










Worklist: 1627

central data reviewed 3/27/17



3/27/2017

<u>LAB CASE</u>	<u>ITEM</u>	<u>TASK ID</u>	<u>DESCRIPTION</u>	
C2017-0446	1	80289	AM 27 Blood THC Quant by LC	
C2017-0454	1	80288	AM 27 Blood THC Quant by LC	
C2017-0465	1	80296	AM 27 Blood THC Quant by LC	
C2017-0500	1	80293	AM 27 Blood THC Quant by LC	
C2017-0512	1	80294	AM 27 Blood THC Quant by LC	
M2017-0858	1	80290	AM 27 Blood THC Quant by LC	
M2017-1017	1	80291	AM 27 Blood THC Quant by LC	
M2017-1143	2	80292	AM 27 Blood THC Quant by LC	
P2017-0559	1	80295	AM 27 Blood THC Quant by LC	



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

Extraction Date: 3-23-17

Analyst: Anne Nord

PRE-ANALYTIC

0499102

1-28-2018

Plate Lot# Custom - 0490364 A Plate Exp. 9-21-2017 * 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: 3-23-17 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: 32317 thc 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 - Cd'A Instrument # 62340

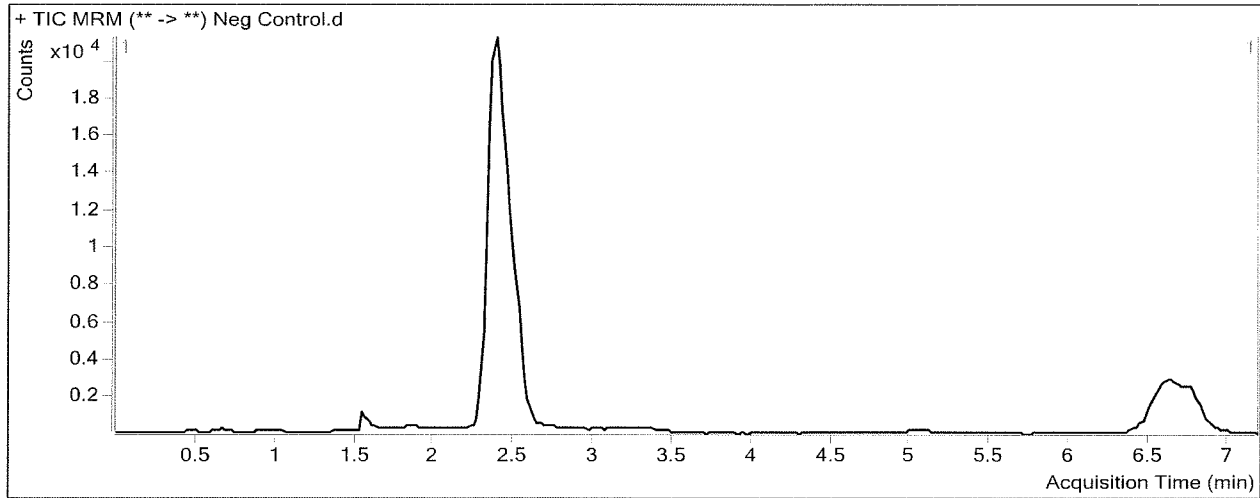
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\3-23-17 THC Quant\QuantResults\32317 thc quant.batch.bin
Analysis Time 3/26/2017 9:32 AM **Analyst Name** ISP Tox
Report Time 3/26/2017 10:12 AM **Reporter Name** ISP Tox
Last Calib Update 3/26/2017 9:32 AM **Batch State** Processed

Analysis Info

Acq Time 2017-03-23 17:52 **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

