

REVIEWED

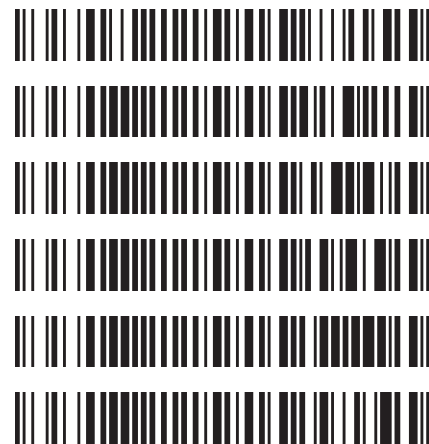
By Britany Wylie at 4:25 pm, Jun 04, 2019

4/30/2019

cg

Worklist: 3349

<u>LAB_CASE</u>	<u>ITEM</u>	<u>TASK_ID</u>	<u>DESCRIPTION</u>
C2019-0684	2	150225	AM 3 Urine Carboxy-THC
P2019-1130	1	150226	AM 3 Urine Carboxy-THC
P2019-1173	1	150227	AM 3 Urine Carboxy-THC
P2019-1204	1	150228	AM 3 Urine Carboxy-THC
P2019-1262	1	150229	AM 3 Urine Carboxy-THC
P2019-1263	1	150230	AM 3 Urine Carboxy-THC



AM 3: Carboxy-THC Urine Extraction

Extraction Date: 05/08/2019

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- 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.

