Determination of Cannabinoids (THC) in Biological Samples

Application Note

Forensic Toxicology

Authors

Joe Crifasi
Saint Louis University
Forensic Toxicology Laboratory
Saint Louis, MO, USA

Ron Honnold
Agilent Technologies, Inc.
Santa Clara, CA, USA

Robert Kubas,
Agilent Technologies, Inc.
Wood Dale, IL, USA

Abstract

A method has been developed on the Agilent 220 Quadrupole Ion Trap using EI-MS/MS for the identification and quantification of Delta-9-THC, 11-Hydroxy-THC, and 11-Nor-Delta-9-THC-COOH in biological samples. A working range of 2.5–25.0 ng/mL for Delta-9-THC and 11-OH-THC and 5.0–150 ng/mL for THC-COOH shows the method linearity. In the analysis of Cannabinoids, the benefits of using GC Quadrupole Ion Trap MS\MS cannot be underestimated, in terms of reducing sample matrix interference, improving signal-to-noise and coupling its high selectivity and sensitivity.

Introduction

The major psychoactive component of marijuana, delta-9-tetrahydrocannabinol (Delta-9-THC) is quickly cleared from the blood detection of the hydroxyl-metabolite (11-OH-THC) and its ratio to the parent compound can be used to interpret the approximate time of use. The carboxylic acid metabolite (THC-COOH) may be detected in blood and urine for days after use of marijuana, and merely indicates past use of marijuana.

Agilent Technologies

This application note describes a method for the analysis of serum, whole blood, vitreous fluid, urine, or tissue homogenates. A minimum of 2.0 mL of sample is required for analysis.

The Delta-9-THC, 11-OH-THC, and the THC-COOH metabolites are extracted from deproteinized whole blood, serum, or hydrolyzed urine, separated by liquid: liquid extraction, concentrated, and derivitized with BSTFA. Ion fragmentation for the resultant silyl-derivatives and the respective deuterated internal standards by GC/MS facilitates the identification and quantitation of both the parent compound and the primary metabolites.

Experimental

Standards and reagents

Reagents - Methanol, Hexane, Ethyl Acetate - Nanopure, Acetonitrile- HPLC grade, Glacial Acetic acid, Sodium Hydroxide: 0.5 N NaOH, 100 mM Phosphate Buffer- pH 6.8, Beta-glucuronidase enzyme (E.coli, Type IX)(Sigma Chemical Co.)

- Reconstitute the lyophilized enzyme so that 0.1 mL contains 2,500 units.
- Dilute the 25,000 unit vial with 10 mL deionized H_2O or the 1 million unit vial with 40 mL of deionized H_2O.
- Make aliquots of the solution in 1.5 mL plastic tubes

(Stable one year, stored frozen)

Extraction solvent - (7:1 Hexane: Ethyl Acetate) (Stable at room temperature for 1 year), BSTFA+ 1% TMS (United Chemical Technologies or Sigma Chemicals)

Standards - Delta-9-THC, 9-Carboxy-11-Nor-Delta-9-THC, 11-Hydroxy-Delta-9-THC, d-3 Delta-9-THC, d-3 9-Carboxy-11-Nor-Delta-9-THC, d-3 11-Hydroxy-THC standards were purchased from Cerilliant. Quality Control stocks were purchased from Grace (Altech).

Intermediate calibration and QC standards - 1,000 ng/mL and 100 ng/mL

Working internal standard - 2.0 µg/mL

Calibration standards were then made from the intermediate standards:

Calibrator 1 - (2.5 ng/mL of Delta-9-THC, 11-OH-THC and 5.0 ng/mL THC-COOH)

Calibrator 2 - (5.0 ng/mL of Delta-9-THC, 11-OH-THC and 10.0 ng/mL THC-COOH)

Calibrator 3 - (10.0 ng/mL of Delta-9-THC, 11-OH-THC and 25.0 ng/mL THC-COOH)

Calibrator 4 - (25.0 ng/mL of Delta-9-THC, 11-OH-THC and 75.0 ng/mL THC-COOH)

Controls and Calibration Standards

Negative control - Drug free whole blood obtained from American Red Cross, dilute 1:1 with normal saline (0.9%) store at –20 °C, stable for 1 year.

Negative control - Drug free urine

Low control - (5.0 ng/mL) 100 µL of the 100 ng/mL Intermediate QC standard into 2.0 mL negative whole blood/urine, prepare fresh for each run.

