UCT

FORENSIC
SAMPLE ANALYSIS

unitedchem.com

FORENSICS

FORENSIC
SAMPLE ANALYSIS

	Solid Phase Extraction of Novel Synthetic 2-Benzylbenzimidazole Opioid Compounds “Nitazenes”
	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 and SelectraCore® C18 Column on LC-MS/MS
	Analysis of Natural Cannabinoids and Metabolites from Urine Using Styre Screen® HLB and SelectraCore® C18 Column on LC-MS/MS
	Simultaneous Analysis of 19 Novel Synthetic Cannabinoids in Urine Using SPE and LC-MS/MS
	Analysis of 26 Natural and Synthetic Opioids in Blood and Urine Using Clean Screen® DAU SPE and a Selectra® DA UHPLC Column
	Amphetamines in Blood, Plasma/Serum, Urine, or Tissue using Clean Screen® DAU SPE and LC-MS/MS analysis

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

UCT Part Numbers

CSDAU133
Clean Screen® DAU
3 mL, 130 mg

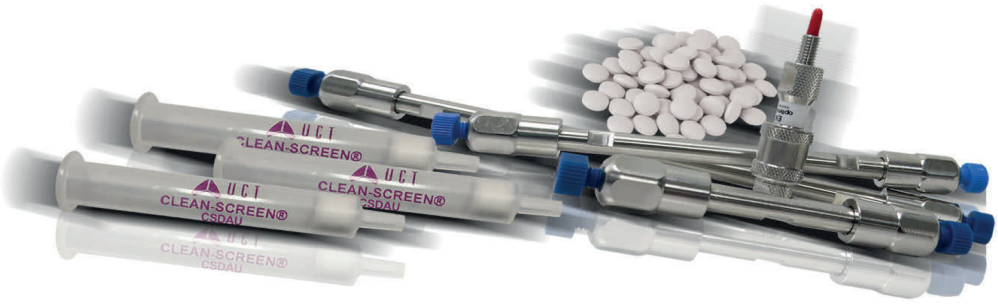
SPHPO7001-10
Select pH buffer pouch 100mM
Phosphate buffer pH 7.0

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

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

SLGRDHLDR-HPOPT
Selectra® Direct Connect Guard Holder

CLINICAL FORENSICS



Summary:

A new class of synthetic opioids is emerging called benzylbenzimidazole-opioids also known as "nitazenes". Nitazene compounds were first synthesized in the 1950s as a potential analgesic.[1] These compounds were never approved for clinical use, but they are re-emerging as a new threat in the ongoing opioid epidemic. These potent synthetic opioids range from three to twenty times more potent than fentanyl.[2] Isotonitazene was the first nitazene analog detected in biological samples in the United States by the CFRSE in July 2019.[3] The number of cases across the country continues to rise. In total eight nitazenes have been temporarily added to schedule I by the Drug Enforcement Administration (DEA).[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.

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:**

   1 x 3 mL methanol (MeOH)

   1 x 3 mL phosphate buffer pH 7

2. **Load Sample:**

   Load at 1 to 2 mL/minute

3. **Wash Column:**

   1 x 3 mL deionized water

   1 x 3 mL 50:50 MeOH:H_2O

4. **Dry Column:**

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

5. **Elute:**

   1 x 3 mL of MeOH:NH_4OH (98:2)

   Note: prepare elution solvent daily

6. **Evaporate:**

   Add 250 µL of 10% HCl in methanol and vortex

   Evaporate eluate at 10 psi, 35°C

7. **Reconstitute:**

   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, samples can be evaporated at 30°C, 5 psi

CLINICAL

FORENSICS

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-C18021)
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
Mobile Phase A: 0.1% formic acid in water
Mobile Phase B: 0.1% formic acid in methanol

Gradient Program:

Time (min)	Mobile Phase A (%)	Mobile Phase B (%)
0	90	10
2.5-3.5	57	43
7	30	70
8-11	0	100
11.3-15	90	10

MRM Table:

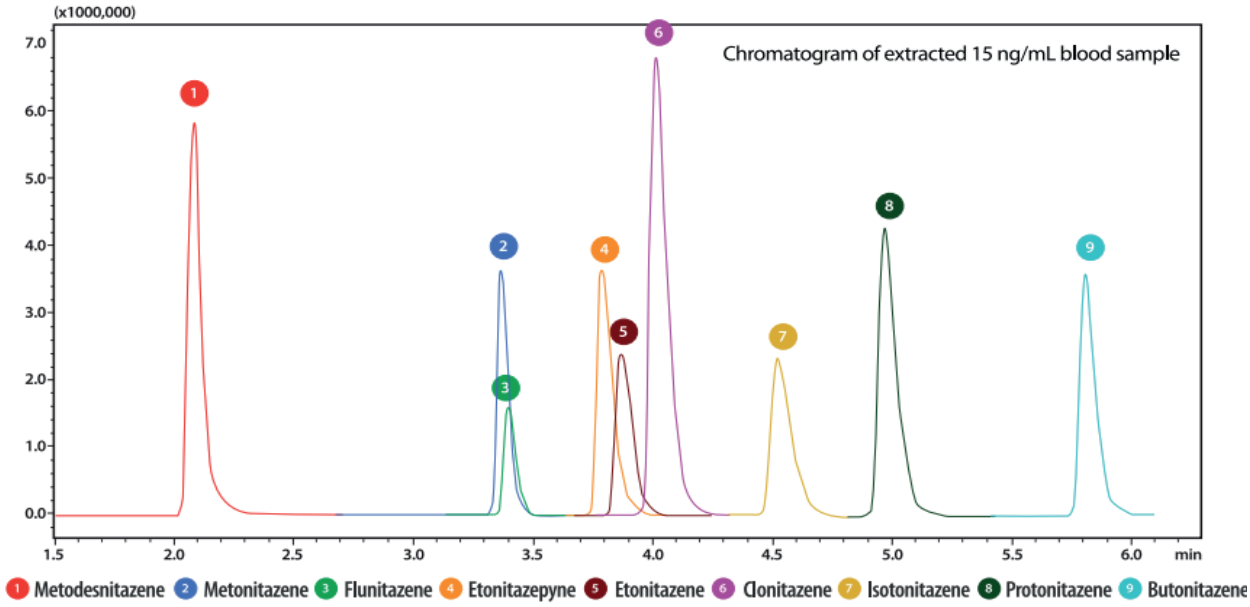
Analyte	Parent Ion (m/z)	Product Ion 1 (m/z)	CE (eV)	Product Ion 2 (m/z)	CE (eV)	RT (min)
Butonitazene	425.5	100.1	-23	72.1	-45	5.83
Clonitazene	386.5	100.1	-26	125.1	-36	4.03
Etonitazene	397.4	100.1	-21	72.0	-36	3.88
Etonitazepyne	395.6	98.1	-23	56.1	-55	3.80
Flunitazene	371.3	100.1	-23	73.1	-26	3.41
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

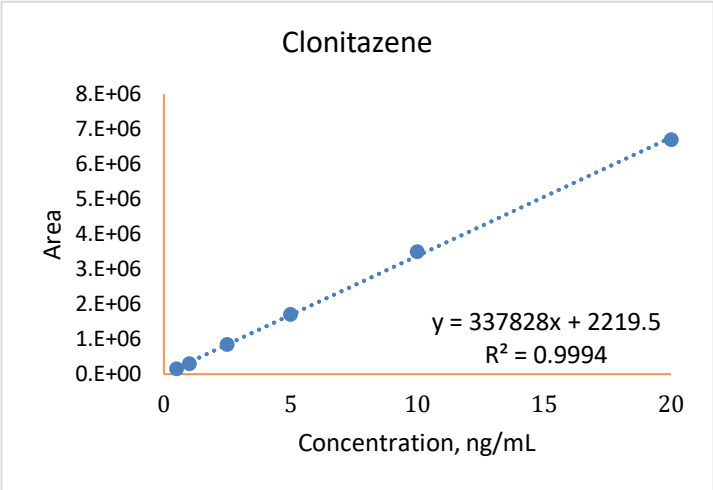
CLINICAL

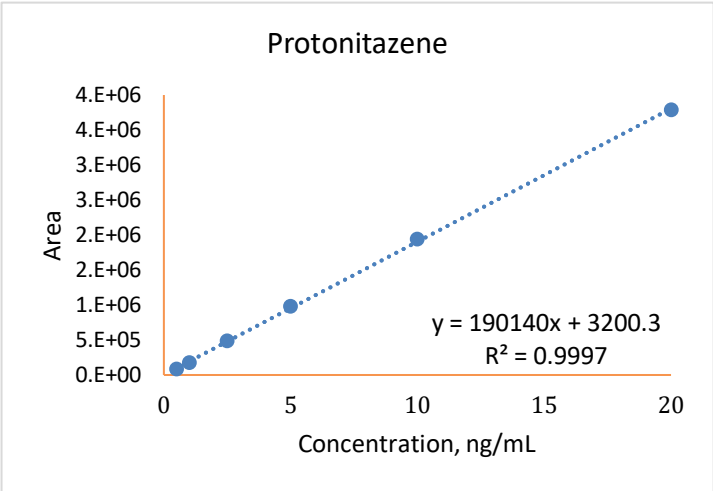
FORENSICS

Chromatogram:



Example Solvent Calibration Curves:





CLINICAL

FORENSICS

Results:

Urine (n=5)

Analyte	1 ng/mL			5 ng/mL			15 ng/mL		
	% Recovery	% RSD	Matrix Effect	% Recovery	% RSD	Matrix Effect	% Recovery	% RSD	Matrix Effect
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)

Analyte	1 ng/mL			5 ng/mL			15 ng/mL		
	% Recovery	% RSD	Matrix Effect	% Recovery	% RSD	Matrix Effect	% Recovery	% RSD	Matrix Effect
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.

CLINICAL

FORENSICS

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 that were fully dried and reconstituted. Formulas for recovery and matrix effect are shown below:

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

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

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%.

