Response to reviews for the paper "Statistical precision of the intensities retrieved from constrained fitting of overlapping peaks in high-resolution mass spectra" by Cubison, M.J. et al.

We thank the reviewers for their comments on our paper. To facilitate the revision process we have copied the reviewer comments in black text. Our responses are in regular blue font. We have responded to all the referee comments and made alterations to our paper (**in bold text**).

Anonymous referee #1

R1.0 Cubison et al provide a theoretical piece on the achievable precision of least square retrievals of the intensities of unresolved peaks in time of flight mass-spectra. While the retrieved intensities are sensitive to a number of parameters, the authors focus on the uncertainty in mass scale calibration and counting statistics. In principle this work is publishable and of good service to an increasing community of TOF users in the field of atmospheric sciences. While the general structure and most Figures are useful, the manuscript is poorly written (insufficient/sloppy, complicated and/or ambiguous explanations) and cannot be published without a major make over.

General comments

R1.1 In addition there are many odd sentences, so thorough proofreading by English native speakers is required as well.

The manuscript has been proofread by an English native speaker.

R1.2 The authors should try to further reduce their results to distill the essence of their work. For example, Figures 5 and 6 are largely redundant and could be combined. The same holds for Fig 7a&c and Fig 7b&d, respectively. There are a number of Figures in the supplementary section, which, in my opinion, are not needed. In the spirit of community service it would be useful to provide a tool (short script or even spreadsheet) allowing users to estimate the precision of their modeled peaks (input parameters mass and modeled intensities of parent and child, resolution).

We see how Figures 5 and 6 could be viewed as redundant. As we have chosen to represent the experimental data in Fig. 6, we choose to keep this figure and remove Fig 5. from the manuscript. It is possible, as the reviewer notes, to directly refer to the Fig 6. in the discussion with only minor alterations to the text.

Regarding Figure 7, we prefer to keep the figure but have removed the bottom two panels as suggested. Representation of the data in 2D space gives rapid visual confirmation that our parameterisation of the transition between uncertainty regimes does indeed split two distinctly different areas of the plot. Without this figure it would not be so simple for the reader to visualise the different regimes.

Regarding the supplementary figures: the observation that some figures are redundant was also made by reviewer 2. We have removed S3-7 and condensed this information into a single table as explained in our response to comment R2.14 below.

We additionally present the equation used to generate Figure 6, which the reader may utilise to directly estimate the minimum intensity precision expected as a result of *m/Q* calibration imprecision.

Specific comments

R1.3 12621,20-22: incomplete sentence

The sentence was removed.

R1.4 12623, 7-9: The sentence 'It is chosen. . .' is very confusing and refers to several unexplained facts: which are the peak parameters? What is the iTOF space? How is m/Q transferred to iTOF (give equation!) and wherefrom comes the non-linearity?

Reworded for clarity as:

"The peak position and width parameters are given as function of iToF rather than *m/Q*, for the mass axis goes as the square-root of iToF and a perfect Gaussian shape observed in iToF does not maintain its symmetry in *m/Q*. The fits are thus conducted in iToF, the axis in which the measurement is taken."

R1.5 12623, 21-23: The sentence 'This perturbation was applied consistently to both fitted peaks for a given fit, but varied from one fit iteration to the next.' does not make sense.

The point is that when perturbing the mass calibration, an equal perturbation in position should be applied to all peaks in the overlapping fit, as this reflects the real situation. I.e. the *m/Q* calibration errors are the same for all peaks at an integer mass in a given spectrum. However, when iterating over many fits, a different perturbation would be used at each iteration. But at each step, the perturbation applied to all peaks should be the same. Reworded for clarity thus:

"For each iteration of the fitting procedure, this perturbation was applied consistently to both fitted peaks (i.e. the calibration parameters remain equal for all peaks). However, the perturbation applied varied from one iteration to the next."

R1.6 12625,4-5: The sentence 'δt the separation of the discrete data points in iToF space' is hardly understandable.

Given that it is already mentioned when introducing the equations that they relate to ion time-of-flight (iToF), the addition here of "in iToF space" is perhaps extraneous and leading to confusion. These words were thus removed. This should help the reader to concentrate on the meaning of delta - the gap between the measurement points. The subscript already denotes this is in time.

R1.7 12625, 14: odd sentence: 'The precision arising from counting error is unavoidable'

Reworded for clarity:

"The minimum attainable precision is that arising from counting error"

R1.8 Figure 1: I don't understand what is displayed in the insert of Figure 1. Needs better explanation in the Figure caption.

This is the relationship between the width of the histograms shown in the main figure and the intensity of the peak being fitted. Reworded the caption for clarity as:

"Shown inset, the linear relationship between the histogram width, given as the standard deviation in Δ, σ_C, and the fitted peak intensity."

