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IMPROVEMENTS IN MASS MEASUREMENT ACCURACY AND REPORTING OVER INCREASED DYNAMIC RANGE USING ORTHOGONAL TOF (TIME OF FLIGHT) MASS SPECTROMETERS

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OVERVIEW

Purpose

- Define sources of error in exact mass measurement.
- Predict probable error range for individual measurements.
- Investigate automated assignment of error.

Method

- Compare theoretically calculated error range to experimental data taken under varying conditions.
- Full spectra exact mass using LC/MS TOF with enhanced resolution and transmission.

Results

- Prediction of error from ions statistics and calibration uncertainty.
- Demonstration of correlation between predicted and experimental error.

INTRODUCTION

Exact mass measurement using orthogonal TOF (Time of Flight) -MS is a well-established technique.

Exact mass may be used to restrict the number of proposed elemental compositions for unknown analytes or to aid confirmation of the presence of target compounds with known elemental composition.

An estimation of the 'confidence' in the mass assignment for an individual mass measurement is essential if this information is to be used to infer information about elemental composition.

This paper describes an approach to error analysis during mass measurement. Predicted error ranges for individual mass measurements are calculated and compared to experimental results.

INSTRUMENTATION

All results presented were obtained using a LCT Premier™ LC/MS orthogonal TOF mass spectrometer. Figure 1.

Figure 1. Waters® Micromass® LCT Premier™ LC/MS TOF Mass Spectrometer.

The LCT Premier is a benchtop LC/MS instrument incorporating a high transmission ion source and high transmission analyzer optics providing increased sensitivity.

The LCT Premier is capable of exact mass measurement with a single internal reference peak at 5000 resolution (FWHM) 'V' mode, or 10,000 resolution (FWHM) 'W' mode of operation.

All results were obtained with Electrospray ionization at a resolution of (10,000) FWHM.

ERRORS IN EXACT MASS MEASUREMENT

Errors in repeated measurements are commonly described by:

- The standard deviation (σ), the scatter around the mean of the results. This describes the 'precision' of measurement.
- The RMS (root mean squared), the scatter of results around the true value. This value encompasses the precision and accuracy of the measurement providing the true value is known.

Commonly, however, only a single mass measurement is made. To arrive at a representative prediction of precision and accuracy for this measurement, as many sources of error as possible should be estimated and combined.

Based on these estimates an error range around the measurement may be reported within which there is a finite probability that the true m/z value of the analyte will fall. Often a range of ± 2 standard deviations is quoted with a 95% probability of containing the true m/z value.

Quantifiable Errors

I. Errors due to the random arrival of ions (ion Statistics)

II. Errors recorded during mass calibration

III. Computational errors (rounding errors)

Computational Error

The standard deviation error associated with computational/rounding errors using the MassLynx™ Software may be estimated to be in the order of 0.05 ppm. These errors can be ignored as insignificant.

Errors Due to Ion Statistics

The random nature of ion arrival is generally the dominant source of error in mass measurement. The precision of mass measurement increases as the number of ions recorded increases.

Using a Gaussian model to approximate the distribution of ions a mass spectral peak the precision (standard deviation) of determination of m/z value in parts per million (σ_{ppm}) is given by equation 1.

$$\sigma_{ppm} = \frac{10^6}{R\sqrt{5.5N_{ions}}}$$

Where

R = The mass resolution (FWHM definition)

N_{ions} = The total number of ion arrivals recorded in the peak

However, it has been found experimentally that this expression must be modified to include an extra fixed error term - Equation 2

$$\sigma_T = \sqrt{\sigma_{ppm}^{\ 2} + \sigma_{inst}^{\ 2}}$$

Where σ_{inst} = an experimentally determined error term.

σ_{inst} represents error arising from sources including electronic noise and peak detection limitations.

From experimental data (see Figure 2) σ_{inst} = 0.65 ppm.

This expression represents the expected scatter around a mean value for repeated measurements (precision). If this were the only source of error it can be estimated that 95% of all measurements will fall within the range ±2σT.