CLINICAL

FORENSICS

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 *N*-pyrrolidino 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)

CLINICAL

FORENSICS

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

UCT

FORENSIC
SAMPLE ANALYSIS

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

- Download Application Note
- Review Poster
- Learn more about SelectraCore®
- Learn more about Clean Screen® DAU

Return to Navigation

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

UCT Part Numbers

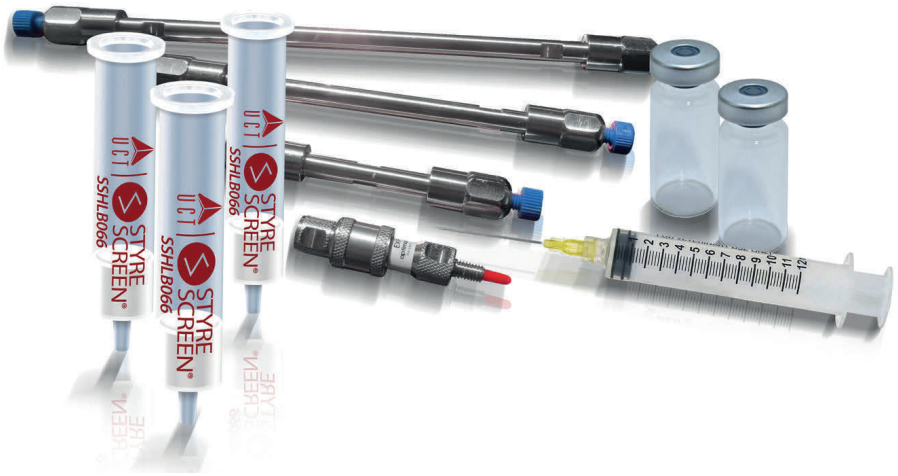
SSHLB066
Styre Screen® HLB
6 mL, 60 mg sorbent

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

CLINICAL FORENSICS



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 only. 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 poster 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.

SPE Procedure:

1) **Sample Preparation**

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

2) **Condition**

1 x 3 mL methanol
1 x 3 mL DI water

3) **Load sample**

Load the samples at 1 to 2 mL/min

4) **Wash**

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

5) **Dry**

Dry columns for 10 mins at full vacuum or pressure

6) **Elute**

1 x 3 mL 50:50 methanol:acetonitrile

7) **Evaporate and Reconstitute**

Evaporate to full dryness at 10 psi, 40°C
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**).

CLINICAL

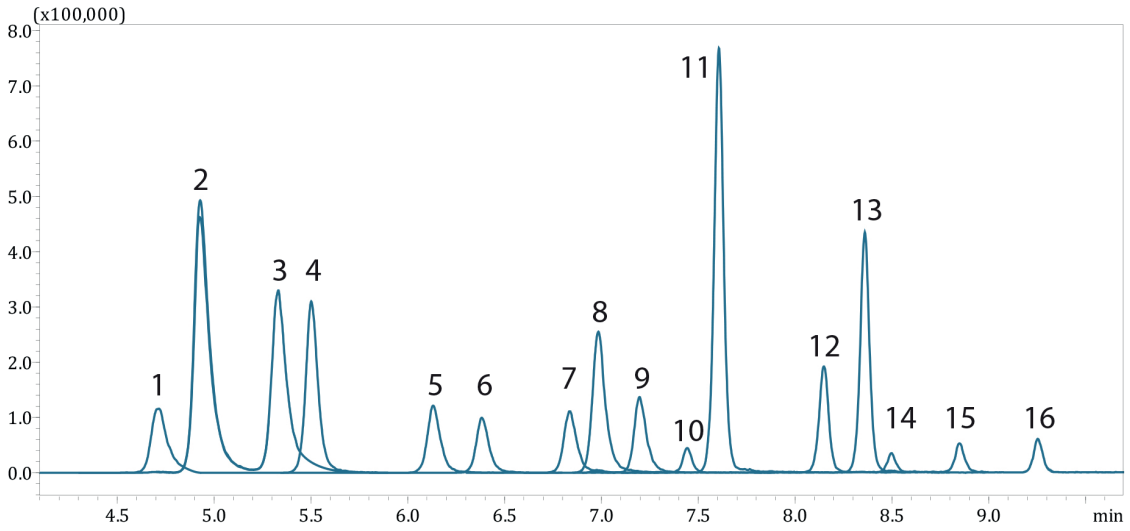
FORENSICS

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. 17b-estradiol-17-sulphate
2. 17b-estradiol-3-sulphate
3. 17a-estradiol Sulphate
4. Boldenone Sulphate
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

CLINICAL

FORENSICS

MRM Table:

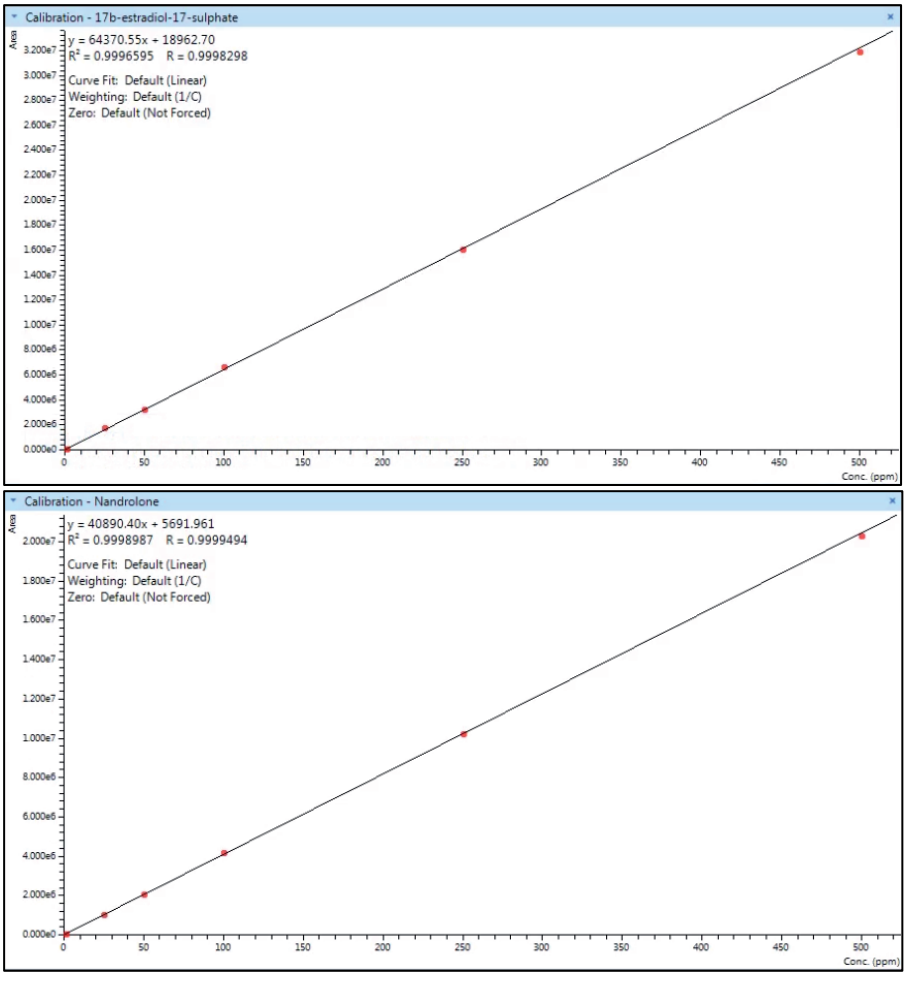
Analyte	Parent ion	Product ion 1	CE	Product ion 2	CE	RT (mins)
17β-estradiol-17-sulfate	351.1	97.0	39	80.0	55	4.70
17β-estradiol-3-sulfate	351.1	271.2	35	145.1	55	4.92
17α-estradiol Sulfate	351.1	271.2	35	145.1	54	5.33
Boldenone Sulfate	365.1	350.2	30	96.9	49	5.50
Nandrolone Sulfate	353.1	97.0	40	79.9	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 2: Calibration Curve Examples (1, 25, 50, 100, 250 & 500 ng/mL)



CLINICAL

FORENSICS

Results:

Urine Extraction									
Analyte	5 ng/mL (n=5)			50 ng/mL (n=5)			250 ng/mL (n=5)		
	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									
Analyte	5 ng/mL (n=5)			50 ng/mL (n=5)			250 ng/mL (n=5)		
	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).

CLINICAL

FORENSICS

Results:

Blood Extraction									
Analyte	5 ng/mL (n=5)			50 ng/mL (n=5)			250 ng/mL (n=5)		
	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).

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

CLINICAL

FORENSICS

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

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.

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.

CLINICAL

FORENSICS

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

UCT

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 Styre Screen® HLB

Return to Navigation

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

UCT Part Numbers

CSTHC206
Clean Screen® THC
6mL, 200mg sorbent

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

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

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

SLGRDHLDR-HPOPT
UHPLC Direct Connect
Guard Holder

CLINICAL FORENSICS

Introduction:

Marijuana is parts or products derived from the *Cannabis* plant with Δ^9-THC content greater than 0.3%. The *Cannabis* plant contains several compounds called cannabinoids, but the most desired is Δ^9-Tetrahydrocannabinol (Δ^9-THC). The resulting effects of this drug includes alteration of perception of time and space, euphoria, and increased appetite.[1] As more states legalize marijuana for recreational and medical use, each has written their own marijuana drug-impaired driving laws. For this reason, it is important for forensic laboratories to have accurate and precise testing protocols.

Protein precipitation is a commonly used method to help remove matrix interferences from blood. The proteins in blood can be precipitated by either changing the pH or the hydrophobicity of the aqueous environment. Common reagents for protein precipitation include acids, organic solvents, salts, and metals.

This application note outlines a protein precipitation sample preparation followed by a solid phase extraction (SPE) method for four natural cannabinoids and the two major metabolites of Δ^9-THC from blood. Analytes were extracted from blood using the Clean Screen® THC SPE column. 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.

