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Comprehensive Investigation of Polymer Oxidation using Interactive Visualizations and User Modified Libraries in PolyMatch Suite

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Introduction

Polysorbate 20, polysorbate 80, and poloxamer, commonly found in protein therapeutics and AAVs respectively are prone to oxidation on the polyoxyethylene groups or polysorbate fatty acid unsaturated bonds. Oxidation occurs at room temperature and is initiated by metal ions such as iron or residual peroxides, both of which are ubiquitously found in formulations.¹ Oxidation of polysorbates and other surfactants is a radical process and can propagate, forming small acids, aldehydes, ketones, and other by-products. In addition, the therapeutic itself can be oxidized. The oxidation products can significantly impact drug efficacy and potentially pose safety risks. While there are numerous peer-reviewed papers exploring surfactant oxidation, there is not a well-defined way to analyze this complex dataset.

To address this analytical challenge, we have adapted an open-source software (PolyMatch) designed to streamline the identification and annotation of polymers by leveraging their repetitive structure and characteristic fragmentation patterns to discover oxidized polymeric series. This approach aims to improve our understanding of the formation and potential safety impacts of these oxidation products.

Experimental

Sample Preparation and LC/Q-TOF Conditions

Polysorbate 80 was diluted to a final w/v of 0.02% in mobile phase A. Polysorbate 80 was incubated with 3% (w/v) hydrogen peroxide overnight in a water bath heated to 40 °C.

Polysorbate components were separated on a 2.1 x 100 mm AdvanceBio Surfactant Profiling column over a 15 minute gradient.

LC/MS/MS analysis was performed on a 1290 Infinity II UHPLC coupled to a 6546 LC/Q-TOF system. Data was collected with the Agilent MassHunter acquisition (11.00) workstation software, using an Auto MS/MS method. Suspect screening analysis was processed with PolyMatch Flow and Visualizer.²

PolyMatch Suite

PolyMatch integrates a wide range of evidence to classify polymers, including mass defect, retention time, and accurate mass, which can be used alongside homologous series to compile groups of chemicals that likely belong to the same class.

MS/MS evidence can provide structural information pertaining to class- or species-level assignments.²

Experimental

PolyMatch Suite Continued

PolyMatch can be used for the detection of unknown polymers, including oxidized species, through fragment screening, homologous series detection, and a “generalized” library. Homologous series detection with fragment screening for polyethylene glycol-related fragments will pull out all respective polymers with pegylated units, regardless of whether they are contained in the PolyMatch rule-based libraries containing known polymer structures. Furthermore, the “generalized” library will also capture oxidized polymers (Figure 1).

