reviewed 2/22/18 amn

2/16/2018

Worklist: 2195

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
M2018-0220	1	107771	AM 27 Blood THC Quant by LC
M2018-0229	1	107772	AM 27 Blood THC Quant by LC
P2018-0245	2	107773	AM 27 Blood THC Quant by LC
P2018-0332	1	107774	AM 27 Blood THC Quant by LC
P2018-0372	1	107775	AM 27 Blood THC Quant by LC
P2018-0381	1	107777	AM 27 Blood THC Quant by LC
P2018-0462	1	107783	AM 27 Blood THC Quant by LC

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

Extraction Date: 02/15/18 Analyst: Sarah Pickle

Plate lot#: 0515037 Plate Expiration: 09/28/18

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: 361331-3 **Column**: UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 59740

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: #27** in wells of analytical (standards) plate.
- [x] 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 067105*
- [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: 067104
- [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: 067103*
- [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: 021518 THC Quant SP Batch Name: THC Quant SP 021518
- [x] 2. Make any necessary integration changes, r^2 values ≥0.98 for each analyte.
- [x] 3. Did all QCs pass for each analyte? Y / N Enter QCs into control charting?
- [x] 4. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS:

Curve Range Limited: 5-250ng for THC-COOH

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 THC, 100ug/ml 100ul THC-OH, C-THC in 9790 ul MeOH (Fisher Lot # 172516)
Prepared: 02/07/18 Expires: 02/07/19 By:TS

Drug	Lot	Expiration
C-THC	497429	N/A
THC	FE04231406	4/30/2019
THC-OH	FE01121503	1/31/2020

AM 27 control 100 ul working solution lot (WS020718) in 9900ul blood (Hemostat Lot# 361331-2)
Prepared: 02/07/18 Expires: 02/07/19 By: TS

ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

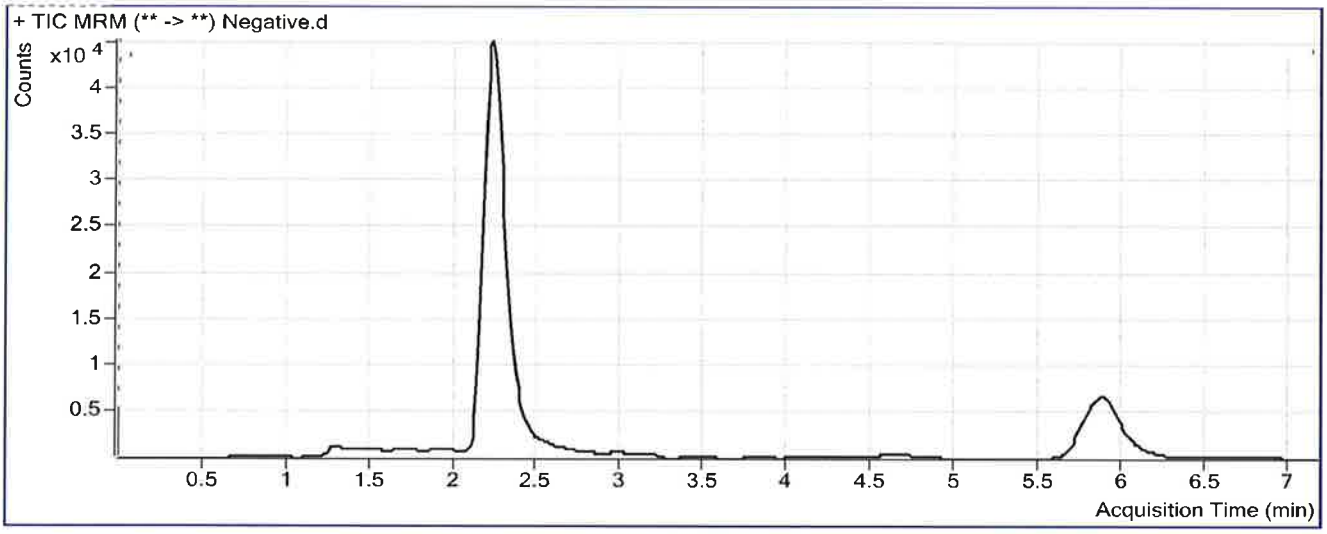
S

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:37 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 13:12	**Data File**	Negative.d
Sample Type	Sample	**Sample Name**	Negative
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-H11	**Sample Info**	
Inj Vol	-1	**Comment**	Hemostat 361331-3

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.025	716	299063	0.0024	0.5453 < 3
THC-COOH	THC-COOH-D9	2.299	3743	87486	0.0428	0.1554 < 10

ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

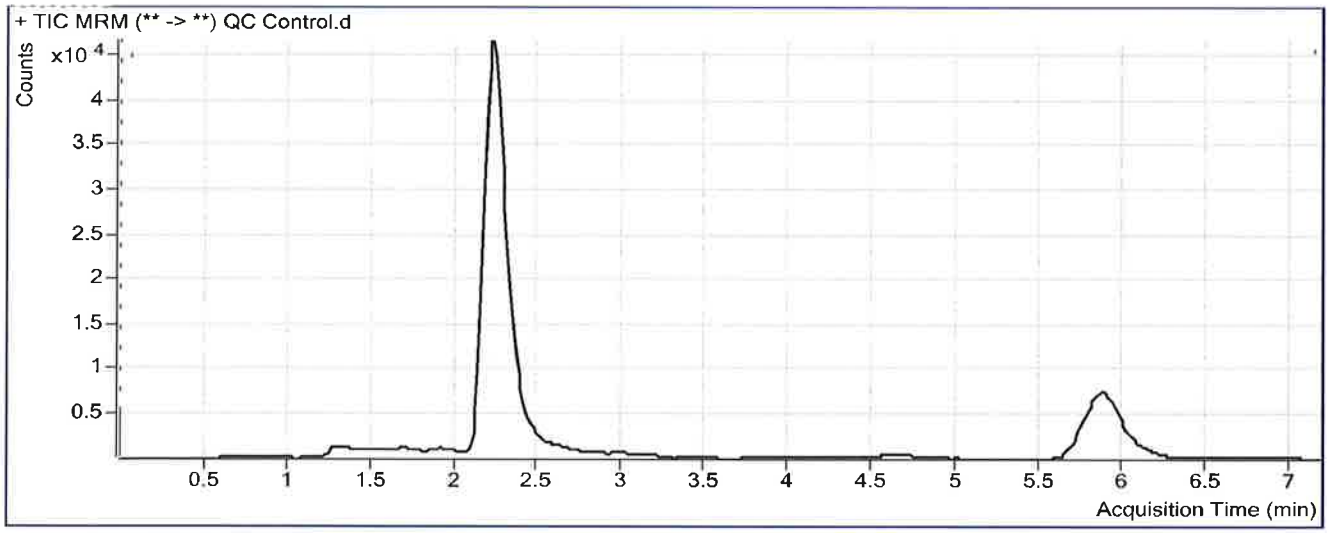
P

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:36 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 12:49	**Data File**	QC Control.d
Sample Type	Sample	**Sample Name**	QC Control
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-A12	**Sample Info**	
Inj Vol	-1	**Comment**	

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.239	33818	273974	0.1234	10.9096
THC-COOH	THC-COOH-D9	2.325	20547	79963	0.2570	10.5171
THC	THC-D3	5.919	13048	94663	0.1378	11.1901

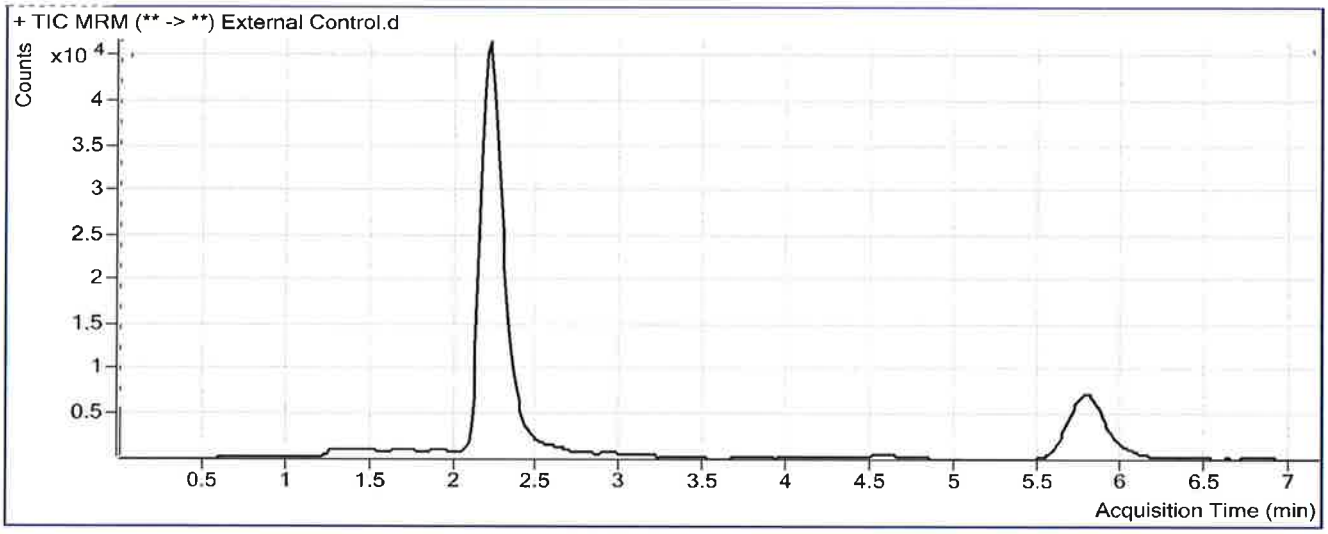
ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:37 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 13:36	**Data File**	External Control.d
Sample Type	Sample	**Sample Name**	External Control
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-G11	**Sample Info**	
Inj Vol	-1	**Comment**	Hemostat 361331-3 + WS 020718

