Ion Exclusion Chromatography of Aromatic Acids

Fotouh R. Mansour¹, Christine L. Kirkpatrick² and Neil D. Danielson²*

¹Department of Pharmaceutical Analytical Chemistry, Tanta University, Egypt, 31111 and ²Department of Chemistry and Biochemistry, Miami University, Oxford, OH, 45056

*Author to whom correspondence should be addressed. Email: danielnd@muohio.edu

Received 2 October 2012; revised 24 December 2012

The determination of aromatic acids by ion exclusion chromatography is challenging due to peak tailing and the long retention time of hydrophobic solutes. This review discusses the retention mechanisms and the factors affecting retention, eluents and detection methods used in ion exclusion chromatography of aromatic acids such as mono-, di-, tri- and tetra-carboxylic acids, amino acids, sulfonates and phenol. In addition, the different approaches used to improve the chromatographic separation of these compounds are also discussed. These approaches include introducing an internal gradient of the ionic strength, using vacancy ion exclusion chromatography, employing a hydrophobic cation exchange resin or adding a modifier such as heptanol to the dilute sulfuric acid mobile phase. The applications of these methods in the analysis of aromatic acids are provided with a table summarizing the stationary phases, the mobile phases and the detection methods.

Introduction

Ion chromatography (IC) represents a range of techniques used for the separation of ionic and low molecular weight acids or bases (1) based on their electrostatic interaction with the charged stationary phase. The separation mechanisms in IC include (i) ion exchange due to attraction between the oppositely charged analyte and stationary phase, and (ii) ion exclusion due to repulsion of the similarly charged analyte and stationary phase (2). The abbreviation "IELC" is usually used to refer to ion exclusion chromatography, although it has sometimes been used for ion exchange chromatography (3), which is often abbreviated IEX. In this review, IELC is used to refer to ion exclusion chromatography to avoid this overlap.

Since its introduction by Wheaton and Bauman in 1953 (4), IELC has been efficiently used to separate not only short chain aliphatic organic acids (C1–C5), but also sugars and alcohols. IELC has several advantages: (i) the eluent is water or aqueous acid or base with little or no organic modifier, which offers environmental and economic benefits in addition to compatibility with aqueous sample matrices; (ii) difficult separations such as simple aliphatic carboxylic acids, e.g., formate, acetate, propionate and butyrate are possible; (iii) it is suitable for samples of high ionic strength because strong acid anions are eluted in the void volume; (iv) it is compatible with a wide range of detection methods; (v) the columns can be ion exchange columns, reversed-phase or normal-phase columns, or dynamically modified reversed-phase with an ion pairing agent (5); and (vi) it is stable for long-term analysis of complex samples such as wine or mustard (6–8).

On the other hand, IELC suffers from some limitations. One of these is the determination of hydrophobic analytes such as aromatic acids, because the peaks are tailing and highly retained due to the π–π interaction of these analytes with the aromatic rings of the polymeric stationary phase (9). Several attempts have been made to overcome this problem and to develop an efficient method for the separation of aromatic acids. The aim of this report is to discuss these methods and their applications for long chain conjugated aliphatic acids and aromatic acids. Very few review articles have been reported for IELC. In 1991, Fritz reviewed the principles of IELC and summarized selected applications involving inorganic anions, short chain carboxylic acids, sugars, polar molecular compounds and even an indirect approach for water analysis (10). Approximately 10 years later, a review about short chain (C≤6) aliphatic carboxylic acids was published by Fischer (11). This review complements Fischer’s work and refers to the most recent advances in this field, updating IELC research and applications.

Discussion

IELC versus other ion chromatographic techniques

The separation of ionic or partially ionized species can be conducted by various techniques: ion exchange, ion exclusion and ion pair (or ion interaction) chromatography. In IEC, charged solute molecules are separated by differential adsorption to oppositely charged porous resin particles (8, 12). Compared to ion exchange, ion exclusion is preferred for the determination of weak organic acids in the presence of a high ionic strength sample matrix, such as the determination of organic acids in sea water, because the chloride ion will elute in the void volume. If ion exchange is used, the high chloride ion concentration will cause serious interference (13).

Ion pair chromatography involves a reversed-phase column and an ion pairing reagent that can adsorb to the stationary phase and act as a site of ion exchange, or combine with the ionic solute to form a neutral species that can interact with the hydrophobic stationary phase (14, 15). However, the dynamic modification of a reversed-phase column can shorten the lifetime of the silica based stationary phase, the equilibration of the mobile phase can be lengthy and the reproducibility depends on the stability of dynamic modification (16). Minimal, if any, organic solvent modifier can be tolerated in the mobile phase. On the other hand, polymer based ion-exchange resins, which are generally used as stationary phases in IELC, are very stable against extreme mobile phase conditions. Techniques other than IC have been employed to separate different aromatic acids. These techniques include reversed-phase liquid...
chromatography (RPLC) (17, 18), gas chromatography (GC) (19, 20) and capillary electrophoresis (CE) (21). In RPLC, gradient elution is required to separate structurally related aromatic acids (17) and organic solvents have to be incorporated in the mobile phase (otherwise, the elution power is too weak) (18). GC has also been used, but headspace microextraction (19) or chemical derivatization (20) is required before performing the analysis. The separation of aromatic acids by different CE techniques (21–24) has been reported, but the reproducibility problems and limited capabilities in preparative applications compromise these electrophoretic methods.

