reviewed 12/22/17 12/21/2017

Worklist: 2090

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-2481	1	103261	AM 27 Blood THC Quant by LC
C2017-2492	1	103260	AM 27 Blood THC Quant by LC
C2017-2493	1	103259	AM 27 Blood THC Quant by LC
M2017-5347	2	103262	AM 27 Blood THC Quant by LC
M2017-5507	1	103263	AM 27 Blood THC Quant by LC

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

Extraction Date: 12-19-17 Analyst: Anne Nord

Plate lot#: 0515037 Plate Expiration: 9/28/18

Mobile phase A: 0.1% Formic Acid in LCMS Water **Mobile phase B:** 0.1% Formic acid in Acetonitrile
MTBE LCMS Methanol Hexane
Blank Blood Lot: 17J20718 **Column**: UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 62340

Pre-Analytic:

- [x] 1. Check levels of mobile phases and needle wash refill as needed. Ensure waste is not full.
- [x] 2. Ensure correct column is installed and begin mobile phase flow allow to equilibrate ~ 30 minutes.
- [x] 3. Create worklist:

Analytic:

- [x] 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- [x] 2. Pipette **1000µL blood (calibrated pipette) Pipette ID: 2609543** in wells of analytical (standards) plate.
- [x] 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- [x] 4. Pipette **500µL 0.1% formic acid in water** in wells of analytical plate.
- [x] 5. Place on shaking incubator at ambient temp., 900rpm for 15 minutes.
- [x] 6. Transfer **800µL of blood+acid** mixture to corresponding wells of SLE+ plate.
- [x] 7. Apply positive pressure for approx. 10-15 seconds (or until no liquid remains on top of sorbent). ***(Load at 85-100 PSI- Selector to the right)*** Manifold ID: 66792
- [x] 8. Wait 5 minutes.
- [x] 9. Add **2.25mL MTBE. (*Add in 3 increments of 750uL)***
- [x] 10. Wait 5 minutes.
- [x] 11. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
- [x] 12. Add **2.25mL Hexane. (*Add in 3 increments of 750uL)***
- [x] 13. Wait 5 minutes.
- [x] 14. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
- [x] 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. *SPE Dry ID: 66819*
- [x] 16. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- [x] 1. Create batch and process data.
  Worklist path: 122017 cann quant Batch Name: 12/19/17 cann quant
- [x] 2. Make any necessary integration changes, r^2 values ≥0.98 for each analyte.
- [x] 3. Did all QCs pass for each analyte? Y / N Enter QCs into control charting?
- [x] 4. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: _

Toxicology AM method 27 external prep information
working solution 1 ug/ml in meoh C-THC, THC-OH, THC
Stock solution 1mg/ml 10 ul each THC, THC-OH 100 ug/ml 100 ul C-THC in 9880 ul meOH lot (Fisher 168427)
Ppd 8/17/17 Exp: 2/17/18 lot 21718 by AMN

Drug	lot (cerilliant)	expiration
C-THC	FE03121501	3/1/2020
THC-OH	FE01141502	1/1/2020
THC	FE04231406	4/1/2019

AM 27 control 100 ul working solution lot (21717) in 9990 ul blood lot (321632)
ppd 8/17/17 Exp 2/17/18 by AMN Concentration 10 ng/ml each

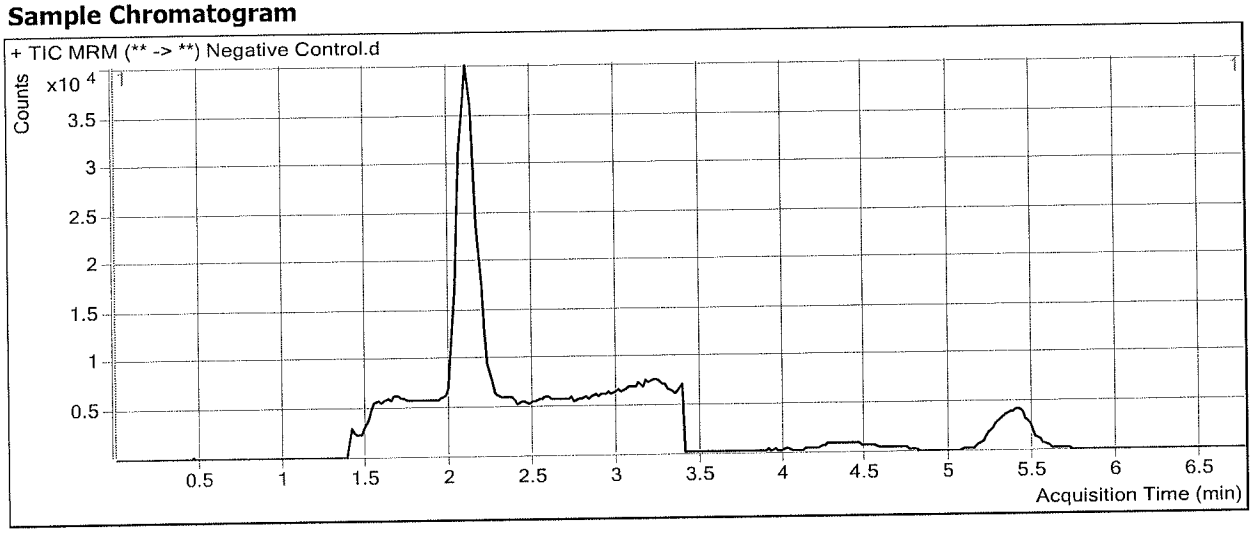
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 19:48	**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-OH	THC-OH-d3	1.975	1578	201908	0.0078	1.6063

