Chromatographic Properties of the Ion-Exclusion Column IonPac ICE-AS6 and Application in Environmental Analysis Part I: Chromatographic Properties

K. Fischer[1,2],*, **J. Kotalik**[1], and **A. Kettrup**[1]

[1]GSF-National Research Center for Environment and Health, Institute of Ecological Chemistry, Ingolstädter Landstr. 1, D-85764 Neuherberg, Germany, and [2]University of Trier, Department of Analytical and Ecological Chemistry, Universitätsring 15, D-54286 Trier, Germany

Abstract

The chromatographic performance of the Dionex IonPac ICE-AS6 ion exclusion column is investigated. Therefore, capacity factors, efficiency, peak symmetry, resolution, and selectivity are determined for various mono- and polyfunctional aliphatic carboxylic acids under selected chromatographic conditions. Except for the stronger acids ($pK_{a1} < 3.75$), the highest chromatographic efficiency is achieved at a column temperature of 40 or 50°C, and peak shape is found to be optimal at approximately 60°C. The separation of the stronger acids is favored by an eluent pH below 3.0 and column temperatures below 40°C. The maximal effective plate numbers range between 126 (tartronic acid) and 6380 (4-oxovaleric acid). Hydroxy-substituted acids are less retained and less influenced by temperature changes than the unsubstituted compounds. It is estimated that size exclusion effects take part in the separation of aldonic acids. The addition of 1% isopropanol to the acidic eluent increases the chromatographic efficiency generally, whereas higher concentrations reduce the retention of several acids drastically.

Introduction

High-performance ion-exclusion chromatography (IEC) is a well established method for the separation of various groups of highly and moderately hydrophilic organic acids with low molecular weights in a broad range of aqueous matrices. Usually, high-capacity cation-exchange resins are applied in IEC. Most of these resins are based on polymers, such as polystyrene-divinylbenzene, and functionalized by sulfonation. Widely used columns belonging to this category include the Bio-Rad Aminex HPX-87 H, the Interaction ORH-801, the Dionex IonPac ICE-AS1, and several others (1). Mechanisms of analyte retention and separation are electrostatic interactions (Donnan exclusion), hydrophobic ("reversed phase") interactions, and size exclusion (2). Therefore, these columns are best suited for the analysis of short-chain fatty acids, dicarboxylic acids, and sugar acids differing in molecular size and acidity (3).

Demands for an improved separation of hydroxycarboxylic acids and krebs cycle acids led to the development of IEC columns based on cross-linked styrene–methacrylate–divinylbenzene polymer resins functionalized with sulfonate and carboxylate groups (4,5). Such columns (e.g., Dionex IonPac ICE-AS5 and ICE-AS6) facilitate higher retention and selectivity for aliphatic di-, tri-, and hydroxycarboxylic acids, which usually elute between the exclusion volume and the total permeation volume on fully sulfonated cation exchangers.

It is to be supposed that hydrogen bonding between the carboxylate groups of the methacrylate units of the stationary phase and the alcoholic OH groups of the analytes are responsible for an increased retardation of hydroxycarboxylic acids on this resin (5).

The Dionex IonPac ICE-AS6 is one of the newest prototypes of this kind of column. Introduced in 1994 (6), it surpasses its predecessor, the IonPac ICE-AS5, with higher solvent compatibility (up to 20%) and increased peak efficiency. Recommended areas of application are various types of aqueous samples containing significant amounts of polyfunctional acids such as beverages, food, pharmaceutical, and chemical products (7). Additionally, many environmental and environmental technical matrices (e.g., soil solutions, groundwater, precipitation, silage effluents, and waste waters) are suited for IEC analysis with special attention to polyfunctional acids (8–12). Recently, an ion chromatography unit equipped with the ICE-AS6 column was hyphenated to a LC–MS detector for the determination of organic acids in aqueous samples (13).

Despite its broad applicability in organic acid analyses, no systematic attempt has been undertaken thus far to investigate the basic chromatographic properties of IonPac ICE-AS6. Therefore,

* Author to whom correspondence should be addressed: e-mail fischerk@uni-trier.de.

Reproduction (photocopying) of editorial content of this journal is prohibited without publisher's permission.

some efforts were made to determine several parameters such as capacity factors, efficiency, peak symmetry, resolution, and selectivity under various chromatographic conditions for a set of 25 aliphatic carboxylic and hydroxy acids. Because the practical aspects of the present authors' analytical studies are orientated towards environmental issues, several examples for the application of the IonPac ICE-AS6 column in environmental analysis will be presented in the second part of this article.

Experimental

Chemicals

All organic acids were of analytical reagent quality (purity > 99%) and purchased from Fluka (Buchs, Switzerland), Merck (Darmstadt, Germany), or Sigma (St. Louis, MO). Tetrabutylammonium hydroxide (TBAOH, suppressor regenerant) was obtained from Riedel-de Haen (Seelze, Germany). All aqueous solutions and dilutions were prepared with ultrapure Milli-Q water (Millipore, Eschborn, Germany).

IEC system and conditions

Separations were performed with a Dionex IonPac ICE-AS6 ion-exclusion column (250 × 9.0 mm, 8-µm particle size, 27 mequiv ion-exchange capacity) containing a cross-linked (8%), microporous, intermediate hydrophobic resin that had been sulfonated. The column was coupled to an AMMS-ICE micromembrane suppressor. Perfluorbutyric acid (PFBA) served as a mobile phase in concentrations of 0.4 (pH 3.4), 0.8 (pH 3.1), 1.6 (pH 2.8), and 2.4 mM/L (pH 2.64). The flow rate was 1.0 mL/min. The column temperature was varied between 20° and 60°C and maintained by a thermostat (Industrial Electronics, Langenzersdorf, Austria). The injection volume was 25 µL. The suppressor regenerant was an aqueous TBAOH solution (5 mM/L) with a flow rate of approximately 3.0 mL/min. The analytical system employed was a Dionex DX-500 chromatographic unit comprised of a GP-40 dual piston high-pressure pump, a quaternary gradient forming module, a He degassing/purge unit for eluent and suppressor regenerant, a Dionex ASM autosampler, an LC-20 chromatography module with sample injection port and sample loop, and an ED-40 electrochemical detector operated in the conductivity mode. Remote system control, data acquisition, and processing was handled by the Dionex PeakNet software.

If not stated otherwise, the concentration of the tested substances ranged between 0.5 and 2.0 mM/L.

Chromatographic parameters were calculated using groundlaying theoretical principles and equations (14). The parameters "asymmetry" and "resolution" were directly computed by the PeakNet system suitability report module.

