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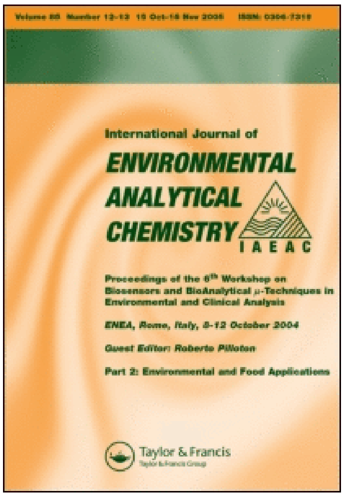

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Liquid Chromatography of Organophosphorus Acids

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The applicability of different liquid chromatographic systems such as ion-exchange, ion-exclusion, reversed phase and ion-pair partition was studied for the analysis of a number of simple structurally related organophosphorus acids which lack a chromophoric group.

Preliminary experiments based on these chromatographic systems were carried out on conventional LC columns with indirect UV or differential refractometric detection. Some promising results were obtained using the combination of micro-liquid chromatography columns and a flame photometric detector.

KEY WORDS: HPLC, micro LC, organophosphorus acids, specific detection.

INTRODUCTION

Generally, organophosphorus acids are non-volatile and strongly polar compounds, being completely dissociated at neutral pH-values. As a consequence it will be difficult to analyse these compounds as such by gas chromatography. This problem can be overcome by applying a derivatization reagent which transforms the acids into more volatile products. However, all derivatization methods have the disadvantage of an additional time consuming sample treatment. This results in the fact that qualitative and quantitative analysis of the original compounds have to be carried out in an indirect way.

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Analysis by liquid chromatography (LC) of the organophosphorus acids seems more straightforward. However, compounds such as alkyl methylphosphonic acids and alkyl phosphoric acids lack a chromophoric group. As a consequence the use of direct UV detection will be impossible. Therefore derivatization procedures have also been described for LC analysis of alkyl methylphosphonic acids, converting the acids into compounds containing a chromophoric group.[1] Bossle *et al.*[2] demonstrated the application of ion-pair reversed phase ion chromatography for the analysis of alkyl methylphosphonic acids in aqueous matrices using a non-selective conductivity detector.

The aim of this work was to evaluate the applicability of different liquid chromatographic systems in combination with the development of a sensitive and specific detection without the use of derivatization reagents. Ion-exchange, ion-exclusion, reversed phase and ion-pair partition were studied. The ultimate goal will be the use of an LC system coupled with a flame photometric detector (FPD) as well as with a mass spectrometer equipped with a thermospray interface (TSP-MS). The applicability of μLC-FPD was studied using the recently improved interface of which the design has been published.[3] Since ammonium counter ions are frequently used for ion-pair chromatography of acids and TSP-MS needs a volatile electrolyte, experiments were carried out with an ammonium acetate-containing aqueous eluent. The concerning LC-TSP-MS results are published by Wils *et al.*[4]

In this paper some preliminary results of the different approaches in the LC analysis concerning both the separation and the selective detection of the organophosphorus acids are presented.

EXPERIMENTAL PART

A liquid chromatographic system consisting of an M6000A pump and either a variable wavelength UV detector model 450 or a differential refractometer model R401 (Waters Ass., Millipore Corp., Milford, MA (USA)) was used. The micro-liquid chromatograph consisted of an LC-5A pump (Shimadzu, Kyoto, Japan) and a flame photometric detector (FPD) model 380 (Carlo Erba, Milan, Italy).

The following columns were used:

1) Partisil SAX, 10 μm (Maidstone, U.K.), 250 mm × 5 mm, home-made.[5]

2) Lichrosorb RP18, 5 μm (Darmstadt, F.R.G.) 250 mm × 5 mm, home-made.[5]
3) Fast Fruit Juice column, 150 mm × 7.8 mm, (Waters Ass.) containing a sulphonated resin combined with the corresponding Guard-Pack cartridge.
4) Fused silica (FS) column, 250 mm × 0.32 mm, packed with Hamilton PRP1 styrene-divinylbenzene polymer (RSL-Alltech, Eke, Belgium) according to Gluckman.[6]
5) FS column, 250 mm × 0.32 mm, containing Rogel SO_3H-1, 6 μm, (Alltech).

