Liquid Chromatography Characterisation of Wood Sugars

S.C.PURI AND S.M.ANAND
Regional Research Laboratory,
Canal Road, Jammu Tawi-180001

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Summary: A method has been developed for analysis of wood sugars in samples from the pulp and paper industry. This high performance liquid chromatography (HPLC) method makes it possible to separate sugars using isocratic elution with water/methanol/ethyl formate. The separation of free glucose, mannose, galactose, arabinose and xylose present in quite unequal proportions in black liquor has been achieved.

There is a need in the field of pulp and paper industry for a rapid and more accurate method for the determiantion of free sugars normally obtained by hydrolysis of wood products. The traditional paper chromatography method [1] is laborious and time consuming for separating arabinose, xylose, mannose, glucose and galactose from the high concentrations of glucose normally encountered as a result of cellulose degradation. If the concentration of single sugar exceeds 4 mg/ml in the mixture, the original solution has to be diluted and re-run before conclusive reflectance measurements can be taken [2]. The major drawbacks of this method are the length of analysis time and insufficient resolution.

Gas chromatographic analysis of monosaccharides has received [3-5] much attention due to a result of pioneering work of Sweely and coworkers with trimethylsilyl derivatives. There are also reports of gas chromatographic separation of alditol acetates. The above methods are tedious because of extensive sample preparation procedures for analysis.

This paper describes separation of wood sugars by HPLC which offers potential advantages over GC. Liquid chromatography of sugars has recently been reported for being able to separate the sugars present in varying ratios more efficiently and accurately. In general it does not require derivatisation and preparation of samples. Earlier methods were reported in which the separation of sugars had been achieved using ion-exchange resin and borate buffers. The corrosiveness of these reagents posed [7-8] problems in routine work. There are reports in which the separation of unmodified sugars by HPLC have employed packings of amine or nitrile bonded phases [9-13] amine-treated silicic acid [14-15] and cation exchange materials [16]. The sugars have also been analysed using refractive index detection [14-15], uv absorption [17] at short wave lengths, post column derivatisation [9] liquid scintillation counting [18,19] or mass spectrometric detections [10]. The derivatised sugars carrying aromatic substituents have excellent sensitivity to uv absorption [20] at 254 nm or flourscence detection [21] which can be analysed by reverse phase HPLC. The present work on HPLC is directed towards the establishment of an efficient and quick method for the separation of

wood sugars that occur in close ratios using a μ-Bendapak/carbohydrate column.

Experimental

Equipment and column packing.

All the work was done on HPLC (Water ALC/GPC-244) equipped with a Model 6000A solvent delivery system; R-400 differential refractometer detector sensitive to a change of $1x10^{-7}$ refractive index units; attenuation 1/4 64x; and a Model UK Universal injector, Waters Associates, Inc. Milford, MA. The elution was done with water/methanol/ethyl formate 1:2:6 with a flow rate 2 ml/min.

A normal phase liquid chromatography was carried out using packing material -BONDAPAK/carbohydrate, stainless steel column (300 mm x 3.9 mm ID), Waters Associates.

Materials

The sugar standards were of highest purity obtained from Fluka chemicals, Switzerland. The pulping liquors were obtained from a commercial straw based paper mill. All the chemicals used were of A.R. grade. Methanol and ethyl formate of spectrophotomeric grade were obtained from E. Merck Reagents. The water was distilled in glass, deionised, and redistilled. All the standards were prepared in concentrations and ratios approximate to those expected to permit accurate integration and calculation of sugars in samples. Fresh standards were prepared by dilution of frozen stock solution.

Procedure

100 ml of pulping liquor was acidified with dil. acetic acid to pH 7 and filtered. The filtrate was evaporated to dryness on steam bath under reduced pressure. The residue was titurated with 3ml cold 72% H_2SO_4 for 2 hours. The reaction contents were diluted to 150 ml with water and refluxed for 4 hours and filtered. The excess of acid in the filtrate was removed by adding $BaCO_3$ solution till all the sulphate ions were precipitated as $BaSO_4$. The filtrate evaporated to dryness in rotary film evaporator and dried under vacuum. To 5 mg of the residue was added 3 ml of H_2O and filtred through a whatman filter paper. The filtrate was diluted to 5 ml with H_2O and subjected to HPLC for analysis.

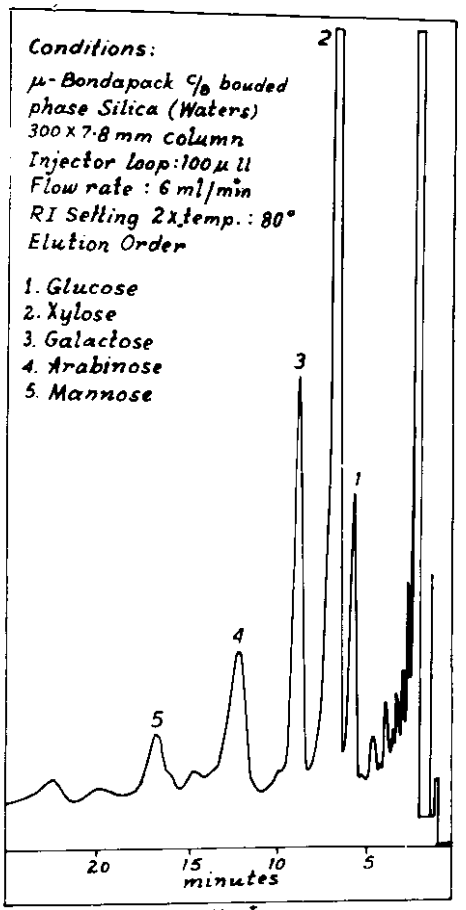

Fig. I

Standard HPLC Chromatogram of five wood Sugars.