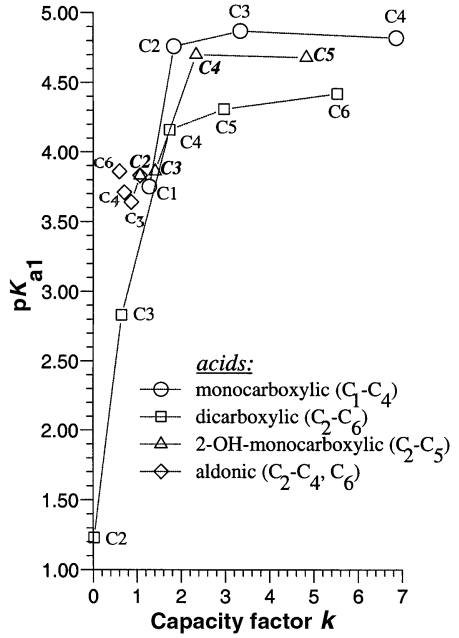

Figure 1. Correlation between pK_{a1} values and capacity factors for several groups of aliphatic acids. Chromatographic conditions: eluent, 1.6mM PFBA (pH 2.80); column temperature, 60°C; flow rate 1.0 mL/min. pK_a of threonic acid and 2-OH-valeric acid were estimated.

Results and Discussion

Correlation between first acid dissociation constants and capacity factors

The electrostatic interactions between the analytes and stationary phase are ruled in IEC mainly by the degree of ionization of the solutes, which is a function of their acidity and of eluent pH. Using an eluent with low or moderate proton concentration, the sulfonic acid groups of the resin can be considered fully deprotonated and therefore able to exclude negatively charged compounds from penetration into the pores of the stationary phase. A plot of capacity factors k versus pK_{a1} for a range of solutes is given in Figure 1. Because the pH value of the eluent was 2.80, it can be concluded that only oxalic acid and malonic acid were largely or partially dissociated, whereas the other compounds remained more or less undissociated.

As Figure 1 elucidates, a distinct correlation between pK_{a1} and k exists only for the short-chain monocarboxylic (C_1, C_2) and dicarboxylic (C_2–C_4) acids. With increasing chain lengths, the pK_{a1} differences decrease, but the k differences increase, indicating the effect of hydrophobic interactions. The comparison between the chromatographic behavior of butyric and valeric acid and of their α-OH-compounds is interesting. Under given conditions, the k value of valeric acid (not included in Figure 1) is approximately 13.75, and pK_a is 4.82. Despite the small pK_a differences of approximately 0.12, the α-hydroxy acids have considerably lower capacity factors than the corresponding unsubstituted acids. This might be a hint that additional hydrogen bonding cannot compensate for weaker hydrophobic bonding forces.

The retention of aldonic acids, represented by the formula CH_2OH–$(CHOH)_n$–COOH, seems not to be ruled by their pK_a values. The component with the lowest acidity (gluconic acid, "C_6") has the lowest k value as well. In fact, the k values decrease with increasing carbon number: glycolic acid ("C_2") > glyceric acid ("C_3") > threonic acid ("C_4") > gluconic acid ("C_6"). With regard to the microporous structure of the resin, it can be

assumed that size exclusion effects take part in the phase distribution of these compounds. Because data about the pore sizes or pore size distribution of the column packing particles are not available, an evaluation of this hypothesis is impeded. Nevertheless, it should be mentioned that various authors have suggested size exclusion effects to be responsible for a reduced retention of medium-sized dicarboxylic acids and for the elution of neutral lactones in order of decreasing molecular size (1). A similar retention sequence of aldonic acids was ascertained by applying a conventional ion-exclusion column without methacrylate groups (3).

Generally, the limited correlation between the dissociation constants of the organic acids and their capacity factors is due to the fact that analyte distribution between the stationary phase and the mobile phase in IEC is accomplished by the combined action of several separation mechanisms. The selectivity of the IonPac ICE-AS6 reflects a specific balancing of these separation factors. For instance, strong hydrophobic interactions between nonpolar molecule regions and the stationary phase facilitate the complete separation of compounds having almost identical pK_a values. On the other hand, a poor resolution of analytes with different pK_a values can occur even under optimized pH conditions. This situation is illustrated for the analyte pair malonic and gluconic acids in Figure 1.

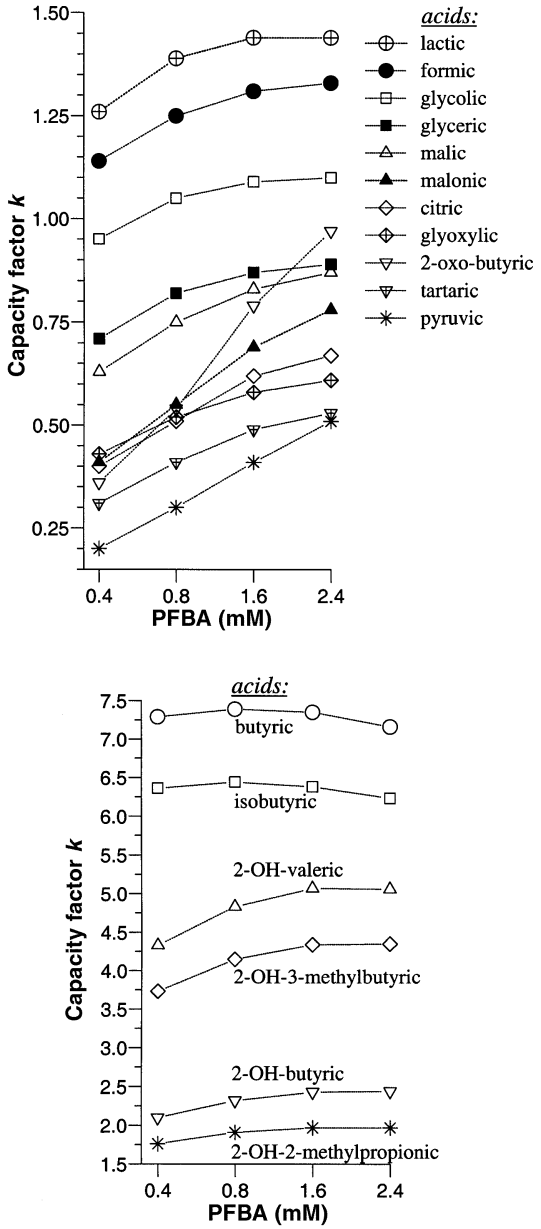

Figure 2. Effect of eluent concentration (eluent pH) on capacity factors: capacity factors less than (A) and greater than (B) 1.5. Chromatographic conditions: column temperature, 60°C; eluent flow rate, 1.0 mL/min.

