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GRADIENT ELUTION OF ALIPHATIC CARBOXYLIC ACIDS BY ION CHROMATOGRAPHY IN THE ION-SUPPRESSION MODE

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SUMMARY

The gradient ion chromatography separation and detection of aliphatic carboxylic acids in the homologous series of butyric acid through stearic acid as well as some unsaturated fatty acids are presented. The separation mode requires the ion suppression of the weak acid analytes for a reversed-phase interaction while detection by electrical conductivity requires analyte ionization through a cation-exchange micromembrane suppressor device.

INTRODUCTION

Liquid chromatographic methods for the determination of aliphatic carboxylic acids include reversed-phase liquid chromatography[1], and ion chromatography in the anion-exchange or ion-exclusion modes[2]. Traditional high-performance liquid chromatography methods use refractive index (RI), ultraviolet (UV) absorption and derivatization–fluorescence[3] techniques for the detection of the acids. The RI and UV methods are bulk property detection schemes and tend to yield more complex chromatograms than those obtained by ion solute-specific detection, such as conductivity. Some pre-derivatization fluorescence methods yield good selectivity and sensitivity but require extensive sample preparation.

The anion-exchange and ion-exclusion modes of ion chromatography cannot provide the complete chromatogram of this homologous series of weak carboxylic acids. It is essentially impossible to elute the acids separated by this technique in the ion-exclusion mode or to resolve them by anion-exchange chromatography.

The combination of ion suppression separation and electrical conductivity detection presented in this paper is new in ion chromatography and offers several advantages over existing techniques for the determination of butyric through stearic acids. Eluent and gradient design is straightforward, sample preparation is minimal, and detection is selective and sensitive. Gradient elution times are short relative to other techniques and detection limits are as good as or better than those obtained with other types of detection except fluorescence.

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EXPERIMENTAL

Apparatus

The 4000i gradient ion chromatograph (Dionex, Sunnyvale, CA, U.S.A.) was used for all work with either a Dionex MPIC-NS1 or a Dionex RPIC-C_{18} separator column and a Dionex anion micromembrane suppressor for ion-exclusion chromatography (AMMS-ICE). A helium headspace was maintained on the eluents by means of a Dionex eluent degas module. A Dionex AI310 data system was used for data reduction and baseline subtraction.

Reagents

Acetonitrile, methanol and 2-propanol were HPLC grade from J. T. Baker (Phillipsburg, NJ, U.S.A.). Water was 17.8 MΩ-cm from a Barnstead Nanopure unit (Barnstead, Boston, MA, U.S.A.). Hydrochloric acid was J. T. Baker Instra-analyzed grade. Potassium hydroxide was reagent grade from J. T. Baker.

Standards were obtained from Sigma (St. Louis, MO, U.S.A.).

Chromatography

Separator columns were cleaned prior to use with 90% aq. acetonitrile at 1 ml/min for 1 h. A general gradient separation was developed for butyric acid through stearic acid, based on conventional gradient method development techniques. The major considerations were chromatographic efficiency, sensitivity, sample solubility, and baseline profile.

Chromatograms, such as that shown in Fig. 1, were generated with a gradient program of 100% eluent A to 100% eluent B in 20 min, holding 100% B an additional 20 min to allow elution of the fatty acids through stearic acid. Eluent A was composed of 24% acetonitrile and 6% methanol in 0.03 m*M* hydrochloric acid. Eluent B consisted of 60% acetonitrile and 24% methanol in 0.05 m*M* hydrochloric acid.

A helium headspace was maintained on the degassed eluents to eliminate carbonate interference and minimize baseline drift. Optimized isocratic conditions (Table I) for three groups of acids within the full series, *e.g.*, butyric acid through heptanoic acid, heptanoic acid through decanoic acid and decanoic acid through stearic acid, were chosen from the gradient chromatogram. Sample preparation con-

TABLE I

ISOCRATIC ELUENT CONDITIONS

Separator, MPIC-NS1; flow-rate 1 ml/min; detection, AMMS-ICE suppressor with electrical conductivity, 30 μS f.s.d.; regenerant, 2.5 m*M* potassium hydroxide, 1 ml/min; temperature, ambient, except for $C_9H_{19}COOH$–$C_{17}H_{35}COOH$, 45°C.

