UCT

FORENSIC
SAMPLE ANALYSIS

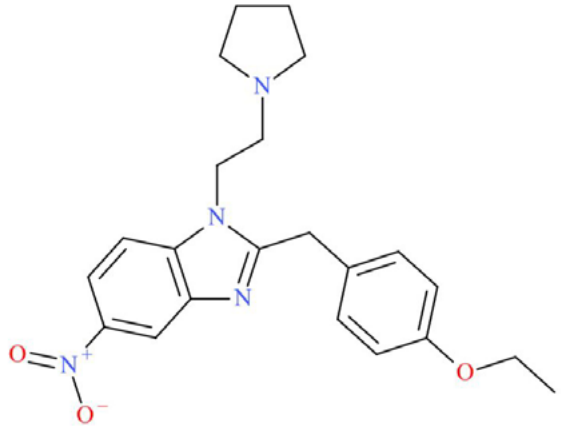
unitedchem.com

FORENSICS

FORENSIC
SAMPLE ANALYSIS

	Solid Phase Extraction of Novel Synthetic 2-Benzylbenzimidazole Opioid Compounds “Nitazenes”
	Amphetamines in Blood, Plasma/Serum, Urine, or Tissue Using Clean Screen® DAU SPE and LC-MS/MS Analysis
	Analysis of 26 Natural and Synthetic Opioids in Blood and Urine Using Clean Screen® DAU SPE and a Selectra® DA UHPLC Column
	Simultaneous Analysis of Free Steroids and Sulfate Conjugates by Solid-Phase Extraction and LC-MS/MS
	Analysis of Natural Cannabinoids and Metabolites from Blood Using Clean Screen® THC SPE Column and SelectraCore® C18 on LC-MS/MS
	Analysis of Natural Cannabinoids and Metabolites from Urine Using Styre Screen® HLB SPE Column and SelectraCore® C18 on LC-MS/MS
	Enhanced LC-MS/MS Separation of Δ^8 and Δ^9 -THC Metabolites and Other Cannabinoids Extracted from Urine Samples using a Polymeric SPE Column
	Analysis of Xylazine, Opioids, and Other Common Adulterants in Blood and Urine by SPE and LC-MS/MS

Solid Phase Extraction of Novel Synthetic 2-Benzylbenzimidazole Opioid Compounds "Nitazenes"



UCT Part Numbers

CSDAU133
Clean Screen® DAU
3 mL, 130 mg

SCS27-C18GDC21
SelectraCore® C18
Guard Column
5 x 2.1 mm, 2.7 µm

SPHPHO7001-10
Select pH buffer
pouch 100 mM
Phosphate buffer pH 7.0

SCS27-C181021
SelectraCore® C18 Column
100 x 2.1 mm, 2.7 µm

SLGRDHLDR-HPOPT
Selectra® Direct
Connect Guard Holder

Summary:

A novel group of synthetic opioids, known as benzylbenzimidazole-opioids or "nitazenes," is emerging. Originally synthesized in the 1950s as a potential analgesic, these compounds were never approved for medical use.[1] However, they are now resurfacing as a significant threat within the ongoing opioid epidemic. These potent synthetic opioids range from three to twenty times more potent than fentanyl.[2] The CFRSE detected the first nitazene analog, isotonitazene, in biological samples in the United States back in July 2019.[3] Since then, the number of cases nationwide has continued to increase. To address this issue, the Drug Enforcement Administration (DEA) has temporarily classified eight nitazenes as schedule I substances.[4] This application note introduces a simple targeted extraction method for the analysis of nine nitazene compounds from urine and blood utilizing UCT's flagship Clean Screen® DAU column and new SelectraCore® C18 core-shell column.

FORENSICS CLINICAL

UCT

Sample Pretreatment:

- In a test tube add 0.5 mL of sample, internal standard, 200 µL of acetonitrile (ACN), and 1.3 mL of 100 mM phosphate buffer pH 7.
- Vortex and centrifuge samples for 10 minutes at 3000 rpm.

SPE Procedure:

1. Condition Column

a) 1 x 3 mL MeOH
b) 1 x 3 mL phosphate buffer pH 7

2. Load Sample

a) Load at 1 to 2 mL/minute

3. Wash Column

a) 1 x 3 mL DI H_2O
b) 1 x 3 mL 50:50 MeOH:H_2O

4. Dry Column

a) Dry column for at least 10 minutes under full pressure or vacuum

5. Elute Analytes

a) 1 x 3 mL of MeOH:NH_4OH (98:2)
Note: Prepare elution solvent daily

6. Evaporate

a) Add 250 µL of 10% HCl in methanol and vortex
b) Evaporate eluate at 35°C, 10 psi

7. Reconstitute

a) 1 mL of 50:50 MeOH:H_2O

Notes:

- Centrifuging blood samples causes a decrease in sample recovery, but improves visual cleanliness of sample at the end of SPE
- As an alternative to adding 10% HCl before evaporation, samples can be evaporated at 30°C, 5 psi

LC-MS/MS Parameters	
LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® C18 Column 100 x 2.1 mm, 2.7 µm (PN: **SCS27-C181021**)
Guard Column	SelectraCore® C18 Guard Column 5 x 2.1 mm, 2.7 µm (PN: **SCS27-C18GDC21**)
Column Temperature	40°C
Flow Rate	0.45 mL/min
Injection Volume	5 µL

Gradient Program		
Time (min)	**% Mobile Phase A: 0.1% formic acid in Water**	**% Mobile Phase B: 0.1% formic acid in Methanol**
0	90	10
2.5-3.5	57	43
7	30	70
8-11	0	100
11.3-15	90	10

MRM Table:

Analyte	Parent Ion (m/z)	Product Ion 1 (m/z)	CE (eV)	Product Ion 2 (m/z)	CE (eV)	RT (min)
Butonitazene	425.5	100.1	-23	72.1	-45	5.83
Clonitazene	386.5	100.1	-26	125.1	-36	4.03
Etonitazene	397.4	100.1	-21	72.0	-36	3.88
Etonitazepyne	395.6	98.1	-23	56.1	-55	3.80
Flunitazene	371.3	100.1	-23	73.1	-26	3.41
Isotonitazene	411.5	100.1	-21	72.2	-45	4.53
Metodesnitazene	339.2	100.1	-21	72.1	-40	2.09
Metonitazene	383.5	100.1	-22	72.2	-39	3.38
Protonitazene	411.7	100.1	-24	72.1	-39	4.98

* CE = collision energy, RT = retention time

FORENSICS CLINICAL

UCT

Chromatogram:

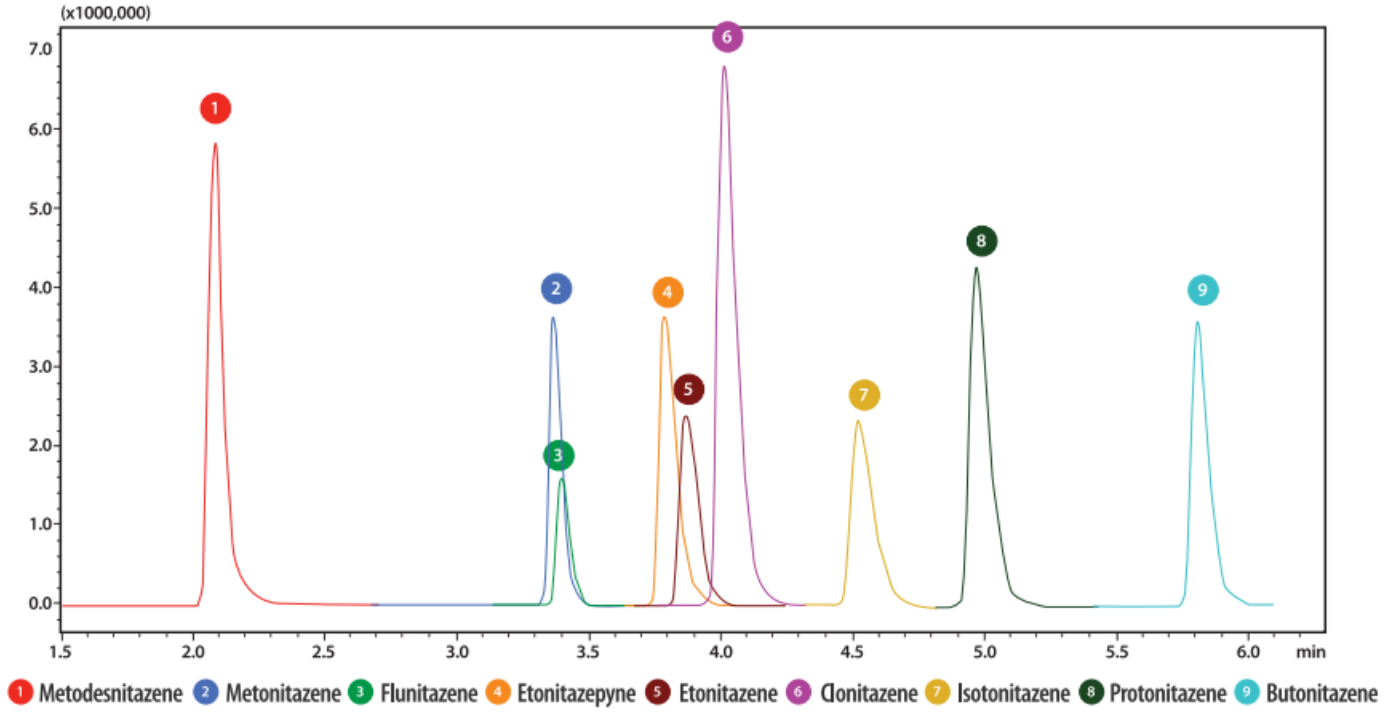


Figure 1: Chromatogram of extracted 15 ng/mL blood sample

Example Solvent Calibration Curves:

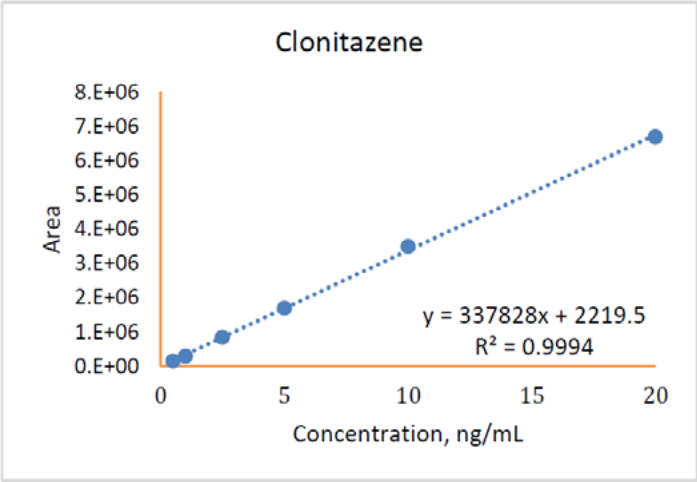


Figure 2a: Example of a 6-point solvent calibration curve for Clonitazene with linear equation & R^2 value (0.5, 1, 2.5, 5, 10 & 20 ng/mL)

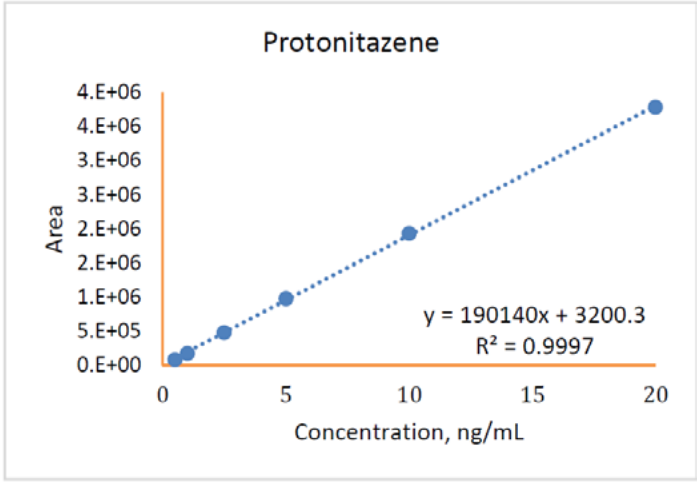


Figure 2b: Example of a 6-point solvent calibration curve for Protonitazene with linear equation & R^2 value (0.5, 1, 2.5, 5, 10 & 20 ng/mL)

FORENSICS CLINICAL

UCT

Results:

Urine (n=5)									
	1 ng/mL			5 ng/mL			15 ng/mL		
Analyte	Recovery (%)	RSD (%)	Matrix Effects (%)	Recovery (%)	RSD (%)	Matrix Effects (%)	Recovery (%)	RSD (%)	Matrix Effects (%)
Butonitazene	97	4	-14	93	2	-12	93	2	-12
Clonitazene	101	6	-5	93	2	0	95	2	-3
Etonitazene	103	6	-5	94	1	-1	99	1	-6
Etonitazepyne	104	6	-2	95	1	4	96	3	-1
Flunitazene	102	5	-5	100	4	1	98	2	-2
Isotonitazene	100	4	-4	94	2	1	98	2	-1
Metodesnitazene	98	2	8	93	4	5	106	4	-10
Metonitazene	97	3	0	92	2	3	98	1	4
Protonitazene	100	4	-5	94	0	1	96	1	-3

Blood (n=5)									
	1 ng/mL			5 ng/mL			15 ng/mL		
Analyte	Recovery (%)	RSD (%)	Matrix Effects (%)	Recovery (%)	RSD (%)	Matrix Effects (%)	Recovery (%)	RSD (%)	Matrix Effects (%)
Butonitazene	75	5	9	74	6	14	81	3	0
Clonitazene	87	3	6	83	5	12	89	3	-3
Etonitazene	93	4	0	87	5	8	94	1	-5
Etonitazepyne	94	2	13	89	4	18	97	1	0
Flunitazene	96	5	-3	92	3	5	98	2	-11
Isotonitazene	85	4	8	83	5	15	90	4	-1
Metodesnitazene	95	6	11	89	4	17	94	6	-6
Metonitazene	95	4	8	89	4	16	98	3	-1
Protonitazene	87	4	3	81	3	10	87	3	-5

Recovery was calculated by comparing peak area of pre-spiked samples to peak area of post-spiked samples. Matrix effects were calculated by comparing peak area of post-spiked samples to peak area of evaporated solvent standards. A negative matrix effect indicates ion suppression while a positive matrix effect indicates ion enhancement.

FORENSICS CLINICAL

UCT

Conclusion/Discussion:

A simple extraction method was developed for the extraction of nine nitazene compounds from urine and blood. Analytes were extracted using UCT's flagship column Clean Screen® DAU and analyzed on a LC-MS/MS equipped with UCT's new SelectraCore® C18 core-shell column. All analytes were separated in 6 minutes with a short total run time of 15 minutes. Isomers protonitazene and isotonitazene were successfully separated on the core-shell column. Due to these compounds' novelty and potency, developing an extraction with a low limit of quantitation was crucial and challenging.

A sizeable amount of the non-polar analytes, particularly butonitazene and isotonitazene, remain in the test tube after loading the sample onto the SPE cartridge. To better retain the analytes, 200 µL of acetonitrile was added during sample preparation. This is vital for detection and quantitation at low concentrations. Another discovery made during method development was that free-base nitazene compounds are volatile. It was difficult to avoid the evaporation step after extraction as these compounds are present at low concentrations in biological samples. Like amphetamines, hydrochloride acid was added to the elution solvent before evaporation to create more stable salt forms.

The extraction method was evaluated using quality control samples prepared at low, medium, and high concentrations. Recovery and matrix effect for each analyte were calculated using pre-spiked samples, post-spiked samples, and evaporated solvent standards. Pre-spiked samples are extracted biological samples spiked during sample preparation. Post-spiked samples are extracted biological samples spiked after the extraction into the elution solvent. Evaporated solvent standards are spiked elution solvent samples with 10% HCl in methanol that were fully dried and reconstituted. Formulas for recovery and matrix effect are shown below:

$$\%\ Recovery = \frac{Peak\ Area\ of\ Pre\text{-}Spiked\ Samples}{Peak\ Area\ of\ Post\text{-}Spiked\ Samples} \times 100$$

$$Matrix\ Effect = \left(\frac{Peak\ Area\ of\ Post\text{-}Spiked\ Samples}{Peak\ Area\ of\ Evaporated\ Samples}\right) \times 100$$

Extraction recoveries of analytes from urine ranged from 93-106% with relative standard deviations less than 10%. Matrix effects for all analytes were within ± 25% making it easy to implement and validate this method in laboratories that follow ANSI/ASB Standard 036. Extraction recoveries of analytes from blood ranged from 74-96% with relative standard deviations less than 10%. Matrix effects for all analytes in blood were also within ± 25%.

FORENSICS CLINICAL

UCT

References:

1. Diversion Control Division, Benzimidazole-Opioids Other Name: Nitazenes (2022).

2. Vandeputte, M.M., Krotulski, A.J., Walther, D. et al. Pharmacological evaluation and forensic case series of Npyrrolidino etonitazene (etonitazepyne), a newly emerging 2-benzylbenzimidazole 'nitazene' synthetic opioid. Arch Toxicol 96, 1845–1863 (2022). https://doi.org/10.1007/s00204-022-03276-4

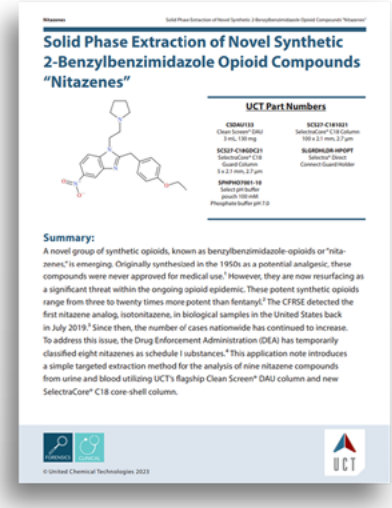
3. Alex J Krotulski, Donna M Papsun, Sherri L Kacinko, Barry K Logan, Isotonitazene Quantitation and Metabolite Discovery in Authentic Forensic Casework, Journal of Analytical Toxicology, Volume 44, Issue 6, July 2020, Pages 521–530, https://doi.org/10.1093/jat/bkaa016

4. Seven Benzimidazole-Opioids: Butonitazene, Etodesnitazene, Flunitazene, Metodesnitazene, Metonitazene, N-Pyrrolidino Etonitazene, and Protonitazene, 86 Fed. Reg. 69183-69186 (December 7, 2021)

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 • 800.385.3153 • 215.781.9255
www.unitedchem.com • Email: methods@unitedchem.com

FORENSIC
SAMPLE ANALYSIS

Solid Phase Extraction of Novel Synthetic 2-Benzylbenzimidazole Opioid Compounds “Nitazenes”


Solid Phase Extraction of Novel Synthetic 2-Benzylbenzimidazole Opioid Compounds “Nitazenes”

UCT Part Numbers

Summary:
A novel group of synthetic opioids, known as benzylbenzimidazole-opioids or “nitazenes,” is emerging. Originally synthesized in the 1950s as a potential analgesic, these compounds were never approved for medical use.[1] However, they are now resurfacing as a significant threat within the ongoing opioid epidemic. These potent synthetic opioids range from three to twenty times more potent than fentanyl.[2] The CFRSE detected the first nitazene analog, isotonitazene, in biological samples in the United States back in July 2019.[3] Since then, the number of cases nationwide has continued to increase. To address this issue, the Drug Enforcement Administration (DEA) has temporarily classified eight nitazenes as schedule I substances.[4] This application note introduces a simple targeted extraction method for the analysis of nine nitazene compounds from urine and blood utilizing UCT’s flagship Clean Screen® DAU column and new SelectraCore® C18 core-shell column.