Effect of eluent concentration (eluent pH) on capacity factors and effective plate numbers N_{eff}

The repression of the analyte dissociation by an increase of the eluent concentration (i.e., pH decrease) lessens the exclusion effectiveness of the Donnan membrane, thus allowing the analytes to enter the micropores of the resin and to get retained by various modes of interaction with the resin surface. Additionally, the protonation of the organic acids lowers their water solubility and increases their affinities to hydrophobic phases. Therefore the retention of most of the organic acids increases with decreasing eluent pH. For the relatively narrow pH range

Table I. Effect of PFBA Eluent Concentration on Effective Plate Number N_{eff}

	20°C			40°C			
Acid	0.4 mM	0.8 mM	1.6 mM	0.4 mM	0.8 mM	1.6 mM	k*
Glucaric	285	376	490	387	479	664	0.39
Pyruvic	77	236	445	67	217	427	0.40
Oxosuccinic	70	189	422	57	190	373	0.40
Gluconic	115	193	266	237	402	513	0.60
Glyoxylic	415	852	1086	468	1132	1634	0.57
Tartaric	128	346	798	114	302	929	0.53
Threonic	697	1305	1293	798	1764	1917	0.73
Isosaccharinic	1116	1228	1229	1469	1644	nd†	nd†
Isocitric	319	798	924	317	860	1281	0.72
Glyceric	1253	1803	1693	1467	2374	2635	0.90
Malonic	154	460	1159	139	486	1165	0.74
Citric	228	609	970	198	558	1247	0.74
Malic	606	1424	1729	578	1709	2333	0.93
Glycolic	2225	2588	2544	2834	3542	3463	1.13
Formic	1271	3055	3064	1177	3367	3902	1.35
Lactic	2345	3027	2858	2561	4108	4366	1.49
2-OH-methylpropionic	3072	3054	2963	4285	4584	4624	2.04
Acetic	4888	4504	4097	6070	5857	5035	2.01
Succinic	3525	3421	3778	4718	4779	4642	2.16
2-OH-butyric	3201	3829	3685	3835	5230	5364	2.62
Propionic	4704	4446	4768	7270	6745	6236	3.95
2-OH-3-methylbutyric	3663	3865	3346	5377	5693	5282	4.96
Glutaric	3748	3618	4172	5594	5423	4837	4.11
4-Oxovaleric	5342	5352	4813	7154	7050	6377	4.66
2-OH-valeric	4144	4326	3476	6139	6197	5970	5.99
Isobutyric	3169	3097	nd†	5944	5534	5299	7.52
Butyric	2445	2348	nd†	4818	4456	4626	8.90

* 40°C, 1.6mM PFBA.
† nd, not determined.

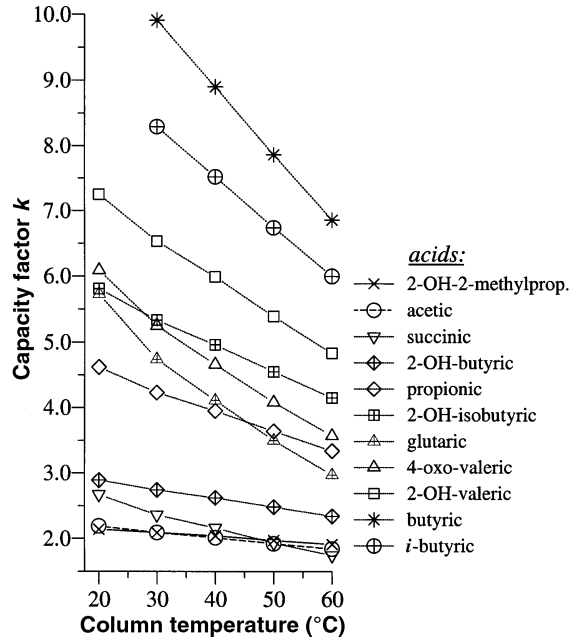

Figure 3. Effect of temperature on capacity factors. Chromatographic conditions: eluent, 1.6mM PFBA (pH 2.80); flow rate, 1.0 mL/min.

Table II. Temperature-Dependent Changes of Capacity Factors of Two Series of Homologous Compounds*

Acid	k (20°C)	Δk (20/60°C)	Δk (%)
Monocarboxylic acids			
Butyric	(11.00)†	–4.10	37.3
Propionic	4.62	–1.28	27.7
Acetic	2.19	–0.35	16.0
Formic	1.44	–0.16	11.1
α-Hydroxy carboxylic acids			
OH-valeric	7.25	–2.42	33.3
OH-butyric	2.89	–0.55	19.0
Lactic	1.56	–0.15	9.6
Glycolic	1.18	–0.11	9.3

* Eluent, 1.6mM PFBA (pH 2.80).
† Extrapolated.

Table III. Temperature-Dependent Changes of Capacity Factors of Various Types of C_4 Compounds*

Structural type	Compound	k (20°C)	Δk (20/60°C)	Δk (%)
Monocarboxylic acid	butyric acid	(11.00)†	–4.10	37.3
Branched monocarboxylic acid	*i*-butyric acid	(9.15)†	–3.15	34.4
Dicarboxylic acid	succinic acid	2.67	–0.93	34.8
2-OH-monocarboxylic acid	2-OH-butyric acid	2.89	–0.55	19.0
2-OH branched monocarboxylic acid	2-OH-2-methyl-propionic acid	2.14	–0.23	10.7
Monohydroxy dicarboxylic acid	malic acid	1.04	–0.22	21.2
2,3-Dihydroxy dicarboxylic acid	tartaric acid	0.69	–0.11	15.9
1,2,3-Trihydroxy monocarboxylic acid	threonic acid	0.75	–0.04	5.3

* Eluent, 1.6mM PFBA (pH 2.80).
† Extrapolated.

between 2.64 and 3.4 (PFBA concentration between 2.4 and 0.4 mM), this situation is illustrated in Figure 2. The following acids under investigation, characterized by low shifts of their k values ($\Delta k < 0.15$), were omitted from graphic illustration: acetic acid, propionic acid, 3-hydroxybutyric acid, oxalic acid, tartronic acid, succinic acid, and glutaric acid.

As the figures reveal, notable changes in k values are almost restricted to those compounds having pK_a values within or near the tested pH range. Again, the structural properties of the solutes superimpose the significance of their acidity. Despite similar pK_{a1} values, the increase in retention of the α-ketocarboxylic acids pyruvic acid (pK_a, 2.26; Δk, 0.31) and 2-oxobutyric acid (pK_a, 2.0; Δk, 0.61) surpasses the one of tartronic acid (pK_1, 2.37; Δk, 0.14) by far. The enhanced retention of 2-oxobutyric acid in comparison with pyruvic acid might be a result of its longer carbon chain, favoring hydrophobic bonding of the permeated molecules. As a consequence of the elevated ion strength of the eluent, the retention of butyric acid and isobutyric acid is slightly depressed as the eluent pH decreases.

