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NEW ION-EXCLUSION PHASE FOR ORGANIC ACIDS

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SUMMARY

Using a PRP-X300 (150 mm × 4.1 mm I.D.) column, packed with a medium-capacity cation-exchange resin, short-chain carboxylic acids have been separated in less than 5 min. To describe the selectivity, a mathematical model was used allowing for the calculation of window diagrams. By comparison of the separation characteristics with other columns of the PRP series, the separation mechanism was shown to be controlled by hydrophobic interaction, hydrogen bonding and ion exclusion. PRP-X300 was found to be suitable for a new approach to carboxylic acid separation due to its unique variability in selectivity.

INTRODUCTION

Many different separation mechanisms involving ionic sample forms are known. Three main principles are most popular in ion chromatography (IC): ion-exchange chromatography (IEC), ion chromatographic exclusion (ICE) and ion-pair chromatography (IPC)[1].

Ideally, in IEC the chromatographic process is controlled by ionic forces alone. The total molar ion concentration is constant along the column axis, either within a sample peak or in between. An exception is made for so-called system peaks. As a consequence of this basic principle of complementarity in IEC, ion focusing is possible and low concentration samples can be injected in large volumes of, *e.g.*, 10 ml without significant loss in efficiency. Attainable detection limits are in the low ppb (10^{-9}) range.

In exclusion chromatography, a substance is eluted between the excluded volume and the void volume. In analogy to gel permeation chromatography, also in classical ICE the columns have a very low apparent porosity. To achieve acceptable separation, rather large column diameters are used[2]. All common packings used in ICE are based on cross-linked polystyrene, sulphonated to an exchange capacity of about 5 mequiv./g. In the following these packings will be referred to as the classical form of ICE. The high mobility of hydrogen ions of resins in the acid form leads to a negatively charged Donnan surface layer which repels anions. In the classical form, also weak carboxylic acids are eluted before the eluent, although strong acids are

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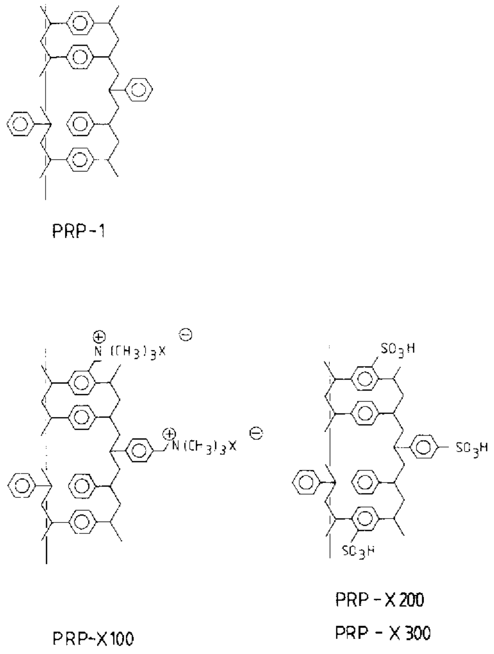
used as the eluents. The organic acids do not penetrate the porous fraction of the packing material as does the eluent and consequently the capacity factors are negative.

In contrast to IEC, the total eluent and sample concentration are additive as in conventional high-performance liquid chromatography (HPLC). In this case, the total molar concentration is higher in the sample peak than in the eluent. Therefore, the sensitivity is not directly dependent on the background signal associated with the eluent. On the other hand, in IEC the detector response always corresponds to the difference of sample and eluent molar sensitivity. As mentioned before the complementarity of sample ion concentration and eluent driving ion concentration is a fundamental boundary condition in IEC[3].

In IPC, ionic compounds form an uncharged complex with a counter ion of the eluent and are separated by non-ionic forces, typically in the reversed-phase mode. A special form of IPC is dynamic ion chromatography (DIC), where the counter ion is not only present in the eluent but forms a reversible ion-exchange phase by adsorption on the column matrix.

