reviewed 10/30/17 10/27/2017

Worklist: 1978

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2017-2094	1	98738	AM 27 Blood THC Quant by LC
C2017-2142	1	98739	AM 27 Blood THC Quant by LC
C2017-2143	1	98740	AM 27 Blood THC Quant by LC
C2017-2146	1	98741	AM 27 Blood THC Quant by LC

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

Extraction Date: 10-24-17 Analyst: Amc Nord

Plate lot#: 0499102 Plate Expiration: 1/29/2018

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: 321632-1 **Column**: UCT Selectra DA 100 x 2.1mm 3um
LCMS-QQQ ID: 62340

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: 2609543** in wells of analytical (standards) plate.
- [x] 3. Place on shaking incubator at ambient temp., 900rpm for 15 minutes. *Shaker ID: 66759*
- [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: 66729
- [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: 66819*
- [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: 102417 cann quant Batch Name: 102417 cann quant
- [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? - See comments
- [x] 4. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports

COMMENTS: Click here to enter text.

THC-OH Not evaluated No samples screened positive for THC-OH.

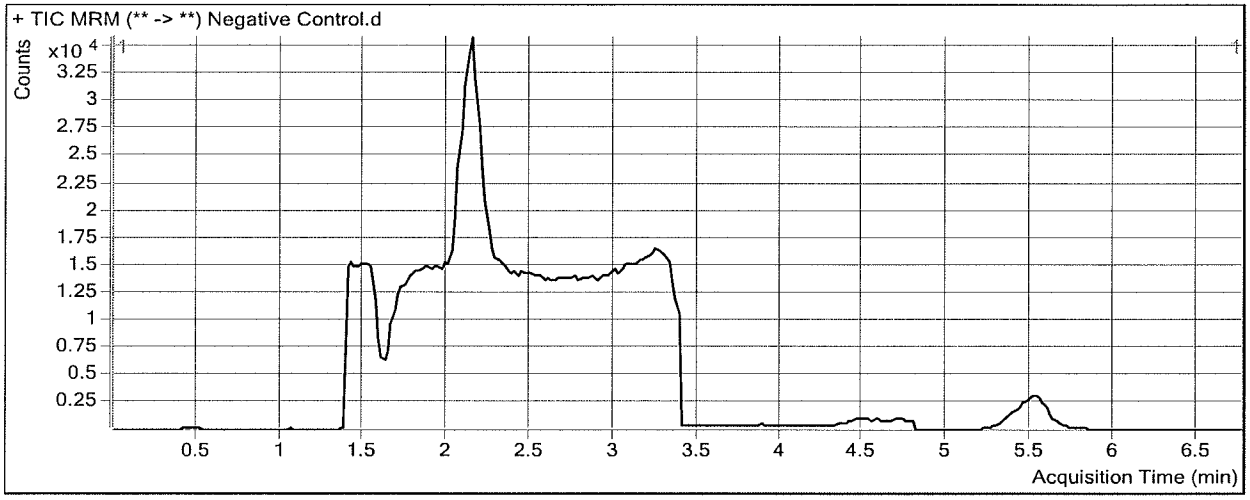
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 12:15	**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



Results

Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
~~THC-OH~~	~~THC-OH-d3~~	~~1.895~~	~~122453~~	~~131870~~	~~0.9286~~	~~92.8280~~
THC-COOH	THC-COOH-d9	2.305	5088	40717	0.1249	0.0000

