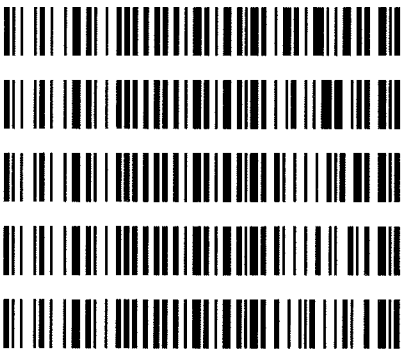
REVIEWED
By Tamara Salazar at 2:54 pm, Feb 11, 2019

2/11/2019

Worklist: 2941

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2019-0109	1	139399	AM 27 Blood THC Quant by LC-QQQ
C2019-0182	1	139400	AM 27 Blood THC Quant by LC-QQQ
C2019-0209	1	139401	AM 27 Blood THC Quant by LC-QQQ
C2019-0210	1	139402	AM 27 Blood THC Quant by LC-QQQ
C2019-0218	1	139403	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 2/7/19 Analyst: Anne Nord
Plate lot#: 0539904 Plate Expiration: 09/10/19

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: 18G207D7 **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: k52558g** 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: 2019 Data\AM 27\020719 Batch Name: cann quant
☒ 2. Make any necessary integration changes, Curve weighting of Linear 1/x with r^2 values ≥0.98 for each analyte
☒ 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
☒ 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.
☒ 5. Did all QCs pass for each analyte? Y / N
☒ 6 Enter QCs into control charting.
☒ 7 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 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
ppd 9/20/18 Exp 4/1/19	lot 92018	Concentration 10 ng/ml each	by AMN
ppd 01/14/18 Exp 4/1/19	lot 011419	Concentration 10 ng/ml each	by AMN

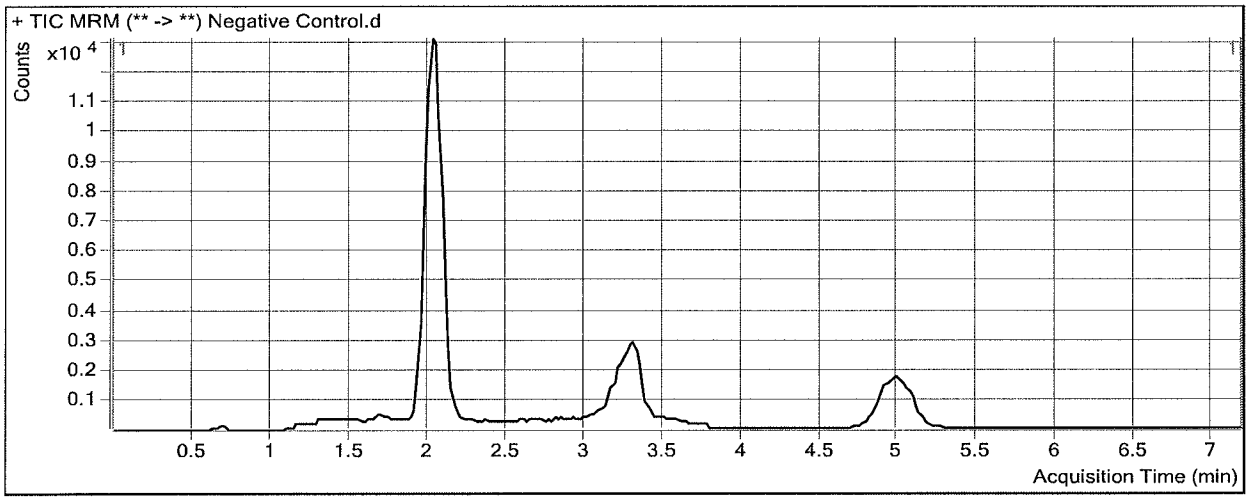
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 16:00	**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	2.256	395	68828	0.0057	1.3601

