Improved Extraction of Cannabinoids from Oral Fluid using Strata™-X-Drug B Solid Phase Extraction (SPE) and Kinetex® Core-Shell Phenyl-Hexyl HPLC/UHPLC Columns

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The simple, noninvasive nature of sample collection of oral fluids has lead to it being increasingly incorporated into drug testing programs. This is further aided by its improved indication of recent drug use when compared to blood or urine sample analysis. A potential drawback is that Δ9-tetrahydrocannabinol (THC), a commonly used illicit drug, is challenging to detect due to small sample size, low concentration of analyte and low detector response. It is with this potential drawback in mind that we developed a simple yet effective solid phase extraction (SPE) protocol for THC and its metabolites from oral fluid.

Introduction

The workplace testing of employees is an important issue in terms of providing a safe working environment and also in terms of regulatory compliance. Oral fluid collection is gaining popularity in such testing protocols due to its simplicity and the fact that samples can be collected under close employee supervision eliminating the possibility of adulteration or switching of samples. Oral fluid sampling is therefore becoming widespread amongst employers, law enforcement and government agencies.

Studies have shown that Δ9-tetrahydrocannabinol (THC), the active constituent of marijuana, is one of the most commonly used illicit drugs. An advantage of oral fluid analysis over urine or plasma sample analysis is that drug levels fall faster in oral fluids. Oral fluid analysis therefore provides a more reliable indication of recent drug use. This does also place more stringent requirements on the analytical protocol used, as sample volumes and analyte concentration will be low. For this reason, efficient sample cleanup is essential to provide accurate, reliable results. Our goal was to develop a simple yet effective solid phase extraction (SPE) protocol for THC and its metabolites from oral fluid as well as an efficient LC/MS/MS analysis method.

List of Analytes Used in this Study

- Δ9-tetrahydrocannabinol (THC)
- 11-nor-9-carboxy-Δ9-tetrahydrocannabinol (THC-COOH)
- 11-Hydroxy-Δ9-tetrahydrocannabinol (THC-OH)
- AM694
- JWH 073
- JWH 073-(3-hydroxybutyl) metabolite (JWH 073-(3-hb))
- JWH 018
- JWH 018-(5-hydroxypentyl) metabolite (JWH 018-(5-hp))

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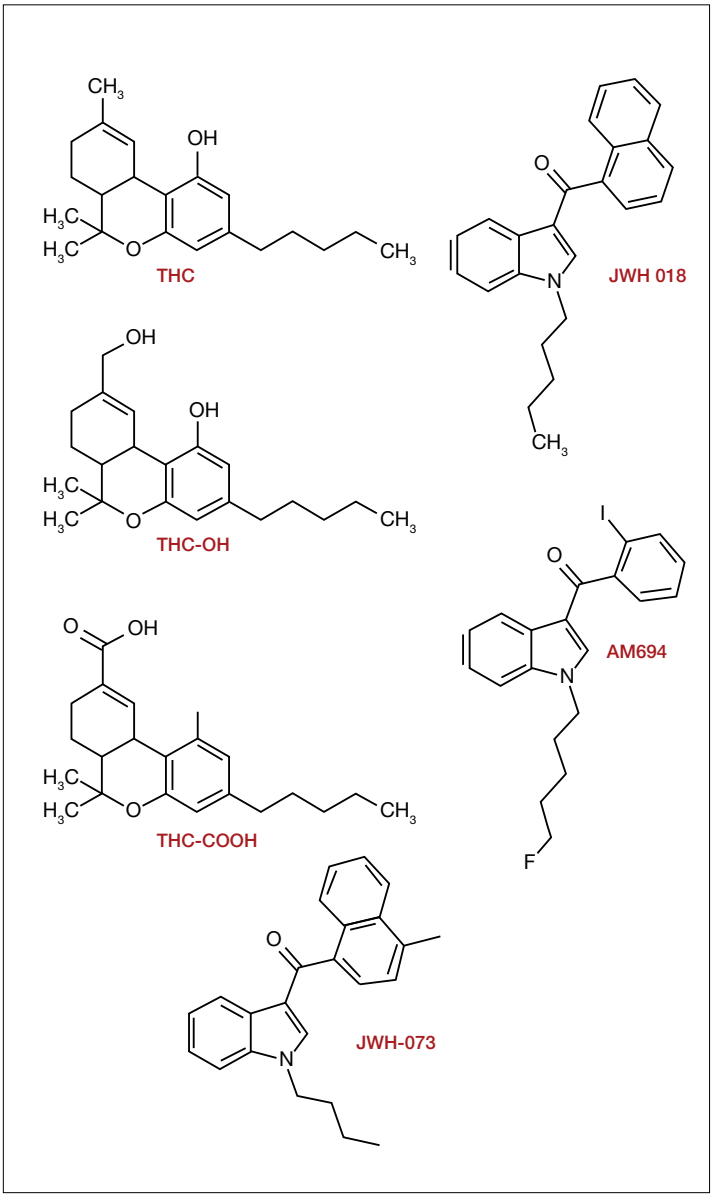
Figure 1.
Structures of select analytes



