Are Raw Material Contaminants Compromising Your Final Product?

Rapid Identification of Unknown Plastics Raw Material Impurities by Accurate Mass Spectrometry and MassFrontier Spectral Interpretation Software

Rationale:

Plastics are fundamental constituents of biomedical devices and of containers and closures (C&Cs) used in packaging of parenteral therapeutics. Leachables and extractables (L&E) residues from these plastics are a common concern of regulated medical industries. Pharmaceutical, biomedical device and therapeutic biologics all require in depth characterization of residual analytes (ISO 10993-18:2005 and 10993-13:2010) detected in their plastic component leachates and extractables.

Plastic additives such as antioxidants, heat stabilizers, UV absorbers, and mold release agents are common L&E residuals released by plastics. Another obvious, but somewhat neglected source of plastic residuals are plastic contaminants - not prescribed additives – but compounds inadvertently introduced during plastics manufacturing. This case study focuses on the chemical identification and quantification of an acrylic plastic L&E residual compound which was introduced into the plastic as a low level contaminant of one of the monomer raw materials used in the acrylic polymerization manufacturing step. The study highlights how a low level contaminant of a raw material can become a prominent L&E residual, and how mining of analytical data with sophisticated software tools helps to uncover its origin and structure.

In practice, a minimum analytic characterization of plastics extracts for regulatory purposes includes liquid chromatography with UV detection (LC/UV). Low concentration plastic residuals analyzed with LC/UV can yield intense UV signals. However, identification and quantification, which are often needed to support product risk analysis requirements codified in numerous international regulatory guidances (ISO 14971:2007, USP<1663> and USP<1664>), are limited with LC/UV. Often, LC/MS provides the only practical analytical avenue for chemical identification of ppm level L&E analytes.

This case study highlights the analytical power of “accurate mass” mass spectrometry in chemical identification of an unknown ppm level analyte extracted from a long-term implantable (Class III) acrylic ophthalmic device. The LC/UV-PDA/QTof-MS instrument used in this study is an effective “qual/quant” tool for this application.

The Qtof-MS which delivered accurate mass determinations to less than 5 ppm from theoretical affords high confidence elemental composition matching. Elemental composition in combination with a corresponding UV spectrum (from in-line UV detector) , molecular ion fragment MS/MS data and other advanced high resolution mass spectrometry related techniques such as isotopic distribution matching, enabled structural identification from the elemental composition.

Mass Frontier spectral interpretation software capable of chemically intelligent structural elucidation of small molecule fragments and mechanisms of their formation significantly increased the confidence of final structural identification.

Case Study:

Determination of Low Level UV absorber Contaminant in Acrylic Extract By Ultra High Performance Liquid Chromatography (UPLC) - UV - Quadrupole Time of Flight (qTof) - Mass Spectrometry (MS)

- Acquity I-class UPLC with PDA UV Detector (Waters)
- Xevo G2S qTof (Waters)
- MassLynx Data Acquisition and Processing (Waters)
- MassFrontier (HighChem, Thermo)

UV absorbing molecules are an integral component of intraocular lens (IOL) materials. These molecules (UV absorber monomers) are tailored for covalent incorporation into the acrylate or silicone lens polymer. UV absorber monomers may contain low (<5%) levels of impurities, which , if not covalently incorporated into the lens material, can be detected as L&E residuals.

Isopropanol (IPA) soxhlet extracts of an IOL material were prepared along with IPA blanks. Material extracts and IPA blanks were analyzed by UPLC/UV/qTof-MS. Signals that were detected in UV chromatograms and MS total ion chromatograms (TICs) of the test extract but not detected in the blank extract were identified as "residual analytes". An unknown residual analyte was detected at 330nm UV wavelength. The analyte and the UV absorber monomer both absorb at 330 nm UV, an identifying feature of UV-absorbing activity, yet differ in mass and retention time. The residual analyte was either introduced into the material with the UV absorber monomer (as a contaminant) or it was generated from the monomer as a degradant.

