Evaluation of Newer Technologies for the LC/MS/MS Analysis of Amphetamines using Strata™-X-Drug B Solid Phase Extraction (SPE) and Kinetex® Core-Shell HPLC / UHPLC Columns

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This study evaluates the performance of four different HPLC columns used to analyze amphetamines, a commonly tested drug class. The work also demonstrates an improved analysis speed when coupling a simplified and effective SPE procedure with a core-shell HPLC column and LC/MS/MS detection.

Introduction

Amphetamines are a class of illegal drugs that are routinely subject to workplace drug testing and illicit drug screening. Recently, the Substance Abuse and Mental Health Services Administration (SAMHSA) has lowered the amphetamine drug class cutoff level and has added MDMA, MDA, and MDEA to the panel. As a result, there will undoubtedly be an increase in positive result conformational testing, placing additional stress on the toxicology laboratory. In our work we screen several HPLC columns and demonstrate how a specialized SPE sorbent and method, together with a high efficiency core-shell HPLC column and LC/MS/MS can decrease analysis time and increase sample throughput.

Materials and Methods
Sample Preparation:

Urine Samples were Prepared as Follows:

1. To 2 mL urine, add 1000 µL 100 mM Phosphate buffer (pH 6.0) and 1000 µL 0.35 M Sodium periodate.
2. Mix/vortex.
3. Incubate at room temperature for 20 minutes, ensure pH is approximately 5.5-6.5.

Solid Phase Extraction

The pre-treated urine sample is further cleaned up and concentrated using SPE.

Cartridge:	Strata-X-Drug B, 60 mg/6 mL
Part No.:	8B-S128-UCH
Condition:	NOT REQUIRED
Equilibrate:	NOT REQUIRED
Load:	Pre-treated urine sample
Wash 1:	2 mL 100 mM Sodium acetate buffer (pH 5.0)
Wash 2:	2 mL Methanol
Dry:	10 min at 10 in. of Hg
Elute:	2 mL Ethyl acetate:Isopropanol:Ammonium hydroxide (70:20:10)
Dry down:	After eluting add 300 µL of 0.5 N Methanolic hydrochloride to each sample. Samples can then be blown down under a stream of nitrogen gas at < 40 ºC until dry.
Reconstitute:	Reconstitute samples with 1 mL of 10 % Methanol in 0.1 % Formic acid. Before injecting onto the LC/MS/MS, dilute the samples by a factor of 20 to bring the concentration into a suitable range for analysis.

Analyses were performed using an HP 1100 LC system (Agilent Technologies, Palo Alto, CA, USA) with an upper pressure limit of 400 bar, equipped with an API 3000™ LC/MS/MS detector.

LC/MS/MS

All column dimensions were 50 x 2.1 mm.
HPLC conditions were identical for all columns.
All analytes were present at a concentration of 10 ng/mL each and are listed in order of elution.

Mobile Phase: A: 5 mM Ammonium formate with 0.1 % Formic acid
B: Methanol with 0.1 % Formic acid

Gradient:

Time (min)	% B
0.00	10
1.00	70
3.00	70

Flow Rate: 0.4 mL/min
Detection: API 3000™ MS/MS, ESI negative (ESI-)
Sample:

1. D11-Amphetamine	6. MDA
2. Amphetamine	7. D5-MDMA
3. D14-Methamphetamine	8. MDMA
4. Methamphetamine	9. D5-MDEA
5. D5-MDA	10. MDEA

The HPLC columns that were screened included:

1. Kinetex XB-C18 2.6 µm (Phenomenex, Torrance, CA, USA)
2. Kinetex C8 2.6 µm (Phenomenex, Torrance, CA, USA)
3. XSelect™ CSH™ C18 3.5 µm (Waters Corp., Milford, MA, USA)
4. HALO® C18 2.7 µm (Advanced Materials Technology, MA, USA)

Results and Discussion

The additional stress placed on the toxicology lab to obtain higher sample throughput requires rapid sample preparation and analysis times. Sample preparation time, a typical lab bottleneck, was improved by utilizing the Strata-X-Drug B SPE column, a specialized media and packing recipe developed to optimize the extraction of basic drugs from complex matrices.

