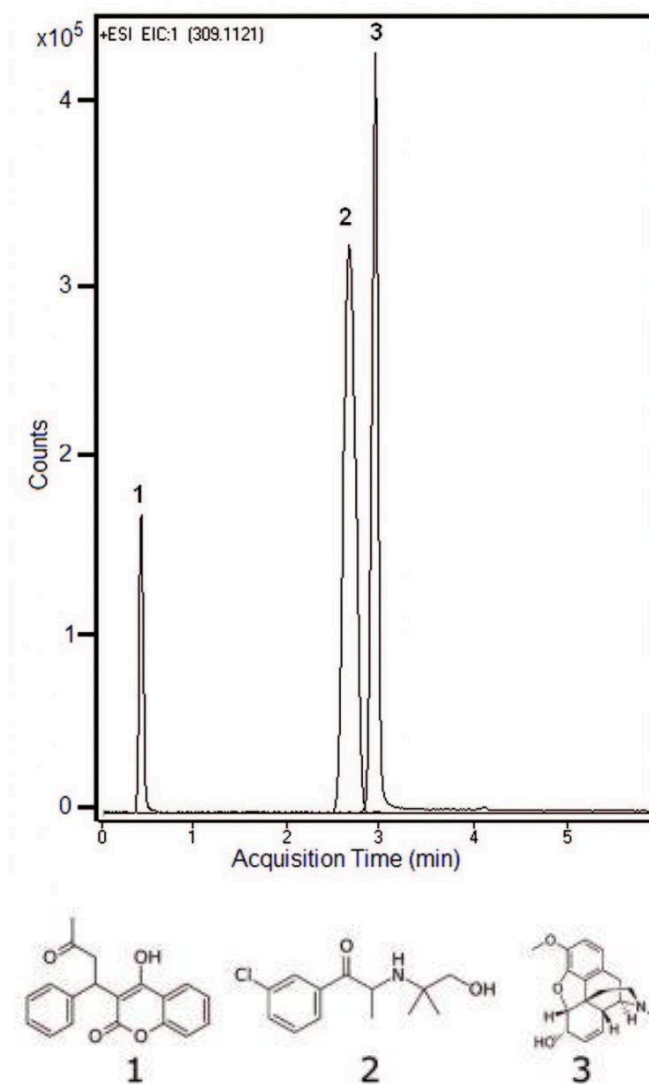


Warfarin, Hydroxybupropion, and Codeine by LCMS - AppNote

Analysis in Spiked Blood Samples

In this application, three pharmaceuticals/metabolites are separated using the Cogent Diol 2.0 Column. The blood sample illustrates the suitability of the Column for analysis of more complex matrices.

Use of Acetone in the Mobile Phase was found to be appropriate for this LC-MS method. As a less expensive and less toxic alternative to Acetonitrile, Acetone presents several advantages to the chromatographer.



Peaks:

1. Warfarin, m/z 309.1121 $[M + H]^+$
2. Hydroxybupropion, m/z 256.1099 $[M + H]^+$
3. Codeine, m/z 300.1594 $[M + H]^+$

Method Conditions

Column: Cogent Diol 2.0™, 2.2µm, 120Å

Catalog No.: [40260-05P-2](#)

Dimensions: 2.1 x 50mm

Mobile Phase:

A: DI Water / 0.1% Formic Acid (v/v)

B: Acetone / 0.1% Formic Acid (v/v)

Gradient:

Time (minutes)	%B
0	90
3	30
5	30
6	90

Flow rate: 0.4 mL/minute

Detection: ESI – POS - Agilent 6210 MSD TOF Mass Spectrometer

Injection vol.: 1µL

Sample Preparation: Stock solutions of each analyte were prepared at 1 mg / mL concentrations using a Methanol diluent. Working solutions were then prepared from the stock solutions at concentrations of 1 micro g / mL. All solutions were stored at -20°C. Solutions used for spiking were prepared at 0.500 micro g / mL concentrations. For Blood samples, 0.2 mL Blood in a 2 mL plastic tube was mixed with 0.2 mL Methanol and 0.2 mL spiking solution. The samples were vortexed for 1 minute and centrifuged for 10 minutes at 13,000 rpm. The final solutions were prepared by diluting 0.2 mL supernatant with 0.5 mL Water / 0.1% Formic Acid.

Note: Warfarin is used in the prevention of Thrombosis. Hydroxybupropion is the active metabolite of Bupropion, a smoking cessation aid. Codeine is an opiate compound that is a common ingredient in many Cough Syrup formulations.



Attachment No 328 Warfarin, Hydroxybupropion, and Codeine by LC-MS pdf 0.3 Mb [Download File](#)

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