Featured Application: *Amphetamine and Methamphetamine on Raptor C18*

Analysis of d- and l-Amphetamine and Methamphetamine Enantiomers for High-Throughput Labs

- Separate d- and l- enantiomers without chiral columns and dedicated instruments.
- Simple precolumn derivatization and dilution sample preparation.
- Accurately distinguish and quantify licit vs. illicit methamphetamine.

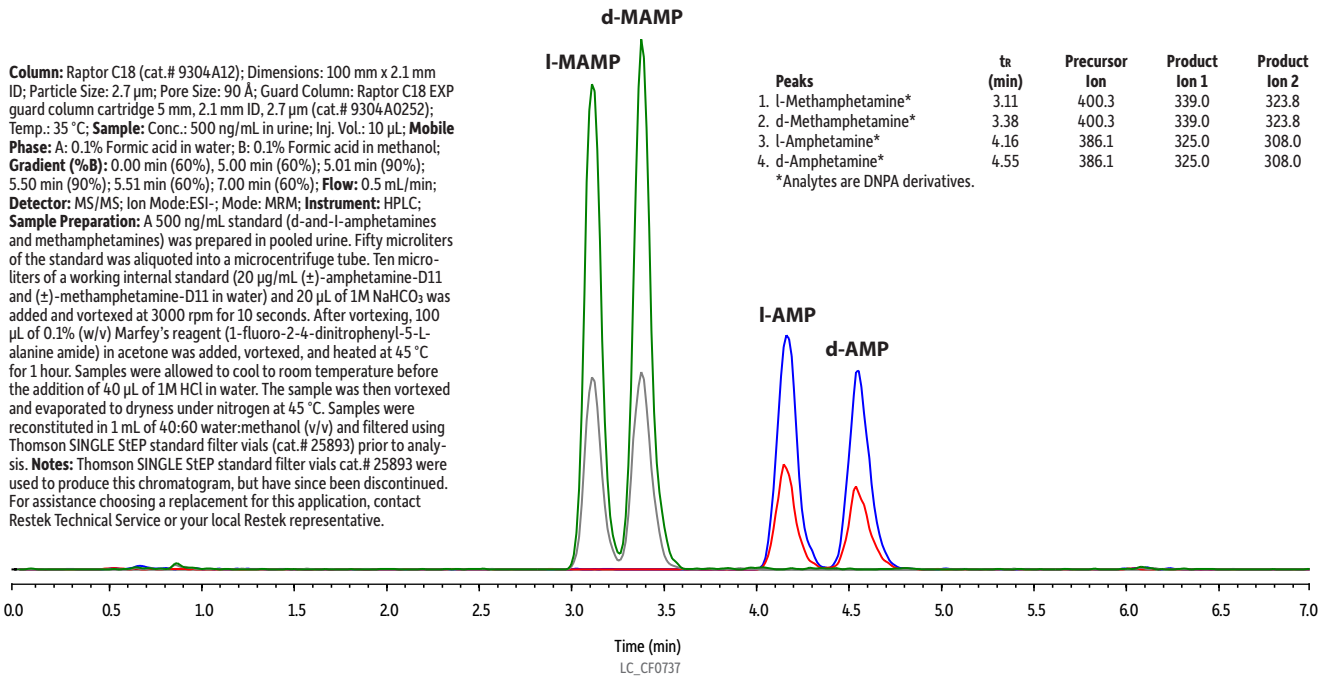
Amphetamine and methamphetamine are psychostimulant drugs and occur as two enantiomers, dextrorotary and levorotary, as a result of their chiral center. The dextro-methamphetamine (d-isomer) form is highly abused and is typically found in illicit preparations. However, detection of abuse is complicated because consumption of over-the-counter and prescription medications may yield positive results if the analytical method used cannot distinguish between the enantiomers. Chiral separation of d- and l-methamphetamine and their metabolites d- and l-amphetamine can help determine whether the source was licit or illicit, but chiral columns can be expensive, may necessitate a dedicated instrument, and are not as broadly useful as ubiquitous C18 columns.

