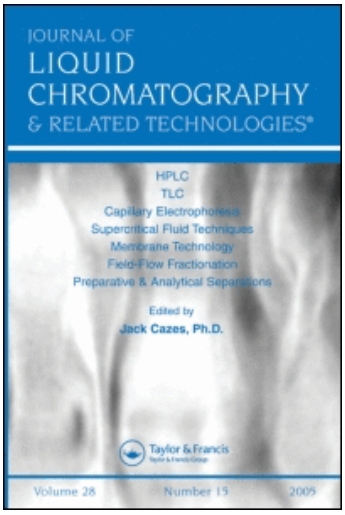

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S. R. Mirmira[a]; M. A. Schreiber[a]; S. A. Guessford[a]
[a] Analytical Research and Development The Du Pont Merck Pharmaceutical Company, Wilmington, Delaware

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HIGH PERFORMANCE ION CHROMATOGRAPHIC ANALYSIS OF GLUCOSE, ISOMALTOSE, AND MALTOSE IN HYDROXYETHYL STARCH

S. R. MIRMIRA, M. A. SCHREIBER, AND S. A. GUESSFORD
Analytical Research and Development
The Du Pont Merck Pharmaceutical Company
Wilmington, Delaware 19880-0353

ABSTRACT

A High Performance Ion Chromatographic (HPIC) method for the quantitation of glucose, isomaltose and maltose in hydroxyethyl starches is described. Hydroxethyl Starches are a family of amylopectin which has been ethoxylated. During the manufacturing and the shelf life of the finished product solutions, mono- and di-saccharide units may be generated as degradation products from the hydrolysis of starch. Those found are maltose from the 1,4-linkage, isomaltose from the 1,6-linkage, and glucose. These are non-toxic substances but are of concern if high levels are administered to diabetic patients. Separations were performed using anion exchange chromatography with a Carbopac PA1 column and 100 mM sodium hydroxide as the mobile phase. The method was found to be linear over the range of 0.1 to 20.0 µg/mL for glucose, 0.2 to 20.0 µg/mL for isomaltose, and 0.5 to 20.0 µg/mL for maltose. The limits of quantitation were found to be 0.5 µg/mL for glucose and isomaltose and 1.0 µg/mL for maltose. Method precision of 2% RSD was found at the limit of quantitation for each component.

INTRODUCTION

Hydroxyethyl starches (HES) are a family of semi-synthetic colloids currently marketed as plasma volume expanders (1) and is an adjunct in the treatment of shock in trauma patients due to blood loss. Pentastarch and hetastarch are the names which refer to the low and

high molecular weight (MW) and low and highly substituted HES, respectively. HES are multi-branched molecules manufactured from amylopectin which is hydrolyzed and hydroxyethylated. The weight average molecular weight of pentastarch is between 150,000 and 350,000 and that of hetastarch is 400,000 to 550,000. During the manufacturing process, mono- and di-saccharide units may be generated from the hydrolysis of starch. These are maltose from the 1,4-linkage, isomaltose from the 1,6-linkage, and glucose. In order to measure mono- and di-saccharides (related substances) in hydroxyethyl starch drug substance and solutions, an easily automated procedure with limited sample preparation was needed. A High Performance Ion Chromatography (HPIC) method has been developed to quantitatively measure these HES related substances.

Several analytical methods for the quantitation of mono- and di-saccharides have been described in the literature. These methods include analyses by HPLC (2-5), gas chromatography(6,7), GLC/MS (8), and anion-exchange HPLC with pulsed amperometric detection (9-13). However, they involve extensive time-consuming sample preparation such as derivatization, deproteinization, sample purification, colorimetric reaction, and extraction to achieve better separations and detection. Anion-exchange HPLC with a sodium hydroxide mobile phase at pH greater than 12, using pulsed amperometric detection is a superior method for separation and quantitation of mono- and di-saccharides in the presence of HES. The reported method involves a straight dilution, separation with an anion-exchange column, followed by pulsed amperometric detection. This method has high selectivity without interference from HES.

EXPERIMENTAL

Reagents

Glucose USP, isomaltose (98%; Sigma) and maltose (98%; Sigma) were used as standards. The sodium hydroxide 50% (w/w) solution, (Certified, Fisher Scientific), sodium acetate trihydrate, (HPLC grade, J. T. Baker) and water (Milli-Q™ grade) were used.

