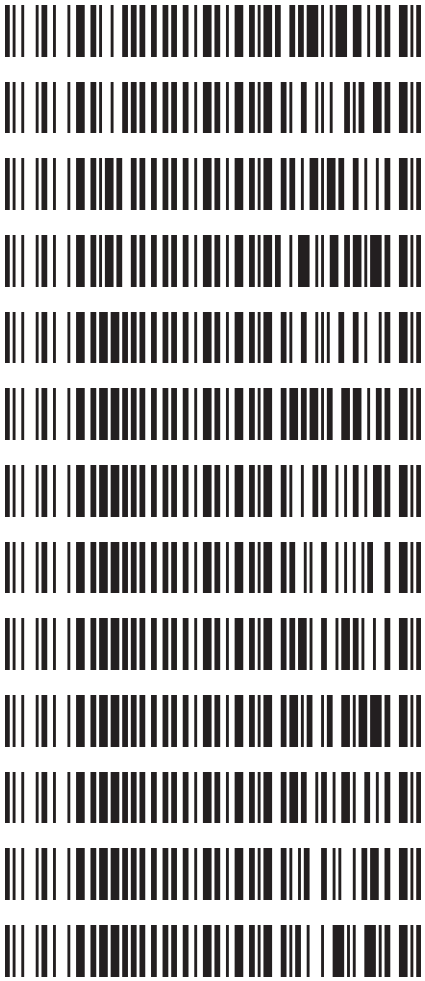
REVIEWED
By Anne Nord at 3:46 pm, Apr 02, 2019

4/1/2019

Worklist: 3133

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2019-0323	2	145929	AM 3 Urine Carboxy-THC
C2019-0445	1	145924	AM 3 Urine Carboxy-THC
M2019-0876	3	145931	AM 3 Urine Carboxy-THC
M2019-0882	3	145935	AM 3 Urine Carboxy-THC
P2019-0333	2	145936	AM 3 Urine Carboxy-THC
P2019-0670	1	145937	AM 3 Urine Carboxy-THC
P2019-0702	1	145938	AM 3 Urine Carboxy-THC
P2019-0746	1	145939	AM 3 Urine Carboxy-THC
P2019-0813	1	145940	AM 3 Urine Carboxy-THC
P2019-0848	1	145941	AM 3 Urine Carboxy-THC
P2019-0872	1	145921	AM 3 Urine Carboxy-THC
P2019-0883	1	145922	AM 3 Urine Carboxy-THC
P2019-0924	1	145928	AM 3 Urine Carboxy-THC



AM 3: Carboxy-THC Urine Extraction

Extraction Date: 03/28/19
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-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 3 worklist 3133

☒ 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 3 worklist 3133\
Data File : THC-PC1.D
Acq On : 28 Mar 2019 13:00
Operator : ISP\datastor
Sample : Spiked Positive c-THC Control
Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3133
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	6.564	323	340	357	VB 2	703	13451	100.00%	100.000%

Sum of corrected areas: 13451

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	6.565	324	341	359	BB 2	291	5484	100.00%	100.000%

