reviewed 6/7/17

5/31/2017

Worklist: 1742

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-0745	1	85915	AM 27 Blood THC Quant by LC
C2017-0855	1	85916	AM 27 Blood THC Quant by LC
C2017-0856	1	85917	AM 27 Blood THC Quant by LC
C2017-0894	1	85918	AM 27 Blood THC Quant by LC
C2017-0895	1	85919	AM 27 Blood THC Quant by LC
C2017-0919	1	85920	AM 27 Blood THC Quant by LC
C2017-0945	1	85921	AM 27 Blood THC Quant by LC
C2017-0946	1	85922	AM 27 Blood THC Quant by LC
M2017-2187	2	85923	AM 27 Blood THC Quant by LC
M2017-2344	5	85924	AM 27 Blood THC Quant by LC

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

Extraction Date: 5-30-17 **Analyst:** Anne Nord

PRE-ANALYTIC 0499102 1-28-2018

Plate Lot# Custom ~~0490364~~ A Plate Exp. ~~9-21-2017~~ A **External QC** Lot 61317, exp 6-13-17

✓ 1. Ensure all solutions are within expiration date.

- Mobile Phase A: ***0.1% Formic Acid in LCMS Water***
- Mobile Phase B: ***0.1% Formic Acid in LCMS Acetonitrile***
- ***LCMS Methanol***
- **Blank/Negative Blood: Lot *321632-1***
- ***0.1% Formic Acid in water***
- ***MTBE***
- ***Hexane***

Column: ***UCT Selectra DA 100 x 2.1 mm 3um***

✓ 2. Check levels of mobile phases and needle wash and refill as necessary. Ensure waste is not full.

✓ 3. Purge Pump and Load appropriate Acq. Method, allow system to equilibrate for approx. 30 min.

✓ 4. Create worklist. Data path name: 5-30-17 THC Quant

ANALYTIC

✓ 1. Remove standards plate, blood, and samples from cold storage. Allow to reach room temperature.

✓ 2. Add ***1000 µL blood*** to wells of analytical (standards) plate. Mix via aspirate and dispense. Place cover on Plate
Blank blood for locations containing standards/QCs and internal standards
Sample blood for locations containing only internal standards

✓ 3. Place on shaking incubator at ambient temp., **900rpm for 15 minutes**. *Shaker ID 66759*

✓ 4. Pipette ***500µL 0.1% formic acid*** to all wells of standards 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). Wait 5 min. ***(Load blood samples at 85- 100 PSI- Selector to Right)***

✓ 8. Add ***2.25mL MTBE*** and allow to flow under gravity for 5 minutes. ***(add in 3 increments of 750uL)***

✓ 9. Apply positive pressure for approx. 15 seconds ***(10-15 PSI- Selector to left -.***

✓ 10. Add ***2.25mL Hexane*** and allow to flow under gravity for 5 minutes.. ***(add in 3 increments of 750uL)***

✓ 11. Apply positive pressure for approx. 15 seconds. ***(10-15 PSI Selector to the left)***

✓ 12. Remove collection plate containing eluate.

✓ 13. Place collection plate on SPE Dry and evaporate to dryness at approx. 35°C. *SPE Dry ID 66819*

✓ 14. Reconstitute in ***100 µL MeOH*** and heat seal plate with foil. Place in autosampler and run worklist.

POST-ANALYTIC

✓ 1. Open quantitation software and create a new quantitation batch.
Batch name: 53017 cann quant

✓ 2. Make any necessary integration changes. Limit curves based on validated linear ranges (3-50ng/mL).

✓ 3. Were all appropriate standards used in the curve for each analyte? Y / N
Are r^2 values ≥0.98 for each analyte? Y / N

✓ 4. Did all QCs pass for each analyte? Y / N Were QCs entered into QC charting? Y / N

✓ 5. Central File Packet to include: ✓ LIMS Worklist: ✓ Method Checklist ✓ Calibration and Control Reports

