Waters

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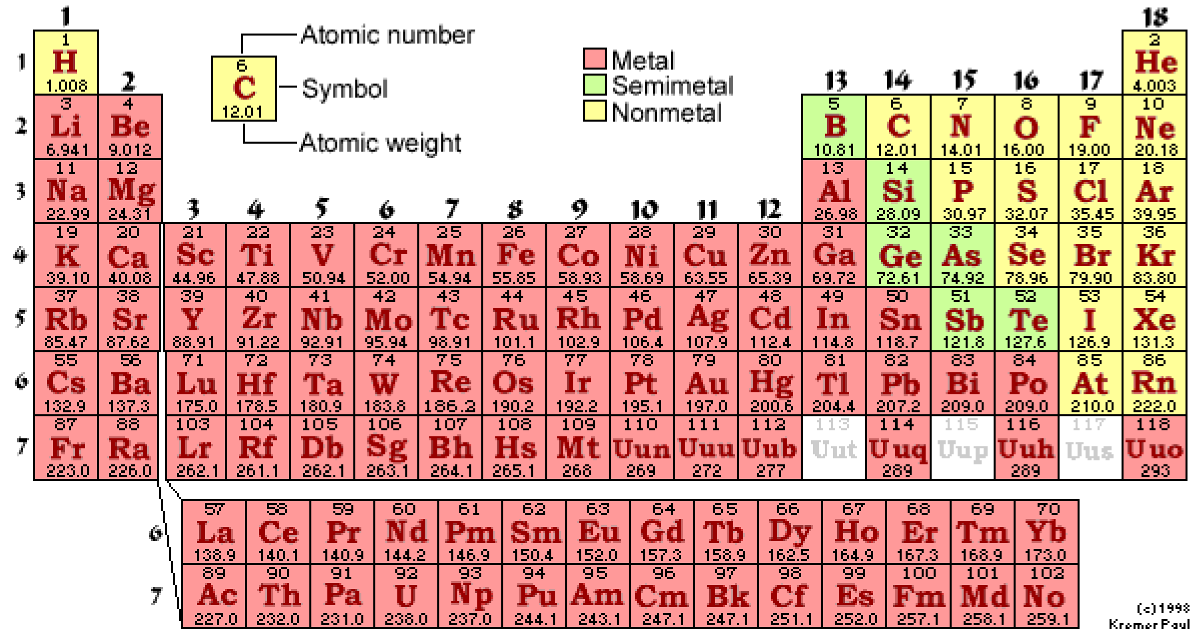
Exact Mass Tools and Technologies for Qualitative Analysis

For Complete Confidence

Presentation overview

- Principles and definitions of exact mass
- Types of instrumentation used for exact mass measurement
- Practical aspects of exact mass measurement
- Routine exact mass measurement on a Q-Tof Premier™
- I-FIT™ elemental composition determination
- Conclusion

Introduction



- Every element found in nature has a unique mass
- Elements are combined to produce compounds with distinct masses and physical properties
- Compounds can be detected by mass spectrometry and thus their masses measured
- If a compound mass can be measured with sufficient accuracy, a unique elemental composition can be inferred – the benefit of exact mass

Definitions

- There are three main types of mass measurement;

- **Nominal** Sum of the inter masses of the most abundant naturally occurring isotopes of which the ion is comprised
- **Average** Calculated using all the isotopes of each element from which the ion is comprised
- **Exact** Calculated by summing the distinct mass of the individual, most abundant, isotopes of which the ion is comprised

Definitions

CO	=	27.9949
N_2	=	28.0061
C_2H_4	=	28.0313

- These elemental combinations have the same nominal mass but different exact mass
- A nominal mass measurement cannot distinguish these
- If any compounds differ in their elemental compositions by substitution of any of these elements, then the exact mass measurement will show this

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Definitions

- The accuracy of the measurement is quoted as the difference (error) between the measured mass and the calculated mass
- The accuracy is measured in
  - milliDaltons (1mDa = 0.001 mass units)
  - ppm = parts per million = $\Delta m/m \times 10^6$

Example:

True' mass	= 400.0000	
Measured mass	= 400.0020	
Difference	= 0.0020	(2 mDa)

$$\text{ppm error} = \frac{0.002}{400} \times 10^6 = 5 \text{ ppm}$$

Definitions

Sulphamethazine $C_{12}H_{14}N_4O_2S$

Nominal	Exact	Average
278	278.0837	278.3313

Definitions

Ketaconazole $C_{26}H_{28}Cl_2N_4O_4$

Nominal	Exact	Average
530	530.1482	531.4306

The contribution of the ^{37}Cl isotope accounts for much of the difference between the average and exact masses

Definitions

Ubiquitin $C_{376}H_{630}N_{105}O_{118}S$

Nominal	Exact	Average
8556	8560.6254	8565.8730

Every 130 H atoms present increases the exact mass by one unit over the nominal mass

Benefits of Exact Mass

- Measurement of mass to 4 decimal places
- High confidence in confirming expected compounds
  - Distinguishes them from compounds of similar mass
- Compound identification
  - Prediction of elemental composition
- Patent submission and publication
  - ACS require better than 5ppm mass accuracy for publication

Instrumentation

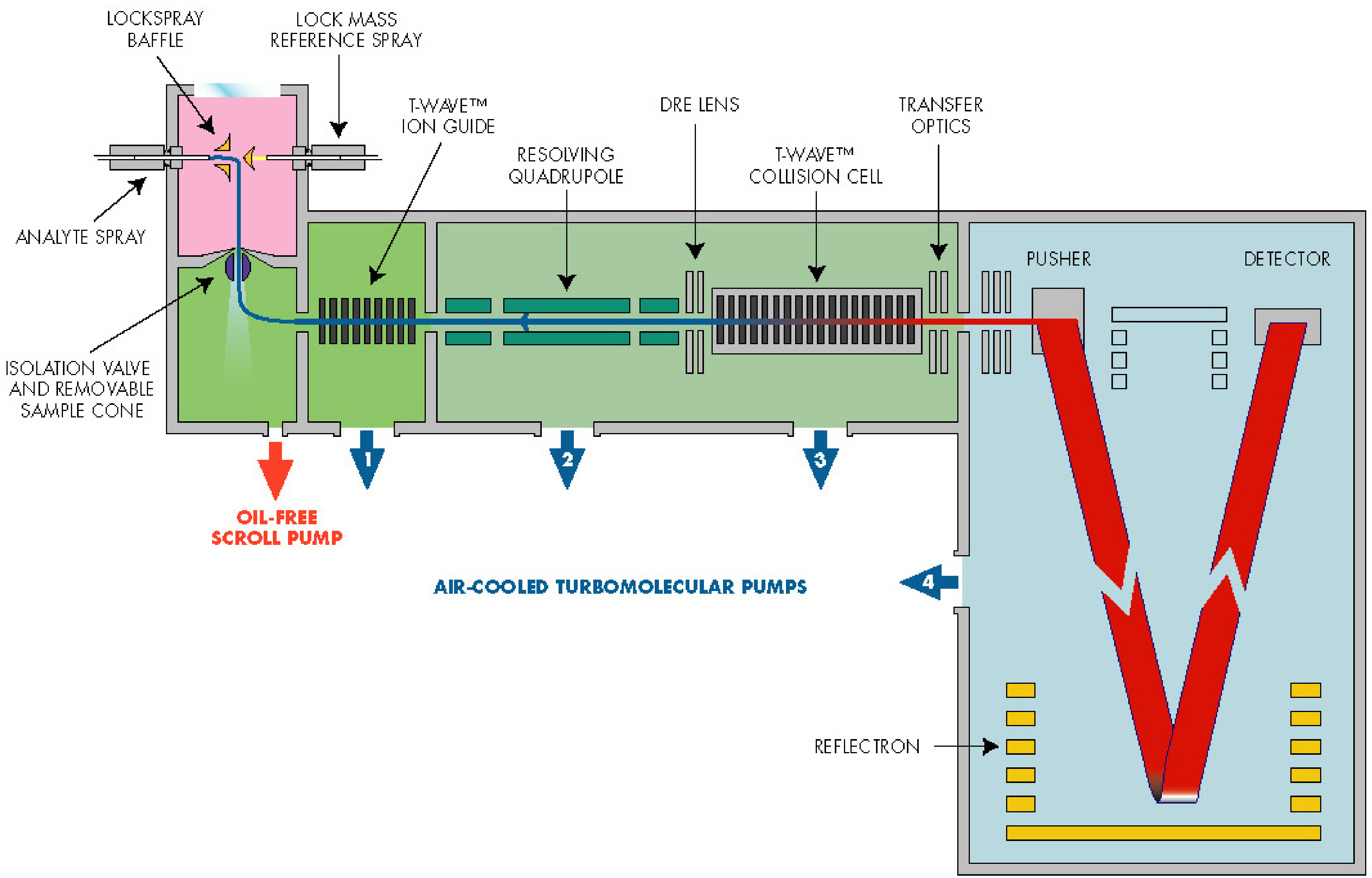
- Magnetic sector
  - Traditionally used for exact mass measurement
  - Requires experience to produce best results
- ICR-FTMS
  - Can produce excellent results, 1-2 ppm
  - Requires experience to produce best results
- oa-Tof
  - Can produce excellent results, >3ppm RMS
  - Latest systems offer routine exact mass measurement

Premier oa-Tof instrumentation

LCT Premier
Benchtop oa-TOF MS

QTOF Premier
oa-TOF MSMS

Q-Tof Premier



Practical aspects of exact mass measurement

- Ion abundance
- Resolving power
- Calibration
  - External
  - Internal
- Data manipulation

