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Combining mass spectra, retention time modelling, and charged aerosol detection for unambiguous peak annotation and uniform-response quantitation in polysorbate profiling

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ABSTRACT

Purpose: Provide a UHPLC method with simultaneous CAD and MS detection to monitor polysorbate-80 (PS80) component abundance, variation due to degradation, or batch-to-batch differences

Methods: a multi-step gradient was run on a reversed phase column. A make-up flow was applied post-column to maintain a constant solvent composition (inverse gradient). The flow was split with an approximate ratio of 1:1 to a CAD and a MS detector

Results: Polysorbate composition can be monitored at the sub-class and single-component level.

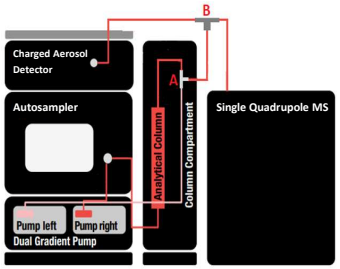

Figure 1. Schematic view of the UHPLC set-up for multi-detection with inverse gradient. To improve mixing of analytical and compensation gradient, a capillary mixer is used to connect mixing point A to split point B

INTRODUCTION

Polysorbates are a class of non-ionic surfactant widely used in drug parenteral formulations. They consist of complex mixtures that include polyoxyethylene sorbitan and isosorbide, esterified with fatty acids. In the case of polysorbate-80 (PS80), which is one of the most used polysorbate types, esterification is mainly based on oleic acid. Polysorbate components lack a chromophore, and the HPLC assays and profiling methods rely on aerosol-based detection, primarily Charge Aerosol Detection (CAD) and MS. HPLC profiling methods aim at separating PS80 sub-classes of compounds and are widely used for stability studies and to compare PS80 from different vendors or production batches. When these methods are combined with CAD and inverse gradient, an accurate estimation of the relative abundance of compound sub-classes can be assessed. In this work, we have extended the CAD inverse gradient set-up with an MS detector. Simultaneous CAD/MS detection enabled the detailed study of PS80 degradation.

MATERIALS AND METHODS

Sample Preparation

Polysorbate 80 was weighed and diluted with water to a concentration of 2 mg/mL. Digestion with Recombinant Human Lipoprotein Lipase (rhLPL) was carried out for 5 days at 37°C (no light exposure).

Chromatography Data System

Thermo Scientific Chromeleon 7.3

Instrumentation

Thermo Scientific™ Vanquish™ Flex Duo UHPLC System for Inverse Gradient, with Vanquish Charge Aerosol Detector H, and ISQ™ EM single quadrupole mass spectrometer.

Table 2. Chromatographic conditions

Column	Thermo Scientific Accucore C18 2.1x100 mm, 2.6 µm (p/n 17126-152130)
Column temperature:	50 °C (forced air), active pre-heater
Injection volume:	10 µL
Flow rate:	0.4 mL/min
Mobile phase:	A: 5 mM ammonium formate, adjusted to pH 4.8 with formic acid
	B: 50/50 acetonitrile / isopropanol (v/v)

Figure 2. Analytical gradient (top) and inverse gradient (bottom)

Table 2. Detectors setting

CAD	Data acquisition rate: 20Hz, Filter 3.6
	Evaporator Temperature 50 °C
	Power Function: 1.5
ISQ EM	Ion polarity : + @ 3kV (- 3kV for oleic acid determination)
	Full scan: 350-2000 m/z (200-600 for oleic acid determination)
	Vaporizer : 227 °C; Ion transfer tube: 150 °C
	Sheath gas: 42.9 psig; Aux gas: 4.8 psig

RESULTS

Mixing point A and split point B of **Figure 1** are preferably connected with a capillary mixer, rather than a standard open capillary. The mixer ensures that a homogeneous solvent reaches the point B where the flow is split toward the two detectors (**Figure 2**). Solvent homogeneity is crucial to ensure stable split-ratio and detectors response.

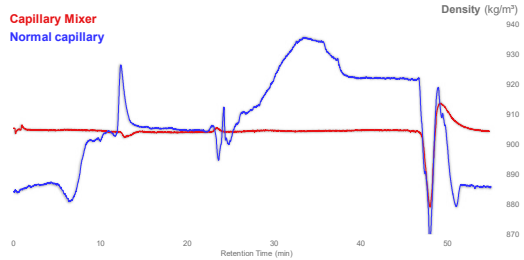

Figure 3. Solvent density measured after split point B (MS detector branch). Constant density is reached with the capillary mixer, but not with the standard capillary. Efficient mixing of the analytical and make-up flow are essential to achieve stable detector response.

