

Evaluation of Methods for the Characterization and Quantification of Polysorbates and Impurities Along with Other Surfactants and Emulsifiers Used in the Food and Pharmaceutical Industries

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Abstract

Polysorbates (e.g., Tween® 20, 60, and 80) are used widely throughout the food and pharmaceutical industries. With foods, polysorbates act as emulsifiers and are added, for example, to ice-cream to make it smoother, easier to handle, and prevent melting. Although the consumption of small quantities of polysorbates is considered to be safe, there is a small subset of the population who show hypersensitivity to these compounds. This appears not to be due to the polysorbate per se but due to the formation of peroxidation products such as hydroperoxides/peroxides and aldehydes (e.g., formaldehyde), as high purity polysorbates do not elicit such a response. Measurement of these impurities uses fluorescence and other techniques but do not characterize the polysorbate. The characterization of polysorbate is extremely challenging as polysorbates are complex, containing polydispersed heterogeneous mixtures of large non-UV active molecules. The first part of this study evaluates both HPLC- and UHPLC-based methods with charged aerosol detection to determine lot-to-lot variability of polysorbates and how these results are affected by the impurity level.

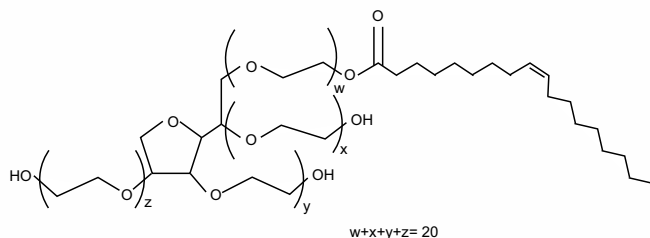
The pharmaceutical industry also relies heavily on the use of polysorbates to help solubilize and stabilize a variety of drugs. In addition to the characterization methods described above, quantification of polysorbates and other nonionic surfactants is also important. The second part of this study evaluates the use of more aggressive mobile phases to shorten run times and decrease the complexity of the chromatography. The development of an on-line dilution method, using ×2 gradient UHPLC system to simultaneously monitor the active pharmaceutical ingredient (API) and the polysorbate during dissolution studies, is also presented.

These analytical approaches solve some of the important challenges encountered with the characterization and quantitation of polysorbates and other nonionic surfactants which are beneficial to processes in the food and pharmaceutical industries.

Introduction

Polysorbates are used in large quantities throughout the food and pharmaceutical industries. Because these products have a significant demand across industries they are often produced in large lots with varying limits of impurities (peroxides, carbonyls, and metals) reported. The characterization and quantification of polysorbates is difficult because these compounds are heterogeneous mixtures with no chromophore (Figure 1). As a result, physical tests and testing for impurities are typically used for release criteria. While this testing is sufficient for the manufacturers to release quality material, it may not be sufficient for the end user. At the point of use, physical characterization such as color change may indicate chemical composition changes which could impact the final product, but the inability to test for composition along with the varying levels of impurities, make lot-to-lot consistency difficult to quantify.

FIGURE 1. Structure of Polysorbate 80.



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The consumption of polysorbate 80 and other polysorbates in both food and drug formulations is generally considered safe. Allergies and other side effects have been reported in the literature, but it is not clear if this is a result of the Tween itself or related impurities. In pharmaceutical dissolution testing, it has been reported that lot-to-lot variability and stability in solution of Tween 80 products have a large impact on the dissolution results.¹ As regulatory agencies begin to ask for more methods to quantify the amounts of these surfactants and excipients in final products, companies are turning to newer detection technologies for analytical solutions.

The Thermo Scientific Dionex Corona® Charged Aerosol Detector (CAD®) uses a mass-sensitive detection technique able to measure nonvolatile and some semivolatile analytes independent of physical characteristics like absorbance, light scattering, and ionization potential. The mechanism for this detection technique is described in Figure 2. Its ability to measure both small molecular weight compounds and large molecular weight polymers with a similar response factor results in a powerful detection method for compounds such as polysorbates. The development and validation of a fast chromatographic method for the analysis of Polysorbate 80 in solutions containing proteins using charged aerosol detection is described in Fekete et al.² The work shown here develops new chemistries to meet both characterization and quantification needs of polysorbates in both the food and pharmaceutical industries. Several approaches to analysis of Tween formulations are described:

- A full gradient HPLC method for Tween and low molecular weight impurities for full characterization.
- A partial gradient method that enables resolution of the major components without the low molecular weight impurities resolved.
- A rapid separation liquid chromatography (RSLC) method that decreases analysis time by tenfold and is used for partial characterization and quantitation.
- An on-line dilution method to measure APIs at low concentration while quantifying Tween products with the capability of delivering a single peak for the major components of Tween.

