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Application Note

Extractables Analysis of Nasal Spray Devices Using Gas Chromatography and High-Resolution Mass Spectrometry With Soft Ionization

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Abstract

Due to concern about the safety of components from plastic, it is crucial to screen for and identify potential extractables and leachables (E&L) in pharmaceutical packaging and medical devices. 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 sensitivity of EI-MS is not sufficient, the identification process becomes challenging.

Here, we describe an E&L screening experiment using gas chromatography and a quadrupole time of flight high-resolution mass spectrometer (QToF-HRMS) with atmospheric pressure gas chromatography (APGC) for soft ionization. A data independent acquisition (DIA) strategy is utilized to aid screening and elucidation which are combined in a screening software solution.

Benefits

- 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

- The UNIFI™ application within the waters_connect™ platform provides customized workflows to simplify screening and structural elucidation in complex datasets
- The Xevo™ G3 QTof Mass Spectrometer enables confident identification of E&L components in complex matrices through novel ion optics and detection system which maximize transmission
- Soft ionization using the APGC™ source allows for the detection of molecular ions from which elemental compositions can be derived to aid compound identification and confirmation
- MS^E, a data independent acquisition, increases confidence in identifications when screening against a library and provides additional information to aid structural elucidation

Introduction

Medical devices, pharmaceutical container closure systems, and manufacturing components, contain different chemicals, including polymers, polymer additives, colorants, and other compounds. These chemicals, their impurities, and degradation products can migrate out of the materials resulting in potentially unsafe substances. To ensure safety for the consumer it is therefore crucial to screen for and identify potential extractables and leachables (E&L).[1–3]

For non-volatile E&L compounds, liquid chromatography-QToF-MS (LC-QToF-MS) is the most common analytical platform whereas for volatile, and semi-volatile compounds, gas chromatography-mass spectrometry (GC-MS) with electron ionization (EI) is typically used. Using this technique, compounds are commonly determined using scientific libraries such as NIST mass spectral libraries.[4] Traditional EI is a very energetic process that produces fragmentation in nearly all cases and often, the molecular ion is small or not present in the spectrum. The real challenge is for unknown compounds that are not included in the library or where the high energy of EI leads to insufficient sensitivity.

High-resolution mass spectrometry (HRMS) with soft ionization is a useful tool in this field to help address the limitations seen with vacuum source EI. Atmospheric pressure gas chromatography (APGC) utilizes an ionization technique similar to atmospheric pressure chemical ionization, which uses a corona discharge enabling softer ionization. This ionization is much softer than EI and results 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. This is a data independent acquisition (DIA) strategy where 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.[5]

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 APGC for soft ionization (Figure 1). A data independent acquisition (DIA) strategy is utilized to aid screening and elucidation which are combined in a screening software workflow solution.

Figure 1. GC coupled to the APGC and Xevo G3 QTof Mass Spectrometer.

Experimental

Sample Description

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 LC-QToF-MS.[6] 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.

Method Conditions

APGC can undergo two primary mechanisms of ionization, charge transfer and protonation. The instrument was run under mixed mode conditions, whereby both the radical cation and protonated species can be seen.[7]

GC Conditions

GC system:	Agilent 8890
Autosampler:	PAL RSI (CTC Analytics)
Inlet mode:	Splitless
Inlet temperature:	300 °C
Septum purge flow:	3 mL/min
Column:	Rtx–5MS, 30 m x 0.25 mm x 0.25 µm (available from RESTEK)
Column flow:	1 mL/min (He)
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

MS Conditions

MS system:	Xevo G3 QTof mass spectrometer
Ionization mode:	APGC
Corona current:	2 µA
Sampling cone:	5 V
Source temperature:	150 °C
Mass range:	*m/z* 50-1200
Scan time:	0.1 s
Cone gas:	140 L/h
Auxillary gas:	250 L/h
MSE collision energy:	Low 6 V High 15 to 45 V
GC interface temperature:	300 °C

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

Results and Discussion

The extracted samples were previously acquired on the LC-QToF-MS platform, with non-volatile compound data reported.[6] Here, GC-QToF MS with APGC was used as an orthogonal technique to increase the overall compound coverage. As with the LC data, the UNIFI application was used for extractables data analysis within

an E&L specific workflow (Figure 2A). The workflow can be customized to meet user requirements and helps to streamline the analysis of complex datasets. Here we describe, system suitability review, screening against a library, and elucidation of unknowns.

