APPROVED
By Anne Nord at 10:31 am, Sep 13, 2019

9/4/2019

**Worklist: 3651**

| LAB_CASE | ITEM | TASK_ID | DESCRIPTION |
|---|---|---|---|
| C2019-1463 | 1 | 162299 | AM 3 Urine Carboxy-THC |
| C2019-1606 | 2 | 162300 | AM 3 Urine Carboxy-THC |
| M2019-3369 | 7 | 162301 | AM 3 Urine Carboxy-THC |
| M2019-3613 | 4 | 162303 | AM 3 Urine Carboxy-THC |
| M2019-3816 | 3 | 162305 | AM 3 Urine Carboxy-THC |
| P2019-2437 | 1 | 162307 | AM 3 Urine Carboxy-THC |
| P2019-2546 | 1 | 162308 | AM 3 Urine Carboxy-THC |
| P2019-2627 | 1 | 162309 | AM 3 Urine Carboxy-THC |
| P2019-2636 | 1 | 162310 | AM 3 Urine Carboxy-THC |

# AM 3: Carboxy-THC Urine Extraction

**Extraction Date:** 09/04/2019
**Analyst:** Celena Shrum
**Negative Urine Lot**: POC031319
**GC/MS ID**: Major Mass Spec
**Positive Control Working Solution Lot:** WS012319
**BioRad C3 Control Lot:** 68460
**1N KOH Lot:** 091817
**Hexane Lot:** BDH 121015A
**Ethyl Acetate Lot:** BDH 121615D
**BSTFA + 1% TMCS Lot:** Cerilliant FN08231301
**Potassium Phosphate Buffer Lot:** 020118

## 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
GCMS Data path: D:\DATA\CDS\2019\am 2 worklist 3668 second run and am 3 worklist 3651

☒ 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, and Control sample GCMS data printouts

Comments:

# Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 2 worklist 3668 second run and am 3 worklist 3651\
Data File : MJ-THC-PC1.D
Acq On : 04 Sep 2019 21:06
Operator : ISP\datastor
Sample : Spiked Positive c-THC Control
Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3668
ALS Vial : 32 Sample Multiplier: 1

Integration Parameters: events.e
Integrator: ChemStation

Method : C:\gcms\1\methods\TOX.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): MJ-THC-PC1.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.193 | 337 | 385 | 434 | BV | 10824 | 205536 | 100.00% | 100.000% |

Sum of corrected areas: 205536

Signal : EIC Ion 473.00 (472.70 to 473.70): MJ-THC-PC1.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.194 | 370 | 386 | 411 | BB | 3624 | 62913 | 100.00% | 100.000% |

