

THE PAPER CHROMATOGRAPHY OF ESTERS OF PHOSPHORIC AND PHOSPHOROUS ACIDS

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INTRODUCTION

Few methods have been published for the analysis of trialkyl or triaryl phosphites. BERNHART AND RATTENBURY¹ described an acid-alkali titration procedure for determining mono-, di- and trialkyl phosphites in mixtures of these compounds, while a method for titrating trialkyl phosphites with a methanolic solution of iodine in the presence of pyridine has been described by THOMPSON². With either method the reaction proceeds slowly with triaryl phosphites, while neither will differentiate between several trialkyl phosphites together in a mixture. In addition certain phosphites, such as tris-2-chloroethyl phosphite, lose chloride with alkali, leading to erroneous results if the acid-alkali titration procedure is used.

Acid esters of phosphoric acid can be analysed by acid-alkali titration methods, which make use of the several end points of phosphoric acid and its esters, but if trialkyl phosphate is present this does not react, being neutral. Further, if esters of pyrophosphoric acid are present, as may often be the case, the results obtained by titration are difficult to interpret. Similarly, the 2-chloroethyl esters lose chloride with alkali.

Separation on paper of phosphoric acid esters, such as hexose monophosphates and adenosine triphosphate was described by HANES AND ISHERWOOD³, who used various mobile phases including alcohol-water-ammonia mixtures. Their method of revealing the spots was to spray the papers with an acid molybdate solution, and then to expose them to hydrogen sulphide gas. Since this paper was published there have been many papers dealing with the separation on paper of mixtures of phosphoric acid esters of biological importance.

The separation of phosphoric acid, monobutyl phosphate and dibutyl phosphate has been reported by several workers. CERRAI, CESARANO AND GADDA⁴ used a *n*-butanol-formic acid-water solvent, and revealed the spots by the HANES-ISHERWOOD³ method, or by spraying with bromophenol blue solution. HARDY AND SCARGILL⁵ employed a *n*-butanol-acetone-water-ammonia solvent mixture, and by spraying with a ferric iron solution followed by a sulphosalicylic acid solution, as recommended by WADE AND MORGAN⁶, obtained colourless spots on a mauve background. SHVEDOV AND ROSYANOV⁷ used several mobile phases, including alcohol-water-ammonia mixtures, and showed up the spots by the HANES-ISHERWOOD³ technique.

PLAPP AND CASIDA⁸ have described the separation of certain mono- and dialkyl

phosphates derived from the breakdown of insecticides. They used isopropanol-water-ammonia mobile phases, and to reveal the spots they sprayed the papers with an acid molybdate solution and then exposed them to ultraviolet light, as recommended by CROWTHER⁹.

WEIL¹⁰ employed a mobile phase containing *n*-butanol, water and ammonia to separate mixtures of phosphonous, phosphonic and phosphinic acids and certain of their esters, showing up the spots by spraying with a saturated alcoholic solution of silver nitrate, or with a slightly alkaline phenol red solution.

We have utilised paper chromatographic techniques to separate technical samples of a number of esters of phosphorous acid. These include tris-2-chloroethyl phosphite, octyl diphenyl phosphite, triallyl phosphite and trioctyl phosphite. Methods have also been developed for the separation of alkyl phosphate samples, which may contain mono-, di- or trialkyl-phosphates, as well as free phosphoric and pyrophosphoric acids and esters of pyrophosphoric acid. The separated components may be determined quantitatively by wet ashing the spots and determining the phosphorus, as suggested by HANES AND ISHERWOOD³.

EXPERIMENTAL

Reagents

All reagents were AnalaR or AR quality wherever possible.

1. Stationary phases

20 % v/v liquid paraffin in ether: Mix 10 ml of liquid paraffin (sp. gr. 0.865–0.890) with 40 ml of ether as required.

Alkaline 20 % v/v polyethylene glycol 400 in methanol: Mix 10 ml of polyethylene glycol 400 with 35 ml of methanol and 5 ml of 0.5 *N* ethanolic potassium hydroxide solution as required.

Sodium carbonate solution: Mix 15 ml of 1 % w/v aqueous sodium carbonate solution with 35 ml of methanol as required.

2. Mobile phases

90/10 petrol-*n*-butanol mixture: Mix 90 ml of 100°–120° petroleum spirit with 10 ml of *n*-butanol.

80/20 petrol-chloroform mixture: Mix 80 ml of 100°–120° petroleum spirit with 20 ml of chloroform.

Nitromethane.

2-Methoxyethanol: Redistilled. Boiling range 122°–124°.

