

CHROMATOGRAPHY OF PHOSPHATE ESTERS

Part II. Separation by Thin Layer Chromatography

M. YOUNAS and M. AKHTAR

Institute of Chemistry, Punjab University, Lahore

(Received February 21, 1977; revised July 20, 1977)

Abstract. Phosphate diesters have been separated by thin layer chromatography on silica gel GF 254 (type 60) at 25°C with chloroform: methanol (65:35) as an irrigating solvent system. R_f values of the esters in the pure form as well as in mixture have been reported.

This communication is an extension [1] of chromatographic study of phosphate esters. The increasing scope [2] of thin layer chromatography (TLC) stimulated its application in the field of phosphate esters. In this investigation an attempt has been made to establish definite conditions and environments for the separation of phosphate diesters by TLC. In view of the growing interest [3-6] in the study of phosphate esters there seems to be a need to standardise the conditions under which the reactions of phosphate esters can be followed by TLC and also when necessary a mixture of phosphate esters could be separated by this technique. TLC study has been carried out on the same model phosphate diesters which were studied by paper chromatography [1] and are listed below.

- A. Diphenyl phosphate
- B. 4-Nitrophenylmethyl phosphate
- C. 2-Nitro-4-chlorophenylmethyl phosphate
- D. Di-4-nitrophenyl phosphate
- E. Di-3-nitrophenyl phosphate
- F. Di-2,4-dinitrophenyl phosphate
- G. Di-2-nitro-4-acetylphenyl phosphate
- H. Di-2-nitrophenyl phosphate

Although all these compounds are diesters, they represent a broad spectrum of the compounds of this class because some are structurally very similar while the others are quite different from each other. So the conditions of TLC established for the separation of these compounds can be considered as of fairly general application for this class of compounds.

Experimental

Choice of Adsorbent. Most commonly used adsorbents for TLC are alumina and silica gel. Therefore, these two adsorbents were tried in the present investigation. It was observed that the substance refused to move when alumina was used in any form but on the other hand silica gel GF 254 (type 60) was found to be the most useful adsorbent in TLC of phosphate esters. Therefore, this adsorbent was used in the present work.

The thickness of the adsorbent layer had little effect on R_f values. In the present investigation a layer of 0.4 mm thickness was used. Moisture content does influence the R_f values.

Application of Samples. Solution of the phosphate esters were prepared in acetone to put the spots on the chromatographic plate. The samples were applied on the starting point on a line which was situated at a distance of about 3.0 cm from the immersed end of the plate and the spots were about 2.0 cm apart. It was noticed that the amount of the sample did not have much effect on the R_f values so long as it is not used in very large quantities.

Solvent for Irrigation. Solvents of reagent grade were generally used but were purified before use when felt necessary and fresh solvents were used every time. In the beginning, the solvents used by Hanes and Isherwood [7] in paper chromatography of sugar phosphates, were tried for the TLC of our phosphate esters. These solvent systems gave very high R_f values (>0.9) for most of the esters. Some esters moved even with the solvent front rendering separation impossible. Then other aqueous solvent systems used in our paper chromatographic studies of these phosphates [1] were tried in varying proportions of the components but in each case an undesirable trailing of the spots and high R_f values with little separation were obtained. Then attention was focussed on neutral organic solvents and the following solvent systems were tried in all possible proportions.

1. Benzene + methanol
2. Chloroform + methanol + carbon tetrachloride
3. Chloroform + methanol

Although the second solvent system was found to be satisfactory, best results were obtained with the last solvent system, i.e. chloroform + methanol in 65:35 ratio.

As the irrigating solvent system consisted of organic solvents differing in volatility, mere opening of the chromatographic chamber changed the proportion of the solvents in the system resulting in the change of R_f values. It was, therefore, necessary to saturate the chamber with vapours of the solvent system, before placing the chromatographic plate in it, to get consistent R_f values. This was best achieved by putting the solvents into the chamber, placing the lid on it and leaving it for at least 2 hr, before use. Such saturation speeds up the irrigation, gives consistent R_f values, produces a straight solvent front and round spots with better separation.

Detection of Spots. As activated silica gel with

fluorescent material was used as an adsorbent and as the phosphate esters show strong absorption in the UV region, the spots were visible, and were marked, by simply placing the irrigated and dried chromatographic plate directly under the UV light from Mineralight Short Wave UVS-12. All the phosphate esters were detected by this method.

Temperature. As the components of the solvent system differ in volatility, the temperature was kept constant within $\pm 0.5^\circ\text{C}$ to avoid any change in the relative proportion of the components.

Results and Discussion

R_f values of the phosphate diesters (A-H) in the pure form as well as in a mixture of six phosphate diesters (A+C+D+F+G+H) under the standardised conditions for TLC are given in the Table 1.

A mixture of up to six phosphate diesters has been separated successfully and the R_f values of these esters in the mixture are not very different from those of

TABLE 1. R_f VALUES OF PHOSPHATE DIESTERS MEASURED BY TLC ON SILICA GEL GF₂₅₄ (TYPE 60), AT 25°C WITH CHLOROFORM: METHANOL (65:35) AS AN IRRIGATING SOLVENT SYSTEM.

Phosphate diesters	R_f values	
	Pure	In mixture
A. Diphenyl phosphate	0.27	0.26
B. 4-Nitrophenylmethyl phosphate	0.31	
C. 2-Nitro-4-chlorophenylmethyl phosphate	0.35	0.34
D. Di-4-nitrophenyl phosphate	0.52	0.53
E. Di-3-nitrophenyl phosphate	0.58	
F. Di-2,4-dinitrophenyl phosphate	0.64	0.63
G. Di-2-nitro-4-acetylphenyl phosphate	0.70	0.67
H. Di-2-nitrophenyl phosphate	0.81	0.84

the same esters in the pure form as shown in Table 1. The R_f values of a given substance is not expected to be exactly the same in mixture as it is in the pure state [8].

While exploring different solvent systems, it was noticed that different solvent systems not only give different R_f values but they also alter the relative order of the R_f values of different esters. However, it was possible by adjusting the proportion of the components in a solvent system to alter markedly the R_f values of the esters without changing their relative order. With most of the solvent systems adequate differentiation was only possible when the composition was adjusted to higher R_f values. The compositions of the solvent system should be adjusted according to which component of the mixture is desired to be separated.

It was observed, as is usually the case in TLC, that R_f values were not exactly reproducible but by controlling all the factors which could influence the R_f values, i.e. nature and thickness of the adsorbent, composition of the irrigating solvent, saturation of the chamber, temperature and amount of the sample, it was possible to narrow down the deviation from the actual R_f values.

Acknowledgement. We wish to thank Dr. M. Umar for his very useful comments and suggestions during these investigations.

References

1. M. Younas and S.A. Zahid, Pakistan J.Sci. Ind. Res. (accepted).
2. E. Stahl, *Thin Layer Chromatography* (Academic Press, London, 1965).
3. J.R. Cox Jr. and O.B. Ramsay, Chem. Rev., **64**, 317 (1964).
4. T.C. Bruice and S.J. Benkovic, *Bioorganic Mechanisms* (Benjamin, New York, 1966), vol II.
5. M. Younas, Pakistan J. Sci., **24**, 100 (1972).
6. M. Younas, *ibid*, **25**, 280, (1973).
7. C.S. Hanes and F.A. Isherwood, Nature, **164**, 1107 (1949).
8. G. Pastuska, Z. Anal. Chem., **179**, 355 (1961).