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

Date: 1-3-17 Analyst: Anne Nord

PRE-ANALYTIC

✓ 1. ~~Std~~. Lot# ~~70103~~ 0490364 Std. Exp. 9-21-17 QC Lot# 31317 QC Exp. 3-13-17
Plate

Mobile Phase A	0.1% Formic Acid in water	
Mobile Phase B	0.1% Formic Acid in water	
Methanol	Hexane	MTBE

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

✓ 3. Begin mobile phase flow and allow system to equilibrate for approx. 1 hour.

✓ 4. Create worklist. Data path name: 1-3-17 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. Mix via aspirate and dispense.
Blank blood for locations containing standards/QCs and internal standards (Blank Blood Used 321632-1)
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. 4 seconds (or until no liquid remains on top of sorbent). Wait 5 min.

✓ 8. Add 2.25mL MTBE and allow to flow under gravity for 5 minutes.

✓ 9. Apply positive pressure for approx. 15 seconds.

✓ 10. Add 2.25mL hexane and allow to flow under gravity for 5 minutes.

✓ 11. Apply positive pressure for approx. 15 seconds.

✓ 12. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C.

✓ 13. SPE Dry ID 66819

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

POST-ANALYTIC

✓ 1. Open quantitation software and create a new quantitation batch. Batch name: 1317 Cann quant

✓ 2. Make any necessary integration changes. Limit curves based on validated linear ranges.

✓ 3. Were all appropriate standards used in the curve for each analyte? (Y) / N
Are r^2 values ~~≥0.99~~ 0.98 per method for each analyte? Y / N

✓ 4. For unknown samples, are the response ratios for each analyte within ~~±30%~~ ±20% of the average ratios of the standards?

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

✓ 6. Print the following items: ✓ Method Checklist ✓ Worklist ✓ Data Report

COMMENTS

Worklist: 1456

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2016-1405	1	73518	AM 27 Blood THC Quant by LC
C2016-2151	1	73536	AM 27 Blood THC Quant by LC
M2016-3278	2	73022	AM 27 Blood THC Quant by LC
M2016-3640	1	73523	AM 27 Blood THC Quant by LC
M2016-3677	1	73519	AM 27 Blood THC Quant by LC
M2016-3677	2	73520	AM 27 Blood THC Quant by LC
M2016-3792	1	73521	AM 27 Blood THC Quant by LC
M2016-3948	1	73522	AM 27 Blood THC Quant by LC
M2016-4114	1	73524	AM 27 Blood THC Quant by LC
M2016-4158	1	73534	AM 27 Blood THC Quant by LC
M2016-4477	1	73526	AM 27 Blood THC Quant by LC
M2016-4536	1	73527	AM 27 Blood THC Quant by LC
M2016-4557	2	73535	AM 27 Blood THC Quant by LC
M2016-4741	1	73528	AM 27 Blood THC Quant by LC
M2016-4866	1	73529	AM 27 Blood THC Quant by LC
M2016-4969	1	73530	AM 27 Blood THC Quant by LC
M2016-5099	2	73525	AM 27 Blood THC Quant by LC
P2016-1858	1	73531	AM 27 Blood THC Quant by LC
P2016-2556	1	73532	AM 27 Blood THC Quant by LC
P2016-2624	1	73533	AM 27 Blood THC Quant by LC