High control - (20.0 ng/mL) 40 µL of the 1,000 ng/mL Intermediate QC standard into 2.0 mL negative whole blood/urine, prepare fresh for each run.

Urine control - (18 ng/mL) Confirmation QC (C3); purchased from Bio-RAD Corporation.

Sample Preparation

Prepare a calibration curve using the following and drug free blood/urine in 16 × 100 mm culture tubes.

	Internal std.	THC/OH-THC 100 ng/mL	THC/OH-THC 1,000 ng/mL	THC-COOH 100 ng/mL	THC-COOH 1,000 ng/mL	Drug free blood/urine
Calibrator 1	50 µL	50 µL		100 µL		2 mL
Calibrator 2	50 µL	100 µL			20 µL	2 mL
Calibrator 3	50 µL		20 µL		50 µL	2 mL
Calibrator 4	50 µL		50 µL		150 µL	2 mL

Procedure

1. To each tube containing urine, pipet 200 µL working beta-glucuronidase enzyme and 0.5 mL of pH 6.8 phosphate buffer.
2. To each urine and 2 mL aliquot of samples or controls, add 50 µL of the working internal standard.
3. Cap, mix, and place urine samples in a 37 ° incubator overnight.
4. Place capped tubes with blood samples into a refrigerator for temporary storage.
5. Remove tubes from the incubator and refrigerator, allow to reach room temperature.
6. Pipet 4.0 mL of Acetonitrile to each tube containing blood while vortexing the tube.
7. Vortex mix until deproteinization is complete.
8. Centrifuge the blood tubes and decant the supernatant into clean 16 × 100 mm test tubes.
9. Concentrate to approximately 1.0 mL by evaporating the supernatant at 60 °C.
10. To the concentrated blood extracts and the urine samples, add 0.5 mL of 0.5 N NaOH and 4 mL of 7:1 hexane:ethyl acetate extraction solvent.
11. Cap and rotate mix the tubes for 10 minutes.
12. Centrifuge and transfer the organic layers (upper) to clean 16 × 100 mm disposable culture tubes.
13. Add 0.5 mL of glacial acetic acid to the extraction tube, cap and rotate 10 minutes.
14. Transfer the organic layers to the labeled corresponding tubes containing the organic from the previous basic extraction.
15. Concentrate the organic solvents under a stream of nitrogen at 40 °C.
16. Add 100 µL BSTFA with 1% TMS to each dried extract and heat for 15 minutes at 70 °C.
17. Transfer the BSTFA to auto-sampler vials with inserts, cap and transfer to GCMS for analysis.

GC/MS Ion Trap Analysis

Column	Agilent DB-5ms Ultra Inert or equivalent 25 m × 200 mm, 0.33 µm
Injection volume	1 µL
Injection mode	Splitless
Inlet temperature	250 °C
Carrier gas	Helium
Column flow	1.3 mL/min
Oven program	160 °C; 1.0 minute hold 25 °C/min to 260 °C, 2.0 minute hold 5 °C/min to 300 °C, 1.0 minute hold

Quadrupole Ion Trap MS Conditions

Tune	Auto-tune
Acquisition	EI-MS/MS 200-380 da
Solvent delay	7.0 minutes
MS temperatures	Trap 210 °C, manifold 50 °C, transfer line 310 °C

Compound	Rt(min)	Precursor	Quant ion	Qualifiers	Excit volt	Filament	Multiplier	Target
THC d-3	7.921	374	308	292/268	0.4 V	50 µA	+100 V	5,000
THC	7.944	371	305	289/265	0.4 V	50 µA	+100 V	5,000
OH-THC d-3	10.193	374	308	292/268	0.4 V	50 µA	+100 V	5,000
OH-THC	10.225	371	305	289/265	0.4 V	50 µA	+100 V	5,000
THC-COOH d-3	11.731	374	308	292/268	0.4 V	50 µA	+100 V	5,000
THC-COOH	11.765	371	305	289/265	0.4 V	50 µA	+100 V	5,000

Results and Discussion

The following criteria are used to determine the presence and amount of THC,OH-THC and THC-COOH:

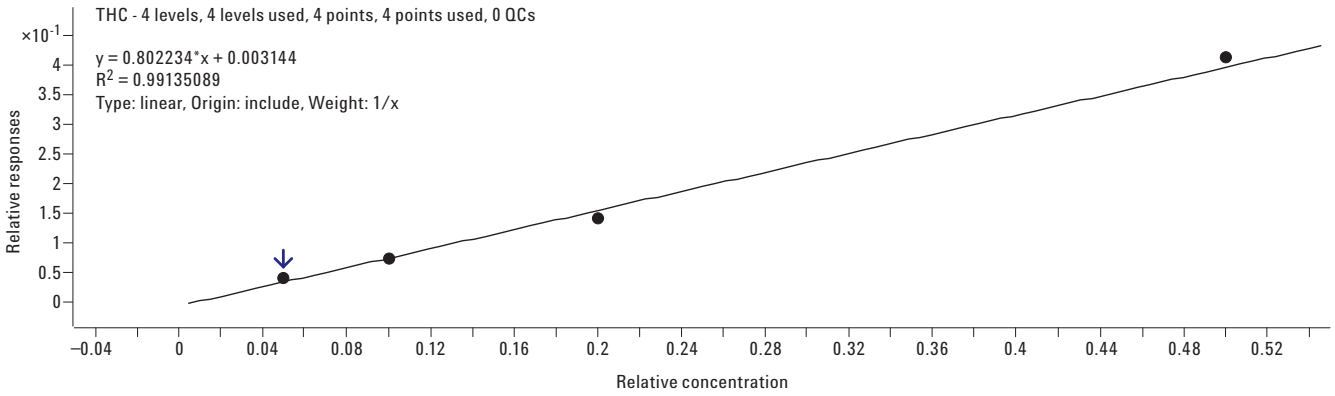
- The chromatography is acceptable (peak resolution, peak symmetry, absence of carryover).
- The selected ions for quantitation and qualification are present.
- Ion ratios are within 20% of the target values determined from the calibration.
- The retention times of the presumed Cannabinoids from the test specimen are within ± 2% of the retention times for the latest calibration.

The area of the Cannabinoids and the internal standard quantitative ions are used for quantitative analysis. Quantitation is accomplished by comparison of the relative response of unknowns and controls against a calibration curve produced from the relative responses for each calibrator concentration. The positive controls must be within their target ranges and the Cannabinoids must be absent in the negative control.

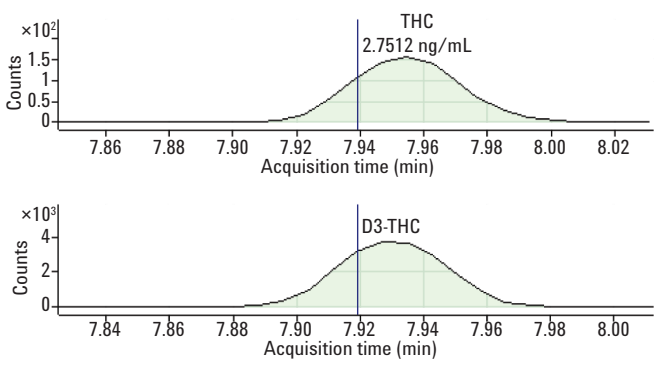
Method Limits

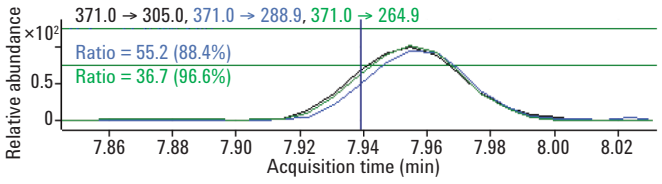
Linearity	2.5–25.0 ng/mL for Delta-9-THC and 11-OH-THC 5.0–150 ng/mL for THC-COOH
Limit of Detection (LOD)	1.0 ng/mL – THC and OH-THC 5.0 ng/mL – THC-COOH
Limit of Quantitation (LOQ)	2.5 ng/mL – THC and OH-THC 10.0 ng/mL – THC-COOH
Carryover	No carryover noted
Interferences	None known

