reviewed 7/2/18

6/26/2018

Worklist: 2524

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2018-1077	1	119750	AM 27 Blood THC Quant by LC-QQQ
C2018-1081	1	119751	AM 27 Blood THC Quant by LC-QQQ
C2018-1136	1	119752	AM 27 Blood THC Quant by LC-QQQ
C2018-1186	1	119753	AM 27 Blood THC Quant by LC-QQQ
C2018-1206	1	119754	AM 27 Blood THC Quant by LC-QQQ
M2018-2862	3	119755	AM 27 Blood THC Quant by LC-QQQ

AM# 27: Quantitation of THC and Metabolites in Blood by LC-MS/MS

Extraction Date: 6-26-18 Analyst: Anne Nord
Plate lot#: 0515037 Plate Expiration: 9/28/2018

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: 17J0718 **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: k52558g** 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: 06272018 cann quant Batch Name: cann quant
- [x] 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥0.98 for each analyte
- [x] 3. RT +/- 3% or 0.100 min, whichever is greater, +/- 20% Accuracy for greater than (+/- 30% for 10ng/ml or less). Ion ratios must be within +/- 20% of the averaged calibrators
- [x] 4. Case sample response for THC and OH-THC 3ng/mL (quantitative), Carboxy-THC: 10ng/mL (qualitative only) will be reported. Samples with a THC or OH-THC response over 50 ng/mL will be reported out as greater than 50 ng/mL.
- [x] 5. Did all QCs pass for each analyte? Y / N– QC internal control THC-OH did not pass. THC-OH will not be evaluated in this run.
- [x] 6 Enter QCs into control charting.
- [x] 7 Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: curves limited 3-100 ng THC and Carboxy-THC

THC-OH not evaluated internal control failed.

I started the run and evaluated the initial data. The calibrators, negative control, internal control, and external control were re-injected, THC was not coming out in the acquisition window.

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 9890 ul meOH
Ppd 6/5/18 Exp: 4/1/19 lot 6518 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 50 ul working solution lot (6518) in 4950 ul blood lot (17J20718)
ppd 6/5/18 Exp 4/1/19 lot 6518 Concentration 10 ng/ml each by AMN

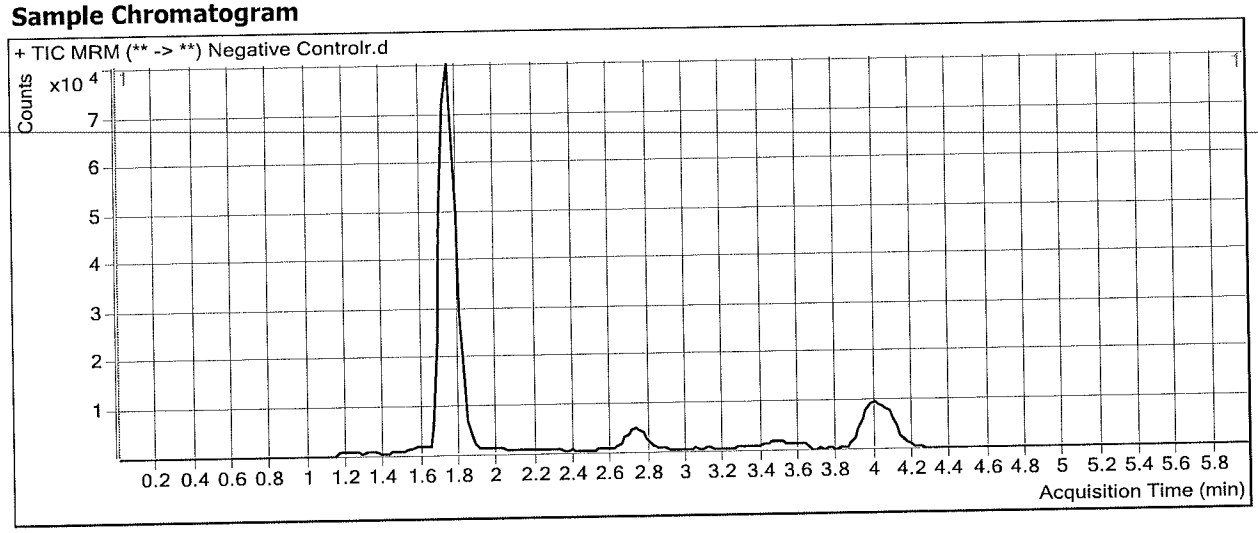
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 16:53	**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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-COOH	THC-COOH-d9	1.585	3536	118086	0.0299	1.1155 L/O

