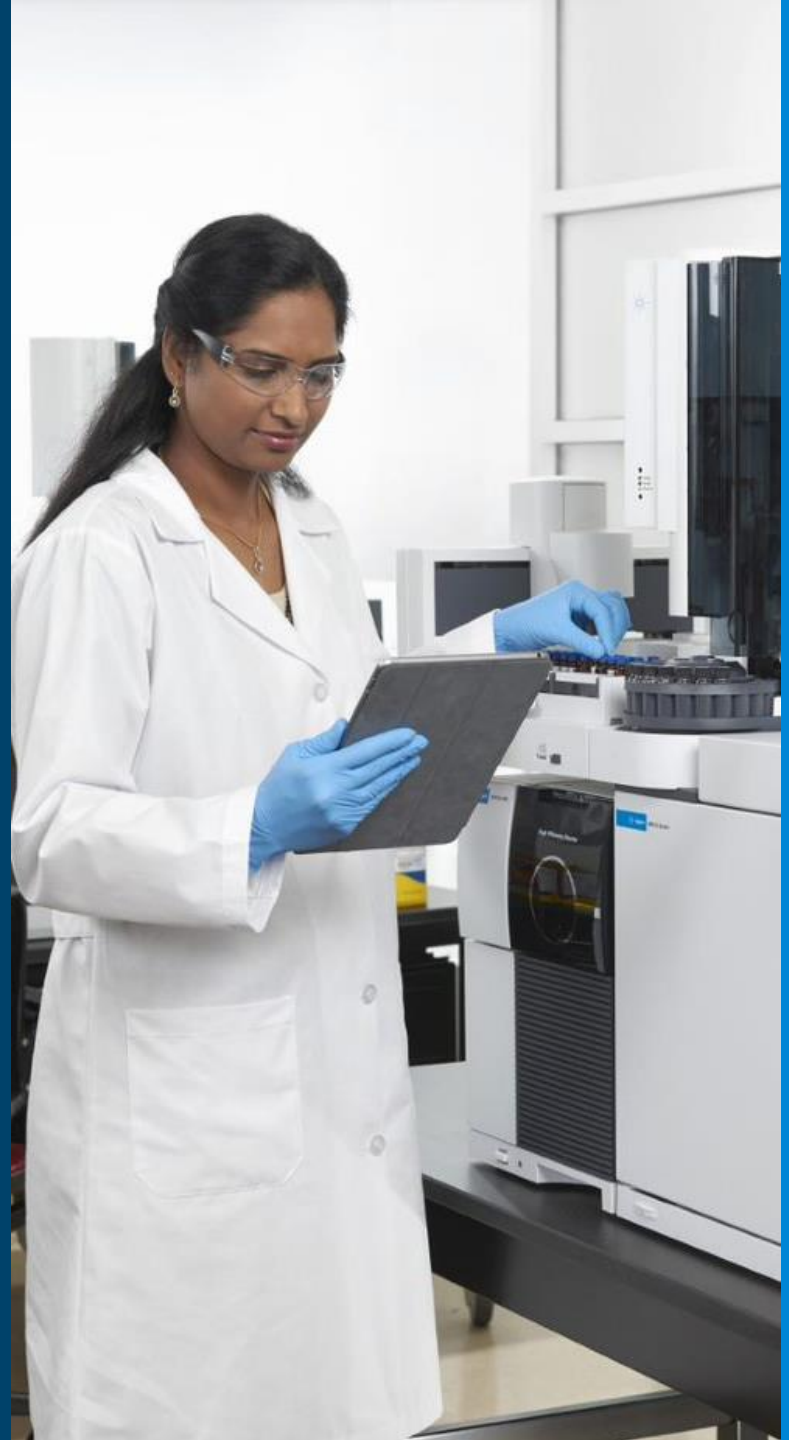
Tune into Your Mass Spectrometer

Troubleshooting GC/MS and tune report interpretation

Angela Smith Henry, Ph.D.
Applications Chemist
CSD Supplies Division

24 June 2021

DE44363.5399305556



Agilent
Trusted Answers

I Have a Tune Report…Now What?

Autotune - 5977

Tune timestamp: 1/28/2021 7:05 AM (UTC-05:00) — Obi-Wan Kenobi

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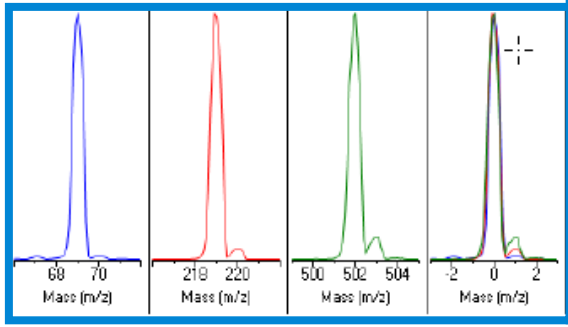
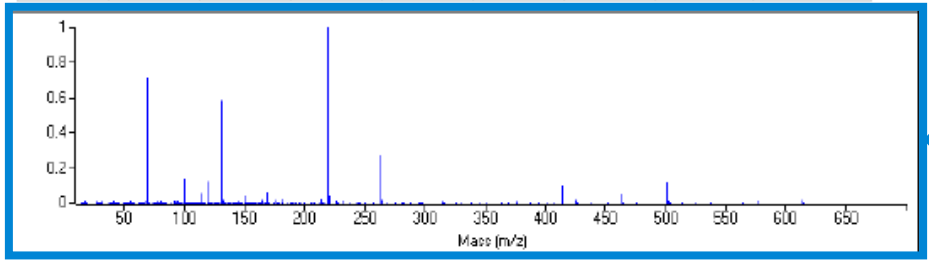


Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	17
Electron Energy	70.0	Mass Offset	-25
Filament	1	Amu Gain	2415
Repeller	30.91	Amu Offset	135.69
Ion Focus	79.5	Width219	-0.033
Entrance Lens	20.2	DC Polarity	Pos
Ent Lens Offset	13.23	HED Enable	On
Ion Body	0.00	EM Volts	989.4
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
69.00	343,938	100.0%	0.60
218.90	467,811	136.0%	0.62
502.00	57,672	16.8%	0.62

Temperatures and Pressures			
MS Source	230	Turbo Speed	100.0
MS Quad	150	Hi Vac	N/C

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	263	219.00	459,136	1,825,819



Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	328,448	100.0%	70.00	4,839	1.5%
219.00	219.00	459,136	139.8%	220.00	19,640	4.3%
502.00	502.00	55,424	16.9%	503.00	4,669	8.4%

Air/Water Check: H20 ~1.3% N2 ~1.4% O2 ~0.2% CO2 ~0.5% N2/H20 ~108.0%

Column(1) Flow: 1.20 Column(2): 0.00 ml/min Interface Temp: 250

Ramp Criteria:

Ion Focus maximum 90 volts using ion 502; Electron Multiplier Gain 100464.862

Repeller maximum 35 volts using ion 219; Gain Factor 1.0046

Mass Gain Values(Scan Speed): 23(3) 34(2) 41(1) 66(0) 118(FS1) 126(FS2)

TARGET MASS:	50	69	131	219	414	502	1050
Amu Offset	135.7	135.7	135.7	135.7	135.7	135.7	135.7
Entrance Lens Offset	13.2	13.2	13.2	13.2	13.2	13.2	13.2

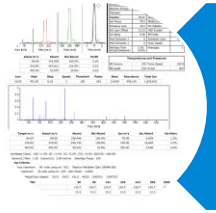
Do I care about these numbers or peaks?

Is all of this normal?!

What is this a mass spectrum of?

I don’t know what this is saying…

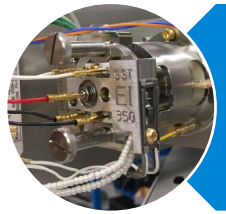
Today



Demystify tuning and tune reports

How often should I tune?

…and how do I determine the frequency of tuning?



Troubleshoot problems using the mass spectrometer

Autotune - 5977

Tune timestamp: 1/28/2021 7:05 AM (UTC-05:00) Obi-Wan Kenobi

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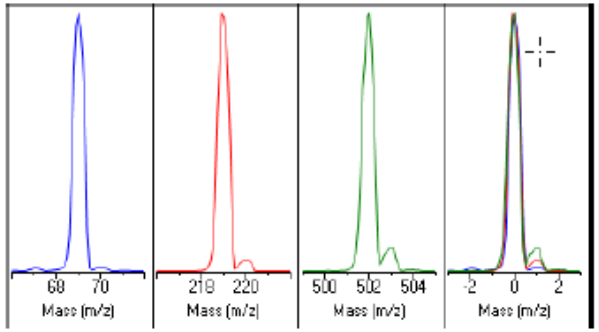

Ion Polarity	Pos	PFTBA	Open
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Electron Energy	70.0	Mass Offset	-25
Filament	1	Amu Gain	2415
Repeller	30.91	Amu Offset	135.69
Ion Focus	79.5	Width219	-0.033
Entrance Lens	20.2	DC Polarity	Pos
Ent Lens Offset	13.23	HED Enable	On
Ion Body	0.00	EM Volts	989.4
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
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Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
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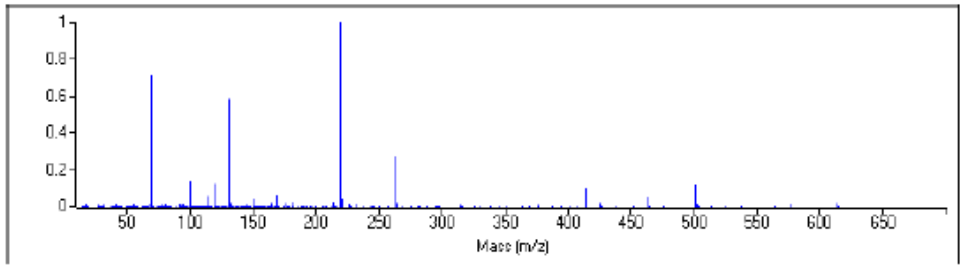

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Mass Gain Values(Scan Speed): 23(3) 34(2) 41(1) 66(0) 118(FS1) 126(FS2)

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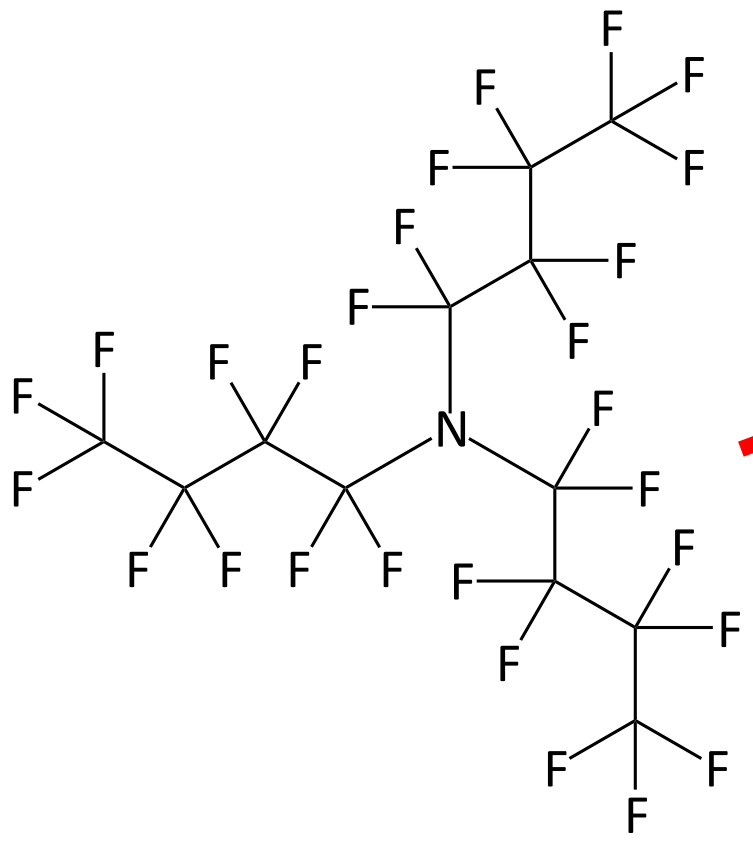
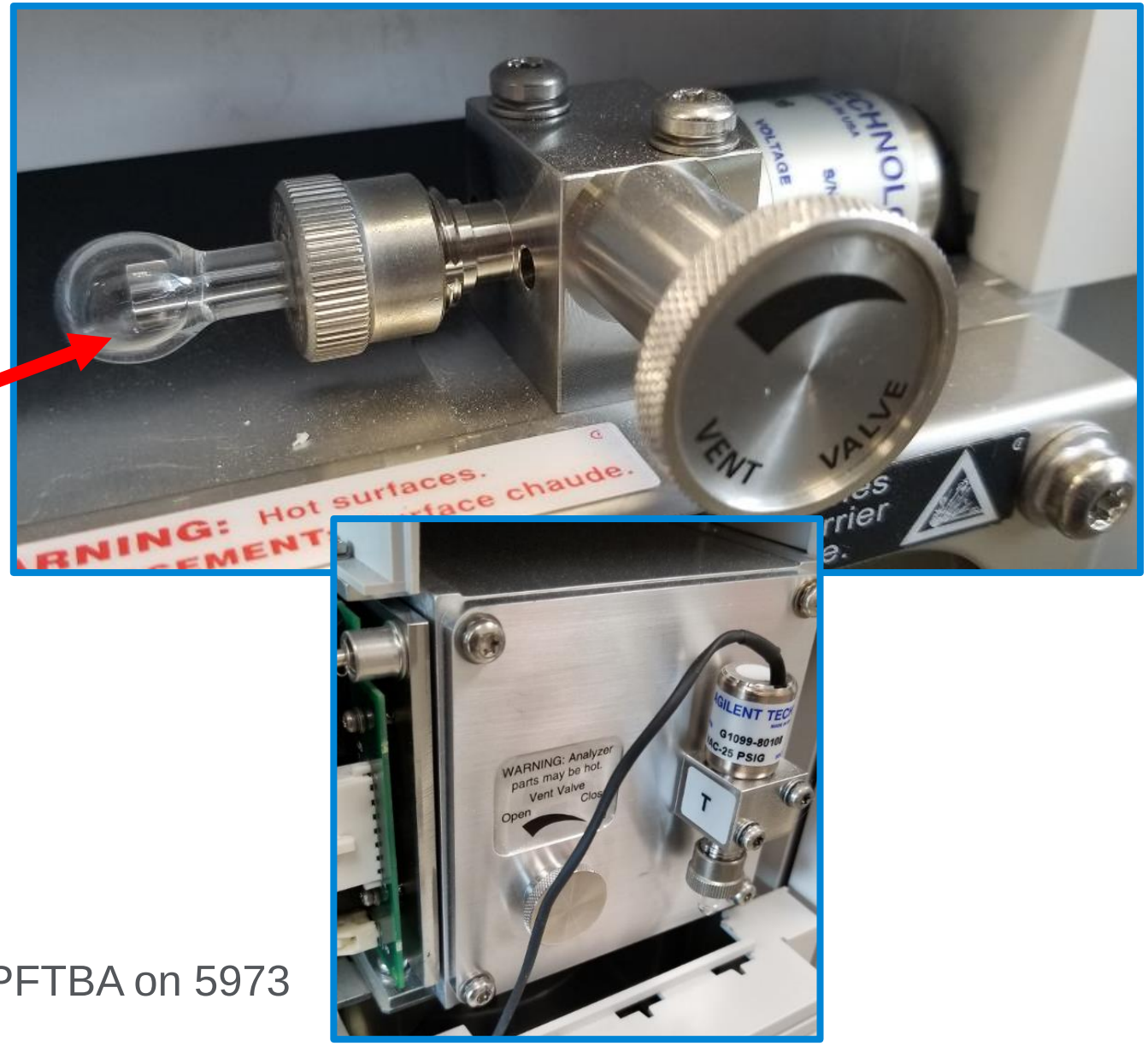
Amu Offset	135.7	135.7	135.7	135.7	135.7	135.7	135.7
Entrance Lens Offset	13.2	13.2	13.2	13.2	13.2	13.2	13.2