COMMENTS

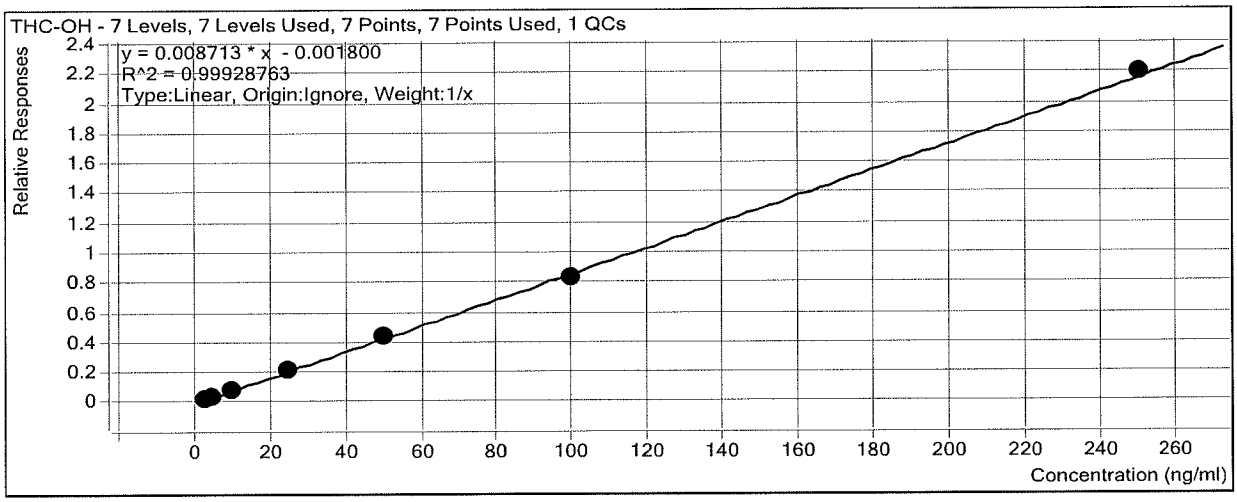
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin

Last Calib Update 6/1/2017 4:26 PM **Analyst Name** ISP TOX

Target Compound *THC-OH*

Internal Standard *THC-OH-d3*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1- 3ng/mL	1	☑	3	3.1	104.1
cal 2- 5ng/mL	2	☑	5	4.9	98.2
cal 3 - 10ng/mL	3	☑	10	9.7	96.6
QC 10	3	☑	10	10.0	99.8
cal 4 - 25ng/mL	4	☑	25	25.4	101.4
cal 5 - 50ng/mL	5	☑	50	51.4	102.7
cal 6 - 100ng/mL	6	☑	100	95.8	95.8
cal 7 - 250ng/mL	7	☑	250	252.8	101.1

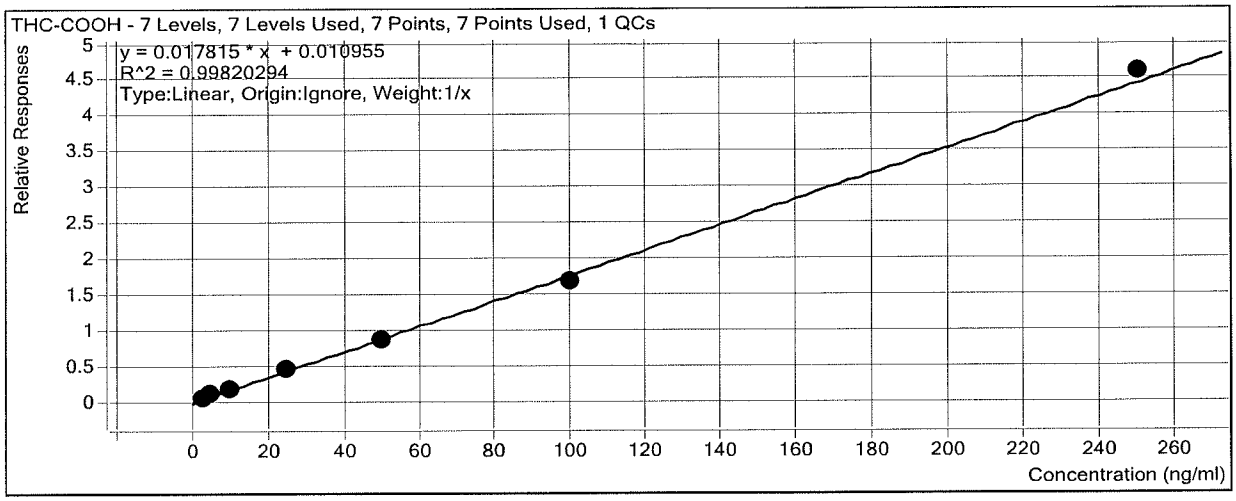
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin

Last Calib Update 6/1/2017 4:26 PM **Analyst Name** ISP TOX

Target Compound *THC-COOH*

Internal Standard *THC-COOH-d9*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1- 3ng/mL	1	☑	3	3.0	101.2
cal 2- 5ng/mL	2	☑	5	5.4	108.6
cal 3 - 10ng/mL	3	☑	10	9.7	97.1
QC 10	3	☑	10	10.1	101.0
cal 4 - 25ng/mL	4	☑	25	24.5	98.2
cal 5 - 50ng/mL	5	☑	50	49.1	98.1
cal 6 - 100ng/mL	6	☑	100	93.8	93.8
cal 7 - 250ng/mL	7	☑	250	257.4	103.0