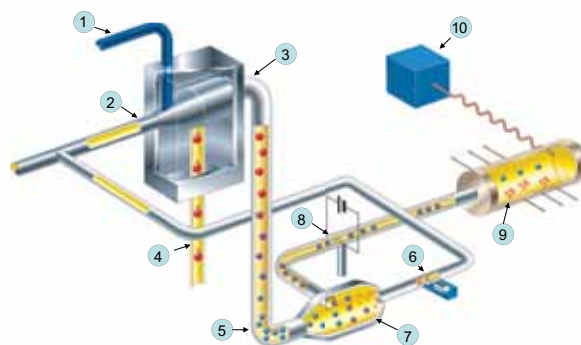
HPLC Method

Conditions

Column: Thermo Scientific Acclaim™ 300 C18, 3 µm, 300 Å 4.6 × 150 mm
 Mobile Phase A: Acetonitrile/methanol/DI water/trifluoroacetic acid (8/2/90/0.1)
 Mobile Phase B: Acetonitrile/methanol/DI water/trifluoroacetic acid (72/18/10/0.1)
 System: Thermo Scientific Dionex UltiMate™ 3000 RSLC with DAD and Dionex Corona CAD
 Sample Solvent: DI water
 Injection Volume: 2 µL
 CAD Settings:
 Gas: 35 psi
 Filter: none
 DAD Wavelengths: 210, 230, 254, and 280 nm

Full Gradient Method		Partial Gradient Method	
Time (min)	% Pump B	Time (min)	% Pump B
0	0	0	50
1	0	1	50
33	100	16	100
57	100	26	100
59	0	27	50
64	0	32	50

FIGURE 2. Flow schematic for charged aerosol detection.



The liquid eluent from the HPLC column enters the Corona (1) where it undergoes pneumatic nebulization by nitrogen or air (2). Small droplets enter the drying tube (3) and form particles while large drops exit the drain (4) to waste.

Dried particles enter the mixing chamber (5). Another gas stream passes over a charged Corona Needle (6). Charged gas then mixes with the dried particles forming charged particles (7).

High mobility species are removed by an ion trap (8) while the remaining charged particles pass to a collector where the charge is measured with a very sensitive electrometer (9). Signal is transferred to chromatographic data software (10).

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Short Analysis Method

RSLC Methods

Column: Acclaim C18, 2.2 μm , 120 \AA , 3.0 \times 50 mm
Mobile Phase A: 20% Methanol
Mobile Phase B: Acetonitrile
Mobile Phase C: Isopropanol
Flow Rate: 1.6 mL/min
System: Dionex UltiMate 3000 RSLC with DAD and Dionex Corona CAD
Sample Solvent: DI water
Injection Volume: 2 μL
Dionex Corona *ultra*[™]
Gas: 35 psi
Filter: High

Full Gradient Method			
Time (min)	% Pump A	% Pump B	% Pump C
0.00	100	0	0
0.50	100	0	0
1.00	5	95	0
1.50	5	95	0
2.00	5	0	95
3.50	100	0	0
3.75	100	0	0

On-Line Dilution Method

System Components

Dual Gradient Pump: DGP-3600RS
Autosampler: WPS-3000TRS
Column Compartment: TCC-3000RS
Diode Array: DAD-3000RS
Charged Aerosol Detector: Dionex Corona *ultra*
Switching Valves: Two 6-port 2-position, high-pressure valves
Adjustable Flow Splitter: 1-2 to 1-20
Green Column: C18 Trap Column
Yellow Column: Analytical Column (Dependent on analyte of interest. For analysis of Naproxen and sodium in dissolution solution, Thermo Scientific Acclaim[™] Trinity[™] P1 was used.)

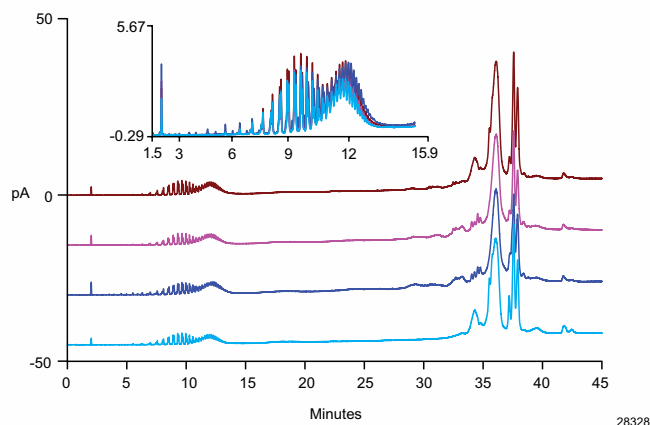
Method Overview

- The sample containing both large quantities of Tween product and small quantities of the API is injected onto the trap column.
- The API elutes the Tween from the trap column onto the analytical column.
- The right pump, using gradient elution, separates the API for detection
- Both valves switch to the 1-2 position.
- A step gradient to 95% isopropanol is used to elute the Tween from the trap column.
- The flow splitter allows only a fraction of the material to the analytical stream where it is joined with a large make-up flow from the right pump.
- The remaining Tween product is sent to waste.