The different organophosphorus compounds were synthesed in this laboratory. The composition of the mobile phases used is described in the legends of the figures. The concerning chemicals were of analytical grade as delivered by Merck.

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RESULTS AND DISCUSSION

Ion-exchange

In ion-exchange chromatography the elution of ions depends on the relative affinities of these ions and the counter-ions toward the ionogenic groups on the resin as well as on the ionic concentrations present in the mobile phase.

To analyse the UV-nonabsorbing organophosphorus acids phthalate was used as counter-ion according to the method of Schmall.[7] The pH and the concentration of potassium hydrogen phthalate in the mobile phase were varied. The wavelength was adjusted to 280–290 nm which differ from the maximum phthalate absorption at 272 nm to suppress the background absorption. The applicability of this chromatographic system was tested with methylphosphinic acid [$CH_3P(O)(OH)H$, I] and methylphosphonic acid [$CH_3P(O)(OH)_2$, II] as model compounds. The ion-exchange system was composed of Partisil SAX-10 μm as stationary phase. In the pH range 4–6 and at potassium hydrogen phthalate concentrations above 5 mM the behaviour of these simple organophosphorus acids as well as of other similar acids was found to be generally characterized by short retention times and a poor resolution. Additionally, solvent and system peaks (S) interfered in the detection. In Figure 1 the pH of the mobile phase is plotted against the retention of I and II. Inversion of the retention at pH 4.7 as well as the close elution of I

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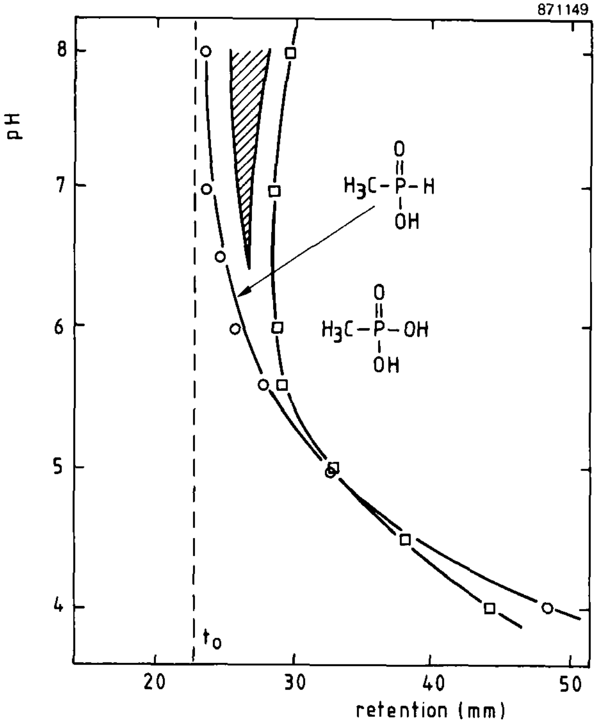

Figure 1 Plot of pH versus retention of methylphosphinic acid and methylphosphonic acid. Mobile phase: 15 mM potassium hydrogen phthalate. Column: no. 1, see Experimental Part. Baseline separation is indicated by shading.

and the solvent (t_0) peak above pH 6.5 can be seen. At phthalate concentrations below 1 mM a better resolution was obtained although broad peaks with tailing occurred (Figure 2). Other comparably charged organophosphorus acids with different alkyl groups were found to elute close to I and II. From this small difference in retention behaviour it can be concluded that the main separation mechanism is governed by ionic interactions of the dissociated species with the positivily charged groups on the stationary phase.

Nicotinic acid and *p*-nitrobenzoic acid have been proposed as possible alternative counter-ions[8] but did not give significant improvement in terms of peak tailing and resolution of the organophosphorus acids. It is concluded that for the analysis of mixtures containing such closely related organophosphorus acids ion-

