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Analysis of low-molecular-mass inorganic and organic anions by ion chromatography–atmospheric pressure ionization mass spectrometry

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Abstract

Different nonvolatile mobile phases have been tested for the combination of ion-exchange chromatography combined with mass spectrometric detection of anions and organic acids. Buffer systems based on carbonate, sulfate, oxalate and citrate as the eluting species have been used. Among these, citrate proved to be the most versatile eluent allowing the separation of anions with absolute detection limits between 0.4 and 0.7 ng and of organic acids with detection limits between 0.4 and 4 ng in the non-suppressed mode. In the suppressed eluent mode iodate, bromate and chlorate could be separated using sodium carbonate as the mobile phase resulting in detection limits of 50 pg. The method was applied to the analysis of water samples containing oxyhalides originating from ozonization. Additionally, organic acids were separated by chromatographic separation techniques like reversed-phase, ion-pair or ion-exclusion chromatography and the compatibility with mass spectrometry was investigated with special respect to sensitivity of this detection mode.

Keywords: Water analysis; Inorganic anions; Organic acids; Oxyhalides

1. Introduction

In recent years mass spectrometry (MS) has become a versatile detection technique for high-performance liquid chromatography, offering the advantage of a sensitive and mass selective detection as well as the possibility of acquiring online mass spectra for the identification of unknown compounds. The demand on the combination of a liquid chromatographic separation system with MS has led to the development of several different interfaces. During the last years techniques such as particle beam interfacing, thermospray and sonic spray have continuously lost importance due to the rapid development of atmospheric pressure ionization (API) techniques utilizing high electric fields for nebulization and vaporization. A detailed overview about these developments is given elsewhere [1–5]. The API interface is state of the art for the determination of large molecules such as peptides and proteins but only little work has been done on the determination of small inorganic and organic ions eluting from an ion chromatographic (IC) separation system.

Both suppressed and non-suppressed IC have successfully been coupled with MS using an API interface. Beside volatile mobile phases based on formate and acetate as the eluting species also a few non-volatile eluents have been investigated for IC–API-MS measurements such as solutions of am-

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monium nitrate [6] and ammonium sulfate [7] in the non-suppressed mode. Sodium hydroxide [8] and sodium carbonate [9] have been reported for IC–API-MS experiments with eluent suppression. The investigations presented in this paper aimed at the determination of some in water samples commonly analyzed anions as well as some oxyhalides in both the non-suppressed and the suppressed IC mode with API-MS using a pneumatically assisted electrospray interface. Special attention was paid to the influence of the mobile phase, especially the kind and concentration of the electrolytes, on the sensitivity of MS detection and the optimization of the operating conditions for IC and MS.

Besides inorganic anions, the investigations were also extended to the determination of organic anions. In contrast to inorganic anions, the nature of organic acids (with special respect to their lower acidity) generally allows the employment of various separation techniques such as ion-exclusion [10,11] and ion-exchange chromatography [12–19] beside reversed-phase and ion-pair chromatography with UV or conductivity detection. MS detection in combination with ion-exclusion chromatography has been reported using a particle beam interface with diluted sulfuric and hydrochloric acid as the mobile phase [20] and an API interface with trifluoroacetic acid as the eluent [21]. Ion-exchange chromatography was successfully coupled to a particle beam interface with either sodium chloride or sodium hydroxide containing acetonitrile as the eluent utilizing a membrane suppressor [22]. In the present work the different separation techniques were investigated and evaluated in terms of compatibility with MS and sensitivity of MS detection depending on the kind and concentration of the mobile phase.

2. Experimental

2.1. Instrumentation

IC was performed on a HP 1100 HPLC System equipped with a vacuum degasser, quaternary pump, UV–Vis diode array detector and a HP 1050 autosampler (all Hewlett-Packard, Palo Alto, CA, USA); MS measurements were done on a quadrupole system HP 5989B using an atmospheric pressure ionization (pneumatically assisted electrospray) interface HP 59987A (Hewlett-Packard) equipped with a radio frequency (RF)-only hexapole (Analytica of Branford, Branford, CT, USA).

