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Applikationsbericht

Determination of Fatty Acids in Polysorbate 80 Pharmaceutical Raw Materials by HPLC With Mass Detection

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Abstract

Polysorbate 80 is a non-ionic surfactant widely used as an excipient in pharmaceutical formulations. The quality and purity of excipients are critical to the safety of the final drug product and must be monitored using suitable analytical test methods. The U.S. Pharmacopoeia (USP) recommends a gas chromatography (GC) with flame ionization detection (FID) method for quality assessment of the polysorbate 80 based on the composition of fatty acids (USP–NF 2021 Issue 1). This procedure requires hydrolysis of the polysorbate 80 and conversion of methylated acids to free fatty acids. In this work, an HPLC method coupled with mass spectrometry (MS) for analysis of fatty acids in the polysorbate 80 pharmaceutical raw material is presented. The new HPLC method offers fast and accurate determination of fatty acids by direct analysis, eliminating the need for a complex sample preparation procedure and GC instrumentation.

Benefits

- Fast quality assessment of the polysorbate 80 pharmaceutical raw material based on fatty acids composition by direct analysis using HPLC with mass detection
- Analysis of non-chromophoric fatty acids by mass detection with the ACQUITY QDa Mass Detector
- Robust, reliable, and reproducible performance of an Arc HPLC System

Introduction

Polysorbate 80, also known as Tween 80, is a common non-ionic surfactant widely used as a pharmaceutical excipient or inactive ingredient in drug products.[1–3] Its main function is to enhance the solubility of poorly soluble drugs and to stabilize aqueous formulations of medications for parenteral administration or vaccinations.

Polysorbate 80 is a mixture of saturated and unsaturated fatty acid esters and sorbitol anhydrides copolymerized with approximately 20 moles of ethylene oxide.[2] Several analytical techniques have been described in the literature for the quantification and characterization of polysorbate 80 using liquid chromatography (LC) combined with charged aerosol detector (CAD), evaporative light scattering detector (ELSD), and high-resolution mass spectrometry.[1–4] Other reported methods utilize an ion mobility separation coupled to tandem mass spectrometry, supercritical fluid chromatography, and nuclear magnetic resonance technologies.[3–4] However, the analysis of complex data generated by using these techniques is a time-consuming and difficult task. Additionally, methods that allow quantification of polysorbate 80 as a single peak do not provide specificity for all fatty acids.[2] Since polysorbate 80 does not have a strong chromophore, the UV-based methods are unsuitable. The USP specifies a GC-FID method for quality assessment via hydrolysis of the polysorbate 80 and conversion of methylated acids to free fatty acids.[5] This is a complex and time-consuming procedure, not ideal for routine testing.

In this work, an HPLC-MS method was developed for the determination of fatty acids composition in the polysorbate 80 pharmaceutical raw material by direct analysis of hydrolyzed samples. Analysis of different batches revealed presence of an additional fatty acids currently not specified by the USP. The identity of these fatty acids was verified by Quadrupole Time-of-flight (QTof) Mass Spectrometer coupled to a UPLC system.

The new HPLC-MS method offers fast quality assessment of the polysorbate 80 pharmaceutical raw materials by direct analysis of hydrolyzed samples, eliminating a complex sample preparation procedure and the need for GC instrumentation.

Experimental

Fatty acids were purchased from Sigma and Nu-Chek Prep, Inc. Polysorbate 80 and mass spectrometry grade solvents were obtained from Sigma.

Sample Description

Fatty acids stock standard solutions were prepared in ethanol at 1 mg/mL concentrations. Stock standard solutions were diluted with a mixture of water/ethanol (50:50, v/v) to 10 µg/mL.

Polysorbate 80 test samples were hydrolyzed with 1 M potassium hydroxide (KOH) in water to release fatty acids. The test samples prepared in 1 M KOH at 1.5 mg/mL were incubated for 6 hours at 40 °C. Solutions were then cooled to room temperature, neutralized with equal volume of 1 M formic acid, and diluted with mixture of water/ethanol (50:50, v/v) to 0.1 mg/mL. All test sample solutions were filtered through GHP syringe filters (p/n: WAT097962 <https://www.waters.com/nextgen/us/en/shop/sample-preparation--filtration/wat097962-acrodiscghp13mm-02-m-w-minispike-100-pk.html>) prior analysis.