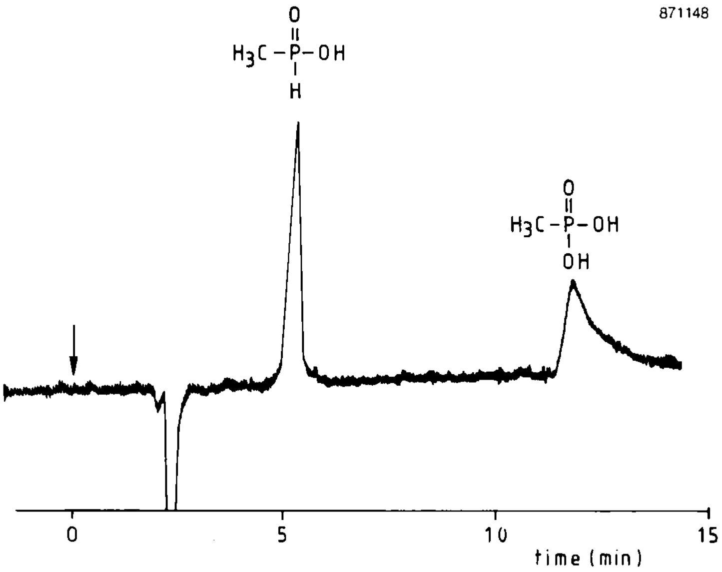

Figure 2 Ion-exchange chromatogram of methylphosphinic acid and methylphosphonic acid at pH 6.5 and 0.5 mM potassium hydrogen phthalate. Column: no. 1, see Experimental Part.

exchange chromatography is unattractive because of the small difference in retention values.

ION-EXCLUSION

In ion-exclusion chromatography the pH of the mobile phase is adjusted to such a low value that undissociated forms of the acids to be analysed result. These neutral species can penetrate the pores of the stationary phase consisting of a cation-exchange material giving adsorption whereas dissociated or anionic forms are excluded from the pores resulting in a retention time close to t_0.[9] The first and essential pK_a value of the organophosphorus acids studied lies around 2.5. As a consequence the pH of the mobile phase has to be adjusted to about 1 to obtain an almost complete protonation of the acids. Because such acidic mobile phases normally affect silica based packing materials organic polymeric cation materials were selected.

The initial experiments were carried out with a commercially available sulphonated resin material (Waters Ass.). A similar station-

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ary phase was used with dilute HCl as mobile phase for the separation of organic acids analysed by TSP-LC-MS.[10] The manufacturer of the Fast Fruit column advised to avoid corrosive mobile phase additives such as HCl, but HNO_3 is allowed.

In Figure 3 a plot of *k'* values against the HNO_3 molarity of the mobile phase is presented. It shows that in the concentration range studied *k'* values of the acids are relatively small and remain unchanged above 0.04 M. The retention differences are mainly determined by the number of carbon atoms in the alkyl group of the organophosphorus acids. The neutral species are retained by the interaction with the organic polymer. As can be seen from Figure 4 the peaks of some representative compounds are symmetrical and a

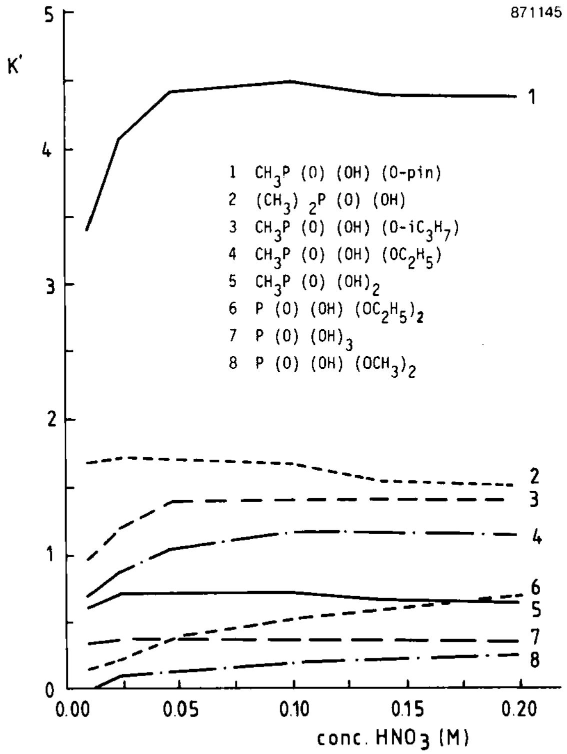

Figure 3 Plot of *k'* versus different conc. HNO_3 (M) as mobile phase and column 3 (see Experimental Part).

