underivatized form. Fatty acids containing unusual structural features, such as cyclopropane rings or epoxy groups, are constituents of some edible vegetable oils and are suspected of being health hazards. Hence they have been analysed in foods by capillary GC as FAMEs. Such studies have provided a basis for identifying components in blends of vegetable oils with potential application to detecting adulteration. Similar studies have been carried out to determine brominated acid constituents in vegetable oils that are added to disperse flavouring constituents in citrus-based beverages. Clinical and epidemiological findings of the beneficial effects of fish oils have led to GC methods, effected on polar capillary columns, for determining ω-fatty acids such as eicosapentaenoic and docosahexaenoic acids in foods. *Trans* isomers of fatty acids have a possible link with cardiovascular diseases. Hence the occurrence of *trans* isomers in relatively large concentrations in margarines, shortenings and similar food products has stimulated development of methods for resolving geometrical isomers. The solution of this problem is very difficult by GC alone and has required the use of very long capillary columns and preliminary separation steps. It may be cited as an existing challenge to GC in the analysis of acids.

Conclusion

GC continues to be the method of choice for the analysis of acids because of its speed, efficiency and sensitivity. However, very complex mixtures still pose serious challenges. Future developments may entail use of shorter, narrower capillary columns for greater speed and, in conjunction with routine MS detection, for more definitive identification. Automation of sample preparation, perhaps in conjunction with microwave irradiation in lieu of conventional heating, will shorten derivatization times, relieve the tedium of manual manipulations and reduce total analysis times.

See also: **II/Chromatography: Gas:** Derivatization; Detectors: Mass Spectrometry; Detectors: Selective. **III/Oils, Fats and Waxes:** Supercritical Fluid Chromatography. **Triglycerides:** Liquid Chromatography; Thin Layer (Planar) Chromatography. **Volatile Organic Compounds in Water:** Gas Chromatography.

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Liquid Chromatography

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Introduction

The determination of carboxylic acids is important in many areas of application, including environmental samples, foods and beverages, and pharmaceutical and biological materials. The modes of high performance liquid chromatography (HPLC) used most frequently in the separation of carboxylic acids are ion suppression chromatography, reversed-phase ion interaction chromatography, ion exclusion chromatography and ion exchange chromatography.

In ion suppression chromatography, a buffer of appropriate pH is added to the mobile phase in order to suppress the ionization of the carboxylic acids so that they can be retained on nonpolar stationary phases and eluted in order of increasing hydrophobicity. Ion interaction (or ion pair) chromatography has been used for the separation of carboxylic acids under isocratic or gradient conditions and involves the complete ionization of the solute and the addition to the mobile phase of an ion interaction reagent (IIR), consisting of lipophilic ions of opposite charge to the solute. Ion exclusion chromatography (i.e. the separation of partially ionized carboxylic acids on a cation exchange stationary phase using amperometry, coulometry, ultra-

violet, refractive index and both suppressed and nonsuppressed conductivity detection) is the most commonly used mode of liquid chromatography for the separation of carboxylic acids. Finally, anion exchange chromatography can be used for the separation of carboxylic acids, after conversion of these species to anions. Detection is usually achieved by suppressed or nonsuppressed conductivity or by indirect photometry.

Ion Suppression Chromatography

Background

Ion suppression chromatography is a technique for the separation of ionizable solutes which functions by suppressing the ionization of these solutes, thus increasing their retention on nonpolar stationary phases. In the separation of carboxylic acids, an acidic buffer is added to the mobile phase to suppress the ionization of the solutes, which are then separated on nonpolar polymeric or silica-based (usually C_{18}) stationary phases.

This method is only applicable to those acids for which the ionization can be suppressed using buffers having pH values in the range 3–8, since the C_{18} stationary phases are unstable outside this pH range. However, these restrictions do not apply to the use of polymeric stationary phases, which can be used for the separation of a wider variety of solutes. The mobile phase is usually an acidic buffer of the appropriate pH. Commonly used buffers include phosphoric acid, sodium or potassium phosphate, sodium hydrogen sulfate, acetic acid and citric acid. Organic modifiers such as methanol or acetonitrile can also be added to the mobile phase to improve the separation.