**Mechanisms of separation in IELC**

Studying the retention mechanisms in IELC is important to understand the problems associated with the separation of long chain or aromatic acids and to propose possible solutions. Upon using a cation exchange resin to separate acids by IELC, the negatively charged functional groups on the resin form a shield known as the Donnan membrane and part of the mobile phase will be occluded or trapped in the interior of the resin and act as a stationary phase. At this time, the Donnan membrane separates the mobile phase from the stationary phase. When a fully negatively charged ion (A<sup>-</sup>) approaches the resin, it will be repelled by the Donnan membrane and elute in the void volume, as shown in Figure 1A (25). However, when a hydrophobic neutral molecule (HA) approaches the resin, it can penetrate the hypothetical Donnan membrane, enter the occluded stationary phase, and get adsorbed on the unfunctionalized part of the resin. These molecules will be extremely retained on the column. Molecules that are partially dissociated will show retention between that of fully ionized and non-polar solutes. Thus, analytes are separated by (i) exclusion or repulsion if they are ionized and have the same charge as the resin, or (ii) adsorption if they are non-polar or partially ionized.

This mechanism could not explain the peak fronting that occurs for all analytes when water is used as an eluent. In addition, if the ion exchange polymer is fully sulfonated, the number of sites available for adsorption will be small because the adsorption sites in this theory are not charged. Novic and Haddad (25) proposed another mechanism in which the pores are also charged, but the charges are neutralized by occluded water, which acts as a stationary phase (Figure 1B). Two processes control the retention of solutes in this case: (i) the diffusion process due to the concentration gradient of the solute between the mobile phase and the occluded stationary phase, and (ii) repulsion effects between the solute and the similarly charged stationary phase. This explanation excluded the hydrophobic adsorption mechanism and suggested an additional retention process that has not been yet identified. Most articles use the original mechanism to explain the long retention of hydrophobic analytes.

![Figure 1. Schematic representation of the mechanisms for IELC: current mechanism (A); alternative mechanism (B). Reprinted from Journal of Chromatography A, 1118, Novic, M., Haddad, P.R., Analyte-stationary phase interactions in ion-exclusion chromatography, Pages 20 & 25, Copyright (2006), with permission from Elsevier.](https://academic.oup.com/chromsci/article-abstract/51/7/655/472405)
Factors affecting retention in IELC
Retention in IELC has been affected by many factors (1). These factors can be classified into four categories: (i) factors related to the solute, such as the molecular size, structure and ionization constant; (ii) factors related to the mobile phase, such as pH, ionic strength, mobile phase modifiers, eluent type and eluent concentration; (iii) factors related to the stationary phase, such as functional group, ionic form, column capacity and the degree of cross-linking; and (iv) factors related to chromatographic conditions, such as temperature, flow rate and injection volume. Among these factors, the degree of ionization is the most important one affecting retention in IELC. As the solute becomes more ionized, the retention time decreases. The degree of ionization changes with the ionization constant, eluent pH and mobile phase composition. For solutes with multiple ionization constants, the retention is related to the first ionization constant. The elution order of geometrical isomers can even be predicted from the pKa values, as shown in Table I, in which isomers with a smaller pKa elute first (26).

Methods used to improve the performance of IELC for aromatic acids
Sample-induced internal gradient of ionic strength
Because the ionic strength is one of the factors that affect the retention of aromatic acids in IELC, Šlais (5) developed a method to generate an internal gradient by decreasing the ionic strength. By injecting a concentrated ammonium sulfate solution (pH 7) into a mobile phase of dilute ammonium sulfate, an internal gradient of decreasing ionic strength was obtained. This gradient profile was induced because the solute equilibration between the mobile and stationary phases was faster than the washing out of ammonium sulfate. By changing the concentration of ammonium sulfate that was injected, the gradient time and steepness could be controlled. Interestingly, this work was performed on a C18 silica column dynamically modified with sodium dodecyl sulfate (SDS) instead of using a regular ion exchange column. Figure 2 shows the effect of the concentration of ammonium sulfate on the induced gradient profile used for separation of different aromatic sulfonates.

