Non-Targeted Suspect Screening Strategies Using Low Resolution LC-MS-MS

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ESB

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Outline

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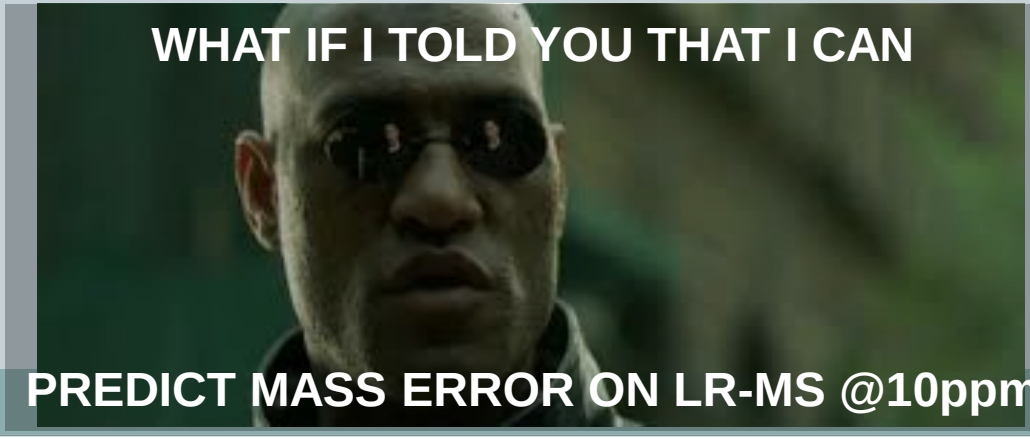
- What, Why, When pertaining to non-Targeted analysis
- Scope of Application
- Data Acquisition
- Data Processing (Example)
- Conclusions/Summary



What, Why When

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What

- Non-Targeted – no assumptions made about what is present. (TICs ver. 2.0)

Why

- Screening for 100-1000s of analytes using reference standards is prohibitively expensive and not practical

When

- Non-regulatory work. Client needs help/guidance

Purely Discovery

Scope

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- High-res accurate mass systems are used for this type of work
- However, TQMS is the best technology <u>available in our lab</u>
- **Can I predict mass accuracy on a Triple-Quad?**

Challenges/Solutions

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- Data is NOT high-res
- Isobaric interference is possible

  Solution - Apply to limited number of unknowns; less complex matrices
- Masses may still not be accurate <u>enough</u>

  Solution – Limit analyte suspects to "known unknowns" using a database.

Suspect Database

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- Limit suspects to ESI responders
  - Internal DB has 6054 ESI responders
  - 2351 have known MS2 transitions
- Reverse search the observed molecular ion as being any possible adduct of any possible analyte.
- Search Chemspider/Pubchem using MassSpec API as a last resort (ie: if no candidates match molecular ion spectrum)

Challenges/Solutions

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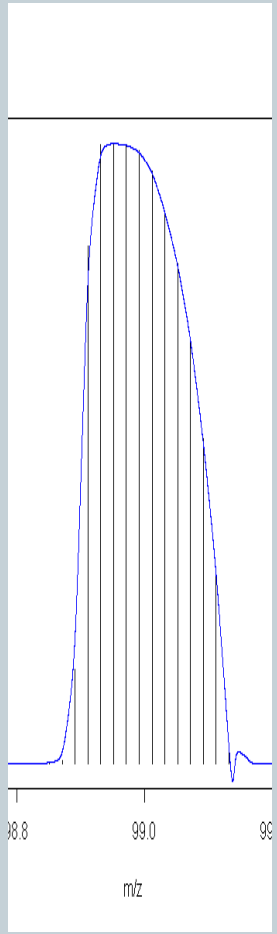
- Quadrupoles are not created equally
- Built-in centroiding algorithm is fast but <u>not accurate nor precise in both mass and intensity</u>
- Profile MS data reveals a distinct "mass shape" asymmetry



Solution: Develop non-parametric regression model of profile peak across a mass range to improve centroiding accuracy

Triple Quad Limitations

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- Mass shape also dependent on acquisition parameters (scan range, data rate, etc.)

  Solution: Carefully acquire sample data similarly to mass calibration verification

