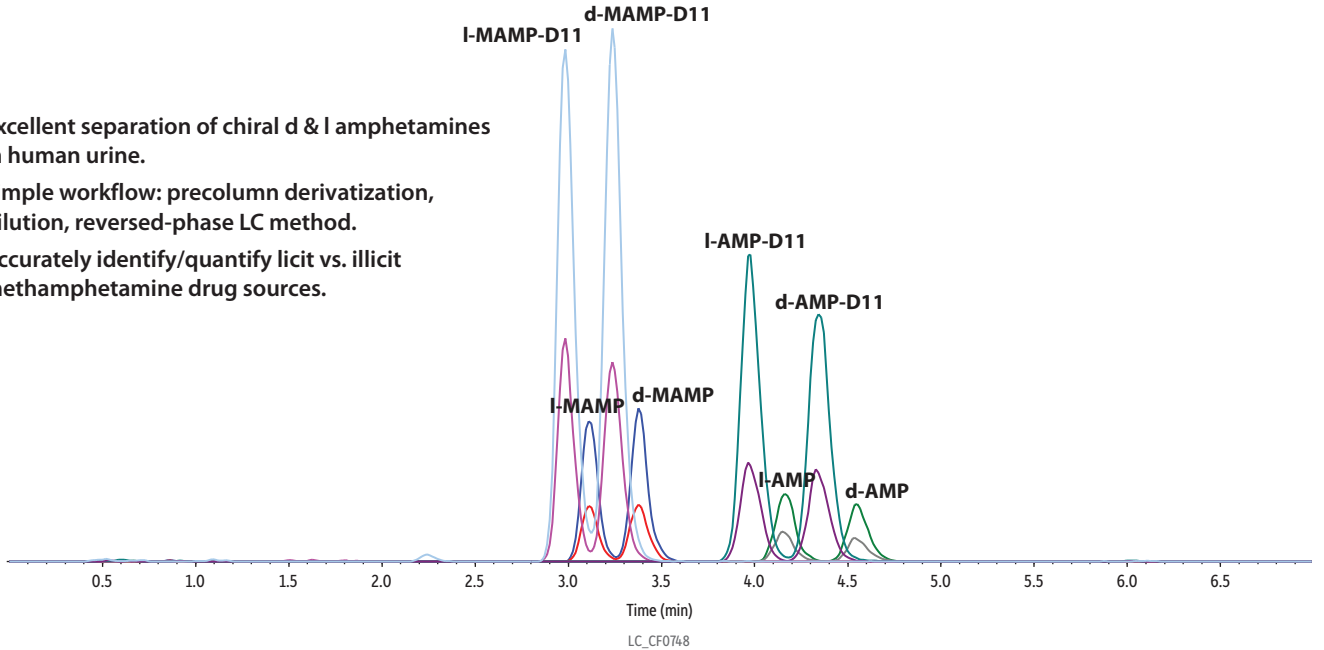
d & l Amphetamines (DNPA Derivatives) TIC in Urine on Raptor C18 by LC-MS/MS

- Excellent separation of chiral d & l amphetamines in human urine.
- Simple workflow: precolumn derivatization, dilution, reversed-phase LC method.
- Accurately identify/quantify licit vs. illicit methamphetamine drug sources.



Peaks	tR (min)	Conc. (ng/mL)	Precursor Ion	Product Ion	Product Ion
1. l-Methamphetamine-D11 (l-MAMP-D11)	2.98	200	411.2	350.2	335.3
2. l-Methamphetamine (l-MAMP)	3.11	500	400.3	339.0	323.8
3. d-Methamphetamine-D11 (d-MAMP-D11)	3.24	200	411.2	350.2	335.3
4. d-Methamphetamine (d-MAMP)	3.38	500	400.3	339.0	323.8
5. l-Amphetamine-D11 (l-AMP-D11)	3.97	200	397.2	336.0	319.0
6. l-Amphetamine (l-AMP)	4.16	500	386.1	325.0	308.0
7. d-Amphetamine-D11 (d-AMP-D11)	4.34	200	397.2	336.0	319.0
8. d-Amphetamine (d-AMP)	4.55	500	386.1	325.0	308.0

All 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
Diluent: 40:60 Water:methanol (v/v)
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 Source: Electrospray
Ion Mode: ESI-
Instrument HPLC
Sample Preparation A 500 ng/mL standard (d- and l-amphetamines and methamphetamines) was prepared in pooled urine. 50 µL of the standard was aliquoted into a microcentrifuge tube. 10 µL of a working internal standard (20 µg/mL (±)-amphetamine-D11 and (±)-methamphetamine-D11 in water) and 20 µL of 1M $NaHCO_3$ 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.