SCREENING FOR EXTRACTABLES AND LEACHABLES IN NASAL SPRAY DEVICES USING HIGH-RESOLUTION MASS SPECTROMETRY COMBINED WITH A DATA INDEPENDENT INFORMATICS STRATEGY

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INTRODUCTION

It is crucial to detect and identify potential extractables and leachables (E&L) through screening studies due to the potential of harmful chemical species migrating from medical devices, pharmaceutical container closure systems, and manufacturing components.

Regulations and standards are in place to ensure safety limits are met for devices such as nasal drug products and there are several challenges when undertaking these studies to meet these regulatory requirements[1,2,3] For example, analytical instrumentation needs to be highly sensitive to detect low level chemical species to meet expected screening thresholds. Additionally, the ability to screen for E&L compounds and elucidate unknowns on the same analytical platform is important.

Here, we describe an E&L screening experiment using ultra-performance liquid chromatography and a quadrupole time of flight high-resolution mass spectrometer (UPLC-QToF HRMS). A data independent acquisition (DIA) strategy is utilized to aid screening and elucidation combined in one informatics workflow solution. The quadrupole time-of-flight mass spectrometer (QToF MS) can acquire data in MS^E mode, whereby both low and high collision energy spectra are simultaneously acquired. Using this technique, the accurate mass of both precursor and fragment ions are available, both of which aid structural elucidation and, ultimately, compound identification.

Figure 1. ACQUITY Premier System with the Xevo G3 QTof mass spectrometer.

METHODS

Sample Preparation

Three commercial nasal sprays were purchased due to the complexity of materials in the container closure system The nasal container closure system was extracted with isopropanol for 72 hours at 40 °C along with a procedural blank. The procedural blank and extracted samples were injected in triplicate on the instrument. An E&L system suitability (SST) mix was also injected at the start and end of the acquisition. Data was acquired and processed in one screening workflow informatics platform.

METHODS

LC Conditions

LC system:	ACQUITY™ Premier System
Column:	ACQUITY CORTECS™ C18, 90 Å (1.6 µm, 2.1 x 100 mm column)
Mobile Phase A:	Water + 1 mM ammonium acetate + 0.1% formic acid
Mobile Phase B:	Methanol
Column temp.:	50 °C
Injection volume:	1 µL
Gradient:	

Time (min)	Flow Rate (mL/min)	% MPA	% MPB	Curve
0.0	0.3	98	2	Initial
0.5	0.3	98	2	6
6.0	0.3	1	99	6
13.0	0.3	1	99	6
13.1	0.3	98	2	6
15.0	0.3	98	2	6

MS Conditions

MS system:	Xevo™ G3 Qtof mass spectrometer
Ionization:	ESI+
Acquisition mode:	MS^E
Source temperature:	120 °C
Desolvation temp.:	600 °C
Desolvation gas:	800 L/h
Cone gas:	50 L/h
Acquisition range:	*m/z* 50-2000
Acquisition scan time:	0.2 s
Capillary voltage:	ESI+ 1.0 kV
Collision energy:	ESI+ Low energy 6 eV
	ESI+ High energy ramp 20-40 V

Data Management

The UNIFI™ application within the waters_connect™ platform was used for acquisition and data processing.

DIA

The XS collision cell in the Xevo G3 QTof, highlighted in Figure 2, lends us the capability to run MS^{E,S} Alternating between low and high collision energy enables the simultaneous acquisition of precursor and fragment ions throughout the entire chromatographic run. During data processing, precursor and fragment ions are precisely aligned based on their retention time. Every peak has the exact mass of the compound and its corresponding fragments. This information helps for screening and in the structural elucidation of unknowns.

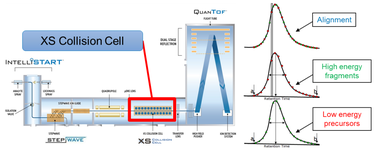

Figure 2. Xevo G3 QTof schematic highlighting the XS collision cell providing MS^E capabilities.

