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アプリケーションノート

Performance Verification of a Proposed USP Monograph for Sorbitan Monooleate Using a Gel Permeation Chromatography (GPC) Method With Refractive Index (RI) Detection

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Abstract

The United States Pharmacopeia (USP) proposed revisions to the monograph for sorbitan monooleate to update the procedure for assay and add a limit for organic impurities test, employing a GPC method (USP-PF 50(2)). In this work, the proposed GPC method was run on an Arc™ HPLC System with a strong solvent compatibility kit and RI detector. The GPC results met the USP system suitability requirements and acceptance criteria for both the assay and organic impurities analysis in sorbitan monoleate.

Benefits

- Excellent performance of a proposed USP monograph for sorbitan monooleate by meeting the requirements for the assay and limit of organic impurities analysis
- Reliable GPC analysis using the Arc HPLC System with a strong solvent compatibility kit and RI detector

Introduction

Sorbitan monooleate, also known as Span 80, is a nonionic surfactant used as a stabilizer and emulsifier in cosmetic, pharmaceutical, and food products.[1] It helps to stabilize formulations by creating a stable mixture between immiscible ingredients, such as oil and water. Additionally, sorbitan monooleate increases stability of food products and helps to elevate their texture and consistency.[1]

The USP is updating monographs for Chemical Medicines and excipients across the compendia with new methodologies and technologies. As part of the modernization efforts, the USP proposed revisions to the monograph for sorbitan monooleate.[2] The proposed USP monograph updates an assay procedure with a GPC method designed for analysis of sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoesters. Additionally, the USP proposes addition of a limit test for organic impurities utilizing the same GPC method conditions as that used for the assay.

In this work, the proposed USP monograph for sorbitan monooleate was run on a GPC system composed of an Arc HPLC System with a strong solvent compatibility kit and RI detector. The experimental work, chromatographic separation, and calculations were performed according to the proposed USP monograph assay and limit of organic impurities procedures. The success of the analysis was measured by comparing GPC results against the USP requirements and acceptance criteria for assay and organic impurities analysis. Empower™ Software was used for data acquisition and analysis.

Experimental

Solutions preparation and experimental conditions proceeded as described in the proposed USP monograph for sorbitan monooleate.[2]

Materials

Tetrahydrofuran (THF) HPLC grade, no preservatives, purchased from Fisher Chemicals, Catalog No.: T425–4. Isopropyl alcohol (IPA) purchased from Honeywell, catalog number LC323–4. Sorbitan monooleate purchased form Sigma-Aldrich.

Sample Description

Standard Solutions

Standard solution for the assay was prepared by dissolving each of oleic acid, 1,4-sorbitan, and isosorbide in THF at 1.0 mg/mL. For organic impurities, preparation of the standard solution proceeded as described for the

assay.

Sample Solutions

Sample solution for the assay testing was prepared by dissolving sorbitan monooleate in THF at 1.0 mg/mL. For organic impurities, preparation of the sample solution proceeded as described for the assay.

Method Conditions

System:	Arc HPLC System with quaternary solvent manager (QSM), flow through needle (FTN) sample manager, and strong solvent compatibility kit (p/n: 205002572). Column heater/cooler (p/n: 186179100)
Detector:	Refractive Index (RI) · Flow cell temperature: 30 °C · Sampling rate: 10 pts/sec · Polarity: positive
Mobile phase:	Tetrahydrofuran
Separation:	Isocratic
Columns:	Columns with 7.8 x 300 mm with 5 µm, connected in series using a joining tube (p/n: WAT084080) supplied with columns. 1. Styragel™ HR 1, 100 Å, molecular weight range: 100–5,000 (p/n: WAT044234) 2. Styragel HR 0.5, 50 Å, molecular weight range: 0–1,000 (p/n: WAT044231)
Column temperature:	30 °C
Sample temperature:	25 °C

Flow rate:	0.9 mL/min
Injection volume:	20 µL
Run time:	30 minutes
Vials:	LCMS Maximum Recovery 2 mL volume (p/n: 600000670CV)
Wash solvents:	Sample manager/purge wash: tetrahydrofuran Seal wash: isopropyl alcohol

The assay and organic impurities procedures operated under the same chromatographic conditions.

Data Management

Chromatography software:	Empower 3 Feature Release 5 Service Release 5 (FR5 SR3) for data acquisition and analysis.

Results and Discussion

The proposed USP monograph for sorbitan monooleate describes a revised assay procedure and adds a limit for organic impurities testing, both utilizing a GPC method.[2] The procedure for organic impurities employs the same standard solution, sample solution and chromatographic conditions as that for the assay. Waters Styragel HR 0.5 and HR 1 columns are recommended for the GPC separation with THF as a mobile phase.

In this work, the GPC analysis was performed following the updated procedures in proposed USP monograph for sorbitan monooleate. Columns were connected in series with the larger pore size column first to reduce back pressure. Calculations were performed using Empower Software.