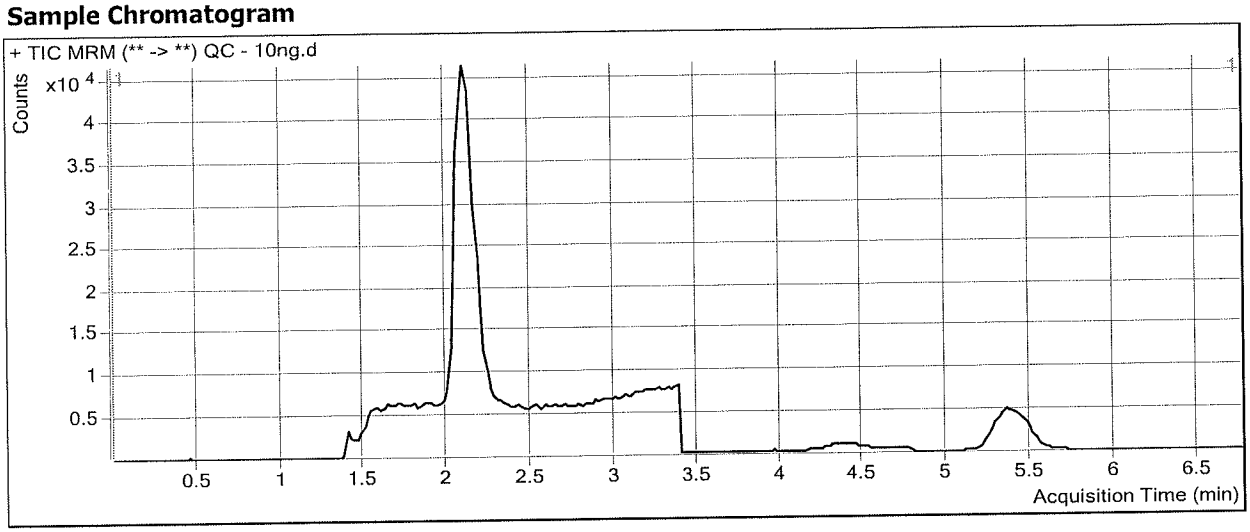
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 20:00	**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	17524	207318	0.0845	9.2233
THC-COOH	THC-COOH-d9	2.205	13298	70074	0.1898	9.3469
THC	THC-d3	5.452	8042	60144	0.1337	10.6973

