reviewed 2/6/17

2/1/2018

Worklist: 2165

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-2547	1	106631	AM 27 Blood THC Quant by LC
C2018-0013	1	106632	AM 27 Blood THC Quant by LC
C2018-0065	1	106633	AM 27 Blood THC Quant by LC
C2018-0101	1	106634	AM 27 Blood THC Quant by LC
C2018-0109	1	106635	AM 27 Blood THC Quant by LC
C2018-0119	1	106636	AM 27 Blood THC Quant by LC
C2018-0148	1	106637	AM 27 Blood THC Quant by LC

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

Extraction Date: 2-1-18 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: 02012018 cann quant Batch Name: 02012018 cann 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 limited 5- 250ng for THC-OH Limit of confirmation 5 ng/ml poor response and signal to noise at lower concentration.

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

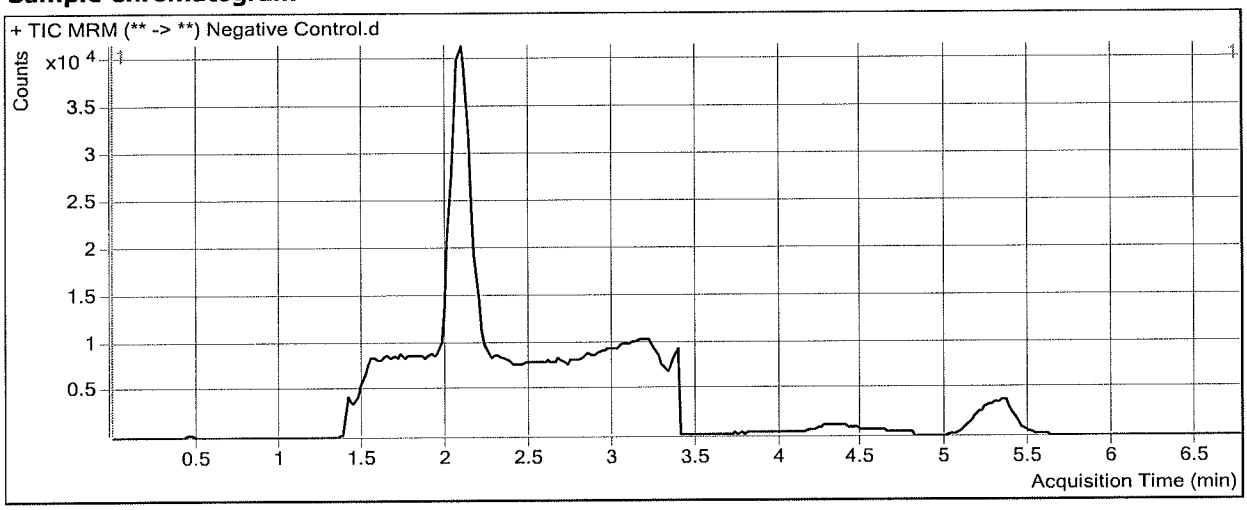
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 13:13	**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	1.995	0	209423	0.0000	0.0000

