The next frontier in extractables screening analyses: Increased identification confidence with a benchtop multi-reflecting time-of-flight mass spectrometer

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INTRODUCTION

- **Due to concern about the safety of components from plastic, it is crucial to screen for potential extractables in pharmaceutical packaging and medical devices.[1,2,3]**
- **Compounds found at levels above the analytical evaluation threshold must be identified and reported for toxicological assessment.[4]**
- **The confidence level of these identifications must be as high as possible which can be challenging due to the complexity of samples, possibility of false positives, and the number of possible compound identifications for each chromatographic peak within the data.**
- **Here we report an extractables analysis using a benchtop multi-reflecting time-of-flight mass spectrometer with high mass accuracy combined with high mass resolution for precursor and fragment ion data which is critical to ensure increased confidence in screening and identifications.**

Figure 1. Waters Xevo MRT Mass Spectrometer and ACQUITY Premier System

METHODS

Sample Preparation

Three cuttings of three different types of plastic packaging were thermally shaken at 50 °C in IPA for 24 hours alongside a negative control and a pooled QC. Samples were spiked with the Waters E&L SST before injection onto the instrument in triplicate.

LC Conditions: ACQUITY™ Premier System

Column: ACQUITY CORTECS™ C18, 90 Å
(1.6 µm, 2.1 x 100 mm Column)
Mobile Phase A / B: Water + 1 mM ammonium acetate + 0.1% formic acid / Methanol
Flow Rate: 0.3 mL/min
Column Temperature: 50 °C
Injection volume: 1 µL
Gradient : Mobile phase B was held at 2% for 0.5 minutes before it was ramped to 98% over 5 and a half minutes then held for 7 minutes. It was then dropped to 2% for 2 minutes.

MS Conditions System: Xevo™ MRT MS

Ionization mode: ESI+
Acquisition mode: MS^E
Source temp: 120 °C
Desolvation temp: 550 °C
Desolvation gas flow: 800 L/hr
Cone gas flow: 50 L/hr
Acquisition range: m/z 50-1200
Capillary voltage: ESI+ 2.5 kV
Collision energy:
Low energy: 6 eV
High energy ramp: 20-40 eV

Data Management

The waters_connect™ platform was used for data acquisition and the UNIFI™ Application out of the waters_connect™ software platform was used for data processing. All statistical analyses were undertaken with EZInfo™ 3.0.

Figure 2. Plastic and foil packaging from three different antihistamine packaging.

INTRODUCTION

The Xevo MRT MS has high mass accuracy. For all compounds in the standard across all injections the RMS was 0.81 ppm for mass errors across all injections.

Figure 3. Component review for E&L SST.

STANDARD REVIEW

Data were acquired using the MS^E mode of acquisiton, providing the high mass accuracy of associated fragment ions for all compounds.[5]

Figure 4. Mass error of diethyl phthalate fragments as a pure standard.

SST spiked into the sample shows mass accuracy across standards and samples to be ≤1 ppm.

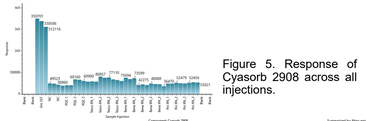


Figure 5. Response of Cyasorb 2908 across all injections.

Figure 6. Mass accuracy (ppm) of Cyasorb 2908 across all injections.

SAMPLE SCREENING

Accurate mass of diethyl phthalate fragments and associated fragment ions is ≤1 ppm.

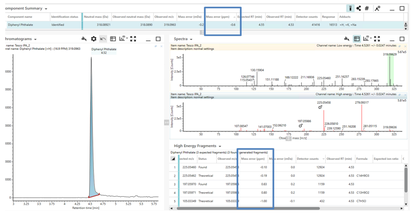


Figure 7. Mass error of diethyl phthalate fragments injected into samples.

Response of bis(2-ethylhexyl)adipate identified in the Waters E&L library.[6] RMS of 0.77 ppm mass errors across all injections.

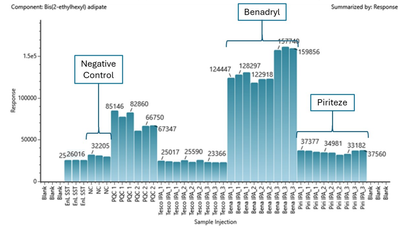
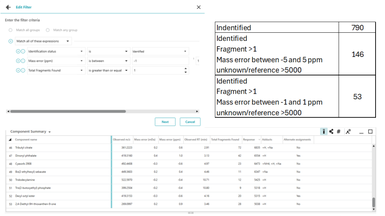


Figure 8. Bis(2-ethylhexyl)adipate elevated in Benadryl and slightly elevated in Piriteze, compared to the negative control.

Filters can be applied to significantly reduce the number of potential possible candidates.



Indentified	790
Identified Fragment >1 Mass error between -5 and 5 ppm unknown/reference >5000	146
Identified Fragment >1 Mass error between -1 and 1 ppm unknown/reference >5000	53

Figure 9. Filters can be applied to the data. Data can also be filtered to only include components elevated or unique to the sample compared to the negative control.

STATISTICAL ANALSYIS AND ELUCIDATION

Principal Components Analysis (PCA) plot highlighting variation between the three samples types, negative control, and the pooled QC.

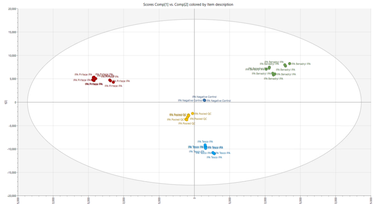


Figure 10. PCA plot showing excellent technical reproducibility between samples and the variation between sample groups.

OPLS-Da shows the variation between the negative control and one of the samples. The markers furthest away on the S-Plot are more statistically significant. These can be selected for elucidation in the UNIFI Application.

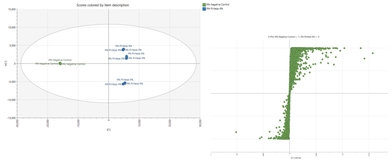


Figure 11. OPLS-Da and S-Plot analyses of the negative control and Piritize sample extractions. The inter group variation of the sample is expected as the samples were not milled down.

The markers are then displayed using the Discovery Tool in the UNIFI Application. The trend plot for one of the elevated markers is displayed.

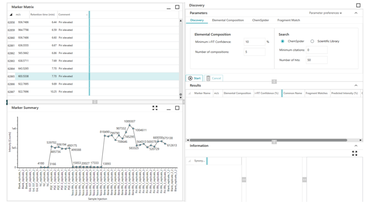


Figure 12. The UNIFI Discovery Tool and the markers selected using statistical analysis.

The Discovery Tool uses the accurate mass and isotope pattern to predict chemical formulas which it then searches against databases for structures before preforming in-silico fragment matching.[7] The highly accurate mass of the precursor and fragment ions are utilized for this process.

Figure 13. Example of a predicted identification of N,N'-1,12-dodecanediyldidodecanamide (mass error –0.77 ppm). A standard would be needed for a 100% match but this greatly reduces the processing time for the analyst.

CONCLUSION

- **Screening with the Xevo MRT Mass Spectrometer is a highly specific tool for E&L screening analyses.**
- **Using MS^E provides highly accurate mass data of both precursor and fragment ion data in sample matrix.**
- **This greatly increases confidence in identifications whilst also reducing the false positive rate and significantly reducing the possible candidate matches.**
- **Software tools are utilized to review highly accurate mass data within complex datasets, allowing for confident extractables screening analyses.**

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