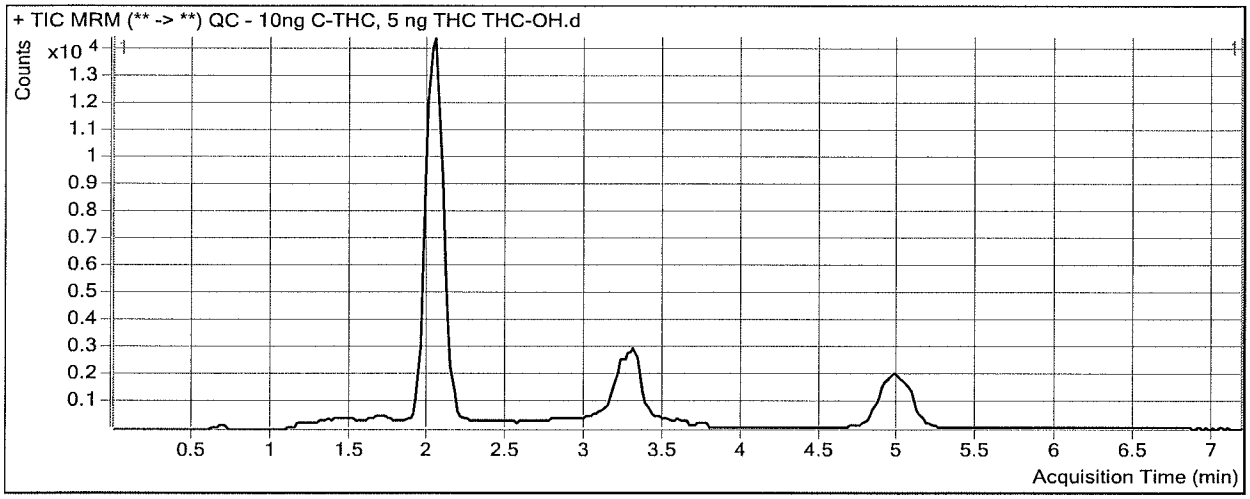
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 16:12	**Data File**	QC - 10ng C-THC, 5 ng THC THC-OH.d
Sample Type	QC	**Sample Name**	QC - 10ng C-THC, 5 ng THC THC-OH
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	2.035	2761	70473	0.0392	5.6513
THC-COOH	THC-COOH-d9	2.105	4622	30919	0.1495	9.9784
THC	THC-d3	5.032	1330	26954	0.0493	4.9038

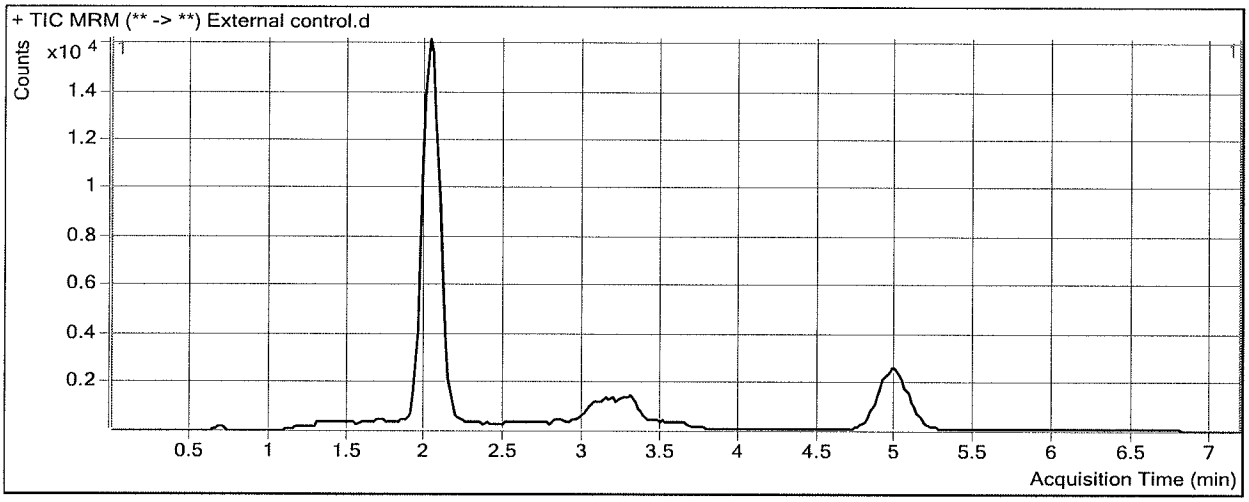
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 16:24	**Data File**	External control.d
Sample Type	Sample	**Sample Name**	External control
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