R1.9 12629,14-16: The given numbers do not seem to agree with Fig 3. The ppm precisions should compare to resolutions of 1000, 2000, 4000, and 8000.

The use of iToF resolving power in the figure, but m/Q resolving power in the text, has understandably led to confusion. The figure legend has been updated to report m/Q resolving power to be consistent with the text. The mass resolving powers are also updated to reflect the comment of reviewer 2 in R2.4 below.

R1.10 Fig S5: give peak intensities in decimal powers

Figure updated as requested.

R1.11 12632,20 – 12633,4: Even after carefully reading this graph three times, I don't understand the idea behind this.

The authors accept that the explanation surrounding the peak shape and its potential application in the figure can be confusing for researchers who are not very familiar already with this type of fit. We have chosen to remove these lines from the plot so as not to distract from the central message, and remove accordingly the corresponding paragraph in the manuscript.

R1.12 Supplementary information: The supplementary information is presented in a very inconvenient format and should be compiled into one pdf.

This has been done as requested.

R1.13 S1. The manuscript refers to S1a which is not labeled in the Figure. Furthermore, I do have problems connecting the caption text to what is shown on the Figure. This clearly needs improvement: What is displayed on x and y? What on the main chart and insert, respectively? What is the difference between the different datasets displayed?

As the text only refers to one of the two peaks in Figure S1, the figure has been updated only to show one peak. The caption was reworded for clarity:

"An example synthesised discrete measurement distribution shown as ion signal in counts as a function of ion time-of-flight (iToF). This consists of a single Gaussian peak centred at 2000 ns and data-point spacing 0.2 ns. The peak width FWHM is 1 ns."

R1.14 Section 3.1. In Table 1 I miss sigma_A and sigma_t

These were added to the glossary table accordingly. They were also renamed to S_A and S_t as per comment R2.9 below.

Anonymous referee #2

R2.0 The paper by Cubison et al. addresses the estimation of the statistical precision of peak intensities obtained upon deconvolution of two overlapping Gaussian peaks. Such statistical precision depends on several factors, among which this study considers only the m/Q calibration and the noise in the measurement distribution. The general idea of the paper is useful to the atmospheric science communty given the increasing popularity of high resolution mass spectrometers but the paper is not publishable in the present form and needs major revisions.

General comments

R2.1 The result section should be condensed, avoiding unecessary and redundant figures, focusing on meaningful points, and providing quantitative data to support the conclusions. Many considerations are only supported by qualitative graphical observations and therefore may not be rubust. Examples: Section 3.2, Fig. S3-S7.

We have condensed and re-arranged the results section as suggested. As detailed below in response to R2.14, rather than rely on the graphical observations supported previously by Figures S3-S7, we have used a quantitative scalar metric to describe the plotted curves and are thus able to compare the different simulations in a single table. The image plot (was Fig. 7, now Fig. 5) is intended only to help the reader visualise the different fitting regimes and its previous position in the manuscript (amongst the results) could have led to the impression that the quoted precision estimates were based on graphical interpretation. We now introduce this figure during the discussion of the different fitting regimes, where its impact can be of most benefit. We also condense this figure for simplicity and remove the previously redundant figure 6.

R2.2 Generally speaking the paper should be presented employing a more “ready to use” approach, otherwise its usefullness for the audience would be limited. A more straightforward way to estimate the statistical precision of retrieved peak intensities (taking in input suitable parameters from constrained fitting) should be provided.

Please see response to comment R2.15 below.

Specific comments

R2.3 12623, 8: give a definition for iToF space, although the meaning is generally understandable from the context

This text was altered, also in part to address comment R1.2 above:

“The peak position and width parameters are given as function of iToF rather than m/Q, for the mass axis goes as the square-root of iToF and a perfect Gaussian shape observed in iToF does not maintain its symmetry in m/Q. The fits are thus conducted in iToF, the axis in which the measurement is taken.”

R2.4 12623, 11: the mass resolving power should be $m/\Delta m = (t/\Delta t)/2 = 2000/2 = 1000$

Text altered accordingly throughout the paper.

R2.5 12623,16-23: the equation from which the calibration parameters are determined should explicitly be provided. Moreover the way the perturbation on m/Q values is applied should be better explained

The equation was inserted into the text:

"In this work we use the equation iToF = *A*+*Bsqrt(*m/Q*), where *A* and *B* are constants."**

The description on the perturbation on m/Q values was updated (see also response to R1.5 above) thus:

"For each iteration of the fitting procedure, this perturbation was applied consistently to both fitted peaks (i.e. the calibration parameters remain equal for all peaks). However, the perturbation applied varied from one iteration to the next."

