

# Chromatographic Properties of the Ion-Exclusion Column IonPac ICE-AS6 and its Application in Environmental Analysis, Part II: Application in Environmental Analysis

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## Abstract

Biological processes, geochemical reactions, anthropogenic emissions, and transformation reactions of xenobiotics are responsible for the widespread occurrence of aliphatic carboxylic acids in the environment. To study the performance of the ion-exclusion chromatography column IonPac ICE-AS6 in the analysis of environmental and environmental-technical samples, organic acids are investigated in composting seepage, silage effluents, aqueous extracts of sewage sludge, molasses hydrolysate, and alkaline cellulose hydrolysates. With respect to the diverse sample matrix and composition, different chromatographic conditions are applied. It is possible to determine various volatile fatty acids, dicarboxylic acids, (poly)hydroxy acids, and keto acids as main and trace components in samples with very high and low dissolved organic carbon content. Low baseline noise allows the determination of malic and succinic acid in the concentration range of approximately 1 µM/L in the presence of higher concentrations of fully ionized compounds. The applicability of the column in environmental analysis may be limited by the poor retardation of strong organic acids, insufficient separation of some relevant substance combinations (i.e., citric and isocitric acid), and very strong hydrophobic interactions with straight-chain monocarboxylic acids containing four or more carbon atoms.

## Introduction

Aliphatic carboxylic acids are ubiquitous in the environment. They are released by many organisms and generated during a multitude of biogeochemical reactions. The main anthropogenic sources are residues and byproducts from agriculture and food processing as well as wastes,

wastewaters, and other related technical processes. Furthermore, organic acids are generated in the environment by biochemical transformations (i.e., oxidation) of released hydrocarbons, xenobiotics, and other organic pollutants.

The important environmental matrices containing aliphatic carboxylic acids are soils, sediments and their pore waters, surface waters, fog, and precipitation. Almost all technical processes for the treatment of organic wastes and sewage sludges (such as dumping, fouling, composting, fermentation, and solidification) trigger the formation of leachates or process waters enriched with organic acids. Furthermore, acidic compounds may be generated during the remediation of organically polluted soils. Manure and silage seepage waters are important agricultural sources and lead to elevated acid concentrations in soil after application.

As illustrated in Figure 1, most of the referred to matrices are suited for ion-exclusion chromatography (IEC) analysis. Therefore, it is not surprising that environmental issues have

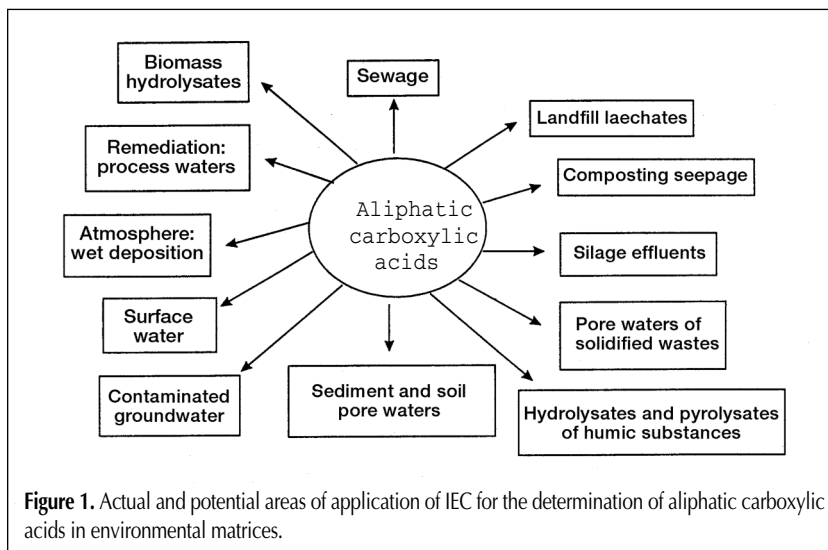


Figure 1. Actual and potential areas of application of IEC for the determination of aliphatic carboxylic acids in environmental matrices.

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been addressed using this chromatographic method since the mid 1980s. In the last several years, IEC has been applied to determine aliphatic carboxylic acids in air (1), soils and soil solutions (2–4), groundwater (5,6), landfill leachates (7,8), silage effluents (9), and biomass hydrolysates (10,11). In addition, carboxylic acids have been monitored during the biodegradation of xenobiotics (12).

