GLC Determination of Sorbitol in Irrigating Solutions

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Abstract □ A GLC method for determining sorbitol in aqueous irrigating solutions is described which has advantages over the USP adsorption column chromatography procedure. It is based on a reported method using the hexaacetate derivative. Samples are chromatographed on a trifluoropropyl silicone on a support of silanized, flux-calcined diatomite. Bis(2-ethylhexyl) sebacate serves as the internal standard. Mannitol and other polyhydric alcohols do not interfere and may be determined concomitantly if desired. The GLC method is recommended for consideration as a replacement for the USP procedure.

Keyphrases □ Sorbitol irrigating solutions—GLC determination, compared to USP XVIII column chromatographic method □ GLC—determination, sorbitol in aqueous irrigating solutions □ Bis(2-ethylhexyl) sebacate—GLC internal standard, determination of sorbitol in aqueous solutions

Sorbitol, a naturally occurring hexahydric alcohol, is a common ingredient in various pharmaceutical products. It is referred to in USP XVIII (1) as a pharmaceutic aid or flavored vehicle, and other relatively minor drug uses have been reported (2, 3). However, the use of sorbitol as a component in urological solutions is of continuing and increasing interest (4, 5).

Analytical methods were sought which could be used to assure the potency, quality, stability, and identity of sorbitol in 3% aqueous irrigating solutions and other related formulations, including some with mannitol as a component. Several procedures are described in the literature with possible utility for such products, but most lack specificity, precision, and ease of use. Methods have been reported for the quantitation or estimation of sorbitol by: titration using periodate titrant (6–8); polarimetry of molybdate complexes (9, 10); colorimetry (9, 11); coulometry with cerium^{+4} (12); chromatography methods using media such as paper (13), ion-exchanging resins (7, 8), and thin-layer plates with several means of quantitation suggested (14–16); adsorption column chromatography (1, 17); and GLC (18).

Chromatographic methods offer the only practical way for determining sorbitol in the presence of mannitol, other polyhydric alcohols, and carbohydrates often encountered in sorbitol-containing drug products. Of these procedures, GLC gives the most useful information and requires relatively less time and effort.

The technique of Hause *et al.* (18) was modified and improved to provide a suitable method for use with dilute irrigating solutions of sorbitol. It was first necessary to establish an internal standard other than mannitol as proposed by those authors, since mannitol is present in most commercially available sorbitol or in the formulated irrigating solutions. Also, it is often desirable or necessary to determine mannitol *per se* in such solutions.

Bis(2-ethylhexyl) sebacate was selected as an internal standard since it has the desired purity, response, and retention time characteristics and it is readily available.

Calculation of sorbitol content by electronic integration of peak areas was considered preferable to the measurement of peak heights, the method used by Hause *et al.* (18). Sample handling was simplified considerably by use of a smaller sample aliquot, completion of the sample workup in the original flask, and elimination of unnecessary intermediate sample transfer and dilution steps. In addition, the unsatisfactory pyridine azeotrope step used previously to remove water from samples was discarded in favor of a simple rotary vacuum evaporation. The latter method was found to be more dependable in assuring sufficient water elimination as well as requiring less operator attention. Since only 2-ml. aliquots of 3% sorbitol solutions are normally required, rotary vacuum evaporation is a relatively quick and simple workup technique.

Application of the method described here to mannitol and other polyhydric alcohols requires only the preparation of suitable reference standard solutions for the determination of response factors. Additional tests for sorbitol needed to assure purity and identity of the irrigating solutions are also discussed.

EXPERIMENTAL

Instrumentation—The following were used: a gas chromatograph[1] fitted with a hydrogen flame-ionization detector (FID); and a 1-mv., 1-sec., 28-cm. (11-in.) strip-chart recorder.

Materials—Pyridine, acetone, and acetic anhydride were reagent grade and required no further purification. The prepared column packing material, Silicone QF-1, 3% on Gas-Chrom Q, and the internal standard, bis(2-ethylhexyl) sebacate (19), are commercially available[2].

Sample Preparation—Transfer 2.0 ml. of the 3% aqueous solution, or an aliquot of sample containing about 60 mg. of sorbitol, to a 50-ml. conical flask with a 19/38 standard taper neck. Remove water in a rotary vacuum evaporator at 60°. When dry, add 2 ml. each of pyridine and acetic anhydride. Attach a water-cooled condenser with a 19/38 standard taper fitting and reflux for 1 hr. Cool and add 5 ml. of a 1% v/v solution of bis(2-ethylhexyl) sebacate in acetone. Mix and inject 1-μl. aliquots directly into the chromatograph.

