Worklist: 2103


Extraction Date: $12-18$
Plate lot\#:0515207

Analyst: AnคC Nord
Plate Expiration:10/18/2018
Mobile phase B 0.1\% Formic acid in MeOH Ethyl Acetate
Column: Phenomenex Phenyl Hexyl (4.6x50mm: 2.6 um)

Mobile phase A: 10 mM Ammonium Formate 0.5M Ammonium Hydroxide

Blank Blood Lot: 17J20718
LCMS-QQQ ID:62340

## Pre-Analytic:

(4 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 $\sim 30$ minutes.
\& 3. Create worklist:

## Analytic:

1. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
2. Pipette $\mathbf{2 5 0} \boldsymbol{\mu}$ L blood (calibrated pipette) Pipette ID: 1926134 in wells of analytical (standards) plate.
$\boxtimes$ 3. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes. Shaker ID66759
3. Pipette $\mathbf{2 5 0} \boldsymbol{\mu} \mathrm{L} \mathbf{0 . 5 M}$ ammonium hydroxide buffer in wells of analytical plate.

区 5. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
6. Transfer $\mathbf{3 0 0} \boldsymbol{\mu} \mathrm{L}$ of blood+base mixture to corresponding wells of SLE + plate.
$\boxtimes$ 7. Apply positive pressure for approx. 4 seconds (or until no liquid remains on top of sorbent).
(Load at 85-100 PSI- Selector to the right) Manifold ID: 66729 Wait 5 minutes
( 8. Add $900 \mu \mathrm{~L}$ ethyl acetate and allow to flow for 5 minutes under gravity.
Q 9. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left)
$\boxtimes 10$. Add $900 \mu \mathrm{~L}$ ethyl acetate and allow to flow for 5 minutes under gravity.
© 11. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left)
(12. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. $35^{\circ} \mathrm{C}$. SPE Dry ID66819
(区 13. Reconstitute in $\mathbf{1 0 0 \mu} \mathrm{L} \mathbf{1 0 0 \%} \mathbf{M e O H}$ and heat seal plate with foil. Place in autosampler and run worklist.

## POST- ANALYTIC

Q. Create batch and process data. 01022018 mds pl Worklist path: 01022018 blood seccir Am250 Batch Name: Oi 022018 mis PQ
\#7 2. Evaluate samples, $\mathrm{S} / \mathrm{N}$ of primary transition $>5$ and $\mathrm{S} / \mathrm{N}$ of secondary transition $>3$ or evaluation of peak symmetry and resolution. Within $+/-2 \%$ or 0.1 min RT of administrative control. $>1 / 5$ the response of administrative control.
3. Did all QCs pass for each analyte? $Y$ N
4. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports
200/ng/ml morphine
Concentration $100 \mathrm{ng} / \mathrm{ml}$ amitriptyline, codeine, diphenhydramine, mirtazapine









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| $\begin{aligned} & \overrightarrow{8} \\ & \underset{\sim}{\mathrm{~N}} \end{aligned}$ | $\left[\begin{array}{l} \overrightarrow{0} \\ 0 \\ 0 \\ 1 \end{array}\right.$ | $\stackrel{\rightharpoonup}{\circ}$ | $\stackrel{\infty}{\stackrel{\infty}{9}}$ | $\begin{aligned} & \mathrm{N} \\ & \mathrm{H} \\ & \mathrm{H} \end{aligned}$ | $\frac{3}{9}$ |  | 总號 |  |  | $\begin{array}{l\|l} \omega \\ 0 & \text { 总 } \\ \hline \end{array}$ |  | $\begin{array}{l\|l} \substack{c \\ N \\ \infty \\ \infty \\ \hline} \\ \hline \end{array}$ | $0$ | $\begin{gathered} 9 \\ \vdots \\ 0 \\ 0 \\ 8 \end{gathered}$ | ${ }^{\text {N }}$ |  | $\begin{aligned} & \vec{N} \\ & y \\ & y \end{aligned}$ | $\left\lvert\, \begin{aligned} & r \\ & \underset{\infty}{\infty} \\ & \underset{\sim}{2} \end{aligned}\right.$ |  | + | o |  | O10 |  | － |  |  |  |  |  | $\underset{\sim}{0}$ | $\begin{gathered} 9 \\ \underset{N}{2} \end{gathered}$ | $\begin{gathered} 9 \\ 0 \\ y \\ \hline \end{gathered}$ | － |  | $\left\lvert\, \begin{aligned} & \substack{0 \\ \infty \\ \infty \\ \infty} \end{aligned}\right.$ | $\begin{aligned} & \stackrel{\rightharpoonup}{\stackrel{N}{N}} \end{aligned}$ |  |  |  |  |  |
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# THC and Metabolites Screen in Blood by LC-MS/MS 

