Silylation and Methanol as an Alternative GC Solvent (W2, Winter)

1/12/2020

Notebook	openEst
Concept	
Labhead	
Program	
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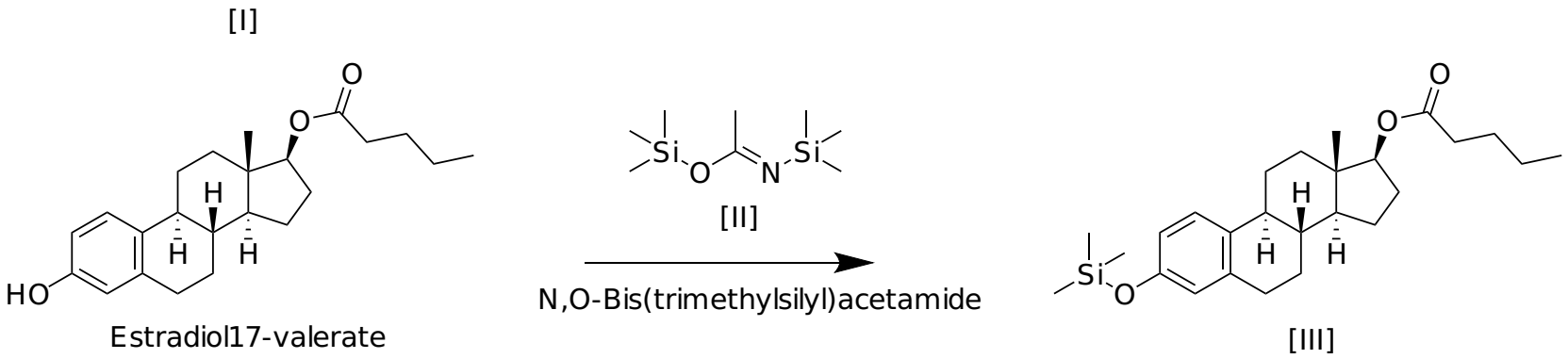
Modified	Gwyn Uttmark uttmark@stanford.edu Jan 16, 2020 12:02 PM PST

Status: Active

Write up

BSA Silylation



Reactants

Rxn ID	Reactant	MF	FM	MW	EM	Limit?	Eq
I	(8R,9S,13S,14S,17S)-3-hydroxy-13-methyl-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl pentanoate	$C_{23}H_{32}O_3$	356.51 g/mol	356.51 g/mol	356.23514	✓	1
II	trimethylsilyl (E)-N-(trimethylsilyl)acetimidate	$C_8H_{21}NOSi_2$	203.43 g/mol	203.43 g/mol	203.11617		1

Products

Rxn ID	Product ID	Product	MF	FM	MW	EM
III	P1	(8R,9S,13S,14S,17S)-13-methyl-3-((trimethylsilyl)oxy)-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyclopenta[a]phenanthren-17-yl pentanoate	$C_{26}H_{40}O_3Si$	428.69 g/mol	428.69 g/mol	428.27467

In Experiment Notes

1. weigh pill: 0.1420g
2. powder in sin vial
3. add 10ml acn and vortex
4. add 10ml more acn and vortex
5. allow to sit for ~10 min
6. weigh second pill: 0.1319g
7. powder in sin vial
8. add 20ml methanol via pipette
9. vortex both

this forms a 100ppm solution - 0.1mg/ml - this is too little, will re-constitute

1. heat two 8ml vials to 60C
2. add ~4mL and evaporate to ~1/2 volume\

while that runs, prepare more dilute gcms samples:

1. pipette 1ml of methanol sample into M1 gcms vial ******DO NOT DO THIS *******

the methanol has an alcohol..... noticed something was off when the reaction gave off lots of heat.

anyways, do this with A1

1. add 1ml acn solution prepped above
2. add .5ml n,o-bis(trimethylsilyl)acetamide
3. mix multiple times, let sit at least 10 min
4. remove top phase and gcms

(at this point, methanol erupted from the methanol tube - the heating block exceded the bp of the sample. even the acn was bubbling)

then prep normal gcms samples:

1. 750ul into whattman filter - M2 and A2 respectively

when the evaporated samples are <1ml transfer to whattman gcms vials, label A3 and M3 respectively (won't be quantitative, but will be helpful for testing

prep two blanks (methanol and acn, BM and BA respectively)

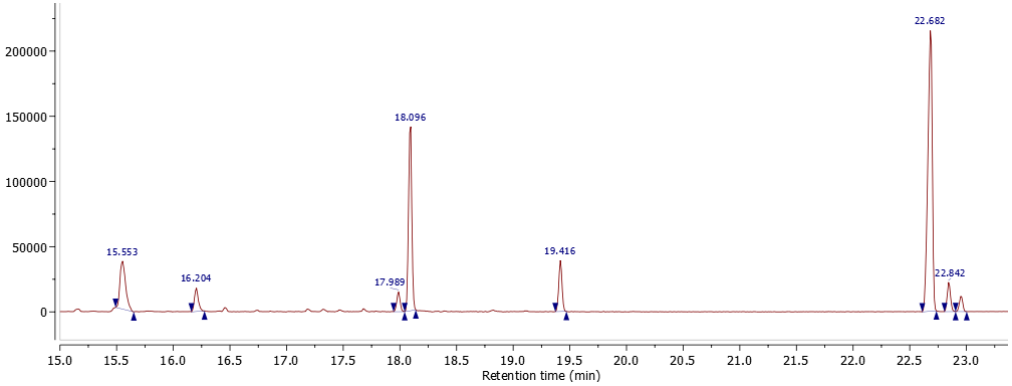
Sample Number	Label	Description
1	BA	Blank AcN
2	BM	Blank Methanol
3	A1	silylated dilute sample
4	A2	2mg/20ml AcN sample
5	M2	2mg/20ml methanol sample
6	M3	evaporated methanol sample
7	A3	evaporated AcN sample

After Experiment Notes

Sample Number	Label	Description
1	BA	Acetonitrile
2	BM	Methanol
3	A1	1mL (0.1mg EV/mL Acetonitrile) Silylated with (N,O-bis(trimethylsilyl)acetamide, 0.5mL) (not acid-catalyzed)
4	A2	1mL (0.1mg EV/mL Acetonitrile)
5	M2	1mL (0.1mg EV/mL Methanol)
6	M3	~1mg EV/mL Methanol
7	A3	~1mg EV/mL Acetonitrile

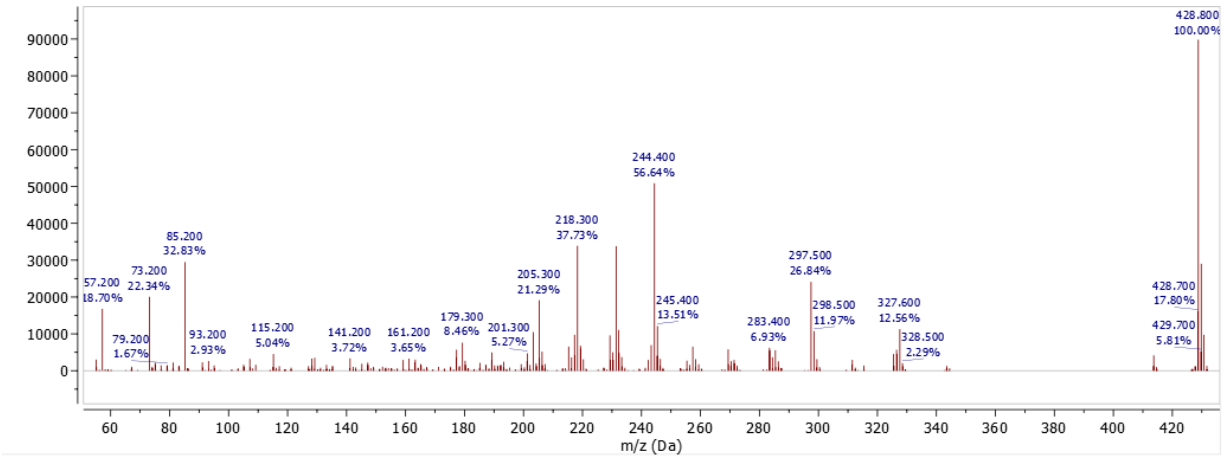
GCMS Runs interrupted by computer issue.