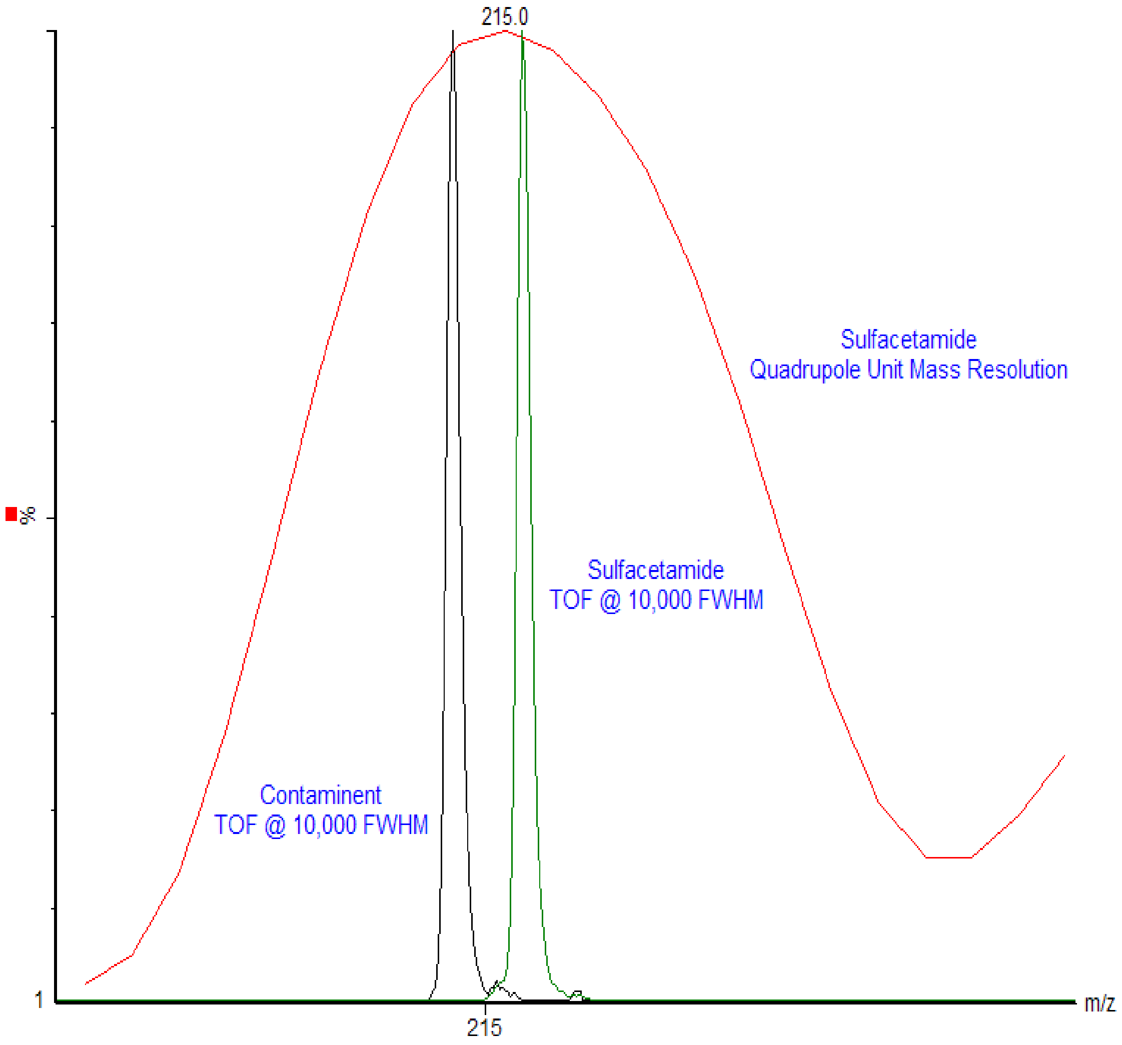
Ion Abundance

- Ion Abundance
  - The signal must be above a certain level in order to obtain good ion statistics and peak shape
  - The signal must not be too strong as to saturate the detector of the instrument
  - The latest oa-Tof instruments have considerably increased dynamic range performance
  - This delays the onset of detector saturation increasing the sample concentration range over which exact mass measurement can be acquired

Resolving power

- Resolving power
  - The resolving power of a mass spectrometer describes it's ability to separate two ions from one another with less than a defined amount of overlap
  - The resolving power required for an exact mass measurement is determined by the m/z values to be resolved and the present of back ground interferences
  - It is good practical to operate an oa-Tof instrument at its specified resolving power as this will offer the best compromise between sensitivity, resolution and peak shape

Resolution & Exact Mass



- Unit mass resolution is not sufficient to differentiate these two compounds
- ToF data with a resolving power of >10,000, clearly shows two distinct peaks.
- These can be accurately mass measured to < 3ppm

Calibration

- Two types of calibration to be considered
  - External
  - Internal

- External
  - Performed prior to analysis
  - Calibrant should have several reference ions, particularly in m/z range of interest, if possible be of same charge state
  - Should cover the required calibrated range, extrapolation generally leads to inferior results
  - Choice of calibrant is generally dictated by the class of samples to be analyzed

Typical calibrants

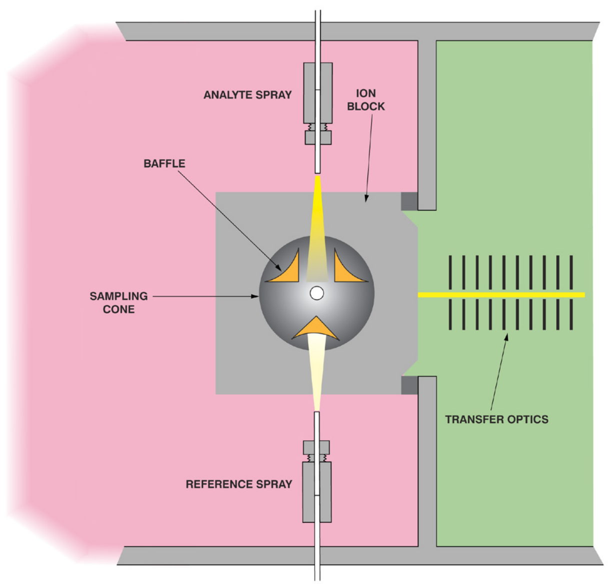
Chemical name	m/z range	Uses
Sodium iodide and Caesium iodide mixture	20 - 4000	ESI pos and neg
Peptide mixture	200 – 3000	ESI pos
Horse heart myoglobin	600 – 16000	ESI pos

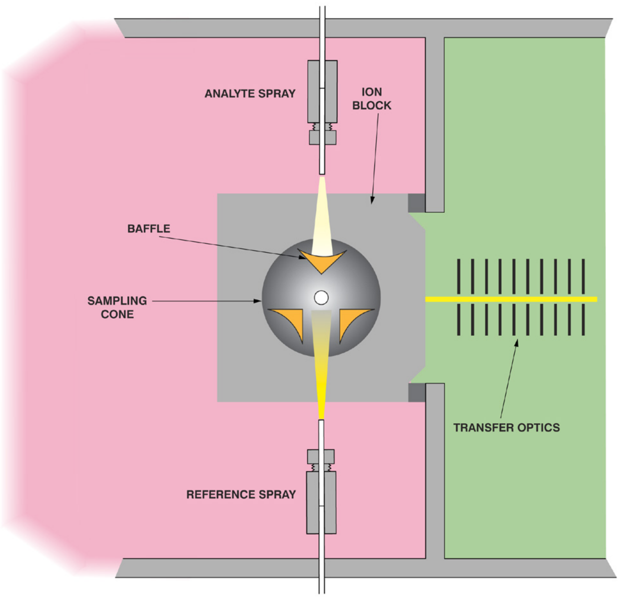
- Internal calibration

  - Introduced during sample analysis
  - For oa-Tof instruments a single point ‘Lockmass’ is sufficient
  - Main purpose is to correction for thermal changes to the environment of the instrument
  - It is very important that the intensity of the Lockmass ion is of a suitable intensity. This can be quite changeling, particularly when the sample introduction is dynamic, for example HPLC or UPLC-MS
  - Waters has a unique solution;

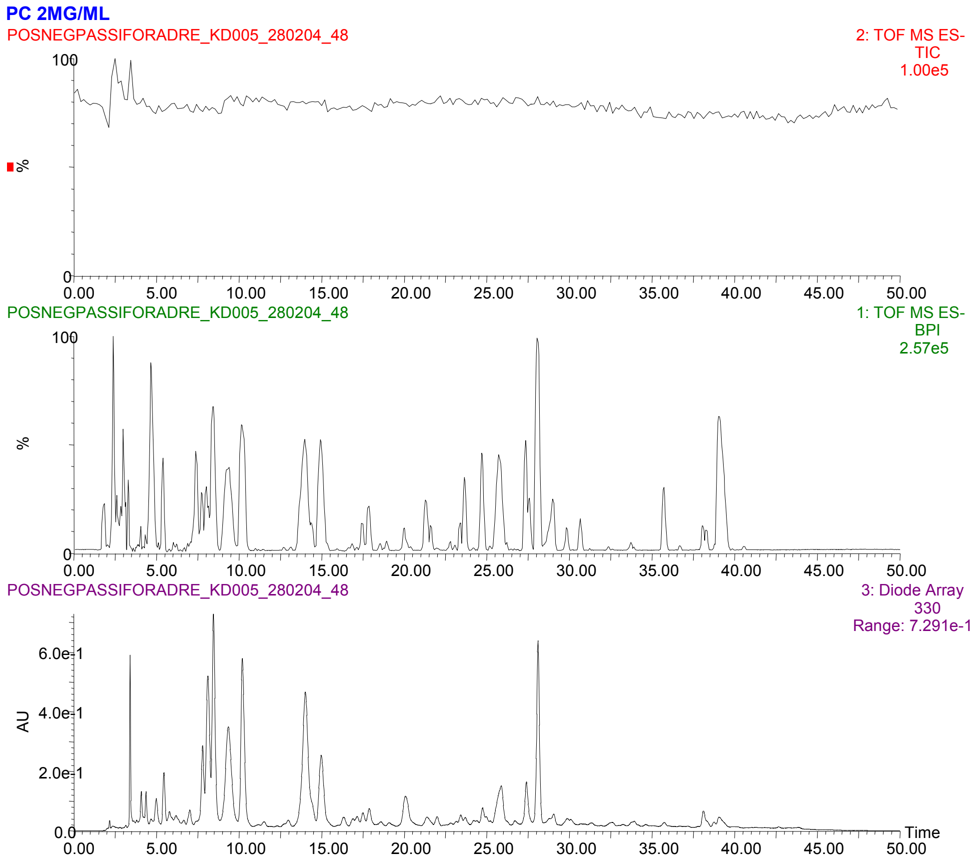
LockSpray – validated exact mass

- Introduces a lockmass, at user defined intervals, throughout the course of an experiment
- Available in nanoflow versions





Integrated LockSpray Ion Source



- Easy to use and setup for dedicated exact mass
- No Teeing in post-column removing plumbing issues
- No ion suppression of reference mass
- No interference of masses
- No change in ionisation of reference with LC gradient
- Every spectrum is validated with a reference, removing issues associated with lab conditions and power supply fluctuation

Data manipulation

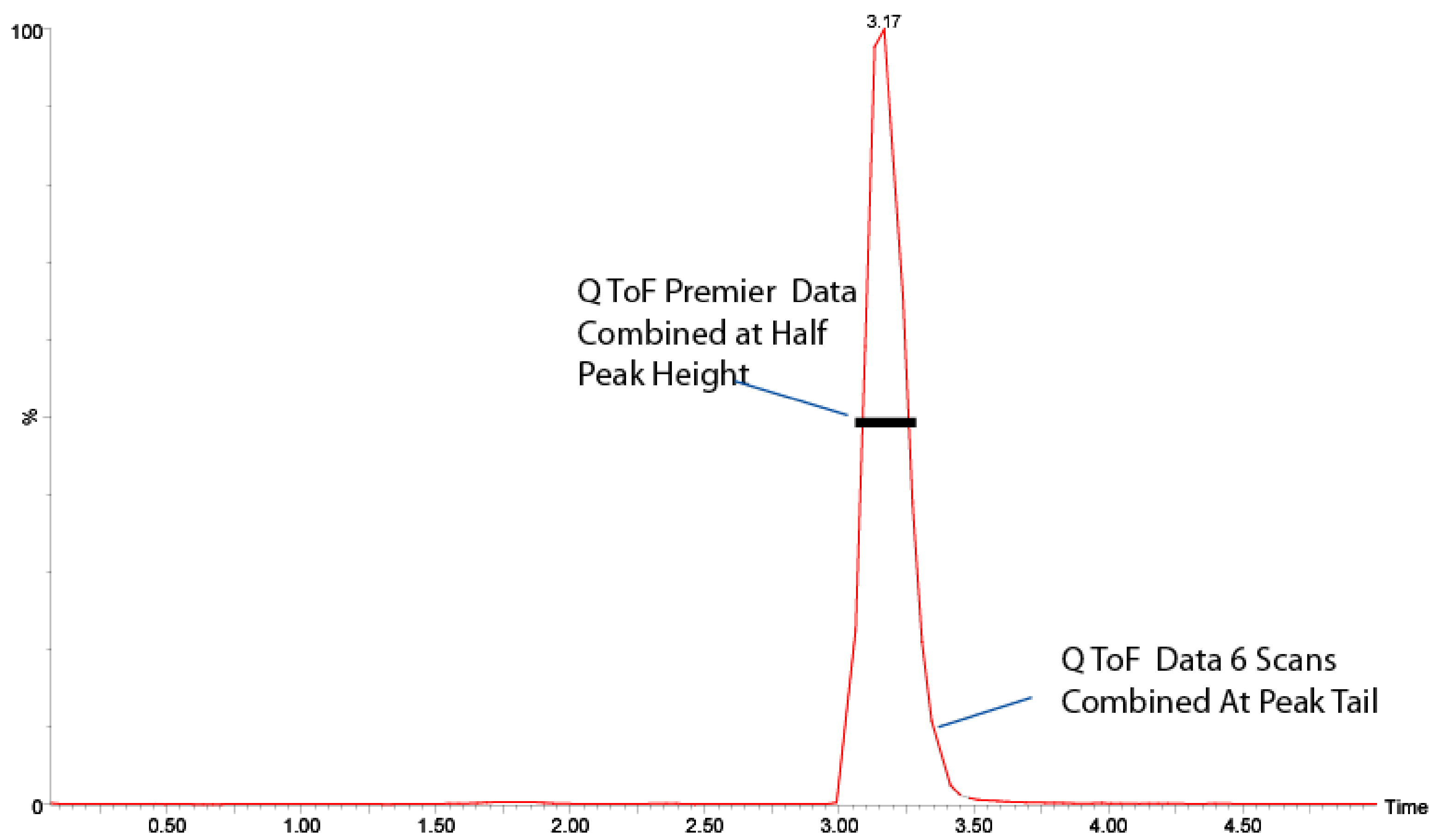
- Data manipulation

- Acquire data
- Baseline subtract to remove interferences near ion of interest
- Centroid ion of interest
- Utilise lockmass for correction of m/z range
- Produce list of potential elemental formulae
- Latest systems automate this procedure

Routine exact mass measurement

ACQUITY UPLC and Q-Tof Premier

Selection of Q ToF P pDRE Data



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MS Experimental

- Mass Spectrometer: Waters Micromass Q ToF Premier™
- Ionization Mode: ESI+ at 3 kV; Sample cone voltage: 30V
- Internal calibration: Sodium formate
- External calibration: Leucine enkephalin, $[M+H]^+$ 556.2771.

