reviewed 8/15/17

8/15/2017

Worklist: 1847

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-1459	2	92399	AM 27 Blood THC Quant by LC
C2017-1467	1	92400	AM 27 Blood THC Quant by LC
C2017-1488	1	92403	AM 27 Blood THC Quant by LC
C2017-1493	1	92401	AM 27 Blood THC Quant by LC
C2017-1495	1	92402	AM 27 Blood THC Quant by LC
C2017-1520	1	92404	AM 27 Blood THC Quant by LC
M2017-3455	1	92405	AM 27 Blood THC Quant by LC
P2017-1759	1	92406	AM 27 Blood THC Quant by LC

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

Extraction Date: 8-8-17 ★ **Analyst:** Anne Nard

PRE-ANALYTIC

Plate Lot# Custom - 499102 Plate Exp. 1/29/2018 **External QC** Lot 91317, exp 9-13-17

√ 1. Ensure all solutions are within expiration date.

- Mobile Phase A: ***0.1% Formic Acid in LCMS Water***
- Mobile Phase B: ***0.1% Formic Acid in LCMS Acetonitrile***
- ***LCMS Methanol***
- **Blank/Negative Blood: Lot *321632-1***
- ***0.1% Formic Acid in water***
- ***MTBE***
- ***Hexane***

√ 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: 8917 cann 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. 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: 8917 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 r^2 values ≥0.98 for each analyte? (Y) / N

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

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

COMMENTS

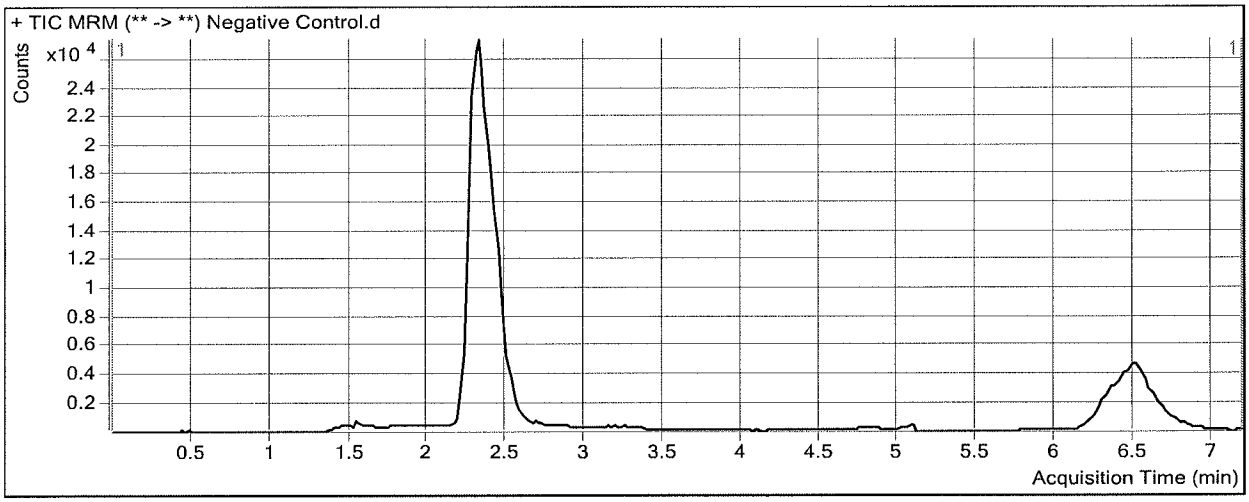
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 11:04	**Data File**	Negative Control.d
Sample Type	Sample	**Sample Name**	Negative Control
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-A2	**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-COOH	THC-COOH-d9	2.366	6243	74804	0.0835	1.3723

