A Simplified and Streamlined Approach to Solid Phase Extraction using Strata™-X-Drug B for SAMHSA's Low Cutoff Drugs of Abuse Panel

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Utilizing a single Solid Phase Extraction (SPE) sorbent and only 3 extraction methods, 11 drugs from 6 different Substance Abuse and Mental Health Services Administration (SAMHSA) regulated drug classes were effectively extracted and analyzed by LC/MS and GC/MS. The SPE sorbent, Strata-X-Drug B, was specifically designed and QC tested for the forensic toxicology environment and as a result was able to provide consistent high recoveries of each drug analyzed that conformed to the new lower cutoff levels that SAMHSA has recently enforced.

Introduction

Workplace drug testing has become routine in the job application process. Corporations are also adopting workplace substance abuse policies which require employees to submit to random drug testing. Drug testing is not only increasing in the workplace but it also plays a large role in rehabilitation facilities and law enforcement agencies.[1] With drug testing on the rise, it has become difficult to establish analytical methods for each of the many drugs that are regulated by SAMHSA because each drug class is vastly different from the next and the regulated cutoff levels are getting lower and lower **(Table 1)**. The goal of this work was to develop extraction methods using a single sorbent for all SAMHSA regulated drugs that meet the most recent SAMHSA dictated cutoff levels. Not only was it important to use a single sorbent but it was also important to develop as few extraction methods as possible in order to reduce user error and technician training times while still producing consistent, optimal results. These requirements led to the creation of a Solid Phase Extraction (SPE) sorbent, Strata-X-Drug B, which is able to extract all drugs regulated by SAMHSA in 3 short methods. To ensure consistent recoveries and reliable results, Strata-X-Drug B is also specifically quality control tested for the forensic toxicology environment by testing recoveries with drugs of abuse probes from urine as well as testing the sorbent to be certain that it does not promote interconversion of norcodeine and normorphine to their parent compounds, which can sometimes happen with strong cation-exchange SPE sorbents.

Table 1.
SAMHSA Regulated Cutoff Levels[2]

Class	Analyte	Cutoff
Marijuana Metabolites	Delta-9-tetrahydrocannabinol-9-carboxylic acid (THCA)	15 ng/mL
Cocaine Metabolites	Benzoylecgonine	100 ng/mL
Opiate Metabolites	Codeine	2,000 ng/mL
	Morphine	2,000 ng/mL
6-MAM	6-Acetylmorphine	10 ng/mL
PCP	Phencyclidine	25 ng/mL
Amphetamines	Amphetamine	250 ng/mL
	Methamphetamine	250 ng/mL
MDMA	Methylenedioxymethamphetamine (MDMA)	250 ng/mL
	Methylenedioxyamphetamine (MDA)	250 ng/mL
	Methylenedioxyethylamphetamine (MDEA)	250 ng/mL

Experimental Conditions

Several drug classes under the expanded SAMHSA regulations were analyzed by LC/MS and GC/MS. The drug classes include opiates, 6-MAM, PCP, amphetamines, marijuana metabolites, and cocaine metabolites. Standards were obtained from Cerilliant® Corporation (Round Rock, TX). Urine samples were prepared for each drug class and were spiked at 40, 100, and 125 % of the SAMHSA cutoff level. The prepared urine samples were then subjected to various pre-treatment steps, depending on the drug that was spiked into each **(Table 2)**. Pre-treated samples were then subjected to SPE using 60 mg/6 mL Strata-X-Drug B tubes (Phenomenex, Inc. Torrance, CA, USA) as specified in **Table 3**. After extraction, each of the 6 drug classes were analyzed by either GC/MS or LC/MS under the conditions specified in **Figures 1-6**. GC/MS analysis was performed on a Zebron™ ZB-Drug-1 column (Phenomenex, Inc. Torrance, CA, USA) with the MS operated in SIM mode while LC/MS analysis was performed either using a Kinetex® C18 or a PFP core-shell HPLC column (Phenomenex, Inc. Torrance, CA, USA) with the MS operating in ESI+ mode.

