Analysis of Fatty Acids in Polysorbate 80 Using High-Performance Liquid Chromatography (HPLC) with Charged Aerosol Detection (CAD)

Authors: Margaret Maziarz, Stephanie Harden, Paul Rainville, Jinchuan Yang
Affiliations: Waters Corporation, Milford, United States

INTRODUCTION

Polysorbate 80 is a common non-ionic surfactant widely used in the preparation of pharmaceutical, food and cosmetic products [1]. The complex heterogeneous nature and lack of chromophore present challenges for the analysis and quality control of Polysorbate 80. The U.S. Pharmacopeia (USP) procedure relies on a time-consuming reflux reaction and GC-FID analysis.

In this study, we demonstrate a high-throughput HPLC-CAD method for the determination of fatty acid composition in Polysorbate 80 [2].

METHODS

Standard Solutions Preparation
Fatty acids stock standard solutions were prepared in methanol at 1 mg/mL and diluted with water/acetonitrile (75:25, v/v) to make solutions for linearity and LOQ determination, and system suitability.

Polysorbate 80 Samples
Sample preparation performed based on previously published study [1]. Polysorbate 80 samples were prepared in 90:10 of 1 M potassium hydroxide (KOH)/methanol at 1.5 mg/mL and incubated for 6 hours at 40°C. After saponification, 250 µL of sample solution was transferred to a glass centrifuge tube, followed by an addition of 50 µL of 100% formic acid, and 500 µL of MTBE. The mixture was vortexed and centrifuged at 2700 rpm for 5 minutes. The organic layer was collected and dried under a nitrogen gas. The residue was reconstituted with 75:25 acetonitrile/water (v/v).

System	Arc™ HPLC System with Column Heater/Cooler and Active Pre-heater Waters Charged Aerosol Detector
Mobile phase	Solvent A: 0.05% formic acid in water Solvent B: 0.05% formic acid in acetonitrile
Column	XBridge® BEH™ C_{18} Column, 4.6 x 100 mm, 3.5 µm, at 60 °C
Flow rate	1.5 mL/min
Injection vol.	35.0 µL
Sample temp.	10 °C
Gradient	Time (min) / %A / %B / Curve Initial 25.0 75.0 6 1.00 25.0 75.0 6 10.00 15.0 85.0 6 10.20 5.0 95.0 6 11.00 5.0 95.0 6 11.20 25.0 75.0 6 14.00 25.0 75.0 6
Wash solvents	Purge/sample wash: 50/50 water/acetonitrile Seal wash: 90:10 water/acetonitrile
CAD Detector settings	• Power function value (PFV): 1.00 • Sampling rate: 10 pts/sec • Filter time constant: 1.4 seconds • Ion trap voltage: 20 V
Data analysis	Empower™ Software, version 3.6.0 was used for data acquisition, processing and reporting.

Table 1. HPLC-CAD method conditions.

RESULTS

Fatty acids lack a strong chromophore required for UV detection but produce a robust signal with CAD (Figure 1).

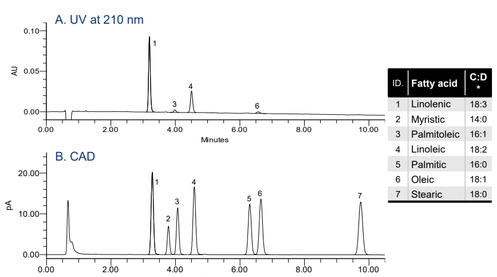

*Figure 1. Chromatographic analysis of fatty acids mixture (10 µg/mL) using UV at 210 nm (A) and CAD Detector (B). * C:D - carbon to carbon chain length: number of double bonds.*

Evaporator Temperature Optimization

The impact of the evaporator temperature on the fatty acids was investigated by evaluating the signal-to-noise ratios (Figure 2).

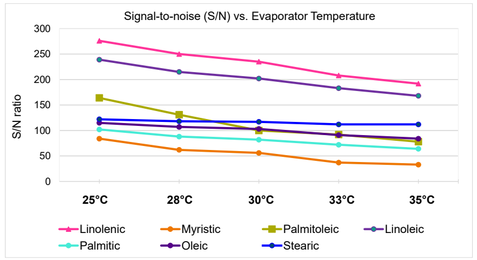

Figure 2. CAD evaporator temperature optimization. The signal decreased with increase of the evaporator temperature, 25ºC proving the highest response. Sample at 0.5 µg/mL.

