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Screening Workflow Using Ion Mobility-Mass Spectrometry for The Analysis of Extractables and Leachables

Jane Cooper, Rachel Sanig, Gitte Barknowitz, Baiba Cabovska

Waters Corporation

Abstract

Identification and characterization of extractable and leachable (E&L) components in various industries is growing in importance due to increasing global regulations. A typical E&L analysis workflow includes a targeted screening step based on a library or database of components, where the quality of the library is a critical factor for reliable screening. The workflow also includes a non-targeted screening step with subsequent characterization of any unknown components found which is typically complex and time-consuming but can be greatly improved using comparison and elucidation software tools such as those provided by the UNIFI Scientific Information System.

High-Resolution Mass Spectrometry (HRMS) techniques are well suited for dealing with large compound lists in both targeted and non-targeted screening analyses. Ion mobility coupled with HRMS affords an additional separation of ions based on size, shape, and charge. This can help determine the collisional cross section (CCS, units of $Å^2$) value of an ion; a distinguishing characteristic of an ion in the gas phase, related to its chemical structure and three-dimensional conformation. Using CCS values as an additional data point can improve the quality of the screening library for a more reliable targeted screening application. Spectral cleanup is another benefit of using ion mobility and aids the elucidation of unknown compound spectra and confirmation of known compounds.

This application note outlines how using the UNIFI Scientific Information System within an E&L workflow allows for the incorporation of both targeted and non-targeted screening within one application, as well as how to create a scientific library, perform a binary comparison, and use the elucidation tool and other additional tools.

Benefits

- Simple LC-MS methodology with high-resolution mass spectrometry for cosmetics, food, and pharmaceutical packaging extractable applications
- Routine ion mobility with CCS processing for increased confidence in targeted screening and identification
- UNIFI Scientific Information System Software provides customized workflows to streamline and simplify structural elucidation of unknown compounds

Introduction

Product packaging or contact materials are made of different chemicals, which can include polymers, polymer additives such as antioxidants, slip agents, colorants, and other compounds. These chemicals, their impurities, and degradation products can migrate into the consumer products they were designed to protect; adding unwanted and potentially unsafe substances into food, medicine, cosmetics, drug delivery systems, implantable medical devices, and other products.

Identification and characterization of extractable and leachable (E&L) components in various industries is growing in importance due to increasing global regulations.[1-7] A typical E&L analysis workflow initially starts with a targeted screening step. The screening is based on a library or database of components where matches are made against accurate mass, retention times, and fragment mass data. The quality of these libraries is critical for a reliable screening application. Next in the workflow is a non-targeted screening step with subsequent characterization of any unknown components found. This step is typically complex and time-consuming; however, comparison, and elucidation software tools can greatly aid and speed up the process.

High-Resolution Mass Spectrometry (HRMS) techniques are well suited for dealing with large compound lists in targeted and non-targeted screening analyses. Ion mobility spectrometry (IMS) coupled with HRMS affords an additional separation of ions, based on size, shape, and charge as they drift through gas in a mobility cell. The collisional cross section (CCS, units of $Å^2$) value of an ion may be determined using its drift time through an IMS device. CCS is a distinguishing characteristic of an ion in the gas phase, being related to its chemical structure and three-dimensional conformation. Using CCS values as an additional data point can further improve upon the quality of these libraries for targeted screening.[8] Spectral cleanup is another benefit of using IMS and aids the elucidation of unknown compound spectra and confirmation of known compounds.[9]

This application note outlines a simple workflow that includes both targeted and non-targeted screening within one application, as well as scientific library creation, binary comparison, elucidation, and additional tools. Also described are the benefits of ion mobility using the Vion IMS QTof (Figure 1) for the application of extractables and leachables screening.

Figure 1. The Vion IMS QTof Mass Spectrometer.

Experimental

Sample Preparation

Sample extracts were prepared by cutting packaging materials into 5 mm^2 pieces and sonicating 1 g of the pieces in 10 mL of 2-propanol for nine hours at an average temperature of 45 °C.

LC Conditions

LC system:	ACQUITY UPLC I-Class FTN
Column(s):	CORTECS C_{18} Column, 90Å, 1.6 µm, 2.1 mm X 100 mm (p/n: 186007095)
Column temp.:	40 °C
Injection volume:	1 µL
Flow rate:	0.4 mL/min
Mobile phase A:	Water + 1 mM ammonium acetate + 0.1% acetic acid
Mobile phase B:	Methanol
Gradient:	Mobile phase gradient is detailed in Table 1

Gradient

Time (min)	%A	%B	Curve
0.0	98	2	Initial
0.5	98	2	6
6.0	1	99	6
12.0	1	99	6
12.1	98	2	6
14.0	98	2	6

Table 1. ACQUITY UPLC mobile phase gradient.

MS Conditions

MS system:	Vion IMS QTof
Ionization mode:	ESI+, ESI-
Acquisition mode:	$HDMS^E$
Acquisition range:	50–1500 *m/z*
Scan time:	0.2 sec
Source temp.:	120 °C
Desolvation temp.:	500 °C

Desolvation gas:	1000 L/hr
Cone gas:	50 L/hr
Reference mass:	Leucine enkephalin $[M+H]^+$ *m/z* 556.276
Capillary voltage:	0.6 kV
Collision energy:	ESI+ low energy: 6 eV high energy ramp: 20-40 eV ESI- low energy: 6 eV high energy ramp: 30-70 eV

Data Management

Chromatography software:	UNIFI Scientific Information System v1.9.4
MS software:	UNIFI Scientific Information System v1.9.4
Informatics:	UNIFI Scientific Information System v1.9.4

Analysis Protocol

Bracketing standards, which were analyzed before, during and after the analysis were used to check mass accuracy and CCS values of components identified. Acceptance criteria for the identified compounds were set as ≤3% ppm for mass accuracy and ≤2% delta for CCS values.

