REVIEWED
By Britany Wylie at 3:38 pm, Mar 26, 2019

3/26/2019

Worklist: 3156

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2019-0389	1	146188	AM 27 Blood THC Quant by LC-QQQ
C2019-0421	1	146189	AM 27 Blood THC Quant by LC-QQQ
C2019-0446	1	146190	AM 27 Blood THC Quant by LC-QQQ
C2019-0461	1	146191	AM 27 Blood THC Quant by LC-QQQ

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

Extraction Date: 3/22/19 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: 19A207P3 **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\032219 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 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 75 ulTHC-OH in 9767.5 ul meOH
Ppd 3/13/19 Exp: 9/13/19 lot 91319 by AMN

Drug	lot	expiration
C-THC	FE01061702 cerillient	3/1/2022
THC-OH	318.1b18.1L1a	12/1/2021
THC	135.1b71.0L6	11/1/2021

AM 27 control 100 ul working solution lot (91319) in 9900 ul blood lot (19A207p3)
ppd 3/13/19 Exp 9/13/19 lot 31319 Concentration 7.5 ng/ml THC, THC-OH and 15 ng/ml C-TH by AMN

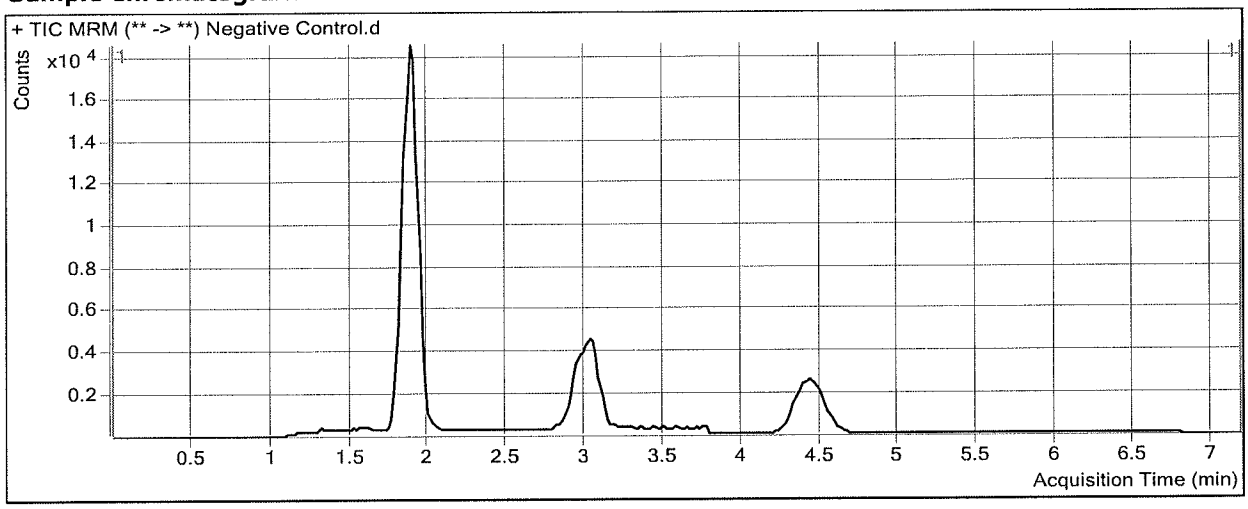
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 13:29	**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-B2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 Cannabinoid Confirmation