Methanol.

n-Propanol-water-ammonia mixture: Mix 70 ml of *n*-propanol with 30 ml of 2 *N* aqueous ammonia.

n-Propanol-*n*-butanol-water-ammonia mixture: Mix 50 ml of *n*-propanol, 20 ml of *n*-butanol and 30 ml of 2 *N* aqueous ammonia.

3. Spray reagents

Alkaline potassium permanganate solution: Dissolve 0.15 g potassium permanganate and 0.1 g anhydrous sodium carbonate in 100 ml of water.

Alkaline universal indicator solution: Dissolve 0.1 g anhydrous sodium carbonate

in 100 ml of Universal Indicator solution, as supplied by British Drug Houses, Ltd., England.

Ferric thiocyanate solution: Mix 10 ml of 2.5 % w/v ferric ammonium sulphate solution with 40 ml of 5 % w/v ammonium thiocyanate solution, add 1 ml of *N* hydrochloric acid solution and dilute to 100 ml with water.

Preparation of papers

Whatman No. 54 papers are usually employed, since they have good wet strength and rapid flow characteristics; a 2 in. wide strip has been found convenient. Where an added stationary phase is necessary the paper is dipped in the appropriate stationary phase solution, the solvent is dried off with an air current and the paper is rolled and stored in an air tight vessel. In the case of the polyethylene glycol 400 and sodium carbonate stationary phases a small dish of water is placed in the vessel, to maintain a water saturated atmosphere. Where no added stationary phase is used the papers are allowed to equilibrate in the chromatographic tank for 2-3 h before use.

General chromatographic procedure

Papers are cut from the prepared roll in lengths of about 18 in. to which is applied a droplet of sample (about 0.25-1.0 μ l); the descending technique is used. The time taken for the solvent to flow varies considerably: with sodium carbonate papers it may be 1-1½ h, while with paraffin papers it may be 7-8 h. When the solvent front has travelled to within 2 in. of the lower edge the paper is removed from the tank and the solvent is evaporated by a current of air. The spots are revealed by spraying the papers.

Phosphites are revealed as yellow spots on a pink background by the use of alkaline permanganate: the background colour fades rapidly, and the spots must be outlined as soon as possible. For acid phosphates alkaline Universal Indicator shows the spots distinctly and is quite sensitive. The traces are yellow on a blue-green background, fading slowly as the paper dries to red on a yellow background.

If trialkyl phosphates are suspected the paper should be sprayed with the ferric thiocyanate solution. Trialkyl phosphates from C₃ upwards show as bright red spots, while acid phosphate esters give colourless spots on a brown-pink background. This reagent is not as sensitive as Universal Indicator to the acidic components.

Individual procedures

I. Esters of phosphorous acid. Samples of tris-2-chloroethyl phosphite or tris-2-chloropropyl phosphite may be separated on sodium carbonate papers with 90/10 petrol-*n*-butanol mixture as mobile phase. The sodium carbonate serves only to maintain a mildly alkaline environment for the sample, which is sensitive to acids. As might be expected the moisture content of the paper affects the R_F' values, and Table I gives typical values obtained with papers treated as described above.

Technical octyl diphenyl phosphite may contain a variety of chemical entities, including trioctyl phosphite, dioctyl phenyl phosphite, octyl diphenyl phosphite, diphenyl phosphite, triphenyl phosphite and phenol. A complete separation of these compounds can be achieved by the use of methanol as mobile phase and liquid paraffin as stationary phase, while nitromethane will separate all but di- and triphenyl phosphite. The appropriate R_F' values are given in Table II.

TABLE I
 R_F' VALUES FOR ESTERS OF PHOSPHOROUS ACID
 (Sodium carbonate stationary phase, 90/10 petrol-*n*-butanol mobile phase)

Tris-2-chloroethyl phosphite	0.95
Bis-2-chloroethyl phosphite	0.35
Tris-2-chloropropyl phosphite	1.00
Bis-2-chloropropyl phosphite	0.73

Some trialkyl phosphites, such as trioctyl and trilauryl phosphites, can be separated on liquid paraffin papers with 2-methoxyethanol as mobile phase. The usual impurity in these materials is dialkyl phosphite, sometimes with traces of alkyl diphenyl phosphite and phenyl dialkyl phosphite. The R_F' values for these compounds will also be found in Table II.

Papers with alkaline polyethylene glycol 400 as stationary phase will effect a separation of technical triallyl phosphite, as well as tris-2-chloroethyl phosphite and tris-2-chloropropyl phosphite. The alkali, which rapidly becomes carbonate, maintains mildly alkaline conditions. In this case the mobile phase is 80/20 petrol-chloroform solution, and the R_F' values from typical samples are listed in Table III.