PRP columns are a group of products based on a macroporous poly(styrene–divinylbenzene): PRP-1 (Fig. 1)[4]. By derivation of the backbone, IC columns are obtained: PRP-X100, provided with trimethylammonium exchange groups for inorganic anion separation[5], PRP-X200 for inorganic cation separation and PRP-X300 for ICE of carboxylic acids[6]. PRP-X200 and PRP-X300 are derivatized by sulphonation to different extent. PRP-X300 shows a moderate sulphonation degree in comparison to the classical ICE columns. All phases are rigid spheres withstanding alkaline and acid solutions as well as organic eluents.



PRP-1

PRP-X100

PRP-X200
PRP-X300

Fig. 1. Chemical structures of PRP packing materials.

In this work the chromatographic properties of PRP-X300 were examined and compared to the columns mentioned before in respect to carboxylic acid separation. It is shown that PRP-X300 has been tailored to provide rapid carboxylic acid separation at room temperature. The principles controlling the selectivity have been examined in detail.

THEORETICAL

In IEC of fully dissociated ions the dependence of the capacity factor, k', on eluent concentration, C_E, is given by the well-known equation describing the driving force of the eluent

$$\log k' = a - \frac{x}{y} \log C_E \tag{1}$$

where x is the charge number of the sample ion and y the charge number of the eluent ion having the same charge[7].

Also in reversed-phase chromatography charged species are excluded from the chromatographic process. If there is a dissociation equilibrium as in the case of a weak acid HA, it has been shown[8] that the retention relationship can be expressed by:

$$k' = a \frac{[\mathrm{HA}]}{[\mathrm{HA}]_{\mathrm{tot}}} \tag{2}$$

Introducing the dissociation constant, K_a, this becomes

$$k' = \frac{a}{1 + \frac{K_a}{[\mathrm{H}]}} \tag{3}$$

where [H] is the concentration of strong acid in the eluent. In eqns. 1–3 the constant a represents the corresponding partition coefficients. In IEC the chromatographic process is dominated by the ion-exchange equilibrium, in reversed-phase chromatography by hydrophobic interactions and on polar phases by hydrogen bonding.

EXPERIMENTAL

Apparatus

A modular instrument was used combining a SP8700 pump (Spectra Physics, Santa Clara, CA, U.S.A.), a 7025 loop injector (Rheodyne, Berkeley, CA, U.S.A.), a conductivity detector 213A (Wescan, Santa Clara, CA, U.S.A.) connected in series to a SP 8400 UV detector (Spectra Physics) equipped with a 5-mm pathlength 10-μl cuvette and a PM 8224 one-to-four channel strip-chart recorder (Philips, Eindhoven, The Netherlands).

Injections were performed with a 1-ml syringe Type 1001 TLL (Hamilton Bonaduz, Bonaduz, Switzerland) provided with a Luer hubbed needle for Rheodyne injection valves. The loop sizes used were 10 and 100 μl.

TABLE I

PHYSICAL PROPERTIES OF COLUMNS

Column type	*Particle size (μm)*	*Exchange capacity (mequiv./g)*	*Typical plate number for acetic acid (N/m)*
PRP-1	10*	0	10 000
PRP-X100	10	0.19	10 000
PRP-X200	10	0.04	3000
PRP-X300	7	0.17	14 000
Classical ICE column	5–10	1.7–4.2	15 000–41 000**

* 5 μm is also available.

** According to refs. 2 and 9.

Columns

Hamilton steel columns (150 mm × 4.1 mm) were used. The void volume was 1.3 ml as determined gravimetrically. Table I features some physical properties of the columns, including classical ICE columns. All chromatograms were operated at room temperature. The columns were kept in an insulating box together with the conductivity detector.

Reagents and solutions

All chemicals were of reagent-grade purity and dissolved in deionized water. Food samples were filtered through 0.45-μm PTFE filter cartridges before injection.