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.212	28700	275771	0.1041	9.2516
THC-COOH	THC-COOH-D9	2.312	19011	77968	0.2438	9.8818
THC	THC-D3	5.812	11417	95101	0.1200	9.6547

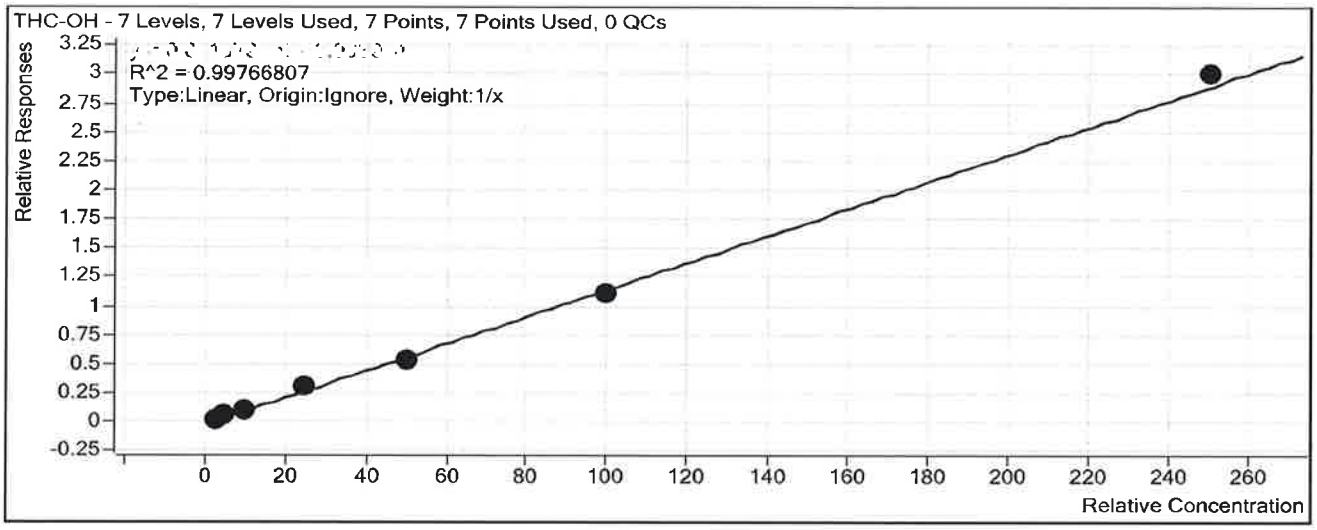
ISP Forensics
Calibration Curve Report

Batch Data Path C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin

Last Calib Update 2/16/2018 10:34 AM **Analyst Name** ISP TOX

Target Compound *THC-OH*

Internal Standard *THC-OH-D3*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☑	3	3.0	99.4
Cal 2	2	☑	5	5.2	104.6
Cal 3	3	☑	10	10.0	100.4
Cal 4	4	☑	25	26.4	105.6
Cal 5	5	☑	50	45.9	91.9
Cal 6	6	☑	100	95.1	95.1
Cal 7	7	☑	250	257.3	102.9