Not evaluated Noise

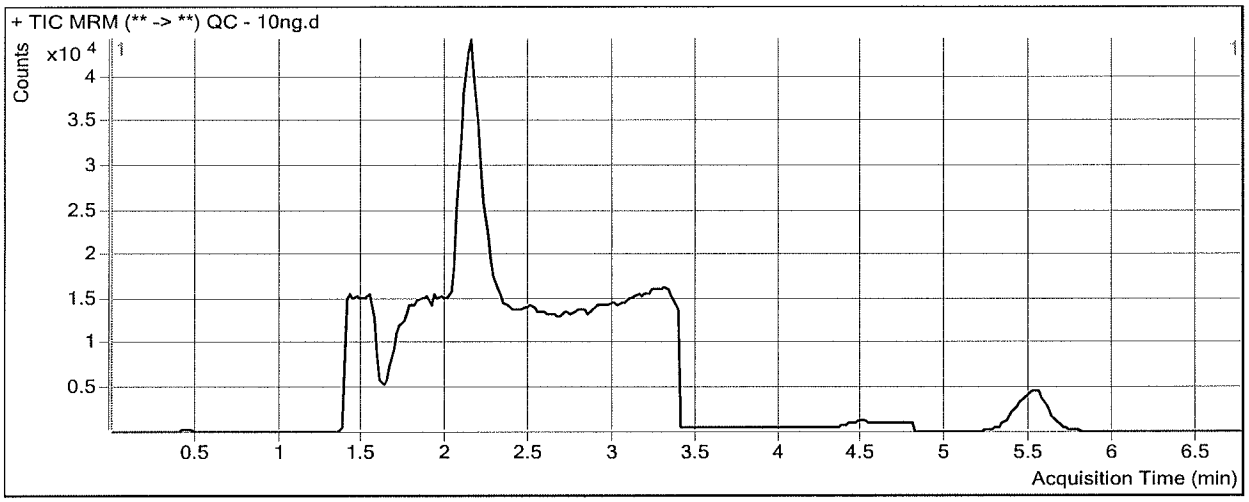
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 12:27	**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	2.135	0	162237	0.0000	0.0000
THC-COOH	THC-COOH-d9	2.245	20242	50310	0.4024	11.2093
THC	THC-d3	5.552	7961	51684	0.1540	10.9249

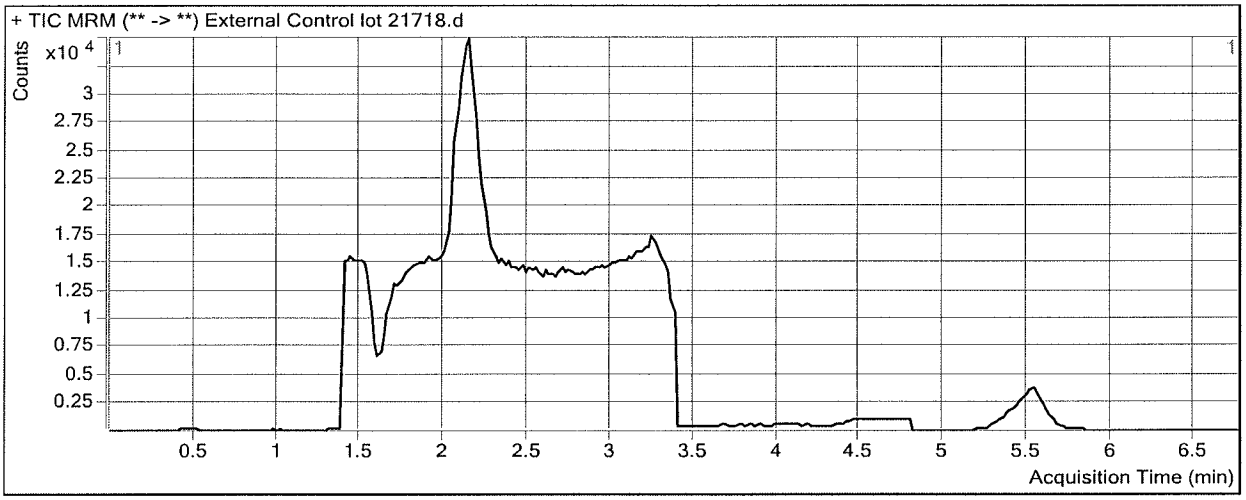
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 12:39	**Data File**	External Control lot 21718.d
Sample Type	Sample	**Sample Name**	External Control lot 21718
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	1.915	0	124491	0.0000	0.0000
THC-COOH	THC-COOH-d9	2.245	10958	37432	0.2927	5.7985
THC	THC-d3	5.532	4687	42012	0.1116	7.2823