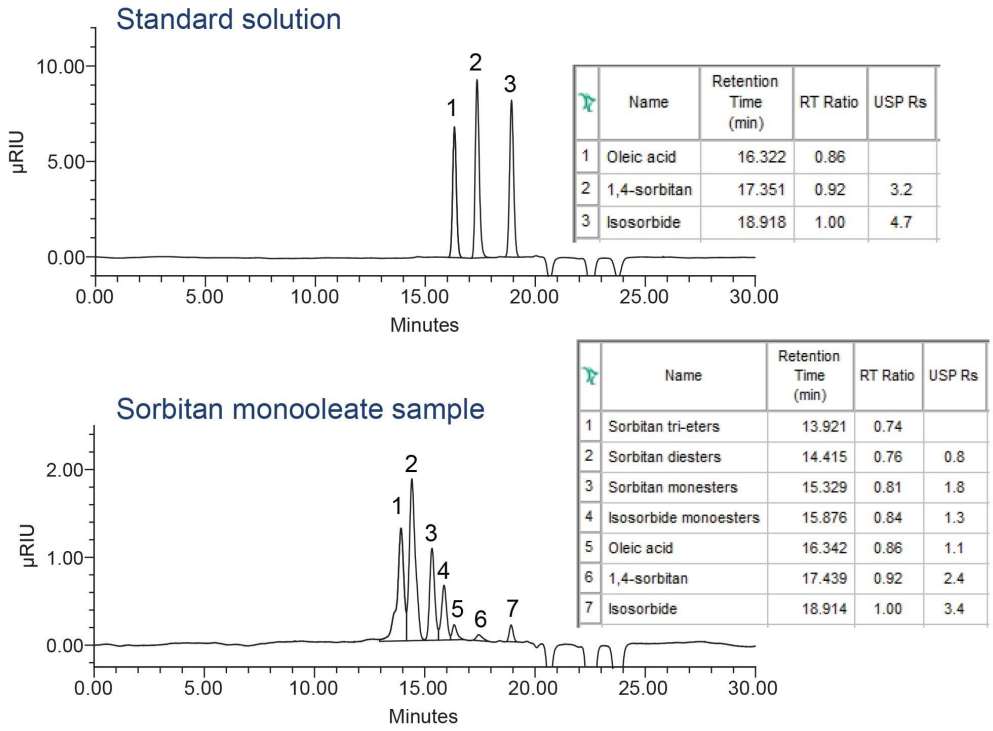
Peak Assignment

The USP lists relative retention times (RRT) to aid in peak assignment and identification of components for the

assay and organic impurities testing (Table 1). These values were used to identify peaks in the chromatographic separation of standard and sample solutions (Figure 1).

Procedure	Peak	RRT
Assay	Sorbitan tri-/higher esters	0.73
	Sorbitan diesters	0.75
	Sorbitan monoesters	0.80
	Oleic acid	0.86
	1,4-Sorbitan	0.91
	Isosorbide	1.0
Organic impurities	Isosorbide monoesters	0.83
	Fatty acid (oleic acid)	0.86
	1,4-Sorbitan	0.91
	Isosorbide	1.0

Table 1. Relative retention time (RRT) to aid in peak assignment for assay and limit of organic impurities testing according to the proposed USP monograph for sorbitan monooleate.[2]



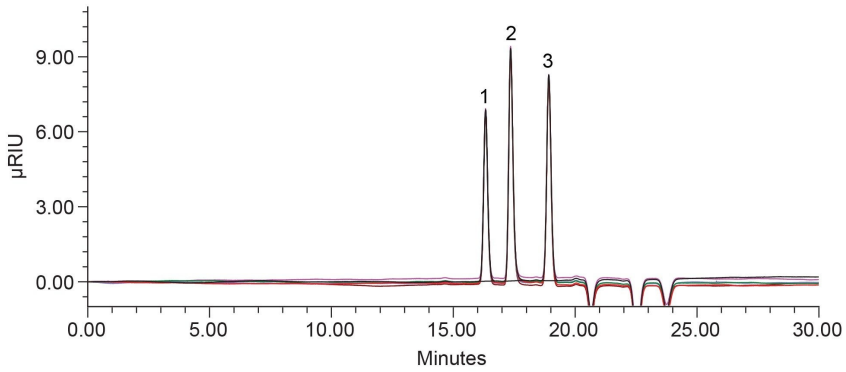
	Name	Retention Time (min)	RT Ratio	USP Rs
1	Oleic acid	16.322	0.86	
2	1,4-sorbitan	17.351	0.92	3.2
3	Isosorbide	18.918	1.00	4.7

	Name	Retention Time (min)	RT Ratio	USP Rs
1	Sorbitan tri-eters	13.921	0.74	
2	Sorbitan diesters	14.415	0.76	0.8
3	Sorbitan monesters	15.329	0.81	1.8
4	Isosorbide monoesters	15.876	0.84	1.3
5	Oleic acid	16.342	0.86	1.1
6	1,4-sorbitan	17.439	0.92	2.4
7	Isosorbide	18.914	1.00	3.4

Figure 1. GPC separation of the standard solution and sorbitan monooleate sample using an Arc HPLC System with a strong solvent compatibility kit and RI detector. Empower RT ratio: relative retention time (RRT).

