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Note

Estimation of sugar alcohols by gas—liquid chromatography using a modified acetylation procedure

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The measurement of sugar alcohols in urine is of interest in patients with diabetes, and renal failure [1]. Urinary galactitol has been studied in patients with galactosaemia [2], and mannitol excretion for determining glomerular filtration rates [3] and in studies of intestinal permeability [4].

Although gas—liquid chromatography (GLC) has been used to measure sugar alcohols, resolution of isomeric mixtures may be difficult, particularly if packed columns are used. We recently described a procedure for mannitol estimation in which trimethylsilyl ethers were formed [5]. Although mannitol was separated from sorbitol and galactitol, the latter two hexitols were not resolved.

The present report describes a modified procedure for the measurement of sugar alcohols as acetyl esters. Isomeric hexitols are resolved and interferences due to monosaccharides overcome by forming methyloxime-acetyl derivatives of reducing sugars.

EXPERIMENTAL

Reagents

Sugar alcohols. Arabitol erythritol, inositol, pentaerythritol, perseitol (α-mannoheptitol), ribitol and xylitol were obtained from Sigma London (Poole, Great Britain). Mannitol and sorbitol were supplied by Koch-Light Labs. (Colnbrook, Great Britain).

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Sugars. D-Arabinose, D-fructose and D-fucose were obtained from Sigma, D-galactose and D-ribose from BDH (Poole, Great Britain). D-Glucose was supplied by Hopkins and Williams (Chadwell Heath, Great Britain) and D-xylose by Koch-Light Labs.

Chemicals. Analytical grade solvents were used. Pyridine was refluxed over potassium hydroxide pellets and redistilled before use. Zerolit DM-F (a mixed ion-exchange resin) was obtained from BDH, and converted to the H^+ acetate$^-$ form [5]. Methoxylamine hydrochloride was supplied by Pierce & Warriner (UK) (Chester, Great Britain).

Chromatograph

An F33 gas chromatograph (Perkin-Elmer, Beaconsfield, Great Britain) with a flame ionisation detector was used. This was fitted with a coiled glass column (2 m × 1.75 mm I.D.) which had been siliconised with 5% dimethyldichlorosilane in toluene, and filled with 3% XE-60 on Gas-Chrom Q (80—100 mesh), obtained from Phase Separations (Queensferry, Great Britain). The column was conditioned before use by heating overnight at 250°C with a carrier gas flow-rate of 50 ml/min.

Test procedure

To 2 ml urine, test solution or standard was added 1 ml internal standard solution (pentaerythritol, 50 mg/l). For quantitative studies a standard solution containing 20 mg/l of erythritol, xylitol, sorbitol and galactitol was used. Resin was added to occupy, after expansion, 60% of the total volume. The specimens were shaken for 3 min and centrifuged at 2000 *g* for 5 min. An aliquot (1 ml) of the clear supernate was transferred to a 10-ml conical glass tube in a water bath at 70°C, and evaporated to dryness under a stream of air. The tubes were transferred to a desiccator containing phosphorus pentoxide which was evacuated for a minimum period of 1 h. After removing the tubes from the desiccator, 0.5 ml methoxylamine hydrochloride in pyridine (10 mg/ml) was added to each sample, followed by incubation at 70°C for 30 min in a heating block (Techne Dri-Block DB-3; Techne, Duxford, Great Britain). The specimens were acetylated by adding 0.5 ml acetic anhydride to each and incubating for a further 10 min at 70°C. Two drops of methanol were added and the solvents evaporated by placing the tubes in a water bath at 30°C under a stream of air. The residues were desiccated for at least 1 h and then dissolved in 50 μl methanol. Using a microsyringe, 2 μl were injected onto the chromatographic column.

The chromatograph was operated with the injector port and detector oven at 250°C and a column temperature of 230°C. Nitrogen was used as carrier gas with a flow-rate of 50 ml/min. The inlet pressures of hydrogen and air were 1.1 bar and 1.6 bar respectively. The amplifier attenuation was 8×10^2. The chromatograph was linked to a Rikadenki Recorder (Rikadenki Kogyo Co., Tokyo, Japan).

Polyhydric alcohols were quantified by comparing the peak height ratios in standard and test samples.

Specimens from normal subjects were collected from ten healthy adult subjects for determination of sugar alcohol excretion. Urine was collected

for 5 h after an overnight fast; volumes were recorded and aliquots, preserved with thiomersal (100 mg/l), stored at 4°C prior to analysis.