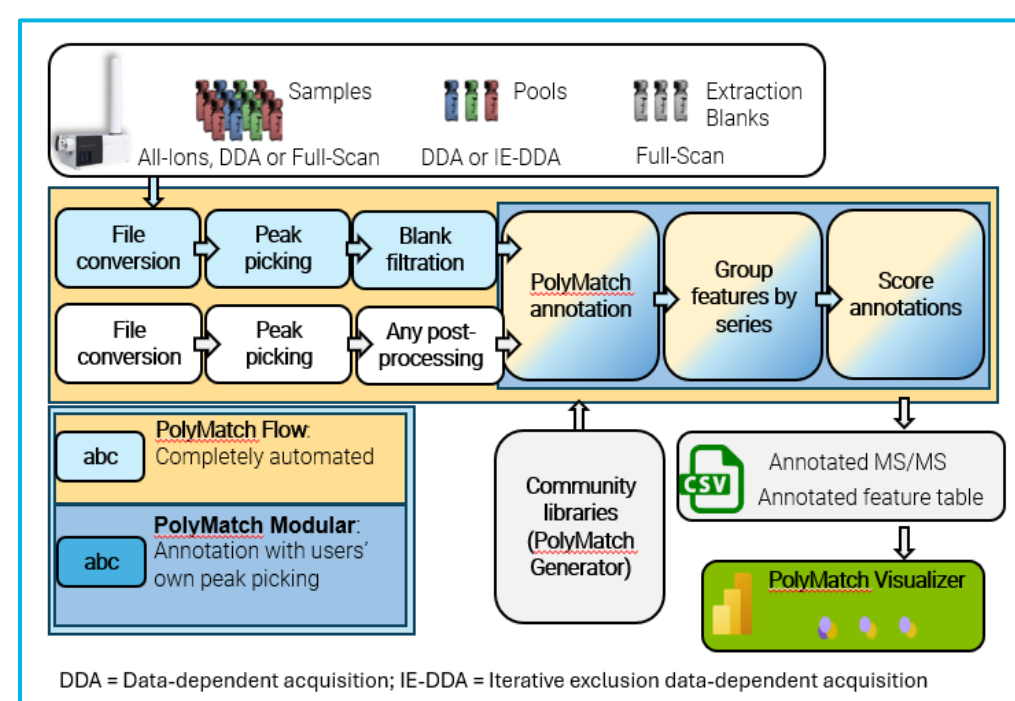


Figure 1. An overview of the PolyMatch workflow. The fully automated PolyMatch Flow interface was used in this experiment..

This general library contains polymers characterized by double bond equivalents, the addition of various functional groups and adducts, the size of pegylated units, and the presence of CH₂ groups. The PolyMatch library will be expanded to include specific oxidized polysorbate species and their fragmentation patterns. The software program includes automatic peak picking, blank filtering, annotation, and visualization.²

Example	Definition	Built-In Range
n = 3	Number of POE/PEG units	3–44
NH ₄ ⁺	Adduct	NH ₄ ⁺ , Na ⁺ , H ⁺
CH ₂ = 6	Number of carbons (non-PEO/PEG)	0–19
DBE = 4	Double bond equivalents	0–6
(4-OH)	Functional groups	OH (0–4), NH ₃ (0–2), =O (0–2)

Table 1. The generalized PolyMatch library contains structures and functional groups such as polyethylene glycol and CH₂.

Results and Discussion

Nomenclature

Note that polyethylene glycol (PEG) and polyoxyethylene (POE) are chemical synonyms. The groups bound to the sorbitan in polysorbates are commonly called POE. PolyMatch uses the term PEG, as the software program was designed to analyze all polymers. Therefore, both terms are used interchangeably throughout the poster.