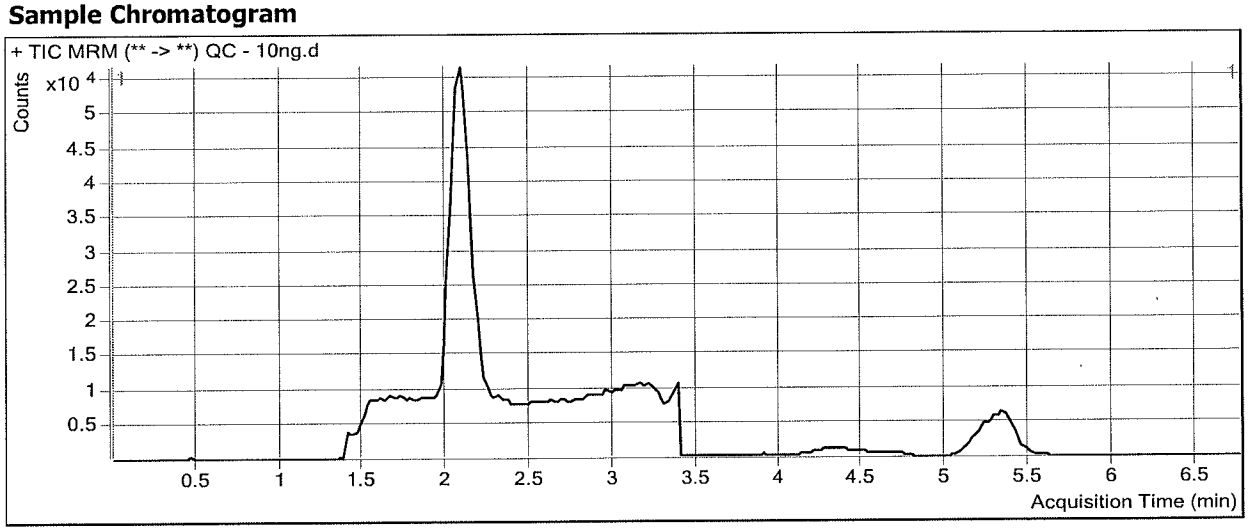
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 13:25	**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	24641	276005	0.0893	10.1192
THC-COOH	THC-COOH-d9	2.165	14099	81062	0.1739	9.1805
THC	THC-d3	5.332	9621	81342	0.1183	9.8720

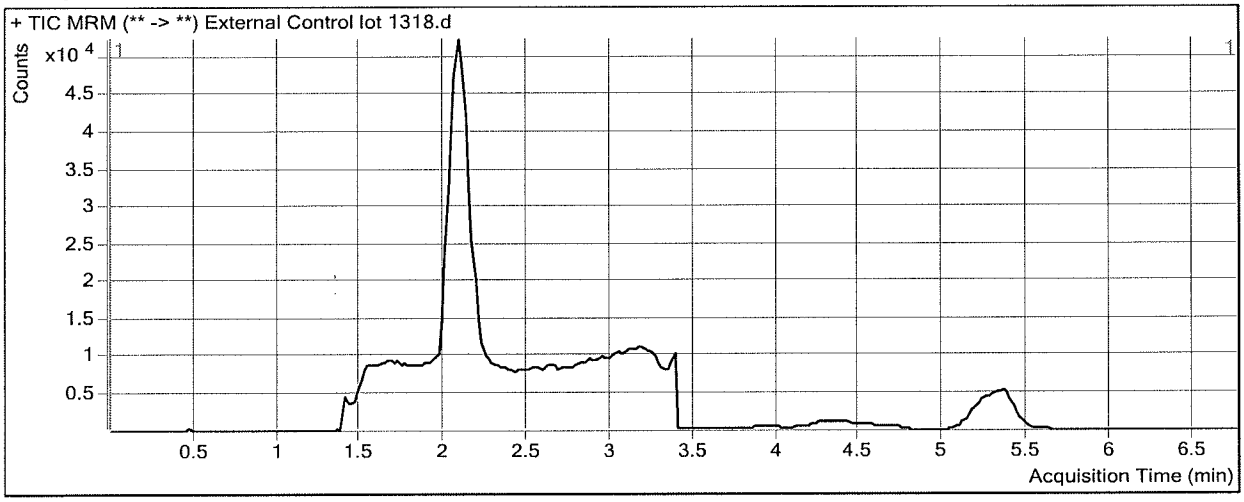
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 13:37	**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

