Simultaneous analysis of polysorbate 80 and poloxamer 188 in biopharmaceutical formulations using charged aerosol detector and single quadrupole mass spectrometer

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Abstract

Purpose: Development and validation of an accurate, sensitive, and reproducible method for the simultaneous determination of polysorbate 80 (PS80) and poloxamer 188 (P188) in biopharmaceutical formulations.

Methods: A HPLC-CAD system with a Thermo Scientific™ Acclaim™ Surfactant Plus column was used to quantify the polysorbate 80 and poloxamer 188 in recombinant protein romiplostim, impurities in polysorbate 80 were identified using Thermo Scientific™ ISQ™ EM single quadrupole mass detector.

Results: This method was demonstrated with a wide linearity range, low LOQ and LOD, and good accuracy and reproducibility. The validation results indicate that the method is well suited for the polysorbate 80 and poloxamer 188 analysis in biopharmaceutical products.

Introduction

Surfactants play a key role in stabilizing protein-based formulations through manufacturing, storage, and transportation. As of 2018, more than 75% of the European Medicines Agency approved liquid protein formulations contained surfactants. Almost 50% of these liquid products are formulated with polysorbate 80, 40% with polysorbate 20, and approximately 10% with poloxamer 188[1]. However, ester bonds as well as unsaturated moieties in polysorbates make them susceptible to degradation by hydrolysis and oxidation in liquid formulations. The concerns regarding the stability and degradation products of polysorbates raised rapidly in recent years[2,3]. Although not as widely used as polysorbates, poloxamer 188 has emerged as an alternative solubilizing agent and surfactant used in biopharmaceutical products, regarded as more stable and safer in formulations [4]. To reduce the potential risk caused by a single surfactant, the use of a mixture of polysorbate and poloxamer 188 in biopharmaceutical formulations has also been reported[5].

To ensure the safety and efficacious quality control of surfactants containing drug products and meet the regulatory requirements to specify the composition and content of drug products, the accurate and sensitive quantification of these surfactants is particularly important. However, it's challenging to develop methods for the quantification of polysorbate and poloxamer 188. There are no chromophores in their structures. Additionally, both commercially available poloxamer 188 and polysorbate 80 are complex mixtures of different chemical variations of the parent structures. To improve the sensitivity, it's preferred to elute them as single peaks respectively in the method. Here, we demonstrate a sensitive HPLC-CAD method for the simultaneous quantification of P188 and PS80 in biopharmaceutical formulations.

Materials and methods

Sample preparation

It is recommended to use glass pipettes, inserts, vials, and bottles to transfer, prepare and store polysorbate 80 and poloxamer 188 solutions/samples, as there is adsorption for polysorbate 80 using plastic pipettes and vials.

Stock solutions were prepared in a 5 mL brown glass bottle with a final concentration of 5.0 mg/mL by diluting the polysorbate 80 and poloxamer 188 with deionized water. Standard solutions were prepared by diluting the stock solution with deionized water.

Recombinant protein samples romiplostim were prepared by diluting romiplostim with deionized water to an appropriate concentration.

Instrumentation

Thermo Scientific™ Vanquish™ Core HPLC system consisting of:

Vanquish Quaternary Pump CN (P/N VC-P21-A)

Vanquish Split Sampler CT (P/N VC-A12-A)

Vanquish Column Compartment C (P/N VC-C10-A)

Vanquish Charged Aerosol Detector F (P/N VF-D20-A)

Vanquish 6-position,7-port Switching Valve (P/N 6036.2530)

Thermo Scientific™ ISQ™ EM single quadrupole mass spectrometer (P/N ISQ EM-ESI)

Chromatography Data System

The Thermo Scientific™ Chromeleon™ Chromatography Data System version 7.3.1 (CDS) was used for data acquisition and analysis.