With respect to their low acidity, the investigated C_4/C_5-α-OH-carboxylic acids should not be influenced by the adjusted eluent concentration or behave like the corresponding monocarboxylic acids. Deviating from this expectation, the retention of these compounds obviously inclines with the PFBA concentration. Given the same carbon number, the effect is more pronounced for the unbranched molecules. It is unlikely that an increase in the eluent concentration has led to intensified electrostatic and hydrophobic interactions between the α-hxdroxy carboxylic acids and the stationary phase, resulting in enhanced analyte retardation. Because hydrogen bonding takes place in the retention of hydroxy compounds, it can be resumed that a decrease in the eluent pH promotes this bonding mode by a repression of the dissociation of methacrylate units, which are part of the stationary phase.

Considering the interrelation between the effective plate number (N_{eff}) and the capacity factor, it seems reasonable to assume on the basis of the previously discussed results that N_{eff} will increase with increasing eluent concentration for most of the compounds. As it can be deduced from the data combined in Table I, the highest N_{eff} values correlate with the maximum tested PFBA concentration for about two-thirds of the analytes at both temperatures. The relative increase of the N_{eff} values with the PFBA concentration was highest for those acids marked by low adjusted retention times. This effect was more pronounced at 40°C than at 20°C. The narrower peak widths at the higher temperature are likely due to the reduced viscosity of the mobile phase leading to improved mass transfer effects. Enhanced retention at higher concentrations of PFBA is probably the result of more effective protonation of the acidic solutes leading to added hydrophobic effects for the more neutral species.

With increasing capacity factors of the analytes (caused mainly by enhanced hydrophobic interactions), the separation efficiency tends to reach maximal values at low or medium eluent concentration. At 40°C, this includes all volatile fatty

acids with at least two carbon atoms. The peak widths of these components were found to decline with decreasing eluent concentration.

As a consequence, the separation efficiency of the IonPac ICE-AS6 must be optimized with regard to the structural composition of the analytes and the dependent modes of interaction with the stationary phase. If Donnan exclusion and hydrogen bonding are the most important modes of interaction, a relatively high PFBA concentration is recommended to attain high effective plate numbers. If hydrophobic interactions prevail, the use of a diluted eluent might be advantageous.

Effect of temperature on capacity factors and resolution

The retention of most of the organic acids decrease with increasing column temperature, reflected by a corresponding decline of the related capacity factors. Greater effects were restricted to solutes having medium or high affinities for the stationary phase, which are included in Figure 3.

The plot of capacity factors versus column temperature yields straight lines with different negative slopes. The magnitude of the negative slope is related to the molecular structure of the analytes and generally increases as their carbon chain lengths increase (Table II). As Figure 3 reveals, strong effects are provoked in the case of mono- and dicarboxylic acids.

Although it cannot be generalized, a certain correlation between the molecular structure and the temperature dependence of the *k* values may be illustrated with the example of C_4 compounds listed in Table III. At least for the monocarboxylic acids, it can be concluded that the percentage differences of the *k* values (last column in Table III), provoked by changes of the column temperature, decrease with increasing degrees of hydroxylation. Despite great differences in their *k* values at 20°C, the relative changes of this parameter in the temperature range between 20 and 60°C are almost the same for butyric, isobutyric, and succinic acids.

As it follows from the data shown in Figure 3, the differences in the slopes of the plots have several consequences for the chromatographic separation of various analyte combinations. For instance, glutaric acid, 4-oxovaleric acid, and 2-hydroxy-3-

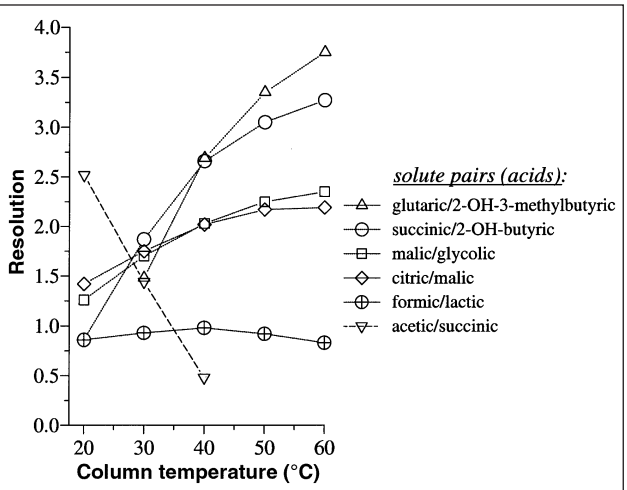

Figure 4. Effect of temperature on resolution. Chromatographic conditions: eluent, 1.6mM PFBA; flow rate, 1.0 mL/min.