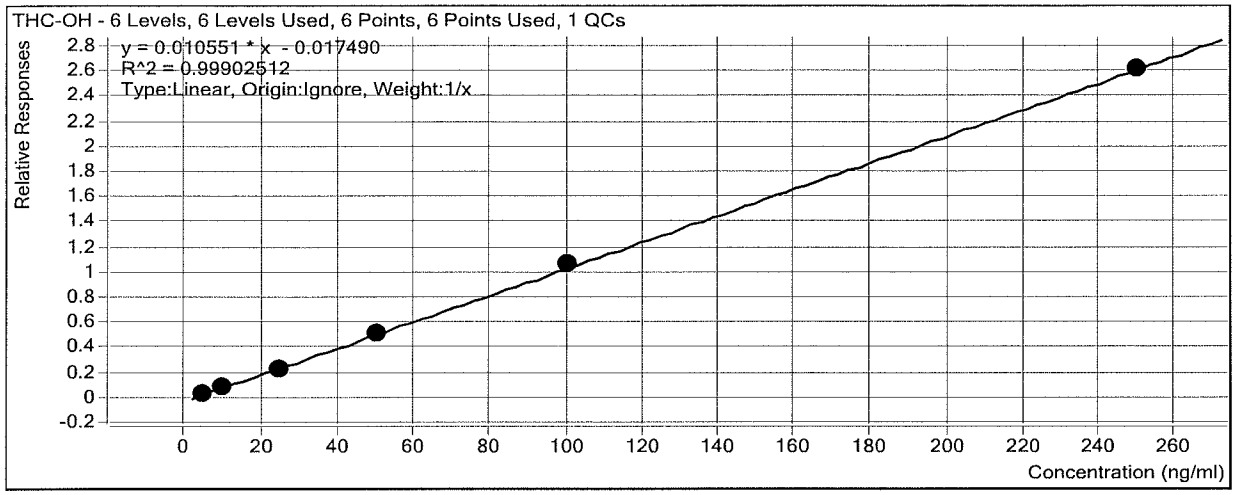
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.115	17668	251248	0.0703	8.3227
THC-COOH	THC-COOH-d9	2.165	11532	77505	0.1488	7.9271
THC	THC-d3	5.352	6685	73100	0.0915	7.7412

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin

Last Calib Update 2/5/2018 10:26 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	0.0	0.0
Cal 2 - 5ng	2	☑	5	5.3	105.1
Cal 3 - 10ng	3	☑	10	10.2	102.1
QC - 10ng	3	☑	10	10.1	101.2
Cal 4 - 25ng	4	☑	25	22.4	89.7
Cal 5 - 50ng	5	☑	50	50.6	101.2
Cal 6 - 100ng	6	☑	100	102.1	102.1
Cal 7 - 250ng	7	☑	250	249.4	99.8

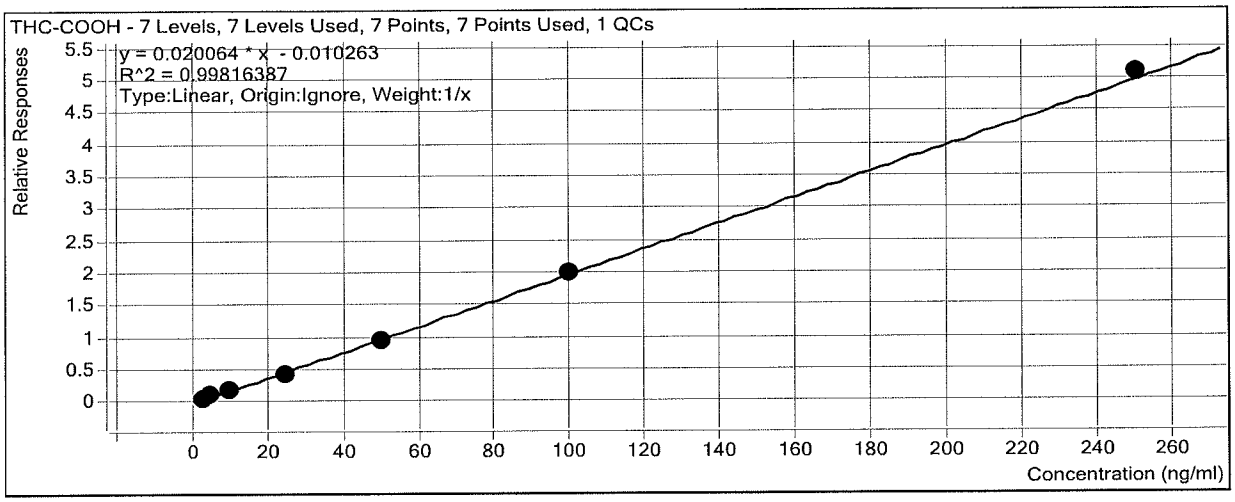
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin

Last Calib Update 2/5/2018 10:26 AM **Analyst Name** ISP TOX

Target Compound *THC-COOH*

Internal Standard *THC-COOH-d9*



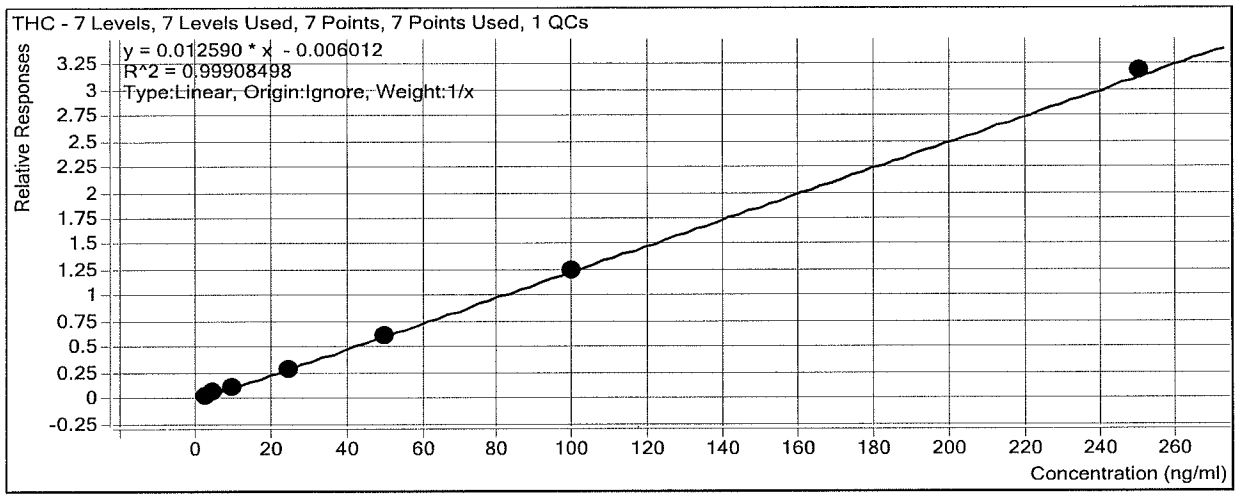
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.3	111.5
Cal 2 - 5ng	2	☑	5	5.4	108.8
Cal 3 - 10ng	3	☑	10	9.4	94.1
QC - 10ng	3	☑	10	9.2	91.8
Cal 4 - 25ng	4	☑	25	21.8	87.1
Cal 5 - 50ng	5	☑	50	48.0	96.0
Cal 6 - 100ng	6	☑	100	100.8	100.8
Cal 7 - 250ng	7	☑	250	254.2	101.7

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin

Last Calib Update 2/5/2018 10:26 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	3.2	108.0
Cal 2 - 5ng	2	☑	5	5.2	104.5
Cal 3 - 10ng	3	☑	10	9.8	98.2
QC - 10ng	3	☑	10	9.9	98.7
Cal 4 - 25ng	4	☑	25	22.5	90.1
Cal 5 - 50ng	5	☑	50	48.9	97.7
Cal 6 - 100ng	6	☑	100	100.2	100.2
Cal 7 - 250ng	7	☑	250	253.1	101.2

