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Abstract

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Thin-Layer Chromatography of Carbohydrates on Gypsum

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The development of methods for the rapid determination of carbohydrates and the separation of their mixtures is an important problem in the analytical chemistry of sugars. Paper chromatography, used for these purposes, despite all its advantages, usually takes a great deal of time.

Recently, for the separation of various classes of organic compounds, a method of thin-layer chromatography on plates has been proposed ([1]). As Kochetkov, Dmitriev, and Usov ([2]) have shown, this method is acceptable for the determination of fully or partially substituted carbohydrates. The aluminum oxide used in this procedure, however, does not give favorable results in the separation of free carbohydrates. Dyatlovitskaya, Voronkova, and Bergelson established the possibility of identifying some sugars by thin-layer chromatography on silica gel ([3]); however, this process takes a long time (up to 4 hours), and the sensitivity of the method is 2-5 times lower than in chromatography on paper. These same authors proposed a method for separating polyhydroxy compounds in a thin layer of powdered cellulose ([4]).

Table 1

Compound	Chloroform–methanol 19:2	Chloroform–methanol 19:3
D-ribose	0.79	0.86
D-xylose	0.58	0.88
L-arabinose	0.63	0.75
D-glucose	0.27	0.77
D-mannose	0.33	0.62
D-galactose	0.30	0.40
D-fructose	0.48	0.91
L-rhamnose	0.88	0.95
L-sorbose	0.73	0.95

We investigated the possibility of chromatographic determination of mono- and disaccharides and polyhydric alcohols in a thin layer of calcium sulfate. This method makes it possible to determine carbohydrates within 40-45 min.

The chromatographic determination of mono- and disaccharides and polyhydric alcohols was carried out on preprepared plates coated with a layer of calcium sulfate. The plates are made as follows: 10 g of anhydrous, sifted calcium sulfate (30-60 mesh) is ground in a porcelain mortar with 20 ml of distilled water for 5-7 min until a homogeneous mass is obtained. The resulting paste is applied in a thin layer to glass plates (6 × 18 cm) and dried in air at room temperature for 20 h. The gypsum layer obtained in this way is very durable.

For the separation of carbohydrates, various solvents were used. The best solvents for separating monosaccharides proved to be chloroform–methanol 19:2 and chloroform–methanol 19:3. The results of the chromatographic determination of the R_f values of these compounds are given in Table 1.

For the separation of certain disaccharides (sucrose, maltose, lactose) and polyhydric alcohols (inositol, sorbitol, mannitol, arabitol), the following systems were used: for the former, chloroform–methanol 19:5; for the latter, chloroform–methanol 9:1. The R_f values for these compounds are given in Table 2.

The use of the solvent chloroform–methanol 19:5 for the determination of polyhydric alcohols, as well as the use of the chloroform–methanol mixture

9:1, for the determination of disaccharides, does not lead to success, since in the first case the substances move with the solvent front, while in the second they remain on the starting line.

The rather considerable differences in the R_f values obtained in the separation of monosaccharides were used by us to investigate the possibility of separating artificially prepared mixtures of monosaccharides. For this purpose, 1% alcoholic solutions of monosaccharides were prepared in advance and mixed in a 1:1 ratio by volume.

Table 2

Compound	Chloroform–methanol 19:5	Chloroform–methanol 9:1
Sucrose	0.61	–
Maltose	0.26	–
Lactose	0.46	–
i-Inositol	–	0.11
Sorbitol	–	0.42
Mannitol	–	0.06
L-Arabitol	–	0.52

For the separation, mixtures of the following sugars were used:
D-glucose–*L*-rhamnose, *L*-arabinose–*D*-galactose, *D*-fructose–*D*-galactose, *L*-rhamnose–*D*-mannose.

Separation of the mixtures was carried out in the solvent chloroform–methanol 19:2. The R_f values obtained are given in Table 3.

The success of the determination and separation of sugars depends on the size of the spot applied—its diameter should be no more than 5 mm. For detecting sugars on chromatograms, the usual reagents for the α-glycol grouping were used. These reagents were prepared as follows:

Table 3

Mixtures	1	2
D-glucose—*L*-rhamnose	0.26	0.86
L-arabinose—*D*-galactose	0.61	0.29
D-fructose—*D*-galactose	0.49	0.29
L-rhamnose—*D*-mannose	0.88	0.33

1. An alkaline solution of potassium permanganate and sodium metaperiodate. To 10 ml of a 2% aqueous solution of sodium carbonate are added 40 ml of a 2% aqueous solution of sodium metaperiodate, and 0.5 g of potassium permanganate is dissolved in the resulting mixture. After spraying, the chromatogram is kept at room temperature for 15-20 min, as a result of which yellow spots of sugars appear on a pink background.
2. A water-acetone solution of silver nitrate. The reagent is prepared by mixing 0.1 ml of a saturated aqueous solution of silver nitrate with 20 ml of acetone. The chromatogram is first sprayed with this reagent, and then, after several minutes, with a saturated aqueous solution of caustic soda. Polyhydroxy compounds appear as black spots on a gray background.
3. For detecting sugars it is convenient to use spraying of the chromatograms with concentrated sulfuric acid followed by heating under an infrared lamp, as a result of which the sugars form dark spots. The latter method is also fairly sensitive; the substance is determined in amounts down to tenths of a γ.

Although we obtained well reproducible results, it must be taken into account that the method described here for the chromatographic determination of polyhydroxy compounds in a thin layer of gypsum, as also in chromatography in a thin layer of aluminum oxide, gives approximate results, since the R_f values apparently depend on the thickness of the applied layer of carrier, temperature, and other factors.

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