CHROM. 3548

## Quantitative determination of 5-hydroxymethyl-2-furaldehyde

In his comprehensive review entitled *5-Hydroxymethyl-furfural* MOYE[1] discusses the qualitative and quantitative methods for determining 5-hydroxymethyl-furfural (HMF). The available methods are spectrophotometric and colorimetric determinations or chromatographic separations. In all cases the procedures are time-consuming and interferences by furfural or parent carbohydrates remain a strong possibility.

The use of gas chromatography in the study of carbohydrates has increased after SWEELY *et al.*[2] introduced the technique of trimethylsilylation of sugars. Using this derivatization technique a method was developed for the quantitative determination of HMF by gas chromatography. The procedure is rapid, quantitative, reproducible and free of interference from furfural or carbohydrates.

*Experimental*

The 5-hydroxymethyl-2-furaldehyde was purchased from Aldrich Chemical Co., Milwaukee, Wisc. The material was found to be chromatographically pure and was used as received. A Perkin-Elmer 800 gas chromatography unit with a flame ionization detector was used in this work. The column was a 6 ft. by 1/8 in. O.D. stainless steel tube. The coating was 8 % neopentylglycol adipate on a silanized Chromosorb W support. The column temperature was 139°, the injection block temperature 230°. Nitrogen flow was 35 ml per min with the stream split 80–20 in favor of the atmosphere. The determinations were carried out isothermally. A Honeywell single pen recorder of 1 mV sensitivity with full-scale deflection in 2 sec was used. The chart speed was 0.5 in. per min.

The HMF weighed to the nearest 0.1 mg was mixed with spectrophotometrically pure benzene and made up to volume. Aliquots of the solution were mixed with known volumes of TRI-SIL (Pierce Chem. Co., Ill.) and left for 3 min at room temperature. The trimethylsilyl ether derivative of the HMF was then examined gas chromatographically. A Hamilton 10 $\mu$l syringe was used and the injection volume was kept constant at 2 $\mu$l. The peak areas were determined with a planimeter.

*Results and discussion*

The relation between peak area and HMF quantity is shown in Table I. The

TABLE I

QUANTITATIVE DETERMINATION OF THE TRIMETHYLSILYL ETHER OF 5-HYDROXYMETHYL-2-FURALDEHYDE

| *HMF* ($\mu$g/$\mu$l) | *Trimethylsilyl HMF* ($\mu$g) | *Peak area units* |
|---|---|---|
| 0.39 | 0.78 | 18 |
| 0.72 | 1.44 | 35 |
| 1.44 | 2.88 | 69 |
| 2.16 | 4.32 | 103 |
| 2.40 | 4.80 | 113 |
| 2.88 | 5.76 | 144 |

TABLE III

DETERMINATION OF 5-HYDROXYMETHYL-2-FURALDEHYDE IN THE PRESENCE OF CARBOHYDRATES AND FURFURAL

| *Substance* | *Retention time (min)* | *Observations* |
|---|---|---|
| HMF | 7.0 | |
| D-Fructose | 8.0 | Second peak appears after 15 min when column temperature programmed to 180°. |
| D-Arabinose | 4.0 | |
| L-Rhamnose | 4.0 | |
| D-Lyxose | 5.0 | |
| 2-Deoxyribose | 3.0 | |
| D-Ribose | 6.0 | Second smaller peak appears at 6.5–7.0 min. |
| α-D-Glucose | 13.0 | |
| β-D-Glucose | 26.0 | |
| Sucrose | — | Appears when column temperature is programmed to 210°. |
| D-Xylose | 7.5 | Some interference but the peaks can be resolved. |
| Furfural | — | Does not appear. |

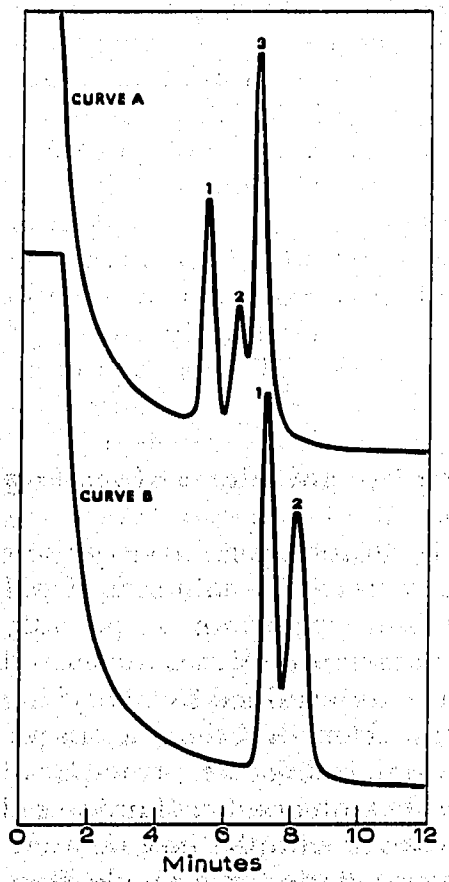


Fig. 2. Chromatographic separation of HMF from D-ribose and D-xylose. Curve A: 1 = D-ribose; 2 = D-ribose; 3 = HMF. Curve B: 1 = HMF; 2 = D-xylose.

The effect of carbohydrates on the quantitative determination of HMF was also examined. The experimental procedure, in this case, was as follows: A single sugar or a combination of carbohydrates were mixed with a known volume of TRI-SIL and left for 5 min at room temperature. To the mixture was then added a known amount of a benzene solution of HMF and after the appropriate waiting period the material was examined by gas chromatography. A typical chromatogram of a mixture containing several sugars and HMF is shown in Fig. 1. The retention times of various sugars, as compared to HMF, are shown in Table III. The retention times show that of the sugars tested only xylose and ribose interfere, but even in these two cases the peaks can easily be resolved. A chromatogram showing the level of interference by the two sugars is presented in Fig. 2.

Furfural has no hydroxyl group and, therefore, cannot form an ether derivative with the TRI-SIL reagent. Under the experimental conditions used furfural does not interfere with the HMF determination even when the concentration of furfural is three times that of HMF.

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## Analytical fractionation of plant and animal proteins by gel electrofocusing

The technique known as isoelectric focusing or electrofocusing involves the migration of protein molecules to the regions of their isoelectric pH values in a pH gradient. In the original procedure[1,2], the pH gradient is produced in a specially designed column by applying a voltage to a mixture of carrier ampholytes stabilized in a sucrose density gradient. The result of the fractionation is determined by analysing fractions drained from the column. The present communication describes a simple procedure for the rapid analysis of small amounts of protein using the principle of isoelectric focusing. By performing the fractionation in tubes of polyacrylamide gel containing protein sample and carrier ampholytes, a number of samples can be analysed simultaneously in several hours, compared with several days for a single fractionation by the original procedure.