Worklist: 2109

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-2578	1	104106	AM 27 Blood THC Quant by LC
C2017-2588	1	104105	AM 27 Blood THC Quant by LC
C2018-0001	1	104104	AM 27 Blood THC Quant by LC
M2017-5510	1	104103	AM 27 Blood THC Quant by LC
M2017-5531	1	104102	AM 27 Blood THC Quant by LC
M2017-5640	1	104101	AM 27 Blood THC Quant by LC
M2017-5862	2	104100	AM 27 Blood THC Quant by LC
M2017-5914	2	104099	AM 27 Blood THC Quant by LC

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

Extraction Date: 1-3-2018 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:

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

Analytic:

- ☑ 1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
- ☑ 2. Pipette **1000µL blood (calibrated pipette) Pipette ID: 2609543** in wells of analytical (standards) plate.
- ☑ 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- ☑ 4. Pipette **500µL 0.1% formic acid in water** in wells of analytical 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). ***(Load at 85-100 PSI- Selector to the right)*** Manifold ID: 66792
- ☑ 8. Wait 5 minutes.
- ☑ 9. Add **2.25mL MTBE. (*Add in 3 increments of 750uL)***
- ☑ 10. Wait 5 minutes.
- ☑ 11. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
- ☑ 12. Add **2.25mL Hexane. (*Add in 3 increments of 750uL)***
- ☑ 13. Wait 5 minutes.
- ☑ 14. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI- Selector to the left).***
- ☑ 15. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. 35°C. *SPE Dry ID: 66819*
- ☑ 16. Reconstitute in **100µL 100% MeOH** and heat seal plate with foil. Place in autosampler and run worklist.

Post-Analytic

- ☑ 1. Create batch and process data.
  Worklist path: 01032018 conn quant Batch Name: 01032018 conn quant
- ☑ 2. Make any necessary integration changes, r^2 values ≥0.98 for each analyte.
- ☑ 3. Did all QCs pass for each analyte? Y / N Enter QCs into control charting?
- ☑ 4. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: curve for THC-OH 5-250 limit of confirmation 5ng/ml

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 9900 ul blood lot (321632)

ppd 8/17/17 Exp 2/17/18	lot 81717	Concentration 10 ng/ml each	by AMN
ppd 1/3/18 Exp 2/17/18	lot 1318	neg blood lot 17J20718	by AMN

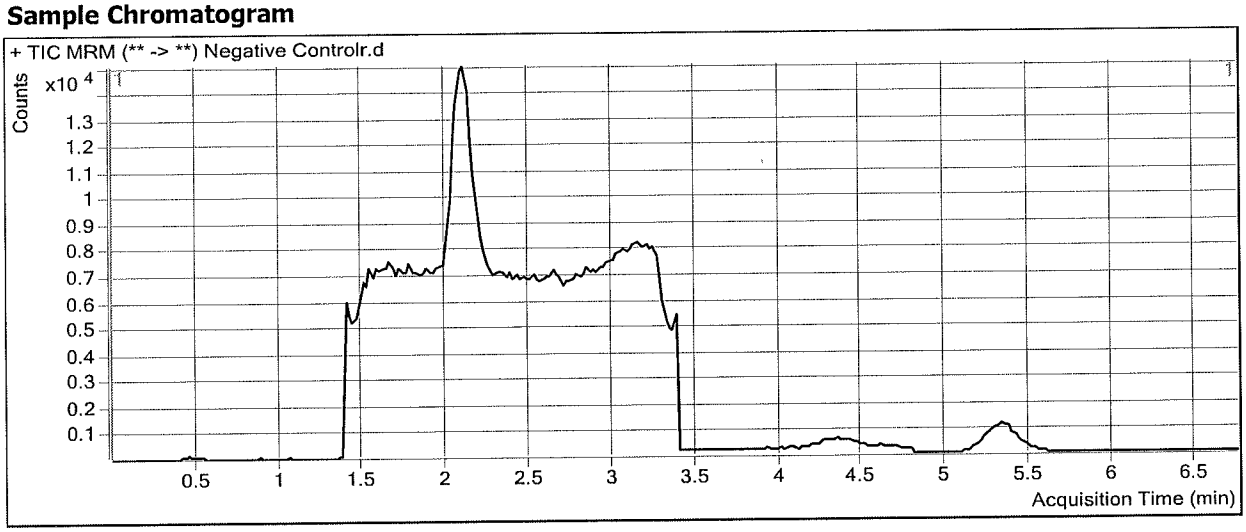
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 13:08	**Data File**	Negative Controlr.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