Vacancy ion exclusion chromatography
Vacancy ion exclusion chromatography (VIELC) was developed by Tanaka et al. in 2002 (27). In this technique, the sample is used as the mobile phase and water is to be injected in the cation exchange column. Negative or vacant peaks appear for each analyte and the retention times are the same as those obtained by conventional IELC when water is used as the mobile phase.

In conventional IELC with conductimetric detection, a vacant or eluent dip peak is observed due to adsorption of the eluent acid, as shown in Figure 3A. By using the acid sample as a mobile phase, the solutes in the running eluent will equilibrate between the stationary phase and the mobile phase, leading to a constant detector response and a stable baseline. By injecting water, this established equilibrium will be disturbed and the solutes will elute one after the other according to their retention order on the employed stationary phase (Figure 3B).

VIELC has been applied using both strong (27) and weak (28–31) cation exchange resins and the applications were extended to haloacids, inorganic acids and aliphatic amines using a weak anion exchange resin. As for aromatic acids, VIELC was employed to determine three different aromatic carboxylic acids: naphthalenetetracarboxylic acid (NTCA), phthalic acid (PA), and benzoic acid (BA) dissolved in either pure water or an aqueous 10% methanol solution. The added methanol did not change the peak retention, but improved the baseline stability and enhanced the peak shape and resolution, as shown in Figures 4A and 4B. The analytical data for these three acids are shown in Table II.

Hydrophilic cation exchange resin
To avoid the π–π interaction between the aromatic solutes and hydrophobic stationary phase, Ohta et al. (32) used
unmodified silica gel (Develosil 30-5) to separate different benzenecarboxylic acid derivatives. The silanol group in the Develosil 30-5 can act as a weak cation exchanger, even at pH 2.0. In addition, the strong hydrophilic properties of the silica overcame the problem of strong solute adsorption due to hydrophobic interactions.

The retention volumes obtained by IELC using the unmodified silica gel were much less than those obtained by polymethacrylate-based weak (TSKgel OA-Pak A) or styrene-divinylbenzene co-polymer (PS/DVB) based strong (TSKgel SCX) cation exchangers, as shown in Figures 5A, 5B and 5C. The retention of benzenecarboxylic acid was decreased by increasing the pH of the eluent due to increased dissociation, which accentuates the exclusion effect. However, the retention of phenol was almost independent of the pH because phenol is primarily retained by adsorption.

Not all of the commercially available silica gel columns have produced good results with aromatic compounds, which was attributed to the inconsistency in the composition of the metal impurities. By analyzing metal impurities in different silica gel columns, it was concluded that the columns that contain high Al content (4.9 μmol/g silica gel or more) displayed cation exchange characteristics in strongly acidic conditions (33). To avoid the irreproducibility of the stationary phase composition in the commercially available columns, Ohata and Tanaka (34) used laboratory made aluminum-modified silica gel (Al-silica) to separate various carboxylic acids. As shown in Figure 6, the separation of eight different benzenecarboxylic acids and phenol generally improved as the mobile phase concentration of sulfuric acid increased; the optimum concentration was 2.5 mM H₂SO₄. The enhancement in the performance of the Al-silica was because the acidity of the silanol group could be improved by adding certain polyvalent cations (35–38).

The retention of benzenecarboxylic acid was decreased by increasing the pH of the eluent due to increased dissociation, which accentuates the exclusion effect. However, the retention of phenol was almost independent of the pH because phenol is primarily retained by adsorption.

Not all of the commercially available silica gel columns have produced good results with aromatic compounds, which was attributed to the inconsistency in the composition of the metal impurities. By analyzing metal impurities in different silica gel columns, it was concluded that the columns that contain high Al content (4.9 μmol/g silica gel or more) displayed cation exchange characteristics in strongly acidic conditions (33). To avoid the irreproducibility of the stationary phase composition in the commercially available columns, Ohata and Tanaka (34) used laboratory made aluminum-modified silica gel (Al-silica) to separate various carboxylic acids. As shown in Figure 6, the separation of eight different benzenecarboxylic acids and phenol generally improved as the mobile phase concentration of sulfuric acid increased; the optimum concentration was 2.5 mM H₂SO₄. The enhancement in the performance of the Al-silica was because the acidity of the silanol group could be improved by adding certain polyvalent cations (35–38). A laboratory-made zirconium-modified silica gel (Zr-silica) column was also used as a cation exchanger to separate different benzenecarboxylic acids (39). A mobile phase of 10 mM tartaric acid was used at pH 2.5 to separate seven different aromatic acids in 20 min. A range of detection limits as low as 0.036–1.1 μM was achieved for all tested analytes.

