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Note

Determination of α-2-deoxy-D-glucose in topical formulations by high-performance liquid chromatography with ultraviolet detection

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α-2-Deoxy-D-glucose (structure I, Fig. 1) has been investigated experimentally as an antiviral agent. It is an antimetabolite of glucose (structure II, Fig. 1). Its effect on influenza virus multiplication, herpes simplex virus and human genital herpes infection has been investigated[1–3].

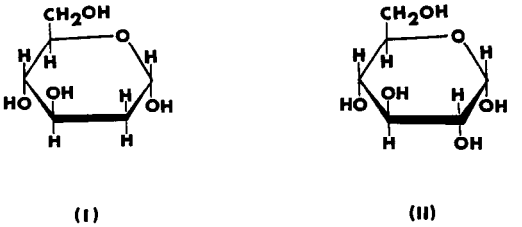

Fig. 1. Structures of α-2-deoxy-D-glucose (I) and glucose (II).

Glucose and glucose analogues present a unique set of difficulties to high-performance liquid chromatographic (HPLC) analysis. Glucose solutions often do not absorb sufficiently above 200 nm to allow routine analysis, hence refractive index detection is usually chosen with a concomitant loss in sensitivity. Chromatographic separation on reversed-phase systems is usually accomplished only by use of relatively expensive and short-lived specialty (*e.g.* carbohydrate) columns. Methods of this type are available for a variety of matrices[4–6]. Topical formulations complicate the analysis since they frequently contain a variety of excipients with chromatographic polarities similar to those of glucose.

This paper describes the determination of α-2-deoxy-D-glucose by HPLC using a standard amine (NH_2) column and ultraviolet detection at 195 nm. Sample analysis of this type has not appeared previously in the literature. Excipients in the model topical formulations analyzed did not interfere with the analysis.

EXPERIMENTAL

Instrumentation

An HPLC apparatus (IBM LC/9533 ternary gradient liquid chromatograph, IBM Instruments, Danbury, CT, U.S.A.) with a WISP autoinjector (Waters Assoc., Milford, MA, U.S.A.) was used in this analysis.

The mobile phase was passed through a μBondapak 10 μm NH_2, 30 × 0.4 cm I.D. (Part No. 84040, Waters Assoc.) column (column 1) or a Varian Micropak 10 μm NH_2 (Part No. 03-912153-44, Varian, Florham Park, NJ, U.S.A.) column (column 2) at a flow-rate of 2 ml/min.

Reagents, materials and mobile phase

α-2-Deoxy-D-glucose (99+%) was obtained from Thiokol (Danvers, ME, U.S.A.). The mobile phase consisted of acetonitrile–water (85:15). The mobile phase was degassed under vacuum and sonicated prior to use.

Sample and standard preparation

The standard solution was prepared by accurately weighing *ca.* 10 mg of α-2-deoxy-D-glucose into a 50-ml volumetric flask. The analyte was then diluted to volume with mobile phase.

The sample was prepared by accurately weighing 0.5 g of a topical formulation containing *ca.* 20 mg/g α-2-deoxy-D-glucose into a 50-ml volumetric flask. The solution was then diluted to volume with mobile phase. A volume of 20 ml of this solution was then centrifuged.

Zinc analysis

Zinc acetate was added to the starch glycerite formulations and used as a measure of sample homogeneity. It was determined by a modification of the standard EDTA complexometric scheme[7]. An amount of 1 g of the formulation, *ca.* 30 ml of water, and 5 ml of a pH 10 buffer (70 g ammonium chloride plus 570 ml of ammonium hydroxide diluted to 1 l) were added to a 150-ml beaker. One drop of 10 mg/ml Eriochrome Black T was added and the solution titrated to a light blue endpoint with 1 mg/ml disodium EDTA.

Sample analysis

The amount of α-2-deoxy-D-glucose present in the formulation expressed in percent by weight, was calculated from the following equation:

$$\text{percent α-2-deoxy-D-glucose} = \frac{\text{peak area sample}}{\text{peak area standard}} \times \text{weight of standard (mg)}$$

Placebo and topical samples "A" and "B" were starch glycerite formulations.