Experimental Conditions:

HPLC Conditions:

Column: Kinetex® 2.6µm Phenyl-Hexyl
Dimensions: 50 x 4.6 mm
Part No.: 00B-4495-E0
Mobile Phase: A: 10 mM Ammonium formate
B: 0.1 % Formic Acid in Methanol
Gradient:

Time (min)	% B
0.0	5
4	100
6	100

Flow Rate: 0.6 mL/min
Temperature: Ambient
Injection Volume: 1 µL
Detection: AB SCIEX API 3200™ MS/MS, ESI+
Instrument: Agilent®1200

MRM Conditions

ID	Q1 Mass	Q3 Mass	DP (V)	CE (V)	EP (V)	CXP (V)
THC-COOH	345.4	229.1	70	26	10	12
	345.2	327	55	29	10	10
AM694	436.1	231	50	39	10	12
	436.1	309.2	50	31	10	12
JWH 018	342.2	155.1	70	36	10	12
	342.2	214.2	70	31	10	12
JWH 018-(5hp)	358.3	155.2	70	31	10	12
	358.3	214.2	70	31	10	12
JWH 073	328.2	155.2	70	33	10	12
	328.2	200.2	70	31	10	12
JWH 073-(3hb)	344.2	155.2	70	29	10	12
THC-OH	331.4	193.2	100	33	10	10
	331.4	201.2	100	1	10	10
THC	315.2	193.2	60	35	10	14
	315.2	259.2	60	20	10	14
THC-D3	318	196.5	85	32	10	13
	318	123.1	90	41	10	13
JWH 073-D5	363.1	155.1	70	33	10	13

SPE Conditions

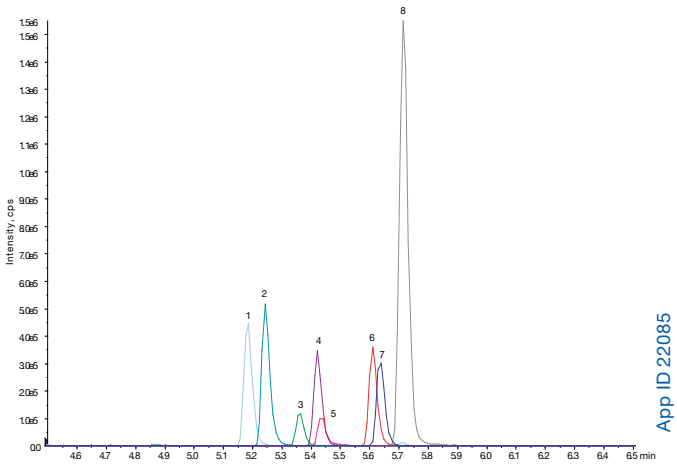
Sample Pretreatment: Dilute 500 µL oral fluid spiked with analyte mix with 1 mL Acetonitrile/100 mM Sodium acetate buffer (pH 5.0) (30:70)