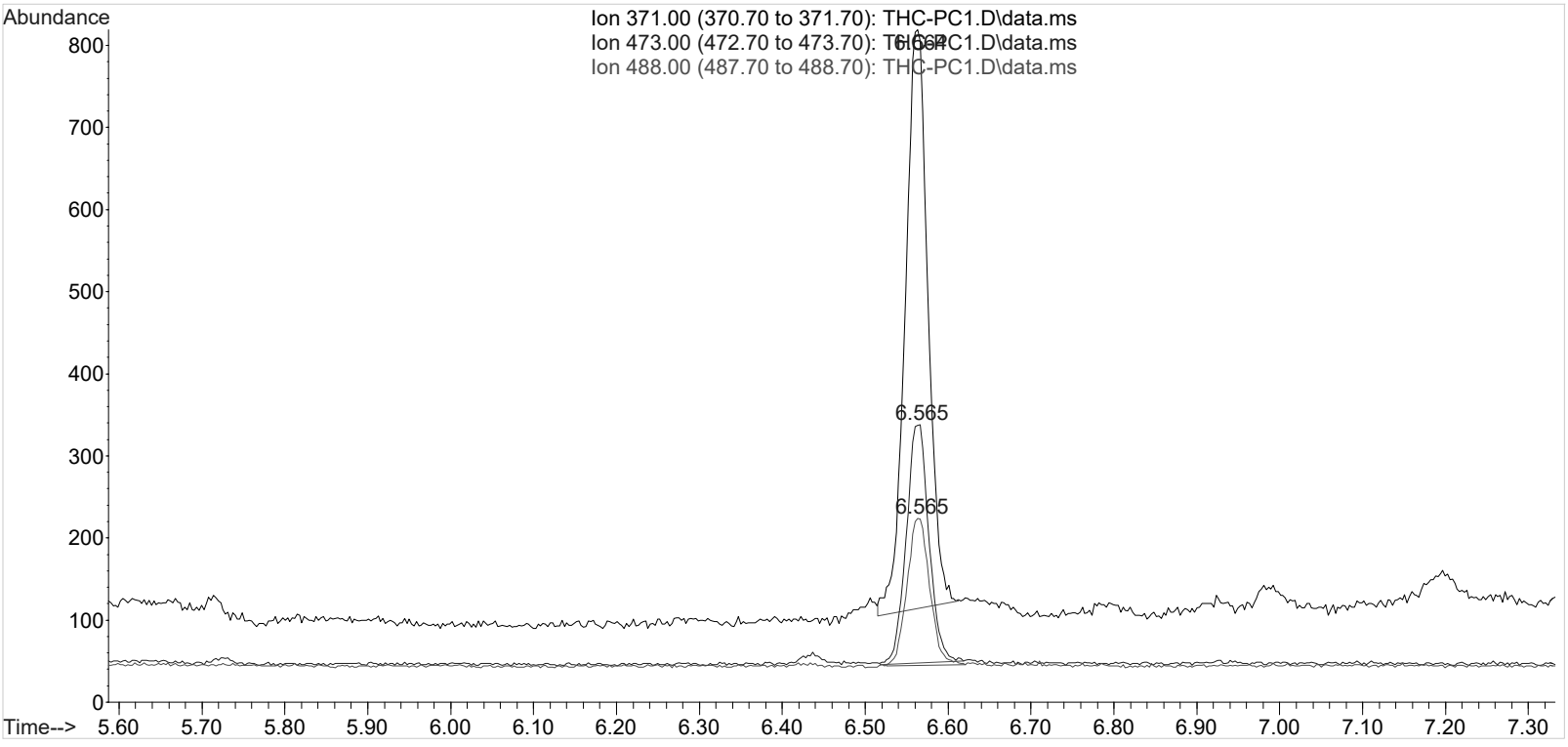
Sum of corrected areas: 5484

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	6.565	327	341	360	BB 2	179	3395	100.00%	100.000%

Sum of corrected areas: 3395

TOX.M Fri Mar 29 08:30:13 2019



Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3133\
Data File : THC-PC2.D
Acq On : 28 Mar 2019 23:54
Operator : ISP\datastor
Sample : Spiked Positive c-THC Control
Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3133
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	6.556	320	337	356	VV	1191	21867	100.00%	100.000%

Sum of corrected areas: 21867

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	6.556	322	337	367	BB	518	9550	100.00%	100.000%

