

DETERMINATION OF THE ANOMERIC COMPOSITION  
OF GLYCOSIDE MIXTURES BY HIGH PERFORMANCE  
LIQUID CHROMATOGRAPHY (HPLC)

V.R.N. Munasinghe and S. Abeysinghe  
Dept. of Chemistry, University of Colombo

In general  $^1\text{H}$  NMR methods are routinely available to determine the composition of methyl glycoside mixtures in carbohydrate chemistry. However in the absence of high field NMR spectrometers we decided to investigate such compositions using HPLC. Though several publications are available for separation of free sugars<sup>1</sup> only a few are documented for separation of methyl glycosides<sup>2</sup>. In this communication we report the separation of  $\alpha$  and  $\beta$  mixtures of methyl D-arabinofuranosides and ribofuranosides as their benzoates on a commonly available HPLC column (silica gel;  $\mu$ -perasil column) in Sri Lankan laboratories. An eluant system of hexane : ethyl acetate (5:1) at a flow rate of 1 ml/minute was used. Both U.V. and R.I. detectors were employed.

Since both  $\alpha$  and  $\beta$  Me glycoside benzoates were required as reference markers, we also present a flash column chromatographic technique for a rapid separation of the 2 anomers from glycoside mixtures. This method readily made available the more inaccessible anomer of such mixtures. In addition to the furanosides, the  $\alpha$ : $\beta$  methyl - D - glucopyranoside tetrabenzoates were also separated by HPLC. The anomeric ratios obtained by HPLC (for the benzoates) were comparable to the  $^1\text{H}$  NMR ratios (for the benzoates) and hence to the  $^1\text{H}$  NMR ratios of the Me glycosides prior to benzylation. In conclusion we find that HPLC could be used as an alternative method to  $^1\text{H}$  NMR to determine the anomeric composition of methyl glycoside mixtures.

References:

1. Conrad E.C. (1976) Food Technol., 30:84
2. Cheetham N.W.H. and Sirimanne, P. (1981) J. Chromatogr., 208 100