Discussion

The first two analytical methods use a 300 \AA analytical column and a standard binary gradient system to fully characterize Polysorbate 80. The method described as the full gradient method enables the resolution of the low molecular weight components at the beginning of the chromatogram as well as the larger main components that elute with high organic content. The examination of four lots of Tween 80 using the full gradient method illustrates similarity in the low molecular weight material with several noticeable differences in the profile and in the relative abundance of peaks at ~34 and 37 min, respectively (Figure 3). This approach can be used for impurity measurements and lot-to-lot variability.

FIGURE 3. Stacked plot of four commercially available Polysorbate 80 products using the full gradient method with charged aerosol detection. Low molecular weight components are seen between 2.5 and 15 min and the major components elute between 30 to 45 min. Inset: Low molecular weight components overlaid.



The second method uses all the same method conditions as the first with a slight change to the gradient profile resulting in a shorter analysis time and a single peak for the low molecular weight, early eluting material, while maintaining resolution between the major components of the Tween 80 product. The reproducibility for replicate injections of a sample in a saline matrix was examined with the partial gradient method (Figure 4). The variability of the three major components was $\leq 1\%$ for the five injections. The correlation for seven concentration points of standard material over almost three orders of magnitude was >0.9999 (Figure 5). The reproducibility and sensitivity of this approach allows for a method to characterize and quantify the major components.

FIGURE 4. Overlay of five injections of Tween 80 sample in a saline matrix using partial gradient method.

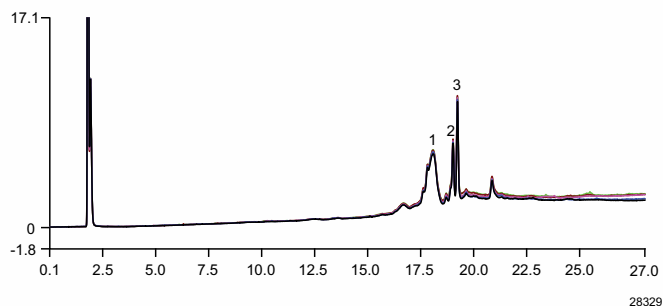
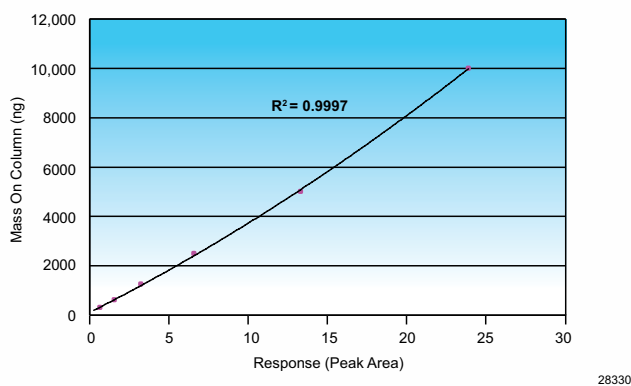


FIGURE 5. Response curve for mass on column vs total area of the three major components, fit with an inverse, second order polynomial fit using seven concentration levels ranging from 156 ng to 10 μ g on column.



After observing lot-to-lot chromatographic differences in the four batches of material using longer analytical methods, a faster RSLC method was developed to determine if similar characterization was achievable with a shorter analysis time. An additional source of Tween 80 was included in the testing (five total) and the physical properties as well as vendor specifications were reviewed in conjunction with the new RSLC chromatographic method. The vendors reported that these materials all had similar specifications with different test levels for peroxides carbonyls and metals. The color specification for the materials varied from yellow to yellow with a green cast. The actual materials had very different colored hues, with two samples observed to be nearly transparent (Figure 6). It was interesting to note that the two materials with the least yellow color (samples 2 and 4) were also the only two materials packaged in clear plastic bottles, while all other materials were packaged in brown amber glass bottles. The chromatographic results correlated to this physical observation as follows:

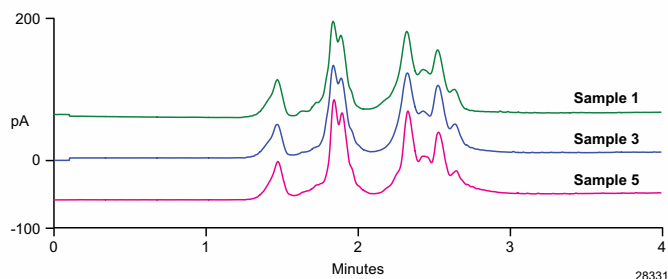
- Samples 1, 3, and 5 exhibited similar profiles with three major groups of chromatographic peaks (Figure 7).
- The samples with less color (2 and 4) had much less definition between those groups with additional peaks observed in the area of ~1.6 and 2.1 min (Figure 8).
- The peaks observed in samples 1,3, and 5 at ~2.7 min were absent as a quantifiable peak in samples 2 and 4.