HPLC-MS Method Conditions

LC system:	Arc HPLC System with column heater/cooler with passive pre-heater, ACQUITY QDa Mass Detector, Isocratic Solvent Manager (ISM)
Vials:	LCMS Maximum Recovery 2 mL volume (p/n: 600000670CV)
Column(s):	XBridge BEH C_{18}, 4.6 x 100 mm, 3.5 µm (p/n: 186003033)
Column temp.:	60 °C
Sample temp.:	10 °C
Injection volume:	25 µL
Flow rate:	2.0 mL/min
Mobile phase:	Solvent A: 10 mM Ammonium acetate in water Solvent B: Acetonitrile Solvent C: Isopropyl alcohol (used for system

	wash)
Wash solvents:	Purge/Sample Wash: 60:40 Water/acetonitrile Seal Wash: 90:10 Water/acetonitrile

Gradient Table

Time (min)	%A	%B	%C	Curve
Initial	60.0	40.0	0.0	6
1.00	60.0	40.0	0.0	6
14.00	20.0	80.0	0.0	6
14.10	0.0	50.0	50.0	6
16.00	0.0	50.0	50.0	6
16.10	60.0	40.0	0.0	6
20.00	60.0	40.0	0.0	6

UPLC-QTof Method Conditions

LC system:	ACQUITY UPLC I-Class PLUS (FTN) with Xevo G2-XS QTof
Column(s):	XBridge BEH C_{18}, 2.1 x 150 mm, 1.7 µm (p/n: 186002353)
Column temp.:	60 °C
Sample temp.:	10 °C
Injection volume:	2.0 µL

Flow rate:	0.3 mL/min
Mobile phase:	Solvent A: 10 mM Ammonium acetate in water Solvent B: Acetonitrile
Wash solvents:	Purge/Sample Wash: 60:40 Water/acetonitrile Seal Wash: 90:10 Water/acetonitrile

Gradient Table

Time (min)	%A	%B	Curve
Initial	60.0	40.0	6
2.10	60.0	40.0	6
30.00	20.0	80.0	6
30.10	5.0	95.0	6
32.00	5.0	95.0	6
32.10	60.0	40.0	6
36.00	60.0	40.0	6

MS Conditions

MS system:	Xevo G2-XS QTof Mass Spectrometer
Ionization mode:	ESI-
Acquisition range:	50–1200 *m/z*

Analyzer mode:	Resolution
Capillary voltage:	2.0 kV
Sampling cone:	80
Source offset:	50
Desolvation temperature:	600 °C
Source temperature:	120 °C
Cone gas flow:	10 L/h
Desolvation gas:	1000 L/h
Lock mass:	Leucine Enkephalin (556.2271 *m/z*)

Data Management

Chromatography software:	MassLynx v4.2 SCN996

Results and Discussion

Analysis of fatty acids was performed using an ACQUITY Arc System with ACQUITY QDa Mass Detector. The Isocratic Solvent Manager (ISM)[6] was used to split and dilute the flow entering the ACQUITY QDa Detector. The ISM make-up (dilution) solvent was added post-column and mixed with the flow entering the source.

The fatty acids specified by the USP[5] are shown in Table 1. The saturated fatty acids lack chromophores (or double bonds) required for UV detection but produce a robust MS signal on the ACQUITY QDa Detector. The developed HPLC-MS method successfully separated all the USP-specified fatty acids (Figure 1). The mass spectral data enabled quick identification of the fatty acids (Figure 1A), while the Single Ion Recording (SIR) was used for quantitative analysis (Figure 1B).