RESULTS

Using the UNIFI application, the data were processed within an E&L specific workflow (Figure 3A). The E&L workflow can be customized to user requirements and helps to streamline the data analysis. An E&L system suitability test (SST) mix was injected to benchmark the system. The mass spectrometer has had updates to the ion optics and detection system to maximize transmission and proved to be highly sensitive (10 fold increase in response compared to previous instrument iterations) and reproducible for the SST mix (0.01% RSDs for retention time) (Figure 3C). This increase in sensitivity helps with the challenge of achieving trace level identification in E&L studies. Mass accuracy for all detected compounds had a mass error of less than 3 ppm (Figure 3B). Mass accuracy aids library matching and elemental composition calculation to ultimately aid full characterization.

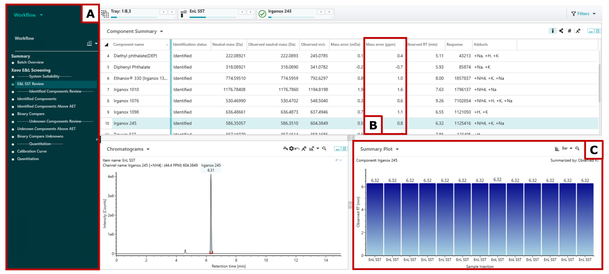

Figure 3. The SST results are displayed for easy data interpretation, including experimental results for each analyte in the SST mix, the extracted ion chromatogram for Irganox 245, and a summary plot for this compound. [A] Example of the customizable UNIFI workflow. [B] Mass ppm error for each analyte. [C] Retention time for Irganox 245 for each injection of the SST mix.

As the Xevo G3 QTof was used in MS^E mode for DIA, this enabled full acquisition of the accurate mass information of both precursor and fragment ions (Figure 4). Having the accurate mass information available for each peak in the chromatogram increases confidence when identifying compounds against a library if MS/MS spectra are included.

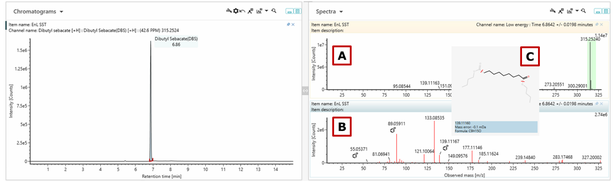

Figure 4. *An example of MS^E data for dibutyl sebacate. [A] Low energy spectra with the protonated precursor ion ($C_{18}H_{34}O_4$, mass error –1.0 ppm). [B] High energy spectra with the fragment ions. [C] Hovering over the symbols in the high energy spectra displays the predicted fragment ion for that mass and the mass error associated with it.*

The samples were then investigated by screening against the Waters E&L scientific library[4] to find matches for accurate mass, retention times, and mass fragments. With these parameters available in a library or database for each compound entry, the possibility of false positives is reduced and confidence in any identifications made is increased.

The analytical evaluation threshold (AET) level can be incorporated into the analysis and any compounds below the AET can be filtered out to make data interpretation easier. The AET is defined as the level below which identification and quantification is not required.[4] In Figure 5, a compound identified at retention time 5.83 minutes has been identified against the library with matches for accurate mass (mass error -0.7 ppm), retention time, and fragment ions. Using the summary plot, the identified compound can be seen present in the extracted profiles of all the nasal sprays but not in the extracted blanks.

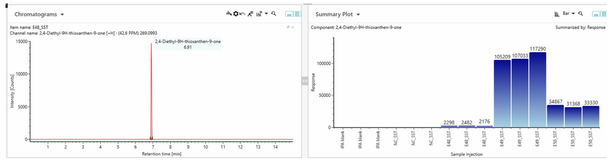

Figure 5. The extracted ion chromatogram of 2,4-Diethyl-9H-thioanthen-9-one identified using accurate mass (mass error -0.7 ppm), retention time, and fragment ions and the response of this compound in each sample. NC is the negative control (extracted blank) and E48, E49, and E50 are the three extracted nasal sprays).

Any peaks above the AET that cannot be identified by screening against the library, need to be elucidated. The comparison feature and elucidation toolkit from within the UNIFI application can both be used to find and characterize unidentified components. Binary compare is used to compare the samples to the procedural blank to find components that are unique to the sample or elevated in the sample compared to the blank (Figure 6).

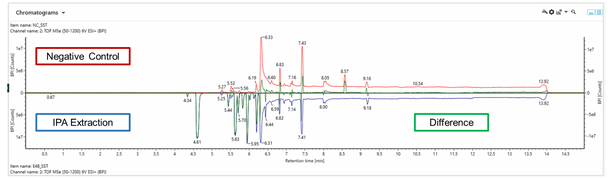

Figure 6. A difference plot of the base peak intensity chromatograms for an extracted sample and the extracted blank.

