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Non-Targeted Screening Analysis of Packaging Extracts Using the UNIFI Scientific Information System

Baiba Čabovska
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Simple LC-MS methodology leverages high-resolution mass spectrometry that can be adopted for cosmetics, food, and pharmaceutical packaging extractable applications.
- Streamlines the structural elucidation process for packaging extracts by utilizing MS^E data of accurate mass precursor and fragment ion information on a single software platform.
- Rapidly evaluate information for an unknown component (*m/z*) by ranking the possible elemental compositions and performing database searches for likely structures ranked based on fragmentation matching.

WATERS SOLUTIONS

ACQUITY UPLC® I-Class System

UNIFI® Scientific Information System

Xevo® G2-XS QTof Mass Spectrometer

CORTECS® C_{18} Column

KEY WORDS

Extractables, leachables, packaging, cosmetics, screening, elucidation, accurate mass, QTof, non-targeted analysis, informatics

INTRODUCTION

Characterization of packaging in various industries has become more important due to ever-increasing global regulations. The first regulations for plastics used in food packaging and contact materials were established in 1982 in Europe,[1] which have been expanded in recent years.[2] In the pharmaceutical field the need for extractables testing was recognized in the 1990s.[3] Manufacturers are required to evaluate packaging for the possible migration of additives and ingredients into the final product because of the potential impact extractables and leachables can have on patients' health.[4,5] Extractables in the pharmaceutical industry are defined as compounds that can be extracted from packaging materials or devices under controlled experimental conditions. Leachables, a subset of extractables, are compounds that actually migrate into the final product during expected shelf or contact time. The latest addition to the industries that require testing of packaging is the cosmetics industry. The most recent regulations for the cosmetics industry in Europe (EU Regulation 1223/2009) Annex 1 states that "impurities, traces, information about the packaging material must be determined".[6] For the cosmetics industry the impact from leachables would depend on the route of application. For example, it would be less critical for cosmetic products that are applied to the skin such as body creams than it would for products that can be ingested or absorbed through the eyes, such as lipstick or mascara.

The initial step for characterizing extractables from packaging involves targeted screening, *i.e.*, testing the extracts for known compounds. This is a well-established process that can be performed using various analytical techniques ranging from GC-FID-MS to LC-UV/MS. However, the final packaging may have impurities present from the starting materials and additional degradants such as those formed during the molding process. The first step in ensuring that these compounds do not pose any toxicological risks to the consumer is to identify the extractables, or at least their structural class. The structural elucidation of unknowns is typically a very complex and time-consuming process that requires the analyst to have a higher level of expertise. Waters® UNIFI Scientific Information System utilizes accurate mass and fragment information to simplify data review and facilitate the decision-making process. It allows analysts to evaluate complex data in a more efficient way and quickly make decisions about the possible identity of an unknown compound.

EXPERIMENTAL

UPLC conditions

UPLC system:	ACQUITY UPLC I-Class
Separation mode:	Gradient
Column:	CORTECS UPLC C_{18} 90Å, 1.6 µm, 2.1 mm x 100 mm
Column temp.:	40 °C
Injection volume:	5 µL
Flow rate:	0.5 mL/min
Mobile phase A:	0.1% formic acid in water
Mobile phase B:	0.1% formic acid in methanol
Gradient:	60% B held for 30s, increased to 99% over 2.5 min, held at 99% for 5 min, then re-equilibrated back to 60%

MS conditions

MS system:	Xevo G2-XS QTof
Ionization mode:	ESI +
Capillary voltage:	3.0 kV
Desolvation temp.:	450 °C
Source temp.:	150 °C
Cone voltage:	25 V
Collision ramp:	10 to 40 eV
MS scan range:	50 to 1200 *m/z*

Data acquisition and processing

UNIFI Software was used for acquisition and data processing.

Sample preparation

Mascara packaging made of polypropylene, lipstick packaging and tonal cream packaging made of polyethylene were chosen as samples. The cosmetics products were removed from the packaging, which was subsequently cut into 1x1 cm pieces. Sample extracts were prepared in isopropanol (IPA) by extracting ~2 g in 5 mL of IPA by sonication in glass scintillation vials for 6 hours.

