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Quality assessment of Polysorbates 80 and 20 Pharmaceutical Raw Materials by Measuring Fatty Acids Composition using HPLC with Mass Detection

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PURPOSE

Polysorbates are non-ionic surfactants widely used as excipients or inactive ingredients in many pharmaceutical products[1,2]. To assure safety of the finished drug products, the quality and purity of excipients must be assessed using suitable and reliable test methods. A gas chromatography (GC) with flame ionization detector (FID) procedures for the polysorbate 80 and 20 based on the fatty acids composition are recommended[3,4]. These procedures require hydrolysis and derivatization of the polysorbates to free fatty acids.

In this work, simple and fast HPLC-mass spectrometry (MS) methods were developed for the determination of fatty acids composition in the polysorbates 80 and 20 by direct analysis of the hydrolyzed samples.

METHODS

Standard solutions preparation

Individual fatty acid standard solutions were prepared in ethanol at 1 mg/mL. The stock standard solutions were diluted with water/ethanol diluent (50:50, v/v) to make two separate standard mixtures containing free fatty acids specified by the USP in polysorbates 80 and 80 monographs[3,4], respectively.

Sample solutions preparation

Polysorbates 80 and 20 test samples were hydrolyzed with 1 M potassium hydroxide solution by incubation at 6 hours at 40°C, neutralized with equal volume of 1 M formic acid, and diluted with water/ethanol (50:50, v/v) to 0.1 mg/mL.

HPLC method

- Chromatographic separation was performed using XBridge™ BEH™ C_{18} (4.6 x 100 mm, 3.5 µm) column, operated at 60°C, run on Arc™ HPLC System. The mobile phase consisting of 10 mM ammonium acetate in water (A) and acetonitrile solvent (B) was delivered under gradient elution with a flow rate of 2 mL/min. Isopropyl alcohol (C) was used to wash the system between injections.

Gradient for polysorbate 80 analysis

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

Gradient for polysorbate 20 analysis

Time (min)	%A	%B	Curve
Initial	95.0	5.0	6
1.00	95.0	5.0	6
1.10	60.0	40.0	6
14.00	5.0	95.0	6
16.00	5.0	95.0	6
16.10	95.0	5.0	6
20.00	95.0	5.0	6

MS Detection

- ACQUITY™ QDa™ Detector
- Isocratic solvent manager (ISM) make-up (dilution) solvent was added post-column and mixed with the flow entering the source to enhance the MS signal.

Ionization mode: Electrospray negative (ESI-)
MS Acquisition: range: 75 – 350 m/z, Single Ion Recording (SIR) for quantitation
Probe temp.: 600°C; Capillary Voltage: 0.5 kV, Cone Voltage: 10 V

ISM makeup solvent: 50:50 water/acetonitrile with 1 mM ammonium acetate
Flow rate: 0.2 mL/min, with 10:1 split and dilute ratio

RESULTS

Fatty acids in polysorbates

The United States Pharmacopeia (USP) procedure for analysis of polysorbate 80 and 20 is based on the fatty acids composition using GC-FID instrumentation[3,4].

Fatty acids in polysorbate 80 according to the USP monograph[3]

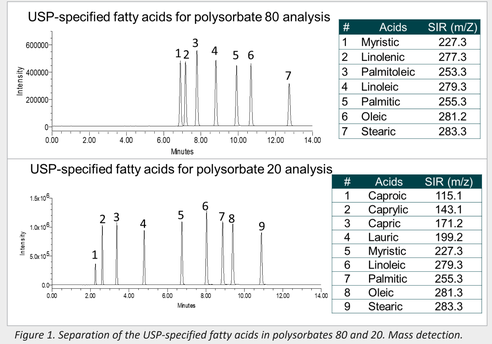
Acid	C:D *	Monoisotopic mass (Da)
Myristic	14:0	228.21
Palmitic	16:0	256.24
Palmitoleic	16:1	254.22
Stearic	18:0	284.27
Oleic	18:1	282.26
Linoleic	18:2	280.24
Linolenic	18:3	278.22