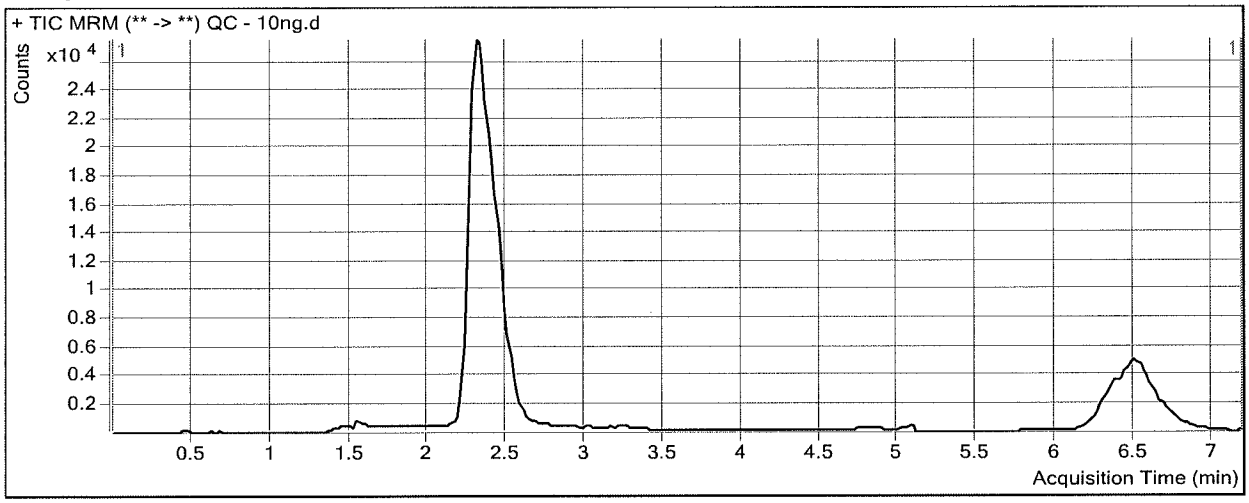
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 11:16	**Data File**	QC - 10ng.d
Sample Type	QC	**Sample Name**	QC - 10ng/mL
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-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	2.336	20548	206921	0.0993	10.5906
THC-COOH	THC-COOH-d9	2.466	20976	70373	0.2981	11.8664
THC	THC-d3	6.493	11413	88303	0.1293	10.8835

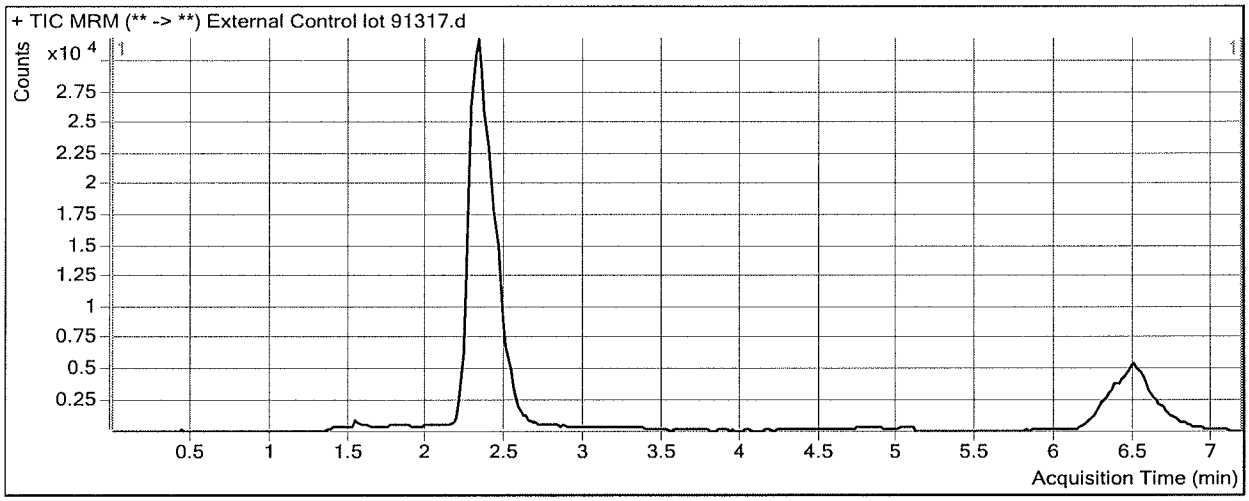
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 11:28	**Data File**	External Control lot 91317.d
Sample Type	Sample	**Sample Name**	External Control lot 91317
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-B2	**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	2.336	21155	239785	0.0882	9.3779
THC-COOH	THC-COOH-d9	2.446	14889	70815	0.2103	7.5723
THC	THC-d3	6.493	8206	94704	0.0867	7.1800