Extraction Date: $1-2-18$
Analyst: $\qquad$
Plate lot\#: 0515037
Plate Expiration: 9/28/2018
Mobile phase A: 10 mM Ammonium Formate Mobile phase B: $0.1 \%$ Formic acid in MeOH
$0.1 \%$ Formic Acid in Water
MTBE
Hexane

Blank Blood Lot: 17J20718
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 $\sim 30$ minutes.
3. Create worklist:

## Analytic:

囚. Remove standards, plate, controls, and samples from cold storage. Allow to reach room temperature.
$\triangle$ 2. Pipette $1000 \mu \mathrm{~L}$ blood (calibrated pipette) Pipette ID: $\mathbf{2 6 0 9 5 4 3}$ in wells of analytical (standards) plate.

- Blank blood for locations containing standards/QCs and internal standards
- Sample blood for locations containing only internal standards
$\pm$ 3. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes. Shaker ID: 66759

4. Pipette $\mathbf{5 0 0} \boldsymbol{\mu} \mathbf{L} \mathbf{0 . 1 \%}$ formic acid in wells of analytical plate.
5. Place on shaking incubator at ambient temp., 900 rpm for 15 minutes.
6. Transfer $800 \mu \mathrm{~L}$ of blood+base mixture to corresponding wells of SLE+ plate.
7. Apply positive pressure for approx. 4 seconds (or until no liquid remains on top of sorbent).
(Load at 85-100 PSI- Selector to the right) Manifold ID: 66792
$\boxtimes 8$. Wait 5 minutes.
8. Add 2.25 mL MTBE (add in 3 increments of 750 uL ).
$\searrow$ 10. Wait 5 minutes.
(11. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
(T) 12. Add 2.25 mL hexane (add in 3 increments of 750 uL ).
9. Wait 5 minutes.
( 14. Apply positive pressure for approx. 15 seconds. (10-15 PSI- Selector to the left).
$\boxtimes 15$. Remove plate containing eluate. Place on SPE Dry and evaporate to dryness at approx. $35^{\circ} \mathrm{C}$. SPE Dry ID: 66819
$\boxtimes 16$. Reconstitute in $\mathbf{1 0 0} \boldsymbol{\mu} \mathrm{L} \mathbf{1 0 0 \%} \mathbf{~ M e O H}$ and heat seal plate with foil. Place in autosampler and run worklist.

## Post-Analytic

1. Create batch and process data.

\$ 2. For unknown samples, calculated concentration $>3 \mathrm{ng}$ THC, THC-OH and $>5 \mathrm{ng}$ Carboxy-THC and $+/-2 \%$ or $+/-0.100 \mathrm{~min}$ (whichever is greater) retention time of calibrators?
( 3. Did all QCs pass for each analyte? Y/N
龱 4. Central File Packet to include: LIMS Worklist, Method Checklist, Calibration and Control Reports
COMMENTS:


6LOZ／兀／t 90tIとてt0ヨコ JH」 C－THC
THC－OH
Ppd 8／17／17 Exp：2／17／18 lot 21718 by AMN
Stock solution $1 \mathrm{mg} / \mathrm{ml} 10 \mathrm{ul}$ each THC，THC－OH $100 \mathrm{ug} / \mathrm{ml} 100 \mathrm{ul} \mathrm{C-THC} \mathrm{in} 9880 \mathrm{ul} \mathrm{meOH}$ lot（Fisher 168427）
Toxicology AM method 27 external prep information
working solution $1 \mathrm{ug} / \mathrm{ml}$ in meoh $\mathrm{C}-\mathrm{THC}, \mathrm{THC}-\mathrm{OH}, \mathrm{THC}$

## ISP FORENSICS - Cd'A Instrument \#62340 <br> Cannabinoid Screen Report



Analysis Info

| Acq Time | 2018-01-02 13:07 |
| :--- | :--- |
| Sample Type | Sample |
| Dilution | 1 |
| Position | P1-a2 |
| Inj Vol | -1 |

D: \2018 Data\01022018 blood screen AM 25-26\QuantResults\01022018 cann screen.batch.bin 1/2/2018 3:57 PM Analyst Name ISP Tox 1/3/2018 8:37 AM Reporter Name ISP Tox 1/2/2018 3:57 PM Batch State Processed

Data File $\quad \mathrm{Neg}$ Control.d
Sample Name Neg Control
Acq Method Screen THC 8-2017.m
Sample Info
Comment

Sample Chromatogram


Results
Compound
THC-COOH

RT
3.546

Response 18783

ISTD Resp 442810

Resp Ratio 0.0424

Final Conc $2.1075<5$

## ISP FORENSICS - Cd'A Instrument \#62340

Cannabinoid Screen Report

| Batch Data Path | D:\2018 Data ${ }^{\text {a }} 1022018$ blood screen AM 25-26\QuantResults $\backslash 01022018$ cann screen.batch.bin |  |  |
| :---: | :---: | :---: | :---: |
| Analysis Time | 1/2/2018 3:57 PM | Analyst Name | ISP Tox |
| Report Time | 1/3/2018 8:37 AM | Reporter Name | ISP Tox |
| Last Calib Update | 1/2/2018 3:57 PM | Batch State | Processed |
| Analysis Info |  |  |  |
| Acq Time | 2018-01-02 13:27 | Data File | QC $10 \mathrm{ng} . \mathrm{d}$ |
| Sample Type | QC | Sample Name | QC 10 ng |
| Dilution | 1 | Acq Method | Screen THC 8-2017.m |
| Position | P1-H1 | Sample Info |  |
| Inj Vol | -1 | Comment | AM 26 Cannabinoid screen |

## Sample Chromatogram



## Results

| Compound | RT | Response | ISTD Resp | Resp Ratio | Final Conc |
| :--- | :--- | :--- | :--- | :--- | :--- |
| THC-OH | 3.473 | 49585 | 500407 | 0.0991 | 9.6881 |
| THC-COOH | 3.546 | 25782 | 124556 | 0.2070 | 10.6815 |
| THC | 3.820 | 3809 | 37667 | 0.1011 | 9.8916 |



## ISP Forensics

## Calibration Curve Report



| Sample | Level | Enabled | Exp Conc | Final Conc | Accuracy |
| :---: | :---: | :---: | :---: | :---: | :---: |
| cal $2-5 \mathrm{ng}$ | 2 | 区 | 5 | 4.8 | 95.7 |
| cal 3-10ng | 3 | V | 10 | 10.2 | 101.7 |
| QC 10 ng | 3 | 『 | 10 | 9.7 | 96.9 |
| cal 4-25ng | 4 | $\square$ | 25 | 24.7 | 99.0 |
| cal 5-50ng | 5 | W | 50 | 51.2 | 102.4 |
| cal 6-100ng | 6 | ■ | 100 | 102.6 | 102.6 |
| cal 7-250ng | 7 | ■ | 250 | 246.5 | 98.6 |