- Sensitivity in full scan is not so good

  Solution: Limit applicable concentration ranges to >100ug/L

Redefining Raw Data

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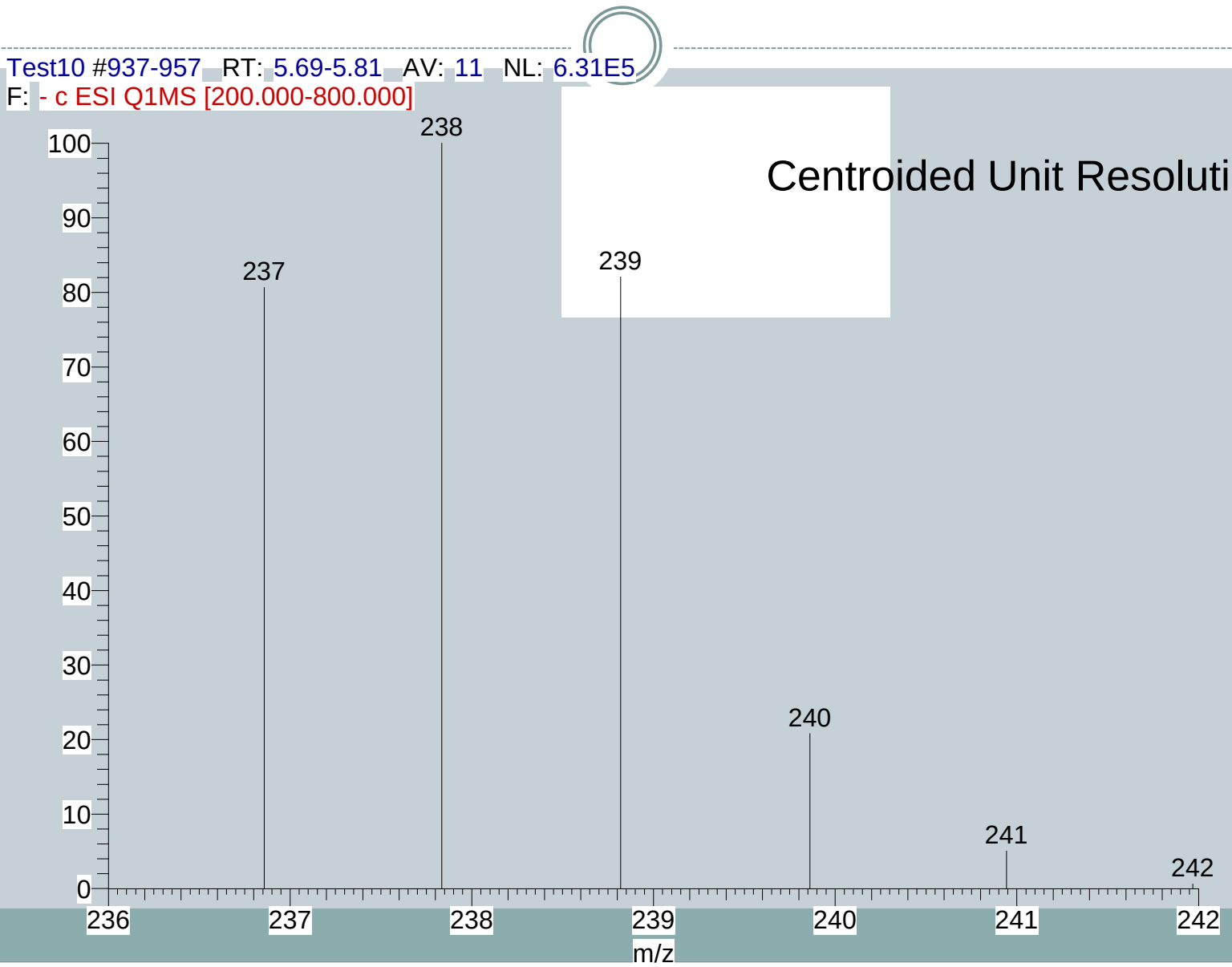

Redefining Raw Data

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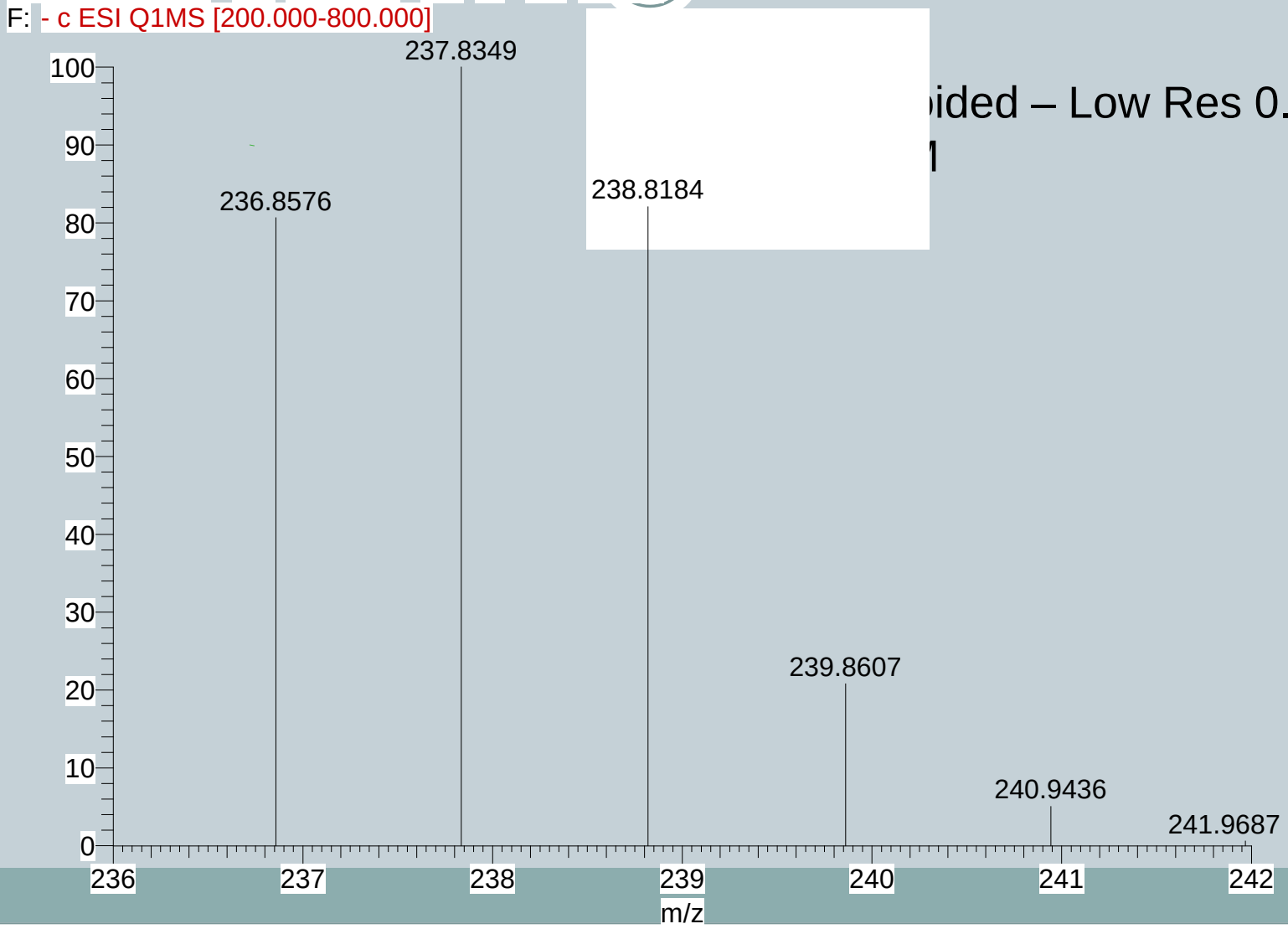