System Suitability Review

An 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.[8] This proved to be highly sensitive and reproducible as shown here for the SST mix (0.01% RSDs for retention time).

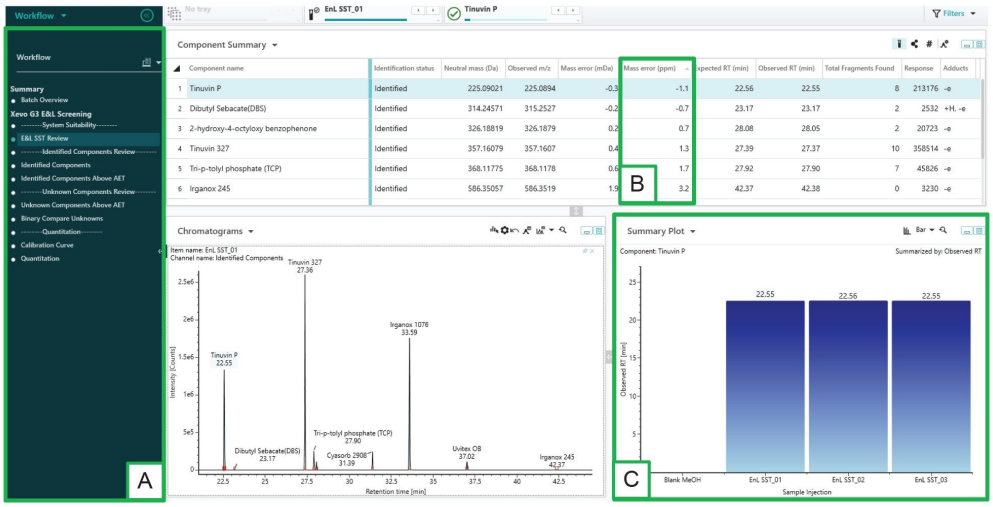

Figure 2. The SST results displayed for easy data interpretation, including experimental results for each analyte, the extracted ion chromatogram of all the identified analytes, and a summary plot. [A] Example of the customizable UNIFI workflow. [B] Mass accuracy for each analyte. [C] Retention time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

Screening Against an E&L Library

The samples were investigated by screening against the Waters E&L scientific library,[9] (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.[10]

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.[6] Figure 3 shows one of these compounds identified at retention time 34.95 min ([-e], mass error 2.6 ppm). Using the summary plot, the identified compound can be seen present in the profiles of the nasal sprays but not in the extracted blanks.

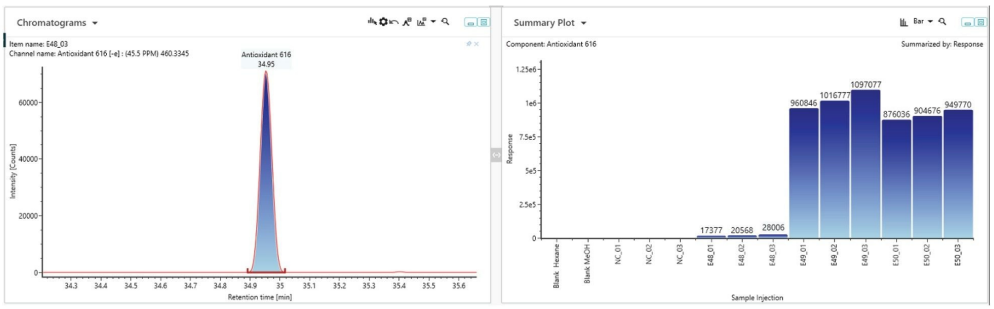

Figure 3. The chromatogram of identified antioxidant 616 and the response of this compound in each sample. NC is the negative control (extracted blank) and E48, E49, and E50 are the three extracted nasal sprays.

With the soft ionization of APGC the intact molecular ion is often present and can therefore be used to screen against 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.[5] The UNIFI application uses theoretical fragment matching to ensure confidence in identifications. Figure 4 shows the annotated spectra for the identified compound antioxidant 616.

