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Application Note

Screening for Cannabinoids Using the Waters Forensic Toxicology Application Solution with UNIFI

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For forensic toxicology use only.

Abstract

This application note demonstrates the sensitivity and selectivity of the expanded Forensic Toxicology Application Solution with UNIFI using negative ionization in providing a consistent comprehensive determination of cannabinoids. It can be applied as both a screen for selected cannabinoids and a method suitable for quantifying these analytes, at levels below the current EWDTS urine screening cut-off (50 ng/mL for cannabis metabolites), using a simple five-fold dilution. The excellent linear dynamic range of this system is demonstrated by simple automatically generated calibration plots.

Benefits

Expanded Forensic Toxicology Application Solution with UNIFI enabling the detection and quantitation of negative ionizing cannabinoids in urine.

Introduction

Cannabis is the most widely used illicit substance in the world and long-term use can lead to dependency. Cannabinoids are one of the most commonly detected classes of illegal drugs; consequently their analysis is of key importance in both forensic and workplace testing. Δ-9 tetrahydrocannabinol (THC) and cannabinol (CBN) are psychoactive elements present in the plant *Cannabis sativa*.[1] THC produces a number of metabolites such as 11-nor-9-hydroxy-Δ9 tetrahydrocannabinol (THC-OH), but the most significant metabolite for urine drug testing is 11-nor-9-carboxy-Δ9 tetrahydrocannabinol (cTHC), which is the major metabolite eliminated in urine, as the free acid or the ester-linked β-glucuronide.[2] Cannabidiol (CBD) is a major constituent of cannabis resin but is believed to have limited psychoactive properties.

The Waters Forensic Toxicology Application Solution with UNIFI currently comprises acquisition of accurate mass MS^E data on an orthogonal acceleration time-of-flight mass spectrometer operating in electrospray positive ionisation mode (ESI+), followed by comparison of the data with a comprehensive library containing more than 1000 toxicologically-relevant substances.[3,4,5] A number of compounds, such as the cannabinoids, also ionise in negative electrospray mode (ESI-) and the aim of the recent work was to further extend the Forensic Toxicology Application Solution to include those compounds. The new method was used to

determine the presence of cannabinoids in urine, particularly at concentrations, below the current screening cut-off,[6] and to compare the values obtained using this method with a recently published fully validated UPLC-MS/MS assay.[7]

Experimental

ACQUITY UPLC conditions

UPLC system:	ACQUITY UPLC I-Class (FTN)
Column:	ACQUITY UPLC HSS, 100Å, 1.8 µm, C_{18}, 2.1 mm x 150 mm (p/n 186003534)
Vials:	Maximum Recovery Vials (p/n 186000327C)
Column temp.:	50 °C
Sample temp.:	10 °C
Injection vol.:	10 µL
Flow rate:	0.4 mL/min
Mobile phase A:	Water containing 0.001% formic acid
Mobile phase B:	Acetonitrile containing 0.001% formic acid
Gradient:	Isocratic at 87% A for 0.5 min, then to 5% A at 4.5 min, hold for 1 min before switching to 87% A
Run time:	7.5 min

MS conditions

MS system:	Xevo G2-S QTof
Ionization mode:	ESISource
temp.:	150 °C
Desolvation temp.:	400 °C
Desolvation gas:	800 L/h
Reference mass:	Leucine enkephalin [M-H]- *m/z* = 554.2620 Acquisition range *m/z* 50–1000
Scan time:	0.1 s
Capillary voltage:	1.5 KV
Cone voltage:	20 V
Collision energy:	Function 1: 6 eV Function 2: ramped 10 to 40 Ev

Materials

Reference standards THC, CBD, CBN, THC-OH, and cTHC (1 mg/mL) were purchased from LGC Standards (Teddington, UK); cTHC-glucuronide and the deuterated (d-3) analogue of cTHC (for use as internal standard; ISTD), were obtained from the same supplier at 0.1 mg/mL.

Prior to use the individual standards were diluted to 5000 ng/mL in acetonitrile and the internal standard was diluted to 100 ng/mL in 0.001% formic acid.

Bio-Rad normal control urine and Bio-Rad Liquichek Urine Toxicology Controls Level C2 and Level S10 reference urines were obtained from Bio-Rad Laboratories (Hemel Hempstead, UK).

All other chemicals used were of the highest grade available and stored according to the supplier's instructions.

Standards preparation

Standards (0.1 mL) were added to 0.9 mL 0.001% formic acid in a Maximum Recovery Vial and vortex-mixed to give a concentration of 500 ng/mL.