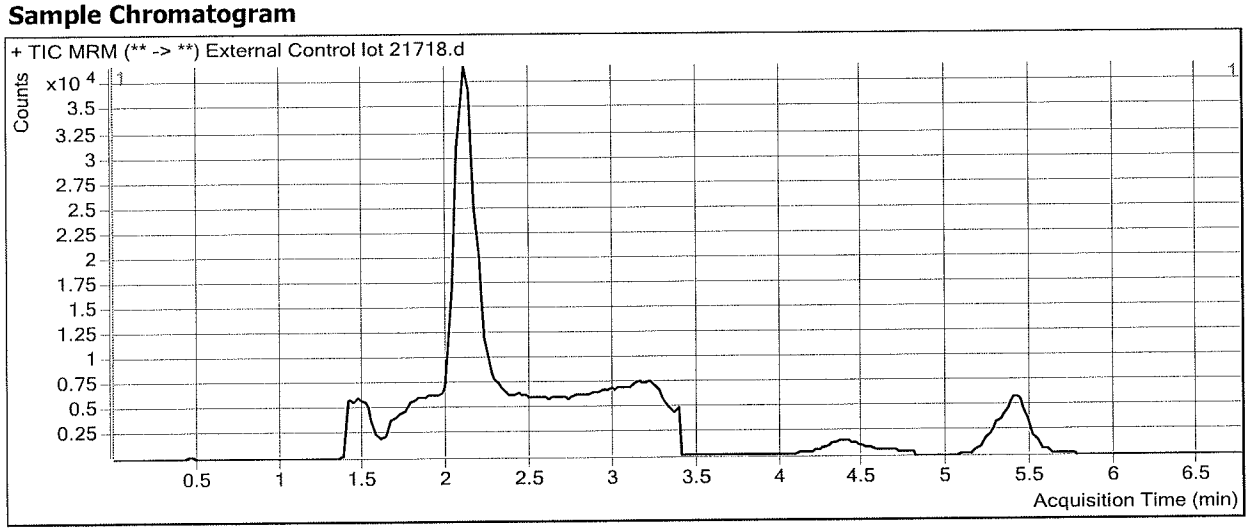
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 4:00 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 4:01 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 4:00 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 20:12	**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	2.115	11538	181064	0.0637	7.1575
THC-COOH	THC-COOH-d9	2.205	16384	65169	0.2514	12.4531
THC	THC-d3	5.412	7109	66605	0.1067	8.5197

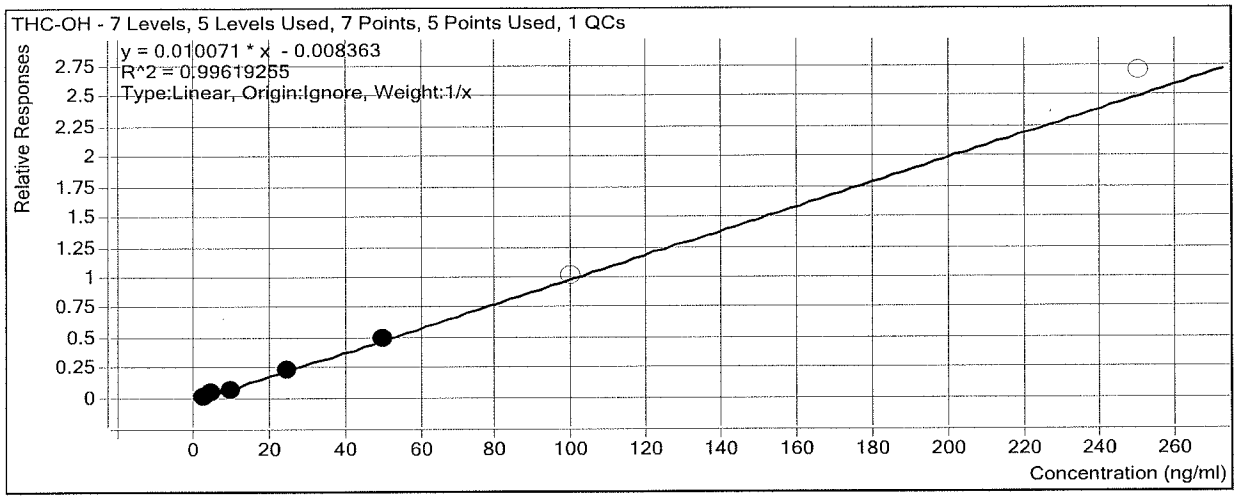
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin

Last Calib Update 12/21/2017 12:38 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	1	☑	3	3.1	103.6
Cal 2 - 5ng	2	☑	5	5.4	107.8
Cal 3 - 10ng	3	☑	10	8.7	86.9
QC - 10ng	3	☑	10	9.2	92.2
Cal 4 - 25ng	4	☑	25	25.1	100.2
Cal 5 - 50ng	5	☑	50	50.8	101.5
Cal 6 - 100ng	6	☐	100	101.5	101.5
Cal 7 - 250ng	7	☐	250	268.9	107.6