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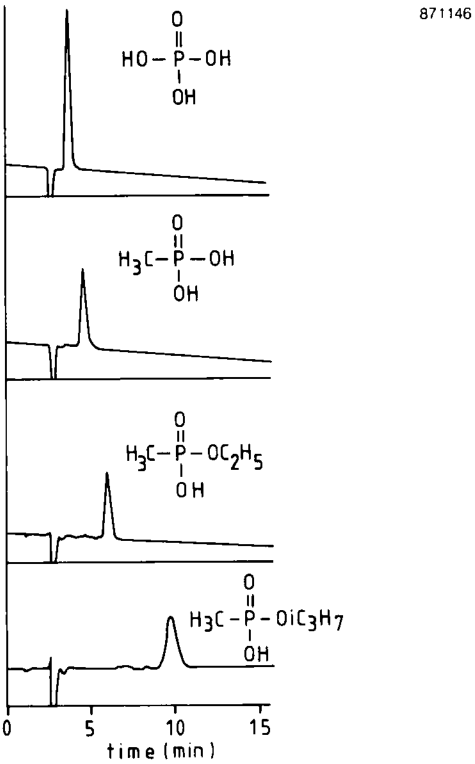

Figure 4 Ion-exclusion chromatogram of a number of organophosphorus acids. Mobile phase: 0.14 M HNO_3; column: no. 3, see Exp. Part; detection: differential refractrometer.

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reasonable separation efficiency (8500 plates pro metre) was obtained.

Figure 5 shows the results of a comparable μLC version of this ion-exclusion separation system in which the differential refractometric detector of the conventional system was replaced by an FPD detector. The excessive peak width is probably due to the incomplete wettability of this stationary phase.

These experiments indicate that ion-exclusion chromatography offers sufficient possibilities for the separation of the organophosphorus acids and that it can be used in the combination of μLC-FPD. However, the use of strong acids have some drawbacks.

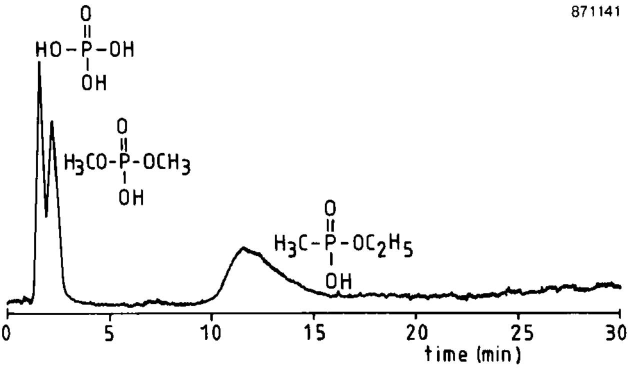

Figure 5 Ion-exclusion chromatogram of three organophosphorus acids. Mobile phase: 0.1 M HNO_3; no. 5, see Experimental Part; detection: FPD.

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Especially when combined with mass spectrometry decomposition in the heated TSP interface could be expected for thermally unstable organophosphorus acids such as pinacolyl methylphosphonic acid.

REVERSED PHASE SEPARATION

Based on the ion-exclusion experiments with the undissociated acids it was concluded that an important effect in the separation is the interaction between the alkyl groups and the lipophilic part of the resin. To prove this assumption a reversed phase ion-suppression system was applied using a PRP column.

A characteristic chromatogram is presented in Figure 6 showing the separation of a mixture consisting of phosphoric acid, dimethyl phosphoric acid and ethyl methylphosphonic acid with a mobile phase at pH 1. The influence of the alkyl groups is obvious as well as the probably incomplete wettability of the stationary phase, comparable to the results presented in Figure 5.

ION-PAIR PARTITION

For the separation of organic acids ion-pair chromatography will frequently be carried out on a reversed phase stationary phase (e.g. Lichrosorb RP18) and an ammonium counterion in the mobile phase. A number of experiments was carried out using ammonium

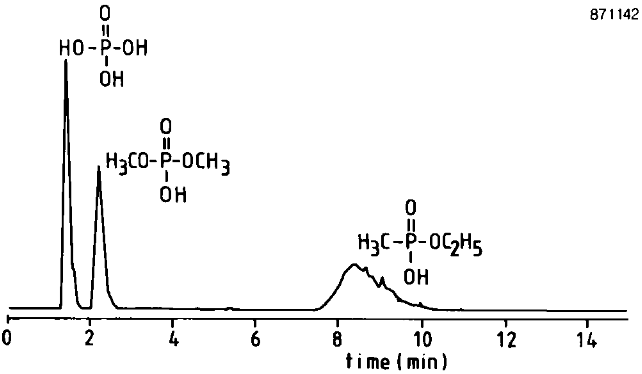

Figure 6 Reversed phase ion-suppression chromatogram of three organophosphorus acids. Mobile phase: HNO_3, pH 1; column: no. 4, see Experimental Part; detection: FPD.

