ENSURING CONFIDENT ANALYSIS OF EXTRACTABLES AND LEACHABLES USING HIGH-RESOLUTION QUADRUPOLE TIME OF FLIGHT TECHNOLOGY

Gordon Kearney[1], Rachel Sanig[1], Jayne Kirk[1], Cristian Cojocariu[1], Andrew Feilden[2]

Waters Corporation,, Altrincham Road, Wilmslow, Cheshire, SK9 4AX, UK[1], Hall Analytical Laboratories Ltd., Waterside Court, 1 Crewe Road, Manchester, M23 9BE, UK[2]

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 chemicals, it is crucial to screen for and identify potential extractables and leachables (E&L). There are a number of regulations and standards in place to ensure safety limits are met and there are several challenges when undertaking these studies to meet the regulatory requirements[1,2,3] For example, analytical instrumentation needs to be highly sensitive to detect low levels of components to meet the expected screening thresholds. Additionally, the ability to identify and quantify E&L compounds from the screening step 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 (LC-QToF HRMS) (Figure 1). Both screening and quantitation can be undertaken on the same platform using the screening software solution.

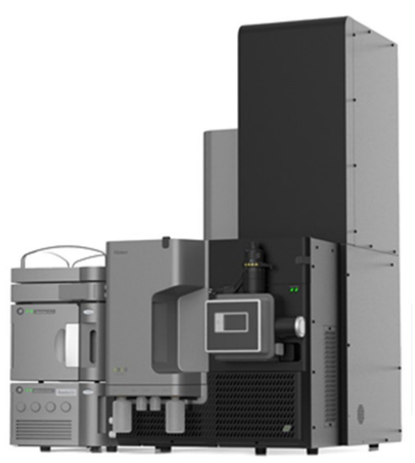

Figure 1. ACQUITY Premier System with the Xevo G3 QTof Mass Spectrometer.

METHODS

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, extracted samples, and neat solutions were spiked with an internal standard and injected in triplicate on the instrument. Additionally, an E&L system suitability (SST) mix (p/n 186008063) was injected onto the instrument.

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

Ionization: ESI+, ESI-

Acquisition mode: MS^E

Source temperature: 120 °C

Desolvation temp.: 600 °C

Acquisition range: *m/z* 50-2000

Acquisition scan time: 0.2 s

Collision voltage: ESI+ 1.0 kV, ESI- 0.8 kV

Collision energy: ESI+ Low energy 6 eV

ESI+ High energy ramp 20-40 V

ESI- Low energy 6 eV

ESI- High energy ramp 30-70 V

Data Management

The UNIFI™ Application within the waters_connect™ Platform was used for acquisition and data processing.

RESULTS AND DISCUSSION

Using the UNIFI Application, the data was processed within an E&L specific workflow (Figure 2). The E&L workflow can be customized to user requirements and helps to streamline the data analysis.

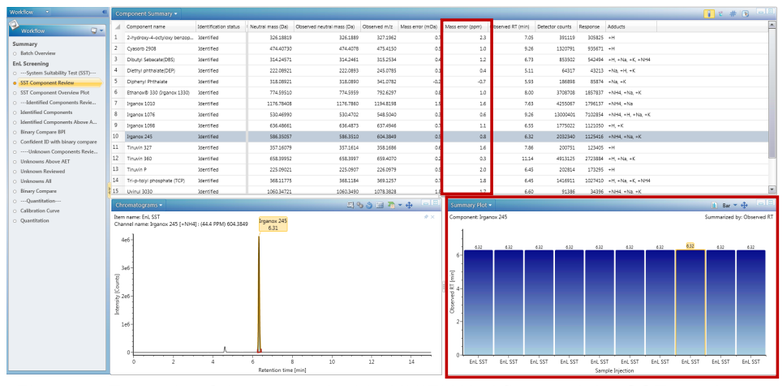

Figure 2. Example of the customizable UNIFI Workflow and the SST results displayed for easy data interpretation.

An E&L system suitability test (SST) mix was injected to benchmark the system (Figure 2). 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) and reproducible for the SST mix (0.01% RSDs for retention time). 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. Mass accuracy aids library matching and elemental composition calculation to ultimately aid full characterization.