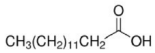
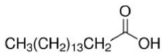
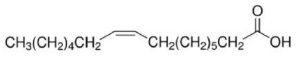
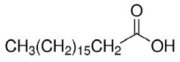
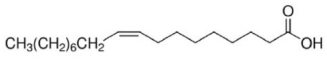
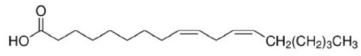
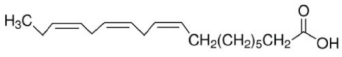
Acid	C:D*	Monoisotopic mass (Da)	Structure
Myristic	14:0	228.21	
Palmitic	16:0	256.24	
Palmitoleic	16:1	254.22	
Stearic	18:0	284.27	
Oleic	18:1	282.26	
Linoleic	18:2	280.24	
Linolenic	18:3	278.22	

Table 1. Fatty acids specified in the USP monograph for polysorbate 80.[5]

** C:D - carbon to carbon chain length: number of double bonds.*

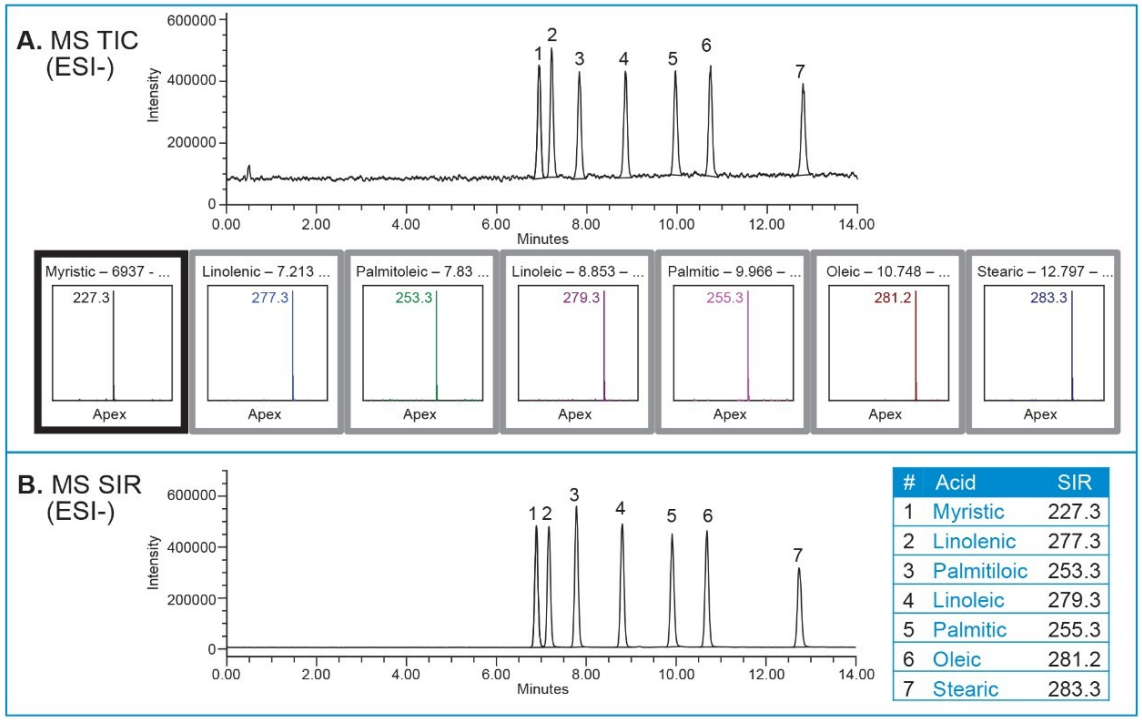


Figure 1. Chromatographic separation of USP-specified fatty acids by an ACQUITY Arc HPLC System with ACQUITY QDa Mass Detector. Standard solution at 10 µg/mL. Total ion chromograph (TIC) with mass spectral data (A) and overlay of Single Ion Recording (SIR) channels (B).

Repeatability of the method was evaluated using six replicate injections of the 10 µg/mL fatty acid standard (Figure 2). The percent relative standard deviation (RSD) for the retention times and peak areas ranged from 0.12

to 0.15 and 1.77 to 4.28 %RSD, respectively. The USP resolution between the least resolved pair of peaks was 2.0.