Sample Chromatogram



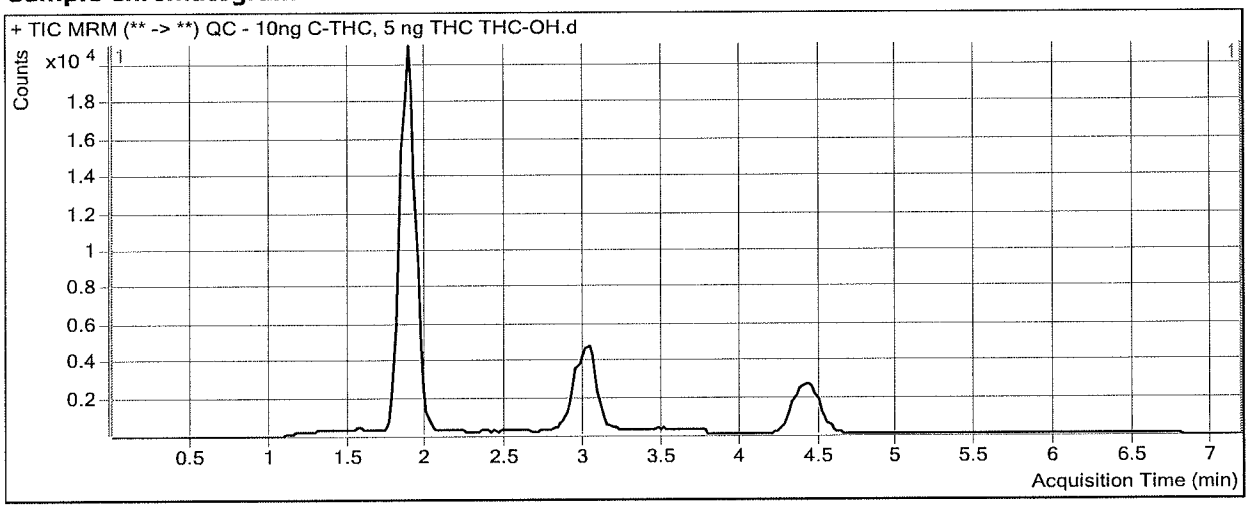
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 13:41	**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	1.895	3913	95357	0.0410	4.9462
THC-COOH	THC-COOH-d9	1.945	6345	37991	0.1670	9.7563
THC	THC-d3	4.452	1413	31526	0.0448	4.4887

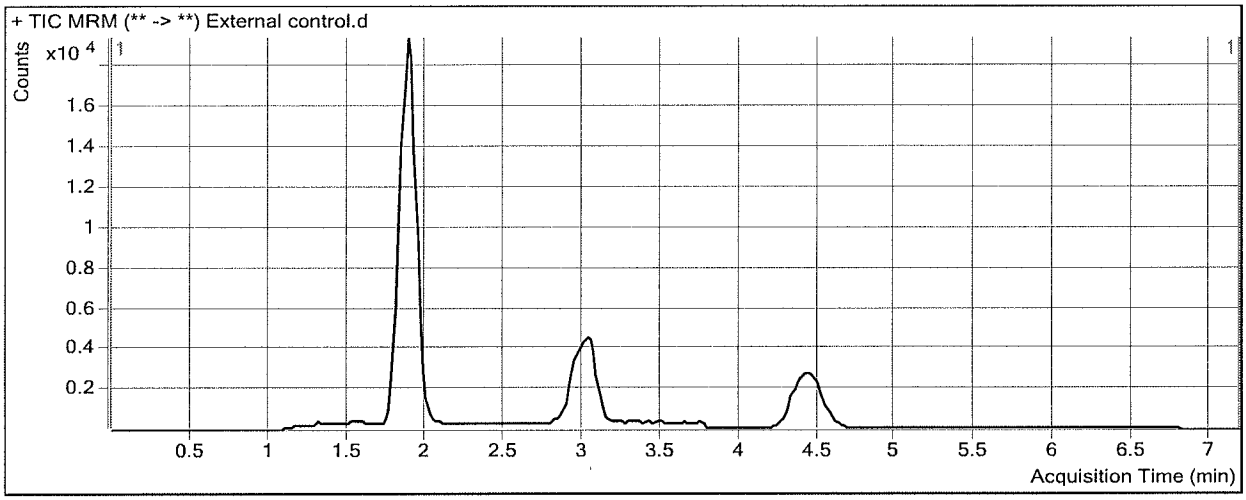
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 13:53	**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-C2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 Cannabinoid Confirmation