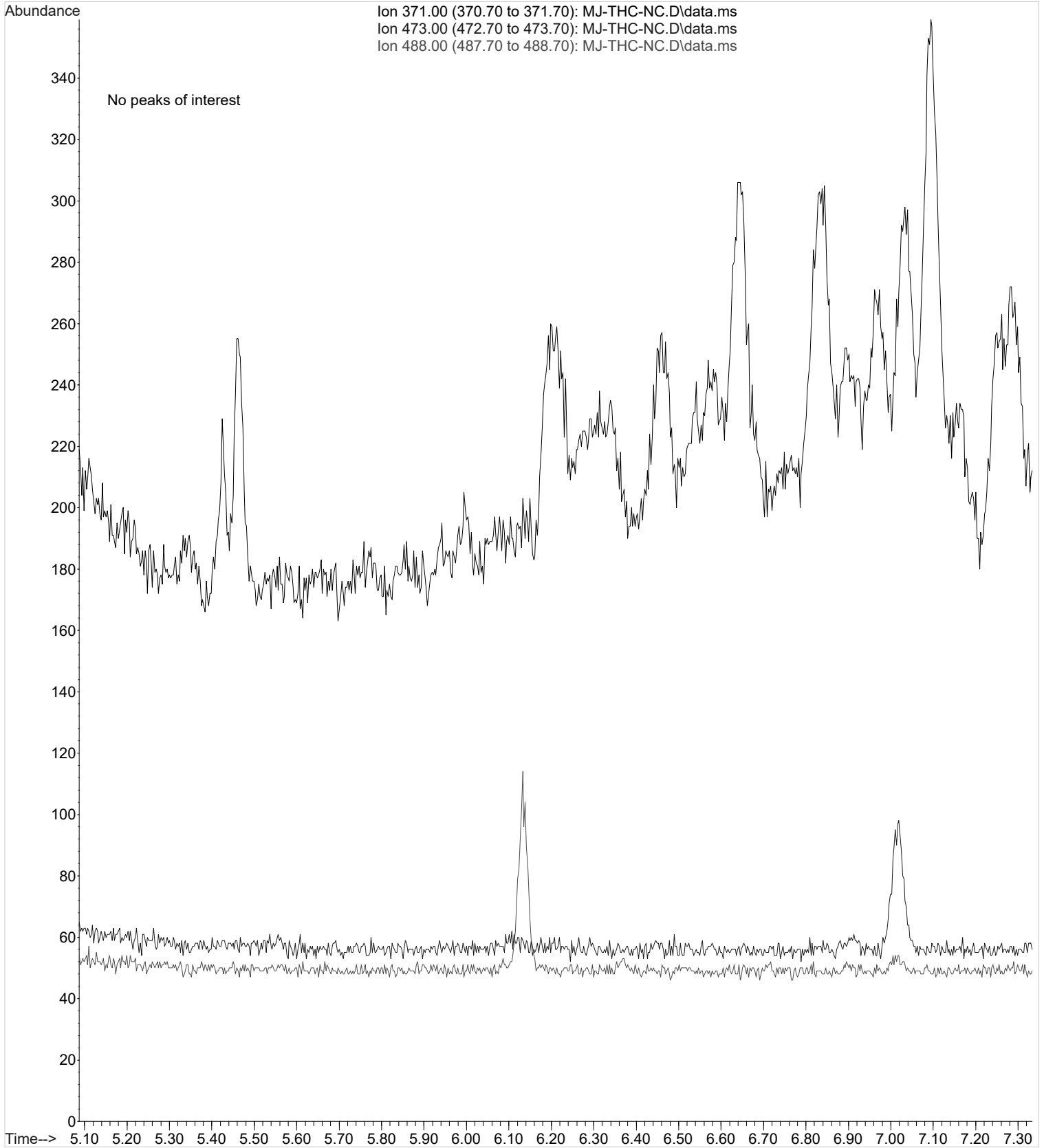
Post-Analytic

- 1. Complete Data analysis on all samples and corresponding sample blanks
GCMS Data path: D:\DATA\CDS\2019\am 2 and am 3 worklists 3334 and 3349 CS
- 2. Did positive and negative control samples provide intended response? Y / N
- 3. **Criteria for ID:** RT +/- 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:

Samples were originally extracted and run on 04/30/19 but the instrument was found to be not working properly. The instrument was placed out of service and maintenance was performed. The samples were re-extracted and run on 05/08/19, after it was determined that the instrument was in good working order. There was an issue with the sequence that caused the instrument to stop part way through the sequence. The sequence was restarted with no issues.

File :C:\My Files\cshrum\Desktop\Consolidated\Data\URINE\am 2 and
... 3 worklists 3334 and 3349 CS\MJ-THC-NC.D
Operator : ISP\datastor
Instrument : Major Mass Spec
Acquired : 09 May 2019 06:07 using AcqMethod U-CANN 7-2018.M
Sample Name: Negative Control
Misc Info : Lot # POC031319; AM #3 Worklist 3349