Manipulation of Retention of Acids in Ion Suppression Chromatography

Solute retention results from solvophobic effects occurring between the mobile phase, the stationary phase and the solutes. For the separation of monocarboxylic acids, the pH of the mobile phase influences the retention behaviour according to the following equation:

$$k' = \frac{k_0 - k_{-1}\dfrac{K_a}{[H^+]}}{1 + \dfrac{K_a}{[H^+]}} \qquad [1]$$

where k_0 is the retention factor of the undissociated acid, k_{-1} is the retention factor of the conjugate base, and K_a is the acid dissociation constant in the mobile phase. This retention behaviour is illustrated in **Figure 1**, which shows the retention factor of a weak acid versus (pH–pK_a). The curve is sigmoidal in shape and the inflection point is located at the point where the pH of the mobile phase is equal to the pK_a of the solute in the mobile phase. At pH values substantially less than its pK_a value, the acid is present in its neutral form and has a large retention factor. Further decreases in mobile-phase pH show no effect on retention factor, since there will be no further change in the ionization of the acid. Conversely, mobile-phase pH values substantially greater than the pK_a value will result in complete ionization of the solute, leading to a small retention factor. At intermediate pHs, the solute charge, and hence its retention, will be dependent on the particular pH used and its proximity to the pK_a value.

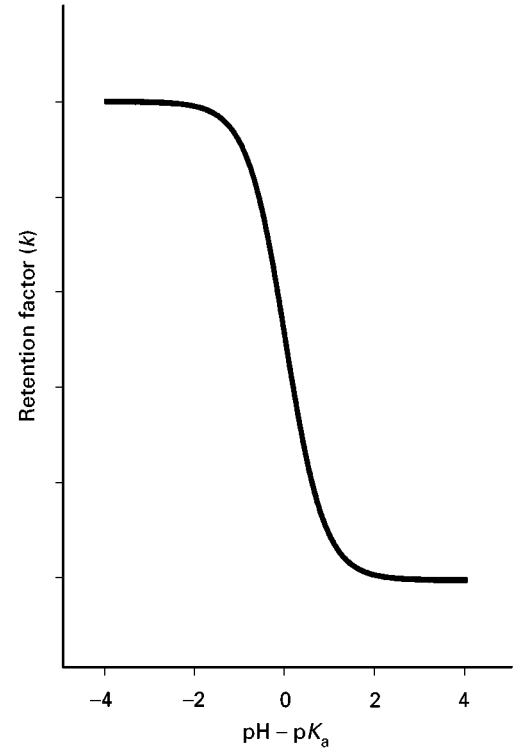

Figure 1 Plot of the retention factor of a weak monoprotic acid vs. (pH − pK_a).

In the case of dicarboxylic acids, the shape of the curve is largely determined by the difference between the two pK_a values. When pK_{a1} and pK_{a2} are very close, sigmoidal curves are obtained and the behaviour of dicarboxylic acids is almost the same as that of monocarboxylic acids. When the two pK_a values are well separated, the curve is a composite of two sigmoidal curves.

Both the ionic strength and organic modifier content of the mobile phase may be varied in order to manipulate retention in ion suppression chromatography. Increasing the ionic strength of the mobile phase causes an apparent increase in the dissociations, leading to a decrease in the retention factor.

This effect is more pronounced in nonaqueous media. In the approximate range of ionic strengths from 0 to 0.5 mol L^{-1}, the higher the ionic strength of the mobile phase, the greater the increase in ΔpK_a. The addition of organic modifiers influences retention behaviour in two ways. Firstly, increasing the organic modifier content of the mobile phase decreases the retention factor, as is generally the case in reversed-phase liquid chromatography. However, the apparent pK_a of the solute increases as organic modifier is added to the mobile phase, leading to an increase in the degree of ionization of the solute and therefore reduced retention.

Applications

The utility of ion suppression on polymeric stationary phases may be appreciated by considering the separation of the homologous series of aliphatic carboxylic acids. Neither ion exchange nor ion exclusion chromatography yields a complete separation of these species. However, ion suppression coupled with gradient elution and conductivity detection enables the separation of butyric through to stearic acid, as illustrated in **Figure 2**. The gradient used involved an increase in the percentage of organic modifier in the mobile phase and a decrease in mobile-phase pH. Carboxylic acids more hydrophilic than butyric acid are eluted in a single peak at the column void volume.

Ion Interaction Chromatography

Background

Ion interaction chromatography involves the addition of an ion interaction reagent (IIR) to the mobile phase. The IIR is usually a lipophilic ion of opposite charge to the analyte ions. In the case of the separation of carboxylic acids, cationic IIRs such as tetraalkylammonium salts are used.

The mechanism of ion interaction chromatography is considered to begin with the establishment of a dynamic equilibrium between IIR in the mobile phase and IIR adsorbed onto the stationary phase:

$$IIR^{+}_{(M)} \leftrightharpoons IIR^{+}_{(S)} \qquad [2]$$

where the subscripts M and S refer to the mobile and stationary phases. This results in the formation of an electrical double layer at the stationary phase surface. The adsorbed IIR ions constitute a primary layer of charge, to which is attracted a diffuse, secondary layer of oppositely charged ions. This secondary layer of charge consists chiefly of the counter-ions of the IIR. The double layer is shown schematically in **Figure 3**A. A solute anion can compete for a position in the secondary charged layer, from which it will tend to move into the primary layer as a result of electrostatic attraction and, if applicable, reversed-phase solvophobic effects. The presence of such a solute anion in the primary layer causes a decrease in the total charge of this layer, so to maintain charge balance a further IIR ion must enter the primary layer. The result is that solute retention involves the adsorption of a solute anion accompanied by the adsorption of an IIR ion, shown schematically in **Figure 3**B.