The unknowns above the AET isolated using binary compare can then be investigated using the Discovery Tool in the UNIFI application.[7] As the data were collected in MS^E mode, the accurate mass of both precursor and fragments ions were available for the interpretation of each unknown. A compound found at *m/z* 368.4253 that was unique to the samples was tentatively assigned as a surfactant using the structural elucidation toolkit (Figure 7).

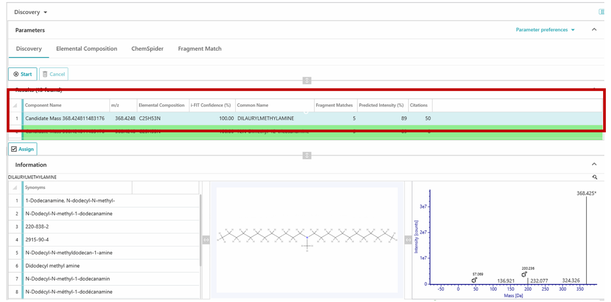

Figure 7. The UNIFI elucidation toolkit can be used for the tentative identification of unknown peaks identified in a sample using the accurate mass and fragmentation data that was acquired with MS^E. Tentative identification of an unknown as a surfactant with protonated m/z 368.4248 (mass error -0.75 ppm). Results include the predicted elemental composition for this marker, i-FIT confidence (algorithm used to score each formula), common name for the compound, number of fragment matches, and the number of citations. Synonyms, structure, and high energy spectrum for this compounds are also displayed.

CONCLUSION

- **With the Xevo G3 QTof, confident identification of E&L components in complex matrices is enabled through novel ion optics and detection system which maximize transmission.**
- **Increased sensitivity of this instrument assists with detection of low level components to meet regulatory screening thresholds.**
- **Using the data independent acquisition, MS^E, the accurate mass of precursor ions and its corresponding fragments ions can both be acquired for all peaks in the chromatogram.**
- **MS^E data increases confidence in identifications of components when screening against a library which contains MS/MS data, whilst also reducing the chance of false positives.**
- **MS^E data also assists with structural elucidation of unknowns utilizing the accurate mass of the compound and the corresponding fragments ions to ultimately aid full characterization.**
- **The UNIFI application within the waters_connect platform enables all steps within an E&L analysis to be included in one workflow that can be customized depending on regulatory needs. It simplifies screening and structural elucidation of complex datasets.**
- **For the extractables analysis of nasal sprays the UNIFI application provided SST benchmarking, screening against a library, summary plots to identify trends, filtering of AET levels, binary compare to isolate relevant unknowns, and a Discovery Tool for elucidation of unknowns.**

References

1. USP-NF/PF, <1664> Assessment of Drug Product Leachables Associated with Pharmaceutical Packaging/Delivery Systems. https://doi.usp.org/USPNF/USPNF_M7127_03_01.html
2. USP-NF/PF, <1663> Assessment of Extractables Associated with Pharmaceutical Packaging/Delivery Systems https://doi.usp.org/USPNF/PNF_M7126_03_01.html
3. Norwood D., Paskiet D., Ruberto M., Feinberg T., Schroeder A., Poochikian G., Wang Q., Deng T., Degrazio F., Munos M., Nagao L. Best Practices for Extractables and Leachables in Orally Inhaled and Nasal Drug Products: An Overview of the PQRI Recommendations. Pharmaceutical research. 25. 727-39, 2008.
4. ISO 10993-18:2020 Biological evaluation of medical devices — Part 18: Chemical characterization of medical device materials within a risk management process, https://www.iso.org/standard/64750.html
5. Stevens D., Cabovska B., Bailey A. Detection and Identification of Extractable Compounds from Polymers. Waters Application Note 720004211. January 2012.
6. McCullagh M., Mortishire-Smith R. J., Goshawk J., Cooper J., Obkircher M., Sprecher H., Koehling R., Nold m., Jacobsen J., Sanig R., Extractables, Leachables, and Contact Materials: The Invaluable Benefit of Ion Mobility-Enhanced Mass Spectrometry Libraries, Application Note 720007655, June 2022.
7. Cabovska B. Screening Workflow for Extractable Testing Using the UNIFI Scientific Information System, Technical Note 720005688, April 2016.

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