

reviewed 12/8/17

12/7/2017

*BWylee*

**Worklist: 2067**

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>
C2017-2307	1	101944	AM 27 Blood THC Quant by LC
C2017-2361	1	101945	AM 27 Blood THC Quant by LC
C2017-2411	1	101946	AM 27 Blood THC Quant by LC
C2017-2435	1	101947	AM 27 Blood THC Quant by LC
M2017-5023	1	101948	AM 27 Blood THC Quant by LC
M2017-5037	2	101949	AM 27 Blood THC Quant by LC



*[Handwritten signature]*

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

Extraction Date: 12-5-17

Analyst: Anne Nord

Plate lot#: 0515037

Plate Expiration: 9/28/18

**Mobile phase A:** 0.1% Formic Acid in LCMS Water  
MTBE

**Mobile phase B:** 0.1% Formic acid in Acetonitrile  
Hexane

**Blank Blood Lot:** 17J20718

**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: 2609543** 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: 120517 cann quant Batch Name: 120517 cann quant
- 2. Make any necessary integration changes,  $r^2$  values  $\geq 0.98$  for each analyte.
- 3. Did all QCs pass for each analyte? Y/N Enter QCs into control charting?
- 4. 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 9880 ul meOH lot (Fisher 168427)  
Ppd 8/17/17 Exp: 2/17/18 lot 21718 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 100 ul working solution lot (21717) in 9990 ul blood lot (321632)  
ppd 8/17/17 Exp 2/17/18 by AMN Concentration 10 ng/ml each



# ISP FORENSICS - Cd'A Instrument # 62340

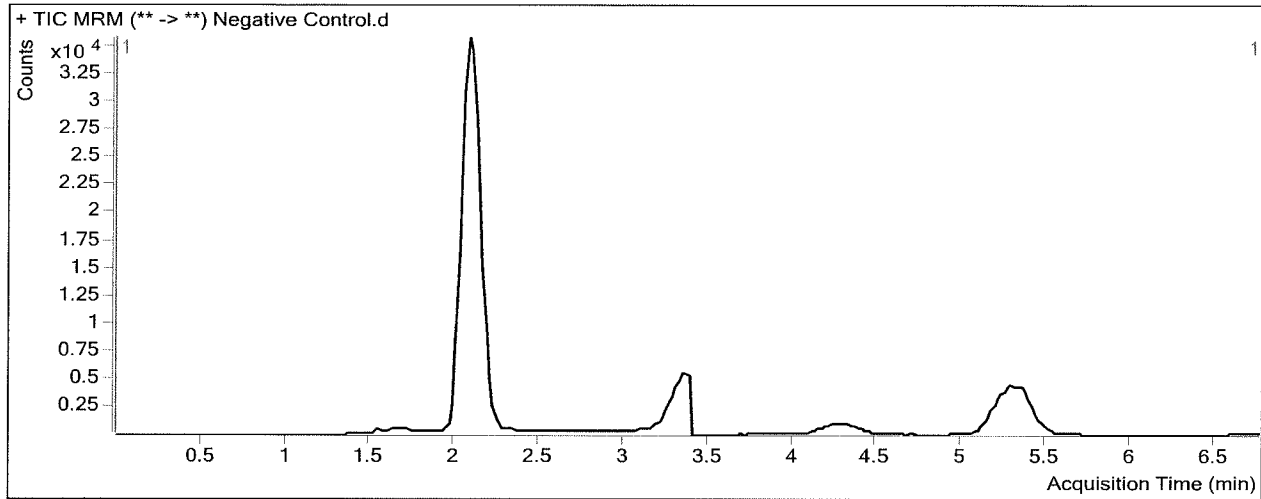
## Cannabinoids Analysis Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.ba  
**Analysis Time** 12/6/2017 8:53 AM **Analyst Name** anord  
**Report Time** 12/6/2017 9:06 AM **Reporter Name** ISP Tox  
**Last Calib Update** 12/6/2017 8:53 AM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-12-05 16:53 **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