Standard Solution—A selected lot of sorbitol USP[3] was used as the working standard since an official reference standard is not available. This material was characterized by application of current pharmacopeial tests, and a potency value was assigned based on data obtained using the official adsorption column chromatography method (1). The potency of irrigating solutions thus can be related to sorbitol USP. Sorbitol of relatively high purity—about

[1] Hewlett Packard (FM) model 810 or equivalent.
[2] Applied Science Laboratories, Inc., State College, Pa.
[3] Sorbitol USP and "pure" crystalline grade sorbitol were obtained from Atlas Chemical Industries, Chemical Division, Wilmington, Del.

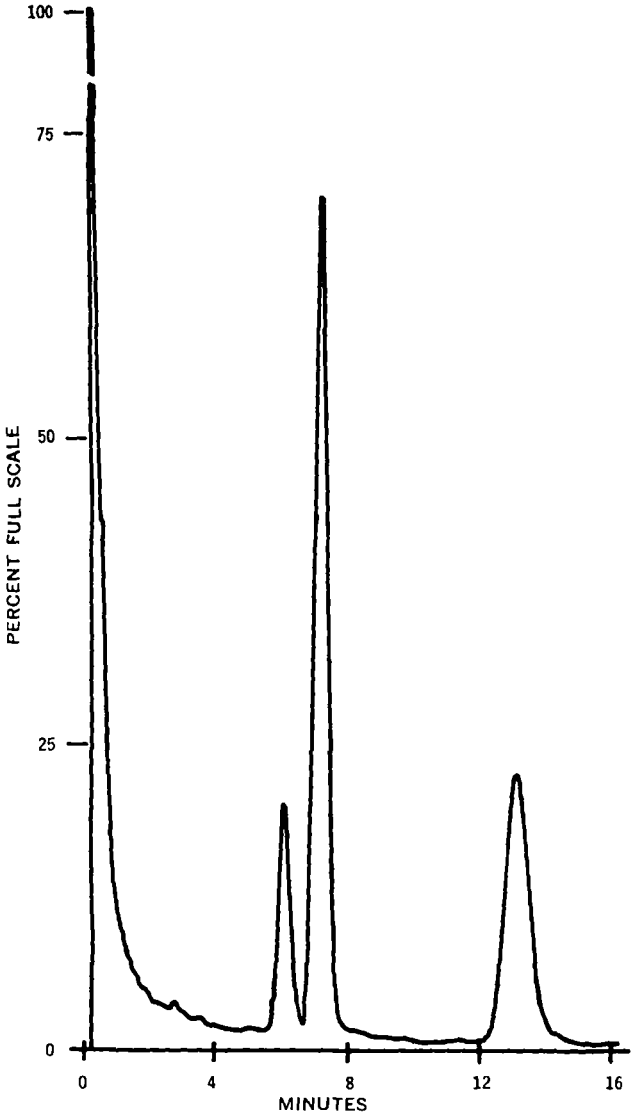

Figure 1—*Chromatogram of sorbitol as the hexaacetate (7 min.), mannitol hexaacetate (6 min.), bis(2-ethylhexyl) sebacate (13 min.), and impurities (2–4 min.) in acetone-pyridine-acetic anhydride on a Silicone QF-1, 3%, Gas-Chrom Q column at 220°.*

97%—is commercially available[3] and may be of value as a standard when quantitating sorbitol and other polyhydric alcohols simultaneously; otherwise, the selected USP grade material should prove satisfactory when determining only sorbitol.

The working standard used to obtain GLC response factors was prepared by transferring about 60 mg. of the working standard, accurately weighed, to a 50-ml. conical flask with a 19/38 standard taper neck. A 2-ml. aliquot of pyridine was added to dissolve the sorbitol, and then 2 ml. of acetic anhydride was added. The solution was refluxed and sampled for chromatography as described under *Sample Preparation*, including addition of the internal standard.

The response factor, F, is the ratio of internal standard peak area to the sorbitol standard peak area, multiplied by the weight of sorbitol standard taken, in milligrams. It is suggested that standard aliquots be injected before and after a series of samples and also interspersed occasionally throughout the sample runs to ensure optimum accuracy and precision of results. A mean value of F calculated from the several standard injections may then be used to calculate sample concentration.

GLC Procedure—The analytical column consisted of a 200 × 0.635-cm. (0.25-in.) o.d. tubing packed with the prepared column material. Operating conditions were: column, 220°; detector, 250°; injector, 250°; and carrier gas flow rate, approximately 80 ml./min. The column was of coiled-copper tubing (although glass, stainless steel, or copper tubing are satisfactory). The packed column is preconditioned for about 12 hr. at 250° with a carrier gas flow rate of 20 ml./min. Nitrogen was used as the carrier gas. Compressed air and hydrogen gas flow rates were 300 and 40 ml./min., respectively. The sensitivity was set at 10^2 and attenuation at "8" or "16." The chart speed was 0.635 cm./min. (0.25 in./min.). Peak areas were determined by use of an electronic integrator.