2.2. Chemicals

Analytical grade chemicals from various suppliers were used throughout this work. High-purity water was prepared by a Milli-Q water purification system (Millipore, Milford, MA, USA). 100 mg/l stock solutions of inorganic anions (bromate, iodate, chlorate, bromide, iodide, sulfate and thiosulfate) were prepared from their analytical grade sodium salts by dissolving the appropriate amount in Milli-Q water; 100 mg/l stock solutions of organic anions (propionate, butyrate, lactate, ascorbate, malonate, succinate, glutarate, fumarate, malate, tartrate, citrate and gallate) were prepared from their corresponding acids.

2.3. Ion chromatography

2.3.1. Separation of inorganic anions in the non-suppressed mode

For the separation of inorganic anions in the non-suppressed IC mode a 75×4.6 mm I.D. Waters IC-Pak Anion HR Column (Waters, Milford, MA, USA) was used throughout this work. As mobile phases carbonate and sulfate eluents (prepared from their ammonium salts as 1 m*M* solutions), an oxalate eluent (prepared from 1 m*M* oxalic acid) and a citrate eluent (prepared from 0.5 m*M* citric acid) were employed. Adjustment of pH was done using 1 *M* ammonia. These solutions were mixed with acetonitrile at a ratio of 90:10 (v/v) so that the final concentration of the carbonate, sulfate and oxalate eluents was 0.9 m*M* and 0.45 m*M* for the citrate eluent. All experiments with these eluents in the non-suppressed mode were performed at pH 9 if not stated otherwise. The flow-rate of 1 ml/min was split so that only 120 μl/min entered the MS interface. An injection volume of 100 μl was applied if not stated otherwise.

2.3.2. Separation of inorganic anions in the suppressed mode

For suppressed IC a 130×2 mm I.D. stainless steel column was packed with the stationary phase of a

Waters IC-Pak Anion HR column using the slurry packing technique. A 5 m*M* sodium carbonate solution was employed as the suspension and packing liquid and the packing process was performed at a constant pressure of 160 bar. When no further decrease of the flow was observed, the flow was kept constant for approximately 1 h at about 0.5 ml/min. Afterwards the column was conditioned with the mobile phase for the suppressed IC separation, i.e. 5 m*M* sodium carbonate containing 10% acetonitrile. The flow-rate was 150 μl/min and the injection volume 100 μl.

2.3.3. Separation of organic acids

Organic acids were analyzed employing a YMC 250×2 mm I.D. ODS column (5 μm, 120 A) (YMC, Kyoto, Japan) for reversed-phase and ion-pair chromatography. A 100×7.8 mm I.D. Fast Acid Analysis Column HPAH (Bio-Rad, Hercules, CA, USA) was used for ion exclusion chromatography, whereas a home-made 200×1 mm I.D. micro column packed with Nucleosil 100-5 $N(CH_3)_2$ (Machery–Nagel, Düren, Germany) was employed for the separations in the ion-exchange mode. The latter column was packed by the slurry packing technique using a mixture of 50% 1 m*M* ammonium citrate pH 4.8 and 50% acetonitrile as both the suspension and packing medium. The packing was performed at a constant pressure of 400 bar employing a pneumatic pump.

The suppressor column for IC of inorganic anions in the suppressed mode was a stainless steel column packed with 50% crosslinked porous sulfonated poly(styrene–divinylbenzene) (mean particle size: 4 μm, ion-exchange capacity: 1.3 mequiv./g). The slurry packing procedure was performed with methanol as both the suspension and packing medium at a constant pressure of 400 bar. The column was brought into the H^+ form by purging with 50 m*M* sulfuric acid at a flow of 0.8 ml/min for about 30 min. It was washed with Milli-Q water containing 10% acetonitrile for 45 min prior to use.