Figure 2 shows a plot of experimentally determined standard deviations for repeated measurements at 10,000 resolution (FWHM) with varying ion counts. The predicted error σ_T is plotted for comparison.

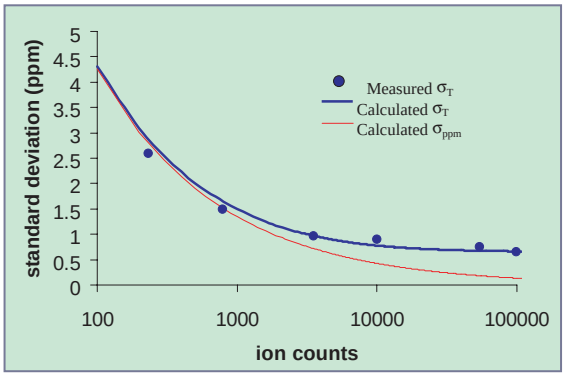

Figure 2. Theoretical and experimentally determined standard deviation due to ion statistics.

Improvements in Precision

From equation 1 it can be seen that the precision of mass measurement may be improved by:

a) Increasing R, the mass resolution of the system.
b) Increasing N_{ions} by increasing the transmission of the instrument.

- Improvements in Mass Resolution

The LCT Premier analyzer includes an additional small mirror allowing the instrument to be operated in two modes, 'V' and 'W'.[1] In 'W' mode ions are allowed an extra pass through the analyzer. The increased flight path in 'W' mode results in an increase in mass resolution of approximately times 2 between 'V' and 'W' modes. This feature improves the statistical precision of measurements and reduces the likelihood of mass interferences.

Figure 3 shows the $[M+H]^+$ molecular ion of Leucine Enkephalin in 'V' mode resolution = 5500 (FWHM) and 'W' mode resolution = 11,000FWHM.

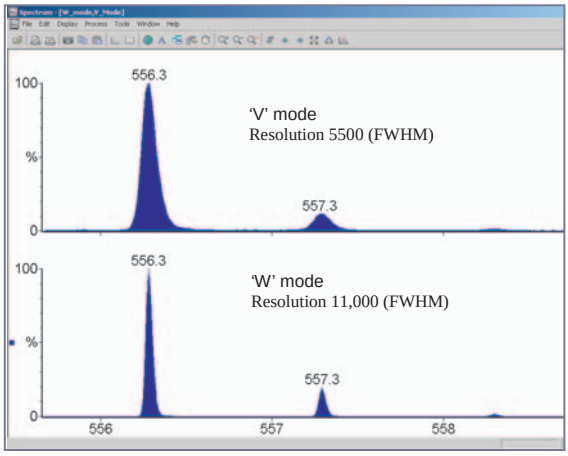

Figure 3. Comparison of 'V' and 'W' mode using LCT Premier.

- Improvements in Transmission

a) Source transfer optics
The source transfer region of the LCT Premier includes dual ion guide transfer optics and three differentially pumped regions prior to the analyzer. This design allows the orifices through which ions pass to be increased in diameter increasing ion transmission.

b) TOF Analyzer
The LCT Premier includes high transmission grids in the orthogonal acceleration region, to give a further increase in ion transmission.[2]
In combination these improvements result in a significant increase in transmission compared to previous designs improving ion statistics for exact mass measurement.

Experimental Results (Precision)

A single mass measurement was made of the $[M+H]^+$ ion of Sulfadimethoxine (m/z = 311.0814) and the predicted precision calculated from equation 2.

This predicted value was tested against the experimentally determined precision from 17 repeat injections of Sulfadimethoxine under identical conditions using Leucine Encephalin as an internal lockmass. Figure 4 shows a comparison between predicted and experimental precision.

A predicted precision for 95% ($\pm 2\sigma_{precision}$) of results of ± 4.14ppm was calculated using equation 2. Where

$$\sigma_{precision} = \sqrt{(\sigma_{T(analyte)})^2 + (\sigma_{T(LockMass)})^2}$$

The experimentally determined precision for 17 repeat measurements was ±4.08ppm.

