Polymer StudioTM: a novel software for fast profiling and identifying complex pharmaceutic excipients by UHPLC-HRMS/MSn technique

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Introduction

Pharmaceutical excipients are an important part of medicine. Their function and safety are closely related to their profiles of components and impurities, including composition, structure, proportion. Due to their complex composition and similar skeletons, it is difficult and time-consuming to profile some complex pharmaceutic excipients, such as polysorbates (Tween), sorbitans (Span), oil excipients (soybean oil, olive oil), and soya lecithins. A UHPLC-HRMS/MS^n technique can be used to profile and identify their components and impurities. However, it relies on the analysis of complex data. This process is a time- and labor-intensive task. It is important to develop a novel strategy and data analysis software for profiling and identifying complex pharmaceutic excipients quickly and comprehensively in the routine control.

In this study, a novel software, Polymer Studio (containing Polymer Builder and Polymer Pattern), was developed for fast profiling and identifying components and impurities in complex pharmaceutic excipients using a UHPLC-HRMS/MS^n technique.
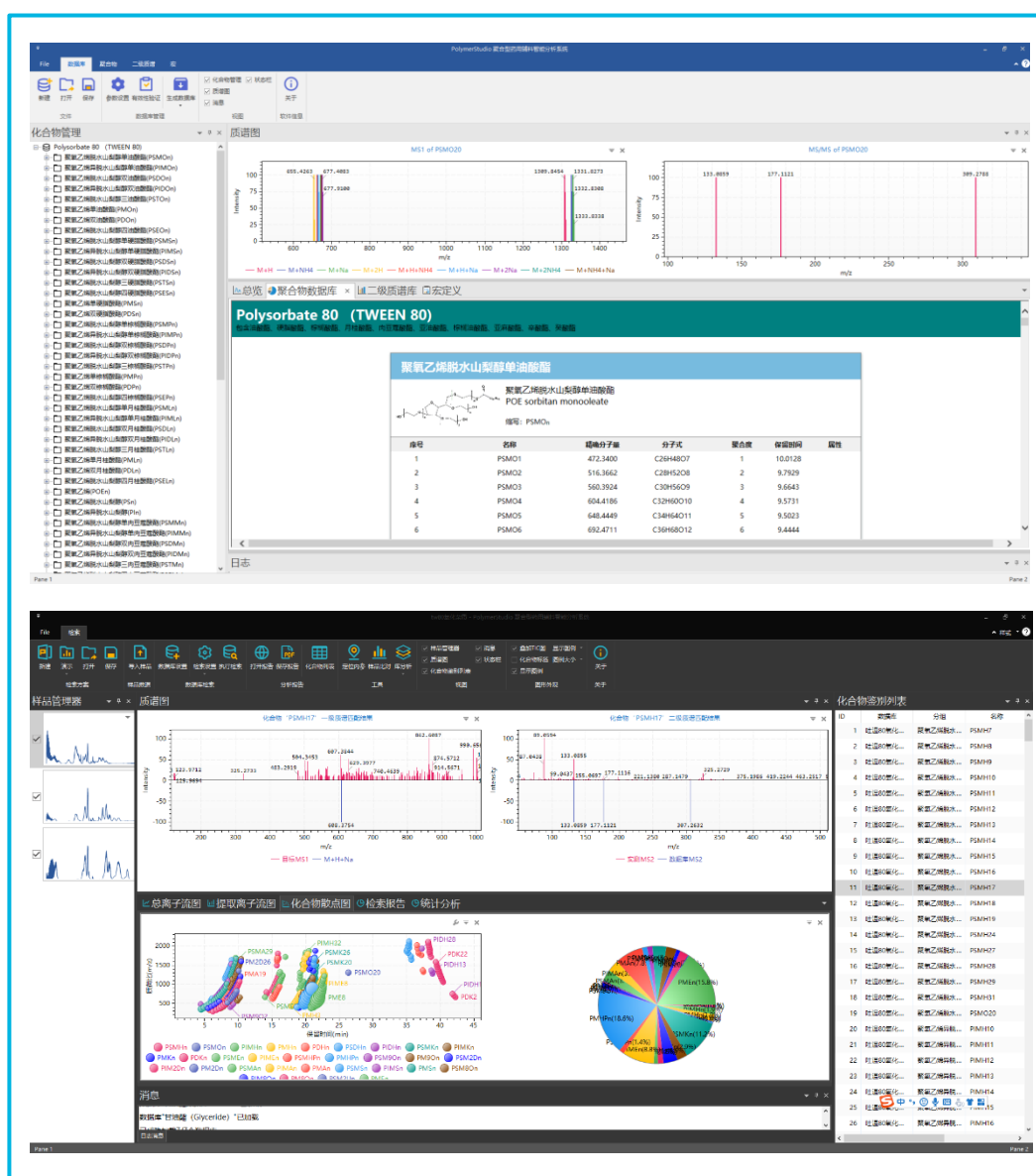