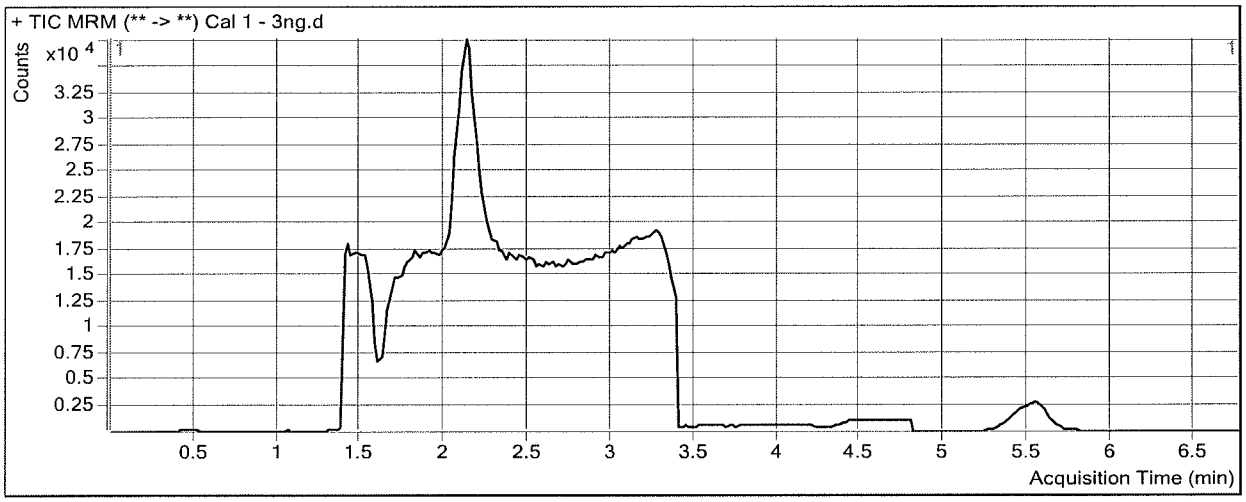
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 10:40	**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	2.035	38174	121261	0.3148	29.8301
THC-COOH	THC-COOH-d9	2.245	8561	35972	0.2380	3.0965
THC	THC-d3	5.552	1990	36333	0.0548	2.4102

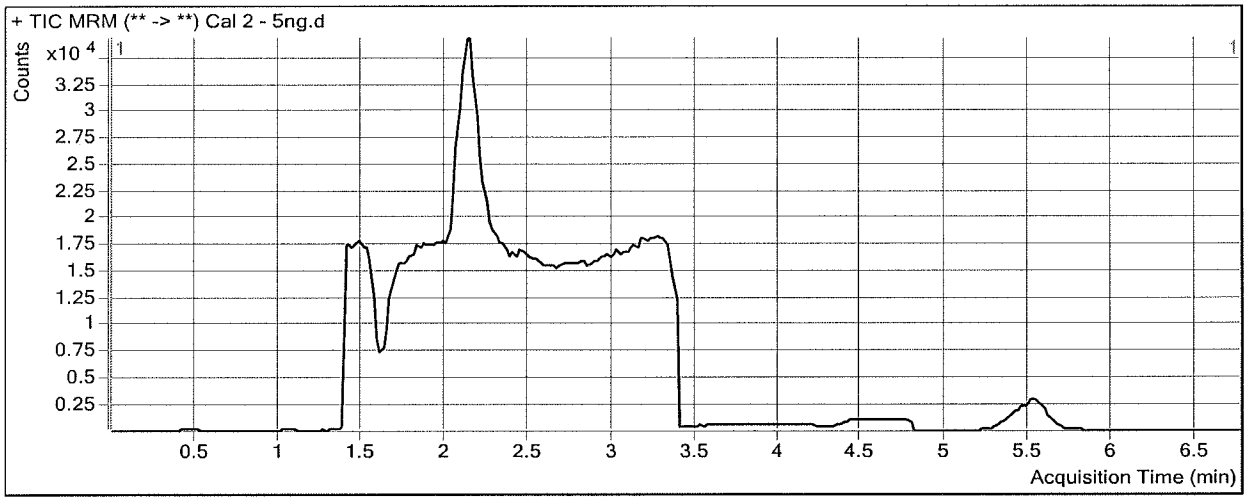
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 10:52	**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	1.995	50558	118639	0.4262	41.2582
THC-COOH	THC-COOH-d9	2.245	9571	35491	0.2697	4.6605
THC	THC-d3	5.552	2867	37739	0.0760	4.2303