Analyte Empirical Formula = $C_{12}H_{14}N_4O_4S$ calculated m/z = 311.0814

Single Measurement		Measured m/z = 311.0814	
	Ion Counts	σ_{ppm}	$\sigma_T = \sqrt{\sigma_{ppm}^2 + \sigma_{mz}^2}$
Analyte	4600	0.63 ppm	0.90 ppm
Lock Mass 556.2771	600	1.74 ppm	1.86 ppm
Predicted Precision $\sigma_{precision} = \sqrt{(\sigma_{T(analyte)})^2 + (\sigma_{T(LockMass)})^2}$			2.07 ppm
Predict precision $_{Precision}$ for repeated measurements of			**2.07 ppm**
Predict error spread for 95% of measurements within			**4.14 ppm**

Multiple measurements							No. of measurements 17
311.0820	311.0811	311.0812	311.0803	311.0819	311.0806	311.0817	311.0824
311.0826	311.0812	311.0808	311.0819	311.0820	311.0814	311.0818	311.0817
Mean m/z = 311.0815							
Measured precision (One standard deviation about the mean) = 2.04 ppm							

Comparison of results		Predicted	Measured
Number of results within $\pm\sigma_{Precision}$	**2.07ppm**	**68%**	**70%**
Number of results within $\pm 2\sigma_{Precision}$	**4.14 ppm**	**95%**	**100%**
Number of results within $\pm 2.5\sigma_{Precision}$	**5.18ppm**	**99%**	**100%**

Figure 4. Comparison of calculated and experimentally determined precision.

Errors Recorded During Mass Calibration

The purpose of mass calibration is to correct systematic differences between theoretical ion arrival times and the arrival times for ions of known reference materials. Any residual error after calibration may be treated as random error and combined with other statistical errors. However, for a single measurement an error in calibration will result in a systematic displacement of the measured value from the true mass. Any additional statistical variation will be superimposed around this displaced value.

Commonly a low order polynomial linear regression is used to generate a calibration function. One way of estimating the uncertainty of this calibration is to calculate a 'confidence interval'. The general expression for calculation of the 95% confidence interval is given in equation 3.[3]

$$\hat{y} \pm t_{\alpha/2} S_{\varepsilon} \sqrt{\frac{1}{n} + \frac{(X_i - \overline{X})^2}{S_x^{\,2}}}$$

n = number of data points

Se = The standard error of estimate

S_x = the sum of the squared deviation in x from the mean value of the x data

$t_{\alpha}/2$ = Probability from the student T distribution. For 95% interval $\alpha/2 = 0.025$.

When applied to calibration this statistic provides an interval which has an estimated 95% confidence of containing the underlying calibration expression. This error estimate may be used to predict the likely error in a subsequent mass measurement based on this calibration.

This approach provides a tool for investigating the optimum order of polynomial calibration with respect to the spacing of the calibration points, the number of calibration points, and the ion statistics of each calibration point.

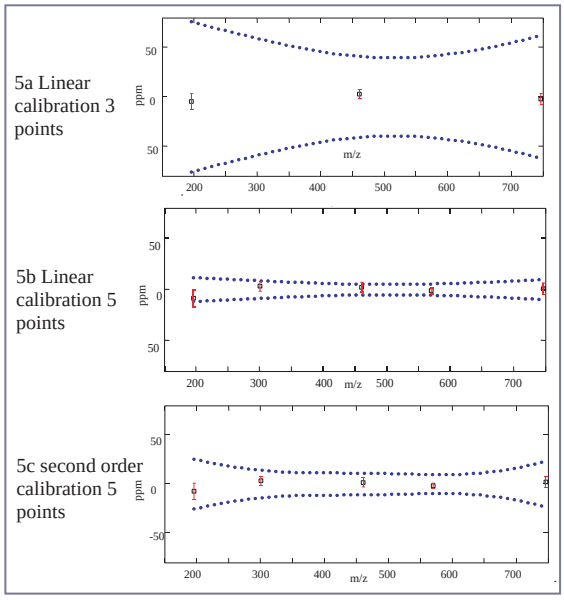

Figure 5. Behavior of 95% confidence intervals with calibration data.

