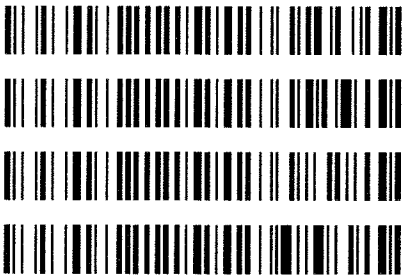
reviewed 8/16/18 BWylee

8/9/2018

Worklist: 2626

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2018-1446	1	123958	AM 27 Blood THC Quant by LC-QQQ
C2018-1447	1	123959	AM 27 Blood THC Quant by LC-QQQ
C2018-1450	1	123960	AM 27 Blood THC Quant by LC-QQQ
C2018-1494	1	123961	AM 27 Blood THC Quant by LC-QQQ



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

Extraction Date: 8/8/18 Analyst: Anne Nord
Plate lot#: 0515037 Plate Expiration: 09/28/2018

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: 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: 08082018 cann quant 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

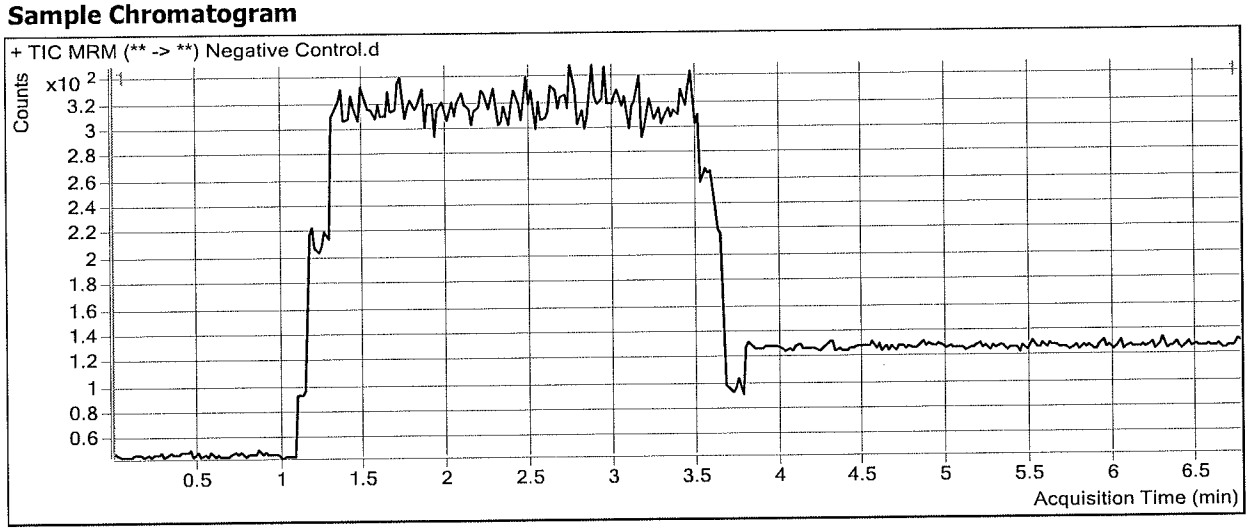
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 16:27	**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



Sample did not inject - reconstituted and re-injected.

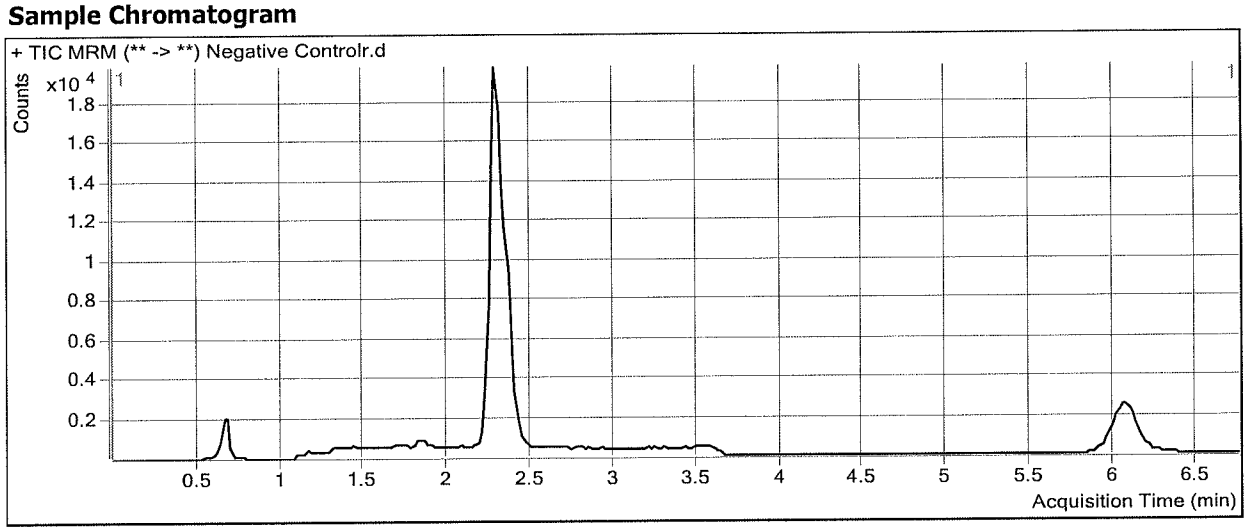
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-10 09:00	**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