Let’s Start at the Beginning of Tuning

Tuning Compound: Perfluorotributylamine (PFTBA)

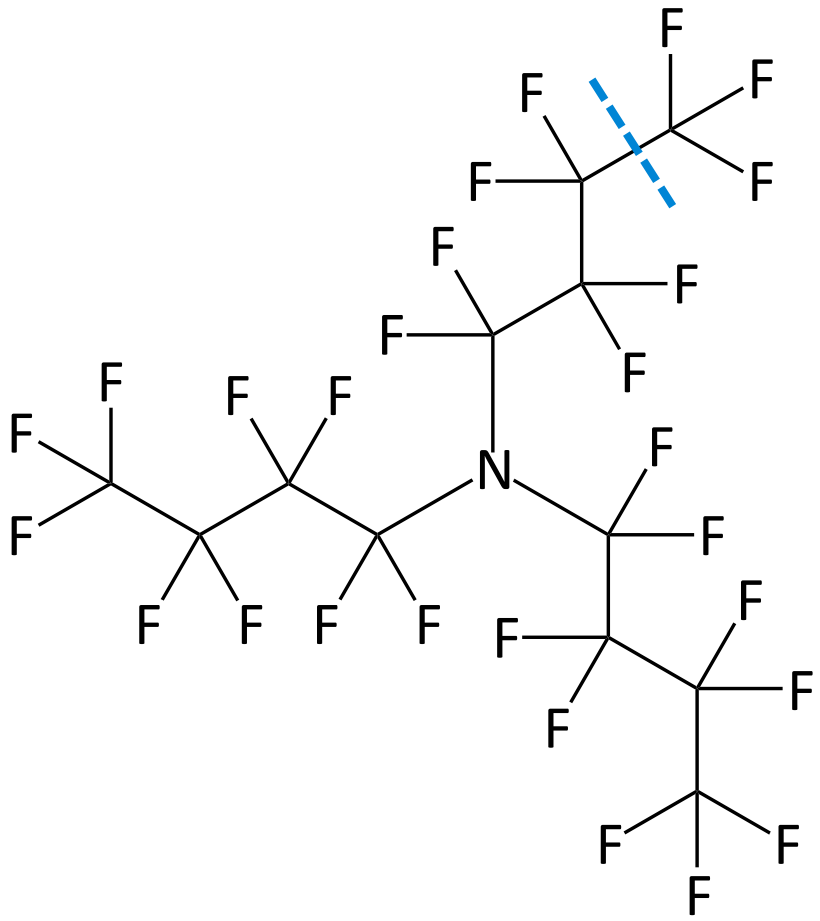


Perfluorotributylamine



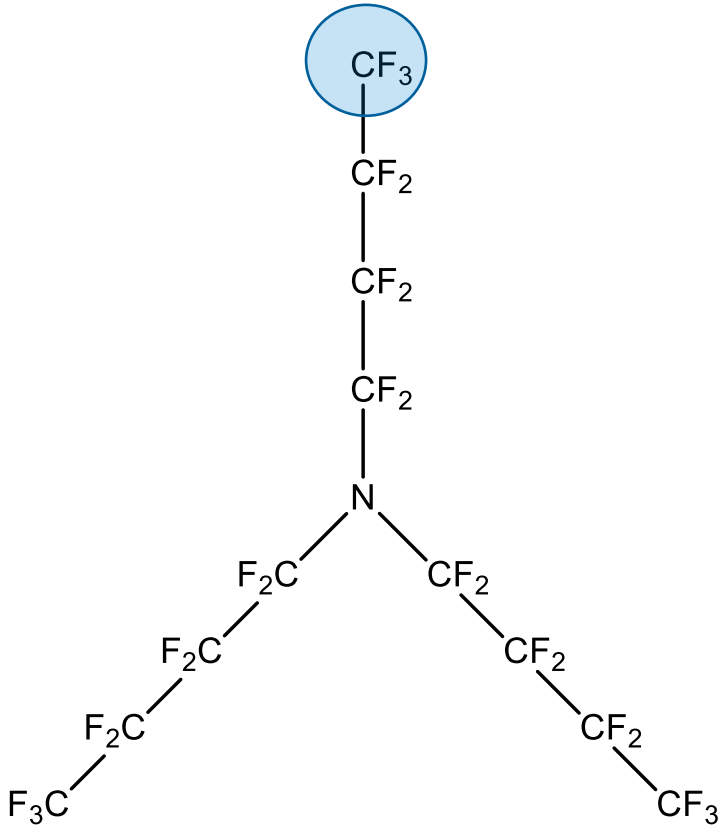
Location of PFTBA on 5973

Tune Report: PFTBA Fragments

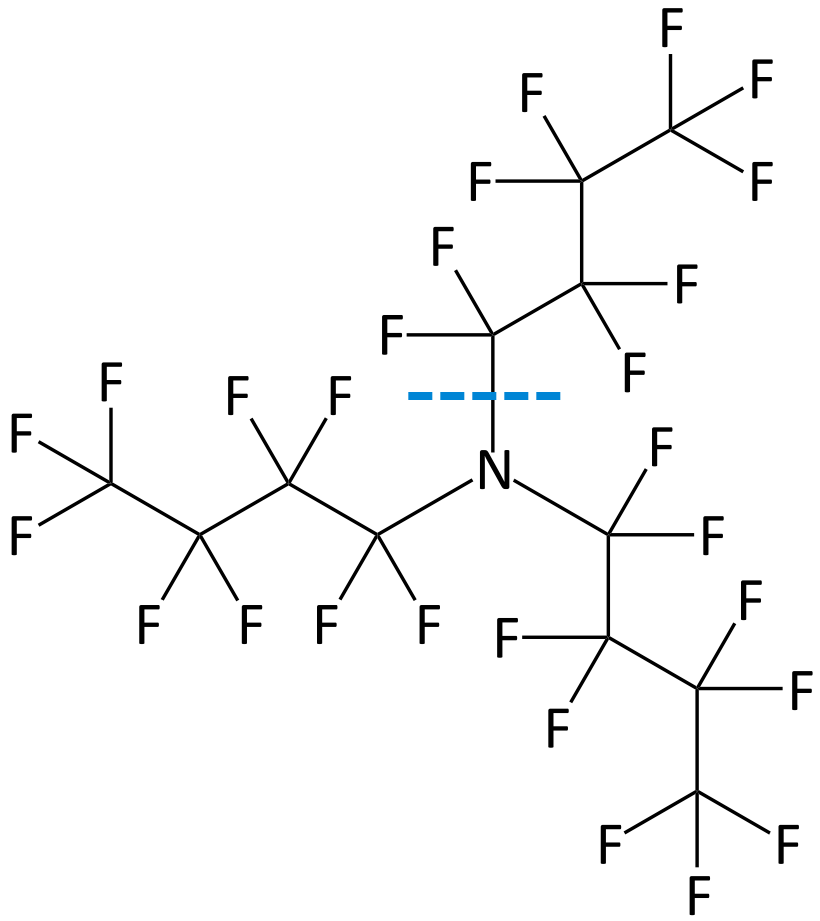


Perfluorotributylamine

Formation of 69

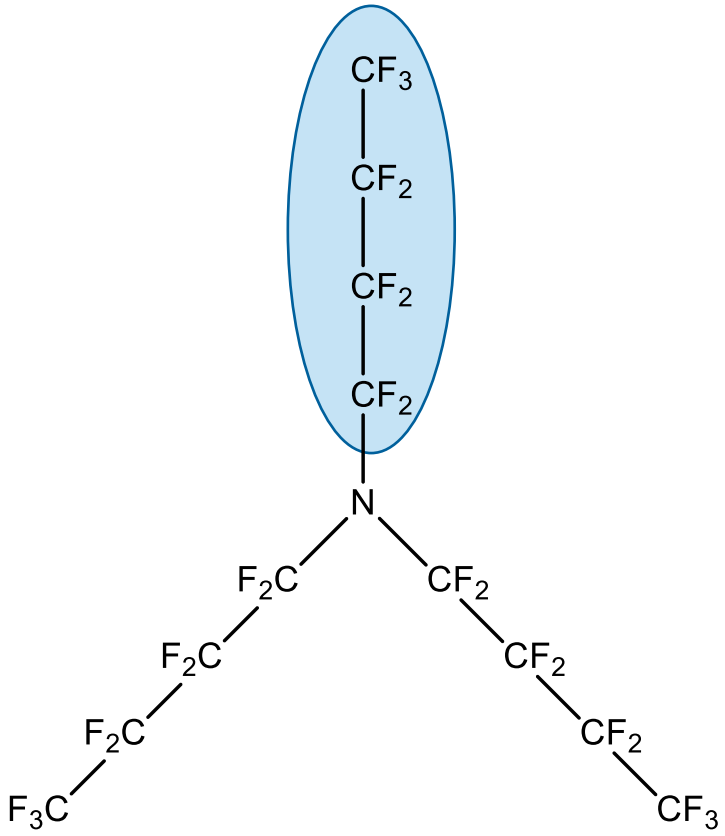


Tune Report: PFTBA Fragments

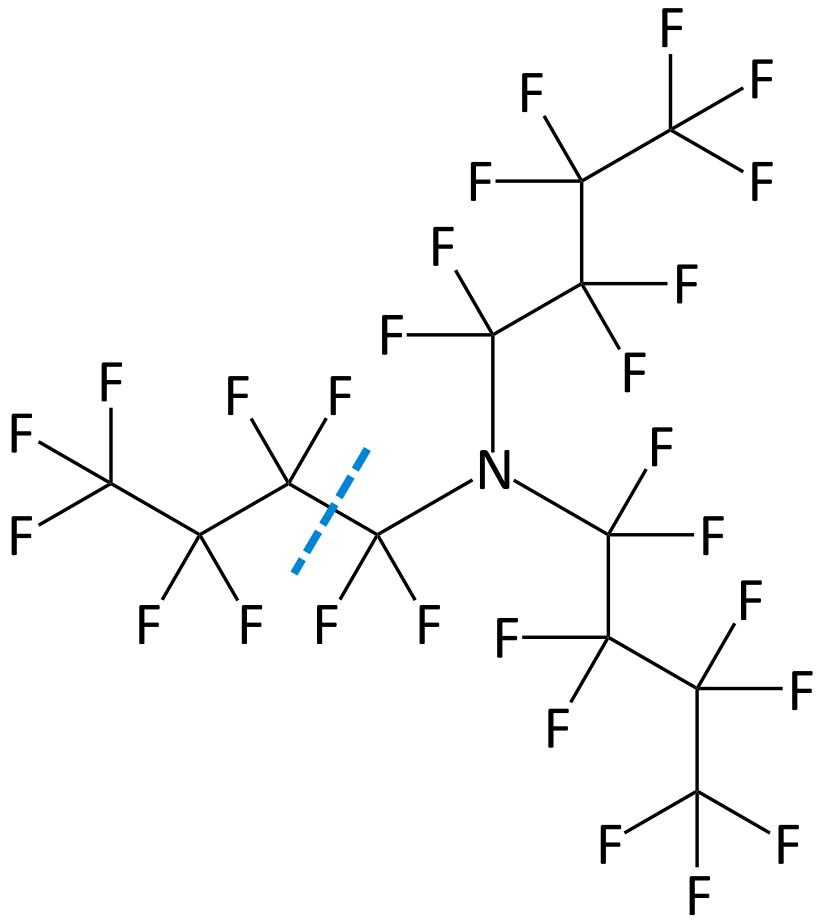


Perfluorotributylamine

Formation of 219

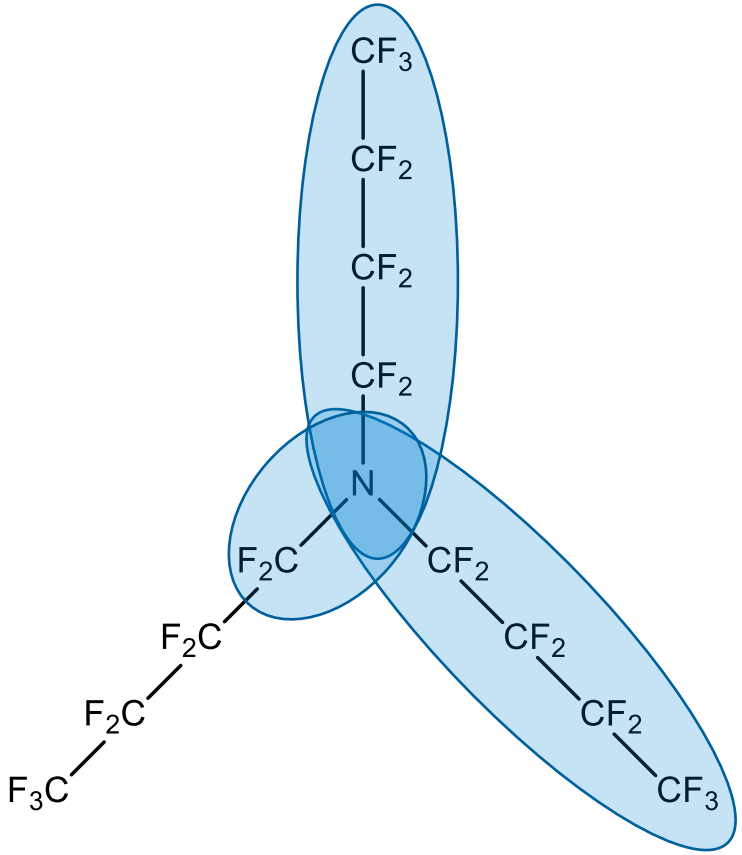


Tune Report: PFTBA Fragments

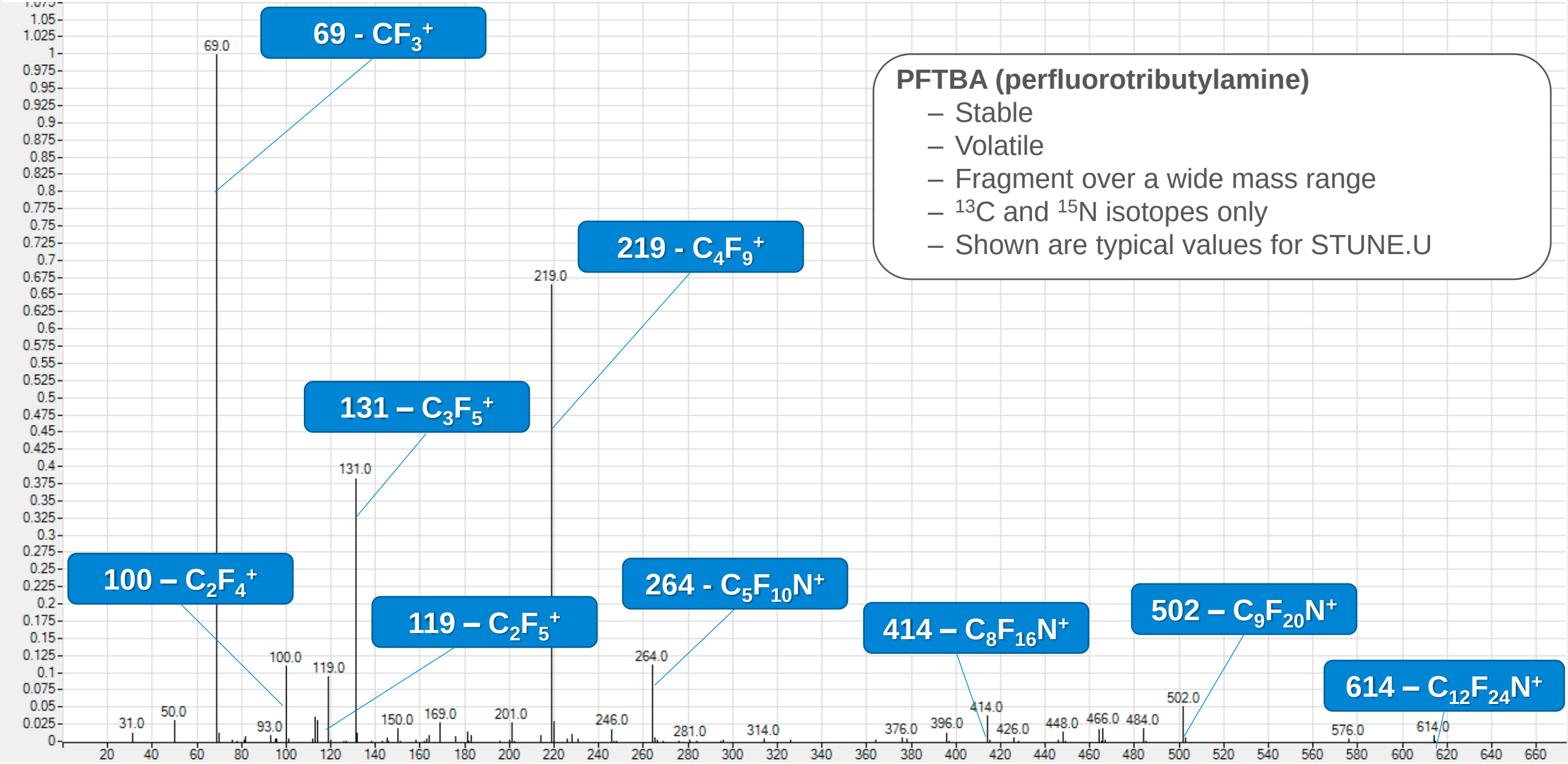


Perfluorotributylamine

Formation of 502



Tuning Compound: Perfluorotributylamine (PFTBA)



PFTBA (perfluorotributylamine)

- Stable
- Volatile
- Fragment over a wide mass range
- ^{13}C and ^{15}N isotopes only
- Shown are typical values for STUNE.U

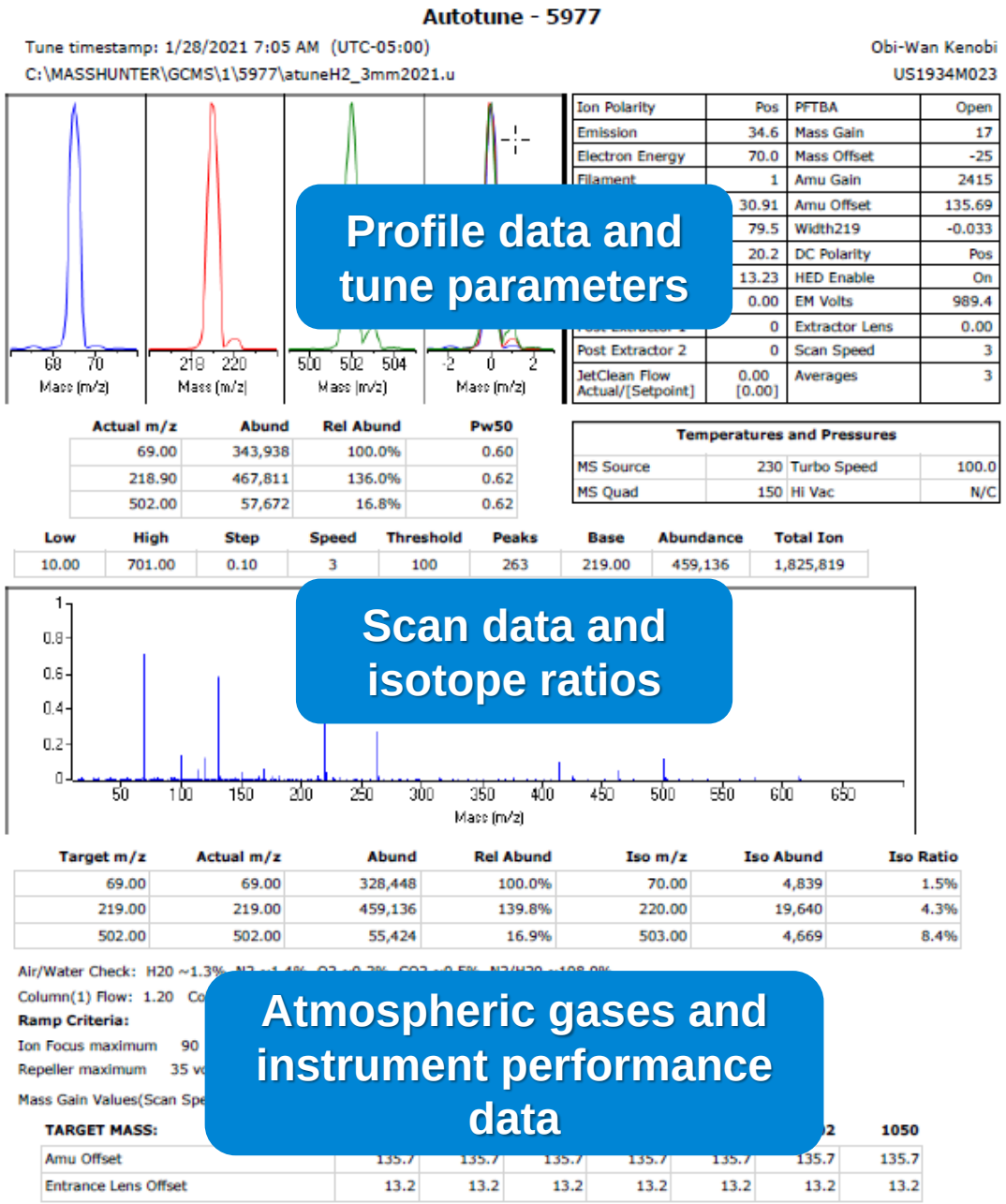
Reviewing a Tune Report

Review in sections

Each section contains important information:

- Profile data and tune parameters
- Scan data and isotope ratios
- Atmospheric gases and instrument performance data

The quality or validity of a tune should not be judged by a single parameter.



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Section 1: Profile Data

Autotune - 5977

Tune timestamp: 02-Feb-18 3:44 PM (UTC-05:00)

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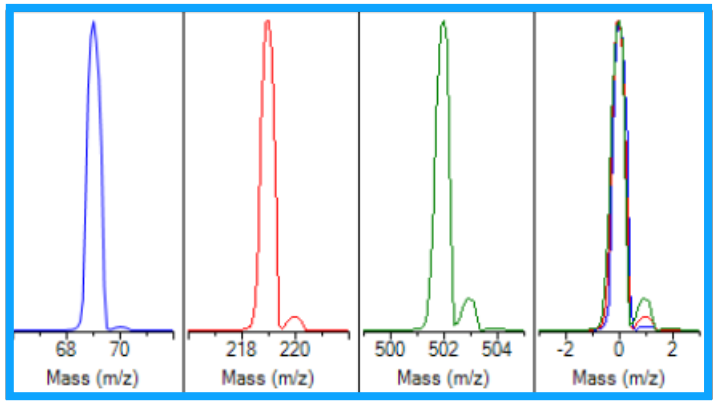
Leakmaster

US1420L215



Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	-669
Electron Energy	70.0	Mass Offset	-35
Filament	1	Amu Gain	1145
Repeller	30.91	Amu Offset	123.00
Ion Focus	90.3	Width219	-0.023
Entrance Lens	22.7	DC Polarity	Neg
Ent Lens Offset	14.11	HED Enable	On
Ion Body	0.00	EM Volts	1139.0
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
69.00	589,861	100.0%	0.60
219.00	496,089	84.1%	0.60
502.00	48,127	8.2%	0.60

Temperatures and Pressures

MS Source	230	Turbo Speed	100.0
MS Quad	150	Hi Vac	9.01e-06

Visually confirm that the peaks are:

1. Symmetrical
2. Without spikes or noise
3. Without splitting, tailing or fronting
4. Peak widths are optimal—0.6 m/z at 50% height, and 1.0 m/z at 0% height

Section 1: Profile Data

Autotune - 5977

Tune timestamp: 02-Feb-18 3:44 PM (UTC-05:00)

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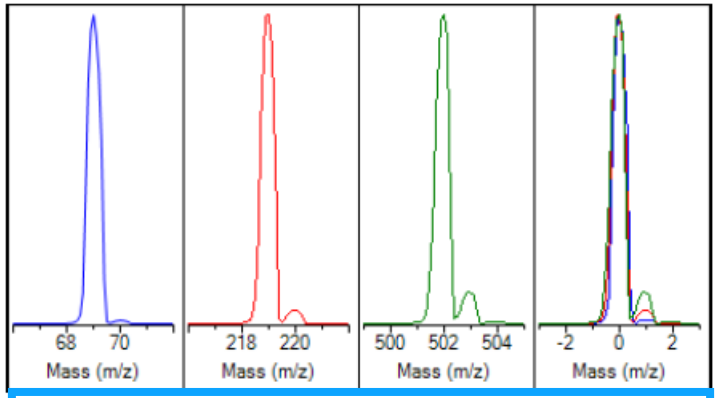
Leakmaster

US1420L215



