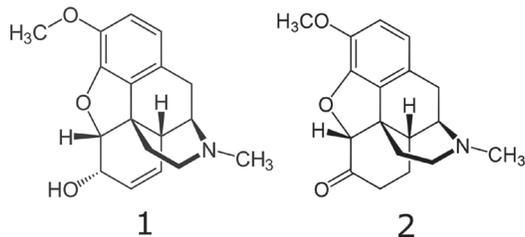
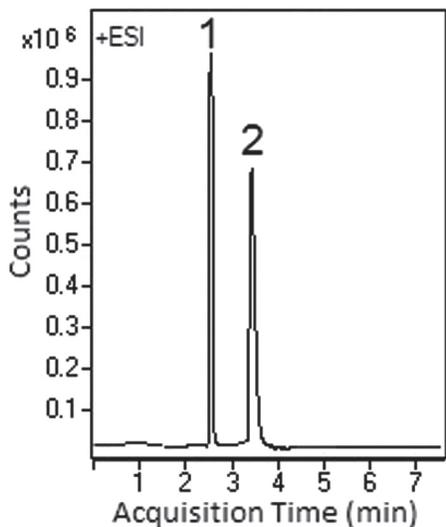


Codeine and Hydrocodone

LC-MS separation of isobaric drugs in urine



Note: Codeine is an opiate used widely because of its antitussive properties as well as others. It is extensively used in cough syrup but it can cause drug addiction if abused. Hydrocodone is a semi-synthetic opioid drug used as a narcotic pain-killer. It is related to codeine but more potent.

Method Conditions

Column: Cogent Bidentate C18 2.0™, 2.2µm, 120Å

Catalog No.: 40218-05P-2

Dimensions: 2.1 x 50 mm

Mobile Phase: A: DI H₂O / 0.1% formic acid (v/v)

B: 50% acetonitrile / 50% methanol / 0.1% formic acid (v/v)

Gradient:	time (min.)	%B
	0	5
	4	50
	5	80
	6	80
	7	5

Post Time: 3 min

Injection vol.: 1µL

Flow rate: 0.4mL/min

Detection: ESI - POS - Agilent 6210 MSD TOF mass spectrometer

Sample: The drugs were spiked into urine at the level of 50 ng/mL.

Extraction method: Spiked urine sample was loaded into SPE cartridge I (Clean Screen Xcel™ purchased from UCT Bristol, PA, USA) and eluted with 0.78 mL of acetonitrile, 200 µL of 2-propanol, 20 µL of ammonia. After the elution, the sample was dried under N₂ gas and dissolved in 100 µL of 50% methanol / 50% DI H₂O / 0.1% formic acid. Before injection, the sample was filtered through a 0.45 µm nylon syringe filter (MicroSolv Tech Corp.).

Peaks: 1. Codeine 300.1594 m/z [M+H]⁺

2. Hydrocodone 300.1594 m/z [M+H]⁺

Discussion

Codeine and hydrocodone which are isobaric drugs (i.e. the same m/z value) were separated using the Cogent Bidentate C18 2.0 column. Multiple samples (n=5) were prepared and analyzed. The results showed excellent reproducibility (RSDs < 2.5%). The presented procedure after validation can be used in forensic toxicological laboratories to determine recent exposure to drugs. Urine collection is a non-invasive procedure and drug concentration in this media is usually much higher than in other matrices.