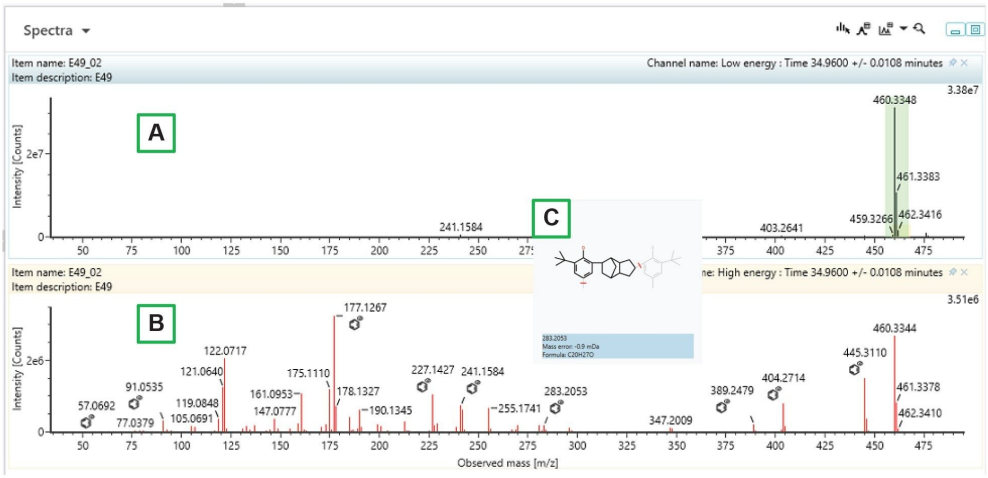

Figure 4. [A] Low energy spectra with the precursor ion ($C_{32}H_{44}O_2$, [-e], mass error 2.6 ppm) for antioxidant 616. [B] High energy spectra with the fragment ions. [C] Selecting the symbols in the high energy spectra displays the predicted fragment ion for that mass and the mass error associated with it.

Identification of Unknowns

Any peaks above the AET that cannot be identified by screening against the library, need to be elucidated. The comparison feature and elucidation toolset within the UNIFI application can be used together to find and characterize unidentified components. The binary compare feature compares the samples to the procedural blank and finds components that are unique to the sample or elevated in the sample (Figure 5).

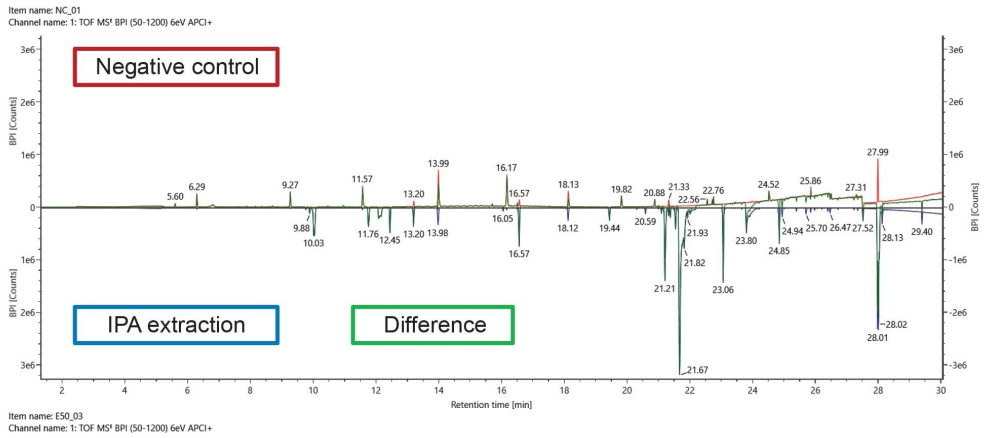

Figure 5. Difference plot of the base peak intensity chromatograms. Red trace is the negative control, blue trace is sample E50, and the green trace is the difference.

Any unknowns above the AET can then be investigated using the Discovery Tool in the elucidation toolset.[11] 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 with *m/z* 284.2703, at 21.52 minutes, that was unique to the samples was tentatively assigned as N-(2-(1-piperazinyl)ethyl)decanamide ([+H], mass error 2.33 ppm) using the Discovery Tool (Figure 6).