TABLE II
 R_F' VALUES FOR ESTERS OF PHOSPHOROUS ACID
 (Liquid paraffin stationary phase)

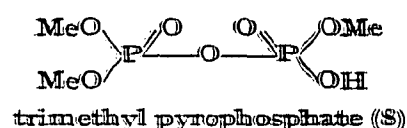
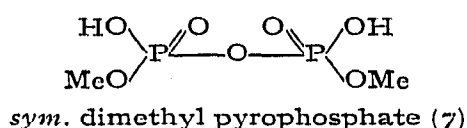
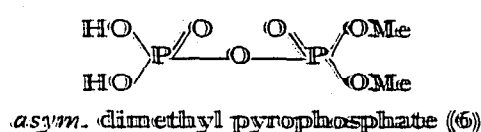
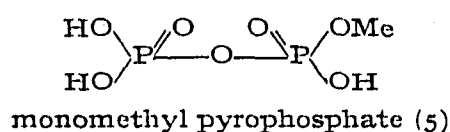
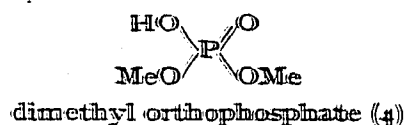
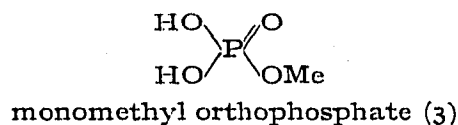
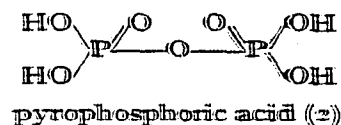
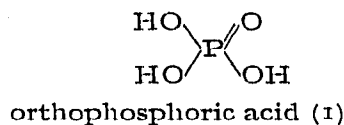
		Mobile phase		
		Methanol	Nitromethane	2-Methoxyethanol
Trioctyl phosphite	$(C_8H_{17}O)_3P$	0.07	0.03	0.12
Phenyl dioctyl phosphite	$C_6H_5OP(OC_8H_{17})_2$	0.20	0.12	0.40
Octyl diphenyl phosphite	$C_8H_{17}OP(OC_6H_5)_2$	0.36	0.51	0.74
Triphenyl phosphite	$(C_6H_5O)_3P$	0.65	0.92	0.96
Dioctyl phosphite	$(C_8H_{17}O)_2POH$	DNS*	DNS	0.88
Diphenyl phosphite	$(C_6H_5O)_2POH$	0.80	1.00	0.96
Trilauryl phosphite	$(C_{12}H_{25}O)_3P$	0.03	0.00	0.03
Dilauryl phosphite	$(C_{12}H_{25}O)_2POH$	DNS	DNS	0.55
Phenol	C_6H_5OH	1.00	1.00	0.96

* DNS = Did not show.

TABLE III
 R_F' VALUES FOR ESTERS OF PHOSPHOROUS ACID AND ASSOCIATED IMPURITIES
 (Polyethylene glycol 400 stationary phase, 80/20 petrol-chloroform mobile phase)

Ester	R_F' value
Triallyl phosphite	$(CH_2=CH\cdot CH_2O)_3P$ 0.97
Diallyl phosphite	$(CH_2=CH\cdot CH_2O)_2POH$ 0.43
Triallyl phosphate	$(CH_2=CH\cdot CH_2O)_3PO$ 0.67
Diallyl phosphate	$(CH_2=CH\cdot CH_2O)_2POOH$ 0.31
Tris-2-chloroethyl phosphite	$(ClCH_2\cdot CH_2O)_3P$ 0.56
Bis-2-chloroethyl phosphite	$(ClCH_2\cdot CH_2O)_2POH$ 0.09
Tris-2-chloropropyl phosphite	$[CH_2\cdot CH(Cl)\cdot CH_2O]_3P$ 0.93
Bis-2-chloropropyl phosphite	$[CH_2\cdot CH(Cl)\cdot CH_2O]_2POH$ 0.25

II. Esters of phosphoric acid. Technical samples of acid alkyl phosphate esters may contain monoalkyl orthophosphate and dialkyl orthophosphate with some free orthophosphoric acid. In addition there may be free pyrophosphoric acid and esters of pyrophosphoric acid. The possible components of, for example, acid methyl phosphates are:



Further, trialkyl phosphate may contain dialkyl phosphate as impurity. The components of such samples can be separated very efficiently using Whatman 54 paper with no prior treatment, apart from equilibration in the chromatographic tank, and 70/30 *n*-propanol-2 *N* aqueous ammonia mixture as mobile phase. This solvent was recommended by HOWE¹¹ for the separation of organic acids.