Typical stationary phases used in ion interaction chromatography include neutral poly(styrene-divinylbenzene) (PS-DVB) polymers and bonded silica materials with C_{18}, C_8, phenyl and cyanopropyl groups as the chemically bound functionality. The

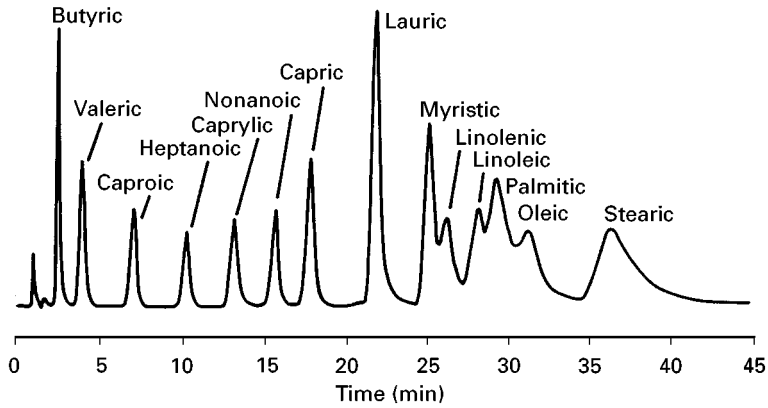

Figure 2 Gradient elution ion suppression chromatography of carboxylic acids, obtained on a polymeric reversed-phase column. A Dionex MPIC-NS1 column was used with a gradient of 100% mobile phase A ($t = 0$) to 100% mobile phase B ($t = 20$ min), with maintenance of mobile phase B after this time. Mobile phase A was 24% acetonitrile and 6% methanol in 0.03 mmol L^{-1} HCl; mobile phase B was 60% acetonitrile and 24% methanol in 0.05 mmol L^{-1} HCl with detection by suppressed conductivity. The baseline conductance for a blank gradient has been subtracted in the chromatogram shown. (Reprinted with permission from Slingsby RW (1986) Gradient elution of aliphatic carboxylic acids by ion chromatography in the ion-suppression mode. *Journal of Chromatography* 371: 373–382.)

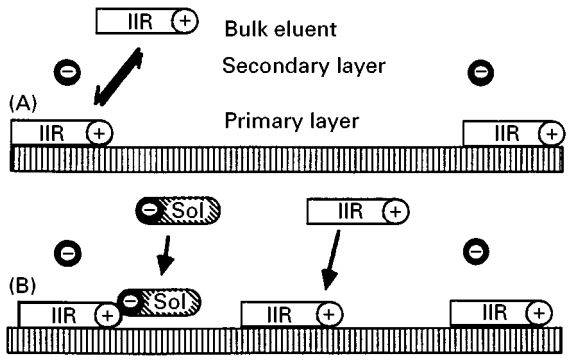

Figure 3 Schematic illustration of the ion interaction model for the retention of anionic solutes in the presence of a lipophilic cationic IIR. The solute and the IIR are labelled on the diagram. The long hatched boxes represent the lipophilic stationary phase, the black circles with negative charges represent the counter-anion of the IIR, whilst the white circles with positive charges represent the counter-cation of the solute. (Reprinted with permission from Haddad and Jackson, 1990.)

choice between stationary phases is usually based on considerations such as chromatographic efficiency, pH stability and particle size. However, the elution position of certain ions can differ between different stationary phases. Further factors to be considered in the selection of a stationary phase for ion interaction chromatography are specific interactions existing between the stationary phase and either the IIR or the solutes, and the role of residual silanol groups on silica-based stationary phases.

The most important component of the mobile phase in ion interaction chromatography is the IIR itself. The requirements of the IIR are that its charge is unaffected by mobile-phase pH, it has suitable lipophilicity to permit adsorption onto nonpolar stationary phases, and it is compatible with other mobile-phase components and the desired detection system.

In the separation of carboxylic acids by dynamic coating ion interaction chromatography, moderately hydrophobic strong base cations, such as tetrabutylammonium ions, are used as the IIR. The IIR is present at a constant, specified concentration in the mobile phase in order to maintain a desired concentration of IIR on the stationary phase. The lipophilicity of the IIR governs the degree to which it is adsorbed onto the stationary phase, which in turn governs the effective ion exchange capacity of the column and hence the retention times of solute ions.