# ISP FORENSICS - Cd'A Instrument # 62340

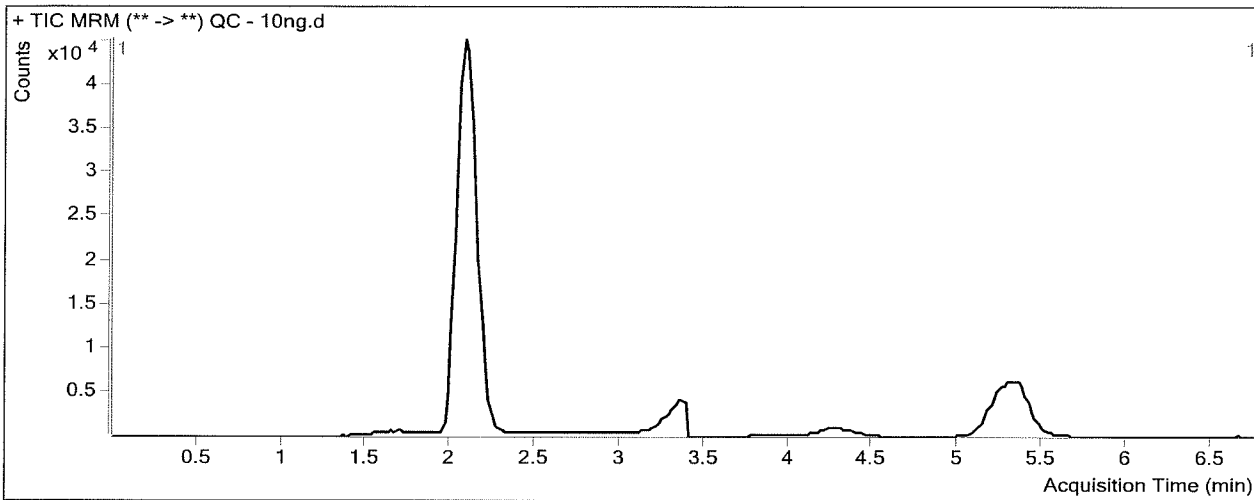
## Cannabinoids Analysis Report

<b>Batch Data Path</b>	C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin		
<b>Analysis Time</b>	12/6/2017 8:53 AM	<b>Analyst Name</b>	anord
<b>Report Time</b>	12/6/2017 9:06 AM	<b>Reporter Name</b>	ISP Tox
<b>Last Calib Update</b>	12/6/2017 8:53 AM	<b>Batch State</b>	Processed

### Analysis Info

<b>Acq Time</b>	2017-12-05 17:05	<b>Data File</b>	QC - 10ng.d
<b>Sample Type</b>	QC	<b>Sample Name</b>	QC - 10ng
<b>Dilution</b>	1	<b>Acq Method</b>	AM 27 Quant THC 7-2017.m
<b>Position</b>	P1-H1	<b>Sample Info</b>	
<b>Inj Vol</b>	-1	<b>Comment</b>	AM 27 Cannabinoid Confirmation

### Sample Chromatogram



### Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.095	17480	238427	0.0733	8.2964
THC-COOH	THC-COOH-d9	2.185	12153	91308	0.1331	7.8820
THC	THC-d3	5.332	7613	87698	0.0868	8.5464

# ISP FORENSICS - Cd'A Instrument # 62340

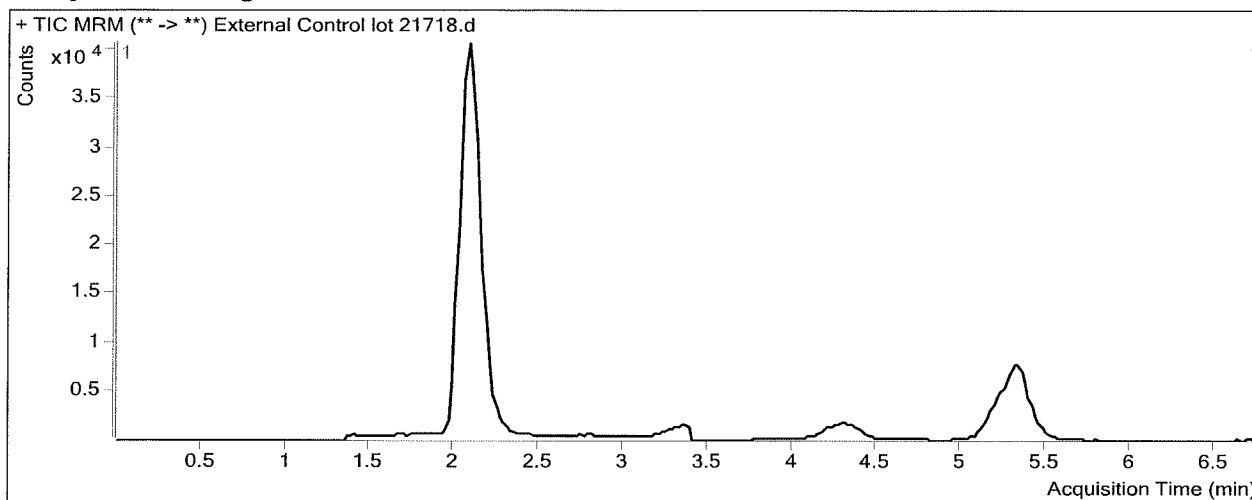
## Cannabinoids Analysis Report

<b>Batch Data Path</b>	C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin		
<b>Analysis Time</b>	12/6/2017 8:53 AM	<b>Analyst Name</b>	anord
<b>Report Time</b>	12/6/2017 9:06 AM	<b>Reporter Name</b>	ISP Tox
<b>Last Calib Update</b>	12/6/2017 8:53 AM	<b>Batch State</b>	Processed

