

Comprehensive workflow for sensitive detection and confident identification of extractable and leachable (E&L) impurities

Featuring an extensive library for confident identification of E&L impurities, including critical polymers in drug formulation packaging

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This technical note highlights a comprehensive LC-MS/MS workflow for the accurate identification of E&L impurities. An extensive library containing 675 compounds, including common polymers such as polypropylene glycol (PPG), polyethylene glycol (PEG), nylon oligomers and their degradants, polyethylene terephthalate (PET), polytetramethylene glycol (PTMG) and hydroxy-terminated polyether (HTPE), was generated. The library was applied to detect and identify the critical polymer impurities that could challenge the safety and efficacy of the final drug formulation.

E&L compounds from single-use technology (SUT) have attracted increasing attention in the biopharmaceutical industry because they can alter the safety and quality of pharmaceutical products. Several regulatory agencies, including the USP, EPA and NMPA, have published guidelines for E&L impurity studies. Extractable compounds are known to migrate from polymer resins and consist of degraded oligomers that exhibit a range of

molecular weights. Some extractable compounds are molecular analogs of additives that originate from the resin formulation. As a result, the complexity of extractables makes compound analysis and identification significantly challenging.

In 2014, the BioPhorum Operations Group (BPOG) published standard guidelines for performing extractable analysis. Since then, the protocol has played a crucial role in standardizing testing for extractables. Previously, Pall Corporation established BPOG extractables datasets for several in-house SUT and filter products. In this technical note, Pall Corporation collaborated with SCIEX to implement the X500R QTOF system for analysis of extractables following the BPOG testing workflow.

Here, a data processing strategy was applied to detect and identify all critical E&L compounds, including previously unknown analytes (Figure 1). The primary emphasis of the results was on extractable analysis of polymer degradants.

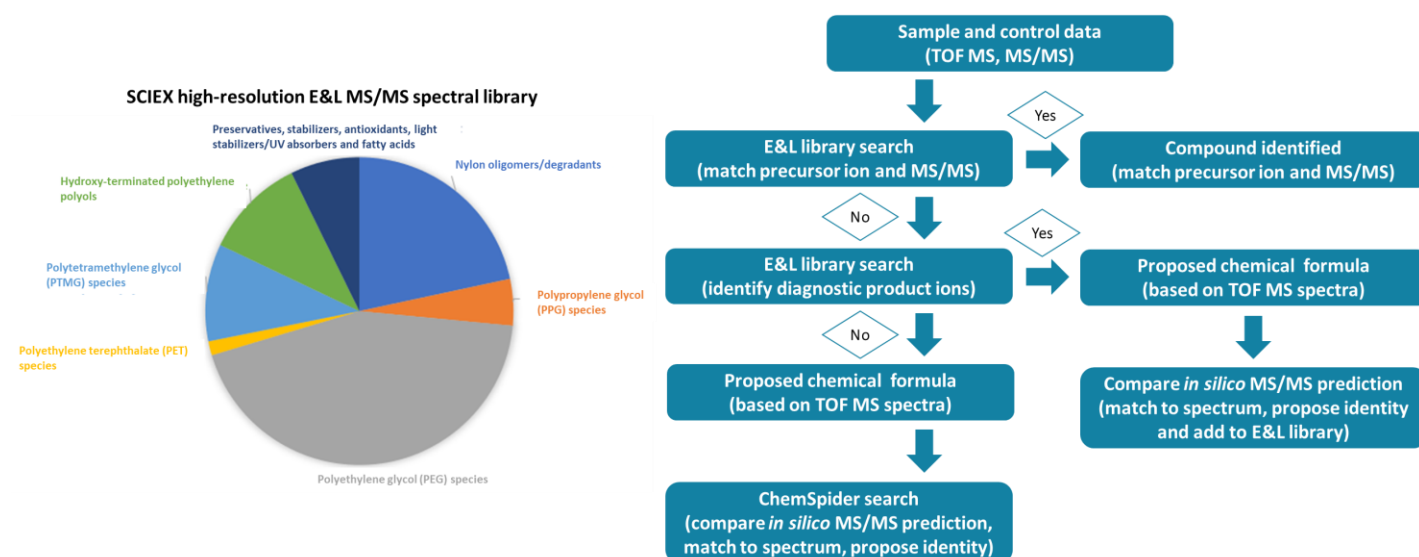


Figure 1. The SCIEX E&L library and the workflow for data processing. The SCIEX E&L library covers 675 compounds based on a high-resolution MS/MS dataset (left). The workflow for E&L analysis using the X500R QTOF system involved 3 data processing strategies for complete identification of all components present in samples (right).

