APPLICATIONS

Novel Approach to Separating Amphetamine Enantiomers from Urine Using Lux® 3 µm AMP Chiral Column

Daniel Spurgin[1], Matthias Fruebis[1], Stefan König[2], and Marianne Hädener[2]
[1] Phenomenex, Inc., 411 Madrid Ave., Torrance, CA 90501 USA
[2] Institute of Forensic Medicine, University of Bern, Switzerland

Daniel Spurgin
Global Product Manager
Danny is a self-proclaimed font enthusiast. His hobbies include gardening, sunscreen, wearing sweaters around his shoulders, and doing donuts in his station wagon while heckling people for using Comic Sans.

Overview

- Rapidly uncover illicit use of amphetamine
- On-line sample prep using novel column-switching technique
- Fast reversed phase LC method without need for derivatization

Introduction

In this technical note, we discuss a novel approach to quantification of amphetamine enantiomers from diluted urine, utilizing a Gemini® C18 trapping column and Lux 3 µm AMP analytical column. Column switching setup allows for accelerated sample throughput without carryover or premature column failure.[1]

Amphetamine (AMP) is a psychostimulant that is commonly prescribed for the treatment of ADHD and other disorders.[2] However, it is widely abused and therefore illicitly produced. Prescribed AMP is manufactured at specific enantiomeric ratios (S-: R-). For example, Adderall® is manufactured at a 3:1 enantiomeric ratio. Illegally produced AMP generally yields an enantiomeric ratio of 1:1. By looking at this ratio forensic scientists can uncover illicit use of this powerful psychostimulant.[3]

The gold standard for the enantiomeric separation of amphetamines is off-line derivatization followed by GC/MS or LC/MS. This is problematic for two reasons: it is time consuming and there is risk that the derivatization agent used may not be 100% enantiomerically pure.

Lux 3 µm AMP LC columns were specifically designed to carry out the reversed phase separation of amphetamine and substituted amphetamines without the need for derivatization. The media used is a pH stable polysaccharide coated chiral phase that is QC tested for the separation of amphetamine and methamphetamine enantiomers.

Materials and Methods

Reagents and Chemicals

Standards were obtained from Cerilliant (Round Rock, TX, USA). Acetonitrile (99.9 %, HPLC gradient grade) was purchased from Acros Organics (Chemie Brunschwig, Basel, Switzerland) and ammonium hydroxide solution from Merck (Grogg Chemie, Stettlen, Switzerland). Water purification via Milli-Q® water system from Millipore (Zug, Switzerland). Blank urine was provided by healthy and abstinent volunteers. HPLC System used was a Thermo UltiMate® 3000 HPLC (Waltham, MA, USA).

Sample Pretreatment

Add 1.41 mL of water and 15 µL of internal standard solution (10 mg/L AMP-d_5) to 75 µL of urine and vortex.

Calibration Solutions

Serial dilutions of racemic AMP standard solution in acetonitrile were used to create working solutions at the following mg/L concentrations: 0.75, 1.5, 7.5, 15, 75, 150, and 375. Adding 5 µL of working solution to 70 µL of blank urine created calibration samples at 0.05, 0.1, 0.5, 1, 5, 10, and 25 mg/L.

