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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ELUTION BEHAVIOUR OF ALCOHOLS, ALDEHYDES, KETONES, ORGANIC ACIDS AND CARBOHYDRATES ON A STRONG CATION-EXCHANGE STATIONARY PHASE

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SUMMARY

The high-performance liquid chromatographic separation of alcohols, aldehydes, ketones, carboxylic acids and carbohydrates on a polystyrene-based strong cation-exchange resin is described. The column temperature was found to be a very important parameter for optimizing separations of these substances. The effect of different functional groups on the elution behaviour is discussed.

INTRODUCTION

High-performance liquid chromatography (HPLC) is a routine method for the rapid analysis of short-chain organic acids, aliphatic aldehydes, ketones, alcohols and carbohydrates, which are important compounds in food chemistry and in clinical, biological and biomass research. The analyses of short-chain aliphatic acids may be performed using bare silica[1,2], but more frequently these compounds are separated underivatized[3–5] or as their phenacyl derivatives[6,7] on reversed-phases. The determination of aldehydes and ketones can be achieved after derivatization to 2,4-dinitrophenylhydrazones[8–11], which is a very sensitive method. Furfurals, relevant compounds for food chemistry, can be rapidly analysed without derivatization on reversed stationary phases[12–14].

The analysis of alcohols is mainly performed using gas chromatography, whereas the determination of carbohydrates usually consists in elution from amino bonded phases[15–24] or from silica and amino modifiers[25–28] with acetonitrile–water mixtures.

Ion-exchange materials have been used in many applications as stationary phases for the liquid chromatography of the above compounds. Reviews by Jandera

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and Churacek included a variety of methods for the separation of organic acids, aldehydes, ketones, alcohols and carbohydrates used in the 1960s and early 1970s[29,30]. Owing to the development of new ion-exchange materials of small particle size and narrow size range, which are stable at higher pressures, improvements in the column efficiency and reductions in retention times were possible. Polystyrene–divinylbenzene cation-exchange resins with a metallic counter ion and water as the eluent proved useful for the separation of carbohydrates and oligomers, whereas the H^+-form with an acidic eluent was employed for organic acid analysis[31–41]. Subsequently, pre-packed columns filled with efficient ion-exchange materials became commercially available and especially columns of the HPX series (Bio-Rad Labs., Richmond, CA, U.S.A.) were used for carbohydrate[42–52] and organic acid separations[53–60].

In this work, the chromatographic behaviour of 63 substances on HPX-87-H and the capability of this column for the separation of acids, aldehydes, ketones, alcohols and carbohydrates were investigated. In addition, optimized methods for the separation of the above substance classes were devised.

EXPERIMENTAL

Apparatus

A Model SP 8000 B high-pressure liquid chromatograph (Spectra-Physics, Santa Clara, CA, U.S.A.) with an integrated oven compartment and data system was used in combination with a Model R 401 refractive index detector (Waters Assoc., Milford, MA, U.S.A.). Sample injection was performed using a sample loop valve equipped with a 25-μl loop (Valco Instruments, Houston, TX, U.S.A.). The column was a pre-packed Aminex HPX-87-H strong cation-exchange resin column (300 × 7.8 mm I.D.), fitted with an ion-exclusion Micro-Guard refill cartridge (Bio-Rad Labs.).

Reagents

The eluent was 0.01 *N* sulphuric acid, prepared by dilution of 0.1 *N* analytical-reagent grade sulphuric acid. During chromatography it was degassed with helium. The reference substances were obtained from Fluka (Buchs, Switzerland), Merck (Darmstadt, F.R.G.), Sigma (St. Louis, MO, U.S.A.) and Serva (Heidelberg, F.R.G.). For injection the samples were dissolved in doubly distilled water. The investigated compounds are listed in Table I.

RESULTS

Separations employing the HPX-87-H column may be controlled by varying the composition of the eluent (pH value or addition of acetonitrile), column temperature and flow-rate. Preliminary studies showed that variation of the pH in the range 1.8–2.3 has little influence on the retention times of most of the substances studied. The number of plates obtained on HPX-87-H normally increases on increasing the column temperature[61], which is advantageous for analyses at elevated temperatures. It was observed, however, that the retention times of several substances react differently to temperature changes. Therefore, the selection of a particular column temperature may frequently enhance the resolution for overlapping substances

