A RAPID ACQUISITION AND EXTRACTION TOOL FOR PROCESSING OF DESI SCREENING APPLICATION DATA

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INTRODUCTION

DESI is a technique typically used for imaging style applications. Traditionally investigating compound localization within a sectioned tissue or on the surface of an object such as a leaf.

However, as well as being a successful mass spectrometry imaging inlet, DESI can also be utilized as a rapid sample delivery source for screening a variety of samples.

For example here we demonstrate the feasibility of using a DESI imaging source as a suitable method for direct analysis of blood product lipid profiling, drug library screening, and cell culture monitoring.

This technique could also potentially increase speed of sample analysis in applications such as real time reaction monitoring or bioreactor upstream and downstream sample analysis.

In order to maximize sample throughput, we have found it is best to acquire all sample data within a single line pass, rather than as individual acquisitions per sample. As such, all samples are acquired within the same raw data file.

Consequently this can complicate subsequent data analysis, as most processing software will expect each sample to be a unique data-file. Initially a perl script based solution was prototyped allowing the segmentation of data based on predicted time. However, the sample spot is generally smaller than the sample well, and the exact position of the spot can vary, as such additional non sample related spectra were also included. Here we present a new software tool in development which allows for data segmentation Based on TIC/XIC and facilitates downstream processing for multiple applications.

Figure 1 A DESI XS source

METHODS

A variety of sample types have been analyzed and subsequently processed using the Chrom Splitter applications.

All sample types were analysed on slides with PTFE sample wells spotting 1-2 µL of analyte per spot.

Figure 2: The data were acquired in a single pass through the centre of the spots on the slides as single lines (n=44, with 11 spots per line).

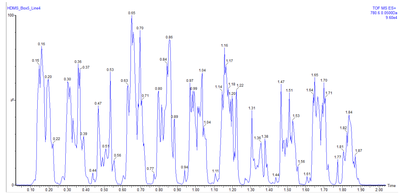

Figure 3: Example XIC (displayed in MassLynx) showing a line of 11 IPA crashed plasma samples. Extracted mass: m/z 780.6 putatively identified as PC 37:3 (mass accuracy -2 ppm).

Data were acquired using a DESI XS source on: a standard QToF mass spectrometer, an ion mobility enabled Q-ToF mass spectrometer and a high resolution (>200,000 FWHM) multi reflecting Q-ToF mass spectrometer. After acquisition data can be investigated manually within MassLynx™.

To perform library searching or statistical analysis on the samples each data file (containing multiple sample data) was processed through the Chrom Splitter app. The resulting segmented files processed with several different software packages:

- Progenesis™ *(Figure 5)*
- UNIFI™ *(Figure 6)*
- Skyline (1) *(Figure 7)*
- DriftScope™ *(Figure 8)*

RESULTS & DISCUSSION

To facilitate rapid sample screening using direct sample analysis on a DESI source, it is beneficial to acquire data as a single raw file containing data for multiple samples. An example TIC containing 10 samples and a QC can be seen in *Figure 3.*

The majority of commercially available software packages for subsequent data analysis expect each sample acquisition to be in individual raw files. This can mean that the time gained from fast acquisition is lost in data processing due to the complex manual nature of sub-dividing the data. The file will also contain redundant scans generated from areas between spots, which may lead to inaccuracies in data comparison.

To provide a user-friendly workflow we have developed an app that allows data acquired in this format to be sub-divided into individual sample files. A new software solution (Chrom Splitter) has been developed which provides sample detection and extraction based on: Total Ion count (TIC), Extracted ion Chromatogram (XIC) of a specified mass range, or the sum ion count of a set of target masses, depending upon user preference. There is an additional option for smoothing to compensate for fluctuations in ion signal. *(Figure 4)*

Example TIC's produced by Chrom Splitter can be seen in *Figure 4*, this shows the segmented raw chromatograms for the first four spots of a IPA crashed human plasma sample. Gaps between the traces show where redundant scans have been discarded thereby reducing the overall size of the data sets and allowing samples to be loaded independently into a range of software applications.

