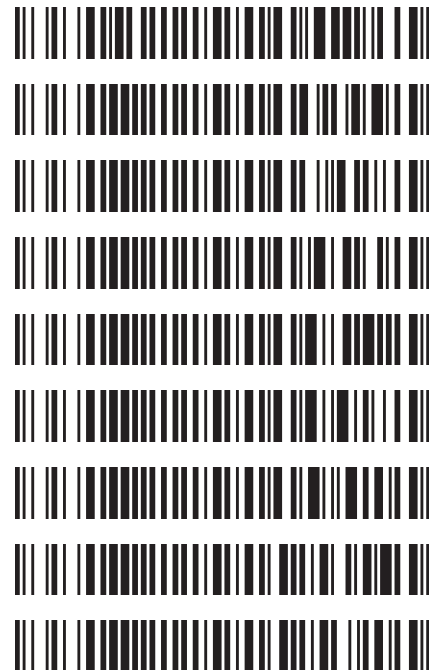


Worklist: 3247

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>
M2019-1340	4	147728	AM 3 Urine Carboxy-THC
P2019-0866	1	147729	AM 3 Urine Carboxy-THC
P2019-0950	1	147731	AM 3 Urine Carboxy-THC
P2019-0997	1	147733	AM 3 Urine Carboxy-THC
P2019-1006	1	147734	AM 3 Urine Carboxy-THC
P2019-1017	1	147736	AM 3 Urine Carboxy-THC
P2019-1024	1	147737	AM 3 Urine Carboxy-THC
P2019-1093	1	147738	AM 3 Urine Carboxy-THC
P2019-1094	1	147739	AM 3 Urine Carboxy-THC



AM 3: Carboxy-THC Urine Extraction

Extraction Date: 04/09/2019
Negative Urine Lot: POC031319
Positive Control Working Solution Lot: WS012319
1N KOH Lot: 091817
Ethyl Acetate Lot: BDH 121615D
Potassium Phosphate Buffer Lot: 020118

Analyst: Celena Shrum
GC/MS ID: Major Mass Spec
BioRad C3 Control Lot: 68460
Hexane Lot: BDH 121015A
BSTFA + 1% TMCS Lot: Cerilliant FN08231301

Pre-Analytic:

- 1. *Positive Control Working Solution Preparation Instructions:*
 Add 180uL of 100ug/mL 11-nor-9-carboxy- Δ 9-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 3 worklist 3247
- 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

CS

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3247\
Data File : THC-PC1.D
Acq On : 09 Apr 2019 12:54
Operator : ISP\Datastor
Sample : Spiked Positive c-THC Control
Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3247
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-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	5.670	18	30	40	BV	116	1713	6.66%	5.626%
2	6.557	297	338	356	PV	1347	25714	100.00%	84.468%
3	6.613	356	358	365	VV 2	17	187	0.73%	0.614%
4	6.789	395	419	430	PV	31	856	3.33%	2.811%
5	6.982	475	486	497	VB	38	746	2.90%	2.451%
6	7.198	534	561	571	BV	43	1227	4.77%	4.031%

Sum of corrected areas: 30442

Signal : EIC Ion 473.00 (472.70 to 473.70): 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	6.558	317	338	364	BB	580	10642	100.00%	100.000%