### Analysis Info

<b>Acq Time</b>	2017-12-05 17:17	<b>Data File</b>	External Control lot 21718.d
<b>Sample Type</b>	Sample	<b>Sample Name</b>	External Control lot 21718
<b>Dilution</b>	1	<b>Acq Method</b>	AM 27 Quant THC 7-2017.m
<b>Position</b>	P1-B2	<b>Sample Info</b>	
<b>Inj Vol</b>	-1	<b>Comment</b>	AM 27 Cannabinoid Confirmation

### Sample Chromatogram



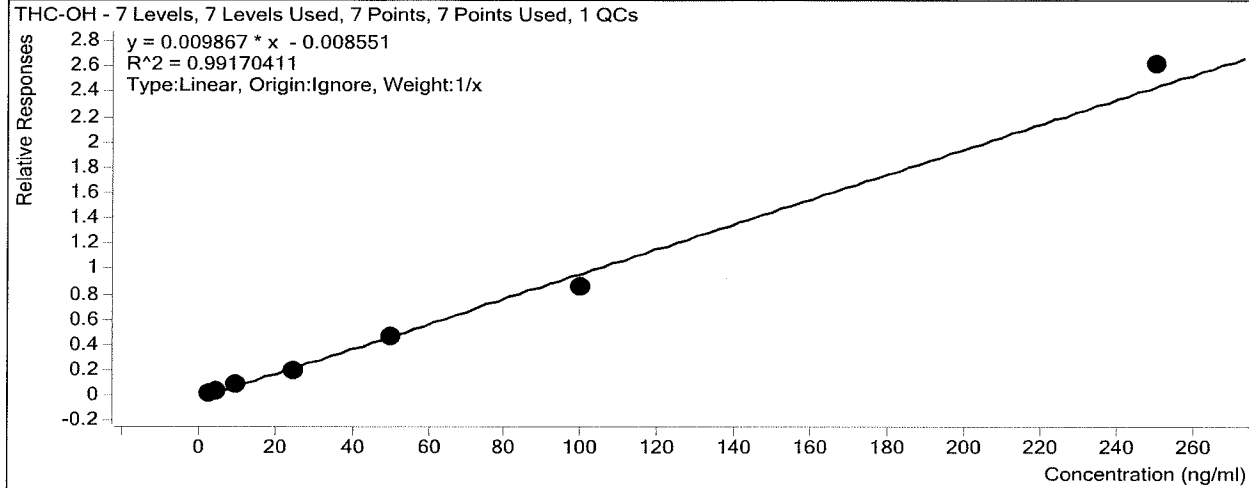
### Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.095	16845	212887	0.0791	8.8854
THC-COOH	THC-COOH-d9	2.185	17754	77053	0.2304	13.8857
THC	THC-d3	5.352	9650	86008	0.1122	11.0277

# ISP Forensics Calibration Curve Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Last Calib Update** 12/6/2017 8:53 AM **Analyst Name** ISP TOX