Traditionally, toxicology labs have used silica-based mixed-mode SPE sorbents that contain a C8 group and a strong cation-exchange group (SCX) for cleanup. This traditional method consists of 9 steps which could potentially slow down a high-throughput laboratory. Alternatively, the Strata-X-Drug B SPE procedure consists of only 5 steps, a load, two washes, dry, and an elution step. Conditioning and equilibrating the sorbent was not required and had no adverse effects on recoveries. See **Table 1** for method comparisons and **Tables 2** and **3** for a summary of standard time and solvent savings that resulted from using Strata-X-Drug B as compared to common silica-based mixed-mode SPE sorbents.

Comparative separations may not be representative of all applications.

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Table 1.
Reduction of steps required for sample preparation using Strata-X-Drug B

Step	Strata-X-Drug B 60 mg/6 mL, part no. 8B-S128-UCH	Traditional C8+SCX SPE 200 mg/10 mL
Condition	NOT REQUIRED	3 mL Methanol
Equilibrate 1	NOT REQUIRED	3 mL Water
Equilibrate 2	NOT REQUIRED	1 mL Phosphate buffer
Load	Pre-treated Sample	
Wash 1	2 mL Sodium acetate buffer	3 mL Water
Wash 2	2 mL Methanol	1 mL Acetic acid
Wash 3	NOT REQUIRED	3 mL Methanol
Dry	10 minutes	5 minutes
Elute	2 mL Ethyl acetate:Isopropanol: Ammonium hydroxide (70:20:10)	3 mL Dichloromethane:Isopropanol: Ammonium hydroxide

Table 2.
Time savings of Strata™-X-Drug B vs. a traditional C8+SCX sorbent

Step	Time Requirements		Strata-X-Drug B Time Savings
	Strata-X-Drug B 60 mg/6 mL	C8+SCX 200 mg/10 mL	
Condition	NOT REQUIRED	3 minutes	3 minutes
Equilibrate 1	NOT REQUIRED	3 minutes	3 minutes
Equilibrate 2	NOT REQUIRED	3 minutes	3 minutes
Load	No difference in time requirements		
Wash 1	No difference in time requirements		
Wash 2	No difference in time requirements		
Wash 3	NOT REQUIRED	3 minutes	3 minutes
Dry	10 minutes	5 minutes	- 5 minutes
Elute	No difference in time requirements		
TOTAL TIME SAVINGS			7 minutes per sample

Table 3.
Solvent savings of Strata-X-Drug B vs. a traditional C8+SCX sorbent

Step	Solvent Requirements		Strata-X-Drug B Solvent Savings
	Strata-X-Drug B 60 mg/6 mL	C8+SCX 200 mg/10 mL	
Condition	0 mL	3 mL	3 mL
Equilibrate 1	0 mL	3 mL	3 mL
Equilibrate 2	0 mL	1 mL	1 mL
Load	—	—	—
Wash 1	2 mL	3 mL	1 mL
Wash 2	2 mL	1 mL	-1 mL
Wash 3	0 mL	3 mL	3 mL
Dry	—	—	—
Elute	2 mL	3 mL	1 mL
TOTAL SOLVENT SAVINGS			11 mL per sample

All extractions were then diverted to LC/MS/MS analysis where screening of the different column technologies was performed. Chromatograms of the results are shown in **Figures 1-5**.