Carboxylic acid group	*Eluent component (%)*		
	0.1 mM Hydrochloric acid	*Acetonitrile*	*Methanol*
C_3H_7COOH–$C_6H_{13}COOH$	70	24	6
$C_6H_{13}COOH$–$C_9H_{19}COOH$	50	45	5
$C_9H_{19}COOH$–$C_{17}H_{35}COOH$	16	60	24

sisted of adjusting pH to below 4 and heating to 40–45°C for dissolution. Samples were diluted in methanol as necessary. For detection by electrical conductivity, which requires analyte ionization, the AMMS-ICE suppressor was inserted ahead of the detector. The regenerant used for the AMMS-ICE was 2.5 m*M* potassium hydroxide, flowing at 1 ml/min for continuous regeneration of the cation-exchange sites within this suppressor.

RESULTS AND DISCUSSION

Separation

Many separation schemes are available for various groups of carboxylic acids. When choosing separation schemes for underivatized carboxylic acids, there are three basic characteristics of the analytes which are considered. These are pK_a values, aromatic–aliphatic character, and valency. Carboxylic acids with strong ionic-type interactions can be considered as more hydrophilic while acids with primarily adsorptive interactions can be categorized as more hydrophobic. The more "hydrophilic" carboxylic acids are those with pK_a values below 4, possibly di- or triprotic, and substituted aromatic or aliphatic character, such as the hydroxy-substituted acids citric, malic, lactic, etc.

Separation modes for carboxylic acids can be based on their ionic character as the main interaction mechanism, their adsorption behavior, or both. Many hydrophilic carboxylic acids can be separated by anion-exchange or ion-exclusion chromatography, as their ionic character is their main basis for separation, as opposed, for example, to adsorption. Anion-exchange chromatography is often the method of choice for the most hydrophilic of the acids, *e.g.*, triprotic acids such as citric and isocitric acid, because selectivity among these acids is very high in this mode. However, resolution of the weakest acids, such as saturated monoprotic acids, is limited. Gradient-elution anion-exchange chromatography also yields separation of inorganic anions with many mono-, di- and triprotic carboxylic acids[4].

The ion-exclusion chromatogram includes unretained strong inorganic anions, *e.g.*, chloride, sulfate, etc., and retained carboxylic acids which are generally eluted in the order of increasing pK_a value. This chromatogram extends into the hydrophobic acid region, consisting of monoprotic acids to approximately valeric acid. Carboxylic acids larger than this have impractically long retention times. This limitation is due primarily to sorption processes of the hydrophobic portions of these molecules to the styrene-based ion-exclusion resins. This type of resin is incompatible with organic solvents that are needed to elute the larger hydrophobic acids. As sorption processes of the more hydrophobic acids limit the chain length of the acids that can be separated by ion-exclusion chromatography, a logical choice for the separation of these acids, (*e.g.*, butyric through stearic), is ion-suppression separation. By removing the small amount of competing hydrophilic character in these acids (*e.g.*, conjugate bases) by ion suppression peak efficiency improves significantly.

Eluent components were chosen for specific functions in the separation. The hydrochloric acid not only helps to maintain protonation of the analytes, but also maintains ion exchange in the AMMS-ICE, which aids baseline stability. The methanol, as well as elevated temperature, mainly aid analyte solubility while the acetonitrile–water performs the major separation, based on partition, on either an