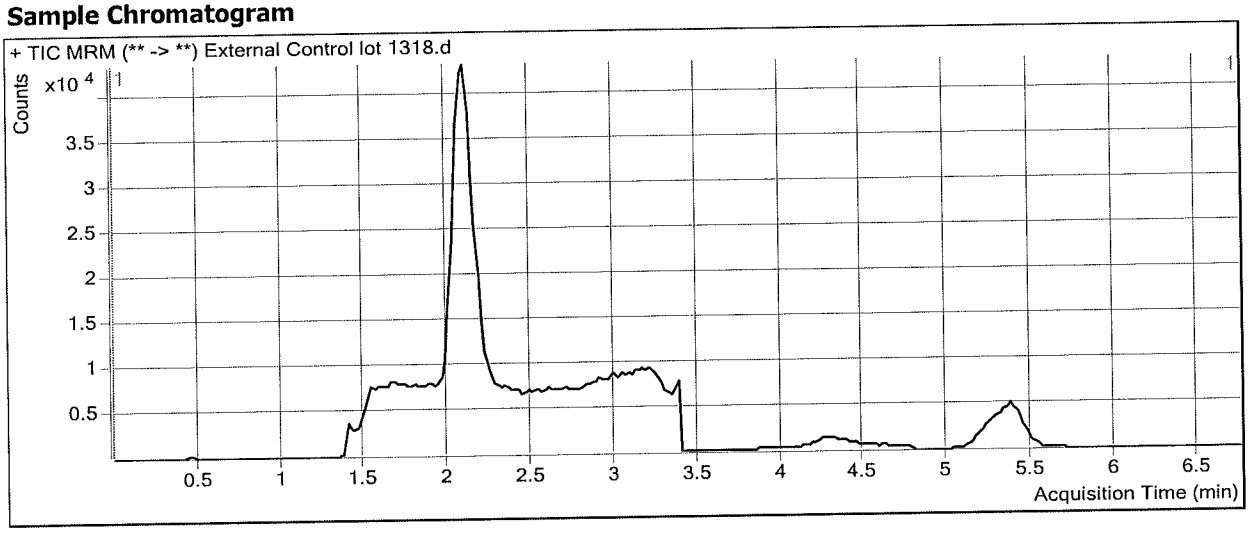
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 11:21	**Data File**	External Control lot 1318.d
Sample Type	Sample	**Sample Name**	External Control lot 1318
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	2.075	13709	200394	0.0684	7.6437
THC-COOH	THC-COOH-d9	2.185	9035	71801	0.1258	6.6126
THC	THC-d3	5.392	5820	63995	0.0909	7.5887

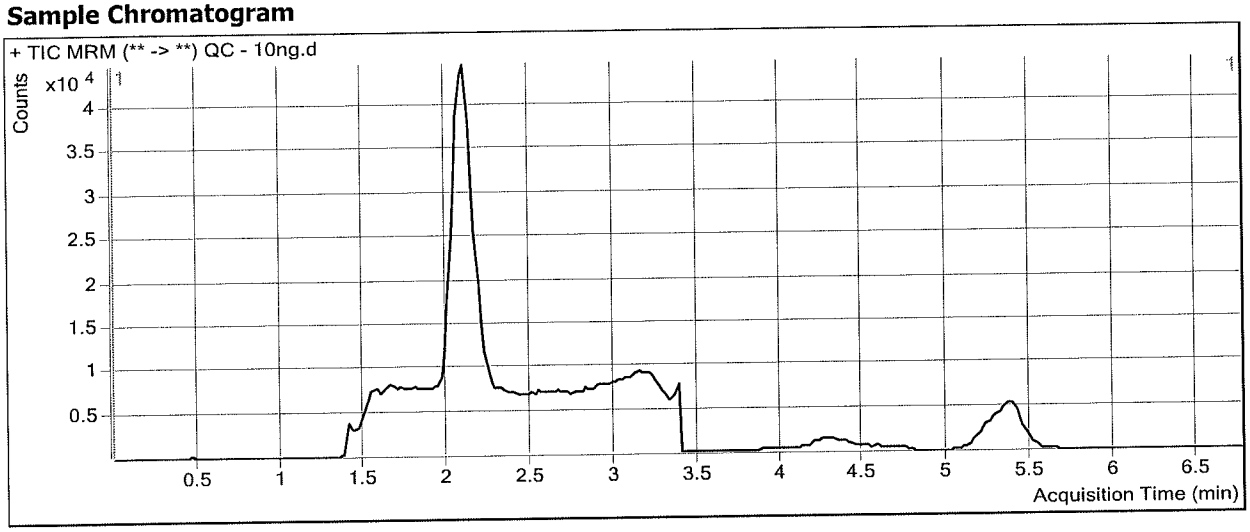
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 11:10	**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	20711	202418	0.1023	10.8687
THC-COOH	THC-COOH-d9	2.185	13905	69199	0.2009	10.5596
THC	THC-d3	5.392	8234	68049	0.1210	10.1836

