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Application Note

Screening for Extractables and Leachables in Nasal Spray Devices Using Liquid Chromatography and High-Resolution Mass Spectrometry With a Data Independent Informatics Strategy

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Abstract

It is crucial to detect and identify potential extractables and leachables (E&L) through screening studies due to the potential for 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 challenges when undertaking these studies to meet these regulatory requirements. 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 high-performance liquid chromatography and a quadrupole time of flight high-resolution mass spectrometer (UHPLC-QToF HRMS). A data independent

acquisition (DIA) strategy is utilized to aid screening and elucidation combined in one informatics workflow solution.

Benefits

- The ACQUITY™ Premier System features MaxPeak™ High Performance Surfaces (HPS) Technology which offers improvements in separation and detection to minimize the risk of undetected analytes
- The UNIFI™ application within the waters_connect™ platform provides customized workflows to simplify screening and structural elucidation in complex datasets
- The Xevo™ G3 QTof enables confident identification of E&L components in complex matrices through novel ion optics and detection system which maximize transmission
- 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 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. To ensure safety for the consumer it is therefore crucial to screen for and identify potential extractables and leachables (E&L).

There are regulations, standards, and guidance in place to ensure safety limits are met for devices such as nasal drug products. For example, the USP (United States Pharmacopeia) sets out a framework for this analysis in chapters <1663> and <1664> and the PQRI (Product Quality Research Institute) details recommendations for Orally Inhaled and Nasal Drug Products.[1,2,3]

There are challenges when undertaking these studies to meet the regulatory requirements. One of the critical challenges comes with the analytical instrumentation used to undertake these studies, as they need to be sensitive to detect low levels of components to meet the expected screening thresholds. Additionally, the ability to screen for E&L compounds, elucidate unknowns, and quantify component levels on the same analytical platform is also important.

To address these challenges, here we describe an E&L screening experiment using liquid chromatography and a benchtop high-resolution quadrupole time of flight mass spectrometer, Xevo G3 QTof (Figure 1). A data independent acquisition (DIA) strategy is utilized to aid screening and elucidation combined in one screening software workflow solution. The Xevo G3 QTof 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.