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acetate as mobile phase additive and a differential refractometer as detector.

Some results are presented in Figure 7 showing a representative set of chromatograms. Due to the short retention times of the chosen organophosphorus compounds and the nonselective refractometric detection the peaks of the acids coincide with the solvent and system peaks. Preliminary experiments using similar mobile and stationary phases were carried out with μLC-FDP (Figure 8).

Due to the selective detection mode no disturbance due to the solvent or system peaks was observed. The μLC system shows a nice baseline separation. Comparable results could be obtained by TSP-LC-MS as published by Wils *et al.*[4] In this case mixtures of several structurally related oxygen- as well as sulphur-containing organophosphorus acids were separated. The micro-LC column and the conventional column show a similar retention sequence for the corresponding compounds. The μLC experiments have shown promising starting points for further investigations.

CONCLUSION

Ion-exclusion, reversed phase ion-suppression and ion-pair chromatography can be applied for the separation of mixtures of organophosphorus acids. The chromatographic behaviour of structurally related compounds showed a comparable retention sequence indicat-

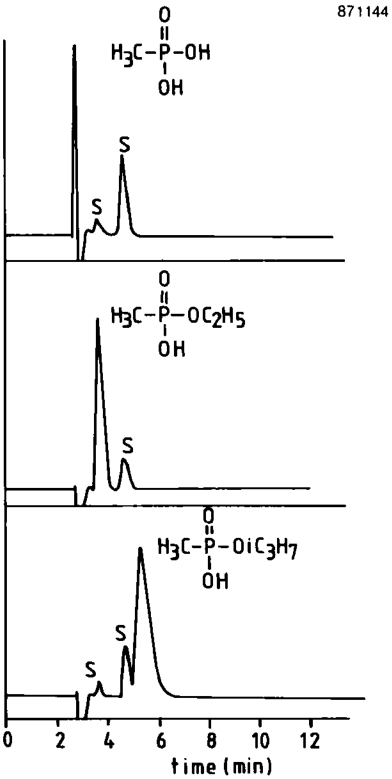

Figure 7 Ion-pair partition chromatogram of a number of organophosphorus acids. Mobile phase: 0.1 M ammonium acetate, pH 5; column: no. 2, see Exp. Part; detection: differential refractometer. S = system peaks.

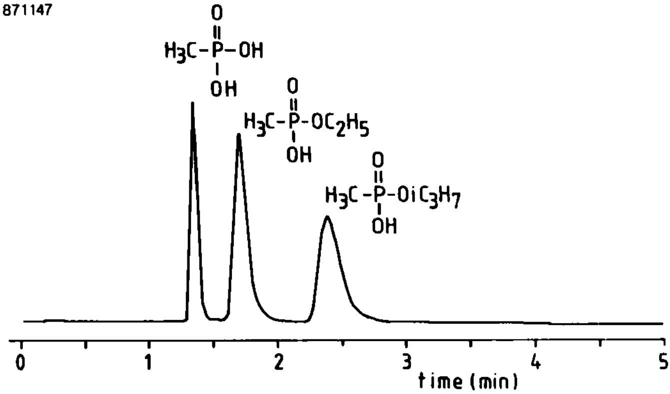

Figure 8 Ion-pair partition chromatogram of a number of organophosphorus acids. Mobile phase: 0.05 M ammonium acetate, pH 5; column: no. 4, see Exp. Part; detection: FPD.

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ing that the retention is based on a reversed phase partition mechanism. Ion-exchange chromatography can be used to a lesser extent unless the predominant role of the charged ionic species can be sufficiently compensated by hydrophobic interactions and by more efficient or longer columns which may be attainable in μLC. In addition to their respective further optimization it can be stated that some of these LC-systems combined with selective detection offered by a flame photometric detector have shown promising results.

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