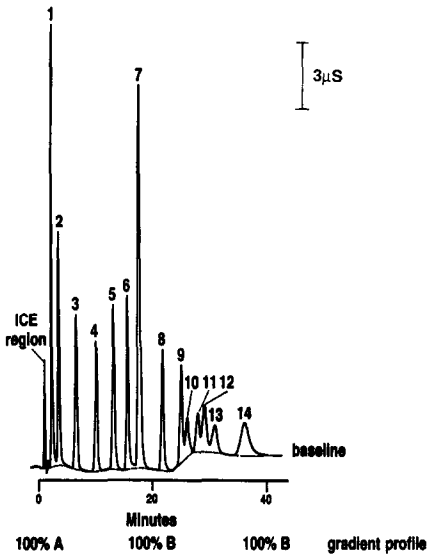

Fig. 1. Gradient ion-suppression separation of the homologous series of butyric through stearic acids, including some unsaturated fatty acids. Peaks: "ICE Region" acids more hydrophilic than butyric acid; 1 = 100 ppm butyric; 2 = 100 ppm valeric; 3 = 100 ppm caproic; 4 = 100 ppm enanthic; 5 = 150 ppm caprylic; 6 = 175 ppm pelargonic; 7 = 200 ppm capric; 8 = 250 ppm lauric; 9 = 250 ppm myristic; 10 = 250 ppm linolenic; 11 = 250 ppm linoleic; 12 = 375 ppm palmitic; 13 = 375 ppm oleic; 14 = 500 ppm stearic. Chromatographic conditions: Column, MPIC-NSl, 20 × 0.4 cm I.D.; eluent A, 24% acetonitrile and 6% methanol in 0.03 m*M* hydrochloric acid; eluent B, 60% acetonitrile and 24% methanol in 0.05 m*M* hydrochloric acid; flow-rate, 1.0 ml/min; detection, electrical conductivity, 30 μS f.s.d.; chart speed, 0.25 cm/min; injection volume, 50 μl; temperature, 42°C; gradient profile indicated below chromatogram.

aromatic stationary phase or an alkyl-bonded stationary phase. As adsorption capacity is higher on RPIC-C_{18}, 2-propanol was added to eluent B to elute stearic acid (Fig. 2).

Fig. 1 shows a chromatogram of butyric acid through stearic acid on the aromatic stationary phase of the MPIC-NSl separator. The term "ICE Region" refers to the void volume elution of acids more hydrophilic than butyric acid, *e.g.*, those acids best separated by ICE. Fig. 2 shows a gradient profile of decanoic through stearic acid on the C_{18} bonded phase of the RPIC-C_{18} separator. In terms of selectivity, it is evident from Fig. 1 and 2 that the aromatic packing material has a higher selectivity for unsaturated fatty acids than the C_{18} column. Impurities in the linolenic acid standard are fairly well resolved from linolenic acid on RPIC-C_{18}. In some applications the superior resolution on C_{18} bonded phase in the lauric acid to palmitic acid region may prove advantageous. The disadvantages of RPIC-C_{18} are the limited resolution of palmitic and oleic acids and the higher organic solvent–water ratio required for elution, which raises detection limits.

Detection

Detection based on electrical conductivity is desirable, because the technique is selective and sensitive for ionized analytes. In order to couple conductivity detec-

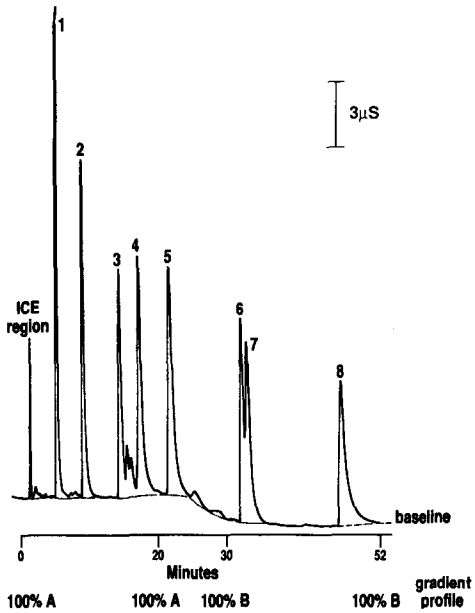

Fig. 2. Gradient ion-suppression separation of fatty acids. Peaks: 1 = 100 ppm capric; 2 = 200 ppm lauric; 3 = 500 ppm linolenic; 4 = 500 ppm myristic; 5 = 600 ppm linoleic; 6 = 1000 ppm palmitic; 7 = 1000 ppm oleic; 8 = 2000 ppm stearic. Chromatographic conditions: separator, RPIC-C_{18}, 25 × 0.44 cm I.D.; eluent A, 60% acetonitrile and 24% methanol in 0.05 m*M* hydrochloric acid; eluent B, 60% acetonitrile, 22% methanol and 5% 2-propanol in 0.05 m*M* hydrochloric acid; flow-rate, 1.0 ml/min; detection, electrical conductivity, 30 μS f.s.d.; chart speed, 0.25 cm/min; injection volume, 50 μl; Temperature, 45°C. Gradient profile indicated below chromatogram.

tion with ion-suppression in ion chromatography, an ion-micromembrane suppressor, designed for ion-exclusion applications (AMMS-ICE) was used. The AMMS-ICE contains cation-exchange sites, which exchange the hydronium ion of the acids for potassium ion, thus causing complete ionization of the acids and allowing detection (Fig. 3a).