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

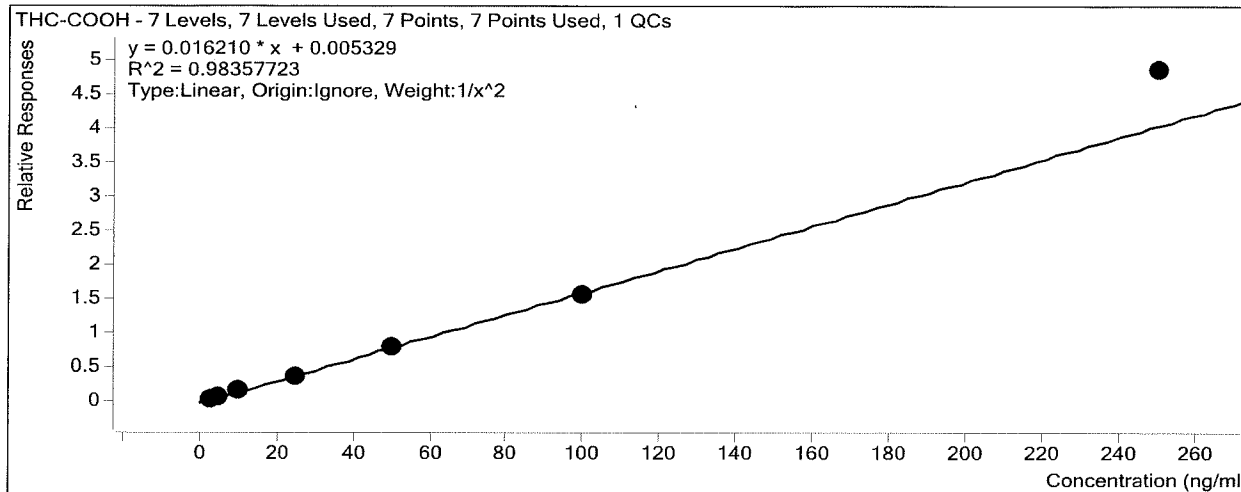


Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.7	121.9
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	5.0	99.9
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.2	102.3
QC - 10ng	3	<input checked="" type="checkbox"/>	10	8.3	83.0
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	21.0	84.1
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	48.2	96.5
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	88.8	88.8
Cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	266.1	106.4

# ISP Forensics Calibration Curve Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Last Calib Update** 12/6/2017 8:53 AM **Analyst Name** ISP TOX

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



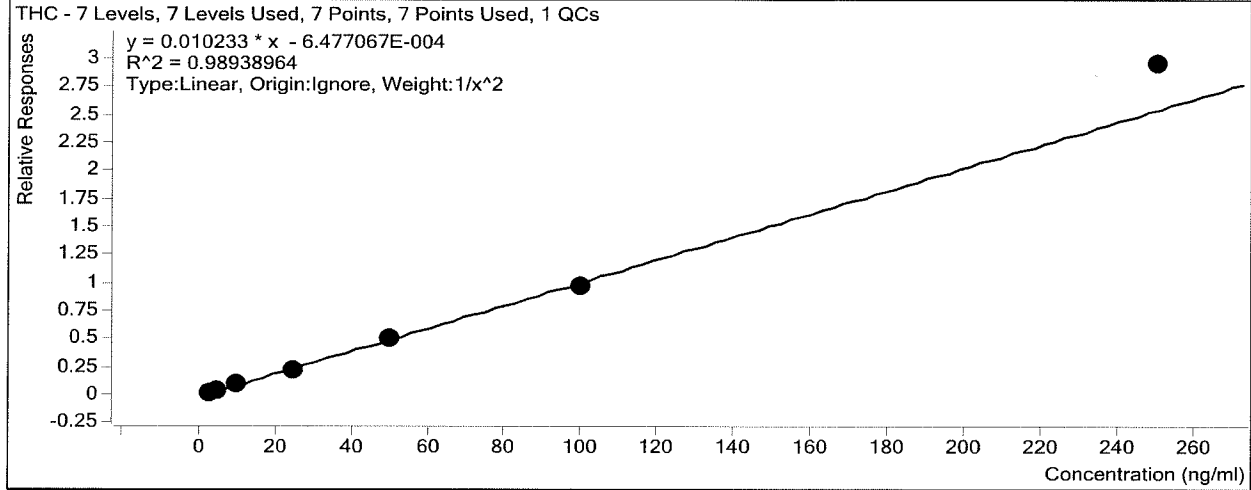
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.2	107.4
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	4.6	91.2
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	9.7	96.7
QC - 10ng	3	<input checked="" type="checkbox"/>	10	7.9	78.8
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	22.2	88.8
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	50.2	100.4
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	96.1	96.1
Cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	298.3	119.3



# ISP Forensics Calibration Curve Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Last Calib Update** 12/6/2017 8:53 AM **Analyst Name** ISP TOX

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



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1 - 3ng	1	<input checked="" type="checkbox"/>	3	3.1	104.5
Cal 2 - 5ng	2	<input checked="" type="checkbox"/>	5	4.7	93.8
Cal 3 - 10ng	3	<input checked="" type="checkbox"/>	10	10.1	101.2
QC - 10ng	3	<input checked="" type="checkbox"/>	10	8.5	85.5
Cal 4 - 25ng	4	<input checked="" type="checkbox"/>	25	22.6	90.2
Cal 5 - 50ng	5	<input checked="" type="checkbox"/>	50	50.2	100.3
Cal 6 - 100ng	6	<input checked="" type="checkbox"/>	100	94.6	94.6
Cal 7 - 250ng	7	<input checked="" type="checkbox"/>	250	288.4	115.4

