Intelligent internal recalibration

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Abstract

Using the new Beacon ionisation source (Vibrat-Ion Ltd, UK), we have successfully analysed complex organic samples (Whisky and Suwannee River Fulvic Acids) using in-house developed automated internal recalibration. The Beacon ionisation source uses a piezoelectric device to introduce calibrant into conventional analysis techniques (e.g. electrospray ionisation) at defined intervals. The software then identifies the points at which calibrant has been introduced, and uses these to perform automated internal recalibration, eliminating the need to manually identify calibrant peaks within the spectra.

Introduction

It is common practice to have to use internal mass recalibration to get the best mass accuracy. Then, this mass accuracy is used to assist in the process of peak assignment. For complex spectra, we commonly have to use manual assignment steps in order to find and use the internal recalibration peaks – that is, if there are confidently known, positively identifiable peaks to use. But, even if such peaks are available in your data, if you have a large number of spectra to process, then this manual assignment step is both slow and can result in accidental errors. If you add additional internal recalibration compounds to your sample then this can introduce other issues. The Beacon ionisation source, presented herein, overcomes these limitations.

Methods

Sample Preparation:

The whisky sample was prepared using a 1:10 dilution with 50:50 ACN:H_2O. The SRFA sample was diluted to 0.1 mg/mL in 50:50 ACN:H_2O. The calibrant for the Whisky analysis was a 0.5 mM Sodium Formate solution. Triton, a PEG based polymer series, was chosen for the SRFA analysis calibrant, which was diluted to 0.1 mg/mL.

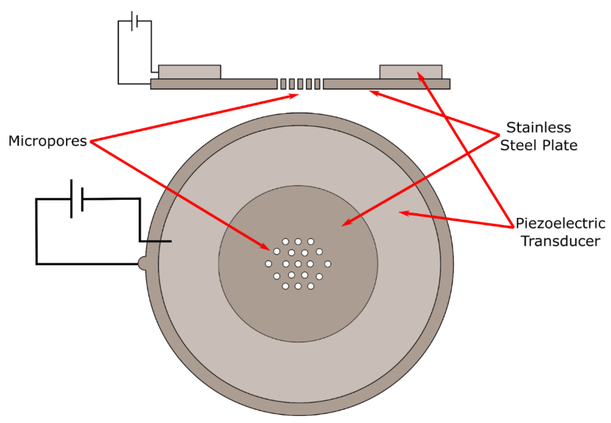


Figure 1 – PUPP Schematic

Instrumentation:

Bruker solariX 12T FT-ICR with 200 ms transient length. Both samples infused through a 75 µm fused silica ESI needle, which was manufactured using an in-house developed 3D printed grinding system

Beacon Ion Source:

The beacon ionisation source uses a piezoelectric device to introduce plumes of microdroplets (≈ 5 µm) of calibrant onto the tip of an electrospray needle, which subsequently co-electrosprays with the sample. A schematic of the device used is shown in Figure 1, and the set up of the system with the FT-ICR is shown in Figure 2.

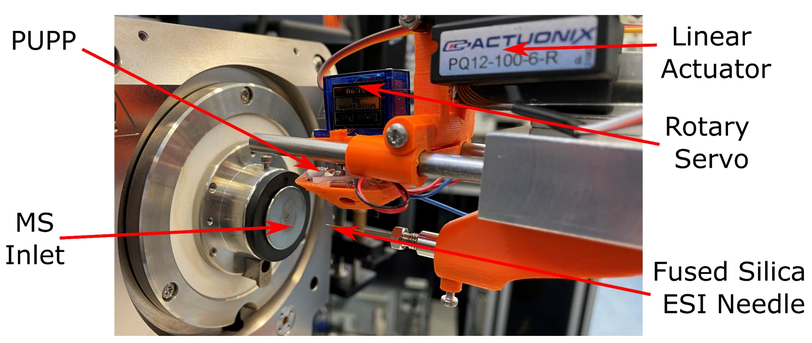


Figure 2 – Beacon ion source coupled to 12T Bruker FT-ICR

The ion source was set to introduce 50 millisecond bursts of calibrant during every 5th ion accumulation event. A typical plume of microdroplets generated by the PUPP is shown below in Figure 3. The ion source and the ion accumulation events were synchronized by using a trigger from the ICR.