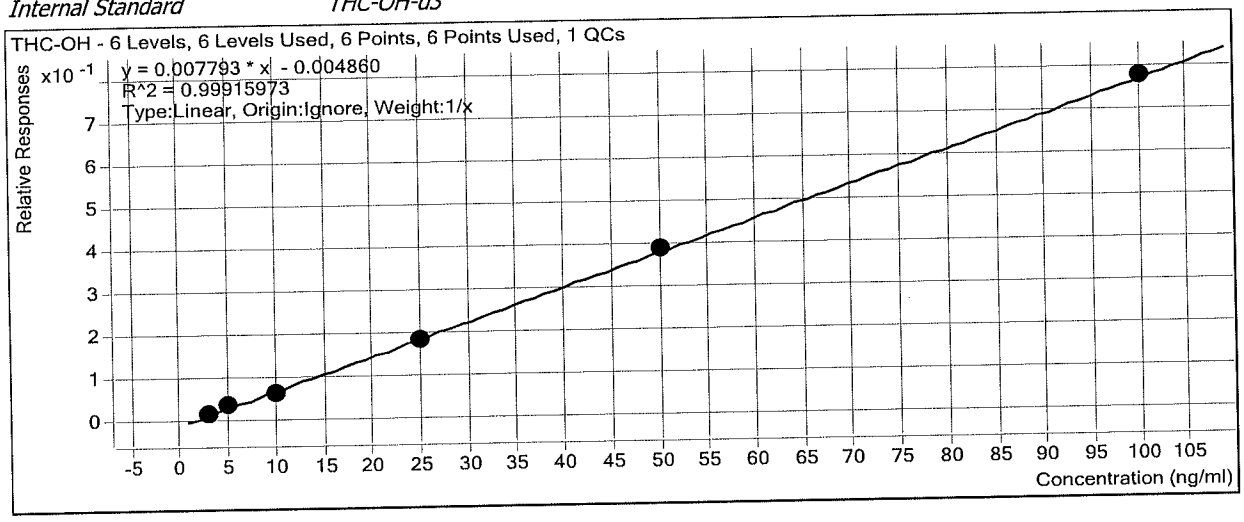
Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.055	5836	77293	0.0755	10.3127
THC-COOH	THC-COOH-d9	2.085	4812	32679	0.1472	9.8182
THC	THC-d3	4.992	3420	30349	0.1127	11.4986

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin
Last Calib Update 2/8/2019 10:24 AM **Analyst Name** ISP TOX