Sample Chromatogram



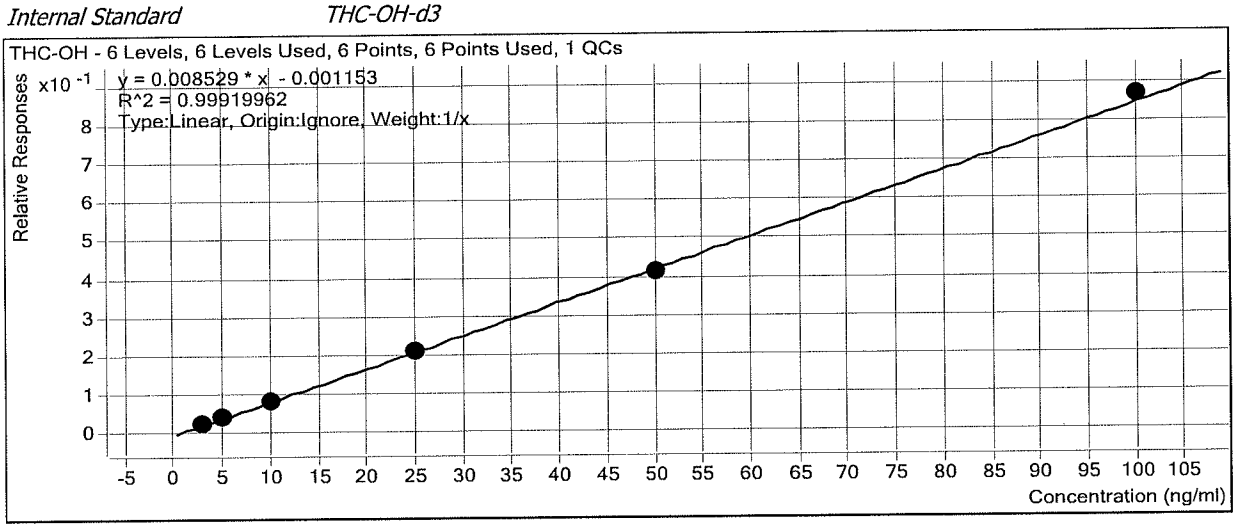
Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	1.875	4879	90678	0.0538	6.4440
THC-COOH	THC-COOH-d9	1.945	7498	35577	0.2108	12.5398
THC	THC-d3	4.452	2643	32599	0.0811	8.2470

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin
Last Calib Update 3/23/2019 9:41 AM **Analyst Name** ISP TOX

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

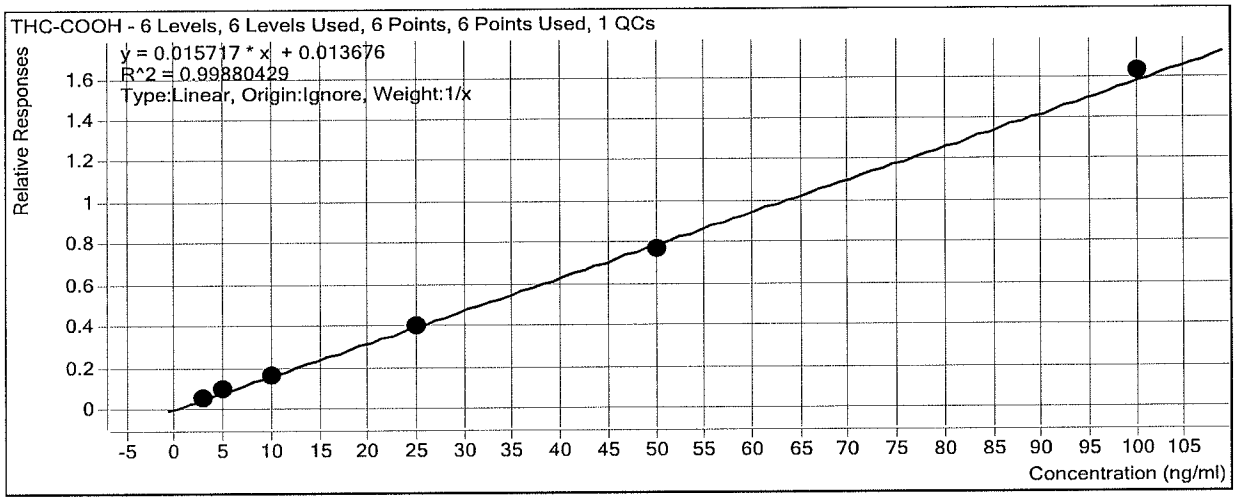


Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.2	107.6
Cal 2 - 5ng	2	☑	5	4.8	96.1
Cal 3 - 10ng	3	☑	10	9.9	98.8
Cal 4 - 25ng	4	☑	25	24.6	98.6
Cal 5 - 50ng	5	☑	50	48.5	97.0
Cal 6 - 100ng	6	☑	100	101.9	101.9
QC - 10ng C-THC, 5 ng THC THC-OH	7	☑	5	4.9	98.9

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin
Last Calib Update 3/23/2019 9:41 AM **Analyst Name** ISP TOX