TABLE I

PEAK IDENTIFICATION OF THE INVESTIGATED COMPOUNDS

Peak No.	*Compound*	*Peak No.*	*Compound*
1	Glycerol	33	Glycolic acid
2	1,2,4-Butanetriol	34	Lactic acid
3	1,2-Propanediol	35	Formic acid
4	1,3-Propanediol	36	Levulinic acid
5	2,3-Butanediol	37	Acetic acid
6	Methanol	38	Propanoic acid
7	1,3-Butanediol	39	2-Furancarboxylic acid
8	1,4-Butanediol	40	Cellobiose
9	Ethanol	41	D-Galacturonic acid
10	2-Propanol	42	D-Glucose
11	*tert.*-Butanol	43	L-Sorbose
12	1-Propanol	44	*meso*-Inositol
13	2-Butanol	45	D-Galactose
14	Isobutanol	46	D-Mannose
15	1-Butanol	47	D-Xylose
16	Glyceraldehyde	48	D-Fructose
17	Methylglyoxal	49	D-Xylulose
18	Glycolaldehyde	50	D-Sorbitol
19	Dihydroxyacetone	51	2-Deoxy-D-glucose
20	Formaldehyde	52	L-Rhamnose
21	Acetaldehyde	53	Dulcitol
22	Acetone	54	L-Arabinose
23	Propanal	55	Ribitol
24	Methyl ethyl ketone	56	D-Ribose
25	5-Hydroxymethylfurfural (HMF)	57	D-Arabitol
26	Butanal	58	D-Xylitol
27	Furfural	59	2-Deoxy-D-galactose
28	5-Methylfurfural	60	6-Deoxy-D-galactose
29	Oxalic acid	61	*meso*-Erythritol
30	2-Oxoglutaric acid	62	1,6-Anhydro-β-D-glucose
31	Glyoxylic acid	63	2-Deoxy-D-ribose
32	Glyceric acid		

and may substantially reduce the analysis time in some instances (*e.g.*, furfurals). Hence the control of the column temperature is important for optimizing analyses using HPX-87-H. The temperature dependence of the capacity factors (k) of each substance (Table I) was determined between 40 and 80°C under standardized conditions (flow-rate, 0.5 ml/min; eluent, 0.01 N sulphuric acid). In addition, the peak width at half-height ($w_{0.5}$) was measured for each peak to calculate the resolution (R_s) as a function of the column temperature.

Fig. 1 shows a plot of the capacity factors *versus* column temperature for all the alcohols investigated. The retention times of alcohols eluted above $k = 2$ increase slightly at first, but at higher temperatures they decrease again. In particular, the capacity factors of highly retarded alcohols (isobutanol, 1-butanol) decrease appreciably at higher temperatures. For some alcohol pairs increases in the differences in the retention times are observed at low column temperature (2-butanol–isobutanol), for others at high temperatures (*e.g.*, ethanol–1,4-butanediol). To illustrate the sep-