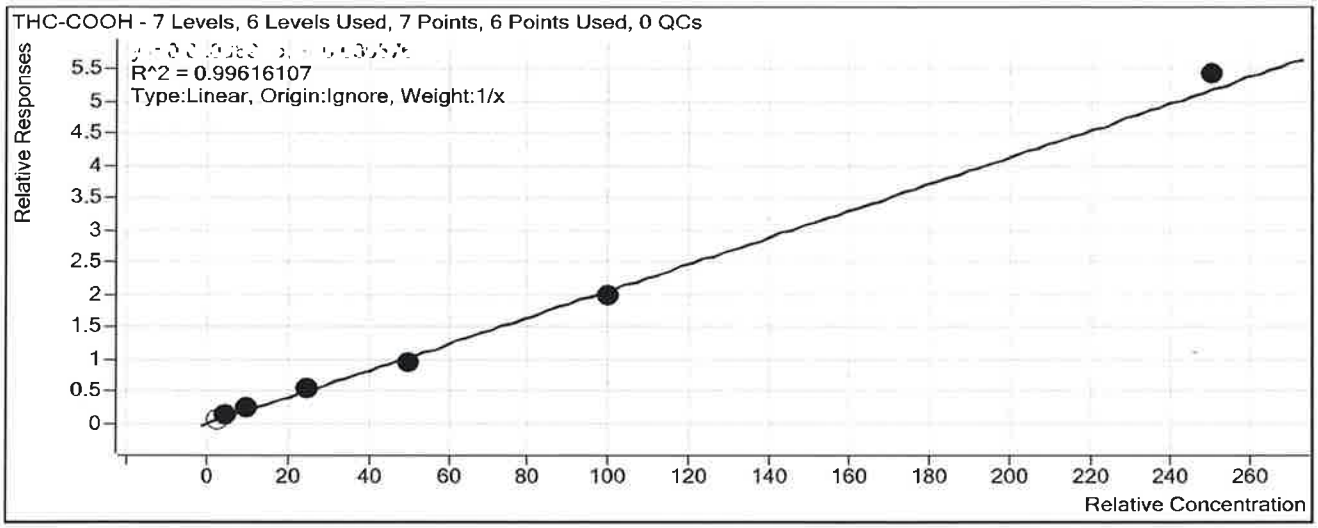
ISP Forensics
Calibration Curve Report

Batch Data Path C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin

Last Calib Update 2/16/2018 10:34 AM **Analyst Name** ISP TOX

Target Compound *THC-COOH*

Internal Standard *THC-COOH-D9*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☐	3	2.5	81.8
Cal 2	2	☑	5	5.6	112.2
Cal 3	3	☑	10	10.0	99.7
Cal 4	4	☑	25	25.1	100.2
Cal 5	5	☑	50	44.5	89.0
Cal 6	6	☑	100	95.0	95.0
Cal 7	7	☑	250	259.9	104.0

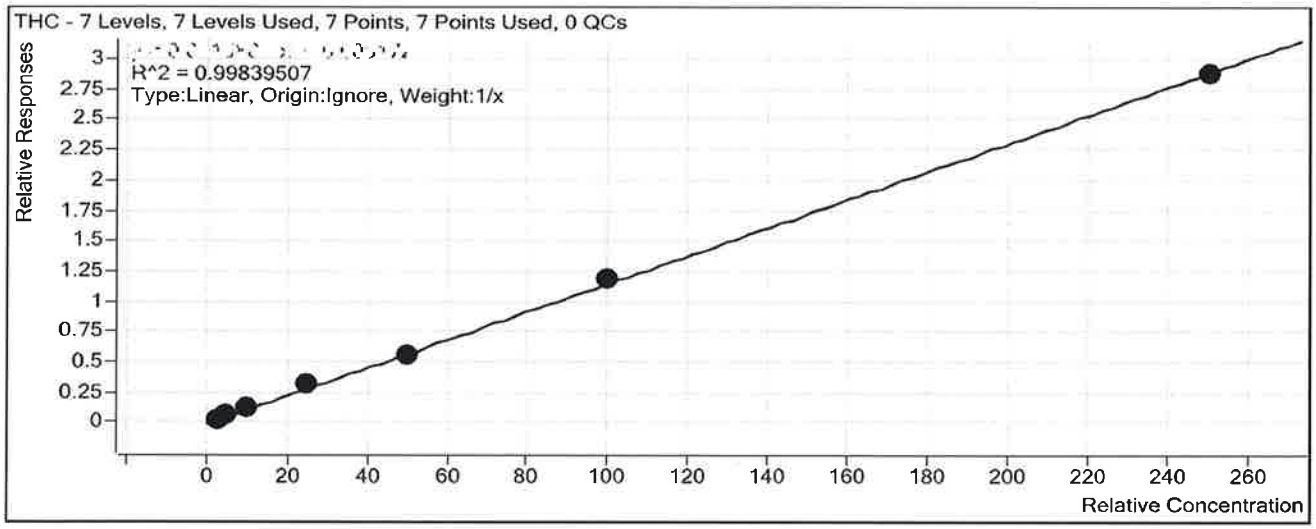
ISP Forensics
Calibration Curve Report

Batch Data Path C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin

Last Calib Update 2/16/2018 10:34 AM **Analyst Name** ISP TOX

Target Compound *THC*

Internal Standard *THC-D3*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	☑	3	2.5	82.2
Cal 2	2	☑	5	5.2	104.7
Cal 3	3	☑	10	10.5	105.1
Cal 4	4	☑	25	27.9	111.6
Cal 5	5	☑	50	48.0	95.9
Cal 6	6	☑	100	101.5	101.5
Cal 7	7	☑	250	247.4	99.0