Sample Pretreatment:

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

Extraction Procedure:

1. **Condition Column**
    - 1 x 2mL of MeOH
    - 1 x 2mL of pH 7 phosphate buffer
2. **Load Sample**
    - Load at 1 to 2 mL/minute
3. **Wash Column**
    - 2 x 3mL deionized water
    - 2 x 3mL 40% MeOH in deionized water
4. **Dry Column**
    - Dry column for at least 10 minutes under full pressure or vacuum
5. **Elution**
    - 1 x 3mL of ACN:MeOH:Acetic Acid (89:9:2)
6. **Dry Eluate**
    - Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C
7. **Reconstitute**
    - Reconstitute in 1mL of MeOH
    - Alternative compatible solvents and volumes can be used

CLINICAL

FORENSICS

LC-MS/MS Parameters:

LC-MS/MS System: Shimadzu Nexara LC-30AD w/MS-8050
UHPLC Column: SelectraCore® C18 HPLC Column 100 x 2.1 mm, 2.7 µm
Guard Column: SelectraCore® C18 5 x 2.1 mm, 2.7 µm
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	Product ion 1	CE	Product ion 2	CE	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

CLINICAL

FORENSICS

Chromatogram:

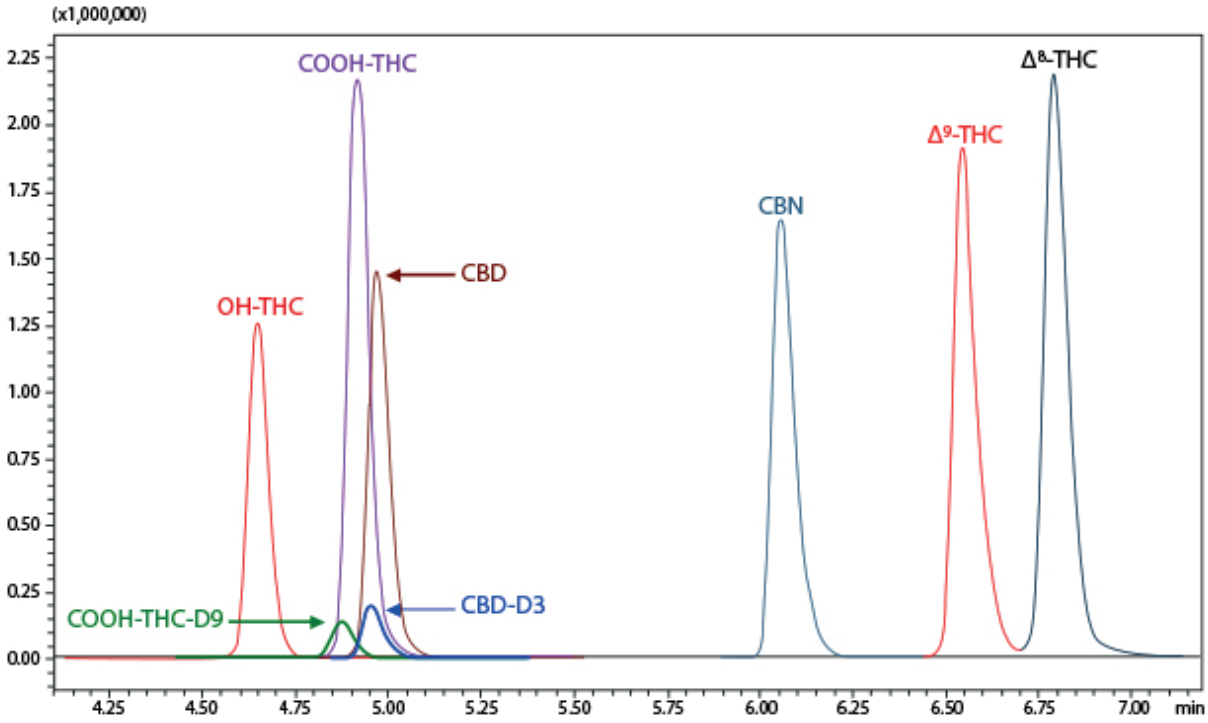


Figure 1. Chromatogram of extracted 50 ng/mL blood sample

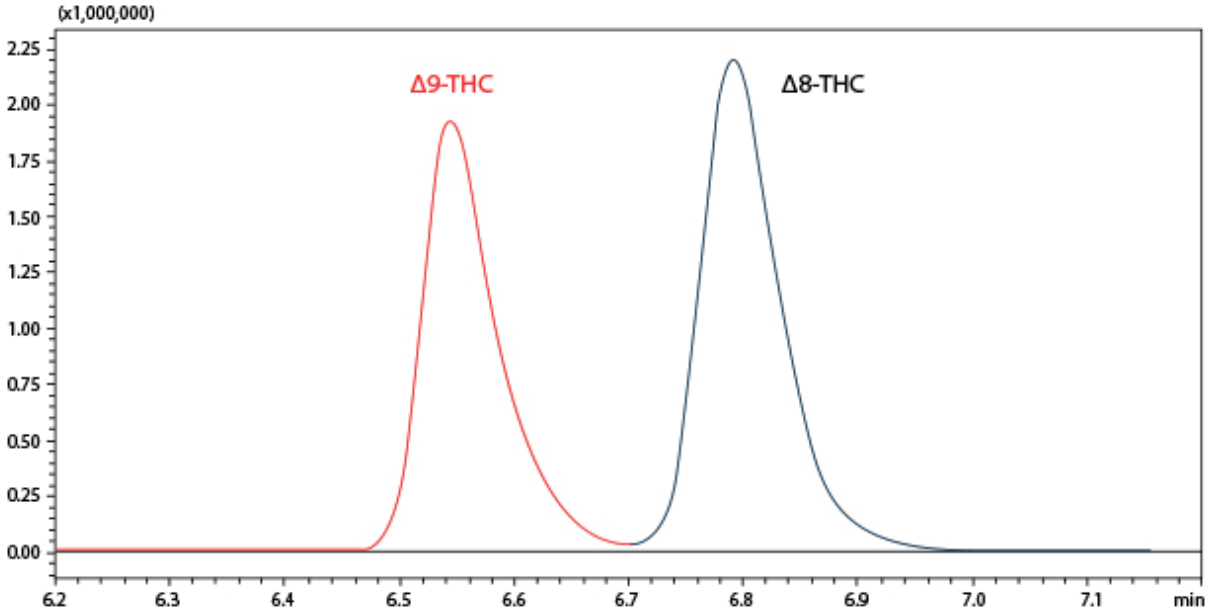


Figure 2. Zoomed in chromatogram of 50 ng/mL extracted blood sample showing separation of THC isomers

CLINICAL

FORENSICS

Calibration Curves:

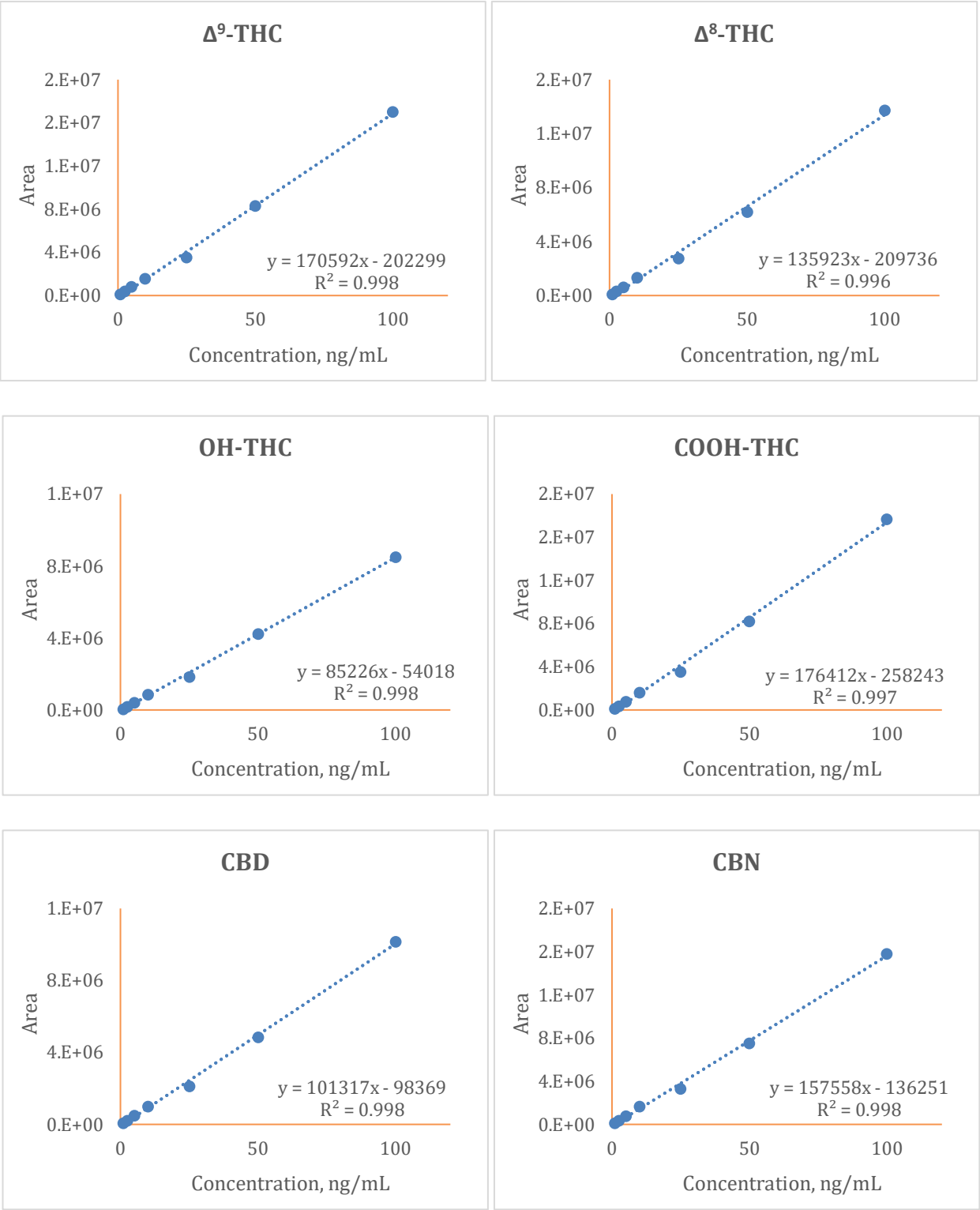


Figure 3. 7-point solvent calibration curve for all analytes with linear equation and R^2 value. [1, 2.5, 5, 10, 25, 50, 100 ng/mL]

CLINICAL

FORENSICS

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 Effect (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%.

CLINICAL

FORENSICS

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

CLINICAL

FORENSICS

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

UCT

FORENSIC SAMPLE ANALYSIS

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

- 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 and SelectraCore® C18 Column on LC-MS/MS

UCT Part Numbers

SSHLB063
Styre Screen® HLB
3mL, 60mg sorbent

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

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

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

SLGRDHLDR-HPOPT
UHPLC Direct Connect
Guard Holder

Introduction:

Natural cannabinoids are compounds found in the Cannabis plant. More than a hundred cannabinoids have already been identified. The legal definition of marijuana is all parts of the Cannabis plant whether growing or not, have Δ9-tetrahydrocannabinol (Δ9-THC) more than 0.3% dry weight. [1] Known for its psychoactive and euphoric effects, Δ9-THC has the highest levels in the subspecies Cannabis Sativa. [2] Marijuana is one of the most popular drugs in the United States and around the world. In the United States, marijuana is a federally illegal drug. However, in 2012 Colorado became the first state to legalize marijuana for recreational use. Many other states have also legalized it for recreational and medical use. With new state laws, it is important to be able to accurately and precisely quantitate cannabinoids from biological matrices.

This application note outlines a solid phase extraction (SPE) procedure from urine and a 12-minute liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for four natural cannabinoids and the metabolites of Δ9-THC. Included in the panel are isomers Δ9-THC and Δ8-THC, which were successfully separated using the new SelectraCore® C18 core-shell column.

