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Article

An Isocratic HPLC Method for the Determination of Sorbitol and Glycerol in Pharmaceutical Formulations

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Sorbitol and glycerol, along with other inactive ingredients such as preservatives and dyes, are commonly used in various pharmaceutical and personal care products. To accurately assess the effectiveness of various formulations containing sorbitol and/or glycerol, their quantitative determination is essential. In the current study, two types of detectors (a Varian evaporative light scattering detector and an Agilent ultraviolet-visible detector) are evaluated for the assay of working sample solutions. The two detection techniques are complimentary, and a comparison of the results obtained using the two detectors is presented here. The current method is shown to be stability-indicating and free from interference from any of the formulation excipients and potential degradation products. The method is reproducible, accurate, sensitive and selective. It provides enhanced detection sensitivity for sorbitol and comparable sensitivity for glycerol versus similar methods reported in the literature that utilize a refractive index detector for the analysis of either of the two polyols.

Introduction

Glycerol has many uses in pharmaceutical and personal care products, including providing lubrication and serving as a humectant. Similar to glycerol, sorbitol is commonly used as a humectant, and is also used as a thickener and a sweetener, among its other uses.

Small variations in the concentration of sorbitol and glycerol used as inactive ingredients in pharmaceutical formulations may potentially affect the usefulness of these formulations. High-performance liquid chromatography (HPLC) coupled with an ultraviolet-visible (UV-VIS) detector or an evaporative light scattering detector (ELSD) allows for a sensitive and quantitative assay of the two polyols in various formulations in the presence or absence of the active drug substance.

In comparison to refractive index detection (1–7), ELSD provides better sensitivity (e.g., for sorbitol) and is gradient-compatible with stable baselines and no solvent front peaks.

With the use of an ELSD, in comparison to a UV-VIS detector, solvent is not selected for spectral properties, detection is independent of absorbance characteristics, and derivatization is not required for non-chromophoric compounds [e.g., sorbitol (8–9)].

Among the various methods that have been used to separate and quantify polyols in addition to those described earlier are those that use the relatively costly gas chromatography–mass spectrometry (GC–MS) equipment or GC only. Both techniques almost invariably include a derivatization step (10–11). Advantages of an ELSD relative to other detection techniques have also been referenced previously (12).

The procedure described here represents an alternate method to accurately quantify sorbitol and glycerol in aforementioned formulations using simple isocratic elution coupled with the use of the techniques of light scattering and UV-VIS detection.

Experimental

Reagents

Propyl paraben (N.F., E.P., B.P., J.P.), methyl paraben, sorbitol (D-glucitol, powder, N.F.) and glycerol (natural, U.S.P., E.P., B.P., J.P.) were purchased from Spectrum Chemical MFG (Gardena, CA) HPLC-grade water was obtained from J.T. Baker Chemical Co. (Phillipsburg, NJ). HPLC-grade acetonitrile was purchased from EMD Chemicals (Gibbstown, NJ).

Preparation of samples and standards

Inasmuch as the regression plot for each analyte using ELSD can usually be best described by a second-order polynomial, to enhance the accuracy of results, a minimum of eight binary working standards were prepared and chromatographed for each analysis. Each working standard consisted of glycerol and sorbitol, each in the approximate concentration range of 30–70 μg/mL. Various-sized aliquots of the stock standard solution of each analyte (approximately 1 mg/mL in diluent) were mixed and diluted to known volume with diluent to prepare the working standard solutions. The composition of the diluent was the same as the mobile phase [acetonitrile–water (78:22 *v*/*v*)]. The current method was tested for the assay of the two polyols in a commercial formulation (suspension) containing the active drug substance as well as inactive ingredients such as methyl paraben, dye and methyl cellulose. The method was also tested for the assay of the two polyols in an in-house formulation containing the active drug substance, propyl, as well as methyl paraben and the other inactive ingredients listed in the preceding for the commercial formulation. The formulations analyzed in this work were typically suspensions, which were initially centrifuged, thus removing one or more of the insoluble excipients. The supernatant contained one or more of the soluble excipients in addition to glycerol and sorbitol. The working sample solutions were prepared by diluting the

supernatant consisting of approximately 111 mg/mL in sorbitol and approximately 80 mg/mL in glycerol by a factor of 2,000× with diluent.

Instrumentation

The HPLC system used was an Agilent 1100 series (Agilent Technologies, Santa Clara, CA), consisting of a quaternary pump, vacuum degasser, column oven, thermostatted autosampler and Agilent software (Chemstation). The detectors used were a Varian 380-LC evaporative light scattering detector with a 35900E Agilent interface box and an Agilent 1100 diode array UV-VIS detector. The analytical column used was an Inertsil amino, 250 × 4.6 mm, 3-μm (GL Sciences, Torrance, CA).

