reviewed 7/31/17

7/28/2017

Worklist: 1822

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-1390	1	91098	AM 27 Blood THC Quant by LC
C2017-1422	1	91104	AM 27 Blood THC Quant by LC
M2017-2859	1	91099	AM 27 Blood THC Quant by LC
M2017-2860	3	91100	AM 27 Blood THC Quant by LC
M2017-3021	1	91101	AM 27 Blood THC Quant by LC
M2017-3091	1	91102	AM 27 Blood THC Quant by LC
M2017-3315	2	91103	AM 27 Blood THC Quant by LC

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

Extraction Date: 7-27-17 **Analyst:** Anne Nord

PRE-ANALYTIC

Plate Lot# Custom - 0490364 Plate Exp. 9-21-2017 **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: 72717 cam 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: 72717 cam 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

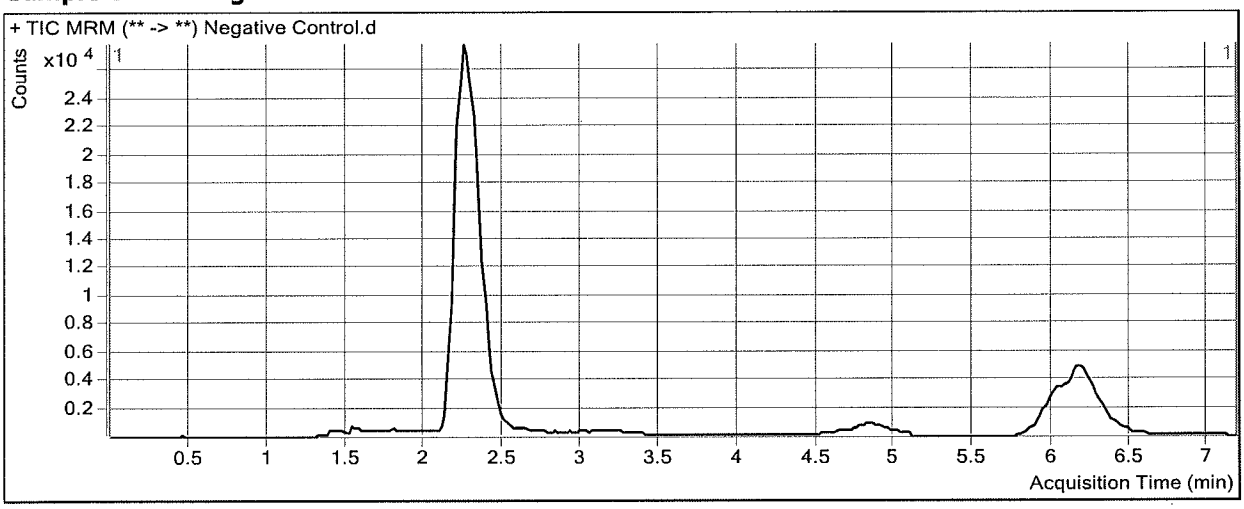
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 13:06	**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