The R_F' values of the components of various acid alkyl phosphates are given in Table IV. Those components which have been identified by the use of reference com-

TABLE IV
 R_F' VALUES FOR ESTERS OF PHOSPHORIC ACID
(70/30 *n*-propanol-2 *N* aqueous ammonia as mobile phase)

Alkyl radicle	Mono-alkyl	Di-alkyl	Tri-alkyl	Pyro acid	Ortho acid	Other traces (probably esters of pyro acid)
Methyl	0.22	0.65	1.00	0.04	0.12	0.08, 0.31, 0.42, 0.72
Ethyl	0.27	0.77	1.00			0.18, 0.47
Isopropyl	0.33	0.86	1.00			0.20, 0.61
<i>n</i> -Butyl	0.48	0.94	1.00			
<i>n</i> -Amyl	0.55	0.95	1.00			0.14, 0.28, 0.39
<i>n</i> -Hexyl	0.62	0.95	1.00			
Octyl	0.68	0.95	1.00			
Nonyl	0.75	0.98	—			
Lauryl	0.79	0.98	—			
2-Chloroethyl	0.33	0.84	—			0.19, 0.59
2-Hydroxyethyl	0.20	0.49	—			0.29

pounds are indicated. The identity of the other traces is conjectural, but they are probably esters of pyrophosphoric acid. Thus a sample of acid methyl pyrophosphate gave eight spots, which were considered to be as follows:

0.04	pyrophosphoric acid	(2) above
0.08	monomethyl pyrophosphate	(5) above
0.13	orthophosphoric acid	(1) above
0.21	monomethyl orthophosphate	(3) above
0.31	dimethyl pyrophosphate (<i>sym.</i>)	(7) above
0.42	dimethyl pyrophosphate (<i>asym.</i>)	(6) above
0.62	dimethyl orthophosphate	(4) above
0.72	trimethyl pyrophosphate	(8) above

As can be seen from Table IV the trialkyl phosphates travel with the solvent front in all cases. A slightly better separation of trialkyl phosphate from dialkyl phosphate can be achieved by substituting 50 : 20 : 30 *n*-propanol-*n*-butanol-2 *N* aqueous ammonia solution as mobile phase.

Quantitative analyses

The spots which have been revealed by the spray reagent can be cut out and wet ashed, using nitric, sulphuric and perchloric acids, and the phosphorus content determined. This technique has been used on a routine basis for the analysis of phosphite and phosphate esters.

The experimental procedure is the same as has been described for the qualitative separations, except that in order to obtain enough phosphorus to permit reasonably accurate determinations the sample weight must be about 3-4 mg. Such an amount of sample cannot be applied as a single spot, and it is our practice to streak the sample on to the starting line, as evenly as possible, from a weighed glass capillary tube. The sample weight is then obtained simply by reweighing the capillary. A micro balance should be used for this purpose.

Duplicate determinations normally agree to within $\pm 3\%$, but for complete recovery the sample must be substantially involatile. This is not the case with, for example, triallyl phosphite or the lower trialkyl phosphites. Most of the phosphites and acid alkyl phosphates mentioned in the paper can be determined in this manner.

SUMMARY

Procedures are described for the separation on paper of the components of technical samples of alkyl and alkyl-aryl phosphites and of esters of phosphoric acid. Suitable stationary and mobile phases, as well as spray reagents, are noted, and typical R_F' values are presented. The quantitative analysis of these esters, by wet ashing the spots and determining the phosphorus content, is briefly indicated.

REFERENCES

- ¹ D. N. BERNHART AND K. H. RATTENBURY, *Anal. Chem.*, 28 (1956) 1765.
- ² Q. E. THOMPSON, *J. Am. Chem. Soc.*, 83 (1961) 845.
- ³ C. S. HANES AND F. A. ISHERWOOD, *Nature*, 164 (1949) 1107.
- ⁴ E. CERRAI, C. CESARANO AND F. GADDA, *Energia Nucleare*, 4 (1957) 405.
- ⁵ C. J. HARDY AND D. SCARGILL, *J. Inorg. Nucl. Chem.*, 10 (1959) 323.
- ⁶ H. E. WADE AND D. M. MORGAN, *Biochem. J.*, 60 (1955) 264.

- ⁷ V. P. SHVEDOV AND S. P. ROSYANOV, *Zhur. Anal. Khim.*, 14 (1959) 507.
- ⁸ F. W. PLAPP AND J. E. CASIDA, *Anal. Chem.*, 30 (1958) 1622.
- ⁹ J. P. CROWTHER, *Anal. Chem.*, 26 (1954) 1383.
- ¹⁰ T. WEIL, *Helv. Chim. Acta*, 38 (1955) 1274.
- ¹¹ J. R. HOWE, *J. Chromatog.*, 3 (1960) 389.

J. Chromatog., 11 (1963) 77-83