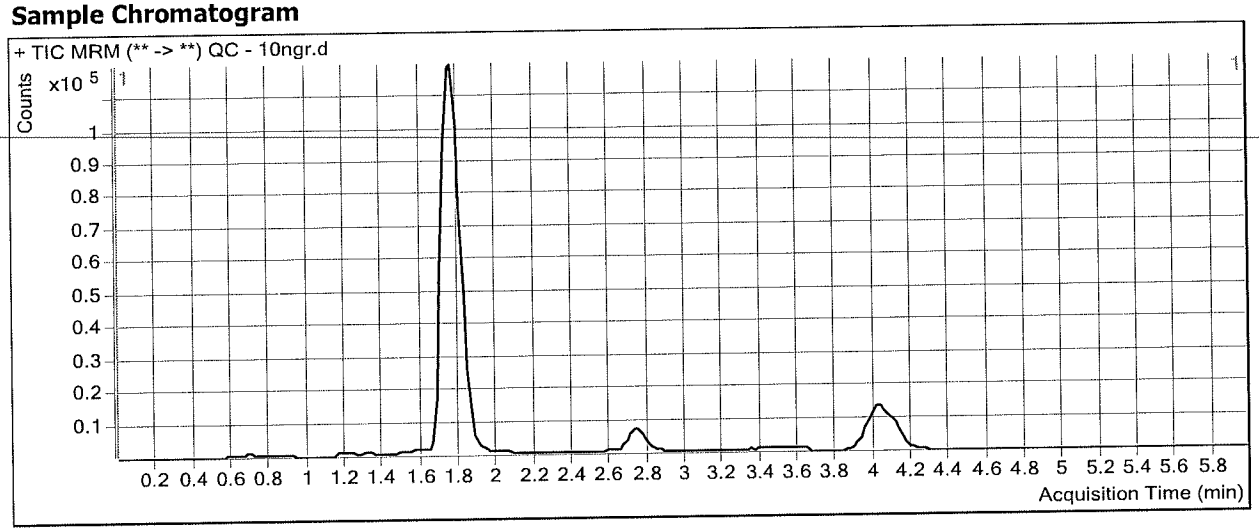
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 17:05	**Data File**	QC - 10ngr.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	1.755	45466	464020	0.0980	8.7963 - ratio out
THC-COOH	THC-COOH-d9	1.805	98534	141670	0.6955	29.7347
THC	THC-d3	4.091	16251	125310	0.1297	9.3139

THC-OH will not be evaluated in this run the qualifier ratio is out of range in this control.

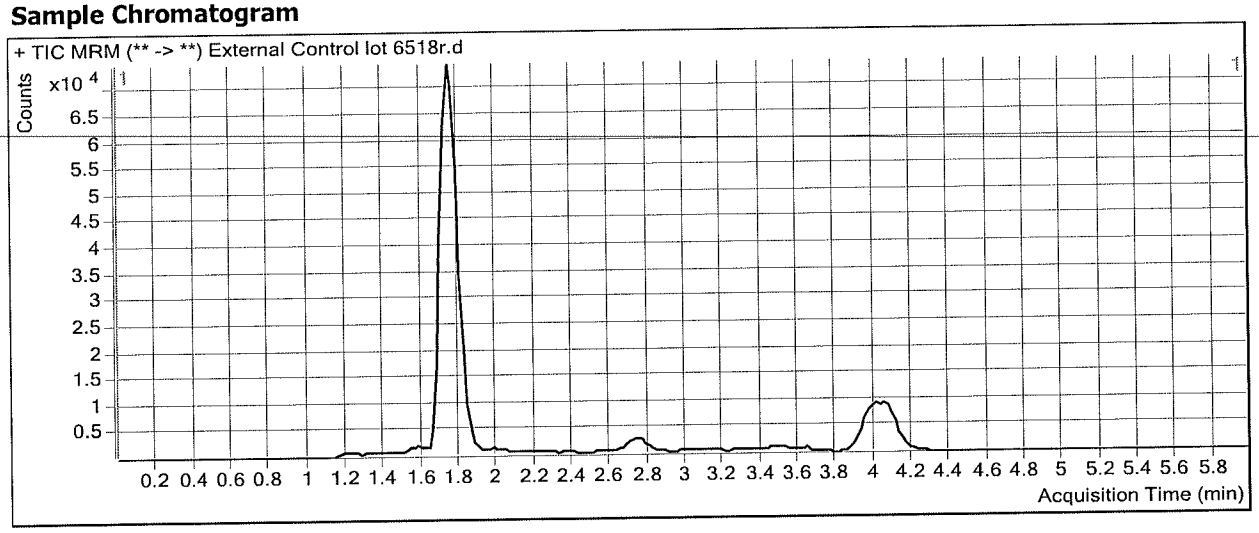
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 17:17	**Data File**	External Control lot 6518r.d
Sample Type	QC	**Sample Name**	External Control lot 6518
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	1.755	25443	309997	0.0821	7.4257 - Not evaluated
THC-COOH	THC-COOH-d9	1.805	17246	102990	0.1675	7.0283
THC	THC-d3	4.051	12121	95202	0.1273	9.1482