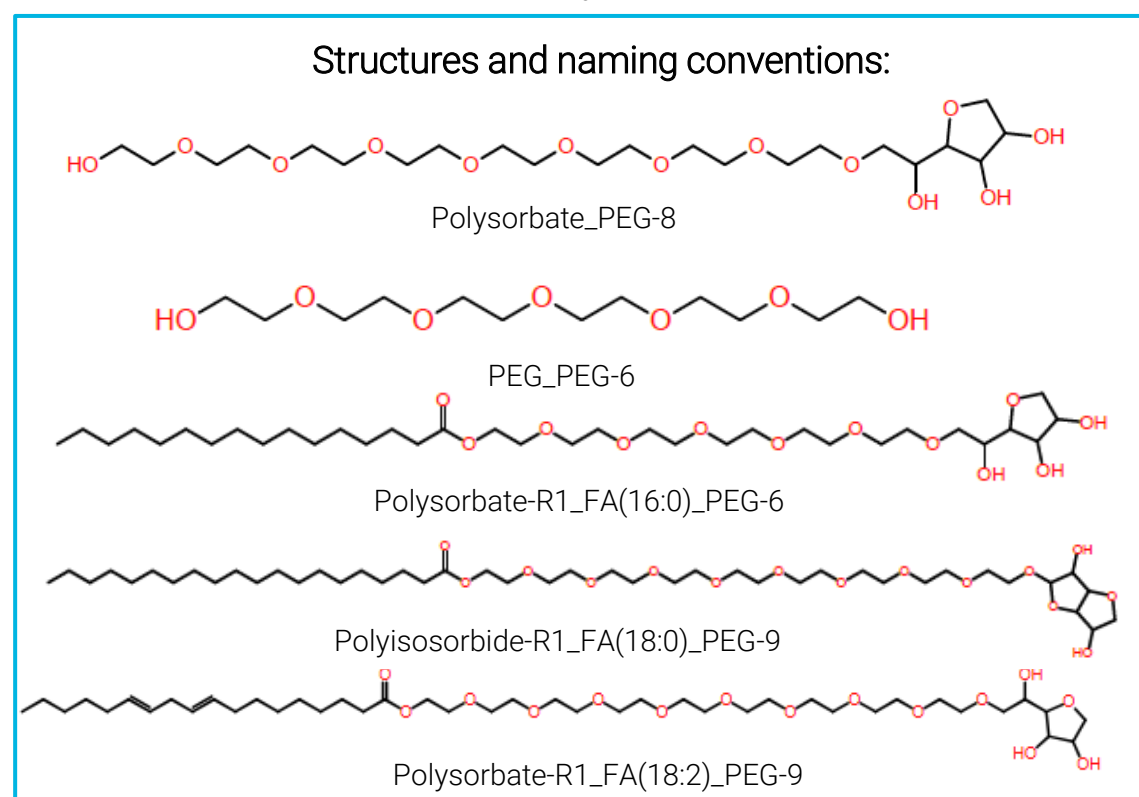


Figure 2. Examples of structures and naming conventions that were identified with high scores in PolyMatch. Structures were generated from SMILES files.

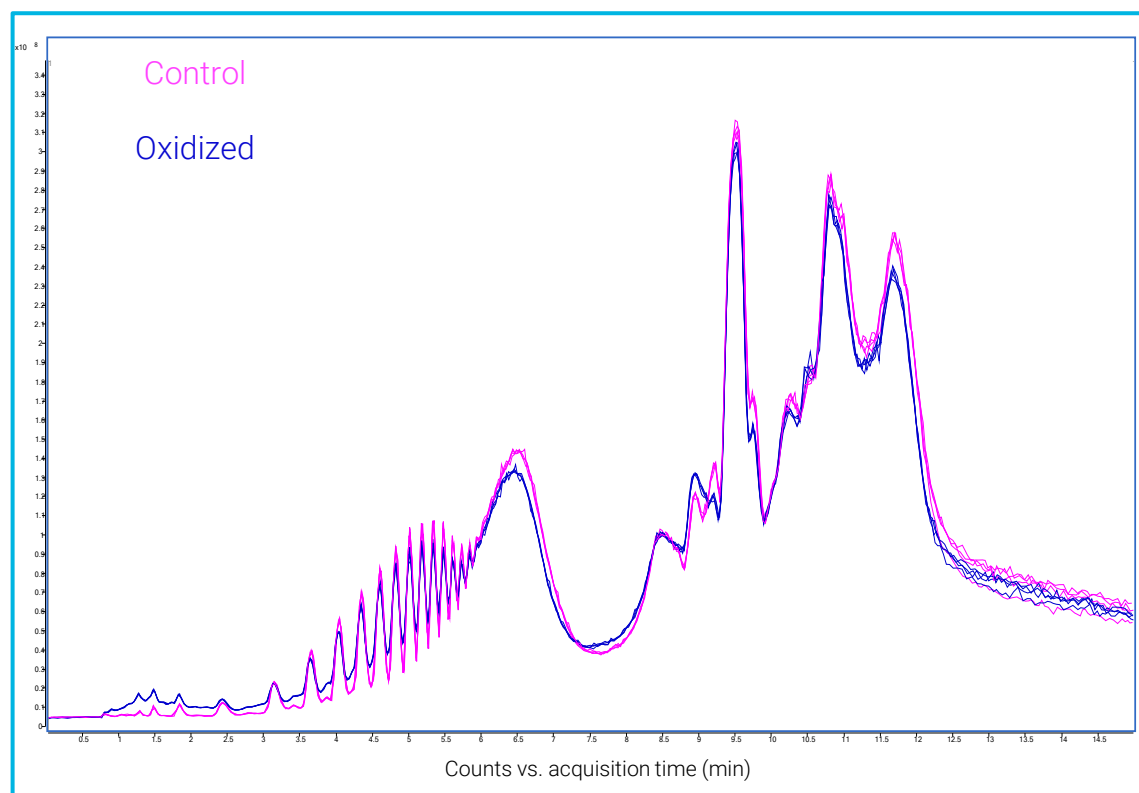


Figure 3. A comparison of total ion chromatograms between the control and oxidized samples indicates that some degradation has occurred. Oxidation is higher between 1-2.5 minutes and around 9 minutes. The polysorbate mono, di, and triester peaks have decreased after oxidation.

Identified Polysorbate Components

This poster is largely focused on one set of polysorbate components, but many other components were detected. PolyMatch uses a scoring system with letters from A to E. Compounds with an A score are considered very confident identifications, based on mass defect, exact mass and a class-specific fragmentation pattern. There were 261 compounds with an A score.