For this analysis, two different system suitability standard mixtures were used, the Waters LC-MS QC reference standard (p/n: 186007362 <https://www.waters.com/nextgen/in/en/shop/standards--reagents/186007362-quad-lcms-qc-reference-material.html>), with established CCS values, and the Waters E&L system suitability mixed standard (p/n: 186008063 <https://www.waters.com/nextgen/in/en/shop/standards--reagents/186008063-extractables--leachables-

screening-standard.html>)[10]. The E&L standard contains 18 compounds, representative of common industry polymer additives and preservatives, which cover a wide mass range, and includes compounds that ionize in both negative and positive ESI modes.

Results and Discussion

For this study, an application specific UNIFI library was created for the targeted screening of E&L compounds containing over 100 entries. Initially, the library was populated with key information about each component including compound name, IUPAC name, tags, and the associated .mol-files. This was then supplemented with experimentally derived detection results from the analysis of standards.

Stock standards containing common compounds from inks, dyes, antioxidants, and UV filters were prepared for over 100 typical E&L compounds and pooled into mixes (9–10 compounds per mix). The mixed standards, at two concentration levels (5 ppm and 0.5 ppm), were injected seven times per mix using the method outlined in the experimental section. From the results generated (Table 2), average CCS and retention time values were imported into the UNIFI library. Additional adduct and fragment ion information was also sent to the scientific library for each library component analyzed (Figure 2). Extensive and accurate information for each library component can reduce the number of false positives during the targeted screening analysis. This can help to increase the user's confidence in the identification of the targeted component.

Component name	Neutral mass (Da)	tR (min)	%RSD tR	Average mass error (ppm)	Average CCS	%RSD CCS	Adduct	ESI +ve or -ve
Methylparaben	152.0473	3.63	0.21	1.09	126.26	0.18	-H	-ve
Propylparaben	180.0786	4.65	0.14	0.94	140.69	0.1	-H	-ve
Diethylphthalate	222.0892	4.76	0.15	0.28	159.54	0.25	+Na	+ve
Tinuvin P	225.0902	6.15	0.11	0.6	146.81	0.12	+H	+ve
Dibutyl sebacate (DBS)	314.2457	6.49	0.08	0.86	196.83	0.11	+Na	+ve
				-	188.18	0.1	+H	
				-	199.34	0.21	+K	
				-	199.29	0.17	+Li	
Diphenyl phthalate	318.0892	5.62	0.11	-0.16	181.74	0.18	+Na	+ve
				-	180.28	0.24	+K	
				-	185.52	0.64	+Li	
Irgafos 168	646.4515	10.71	0.17	0.45	283.57	0.23	+H	+ve
				-	281.32	0.33	+Na	
					286.45	0.62	+K	

Table 2. Generated average CCS and retention times data for a selection of the compounds in the E&L library created for this study.

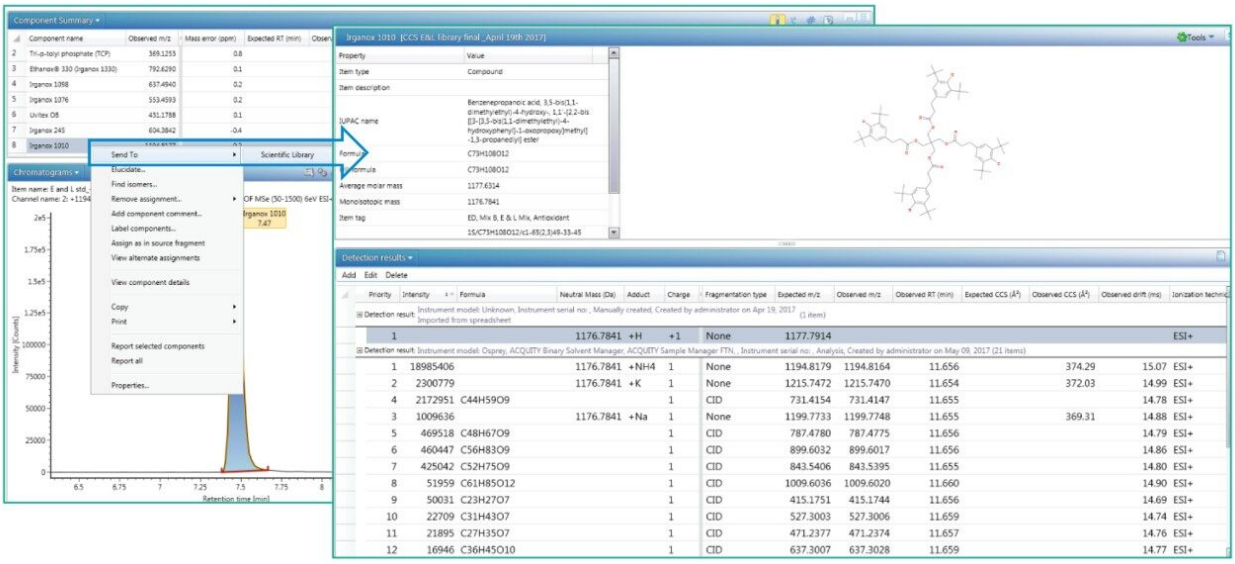


Figure 2. Example of adduct and fragment ion information sent to the scientific E&L library, illustrated for Irganox 1010. Library entry layout in UNIFI on the right side of the illustration including the observed CCS, retention time values, ionization mode information, and adducts.