But the first two A1 runs went through (poster and oct-25 (a modified poster protocol), coming up with very similar chromatographs:

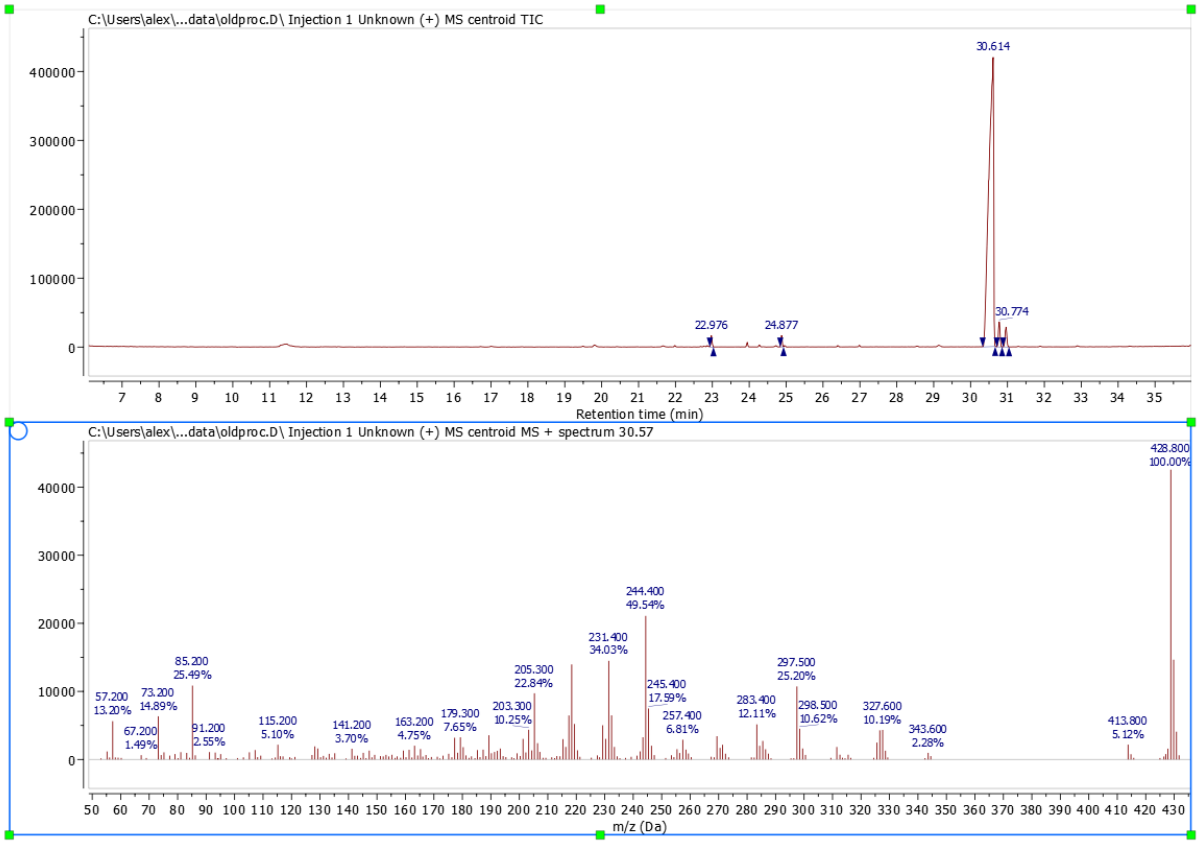


which altogether were quite confusing.

No peaks for the estradiol M+ peak (356g/mol) were located, but a variety of 326 peaks (15.53, 16.20). The 17.989,18.096 and 19.426 peaks are unexplained. The 22.68 cluster of peaks represented the silylated estradiol valerate with a clear 428.8 M+ peak:



which is very similar to previous silylated estradiol valerate peaks both in mass spec and peak clustering [slack]:



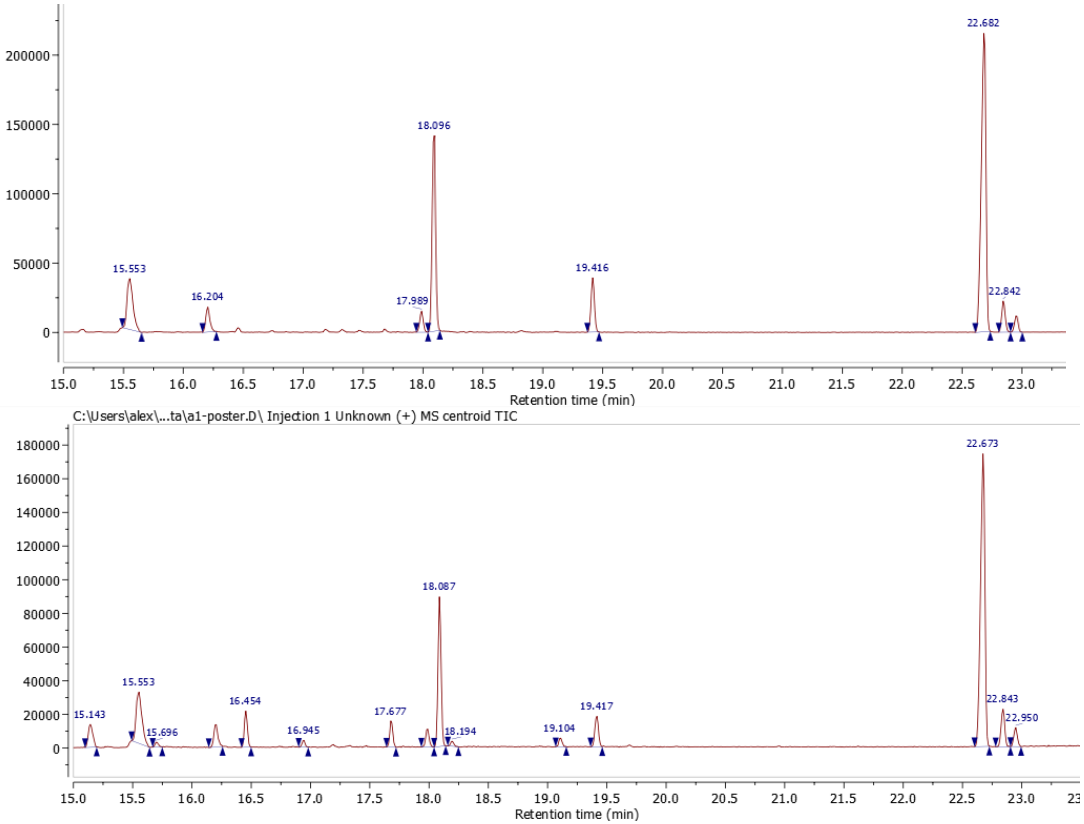
Possibly, allowing the mixture to react further or adding acid might help drive a complete reaction. A ThermoScientific procedure [s] uses a similar procedure to this but heats @70C for 15 minutes (and doesn't use acid catalysis). Further GCMS results will aid in analysis, but excitedly, the very dilute sample was clearly visible in the chromatographs.

After Experimental Notes (cont. a1 analysis)

With more data, more understanding follows ... if all goes well.