Columns used for these analytical purposes were Dionex (Sunnyvale, CA) IonPac ICE-AS1, ICE-AS5, ICE-AS6, BioRad (Krefeld, Germany) HPX-87H, Supelco (Bellefonte, PA) Supelcogel C610-H, Shimadzu (Duisburg, Germany) SCR-102H, Interaction Chemicals (Mountain View, CA) ORH-801, and Merck (Darmstadt, Germany) Polyspher OA-HY.

According to the manufacturer's statement, the ICE-AS6 was specially designed to facilitate improved selectivity for hydroxy-carboxylic acids and Krebs cycle acids. The specific properties of the column have been described previously (13). Because multi-

functional acids are of great importance for biological processes and for the speciation of heavy metals in environmental compartments, IEC should offer the capability to investigate this class of compounds in different matrices.

In this context, various applications of the IonPac ICE-AS6 column are presented, which allows for the evaluation of its versatility and performance in the analysis of environmental samples with emphasis on the detection of multifunctional aliphatic carboxylic acids. Therefore, the following matrices were chosen: composting seepage, alkaline cellulose hydrolysate, molasses hydrolysate, silage effluent, and water extracts of aged stabilized sewage sludge.

## Experimental

### Chemicals

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

### Instrumental

The analysis was performed by a Dionex DX-500 system, which was composed of a GP-40 gradient pump, an He degasser unit for eluent and suppressor regenerant, an LC-20 chromatography module with injection valve and 25- $\mu$ L sample loop, an ED-40 electrochemical detector operated in the conductivity mode, and a Dionex ASM autosampler. The IonPac ICE-AS6 column was housed in a thermostat (Industrial Electronics, Langenzersdorf, Austria) in order to adjust separation temperatures between 10°C and 60°C. Various dilutions of perfluorobutyric acid (PFBA) served as an eluent at a flow rate of 1.0 mL/min. A Dionex anion micromembrane suppressor (AMMS-ICE) was installed between the column and the detector cell in order to suppress the background conductivity of the eluent. The suppressor regenerant used was an aqueous TBAOH solution (5mM/L) with a flow rate of approximately 3.0 mL/min.

Remote system control, data acquisition, and processing were handled by Dionex PeakNet software. To enhance the chromatographic versatility and certainty of substance identification by comparing their retention times with those of reference compounds, most of the samples were analyzed in two chromatographic conditions using the structure-dependent variations of the analyte retentions as affected by changes in the column temperature and eluent (proton) concentration (13).

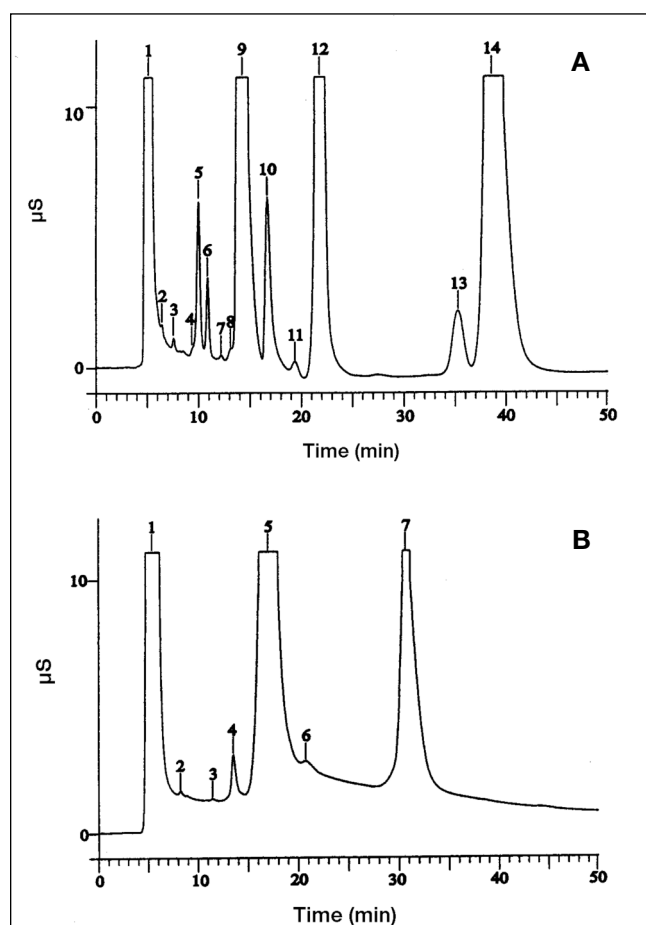
### Sample preparation or sampling and sample pretreatment

#### Composting seepage

The sample was taken from a seepage collection tank and then filled into a PE screw-top tube and frozen at -18°C.

