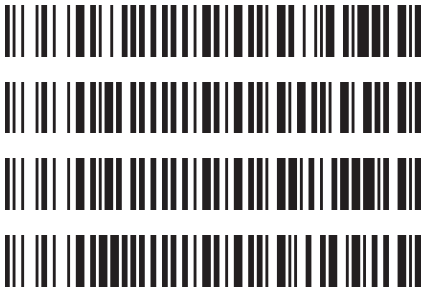
REVIEWED
By Anne Nord at 4:41 pm, Aug 19, 2019

8/9/2019

Worklist: 3600

LAB_CASE	ITEM	TASK_ID	DESCRIPTION
C2019-1460	2	159730	AM 3 Urine Carboxy-THC
M2019-3195	2	159735	AM 3 Urine Carboxy-THC
M2019-3289	2	159733	AM 3 Urine Carboxy-THC
P2019-2262	1	159736	AM 3 Urine Carboxy-THC



A method revision occurred after this batch had been done. The revision that applies to this batch is revision 8.

AM 3: Carboxy-THC Urine Extraction

Extraction Date: 08/09/2019
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 3 worklist 3600

☒ 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 3600\
Data File : THC-PC1.D
Acq On : 09 Aug 2019 12:21
Operator : ISP\Datastor
Sample : Spiked Positive c-THC Control
Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3600
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	6.200	307	388	406	BV	9831	172666	100.00%	100.000%

Sum of corrected areas: 172666

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	6.201	373	388	413	BB	3260	56781	100.00%	100.000%