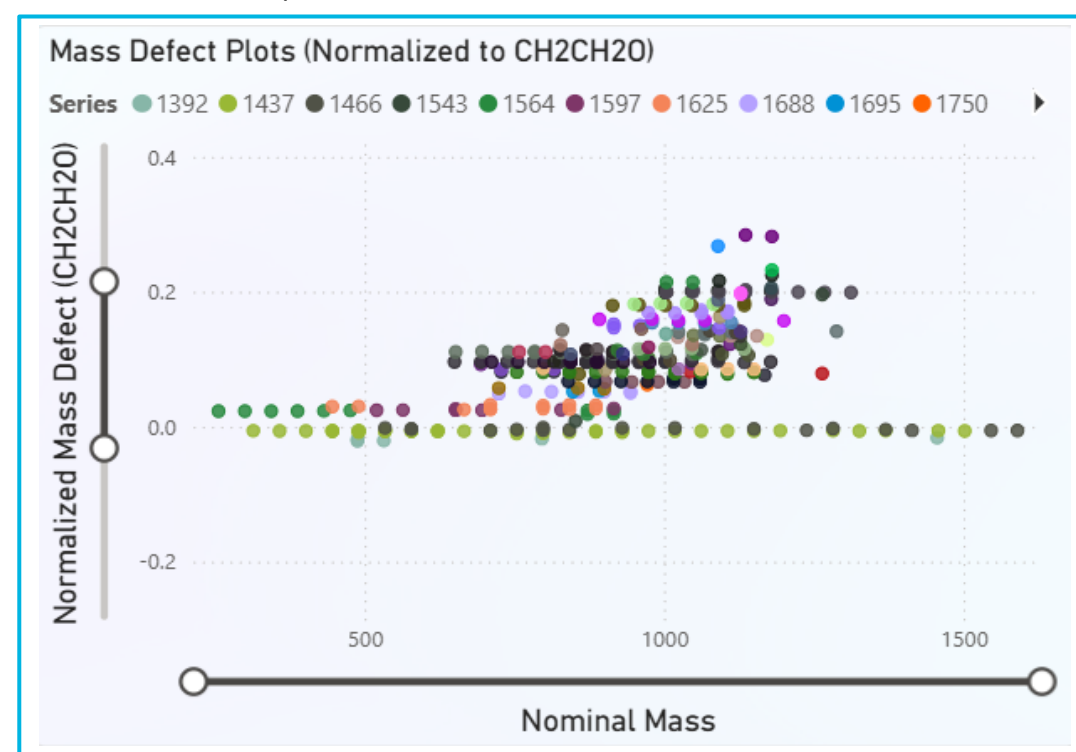


Figure 4. The mass defect plot for all high-scoring compounds. Multiple series were discovered, for a set of 261 compounds in total.

The Power of Mass Defect

The use of mass defect is particularly powerful because it leverages the repeating POE groups in polysorbates and is normalized to these groups. PolyMatch can confirm the degradation products' series and offers a clear visual representation, making it easy to exclude false positives.

