

# Accurate Mass Measurement in a Service Environment

Christopher M. Williams, Bridget K. Stein and A. Gareth Brenton

EPSRC National Mass Spectrometry Service Centre (NMSSC), Institute of Mass Spectrometry, School of Medicine, Swansea University, Singleton Park, Swansea SA2 8PP, U.K.

## Summary

- Robust method for accurate mass measurement is required by the National Service.
- Traditional method uses a 2-stage process on two different spectrometers.
- New instrumentation provides a 1-stage process with full-scan accurate mass.
- Target is to use new instrumentation to improve efficiency whilst maintaining robustness and ease-of-use of traditional method.

## Introduction & History of the 2-stage process

- The NMSSC provides an accurate mass service to the UK university community.
- Traditionally, the NMSSC has performed these analyses by peak-matching experiments using sector instruments with a resolution  $>8,000$ .
- In the last 10 years, electrospray has become the technique of choice due to ease and speed of sample introduction, increasing performance over traditional high-vacuum sources.
- Peak-matching requires a characterisation, (low-resolution) spectrum to establish the presence of the expected ion.

## Step 1: Characterisation Scan

- Low-resolution characterisation scans were obtained using a Fisons Quattro II (EI/CI) with Roboprobe autosampler or Waters ZQ4000 with Advion NanoMate (ESI).

## Step 2: Peak-Matching

- Accurate mass measurements were obtained using the Finnigan MAT 900XL mass spectrometer fitted with an electrospray source and operated with the standard operating procedures<sup>1</sup>.
- Xcalibur v1.4 SR1 with high resolution mass spectrometry programs v1.4 software was used to produce lists of elemental formulae.
- Typical Source Settings used:
  - Accelerating voltage: 5kV;
  - Capillary temp.: 230°C;
  - Spray voltage: positive ionisation; +3.0kV.
- Samples were loop injected into a stream of water / methanol (1:1) flowing at 30 to 60µL/min.
- Polyethyleneimine (PEI) is introduced to the system via the 'Sheath Liquid' port on the ESI source, typically at a flow rate of 10µL/min.

## Results output: Peak-matching

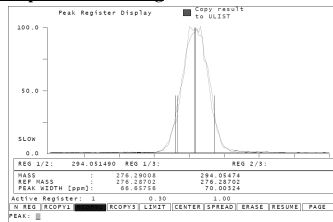


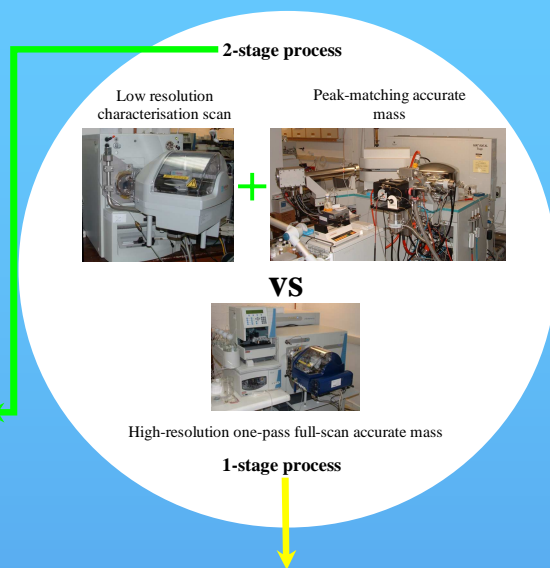
Figure 1: Peak-matching of a sample ion and reference ion

## Chemicals

PEI (low-molecular weight), ammonium acetate ( $\text{NH}_4\text{OAc}$ ), diethylamine (DEA) and caffeine were purchased from Aldrich (Dorset, UK). HPLC grade dichloromethane (DCM), methanol (MeOH) and water were purchased from Fisher Scientific (Loughborough, UK). MRFA was purchased from Research Plus, Inc. (New Jersey, USA). Ultramark 1621 was purchased from Alfa Aesar (Massachusetts, USA). Samples were obtained as part of the routine service of the NMSSC.

## Accuracy of measurements

- Sector instruments have been proven to be highly accurate<sup>2</sup>
- The Orbitrap is a relatively new analyser, which has been reported to have good mass accuracy and dynamic range<sup>3</sup>
- Data analysis has been performed on the measurements made using both instruments.
- Peak-matching showed a characteristic distribution about 0
- One-pass accurate mass showed a wider distribution of measurements from the calculated mass.
- RMS mass error is 0.35mDa (peak-matching) and 0.43 mDa (one-pass accurate mass), see Figure 4.



## New 1-stage process - Full-scan Accurate Mass

- Accurate mass measurements were obtained using the ThermoFisher LTQ Orbitrap XL mass spectrometer fitted with an Advion Nanomate Triversa nano-electrospray source.
- Accurate mass and characterisation spectra were obtained simultaneously at a resolution of  $10^5$  (at  $m/z$  400).
- Typical Source Settings used:
  - Nano-electrospray voltage: 1.4kV;
  - Capillary temp.: 200°C;
- External mass calibration was performed each day; Xcalibur v2.0.7 software used to process data.
- With a few exceptions<sup>4</sup>, samples for positive mode analysis were diluted into 1ml MeOH (10%  $\text{NH}_4\text{OAc}$ ) before loading into the NanoMate tray.
- Samples requiring confirmation of anion were analysed by switching polarity on the LTQ – due to the limitation of the NanoMate this required a second analysis.
- Using the standard electrospray source with loop-injection or LC autosampler, polarity-switching can be performed in one pass.
  - Salts, organometallics, water sensitive compounds