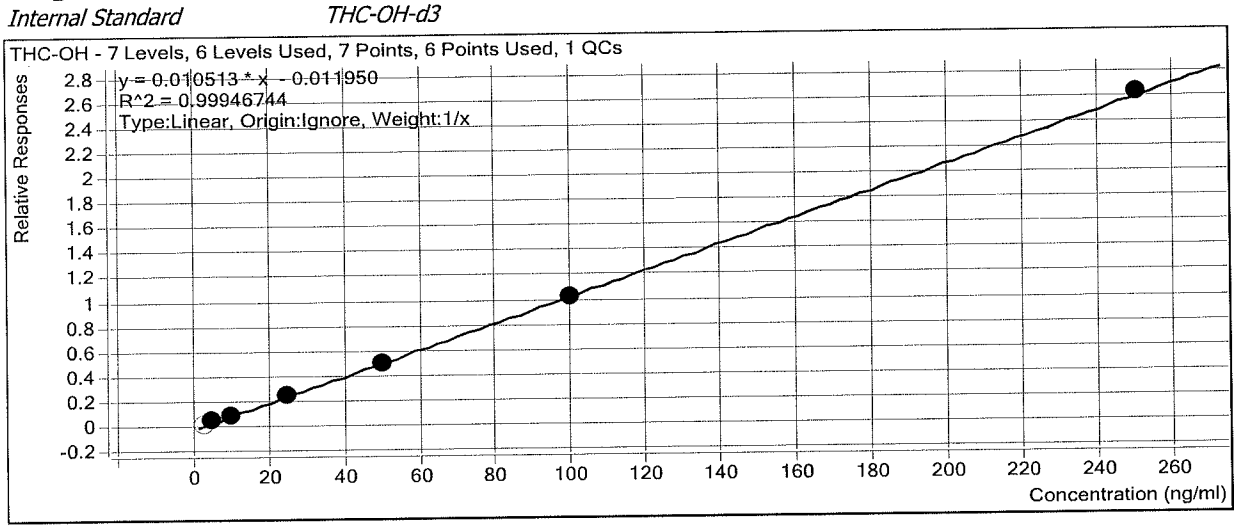
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin

Last Calib Update 1/4/2018 4:26 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	3.2	106.6
Cal 2 - 5ng	2	☑	5	5.6	111.6
Cal 3 - 10ng	3	☑	10	9.0	90.0
QC - 10ng	3	☑	10	10.9	108.7
Cal 4 - 25ng	4	☑	25	24.7	98.8
Cal 5 - 50ng	5	☑	50	49.8	99.5
Cal 6 - 100ng	6	☑	100	99.5	99.5
Cal 7 - 250ng	7	☑	250	251.5	100.6

