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Note

Simple rapid method for the separation and quantitative analysis of carbohydrates in biological fluids

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The concentrations of several mono- and disaccharides in body fluids may be changed in some anomalies of carbohydrate metabolism; therefore the quantitative estimation of sugars in biological fluids could be of great clinical importance.

Thin-layer chromatography (TLC) is the method used most often for quantitative sugar analysis [1—9]. The screening method of Kraffczyk et al. [10] is widely used in clinical laboratories, but it does not suit requirements for quantitative results. All other standard methods have a few major problems: interference by normally encountered urinary substances, time-consuming pre-treatment, insufficient separation or a long development time.

The present method eliminates all these disadvantages and allows the identification and estimation of most of the commonly occurring sugars in biological fluids by means of one-dimensional TLC.

MATERIALS AND METHODS

TLC plates, 20 × 20 cm aluminium-backed silica gel 60 plates were from Merck (Merck No. 5553, Darmstadt, G.F.R.). Diphenylamine, aniline, acetone, chloroform, ethanol and orthophosphoric acid (85%) were of analytical grade and supplied by Merck. Charcoal, Darco G 60, was supplied by Fluka, Buchs, Switzerland.

Sugar stock solutions were prepared by dissolving 500 mg of each sugar (sugar standards, collection A and B, Merck No. 8005 and No. 8002) in 100 ml of 20% ethanol. Working standards ranging from 10 mg/100 ml to 500 mg/100 ml were prepared by diluting a stock solution of each sugar with 20% ethanol.

The chromatogram developing solvent was acetone—chloroform—water (85:10:5). The detection reagent was prepared by dissolving 2 g of diphenylamine and 2 ml aniline in 100 ml of acetone and adding 15 ml of 85% orthophosphoric acid. This was made up fresh daily.

Preparation of samples

Prior to chromatography all urine samples were tested with Benedict's reagent to ensure that the concentration was not higher than 500 mg/100 ml. To 10 ml of the sample 1 ml of ethanol is added and the mixture is treated with about 100 mg of charcoal. Finally the mixture is filtered and the filtrate is ready for application.

Plasma has to be filtered on a Sartorius SM-11310 filtration membrane (Sartorius, Göttingen, G.F.R.), and is then ready for application.

Cerebrospinal fluid can be applied directly to the TLC plate.

Application and development

Two to ten microlitres of the sample are applied to the plate as 0.5-cm stripes using a Hamilton microsyringe. The distance between two samples is 1 cm and the application line is placed 2 cm from the edge of the plate. A stream of warm air is used during the application.

Chromatographic development is performed in a vapour-saturated chromatographic tank at room temperature. The plate is developed twice in order to obtain a better separation. After each development the plate is dried in the oven for 2 min at 110°. Running time is 70 min and solvent migration is 17.5 cm.

Detection

The dried plates are sprayed with diphenylamine—aniline—phosphoric acid reagent, and heated in the oven for 20 min at 110°. The sugars are estimated by scanning the plates in a spectrodensitometer KM-3 (Carl Zeiss, Oberkochen, G.F.R.). If not scanned immediately, the plates should be stored in the dark at 4° for a maximum of 5 days.

RESULTS AND DISCUSSION

Aluminium-backed silica gel plates were found to yield the best separation of sugars (Fig. 1). The carbohydrates appear well defined as coloured spots, being very compact. Because of different absorption maxima each sugar has to be measured separately, but this handicap can be minimalised by rectangular measurement.

R_F values, colour, absorption maxima, sensitivity and normal values are summarised in Table I.

The precision of the method was estimated from 22 measurements of the same standard mixture over a period of several weeks, averaging 91.1 mg/100