# ISP FORENSICS - Cd'A Instrument # 62340

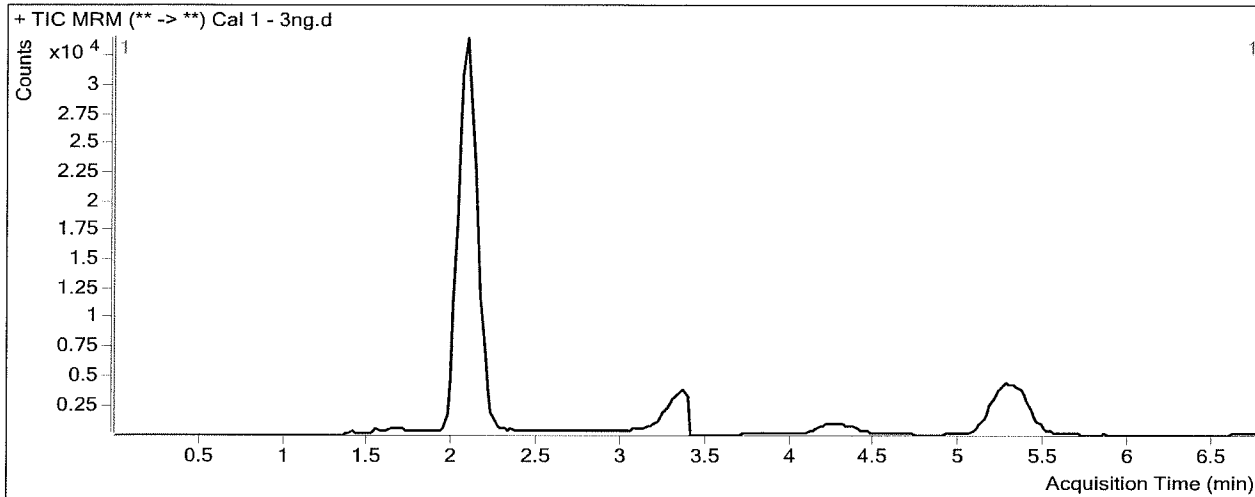
## Cannabinoids Analysis Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Analysis Time** 12/6/2017 8:53 AM **Analyst Name** anord  
**Report Time** 12/6/2017 9:06 AM **Reporter Name** ISP Tox  
**Last Calib Update** 12/6/2017 8:53 AM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-12-05 15:19 **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	2.095	4945	179595	0.0275	3.6571
THC-COOH	THC-COOH-d9	2.185	3957	68726	0.0576	3.2235
THC	THC-d3	5.372	2051	65233	0.0314	3.1352

# ISP FORENSICS - Cd'A Instrument # 62340

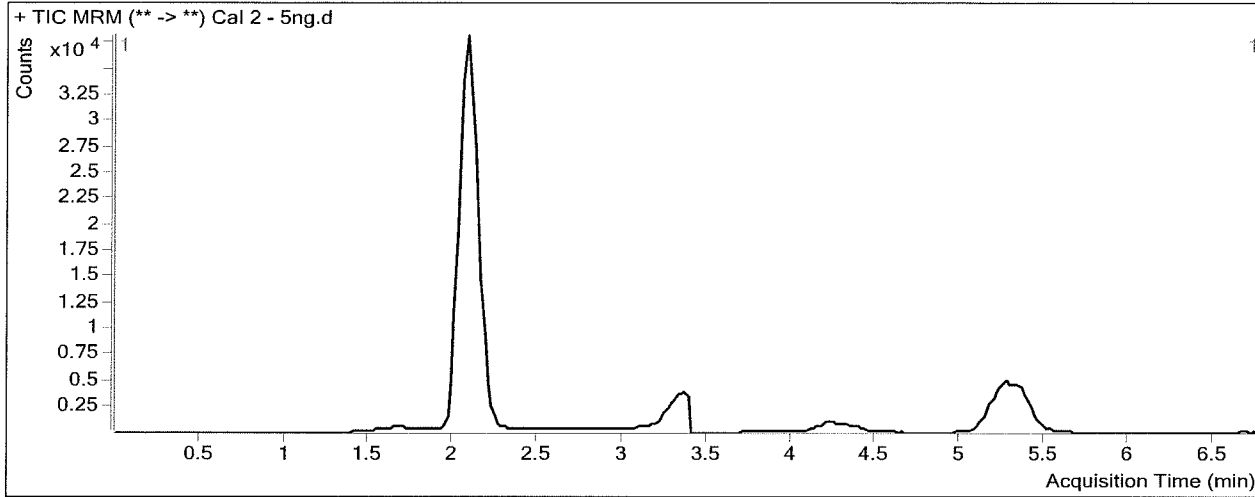
## Cannabinoids Analysis Report

<b>Batch Data Path</b>	C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin		
<b>Analysis Time</b>	12/6/2017 8:53 AM	<b>Analyst Name</b>	anord
<b>Report Time</b>	12/6/2017 9:06 AM	<b>Reporter Name</b>	ISP Tox
<b>Last Calib Update</b>	12/6/2017 8:53 AM	<b>Batch State</b>	Processed