CLINICAL FORENSICS

Sample Pretreatment:

To 1mL of urine add internal standard, 1mL of acetonitrile, and 1mL 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

Extraction Procedure:

1. Condition Column
   - 1 x 2mL of MeOH
   - 1 x 2mL of pH 7 phosphate buffer

2. Load Sample
   - Load at 1 to 2 mL/minute

3. Wash Column
   - 1 x 3mL deionized water
   - 1 x 3mL 50% MeOH in deionized water

4. Dry Column
   - Dry column for at least 10 minutes under full pressure or vacuum

5. Elution
   - 1 x 3mL of 60:40 MeOH: Hexane

   Note: shake or vortex elution solvent well before use

6. Dry Eluate
   - Evaporate eluate under a constant gentle stream of nitrogen ≤ 40°C

7. Reconstitute
   - Reconstitute in 1mL of MeOH
   - Alternative compatible solvents or volumes can be used

CLINICAL

FORENSICS

LC-MS/MS Parameters:

LC-MS/MS System: Shimadzu Nexara LC-30AD w/MS-8050
UHPLC Column: SelectraCore® C18 Column 100 x 2.1 mm, 2.7 µm
Guard Column: SelectraCore® C18 5 x 2.1 mm, 2.7 µm
Column Temperature: 40°C
Flow Rate: 0.4 mL/min
Injection Volume: 10 uL

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	Product ion 1	CE	Product ion 2	CE	RT (min.)
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

CLINICAL

FORENSICS

Chromatogram:

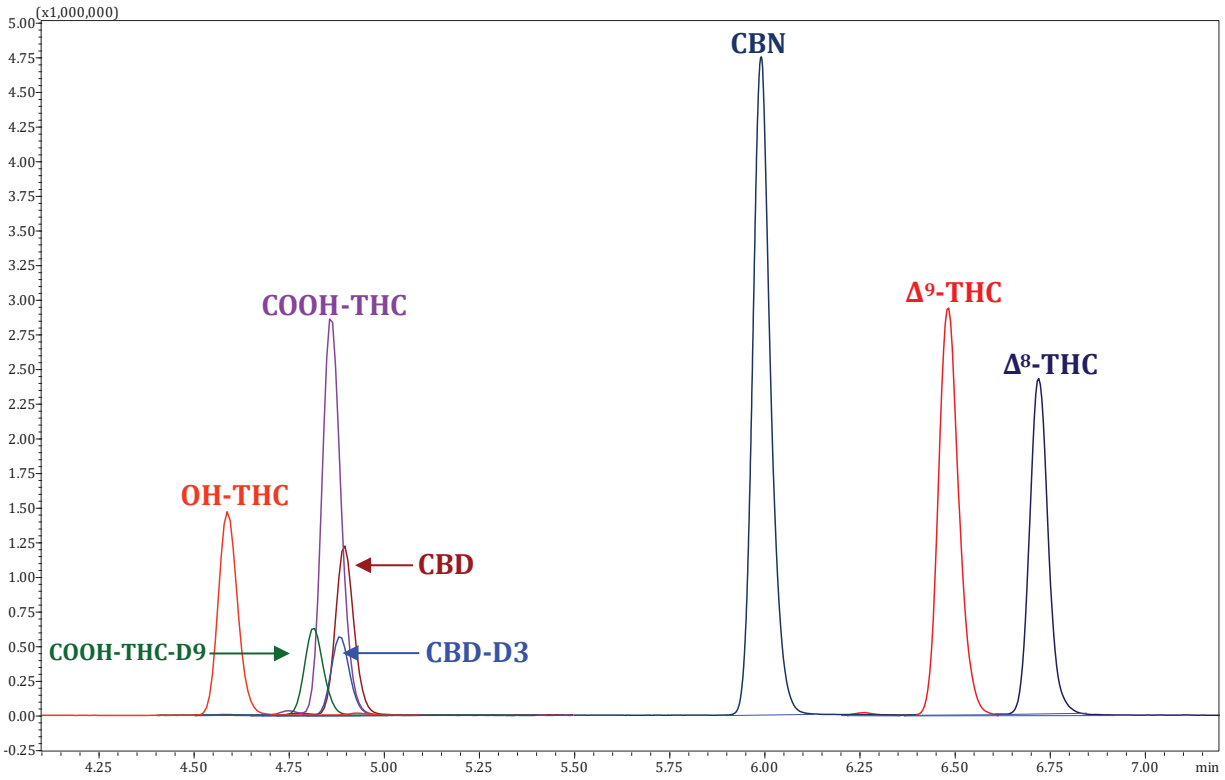


Figure 1. Chromatogram of extracted 50 ng/mL urine sample, minutes 4.10 – 7.20

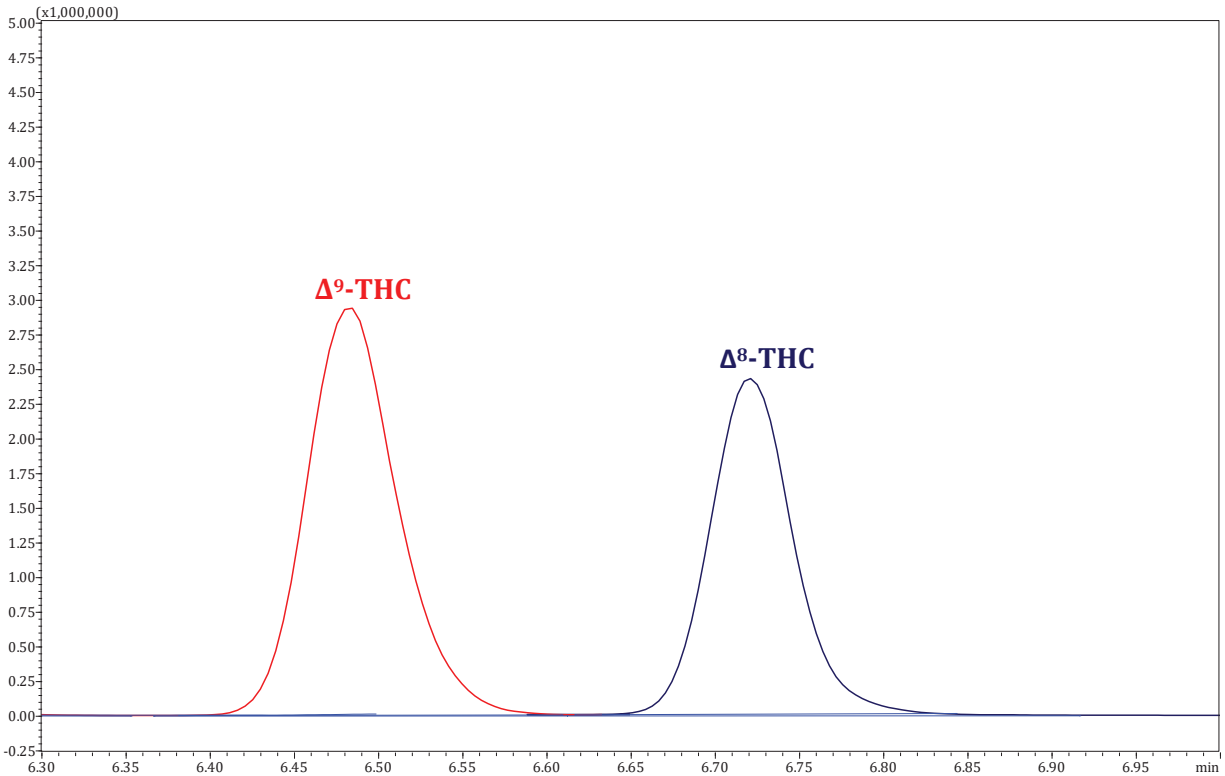


Figure 2. Zoomed in chromatogram of extracted 50 ng/mL urine sample showing the complete separation of the THC isomers, minutes 6.30 – 7.00

CLINICAL

FORENSICS

Calibration Curves:

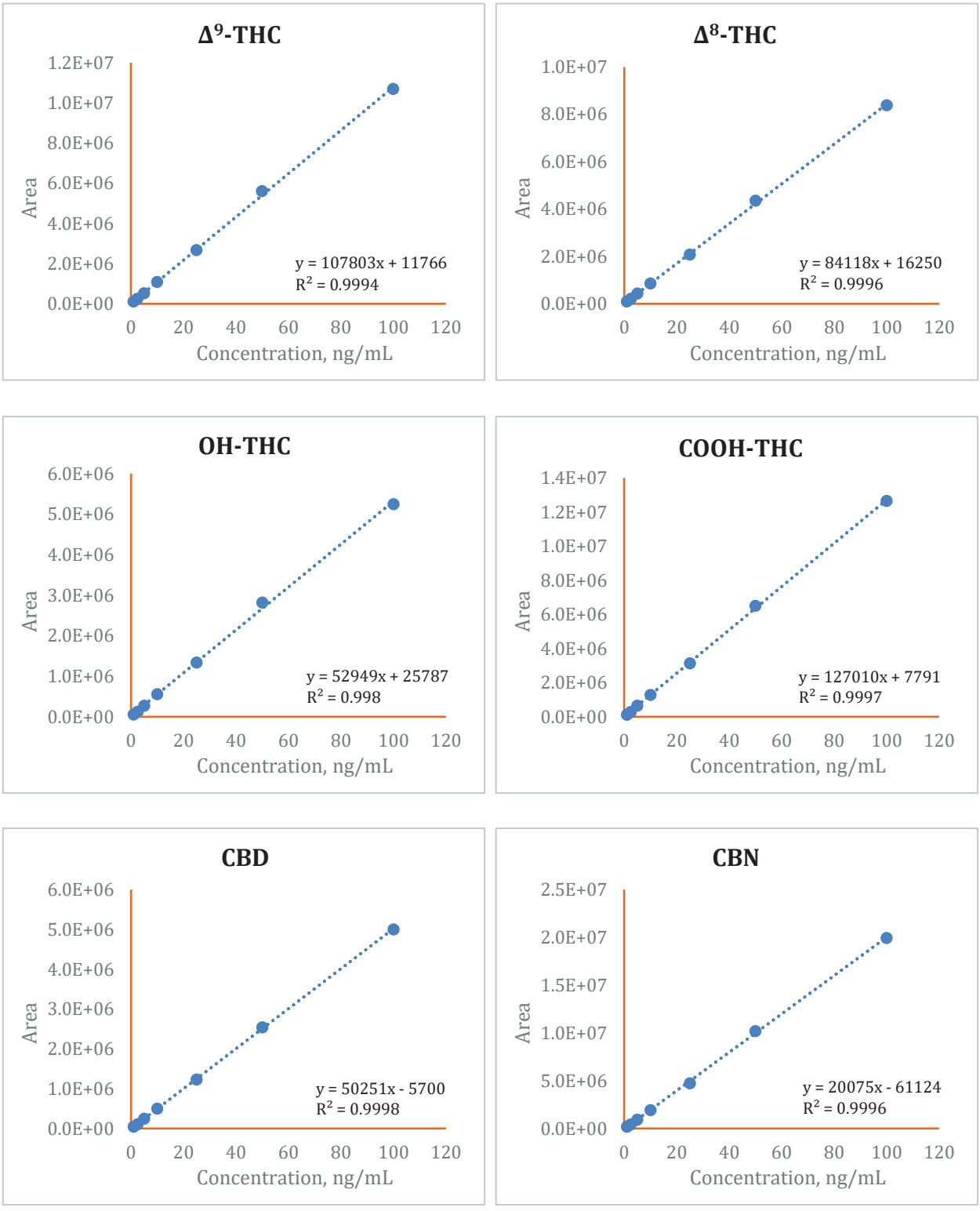


Figure 3. 7-point neat calibration curve for all analytes with linear equation and R^2 value. [1, 2.5, 5, 10, 25, 50, 100 ng/mL]

