reviewed 10/16/17

10/12/2017

Worklist: 1943

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-1925	1	97412	AM 27 Blood THC Quant by LC
C2017-1938	1	97413	AM 27 Blood THC Quant by LC
C2017-1969	1	97415	AM 27 Blood THC Quant by LC
C2017-1974	1	97414	AM 27 Blood THC Quant by LC
C2017-2016	1	97416	AM 27 Blood THC Quant by LC
C2017-2030	1	97417	AM 27 Blood THC Quant by LC
M2017-4292	1	97418	AM 27 Blood THC Quant by LC

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

Extraction Date: 10-11-2017 **Analyst:** Anne Nord

PRE-ANALYTIC

Plate Lot# Custom - 499102 Plate Exp. 1/29/2018 **External QC** Lot 21718 exp 2-17-18

✓ 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: 101117 cann quant r

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: 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 Samples run in data path 101117 cann quant. 10-12-17 A The blank run did not have internal standard in it. Samples were reconstituted and run 10-13-17. A

A

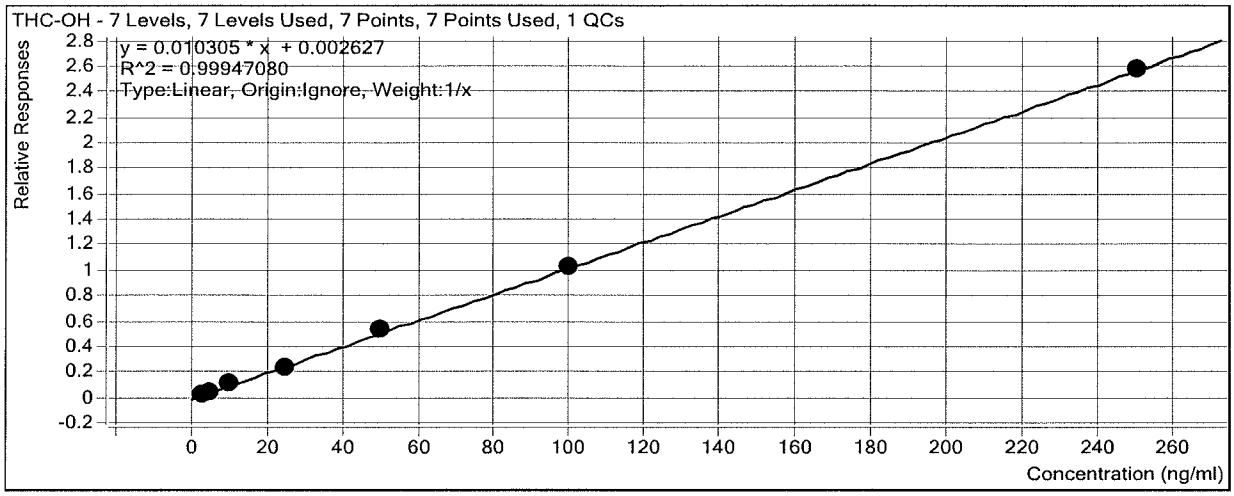
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin

