Improved Extraction and LC/MS/MS Analysis of Barbiturates from Urine Samples using Strata™-X-Drug N SPE and Kinetex® Core-Shell HPLC/UHPLC Columns

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Utilizing a fast and simplified Solid Phase Extraction (SPE) procedure with Strata-X-Drug N, barbiturates from urine samples were concentrated and cleaned prior to LC/MS/MS analysis. This SPE sorbent was specifically designed and QC'd for neutral drugs of abuse analysis and does not require conditioning or equilibrating steps, saving both time and solvent while providing high recoveries at the 300 ng/mL cutoff level. After cleanup, excellent resolution and separation were achieved with a Kinetex 2.6 µm C18 core-shell HPLC/UHPLC column for 5 barbiturate analytes, including resolution between pentobarbital and amobarbital which have been historically difficult to separate because they are structural isomers.

Introduction

Barbiturates are a class of antidepressants whose abuse and addiction can lead to chronic symptoms including memory loss, irritability, changes in alertness, and decreased interpersonal functioning. This class of drugs is often prescribed to remedy seizure disorders or pain syndromes, however, widespread addiction of these drugs by recreational users has become a problem. For this reason drug tests sometimes include a screen for barbiturates in addition to other abused drugs. In our work we strived to streamline this screening process to provide a fast, cost-effective, and reproducible method from start to finish for forensic labs who are involved in high-throughput processing of barbiturate screens.

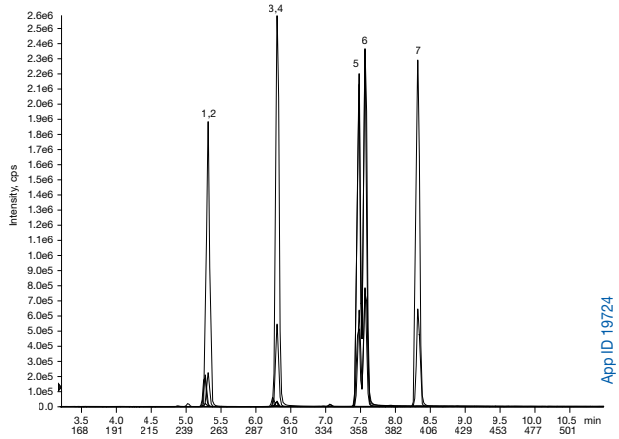
Experimental Conditions

Phenobarbital, butalbital, pentobarbital, amobarbital, and secobarbital were analyzed at a cutoff level of 300 ng/mL. Urine samples were spiked with standards at 40, 100, and 125 % of cutoff level. The prepared urine samples were then subjected to a pre-treatment which involved adding 2 mL of 100 mM Sodium acetate buffer (spiked with internal standard at 300 ng/mL) to 2 mL of urine. Pre-treated samples were then cleaned and concentrated via SPE on a 100 mg/6 mL Strata-X-Drug N tube as specified in **Table 1**. After extraction, the extracted barbiturates were analyzed by LC/MS/MS using a Kinetex 2.6 µm C18 100 x 2.1 mm core-shell HPLC/UHPLC column with the MS operating in APCI negative mode (**Figure 1**).

Table 1.
Solid Phase Extraction

Cartridge:	Strata-X-Drug N, 100 mg/6 mL
Part No.:	8B-S129-ECH
Condition:	Not Required
Equilibrate:	Not Required
Load:	Pre-treated urine samples
Wash 1:	2 mL 0.1 N Hydrochloric acid (HCl)
Wash 2:	2x 2 mL Methanol/0.1 N HCl (30:70)
Dry:	10 minutes at 10 in. of Hg
Elute:	2 mL Ethyl acetate/Isopropanol (85:15)
Dry down:	To dryness at 50 °C
Reconstitute:	1 mL of 10 % Acetonitrile

Figure 1.
LC/MS/MS Analysis of Barbiturates



Column:	Kinetex 2.6 µm C18
Dimensions:	100 x 2.1 mm
Part No.:	00D-4462-AN
Mobile Phase:	A: 5 mM Ammonium acetate buffer B: Acetonitrile

Gradient:

Time (min)	B (%)
0	10
10	45
10.01	90
12	90
12.01	10
16	10

Flow Rate:	400 µL/min
Detection:	Negative mode (APCI)
Temperature:	Ambient
Sample:	1. Phenobarbital-D5 2. Phenobarbital 3. Butalbital-D5 4. Butalbital 5. Pentobarbital 6. Amobarbital 7. Secobarbital