System Suitability Report

Sample Set ID: 13861 Result Set Id: 13952

Processed Channel Descr.: [QDa 1: SIR Ch1]+[QDa 1: SIR Ch2]+[QDa 1: SIR Ch3]+[QDa 1: SIR Ch4]+[QDa 1: SIR

Peak Results

	Name	# of Inj.	Ave RT	%RSD RT	%RSD Peak Areas	Ave USP Resolution	Ave USP Tailing
1	Myristic	6	6.918	0.15	2.33		1.0
2	Linolenic	6	7.199	0.14	1.85	2.0	1.0
3	Palmitoleic	6	7.810	0.15	1.77	4.3	1.1
4	Linoleic	6	8.831	0.16	4.28	7.0	1.0
5	Palmitic	6	9.943	0.13	3.20	7.3	1.1
6	Oleic	6	10.713	0.12	2.58	4.9	1.1
7	Stearic	6	12.774	0.12	2.35	12.8	1.1

Figure 2. Six replicate injections of fatty acids standard solution at 10 µg/mL. ACQUITY Arc System with ACQUITY QDa Mass Detector. MS SIR data.

Quality assessment was performed by measuring composition of the fatty acids released from the polysorbate 80 batches after hydrolysis. Different reaction media were investigated during the study to ensure complete extraction of all fatty acids from the test samples. These included water, water/ethanol (50:50, v/v), 1 M sodium hydroxide (NaOH), and 1 M potassium hydroxide (KOH). Sample solutions were incubated for 6 hours at 40 °C. The study showed that hydrolysis with base released more fatty acids compared to hydrolysis with water and water/ethanol media (Figure 3). Additionally, previous studies described the use of base hydrolysis in preparation of polysorbate 80 samples.[1] Therefore, all samples were hydrolyzed with 1 M KOH, neutralized with formic acid, and diluted with water/ethanol (50:50, v/v) to 0.1 mg/mL. Analysis of the polysorbate 80 samples revealed presence of unknown peaks around 9 and 11 minutes with the same *m/z* values as the linoleic (18:2) and oleic (18:1) acids of 279.2 and 281.3, respectively (Figure 4). It was concluded that the unknown peaks were positional isomers of linoleic and oleic acids. The identity of these peaks was confirmed via retention times and analysis of isomers standards (purchased from Nu-Chek Prep. Inc.) using a Xevo G2-XS QTof Mass Spectrometer coupled to a UPLC system. For separation by UPLC, the HPLC conditions were scaled to a 1.7 µm particle size column with 2.1 x 150 mm dimension.