The construction and eluent suppresion function of the micromembrane suppressors for anion-exchange applications as well as other modes of ion chromatography have been described in detail in the literature[5]. The patented[6] chemical function of analyte ionization–conductivity signal enhancement and reversed eluent suppression operating in AMMS-ICE are illustrated in Fig. 3a and b. Analyte ionization and signal enhancement are derived from the exchange of hydronium ion for potassion ion from the AMMS-ICE regenerant. Referring to Fig. 3a, the AMMS-ICE entails three flow paths: a central, low-volume eluent path sandwiched between two cation-exchange membranes, and two regenerant paths, formed between the membranes and the walls of the device. All three of these paths contain cation-exchange screens, which increase the ion-exchange capacity of the device and minimize band dispersion. The exchange sites of the membranes and screens are continuously regenerated to the potassium form by the continuously flowing potassium hydroxide regenerant solution. Similarly, the background conductivity of the system generated

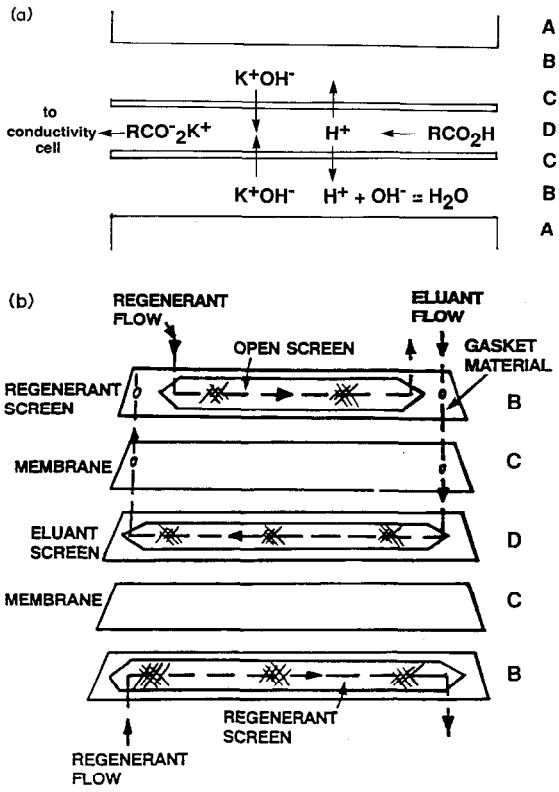

Fig. 3. (a) Simplified schematic diagram of analyte ionization in AMMS-ICE. A = Suppressor body; B = flowpaths for potassium hydroxide regenerant; C = cation-exchange membrane; D = flowpath for eluent and analyte. Suppression of eluent conductivity occurs simultaneously as hydrochloric acid eluent is exchanged for potassium chloride. (b) Flowpaths in AMMS-ICE suppressor. Identifications as in (a). Gasketed cation-exchange screens form flowpaths between cation-exchange membranes.

by the acidic eluent is reduced by replacement of the hydronium ion of the eluent by potassium ion of the regenerant. The driving forces for the exchange of hydronium ion for potassium ion in this system are the neutralization reaction that occurs in the regenerant paths and the concentration gradient of the cations across the membrane. The background conductivity, generated for Fig. 1 averages 8 μS/cm during the gradient.

Table II shows an example of the net lowering of background conductivity (conductivity generated by the eluent) in the AMMS-ICE. This reduction is significant because of the effect of background conductivity on system noise. As background conductivity is lowered, chemical noise is lowered and the signal-to-noise ratio of the analytes is increased. This effect on signal enhancement adds to the signal enhancement by the ionization of the weak acid analytes.

For this application, there were several considerations for choosing potassium hydroxide as the regenerant base for the AMMS-ICE suppressor. Analyte ionization and the specific conductance of the resulting analyte band determine the sensitivity

TABLE II

CONDUCTANCE CALCULATIONS

Eluent in water	*Specific conductance (25°C) (μS/cm)*	*Background conductivity (calc.) (with AMMS-ICE, KOH regenerant)*
HCl, 0.1 m*M*	42.5	15
HCl, 1.0 m*M*	425	150

and detection limits of this system. Referring to Fig. 3a, it can be seen that the carboxylic acids are detected as potassium carboxylates. It is evident that the regenerant cation, *e.g.*, K^+, contributes directly to analyte conductivity as well as the background conductivity produced by neutralization of the hydrochloric acid eluent with potassium hydroxide regenerant. A compromise is made in the signal-to-noise ratio between producing the analyte salt with the highest possible conductance and the eluent salt with the lowest possible conductance.