UNIFI Workflow

A workflow is a series of steps that are designed to enable thorough visualization of the entire dataset, so that the information required to make decisions can be easily accessed with minimal user intervention. Within UNIFI, users can define how their data is processed and displayed with workflows that are customizable to individual user requirements. A typical example of an E&L method-specific analysis was created with multiple steps included within one workflow (Figure 3), which could be customized further depending on the user's needs. Here, we describe the following steps: system suitability review; identified components review against a developed library (targeted screening); unknown compound identification using binary compare (non-targeted screening); and elucidation discovery tools. Samples were analyzed using the method outlined in the experimental.

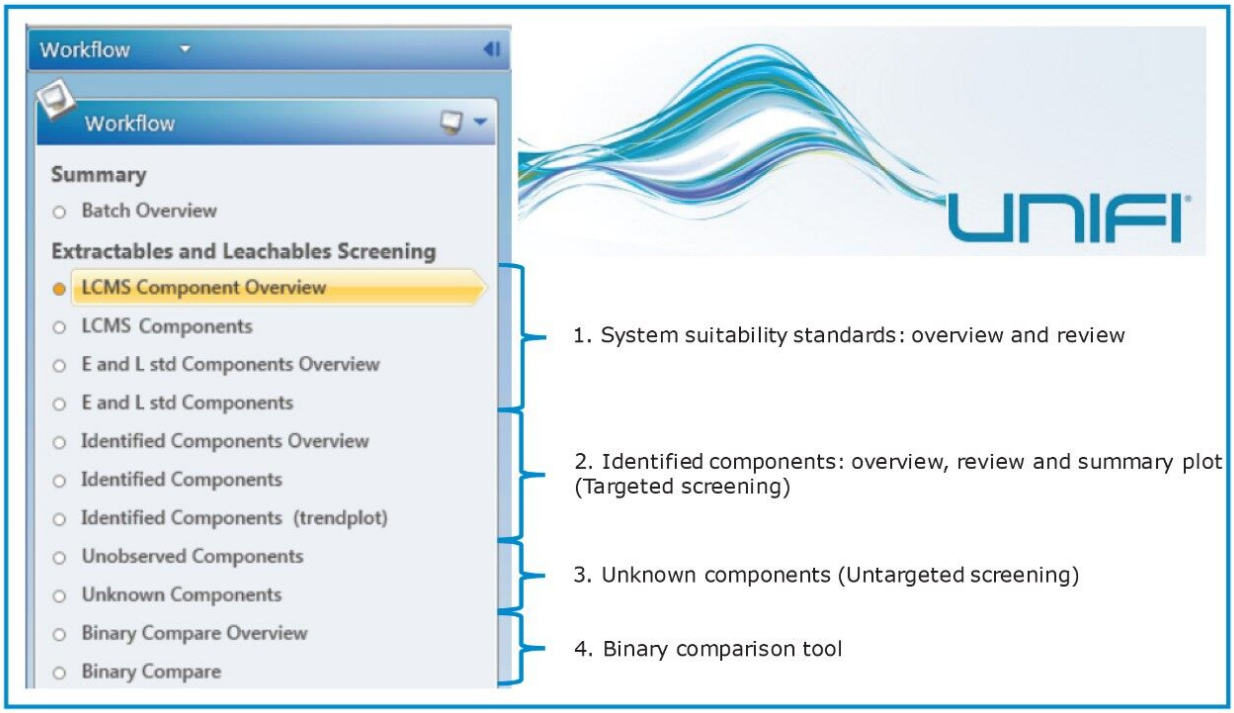


Figure 3. Example of the customizable UNIFI workflow, highlighting the different techniques that can be employed within one workflow and the different ways to view the data.

1. System Suitability Standard Overview and Review

The first step in this UNIFI example workflow aids the review and acceptance of system suitability standards, checking the performance of the whole system. Data can be viewed as a component overview plot (see Figure 4) or as a component summary.