ided – Low Res 0.4Da

Redefining Raw Data

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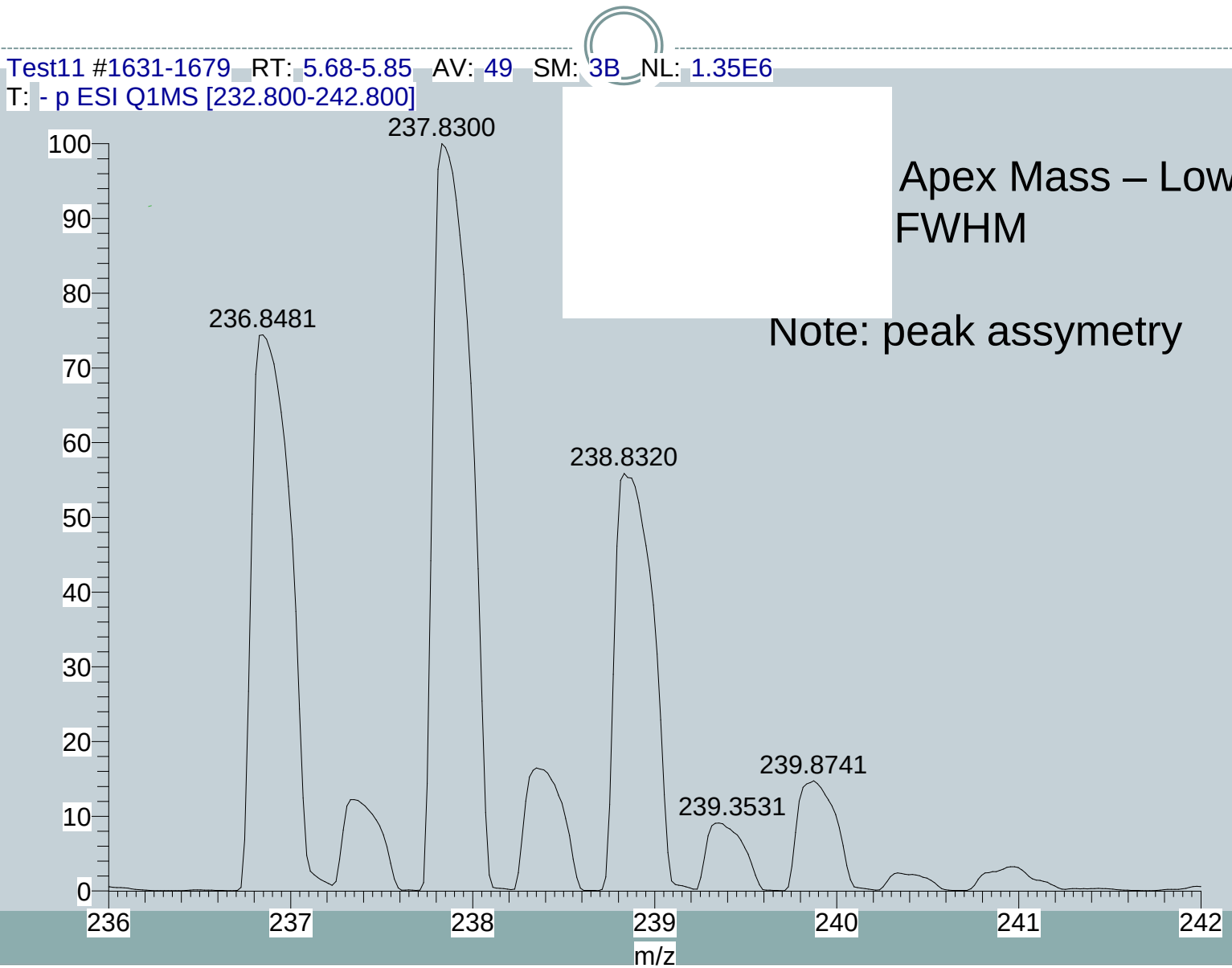