Last Calib Update 10/15/2017 11:35 AM **Analyst Name** ISP TOX

Target Compound *THC-OH*

Internal Standard *THC-OH-d3*



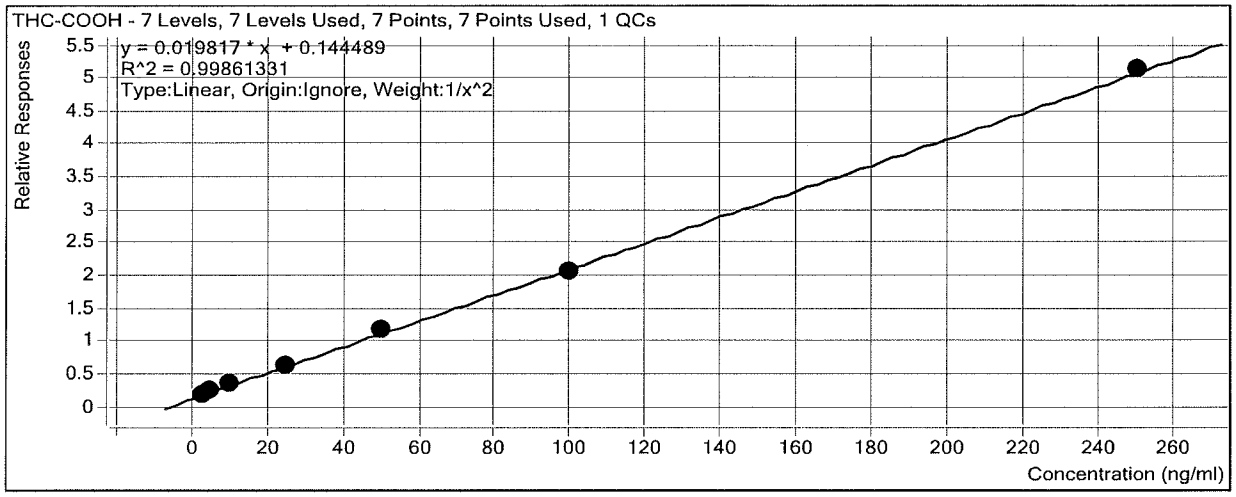
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.3	109.5
Cal 2 - 5ng	2	☑	5	4.4	88.3
Cal 3 - 10ng	3	☑	10	10.4	104.0
QC - 10ng	3	☑	10	9.3	92.9
Cal 4 - 25ng	4	☑	25	23.9	95.5
Cal 5 - 50ng	5	☑	50	51.3	102.6
Cal 6 - 100ng	6	☑	100	100.1	100.1
Cal 7 - 250ng	7	☑	250	249.6	99.8

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin

Last Calib Update 10/15/2017 11:35 AM **Analyst Name** ISP TOX

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



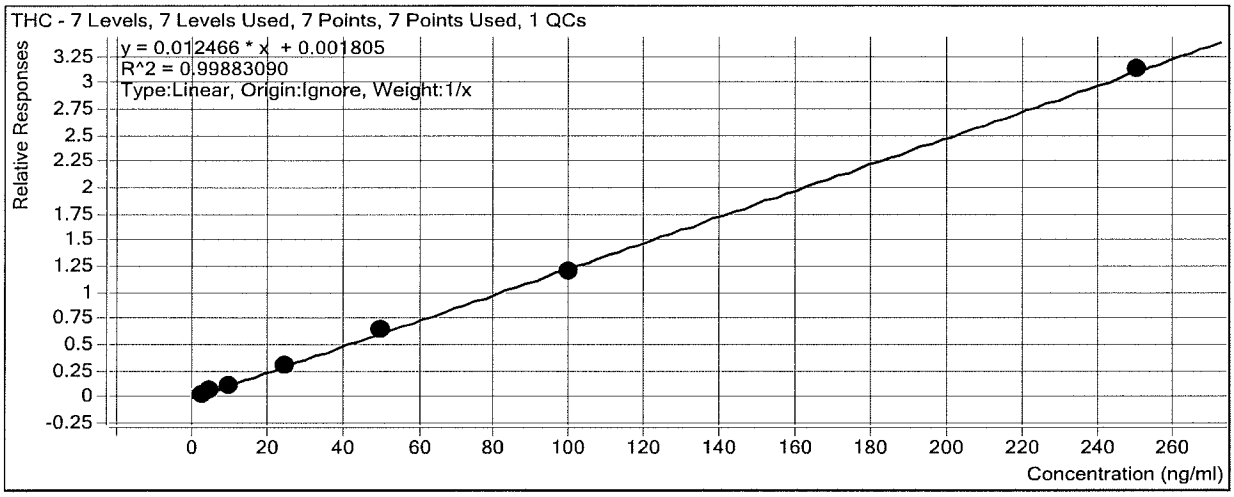
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	2.9	97.6
Cal 2 - 5ng	2	☑	5	5.2	103.5
Cal 3 - 10ng	3	☑	10	10.2	102.0
QC - 10ng	3	☑	10	10.9	109.1
Cal 4 - 25ng	4	☑	25	24.1	96.3
Cal 5 - 50ng	5	☑	50	51.5	103.0
Cal 6 - 100ng	6	☑	100	97.0	97.0
Cal 7 - 250ng	7	☑	250	251.6	100.6