© United Chemical Technologies 2023

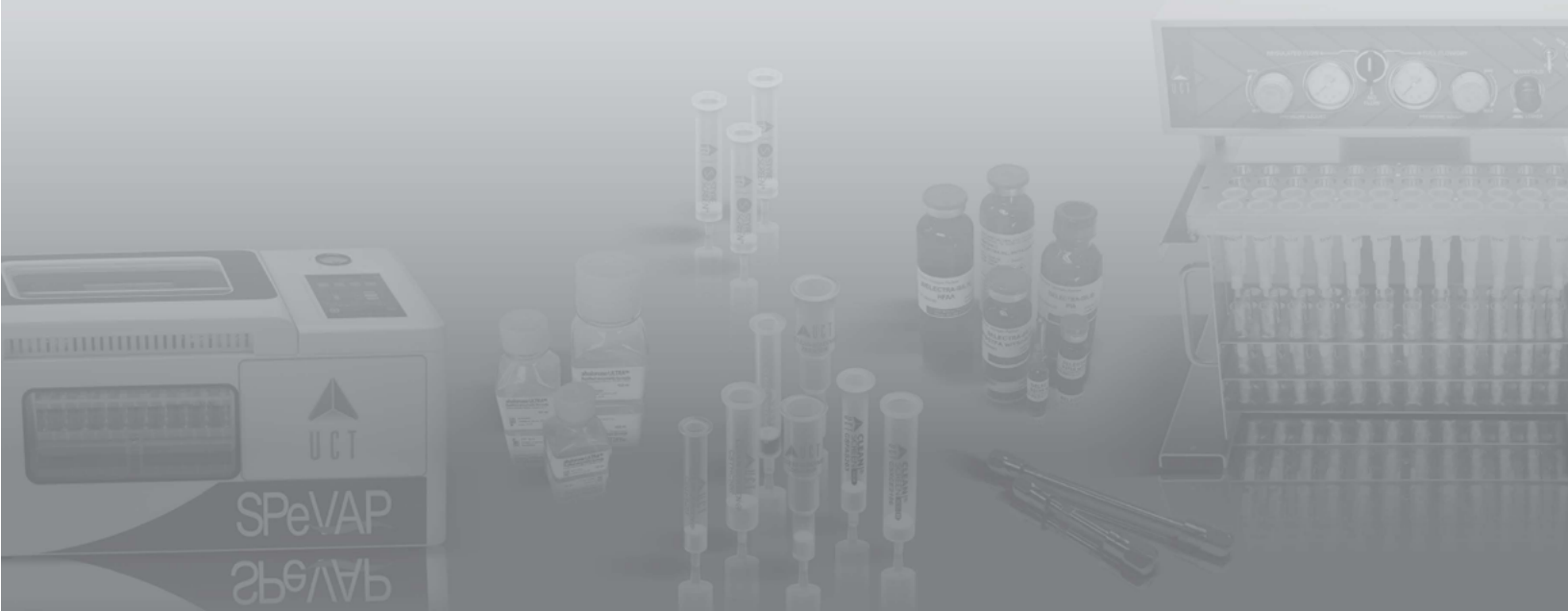
> Download Application Note

> Review Poster

> Learn more about SelectraCore®

> Learn more about Clean Screen® DAU

Return To Navigation



Amphetamines in Blood, Plasma/Serum, Urine, or Tissue Using Clean Screen® DAU SPE and LC-MS/MS Analysis

UCT Part Numbers

CSDAU206
Clean Screen® DAU
200 mg, 6 mL Column

SPHPHO6001-10
Select pH Buffer Pouch
100 mM Phosphate, pH 6.0

SLPFPP50ID21-18UM
Selectra® PFPP UHPLC Column
50 X 2.1 mm, 1.8 µm

SLPFPPGDC20-18UM
Selectra® PFPP Guard Column
10 X 2.0 mm, 1.8 µm

SLGRDHLDR-HP
Guard Column Holder

Summary:

Amphetamines are a group of drugs that stimulate the central nervous system (CNS). These drugs can be administered in the body through many ways. While oral consumption is the most common route, they can also be snorted, smoked and injected intravenously. Increasing abuse potential and dependence liability of amphetamine & methamphetamine have caused the DEA/FDA to classify these drugs as Schedule II controlled substances. The ease of manufacturing has made methamphetamine one of the most frequently encountered substance in drug related cases. Designer drugs metylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA) are methylenedioxy derivatives of methamphetamine and amphetamine respectively.

Phentermine is a schedule IV drug that is not heavily abused but is known to exert effects that are similar to amphetamine.

This application note describes a simple and robust solid-phase extraction (SPE) procedure for amphetamines in blood, plasma/serum, urine and tissue samples. The mixed-mode functionality of the Clean Screen® DAU SPE cartridge ensures efficient extraction of the amphetamines while removing undesired matrix components and yielding clean extracts. UHPLC separation was carried out using a Selectra® PFPP column prior to detection by tandem mass spectrometry (MS/MS). The PFPP (pentafluorophenylpropyl) stationary phase can undergo dipole–dipole and pi–pi interactions, imparting unique selectivity and retention mechanisms. In this application excellent retention of the polar amphetamines, including baseline separation of the isobaric methamphetamine and phentermine was obtained in less than 4.5 minutes.

FORENSICS

UCT

Sample Preparation:

- Add appropriate volumes of internal standard to 1 -2 mL of blood, plasma/ serum, urine, or 1 g (1:4) tissue homogenate
- Mix/vortex briefly and let stand for 5 minutes
- Add 3 mL of 100 mM phosphate buffer (pH 6.0)
- Mix/vortex briefly
- For blood, plasma/ serum tissue homogenate samples, centrifuge for 10 minutes at 2000 rpm (discard pellet after loading sample onto SPE column)

SPE Procedure:

1. Condition Column

a) 1 x 3 mL methanol
b) 1 x 3 mL 100 mM phosphate buffer (pH 6.0)

2. Apply Sample

a) Load sample at 1-2 mL/minute

3. Wash Column

a) 1 x 3 mL 0.1 M HCl
b) 1 x 3 mL methanol
c) Dry SPE column for 2 mins at 80-100 psi

4. Analytes

a) 1 x 3 mL ethyl acetate/ IPA/ NH_4OH (78:20:2)
b) Collect eluate at 1-2 mL/minute

5. Dry Eluate

a) Evaporate the eluate for 5 minutes to remove NH_4OH (40°C, gentle stream of N_2)
b) Add 100 µL of 1% HCl in methanol to prevent volatization of the drugs and loss during evaporation

Note: It is important to remove the NH_4OH prior to adding 1% HCl in methanol, otherwise a white precipitate (NH_4Cl) will form.

6. Reconstitute

a) Reconstitute samples in 100 µL of mobile phase (alternative volumes may also be used)

FORENSICS

UCT

LC-MS/MS Parameters

System	Shimadzu Nexera LC-30 AD with MS-8050
UHPLC Column	Selectra® PFPP (50 X 2.1 mm, 1.8 µm) UCT P/N: (**SLPFPP50ID21-18UM**)
Guard Column	Selectra® PFPP (10 X 2.0 mm, 1.8 µm) UCT P/N: (**SLPFPPGDC20-18UM**)
Column Temperature	40 °C
Flow Rate	0.5 mL/min
Injection Volume	2 µL
Autosampler Temperature	10 °C

Gradient Program

Time (min)	Mobile Phase A (%) (0.1% Formic Acid in Water)	Mobile Phase B (%) (0.1% Formic Acid in Methanol)
0.0	100	0
0.5	70	30
3.0	60	40
3.5	0	100
4.5	0	100
4.6	100	0
6.0	100	0

Results

Recovery - Blood				
Analyte	**10 ng/mL (n=3)**	**Rel. Std Dev (%)**	**100 ng/mL (n=3)**	**Rel. Std Dev (%)**
Amphetamine	96%	2.55	92%	0.78
Methamphetamine	95%	2.59	92%	1.72
Phentermine	104%	3.37	95%	8.16
MDA	100%	4.80	94%	1.16
MDMA	97%	3.36	94%	0.64
MDEA	93%	1.87	91%	0.36

FORENSICS

UCT

Recovery - Urine				
Analyte	**10 ng/mL (n=3)**	**Rel. Std Dev (%)**	**100 ng/mL (n=3)**	**Rel. Std Dev (%)**
Amphetamine	104%	2.85	95%	2.02
Methamphetamine	103%	3.38	93%	3.03
Phentermine	117%	4.08	107%	5.25
MDA	106%	2.27	96%	3.04
MDMA	105%	2.82	96%	2.62
MDEA	102%	2.16	94%	2.41

Chromatograms

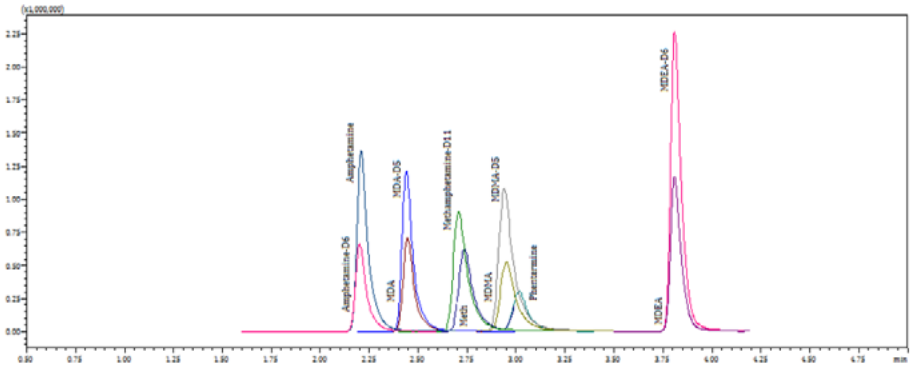


Figure 1: Chromatogram of a 10 ng/mL extracted sample.

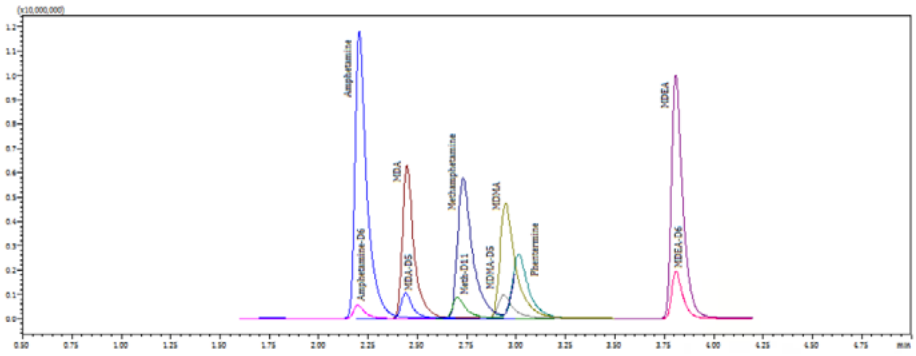


Figure 2: Chromatogram of a 100 ng/mL extracted sample.

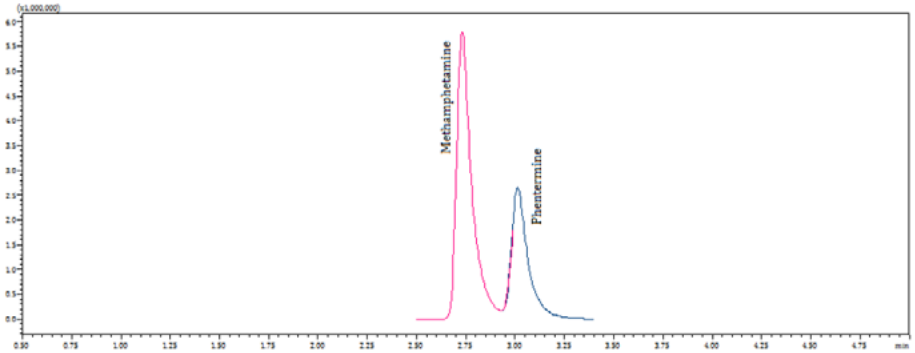


Figure 3: Chromatogram showing excellent baseline separation of methamphetamine & phentermine

Calibration Curves:

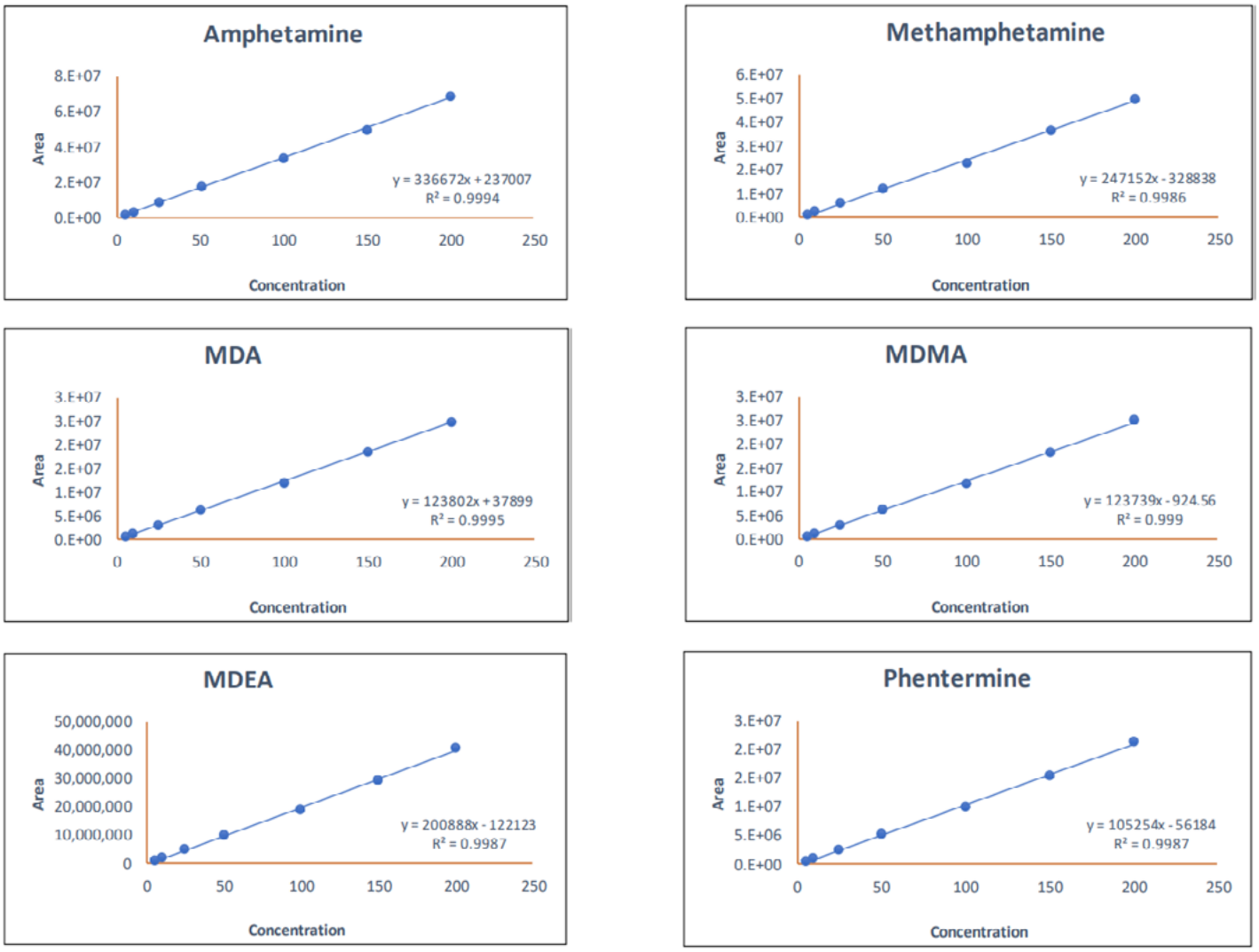


Figure 4: Calibration curve for the six amphetamines (5, 10, 25, 50, 100, 150 & 200 ng/mL).

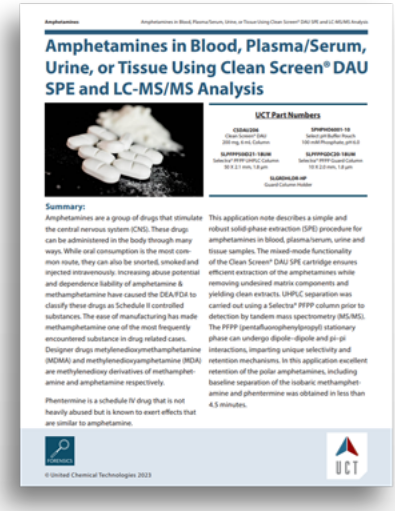
Note: For accurate quantitation of recoveries and to prevent saturation of the MS detector, a calibration curve ranging from 5-200 ng/mL was utilized for this study. Depending upon the requirements of an individual testing lab, a calibration curve with a wider concentration range may be required for routine analysis.