Equipment

All experiments were carried out on a system consisting of an autosampler (Perkin Elmer LC 600), Dionex GPM-II pump, and a Dionex PAD-II detector. The PAD consisted of a gold working electrode with reference electrode filled with mobile phase. The applied potentials were E1=0.05V, E2=0.6V and E3= -0.80V with pulsed duration of 300, 120, and 300 ms, respectively. All data were collected and analyzed on a Fisons Multichrom™ data acquisition system.

Standard Preparation

Standards were prepared to achieve concentrations of 0.5, 1.0, and 2.0 μg/mL for glucose and isomaltose and 1.0, 2.0, and 4.0 μg/mL maltose. These standards are stable for 48 hours at 4°C.

Sample Preparation

1.00 g of hydroxethyl starch drug substance was dissolved in 100 mL water. 10.0 mL of pentastarch 10% (w/w) Pentaspan® and 8.0 mL of hetastarch 6% (w/w) Hespan® solutions were pipetted into separate 100 mL volumetric flasks and diluted to volume with water. These samples were thoroughly mixed and injected on the HPIC.

Chromatography

All experiments were conducted at ambient temperatures and separations were performed using anion-exchange chromatography with a Carbopac PA1 column. The mobile phase consisted of 100 mM sodium hydroxide. The mobile phase was filtered and degassed through a 0.45 μm filter (Millipore HA 0.45 μm) under vacuum. The mobile phase was stored in a polyethylene container and sparged with a low flow of helium during the run. The mobile phase flow rate for the

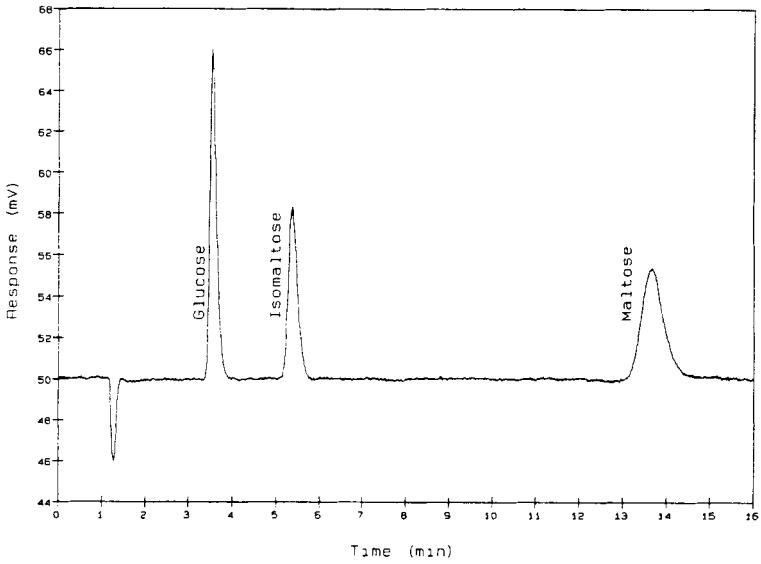

FIGURE 1. High Performance Ion Chromatogram of glucose, isomaltose, and maltose on a Carbopac PA1 column demonstrating the separation by this method.

analysis was 1.0 mL/min. After each run, the column was rinsed with 1 M sodium acetate for 2 to 3 hours. During this wash cycle, the column was detached from the detector. This wash cycle removes any compounds that do not elute under these chromatographic conditions. Since hydroxyethyl starches are a heterogeneous multi-branched molecule containing a wide range of molecular weights, some of the molecules are retained on the column and cause the retention time to decrease over time. A chromatogram demonstrating the separation is presented in Figure 1.

RESULTS AND DISCUSSION

The recovery of the method was determined by spiking known concentrations of glucose, isomaltose and maltose in hydroxyethyl starch

TABLE 1

Mean Recoveries for Glucose, Isomaltose and Maltose at the Three Spiked Levels

	% GLUCOSE	% ISOMALTOSE	% MALTOSE
Low	99.3	100.3	102.2
Middle	99.3	100.5	106.8
High	98.1	101.2	101.6
Grand Mean	99.1	101.3	103.5
Std. Dev.	1.0	0.9	2.8
% RSD	1.0	0.9	2.7

samples. Samples were accurately prepared in triplicate at approximate concentrations of 0.5, 1.0 and 2.0 μg/mL for glucose and isomaltose, and 1.0, 2.0, and 3.0 μg/mL maltose. The samples were chromatographed by the procedure described with a mean recovery of 99.1% for glucose, 101.3% for isomaltose and 103.5% for maltose for each each component (Table 1). Calibration curves were determined over the range of 0.5 to 2.0 μg/mL for glucose and isomaltose, and 1.0 to 4.0 μg/mL for maltose. Linear correlation coefficients of 0.99995 for glucose, 0.99996 for isomaltose, and 0.99999 for maltose were achieved .