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin

Last Calib Update 10/15/2017 11:35 AM **Analyst Name** ISP TOX

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



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	2.9	97.3
Cal 2 - 5ng	2	☑	5	5.6	111.2
Cal 3 - 10ng	3	☑	10	8.9	88.6
QC - 10ng	3	☑	10	10.6	106.1
Cal 4 - 25ng	4	☑	25	24.9	99.8
Cal 5 - 50ng	5	☑	50	52.8	105.7
Cal 6 - 100ng	6	☑	100	97.2	97.2
Cal 7 - 250ng	7	☑	250	250.7	100.3

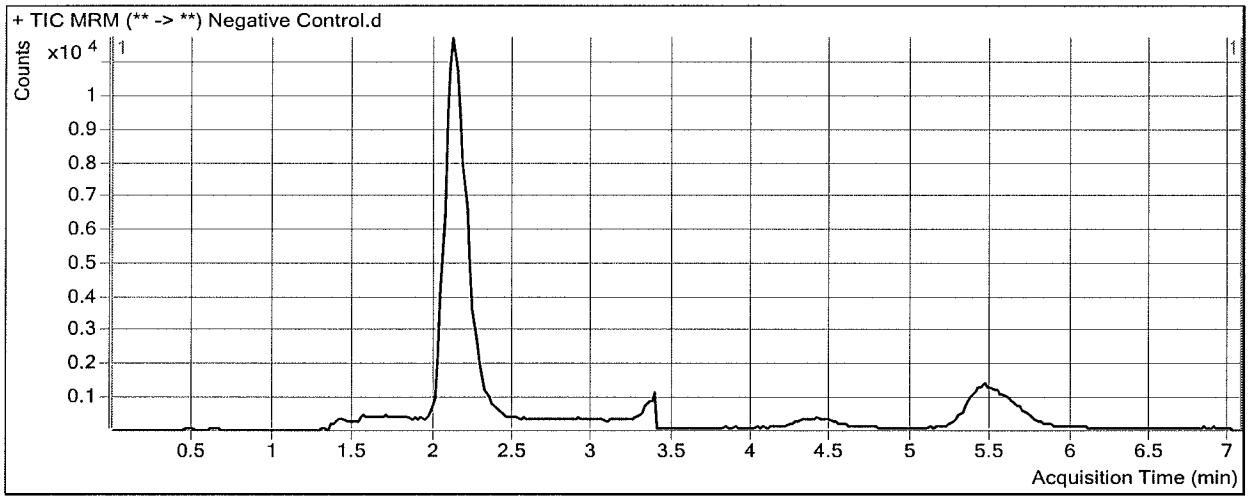
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 12:05	**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	2.285	4371	29136	0.1500	0.2790

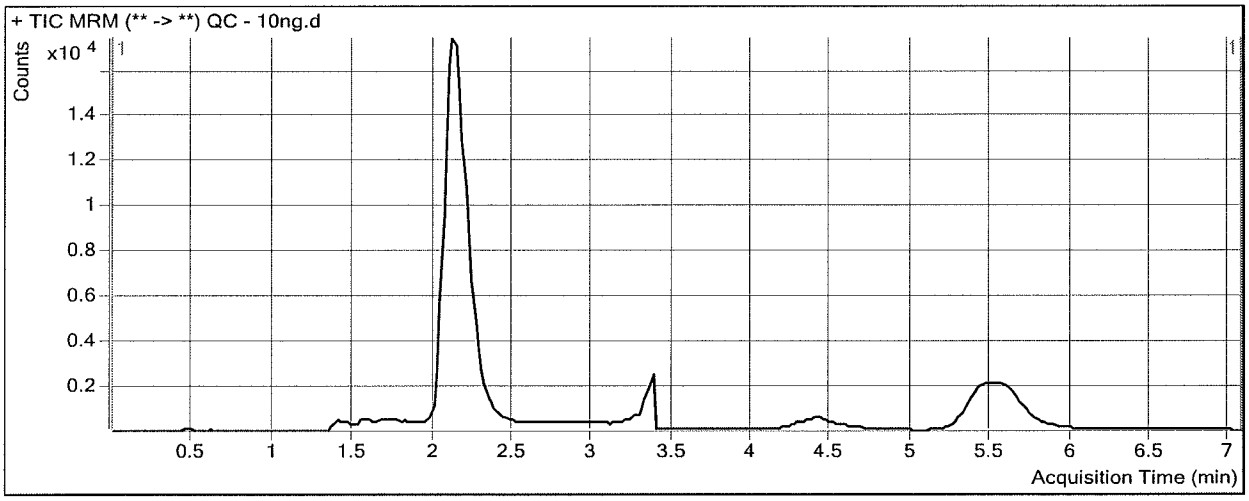
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 12:17	**Data File**	QC - 10ng.d
Sample Type	QC	**Sample Name**	QC - 10ng
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	2.115	10537	107151	0.0983	9.2875
THC-COOH	THC-COOH-d9	2.245	14259	39525	0.3608	10.9128
THC	THC-d3	5.572	5237	39074	0.1340	10.6066