**Organic modifiers**

Li and Fritz (40) discussed the use of organic modifiers to improve the separation of organic acids and bases in liquid chromatography. Solvents such as methanol (41–45), ethanol (46), acetone (47) and acetonitrile (48) have been extensively used to decrease the retention of hydrophobic solvents. However, very few reported IELC articles have used organic modifiers to improve the separation of aromatic carboxylic acids. When 1% (v/v) acetonitrile was added to an eluent of 10 mM methanesulfonic acid, a run time of 95 min was required to elute six different aromatic acids (48). Ohta et al. (49) studied the effect of chain length of the added alcohol on the retention time and found that long chain alcohols such as pentanol and heptanol were more effective in enhancing peak symmetry and decreasing the retention time of aliphatic carboxylic acids. Longer chain alcohols were not usable due to their limited miscibility.

**Figure 3.** Chromatograms of six aliphatic carboxylic acids: conventional chromatography (A); VIELC (B). Column: TSKgel SCX (150 × 6 mm i.d.); column temperature: 35°C; mobile phase: 2 mM benzoic acid at pH 3.32 (Figure 3A), mixture of 8 mM oxalic acid, 8 mM formic acid, 40 mM AA, 40 mM propionic acid, 92 mM butyric acid and 120 mM valeric acid; flow rate: 1.0 mL/min; detection: conductivity; injection volume: 100 μL; peaks: 1, oxalic acid; 2, formic acid; 3, AA; 4, propionic acid; 5, butyric acid; 6, valeric acid. Reprinted from Analytica Chimica Acta, 474, Tanaka, K., Ding, M.Y., Takahashi, H., Helaleh, M.I.H., Taoda, H., Hu, et al.; Vacancy ion-exclusion chromatography of carboxylic acids on a strongly acidic cation-exchange resin, Page 34, Copyright (2002), with permission from Elsevier.
Cyclodextrins are a class of chiral, organic compounds that are made up of D-glucose monomers arranged in a hydrophilic circle with a hydrophobic cavity. Typical cyclodextrins contain six, seven, or eight glucose monomers and are named α, β and γ-CD, respectively. Tanaka et al. (50) used β-CD as a mobile phase modifier to decrease the retention of highly retained carboxylic acids. The peak fronting effect was also minimized because of the increased hydrophilicity of the cation exchange resin due to adsorption of the hydroxyl groups of the β-CD to the resin surface. By increasing the concentration of β-CD, the retention of some aromatic organic acids was decreased (51). As shown in Figure 7, the first five acids were not affected: 2-naphthol-6-sulphonic, sulfanilic, barbituric, gallic and o-nitrobenzoic acids, whereas acetylsalicylic, salicylic and m-nitrobenzoic acid showed decreases in retention with increased β-CD concentration. The retention profiles of the two positional isomers (o-nitrobenzoic and m-nitrobenzoic acid) were obviously different. This was explained by the significant difference in the distribution coefficients of o-nitrobenzoic and m-nitrobenzoic acid in the presence of β-CD (1.4 and 7.7, respectively) (52).

**Eluent**

In IELC of aromatic organic acids, water can be used as an eluent (7, 53). Although water can be considered a strong eluent, the peak fronting observed when pure water is used as the mobile phase restricts its use to few applications. This peak fronting is due to the partial dissociation of weak organic acids in pure water. In addition, the retention can be too low to resolve the peaks. For these reasons, the use of an acidic eluent such as sulfuric acid (9, 25, 34, 54–56), tartaric acid (39), camphorsulphonic acid (52) or octanesulfonic acid (57) is very common in the analysis of aromatic acids. Organic solvents like acetonitrile (58) and heptanol (34) have been used to decrease the retention of such hydrophobic solutes and to enhance peak shape. As previously discussed, the eluent in VIELC is the sample itself, whereas water or water–alcohol is the injectate. However, very similar chromatograms can be obtained when different acid eluents are used at the same pH, so the detection method is the primary factor controlling the choice of the best acid eluent (1).

**Detection Methods**

Different detection methods have been reported for organic aromatic acids, but conductimetric detection is the most commonly used approach for IELC (59–62). However, the high background reading due to the high conductivity of the frequently used strong mineral acids, such as sulfuric acid, compromises the sensitivity. The strong chromophore in aromatic organic acids allows ultraviolet (UV) detection at reasonably high wavelengths to avoid interferences from the solvent and non-conjugated double bond species (54–56). In VIELC, the detector monitors the decreased detector response because the mobile phase itself is UV active (Figure 3). Although the peaks are negative, which is a common pattern for indirect detection, the detection is considered to be direct because the measured absorbance is the absorbance from the sample components although it is running as the mobile phase. Using two detectors at the same time can overcome the limitations associated with each detector and allows for the analysis of...
Figure 5. Effect of eluent pH on the retention volumes of the benzenecarboxylic acids and phenol with the various columns: Develosil 30-5 (A); TSKgel SCX (B); TSKgel OA-Pak A (C). All column dimensions: 300 × 7.8 mm i.d.; column temperature: 35 °C; flow rate: 1 mL/min; detection: UV at 200 nm; injection volume: 100 μL; sample concentration: 0.01 mM; peaks: 1, pyromellitic acid; 2, trimellitic acid; 3, hemimellitic acid; 4, PA; 5, m-phthalic acid; 6, o-phthalic acid; 7, phenol; 8, SA; 9, BA. Reprinted from Journal of Chromatography A, 782, Ohta, K., Tanaka, K., Haddad, P.R., Ion-exclusion chromatography of benzenecarboxylic acids on an unmodified silica-gel column, Pages 36 & 37, Copyright (1997), with permission from Elsevier.