Apex Mass – Low Res FWHM

Note: peak assymetry

Redefining Raw Data

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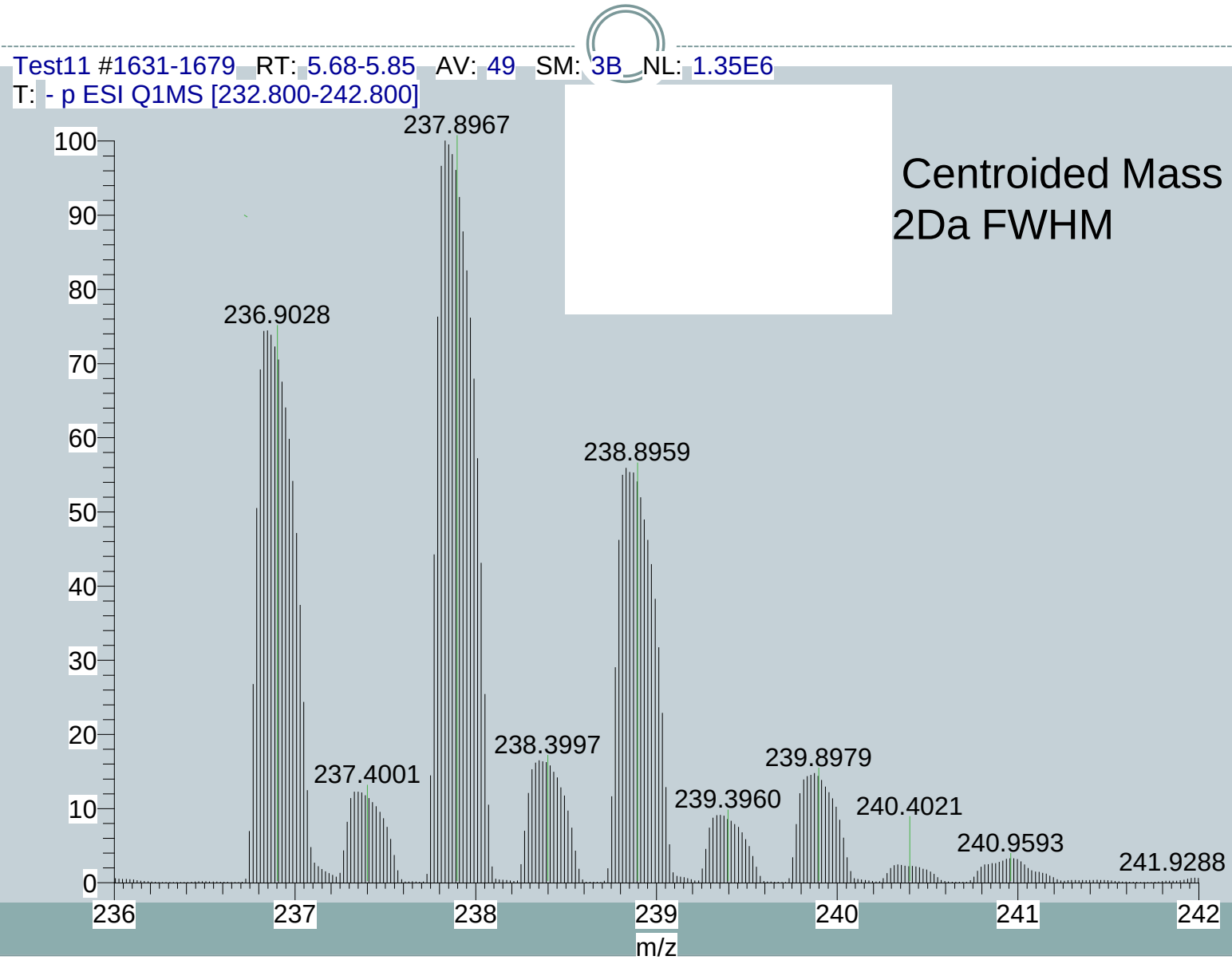
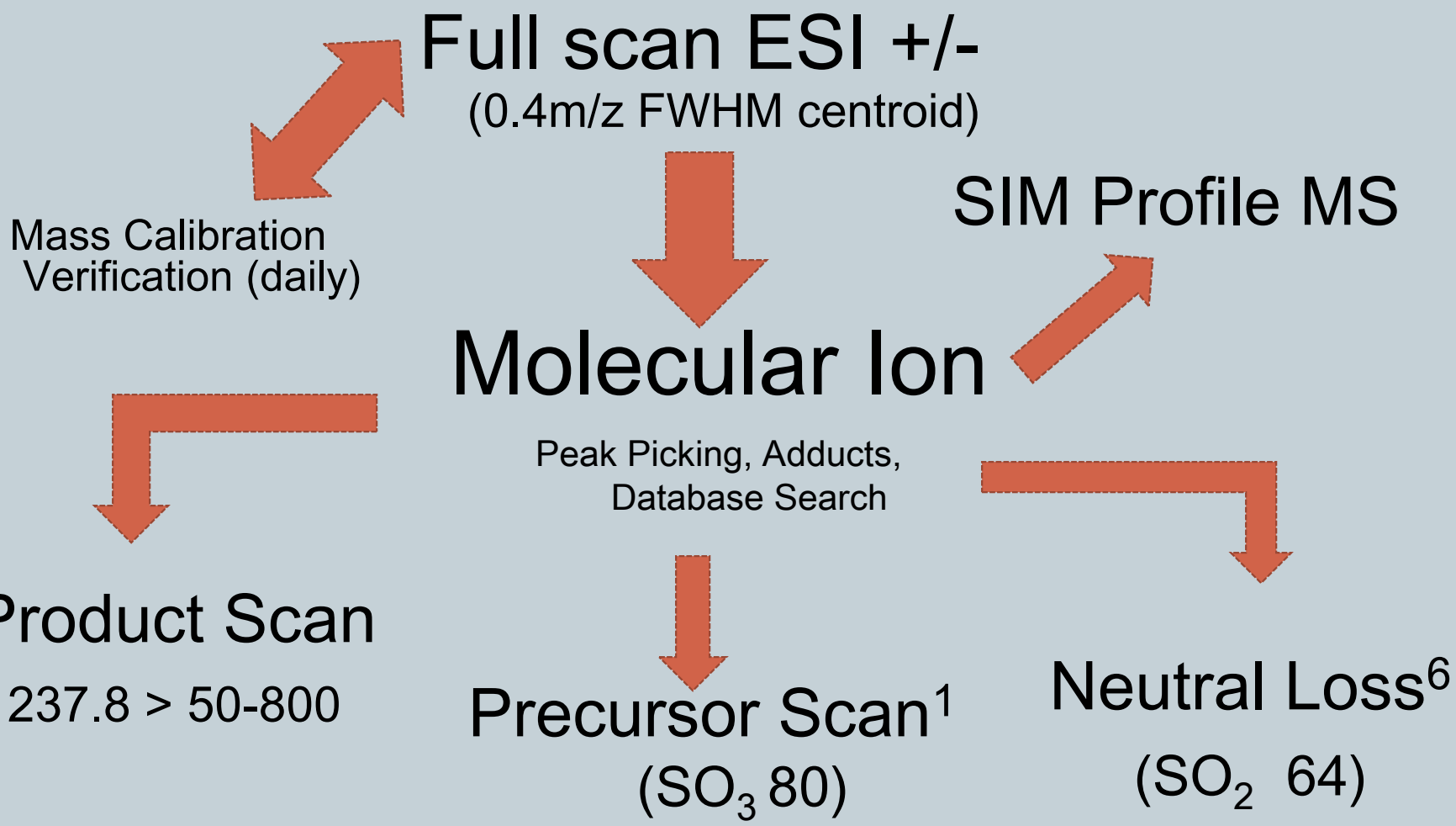


