**OVERVIEW**

- **SONAR**® DIA and UDMSE® operated alternately on the same instrument platform.
- Characteristics of the two methods are compared.
- **SONAR**® DIA analysis of samples derived from patients with different respiratory conditions.

**INTRODUCTION**

In discovery applications, Q-Tof's are generally operated in data-dependent or data-independent acquisition modes. SONAR is a data-independent acquisition (DDA) and data-independent (UDMSE®) technique that can be used to analyze the spectra of plasma samples of COPD, Asthma and Control patients. Initially, we investigate the different methods to be employed in alternate experiments on a single MS platform. We describe here the use of new SONAR and UDMSE® methods.

**METHODS**

Samples
- MPS Mixture 1, four protein tryptic digests. (Hawkes)
- MPS Oxidative (dig) (Waters)
- UDMSE ‘ ’

**RESULTS AND DISCUSSION**

**Specificity and Data Extraction**

- **Sonar and Udmse**
- **Mobility Separation**

**Mass Spectrometry**
- Synapt G2Si with Nanodrop and PicoTorr® inlet.

**Integration Time**
- 0.5s for alternating low (LE) and elevated (EE) scans.

**Collision Energy**
- 25.5% for UDMSE®.

**Energy Bin Number**
- Masses for each experiment and separated using a 90-minute gradient.

**Integration Lengths**
- 30, 45 and 90 minutes + reequilibration.

**Energy**
- 445.112

**Mobility**
- 519.3

**RESULTS**

- A total of 292 proteins are quantified within the plot and, whilst there appears to be no definitive groupings of the COPD and Asthma samples, they appear to be mainly separated from the Control and QC samples which are well grouped in the middle of the plot. Further exploration of the sample metabolites and ion mobility matrix comparisons between different groups described therein may prove interesting. A much larger sample number would be beneficial to fully investigate potential sample differences.

**CONCLUSION**

- **SONAR**® DIA and UDMSE® methods have been used alternating on the same instrument platform and automatically reorganizing the acquisition system.
- **SONAR**® DIA and UDMSE® acquisition provide multi-dimensional data sets exhibiting excellent specificity, allowing spectra to be obtained which may have interfered with other techniques.

**References**
2. Richardson et al, ASMS 2015

**Figure 5.** Normalised **SONAR**® DIA ion energy TIC chromatograms from the increasing window size. 100-400 Da in 400 Da windows was selected on 400 Da for each experiment and repeated using a 10-minutes gradient. **SONAR**® DIA in the same load for 40 Da and 100 Da. The data suggests that whilst a narrower window gives more specificity, the consequent loss in signal due to the duty cycle (peak range/peak width) has an effect on protein identifications.

**Figure 6.** Comparison of **SONAR** and UDMSE in E Cof Protein Scans vs 100-400 Da. UDMSE shows an increase in protein peaks based upon a peak window size of 100-400 Da which appears to the optimum. Protein identifications for **SONAR**® DIA’s samples optimise at much lower loadings and may be explained by the advantage of EIC (energy integrations) on each sample amount are not limited. Also, data file sizes are approximately 50% less for **SONAR** compared to UDMSE®.

**Figure 7.** COPD vs Normal vs Asthma Airways

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<tr>
<td>Mobility Bin Number</td>
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**Figure 8.** PCA Fits for COPD, Asthma, Control and QC