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Improvements to PS80 Detection in Drug Product Formulations:

A Mixed-Mode High-Performance Liquid Chromatography Method for the Determination of Polysorbates Offers Notable Improvements Over a Conventional Reverse Phase HPLC Method

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Goal

To present innovative enhancements to the conventional reverse phase (RP) High Performance Liquid Chromatography (HPLC) method utilized for the analysis of polysorbates, including Polysorbate 80 (PS80). The new and improved approach to polysorbate analysis involves mixed-mode HPLC. Mixed-mode HPLC offers several advantages over the RP method, including reduced run times, improved signal resolution, minimal sample preparation, and decreased reliance on consumables. These enhancements not only improve data analysis, but also lead to substantial cost savings compared to the RP method, while also reducing the potential errors associated with extensive sample handling.

Introduction

Excipients play an important role in drug preparation — they serve as binders, disintegrants, stabilizers, or pH adjustors for proper performance of the dosage forms. Some of the most common excipients found in biotherapeutic formulations are non-ionic surfactants, such as polysorbates, specifically PS80 and PS20 (Tween 80 and Tween 20). For quality control and overall public health and safety, it is important to have analytical assays that can accurately measure excipient levels in samples of interest. As it is, the analysis of these small molecules is typically riddled with challenges. Polysorbates are highly heterogeneous in nature. For example, PS80 is comprised of structurally diverse polymeric species, containing polyoxyethylenes and fatty acid esters, with or without a carbohydrate core. Additionally, these molecules lack a chromophore, making UV detection impractical.

Prolytix has successfully quantified PS80 levels in customer protein drug products using an RP HPLC method with a charged aerosol detector (CAD).

However, the method requires time-consuming solid phase extraction (SPE) as part of sample preparation. The R&D team at Prolytix, in partnership with Medexus Pharma Inc., developed a mixed-mode HPLC method with a CAD detector to optimize and improve the previous approach. The new method showcases decreased run time, requires minimal sample handling and preparation, and offers superior resolution from matrix components. This paper summarizes the development of the mixed-mode method.

Sample Preparation and Run Time

RP HPLC method for PS80 analysis relies on either extracting proteins from the sample or employing sample derivatization to eliminate interferences from other Drug Product (DP) components in the matrix. Not only is this approach time-consuming, but the synthetic compounds used in these pretreatment steps can be toxic, increasing the risk to the technician and cost for hazardous waste.

While SPE was employed in the RP HPLC method previously used at Prolytix, there was a desire to improve the process by reducing pre-column sample handling and reducing the time demands on technicians. Toward that end we developed a mixed-mode HPLC method.

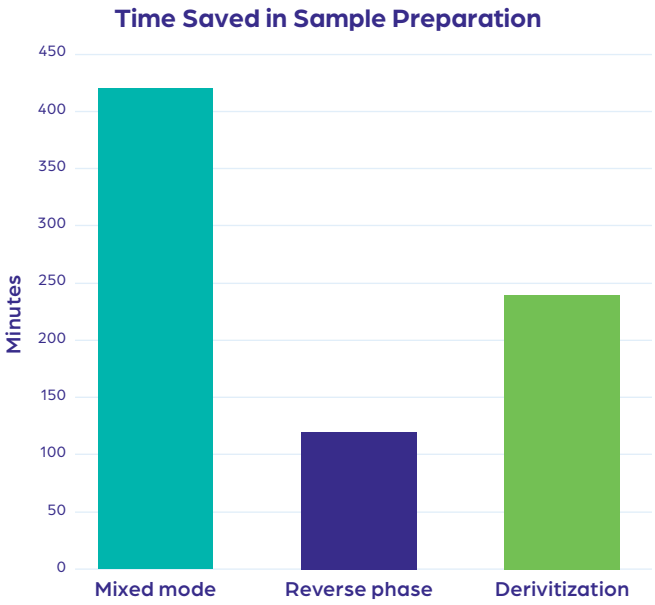


Figure 1: Time saved in an 8-hour workday by using mixed-mode technology.

A mixed-mode column offers many advantages over the traditional RP method. First and foremost, the removal of proteins is now performed by the analytical column of the instrument, rather than through the pre-column SPE step. In the acidic mobile phase, the protein becomes positively charged, which facilitates its separation by the anionic exchange mode solid phase of the column (rather than SPE). Moreover, any matrix components of the DP are also simultaneously removed with the void volume.