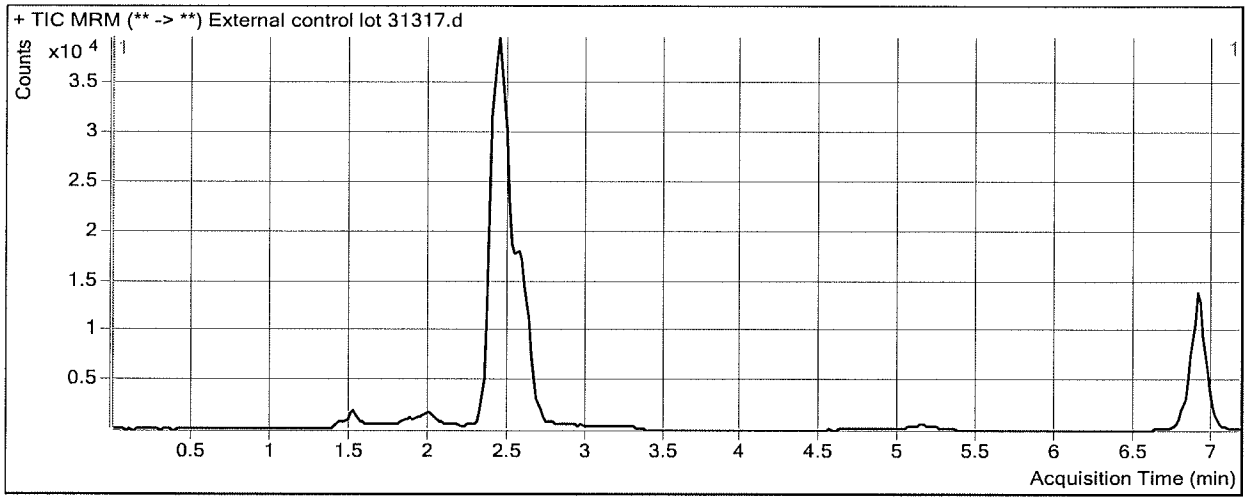
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 14:45	**Data File**	External control lot 31317.d
Sample Type	Sample	**Sample Name**	External control lot 31317
Dilution	1	**Acq Method**	Quant THC 12-15-16.m
Position	p1b2	**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	2.476	28680	263853	0.1087	11.6601
THC-COOH	THC-COOH-d9	2.626	16367	96058	0.1704	11.4684
THC	THC-d3	6.913	8411	85315	0.0986	9.6196

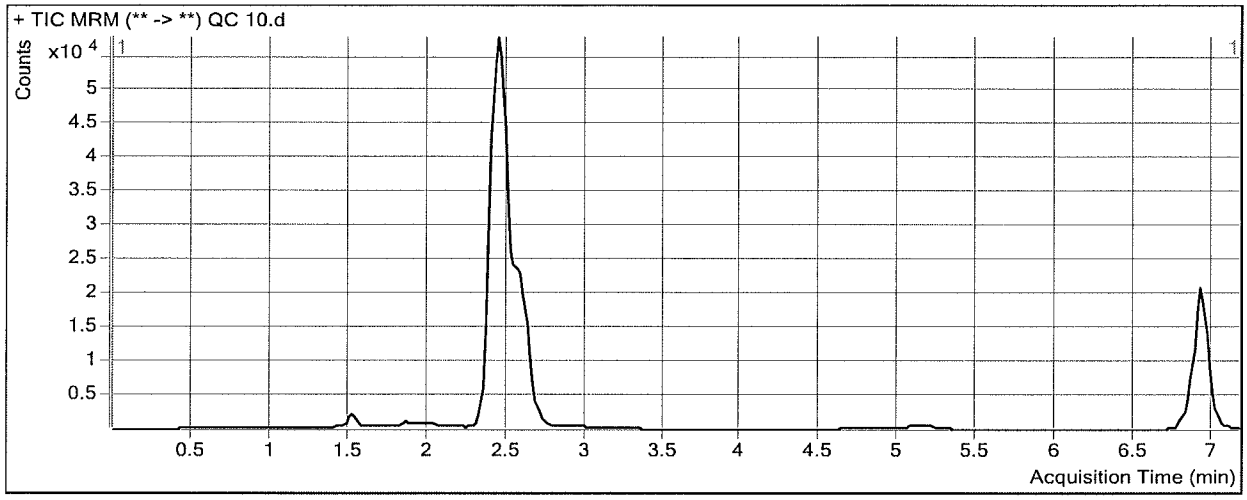
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 14:33	**Data File**	QC 10.d
Sample Type	QC	**Sample Name**	QC 10
Dilution	1	**Acq Method**	Quant THC 12-15-16.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	2.476	31590	398667	0.0792	8.9248
THC-COOH	THC-COOH-d9	2.626	20119	126550	0.1590	10.6331
THC	THC-d3	6.913	11542	128091	0.0901	8.9006