Filter Time Constant Optimization

The impact of the filter time constant on fatty acid signal was assessed by evaluating signal-to-noise (S/N) values over a range of 0.9 to 2.0 seconds. (Figure 3).

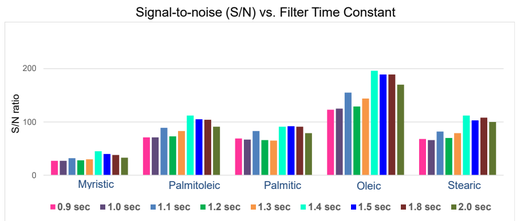

Figure 3. Filter time constant optimization. Value of 1.4 seconds provided the highest S/N for fatty acids. Sample at 0.5 µg/mL.

Power Function Value Optimization

The power function value (PFV) can be used to optimize a non-linear CAD response through digital signal processing.

The impact of PFV on linearity of fatty acids was evaluated using standards ranging from 0.05 to 25 µg/mL (Figure 4).

A. Correlation coefficients (R^2)

Fatty acid	Power Function Value (PFV)			
	0.80	0.90	1.00	1.10
Linolenic	0.9532	0.9777	0.9927	0.9988
Myristic	0.9986	0.9919	0.9774	0.9602
Palmitoleic	0.9893	0.9983	0.9981	0.9896
Linoleic	0.9671	0.9873	0.9975	0.9986
Palmitic	0.9744	0.9868	0.9915	0.9897
Oleic	0.9782	0.9940	0.9991	0.9948
Stearic	0.9801	0.9911	0.9928	0.9871

B. Calibration plots for oleic acid

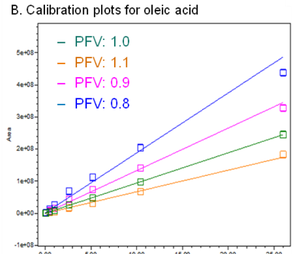

Figure 4. Power function value optimization. R^2 for linearity data processed with different PFV values (A), calibration plots for oleic acid with peak area vs. concentration (B). Linear fit with 1/x weighting. PVF of 1.00 produced R^2 ≥0.99 for most fatty acids, except for myristic.

Limit of quantitation (LOQ)

LOQ were determined based on the S/N criteria of 10:1 based. The LOQ for fatty acids ranged from 0.9 to 3.5 ng on column (Figure 5).

A. LOQs

Fatty acid	LOQ ng on column
Linolenic	0.9
Myristic	3.5
Palmitoleic	1.8
Linoleic	1.1
Palmitic	2.0
Oleic	1.8
Stearic	2.5

B. Chromatographic data

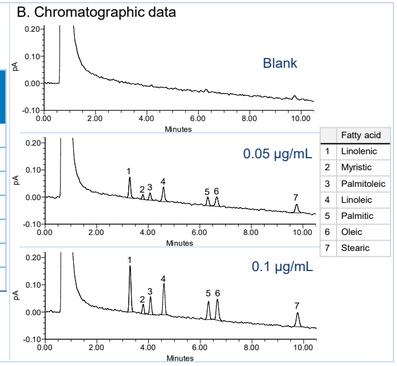

Figure 5. LOQ determination for fatty acids based on S/N ≥ 10. LOQ concentrations, average of n=6 (A) and chromatograms at the LOQ level (B).

System suitability

Ten replicate injections of a standard solution at 10 µg/mL of each fatty acid were evaluated (Figure 6).

System Suitability
Sample Set ID: 15466 Result Set Id: 15707
Processed Channel Descr.: CAD Ch1 - PFV=1

Peak Results

	Name	Inj. #	Ave_RT	% RSD RT	%RSD Peak Areas	Ave USP Resolution	Ave USP Tailing
1	Linolenic	10	3.273	0.02	0.41		1.1
2	Myristic	10	3.774	0.02	0.48	3.7	1.0
3	Palmitoleic	10	4.059	0.01	0.40	2.0	1.0
4	Linoleic	10	4.575	0.02	0.29	3.3	1.0
5	Palmitic	10	6.289	0.01	0.38	9.9	1.0
6	Oleic	10	6.634	0.01	0.35	1.8	1.0
7	Stearic	10	9.727	0.02	0.42	14.6	1.0

Figure 6. System suitability determination using 10 injections of 10 µg/mL standard mixture. The %RSD of peak areas and retention times were ≤ 0.48% and ≤ 0.02%, respectively.