Column-Switching Setup

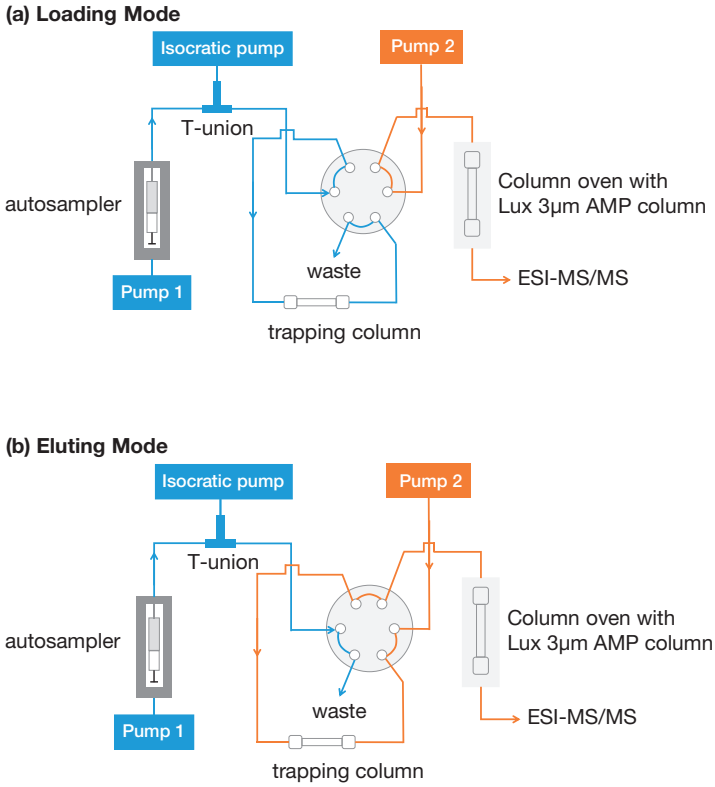

Figure 1.
Configuration of Column-Switching LC/MS/MS system. (a) Loading mode (b) Eluting mode

APPLICATIONS

LC Conditions

Trapping Column: Gemini® 5 µm C18 110Å
Dimension: 10 x 2.0 mm
Part No: 00N-4435-B0-CE
Cartridge Holder: CH0-5846
Analytical Column: Lux® 3 µm AMP
Dimension: 150 x 3.0 mm
Part No.: 00F-4751-Y0
Guard: KrudKatcher™ Ultra
Part No: AF0-8497
Mobile Phase: A: 0.1M Aqueous Ammonia (pH 11)
B: Acetonitrile
Flow Rate: Variable (see Table 1)
Temperature: Ambient for trapping column. 30° C for analytical column
Injection Volume: 1.0 µL

MS/MS Conditions

Detector: SCIEX 5500 QTRAP®
Mode: Positive electrospray ionization
Scan Type: SRM
Curtain Gas (CUR): 40
Gas 1 (GS1): 40
Gas 2 (GS2): 60
IS: 3500 V
Temperature (TEM): 600 °C
Interface Heater: ON
Collision Gas (CAD): Medium
Collision Energy: 26 eV
Declustering Potential: 45 V
Entrance Potential (EP): 10 V
Collision Cell Exit Potential: 8 V
Dwell Time: 150 ms

Table 1.
HPLC program

	Binary pump 1 (µL/min)	Isocratic pump (µL/min)	Binary pump 2 (µL/min)	Position of switching valve
% Mobile Phase B	20%	0%	25%	
Time (min)				
0.0	400	200	500	Loading Mode
1.0	400	200	500	Eluting Mode
1.1	20	20	500	
4.9	20	20	500	Loading Mode
5.0	400	200	500	
6.0	400	200	500	

Results and Discussion

The dilute sample is first injected on to the C18 trapping column for preparation (**Figure 1a**), then in backflush mode an additional pump pushes the sample to the Lux AMP column for chiral separation (**Figure 1b**). By using this technique, the subsequent sample can be injected and cleaned up, before the first sample has completed eluting on the analytical column. With on-line sample prep, column switching and the elimination of the derivatization step, this novel method allows for greater sample throughput and decreased active time spent on sample preparation.

This technique shows a good separation of enantiomers. Representative chromatograms for AMP racemate and S-AMP are shown in **Figures 2 and 3** respectively.

APPLICATIONS

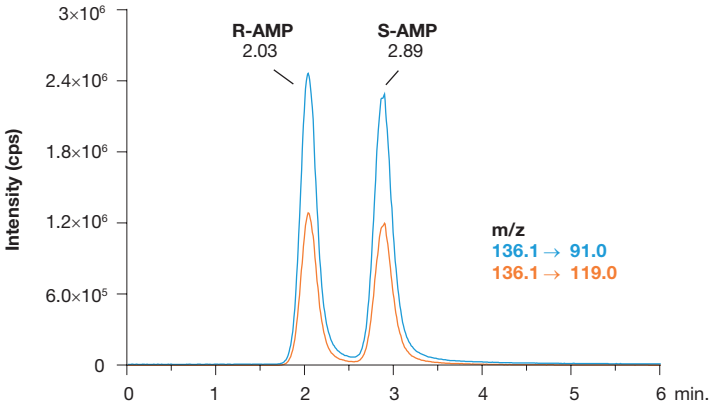

Figure 2.
Representative extracted ion chromatogram of a urine sample spiked with racemic (±)-AMP at 50 mg/L (25 mg/L per enantiomer).