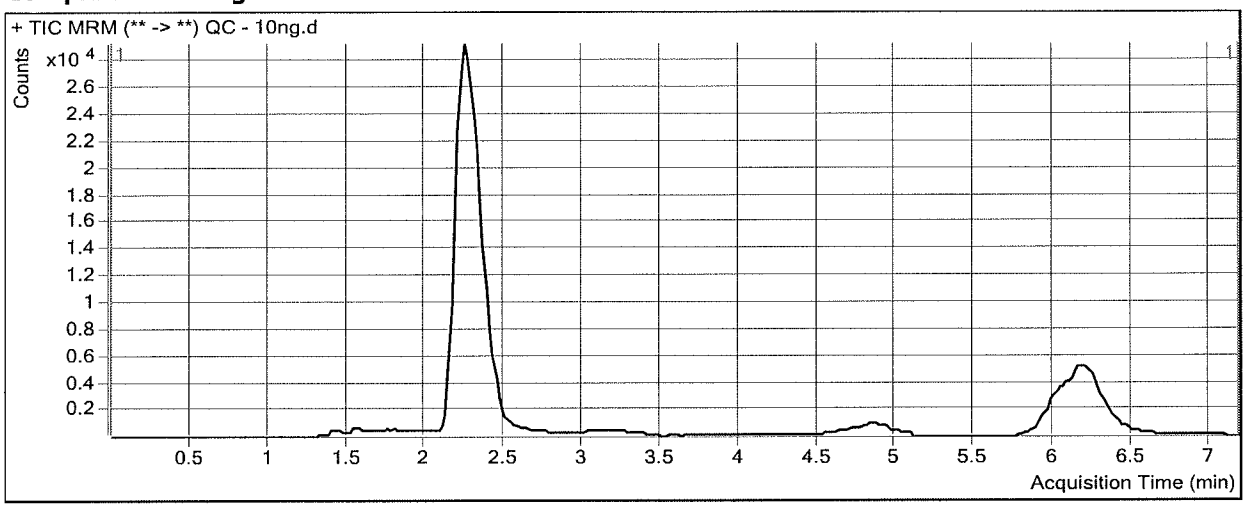
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 13:18	**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	2.255	18739	208329	0.0899	10.1336
THC-COOH	THC-COOH-d9	2.386	14398	77133	0.1867	9.7112
THC	THC-d3	6.172	10155	94914	0.1070	9.9853

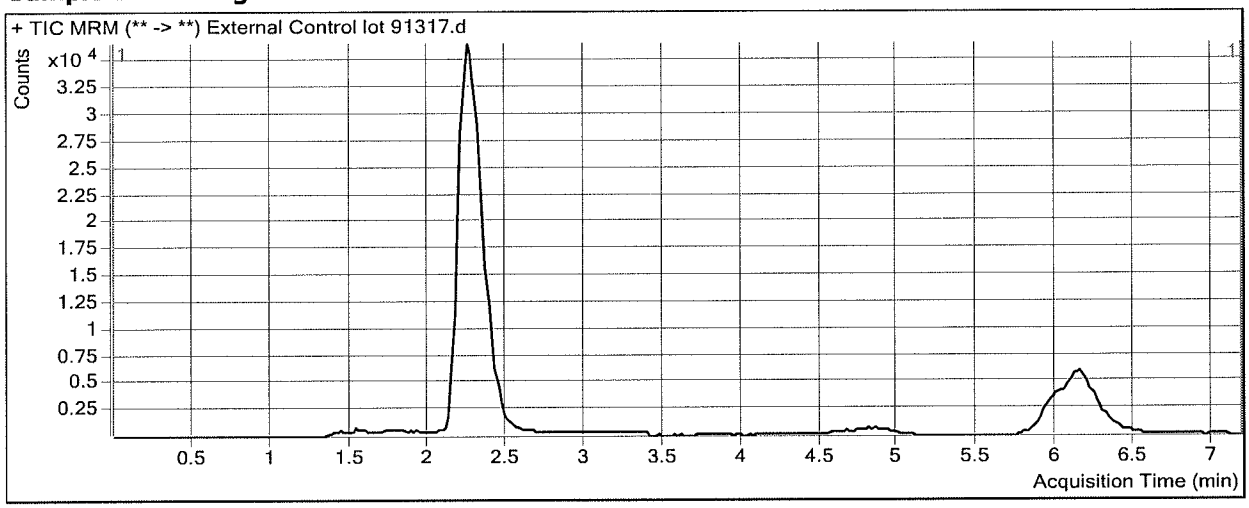
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 13:30	**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	2.255	22754	266900	0.0853	9.6295
THC-COOH	THC-COOH-d9	2.386	13686	85907	0.1593	8.1720
THC	THC-d3	6.152	8006	105689	0.0758	7.1060