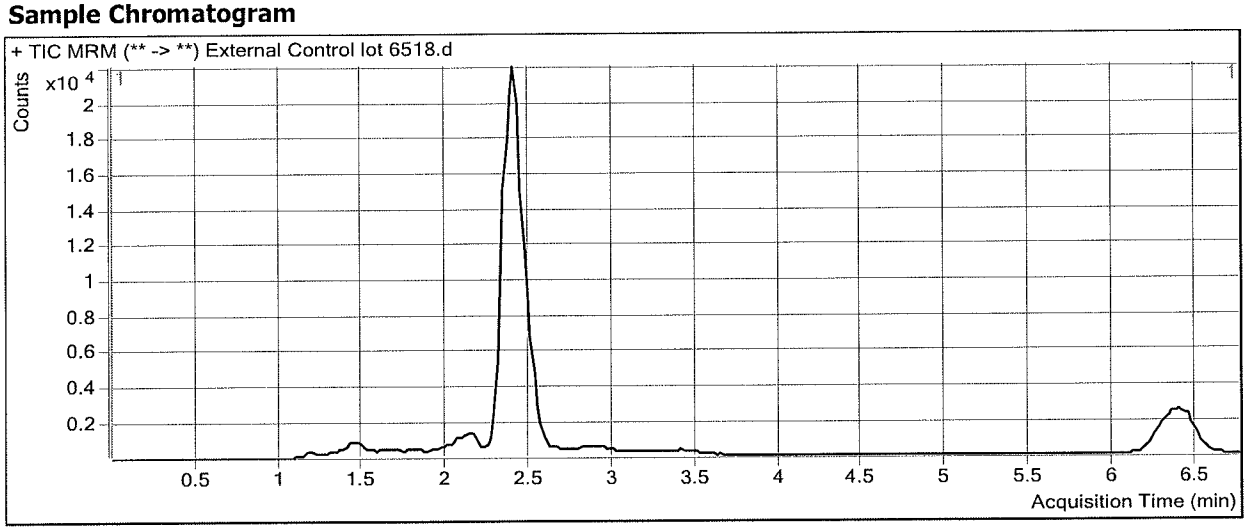
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 16:51	**Data File**	External Control lot 6518.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	2.416	9784	126127	0.0776	7.2567
THC-COOH	THC-COOH-d9	2.506	8442	46356	0.1821	8.1300
THC	THC-d3	6.453	3862	34031	0.1135	9.6174