ISP Forensics
Calibration Curve Report

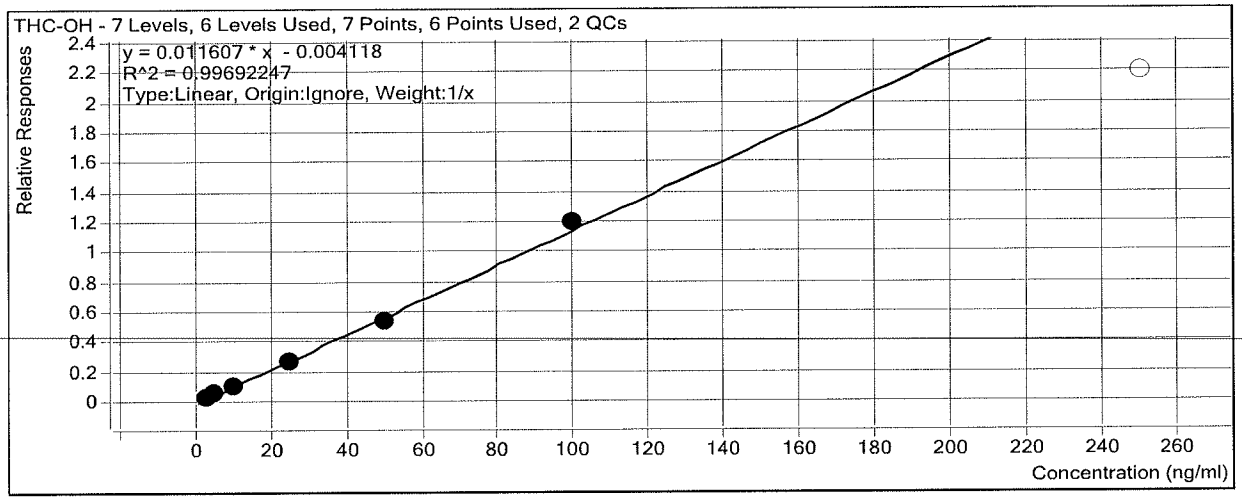
*Not Evaluated in this batch

Batch Data Path D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update 6/28/2018 7:47 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	3.3	109.6
Cal 2 - 5ng	2	☑	5	5.0	100.9
Cal 3 - 10ng	3	☑	10	9.5	95.4
QC - 10ng	3	☑	10	8.8	88.0
External Control lot 6518	3	☑	10	7.4	74.3
Cal 4 - 25ng	4	☑	25	24.0	95.8
Cal 5 - 50ng	5	☑	50	47.0	94.1
Cal 6 - 100ng	6	☑	100	104.1	104.1
Cal 7 - 250ng	7	☐	250	189.9	76.0