As an example Figure 5 shows the residual error in ppm, and the 95% confidence interval, after a first order linear calibration. The vertical error bars represent ±2σT or 95% error limits from ion statistics. The data is taken from a continuous infusion of Polyethylene Glycol (PEG).

Figure 5a includes only three reference peaks and exhibits large uncertainty reflecting the limited amount of data.

In Figure 5b two more data points have been added and the uncertainty has dramatically decreased.

Figure 5c shows the same data as in Figure 5b however a second order polynomial has been fitted. The uncertainty has increased suggesting that the order of the polynomial is too high for such a small set of data.

For illustration Figure 6 shows a complete calibration including 30 data points fitted with a second order calibration. The 95% confidence interval is in the order of ±0.5ppm.

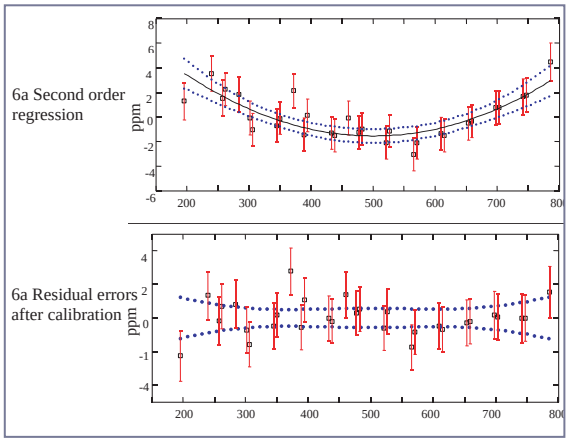

Figure 6. Example of second order PEG calibration with 95% confidence intervals.

Combination of Errors

To arrive at an estimate of error for a single mass measurement the errors calculated at each stage of the measurement must be combined into a final figure. Equation 4.

$$\sigma_{predicted} = \sqrt{(\sigma_{T(analyte)})^2 + (\sigma_{T(lockMass)})^2 + (\sigma_{Cal(Analyte)})^2 + (\sigma_{Cal(LockMass)})^2}$$

Where

$\sigma_{T(analyte)}$ = the predicted error from equation 2 for the analyte.

$\sigma_{T(LockMass)}$ = the predicted error from equation 2 for the lockmass.

$\sigma_{Cal(Analyte)}$ = the predicted calibration error for the analyte.

$\sigma_{Cal(LockMass)}$ = the predicted calibration error for the lockmass.

Results: Error Prediction

Polyethylene Glycol was continuously infused and a first order calibration generated using nine evenly spaced reference peaks. Twenty, different peaks from the same sample, spanning the whole calibration range, were then mass measured using

an internal lockmass at m/z 745.41977. The total predicted error for each m/z was compared to the experimentally determined error.

This experiment was repeated three times using the conditions.

1. Calibration 1 (Figure 7) with intense analyte and lockmass.
2. Calibration 2 (Figure 8) with intense analyte and lockmass.
3. Calibration 2 (Figure 8) with weak analyte and lockmass.

Calibration 1 was generated from peaks with much lower statistical precision than in Calibration 2. This is indicated by the vertical 95% error bars shown in Figure 7 and 8.

The results of the three analysis are presented in Figure 9, Figure10, and Figure 11 respectively.

