Worklist: 1661

LAB CASE	ITEM	TASK ID	DESCRIPTION	
C2017-0556	1	82011	AM 27 Blood THC Quant by LC	
C2017-0557	1	82012	AM 27 Blood THC Quant by LC	
C2017-0601	1	82013	AM 27 Blood THC Quant by LC	
C2017-0608	1	82014	AM 27 Blood THC Quant by LC	
M2017-1280	1	82015	AM 27 Blood THC Quant by LC	
M2017-1308	2	82016	AM 27 Blood THC Quant by LC	

Quantitation of THC and Metabolites in Blood by LC-MS/MS

Extraction Date: 4-7X-17 13   **Analyst:** Anne Nord

PRE-ANALYTIC   0499102

Plate Lot# Custom - ~~0490364~~ *A*   **Plate Exp.** ~~9-21-2017~~ *A* 1-28-2018   **External QC** Lot 61317, exp 6-13-17

√ 1. Ensure all solutions are within expiration date.

- Mobile Phase A: *0.1% Formic Acid in LCMS Water*   • *0.1% Formic Acid in water*
- Mobile Phase B: *0.1% Formic Acid in LCMS Acetonitrile*   • *MTBE*
- *LCMS Methanol*   • *Hexane*
- **Blank/Negative Blood: Lot** *321632-1*
  Column: *UCT Selectra DA 100 x 2.1 mm 3um*

√ 2. Check levels of mobile phases and needle wash and refill as necessary. Ensure waste is not full.

√ 3. Purge Pump and Load appropriate Acq. Method, allow system to equilibrate for approx. 30 min.

√ 4. Create worklist. Data path name: 4-13-17 THC Quant

ANALYTIC

√ 1. Remove standards plate, blood, and samples from cold storage. Allow to reach room temperature.

√ 2. Add *1000 µL blood* to wells of analytical (standards) plate. Mix via aspirate and dispense. Place cover on Plate
Blank blood for locations containing standards/QCs and internal standards
Sample blood for locations containing only internal standards

√ 3. Place on shaking incubator at ambient temp., **900rpm for 15 minutes.** *Shaker ID 66759*

√ 4. Pipette *500µL 0.1% formic acid* to all wells of standards plate.

√ 5. Place on shaking incubator at ambient temp., **900rpm for 15 minutes.**

√ 6. Transfer *800µL of blood+acid mixture* to corresponding wells of SLE+ plate.

√ 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent). Wait 5 min.
(*Load blood samples at 85- 100 PSI- Selector to Right*)

√ 8. Add *2.25mL MTBE* and allow to flow under gravity for 5 minutes. *(add in 3 increments of 750uL)*

√ 9. Apply positive pressure for approx. 15 seconds *(10-15 PSI- Selector to left -.*

√ 10. Add *2.25mL Hexane* and allow to flow under gravity for 5 minutes. *. (add in 3 increments of 750uL)*

√ 11. Apply positive pressure for approx. 15 seconds. *(10-15 PSI Selector to the left)*

√ 12. Remove collection plate containing eluate.

√ 13. Place collection plate on SPE Dry and evaporate to dryness at approx. 35°C. *SPE Dry ID 66819*

√ 14. Reconstitute in *100 µL MeOH* and heat seal plate with foil. Place in autosampler and run worklist.

POST-ANALYTIC

√ 1. Open quantitation software and create a new quantitation batch.
Batch name: 41317 cann quant

√ 2. Make any necessary integration changes. Limit curves based on validated linear ranges (3-50ng/mL).

√ 3. Were all appropriate standards used in the curve for each analyte? Y / N
Are r² values ≥0.98 for each analyte? Y / N

√ 4. Did all QCs pass for each analyte? Ⓨ/ N   Were QCs entered into QC charting? Y / N

√ 5. Central File Packet to include: √ LIMS Worklist: √ Method Checklist √ Calibration and
Control Reports

