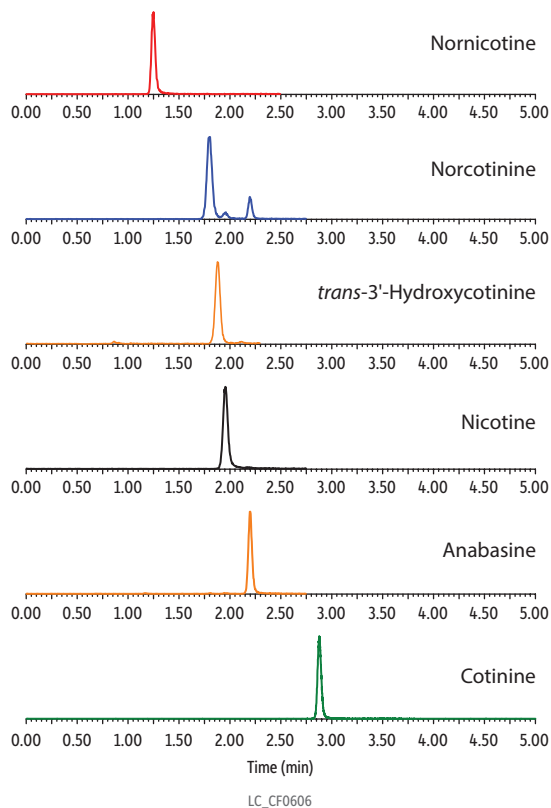


Nicotine-Related Compounds in Urine on Raptor Biphenyl by LC-MS/MS



| Peaks | tr (min) | Precursor Ion | Product Ion |
|-------------------------------------|----------|---------------|-------------|
| 1. Nornicotine | 1.25 | 149.10 | 80.05 |
| 2. Norcotinine | 1.81 | 163.09 | 80.05 |
| 3. <i>trans</i> -3'-Hydroxycotinine | 1.88 | 193.12 | 80.05 |
| 4. Nicotine | 1.95 | 163.15 | 132.10 |
| 5. Anabasine | 2.20 | 163.13 | 91.63 |
| 6. Cotinine | 2.88 | 177.12 | 98.07 |

Column Raptor Biphenyl (cat.# 9309512)
 Dimensions: 100 mm x 2.1 mm ID
 Particle Size: 5 µm
 Pore Size: 90 Å
 Guard Column: Raptor Biphenyl EXP cartridges 5 mm, 2.1 mm ID, 5 µm (cat.# 930950252)
 Temp.: 30 °C

Standard/Sample
 Diluent: Water
 Conc.: 25 ng/mL (fortified human urine)
 Inj. Vol.: 5 µL

Mobile Phase
 A: 0.1% Formic acid, 5 mM ammonium formate in water
 B: 0.1% Formic acid in methanol

| Time (min) | Flow (mL/min) | %A | %B |
|------------|---------------|----|----|
| 0.00 | 0.4 | 90 | 10 |
| 1.00 | 0.4 | 90 | 10 |
| 2.00 | 0.4 | 70 | 30 |
| 3.00 | 0.4 | 30 | 70 |
| 3.01 | 0.4 | 90 | 10 |
| 5.00 | 0.4 | 90 | 10 |

Detector MS/MS
 Ion Mode: ESI+
 Mode: MRM
 Instrument UHPLC

Sample Preparation The urine from a non-tobacco user was fortified with analytes to a final concentration of 25 ng/mL and prepared by a liquid-liquid extraction method. A 250 µL urine sample was mixed with 50 µL of 5N NaOH and extracted with 1.5 mL of 50/50 methylene chloride/diethyl ether. A portion of organic phase (1.0 mL) was dried and reconstituted with 200 µL of water for analysis.