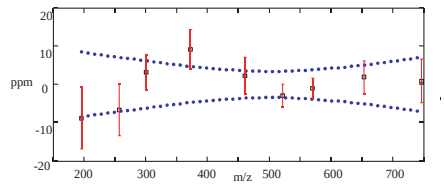

Figure 7. Calibration1 residuals and 95% confidence limits

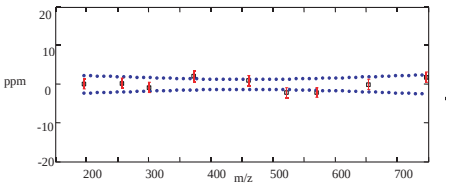

Figure 8. Calibration2 residuals and 95% confidence limits

m/z Calculated	m/z measured	$\sigma_{(ppm)}$	$\sigma_{T(ppm)}$	$\sigma_{cal(ppm)}$	$\sigma_{(predicted)}$ (ppm)	$2\sigma_{(predicted)}$ 95% range	ppm error measured
239.1495	239.1484	0.16	0.67	3.8	5.22	10.43	-4.43
261.1314	261.1301	0.07	0.65	3.5	5.04	10.07	-5.02
283.1757	283.1742	0.17	0.67	3.3	4.87	9.74	-5.23
305.1576	305.1555	0.07	0.65	3.0	4.70	9.41	-6.95
344.2274	344.2264	0.10	0.66	2.6	4.46	8.92	-5.93
349.1838	349.1822	0.06	0.65	2.6	4.43	8.86	-4.70
388.2547	388.2524	0.08	0.66	2.3	4.26	8.53	-5.82
393.2101	393.2083	0.06	0.65	2.2	4.24	8.47	-4.45
432.2809	432.2786	0.08	0.65	1.9	4.09	8.18	-5.25
437.2363	437.2339	0.06	0.65	1.9	4.09	8.18	-5.42
476.3071	476.305	0.07	0.65	1.8	4.02	8.05	-4.39
481.2625	481.2607	0.06	0.65	1.8	4.02	8.04	-3.70
525.2887	525.2866	0.06	0.65	1.8	4.02	8.04	-4.00
564.3595	564.3569	0.08	0.65	1.9	4.09	8.18	-4.64
608.3857	608.3839	0.09	0.66	2.2	4.21	8.42	-3.01
613.3395	613.3391	0.07	0.65	2.2	4.24	8.48	-3.31
657.3673	657.3657	0.09	0.66	2.5	4.40	8.80	-2.49
696.4382	696.4374	0.14	0.66	3.0	4.70	9.41	-1.09
701.3936	701.393	0.11	0.66	3.0	4.70	9.41	-0.80
740.4664	740.4657	0.17	0.67	3.5	5.04	10.08	1.78
Lock Mass	745.41977	0.13	0.65	3.5			RMS=4.4

Comparison of results	Predicted	Measured
Number of results within $\pm\sigma_{Predicted}$	68%	50%
Number of results within $\pm 2\sigma_{Predicted}$	95%	100%
Number of results within $\pm 2.5\sigma_{Predicted}$	99%	100%

Figure 9. Calibration[1] with intense analyte and lockmass

m/z Calculated	m/z measured	$\sigma_{(ppm)}$	$\sigma_{T(ppm)}$	$\sigma_{cal(ppm)}$	$\sigma_{(predicted)}$ (ppm)	$2\sigma_{(predicted)}$ 95% range	ppm error measured
239.1495	239.1496	0.16	0.67	1	1.86	3.71	0.42
261.1314	261.1313	0.07	0.65	1	1.85	3.70	-0.38
283.1757	283.1754	0.17	0.67	1	1.86	3.72	-1.06
305.1576	305.1566	0.07	0.65	0.85	1.78	3.55	-3.28
344.2274	344.2274	0.10	0.66	0.75	1.73	3.46	0.00
349.1838	349.1833	0.06	0.65	0.75	1.73	3.46	-1.43
388.2547	388.2534	0.08	0.66	0.7	1.71	3.42	-3.35
393.2101	393.2092	0.06	0.65	0.7	1.71	3.42	-2.29
432.2809	432.2795	0.08	0.65	0.65	1.69	3.38	-3.24
437.2363	437.2347	0.06	0.65	0.65	1.69	3.38	-3.66
476.3071	476.3058	0.07	0.65	0.65	1.69	3.38	-2.73
481.2625	481.2614	0.06	0.65	0.65	1.69	3.38	-2.29
525.2887	525.2872	0.06	0.65	0.7	1.71	3.42	-2.86
564.3595	564.3574	0.08	0.65	0.75	1.73	3.46	-3.72
608.3857	608.3842	0.09	0.66	0.85	1.78	3.55	-2.47
613.3395	613.3395	0.07	0.65	0.85	1.78	3.55	0.00
657.3673	657.3660	0.09	0.66	0.95	1.83	3.65	-1.98
696.4382	696.4376	0.14	0.66	1.1	1.91	3.82	-0.86
701.3936	701.3931	0.11	0.66	1.1	1.91	3.82	-0.71
740.4664	740.4656	0.17	0.67	1.2	1.97	3.95	-1.08
Lock Mass	745.41977	0.13	0.65	1.25			RMS=2.25