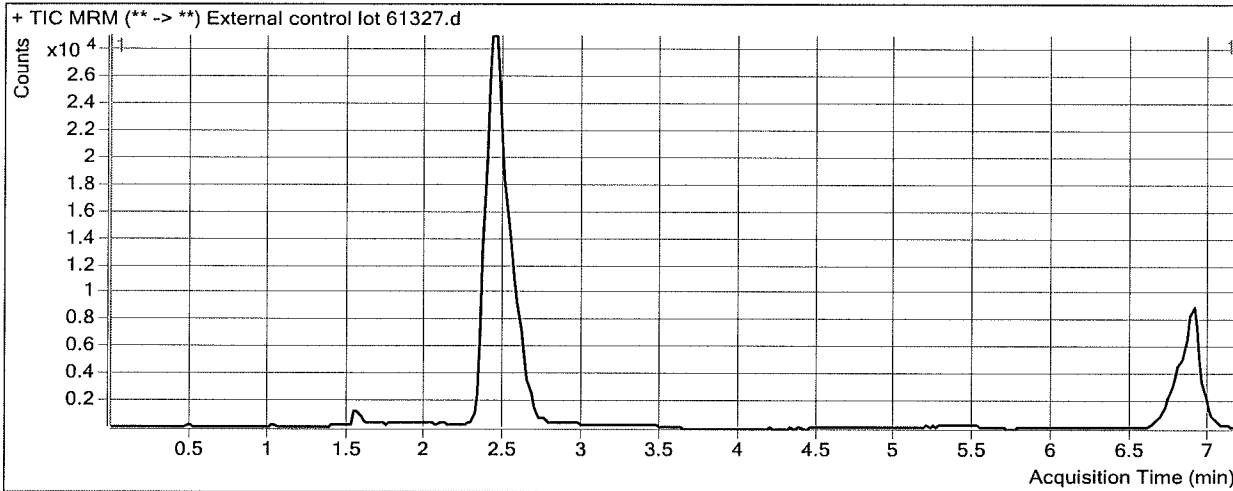COMMENTS

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path   D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin

Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 13:10	**Data File**	External control lot 61327.d
Sample Type	Sample	**Sample Name**	External control lot 61327
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	p2c2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation 10 ng

Sample Chromatogram

+ TIC MRM (** -> **) External control lot 61327.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	23141	202980	0.1140	12.5124
THC-COOH	THC-COOH-d9	2.586	10352	59261	0.1747	9.2082
THC	THC-d3	6.893	7695	74923	0.1027	10.2817

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin		
Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 12:58	**Data File**	QC 10.d
Sample Type	QC	**Sample Name**	QC 10
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-H1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation

Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	17831	197705	0.0902	9.8934
THC-COOH	THC-COOH-d9	2.606	9051	58205	0.1555	8.1708
THC	THC-d3	6.913	7198	67939	0.1059	10.6237

ISP FORENSICS - Cd'A Instrument # 62340
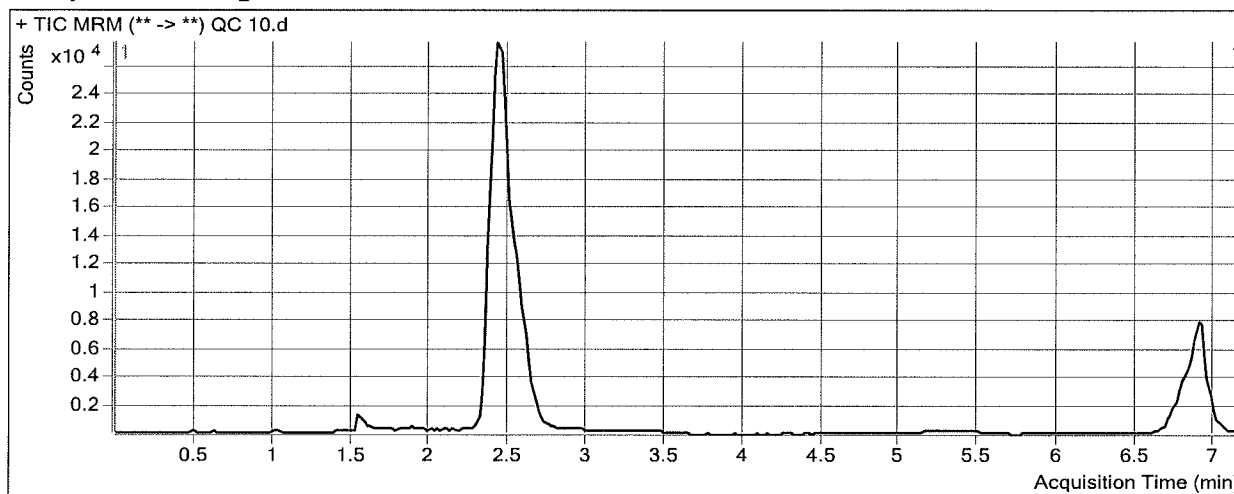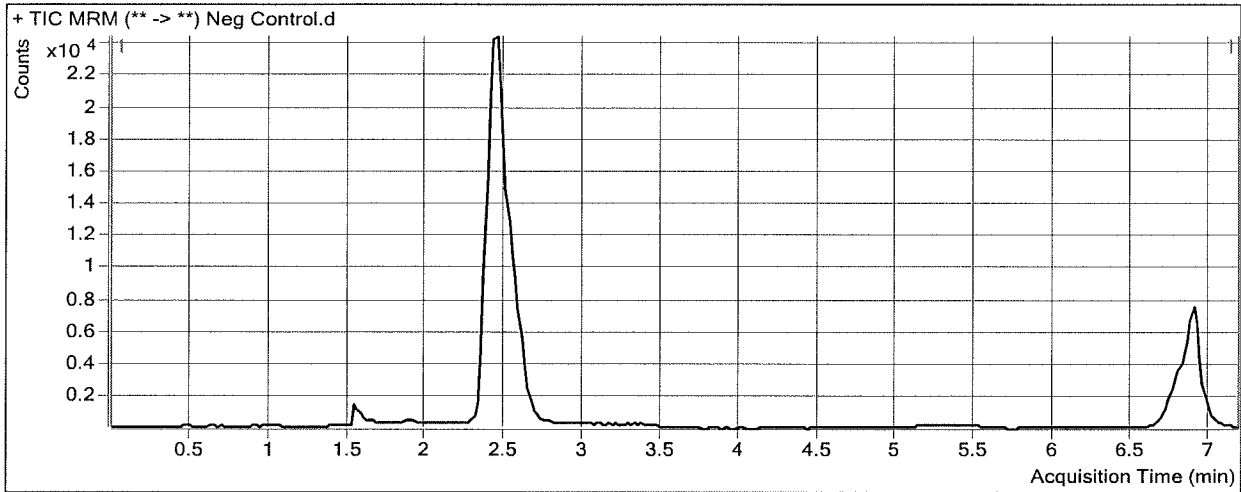# Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin

Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 12:46	**Data File**	Neg Control.d
Sample Type	Sample	**Sample Name**	Neg Control
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-a2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation

Sample Chromatogram

ISP Forensics
Calibration Curve Report

Batch Data Path   D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin

Last Calib Update   4/15/2017 7:11 AM   **Analyst Name**   ISP TOX

Target Compound   *THC-OH*
Internal Standard   *THC-OH-d3*

THC-OH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs

$y = 0.009094 * x + 2.201921E\text{-}004$
$R^2 = 0.99872022$
Type:Linear, Origin:Ignore, Weight:1/x

Relative Responses vs *Concentration (ng/ml)*

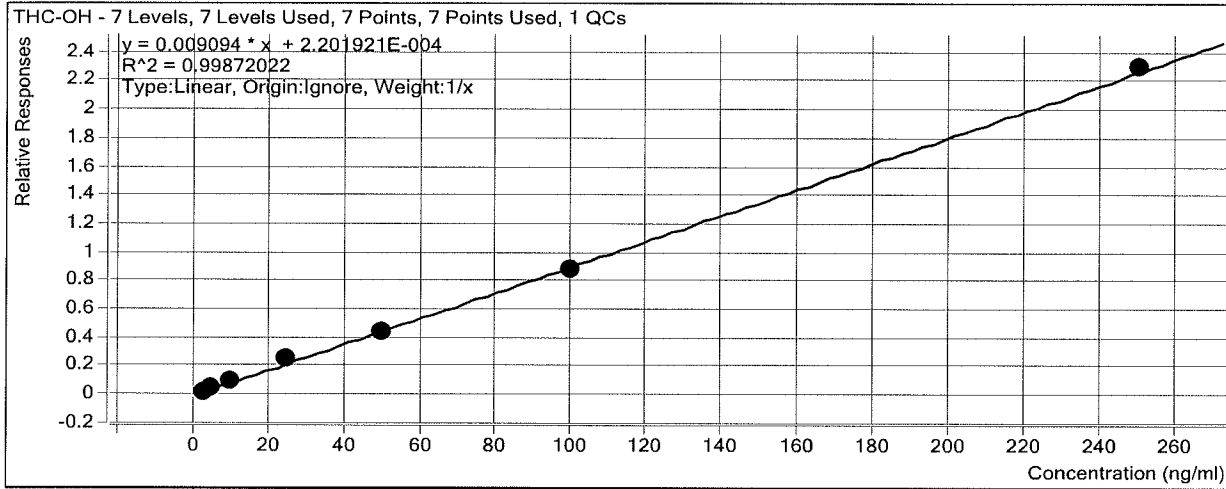| Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☑	3	2.8	92.3
cal 2	2	☑	5	5.2	103.5
cal 3	3	☑	10	10.0	99.8
QC 10	3	☑	10	9.9	98.9
cal 4	4	☑	25	27.6	110.3
cal 5	5	☑	50	48.1	96.2
cal 6	6	☑	100	97.0	97.0
cal 7	7	☑	250	252.4	101.0