In this application, the eluent acid concentration is so low that the main consideration in the choice of a regenerant is the analyte conductivity. Potassium ion has a relatively high limiting equivalent ionic conductance (75 S · cm^2/equiv.) among cations other than the hydronium ion, and fast ion-exchange through the membrane. Tetrabutylammonium ion, which has a limiting equivalent ionic conductance of 19 S · cm^2/equiv., is the standard additive in ion-exclusion applications where eluent acid concentrations are usually more than 1 mM and background conductivities are much higher than in this application. Divalent cations are poor choices for regenerant cations in the AMMS-ICE because they have high affinity for the cation-exchange membrane, and this leads to slow H^+ exchange.

Detection limits for the MPIC-NSl separator under isocratic conditions range from 50 ppb (butyric acid) to 50 ppm (stearic acid). Detection limits under gradient conditions are on the average 10% higher, due to higher baseline noise. Detection limits for RPIC-C_{18} are 10–20% higher, due to the solvent levels required for elution. Conductivity of the analyte decreases at higher organic solvent concentrations, because the solvents have lower dielectric constants than water, and the analyte ions have decreased ionic mobilities.

Baseline

In order to produce a relatively flat baseline several factors had to be considered: (a) balancing the specific conductance of the mixed eluent during gradient elution, (b) control of carbonate, which builds up and is eluted from the separator, and (c) control of the membrane-suppressor permeability during large changes in organic solvent concentration.

(a) With pre-acidified samples, a slightly acidic eluent serves to maintain ion exchange in the cation-exchange membrane of the AMMS-ICE and baseline stability. As the water content decreases in gradient elution, the conductivity measured decreases due to decreased ionic mobility/lower solvent dielectric constant. Therefore,