Limit of confirmation 5ng/ml

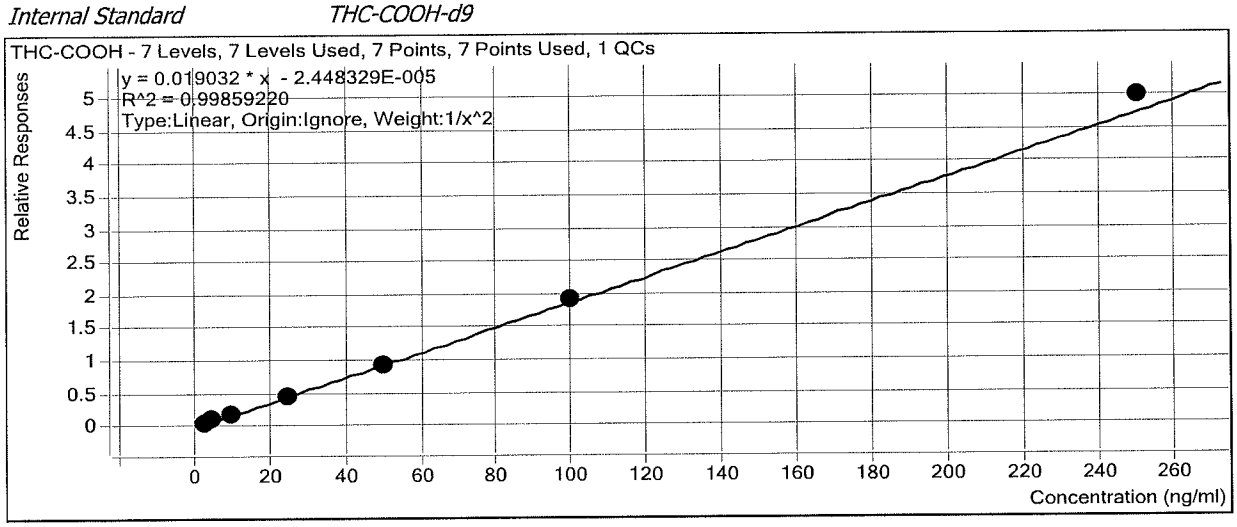
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin

Last Calib Update 1/4/2018 4:26 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	99.6
Cal 2 - 5ng	2	☑	5	5.1	102.3
Cal 3 - 10ng	3	☑	10	9.9	98.6
QC - 10ng	3	☑	10	10.6	105.6
Cal 4 - 25ng	4	☑	25	23.8	95.4
Cal 5 - 50ng	5	☑	50	49.2	98.4
Cal 6 - 100ng	6	☑	100	100.9	100.9
Cal 7 - 250ng	7	☑	250	262.1	104.8

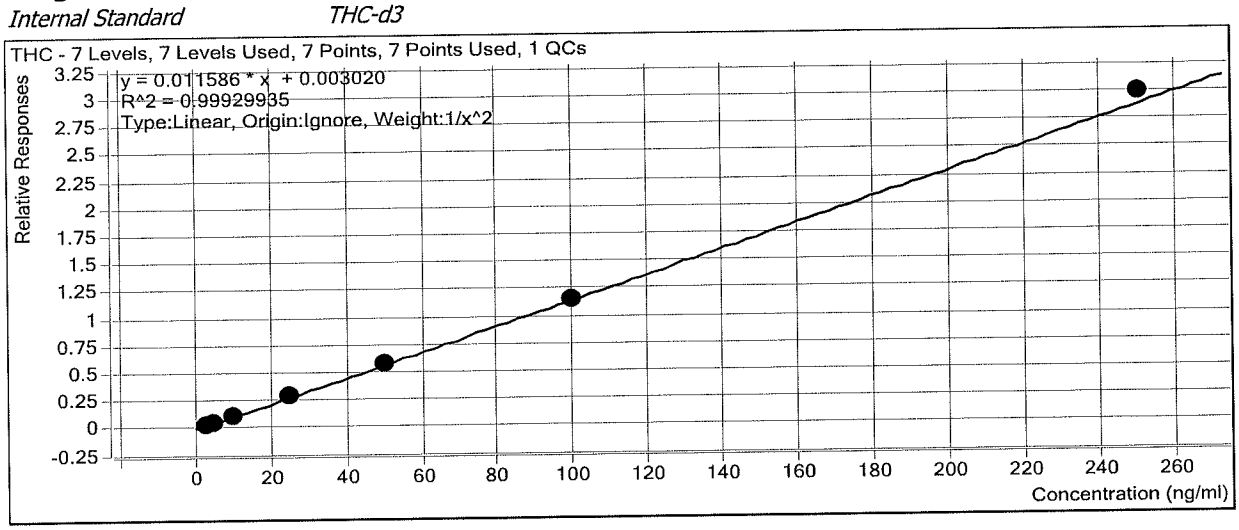
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin

Last Calib Update 1/4/2018 4:26 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	101.5
Cal 2 - 5ng	2	☑	5	5.0	99.0
Cal 3 - 10ng	3	☑	10	9.7	97.3
QC - 10ng	3	☑	10	10.2	101.8
Cal 4 - 25ng	4	☑	25	24.5	98.0
Cal 5 - 50ng	5	☑	50	50.7	101.5
Cal 6 - 100ng	6	☑	100	99.4	99.4
Cal 7 - 250ng	7	☑	250	258.1	103.2