Key features of the E&L analysis using the X500R QTOF system

- **Fast scanning accurate mass spectrometer:** Perform fast analysis with exceptional data quality and sensitive results using the X500R QTOF system
- **Comprehensive library including polymer degradants:** The SCIEX E&L library contains 675 compounds, including nylon oligomers and their degradants, PPG species, PEG species, piperidinones, PET species, PTMG species, hydroxy-terminated polyethylene polyols, other polymer components, preservatives, stabilizers, antioxidants, light stabilizers/UV absorbers and fatty acids
- **High-resolution MS/MS spectral data:** Accomplish confident compound identification using a comprehensive library including positive and negative ionization MS/MS data
- **Ease of data analysis:** Accurately and efficiently identify critical E&L compounds with a streamlined data processing and management using SCIEX OS software

Methods

Sample description: Sample pre-treatment followed the BPOG guidelines.

Chromatography: Separation was performed on an ExionLC AD system with a UV detector using a HSS T3 column (50 mm x 2.1 mm, 1.7 µm, 100 Å). A 10 min run time was used with a flow rate of 0.45 mL/min. Mobile phase A was 0.01% formic acid and 3 mM ammonium formate in water. Mobile phase B was 0.01% formic acid and 3 mM ammonium formate in methanol.

Table 1. DDA parameters.

Parameter	Setting
Method duration	10 min
TOF MS start-stop mass	100–1400 Da
Maximum candidate ions	8
Accumulation time (TOF MS)	0.15 s
TOF MS/MS start-stop mass	50–1400 Da
Accumulation time (TOF MS/MS)	0.60 s
Collision energy	40 V
Collision energy spread	20 V

Mass spectrometry: Data acquisition was performed using an X500R QTOF system operating in data dependent acquisition (DDA) mode (Table 1).

Table 2 summarizes the source, gas and MS conditions.

Table 2. Source, gas and MS conditions.

Parameter	Setting
Curtain gas	35 psi
Ion source gas 1	55 psi
Ion source gas 2	60 psi
CAD gas	8
Ion spray voltage	5500 V, -4500 V
Source temperature	600°C

Data processing: Sample and control data were processed using the Analytics module in SCIEX OS software.

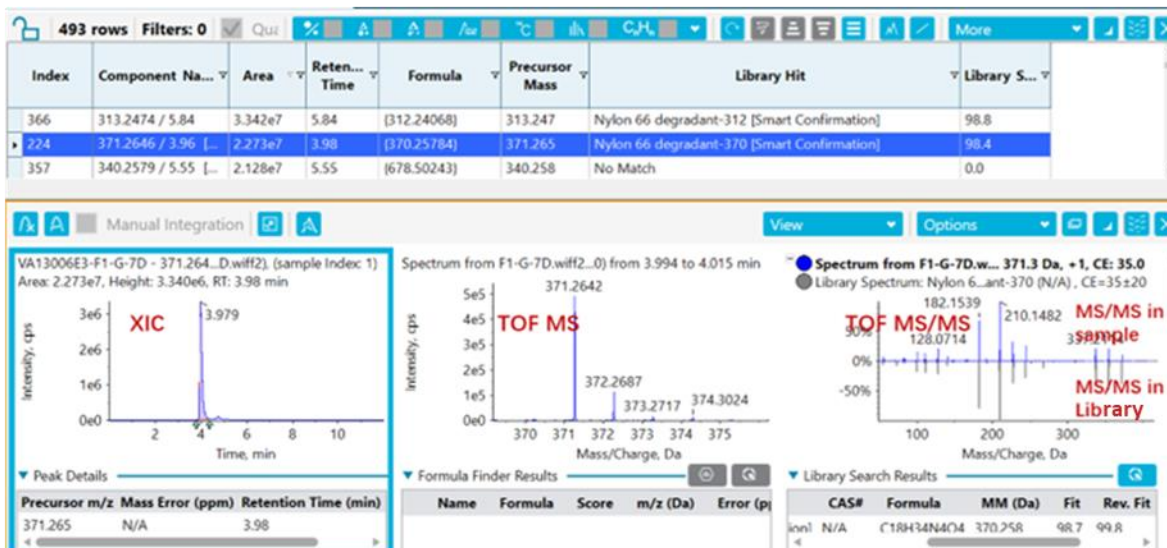


Figure 2. Use of precursor ion matching for E&L compound identification. A representative peak at a retention time of 3.98 min was used to demonstrate the first strategy. The MS/MS spectra at a retention time of 3.98 min was searched against the E&L library using precursor ion match with mass tolerance of ± 0.1 Da. The library search identified the peak as a nylon 66 degradant with a library score of 98.4%.