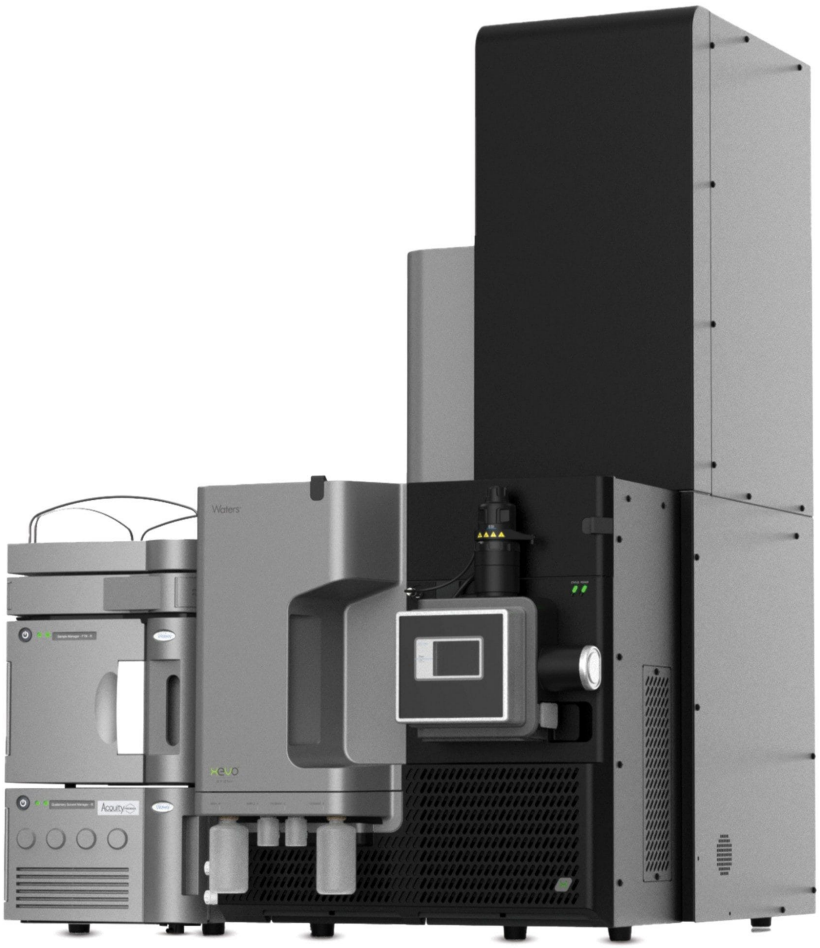

Figure 1. ACQUITY Premier System with the Xevo G3 QTof Mass Spectrometer.

Experimental

Sample Preparation

Three commercial nasal sprays were purchased. The neat solution (leachables) was removed for analysis. The nasal container closure system was then extracted with isopropanol for 72 hours at 40 °C (extractables), along with a control blank. The procedural blank, neat samples, and extracted samples, were spiked with an internal standard (metafluzimone, Merck, Germany) and injected in triplicate on the instrument. Additionally, an E&L system suitability test mix (SST) mix (p/n: 186008063 < https://www.waters.com/nextgen/global/shop/standards--reagents/186008063-extractables--leachables-screening-standard.html>) was injected at the start and end of the sample sequence acquisitions.

LC Conditions

LC system:	ACQUITY Premier System
Column(s):	ACQUITY CORTECS™ C_{18}, 90 Å (1.6 µm, 2.1 mm x 100 mm)
Column tempertaure:	50 °C
Sample temperature:	4 °C
Injection volume:	1 µL
Flow rate:	0.3 mL/min
Mobile phase A:	Water +1 mM ammonium acetate +0.1% formic acid
Mobile phase B:	Methanol

Gradient Table

Time (min)	%A	%B	Curve
0.0	98	2	Initial
0.5	98	2	6
6.0	1	99	6
13.0	1	99	6
13.1	98	2	6
15.0	98	2	6

MS Conditions

MS system:	Xevo G3 QTof
Reference mass:	Leucine enkephalin [M+H]+ at *m/z* 556.2771 and [M-H]- at *m/z* 554.2615
Source temperature:	120 °C
Desolvation temperature:	600 °C
Desolvation gas:	800 L/h
Cone gas:	50 L/h
Ionization mode:	ESI+, ESI-
Acquisition mode:	MS^E
Acquisition range:	*m/z* 50–1200
Acquisition time:	0.2 s

Capillary voltage:	1 kV
Cone voltage:	40 V
Collision energy:	ESI+ Low energy: 6 eV ESI+ High energy ramp: 20–40 eV ESI- Low energy: 6 eV ESI- High energy ramp: 30–70 eV

Data Management

The UNIFI Application within the waters_connect platform was used for acquisition and processing (version 3.1.0.16).

Results and Discussion

A complete extractables analysis can be undertaken using the UNIFI Application within the waters_connect platform as the data can be processed 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.

System Suitability Review

The first step undertaken in the workflow is to review the E&L SST mix results, used to benchmark the system (Figure 2). The E&L SST contains common polymer additives over a range of molecular weights, polarities, and that ionize in either positive or negative mode.

The mass spectrometer has had updates to the ion optics and detection system to maximize transmission and proved to be highly sensitive and reproducible, as shown for the SST mix (0.01% RSDs for retention time, Figure 2C). This increase in sensitivity, compared to its predecessor, helps with the challenge of achieving trace level identification in E&L studies. Mass accuracy for all detected compounds was <3 ppm (Figure 2B). Consistent high mass accuracy aids library matching and elemental composition calculation to support full characterization.