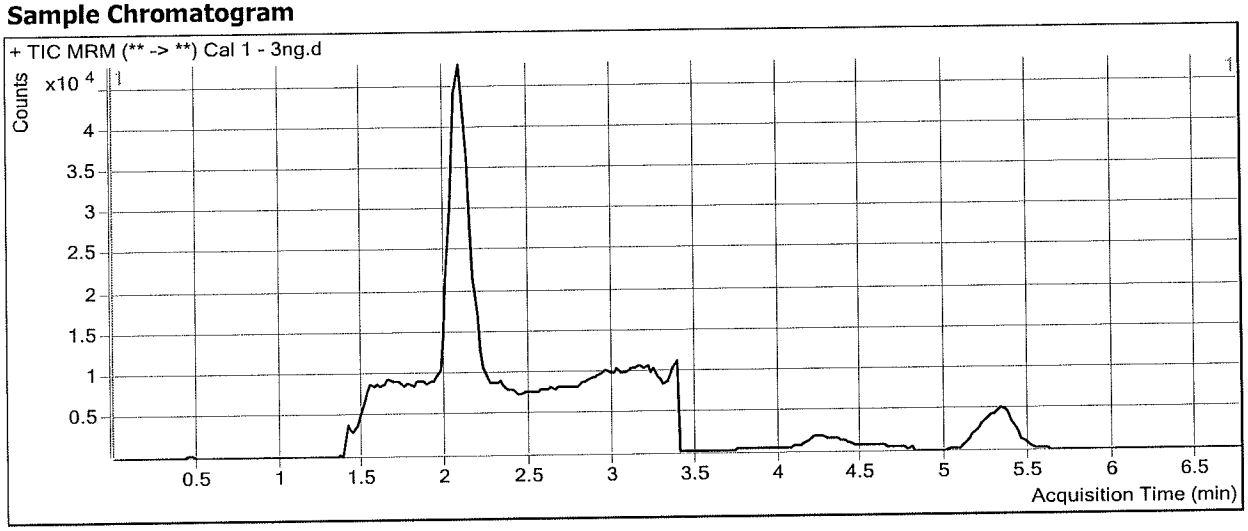
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 09:23	**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	2.095	4960	228926	0.0217	3.1975
THC-COOH	THC-COOH-d9	2.185	4099	72105	0.0568	2.9883
THC	THC-d3	5.332	2633	68735	0.0383	3.0458

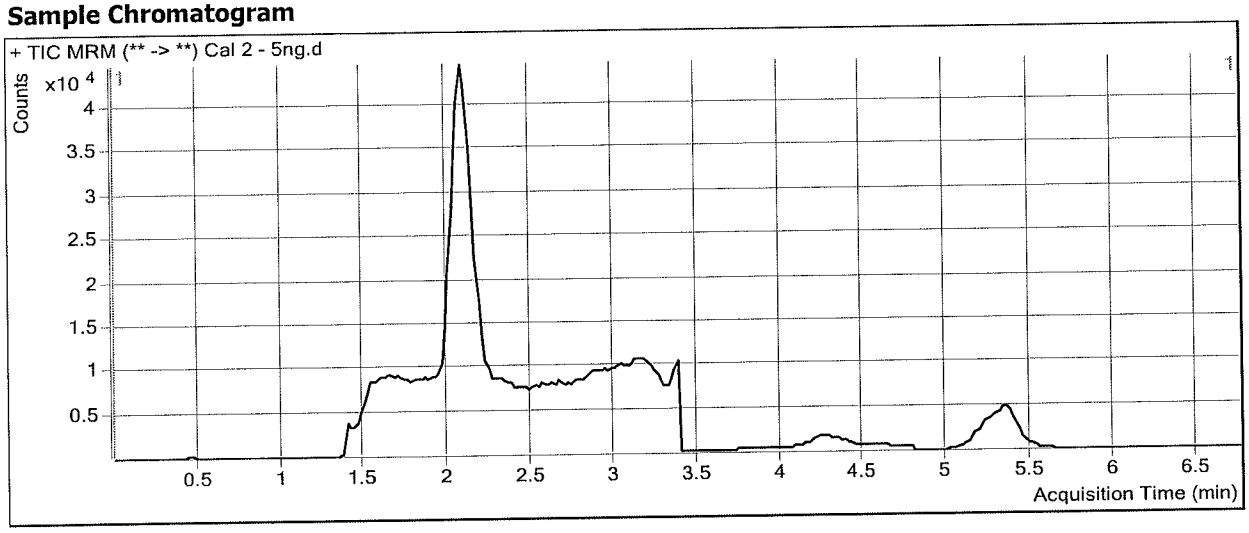
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 09:35	**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	2.095	9647	206477	0.0467	5.5806
THC-COOH	THC-COOH-d9	2.165	6745	69309	0.0973	5.1149
THC	THC-d3	5.332	3939	65225	0.0604	4.9513