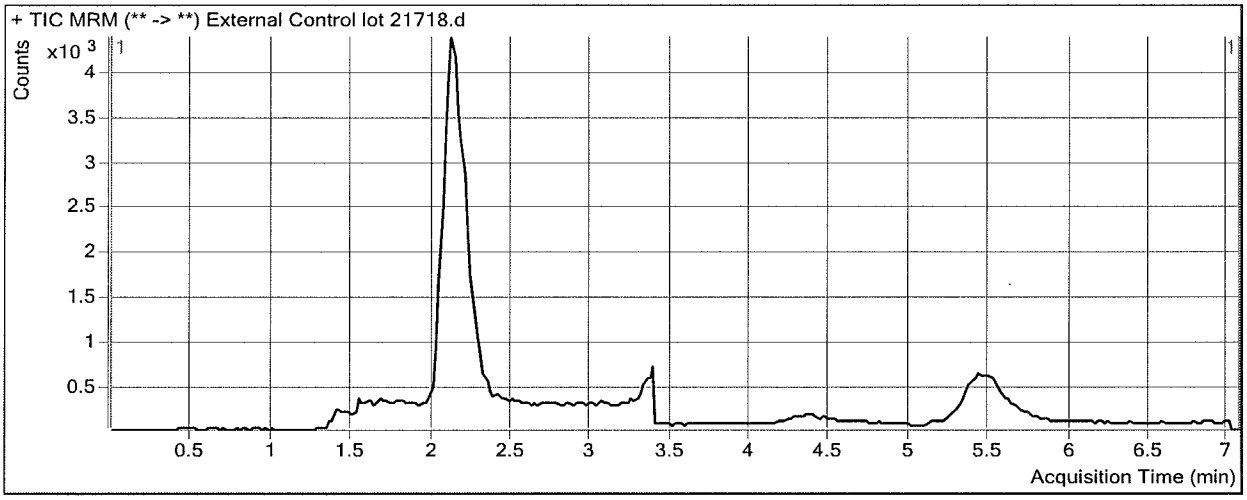
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 12:29	**Data File**	External Control lot 21718.d
Sample Type	Sample	**Sample Name**	External Control lot 21718
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	2.135	2076	24928	0.0833	7.8283
THC-COOH	THC-COOH-d9	2.205	3351	9980	0.3358	9.6542
THC	THC-d3	5.412	909	8329	0.1091	8.6099