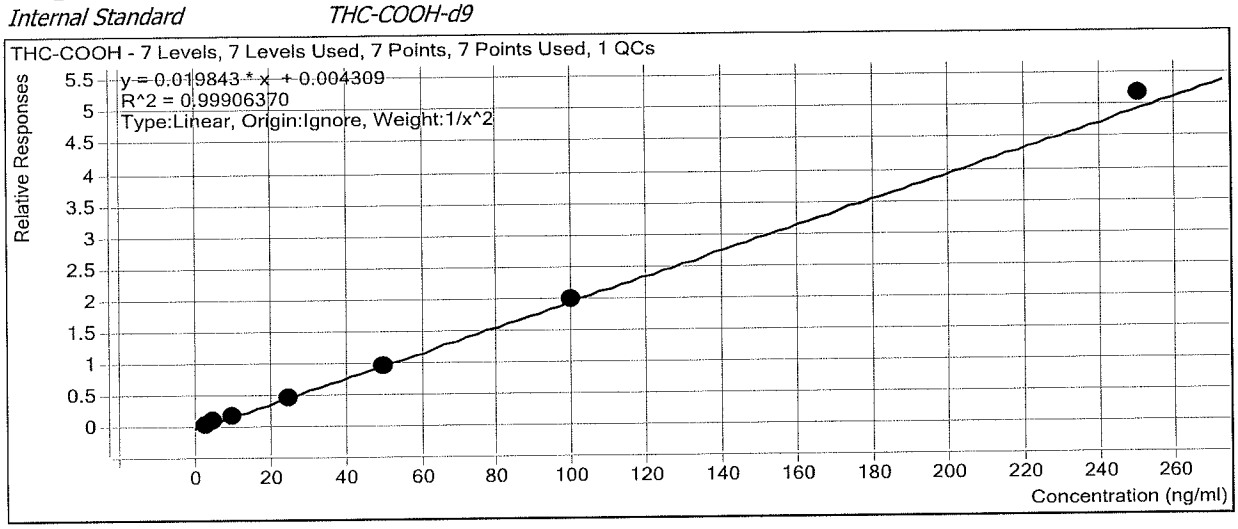
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin

Last Calib Update 12/21/2017 12:38 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	1	☑	3	3.0	99.8
Cal 2 - 5ng	2	☑	5	5.1	101.4
Cal 3 - 10ng	3	☑	10	9.9	99.2
QC - 10ng	3	☑	10	9.3	93.5
Cal 4 - 25ng	4	☑	25	24.2	96.7
Cal 5 - 50ng	5	☑	50	48.9	97.8
Cal 6 - 100ng	6	☑	100	100.7	100.7
Cal 7 - 250ng	7	☑	250	260.7	104.3

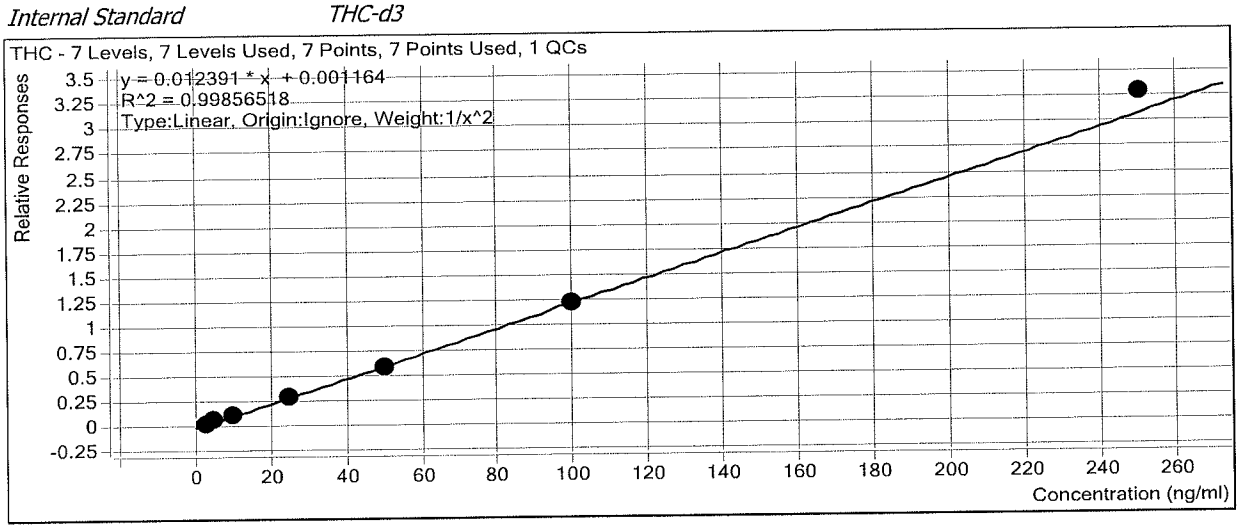
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin