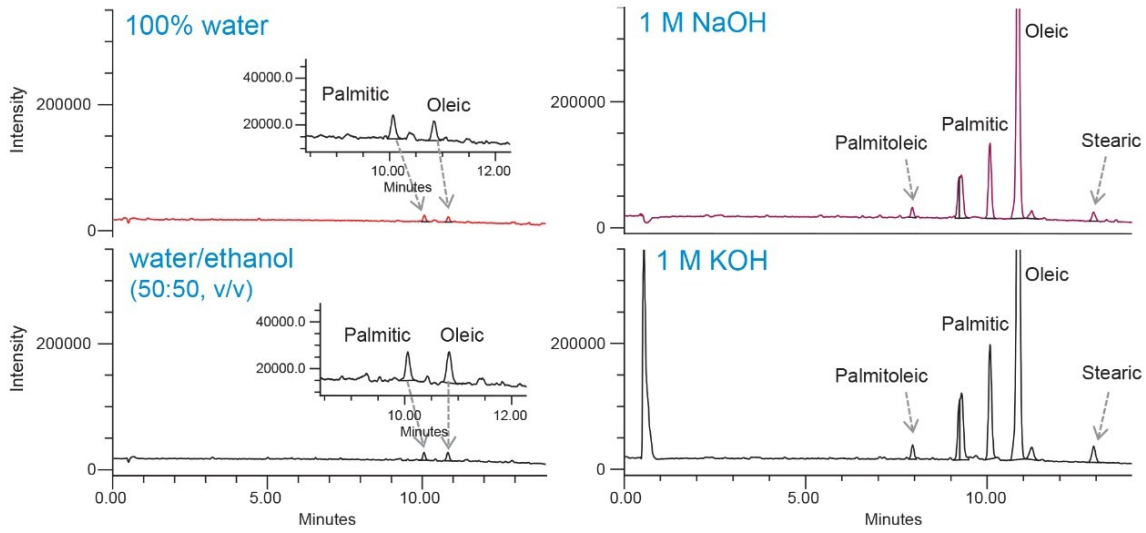


Figure 3. Hydrolysis study of polysorbate 80 in different reaction media to release fatty acids. ACQUITY Arc System with an ACQUITY QDa Mass Detector, MS SIR data.