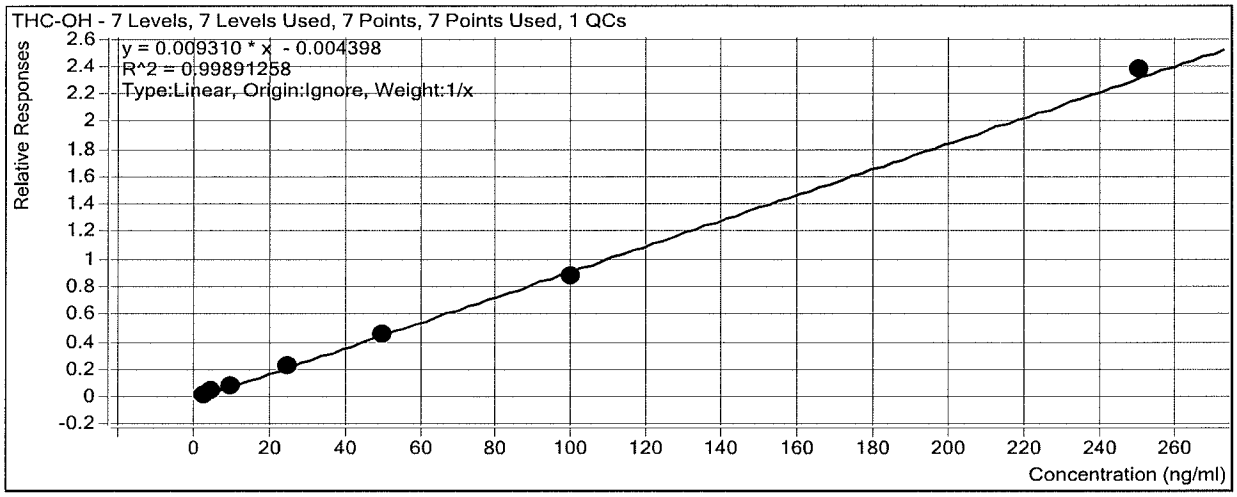
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin

Last Calib Update 7/28/2017 8:55 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/mL	1	☑	3	3.1	103.9
Cal 2 - 5ng/mL	2	☑	5	5.1	102.2
Cal 3 - 10ng/mL	3	☑	10	9.8	98.1
QC - 10ng/mL	3	☑	10	10.1	101.3
Cal 4 - 25ng/mL	4	☑	25	24.6	98.6
Cal 5 - 50ng/mL	5	☑	50	50.1	100.3
Cal 6 - 100ng/mL	6	☑	100	94.8	94.8
Cal 7 - 250ng/mL	7	☑	250	255.4	102.2

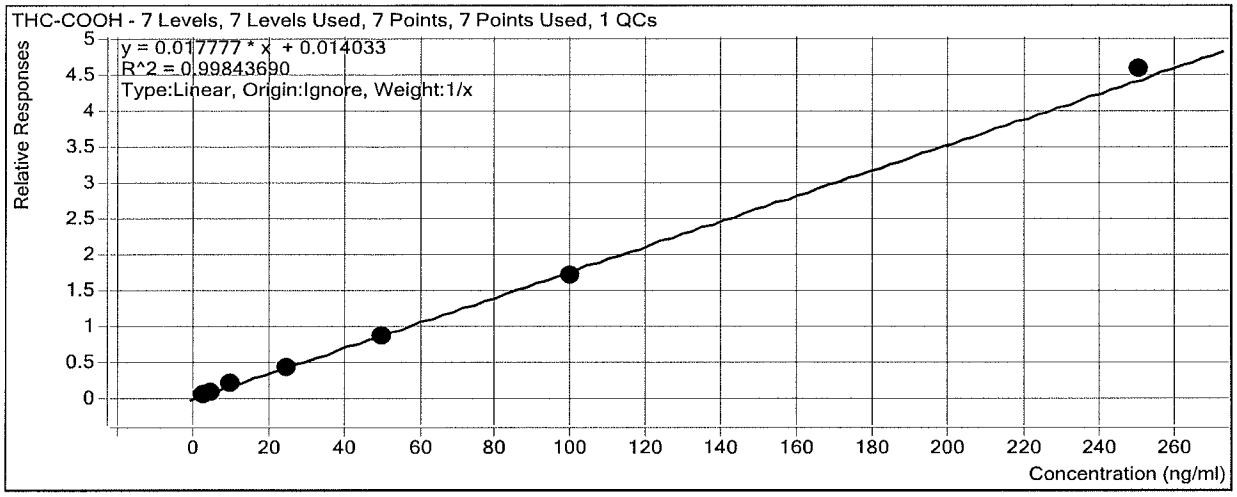
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin

Last Calib Update 7/28/2017 8:55 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/mL	1	☑	3	3.2	106.7
Cal 2 - 5ng/mL	2	☑	5	5.0	99.4
Cal 3 - 10ng/mL	3	☑	10	10.4	104.2
QC - 10ng/mL	3	☑	10	9.7	97.1
Cal 4 - 25ng/mL	4	☑	25	23.9	95.7
Cal 5 - 50ng/mL	5	☑	50	47.9	95.7
Cal 6 - 100ng/mL	6	☑	100	95.4	95.4
Cal 7 - 250ng/mL	7	☑	250	257.2	102.9

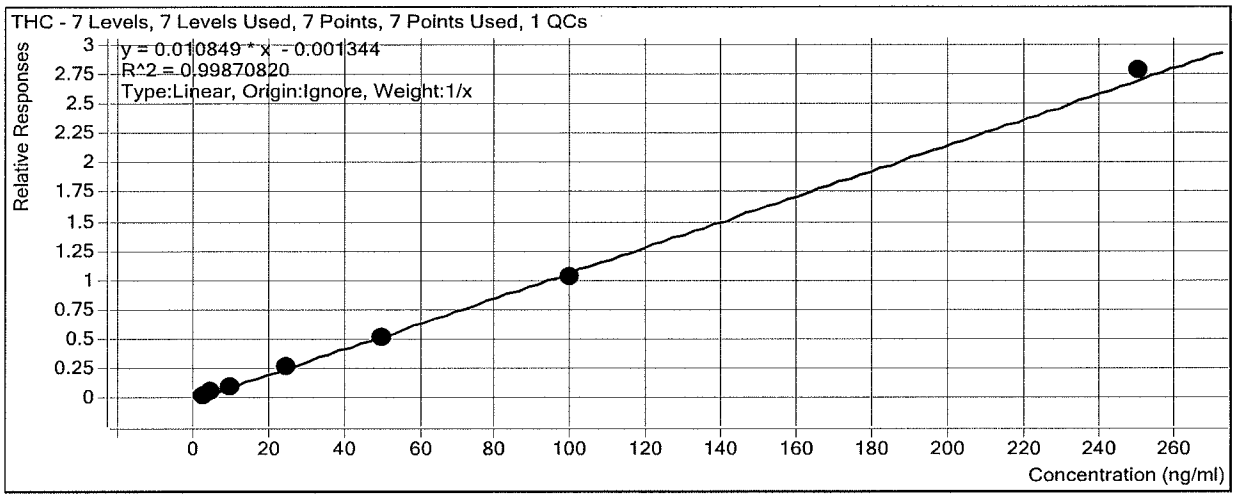
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin

Last Calib Update 7/28/2017 8:55 AM **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	3.0	101.3
Cal 2 - 5ng/mL	2	☑	5	5.2	103.6
Cal 3 - 10ng/mL	3	☑	10	10.0	100.4
QC - 10ng/mL	3	☑	10	10.0	99.9
Cal 4 - 25ng/mL	4	☑	25	25.1	100.5
Cal 5 - 50ng/mL	5	☑	50	48.4	96.9
Cal 6 - 100ng/mL	6	☑	100	94.8	94.8
Cal 7 - 250ng/mL	7	☑	250	256.3	102.5