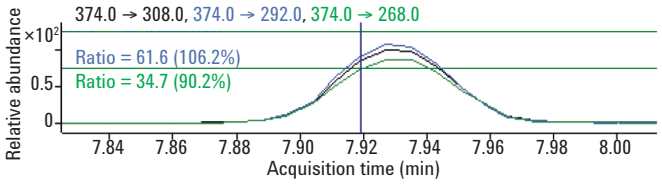
THC Calibration



THC Low Standard 2.5 ng/mL





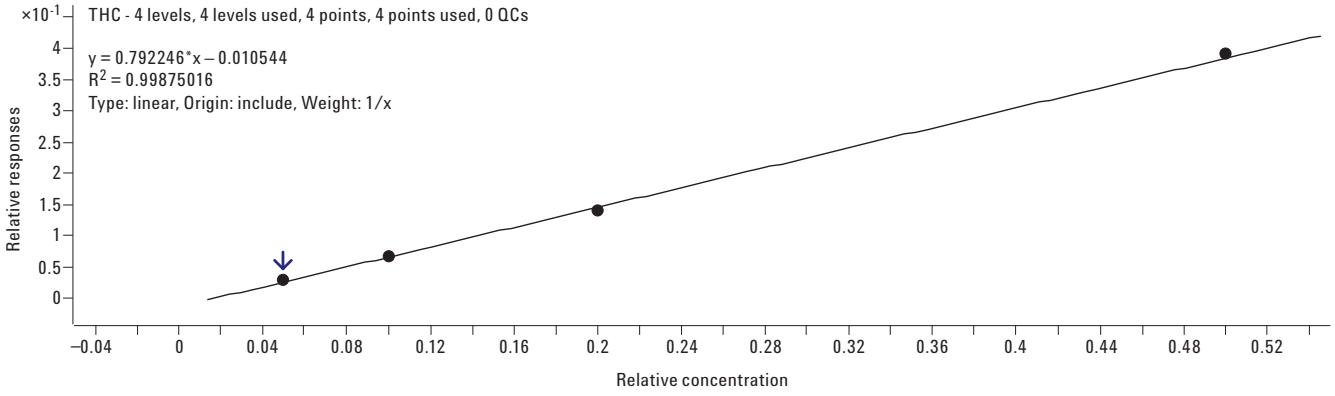


Batch Results

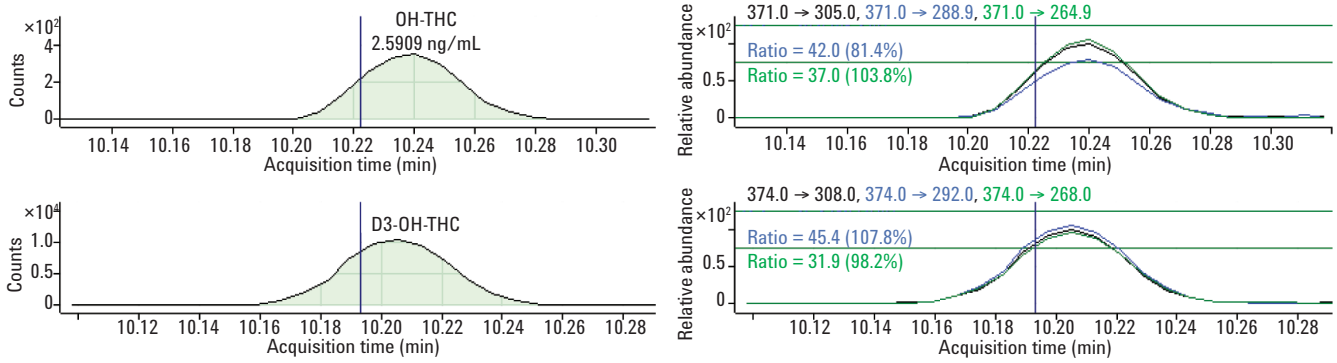
Sample							THC Met...	THC Results						Qualifier...		Qualifier...		D3-THC (ISTD) Re...		Qualifier...		Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
		C1	C1 4-13-2012 1-30-44 PM.SMS.D	Cal	1	4/13/2012 11:30 AM	2.5000	7.955	389		2.7512	2.7512	110.0	55.2		36.7		7.927	9492	61.6		34.7	
		C2	C2 4-13-2012 1-51-14 PM.SMS.D	Cal	2	4/13/2012 11:51 AM	5.0000	7.947	1017		4.8335	4.8335	96.7	62.6		38.6		7.920	13664	52.7		35.3	
		C3	C3 4-13-2012 2-11-55 PM.SMS.D	Cal	3	4/13/2012 12:11 PM	10.0000	7.945	1970		8.9367	8.9367	89.4	60.3		37.8		7.919	14046	46.8		33.3	
		C4	C4 4-13-2012 2-32-20 PM.SMS.D	Cal	4	4/13/2012 12:32 PM	25.0000	7.945	4383		25.9786	25.9786	103.9	62.6		42.5		7.919	10596	57.8		38.4	
		NEG	NEG 4-13-2012 2-53-03 PM.SMS.D	Sample		4/13/2012 12:53 PM												7.918	11371	59.0		31.2	
		LOW	LOW 4-13-2012 3-13-39 PM.SMS.D	Sample		4/13/2012 1:13 PM		7.946	1070		5.3850	5.3850		59.5		40.6		7.919	12851	53.7		36.7	
		HIGH	HIGH 4-13-2012 3-34-11 PM.SMS.D	Sample		4/13/2012 1:34 PM		7.941	3026		23.0828	23.0828		62.0		34.4		7.914	8240	56.5		35.2	
		CNF	CNF 4-13-2012 3-54-39 PM.SMS.D	Sample		4/13/2012 1:54 PM												7.917	9670	55.6		36.4	
		BLK	BLK 4-13-2012 4-15-19 PM.SMS.D	Sample		4/13/2012 2:15 PM																	