Fatty acids in polysorbate 20 according to the USP monograph[4]

Acid	C:D *	Monoisotopic mass (Da)
Caproic	6:0	116.08
Caprylic	8:0	144.11
Capric	10:0	172.14
Lauric	12:0	200.11
Myristic	14:0	228.21
Palmitic	16:0	256.24
Stearic	18:0	284.27
Oleic	18:1	282.26
Linoleic	18:2	280.24

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

Herein, the HPLC-MS method developed in this work successfully separated all the USP-specified fatty acids for polysorbates 80 and 20.



#	Acids	SIR (m/Z)
1	Myristic	227.3
2	Linolenic	277.3
3	Palmitoleic	253.3
4	Linoleic	279.3
5	Palmitic	255.3
6	Oleic	281.2
7	Stearic	283.3

#	Acids	SIR (m/z)
1	Caproic	115.1
2	Caprylic	143.1
3	Capric	171.2
4	Lauric	199.2
5	Myristic	227.3
6	Linoleic	279.3
7	Palmitic	255.3
8	Oleic	281.3
9	Stearic	283.3

Figure 1. Separation of the USP-specified fatty acids in polysorbates 80 and 20. Mass detection.

Sample preparation study

Different reaction media were investigated during the study to ensure complete extraction of all fatty acids from the polysorbate test samples. Hydrolysis with base released most free fatty acids. The 1 M KOH media was chosen for preparation of all samples tested in this work.

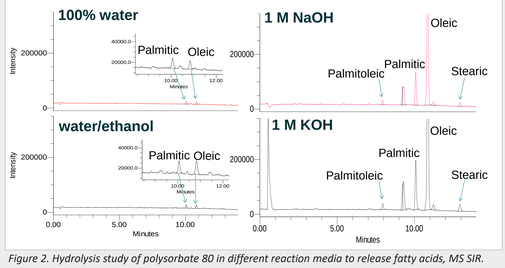


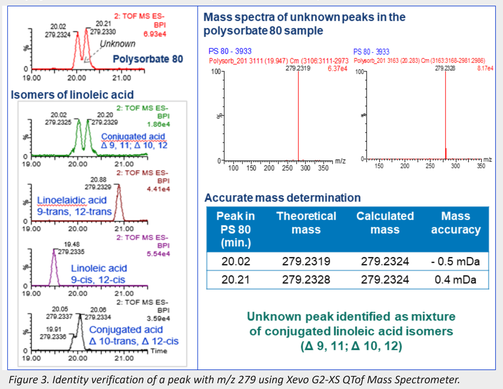
Figure 2. Hydrolysis study of polysorbate 80 in different reaction media to release fatty acids, MS SIR.

Analysis of polysorbate test samples

Identification of unknown peaks

Analysis of the polysorbate 80 sample (batch 1) revealed presence of unknown peaks with the same *m/z* values as the linoleic (18:2) and oleic (18:1) acids of 279.2 and 281.3, respectively. Identity of the unknown peaks was verified via retention times and accurate mass determination by comparison with the reference isomers standards (purchased from Nu-Chek Prep. Inc.). The analysis was performed using a Xevo™ G2-XS QTof Mass Spectrometer coupled to a UPLC™ system. For UPLC separation, the HPLC conditions were scaled to a 1.7 µm particle size column with 2.1 x 150 mm dimension.

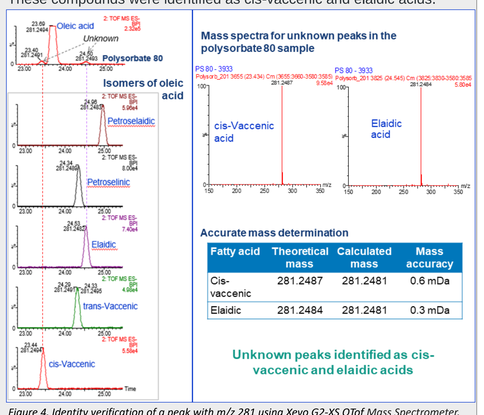
The unknown peak with *m/z* 279 was identified as a mixture of conjugated linoleic acid isomers (Δ 9, 11; Δ 10, 12).