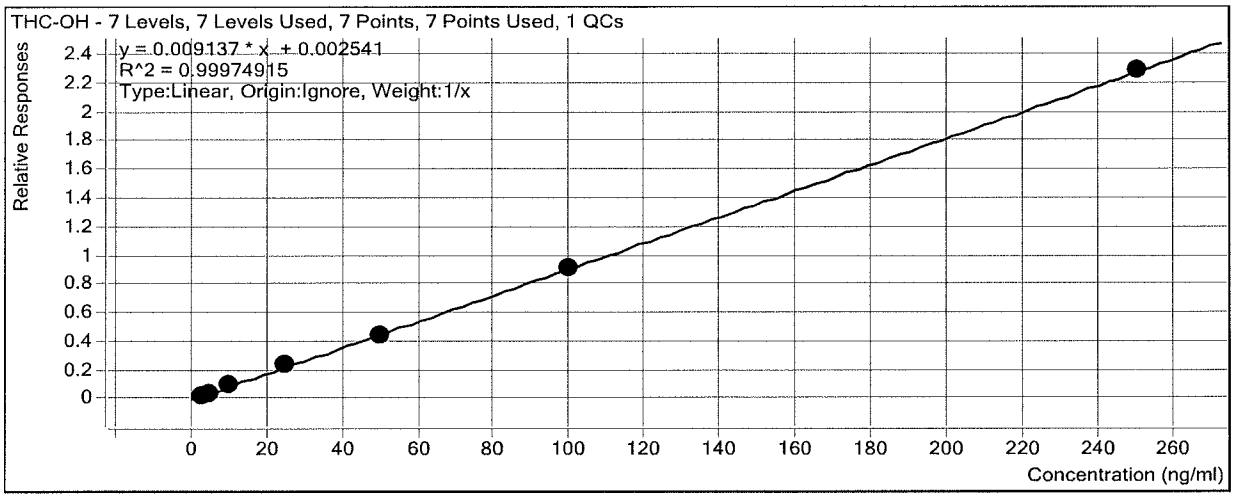
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin

Last Calib Update 8/10/2017 3:47 PM **Analyst Name** ISP TOX

Target Compound *THC-OH*

Internal Standard *THC-OH-d3*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng/mL	1	☑	3	2.9	97.4
Cal 2 - 5ng/mL	2	☑	5	4.7	94.6
Cal 3 - 10ng/mL	3	☑	10	10.7	107.4
QC - 10ng/mL	3	☑	10	10.6	105.9
Cal 4 - 25ng/mL	4	☑	25	25.6	102.2
Cal 5 - 50ng/mL	5	☑	50	49.3	98.6
Cal 6 - 100ng/mL	6	☑	100	99.9	99.9
Cal 7 - 250ng/mL	7	☑	250	249.9	100.0

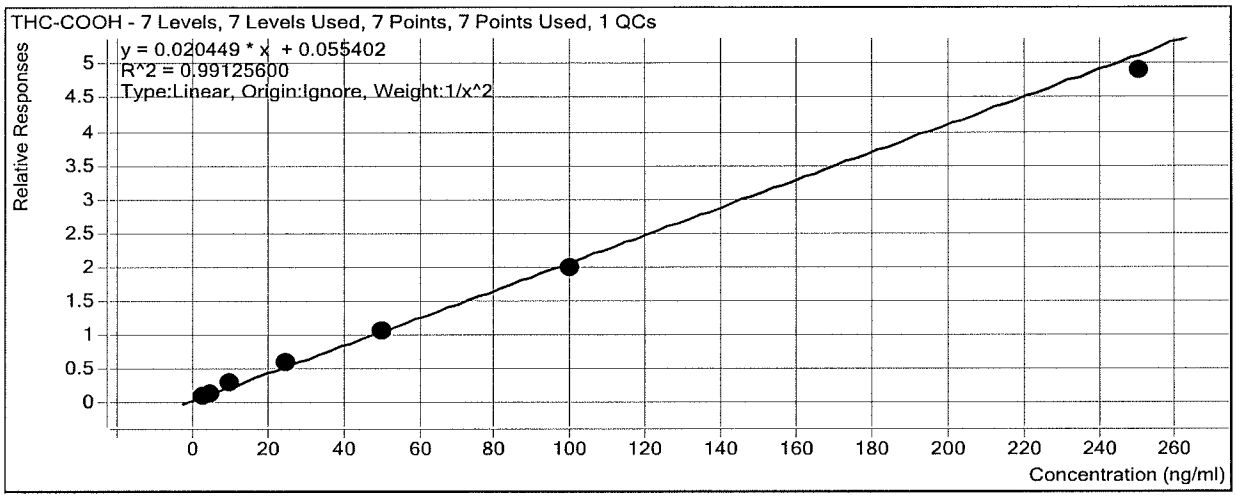
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin

Last Calib Update 8/10/2017 3:47 PM **Analyst Name** ISP TOX

Target Compound *THC-COOH*

Internal Standard *THC-COOH-d9*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng/mL	1	☑	3	3.0	100.2
Cal 2 - 5ng/mL	2	☑	5	4.6	92.1
Cal 3 - 10ng/mL	3	☑	10	11.4	114.1
QC - 10ng/mL	3	☑	10	11.9	118.7
Cal 4 - 25ng/mL	4	☑	25	26.4	105.5
Cal 5 - 50ng/mL	5	☑	50	49.1	98.2
Cal 6 - 100ng/mL	6	☑	100	95.1	95.1
Cal 7 - 250ng/mL	7	☑	250	236.9	94.8

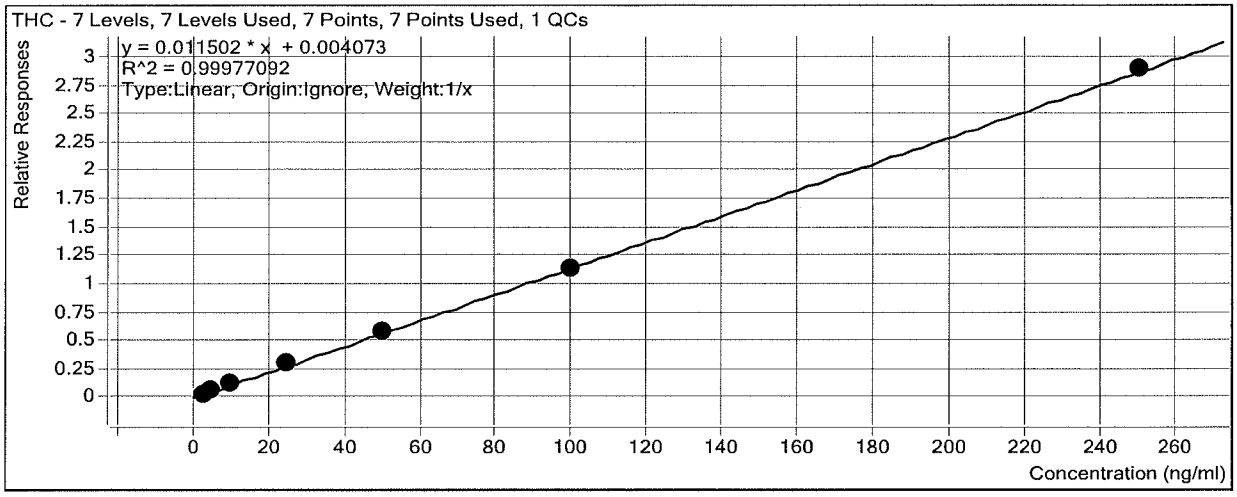
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin

Last Calib Update 8/10/2017 3:47 PM **Analyst Name** ISP TOX

Target Compound *THC*

Internal Standard *THC-d3*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng/mL	1	☑	3	2.9	96.8
Cal 2 - 5ng/mL	2	☑	5	5.0	100.2
Cal 3 - 10ng/mL	3	☑	10	10.1	101.0
QC - 10ng/mL	3	☑	10	10.9	108.8
Cal 4 - 25ng/mL	4	☑	25	25.9	103.6
Cal 5 - 50ng/mL	5	☑	50	50.1	100.1
Cal 6 - 100ng/mL	6	☑	100	97.9	97.9
Cal 7 - 250ng/mL	7	☑	250	251.1	100.5