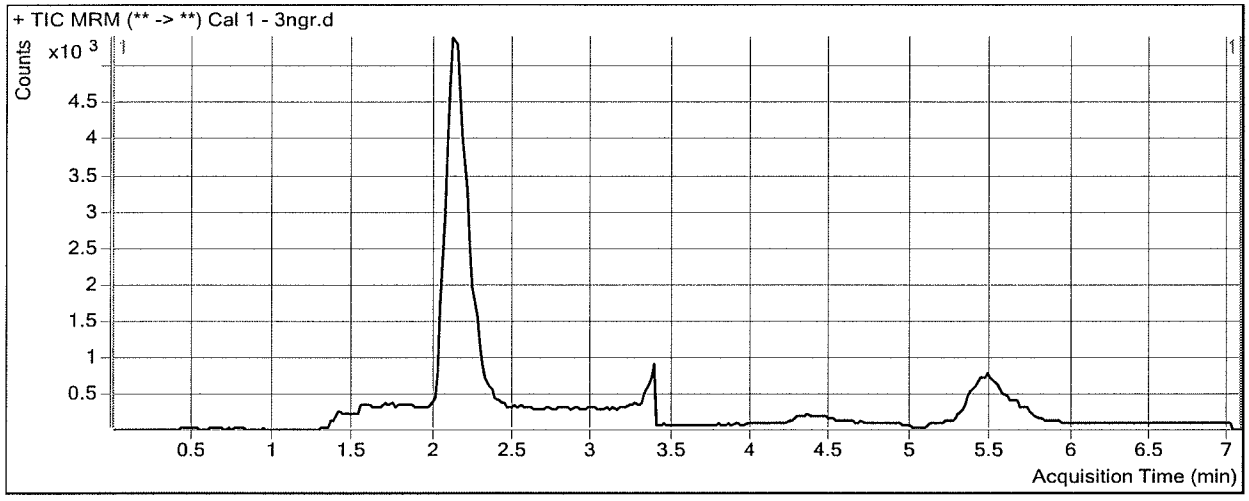
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 11:18	**Data File**	Cal 1 - 3ngr.d
Sample Type	Calibration	**Sample Name**	Cal 1 - 3ng
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	2.155	1178	32280	0.0365	3.2864
THC-COOH	THC-COOH-d9	2.265	2659	13130	0.2025	2.9290
THC	THC-d3	5.532	417	10922	0.0382	2.9186

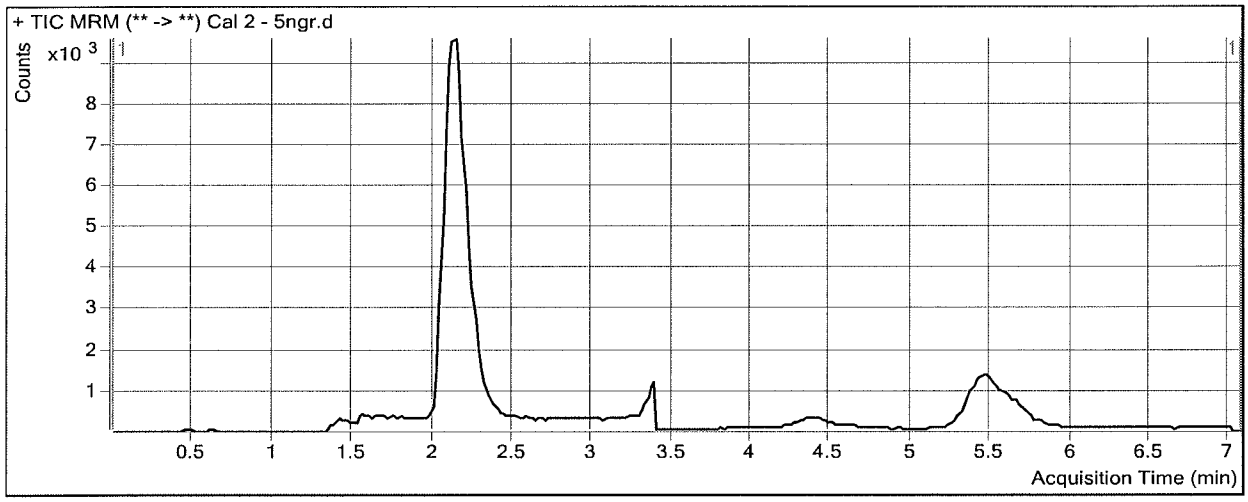
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 11:29	**Data File**	Cal 2 - 5ngr.d
Sample Type	Calibration	**Sample Name**	Cal 2 - 5ng
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	2.155	2912	60495	0.0481	4.4164
THC-COOH	THC-COOH-d9	2.265	5915	23939	0.2471	5.1763
THC	THC-d3	5.492	1603	22527	0.0711	5.5616