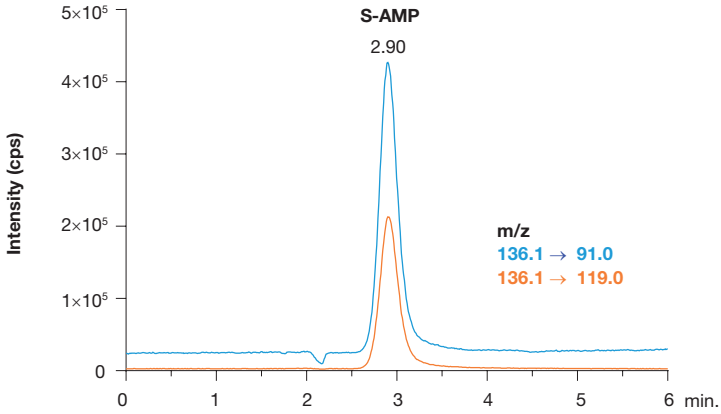

Figure 3.
Representative extracted ion chromatogram of a urine sample spiked with S-(+)-AMP at 1.6 mg/L.

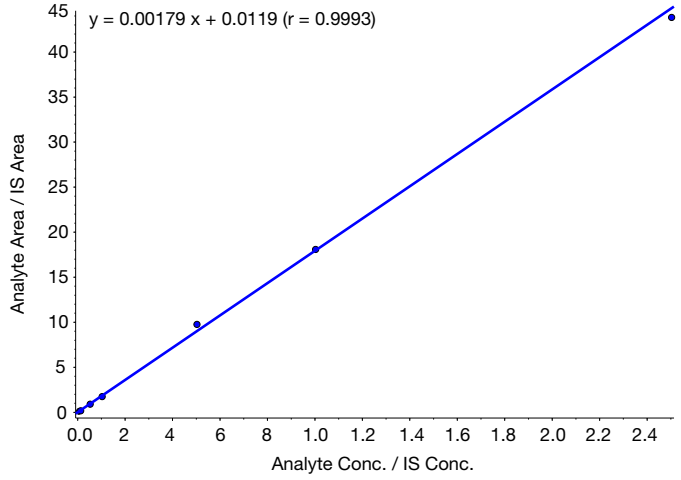

Figure 4.
Seven point calibration curve for R-AMP in urine at concentrations between 0.05 and 25 mg/L (linear least-squares regression with 1/x weighting). R=0.9993.

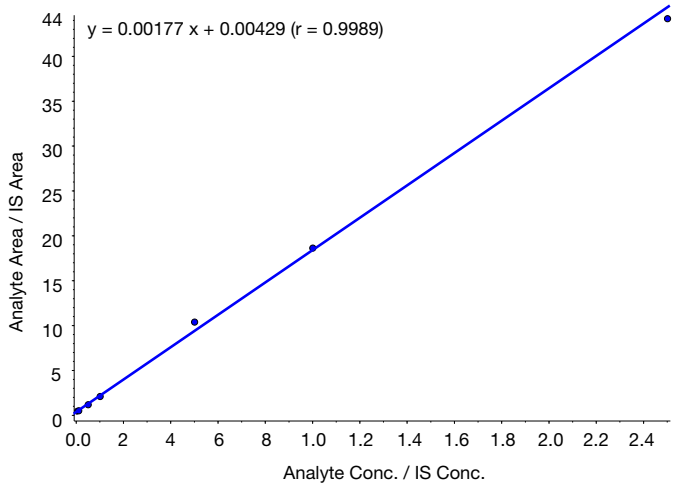

Figure 5.
Seven point calibration curve for S-AMP in urine at concentrations between 0.05 and 25 mg/L (linear least-squares regression with 1/x weighting). R = 0.9989.

This method was assessed for linearity using a seven-point calibration curve (**Figures 4 and 5**) with correlation coefficient (R) greater than 0.99 in both cases. Calibration curves for diluted samples in this study covered a range from 0.05 mg/L to 25 mg/L. This method was validated for precision and accuracy, selectivity, matrix effect, carry-over, dilution integrity, and re-introduction reproducibility. Additionally, it was applied to 67 samples from forensic cases. For complete validation methodology please refer to M. Hädener *et al.*[1]

Conclusion

In this technical note we demonstrated a novel column-switching method developed by Hädener et al. at University of Bern. This technique allows for rapid on-line sample clean up and higher throughput. The Lux® 3 µm AMP LC column was selected to overcome common setbacks in the analysis of enantiomeric amphetamines.