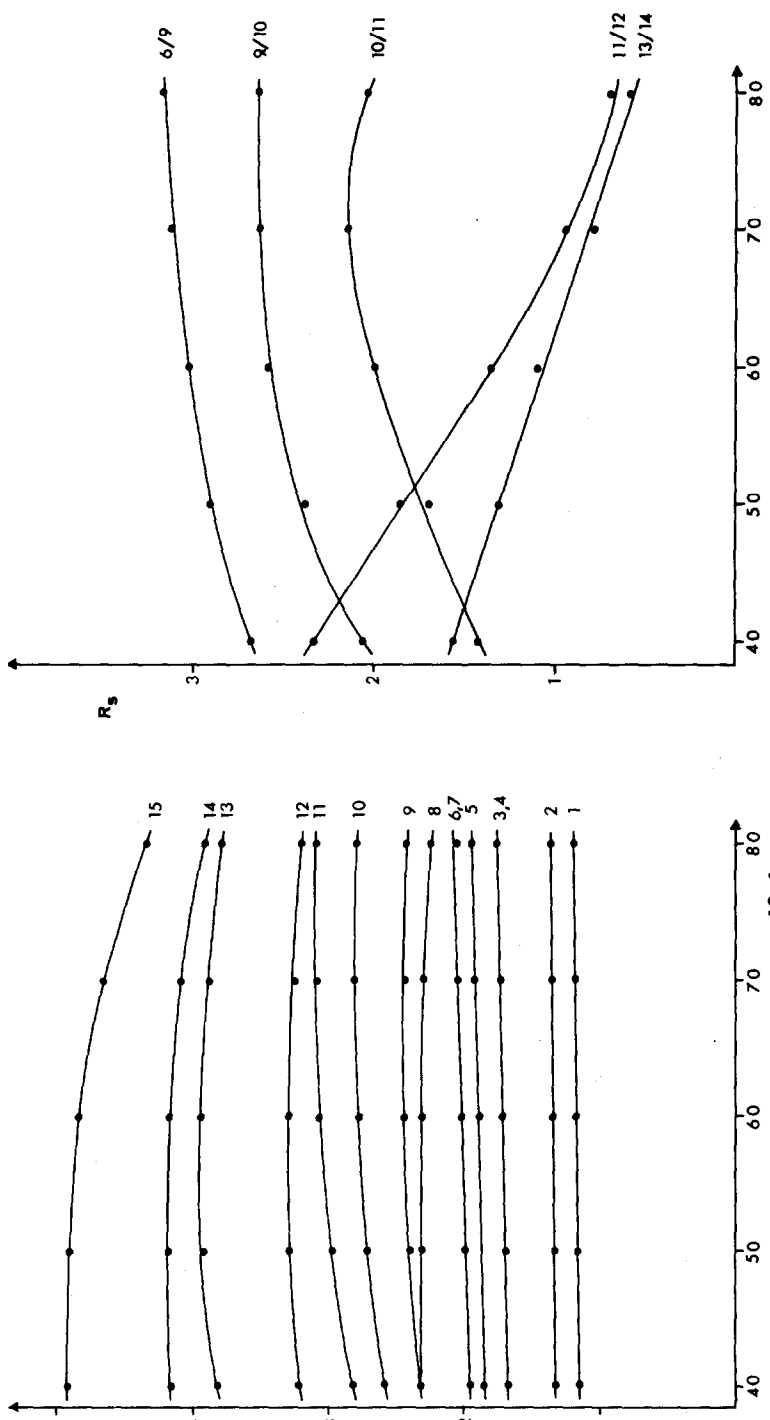

Fig. 1. Effect of column temperature (T) on the capacity factors (k) of the investigated alcohols. Column, HPX-87-H (300 × 7.8 mm I.D.); mobile phase, 0.01 N sulphuric acid; flow-rate, 0.5 ml/min. For substance identification, see Table I.

Fig. 2. Effect of column temperature (T) on the resolution (R_s) of some alcohol pairs. Chromatographic conditions as in Fig. 1. For substance identification, see Table I.

aration of alcohols under optimal conditions, a mixture of monovalent alcohols and glycerol was chosen.

Fig. 2 shows the resolution as a function of column temperature for some alcohol pairs in a sample mixture. 2-Butanol–isobutanol and *tert.*-butanol–1-propanol form critical substance pairs because, with increasing temperature, their R_s values drop below 1. The resolution of the other investigated combinations of alcohols, however, already shows an appreciable increase in the low temperature range. At 50°C, baseline separation for the highest possible number of alcohol pairs was achieved.

For the sample studied, the flow-rate of the chromatographic system was optimized at 50°C by calculating the height equivalent to a theoretical plate, H, at different linear velocities, u. Fig. 3 shows the plot of H/u *versus* linear velocity for *tert.*-butanol and 1-butanol. H/u decreases rapidly up to a linear velocity of about 5.5 cm/min, corresponding to a flow-rate of 0.7 ml/min, and then flattens out. A flow-rate of 0.7 ml/min also proved optimal for the other substance classes investigated. Fig. 4 shows the temperature- and flow-rate-optimized separation of the eight monovalent alcohols and glycerol. With the exception of 2-butanol and isobutanol, all alcohols were separated completely. The shape of the peaks and the analysis time of about 30 min are satisfactory.

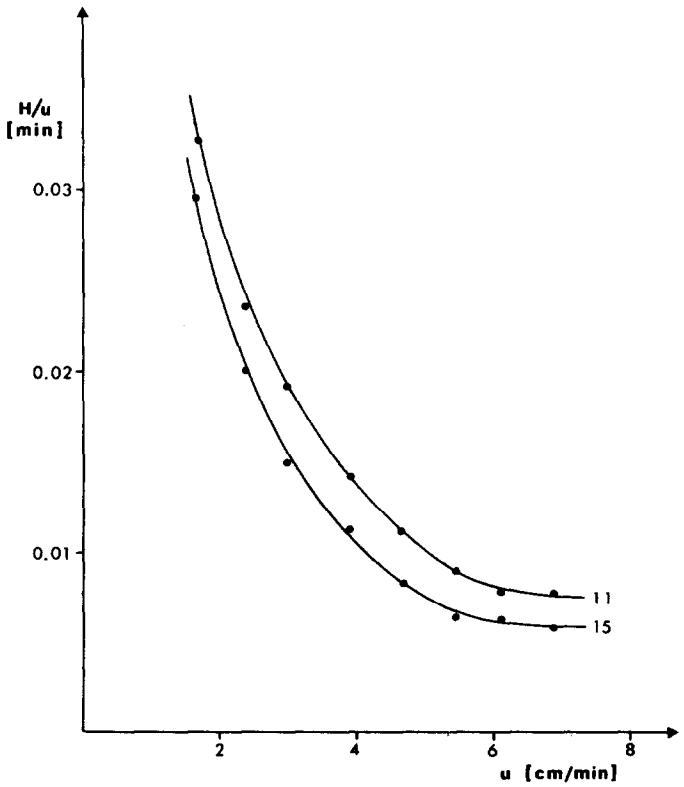

Fig. 3. Optimization of the flow-rate: plot of H/u *versus* linear velocity (u) for *tert.*-butanol (11) and *n*-butanol (15). Column temperature: 50°C.