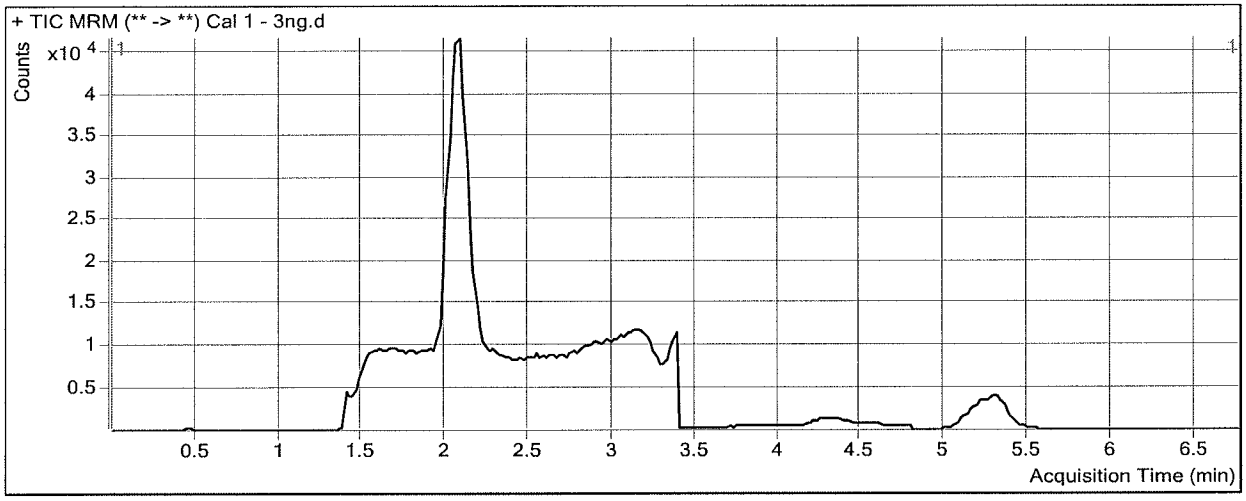
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 11:38	**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-COOH	THC-COOH-d9	2.145	3813	67096	0.0568	3.3442
THC	THC-d3	5.312	1983	57020	0.0348	3.2399

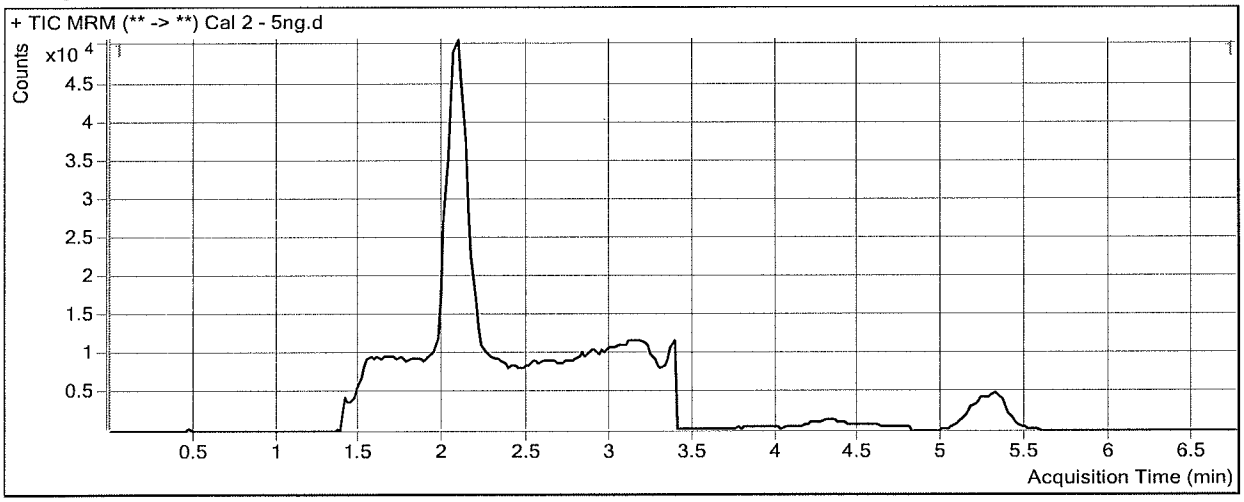
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 11:50	**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	2.055	9397	247618	0.0379	5.2545
THC-COOH	THC-COOH-d9	2.165	7220	73013	0.0989	5.4403
THC	THC-d3	5.312	4044	67621	0.0598	5.2274