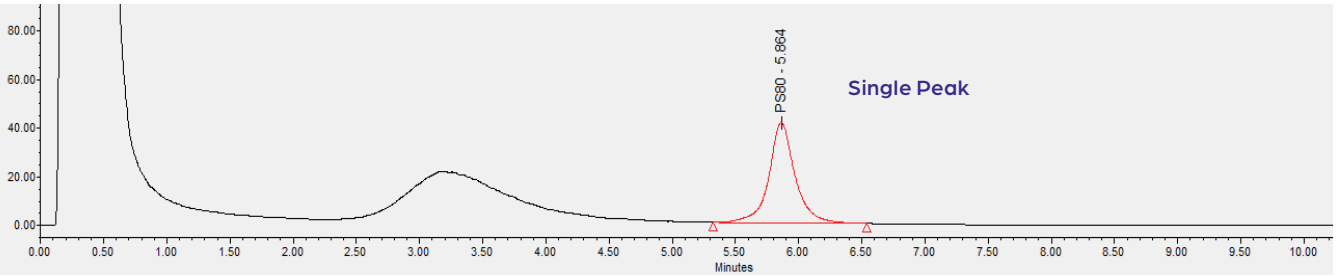
This new method enables researchers to significantly reduce the amount of time required for sample preparation — from hours to minutes **(Fig. 1)**. Instead of a lengthy and cumbersome SPE step, the method is dramatically simplified to what is essentially a "dilute and shoot" protocol; the sample undergoes a minimum required dilution, followed by injection onto the HPLC system.

Removal of the SPE step with mixed-mode HPLC not only increases sample preparation time, but decreases sample handling, potential human error, and variability between technicians performing the extraction. Additionally, assay run times are also decreased by >20%.

Matrix Interference and Signal Resolution

Specificity experiments using a DP sample with the mixed-mode approach demonstrate a well-resolved PS80 peak with little to no background interference **(Fig. 2, upper chromatogram)**, compared to the overlapping and suboptimal resolution results generated by SPE/RP HPLC approach **(Fig. 2, lower chromatogram)**. The mixed-mode method is shown to enhance PS80 peak shape and resolution by reducing matrix components. The PS80 peak is now single and well-defined, with no "tailing" or other types of unwanted artifacts. Low interference and high signal resolution allows for consistent integration and therefore reliable quantification!

MM–HPLC DP Sample



RP–HPLC DP Sample

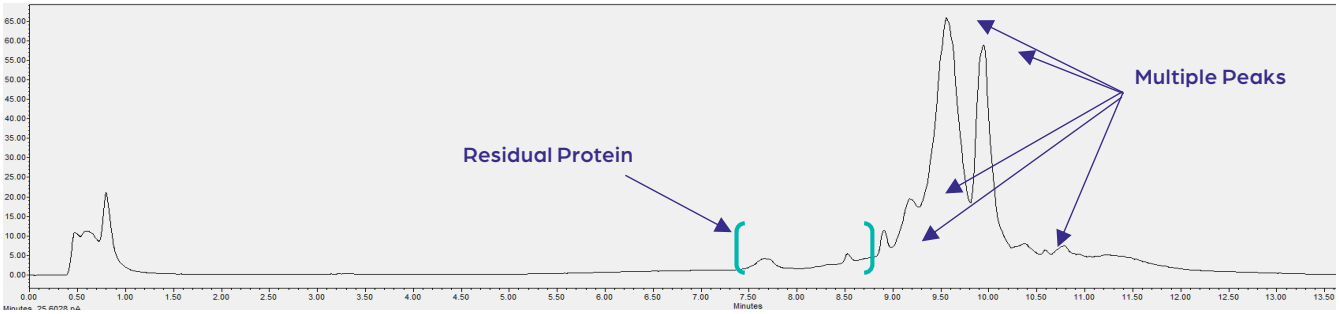


Figure 2: Mixed-mode (top) and reverse phase (bottom) comparison of PS80 from a DP sample.

Cost Improvements

Reverse Phase Materials	Mixed–Mode Materials
HPLC solvent 1	HPLC solvent 1
HPLC solvent 2	HPLC solvent 2
Organic acid	Organic acid
PS80	PS80
Nitrogen gas	Nitrogen gas
SPE cartridges	
Luer lock syringes	
Large centrifuge tubes	
Glass pipettes	
Vial adaptors	
Microfuge tubes and caps	

Table 1. List of materials needed for the RP and mixed–mode methods.

In addition to the efficiencies gained in sample processing time and improved assay performance, switching to the mixed–mode HPLC method comes with substantial cost savings.

By removing SPE from the method entirely, more than half of the required materials are no longer needed. SPE cartridges can be fairly expensive, especially when used on a regular basis as a consumable product. On the flip side, the mixed–mode method requires no costly consumables and only standard reagents; even the Minimum Required Dilution (MRD) for the sample itself is done using one of the mobile phases.

Assay Characteristics

The mixed-mode method is in final stages of development for the analysis of DP samples. Preliminary results, interrogating various dosages and formulations, show a wide range of potential and applicability in diverse settings for this new method. Utilizing a calibration curve and linear regression, the method can quantify the levels of specific polysorbates with a high degree of certainty **(Fig. 3)**. Validation data support excellent precision in both methods; between-run precision is <10% CV and within-run precision is 2% CV for the mixed-mode approach, vs. 3% CV in the RP method. Additionally, with a limit of detection of about 3 µg/mL and a limit of quantification of 10 µg/mL, this mixed-mode approach could be adapted for a wide range of products. This method also shows specificity, accuracy, precision, and linearity parameters that would fully comply with ICH guidelines Q2(R2).