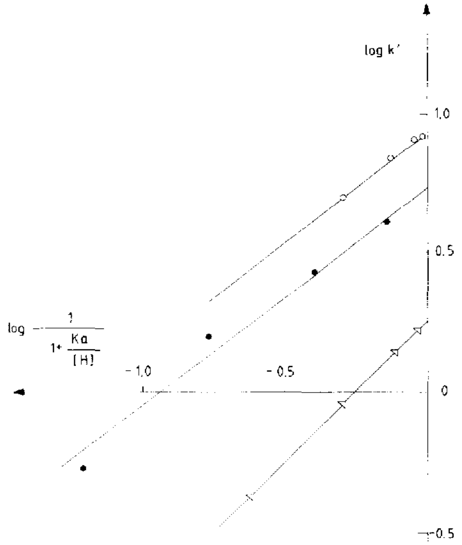


Fig. 2. Dependence of the capacity factor on concentration of the sulphuric acid eluent pH 1.4–3 on PRP-X300. Data taken from ref. 6. ●—●, Maleic acid, $\log k' = 0.73 - 0.77 \log (1 + K_a/[H])$, $pK_a = 1.8$; △—△, pyruvic acid, $\log k' = 0.25 - 1.00 \log (1 + K_a/[H])$, $pK_a = 2.5$; ○—○, fumaric acid, $\log k' = 0.93 - 0.80 \log (1 + K_a/[H])$, $pK_a = 3.0$.

RESULTS AND DISCUSSION

Retention equation

The relationship 3 was transferred to the chromatographic data of PRP-X300. Fig. 2 clearly demonstrates the decrease in retention with decreasing [H] for three relatively strong carboxylic acids. By simplification, only the first dissociation constant was considered. The slopes of the logarithmic plots were from 0.77 to 1, which would be the predicted value.

In contrast to this clear statement, with classical ICE columns very little dependence of k' *versus* sulphuric acid concentration has been reported[9]. This indicates again that in classical ICE the exclusion effect is effective also for species present in the eluent in the protonated state.

Selectivity

From eqn. 3 the selectivity coefficient α, is obtained:

$$\alpha = \frac{k'_2}{k'_1} = a_{1,2} \cdot \frac{K_{a_1} + [H]}{K_{a_2} + [H]} \tag{4}$$

Therefore a window diagram may be calculated easily from one chromatogram only. Table II shows a very good agreement between calculated and experimental selectivity. Three typical cases were selected.

(a) For the pair citric–succinic acid the selectivity increases with pH.

(b) For the pair malic–citric acid the selectivity decreases with pH.

(c) For the pair lactic–citric acid the selectivity coefficient passes through 1. In other words, the selectivity ratio changes with pH and therefore also does the elution order.

In all cases the coincidence of predicted and experimental selectivity was obvious.

TABLE II

COMPARISON OF EXPERIMENTAL AND CALCULATED SELECTIVITIES ON PRP-X300

Experimental data taken from ref. 6.

	pH of eluent (sulphuric acid)			
	*1.4**	*2*	*2.5*	*3*
Citric succinic acid				
α (calc.)	2.7	2.8	3.3	4.5
α (exptl.)	2.7	2.8	3.6	5.1
Malic–citric acid				
α (calc.)	2.2	2.1	2.0	1.6
α (exptl.)	2.2	2.2	1.9	1.8
Lactic citric acid				
α (calc.)	1.14	1.09	0.96	0.73
α (exptl.)	1.14	1.10	0.93	0.61

* First chromatogram for the calculation of $a_{1,2}$.

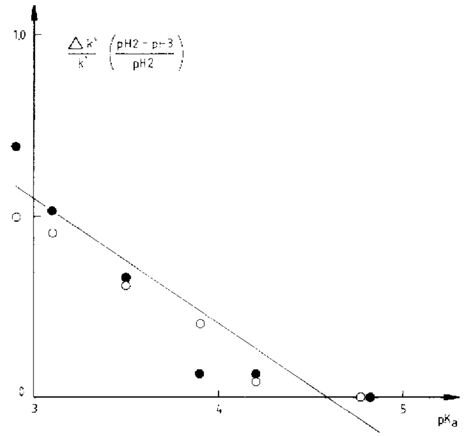


Fig. 3. Relative increase in k' caused by a change in the pH of the sulphuric acid eluent from 3 to 2 for the acids listed in Table III *versus* pK_a. Data taken from ref. 6. Columns: PRP-X300 (●); PRP-1(○).