### Analysis Info

<b>Acq Time</b>	2017-12-05 15:30	<b>Data File</b>	Cal 2 - 5ng.d
<b>Sample Type</b>	Calibration	<b>Sample Name</b>	Cal 2 - 5ng
<b>Dilution</b>	1	<b>Acq Method</b>	AM 27 Quant THC 7-2017.m
<b>Position</b>	P1-B1	<b>Sample Info</b>	
<b>Inj Vol</b>	-1	<b>Comment</b>	AM 27 Cannabinoid Confirmation

### Sample Chromatogram



### Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.095	8212	201476	0.0408	4.9972
THC-COOH	THC-COOH-d9	2.185	6069	76548	0.0793	4.5620
THC	THC-d3	5.352	3346	70699	0.0473	4.6884

# ISP FORENSICS - Cd'A Instrument # 62340

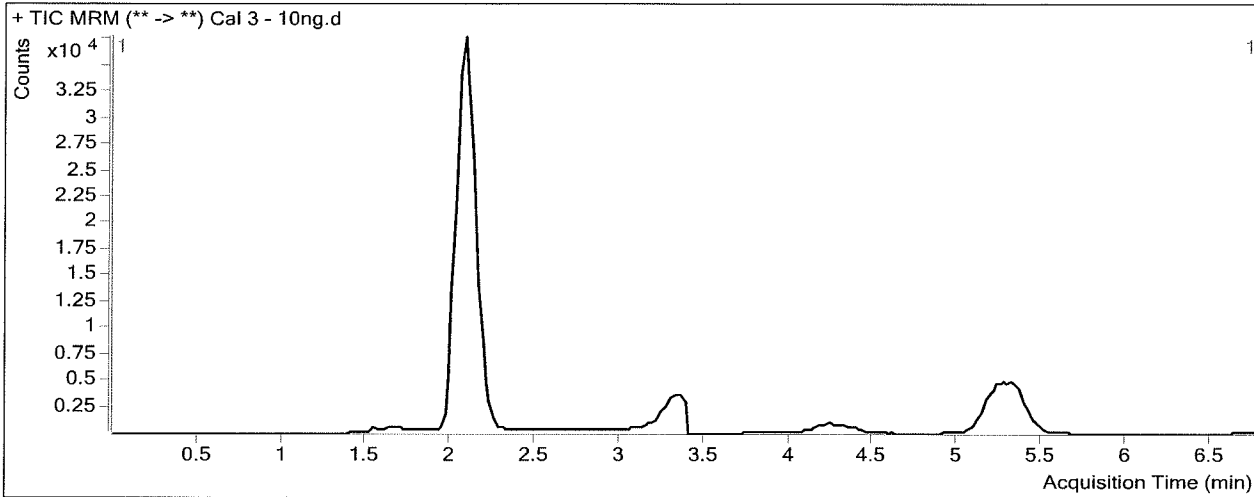
## Cannabinoids Analysis Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Analysis Time** 12/6/2017 8:53 AM **Analyst Name** anord  
**Report Time** 12/6/2017 9:06 AM **Reporter Name** ISP Tox  
**Last Calib Update** 12/6/2017 8:53 AM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-12-05 15:42 **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	2.095	17434	188630	0.0924	10.2334
THC-COOH	THC-COOH-d9	2.185	11529	71129	0.1621	9.6702
THC	THC-d3	5.352	7089	68882	0.1029	10.1209