CLINICAL

FORENSICS

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

CLINICAL

FORENSICS

Conclusions:

A LC-MS/MS and SPE extraction method was developed for the analysis of four natural cannabinoids and the two major Δ9-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, Δ9-THC and Δ8-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

CLINICAL

FORENSICS

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

UCT

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 and SelectraCore® C18 Column on LC-MS/MS

UCT Part Numbers

Introduction:

- Download Application Note
- Review Poster
- Learn more about SelectraCore®
- Learn more about Styre Screen® HLB

Return to Navigation

Simultaneous Analysis of 19 Novel Synthetic Cannabinoids in Urine Using SPE and LC-MS/MS

UCT Part Numbers

SSHLD063
Styre Screen® HLD
60 mg, 3mL Column

SPHPHO6001-10
Select PH Buffer Pouches 100 mM Phosphate pH 6.0

SLC-18100ID21-18UM
Selectra® C18 HPLC Column
100 X 2.1 mm, 1.8 µm

SLC-18GDC20-18UMOPT
Selectra® C18 Guard Column
10 X 2.0 mm, 1.8 µm

SLGRDHLDR-HPOPT
UHPLC Guard Column Holder

CLINICAL FORENSICS

Summary:

Newly identified synthetic cannabinoids pose a significant threat to public health and safety, as their implications in drug overdose and adverse events continue to rise in the United States and around the world. The diverse chemical structures of these compounds have a great impact on their potency and side effects. These synthetic cannabinoids were previously un-reported in forensic toxicology casework in the United States. There are currently few published methods available for the analysis of these novel compounds. However, the importance of identifying and extracting these compounds from various biological matrices is becoming more critical for accurate forensic criminal investigations and clinical diagnostics.

This application note outlines a solid-phase extraction (SPE) and LC-MS/MS method for the analysis of 19 synthetic cannabinoids in urine. These specific compounds were selected based on positivity rates from several key testing labs in the area. The use of UCT's Styre Screen® HLD highly crosslinked polymeric SPE sorbent ensures efficient extraction of the synthetic cannabinoids while removing undesired matrix components and yielding clean extracts. LC separation was carried out using a Selectra® C18 UHPLC column which resulted in excellent retention and baseline separation of the critical isobaric compounds ADBICA N-pentanoic acid and ADB-PINACA N-pentanoic acid metabolite in under 10 minutes.

SPE Procedure:

1) **Sample Preparation:**
   - To 1 mL of urine add 1 mL of pH 6 phosphate buffer (0.1M) and internal standard(s).
   - Mix/vortex briefly.
     - **Note:** A hydrolysis protocol may be required if conjugated compounds are to be included into the above drug panel.
2) **Condition Cartridge**
   - 1 x 1 mL MeOH
   - 1 x 1 mL DI H_2O
3) **Apply Sample:**
   - Load sample at 1- 2 mL/minute.
4) **Wash Cartridge**
   - 1 x 2 mL DI H_2O
   - 1 x 2 mL ACN: H_2O (20:80, v/v) containing 1% Formic Acid.
   - Dry cartridges under full vacuum or pressure for 5 minutes.
5) **Elute Analytes**
   - 1 x 3 mL Ethyl Acetate
   - Collect at 1-2 mL/minute.
6) **Dry Eluate**
   - Evaporate to dryness at < 40°C.
7) **Reconstitute**
   - Reconstitute sample in 1 mL of mobile phase or other appropriate organic solvent.

LC-MS/MS PARAMETERS

LC-MS/MS PARAMETERS		
System: Shimadzu LCMS-8050		
UHPLC Column: Selectra® C18 (100 X 2.1 mm, 1.8 µm)		
Guard Column: Selectra® C18 (10 X 2.0 mm, 1.8 µm)		
Column Temperature: 50°C		
Flow Rate: 0.4 mL/min		
Injection Volume: 5 µL		
Gradient Program:		
	% Mobile Phase A (0.1% Formic Acid in Water)	**% Mobile Phase B** (0.1% Formic Acid in ACN)
0	70	30
3.5	55	35
9	0	100
11	0	100
11.1	70	30
14	70	30

CLINICAL FORENSICS

Chromatogram:

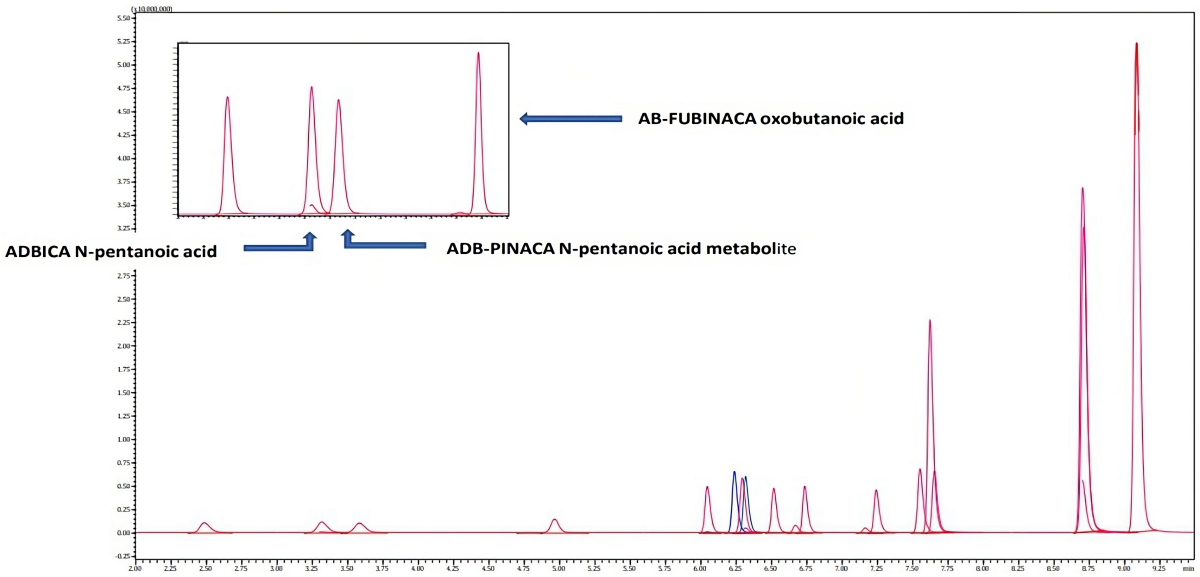


Figure 1: Chromatogram of 25 ng/mL extracted sample demonstrating the isobaric separation of ADBICA N-pentanoic acid and ADB-PINACA N-pentanoic acid metabolite.

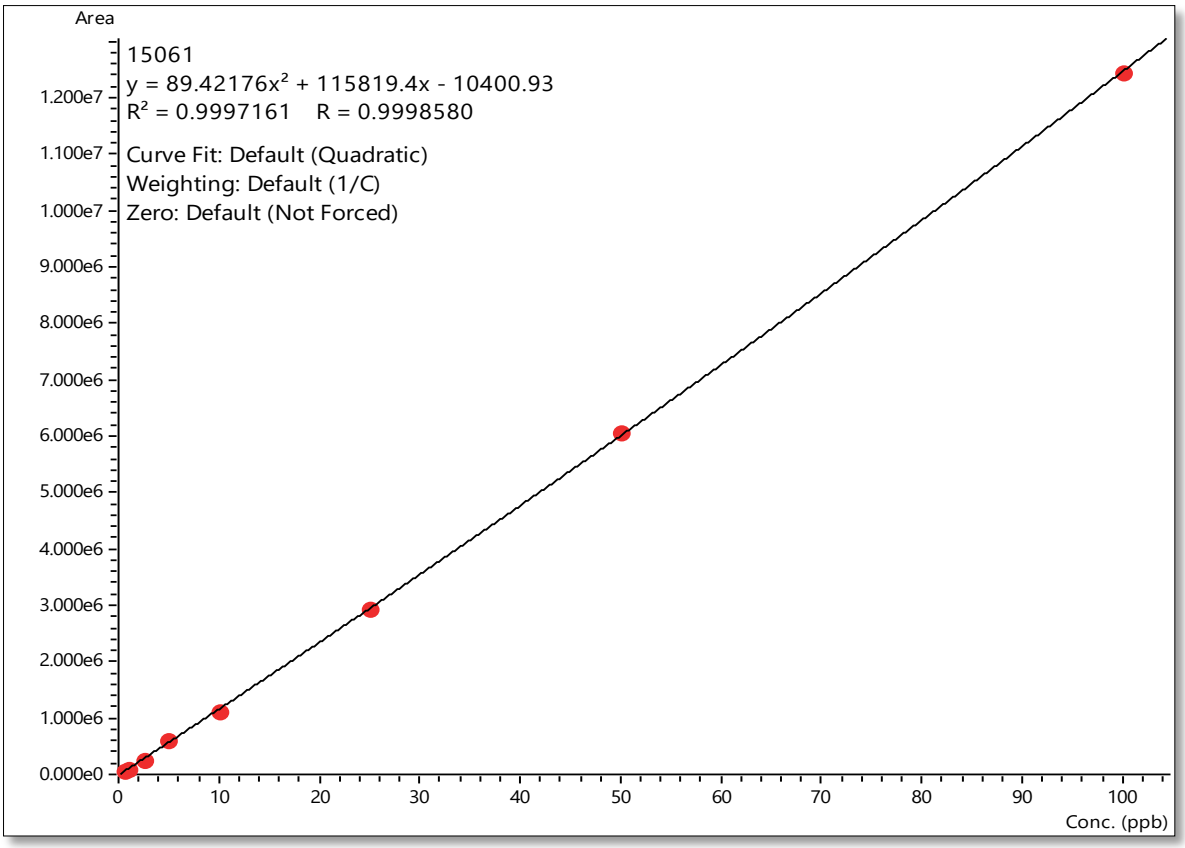
Results:

Analyte	Recovery (%, n=5)			
	2.5 ng/mL	RSD (%)	10 ng/mL	RSD (%)
AB-PINACA N-Pentanoic Acid Metabolite	102%	0.16	110%	0.32
ADBICA N-Pentanoic Acid	115%	0.12	108%	0.71
ADB-PINACA N-Pentanoic Acid Metabolite	87%	0.12	113%	1.04
AB-FUBINACA Oxobutanoic Acid	92%	0.06	97%	0.14
5-Fluoro ADBICA	91%	0.08	106%	0.17
ADB-BICA	88%	0.09	101%	0.19
4-cyano CUMYL-BUTINACA	105%	0.09	112%	0.28
ADB-FUBICA	86%	0.12	106%	0.13
5-Fluoro MDMB-PICA	100%	0.11	109%	0.25
PB-22 3-Carboxyindole Metabolite	97%	0.12	107%	0.18
MDMB-FUBICA	98%	0.09	108%	0.19
BB-22-Carboxyindole Metabolite	103%	0.06	106%	0.18
UR-144 (XLR11) N-Pentanoic Acid Metabolite	95%	0.11	105%	0.20
AKB-48 N-Pentanoic Acid Metabolite	100%	0.09	109%	0.12
MDMB-FUBICA	93%	0.24	95%	0.30
AB-CHMINACA 3-methyl Butanoic Acid	99%	0.11	110%	0.24
BB-22	93%	0.27	103%	0.66
MA-CHMINACA	95%	0.38	97%	0.49
MDMB-CHMINACA	100%	0.43	100%	1.02

CLINICAL

FORENSICS

Representative Calibration Curve (ADBICA N-Pentanoic Acid):



Conclusions:

This application note outlines a simple SPE procedure for the analysis of 19 synthetic cannabinoids in urine using UCT's Styre Screen® HLD highly crosslinked polymeric SPE cartridges. All 19 compounds were analyzed in under 10 minutes using LC-MS/MS. The use of a Selectra® C18 UHPLC column resulted in excellent peak shape for all the compounds included in the method, including baseline separation of any isobaric compounds. The recoveries obtained in this research study are satisfactory for the vast majority of analytes despite their diverse chemical structures. Extracted urine samples fortified at two concentrations (2.5 and 10 ng/mL) had, on average, recoveries in the range of 85-110% and corresponding RSD values less than 5%. The quality control concentrations of 2.5 ng/mL and 10 ng/mL were chosen to ensure low-level accurate detection based on heightened potency for this class of compounds at exceptionally low biological levels. This method will be beneficial to any lab looking to implement testing of these novel synthetic cannabinoids.

CLINICAL

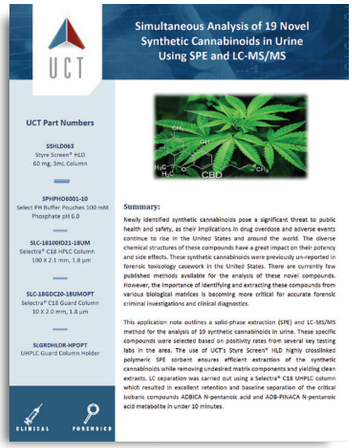
FORENSICS

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

UCT

FORENSIC SAMPLE ANALYSIS

Simultaneous Analysis of 19 Novel Synthetic Cannabinoids in Urine Using SPE and LC-MS/MS


Simultaneous Analysis of 19 Novel Synthetic Cannabinoids in Urine Using SPE and LC-MS/MS

UCT Part Numbers

CBD

Summary:

Newly identified synthetic cannabinoids pose a significant threat to public health and safety, as their implications in drug overdose and adverse events continue to rise in the United States and around the world. The diverse chemical structures of these compounds have a great impact on their potency and side effects. These synthetic cannabinoids were previously un-reported in forensic toxicology casework in the United States. There are currently few published methods available for the analysis of these novel compounds. However, the importance of identifying and extracting these compounds from various biological matrices is becoming more critical for accurate forensic criminal investigations and clinical diagnostics.

This application note outlines a solid-phase extraction (SPE) and LC-MS/MS method for the analysis of 19 synthetic cannabinoids in urine. These specific compounds were selected based on positivity rates from several key testing labs in the area. The use of UCT's Styre Screen® HLD highly crosslinked polymeric SPE sorbent ensures efficient extraction of the synthetic cannabinoids while removing undesired matrix components and yielding clean extracts. LC separation was carried out using a Selectra® C18 UHPLC column which resulted in excellent retention and baseline separation of the critical isobaric compounds ADBICA N-pentanoic acid and ADB-PINACA N-pentanoic acid metabolite in under 10 minutes.

CLINICAL FORENSICS

- Download Application Note
- Review Poster
- Learn more about Selectra® C18 HPLC Column
- Learn more about Styre Screen® HLD

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 Cartridge

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

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

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

SLGRDHLDR-HPOPT
Guard Column Holder

CLINICAL FORENSICS

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 (UHPLC-MS/MS). Clean Screen® DAU is UCT's flagship SPE cartridge 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 included in the method, 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.

Sample Pretreatment:

Urine Specimens*:

- To 1 mL of urine add 1 mL of pH 6 phosphate buffer (0.1M) and the 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 the 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 cartridge)

SPE Procedure:

1. **Condition Cartridge:**
   - 1 x 3 mL MeOH
   - 1 x 3 mL pH 6 phosphate buffer (0.1M)
2. **Apply Sample:**
   - Load at 1-2 mL/minute
3. **Wash Cartridge:**
   - 1 x 3 mL 1% Formic Acid in DI H_2O
   - 1 x 3 mL MeOH
   - Dry cartridges under full vacuum or pressure for 1 minute to remove residual MeOH
4. **Elute Analytes:**
   - 1 x 3 mL MeOH + 5% Ammonium Hydroxide (MeOH:NH_4OH, 95:5, v/v)
   - Collect at 1-2 mL/minute
5. **Dry Eluate:**
   - Evaporate to dryness under a gentle stream of nitrogen at <40°C
6. **Reconstitute:**
   - Reconstitute sample in 1 mL of mobile phase (alternative volumes may also be used)

CLINICAL

FORENSICS

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)
Guard Column: Selectra® DA Guard Column (5 X 2.1 mm, 1.8 µm)
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

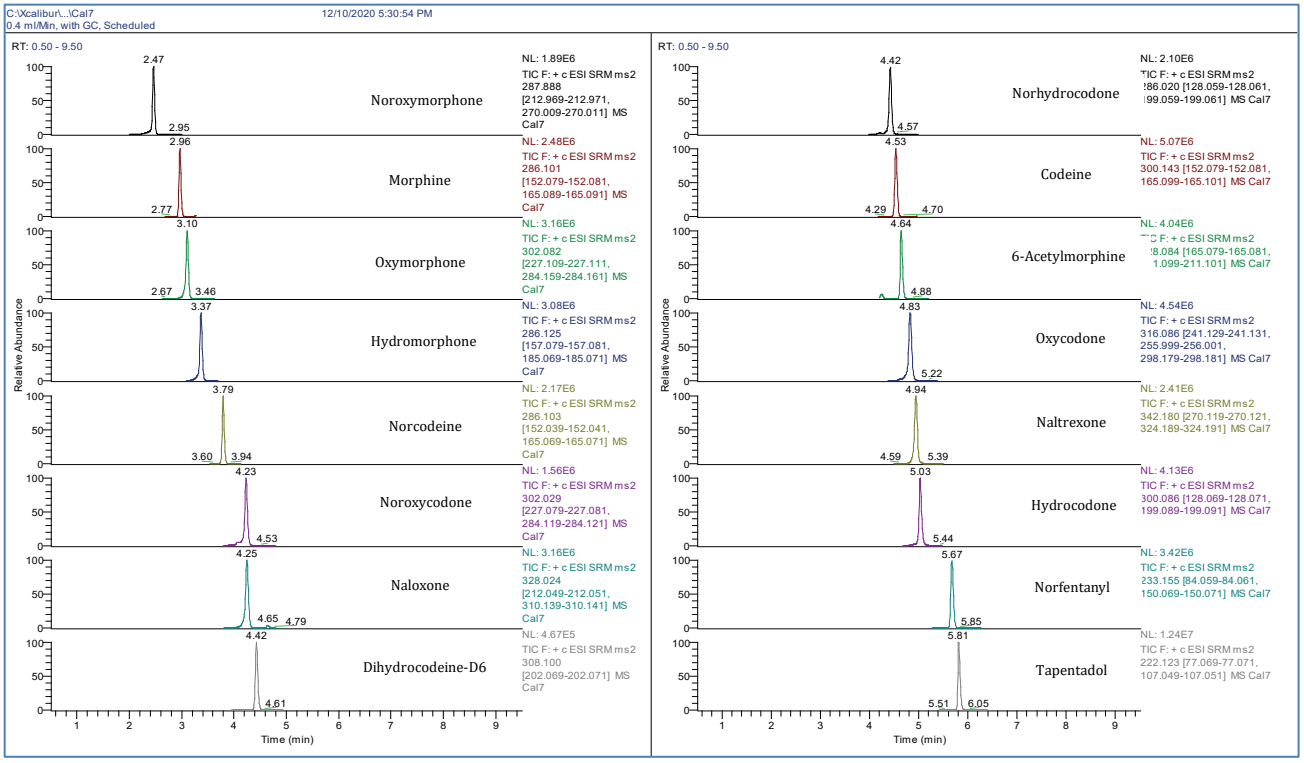
MRM Table:

Analyte	RT (min)	MRM					Internal Standard
		Parent Ion	Product Ion 1	CE	Product Ion 2	CE	
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

CLINICAL

FORENSICS

Chromatograms:



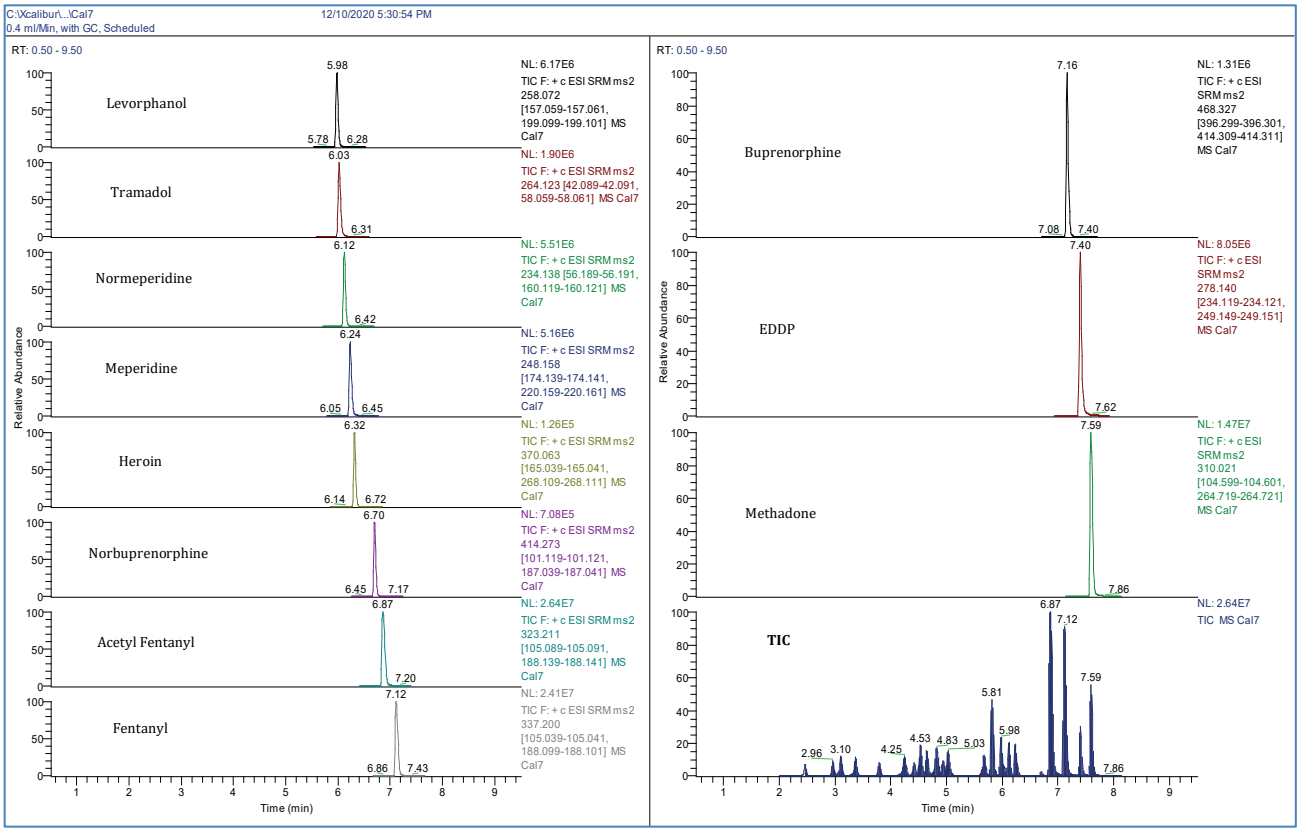


Figure 1: Exemplary chromatogram of the separation obtained using the Selectra® DA UHPLC column. All analytes included in the method eluted in less than 8 minutes. The TIC represents the total ion chromatogram for a single injection.

CLINICAL

FORENSICS

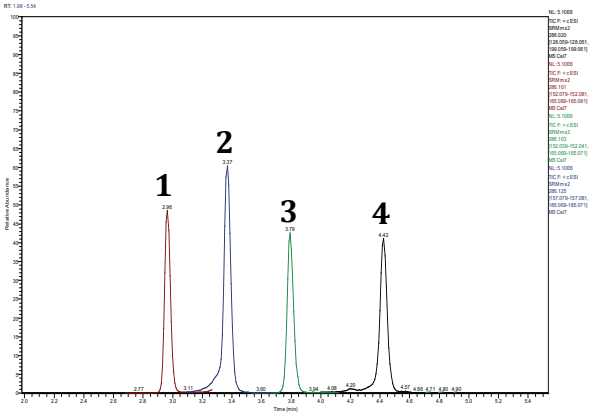


Figure 2a: Complete separation of critical isobaric compounds 1) Morphine, 2) Hydromorphone, 3) Norcodeine, and 4) Norhydrocodone.

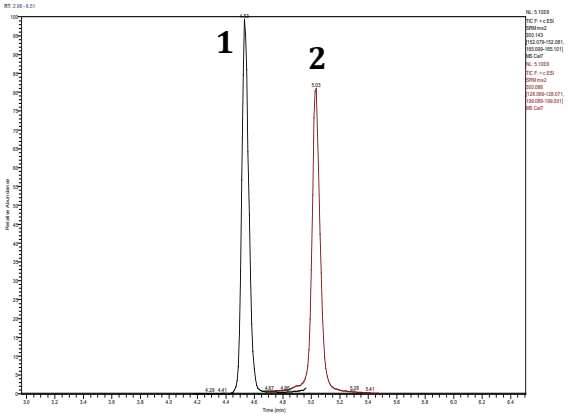


Figure 2b: Complete separation of critical isobaric compounds 1) Codeine and 2) Hydrocodone.

Calibration Curves:

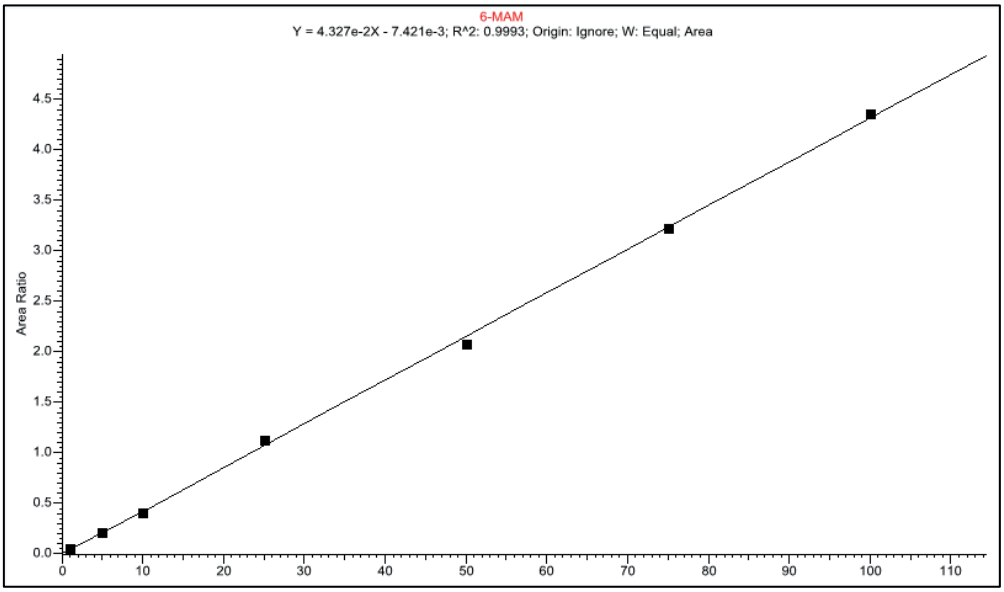


Figure 3a: Example of a 7-point calibration curve for 6-MAM with R^2 of 0.9993 (1, 5, 10, 25, 50 & 100 ng/mL). ng/mL).

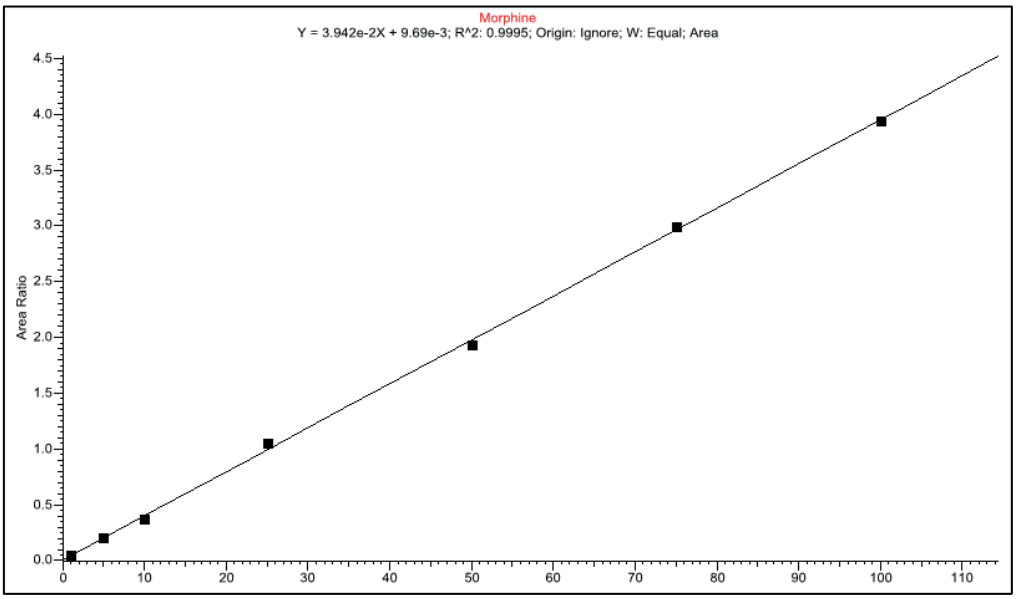


Figure 3b: Example of a 7-point calibration curve for Morphine with R^2 of 0.9995 (1, 5, 10, 25, 50 &100 ng/mL).

CLINICAL

FORENSICS

Results:

Urine:

Analyte	Recovery (n=5)					
	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

Blood:

Analyte	Recovery (n=5)					
	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

CLINICAL

FORENSICS

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

UCT

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

- Download Application Note
- Learn more about Clean Screen® DAU
- Learn more about Abalonase™ Ultra

Return to Navigation

AMPHETAMINES IN BLOOD, PLASMA/SERUM, URINE, OR TISSUE USING CLEAN SCREEN® DAU SPE AND LC-MS/MS ANALYSIS

UCT Part Numbers

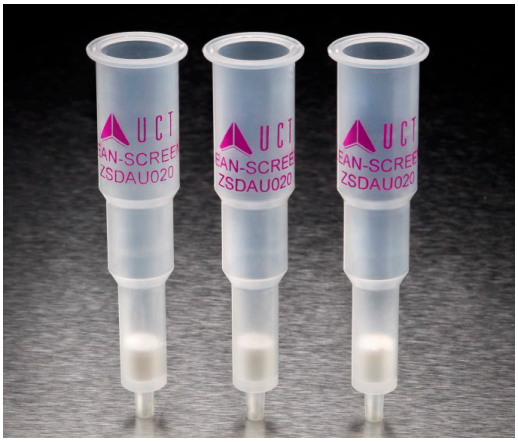
CSDAU206
Clean Screen® DAU
200 mg, 6 mL Column

SLPFPP50ID2.1-1.8UM
Selectra® PFPP UHPLC Column
50 X 2.1 mm, 1.8 µm

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

SLGRDHLDR-HP
Guard Column Holder

CLINICAL FORENSICS



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 also 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 to the column that distinguish it from other stationary phases. 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.