Figure 6. Chromatogram of benzenecarboxylic acids by IELC using various concentrations of sulfuric acid using a mobile phase of sulphuric acid (pH 2.36): 0.05 mM (A); 0.5 mM (B); 2.5 mM (C); 5 mM (D). Column: Al-silica (250 × 4.6 mm i.d.); column temperature: 35 °C; flow rate: 1 mL/min; detection: UV at 200 nm; injection volume: 50 μL; sample concentration: 0.01 mM; peaks: 1, pyromellitic acid; 2, trimellitic acid; 3, hemimellitic acid; 4, p-phthalic acid; 5, o-phthalic acid; 6, m-phthalic acid; 7, phenol; 8, SA; 9, BA. Reprinted from Journal of Chromatography A, 850, Ohta, K., Tanaka, K., Ion-exclusion chromatography of carboxylic acids on silica gel modified with aluminum, Pages 183 & 184, Copyright (1999), with permission from Elsevier.
mixtures that have completely different natures. Coupling UV detection with either conductimetric (63, 64) or refractive index (65), or coupling conductimetric detection with refractive index (66), have been reported. Mass spectrometry detection can solve the problem of co-eluted analytes by deconvolution through monitoring selected fragments (67).

Applications for Aromatic Acids

Carboxylic acids

Determination of monocarboxylic acids

Different monocarboxylic aromatic acids have been determined by IELC. Lehotay and Traiter (53) used a strong cation exchanger on a PS/DVB resin to separate benzoic, p-hydroxybenzoic and salicylic acids. When water was used as the eluent, the peaks were extremely fronted. Better results were obtained by Glod and Kemula (7) using a strong cation exchanger on silica gel. The separation of benzoic acid from other ionic components in mustard was performed in less than 5 min. The same method could resolve nitrobenzoic acid, which is more retained due to increased hydrophobicity. Ohta et al. (32) used Develosil 30-5 silica gel to separate salicylic and benzoic acids from other aromatic compounds and compared the results to other columns of different functional groups and different resin structures. The retention times observed for salicylic and benzoic acids using 5 mM \( \text{H}_2\text{SO}_4 \) on this Develosil 30-5 column (300 × 8 mm) were approximately 30 and 38 min, respectively. The TSKgel SCX column (300 × 8 mm) retained benzoic acid for 300 min, whereas the TSKgel OA-Pak A column (300 × 8 mm) showed a retention time of 170 min. The significant decrease in the retention time of benzoic acid using Develosil 30-5 silica gel compared to TSKgel SCX and TSKgel OA Pak A was due to the hydrophilic nature of the silica gel. The same group (34) achieved a good separation of benzoic and salicylic acids that eluted at 7.3 and 5.0 min, respectively, using 2.5 mM sulfuric acid on Al-silica. Upon using a mobile phase of 50 mM tartaric acid at pH 2.2 and Zr-silica, salicylic and benzoic acids were more retained and the sensitivity of the method was decreased (39). Other monocarboxylic acids such as methoxyphenol acetic acid, acetylsalicylic acid and gallic acid have been determined by IELC. The type of column, temperature, mobile phase and detection methods are shown in Table III.

Determination of dicarboxylic acids

Isophthalic acid was retained by strong cation exchange resin using a mobile phase of water and conductimetric detection (53). The three different isomers, \( \alpha \), \( m \) and \( \beta \)-phthalic acids, were separated in less than 20 min using 5 mM sulfuric acid (32). Lower concentrations of sulfuric acid failed to resolve \( \alpha \) and \( \beta \)-phthalic acids. A relatively better separation was achieved using an Al-silica column and 2.5 mM sulfuric acid (pH 2.36) (34). The resolution was enhanced by using 5 mM sulfuric acid containing 0.07% heptanol on a TSKgel SCX column (49, 55). However, the best separation was obtained on a silica gel column functionalized with carboxymethyl groups (TSKgel CM-2SW) using 2.5 mM sulfuric acid at pH 2.4 (54).