- Acquisition Parameters:
  - 50-900 m/z
  - 0.1 second/spectrum;0.05 second inter scan delay
  - Resolution = >8000 FWHM (V mode) and 15000 FWHM (W mode)

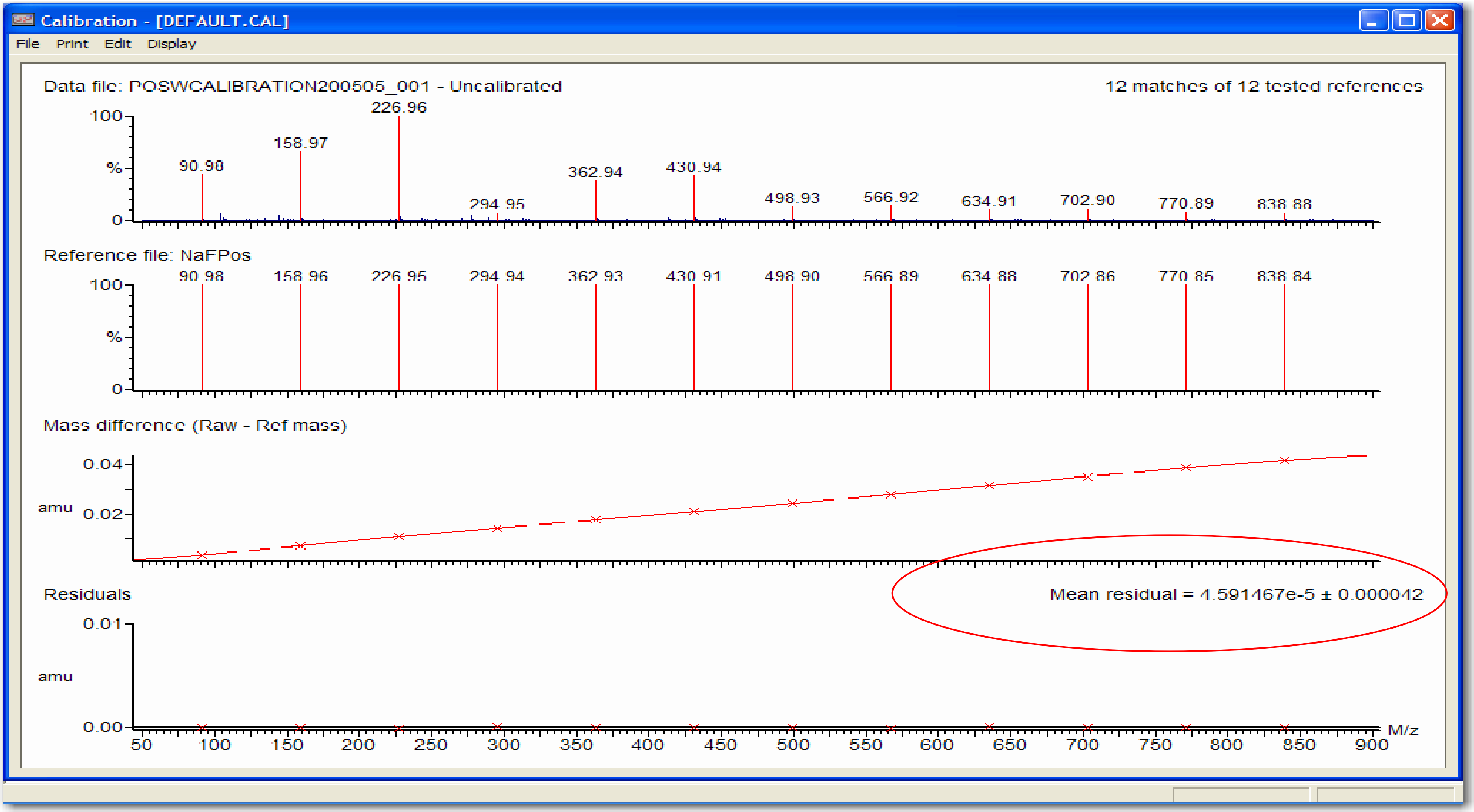
UPLC Experimental

- System: Waters ACQUITY UPLC™
- Column: Waters ACQUITY UPLC™ BEH C_{18} (100 mm x 2.1 mm, 1.7 μm particle size)
- Mobile phase: MeCN (B) : H_2O (0.1% HCOOH) (A)
- Isocratic: 50(A):50 (B)
- Column temperature: 45°C Flow: 0.4 mL/min
- Analyte: Temazepam

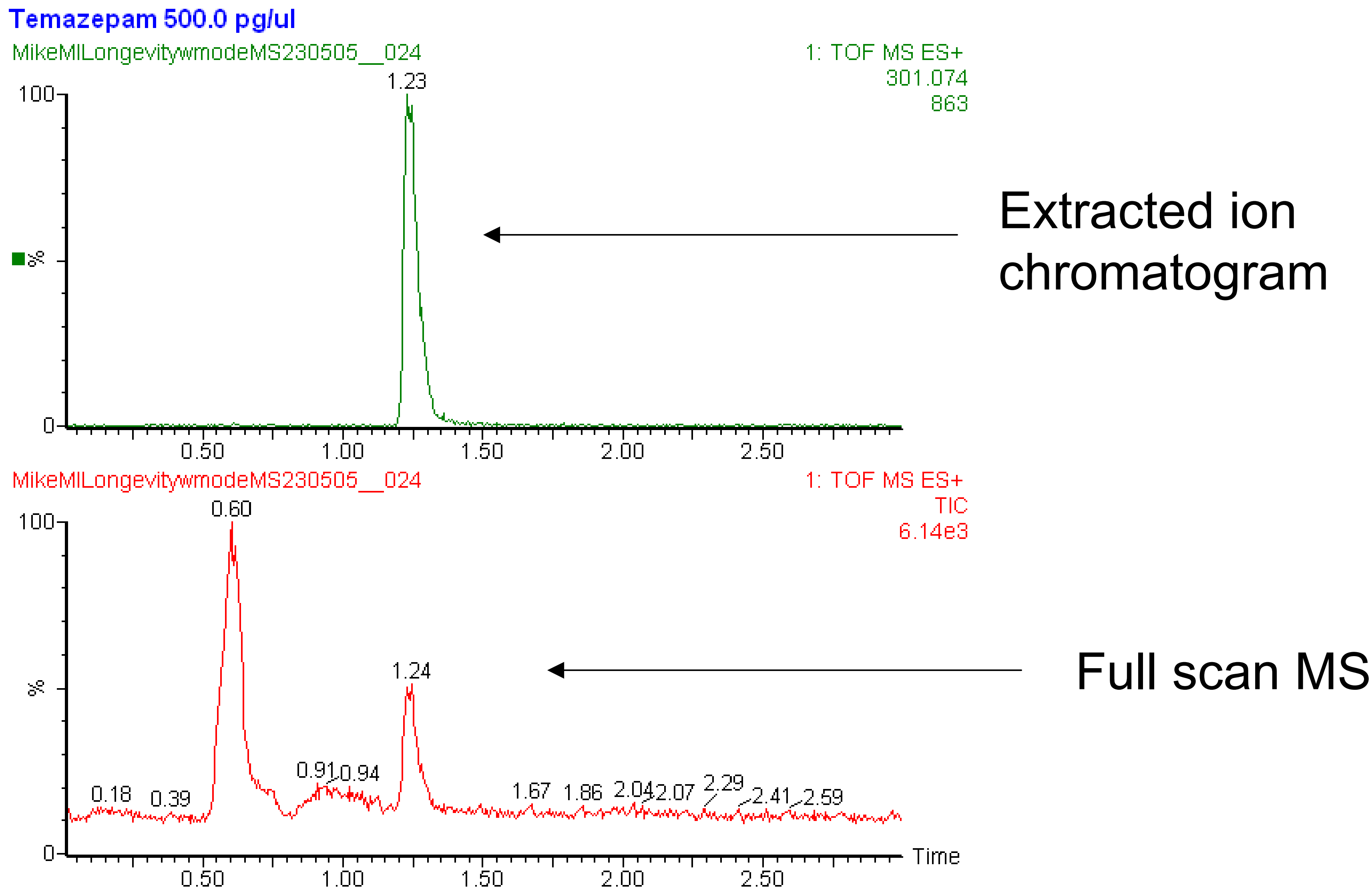
Longevity/Linearity Experiment

- Q TOF calibrated once in V mode and W mode
- 8 Day Experiment
- 500 samples analysed in V mode
- 630 samples analysed in W mode
- Longevity data processed using MetaboLynx
- Temazepam Linearity Series
  - 0.05 pg/µL to 5000 pg/µL (triplicate injection)

