reviewed 8/24/17 8/22/2017

Worklist: 1851

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-1469	2	92968	AM 27 Blood THC Quant by LC
C2017-1519	1	92965	AM 27 Blood THC Quant by LC
C2017-1656	1	92966	AM 27 Blood THC Quant by LC
M2017-3500	1	92967	AM 27 Blood THC Quant by LC
M2017-3613	1	92964	AM 27 Blood THC Quant by LC

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

Extraction Date: 8-22-17 **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: 82217 conn screen 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: 82217 conn 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

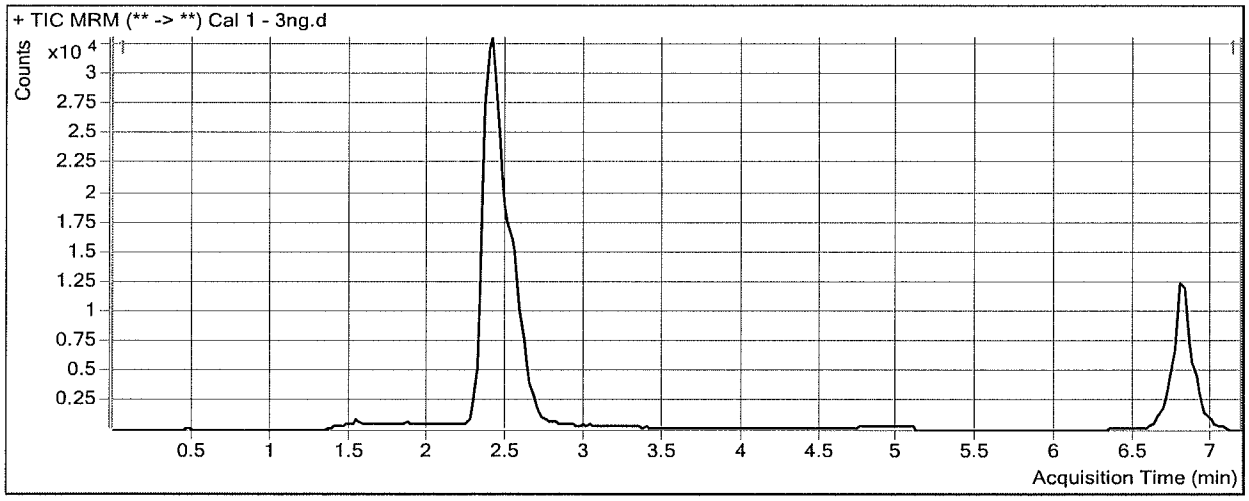
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 17:47	**Data File**	Cal 1 - 3ng.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	2.416	6856	263226	0.0260	2.8415
THC-COOH	THC-COOH-d9	2.566	5826	86977	0.0670	2.9381
THC	THC-d3	6.813	3640	101956	0.0357	2.8331

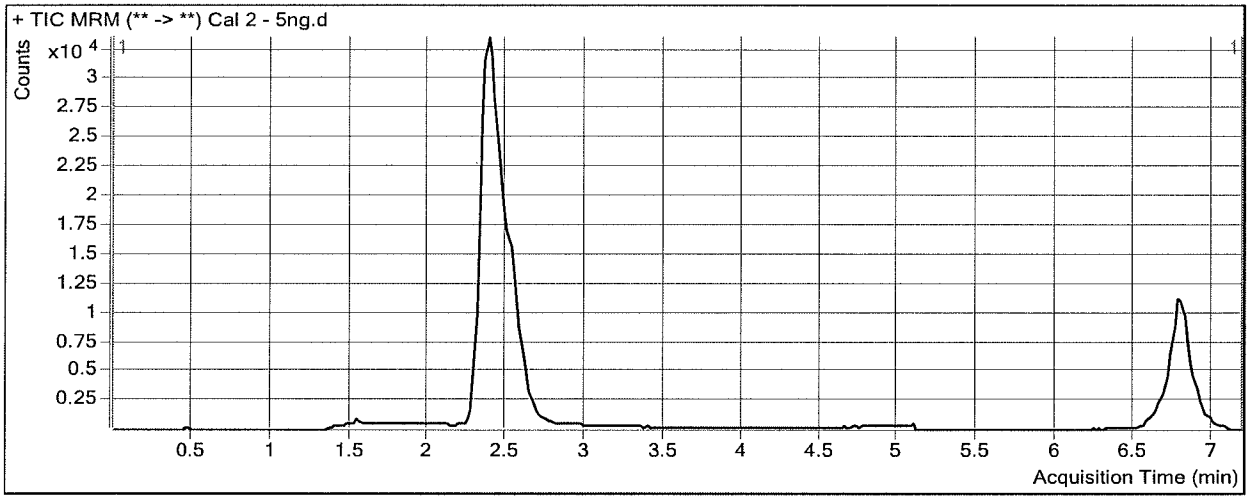
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 17:59	**Data File**	Cal 2 - 5ng.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	2.416	11924	264791	0.0450	4.7829
THC-COOH	THC-COOH-d9	2.586	10029	87899	0.1141	5.0523
THC	THC-d3	6.793	6073	99858	0.0608	4.8926