ISP Forensics
Calibration Curve Report

Batch Data Path　　　　D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin

Last Calib Update　　　4/15/2017 7:11 AM　　　　**Analyst Name**　　　ISP TOX

Target Compound　　*THC-COOH*
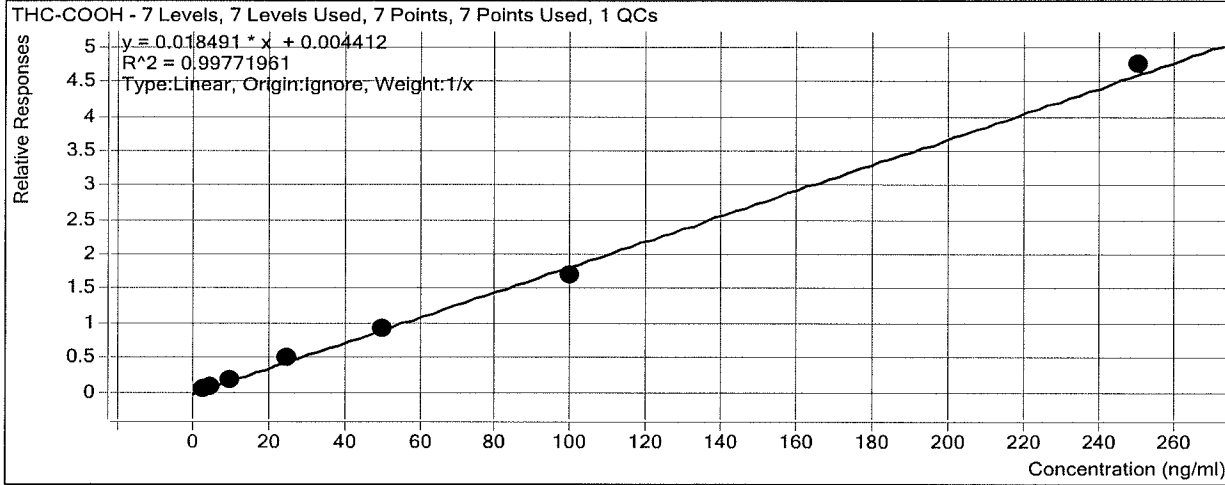*Internal Standard*　　　*THC-COOH-d9*

THC-COOH - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs

$y = 0.018491 * x + 0.004412$
$R^2 = 0.99771961$
Type:Linear, Origin:Ignore, Weight:1/x

Relative Responses vs Concentration (ng/ml)

Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☑	3	3.2	105.7
cal 2	2	☑	5	4.7	93.6
cal 3	3	☑	10	10.1	101.5
QC 10	3	☑	10	8.2	81.7
cal 4	4	☑	25	26.2	105.0
cal 5	5	☑	50	49.5	99.1
cal 6	6	☑	100	92.4	92.4
cal 7	7	☑	250	256.8	102.7

ISP Forensics
Calibration Curve Report

Batch Data Path   D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin

Last Calib Update   4/15/2017 7:11 AM   **Analyst Name**   ISP TOX

Target Compound *THC*
Internal Standard   *THC-d3*
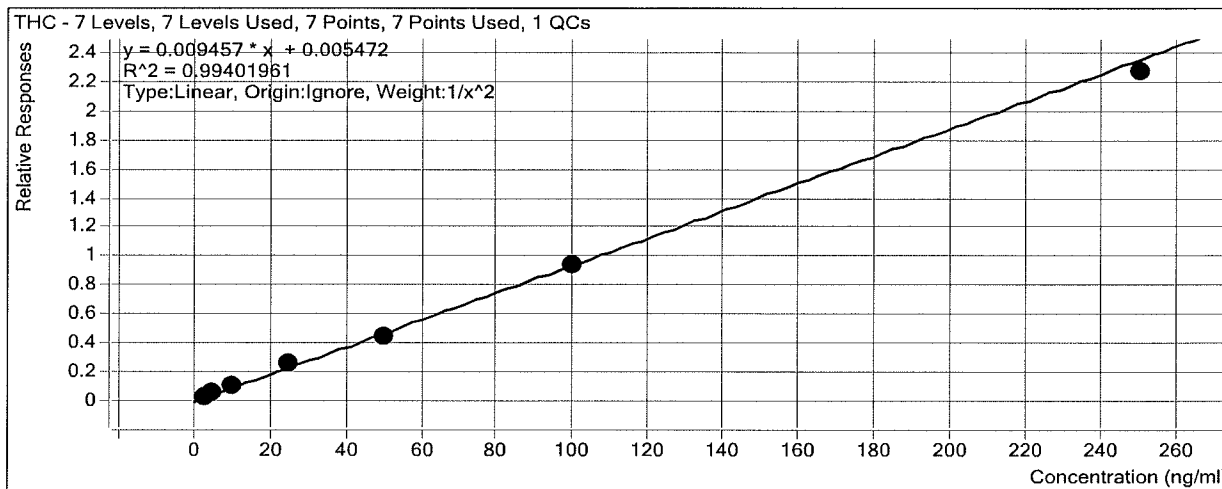
THC - 7 Levels, 7 Levels Used, 7 Points, 7 Points Used, 1 QCs