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

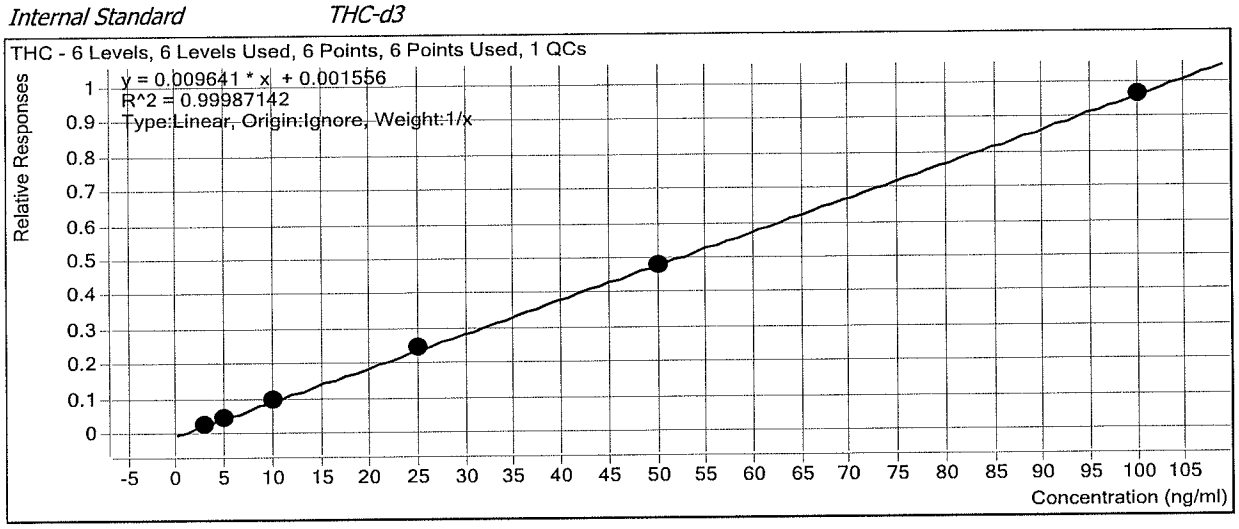


Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	☑	3	3.1	102.8
Cal 2 - 5ng	2	☑	5	5.3	105.3
Cal 3 - 10ng	3	☑	10	9.5	94.8
Cal 4 - 25ng	4	☑	25	24.5	98.2
Cal 5 - 50ng	5	☑	50	48.3	96.5
Cal 6 - 100ng	6	☑	100	102.4	102.4
QC - 10ng C-THC, 5 ng THC THC-OH	7	☑	10	9.8	97.6

ISP Forensics
Calibration Curve Report

Batch Data Path D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin
Last Calib Update 3/23/2019 9:41 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	3.1	101.8
Cal 2 - 5ng	2	☑	5	4.9	98.0
Cal 3 - 10ng	3	☑	10	9.8	98.4
Cal 4 - 25ng	4	☑	25	25.5	102.2
Cal 5 - 50ng	5	☑	50	49.9	99.9
Cal 6 - 100ng	6	☑	100	99.7	99.7
QC - 10ng C-THC, 5 ng THC THC-OH	7	☑	5	4.5	89.8