Data Path : C:\My Files\cshrum\Desktop\Consolidated\Data\URINE\am 2 and 3 worklists 3334 and 3349
 ... CS\
 Data File : MJ-THC-PC1.D
 Acq On : 09 May 2019 06:18
 Operator : ISP\datastor
 Sample : Spiked Positive c-THC Control
 Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3349
 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	5.348	80	92	103	PV	41	773	0.51%	0.295%
2	5.429	109	120	125	VV	58	852	0.56%	0.325%
3	5.468	125	133	154	VV	309	4807	3.17%	1.832%
4	5.562	160	166	168	PV 2	31	264	0.17%	0.101%
5	5.593	175	177	189	VB 3	32	301	0.20%	0.115%
6	5.647	189	195	200	BV	39	418	0.28%	0.159%
7	5.667	200	202	214	VV 2	31	508	0.34%	0.194%
8	5.742	227	228	234	VV 3	28	229	0.15%	0.087%
9	5.773	234	239	253	VB	40	718	0.47%	0.274%
10	5.836	254	261	275	BV 3	27	614	0.40%	0.234%
11	5.948	292	300	309	PV	27	521	0.34%	0.198%
12	6.004	309	320	332	VV	73	1386	0.91%	0.528%
13	6.050	332	336	339	VV 2	31	302	0.20%	0.115%
14	6.073	339	344	348	VV 2	44	502	0.33%	0.191%
15	6.093	348	350	358	VV 2	61	801	0.53%	0.305%
16	6.140	358	367	375	VV	64	1325	0.87%	0.505%
17	6.207	375	390	393	VV	249	5170	3.41%	1.970%
18	6.268	393	411	459	VV	7770	151624	100.00%	57.776%
19	6.462	459	478	494	VV	233	8226	5.43%	3.134%
20	6.589	494	523	531	VV	214	8811	5.81%	3.357%
21	6.650	531	544	569	VV	383	12929	8.53%	4.927%
22	6.842	575	610	625	VV	388	15899	10.49%	6.058%
23	6.908	625	633	646	VV 2	196	6023	3.97%	2.295%
24	6.979	646	658	668	VV	231	6837	4.51%	2.605%
25	7.040	668	679	689	VV 2	327	8732	5.76%	3.327%
26	7.102	689	701	718	VV	527	14311	9.44%	5.453%
27	7.162	718	722	740	VV	140	2877	1.90%	1.096%
28	7.258	740	755	761	PV	168	3165	2.09%	1.206%
29	7.299	761	769	776	VBA	169	3510	2.31%	1.337%

Sum of corrected areas: 262435

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	6.269	394	412	435	BV	2287	39577	100.00%	96.654%
2	7.024	646	674	694	BB 2	69	1370	3.46%	3.346%