0204-01-01

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

FORENSIC
SAMPLE ANALYSIS

Amphetamines in Blood, Plasma/Serum, Urine, or Tissue Using Clean Screen® DAU SPE and LC-MS/MS Analysis


Amphetamines

Amphetamines in Blood, Plasma/Serum, Urine, or Tissue Using Clean Screen® DAU SPE and LC-MS/MS Analysis

Amphetamines in Blood, Plasma/Serum, Urine, or Tissue Using Clean Screen® DAU SPE and LC-MS/MS Analysis

UCT Part Numbers

Summary:

Amphetamines are a group of drugs that stimulate the central nervous system (CNS). These drugs can be administered in the body through many ways. While oral consumption is the most common route, they can also be snorted, smoked and injected intravenously. Increasing abuse potential and dependence liability of amphetamine & methamphetamine have caused the DEA/FDA to classify these drugs as Schedule II controlled substances. The ease of manufacturing has made methamphetamine one of the most frequently encountered substance in drug related cases. Designer drugs metylenedioxymethamphetamine (MDMA) and methylenedioxyamphetamine (MDA) are methylenedioxy derivatives of methamphetamine and amphetamine respectively.

Phentermine is a schedule IV drug that is not heavily abused but is known to exert effects that are similar to amphetamine.

This application note describes a simple and robust solid-phase extraction (SPE) procedure for amphetamines in blood, plasma/serum, urine and tissue samples. The mixed-mode functionality of the Clean Screen® DAU SPE cartridge ensures efficient extraction of the amphetamines while removing undesired matrix components and yielding clean extracts. UHPLC separation was carried out using a Selectra® PFPP column prior to detection by tandem mass spectrometry (MS/MS). The PFPP (pentafluorophenylpropyl) stationary phase can undergo dipole–dipole and pi-pi interactions, imparting unique selectivity and retention mechanisms. In this application excellent retention of the polar amphetamines, including baseline separation of the isobaric methamphetamine and phentermine was obtained in less than 4.5 minutes.

© United Chemical Technologies 2023

> Download Application Note

> Learn more about Clean Screen® DAU

> Learn more about Selectra® PFPP UHPLC Column

Return To Navigation

Analysis of 26 Natural and Synthetic Opioids in Blood and Urine Using Clean Screen® DAU SPE and a Selectra® DA UHPLC Column

UCT Part Numbers

CSDAU206
Clean Screen® DAU
200 mg, 6mL Column

Abalonase™ Ultra Purified β-Glucuronidase
UASBETA-GLUC-10

SLDAGDC20-18UMOPT
Selectra® DA Guard Column
5 X 2.1 mm, 1.8 µm

SPHPHO6001-10
Select pH Buffer Pouch
100 mM Phosphate, pH 6.0

SLDA50ID21-18UM
Selectra® DA UHPLC Column
50 X 2.1 mm, 1.8 µm

SLGRDHLDR-HPOPT
Guard Column Holder

Summary:

In recent years, drug abuse has become one of the leading causes of accidental deaths across the country. The opioid crisis was one of the main manifestations of drug-related addictions that caused severe dependency and, in too many cases, fatal overdoses. In this application note, UCT offers a simple yet effective procedure to extract and monitor an extensive panel of opiates in blood and urine using solid-phase extraction (SPE) combined with ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLCMS/MS). Clean Screen® DAU is UCT's flagship SPE column that can be used to extract a wide range of natural and synthetic opioids with excellent recoveries and overall precision. In addition, UCT's Selectra® DA UHPLC column provides excellent retention and peak shape for all the opioids in the panel, including baseline separation of the critical isobaric compounds. Furthermore, all compounds eluted in less than 8 minutes from the Selectra® DA UHPLC column. The simple protocol outlined in this application note can be readily implemented in pain management, clinical diagnostics, and forensic analysis.

FORENSICS

UCT

Sample Pretreatment:

Urine Specimens*:

- To 1 mL of urine add 1 mL of pH 6 phosphate buffer (0.1M) and appropriate volume of internal standard(s)
- Mix/vortex briefly

***A hydrolysis protocol is required if conjugated compounds are added into the drug panel**

Blood Specimens:

- To 1 mL of blood add 4 mL of pH 6 phosphate buffer (0.1M) and appropriate volume of internal standard(s)
- Mix/vortex briefly
- If necessary (e.g. postmortem blood), centrifuge the sample for 10 minutes at 3000 rpm (discard pellet after loading sample onto SPE column)

SPE Procedure:

1. Condition Column

a) 1 x 3 mL MeOH
b) 1 x 3 mL pH 6 phosphate buffer (0.1M)

2. Apply Sample

a) Load at 1-2 mL/minute

3. Wash Column

a) 1 x 3 mL 1% Formic Acid in DI H_2O
b) 1 x 3 mL MeOH
c) Dry cartridges under full vacuum or pressure for 1 minute to remove residual MeOH

4. Elute Analytes

a) 1 x 3 mL MeOH + 5% Ammonium Hydroxide (MeOH:NH_4OH, 95:5, v/v)
b) Collect at 1-2 mL/minute

5. Dry Eluate

a) Evaporate to dryness under a gentle stream of nitrogen at <40°C

6. Reconstitute

a) Reconstitute sample in 1 mL of mobile phase (alternative volumes may also be used)

LC-MS/MS Parameters:

HPLC Parameters	
MS System	Thermo Scientific TSQ Vantage
HPLC System	Thermo Scientific Dionex Ultimate 3000
UHPLC Column	Selectra® DA (50 X 2.1 mm, 1.8 µm) (UCT P/N: **SLDA50ID21-18UM**)
Guard Column	Selectra® DA Guard Column (5 X 2.1 mm, 1.8 µm) (UCT P/N: **SLDAGDC20-18UMOPT**)
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection Volume	5 µL

Gradient Program		
Time (min)	**% Mobile Phase A (0.1% FA in Water)**	**% Mobile Phase B (0.1% FA in Methanol)**
0	100	0
0.5	85	15
3.5	70	30
7.5	0	100
8.5	0	100
8.6	100	0
11	100	0

FORENSICS

UCT

MRM Table:

MRM							
Analyte	RT (min)	Parent Ion	Product Ion 1	CE	Product Ion 2	CE	Internal Standard
6-Acetylmorphine	4.64	328.1	165.1	36	211.1	25	6-Acetylmorphine-D6
Acetyl fentanyl	6.87	323.2	105.1	33	188.1	21	Fentanyl-D5
Buprenorphine	7.16	468.4	396.3	37	414.3	32	Buprenorphine-D4
Codeine	4.53	300.1	152.1	63	165.1	41	Codeine-D6
EDDP	7.40	278.1	234.1	31	249.2	23	Methadone-D9
Fentanyl	7.12	337.2	105.0	34	188.1	22	Fentanyl-D5
Heroin	6.32	370.1	165.0	46	268.1	27	Heroin-D9
Hydrocodone	5.03	300.1	128.1	56	199.1	29	Hydrocodone-D6
Hydromorphone	3.37	286.1	157.1	40	185.1	29	Hydromorphone-D6
Levorphanol	5.98	258.1	157.1	37	199.1	26	Morphine-D3
Meperidine	6.24	248.2	174.1	19	220.2	20	Meperidine-D4
Morphine	2.96	286.1	152.1	64	165.1	43	Morphine-D3
Methadone	7.59	310.0	104.6	28	264.7	13	Methadone-D9
Naloxone	4.25	328.0	212.1	37	310.1	18	Fentanyl-D5
Naltrexone	4.94	342.2	270.1	26	324.2	19	Fentanyl-D5
Norcodeine	3.79	286.1	152.0	56	165.1	43	Morphine-D3
Norbuprenorphine	6.70	414.3	101.1	36	187.0	35	Norbuprenorphine-D3
Norfentanyl	5.67	233.2	84.1	17	150.1	17	Norfentanyl-D5
Norhydrocodone	4.42	286.0	128.1	55	199.1	27	Norhydrocodone-D3
Normeperidine	6.12	234.1	56.2	23	160.1	16	Normeperidine-D4
Noroxycodone	4.23	302.0	227.1	28	284.1	16	Noroxycodone-D3
Noroxymorphone	2.47	287.9	212.9	29	270.0	17	Morphine-D3
Oxycodone	4.83	316.1	298.2	18	241.1	27	Oxycodone-D6
Oxymorphone	3.10	302.1	227.1	28	284.2	19	Morphine-D3
Tapentadol	5.81	222.1	77.1	45	107.1	29	Norfentanyl-D5
Tramadol	6.03	264.1	42.1	79	58.1	16	Fentanyl-D5

FORENSICS

UCT

Chromatograms:

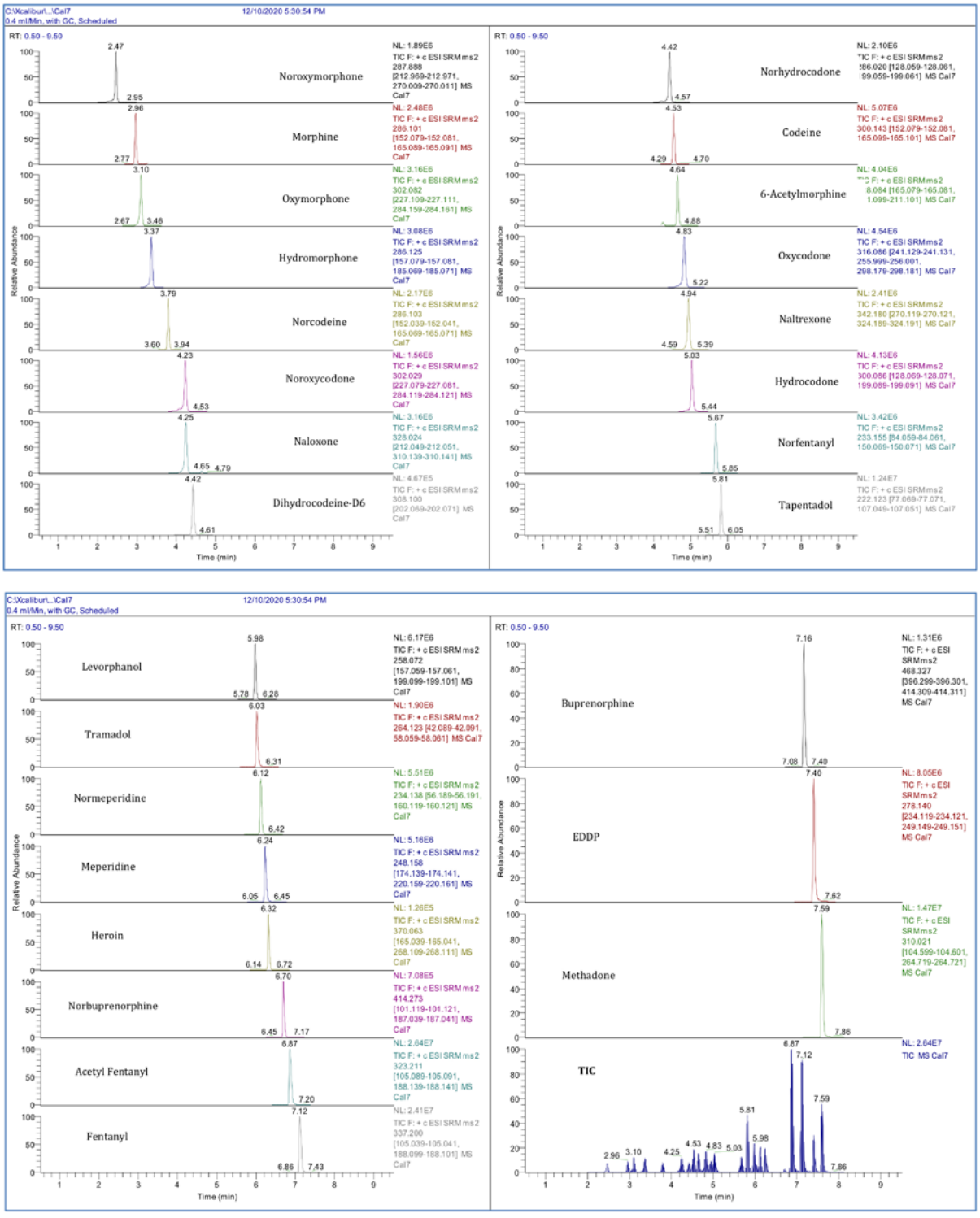


Figure 1: Exemplary chromatogram of the separation obtained using the Selectra® DA UHPLC column. All analytes included in the method eluted in less than 8 minutes. The TIC represents the total ion chromatogram for a single injection.

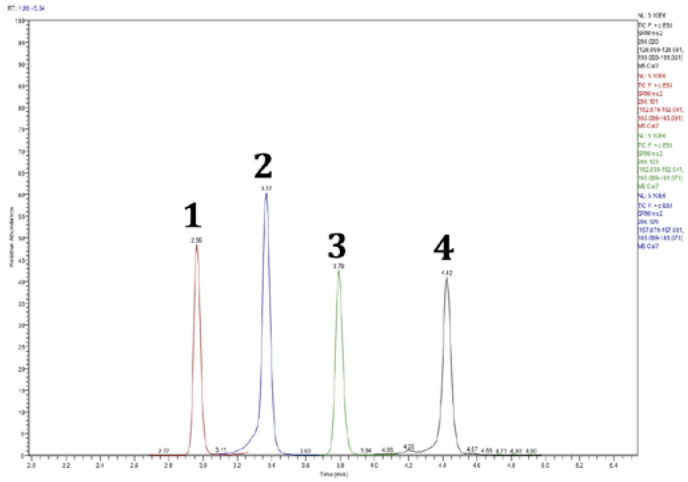


Figure 2a: Complete separation of critical isobaric compounds 1) Morphine, 2) Hydromorphone, 3) Norcodeine, and 4) Norhydrocodone

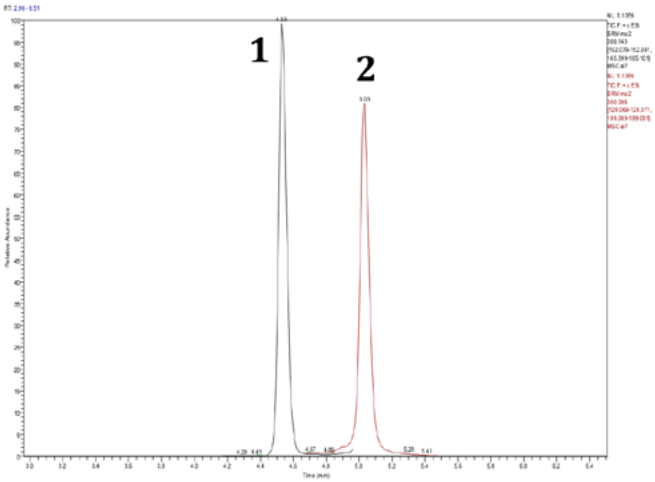


Figure 2b: Complete separation of critical isobaric compounds 1) Codeine and 2) Hydrocodone

Calibration Curves:

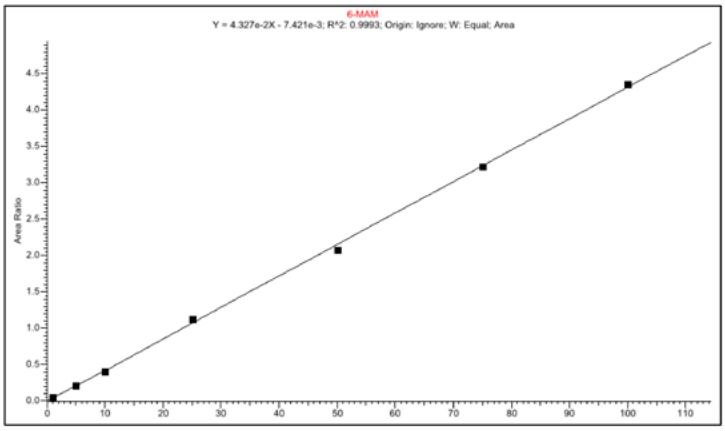


Figure 3a: Example of a 7-point calibration curve for 6-MAM with R^2 of 0.9993 (1, 5, 10, 25, 50 & 100 ng/mL).

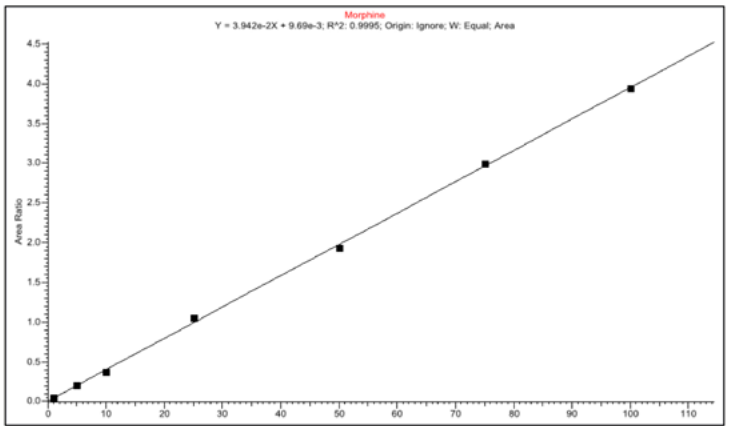


Figure 3b: Example of a 7-point calibration curve for Morphine with R^2 of 0.9995 (1, 5, 10, 25, 50 & 100 ng/mL).