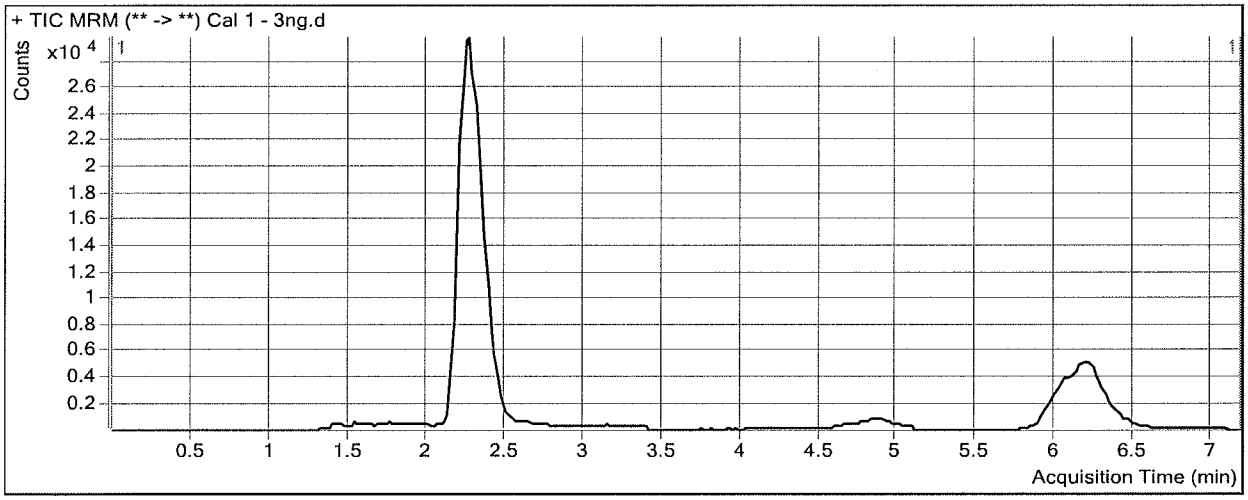
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 11:32	**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	2.255	5628	228706	0.0246	3.1156
THC-COOH	THC-COOH-d9	2.406	5829	82150	0.0709	3.2017
THC	THC-d3	6.192	3210	101544	0.0316	3.0378

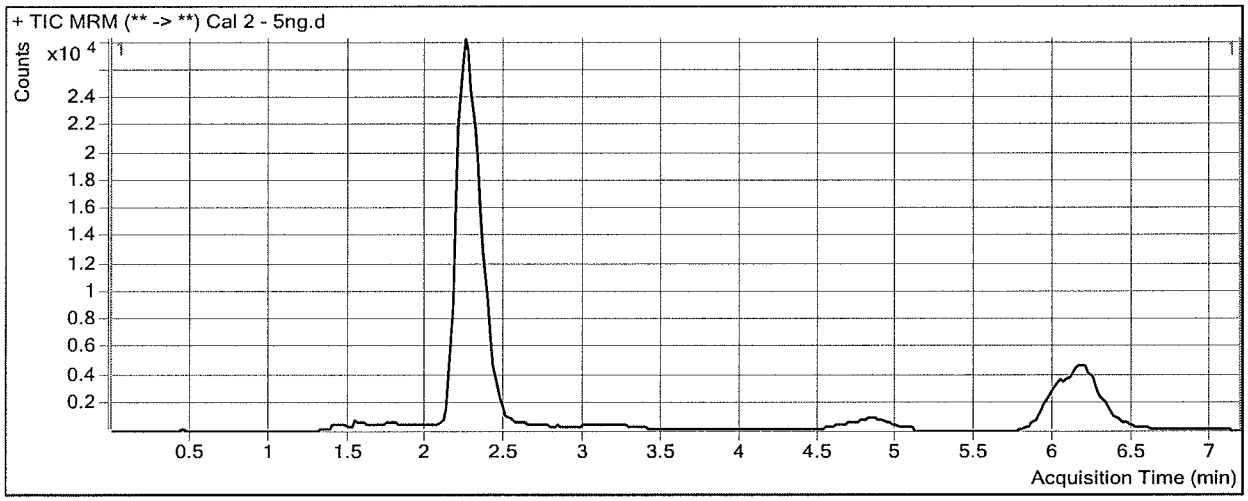
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 11:43	**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	2.275	8890	205806	0.0432	5.1120
THC-COOH	THC-COOH-d9	2.366	7557	73824	0.1024	4.9690
THC	THC-d3	6.172	4956	90396	0.0548	5.1776