After checking the SST mix, the samples were investigated by screening any compounds found in the samples against a library to find matches for accurate mass, retention times, and mass fragments. As the Xevo G3 QTof MS was used in MS^E mode, this enabled full acquisition of the accurate mass information of both precursor and fragment ions which increases confidence when identifying compounds against a library if MS/MS spectra are included (Figure 3).

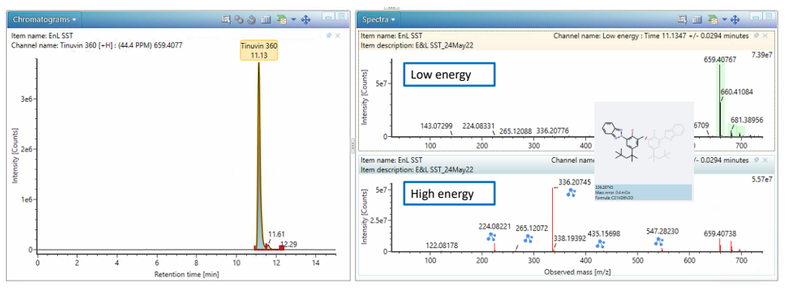

Figure 3. An example of MS^E data for Tinuvin 360. Both high and low energy data is acquired for the accurate mass of both precursor and fragment ions.

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 quantification is not required.[3] Here we can see a compound detected at retention time 5.77 minutes. Using the trend plots in the UNIFI Application we can see that the compound is present in the extracted profiles of two of the nasal sprays and the corresponding neat solution but not in the procedural blank (Figure 4).

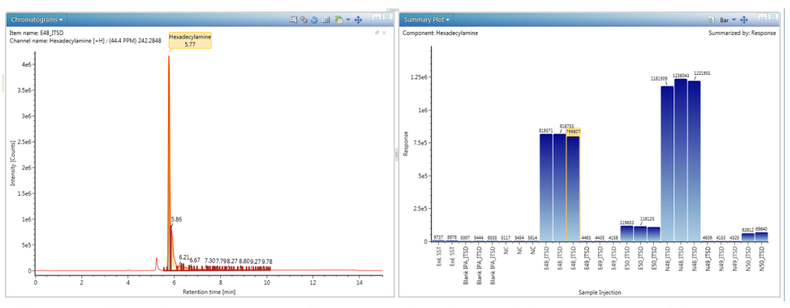

Figure 4. The chromatogram of hexadecylamine and the response of this compound in each sample. (NC = negative control)

The comparison feature and elucidation toolkit from within the UNIFI Application were employed to find and characterize unidentified components. Binary compare can be use to compare the samples to the procedural blank and find the components that are unique or elevated to the sample (Figure 5).

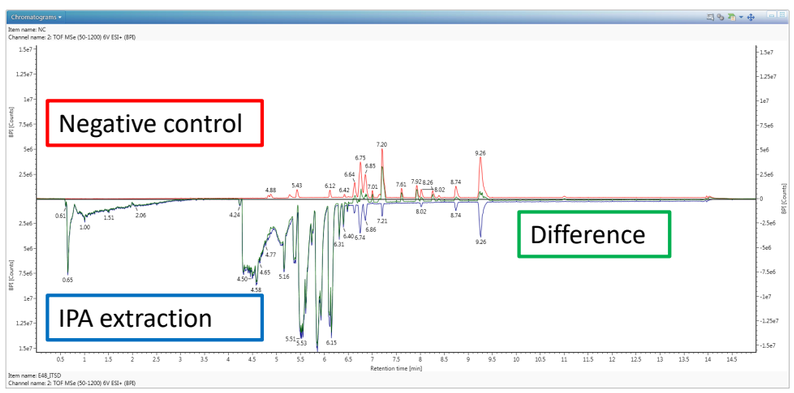

Figure 5. A difference plot of the base peak intensity chromatograms.