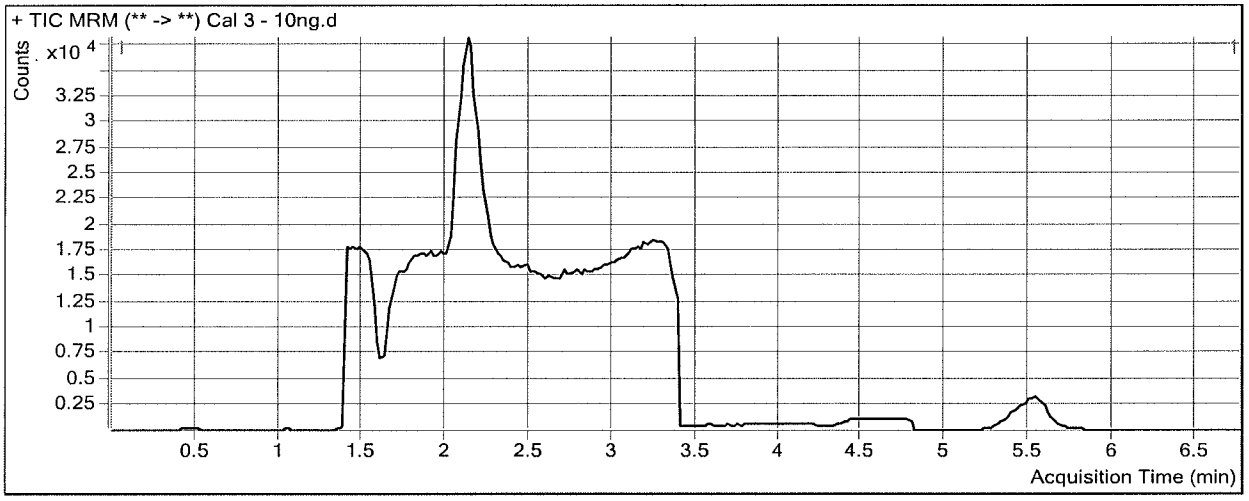
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 11:04	**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	15051	116680	0.1290	10.7583
THC-COOH	THC-COOH-d9	2.245	13260	34731	0.3818	10.1936
THC	THC-d3	5.552	6343	35843	0.1770	12.8912

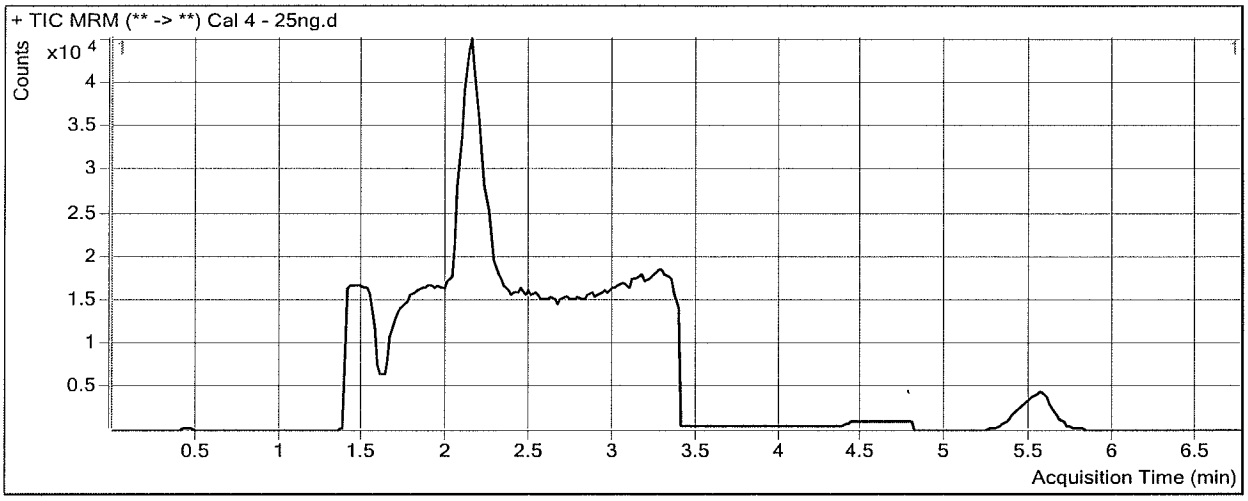
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 11:16	**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	2.155	35803	142656	0.2510	23.2781
THC-COOH	THC-COOH-d9	2.245	28128	41282	0.6814	24.9809
THC	THC-d3	5.572	13616	42358	0.3214	25.2844