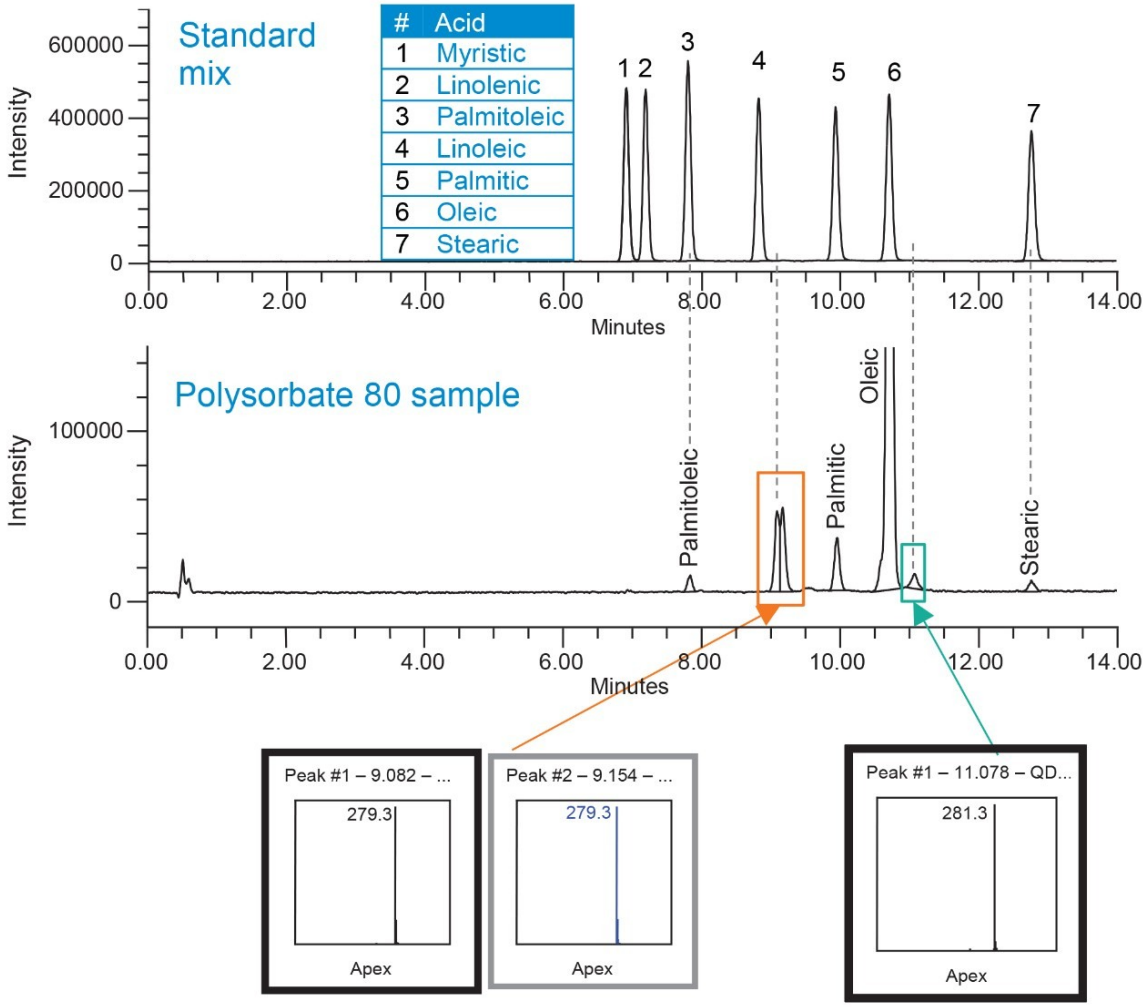
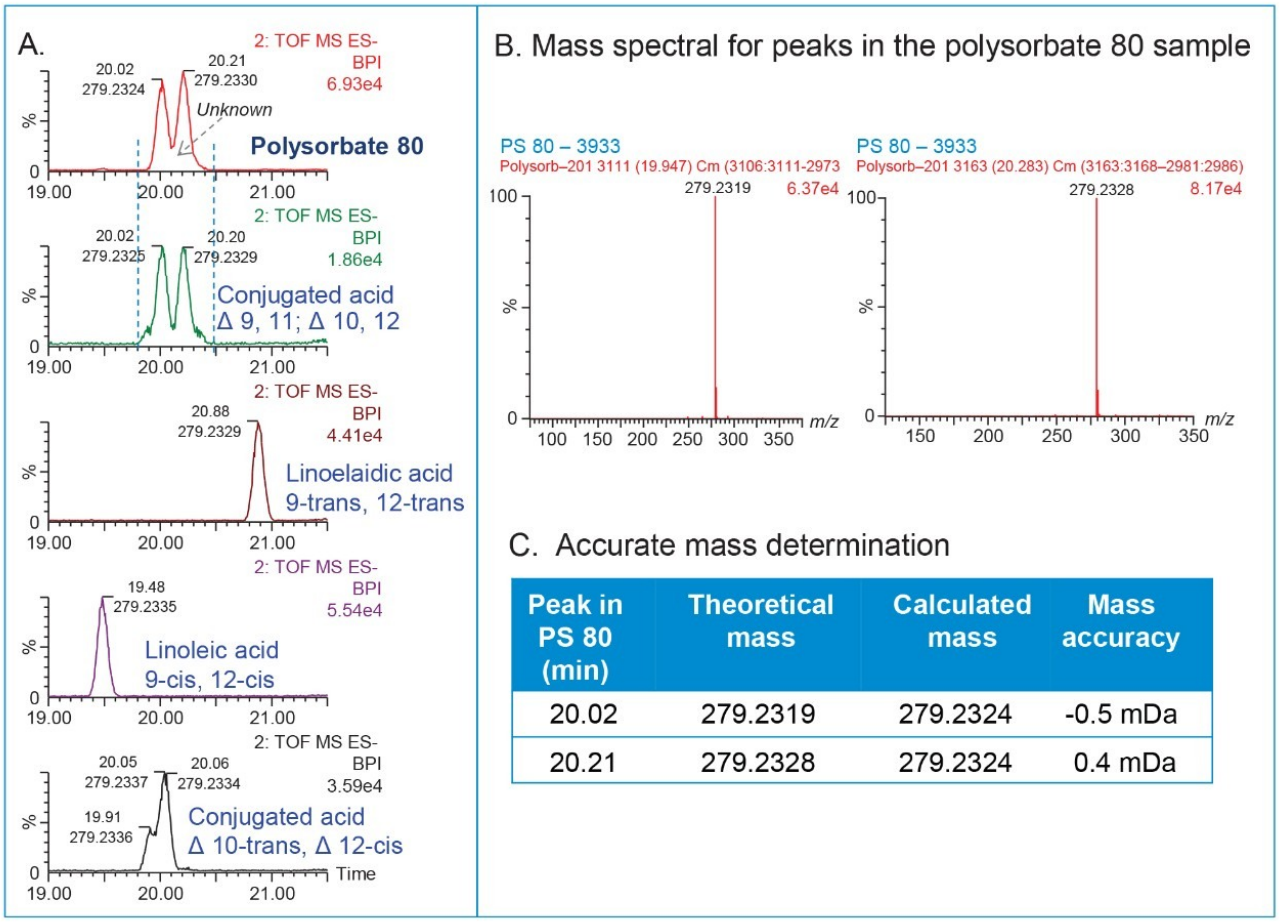


