Journal of Chromatography, 202 (1980) 319–322
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,217

Note

High-performance liquid chromatographic method for the analysis of D-arabino-2-hexosulose (D-glucosone)

JOHN GEIGERT*, DAVID S. HIRANO and SAUL L. NEIDLEMAN
Cetus Corporation, 600 Bancroft Way, Berkeley, CA 94710 (U.S.A.)
(First received July 11th, 1980; revised manuscript received August 5th, 1980)

D-Glucosone is the key chemical intermediate in a new process for manufacturing crystalline fructose from glucose. This process involves a combined enzymatic oxidation and chemical reduction reaction run in an aqueous system[1].

Reliable measurement of glucosone has been limited. A colorimetric assay —the reaction of triphenyltetrazolium chloride— has been developed but interference from D-glucose, D-gluconic acid and other reductants occurs[2]. A gas chromatographic assay —analysis of the trimethylsilylated derivative— has also been developed but it is time consuming (rigorous removal of water from the samples is required) and qualitative at best, owing to the heat lability of the derivatives formed[3].

In this paper is reported a reliable, rapid high-performance liquid chromatographic (HPLC) method for the measurement of D-glucosone.

EXPERIMENTAL

High-performance liquid chromatography

A Spectra-Physics 8000 high-performance liquid chromatograph equipped with dual detectors —refractive index (RI) and ultraviolet (UV) at 192 nm— was used. Aqueous samples (10 μl) were injected onto a 30 cm × 4.6 mm I.D. Waters Assoc. (Milford, MA, U.S.A.) carbohydrate analysis column (10 μm). The mobile phase was 20% aqueous acetonitrile containing 0.003 *M* (final) potassium phosphate buffer (pH 6.0). Flow was set at 2.0 ml/min and column temperature was set at 25°C. Attenuation was 8 × on the RI detector and 0.25 a.u.f.s. on the UV detector.

Standards

The sugar-type compounds shown in Table I were used. All standards were prepared in aqueous solution. Concentrations were 30 mg/ml, unless otherwise noted.

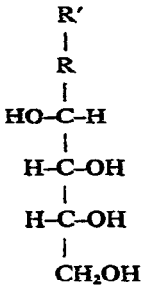
RESULTS AND DISCUSSION

Separation of sugars on the carbohydrate analysis column using an aqueous acetonitrile mobile phase is a well-documented method[4]. Although D-glucosone also eluted under these standard conditions, loss of component was occasionally observed, especially when a new column was used. It is possible for D-glucosone, which has a

0021-9673/80/0000–0000/$02.25

TABLE I

STRUCTURE AND SOURCE OF SUGAR-TYPE COMPOUNDS



No.	*Name*	*R*	*R'*	*Source*
I	D-Glucose	H–C–OH	CHO	Applied Science Corp., State College, PA, U.S.A.
II	D-Gluconic acid	H–C–OH	CO_2H	Applied Science Corp.
III	D-Fructose	C=O	CH_2OH	Applied Science Corp.
IV	D-Glucosone	C=O	CHO	Enzymatically synthesized from glucose[4]
V	D-2-Ketogluconic acid	C=O	CO_2H	Sigma, St. Louis, MO, U.S.A.

reactive aldehyde functional group, to form Schiff bases with the column packing resulting in irreversible adsorption[5]. Also, it is known that D-glucosone, which is chemically unstable, decomposes in the presence of traces of base[6]. To overcome these problems, the mobile phase was buffered on the acidic side (pH 6.0). Reliable measurements were then possible. Fig. 1 shows the measurement of D-glucosone using RI detection and UV absorption at 192 nm.

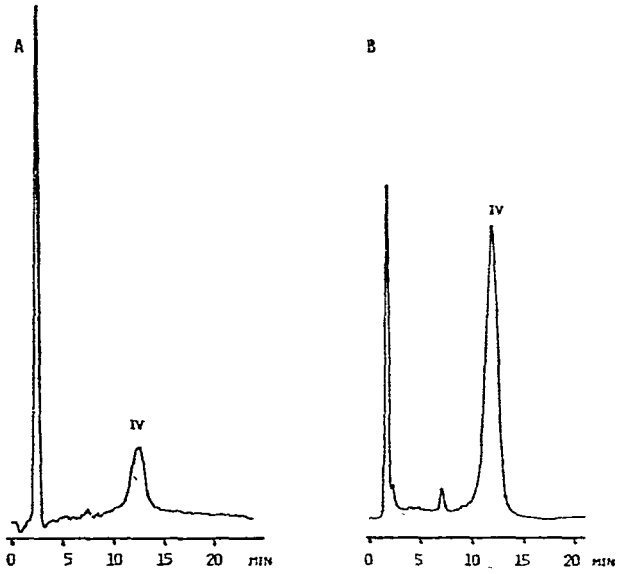


Fig. 1. Chromatograms of D-glucosone (IV) using RI detection (A) and UV detection at 192 nm (B).