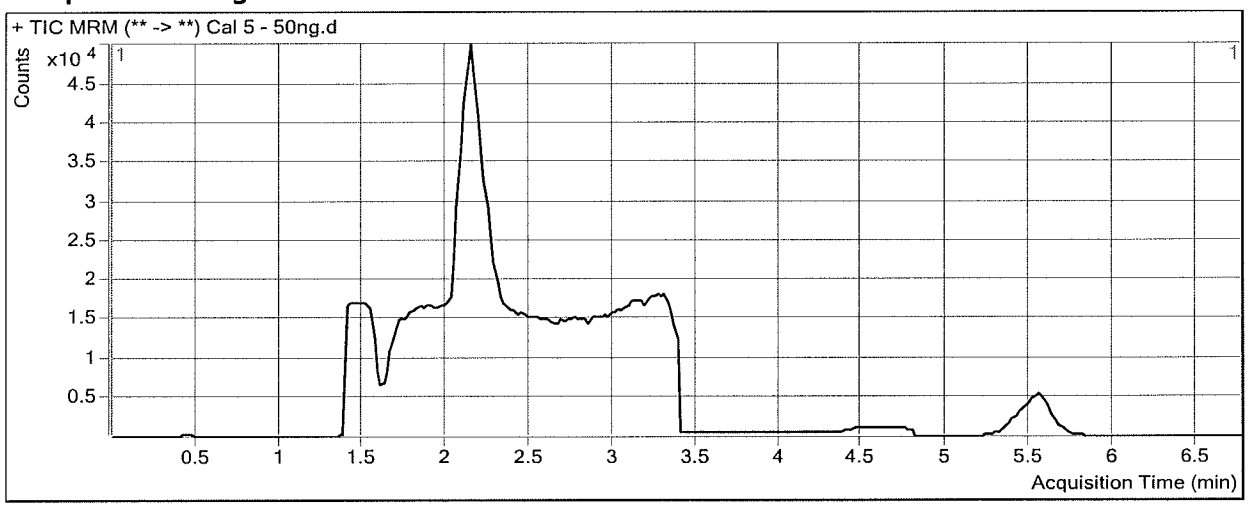
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 11:27	**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	2.155	60705	132347	0.4587	44.5972
THC-COOH	THC-COOH-d9	2.245	47369	38005	1.2464	52.8696
THC	THC-d3	5.552	25540	39360	0.6489	53.3687