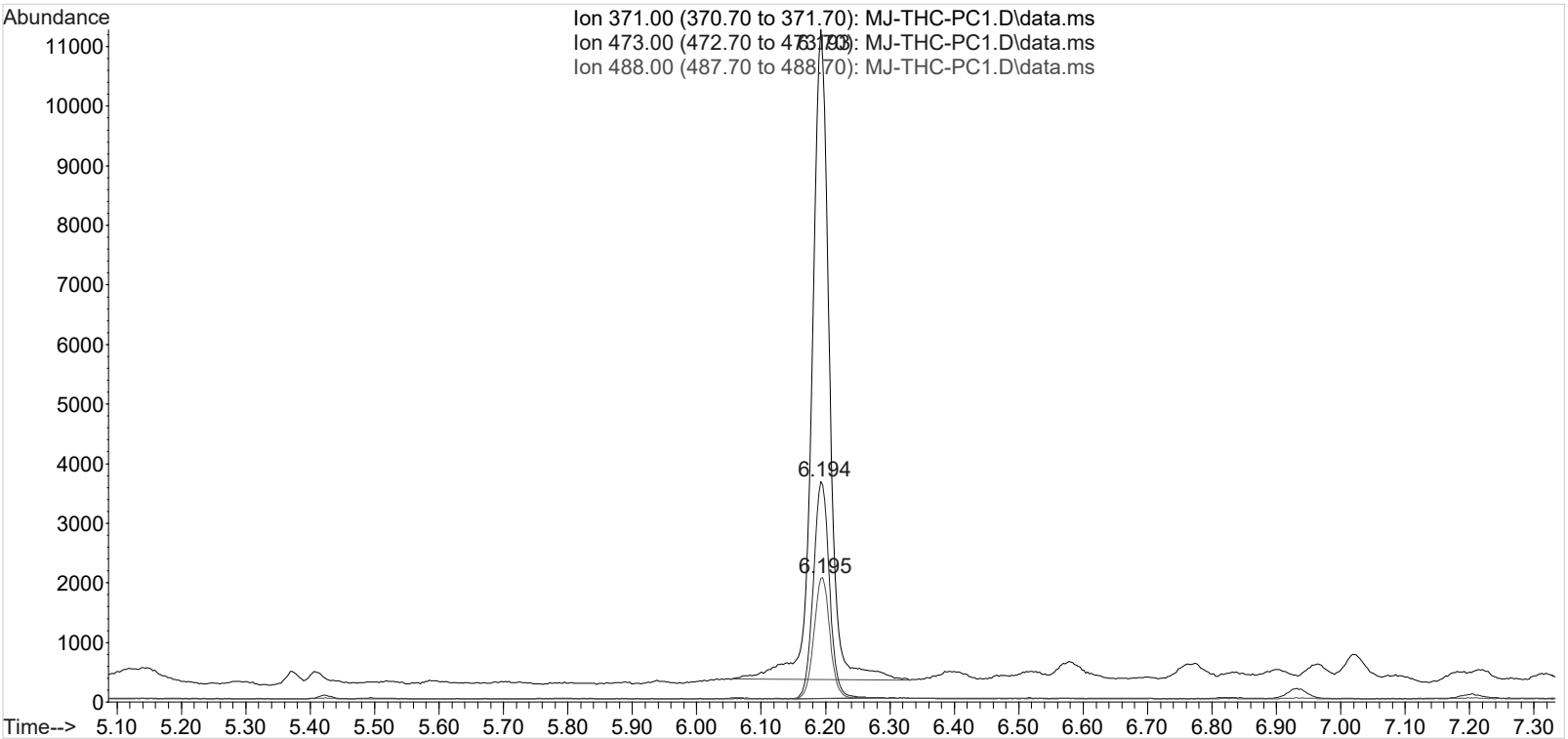
Sum of corrected areas: 62913

Signal : EIC Ion 488.00 (487.70 to 488.70): MJ-THC-PC1.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.195 | 370 | 386 | 409 | BB | 2032 | 35048 | 100.00% | 100.000% |

Sum of corrected areas: 35048

TOX.M Thu Sep 05 12:08:52 2019



# Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 2 worklist 3668 second run and am 3 worklist 3651\
Data File : THC-PC2.D
Acq On : 05 Sep 2019 02:33
Operator : ISP\datastor
Sample : Spiked Positive c-THC Control
Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3668
ALS Vial : 32 Sample Multiplier: 1

Integration Parameters: events.e
Integrator: ChemStation

Method : C:\gcms\1\methods\TOX.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): THC-PC2.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.196 | 287 | 386 | 439 | BV | 12194 | 236940 | 100.00% | 100.000% |

Sum of corrected areas: 236940

Signal : EIC Ion 473.00 (472.70 to 473.70): THC-PC2.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.197 | 369 | 387 | 410 | BB | 3994 | 69062 | 100.00% | 100.000% |