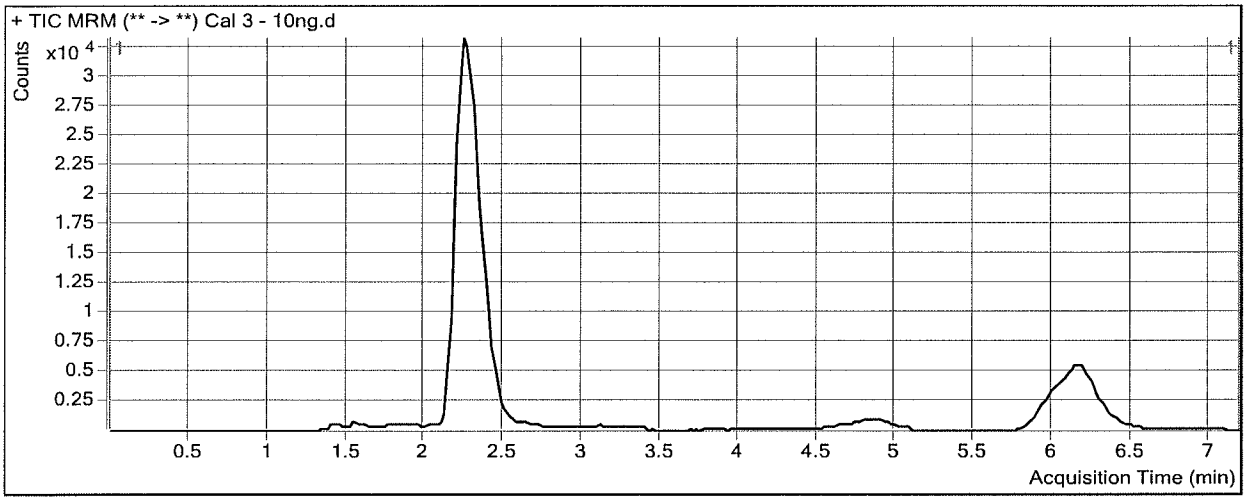
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 11:55	**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	2.255	21154	243283	0.0870	9.8119
THC-COOH	THC-COOH-d9	2.366	16168	81169	0.1992	10.4153
THC	THC-d3	6.172	10524	97788	0.1076	10.0437

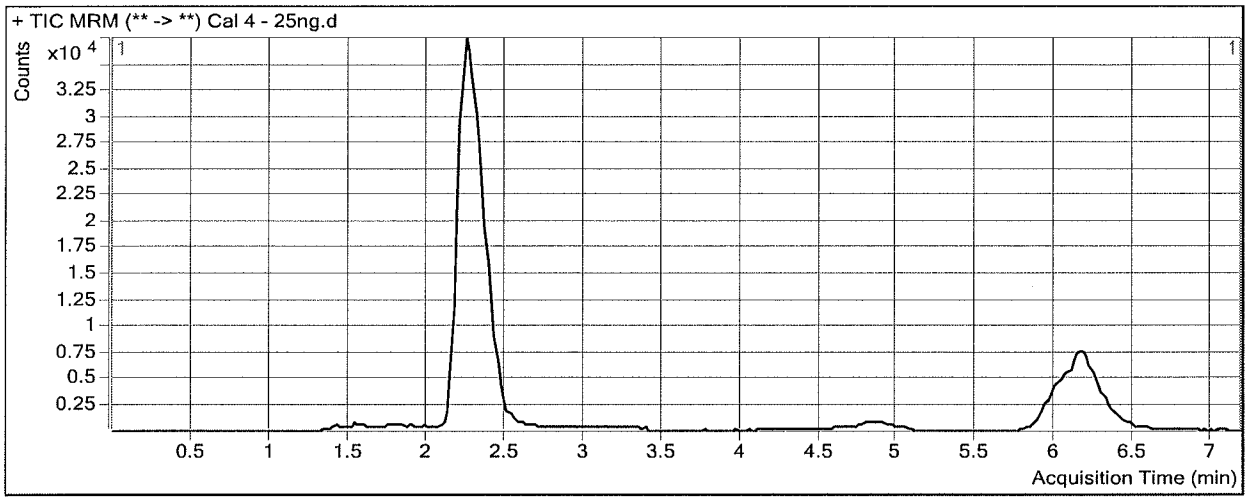
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 12:07	**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	2.255	52753	234415	0.2250	24.6440
THC-COOH	THC-COOH-d9	2.386	37111	84452	0.4394	23.9296
THC	THC-d3	6.132	28938	106689	0.2712	25.1237