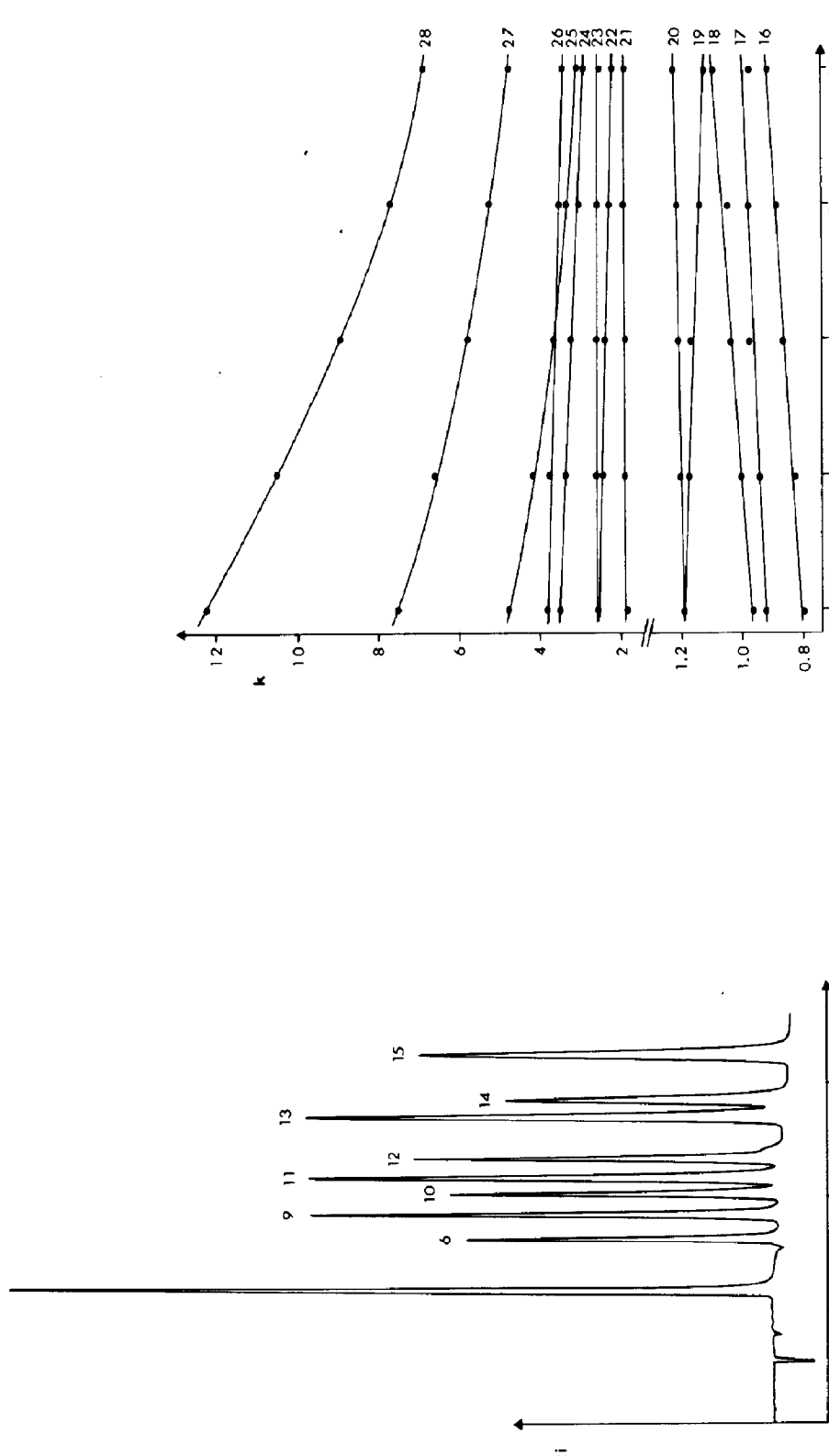

Fig. 4. Optimized separation of alcohols. Column, HPX-87-H (300 × 7.8 mm I.D.); column temperature, 50°C; mobile phase, 0.01 *N* sulphuric acid; flow-rate, 0.7 ml/min; refractive index detection. For peak identification, see Table I.

Fig. 5. Effect of column temperature (T) on the capacity factors (k) of the investigated aldehydes and ketones. Chromatographic conditions as in Fig. 1. For