Target Compound *THC-OH*
Internal Standard *THC-OH-d3*

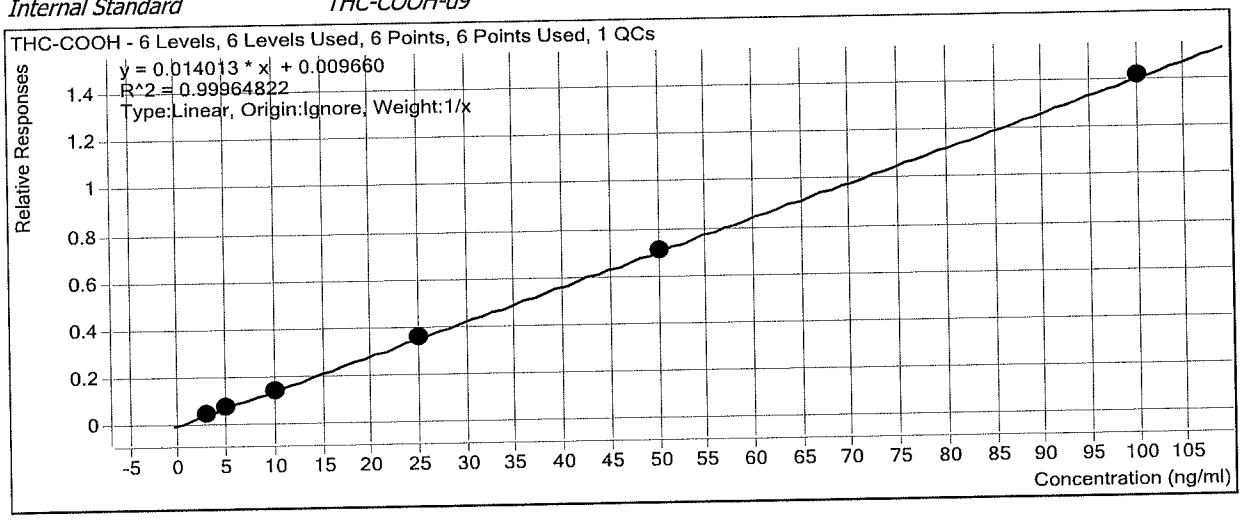


Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.1	103.5
Cal 2 - 5ng	2	☑	5	5.3	105.7
Cal 3 - 10ng	3	☑	10	9.1	90.7
Cal 4 - 25ng	4	☑	25	24.6	98.6
Cal 5 - 50ng	5	☑	50	50.6	101.2
Cal 6 - 100ng	6	☑	100	100.3	100.3
QC - 10ng C-THC, 5 ng THC THC-OH	7	☑	5	5.7	113.0

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin
Last Calib Update 2/8/2019 10:24 AM **Analyst Name** ISP TOX