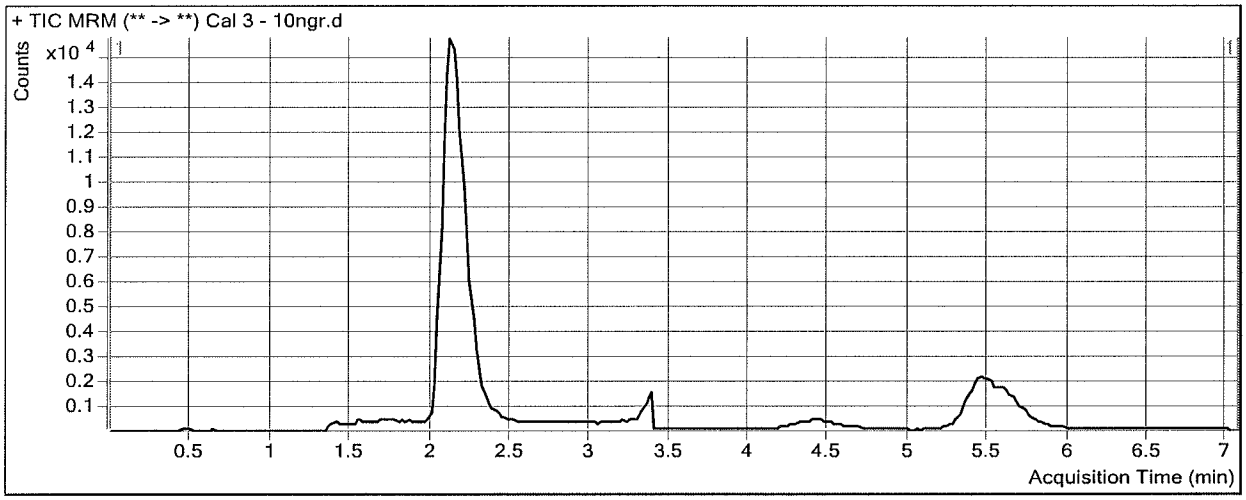
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 11:41	**Data File**	Cal 3 - 10ngr.d
Sample Type	Calibration	**Sample Name**	Cal 3 - 10ng
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	2.135	10340	94146	0.1098	10.4031
THC-COOH	THC-COOH-d9	2.245	12365	35669	0.3467	10.2019
THC	THC-d3	5.592	3937	35095	0.1122	8.8552

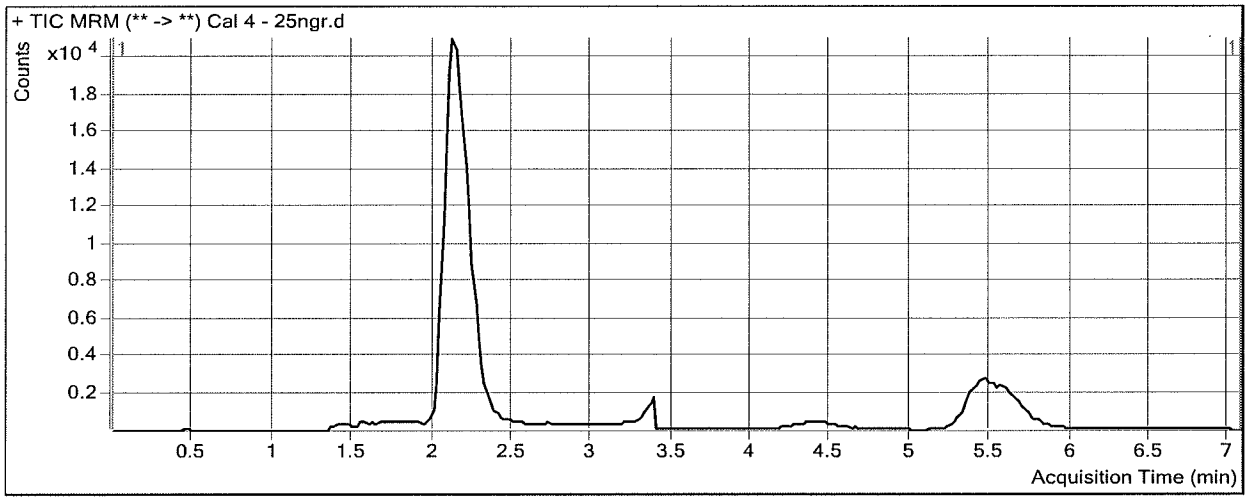
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 11:53	**Data File**	Cal 4 - 25ngr.d
Sample Type	Calibration	**Sample Name**	Cal 4 - 25ng
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	2.135	27135	109116	0.2487	23.8773
THC-COOH	THC-COOH-d9	2.225	26413	42510	0.6213	24.0627
THC	THC-d3	5.552	12218	39061	0.3128	24.9473