RESULTS AND DISCUSSION

Typically, screening experiments for packaging extracts are performed using generic gradient LC-MS methods. As it is not known what kind of chromatographic profile the extract might have, the screening methods are not optimized for each individual packaging material at this initial stage in R&D. If the chromatogram only has one or two peaks, it is easy for analysts to decide where to start their investigation. However, if the extract has a multiple chromatographic peaks that are not completely resolved, or if several groups of samples must be compared, the analyst needs to determine which compounds are unique to the extract and are not present in the extraction blank (Figure 1). Furthermore, less intensively ionized compounds or trace-level compounds of toxicological concern may not be visible in the total ion current (TIC) chromatogram, or even in the base peak intensity (BPI) chromatogram.

Binary compare

In cases where only two samples must be compared, for example a blank extract (reference) and a sample (unknown), UNIFI Software's binary comparison feature allows the analyst to directly compare the chromatographic and spectral results of an analyte sample with those of a reference sample. Masses (*m/z*) in the reference and unknown spectra are considered to be the same component if they are within the user-specified mass, retention time, and intensity difference tolerance. The comparison can be presented graphically as a mirror image of BPI or TIC chromatograms, or as a table of Candidate Masses (Figure 2). The candidates are accurate mass and retention time pairs which have common peak features in the raw data. They are grouped according to retention time alignment and isotope spacing.

UNIFI shows a comparison between the mass spectrum of the compound in the unknown sample with the reference sample, and displays any differences. Figure 2 shows the comparison between an IPA blank extract "Reference sample" and lipstick packaging extract "Unknown sample" with the column "Match type" highlighting if the candidate is present in only the unknown sample, the reference sample, or both - the corresponding match types would be Unknown Unique, Reference Unique or Common. In this case, the most interesting candidates for further evaluation would be those that are not present in the extraction blank- Unknown Unique.

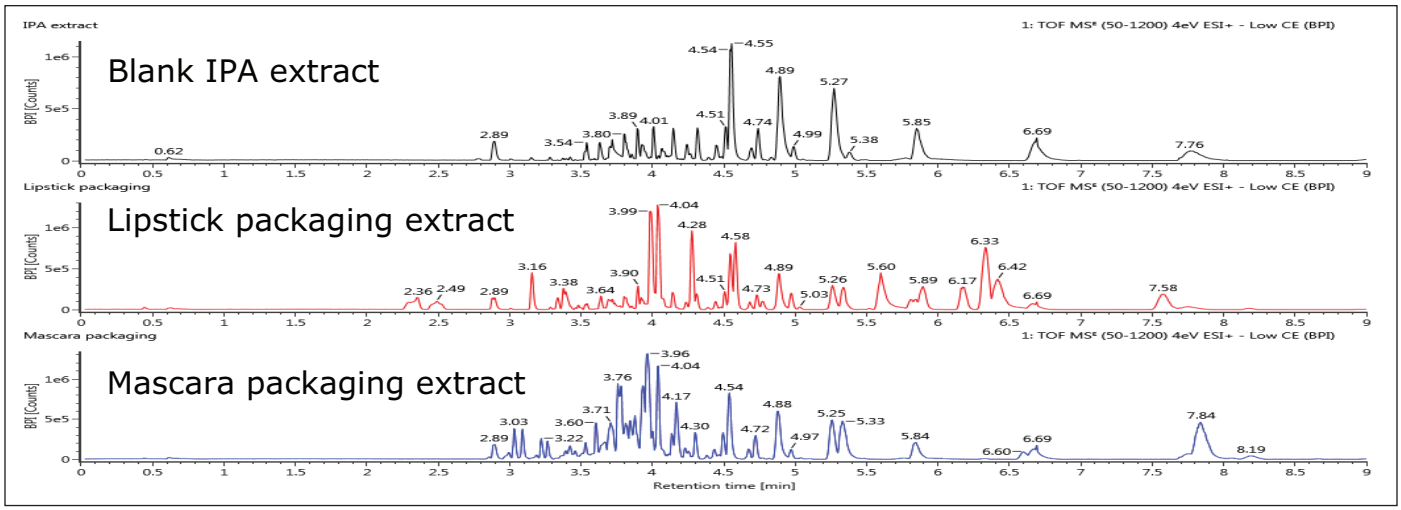

Figure 1. Mass chromatograms for packaging extracts and a blank extract.