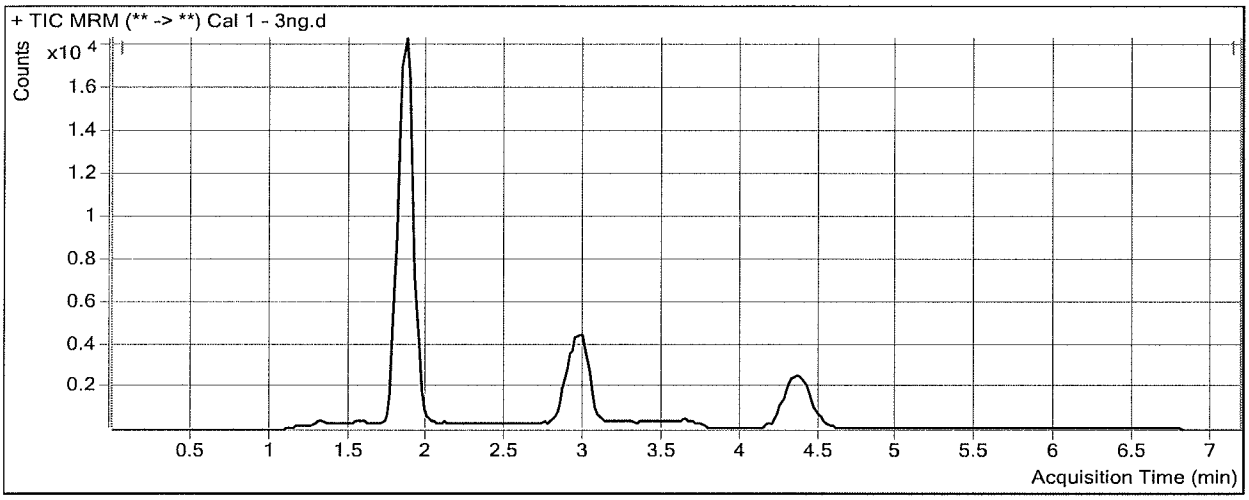
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 12:06	**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	1.875	2239	84908	0.0264	3.2266
THC-COOH	THC-COOH-d9	1.905	2136	34355	0.0622	3.0852
THC	THC-d3	4.392	913	29467	0.0310	3.0535

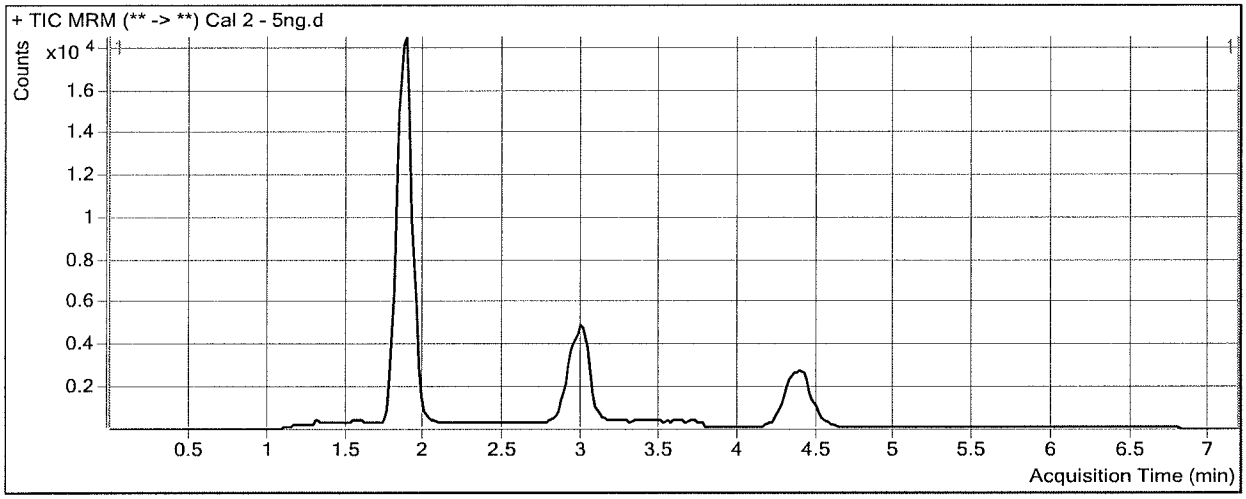
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 12:18	**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	1.875	3399	85354	0.0398	4.8047
THC-COOH	THC-COOH-d9	1.945	3223	33416	0.0964	5.2661
THC	THC-d3	4.392	1470	30116	0.0488	4.9005