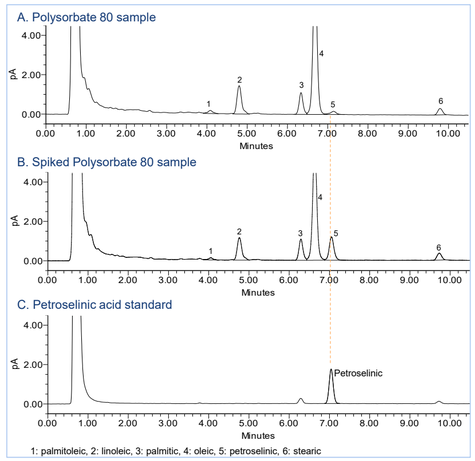

Figure 7. Analysis of polysorbate 80 batch 1 (A), sample spiked with 1 µg/mL of petroselinic acid (B), and petroselinic acid standard (C). Based on the retention time and area increase in the spiked test sample, it was concluded that the addition peak is petroselinic acid.

Polysorbate 80 Samples

The polysorbate 80 samples were analyzed for composition of fatty acids. An additional peak eluting after oleic acid (Figure 7) was detected. A previously published study identified this peak as a petroselinic acid[1]. To confirm the identity, a polysorbate sample was spiked with a petroselinic acid standard and compared with the injections of an un-spiked test sample and a standard solution (Figure 7).

Composition of the fatty acids was determined by comparing peak area of each fatty acid to the total area of all peaks related to fatty acids, as detailed in the USP monograph for polysorbate 80 [3]. The calculations included petroselinic acid (Table 1).

Acid	*USP Criteria* *	% Acid Batch 1	% Acid Batch 2	% Acid Batch 3
Myristic	NMT 5.0%	ND	ND	ND
Linolenic	NMT 4.0%	ND	ND	ND
Palmitoleic	NMT 8.0%	1.6	1.4	1.7
Linoleic	NMT 18.0%	14.5	10.9	6.3
Palmitic	NMT 16.0%	9.9	4.2	4.9
Oleic	NLT 58.0%	68.3	80.1	82.4
Petroselinic	N/A	2.3	2.0	2.9
Stearic	NMT 6.0%	3.3	1.4	1.8

Table 1. Composition of fatty acids in polysorbate 80, calculations performed using Empower Software. Results met the USP criteria.
*NMT: not more than, NLT: not less than, * USP monograph for polysorbate 80[3].*

CONCLUSION

- A high-throughput HPLC method using Waters CAD was developed for the determination of fatty acid composition in polysorbate 80 raw materials, utilizing an Arc HPLC System controlled by Empower Software.
- Optimization of detector parameters including evaporator temperature, filter time constant, and power function value was critical to enhancing sensitivity, linearity, and repeatability.
- The developed method enabled reliable determination of fatty acids, eliminating the need for a complex and time-consuming sample reflux reaction procedure and analysis by GC/FID.
- Seamless integration with Empower Software, CAD enabled a compliance-ready solution for quality control of polysorbate 80 raw materials.

References

1. Ilko D, Braun A, Germershaus O, Meinel L, Holzgrabe U. Fatty Acid Composition Analysis in Polysorbate 80 with High Performance Liquid Chromatography Coupled to Charged Aerosol Detection. European Journal of Pharmaceutics and Biopharmaceutics, 94 (2015) 569-574.
2. Maziarz M, Harden S, Rainville R. Determination of Fatty Acid Composition in Polysorbate 80 using HPLC with Charged Aerosol Detection. Waters Application Note 720009340, 2026.
3. USP Monograph for Polysorbate 80, United States Pharmacopoeia, USP–NF 2021 Issue 1. The United States Pharmacopoeia Convention, Official 01-May-2020.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS

720009439EN