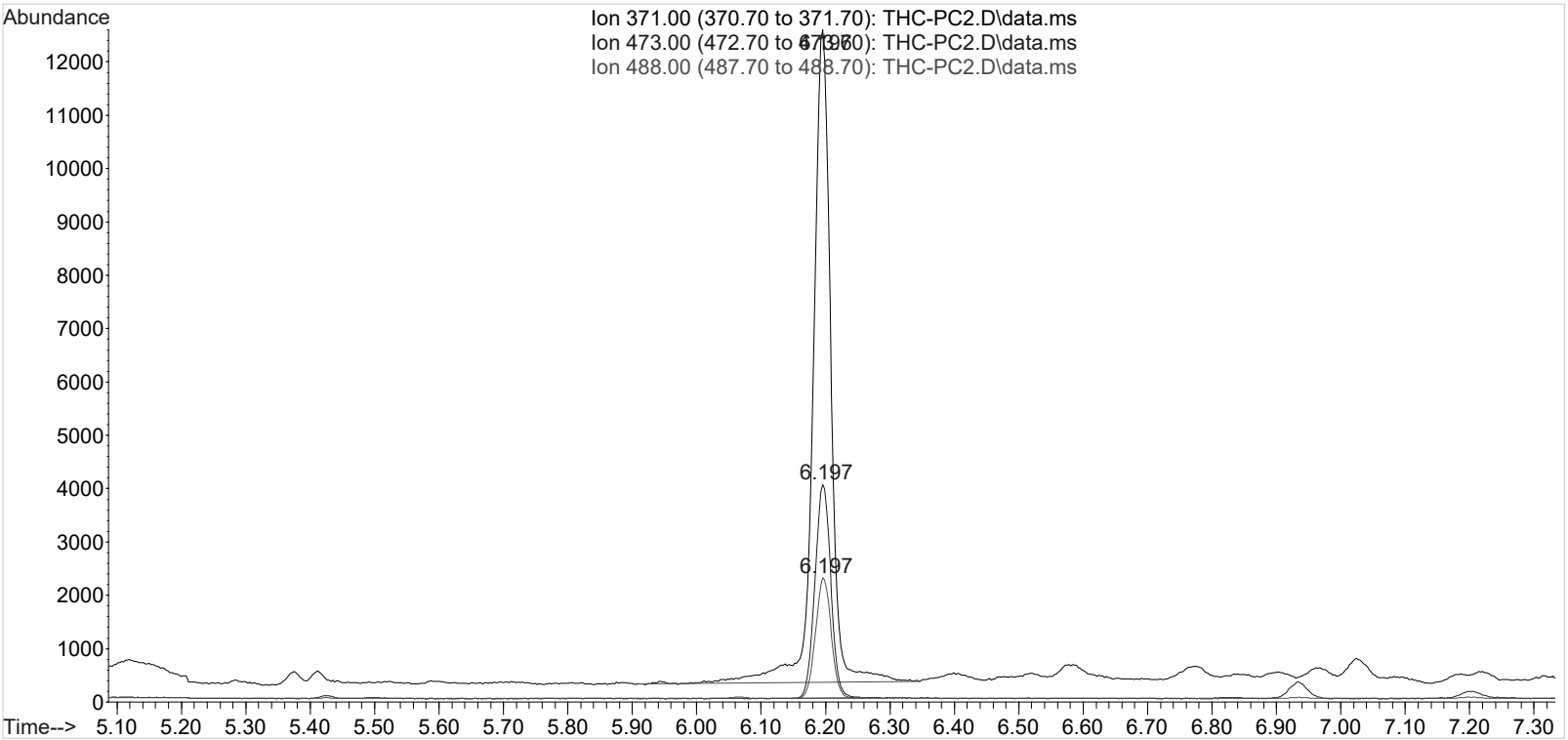
Sum of corrected areas: 69062

Signal : EIC Ion 488.00 (487.70 to 488.70): THC-PC2.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.197 | 370 | 387 | 408 | BB | 2250 | 38709 | 100.00% | 100.000% |

Sum of corrected areas: 38709

TOX.M Thu Sep 05 12:09:24 2019



Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 2 worklist 3668 second run and am 3 worklist 3651\
Data File : MJ-THC-NC.D
Acq On : 04 Sep 2019 20:55
Operator : ISP\datastor
Sample : Negative Control
Misc : Lot # POC031319; AM #3 Worklist 3668
ALS Vial : 31 Sample Multiplier: 1

Integration Parameters: events.e
Integrator: ChemStation

Method : C:\gcms\1\methods\TOX.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): MJ-THC-NC.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|

No peaks were detected using the method integration parameters!

Signal : EIC Ion 473.00 (472.70 to 473.70): MJ-THC-NC.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|