More confident compound identification with high-resolution MS/MS

A comprehensive workflow for a high-resolution LC-MS/MS method to identify E&L impurities was demonstrated using the X500R QTOF system. Three methods were applied to identify all E&L components in the samples. The methods were applied in combination to extract the most information and identify all E&L components from the sample set. In this technical note, specific focus was dedicated to highlighting identification of critical polymer degradants. The SCIEX E&L library was used to confidently identify all critical targets present in the sample. A library score of $>90\%$ was defined as confident identification. For

library scores $<90\%$, fragment ion match was performed to confirm the identity of the structure.

In the first method, a non-targeted peak finding algorithm was used to identify and integrate signals from the TOF MS data that were identified as peaks. Once peaks were identified, the precursor ion and the corresponding MS/MS spectra were searched against a library for identification. Figure 2 shows a representative example of a peak that occurred at a retention time of 3.98 min and was identified using TOF MS data. The precursor ion of m/z 371.2642 and fragment ions were matched against the E&L compound library. The compound was identified as nylon 66 with a library score of 98.4%.

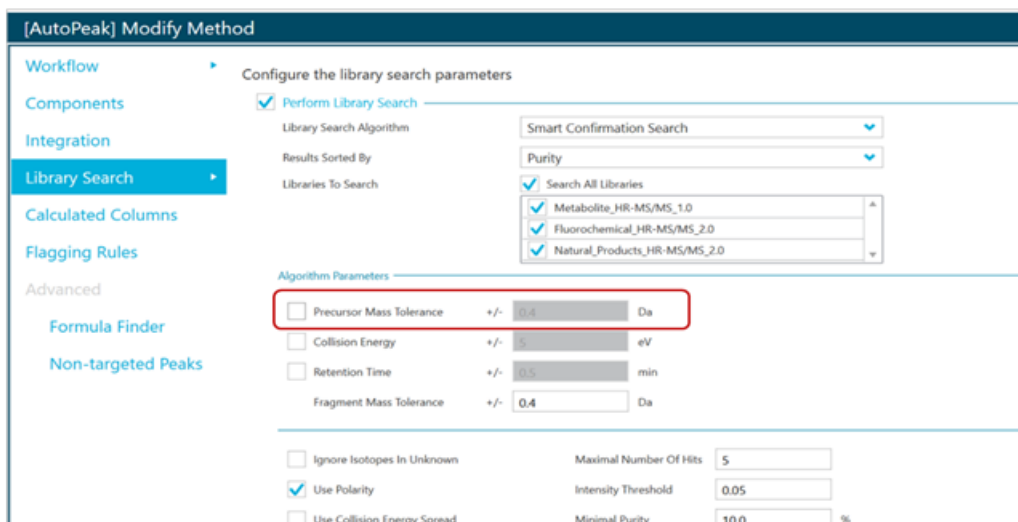


Figure 3. Matching diagnostic product ions using TOF MS/MS data. In this case, the library matching was based on the product ion spectra of the observed data and the library. As a result, the precursor mass tolerance was not selected.