Ion-exclusion effect

The ion-exclusion effect was compared between PRP-X300 and PRP-1. The relative increase in k' from pH 3 to 2 in the eluent was plotted against the pK_a values of the components (Fig. 3). Surprisingly, no distinct difference was discerned. It might be concluded that the exclusion effect with PRP-X300 is largely governed by the apolar backbone of the resin, while ion exclusion from the cation-exchange sites is inferior. However, because the inorganic anions give negative k' values, also ion exclusion must be effective with PRP-X300[6]. For carboxylic acids the exclusion effect is complete only if the pH of the eluent is at least one unit above the pK_a value. This finding corresponds well to the smaller exchange capacity in comparison to classical ICE materials.

Hydrogen-bonding effect

Because the k' of carboxylic acids decreased in the same way both for PRP-X300 and PRP-1 according to Fig. 3, a criterion was needed in order to exemplify

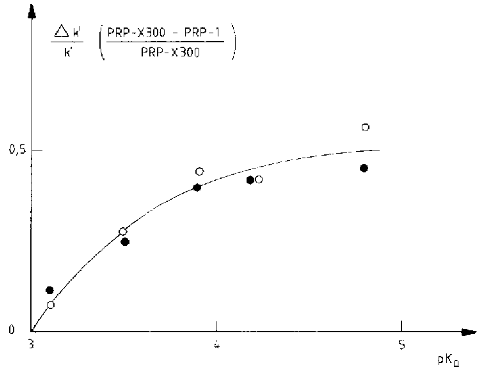


Fig. 4. Relative increase in k' on PRP-X300 in comparison to PRP-1 for the acids listed in Table III, except for tartaric acid, *versus* pK_a. Data taken from ref. 6 for sulphuric acid eluent. pH: 2 (●); 3 (○).

the specific interaction of PRP-X300 in addition to the ion-exclusion effect. For this purpose the relative difference in k' between PRP-X300 and PRP-1 was evaluated as a function of the pK_a of the acid. While ion exclusion alone would lead to negative k' values, PRP-X300 shows longer retention times than PRP-1 at the same pH. Referring to Fig. 4, this parameter of relative retention increase correlates well with the pK_a, either at pH 2 or 3. This remarkable correlation could be explained by a synergistic effect between hydrogen bonding and hydrophobic interaction with the particle surface. The reversed-phase effect of a sample subject to hydrogen bonding is more marked on the polar–apolar column PRP-X300 in comparison to the apolar PRP-1 column. For the less acidic components the contribution of hydrogen bonding is prevalent over a relatively large pH range.

Elution order

In ICE the pK_a of the component gives a rough rule of the elution order and in an homologous series also the molecular weight. Because the natural carboxylic acids are so different in structure, the pK_a rule is not absolute. The elution order of a group of six common carboxylic acids on all PRP columns with a strongly acidic eluent was assessed to obtain additional information on the separation mechanism. Table III summarizes the experimental data obtained from PRP-1, PRP-X100, PRP-X200 and PRP-X300.

The separation of fruit acids by IPC on PRP-1 has been described elsewhere[10] and was not considered in this study.

PRP-1 has the lowest k' values. PRP-X100 is a more polar column, but the peaks show tailing. It is expected that at higher pH the retention would increase considerably, because the anion-exchange mechanism would start to work. The cation-exchange column PRP-X200 shows a complete rearrangement of elution order. This was surprising considering the chemical similarity to PRP-X300. A strict order is observed with k' increasing with increasing number of carboxyl groups per molecule. A possible explanation could be a ligand-exchange mechanism caused by metallic impurities in the column. Transition metals are immobilized by the cation-exchange group. As a consequence, organic acids can complex the metal ions de-

TABLE III

COMPARISON OF ELUTION ORDER OF SIX CARBOXYLIC ACIDS WITH ACIDIC ELUENTS

Abbreviations: A = acetic; C = citric; L = lactic; M = malic; S = succinic; T = tartaric acid.