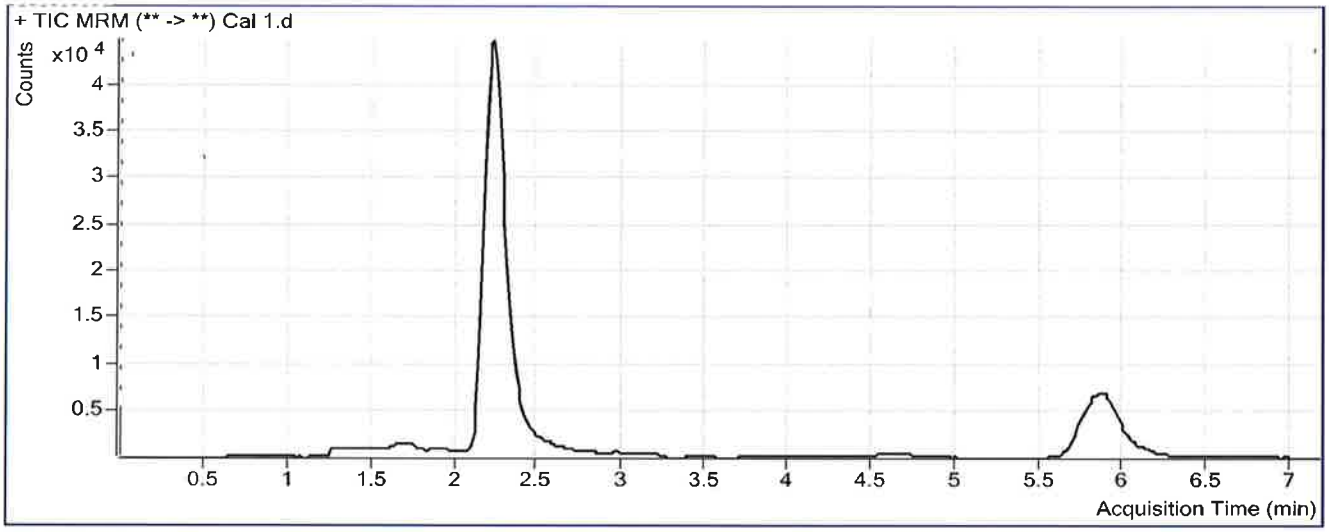
ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:36 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 11:14	**Data File**	Cal 1.d
Sample Type	Calibration	**Sample Name**	Cal 1
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-H12	**Sample Info**	
Inj Vol	-1	**Comment**	

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.225	8838	286513	0.0308	2.9816
THC-COOH	THC-COOH-D9	2.325	7980	88380	0.0903	2.4538
THC	THC-D3	5.892	3815	103823	0.0367	2.4660

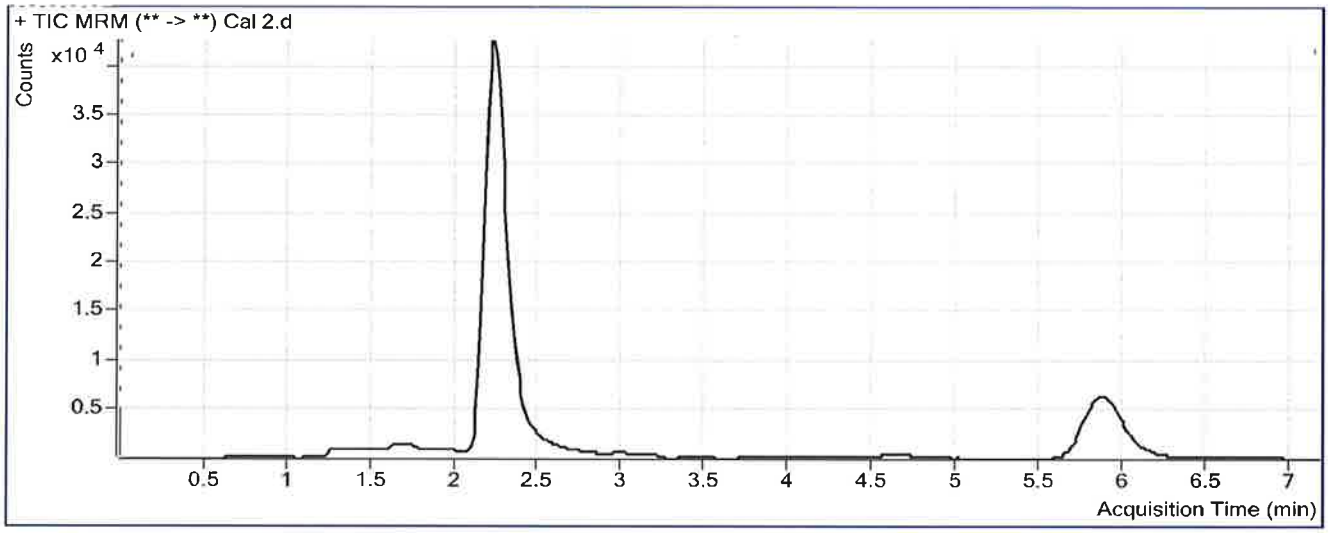
ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:36 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 11:26	**Data File**	Cal 2.d
Sample Type	Calibration	**Sample Name**	Cal 2
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-G12	**Sample Info**	
Inj Vol	-1	**Comment**	

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.239	15325	268266	0.0571	5.2319
THC-COOH	THC-COOH-D9	2.325	12285	79014	0.1555	5.6078
THC	THC-D3	5.932	6275	91148	0.0688	5.2356