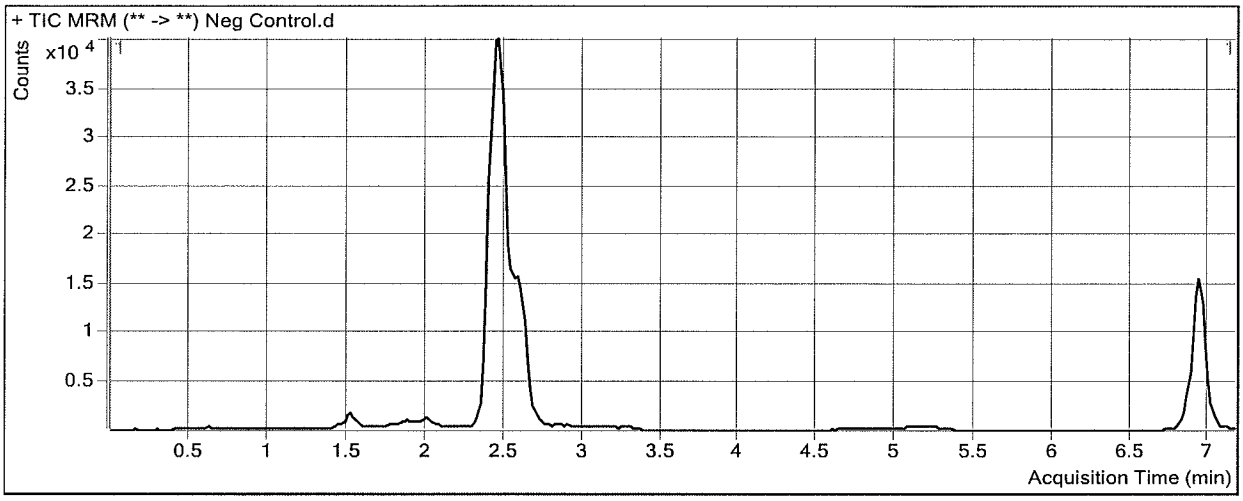
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 14:21	**Data File**	Neg Control.d
Sample Type	Sample	**Sample Name**	Neg Control
Dilution	1	**Acq Method**	Quant THC 12-15-16.m
Position	P1-a2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation

Sample Chromatogram



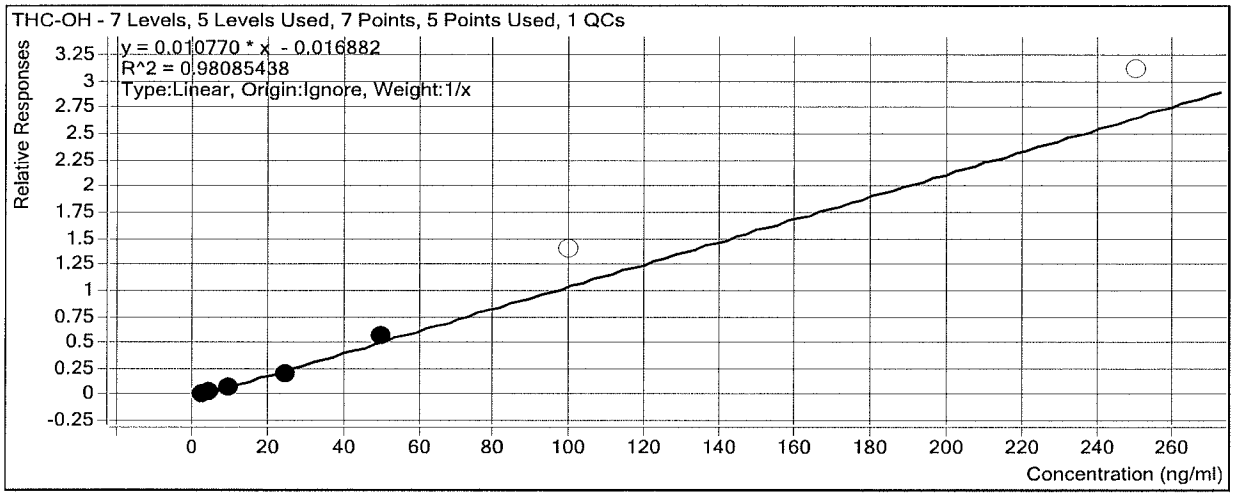
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin

Last Calib Update 1/4/2017 10:17 AM **Analyst Name** ISP TOX

Target Compound *THC-OH*

Internal Standard *THC-OH-d3*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☑	3	3.4	112.1
cal 2	2	☑	5	5.3	105.4
cal 3	3	☑	10	8.9	89.2
QC 10	3	☑	10	8.9	89.2
cal 4	4	☑	25	21.2	84.8
cal 5	5	☑	50	54.2	108.5
cal 6	6	☐	100	132.6	132.6
cal 7	7	☐	250	290.7	116.3

