

# Analysis for Polysorbate 80 Using New High-Performance Liquid Chromatography (HPLC) Method with Charged Aerosol Detection (CAD)

By Lindsay McNalley, Senior Analytical Chemist



## About Impact Analytical

Impact Analytical is a contract analytical testing laboratory that provides testing for four main sectors: specialty chemicals including polymers and silicon materials, medical devices, pharmaceuticals, and agricultural products. We provide release testing, R&D support, method development and validation, extractable/leachable studies, stability studies, actives quantitation, raw materials testing, problem solving, and competitive analysis. Impact Analytical is ISO 9001:2015 certified, DEA licensed, FDA registered, cGMP and GLP compliant.

## Introduction

Polysorbate 80 (polyoxyethylene (20) sorbitan monooleate) is a common nonionic surfactant, emulsifier and solubilizer that is used in a wide variety of industries and applications. Polysorbate 80 is typically used in food products, cosmetics, medications, vitamins and vaccines. There can be many reasons a company may need to determine the exact amount of polysorbates in their material. For example, companies may need to determine AIG limits in their products, validate analytical methods for product production or require the information for reverse engineering purposes. Thus, a well-developed analytical method is crucial for accurately quantitating polysorbates.

Due to the chemical structure and chemical properties of polysorbate 80, it cannot be accurately quantitated by analytical methods such as gas chromatography or liquid chromatography with ultraviolet absorption. Since polysorbates do not contain a UV chromophore, this does not provide sufficient chromatography. The polysorbate material elutes over a long period of time during the analysis, making it difficult to quantitate accurately with the lack of a single peak. However, Charged Aerosol Detection (CAD) can provide a universal detection that is independent of the chemical structure for many chemicals, such as polysorbates. This paper discusses an analytical method developed by Impact Analytical to provide a single peak for quantitating polysorbate 80. The analytical method that was developed is able to quantitate the amount of polysorbate 80 in a pharmaceutical formulation containing polysorbate 80, active ingredient, microcrystalline cellulose (CMC) and sodium carboxymethyl cellulose (NaCMC).

## Analytical Method

A previous analytical method using High Performance Liquid Chromatography (HPLC) with Charged Aerosol Detection (CAD) did not provide sufficient chromatography to quantitate for polysorbate 80. The polysorbate 80 standards and samples were prepared in methanol. A Cadenza CD-C18, 3  $\mu\text{m}$ , 4.6 x 50 mm column was used for the method with a 0.5 mL/minute flow rate for a total run time of 23 minutes. A methanol and water gradient was used for the analysis. The calibration curve used for quantitation with this method provided a linear curve and a R2 value of 0.9850. An example chromatogram of a polysorbate 80 USP standard generated from this method is located in **Figure 1**.

A polysorbate 80 USP standard was used to develop the analytical method further to obtain a single peak by using HPLC/CAD. The polysorbate 80 USP standard was used to prepare standards ranging from 107  $\mu\text{g}/\text{mL}$  to 323  $\mu\text{g}/\text{mL}$  in acetonitrile. The calibration curve used to quantitate polysorbate 80 is presented in **Figure 2**. The calibration curve shows linearity and a R2 value of 0.9988. The instrument method used an Inertsil ODS-3, 5  $\mu\text{m}$ , 4.6 x 150 mm column controlled at a temperature of 40 °C. The mobile phase was isocratic with 50% acetonitrile and 50% water with 2 mM ammonium acetate. The flow rate was 1 mL/minute and a total run time of 6 minutes. An example chromatogram of a polysorbate 80 USP standard at a concentration of 215  $\mu\text{g}/\text{mL}$  is shown in **Figure 3**. The Polysorbate 80 USP standard provided a single peak to quantitate the amount of polysorbate 80.

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A formulation sample was analyzed with the improved analytical method to show a single peak. An example chromatogram of a formulation sample prepared in methanol using the previous analytical method is presented in **Figure 4**. An example chromatogram of a formulation sample prepared in acetonitrile using the new developed analytical method is presented in **Figure 5**.