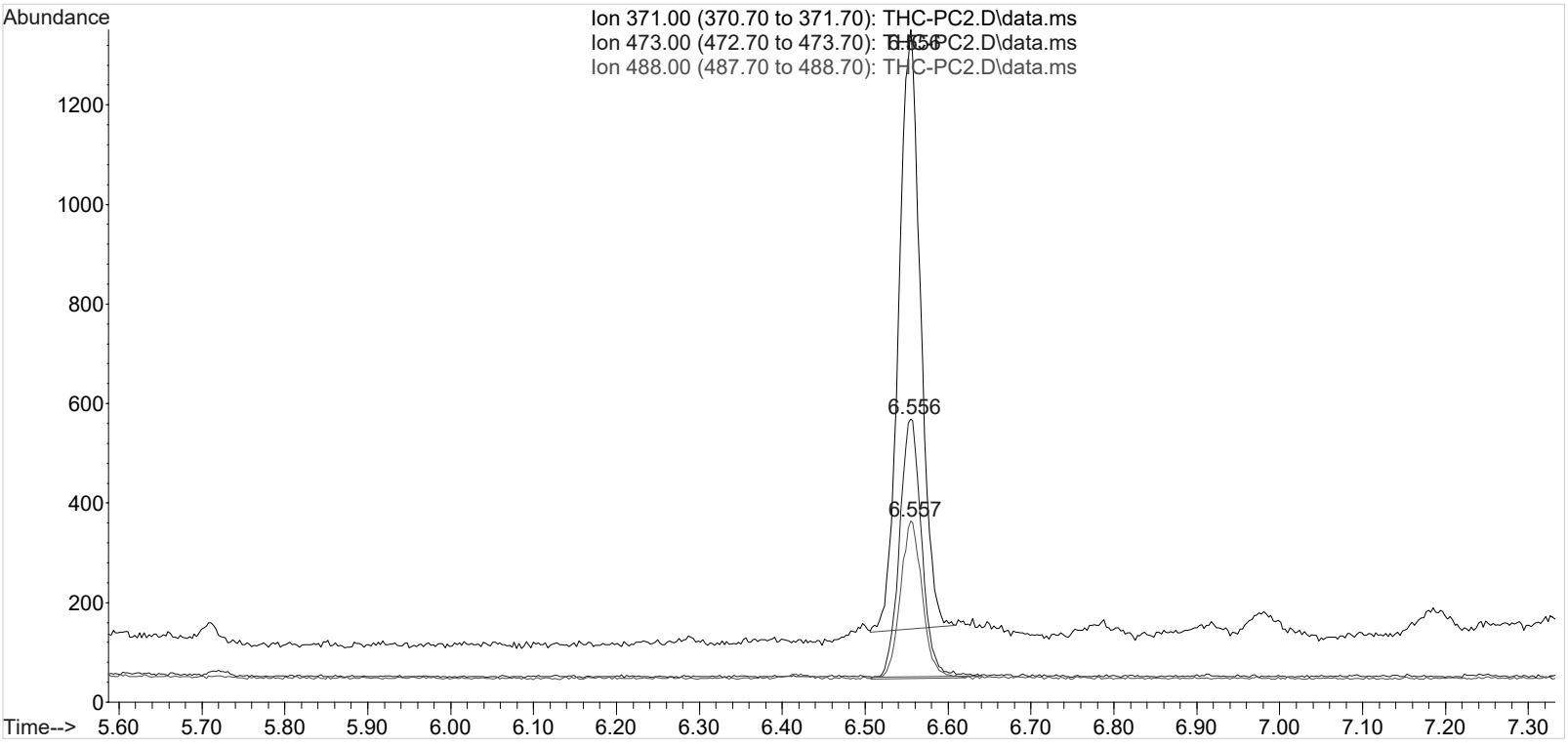
Sum of corrected areas: 9550

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	6.557	320	338	360	BB	315	5789	100.00%	100.000%

Sum of corrected areas: 5789

TOX.M Fri Mar 29 08:32:45 2019



Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3133\
Data File : THC-NC.D
Acq On : 28 Mar 2019 12:49
Operator : ISP\datastor
Sample : Negative Control
Misc : Lot # POC031319; AM #3 Worklist 3133
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

No peaks were detected using the method integration parameters!

