



# A Study of the Factors & Protocols that Influence Accurate Mass Measurement by FT-ICR MS to Improve the Confidence of Assignment of Elemental Composition

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## Abstract

- The confidence in a single mass measurement, rather than an averaged mass measurement is the critical factor when defining elemental formulae candidates following an accurate mass measurement<sup>1</sup>.
- A high confidence level, coupled with good accuracy and precision, allows limits to be defined to restrict the number of candidates in this list<sup>2</sup>.
- Here, an experimental protocol is discussed to determine the frequency of accurate mass measurements using a Bruker Apex III FT-ICR MS (no automatic gain control, AGC).
- This study expands previous work to encompass the presence of 2 compounds at varying concentrations rather than a single species at 3 set concentrations.
- External calibration is used at all times in order to decrease analysis time, a particularly crucial factor in a high throughput (HT) environment.

## Previous Work

- The original work comprised of 1 compound at 3 set concentrations, externally calibrated with solutions of 4 different concentrations
- If sample ion abundance is low, then the accuracy and precision of accurate mass measurement is more tolerant of a wider range of calibration ion abundances, a valuable consideration for an HT environment
- This is at variance with the commonly accepted view that matching of ion populations is critical to achieving the best accuracy for externally calibrated accurate mass measurement
- If the sample ion abundance is high, then the calibration ion population must also be high to maintain the same level of confidence in the individual mass measurement

## Experimental

- Instrumentation**
  - Apex III FT-ICRMS, Bruker Daltonics (Billerica, MA)
  - Equipped with ;
    - A 4.7 Tesla actively shielding superconducting magnet
    - An Infinity cylindrical analyzer cell
    - An Apollo electrospray ionisation source
- Samples, Standards and Reagents**
  - Two unknowns ( $m/z$  218 & 224 [M+Na]<sup>+</sup>), at 3 different concentrations; 0.1, 1, & 10  $\mu\text{g mL}^{-1}$  in methanol were cross mixed in a 1:1 ratio to give nine solutions: 0.1/0.1, 0.1/1, 1/0.1, 1/1, 0.1/10, 10/0.1, 1/10, 10/1, & 10/10  $\mu\text{g mL}^{-1}$
  - Soton Mix was used as the calibrant at 0.1, 1, 10 & 100  $\mu\text{g mL}^{-1}$  in methanol
- External Calibration & Sample Analysis**
  - Calibration solutions & samples were infused using a syringe pump (Harvard Apparatus, Holliston, MA) at a constant rate of 3  $\mu\text{L min}^{-1}$
  - Accurate mass measurements were performed on the 9 solutions (x 5) & externally calibrated with the 4 calibration files = 180 sets of data per experiment

## Results & Discussion

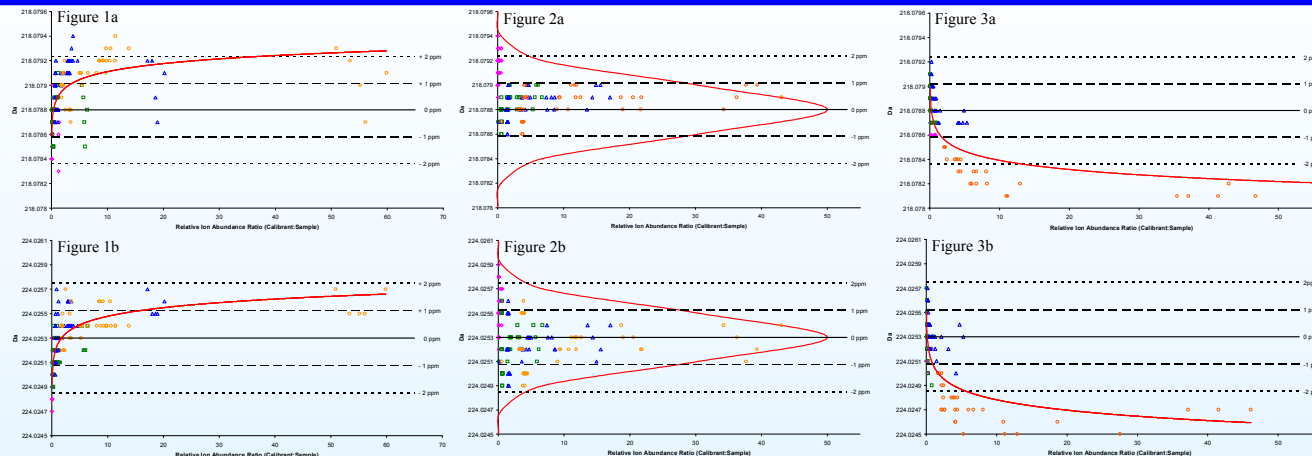
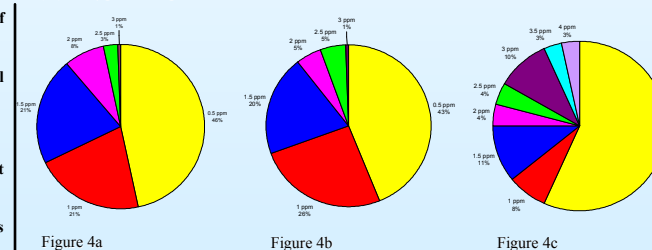


Figure series a & series b relate to ion 218.0788 & 224.0253 respectively. Experiments were acquired over a 6 month period; (1) DS1 [Xmass], (2) DS2 [Xmass & DA] & (3) DS3 [Apex & DA], with software shown in brackets. Calibrant concentration: ( $\diamond$ ) 0.1  $\mu\text{g mL}^{-1}$ , ( $\square$ ) 1  $\mu\text{g mL}^{-1}$ , ( $\Delta$ ) 10  $\mu\text{g mL}^{-1}$ , & ( $\circ$ ) 100  $\mu\text{g mL}^{-1}$ . Figure 4 shows the % error recorded for ion  $m/z$  224.0253 over the 3 experiments (a), (c) as before.

Plots were constructed of accurate mass measurement against the relative abundance (RA) of sample:calibrant

- The distribution of errors vary over time; from an upwards trend (Fig 1), to a normal distribution (Fig 2), and then to a downward trend (Fig 3)
- Generally, the higher sample-to-calibrant ratios give larger mass measurement errors
- However, the converse is not always true; the lower sample-to-calibrant ratios does not always give smaller mass measurement errors
- The % of errors have remained constant throughout, except DS3 where the error range was up to 4 ppm, which was not seen in DS1 & DS2



## Conclusions

- A high RA of sample:calibrant does not always give large mass measurement errors; inferring that ion population matching is not necessary to give the best accuracy
- Although the spread of errors varies with time, the actual number of errors <0.5 ppm does not vary to the same degree over time
- Only DS3 data fits with the previous work, i.e. with low sample abundance being more tolerant to a wide range of calibrant abundance

## Future Work

- Repeat the experiments, including acquiring the data over a shorter period of time in order to assess if the results reflect the previous findings
- Alter the mass range and position of the analytes on the mass scale (i.e. the frequency scale) & repeat these experiments. Repeat the measurements in the presence of a high mass ion, e.g. a small protein
- Further increase the complexity of the sample to reflect real sample issues to assess the extent of the space charge effects.
- Determine what drives these different mass error trends

## References

- J. M. Herniman, G. J. Langley, T. W. T. Bristow and G. O'Connor, *J. Am. Soc. Mass Spectrom.*, **16**, 1100 (2005)
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