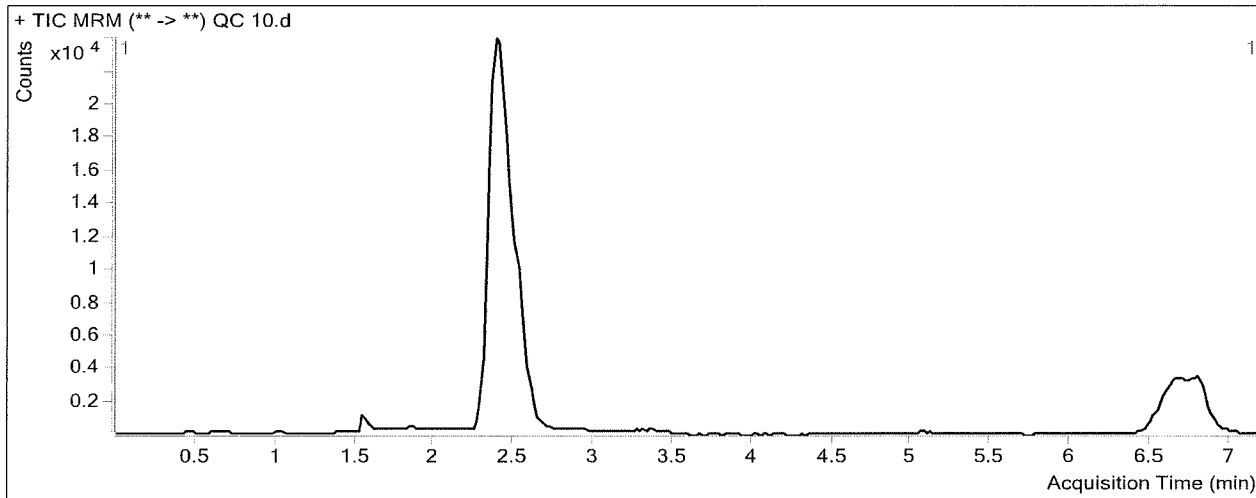
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\3-23-17 THC Quant\QuantResults\32317 thc quant.batch.bin
Analysis Time 3/26/2017 9:32 AM **Analyst Name** ISP Tox
Report Time 3/26/2017 10:12 AM **Reporter Name** ISP Tox
Last Calib Update 3/26/2017 9:32 AM **Batch State** Processed

Analysis Info

Acq Time 2017-03-23 18:04 **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	2.416	15026	165462	0.0908	9.7508
THC-COOH	THC-COOH-d9	2.546	10869	53752	0.2022	10.3431
THC	THC-d3	6.773	6516	55986	0.1164	10.1609

ISP FORENSICS - Cd'A Instrument # 62340

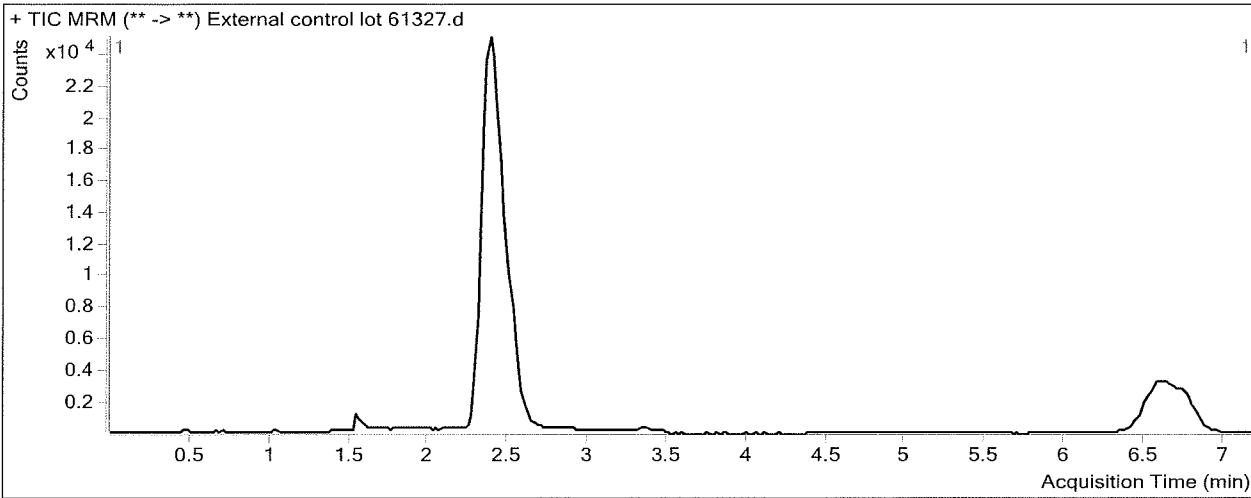
Cannabinoids Analysis Report

Batch Data Path D:\2017 Data\3-23-17 THC Quant\QuantResults\32317 thc quant.batch.bin
Analysis Time 3/26/2017 10:23 AM **Analyst Name** ISP Tox
Report Time 3/26/2017 10:24 AM **Reporter Name** ISP Tox
Last Calib Update 3/26/2017 10:23 AM **Batch State** Processed

Analysis Info

Acq Time 2017-03-23 18:16 **Data File** External control lot 61327.d
Sample Type Sample **Sample Name** External control lot 61327
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