Comparison of results	Predicted	Measured
Number of results within $\pm\sigma_{Predicted}$	68%	45%
Number of results within $\pm 2\sigma_{Predicted}$	95%	90%
Number of results within $\pm 2.5\sigma_{Predicted}$	99%	100%

Figure 10. Calibration[2] with intense analyte and lockmass

m/z Calculated	m/z measured	$\sigma_{(ppm)}$	$\sigma_{T(ppm)}$	$\sigma_{cal(ppm)}$	$\sigma_{(predicted)}$ (ppm)	$2\sigma_{(predicted)}$ 95% range	ppm error measured
239.1495	239.15	2.89	2.96	1	4.40	8.80	0.84
261.1314	261.13	1.20	1.36	1	3.53	7.06	5.36
283.1757	283.18	3.10	3.17	1	4.54	9.09	3.53
305.1576	305.16	1.20	1.36	0.85	3.49	6.98	-1.31
344.2274	344.23	1.80	1.92	0.75	3.72	7.44	3.49
349.1838	349.18	1.14	1.31	0.75	3.45	6.89	2.86
388.2547	388.25	1.55	1.68	0.7	3.59	7.18	0.00
393.2101	393.21	1.06	1.24	0.7	3.41	6.82	3.56
432.2809	432.28	1.42	1.56	0.65	3.53	7.06	-2.54
437.2363	437.24	1.05	1.23	0.65	3.40	6.79	-0.46
476.3071	476.31	1.41	1.55	0.65	3.52	7.05	-1.89
481.2625	481.26	1.02	1.21	0.65	3.39	6.78	1.04
525.2887	525.29	1.06	1.24	0.7	3.41	6.82	-0.19
564.3595	564.36	1.54	1.67	0.75	3.60	7.19	1.42
608.3857	608.39	1.72	1.84	0.85	3.70	7.40	3.62
613.3395	613.34	1.39	1.54	0.85	3.56	7.12	2.61
657.3673	657.37	1.58	1.71	0.95	3.66	7.32	-0.15
696.4382	696.44	2.49	2.57	1.1	4.17	8.35	4.31
701.3936	701.40	1.99	2.09	1.1	3.90	7.79	2.14
740.4664	740.47	3.22	3.29	1.2	4.67	9.35	4.73
Lock Mass	745.41977	2.76	2.83	1.25			RMS=2.8

Comparison of results	Predicted	Measured
Number of results within $\pm\sigma_{Predicted}$	68%	80%
Number of results within $\pm 2\sigma_{Predicted}$	95%	100%
Number of results within $\pm 2.5\sigma_{Predicted}$	99%	100%

Figure 11. Calibration[2] with weak analyte and lockmass

Discussion

- The results presented show generally good correlation between predicted and experimentally determined error ranges.
- Calibration uncertainty and the fixed error sinst have been added for both the analyte and lockmass peak.
- The measured error in Figure 10 clearly changes systematically with m/z indicating an underlying second order characteristic in calibration. Because there were insufficient data points to characterize this in calibration 2 a first order calibration was performed. However the 95% confidence interval technique has still characterized the uncertainty in the calibration with reasonable accuracy. 90% of all results falling within 2 $\sigma_{predicted}$.
- It is envisaged that individual mass measurements could be annotated automatically with a 95% confidence range using the criteria outlined.