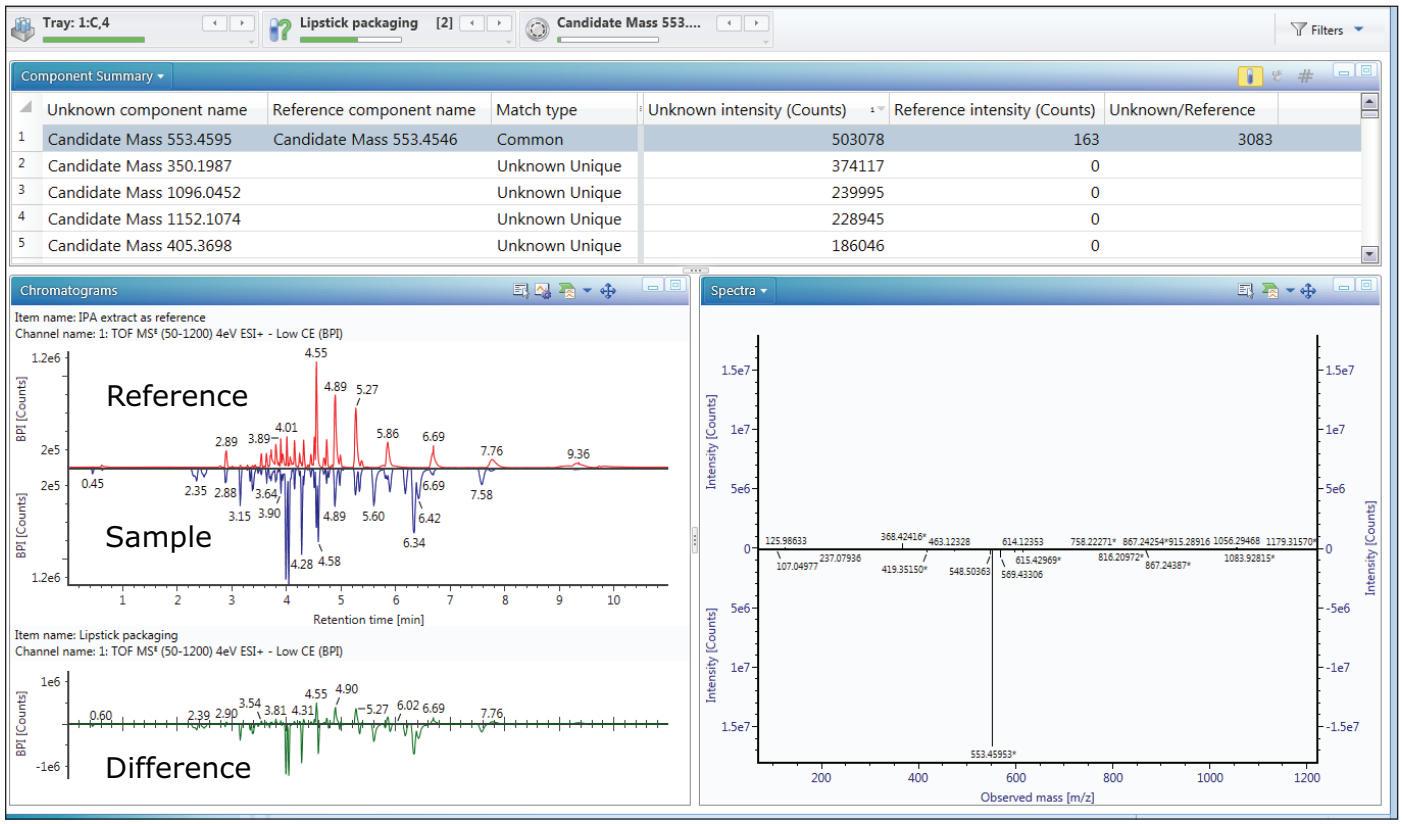

Figure 2. Binary compare results window for the IPA reference sample extract and lipstick packaging sample. The red trace shows the BPI chromatogram of the reference sample (IPA blank extract); the blue trace shows the BPI chromatogram of the lipstick packaging extract; and the green trace shows the difference between the samples.