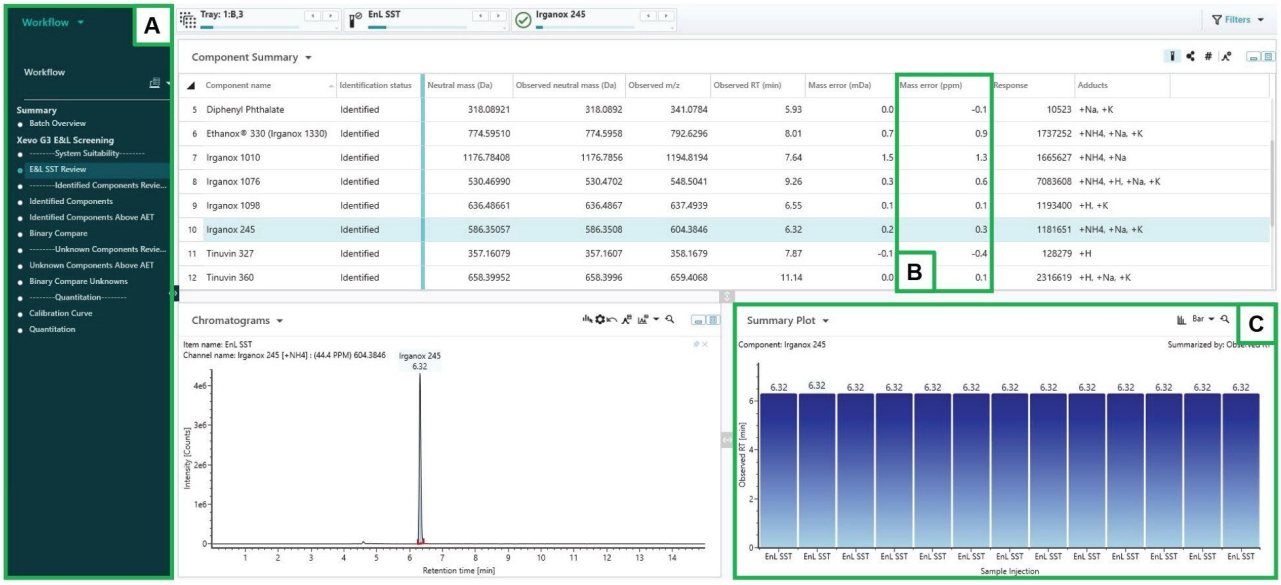

Figure 2. The SST results displayed for easy data interpretation. Shown here is the component summary of the experimental results for each analyte, the extracted ion chromatogram for Irganox 245, and a summary plot for this analyte across all injections. [A] Example of the customizable UNIFI workflow. [B] Mass accuracy 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,[4] low and high collision energy is alternated enabling simultaneous acquisition of the accurate mass information of both precursor and fragment ions throughout the entire chromatographic experiment (Figure 3).[4] This increases confidence when identifying compounds against a library when MS/MS spectra are included.

Figure 3. An example of MS^E data for dibutyl sebacate. [A] Low energy spectra with the precursor ion ($C_{18}H_{34}O_4$, exact mass 315.2535, mass accuracy: –1.0 ppm). [B] High energy spectra with the fragment ions. [C] The symbols in the high energy spectra indicate there is an assignment for the fragment ions and the mass accuracy associated with it. The charge retaining fragment is highlighted.

Screening Against an E&L Library

After verifying the SST mix, the samples were investigated by screening against an E&L library to find matches for accurate mass, retention time, and mass fragments.[5] With these parameters available in a library or database for each compound entry, the possibility of false positives is reduced and confidence in any identification made is increased. Using the UNIFI application, 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 quantitation is not required.[6]

Here, a compound identified as 2,4-Diethyl-9H-thioanthen-9-one with retention time 6.91 minutes (Figure 4) is shown with library matches for accurate mass measurement (mass accuracy: -0.7 ppm), retention time, and fragment ions. Using the UNIFI trend plots we can see that the compound is present in the extracted profiles of the nasal sprays but not in the procedural blank (Figure 4).