#### Silage effluent

Effluents from freshly ensiled maize plants were collected from



**Figure 2.** Chromatograms of a compost leachate. Chromatographic conditions (A): eluent, 0.4mM PFBA; column temperature, 60°C; and eluent flow, 1.0 mL/min. Analytes (acids): void volume, 1; pyruvic (not quantified), 2; unknown, 3; glycolic (not quantified), 4; formic (1.1mM), 5; lactic (0.6mM), 6; unknown, 7; succinic (not quantified), 8; acetic (72.7mM), 9; unknown, 10; glutaric (0.1mM), 11; propionic (21.9mM), 12; *i*-butyric (2.2mM), 13; and *n*-butyric (28.9mM), 14. Chromatographic conditions (B): eluent, 1.6mM PFBA; column temperature, 10°C; and eluent flow, 1.0 mL/min. Analytes (acids): void volume, 1; pyruvic (not quantified), 2; glycolic (not quantified), 3; lactic (0.6mM), 4; acetic (not quantified), 5; succinic (not quantified), 6; and propionic (20.1mM), 7.

Bavarian farms in 1991 and 1998. Prior to analysis, the samples taken in 1991 were stored in completely filled PE screw-top tubes or in tubes with a headspace of nitrogen and refrigerated at 4°C. Samples taken in 1998 were frozen at -20°C.

#### Sewage sludge extract

Twenty-year-old stabilized sewage sludge from the upper 25 cm of a sewage sludge dump was directly cut out with a laboratory column (250 × 90 mm, filling amount approximately 0.5 kg) and percolated in reverse direction with deionized water for a period of 6 weeks. The percolating rate was 43 mL/day. One sample was taken every week.

#### Molasses hydrolysate

Sugar beet molasses is a residue of the technical saccharose processing and contains about 51% (w/w) of saccharose. Five grams of molasses was added to 20 mL of 30% HNO<sub>3</sub> in a round-bottomed flask and digested for 4 h at 85°C. Particles that were possibly remaining were removed by filtration.

#### Alkaline cellulose hydrolysate

In order to mimic chemical conditions prevailing in cementified wastes containing higher amounts of cellulosic material, pure cellulose powder (20- $\mu$ m spheres, Aldrich article, 31,069-7) was transferred into artificial cement pore water (ACW) and degraded in the dark at 25°C in the absence of oxygen. The ACW used was a NaOH-KOH solution saturated with respect to Ca(OH)<sub>2</sub> containing 114mM of Na, 180mM of K, and 2.3mM of Ca and having a pH level of 13.3. After the desired reaction times (several weeks or months), the suspensions were filtered through a prewashed membrane filter (Teflon filter type FH 0.5  $\mu$ m, Millipore). Further details are given elsewhere (14).

If not stated otherwise, the sample preparation procedure included three steps: (a) if necessary, removal of suspended particles performed by centrifugation at approximately 10,000 rpm in a Biofuge 22 R (Heraeus-Sepatech, Osterode, Germany); (b) filtration through 0.1- or 0.2- $\mu$ m cellulose nitrate membranes (Sartorius, Göttingen, Germany) prewashed by the sample liquid (the possible release of interfering organic compounds from the

filter was controlled by the analysis of blank and standard solutions); and (c) removal of strongly adsorbing humus-like substances by extraction with Dionex Ongaard-P PVP cartridges (tests with standard solutions and spiked samples have established that PVP cartridges do not adsorb hydrophilic acids under recommended application conditions).