		2508	2508 4-13-2012 4-35-39 PM.SMS.D	Sample		4/13/2012 2:35 PM		7.975	1116		7.0053	7.0053		55.7		33.9		7.948	10218	52.1		34.6	
		2512	2512 4-13-2012 4-56-24 PM.SMS.D	Sample		4/13/2012 2:56 PM		7.958	1205		8.9847	8.9847		63.7		37.2		7.939	8542	41.5		32.7	
		2263 B	2263 B 4-13-2012 5-16-51 PM.SMS.D	Sample		4/13/2012 3:16 PM		7.958	829		6.9038	6.9038		56.8		29.9		7.930	7706	55.3		31.2	
		2263 UR	2263 UR 4-13-2012 5-37-13 PM.SMS.D	Sample		4/13/2012 3:37 PM		7.959	257		2.0443	2.0443		52.6		39.1		7.924	8655	53.8		33.2	
		2320 BX4	2320 BX4 4-13-2012 5-57-29 PM.SMS.D	Sample		4/13/2012 3:57 PM												7.921	8127	53.8		35.9	
		2336	2336 4-13-2012 6-18-09 PM.SMS.D	Sample		4/13/2012 4:18 PM		7.968	1377		14.1312	14.1312		57.3		33.3		7.941	6157	51.4		29.6	
		2347	2347 4-13-2012 6-38-30 PM.SMS.D	Sample		4/13/2012 4:38 PM		7.967	435		4.8902	4.8902		60.6		35.6		7.947	5782	54.3		30.3	
		2370	2370 4-13-2012 6-58-29 PM.SMS.D	Sample		4/13/2012 4:58 PM		7.953	1719		10.0982	10.0982		62.6		37.9		7.927	10822	55.6		33.9	
		2371	2371 4-13-2012 7-18-15 PM.SMS.D	Sample		4/13/2012 5:18 PM		7.945	1553		7.9919	7.9919		58.1		42.6		7.919	12414	44.8		33.3	
		2373	2373 4-13-2012 7-38-47 PM.SMS.D	Sample		4/13/2012 5:38 PM		7.949	687		5.3878	5.3878		55.7		38.3		7.922	8250	47.2		28.1	
		2417	2417 4-13-2012 7-59-08 PM.SMS.D	Sample		4/13/2012 5:59 PM												7.934	3496	55.3		32.9	

Note tags for outliers and below calibration.