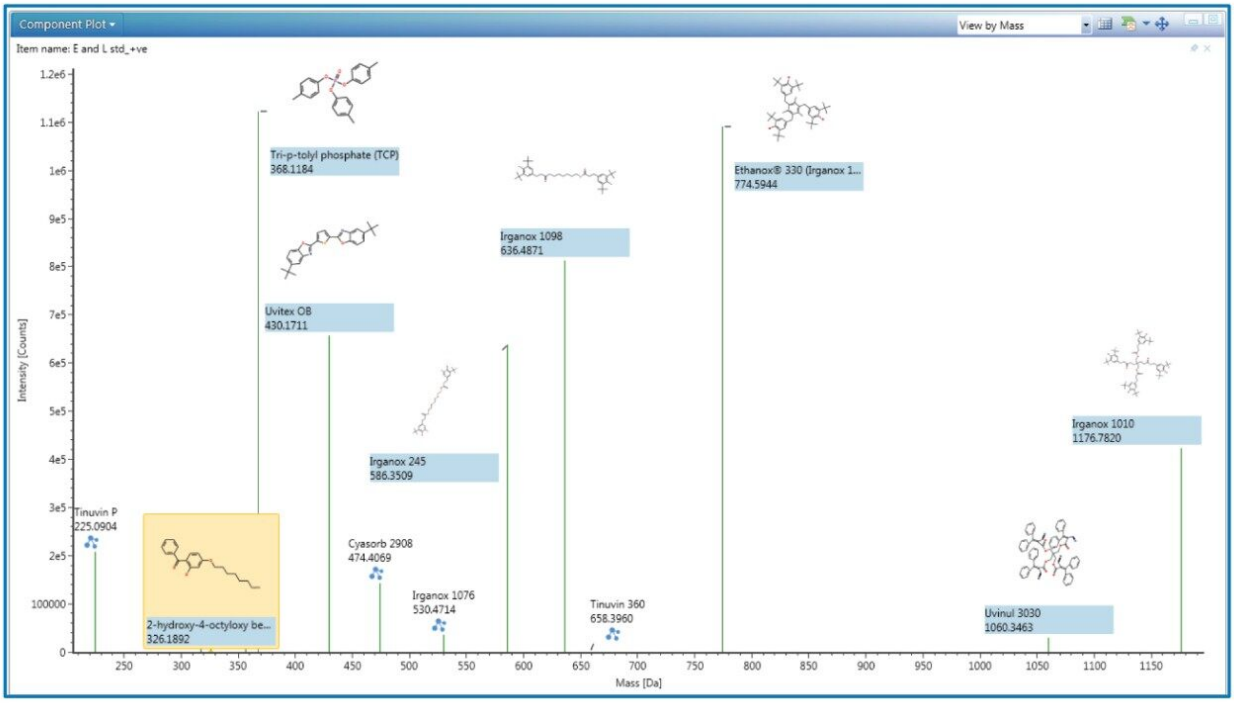


Figure 4. Component overview plot for the analysis of an E&L system suitability standard used to check system performance.

2. Identified Component Overview, Review, and Summary Plot

The next stage in the UNIFI workflow includes steps to aid with the review and acceptance of components identified from the targeted screening analysis against the UNIFI scientific library. These results can be viewed as a component overview plot or component summary (Figure 5). Multiple identification points are important to help avoid false positives and enhance confidence in the identification, all reducing the time required to review the results. Accurate mass, retention time, mass fragments, and CCS can all be used as identification points for targeted screening.

The CCS values, obtained using ion mobility spectrometry, can be advantageous as an additional identification point, helping to reduce false positives. Unlike retention times, CCS values are not affected by changes to mobile phase, column, etc. or by matrix effects. Furthermore, where accurate mass alone would fail to separate isomers, CCS values could potentially be used to separate and identify any isomers present. Therefore, the addition of CCS values to the extractables and leachables screening library can help aid successful targeted screening.

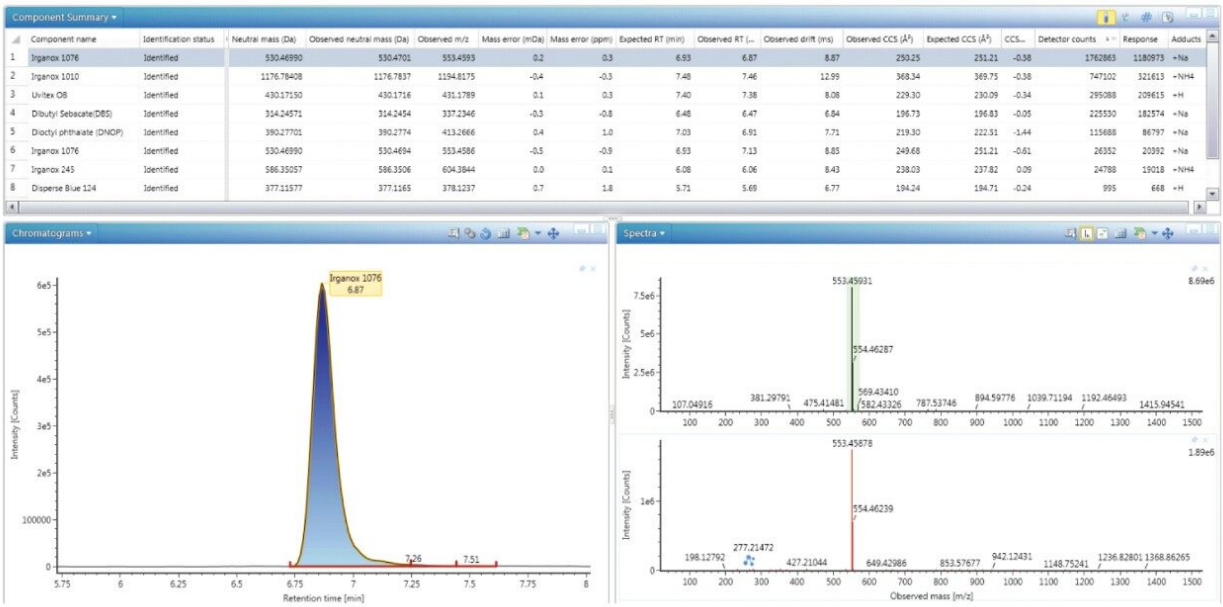


Figure 5. Identified component summary view for an extracted sample. The view can be customized to suit a user's needs. Here the identified component summary table and a selected component's chromatogram and spectra are visible.