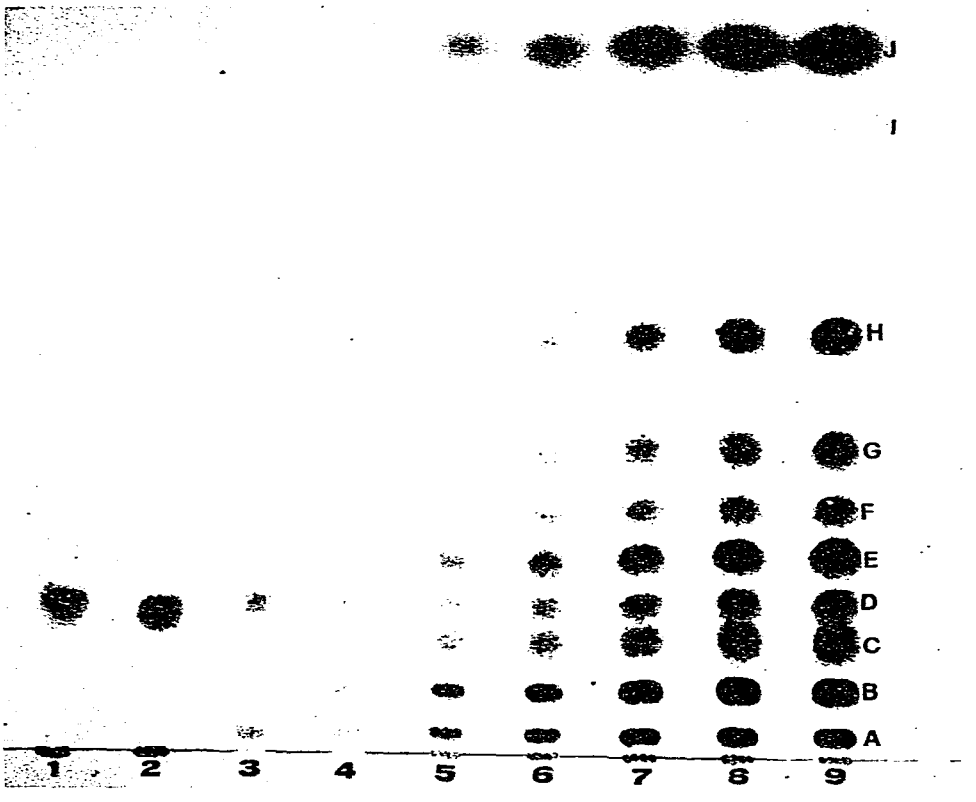

Fig. 1. Chromatogram showing TLC separation of sugars from normal plasma and urine (1, 4), plasma and urine from a patient with diabetes mellitus (2, 3) and sugar standards (5—9) as follows: A, lactose; B, saccharose; C, galactose; D, glucose; E, fructose; F, sorbose; G, arabinose; H, xylose; I, rhamnose; and J, 2-deoxy-D-ribose.

TABLE I

R_F VALUES, COLOUR, ABSORPTION MAXIMA, SENSITIVITY AND NORMAL VALUES OF THE CARBOHYDRATES STUDIED

Sugar	Colour	R_F × 100	Absorption maxima (nm)	Sensitivity limit (mg/100 ml)	Normal values	
					Urine (mg/24 h)	Plasma (mg/100 ml)
Lactose	Blue	4	600	5	0—100	—
Saccharose	Grey—violet	12	540	5	0—20	—
Galactose	Blue	16	650	10	0—20	—
Glucose	Blue	22	650	10	15—150	70—110
Fructose	Orange—red	27	530	5	—	—
Sorbose	Light-brown	32	550	10	—	—
Arabinose	Blue	36	650	10	—	—
Xylose	Blue	48	650	5	10—100	—
Rhamnose	Yellow—green	63	370	15	—	—
2-Deoxy-D-ribose	Red	71	520	5	—	—

ml (S.D. 4.4), with a coefficient of variation of 4.8%. Relationship of peak height to concentration is linear up to 500 mg/100 ml and the sensitivity limit is 5 mg/100 ml.

It has been recognized only recently that normal urine contains many sugars in very low concentration, the clinical significance of which has not yet been established [11]. The results of our method of carbohydrate analysis of normal human urine and plasma (Table I) are identical with those published previously [12, 13].

With a growing interest in carbohydrate metabolism in diagnosing disorders involving carbohydrates, there is an increased need for a more sensitive and simple assay method. We have developed a simple TLC method for carbohydrates, after investigating various steps in the analysis, which is sufficiently sensitive and reproducible for clinical purposes as well as for research work. Specifically, this method can be used as a diagnostic aid in most known clinical syndromes, such as diabetes mellitus, glycosuria, galactosuria, ribosuria, lactose intolerance, fructosuria, fructose intolerance, essential and alimentary pentosuria.

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