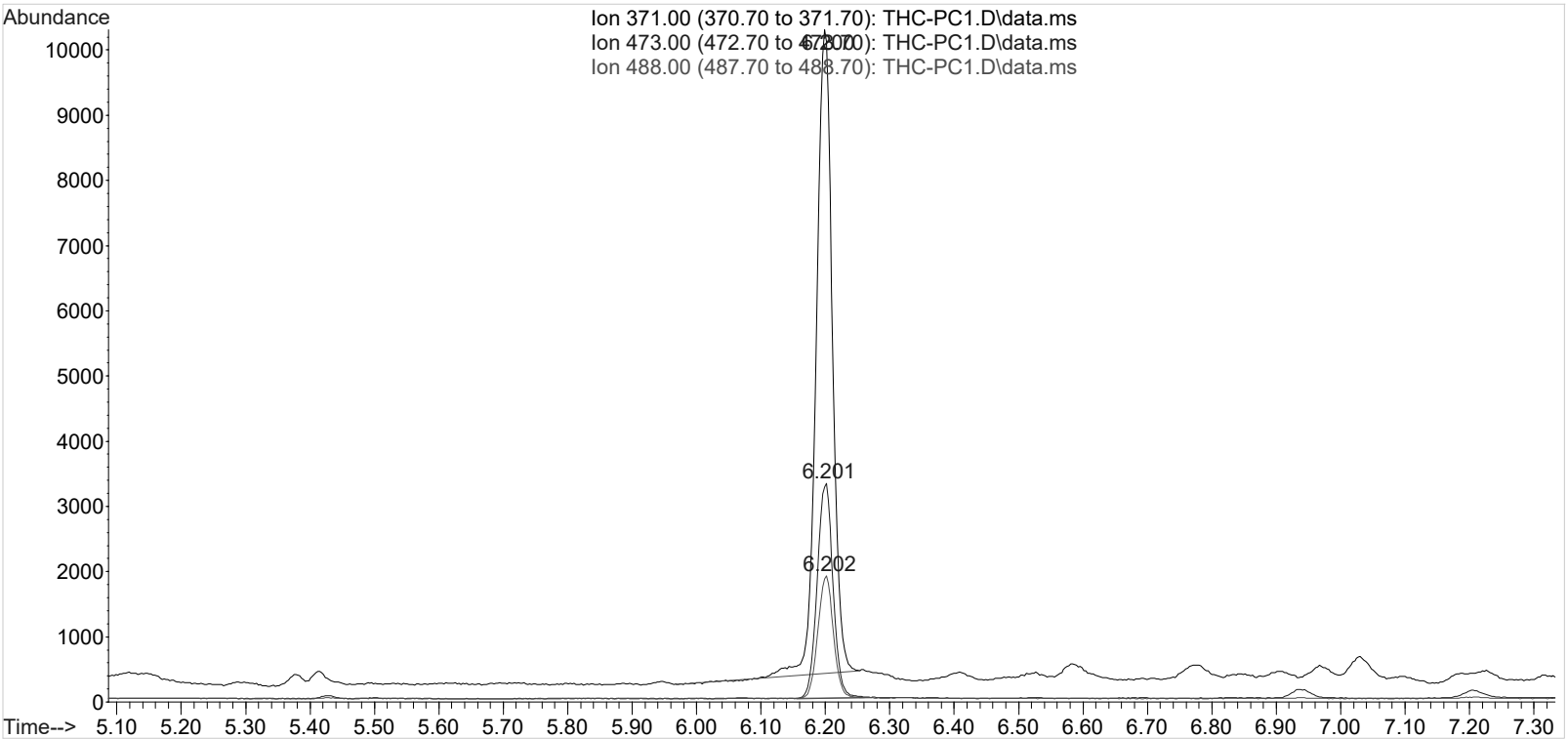
Sum of corrected areas: 56781

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	6.202	372	388	409	BB	1865	31938	100.00%	100.000%

Sum of corrected areas: 31938

TOX.M Mon Aug 12 19:28:55 2019



Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3600\
Data File : THC-PC2.D
Acq On : 09 Aug 2019 15:05
Operator : ISP\Datastor
Sample : Spiked Positive c-THC Control
Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3600
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	6.200	315	387	434	BB 2	12670	237703	100.00%	100.000%

Sum of corrected areas: 237703

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	6.201	372	388	411	BB 2	4262	72242	100.00%	100.000%

