REVIEWED
By Anne Nord at 1:30 pm, Mar 12, 2020

3/10/2020

**Worklist: 4075**

| LAB_CASE | ITEM | ITEM_TYPE | DESCRIPTION |
|---|---|---|---|
| C2020-0441 | 1 | UCK | AM 3 Urine Carboxy-THC |

# AM 3: Carboxy-THC Urine Extraction

Extraction Date: 3/10/2020

Analyst: Britany Wylie

**Negative Urine Lot:** 11420
**Positive Control Working Solution Lot:** 31020
**Methanol Lot:** 156796
**Ethyl Acetate Lot:** 020419

**GC/MS ID:** 65198

**Hexane Lot:** 142712
**Silylating Agent Lot:** FN08181601

## Pre-Analytic:

☒ 1. *Positive Control Working Solution Preparation Instructions:*
*Add 180uL of 100ug/mL 11-nor-9-carboxy-Δ9-THC Stock Solution to 9.82mL Methanol. Other volumes may be prepared. Solution is stable for 1-year or the expiration of the stock reference material (whichever is sooner). Store under refrigeration*

☒ 2. Verify Tune and Tune evaluation completed within the previous 7 days. Tune and Tune evaluation reports initialed and filed.

☒ 3. Create GCMS sequence to include controls, case blanks and case samples.

## Analytic:

☒ 1. Remove working solutions, external control, negative urine and case samples from cold storage.

☒ 2. To each labeled round bottomed tube add 3mL sample, using negative urine sample for both negative and positive control. Positive control: spike negative urine with 100uL positive control working solution.

☒ 3. Add 500uL 1N KOH to all tubes. Check pH. *(If pH <12, add additional 500ul 1N KOH). (Note: put a mark on the tube or separate the tubes that have a pH<12 as you will need to know this in step 5).*

☒ 4. Place tubes in 40C water bath for ~15 min. Remove and allow to cool.

☒ 5. If original pH was >12, add 1.5mL pH 1.8 Saturated Phosphate Buffer and 3mL Hexane/Ethyl Acetate *(87:13)*
If original pH was <12, add 3mL pH 1.8 Saturated Phosphate Buffer and 4mL Hexane/Ethyl Acetate *(87:13)*

☒ 6. Rock at ambient temp for ~ 10 minutes.

☒ 7. Centrifuge for ~ 10 min at ~3500rpm.

☒ 8. Transfer solvent to tapered bottom tube and evaporate to dryness under nitrogen @ 37C.

☒ 9. Add 50uL Ethyl Acetate and 50uL silylating reagent, cap and vortex. Heat @ 95C for 15min, then allow to cool.

☒ 10. Transfer sample to labeled ALS vial with insert.

☒ 11. Place ALS Vials in appropriate location on GCMS rack and run using appropriate GCMS method.

## Post-Analytic

☒ 1. Complete Data analysis on all samples and corresponding sample blanks
☒ 2. Did positive and negative control samples provide intended response? Y / N
☒ 3. **Criteria for ID**: RT +/1 0.1 min., Ion Ratio of 347:473 & 371:488 within +/- 20%.
Sample response greater than Min Corrected Area, Diluted samples
☒ 4. Central File Packet to include: LIMS Worklist, Method Checklist, Working solution prep sheet, and Control sample GCMS data printouts

Comments:

Area Percent Report

Data Path : D:\DATA\2020\am 2\31020 am 2 and am3\
Data File : 00701011.D
Acq On : 10 Mar 2020 13:55
Operator : Instrument 65198
Sample : NCMJ
Misc :
ALS Vial : 7 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 3 % of largest Peak
Start Thrs: 0.2 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : D:\MassHunter\GCMS\1\METHODS\DEFAULT.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): 00701011.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.246 | 3 | 12 | 31 | rVB | 10056 | 46250 | 100.00% | 53.465% |
| 2 | 4.520 | 54 | 63 | 70 | rBV | 24992 | 40255 | 87.04% | 46.535% |

Sum of corrected areas: 86505

Signal : EIC Ion 473.00 (472.70 to 473.70): 00701011.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.499 | 53 | 59 | 69 | rVB | 718 | 1954 | 100.00% | 47.531% |
| 2 | 4.585 | 71 | 75 | 91 | rVB2 | 268 | 734 | 37.56% | 17.855% |
| 3 | 5.031 | 152 | 158 | 165 | rVB2 | 692 | 1267 | 64.84% | 30.820% |
| 4 | 5.171 | 173 | 184 | 185 | rBV2 | 45 | 156 | 7.98% | 3.795% |

