Chemistry

1961

Abstract

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Chemistry

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Detection of α-Glycol Groups in Thin-Layer Chromatography on Silica Gel

(Presented by Academician M. M. Shemyakin, 12 VI 1961)

Thin-layer chromatography ([1,2]), discovered by N. A. Izmailov and M. S. Shraiber ([3]) as early as 1938, has in recent years found wide application for the separation of lipophilic substances ([4–11]). This method has also been extended to certain hydrophilic substances (amino acids ([12–14]), amines ([15])), but until now it has not yet been used for the separation of polyhydroxy compounds and, in particular, carbohydrates.*

In the present work, undertaken by us in connection with the study of the chemistry of macrolide antibiotics, the possibility of detecting polyhydroxy compounds during chromatography in a thin layer of silica gel was investigated. The objects of the study were carbohydrates and related substances (glucose, lactose, glucosaccharin), polyhydric alcohols (ethylene and propylene glycols, glycerol, 3-methylhexane-1,3,4-triol) and polyhydroxy acids (tartaric and 2,3-dihydroxy-2-methylpentanoic acid). Chromatography was carried out on plates (13 × 18 cm) coated with a layer of KSK silica gel (150–200 mesh), mixed with gypsum and water (6 g silica gel, 0.35 g gypsum, 15 ml water). After application of the paste, the plates were dried for 6–12 hr in air and for 40 min at 104–106°. For the detection of polyhydroxy compounds on chromatograms, reagents for the α-glycol group usually used in paper chromatography were tested: a mixture of 5% aqueous silver nitrate solution with 25% ammonia (A) ([17]), an alkaline solution of sodium periodate and potassium permanganate (B) ([18]), lead tetraacetate (with subsequent spraying with rosaniline) (C) ([19,20]), and potassium periodate with benzidine (D) ([21]).

We studied the dependence of the sensitivity of these reagents both on the nature of the substances chromatographed and on the developing system (see Table 1)**. The most sensitive reagent proved to be the ammoniacal silver nitrate solution, which gives distinct brown spots on a light background. A drawback of this reagent is the formation of a broad dark band (1–2 cm) at the front line, which makes it difficult to determine substances with a large R_f value. Somewhat less sensitive is the method based on oxidation with a solution of lead tetraacetate in chloroform. The white spots formed on a brown background in some cases (2,3-dihydroxy-2-methylpentanoic acid, glucosaccharin) become sharper upon subsequent treatment of the chromatograms with rosaniline. A

mixture of sodium periodate and potassium permanganate in alkaline solution makes it possible to detect the α-glycol group by the appearance on silica gel of white spots on a pink background. However, these spots appear relatively slowly (in a number of cases only after 40–50 min). The least suitable proved to be the method based

* When the present article was being prepared for publication, a paper by Stahl et al. ([16]) appeared on the separation of carbohydrates in a thin layer of Kieselguhr.

** The table gives the smallest amounts of substances detectable in the form of a distinct spot. The R_f values are averages of four determinations. Discrepancies between the data of individual determinations, as a rule, did not exceed $±15%.

of oxidation with potassium periodate followed by spraying with benzidine. Although this method is known to be very sensitive in the chromatography of carbohydrates on paper, under thin-layer chromatography conditions most of the substances we studied were detected only in amounts of 20–50 γ.

The data given in Table 1 show that glycerol, glucose, lactose, and tartaric acid are detected most readily; glycols and, in particular, compounds with branched chains containing tertiary hydroxyl groups are detected with greater difficulty. The sensitivity of the detection reaction depends strongly on the developing system and varies within fairly wide limits, which apparently is connected with the different sizes of the spots (i.e., with differences in the concentration of the substances after chromatography). We found that the systems ethyl acetate–methyl benzoate–formic acid and butanol–ethanol–formic acid, used in paper chromatography for the development of acids and carbohydrates, respectively, are practically inapplicable in thin-layer chromatography.

Table 1

Minimum amounts of substances (in γ) detected in various systems

Compounds	Rf	Reagents A	Reagents B	Reagents C	Reagents D
Methanol–chloroform (1 : 9)					
Ethylene glycol	0.3	10	10	50	50
1,2-Propylene glycol	0.35	10	20	40	40
Glycerol	0.1	2	4	10	6