Last Calib Update 12/21/2017 12:38 PM **Analyst Name** ISP TOX

Target Compound *THC*

Internal Standard *THC-d3*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.0	100.7
Cal 2 - 5ng	2	☑	5	5.0	100.3
Cal 3 - 10ng	3	☑	10	9.8	98.1
QC - 10ng	3	☑	10	10.7	107.0
Cal 4 - 25ng	4	☑	25	24.8	99.3
Cal 5 - 50ng	5	☑	50	48.3	96.5
Cal 6 - 100ng	6	☑	100	98.9	98.9
Cal 7 - 250ng	7	☑	250	265.6	106.2

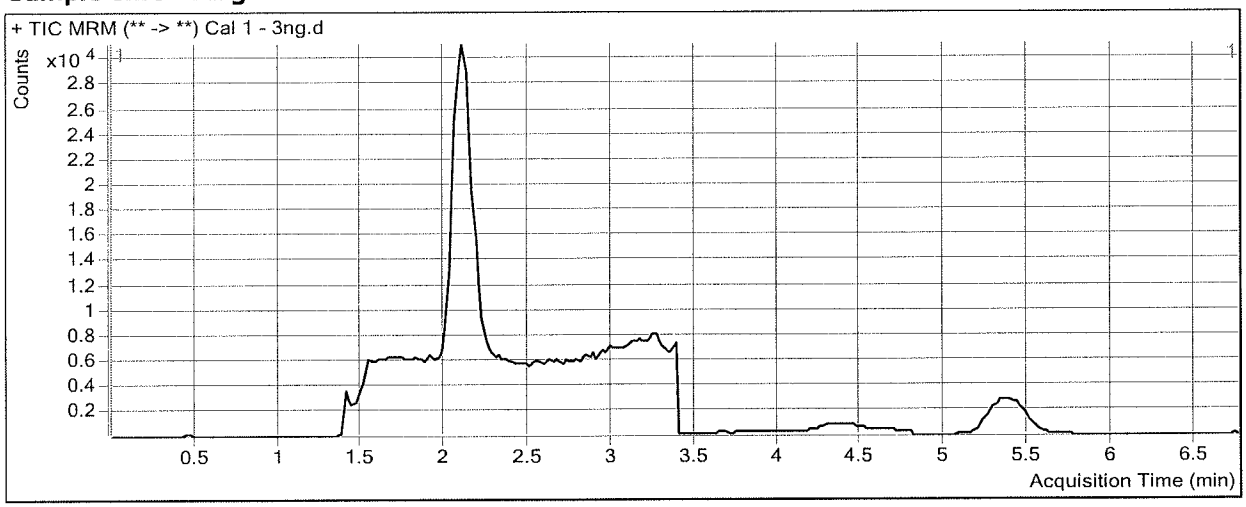
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 18:13	**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	2.115	3281	142998	0.0229	3.1087
THC-COOH	THC-COOH-d9	2.205	3091	48489	0.0637	2.9954
THC	THC-d3	5.432	1645	42640	0.0386	3.0195

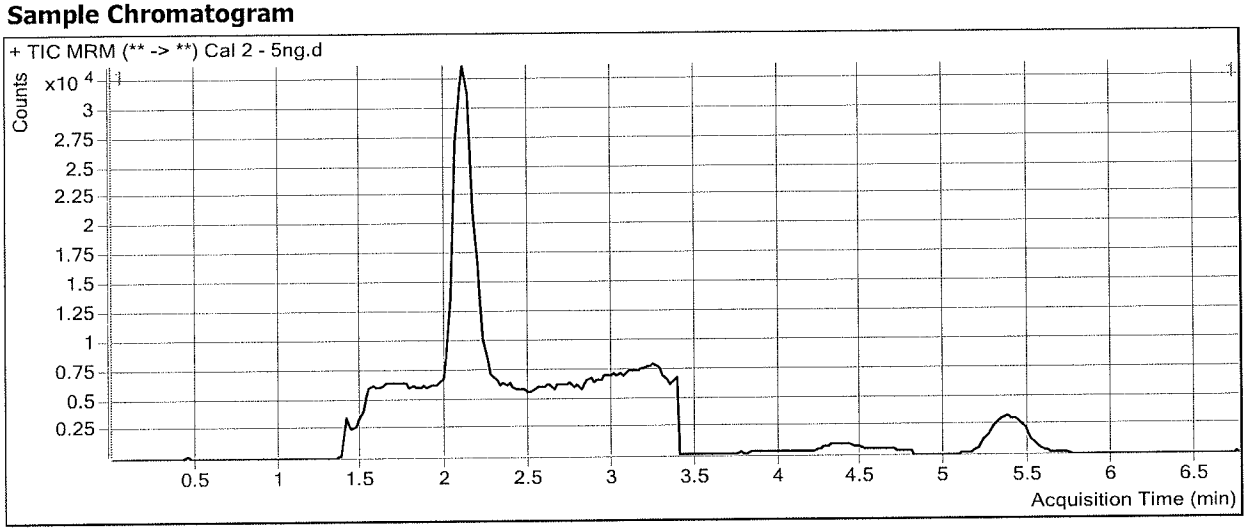
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 18:25	**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	2.135	7020	152959	0.0459	5.3876
THC-COOH	THC-COOH-d9	2.205	5392	51387	0.1049	5.0704
THC	THC-d3	5.412	2936	46386	0.0633	5.0136