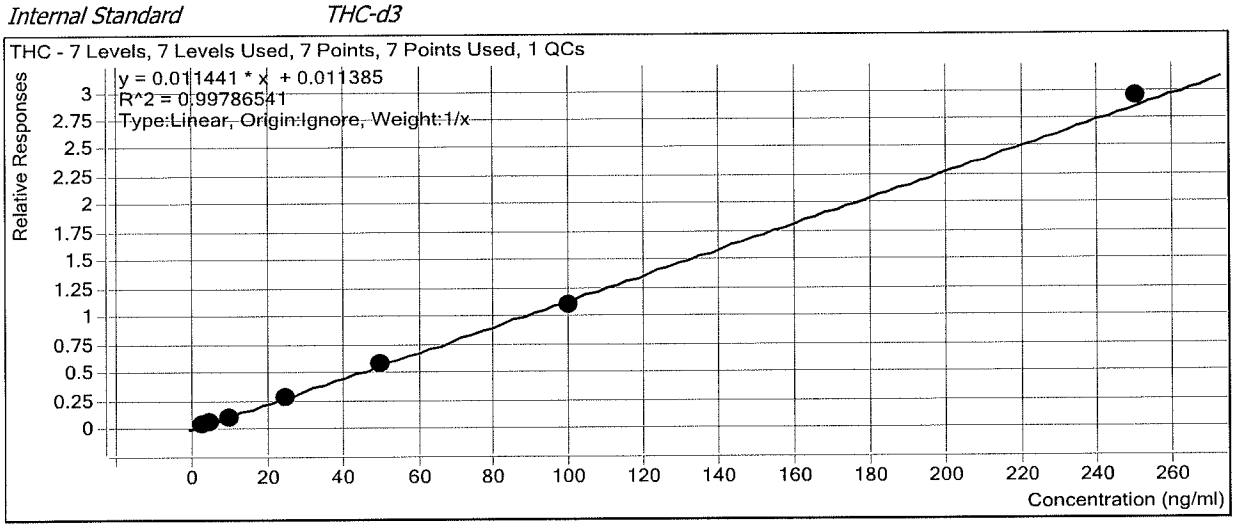
ISP Forensics
Calibration Curve Report

Batch Data Path D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin

Last Calib Update 6/1/2017 4:26 PM **Analyst Name** ISP TOX

Target Compound *THC*

Internal Standard *THC-d3*



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1- 3ng/mL	1	☑	3	3.8	126.7
cal 2- 5ng/mL	2	☑	5	4.4	87.7
cal 3 - 10ng/mL	3	☑	10	9.0	90.4
QC 10	3	☑	10	9.3	92.7
cal 4 - 25ng/mL	4	☑	25	24.3	97.2
cal 5 - 50ng/mL	5	☑	50	50.3	100.5
cal 6 - 100ng/mL	6	☑	100	95.1	95.1
cal 7 - 250ng/mL	7	☑	250	256.2	102.5

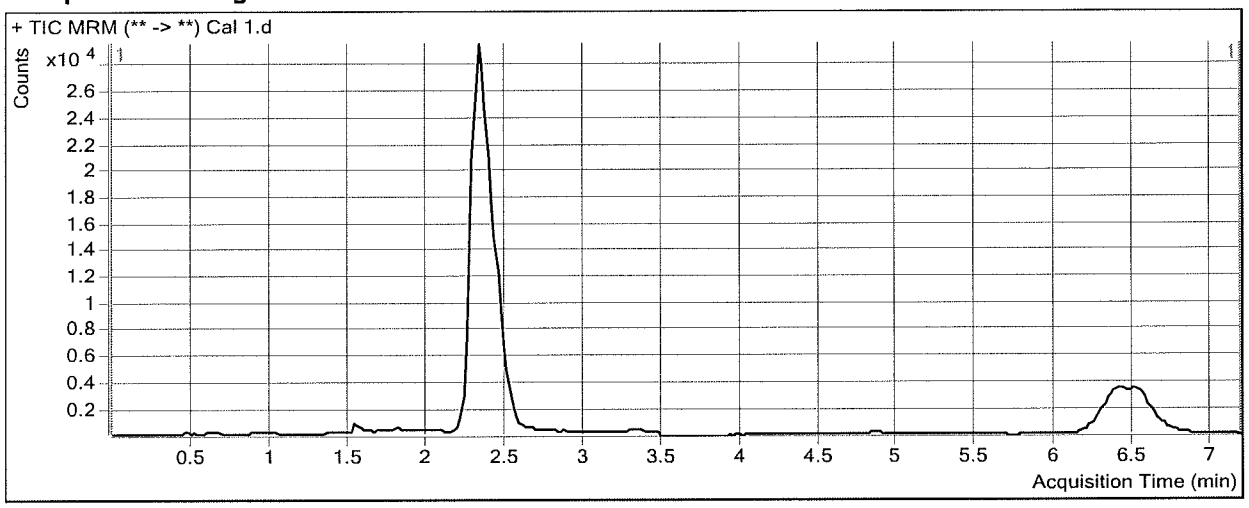
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 11:59	**Data File**	Cal 1.d
Sample Type	Calibration	**Sample Name**	Cal 1- 3ng/mL
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-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	2.356	5409	212945	0.0254	3.1219
THC-COOH	THC-COOH-d9	2.486	4268	65619	0.0650	3.0360
THC	THC-d3	6.513	2648	48250	0.0549	3.8012