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Emission	34.6	Mass Gain	-669
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Filament	1	Amu Gain	1145
Repeller	30.91	Amu Offset	123.00
Ion Focus	90.3	Width219	-0.023
Entrance Lens	22.7	DC Polarity	Neg
Ent Lens Offset	14.11	HED Enable	On
Ion Body	0.00	EM Volts	1139.0
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Temperatures and Pressures

MS Source	230	Turbo Speed	100.0
MS Quad	150	Hi Vac	9.01e-06

Review:

Peak widths are optimal – 0.6 m/z at 50% height, and 1.0 m/z at 0% height

Relative abundance – 69 and 219 m/z > 400,000 (400,000 to 600,000 counts), 502 m/z ~ 10% of base peak

- For etune, 219 and 69 m/z may be similar in abundance

Section 1: Tune Parameters

Autotune - 5977

Tune timestamp: 02-Feb-18 3:44 PM (UTC-05:00) Leakmaster

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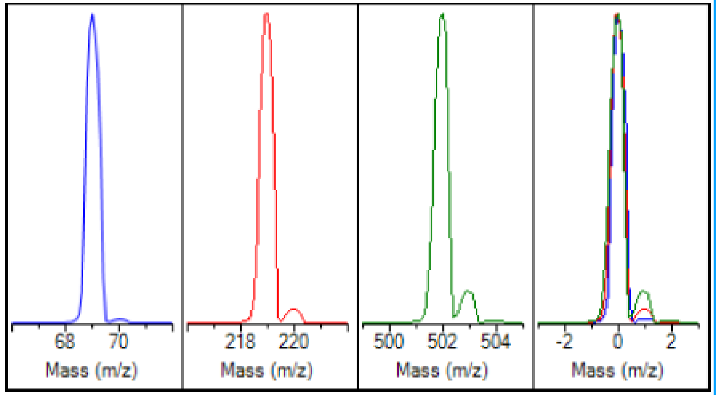

Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	-669
Electron Energy	70.0	Mass Offset	-35
Filament	1	Amu Gain	1145
Repeller	30.91	Amu Offset	123.00
Ion Focus	90.3	Width219	-0.023
Entrance Lens	22.7	DC Polarity	Neg
Ent Lens Offset	14.11	HED Enable	On
Ion Body	0.00	EM Volts	1139.0
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
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Temperatures and Pressures

MS Source	230	Turbo Speed	100.0
MS Quad	150	Hi Vac	9.01e-06

List of tune parameters for the **source**, **quadrupole**, and **detector**

Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	-669
Electron Energy	70.0	Mass Offset	-35
Filament	1	Amu Gain	1145
Repeller	30.91	Amu Offset	123.00
Ion Focus	90.3	Width219	-0.023
Entrance Lens	22.7	DC Polarity	Neg
Ent Lens Offset	14.11	HED Enable	On
Ion Body	0.00	EM Volts	1139.0
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	-669
Electron Energy	70.0	Mass Offset	-35
Filament	1	Amu Gain	1145
Repeller	30.91	Amu Offset	123.00
Ion Focus	90.3	Width219	-0.023
Entrance Lens	22.7	DC Polarity	Neg
Ent Lens Offset	14.11	HED Enable	On
Ion Body	0.00	EM Volts	1139.0
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Ion Polarity	Pos	PFTBA	Open
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Ent Lens Offset	14.11	HED Enable	On
Ion Body	0.00	EM Volts	1139.0
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Reviewing Atune and Etune Profile Data

How do additional lenses affect the tune data?

