

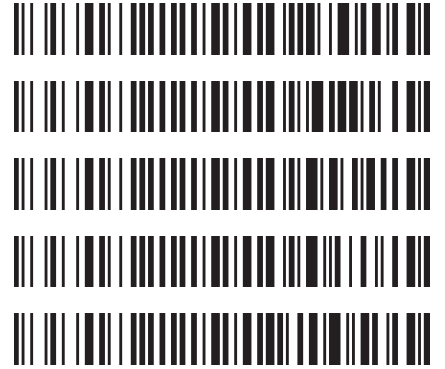
REVIEWED

By Brittany Wylie at 8:14 am, Sep 29, 2020

9/28/2020

Worklist: 4543

<u>LAB_CASE</u>	<u>ITEM</u>	<u>ITEM_TYPE</u>	<u>DESCRIPTION</u>
C2020-1667	1	UCK	AM 3 Urine Carboxy-THC
C2020-1803	1	UCK	AM 3 Urine Carboxy-THC
C2020-1823	2	UCK	AM 3 Urine Carboxy-THC
C2020-1830	1	UCK	AM 3 Urine Carboxy-THC
C2020-1883	1	UCK	AM 3 Urine Carboxy-THC



AM 3: Carboxy-THC Urine Extraction

Extraction Date: 9/25/20

Analyst: Anne Nord

Negative Urine Lot: 73020

GC/MS ID: 65198

Positive Control Working Solution Lot: working solution 82620 120 ul working solution to 2880 ul negative urine

Hexane Lot: fisher 42712 Ethyl Acetate Lot: fisher 020419

Silylating Agent Lot: Cerilliant FN08181601

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: added working solution to equal approximately 60 ng/ml Carboxy-THC
- 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
- 2. Did positive and negative control samples provide intended response? yes
- 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, Working solution prep sheet, and Control sample GCMS data printouts

Comments:

Toxicology AM method 27/26 external prep information

A

15 ug/ml C-THC and THC-OH 7.5 ug/ml THC ^{9/28/20} A

working solution ~~1 ug/ml in meoh C-THC, THC-OH, THC~~

Stock solution 1mg/ml 7.5 ul each THC, 100 ug/ml 150 ul C-THC, 150 ul THC-OH in 9692.5 ul meOH
 Ppd 8/26/20 Exp: 7/1/21 lot 82620 by AMN

Drug	lot	expiration
C-THC	FE01061702	3/1/2022
THC-OH	FE07221601	7/1/2021
THC	FE01041701	3/1/2022

AM 27/26 blood control 100 ul working solution lot () in 9900 ul blood lot ()

		Concentration 7.5 ng/ml THC, 15 ng/ml C-THC, THC-OH	
--	--	--	--

AM 27/26 urine control 400 ul working solution lot (82620) in 9600 ul urine lot (73020)

out of use

ppd 8/26/20 Exp 7/1/21	lot u82620	Concentration 30 ng/ml THC, and 60 ng/ml C-THC, THC-OH	by amn	



Run Date: 9/25/2020

Worklist #: 4543

Laboratory Case #: BRC3 lot 68460

Case Calculations:

EIC Ion 371 Corr. Area: 115264

EIC Ion 473 Corr. Area: 52413

EIC Ion 488 Corr. Area: 34349

473:371 Ratio: **0.455**

488:371 Ratio: **0.298**

Quality Control Calculations:

	1st Control	2nd Control
EIC Ion 371 Corr. Area:	278301	228052
EIC Ion 473 Corr. Area:	130528	105028
EIC Ion 488 Corr. Area:	81269	65595
Ratio of 473:371:	0.469	0.461
		Average: 0.465
Ratio of 488:371:	0.292	0.288
		Average: 0.290

Acceptable Retention Time Range: 4.496 to 4.653

Approximate Minimum Corrected Area of 371 Ion: 55660

Acceptable 473:371 Ratio Range: 0.372 to 0.558

Acceptable 488:371 Ratio Range: 0.232 to 0.348



Data Path : D:\DATA\2020\am 2\092520 am2\
 Data File : 01301015.D
 Acq On : 25 Sep 2020 18:24
 Operator : Instrument 65198
 Sample : BRC3
 Misc : am 3
 ALS Vial : 13 Sample Multiplier: 1