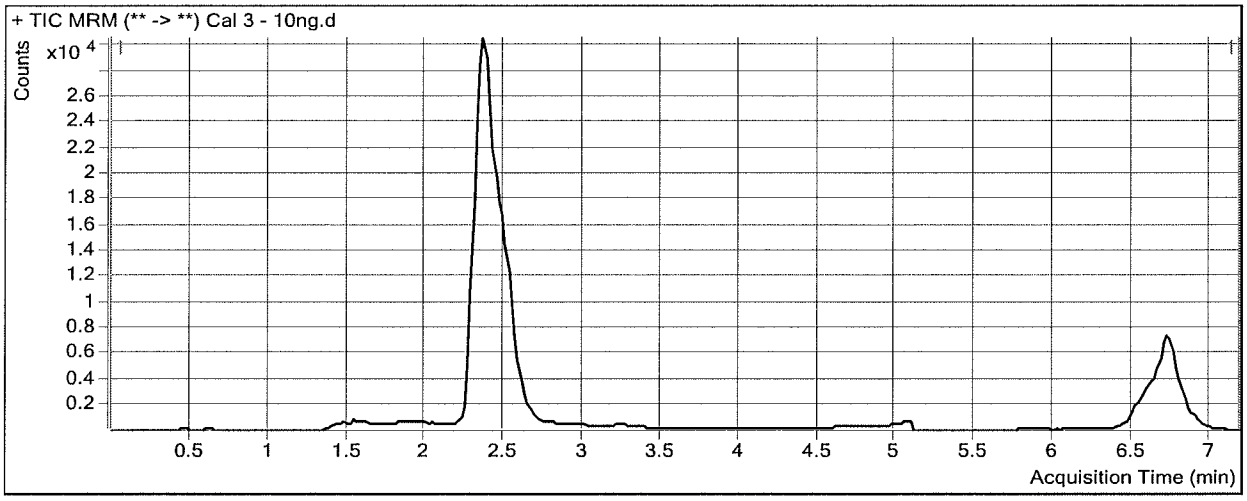
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 18:11	**Data File**	Cal 3 - 10ng.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	2.376	22804	224255	0.1017	10.5753
THC-COOH	THC-COOH-d9	2.526	17223	77311	0.2228	9.9293
THC	THC-d3	6.733	9758	78433	0.1244	10.1073