Due to increases in instrument sensitivity and the ubiquitous presence of many extractables in LC-MS solvents, extraction vessels, plastic pipette tips, *etc.*, it is often difficult to obtain a clean blank. It is useful to evaluate the compounds where the candidate intensity in the unknown sample is much higher than in the reference sample. The column labeled Unknown/Reference (Figure 2) shows a ratio for common components, allowing users to quickly identify common extractables that may be persistent, but have a fold change that is significant. For candidate mass *m/z* 553.4595 the response ratio is over 3000 which indicates potential presence of the candidate in the extraction blank or a carryover.

High resolution mass spectrometry provides very comprehensive, high-quality information, but interpreting the data sets manually can be challenging. Therefore data processing software is of utmost importance for managing and reviewing data in an more efficient way. UNIFI Software allows users to set up their workflow in order to facilitate visualization of their data in the most productive way, and only display data that is relevant – all with a single click. The processed data can then be filtered using criteria defined by the user. In this case, to make the information in the table easier to manage the data was filtered based on specifications that showed Unknown Unique candidate masses with an intensity over 10,000 counts and Common candidate masses with a response ratio of Unknown/Reference of at least 300.

Once the data has been organized in a way that is most appropriate for the analyst, the next step is to proceed to elucidation of the candidates of interest (most intense for example) by utilizing the accurate mass information and high-collision energy fragment information.

Multivariate analysis (MVA)

Binary compare is useful for comparing two samples, but when multiple samples or sample groups need to be compared, the use of multivariate statistical analysis tools such as principal component analysis (PCA) facilitate the identification of differences between samples or groups. UNIFI can generate marker matrices based upon user-defined criteria which can then be automatically transferred to EZInfo 3.0.3 for MVA. PCA is a statistical tool that reduces a large set of multivariate data into uncorrelated variables called principal components. If additional discrimination among the investigated sample groups is required, the differences can be emphasized by using a Projection to Latent Structures Discriminant Analysis (PLS-DA) model (Figure 3). PLS-DA creates models of the quantitative relationships between the variables X (predictors) and Y (responses) for all sample groups. However, in these plots, each sample is presented by a single point, which does not allow individual markers contributing to the differences between the groups to be observed.