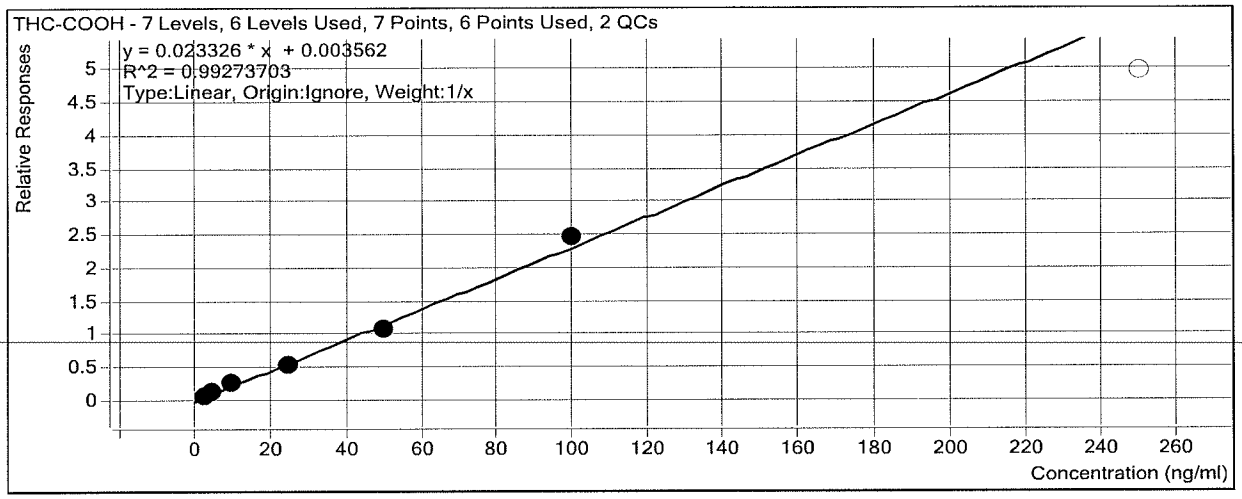
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update 6/28/2018 7:47 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	3.0	99.6
Cal 2 - 5ng	2	☑	5	4.9	98.8
Cal 3 - 10ng	3	☑	10	11.3	113.0
QC - 10ng	3	☑	10	30.2	302.1
External Control lot 6518	3	☑	10	7.0	70.3
Cal 4 - 25ng	4	☑	25	23.2	92.6
Cal 5 - 50ng	5	☑	50	45.3	90.6
Cal 6 - 100ng	6	☑	100	105.3	105.3
Cal 7 - 250ng	7	☐	250	212.6	85.0

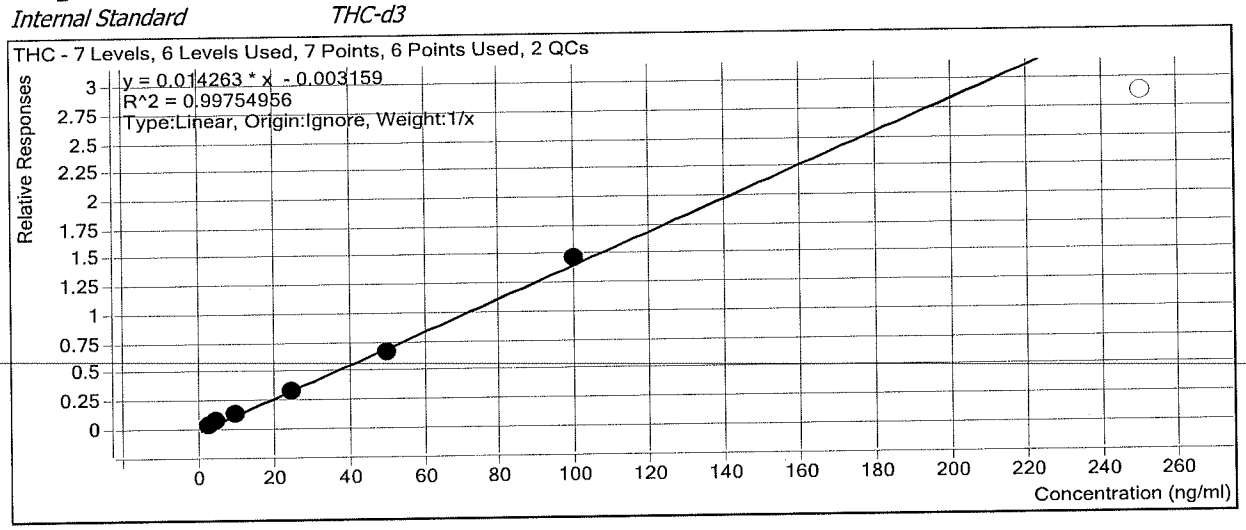
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update 6/28/2018 7:47 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	106.4
Cal 2 - 5ng	2	☑	5	5.1	102.9
Cal 3 - 10ng	3	☑	10	9.7	96.7
QC - 10ng	3	☑	10	9.3	93.1
External Control lot 6518	3	☑	10	9.1	91.5
Cal 4 - 25ng	4	☑	25	23.9	95.6
Cal 5 - 50ng	5	☑	50	47.4	94.8
Cal 6 - 100ng	6	☑	100	103.7	103.7
Cal 7 - 250ng	7	☐	250	201.8	80.7