Atune.u

Autotune - 5977

Tune timestamp: 3/4/2021 4:28 PM (UTC-05:00) — Obi-Wan Kenobi

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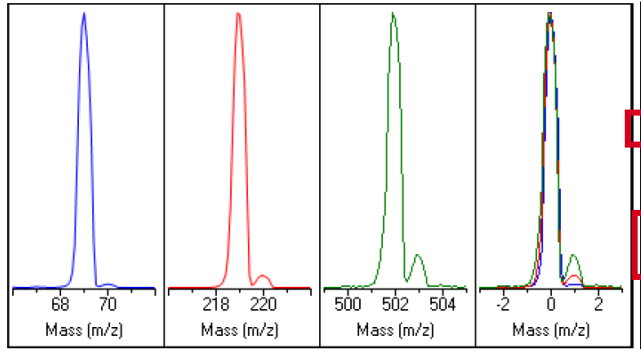

Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	9
Electron Energy	70.0	Mass Offset	-25
Filament	1	Amu Gain	2404
Repeller	34.90	Amu Offset	136.06
Ion Focus	90.3	Width219	-0.034
Entrance Lens	10.1	DC Polarity	Pos
Ent Lens Offset	14.94	HED Enable	On
Ion Body	0.00	EM Volts	918.9
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
69.00	538,135	100.0%	0.60
218.90	366,411	68.1%	0.59
501.90	20,333	3.8%	0.62

Temperatures and Pressures			
MS Source	300	Turbo Speed	100.0
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Etune.u

Extraction Source Autotune - 5977

Tune timestamp: 3/11/2021 11:03 AM (UTC-05:00) — Obi-Wan Kenobi

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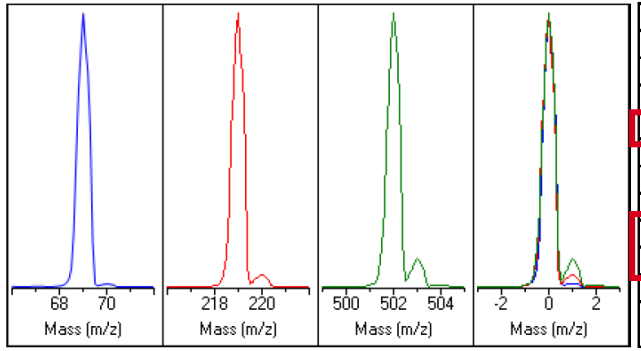

Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	21
Electron Energy	70.0	Mass Offset	-26
Filament	1	Amu Gain	2417
Repeller	1.00	Amu Offset	135.00
Ion Focus	89.8	Width219	-0.031
Entrance Lens	7.5	DC Polarity	Pos
Ent Lens Offset	15.10	HED Enable	On
Ion Body	10.00	EM Volts	863.6
Post Extractor 1	0	Extractor Lens	-2.60
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
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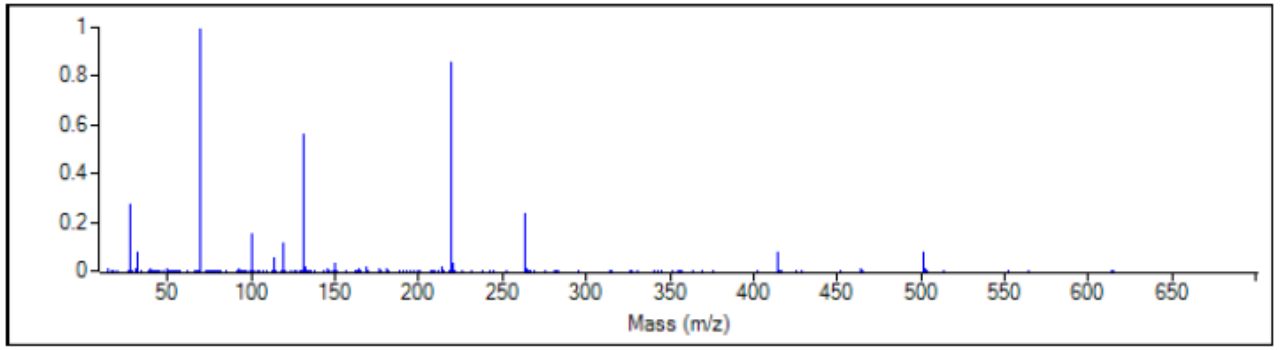
219.00	447,048	93.4%	0.62
502.00	29,005	6.1%	0.62

Section 2: Scan Data and Isotope Ratios

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	162	69.00	562,368	2,172,117

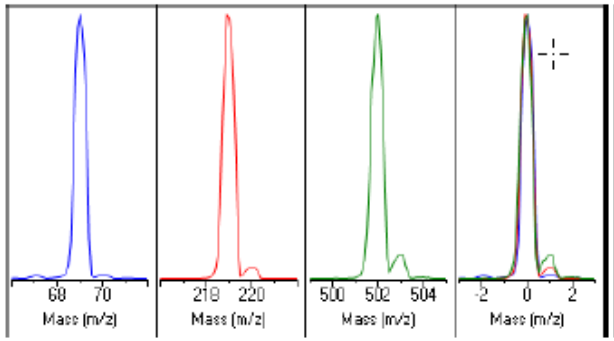


Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	562,368	100.0%	70.00	6,281	1.1%
219.00	219.00	481,152	85.6%	220.00	21,056	4.4%
502.00	502.00	46,664	8.3%	503.00	4,612	9.9%

Autotune - 5977

Tune timestamp: 1/28/2021 7:05 AM (UTC-05:00)
C:\MASSHUNTER\GCMS\1\5977\atuneH2_3mm2021.u

Obi-Wan Kenobi
US1934M023

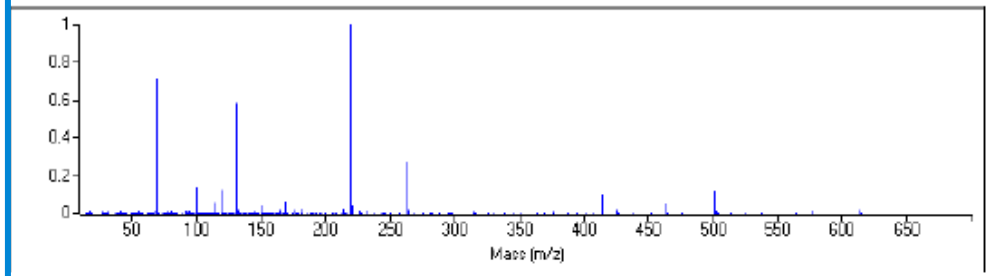


Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	17
Electron Energy	70.0	Mass Offset	-25
Filament	1	Amu Gain	2415
Repeller	30.91	Amu Offset	135.69
Ion Focus	79.5	Width219	-0.033
Entrance Lens	20.2	DC Polarity	Pos
Ent Lens Offset	13.23	HED Enable	On
Ion Body	0.00	EM Volts	989.4
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
69.00	343,938	100.0%	0.60
218.90	467,811	136.0%	0.62
502.00	57,672	16.8%	0.62

Temperatures and Pressures			
MS Source	230	Turbo Speed	100.0
MS Quad	150	Hi Vac	N/C

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	263	219.00	459,136	1,825,819



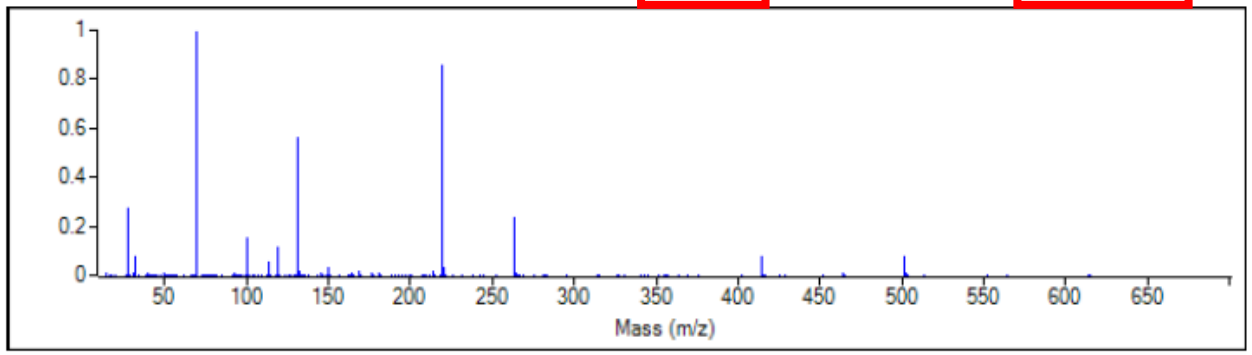
Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	328,448	100.0%	70.00	4,839	1.5%
219.00	219.00	459,136	139.8%	220.00	19,640	4.3%
502.00	502.00	55,424	16.9%	503.00	4,669	8.4%

Air/Water Check: H20 ~1.3% N2 ~1.4% O2 ~0.2% CO2 ~0.5% N2/H20 ~108.0%
Column(1) Flow: 1.20 Column(2): 0.00 ml/min Interface Temp: 250
Ramp Criteria:
Ion Focus maximum 90 volts using ion 502; Electron Multiplier Gain 100464.862
Repeller maximum 35 volts using ion 219; Gain Factor 1.0046
Mass Gain Values(Scan Speed): 23(3) 34(2) 41(1) 66(0) 118(FS1) 126(FS2)

TARGET MASS:	50	69	131	219	414	502	1050
Amu Offset	135.7	135.7	135.7	135.7	135.7	135.7	135.7
Entrance Lens Offset	13.2	13.2	13.2	13.2	13.2	13.2	13.2

Section 2: Scan Data and Isotope Ratios

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	162	69.00	562,368	2,172,117



Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	562,368	100.0%	70.00	6,281	1.1%
219.00	219.00	481,152	85.6%	220.00	21,056	4.4%
502.00	502.00	46,664	8.3%	503.00	4,612	9.9%

Peaks: Between 100 to 250 when the system is clean and well equilibrated.

After maintenance, peaks may be elevated (300 to 400)

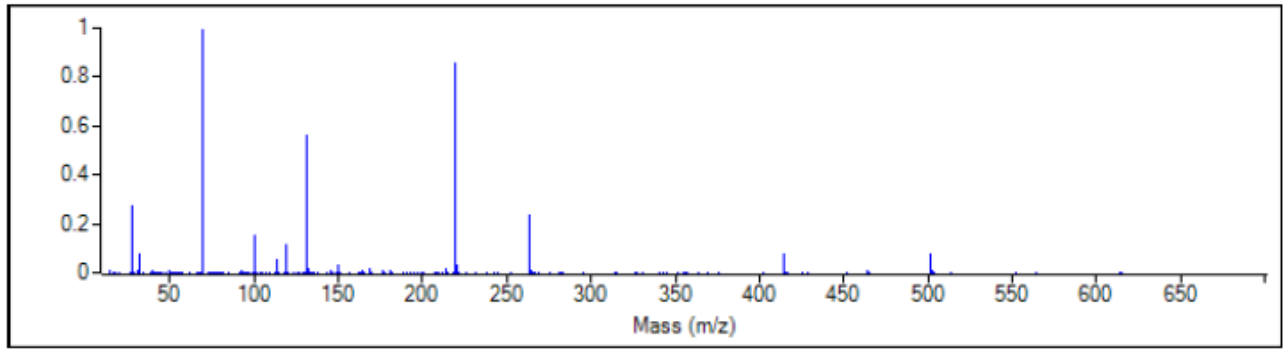
- As the system equilibrates, the number of peaks will decrease

High number of peaks (>600) = Detector noise or some form of contamination

Total ion: Summation of all ion fragment abundances. A high total ion current = leak or contamination.

Section 2: Scan Data and Isotope Ratios

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	162	69.00	562,368	2,172,117



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502.00	502.00	46,664	8.3%	503.00	4,612	9.9%

Isotope ratios: Used to confirm instrument performance

Mass Comparison	Typical isotope ratio (±20%)
69/70	1.1%
219/220	4.4%
502/503	10.1%

Section 3: Atmospheric Gases and Instrument Performance Data

Air/Water Check: H20 ~1.3% N2 ~1.4% O2 ~0.2% CO2 ~0.5% N2/H20 ~108.0%
Column(1) Flow: 1.20 Column(2): 0.00 ml/min Interface Temp: 250

Ramp Criteria:

Ion Focus maximum 90 volts using ion 502; Electron Multiplier Gain 100464.862
Repeller maximum 35 volts using ion 219; Gain Factor 1.0046

Mass Gain Values(Scan Speed): 23(3) 34(2) 41(1) 66(0) 118(FS1) 126(FS2)

TARGET MASS:	50	69	131	219	414	502	1050
Amu Offset	135.7	135.7	135.7	135.7	135.7	135.7	135.7
Entrance Lens Offset	13.2	13.2	13.2	13.2	13.2	13.2	13.2

- Atmospheric gases (water, nitrogen, oxygen, and carbon dioxide) should be as low as possible.
  - The ratio of nitrogen to oxygen in atmospheric air is 3.7:1.
  - If the water is high, there will also be air present. Be patient and do not tune or acquire data until equilibrated.
- Why are two GC parameters included?
  - Tune parameters depend on flow rate and MS transfer line temperature
- For single quadrupole instruments, the gain factor is the detector gain needed to achieve the target PFTBA abundance.
  - Gain will be discussed in detail in a future webinar.

What to Look For?

Symmetrical smooth peak shapes

Consistent mass peak widths

Low water and air

Correct mass assignments

Acceptable column flow rate

Reasonable EM voltage

Proper absolute abundance

Typical relative abundance

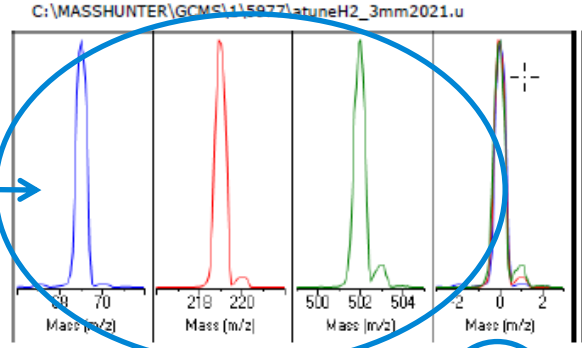
Proper isotope ratios

Gain Factor

Autotune - 5977

Tune timestamp: 1/28/2021 7:05 AM (UTC-05:00) Obi-Wan Kenobi

C:\MASSHUNTER\GCMS\1\5977\atuneH2_3mm2021.u US1934M023



Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	17
Electron Energy	70.0	Mass Offset	-25
Filament	1	Amu Gain	2415
Repeller	30.91	Amu Offset	135.69
Ion Focus	79.5	Width219	-0.033
Entrance Lens	20.2	DC Polarity	Pos
Ent Lens Offset	13.23	HED Enable	On
Ion Body	0.00	EM Volts	989.4
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
69.00	343,938	100.0%	0.60
218.90	467,811	136.0%	0.62
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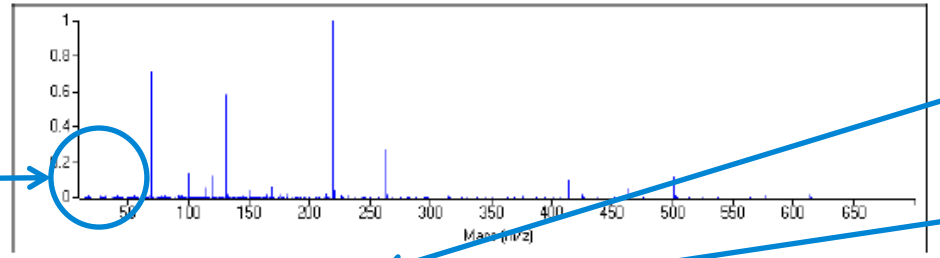
Temperatures and Pressures			
MS Source	230	Turbo Speed	100.0
MS Quad	150	HI Vac	N/C

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	263	219.00	459,136	1,825,819



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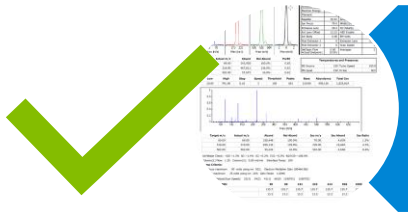
Entrance Lens Offset	13.2	13.2	13.2	13.2	13.2	13.2	13.2

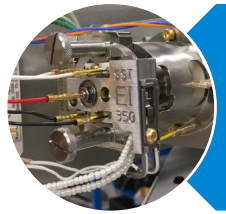
Today



Demystify tuning and tune reports

Tuning regularity

…and how do I determine the frequency of tuning?