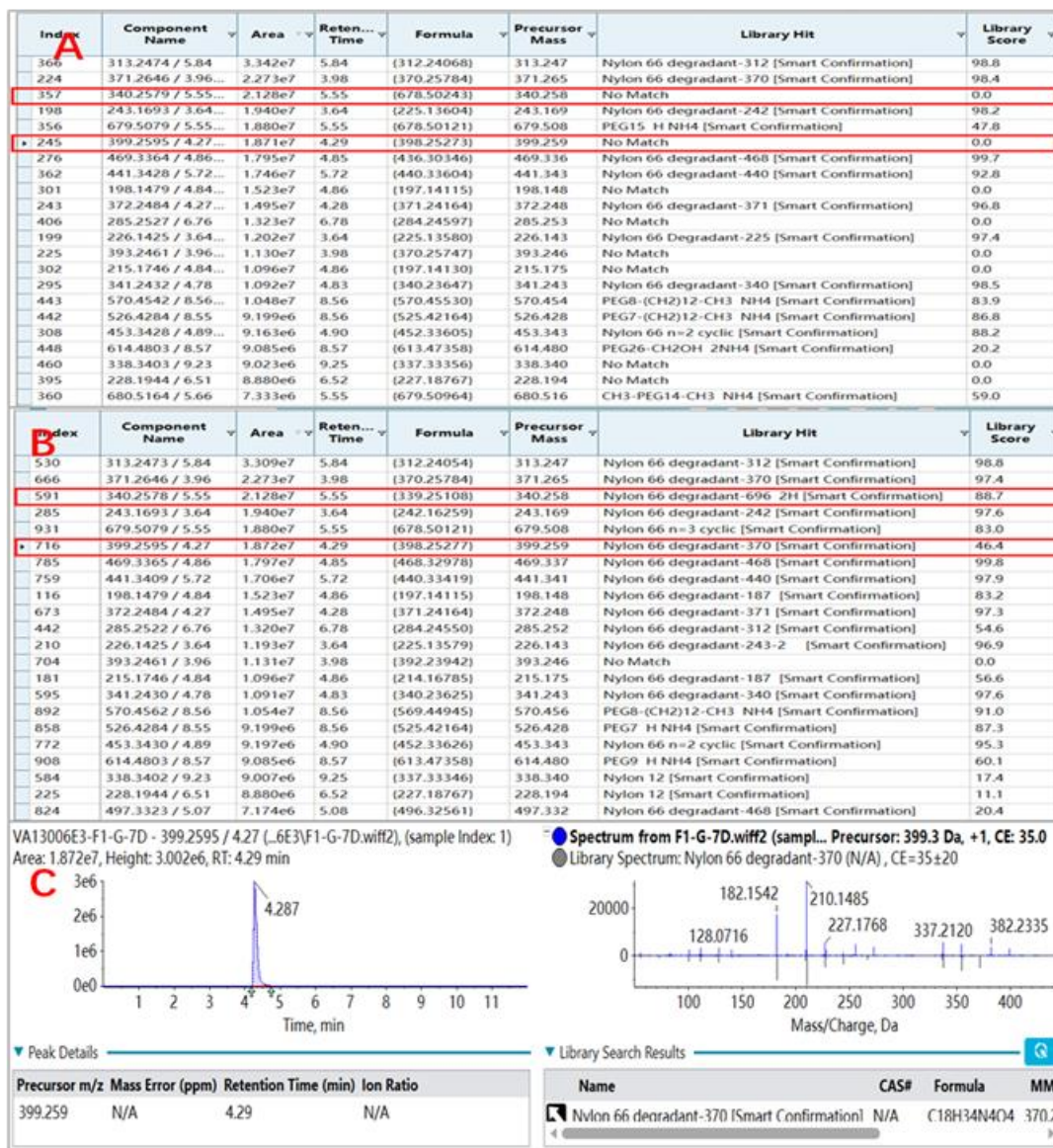


Figure 4. Search result of a nylon 66 filter extract in pH 3 solvent. Data highlighted in the red boxes of panel A demonstrate that the library identified most nylon- and PET-related compounds using the first strategy tested. The library score match information was easily accessible in the SCIEX OS software. Prominent peaks at retention times of 4.29 min and 5.55 min were not identified using the first strategy. Data highlighted in the red boxes of panel B displayed the results from the second strategy based on the fragment ion match. Using this strategy, the peaks at 4.29 min and 5.55 min matched with nylon 66 degradant compounds. Data highlighted in panel C showed the detailed match information and indicated that the MS/MS spectrum at a retention time of 4.29 min was matched successfully with a nylon degradant compound in the library.

In the second method, TOF MS/MS spectra were acquired and used for library search to match characteristic product ions of certain polymers (Figure 3). The resulting output used the MS/MS spectra match to identify the category to which the polymer degradant belonged.

Figure 4 demonstrates the output for compound identification using both the first and second strategy. Figure 4A shows that using precursor ion-based matching, there were 2 precursor ions that were left unmatched (highlighted in red), which included m/z 340.258 and m/z 399.259. Involving the TOF MS/MS spectra-

based matching enabled confident identification of the 2 target degradants, as shown in Figure 4B. Both target degradants were identified as nylon 66 degradants with library scores of 88.7% for m/z 340.258 and 46.4% for m/z 399.259.