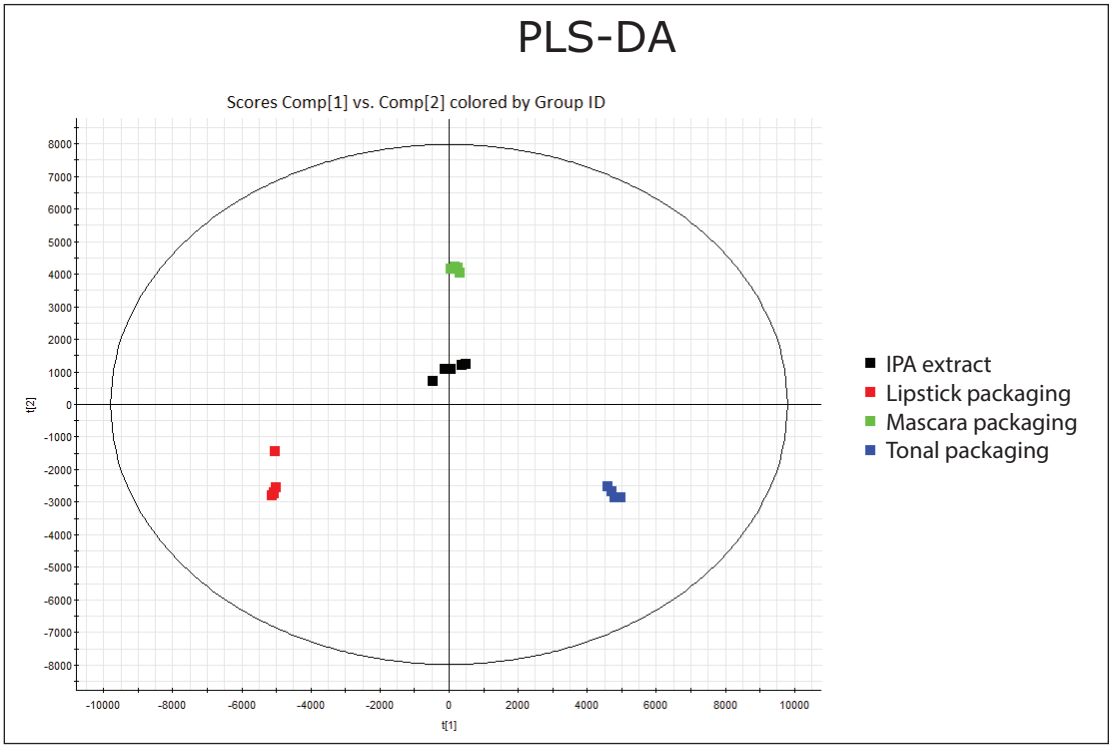

Figure 3. PLS-DA model for all of the packaging sample groups.

In order to investigate group differences down to individual markers, a loadings plot can be used. The loadings plot displays how the X variables correlate to each other, with points further away from the center being the most dissimilar between the sample groups (Figure 4). The data points in these plots are called Accurate Mass/Retention Time (AMRT) pairs. The quadrants in the loadings plot correspond to the PLS-DA model, thus the AMRTs in the lower left quadrant represent the unique markers in the lipstick packaging. Markers selected in red contribute most to the difference between the lipstick packaging and all the other packaging samples.

The differences between the groups can come from analytes that are not present in one of the groups, or from analytes with the greatest change in intensity (concentration) between the groups.

The individual markers that represented the biggest differences between the lipstick packaging and the rest of the group were selected (highlighted in red in Figure 4) and transferred back into UNIFI's Discovery tool for elucidation. When transferring selected markers from the loadings plot, labels can be added to make the data easier to sort and keep track of markers from different sample groups (Figure 5). When an individual marker is selected from the Marker Matrix table, a trend plot is displayed which allows users to quickly evaluate its presence in the other samples or injections.