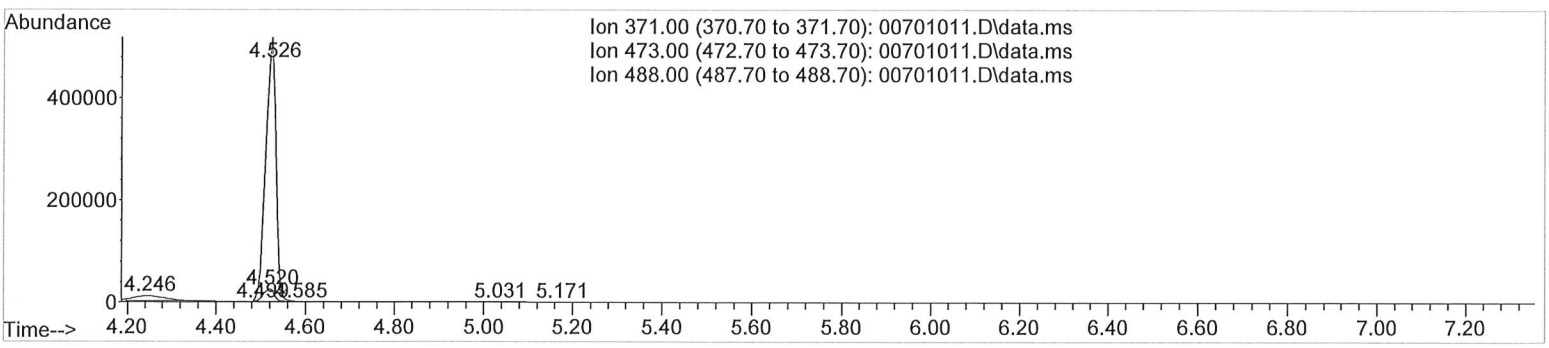
Sum of corrected areas: 4111

Signal : EIC Ion 488.00 (487.70 to 488.70): 00701011.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.526 | 53 | 64 | 98 | rBV | 517832 | 810158 | 100.00% | 100.000% |

Sum of corrected areas: 810158

DEFAULT.M Tue Mar 10 14:30:29 2020



Area Percent Report

Data Path : D:\DATA\2020\am 2\31020 am 2 and am3\
Data File : 00601010.D
Acq On : 10 Mar 2020 13:46
Operator : Instrument 65198
Sample : PC MJ
Misc : am 3
ALS Vial : 6 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 3 % of largest Peak
Start Thrs: 0.2 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : D:\MassHunter\GCMS\1\METHODS\DEFAULT.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): 00601010.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.251 | 3 | 13 | 31 | rVB | 7355 | 35994 | 11.34% | 9.108% |
| 2 | 4.520 | 54 | 63 | 67 | rBV | 25504 | 41698 | 13.13% | 10.552% |
| 3 | 4.579 | 67 | 74 | 94 | rVB | 183119 | 317491 | 100.00% | 80.340% |

Sum of corrected areas: 395183

Signal : EIC Ion 473.00 (472.70 to 473.70): 00601010.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.579 | 66 | 74 | 95 | rVB | 88367 | 149252 | 100.00% | 100.000% |