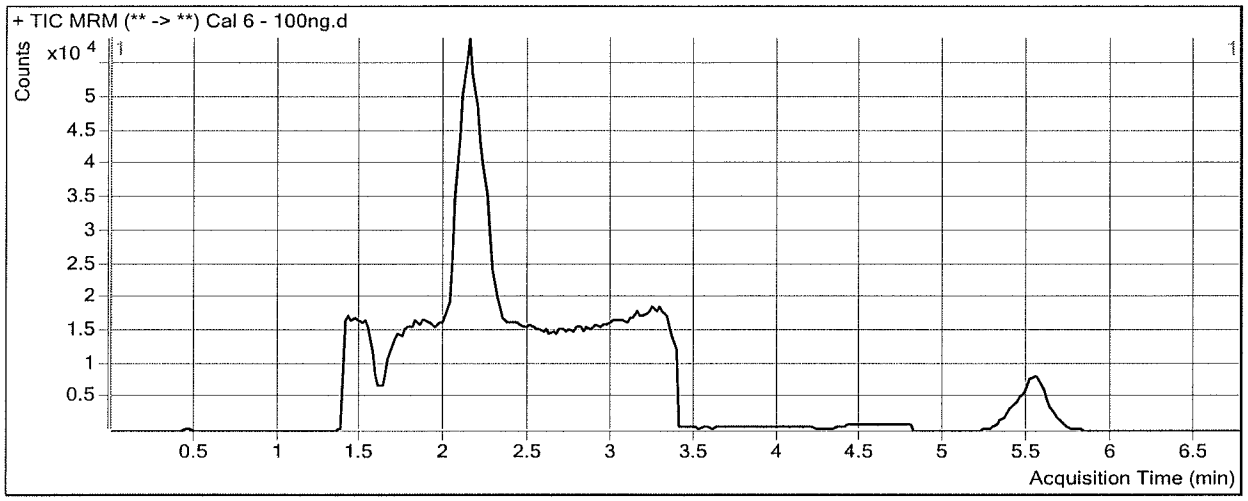
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 11:39	**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	2.155	143138	127654	1.1213	112.6066
THC-COOH	THC-COOH-d9	2.225	82431	37637	2.1902	99.4502
THC	THC-d3	5.552	49060	40911	1.1992	100.5665

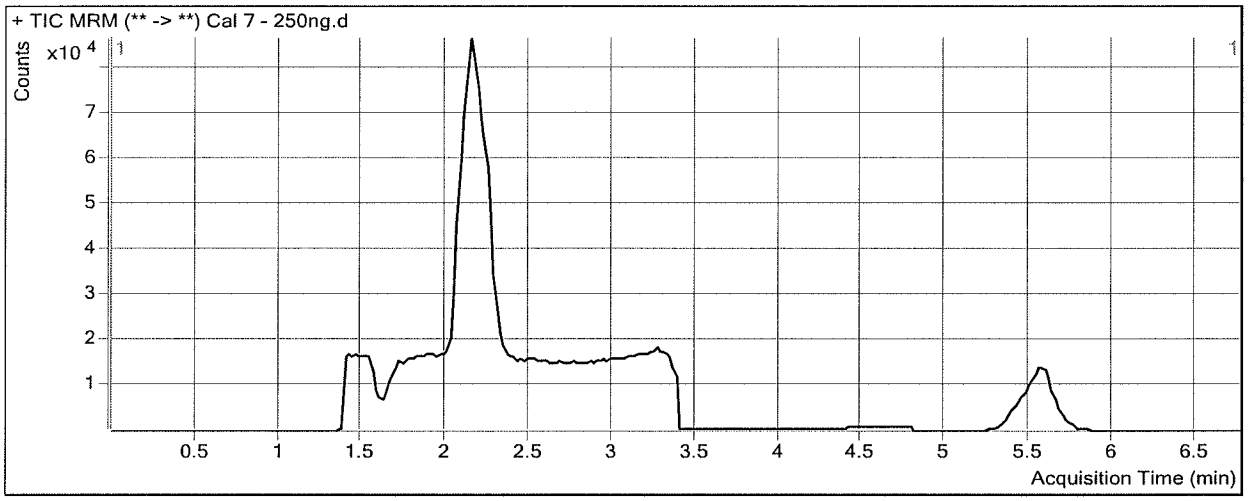
ISP FORENSICS - Cd'A Instrument # 62340
Cannabinoids Analysis Report

Batch Data Path	C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin		
Analysis Time	10/26/2017 8:52 AM	**Analyst Name**	anord
Report Time	10/26/2017 9:11 AM	**Reporter Name**	ISP Tox
Last Calib Update	10/26/2017 8:52 AM	**Batch State**	Processed

Analysis Info

Acq Time	2017-10-25 11:51	**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	2.155	293751	122442	2.3991	243.7599
THC-COOH	THC-COOH-d9	2.245	179017	35349	5.0643	241.3105
THC	THC-d3	5.572	110542	38458	2.8744	244.2487