Table 2.
Sample Pre-treatments

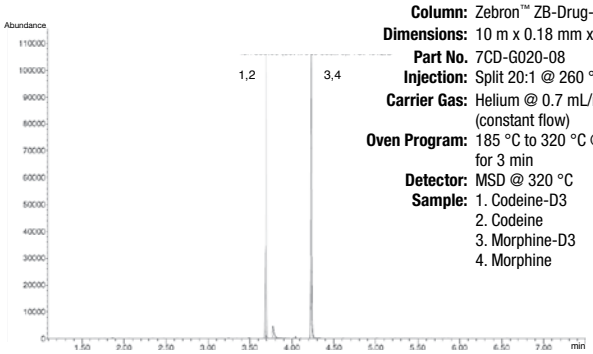
Opiates	To each 2 mL urine sample, add 500 µL of concentrated Hydrochloric acid. Heat at 90 °C for 2 hours. Add 2 mL of 200 mM Sodium acetate buffer (pH 4.0). Add 1 mL of 6 N Potassium hydroxide then vortex. Centrifuge for 5 minutes at 5000 rpm. Verify that pH is between 4.0-6.0.
6-MAM	To each 2 mL urine sample, add 1000 µL of ß-glucuronidase solution (contains 5000 F units/mL Patella vulgata in 100 mM Acetate buffer, pH 5.0) and vortex. Hydrolyze for 3 hours at 60 °C. Let cool and add 1000 µL of 100 mM Phosphate buffer (pH 6.0). Verify that pH is between 5.5 and 6.5. Centrifuge for 5 minutes at 5000 rpm and discard pellet.
PCP	To each 2 mL urine sample, add 2 mL of 100 mM Sodium acetate buffer (pH 5.0) then vortex. Verify that pH is between 4.0 and 6.0.
Amphetamines	To each 2 mL urine sample, add 1000 µL of 100 mM Phosphate buffer (pH. 6.0) and 1000 µL of 0.35 M Sodium periodate. Vortex and incubate at room temperature for 25 minutes. Verify that the pH is between 5.5 and 6.5.
Marijuana Metabolites	To each 2 mL urine sample, add 100 µL of 11.8 N Potassium hydroxide. Vortex then incubate at 60 °C for 20 minutes. Cool and add ~450 µL glacial acetic acid then vortex. Verify that the pH is between 4.0 and 6.0.
Cocaine Metabolites	To each 2 mL urine sample, add 2 mL of 100 mM Sodium acetate buffer (pH 5.0) then vortex. Verify that the pH is between 4.0 and 6.0.

Table 3.
Protocols
Strata™-X-Drug B 60 mg/6 mL (part no. 8B-S128-UCH)

	Opiates, 6-MAM, PCP, and Amphetamines	Marijuana Metabolites	Cocaine Metabolites
Condition/ Equilibrate	No Conditioning Required		
Load	Pre-treated urine sample	Pre-treated urine sample	Pre-treated urine sample
Wash 1	2 mL of 100 mM Sodium acetate buffer (pH 5.0)	2 mL of 100 mM Sodium acetate (pH 5.0)	2 mL of 0.1 N Hydrochloric acid
Wash 2	2 mL Methanol	2 mL of (30:70) Acetonitrile/100 mM Sodium acetate (pH 5.0)	2 mL Methanol
Dry	10 minutes under full vacuum	15 minutes under full vacuum	10 minutes under full vacuum
Elute	2 mL of Ethyl acetate/ Isopropanol/ Ammonium hydroxide (70:20:10)	2 mL of Ethyl acetate/ Isopropanol (85:15)	2 mL of Ethyl acetate/ Isopropanol/ Ammonium hydroxide (70:20:10)

Figure 1.
GC Analysis of Opiates

Extracted samples were evaporated to dryness at 50 °C then reconstituted in 50 µL of propionic anhydride (PIA) and 50 µL of acetonitrile. Samples were heated for 30 minutes at 60 °C and were then evaporated to dryness at 50 °C. Samples were again reconstituted in 100 µL of ethyl acetate.