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

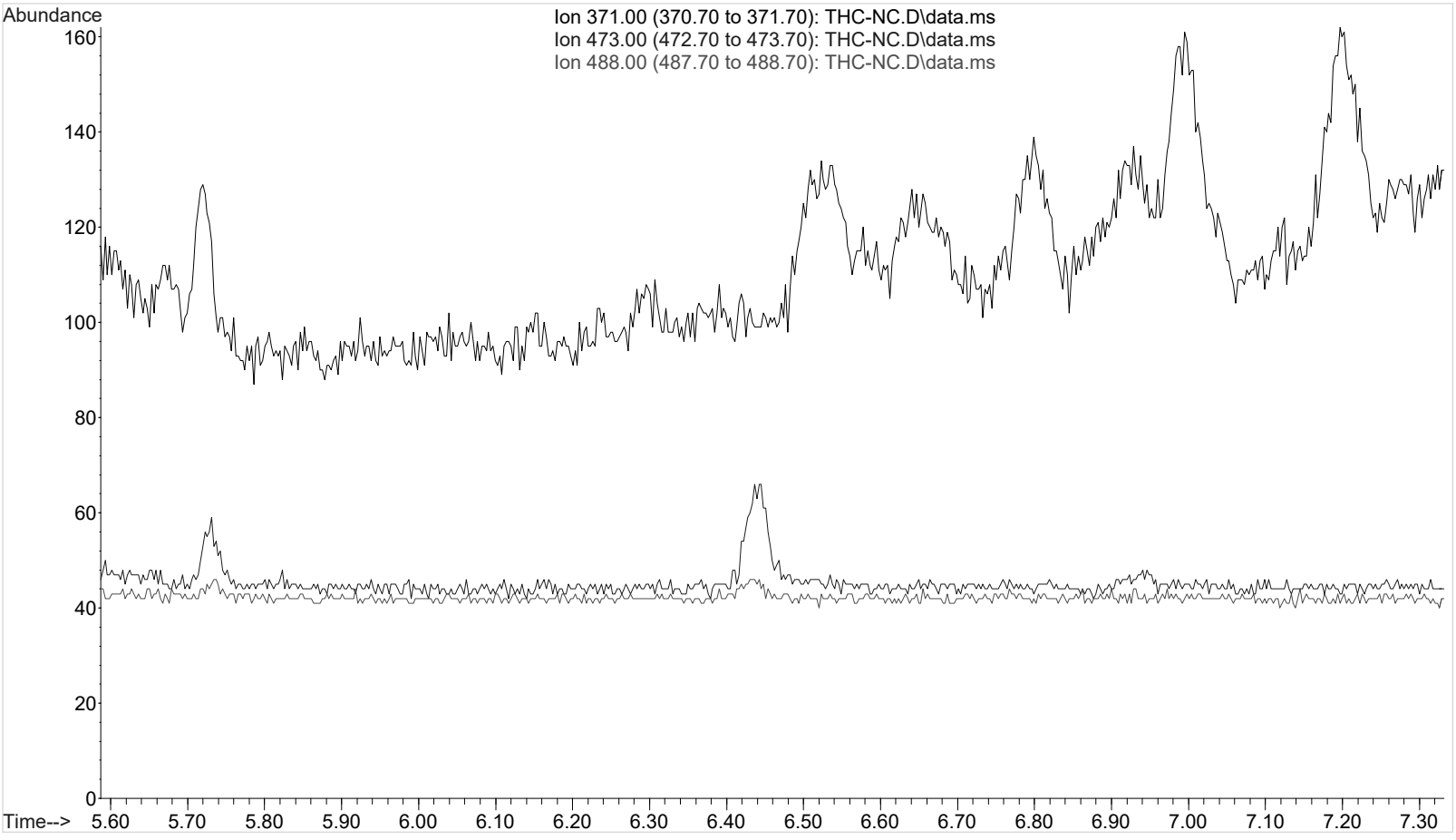
No peaks were detected using the method integration parameters!

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

No peaks were detected using the method integration parameters!

TOX.M Fri Mar 29 08:33:12 2019



Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3133\
Data File : THC-C3.D
Acq On : 29 Mar 2019 08:42
Operator : ISP\datastor
Sample : Biorad C3 Control
Misc : C3 Lot # 68460; Worklist 3133
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): 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	6.653	320	371	391	BV 2	447	9051	100.00%	100.000%

Sum of corrected areas: 9051

Signal : EIC Ion 473.00 (472.70 to 473.70): 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	6.653	356	371	392	BB 2	180	3419	100.00%	100.000%