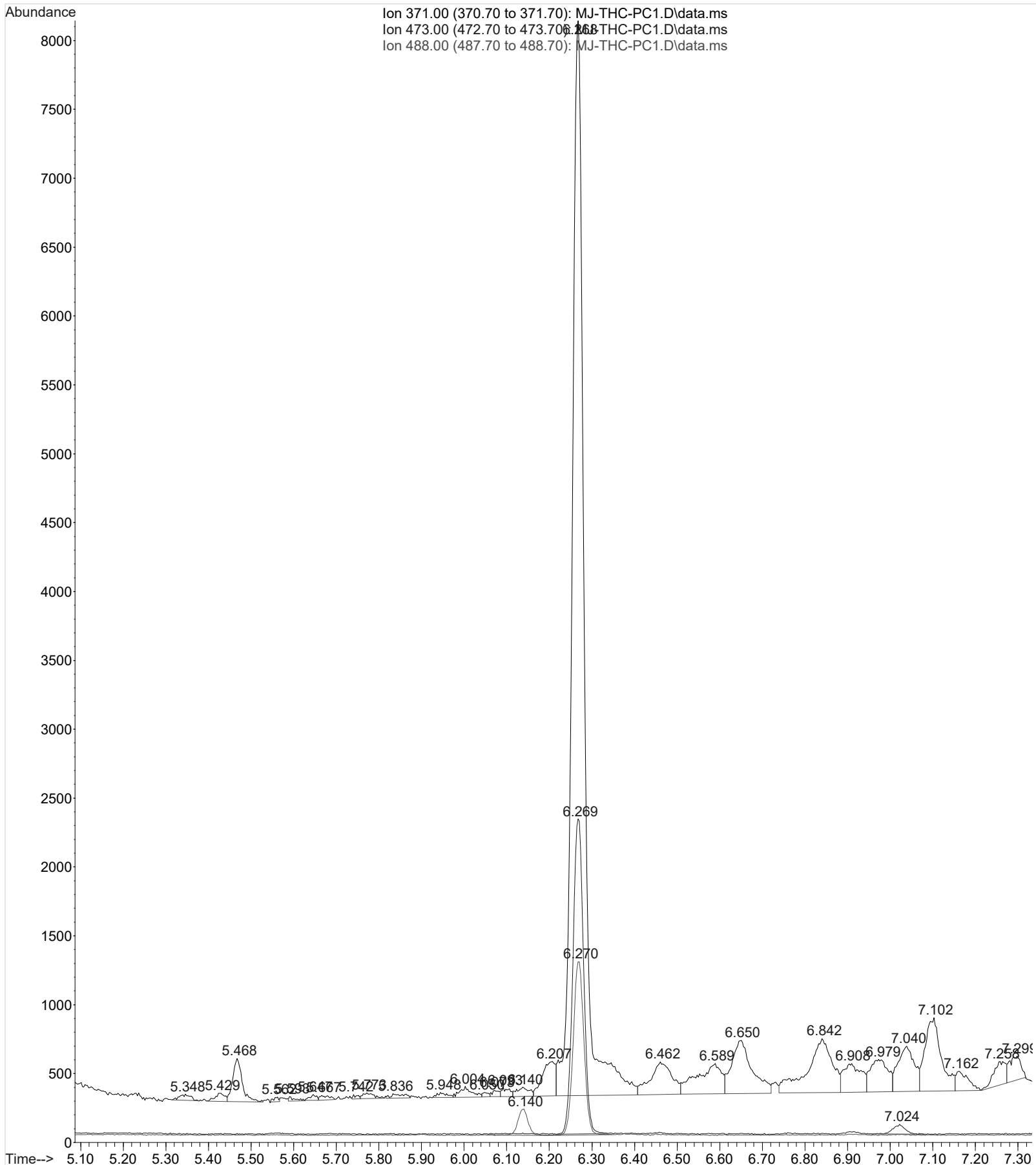
Sum of corrected areas: 40947

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	6.140	337	367	386	BV 2	188	3159	14.47%	12.641%

Sum of corrected areas: 24993

TOX.M Tue May 28 09:51:45 2019



Data Path : C:\My Files\cshrum\Desktop\Consolidated\Data\URINE\am 2 and 3 worklists 3334 and 3349
 ... CS\
 Data File : MJ-THC-PC2.D
 Acq On : 10 May 2019 12:08
 Operator : ISP\datastor
 Sample : Spiked Positive c-THC Control
 Misc : c-THC lot # 0497429 in Negative Lot # POC031319; Worklist 3349
 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-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	5.217	29	46	48	PV 3	47	632	0.30%	0.179%
2	5.225	48	49	55	VV 2	46	445	0.21%	0.126%
3	5.353	73	93	105	PV	62	1775	0.85%	0.502%
4	5.426	108	119	125	VV	115	1745	0.84%	0.494%
5	5.465	125	132	149	VV	364	5689	2.74%	1.610%
6	5.565	158	167	177	PV 3	16	333	0.16%	0.094%
7	5.643	184	194	220	BV 3	33	793	0.38%	0.224%
8	5.768	220	238	251	PV 2	42	1343	0.65%	0.380%