RESULTS AND DISCUSSION

Although standard NH_2 columns are usually not useful for sugar analyses, a typical column was able to effect a suitable separation of α-2-deoxy-D-glucose in

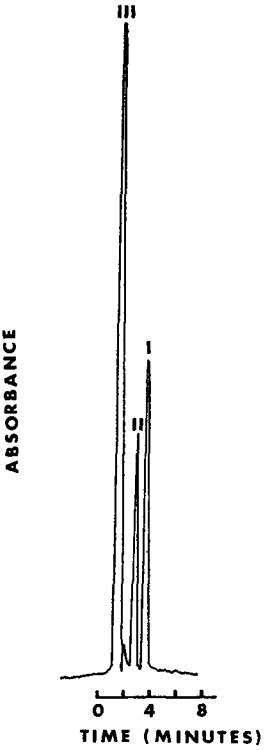

Fig. 2. Chromatogram of α-2-deoxy-D-glucose (I) in a topical formulation; (II) and (III) are excipient peaks.

topical formulations. The α-2-deoxy-D-glucose peak was separated from several excipient peaks (Fig. 2). Linearity of standards was acceptable from 50 μg/ml to 2.5 mg/ml. To determine the accuracy of the method, four analytically prepared samples of formulation "A" were assayed and the results appear in Table I. The average recovery was 5.07% or 101% of the 5.00% nominal value. The 1σ relative standard

TABLE I

METHOD VALIDATION DATA FOR THE DETERMINATION OF α-2-DEOXY-D-GLUCOSE (DDG) IN TOPICAL FORMULATIONS

Added % DDG	*Found % DDG*	*Formulation "A" recoveries, FN "A"*				*Precision and homogeneity, FN "B"*			
		Label % DDG	*Actual % DDG*	*Label % zinc*	*Actual % zinc*	*Label % DDG*	*Actual % DDG*	*Label % zinc*	*Actual % zinc*
5.00	5.00	2.00	1.84	1.40	1.45	8.00	8.17	1.40	1.37
5.00	5.21	2.00	2.02	1.40	1.35	8.00	7.98	1.40	1.43
5.00	5.00	2.00	5.24	1.40	1.49	5.00	5.04	1.40	1.31
5.00	5.10*	5.00	5.22	1.40	1.41	5.00	5.04**	1.40	1.36

* $\bar{x}$ = 5.07% (5.00% nominal), 101% recovery, relative standard deviation = 1.6%.
** Average relative standard deviation = 2.1%.

deviation for the five samples was 1.6%. The average relative standard deviation of the eight samples listed in Table I containing from 2 to 8% α-2-deoxy-D-glucose was 2.1%. For the batch prepared samples, homogeneity was inferred by analysis for zinc. The precision of the method itself is probably more realistically represented by the analytically prepared samples since the set of precision samples was batch prepared and analysis of the sample reflected some error due to inhomogeneity.

SELECTIVITY

Formulations "A" and "B" were thermally-stressed for 48 h at 50°C and stored at room temperature for 2 weeks. The thermally-stressed samples contained less α-2-deoxy-D-glucose than the initial samples.

The chromatograms of the stressed samples contained additional peaks, some of which interfered with the assay when column 2 was used. The same solutions were then chromatographed using the column 1 and satisfactory results were obtained. Both columns were new at the beginning of the study. This may suggest that not all the amine columns are suitable for analysis of this type. Column 1 continued to perform satisfactorily to the end of the study (*ca.* 100 injections). As was noted before, topical formulations present a particularly complex matrix to a chromatographic system, especially when the crude sample is introduced, and hence the relative merits of the two columns presented here may not be representative of simpler systems.

In summary, the method presented is sufficiently accurate and precise to determine α-2-deoxy-D-glucose in topical formulations using a standard amine column and ultraviolet detection.

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