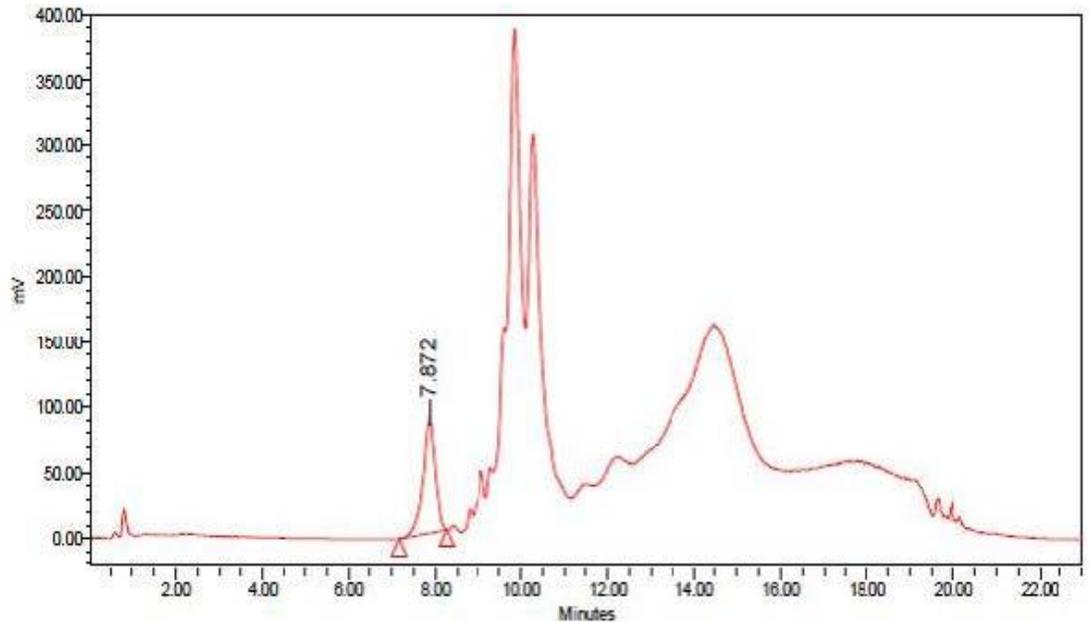


Figure 1. Example HPLC/CAD chromatogram of a polysorbate 80 USP standard at a concentration of 338 µg/mL.

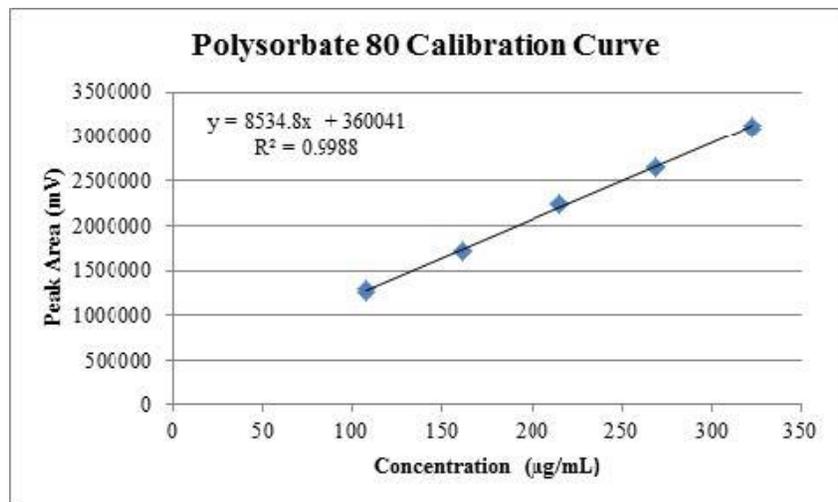


Figure 2. Calibration curve used to quantitate polysorbate 80.

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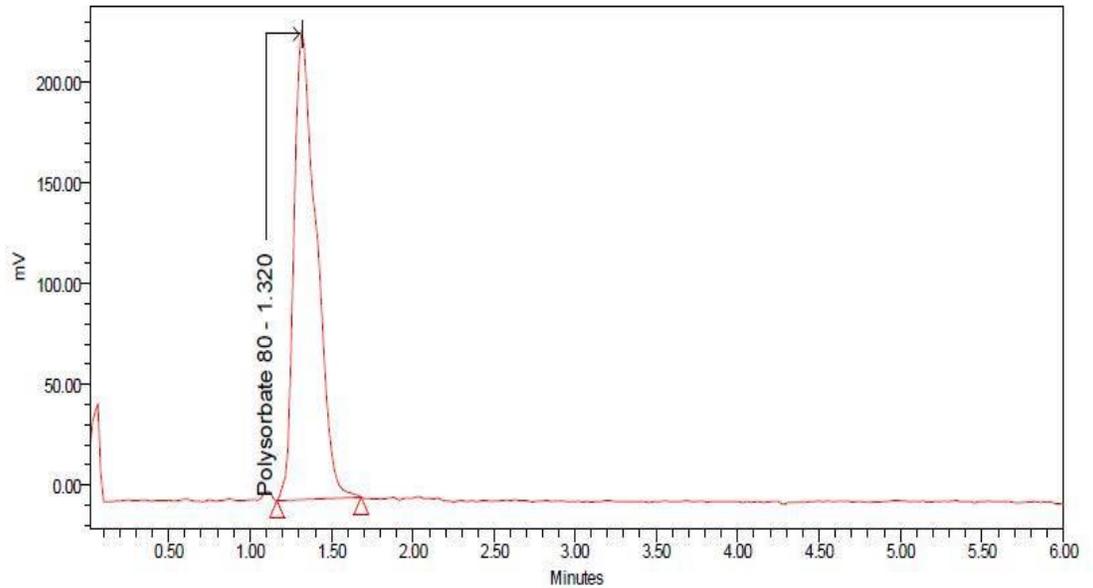


Figure 3. Example HPLC/CAD chromatogram of a polysorbate 80 USP standard at a concentration of 215 µg/mL located at a retention time of 1.32 minutes.