Determination of tri- and tetra-carboxylic acids

When water was used as an eluent, trimellitic and trimesic acids overlapped together and the peak for the latter component was too fronted (53). The two acids could be resolved and separated from the tetracarboxylic acid, pyromellitic acid, in less than 20 min on the Develosil 30-5 column at 35 °C using 5 mM sulfuric acid at pH 2 (32). The time required to separate these three acids was halved using an Al-silica column (34). Similar results were obtained with a Zr-silica column using 10 mM tartaric acid as an eluent (39). A further decrease in the retention was achieved by using a silica gel column with sulfopropyl functional groups (TSKgel SP-2SW) and a mobile phase of 2.5 mM sulfuric acid at pH 2.4 (54). Upon adding heptanol to the mobile phase, the detection limits were calculated and found to be 3.2, 3.1 and 4.5 nM for pyromellitic, trimellitic and hemimellitic acids, respectively (49, 55). NTCA was separated from phthalic and benzoic acid by VIILC on a TSKgel OA Pak A column using the sample as a mobile phase with pH adjusted to 4.1 (30).

Amino acids and amino acid derivatives

Some aromatic amino acids have been determined by IELC using water as a mobile phase and LiChrosorb KAT as a support (7). Hippuric and orotic acid were determined using photodiode array detection and an HPX-87H column. The two peaks of hippuric and orotic acids were monitored at 210 and 280 nm, respectively. By using dual wavelength detection, the intensity of the hippuric acid peak was improved and the interference of other aliphatic compounds with orotic acid was avoided (56).
## Table III

**Determination of Different Aromatic Carboxylic Acids by ELC**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Column</th>
<th>Temperature</th>
<th>Mobile phase</th>
<th>Detection</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aromatic carboxylic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BA and SA</td>
<td>LChrosorb KAT 150 × 4 mm i.d.</td>
<td>22°C</td>
<td>Water</td>
<td>Conductimetric</td>
<td>6</td>
</tr>
<tr>
<td>Aromatic carboxylic acids</td>
<td>Dowelosil 30-5 300 × 7.8 mm i.d.</td>
<td>35°C</td>
<td>5 mM Sulfuric acid (pH 2.0)</td>
<td>UV at 200 nm</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Al-silica 250 × 4.6 mm i.d.</td>
<td>35°C</td>
<td>2.5 mM Sulfuric acid</td>
<td>UV at 200 nm</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Zr-silica 250 × 4.6 mm i.d.</td>
<td>35°C</td>
<td>10 mM Tartaric acid pH 2.5</td>
<td>UV at 254 nm</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Dowex 50W× 2 4.98 × 10.2 mm i.d.</td>
<td>25°C</td>
<td>Water</td>
<td>Conductimetric</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>TSKgel SP-SWP 150 × 6 mm i.d.</td>
<td>35°C</td>
<td>2.5 mM Sulfuric acid (pH 2.4)</td>
<td>UV at 200 nm</td>
<td>54</td>
</tr>
<tr>
<td>m- and p-Nitro-benzoic acid</td>
<td>LChrosorb KAT 150 × 4 mm i.d.</td>
<td>35°C</td>
<td>5 mM Sulfuric acid (pH 2.0)</td>
<td>UV at 200 nm</td>
<td>8</td>
</tr>
<tr>
<td>Methoxyl-phenolic acid</td>
<td>Dowelosil 30-5 300 × 7.8 mm i.d.</td>
<td>35°C</td>
<td>2.5 mM Sulfuric acid pH 2.4</td>
<td>UV at 200 nm</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Al-silica 250 × 4.6 mm i.d.</td>
<td>35°C</td>
<td>5 mM Sulfuric acid containing 0.075% heptanol</td>
<td>UV at 200 nm</td>
<td>49, 55</td>
</tr>
<tr>
<td></td>
<td>Dowex 50W×2 4.98 × 10.2 mm i.d.</td>
<td>25°C</td>
<td>Water</td>
<td>Conductimetric</td>
<td>6</td>
</tr>
<tr>
<td><strong>Dicarboxylic acids</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>m- and p-Phthalic acid</td>
<td>Al-silica 250 × 4.6 mm i.d.</td>
<td>35°C</td>
<td>2.5 mM Sulfuric acid</td>
<td>UV at 200 nm</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Dowelosil 30-5 300 × 7.8 mm i.d.</td>
<td>35°C</td>
<td>5 mM Sulfuric acid (pH 2.0)</td>
<td>UV at 200 nm</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>TSKgel SP-SWP 150 × 6 mm i.d.</td>
<td>35°C</td>
<td>2.5 mM Sulfuric acid (pH 2.4)</td>
<td>UV at 200 nm</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>TSKgel SC× 150 × 6 mm i.d.</td>
<td>35°C</td>
<td>5 mM Sulfuric acid containing 0.075% heptanol</td>
<td>UV at 200 nm</td>
<td>49, 55</td>
</tr>
<tr>
<td><strong>Trimesic acid</strong></td>
<td>Dowelosil 30-5 300 × 7.8 mm i.d.</td>
<td>35°C</td>
<td>5 mM Sulfuric acid (pH 2.0)</td>
<td>UV at 200 nm</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Dowex 50W×2 4.98 × 10.2 mm i.d.</td>
<td>25°C</td>
<td>Water</td>
<td>Conductimetric</td>
<td>53</td>
</tr>
<tr>
<td>TzCA</td>
<td></td>
<td>35°C</td>
<td>50 μM TzCA</td>
<td>Conductimetric</td>
<td>30</td>
</tr>
<tr>
<td>Amino acids and amino acid derivatives</td>
<td></td>
<td>22°C</td>
<td>Water</td>
<td>Conductimetric</td>
<td>6</td>
</tr>
<tr>
<td>Histidine, tyrosine and tryptophan</td>
<td>LChrosorb KAT 150 × 4 mm i.d.</td>
<td>HPX-87H, H2-75 300 × 7.8 mm i.d.</td>
<td>65°C</td>
<td>10 mM Sulfuric acid solution</td>
<td>PDA at 210 and 280 nm</td>
</tr>
<tr>
<td>Sulfonic acid derivatives</td>
<td>Aminex HPX-87 300 × 7.8 mm i.d.</td>
<td>25°C</td>
<td>5 mM Sulfuric acid containing 0.075% heptanol</td>
<td>UV at 200 nm</td>
<td>49, 55</td>
</tr>
<tr>
<td>2-Naphthol-benzenesulfonic and sulfuric acid</td>
<td>Aminex HPX-87 300 × 7.8 mm i.d.</td>
<td>25°C</td>
<td>5 mM Sulfuric acid containing 0.075% heptanol</td>
<td>UV at 200 nm</td>
<td>49, 55</td>
</tr>
<tr>
<td>Phenol</td>
<td>Dowelosil 30-5 300 × 7.8 mm i.d.</td>
<td>35°C</td>
<td>5 mM Sulfuric acid (pH 2.0)</td>
<td>UV at 200 nm</td>
<td>32</td>
</tr>
<tr>
<td></td>
<td>Al-silica 250 × 4.6 mm i.d.</td>
<td>35°C</td>
<td>2.5 mM Sulfuric acid</td>
<td>UV at 200 nm</td>
<td>34</td>
</tr>
<tr>
<td></td>
<td>Zr-silica 250 × 4.6 mm i.d.</td>
<td>35°C</td>
<td>10 mM Tartaric acid pH 2.5</td>
<td>UV at 254 nm</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>TSKgel SP-SWP 150 × 6 mm i.d.</td>
<td>35°C</td>
<td>2.5 mM Sulfuric acid (pH 2.4)</td>
<td>UV at 200 nm</td>
<td>54</td>
</tr>
<tr>
<td>Barbituric acid</td>
<td>Aminex HPX-87 300 × 7.8 mm i.d.</td>
<td>25°C</td>
<td>5 mM Sulfuric acid containing 0.075% heptanol</td>
<td>UV at 200 nm</td>
<td>49, 55</td>
</tr>
<tr>
<td>Hydroquinone</td>
<td>HPX-87H 300 × 7.8 mm i.d.</td>
<td>50°C</td>
<td>Acetophenone/sulfuric acid (0.0038M, pH 2.25, 7.5:92.5)</td>
<td>UV from 199 to 309 nm</td>
<td>58</td>
</tr>
</tbody>
</table>