Sample preparation

Acetonitrile (0.1 mL) was added to 0.2 mL urine and ISTD (0.7 mL). The sample was vortex-mixed, for 5 min at 1200 rpm, and then centrifuged at 8000 *g* for 10 min. Supernatant was transferred to a Maximum Recovery Vial.

Results and Discussion

The cannabinoids investigated in this analysis are listed in Table 1, along with their exact neutral mass, high energy fragment ions, and UPLC retention times.

Analyte	Neutral monoisotopic mass	Fragment ions (*m/z*)		Retention time (min)
cTHC-glucuronide	520.2308	343.1915	299.2017	4.7
		245.1547	175.0248	
cTHC	344.1988	299.2017	245.1547	5.0
		191.1078	179.1078	
THC-OH	330.2190	311.2017	281.1547	5.1
		268.1469	267.1391	
Cannabidiol	314.2246	245.1547	229.1234	5.4
		179.1078	135.1179	
Cannabinol	310.1933	279.1391	252.1156	5.6
		222.0686	159.0815	
THC	314.2246	245.1547	229.1234	5.8
		191.1078	149.0972	
cTHC-d3	347.2176	302.2191	248.1739	5.0

Table 1. Analyte neutral mass, high energy fragment ions, and retention times.

The acceptance criteria for a positive identification of each analyte was as follows: three dimensional (3D) low energy ion count intensity greater than 250, retention time to be within 0.35 min of reference, and the observed precursor mass to be within 5 ppm of expected. For additional confirmation, a minimum of one diagnostic fragment ion had to be found in the high energy function. Chromatographic separation of

cannabinoid standards at 100 ng/mL (50 ng/mL for cTHC-glucuronide and 500 ng/mL for cannabinol) is shown in Figure 1.

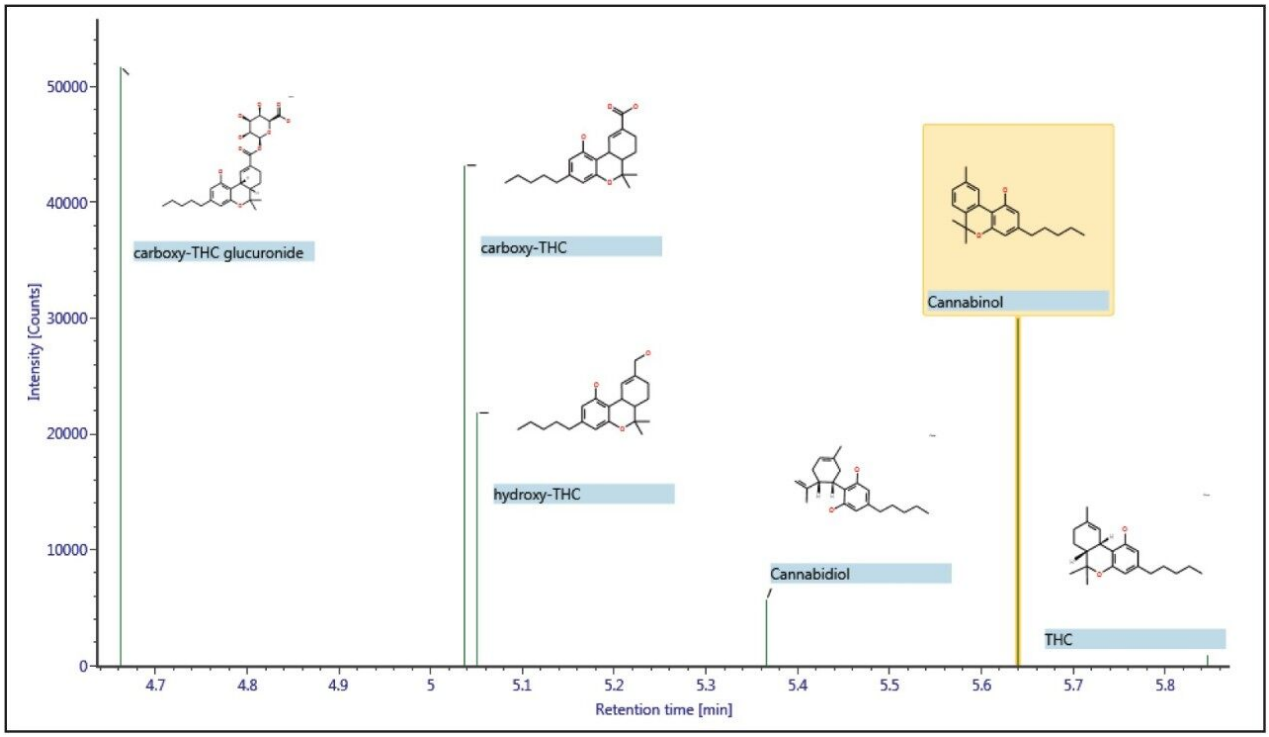

Figure 1. Component plot showing positively identified cannabinoids in a mixture of standards (ISTD not shown). The plot shows chromatographic separation for the isomers cannabidiol and THC.

