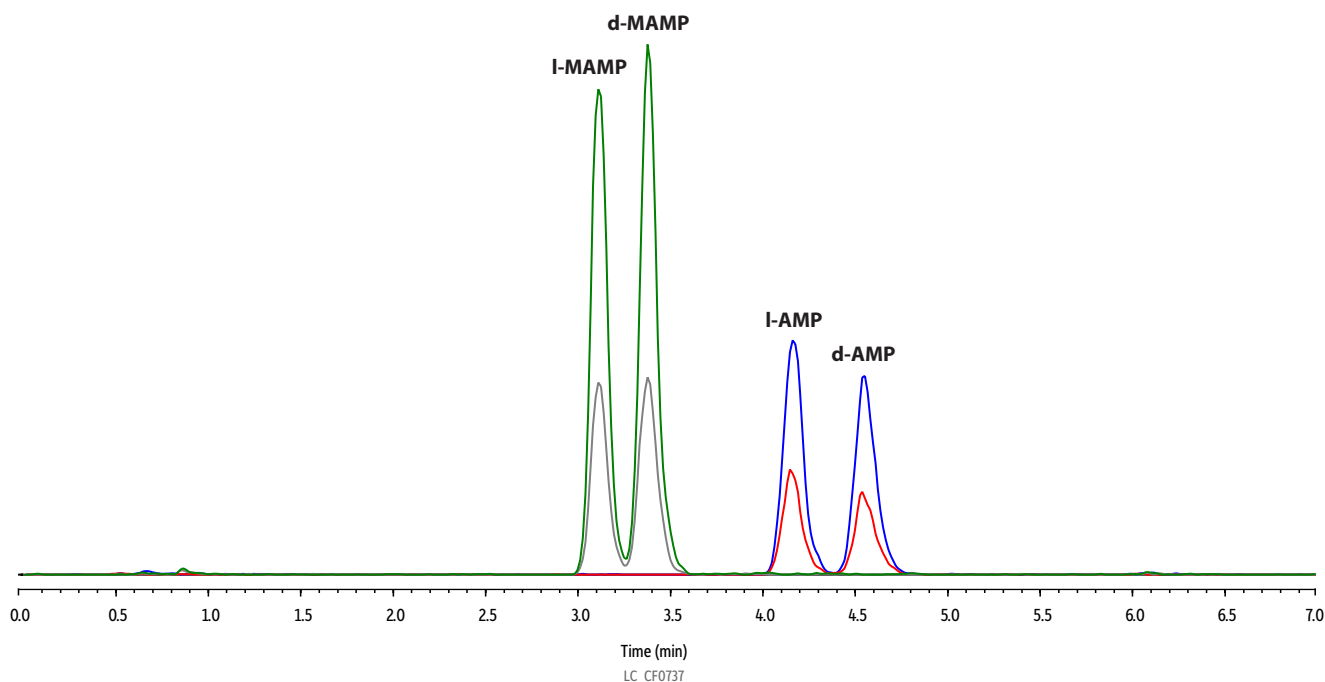


d & l Amphetamines (DNPA Derivatives) in Urine by LC-MS/MS



Peaks	tr (min)	Precursor Ion	Product Ion 1	Product Ion 2
1. l-Methamphetamine*	3.11	400.3	339.0	323.8
2. d-Methamphetamine*	3.38	400.3	339.0	323.8
3. l-Amphetamine*	4.16	386.1	325.0	308.0
4. d-Amphetamine*	4.55	386.1	325.0	308.0

*Analytes are DNPA derivatives.

Column Raptor C18 (cat.# 9304A12)
 Dimensions: 100 mm x 2.1 mm ID
 Particle Size: 2.7 µm
 Pore Size: 90 Å
 Guard Column: Raptor C18 EXP guard column cartridge 5 mm, 2.1 mm ID, 2.7 µm (cat.# 9304A0252)
 Temp.: 35 °C

Standard/Sample
 Conc.: 500 ng/mL in urine
 Inj. Vol.: 10 µL

Mobile Phase
 A: 0.1% Formic acid in water
 B: 0.1% Formic acid in methanol

Time (min)	Flow (mL/min)	%A	%B
0.00	0.5	40	60
5.00	0.5	40	60
5.01	0.5	10	90
5.50	0.5	10	90
5.51	0.5	40	60
7.00	0.5	40	60

Detector MS/MS
 Ion Mode: ESI-
 Mode: MRM
Instrument HPLC

Sample Preparation A 500 ng/mL standard (d- and l-amphetamines and methamphetamines) was prepared in pooled urine. Fifty microliters of the standard was aliquoted into a microcentrifuge tube. Ten microliters of a working internal standard (20 µg/mL (±)-amphetamine-D11 and (±)-methamphetamine-D11 in water) and 20 µL of 1M NaHCO₃ was added and vortexed at 3000 rpm for 10 seconds. After vortexing, 100 µL of 0.1% (w/v) Marfey's reagent (1-fluoro-2-4-dinitrophenyl-5-L-alanine amide) in acetone was added, vortexed, and heated at 45 °C for 1 hour. Samples were allowed to cool to room temperature before the addition of 40 µL of 1M HCl in water. The sample was then vortexed and evaporated to dryness under nitrogen at 45 °C. Samples were reconstituted in 1 mL of 40:60 water:methanol (v/v) and filtered using Thomson SINGLE STEP standard filter vials (cat.# 25893) prior to analysis.

Notes Thomson SINGLE STEP standard filter vials cat.# 25893 were used to produce this chromatogram, but have since been discontinued. For assistance choosing a replacement for this application, contact Restek Technical Service or your local Restek representative.