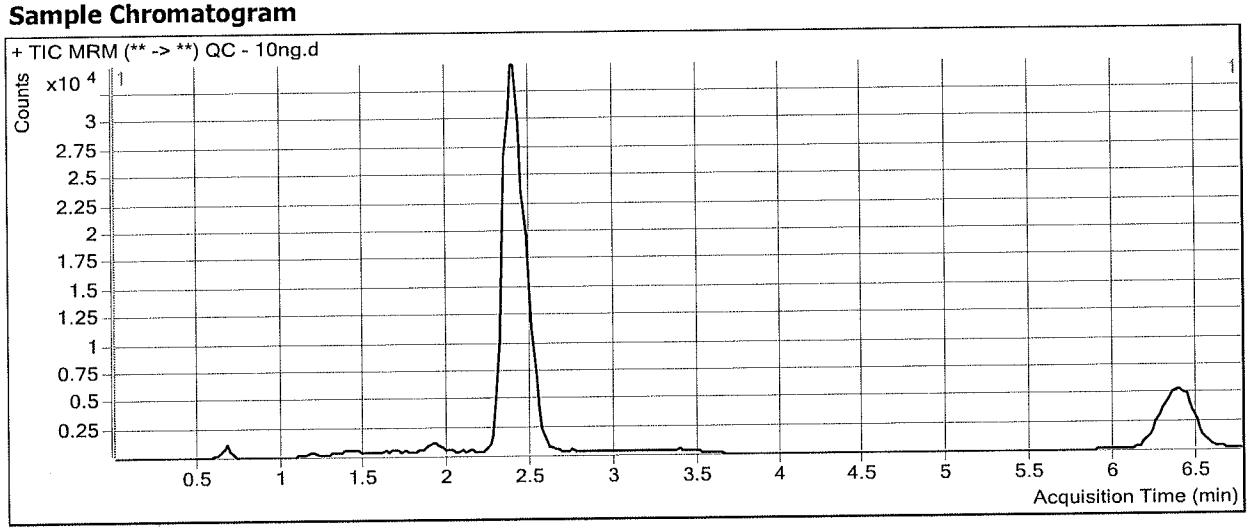
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 16:39	**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	2.396	21652	196060	0.1104	10.3163
THC-COOH	THC-COOH-d9	2.506	18570	80785	0.2299	10.5214
THC	THC-d3	6.393	8419	67268	0.1252	10.6192

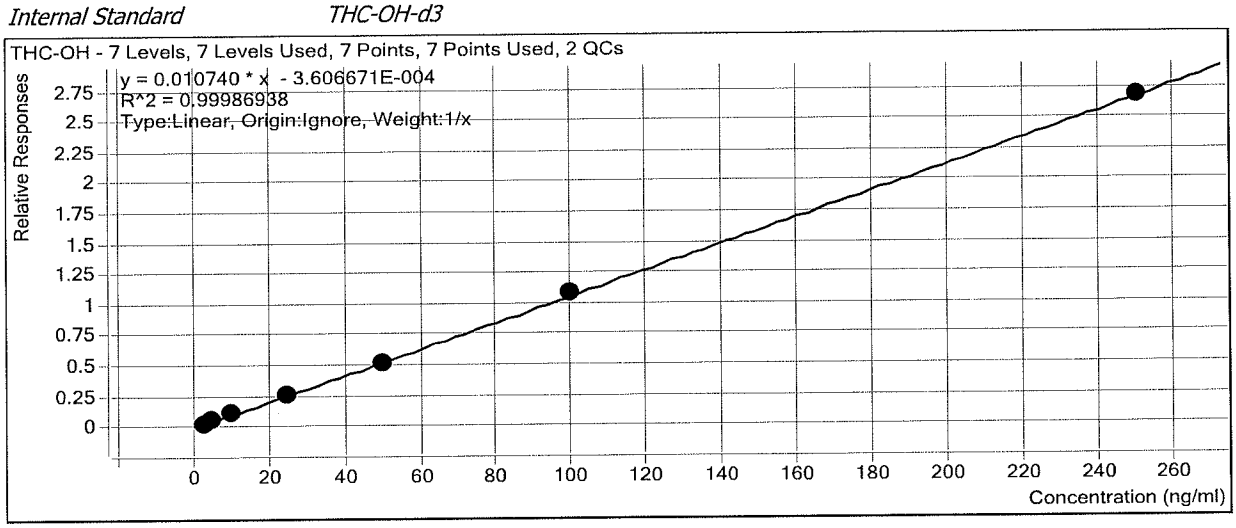
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update 8/10/2018 9:19 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	3.0	99.3
Cal 2 - 5ng	2	☑	5	5.2	103.0
Cal 3 - 10ng	3	☑	10	10.0	100.1
QC - 10ng	3	☑	10	10.3	103.2
External Control lot 6518	3	☑	10	7.3	72.6
Cal 4 - 25ng	4	☑	25	24.4	97.7
Cal 5 - 50ng	5	☑	50	49.3	98.6
Cal 6 - 100ng	6	☑	100	101.5	101.5
Cal 7 - 250ng	7	☑	250	249.7	99.9