An alternative to the above method is permanent coating ion interaction chromatography, where a very lipophilic IIR is used initially to equilibrate the stationary phase and is then removed from the mobile phase in the separation step. The coating persists for long periods of subsequent use. Permanent coating of the column is achieved by passing a solution of the IIR (approximately 10^{-3} mol L^{-1}) in dilute (5%) methanol or acetonitrile through the column for about 20 min. The purpose of the organic solvent is to wet the surface of the lipophilic stationary phase in order to improve binding of the IIR.

The counter-ion of the IIR is important in dynamic coating ion interaction chromatography of anionic solutes. This counter-ion usually acts as an ion exchange competing anion and is responsible for the elution (and in many cases also the detection) of the solute anions. The nature of the counter-ion determines the type of separation which is required and the mode of detection applicable.

Manipulation of Retention of Acids in Ion Interaction Chromatography

The parameters which affect the adsorption of the IIR onto the stationary phase and hence the retention of solutes include the nature of the stationary phase, the lipophilicity of the IIR, the concentration of the IIR in the mobile phase, the ionic strength of the mobile phase, the nature and concentration of any competing ion added to the mobile phase, and the mobile-phase pH.

The first four of these factors will determine the surface concentration of the IIR on the stationary phase, and hence the surface charge density and the effective ion exchange capacity. The higher the surface concentration of IIR, the greater the solute retention. Thus, retention times will increase as the lipophilicity of the IIR is increased and as the percentage of modifier in the mobile phase is decreased. Solute retention generally increases with the concentration of IIR in the mobile phase, but there is a threshold concentration above which solute retention decreases with further increase in the concentration of IIR. The stationary phase becomes saturated with IIR and any further addition to the mobile phase results in decreased retention because of the increased concentration of the IIR counter-ion.

The nature and concentration of any competing ion added to the mobile phase will determine the retention times and elution order for solute ions. Increases in the concentration of the mobile phase competing ion will result in decreased solute retention, in the same manner as observed for ion exchange separations. Finally, the mobile phase pH may influence the charges on the competing ion and the solutes. An example of this effect is the influence of pH in an ion interaction chromatographic system using tetrabutylammonium as the IIR and phthalate as the competing anion. Increases in mobile-phase pH over the range 4.0–6.0 cause a decrease in the solute retention as a result of increased ionization of phthalate,

leading to the formation of a strong, divalent competing anion.

Applications

Carboxylic acids are usually separated by ion interaction chromatography using a reversed-phase column with quaternary ammonium salts as the IIR and water–methanol or water–acetonitrile as the mobile phase. The more lipophilic the quaternary ammonium ion, the more the acid is retained on nonpolar stationary phases. Such separation systems have been used for the determination of ascorbic acid in fruits and vegetables, as well as carboxylic acids in beverages such as wine, beer and fruit juices.

Gradient elution ion interaction chromatography is also possible. The concentration of the organic modifier or the pH of the mobile phase may be varied to optimize the separation. **Figure 4** shows an example of the separation of carboxylic acids on a reversed-phase column by gradient elution using tetrabutylammonium hydroxide as the ion interaction reagent.

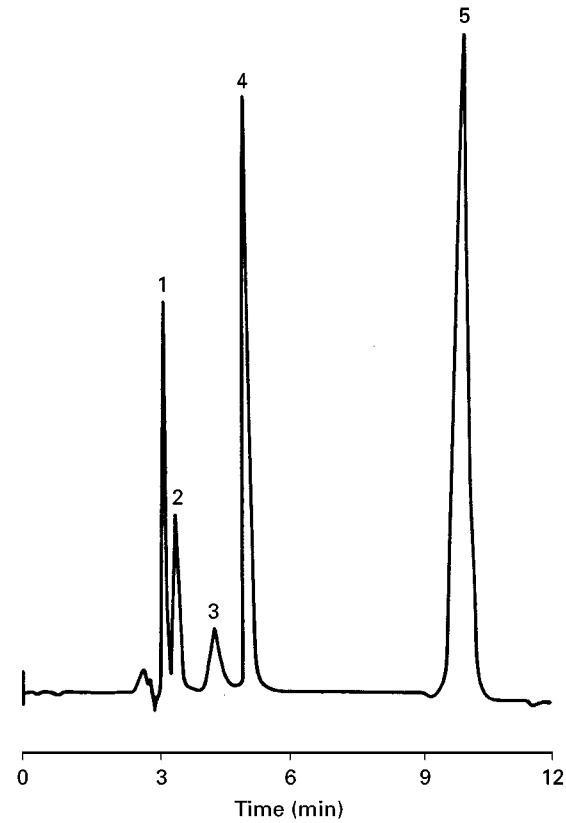

Figure 4 Ion interaction chromatography of carboxylic acids on a LiChrosorb RP-8 column with a mobile phase of aqueous tetrabutylammonium hydroxide (1 g L^{-1}) and methanol using gradient elution. Detection was at 254 nm. Carboxylic acids are: 1, ascorbic; 2, oxalic; 3, pyruvic; 4, fumaric; 5; maleic. Chromatogram courtesy of Alltech Chromatography Catalog (1997) 610.