## Results output: One-pass Accurate Mass

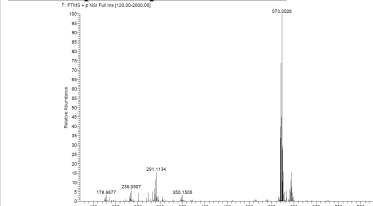


Figure 2: Sample output from LTQ Orbitrap XL. Data acquired at R=100,000

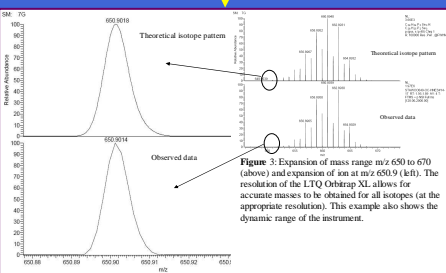


Figure 3: Expansion of mass range  $m/z$  650 to 670 (above) and expansion of ion at  $m/z$  650.9 (left). The resolution of the LTQ Orbitrap XL allows for accurate masses to be obtained for all isotopes (at the appropriate resolution). This example also shows the dynamic range of the instrument.

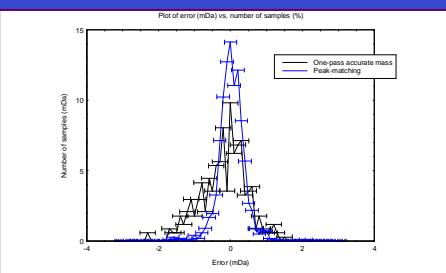


Figure 4: Plot of error (mDa) vs. number of samples (%). Number of samples for peak matching 1004, number of samples for one-pass accurate mass 336.

## Examples of analyses facilitated by the new method:

### Example 1 – Polyphosphates / polysulphonates

- Addition of DEA enhances analysis in negative ion mode<sup>5</sup>.
- Previous method required  $\text{Na}^+$  TFA- to be run concurrently with the sample as a reference compound, negating the DEA effect and rendering no molecular ion.
- New method allows effective addition of DEA to observe and measure  $[\text{M}-\text{H}]^-$ :

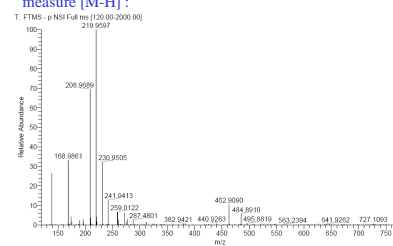


Figure 5: Spectrum of a polyphosphate analyte resulting from the addition of DEA

- The above example shows a polyphosphate with ion series  $[\text{M}-n\text{Na}+(n-1)\text{H}]^-$

### Example 2 - Characterisation of contaminant

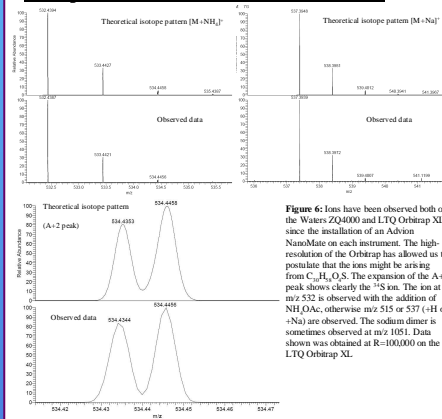


Figure 6: Ions have been observed both on the Waters ZQ4000 and LTQ Orbitrap XL since the installation of an Advion NanoMate on each instrument. The high-resolution of the Orbitrap has allowed us to postulate that the ions might be arising from  $\text{C}_8\text{H}_8\text{O}_8\text{S}$ . The expansion of the A-2 peak shows clearly the  $^{35}\text{S}$  ion. The ion at  $m/z$  532 is observed with the addition of  $\text{NH}_4\text{OAc}$ , otherwise  $m/z$  515 or 537 (+H or +Na) are observed. The sodium dimer is sometimes observed at  $m/z$  1051. Data shown was obtained at  $R=100,000$  on the LTQ Orbitrap XL.

## Discussion

- Both old and new methods have proved to be robust and effective.

### Advantages of new method:

- Improved laboratory efficiency, throughput and cost effectiveness.
- Use of Triversa NanoMate allows high sample throughput, with minimal repeats or carry-over (4 analyses per minute).
- Facility for accurate mass MS/MS and  $\text{MS}^n$  adds value.
- High-resolution with accurate mass over the full-scan mass range gives increased confidence for characterisation due to:
  - Accurate masses of all isotopes
  - Elemental identification (e.g.  $^{34}\text{S}$ )
- Improved efficiency (ca. 30%) and cost-effectiveness.
- Faster sample turnaround.
- Improved analytical quality and extended capability.

### Advantages of old method:

- A broad range of ionisation modes is required for samples unsuited to electrospray.
- If a characterisation spectrum is provided, the high speed of the traditional method still makes it useful.

- Both old and new methods will continue to be used for the foreseeable future.

## Acknowledgements

We wish to thank EPSRC for funding this work (grant nos. GR/M13992, GR/R70088/01, EP/F014341/1).

## References

1. NMSSC website, [www.swansea.ac.uk/nmssc](http://www.swansea.ac.uk/nmssc)
2. Bristow, A.W.T. and Webb, K.S., *J. Am. Soc. Mass Spectrom.*, **14**, 1086-1098 (2003)
3. Makarov, A., Denisov, E., Lange, O. and Horning, S., *J. Am. Soc. Mass Spectrom.*, **17**, 977-982 (2006)
4. Slater, P.S., Ballantine, J.A. and Games, D.E., *Rapid Commun. Mass Spectrom.*, **11**, 624-629 (1997)