Centroided Mass – Low 2Da FWHM

Data Acquisition



Data Acquisition Summary

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- Full scan centroid at 0.4 FWHM in both ESI+/- from 90-1000mz
  - Profile MS is duty cycle limited
- Perform Peak Picking
- Acquire SIM profile data at 0.2 FWHM
- Apply mass correction based on daily check data
- Database search mass/intensity

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Mass Calibration

- Using 0.1% Phosphoric acid in 50:50 MeOH:Water as mass calibrant
- Full mass calibration weekly ~ 2 hours (25mins/Quad/polarity)
- Daily mass check in septuplicate (5mins) using mass calibrant at 10 masses
  - Used to build mass correction curve and upper/lower bounds for suspects
- Manual Tune Tweaks (Voltages, Fits)

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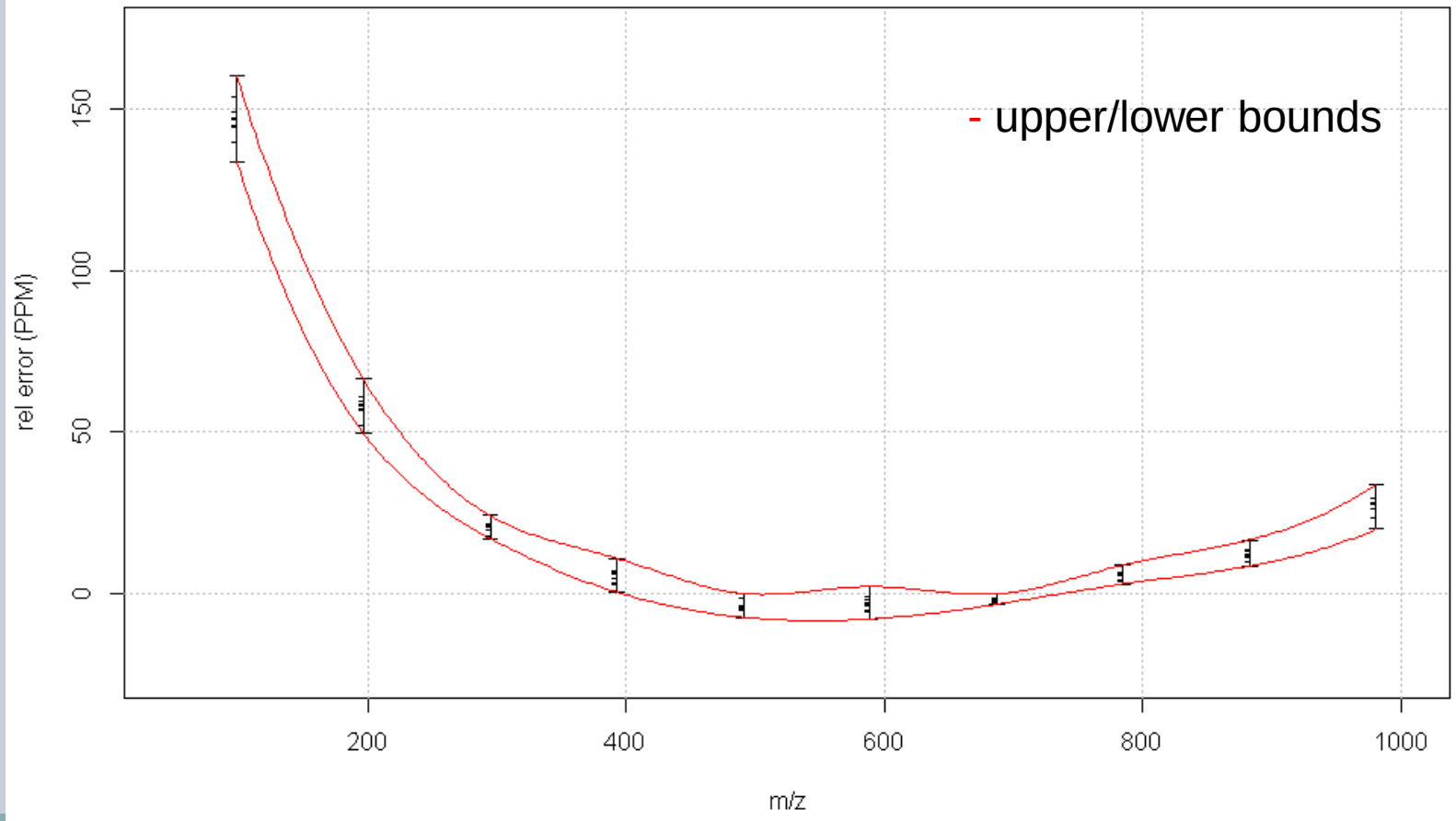
Mass Error Prediction Curve