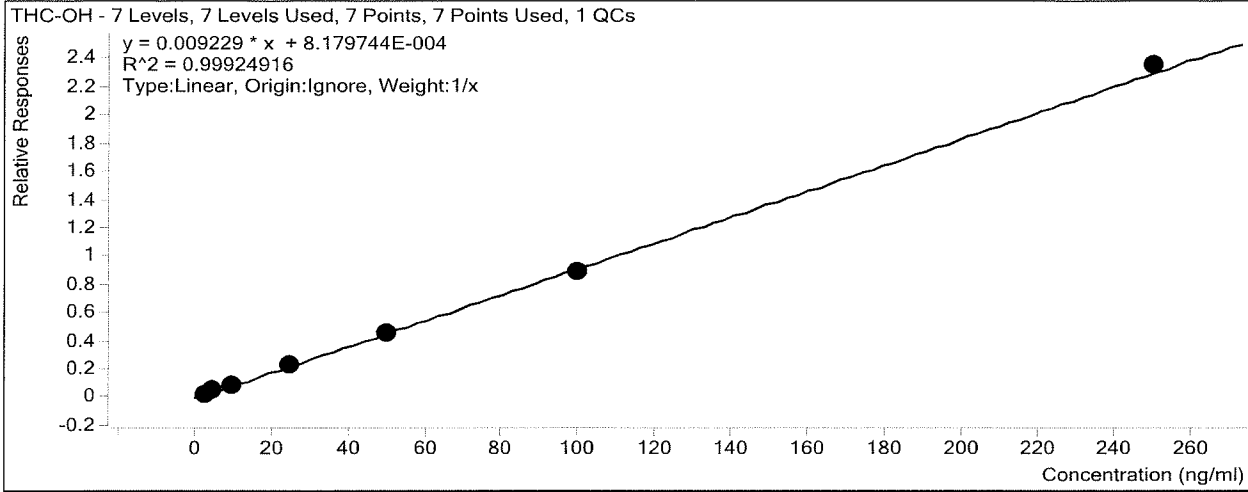
Compound	ISTD Compound	RT	Response	ISTD Resp	Resp Ratio	Final Conc
THC-OH	THC-OH-d3	2.396	19886	168749	0.1178	12.6795
THC-COOH	THC-COOH-d9	2.526	8862	50978	0.1738	8.8688
THC	THC-d3	6.713	6809	55320	0.1231	10.7502

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\3-23-17 THC Quant\QuantResults\32317 thc quant.batch.bin

Last Calib Update 3/26/2017 9:32 AM **Analyst Name** ISP TOX

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



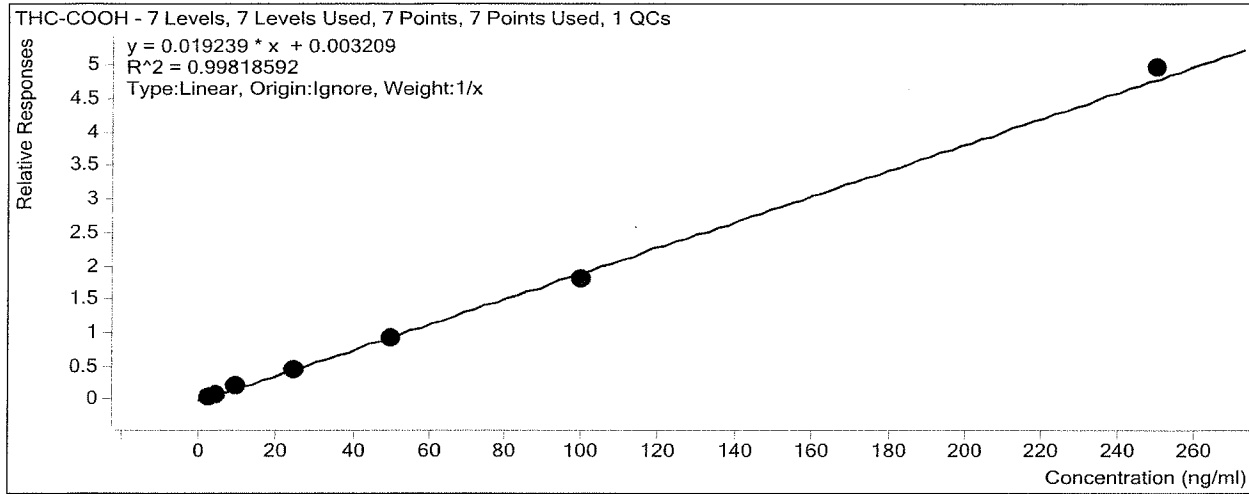
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	<input checked="" type="checkbox"/>	3	3.0	101.4
cal 2	2	<input checked="" type="checkbox"/>	5	5.1	102.5
cal 3	3	<input checked="" type="checkbox"/>	10	10.0	99.6
QC 10	3	<input checked="" type="checkbox"/>	10	9.8	97.5
cal 4	4	<input checked="" type="checkbox"/>	25	25.1	100.4
cal 5	5	<input checked="" type="checkbox"/>	50	49.1	98.3
cal 6	6	<input checked="" type="checkbox"/>	100	95.9	95.9
cal 7	7	<input checked="" type="checkbox"/>	250	254.8	101.9

