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A RAPID QUANTITATIVE THIN-LAYER CHROMATOGRAPHIC SEPARATION OF FUCOSE FROM OTHER NEUTRAL MONOSACCHARIDES

AN APPLICATION TO SEPARATE OTHER SUGARS OF PHYSIOLOGICAL INTEREST

M. Q-K. TALUKDER
Physiology Department, University Medical School, Edinburgh EH8 9AG (Great Britain)
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SUMMARY

Fucose can be completely separated in 65 min from seven other neutral monosaccharides of biological interest on thin layers of Kieselguhr G buffered with 0.15 *M* sodium dihydrogen orthophosphate. It can be determined within the range 5 to 80 μg. Using a plate 35 cm long, each of the eight individual sugars can be well separated, one from another, in 9 h. This procedure is limited to mixtures containing an upper level of 15 μg of xylose and ribose. Mixtures of fucose and xylose can be determined quantitatively by the method described.

INTRODUCTION

BELL AND TALUKDER[1] have reported a quantitative thin-layer chromatographic (TLC) separation of arabinose, xylose and ribose from rhamnose, fructose, glucose and galactose; they found it impossible to separate fucose from xylose. Little information is available on the chromatographic separation of fucose (paper or thin layer); however, since BELL AND TALUKDER (unpublished) found that free fucose is invariably present in urine from fasting normal human subjects it became important to separate this sugar from all accompanying monosaccharides. As far as the author is aware, the only separation of fucose from xylose so far reported is the slow running paper/phenol–ammonia procedure of PARTRIDGE[2]. Here however rhamnose and ribose coincide and the close R_F values of ribose and fucose may not permit quantitative measurements. A two-dimensional TLC procedure[3] which separates fucose from other monosaccharides obtained on hydrolysis of glycoproteins, does not deal with other aldopentoses which occur in association with connective tissue. LATO *et al.*[4], in an intensive study using silica gel impregnated by either sodium acetate, sodium dihydrogen phosphate or disodium hydrogen phosphate, show a few separations between xylose and fucose with respect to differences in their R_F values. These values are small and in such instances the sugar spots are reported to be diffuse or tailing. Other sugars in urine would tend to coincide either with xylose or fucose or both. Moreover, the closeness or overlapping of the spots of the various sugars would militate against quantitative applications of these systems. In their illustration (Fig. 1 p. 416) the only run with both fucose and

xylose shows convergence of the fucose and ribose spots, although in this instance xylose and fucose are clearly separated.

Fucose, as tritiated fucitol, has been separated[5] from the corresponding alditols resulting from the reduction of ribose, mannose and galactose; again other monosaccharides of interest were not considered.

Having found[1] that plates coated with Kieselguhr G buffered with sodium acetate and suitably developed will effectively separate the eight monosaccharides of interest, with the exception of xylose and fucose, the observations of OVODOV *et al.*[6] were noted. These authors made the point that successful sugar separations (on buffered silica) depended on the nature and concentration of the impregnating salt. TALUKDER (unpublished) examined silica, cellulose and alumina, buffered by borate, sodium acetate, sodium dihydrogen phosphate and disodium hydrogen phosphate without satisfactorily separating fucose from xylose. Success was eventually attained using Kieselguhr G buffered with 0.15 *M* sodium dihydrogen phosphate.

EXPERIMENTAL AND RESULTS

Solvents

Alcohols (either reagent quality or AR) were distilled over sodium hydroxide and silver oxide. If methanol still contained volatile bases, it was re-distilled over potassium hydrogen sulphate. Ethyl acetate was distilled after storage over anhydrous potassium carbonate. Acetic acid was distilled from potassium permanganate.

Sugars

Aldopentoses were purified as described by BELL AND TALUKDER[1]. Rhamnose was recrystallized from ethyl acetate at room temperature. Fucose (D or L) was recrystallised from ethanol at 2°; to ensure freedom from pentose it was spotted on a kieselguhr plate and stained differentially by the 4-aminobenzoic acid–sulphosalicylic acid mixture of BELL[7] with addition of 2 % $SnCl_2$. One commercial sample did indeed contain a pentose. Glucose, galactose and fructose were commercial products.

Stock solutions (5 mg/ml) were prepared in saturated benzoic acid.

Spray reagents

4-Methoxy aniline (*p*-anisidine) hydrochloride was prepared in the laboratory. Other materials were commercial products.

Chromatographic plates

Kieselguhr G (Merck) (one part) was blended for 90 sec with 0.15 *M* sodium dihydrogen orthophosphate (2.5 parts) and the slurry spread to a thickness of 0.5 mm. The plates were allowed to dry at room temperature (18–20°) for at least 24 h and stored in presence of air at a temperature not exceeding 20°. If the storage temperature exceeds 20° the plates become useless.

Application of the sugar solutions

Solutions were applied, from a 5 μl graduated micropipette in streaks of not more than 1 cm in length to the plates; not more than a total of 3 μl was applied uniformly at one time. Spots were dried by a current of warm air (75–80°).

Development

A considerable variety of solvent mixtures were examined; only the following gave satisfactory results: (1) ethyl acetate–methanol–butan-1-ol–water (16:3:3:2); (2) ethyl acetate–methanol–propan-2-ol–butan-1-ol–water (8:1:1:1:1); (3) ethyl acetate–methanol–butan-1-ol–water (16:3:3:1). These solvent mixtures were unsuitable for use after 48 h.