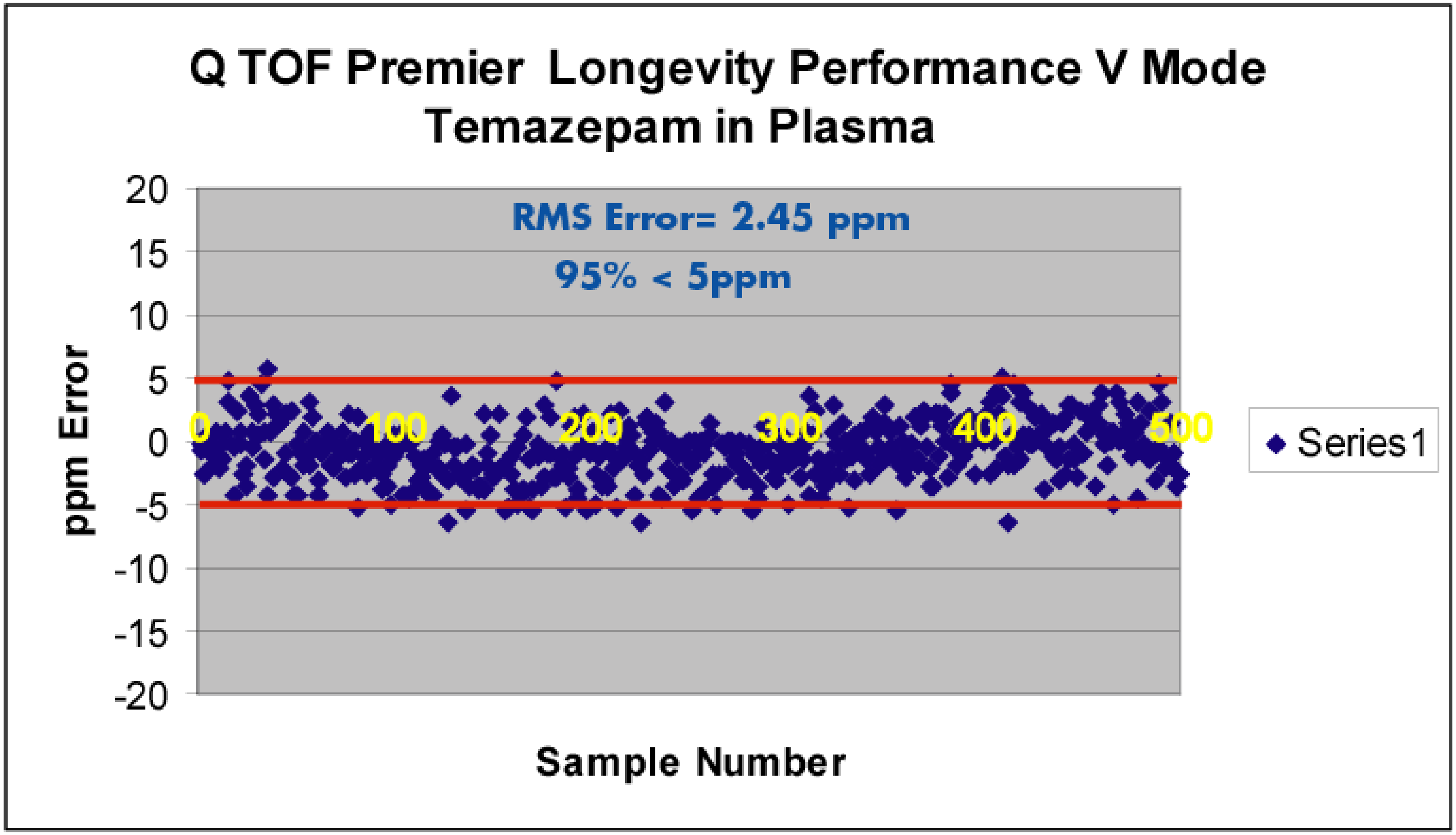
Typical 1 min Q-Tof P Calibration



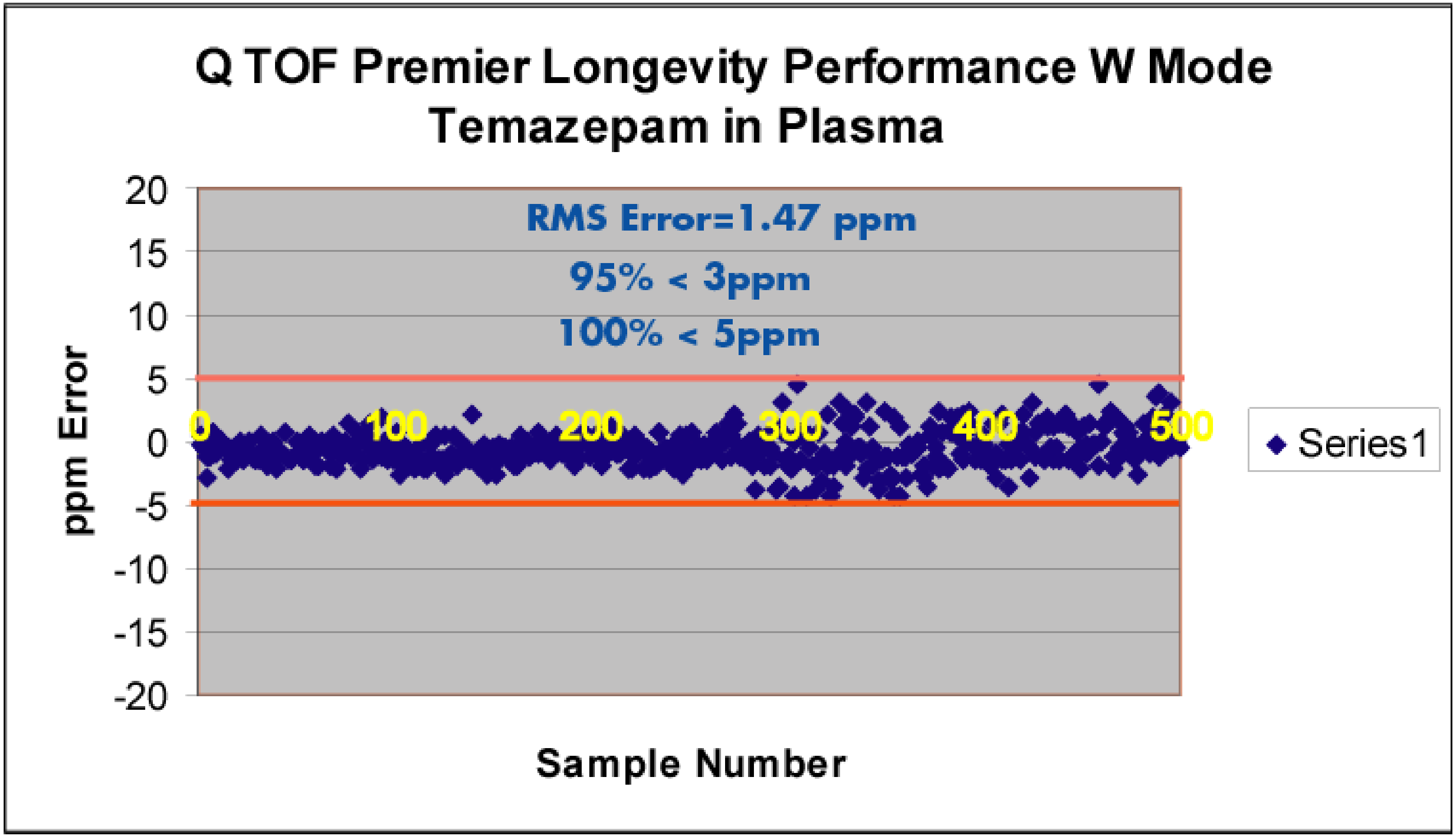
Typical plasma injection for Temazepam



V Mode Longevity 4 Days

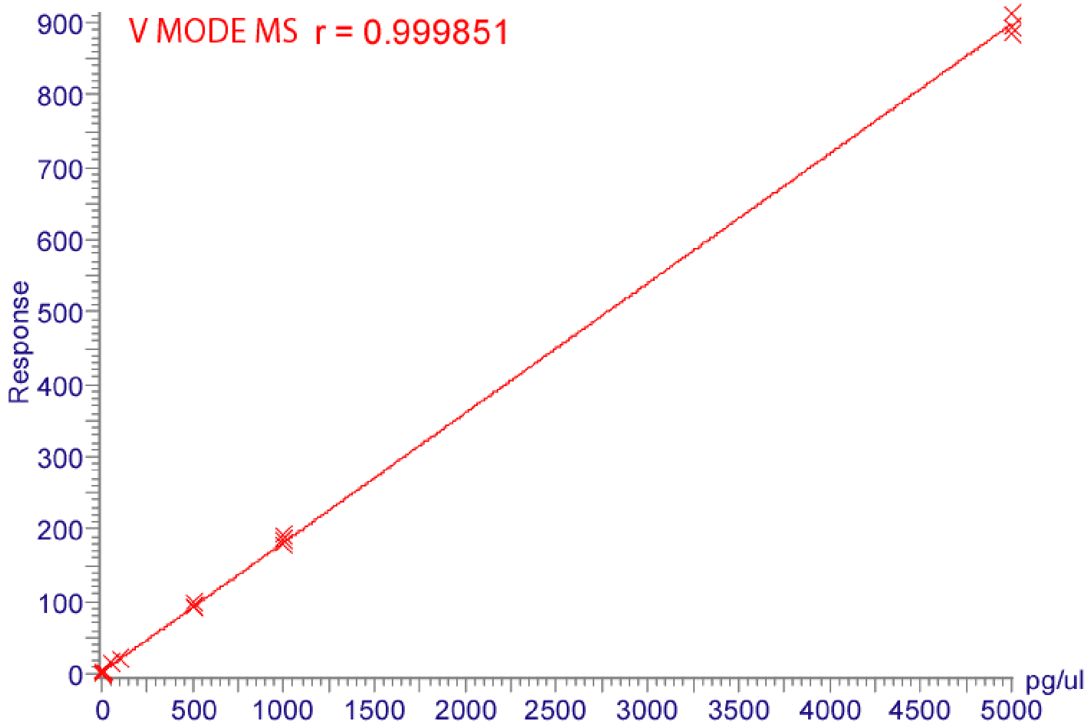


W Mode Longevity 4 Days



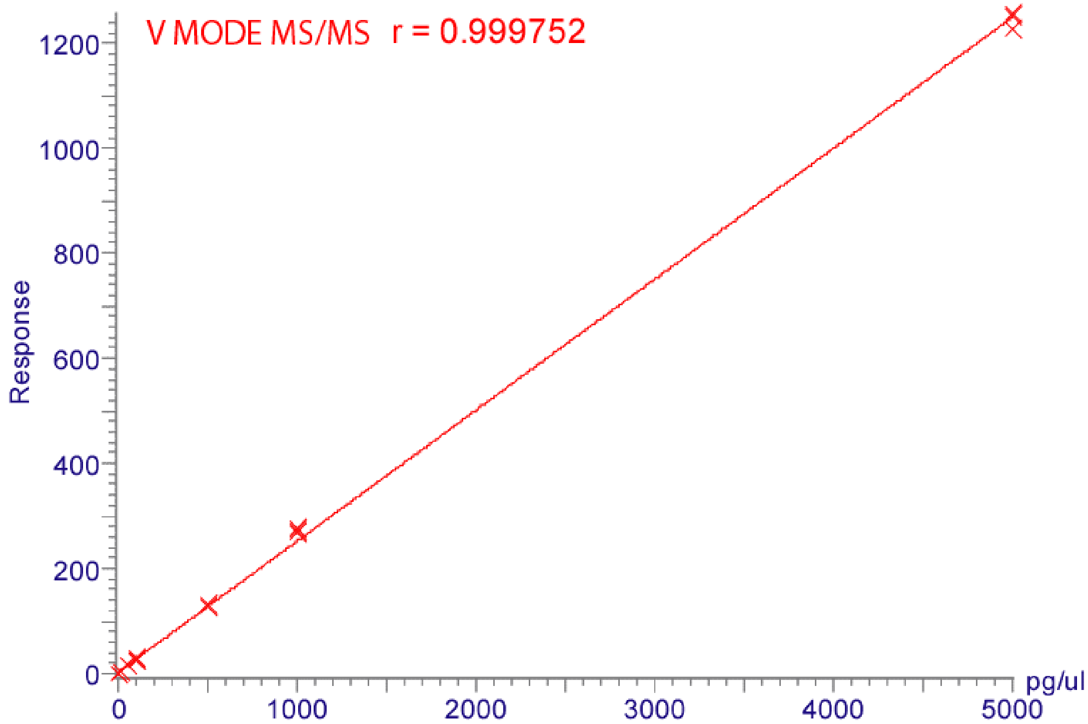
Temazepam MS Linearity

Compound name: temazepam
Correlation coefficient: r = 0.999851, r^2 = 0.999702
Calibration curve: 0.17925 * x + 2.18854
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None



Temazepam MSMS Linearity

Compound name: temazepam
Correlation coefficient: r = 0.999752, r^2 = 0.999505
Calibration curve: 0.249357 * x + 2.91198
Response type: External Std, Area
Curve type: Linear, Origin: Include, Weighting: Null, Axis trans: None