Compounds	*Rf*	Reagents A	Reagents B	Reagents C	Reagents D
3-Methylhexane-1,3,4-diol	0.5	20*	10	40	50
Glucosaccharin	0.15	20	40	50	40
2,3-Dihydroxy-2-methylpentanoic acid	0.05	12	–	–	–
Ethanol–water (95 : 5)					
Tartaric acid	0	1.5	5	10	10
Glucose	0.7	1.5	15	20	15
Lactose	0.5	1.5	15	20	20
Glycerol	0.8	4	15	20	15
Glucosaccharin	0.8	20	40	32	50
2,3-Dihydroxy-2-methylpentanoic acid	0.5	20	40	32	50
Ethanol–acetic acid–water (60 : 0.25 : 10)					
Tartaric acid	0	2	5	10	10
Glucose	0.7	2	15	20	50
Lactose	0.6	2	10	20	50
Glycerol	0.8	4	20	15	50
Glucosaccharin	0.8	20	40	40	50
2,3-Dihydroxy-2-methylpentanoic acid	**	20	40	40	50

Compounds	Rf	Reagents A	Reagents B	Reagents C	Reagents D
Ethanol –25% ammonia solution– water (85 : 5 : 10)					
Tartaric acid	0.05	2	5	10	8
Glucose	0.4	2	12	20	25
Lactose	0.1	2	10	16	32
Glycerol	0.7	3	10	20	16
Glucosaccharin	0.15	15	40	32	40
2,3-Dihydroxy-2-methylpentanoic acid	0.8	15	40	32	40

* Detected as a white spot, which is due to the formation of the ammonia complex of silver acetylenide.
** Does not give a clear spot.

Methyl benzoate and butanol, which are components of the developing systems, are strongly adsorbed by silica gel (they cannot be removed even by prolonged drying at 105–110°*), which creates a background that interferes with detection of the substances.

* For the other systems indicated in Table 1, drying at 95–100° for 1.5–2 hours is sufficient. Development of the chromatogram, as a rule, takes from 2 to 4 hours.

Table 1 also presents some data on the separating ability of the developing systems we studied. For the separation of polyhydric alcohols, the most suitable system proved to be a chloroform–methanol mixture (9 : 1). For the separation of glucose, lactose, and hydroxy acids, the optimal systems are ethanol–water (95 : 5) and ethanol–ammonia–water (16 : 1 : 3).

The results we obtained show that the method of thin-layer chromatography on silica gel is suitable for the separation of polyhydroxy compounds, but the α-glycol grouping of the chromatographed substances is detected only at relatively high concentrations. The sensitivity of all the reagents proved to be 2-

5 times lower than in chromatography on paper, which is probably explained by the formation of strong hydrogen bonds between the hydroxyl groups of the compounds under study and the oxygen atoms of the silica gel.

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Received
8 VI 1961

REFERENCES CITED

1. E. Stahl, *Pharmaz. Rundschau*, No. 2, 1 (1959).
2. E. Demole, *J. Chromatography*, 1, 24 (1958).
3. N. A. Izmailov, M. S. Shraiber, *Farmatsiya*, 1938, 1.
4. J. G. Kirchner, J. M. Miller, G. J. Keller, *Anal. Chem.*, 23, 420 (1951).
5. R. H. Reitsema, *Anal. Chem.*, 26, 960 (1954).
6. M. Barbe, S. I. Zav' yalov, *Izv. AN SSSR, OKhN*, 1960, No. 7, 1309.
7. A. Winterstein, A. Studer, R. Rüegg, *Chem. Ber.*, 93, 2951 (1960).
8. R. Tschesche, F. Lampert, G. Snatzke, *J. Chromatography*, 5, 217 (1961).
9. A. A. Akhrem, A. I. Kuznetsova, *Med. prom.*, No. 2, 57 (1961).
10. M. Barbier, H. Jäger, H. Tobias, E. Wyss, *Helv. chim. acta*, 42, 2440 (1959).
11. M. J. D. van Dam, G. J. de Kleuver, J. G. de Heus, *J. Chromatography*, 4, 26 (1960).
12. E. Mutschler, H. Rochelmyer, *Arch. Pharmaz.*, 292/64, 449 (1959).
13. E. Nürnberg, *Arch. Pharmaz.*, 292/64, 610 (1959).
14. M. Brenner, A. Niederwieser, *Experientia*, 16, 378 (1960).
15. K. Teichert, E. Mutschler, H. Rochelmyer, *Deutsche Apotheker Z.*, 100, 283 (1960).
16. E. Stahl, U. Kaltenbach, *J. Chromatography*, 5, 351 (1961).

17. *Handbuch der Papierchromatographie*, hrsg. J. M. Heis und K. Macek, 1958, Jena, 1, S. 736.

18. R. U. Lemieux, H. F. Bauer, *Anal. Chem.*, 26, 920 (1954).

19. J. G. Buchanan, C. A. Dekker, A. G. Long, *J. Chem. Soc.*, 1950, 3162.

20. K. Sampson, F. Schild, R. J. Wicker, *Chem. and Ind.*, 1961, 82.

21. J. A. Cifonelli, F. Smith, *Anal. Chem.*, 26, 1132 (1954).

Note: Figure translations are in progress. See original paper for figures.