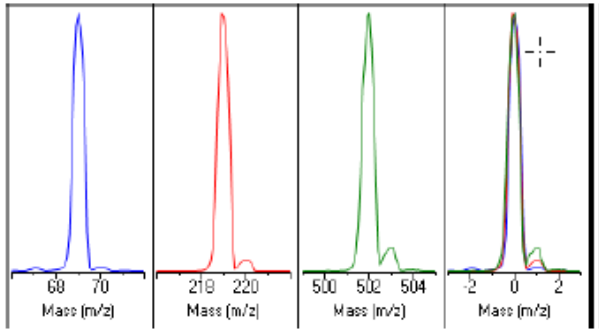


Troubleshoot problems using the mass spectrometer

Autotune - 5977

Tune timestamp: 1/28/2021 7:05 AM (UTC-05:00) — Obi-Wan Kenobi

C:\MASSHUNTER\GCMS\1\5977\atuneH2_3mm2021.u — US1934M023



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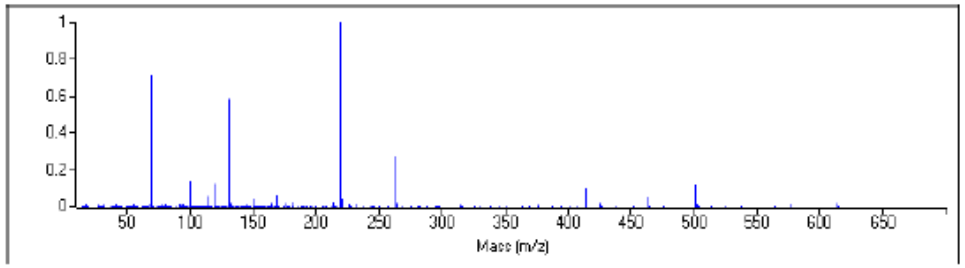

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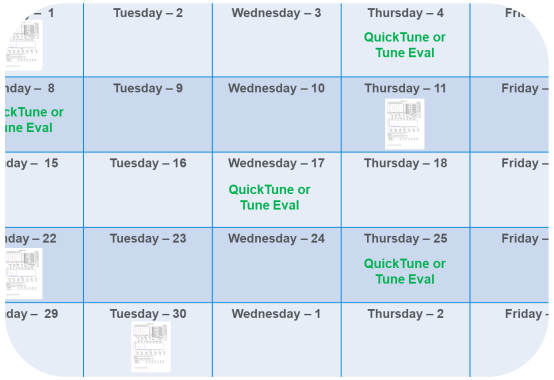
Amu Offset	135.7	135.7	135.7	135.7	135.7	135.7	135.7
Entrance Lens Offset	13.2	13.2	13.2	13.2	13.2	13.2	13.2

Tuning Frequency

Follow lab SOP

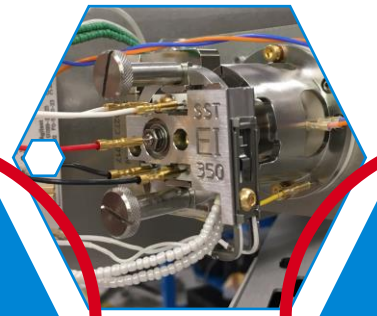
Follow (regulated) method criteria



"It depends" (but not daily)

Major changes

What is a Major Change?



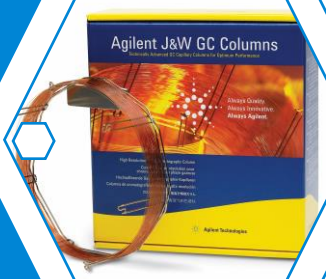
Changing source temperatures

New column installation

Change carrier gas flow rates

After source cleaning

1.0 → 1.5 mL/min



New GC/MS system

Changing carrier gas type (for example, He to H_2)

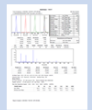
Running JetClean in Online mode

He → H_2

What Should I Do if I Fall into the “It Depends?” Category

You don’t have to tune every day

Example month of tuning

Monday – 1 	Tuesday – 2	Wednesday – 3	Thursday – 4 QuickTune or Tune Eval	Friday – 5
Monday – 8 QuickTune or Tune Eval	Tuesday – 9	Wednesday – 10	Thursday – 11 	Friday – 12
Monday – 15	Tuesday – 16	Wednesday – 17 QuickTune or Tune Eval	Thursday – 18	Friday – 19
Monday – 22 	Tuesday – 23	Wednesday – 24	Thursday – 25 QuickTune or Tune Eval	Friday – 26
Monday – 29	Tuesday – 30 	Wednesday – 1	Thursday – 2	Friday – 3

How often do I tune?

- Depends on usage
- Once a week or less

How can I tune less often?

- Run tune evaluation
- Run Quicktune
- Update detector gain coefficients

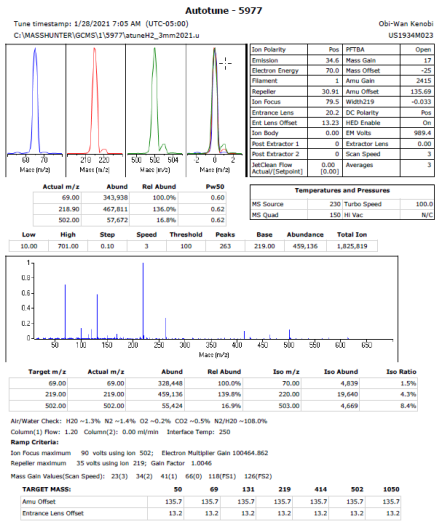
DE44363.5399305556

How Do I Determine that I Need to Tune?

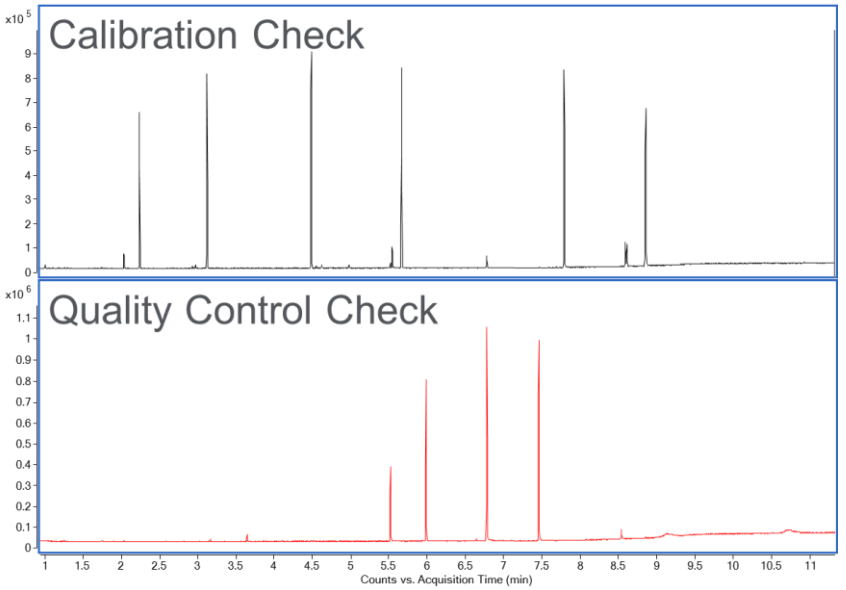
1. Have a Baseline

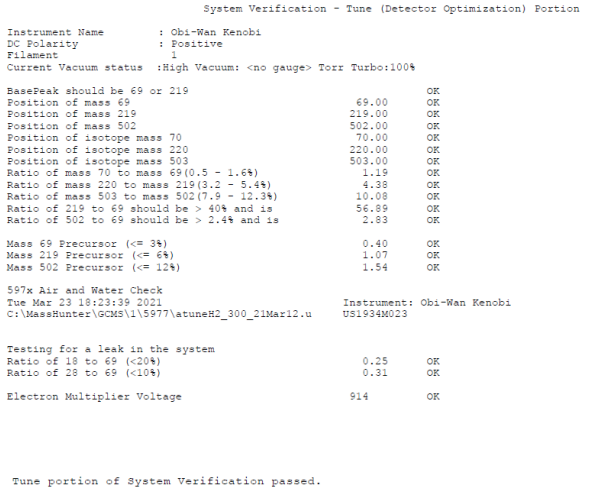
System start-up

- Tune system
- Run Tune Evaluation
- Run QC and cal samples



Tune report





```
                    System Verification - Tune (Detector Optimization) Portion

Instrument Name          : Obi-Wan Kenobi
DC Polarity              : Positive
Filament                 : 1
Current Vacuum status    :High Vacuum: <no gauge> Torr Turbo:100%

BasePeak should be 69 or 219                                    OK
Position of mass 69                                  69.00      OK
Position of mass 219                                219.00      OK
Position of mass 502                                502.00      OK
Position of isotope mass 70                          70.00      OK
Position of isotope mass 220                        220.00      OK
Position of isotope mass 503                        503.00      OK
Ratio of mass 70 to mass 69(0.5 - 1.6%)               1.19      OK
Ratio of mass 220 to mass 219(3.2 - 5.4%)             4.38      OK
Ratio of mass 503 to mass 502(7.9 - 12.3%)           10.08      OK
Ratio of 219 to 69 should be > 40% and is            56.89      OK
Ratio of 502 to 69 should be > 2.4% and is            2.83      OK

Mass 69 Precursor (<= 3%)                             0.40      OK
Mass 219 Precursor (<= 6%)                            1.07      OK
Mass 502 Precursor (<= 12%)                           1.54      OK

597x Air and Water Check
Tue Mar 23 18:23:39 2021                    Instrument: Obi-Wan Kenobi
C:\MassHunter\GCMS\1\5977\atuneH2_300_21Mar12.u    US1934M023


Testing for a leak in the system
Ratio of 18 to 69 (<20%)                              0.25      OK
Ratio of 28 to 69 (<10%)                              0.31      OK

Electron Multiplier Voltage                            914      OK


Tune portion of System Verification passed.
```

Tune eval report

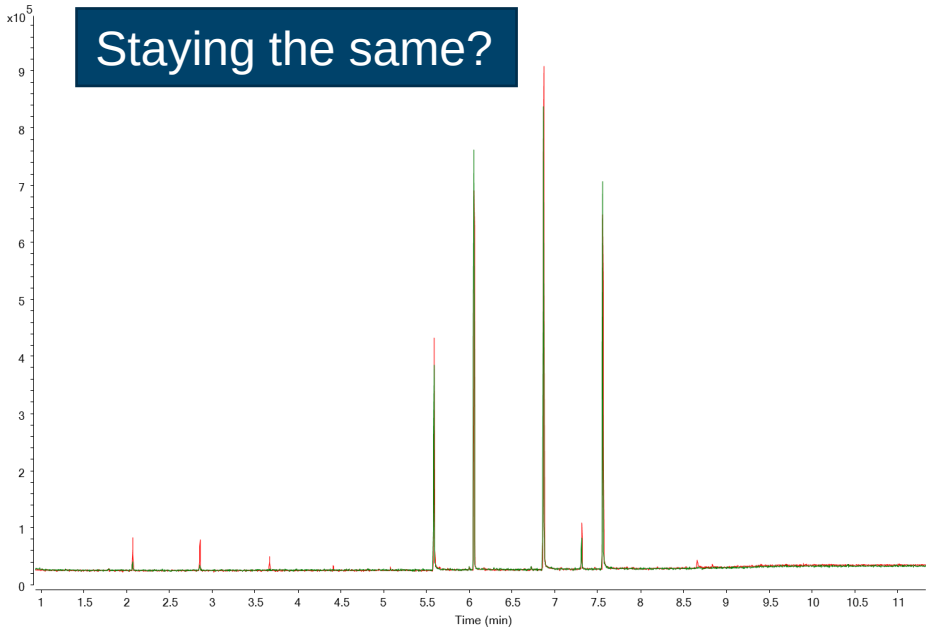
How Do I Determine that I Need to Tune?

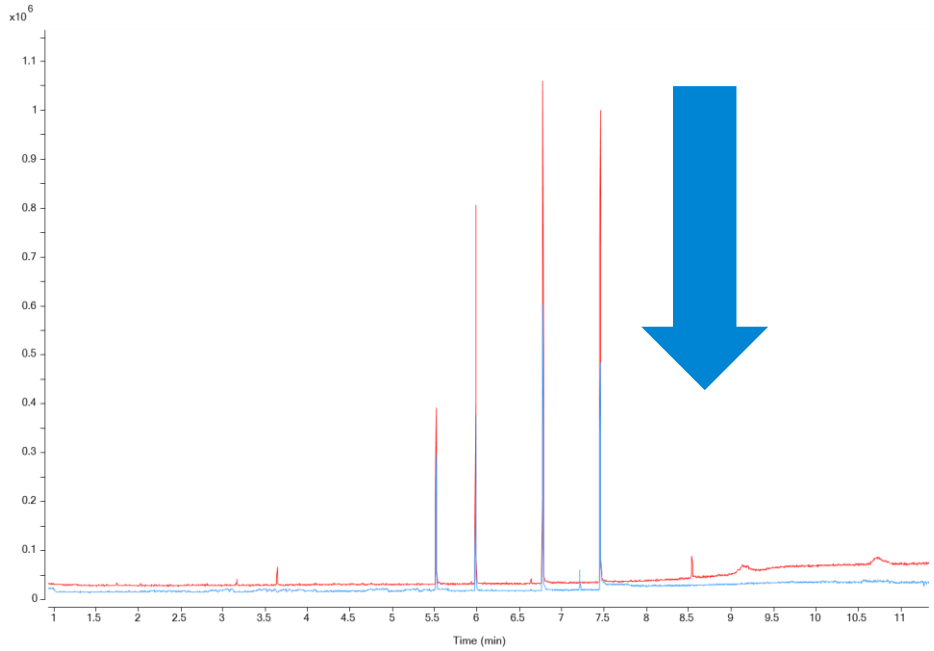
1. Have a baseline

System start-up
- Tune system
- Run Tune Evaluation
- Run QC and cal samples

2. Review QC and CCV

What are peak responses doing?





How Do I Determine that I Need to Tune?