Target Compound *THC-COOH*
Internal Standard *THC-COOH-d9*

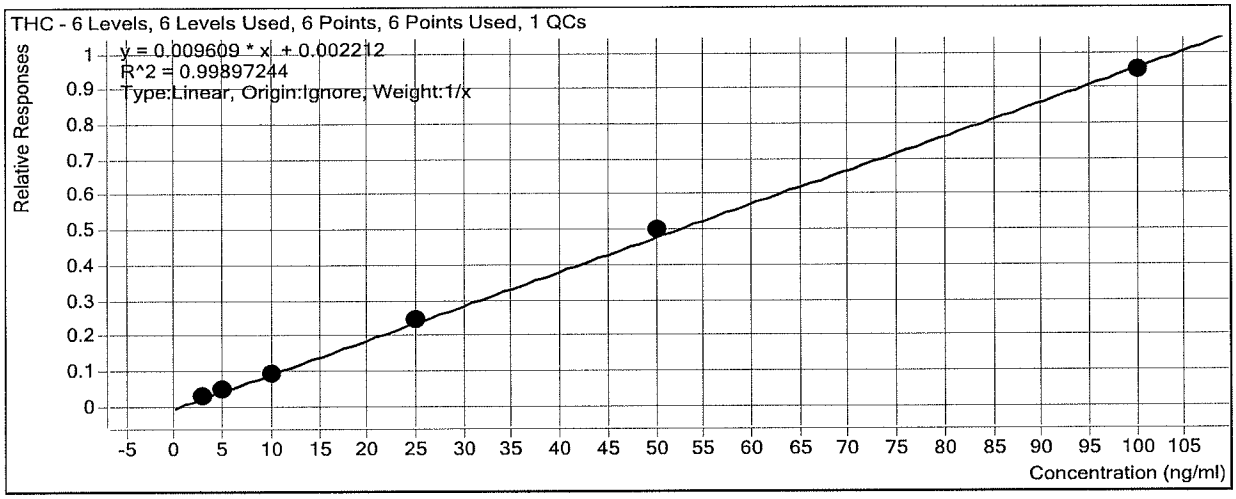


Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.1	104.7
Cal 2 - 5ng	2	☑	5	5.0	100.2
Cal 3 - 10ng	3	☑	10	9.4	94.0
Cal 4 - 25ng	4	☑	25	25.3	101.3
Cal 5 - 50ng	5	☑	50	49.8	99.5
Cal 6 - 100ng	6	☑	100	100.4	100.4
QC - 10ng C-THC, 5 ng THC THC-OH	7	☑	10	10.0	99.8

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin
Last Calib Update 2/8/2019 10:24 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	107.9
Cal 2 - 5ng	2	☑	5	4.7	95.0
Cal 3 - 10ng	3	☑	10	9.4	93.9
Cal 4 - 25ng	4	☑	25	25.3	101.2
Cal 5 - 50ng	5	☑	50	51.7	103.3
Cal 6 - 100ng	6	☑	100	98.7	98.7
QC - 10ng C-THC, 5 ng THC THC-OH	7	☑	5	4.9	98.1

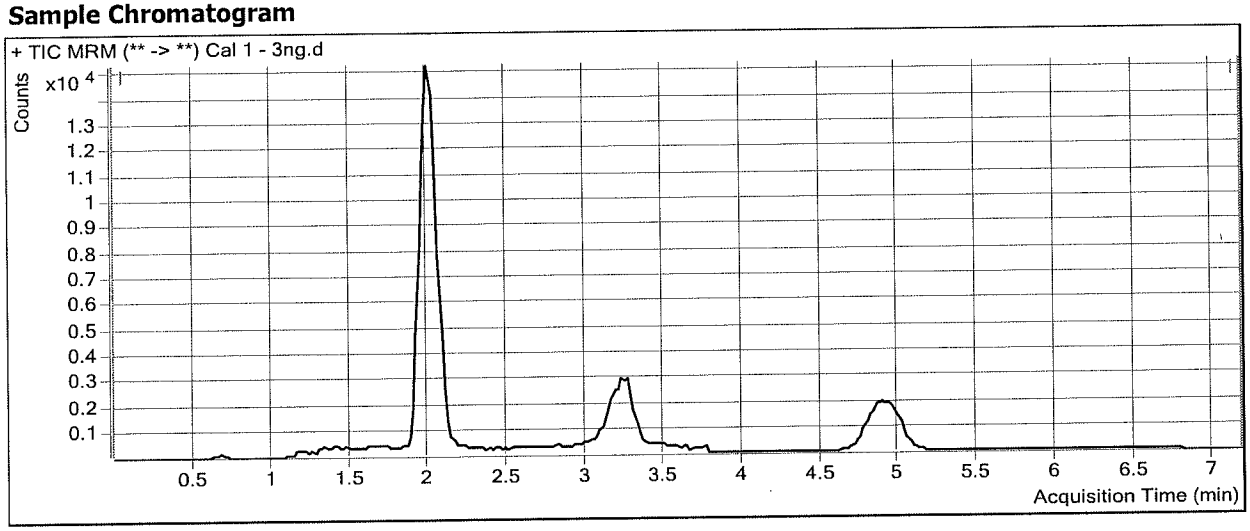
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 14:37	**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-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	2.035	1474	76173	0.0193	3.1060
THC-COOH	THC-COOH-d9	2.045	1790	33362	0.0537	3.1405
THC	THC-d3	4.952	910	27293	0.0333	3.2381