R2.6 12624,1-2: even in modern aquisition systems, for low intensity peaks (e.g. for recorded at high m/Q values in ToF spectra) sometimes the baseline noise and the peak intensity are comparable

We clarify that is it *electronic* baseline noise that is claimed to be small relative to ion counting noise, not the MS baseline itself which is often dominated by ions striking the detector. Reworded for clarity:

"In modern data acquisition systems they are typically small compared to ion counting noise which leads to signal degradation and a non-zero mass-spectrum baseline."

R2.7 12625, 14: "The precision" should probably be substituted by "The uncertainty"

The authors must politely disagree, although the reviewer is correct to note that the expressed quantity is indeed an uncertainty on the reported value. However, the term "uncertainty" is vague and is a combination of all possible sources of error, such as systematic biases and also random errors that lead to the imprecision studied in this work. "Precision", on the other hand, is a well-defined quantity and it is indeed the precision in the reported fitted intensities that we report here. In addition to the IUPAC definitions, Menditto et al (Accred Qual Assur (2007)) make a useful summary of the same point we are making here. (http://link.springer.com/article/10.1007/s00769-006-0191-z)

R2.8 12625, 18: "1000" instead of "4000".

Text altered accordingly.

R2.9 12624, 21-12625,25: σ is used to express SDs (e.g. for σA) or normalized precision (e.g. for σN). Please be consistent.

The standard deviations in equations 1) and 2) are now expressed as S_A and S_t as suggested. The precisions remain denoted by σ.

R2.10 12626: in this section a quantitative determination of σC depending on the two peak intensities (and/or other suitable parameters) should be provided.

This is not something which we addressed whilst running the simulations. We stress that the precision estimates presented here result from a parameterisation of many simulation results, and are not derived from a single formula dependent on all the fit parameters. Whilst σ_C is of conceptual use in presenting the methodology, it is the precision in σ_I which is pertinent to the real-life scenario and thus we have focused on developing a quantitative estimate of that.

R2.11 12629, 16: mass resolving powers should be half (and not double) of TOF resolving power reported in Fig. 3

Text altered throughout.

R2.12 12630: the reason why m/Q calibration errors affect estimation of ion intensities should be better explained. It is because peak positions are constrained in the fits? In this case, why an error in peak position does not affect estimated peak intensity appreciably in the case of “counting-error regime”? Is it because the considered calibration errors are small? The authors should make an effort to quantify their claims (e.g. define “appreciable increase in the precision”)

The reviewer assumes correctly that the fixed position of the fits leads to calibration uncertainties propagating into the fitted intensity values. We have explicitly stated this in the text as:

“We now estimate the achievable precision for the peak intensities resulting from the constrained fitting procedure, combining the precisions from counting- and calibration-errors in an overlapping two-ion system. As the peak positions are held fixed, uncertainties in the calibration propagate to uncertainties in the fitted intensities. Both these effects contribute to the precisions summarised in Figure 4.”

The qualitative description “appreciable increase in the precision” is as suggested replaced with a comparative description:

“Note that for well-separated peak the imprecision in the *m/Q* calibration at the realistic levels used here is small relative to the counting error and does not result in an increase in the precision above that imposed by counting statistics.”

R2.13 12631, 8: A definition of Xd should be provided. When is a value of σI defined to diverge from the counting-error limit?

In our analysis, principally in the plotting of Figure 5 (image plot), this was determined as the point at which σI was 5% greater than the counting-error limit. We thus insert the sentence:

“To enable quantitative determination of this parameter, we define χ_d as the point as which σ_I = 1.05 * σ at χ = 4 ~ σ_C.”

R2.14 12630: The considerations on the parameters from which Xd is independent should be more schematic or in a dedicated sub-section. Moreover, conclusions arising from graphical consideration (S3, S4, S5, S6, and S7) could be more straightforwardly motivated by quantitatively assessing the impact of each parameter on Xd and eliminating redundant figures.

We have followed the reviewer’s recommendation and condensed the information previously given in S3-7 (which we remove) into a single table, where the influence of various parameters upon σ_B is summarised. The description of these independent parameters is given its own dedicated section (3.5) and split from the consequent discussion of precision estimates.

Previously we concentrated on demonstrating that X_d was insensitive to various parameters. Whilst this is true for fixed ion counts, a more straightforward explanation is to demonstrate that it is σ_B, the precision in fitted intensity arising from calibration error, that is insensitive to the fit parameters. This is the key finding for the precision estimates, whereas X_d is useful for visualising the different regimes.