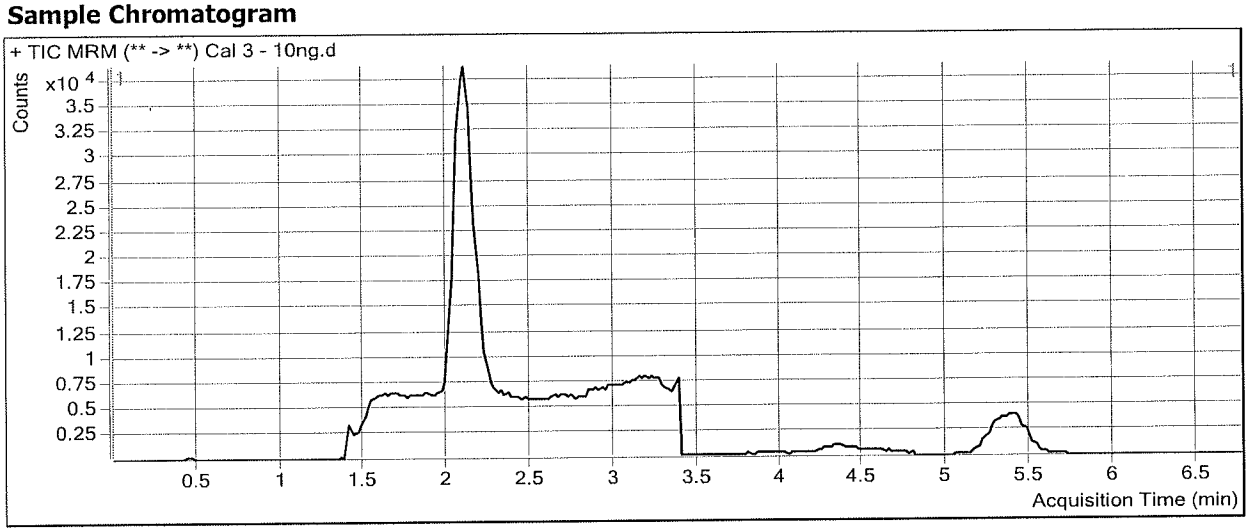
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 18:37	**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	2.115	13729	173460	0.0791	8.6888
THC-COOH	THC-COOH-d9	2.185	11507	57206	0.2012	9.9202
THC	THC-d3	5.412	6564	53470	0.1228	9.8131