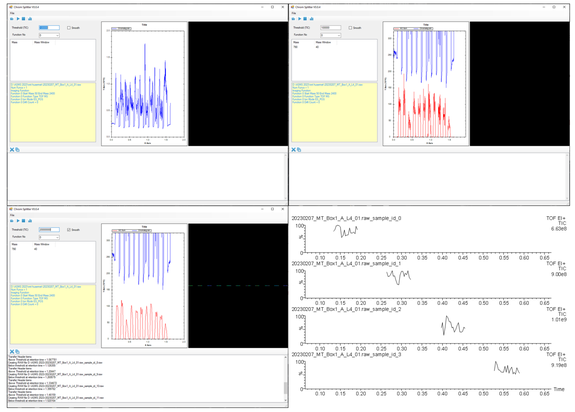

Figure 4. Example of data segmentation in chrom splitter app. Top Left, imported raw chromatogram. Top Right, imported data with XIC trace for m/z 770-810. Bottom Left smoothed XIC. Bottom right extracted spot data TIC in MassLynx. 11 spots were correctly extracted using the XIC cutoff.

The data-file produced by the app can be processed in any processing software that supports traditional Waters™ .raw file format.

Figure 5. An IPA crashed human plasma samples processed through Progenesis QI to provide statistical analysis, looking for features of interest within the cohort.

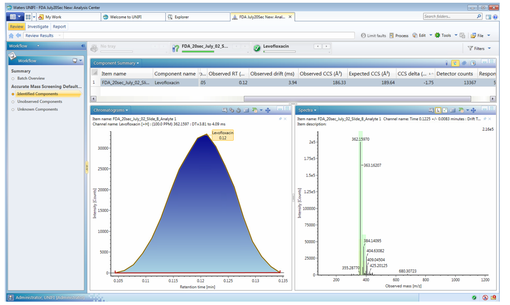

Figure 6. An example of drug library screening being processed through UNIFI providing library searching including CCS information .

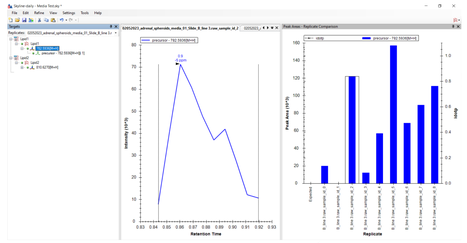

Figure 7. An example of IPA crashed spent cell Media in Skyline([1]), showing a variation in a lipid signal for varying spheroid growth conditions

Chrom splitter will also retain all data functions such as collision cross section information, and the collection of fragmentation data for each sample.

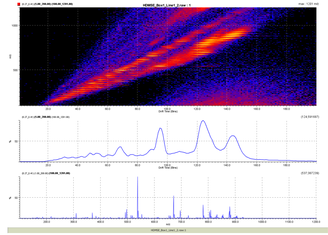

Figure 10. An example of IPA crashed serum HDMS^E data loaded into Driftscope, demonstrates retention of the drift time dimension.

CONCLUSIONS

Initial feasibility assessment of the Chrom Splitter App was performed using a number of representative samples:

- IPA crashed serum samples from the HUSERMET study (Stockport, UK),
- an FDA drug library screen,
- IPA crashed spent cell media.

A number of data acquisition formats and instrument platforms have been tested to ensure compatibility with the app:

- conventional MS,
- high resolution MS
- HDMS^E analysis.

Confirming that the app can be used to generate individual .raw files from a number of different sample types and analysis formats whilst retaining all additional information such as ion mobility when available.

The data-files produced by the app could be imported into a number of different software platforms and successfully processed.

References

1. Brendan MacLean *et al.* Bioinformatics. 2010

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