Results:

Urine Recovery (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	75 ng/mL	RSD
6-Acetylmorphine	98%	0.17	103%	0.88	100%	1.06
Acetyl fentanyl	106%	0.28	104%	0.98	99%	3.68
Buprenorphine	98%	0.23	100%	0.67	99%	1.05
Codeine	96%	0.17	104%	0.58	99%	1.99
EDDP	106%	0.13	104%	2.06	95%	2.59
Fentanyl	84%	0.33	102%	1.31	101%	1.17
Heroin	104%	0.14	100%	0.87	99%	1.40
Hydrocodone	103%	0.14	102%	0.64	100%	1.69
Hydromorphone	97%	0.08	103%	0.74	102%	1.03
Levorphanol	106%	0.23	103%	2.58	91%	4.27
Meperidine	97%	0.13	101%	0.59	100%	0.87
Morphine	95%	0.18	102%	0.80	100%	1.81
Methadone	107%	0.17	97%	0.74	97%	1.86
Naloxone	106%	0.31	108%	1.31	94%	5.52
Naltrexone	109%	0.27	108%	1.20	96%	5.85
Norcodeine	109%	0.24	104%	0.97	97%	4.65
Norbuprenorphine	84%	0.43	99%	0.85	98%	1.42
Norfentanyl	92%	0.23	105%	0.81	101%	1.42
Norhydrocodone	102%	0.20	100%	0.95	97%	0.92
Normeperidine	90%	0.17	102%	0.85	102%	0.84
Noroxycodone	103%	0.26	93%	0.87	95%	4.13
Noroxymorphone	102%	0.25	102%	1.27	100%	3.03
Oxycodone	100%	0.19	103%	0.97	103%	1.78
Oxymorphone	110%	0.22	109%	1.30	97%	4.51
Tapentadol	82%	0.26	106%	1.94	99%	2.65
Tramadol	96%	0.35	109%	1.49	99%	2.85

Results:

Blood Recovery (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	75 ng/mL	RSD
6-Acetylmorphine	101%	0.14	104%	0.82	98%	1.74
Acetyl fentanyl	107%	0.31	95%	3.04	100%	1.88
Buprenorphine	101%	0.13	103%	0.57	97%	1.76
Codeine	89%	0.14	106%	0.99	97%	1.51
EDDP	102%	0.56	98%	3.23	100%	3.17
Fentanyl	103%	0.14	108%	0.65	101%	1.30
Heroin	107%	0.30	105%	1.24	95%	2.82
Hydrocodone	97%	0.14	107%	0.44	99%	1.21
Hydromorphone	93%	0.07	106%	0.62	100%	1.18
Levorphanol	100%	0.10	108%	0.99	93%	2.19
Meperidine	94%	0.12	106%	0.78	99%	1.22
Morphine	96%	0.12	104%	0.64	98%	1.44
Methadone	85%	0.15	106%	1.13	101%	1.55
Naloxone	107%	0.38	105%	1.23	102%	2.64
Naltrexone	106%	0.36	108%	1.59	101%	3.51
Norcodeine	94%	0.11	98%	0.63	98%	2.51
Norbuprenorphine	98%	0.15	104%	0.74	98%	1.33
Norfentanyl	88%	0.14	109%	1.09	102%	1.72
Norhydrocodone	98%	0.12	104%	0.77	99%	0.97
Normeperidine	95%	0.15	108%	0.86	101%	1.47
Noroxycodone	95%	0.26	107%	0.77	100%	1.98
Noroxymorphone	95%	0.12	100%	0.92	98%	2.45
Oxycodone	97%	0.13	108%	0.74	99%	1.72
Oxymorphone	103%	0.07	105%	0.55	97%	2.53
Tapentadol	96%	0.17	106%	0.78	98%	2.00
Tramadol	105%	0.53	103%	1.57	104%	3.03

1202-01-01

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255

www.unitedchem.com Email: methods@unitedchem.com

FORENSIC
SAMPLE ANALYSIS

Analysis of 26 Natural and Synthetic Opioids in Blood and Urine Using Clean Screen® DAU SPE and a Selectra® DA UHPLC Column


Analysis of 26 Natural and Synthetic Opioids in Blood and Urine Using Clean Screen® DAU SPE and a Selectra® DA UHPLC Column

UCT Part Numbers

Summary:
In recent years, drug abuse has become one of the leading causes of accidental deaths across the country. The opioid crisis was one of the main manifestations of drug-related addictions that caused severe dependency and, in too many cases, fatal overdoses. In this application note, UCT offers a simple yet effective procedure to extract and monitor an extensive panel of opiates in blood and urine using solid-phase extraction (SPE) combined with ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLCMS/MS). Clean Screen® DAU is UCT's flagship SPE column that can be used to extract a wide range of natural and synthetic opioids with excellent recoveries and overall precision. In addition, UCT's Selectra® DA UHPLC column provides excellent retention and peak shape for all the opioids in the panel, including baseline separation of the critical isobaric compounds. Furthermore, all compounds eluted in less than 8 minutes from the Selectra® DA UHPLC column. The simple protocol outlined in this application note can be readily implemented in pain management, clinical diagnostics, and forensic analysis.

© United Chemical Technologies 2023

> Download Application Note

> Learn more about Clean Screen® DAU

> Learn more about Abalonase™ Ultra

Return To Navigation

Simultaneous Analysis of Free Steroids and Sulfate Conjugates by Solid-Phase Extraction and LC-MS/MS

UCT Part Numbers

SSHLB066
Styre Screen® HLB
6 mL, 60 mg sorbent

SCS27-DA1021
SelectraCore® DA UHPLC Column
100 X 2.1 mm, 2.7 µm

SCS27-DAGDC21
SelectraCore® DA Guard Column
5 X 2.1 mm, 2.7 µm

SLGRDHLDR-HPOPT
UHPLC Direct Connect Guard Cartridge Holder

Summary:

Steroids are common analytes tested by forensic, clinical, and anti-doping laboratories. Analysis of free steroids generally requires a hydrolysis step, most commonly performed using an enzyme to cleave glucuronide groups. However, recently, there is a growing interest in the direct analysis of steroid sulfate conjugates rather than targeting free steroids originating from glucuronide conjugates after hydrolysis. This is because the ratio between glucuronide and sulfate metabolites is different from person to person and sulfate conjugates may even exceed the glucuronide-bound steroids in some cases, potentially leaving a large degree of analytes unanalyzed.[1] Also, sulfate metabolites are excreted at a slower rate, meaning their abundance is dependent on the time and route of administration.[2] Current research suggests that steroid sulfate markers can increase the detection window for the identification of analytes that are potentially being abused by athletes. Sulfate metabolites increase with time after use and remain present in the body longer than glucuronide metabolites.[2]

This application note outlines a highly efficient method for the simultaneous analysis of free and sulfated steroids from urine, plasma, and blood utilizing solid-phase extraction (SPE) and liquid chromatography-tandem mass spectrometry (LC-MS/MS). Styre Screen® HLB extraction cartridges consist of a highly retentive hydrophilic and lipophilic sorbent which can effectively retain these challenging analytes leading to high recoveries.
The SelectraCore® DA UHPLC column provided exceptional retention and peak shape for the wide range of steroids included in the method.

FORENSICS CLINICAL

UCT

Sample Pretreatment:

Urine
- In a test tube, add 0.5 mL sample, internal standards, 200 µL of methanol, and 1.3 mL of DI water
- Vortex

Blood and Plasma
- In a test tube, add 0.25 mL of sample, internal standards, and 0.75 mL acetonitrile
- Vortex and centrifuge
- Decant supernatant in 5 mL of DI water
- Vortex

SPE Procedure:

1. Condition Column

a) 1 x 3 mL methanol
b) 1 x 3 mL DI water

2. Load Sample

a) Load the samples at 1 to 2 mL/min

3. Wash Column

a) 1 x 3 mL 60 mM HCl in DI water
b) 1 x 3 mL 30% methanol in DI water

4. Dry

a) Dry columns for 10 mins at full vacuum or pressure

5. Elute Analytes

a) 1 x 3 mL 50:50 methanol:acetonitrile

6. Evaporate and Reconstitute

a) Evaporate to full dryness at 10 psi, 40°C
b) Reconstitute in 1 mL 80:20 water:acetonitrile or other appropriate volume and solvent

Notes:

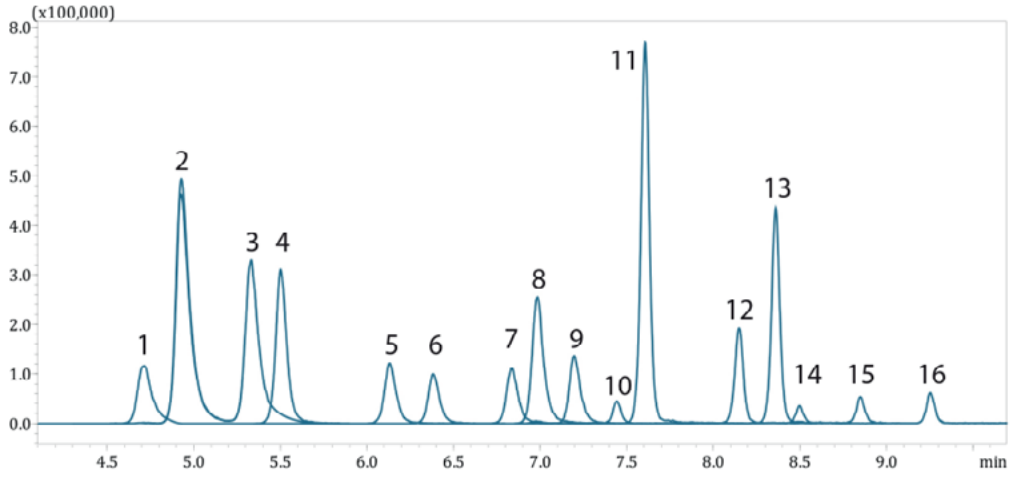
Steroids are endogenous substances; this was accounted for in the following ways:

- Synthetic urine was used as a surrogate matrix for urine
- Surrogate matrices were not used for blood and plasma, results were obtained by using a background subtraction method (See **Equations 1, 2**).

LC-MS/MS Parameters	
System	Shimadzu Nexera LC-30AD w/ MS-8050
UHPLC Column	SelectraCore® DA 100 x 2.1 mm, 2.7 µm (**P/N: SCS27-DA1021**)
Guard Column	SelectraCore® DA 5 x 2.1 mm, 2.7 µm (**P/N: SCS27-DAGDC21**)
Column Temperature	40°C
Column Flow Rate	0.4 mL/min
Injection volume	5 µL

Gradient Program		
Time (min)	**Mobile Phase A (%)** 0.1% Formic Acid in Water	**Mobile Phase B (%)** Acetonitrile
0	80	20
5	70	30
8.5	55	45
10.5	0	100
11.5	0	100
11.6	80	20
15.6	80	20

Chromatogram:



1. 17β-estradiol-17-sulfate
2. 17β-estradiol-3-sulfate
3. 17α-estradiol Sulfate
4. Boldenone Sulfate
5. Nandrolone Sulfate
6. Testosterone Sulfate
7. Estrone-3-sulfate
8. DHEA sulfate
9. Androsterone Sulfate
10. Estradiol
11. Boldenone
12. Nandrolone
13. Testosterone
14. DHEA
15. Estrone
16. Androsterone

Figure 1: Standards mix at 25 ng/mL in 80:20 water:acetonitrile

FORENSICS CLINICAL

UCT

MRM Table:

MRM						
Analyte	**Parent Ion**	**Product Ion 1**	**CE**	**Product Ion 2**	**CE**	**RT (mins)**
17β-estradiol-17-sulfate	351.1	97.0	39	80.0	55	4.70
17β-estradiol-3-sulfate	351.1	271.2	35	145.1	55	4.92
17*α*-estradiol Sulfate	351.1	271.2	35	145.1	54	5.33
Boldenone Sulfate	365.1	350.2	30	96.9	49	5.50
Nandrolone Sulfate	353.1	97.0	40	79.9	54	6.12
Testosterone Sulfate	367.2	97.1	42	351.2	44	6.37
Estrone-3-sulfate	349.1	269.3	34	145.0	53	6.83
DHEA Sulfate	367.1	97.0	33	-	-	6.97
Androsterone Sulfate	369.2	97.0	39	-	-	7.19
Estradiol	255.0	159.0	21	95.5	30	7.44
Boldenone	287.2	121.1	25	135.1	15	7.60
Nandrolone	275.1	109.1	27	257.3	17	8.14
Testosterone	289.2	97.1	27	97.1	23	8.36
DHEA	270.8	253.0	15	97.1	40	8.50
Estrone	270.8	253.0	14	79.1	47	8.84
Androsterone	291.2	273.3	10	255.3	15	9.25

*CE=collision energy, RT=retention time

Note: Free steroid compounds were analyzed in positive mode while the sulfate steroid conjugates were analyzed in negative mode

Representative Calibration Curves:

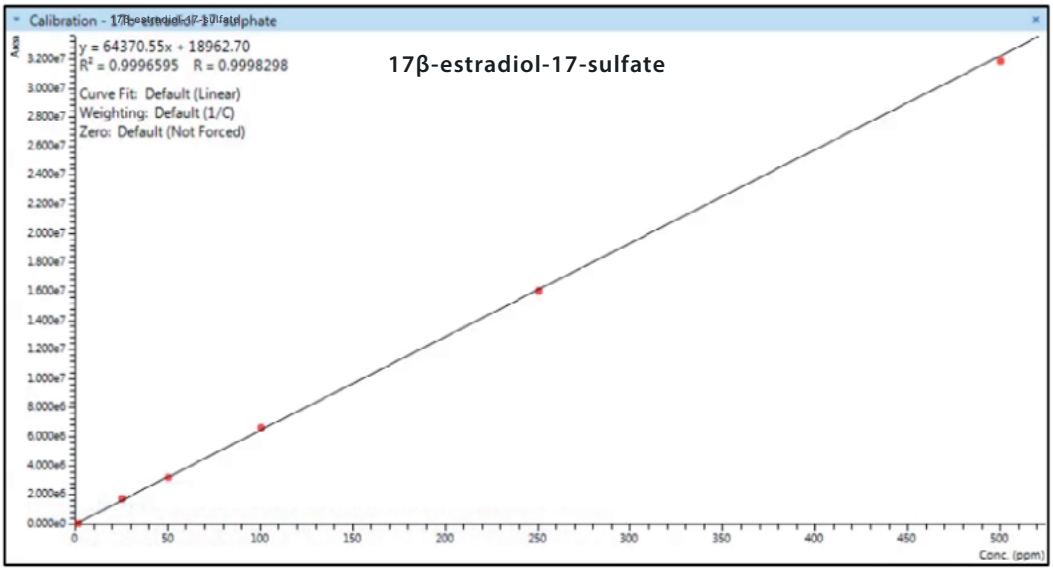


Figure 2a: Example of a 6-point solvent calibration curve for 17β-estradiol-17-sulfate with linear equation & R^2 value (1, 25, 50, 100, 250 & 500 ng/mL)

FORENSICS CLINICAL

UCT

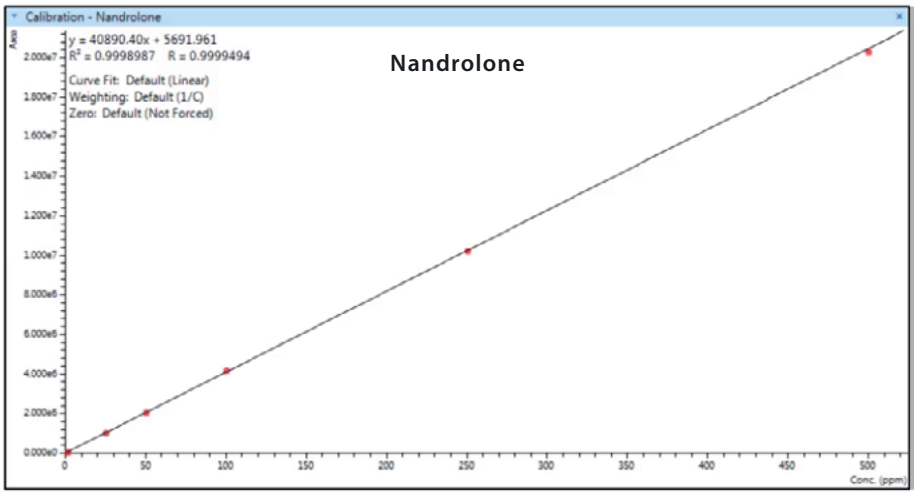


Figure 2b: Example of a 6-point solvent calibration curve for Nandrolone with linear equation & R^2 value (1, 25, 50, 100, 250 & 500 ng/mL)

Results:

Urine Extraction									
	5 ng/mL (n=5)			50 ng/mL (n=5)			250 ng/mL (n=5)		
Analyte	Recovery (%)	RSD (%)	Matrix Effects (%)	Recovery (%)	RSD (%)	Matrix Effects (%)	Recovery (%)	RSD (%)	Matrix Effects (%)
17β-estradiol-17-sulfate	85	3	-5	89	7	-12	86	18	-13
17β-estradiol-3-sulfate	89	3	-1	96	3	-7	93	7	-8
17α-estradiol Sulfate	91	1	0	100	3	-12	96	3	-13
Boldenone Sulfate	92	2	0	98	2	-6	98	3	-9
Nandrolone Sulfate	90	2	1	97	2	-5	98	5	-9
Testosterone Sulfate	91	4	0	97	3	-10	96	14	-12
Estrone-3-sulfate	85	7	9	85	3	13	103	9	-8
DHEA Sulfate	90	2	14	86	5	6	100	12	-9
Androsterone Sulfate	88	4	2	92	5	-7	92	1	-13
Estradiol	87	1	9	97	1	1	99	1	2
Boldenone	92	1	3	97	1	0	100	0	-2
Nandrolone	92	1	13	96	1	6	103	1	-1
Testosterone	91	2	12	96	1	7	102	1	2
DHEA	87	6	11	94	1	9	98	2	2
Estrone	93	4	2	95	2	2	99	1	1
Androsterone	98	4	0	95	1	1	98	7	-1

Plasma Extraction									
	5 ng/mL (n=5)			50 ng/mL (n=5)			250 ng/mL (n=5)		
Analyte	Recovery (%)	RSD (%)	Matrix Effects (%)	Recovery (%)	RSD (%)	Matrix Effects (%)	Recovery (%)	RSD (%)	Matrix Effects (%)
17β-estradiol-17-sulfate	82	2	-5	83	3	-3	80	4	-5
17β-estradiol-3-sulfate	80	2	-4	82	2	-2	81	2	-4
17α-estradiol Sulfate	85	3	-5	88	2	-4	85	2	-5
Boldenone Sulfate	86	2	-4	88	2	-2	89	1	-4
Nandrolone Sulfate	88	2	-5	88	2	-3	89	2	-4
Testosterone Sulfate	85	2	-4	88	2	2	88	2	-3
Estrone-3-sulfate	84	3	-3	84	2	-3	81	2	-5
DHEA Sulfate	59*	2	-77*	90	2	-12	86	1	-5
Androsterone Sulfate	76*	1	-41*	86	2	-9	85	4	-6
Estradiol	87	3	-8	92	3	-8	89	3	-9
Boldenone	89	1	0	89	1	2	92	1	1
Nandrolone	89	1	-1	89	1	2	91	1	-1
Testosterone	87	2	-5	88	1	-1	91	2	-5
DHEA	84	3	3	90	2	-3	90	3	-8
Estrone	86	6	-3	93	2	-8	89	5	-9
Androsterone	84	3	-13	92	3	-17	92	6	-23

*LLOQ must be higher than 5 ng/mL for reproducibility of DHEA sulfate and androsterone due to endogenous background concentrations (See Conclusion/Discussion section).