$y = 0.009457 * x + 0.005472$
$R^2 = 0.99401961$
Type:Linear, Origin:Ignore, Weight:1/x^2

Relative Responses vs Concentration (ng/ml)

| Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☑	3	2.9	95.5
cal 2	2	☑	5	5.2	103.8
cal 3	3	☑	10	10.6	105.5
QC 10	3	☑	10	10.6	106.2
cal 4	4	☑	25	27.3	109.1
cal 5	5	☑	50	45.9	91.7
cal 6	6	☑	100	98.4	98.4
cal 7	7	☑	250	239.6	95.9

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin

Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 11:23	**Data File**	Cal 1a.d
Sample Type	Calibration	**Sample Name**	Cal 1
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-A1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
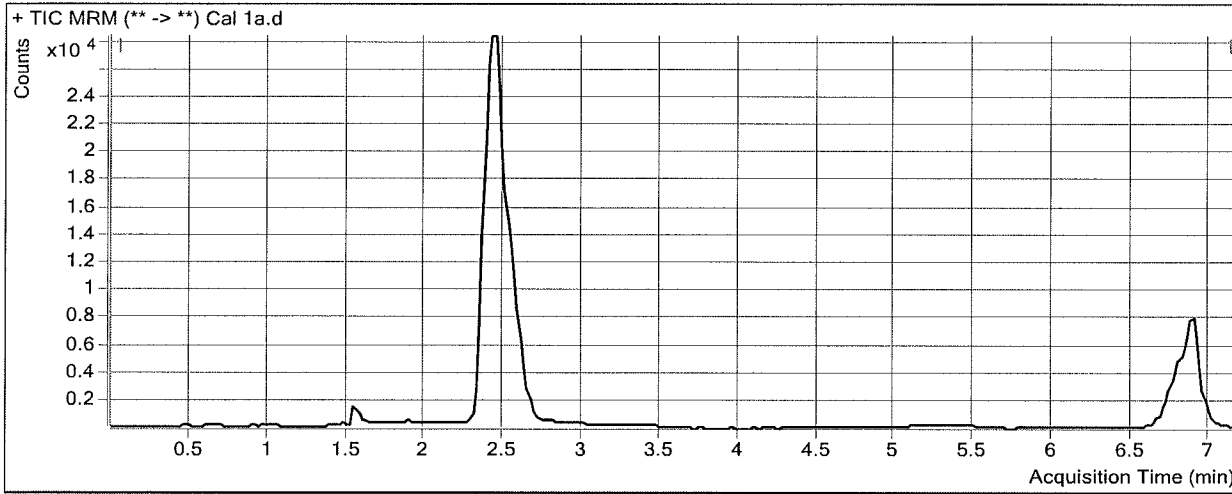
Sample Chromatogram

+ TIC MRM (** -> **) Cal 1a.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	5534	217993	0.0254	2.7675
THC-COOH	THC-COOH-d9	2.606	4166	66072	0.0631	3.1717
THC	THC-d3	6.893	2570	78897	0.0326	2.8660

Batch Data Path	D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin		
Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 11:35	**Data File**	cal 2a.d
Sample Type	Calibration	**Sample Name**	cal 2
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-B1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
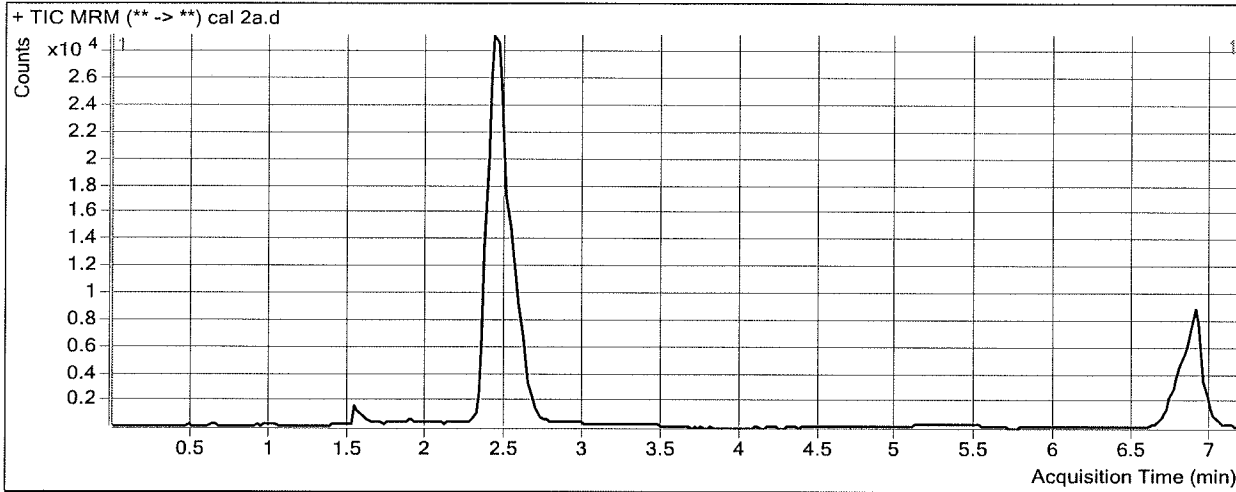
Sample Chromatogram