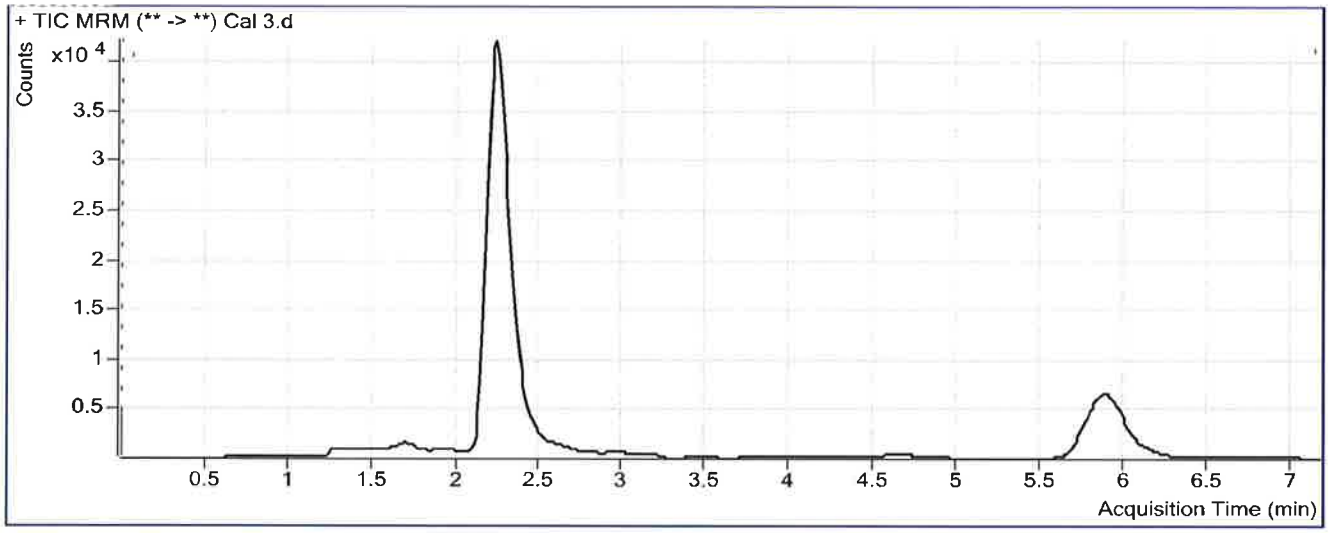
ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:36 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 11:38	**Data File**	Cal 3.d
Sample Type	Calibration	**Sample Name**	Cal 3
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-F12	**Sample Info**	
Inj Vol	-1	**Comment**	

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.239	28403	250651	0.1133	10.0433
THC-COOH	THC-COOH-D9	2.325	18307	74525	0.2457	9.9701
THC	THC-D3	5.892	11095	85384	0.1299	10.5089

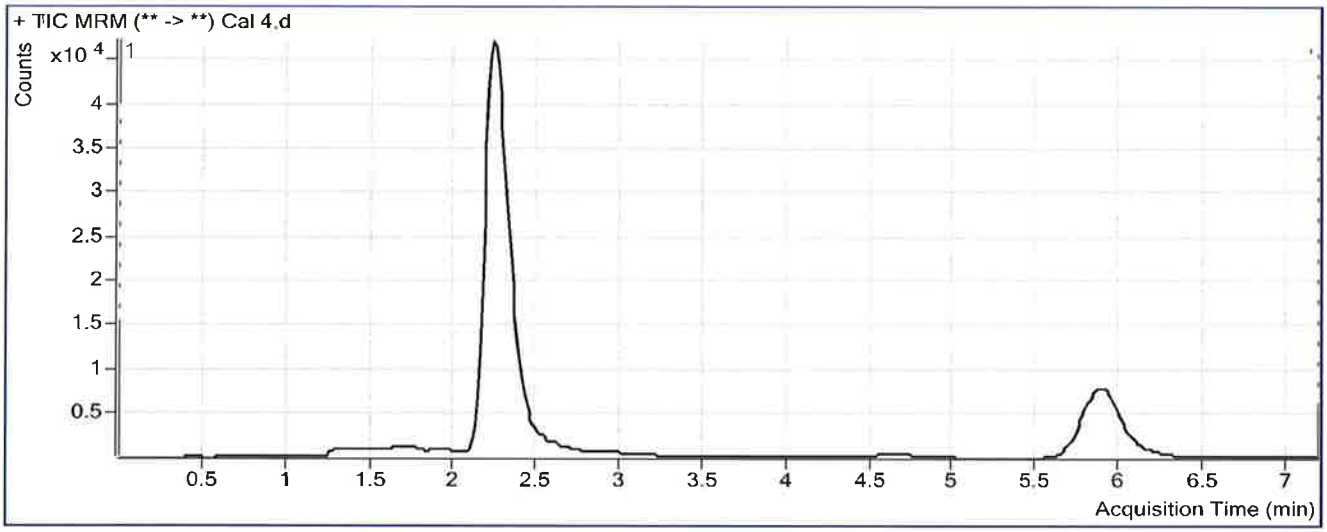
ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:36 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 11:50	**Data File**	Cal 4.d
Sample Type	Calibration	**Sample Name**	Cal 4
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-E12	**Sample Info**	
Inj Vol	-1	**Comment**	