Integration Parameters: rteint.p
 Integrator: RTE
 Smoothing : ON Filtering: 5
 Sampling : 1 Min Area: 3 % of largest Peak
 Start Thrs: 0.2 Max Peaks: 100
 Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
 Peak separation: 5

Method : D:\MassHunter\GCMS\1\METHODS\DEFAULT.M
 Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): 01301015.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.499	52	59	63	rBV	4102	7195	6.24%	5.875%
2	4.553	63	69	94	rVB	67504	115264	100.00%	94.125%

Sum of corrected areas: 122459

Signal : EIC Ion 473.00 (472.70 to 473.70): 01301015.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.558	62	70	91	rVB	30404	52413	100.00%	96.399%
2	4.757	101	107	118	rVB	1003	1958	3.74%	3.601%

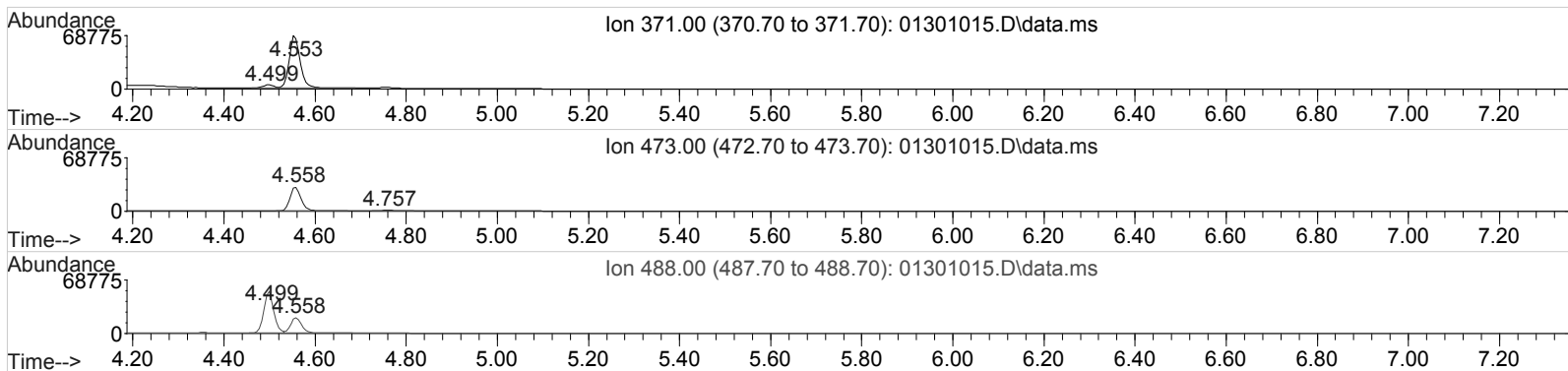
Sum of corrected areas: 54371

Signal : EIC Ion 488.00 (487.70 to 488.70): 01301015.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.499	51	59	65	rBV	53700	82539	100.00%	70.614%
2	4.558	65	70	93	rVB	19539	34349	41.62%	29.386%

Sum of corrected areas: 116888

DEFAULT.M Mon Sep 28 14:18:15 2020





Data Path : D:\DATA\2020\am 2\092520 am2\
Data File : 01101013.D
Acq On : 25 Sep 2020 18:05
Operator : Instrument 65198
Sample : positive control CTCH
Misc : am 3
ALS Vial : 11 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 3 % of largest Peak
Start Thrs: 0.2 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : D:\MassHunter\GCMS\1\METHODS\DEFAULT.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): 01101013.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.553	63	69	92	rVB	129040	228052	100.00%	100.000%

Sum of corrected areas: 228052
Signal : EIC Ion 473.00 (472.70 to 473.70): 01101013.D\data.ms