## Results and Discussion

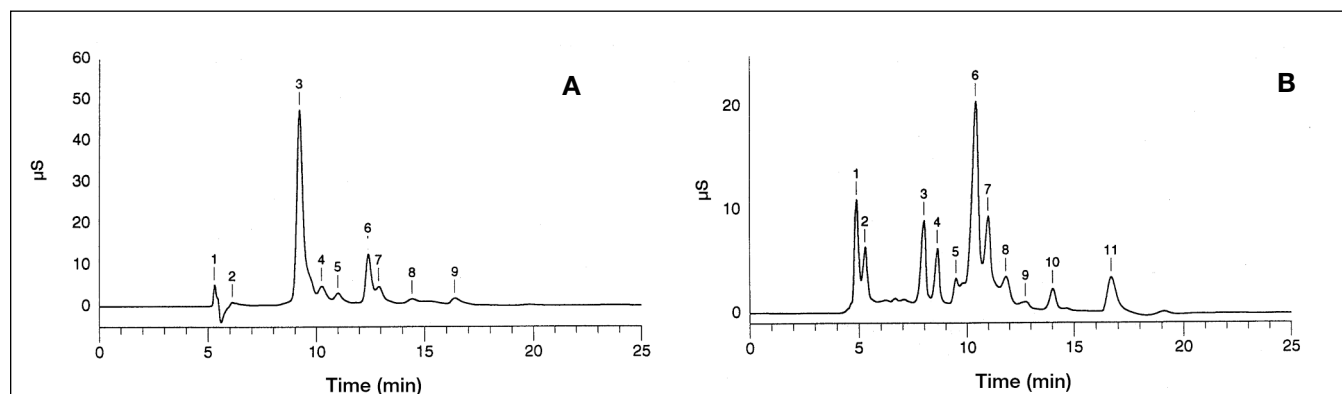
#### Compost leachate

Composting is a common process for the treatment and utilization of biowastes, especially organic garbage and garden litter. Depending on the composting technology, composition, and water content of treated wastes, high amounts (up to 0.5 m<sup>3</sup> per mg solid waste) of seepage are generated during the accelerated rot of the biomass. A portion of the seepage is cycled within the process. Surplus amounts are discharged into sewers or applied to soils. The determination of the carboxylic acid content of compost leachates provides useful information for the control of the composting process and the environmentally responsible disposal of the leachates.

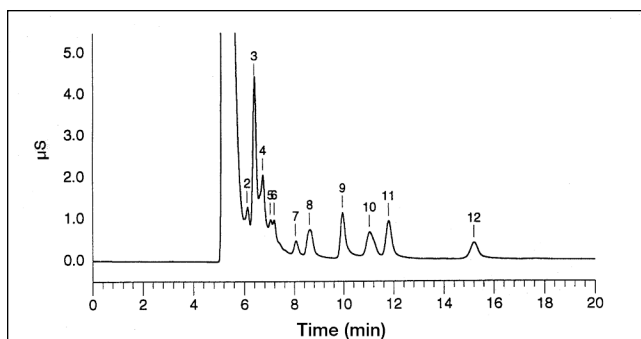
Chromatograms of a compost leachate (recorded under different analytical conditions) are shown in Figures 2A and 2B. In total, ten aliphatic carboxylic acids were detected, including five multifunctional hydroxy-, keto-, and dicarboxylic acids. The main components were the volatile fatty acids. Because of their high retention, monofunctional acids with more than four carbon atoms could not be analyzed at the given recording time. Three chromatographic signals remained unidentified.

As a result of smaller peak widths, shorter retention times, and lower peak tailing, analytical condition A (column temperature 60°C, eluent 0.4mM PFBA) (Figure 2A) offered superior separation efficiency and peak capacity compared with condition B (column temperature 10°C, eluent 1.6mM PFBA) (Figure 2B). Under condition A, solute diffusion was improved for more efficient phase distribution.

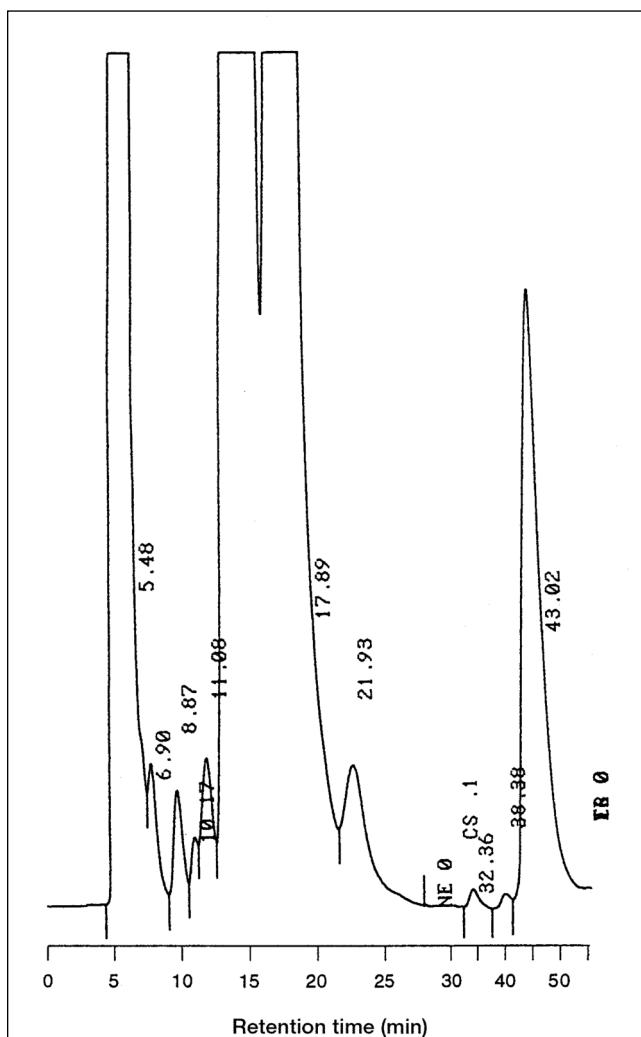
Separation condition B reflected the effect of lower eluent pH