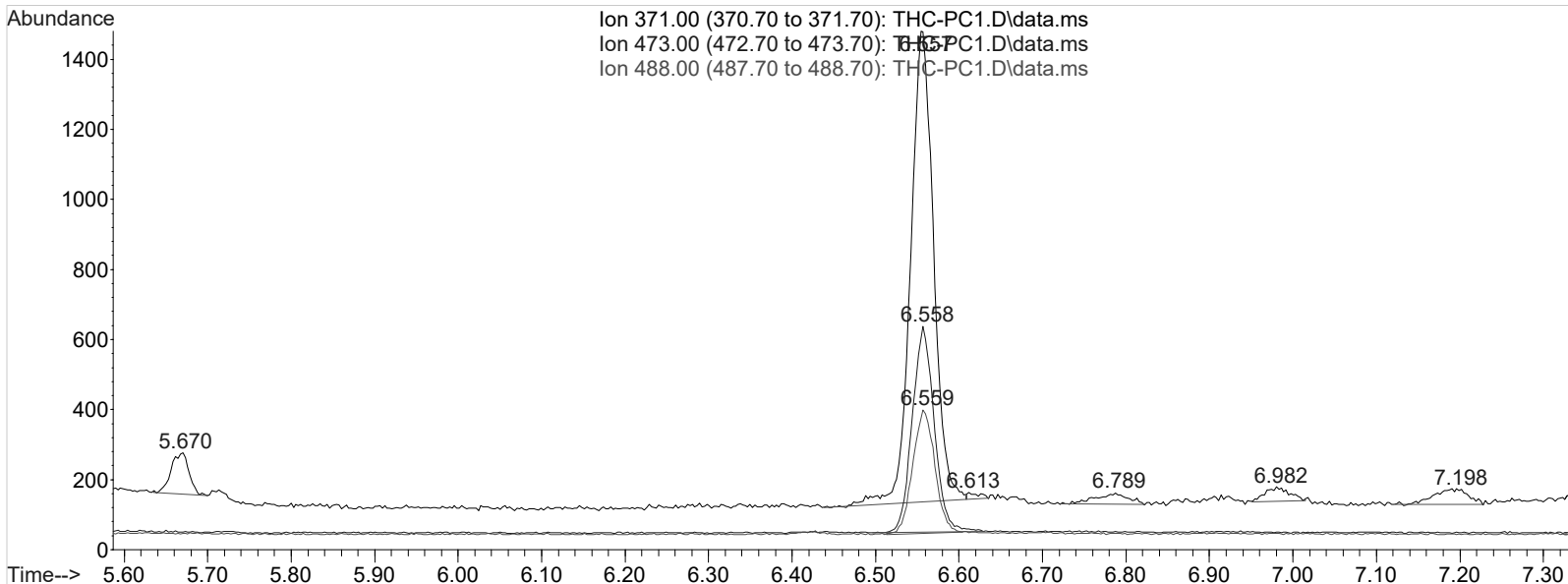
Sum of corrected areas: 10642

Signal : EIC Ion 488.00 (487.70 to 488.70): 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	6.559	323	339	354	BV	350	6432	100.00%	100.000%

Sum of corrected areas: 6432

TOX.M Thu Apr 11 09:37:51 2019



Area Percent Report

9

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3247\
Data File : THC-PC2.D
Acq On : 09 Apr 2019 20:21
Operator : ISP\Datastor
Sample : Spiked Positive c-THC Control
Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3247
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	5.664	11	28	37	BV	76	1198	4.52%	4.038%
2	6.552	292	336	380	PV	1317	26504	100.00%	89.341%
3	6.970	474	481	496	BV	38	879	3.32%	2.963%
4	7.183	531	555	571	PV	38	1085	4.09%	3.658%

Sum of corrected areas: 29666
Signal : EIC Ion 473.00 (472.70 to 473.70): THC-PC2.D\data.ms