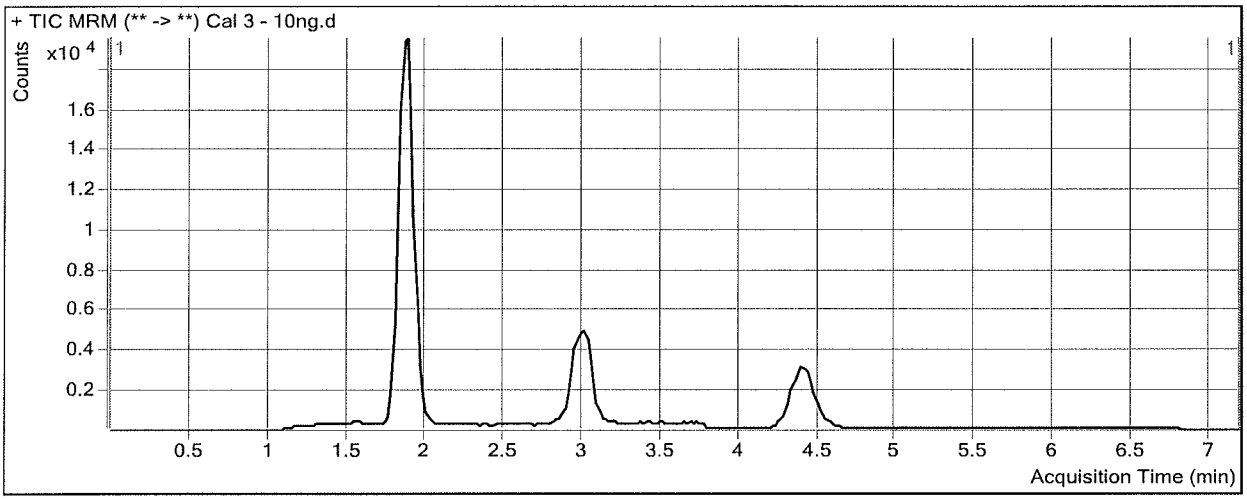
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 12:30	**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	1.875	6889	82852	0.0831	9.8838
THC-COOH	THC-COOH-d9	1.925	5305	32613	0.1627	9.4788
THC	THC-d3	4.432	2833	29377	0.0964	9.8423

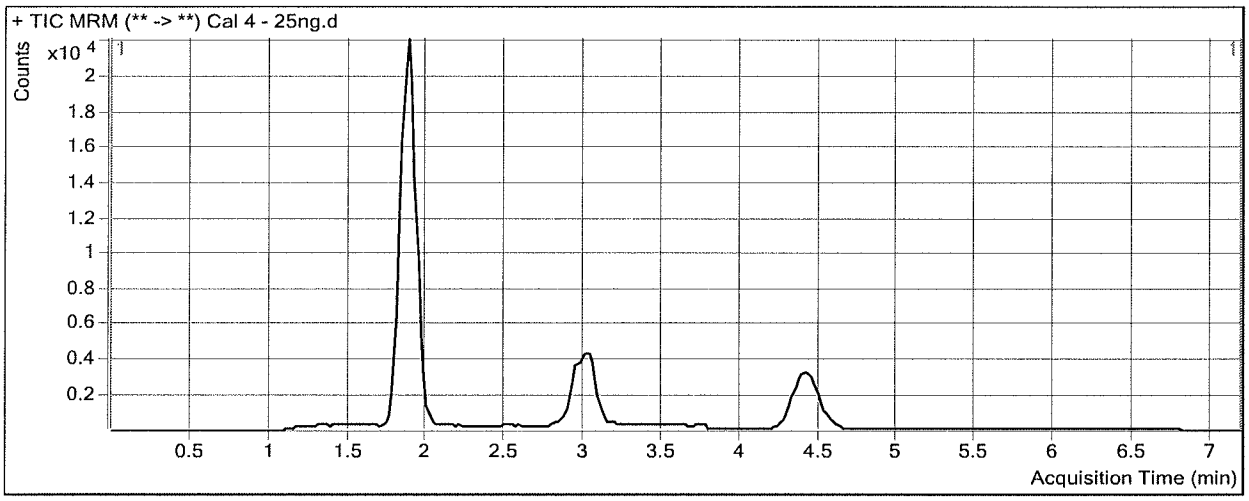
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 12:42	**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	1.895	17611	84260	0.2090	24.6413
THC-COOH	THC-COOH-d9	1.945	13278	33243	0.3994	24.5425
THC	THC-d3	4.432	7207	29077	0.2478	25.5465