Cartridge: Strata™-X-Drug B 30 mg/ 3 mL
Part No.: 8B-S128-UBJ
Condition: 1 mL Methanol
Equilibrate: 1 mL Acetonitrile/100 mM Sodium acetate buffer (pH 5.0) (30:70)
Load: Pre-treated samples
Wash 1: 1 mL 100 mM Sodium acetate buffer (pH 5.0)
Wash 2: 1 mL Acetonitrile/100 mM Sodium acetate buffer (pH 5.0) (30:70)
Dry: 5 minutes under vacuum at 10 inches of Hg
Elute: 2x 0.5 mL Ethyl acetate/Isopropanol (85:15)
Dry down: Dry down @ 45 °C under a stream of nitrogen for 20 minutes
Reconstitute: In 500 µL of mobile phase (A/B, 55:45) spiked with 40 µL of internal standard at 1 µg/mL

Table 1.
Linear regression and R^2 values for analytes used in this study

Analyte	Regression	R^2 Value
THC	0.0257x+0.049	0.9994
THC-COOH	0.00819x+0.173	0.9990
THC-OH	0.0096x+0.0127	0.9992
JWH 018	0.0117x+0.0183	1.0000
JWH 018-(5-hp)	0.00356x+0.00237	0.9999
JWH 073	0.00243x+0.00299	1.0000
JWH 073-(3-hb)	0.00315x+0.00449	0.9999
AM694	0.00229x+0.00359	1.0000

Figure 2.
LC/MS/MS chromatogram of THC and its metabolites and selected synthetic cannabinoids extracted from oral fluid



Sample: 1. JWH 073-3-hydroxybutyl metabolite
2. JWH 018-(5-hydroxypentyl) metabolite
3. THC-OH
4. AM694
5. THC-COOH
6. JWH 073
7. THC
8. JWH 018

Figure 3.
Calibration curve of THC

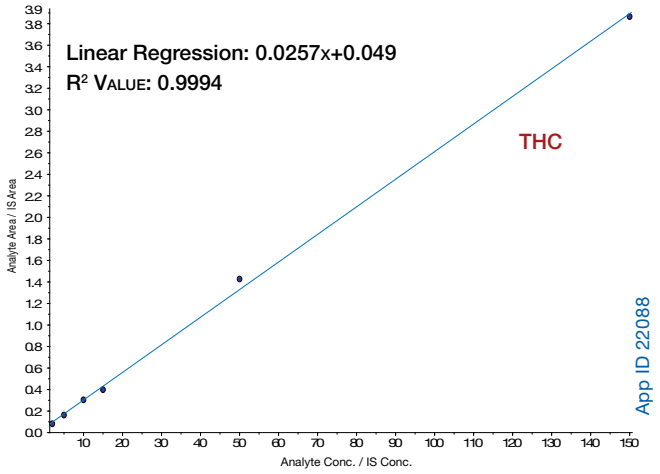


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Figure 3. *continued*
Calibration curve of THC-OH

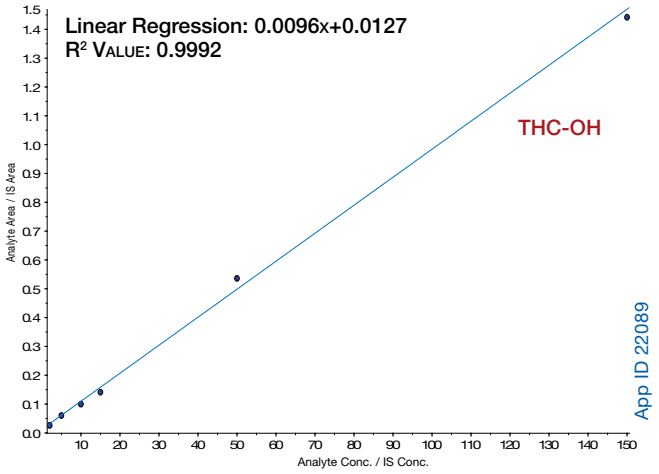

Figure 4.
LC/MS/MS chromatogram of THC and THC-OH extracted from saliva at a concentration level of 2 ppb