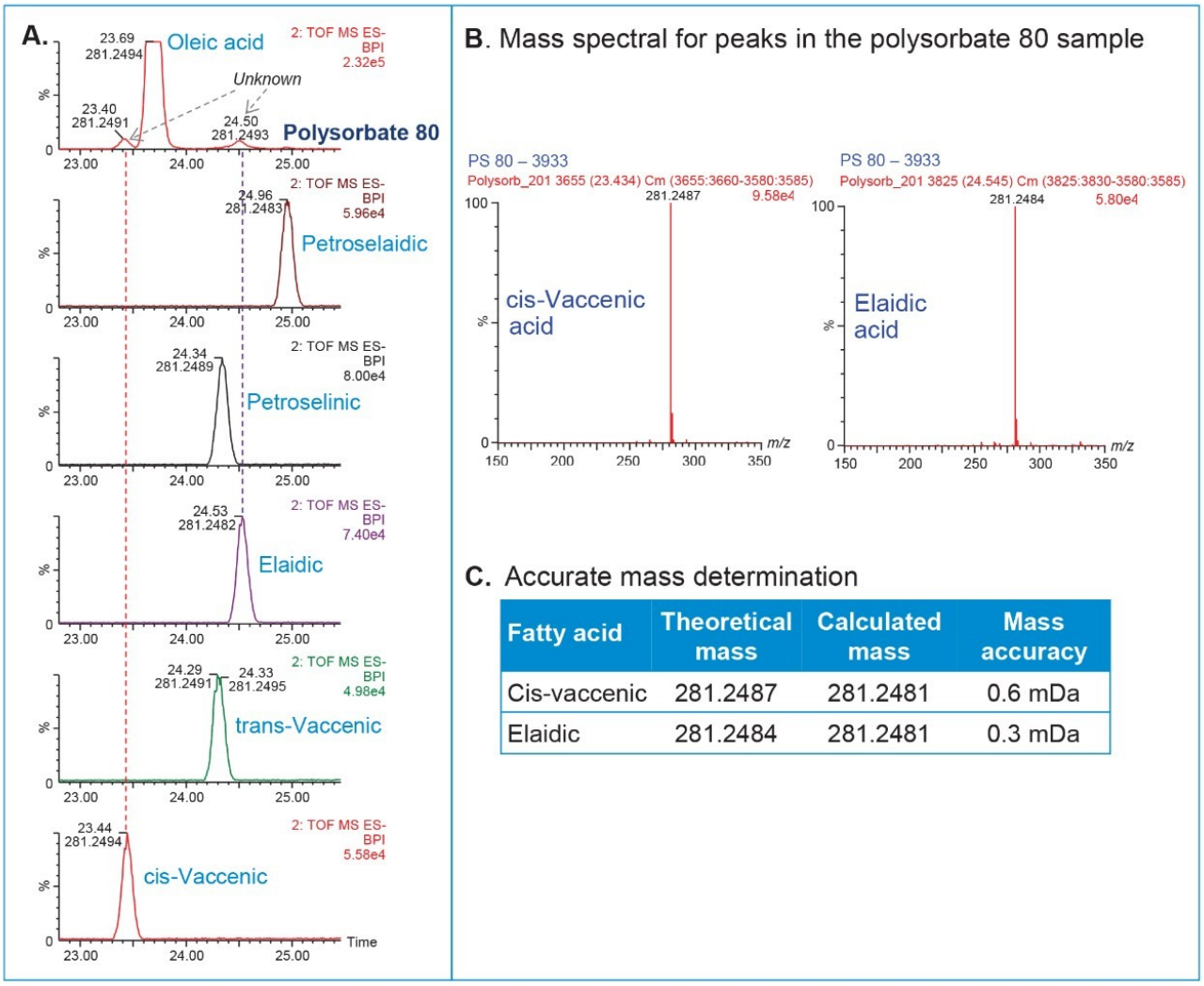
Figure 4. Polysorbate 80 sample analysis by Arc HPLC System with an ACQUITY QDa Mass Detector, MS SIR data.