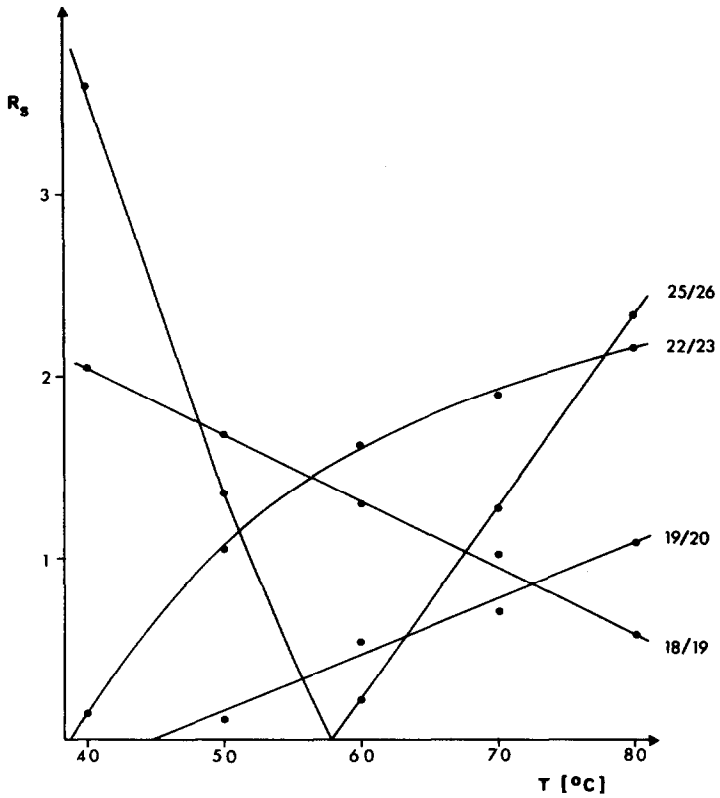

Fig. 6. Effect of column temperature (T) on the resolution (R_s) of some aldehydes and ketones. Chromatographic conditions as in Fig. 1. For substance identification, see Table I.

Fig. 5 shows the temperature dependence of the investigated aldehydes and ketones. The retention time of aldehydes and ketones with additional functional groups, and of formaldehyde, are within a narrow capacity factor range and, except for dihydroxyacetone, increase slightly with increase in temperature. The k values of acetone, methyl ethyl ketone and butanal decrease with increasing temperatures. Furfurals, owing to their aromatic structure, have a high affinity for the matrix of the stationary phase of the column and therefore have long retention times. Moreover, their capacity values are highly temperature dependent, decreasing rapidly with increasing column temperature. This results in a reversal of the elution order of HMF and butanal at *ca.* 60°C. By a suitable choice of the column temperature, the separation of all thirteen aldehydes and ketones was achieved. Fig. 6 shows the resolution *versus* temperature plot for those substance pairs most difficult to separate. It is obvious that, at low column temperatures, a complete separation of the sample cannot be achieved, because the R_s values of acetone–propanal and dihydroxyacetone–formaldehyde are too small. Moreover, the resolution of HMF and butanal decreases towards zero at *ca.* 60°C and then their retention times overlap (Fig. 5). At high column temperatures, glycolaldehyde–dihydroxyacetone and dihydroxyacetone–

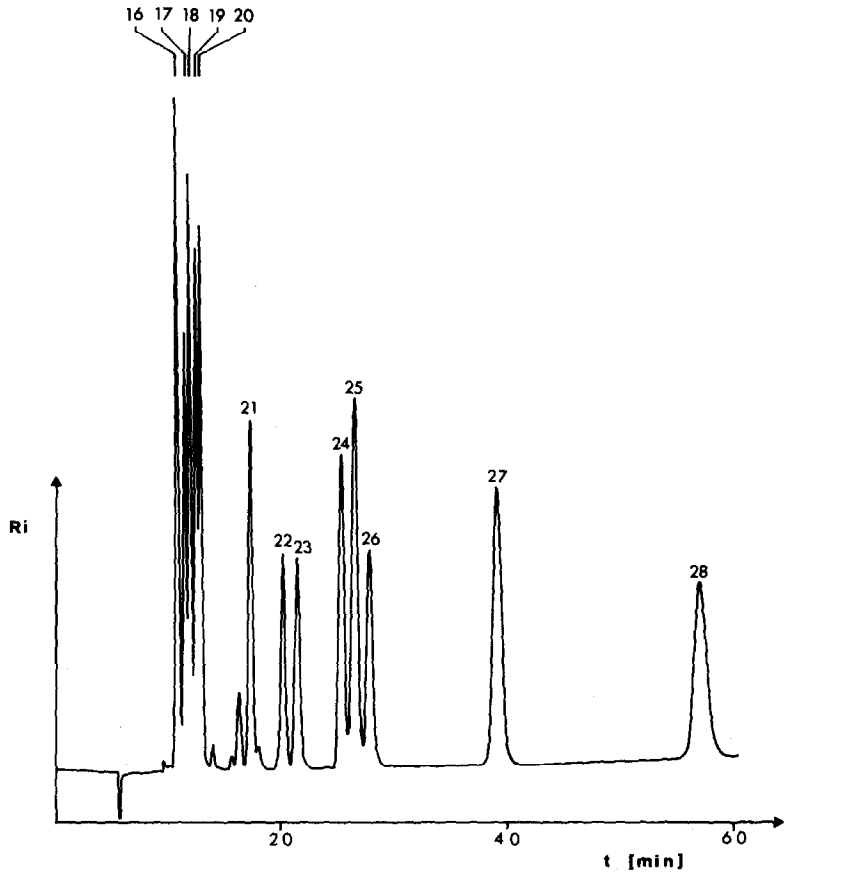

Fig. 7. Optimized separation of aldehydes and ketones. Column, HPX-87-H (300 × 7.8 mm I.D.); column temperature, 70°C; mobile phase, 0.01 *N* sulphuric acid; flow-rate, 0.7 ml/min; refractive index detection. For peak identification, see Table I.

formaldehyde have the smallest R_s values. For these pairs the best resolution is achieved at about 70°C with an R_s value of *ca.* 0.8–0.9.