PRP-1 pH 2.6			*PRP-X100 pH 2.3*			*PRP-X200 pH 3.3*			*PRP-X300 pH 3.0*			*Acid*	*pK_a*
Acid	*k'*	*α*	*Acid*	*k'*	*α*	*Acid*	*k'*	*α*	*Acid*	*k'*	*α*		
T	0.0	–	T	0.5	–	A	0.3	–	T	0.1	–	T	2.9
M	0.4	14.0	M	1.1	2.1	L	0.3	1.0	M	0.5	6.0	C	3.1
L	0.8	1.9	L	1.5	1.4	M	1.3	4.2	C	0.9	1.8	M	3.5
C	1.0	1.3	A	2.4	1.6	S	1.6	1.2	L	1.5	1.7	L	3.9
A	1.2	1.2	C	3.2	1.3	T	3.1	2.0	A	2.8	1.9	S	4.2
S	2.8	2.3	S	5.8	1.8	C	5.1	1.6	S	4.9	1.8	A	4.8

pending on the complexation constants. This effect did not disappear, even when the column was treated by injections of 0.01 M nitric acid 0.1 M and sodium salt of EDTA although the the retention factors became smaller. The alkali metals were eluted at the end of the chromatogram and could be detected by conductivity.

Besides PRP-X200, there are rather small differences in the elution order: tartaric acid as the most acidic component is eluted first. The second acid citric acid appears at variable positions with the different columns. PRP-X300 shows the highest selectivity for the test mixture. Separations can be performed in less than 5 min, *i.e.*,