SPE Procedure:

1) **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 cartridge)

2) **Condition SPE Sorbent:**

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

3) **Apply sample:**

- Load sample at 1-2 mL/minute

4) **Wash Sorbent:**

- 1 x 3 mL 0.1 M HCl
- 1 x 3 mL methanol

5) **Dry Sorbent:**

- Dry SPE cartridge for 2 mins at 80-100 psi

6) **Elute:**

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

7) **Evaporate Eluent:**

- Evaporate the eluent for 5 minutes to remove NH_4OH (40°C, gentle stream of N_2)
- 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.

8) **Reconstitute:**

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

CLINICAL

FORENSICS

LC-MS/MS Parameters:

System: Shimadzu LCMS-8050	
UHPLC Column: Selectra® PFPP (50 X 2.1 mm, 1.8 µm)	
Guard Column: Selectra® PFPP (10 X 2.0 mm, 1.8 µm)	
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
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

CLINICAL

FORENSICS

Chromatograms:

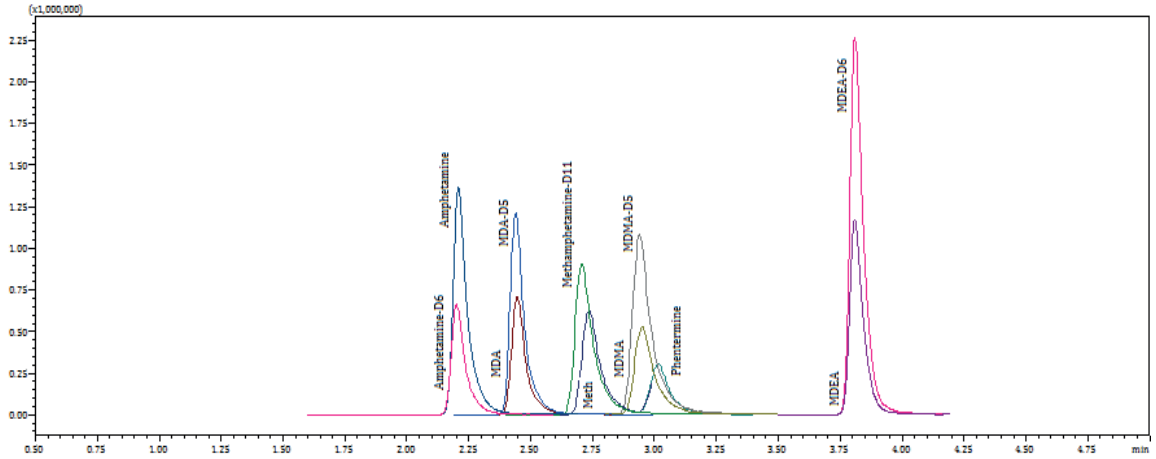


Figure 1. Chromatogram of a 10 ng/mL extracted sample.

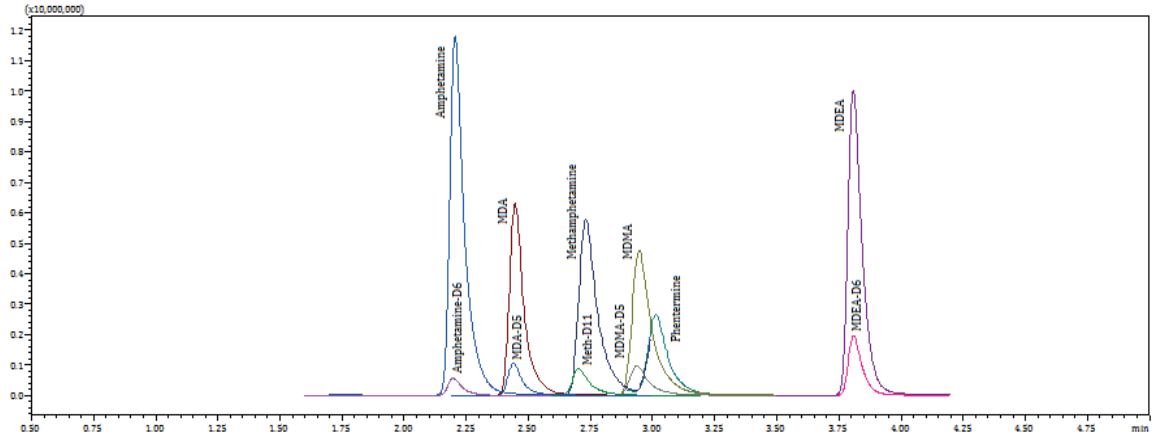


Figure 2. Chromatogram of a 100 ng/mL extracted sample.

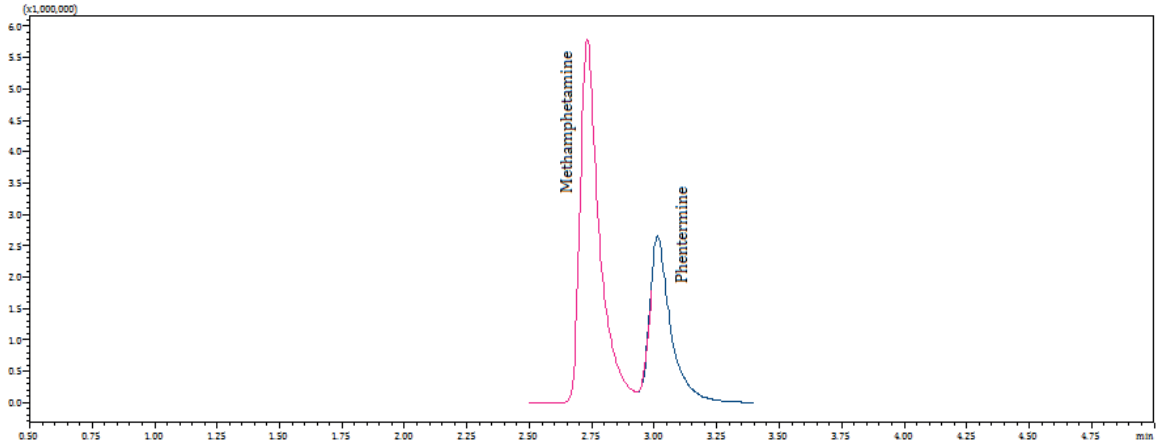


Figure 3. Chromatogram showing excellent baseline separation of methamphetamine & phentermine

CLINICAL

FORENSICS

Calibration Curves:

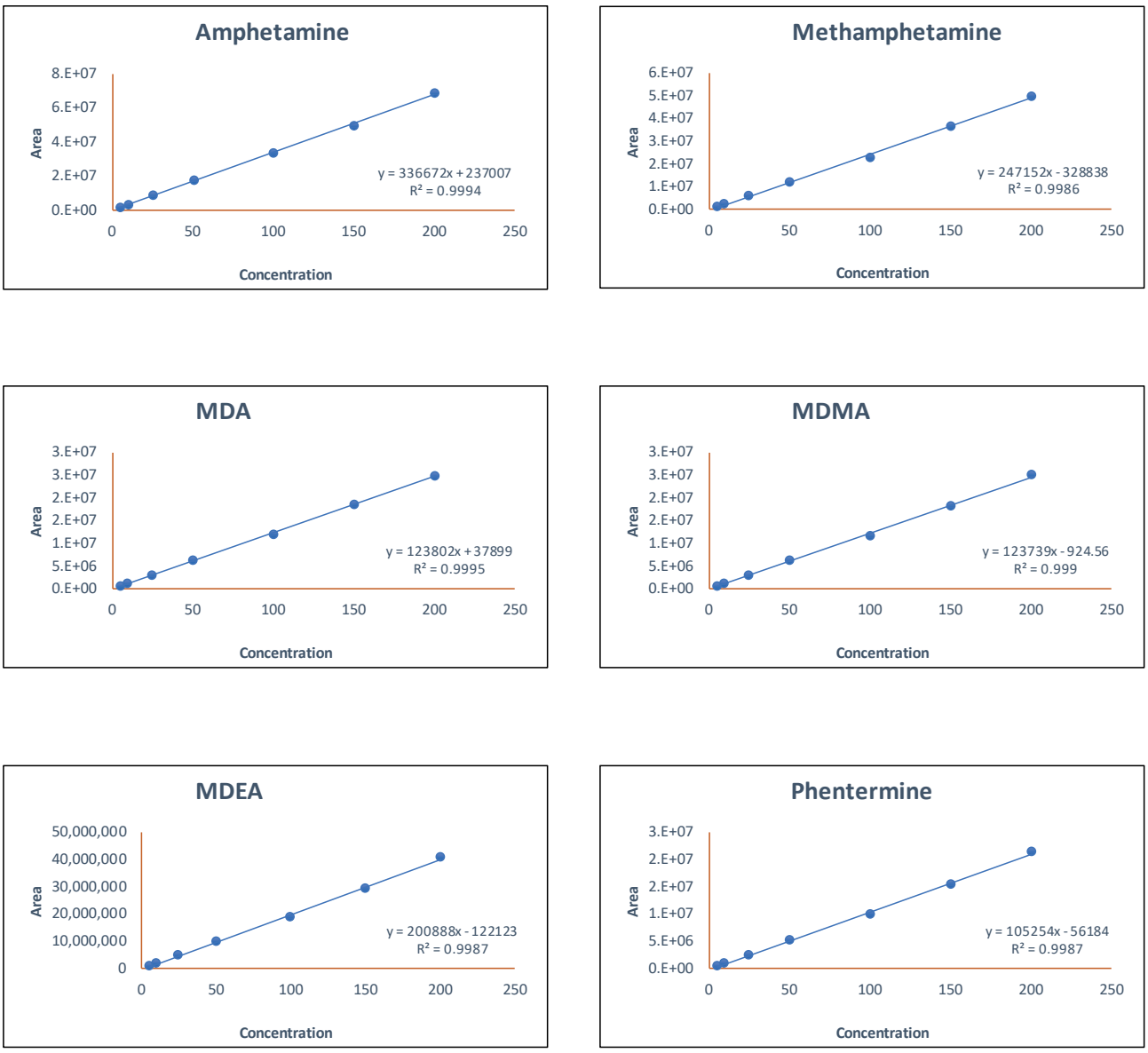


Figure 4. Calibration curve for the six amphetamines (5, 10, 25, 50, 100, 150 & 200 ng/mL).

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.

CLINICAL

FORENSICS

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

UCT

FORENSIC SAMPLE ANALYSIS

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:

- Download Application Note
- Learn more about Clean Screen® DAU
- Learn more about Selectra® PFPP UHPLC Column

Return to Navigation