Fig. 7 shows the optimized separation of all aldehydes and ketones. The separation of the four polyfunctional aldehydes and ketones and formaldehyde, which show small differences in their retention times, was achieved by controlling the temperature precisely. Under the conditions chosen, monofunctional aldehydes and ketones were separated with good selectivity. Due to the high column temperature of 70°C the retention times of furfurals could be kept below 1 h.

The separation of acids is the main use of the HPX-87-H, so we also studied the retention behaviour of eleven acids. Only the retentions of levulinic acid and 2-furancarboxylic acid showed a noticeable temperature dependence, increasing temperature resulting in decreasing capacity values (Fig. 8). As a consequence, the curves of levulinic and acetic acid cross at a high column temperature. On the other hand, the differences in the retention times of lactic and glycolic acid become smaller at low column temperatures, so the calculation of resolution showed 60°C to be optimal.

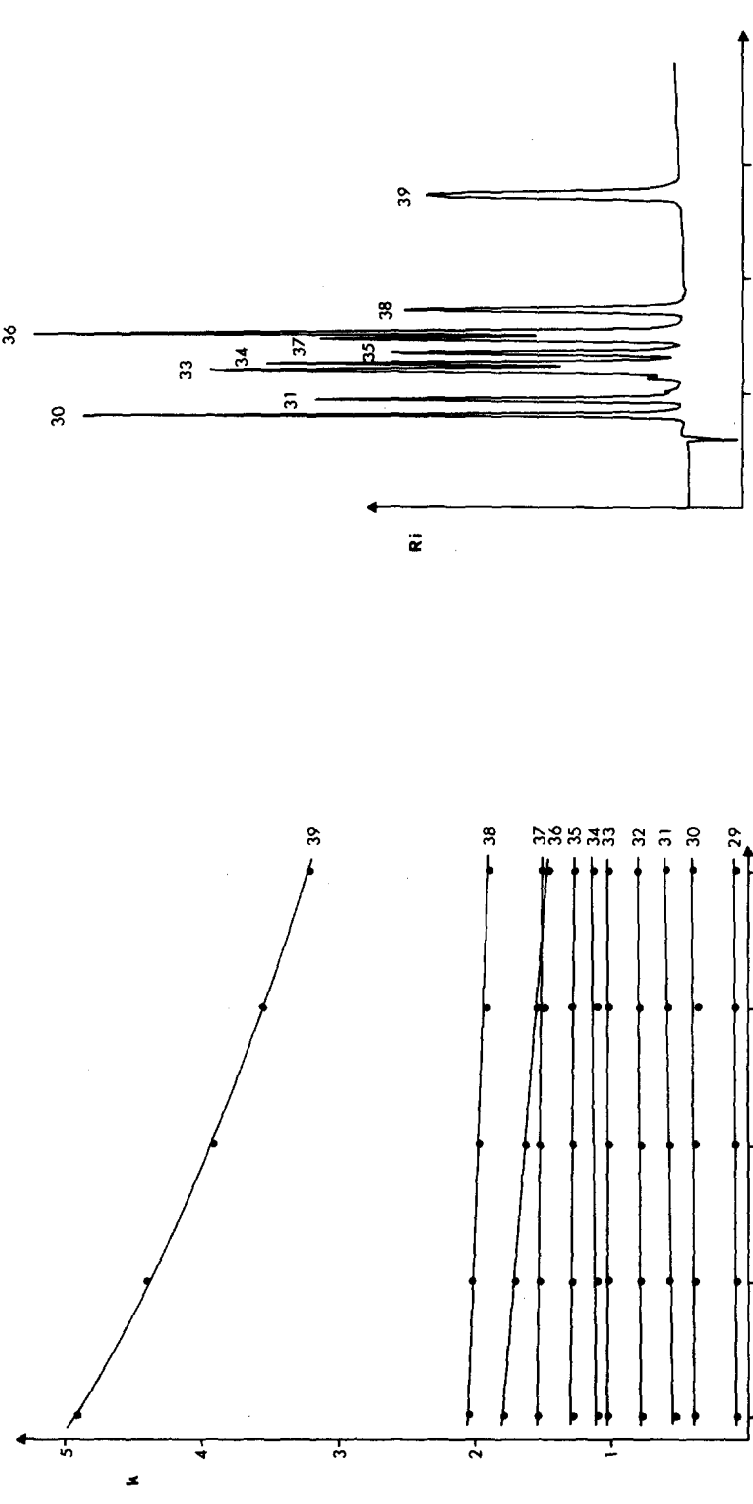

Fig. 8. Effect of column temperature (T) on the capacity factors (k) of the investigated acids. Chromatographic conditions as in Fig. 1. For substance identification, see Table I.