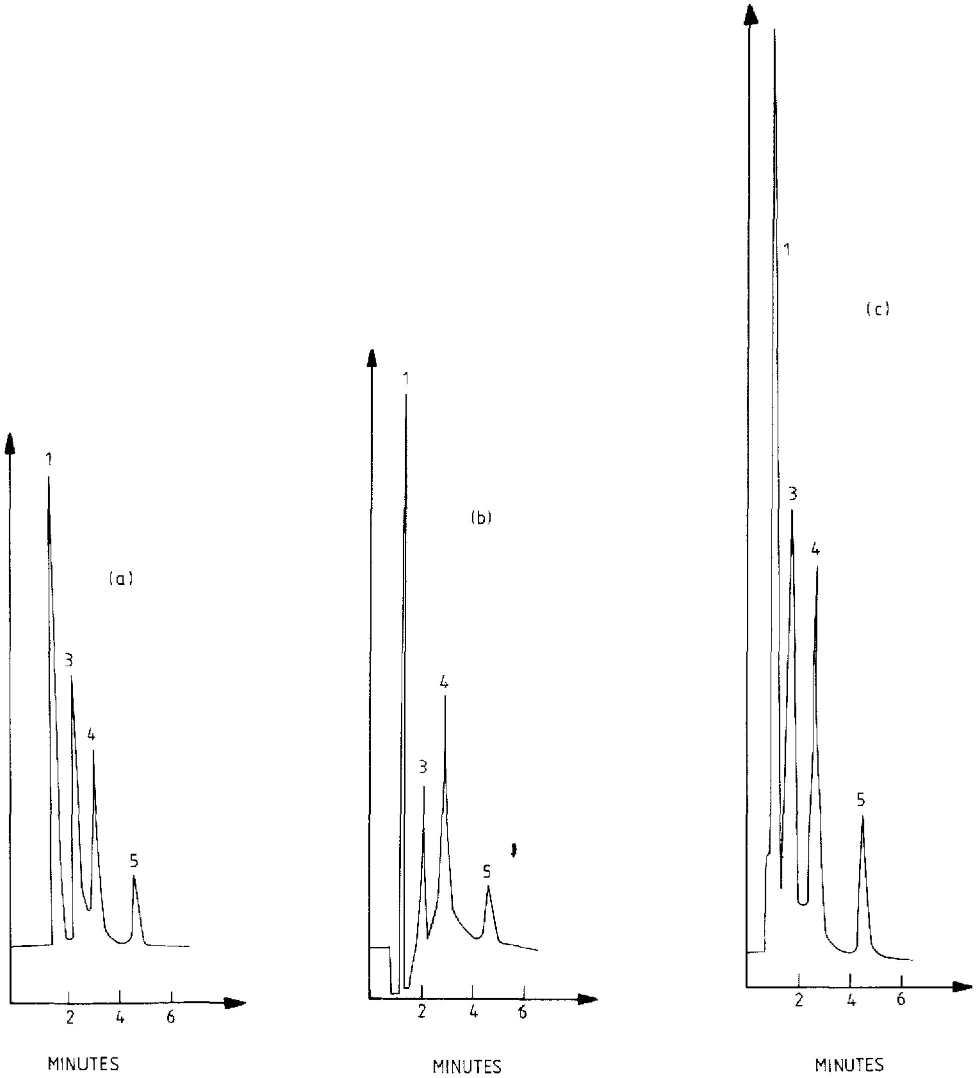


Fig. 5. Separation of tartaric (1), citric (3), lactic (4) and acetic acid (5) on PRP-X300 using different strong acids as eluents. Eluent concentration 0.5 mM. Flow-rate: 1 ml/min. Pressure: 60 bar. Loop: 100 μl. Concentrations: 60 mg/l. UV detection at 210 nm (a, c), 222 nm (b). Eluents: (a) sulphuric; (b) nitric; (c) phosphoric acid.

more rapidly than with existing ICE phases. Regarding the elution order the fundamental partition seems to be PRP-specific for all PRP columns except for PRP-X200.

Eluent selection

With PRP-X300 the use of different strong acids as the eluent was studied. Fig. 5 shows the separation of four components in sulphuric, nitric and phosphoric acid under identical conditions. The results between sulphuric and phosphoric acid compared well, whereas nitric acid of course is less suitable for UV detection. The high background signal leads to a strong water peak. Generally the retention times were identical.

Application

In real samples different metal salts can be present which may undergo ion exchange with the column. In this context the low exchange capacity of PRP-X300 is of special interest. Therefore, the influence of sodium and calcium on the chro-

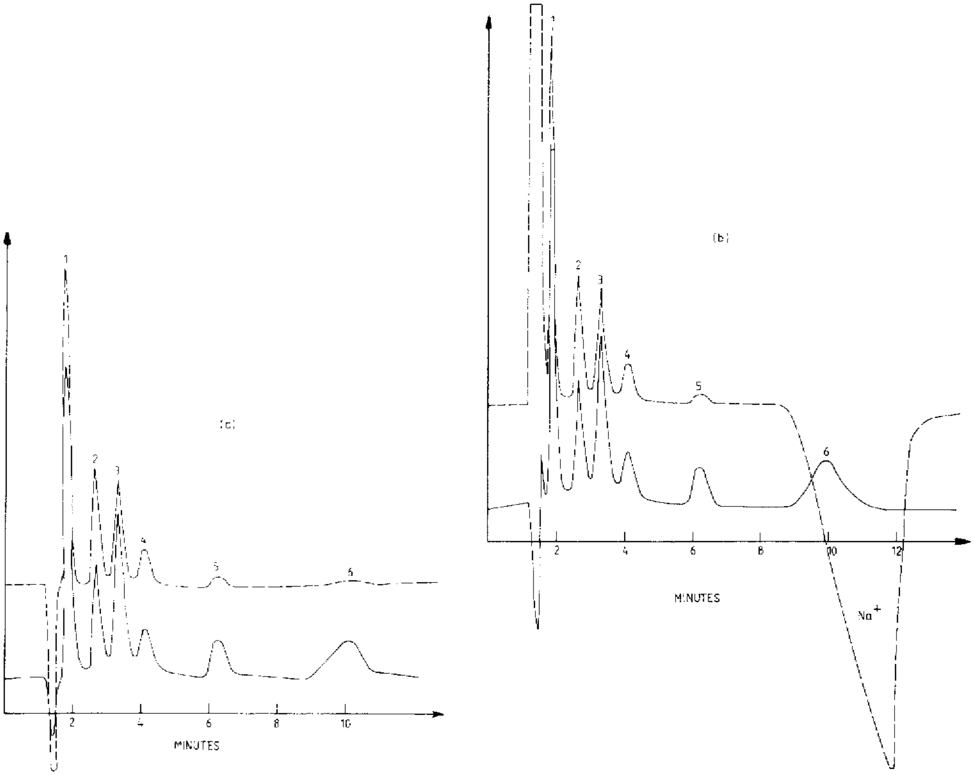


Fig. 6. Effect of addition of sodium sulphate to the sample on the carboxylic acid peaks on PRP-X300. Sodium is coeluted with succinic acid. Eluent: sulphuric acid pH 3. Flow-rate: 0.8 ml/min. Pressure: 48 bar. Loop: 100 μl. Concentration: 60 mg/l. (a) Without sodium sulphate, (b) with 250 mg/l sodium sulphate. Detection: ——, UV 210 nm; ----, conductivity. Carboxylic acids: 1 = tartaric; 2 = malic; 3 = citric; 4 = lactic; 5 = acetic; 6 = succinic.

matograms was studied. Addition of 100–1000 mg/l sodium sulphate had no effect on the chromatogram of the six acids in the UV detection mode. Similarly to PRP-X200, sodium was eluted at the end as traced by conductivity detection (Fig. 6).