References

1. Hädener, M. *et al*. (2016) Accelerated Quantification of Amphetamine Enantiomers in Human Urine using Chiral Liquid Chromatography and On-Line Column-Switching Coupled with Tandem Mass Spectrometry. *Anal Bioanal Chem*, DOI 10.1007/s00216-016-0056-1

2. DrugFacts—Stimulant ADHD Medications: Methylphenidate and Amphetamines (2014, January). Retrieved from https://www.drugabuse.gov/publications/drugfacts/stimulant-adhd-medications-methylphenidate-amphetamines

3. George S, Braithwaite RA. Using amphetamine isomer ratios to determine the compliance of amphetamine abusers prescribed dexedrine. J Anal Toxicol. 2000;24:223–7.

APPLICATIONS

Ordering Information

Lux® 3.0 µm Analytical Columns			SecurityGuard™ Cartridges‡	
Phase	150 x 3.0	150 x 4.6	10/pk	10/pk
AMP	00F-4751-Y0	00F-4751-E0	AJ0-8475	AJ0-8476
			3.0 mm ID	4.6 mm ID

‡ SecurityGuard Cartridges require holder, Part No.: KJ0-4282

Krudkatcher™ Ultra** In-Line Filter (Analytical)		
Part No.	Description	Unit
AF0-8497	HPLC KrudKatcher Ultra Column In-Line Filter, 0.5 µm Depth Filter x 0.004 in ID	3/pk

** Krudkatcher Ultra requires 5/16 inch wrench, Part No.: AQ0-8903

Gemini 5 µm C18 110 Å Trapping Column			
10 x 2.0 mm	MercuryMS™ Holder	20 x 2.0 mm	MercuryMS Holder
00N-4435-B0-CE	CH0-5846	00M-4435-B0-CE	CH0-5845

Australia
t: +61 (0)2-9428-6444
f: +61 (0)2-9428-6445
auinfo@phenomenex.com

Austria
t: +43 (0)1-319-1301
f: +43 (0)1-319-1300
anfrage@phenomenex.com

Belgium
t: +32 (0)2 503 4015 (French)
t: +32 (0)2 511 8666 (Dutch)
f: +31 (0)30-2383749
beinfo@phenomenex.com

Canada
t: +1 (800) 543-3681
f: +1 (310) 328-7768
info@phenomenex.com

China
t: +86 400-606-8099
f: +86 (0)22 2532-1033
phen@agela.com

Denmark
t: +45 4824 8048
f: +45 4810 6265
nordicinfo@phenomenex.com

Finland
t: +358 (0)9 4789 0063
f: +45 4810 6265
nordicinfo@phenomenex.com

France
t: +33 (0)1 30 09 21 10
f: +33 (0)1 30 09 21 11
franceinfo@phenomenex.com

Germany
t: +49 (0)6021-58830-0
f: +49 (0)6021-58830-11
anfrage@phenomenex.com

India
t: +91 (0)40-3012 2400
f: +91 (0)40-3012 2411
indiainfo@phenomenex.com

Ireland
t: +353 (0)1 247 5405
f: +44 1625-501796
eireinfo@phenomenex.com

Italy
t: +39 051 6327511
f: +39 051 6327555
italiainfo@phenomenex.com

Luxembourg
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

Mexico
t: 01-800-844-5226
f: 001-310-328-7768
tecnicomx@phenomenex.com

The Netherlands
t: +31 (0)30-2418700
f: +31 (0)30-2383749
nlinfo@phenomenex.com

New Zealand
t: +64 (0)9-4780951
f: +64 (0)9-4780952
nzinfo@phenomenex.com

Norway
t: +47 810 02 005
f: +45 4810 6265
nordicinfo@phenomenex.com

Puerto Rico
t: +1 (800) 541-HPLC
f: +1 (310) 328-7768
info@phenomenex.com

Spain
t: +34 91-413-8613
f: +34 91-413-2290
espinfo@phenomenex.com

Sweden
t: +46 (0)8 611 6950
f: +45 4810 6265
nordicinfo@phenomenex.com

United Kingdom
t: +44 (0)1625-501367
f: +44 (0)1625-501796
ukinfo@phenomenex.com

USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

All other countries
Corporate Office USA
t: +1 (310) 212-0555
f: +1 (310) 328-7768
info@phenomenex.com

www.phenomenex.com
Phenomenex products are available worldwide. For the distributor in your country, contact Phenomenex USA, International Department at international@phenomenex.com

guarantee

If Phenomenex products in this technical note do not provide at least an equivalent separation as compared to a competing column of the same phase and dimensions, return the product with comparative data within 45 days for a FULL REFUND.

Anal Bioanal Chem, DOI 10.1007/s00216-016-0056-1, 2016, Marianne Hädener, "With permission of Springer"

TN47971216_W