GLC Assay of Sorbitol as Cyclic *n*-Butylboronate

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Abstract □ A simple, rapid, and quantitative GLC procedure was developed for the determination of sorbitol in solid and aqueous solution forms. The method utilizes the ability of *n*-butylboronic acid to convert sorbitol quantitatively to the cyclic *n*-butylboronate ester. Preparation of the sample is simple, and the reaction goes rapidly to completion. Baseline separation and peak symmetry are obtained for sorbitol, mannitol, and galactitol. A procedure for establishing a sorbitol reference standard is presented.

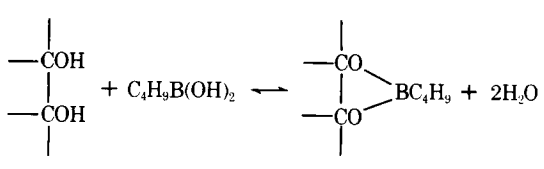
Keyphrases □ Sorbitol—GLC analysis as the cyclic *n*-butylboronate □ *n*-Butylboronic acid—conversion of sorbitol to cyclic *n*-butylboronate ester, GLC analysis □ GLC—analysis, sorbitol as the cyclic *n*-butylboronate

Sorbitol is a naturally occurring, straight-chain hexahydric alcohol commonly incorporated in pharmaceutical products as a humectant and sweetener (1). It is also used as a chemical intermediate in the synthesis of ascorbic acid and is of particular importance as a food additive for dietary purposes (2). Medically, it has been used as an osmotic diuretic and cathartic and for parenteral alimentation (3).

Many reported analytical methods for the quantitative and qualitative determination of sorbitol, sorbitol solutions, and similar alditols are based on refractometry (4, 5), polarimetry of molybdate complexes (6, 7), coulometry (8), colorimetry (9), and titration after periodate oxidation (10). Some of these methods are rapid, others are laborious, and none are specific for sorbitol. Chromatographic methods such as ion exchange (10, 11), paper (12, 13), thin layer (10, 14–16), and adsorption column (1, 17) offer some degree of specificity but are also laborious or time consuming.

DISCUSSION

At the present time, GLC offers the best means of analyzing sorbitol and sorbitol solutions quantitatively with a measure of selectivity and rapidity. Many methods have been proposed based on the conversion of monosaccharides and alditols to silyl and acetate derivatives (18–24), but the numerous disadvantages of these methods have been cited. The silylation procedure necessitates the complete removal of water from the sample before reaction, requires extended reaction times, and does not completely separate sorbitol from mannitol and galactitol. Conversion of the alditols to the hexaacetate derivatives shortens the reaction time considerably, and the derivatives are separated when chromatographed. However, this procedure is still time consuming due to the necessity of either removing water from the sample by rotary evaporation (25) or removing the solvent (pyridine) after reaction to assure that no tailing of peaks will occur (26).



diol + *n*-butylboronic acid dioldibutylboronate

Scheme I

Alkaneboronic acids were recently introduced as aids for the volatilization of various organic compounds (27, 28). Eisenberg (29) reported the successful application of *n*-butylboronic acid for GLC of several sugars and alditols, including sorbitol, mannitol, and galactitol. The proposed reaction is shown in Scheme I (29).

This paper describes the determination of sorbitol in powder form and in aqueous solutions as the cyclic *n*-butylboronate. The sorbitol boronate is formed readily by combining a known weight of sample with a solution of *n*-butylboronic acid in pyridine, containing methyl nonadecanoate as the internal standard. Studies performed in this laboratory indicate that the reaction goes to completion almost immediately. The liquid phase used is a recently introduced cyanoalkyl phenyl silicone which is versatile and stable and exhibits virtually no bleeding of the liquid phase when used at the operating parameters described.

The absence of a suitable sorbitol reference standard of verified composition has hindered the development of a truly quantitative method for the determination of sorbitol. Therefore, a selected lot of crystalline sorbitol was analyzed for total polyhydric alcohol content, using sodium arsenite as the titrant after periodate oxidation, and then for the sorbitol portion of the total by GLC without the internal standard. Such a standard, when properly stored, is stable for several months.