A compound detected at *m/z* 368.4253 that was unique to the samples was putatively assigned as a surfactant using the structural elucidation toolkit (Figure 6).[4]

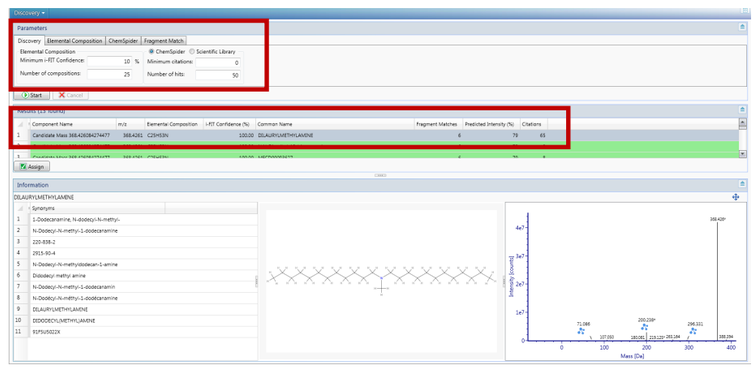

Figure 6. The elucidation toolkit in the UNIFI Application can be used for the tentative identification of unknown peaks identified in a sample using the accurate mass and fragmentation data that was acquired on the instrument.

Implementing quantitation into the workflow was also investigated. The internal standard, metafluzimone, was spiked in the neat solutions at 250 ng/mL to assess the platform for quantifying leachables alongside extractables. A calibration curve was created with the standard from 5 to 1000 ng/mL (Figure 7).

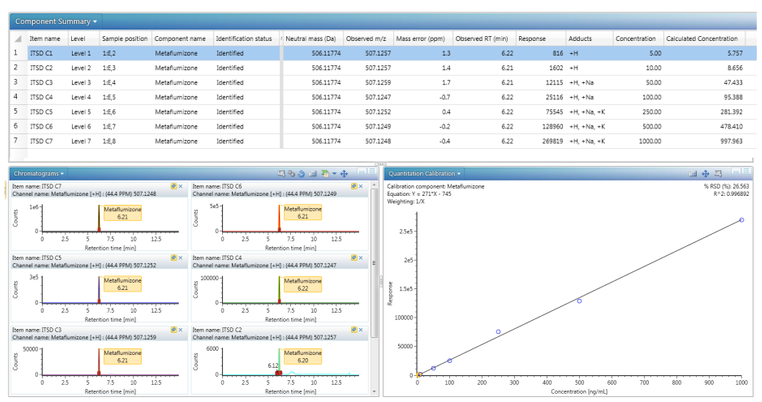

Figure 7. Calibration curve results for the internal standard. The calibration curve covers 4 orders of magnitude with a R^2 value of 0.999.

Using the calibration curve of the internal standard, the concentration of the internal standard spiked into the samples could be calculated within 8% of the known value (Figure 8).This demonstrates that if a calibration curve is created for any analytes of interest, quantitation can be done along side the experiment for any expected extractables.

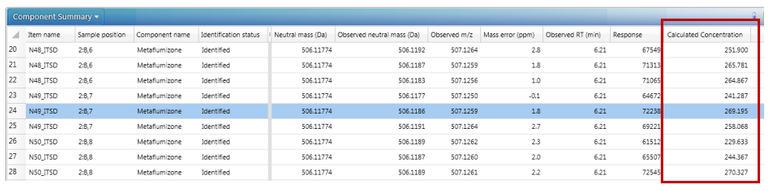

Figure 8. The calculated concentration of the spiked internal standard was calculated within 8% of the known value.

Using an internal standard or standards, response factors can also be included in the UNIFI Application for semi-quantitation. Response factors and relative response factors can be used to estimate the concentration.[5] For example, the concentration of Irganox™ 1010 Antioxidant could be calculated within 5% of the known value (125 µL) using metafluzimone as the internal standard (Figure 9).

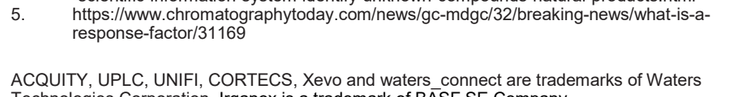

Figure 9. The calculated response of Irganox 1010 Antioxidant using response factors.

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.**
- **Increased sensitivity assists with detection of low level components to meet screening thresholds.**
- **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.**
- **Quantitation and semi-quantitation of components can be included on the platform through calibration curves or response factors.**
- **The UNIFI Application enables all steps within an E&L analysis to be included in one workflow that can be customized depending on regulatory needs.**

References

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