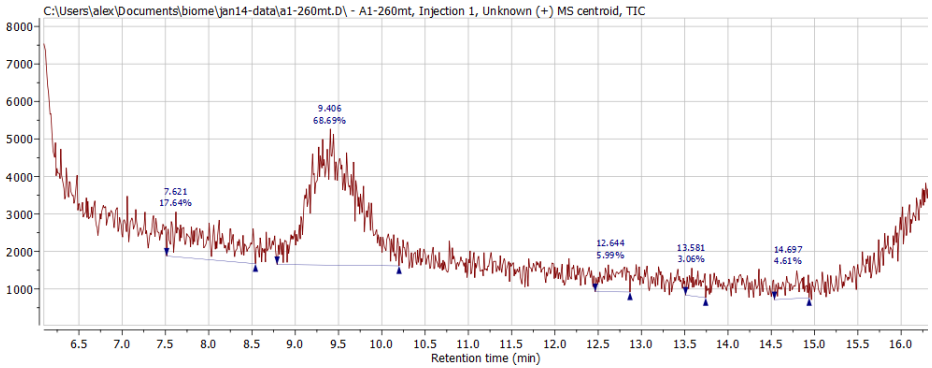
Few highlights on the remaining a1 data:

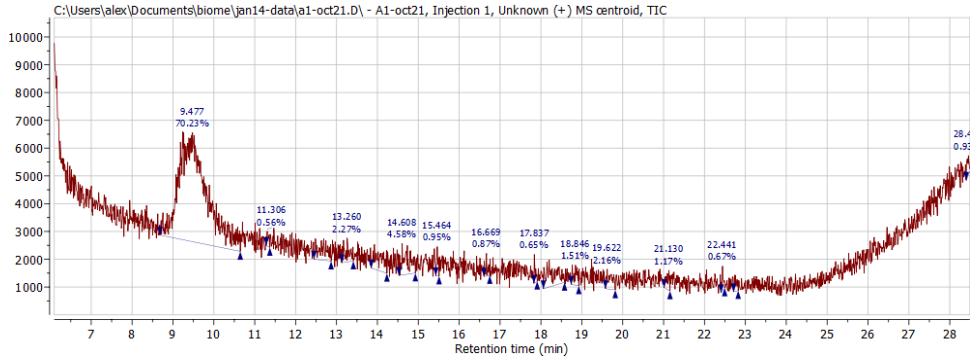
- the samples' noise floor and anomalous peaks increased the second day - maybe they're a result of the Whatman filter? (this would explain why they weren't present before, I simply used the normal no-filter vials)
- the peak attributed to EV appears to have decreased in magnitude relative to the EVS (estradiol valerate silylated) peak - the reaction didn't go to completion yet



(jan13, a1, poster) vs (jan14, a1, poster)

- the monotemp/oct21 procedures were much worse than the poster/oct25 procedures, eluting only the EVS at 9.406 and 9.477 respectively:

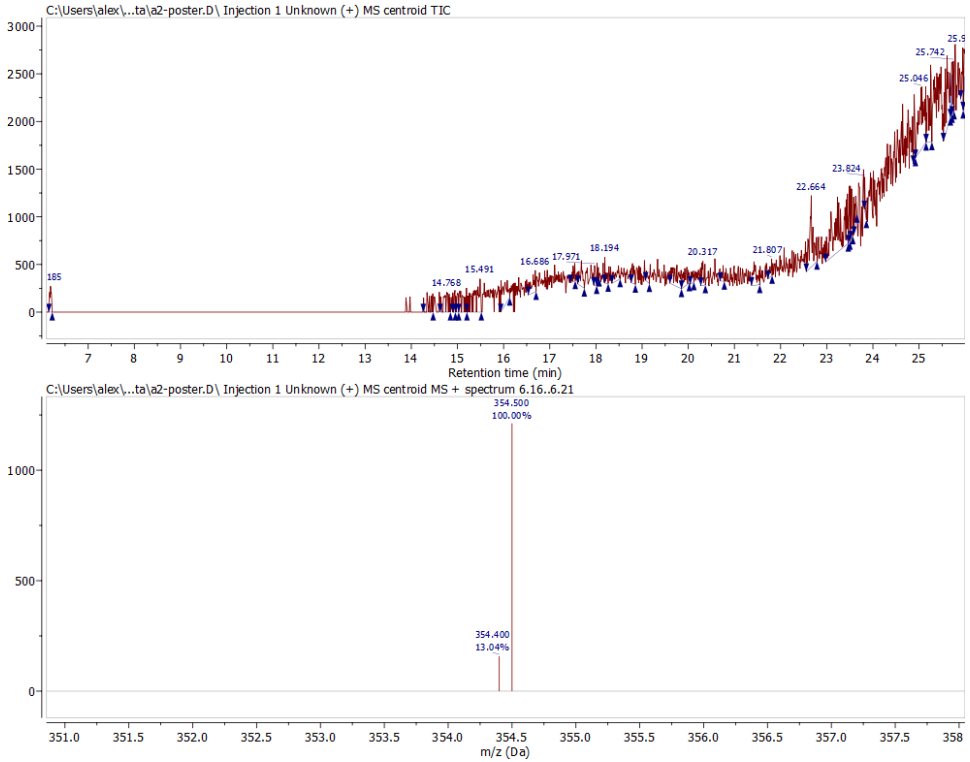


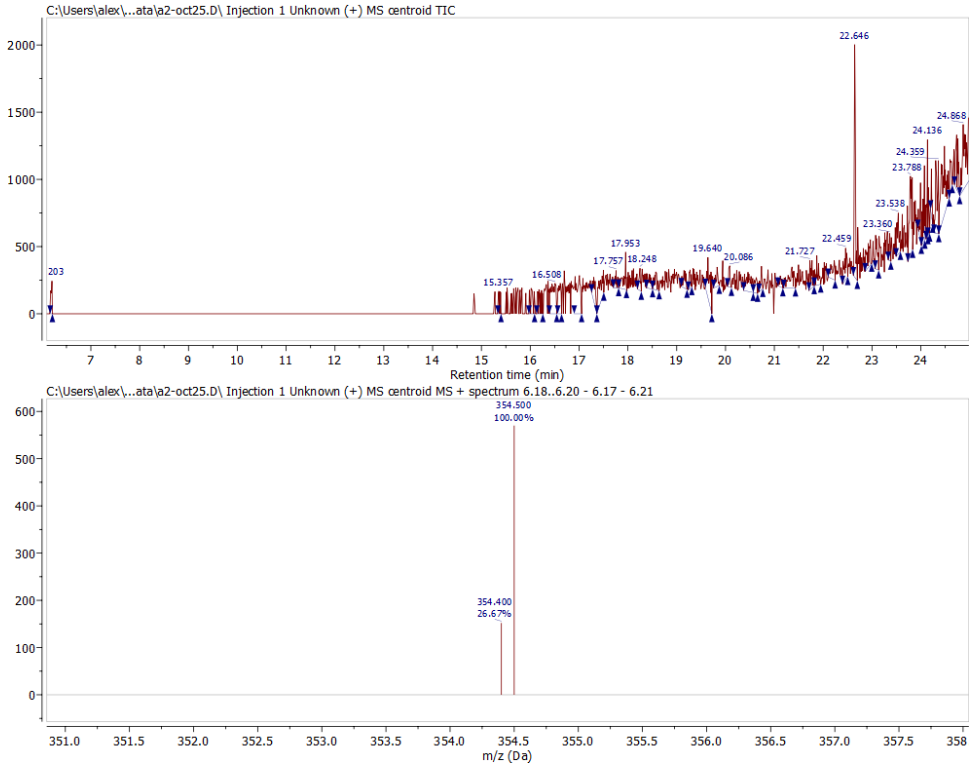


(jan14, a1, mt260) and (jan14, a1, oct21)

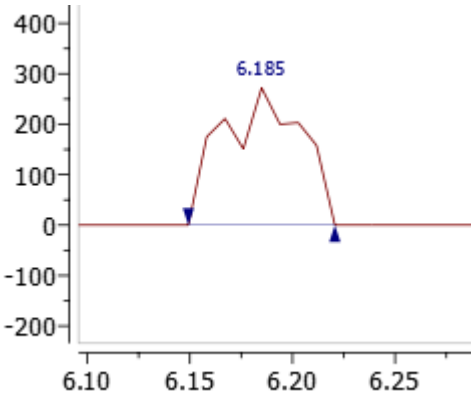
After Experimental Notes (a2 analysis)

The charts from a2 were much lower in peak intensity, but very low background noise (except oct21). Elution of EV happened right after the solvent delay, so a re-run with a shorter delay might be useful to ensure no prior peaks eluted. Some EVS occurred in oct25 (22.646) even though it ran 4 samples after any a1, indicating column sticking. It is also found in a3 samples (most), demonstrating impressive column retention (see a3 section for good illustration of this).