Results:

Blood Extraction									
	5 ng/mL (n=5)			50 ng/mL (n=5)			250 ng/mL (n=5)		
Analyte	**Recovery (%)**	**RSD (%)**	**Matrix Effects (%)**	**Recovery (%)**	**RSD (%)**	**Matrix Effects (%)**	**Recovery (%)**	**RSD (%)**	**Matrix Effects (%)**
17β-estradiol-17-sulfate	76	2	3	73	2	1	77	6	0
17β-estradiol-3-sulfate	77	1	-1	77	1	2	78	3	0
17α-estradiol Sulfate	78	1	2	80	2	1	82	3	0
Boldenone Sulfate	85	1	1	82	1	2	86	2	0
Nandrolone Sulfate	84	1	0	81	1	0	86	3	-1
Testosterone Sulfate	84	10	24	81	2	2	87	3	2
Estrone-3-sulfate	77	3	3	77	2	0	79	4	-1
DHEA Sulfate	113*	2	27*	79	1	6	86	4	3
Androsterone Sulfate	101*	3	10*	76	1	3	83	4	1
Estradiol	81	2	-2	77	3	-1	84	3	-3
Boldenone	82	1	-2	83	2	0	86	3	0
Nandrolone	85	1	10	83	2	1	86	2	0
Testosterone	85	2	4	83	1	-1	87	2	-1
DHEA	89	1	1	84	2	-6	85	2	-4
Estrone	83	2	-3	78	3	-8	84	1	-6
Androsterone	80	4	5	82	2	-4	86	1	-3

*LLOQ must be higher than 5 ng/mL for reproducibility of DHEA sulfate and androsterone due to endogenous background concentrations (See Conclusion/Discussion section).

FORENSICS CLINICAL

UCT

Conclusion/Discussion:

A method was developed for the simultaneous extraction of both free and sulfated steroids in urine, plasma, and blood. Steroids are inherently difficult to work with because they are endogenous, especially DHEA sulfate, androsterone, and testosterone, making accurate analysis challenging. As a solution to this, synthetic urine was used as a surrogate matrix for urine. For blood and plasma, a suitable surrogate matrix is not available, so background subtraction was employed to determine recoveries. This was done by extracting a set of blank matrices simultaneously with the samples and subtracting the steroids present in the blanks from the samples. This was particularly necessary for DHEA sulfate, androsterone sulfate, and testosterone since they are present in the blank matrix in the highest amounts. Recoveries were calculated using **Equation 1** and matrix effects were determined using **Equation 2**.[3] However, for a background subtraction method to be reproducible, the lower limit of quantitation (LLOQ) for endogenous analytes should be at least 15-20% of the background peak concentrations.[4] For this reason, the results for DHEA sulfate and androsterone spiked at 5 ng/mL are irreproducible and insignificant in practice. A higher LLOQ would be required for these two analytes specifically because they are limited by the endogenous background concentrations rather than the analytical sensitivity of the method.

$$\%\ recovery = \frac{average\ area\ pre\ spiked\ samples - average\ area\ blank\ matrix}{average\ area\ post\ spiked\ samples - average\ area\ blank\ matrix}$$

Equation 1: Determination of percent recovery of endogenous substances

$$\%\ matrix\ effects = \left(\frac{average\ area\ post\ spiked\ samples - average\ area\ blank\ matrix}{average\ area\ solvent\ calibrator}\right) - 1$$

Equation 2: Determination of percent matrix effects of endogenous substances

Working with both free and sulfated steroids was further challenging because the sulfate conjugates are always negatively charged while the free steroids are always neutral, making reverse phase SPE an evident strategy for these analytes. Additionally, during LC-MS/MS analysis, sulfated steroids were ionized in negative mode, while the free steroids were ionized in positive mode. Meaning, the LC-MS/MS method required polarity switching for the simultaneous detection of target ions in both positive and negative ionization modes.

The steroid sulfates are also different from free steroids because the sulfate conjugates are highly protein bound, making the protein precipitation step necessary in order to analytically observe these substances. This part of the method also helps to clean up the sample and remove potential matrix interferences before it is even introduced to the SPE cartridge, which is preferable for blood matrices extracted on a polymeric cartridge, like the Styre Screen® HLB cartridge utilized in this method.

The full SPE method including the protein crash, wash solvents, and elution solvents were optimized to achieve the highest recoveries with the lowest matrix effects. Recovery for analytes at low, medium, and high concentrations in all three matrices ranged from 73-103% with low relative standard deviations (RSD) and matrix effects. Negative and positive matrix effects represent ion suppression and ion enhancement respectively. A robust analysis method for steroids was developed that can readily be implemented by clinical, forensic, and anti-doping labs.

FORENSICS CLINICAL

UCT

References:

[1] Gomes, R. L., Meredith, W., Snape, C. E., & Sephton, M. A. (2009). Analysis of conjugated steroid androgens: deconjugation, derivatization and associated issues. Journal of pharmaceutical and biomedical analysis, 49(5), 1133–1140. https://doi.org/10.1016/j.jpba.2009.01.027

[2] Esquivel, A., Alechaga, É., Monfort, N., & Ventura, R. (2018). Sulfate metabolites improve retrospectivity after oral testosterone administration. Drug Testing and Analysis. doi:10.1002/dta.2529

[3] Hess, C., Sydow, K., Kueting, T., Kraemer, M., & Maas, A. (2018). Considerations regarding the validation of chromatographic mass spectrometric methods for the quantification of endogenous substances in forensics. Forensic science international, 283, 150-155.

[4] Thakare, R., Chhonker, Y. S., Gautam, N., Alamoudi, J. A., & Alnouti, Y. (2016). Quantitative analysis of endogenous compounds. Journal of pharmaceutical and biomedical analysis, 128, 426-437.

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

FORENSIC
SAMPLE ANALYSIS

Simultaneous Analysis of Free Steroids and Sulfate Conjugates by Solid-Phase Extraction and LC-MS/MS


Simultaneous Analysis of Free Steroids and Sulfate Conjugates by Solid-Phase Extraction and LC-MS/MS

UCT Part Numbers

Summary:

> Download Application Note

> Review Poster

> Learn more about SelectraCore®

> Learn more about Clean Screen® DAU

Return To Navigation

Analysis of Natural Cannabinoids and Metabolites from Blood Using Clean Screen® THC SPE Column and SelectraCore® C18 on LC-MS/MS

UCT Part Numbers

CSTHC206
Clean Screen® THC
6 mL, 200 mg sorbent

SCS27-C181021
SelectraCore® C18 Column
100 X 2.1 mm, 2.7 µm

SPHPHO7001-10
Select pH buffer pouch
100 mM phosphate pH 7.0

SLGRDHLDR-HPOPT
UHPLC Direct Connect
Guard Holder

SCS27-C18GDC21
SelectraCore® C18 Guard Column
5 X 2.1 mm, 2.7 µm

Introduction:

Marijuana refers to parts or products derived from the *cannabis* plant that contains a concentration of Δ^9-THC greater than 0.3%. The *cannabis* plant contains various cannabinoids, with Δ^9-Tetrahydrocannabinol (Δ^9-THC) being the most desired compound due to the resulting effects that include altered perception of time and space, euphoria, and increased appetite.[1] With the growing number of states legalizing marijuana for recreational and medical purposes, each state has established its own laws regarding drug-impaired driving while under the influence of marijuana. Consequently, it is crucial for forensic laboratories to develop accurate and precise testing protocols.

Protein precipitation is a widely utilized technique to eliminate interferences from blood samples. It involves precipitating the proteins in the blood by modifying the pH or the hydrophobicity of the aqueous environment. Common reagents used for protein precipitation include acids, organic solvents, salts, and metals.

This application note provides a detailed procedure for protein precipitation sample preparation followed by a solid phase extraction (SPE) method to extract four natural cannabinoids and the two major metabolites of Δ^9-THC from blood samples. The analytes were extracted using the Clean Screen® THC SPE column. The LC-MS/MS parameters are also outlined which were optimized for the separation of isomers Δ^8-THC and Δ^9-THC using a SelectraCore® C18 core-shell column.

FORENSICS CLINICAL

UCT

Sample Pretreatment:

- To 0.5 mL of blood add internal standard(s) and 2 mL of ACN:Acetone (75:25)
- Vortex well and centrifuge for 10 minutes at 3000 rpm
- Decant sample into 3 mL of pH 7 phosphate buffer leaving behind blood pellet
- Vortex sample

SPE Procedure:

1. Condition Column

a) 1 x 2 mL of MeOH
b) 1 x 2 mL of pH 7 phosphate buffer

2. Load Sample

a) Load at 1 to 2 mL/minute

3. Wash Column

a) 2 x 3 mL deionized water
b) 2 x 3 mL 40% MeOH in deionized water

4. Dry Column

a) Dry column for at least 10 minutes under full pressure or vacuum

5. Elute Analytes

a) 1 x 3 mL of ACN:MeOH:Acetic Acid (89:9:2)

6. Dry Eluate

a) Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C

7. Reconstitute

a) Reconstitute in 1 mL of MeOH
Note: Alternative compatible solvents and volumes can be used

FORENSICS CLINICAL

UCT

LC-MS/MS Parameters	
LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® C18 Column 100 x 2.1 mm, 2.7 µm (UCT P/N: **SCS27-C181021**)
Guard Column	SelectraCore® C18 5 x 2.1 mm, 2.7 µm (UCT P/N: **SCS27-C18GDC21**)
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection volume	10 µL

Gradient Program		
Time (min)	**% Mobile Phase A: 0.1% formic acid in DI H_2O**	**% Mobile Phase B: 0.1% formic acid in MeOH**
0	50	50
3	20	80
7.5	10	90
8	0	100
9	0	100
9.1	50	50
12	50	50

MRM Table:

Analyte	Parent ion (m/z)	Product ion 1 (m/z)	CE (v)	Product ion 2 (m/z)	CE (v)	RT (mins)
Δ^9-THC	314.9	193.1	24	283.1	11	6.57
Δ^8-THC	314.9	193.1	23	123.1	35	6.81
COOH-THC	344.9	327.2	17	299.2	19	4.94
OH-THC	330.9	201.2	23	193.0	26	4.65
Cannabidiol (CBD)	314.9	193.2	23	282.9	14	4.98
Cannabinol (CBN)	311.2	223.2	21	241.1	18	6.08
COOH-THC D9	354.2	336.0	16	308.2	21	4.88
CBD-D3	318.2	196.1	23	122.9	30	4.97

*CE=collision energy, RT=retention time

FORENSICS CLINICAL

UCT

Chromatogram:

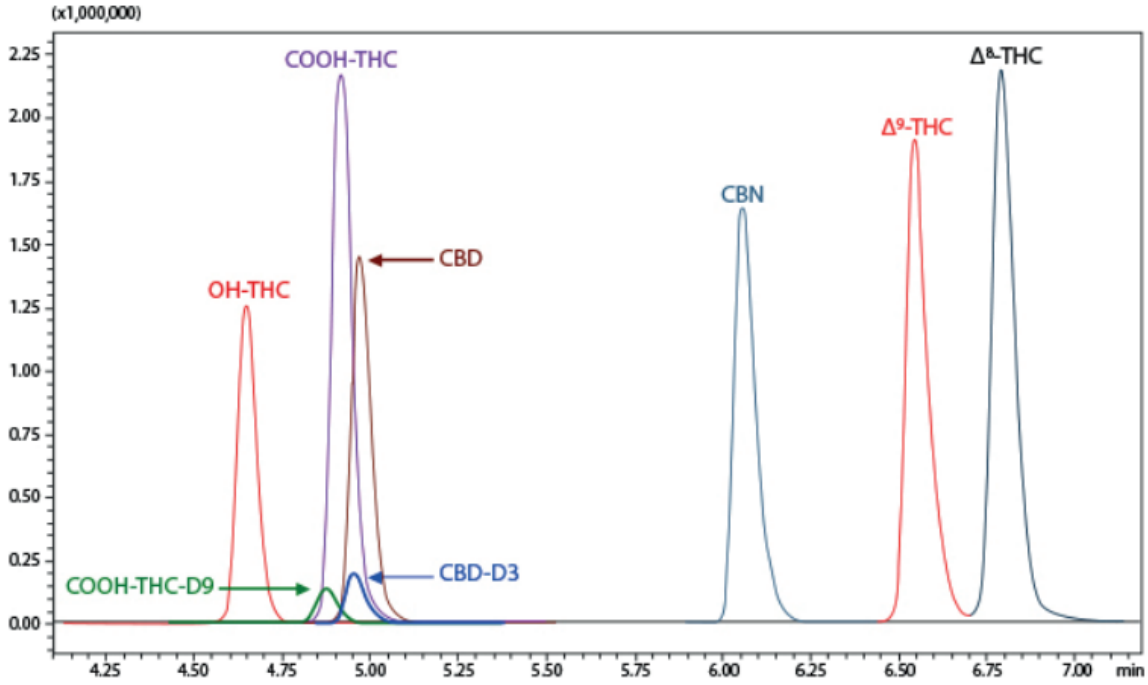


Figure 1. Chromatogram of extracted 50 ng/mL blood sample, minutes 4.10-7.20

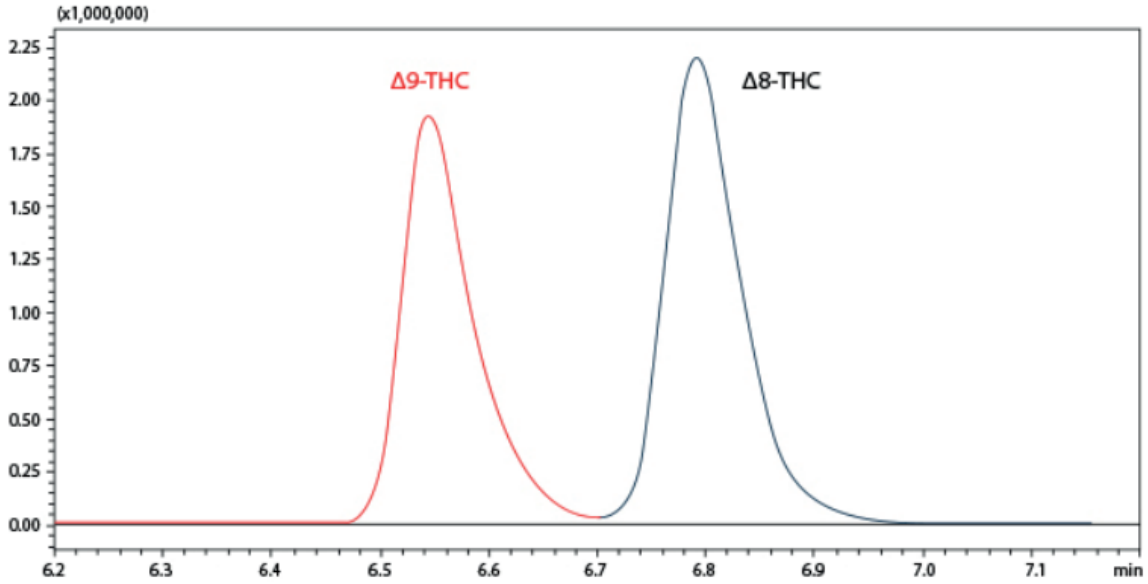


Figure 2. Zoomed in chromatogram of 50 ng/mL extracted blood sample showing separation of THC isomers, minutes 6.20-7.20

FORENSICS CLINICAL

UCT

Calibration Curves:

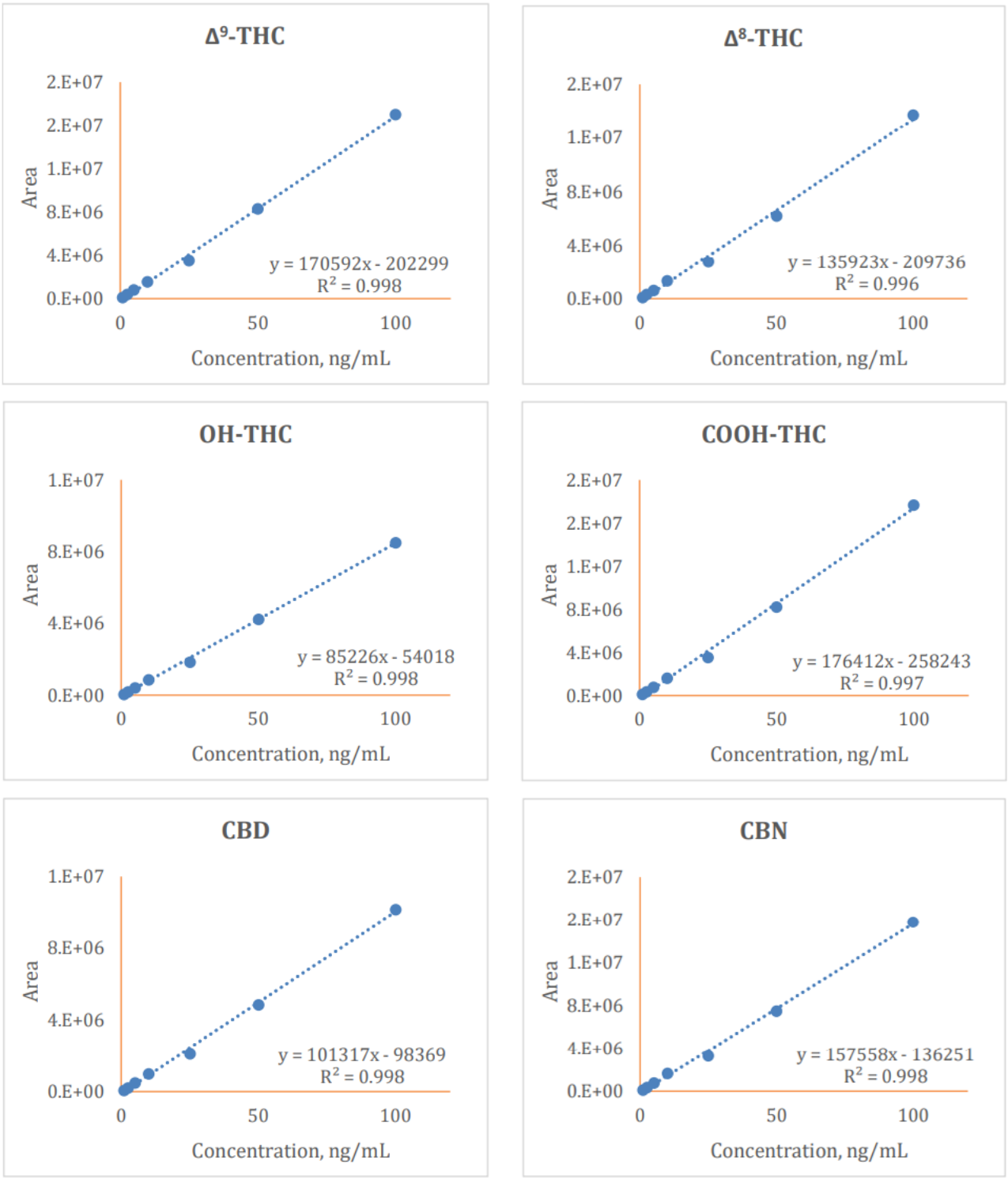


Figure 3. 7-point solvent calibration curve for all analytes with linear equation and R^2 value. [1, 2.5, 5, 10, 25, 50 and 100 ng/mL]

FORENSICS CLINICAL

UCT

Results:

Recovery (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	85%	2%	74%	3%	76%	2%
Δ^8-THC	85%	1%	74%	3%	75%	1%
OH-THC	89%	3%	84%	2%	87%	3%
COOH-THC	85%	2%	80%	1%	80%	2%
CBD	84%	2%	80%	3%	81%	2%
CBN	83%	3%	75%	2%	76%	2%

Table 1: The peak areas of pre-spiked samples were compared to the peak area of post-spiked samples

Matrix Effects (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	-26%	4%	-8%	3%	-3%	5%
Δ^8-THC	-26%	3%	-7%	3%	0%	5%
OH-THC	-16%	1%	-6%	2%	0%	3%
COOH-THC	-10%	1%	6%	3%	11%	3%
CBD	-23%	3%	-5%	2%	-2%	6%
CBN	-25%	4%	-4%	2%	2%	3%

Table 2: The peak areas of post-spiked samples were compared to the respective solvent standard in the calibration curve

Conclusions:

An extraction method was developed for the detection of four cannabinoids and the two major Δ^9-THC metabolites in blood (OH-THC and COOH-THC). The sticky nature of these compounds can make them difficult to work with and result in low recoveries. The acetonitrile: acetone (75:25) protein precipitation in the sample preparation has two purposes: First, as a solvent to precipitate and remove potential matrix interferences from blood. Second, to prevent the cannabinoids from sticking to the test tube when transferring the sample to the SPE column. An LC-MS/MS method was optimized that allowed successful analysis of samples in 12 minutes. Additionally, UCT's new SelectraCore® C18 core-shell column was able to separate isomers, Δ^9-THC, and Δ^8-THC eliminating the need for a chiral column. Washes were optimized to produce the highest recoveries with the lowest matrix effects. Recovery for analytes at low, medium, and high concentrations range from 74-89% with low relative standard deviation < 6%. Matrix effects for blood samples were within ± 26%.