Table 1. Final chromatographic conditions for HPLC-CAD analysis

Column:	Acclaim Surfactant Plus column, 4.6 × 150 mm, 3 µm (P/N 078950)		
Mobile Phase:	A: 0.1% formic acid in water B: 0.1% formic acid in isopropanol		
Gradient:	Time (min)	Mobile phase A (%)	Mobile phase B (%)
	0	80	20
	1.8	80	20
	2.0	67	33
	3.0	67	33
	3.5	0	100
	8.5	0	100
	9.0	80	20
	17	80	20
Flow Rate:	0.6 mL/min		
Column Temperature:	25℃		
Autosampler Temperature:	4℃		
Injection Volume	10 µL		
Needle Wash Solvent	100% water		
CAD:	Evaporation temperature: 50℃ Power function value: 1.25 Data collection rate: 10 Hz		

Table 2. Conditions for HPLC-CAD-ISQ analysis

Column:	Thermo Scientific™ Accucore™ C18, 150 × 2.1 mm, 2.6 µm (P/N 17126-152130)			
Mobile Phase:	A : 5 mM ammonium formate, pH 4.8 B : 50/50 isopropanol/acetonitrile (v/v)			
Gradient:	Time (min)	B%	Time(min)	B%
	0	9	26	85
	3	9	35	100
	10	22	45	100
	10	57	46	9
	21	69	56	9
	21	84		
Flow Rate:	0.4 mL/min			
Column Temperature:	50℃			
Sample Compartment Temperature:	6℃			
Injection Volume	10 µL			
Needle Wash Solvent	10/90 water/isopropanol (v/v)			
CAD	same with Table 1			
ISQ	Ionization mode: HESI Source setting: Easy Mode Method type: Scan Mode Polarity: Positive Mass range: 250-2000 Source CID voltage: 0V Dwell time: 0.5s			

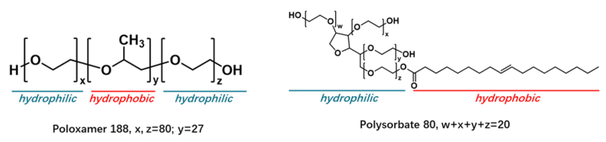


Figure 1: Chemical structures of main compounds of poloxamer 188 and polysorbate 80.

Results

Method development and optimization

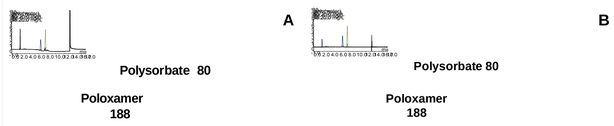


Figure 2: Effects of baseline subtraction

A: Baseline rise affects the peak shape and automatic integration process for low concentration of polysorbate 80.

B: After baseline subtraction in Chromeleon CDS software, the effects of baseline rise were eliminated.

The unknown peak eluted at 2.75 mins was identified by ISQ EM mass detector. Eluate from 2.1 minutes to 3.25 minutes was collected in a glass vial by using column switching valves. And then the solution was injected into HPLC-CAD-ISQ system for identification. The results showed that this peak was a mixture of unesterified polyoxyethylene (POE), polyoxyethylene sorbitan (sorbitan-POE), and polyoxyethylene isosorbide (isosorbide-POE) (Figure 3).

Impurity identification

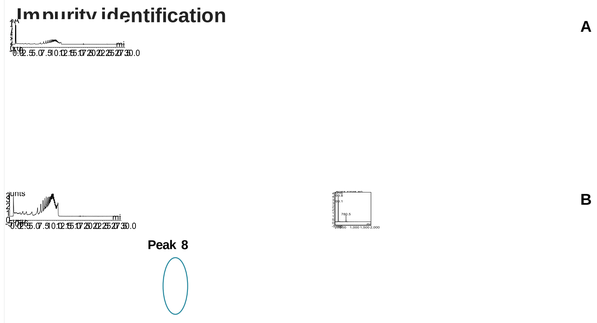
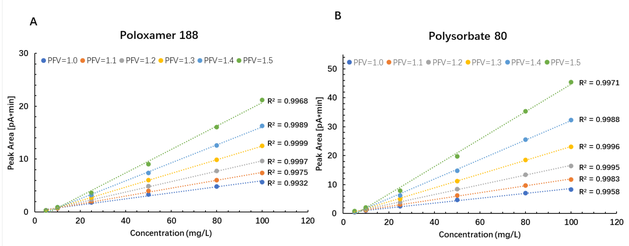


Figure 3. The CAD (A) and TIC (B) response of the unknown compounds in polysorbate 80.