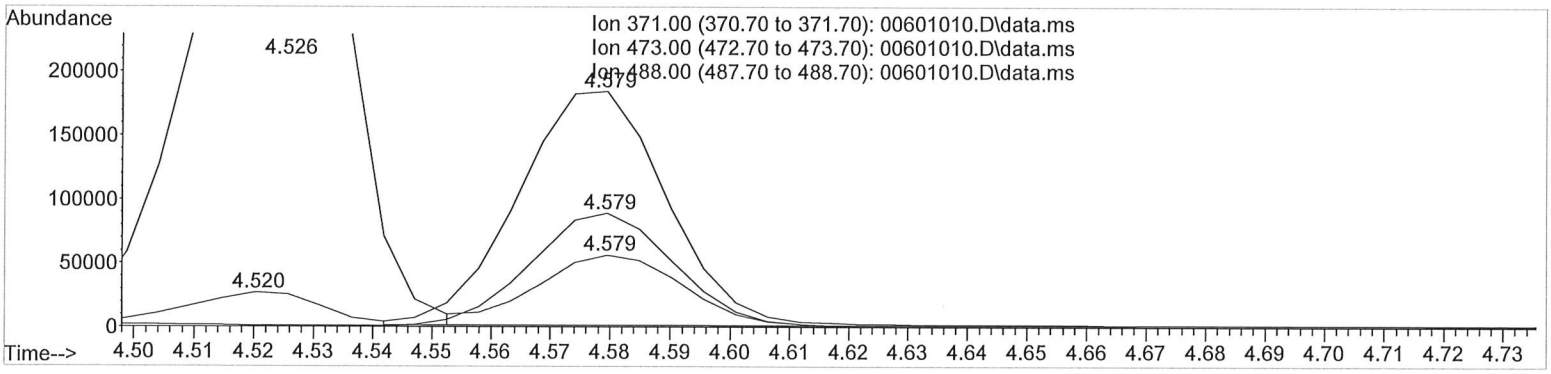
Sum of corrected areas: 149252

Signal : EIC Ion 488.00 (487.70 to 488.70): 00601010.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.526 | 52 | 64 | 69 | rBV | 535861 | 835709 | 100.00% | 89.695% |
| 2 | 4.579 | 69 | 74 | 85 | rVB | 55696 | 96017 | 11.49% | 10.305% |

Sum of corrected areas: 931726

DEFAULT.M Tue Mar 10 14:30:58 2020



Area Percent Report

BW

Data Path : D:\DATA\2020\am 2\31020 am 2 and am3\
Data File : 00601015.D
Acq On : 10 Mar 2020 14:32
Operator : Instrument 65198
Sample : PC MJ
Misc : am 3
ALS Vial : 6 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 3 % of largest Peak
Start Thrs: 0.2 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : D:\MassHunter\GCMS\1\METHODS\DEFAULT.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): 00601015.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.521 | 56 | 63 | 67 | rBV | 30122 | 47221 | 12.92% | 11.443% |
| 2 | 4.580 | 67 | 74 | 96 | rVB | 207062 | 365455 | 100.00% | 88.557% |

Sum of corrected areas: 412676

Signal : EIC Ion 473.00 (472.70 to 473.70): 00601015.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.580 | 66 | 74 | 94 | rVB | 99300 | 170892 | 100.00% | 100.000% |

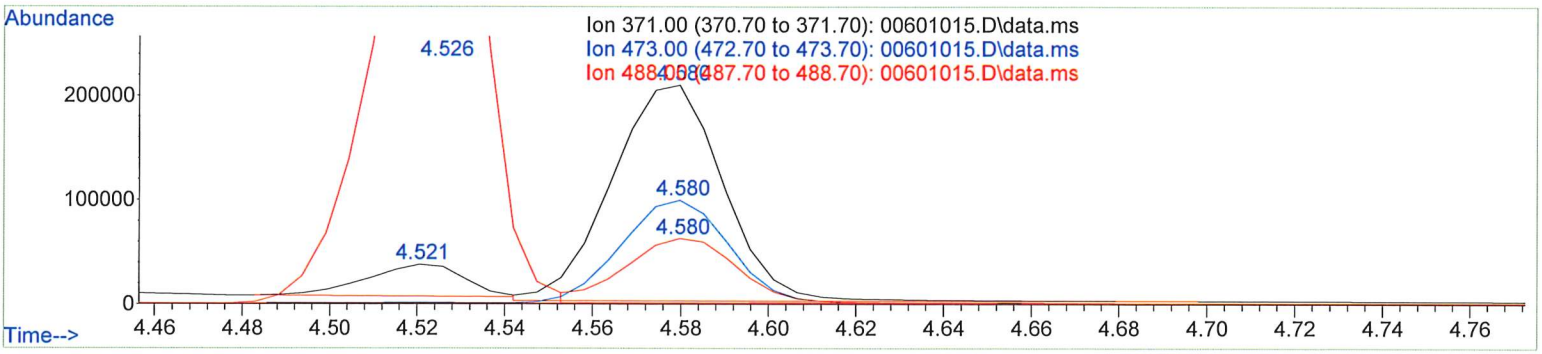
Sum of corrected areas: 170892

Signal : EIC Ion 488.00 (487.70 to 488.70): 00601015.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.526 | 53 | 64 | 69 | rBV | 576593 | 907383 | 100.00% | 89.323% |
| 2 | 4.580 | 69 | 74 | 85 | rVB | 61886 | 108459 | 11.95% | 10.677% |