9	5.867	251	272	281	VV 2	39	1291	0.62%	0.365%
10	5.901	281	284	290	VV 2	39	446	0.21%	0.126%
11	5.937	290	296	299	VV 2	51	609	0.29%	0.172%
12	5.949	299	300	306	VV	53	494	0.24%	0.140%
13	5.998	306	317	329	VV	97	2183	1.05%	0.618%
14	6.091	329	350	357	VV	89	2829	1.36%	0.801%
15	6.139	357	366	373	VV 2	85	1985	0.95%	0.562%
16	6.200	373	387	394	VV	292	7507	3.61%	2.125%
17	6.263	394	409	433	VV	11673	207969	100.00%	58.871%
18	6.338	433	436	457	VV	237	5996	2.88%	1.697%
19	6.461	462	478	496	VV	281	10324	4.96%	2.923%
20	6.536	496	504	508	VV	182	2975	1.43%	0.842%
21	6.583	508	521	530	VV	254	7941	3.82%	2.248%
22	6.645	530	542	569	VV	449	15532	7.47%	4.397%
23	6.762	569	583	592	VV 3	117	4096	1.97%	1.159%
24	6.842	592	610	622	VV	450	14249	6.85%	4.034%
25	6.902	622	631	645	VV 2	234	7710	3.71%	2.183%
26	6.970	645	655	666	VV	285	7837	3.77%	2.218%
27	7.035	666	678	687	VV 2	390	10387	4.99%	2.940%
28	7.096	687	699	712	VV	616	15853	7.62%	4.488%
29	7.160	712	721	740	VV	159	4648	2.23%	1.316%
30	7.289	740	766	775	PBA	206	7651	3.68%	2.166%