Table 3: A typical tentative peak identification of unknown compounds using ISQ EM. (Δm/z= observed m/z – theoretical average m/z)

Peak No	Compound	Formula	Detected ion	Theoretical m/z	Observed m/z	Δ m/z
8	Isorsorbide-POE$_{14}$	$C_{34}H_{66}O_{18}$	$[M+2NH_4]^{2+}$	399.25	399.8	0.55
8	Isorsorbide-POE$_{14}$	$C_{34}H_{66}O_{18}$	$[M+NH_4]^{+}$	780.46	780.5	0.04

The response of CAD over a wide range of analyte concentration is non-linear, while for a narrow range (1.5 to 2 orders), CAD response can be treated as linear. For a given method, it's possible to linearize the response for the analyte concentration range by optimizing the power function value (PFV).



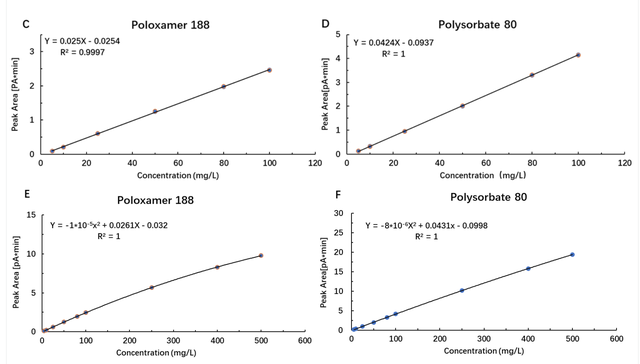


Figure 4 : Calibration curves for poloxamer 188 and polysorbate 80.

A, B: The linearity regression for P188 and PS80 ranged from 5 mg/L to 100 mg/L by using different power function values.

C, D: The linearity regression for P188 and PS80 ranged from 5 mg/L to 100 mg/L with PFV 1.25.

E, F: The nonlinear fit (quadratic fit) for P188 and PS80 ranged from 5 mg/L to 500 mg/L with PFV 1.25.

The linear range was expanded by optimizing the power function value of CAD. This range is suitable for almost all biopharmaceutical formulations in the market.

Method validation

Table 4. Linear range, LOD and LOQ

Item	Poloxamer 188	Polysorbate 80
Linear range	5-100 mg/L, R^2=0.9997	5-100 mg/L, R^2=1.0000
Nonlinear fit (quadratic fit)	5-500 mg/L, R^2=1.0000	5-500 mg/L, R^2=1.0000
LOD	2.0 mg/L	1.0 mg/L
LOQ	5.0 mg/L	2.0 mg/L

Table 5. Repeatability results of poloxamer 188 and polysorbate 80 (n=9)

Level	Poloxamer 188	Polysorbate 80
	RSD of peak area (%)	RSD of peak area (%)
Low concentration	3.56	4.15
Middle concentration	1.95	1.82
High concentration	2.06	2.17

Table 6. Recovery results of poloxamer 188 and polysorbate 80 (n=3)

Level	Poloxamer 188		Polysorbate 80	
	Recovery (%)	RSD (%)	Recovery(%)	RSD (%)
Low concentration	110.0	2.16	95.7	3.14
Middle concentration	96.8	1.58	99.01	0.90
High Concentration	103.1	0.45	98.92	0.66

Sample analysis

To avoid the high content of protein and excipients contaminating the CAD, column switching valves can be used, that switch the eluent before 4.0 minutes to waste.