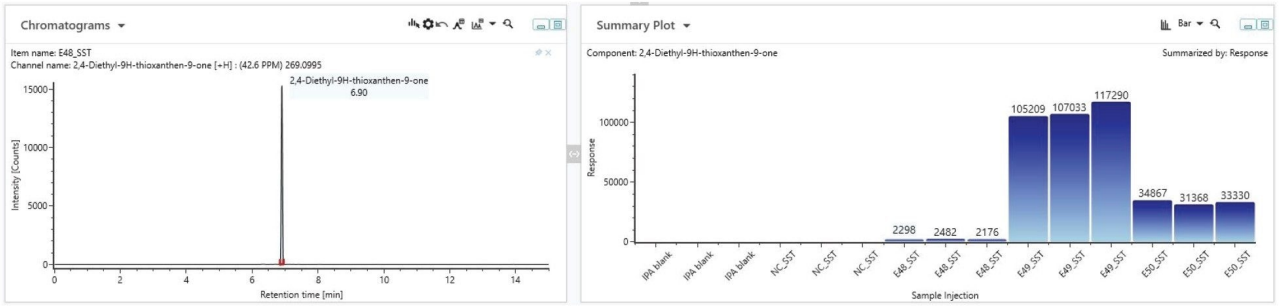

Figure 4. The extracted ion chromatogram of 2,4-Diethyl-9H-thioxanthen-9-one and the response of this compound in each sample. NC is the negative control (extracted blank) and E48, E49, and E50 are the three extracted nasal sprays injected in triplicate.

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 toolkit from within the UNIFI application can both be utilized to find and characterize unidentified components. The Binary Compare tool is used to compare the samples to the extracted blank to find components that are unique to the sample or elevated in the sample compared to the blank (Figure 5).

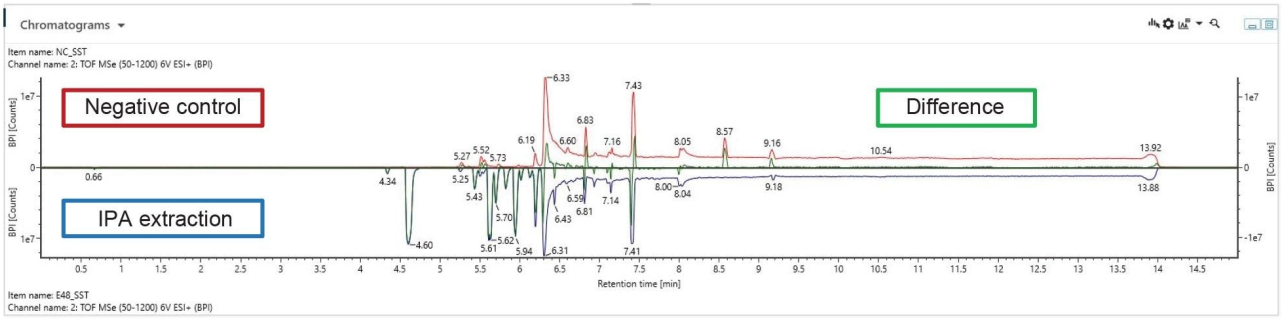

Figure 5. Difference plot of the base peak intensity chromatograms. Red trace is the negative control, blue trace is sample E48, and the green trace is the difference.

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 6).