Figure 1. The interface of Polymer Studio software.

Experimental

Sample preparation

The pharmaceutic excipient samples were dissolved and diluted with 10% acetonitrile in water (v/v) or methanol/chloroform (1:1, v/v). The pharmaceutical preparations were filtered before analysis.

1290 UHPLC system conditions

Polysorbates (Tween) and sorbitans (Span)

Column: C4 column (2.1 × 100 mm, 1.7 μm)

Mobile phase A: 2 mM NH_4CO_2H and 0.1% formic acid in water; Mobile phase B: acetonitrile

Column oven: 35 °C

Flow rate: 0.4 mL min^{-1}

Oil excipients (soybean oil, olive oil) and soya lecithin

Column: C8 column (2.1 × 100 mm, 3.5 μm)

Mobile phase A: 5 mM $NH_4C_2H_3O_2$ and 0.1% formic acid in water; Mobile phase B: acetonitrile/isopropanol (5:2, v/v)

Column oven: 40 °C

Flow rate: 0.35 mL min^{-1}

6550 iFunnel LC/Q-TOF MS conditions

Ion source: AJS ESI , positive and negative mode

Nebulizer gas: 30 psi

Dry gas: 14 L min^{-1}

Dry gas temperature: 200 ^{o}C

Sheath gas: 12 L min^{-1}

Sheath gas temperature: 300 ^{o}C

Nozzle voltage: 500 V (positive and negative)

Capillary voltage: 4000 V (positive and negative)

Data processing and analysis

The mathematical identification models and databases were developed using the Polymer Builder software. The identification and semi-quantification of the components and impurities in the tested pharmaceutic excipients and pharmaceutical preparations were made using the Polymer Pattern software.

① Sample data acquisition

The pharmaceutic excipient samples were first analyzed by the UHPLC-HRMS/MS^n method for collecting data. Then, compounds were identified based on the HRMS and MS/MS data.