Peaks are steady

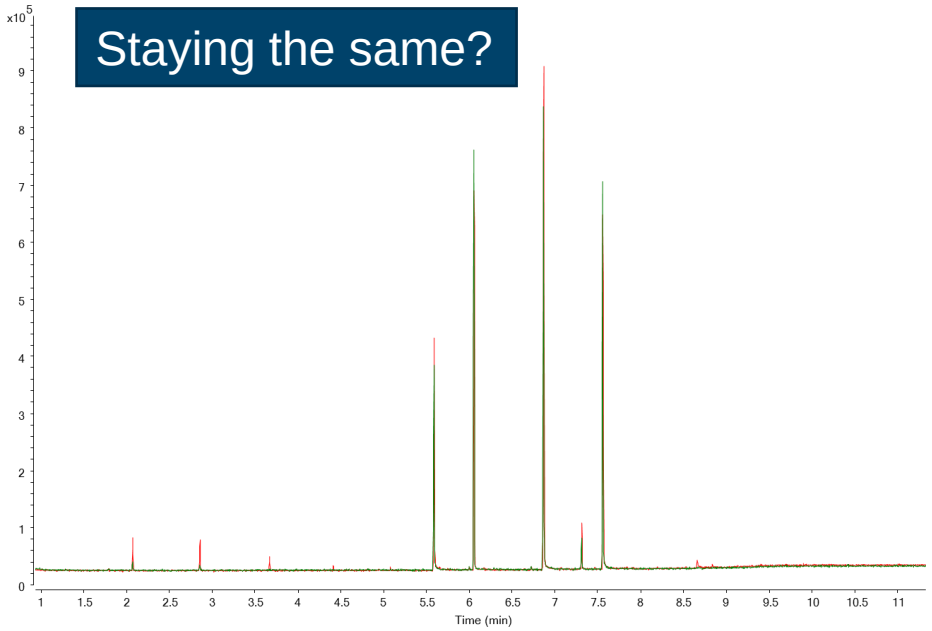
Keep monitoring

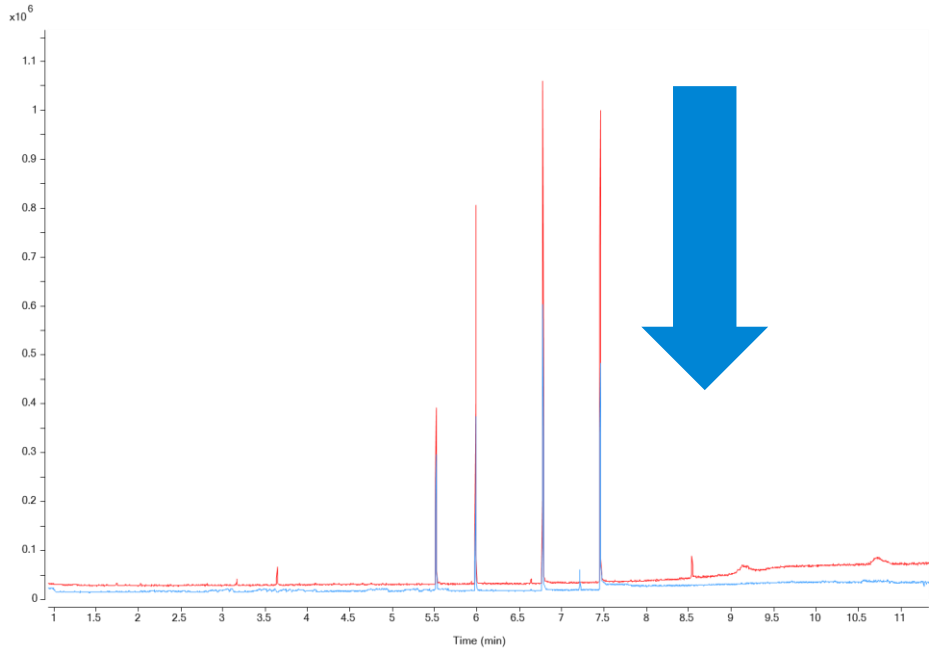
2. Review QC and CCV

What are peak responses doing?

Peak responses drop

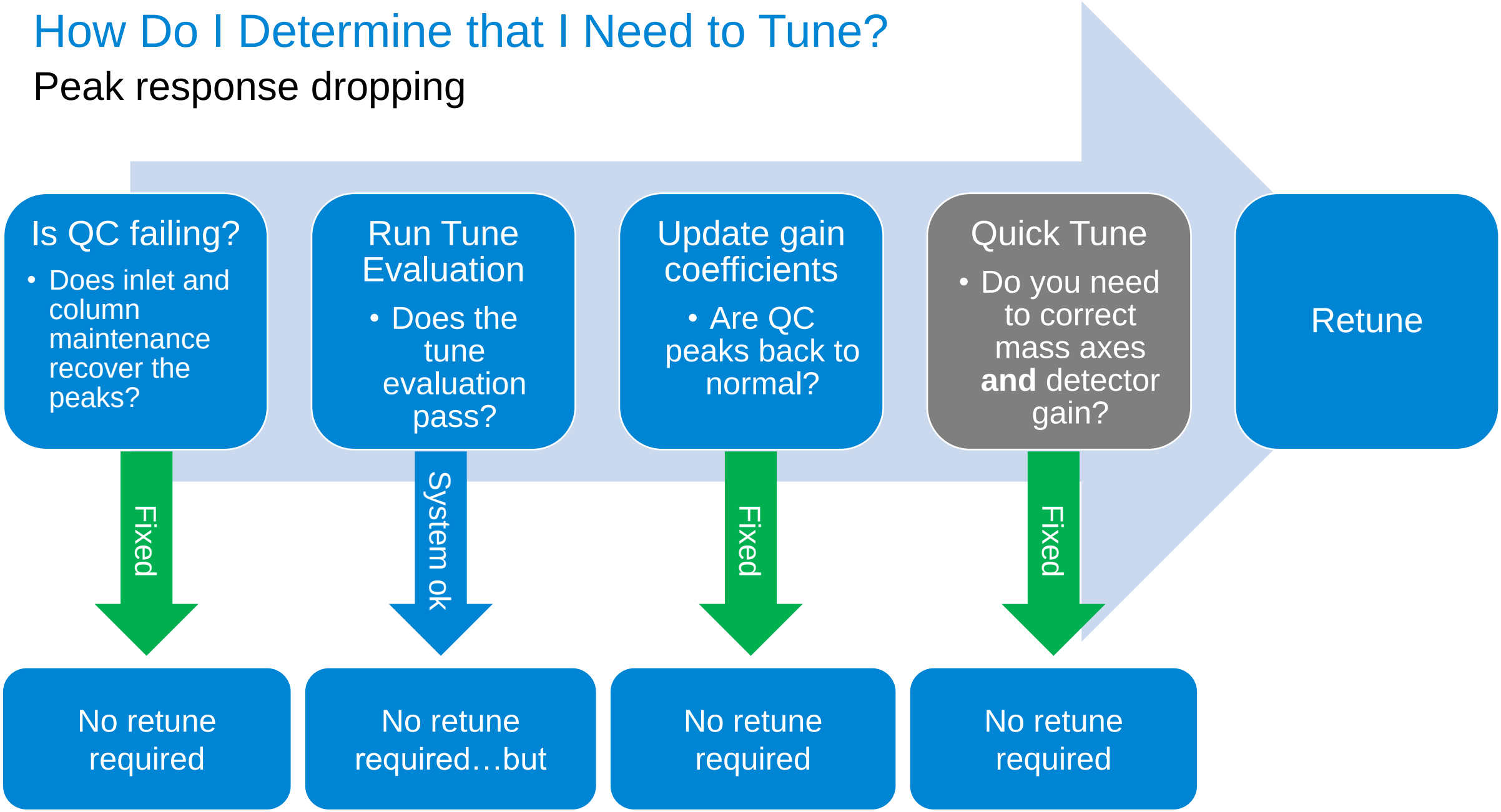
Let’s check a few things





How Do I Determine that I Need to Tune?

Peak response dropping



How Do I Determine that I Need to Tune?

1. Have a Baseline

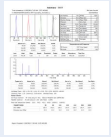
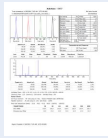
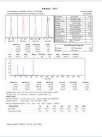
System start-up
- Tune system
- Run Tune Evaluation
- Run QC and cal samples

2. Review QC and CCV

What are peak responses doing?

3. Keep records

Build a profile of tuning occurrence

Monday	Tuesday	Wednesday	Thursday	Friday
Monday – 1  New system start-up	Tuesday – 2	Wednesday – 3	Thursday – 4 GC maintenance	Friday – 5
Monday – 8 Tune Eval + update gain coeff.	Tuesday – 9	Wednesday – 10 GC maintenance	Thursday – 11	Friday – 12 Tune Eval + QuickTune
Monday – 15	Tuesday – 16 GC maintenance + Tune Eval + QuickTune	Wednesday – 17  New tune	Thursday – 18	Friday – 19
Monday – 22 GC maintenance	Tuesday – 23	Wednesday – 24	Thursday – 25	Friday – 26 Tune Eval + update gain coeff.
Monday – 29	Tuesday – 30	Wednesday – 1 Failed ISTD areas = clean source	Thursday – 2  Clean source = new tune	Friday – 3

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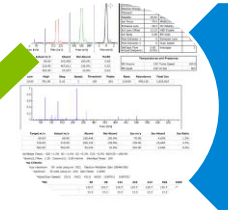
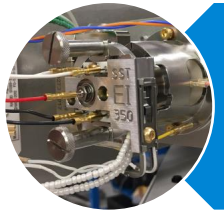
Today



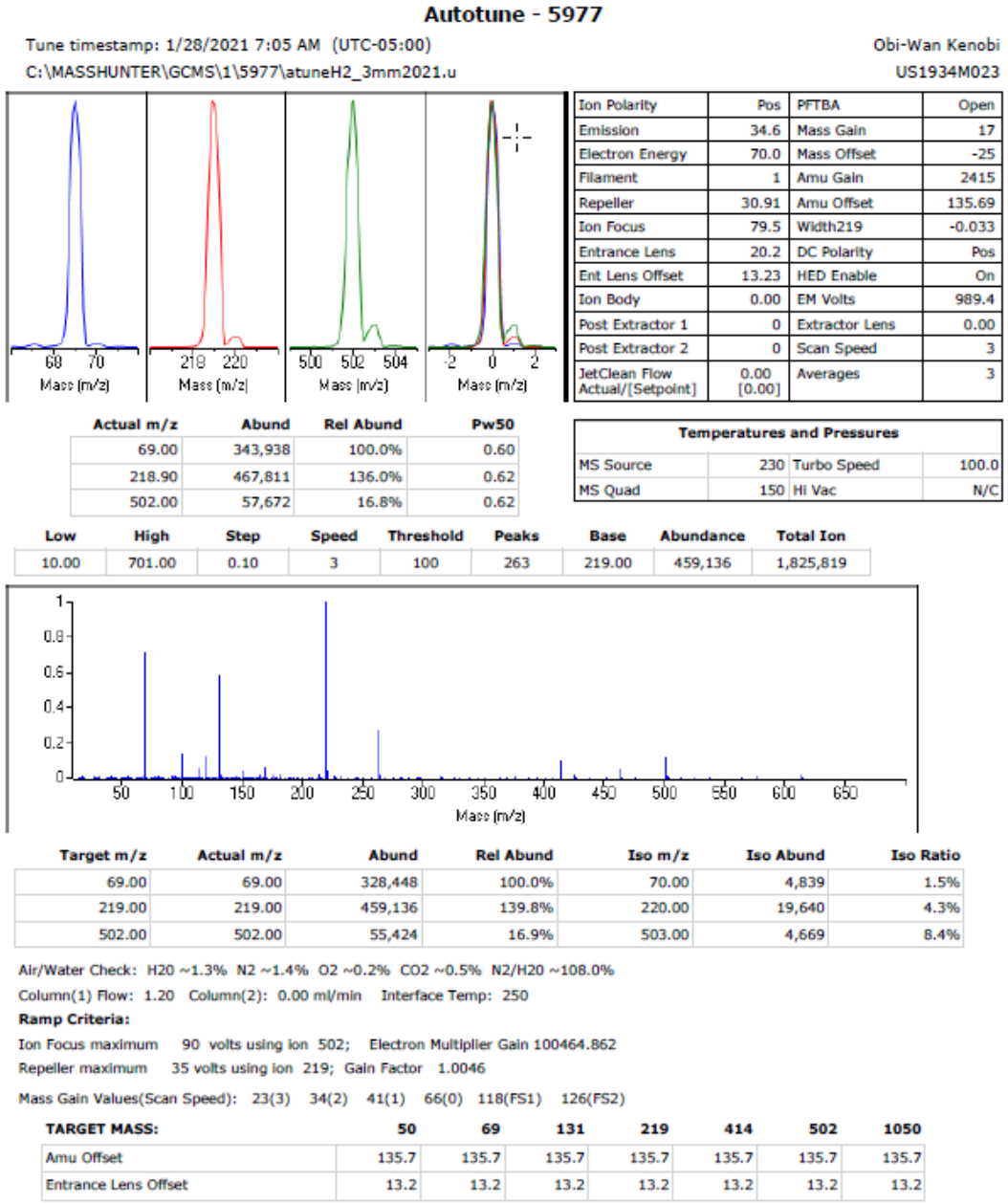
Demystify tuning and tune reports

Tuning regularity

…and how do I determine the frequency of tuning?