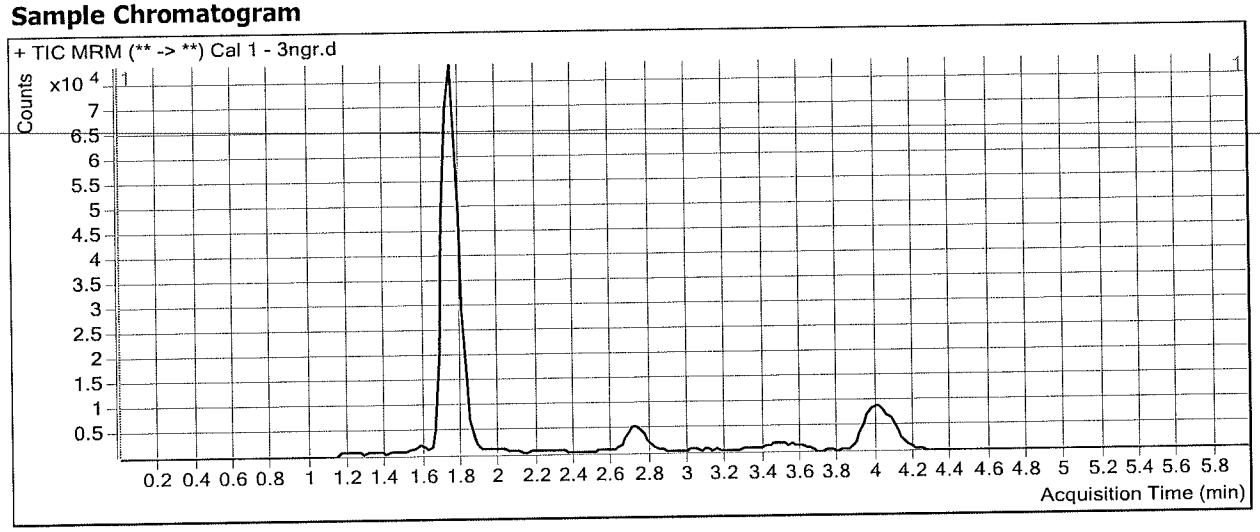
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 15:18	**Data File**	Cal 1 - 3ngr.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	1.755	11347	333268	0.0340	3.2882
THC-COOH	THC-COOH-d9	1.805	8105	110609	0.0733	2.9785
THC	THC-d3	4.071	4099	96776	0.0424	3.1909

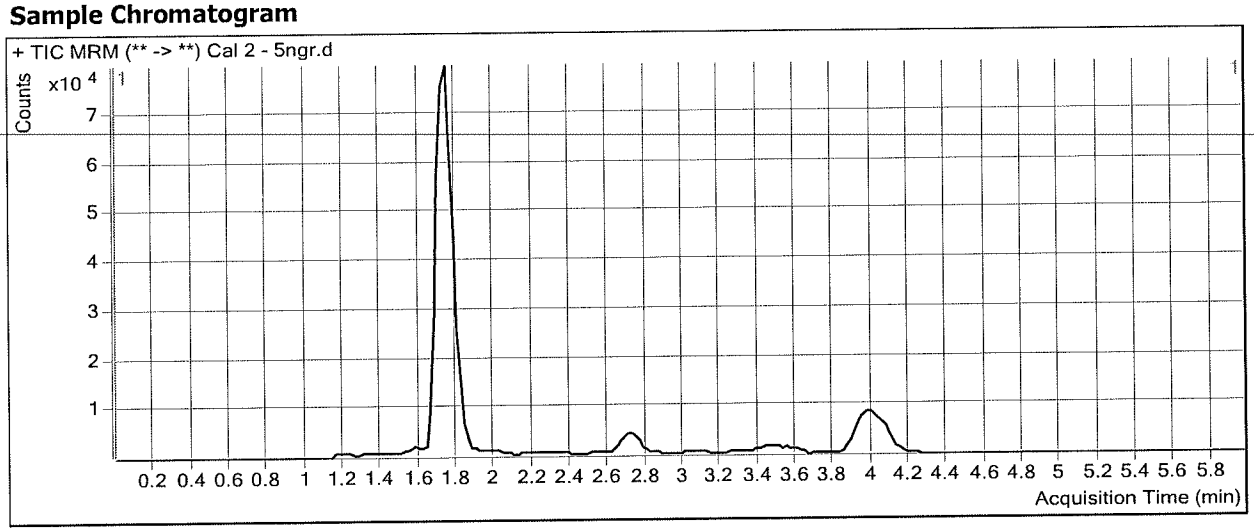
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 15:30	**Data File**	Cal 2 - 5ngr.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	1.735	18211	334388	0.0545	5.0467
THC-COOH	THC-COOH-d9	1.805	13255	111528	0.1188	4.9383
THC	THC-d3	4.051	6382	90913	0.0702	5.1428