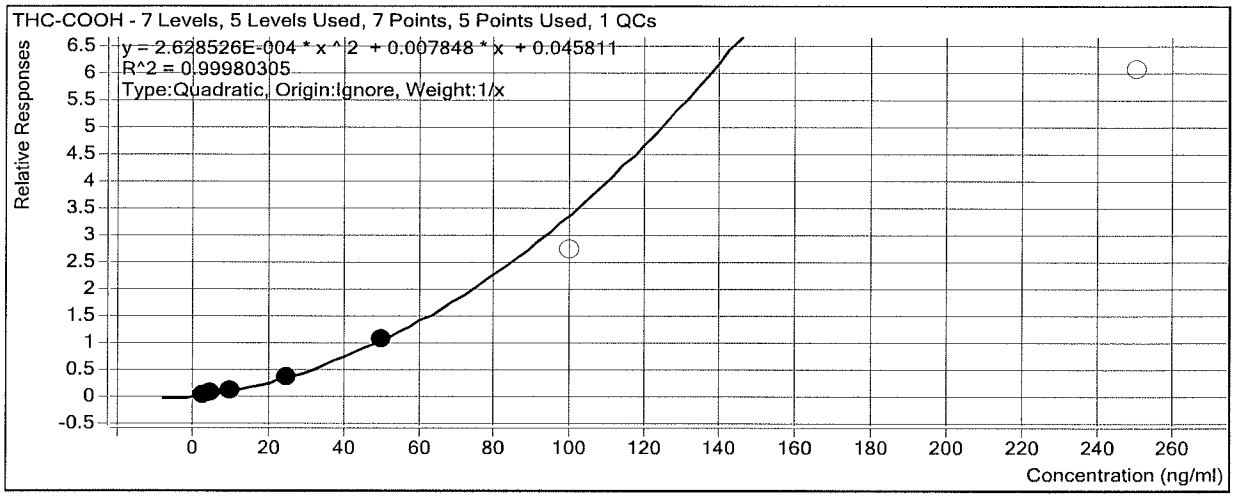
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin

Last Calib Update 1/4/2017 10:17 AM **Analyst Name** ISP TOX

Target Compound *THC-COOH*

Internal Standard *THC-COOH-d9*



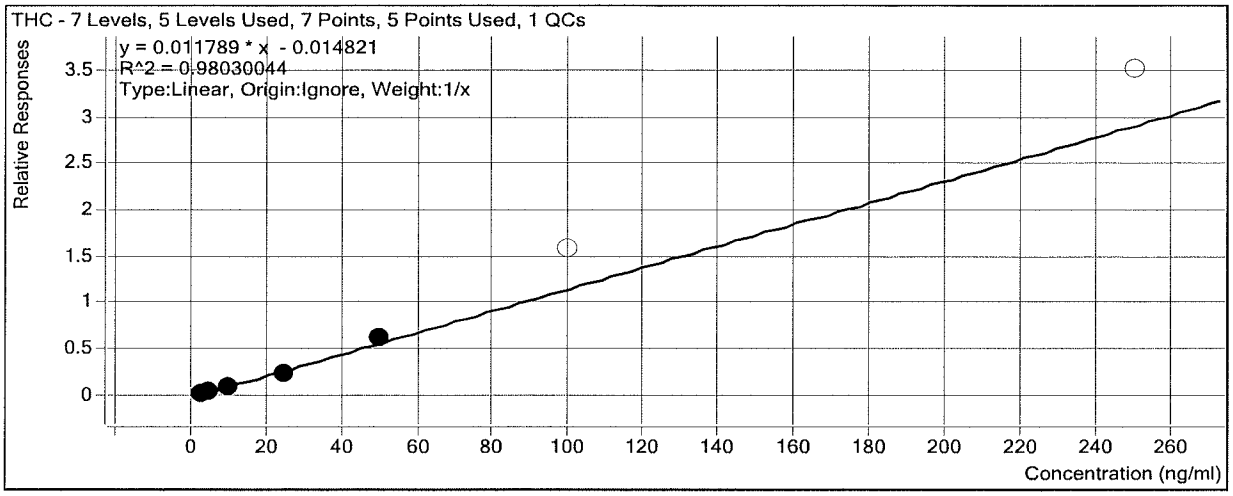
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☑	3	2.9	96.4
cal 2	2	☑	5	5.0	100.6
cal 3	3	☑	10	10.4	103.7
QC 10	3	☑	10	10.6	106.3
cal 4	4	☑	25	24.7	98.8
cal 5	5	☑	50	50.1	100.1
cal 6	6	☐	100	88.0	88.0
cal 7	7	☐	250	137.2	54.9