OH-THC Calibration



OH-THC Low Standard 2.5 ng/mL

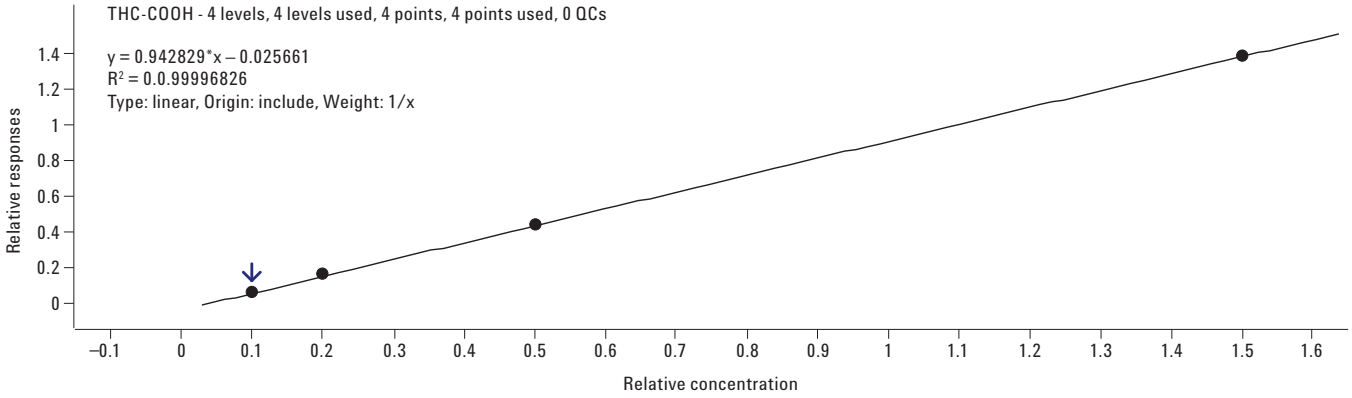


Batch Results

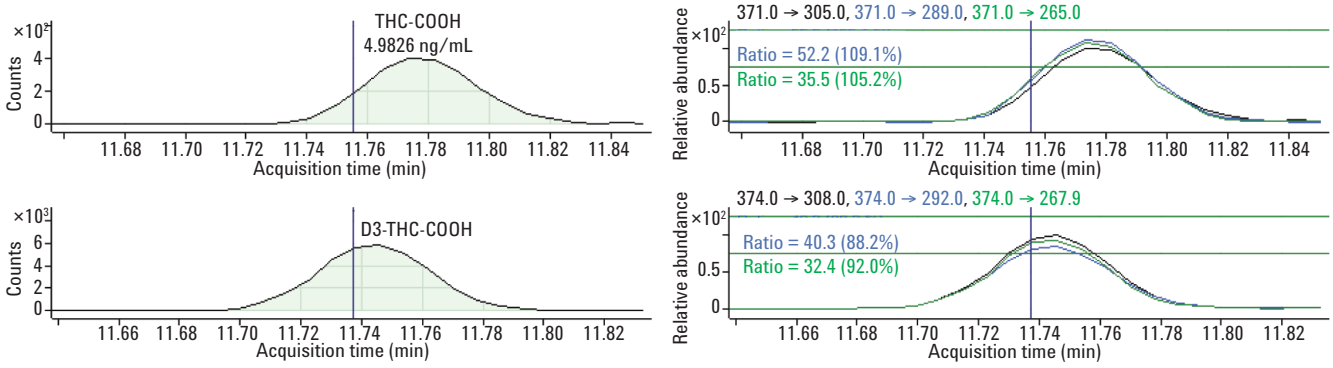
Sample							OH-THC...	OH-THC Results						Qualifier...		Qualifier...		D3-OH-THC (I...		Qualifier...		Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
		C1	C1 4-13-2012 1-30-44 PM.SMS.D	Cal	1	4/13/2012 11:30 AM	2.5000	10.240	827		2.5909	2.5909	103.6	42.0		37.0		10.205	27114	45.4		31.9	
		C2	C2 4-13-2012 1-51-14 PM.SMS.D	Cal	2	4/13/2012 11:51 AM	5.0000	10.228	2285		4.9638	4.9638	99.3	52.9		30.2		10.194	33553	47.0		32.4	
		C3	C3 4-13-2012 2-11-55 PM.SMS.D	Cal	3	4/13/2012 12:11 PM	10.0000	10.223	4943		9.5514	9.5514	95.5	47.5		35.0		10.197	35105	43.9		32.1	
		C4	C4 4-13-2012 2-32-20 PM.SMS.D	Cal	4	4/13/2012 12:32 PM	25.0000	10.227	11132		25.3939	25.3939	101.6	51.8		35.7		10.194	28411	42.0		32.4	
		NEG	NEG 4-13-2012 2-53-03 PM.SMS.D	Sample		4/13/2012 12:53 PM												10.194	30781	44.2		32.7	
		LOW	LOW 4-13-2012 3-13-39 PM.SMS.D	Sample		4/13/2012 1:13 PM		10.222	2517		5.6426	5.6426		49.8		35.1		10.195	31919	41.6		34.5	
		HIGH	HIGH 4-13-2012 3-34-11 PM.SMS.D	Sample		4/13/2012 1:34 PM		10.225	5785		19.2529	19.2529		51.5		36.6		10.191	19641	46.0		32.9	
		CNF	CNF 4-13-2012 3-54-39 PM.SMS.D	Sample		4/13/2012 1:54 PM												10.192	26546	42.8		31.0	
		BLK	BLK 4-13-2012 4-15-19 PM.SMS.D	Sample		4/13/2012 2:15 PM																	