In order to provide labs with a high-throughput assay that effectively separates d- and l-amphetamine and methamphetamine enantiomers in urine without the use of a costly and specialized chiral column, we developed the LC-MS/MS method shown here on a standard reversed-phase Raptor C18 column. Our method employs a simple precolumn derivatization followed by dilution and results in a selective, specific analysis of d- and l-amphetamine and methamphetamine enantiomers that is free from sample matrix interferences. Separation was achieved within a total run time of seven minutes, and quantitation in urine was performed across a linear range of 50-5000 ng/mL. Validation across this range demonstrated that this method provides reliable analysis of d- and l-amphetamine and methamphetamine enantiomers in a workflow and time frame suitable for high-throughput clinical and forensic toxicology labs.

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; **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; **Gradient (%B):** 0.00 min (60%), 5.00 min (60%); 5.01 min (90%); 5.50 min (90%); 5.51 min (60%); 7.00 min (60%); **Flow:** 0.5 mL/min; **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 $NaHCO_3$ 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. **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.

Peaks	t_R (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

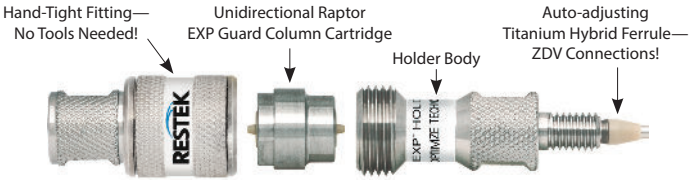
*Analytes are DNPA derivatives.



Raptor C18 LC Columns (USP L1)

Length	2.1 mm cat.#	3.0 mm cat.#	4.6 mm cat.#
1.8 µm Columns			
30 mm	9304232	—	—
50 mm	9304252	930425E	—
100 mm	9304212	930421E	—
150 mm	9304262	—	—
2.7 µm Columns			
30 mm	9304A32	9304A3E	9304A35
50 mm	9304A52	9304A5E	9304A55
100 mm	9304A12	9304A1E	9304A15
150 mm	9304A62	9304A6E	9304A65
5 µm Columns			
30 mm	—	930453E	—
50 mm	9304552	930455E	9304555
100 mm	9304512	930451E	9304515
150 mm	9304562	930456E	9304565
250 mm	—	—	9304575

Raptor EXP Guard Cartridges



Protect your investment and extend the life of our already-rugged LC columns and change guard column cartridges by hand without breaking fluid connections—no tools needed!

EXP Direct Connect Holder

25808

Description	qty.	cat.#
EXP Direct Connect Holder for EXP Guard Cartridges (includes hex-head fitting & 2 ferrules)	ea.	25808

Maximum holder pressure: 20,000 psi (1400 bar)

Raptor C8 EXP Guard Column Cartridge

Description	Particle Size	qty.	5 x 2.1 mm cat.#	5 x 3.0 mm cat.#	5 x 4.6 mm cat.#
Raptor C18 EXP Guard Column Cartridge	UHPLC	3-pk.	9304U0252	9304U0253	
Raptor C18 EXP Guard Column Cartridge	2.7 µm	3-pk.	9304A0252	9304A0253	9304A0250
Raptor C18 EXP Guard Column Cartridge	5 µm	3-pk.	930450252	930450253	930450250

Maximum cartridge pressure: 1034 bar/15,000 psi* (UHPLC), 600 bar/8700 psi (2.7 µm); 400 bar/5800 psi (5 µm)

* For maximum lifetime, recommended maximum pressure for UHPLC particles is 830 bar/12,000 psi.

Intellectual Property: optimizetech.com/patents

Thomson SINGLE StEP Standard Filter Vials

Porosity	Color	qty.	cat.#
Nylon			
0.2 µm	black preslit cap	100-pk.	27896
0.45 µm	pink preslit cap	100-pk.	27897
PES (polyethersulfone)			
0.2 µm	grey preslit cap	100-pk.	27895
PTFE (polytetrafluoroethylene)			
0.2 µm	green preslit cap	100-pk.	28307
0.45 µm	blue preslit cap	100-pk.	28306
PVDF (polyvinyldifluoride)			
0.2 µm	red preslit cap	100-pk.	27894
0.45 µm	yellow preslit cap	100-pk.	27898

Patent No. 7,790,117

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