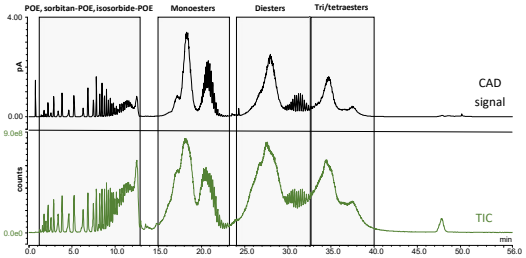

Figure 4. Chromatograms obtained with the multi-detector UHPLC system. CAD chromatogram provides an accurate assessment of the relative abundance of different PS80 sub-species.

Based on CAD's peak-height under inverse gradient conditions, it can be stated that the most abundant species in PS80 are sorbitan-POEn-oleate, eluting in the retention window approximately within 17-19 min (**Figure 4**).

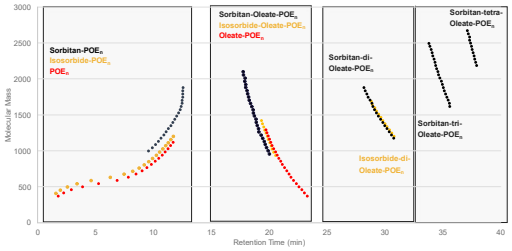

Figure 5. Retention time dependency on the mass of PS80 components. The retention time depends by the degree of esterification, and the compound class (sorbitan or isosorbide). Within a specific class, retention time depends on size, *i.e.* the length of the polyoxyethylene branch

The effect of the degradation is visible in **Figure 6**. The oleic acid peak was observed in the degraded sample. The identity of the oleic acid peak was confirmed by MS in negative polarity ionization. Almost all mono-esters were degraded into the corresponding polyols, whereas higher order esters were not hydrolyzed by the enzyme.

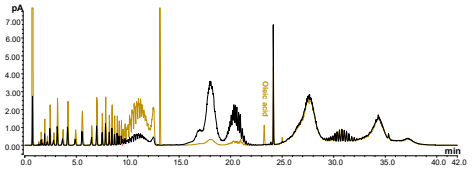

Figure 6. CAD chromatograms showing the effects of lipase-induced degradation of PS80. Comparison between the control sample (black) and degraded sample (blue). Sorbitan and isosorbide mono-esters are hydrolyzed to polyols. The peak of oleic acid released by the hydrolysis is visible in the blue trace.

The dependency between retention time and component is shown in **Figure 5**. The model was built using the PS80 reference sample. Similar plots were generated using average mass or detected m/z (data not shown). The model based on component m/z was incorporated in the Chromatography Data System processing method and used to assign peak identity to sample injections. With this approach, individual component changes due to degradation could be monitored. In **Figure 7** the impact of lipase degradation on sorbitan monoester components can be observed.

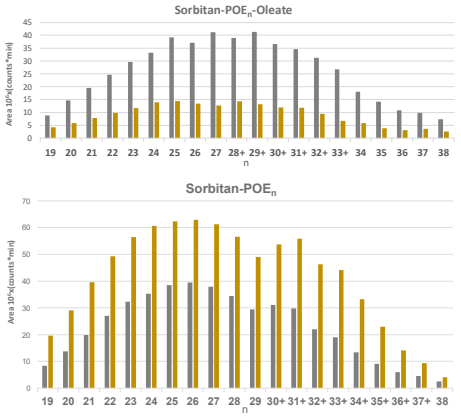

Figure 7. Peak area of components extracted from the MS signal. Component identity assignment was based on the model described in Figure 8. Reference sample (grey) and lipase degradation sample (yellow). X-axis represents the number of oxyethylene monomers *n*; the annotation "+" indicates that the peak area is the sum of multiple charge-states

CONCLUSIONS

- CAD with inverse gradient allows for the accurate determination of PS80 sub-classes mass-balance
- Single component identity assignment is enabled by combining retention time and MS spectra information
- The UHPLC multidetector system enables complete analysis of PS80. The system can be used to elucidate degradation mechanism or comparison of PS80 from different batches
- The UHPLC multi-detector system does not require advanced MS expertise and is operated by with a fully compliant chromatography data system.

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