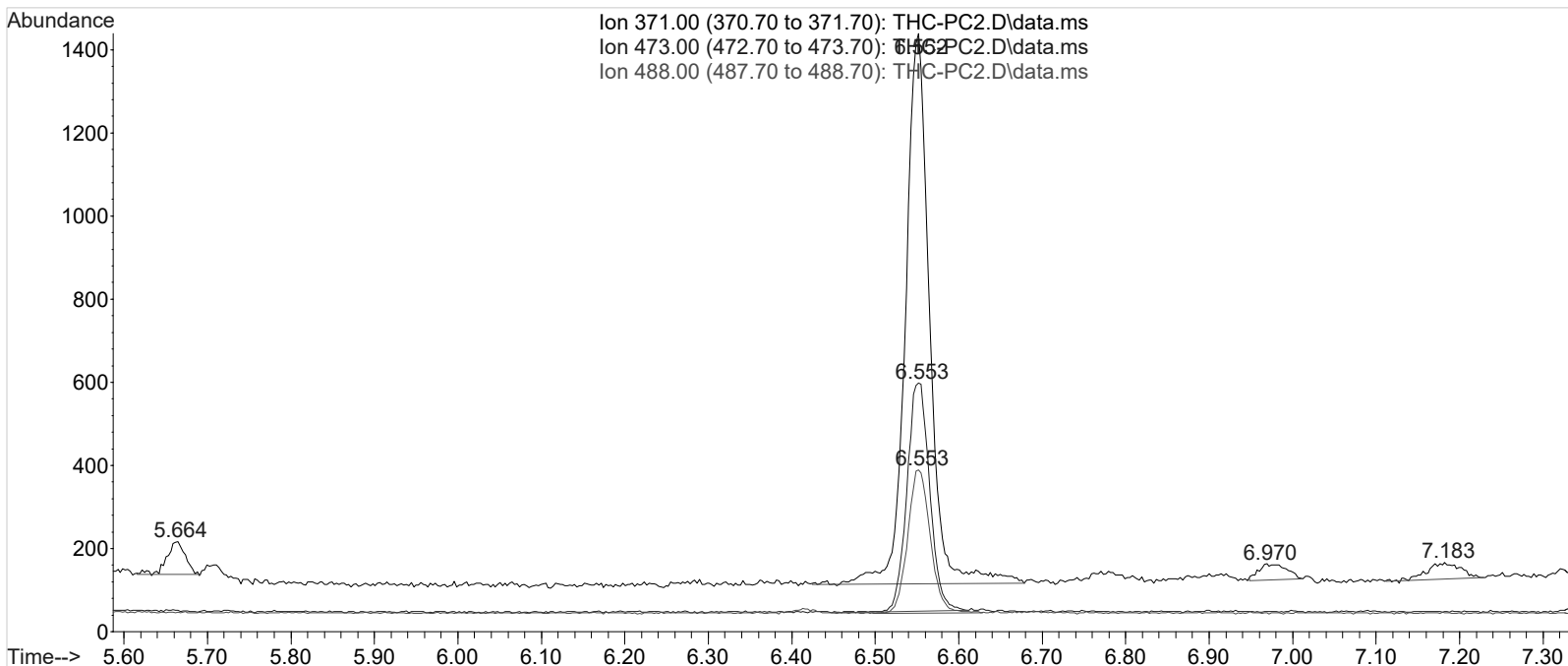
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.553	300	337	357	PV	550	10334	100.00%	100.000%

Sum of corrected areas: 10334
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	6.553	317	336	363	BB	345	6469	100.00%	100.000%

Sum of corrected areas: 6469

TOX.M Thu Apr 11 09:38:22 2019



Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3247\
Data File : THC-NC.D
Acq On : 09 Apr 2019 12:43
Operator : ISP\Datastor
Sample : Negative Control
Misc : Lot # POC031319; AM #3 Worklist 3247
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): 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
1	5.674	7	31	40	BV	58	730	51.36%	16.814%
2	6.501	295	318	339	PB	29	800	56.28%	18.423%
3	6.986	474	487	515	VB	49	1391	97.84%	32.028%
4	7.191	522	558	575	BV	49	1422	100.00%	32.736%

Sum of corrected areas: 4344
Signal : EIC Ion 473.00 (472.70 to 473.70): THC-NC.D\data.ms