Table I—Impurity Content of Commercial Sorbitol USP

Sample Lot Number	Percent Total Peak Area[a]				
	Sorbitol	Mannitol	A	B	C
1	97.8	1.4	0.2	—	0.1
2	98.0	1.0	0.2	0.2	0.1
3	97.5	1.7	0.4	0.2	0.1

[a] Data obtained by the described GLC method.

RESULTS AND DISCUSSION

Figure 1 shows the chromatogram of a typical 3% sorbitol irrigating solution sample taken through the described procedure. Adequate separation and baseline resolution are observed, with minimal tailing of the hexaacetate derivatives of sorbitol and mannitol. Approximate retention times for sorbitol hexaacetate and the internal standard were 460 and 830 sec., respectively.

Precision of the GLC method for a typical 3% sorbitol solution was found to be ±1.2% relative standard deviation based on individual percentage values of 96.2, 95.0, 93.8, 96.1, and 96.7 ($\bar{X}$ equals 95.6%), each value being determined on a separate day.

The impurity content of commercially available sorbitol USP was determined by a total area calculation applied to GLC runs made without an internal standard (Table I).

The values in Table I do not reflect moisture content, which was tested separately and found to be less than 1%. Any impurities present which would not yield detectable peaks by this GLC method could result in an undetermined lowering of the relative percent of sorbitol found in the sample. Peaks A, B, and C represent unidentified impurities in the sample and may possibly be due to other polyhydric alcohols.

By using a sorbitol working standard with an accurately determined potency value and the GLC method with an internal standard, it is possible to achieve reasonably good agreement with the USP procedure when testing 3% sorbitol solutions. Table II lists values found for 12 samples from six batches of solutions tested at various storage intervals and conditions.

The USP procedure appears to give average values about 1.4% higher than the GLC method. Further investigation is needed to establish if this difference is significant and which method might provide the more accurate results.

Other procedures can be used to provide additional data for the quality assurance testing of sorbitol irrigating solutions. Where

Table II—Comparison of GLC and USP Methods Applied to Sorbitol 3% Solutions

Sample Number	Storage Conditions	Sorbitol Found, %	
		GLC	USP
1-a	1 month, 25°	92.5	94.0
1-b	3 months, 50°	93.0	95.3
2-a	1 month, 25°	96.6	95.5
2-b	3 months, 50°	94.2	94.2
3-a	1 month, 25°	96.3	94.3
3-b	3 months, 50°	95.9	96.5
4-a	6 months, 25°	94.6	96.1
4-b	6 months, 50°	94.9	96.8
5-a	Initial	91.0, 90.4 (process)	93.6 (final)
5-b	3 months, 25°	92.1[a]	93.4
6-a	Initial	90.4, 91.9 (process)	92.1 (final)
6-b	3 months, 25°	91.9[b]	93.7

[a] $\bar{X}$, based on individual values of 92.1, 91.6, 90.2, and 93.9 with a standard deviation of ±1.5%. [b] $\bar{X}$, based on individual values of 89.6, 91.1, 93.4, and 90.5 with a standard deviation of ±1.6%.

sorbitol is the only major component, as in the 3% solutions, a periodate titration similar to that specified for mannitol USP (20) may give in-process results more quickly than the GLC method and provide data on total polyhydric alcohol content. A total solids test also gives an added control over extraneous solids or impurities, although the gravimetric determination of solids with vacuum drying at 80° is a rather slow method for in-process use. Sample potency can be determined satisfactorily by using these procedures in combination with the GLC method.

Hause *et al.* (18) stated that methods available at that time, including the USP adsorption column procedure, and used for the separation and quantitation of sorbitol in the presence of other polyols were time consuming, involved complex manipulation, and were prone to errors. Similar observations were made by the present authors—the USP procedure requires about 10-fold more time to perform than the GLC method. Further reduction in analysis time for the GLC method presented here may be possible. For example, it was reported that the acid-catalyzed acetylation of polyhydric alcohols can be accomplished in about 15 min. at room temperature (21).

CONCLUSION

The GLC method described here is considered the preferred method for the determination of sorbitol. It may be combined with other tests to provide assurances of potency, quality, stability, and identity of manufactured sorbitol irrigating solutions.

It is recommended that such a GLC method be evaluated as a replacement for the USP adsorption column chromatography procedure. Work should be initiated to provide a suitable sorbitol reference standard and, eventually, to begin a collaborative study of a GLC or other satisfactory method to be applied to sorbitol USP and sorbitol solution USP. Adoption of a method like the GLC procedure would represent a significant reduction in analysis time and effort, reduce the complexity and manipulations which are potential sources of error, and provide more complete analytical data concerning purity and identity than does the present USP procedure.

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