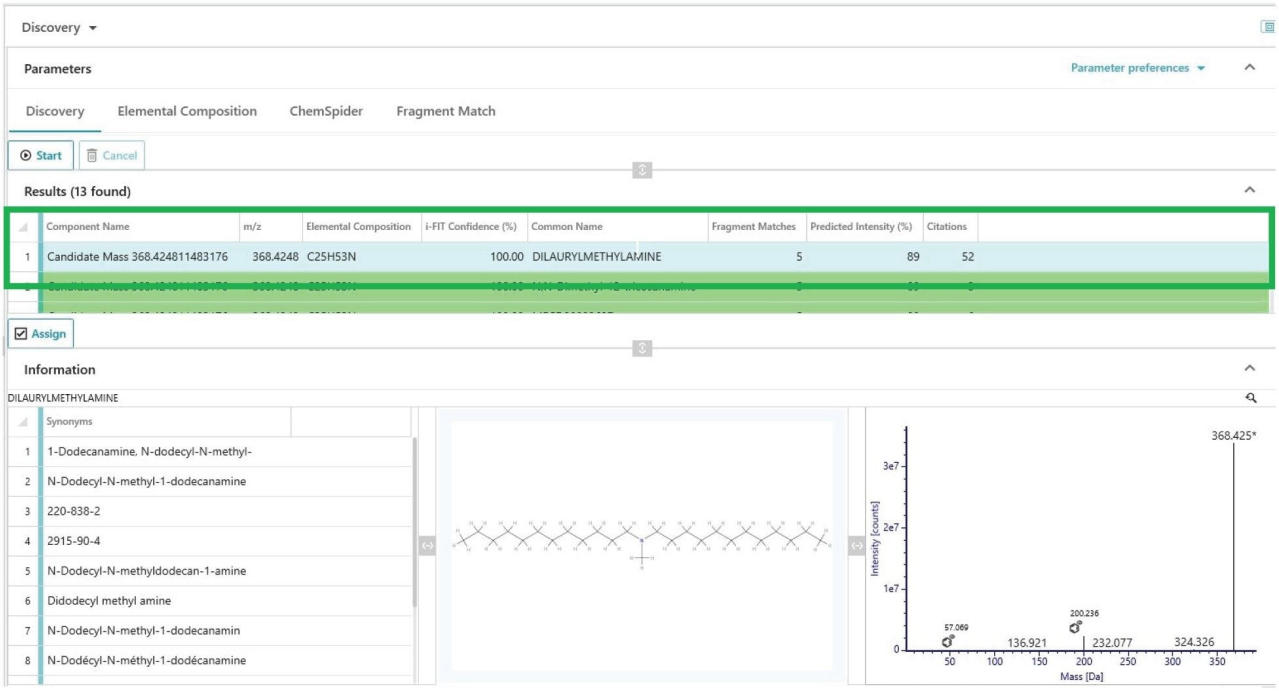

Figure 6. The UNIFI elucidation toolkit can be used for the tentative identification of unknown peaks identified in a sample using the accurate mass and fragmentation data that was acquired with MS^E. An unknown with protonated m/z 368.4248 was identified as a surfactant (mass accuracy -0.8 ppm) by the software. Results include the predicted elemental composition for this marker, 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.

Quantitation

It is important to incorporate quantitation into an E&L screening analysis. Using an internal standard or standards, response factors can be included in the UNIFI application for semi-quantitation of extractables data.

Response factors and relative response factors can be used to estimate the concentration.[8] For example, the E&L SST mix was spiked into the extracted samples and extracted blank, with Ethanox 330 (Irganox 1330) assigned as the internal standard from which relative response factors were calculated. The calculated concentrations of dibutyl sebacate in the extracted blank and the extracted samples are shown below (expected concentration 10 ng/mL) (Figure 7).