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.239	72577	238386	0.3045	26.4095
THC-COOH	THC-COOH-D9	2.339	39495	70854	0.5574	25.0536
THC	THC-D3	5.905	26798	80829	0.3315	27.9061

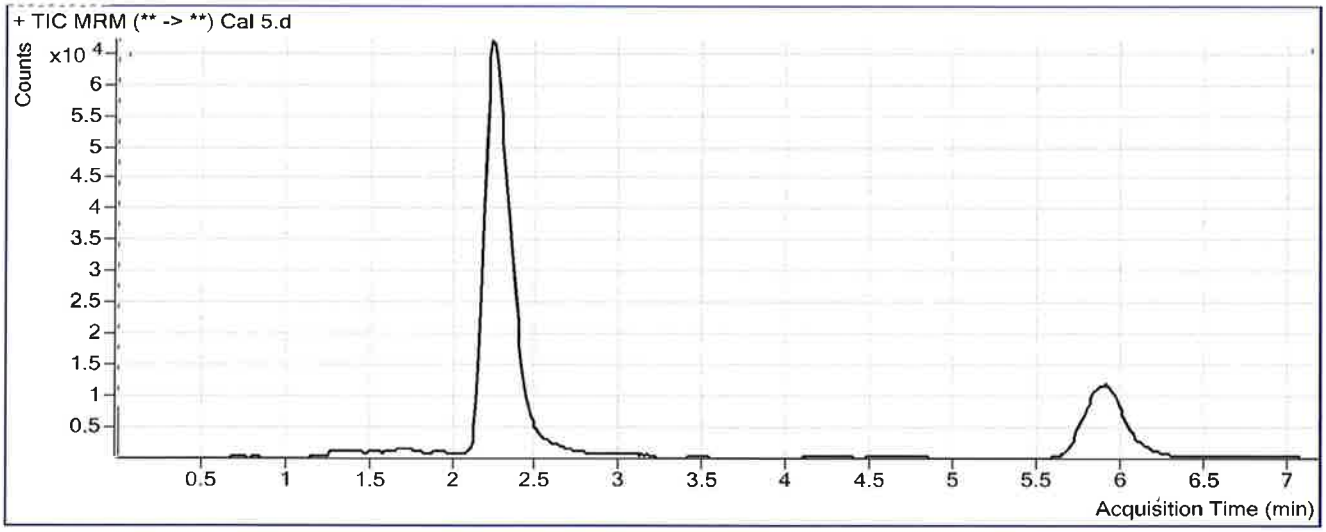
ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:36 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 12:01	**Data File**	Cal 5.d
Sample Type	Calibration	**Sample Name**	Cal 5
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-D12	**Sample Info**	
Inj Vol	-1	**Comment**	

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.239	154373	289820	0.5327	45.9496
THC-COOH	THC-COOH-D9	2.339	80338	83735	0.9594	44.5039
THC	THC-D3	5.919	55805	98960	0.5639	47.9603

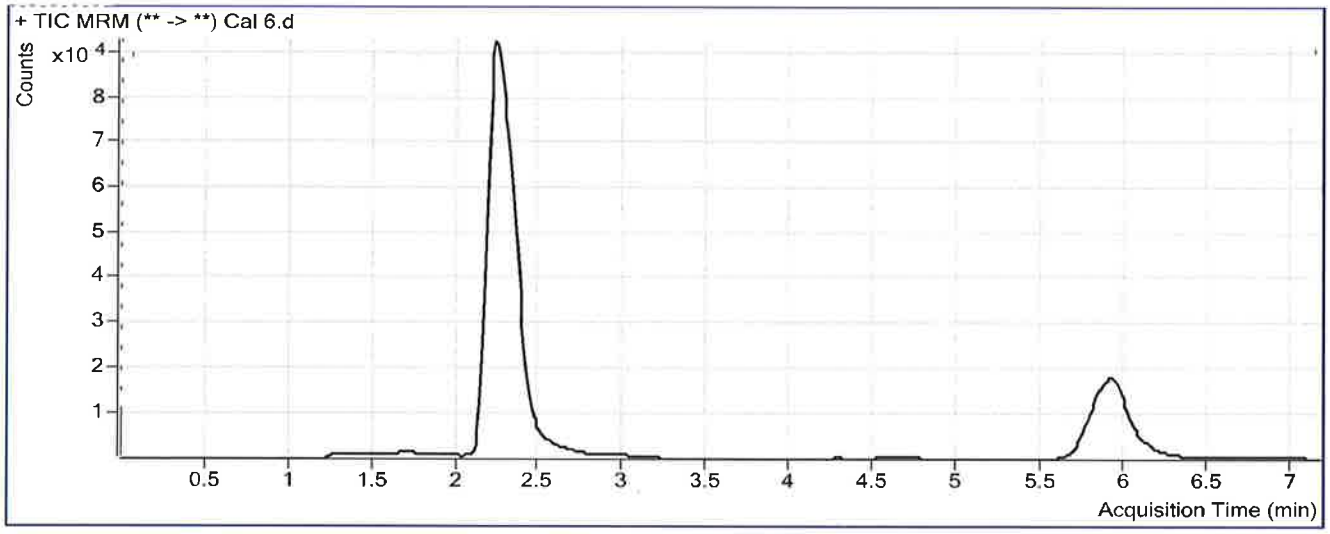
ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:36 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 12:13	**Data File**	Cal 6.d
Sample Type	Calibration	**Sample Name**	Cal 6
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-C12	**Sample Info**	
Inj Vol	-1	**Comment**	