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin

Last Calib Update 1/4/2017 10:17 AM **Analyst Name** ISP TOX

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



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☑	3	3.5	117.2
cal 2	2	☑	5	5.1	101.2
cal 3	3	☑	10	8.8	87.5
QC 10	3	☑	10	8.9	89.0
cal 4	4	☑	25	21.4	85.5
cal 5	5	☑	50	54.3	108.6
cal 6	6	☐	100	135.6	135.6
cal 7	7	☐	250	299.7	119.9

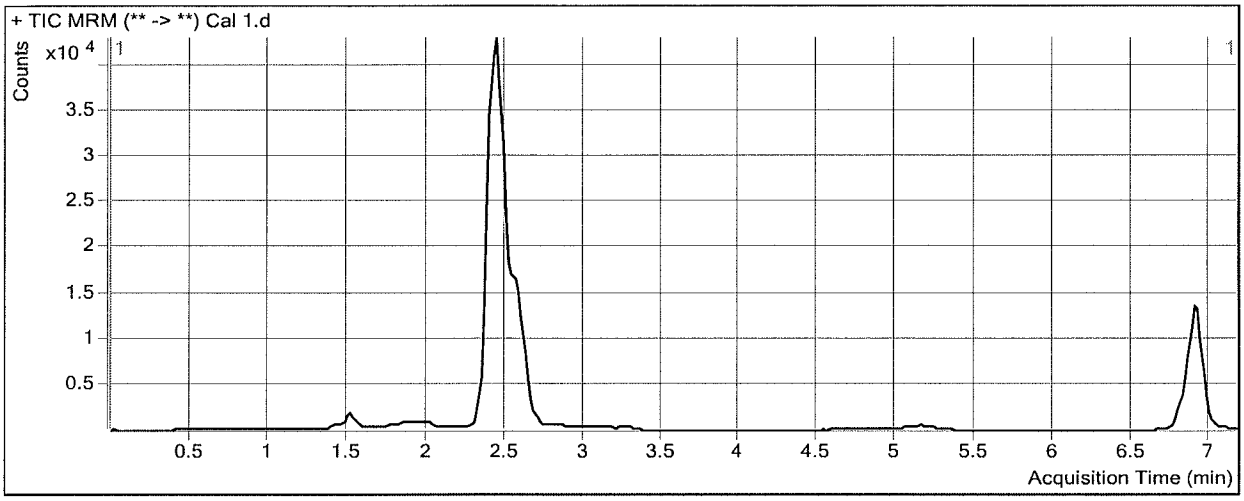
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 12:47	**Data File**	Cal 1.d
Sample Type	Calibration	**Sample Name**	Cal 1
Dilution	1	**Acq Method**	Quant THC 12-15-16.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	2.476	5986	309560	0.0193	3.3629
THC-COOH	THC-COOH-d9	2.606	6715	94979	0.0707	2.8916
THC	THC-d3	6.893	2546	95650	0.0266	3.5147

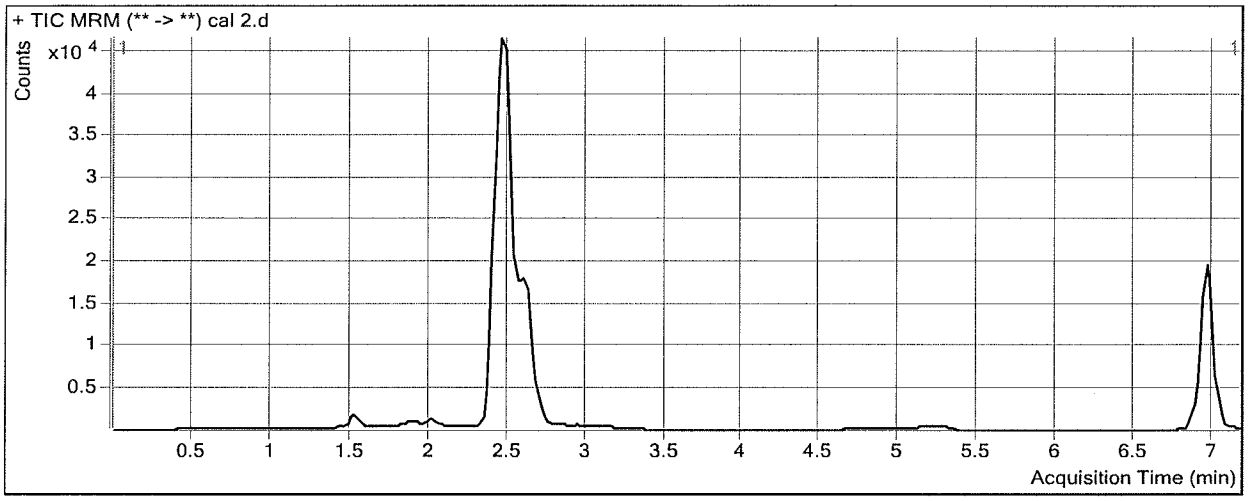
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 12:58	**Data File**	cal 2.d
Sample Type	Calibration	**Sample Name**	cal 2
Dilution	1	**Acq Method**	Quant THC 12-15-16.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	2.496	13503	338737	0.0399	5.2688
THC-COOH	THC-COOH-d9	2.646	9454	102827	0.0919	5.0304
THC	THC-d3	6.953	4931	109921	0.0449	5.0619