Ion Exclusion Chromatography

Background

Ion exclusion chromatography was first introduced by Wheaton and Bauman in 1953. In this mode of chromatography, the negatively charged, partially dissociated carboxylic acids are separated on cation exchange resins comprising silica or a polymer with chemically bound anionic sulfonate or carboxylate functional groups. This is the opposite situation to that occurring in normal ion exchange chromatography.

The chromatographic system consists of three phases: the mobile phase, the resin phase and an occluded liquid phase. The mobile phase passes through the interstitial volume existing between the beads of the ion-exchange resin. An occluded liquid phase is formed by mobile phase that becomes trapped within the pores of the resin phase. This trapped liquid acts as the stationary phase of the system. The resin phase is the solid resin network and functionlized groups, which can be considered to be a semipermeable ion-exchange membrane separating the flowing mobile phase from the stationary occluded liquid inside the resin. The three phases are illustrated schematically in **Figure 5**.

Fully ionized species (A^-) are completely excluded from the interior of the resin due to electrostatic repulsion by the fixed anionic functional groups, in accordance with the Donnan exclusion effect. Therefore, these species are not retained and are eluted at the column void volume. Partially ionized species like weak carboxylic acids ($pK_a = 2.5–6.5$) permeate selectively into the stationary phase (the occluded liquid trapped within the pores of the resin), resulting in some retention of these species, which are then eluted some time later than the fully ionized solutes.

Ion exclusion chromatography was first performed on large particle size, high capacity, fully functionalized PS-DVB polymers. However, separations have also been performed on ploymethacrylate copolymer resins, as well as on silica. Separations of carboxylic acids by modern ion exclusion chromatography are usually carried out on a cation exchange column containing sulfonated functional groups ($-SO_3^-$) or mixed sulfonate and carboxylate functional groups, with the resin most commonly being used in the hydrogen form.

Ion exclusion columns are usually quite large because most sample species are eluted with retention volumes intermediate between the interstitial volume (V_0) and $V_0 + V_i$, where V_i is the occluded (or intraparticle) liquid volume. Large columns contain more

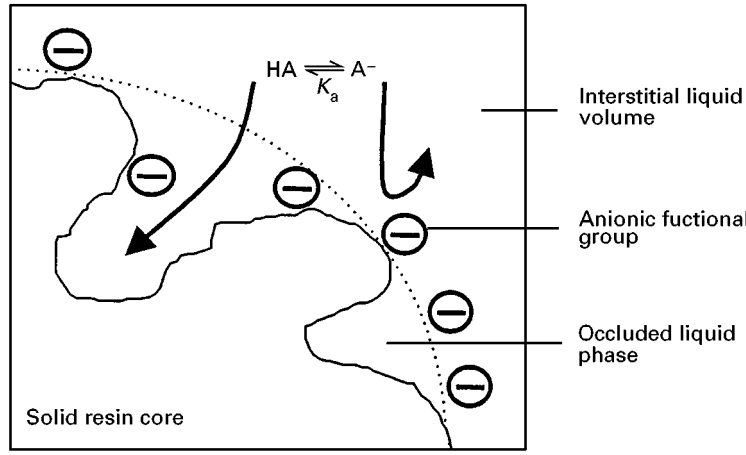

Figure 5 Schematic representation of the ion exclusion mechanism, showing the retention of a weak acid (HA) in the occluded liquid phase and the exclusion of the acid anion (A^-).

resin, thus increasing the amount of occluded liquid and hence also the capacity of the stationary phase. A typical column would be 30 cm in length, with an internal diameter of 7 mm or more.

The mobile phases used in ion exclusion chromatography are often very simple in composition. Most of the early work was performed using water as the mobile phase. However, water has limitations as stronger acids or bases show too great a degree of ionization to be retained and for weaker acids such as carboxylic acids, the separation is slow and the peaks are unsymmetrical.

In modern ion exclusion chromatography it is common to use dilute solutions of strong mineral acids for the elution of carboxylic acids. The dilute mineral acid solution suppresses the ionization of the acids so that they can partition into the occluded liquid phase, resulting in longer retention times and better separation between the stronger carboxylic acids. The choice of acid used in the mobile phase is usually determined by the form of detection being used. Sulfonic acids are used for conductivity detection without suppression since mineral acids have a high background conductance. Sulfuric acid is often used with ultraviolet detection and hydrochloric acid is used with conductivity detection after the mobile phase has been passed through a suitable suppressor. Weak acids such as benzoic acid, phosphoric acid, salicylic acid and carbonic acids have also been used as mobile phases in ion exclusion chromatography when conductivity detection is utilized.