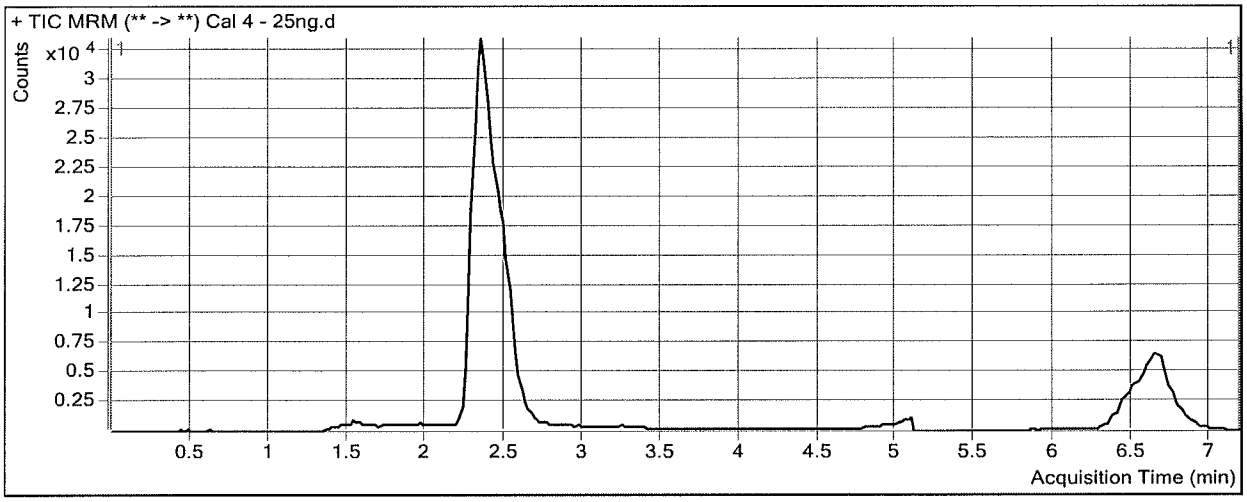
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 18:23	**Data File**	Cal 4 - 25ng.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	2.356	53183	213047	0.2496	25.7005
THC-COOH	THC-COOH-d9	2.486	46808	72772	0.6432	28.7960
THC	THC-d3	6.633	24856	80381	0.3092	25.2603

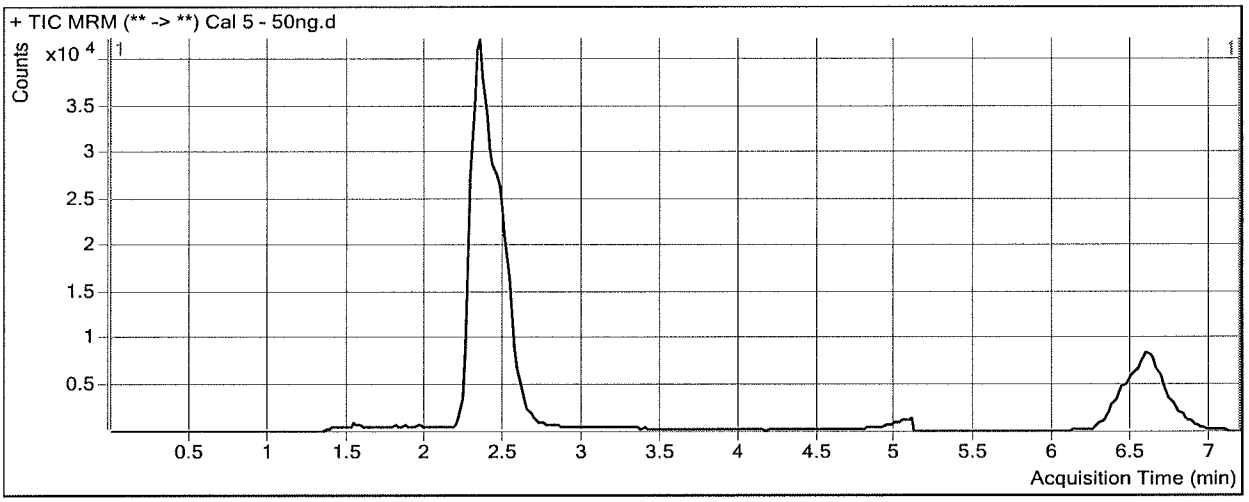
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 18:35	**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	2.356	116454	218360	0.5333	54.7038
THC-COOH	THC-COOH-d9	2.486	85644	73844	1.1598	51.9765
THC	THC-d3	6.593	54824	84238	0.6508	53.2670

