Determination of Glucose and Fructose from Glucose Isomerization Process by High-performance Liquid Chromatography with UV Detection

N A.Rahman

Department of Chemical Engineering & Process

Faculty of Engineering, Universiti Kebangsaan Malaysia

43650 Bangi, Selangor, Malaysia.

E-mail:liza@vlsi.eng.ukm.my

M.Hasan & M.A.Hussain

Department of Chemical Engineering, Faculty of Engineering, Universiti Malaya

50603K.Lumpur, Malaysia

E-mail:masitahhasan@um.edu.my

J Jahim

Department of Chemical Engineering & Process

Faculty of Engineering, Universiti Kebangsaan Malaysia

43650 Bangi, Selangor, Malaysia.

E-mail:liza@vlsi.eng.ukm.my

Abstract

Analysis of fructose and glucose from glucose isomerization process using immobilised glucose isomerase (IGI), {Sweetzyme from Novozymes} are often performed by HPLC methods with refractive index (RI) detector. This study is focused on developing new methods of measuring glucose and fructose using a specific carbohydrate column. The importance of this research, primarily based on the performance of the HPLC with ultra-violet (UV) detection as another alternative of detector rather than using RI . The method was carried out under the following condition; UV detection was made at 195 nm with column temperature of 30^0C , flow rate of 0.6 ml/min and injections of 20μL . The ratio of acetonitrile and the deionised water used was 80% to 20%. From the results, the detection of fructose and glucose by HPLC with acetonitrile and water as solvents can be obtained using UV detection (195nm) instead of the commonly used detector of RI.

Keywords: Isomerization, HPLC, Glucose, Fructose ,UV detection

1. Introduction

Enzymatic reaction is a chemical reaction with enzymes acting as biological catalysts. According to Shuler & Kargi(1992), under ambient conditions, the presence of enzymes result in much higher reactions rates as compared to chemically reactions. The role of enzyme catalysis in organic chemistry and bioprocess technology has increased tremendously in the last decade. According to Harmand et al (2004), two types of biological processes exist, microbiological and enzymatic reactions.

Isomerization of D-glucose to D- fructose by immobilized glucose isomerase is one example of enzymatic reaction. This reaction is a reversible reaction and important industrial process to produce high fructose syrup (HFS) with at least 50% conversion of glucose to fructose. The discovery of glucose isomerase started in 1957 by Marshall & Kooi (1957) who carried out enzymatic isomerization in batch reactors with soluble enzymes of immobilized glucose isomerase (IGI).

Detection of fructose and glucose in this process have widely used HPLC column using RI detector such as by Gram & Bang (1990) followed by several researchers (Crabb & Shetty , Bhosale.; Rao.& Deshpande , Salehi, Sohrabi , Kaghazchi.& Bonakdarpour , Lee. &Hong). Another paper (Rački et al..(1991) reported a Dische-Borenfreund method for determination of fructose concentration.

In the present work, we demonstrate the use of HPLC column by UV detection to measure glucose and fructose using a carbohydrate column, instead of using RI detector. This research differ from the work done by Slimestad &

Vågen. [10] in terms of range of detector and procedure of HPLC. In their study, Slimestad & Vågen (2006) used evaporative light scattering (ELSD) detection of 230±4nm and the solvent gradient consisted of a linear increase in the amount of water in acetonitrile. The ability of the method proposed to analyze fructose and glucose is demonstrated under various operating conditions of the reaction.

2. Materials and methods

The materials for this study are: D-glucose(G), D-fructose(F) and $MgSO_4.7H_2O$, obtained from R&M Chemical,UK; 12g of Immmobilised Glucose Isomerase (IGI) of *S.murinus,*(brown cylindrical shape granules, diameter 0.3 – 1.0 mm, length 1.0- 1.5mm activity 350 IGIU/g) from Sweetzyme, Novozymes; deionised water and acetonitrile (HPLC grade). The standard solutions were prepared in the following ways ; with 2g/100mL each of G and F and diluted with distilled water. All analytical samples were diluted with distilled water and filtered through 0.2μm Nylon filters prior to HPLC-analysis.

The HPLC system in this study is an Agilent 1100 with diode array detector. UV detection was made at 195 nm   with column temperature of 30^0C. The flow rate was set to 0.6mL/min and injections of 20μL were made. The column used was Supelco Kromasil NH_2 column (250mm x 4.5mm,5 μm). The ratio of acetonitrile and the deionised water used was 80% to 20%. A guard column was attached to the inlet of the Kromasil column to prevent clogging.

3. Results and discussion

Table 1 shows the retention time t_R(min) and the area (mAUs) of glucose and fructose by HPLC-UV at different concentrations on a Kromasil NH_2 column.

From Table 1 it can be confirmed that fructose (mainly) and glucose can be determined using UV detector instead of the commonly used RI detector. The average retention time of fructose was 14.2 min, and 16.25 min for glucose. Figure 1 shows the standard curve for fructose and glucose at a specific concentration range. The values of R^2 for both of fructose and glucose confirmed that the results were statistically reliable. Figure 3 shows the HPLC result in this study for detection of fructose and glucose   using the Kromasil column at a 0.5% concentration of fructose and glucose. The detection of fructose was faster compared to the glucose which was similar in trends to given by the supplier (Kromasil) who suggested using RI as the detector, as seen in Figure 2 and the research by Slimestad and Vågen (2006). This occurs because fructose has been described as the first step in the hydrothermal degradation of glucose. Fructose and glucose are isomers which have similar molecular weight but different in terms of the arrangement or configuration of the atoms (Hawley,2001).

Comparable analyses with other method for detection of glucose was made by the DNS method (Miller,1959) for reducing sugar, and using a UV spectrophotometer (Shimadzu) at wavelength of 550nm for a sample obtained from the same operating reaction conditions. The result show that the   glucose concentration using   HPLC –UV was 12.8 g/L whereas for UV spectrophotometer (Shimadzu) was at 14.96 g/L. However the spectrophotometer measurement could not distinguish glucose from fructose, as they exist as isomers in the mixture. Hence resulting in the higher value.

4. Conclusion

The results imply that the detection of fructose and glucose by UV (195nm) with acetonitrile and water as solvents can be obtained using HPLC instead of commonly used detector of RI.

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Table 1. The retention time t_R (min) and the area (mAUs) of glucose and fructose by HPLC-UV at different concentrations on a Kromasil NH_2 column

Conc.	Area [mAU.s]	
[g/100 ml]	Fru	Glu
0	0	0
0.5	2101	721
1	5329	1939
1.5	8532	3080
2	12019	4410
R.time	14.2	16.3

Fructose + Glucose Standard Curve

Figure 1. Standard curve for HPLC result for detection of fructose and glucose using Kromasil column by UV detector.

Figure 2. HPLC result for detection of   fructose and glucose using Kromasil column by RI detector

Figure 3. HPLC result for detection of fructose and glucose at a 0.5% concentration of fructose and glucose using Kromasil with UV detector.