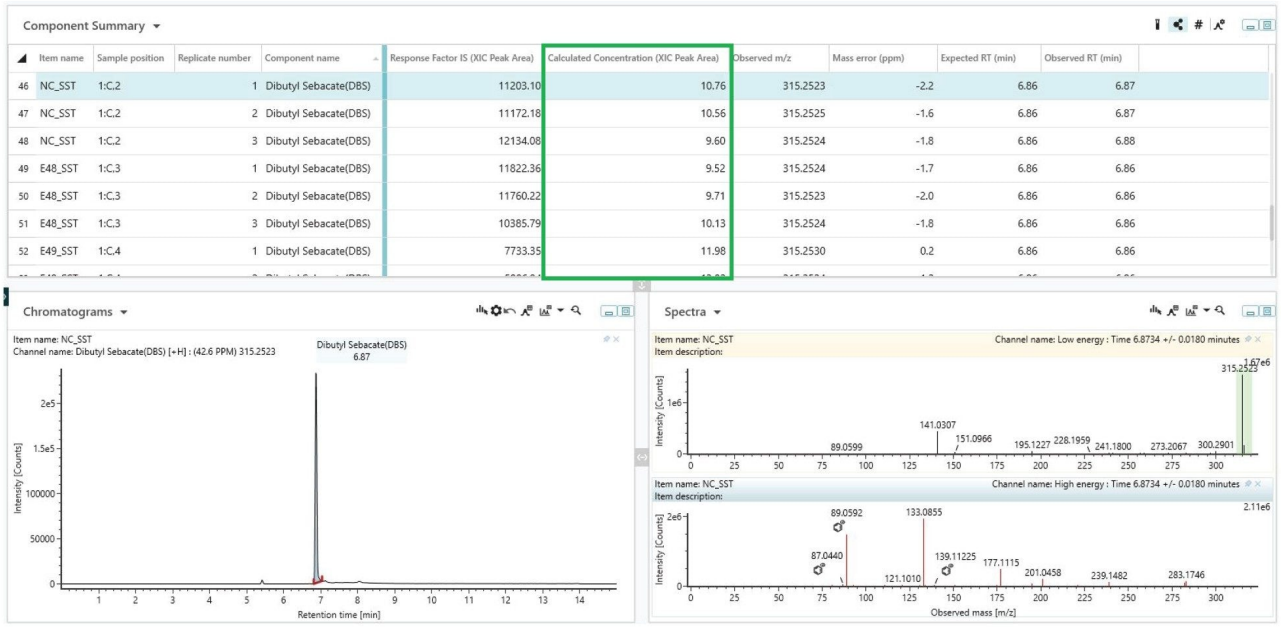

Figure 7. Calculated concentration of dibutyl sebacate using response factors.

Another method to include quantitation within an E&L workflow is to use calibration curves. To assess the platform for quantifying leachables alongside extractables the neat solution was removed from the nasal spray devices and an internal standard, metafluzimone, was spiked in at 100 ng/mL. A calibration curve was created with the standard from 5 to 1000 ng/mL (Figure 8).

