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Application Note

Demonstrating Compliance of a Gel Permeation Chromatography (GPC) Method With the Requirements in a Proposed USP Monograph for Sorbitan Sesquioleate

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Abstract

The United States Pharmacopeia (USP) proposed changes to the monograph for sorbitan sesquioleate to udpate assay procedure and add a limit test for organic impurities (USP-PF 50(2)). Both test procedures utilize a GPC method with a refractive index (RI) detector. In this work, the proposed GPC method was run on an Arc™ HPLC System with a strong solvent compatibility kit. The results met all the system suitability specifications and acceptance criteria for both the assay and organic impurities analysis in sorbitan sesquioleate, demonstrating compliance with the USP requirements.

Benefits

- Successful implementation of a proposed USP monograph for sorbitan sesquioleate by demonstrating compliance with all the requirements for system suitability and acceptance criteria for the assay and organic impurities testing
- Reliable GPC analysis using the Arc HPLC System with a strong solvent compatibility kit and RI detector

Introduction

The USP is modernizing monographs for drug substance, drug products, and excipients across the compedia to take advantage of new metholodogies and technogies.[1] As part of the initiative, USP proposed updates to the monograph for sorbitan sesquioleate.[2] For assay, USP proposed a GPC method for analysis of sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoester to replace current tests for fatty acids and polyols testing. For impurities, a limit of organic impurities test is added, utilizing similar method as for the assay.

Sorbitan sesquioleate is a non-ionic surfactant and emulsifier used as excipients in cosmetic, food, veterinary products, household items, pharmaceutical ointments, and creams.[3–4] It is primarily used to create stable oil-in-water emulsions and improve the texture and appearance of products. As a surfactant, it reduces surface tension and facilitates the spread of liquids. Sorbitan sesquioleate is synthesized by the esterification reaction of sorbitol and oleic acid.[4]

Herein, the GPC method described for the assay and organic impurities in the proposed USP monograph for sorbitan sesquioleate was run an Arc HPLC System with a strong solvent compatibility kit and RI detector. [2] The results generated by the method were compared against the USP requirements for system suitability and acceptance criteria for assay and limit of organic impurities. The GPC analysis and implementation of a proposed USP monograph for sorbitan monooleate is shown in a previously published Waters™ Application Note.[5]

Experimental

Solutions preparation and experimental conditions proceeded as described in the proposed USP monograph for sorbitan sesquioleate.

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 sesquioleate purchased form Sigma-Aldrich.

Sample Description

Standard Solutions

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

Sample Solutions

Sample solution for assay testing prepared by dissolving sorbitan sesquioleate in tetrahydrofuran at 1.0 mg/mL. For limit of organic impurities, proceeded as described in 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, starting with larger pore size 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

Assay and limit of organic impurities procedures operated under the same chromatographic conditions.

Data Mangement

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

Results and Discussion

The procedures for assay and a limit test of organic impurities described in the proposed USP monograph for sorbitan sesquioleate operate under the same GPC method conditions using the Waters Styragel HR 0.5 and HR

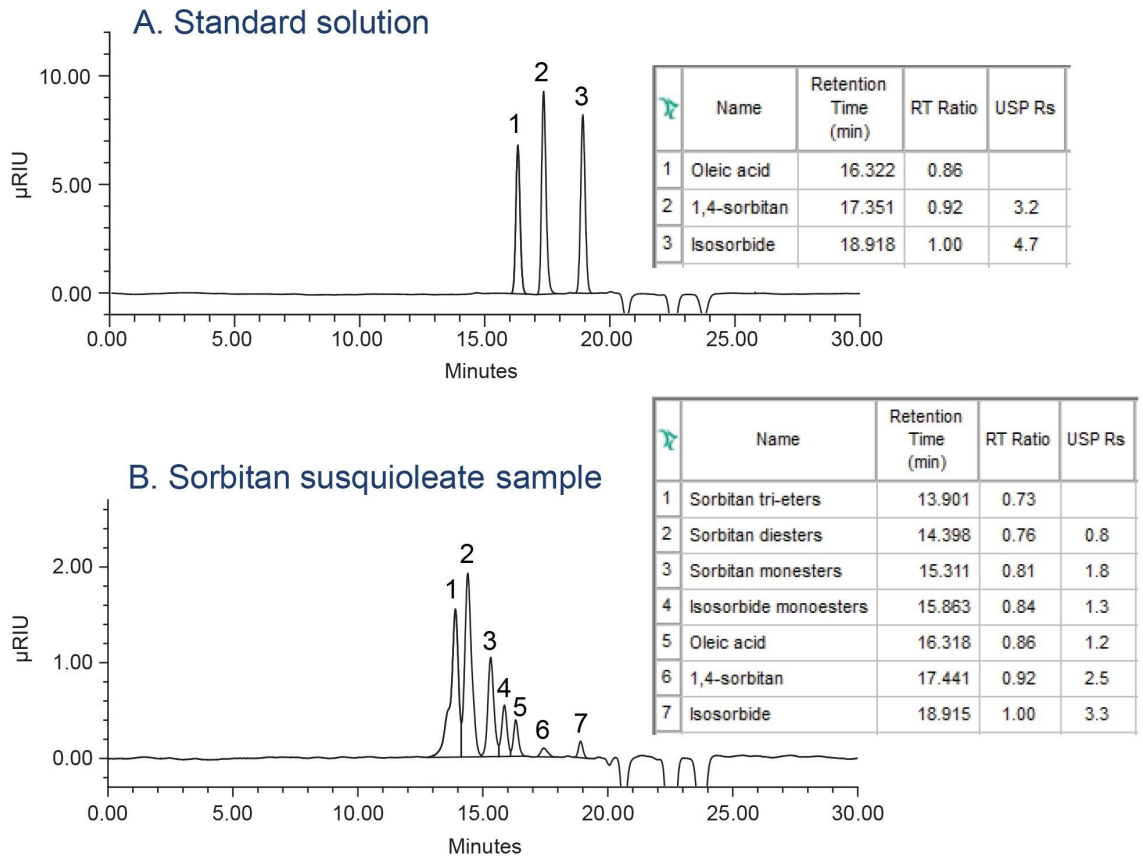
1 columns.[2] Additionally, both procedures follow the same preparation scheme for standard and sample solutions.