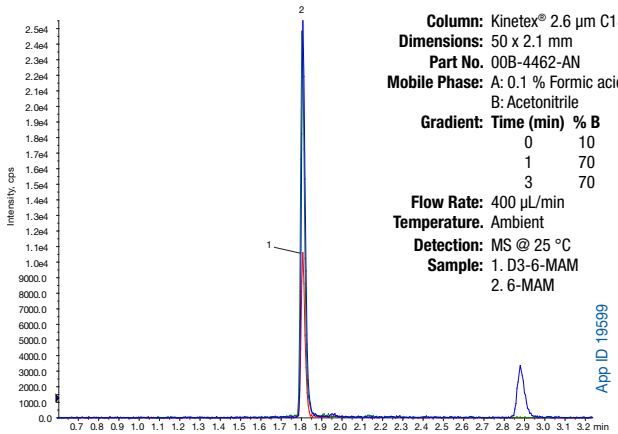


Column: Zebron™ ZB-Drug-1
Dimensions: 10 m x 0.18 mm x 0.18 µm
Part No. 7CD-G020-08
Injection: Split 20:1 @ 260 °C, 1 µL
Carrier Gas: Helium @ 0.7 mL/min (constant flow)
Oven Program: 185 °C to 320 °C @ 30 °C/min for 3 min
Detector: MSD @ 320 °C
Sample: 1. Codeine-D3
2. Codeine
3. Morphine-D3
4. Morphine

App ID 19600

Figure 2.
HPLC Analysis of 6-MAM

Extracted samples were evaporated to dryness at 50 °C then reconstituted in 1 mL of 10 % Methanol / 90 % Formic acid (0.1 %).

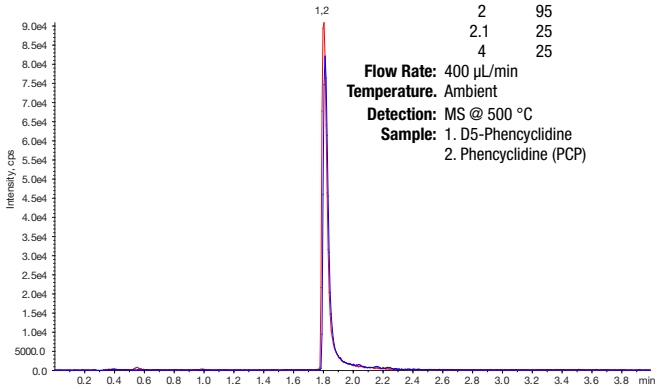


Column: Kinetex® 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No. 00B-4462-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: Acetonitrile
Gradient: **Time (min) % B**
0 10
1 70
3 70
Flow Rate: 400 µL/min
Temperature. Ambient
Detection: MS @ 25 °C
Sample: 1. D3-6-MAM
2. 6-MAM

App ID 19599

Figure 3.
HPLC Analysis of PCP

Extracted samples were evaporated to dryness at 50 °C then reconstituted in 1 mL of 25 % Methanol / 75 % Formic acid (0.1 %).

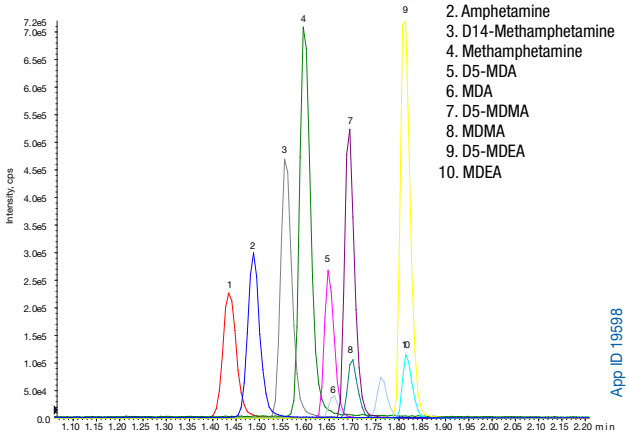


Column: Kinetex 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No. 00B-4462-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol
Gradient: **Time (min) % B**
0 25
1 95
2 95
2.1 25
4 25
Flow Rate: 400 µL/min
Temperature. Ambient
Detection: MS @ 500 °C
Sample: 1. D5-Phencyclidine
2. Phencyclidine (PCP)