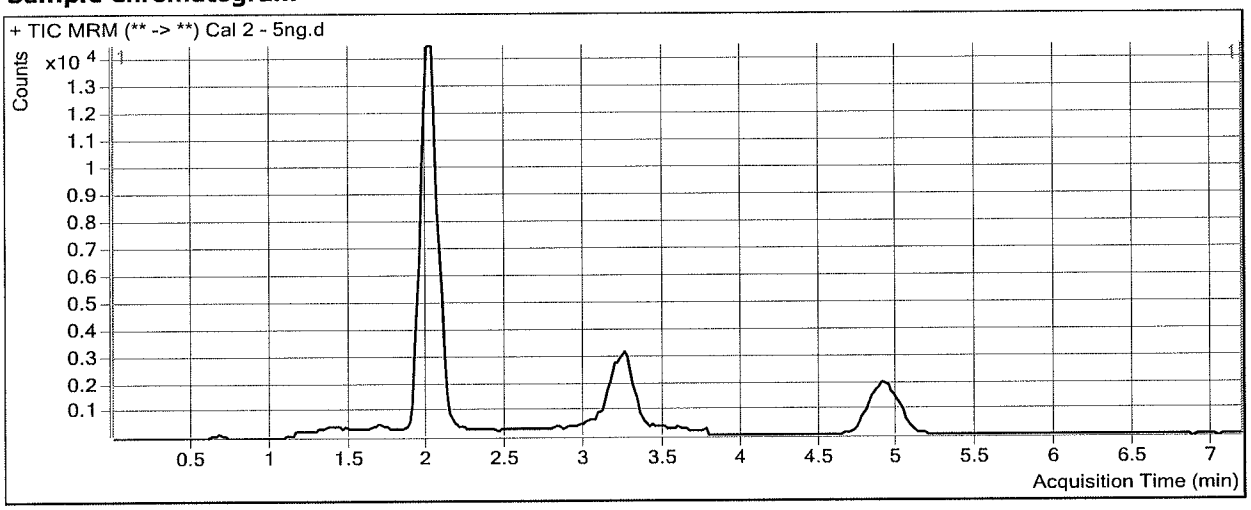
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 14:49	**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-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	2.015	2561	70526	0.0363	5.2836
THC-COOH	THC-COOH-d9	2.085	2502	31341	0.0798	5.0081
THC	THC-d3	4.952	1201	25107	0.0478	4.7491

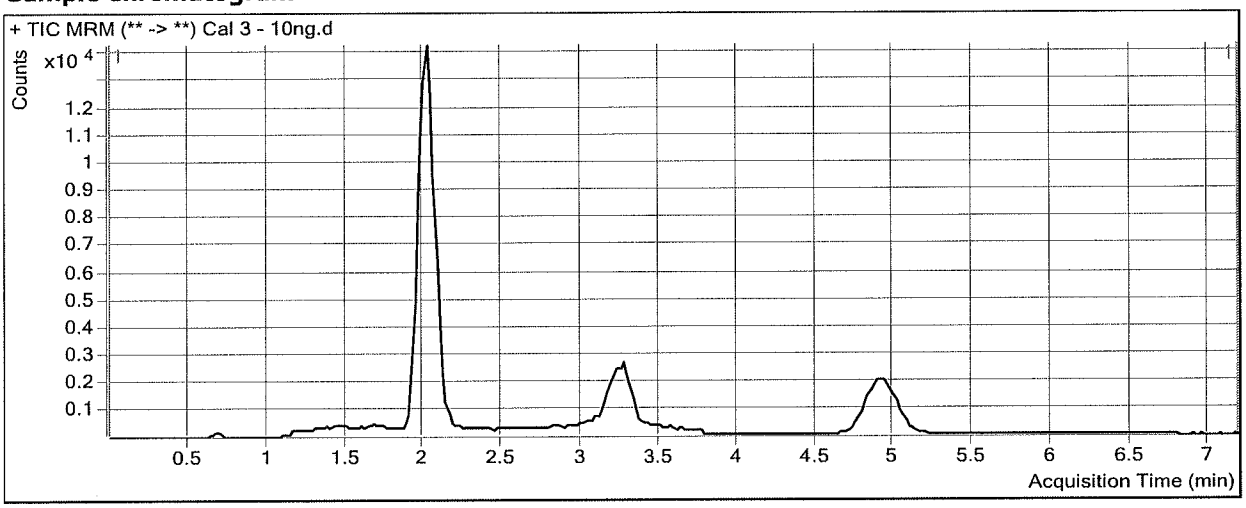
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 15:01	**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-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	2.015	4261	64729	0.0658	9.0709
THC-COOH	THC-COOH-d9	2.085	4129	29221	0.1413	9.3958
THC	THC-d3	4.952	2281	24686	0.0924	9.3873

