

Verifying Performance and Compliance of a Gel Permeation Chromatography (GPC) Method With the Requirements in a Proposed USP Monograph for Sorbitan Monostearate

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Abstract

The proposed United States Pharmacopeia (USP) monograph for sorbitan monostearate utilizes a GPC method with refractive index (RI) detector for the assay and a limit test of organic impurities (USP-PF 50(2)). This application note demonstrates performance and implementation of a proposed USP monograph for sorbitan monostearate on an Arc HPLC™ System with a strong solvent compatibility kit and RI detector. Results generated by the method met all the USP requirements for system suitability and acceptance criteria for the assay and organic impurities testing in sorbitan monostearate.

Benefits

- Successful performance verification of a proposed USP monograph for sorbitan monostearate by demonstrating compliance with all the requirements

- Excellent GPC results generated on the Arc HPLC System with a strong solvent compatibility kit and RI detector, meeting the USP specifications for system suitability and acceptance criteria for the assay and organic impurities testing

Introduction

Sorbitan monostearate (Span 60), is a non-ionic surfactant with emulsifying, dispersing, and wetting properties used in the production of food and health care products.¹⁻² It is primarily used as an emulsifier to prevent ingredients from separating in many food products such as margarine, chocolate cream, and similar dairy products.² In yeast manufacturing, it protects the yeast from excess drying and also helps rehydrate the yeast cells.¹ Sorbitan monostearate is a synthetic ester made from sorbitol and stearic acid.

The USP has launched an initiative to update and improve its outdated monographs for drug substances, drug products and excipients published in the United States Pharmacopeia—National Formulary (USP–NF) compendia.³⁻⁴ As part of the modernization effort, the USP proposed revisions to the monograph for sorbitan monostearate to update assay procedure and add a limit of organic impurities testing.⁵ The assay procedure is designed to replace current tests for fatty acids and polyols testing with a GPC method for analysis of sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoester. The limit of organic impurities procedure operates under the same GPC chromatographic conditions as the assay.

In this work, a GPC method outlined in the proposed USP monograph for sorbitan monostearate was run on an Arc HPLC with a strong solvent compatibility kit and RI detector. To demonstrate compliance with the proposed monograph, the results were compared against all requirements for system suitability and acceptance criteria for both the assay and organic impurities testing. Performance verification of the USP proposed monographs for sorbitan monooleate and sorbitan sesquioleate is demonstrated in a previously published application notes.^{6,7}

Experimental

Solutions preparation and experimental conditions proceeded as described in the proposed USP monograph for sorbitan monostearate.⁵

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 monostearate purchased from Sigma-Aldrich.

Sample Description

Standard solutions

Standard solution for assay prepared by dissolving each of stearic 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 monostearate 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) <ul style="list-style-type: none">· 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 Management

Results and Discussion

The assay and limit for organic impurities procedures outlined in the proposed USP monograph for sorbitan monostearate utilize the same GPC separation conditions.⁵ The USP recommends Waters Styragel HR 0.5 and HR 1 columns with THF as a mobile phase delivered isocratically. Additionally, preparation of standard, and sample solutions for organic impurities is the same as that for the assay.

Peak Assignment

The identification of the sample components for the assay and organic impurities testing was performed according to the USP recommendation of relative retention times to aid peak assignment (Table 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	0.83
	Fatty acid (stearic acid) plus isosorbide monoesters	0.84
	Stearic acid	0.87
	1,4-Sorbitan	0.92
	Isosorbide	1.0

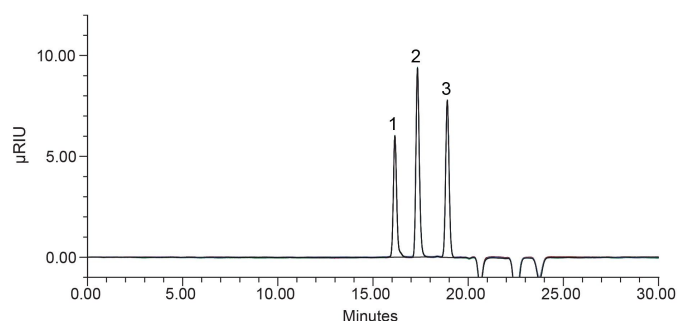
Table 1. Relative retention time (RRT) to aid in peak assignment for assay and limit of organic impurities testing according to the proposed USP monograph for sorbitan monostearate.⁵

System Suitability

System suitability results generated by the method were examined against the USP requirements for relative standard deviation (RSD) and resolution as specified in the proposed USP monograph for sorbitan monostearate.⁵ Solutions for system suitability determination include:

- Assay: standard and sorbitan monostearate sample solutions
- Limit of organic impurities: standard solution

The relative standard deviation (RSD) for assay and organic impurities was assessed using six replicate injections of standard solution. Method produced excellent RSD for peak areas and retention times of $\leq 1.18\%$ and $\leq 0.01\%$, respectively (Figure 1). Resolution for assay measured using sample solution was found to be 1.6, which is above the USP requirement of not less than (NLT) 1.0 (Figure 2). Method also met the resolution requirement for organic impurities of NLT 1.5 between the 1,4-sorbitan and, isosorbide peaks, producing value of 4.7 (Figure 1).