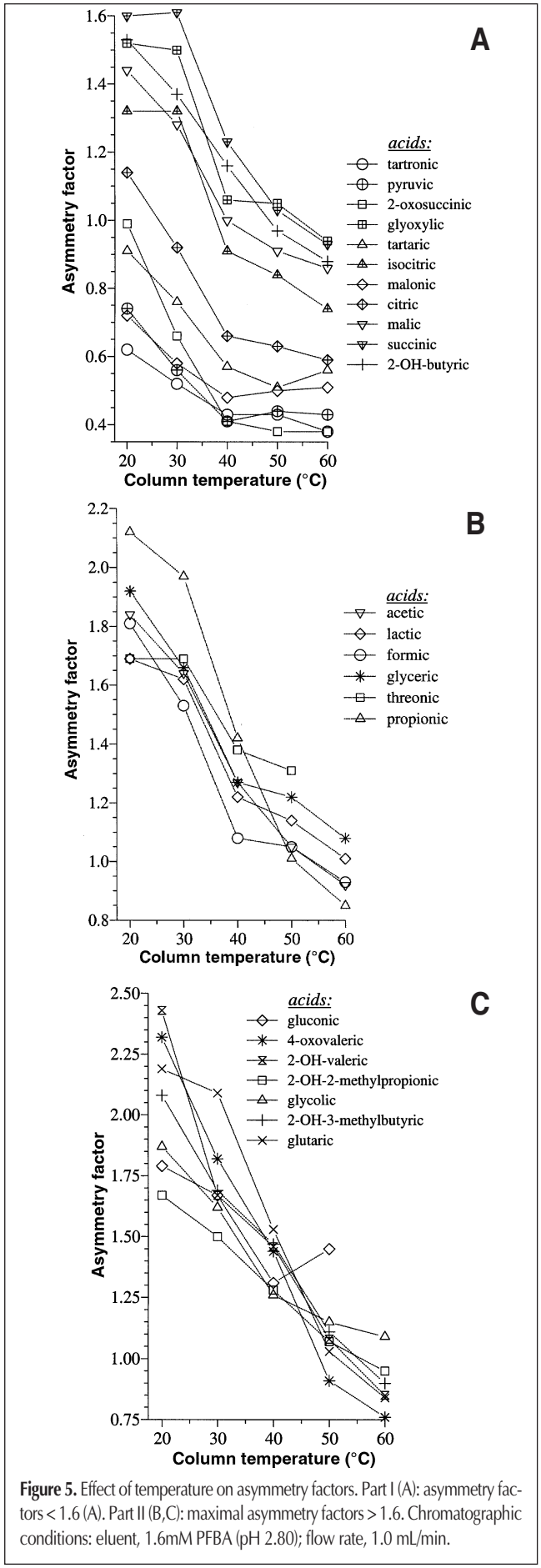

Figure 5. Effect of temperature on asymmetry factors. Part I (A): asymmetry factors < 1.6 (A). Part II (B,C): maximal asymmetry factors > 1.6. Chromatographic conditions: eluent, 1.6mM PFBA (pH 2.80); flow rate, 1.0 mL/min.

methylbutyric acid cannot be separated at 20°C, but separation is possible at 60°C.

A rise in the temperature can affect retention on an IonPac ICE-AS6 under conditions applied in the following manner: (*a*) as far as the sorption of the solutes onto the stationary phase caused by hydrophobic interactions is an exothermic process (release of heat of adsorption, negative adsorption enthalpy), the standard free energy of the phase transfer is diminished; (*b*) the degree of ionization of both the solutes and weak acidic functional groups of the resin is elevated, resulting in an enhanced electrostatic repulsion; (*c*) hydrogen bonds between hydroxy compounds and the functional groups of the resin are weakened by increased thermal agitation.

Under the given chromatographic conditions, the depression of the standard free energy of the phase transfer reaction is the main factor responsible for the reductions of the capacity factors.

Because the standard free adsorption enthalpy is a function of the number of interacting molecular units (i.e., methylene groups), a correlation between the slopes of their temperature plots and the carbon chain length of compounds belonging to a certain homologous series exists.

The temperature influence on the *k* values is slight for those acids, whose retention should be susceptible to changes in ionization equilibria due to the approximate correspondence between their pK_{a1} values and the eluent pH (e.g., tartaric, citric, isocitric, and malonic acids). The shifts of the capacity factors (D*k* values) measured for this group of analytes ranged between 0.11 and 0.29. Similar values (from 0.04 to 0.15) were found for solutes subjected to hydrogen bonding (e.g., glycolic, lactic, glyceric, and threonic acids).

To illustrate the influence of the separation temperature on the resolution of several substance combinations, the following acid pairs were chosen: glutaric acid/2-hydroxy-3-methylbutyric acid, succinic acid/2-hydroxybutyric acid, malic acid/glycolic acid, citric acid/malic acid, formic acid/lactic acid, and acetic acid/succinic acid. The results are combined in Figure 4.

The resolution of four of five combinations that were incompletely separated at low temperature was significantly enhanced as temperature increased. The improved resolution of the solute pairs, including a dicarboxylic acid and a 2-hydroxy monocarboxylic acid, can be traced back to a stronger reduction of the retention of the respective dicarboxylic acid component. The resolution of the pair formic acid/lactic acid cannot be essentially improved neither by a temperature variation nor by a change in the eluent pH.

The substance pair acetic acid/succinic acid provides an example for an impairment of resolution with increasing column temperature.

Effect of temperature on peak symmetry

Peak asymmetries such as fronting or tailing can impair analyte quantitation, especially if peaks are not fully resolved ("rider" peaks) or peak-to-noise signal levels are low (uncertainty of peak delimitation). Therefore, the generation of approximately symmetric peak shapes should be part of the optimization of chromatographic conditions.

Due to the acceleration of mass transfer processes and the promotion of desorption reactions, peak tailing is reduced and

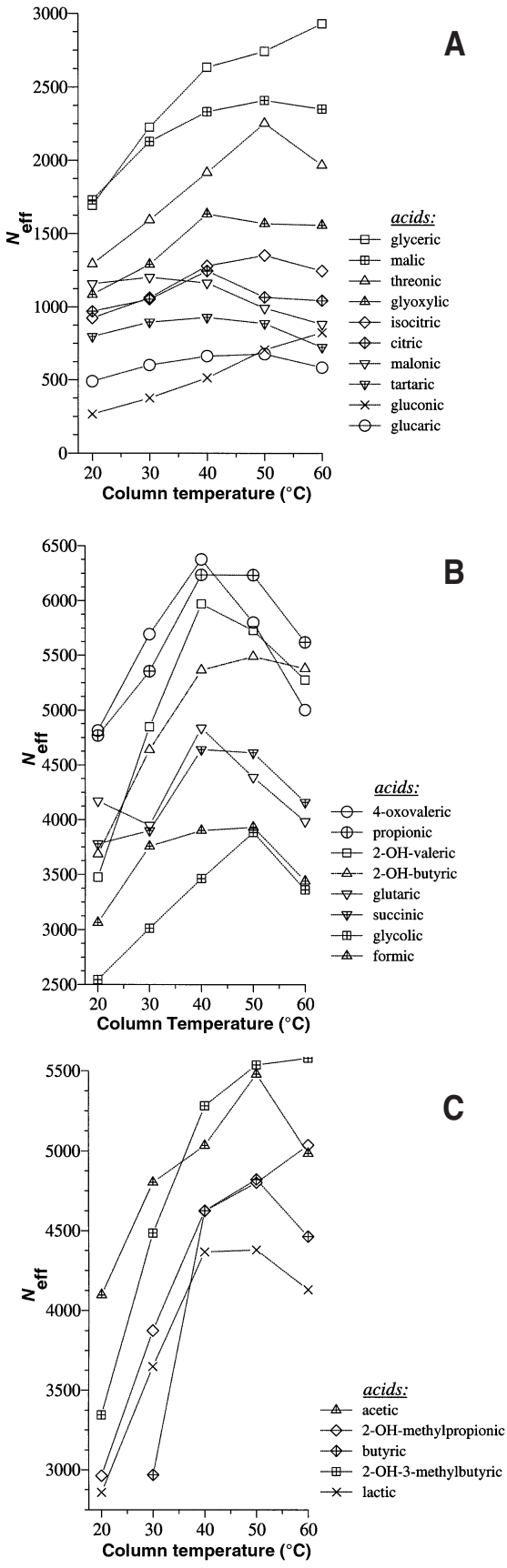

Figure 6. Effect of temperature on effective plate number N_{eff}. Chromatographic conditions: eluent, 1.6mM PFBA; flow rate, 1.0mL/min.

fronting is enhanced as column temperature rises. This situation is depicted in Figure 5. At a low temperature, peak tailing is greatest mainly for solutes retained by hydrophobic interactions, whereas fronting is restricted to compounds subjected to Donnan exclusion and size exclusion effects.