FIGURE 6. Five lots of Polysorbate 80 illustrating various physical properties in different vendor products.



The results indicated that samples with the absence of the yellowish color (samples 2 and 4) are different chromatographically. The reproducibility of detection was 1% RSD for the three groups of sample 1 using this 4 min RSLC method (Figure 7).

FIGURE 7. Overlay of three lots of Polysorbate 80 from glass storage bottles using RSLC method.



The system configuration for on-line dilution was developed to address two industry requests. The first objective is to be able to monitor the Tween content relative to other compounds of interest e.g., API (at >1/100 of the Tween concentration). The second objective was to develop a system that could be used to measure Tween product as a single chromatographic peak. The on-line dilution strategy enables the end user to define the exact portion of the Tween needed to be measured (Figure 8). This approach improves the chromatographic results of the assay.

FIGURE 8. Overlay of two lots of Polysorbate 80 from plastic storage bottles using RSLC method.

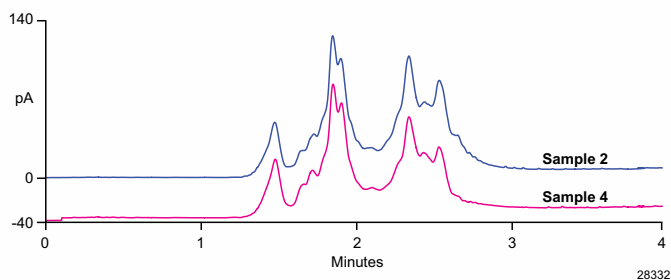


FIGURE 9. Overlay of three 1 μ L injections of Polysorbate 80 with RSLC method.

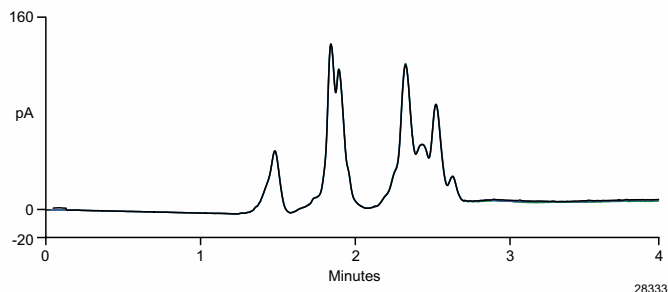
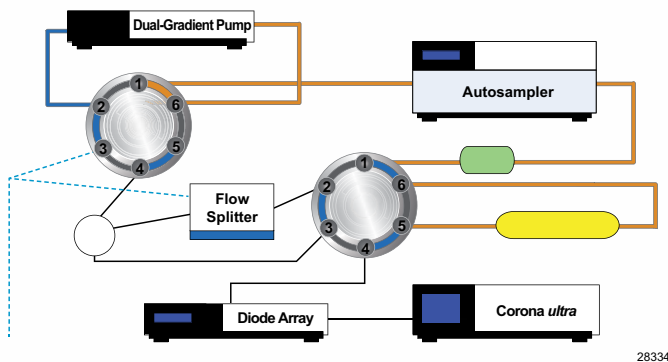


FIGURE 10. Schematic of the flow path for on-line dilution of Tween from API solution.



Conclusion

Several sensitive and robust approaches for characterizing and quantifying Tween 80 and other polysorbates are presented here. Use of the Dionex Corona CAD enabled the determination of lot-to-lot variability in off the shelf Tween 80 products. Some of the physical characteristics observed in multiple lots can be correlated to chemical composition differences determined by the short RSLC chromatographic method.

Reference

1. Zhao, H. *Methodology to Measure the Ability of a Surfactant to Solubilize a Model Compound, Application to the Control of Surfactants in Pharmaceutical Dissolution Testing*. Report for Boehringer Ingelheim Pharmaceuticals, Inc. Oral Presentation by Stephen Cafiero and Kevin C. Bynum. Pittcon 2010, Orlando, FL.
2. Fekete S., et al., Fast and Sensitive Determination of Polysorbate 80 in Solutions Containing Proteins. *J.Pharm. Biomed. Anal.* **2010**.

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