Alongside this, another benefit of CCS for screening includes the spectral clarity that is achieved when components are aligned by their retention time and ion mobility drift time, making it easier to visualize and identify both precursor and fragment ions (Figure 6). Low and high energy spectra become cleaner as chromatographically coeluting species with different ion mobility drifts times are removed, and fragment ions are more easily associated with their precursor ions as their drift times align in the ion mobility dimension.

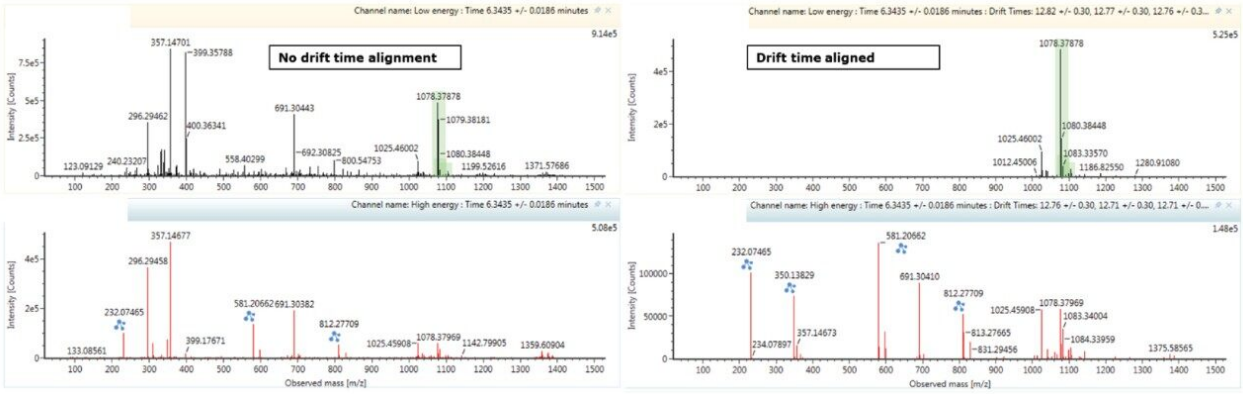


Figure 6. Low and high energy spectra for Uvinul 3030 from the E&L standard mix. On the left you can see the spectra without drift time alignment applied and, on the right, you can see the spectra with the drift time alignment which results in cleaner spectra.

Another way to view the data in UNIFI is via the summary plot feature, which enables users to visualize trends in the results for the selected component over all injections in the analysis run (Figure 7). This can be summarized for any result for the component such as CCS, accurate mass, retention time or response.