Case Study

- MS linearity over 4 orders of magnitude has been shown where r= 0.9998
- MS/MS linearity over 4 orders of magnitude has been shown where r= 0.9997
- Routine, automated exact mass measurement is achievable
  - No recalibration required
  - >1000 spiked plasma samples analysed
  - RMS error V mode = 2.45 ppm
  - RMS error W mode = 1.47 ppm

Using exact mass

- With an exact mass measurement, an elemental composition can be calculated
- The better the accuracy on the measurement (<3ppm), the fewer possible elemental compositions for a given set of elements
- However, the higher you go in mass, the number of possibilities go up (even with good accuracy)
- Results in a long list for users to look through

Monoisotopic Mass, Even Electron Ions
104900 formula(e) evaluated with 148 results within limits (all results (up to 1000) for each mass)
Elements Used:
C: 0-500 H: 0-500 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10 Br: 0-10

Mass	Calc. Mass	mDa	PPM	Formula
556.2771	556.2771	0	0	C24 H43 N9 S2 Cl
	556.2771	0	0	C39 H39 N Cl
	556.2771	0	0	C28 H38 N5 O7
	556.277	0.1	0.2	C16 H39 N15 O3 S Cl
	556.2772	-0.1	-0.2	C13 H42 N13 O7 S2
	556.2772	-0.1	-0.2	C17 H45 N11 O3 Cl3
	556.2773	-0.2	-0.4	C21 H46 N7 O4 S3
	556.2768	0.3	0.5	C21 H48 N3 O9 Cl2
	556.2774	-0.3	-0.5	C25 H49 N5 S Cl3
	556.2768	0.3	0.5	C13 H39 N19 O Br
	556.2768	0.3	0.5	C26 H52 N O4 Cl Br
	556.2775	-0.4	-0.7	C29 H50 N O S4
	556.2767	0.4	0.7	C28 H46 N O6 S2
	556.2776	-0.5	-0.9	C13 H34 N17 O8
	556.2776	-0.5	-0.9	C24 H35 N13 O Cl
	556.2765	0.6	1.1	C31 H43 N3 O2 S Cl
	556.2765	0.6	1.1	C20 H42 N7 O9 S
	556.2765	0.6	1.1	C16 H47 N11 O2 S3 Cl
	556.2778	-0.7	-1.3	C21 H38 N11 O5 S
	556.2763	0.8	1.4	C12 H38 N13 O12
	556.2763	0.8	1.4	C23 H39 N9 O5 Cl
	556.2763	0.8	1.4	C28 H43 N7 Br
	556.278	-0.9	-1.6	C14 H46 N13 O2 S4
	556.278	-0.9	-1.6	C29 H42 N5 O2 S2
	556.2761	1	1.8	C11 H40 N19 O S Cl2
	556.2781	-1	-1.8	C22 H44 N7 O5 Cl2
	556.2782	-1.1	-2	C27 H48 N5 Cl Br
	556.2783	-1.2	-2.2	C30 H48 N O2 S Cl2
	556.2759	1.2	2.2	C27 H50 N3 Cl4
	556.2783	-1.2	-2.2	C19 H47 N5 O9 S Cl
	556.2758	1.3	2.3	C25 H30 N15 O
	556.2758	1.3	2.3	C23 H47 N5 O4 S2 Cl
	556.2784	-1.3	-2.3	C24 H51 N3 O4 S Br
	556.2758	1.3	2.3	C27 H42 N O11
	556.2785	-1.4	-2.5	C14 H38 N17 O3 S2
	556.2757	1.4	2.5	C20 H47 N9 O2 S Br
	556.2785	-1.4	-2.5	C29 H34 N9 O3
	556.2756	1.5	2.7	C26 H44 N7 S Cl2
	556.2756	1.5	2.7	C15 H43 N11 O7 S Cl
	556.2786	-1.5	-2.7	C37 H38 N3 S
	556.2755	1.6	2.9	C12 H43 N15 O5 Br

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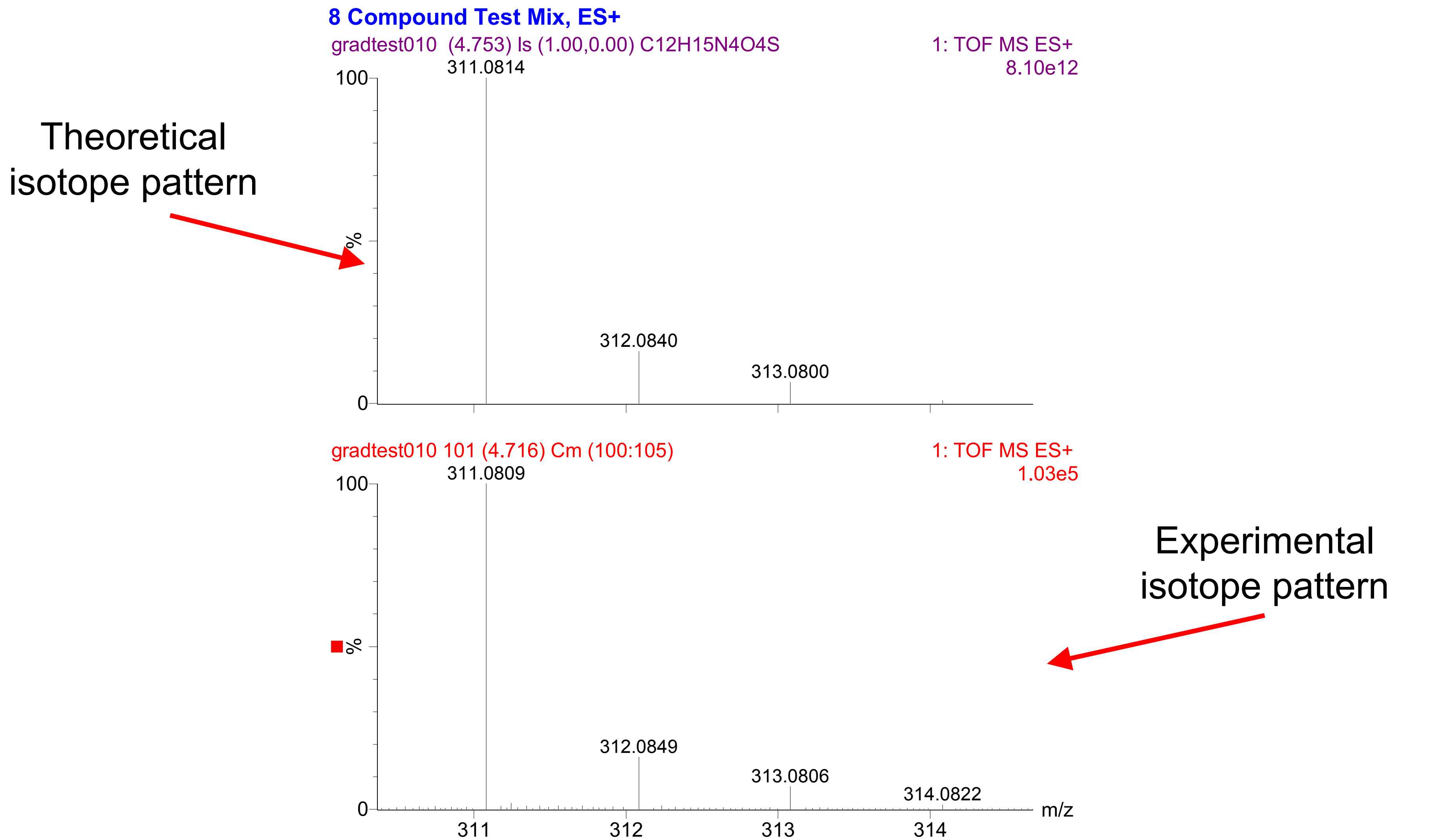
I-FIT elemental composition calculator

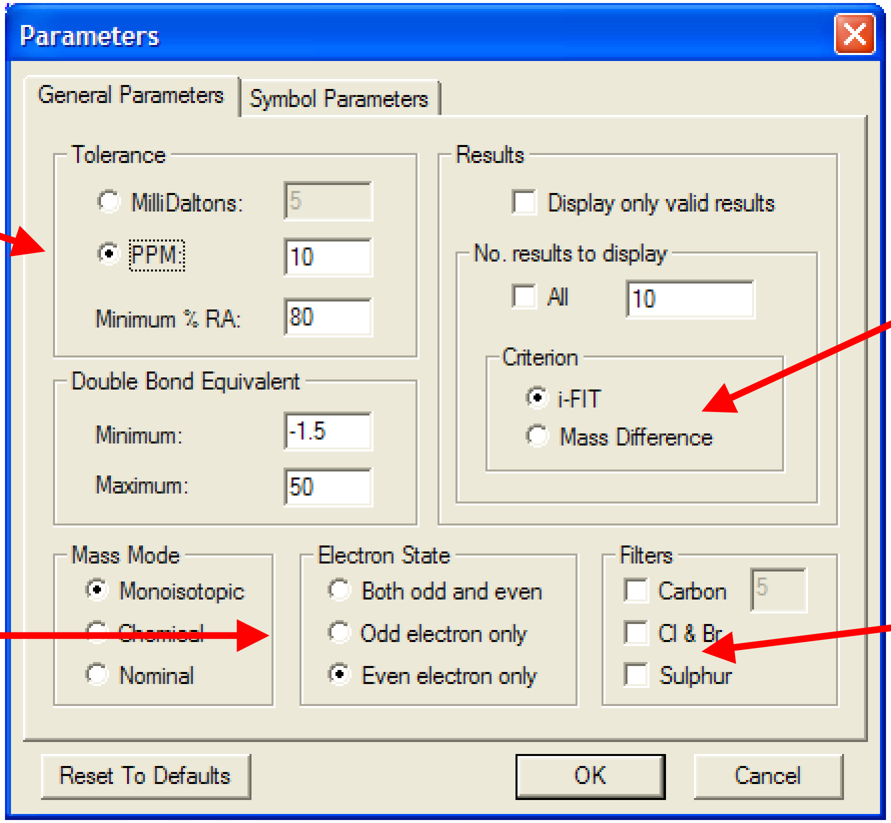
- For every elemental composition determined an i-FIT™ value is calculated
  - Calculation uses both **isotope pattern** and **mass**
  - Generally, lower the i-FIT™, better the match
- Valency state is set automatically for ESI & APcI
  - ESI & APcI generates even electron state for molecular ion (N-Rule)
- Applies filtering to aid simplification of results list
  - Carbon, Cl & Br
  - Simplifies the results list by aiding the removal of incorrect answers

Correct Isotope Patterns



Elecomp Parameters

Set a mass measure tolerance

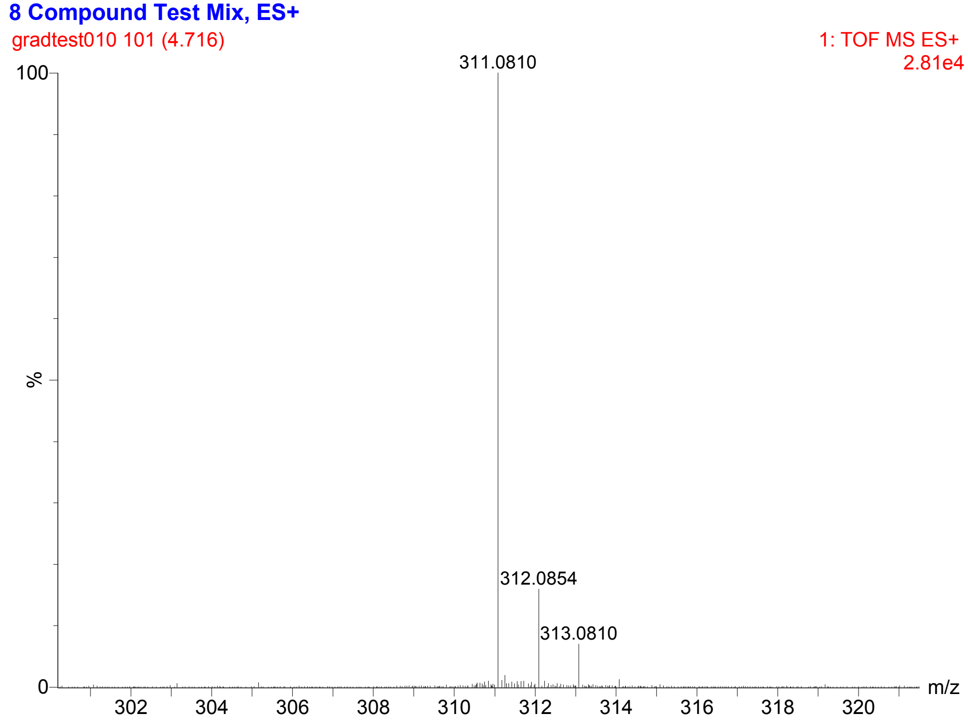


Rank results on Mass Difference or i-FIT™

Automatically assign the valency state based on data type

Apply filtering technology to aid simplification of the results list

Example



- An exact mass spectrum for a particular ion is generated
- The monoisotopic peak is selected for elemental composition calculation (note, the correct mass is m/z 311.0814)