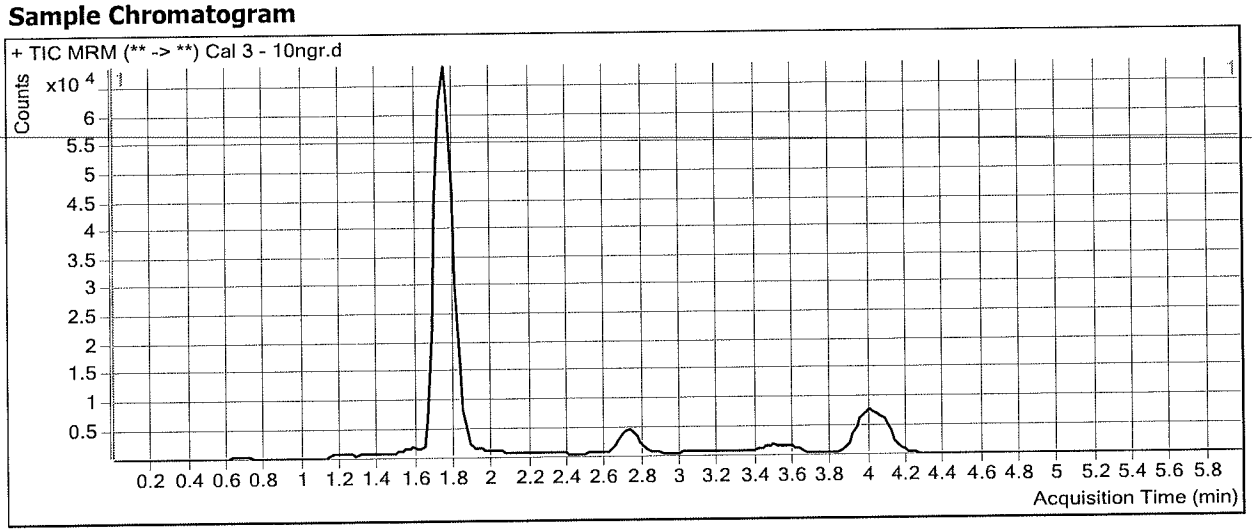
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 15:42	**Data File**	Cal 3 - 10ngr.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	1.755	29826	279685	0.1066	9.5422
THC-COOH	THC-COOH-d9	1.805	25234	94437	0.2672	11.3174
THC	THC-d3	4.071	10302	76460	0.1347	9.6676

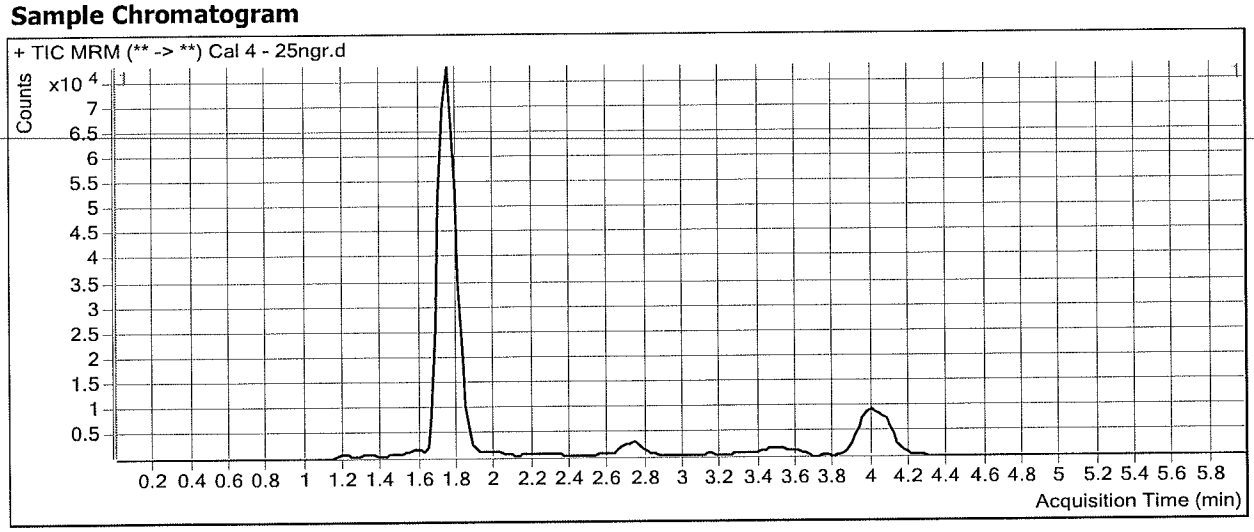
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 15:54	**Data File**	Cal 4 - 25ngr.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	1.755	72982	266390	0.2740	23.9577
THC-COOH	THC-COOH-d9	1.805	48918	89976	0.5437	23.2058
THC	THC-d3	4.051	25268	74801	0.3378	23.9045