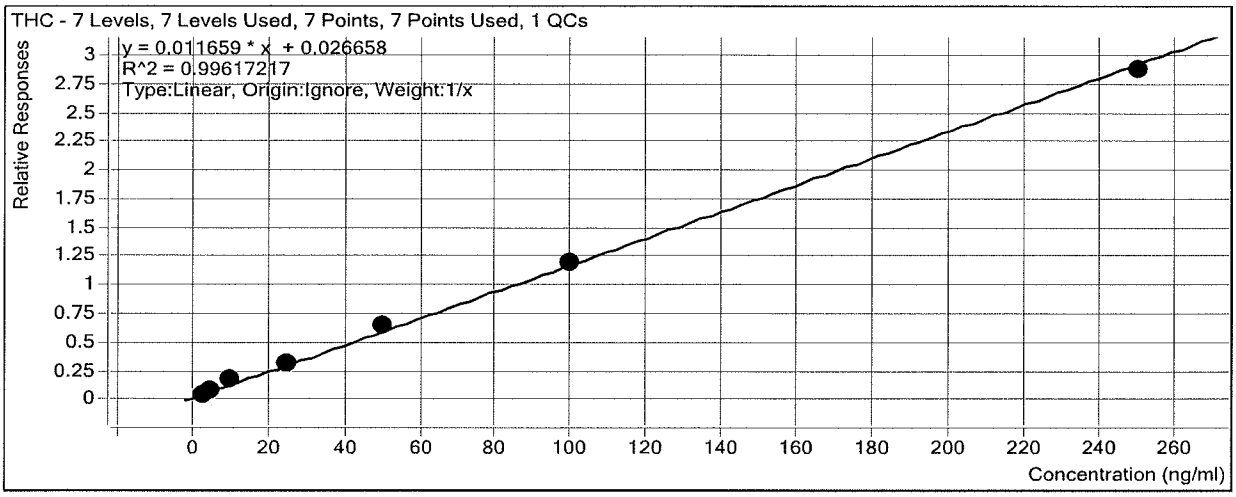
ISP Forensics
Calibration Curve Report

Batch Data Path C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin

Last Calib Update 10/26/2017 8:52 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	2.4	80.3
Cal 2 - 5ng	2	☑	5	4.2	84.6
Cal 3 - 10ng	3	☑	10	12.9	128.9
QC - 10ng	3	☑	10	10.9	109.2
Cal 4 - 25ng	4	☑	25	25.3	101.1
Cal 5 - 50ng	5	☑	50	53.4	106.7
Cal 6 - 100ng	6	☑	100	100.6	100.6
Cal 7 - 250ng	7	☑	250	244.2	97.7