Non-Quantifiable Errors

In some cases it is not possible to adequately quantify the magnitude of error. If error of this type is present no estimate of the error range of the result can be presented.

I. Errors due to instrument instability or drift. During or between analysis instrument conditions may change to such an extent that the calibration is no longer valid. This could be because of user intervention, power supply instability or excessive environmental change.

The use of internal lockmass correction provides an excellent check of the stability of the instrument during analysis. Short-term variation in the calculated correction factor, not accounted for by ion statistics or instrument drift, may be used to give an indication of instrument instability.

II. Errors due to mass interference.

It may be possible to recognize gross distortions of peak shape due to mass interference using specialized peak detection algorithms. However, at the very high levels of mass accuracy demanded this becomes increasing difficult.

As an example Figure 12a shows an idealized mass spectral peak at m/z 500 'peak1' with a resolution of 10,000 FWHM. In this model interference peak 'peak2' at fixed % intensity relative to 'peak1' is introduced. The area of the combined peak (Peak1+Peak2) above a 20% intensity threshold was used to calculate the peak centre. The effect on the calculated m/z of 'Peak1' is plotted against the separation (peak2-peak1) for different relative intensities of 'peak 2'. Figure 12b.

From this example it can be seen that the ratio of the signal intensity (peak1) to chemical background (peak2) for the analyte and the lockmass peak will limit mass measurement accuracy. It is possible to assign a notional standard deviation arising from mass interference $\sigma_{(interference)}$. This may be included in equation 4 to yield a value $\sigma_{(predicted)}$ incorporating this source of error.

Maximum ppm error and estimated $\sigma_{(interference)}$ is given for different analyte to background interference ratios in the following table.

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Ratio Signal : Background	Maximum error	$\sigma_{(interference)}$
100 : 1	0.4 ppm	0.20 ppm
50:1	0.7 ppm	0.35 ppm
25:1	1.9 ppm	0.95 ppm
10:1	3.8 ppm	1.90 ppm
5:1	8.3 ppm	4.15 ppm

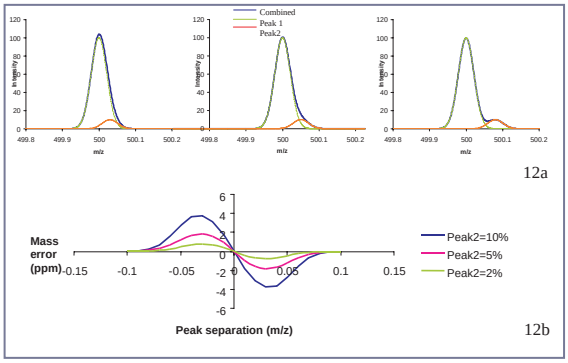

Figure 12. Effect of mass interference on mass accuracy.

Conclusions

- Strategies for quantifying statistical error and calibration uncertainty have been developed and tested.
- The effect of mass interference on mass accuracy may be crudely quantified and may be used to give a guide to potential errors.
- Error ranges for single, exact mass measurements may be predicted and used to qualify elemental composition results.
- Further work includes refining the prediction of uncertainty in calibration and automatically guiding selection of calibration criteria.
- This method may be easily automated to provide an estimation of mass accuracy. This facility will increase confidence in results, help to avoid incorrect empirical formula being assigned and help to reject improbable elemental composition candidates.

References

1. High Resolution Analyser for an Orthogonal TOF Mass Spectrometer.
   J. Hoyes, R. Bateman, J. Wildgoose.
   Proceedings of the 15th IMSC, Barcelona, Spain.
2. Ion Dspersion near grids in TOFMS.
   M. lewin, M. Guilhaus, F. Read, J. Wildgoose, J. Hoyes, R. Bateman.
   Proceedings of the 49th ASMS conference.
3. Applied Linear Statistical Models
   Neter, Kutner, Nachtsheim, Wasserman.
   McGraw-Hill

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