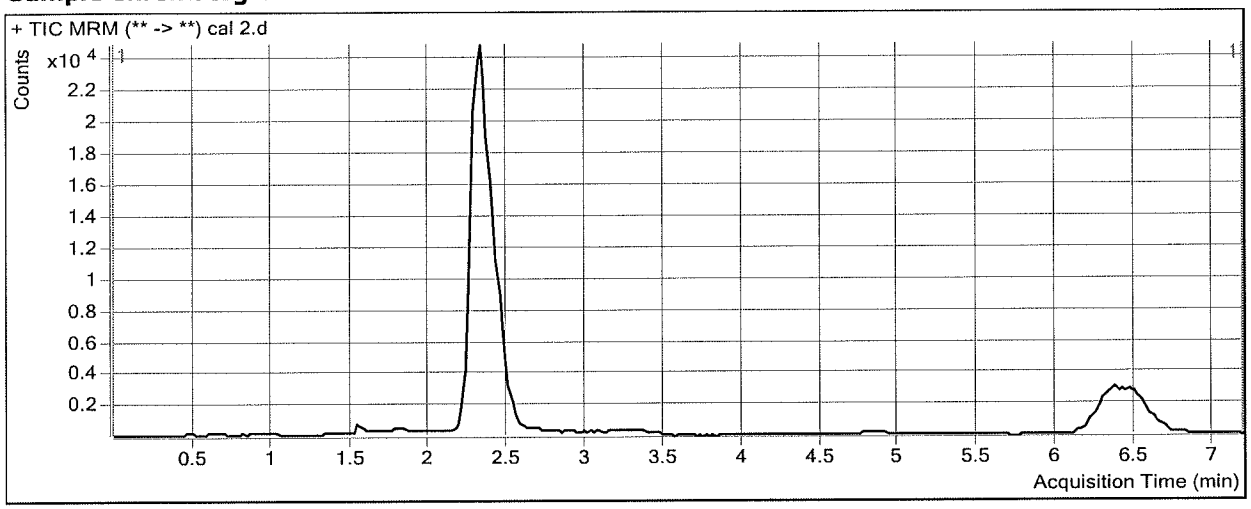
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 12:11	**Data File**	cal 2.d
Sample Type	Calibration	**Sample Name**	cal 2- 5ng/mL
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-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	2.356	7233	176447	0.0410	4.9114
THC-COOH	THC-COOH-d9	2.466	5910	54887	0.1077	5.4295
THC	THC-d3	6.453	3634	59042	0.0615	4.3841

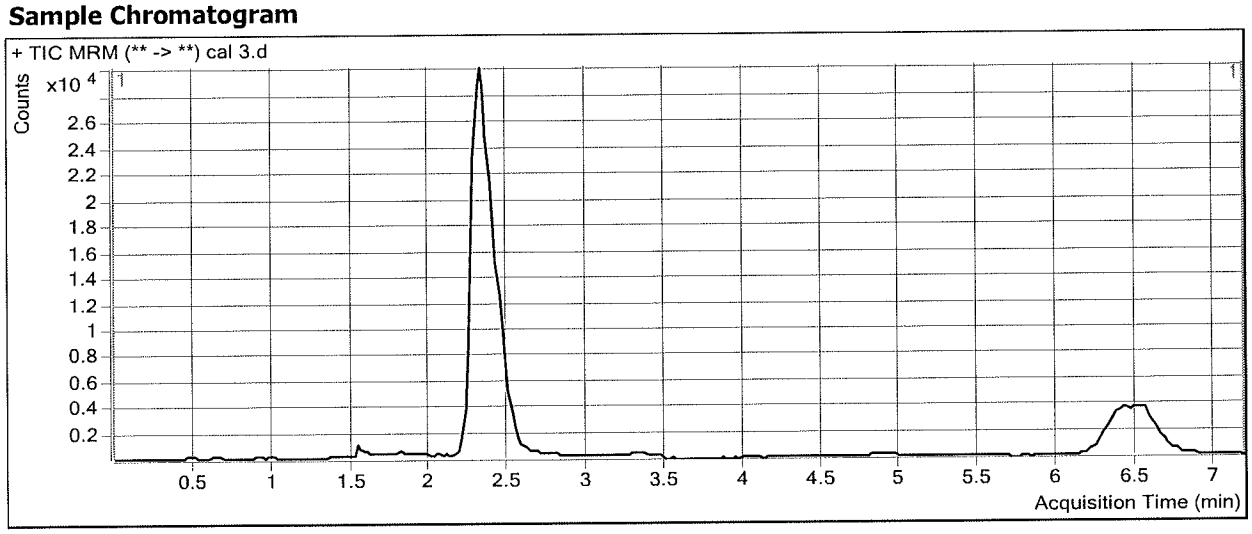
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 12:23	**Data File**	cal 3.d
Sample Type	Calibration	**Sample Name**	cal 3 - 10ng/mL
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-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	2.336	17253	209377	0.0824	9.6644
THC-COOH	THC-COOH-d9	2.466	11606	63078	0.1840	9.7134
THC	THC-d3	6.513	8267	72009	0.1148	9.0399