Manipulation of Retention of Acids in Ion Exclusion Chromatography

The dominating factor which determines retention is the degree to which the acid is ionized. Separation is based on the electrostatic repulsion between the solute ions and fixed functional groups of the resin. Therefore, ionic species are excluded from the stationary phase while partially ionized or uncharged species partition between the mobile phase and the occluded liquid within the resin pores. Assuming this is the only mechanism, the solute retention time, t_R, is given by:

$$t_R = t_0 + D_A t_i \qquad [3]$$

where t_0 is the time taken for the interstitial volume of mobile phase (i.e. the volume of mobile phase flowing between the resin beads) to be eluted, t_i is the time taken for the volume of occluded liquid inside the pores of the resin to be eluted, and D_A is the distribution constant for the solute between the interstitial mobile phase and the occluded liquid. The value of D_A is dependent on the degree of ionization of the solute.

If a solute cannot enter the stationary phase because it is fully ionized (ion exclusion), $D_A = 0$. Therefore, the retention time of fully ionized solutes is equal to t_0, whilst for an uncharged solute which is free to enter the stationary phase, $D_A = 1$, and its retention time is equal to t_i. Thus, in the separation of carboxylic acids, the retention times of the acids depend on their first dissociation constants (pK_a). Since the fraction of the ionized solute molecules increases with increasing pH, an increase in the mobile phase pH will reduce the retention time.

The retention times of monocarboxylic acids larger than acetic acid, and dicarboxylic acids larger than succinic acid, show an increase with increasing carbon number, even for solutes with similar pK_a values. This increased retention can be attributed to hydrophobic adsorption of the solutes on to the neutral, unfunctionalized regions of the polymeric resin, in a manner similar to that observed in reversed-phase

HPLC. Hydrophobic adsorption effects can be expected to increase in magnitude as the alkyl chain length of the solute is increased, leading to larger retention times. In the case of aromatic acids, the interaction of π-electrons of the benzene ring of the acid with those of the ion exchanger (such as styrene-divinylbenzene packing materials) leads to much higher retention times than expected from their pK_a values. The existence of hydrophobic adsorption effects creates the possibility for manipulation of solute retention by adding typical reversed-phase organic modifiers, such as methanol or acetonitrile, to the mobile phase.

Ion exclusion chromatography is usually carried out on a high-capacity sulfonated PS-DVB resin in the H^+ form. However, recently work has also been carried out using a polymethacrylate resin in the H^+ form with carboxylate functional groups, bare silica (where the silanol group on the surface of the silica acts as the anionic functional group) and also on silica-based cation exchangers functionalized with alkylsulfonic acid or phenylsulfonic acid groups. Since silica gel is chemically stable and inert to organic solvents, silica-based cation exchangers offer the advantage that high concentrations of organic modifiers can be used. Also, aromatic acids which adsorb strongly on to PS-DVB resin due to π-electron interactions between the aromatic ring and the solid resin network are eluted earlier when using a silica gel column.

Other factors which play a part in the retention process of carboxylic acids in ion exclusion chromatography include the addition of other mobile phase modifiers such as polyols, sugars and inclusion compounds (e.g. β-cyclodextrin), as well as resin characteristics such as the pore size, the degree of cross-linking, the ion exchange capacity and the ionic form of the resin.

Applications

The separation of carboxylic acids is the most common application of ion exclusion chromatography. When coupled with spectrophotometric detection at low wavelength (e.g. 210 nm), ion exclusion chromatography yields excellent separations and relatively clean chromatograms for a wide variety of complex sample matrices, such as urine, plasma, foods and beverages and pharmaceuticals. **Figure 6** shows a chromatogram for a urine sample, without sample pretreatment. Ion exclusion chromatography has also found increasing use for the determination of anions of weak inorganic acids. It is especially attractive as an adjunct to ion exchange chromatography since the selectivities obtained by these two techniques are quite different. Solutes such as fluoride, carbonate, cyanide, borate and sulfite have been determined using this approach. Interference from strongly ionized species is minimal because these solutes are unretained and appear at the column void volume. Ion exclusion chromatography can therefore readily separate weakly ionized solutes in samples containing high concentrations of ionic species, e.g. sea water and oil reservoir brines.

Ion Exchange Chromatography

Background

Ion exchange chromatography of carboxylic acids can be performed using an anion exchange stationary phase. The capacity of this anion exchanger is important since the capacity needs to be sufficiently high to separate carboxylic acids of similar charge, but low enough for the ionic strength of the mobile phase to permit the use of conductivity detection. The development of new high-efficiency, low- and medium-capacity columns combined with a new generation of micromembrane suppressors capable of handling concentrated mobile phases has made the determination of carboxylic acids by anion exchange chromatography a practical proposition.

