

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 measurement¹

A high confidence level, coupled with good accuracy and precision, allows limits to be defined to restrict the number of candidates in this list²

Here, an experimental protocol is discussed to improve the confidence 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 environment³

Background

The original work comprised of 1 compound at 3 set concentrations, externally calibrated with solutions of 4 different concentrations¹

It was found that if sample ion abundance is low, then the accuracy and precision of accurate mass measurements is more tolerant of a wider range of calibration ion abundances, a valuable consideration for an HT environment¹

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

Instrumentation

Apex III FT-ICR MS using Apex & DA 3.3, Bruker Daltonics (Billerica, MA)

Equipped with: A 4.7 Tesla actively shielding superconducting magnet

An Infinity cylindrical analyzer cell

An Apollo electrospray ionisation source; direct infusion (3µL min⁻¹)

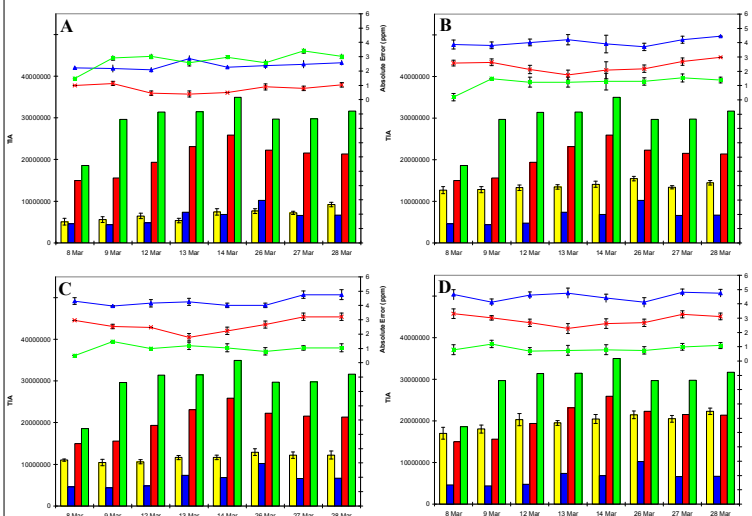
Experimental

Samples, standards and reagents: Two unknowns ([M+H]⁺ *m/z* 196 & 202), at 2 different concentrations; 1 & 10 µg mL⁻¹ in methanol. These were cross mixed in a 1:1 ratio to give four solutions: 1/1, 1/10, 10/1, & 10/10 µg mL⁻¹

'Soton Mix'¹ was used as the external calibrant at 1, 10 & 100 µg mL⁻¹ in methanol

Results & Discussion

The study was conducted over 8 days, recording the accurate mass measurements of 4 ions of interest; 196, 202, 218 & 224. MME (mass measurement error), the total ion abundance (TIA) of each sample & calibrant were also recorded



Figures A – D: The TIA of calibrants & samples with their associated MME for ion *m/z* 202. The 4 plots (A – D) are of solutions 1/1, 1/10, 10/1 & 10/10 respectively. Each coloured TIA column has a MME line in the same colour.

- The graphs show how TIA sample & TIA calibrant are linked in achieving minimum MME
- Minimum MME is never achieved when TIA Sample > TIA Calibrant
- Matching the TIA Sample to TIA Calibrant does not give the best MME
- Minimum MME is accomplished with a TIA calibrant:sample of ~3:1 (A), ~5:2 (B), ~3:1 (C) & ~3:2 (D)

All the MME & corresponding TIA calibrant:sample for ion *m/z* 202 were sorted into TIA calibrant:sample bins

The total number of MME, MME above & below 2.5 ppm per bin were recorded

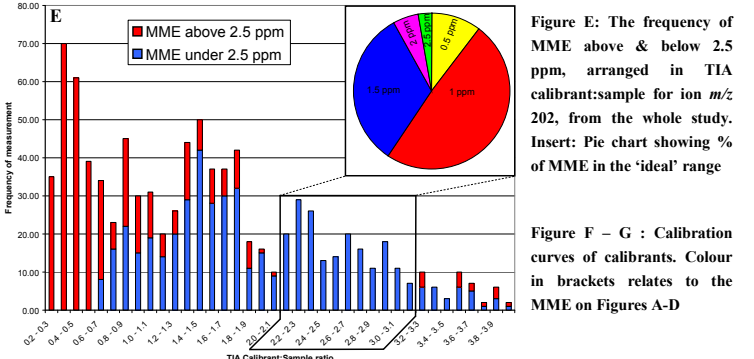


Figure E: The frequency of MME above & below 2.5 ppm, arranged in TIA calibrant:sample for ion *m/z* 202, from the whole study. Insert: Pie chart showing % of MME in the 'ideal' range

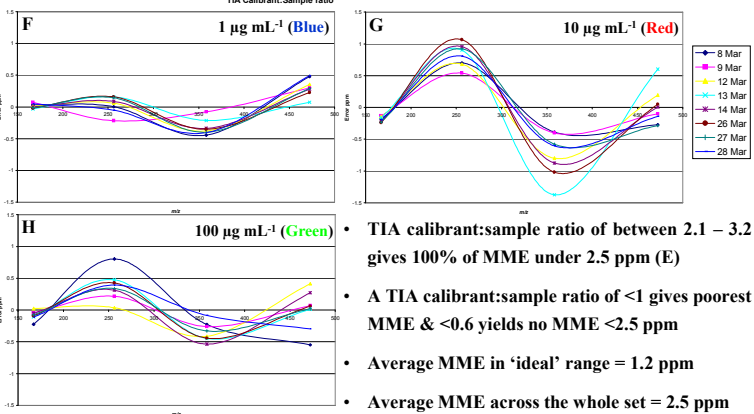


Figure F – G : Calibration curves of calibrants. Colour in brackets relates to the MME on Figures A-D

- TIA calibrant:sample ratio of between 2.1 – 3.2 gives 100% of MME under 2.5 ppm (E)
- A TIA calibrant:sample ratio of <1 gives poorest MME & <0.6 yields no MME <2.5 ppm
- Average MME in 'ideal' range = 1.2 ppm
- Average MME across the whole set = 2.5 ppm

Conclusions

- A protocol working towards high throughput external calibrated accurate mass measurements has been investigated
- Accurate mass measurements of 2.5 ppm & below can be routinely achieved without the need for extra equipment, new calibration protocols or addition software design
- Matching ion population of calibrant:sample does not give the best MME
- The TIA calibrant > TIA sample to achieve minimal MME

Future Work

- To explore the link between the calibration curves & MME achieved
- Expand the study to higher relative molecular mass compounds; ~ 400, 800 & 1500+
- Tailor make solutions to a specific calibrant:sample ratio & record MME
- Apply the protocol to flow injection & online HPLC experiments
- Ensure reproducibility by replicate experiments on a sister system

References

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