**Figure 3.** Chromatograms of an alkaline hydrolysate of pure cellulose. Chromatographic conditions (A): eluent, 1.6mM PFBA; column temperature, 20°C; and eluent flow, 1.0 mL/min. Analytes (acids): void volume, 1; unknown, 2; isosaccharinic (36.0mM), 3; glyceric (0.7mM), 4; glycolic (0.8mM), 5; formic (2.1mM), 6; lactic (1.0mM), 7; unknown, 8; and acetic (0.6mM), 9. Chromatographic conditions (B): eluent, 0.4mM PFBA; column temperature, 60°C; and eluent flow, 1.0 mL/min. Analytes (acids): void volume, 1; unknown, 2; glyceric (1.4mM), 3; unknown, 4; glycolic (0.4mM), 5; formic (4.0mM), 6; lactic (1.8mM), 7; unknown, 8; succinic (0.1mM), 9; acetic (0.6mM), 10; and unknown, 11.



**Figure 4.** Chromatogram of products from the oxidative degradation of sugar beet molasses by nitric acid. Chromatographic conditions: eluent, 0.8mM PFBA; column temperature, 40°C; and eluent flow, 1.0 mL/min. Analytes (acids): void volume, 1; unknown, 2; glucaric (saccharic) (31.4mM), 3; tartaric (6.1mM), 4; unknown, 5; unknown, 6; threonic (4.2mM), 7; glyceric (10.8mM), 8; glycolic (16.4mM), 9; formic (13.5mM), 10; lactic (12.3mM), 11; and acetic (9.4mM), 12.



**Figure 5.** Chromatogram of a maize silage effluent (sampled 1991, 10-fold diluted). Chromatographic conditions: IonPac ICE-AS5 column; eluent, 0.9mM PFBA; column temperature, 10°C; eluent flow, 0.3 mL/min; and 10- $\mu$ S full-scale detection. Analytes (acids): void volume, 5.48; pyruvic, 6.90; unknown, 8.87; formic, 10.17; unknown, 11.08; lactic and acetic, 17.89; isocitric, 21.93; unknown, 32.36; propionic, 38.38; and succinic, 43.02.

and decreased column temperature on the analyte retention. Reduced dissociation of the acids, elevated bonding forces between the analytes and stationary phase, and slower phase transfer processes of the analytes resulted in increased analyte retardation, peak broadening, and peak tailing. The gained overall analytical yield was relatively low. Despite these drawbacks, condition B provided valuable chromatographic information. To begin with, the increased proton concentration of the eluent lowered the dissociation of those organic acids especially whose  $pK_a$  values were in the range of the eluent pH. As a consequence, the separation of these acids from each other and from the void volume was improved. This effect, together with the elevated retention of acetic acid, caused the opening of a broader analytical window for keto- and hydroxy acids with small  $k$  values. As illustrated in Figure 2B, the interaction of these factors lead to an improved separation of pyruvic and glycolic acid. Also, the selectivity, which is ruled by parameters that have been discussed previously (13), was modified by alterations in the chromatographic conditions. Under condition B, succinic acid eluted after acetic acid and the resolution between the two components was improved. The alteration of the elution sequence and the retention time of succinic acid offered an additional indication for the identity of this analyte. Lactic and propionic acid were the only acids quantified under both chromatographic conditions, which resulted in a good agreement between the respective measurements.

#### Alkaline cellulose hydrolysate

Low- and intermediate-level radioactive waste often contains high amounts of cellulosic materials (i.e., tissues, clothes, and paper) (15).