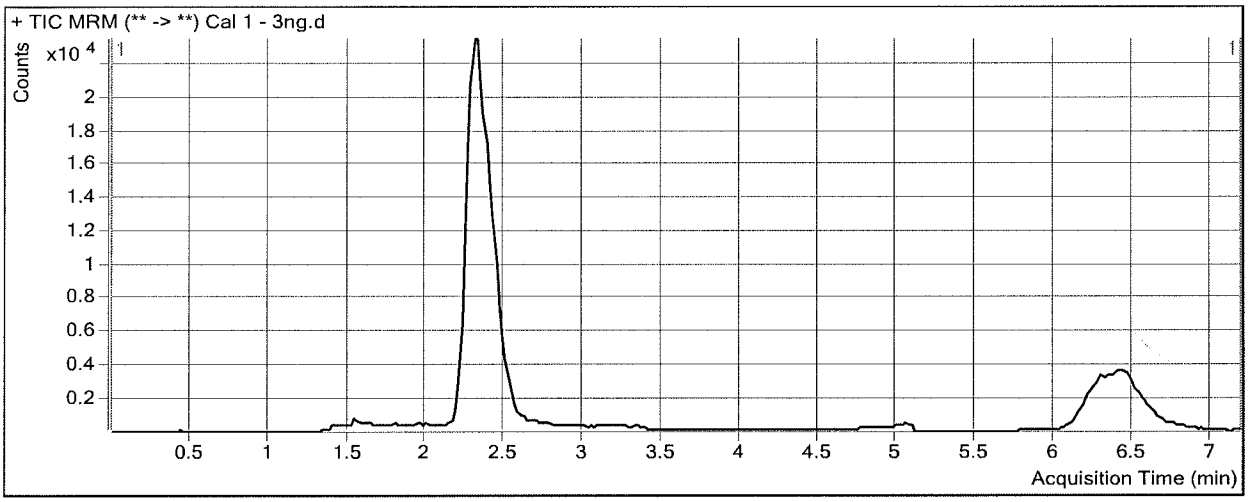
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 09:29	**Data File**	Cal 1 - 3ng.d
Sample Type	Calibration	**Sample Name**	Cal 1 - 3ng/mL
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-A1	**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	2.315	5500	188138	0.0292	2.9217
THC-COOH	THC-COOH-d9	2.426	6944	59420	0.1169	3.0054
THC	THC-d3	6.453	2888	77093	0.0375	2.9027

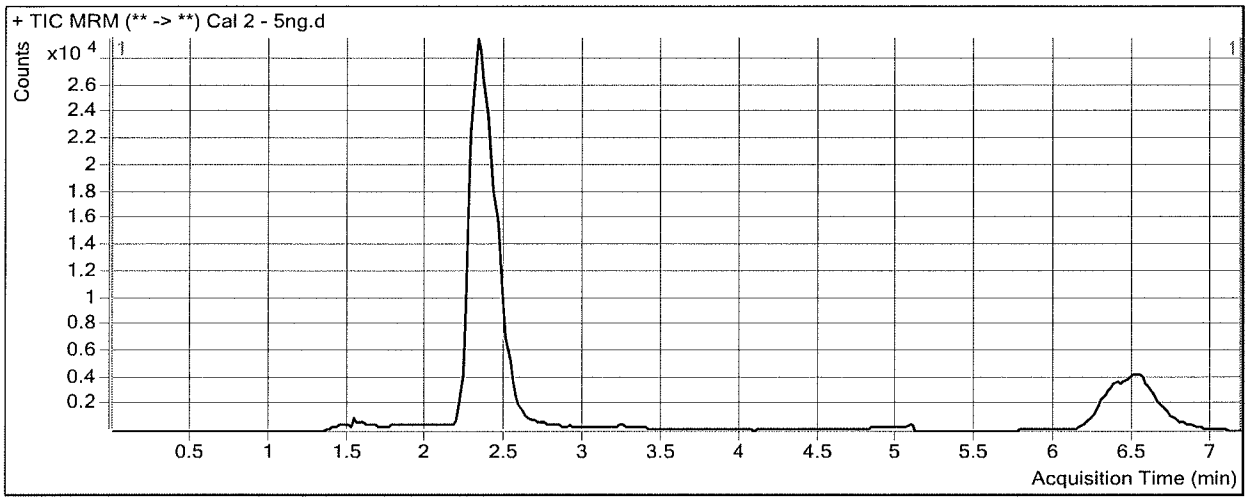
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 09:41	**Data File**	Cal 2 - 5ng.d
Sample Type	Calibration	**Sample Name**	Cal 2 - 5ng/mL
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-B1	**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	2.336	10839	236903	0.0458	4.7297
THC-COOH	THC-COOH-d9	2.446	10718	71674	0.1495	4.6031
THC	THC-d3	6.513	5456	88443	0.0617	5.0094