Figure 1.
Amphetamines by LC/MS/MS using a Kinetex® XB-C18 2.6 µm, 50 x 2.1 mm

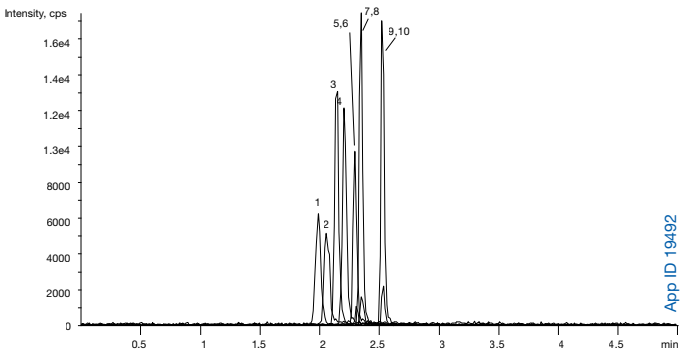

Figure 2.
Amphetamines by LC/MS/MS using a Kinetex C8 2.6 µm, 50 x 2.1 mm

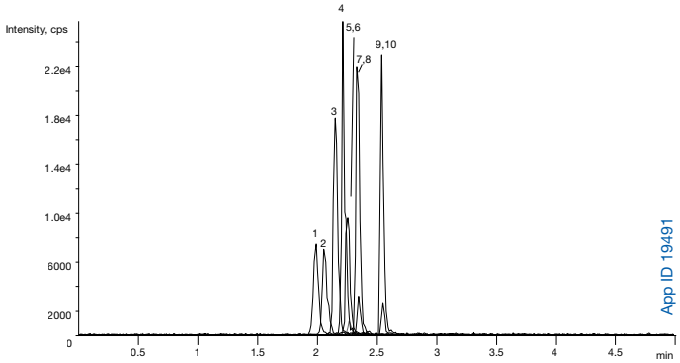

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Figure 4.
Amphetamines by LC/MS/MS using a HALO® C18 2.7 µm, 50 x 2.1 mm

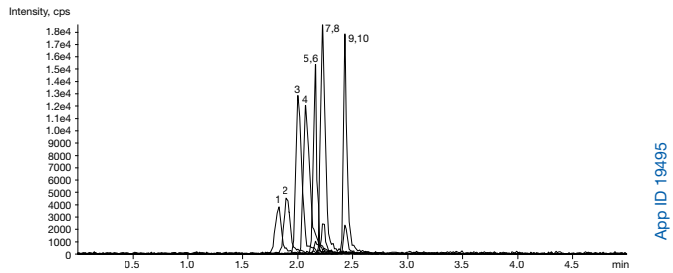
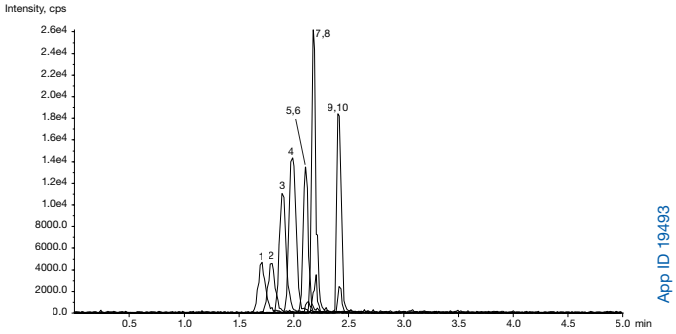


Figure 3.
Amphetamines by LC/MS/MS using a XSelect™ CSH™ C18 3.5 µm, 50 x 2.1 mm



Figures 1 and **2** demonstrate the ability of Kinetex® 2.6 µm XB-C18 and C8 columns to rapidly screen amphetamines while providing efficient conformational results. In this separation method, conditions and column dimensions remained equal while column chemistries and particle design were the only variables. Therefore, measurable performance criteria of peak height and peak capacity were also evaluated to determine the best chromatographic performance **(Table 4)**.

Table 4.
Amphetamine peak capacities and peak height across columns

Column	Peak Width	Peak Capacity	Peak Height
Kinetex XB-C18 2.6 µm	0.147	6.80	5030
Kinetex C8 2.6 µm	0.160	6.25	7010
XSelect™ CSH™ C18 3.5 µm	0.227	4.41	4570
HALO® µm C18 2.7 µm	0.253	3.95	4550

Kinetex 2.6 µm XB-C18 and C8 columns showed the highest peak capacity and height out of all of the columns screened. Peak capacity is the best measure of column efficiency under gradient separations. Sharper, taller peaks provide greater sensitivity, resolving power, and yield more reliable data output. Thus, analytical runs can be performed faster in less than 6 minutes with room to decease further.