App ID 19595

Figure 4.
HPLC Analysis of Amphetamines

300 µL of 0.5 N Methanolic hydrochloride (0.5 N HCl in Methanol) was added to each sample. Samples were then evaporated to dryness at < 35 °C then reconstituted with 1 mL of 10 % Methanol / 90 % Formic acid (0.1 %). Before injecting onto the LC/MS, samples were diluted by a factor of 20 to bring the concentration into a suitable range for analysis.



Column: Kinetex 2.6 µm C18
Dimensions: 50 x 2.1 mm
Part No. 00B-4462-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol
Gradient: **Time (min) % B**
0 10
1 70
3 70
Flow Rate: 400 µL/min
Temperature. Ambient
Detection: MS @ 25 °C
Sample: 1. D11-Amphetamine
2. Amphetamine
3. D14-Methamphetamine
4. Methamphetamine
5. D5-MDA
6. MDA
7. D5-MDMA
8. MDMA
9. D5-MDEA
10. MDEA

App ID 19598

Figure 5.
GC Analysis of Marijuana Metabolites

Extracted samples were evaporated to dryness at 50 °C then reconstituted in 25 µL of ethyl acetate and 50 µL of BSTFA. Samples were heated for 20 minutes at 70 °C.

Column: Zebron™ ZB-Drug-1
Dimensions: 10 m x 0.18 mm x 0.18 µm
Part No. 7CD-G023-08
Injection: Split 10:1 @ 280°C, 1 µL
Carrier Gas: Helium @ 0.7 mL/min (constant flow)
Oven Program: 220 °C to 340 °C @ 30 °C/min
Detector: MS @ 340 °C
Sample: 1. D9-Carboxy-THC 380
2. Carboxy-THC 371

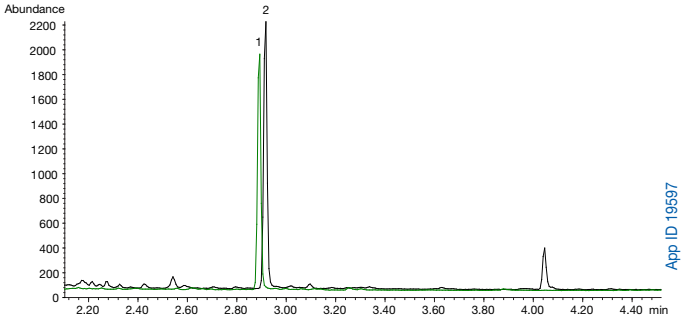


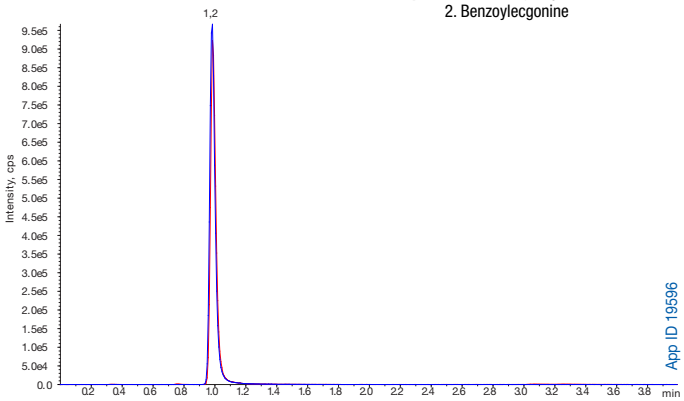
Figure 6.
HPLC Analysis of Cocaine Metabolites

Extracted samples were evaporated to dryness at 50 °C then reconstituted in 1 mL of 95 % Methanol / 5 % Formic acid (0.1 %).