## ISP Forensics Calibration Curve Report



## ISP Forensics <br> Calibration Curve Report



# ISP FORENSICS - Cd'A Instrument \#62340 <br> Cannabinoid Screen Report 

## Batch Data Path Analysis Time Report Time Last Calib Update

| D: $\backslash 2018$ Data $\backslash 01022018$ | blood screen AM | 25-26\QuantResults $\backslash 01022018$ cann screen.batch.bin |
| :--- | :--- | :--- |
| 1/2/2018 3:57 PM | Analyst Name | ISP Tox |
| 1/3/2018 8:37 AM | Reporter Name | ISP Tox |
| 1/2/2018 3:57 PM | Batch State | Processed |
|  |  |  |
|  |  |  |
| 2018-01-02 12:34 | Data File | cal 2-5ng.d |
| Calibration | Sample Name | cal 2-5ng |
| 1 | Acq Method | Screen THC 8-2017.m |
| P1-B1 | Sample Info |  |
| -1 | Comment | AM 26 Cannabinoid screen |

Sample Chromatogram


| Results |  |  |  |  | Response |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Compound | RT | RestD Resp | Resp Ratio | Final Conc |  |
| THC-OH | 3.473 | 18859 | 371878 | 0.0507 | 4.7843 |
| THC-COOH | 3.546 | 10018 | 103313 | 0.0970 | 4.9495 |
| THC | 3.820 | 1453 | 29603 | 0.0491 | 4.9879 |

## ISP FORENSICS - Cd'A Instrument \#62340 <br> Cannabinoid Screen Report

| Batch Data Path | D: \2018 Data\01022018 blood screen AM 25-26\QuantResults\01022018 cann screen.batch.bin |  |  |
| :---: | :---: | :---: | :---: |
| Analysis Time | 1/2/2018 3:57 PM | Analyst Name | ISP Tox |
| Report Time | 1/3/2018 8:37 AM | Reporter Name | ISP Tox |
| Last Calib Update | 1/2/2018 3:57 PM | Batch State | Processed |
| Analysis Info |  |  |  |
| Acq Time | 2018-01-02 12:40 | Data File | cal 3-10ng.d |
| Sample Type | Calibration | Sample Name | cal 3-10ng |
| Dilution | 1 | Acq Method | Screen THC 8-2017.m |
| Position | P1-C1 | Sample Info |  |
| Inj Vol | -1 | Comment | AM 26 Cannabinoid screen |

## Sample Chromatogram



## Results

| Compound | RT |
| :--- | :--- |
| THC-OH | 3.473 |
| THC-COOH | 3.546 |
| THC | 3.820 |

Response
86417
76359
3376
ISTD Res
831888
383774
32113
Resp Ratio
0.1039
0.1990
0.1051

## Final Conc

10.1738
10.2635
10.2685

## ISP FORENSICS - Cd'A Instrument \#62340 <br> Cannabinoid Screen Report

| Batch Data Path | D:\2018 Data\010220 | ood screen AM 25 | 5-26\QuantResults\010220 |
| :---: | :---: | :---: | :---: |
| Analysis Time | 1/2/2018 3:57 PM | Analyst Name | ISP Tox |
| Report Time | 1/3/2018 8:37 AM | Reporter Name | ISP Tox |
| Last Calib Update | 1/2/2018 3:57 PM | Batch State | Processed |
| Analysis Info |  |  |  |
| Acq Time | 2018-01-02 12:47 | Data File | cal 4-25ng.d |
| Sample Type | Calibration | Sample Name | cal 4-25ng |
| Dilution | 1 | Acq Method | Screen THC 8-2017.m |
| Position | P1-D1 | Sample Info |  |
| Inj Vol | -1 | Comment | AM 26 Cannabinoid screen |

## Sample Chromatogram



| Results |  |  |  |  | Response |
| :--- | :--- | :--- | :--- | :--- | :--- |
| Compound | RT | ISTD Resp | Resp Ratio | Final Conc |  |
| THC-OH | 3.473 | 233022 | 941191 | 0.2476 | 24.7402 |
| THC-COOH | 3.546 | 188054 | 407521 | 0.4615 | 23.9387 |
| THC | 3.820 | 8083 | 31886 | 0.2535 | 24.2542 |