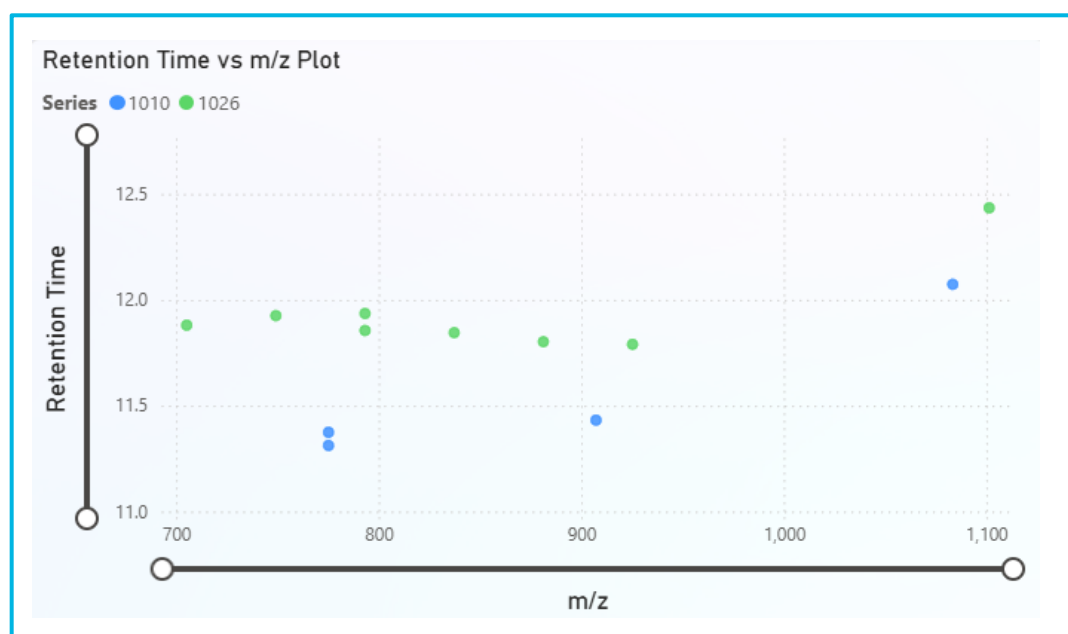


Figure 5. Two series with similar mass defects but no clear retention time vs. m/z patterns, indicating false positives. In addition, both series exhibit low scores.

Results and Discussion

Chromatographic Solutions for Isomeric Separations

The advantage of the AdvanceBio Surfactant Profiling column coupled with the PolyMatch software is highlighted by the right panel of Figure 6. While the left panel shows a single series of related polysorbate components, the right panel reveals a set of isomers that have been chromatographically separated within the mass defect series. The selectivity of the LC column separates the isomers. The bottom MS/MS spectrum in Figure 7 differs greatly from the top as it includes a highly abundant not-yet annotated peak of m/z 323.2576. The literature indicates that this MS/MS ion is a keto oleic acid, which provides evidence of polysorbate oxidation.

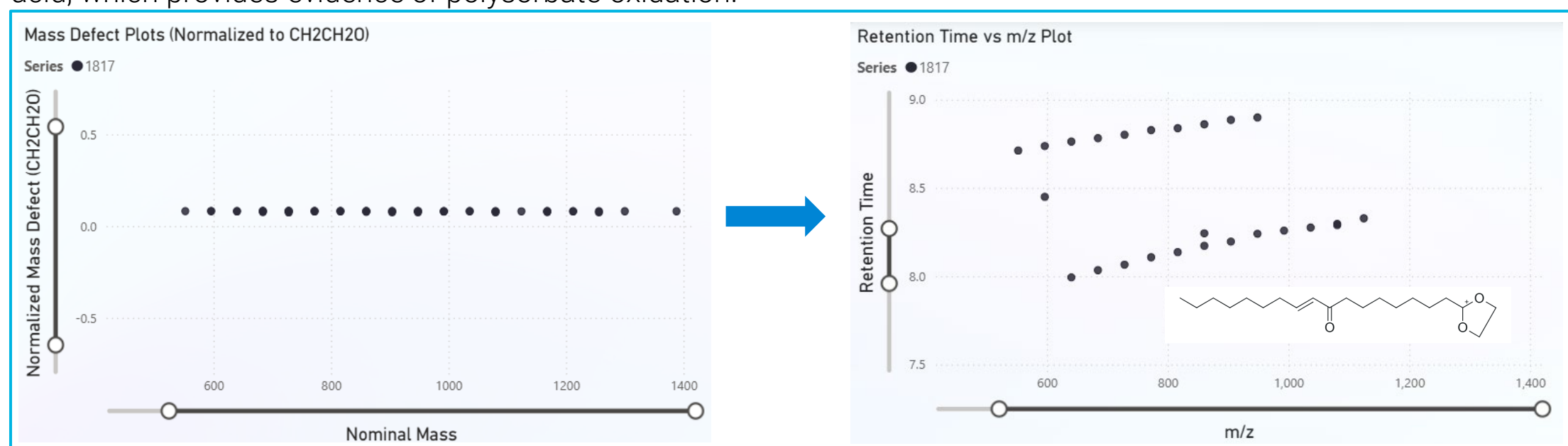


Figure 6. The Kendrick mass plot (left), shows a series of polysorbate components most confidently identified as Polysorbate-R1_FA(16:0)-PEG(5–18). The retention time versus m/z plot (right) shows a set of isomers within the mass defect series that have been chromatographically separated. The bottom series differs by a unique fragment ion, shown in the right panel.