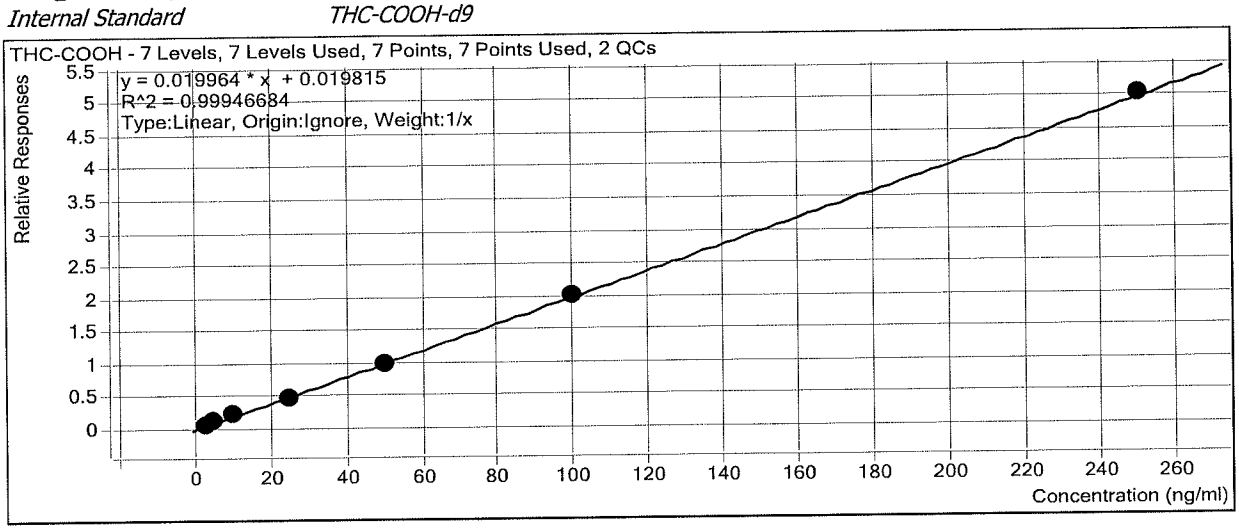
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update 8/10/2018 9:19 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	101.0
Cal 2 - 5ng	2	☑	5	5.2	104.0
Cal 3 - 10ng	3	☑	10	10.3	102.8
QC - 10ng	3	☑	10	10.5	105.2
External Control lot 6518	3	☑	10	8.1	81.3
Cal 4 - 25ng	4	☑	25	23.1	92.5
Cal 5 - 50ng	5	☑	50	49.0	98.0
Cal 6 - 100ng	6	☑	100	101.3	101.3
Cal 7 - 250ng	7	☑	250	251.1	100.4

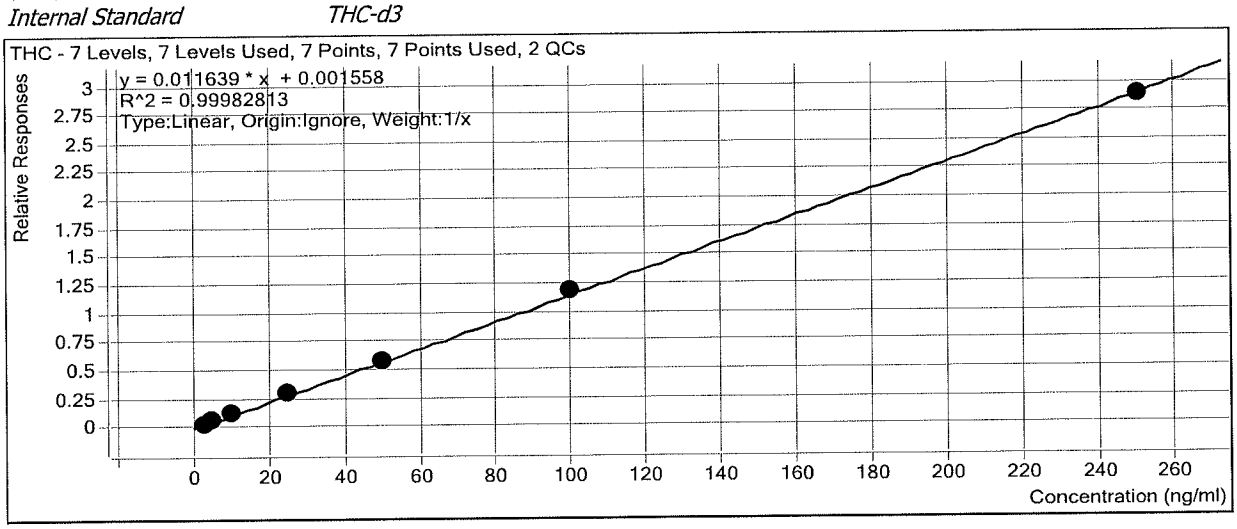
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin

Last Calib Update 8/10/2018 9:19 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	2.9	98.1
Cal 2 - 5ng	2	☑	5	5.0	99.7
Cal 3 - 10ng	3	☑	10	10.0	99.8
QC - 10ng	3	☑	10	10.6	106.2
External Control lot 6518	3	☑	10	9.6	96.2
Cal 4 - 25ng	4	☑	25	25.2	100.8
Cal 5 - 50ng	5	☑	50	50.4	100.8
Cal 6 - 100ng	6	☑	100	101.9	101.9
Cal 7 - 250ng	7	☑	250	247.6	99.0

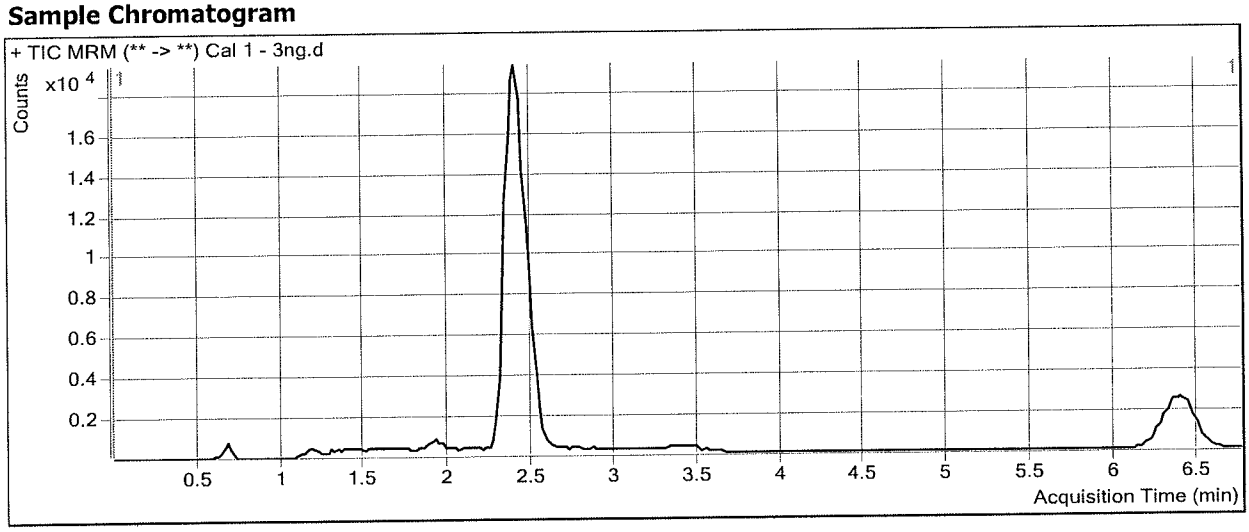
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 14:52	**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	2.416	3589	113485	0.0316	2.9779
THC-COOH	THC-COOH-d9	2.506	3993	49708	0.0803	3.0308
THC	THC-d3	6.413	1262	35262	0.0358	2.9418

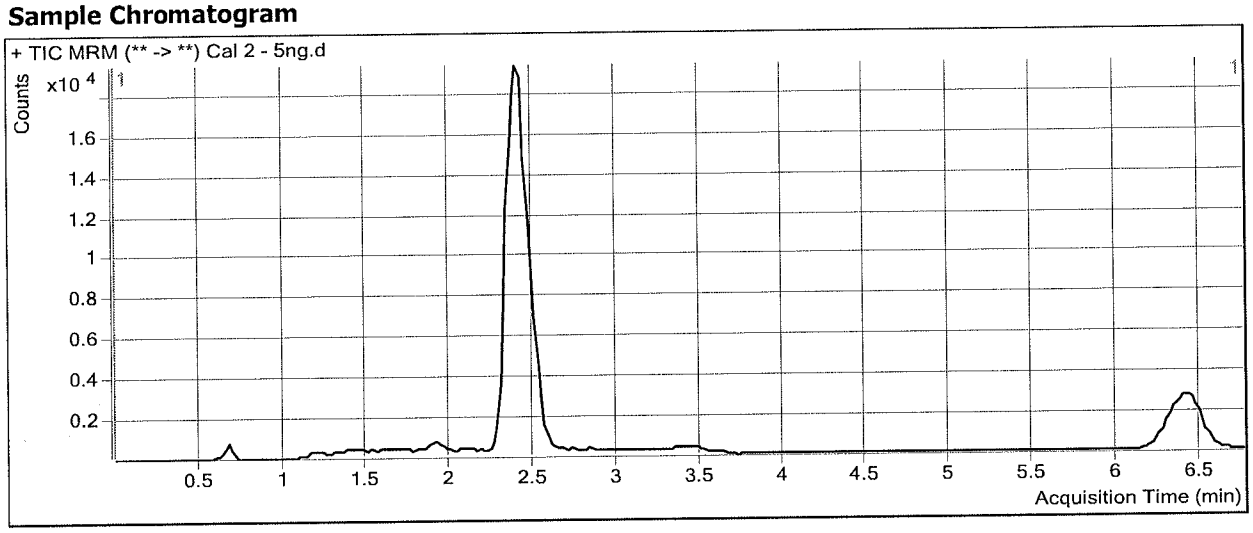
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 15:04	**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	2.436	6162	112125	0.0550	5.1504
THC-COOH	THC-COOH-d9	2.546	5905	47773	0.1236	5.1984
THC	THC-d3	6.433	2197	36888	0.0596	4.9831