Data demonstrating the linearity of response with changes in concentration, reproducibility, and completeness of recovery are presented.

EXPERIMENTAL

Operational Parameters—A gas chromatograph[1] with hydrogen flame-ionization detectors was used. The column was glass with a U-type configuration, 183 cm long by 4 mm i.d., packed with 3% cyanoalkyl phenyl silicone liquid phase on a silanized diatomaceous earth support, 80–100 mesh. The column was conditioned for 18 hr at 250° with carrier gas flow. Operating parameters were: column temperature, 205°; detector temperature, 260°; injection port temperature, 245°; and carrier gas (nitrogen) flow rate, 50 ml/min. Hydrogen and air flows were adjusted to obtain maximum efficiency as recommended by the manufacturer; the range selector was set at 10 and attenuation was adjusted to obtain at least a 50% recorder scale response for major peaks. All peak areas were measured with an electronic integrator[2].

Reagents and Chemicals—The liquid phase, 50% cyanoalkyl 50% phenyl silicone (SILAR 5CP)[3], the silanized diatomaceous earth support (Gas Chrom Q)[3], and methyl nonadecanoate were used as supplied[3]. Sorbitol standard was crystalline USP grade[4]. *n*-Butylboronic acid, 99+%[5], was kept moist with water until ready for use. Pyridine (ACS reagent grade) was used without further purification.

Reagent–Internal Standard Preparation—An amount of *n*-butylboronic acid (from which the excess water had been removed by light vacuum filtration) was dissolved in sufficient pyridine to yield a final concentration of 10 mg/ml. Methyl nonadecanoate was added to a portion of the *n*-butylboronic acid stock solution so that the concentration of internal standard in the reagent solution was 2 mg/ml.

Sample Preparation—An accurately weighed sample, containing 130–160 mg of sorbitol, was transferred to a 100-ml volumetric flask and was dissolved in and diluted to volume with water. A 1.0-ml aliquot, transferred to a suitable glass vial, was taken to

[1] Model 402, Hewlett-Packard.
[2] Model CSI-208E, Columbia Scientific Industries.
[3] Applied Science Laboratories, State College, Pa.
[4] Pfizer, Inc., New York, N.Y.
[5] Aldrich Chemical Co., Milwaukee, Wis.

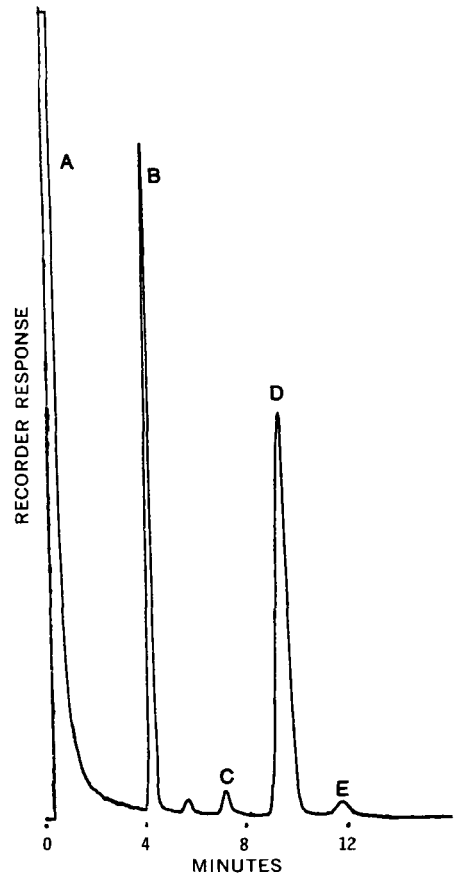


Figure 1—*Sample chromatogram showing relative retention times of sorbitol, mannitol, and galactitol as the* n*-butylboronates and the internal standard methyl nonadecanoate. Key: A, solvent; B, methyl nonadecanoate; C, mannitol; D, sorbitol; and E, galactitol.*

dryness in a vacuum oven at 40–60°. The residue was reconstituted with a 1.0-ml aliquot of the reagent–internal standard preparation. A 1.0-μl sample was injected into the chromatograph and allowed to elute for about 12 min (Fig. 1).