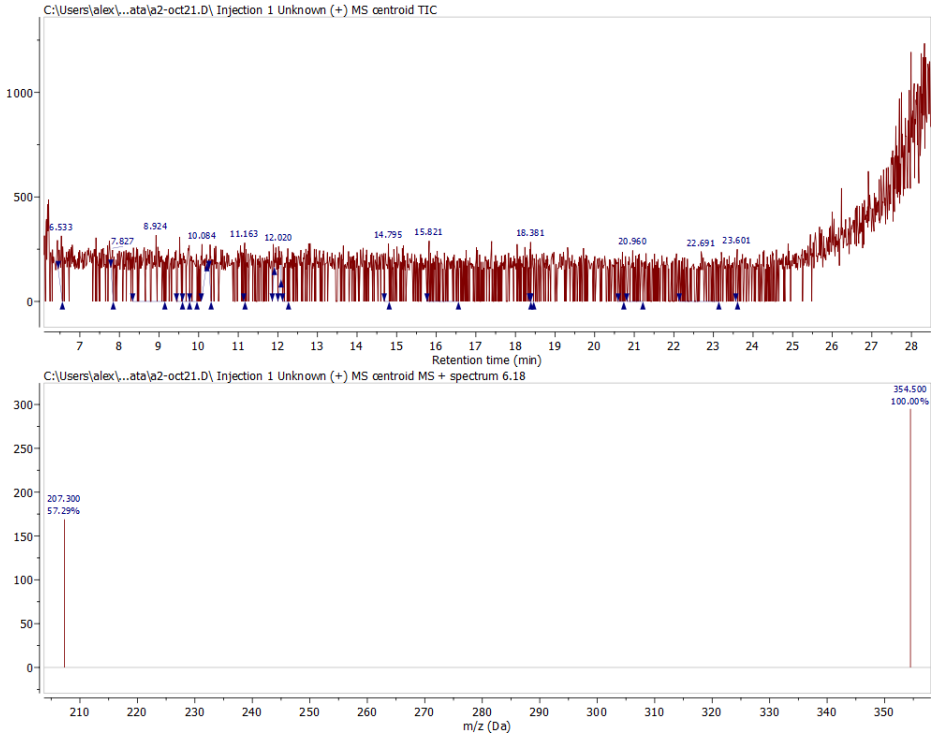

C:\Users\alex\...ta\a2-poster.D\ Injection 1 Unknown (+) MS centroid TIC
185
14.768
15.491
16.686
17.971
18.194
20.317
21.807
22.664
23.824
25.046
25.742
Retention time (min)
C:\Users\alex\...ta\a2-poster.D\ Injection 1 Unknown (+) MS centroid MS + spectrum 6.16..6.21
354.500
100.00%
354.400
13.04%
m/z (Da)



(jan14, a2, poster) and (jan14, a2, oct25)



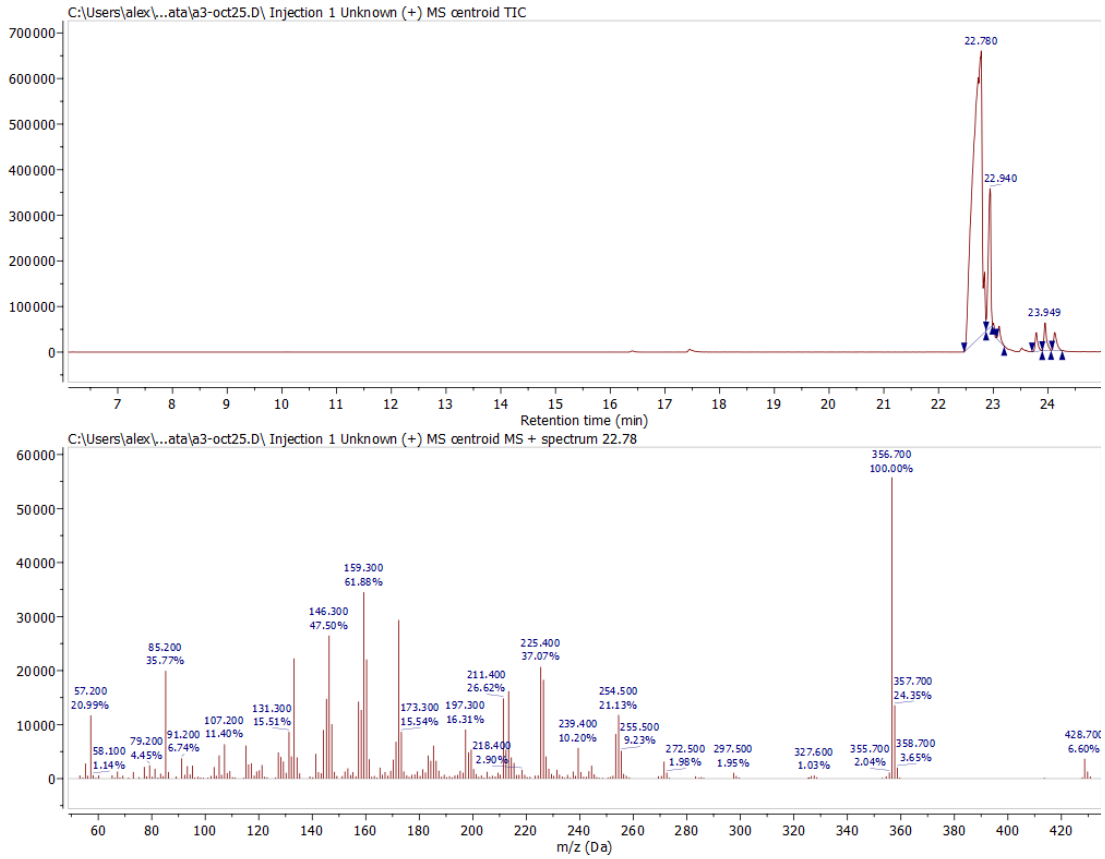
(jan14, a2, poster (zoomed in))

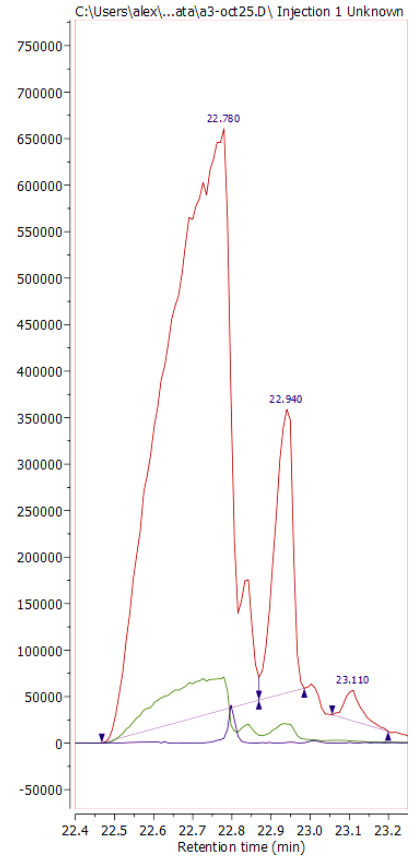


(jan14, a2, oct21)

After Experimental Notes (a3 analysis)

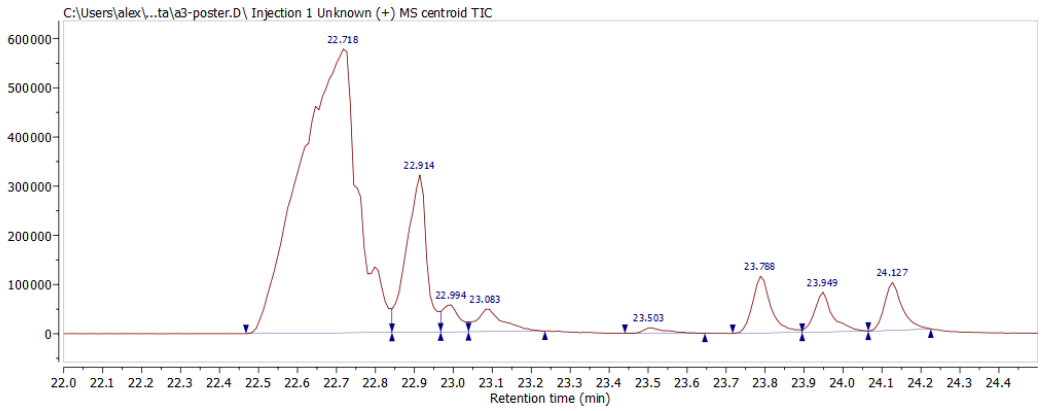
A3 was mostly unsurprising with moderately "noisy" peaks around 22.8 with strong EV character. Interestingly, they also still had clear EVS co-elution with a3 samples - a worrisome trend when it comes to quantification.