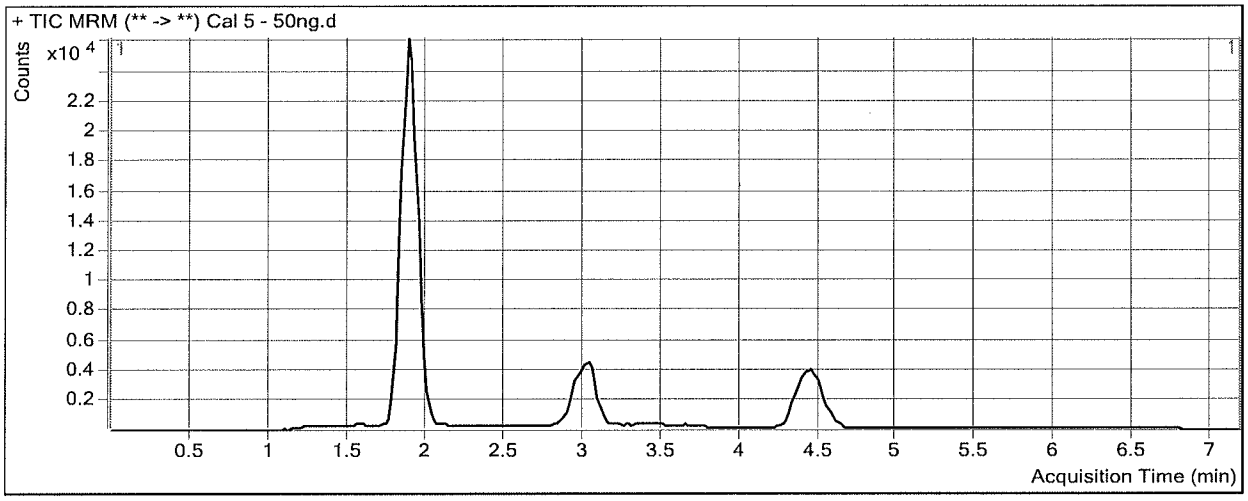
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 12:54	**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	1.895	34492	83610	0.4125	48.5043
THC-COOH	THC-COOH-d9	1.945	24799	32121	0.7721	48.2510
THC	THC-d3	4.452	14336	29680	0.4830	49.9390

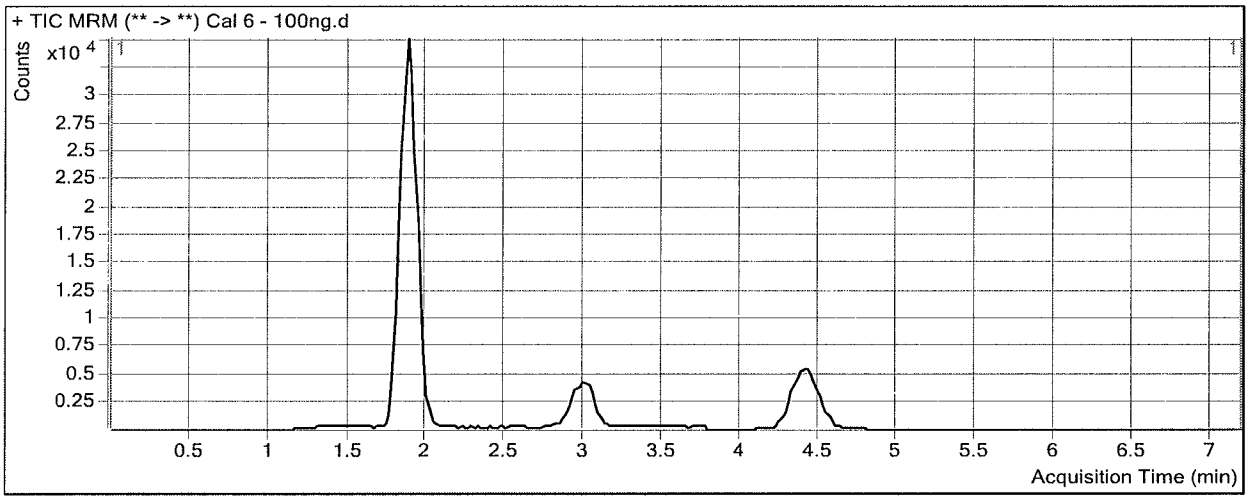
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2019 Data\AM 27\032219\QuantResults\cann quant.batch.bin		
Analysis Time	3/23/2019 9:41 AM	**Analyst Name**	ISP Tox
Report Time	3/23/2019 9:43 AM	**Reporter Name**	ISP Tox
Last Calib Update	3/23/2019 9:41 AM	**Batch State**	Processed

Analysis Info

Acq Time	2019-03-22 13:06	**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	1.875	70557	81263	0.8683	101.9393
THC-COOH	THC-COOH-d9	1.945	51032	31448	1.6228	102.3763
THC	THC-d3	4.432	27865	28937	0.9629	99.7180