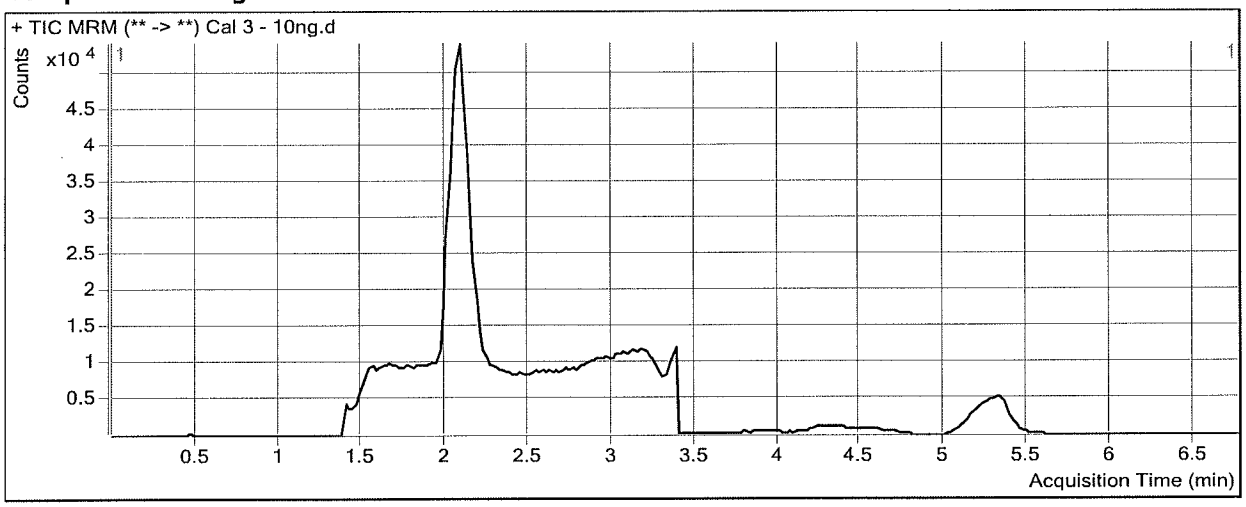
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 12:02	**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	22228	246242	0.0903	10.2134
THC-COOH	THC-COOH-d9	2.165	12747	71379	0.1786	9.4119
THC	THC-d3	5.332	8065	68603	0.1176	9.8154

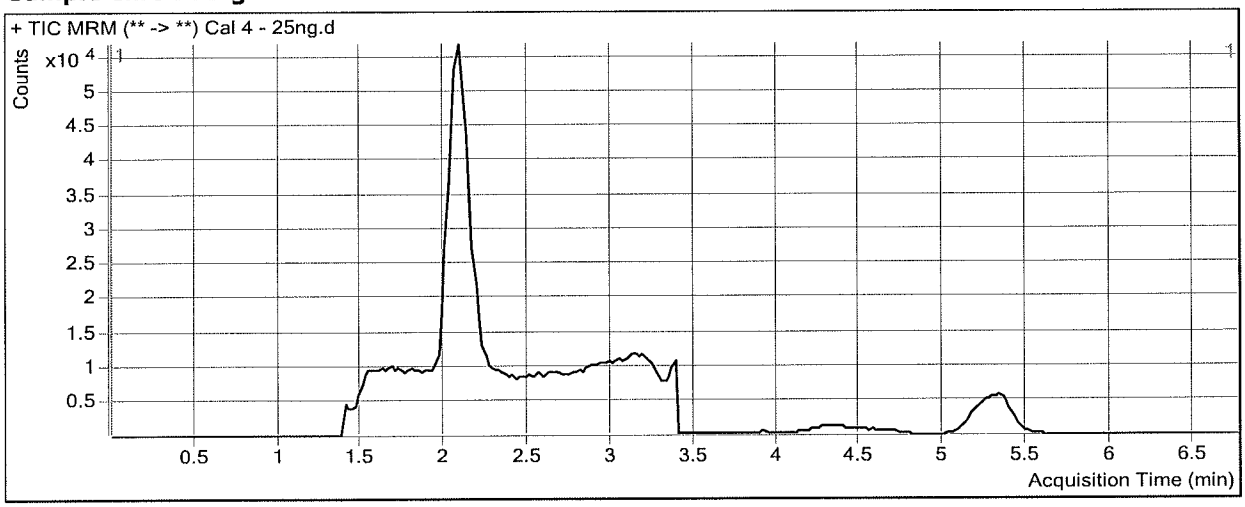
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 12:14	**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	50943	232481	0.2191	22.4263
THC-COOH	THC-COOH-d9	2.185	29117	68278	0.4265	21.7661
THC	THC-d3	5.352	18036	64972	0.2776	22.5263