Using QTof data, the unknown peak with *m/z* 279.2 was identified as a mixture of conjugated linoleic acid isomers (Δ 9, 11; Δ 10, 12). (Figure 5). Mass accuracy was found to be -0.5 and 0.4 mDa (Figure 5C), respectively. Furthermore, the analysis showed presence of two positional isomers of oleic acid, eluting before and after the oleic peak (Figure 6). These compounds were identified as cis-vaccenic and elaidic acids, with mass accuracy of 0.7 mDa and 0.8 mDa, respectively (Figure 6C).



Peak in PS 80 (min)	Theoretical mass	Calculated mass	Mass accuracy
20.02	279.2319	279.2324	-0.5 mDa
20.21	279.2328	279.2324	0.4 mDa

Figure 5. Xevo G2-XS QTof data. Isomers of linoleic acid (A), mass spectral data (B), and accurate mass determination (C) for peaks in polysorbate 80.



Fatty acid	Theoretical mass	Calculated mass	Mass accuracy
Cis-vaccenic	281.2487	281.2481	0.6 mDa
Elaidic	281.2484	281.2481	0.3 mDa

Figure 6. Xevo G2-XS QTof data. Isomers of oleic acid (A), mass spectral data (B), and accurate mass determination (C) for peaks in polysorbate 80.

Composition of the fatty acids in the polysorbate 80 batches was determined by comparing peak area of each fatty acid to the total area of all fatty acids found in the chromatographic injection, as indicated in the USP.[5] In this case, the calculations included the USP-specified fatty acids found in the test samples and isomers of linoleic and oleic acids detected by the new HPLC-MS method, all performed using Empower Software (Table 2). For all three batches tested, the amount of oleic acid ranged from 70.8 to 79.8%, meeting the USP criteria of not less than (NLT) 58.0%. Linoleic acid was found in batch one at 0.2%, while the mixture of conjugated linoleic acid isomers (Δ 9, 11; Δ 10, 12) was detected in all batches at 11.5 to 12.2% range. Overall, the USP-specified fatty acids found in all batches were within the USP criteria limits.

Acid name	SIR (*m/z*)	% Acid batch 1	% Acid batch 2	% Acid batch 3	*USP criteria**
Myristic	227.3	0.1	0.5	Not detected	*NMT 5.0%*
Linolenic	277.3	Not detected	Not detected	Not detected	*NMT 4.0%*
Palmitoleic	253.3	1.2	1.1	1.0	*NMT 8.0%*
Linoleic	279.3	0.2	Not detected	Not detected	*NMT 18.0%*
Conjugated Δ 9, 11; Δ 10, 12	279.3	11.5	12.2	11.6	*N/A*
Palmitic	255.3	11.4	4.2	4.3	*NMT 16.0%*
Cis-vaccenic	281.2	1.1	Not detected	Not detected	*N/A*
Oleic	281.2	70.6	79.2	79.8	*NLT 58.0%*
Elaidic	281.2	1.9	1.3	2.0	*N/A*
Stearic	283.3	2.0	1.7	1.1	*NMT 6.0%*

*Table 2. Determination of fatty acids composition (% acid) in polysorbate 80 batches using an ACQUITY Arc System with ACQUITY QDa Mass Detector. NMT: not more than, NLT: not less than. * USP monograph for polysorbate 80.*[5]

Conclusion

The developed HPLC-MS method offers fast determination of fatty acids composition in hydrolyzed polysorbate 80, eliminating the need for a complex sample pretreatment procedure required for analysis by GC. Additionally, the new method separates additional fatty acids that are not specified in the GC-FID procedure listed in the current USP monograph for polysorbate 80 (USP–NF 2021 Issue 1). These fatty acids were identified as isomers of linoleic and oleic acids using data from the QTof Mass Spectrometer.

Overall, the Arc HPLC System coupled with an ACQUITY QDa Mass Detector provides accurate and reliable results, making this technology suitable for routine testing of pharmaceutical raw materials in the QC laboratory.

References

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