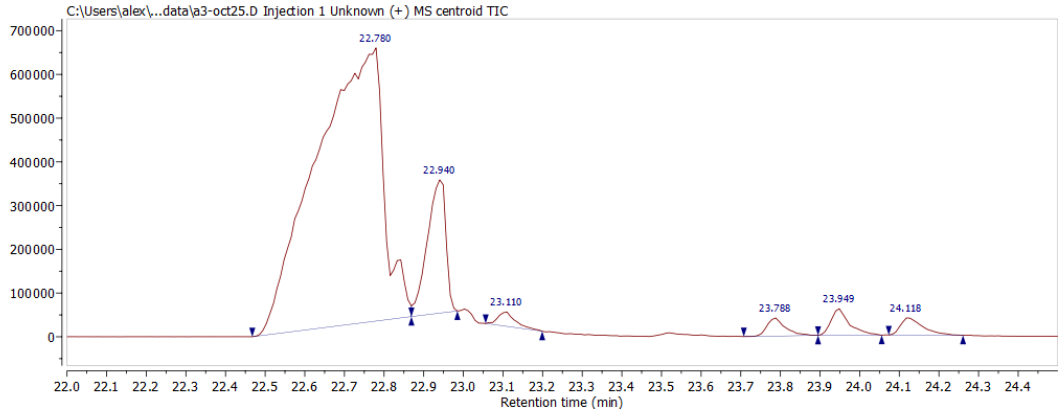




(jan14, a3, oct25) (left: full-spectrum, right: zoom with green representing EV levels and blue representing EVS levels)

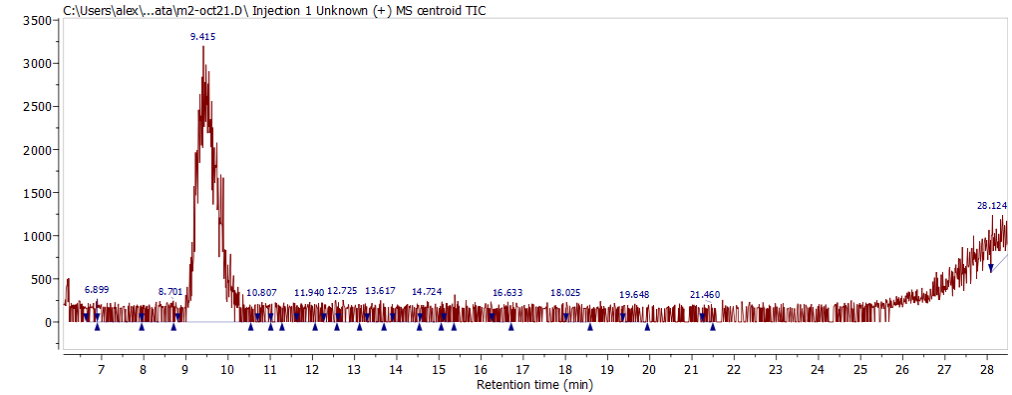
As usual, by now, the poster and oct25 tracked each other, with poster having slightly better peak shape *but* more noise peaks.

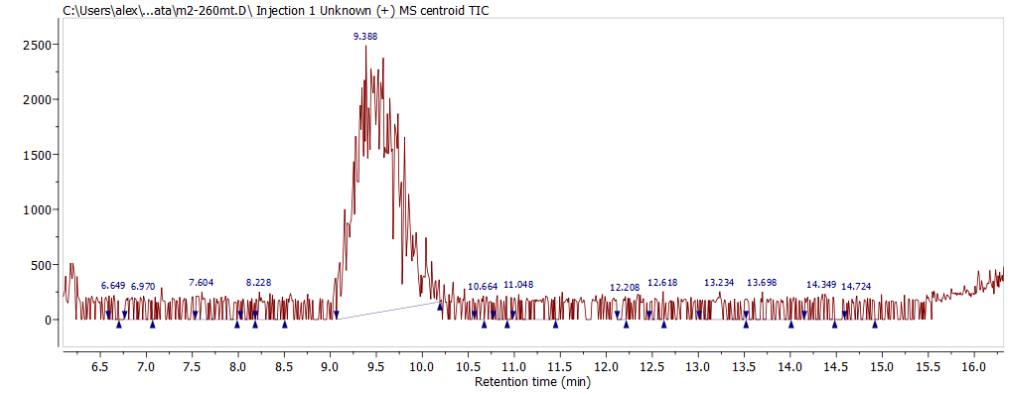




left:(jan14, a3, poster) right:(jan14, a3, oct25)

Oct21 and 260mt both had lower intensity readings (even with the quite concentrated a3) with oct21 having a better peak shape:



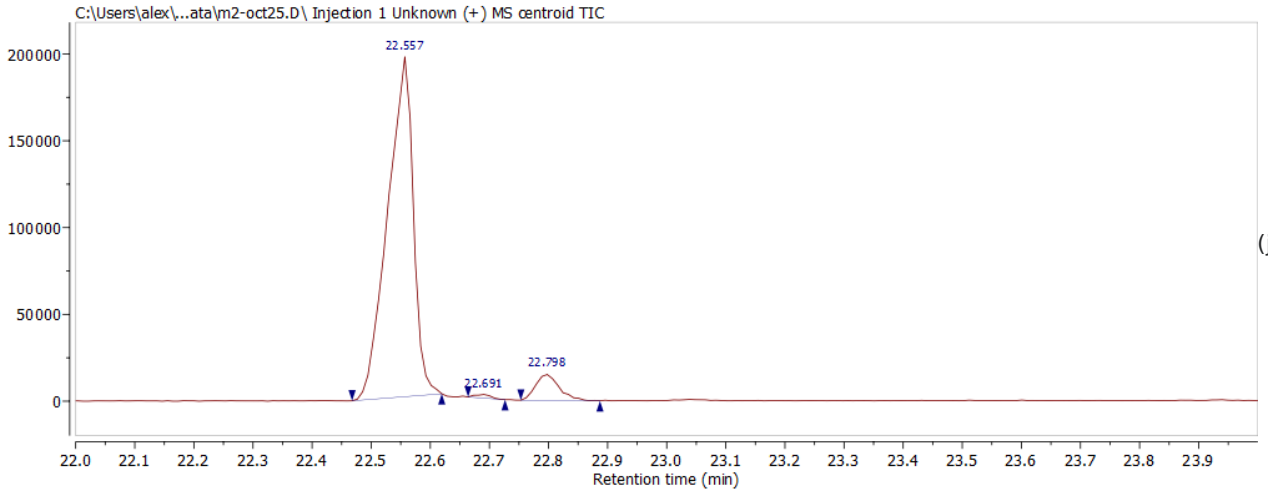


left:(jan14, a3, oct21) right:(jan14, a3, 260mt)