*Note: Not reported (NR).
**Sulfonic acid derivatives**

Sulfanilic and 2-naphthol-6-sulfonic acids were separated on an Aminex HPX-87H column using 1 mM camphorsulphonic acid and 10 mM β-CD in ethanol–water (20:80, v/v). The addition of β-CD decreased the retention and improved the resolution and baseline stability (52).

**Other acids**

The interconversion of hydroquinone and benzoquinone was studied using a PS/DVB resin, but this rapid reaction precluded their separate quantification, and thus, RPLC had to be used (58). Barbbituric acid was determined on an Aminex HPX-87H column using 1 mM camphorsulfonic acid and 10 mM β-CD in ethanol–water (20:80, v/v) with UV detection at 210 nm.

Several papers have been reported for the determination of phenol. Upon using a Develosil 30-5 column and 5 mM H2SO4 (pH 2), phenol was separated after 28 min with a detection limit of 19 nM (55). Using an Al-silica column, phenol was separated from m-phthalic acid by using water or 0.05 mM sulfuric acid as an eluent (34). When a Zr-silica column was used, a detection limit of 0.05 μM was obtained using 10 mM tartaric acid. This phenol peak was separated from α-phthalic acid, the only isomer tried (39). Ohta et al. (54) could separate phenol from the three different isomers of phthalic acid by using different columns, but only TSKgel SP-25W showed good separation and a reasonable analysis time. Similar results were observed when pentanol or heptanol were added to the mobile phase and a detection limit of 28 nM was possible.