+ TIC MRM (** -> **) cal 2a.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	10160	214965	0.0473	5.1729
THC-COOH	THC-COOH-d9	2.586	5863	64481	0.0909	4.6791
THC	THC-d3	6.893	4329	79316	0.0546	5.1923

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin		
Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 11:11	**Data File**	cal 3.d
Sample Type	Calibration	**Sample Name**	cal 3
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-C1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
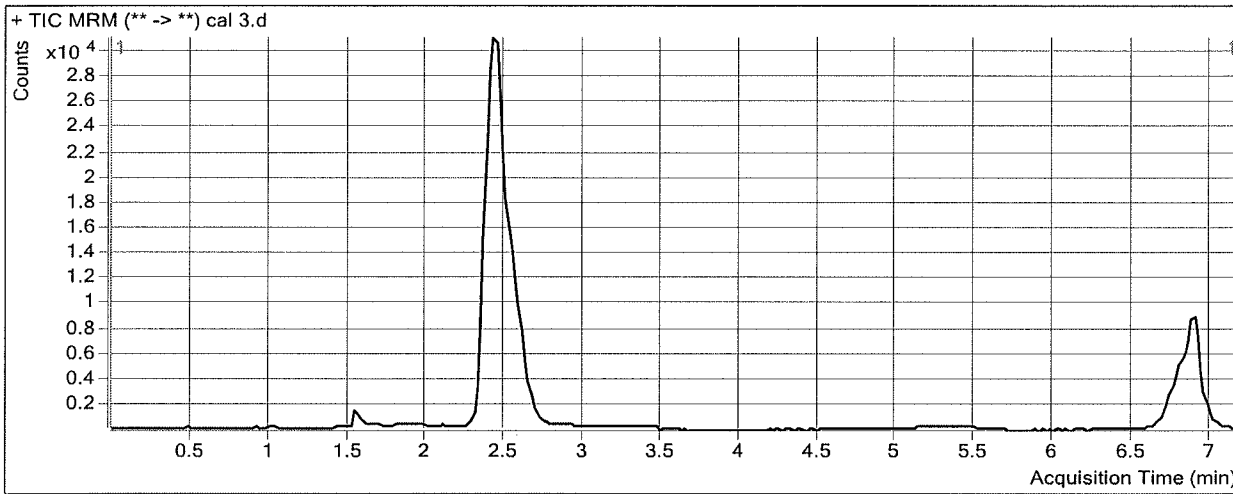
Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	20159	221537	0.0910	9.9820
THC-COOH	THC-COOH-d9	2.586	12713	66189	0.1921	10.1484
THC	THC-d3	6.893	8218	78064	0.1053	10.5530