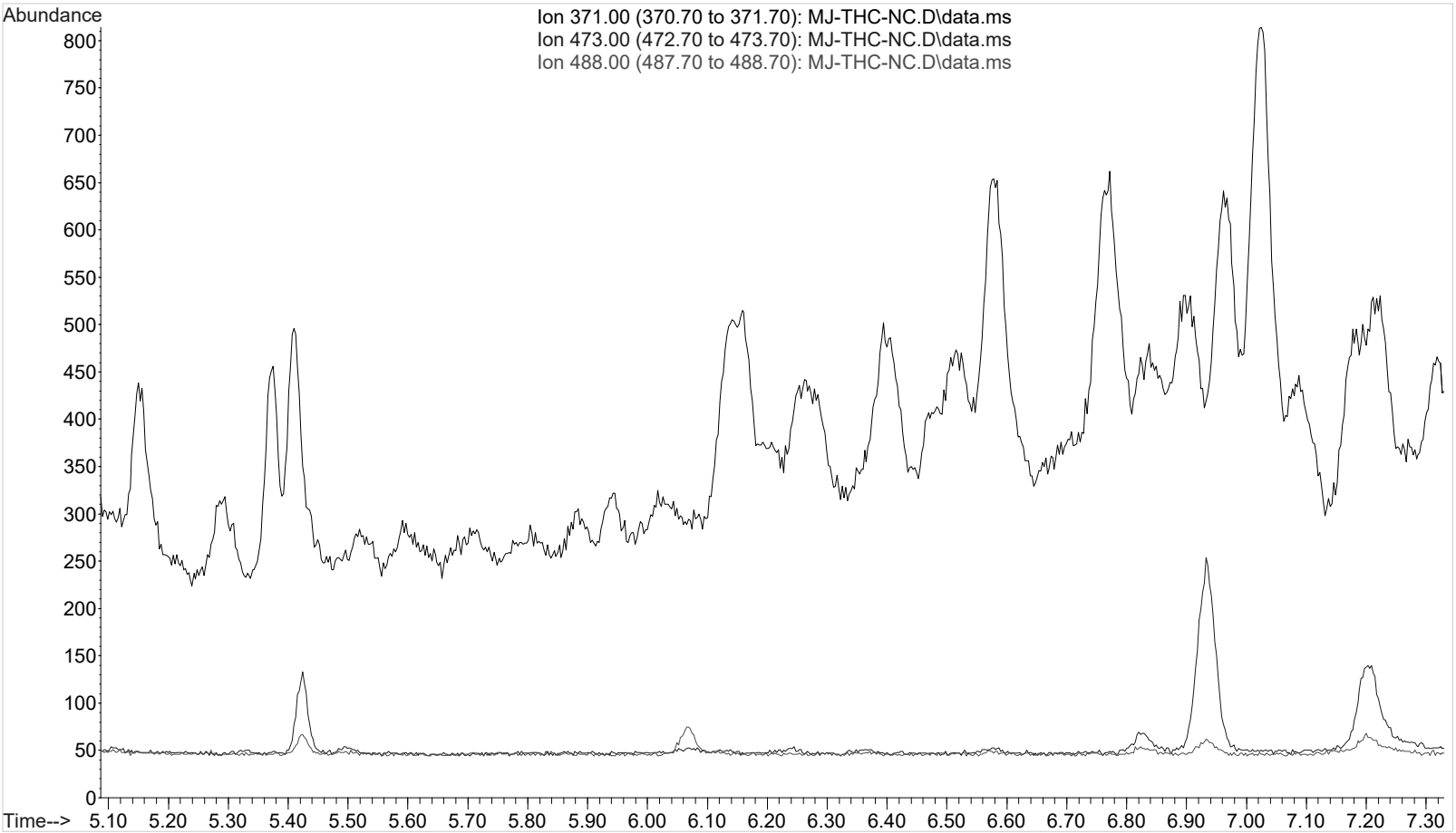
No peaks were detected using the method integration parameters!

Signal : EIC Ion 488.00 (487.70 to 488.70): MJ-THC-NC.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|

No peaks were detected using the method integration parameters!

TOX.M Thu Sep 05 12:09:38 2019



Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 2 worklist 3668 second run and am 3 worklist 3651\
Data File : MJ-THC-C3.D
Acq On : 04 Sep 2019 21:27
Operator : ISP\datastor
Sample : Biorad C3 Control
Misc : C3 Lot # 68460; Worklist 3668
ALS Vial : 33 Sample Multiplier: 1

Integration Parameters: events.e
Integrator: ChemStation

Method : C:\gcms\1\methods\TOX.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): MJ-THC-C3.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.192 | 346 | 385 | 400 | BV 2 | 2257 | 42558 | 100.00% | 100.000% |

Sum of corrected areas: 42558

Signal : EIC Ion 473.00 (472.70 to 473.70): MJ-THC-C3.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.193 | 369 | 385 | 405 | BB 2 | 769 | 13454 | 100.00% | 100.000% |