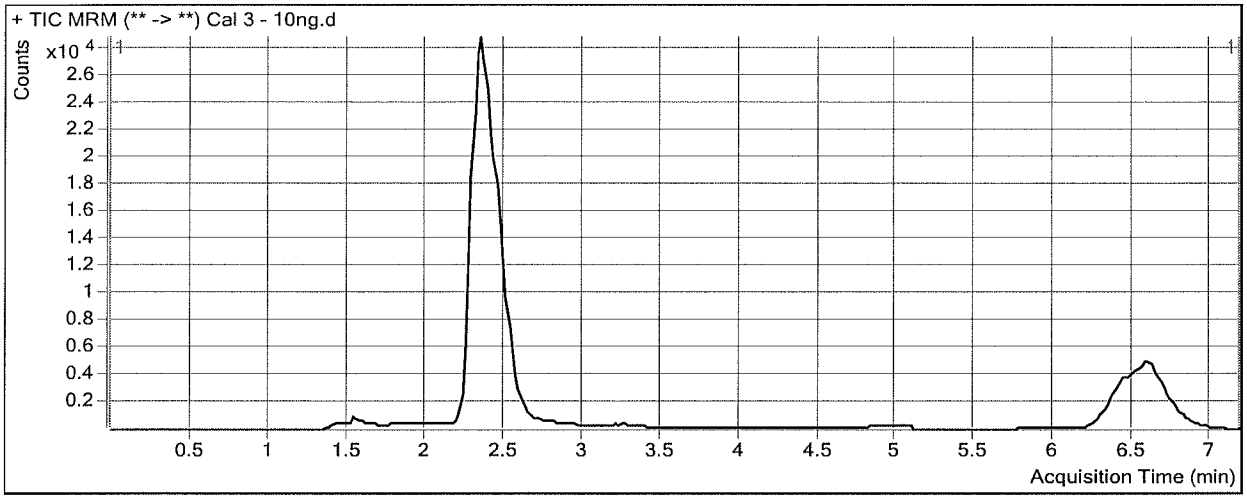
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 09:53	**Data File**	Cal 3 - 10ng.d
Sample Type	Calibration	**Sample Name**	Cal 3 - 10ng/mL
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-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	2.356	21735	215931	0.1007	10.7390
THC-COOH	THC-COOH-d9	2.486	21256	73613	0.2888	11.4113
THC	THC-d3	6.573	10938	90968	0.1202	10.0999

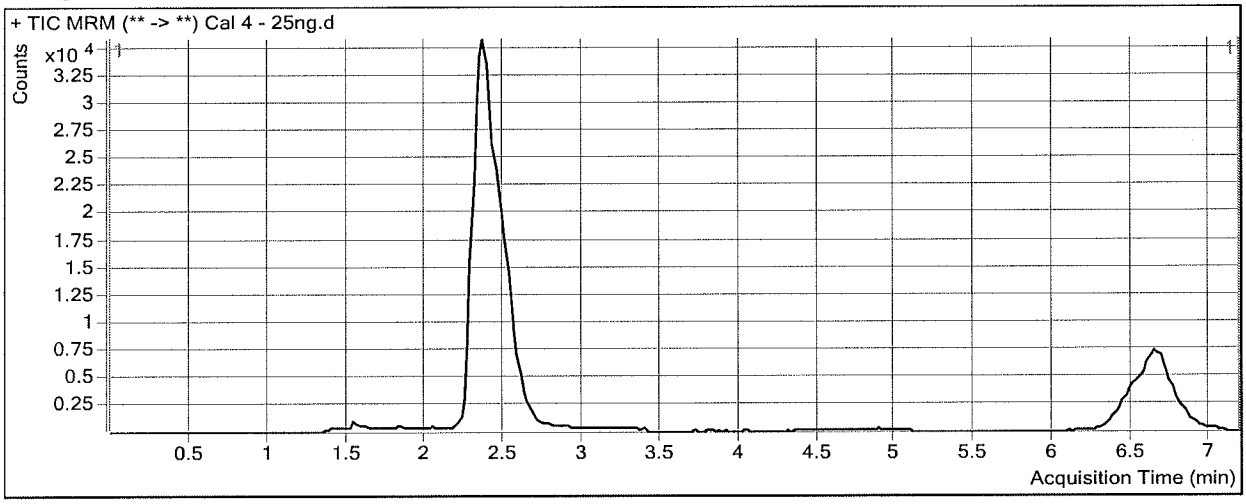
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 10:05	**Data File**	Cal 4 - 25ng.d
Sample Type	Calibration	**Sample Name**	Cal 4 - 25ng/mL
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-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	2.376	56470	239256	0.2360	25.5543
THC-COOH	THC-COOH-d9	2.506	45992	77313	0.5949	26.3813
THC	THC-d3	6.633	28669	94974	0.3019	25.8909