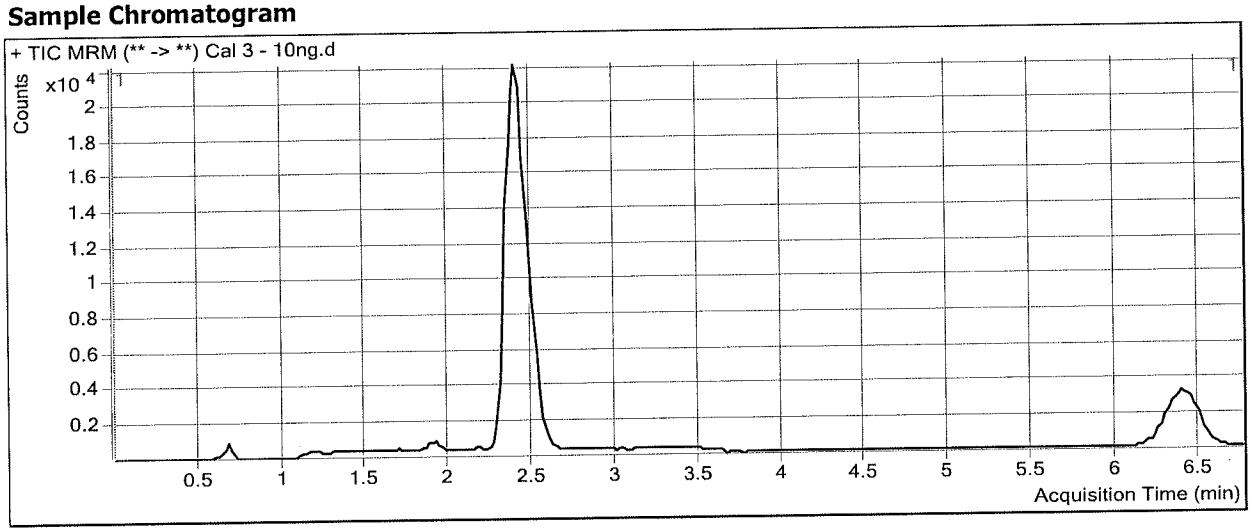
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 15:16	**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	2.416	12826	119744	0.1071	10.0071
THC-COOH	THC-COOH-d9	2.506	11560	51347	0.2251	10.2848
THC	THC-d3	6.453	4645	39467	0.1177	9.9779