One disadvantage of anion exchange chromatography is that groups of mono-, di- and tricarboxylic acids must be analysed separately. However, the use of gradient elution (water, sodium hydroxide and methanol) has made it possible to separate these compounds in a single run, as well as simultaneously separating inorganic anions (**Figure 7**).

Ion exchange chromatography of carboxylic acids has been performed on anion exchange resins in the hydroxyl, carbonate, sulfate, chloride, nitrate, formate, acetate or borate form. The mobile phase usually consists of an alkaline solution such as sodium hydroxide or sodium carbonate and sodium hydrogen carbonate, with detection achieved using a suppressor column and conductivity. Solutes are usually low-molecular-weight, saturated or unsaturated acids and hydroxy acids. Factors which affect retention include molecular dimensions, pK_a and specific adsorption of organic acid molecules on the organic matrix of the ion exchanger.

Manipulation of Retention of Acids in Ion Exchange Chromatography

Apart from the usual electrostatic effects which govern retention in ion exchange chromatography, one of the main factors affecting retention of carboxylic acids is the molecular adsorption of the acid on the anion exchange resin. The presence of

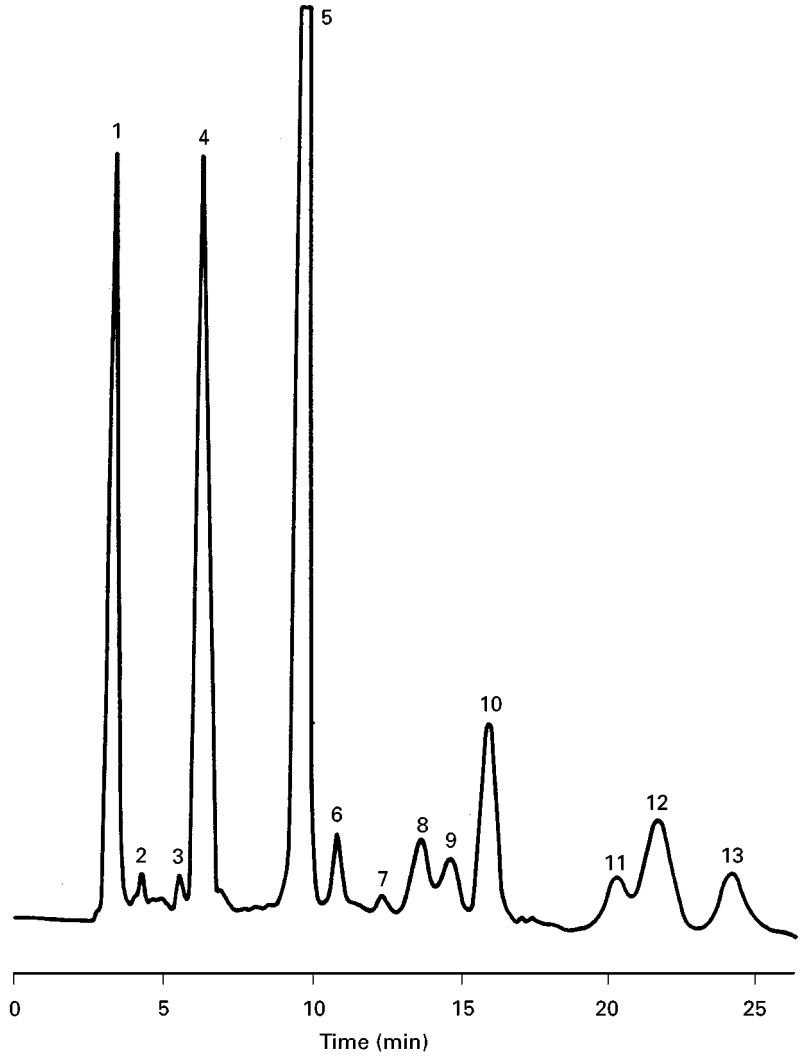

Figure 6 Analysis of human urine using ion exclusion chromatography. An Interaction ORH-801 column was used with a mobile phase comprising 10 mmol L^{-1} H_2SO_4 containing 10% methanol. Detection was at 254 nm. Solute identities: 1, oxalic acid; 2, oxaloacetic acid; 3, α-ketoisovaleric acid; 4, ascorbic acid and α-keto-β-methyl-*n*-valeric acid; 5, β-phenylpyruvic acid; 6, uric acid; 7, α-ketobutyric acid; 8, homoprotocatechuic acid; 9, unknown; 10, unknown; 11, hydroxyphenylacetic acid; 12, *p*-hydroxyphenyllactic acid; 13, homovanillic acid. (Reprinted with permission from Woo DJ and Benson JR (1984) *American Clinical Products Review* Jan: 20.)