# ISP FORENSICS - Cd'A Instrument # 62340

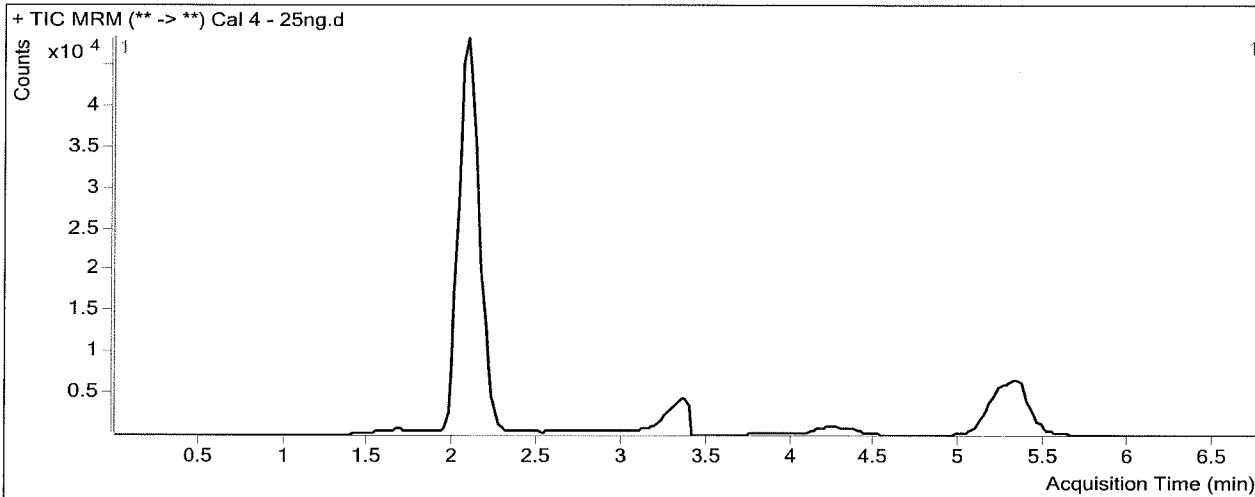
## Cannabinoids Analysis Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Analysis Time** 12/6/2017 8:53 AM **Analyst Name** anord  
**Report Time** 12/6/2017 9:06 AM **Reporter Name** ISP Tox  
**Last Calib Update** 12/6/2017 8:53 AM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-12-05 15:54 **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	2.095	45042	226321	0.1990	21.0359
THC-COOH	THC-COOH-d9	2.165	29310	80243	0.3653	22.2043
THC	THC-d3	5.332	18156	78869	0.2302	22.5602

# ISP FORENSICS - Cd'A Instrument # 62340

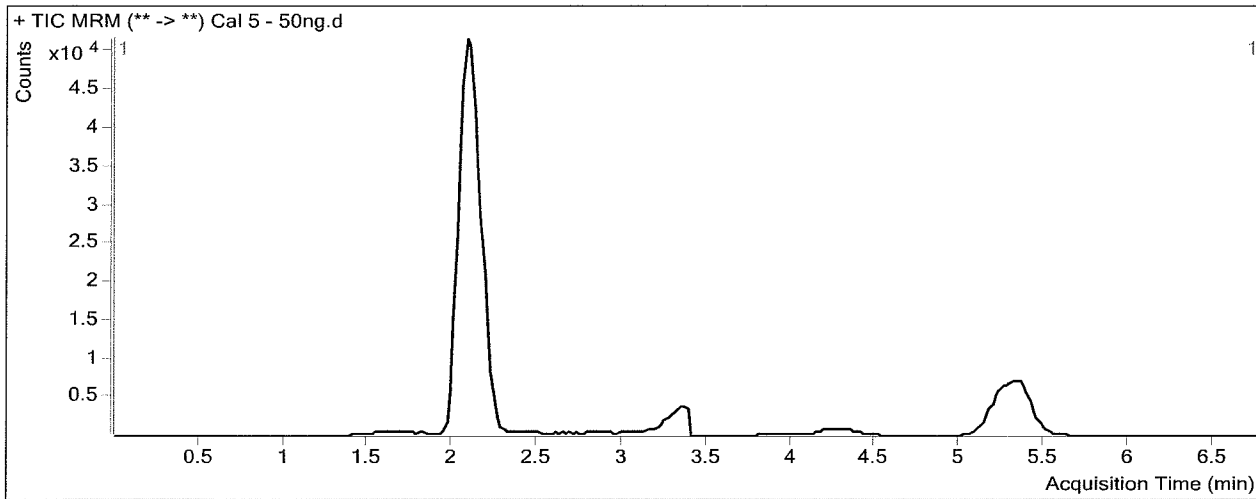
## Cannabinoids Analysis Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Analysis Time** 12/6/2017 8:53 AM **Analyst Name** anord  
**Report Time** 12/6/2017 9:06 AM **Reporter Name** ISP Tox  
**Last Calib Update** 12/6/2017 8:53 AM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-12-05 16:06 **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	2.095	91128	194992	0.4673	48.2290
THC-COOH	THC-COOH-d9	2.165	59331	72431	0.8191	50.2036
THC	THC-d3	5.352	33821	65961	0.5127	50.1709