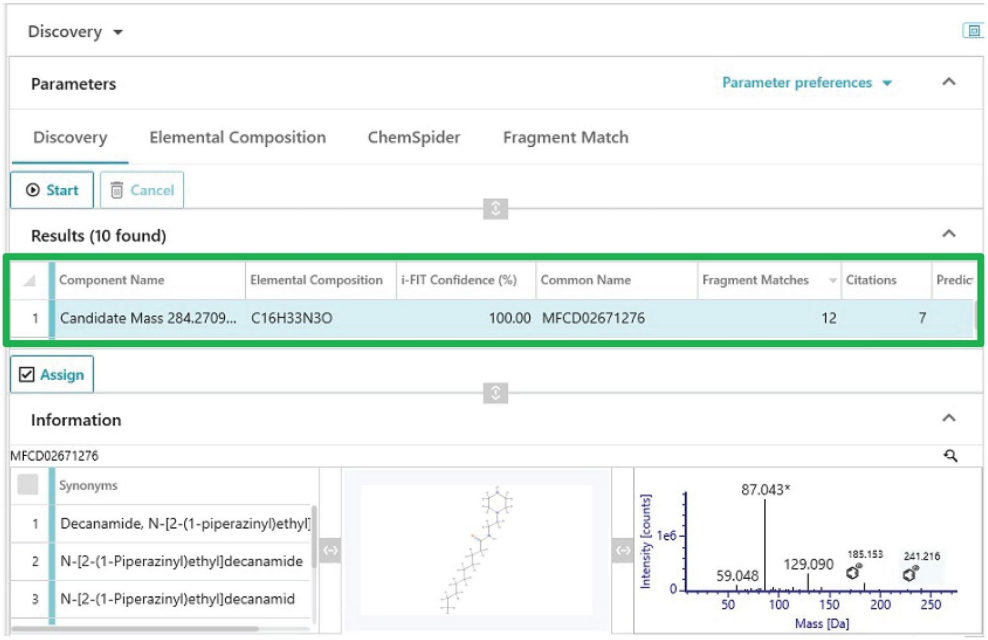

Figure 6. An unknown with protonated m/z 284.2703 was identified as a N-(2-(1-piperazinyl)ethyl)decanamide (mass error 2.33 ppm) by the software. Results include the predicted elemental composition, i-FIT confidence (isotopic pattern algorithm used to score each formula), common name for the compound, number of fragment matches, and the number of citations. Synonyms, structure, and high energy spectrum for this compound are also displayed.

Conclusion

When undertaking E&L screening analyses it is important to use a range of analytical techniques to account for different compound chemistries. GC-QToF MS with APGC as an orthogonal technique to LC-QToF-MS allowed for comprehensive compound coverage with increased sensitivity compared to typical EI techniques. Due to the soft ionization of APGC, the intact molecular ion is often present in the spectra. This combined with a data independent acquisition, MS^E mode, utilizes full spectral acquisition of the accurate mass information of both precursor and fragment ions.

This approach boosts confidence in component identifications when screening against an MS/MS library, while minimizing false positives. For instance, in the LC-QToF-MS there were just under 40 potential compounds matches in sample E48 after considering factors such as retention time match, mass accuracy below 3 ppm, and the presence of at least one fragment ion. A further 38 compound matches in sample E48 were tentatively identified using GC-QToF MS with APGC, with mass accuracy below 3 ppm, and the presence of at least one fragment ion.

MS^E data also aids in the structural elucidation of unknown substances by utilizing accurate mass and corresponding fragment ions, facilitating comprehensive characterization. This data increases confidence in identifications of components and assists with structural elucidation of unknowns to ultimately aid full characterization. For example an unknown with protonated *m/z* 284.2703 was tentatively identified as a N-(2-(1-piperazinyl)ethyl)decanamide (mass error 2.33 ppm) by the software.

For the extractables analysis of nasal sprays the UNIFI application within the waters_connect platform provided SST benchmarking, screening against a library, summary plots to identify trends, filtering of AET levels, binary compare mode to isolate relevant unknowns, and a Discovery Tool for elucidation of unknowns.

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