Standard Preparation—Sorbitol reference standard was accurately weighed and diluted with water to a concentration of 1.5–1.7 mg/ml. A 1.0-ml aliquot was transferred to a suitable vial and was taken to dryness in a vacuum oven at 40–60°. The residue was reconstituted with a 1.0-ml aliquot of the reagent–internal standard solution. A 1.0-μl sample was injected into the chromatograph and allowed to elute for about 12 min.

Sorbitol Standard Potency—A batch of sorbitol USP, crystalline grade, was selected as an arbitrary working standard since an official reference standard of known potency is not available. Total polyhydric alcohol content was determined by titration of a 0.40-mg/ml solution of sorbitol standard in water with 0.05 *N* sodium arsenite, as described in the USP XVIII (1, p. 680). The sorbitol content of the standard was determined by an area normalization calculation applied to GLC determinations made using the operating parameters described but without the internal standard. The purity of the sorbitol standard was calculated as the total polyhydric alcohol content times the sorbitol content determined by GLC without the internal standard.

RESULTS

The analytical procedure was designed so that sorbitol solutions of any concentrations may be easily analyzed. No difficulty with the analysis due to the presence of water was encountered when the reagent–internal standard preparation was reacted with a 1-ml aliquot of a diluted sample solution and injected directly. However, the long-term deteriorating effect of routinely injecting water onto a column with a silanized support was considered. Taking the sample aliquot to dryness (or near dryness) in a vacuum oven allows the assembly and manipulation of a large number of samples, which may then be stored until ready for analysis. The almost immediate completeness of the reaction also suggests the possibility of a semiautomated GLC procedure.

Table I—GLC Analysis of Sorbitol Solution USP

Run Number	Sorbitol, % Found (w/w)
1	65.4
2	65.6
3	65.7
4	65.6
5	65.7
6	65.6
Mean	65.6
σ	±0.1

n-Butylboronic acid, when stored under water (1 ml/g of reagent) to inhibit degradation (30), is quite stable. The reagent–internal standard preparation has been used in this laboratory for as long as a month without signs of degradation. Although excess water is removed before dissolving *n*-butylboronic acid in pyridine, the reagent is still slightly damp. Water is also generated in the reaction of the reagent with the alditols. The small amount of water present has not caused any noticeable change in the column characteristics over several months of repeated use.

Eisenberg and others (29–31) noted that extended storage of *n*-butylboronate derivatives in the reaction medium may lead to possible degradation of the derivative.

Standard solutions of sorbitol, ranging from 25 to 125% of the concentration levels to be expected in a typical analysis, were chromatographed; a plot of the ratio of areas *versus* concentration (Fig. 2) shows that the chromatographic response is linear over the entire range.

Replicate determinations of a commercial sample of sorbitol solution, 70%, USP (Table I), were made. The standard deviation calculated indicates excellent precision for the analytical procedure.

A recovery study was performed by analyzing solutions of known concentration prepared from the reference standard which had been analyzed as previously described (Table II).