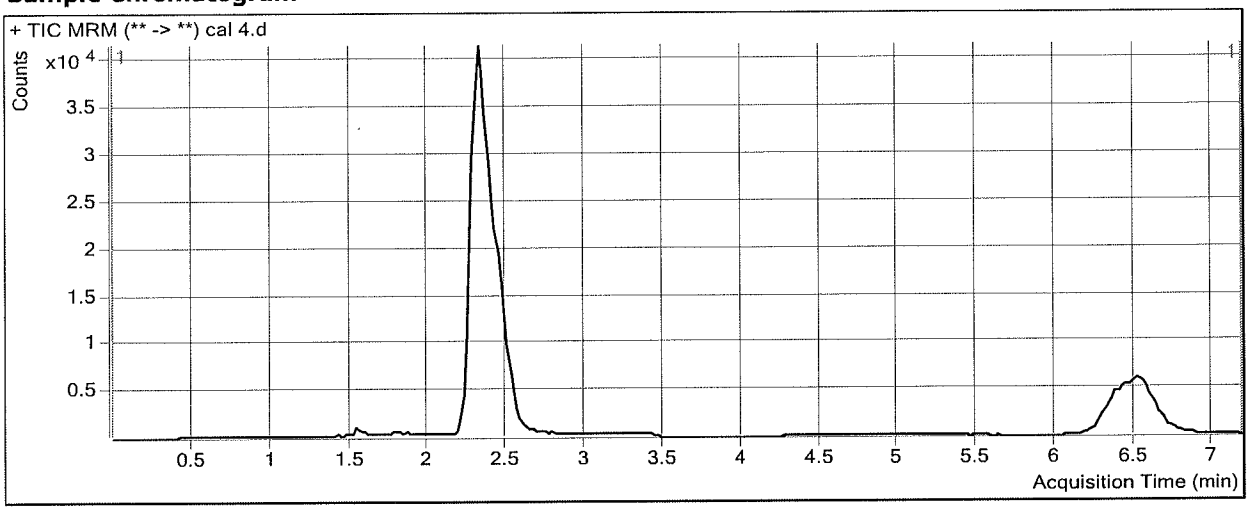
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 12:35	**Data File**	cal 4.d
Sample Type	Calibration	**Sample Name**	cal 4 - 25ng/mL
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-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	2.356	54493	248637	0.2192	25.3617
THC-COOH	THC-COOH-d9	2.466	33540	74827	0.4482	24.5456
THC	THC-d3	6.533	25544	88289	0.2893	24.2928