The minimum detectable quantity of D-glucosone is 20 μg using RI detection and 0.1 μg using UV detection at 192 nm. The precision of this assay method is 10.1% standard deviation and 7.3% standard deviation for D-glucosone standards of 10 mg/ml and 30 mg/ml, respectively; assayed fourteen times over a 48-h time period, using peak areas obtained with the RI detector. A linear relationship is found between peak area and D-glucosone concentration in the range of 8 mg/ml through 100 mg/ml D-glucosone, using the RI detector.

The chromatographic conditions developed also permitted the measurement of the other sugars in the commercial process. Fig. 2 shows the separation of D-glucose and D-fructose in the presence of D-glucosone. To be able to measure simultaneously both D-glucose and D-glucosone is important. Low levels of D-glucose, were they to be carried over in the process, might interfere with the crystallization of D-fructose.

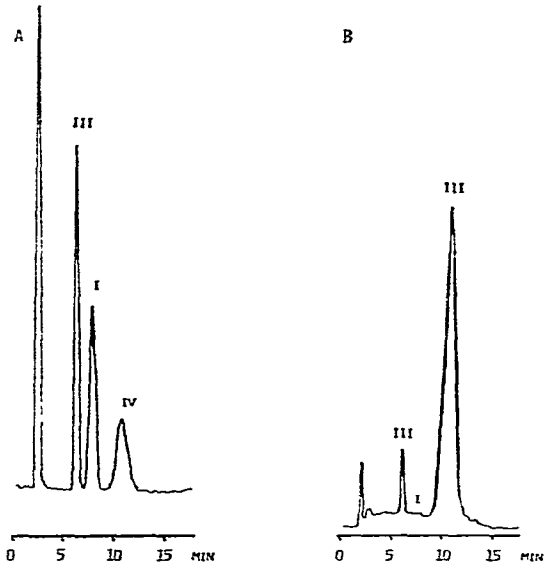
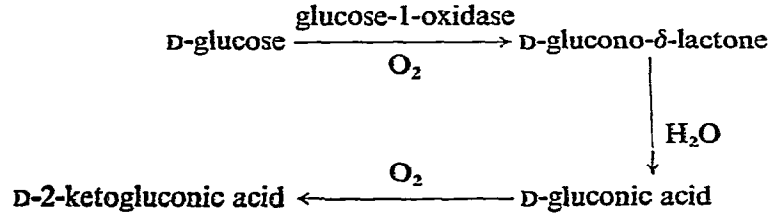


Fig. 2. Chromatograms of D-glucose (I), D-fructose (III) and D-glucosone (IV) using RI detection (A) and UV detection at 192 nm (B).

Since the first step of the process is an enzymatic step, a competing enzymatic reaction on D-glucose was possible. A potential competing enzyme would be glucose-1-oxidase:



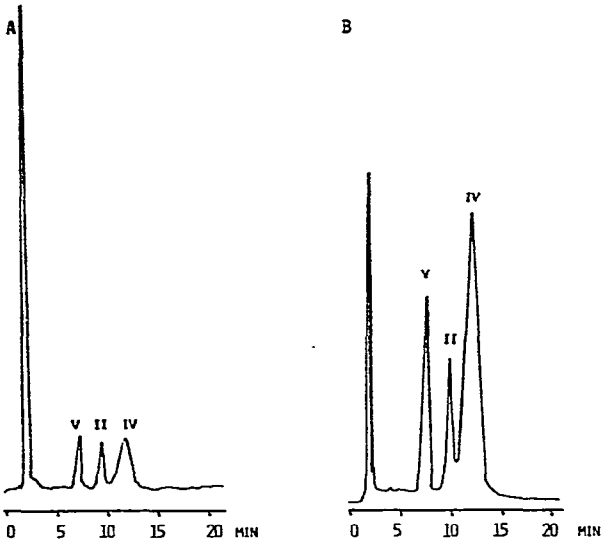


Fig. 3. Chromatograms of D-gluconic acid (II), D-glucosone (IV) and D-2-ketogluconic acid (V) using RI detection (A) and UV detection at 192 nm (B). Compounds II and V are each 10 mg/ml.

Fig. 3 shows the separation of D-gluconic acid and D-2-ketogluconic acid in the presence of D-glucosone. D-Glucono-δ-lactone eluted at 4 min under these conditions. The presence of acidic buffer in the mobile phase is important for these compounds. The absence of acidic buffer in the HPLC mobile phase caused the D-gluconic acid to elute with broad tailing and the D-2-ketogluconic acid to not elute at all.

ACKNOWLEDGEMENT

Our thanks are due to Dr. T. N. Liu for the enzymatic synthesis of D-glucosone.

REFERENCES

1 S. L. Neidleman, W. Amon and J. Geigert, *U.S. Patent*, Application Serial No. 42,219 (1979).
2 J. Volc, P. Sedmera and V. Musilek, *Folia Microbiol.*, 23 (1978) 292.
3 J. Volc, M. Wurst and V. Musilek, *Folia Microbiol.*, 23 (1978) 448.
4 J. K. Palmer, *Anal. Lett.*, 8 (1975) 215.
5 B. B. Wheals and P. C. White, *J. Chromatogr.*, 176 (1979) 421.
6 B. Ericsson, B. O. Lindgren and O. Theander, *Cellulose Chem. Technol.*, 7 (1973) 581.