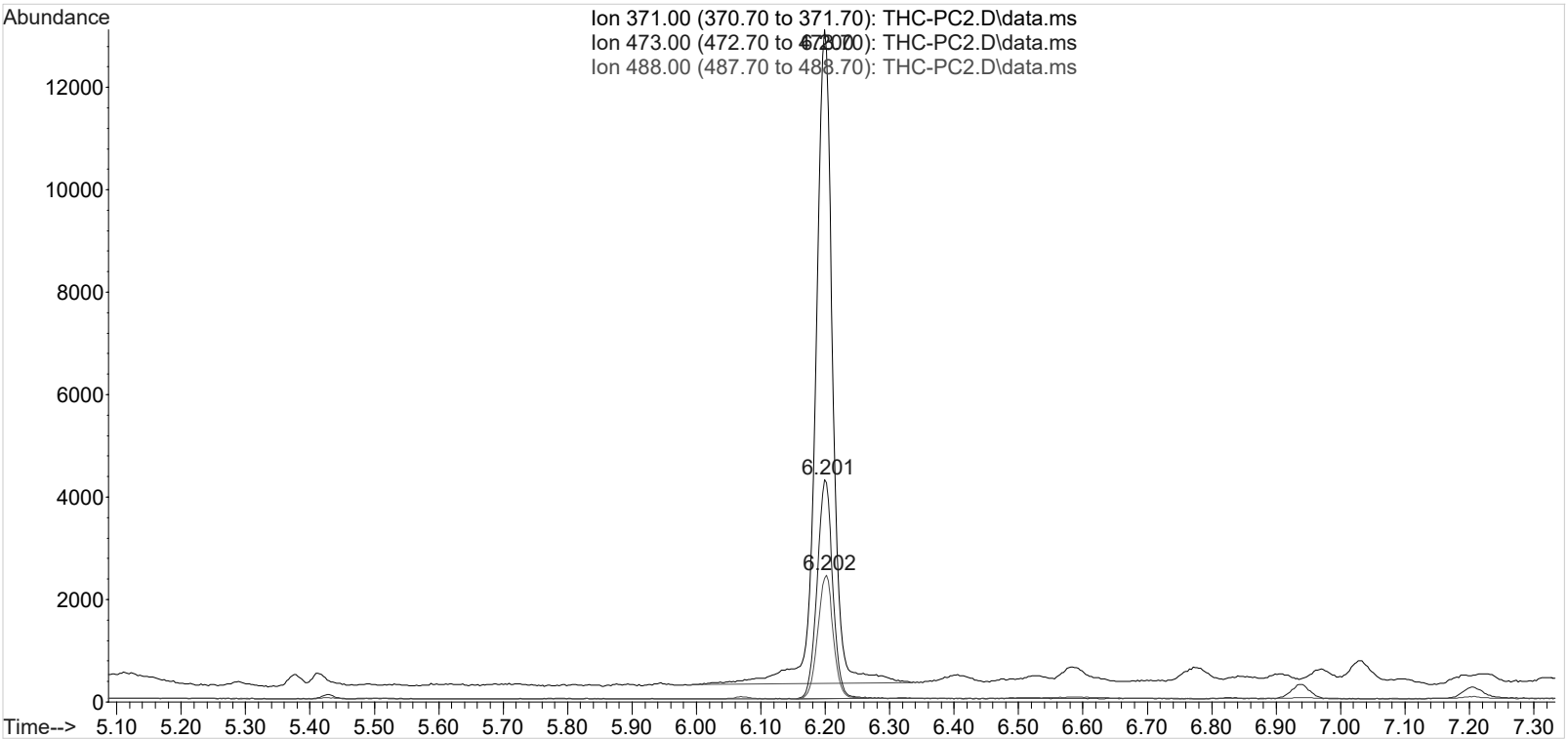
Sum of corrected areas: 72242

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	6.202	373	388	410	BB 2	2396	40590	100.00%	100.000%

Sum of corrected areas: 40590

TOX.M Mon Aug 12 19:30:15 2019



Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3600\
Data File : THC-NC.D
Acq On : 09 Aug 2019 12:11
Operator : ISP\Datastor
Sample : Negative Control
Misc : Lot # POC031319; AM #3 Worklist 3600
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