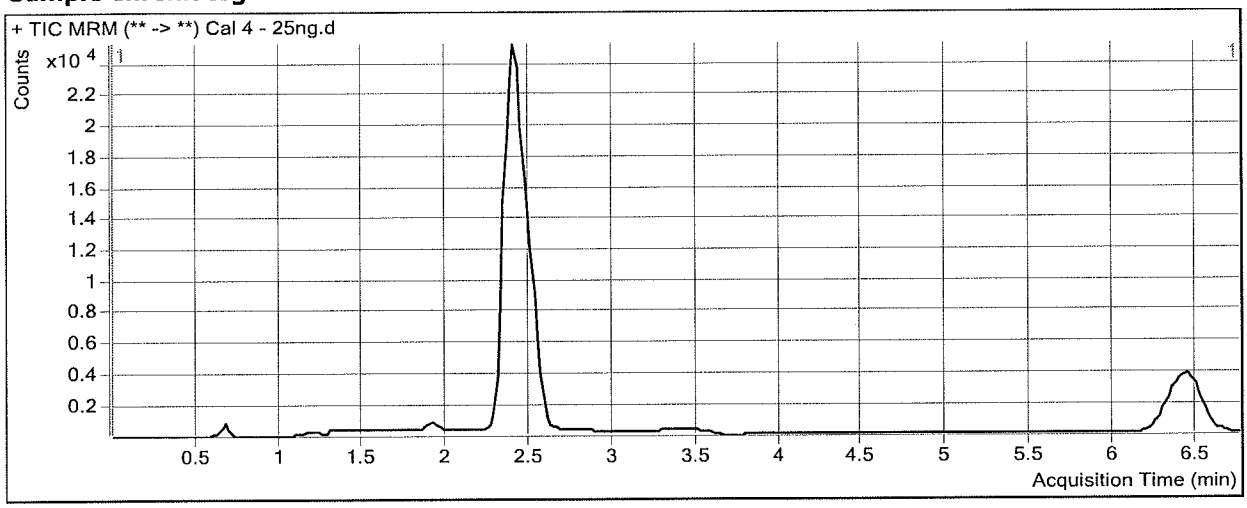
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 15:28	**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	2.416	31223	119190	0.2620	24.4247
THC-COOH	THC-COOH-d9	2.526	24558	51029	0.4813	23.1135
THC	THC-d3	6.453	12061	40923	0.2947	25.1878

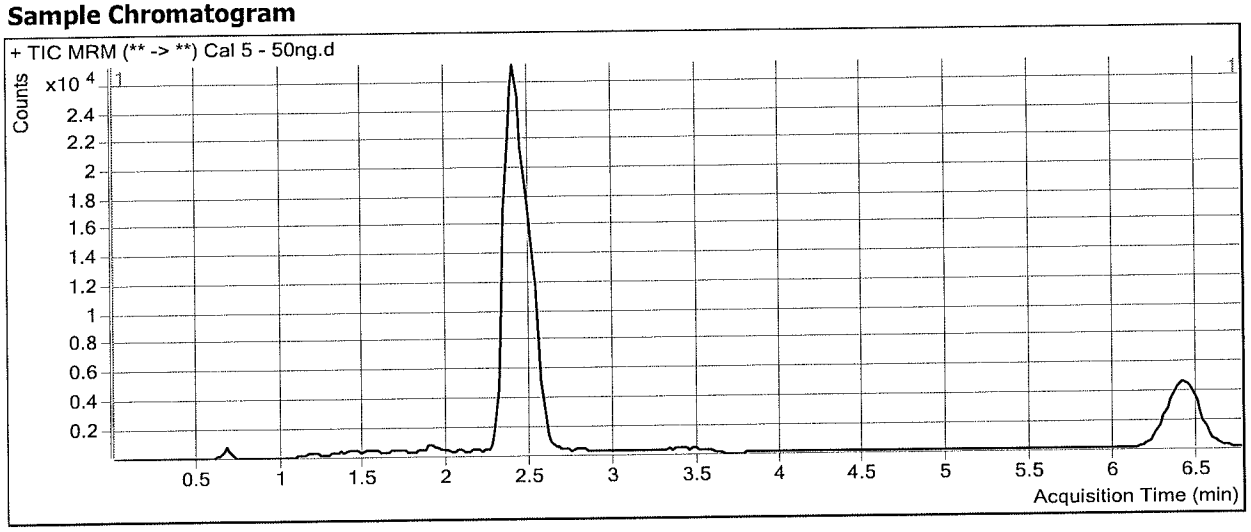
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 15:40	**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	2.416	56161	106101	0.5293	49.3184
THC-COOH	THC-COOH-d9	2.506	44332	44418	0.9981	49.0010
THC	THC-d3	6.433	21870	37177	0.5883	50.4074