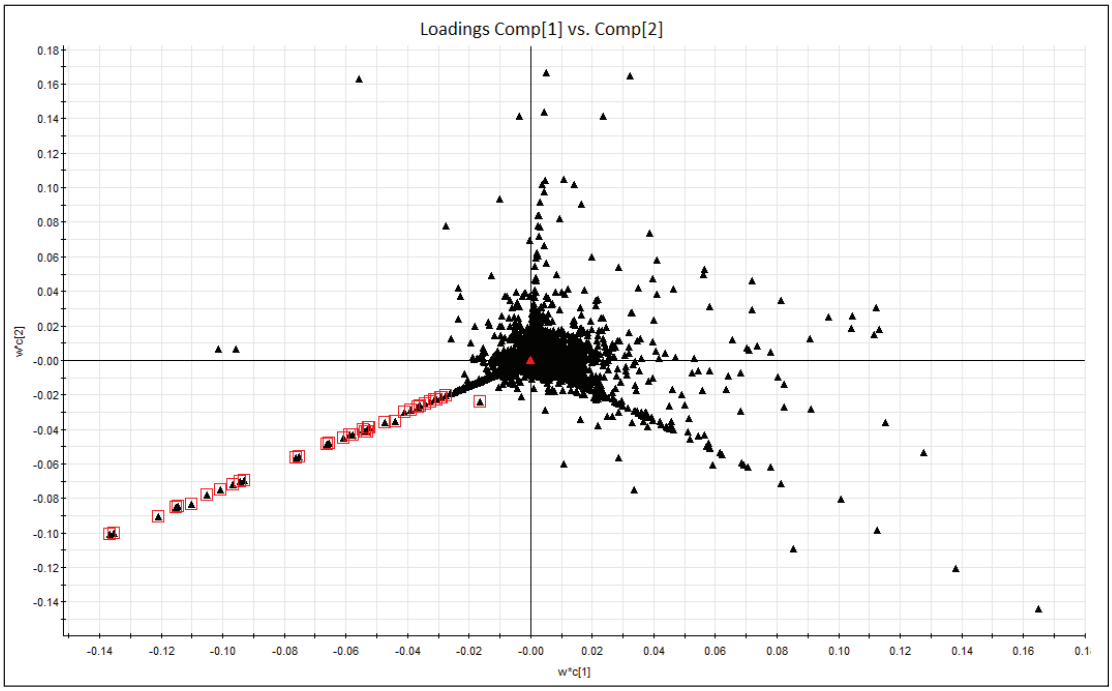

Figure 4. Loadings plot for all of the packaging samples.