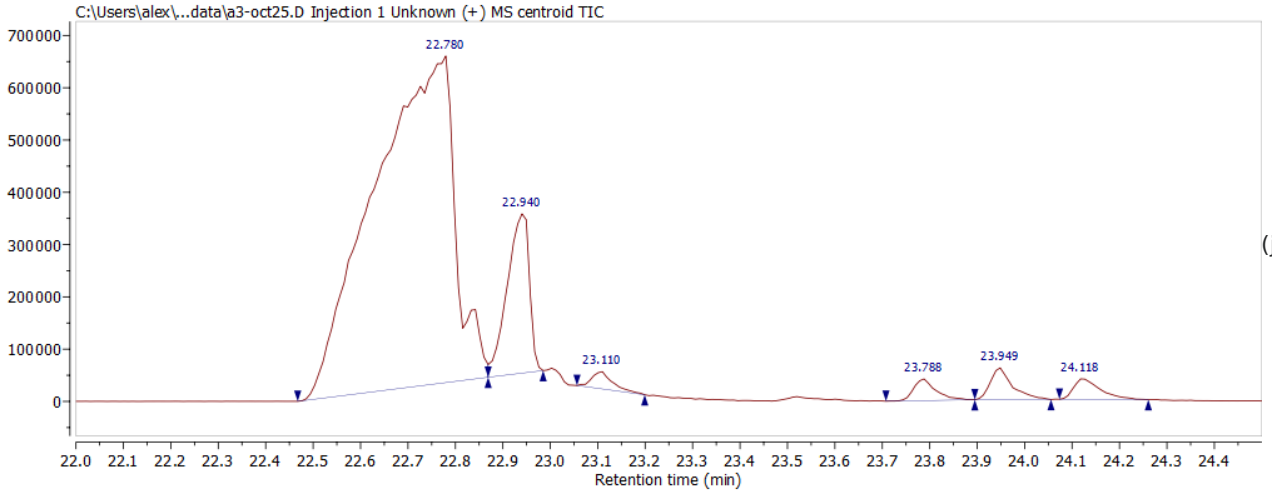
After Experimental Notes (methanol)

The methanol samples, in general, offered better peak shape and, in the case of poster and oct25, lower background noise.

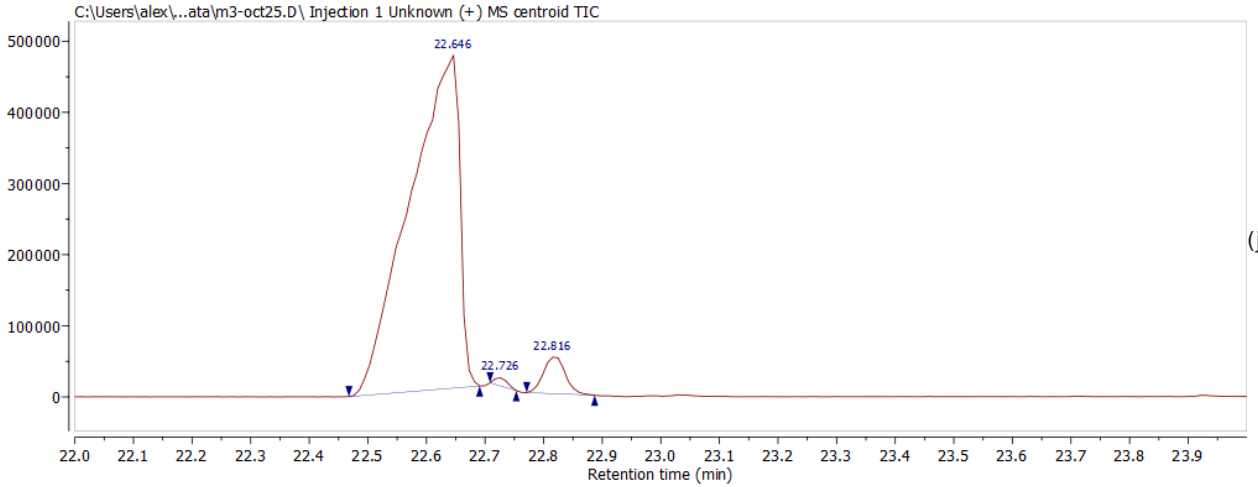
Taking the best results from oct25, we see better elution in both m2 and m3 samples:



(jan14, m2, oct25)

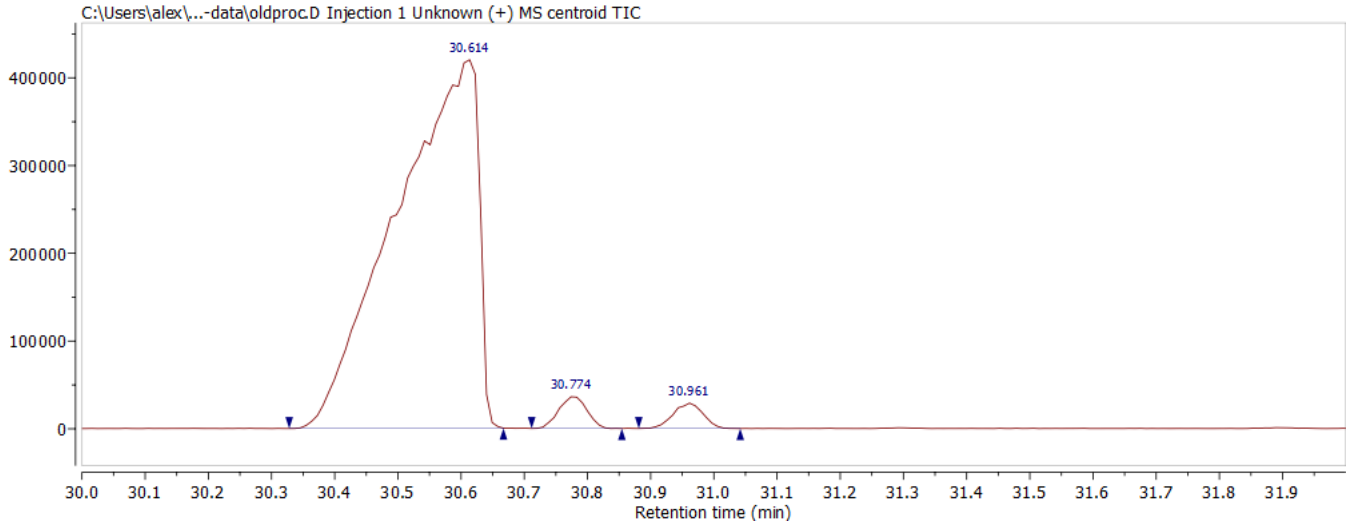


(jan14, a3, oct25)



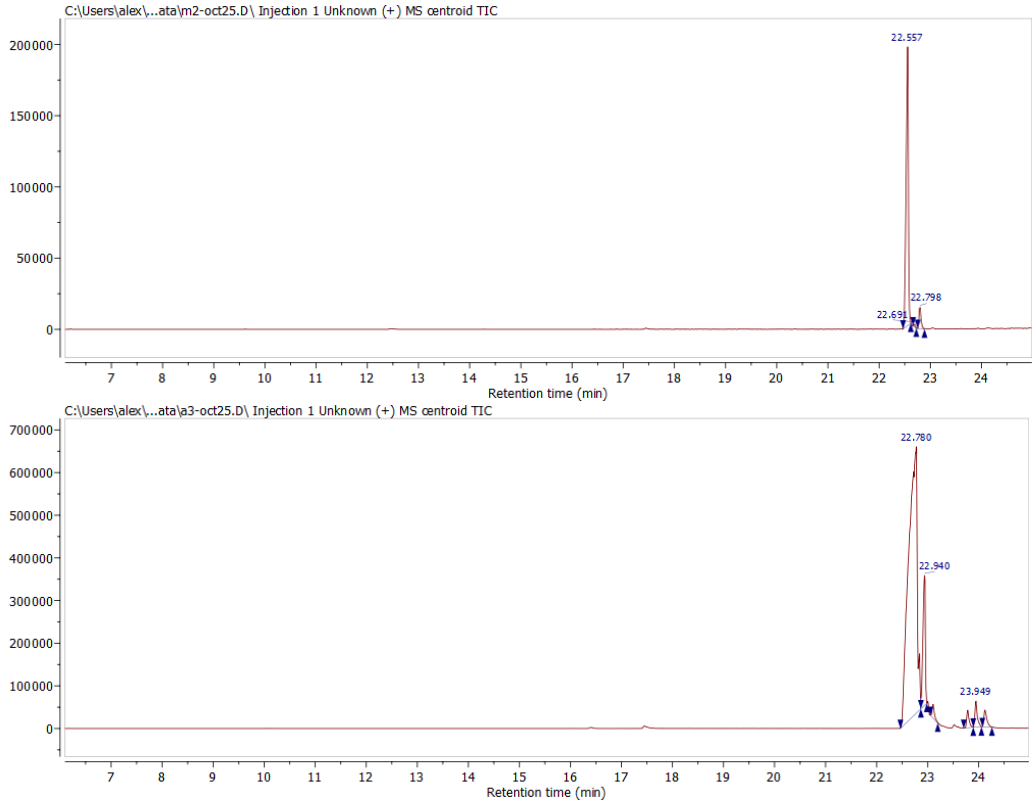
(jan14, m3, oct25)