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin		
Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 11:47	**Data File**	cal 4.d
Sample Type	Calibration	**Sample Name**	cal 4
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-D1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
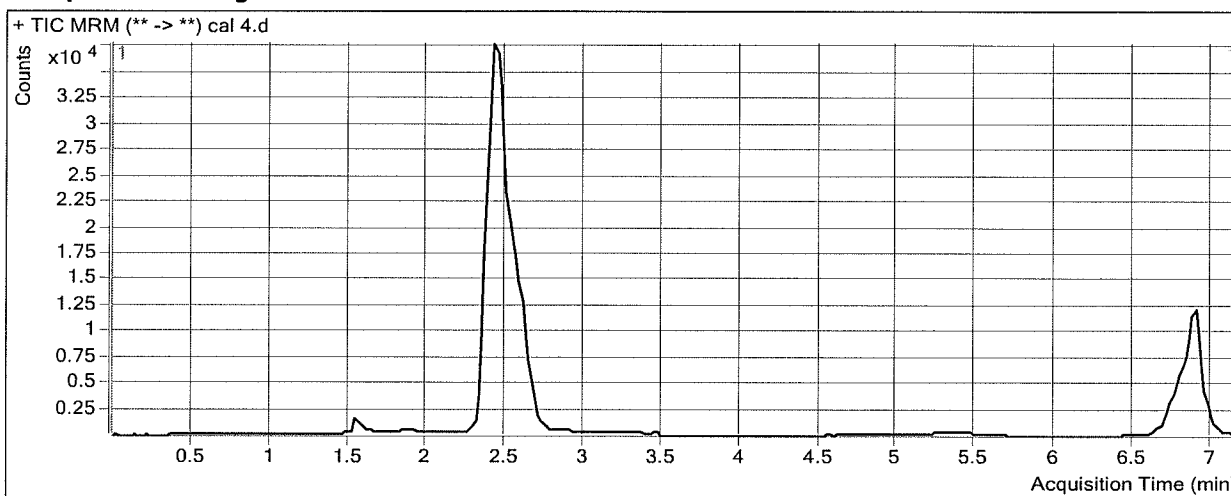
Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	59046	235190	0.2511	27.5831
THC-COOH	THC-COOH-d9	2.606	34885	71225	0.4898	26.2493
THC	THC-d3	6.893	21885	83062	0.2635	27.2812

ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin		
Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 11:59	**Data File**	cal 5.d
Sample Type	Calibration	**Sample Name**	cal 5
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-E1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
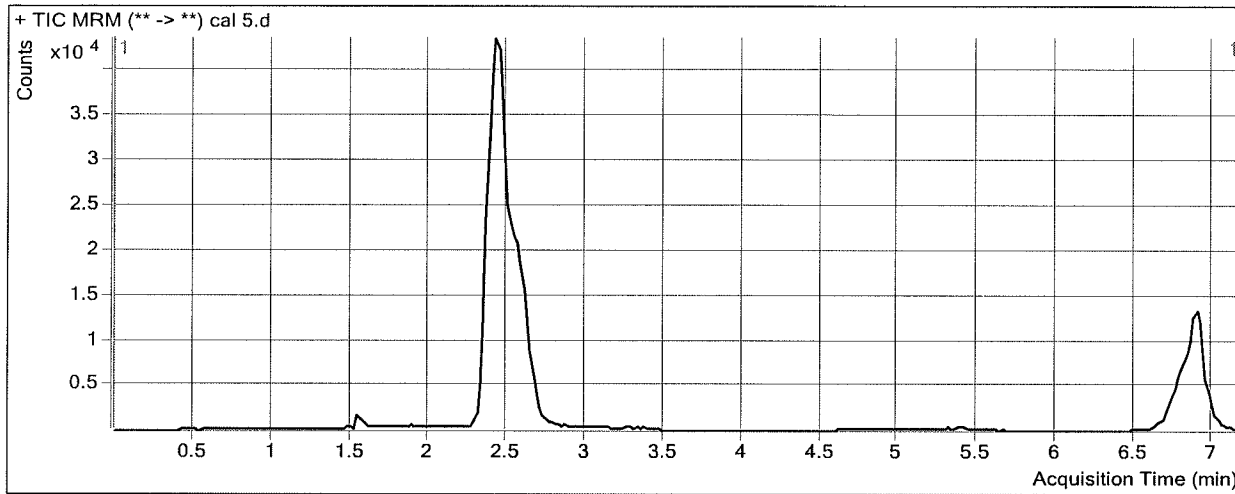
Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	100316	229208	0.4377	48.1034
THC-COOH	THC-COOH-d9	2.586	60524	65770	0.9202	49.5288
THC	THC-d3	6.893	37291	84897	0.4393	45.8678