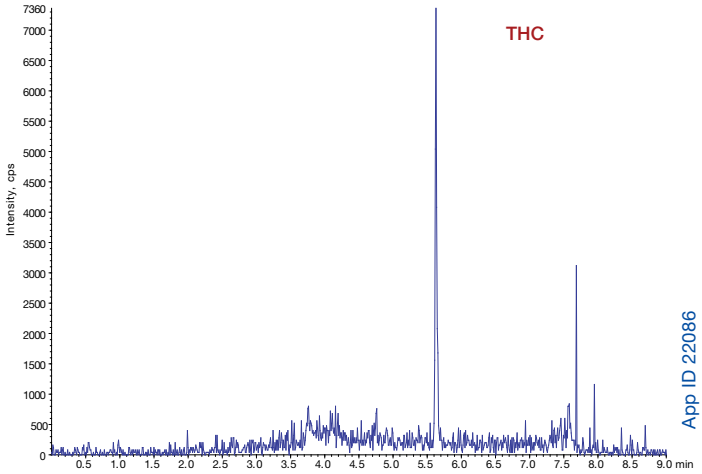

Table 2.
Signal-to-Noise (S/N) ratios from extracted saliva at lowest concentration level (2 ppb)

Analyte	S/N at 2 ppb
THC	24.8
THC-OH	16.9
THC-COOH	55
JWH 018	148.5
JWH 018-(5-hp)	21.6
JWH 073	50.1
JWH 073-(3-hb)	73.8
AM694	182.5

Figure 4. *continued*
LC/MS/MS chromatogram of THC and THC-OH extracted from saliva at a concentration level of 2 ppb

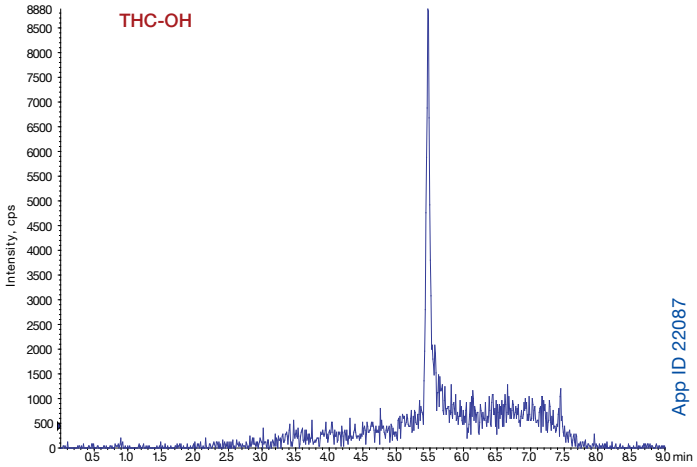

Table 3.
Statistical analysis of quality control (QC) samples at 8 ppb

Analyte	Mean Concentration (ppb)	Standard Deviation	CV (%)	Accuracy (%)
AM694	6.4	0.2	3	80
JWH 018	7.4	0.6	8	92
JWH 018-(5-hp)	7.1	0.8	11	89
JWH 073	6.7	0.6	10	83
JWH 073-(3-hb)	6.9	0.7	10	86
THC	7.1	0.8	11	89
THC-OH	7.1	0.8	11	89
THC-COOH	7.5	0.4	5	93

Table 4.
Statistical analysis of quality control (QC) standards at 80 ppb

Analyte	Mean Concentration (ppb)	Standard Deviation	CV (%)	Accuracy (%)
AM694	72.4	5.1	7	91
JWH 018	75.4	8.7	12	94
JWH 018-(5-hp)	73.7	5.9	8	92
JWH 073	70.4	9.2	13	88
JWH 073-(3-hb)	75.7	5.6	7	95
THC	79.3	4.7	6	99
THC-OH	79.3	4.7	6	99
THC-COOH	77.5	11.5	15	97

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Results and Discussion

The SPE method developed has been shown to deliver high extraction recoveries from saliva. It is possible to detect THC and its metabolites at the 2 ppb level which is lower than that set by the Substance Abuse and Mental Health Services Administration (SAMHSA) cut off (15 ppb). The method also shows good signal to noise ratios for all analytes at the 2 ppb level whilst also showing linearity from 2 ppb to 150 ppb. Coefficients of Variation (CV) were found to range from 3-15 % and accuracies were between 80 and 99 % for Quality Control samples. To validate the methodology, results were compared to urine analysis and excellent correlation was found.