Further, past runs in acetonitrile also show a similar peak shape:



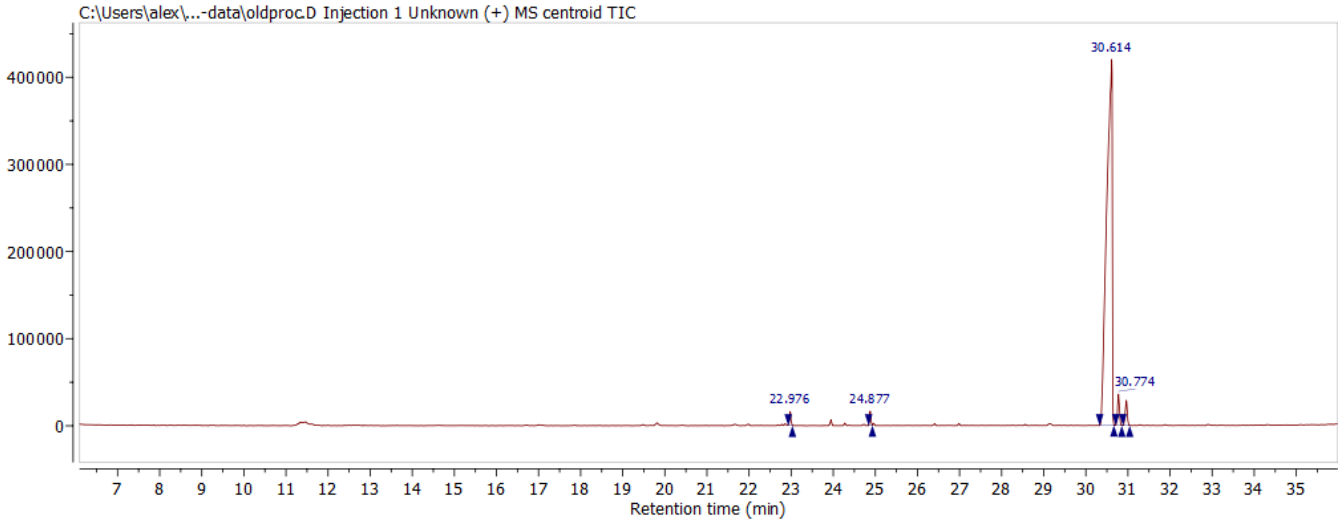
(19-nov10, ?, oldproc)

Methanol generally had lower noise across the entire spectrum:



left:(jan14, m2, oct25) right:(jan14, a3, oct25)

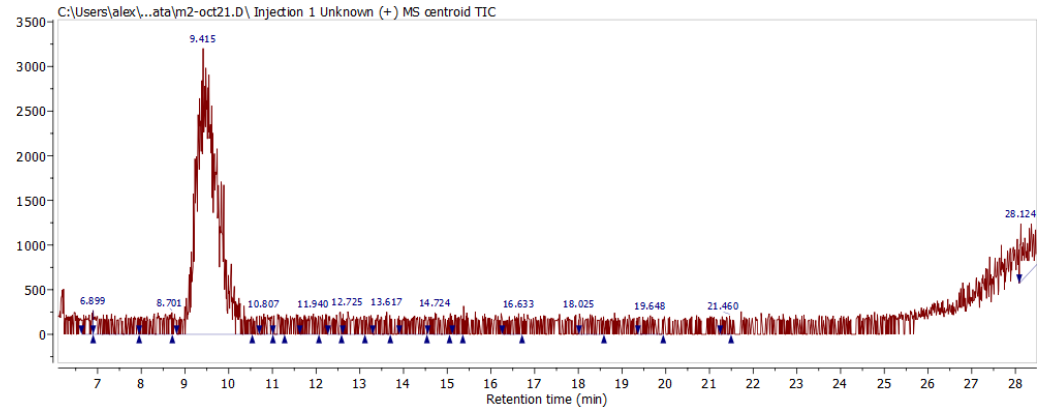
Although older acetonitrile samples do *not* show the same noise issues, pointing to idiosyncrasies of this particular sample as the culprit:

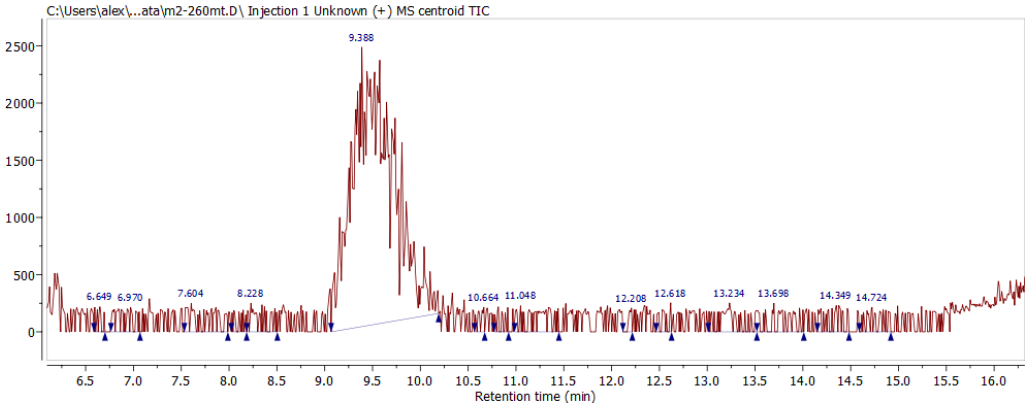


(19-nov10, ?, oldproc)

The m3 oct21 and 260mt chromatograms were decent with a poor peak shape but quick elution -