The raw material contaminant origin was investigated first. Solutions of UV monomer raw material were analyzed by UPLC-UV-qTof MS. We identified an impurity in a high concentration (2000 ppm) UV absorber standard that matched the device impurity in UPLC retention time, UV response, and mass spectrum (Figure 1) below.

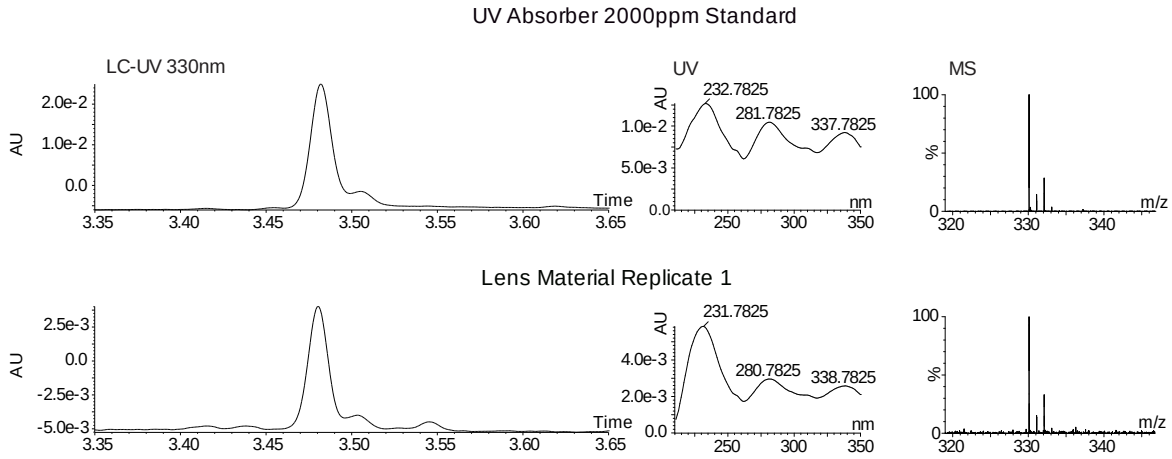


Figure 1. (Top) The UV absorber impurity identified by retention time, UV and MS spectra in analysis of a high concentration (2,000 ppm) standard of the UV absorber raw material. (Bottom) The unknown residual analyte in the lens material was found to match the UV absorber impurity identified in the UV absorber standard in retention time, UV and MS.

After identifying the source of the unknown, its complete chemical identification was the next challenge. Accurate mass data was pivotal in achieving this goal. A key advantage of the qTof MS detector is the capability, with proper calibration and lockmass correction, to output highly accurate mass data. For example, in the mass range of the UV absorber (~330 g/mol) the qTof MS detector routinely affords better than 5 parts per million (0.0005%) mass accuracy determination (*i.e.*, over a mass of 330.0000, the 0.0005% difference accounts for changes in the third decimal place). Such high mass accuracy allows high confidence elemental composition matching. We first characterized the mass spectrum of the UV absorber in Figure 2. The MS spectrum of the UV absorber has a characteristic isotopic pattern due to the presence of the chlorine isotopes ^{35}Cl and ^{37}Cl. The ^{35}Cl containing signal of the UV absorber was detected at 328.1204 g/mol, with a mass accuracy shift of -3.66 ppm (detected mass minus theoretical protonated mass 328.1216, divided by theoretical protonate mass, and multiplied by $1x10^6$).

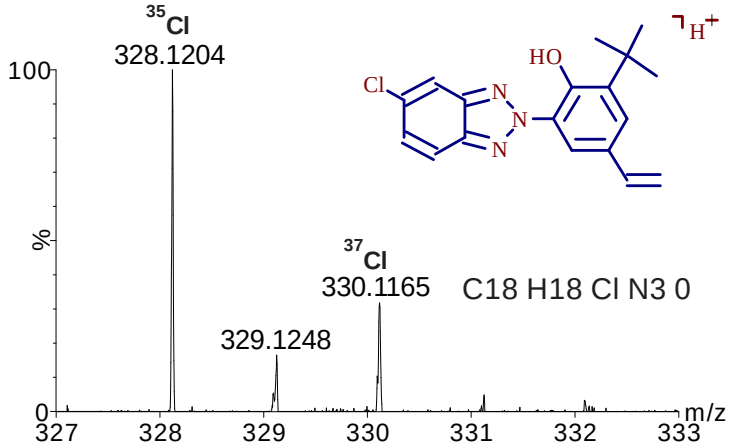


Figure 2. Structure, elemental composition, and MS spectrum of the UV absorber. Notice the reactive vinyl functionality, which allows the UV absorber incorporation into the device material during polymerization.