As found previously[1] no initial vapour saturation was necessary when using paper-lined tanks. The solvent front was allowed to run the full length of the plate at room temperature (65 min for 20 cm long plates with solvent mixtures 1 or 2). The 35 cm long plates required $3^3/_4$ h with solvent mixture 3 for a single run. While a single development sufficed qualitatively to separate fucose from all the other sugars, to obtain sufficient spatial separation of fucose, as well as all the other individual monosaccharides from each other except ribose and xylose, two developments were necessary. Single development showed urinary sugars to run slightly behind the corresponding standards. But a second development overcame this. Moreover, the sugar spots became more compact and well shaped with a second or third development. After each run the plates were dried at room temperature for 15 min and then heated at 100° for 5 min to vaporise the butanol. When cold, the plates were ready either for a subsequent development or for spraying.

Visualisation of the sugar spots

In preliminary investigations a differential spray was used, based on the BELL's reagent[7] modified by the addition of 2 g/100 ml of $SnCl_2$ to stabilize the red colour of the pentoses and the yellows developed with the different classes of hexoses. This reagent cannot yet be used for accurate quantitative work because of small irregularities in the amounts of colour developed in the pentose spots. 6-Deoxyhexose spots showed a distinct pink tinge in the yellow which was formed on phosphate-buffered plates.

As before[1], butanolic 4-methoxyaniline containing sodium dithionite (PRIDHAM[8]) gave stable colours, yellow grey with hexoses and sepia with pentoses, thus differing slightly from the colours on acetate-buffered Kieselguhr G. The sprayed (4 directions) plates were heated at 125° for 15 min.

TABLE I

TYPICAL R_F VALUES FOR EIGHT MONOSACCHARIDES USING A 20 × 20 cm PLATE AND SOLVENT 1

Sugars	$R_F \times 100$	
	Single development	*Double development*
Galactose	10	18
Glucose	14	26
Fructose	20	34
Arabinose	25	42
Xylose	38	60[a]
Ribose	39	62[a]
Fucose	55	77
Rhamnose	80	97

[a] Xylose and ribose do not separate.

R_F values

Using a 20 cm plate, R_F values were markedly increased after a second development with either solvent 1 or 2 allowing quantitative measurements of fucose as well as of galactose, glucose, fructose and arabinose. Rhamnose runs with the solvent front (R_F 0.97); it cannot be satisfactorily measured under these conditions especially if pentuloses, which are very fast running, are present. This system will not separate xylose from ribose (Table I).

On a 35 cm plate and with two developments with solvent 3 the eight monosaccharides were clearly separated as shown by the R_F values (Table II). With this procedure however not more than 15 μg each of xylose and ribose can be separated quantitatively .The longer plate has other disadvantages because each run takes $3^3/_4$ h and with intermediate drying the whole operation occupies 9 h. Moreover the plate is cumbersome for routine clinical laboratory work. But on occasion, this procedure could be of value for quantitative measurements of the relatively large amounts of galactose, glucose and fructose found free in biological fluids (see R_F values in Table II).

Quantitative applications

The procedure was identical with that used by BELL AND TALUKDER[1]. Equal areas of the adsorbent containing the coloured spots were scraped off, the colour extracted by shaking with 3 ml of a mixture of methanol (90 ml) and 5 ml of 1 % (w/v) aqueous stannous chloride. After centrifuging down solids the extinction at

TABLE II

TYPICAL R_F VALUES FOR EIGHT MONOSACCHARIDES ON A 20 × 35 cm PLATE: DEVELOPED TWICE WITH MIXTURE 3

Sugars	$R_F \times 100$[a]
Galactose	28
Glucose	42
Fructose	53
Arabinose	61
Xylose	77
Ribose	83
Fucose	91
Rhamnose	98

[a] All sugars are clearly separated from each other.

TABLE III

TYPICAL EXTINCTIONS FOR FUCOSE AND TWO ALDOPENTOSES STAINED BY PRIDHAM'S REAGENT

Amount (μg)	*Extinction*		
	Fucose	*Xylose*	*Ribose*
5	0.03	0.035	0.035
10	0.065	0.075	0.07
20	0.125	0.14	0.14
40	0.24	0.275	0.28
80	0.48	0.55	0.55

380 nm was measured on an SP 500 spectrophotometer against a blank identically prepared from an equivalent area of adsorbent free from sugar spots. Table III shows typical extinctions in the range of 5 to 80 μg for fucose and also for xylose and ribose run separately under the same conditions.

Determination of fucose and xylose in the same sample

This requires two plates, 20 × 20 cm; one is buffered with sodium dihydrogen phosphate (plate A), as described and the other with 0.03 *M* sodium acetate[1] (plate B). Plate A is developed twice with solvent 1 and the amounts of fucose and (ribose + xylose) determined. Plate B is developed twice with ethyl acetate–propan-2-ol–water (8:2:1)[1] and ribose determined; the amount of ribose is then subtracted from the (xylose + ribose) value obtained from plate A since both pentoses yield the same amount of colour, weight for weight (see Table III).

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REFERENCES

1 D. J. BELL AND M. Q-K. TALUKDER, *J. Chromatogr.*, 49 (1970) 469.
2 S. M. PARTRIDGE, *Biochem. J.*, 42 (1948) 238.
3 K. HOTTA AND M. KUROKAWA, *Anal. Biochem.*, 26 (1968) 472.
4 M. LATO, B. BRUNELLI, G. CIUFFINI AND I. MEZZETTI, *J. Chromatogr.*, 39 (1969) 407.
5 M. MURAKAMI AND R. J. WINZLER, *J. Chromatogr.*, 28 (1967) 344.
6 YU. S. OVODOV, E. V. EVTUSHENKO, V. E. VASKOVSKY, G. OVODOVA AND T. F. SOLOVEVA, *J. Chromatogr.*, 26 (1967) 111.
7 D. J. BELL, *J. Chem. Soc.*, (1966) 1638.
8 J. B. PRIDHAM, *Anal. Chem.*, 28 (1956) 1967.