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Mass Error Prediction Data

	Relative Error (ppm)										
Mass(Da)	**Rep1**	**Rep2**	**Rep3**	**Rep4**	**Rep5**	**Rep6**	**Rep7**	**StdDev**	**Mean**	**99.7% conf interval**	
98.98417	154.4454	147.8787	144.8479	147.3736	149.8992	145.353	140.3017	4.4	147.2	133.9	160.4
196.9611	57.11872	59.14958	58.38801	57.62643	52.54929	61.18044	60.16501	2.8	58.0	49.6	66.4
294.938	20.80291	20.80291	21.9896	18.26	20.29432	20.29432	21.48102	1.2	20.6	17.0	24.1
392.9149	3.15705	3.411558	6.974671	7.356433	6.847417	6.465655	4.938607	1.8	5.6	0.3	10.9
490.8918	-5.00042	-4.59289	-3.88001	-4.28743	-3.98186	-1.23176	-3.47258	1.2	-3.8	-7.5	-0.1
588.8686	-5.21221	-4.61785	-3.00459	-1.47624	-0.45733	-2.49514	-3.59895	1.7	-3.0	-8.0	2.1
686.8455	-1.88535	-1.01179	-2.46772	-1.37577	-1.88535	-1.81255	-2.54051	0.5	-1.9	-3.5	-0.2
784.8224	7.059509	4.192619	5.976462	6.231297	4.765997	6.295005	5.849045	1.0	5.8	2.8	8.7
882.7993	10.11086	12.20647	13.39587	12.37638	11.47018	13.79234	13.79234	1.4	12.4	8.4	16.5
980.7762	23.70595	26.71377	29.92551	27.93729	26.56083	23.96085	28.65101	2.3	26.8	19.8	33.7

Analytical Parameters

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- Suggested Mobile phase: Water with dilute NH_4OH (0.02%) + Methanol (protic).
- Use C8 or C18 column to use SLogP to eliminate candidates based on retention time
- No Acetonitrile: ACN completely suppresses certain analyte classes[5] (pyrethroid esters)
- Direct aqeuous injection – Minimize background and the possibility of missing potential analytes (non-extractables)