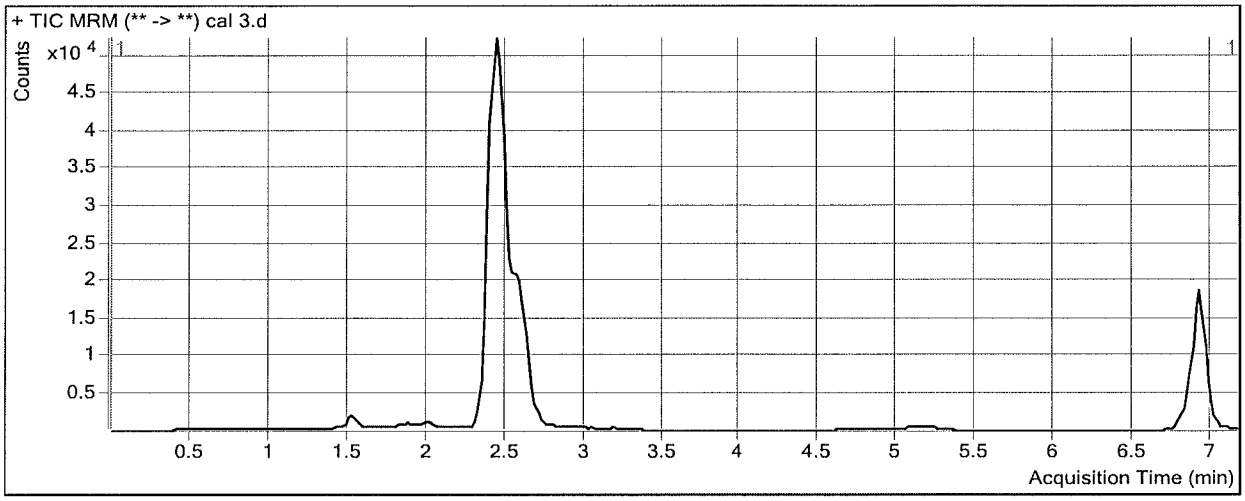
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 13:10	**Data File**	cal 3.d
Sample Type	Calibration	**Sample Name**	cal 3
Dilution	1	**Acq Method**	Quant THC 12-15-16.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	2.476	28551	360385	0.0792	8.9235
THC-COOH	THC-COOH-d9	2.626	17220	110758	0.1555	10.3708
THC	THC-d3	6.933	9934	112390	0.0884	8.7543