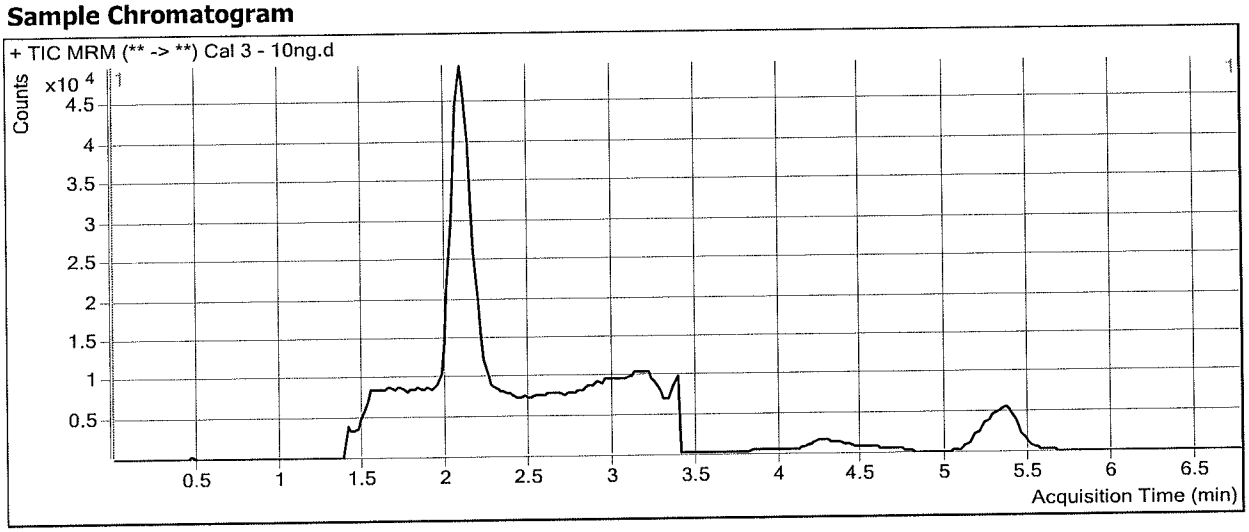
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 09:47	**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	2.095	18659	225703	0.0827	8.9999
THC-COOH	THC-COOH-d9	2.185	13860	73872	0.1876	9.8591
THC	THC-d3	5.352	8378	72395	0.1157	9.7275