Although there is a certain tendency, the capacity factor and asymmetry factor are not strongly correlated. For instance, the capacity factors of malonic acid and isocitric acid are nearly the same at 20°C/1.6mM PFBA, but the isocitric acid peak is tailing and the malonic acid peak is fronting.

Because different bonding modes and bonding strengths are involved in the retardation of the various types of solutes, different temperature ranges exist for peak shape improvement. As far as Donnan exclusion and size exclusion effects rule retention, temperatures of approximately 20°C seem to be advantageous. In all other cases, temperatures of 50 or 60°C are favorable. At 60°C, the asymmetry factors of the test compounds range between 1.09 (glycolic acid) and 0.38 (tartronic and oxosuccinic acid).

Effect of temperature on effective plate number N_{eff}

As displayed in Figure 6, the maximal effective plate number is achieved for most of the solutes at 40 or 50°C. It can be concluded that the decrease in the adjusted retention time (or of the capacity factors) with increasing temperature (see Figure 3) is overcompensated by smaller peak widths, resulting in an enhanced chromatographic efficiency. In the case of malonic acid and a few other acids with very low N_{eff} values (tartronic acid, oxosuccinic acid, and pyruvic acid; data not shown), the decrease in the adjusted retention times is the deciding factor, leading to highest N_{eff} values at 20 or 30°C. In total, the maximal N_{eff} values range between 126 (tartronic acid) and 6380 (4-oxovaleric acid).

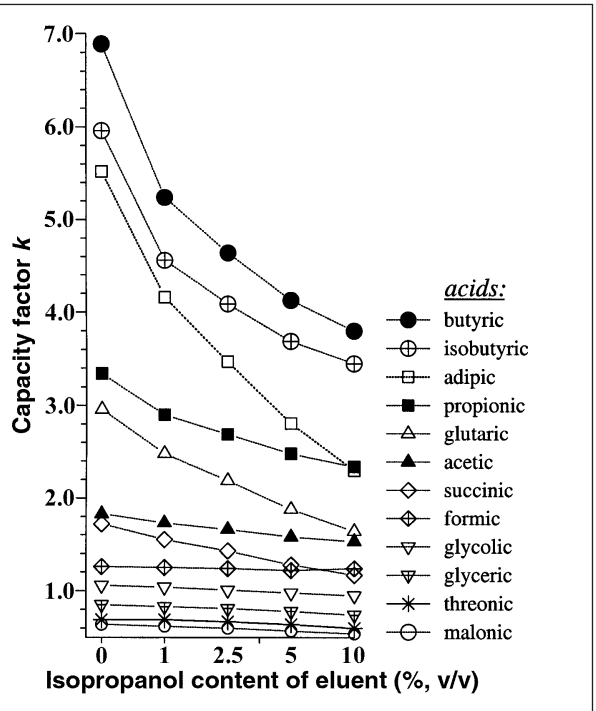

Figure 7. Effect of isopropanol additive on capacity factors. Chromatographic conditions: eluent, 1.6mM PFBA; column temperature, 60°C; flow rate, 1.0 mL/min.

The N_{eff} values of most of the solutes, which were poorly retained (k < 1.0), vary only slightly with temperature. Exceptions are gluconic acid, glyoxylic acid, threonic acid, and glyceric acid (Figure 6A). In the latter cases, a rise in the column temperature diminished peak widths without any significant effect on capacity factors. There is a tendency for temperature dependence of N_{eff} values to increase as the strength of hydrophobic interactions between solute and stationary phase increase. Therefore, a high temperature dependence is demonstrated by the N_{eff} versus temperature plots of butyric acid, 2-hydroxymethylpropionic acid, 2-hydroxy-3-methylbutyric acid, and 2-hydroxyvaleric acid (Figures 6B and 6C).