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 3. Identity verification of a peak with m/z 279 using Xevo G2-XS QTof Mass Spectrometer.

For peak with m/z 281, the analysis showed presence of two positional isomers of oleic acid, eluting before and after the oleic peak. These compounds were identified as cis-vaccenic and elaidic acids.



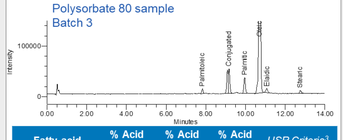
Fatty acid	Theoretical mass	Calculated mass	Mass accuracy
Cis-vaccenic	281.2487	281.2481	0.6 mDa
Elaidic	281.2484	281.2481	0.3 mDa

Figure 4. Identity verification of a peak with m/z 281 using Xevo G2-XS QTof Mass Spectrometer.

Determination of fatty acids composition

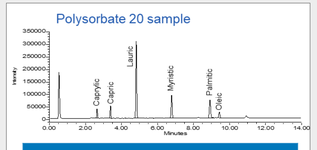
Composition or the percent (%) of each fatty acids in the polysorbate test samples was determined by comparing peak area of each fatty acid to the total area of all fatty acids found in the chromatographic injection. Calculations performed using Empower™ Software following the USP monographs[3,4].

For polysorbate 80 analysis, calculations included the USP-specified fatty acids found in the test samples and the additional isomers detected by the new HPLC-MS method. Results met the USP criteria limits for the specified fatty acids.



Fatty acid	% Acid Batch 1	% Acid Batch 2	% Acid Batch 3	USP Criteria[3]
Myristic	0.1	0.5	ND	*NMT 5.0%*
Linolenic	ND	ND	ND	*NMT 4.0%*
Palmitoleic	1.2	1.1	1.0	*NMT 8.0%*
Linoleic	0.2	ND	ND	*NMT 18.0%*
Conjugated Δ 9, 11; Δ 10, 12	11.5	12.2	11.6	*N/A*
Palmitic	11.4	4.2	4.3	*NMT 16.0%*
Cis-vaccenic	1.1	ND	ND	*N/A*
Oleic	**70.6**	**79.2**	**79.8**	***NLT 58.0%***
Elaidic	1.9	1.3	2.0	*N/A*
Stearic	2.0	1.7	1.1	*NMT 6.0%*

Composition of fatty acids in the polysorbate 20 sample solutions met the USP criteria.



Fatty Acid	% Acid	USP Criteria[4]
Caproic	Not detected	*≤ 1.0*
Caprylic	5.2	*≤ 10.0*
Capric	7.0	*≤ 10.0*
Lauric	51.7	*40-60*
Myristic	18.1	*14-25*
Palmitic	12.7	*7-15*
Stearic	Not detected	*≤ 11*
Oleic	5.3	*≤ 11*
Linoleic	Note detected	*≤ 3*

CONCLUSION(S)

- The developed HPLC-MS method offers fast quality assessment of the polysorbates 80 and 20 pharmaceutical raw materials by measuring fatty acids composition in hydrolyzed samples
  - Direct injection of hydrolyzed samples eliminates the need for a complex sample pretreatment procedure required for analysis by GC.
  - Easy and accurate identification of fatty acids by mass detection using mass spectral data from an ACQUITY QDa Detector.
  - Integrated with a compliant-ready Empower Software, suitable for routine QC testing
- HPLC-MS method separates additional fatty acids not listed in the GC-FID procedure for polysorbate 80 recommended by the USP (USP–NF 2021 Issue 1).
- The QTof mass spectrometer enables accurate identity verification of unknown peaks.

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