Batch Data Path	D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin		
Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 12:10	**Data File**	cal 6.d
Sample Type	Calibration	**Sample Name**	cal 6
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-F1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
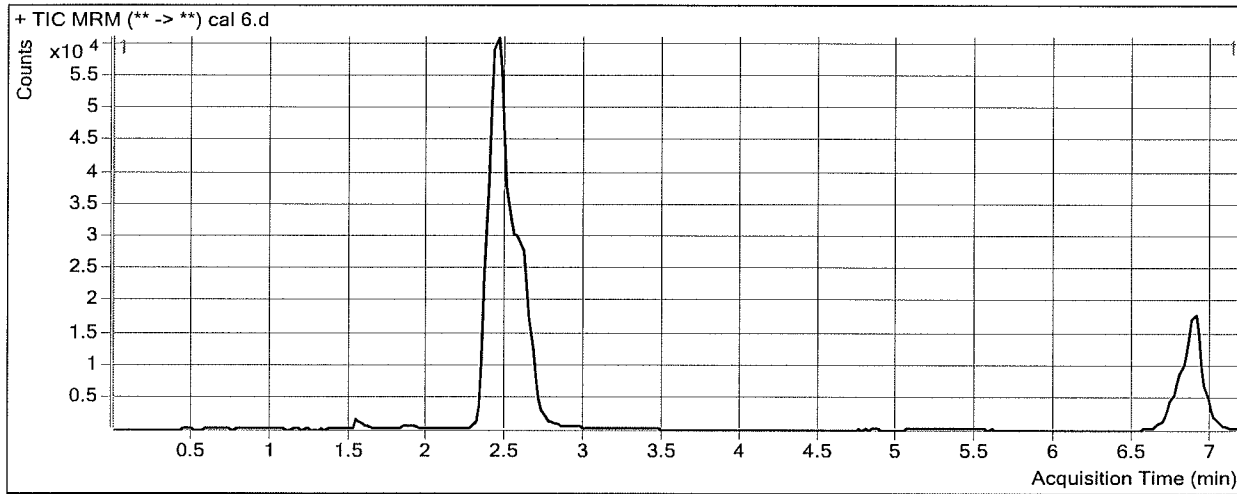
Sample Chromatogram

+ TIC MRM (** -> **) cal 6.d

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	215091	243878	0.8820	96.9602
THC-COOH	THC-COOH-d9	2.606	108577	63355	1.7138	92.4442
THC	THC-d3	6.893	68323	73004	0.9359	98.3802

Batch Data Path	D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin			
Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox	
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox	
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed	

Analysis Info

Acq Time	2017-04-14 12:22	**Data File**	cal 7.d
Sample Type	Calibration	**Sample Name**	cal 7
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-G1	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation
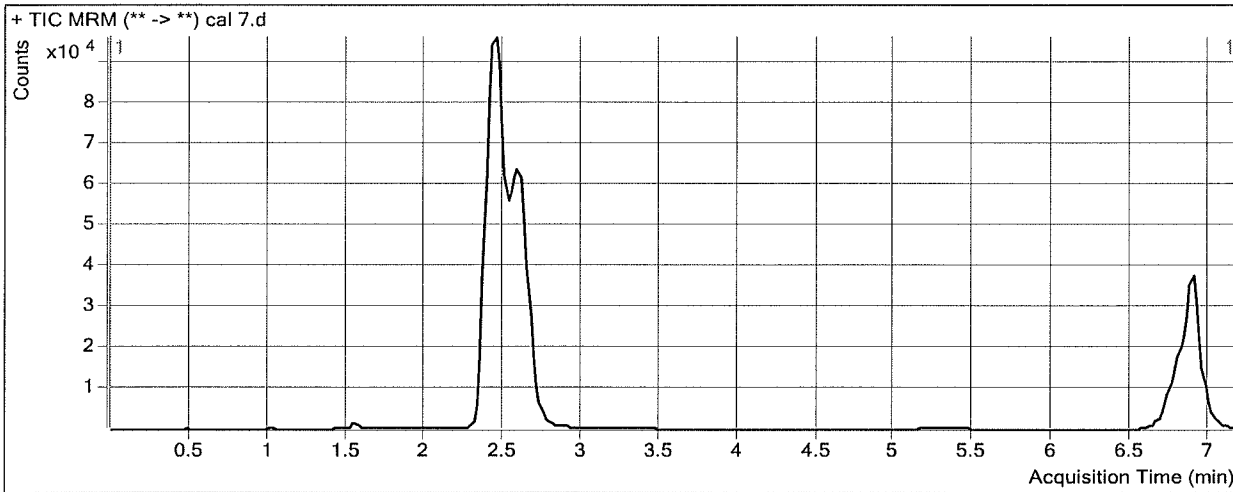
Sample Chromatogram

Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.456	505088	220007	2.2958	252.4308
THC-COOH	THC-COOH-d9	2.606	290627	61153	4.7525	256.7785
THC	THC-d3	6.893	187520	82548	2.2717	239.6265

Batch Data Path   D:\2017 Data\4-13-17 THC Quant\QuantResults\41317 cann quant.batch.bin

Analysis Time	4/15/2017 7:11 AM	**Analyst Name**	ISP Tox
Report Time	4/15/2017 7:13 AM	**Reporter Name**	ISP Tox
Last Calib Update	4/15/2017 7:11 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-04-14 12:34	**Data File**	blank internal std.d
Sample Type	Sample	**Sample Name**	blank internal std
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	Vial 2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation

Sample Chromatogram

+ TIC MRM (** -> **) blank internal std.d