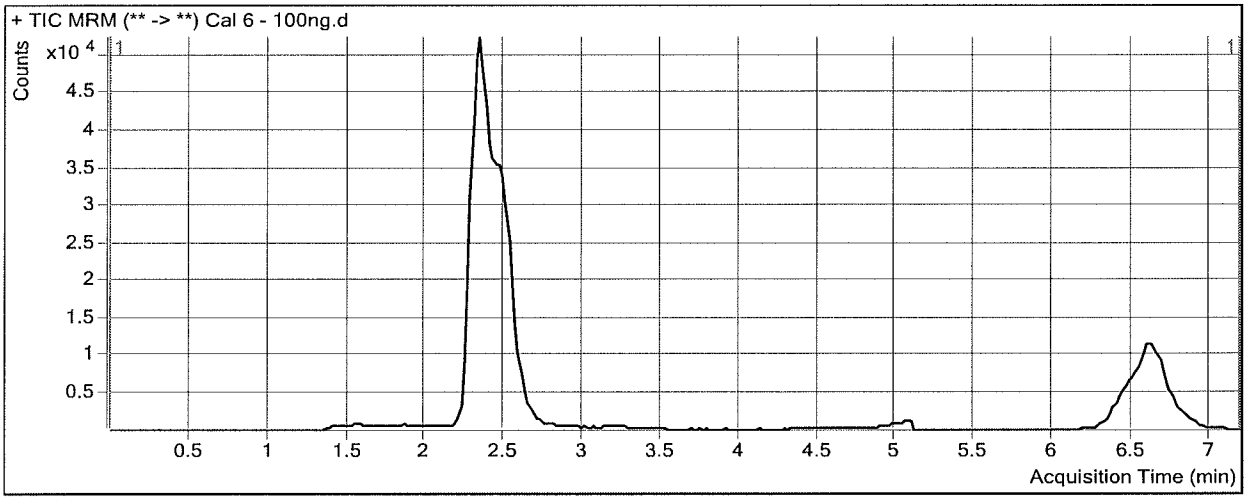
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 18:46	**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	2.356	192630	219648	0.8770	89.8416
THC-COOH	THC-COOH-d9	2.486	138792	70165	1.9781	88.6957
THC	THC-d3	6.613	95626	77804	1.2291	100.6775

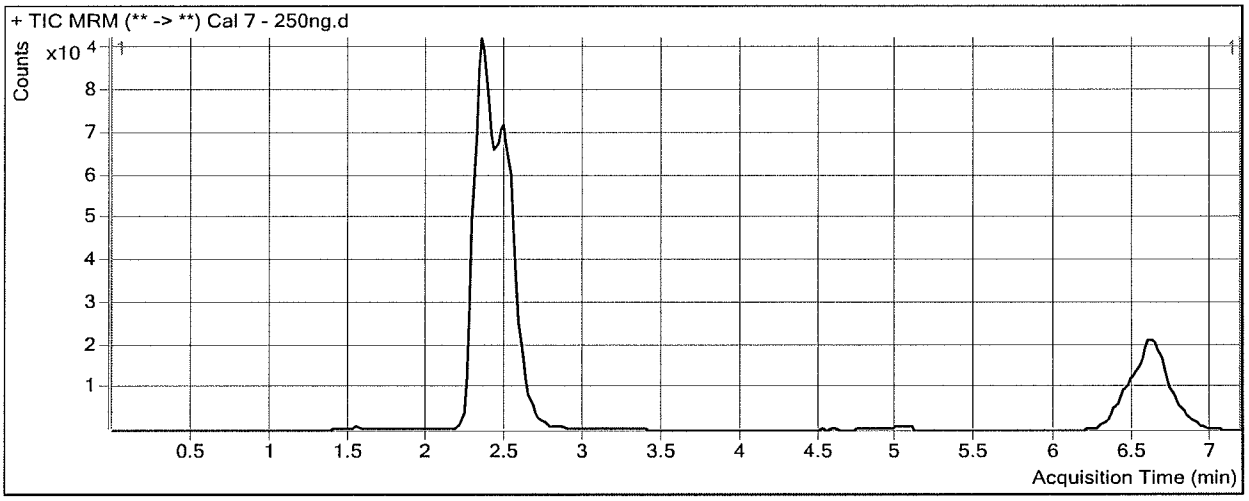
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 18:58	**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	2.356	521937	209777	2.4881	254.5544
THC-COOH	THC-COOH-d9	2.506	337919	64583	5.2323	234.7264
THC	THC-d3	6.613	228443	76121	3.0011	245.9622