Fig. 9. Optimized separation of acids. Column, HPX-87-H (300 × 7.8 mm I.D.); column temperature, 60°C; mobile phase, 0.01 N sulphuric acid; flow-rate, 0.7 ml/min; refractive index detection. For peak identification, see Table I.

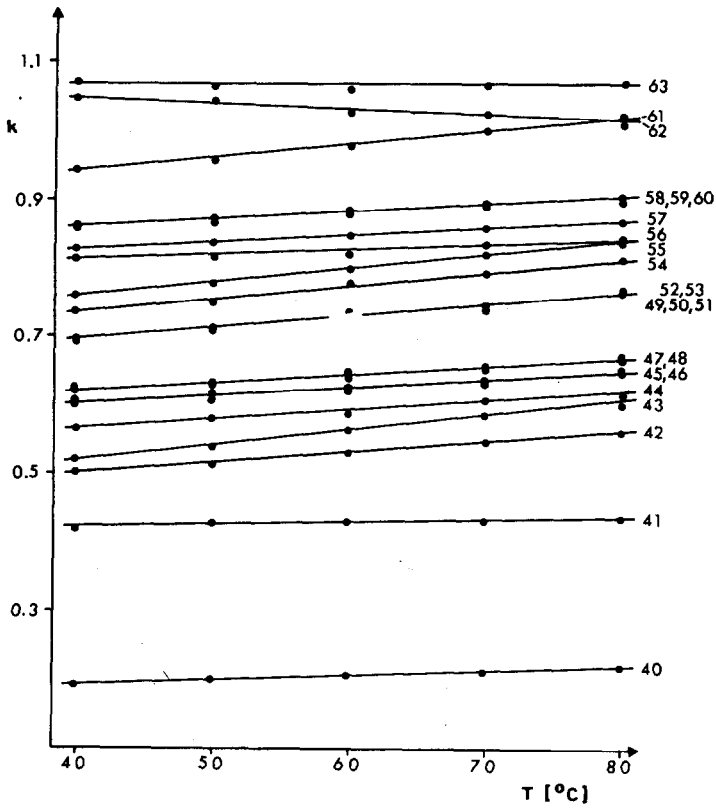

Fig. 10. Effect of column temperature (T) on the capacity factors (k) of the investigated carbohydrates. Chromatographic conditions as in Fig. 1. For substance identification, see Table I.

Despite their differing chemical properties, narrow and symmetrical peaks were obtained for all acids under the optimal conditions. Lactic and levulinic acid show markedly overlapping peaks (Fig. 9).

All 24 carbohydrates are eluted from HPX-87-H in a very narrow capacity factor range. The retention times of several of them overlap over the entire temperature range investigated (Fig. 10). As the retention times of all of these compounds, except 1,6-anhydro-β-D-glucose, increase slightly and uniformly with increasing column temperature, an improvement in analysis cannot be achieved by altering the temperature. Fig. 11 shows the separation of D-glucose, D-galactose and some related compounds, which are of interest in sugar chemistry. The carbohydrates are eluted within 15 min, showing only slight differences in their retention times. Owing to the low selectivity of the column for this substance class, the separation of more than about seven sugars hardly seems possible.

The high efficiency of HPX-87-H for the analysis of polar compounds with different chemical properties is illustrated in Fig. 12, which shows a chromatogram of a sample mixture of organic acids, aldehydes, ketones, alcohols and carbohydrates. As in the separations described above, the optimal column temperature was deter-

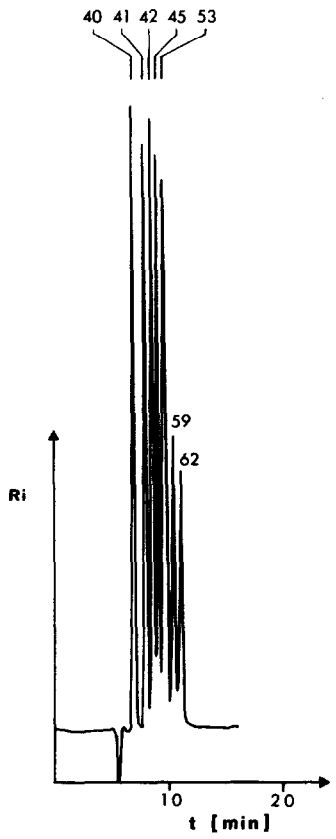

Fig. 11. Optimized separation of carbohydrates. Column, HPX-87-H (300 × 7.8 mm I.D.); column temperature, 80°C; mobile phase, 0.01 *N* sulphuric acid; flow-rate, 0.7 ml/min; refractive index detection. For peak identification, see Table I.

mined by comparing the retention times and calculating the resolution. Twenty-three compounds were separated using this method.