No Filters

Elemental Composition

File Edit View Process Help

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: None

Monoisotopic Mass, Even Electron Ions

7665 formula(e) evaluated with 40 results within limits (up to 10 best isotopic matches for each mass)

Elements Used:

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	S	Cl	Br
311.0810	311.0814	-0.4	-1.3	7.5	C12 H15 N4 O4 S	6.0	12	15	4	4	1		
	311.0801	0.9	2.9	2.5	C11 H19 O8 S	42.9	11	19		8	1		
	311.0827	-1.7	-5.5	12.5	C13 H11 N8 S	68.7	13	11	8		1		
	311.0787	2.3	7.4	8.5	C8 H11 N10 O2 S	77.0	8	11	10	2	1		
	311.0821	-1.1	-3.5	3.5	C5 H15 N10 O2 S2	371.2	5	15	10	2	2		
	311.0780	3.0	9.6	12.5	C15 H11 N4 O4	483.8	15	11	4	4			
	311.0794	1.6	5.1	4.5	C H11 N16 S2	567.4	1	11	16		2		
	311.0839	-2.9	-9.3	3.5	C8 H15 N4 O9	625.1	8	15	4	9			
	311.0794	1.6	5.1	17.5	C16 H7 N8	675.9	16	7	8				

For Help, press F1

- Using No Filters, 40 results were returned within a 10ppm tolerance
- Based on elements $C_{500}H_{500}N_{20}O_{20}S_{10}Cl_{10}Br_{10}$
- No. 1 is the correct elemental composition ($C_{12}H_{15}N_4O_4S$, m/z 311.0814)

Application of Filters

Elemental Composition

File Edit View Process Help

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: Carbon;

Monoisotopic Mass, Even Electron Ions

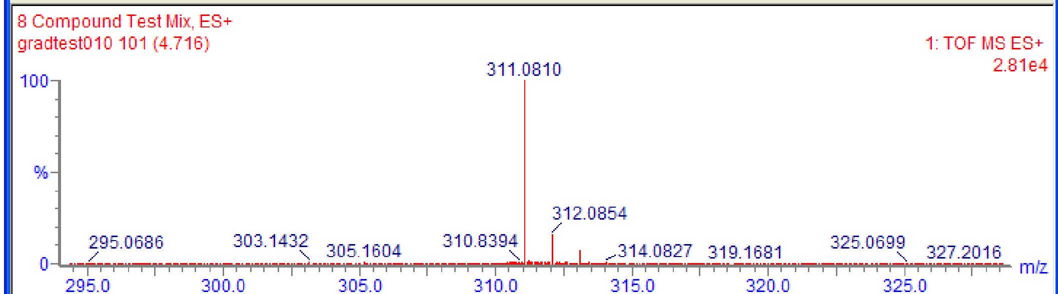
7665 formula(e) evaluated with 12 results within limits (up to 10 best isotopic matches for each mass)

Elements Used:

C: 0-500 H: 0-1000 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10

Br: 0-10

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	S	Cl	Br
311.0810	311.0814	-0.4	-1.3	7.5	C12 H15 N4 O4 S	6.0	12	15	4	4	1		
	311.0801	0.9	2.9	2.5	C11 H19 O8 S	42.9	11	19		8	1		
	311.0827	-1.7	-5.5	12.5	C13 H11 N8 S	68.7	13	11	8		1		
	311.0780	3.0	9.6	12.5	C15 H11 N4 O4	483.8	15	11	4	4			
	311.0794	1.6	5.1	17.5	C16 H7 N8	675.9	16	7	8				
	311.0809	0.1	0.3	1.5	C12 H23 O3 S3	1041.0	12	23		3	3		
	311.0812	-0.2	-0.6	12.5	C15 H12 N6 Cl	5803.5	15	12	6			1	
	311.0799	1.1	3.5	7.5	C14 H16 N2 O4 Cl	6039.8	14	16	2	4		1	
	311.0832	-2.2	-7.1	2.5	C11 H20 N2 O4 S Cl	7136.1	11	20	2	4	1	1	



For Help, press F1

- Application of the Carbon Filter reduces list to 12 hits
- Only considers 12C +/- 4

Application of Filters

Elemental Composition

File Edit View Process Help

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: Carbon; Cl & Br;

Monoisotopic Mass, Even Electron Ions

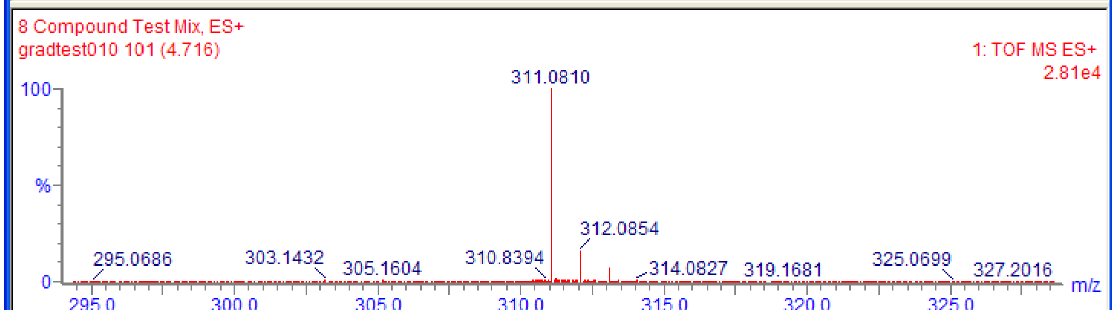
7665 formula(e) evaluated with 6 results within limits (up to 10 best isotopic matches for each mass)

Elements Used:

C: 0-500 H: 0-1000 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10

Br: 0-10

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	S	Cl	Br
311.0810	311.0814	-0.4	-1.3	7.5	C12 H15 N4 O4 S	6.0	12	15	4	4	1		
	311.0801	0.9	2.9	2.5	C11 H19 O8 S	42.9	11	19		8	1		
	311.0827	-1.7	-5.5	12.5	C13 H11 N8 S	68.7	13	11	8		1		
	311.0780	3.0	9.6	12.5	C15 H11 N4 O4	483.8	15	11	4	4			
	311.0794	1.6	5.1	17.5	C16 H7 N8	675.9	16	7	8				
	311.0809	0.1	0.3	1.5	C12 H23 O3 S3	1041.0	12	23		3	3		



For Help, press F1

- Application of the Carbon and Cl & Br Filter reduces list to 6 hits
- In this example removes any entrants that contain Cl and Br

Application of Filters

Elemental Composition

File Edit View Process Help

Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: Carbon; Cl & Br; Sulphur;

Monoisotopic Mass, Even Electron Ions

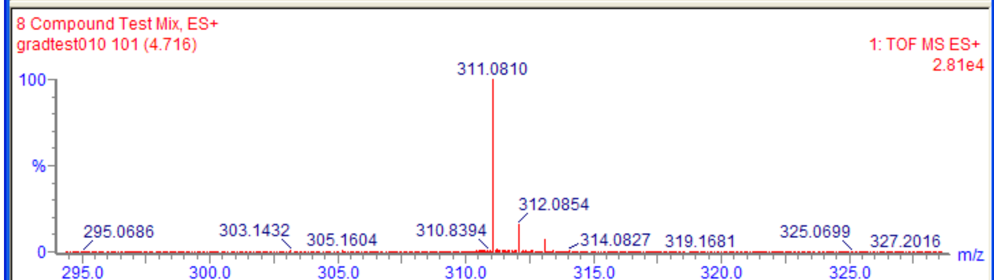
7665 formula(e) evaluated with 3 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-500 H: 0-1000 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10

Br: 0-10

Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	S	Cl	Br
311.0810	311.0814	-0.4	-1.3	7.5	C12 H15 N4 O4 S	6.0	12	15	4	4	1		
	311.0801	0.9	2.9	2.5	C11 H19 O8 S	42.9	11	19		8	1		
	311.0827	-1.7	-5.5	12.5	C13 H11 N8 S	68.7	13	11	8		1		



For Help, press F1

- Application of the Carbon, Cl & Br filters reduces the list to only 3 possibilities !
- No.1 is the correct result
- i-FIT for No.1 is significantly different from other results

Application of Filters

Elemental Composition

File Edit View Process Help

Single Mass Analysis

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0

Selected filters: Carbon; Cl & Br; Sulphur;

Monoisotopic Mass, Even Electron Ions

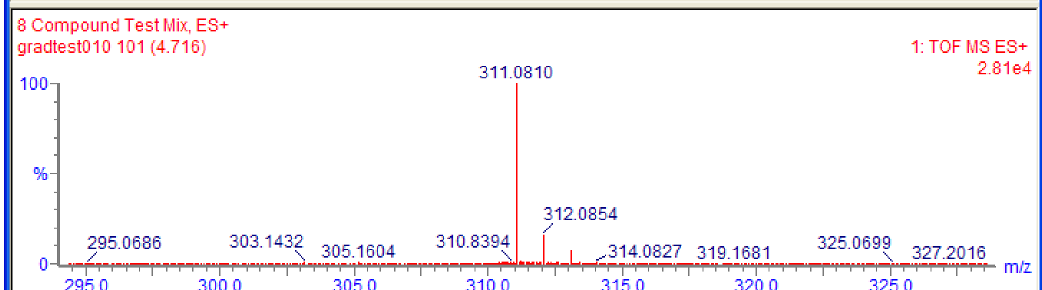
7665 formula(e) evaluated with 2 results within limits (all results (up to 1000) for each mass)

Elements Used:

C: 0-500 H: 0-1000 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10

Br: 0-10

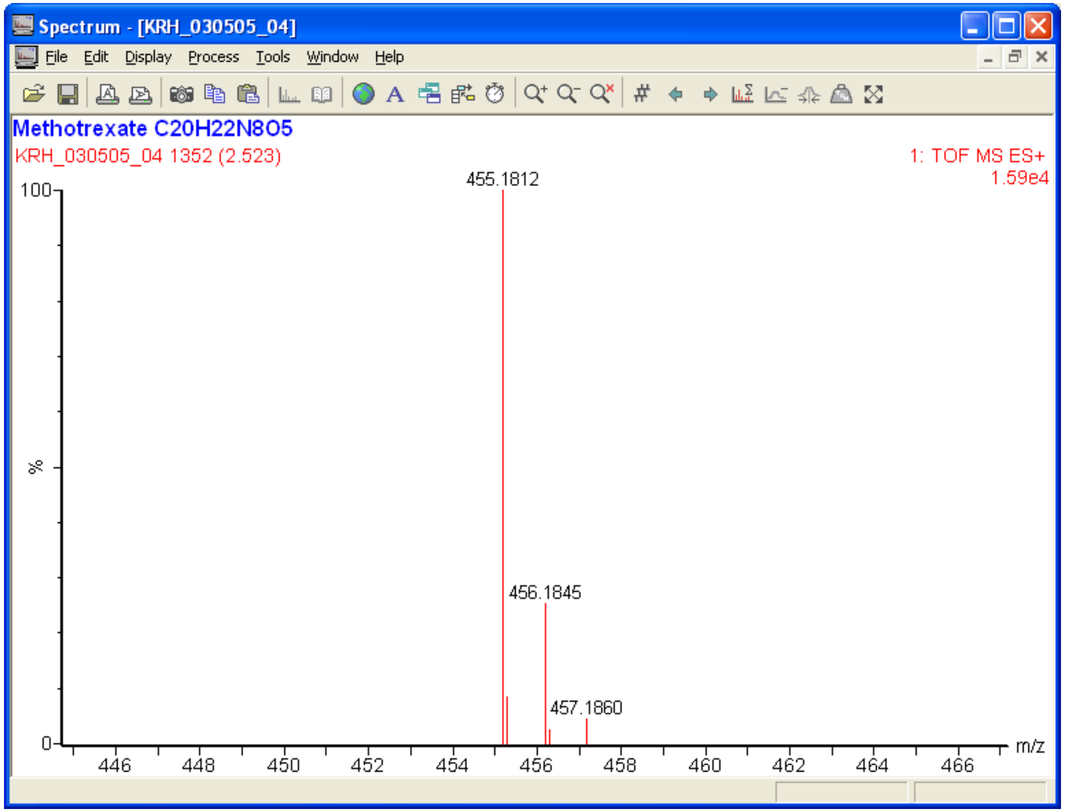
Mass	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	S	Cl	Br
311.0810	311.0814	-0.4	-1.3	7.5	C12 H15 N4 O4 S	6.0	12	15	4	4	1		
	311.0801	0.9	2.9	2.5	C11 H19 O8 S	42.9	11	19		8	1		



For Help, press F1

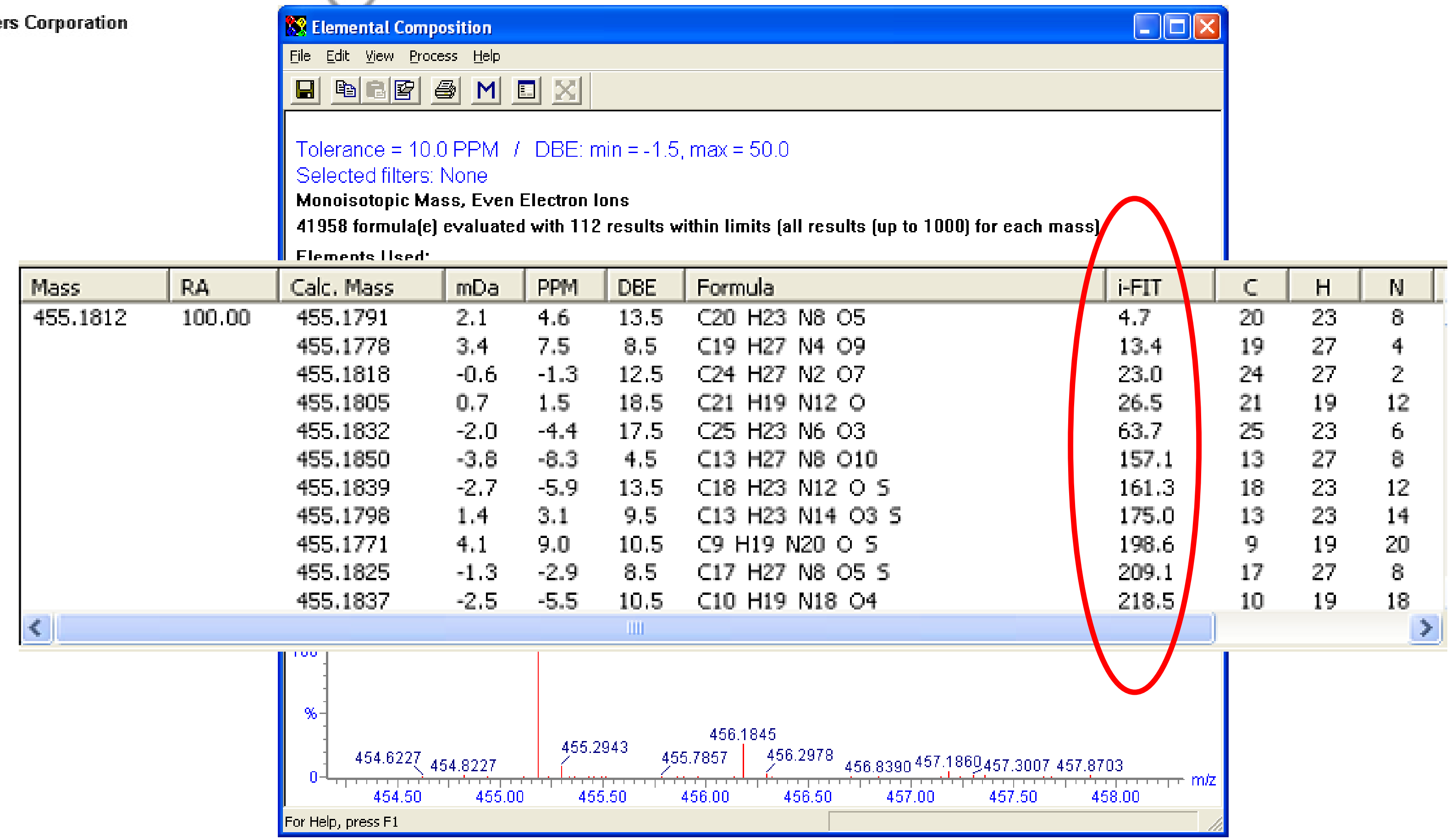
- Set the exact mass tolerance to 5ppm reduces the results list to only 2 possibilities !

Higher m/z example



- The monoisotopic peak is selected for elemental composition calculation (note, the correct mass is m/z 455.1791, $C_{20}H_{23}N_8O_5$)
- The error on the mass measurement is 4.6ppm

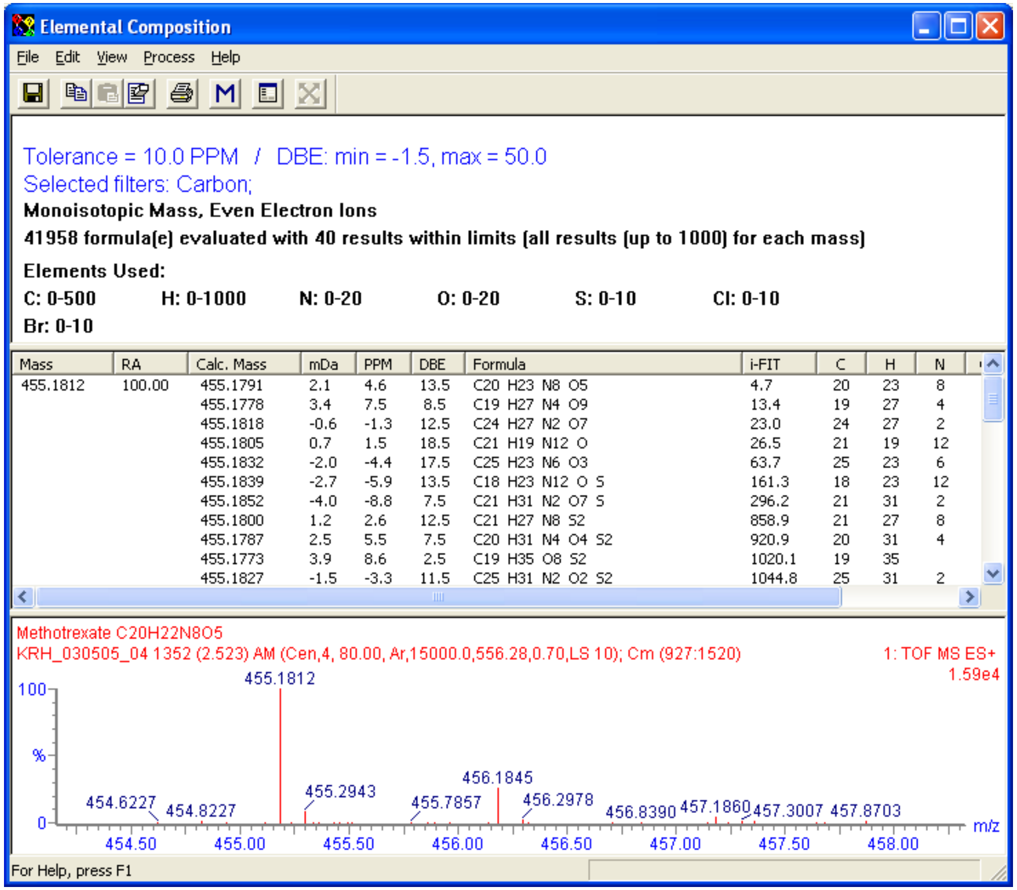
No Filters



Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N
455.1812	100.00	455.1791	2.1	4.6	13.5	C20 H23 N8 O5	4.7	20	23	8
		455.1778	3.4	7.5	8.5	C19 H27 N4 O9	13.4	19	27	4
		455.1818	-0.6	-1.3	12.5	C24 H27 N2 O7	23.0	24	27	2
		455.1805	0.7	1.5	18.5	C21 H19 N12 O	26.5	21	19	12
		455.1832	-2.0	-4.4	17.5	C25 H23 N6 O3	63.7	25	23	6
		455.1850	-3.8	-8.3	4.5	C13 H27 N8 O10	157.1	13	27	8
		455.1839	-2.7	-5.9	13.5	C18 H23 N12 O S	161.3	18	23	12
		455.1798	1.4	3.1	9.5	C13 H23 N14 O3 S	175.0	13	23	14
		455.1771	4.1	9.0	10.5	C9 H19 N20 O S	198.6	9	19	20
		455.1825	-1.3	-2.9	8.5	C17 H27 N8 O5 S	209.1	17	27	8
		455.1837	-2.5	-5.5	10.5	C10 H19 N18 O4	218.5	10	19	18

- 112 results within 10ppm tolerance (using $C_{500}H_{1000}N_{20}O_{20}S_{10}Cl_{10}Br_{10}$)
- Correct answer top with i-FIT of 4.7

Application of Filters



Elemental Composition

File Edit View Process Help

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: Carbon;
Monoisotopic Mass, Even Electron Ions
41958 formula(e) evaluated with 40 results within limits (all results (up to 1000) for each mass)

Elements Used:
C: 0-500 H: 0-1000 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10
Br: 0-10

Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N
455.1812	100.00	455.1791	2.1	4.6	13.5	C20 H23 N8 O5	4.7	20	23	8
		455.1778	3.4	7.5	8.5	C19 H27 N4 O9	13.4	19	27	4
		455.1818	-0.6	-1.3	12.5	C24 H27 N2 O7	23.0	24	27	2
		455.1805	0.7	1.5	18.5	C21 H19 N12 O	26.5	21	19	12
		455.1832	-2.0	-4.4	17.5	C25 H23 N6 O3	63.7	25	23	6
		455.1839	-2.7	-5.9	13.5	C18 H23 N12 O S	161.3	18	23	12
		455.1852	-4.0	-8.8	7.5	C21 H31 N2 O7 S	296.2	21	31	2
		455.1800	1.2	2.6	12.5	C21 H27 N8 S2	858.9	21	27	8
		455.1787	2.5	5.5	7.5	C20 H31 N4 O4 S2	920.9	20	31	4
		455.1773	3.9	8.6	2.5	C19 H35 O8 S2	1020.1	19	35	
		455.1827	-1.5	-3.3	11.5	C25 H31 N2 O2 S2	1044.8	25	31	2

