EXTRACTABLES ANALYSIS OF NASAL SPRAY DEVICES USING GAS CHROMATOGRAPHY AND QUADRUPOLE TIME OF FLIGHT HIGH-RESOLUTION MASS SPECTROMETRY WITH SOFT IONIZATION

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INTRODUCTION

Pharmaceutical packaging or medical devices are made of different chemicals, including polymers, polymer additives such as antioxidants, slip agents, colorants, and other compounds. These chemicals, their impurities, and degradation products can migrate out of the materials resulting in potentially unsafe substances. Due to concern about the safety of these products it is crucial to screen for and identify potential extractables and leachables (E&L). [1,2,3]

For volatile, and semi-volatile compounds, gas chromatography-mass spectrometry (GC-MS) with electron ionization (EI) is typically used. Compounds are determined using scientific libraries; however, where compounds are not listed or where the high energy of electron ionization (EI) leads to insufficient sensitivity the identification process becomes challenging.

GC with soft ionization high resolution mass spectrometry (HRMS) is potentially a useful tool in this field to help address some of the limitations. Atmospheric Pressure Gas Chromatography (APGC) enables softer ionization, resulting in molecular ion detection which can help with the confirmation of a molecular formula for identification. APGC can be coupled to a quadrupole time-of-flight mass spectrometer (QToF MS) on which data can be acquired in MS^E mode. The accurate mass of both precursor and fragment ions are available to provide information for structural elucidation and ultimately aid compound identification.[4]

Here, we describe an E&L screening experiment using gas chromatography and a quadrupole time of flight high-resolution mass spectrometer (GC-QToF-HRMS) with atmospheric pressure gas chromatography (APGC) for soft ionization (Figure 1) combined with a screening software solution.

Figure 1. GC coupled to the APGC and Xevo G3 QTof mass spectrometer.

METHODS

Sample Preparation

Three commercial nasal sprays were purchased. The nasal container closure system was extracted with isopropanol for 72 hours at 40 °C, along with a control blank. Non-volatile data was previously acquired on a liquid chromatography-QToF-MS (LC-QToF-MS) platform.[5] The same MS platform was switched over to GC. The procedural blank and extracted samples were injected in triplicate alongside an E&L system suitability (SST) mix (Waters, p/n 186008063).

Data Management

Data were acquired using MassLynx™ software (version: 4.2) and processed in the UNIFI™ application out of the waters_connect™ platform (version: 3.1.0.16).

Instrument conditions

APGC data were acquired using dry conditions, where nitrogen charge transfer mainly occurs and gives rise to the radical cation molecular ion, $M^{+\cdot}$. Even under dry conditions some structures give rise to the protonated molecular ion, $[M+H]^+$ because moisture cannot be completely eliminated from the source.

GC Conditions	
GC System	Agilent 8890
Autosampler	PAL RSI (CTC Analytics)
Inlet Mode	Splitless
Inlet Temp.	300 °C
Septum Purge Flow	3 mL/min
Column	Rtx-5MS, 30m x 0.25 mm x 0.25 µm (available from RESTEK)
Column Flow	1 mL/min
Oven Gradient	40 °C (5 min hold), up to 330 °C at 10°C/min (14 min hold)
Total GC Run Time	27.75 min

METHODS

MS Conditions	
MS System	Xevo™ G3 mass spectrometer
Ionization Mode	APGC™ +ve
Corona current	2 µA
Sampling cone	5 V
Source Temp.	150 °C
Mass Range	*m/z* 50-1200
Scan Time	0.1 s
Cone gas	140 L/h
Auxillary gas	250 L/h
MS^E collision energy	Low 6 V, high 15 to 45 V
GC Interface Temp	300 °C

RESULTS AND DISCUSSION

Using the UNIFI application, data were processed within an E&L specific workflow (Figure 2A). The E&L workflow can be customized to user requirements and helps to streamline data analysis. The E&L SST mix was injected to benchmark the system (Figure 2). The mass spectrometer has had updates, compared to previous iterations, to the ion optics and detection system to maximize transmission[6] and proved to be highly sensitive and reproducible for the SST mix (0.01% RSDs for retention time).