Sum of corrected areas: 1015842

DEFAULT.M Wed Mar 11 07:13:27 2020



# Area Percent Report

Data Path : D:\DATA\2020\am 2\31020 am 2 and am3\
Data File : 00801012.D
Acq On : 10 Mar 2020 14:04
Operator : Instrument 65198
Sample : BRC3
Misc :
ALS Vial : 8 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 3 % of largest Peak
Start Thrs: 0.2 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : D:\MassHunter\GCMS\1\METHODS\DEFAULT.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): 00801012.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.246 | 3 | 12 | 39 | rVB2 | 10422 | 53993 | 56.51% | 28.318% |
| 2 | 4.520 | 54 | 63 | 67 | rBV | 23846 | 37803 | 39.57% | 19.827% |
| 3 | 4.574 | 67 | 73 | 99 | rVB | 56324 | 95538 | 100.00% | 50.108% |
| 4 | 4.779 | 105 | 111 | 130 | rVB | 1574 | 3330 | 3.49% | 1.747% |

Sum of corrected areas: 190664

Signal : EIC Ion 473.00 (472.70 to 473.70): 00801012.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.499 | 53 | 59 | 66 | rBV | 467 | 1355 | 3.02% | 2.532% |
| 2 | 4.574 | 66 | 73 | 97 | rVB | 25944 | 44934 | 100.00% | 83.957% |
| 3 | 4.779 | 104 | 111 | 120 | rVB | 1058 | 1822 | 4.05% | 3.404% |
| 4 | 5.031 | 146 | 158 | 172 | rVB3 | 1821 | 5409 | 12.04% | 10.107% |