Troubleshoot problems using the mass spectrometer



Autotune - 5977

Tune timestamp: 1/28/2021 7:05 AM (UTC-05:00) — Obi-Wan Kenobi

C:\MASSHUNTER\GCMS\1\5977\atuneH2_3mm2021.u — US1934M023

Ion Polarity	Pos	PFTBA	Open
Emission	34.6	Mass Gain	17
Electron Energy	70.0	Mass Offset	-25
Filament	1	Amu Gain	2415
Repeller	30.91	Amu Offset	135.69
Ion Focus	79.5	Width219	-0.033
Entrance Lens	20.2	DC Polarity	Pos
Ent Lens Offset	13.23	HED Enable	On
Ion Body	0.00	EM Volts	989.4
Post Extractor 1	0	Extractor Lens	0.00
Post Extractor 2	0	Scan Speed	3
JetClean Flow Actual/[Setpoint]	0.00 [0.00]	Averages	3

Actual m/z	Abund	Rel Abund	Pw50
69.00	343,938	100.0%	0.60
218.90	467,811	136.0%	0.62
502.00	57,672	16.8%	0.62

Temperatures and Pressures			
MS Source	230	Turbo Speed	100.0
MS Quad	150	Hi Vac	N/C

Low	High	Step	Speed	Threshold	Peaks	Base	Abundance	Total Ion
10.00	701.00	0.10	3	100	263	219.00	459,136	1,825,819

Target m/z	Actual m/z	Abund	Rel Abund	Iso m/z	Iso Abund	Iso Ratio
69.00	69.00	328,448	100.0%	70.00	4,839	1.5%
219.00	219.00	459,136	139.8%	220.00	19,640	4.3%
502.00	502.00	55,424	16.9%	503.00	4,669	8.4%

Air/Water Check: H20 ~1.3% N2 ~1.4% O2 ~0.2% CO2 ~0.5% N2/H20 ~108.0%

Column(1) Flow: 1.20 Column(2): 0.00 ml/min Interface Temp: 250

Ramp Criteria:

Ion Focus maximum 90 volts using ion 502; Electron Multiplier Gain 100464.862

Repeller maximum 35 volts using ion 219; Gain Factor 1.0046

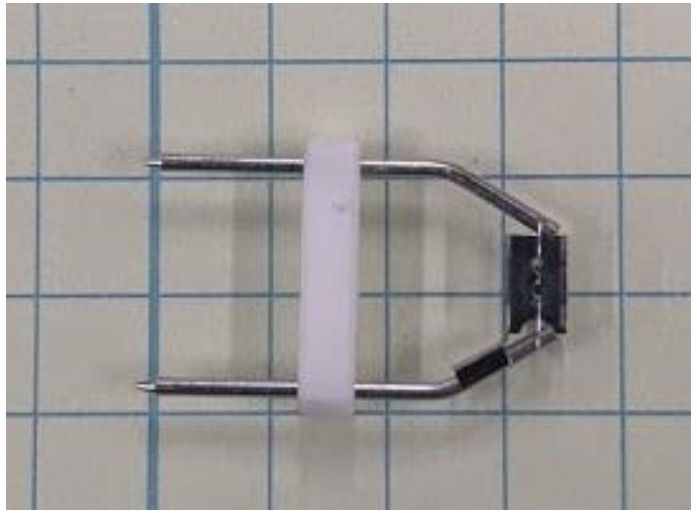
Mass Gain Values(Scan Speed): 23(3) 34(2) 41(1) 66(0) 118(FS1) 126(FS2)

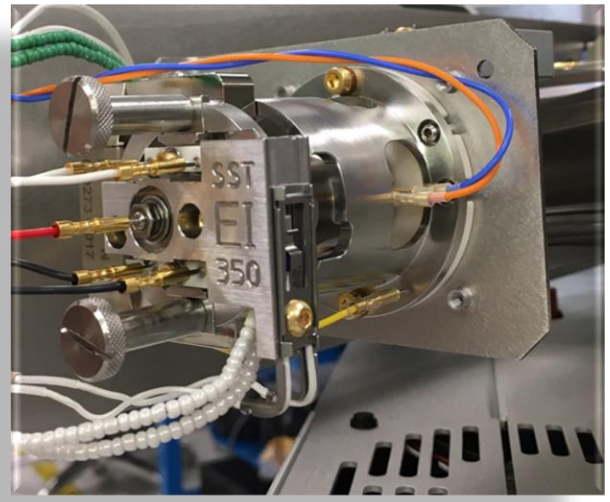
TARGET MASS:	50	69	131	219	414	502	1050
Amu Offset	135.7	135.7	135.7	135.7	135.7	135.7	135.7
Entrance Lens Offset	13.2	13.2	13.2	13.2	13.2	13.2	13.2

Can a Tune Really Tell us About Problems with Our Mass Spectrometer?

What can we learn from a successful tune?

- Are there any leaks?
- Source health
- Filament health/age
- Electron multiplier health/age



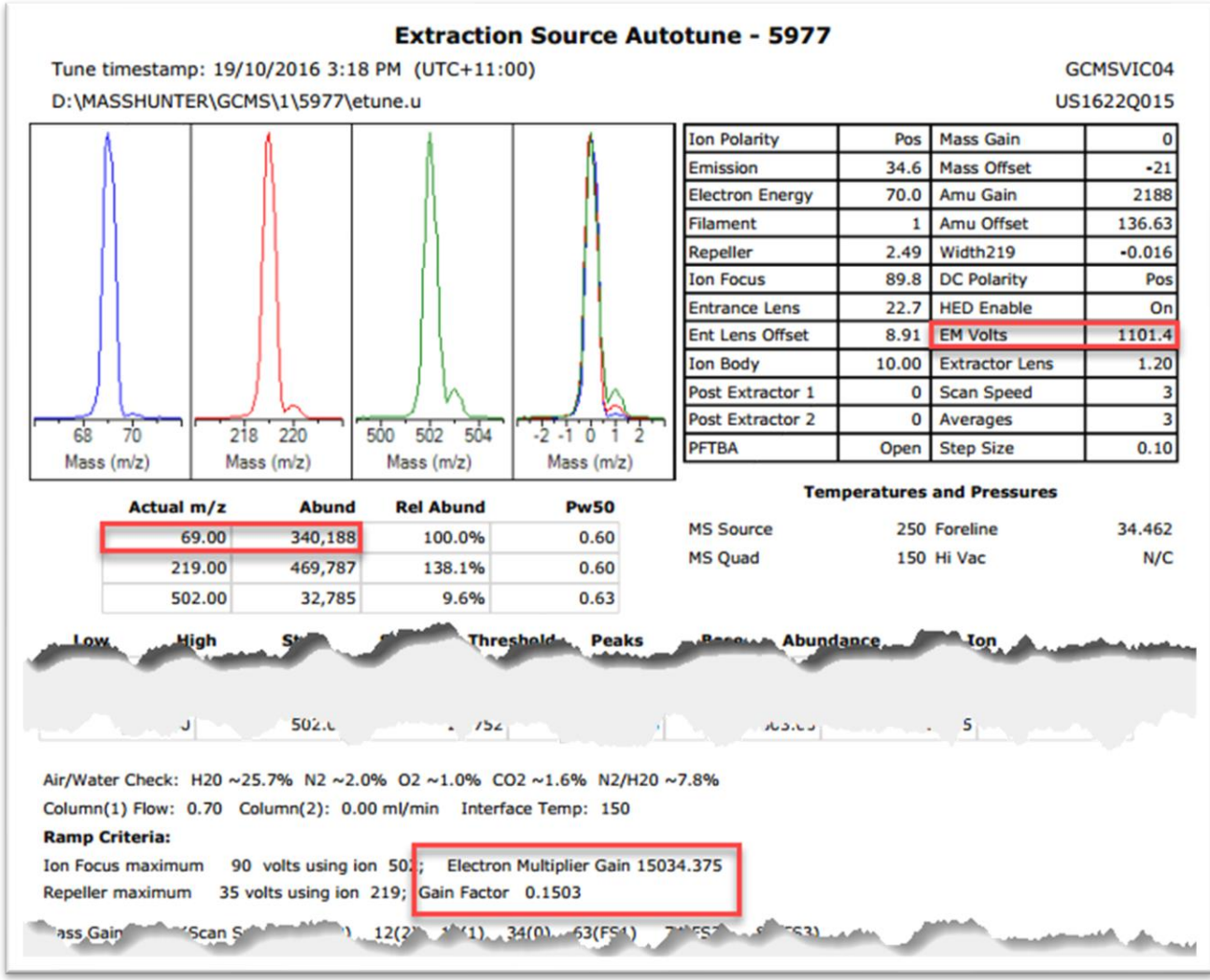


Autotune Report (SQ)

- The single quadrupole autotune process optimizes the source and EMV to achieve between ~350,000 and ~600,000 for m/z 69 (or the base peak out of the three target ions).

- Gain is **not** taken into consideration when it **sets** the autotune EMV.

- However, the gain and gain factor is **calculated** for the EMV at the end of the autotune.

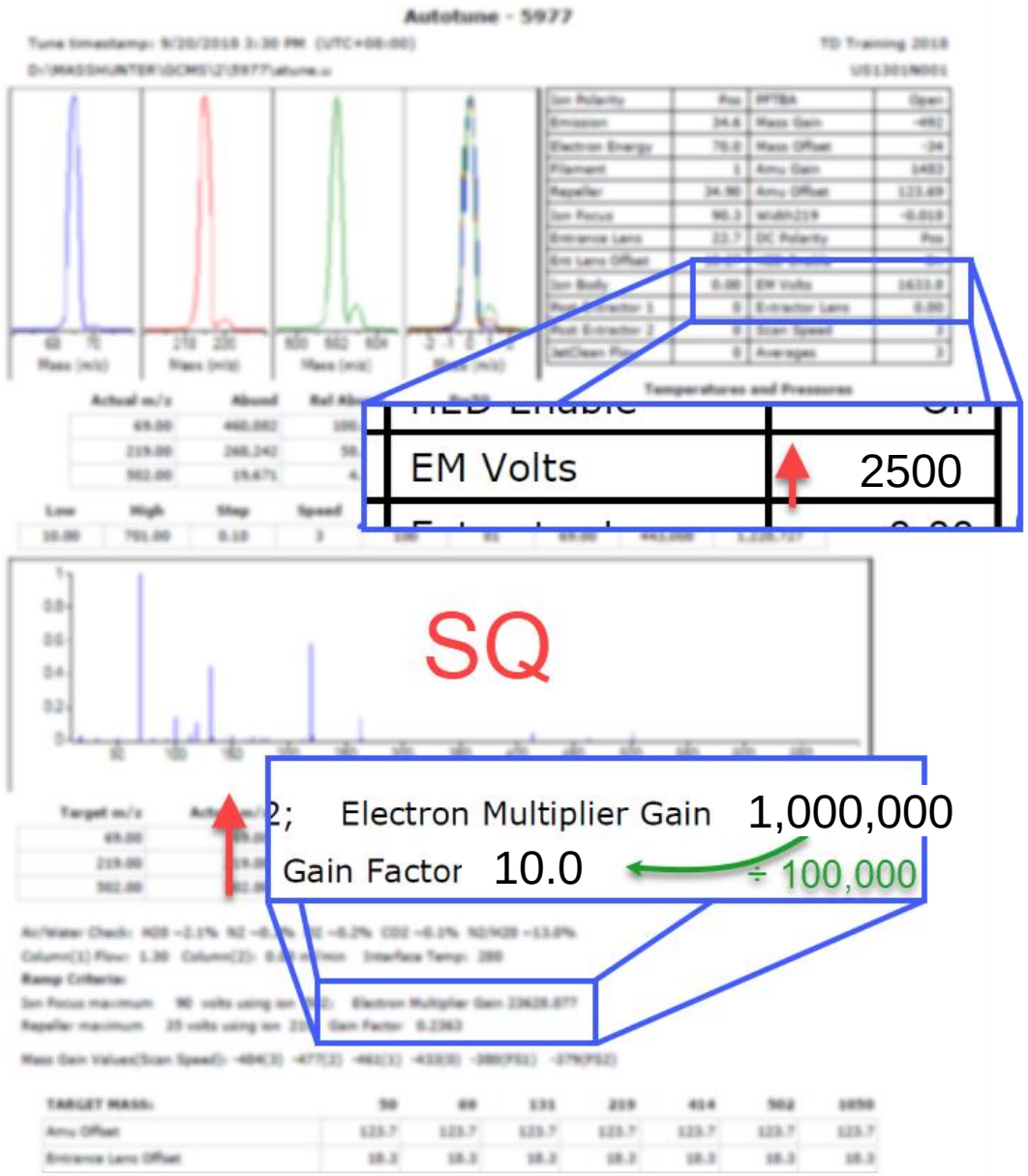


What Can We Learn from Autotune Report (SQ)

- **Increasing Gain Factor… and EMV creeping up…**

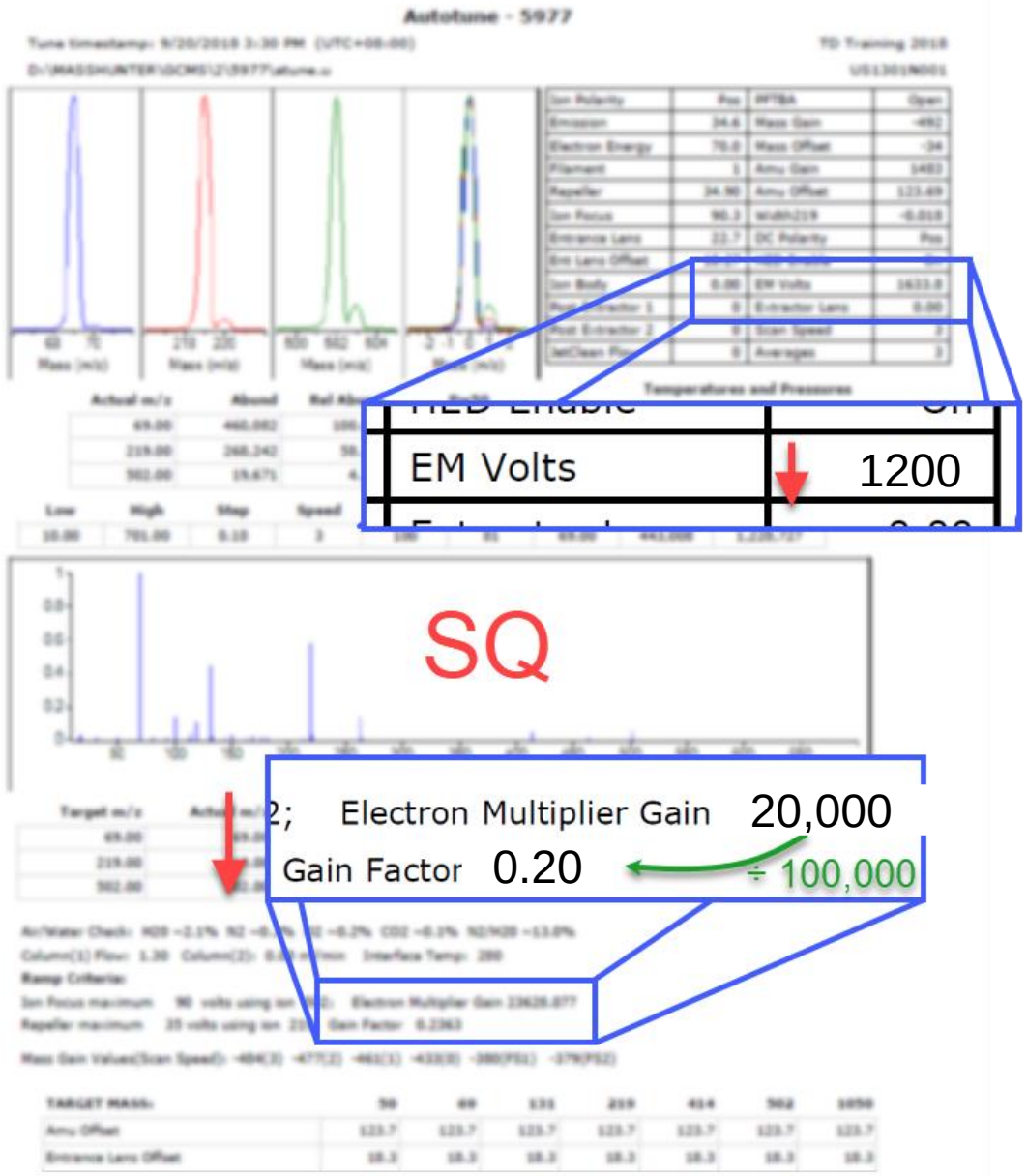
- Generally indicates that the **source is getting dirty**

- Usually happens over a shorter period (depends on the application)



What Can We Learn from Autotune Report (SQ)

- **Cleaning the source** causes the tune algorithm to check and reset tune values including the Gain.