CONCLUSIONS

The increase in retention times with increasing chain length observed with linear acids and with aldehydes and alcohols is caused by a reversed-phase mechanism[62]. Other influences determining the elution order are the positions of the functional groups and branching points in the chain: compounds containing a terminal functional group are more retarded than the secondary or tertiary isomers; branched-chain compounds have shorter retention times than the corresponding straight-chain isomers. This effect on the elution order is demonstrated by the butanol and propanol isomers: *tert.*-butanol < isobutanol < 2-butanol < 1-butanol; 2-propanol is less retarded than 1-propanol. For the linear butanediol isomers the following elution order is observed: 2,3- < 1,3- < 1,4-butanediol. The propanediol isomers, however, have nearly identical retention times in the temperature range investigated.

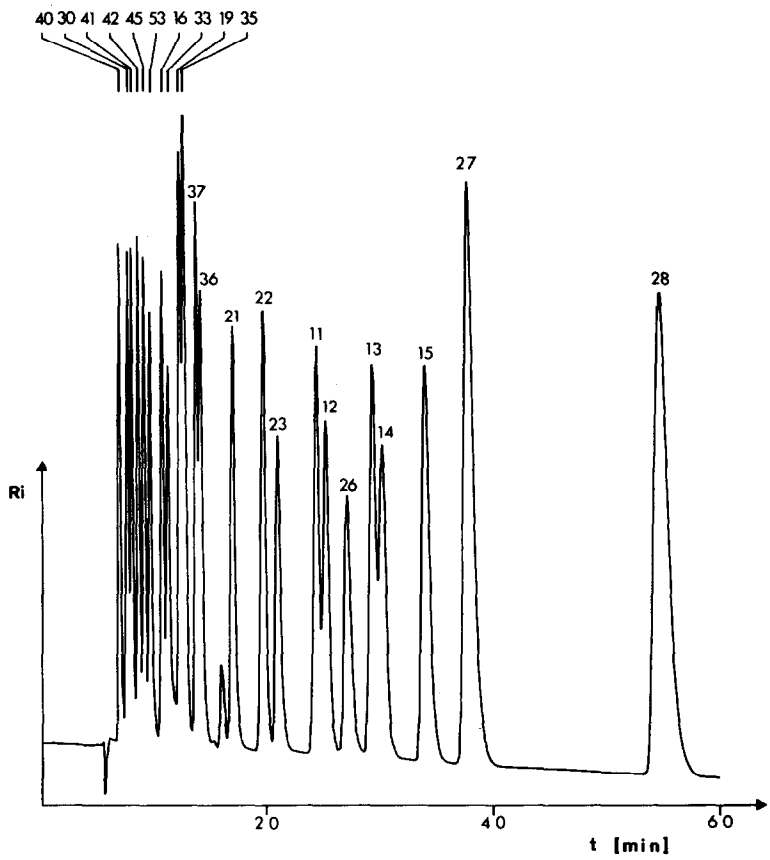

Fig. 12. Optimized separation of a sample mixture of alcohols, aldehydes, ketones, acids and carbohydrates. Column, HPX-87-H (300 × 7.8 mm I.D.); column temperature, 70°C; mobile phase, 0.01 *N* sulphuric acid; flow-rate, 0.7 ml/min; refractive index detection. For peak identification, see Table I.

This relationship between the position of a functional group and the retention time was also found with carbonyl compounds: aldehydes are more retarded than the corresponding ketones. For instance, acetone is eluted earlier than propanal, and methyl ethyl ketone sooner than butanal.

The influence of an increasing number of identical functional groups in a given compound on the elution order is exemplified by alcohols derived from 1-butanol: *meso*-erythritol < 1,2,4-butanetriol < *n*-butanediol isomers < 1-butanol. The type of functional group determines, of course, the capacity factors of compounds with identical chain length also. The elution order RCO_2H < RCHO/RCOR' < ROH is clearly demonstrated by the series propanoic acid < acetone < propanal < propanol isomers and acetic acid < acetaldehyde < ethanol. These sequences remain unchanged, when a carboxylic, carbonyl or hydroxy group is introduced into a molecule already containing a certain functionality. Thus, when acetic acid is regarded as the basic unit, the following elution order applies: oxalic < glyoxylic < glycolic < acetic acid.

By introducing additional functional groups (*e.g.*, OH) into a molecule already containing one other (*e.g.*, CO_2H), analogous effects are observed: glycerinic acid is eluted earlier than lactic acid, which in turn has a shorter retention time than propanoic acid. The same applies to carbohydrates: hexoses and pentoses have shorter retention times than the corresponding deoxysugars, which have one hydroxy group less.

In conclusion, this investigation demonstrates the suitability of HPX-87-H for the separation of polar compounds. In further studies we employed this optimized method for the analysis of hydrothermolysis and biomass degradation products[63,64], with good results.

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