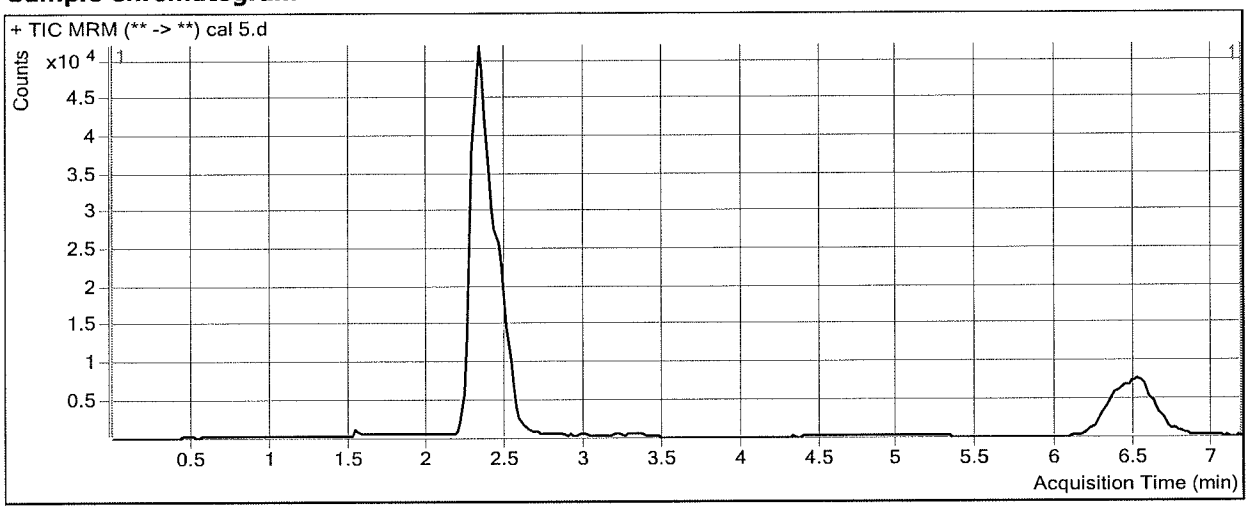
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 12:46	**Data File**	cal 5.d
Sample Type	Calibration	**Sample Name**	cal 5 - 50ng/mL
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-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	2.336	116047	260430	0.4456	51.3500
THC-COOH	THC-COOH-d9	2.466	65877	74445	0.8849	49.0567
THC	THC-d3	6.513	51315	87511	0.5864	50.2573

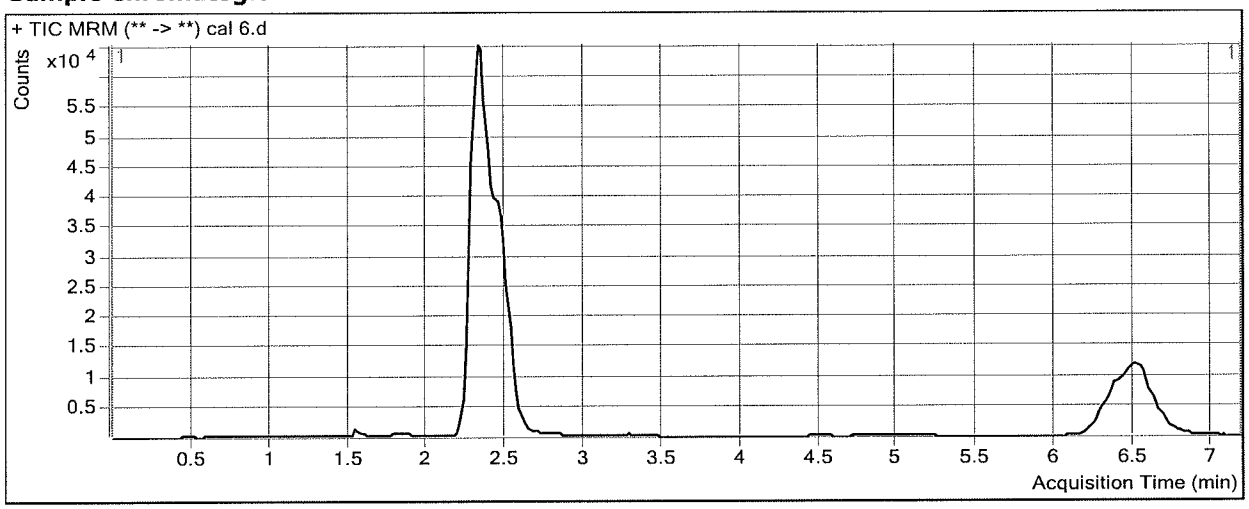
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 12:58	**Data File**	cal 6.d
Sample Type	Calibration	**Sample Name**	cal 6 - 100ng/mL
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-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	2.356	216441	259856	0.8329	95.8057
THC-COOH	THC-COOH-d9	2.466	130534	77591	1.6823	93.8182
THC	THC-d3	6.533	104740	95308	1.0990	95.0598