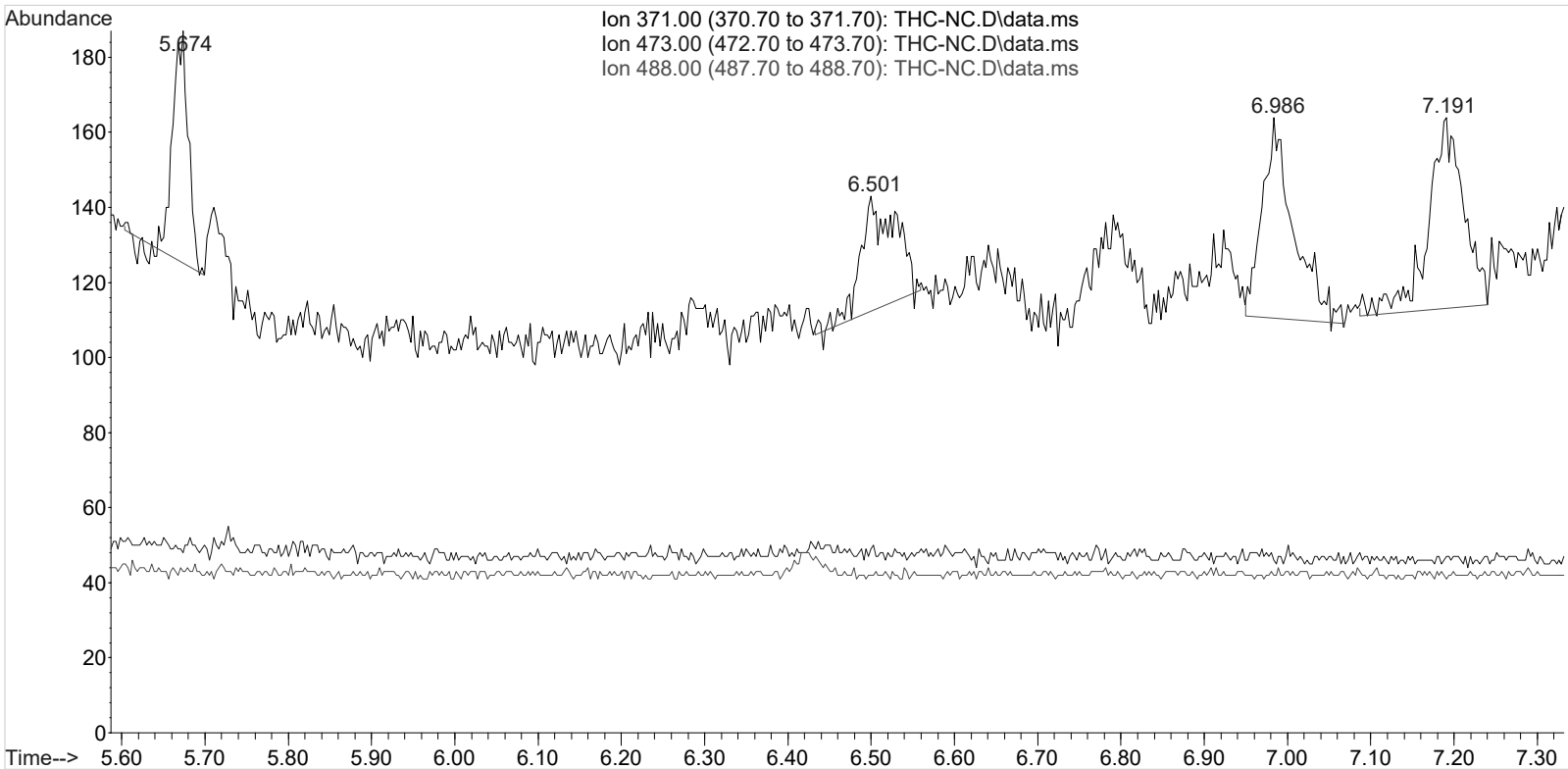
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
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No peaks were detected using the method integration parameters!
Signal : EIC Ion 488.00 (487.70 to 488.70): THC-NC.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
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No peaks were detected using the method integration parameters!

TOX.M Thu Apr 11 09:38:44 2019



Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3247\
Data File : THC-C3-reinject.D
Acq On : 12 Apr 2019 09:17
Operator : ISP\Datastor
Sample : Biorad C3 Control
Misc : C3 Lot # 68460; Worklist 3247
ALS Vial : 33 Sample Multiplier: 1

Sample failed to inject the first time and was reinjected on 4/12/19 with no issues.

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-C3-reinject.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	5.793	64	73	91	PV 2	37	830	4.90%	4.580%
2	6.651	338	371	394	BV 2	870	16933	100.00%	93.414%
3	7.299	578	596	602	PBA2	15	364	2.15%	2.006%

Sum of corrected areas: 18126
Signal : EIC Ion 473.00 (472.70 to 473.70): THC-C3-reinject.D\data.ms