We introduce section 3.5 as follows:

"3.5 Parameterisation of the intensity precision for overlapping ions with *m/Q* calibration error: independent parameters

Since our simulations are complex and time consuming, a parameterisation of the value of σ_B, the limiting precision due to *m/Q* calibration imprecision, is desirable in assessing fitting precision. We note that, in the case of high ion counts, $\sigma_B \sim \sigma_I$, for uncertainty arising from counting error is negligible in comparison to that introduced by calibration imprecision. Furthermore, from Fig. 4, as χ is decreased, the limiting values of σ_I for different peak intensities all fall along the same line (with the exception of the "overlapping counting-error regime"). This implies that σ_B is insensitive to peak intensity. Further simulations were thus conducted to assess the sensitivity of σ_B to various parameters in the fit, altering one parameter at a time and holding the peak intensity ratio R_I fixed at 2. These results are reduced to scalar values in Table 1 by reporting the value of σ_I for the child peak at $\chi = 1.0$ for the default, and altered inputs. By considering only results at high ion counts, we assume $\sigma_B \sim \sigma_I$ and thus effectively report σ_B at $\chi = 1.0$.

Table 1 demonstrates that σ_B is insensitive to changes in the spectrometer resolving power, data acquisition rate (point spacing) and peak intensity. Only increasing the estimate of imprecision in the *m/Q* calibration had any impact on the simulations. Tripling the calibration imprecision roughly doubled σ_B. A conservative estimate of σ_B could therefore be up to twice the values reported in this study (which we consider the best-case scenario), but is almost certainly not going to be larger than that for a well-calibrated system.

Considered together, these observations imply that the precision owing to calibration imprecision, σ_B, can be considered a function of only χ and R_I and is independent of W, δt and I_p.

Parameter adjusted	σ_I at $\chi = 1.0$ for default value	σ_I at $\chi = 1.0$ for adjusted value	
Calibration imprecision	35 % at 5 ppm	64 % at 10 ppm	82 % at 15 ppm
Mass resolving power	35% at 1000	32 % at 500	41 % at 2000
Intensity	35 % at 10^4 counts	35 % at 10^5 counts	35 % at 10^7 counts
Sample interval	35 % at 1 ns	34 % at 0.5 ns	35 % at 2 ns

Table 1: Changes in precision in fitted intensity of the child peak when altering properties of the fitting procedure and its input distributions, whilst holding the peak intensity ratio constant at 2."

R2.15 12632, 6-19: a clear parameterisation of the dependence of σI and Xd on Ip, RI , W, δt should be provided. The way it is presented in the paper is not effective.

We preface our response to this comment with the observation that the precision estimates presented in this work are derived from the results of simulations. It is not our intention to derive a formula for the direct calculation of the intensity precision σ_I, if that is indeed possible. We aim to describe the minimum expected precision arising from two effects, counting error and mass-calibration imprecision, and argue that a reasonable estimate of σ_I is the larger of the two, since each of them dominates in a given regime. Counting error can of course be directly calculated. The reviewer is however absolutely correct that the formula used to parameterise σ_B (σ arising from calibration imprecision) should be presented in the paper and not just utilised to generate the traces shown in Figure 6. We update the text thus:

"Given the weak dependence of σ_B upon I_p, W and δt shown in the previous section, we thus propose that σ_B in the resolving power range considered in this study (<4000) can be empirically parameterised by fitting a polynomial in log σ-space to the *m/Q* calibration-limited regime section of the results for high signal-to-noise (Figure S3). In this region $\sigma_I \sim \sigma_B$, i.e. the imprecision arising from calibration effects dominates and counting error can be considered negligible. This leads to the relationships:

$$\sigma_B(\text{child}) = \frac{R_I}{0.6}\left[10^{\left(0.6-0.41(\chi-0.4)-0.2(\chi-0.4)^2\right)}\right] \quad (3)$$

$$\sigma_B(\text{parent}) = 10^{\left(0.6-0.41(\chi-0.4)-0.2(\chi-0.4)^2\right)} \quad (4)$$

These equations are derived from simulations using the estimated best-achievable calibration precision. Following Table 1, a more conservative approach might choose values up to double those calculated using these formulae, according to the expected imprecision in the *m/Q* calibration. We note that, although the relationships are independent of resolving power, intensity and sample interval as shown in Table 1, for application to the child peak it does depend linearly on the intensity ratio R_I.

Given that σ_N is independent of χ and easily calculated from W, δt, I_p and R_I using eqn. 1, it is possible to describe the minimum estimated precision on I_c as the larger of the calculated σ_N and parameterised σ_B. The two lines cross at χ_d, the value of χ at which σ_I is observed to diverge from the counting-error limit of an isolated peak. For $\chi < \chi_d$, σ_I can be estimated from the parameterisation of σ_B, whereas for $\chi > \chi_d$, σ_I can be calculated directly and is equal to σ_N."

R2.16 Supplementary material: it should be presented in a more convenient way. E.g. I have problems figuring out which figure is S1.

The supplementary info is now combined into one single PDF, as was also requested by reviewer 1.

R2.17 12619, 6 and 12619, 26: the reference Titzmann et al. 2010 is misspelled

The misspelt references were corrected.

R2.18 12621, 22: "of" is not needed

This sentence was removed, as also noted in response to R1.1.