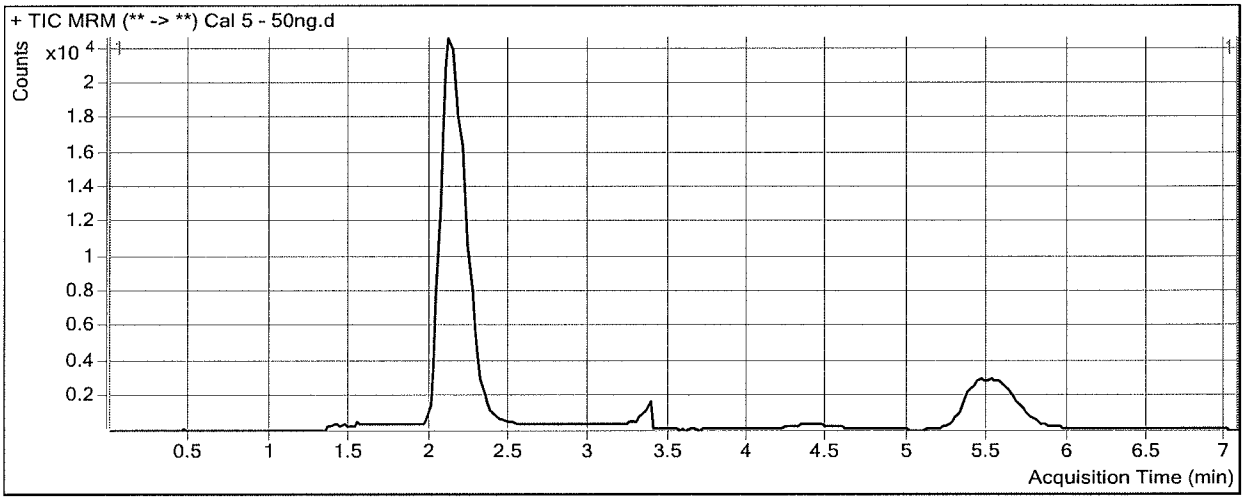
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 10:42	**Data File**	Cal 5 - 50ng.d
Sample Type	Calibration	**Sample Name**	Cal 5 - 50ng
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	2.135	52333	98461	0.5315	51.3230
THC-COOH	THC-COOH-d9	2.225	39503	33912	1.1649	51.4891
THC	THC-d3	5.532	22269	33718	0.6604	52.8346