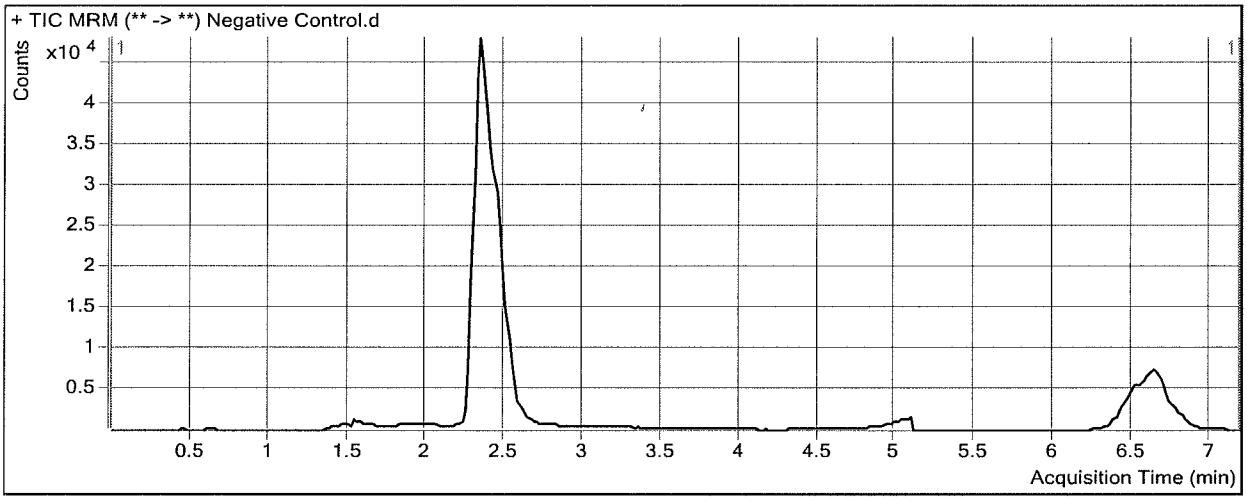
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 19:22	**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	2.406	14779	119770	0.1234	5.4697

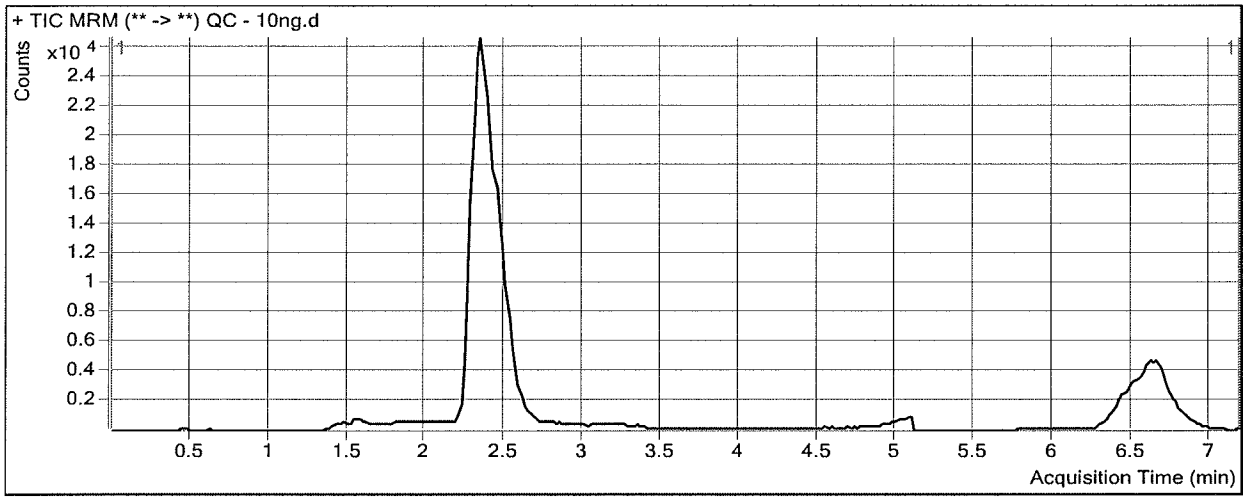
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 19:34	**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	2.356	22418	192586	0.1164	12.0800
THC-COOH	THC-COOH-d9	2.486	16169	63000	0.2567	11.4495
THC	THC-d3	6.653	11338	73029	0.1553	12.6352