A common disposal method of this type of waste is solidifying it by adding cement and storing it in an underground repository. For very long time periods, the cement pore water remains strongly alkaline (pH between 12.5 and 13.3) (16). Under this condition, cellulose will degrade to water-soluble acidic low-molecular-weight compounds, most of them containing hydroxy functions (17–19). The quantitative composition of such degradation products and their chemical properties must be investigated as part of the safety assessment of the underground repository for radioactive waste, because formed polyhydroxy-type ligands such as isosaccharinic acid are able to strongly complex various radionuclides and enhance their mobility (20,21). Therefore, various types of cellulose and cellulosic materials were artificially degraded under conditions relevant for solidified wastes. The formed alkaline hydrolysates were analyzed by IEC and other techniques. More reported details have been given by Glaus et al. (14). Typical chromatograms of an alkaline hydrolysate of pure cellulose are shown in Figures 3A and 3B.

At low separation temperature (Figure 3A), the main peak (peak 3) can be attributed to isosaccharinic acid (ISA). The shape of the ISA peak indicates that at least one other component coelutes with ISA. Furthermore, three hydroxy-carboxylic acids (glyceric, glycolic, and lactic acid) and two monocarboxylic acids (formic and acetic acid) were detected. No chromatographic signal with higher peak area remained unidentified.

Because of the temperature- and pH-dependent acid-lactone equilibrium, the free ISA (a monoprotic acid) was almost com-

pletely converted in its lactone form during the chromatographic run at high column temperature (60°C). With the use of conductivity detection, this chemical species could not be monitored (Figure 3B). The negative correlation between the separation temperature and the ISA peak area using conductivity detection was confirmed by the analysis of ISA standard solutions at various temperatures.

The chromatographic conditions applied to record Figure 3B enhanced peak efficiency and reduced peak widths and retention times. In relation to the previous results (Figure 3A), succinic acid was also determined. Various other peaks were recorded but remained unidentified.

From a critical perspective, it should be noted that the correspondence between the quantitative data gained by the two chromatographic conditions was not satisfactory, except for acetic acid. This could have been caused by the incomplete separation of several components and (in some cases) by coelution with unidentified compounds.

Despite these drawbacks, the IEC analysis supported the conclusion that the amount of ISA is one of the critical factors with respect to the possible influences of cellulose degradation products on the mobility of radionuclides in solidified wastes.

#### Acidic molasses hydrolysate

The use of biomass residues as sources for natural chelating agents (which are able to liberate and remove heavy metals from contaminated solid materials) marks a new concept for the sanitation of metal-polluted soils (11). Most of these residues originate from agriculture and food industry. The oxidation of carbohydrate-containing residues such as molasses, potato-peel sludge, and whey powder by nitric acid yields in hydrolysates rich in (poly)hydroxy acids.

Predominantly, these hydrolysates are analyzed by IEC applying strong acidic inorganic eluents (H<sub>2</sub>SO<sub>4</sub>) and ultraviolet (UV) detection (10,11). If some sugar acids (which were rapidly transformed into their lactones under acidic conditions) must not be quantitated, the analysis could also be performed by IEC combined with conductivity detection, offering an alternative detection selectivity and sensitivity.

An example for the chromatographic determination of organic acids in a sugar beet molasses hydrolysate is given in Figure 4. In total, eight components were identified including two sugar acids (glucaric and threonic acid), four other hydroxy-carboxylic acids, and two volatile fatty acids. Three relevant chromatographic signals remained unidentified. The main component was saccharic acid. Approximately 30% of the mole sum of quantitated acids was apportioned to this compound. Because of the incomplete separation of glucaric and tartaric acid from each other and the void peak, the exact quantitation of these compounds was impeded.

Basically, our findings were comparable with earlier investigations performed by means of an IEC-UV-detection system (10). The main difference was the missing detection of oxalic acid and gluconic acid by our investigation. In this context,