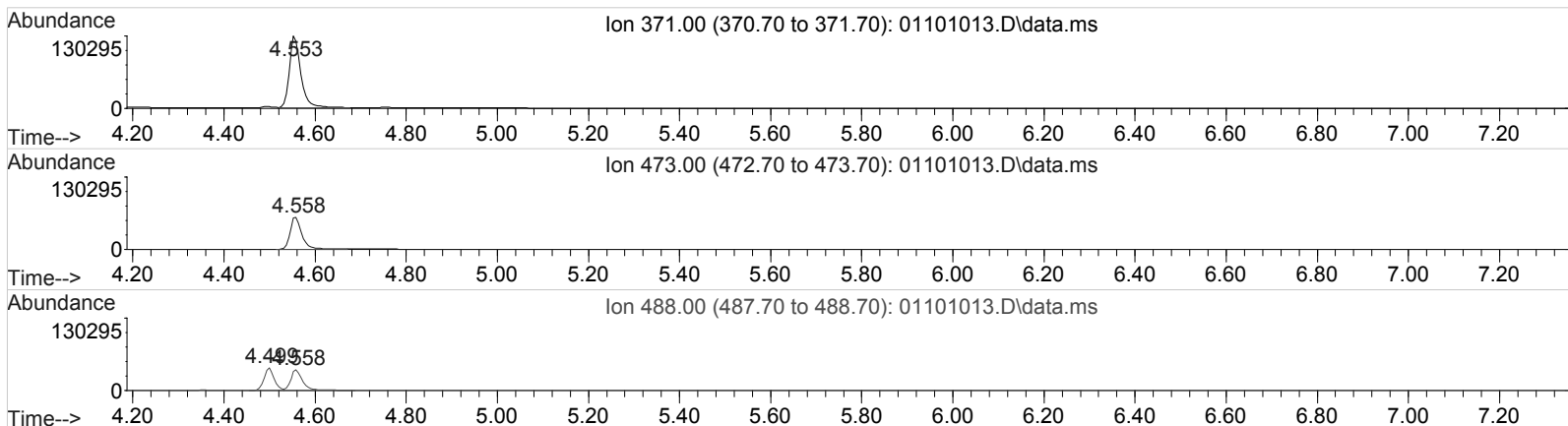
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.558	62	70	91	rBV	57816	105028	100.00%	100.000%

Sum of corrected areas: 105028
Signal : EIC Ion 488.00 (487.70 to 488.70): 01101013.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.499	51	59	65	rBV	40645	63956	97.50%	49.367%
2	4.558	65	70	94	rVB	37259	65595	100.00%	50.633%

Sum of corrected areas: 129551

DEFAULT.M Mon Sep 28 14:17:20 2020





Data Path : D:\DATA\2020\am 2\092520 am2\
Data File : 01101001.D
Acq On : 26 Sep 2020 09:33
Operator : Instrument 65198
Sample : positive control CTCH
Misc : am 3
ALS Vial : 11 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 3 % of largest Peak
Start Thrs: 0.2 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : D:\MassHunter\GCMS\1\METHODS\DEFAULT.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): 01101001.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.596	71	77	100	rVB	159111	278301	100.00%	100.000%

Sum of corrected areas: 278301
Signal : EIC Ion 473.00 (472.70 to 473.70): 01101001.D\data.ms

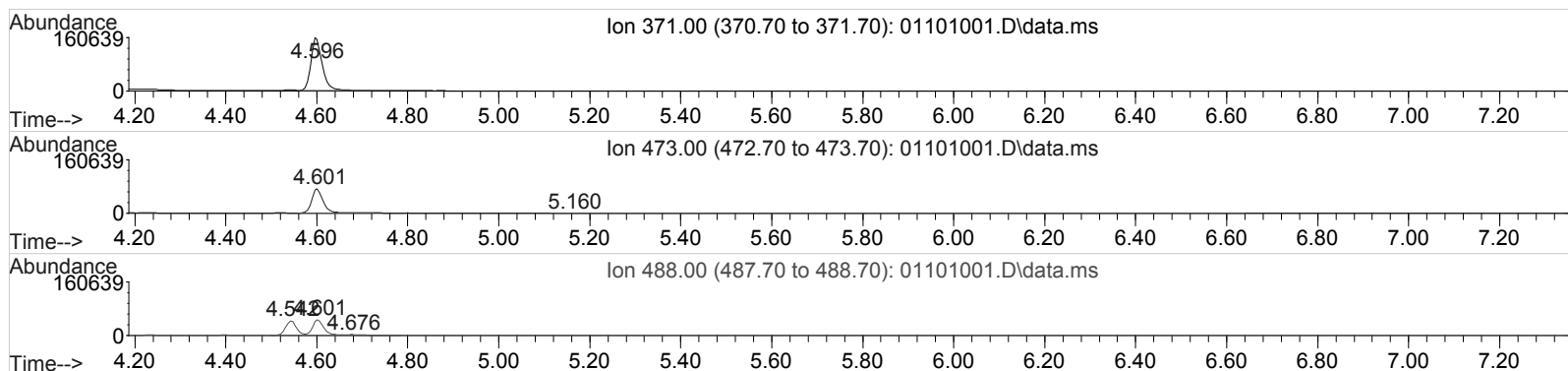
peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.601	70	78	101	rBV	72256	130528	100.00%	97.016%
2	5.160	173	182	457	rBV2	44	4015	3.08%	2.984%