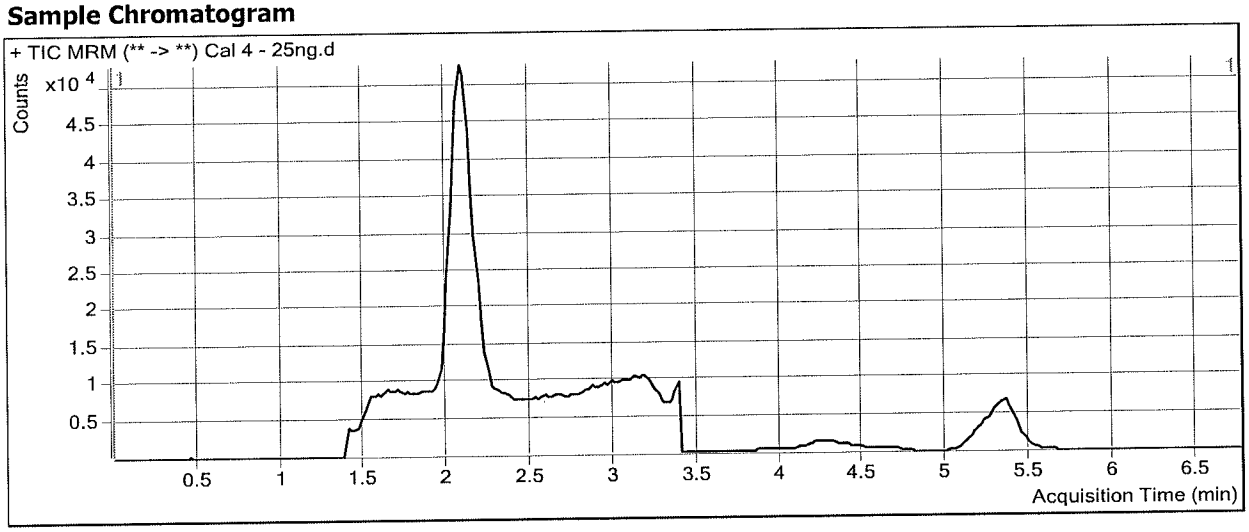
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 09:58	**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	2.095	53007	214048	0.2476	24.6914
THC-COOH	THC-COOH-d9	2.185	32068	70664	0.4538	23.8456
THC	THC-d3	5.332	20283	70677	0.2870	24.5091

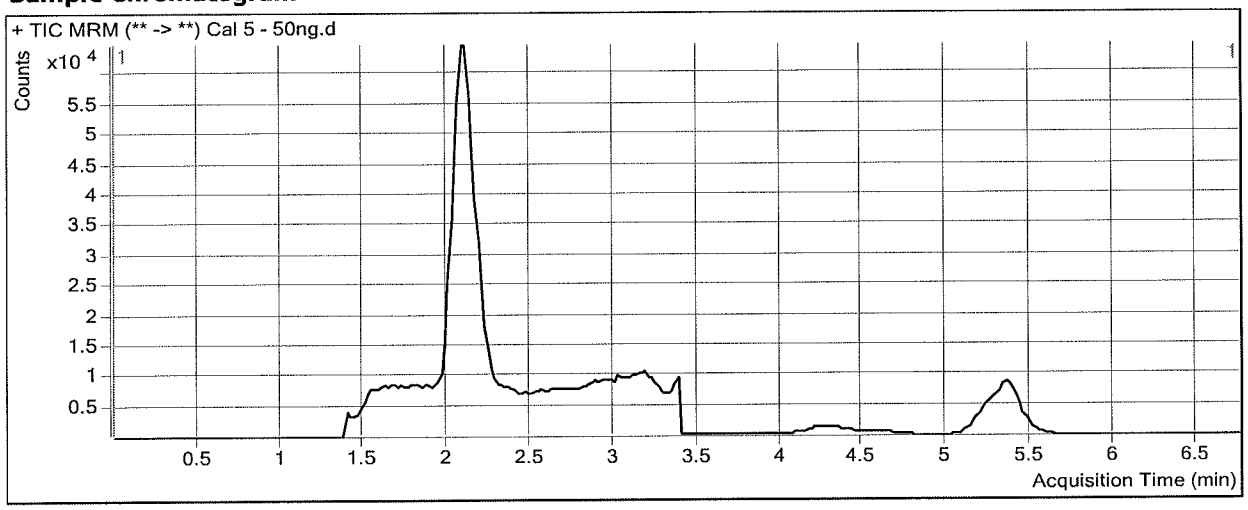
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 10:10	**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	2.095	112578	220196	0.5113	49.7664
THC-COOH	THC-COOH-d9	2.185	65937	70434	0.9362	49.1897
THC	THC-d3	5.372	41735	70632	0.5909	50.7399