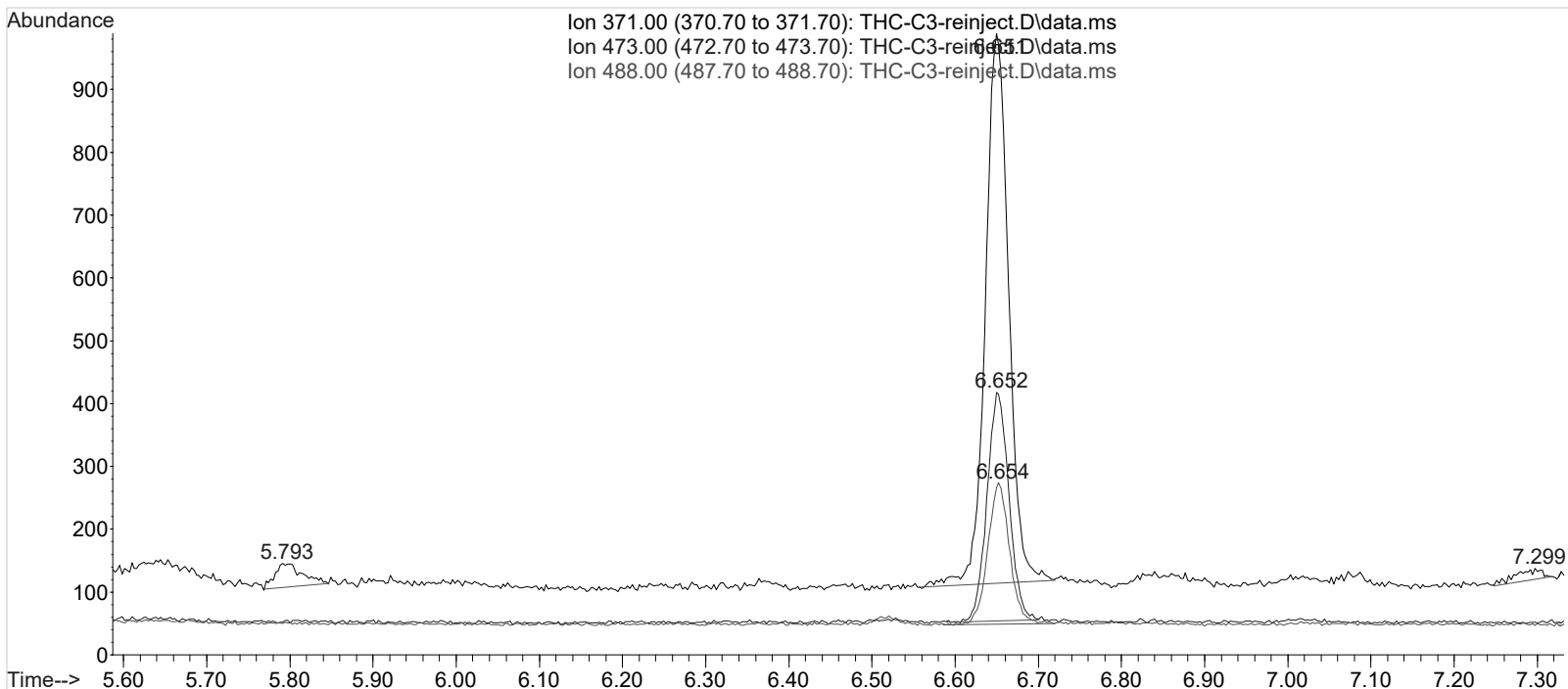
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.652	349	371	388	BV 2	362	6608	100.00%	100.000%

Sum of corrected areas: 6608
Signal : EIC Ion 488.00 (487.70 to 488.70): THC-C3-reinject.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	6.654	348	371	394	BB 2	223	4064	100.00%	100.000%

Sum of corrected areas: 4064

TOX.M Fri Apr 12 10:00:11 2019



AM 3 carboxy-THC Calculations Sheet

Run Date: 4/9/2019

Worklist #: 3247

Laboratory Case #: BioRad C3 68460

Case Calculations:

EIC Ion 371 Corr. Area: 16933

EIC Ion 473 Corr. Area: 6608

EIC Ion 488 Corr. Area: 4064

473:371 Ratio: **0.390**

488:371 Ratio: **0.240**

Quality Control Calculations:

1st Control

2nd Control

EIC Ion 371 Corr. Area: 25714

26504

EIC Ion 473 Corr. Area: 10642

10334

EIC Ion 488 Corr. Area: 6432

6469

Ratio of 473:371: 0.414

0.390

Average: 0.402

Ratio of 488:371: 0.250

0.244

Average: 0.247

Acceptable Retention Time Range: 6.457 to 6.652

Approximate Minimum Corrected Area of 371 Ion: 5143

Acceptable 473:371 Ratio Range: 0.322 to 0.482

Acceptable 488:371 Ratio Range: 0.198 to 0.297