		2508	2508 4-13-2012 4-35-39 PM.SMS.D	Sample		4/13/2012 2:35 PM		10.221	877		3.1229	3.1229		49.8		26.3		10.194	22521	46.0		33.5	
		2512	2512 4-13-2012 4-56-24 PM.SMS.D	Sample		4/13/2012 2:56 PM		10.221	376		2.7540	2.7540		49.5		39.2		10.193	11366	46.7		32.9	
		2263 B	2263 B 4-13-2012 5-16-51 PM.SMS.D	Sample		4/13/2012 3:16 PM		10.224	206		1.4184	1.4184		65.7				10.190	17255	45.3		30.7	
		2263 UR	2263 UR 4-13-2012 5-37-13 PM.SMS.D	Sample		4/13/2012 3:37 PM		10.221	6243		14.4895	14.4895		52.0		32.0		10.195	28501	46.7		32.2	
		2320 BX4	2320 BX4 4-13-2012 5-57-29 PM.SMS.D	Sample		4/13/2012 3:57 PM												10.184	3590	42.4		30.0	
		2336	2336 4-13-2012 6-18-09 PM.SMS.D	Sample		4/13/2012 4:18 PM		10.218	202		2.7980	2.7980		55.5				10.192	5980	40.5		33.7	
		2347	2347 4-13-2012 6-38-30 PM.SMS.D	Sample		4/13/2012 4:38 PM												10.192	11133	42.1		31.2	
		2370	2370 4-13-2012 6-58-29 PM.SMS.D	Sample		4/13/2012 4:58 PM		10.221	3123		4.7635	4.7635		44.2		34.5		10.187	48099	42.8		32.3	
		2371	2371 4-13-2012 7-18-15 PM.SMS.D	Sample		4/13/2012 5:18 PM		10.219	319		1.1324	1.1324		45.8				10.184	43056	46.9		33.8	
		2373	2373 4-13-2012 7-38-47 PM.SMS.D	Sample		4/13/2012 5:38 PM		10.220	510		2.7710	2.7710		52.2		33.6		10.186	15278	39.7		28.2	
		2417	2417 4-13-2012 7-59-08 PM.SMS.D	Sample		4/13/2012 5:59 PM												10.184	7599	46.6		30.3	