It can be concluded by the results that the Kinetex 2.6 µm XB-C18 and C8 core-shell HPLC columns provided a significant advantage in this separation. The core-shell particle design is comprised of a 1.9 µm solid core with a surrounding 0.35 µm porous layer of silica. The result is faster mass transfer of analytes into and out of the stationary phase, minimizing band broadening. Comparatively, the XSelect CSH 3.5 µm C18 fully porous silica column experiences slower diffusion of analyte into and out of the particle, leading to the reduced peak capacity and peak height shown in **Table 4**. The other core-shell particle column, HALO 2.7 µm C18, gave the worst performance out of all columns tested, most likely due to the significant tailing that was seen. This could be an artifact of high silanol activity.

All columns were operated under 400 bar and may therefore be used on any HPLC system without the need for specialized ultra-high pressure equipment.

Conclusions

A rapid analysis for amphetamines was developed which can dramatically improve the efficiency of toxicology laboratories while simultaneously reducing cost due to solvent consumption. By using a specialized SPE media, Strata™-X-Drug B, the sample preparation time was reduced by 7 minutes per sample while solvent consumption was reduced by 11 mL per sample. These time and solvent savings can be multiplied to provide substantial savings when running multiple samples in a high capacity laboratory. Additionally, the use of LC/MS/MS provided additional benefits over GC/MS analysis (which is commonly used in the toxicology industry) including the elimination of a derivatizing step as well as time savings with run times of less than 6 minutes.

The best combination of sample preparation and analytical results was achieved using the Strata-X-Drug B SPE column and either the 2.6 µm Kinetex XB-C18 or C8 analytical column. It should be noted that the use of Kinetex allowed for development of an ultra-high performance liquid chromatography method measured by peak capacity, using an Agilent® 1100 system.

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Ordering Information

Kinetex® 2.6 µm Analytical Columns (mm)

	30 x 4.6	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6	KrudKatcher™ Ultra In-Line Filter* /3pk
XB-C18	—	00B-4496-E0	00C-4496-E0	00D-4496-E0	00F-4496-E0	AF0-8497
C18	00A-4462-E0	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0	AF0-8497
C8	—	00B-4497-E0	00C-4497-E0	00D-4497-E0	00F-4497-E0	AF0-8497
PFP	00A-4477-E0	00B-4477-E0	00C-4477-E0	00D-4477-E0	00F-4477-E0	AF0-8497
HILIC	—	00B-4461-E0	00C-4461-E0	00D-4461-E0	00F-4461-E0	AF0-8497

2.6 µm Minibore Columns (mm)

	30 x 2.1	50 x 2.1	100 x 2.1	150 x 2.1	KrudKatcher Ultra In-Line Filter* /3pk
XB-C18	00A-4496-AN	00B-4496-AN	00D-4496-AN	00F-4496-AN	AF0-8497
C18	00A-4462-AN	00B-4462-AN	00D-4462-AN	00F-4462-AN	AF0-8497
C8	00A-4497-AN	00B-4497-AN	00D-4497-AN	00F-4497-AN	AF0-8497
PFP	00A-4477-AN	00B-4477-AN	00D-4477-AN	00F-4477-AN	AF0-8497
HILIC	—	00B-4461-AN	00D-4461-AN	00F-4461-AN	AF0-8497

*KrudKatcher Ultra requires 5/16 in. wrench. Wrench not provided.

Strata™-X-Drug B

Sorbent Mass	Part No.	Unit
Tube		
10 mg	8B-S128-AAK	1 mL (100/box)
10 mg	8L-S128-AAK†	1 mL (100/box)
30 mg	8B-S128-TAK	1 mL (100/box)
30 mg	8L-S128-TAK†	1 mL (100/box)
30 mg	8B-S128-TBJ	3 mL (50/box)
60 mg	8B-S128-UBJ	3 mL (50/box)
60 mg	8B-S128-UCH	6 mL (30/box)
60 mg	8B-S128-UCL	6 mL (200/bag)
Giga™ Tube		
100 mg	8B-S128-EDG	12 mL (20/box)
96-Well Plate		
10 mg	8E-S128-AGB	2 Plates/Box
30 mg	8E-S128-TGB	2 Plates/Box
60 mg	8E-S128-UGB	2 Plates/Box

† Tab-less tube

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Strata-X is patented by Phenomenex, Inc. U.S. Patent No. 7,119,145

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