double bonds in carboxylic acids leads to higher retention factors, probably due to stronger hydrophobic interactions of the double bond with the polymeric matrix of the resin and also stronger electrostatic interactions between ionic groups. The presence of hydroxy groups in carboxylic acids increases the polarity of the acid and results in stronger interactions both with the aqueous mobile phase (leading to lower retention factors for the acids) and any alkanol substituent of the quaternary ammonium functional group of the anion exchange resin (leading to higher retention factors). Since the adsorption of carboxylic acids plays such an important role in the retention of these acids in ion exchange chromatography, the pK_a values of the acids are also important, as are any parameters which influence the dissociation of the acid, such as the pH of the mobile phase and the concentration of any organic solvent. Additionally, the pH of the mobile phase may also affect its elution strength and hence affect retention as well.

Applications

Compared to other separation methods such as ion exclusion chromatography, anion exchange provides improved selectivity within the three groups of acids: mono-, di- and tricarboxylic acids. This is particularly true among the stronger acids such as most of

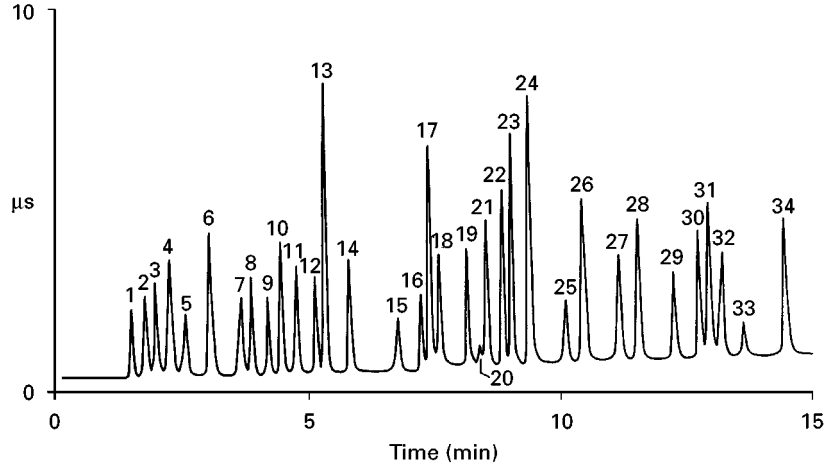

Figure 7 Example of a gradient separation of inorganic and organic acid anions by anion exchange chromatography. A Dionex IonPac AS11 column was used with a mobile phase comprising water and NaOH as a gradient. Detection was by conductivity in the suppressed mode. Solute identities: 1, isopropylethylphosphonic acid; 2, quinate; 3, fluoride; 4, acetate; 5, propionate; 6, formate; 7, methylsulfonic acid; 8, pyruvate; 9, chlorite; 10, valerate; 11, monochloroacetate; 12, bromate; 13, chloride; 14, nitrite; 15, trifluoroacetate; 16, bromide; 17, nitrate; 18, chlorate; 19, selenite; 20, carbonate; 21, malonate; 22, maleate; 23, sulfate; 24, oxalate; 25, ketomalonate; 26, tungstate; 27, phthalate; 28; phosphate; 29, chromate; 30, citrate; 31, tricarballylate; 32, isocitrate; 33, *cis*-aconitate; 34, *trans*-aconitate. Chromatogram courtesy of Dionex Corporation Product Selection Guide (1997–98) 48.

the di- and tricarboxylic acids. Of the di- and tricarboxylic acids which are in the Krebs cycle or are commonly found in foods, there are only two groups of co-eluting acids: malic and malonic, and isocitric and *cis*-aconitic. Another advantage of anion exchange separation is the possibility of simultaneous determination of some inorganic ions, such as fluoride, chloride, and sulfate, with the carboxylic acids.

Applications of anion exchange chromatography of carboxylic acids include the quantification of short chain organic acids and inorganic anions for the biotechnology, chemical or power industries, the separation of the Krebs cycle acids in foods and beverages, and also the separation of aromatic carboxylic acids in chemical process solutions and as impurities in precursors in the polymer industry.

Conclusion

Four modes of HPLC used in the separation of carboxylic acids have been discussed. Ion suppression chromatography, using a buffer to suppress the ionization of the acids, is the simplest separation system for carboxylic acids. Ion interaction chromatography offers the greatest variety of parameters to alter the selectivity of the separation system by changing the properties of the ion interaction reagent. Ion exclusion chromatography is the most commonly used method in the separation of carboxylic acids due to its compatibility with a wide range of sample matrices. Ion exchange chromatography provides improved selectivity within groups of acids but the technique requires the use of gradient elution.

See also: **I/Ion Exchange. II/Chromatography: Liquid:** Mechanisms: Ion Chromatography. **Ion Exchange:** Theory.

Further Reading

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