A standard chromatogram (Fig. 3) and a typical sample chro-

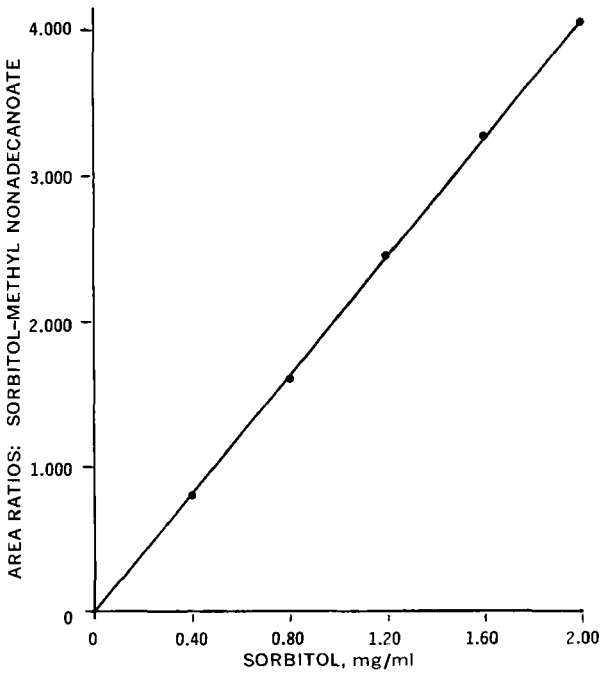


Figure 2—*Linearity of response (ratio of areas) with concentration. Concentration is in milligrams per milliliter with the internal standard as a constant. (See text for other conditions.)*

Table II—Sorbitol Recovery Study

Amount Present, % (w/w)	Experimentally Found, % (w/w)	Recovery, %
70.4	70.8	100.6
70.0	69.7	99.6
68.2	67.8	99.4
70.1	69.6	99.3
70.1	69.7	99.4
69.2	69.5	100.4
Mean	—	99.8

matogram (Fig. 1) show that the sorbitol, mannitol, and galactitol cyclic *n*-butylboronates are well separated and that the peaks show excellent symmetrical configuration. It is believed that the 5- or 6-membered rings formed in the reaction with the *n*-butylboronic acid greatly accentuate the small steric differences between the alditols. A sufficient separation occurs between sorbitol, mannitol, and galactitol, thereby permitting a rapid chromatographic analysis. Relative retention times (to internal standard methyl nonadecanoate, retention time of 4.20 min) are: sorbitol, 2.25; mannitol, 1.71; and galactitol, 2.80. Although linearity response and recovery studies were not done for mannitol or galactitol, quantitative analysis of these two alditols probably could be easily accomplished using the method and parameters described.

The liquid phase chosen is a recently introduced (31) 50% cyanoalkyl 50% phenyl silicone[3] of moderate polarity. It has been found to be stable at relatively high temperatures and exhibits little or no bleeding of the liquid phase at operating temperatures. Despite its use for the analysis of several other compounds and without the use of an oxygen scrubber for the carrier gas, the retention and relative retention times of sorbitol, mannitol, and galactitol, with a particular column, have remained constant over several months.

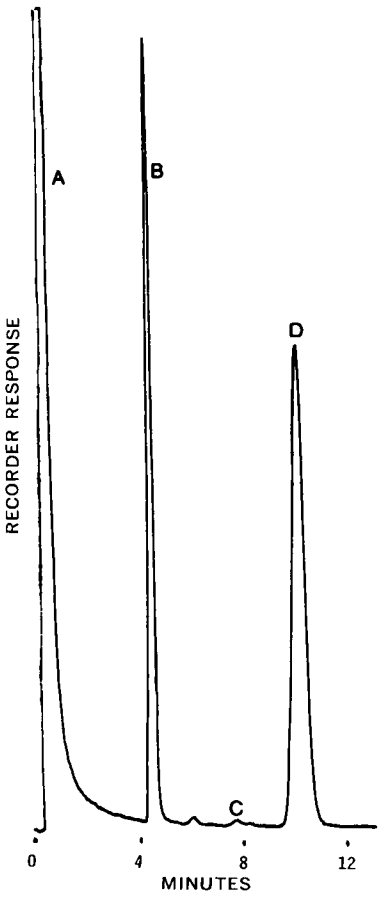


Figure 3—*Standard chromatogram showing relative retention times of sorbitol and mannitol as* n*-butylboronates and the internal standard methyl nonadecanoate. Key: A, solvent; B, methyl nonadecanoate; C, mannitol; and D, sorbitol.*

Table III—Comparison of USP and Proposed GLC Methods

Commercial Sample	Sorbitol Found, % (w/w)	
	GLC	USP
1	68.2	67.7
2	68.4	67.9
3	64.6	64.1
4	64.6	64.3
5	93.9	94.4

Samples from several commercial suppliers were analyzed, and the results were compared to those obtained by the USP (1) procedure (Table III). The USP analyses were performed and certified by the suppliers. The analysis of components of the standard need not be done for each analysis if the standard is kept tightly sealed and placed in a desiccator over silica gel. Total polyhydric alcohol analysis over 6 months has shown a variation of less than 0.05%. Analysis for sorbitol in the reference standard (by GLC without the internal standard) has varied only slightly more over the same period.