FORENSICS CLINICAL

UCT

References:

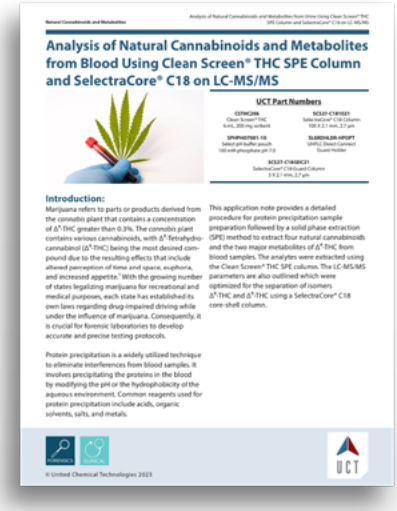
[1] Carlini E. A. (2004). The good and the bad effects of (-) trans-delta-9-tetrahydrocannabinol (Delta 9-THC) on humans. *Toxicon : official journal of the International Society on Toxinology,* 44(4), 461–467. https://doi.org/10.1016/j.toxicon.2004.05.009

6108-01-02

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

FORENSIC
SAMPLE ANALYSIS

Analysis of Natural Cannabinoids and Metabolites from Blood Using Clean Screen® THC and SelectraCore® C18 Column on LC-MS/MS


Analysis of Natural Cannabinoids and Metabolites from Blood Using Clean Screen® THC SPE Column and SelectraCore® C18 on LC-MS/MS

UCT Part Numbers

Introduction:

Marijuana refers to parts or products derived from the *cannabis* plant that contains a concentration of Δ^9-THC greater than 0.3%. The *cannabis* plant contains various cannabinoids, with Δ^9 tetrahydrocannabinol (Δ^9-THC) being the most desired compound due to the resulting effects that include altered perception of time and space, euphoria, and increased appetite.[1] With the growing number of states legalizing marijuana for recreational and medical purposes, each state has established its own laws regarding drug-impaired driving while under the influence of marijuana. Consequently, it is crucial for forensic laboratories to develop accurate and precise testing protocols.

Protein precipitation is a widely utilized technique to eliminate interferences from blood samples. It involves precipitating the proteins in the blood by modifying the pH or the hydrophobicity of the aqueous environment. Common reagents used for protein precipitation include acids, organic solvents, salts, and metals.

This application note provides a detailed procedure for protein precipitation sample preparation followed by a solid phase extraction (SPE) method to extract four natural cannabinoids and the two major metabolites of Δ^9-THC from blood samples. The analytes were extracted using the Clean Screen® THC SPE column. The LC-MS/MS parameters are also outlined which were optimized for the separation of isomers Δ^9-THC and Δ^8-THC using a SelectraCore® C18 core-shell column.

© United Chemical Technologies 2023

> Download Application Note

> Review Poster

> Learn more about SelectraCore®

> Learn more about Clean Screen® THC

Return To Navigation

Analysis of Natural Cannabinoids and Metabolites from Urine Using Styre Screen® HLB SPE Column and SelectraCore® C18 on LC-MS/MS

UCT Part Numbers

SSHLB063
Styre Screen® HLB
3 mL, 60 mg sorbent

SCS27-C181021
SelectraCore® C18 Column
100 X 2.1 mm, 2.7 µm

SPHPHO7001-10
Select pH buffer pouch
100 mM phosphate pH 7.0

SLGRDHLDR-HPOPT
UHPLC Direct Connect
Guard Holder

SCS27-C18GDC21
SelectraCore® C18 Guard Column
5 X 2.1 mm, 2.7 µm

Introduction:

Natural cannabinoids are chemical compounds that can be found in the *Cannabis* plant. Over a hundred different cannabinoids have been identified so far. The legal definition of marijuana encompasses all parts of the *Cannabis* plant, whether they are growing or not, and contain more than 0.3% dry weight of Δ^9-tetrahydrocannabinol (Δ^9-THC).[1] Δ^9-THC is known for its psychoactive and euphoric effects and is abundant in the *Cannabis Sativa* subspecies.[2] Marijuana is widely used as a recreational drug in the United States and across the globe. Although marijuana is federally illegal in the United States, Colorado became the first state to legalize it for recreational use in 2012, and many other states have followed suit by legalizing it for both recreational and medical purposes. As new state laws emerge, it is crucial to have accurate and precise methods for quantifying cannabinoids from biological samples.

This application note presents a solid phase extraction (SPE) procedure for extracting cannabinoids from urine samples, along with a 12-minute liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for analyzing four natural cannabinoids and the metabolites of Δ^9-THC. The panel includes the isomers Δ^9-THC and Δ^8-THC, which were successfully separated using the innovative SelectraCore® C18 core-shell column.

FORENSICS CLINICAL

UCT

Sample Pretreatment:

- To 1 mL of urine add internal standard, 1 mL of acetonitrile, and 1 mL of pH 7 phosphate buffer
- Vortex and centrifuge samples for 10 minutes at 3000 rpm

  Note: Include a hydrolysis procedure if glucuronide compounds are to be recovered

SPE Procedure:

1. Condition Column

a) 1 x 2 mL of MeOH
b) 1 x 2 mL of pH 7 phosphate buffer

2. Load Sample

a) Load at 1 to 2 mL/minute

3. Wash Column

a) 1 x 3 mL deionized water
b) 1 x 3 mL 50% MeOH in deionized water

4. Dry Column

a) Dry column for at least 10 minutes under full pressure or vacuum

5. Elute Analytes

a) 1 x 3 mL of 60:40 MeOH: Hexane
Note: shake or vortex elution solvent well before use

6. Dry Eluate

a) Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C

7. Reconstitute

a) Reconstitute in 1 mL of MeOH
Note: Alternative compatible solvents and volumes can be used

LC-MS/MS Parameters	
LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® C18 Column 100 x 2.1 mm, 2.7 µm (UCT P/N: **SCS27-C181021**)
Guard Column	SelectraCore® C18 5 x 2.1 mm, 2.7 µm (UCT P/N: **SCS27-C18GDC21**)
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection volume	10 µL

Gradient Program		
Time (min)	**% Mobile Phase A: 0.1% formic acid in DI H_2O**	**% Mobile Phase B: 0.1% formic acid in MeOH**
0	50	50
3	20	80
7.5	10	90
8	0	100
9	0	100
9.1	50	50
12	50	50

MRM Table:

Analyte	Parent ion (m/z)	Product ion 1 (m/z)	CE (v)	Product ion 2 (m/z)	CE (v)	RT (mins)
Δ^9-THC	314.9	193.1	24	283.1	11	6.42
Δ^8-THC	314.9	193.1	23	123.1	35	6.72
COOH-THC	344.9	327.2	17	299.2	19	4.86
OH-THC	330.9	201.2	23	193.0	26	4.58
Cannabidiol (CBD)	314.9	193.2	23	282.9	14	4.89
Cannabinol (CBN)	311.2	223.2	21	241.1	18	5.99
COOH-THC D9	354.2	336.0	16	308.2	21	4.81
CBD-D3	318.2	196.1	23	122.9	30	4.88

*CE=collision energy, RT=retention time

FORENSICS CLINICAL

UCT

Calibration Curves:

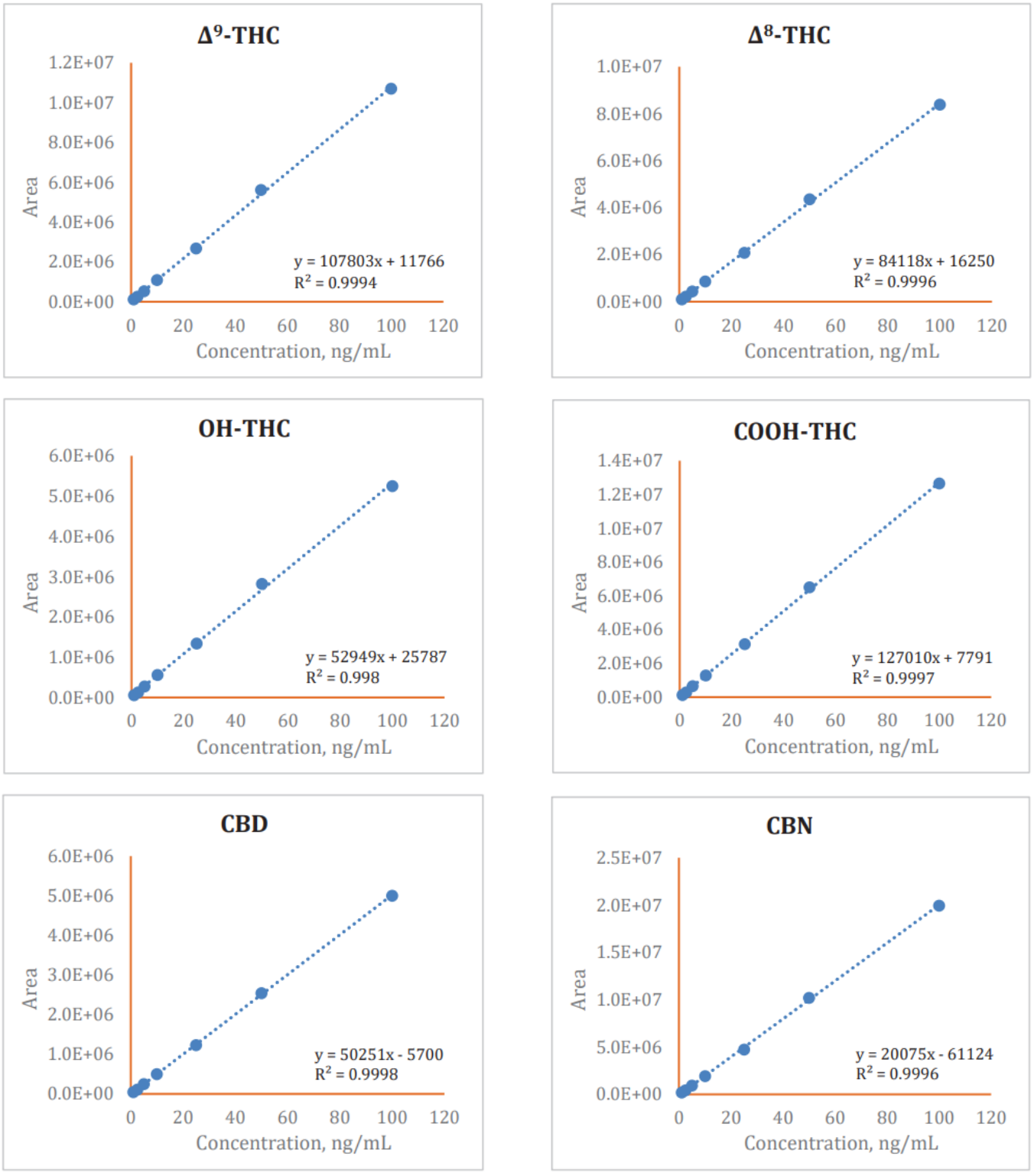


Figure 3. 7-point neat calibration curve for all analytes with linear equation and R^2 value. [1, 2.5, 5, 10, 25, 50, and 100 ng/mL]

FORENSICS CLINICAL

UCT

Results:

Absolute Recovery (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	97%	5%	99%	2%	98%	2%
Δ^8-THC	95%	4%	96%	3%	90%	3%
OH-THC	80%	4%	84%	5%	86%	6%
COOH-THC	95%	5%	97%	4%	94%	3%
CBD	103%	6%	99%	1%	97%	2%
CBN	97%	4%	99%	1%	95%	1%

Table 1: Extracted samples were compared to a solvent calibration curve

Extraction Efficiency (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	98%	4%	97%	2%	99%	2%
Δ^8-THC	93%	4%	94%	2%	95%	1%
OH-THC	103%	3%	99%	1%	105%	3%
COOH-THC	94%	7%	95%	3%	99.7%	2%
CBD	96%	4%	98%	1%	99.8%	1%
CBN	99%	4%	93%	2%	96%	1%
CBD-D3	98%	3%	96%	2%	91%	0%
COOH-THC-D9	93%	8%	94%	4%	92%	2%

Table 2: The Peak area of pre-spiked samples was compared to post-spiked samples

Matrix Effects (n=5)						
Analyte	5 ng/mL	RSD	25 ng/mL	RSD	50 ng/mL	RSD
Δ^9-THC	-1.2%	5%	-0.4%	2%	-5.1%	2%
Δ^8-THC	-4.1%	4%	-2.1%	3%	-8.1%	3%
OH-THC	-19.4%	6%	-15.3%	5%	-22.0%	3%
COOH-THC	-12.9%	3%	-1.8%	4%	-7.6%	5%
CBD	-3.9%	6%	0.8%	1%	-4.4%	2%
CBN	-0.2%	4%	4.1%	1%	-3.2%	1%
CBD-D3	1.3%	2%	3.9%	2%	6.8%	4%
COOH-THC-D9	1.2%	3%	3.2%	2%	7.7%	3%

Table 3: The peak area of post-spiked samples compared to respective solvent standard in curve

FORENSICS CLINICAL

UCT

Conclusions:

A LC-MS/MS and SPE extraction method was developed for the analysis of four natural cannabinoids and the two major Δ^9-THC metabolites in urine (OH-THC and COOH-THC). The sticky nature of these compounds can make them difficult to work with and result in low recoveries. The addition of 1 mL of acetonitrile in the sample preparation helps prevent analytes from sticking to the test tube when transferring the sample to the SPE cartridge. The LC-MS/MS method was able to successfully analyze samples in 12 minutes. Additionally, UCT's new SelectraCore® C18 core-shell column was able to separate THC isomers, Δ^9-THC and Δ^8-THC.

All analytes were extracted from urine using Styre Screen® HLB, a water wettable polymeric sorbent. For all the analytes in the panel, the absolute recovery from urine was equal to or greater than 80% with a relative standard deviation of less than 6%. The extraction efficiency of all analytes at low, medium & high concentrations was greater than 90% with a relative standard deviation of less than 8%. Matrix effects were minimized by washing the sorbent with deionized water and 50% methanol before eluting the compounds. Apart from COOH-THC and OH-THC, all other analytes had matrix effects between +10% and -10%. Although the matrix effects for COOH-THC and OH-THC were significant, the absolute recoveries were found to be equal to or greater than 80%. The simple and robust extraction method described in this application note can be readily implemented in high throughput forensic and clinical laboratories.

References:

[1] 21 U.S.C. § 802 (16) (2022)

[2] Pellati, Federica et al. "Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer." BioMed research international vol. 2018 1691428. 4 Dec. 2018, doi:10.1155/2018/1691428

10022022

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

FORENSIC
SAMPLE ANALYSIS

Analysis of Natural Cannabinoids and Metabolites from Urine Using Styre Screen® HLB and SelectraCore® C18 Column on LC-MS/MS


Analysis of Natural Cannabinoids and Metabolites from Urine Using Styre Screen® HLB SPE Column and SelectraCore® C18 on LC-MS/MS

UCT Part Numbers

Introduction:

Natural cannabinoids are chemical compounds that can be found in the Cannabis plant. Over a hundred different cannabinoids have been identified so far. The legal definition of marijuana encompasses all parts of the Cannabis plant, whether they are growing or not, and contain more than 0.3% dry weight of Δ⁹-tetrahydrocannabinol (Δ⁹-THC).[1] Δ⁹-THC is known for its psychoactive and euphoric effects and is abundant in the Cannabis Sativa subspecies.[2] Marijuana is widely used as a recreational drug in the United States and across the globe. Although marijuana is federally illegal in the United States, Colorado became the first state to legalize it for recreational use in 2012, and many other states have followed suit by legalizing it for both recreational and medical purposes. As new state laws emerge, it is crucial to have accurate and precise methods for quantifying cannabinoids from biological samples.

This application note presents a solid phase extraction (SPE) procedure for extracting cannabinoids from urine samples, along with a 12-minute liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for analyzing four natural cannabinoids and the metabolites of Δ⁹-THC. The panel includes the isomers Δ⁹-THC and Δ⁸-THC, which were successfully separated using the innovative SelectraCore® C18 core-shell column.