- Application of Carbon filter reduces results list to 40

Application of Filters

Elemental Composition

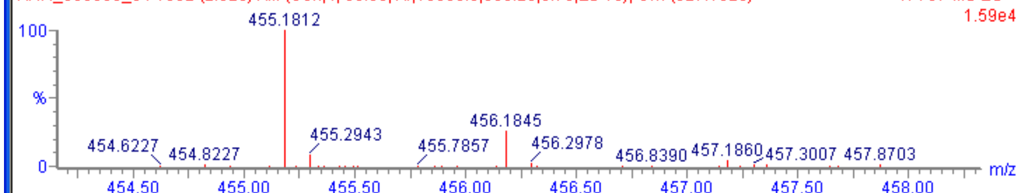
File Edit View Process Help

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: Carbon; Cl & Br;
Monoisotopic Mass, Even Electron Ions
41958 formula(e) evaluated with 13 results within limits (all results (up to 1000) for each mass)

Elements Used:
C: 0-500 H: 0-1000 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10
Br: 0-10

Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O
455.1812	100.00	455.1791	2.1	4.6	13.5	C20 H23 N8 O5	4.7	20	23	8	5
		455.1778	3.4	7.5	8.5	C19 H27 N4 O9	13.4	19	27	4	9
		455.1818	-0.6	-1.3	12.5	C24 H27 N2 O7	23.0	24	27	2	7
		455.1805	0.7	1.5	18.5	C21 H19 N12 O	26.5	21	19	12	1
		455.1832	-2.0	-4.4	17.5	C25 H23 N6 O3	63.7	25	23	6	3
		455.1839	-2.7	-5.9	13.5	C18 H23 N12 O S	161.3	18	23	12	1
		455.1852	-4.0	-8.8	7.5	C21 H31 N2 O7 S	296.2	21	31	2	7
		455.1800	1.2	2.6	12.5	C21 H27 N8 S2	858.9	21	27	8	
		455.1787	2.5	5.5	7.5	C20 H31 N4 O4 S2	920.9	20	31	4	4
		455.1773	3.9	8.6	2.5	C19 H35 O8 S2	1020.1	19	35		8
		455.1827	-1.5	-3.3	11.5	C25 H31 N2 O2 S2	1044.8	25	31	2	2

Methotrexate C20H22N8O5
KRH_030505_04 1352 (2.523) AM (Cen,4, 80.00, Ar,15000.0,556.28,0.70,LS 10); Cm (927:1520)
1: TOF MS ES+
1.59e4



For Help, press F1

- Application of Carbon and Cl & Br filter reduces results list to 13

Application of Filters

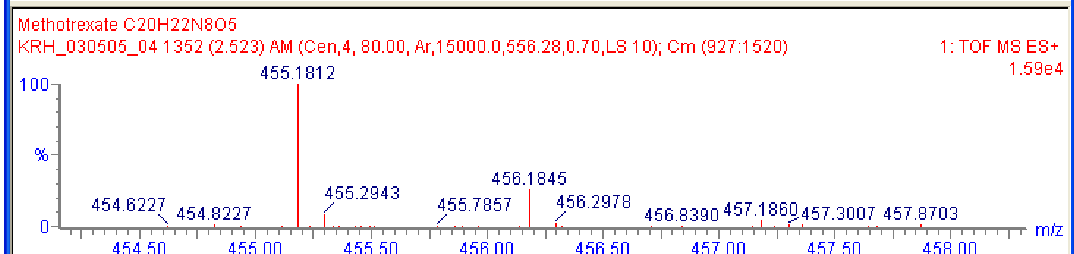
Elemental Composition

File Edit View Process Help

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0
Selected filters: Carbon; Cl & Br; Sulphur;
Monoisotopic Mass, Even Electron Ions
41958 formula(e) evaluated with 5 results within limits (all results (up to 1000) for each mass)

Elements Used:
C: 0-500 H: 0-1000 N: 0-20 O: 0-20 S: 0-10 Cl: 0-10
Br: 0-10

Mass	RA	Calc. Mass	mDa	PPM	DBE	Formula	i-FIT	C	H	N	O	S	Cl	Br
455.1812	100.00	455.1791	2.1	4.6	13.5	C20 H23 N8 O5	4.7	20	23	8	5			
		455.1778	3.4	7.5	8.5	C19 H27 N4 O9	13.4	19	27	4	9			
		455.1818	-0.6	-1.3	12.5	C24 H27 N2 O7	23.0	24	27	2	7			
		455.1805	0.7	1.5	18.5	C21 H19 N12 O	26.5	21	19	12	1			
		455.1832	-2.0	-4.4	17.5	C25 H23 N6 O3	63.7	25	23	6	3			

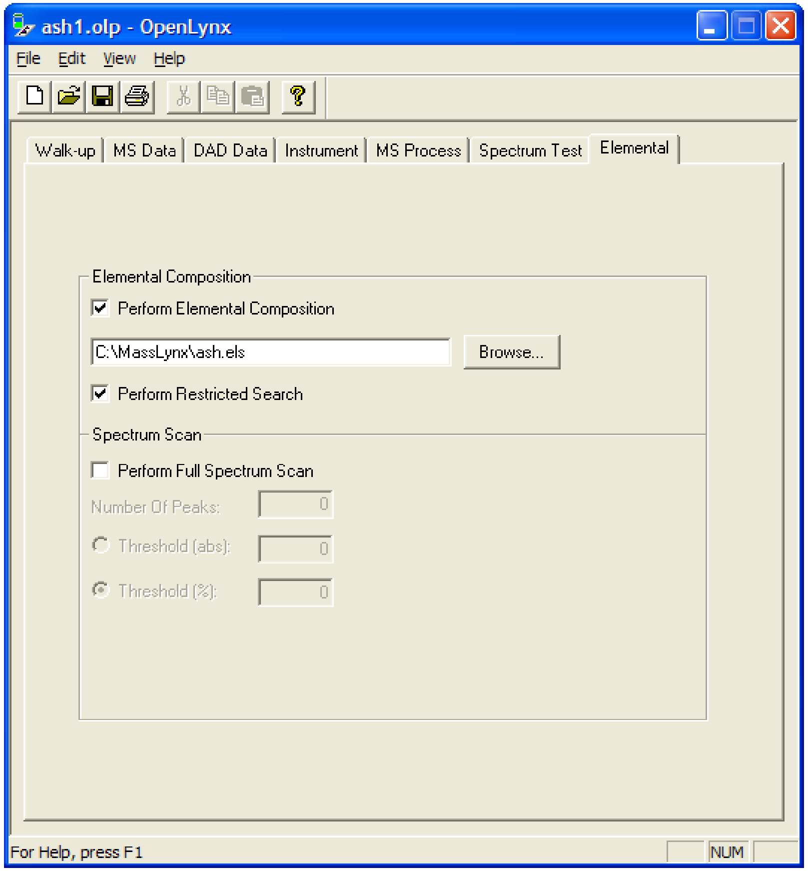


For Help, press F1

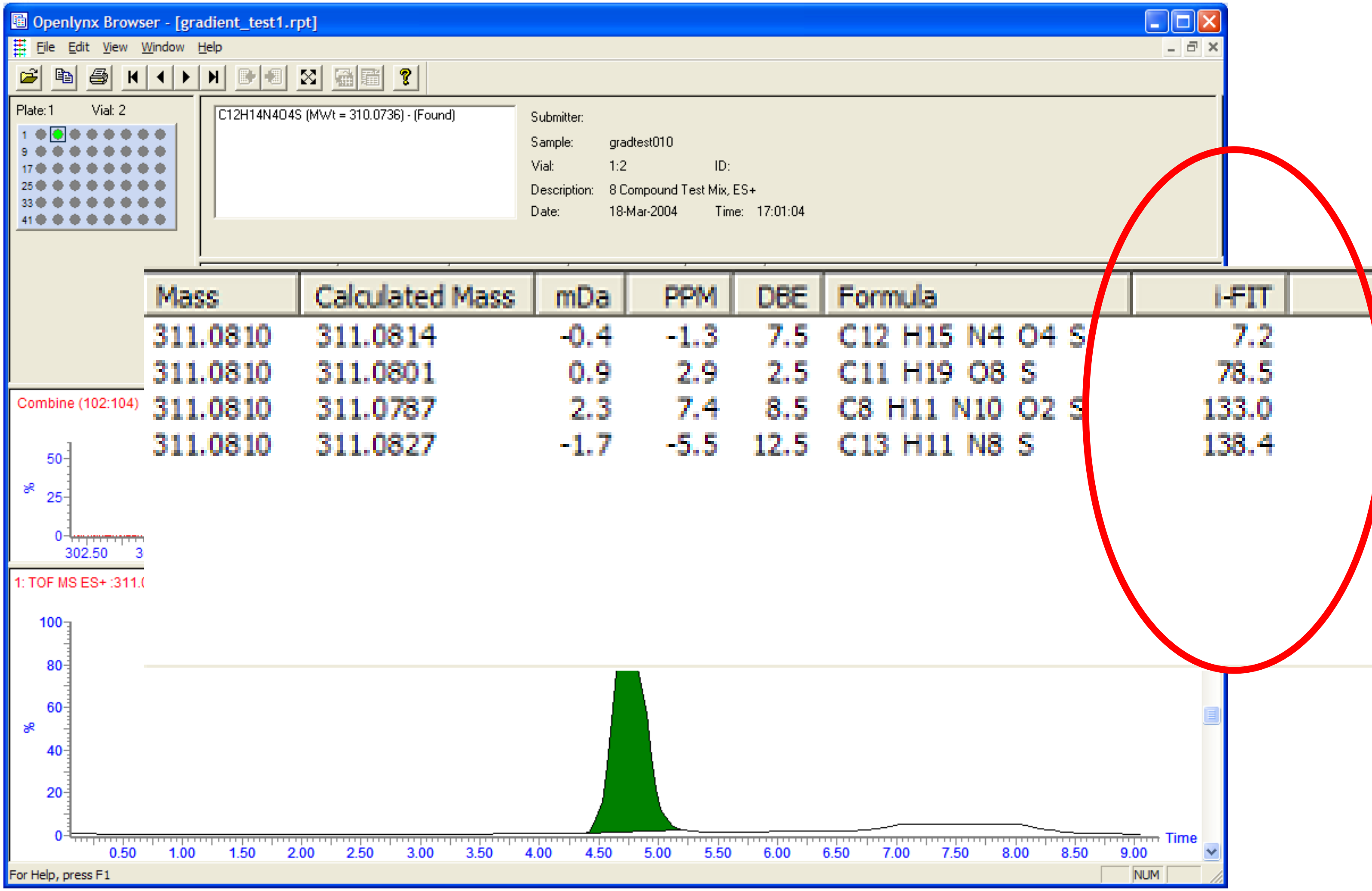
- Application of Carbon, Cl & Br and sulphur filter reduces results list to only 6 results from 112

Can be used in an automated way

- The i-FIT calculation and filtering technology has been applied to OpenLynx Application Manager
- Works in Open Access or batch processing mode
- The functionality is applied to aid simplification of the elemental composition results list



OpenLynx Browser



Mass	Calculated Mass	mDa	PPM	DBE	Formula	i-FIT
311.0810	311.0814	-0.4	-1.3	7.5	C12 H15 N4 O4 S	7.2
311.0810	311.0801	0.9	2.9	2.5	C11 H19 O8 S	78.5
311.0810	311.0787	2.3	7.4	8.5	C8 H11 N10 O2 S	133.0
311.0810	311.0827	-1.7	-5.5	12.5	C13 H11 N8 S	138.4

Conclusions

- Benefits of mass measurement to 4 decimal places
  - High confidence in confirming expected compounds
  - Compound identification
  - Patent submission and publication

- oa-Tof instruments are ideally suited to routine exact mass measurement and semi-quantitative applications

- i-FIT software can provide confident element compositions determinations

Further reading

"Methodology for accurate mass measurement of small molecules"

Available from the British mass spectrometry society

www.bmss.org.uk/