② Establishment of mathematical identification model

Mathematical models were established based on the good logarithmic relationship between the structural characteristics (for example, POE polymerization degree, carbon chain length, degree of unsaturation), and RTs of the identified components and impurities. [1, 2]
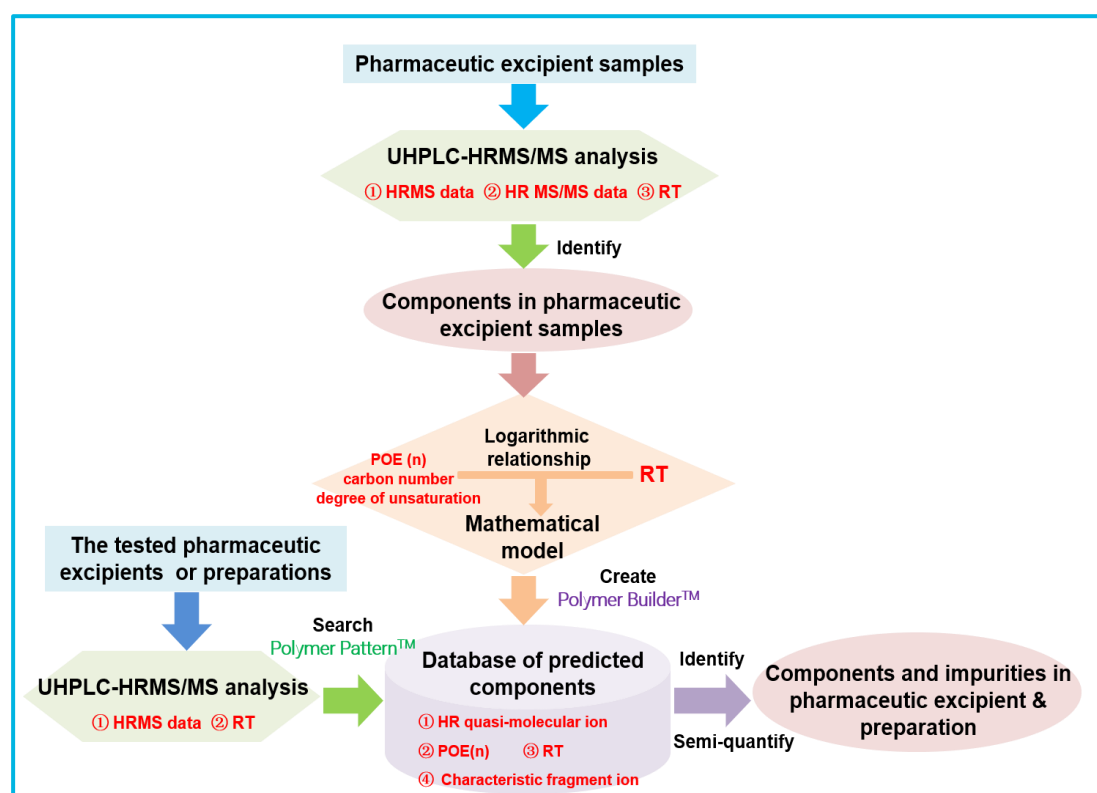

Figure 2. The workflow of the strategy for profiling and identification of pharmaceutic excipient using Polymer Studio.
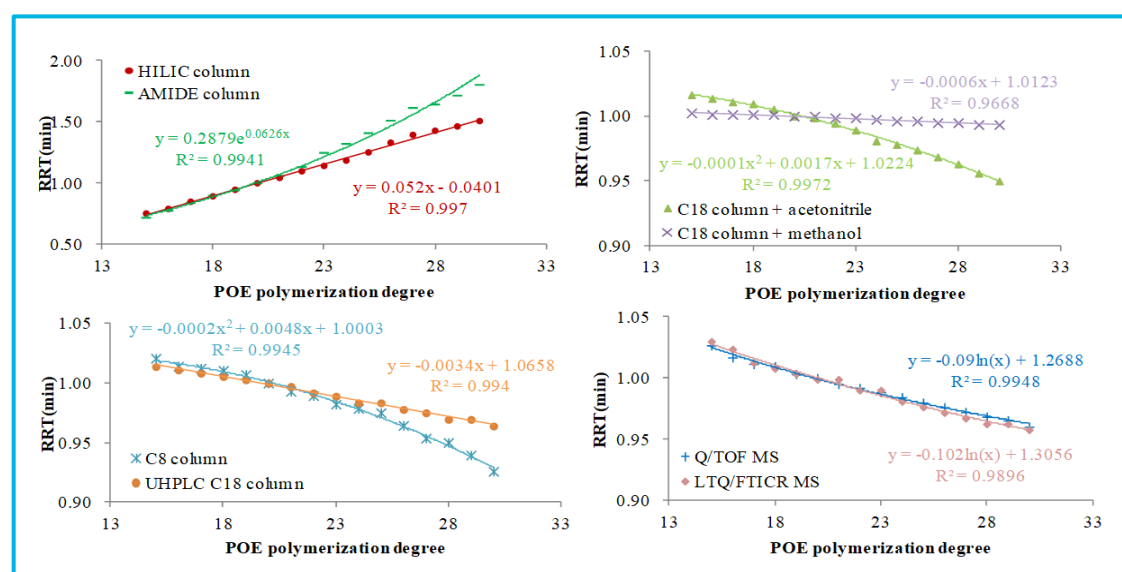

Figure 3. Relationships between the POE polymerization degrees of POE sorbitan monooleates and their RTs in different analytical conditions.