To investigate linearity, Bio-Rad control urine was spiked with cTHC and cTHC glucuronide over a range from 0 to 500 ng/mL (250 ng/mL for cTHC-glucuronide) and prepared in duplicate, as described above. The 3D peak response for each positively identified analyte trace was generated automatically during processing and referenced to the ISTD response; calibration curves were plotted using a 1/x weighting and a linear fit was applied to both analytes. The correlation coefficient (r^2 value) was ≥0.995 for both analytes; a calibration curve for cTHC is shown in Figure 2. The lowest calibrator positively identified, using the criteria detailed above, was 6.25 ng/mL for cTHC-glucuronide and 12.5 ng/mL for cTHC.

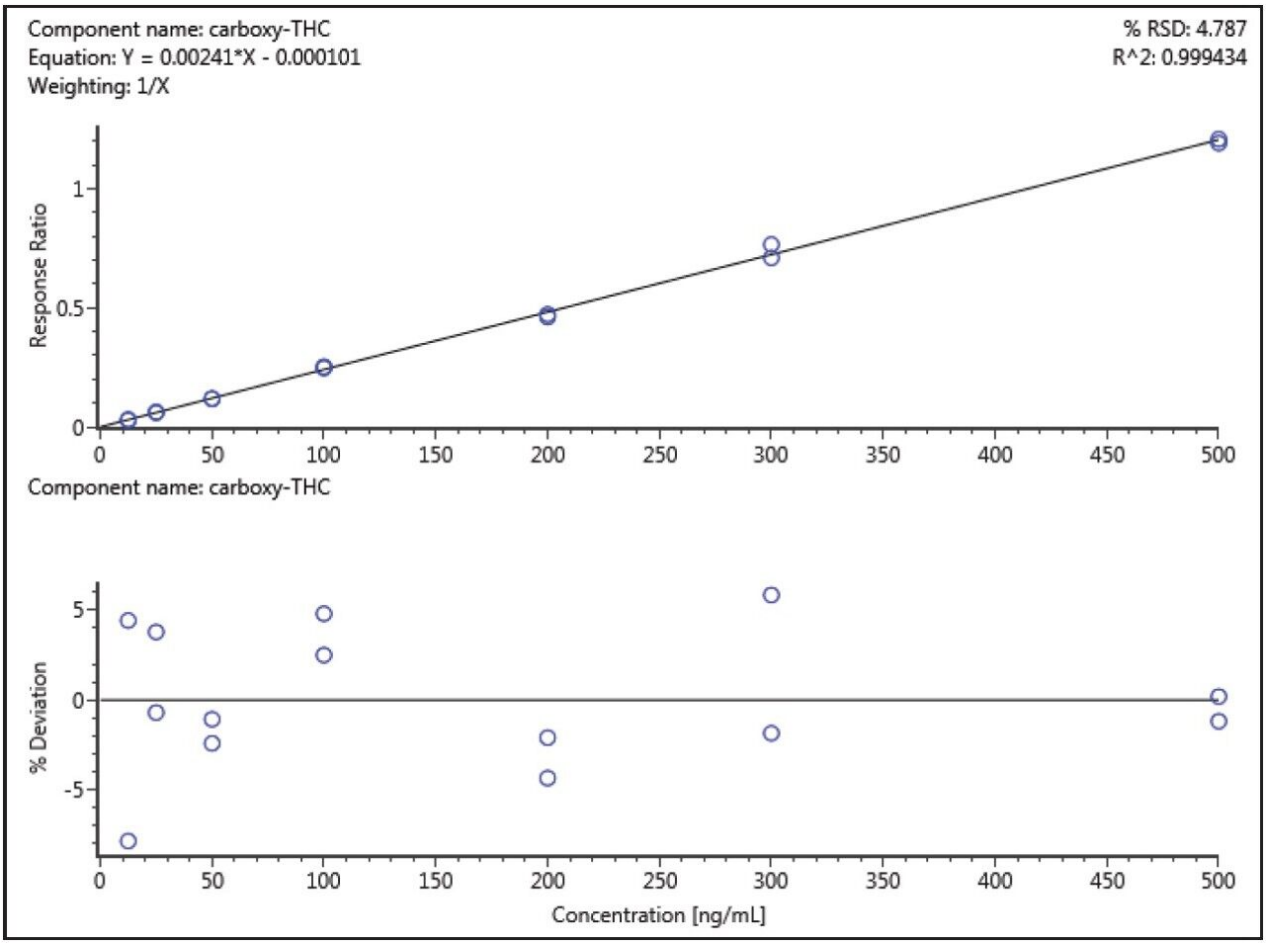

Figure 2. A spiked urine calibration curve for cTHC over the range 0 to 500 ng/mL using a linear fit with 1/x weighting applied.

