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## Note

# Manual and automated determination of ω-deoxy sugars

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Acetaldehyde may be determined spectrophotometrically by its reaction with fructose, resorcinol, and hydrochloric acid[1]. The method is based on the interference[2] of acetaldehyde in the resorcinol assay for fructose[3], in which the wavelength of maximum absorption of the chromophore changes from 480 to 555 nm, and the absorption is greatly enhanced. The method enables[1] the quantitative determination of acetaldehyde in the presence of fixed quantities of fructose, by measurement of absorbances at 555 nm. Determination of the acetaldehyde released from the 1-methyl-1,2-diol system on oxidation with periodate provides a method for the determination of ω-deoxy sugars and their derivatives.

After oxidation of ω-deoxy sugars by periodate, excess reagent was reduced by addition of sodium hydrogen sulphite solution. This gave no apparent interference in the subsequent chromophore development. No chromophore was formed when sodium arsenite was used to terminate the periodate oxidation. Analyses for L-fucose by the manual procedure showed a linear relationship between absorbance at 555 nm and concentration of L-fucose up to 40 μg/ml (Table I); at higher concentrations of L-fucose, loss of linearity was observed. Neither formic acid produced by oxidation of a 1,2,3-triol system with periodate, glyoxylic acid from aldonic acids, nor formaldehyde from terminal *vic*-diols interfered in this procedure. No extra chromophore or inhibition of the acetaldehyde chromophore was observed from hexoses, pentoses, or hexonic acids, whether before or after oxidation with periodate.

TABLE I

TYPICAL CALIBRATION OF THE MANUAL ANALYSIS

| L-Fucose (μg/ml) | 3.5 | 7.0 | 21 | 42 | 63 | 125 |
|---|---|---|---|---|---|---|
| Absorbance (550 nm) | 0.019 | 0.042 | 0.122 | 0.240 | 0.332 | 0.480 |

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The analysis was automated, using standard Technicon modular equipment and reagents identical with those for the manual procedure. Resorcinol and hydrochloric acid were added as a single reagent. For greater stability, a stock solution of resorcinol in water was employed, hydrochloric acid being added immediately prior to commencing a series of analyses. Reproducible results were obtained with chromophore development at 95°. Analyses for L-fucose and L-rhamnose revealed a linear relationship between recorded absorbance at 550 nm and concentration up to 180 μg/ml (Table II). A schematic diagram for the analysis is shown in Fig. 1.

TABLE II

TYPICAL CALIBRATION OF THE AUTOMATED ANALYSIS

| | | | | |
|---|---|---|---|---|
| L-Fucose (μg/ml) | 45 | 90 | 135 | 180 |
| Absorbance (550 nm) | 0.060 | 0.120 | 0.196 | 0.258 |
| L-Rhamnose (μg/ml) | 44 | 88 | 132 | 176 |
| Absorbance (550 nm) | 0.042 | 0.101 | 0.155 | 0.196 |

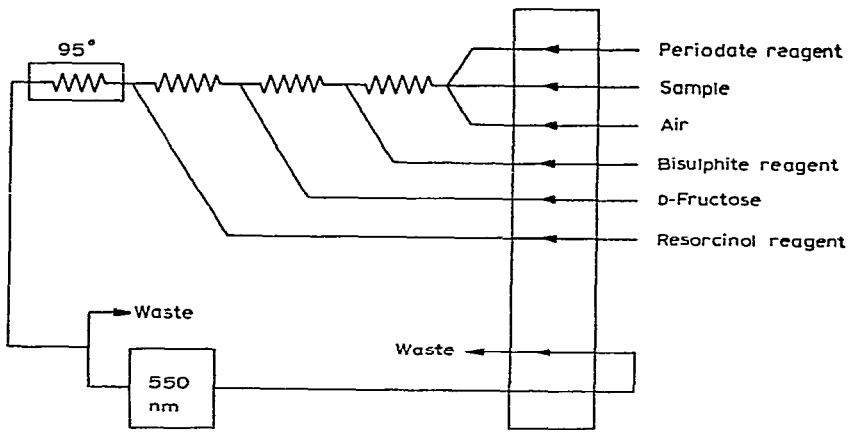


Fig. 1. Schematic diagram of the automated analysis of ω-deoxyhexoses by determination of acetaldehyde produced on oxidation with periodate (reagent composition as in Text).

The foregoing procedure is useful in the analysis of aldonic and saccharinic acids produced by the alkaline degradation of 6-deoxyhexose-containing oligosaccharides, and enables a ready and quantitative distinction of these acids from those derived from hexoses.

## EXPERIMENTAL

*Manual analysis procedure.* — Sample solutions (0.1 ml) containing 6-deoxyhexose (0–126 μg/ml) were pipetted into stoppered test-tubes, and a solution of

periodic acid (25mM) in sulphuric acid (62.5mM; 0.1 ml) was added. Solutions were maintained for 30 min at room temperature, and then oxidation was terminated with sodium metabisulphite (M; 0.05 ml). After a further 5 min, D-fructose (0.4mM; 0.25 ml) was added, followed by a resorcinol reagent (3.0 ml), prepared immediately prior to use by addition of M.A.R.-grade hydrochloric acid (10 vol.) to a stock, aqueous solution of resorcinol (12mM, 1 vol.). Solutions were heated at 80° for 5 min and then cooled to room temperature, and the absorbance of the characteristic red-chromophore was measured at 555 nm.

*Automated analysis procedure.* — A schematic diagram for the analysis is shown in Fig. 1. The reagents employed were identical with those used in the manual procedure. Solutions containing L-fucose or L-rhamnose (0–180 $\mu$g/ml) were sampled at 0.10 ml/min for periods of $\sim$15 min, segmented with air (0.23 ml/min), mixed with periodate reagent (0.10 ml/min), and oxidized for 9 min. Oxidation was terminated by addition of M sodium metabisulphite (0.03 ml/min), and, after further mixing, D-fructose solution (0.23 ml/min) and resorcinol reagent (1.19 ml/min) were added. The stream was passed for 2.5 min through a coil in a bath controlled at 95 $\pm$ 0.5°, then cooled to room temperature, and debubbled. The solution was drawn through the flow cell at 1.19 ml/min, the absorbance of the chromophore being recorded at 550 nm. Resorcinol reagent was not stable for more than one day; the other reagents were stable for several days. Tygon pump and connecting tubing were used for stages up to the addition of resorcinol reagent; Acidflex tubing was employed for the later stages.

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