Results and Discussion

While developing the solid phase extraction method for the application, we strived to achieve high, reproducible recoveries while keeping time and solvent usage to a minimum. Traditional mixed-mode silica-based SPE sorbents that are often used for drugs of abuse analysis require that the sorbent be conditioned and equilibrated, adding substantial volumes to the total solvent used. Silica-based sorbents also have a lower loadability as compared to polymeric sorbents, requiring the need for more sorbent and therefore larger volumes of solvent during the wash and elution steps. In order to minimize the solvent usage, Strata™-X-Drug N was used as the extraction sorbent because it does not require a condition or equilibration step and is a polymeric sorbent which allowed for a smaller sorbent mass to be used.

Strata-X-Drug N not only reduces solvent usage but it also improved the analyte-sorbent interaction when analyzing barbiturates. The sorbent chemistry of Strata-X-Drug N is designed for neutral drugs of abuse analysis and tightly interacts with barbiturates, allowing for a 30 % organic wash to remove any interferences that may be present. This is a substantial improvement over traditional mixed-mode silica-based SPE sorbents because these sorbents typically recommend a weak aqueous wash when extracting neutral drugs, such as water or acid, to avoid washing away target analytes.

Tables 2 and **3** show a side by side comparison of the barbiturate extraction methods on both Strata-X-Drug N and a traditional mixed-mode silica-based SPE sorbent, highlighting the increased wash strength allowed on Strata-X-Drug N as well as the solvent savings that were achieved. The protocol for the mixed-mode silica-based SPE sorbent requires a wash of 100 % hexane prior to the elution. This is most likely to remove any residual acid that was left in the sorbent and does not act as a strong wash solvent.

Table 2.
Increased wash strength of Strata-X-Drug N vs. a traditional mixed-mode silica-based SPE sorbent

	Strata-X-Drug N 100 mg/6 mL Part No. 8B-S129-ECH	Mixed-Mode Silica-Based SPE Sorbent 200 mg/10 mL
Wash 1	2 mL 0.1 N Hydrochloric acid (HCl)	3 mL D.I. Water
Wash 2	**2x 2 mL Methanol/0.1N HCl (30:70)**	1 mL 100 mM Acetic acid
Dry	10 minutes at 10 in. of Hg	5 minutes at > 10 in. Hg
Wash 3	--	2 mL Hexane
Elution	2 mL Ethyl acetate/Isopropanol (85:15)	3 mL Hexane/Ethyl acetate (50:50)

Table 3.
Benefits of Strata-X-Drug N vs. a traditional mixed-mode silica-based SPE sorbent

Step	Strata-X-Drug N 100 mg/6 mL Part No. 8B-S129-ECH	Mixed-Mode Silica-Based SPE Sorbent 200 mg/10 mL	Strata-X-Drug N Solvent Savings
Condition	0 mL	3 mL	3 mL
Equilibrate 1	0 mL	3 mL	3 mL
Equilibrate 2	0 mL	1 mL	1 mL
Load	Pre-treated urine sample	Pre-treated urine sample	--
Wash 1	2 mL	3 mL	1 mL
Wash 2	4 mL	1 mL	-3 mL
Dry	--	--	--
Wash 3	0 mL	2 mL	2 mL
Elute	2 mL	3 mL	1 mL
TOTAL SOLVENT SAVINGS			8 mL per sample

Because Strata-X-Drug N is a polymeric sorbent, the loadability of the sorbent is approximately doubled as compared to a silica-based sorbent. Therefore, 100 mg of Strata-X-Drug N was compared to 200 mg of silica-based sorbent when analyzing a 2 mL urine sample.

The Strata-X-Drug N significantly reduces solvent usage while allowing for a strong wash; however it also provides reproducibly high recoveries (**Table 4**), making it an excellent choice for our barbiturate analysis.