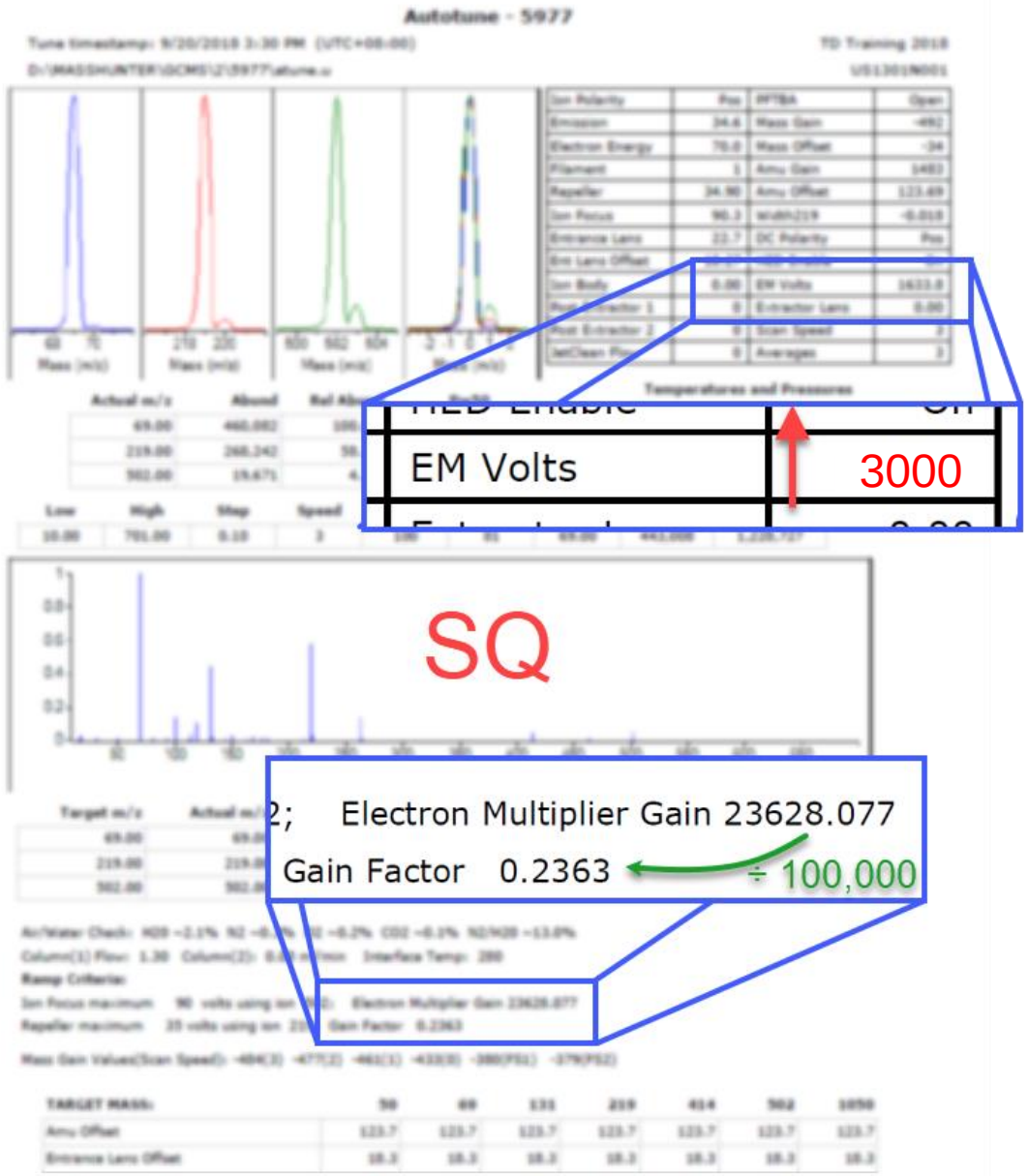
What Can We Learn from Autotune Report (SQ)

- **EMV increasing but Gain Factor remains relatively unchanged**

- Indicates our EM is aging, which happens over several months

- It is able to achieve the ~500,000 without needing to increase the gain; however, an increase of EMV is required to maintain gain factor.

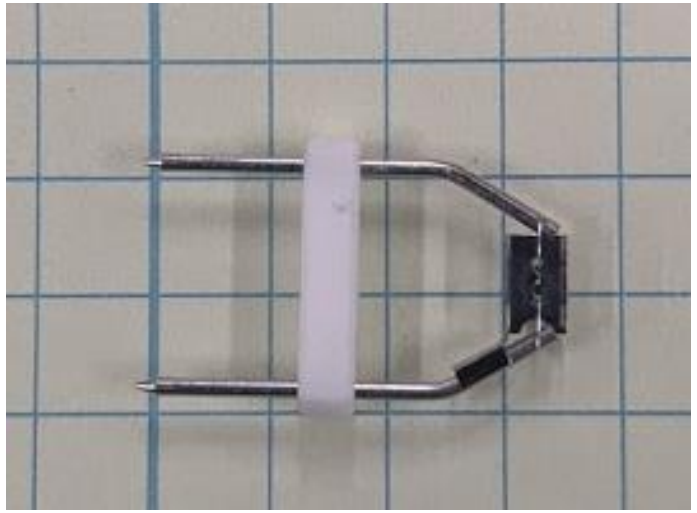
- EM is aging and needs to be replaced

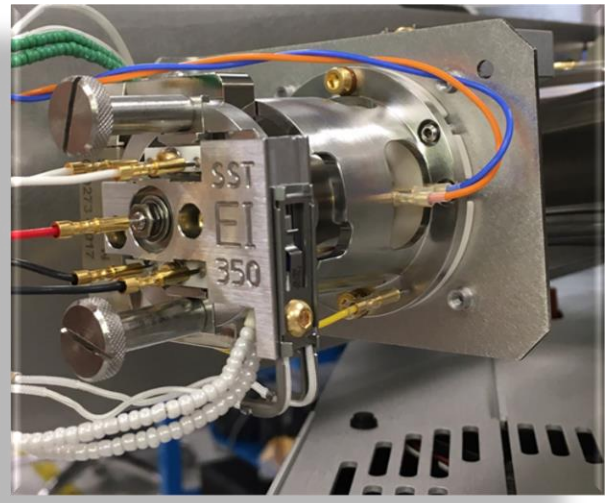


Can a Tune Really Tell us About Problems with Our Mass Spectrometer?

What can we learn from a failed tune?

- Problems with source installation or parts
- Column installation depth
- Are there any leaks?
- Attempting to tune too soon



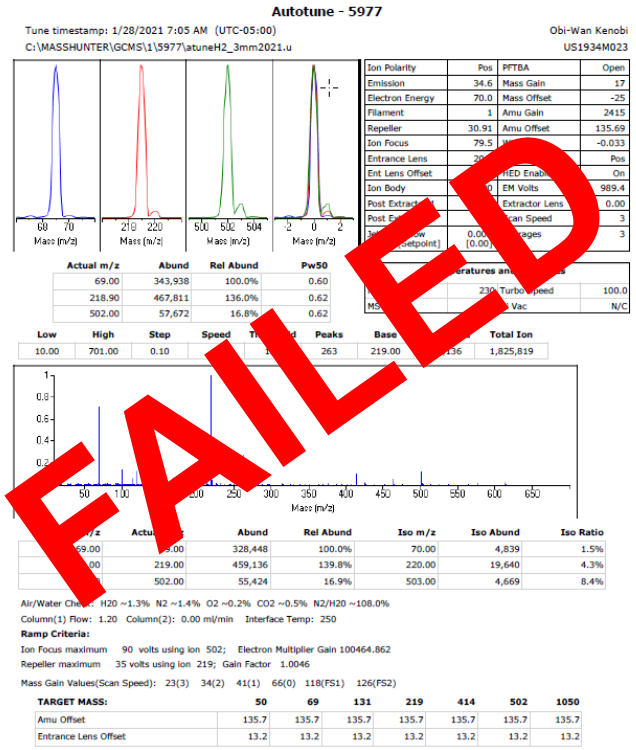


Does Column Installation Length Really Matter?

Installation length: 1-2mm beyond end of transfer line (flush with the ceramic tip)

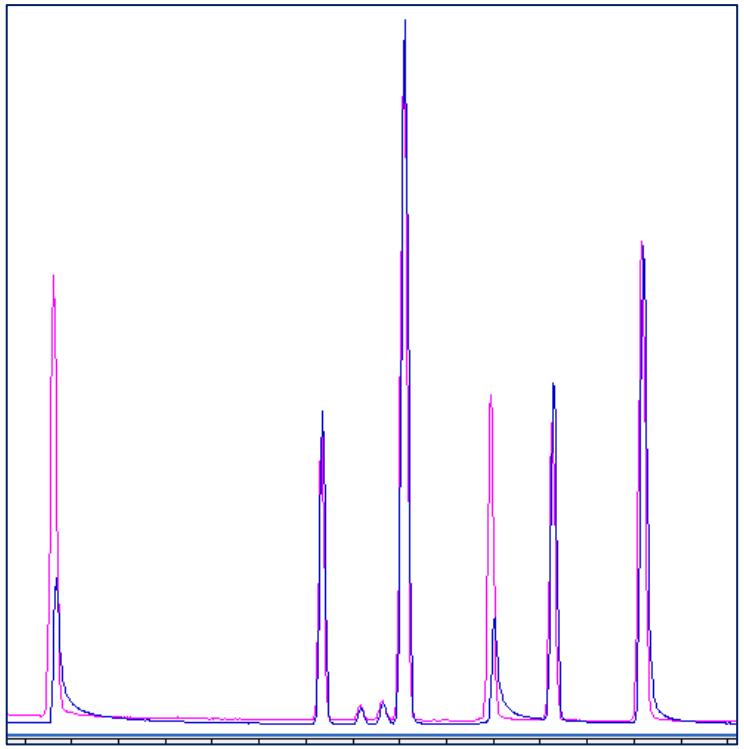
Column installed too far into MS

- Compromised sensitivity
- Problems with tuning



Column installed (very) short in transfer line

Peaks begin to tail and lose response



Pink = Normal column insertion distance

Blue = 50.8 mm pulled back into the transfer line

Other Upcoming Mass Spec Webinars

[Eliminate the Fear of Mass Spec | Agilent]

Title	Date	Time	Presenter
How to clean an Agilent GCMS Ion Source: A comprehensive guide	June 22, 2021	11 AM PT / 2 PM ET	Paul Salverda, GCMS Regional System Specialist, Agilent Technologies, Inc.
Optimizing the GC to Get the Analytes to the MS Efficiently	June 24, 2021	11 AM PT / 2 PM ET	Matt Curtis, GC/MS Applications Scientist. Agilent Technologies, Inc.
The Power of MS and MassHunter Software for GCMS	June 29, 2021	11 AM PT / 2 PM ET	Kirk Lokits, PhD, GC/MS Applications Scientist, Agilent Technologies, Inc.

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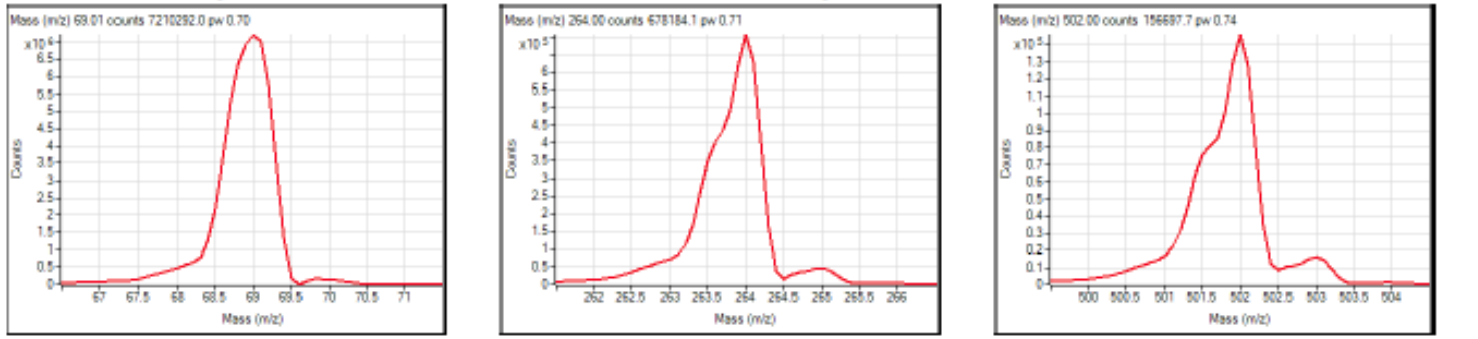
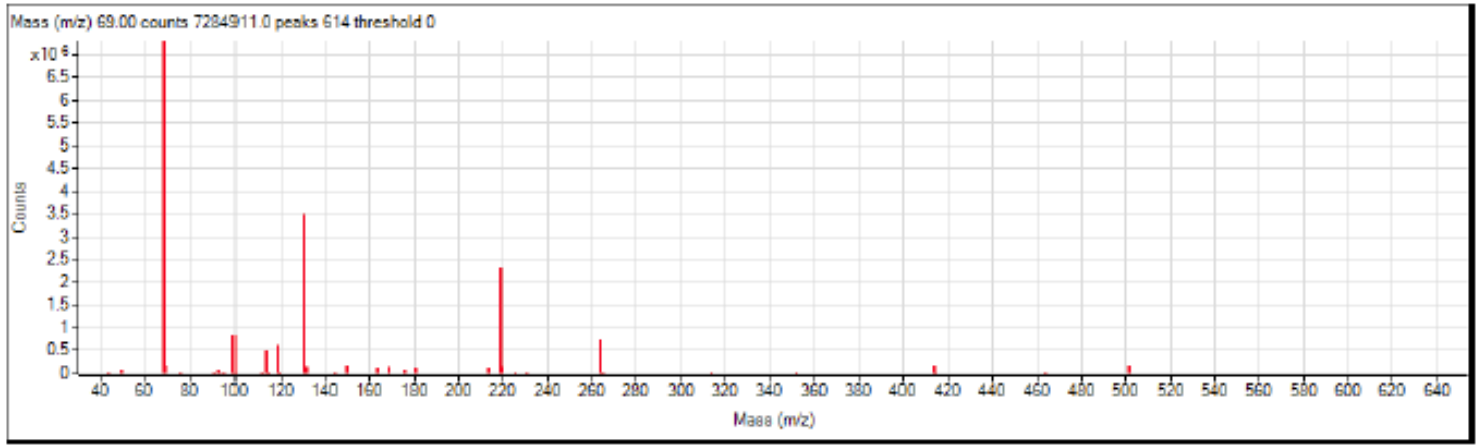
Help Diagnose the Issue with this Tune Report

- What do you see as possible issues?
- What could be the possible causes?

Analyzer: Q1 **Ion Polarity: Positive** **Width: Unit**





m/z	Abundance	Rel Abund	Isotope	Iso Abund	Iso Ratio
69.00	7284910.5	100.0%	69.90	152549.3	2.1%
219.00	2315955.8	31.8%	220.00	117245.3	5.1%
264.00	734633.6	10.1%	265.00	47270.8	6.4%
414.00	167292.4	2.3%	415.00	16569.6	9.9%
502.00	146866.6	2.0%	503.00	15947.6	10.9%