SUMMARY

The analytical procedure described overcomes the difficulties previously encountered in reported GLC analyses of sorbitol. Sample preparation and reaction with the derivatizing agent, *n*-butylboronic acid, are simple and rapidly accomplished. Analyses are reproducible and accurate and compare favorably with the cumbersome compendial (1) procedure. A method that enables each laboratory to certify and maintain its own sorbitol reference standard is presented.

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Effect of Maleic Acid in Compendial UV Absorption Assays for Antihistamine Maleate Salts

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Abstract □ Standard recoveries averaging 89.9% are reported for the NF XIII brompheniramine maleate tablet assay procedure, which employs UV comparison of unidentical sample and standard molecular species in the same medium. The data presented indicate that these low assay values are attributable to the chromophoric properties of the maleic acid moiety and its ability to protonate the α-pyridyl chromophore of the brompheniramine molecule in a neutral organic solvent.

Keyphrases □ Maleic acid—effect on compendial UV absorption assay for antihistamine maleate salts □ Maleate salts of antihistamines—effect of maleic acid on compendial UV absorption assay □ Antihistamine maleate salts—effect of maleic acid in compendial UV absorption assays □ UV absorption—effect of maleic acid in compendial assays for antihistamine maleate salts

Several current compendial monographs contain assay procedures for organic nitrogenous base maleate salts in which the UV absorbance of a sample solution of an extracted base is compared with that of a standard solution of its maleate salt in the same solvent. Recoveries of about 97–98% were previously reported (1) for dexbrompheniramine, dexchlorpheniramine, and chlorpheniramine maleate reference standards obtained by using one method of the Association of Official Analytical Chemists (2) and three monograph assays of NF XIII (3), all of which employ such methodology. These erroneously low assay values, resulting from UV comparisons of unidentical molecular species, have been shown to be caused by the UV absorption properties of maleic acid in 0.1 *N* HCl and 0.1 *N* H_2SO_4, where measurements are made at about 264 nm (1).

In extending these studies of similar antihistamine maleate assays in official monographs, the NF XIII assay for brompheniramine maleate (I) tablets (3, p. 107) was tested for percent recovery values. This NF procedure involves sample extraction of the free amine from alkaline aqueous solution into chloroform. The UV absorbances of the sample solution and of a standard solution of I, diluted directly to the desired concentration in the same medium, are then determined and compared. This method differs from other antihistamine maleate assays previously tested by the authors in that UV measurements of the extracted sample amine base and directly diluted standard amine salt are made in an organic solvent (chloroform) rather than in aqueous acid solution.

Recoveries ranging from 89.3 to 90.5% were ob-

Table I—Recovery Data for Brompheniramine, Chlorpheniramine, and Pheniramine Maleates Reference Standards Using the NF XIII Brompheniramine Maleate Tablet Assay

Salt	Source	Solvent Used for Standard Dissolution	Recovery, %
Brompheniramine maleate	Commercial	Chloroform	90.2
Brompheniramine maleate	Commercial	Chloroform	89.3
Brompheniramine maleate	Commercial	Chloroform (sodium hydroxide treated)	89.7
Brompheniramine maleate	NF	Chloroform (sodium hydroxide treated)	90.5
			Average 89.9
Chlorpheniramine maleate	USP	Chloroform	88.6
Chlorpheniramine maleate	USP	Chloroform (sodium hydroxide treated)	89.0
			Average 88.8
Pheniramine maleate	Commercial	Chloroform	89.5
Pheniramine maleate	Commercial	Chloroform (sodium hydroxide treated)	89.9
			Average 89.7