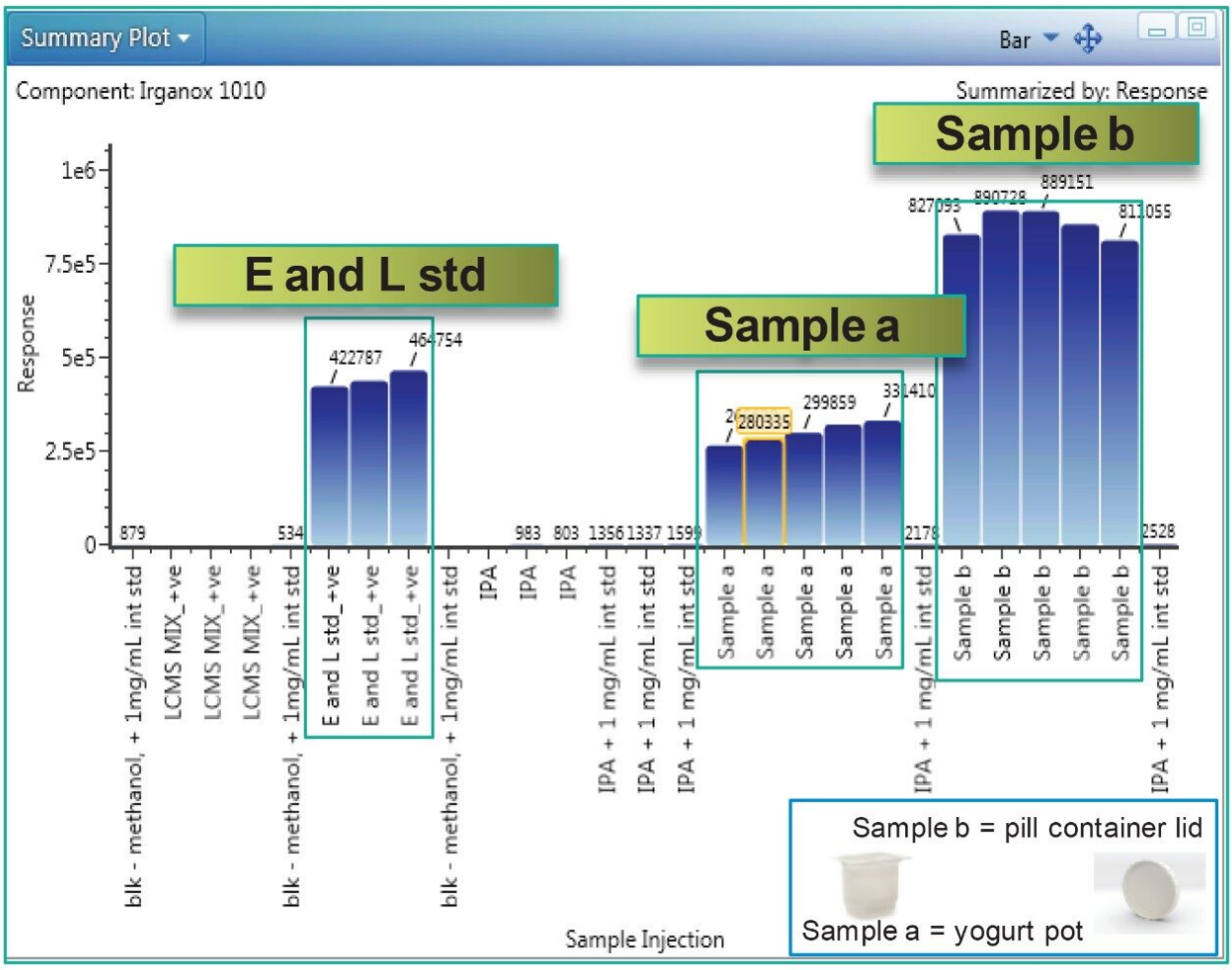


Figure 7. Summary Plot for the response of Irganox 1010 identified in the E&L standards and two different samples (a and b).

3. Unknown Components Review

The identification of unknown peaks in the UPLC chromatogram is a critical part of the analysis of E&L to achieve full characterization of the sample. In this created workflow, there are two steps to review the data. The first step finds and lists the unknown components (Figure 8).

Figure 8. Unknown components listed as candidate masses for an extracted sample.

The second step is to use binary compare. This feature of UNIFI Software allows the analyst to directly compare results of an analyte sample and a reference blank extract and identify key differences. Components that are unique to the sample or highly elevated in the sample can be easily displayed in the component summary for further review (Figure 9). This is an important tool for E&L analyses where background levels of E&L type compounds are often seen. In this workflow, binary compare is highlighted but a multi-variate analysis suite can also be utilized when statistical comparison is required.

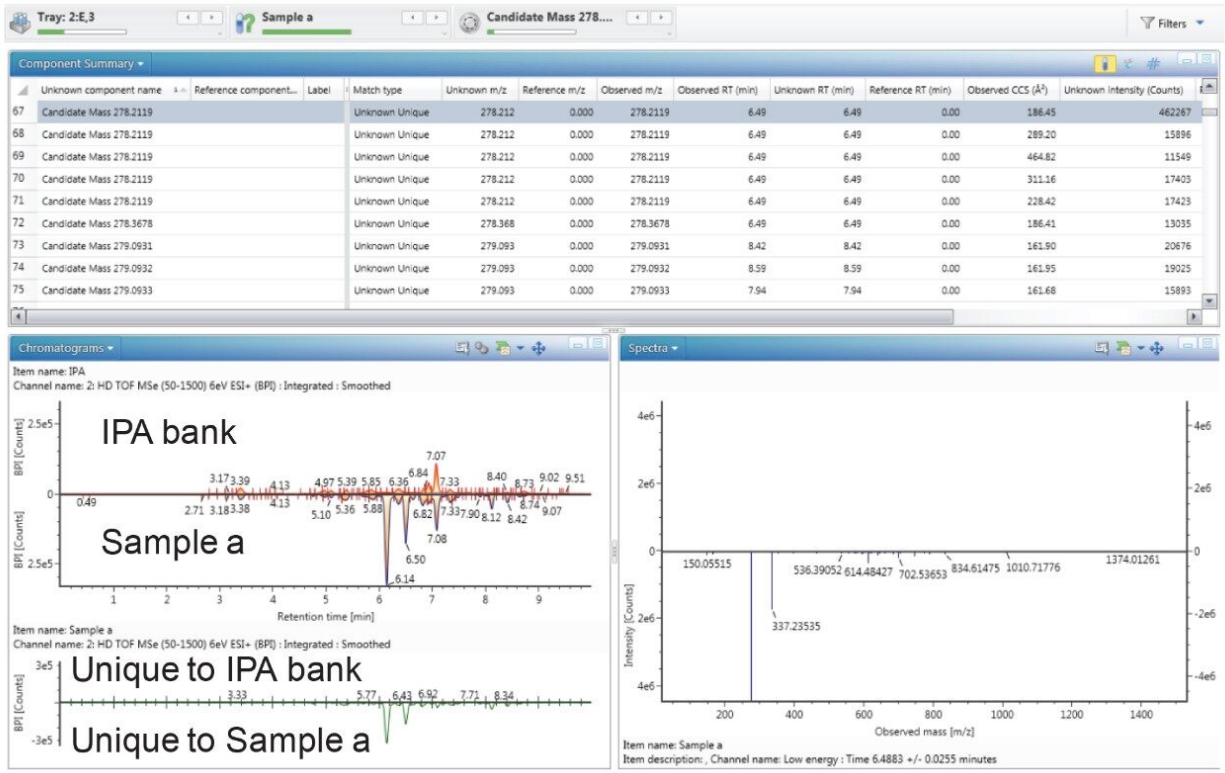


Figure 9. Compounds observed in the sample following the binary comparison between a sample and a reference blank (IPA) are listed as candidate masses. The comparison plots here are of the base peak intensity chromatograms but can be customized to suit the user.