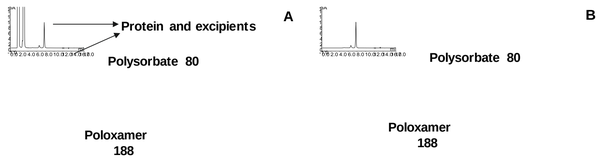


Figure 5: Chromatogram of recombinant protein romiplostim sample.

A: Without switching valve.

B: With switching valve to transfer the eluent before 4.0 mins to waste.

Table 7. Polysorbate 80 test results in recombinant protein sample (n=6)

Sample	Dilution Ratio	Protein amount (mg/g)	Expected PS80 amount (mg/L)	Detected PS80 amount (mg/L)	RSD (%)
Romiplostim 1	1	0.42	20-50	33.2	1.82
Romiplostim 2	1	0.42	20-50	32.5	2.54
Romiplostim 1	2	0.21	10-25	16.5	1.09
Romiplostim 2	2	0.21	10-25	16.1	1.59

Stability analysis

Polysorbate 80 and poloxamer 188 solution were stored under 60℃ for 5 days to do the stability analysis. Results showed that the poloxamer 188 solution was more stable than the polysorbate 80, there was no degradation for poloxamer 188 solution, and a 10% degradation was found for polysorbate 80 solution after 5 days of storage at 60℃.

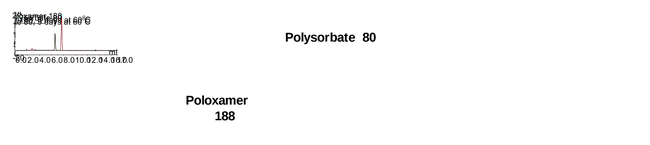


Figure 6: Overlaid Chromatogram of poloxamer 188 and polysorbate 80 solution (0.5 mg/mL) before and after 5 days storage at 60℃.

To get more detailed information of the degradation, the polysorbate 80 solution before and after storage was injected into the HPLC-CAD-ISQ[6].

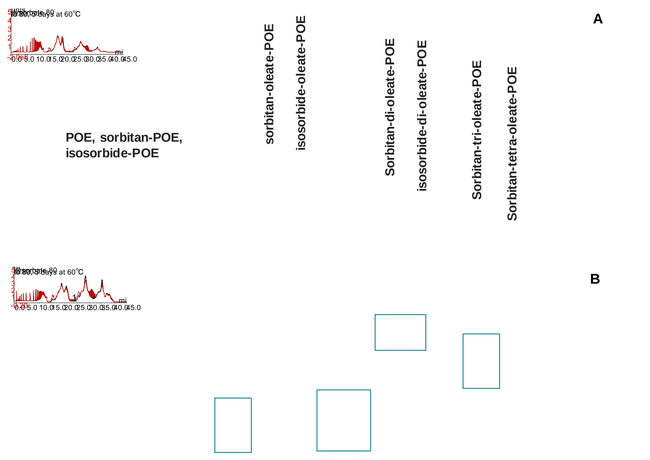


Figure 7: Overlaid CAD response (B) and TIC (A) of polysorbate 80 solution (0.5 mg/mL) before and after 5 days storage at 60℃.

After storage, the signal of sorbitan-di-oleate-POE and sorbitan-tri-oleate-POE was decreased obviously, and the signal of sorbitan-POE was almost the same, which may indicate an oxidative degradation under this condition.

Conclusions

- Using CAD combined with Acclaim Surfactant Plus Column, a single HPLC method was developed for the quantification of poloxamer 188 and polysorbate 80 simultaneously.
- This method was demonstrated with a wide linearity range, low LOQ and LOD, and good accuracy and reproducibility.
- The validation results indicate that this method is well suited for the poloxamer 188 and polysorbate 80 analysis in biopharmaceutical products.
- Confirmation of the identity of the main components of polysorbate 80 is easily achieved by LC-CAD with an ISQ EM mass detector.

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