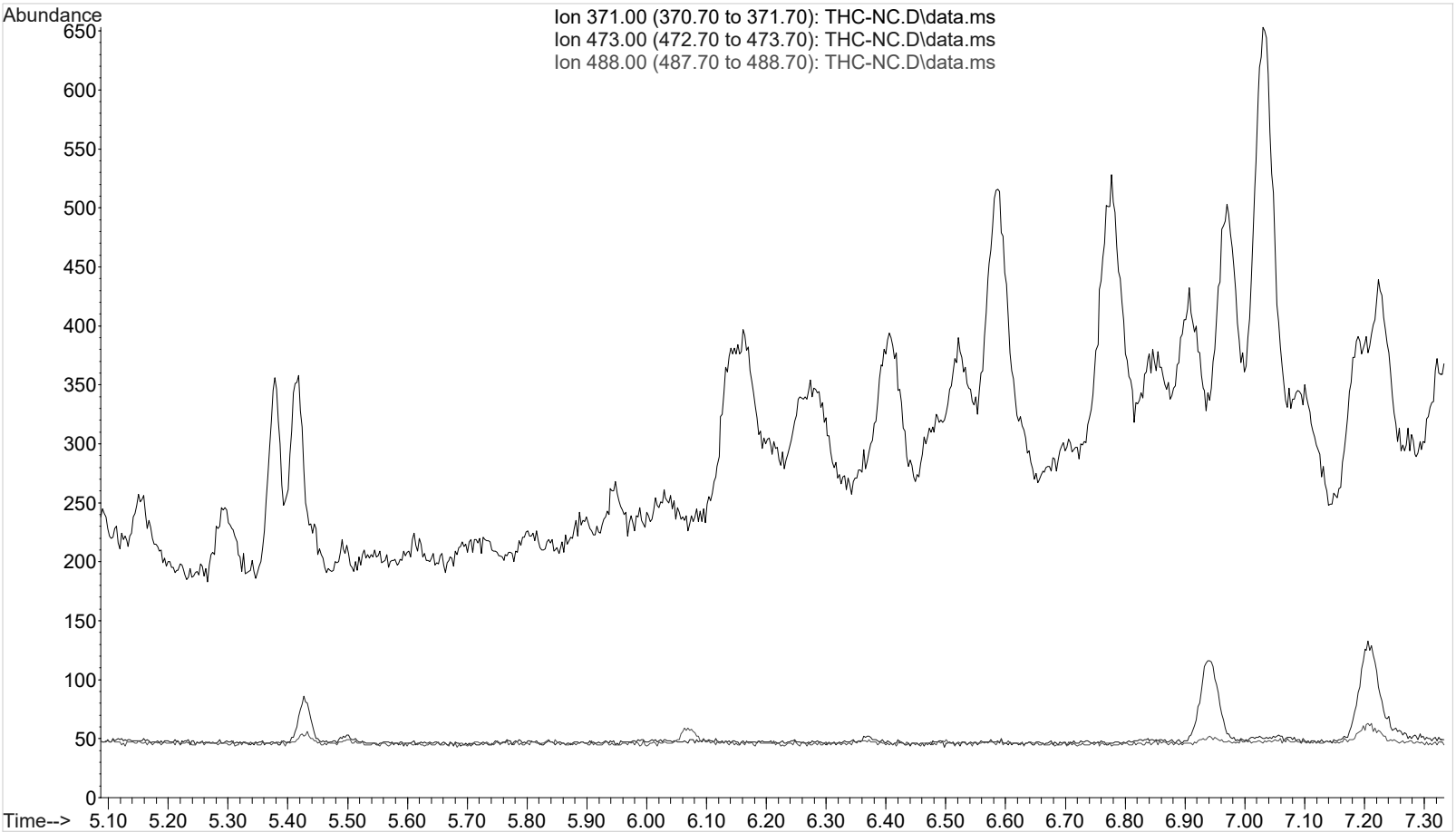
No peaks were detected using the method integration parameters!

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 Mon Aug 12 19:32:18 2019



Area Percent Report

Data Path : G:\TOX\Pocatello\MMS\CDS\2019\am 3 worklist 3600\
Data File : THC-C3.D
Acq On : 09 Aug 2019 12:43
Operator : ISP\Datastor
Sample : Biorad C3 Control
Misc : C3 Lot # 68460; Worklist 3600
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	6.198	338	387	408	BV	2921	50735	100.00%	100.000%

Sum of corrected areas: 50735

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	6.200	374	387	406	BB	979	16627	100.00%	100.000%