System precision was determined by the following experiments. Six replicate determinations of hydroxyethyl starch sample spiked with 0.5, 1.0 and 2.0 μg/mL of glucose and isomaltose, and 1.0, 2.0 and 3.0 μg/mL of maltose were analyzed to obtain a measure of HPIC system precision. Maximum relative standard deviations of 1.5% for glucose, 1.9% for isomaltose, and 1.8% for maltose were obtained. No chromatographic interferences were detected. Limits of quantitation for hydroxyethyl starch powder and solutions are 0.5 μg/mL for glucose and isomaltose, and 1.0 μg/mL for maltose. The detection limits in the sample solutions for glucose, isomaltose, and maltose were 0.05 μg/mL, 0.1 μg/mL, and 0.5 μg/mL, respectively (Figure 2). A typical sample chromatogram is shown in Figure 3.

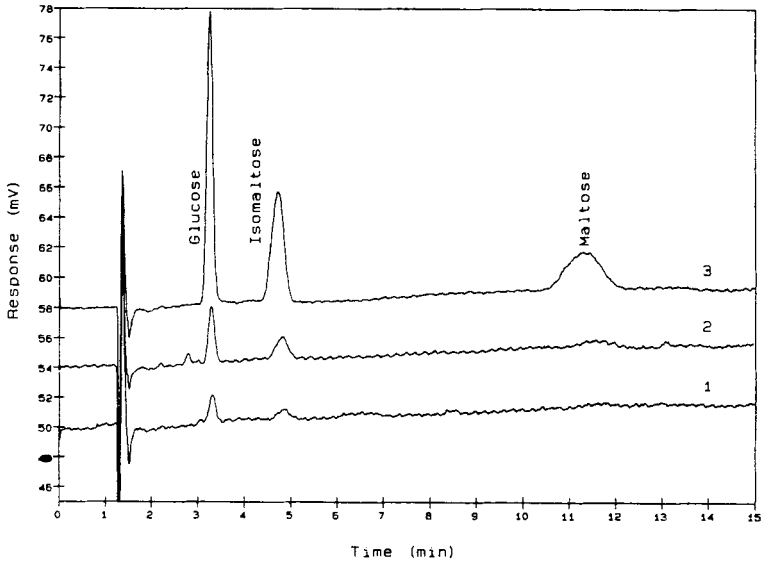

FIGURE 2. Detection limits for (1) glucose, 0.05 µg/mL; (2) isomaltose, 0.1 µg/mL; and (3) maltose, 0.5 µg/mL.

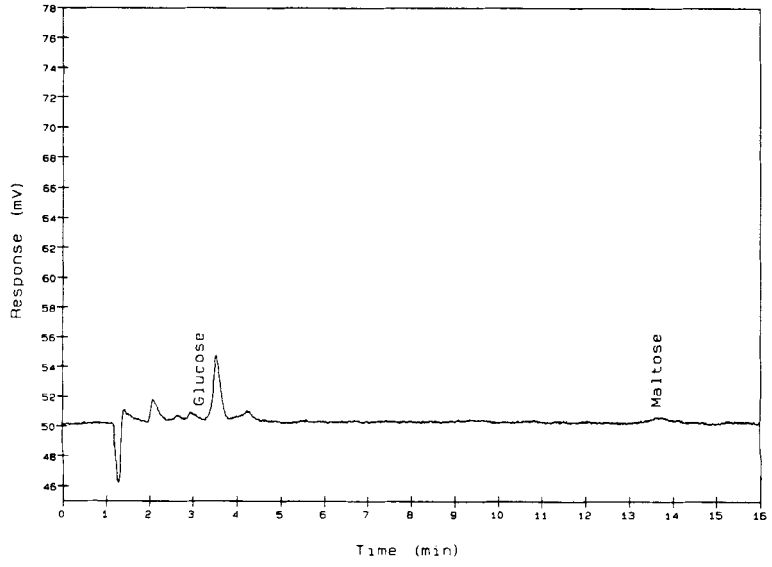

FIGURE 3. Pentastarch sample prepared and analyzed as described in the text.

CONCLUSIONS

This method has been proven to be simple and reliable for the determination of low levels of glucose, isomaltose, and maltose in hydroxyethyl starch powders, 10% pentastarch and 6% hetastarch solutions. The accuracy and precision of the method is suitable for use in a research or quality control laboratory.

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