Finally, if the acquired spectra did not successfully match with any library spectra, further evaluation of the peak was performed. SCIEX OS software integrates with the ChemSpider database. ChemSpider is a free chemical structure database that complements the E&L compound library. Therefore, users can easily access comprehensive chemical data not included in the

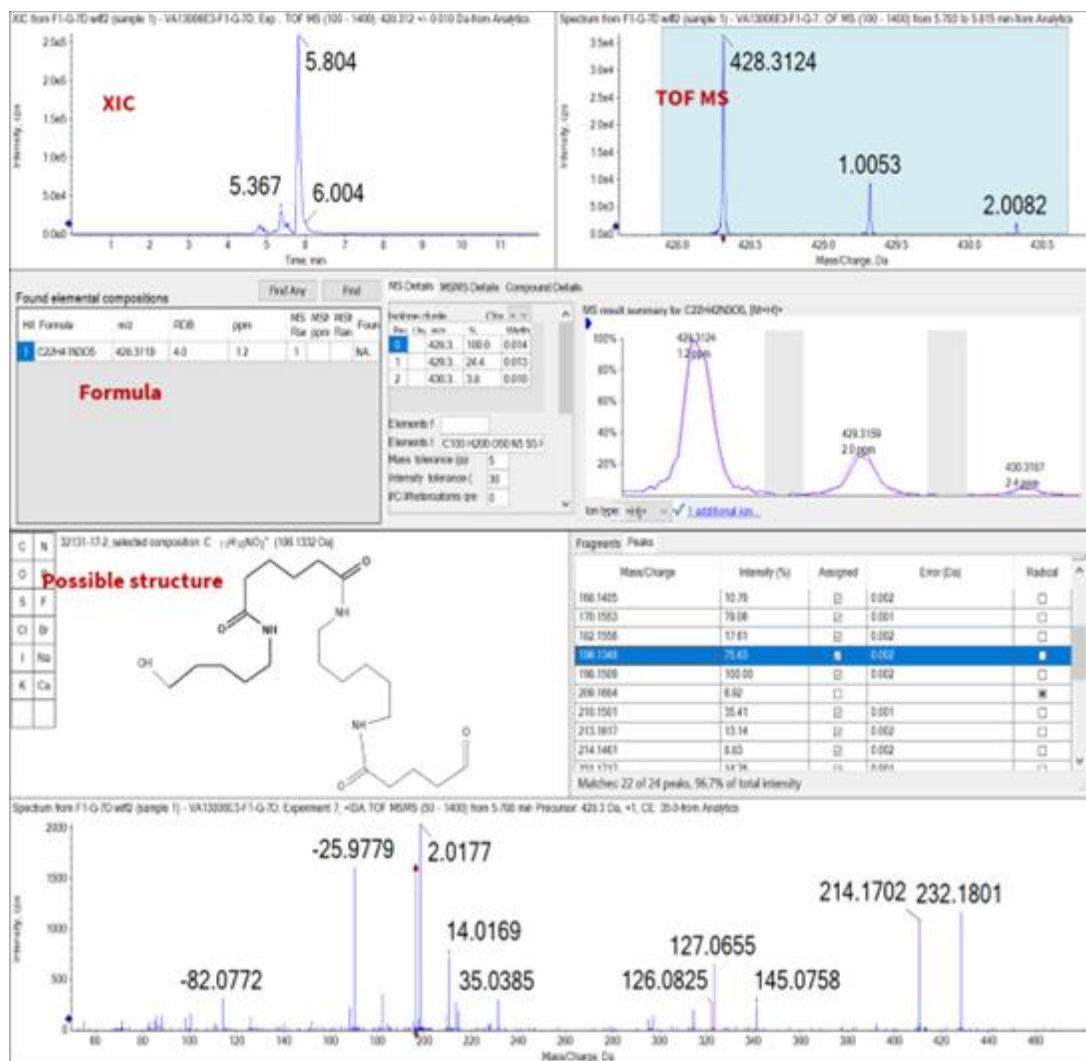


Figure 5. Recommended molecular formula and identified structure for m/z 428.3124 using SCIEX OS software and ChemSpider. The compound was determined to be a nylon 66 degradant.

E&L compound library. A molecular formula was proposed based on the exact mass of the precursor. The molecular formula was searched against ChemSpider, which resulted in *.mol files that were identified as hits. The structures were compared to the acquired MS/MS spectra based on *in silico* fragmentation. The best match was then selected to propose the identity of the compound. Figure 5 demonstrates the identification of the peak at a retention time of 5.80 min. Based on TOF MS, the precursor ion was identified as m/z 428.3124. The exact mass of the precursor was processed through ChemSpider to determine the chemical formula C₂₂H₄₁N₃O₅. *In silico* fragmentation was applied to determine a possible structure. The compound was then identified as a nylon 66 degradant.

Conclusions

- SCIEX E&L compound library was enhanced with hundreds of new compounds that are typically detected in E&L experiments, including critical polymer degradants
- The new processing workflow includes 3 strategies that were streamlined using SCIEX OS software and greatly improved identification efficiency
- A comprehensive and sensitive workflow for a high-resolution LC-MS/MS E&L method was demonstrated with the SCIEX X500R QTOF system

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