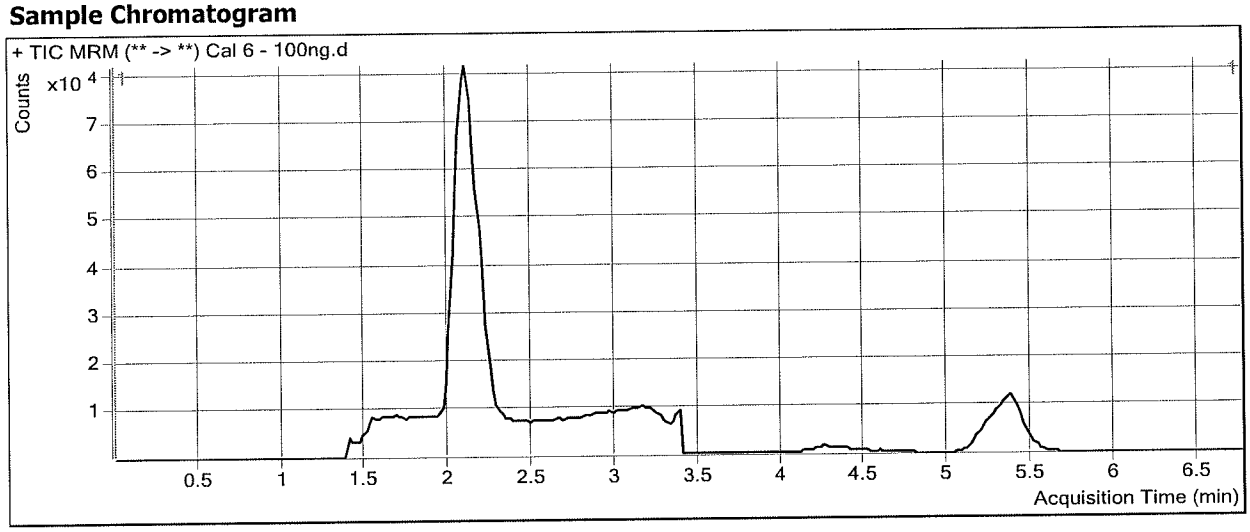
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 10:22	**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	2.095	216705	209531	1.0342	99.5101
THC-COOH	THC-COOH-d9	2.185	127308	66284	1.9206	100.9164
THC	THC-d3	5.372	77386	67010	1.1548	99.4155

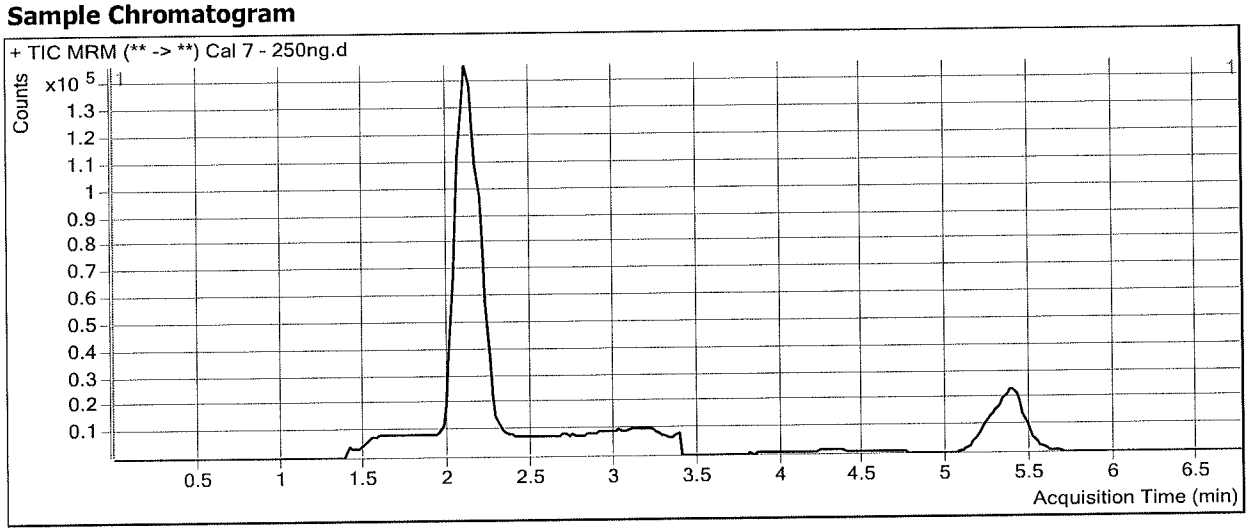
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\01032018 cann quant\QuantResults\01032018 cann quant.batch.bin		
Analysis Time	1/4/2018 4:26 PM	**Analyst Name**	ISP Tox
Report Time	1/4/2018 4:27 PM	**Reporter Name**	ISP Tox
Last Calib Update	1/4/2018 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2018-01-04 10:34	**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	560085	212826	2.6317	251.4516
THC-COOH	THC-COOH-d9	2.185	325323	65227	4.9875	262.0578
THC	THC-d3	5.392	200619	67021	2.9933	258.0994