We found that the accurate monoisotopic mass of the residual analyte from UV absorber impurity was 330.1021. With this mass, expected elemental content and 5ppm mass accuracy search, we were able to determine its elemental composition. Using an element content C, O, H, N, Cl only one chlorine containing chemical formula, C17H16ClN3O2, matched the accurate experimental mass within 5 ppm. In comparison, the UV absorber monomer chemical formula is C18H18ClN3O, a difference of one less oxygen and one more methylene (CH_2) group. Chemical logic suggested the impurity structure, below, in which an aldehyde group replaces the vinyl group of the UV absorber monomer (Figure 3). The presence of the aldehyde group prevents the incorporation of the impurity into the material during polymerization and leads to its detection as residual L&E analyte.

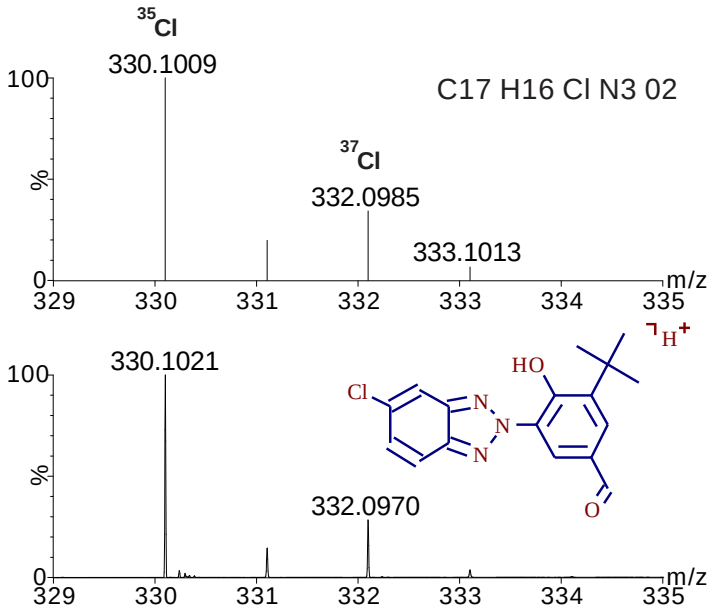


Figure 3. (Top) Proposed elemental composition and respective predicted isotopic pattern of the aldehyde impurity. (Bottom) Experimental MS spectrum of the putative UV absorber impurity and the proposed structure.

Next, we turned to experimental mass fragmentation and MassFrontier (HighChem, Thermo) software to verify the proposed structure of the impurity. MassFrontier is an in silico based, i.e. predictive, fragmentation elucidation software that can generate structures of expected fragments from a given precursor ion. Mass Frontier draws on predictive algorithms as well as fragmentation libraries based on published small molecule fragmentation mechanisms. As shown here, the software predicts fragments as well as the pathways of their formation.

In this study, we first compared experimental molecular ion fragmentation data of the UV absorber and the unknown residual analyte (Figure 4). We then used MassFrontier to generate in silico predicted fragments (blue structures in Figure 4) of the UV absorber monomer and the proposed aldehyde impurity. Using this approach, we were able to assign the key experimentally observed fragments, and obtained definitive evidence that the proposed aldehyde structure of the impurity was correct. Mass Frontier also helped to elucidate how the structural differences of UV absorber and the aldehyde residual contribute to the difference in fragmentation (notice the dominant loss of carbon monoxide in aldehyde impurity fragmentation). The software also generated proposed fragmentation pathways of the key fragments, presented in Figure 5, and illustrates the detailed predictive power inherent in this software.