③ Establishment of prediction component database

An excipient compound database software Polymer Builder^{TM} was developed. After inputting the established mathematical identification models and structural characteristics, the predicted RT of each theoretical compound was calculated, and the theoretically possible compound database containing the structural characteristics, RT, HRMS, and MS/MS data was created in this software.

④ Identification and semiquantification of the components and impurities in the tested samples

Another software Polymer Pattern^{TM} was developed for searching and identifying components and impurities in the test samples. After inputting the created database and the raw data of the test samples, accurate m/z values and corresponding RTs in the database were introduced to search against the chromatographic and HRMS data from the test samples. Only peaks with mass errors less than 10 ppm (compared to the theoretical m/z value) and actual RTs within ±3% deviation of the predicted RTs were accepted as compounds in the test samples. Each compound was also semi-quantified by the peak area normalization method using this software.
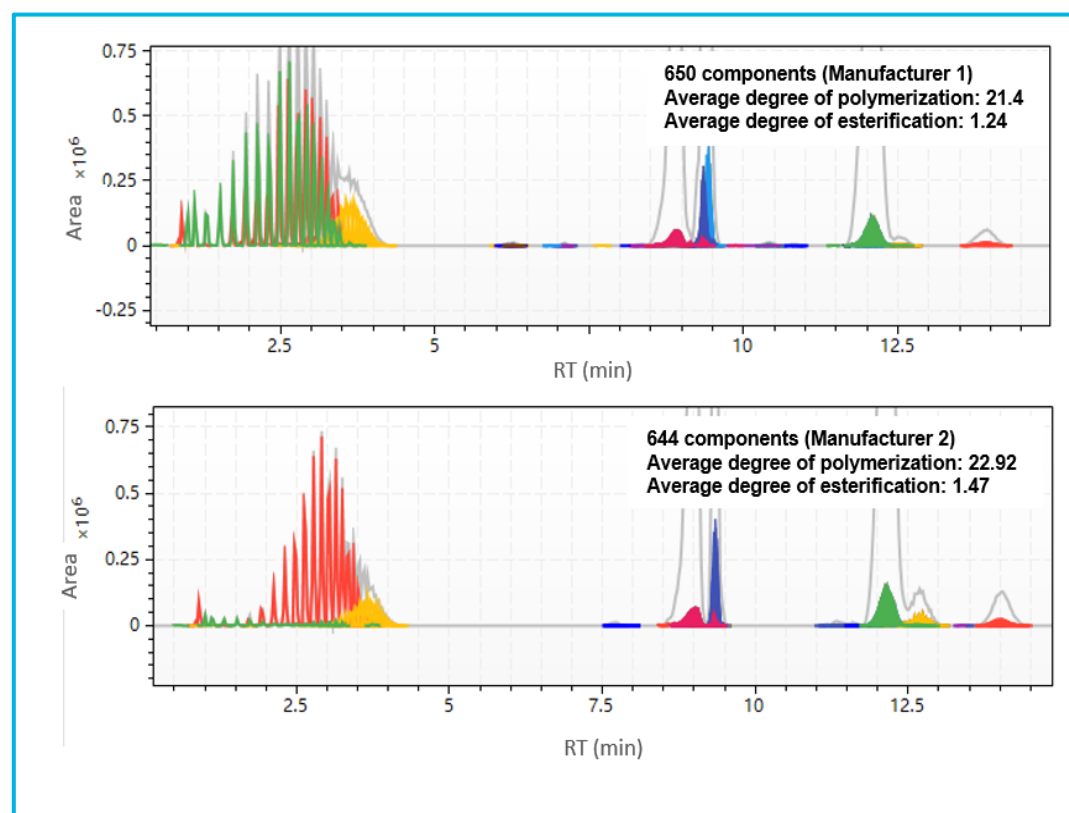

Figure 4. The overlapped EICs of polysorbate components in the same biologic drug from different manufacturers.