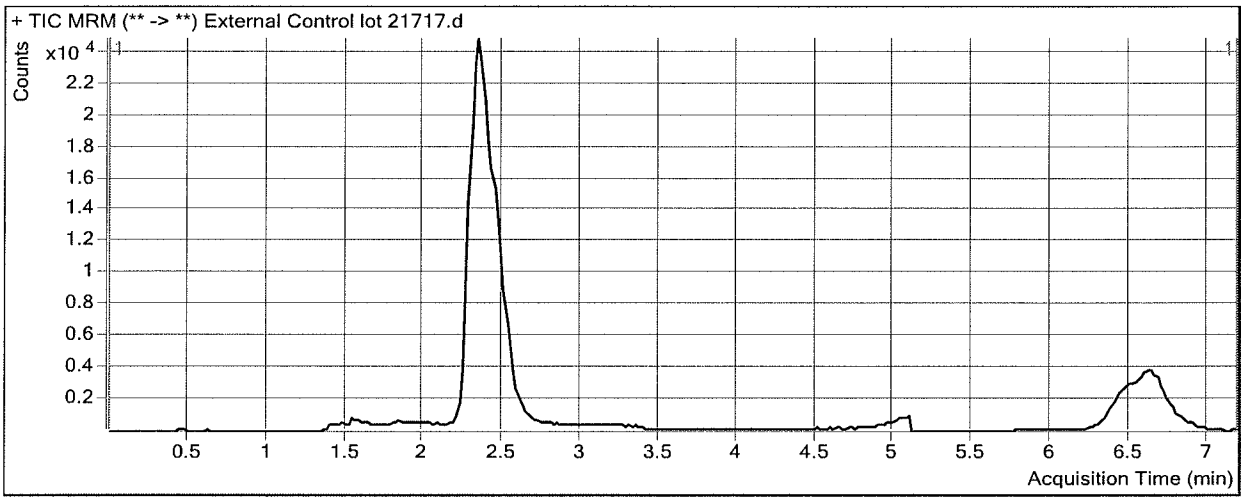
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 19:46	**Data File**	External Control lot 21717.d
Sample Type	Sample	**Sample Name**	External Control lot 21717
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	2.376	15219	185762	0.0819	8.5552
THC-COOH	THC-COOH-d9	2.486	14097	61671	0.2286	10.1898
THC	THC-d3	6.633	7543	67271	0.1121	9.0999

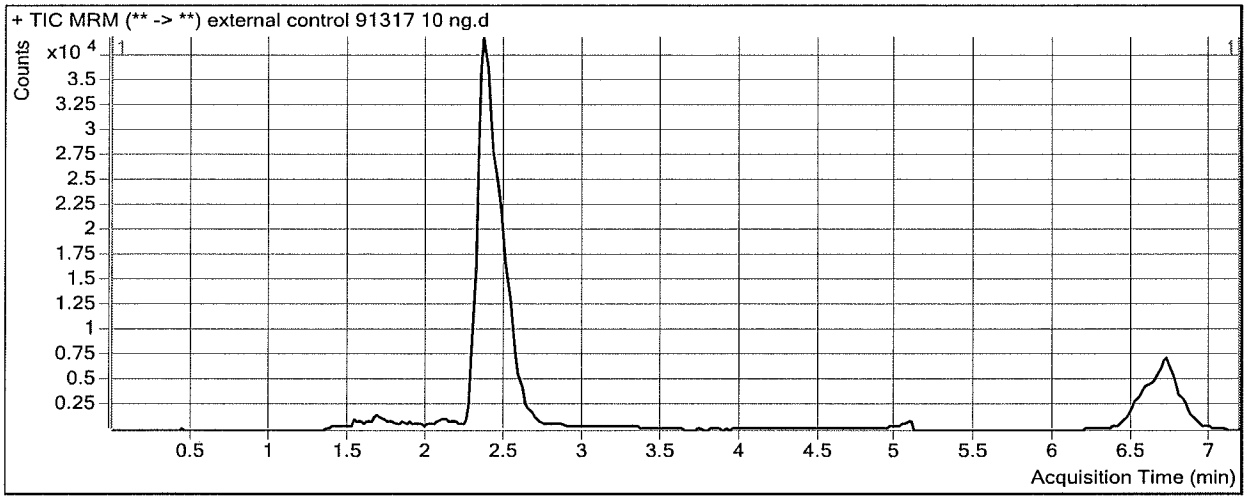
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin		
Analysis Time	8/24/2017 8:48 AM	**Analyst Name**	ISP Tox
Report Time	8/24/2017 8:50 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/24/2017 8:48 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-08-23 21:56	**Data File**	external control 91317 10 ng.d
Sample Type	Sample	**Sample Name**	external control 91317 10 ng
Dilution	1	**Acq Method**	AM 27 Quant THC 7-2017.m
Position	P1-H2	**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	29307	274107	0.1069	11.1101
THC-COOH	THC-COOH-d9	2.526	16604	86868	0.1911	8.5098
THC	THC-d3	6.713	11049	86275	0.1281	10.4065