© United Chemical Technologies 2023

> Download Application Note

> Review Poster

> Learn more about SelectraCore®

> Learn more about Styre Screen® HLB

Return To Navigation

Enhanced LC-MS/MS Separation of Δ[8] and Δ[9] -THC Metabolites and Other Cannabinoids Extracted from Urine Samples using a Polymeric SPE Column

UCT Part Numbers

SSHLB066
Styre Screen® HLB
60 mg, 6 mL

SCS27-PFPGDC21
SelectraCore® PFPP Guard Column 5 x 2.1 mm, 2.7 µm

SPHPHO7001-10
Select pH buffer pouch
100 mM Phosphate Buffer pH 7.0

SCS27-PFP1021
SelectraCore® PFPP Column
100 x 2.1 mm, 2.7 µm

SLGRDHLDR-HPOPT
Selectra® Direct Connect Guard Holder

Introduction:

In recent years, there has been a notable increase in the use and commercialization of Δ[8] -THC products. The rise in numbers is attributed to the legalization of hemp through the Farm Bill of 2018. The main cannabinoid of hemp is CBD which can be converted into Δ[8] -THC.[1] This resulted in drug testing laboratories, needing to be able to separate parent THC isomers Δ[8] and Δ[9] -THC. However, tetrahydrocannabinol (THC) is extensively metabolized by the body into 11-nor-9-carboxyTHC (COOH-THC) and 11-hydroxy-THC (OH-THC). Therefore, quantitation and identification of THC's metabolites are critical for proper identification and interpretation. The newest challenge many laboratories face is the separation of the isomeric metabolites. Δ[8] -COOH-THC and Δ[9] -COOH-THC can be separated on a core-shell C18 column; however, Δ[8] -OH-THC and Δ[9] -OH-THC continue to be a challenge.

Additionally, with the expanding cannabis market, new cannabinoids continue to emerge. One example includes Δ[10] and Δ[6a,10a] -THC which occur naturally in marijuana at low levels but can be artificially synthesized to obtain higher concentrations.[2] THC-O-Acetate (THC-O) is a new semi-synthetic cannabinoid that is synthesized by adding acetic anhydride to THC.[3] These three examples are included in the panel with other emerging cannabinoids. This application note introduces an LC-MS/MS method that separates 16 cannabinoids using UCT's SelectraCore® PFPP column. This includes the separation of the isomeric metabolites of Δ[8] and Δ[9] -THC. A solid phase extraction from urine is also introduced using UCT's Styre Screen® HLB column.

FORENSICS CLINICAL

Sample Pretreatment:

1 mL urine sample + ISTD + 1 mL ACN + 1 mL of 100 mM phosphate buffer pH 7.0, vortex

Note: Include a hydrolysis procedure to recover conjugated analytes.

SPE Procedure:

1. Condition Column:
- a) 1 x 3 mL MeOH
- b) 1 x 3 mL 100 mM phosphate buffer pH 7.0

2. Load Sample:
- a) Load sample and aspirate 1-2 mL/min

3. Wash Column:
- a) 1 x 3 mL DI H_2O
- b) 1 x 3 mL 40:60 MeOH: DI H_2O

4. Dry Column:
- a) Dry for at least 10 minutes at full vacuum or pressure

5. Elute Analytes:
- a) 1 x 3 mL of 60:40 MeOH:Hexane

Note: Make elution solvent daily and shake/vortex well before use

6. Evaporate:
- a) Evaporate samples to dryness at 10 psi and 40°C

7. Reconstitute:
- a) Reconstitute samples in 1 mL of 45:55 MeOH:DI H_2O or other appropriate solvent and volume

Instrument Parameters	
LC-MS/MS System	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® PFPP Column 100 x 2.1 mm, 2.7 µm (PN: **SCS27-PFP1021**)
Guard Column	SelectraCore® PFPP Guard Column 5 x 2.1 mm, 2.7 µm (PN: **SCS27-PFPGDC21**)
Column Temperature	35°C
Flow Rate	0.3 mL/min
Injection Volume	10 µL

Gradient Program		
Time (min)	**Mobile Phase A (%)** 5 mM Ammonium Formate + 0.1% Formic Acid in DI H_2O	**Mobile Phase B (%)** Methanol
0	55	45
6	30	70
19-24.5	36	74
24.6-27.6	55	45

MRM Table						
Analyte	**R.T. (min)**	**Parent Ion (m/z)**	**Product Ion 1 (m/z)**	**CE (V)**	**Product Ion 2 (m/z)**	**CE (V)**
11-OH-Δ⁸ -THC	8.67	330.7	313.1	-16	201.0	-26
11-COOH-Δ⁸ -THC	8.87	344.7	299.2	-21	327.2	-18
11-OH-Δ⁹ -THC	8.93	300.9	313.2	-15	267.2	-20
11-COOH-Δ⁹ -THC	9.33	344.9	327.2	-17	299.2	-19
CBD	9.47	314.9	193.2	-23	123.1	-35
Δ⁸ -THCB	11.61	301.1	179.3	-22	123.0	-35
Δ⁹ -THCB	11.98	301.1	179.1	-24	23.0	-32
exo-THC	12.58	314.7	193.1	-24	123.0	-33
Δ⁸ -THC	13.47	314.9	193.1	-23	123.1	-35
Δ⁹ -THC	13.95	314.9	193.1	-24	123.1	-35
Δ^{10} /$\Delta^{6a,10a}$-THC	15.54	314.9	193.25	-24	123.1	-37
CBN	15.90	311.2	223.2	-21	293.2	-16
Δ⁸ -THCP	18.41	342.7	221.1	-25	122.9	-35
Δ⁹ -THCP	19.10	342.7	221.1	-24	123.0	-35
Δ⁸ -THC-O-Acetate	21.62	356.7	315.2	-18	193.1	-30
Δ⁹ -THC-O-Acetate	22.15	356.7	315.2	-17	193.1	-29

FORENSICS CLINICAL

UCT

Chromatogram:

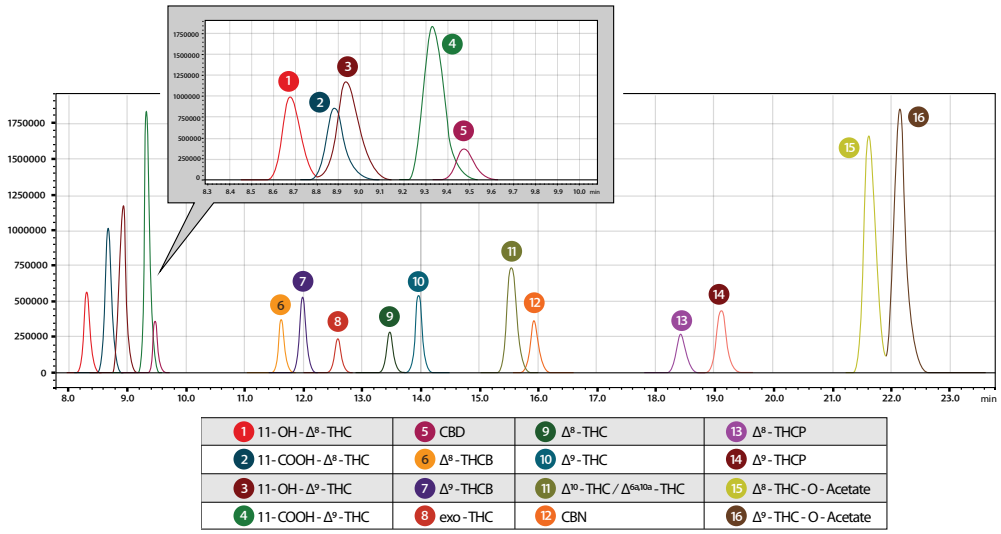
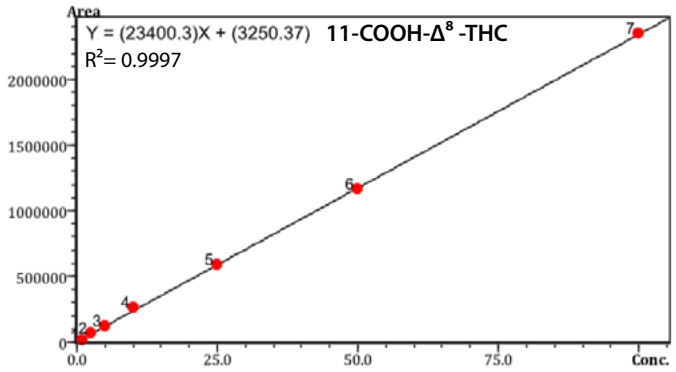
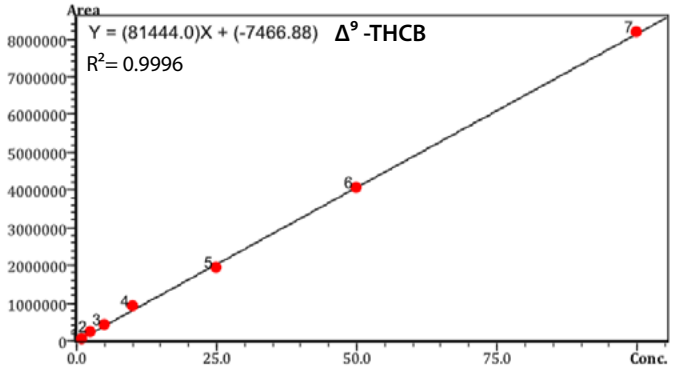


Figure 1: Chromatogram of Solvent Standard Mix Prepared at 25 ng/mL

Example Solvent Calibration Curves:





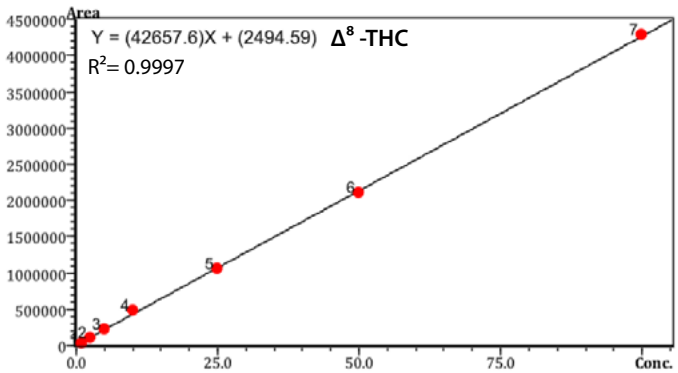


Figure 2: A 7-point solvent calibration curve for 11-COOH-Δ⁸ -THC, Δ⁹ -THCB, and Δ⁸ -THC with linear equation and R^2 Value (1, 2.5, 5, 10, 25, 50, and 100 ng/mL)

FORENSICS CLINICAL

UCT

Results									
n=3	5 ng/mL			25 ng/mL			50 ng/mL		
Analyte	**Recovery**	**Matrix Effects**	**RSD**	**Recovery**	**Matrix Effects**	**RSD**	**Recovery**	**Matrix Effects**	**RSD**
11-OH-Δ⁸-THC	104%	-20%	3%	83%	-6%	11%	88%	-3%	10%
11-COOH-Δ⁸-THC	99%	-16%	4%	83%	-4%	8%	84%	-1%	6%
11-OH-Δ⁹-THC	99%	-11%	2%	83%	0%	14%	86%	6%	8%
11-COOH-Δ⁹-THC	105%	-21%	13%	82%	-9%	10%	86%	-8%	8%
CBD	118%	-24%	6%	85%	-12%	8%	85%	-7%	8%
Δ⁸ -THCB	106%	-14%	4%	85%	-8%	11%	83%	4%	8%
Δ⁹ -THCB	106%	-18%	4%	84%	-9%	13%	84%	7%	9%
exo-THC	104%	-8%	7%	86%	-1%	15%	84%	4%	9%
Δ⁸ -THC	108%	-13%	5%	87%	2%	13%	86%	6%	7%
Δ⁹ -THC	106%	-20%	3%	86%	3%	10%	84%	7%	9%
Δ¹⁰-THC/Δ⁶ᵃ,¹⁰ᵃ-THC	110%	14%	2%	83%	25%	9%	90%	11%	6%
CBN	97%	-2%	3%	80%	5%	13%	78%	3%	7%
Δ⁸ -THCP	111%	-15%	4%	85%	-4%	11%	83%	-2%	9%
Δ⁹ -THCP	109%	-16%	2%	88%	-7%	10%	85%	-6%	9%
Δ⁸ -THC-O-Acetate	117%	-13%	1%	95%	-8%	3%	87%	-11%	3%
Δ⁹ -THC-O-Acetate	117%	-19%	1%	96%	-16%	4%	90%	-3%	4%

**Recoveries were calculated using a pre and post-spiked sample technique. Matrix effects were calculated by comparing post-spiked and solvent standards.*

Conclusion:

UCT's SelectraCore® PFPP column with methanol as mobile phase B proved to be the best combination to achieve separation of the four isomeric metabolites. This method separated a total of 16 cannabinoids. The PFPP column was not able to separate Δ¹⁰-THC and Δ⁶ᵃ,¹⁰ᵃ -THC. Additionally, baseline separation of the THC-O-Acetate isomers was also not achieved. Analytes were extracted from urine utilizing UCT's Styre Screen® HLB solid phase extraction column. After optimization, the extraction's recovery, matrix effect, and relative standard deviation were evaluated at three concentrations (5, 25, and 50 ng/mL). Recoveries for all analytes were above 75% (range 78-118%). Matrix effects and relative standard deviations were within ANSI/ASB Standard 063 guidelines. Matrix effects were within ± 25% and the RSDs were less than 20% (range 1-15%).

FORENSICS CLINICAL

UCT

References:

[1] "5 Things to Know about Delta-8 Tetrahydrocannabinol – Delta-8 THC." U.S. Food and Drug Administration, 4 May 2022, www.fda.gov/consumers/consumer-updates/5-things-know-about-delta-8-tetrahydrocannabinol-delta-8-thc.

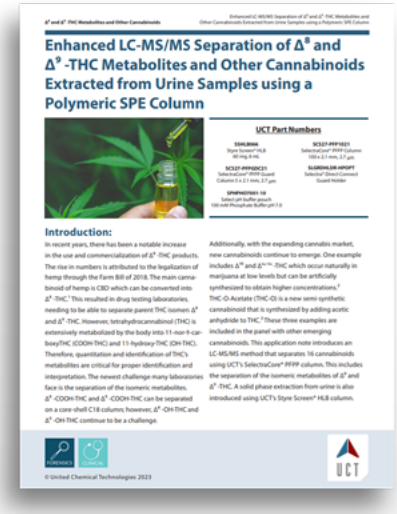
[2] Mallen, Briana. "Is Delta 10 Natural or Synthetic?" Secret Nature, 1 Sept. 2021, secretnaturecbd.com/blogs/cbd/is-delta-10-natural-or-synthetic.

[3] Alaina K Holt and others, Δ⁸ -THC, THC-O Acetates and CBD-di-O Acetate: Emerging Synthetic Cannabinoids Found in Commercially Sold Plant Material and Gummy Edibles, Journal of Analytical Toxicology, Volume 46, Issue 8, October 2022, Pages 940–948, https://doi.org/10.1093/jat/bkac036

UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

FORENSIC
SAMPLE ANALYSIS

Enhanced LC-MS/MS Separation of Δ^8 and Δ^9 -THC Metabolites and Other Cannabinoids Extracted from Urine Samples using a Polymeric SPE Column


Enhanced LC-MS/MS Separation of Δ⁸ and Δ⁹ -THC Metabolites and Other Cannabinoids Extracted from Urine Samples using a Polymeric SPE Column

UCT Part Numbers

Introduction:

> Download Application Note

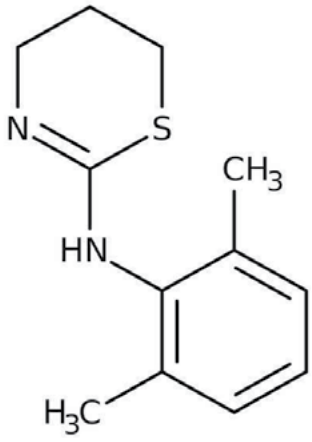
> Review Poster

> Learn more about SelectraCore® PFPP Column

> Learn more about Styre Screen® HLB

Return To Navigation

Analysis of Xylazine, Opioids, and Other Common Adulterants in Blood and Urine by SPE and LC-MS/MS



UCT Part Numbers

CSDAU206
Clean Screen® DAU
200 mg, 6 mL

SCS27-DA1021
SelectraCore® DA Column
100 x 2.1 mm, 2.7 µm

SCS27-DAGDC21
SelectraCore®
DA Guard Column
5 x 2.1 mm, 2.7 µm

SLGRDHLDR-HPOPT
Selectra®
Direct Connect
Guard Holder

SPHPHO6001-10
Select pH Buffer Pouch
100 mM Phosphate Buffer
pH 6.0

Introduction:

Xylazine is a veterinary sedative that has been emerging as a popular adulterant in the illicit drug market. It is most commonly seen with powders and tablets containing fentanyl.[1,2] Drug powders or tablets can easily be "cut" with other substances. Some of these substances have pharmacological activity of their own, adding to the effects intended by the main drug. Substances added to drug samples that are pharmacologically active are considered to be adulterants. It is becoming increasingly popular for fentanyl samples to be adulterated with xylazine. This drug combination is commonly called "Tranq".[1]

Although xylazine is not an opioid, its use with fentanyl is having a significant impact on the opioid epidemic for a number of reasons. It can induce a state of unconsciousness, worsen addiction and potentially increase the risk of fatal overdose. Unfortunately, its effects are not counteracted by naloxone.[1,2] Of all of the fentanyl-positive samples tested by the DEA, 23% of powder samples and 7% of tablet samples also contained xylazine. The trends of xylazine usage parallel to fentanyl, indicating that it is likely to persist.[3] This application note details a robust and effective method for the simultaneous analysis of fentanyl, fentanyl analogs, xylazine, and other common adulterants by SPE and LC-MS/MS.