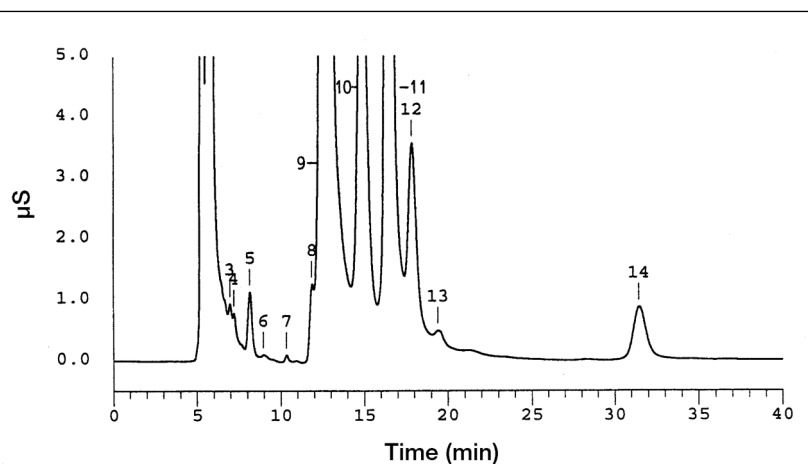
several aspects should be considered. First, the detection of oxalic acid was methodically impossible, because oxalic acid was not separated from the void volume under given chromatographic conditions ( $pK_1$  of 1.23 for oxalic acid and pH of 3.25 for 0.4mM PFBA). Additionally, gluconic acid has a significant tendency to convert into its 1,4-lactone for high temperature and high-proton concentrations (22). Finally, gluconic acid is an intermediate of the oxidation of glucose to glucaric acid. Therefore, the gluconic acid concentration depends on the reaction conditions and the degree of reaction completeness.

#### Maize silage effluent

Silage effluent is the byproduct of anaerobic crop preservation that provides winter feed for various farm animals in the form of silage. Usually, the main constituents are peptides, amino acids, volatile and nonvolatile fatty acids, carbohydrates, mineral salts, and ammonia. Because of this composition, silage effluents have a certain value for animal nutrition and fertilization of soils. Mostly, they are handled as wastes constituting serious disposal problems with the inherent risk of environmental pollution. Therefore, the need for a detailed analytical investigation of silage effluents is diverse.

Because aliphatic carboxylic acids are measured in silage effluents almost exclusively by gas-liquid chromatography (discriminating both very volatile and very hydrophilic components), we undertook the first attempt at analyzing these components by IEC in 1992 (9). At that time, we used a Dionex DX-300 ion chromatograph, equipped with a Dionex IonPac ICE-AS5 ion-exclusion column (250- × 4-mm i.d., 6- $\mu$ m particle size). The main differences between the ICE-AS5 and its successor, the ICE-AS6, consists of the low compatibility of the ICE-AS5 with organic solvents, the relatively high compressibility of its packing material requiring low flow rates, and different chromatographic selectivities resulting from differences in the sulfonate-carboxylate ratio and applied sulfonation conditions.

By comparing the analytical performance of both columns, we recently sampled and measured again a maize silage effluent. The



**Figure 6.** Chromatogram of a maize silage effluent (sampled 1998, 10-fold diluted). Chromatographic conditions: IonPac ICE-AS6 column; eluent, 0.8mM PFBA; column temperature, 25°C; and eluent flow, 1.0 mL/min. Analytes (acids): void volume, 1; unknown, 2; tartaric, 3; unknown, 4; isocitric, 5; unknown, 6; glycolic, 7; formic, 8; lactic, 9; unknown, 10; acetic, 11; succinic, 12; unknown, 13; and propionic, 14.

recorded chromatograms are presented in Figures 5 and 6.

The determination of organic acids in a 10-fold diluted silage effluent (sampled in 1991 and analyzed by ICE-AS5 in combination with AMMS-ICE and conductivity detection) is shown in Figure 5. The chromatogram was recorded by a Chromjet integrator at a full-scale detector of 10  $\mu$ S. The decrease of chart speed after 30 min should be noted. The main components, lactic and acetic acid (which are indicated by maximal signal intensities much higher than 10  $\mu$ S), were recorded as a broad, poorly resolved peak at 17.89 min. Lactic acid was represented in the first part of the peak and acetic acid in the second part. Other relevant compounds were isocitric (21.93 min) and succinic acid (43.02 min). Pyruvic (6.90 min), formic (10.17 min), and propionic acid (38.38 min) were determined in traces.

Because of the higher flow rate (1.0 instead of 0.3 mL/min) and higher column back pressure, the silage effluent sampled in 1998 was analyzed with the ICE-AS6 at a higher temperature (25°C instead of 10°C) and a slightly lower eluent concentration (0.8 instead of 0.9mM/L). As Figure 6 reveals, the important compounds were nearly the same as earlier determined. A further broad unidentified peak at 74.77 min is not shown. Tartaric acid (peak 3, 6.93 min), glycolic acid (peak 7, 10.32 min), and formic acid (peak 8, 11.85 min) were identified as trace components. As it can be deduced from the separation of lactic and acetic acid, the ICE-AS6 column offered an increased peak resolution caused by reduced peak widths and reduced peak tailing of hydroxy acids. This allowed for the fractionation of a third unidentified compound between acetic and lactic acid.