Analysis of authentic urine samples Twenty-six authentic urine samples and two commercial reference urines (Bio-Rad Liquichek Level C2 and S10) were analyzed following the sample preparation method described here. The authentic samples comprised anonymized samples that had previously been quantified using a fully validated UPLC-MS/MS assay.[7] The UPLC-MS/MS results for the 26 authentic samples are shown in Table 2, along with the results from the UPLC-QTof-MS^E assay, showing whether cTHC or cTHC-glucuronide were positively identified based on the criteria stated previously.

Sample	cTHC		cTHC-glucuronide	
	UPLC-MS/MS (ng/mL)	UPLC-QTof-MS^E (positive ID)	UPLC-MS/MS (ng/mL)	UPLC-QTof-MS^E (positive ID)
001	20	YES	40	YES
002	0	NO	33	YES
003	68	YES	285	YES
004	0	NO	0	NO
005	0	NO	0	NO
006	0	NO	0	NO
007	0	NO	0	NO
008	12	YES	70	YES
009	3	NO	16	YES
010	6	NO	33	YES
011	0	NO	0	NO
012	18	YES	49	YES
013	7	NO	21	YES
014	14	YES	206	YES
015	11	YES	10	YES
016	0	NO	12	YES
017	54	YES	14	YES
018	15	YES	76	YES
019	10	YES	177	YES
020	0	NO	0	NO
021	0	NO	0	NO
022	67	YES	120	YES
023	0	NO	0	NO
024	83	YES	233	YES
025	0	NO	0	NO
026	0	NO	7	NO

Table 2. Comparison of individual sample results for either the UPLC-MS/MS or UPLC-QTof-MS^E methods. Positive identification criteria for the UPLC-QTof-MS^E assay were 3D low energy ion count intensity greater than 250, retention time to be within 0.35 min of reference, observed precursor mass to be within 5 ppm of expected and a minimum of one high energy fragment ion detected.

The UPLC-QTof-MS^E method positively identified cTHC in 11 samples and cTHC-glucuronide in 16 samples, and overall demonstrated excellent concordance with the UPLC-MS/MS data (Table 3). This demonstrated

that this method can consistently detect cTHC and cTHC-glucuronide in urine, using a simple fivefold dilution, at concentrations below the current European Workplace Drug Testing Society (EWDTS) screening cut-off of 50 ng/mL for cTHC.[6]

cTHC			cTHC-glucuronide		
UPLC-MS/MS		UPLC-QTof-MS^E	UPLC-MS/MS		UPLC-QTof-MS^E
Blank	12	15 NEG	Blank	9	10 NEG
Positive <10 ng/mL	3		Positive <10 ng/mL	1	
Positive ≥10 ng/mL	11	11 POS	Positive ≥10 ng/mL	16	16 POS

Table 3. Summary of results for 26 authentic urine samples obtained using the quantitative UPLC-MS/MS methodology[7] and the described UPLC-QTof-MS^E assay.

Furthermore the method detected and correctly assigned cTHC in both commercial reference urines. The semi-quantitative results obtained using this method for the analysis for the Bio-Rad Liquichek Level C2 and S10 reference urines were in accordance with the manufacturer's stated reference values, and are shown in Table 4. The additional confirmation provided by the presence of 4 high energy fragments for cTHC in the Bio-Rad Liquichek level S10 reference urine is shown in Figure 3.

Reference urine	GC/MS (ng/mL)	UPLC-QTof-MS^E (ng/mL)
Bio-Rad Liquichek Level C2	11.5	11.1
Bio-Rad Liquichek Level S10	35.3	40.5

Table 4. Comparison between the values obtained using the UPLC-QTof-MS^E method for the analysis of the Bio-Rad Liquichek reference urines and the values stated by the manufacturer.