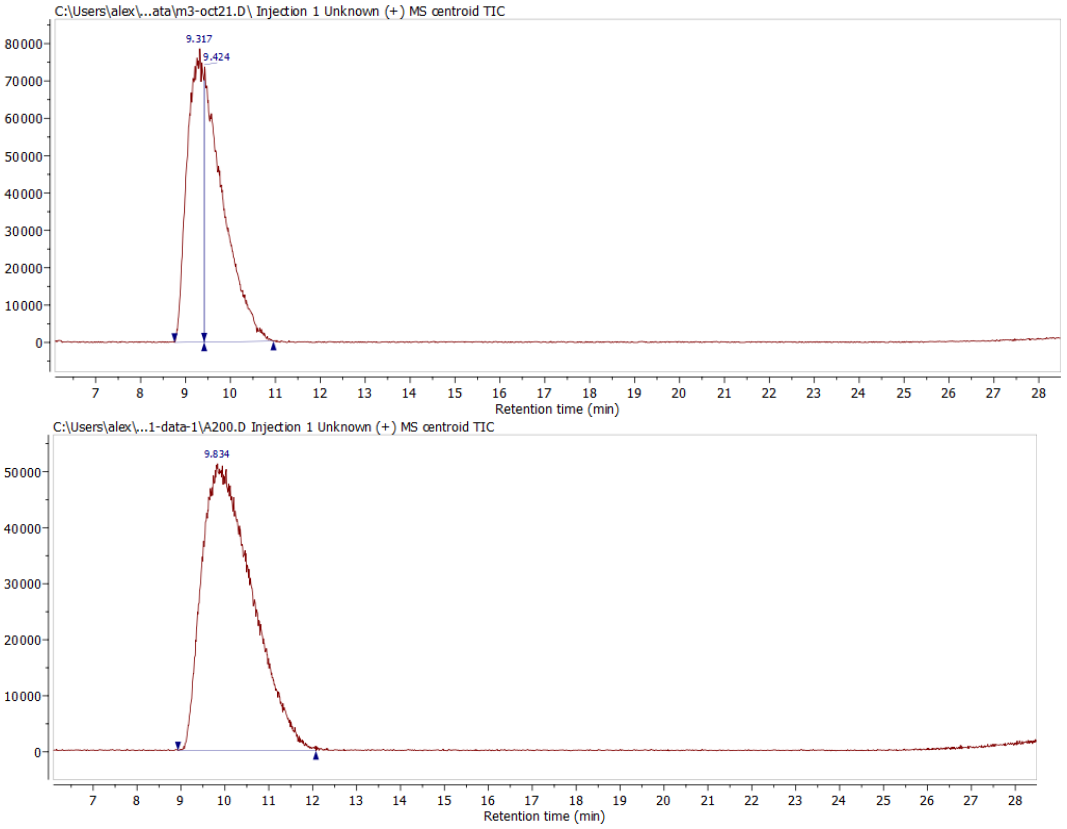
For completeness, I'm including the oct21 and 260mt chromatograms even though they are unremarkable:





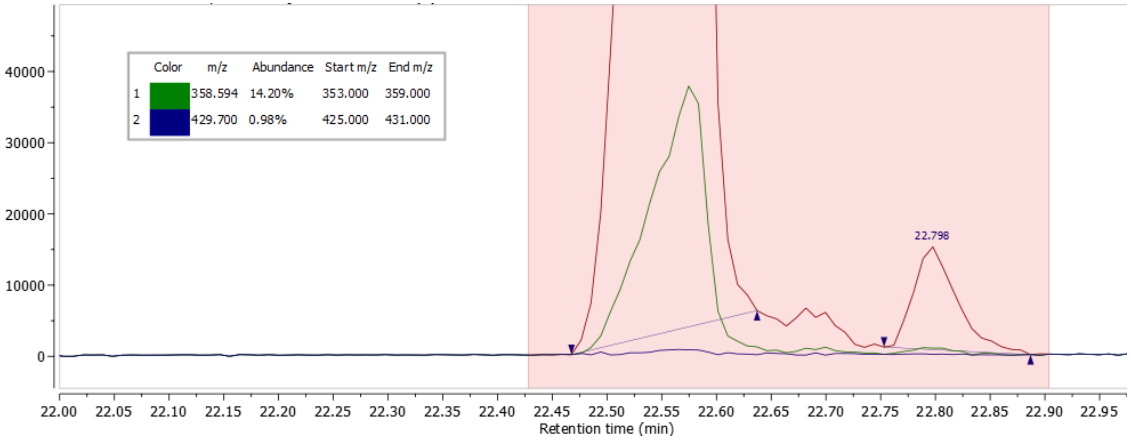
left: (jan14, m2, oct21) right: (jan14, m2, 260mt)

And, the methanol oct21 peak shape is only *slightly* better when compared with previous runs in acetonitrile:



left: (jan14, m3, oct21) right: (oct21-19, A200, oct21)

Last, but not least, we continue to see slight co-elution of the silylated estradiol valterate with the non-silylated estradiol, even in m2 (which is multiple samples after a1, the only silylated compound):



(jan14, m2, poster)

Summary

Key Findings:

- Silylated samples are more easily detectable at lower concentrations
- Silylated Estradiol Valerate continues to elute with estradiol in later samples, which is potentially an issue when it comes to quantification
- EV elution occurs early in A2 samples, which at higher concentrations offers a fast low noise method for quantification (at ~6min!)
- Methanol as a co-solvent offers lower noise (questionable, needs verification) and better peak shape (well-supported herein)

Moving forward, a simple experiment that looks at silylated EV in methanol (just to make sure it's not *amazing*), an experiment investigating a2's interesting low noise (as opposed to a3s runs) with the oct21 and 260mt procedures may be useful.

Overall, this experiment was very informative, but could have been done better if the methanol and acetonitrile samples were prepared from the same solid stock - future experiments of this nature should use stock EV.

Sample Running Order

> 1	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba1	Blank
2	A1-poster-meth	3	D:\est\biome\jan13-methods	...	poster-replicate.M	...	D:\est\biome\jan13-data	...	a1-poster	Sample
3	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba2	Blank
4	A1-oct25	3	D:\est\biome	...	oct25.M	...	D:\est\biome\jan13-data	...	a1-oct25	Sample
5	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba3	Blank
6	A1-oct21	3	D:\est\biome	...	oct21.M	...	D:\est\biome\jan13-data	...	a1-oct21	Sample
7	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba4	Blank
8	A1-260mt	3	D:\est\biome	...	260mt.M	...	D:\est\biome\jan13-data	...	a1-260mt	Sample
9	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba5	Blank
10	A2-poster	4	D:\est\biome\jan13-methods	...	poster-replicate.M	...	D:\est\biome\jan13-data	...	a2-poster	Sample
11	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba6	Blank
12	A2-oct25	4	D:\est\biome	...	oct25.M	...	D:\est\biome\jan13-data	...	a2-oct25	Sample
13	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba7	Blank
14	A2-oct21	4	D:\est\biome	...	oct21.M	...	D:\est\biome\jan13-data	...	a2-oct21	Sample
15	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba8	Blank
16	A2-260mt	4	D:\est\biome	...	260mt.M	...	D:\est\biome\jan13-data	...	a2-260mt	Sample
17	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba9	Blank
18	A3-poster	7	D:\est\biome\jan13-methods	...	poster-replicate.M	...	D:\est\biome\jan13-data	...	a3-poster	Sample
19	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba10	Blank
20	A3-oct25	7	D:\est\biome	...	oct25.M	...	D:\est\biome\jan13-data	...	a3-oct25	Sample
21	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba11	Blank
22	A3-oct21	7	D:\est\biome	...	oct21.M	...	D:\est\biome\jan13-data	...	a3-oct21	Sample
23	blankA	1	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	ba12	Blank
24	A3-260mt	7	D:\est\biome	...	260mt.M	...	D:\est\biome\jan13-data	...	a3-260mt	Sample
25	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM5	Blank
26	M2-poster	5	D:\est\biome\jan13-methods	...	poster-replicate.M	...	D:\est\biome\jan13-data	...	m2-poster	Sample
27	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM6	Blank
28	M2-oct25	5	D:\est\biome	...	oct25.M	...	D:\est\biome\jan13-data	...	m2-oct25	Sample
29	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM7	Blank
30	M2-oct21	5	D:\est\biome	...	oct21.M	...	D:\est\biome\jan13-data	...	m2-oct21	Sample
31	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM8	Blank
32	M2-260mt	5	D:\est\biome	...	260mt.M	...	D:\est\biome\jan13-data	...	m2-260mt	Sample
33	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM9	Blank
34	M3-poster	6	D:\est\biome\jan13-methods	...	poster-replicate.M	...	D:\est\biome\jan13-data	...	m3-poster	Sample
35	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM10	Blank
36	M3-oct25	6	D:\est\biome	...	oct25.M	...	D:\est\biome\jan13-data	...	m3-oct25	Sample
37	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM11	Blank
38	M3-oct21	6	D:\est\biome	...	oct21.M	...	D:\est\biome\jan13-data	...	m3-oct21	Sample
39	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM12	Blank
40	M3-260mt	6	D:\est\biome	...	260mt.M	...	D:\est\biome\jan13-data	...	m3-260mt	Sample
41	BM	2	D:\est\biome	...	nov4-blanking.M	...	D:\est\biome\jan13-data	...	BM13	Blank