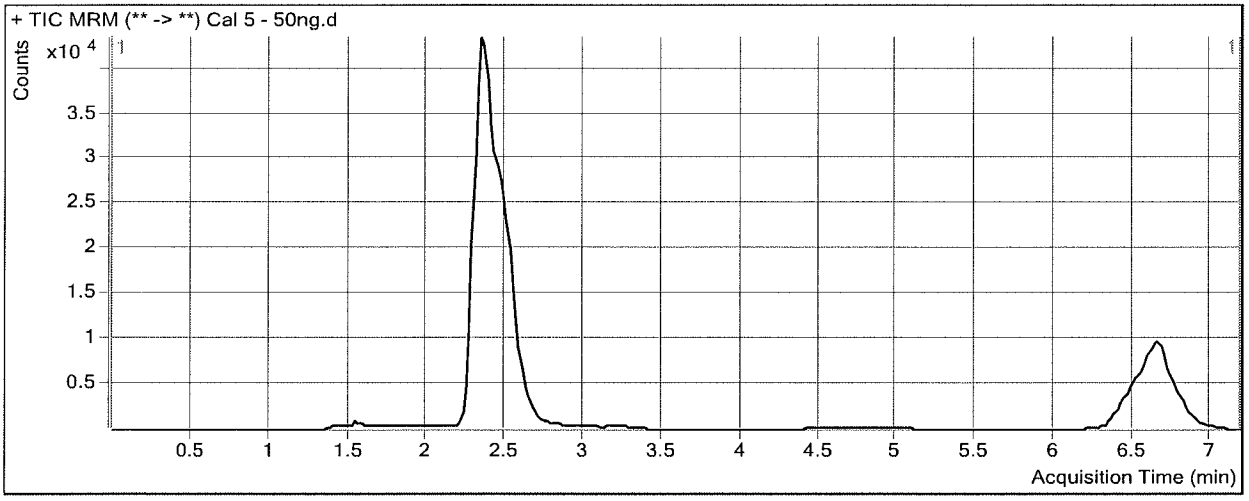
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 10:17	**Data File**	Cal 5 - 50ng.d
Sample Type	Calibration	**Sample Name**	Cal 5 - 50ng/mL
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-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	2.376	111141	245391	0.4529	49.2931
THC-COOH	THC-COOH-d9	2.506	81575	76973	1.0598	49.1158
THC	THC-d3	6.653	56244	96969	0.5800	50.0748