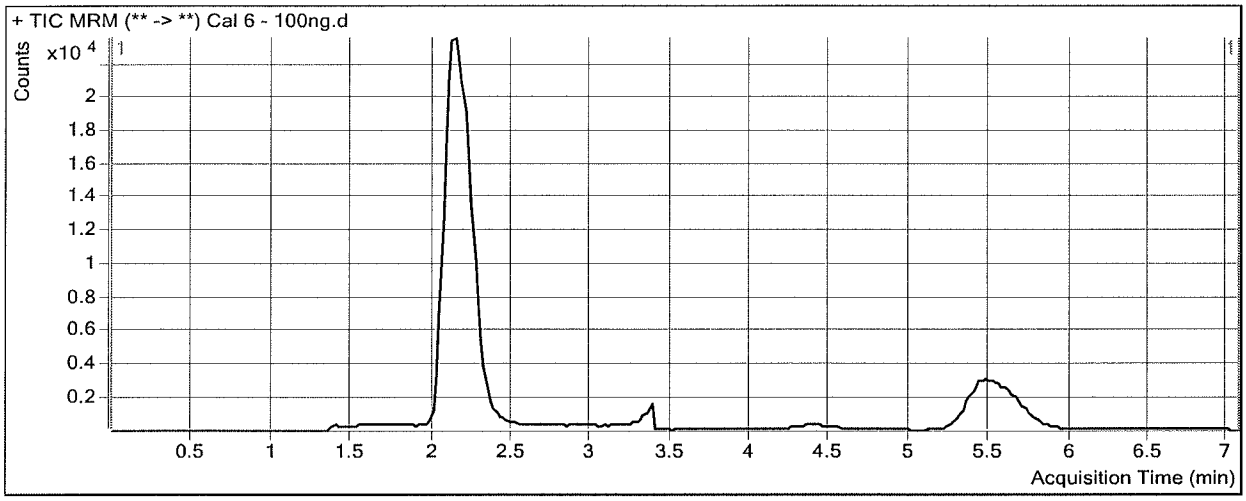
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 10:54	**Data File**	Cal 6 - 100ng.d
Sample Type	Calibration	**Sample Name**	Cal 6 - 100ng
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	2.135	78527	75934	1.0342	100.0997
THC-COOH	THC-COOH-d9	2.225	58210	28174	2.0661	96.9645
THC	THC-d3	5.512	28557	23533	1.2135	97.1997

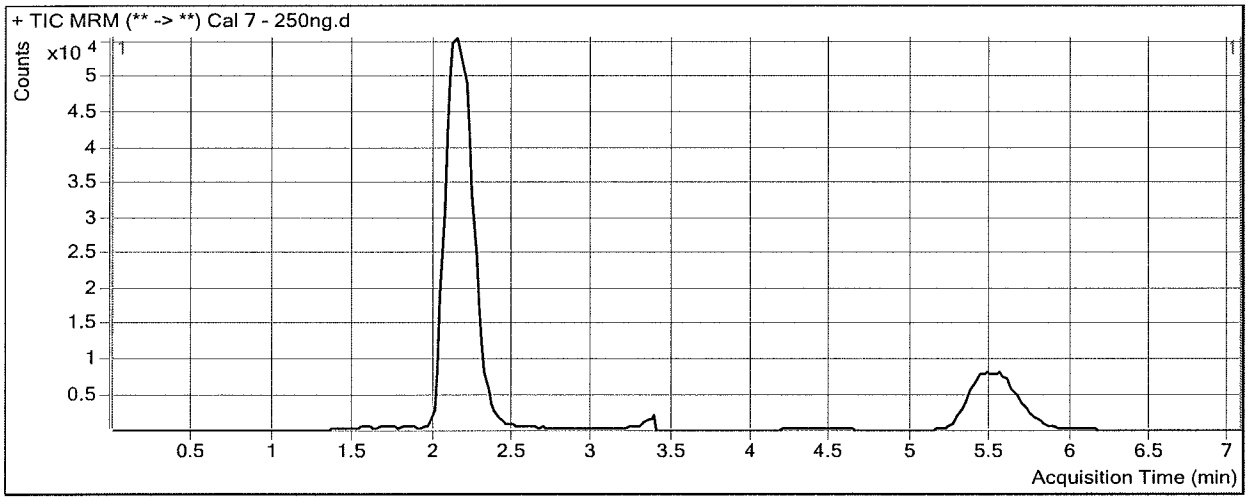
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\101117 cann quant r\QuantResults\cann quant.batch.bin		
Analysis Time	10/15/2017 11:35 AM	**Analyst Name**	ISP Tox
Report Time	10/15/2017 11:37 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/15/2017 11:35 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-13 11:06	**Data File**	Cal 7 - 250ng.d
Sample Type	Calibration	**Sample Name**	Cal 7 - 250ng
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	2.135	256117	99475	2.5747	249.5940
THC-COOH	THC-COOH-d9	2.205	176125	34332	5.1300	251.5714
THC	THC-d3	5.552	106791	34153	3.1269	250.6831