Sum of corrected areas: 353264
 Signal : EIC Ion 473.00 (472.70 to 473.70): MJ-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	6.263	393	410	444	BB	3557	60400	100.00%	94.846%
2	7.017	649	671	700	BB 2	159	3282	5.43%	5.154%

Sum of corrected areas: 63683
 Signal : EIC Ion 488.00 (487.70 to 488.70): MJ-THC-PC2.D\data.ms

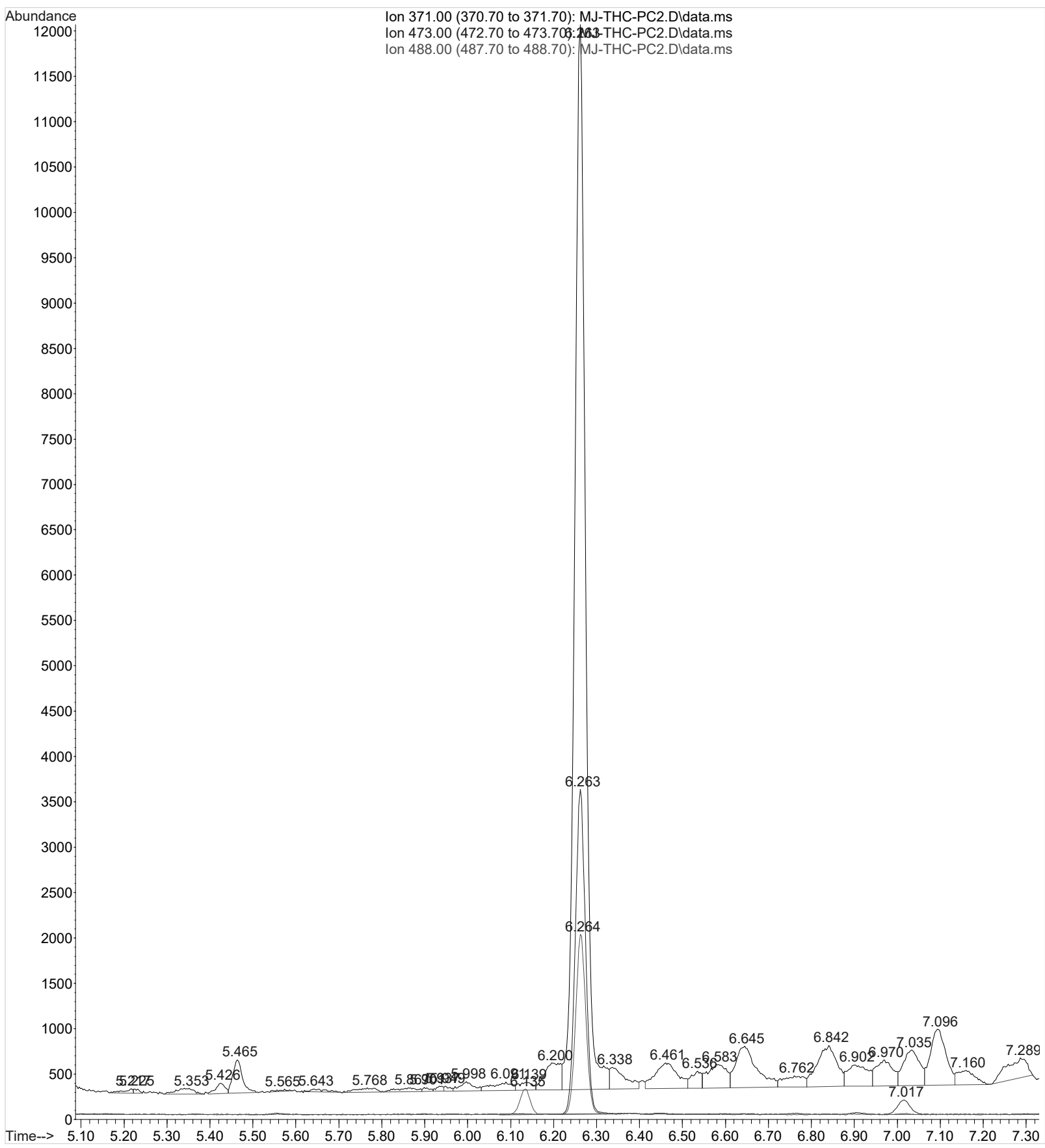
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
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CS