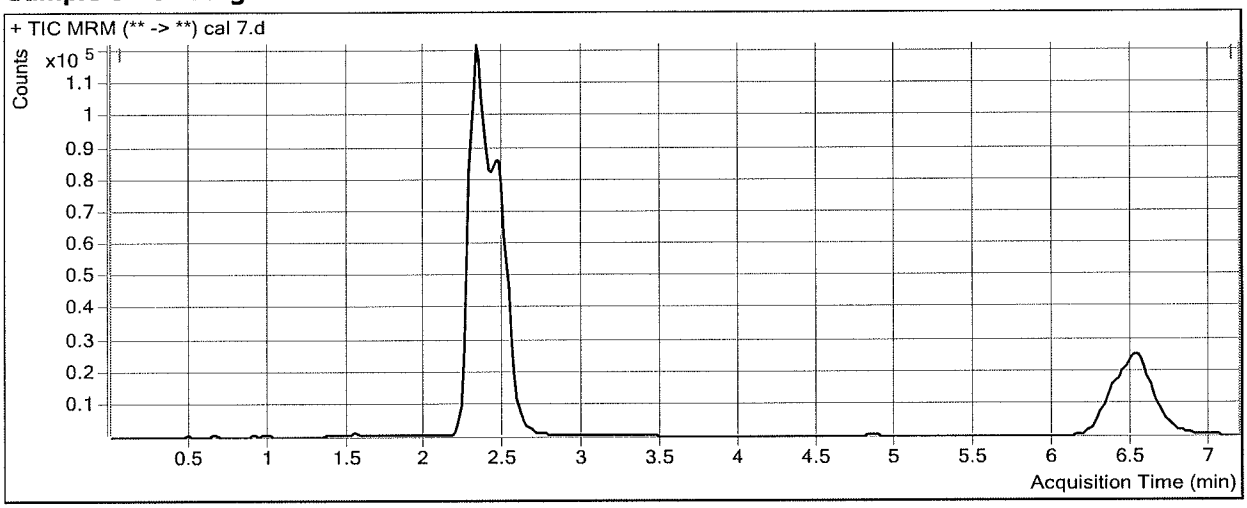
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 13:10	**Data File**	cal 7.d
Sample Type	Calibration	**Sample Name**	cal 7 - 250ng/mL
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-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	2.336	595459	270584	2.2006	252.7850
THC-COOH	THC-COOH-d9	2.466	359606	78233	4.5966	257.4005
THC	THC-d3	6.533	289720	98472	2.9422	256.1649

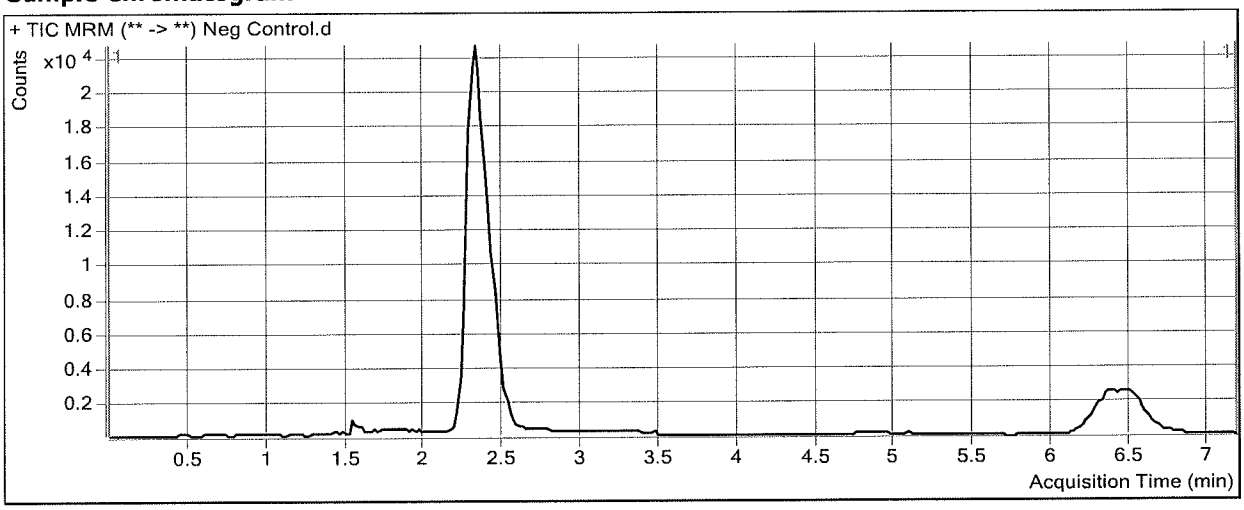
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 13:34	**Data File**	Neg Control.d
Sample Type	Sample	**Sample Name**	Neg Control
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-a2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation

Sample Chromatogram



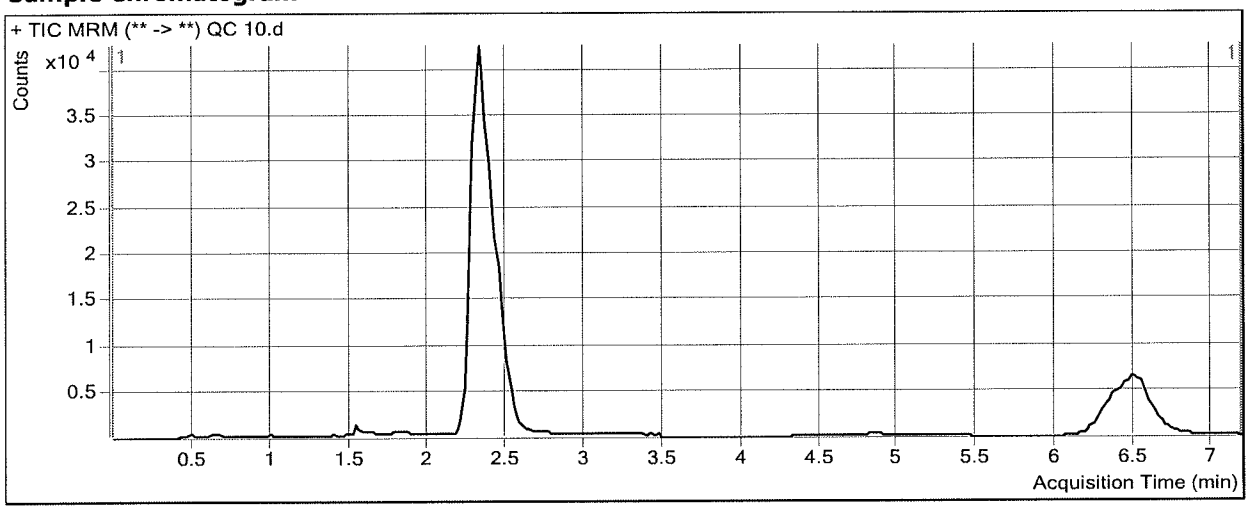
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 13:46	**Data File**	QC 10.d
Sample Type	QC	**Sample Name**	QC 10
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	P2-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	2.336	24759	290892	0.0851	9.9756
THC-COOH	THC-COOH-d9	2.466	17325	90798	0.1908	10.0956
THC	THC-d3	6.493	12817	109125	0.1175	9.2709

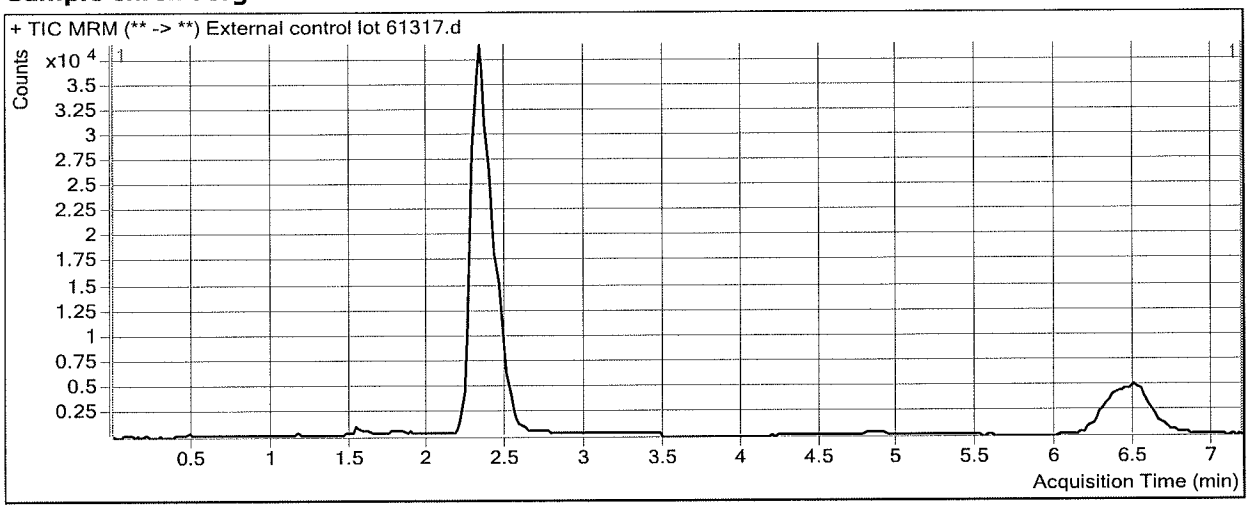
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	D:\2017 Data\5-30-17 THC Quant\QuantResults\53017 cann quant.batch.bin		
Analysis Time	6/1/2017 4:26 PM	**Analyst Name**	ISP Tox
Report Time	6/1/2017 4:28 PM	**Reporter Name**	ISP Tox
Last Calib Update	6/1/2017 4:26 PM	**Batch State**	Processed

Analysis Info

Acq Time	2017-05-30 13:58	**Data File**	External control lot 61317.d
Sample Type	Sample	**Sample Name**	External control lot 61317
Dilution	1	**Acq Method**	Quant THC 2017.m
Position	p2b2	**Sample Info**	
Inj Vol	-1	**Comment**	AM 27 cannabinoid confirmation 10 ng

Sample Chromatogram



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.336	26734	258922	0.1033	12.0572
THC-COOH	THC-COOH-d9	2.466	11204	77298	0.1449	7.5213
THC	THC-d3	6.513	10109	88868	0.1138	8.9477