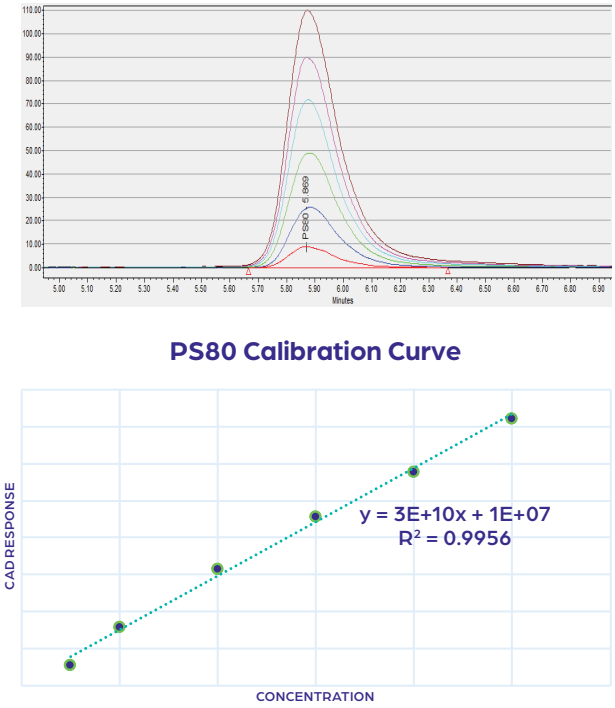


Figure 3: Stacked chromatograms of calibration samples (top). PS80 linear regression curve (bottom).

Other Polysorbates and Surfactants

Currently, the mixed-mode method is employed for detection and quantitation of PS80, though it could also be applied to PS20 analysis **(Fig. 4)**. Ultimately, with minor modifications, this method could be used for a variety of different surfactants used in biotherapeutic development, including PS40 and PS60 **(Fig. 4)**. By utilizing the CAD in conjunction with mixed-mode chromatography, Prolytix has quickly adapted to the evolving needs of its partners, expanding the ability to analyze a broad range of excipients rapidly, efficiently, and with unwavering quality.

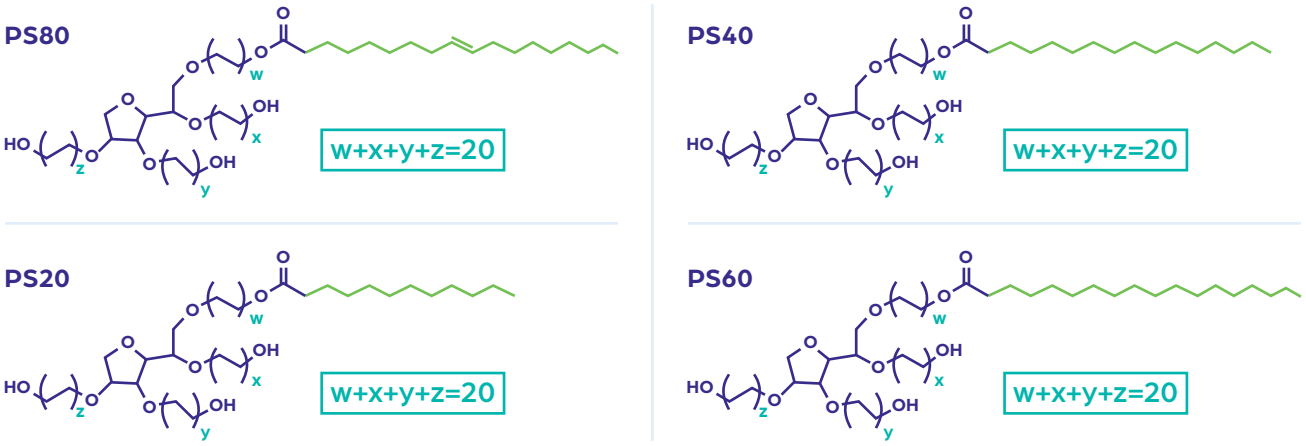


Figure 4: Chemical structures of common polysorbates within drug products including PS80 and PS20.

Conclusions

A novel mixed-mode HPLC chromatography method was developed to improve the determination of PS80. This streamlined process is robust and flexible; it can easily be adapted to other drug products and nonionic surfactants **(Fig. 4)**.

Overall, mixed-mode HPLC analysis of polysorbates offers the following advantages:

- Reduced hands-on labor, freeing up valuable technician time
- Ability to run more samples per batch
- Expedited results
- Reduced cost

Acknowledgments

We would like to extend our thanks to Medexus Pharma Inc. Their contribution to this work was invaluable.

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