System Suitability

System suitability was measured using the standard and sample solutions as indicated in the proposed USP monograph for sorbitan monooleate.[2] The results for six replicate injections of the standards solution, showed excellent relative standard deviations (RSD) for peak areas and retention times of ⩽ 0.68% and ⩽ 0.01%, respectively (Figure 2). A summary of the USP system suitability requirements and results generated by the GPC method for assay and limit of organic impurities are shown in Table 2. The GPC method run on an Arc HPLC System with strong solvent compatibility kit met all the USP acceptance criteria for resolution and RSD of six replicate injections of standard solution.



System Suitability
Sample Set ID: 5340 Result Set Id: 5450
Processed Channel Descr.: W2414 RI

Peak Results

	Name	Inj. #	Ave_RT	% RSD RT	%RSD PeakAreas	Ave USP Resolution	Ave USP Tailing
1	Oleic acid	6	16.323	0.01	0.58		1.1
2	1,4-sorbitan	6	17.351	0.01	0.38	3.2	1.2
3	Isosorbide	6	18.918	0.01	0.68	4.7	1.1

Figure 2. Results for six replicate injections of standard solution. RT: retention time; RSD: relative standard deviation.

Procedure	Parameter	USP requirement[2]	GPC results
Assay	Resolution: between the sorbitan diesters and sorbitan monoesters peaks (sample solution)	Not less than (NLT) 1.0	1.8
Assay and organic impurities	Relative standard deviation: for the oleic acid, 1,4-sorbitan, and isosorbide peaks (six replicate injections of standard solution)	≤5.0%	▪ Oleic acid peak: –RSD of areas: 0.58% ; RSD of RT: 0.01% ▪ 1,4-sorbitan peak: –RSD of areas: 0.38%; RSD of RT: 0.01% ▪ Isosorbide peak: –RSD of areas: 0.68%; RSD of RT: 0.01%
Organic impurities	Resolution: between the 1,4-sorbitan and isosorbide peaks (standard solution)	NLT 1.5	4.7

Table 2. System suitability for assay and limit of organic impurities in sorbitan monooleate. USP requirements and results generated by the GPC method.[2]

Assay: Analysis of Sorbitan Tri-/Higher Esters, Sorbitan Diesters, and Sorbitan Monoesters

The percentage (%) of each sorbitan ester component in the sorbitan monooleate sample was calculated by area normalization as instructed by the USP.[2] The peak area of individual peak was divided by the sum of the relevant peak areas and multiplied by 100. The results generated by the GPC method for the sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoesters met the USP acceptance criteria ranges (Table 3).

Sorbitan ester	USP acceptance criteria: range (%)[2]	GPC results (%)
Sorbitan tri-/higher esters	25.0–40.0	27.4
Sorbitan diesters	30.0–40.0	37.6
Sorbitan monoesters	15.0–20.0	17.6

Table 3. GPC results for the assay of sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoesters in sorbitan monooleate sample (n=6).

Limit of Organic Impurities

The percentage of each impurity peak in the sorbitan monooleate sample was determined by comparing the area of each peak to the sum of the relevant peaks. The GPC results were within the USP limits for organic impurities content (Table 4).

Impurity	USP acceptance criteria: limit, NMT (%)	GPC results (%)
Isosorbide monoesters	15.0	10.6
oleic acid	5.0	2.7
1,4-Sorbitan	2.5	1.8
Isosorbide	3.0	2.4

Table 4. GPC results for limit of organic impurities in sorbitan monooleate sample (n=6). NMT: not more than.

Conclusion

The GPC method described in the proposed USP monograph for sorbitan monooleate was successfully run on

the Arc HPLC System with a strong solvent compatibility kit and refractive index detector, meeting all the requirements for system suitability, assay, and limit of organic impurities testing. The GPC method demonstrated excellent relative standard deviations (RSD) for peak areas and retention times of ⩽0.68% and ⩽0.01%, well below the required acceptance criteria of 5.0%. The USP criteria for resolution were also met.

References

1. https://www.cnchemsino.com/blog/sorbitan-monooleate-span-80-properties-and-uses.html < https://www.cnchemsino.com/blog/sorbitan-monooleate-span-80-properties-and-uses.html> .
2. United States Pharmacopeia (USP) proposed revision to monograph for sorbitan monoleate, publication PF 50(2). https://doi.usp.org/USPNF/USPNF_M77590_30101_01.html < https://doi.usp.org/USPNF/USPNF_M77590_30101_01.html> .

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