⑤ Application to biologic drugs containing polysorbate 80

This novel strategy and software were employed to profile components and impurities in the same biological drug made by two different manufacturers. Over 600 components and approximately 60 oxidized impurities were identified and semi-quantified in each sample. The abundance, degree of polymerization, and degree of esterification of each species of components in the two samples were significantly different.
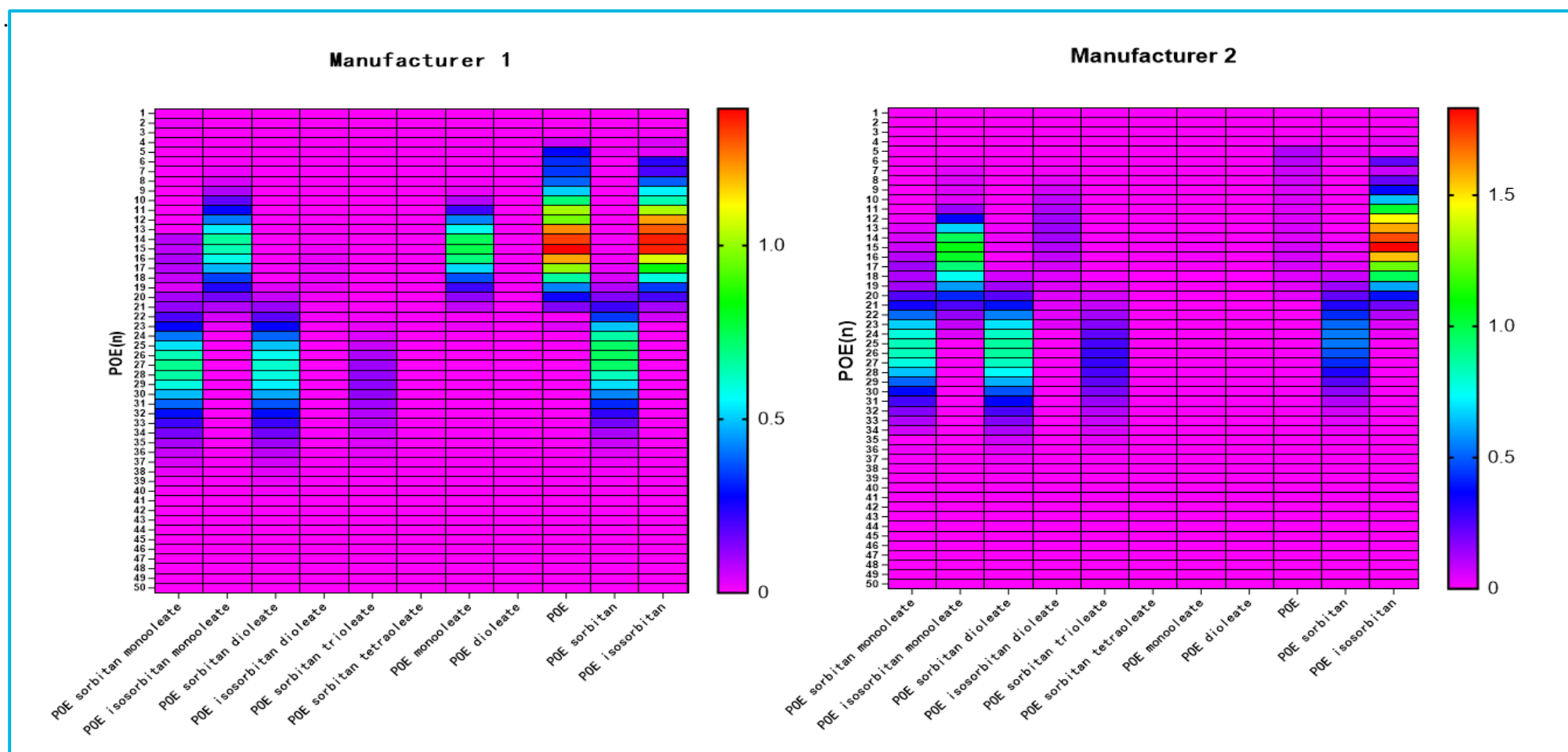