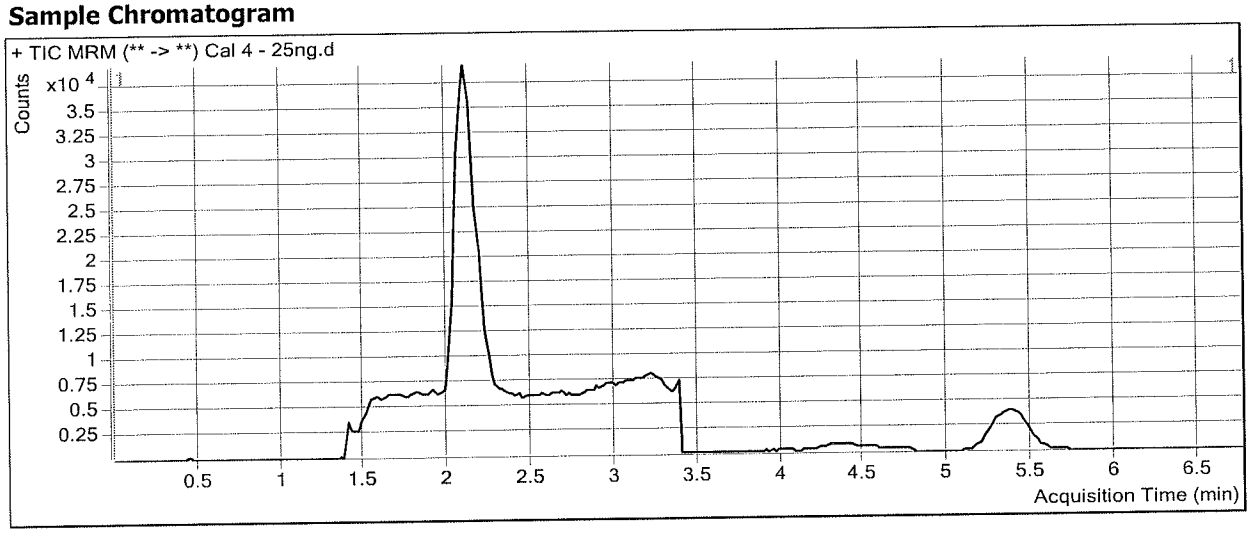
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 18:49	**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	2.115	35531	145647	0.2440	25.0526
THC-COOH	THC-COOH-d9	2.185	24047	49676	0.4841	24.1784
THC	THC-d3	5.432	13657	44245	0.3087	24.8167

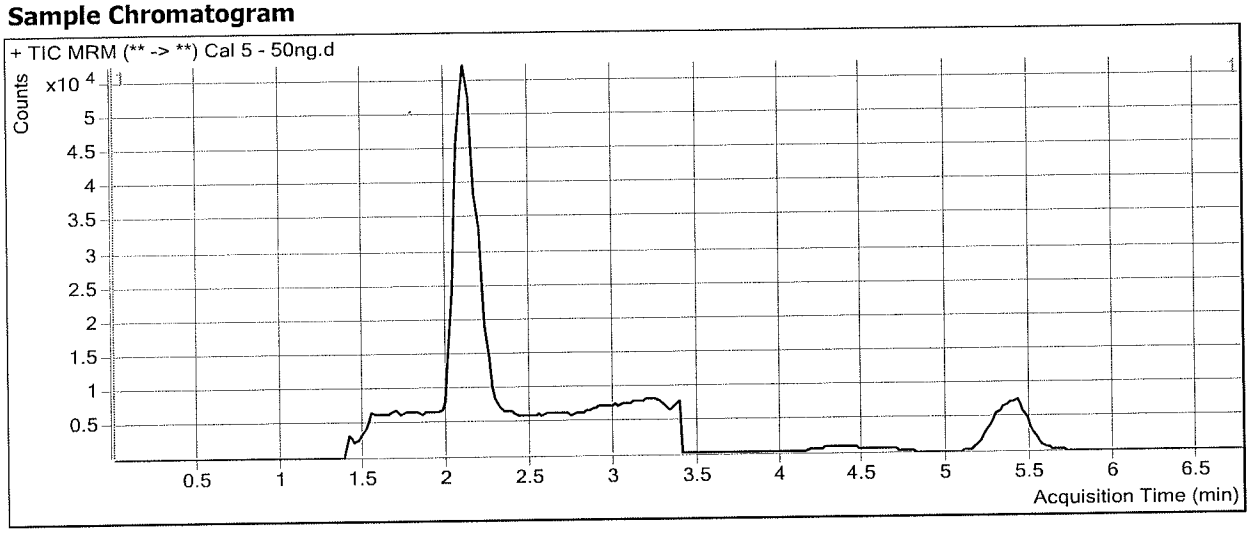
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 19:01	**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	2.115	97385	193653	0.5029	50.7622
THC-COOH	THC-COOH-d9	2.185	61952	63545	0.9749	48.9157
THC	THC-d3	5.412	37246	62145	0.5993	48.2737