The use of Strata™-X-Drug B SPE sorbent allows the method to be used with a wide range of basic drugs which may be present in saliva samples. It provides the ability to perform comprehensive matrix interference removal resulting in extremely clean extracts. This is combined with excellent extraction recoveries of both parent drugs and their metabolites.

Conclusion

THC, its metabolites, and popular synthetic cannabinoids were efficiently extracted from oral fluid with high percent recoveries using the method developed in this study. Linearity of response for all analytes was determined across a broad concentration range and could be detected at concentrations beneath their cutoff limits according to SAMHSA.

Strata™-X-Drug B SPE Ordering Information

Format	Sorbent Mass	Part Number	Unit
Tube			
	10 mg	8B-S128-AAK	1 mL (100/box)
	10 mg	8L-S128-AAK†	1 mL (100/box)
	30 mg	8B-S128-TAK	1 mL (100/box)
	30 mg	8L-S128-TAK†	1 mL (100/box)
	30 mg	8B-S128-TBJ	3 mL (50/box)
	60 mg	8B-S128-UBJ	3 mL (50/box)
	60 mg	8B-S128-UCH	6 mL (30/box)
	60 mg	8B-S128-UCL	6 mL (200/box)
Giga™ Tube			
	100 mg	8B-S128-EDG	12 mL (20/box)
96-Well Plate			
	10 mg	8E-S128-AGB	2 Plates/box
	30 mg	8E-S128-TGB	2 Plates/box
	60 mg	8E-S128-UGB	2 Plates/box

†Tab-less tube

Kinetex® Core-Shell HPLC/UHPLC Ordering Information

5 µm Minibore Columns (mm)			SecurityGuard™ ULTRA Cartridges‡
Phases	**50 x 2.1**	**100 x 2.1**	**3/pk**
Phenyl-Hexyl	00B-4603-AN	00D-4603-AN	AJ0-8788 for 2.1 mm ID

5 µm MidBore™ Columns (mm)			SecurityGuard ULTRA Cartridges‡
Phases	**50 x 3.0**	**100 x 3.0**	**3/pk**
Phenyl-Hexyl	00B-4603-Y0	00D-4603-Y0	AJ0-8781 for 3.0 mm ID

5 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	**50 x 4.6**	**100 x 4.6**	**150 x 4.6**	**250 x 4.6**	**3/pk**
Phenyl-Hexyl	00B-4603-E0	00D-4603-E0	00F-4603-E0	00G-4603-E0	AJ0-8774 for 4.6 mm ID

2.6 µm Analytical Columns (mm)					SecurityGuard ULTRA Cartridges‡
Phases	**50 x 4.6**	**75 x 4.6**	**100 x 4.6**	**150 x 4.6**	**3/pk**
Phenyl-Hexyl	00B-4495-E0	00C-4495-E0	00D-4495-E0	00F-4495-E0	AJ0-8774 for 4.6 mm ID

2.6 µm MidBore™ Columns (mm)			SecurityGuard ULTRA Cartridges
Phases	**100 x 3.0**	**150 x 3.0**	**3/pk**
Phenyl-Hexyl	00D-4495-Y0	00F-4495-Y0	AJ0-8781 for 3.0 mm ID

2.6 µm Minibore Columns (mm)						SecurityGuard ULTRA Cartridges
Phases	**30 x 2.1**	**50 x 2.1**	**75 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
Phenyl-Hexyl	00A-4495-AN	00B-4495-AN	00C-4495-AN	00D-4495-AN	00F-4495-AN	AJ0-8788 for 2.1 mm ID

1.7 µm Minibore Columns (mm)				SecurityGuard ULTRA Cartridges
Phases	**50 x 2.1**	**100 x 2.1**	**150 x 2.1**	**3/pk**
Phenyl-Hexyl	00B-4500-AN	00D-4500-AN	00F-4500-AN	AJ0-8788 for 2.1 mm ID

‡SecurityGuard ULTRA cartridges require holder, Part No.: AJ0-9000

Kinetex Core-Shell Technology is also available in many other dimensions and particle sizes including 5 µm, 2.6 µm 1.7 µm, and 1.3 µm

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guarantee

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