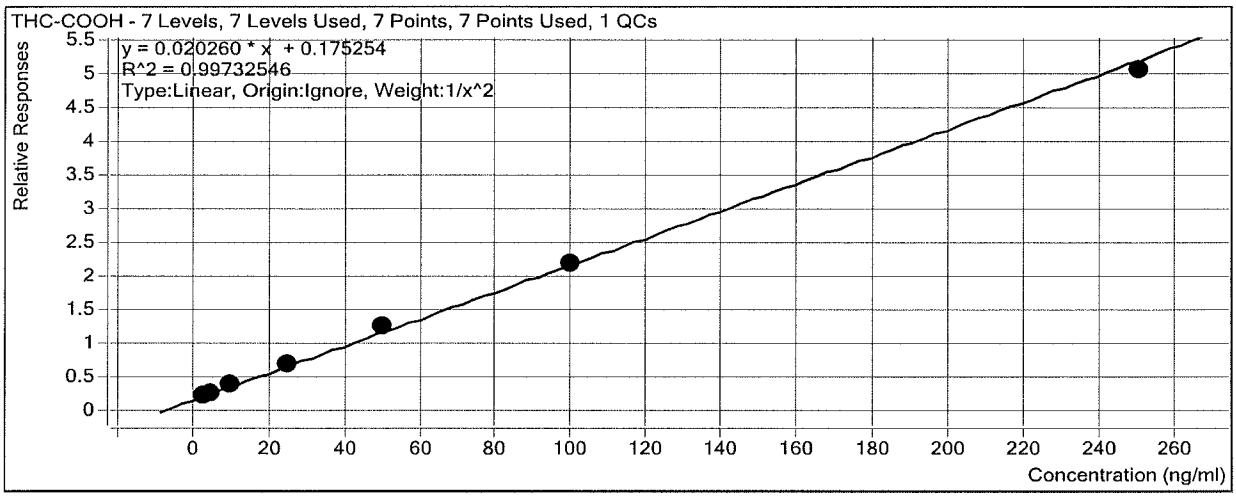
ISP Forensics
Calibration Curve Report

Batch Data Path C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin

Last Calib Update 10/26/2017 8:52 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	103.2
Cal 2 - 5ng	2	☑	5	4.7	93.2
Cal 3 - 10ng	3	☑	10	10.2	101.9
QC - 10ng	3	☑	10	11.2	112.1
Cal 4 - 25ng	4	☑	25	25.0	99.9
Cal 5 - 50ng	5	☑	50	52.9	105.7
Cal 6 - 100ng	6	☑	100	99.5	99.5
Cal 7 - 250ng	7	☑	250	241.3	96.5

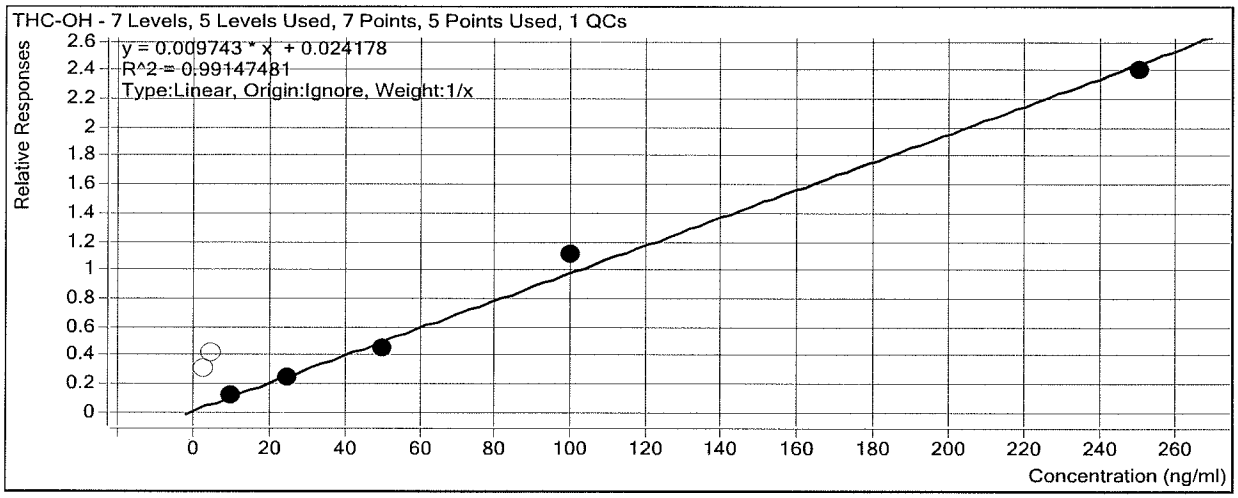
ISP Forensics
Calibration Curve Report

Batch Data Path C:\MyFiles\anord\Documents\blood data\1024 cann quant\QuantResults\102417 cann quant.batch.bin

Last Calib Update 10/26/2017 8:52 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	29.8	994.3
Cal 2 - 5ng	2	☐	5	41.3	825.2
Cal 3 - 10ng	3	☑	10	10.8	107.6
QC - 10ng	3	☑	10	0.0	0.0
Cal 4 - 25ng	4	☑	25	23.3	93.1
Cal 5 - 50ng	5	☑	50	44.6	89.2
Cal 6 - 100ng	6	☑	100	112.6	112.6
Cal 7 - 250ng	7	☑	250	243.8	97.5

Not evaluate too much noise.