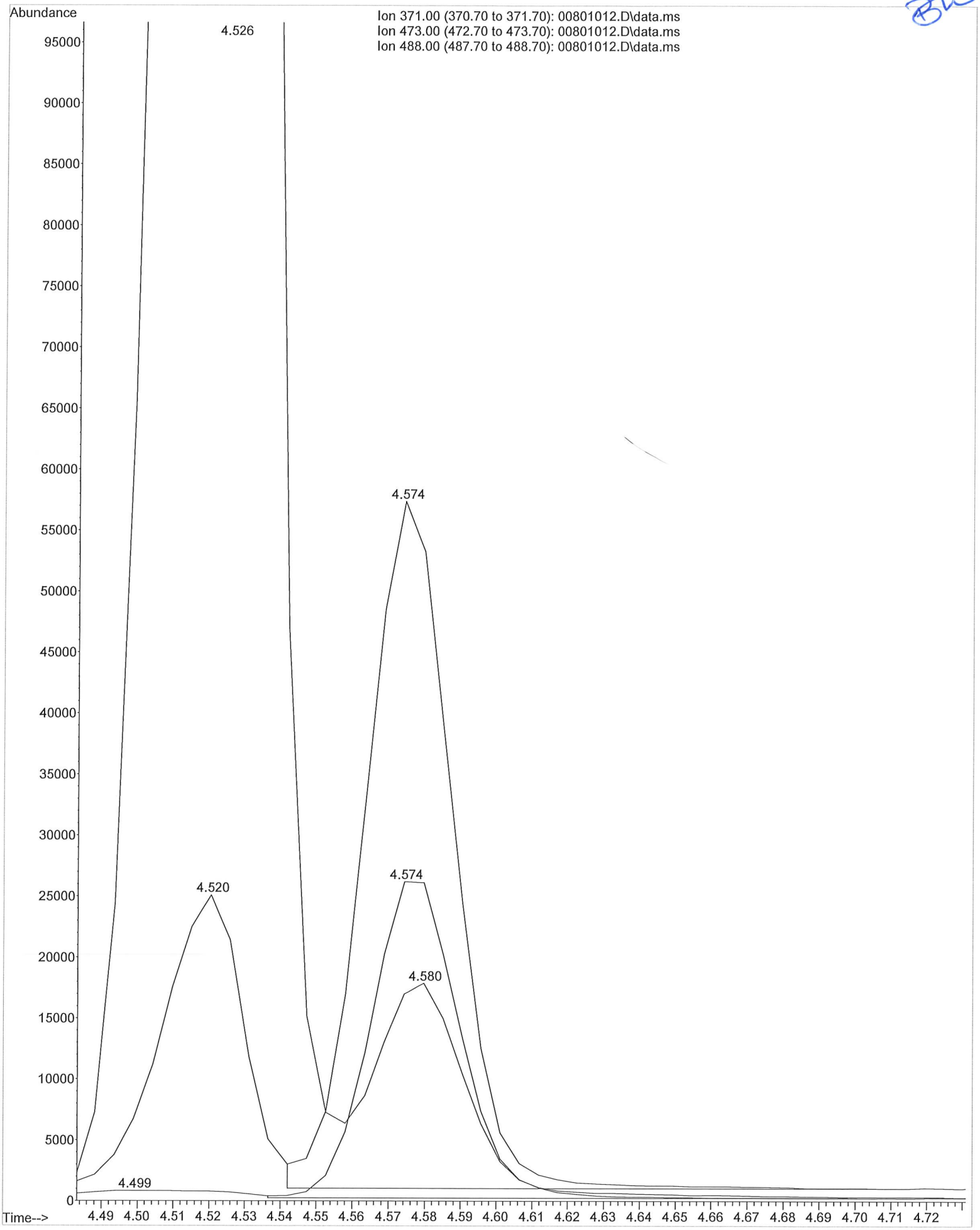
Sum of corrected areas: 53520

Signal : EIC Ion 488.00 (487.70 to 488.70): 00801012.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 4.526 | 52 | 64 | 70 | rBV | 484258 | 768183 | 100.00% | 96.064% |
| 2 | 4.580 | 70 | 74 | 105 | rVB | 17638 | 31477 | 4.10% | 3.936% |

Sum of corrected areas: 799660

DEFAULT.M Tue Mar 10 14:32:09 2020


Abundance
Ion 371.00 (370.70 to 371.70): 00801012.D\data.ms
Ion 473.00 (472.70 to 473.70): 00801012.D\data.ms
Ion 488.00 (487.70 to 488.70): 00801012.D\data.ms
4.526
4.574
4.520
4.574
4.580
4.499
95000
90000
85000
80000
75000
70000
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
15000
10000
5000
0
Time-->
4.49 4.50 4.51 4.52 4.53 4.54 4.55 4.56 4.57 4.58 4.59 4.60 4.61 4.62 4.63 4.64 4.65 4.66 4.67 4.68 4.69 4.70 4.71 4.72

# AM 3 carboxy-THC Calculations Sheet

BW

| | |
|---|---|
| **Run Date:** | 3/10/2020 |
| **Worklist #:** | 4075 |
| **Laboratory Case #:** | BRC3 Lot 68460 |

**Case Calculations:**

| | |
|---|---|
| EIC Ion 371 Corr. Area: | 95538 |
| EIC Ion 473 Corr. Area: | 44934 |
| EIC Ion 488 Corr. Area: | 31477 |

473:371 Ratio: **0.470**

488:371 Ratio: **0.329**

**Quality Control Calculations:**

| | 1st Control | 2nd Control |
|---|---|---|
| EIC Ion 371 Corr. Area: | 317491 | 365455 |
| EIC Ion 473 Corr. Area: | 149252 | 170892 |
| EIC Ion 488 Corr. Area: | 96017 | 108459 |
| Ratio of 473:371: | 0.470 | 0.468 |
| | | Average: 0.469 |
| Ratio of 488:371: | 0.302 | 0.297 |
| | | Average: 0.300 |

| | | | |
|---|---|---|---|
| **Acceptable Retention Time Range:** | 4.480 ~~4.479~~ BW 3-12-20 | to | 4.679 ~~4.680~~ BW 3-12-20 |
| **Approximate Minimum Corrected Area of 371 Ion:** | | | 63498 |
| **Acceptable 473:371 Ratio Range:** | 0.375 | to | 0.563 |
| **Acceptable 488:371 Ratio Range:** | 0.240 | to | 0.360 |

BW

Toxicology AM method 3 external urine preparation information

Stock solution 45 ul (100 ug/ml) C-THC in 2.455 mls neg urine lot 11420
Ppd 3/10/20 Exp: 9/1/20 lot 31020 by baw

| Drug | lot | expiration |
|---|---|---|
| C-THC | FE07171501 | 9/1/2020 |