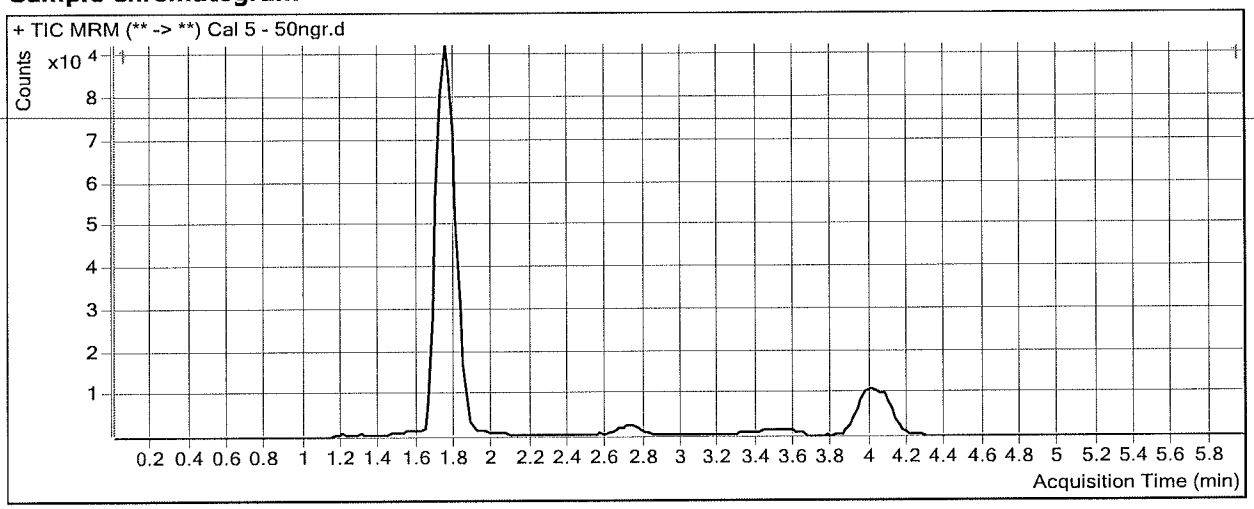
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 16:06	**Data File**	Cal 5 - 50ngr.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	1.755	140632	259519	0.5419	47.0407
THC-COOH	THC-COOH-d9	1.805	93101	87860	1.0597	45.3922
THC	THC-d3	4.071	48173	71600	0.6728	47.3921