Peak Assignment

The relative retention times (RRT) provided in the proposed USP monograph are designed to aid peak assignment for the assay and organic impurities testing (Table 1). Therefore, identification of peaks in the chromatographic analysis of standard and sample solutions on an Arc HPLC System with the strong solvent compatibility kit was performed following the USP specification (Figure 1).

Procedure	Peak	RRT
Assay	Sorbitan tri-/higher esters	0.73
	Sorbitan diesters	0.76
	Sorbitan monoesters	0.81
	Oleic acid	0.86
	1,4-Sorbitan	0.91
	Isosorbide	1.0
Organic impurities	Isosorbide monoesters	0.84
	Fatty acid (oleic acid)	0.86
	1,4-Sorbitan	0.92
	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 sesquioleate.[2]



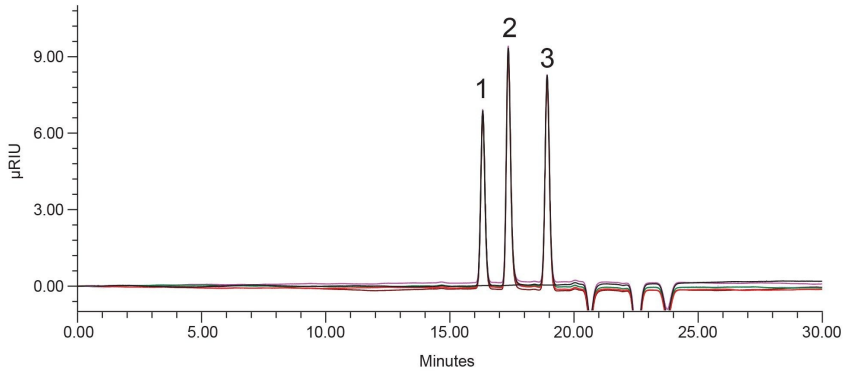
	Name	Retention Time (min)	RT Ratio	USP Rs
1	Oleic acid	16.322	0.86	
2	1,4-sorbitan	17.351	0.92	3.2
3	Isosorbide	18.918	1.00	4.7

	Name	Retention Time (min)	RT Ratio	USP Rs
1	Sorbitan tri-eters	13.901	0.73	
2	Sorbitan diesters	14.398	0.76	0.8
3	Sorbitan monesters	15.311	0.81	1.8
4	Isosorbide monoesters	15.863	0.84	1.3
5	Oleic acid	16.318	0.86	1.2
6	1,4-sorbitan	17.441	0.92	2.5
7	Isosorbide	18.915	1.00	3.3

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

System Suitability

System suitability determination performed as instructed in the proposed USP monograph for sorbitan sesquioleate, under assay and limit of organic impurities.[2] Both standard and sample solutions were used for assay procedure, while standard solution for a limit testing of organic impurities. Six replicate injections of standards solutions showed excellent relative standard deviations (RSD) for peak areas and retention times of ≤0.68 % and ≤0.01 %, respectively (Figure 2). Overall, the system suitability results generated by the GPC method met all the USP requirements specified for the assay and limit of organic impurities (Table 2).



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	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 organic impurities of sorbitan sesquioleate. 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 sesquioleate sample was calculated by area normalization as instructed by the USP.[2] 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).

Peak	USP acceptance criteria: range (%)[2]	GPC results (%)
Sorbitan tri-/higher esters	25.0–40.0	**32.9**
Sorbitan diesters	30.0–40.0	**34.9**
Sorbitan monoesters	15.0–20.0	**16.2**

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

Limit of Organic Impurities

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

Impurity	USP acceptance criteria: limit, NMT (%)[2]	GPC results (%)
Isosorbide monoesters	15.0	**7.6**
Oleic acid	5.0	**4.9**
1,4-Sorbitan	2.5	**1.6**
Isosorbide	3.0	**1.9**

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

Conclusion

The GPC method described in the proposed USP monograph for sorbitan sesquioleate demonstrated excellent performance on the Arc HPLC System with a strong solvent compatibility kit and refractive index detector. The USP requirements for system suitability, and acceptance criteria for assay and limit of organic impurities testing were met. The GPC system delivered reliable and reproducible results, which is critical to assure compliance with the USP requirements.

References

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5. Maziarz M. Performance Verification of a Proposed USP Monograph for Sorbitan Monooleate Using a Gel Permeation Chromatography (GPC) Method With Refractive Index (RI) Detection. Waters Application Note. 720008502. September 2024.

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