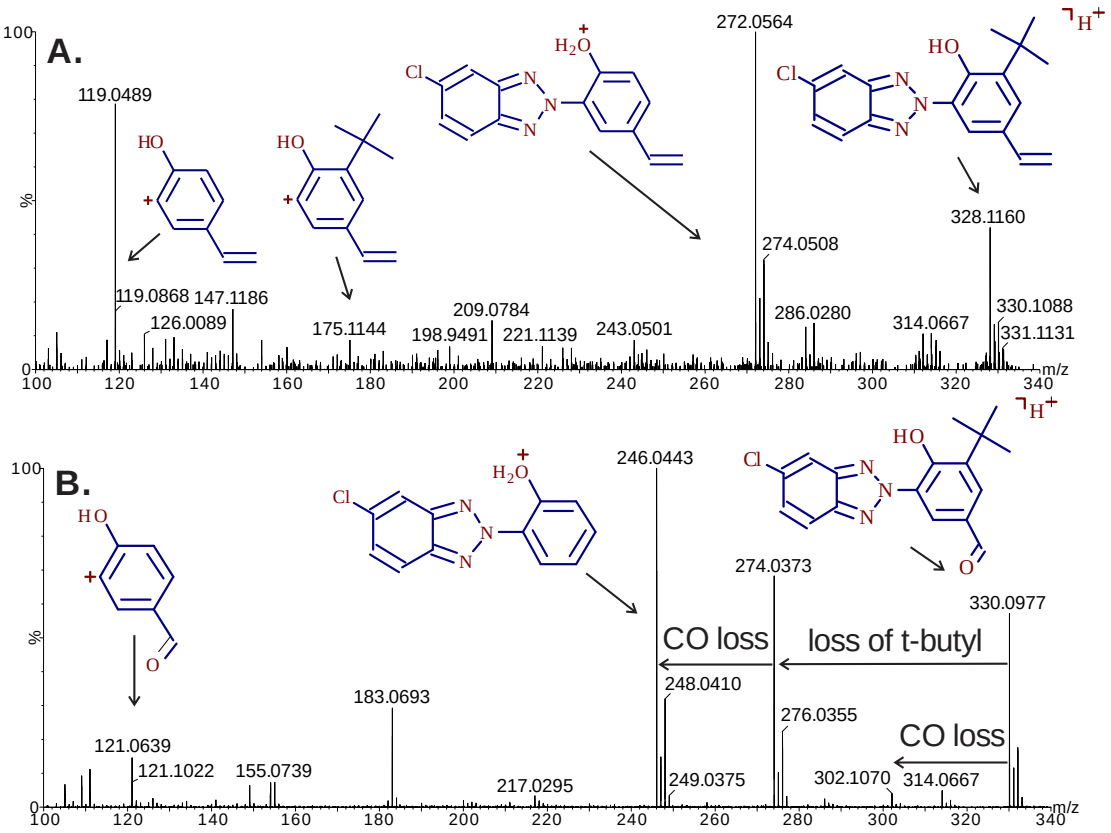


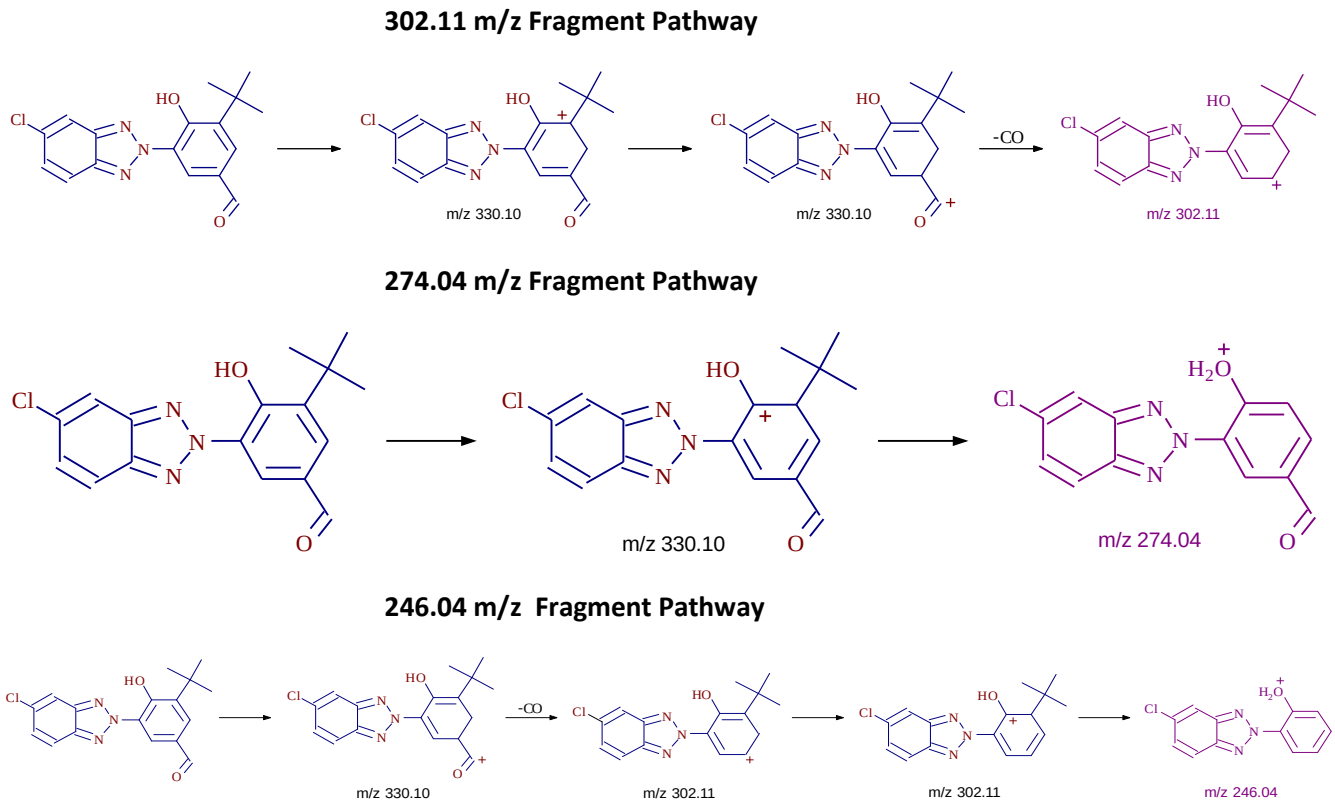
Figure 4. Comparison of UV absorber (top, A) and putative UV absorber contaminant (bottom, B) MS/MS fragmentation spectra. MassFrontier (Thermo, HighChem) software allowed structural assignment of key observed fragments and neutral losses.

Having confirmed the structure of the aldehyde impurity, we performed quantitation of the analyte in the IPA extract based on UV monomer standards – a justified approach given the discovered structural similarity of the UV absorber and the aldehyde impurity. The aldehyde extract residual concentration was found to be 4 ppm, equivalent to approximately 40ppm in the lens material. In this case the lens concentration, though not trace, was found to be relatively low. This is consistent with the fact that the UV absorber monomer is a minor component (approximately 0.5%) of the final IOL acrylic material. In cases where the contribution of the raw material in the final biomedical device is higher, trace level contaminants can become significant and potentially problematic L&E residuals. Importantly, the understanding of the chemical structures of residual analytes may flag those molecules that could be problematic even at relatively low levels, and if needed, may greatly inform even simplify their removal from the raw material.

In summary, unambiguous determination of the impurity's structure and its quantitation were enabled by a formidable analytical and data processing toolkit, a work flow yielding synergistic data including:

1) Non-targeted screening of extract with UV and MS detection
2) Accurate mass and elemental composition determination of molecular ions
3) MSMS spectra
4) Fragment structural elucidation with Mass Frontier

Figure 5. Examples of fragmentation pathways of the key contaminant fragments 302, 274, and 246 generated by MassFrontier.



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