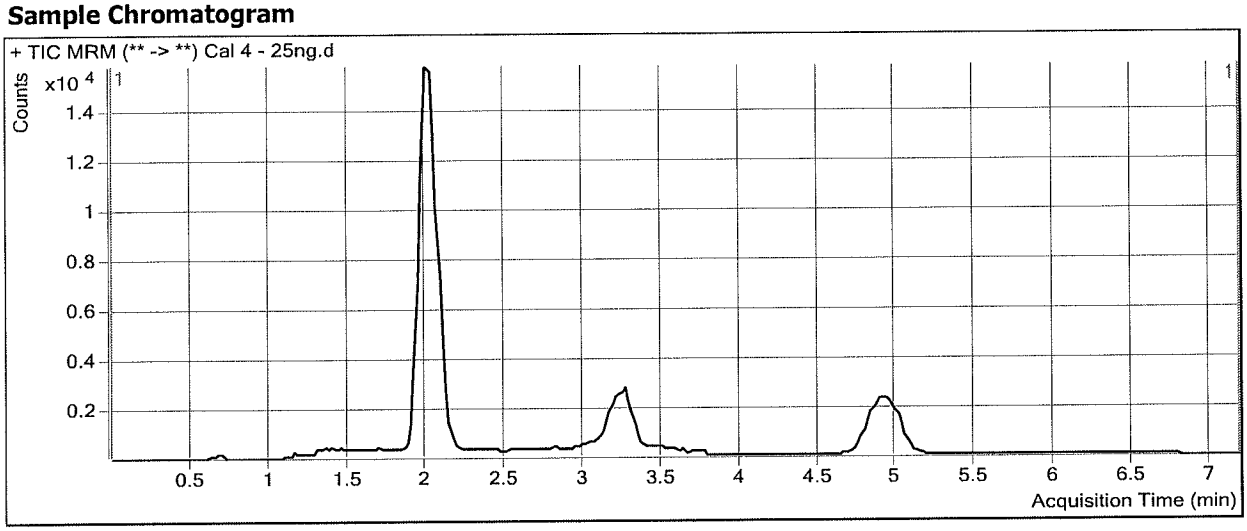
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 15:12	**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-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	2.015	12471	66614	0.1872	24.6468
THC-COOH	THC-COOH-d9	2.085	10235	28075	0.3646	25.3271
THC	THC-d3	4.972	6188	25215	0.2454	25.3087