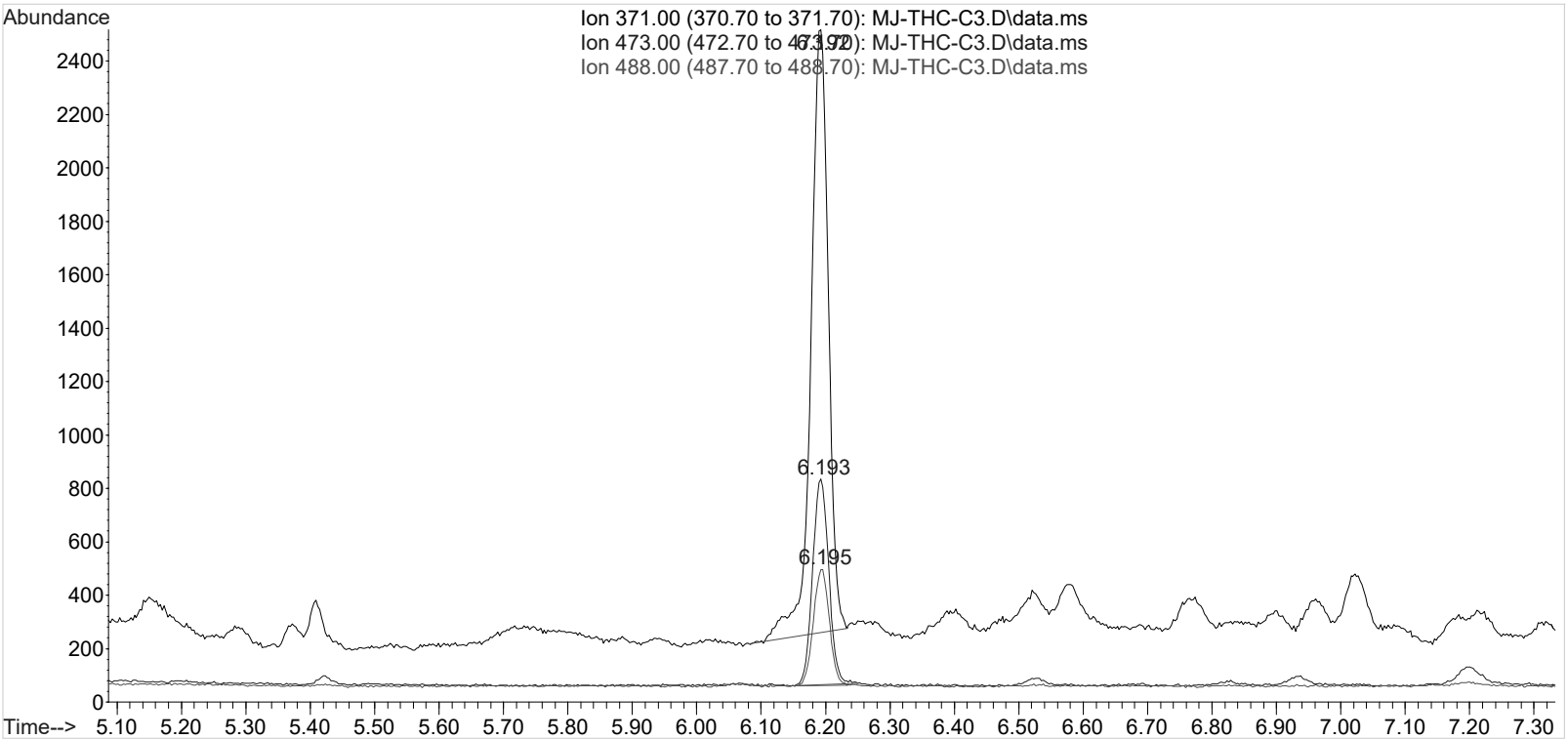
Sum of corrected areas: 13454

Signal : EIC Ion 488.00 (487.70 to 488.70): MJ-THC-C3.D\data.ms

| peak # | R.T. min | first scan | max scan | last scan | PK TY | peak height | corr. area | corr. % max. | % of total |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 6.195 | 374 | 386 | 400 | BB 2 | 434 | 7479 | 100.00% | 100.000% |

Sum of corrected areas: 7479

TOX.M Thu Sep 05 12:09:54 2019



| | |
|---|---|
| **Run Date:** | 9/4/2019 |
| **Worklist #:** | 3651 |
| **Laboratory Case #:** | BioRad C3 Lot: 68460 |

**Case Calculations:**

| | |
|---|---|
| EIC Ion 371 Corr. Area: | *42558* |
| EIC Ion 473 Corr. Area: | *13454* |
| EIC Ion 488 Corr. Area: | *7479* |

473:371 Ratio: **0.316**
488:371 Ratio: **0.176**

**Quality Control Calculations:**

| | 1st Control | 2nd Control |
|---|---|---|
| EIC Ion 371 Corr. Area: | *205536* | *236940* |
| EIC Ion 473 Corr. Area: | *62913* | *69062* |
| EIC Ion 488 Corr. Area: | *35048* | *38709* |
| Ratio of 473:371: | *0.306* | *0.291* |
| | | Average: *0.299* |
| Ratio of 488:371: | *0.171* | *0.163* |
| | | Average: *0.167* |

| | | | |
|---|---|---|---|
| **Acceptable Retention Time Range:** | *6.096* | to | *6.293* |
| **Approximate Minimum Corrected Area of 371 Ion:** | | | *41107* |
| **Acceptable 473:371 Ratio Range:** | *0.239* | to | *0.359* |
| **Acceptable 488:371 Ratio Range:** | *0.134* | to | *0.200* |