Step1: Data partitioning

Separate full scan into 10mz bins and perform peak picking

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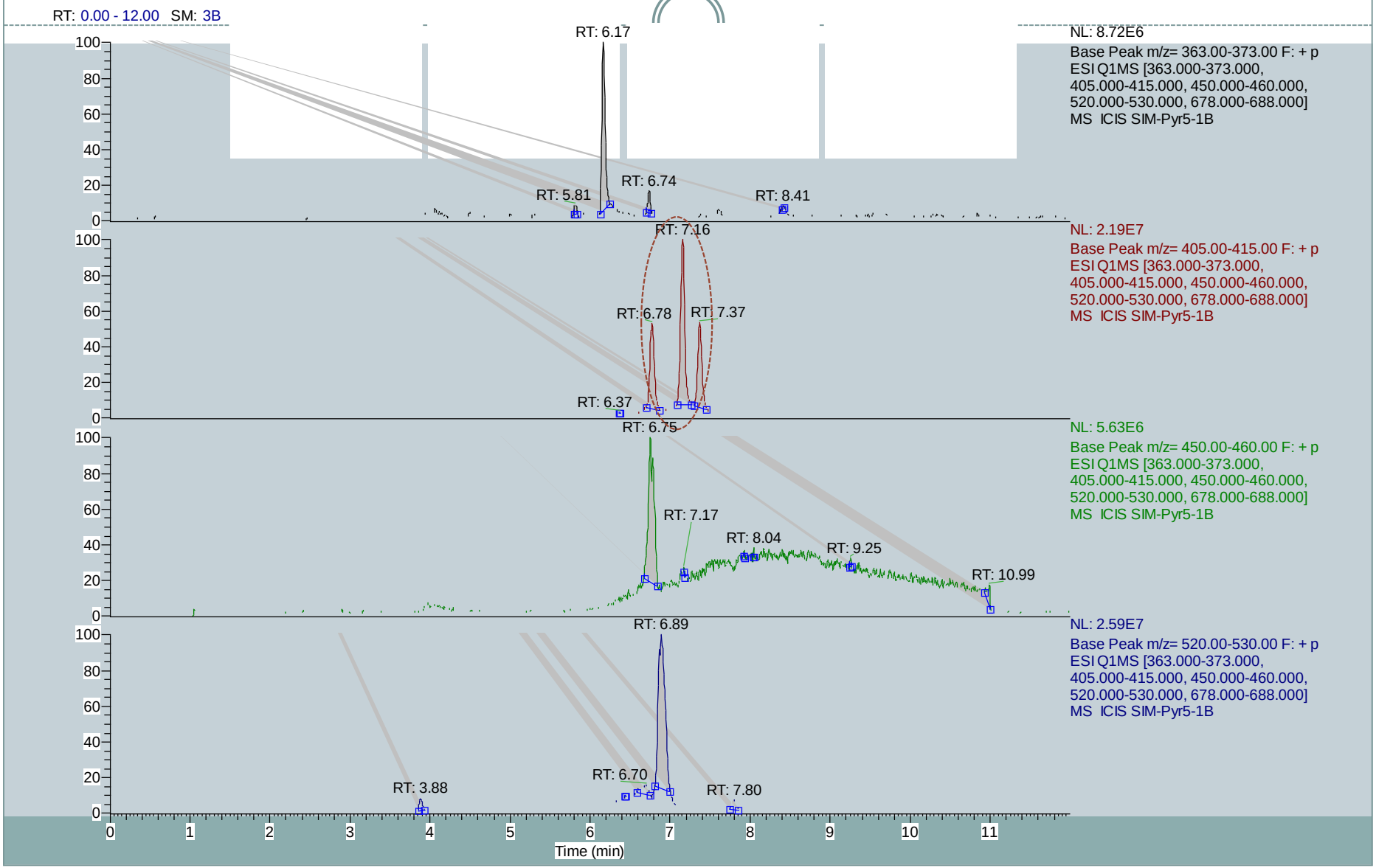

Step 2: SIM Profile

Generate Mass/Intensity List

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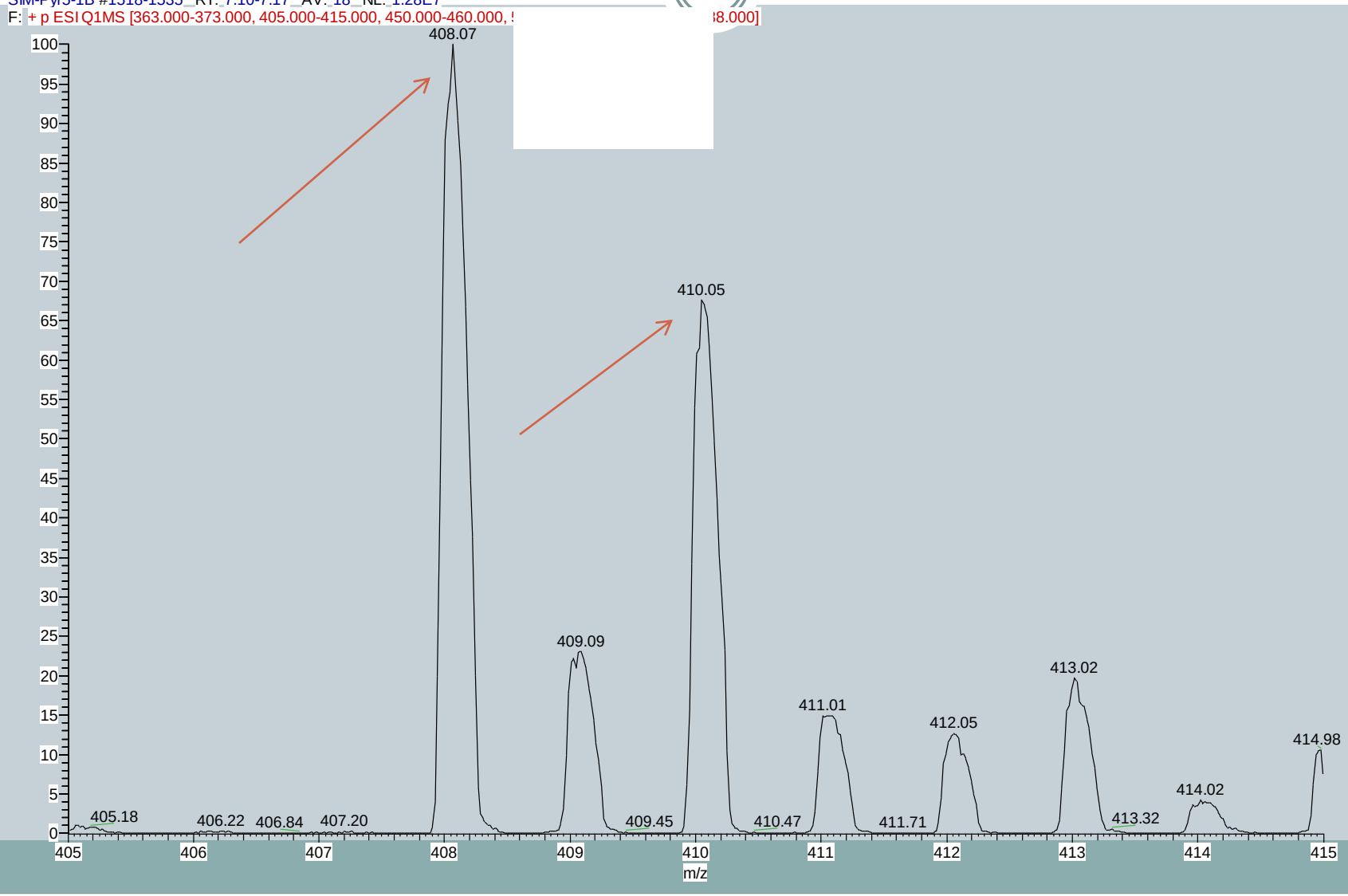