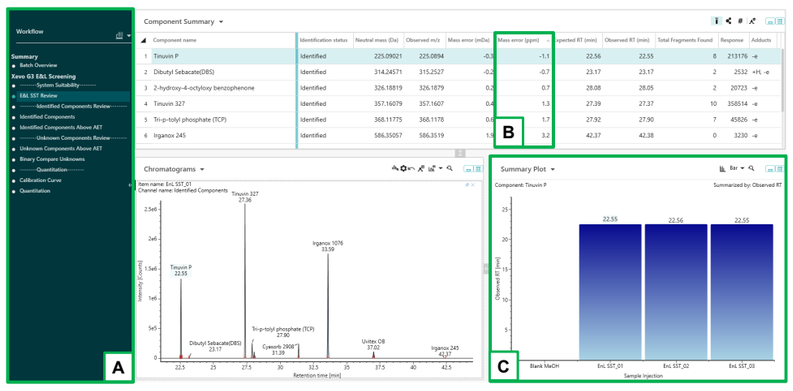

Figure 2. The SST results displayed for easy data interpretation, including experimental results for each analyte, the extracted ion chromatogram of all the identified analytes, and a summary plot. [A] Example of the customizable UNIFI workflow. [B] Mass ppm error for each analyte. [C] Retention time for Tinuvin P across each SST injection.

The samples were investigated by screening against the Waters E&L scientific library[7] (with additional typical GC compounds added). The analytical evaluation threshold (AET) level was incorporated into the analysis with any compounds below the AET filtered out to make data interpretation easier. The AET is defined as the level below which identification and quantification is not required.[8]

Using GC-QToF MS with APGC as an orthogonal technique to LC-QToF-MS found different compounds identified in the extracted nasal sprays, increasing the overall compound coverage.[5] Figure 3 shows one of these compounds identified at retention time 29.45 min ([+H], mass error -0.1 ppm). Using the summary plot, the identified compound can be seen present in the profiles of two of the nasal sprays but not in the extracted blanks.

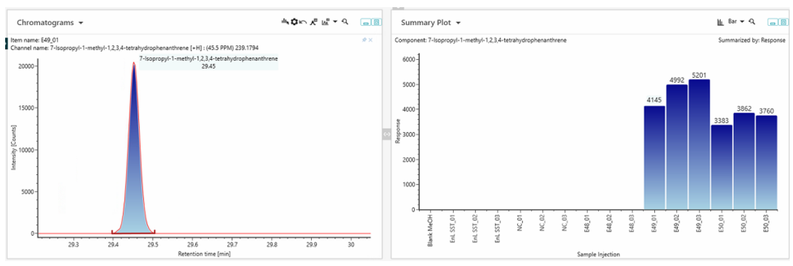

Figure 3. The chromatogram of identified 7-Isopropyl-1-methyl-1,2,3,4-tetrahydrophenanthrene and the response of this compound in each sample. NC is the negative control (extracted blank) and E48, E49, and E50 are the three extracted nasal sprays.

Due to the soft ionization of APGC the intact molecular ion is often present and can therefore be used to screen again the accurate mass. Additionally, the mass spectrometer was used in MS^E mode, which alternates between low and high collision energy and enables the simultaneous acquisition of both precursor and fragment ions throughout the entire chromatographic run.[4] The UNIFI application uses theoretical fragment matching to ensure confidence in identifications (Figure 4).

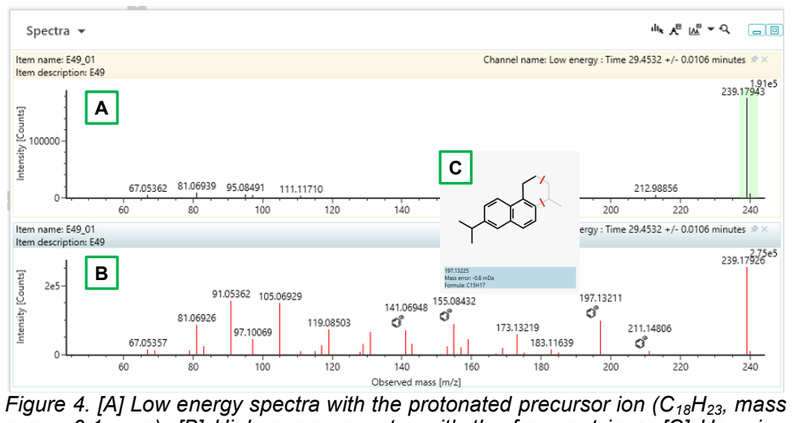

Figure 4. [A] Low energy spectra with the protonated precursor ion ($C_{18}H_{23}$, mass error –0.1 ppm). [B] High energy spectra with the fragment ions. [C] Hovering over the symbols in the high energy spectra displays the predicted fragment ion for that mass and the mass error associated with it.