Note tags for outliers and below calibration.

THC-COOH Calibration



THC-COOH Low Standard 5.0 ng/mL



Batch Results

Sample							THC-CO...	THC-COOH Results						Qualifier...		Qualifier...		D3-THC-COO...		Qualifier...		Qualifier...	
		Name	Data File	Type	Level	Acq. Date-Time	Exp. Conc.	RT	Resp.	MI	Calc. Conc.	Final Conc.	Accuracy	Ratio	MI	Ratio	MI	RT	Resp.	Ratio	MI	Ratio	MI
		C1	C1 4-13-2012 1-30-44 PM.SMS.D	Cal	1	4/13/2012 11:30 AM	5.0000	11.774	1083		4.9826	4.9826	99.7	52.2		35.5		11.746	15855	40.3		32.4	
		C2	C2 4-13-2012 1-51-14 PM.SMS.D	Cal	2	4/13/2012 11:51 AM	10.0000	11.768	3349		10.0907	10.0907	100.9	49.1		34.3		11.734	20344	47.7		32.4	
		C3	C3 4-13-2012 2-11-55 PM.SMS.D	Cal	3	4/13/2012 12:11 PM	25.0000	11.760	8764		24.8270	24.8270	99.3	52.0		31.6		11.734	19806	47.6		33.4	
		C4	C4 4-13-2012 2-32-20 PM.SMS.D	Cal	4	4/13/2012 12:32 PM	75.0000	11.765	21168		75.0997	75.0997	100.1	47.9		33.9		11.739	15224	45.5		35.0	
		NEG	NEG 4-13-2012 2-53-03 PM.SMS.D	Sample		4/13/2012 12:53 PM		11.765	189		1.8879	1.8879						11.737	19027	46.9		33.4	
		LOW	LOW 4-13-2012 3-13-39 PM.SMS.D	Sample		4/13/2012 1:13 PM		11.764	1981		6.5902	6.5902		49.4		37.7		11.729	20092	42.4		32.7	
		HIGH	HIGH 4-13-2012 3-34-11 PM.SMS.D	Sample		4/13/2012 1:34 PM		11.759	5469		23.0919	23.0919		53.3		37.7		11.732	13347	49.1		33.0	
		CNF	CNF 4-13-2012 3-54-39 PM.SMS.D	Sample		4/13/2012 1:54 PM		11.760	4785		15.6866	15.6866		53.0		36.0		11.734	17713	48.1		32.6	
		BLK	BLK 4-13-2012 4-15-19 PM.SMS.D	Sample		4/13/2012 2:15 PM																	
		2508	2508 4-13-2012 4-35-39 PM.SMS.D	Sample		4/13/2012 2:35 PM		11.762	20071		114.3907	114.3907		50.5		33.5		11.728	9417	47.7		34.4	
		2512	2512 4-13-2012 4-56-24 PM.SMS.D	Sample		4/13/2012 2:56 PM		11.759	8771		75.5572	75.5572		43.6		35.8		11.732	6269	46.0		30.5	
		2263 B	2263 B 4-13-2012 5-16-51 PM.SMS.D	Sample		4/13/2012 3:16 PM		11.761	4498		24.2553	24.2553		53.6		39.2		11.726	10419	45.1		30.0	
		2263 UR	2263 UR 4-13-2012 5-37-13 PM.SMS.D	Sample		4/13/2012 3:37 PM		11.763	69047		215.6868	215.6868		45.9		33.2		11.730	17085	50.5		32.2	
		2320 BX4	2320 BX4 4-13-2012 5-57-29 PM.SMS.D	Sample		4/13/2012 3:57 PM		11.759	1147		32.5571	32.5571		52.1		33.6		11.725	1950	41.5		32.3	
		2336	2336 4-13-2012 6-18-09 PM.SMS.D	Sample		4/13/2012 4:18 PM		11.760	1702		30.6614	30.6614		52.3		37.3		11.725	3081	46.2		32.2	
		2347	2347 4-13-2012 6-38-30 PM.SMS.D	Sample		4/13/2012 4:38 PM		11.759	2348		21.7280	21.7280		46.1		35.0		11.723	6114	44.2		32.3	
		2370	2370 4-13-2012 6-58-29 PM.SMS.D	Sample		4/13/2012 4:58 PM		11.755	13738		48.7563	48.7563		46.8		31.1		11.729	15372	43.2		28.6	
		2371	2371 4-13-2012 7-18-15 PM.SMS.D	Sample		4/13/2012 5:18 PM		11.753	23757		86.0749	86.0749		46.5		33.3		11.727	14872	43.5		31.0	
		2373	2373 4-13-2012 7-38-47 PM.SMS.D	Sample		4/13/2012 5:38 PM		11.756	11674		75.4346	75.4346		45.6		33.9		11.730	8358	47.7		36.4	
		2417	2417 4-13-2012 7-59-08 PM.SMS.D	Sample		4/13/2012 5:59 PM		11.753	4399		42.0538	42.0538		52.0		38.4		11.726	5733	48.2		33.6	

Note tags for outliers and below calibration.

Conclusions

This application note presents a sensitive, selective, and robust method to determine Pyrovalerone Analogs in biological samples using Ropivacaine as an internal standard. For the analysis of Pyrovalerone Analogs, the benefits of GC Quadrupole Ion Trap MS\MS cannot be underestimated. In terms of reducing sample matrix interference, improving signal-to-noise and coupling its high selectivity, and sensitivity the GC Quadrupole Ion Trap MS\MS provides a more confidence driven solution for the analysis of Pyrovalerone Analogs. GC Quadrupole Ion Trap MS\MS analysis has the potential to reduce false positive and negatives as well as providing an additional degree of confidence in the results obtained. Using the optimized method listed above, a fast, targeted GC/MS/MS analytical method can be used to solve the current Pyrovalerone Analog analysis problem facing forensic laboratories. Positive controls were used in conjunction with negative controls to assure accurate quantification and rule out false negatives in the unknown biological samples. Low nanogram/mL detection limits were observed for Pyrovalerone Analogs in various sample matrices.

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For More Information

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