Step 3: Gather Information

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- Determine "Corrected" Monoisotopic Mass
  - Look for Multiple Adducts
- Determine Charge: usually 1, but don't assume
- Assemble Mass/Intensity List and do a quick search.
  - Compare observed with theoretical
  - Best to search using automated algorithm

Many suspects will be eliminated at this point

Automated Search Example

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Mass, Abun
408.1046,100
409.1118,23.53
410.1025,68.77
411.1107,15.5
412.0993,12.85

408.1046	Lowest Observed Ion M/Z
0.1	Mass Width(Da)
+	Polarity(+/-)
1	Charge(integer)
O|N	Formula Map (Regex)
100	Ion Rank
100	Error Threshold(ppm) - Hi

ESI only (Overrides Formula Map)

Search

Spectral Data (Mass,Abun) List

```
408.1046,100
409.1118,23.53
410.1025,68.77
411.1107,15.5
412.0993,12.85
```

Chemical	Formula	Principal Mass	Adduct	MZIon	IonRank*	SPDR score	# Ref Ions
Triclopyricarb	$C_{15}H_{13}Cl_3N_2O_4$	389.9943	$[M+NH3+H]^{1+}$	408.0281	725	82.4%	
Flazasulfuron	$C_{13}H_{12}F_3N_5O_5S$	407.0512	$[M+H]^{1+}$	408.0585	6497	43.7%	7
Thiencarbazone methyl	$C_{12}H_{14}N_4O_7S_2$	390.0305	$[M+NH3+H]^{1+}$	408.0643	725	63.7%	
Flupropacil	$C_{16}H_{14}ClF_3N_2O_4$	390.0595	$[M+NH3+H]^{1+}$	408.0933	725	72.6%	
cis-Permethrin	$C_{21}H_{20}Cl_2O_3$	390.0791	$[M+NH3+H]^{1+}$	408.1129	725	98.8%	3
Permethrin	$C_{21}H_{20}Cl_2O_3$	390.0791	$[M+NH3+H]^{1+}$	408.1129	725	98.8%	7
1R-trans-Permethrin	$C_{21}H_{20}Cl_2O_3$	390.0791	$[M+NH3+H]^{1+}$	408.1129	725	98.8%	3
1S-trans-Permethrin	$C_{21}H_{20}Cl_2O_3$	390.0791	$[M+NH3+H]^{1+}$	408.1129	725	98.8%	
Clethodim sulfone, 5-Hydroxy-	$C_{17}H_{26}ClNO_6S$	407.1171	$[M+H]^{1+}$	408.1244	6497	93.2%	3
Sitagliptin	$C_{16}H_{15}F_6N_5O$	407.1181	$[M+H]^{1+}$	408.1254	6497	44.4%	
Polydatin	$C_{20}H_{22}O_8$	390.1315	$[M+NH3+H]^{1+}$	408.1653	725	42.8%	

20ppm Mass Error

http://massspec.info/php/SPDR4MS.php

Spectral Accuracy

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Experimental Data

mz	inten	mzAcc	FWHM	mzCorr	RelInt
408.1087	8808896	408.1071	0.2313	408.1046	100
409.1307	2072994	409.1143	0.239	409.1118	23.53
410.1127	6058035	410.105	0.2286	410.1025	68.77
411.1147	1365217	411.1132	0.2411	411.1107	15.50
412.0966	1131671	412.1018	0.2335	412.0993	12.85

Theoretical

m/z	Abund
408.11297	100
409.11627	23.42
410.11039	67.03
411.11334	15.19
412.10855	12.37
413.1105	2.49

http://massspec.info/php/SpectrumSIM.php

Data Processing Tools

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- ReAdW.exe: Export ThermoSci raw data files to mzXML (github.com/PedrioliLab/ReAdW)
- R packages: XCMS[2], mzR, enviPat[3], npreg
- KNIME: Generate ESI bit-strings based on chemoinformatics (knime.org)
- MS2 Analyzer[4]: Piece together MS2 fragmentation scans

Final Thoughts

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- Mass accuracy can be predicted with known error and precision limits on a reasonable time scale using a triple-quadrupole.
- Some non-targeted suspects can be eliminated based on narrower mass tolerances and whether or not they respond in ESI
- Knowing the limitations of a Low-Res MS system is key to applying mass measurements to sample data

Acknowledgements

- Dr. Creed Jones
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  - Senior Chemist, Babcock Laboratories

Questions

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