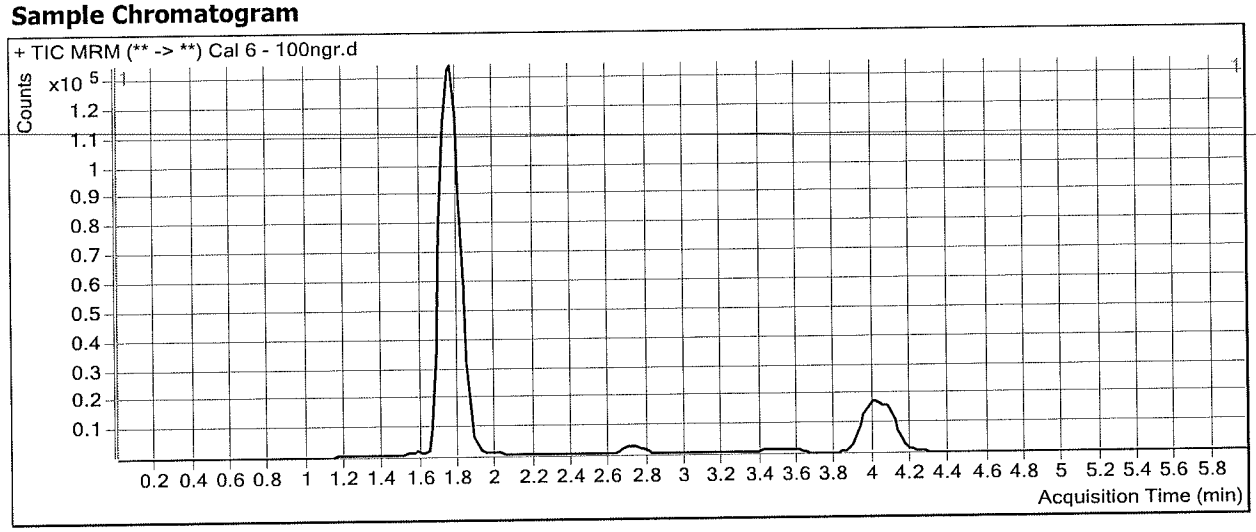
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 16:17	**Data File**	Cal 6 - 100ngr.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	1.755	310323	257642	1.2045	104.1244
THC-COOH	THC-COOH-d9	1.805	203691	83146	2.4498	105.1678
THC	THC-d3	4.031	107059	72534	1.4760	103.7021

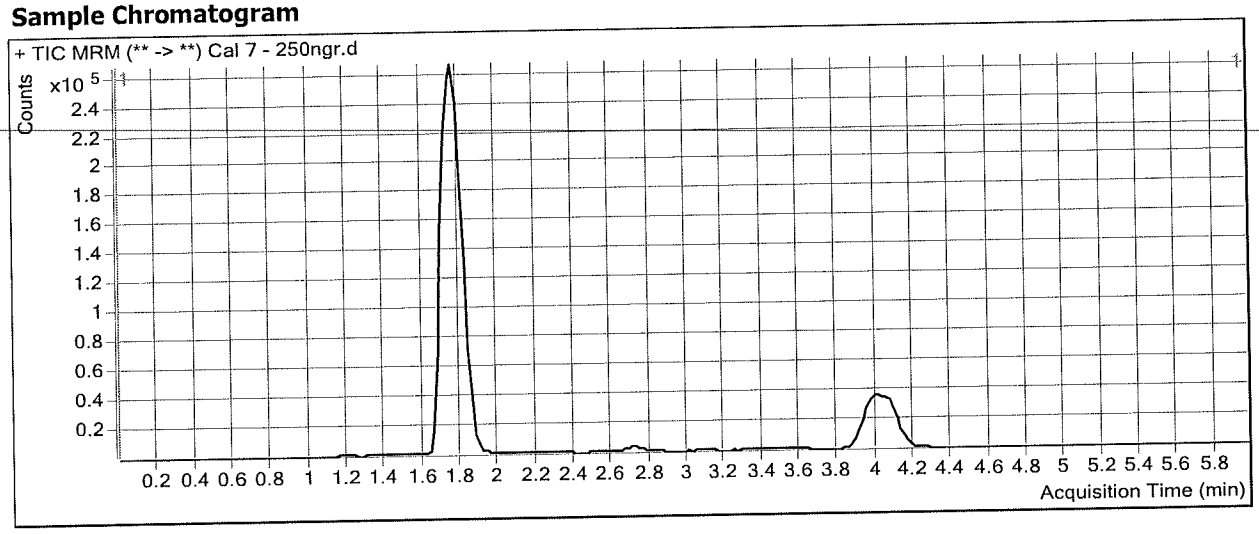
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\06272018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	6/28/2018 10:38 AM	**Analyst Name**	ISP Tox
Report Time	6/28/2018 10:39 AM	**Reporter Name**	ISP Tox
Last Calib Update	6/28/2018 10:38 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-06-27 16:29	**Data File**	Cal 7 - 250ngr.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	1.755	746813	339420	2.2003	189.9149
THC-COOH	THC-COOH-d9	1.805	487631	98268	4.9622	213.2009
THC	THC-d3	4.071	264949	92146	2.8753	201.8096