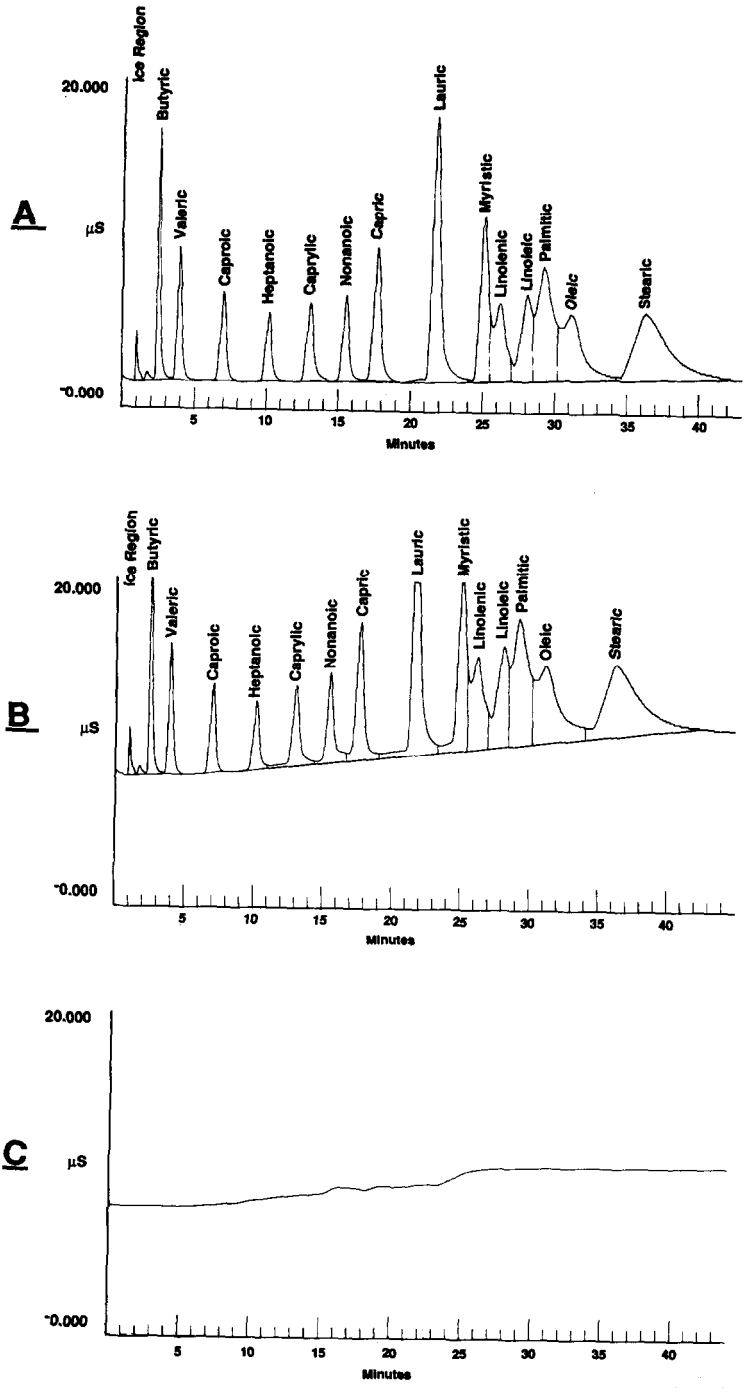

Fig. 4. Baseline subtraction in the gradient elution of butyric acid through stearic acid. (A) Chromatogram with baseline subtracted; (B) untreated chromatogram; (C) baseline; same conditions as Fig. 1.

based solely on conductivity, a higher acid concentration is used in eluent B than in eluent A in order to balance conductivity during the gradient. In practice, the eluents were prepared and the conductivity of each suppressed eluent was determined.

(b) The major effect of carbonate is derived from the adsorption of carbon dioxide from the eluent onto the reversed-phase packings. Each day, before analyses were started, a complete gradient elution was performed to purge the system of carbon dioxide which had permeated the PTFE connecting tubing overnight. During this preparatory elution a broad dip in the baseline was observed which was due to the adsorption of carbon dioxide from the eluents (where it contributes to conductivity as bicarbonate) onto the resin. This effect was more pronounced for the aromatic MPIC-NS1 resin than for the alkyl-bonded phase of RPIC-C_{18} and it was almost completely eliminated by degassing the eluents and maintaining a helium headspace on them. Sparging was found unnecessary.

(c) A third consideration was the increased permeability of the cation-exchange membrane of the AMMS-ICE at higher solvent levels. Although this has been minimized by using special membrane polymers, a minimal shift in the baseline still occurs at the end of the gradient due to non-stoichiometric transfer of regenerant through the membrane.

A method for producing more stable baselines is the use of a baseline subtraction program in the AI310 chromatography software (Fig. 4). Eluent preparation does not require matching the conductivity of eluent A and eluent B and the reso-

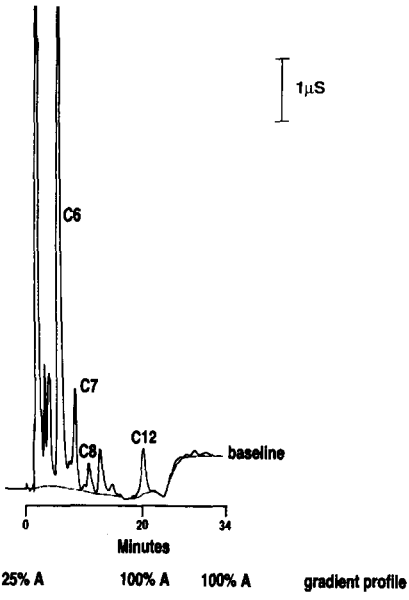

Fig. 5. Gradient ion-suppression separation of acid components of a commercial cologne. Identified peaks are labelled. Chromatographic conditions: separator, MPIC-NS1, 20 × 0.4 cm I.D.; eluent A, 60% acetonitrile and 24% methanol in 0.1 m*M* hydrochloric acid; eluent B, 20% acetonitrile and 5% methanol in 0.03 m*M* hydrochloric acid; flow-rate, 1.0 ml/min; temperature, 42°C; detection, electrical conductivity, 10 μS f.s.d.; chart speed, 0.25 cm/min; injection volume, 50 μl; dilution, 1:100; gradient profile indicated below chromatogram.

lution of analytes in the separation was easily detectable after baseline shifts were subtracted. This technique produces acceptable baselines without affecting quantitation, and eluent conductivities need not be balanced as previously described.

Other applications

Fig. 5 illustrates an aliphatic carboxylic acid profile of a commercial cologne. As the aroma of lower aliphatic acids is not as pleasant as that of the esters and aldehydes of the same moieties, the acid-specific detection of this method yields a profile of considerable interest.

Other types of acids separated by this method include sorbic acid and higher unsaturated and dicarboxylic acids larger than approximately 8 carbon atoms. The retention behaviour of dicarboxylic acids depends on the relative position of the carboxyl groups in the hydrocarbon chain. Retention is shorter as the carboxyl groups interrupt the hydrocarbon chain to form shorter chains and reduce the solvophobic surface area of the solute.

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