

Separation of D-Xylose Methyl Derivatives on Microcrystalline Cellulose

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Received October 26, 1969

This paper describes the chromatographic separation of D-xylose methyl derivatives on microcrystalline cellulose by thin-layer and column technics. Butanol saturated with water has proved to be the best of seven investigated solvent systems. It possesses a better partitioning effect and requires shorter developing times in comparison with paper chromatography. The preparative separation of D-xylose methyl derivatives on microcrystalline cellulose is superior to their separation on the mixture of activated charcoal with celite because it gives higher yields of pure substances.

Thin-layer chromatography may be used for a great number of organic compounds, their qualitative and quantitative determination as well as for preparative purposes. Either silica gel or microcrystalline cellulose is most frequently used for thin-layer preparation. Silica gel has been employed by several authors [1–3] for the qualitative determination and isolation of amino acids, saccharides, and their derivatives. Its drawback consists in the difficulties encountered in preparative separations since the silica gel thin-layers permit only the separation of small amounts.

In this respect, the use of powdered cellulose in thin-layer chromatography has a certain asset because the operation is fast and simple. This material was used for the separation of amino acids [4], neutral sugars and their derivatives [4], and alduronic acids as well [5, 6]. *Wolfrom* and co-workers employed the microcrystalline cellulose „Avicel“ for the separation of amino sugars, methylated hexoses, hydroxy acids, and lactones [7, 8]. The microcrystalline cellulose prepared in our Institute was used for the chromatographic separation of aldopentoses and pentuloses in thin-layer and column [9].

In this study, microcrystalline cellulose was employed for the separation of methylated D-xylose on thin-layer as well as for its preparative fractionation on column. Various solvent systems were correlated with respect to their selectivity and separation speed.

Experimental

A layer 0.3 mm thick of homogeneous suspension of blended microcrystalline cellulose [9] in water was spread on plates (10 × 20 cm). These chromatoplates were dried in air at room temperature.

Solutions used: 3-O-methyl-D-xylose (1 mg/ml), 2,3,4-tri-O-methyl-D-xylose (2 mg/ml), 2,3-di-O-methyl-D-xylose (1.5 mg/ml), and mixture of these solutions with ratio 1 : 1 : 1.

The detection of chromatograms was performed with aniline hydrogen phthalate [10].

A column (4.5 × 50 cm) packed with a suspension of blended microcrystalline cellulose in acetone was used for the preparative fractionation of a mixture of above D-xylose methyl derivatives. The fractions of 12 ml volume were collected in time intervals of 30 minutes by means of an automatic collector of fractions. The elution was performed with the solvent system S₂ which was also employed for washing the column before the use. For comparison, a preparative fractionation of methylated D-xylose was carried out on a column packed with the mixture of active charcoal (Darco G 60) and celite (1 : 1) under equal conditions as in preceding fractionation. For elution, the water solutions with 0–30% of ethanol were used.

Results and Discussion

The use of cellulose as adsorbent for thin-layer chromatography has a great asset because it permits in most cases to apply solvent systems which are customary in paper chromatography. *Wolfrom* and co-workers [7] have successfully employed microcrystalline cellulose among others for the separation of methylated hexoses, too. In this study, we have tried to separate some methyl derivatives of D-xylose on the microcrystalline cellulose which has been prepared in our Institute [9]. These investigations have involved the evaluation of seven solvent systems. It follows from the results referred in Table 1 that the solvent system S₂, *i.e.* butanol saturated with water is the most convenient because it separates distinctly 2,3-di-*O*-methylxylose from 2,3,4-tri-*O*-methylxylose in contrast to the solvent system S₁ which is usual in paper chromatography. Besides, it involves a substantial shortening of developing time in comparison to paper chromatography.

Table 1

Chromatographic mobilities (R_F) of D-xylose methyl derivatives
in seven solvent systems

Substance	$R_F \times 100$ in systems (S)						
	S ₁	S ₂	S ₃	S ₄	S ₅	S ₆	S ₇
D-xylose	43	28	17		49	43	11
2- <i>O</i> -methyl-D-xylose	57	46	42	57	79	78	40
2,3-di- <i>O</i> -methyl-D-xylose	76	73	78	78	95	94	90
2,3,4-tri- <i>O</i> -methyl-D-xylose	95	94	94	95	98	98	98
mixture of D-xylose methyl derivatives	Y	Y	S	S	N	N	N
Average developing time [min]	200	180	55	70	60	65	60

N — no separation.

Y — mixture separated.

S — badly streaked.

S₁: *n*-butanol—ethanol—water 4 : 1 : 5 (upper layer).

S₂: *n*-butanol saturated with water.

S₃: methyl ethyl ketone—acetic acid—methanol 6 : 1 : 2.

S₄: methyl ethyl ketone—acetic acid—methanol 3 : 1 : 1.

S₅: methyl ethyl ketone—acetic acid—methanol 2 : 1 : 3.

S₆: methyl ethyl ketone—acetic acid—methanol—water 6 : 1 : 1 : 1.

S₇: methyl ethyl ketone—water (azeotropic mixture).

The knowledge thus obtained has then been utilized for the preparative separation of a mixture containing methyl derivatives of D-xylose on a column packed with microcrystalline cellulose, the elution having been performed with the system S₂. The course of fractionation has been checked by thin-layer chromatography of correspondent fractions on microcrystalline cellulose using the system S₂ again. The results of fractionation are given in Table 2. For comparison, this table also includes

Table 2

Separation of a mixture containing methyl derivatives of D-xylose.
Separation efficiency of a column packed either with microcrystalline cellulose or with a mixture of active charcoal and celite

Substance	Amounts of investigated substances [mg]	Yields of pure substances [mg]	
		microcrystalline cellulose	active charcoal with celite
3- <i>O</i> -methyl-D-xylose	20	11	6
2,3-di- <i>O</i> -methyl-D-xylose	300	250	126
2,3,4-tri- <i>O</i> -methyl-D-xylose	50	33	—

the results of a preparative separation of methylated D-xylose which have been obtained in a previous study of structural features of 4-*O*-methylglucuronoxylan from the wood of hornbeam [11]. This separation method has involved low yields of pure fractions and has been lengthy owing to the tests of fraction purity by paper chromatography. It follows from the data presented in Table 2 that the separation efficiency on microcrystalline cellulose is higher under equal working conditions since the obtained amounts of pure substances are greater. Further advantage of this technique consists in a considerable shortening of the whole separation process.

The authors are grateful to Ing. L. Kuniak, CSc., who afforded them microcrystalline cellulose for his kindness.

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Translated by R. Domanský