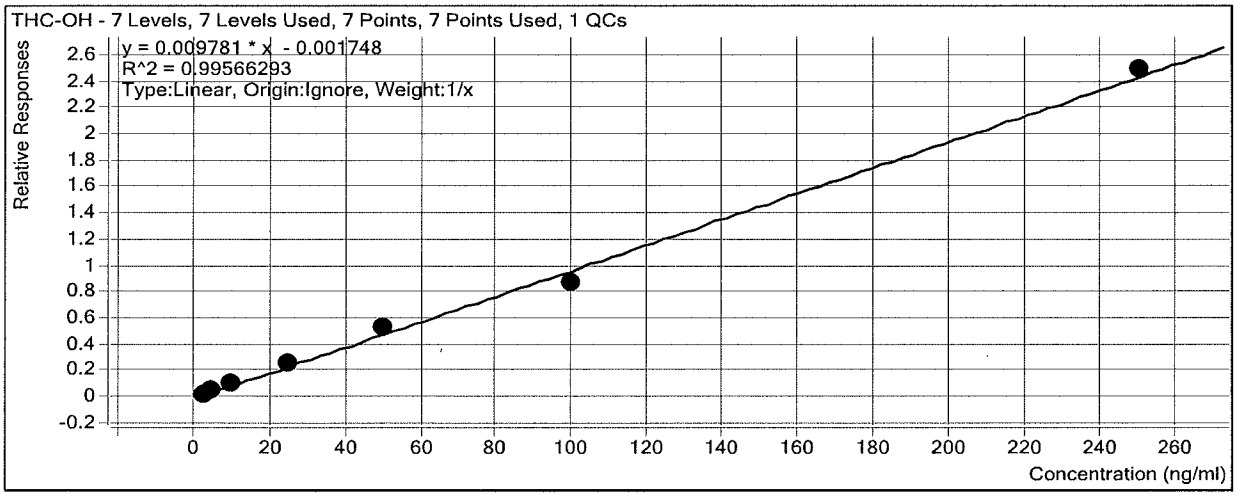
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin

Last Calib Update 8/24/2017 8:48 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	2.8	94.7
Cal 2 - 5ng	2	☑	5	4.8	95.7
Cal 3 - 10ng	3	☑	10	10.6	105.8
QC - 10ng	3	☑	10	12.1	120.8
Cal 4 - 25ng	4	☑	25	25.7	102.8
Cal 5 - 50ng	5	☑	50	54.7	109.4
Cal 6 - 100ng	6	☑	100	89.8	89.8
Cal 7 - 250ng	7	☑	250	254.6	101.8