Optimized chromatographic conditions

The optimized chromatographic conditions were as follows: eluent and diluent composition, acetonitrile–water (78:22 *v*/*v*); flow rate, 1.0 mL/min, column temperature, 30°C; autosampler temperature, 5°C; injection volume, 100 μL. The effluent emerging from the UV-VIS detector (λ: 191 nm) was passed through the ELSD using narrow-bore HPLC tubing with the following ELSD settings: evaporation temperature, 40°C; nebulization temperature, 70°C; nitrogen gas flow rate, 0.90 L/min. The ELSD settings listed here resulted in maximum peak areas for glycerol and sorbitol.

Results and Discussion

Optimization of elution conditions

The effect of mobile phase composition on retention time of glycerol and sorbitol was examined. The variations in mobile phase composition consisted of changes in the acetonitrile to water volume ratio. An increase in the acetonitrile content of the mobile phase resulted in an increase in the retention time of sorbitol and glycerol, presumably due to a decrease in their solubility in a more lipophilic eluent and a corresponding increase in affinity for the polar amino phase (Figure 1). As expected, increasing the column temperature had the effect of a decrease in the retention time of the two polyols. Although glycerol and sorbitol are baseline-resolved using the mobile phase composition in the range of 70:30 (*v*/*v*) to 82:18 (*v*/*v*) acetonitrile–water, and column temperature range of 25—45°C was tested in this work, a mobile phase composition of acetonitrile–water (78:22 *v*/*v*); flow rate, 1.0 mL/min; and column temperature of 30°C were selected as optimized conditions. This combination provided the desired separation in a reasonable amount of time.

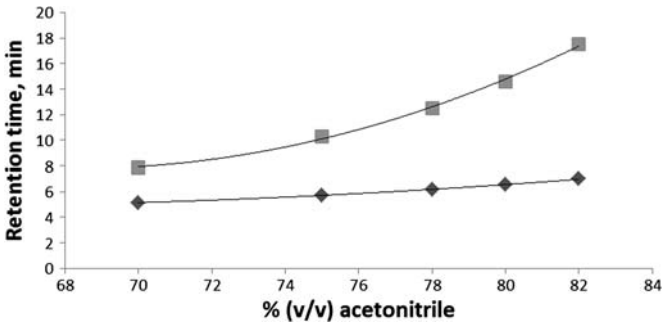

Figure 1. Effect of eluent composition [% (*v*/*v*) acetonitrile] on retention time of glycerol and sorbitol using a flow rate of 1 mL/min and column temperature of 30°C. Diamonds: glycerol; squares: sorbitol.

Figure 2 shows a typical chromatogram of a working sample solution obtained under the optimized HPLC conditions described in the preceding. The amino-bonded silica packing has been widely used for the separation of carbohydrates. When used in the current study, it provided the required separation of sorbitol and glycerol from each other and from other inactive ingredients used in formulations examined in this work containing the two polyols.

Elution of potential degradation products and excipients

Using optimized elution conditions, various excipients (i.e., methyl and propyl parabens) coloring agent and others referenced in the "Preparation of samples and standards" section were observed to elute essentially in the void volume and did not interfere with the elution of sorbitol and glycerol. Also, no chromatographic peaks were observed for any of the possible degradation products generated by thermal stressing (80°C for 20 min) or by treatment of the formulation samples with 30% hydrogen peroxide.

Method validation

The evaluation of method reproducibility consisted of six replicate injections of the working sample solution. With the UV-VIS detector, overall percent relative standard deviations (%RSDs) of ± 1.4% and ± 2.1% for assay results were obtained for sorbitol and glycerol, respectively. Method reproducibility for the same working sample solution using the ELSD was determined to be ± 0.65% and ± 1.8% for sorbitol and glycerol, respectively.

Included in the method validation experiments were those used to determine the limit of quantitation (LOQ) for the two analytes. With optimized detector (ELSD) sensitivity and chromatographic conditions, the LOQs were estimated to be approximately 2 and 1 μg/mL for glycerol and sorbitol, respectively. Using the UV-VIS detector, the LOQs (in mobile phase) were estimated to be approximately 6 and 4 μg/mL for glycerol and sorbitol, respectively. If needed, the LOQs using UV-VIS detection could be further lowered (e.g., 4 to 2 μg/mL for sorbitol) by lowering the flow rate to 0.5 mL/min following the reciprocal ($1/x$) relationship between flow rate and detector response. In this case, a change in the chromatographic conditions [i.e., 65:35 (*v*/*v*) (acetonitrile–water); column temperature, 30°C and flow rate, 0.5 mL/min] versus optimized conditions yielded a chromatogram comparable to that shown in Figure 2, with approximately double the peak areas for glycerol and sorbitol.