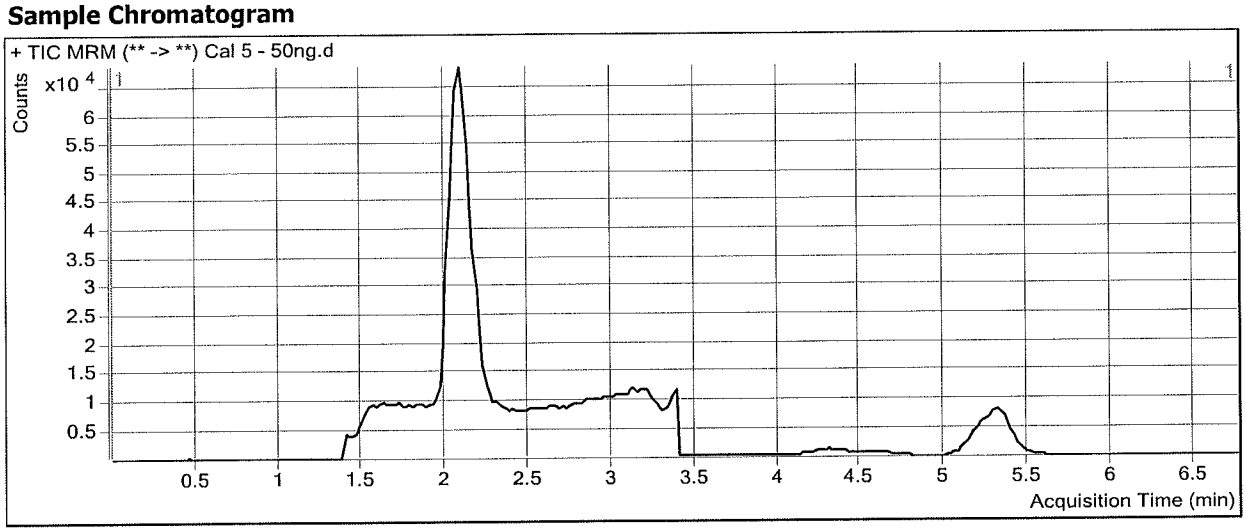
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 12:26	**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	121107	234510	0.5164	50.6035
THC-COOH	THC-COOH-d9	2.165	64901	68100	0.9530	48.0110
THC	THC-d3	5.332	39671	65113	0.6093	48.8691