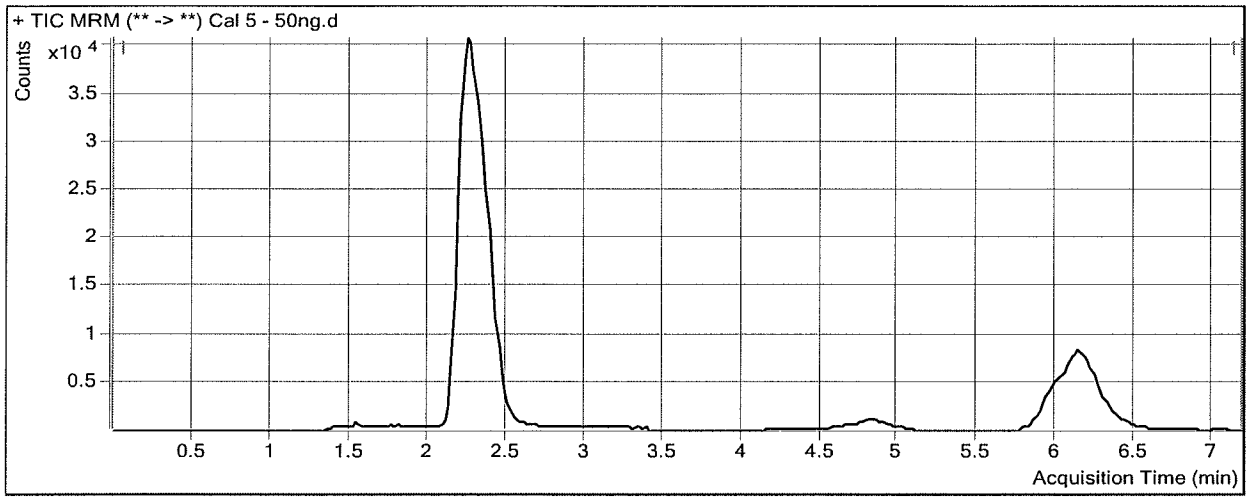
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 12:19	**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	2.255	100290	216882	0.4624	50.1407
THC-COOH	THC-COOH-d9	2.366	68184	78838	0.8649	47.8611
THC	THC-d3	6.132	49913	95215	0.5242	48.4409