Figure 3 – PUPP Droplet Generation

Results

A representative spectra for the SRFA sample analysis has been given in Figure 4, which shows the spectra obtained with and without the calibrant (Triton) present.

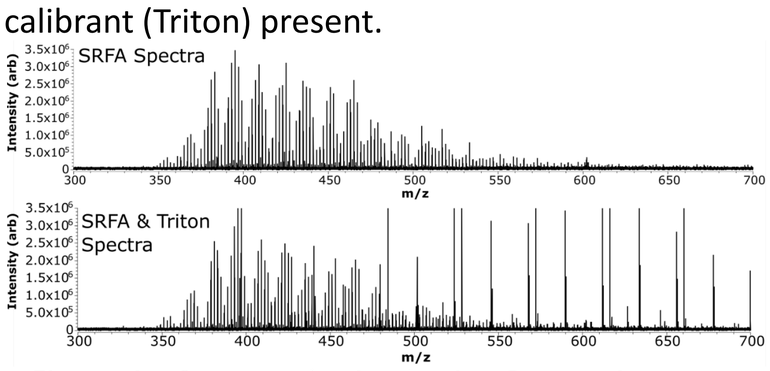


Figure 4 – Spectra obtained using Beacon ion source

The data was subsequently internally recalibrated using the reduced workflow the Beacon software offers. The improvement in mass accuracy of the sample peaks is shown in Figure 5 compared to the conventional analysis data which was not recalibrated.

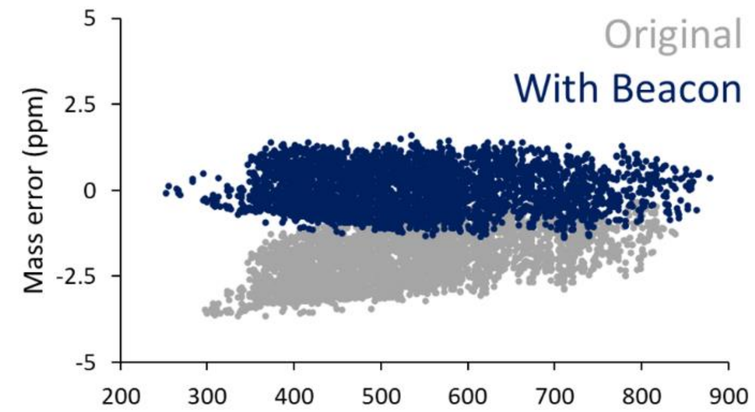


Figure 5 – SRFA Mass Accuracy with and without the Beacon workflow implemented

The intensity of the calibrant peak can be adjusted to suit the intensity of the sample spectra peaks through modification of the ion source duty cycle, as shown in Figure 6.

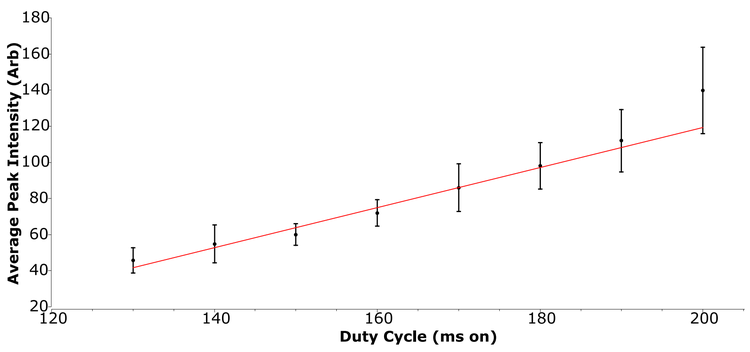


Figure 6 – Effect of duty cycle on calibrant intensity

As mentioned previously, the ion source and FT-ICR accumulation events were synchronized using a trigger. Without this synchronization, an inconsistent number of ions are scanned, and therefore a mass shift between scans is observed due to the space charge effect, shown in Figure 7.