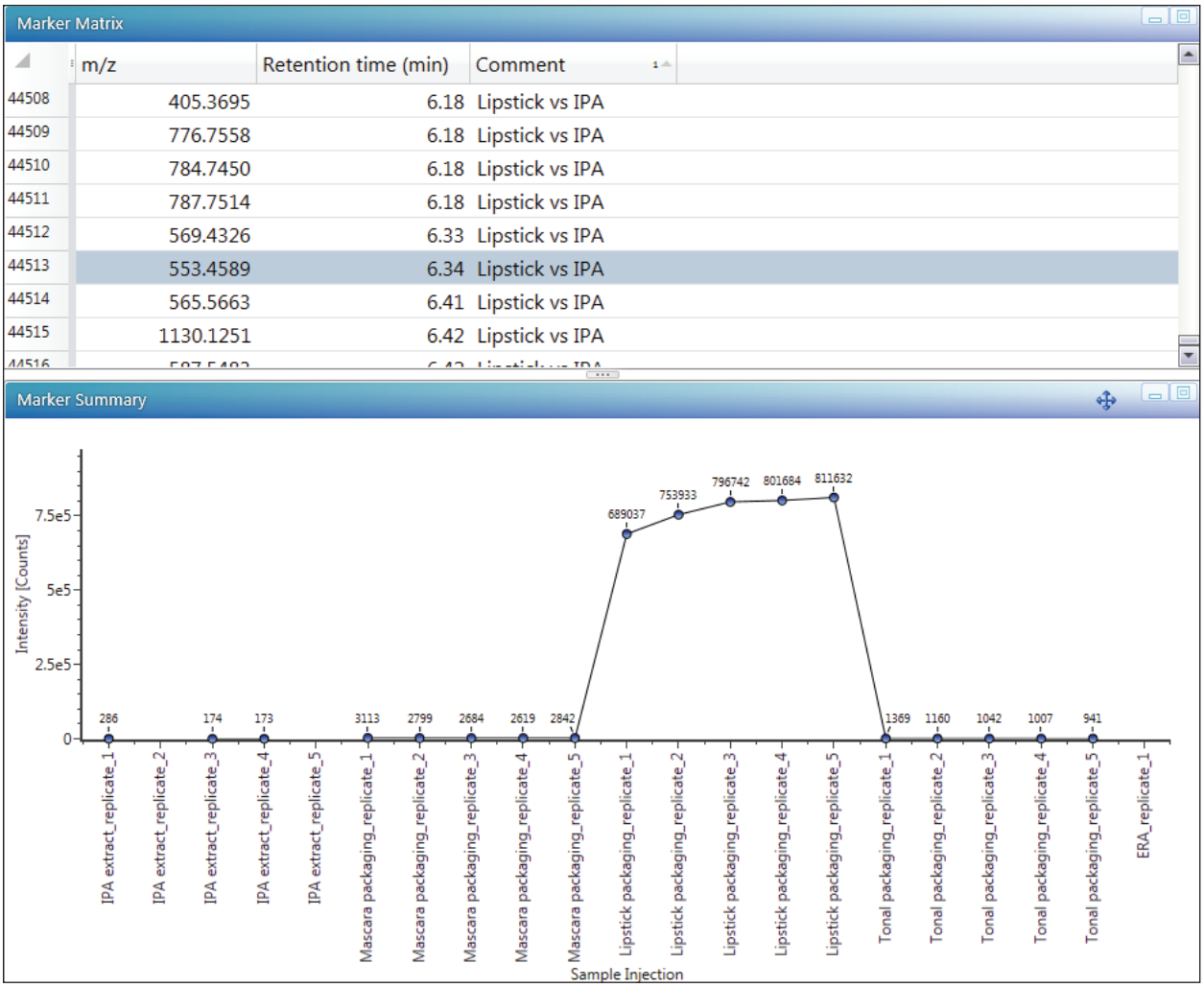

Figure 5. Marker Matrix with labeled markers and a trend plot for a marker 553.4589 at RT 6.34 min.

Discovery tool

Regardless of whether a marker or candidate of interest was obtained by binary compare or multivariate analysis, the next step in the workflow is structural elucidation. The Discovery tools within UNIFI's Elucidation toolset include automated elemental composition, database searching through ChemSpider or UNIFI's configurable Scientific Library, as well as fragment matching of high-collision energy data (Figure 6) of individual or batches of candidates. The best matches are displayed based upon the number of identified high energy fragments, citations from ChemSpider, and mass accuracy. The elemental composition algorithm uses accurate mass and isotope information to calculate the possible compositions for each marker. Using the Discovery tool settings, analysts can specify an acceptable level of isotope match (i-FIT™), elements to be included in the elemental composition search, which libraries to select from ChemSpider (all or specific ones), and minimum number of citations in ChemSpider, among other things.

The final results for the candidate mass *m/z* 360.3236 in the mascara packaging are displayed in a table that lists the elemental compositions within specified limits, possible structures with citations from the ChemSpider database, and how many fragments can be matched to the high collision energy data for each structure (Figure 7).

Many polymer additives form adducts during LC-MS (Na+ being the most common). The adduct ion can be more intense than the protonated species, or the protonated ion can be absent entirely. In this case, the initial evaluation of the mass using +H ion, did not provide a reasonable molecular formula (no i-FIT above 50% and no structure from ChemSpider). Therefore Na+ was selected as an adduct and the Discovery tool process was repeated. As shown in Figure 7, the molecular formula C22H43NO has a 100% i-FIT, meaning that the isotope ratio for the *m/z* is consistent with the proposed composition. ChemSpider returned a lot of possible structural hits for this formula. When sorted by the number of citations, it can be seen that the top choice also has one of the highest number of possible fragment matches in the high energy data. Additionally, common names are returned from the ChemSpider search that can help analysts determine the correct structure. Many polymer additives have common names such as Irganox's or Tinuvin's which are much easier to recognize than just a chemical name. The most cited chemical with the elemental composition C22H43NO has several common names indicating a polymer additive *e.g.* Armoslip E. Researching the identity of the chemical further, it turned out to be erucamide – a fatty acid derivative that is commonly used as a slip agent in packaging materials.

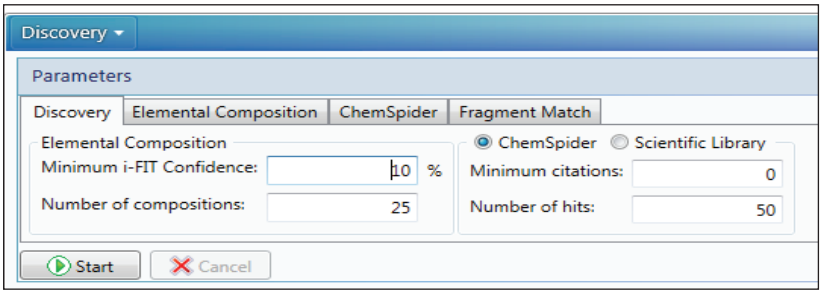

Figure 6. Interface for UNIFI's Discovery tool.