Any peaks above the AET that cannot be identified by screening against the library, need to be elucidated. The comparison feature and elucidation toolkit 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 (Figure 5).

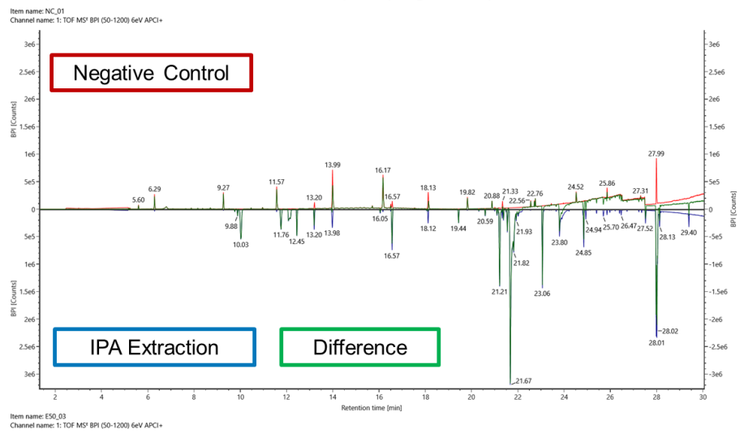

Figure 5. Difference plot of the base peak intensity chromatograms. Red trace is the negative control, blue trace is sample E50, and the green trace is the difference.

Any unknowns above the AET can then be investigated using the Discovery Tool in the UNIFI application.[9] As the data were acquired with soft ionization and 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* 284.2709 that was unique to the samples was tentatively assigned as [(3-methylbutoxy)methyl]benzene using the structural elucidation toolkit (Figure 6).

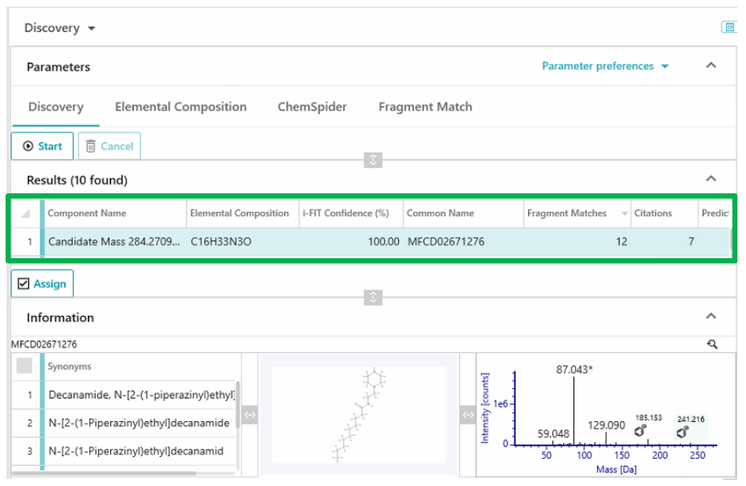

Figure 6. An unknown with protonated m/z 284.2709 was identified as a [(3-methylbutoxy)methyl]benzene (mass error -0.8 ppm) by the software. Results include the predicted elemental composition, i-FIT confidence (isotopic pattern algorithm used to score each formula), common name for the compound, number of fragment matches, and the number of citations. Synonyms, structure, and high energy spectrum for this compound are also displayed.

CONCLUSION

- **With the Xevo G3 QTof MS, confident identification of E&L components in complex matrices is enabled through novel ion optics and detection system which maximize transmission.**
- **GC-QToF MS with APGC as an orthogonal technique to LC-QToF-MS allows for comprehensive compound coverage with increased sensitivity compared to typical EI techniques.**
- **APGC combined with MS^E mode utilizes full spectral acquisition of the accurate mass information of both precursor and fragment ions.**
- **Accurate mass of precursor and fragments ions increases confidence in identifications of components and assists with structural elucidation of unknowns to ultimately aid full characterization.**
- **The screening software solution streamlines data interpretation with library screening, comparison tools, and an elucidation toolkit all within the same E&L workflow. All steps within the workflow can be customized depending on regulatory needs.**

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