# ISP FORENSICS - Cd'A Instrument # 62340

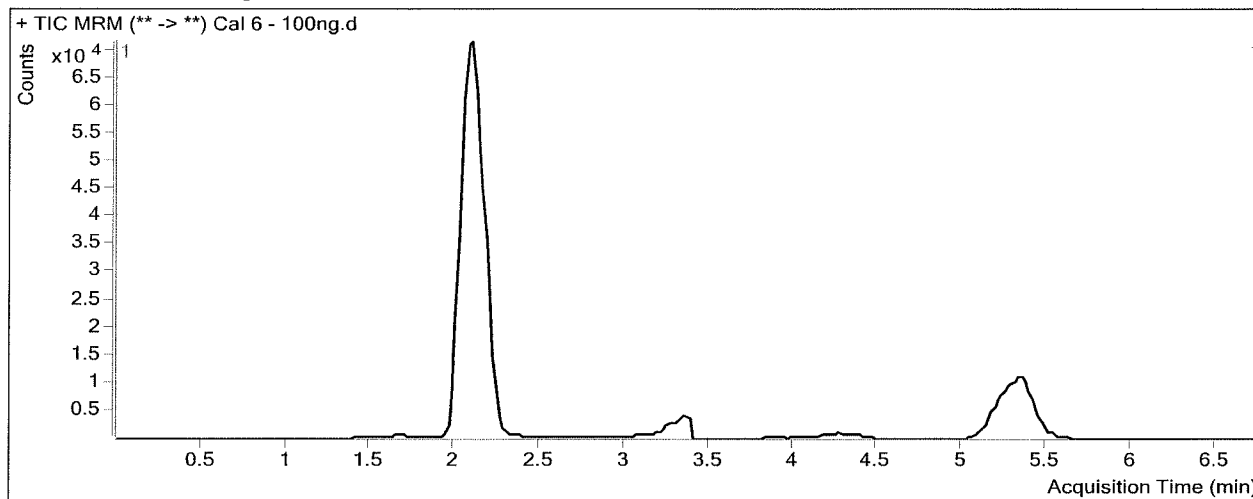
## Cannabinoids Analysis Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Analysis Time** 12/6/2017 8:53 AM **Analyst Name** anord  
**Report Time** 12/6/2017 9:06 AM **Reporter Name** ISP Tox  
**Last Calib Update** 12/6/2017 8:53 AM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-12-05 16:18 **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	2.095	184123	212212	0.8676	88.7966
THC-COOH	THC-COOH-d9	2.165	121344	77656	1.5626	96.0666
THC	THC-d3	5.352	69373	71743	0.9670	94.5591

# ISP FORENSICS - Cd'A Instrument # 62340

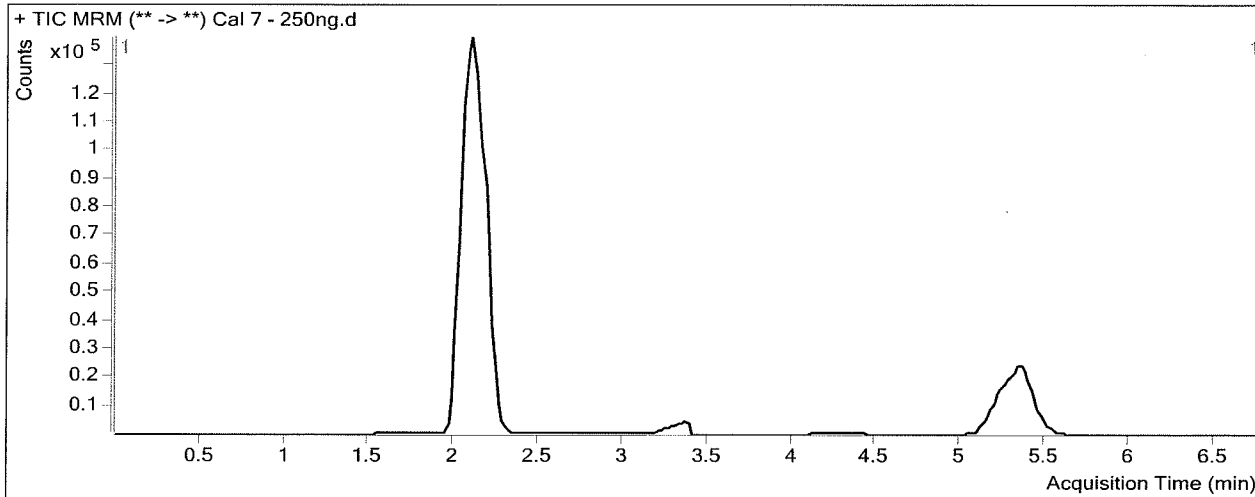
## Cannabinoids Analysis Report

**Batch Data Path** C:\MyFiles\anord\Documents\blood data\120517 cann quant\QuantResults\120517 cann quant.batch.bin  
**Analysis Time** 12/6/2017 8:53 AM **Analyst Name** anord  
**Report Time** 12/6/2017 9:06 AM **Reporter Name** ISP Tox  
**Last Calib Update** 12/6/2017 8:53 AM **Batch State** Processed

### Analysis Info

**Acq Time** 2017-12-05 16:30 **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	2.095	545255	208377	2.6167	266.0508
THC-COOH	THC-COOH-d9	2.185	332732	68736	4.8407	298.2953
THC	THC-d3	5.332	206936	70126	2.9509	288.4386