Table I lists the accuracy (spike-recovery) data for a formulation sample spiked with known amounts of sorbitol and glycerol. For example, when determining the recovery for sorbitol, known amounts of sorbitol were spiked into formulations containing target amounts of glycerol as well as other inactive excipients such as such as methyl and propyl paraben. Working sample solutions prepared from spiked formulations

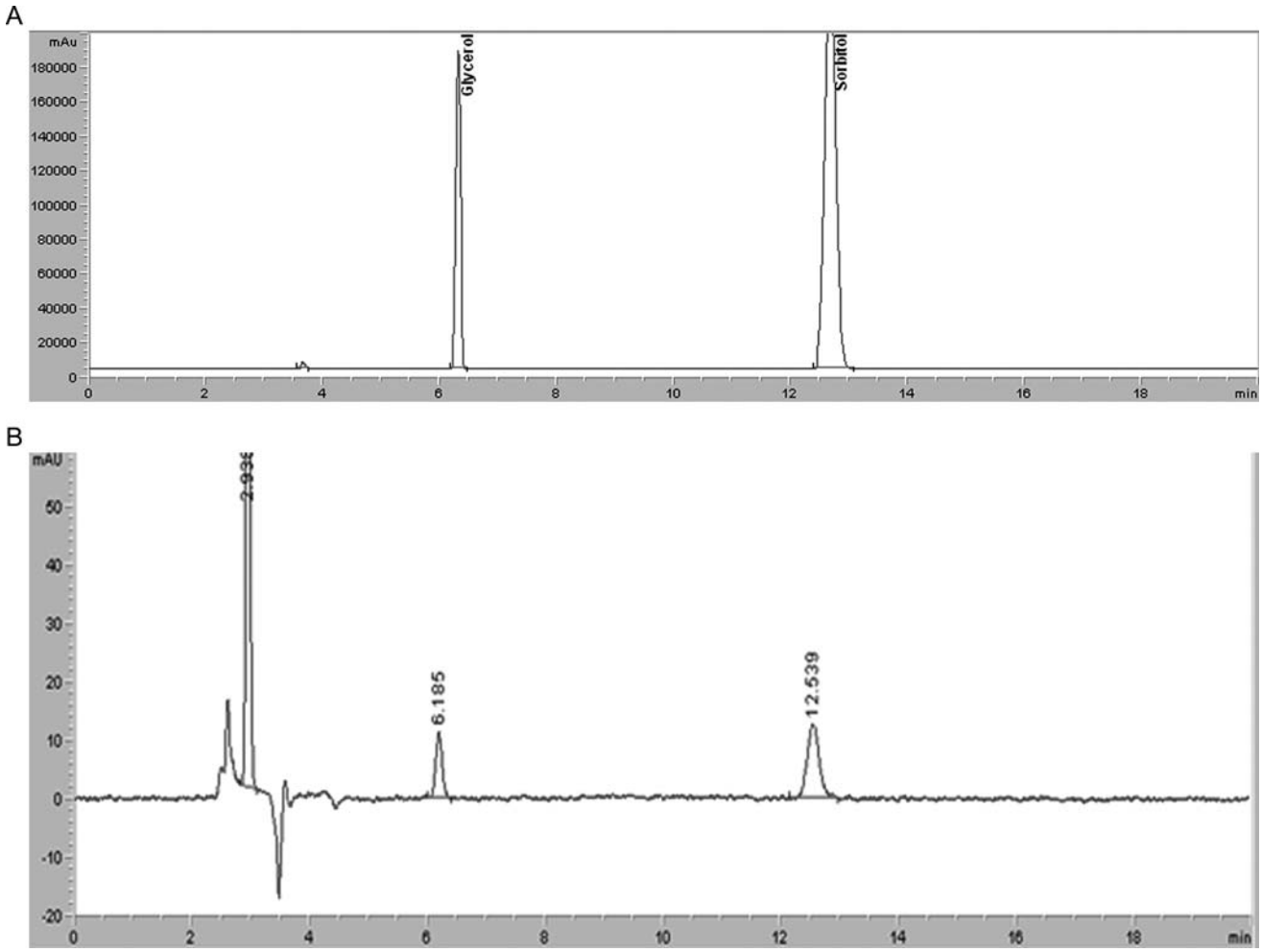

Figure 2. Representative chromatograms of the working sample solution using optimized conditions: ELSD (A); UV-VIS (λ: 191 nm) (B).

Table I
Accuracy (Spike-Recovery) Results for Glycerol and Sorbitol

Level (% of target)	Sample	% Recovery for glycerol	% Recovery for sorbitol
80%	Prep 1	98.4	103.1
	Prep 2	101.2	98.6
	Avg.	99.8	100.9
100%	Prep	102.0	97.5
	1Prep	99.5	99.8
	2Avg.	100.8	98.6
120%	Prep 1	97.6	100.6
	Prep 2	99.7	97.9
	Avg.	98.6	99.3

were then assayed using the UV-VIS detector and optimized chromatographic conditions.

Selectivity was insured using the UV-VIS detector and Agilent peak purity software, coupled with variations in mobile phase composition and ensuring absence of co-eluting impurities/degradants.

Conclusion

An HPLC method for the simultaneous determination of sorbitol and glycerol has been developed and is presented here. The method uses isocratic elution and is shown to be selective, providing good reproducibility, accuracy and sensitivity. It has been applied to the analysis of sorbitol and glycerol in various formulations containing the two polyols.

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