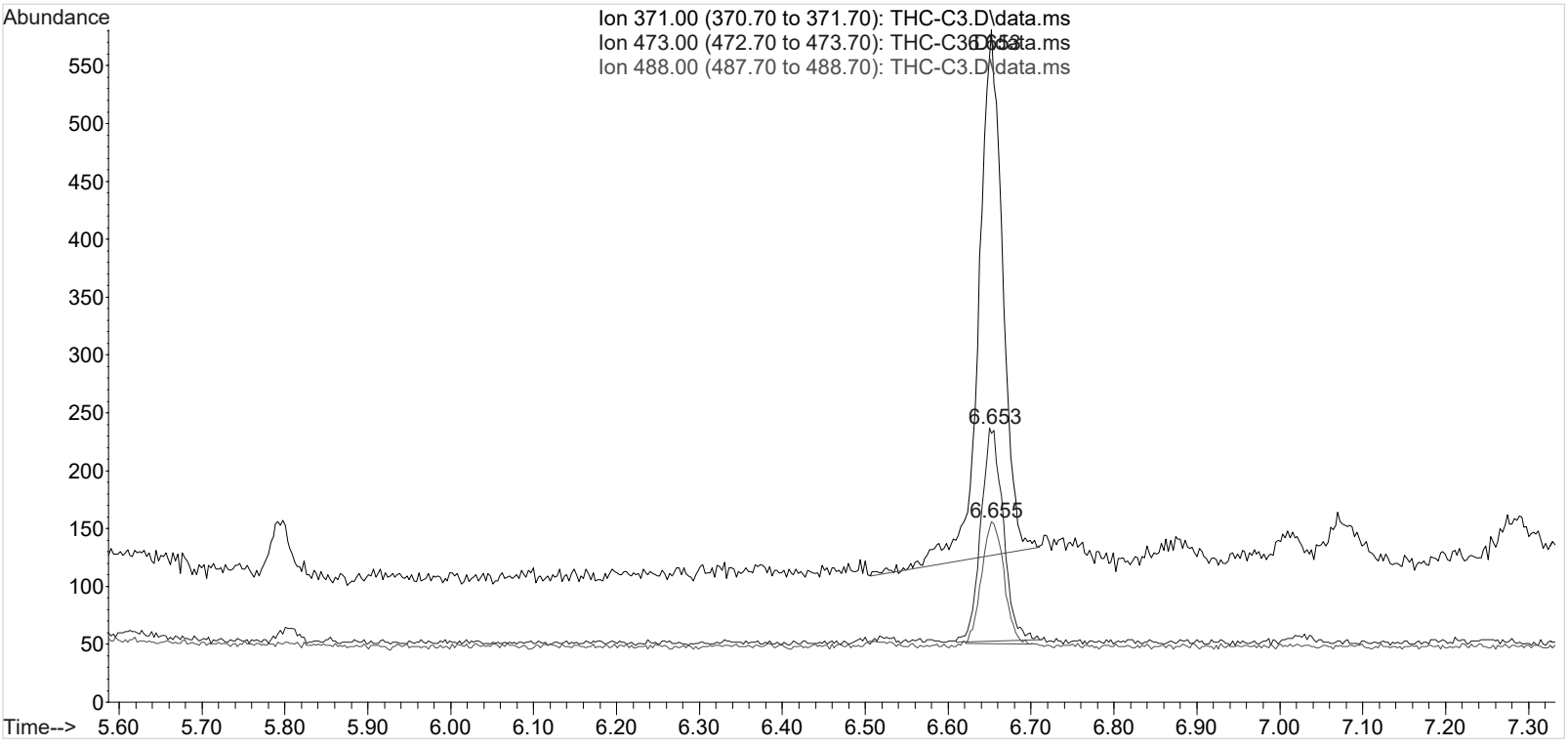
Sum of corrected areas: 3419

Signal : EIC Ion 488.00 (487.70 to 488.70): 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	6.655	360	372	388	BB 2	105	1938	100.00%	100.000%

Sum of corrected areas: 1938

TOX.M Fri Mar 29 08:51:51 2019



Run Date:	3/28/2019
Worklist #:	3133
Laboratory Case #:	BioRad C3 68460

Case Calculations:

EIC Ion 371 Corr. Area:	*9051*
EIC Ion 473 Corr. Area:	*3419*
EIC Ion 488 Corr. Area:	*1938*

473:371 Ratio: **0.378**

488:371 Ratio: **0.214**

Quality Control Calculations:

	1st Control	2nd Control	
EIC Ion 371 Corr. Area:	*13451*	*21867*	
EIC Ion 473 Corr. Area:	*5484*	*9550*	
EIC Ion 488 Corr. Area:	*3395*	*5789*	
Ratio of 473:371:	*0.408*	*0.437*	
		Average:	*0.422*
Ratio of 488:371:	*0.252*	*0.265*	
		Average:	*0.259*

Acceptable Retention Time Range:	*6.464*	to	*6.656*
Approximate Minimum Corrected Area of 371 Ion:			*2690*
Acceptable 473:371 Ratio Range:	*0.338*	to	*0.507*
Acceptable 488:371 Ratio Range:	*0.207*	to	*0.310*