4. Elucidation Toolkit

Once an unknown component has been found, analysts can investigate the identity of the component directly from the workflow via the 'elucidation toolkit' using UNIFI's discovery tools[11] (illustrated in Figure 10). The toolset includes an elemental composition calculator that determines the most likely chemical formulas for an accurate mass peak. An i-FIT algorithm is used to score each formula by the likelihood that the theoretical isotope pattern of the formula matches a cluster of peaks in the spectrum, along with the high-resolution accurate mass measurement. The software then uses the predicted elemental composition to undertake database searches for structures, for example in ChemSpider. Next it will do an *in-silico* fragmentation of the found structure to look for matches of the theoretical fragments with the observed high energy ions. Tentative results are then displayed for the user to evaluate. To achieve a 100% match, a reference standard would be needed. However, UNIFI Software significantly saves on the analyst's time in the elucidation process.

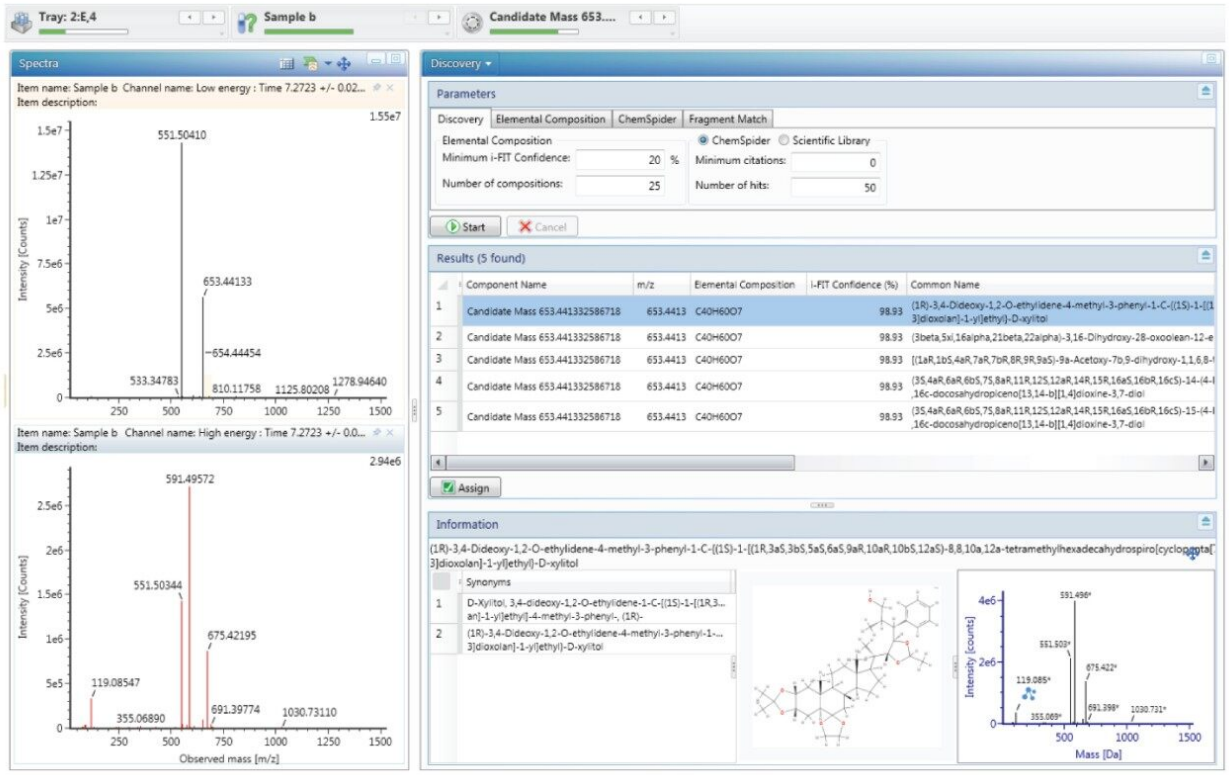
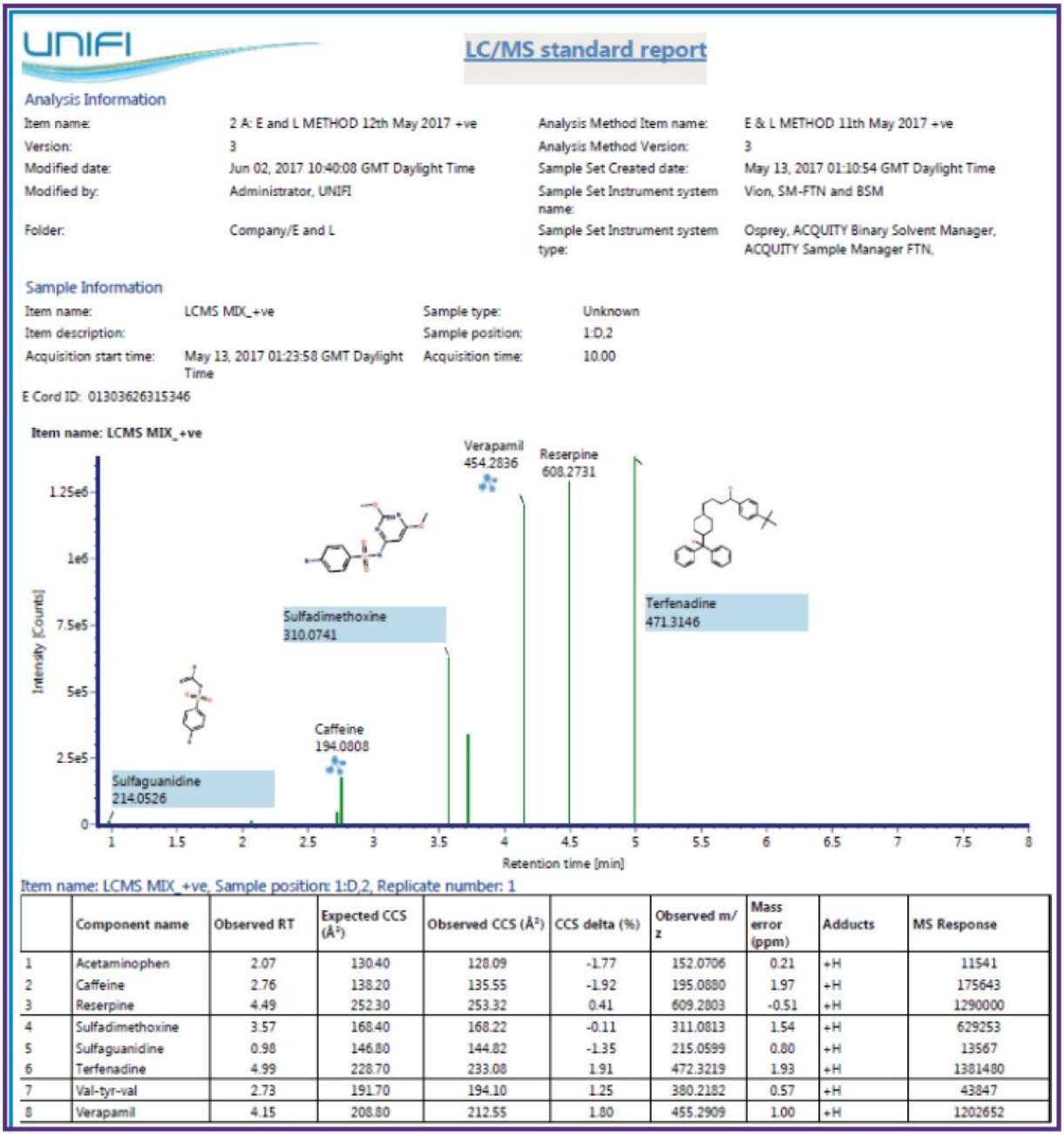