Column: Kinetex® 2.6 µm PFP
Dimensions: 50 x 2.1 mm
Part No. 00B-4477-AN
Mobile Phase: A: 0.1 % Formic acid in Water
B: 0.1 % Formic acid in Methanol
Gradient:

Time (min)	% B
0	95
1	50
2	50
2.1	95
4	95

Flow Rate: 400 µL/min
Temperature. Ambient
Detection: MS @ 500 °C
Sample: 1. D3-Benzoylecgonine
2. Benzoylecgonine



Results and Discussion

While developing the extraction methods, it was discovered that conditioning and equilibrating the Strata-X-Drug B sorbent was not necessary. The elimination of these steps reduced solvent consumption and time without compromising results. The unconditioned sorbent proved to be effective in the extraction of all 11 drugs regulated under SAMHSA by providing high recoveries and consistent results **(Table 4)**. Coupled with efficient GC/MS and LC/MS analysis using either a Zebron ZB-Drug 1 GC column or a Kinetex core-shell HPLC column, it was determined that the extraction and analysis techniques were able to produce clean samples, excellent separation and quantitation, and quick analysis times which conform with SAMHSA regulations.

Table 4.
Relative Recovery, RSD, and Linearity

Analyte	Relative Recovery (%)	RSD % (N=3)	Linearity
Codeine	99.86	0.43	0.999
Morphine	99.80	0.59	0.999
6-MAM	100.5	0.63	0.999
Phencyclidine	99.33	0.63	0.999
Amphetamine	101.8	1.59	0.997
Methamphetamine	97.76	1.99	0.998
MDA	96.65	3.03	0.995
MDMA	100.1	1.20	0.998
MDEA	102.2	1.93	0.999
Carboxy-THC	99.45	0.95	0.999
Benzoylecgonine	99.45	0.95	0.999

Conclusion

Strata-X-Drug B effectively decreased extraction time by eliminating the condition and equilibration steps, reducing the extraction method steps to a load, two washes, and an elution. Strata-X-Drug B also allowed for a streamlined extraction approach by minimizing the number of different extraction protocols required for the expanded SAMHSA analysis to only 3 methods with few variations of solvents between methods. This approach drastically reduced processing time and user error allowing for increased throughput and higher quality data. It was also shown that subsequent analysis by GC/MS and LC/MS proved that both analysis techniques were acceptable under the same SPE extraction methodology. However, LC/MS proved to be ideal as it resulted in much shorter analysis times which allowed for a significant increase in sample throughput.

References

1. www.wikipedia.org/wiki/Drug-Testing
2. www.samhsa.gov

APPLICATIONS

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Ordering Information

Strata™-X-Drug B SPE

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/box)
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

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
PFP	00B-4476-AN	00D-4476-AN	00F-4476-AN

2.6 µm Minibore Columns (mm)

	50 x 2.1	100 x 2.1	150 x 2.1
C18	00B-4462-AN	00D-4462-AN	00F-4462-AN
PFP	00B-4477-AN	00D-4477-AN	00F-4477-AN

2.6 µm Solvent Saver MidBore™ Columns (mm)

	50 x 3.0	100 x 3.0	150 x 3.0
C18	00B-4462-Y0	00D-4462-Y0	00F-4462-Y0
PFP	00B-4477-Y0	00D-4477-Y0	00F-4477-Y0

2.6 µm Analytical Columns (mm)

	50 x 4.6	100 x 4.6	150 x 4.6
C18	00B-4462-E0	00D-4462-E0	00F-4462-E0
PFP	00B-4477-E0	00D-4477-E0	00F-4477-E0

More dimensions and phases available, please inquire.

Zebron™ ZB-Drug-1 GC Columns

ID(mm)	df(µm)	Temp. Limits °C	Part No.
10-Meter			
0.18	0.18	40 to 320/340 °C	7CD-G023-08
15-Meter			
0.25	0.25	40 to 320/340 °C	7EG-G023-11
30-Meter			
0.25	0.25	40 to 320/340 °C	7HG-G023-11

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