Batch Data Path Analysis Time Report Time Last Calib Update

## Analysis Info

Acq Time
Sample Type
Dilution
Position Inj Vol

D: \2018 Data\01022018 blood screen AM 25-26\QuantResults\01022018 cann screen.batch.bin 1/2/2018 3:57 PM Analyst Name ISP Tox 1/3/2018 8:37 AM Reporter Name ISP Tox 1/2/2018 3:57 PM Batch State Processed

2018-01-02 12:53
Calibration
1
P1-E1
-1

Data File
Sample Name
Acq Method
Sample Info
Comment
cal 5-50ng.d
cal 5 - 50 ng
Screen THC 8-2017.m

AM 26 Cannabinoid screen

## Sample Chromatogram



Results

| Results | RT |
| :--- | :--- |
| Compound | 3.473 |
| THC-OH | 3.546 |
| THC-COOH | 3.820 |

Response
409354
366862
16019

| ISTD Resp | Resp Ratio | Final Conc |
| :--- | :--- | :--- |
| 804696 | 0.5087 | 51.2092 |
| 364498 | 1.0065 | 52.3335 |
| 30478 | 0.5256 | 49.8979 |

## ISP FORENSICS - Cd'A Instrument \#62340 <br> Cannabinoid Screen Report

Batch Data Path
Analysis Time
Report Time
Last Calib Update

D:\2018 Data\01022018 blood screen AM 25-26\QuantResults\01022018 cann screen.batch.bin 1/2/2018 3:57 PM Analyst Name ISP Tox
1/3/2018 8:37 AM Reporter Name ISP Tox
1/2/2018 3:57 PM Batch State Processed
Analysis Info
Acq Time
Sample Type
Dilution
Position
Inj Vol

2018-01-02 13:00
Calibration
1
P1-F1
-1

Data File
Sample Name
Acq Method
Sample Info
Comment
cal 6-100ng.d
cal $6-100 \mathrm{ng}$
Screen THC 8-2017.m

AM 26 Cannabinoid screen

Sample Chromatogram


## Results

| Compound | RT |
| :--- | :--- |
| THC-OH | 3.473 |
| THC-COOH | 3.566 |
| THC | 3.820 |


| Response | ISTD Resp | Resp Ratio | Final Conc |
| :--- | :--- | :--- | :--- |
| 830095 | 817274 | 1.0157 | 102.5997 |
| 691971 | 346875 | 1.9949 | 103.8264 |
| 36511 | 34246 | 1.0661 | 100.8460 |



## ISP FORENSICS - Cd'A Instrument \#62340

Cannabinoid Screen Report

Batch Data Path
Analysis Time
Report Time
Last Calib Update
Analysis Info
Acq Time
Sample Type
Dilution
Position
Inj Vol

D:\2018 Data\01022018 blood screen AM 25-26\QuantResults\01022018 cann screen.batch.bin
1/2/2018 3:57 PM Analyst Name ISP Tox
1/3/2018 8:37 AM Reporter Name ISP Tox
1/2/2018 3:57 PM Batch State Processed
2018-01-02 13:13
Calibration
1
P1-G1
-1

2018-01-02 13:13
Calibration
P1-G1
-1

Data File
Sample Name
Acq Method
Sample Info
Comment
cal 7-250ng.d
cal 7-250ng
Screen THC 8-2017.m

AM 26 Cannabinoid screen

Sample Chromatogram


Results
Compound $\mathrm{THC}-\mathrm{OH}$
THC-COOH
THC

RT
3.473
3.546
3.820
Response
2490902
1812345
88164

| ISTD Resp | Resp Ratio | Final Conc |
| :--- | :--- | :--- |
| 1022860 | 2.4352 | 246.4929 |
| 401075 | 4.5187 | 235.3137 |
| 33320 | 2.6459 | 249.7455 |