Sum of corrected areas: 134543
Signal : EIC Ion 488.00 (487.70 to 488.70): 01101001.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.542	59	67	73	rBV	43227	71596	88.10%	45.121%
2	4.601	73	78	88	rVV	46093	81269	100.00%	51.217%
3	4.676	88	92	112	rVB	2447	5812	7.15%	3.663%

Sum of corrected areas: 158677

DEFAULT.M Mon Sep 28 14:16:36 2020





Run Date: 9/25/2020

Worklist #: 4543

Laboratory Case #: Negative control

Case Calculations:

EIC Ion 371 Corr. Area: *no peaks in range*

EIC Ion 473 Corr. Area: 951

473:371 Ratio: **#VALUE!**

EIC Ion 488 Corr. Area: 63737

488:371 Ratio: **#VALUE!**

Quality Control Calculations:

	1st Control	2nd Control
EIC Ion 371 Corr. Area:	278301	228052
EIC Ion 473 Corr. Area:	130528	105028
EIC Ion 488 Corr. Area:	81269	65595
Ratio of 473:371:	0.469	0.461
		Average: 0.465
Ratio of 488:371:	0.292	0.288
		Average: 0.290

Acceptable Retention Time Range: 4.496 to 4.653

Approximate Minimum Corrected Area of 371 Ion: 55660

Acceptable 473:371 Ratio Range: 0.372 to 0.558

Acceptable 488:371 Ratio Range: 0.232 to 0.348

GA

Data Path : D:\DATA\2020\am 2\092520 am2\
Data File : 01201014.D
Acq On : 25 Sep 2020 18:15
Operator : Instrument 65198
Sample : negative control CTCH
Misc : am 3
ALS Vial : 12 Sample Multiplier: 1

Integration Parameters: rteint.p
Integrator: RTE
Smoothing : ON Filtering: 5
Sampling : 1 Min Area: 3 % of largest Peak
Start Thrs: 0.2 Max Peaks: 100
Stop Thrs : 0 Peak Location: TOP

If leading or trailing edge < 100 prefer < Baseline drop else tangent >
Peak separation: 5

Method : D:\MassHunter\GCMS\1\METHODS\DEFAULT.M
Title :

Signal : EIC Ion 371.00 (370.70 to 371.70): 01201014.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.494	52	58	65	rBV	4916	9745	100.00%	37.923%
2	4.757	104	107	116	rVB	3864	6643	68.17%	25.851%
3	4.881	124	130	135	rBV	2664	5412	55.54%	21.061%
4	4.994	147	151	154	rBV	2389	3897	39.99%	15.165%

Sum of corrected areas: 25697
Signal : EIC Ion 473.00 (472.70 to 473.70): 01201014.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.558	64	70	86	rVB	424	951	100.00%	84.759%
2	5.171	173	184	186	rBV2	45	171	17.98%	15.241%

Sum of corrected areas: 1122
Signal : EIC Ion 488.00 (487.70 to 488.70): 01201014.D\data.ms

peak #	R.T. min	first scan	max scan	last scan	PK TY	peak height	corr. area	corr. % max.	% of total
1	4.499	50	59	93	rVB	40281	63737	100.00%	100.000%

Sum of corrected areas: 63737

DEFAULT.M Mon Sep 28 14:17:44 2020