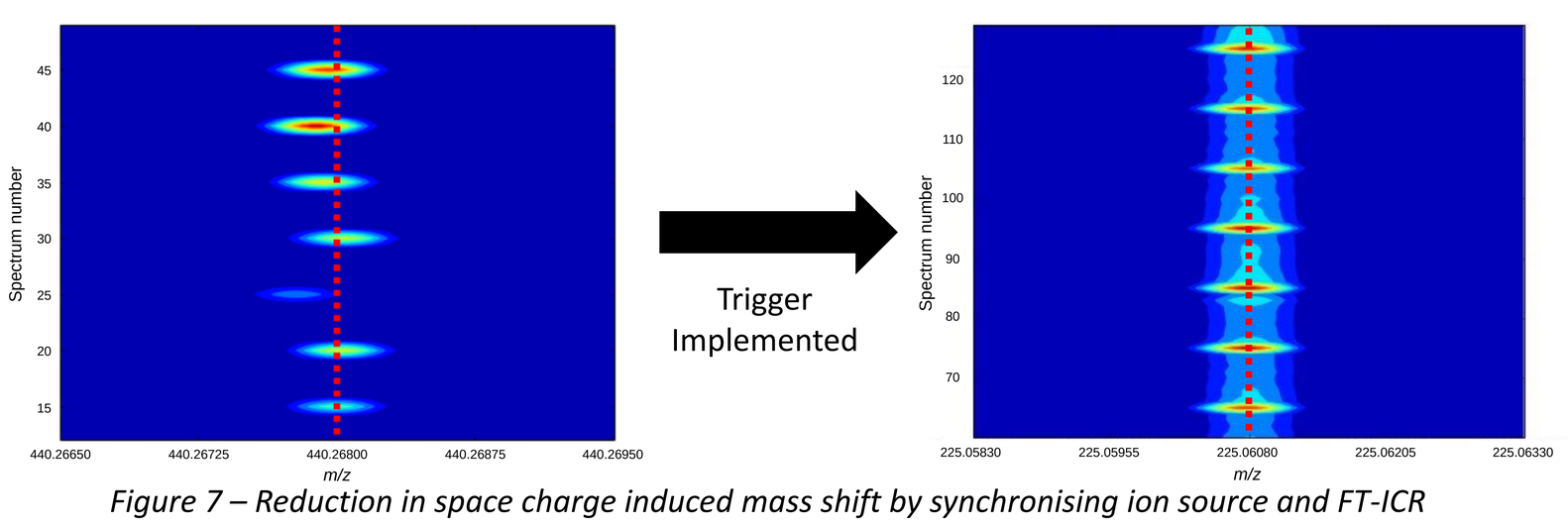


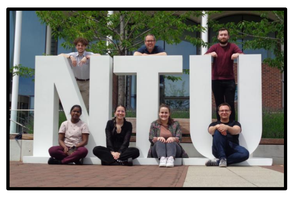
Figure 7 – Reduction in space charge induced mass shift by synchronising ion source and FT-ICR

Other Work and the Future

Alongside the work we have conducted with intelligent internal recalibration, we have also demonstrated the Beacon ionisation source's ability to perform rapid analysis of pharmaceutical, beverage and biological samples, rapid adduct modification and component confirmation through overspraying.

Looking ahead, we are working to demonstrate the ability to perform rapid in-source protein and antibody enzymatic digestions, exploring other charge inducing methods and continuing to improve the existing hardware and software.

If you are interested learning more about any of our work, are interested in having some sample analysis performed, or would like to collaborate in any other way, please speak to any of our team.



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