RESULTS

Relative retention times

The retention times of monosaccarides and polyols relative to that of the internal standard were determined using pure aqueous solutions (1 g/l). The results are shown in Table I. The retention time of pentaerythritol was approximately 4 min. Pentaerythritol was selected as the internal standard because it does not occur naturally, and appears in an otherwise vacant position on the chromatogram. Fig. 1 shows a chromatogram obtained using an aqueous solution containing ten sugar alcohols.

TABLE I

RETENTION TIMES OF SUGAR ALCOHOLS AND SELECTED MONOSACCHARIDES RELATIVE TO THAT OF THE INTERNAL STANDARD, PENTAERYTHRITOL

The retention time of pentaerythritol was approx. 4 min.

Compound	Peak 1	Peak 2
Arabinose	0.64	0.70
Arabitol	1.17	
Erythritol	0.45	
Fucose	0.54	0.60
Fructose	1.84	
Galactitol	2.68	
Galactose	1.57	1.80
Glucose	1.23	1.93
Inositol	3.49	
Lyxose	0.73	
Mannitol	2.44	
3-O-Methylglucose	1.24	1.29
Pentaerythritol	1.00	
Perseitol	5.75	
Ribitol	1.09	
Ribose	0.68	
Sorbitol	3.03	
Xylitol	1.47	
Xylose	0.89	

Recovery

Known amounts of erythritol, xylitol, galactitol and sorbitol were added to urine and the concentrations of each determined in treated and untreated specimens. After subtracting the values for endogenous polyols, the concentrations were expressed as a percentage of the true values. Recoveries varied from 94 to 109%. The results are shown in Table II.

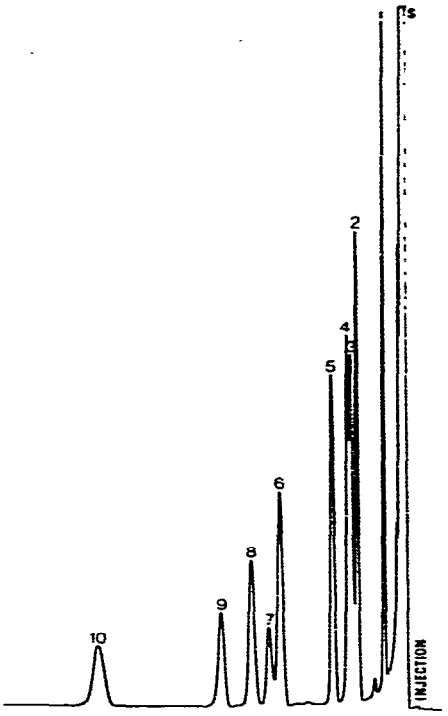

Fig. 1. Chromatogram of a standard solution of ten sugar alcohols. Peaks: S = solvent front; 1 = erythritol; 2 = pentaerythritol; 3 = ribitol; 4 = arabitol; 5 = xylitol; 6 = mannitol; 7 = galactitol; 8 = sorbitol; 9 = inositol; 10 = perseitol. The retention time of pentaerythritol was 4 min.

TABLE II

RECOVERY OF SUGAR ALCOHOLS ADDED TO URINE

Values for endogenous polyols have been subtracted after estimating the amounts in untreated urine.

Amount added (mg/l)	Recovery (%)			
	Erythritol	Galactitol	Sorbitol	Xylitol
20	94	105	109	109
200	98	102	95	105

Precision

Precision was assessed for erythritol, xylitol, galactitol and sorbitol by adding various amounts to urine and estimating the concentrations by single injection of samples which were analysed in three batches. These results are shown in Table III, coefficients of variation of 4.1—9.7% being obtained.

TABLE III

PRECISION OF SUGAR ALCOHOL MEASUREMENT IN URINE

The formula $\sqrt{\frac{\Sigma x^2 - (\Sigma x)^2}{n-1}}$ was used for calculating standard deviation.

Sugar alcohol	Mean (mg/l)	n	S.D.	Coefficient of variation (%)
Erythritol	194.7	15	28.7	9.7
	88.1	21	6.0	6.8
Galactitol	161.5	15	13.0	8.0
	26.9	21	2.3	8.5
Sorbitol	153.5	15	10.3	6.7
	20.2	21	1.4	6.9
Xylitol	179.3	15	7.7	4.3
	33.9	21	1.4	4.1

Linearity

Linearity was assessed for seven sugar alcohols by adding varying amounts to urine to a limit of 1 g/l. The concentration of internal standard solution remained constant (200 mg/l). Inositol measurement was linear to 700 mg/l, erythritol, sorbitol and perseitol to 800 mg/l and xylitol, mannitol and galactitol to 1 g/l.