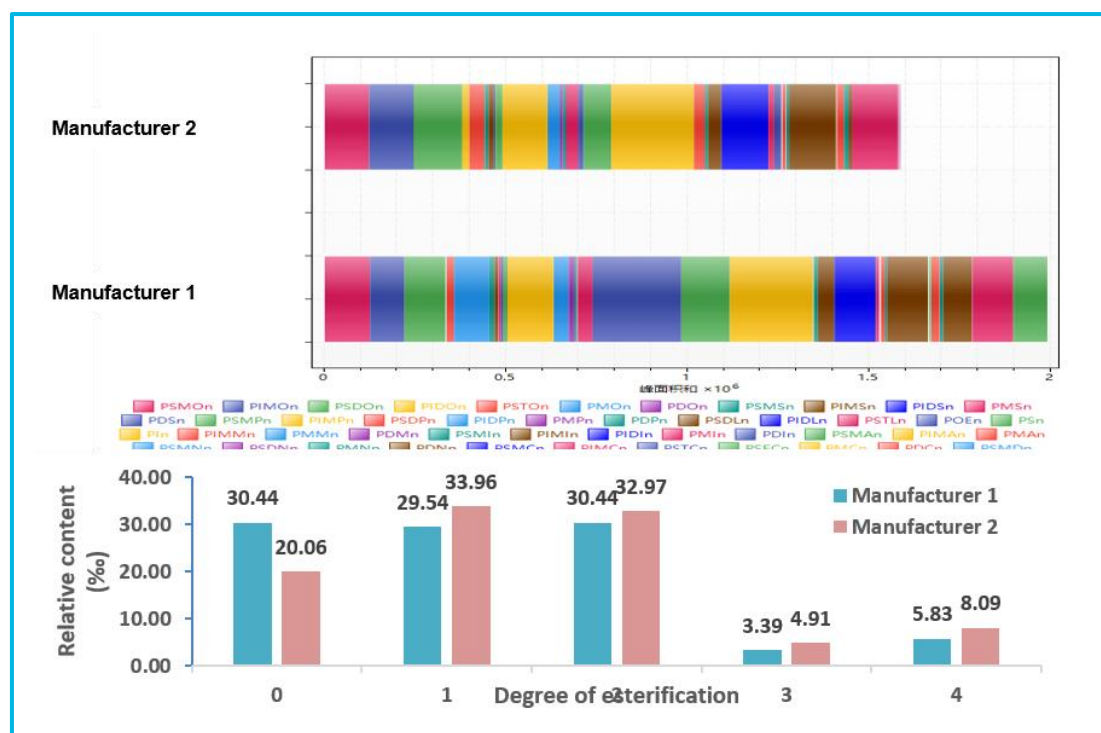Figure 5. Comparison of POE polymerization degree of polysorbate 80 in a biologic drug from different manufacturers.

Figure 6 Comparison of the abundance and degree of esterification of polysorbate in the same biological drug from different manufacturers.

Conclusion

A novel software, Polymer Studio , was developed for fast profiling and identifying components and impurities in complex pharmaceutic excipients and their preparations.

To date, five UHPLC-HRMS/MS^n methods, 124 mathematical models and six databases of 11,727 detected and predicted compounds of polysorbates [1], sorbitans, a soybean oil [2] and a soya lecithin were created.

References

[1] Wang, Z.; Wang, Y.; Tie, C.; Zhang, J. *J Chromatogr A.* 2020; 1609: 460450.

[2] Zheng, H.; Yang, R.; Wang, Z.; Wang, J.; Zhang, J.; Sun, H. *Rapid Commun Mass Spectrom.* 2020; 34: e8557.

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