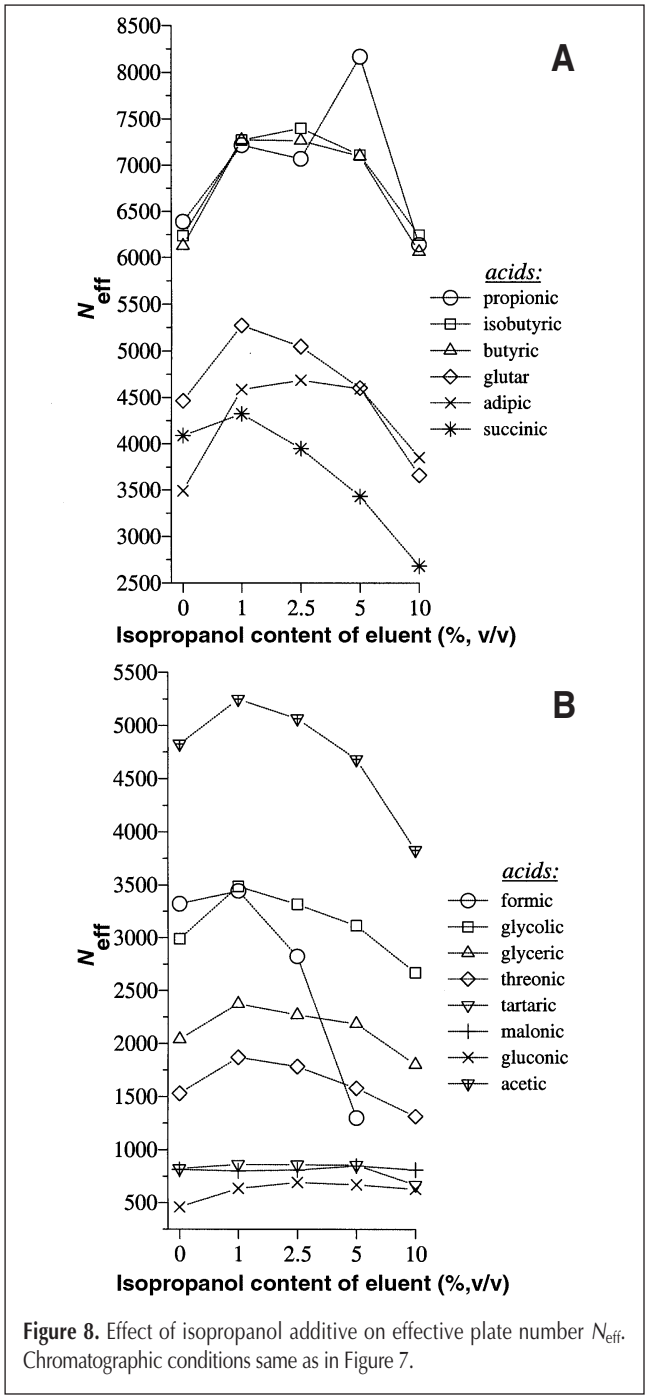

Figure 8. Effect of isopropanol additive on effective plate number N_{eff}. Chromatographic conditions same as in Figure 7.

Effect of isopropanol additive on capacity factor and effective plate number

The addition of typical reversed-phase organic modifiers (such as methanol, acetonitrile, or acetone) counteracts hydrophobic solute adsorption mainly by a competing coverage of the resin surface with the modifier molecules. Alternatively, the polystyrene-based particles could be solvated by the organic modifiers with higher contents of these solvents in the eluent. Figure 7 shows the retention changes of mono-, di-, and various hydroxycarboxylic acids by the addition of isopropanol to the PFBA eluent. As expected, the correlation between the magnitude of lowering of retention and the property of the solute chain length was the same as that found for the influence of temperature on compound-specific retention. Isopropanol had little effect on the retardation of the smallest members of the homologeous series of mono- and dicarboxylic acids and the hydroxy acids. Strong impacts on retention were achieved with the following (percentage decrease of capacity factors at an eluent concentration of 10% isopropanol in parentheses): isobutyric acid (42%), butyric acid (45%), glutaric acid (45%), and adipic acid (58%). These decreases are considerably higher than reported for an acetonitrile–methanesulfonic acid eluent on an Aminex HPX-87H column (2) and a methanol–benzoic acid eluent on a TSK cation-exchange resin (15).

The significant effect of very low isopropanol concentrations (1%) on the retention of the mentioned acids is noteworthy. Attempts to perform gradient runs with increasing isopropanol concentrations failed because of baseline upsets and irreproducible retention times and peak areas. It is assumed that relatively quickly varying isopropanol concentrations affect the membranes of the suppressor, disturbing ion permeation and exchange processes.

The plots of the effective plate numbers versus isopropanol content of the PFBA eluent are summarized in Figure 8. Due to an improvement in the peak shapes (peak narrowing), low eluent concentrations of isopropanol (especially 1%) increase the chromatographic efficiency. This is probably caused by the lower viscosity of the mobile phase with the isopropanol, leading to improved mass transfer of the analytes between the chromatographic phases. A further addition of isopropanol compensates or overcompensates this positive result, because the retention times were more reduced than the peak widths.

Once again, solutes that are primarily exposed to Donnan exclusion and size exclusion (such as malonic acid, tartronic acid, and gluconic acid) exhibit only marginal responses to changes in the isopropanol concentration. The sharp decrease of the chromatographic efficiency for formic acid is caused by an unusual peak broadening. Analytes that are predestined for hydrogen bonding (glycolic acid, glyceric acid, and threonic acid) were somewhat more strongly influenced by variations in the alcohol content.

Example chromatograms and experiences of long-term operation

Figure 9 depicts a chromatogram of a standard mixture of 15 organic acids, including 14 polyfunctional acids, which eluted within 35 min. Oxalic acid (peak 1) elutes before t_0, indicating that this solute is totally excluded from the pores of the column packing. In this case, total ion-exclusion must be involved. The tailing of most of the peaks is obvious but relatively low even for the more retained components. Despite a retention time difference of 0.42 min, the separation of formic acid and lactic acid is incomplete, indicating the beginning of loss of column efficiency, possibly jointly caused by a slight contamination with heavy metal ions. Trivalent ions such as Fe(III) strongly coordinate with the sulfonate groups of the resin especially. The consequence is a partial neutralization of negative charges, leading to a diminished efficiency of the Donnan exclusion mechanism.

Figure 10 shows a chromatogram of a very diluted reference standard. Due to the high detection sensitivity, baseline noise is visible. Because the standard was diluted with Milli-Q water, a large negative peak marks the void volume and a second small negative peak at about 17.4 min occurred, indicating traces of carbonate. The chromatogram clarifies that a sensitive detection of organic acids is possible in the lower micromole-per-liter range and even below, especially, if one considers that no special efforts were made to lower the detection limit (for example, by

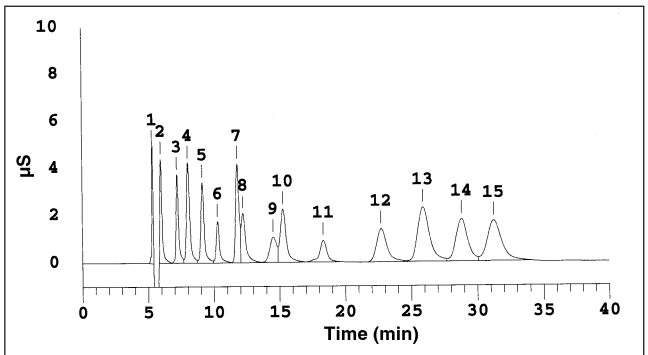

Figure 9. Chromatogram of a multicomponent standard. Chromatographic conditions: eluent, 1.6mM PFBA (pH 2.80); background conductivity, 69 µS; column temperature, 40°C; eluent flow rate, 1.0 mL/min. Analytes (acids, mM/L): 1, oxalic (0.2); 2, tartronic (0.2); 3, tartaric (0.2); 4, citric (0.2); 5, malic (0.2); 6, glycolic (0.6); 7, formic (1.2); 8, lactic (0.45); 9, 2-oxovaleric (0.8); 10, 2-hydroxymethylpropionic (0.8); 11, 2-hydroxy-butyric (0.8); 12, methylsuccinic (0.4); 13, glutaric (0.8); 14, 4-oxovaleric (1.6); 15, 2-hydroxy-3-methylbutyric (2.0).