1	6.135	344	365	378 BV	278	4672	13.87%	12.184%
2	6.264	391	410	430 BV	1984	33671	100.00%	87.816%

Sum of corrected areas: 38342

TOX.M Tue May 28 09:52:39 2019



Data Path : C:\My Files\cshrum\Desktop\Consolidated\Data\URINE\am 2 and 3 worklists 3334 and 3349
...
Data File : MJ-THC-C3.D
Acq On : 09 May 2019 06:40
Operator : ISP\datastor
Sample : Biorad C3 Control
Misc : C3 Lot # 68460; Worklist 3349
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

Table with 10 columns: peak #, R.T. min, first scan, max scan, last scan, PK TY, peak height, corr. area, corr. % max., % of total. Contains 25 rows of peak data.

Sum of corrected areas: 78732
Signal : EIC Ion 473.00 (472.70 to 473.70): MJ-THC-C3.D\data.ms

Table with 10 columns: peak #, R.T. min, first scan, max scan, last scan, PK TY, peak height, corr. area, corr. % max., % of total. Contains 1 row of peak data.

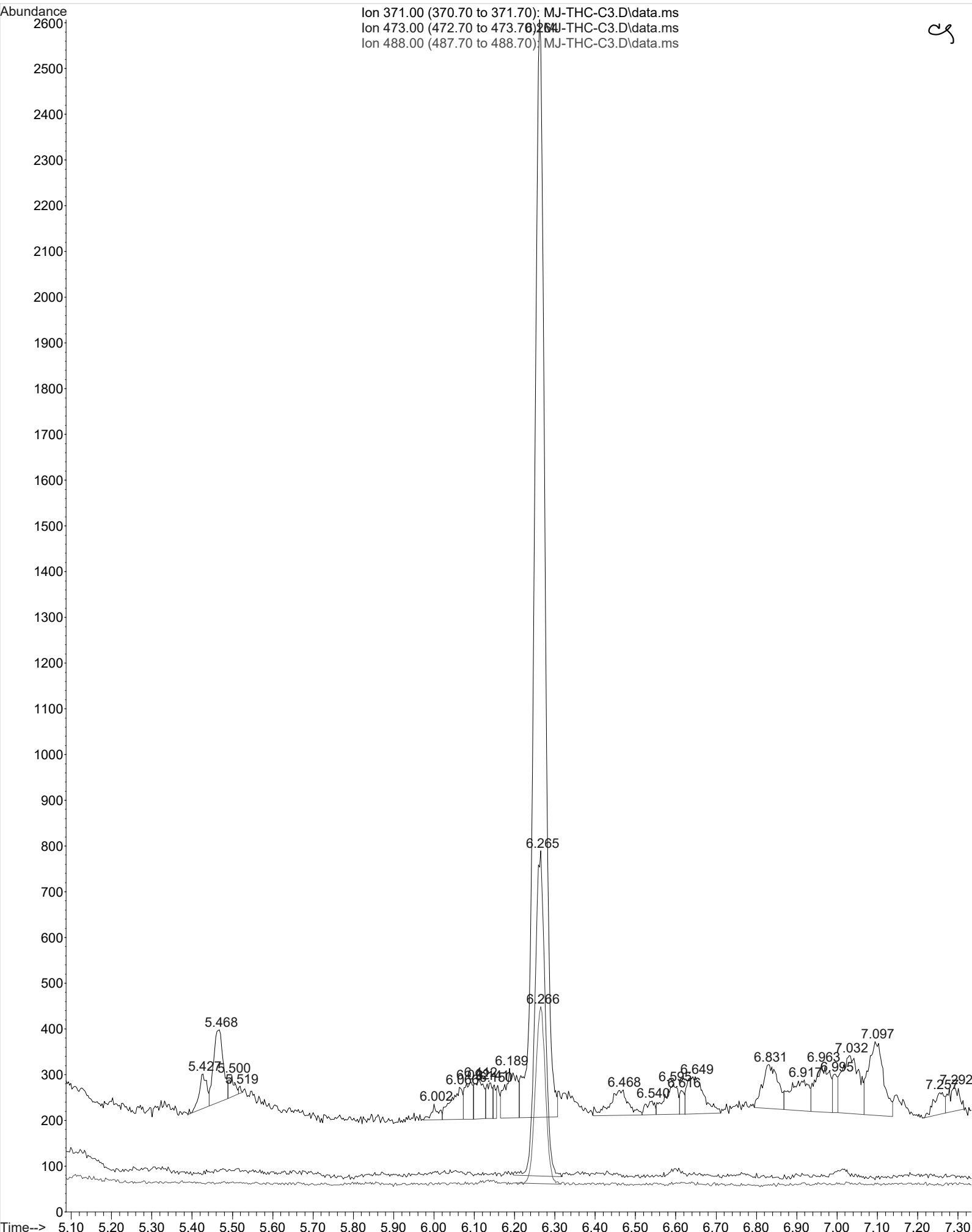
Sum of corrected areas: 12332
Signal : EIC Ion 488.00 (487.70 to 488.70): MJ-THC-C3.D\data.ms

Table with 10 columns: peak #, R.T. min, first scan, max scan, last scan, PK TY, peak height, corr. area, corr. % max., % of total. Contains 1 row of peak data.

Sum of corrected areas: 6665

Ion 371.00 (370.70 to 371.70): MJ-THC-C3.D\data.ms
Ion 473.00 (472.70 to 473.70): MJ-THC-C3.D\data.ms
Ion 488.00 (487.70 to 488.70): MJ-THC-C3.D\data.ms

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AM 3 carboxy-THC Calculations Sheet

Run Date: 5/9/2019

Worklist #: 3349

Laboratory Case #: BioRad C3 Lot: 68640

Case Calculations:

EIC Ion 371 Corr. Area: 43256

EIC Ion 473 Corr. Area: 12332

EIC Ion 488 Corr. Area: 6665

473:371 Ratio: **0.285**

488:371 Ratio: **0.154**

Quality Control Calculations:

1st Control

2nd Control

EIC Ion 371 Corr. Area: 151624

207969

EIC Ion 473 Corr. Area: 39577

60400

EIC Ion 488 Corr. Area: 21833

33671

Ratio of 473:371: 0.261

0.290

Average: 0.276

Ratio of 488:371: 0.144

0.162

Average: 0.153

Acceptable Retention Time Range: 6.168

to 6.363

Approximate Minimum Corrected Area of 371 Ion: 30325

Acceptable 473:371 Ratio Range: 0.221

to 0.331

Acceptable 488:371 Ratio Range: 0.122

to 0.184