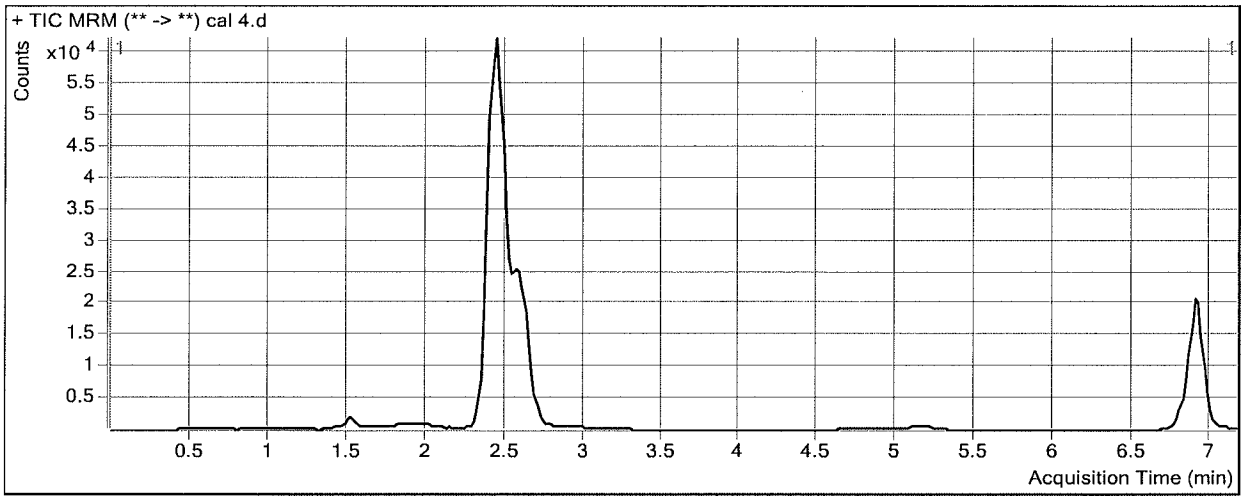
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 13:22	**Data File**	cal 4.d
Sample Type	Calibration	**Sample Name**	cal 4
Dilution	1	**Acq Method**	Quant THC 12-15-16.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	2.456	79043	373794	0.2115	21.2015
THC-COOH	THC-COOH-d9	2.626	46360	115954	0.3998	24.6895
THC	THC-d3	6.913	26620	112304	0.2370	21.3628

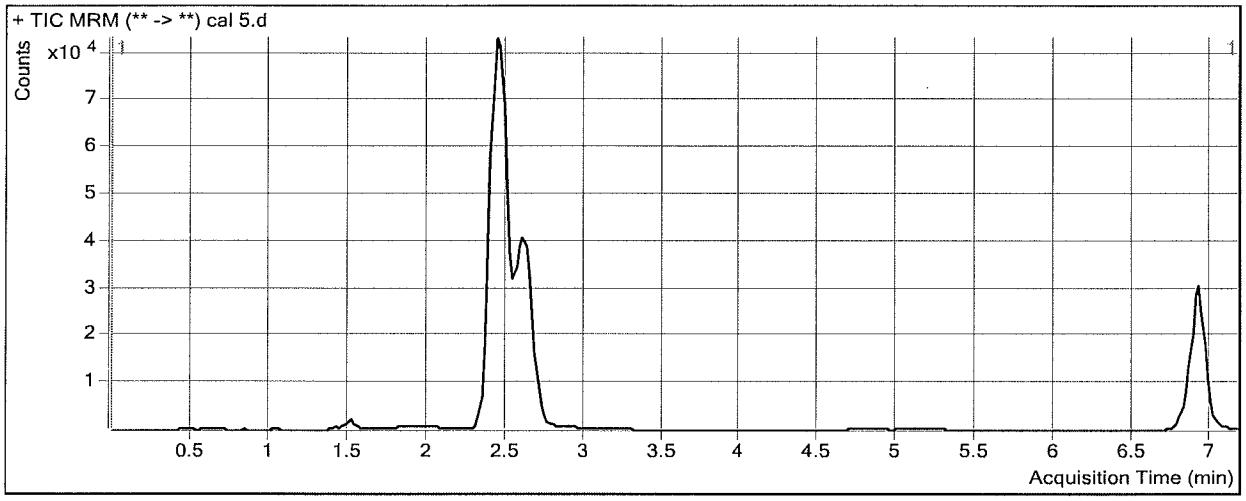
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 13:34	**Data File**	cal 5.d
Sample Type	Calibration	**Sample Name**	cal 5
Dilution	1	**Acq Method**	Quant THC 12-15-16.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	2.476	218453	385057	0.5673	54.2433
THC-COOH	THC-COOH-d9	2.626	130617	118996	1.0977	50.0672
THC	THC-d3	6.913	71747	114719	0.6254	54.3063

