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THIN-LAYER QUANTITATIVE CHROMATOGRAPHY OF ARABINOSE, RIBOSE AND XYLOSE IN THE PRESENCE OF OTHER SUGARS

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SUMMARY

We describe a rapid and unequivocally distinct separation of arabinose, ribose and xylose from all sugars, except fucose, which are normally found in biological fluids. The method can be applied quantitatively, with reasonable accuracy, to amounts of up to 80 μg of each aldopentose.

INTRODUCTION

Having found that arabinose, ribose and xylose are invariably present in the urine of fasting normal human subjects we sought for a simple and rapid way to measure the amounts of these sugars. We considered that the development of a procedure which could be applied in a clinical laboratory might prove of value in studying diseases involving connective tissue since it is now well-established that D-xylose and L-arabinose occur as components of glycoproteins associated with this tissue.

The three aldopentoses are not readily separated on paper, if at all, by the published procedures[1-3] and many hours of development, with concomitant spread of the spots, are required, so that quantitative analyses become difficult. Thin layers of Silica Gel G do not allow good separation between arabinose, xylose and mannose[4-6] and analogous overlapping takes place with crystalline cellulose[7].

This paper describes a slight modification of the thin-layer system of STAHL AND KALTENBACH[8] using Kieselguhr G made up with 0.02 *M* sodium acetate; two developments of 55 min each with suitable proportions of ethyl acetate, propan-2-ol and water give wide spatial separation of the three aldopentoses from one another and from all the other sugars found in normal urine with the exception of fucose. The latter, as far as we can see, occurs only in minor amounts in normal fasting urine but its presence can be detected by a qualitative differential spray reagent. We have not been able to separate xylose from fucose. The clear-cut separation of the aldopentose spots enables quantitative measurements to be made.

Using the unpleasant mixture of ethyl acetate–pyridine–water, it is possible on

cellulose to show separations which are qualitatively satisfactory but the resolution is not sufficient for quantitative purposes.

Our procedure has now been successfully used by colleagues in other laboratories.

EXPERIMENTAL AND RESULTS

Solvents

Alcohols were distilled over potassium hydroxide and silver oxide. Ethyl acetate was distilled after keeping over anhydrous potassium carbonate. Glacial acetic acid was distilled over potassium permanganate.

Sugars

Commercial samples were recrystallised, D-xylose and D-ribose from propan-2-ol and L-arabinose from aqueous propan-2-ol. Stock solutions (5 mg/ml) were prepared in water saturated with benzoic acid.

Reagents

The reagents were commercial samples and usually did not require further purification. 4-Methoxyaniline (*p*-anisidine) hydrochloride was prepared in the laboratory.

Chromatographic plates

Kieselguhr G (Merck) (one part) blended for 1 min with 0.02 *M* sodium acetate (three parts) was spread at a thickness of 0.5 mm. The plates were allowed to dry at room temperature for 24 h and were stored at the humidity and temperature of the laboratory.

Application of sugar solutions

Streaks, about 1.5 cm long, were applied uniformly from a 5-μl graduated pipette. Amounts of 1, 2 and 3 μl were typically used. Water was dried off in a current of warm air.

Development

The tanks were lined with filter paper as a routine but no previous vapour saturation was found to be necessary. The developing solvent, slightly modified in proportions from that of STAHL AND KALTENBACH[8] consisted in ethyl acetate–propan-2-ol–water (4:1:0.5). At room temperature the solvent was allowed to run the full length of the plate (20 cm in 55 min). If fructose was present, or if quantitative results were required, the solvent was evaporated in a current of air (15 min) and a second development was done to separate arabinose from the hexulose.

The developing solvent lasts for 48 h.

Spray reagents and revelation of sugars

Application was made using the Shandon Laboratory Spray Gun.

Attempts to reveal the sugar spots. Sprays commonly used on paper, cellulose powder or silica, when applied to the buffered kieselguhr, despite attempts to over-

come the buffer by increasing the acidity of the reagents, etc., generally proved quite unsatisfactory. Reagents containing aniline salts or salts of 4-aminobenzoic acid[9] gave very faint colours. Naphthoresorcinol[4-6] gave generally blue colours with a poor background. A range of indicators of suitable pK values with boric acid failed completely to show up any sugars. Acid phloroglucinol likewise proved unsatisfactory. However, a new reagent proved to give excellent qualitatively differential results but it was impossible to extract the colours without immediate fading; however, a fairly permanent staining took place with PRIDHAM's[10] *p*-anisidine spray (see below).

New qualitative spray. Stannous chloride added to the reagent used by BELL[9] for assay of hexoses and pentoses in aqueous solution gave intense colours as follows: pentoses, cerise; aldohexoses, brown-yellow; hexuloses, yellow; 6-deoxyaldohexoses, yellow or yellow-pink. The reagent consisted in 4-aminobenzoic acid (2 g), 3-carboxy-4-hydroxybenzenesulphonic acid (3 g) and $SnCl_2$ (1 g) in 100 ml of 80% aqueous acetic acid. The solution was filtered before use. Air-dried plates were sprayed and, without further drying, were heated for 15 min at 100°.

Quantitative spray. PRIDHAM's[10] reagent, devised for paper work, gave a stable colour which could be extracted from the kieselguhr. It consisted in 4-methoxyaniline (*p*-anisidine) hydrochloride (1 g) dissolved in MeOH (5 ml) containing sodium dithionite (100 mg) and butan-1-ol (95 ml). The air-dried plate was sprayed in four directions, allowed to dry in a current of air and then heated at 130° for 15 min when all the classes of sugar showed as brownish spots. (This spray gives selective colours when used on paper.)

R_F and R_G values. Typical values are given in Table I.

TABLE I

R_F AND R_G VALUES FOR EIGHT MONOSACCHARIDES

Sugar	*Typical R_F values × 100*	*Typical R_G values × 100*
Rhamnose	78	536
Ribose	66	460
Fucose[a]	47	324
Xylose[a]	45	312
Arabinose	32	224
Fructose	25	170
Glucose	14	100
Galactose	11	80

[a] Fucose and xylose do not separate completely.

Quantitative measurements

Because the sugar spots were so widely separated it was possible completely to remove the coloured areas of the kieselguhr from the glass surface *in toto*. Equal areas of the kieselguhr which contained the stain were carefully transferred to centrifuge tubes; at the same time equal areas which contained no sugars were treated likewise to serve as blanks. To each sample was added 4 ml of 95% aqueous methanol containing 1 g of $SnCl_2$ per 100 ml. The tubes were stoppered and vigorously shaken for 10 min and the solid then packed on the centrifuge. The extinctions of the supernatant

solutions were then measured at 395 nm (which is the λ_{max}), against the "blank" supernatant, on an SP500 spectrophotometer. The colour was stable for about 3 h and the three aldopentoses gave almost identical extinctions in the range of 5 to 80 μg. Typical results are given in Table II.

TABLE II

TYPICAL EXTINCTIONS FOR THREE ALDOPENTOSES STAINED BY PRIDHAM'S REAGENT

Amount (*μg*)	*Extinctions*		
	Arabinose	*Xylose*	*Ribose*
5	0.045	0.045	0.040
10	0.12	0.12	0.09
20	0.24	0.25	0.23
40	0.58	0.58	0.60
80	1.15	1.15	1.16

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