System Suitability	
Sample SetID: 5290	Result SetId: 5571
Processed ChannelDescr.: W2414 RI	

Peak Results							
	Name	Inj. #	Ave_RT	% RSD RT	%RSD PeakAreas	Ave USP Resolution	Ave USP Tailing
1	Stearic acid	6	16.147	0.01	0.85		1.2
2	1,4-sorbitan	6	17.333	0.01	0.99	3.7	1.2
3	Isosorbide	6	18.899	0.01	1.18	4.7	1.1

Figure 1. Six replicate injections of standard solution for system suitability determination using an Arc HPLC System with strong solvent compatibility test and RI detector. RT: retention time, RSD: relative standard deviation.

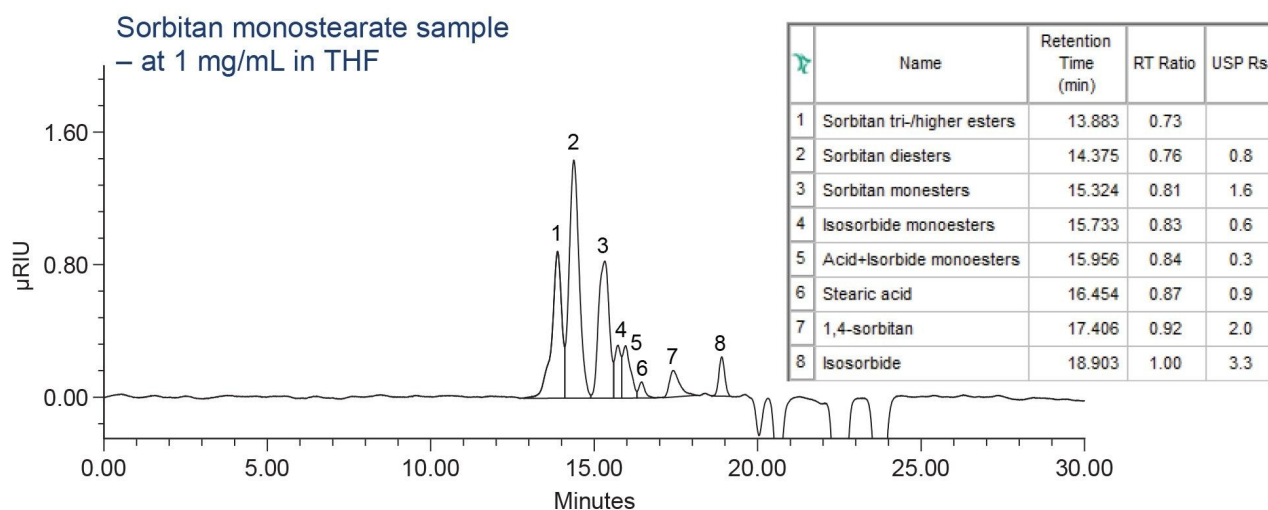


Figure 2. GPC separation of a sorbitan monostearate sample solution using an Arc HPLC System with strong solvent compatibility test and RI detector. RT ratio in Empower: RRT.

Overall, the GPC method on the Arc HPLC System successful met all the USP requirements specified for both procedures, summarized in Table 2.

Procedure	Parameter	USP requirement ⁵	GPC results
Assay	Resolution: between the sorbitan diesters and sorbitan monoesters peaks (sample solution)	Not less than (NLT) 1.0	1.6
Assay and organic impurities	Relative standard deviation: for the stearic acid, 1,4-sorbitan, and isosorbide peaks (six replicate injections of standard solution)	≤5.0%	<ul style="list-style-type: none"> ▪ Stearic acid peak: –RSD of areas: 0.85% ; RSD of RT: 0.01% ▪ 1,4-sorbitan peak: –RSD of areas: 0.99%; RSD of RT: 0.01% ▪ Isosorbide peak: –RSD of areas: 1.18%; 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 according to the USP proposed monograph for sorbitan monostearate.⁵

Sorbitol Monostearate Sample Analysis

Assay: analysis of sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoesters

The percentage (%) of each sorbitan ester component in the sorbitan monostearate sample was calculated by area normalization as instructed by the USP.⁵ Area of individual peak was divided by the sum of the relevant peak areas and multiplied by 100. The assay results were within the USP acceptance criteria ranges (Table 3).

Peak	USP acceptance criteria: Range (%)	GPC results (%)
Sorbitan tri-/higher esters	20.0–30.0	21.8
Sorbitan diesters	30.0–40.0	35.7
Sorbitan monoesters	20.0–30.0	22.1

Table 3. GPC results for assay of sorbitan tri-/higher esters, sorbitan diesters, and sorbitan monoesters in sorbitan monostearate sample (n=6) acquired using an Arc HPLC System with strong solvent compatibility test and RI detector.

Limit of Organic Impurities

To determine the percentage (%) of each impurity in the sorbitan monostearate sample, the area of each individual peak was compared 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 (%)	GPC results (%)
Isosorbide monoesters	5.0	4.9
Stearic acid + isosorbide monoesters	8.0	6.3
Stearic acid	3.0	1.6
1,4-Sorbitan	5.0	4.0
Isosorbide	5.0	3.5

Table 4. GPC results for organic impurities content in sorbitan monostearate sample (n=6) acquired using an Arc HPLC System with strong solvent compatibility test and RI detector. NMT: not more than.

Conclusion

Excellent performance of the GPC method outlined in the proposed USP monograph for sorbitan monostearate was achieved on the Arc HPLC System with a strong solvent compatibility kit and RI detector. The system suitability results met all the USP specification for resolution and RSD of replicate injections. The results for assay and limit of organic impurities in the sorbitan monostearate sample met the acceptance criteria for percentages of relevant components. The GPC system delivered reliable, and reproducible, demonstrating compliance with the requirements and successful implementation of the USP monograph.

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