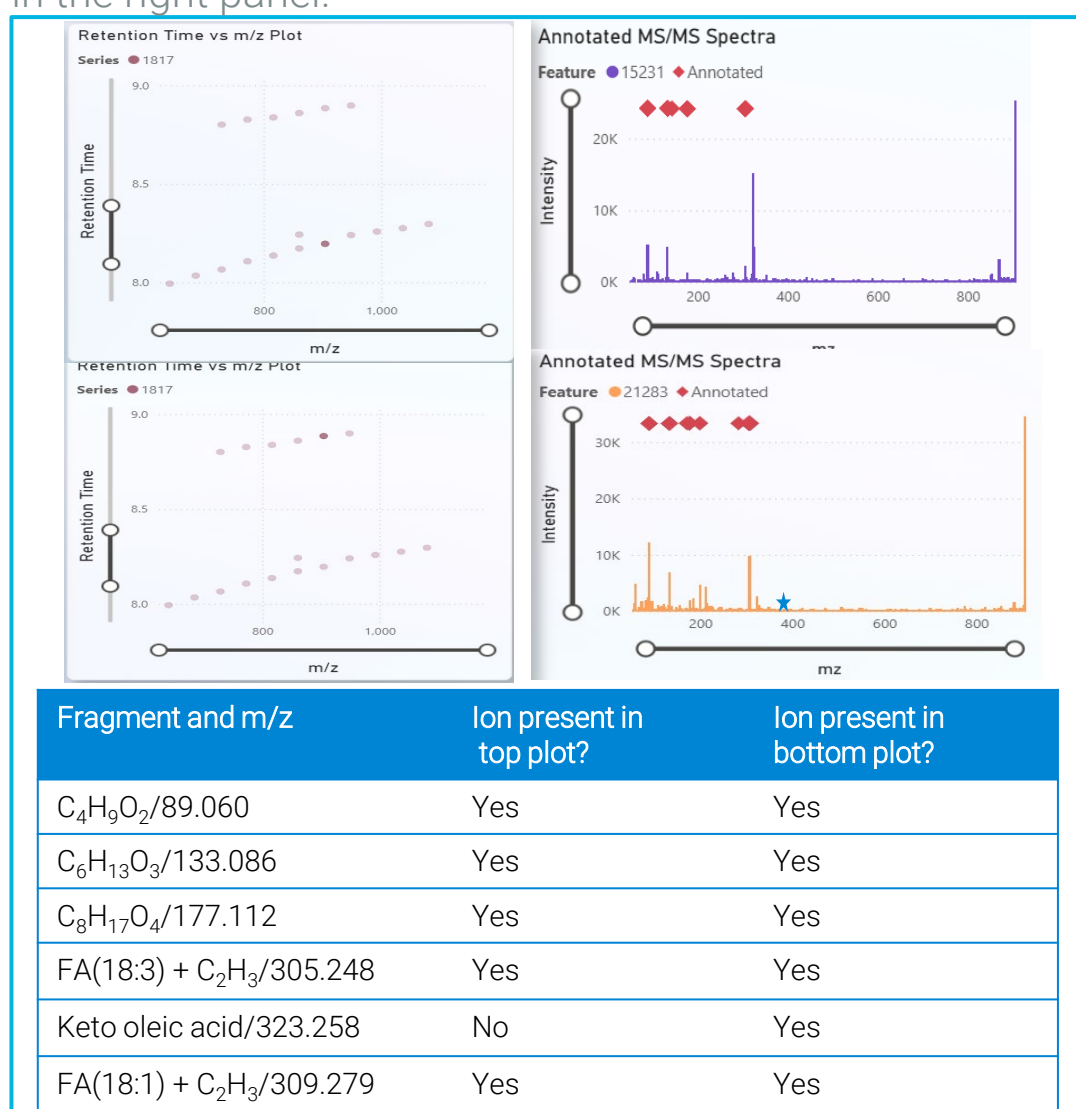


Figure 7/Table 2. Examples of MS/MS spectra and annotated fragments from each set of isomers. MS/MS confirms that the bottom compound is oxidized with the diagnostic ion, m/z 323.258, marked with a blue star.

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Conclusions

- First dedicated open-source software for polymer and oxidized polymer analysis with in-depth interactive visuals covering all aspects of non-targeted analysis
- A novel LC/MS/MS workflow for identifying polysorbate oxidation by-products has been developed as a screening tool for labs monitoring biotherapeutic product and formulation stability.
- The Agilent AdvanceBio Surfactant Profiling column provides high resolution of polysorbate components with a short run time.
- Oxidized species will be added to the PolyMatch library for easier identification.

References

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