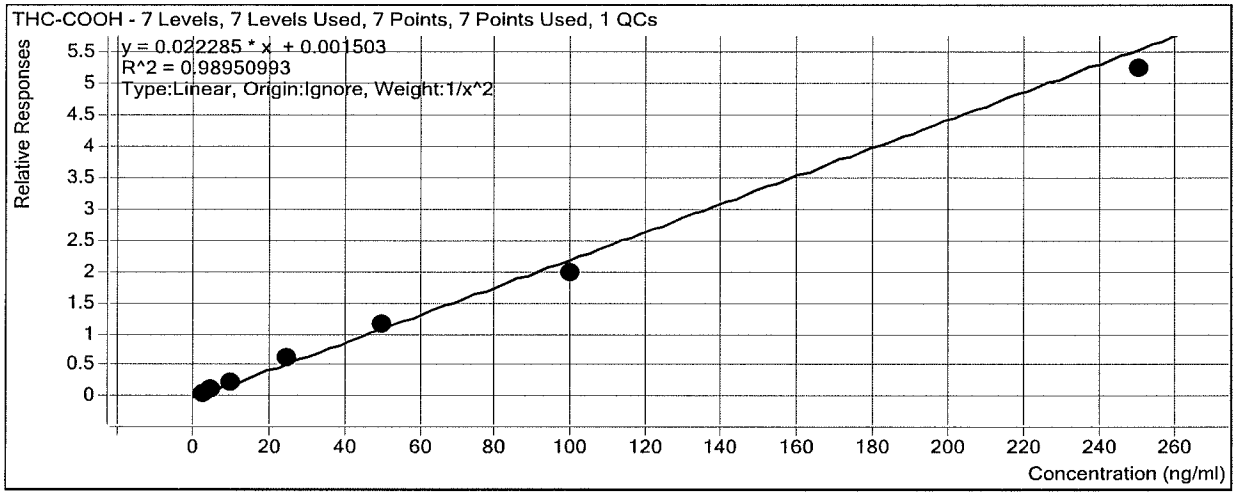
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin

Last Calib Update 8/24/2017 8:48 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	97.9
Cal 2 - 5ng	2	☑	5	5.1	101.0
Cal 3 - 10ng	3	☑	10	9.9	99.3
QC - 10ng	3	☑	10	11.4	114.5
Cal 4 - 25ng	4	☑	25	28.8	115.2
Cal 5 - 50ng	5	☑	50	52.0	104.0
Cal 6 - 100ng	6	☑	100	88.7	88.7
Cal 7 - 250ng	7	☑	250	234.7	93.9

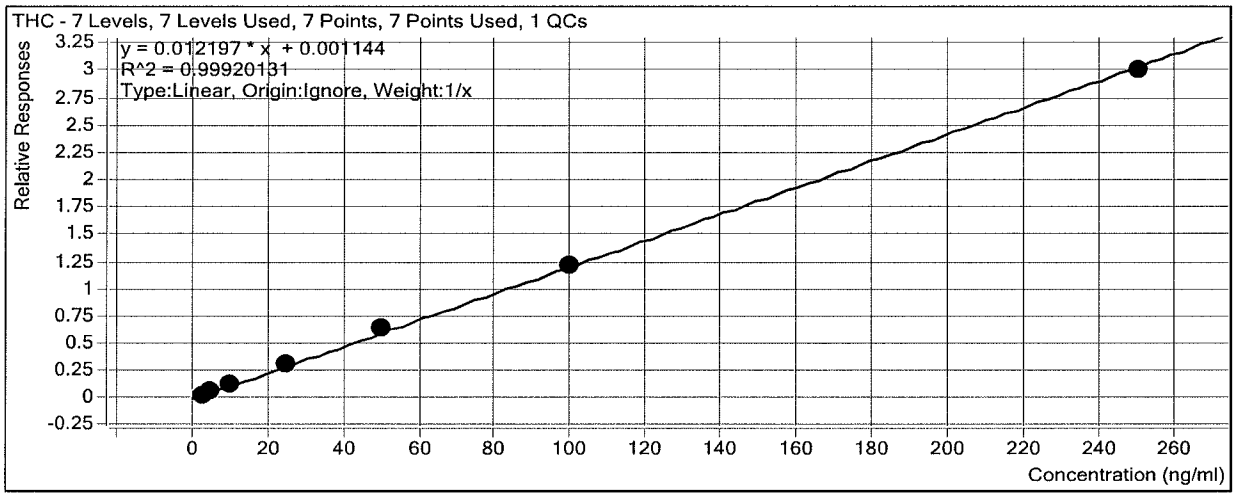
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\82217 cann quant\QuantResults\82217 cann quant.batch.bin

Last Calib Update 8/24/2017 8:48 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	2.8	94.4
Cal 2 - 5ng	2	☑	5	4.9	97.9
Cal 3 - 10ng	3	☑	10	10.1	101.1
QC - 10ng	3	☑	10	12.6	126.4
Cal 4 - 25ng	4	☑	25	25.3	101.0
Cal 5 - 50ng	5	☑	50	53.3	106.5
Cal 6 - 100ng	6	☑	100	100.7	100.7
Cal 7 - 250ng	7	☑	250	246.0	98.4