FORENSICS CLINICAL

UCT

Sample Pretreatment:

Urine: In a test tube add 1 mL urine sample + 500 µL MeOH (optional) + 2.5 mL of 100 mM phosphate buffer pH 6.0 + ISTDs

Note: Include a hydrolysis procedure to recover conjugated analytes

Blood: In a test tube add 0.5 mL whole blood sample + 3 mL of 100 mM phosphate buffer pH 6.0 + ISTDs

SPE Procedure:

1. Condition Column

a) 1 x 3 mL MeOH
b) 1 x 3 mL DI H_2O
c) 1 x 3 mL 100 mM phosphate buffer pH 6

2. Load Sample

a) Load at 1 to 2 mL/minute

3. Wash Column

a) 1 x 3 mL 100 mM HCl in DI H_2O
b) 1 x 3 mL MeOH

4. Dry Column

a) Dry for at least 10 minutes under full pressure or vacuum

5. Elute

a) 1 x 3 mL MeOH:NH_4OH (98:2) or DCM:IPA:NH_4OH (78:20:2)

Note: Make elution solvent fresh daily

6. Evaporate

a) Evaporate eluate at 40°C, starting at 5 psi and increasing the pressure slowly over 30 minutes

7. Reconstitute

a) 1 mL MeOH:H_2O (5:95) or other appropriate solvent and volume

FORENSICS CLINICAL

UCT

LC-MS/MS Parameters	
LC-MS/MS	Shimadzu Nexera LC-30AD with MS-8050
UHPLC Column	SelectraCore® DA Column 100 x 2.1 mm, 2.7 µm (PN: **SCS27-DA1021**)
Guard Column	SelectraCore® DA Guard Column 5 x 2.1 mm, 2.7 µm (PN: **SCS27-DAGDC21**)
Column Temperature	40°C
Flow Rate	0.4 mL/min
Injection Volume	5 µL
Mobile Phase A	0.1% formic acid in water
Mobile Phase B	Methanol

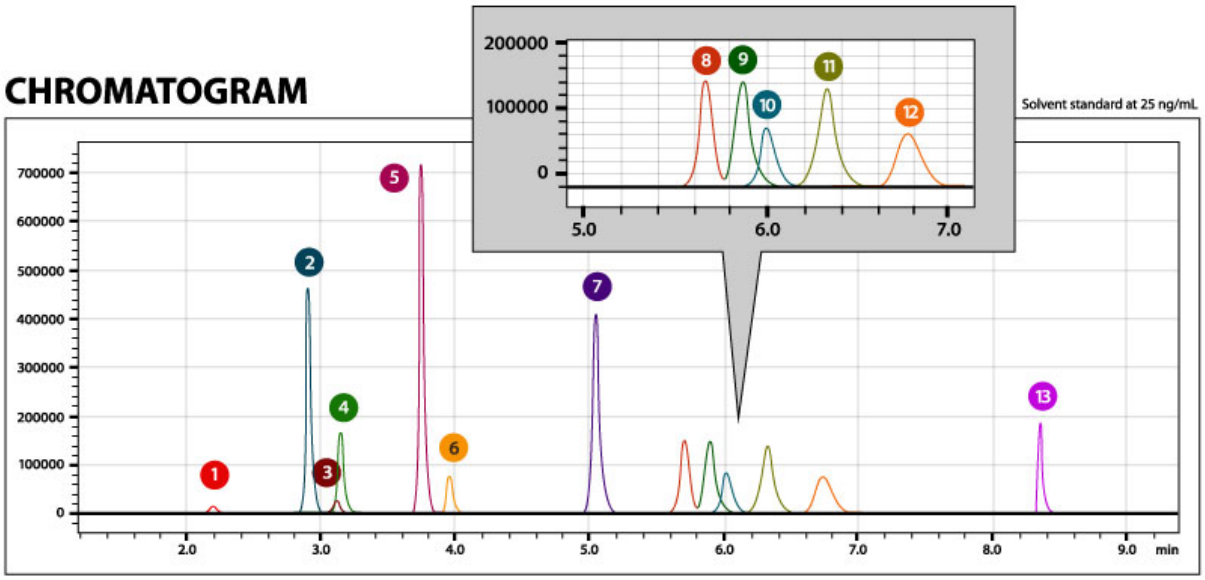
Gradient Program		
Time (min)	**Mobile Phase A (%)**	**Mobile Phase B (%)**
0	95	5
3-7	55	45
8-9	0	100
9-12	95	5

MRM Table						
Analyte	**RT (min)**	**Parent Ion (m/z)**	**Product Ion 1 (m/z)**	**CE (V)**	**Product Ion 2 (m/z)**	**CE (V)**
Morphine	2.17	285.6	165.1	41	152.1	55
Procaine	2.88	236.6	120.0	25	100.2	16
6-MAM	3.12	327.9	165.2	40	211.1	28
Lidocaine	3.13	234.7	86.2	15	58.1	34
Norfentanyl	3.75	233.0	84.1	19	55.1	35
Xylazine	3.94	220.9	89.9	23	164.0	27
4-ANPP	5.02	280.8	188.1	18	105.0	32
para-Fluorofentanyl	5.71	355.0	188.2	25	105.1	40
meta-Fluorofentanyl	5.90	355.0	188.2	24	105.1	39
Fentanyl	6.02	337.3	188.2	20	105.1	37
ortho-Fluorofentanyl	6.31	355.0	188.2	25	105.1	39
Quetiapine	6.75	384.0	253.1	24	221.1	39
Acepromazine	8.35	326.8	86.1	21	58.1	40

FORENSICS CLINICAL

UCT

Chromatogram:



1 Morphine	5 Norfentanyl	9 meta-Fluorofentanyl	13 Acepromazine
2 Procaine	6 Xylazine	10 Fentanyl	
3 6-MAM	7 4-ANPP	11 ortho-Fluorofentanyl	
4 Lidocaine	8 para-Fluorofentanyl	12 Quetiapine	

Figure 1: Chromatogram of a solvent standard mix prepared at 25 ng/mL.

Calibration Curve Examples:

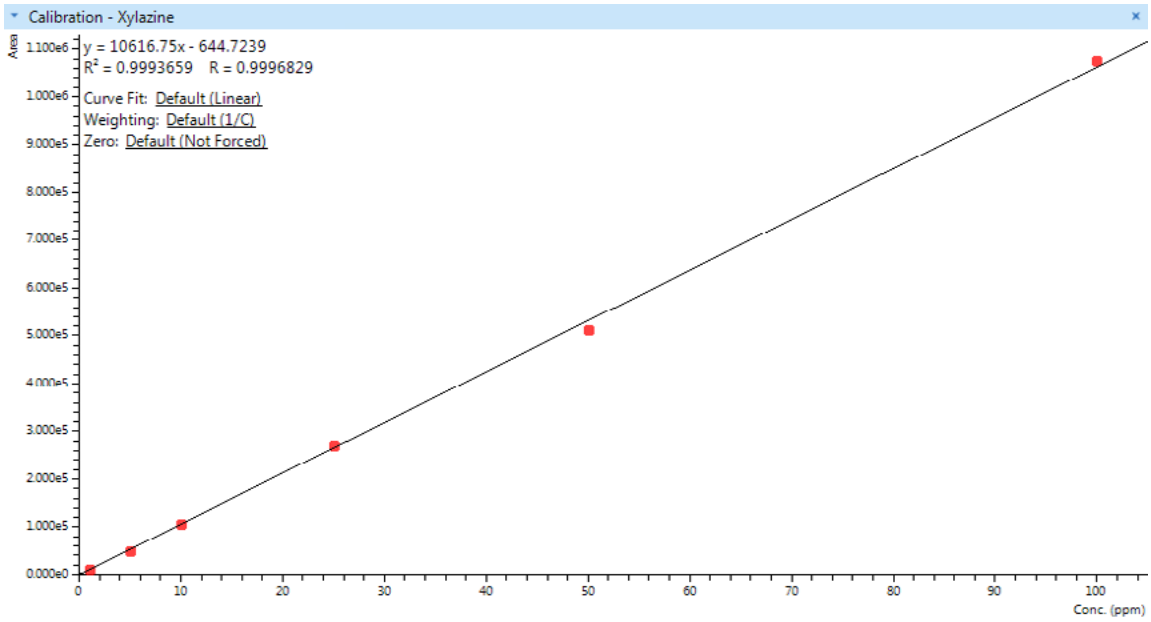


Figure 2a: Example of a 6-point solvent calibration curve for Xylazine with linear equation and R^2 value (1, 5, 10, 25, 50 and 100 ng/mL)

FORENSICS CLINICAL

UCT

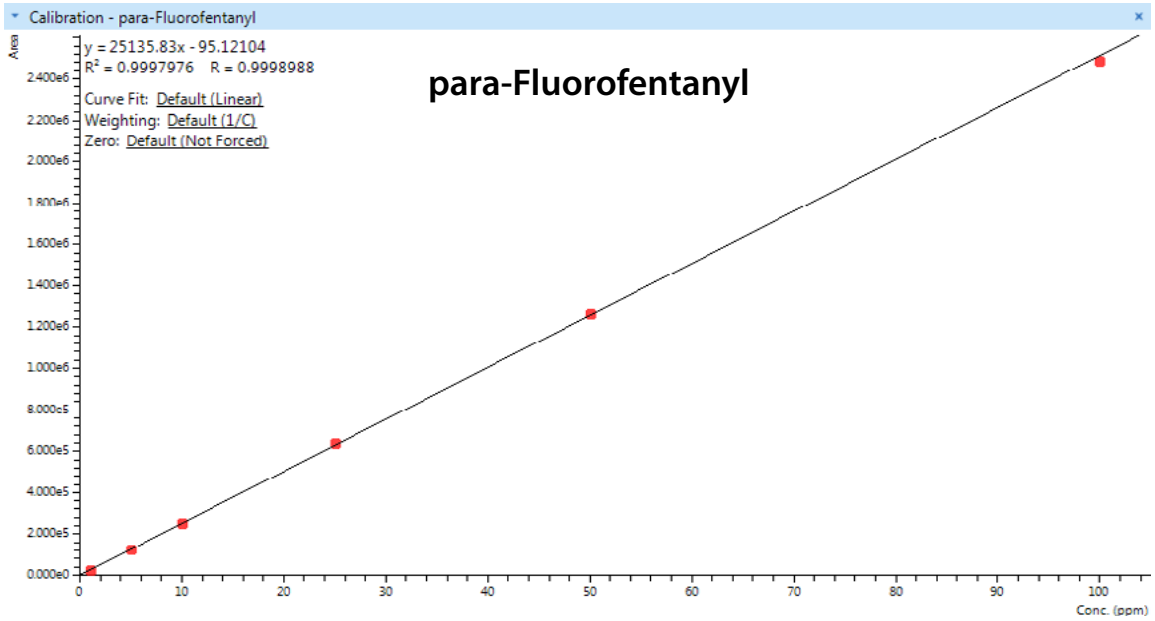


Figure 2b: Example of a 6-point solvent calibration curve for para-Fluorofentanyl with linear equation and R^2 value (1, 5, 10, 25, 50 and 100 ng/mL)

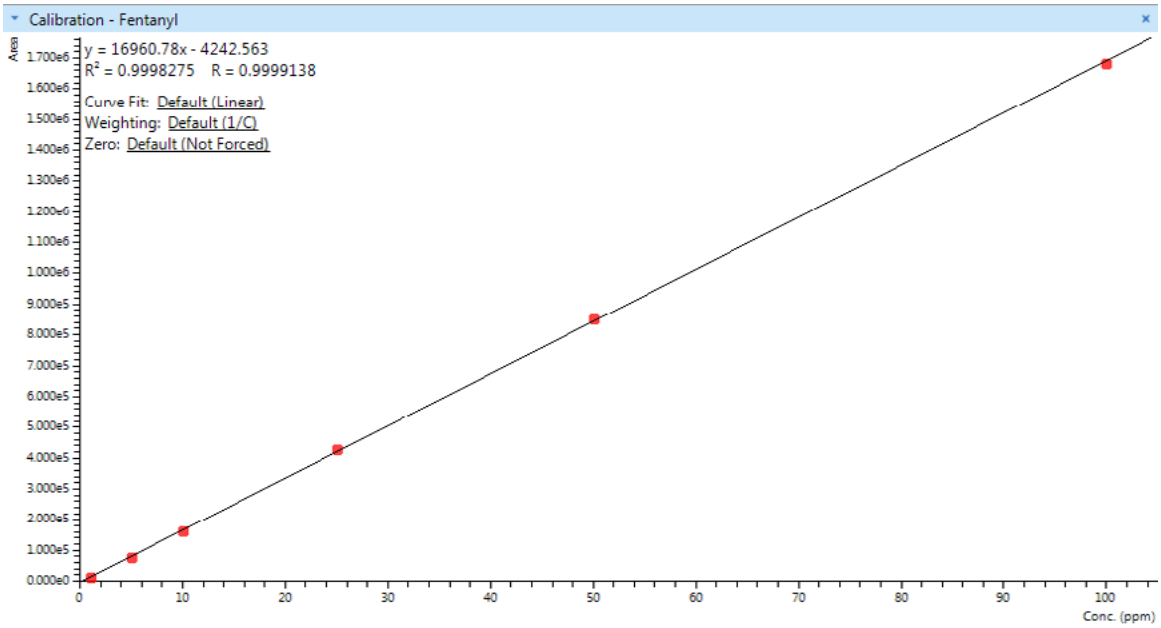


Figure 2c: Example of a 6-point solvent calibration curve for Fentanyl with linear equation and R^2 value (1, 5, 10, 25, 50 and 100 ng/mL)

Results:

Urine									
n=3	5 ng/mL			25 ng/mL			80 ng/mL		
Analyte	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD
Morphine	119%	-15%	8%	90%	-24%	13%	100%	-25%	2%
Procaine	107%	1%	9%	96%	0%	8%	88%	-4%	4%
6-MAM	96%	-20%	17%	96%	-22%	2%	92%	12%	6%
Lidocaine	116%	12%	10%	96%	17%	5%	93%	-9%	5%
Norfentanyl	94%	-23%	19%	90%	-6%	2%	91%	-25%	10%
Xylazine	91%	-17%	2%	109%	-13%	5%	96%	-25%	13%
4-ANPP	89%	-24%	18%	105%	-17%	5%	91%	-5%	17%
Para-Fluorofentanyl	117%	-12%	16%	102%	-17%	7%	91%	-21%	20%
Fentanyl	114%	-5%	14%	104%	-12%	6%	91%	-21%	18%
Quetiapine	98%	-18%	4%	104%	11%	4%	95%	-25%	13%
Acepromazine	114%	-28%	2%	114%	-42%	4%	102%	-44%	12%

**Recoveries were calculated by comparing pre vs. post spiked samples. Matrix effects were calculated by comparing post spiked samples to solvent calibrators.*

Blood									
n=3	5 ng/mL			25 ng/mL			80 ng/mL		
Analyte	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD	Recovery	Matrix Effects	RSD
Morphine	113%	-5%	20%	94%	14%	1%	93%	5%	2%
Procaine	103%	-8%	19%	96%	-16%	2%	104%	-6%	5%
6-MAM	110%	-6%	1%	86%	-18%	8%	86%	6%	2%
Lidocaine	113%	-10%	20%	94%	-4%	2%	93%	-5%	3%
Norfentanyl	101%	-8%	18%	85%	-7%	6%	94%	-22%	8%
Xylazine	101%	-9%	18%	83%	-6%	12%	97%	-21%	11%
4-ANPP	92%	-25%	16%	84%	7%	7%	92%	-25%	6%
Para-Fluorofentanyl	92%	-20%	19%	87%	-16%	8%	91%	-24%	7%
Fentanyl	102%	-23%	20%	84%	-20%	9%	89%	-22%	7%
Quetiapine	109%	-7%	18%	86%	-9%	6%	93%	-11%	5%
Acepromazine	94%	-40%	5%	71%	-48%	12%	91%	-51%	15%

**Recoveries were calculated by comparing pre vs. post spiked samples. Matrix effects were calculated by comparing post spiked samples to solvent calibrators.*

Conclusion/Discussion:

A full SPE and LC-MS/MS method was developed and optimized to achieve the highest recoveries of the analytes with the lowest matrix effects. Like most drugs of abuse, xylazine is slightly basic and ionizable at a pH less than 7, making this veterinary drug easy to integrate into an existing fentanyl or opioid panel that is potentially already in use at drug testing laboratories. It also makes this panel of drugs an excellent candidate for UCT's flagship mixed-mode Clean Screen® DAU, which combines cation exchange and reverse phase functionalities.

This method yields high recoveries ranging from 80 to 119% at low, medium, and high concentrations in both blood and urine. Aside from acepromazine, which was not the focus of this panel, relative standard deviations (RSDs) were ≤20% and matrix effects were within ±25%. The LC-MS/MS method features the separation of the three fluorofentanyl isomers, para-, meta-, and ortho-fluorofentanyl, to ensure that misidentifications will not be made. This application note provides a procedure that can be readily implemented by drug testing laboratories to simultaneously monitor fentanyl, fentanyl analogs, xylazine, and other common adulterants.

References:

[1] Congressional Research Service (2023, February 2) Xylazine: Considerations for Federal Control. CRS Insight. https://www.everycrsreport.com/

[2] Holt, Andrew C, et. al. (2023, January 6) Widespread Distribution of Xylazine Detected Throughout the United States in Healthcare Patient Samples. Journal of Addiction Medicine. https://journals.lww.com/journaladdictionmedicine.

[3] DEA Joint Intelligence Report. (2022 October) The Growing Threat of Xylazine and its Mixture with Illicit Drugs. US DOJ DEA. https://www.dea.gov/.

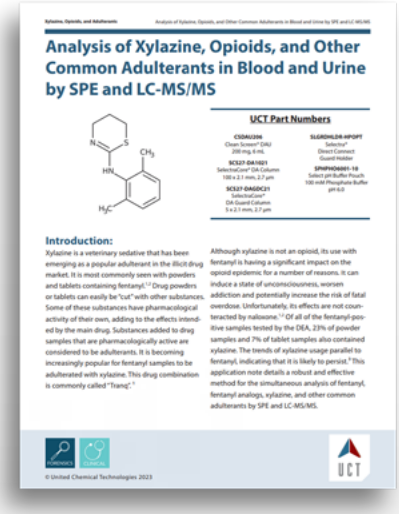
UCT, LLC • 2731 Bartram Road • Bristol, PA 19007 800.385.3153 • 215.781.9255
www.unitedchem.com Email: methods@unitedchem.com

FORENSICS CLINICAL

UCT

FORENSIC
SAMPLE ANALYSIS

Analysis of Xylazine, Opioids, and Other Common Adulterants in Blood and Urine by SPE and LC-MS/MS


Analysis of Xylazine, Opioids, and Other Common Adulterants in Blood and Urine by SPE and LC-MS/MS

UCT Part Numbers

Introduction:

> Download Application Note

> Learn more about SelectraCore®

> Learn more about Clean Screen® DAU

Return To Navigation