Figure 10. UNIFI discovery tools used for the identification of unknown peaks identified in a sample.

5. Additional Tools

There are additional steps that could be utilized in the UNIFI E&L screening workflow that have not been described here. For example, a quantification step can be included, whereby a calibration curve with known standards can be created and utilized to quantify levels of a component within the sample being analyzed.[12] Furthermore, the UNIFI workflow includes a customizable reporting tool that is designed to display data and other information required by the user. Figure 11 illustrates the report output from a system suitability test using the Waters LC-MS QC reference standard.



	Component name	Observed RT	Expected CCS (Å²)	Observed CCS (Å²)	CCS delta (%)	Observed m/z	Mass error (ppm)	Adducts	MS Response
1	Acetaminophen	2.07	130.40	128.09	-1.77	152.0706	0.21	+H	11541
2	Caffeine	2.76	138.20	135.55	-1.92	195.0880	1.97	+H	175643
3	Reserpine	4.49	252.30	253.32	0.41	609.2803	-0.51	+H	1290000
4	Sulfadimethoxine	3.57	168.40	168.22	-0.11	311.0813	1.54	+H	629253
5	Sulfaguanidine	0.98	146.80	144.82	-1.35	215.0599	0.80	+H	13567
6	Terfenadine	4.99	228.70	233.08	1.91	472.3219	1.93	+H	1381480
7	Val-tyr-val	2.73	191.70	194.10	1.25	380.2182	0.57	+H	43847
8	Verapamil	4.15	208.80	212.55	1.80	455.2909	1.00	+H	1202652

Figure 11. UNIFI LC-MS System suitability standard report.

Conclusion

The UNIFI E&L screening workflow provides analysts with a user-definable and established workflow to aid navigation through complex data. Acquisition, processing, and reporting are carried out from a single integrated platform - reducing the time from sample analysis to reporting. Targeted and non-targeted screening can be undertaken within one application while also allowing the user extra steps such as scientific library creation, data comparison, and elucidation needed to complete their specific workflow.

The scientific library application offers the ability to quickly perform searches against a database of entries within the workflow. The user can search with accuracy and confidence against *m/z*, retention time, CCS, and fragment ion information. The informatics-based structural elucidation discovery tool provides a rapid process to evaluate information for an unknown component.

Using ion mobility mass spectrometry allows for an additional dimension of separation of complex samples and the generation of CCS values. For the analysis of extractables and leachables, an additional data point for targeted screening aids the reduction of false positives and increases confidence in identification. The spectral clarity achieved with the drift time alignment makes it easier to identify ions and aids the elucidation of unknown compound spectra and confirmation of known compounds.

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