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\3-23-17 THC Quant\QuantResults\32317 thc quant.batch.bin

Last Calib Update 3/26/2017 9:32 AM **Analyst Name** ISP TOX

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



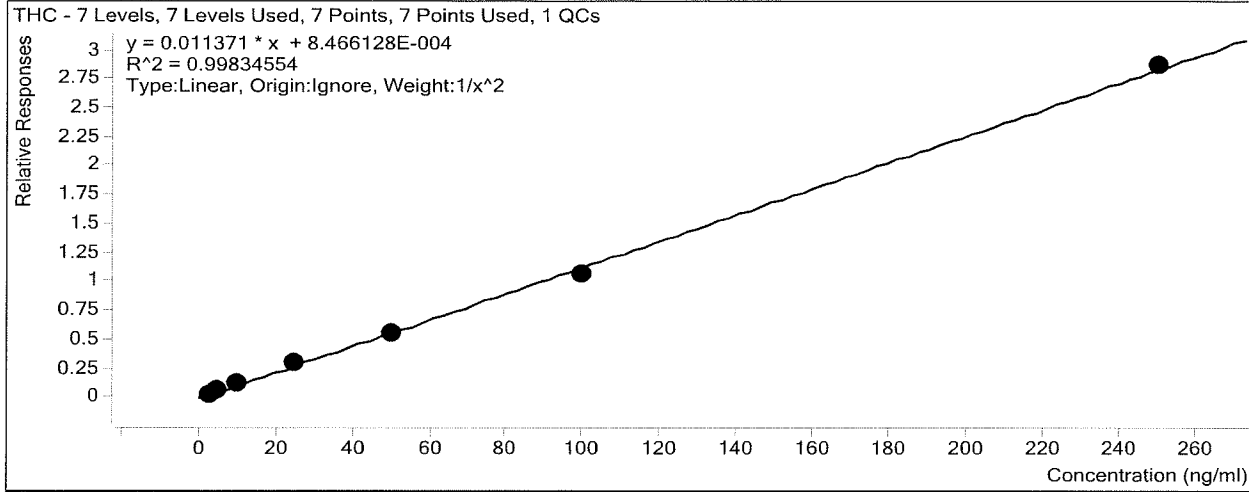
Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	<input checked="" type="checkbox"/>	3	3.1	102.6
cal 2	2	<input checked="" type="checkbox"/>	5	5.0	99.5
cal 3	3	<input checked="" type="checkbox"/>	10	10.7	106.7
QC 10	3	<input checked="" type="checkbox"/>	10	10.3	103.4
cal 4	4	<input checked="" type="checkbox"/>	25	24.3	97.4
cal 5	5	<input checked="" type="checkbox"/>	50	48.3	96.6
cal 6	6	<input checked="" type="checkbox"/>	100	94.2	94.2
cal 7	7	<input checked="" type="checkbox"/>	250	257.4	103.0

ISP Forensics Calibration Curve Report

Batch Data Path D:\2017 Data\3-23-17 THC Quant\QuantResults\32317 thc quant.batch.bin

Last Calib Update 3/26/2017 9:32 AM **Analyst Name** ISP TOX

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



Sample	Level	Enabled	Exp Conc	Final Conc	Accuracy
Cal 1	1	<input checked="" type="checkbox"/>	3	3.0	99.1
cal 2	2	<input checked="" type="checkbox"/>	5	5.0	99.4
cal 3	3	<input checked="" type="checkbox"/>	10	10.3	103.4
QC 10	3	<input checked="" type="checkbox"/>	10	10.2	101.6
cal 4	4	<input checked="" type="checkbox"/>	25	26.1	104.4
cal 5	5	<input checked="" type="checkbox"/>	50	49.3	98.5
cal 6	6	<input checked="" type="checkbox"/>	100	94.5	94.5
cal 7	7	<input checked="" type="checkbox"/>	250	251.9	100.7