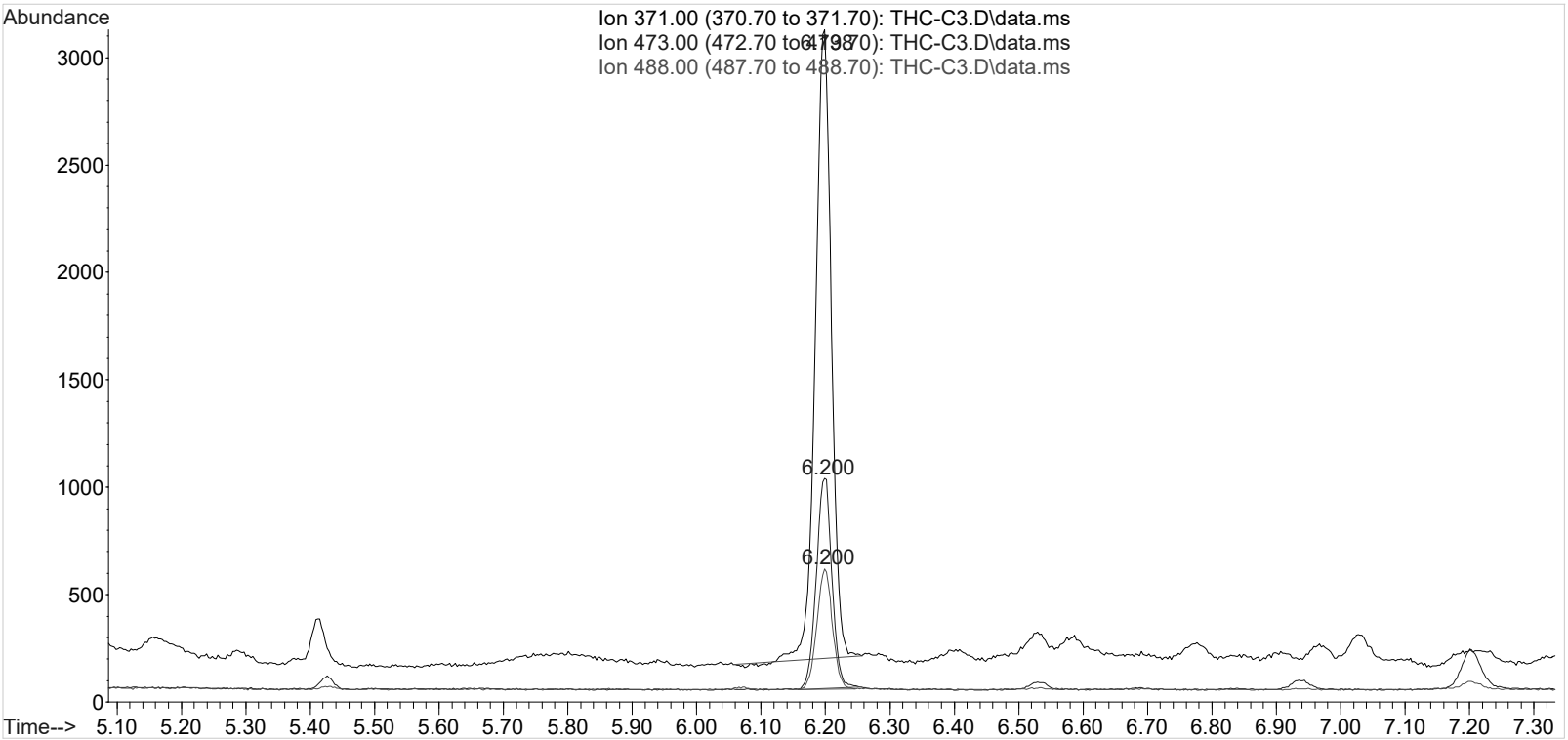
Sum of corrected areas: 16627

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	6.200	373	388	404	BB	556	9512	100.00%	100.000%

Sum of corrected areas: 9512

TOX.M Mon Aug 12 19:31:51 2019



Run Date:	8/9/2019
Worklist #:	3600
Laboratory Case #:	Bioroad C3 Lot 68460

Case Calculations:

EIC Ion 371 Corr. Area:	*50735*		
EIC Ion 473 Corr. Area:	*16627*	473:371 Ratio:	**0.328**
		488:371 Ratio:	**0.187**
EIC Ion 488 Corr. Area:	*9512*		

Quality Control Calculations:

	1st Control	**2nd Control**	
EIC Ion 371 Corr. Area:	*172666*	*237703*	
EIC Ion 473 Corr. Area:	*56781*	*72242*	
EIC Ion 488 Corr. Area:	*31938*	*40590*	
Ratio of 473:371:	*0.329*	*0.304*	
		Average:	*0.316*
Ratio of 488:371:	*0.185*	*0.171*	
		Average:	*0.178*

Acceptable Retention Time Range:	*6.100*	to	*6.300*
Approximate Minimum Corrected Area of 371 Ion:			*34533*
Acceptable 473:371 Ratio Range:	*0.253*	to	*0.380*
Acceptable 488:371 Ratio Range:	*0.142*	to	*0.213*