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.239	323069	291983	1.1065	95.0835
THC-COOH	THC-COOH-D9	2.339	159529	79672	2.0023	94.9598
THC	THC-D3	5.919	114025	96305	1.1840	101.4732

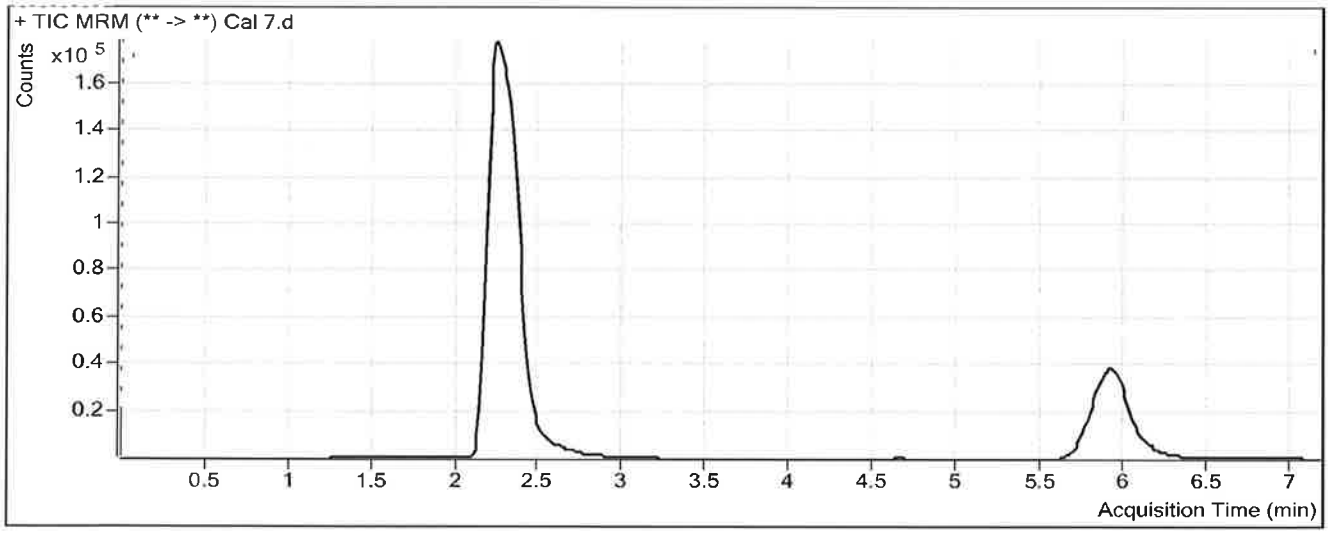
ISP FORENSICS - Pocatello Instrument # 59740
Cannabinoids Analysis Report

Batch Data Path	C:\MassHunter\Data\2018\THC Quant\021518 THC Quant SP\QuantResults\THC Quant SP 021518.batch.bin		
Analysis Time	2/16/2018 10:34 AM	**Analyst Name**	ISPUser
Report Time	2/16/2018 10:36 AM	**Reporter Name**	ISPUser
Last Calib Update	2/16/2018 10:34 AM	**Batch State**	Processed

Analysis Info

Acq Time	2018-02-15 12:25	**Data File**	Cal 7.d
Sample Type	Calibration	**Sample Name**	Cal 7
Dilution	1	**Acq Method**	THC Quant 051517 workingmm.m
Position	P1-B12	**Sample Info**	
Inj Vol	-1	**Comment**	

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-D3	2.239	884341	294690	3.0009	257.3005
THC-COOH	THC-COOH-D9	2.339	433334	80075	5.4116	259.9049
THC	THC-D3	5.919	313326	108964	2.8755	247.4499