The separation of longer chain aliphatic acids (> C6) is also challenging. Very few papers have addressed the separation of these hydrophobic aliphatic acids. With heptanol added to the dilute sulfuric acid mobile phase, Ohta and co-workers separated C1–C7 aliphatic carboxylic acids on a TSKgel SCX column (49). A heptanol modifier at 0.15% was also used with a 0.2 mM pyromellitic acid mobile phase to separate C1–C8 carboxylic acids on Zr-silica in the cation exchange mode (68). The same separation was achieved using Al-silica and a 0.5 mM H2SO4 eluent with 0.15% heptanol (34). Longer chain (C1–C10) carboxylic acids were successfully separated using the same mobile phase (55).

A study was initiated of the separation of short chain aliphatic and aromatic carboxylic acids by IELC (69). Using StatEase optimization software, the analysis times for the baseline separation of acetic acid (AA), acetyl salicylic acid (ASA), and salicylic acid (SA) could be reduced by a factor of approximately two from 40 min. The AA degradation product could be baseline separated from acetylcholine in approximately 4 min using IELC. Recently, IELC was conducted in the ultra-high-performance liquid chromatography (UHPLC) mode using a surfactant-modified reversed-phase column and AA, SA and ASA were separated (a change in retention order) in approximately 6 min.

**Conclusion**

The separation of aromatic acids by IELC has been reviewed. The problems of long retention times and peak asymmetry were overcome by different IELC methods. Water is a strong eluent in IELC, but the peaks are extremely fronted. By using the sample as a mobile phase and water as the injectate, resolved symmetric peaks were eluted at the same retention time obtained by using water as the eluent. When silica and modified silica-based stationary phases were used, the hydrophobic interaction of the aromatic acids was decreased, reducing the analysis time. Adding β-CD to the mobile phase could decrease the retention by increasing the hydrophilicity of the cation exchange resin due to adsorption through the hydroxyl groups of the β-CD to the resin surface. This effect should be observable with other cyclodextrin derivatives, but this study has not yet been conducted. The effect of adding organic modifiers to the mobile phase was also studied, and it was found that long chain alcohols such as heptanol were more effective in decreasing the retention time and enhancing the peak shape for selected aromatic and long chain aliphatic carboxylic acids.

**References**

7. Glod, B.K., Kemula, W.; Separation mechanism and determination of acidic compounds by ion-exclusion liquid chromatography with electrokinetic detection; *Journal of Chromatography A,* (1986); 366: 39–50.
10. Fritz, J.S.; Principles and applications of ion-exclusion chromatography; *Journal of Chromatography,* (1991); 546: 111–118.
17. Casteel, K.V., Geiger, H., Sumere, C.F.; Separation of phenolics (benzoic acids, cinnamic acids, phenylacetic acids, quinic acid esters, benzenzdehydes and acetophenones, miscellaneous
phenolics) and coumarins by reversed-phase high-performance liquid chromatography; *Journal of Chromatography A* (1983); 258: 111–124.


22. Boyce, M.C.; Simultaneous determination of antioxidants, preservatives and sweeteners permitted as additives in food by mixed micellar electrokinetic chromatography; *Journal of Chromatography A* (1999); 847: 369–375.


33. Ohta, K., Morikawa, H., Tanaka, K., Uryu, Y., Puull, B., Haddad, P.R.; Ion chromatographic behavior of alkali and alkaline earth metal cations on silica gel columns with cation exchange characteristics; *Analytica Chimica Acta* (1998); 358: 255–261.


37. Verzele, M., Depotter, D., Gyhse's, J.; Trace elements in HPLC silica gel; *Journal of High Resolution Chromatography & Chromatography Communications* (1979); 2: 151–158.


45. Steffek, R.J., Zelechonok, Y.; Enantioselective ion-exclusion chromatography on teicoplanin aglycone and (+)-(18-crown-6)-2,3,11,12-tetracarboxylic acid stationary phases; *Journal of Chromatography A* (2003); 985: 91–100.


56. Papadakis, E.N., Polychroniadou, A.; Application of a microwave-assisted extraction method for the extraction of organic acids from Greek cheeses and sheep milk yoghurt and subsequent analysis by ion-exclusion liquid chromatography; *International Dairy Journal* (2005); 15: 165–172.

57. Bewsher, A.D., Polya, D.A., Lythgoe, P.R., Bruckshaw, I.M., Manning, D.A.C.; Analysis of fountain solutions for anionic components, including alkylbenzenesulfonates, carboxylates and polyphosphates, by a combination of ion-exchange and ion-exclusion chromatography and inductively coupled plasma atomic emission spectrometry; *Journal of Chromatography A* (2001); 920: 247–253.


69. Mansour, F.R., Kirkpatrick, C.L., Danielson, N.D.; Separation of acetyl salicylic acid from degradation products by conventional and ultra high performance ion exclusion chromatography; *Chromatographia* (2013); 76: 603–609.