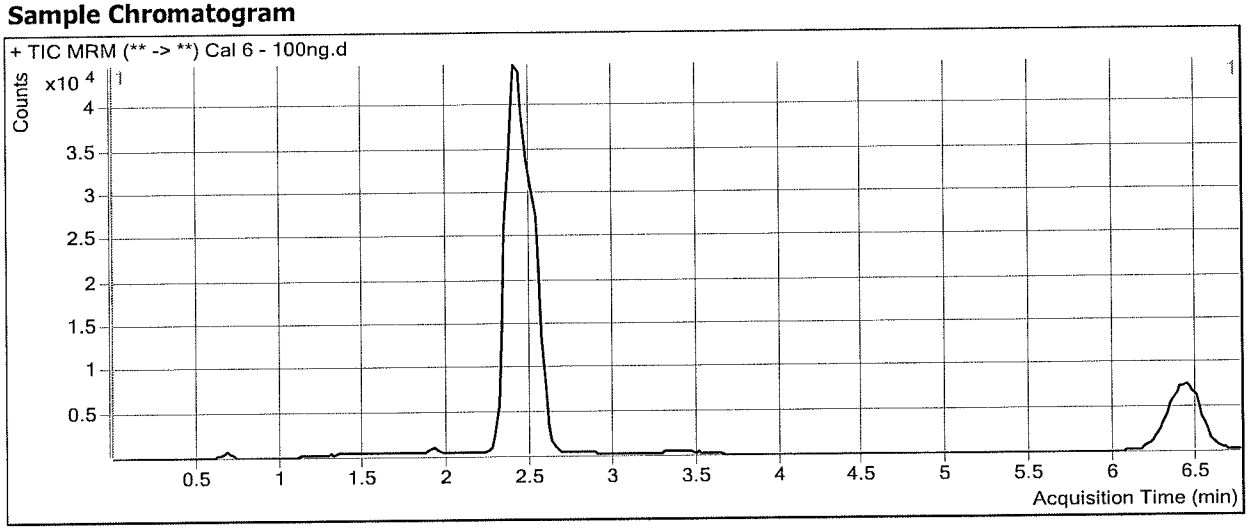
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 15:51	**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	2.416	142376	130710	1.0893	101.4545
THC-COOH	THC-COOH-d9	2.526	108367	53087	2.0413	101.2559
THC	THC-d3	6.433	51933	43736	1.1874	101.8856

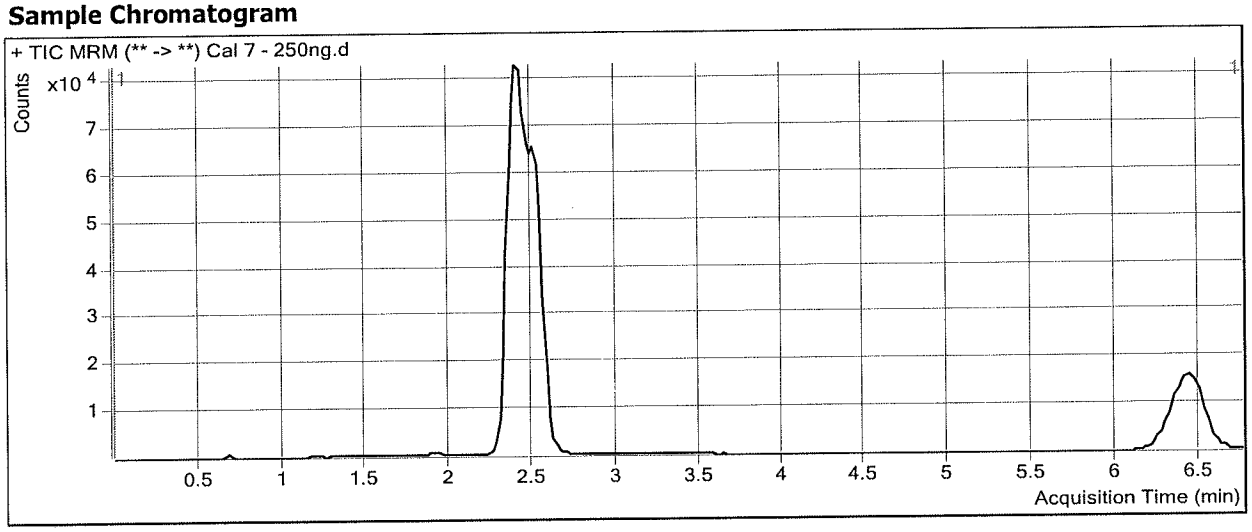
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2018 Data\08082018 cann quant\QuantResults\cann quant.batch.bin		
Analysis Time	8/10/2018 9:19 AM	**Analyst Name**	ISP Tox
Report Time	8/10/2018 9:20 AM	**Reporter Name**	ISP Tox
Last Calib Update	8/10/2018 9:19 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-08-09 16:03	**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	2.416	365797	136438	2.6810	249.6670
THC-COOH	THC-COOH-d9	2.526	264030	52459	5.0331	251.1156
THC	THC-d3	6.453	137528	47693	2.8836	247.6164