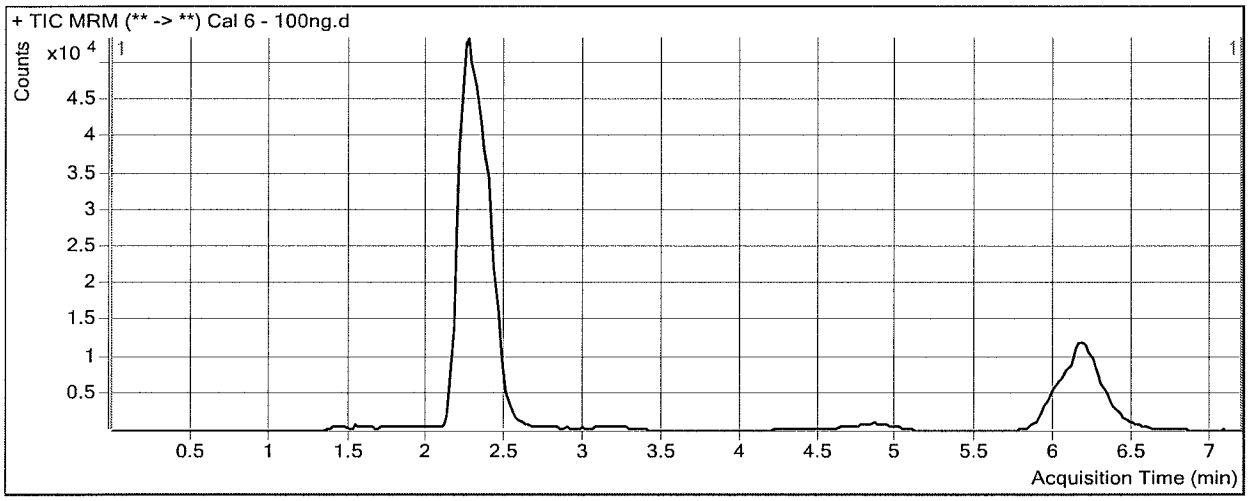
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 12:31	**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	2.255	192416	219179	0.8779	94.7669
THC-COOH	THC-COOH-d9	2.386	130554	76330	1.7104	95.4237
THC	THC-d3	6.172	99125	96463	1.0276	94.8377

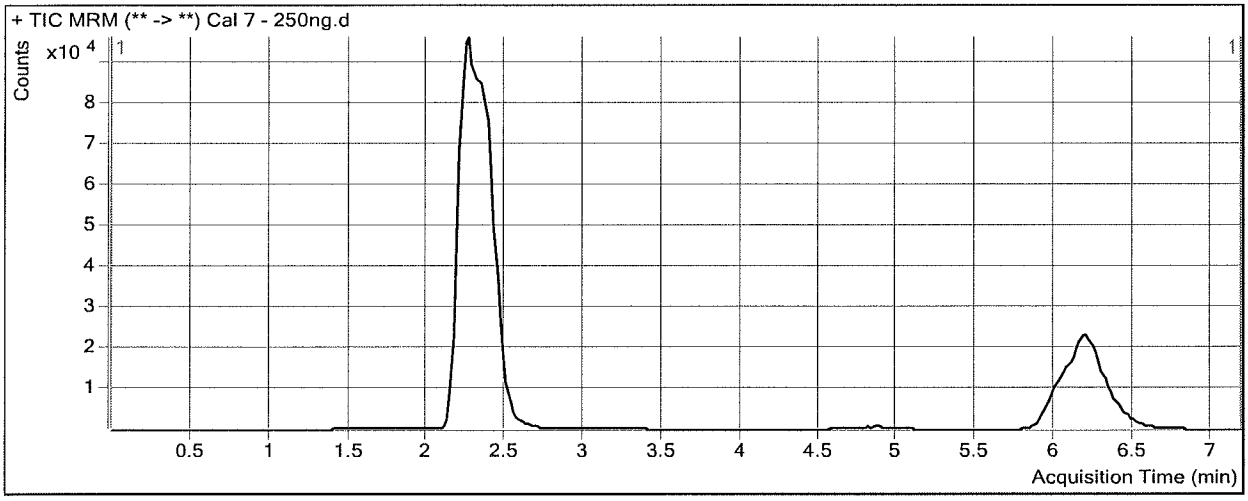
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\72717 cann quant\QuantResults\72717 cann quant.batch.bin		
Analysis Time	7/28/2017 8:55 AM	**Analyst Name**	ISP Tox
Report Time	7/28/2017 8:56 AM	**Reporter Name**	ISP Tox
Last Calib Update	7/28/2017 8:55 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-07-27 12:43	**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	2.255	517071	217852	2.3735	255.4089
THC-COOH	THC-COOH-d9	2.386	339725	74074	4.5863	257.1996
THC	THC-d3	6.192	264923	95303	2.7798	256.3384