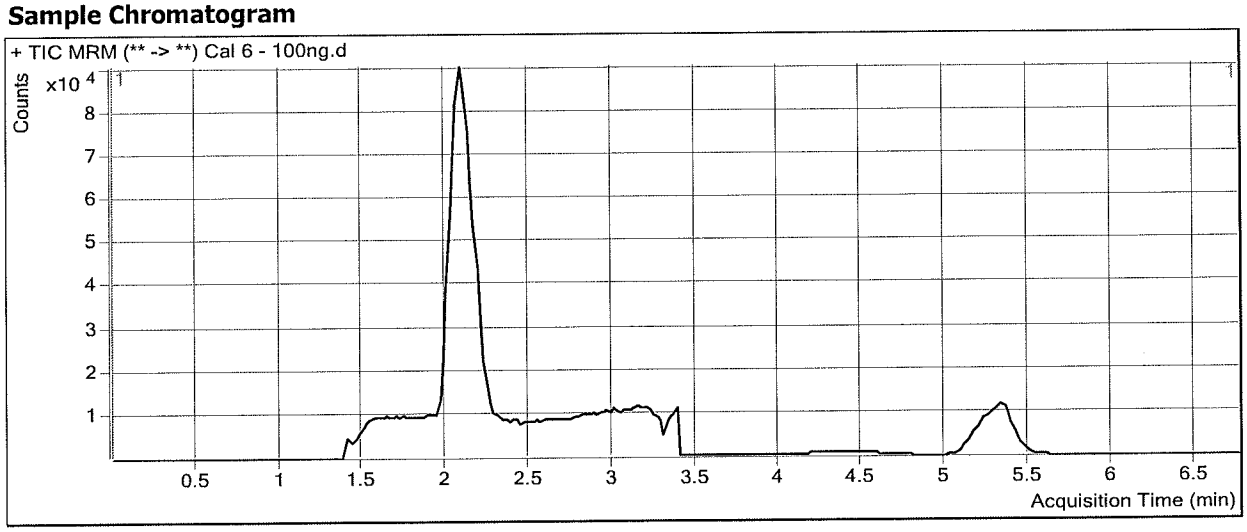
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 12:38	**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	245380	231535	1.0598	102.1034
THC-COOH	THC-COOH-d9	2.165	129789	64476	2.0130	100.8395
THC	THC-d3	5.332	80956	64467	1.2558	100.2187

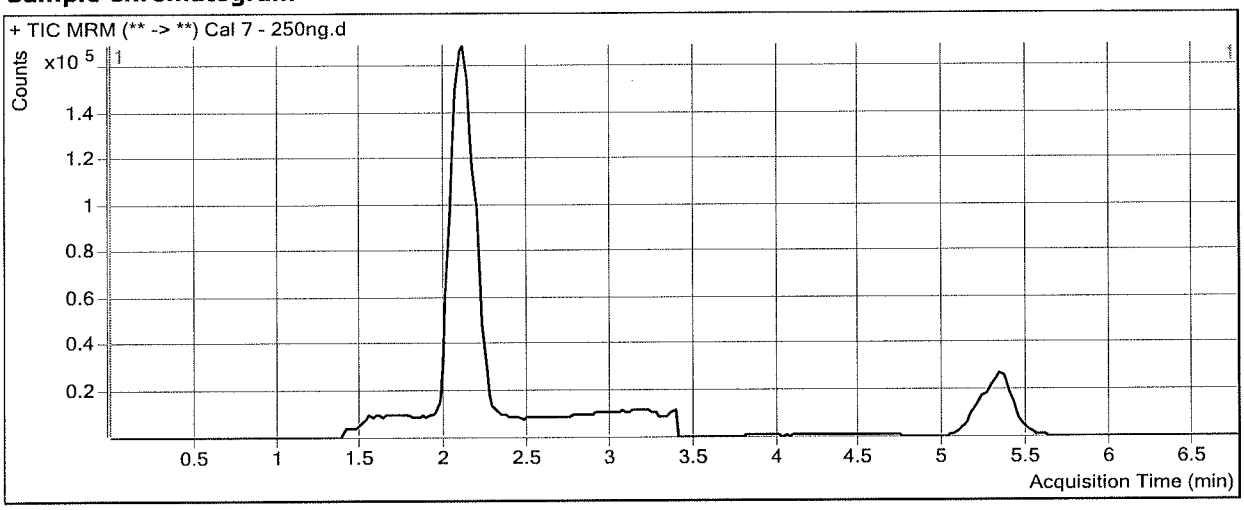
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\02012018 cann quant\QuantResults\02012018 cann quant.batch.bin		
Analysis Time	2/5/2018 10:26 AM	**Analyst Name**	ISP Tox
Report Time	2/5/2018 10:26 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/5/2018 10:26 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-02 12:49	**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	2.095	674280	257960	2.6139	249.3988
THC-COOH	THC-COOH-d9	2.165	360176	70765	5.0897	254.1870
THC	THC-d3	5.352	224794	70675	3.1807	253.1033