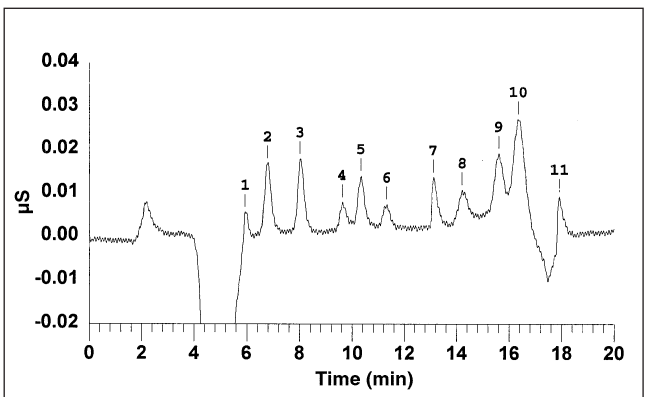

Figure 10. Chromatogram of a very diluted reference standard. Chromatographic conditions: eluent, 0.4mM PFBA (pH 3.4); background conductivity, 19 µS; column temperature, 30°C; eluent flow rate, 1.0 mL/min. Analytes (acids, µM/L): 1, tartaric (0.7); 2, citric (0.7); 3, malic (1.5); 4, glycolic (1.3); 5, formic (2.2); 6, lactic (0.9); 7, unknown; 8, 2-hydroxymethylpropionic (2.9); 9, acetic (4.2); 10, succinic (3.4); 11, unknown.

extension of the injection volume, thermostatization of the conductivity cell, additional pulse damping, or the application of preconcentration techniques).

The experience was that the IonPac ICE-AS6 is a durable and reliable column with long-term stability in chromatographic performance, if prerequisites to avoid contamination by high-affinity organic compounds and heavy metals were taken. Under these conditions, highly loaded samples with complex matrices such as silage effluents and landfill leachates can be analyzed without a significant loss of sensitivity for trace components.

Organic modifiers should be used at fixed concentrations only, because equilibration times are long and retention times may slightly shift over several days.

In this context, the relatively high batch-to-batch variance of the column performance should be critically noted. Test chromatograms of newly delivered columns exhibited significant differences in the resolution of formic acid/lactic acid, as well as in retention times and efficiencies of formic acid and acetic acid. This requires a time-consuming repeating of the chromatographic optimization process after each column exchange, and by doing so, the realization of the needed chromatographic efficiency is not secured.

Conclusion

The IonPac ICE-AS6 is designed for the separation of aliphatic mono- and polyfunctional acids in conjunction with conductivity detection and usage of a micromembrane suppressor. Therefore, the recommended pH range for eluents is very limited. Under tested conditions, only the retention of acids with $pK_{a1} < 3.75$ is ruled mainly by the eluent pH. Here, capacity factors and peak symmetries increase with increasing eluent concentration. The retention of the other acids, which interact with the stationary phase predominantly by hydrophobic bonding, is strongly influenced by the column temperature and the activity of organic modifiers. For this group of analytes, a column temperature of approximately 60°C offers optimal peak shapes, and a temperature between 40 and 50°C achieves the highest effective plate numbers. An addition of small amounts of an organic modifier (i.e., isopropanol) generally increases the chromatographic efficiency.

Generally, optimum chromatographic conditions for the separation of analytes retarded primarily by hydrophobic interactions and those subjected to Donnan exclusion do not coincide. Viewed under this aspect, the IonPac ICE-AS6 allows various practical compromises and offers considerable flexibility for the regulation of the required chromatographic selectivity. Nevertheless, not always satisfying chromatographic efficiencies, the possible resolution of organic acids that are poorly resolved by ion exclusion alone (7) is attended by the possible coelution of analytes that differ in their acidity significantly.

Acknowledgments

The authors thank J.J. Kirkland for his constructive and helpful comments.

References

1. P.R. Haddad and P.E. Jackson. *Ion Chromatography: Principles and Applications*. Elsevier, Amsterdam, The Netherlands, 1990, pp 195–222.
2. R. Widiastuti, P.R. Haddad, and P.E. Jackson. Approaches to gradient elution in ion-exclusion chromatography of carboxylic acids. *J. Chromatogr.* **602:** 43–50 (1992).
3. K. Fischer, H.-P. Bipp, D. Bieniek, and A. Kettrup. Determination of monomeric sugar and carboxylic acids by ion-exclusion chromatography. *J. Chromatogr.* **706:** 361–73 (1995).
4. J. Weiss and S. Reinhard. Aliphatic carboxylic acids—a difficult chromatographic problem? *GIT Fachz. Labor. Spezial Chromatographie* **2:** 105–114 (1995).
5. J. Weiss. *Ionenchromatographie*, 2nd ed. VCH-Verlag, Weinheim, Germany, 1991, pp 217–19.
6. C.A. Pohl and A. Woodruff. Paper 244, 27.02. – 04.03., Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Chicago, IL, 1994.
7. *Installation Instructions and Troubleshooting Guide for the IonPac ICE-AS6 Analytical Column*, Rev. 02. Dionex, Sunnyvale, CA, 1995.
8. E.A. Dietz and K.F. Singley. Determination of fumaric acid, maleic acid, and phthalic acid in groundwater and soil. *J. Liq. Chromatogr.* **17:** 1637–51 (1994).
9. E.A. Elkhatib. Simultaneous determination of low molecular weight organic acids in soil solution by ion chromatography. *Z. Pflanzenernähr. Bodenkd.* **153:** 201–205 (1990).
10. S.R. Bachmann and M.E. Peden. Determination of organic acid anions in precipitation by ion chromatography exclusion. *Water Air Soil Pollut.* **33:** 191–98 (1987).
11. K. Fischer, C. Corsten, P. Leidmann, D. Bieniek, and A. Kettrup. Determination of short chain aliphatic acids in silage effluents by ion exclusion chromatography. *Chromatographia* **38:** 43–51 (1994).
12. K. Fischer, A. Chodura, J. Kotalik, D. Bieniek, and A. Kettrup. Analysis of aliphatic carboxylic acids and amino acids in effluents of landfills, composting plants and fermentation plants by ion-exclusion and ion-exchange chromatography. *J. Chromatogr.* **770:** 229–41 (1997).
13. S.K. Johnson, L.L. Houk, J. Feng, D.C. Johnson, and R.S. Houk. Determination of small carboxylic acids by ion-exclusion chromatography with electrospray mass spectrometry. *Anal. Chim. Acta* **341:** 205–216 (1997).
14. L.S. Ettre. Nomenclature for chromatography. *Pure Appl. Chem.* **65:** 819–72 (1993).
15. Tanaka and J.S. Fritz. Separation of aliphatic carboxylic acids by ion-exclusion chromatography using a weak-acid eluent. *J. Chromatogr.* **361:** 151–60 (1986).

Manuscript accepted November 5, 1999.