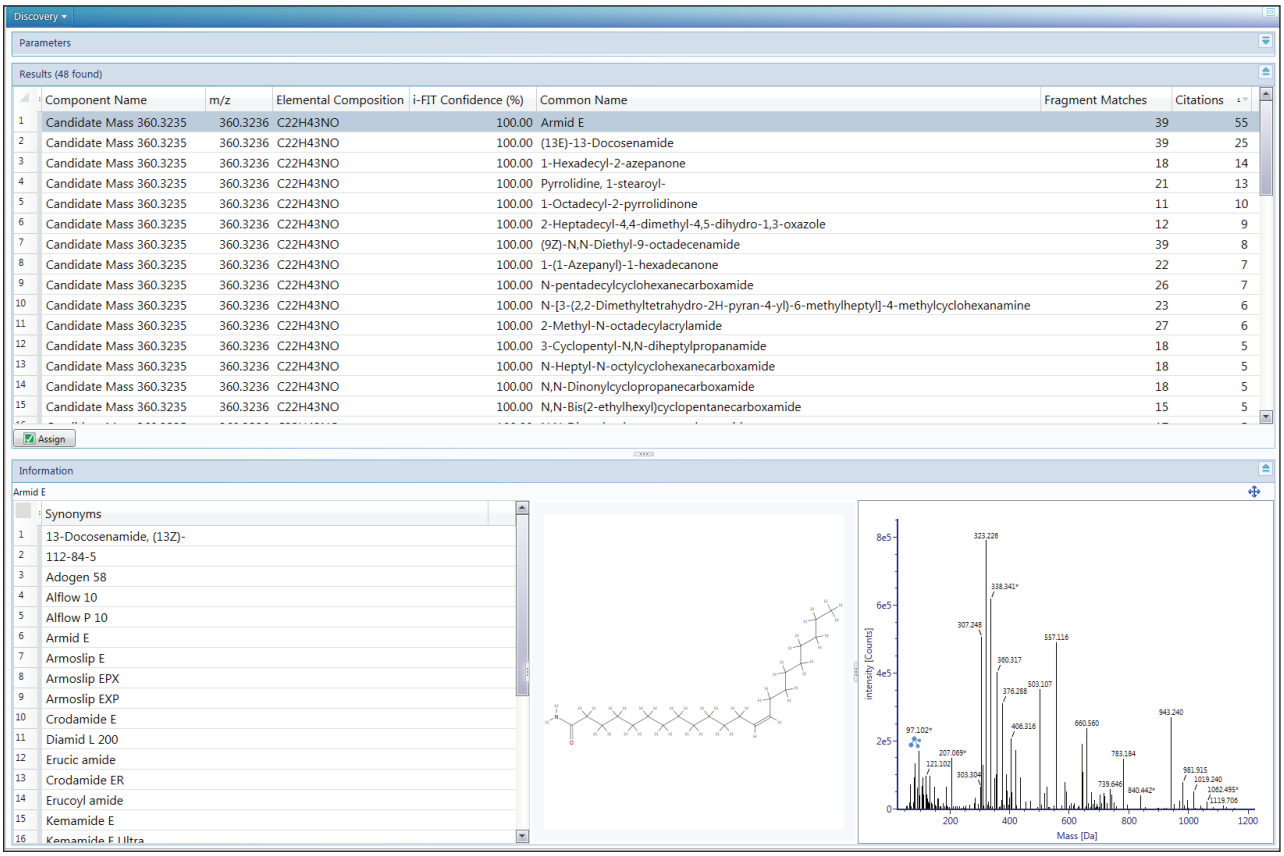

Figure 7. Results from UNIFI's Discovery tool for m/z 360.3236 at RT 4.18 in the mascara packaging.

CONCLUSIONS

Characterizing component spectra in non-optimized LC-MS analysis can be complex, therefore it is advantageous to use automated software tools to quickly evaluate possible structures for candidate masses. The described LC-MS and Informatics workflow, which employs high-resolution mass spectrometry, can be adopted for cosmetics, food, and pharmaceutical packaging extractable applications. Utilization of MS^E data containing accurate mass precursor and fragment ion information on a single software platform streamlines the identification and review process.

An Informatics-based structural elucidation discovery tool provides a rapid process to evaluate information for an unknown *m/z* by ranking the possible elemental compositions and subsequently searching databases for possible structures that are prioritized based on fragmentation matching. The UNIFI Software workflow makes it easy to rank markers of importance and facilitates component identification.

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Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com