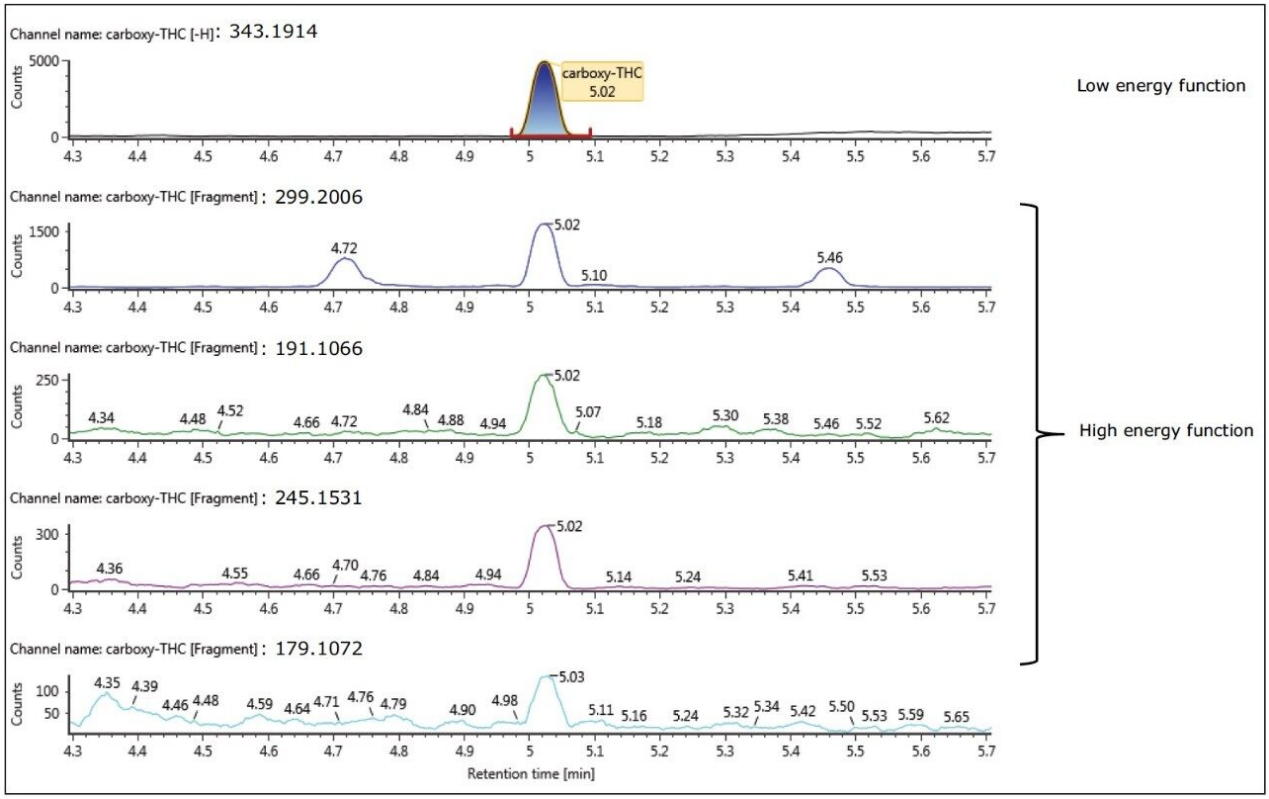

Figure 3. Data for cTHC in the Bio-Rad Liquichek Level S10 reference urine; additional confirmation is achieved by the presence of 4 fragment ions in the high energy function.

Conclusion

This application note demonstrates the sensitivity and selectivity of the expanded Forensic Toxicology Application Solution with UNIFI using negative ionisation in providing a consistent comprehensive determination of cannabinoids. It can be applied as both a screen for selected cannabinoids and a method suitable for quantifying these analytes, at levels below the current EWDTS urine screening cut-off (50 ng/mL for cannabis metabolites), using a simple five-fold dilution. The excellent linear dynamic range of this system is demonstrated by simple automatically generated calibration plots.

Acknowledgements

CEDAM Italia, Bresso, Italy and Bianalisi Analisi Mediche, Carate Brianza, Italy for supplying the anonymized authentic urine samples.

References

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Forensic Toxicology Screening Application Solution with UNIFI <https://www.waters.com/134779723>

Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>

Available for Purchase Online

ACQUITY UPLC HSS C18 Column, 100Å, 1.8 µm, 2.1 mm X 150 mm, 1/pkg < https://www.waters.com/waters/partDetail.htm?partNumber=186003534>

LCGC Certified Clear Glass 12 x 32mm Screw Neck Max Recovery Vial, with Cap and Preslit PTFE/Silicone Septa, 2 mL Volume, 100/pkg < https://www.waters.com/waters/partDetail.htm?partNumber=186000327C>

A full validation by the user would be necessary prior to adoption in a laboratory.

720005413, May 2015