For cases in which the retention times of acetic and lactic acid were almost the same for both columns under chosen conditions, several analytes (especially isocitric and succinic acid) were eluting remarkably faster from ICE-AS6, which expresses an altered column selectivity.

### Sewage sludge leachate

Sewage sludges are often loaded with heavy metals. Their admixture to soils bears the risk of soil pollution. Even deposited in a sewage sludge dump, the long-term behavior of heavy metals has to be controlled with respect to solubilization and translocation processes. The mobilization of heavy metals may be initiated

or enhanced by the decomposition of high-molecular-weight organic constituents of sewage sludge, leading to the formation of short-chain carboxylic acids. Therefore, an indepth knowledge of the organic matter composition of sewage sludge leachate is necessary to assess the long-term stability under disposal conditions and to monitor early processes capable of liberating heavy metals from the solid matrix.

The chromatographic investigation of carboxylic acids in an artificial leachate of a 20-year-old sewage sludge sample is depicted in Figure 7. The low baseline noise allowed for the sensitive detection of organic trace compounds in the presence of relatively high concentrations of fully ionized components (void volume). The determined analytes were malic, glycolic, formic, lactic, and succinic acid. Peak 1 is presumed to be isocitric acid. The estimated concentrations (deduced from former calibration data) were approximately 1 $\mu$ M/L for malic and succinic acid, 7 $\mu$ M/L for glycolic acid, and 10 $\mu$ M/L for formic acid.

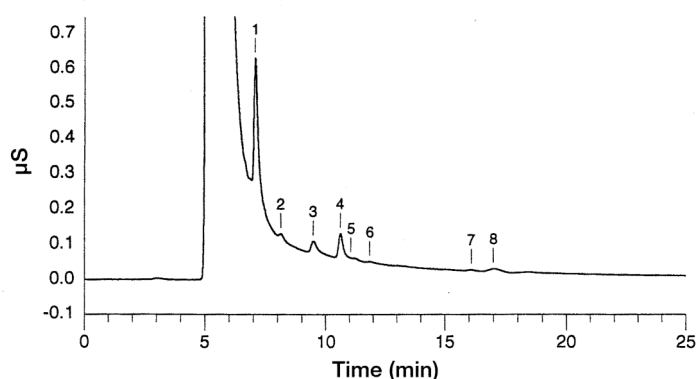
These results indicate that sewage sludge (or inherent microorganisms) is still a source of carboxylic acids 20 years after dumping. With the exception of isocitric acid, the determined aliphatic acids were weak complexing agents. Therefore, it can be estimated that these compounds have no significant influence on the metal mobility at the found concentration levels.

### Conclusion

As illustrated by several examples, the IonPac ICE-AS6 column can be successfully applied in the analysis of aliphatic carboxylic acids in various environmental matrices. Both highly loaded wastewaters and samples with low DOC content are suited for chromatographic determination. The adequate selection of the eluent pH and column temperature allows for the adaptation of the separation conditions to specific sample compositions. The investigation of a silage effluent elucidates improvements of the separation efficiency compared with IonPac ICE-AS5. The applicability of the column in environmental analysis may be limited by the low retention of strong organic acids (i.e., oxalic acid), insufficient separation of some relevant substance combinations (i.e., citric or isocitric acid), and strong adsorption of straight-chain monocarboxylic acids with four or more carbon atoms. The parallel application of two IEC systems (differing in the properties and selectivity of the chromatographic phases and the detection mode) is recommended for the improvement of the separation and identification of analytes in environmental samples showing a complex DOC composition.

### Acknowledgments

The authors would like to thank P. Leidmann for sampling of the silage effluents and D. Klotz for supplying sewage sludge leachate.



**Figure 7.** Traces of organic acids in aqueous leachate of aged sewage sludge. Chromatographic conditions: eluent, 0.4mM PFBA; column temperature, 20°C; and eluent flow, 1.0 mL/min. Analytes: presumably isocitric, 1; malic, 2; glycolic, 3; formic, 4; lactic, 5; unknown, 6; unknown, 7; and succinic, 8.

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Manuscript accepted June 22, 2000.