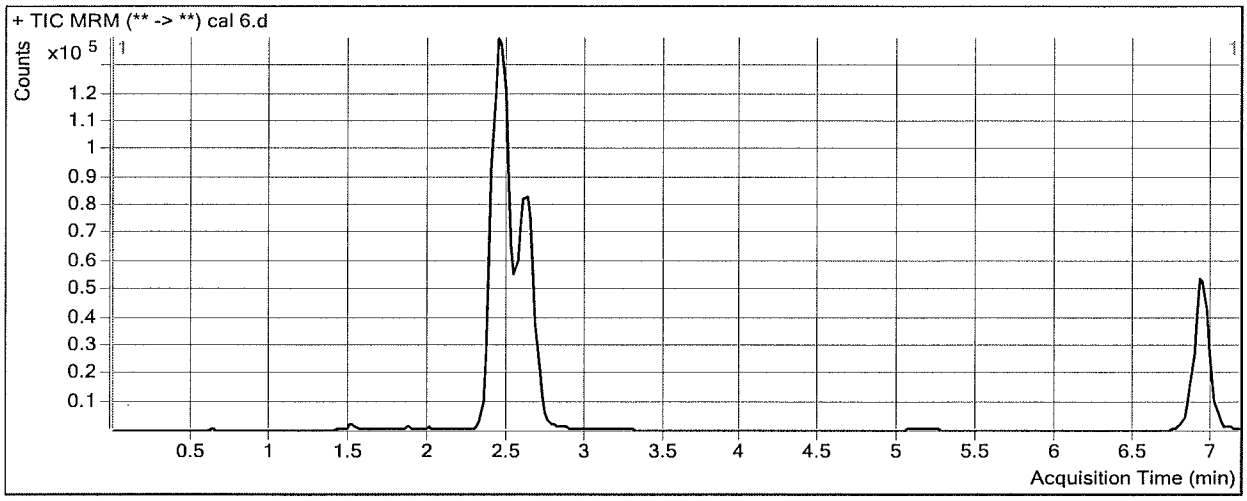
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 13:46	**Data File**	cal 6.d
Sample Type	Calibration	**Sample Name**	cal 6
Dilution	1	**Acq Method**	Quant THC 12-15-16.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	2.476	588718	417236	1.4110	132.5772
THC-COOH	THC-COOH-d9	2.626	341712	123360	2.7700	87.9635
THC	THC-d3	6.933	192490	121579	1.5832	135.5525

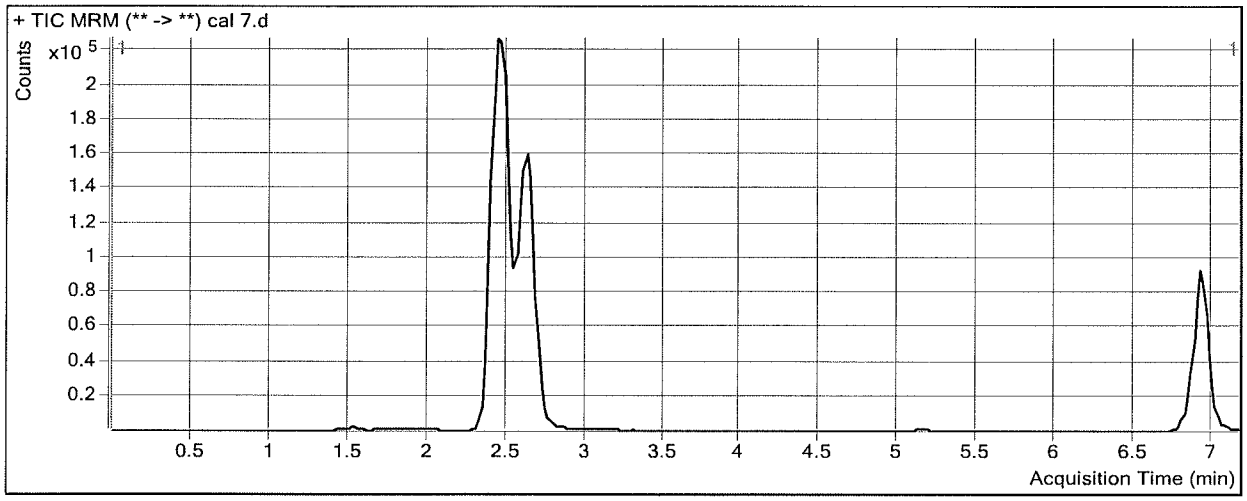
ISP FORENSICS - Cd'A
Cannabinoids Analysis Report

Batch Data Path	D:\2016 Data\1-3-17 cann quant\QuantResults\1317 cann quant.batch.bin		
Analysis Time	1/4/2017 10:17 AM	**Analyst Name**	ISP Tox
Report Time	1/4/2017 10:46 AM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2017 10:17 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-01-03 13:58	**Data File**	cal 7.d
Sample Type	Calibration	**Sample Name**	cal 7
Dilution	1	**Acq Method**	Quant THC 12-15-16.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	2.476	1245714	399995	3.1143	290.7304
THC-COOH	THC-COOH-d9	2.626	702765	115728	6.0726	137.2257
THC	THC-d3	6.913	396071	112559	3.5188	299.7294