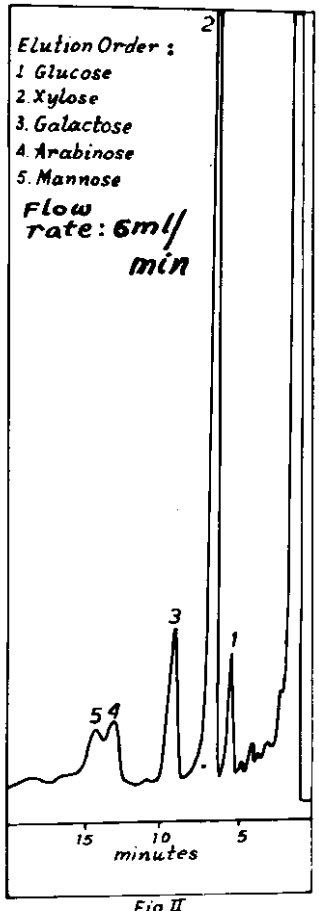

Fig II
HPLC Chromatogram of free Sugar in pulping liquors.

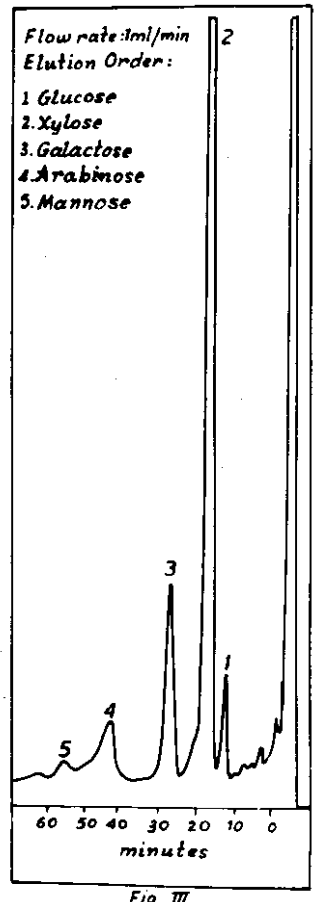

Fig. III
Separation of wood sugars in pulping hydrolysates.

Results and Discussion

A typical chromatogram of standard solution alongwith the chromatographic conditions is shown in Fig.1. The separation of peaks is sufficient for quantification. Fig. II. shows the typical separation of five sugars in which the resolution of first three sugars is closer to the base line. In this column the separation of glucose, xylose and galactose was satisfactory as long as their concentration is same. In our sample the arabinose and mannose are in the ratio of 1:100 and these two sugars could not be separated as arabinose was lost in the mannose peak. Fig. III shows separation of arabinose and mannose in which the flow rate was reduced and the analysis time increased to sixty minutes. By this method all five wood sugars could be resolved even in the presence of traces of lignin.

Conclusion

The HPLC method of analysis of five wood sugars in any ratio has been developed. The advantage of HPLC method is that it is a quick and sensitive technique, thus the samples of wood sugar hydrolysates can be analysed for free sugars on μBondapak/carbohydrate bonded

phase silica (Waters Associates) columns which are commercially available.

References

1. J.E.Jeffery, E.V.Partlow and W.J.Polglase, *Anal.Chem.*, **32**, *1174 (1960)*
2. F.E.Wenz, A.D.Mercey and M.J. Gary, *Chromatogr.Sci.*, **20**, *249 (1982)*
3. C.C.Sweely R.Bentley, M. Makita and W.D.Wells, *J.Am.Chem.Soc.*, **85**, *2497 (1963)*
4. P.K.Davison and R.Young, *J.Chromatogr.*, **41**, *12 (1969)*
5. C.W.Ford, *Anal.Biochem.*, **57**, *413 (1974)*
6. L.A.T. Verhaar and B.F.M. Kuster, *J.Chromatogr.*, **220**, *313 (1981)*
7. J.S.Khym and L.P. Zill, *J.Am.Chem.Soc.*, **73**, *2399 (1951)*
8. J.H.Khym and L.P.Zill, *J.Am.Chem.Soc.*, **74**, *2090 (1952)*
9. R.Macrae and J.Dick, *J.Chromatogr.*, **210**, *138 (1981)*
10. A.Beersma, G.Lamblin, P. Degand and P.Roussel, *Carbohydr.Res.*, **94**, *C7 (1981)*
11. G.J.L.Lee, D.W.Liu, J.W.Pav and H.Tieckelmann, *J.Chromatogr.*, **212**, *65(1981)*
12. K.Aitzetmuller, *J.Chromatogr.*, **156**, *354 (1978)*
13. B.B.Wheals and P.C.White, *J.Chromatogr.*, **176**, *421 (1979)*
14. C.A.White, P.H.Corran, J.F. Kennedy, *Carbohydr.Res.*, **87**, *165 (1981)*
15. L.A.T.Verhaar and B.F.M.Kuster, *J.Chromatogr.*, **210**, *279 (1981)*
16. H.Binder, *J.Chromatogr.*, **189**, *414 (1980)*
17. S.J.Mellis and J.U.Baenzinger, *Anal.Biochem.*, **114**, *276 (1981)*
18. S.J.Turco, *Anal.Biochem.*, **118**, *178 (1981)*
19. B.Kloarge, *J.Chromatogr.*, **236**, *217 (1982)*
20. S.Hase, T. Ikenaka and Y.Matsushima, *J.Biochem.*, **90**, *407 (1981)*