By addition of 1000 mg/l calcium, the peak height of citric acid changed: an increase in UV detection and a decrease in conductivity detection was observed. However, the retention times were unaltered. The column could easily be restored by repeated injections of 0.01 *M* nitric acid which expelled the calcium accumulated on the column. After baseline stability was achieved, the next samples could be analyzed.

Therefore, UV seeems to be the preferred detection mode in ICE, whereas it is conductivity in IEC. Moreover, UV detection is feasible with gradients of strong acids. In a typical range of 5–100 mg/l carboxylic acids a good linearity was confirmed for both detectors with PRP-X300. With lower concentrations band broadening occurred.

In a practical example, the following acids were identified by UV detection in yoghurt serum: citric, lactic, acetic and succinic acid (Fig. 7). The peaks identified could not all be seen by conductivity detection because of the alkali metals. Additional peaks shown on the chromatograms were not identified. Additional applications such as the analysis of wine and an orange juice have been discussed[6], as have the separation of glucose oxidation products and of aliphatic alcohols[11].

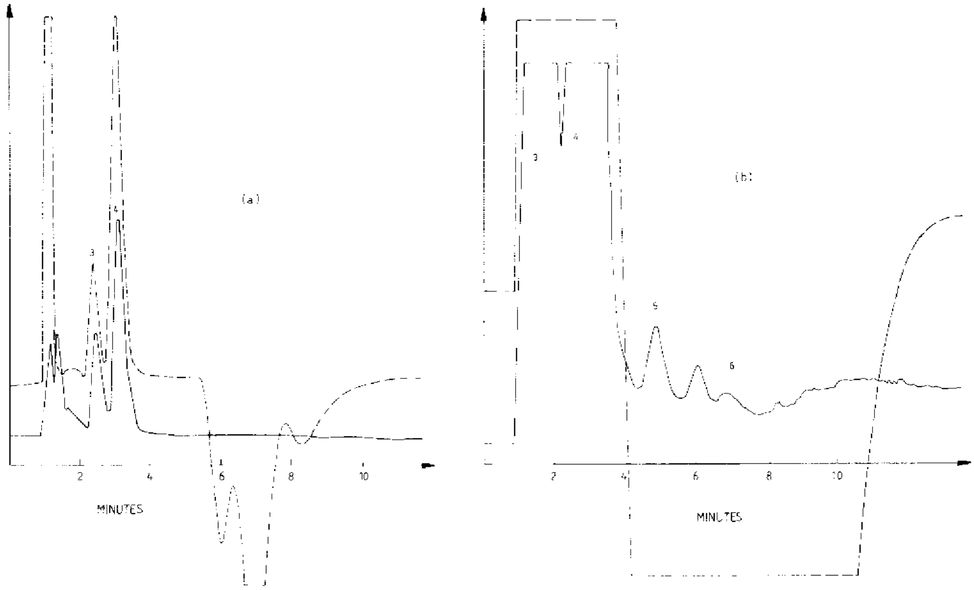


Fig. 7. Analysis of yoghurt serum on PRP-X300. Eluent: sulphuric acid pH 3. Flow-rate: 1.1 ml/min. Pressure: 60 bar. Loop: 10 μl. Detection: UV 210 nm (—); conductivity (----). (a) Diluted sample 1:10. Range: 0.02 a.u.f.s. 2.2 g/l citric acid, 22.5 g/l lactic acid. (b) Undiluted sample. Range: 0.08 a.u.f.s. 0.18 g/l acetic acid, 0.08 g/l succinic acid. Carboxylic acids: 3 = citric; 4 = lactic; 5 = acetic; 6 = succinic.

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