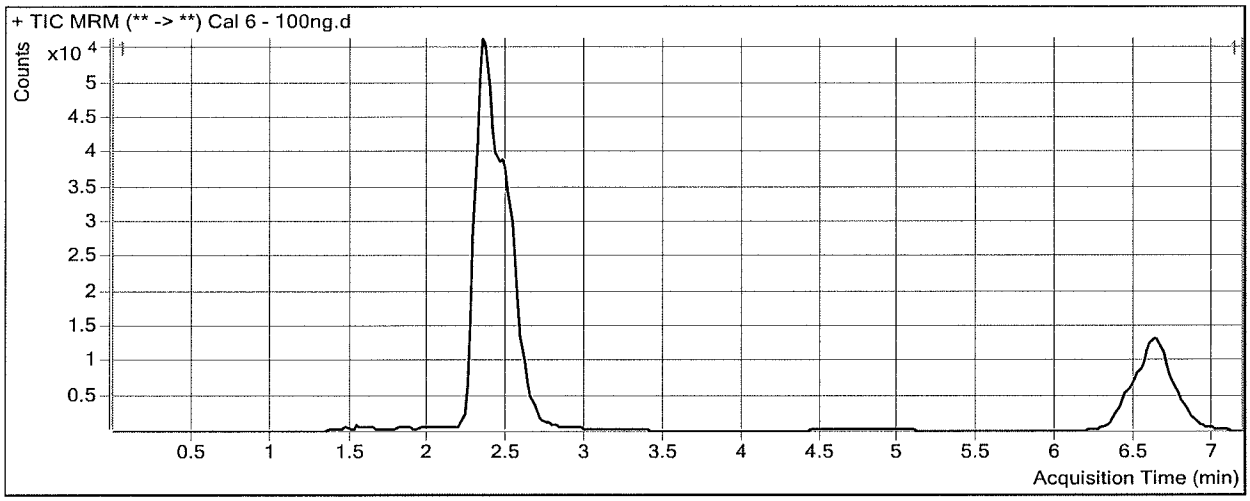
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 10:29	**Data File**	Cal 6 - 100ng.d
Sample Type	Calibration	**Sample Name**	Cal 6 - 100ng/mL
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-F1	**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	2.376	219507	239913	0.9149	99.8623
THC-COOH	THC-COOH-d9	2.506	149959	74959	2.0006	95.1208
THC	THC-d3	6.633	106968	94663	1.1300	97.8910

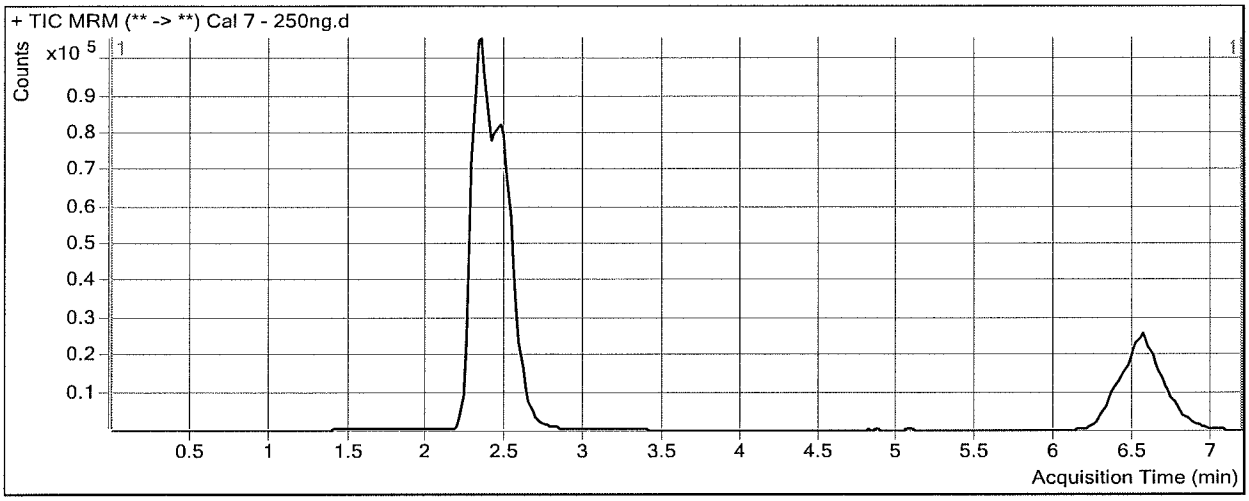
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\8917 cann quant\QuantResults\8917 cann quant.batch.bin		
Analysis Time	8/10/2017 3:47 PM	**Analyst Name**	ISP Tox
Report Time	8/10/2017 3:48 PM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2017 3:47 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-10 10:40	**Data File**	Cal 7 - 250ng.d
Sample Type	Calibration	**Sample Name**	Cal 7 - 250ng/mL
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-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	2.356	590058	258144	2.2858	249.8999
THC-COOH	THC-COOH-d9	2.486	373860	76296	4.9001	236.9152
THC	THC-d3	6.553	295584	102190	2.8925	251.1312