Table 4.
Relative Recovery, RSD, and Linearity of Barbiturates

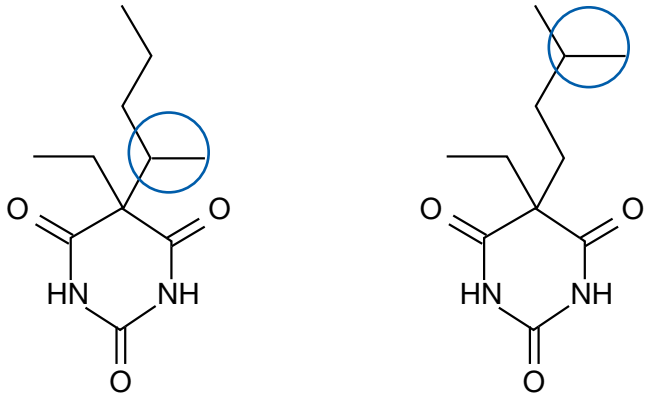
Analyte	Relative Recovery (%)	RSD % (N=3)	Linearity
Phenobarbital	99.6	0.48	0.994
Butalbital	95.7	4.08	0.998
Pentobarbital	96.8	4.50	0.989
Amobarbital	96.4	4.34	0.991
Secobarbital	95.6	6.31	0.990

Downstream LC/MS/MS analysis on the Kinetex® core-shell HPLC/UHPLC column also provided significant benefits as we were able to successfully separate pentobarbital and amobarbital. These two compounds have been historically difficult to se-

APPLICATIONS

parate because their chemical structures differ by the placement of a single methyl group (**Figure 2**). This similarity often results in co-elution of the two analyte peaks making it difficult to quantitate each compound. It is thought that this separation on the Kinetex® core-shell column was made possible by the high peak capacity and efficiency of the Kinetex core-shell particle technology. This is most likely a result of the design of the Kinetex core-shell particles which contain a 1.9 µm solid core surrounded by a 0.35 µm porous layer of silica. This particle design allows the 2.6 µm particle to perform like a sub-2 µm column in terms of resolution, efficiency, and speed without the limiting backpressures that are associated with sub-2 µm particles. This means that any forensic lab could easily adopt this technology without the need to invest in a UHPLC system.

Figure 2.
Chemical Structures of Pentobarbital and Amobarbital



Pentobarbital

Amobarbital

Conclusion

By pairing a streamlined SPE extraction with an efficient LC/MS/MS analysis, it is possible for any forensic lab to improve their output of barbiturate screening. The Strata™-X-Drug N SPE sorbent was able to provide both time and solvent savings which can be multiplied when screening several samples at once, making it faster and more profitable for forensic labs to screen barbiturate samples. By pairing this improved extraction with a sensitive LC/MS/MS method on a Kinetex 2.6 µm C18 core-shell HPLC column, labs can resolve all 5 barbiturates screened including pentobarbital and amobarbital, which has proved difficult using older HPLC methods.

Ordering Information

Strata-X-Drug N SPE

Sorbent Mass	Part No.	Unit
Tube		
10 mg	8B-S129-AAK	1 mL (100/box)
10 mg	8L-S129-AAK*	1 mL (100/box)
30 mg	8B-S129-TAK	1 mL (100/box)
30 mg	8L-S129-TAK*	1 mL (100/box)
30 mg	8B-S129-TBJ	3 mL (50/box)
60 mg	8B-S129-UBJ	3 mL (50/box)
60 mg	8B-S129-UCH	6 mL (30/box)
60 mg	8B-S129-UCL	6 mL (200/box)
100 mg	8B-S129-ECH	6 mL (30/box)
Giga™ Tube		
100 mg	8B-S129-EDG	12 mL (20/box)
96-Well Plate		
10 mg	8E-S129-AGB	2 Plates/box
30 mg	8E-S129-TGB	2 Plates/box
60 mg	8E-S129-UGB	2 Plates/box

* Tab-less tube

Kinetex Core-Shell HPLC/UHPLC Columns

1.7 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1
C18	00B-4475-AN	00D-4475-AN	00F-4475-AN

2.6 µm Minibore Columns (mm)

	50 x 2.1	75 x 2.1	100 x 2.1	150 x 2.1
C18	00B-4462-AN	00C-4462-AN	00D-4462-AN	00F-4462-AN

2.6 µm Solvent Saver MidBore™ Columns (mm)

	50 x 3.0	75 x 3.0	100 x 3.0	150 x 3.0
C18	00B-4462-Y0	00C-4462-Y0	00D-4462-Y0	00F-4462-Y0

2.6 µm Analytical Columns (mm)

	50 x 4.6	75 x 4.6	100 x 4.6	150 x 4.6
C18	00B-4462-E0	00C-4462-E0	00D-4462-E0	00F-4462-E0

guarantee

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

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