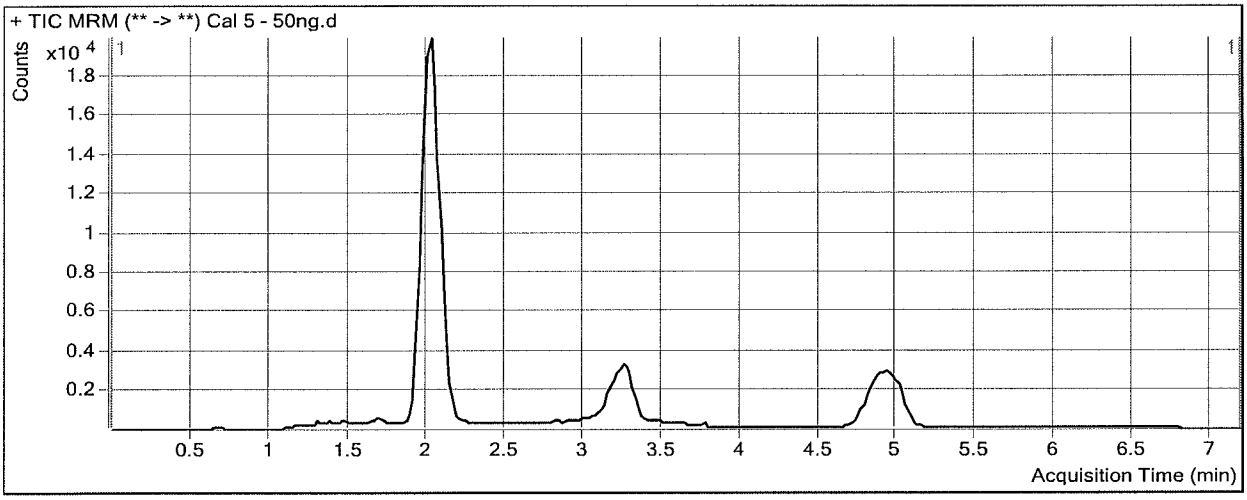
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 15:24	**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-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	2.015	26826	68875	0.3895	50.6031
THC-COOH	THC-COOH-d9	2.085	21207	30000	0.7069	49.7591
THC	THC-d3	4.952	12592	25256	0.4986	51.6588

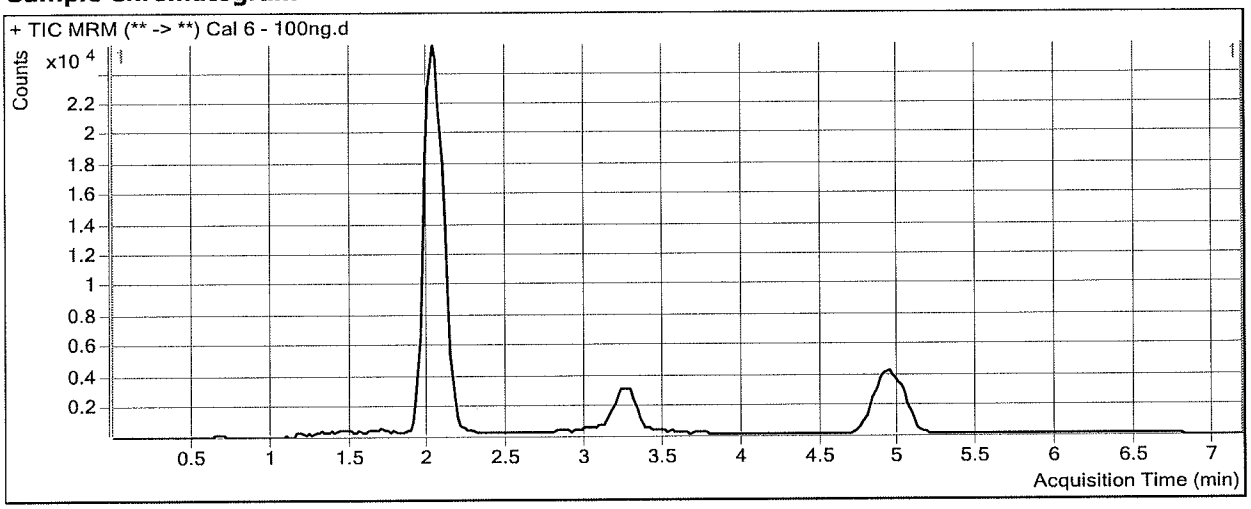
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\020719\QuantResults\cann quant.batch.bin		
Analysis Time	2/8/2019 10:24 AM	**Analyst Name**	ISP Tox
Report Time	2/8/2019 10:25 AM	**Reporter Name**	ISP Tox
Last Calib Update	2/8/2019 10:24 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-02-07 15:36	**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-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	2.015	53053	68307	0.7767	100.2895
THC-COOH	THC-COOH-d9	2.105	41259	29136	1.4161	100.3693
THC	THC-d3	4.952	24094	25357	0.9502	98.6582