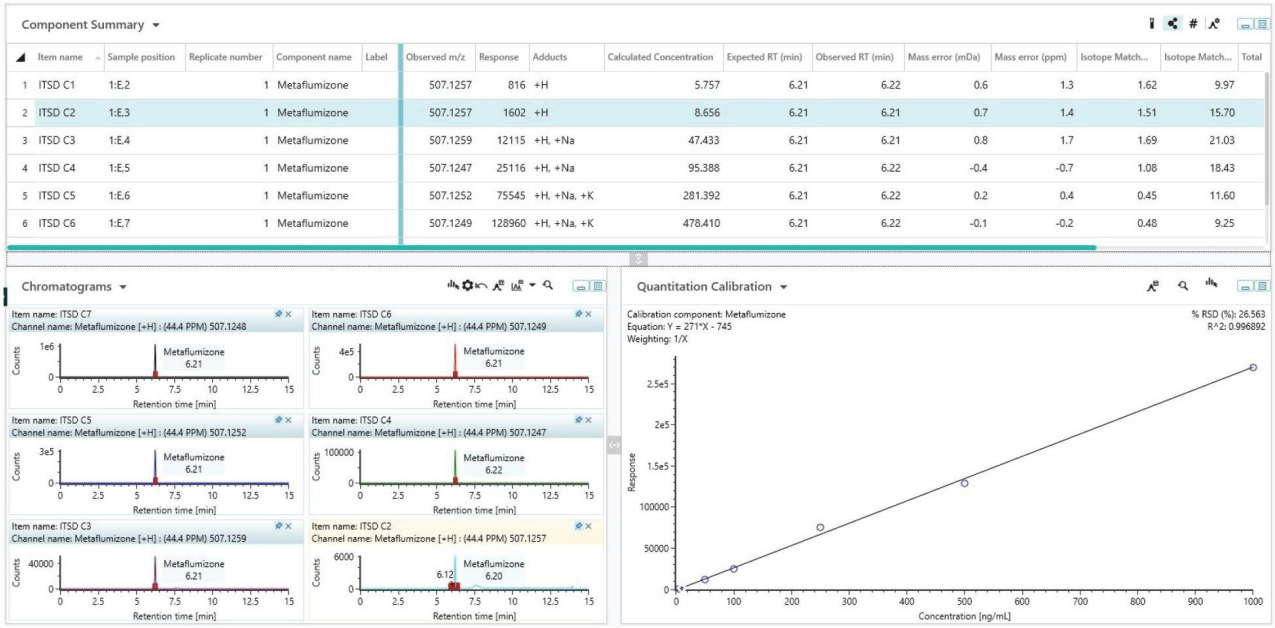

Figure 8. Metafluzimone calibration curve results with a R^2 value of 0.999.

Using the calibration curve of the internal standard, the concentration of the internal standard spiked into the samples could be calculated within 15% of the known value (Figure 9).

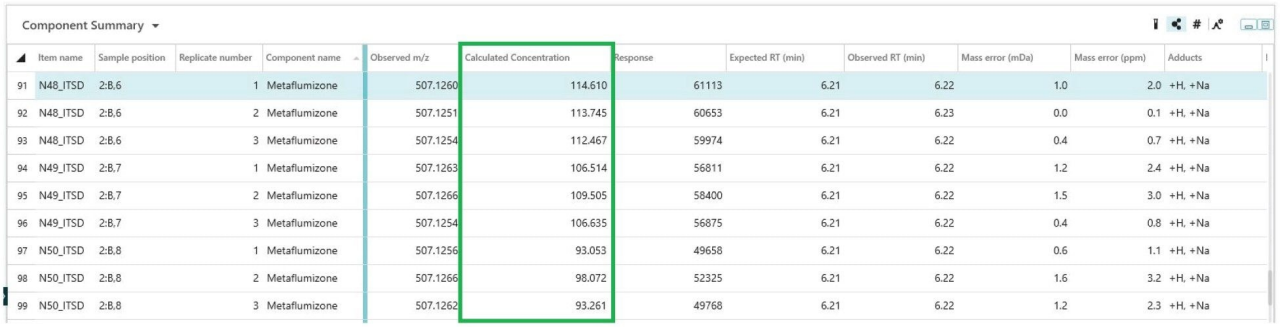

Figure 9. The calculated concentration of the spiked internal standard was calculated within 15% of the known value.

Conclusion

When undertaking E&L screening analyses it is beneficial to be able to screen for E&L compounds at low levels whilst also undertaking elucidation of unknowns and quantitation of component levels. In this study, a data independent acquisition (DIA) strategy is described to aid screening and elucidation combined in one screening software workflow solution.

The ACQUITY Premier System coupled to the Xevo G3 QTof Mass Spectrometer, demonstrated excellent reproducibility when analysing the E&L SST mix. Retention times exhibited a 0.01% relative standard deviation (RSD), while the mass accuracy for all detected compounds remained below 3 ppm. 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.

Data independent acquisition (MS^E) enables accurate mass information for precursor ions and their corresponding fragments in the chromatogram. This approach boosts confidence in component identifications when screening against an MS/MS library, while minimizing false positives. For instance, sample E48 initially yielded over 400 potential matches in the library, but after considering factors such as retention time match, mass accuracy below 3 ppm, and the presence of at least one fragment, the number reduced to less than 40 compounds. Applying the analytical evaluation threshold further reduces this number. MS^E data also aids in the structural elucidation of unknown substances by utilizing accurate mass and corresponding fragment ions, facilitating comprehensive characterization.

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, a Discovery Tool for elucidation of unknowns, and quantitation methods.

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