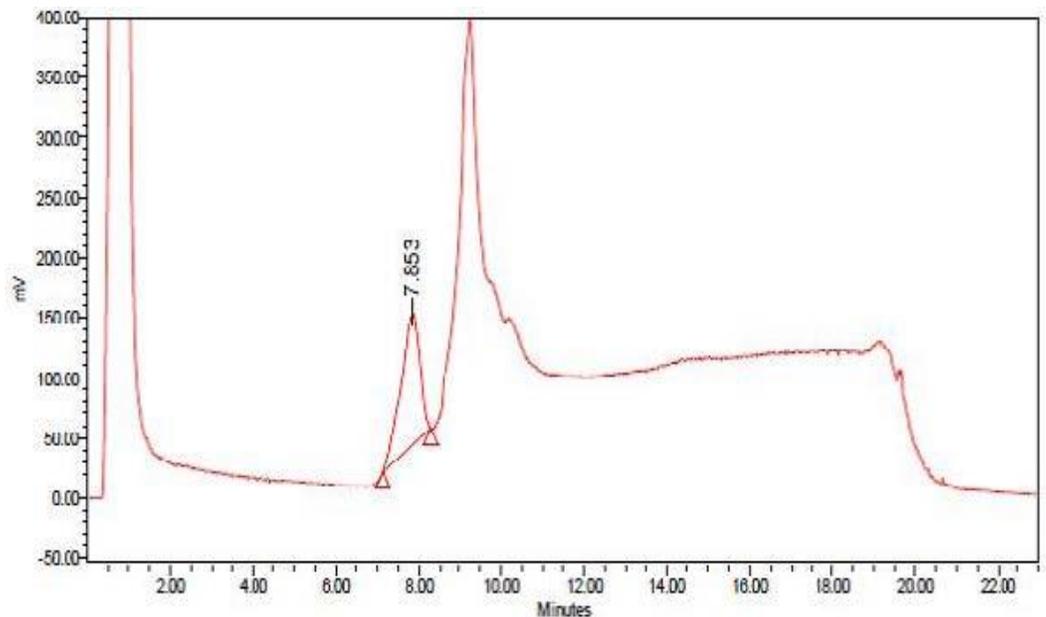


Figure 4. Example HPLC/CAD chromatogram of a formulation sample using the previous analytical method.

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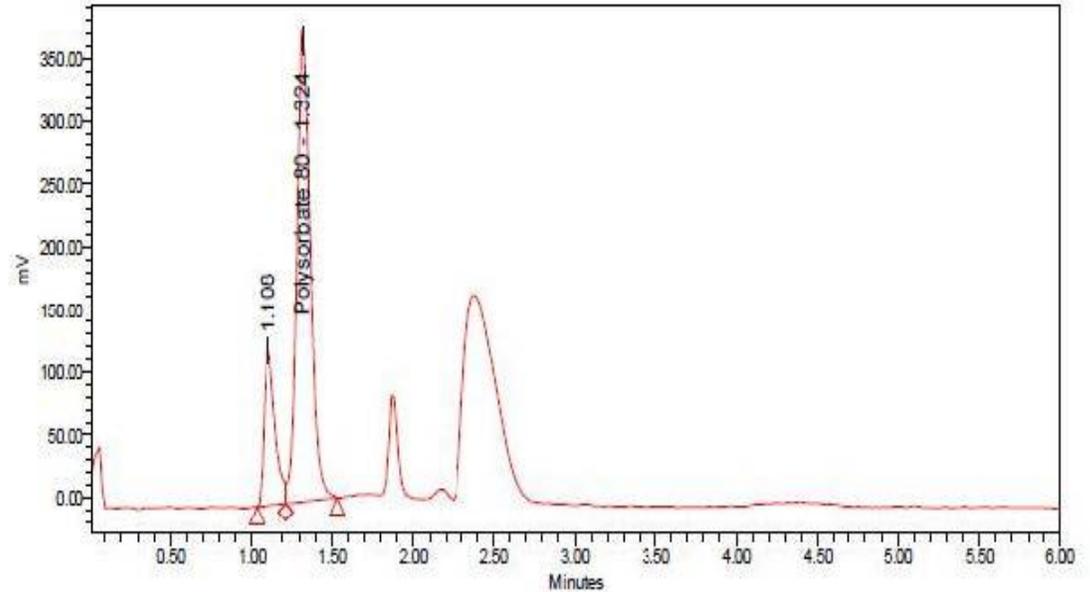


Figure 5. Example HPLC/CAD chromatogram of a formulation sample using the new analytical method.