Sensitivity

For erythritol, galactitol, sorbitol and xylitol, concentrations of 2 mg/l were quantified if the amplifier attenuation was reduced to 1×10^2. Although the limit of sensitivity was not determined absolutely, it is well below this level for most polyols and may be further improved if the sample volume is increased. With concentrations below 2 mg/l, erythritol was not well defined because of its proximity to the solvent peak. However, the resolution of erythritol was improved by temperature programming with the initial temperature 200°C for 4 min, the rate of increase 3°C/min and the final oven temperature 230°C. With such a modification erythritol concentrations lower than 2 mg/l may be measured.

Interferences

If monosaccharides were not converted to the corresponding methyloxime derivatives prior to acetylation, galactose and glucose interfered with galactitol and mannitol estimation, respectively. By including the additional derivatisation procedure the retention times of sugars were reduced and interferences prevented (Fig. 2). Sugars and polyols do not appear to cause interference.

Excretion of sugar alcohols in healthy subjects

Excretion rates of xylitol, mannitol, galactitol and sorbitol were determined, the results being expressed as mg/h (Table IV).

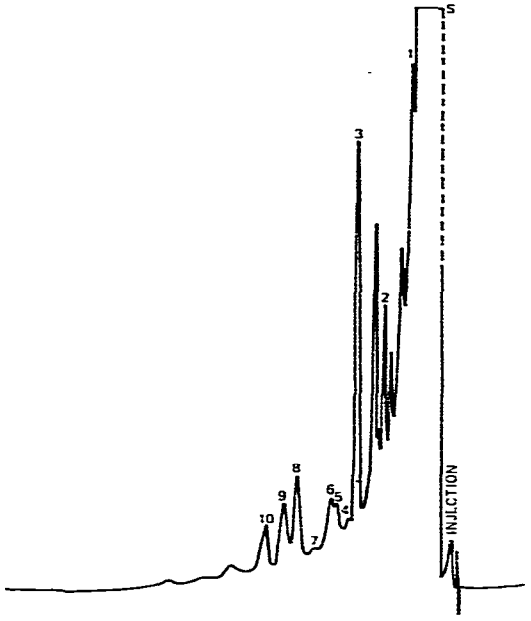

Fig. 2. Chromatogram of urine. Peaks: S = solvent front; 1 = erythritol; 2 = pentaerythritol; 3 = xylitol; 4 and 7 = glucose; 5 and 6 = galactose; 8 = mannitol; 9 = galactitol; 10 = sorbitol. For clarity, sugar alcohols were added to urine to give concentrations of 20 mg/l.

TABLE IV

RATE OF EXCRETION OF SUGAR ALCOHOLS IN TEN HEALTHY SUBJECTS

Sugar alcohol	Excretion (mg per 5 h)	
	Mean	Range
Xylitol	0.72	0.32—2.08
Mannitol	1.24	0.44—2.47
Galactitol	0.51	0.26—0.87
Sorbitol	0.46	0.22—0.86

DISCUSSION

Gas—liquid chromatography has been used to measure the low levels of sugar alcohols which occur in urine, since it is sensitive and capable of resolving the components of complex mixtures. However, isomeric polyols have proved difficult to separate, particularly if trimethylsilyl derivatives are formed. Although these derivatives have been widely used for sugar measurements complete resolution of isomeric trimethylsilyl sugar alcohols does not occur when packed columns are used [5—7].

Oades [8] demonstrated that sugar alcohol mixtures may be more satisfactorily resolved if acetyl esters are prepared and this procedure was adapted for

analysis of polyols in urine by Pitkänen [1]. Because it interfered with sorbitol and galactitol estimation, glucose was removed prior to chromatography by incubating samples with glucose oxidase, the resulting gluconate being removed with an ion-exchange resin. The present procedure avoids interference due to monosaccharides by the less cumbersome method of combining methyloxime formation with acetylation, thus modifying the retention times of sugars which would otherwise co-chromatograph with polyols. The retention times of sugar alcohols are not altered by including derivatisation with methoxylamine hydrochloride in addition to acetylation; thus polyols form only acetyl esters. Sugar alcohol excretion rates are comparable to those obtained by Pitkänen [1].

The method is precise, accurate, sensitive and linear over wide ranges. It appears to be free from interferences.

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