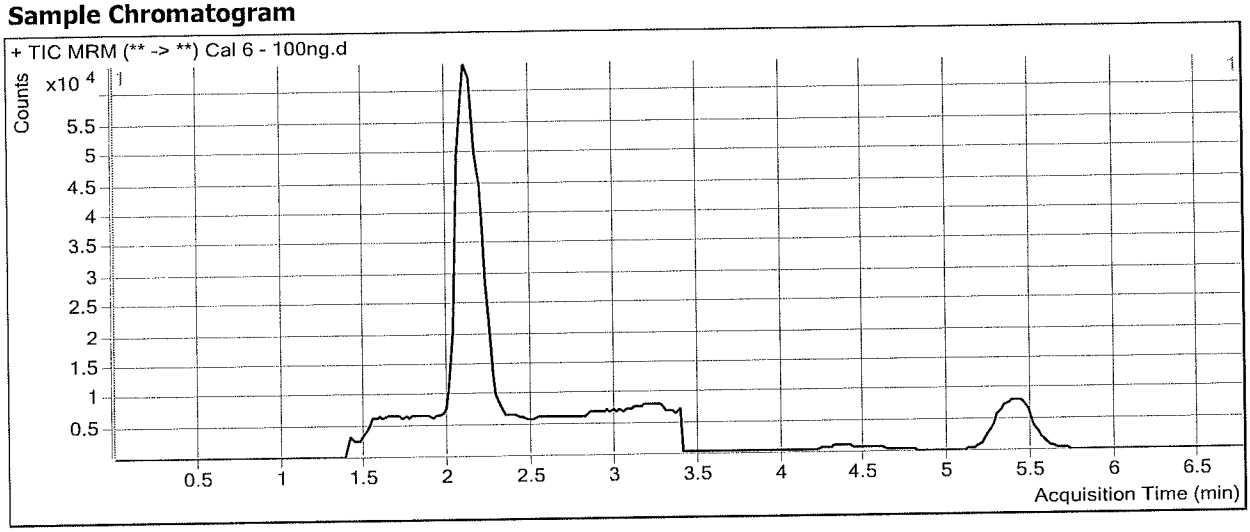
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 19:12	**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	2.115	166314	164032	1.0139	101.5024
THC-COOH	THC-COOH-d9	2.185	105744	52788	2.0032	100.7348
THC	THC-d3	5.412	59461	48475	1.2266	98.8973

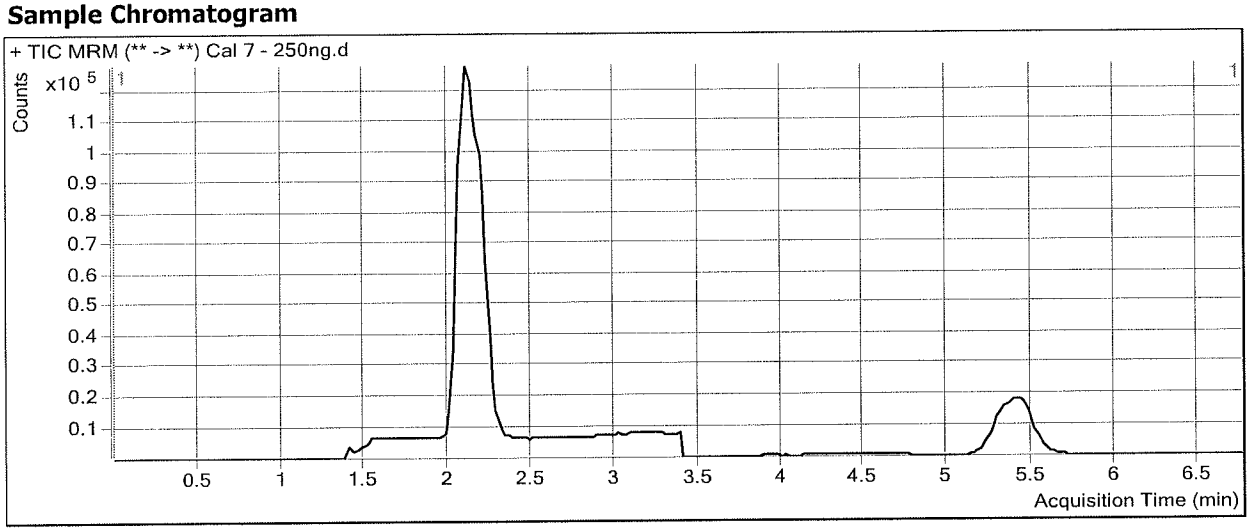
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\121917cann quant\QuantResults\121917 cann quant.batch.bin		
Analysis Time	12/21/2017 12:38 PM	**Analyst Name**	ISP Tox
Report Time	12/21/2017 12:39 PM	**Reporter Name**	ISP Tox
Last Calib Update	12/21/2017 12:38 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-12-20 19:24	**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	2.115	489162	181158	2.7002	268.9343
THC-COOH	THC-COOH-d9	2.185	299995	57952	5.1766	260.6619
THC	THC-d3	5.432	168597	51212	3.2922	265.5870