Eluents tested for the separation of organic acids were either trifluoroacetic or formic acid at various concentrations in the case of reversed-phase chromatography, octylamine at a concentration of 0.5 m*M* in the case of ion-pair chromatography and 3 m*M* formic acid was employed for ion exclusion chromatography. Ion-exchange chromatography of organic anions was performed using a 0.5 m*M* ammonium citrate eluent prepared from 1 m*M* aqueous solution of citric acid (adjusted to pH 4.8 with 1 *M* ammonia) which was mixed with acetonitrile at a ratio of 1/1 so that the final concentration of citrate was 0.5 m*M*. The flow-rates varied depending on the technique employed and are given in the results and discussion section. The injection volume for the determination of the detection limits in the ion-exchange mode was 100 μl, for other experiments the injection volume is given in the results and discussion section.

2.4. Mass spectrometry

A pneumatically assisted electrospray was employed throughout this work. Nitrogen 5.0 was used as both the spraying gas and the drying gas. Data were acquired in the negative selected ion monitoring (SIM) mode except for the collisionally induced dissociation experiments which were performed in the scan mode.

2.5. Sample pretreatment for drinking water

Sample pretreatment of water samples included the removal of sulfate, chloride and hydrogen carbonate by solid-phase extraction using a strong cation-exchange (SCX) material. Beside a commercially available SCX cartridge (Bond Elut, 3 ml, Analytichem International) also laboratory-made cartridges were tested using the same cation-exchange material as for the preparation of the suppressor column. 250 mg of these particles were filled into each of three glass cartridges (I.D. 12 mm) and conditioned by passing 5 ml of methanol, 10 ml of 50 m*M* sulfuric acid and 15 ml of Milli-Q water through the bed. For sulfate removal one cartridge was then flushed with 5 ml of a 50 m*M* barium nitrate solution, for chloride removal another one was treated with 5 ml of a 100 m*M* silver nitrate solution. Finally the cartridges were washed with 30 ml Milli-Q water to remove nitrate and excess barium or silver.

Samples of drinking water were first passed through the cartridge in the barium form, then through the one in the silver form and finally through the cartridge in the H^+ form. The collected samples were injected directly into the HPLC system. One

cartridge could be used for the pretreatment of at least seven samples without notable decrease of efficiency.

3. Results and discussion

3.1. Separation of anions by ion-exchange chromatography

Typically employed eluents for ion-exchange chromatography are in many cases regarded as incompatible with electrospray MS because of contamination and plugging of the interface. Therefore, eluents recommended for any coupling of HPLC to MS generally contain volatile compounds as eluent additives. For this reason in anion-exchange chromatography the possible electrolytes are more or less limited to formate and acetate. The disadvantage of these anions is their low elution strength compared to other organic and inorganic anions. Mobile phases using formate and acetate at low concentrations lead to unduly long retention times and often to assymmetric peak shapes, whereas higher concentrations of these salts (up to 100 m*M*) cause a drastic decrease in sensitivity of the MS detection. To overcome these problems, a range of other electrolytes was evaluated in terms of compatibility and sensitivity of the detector. The stationary phase which promised the best results in this series of experiments was a low capacity anion-exchange resin based on polymethacrylate with alkylammonium as functional group (IC-Pak Anion HR column). Its total exchange capacity was 30 μequiv/g and separations were possible in aqueous media from pH 2 to pH 12 containing not more than 12% acetonitrile.

Experiments with an IC-Pak Anion HR column revealed that the use of non-volatile mobile phases such as solutions of carbonate, sulfate, oxalate and citrate did not result in contamination of the API interface except for the spray shield which could be cleaned very easily after 1 day's work (such a cleaning procedure takes only about 1 min). Fig. 1 shows a comparison of these eluents in terms of retention times and the signal-to-noise ratios of the analytes iodate, bromate and bromide. Strongly retained analyte anions such as sulfate, thiosulfate and iodide were not investigated in these experiments because the low elution strength of carbonate did not allow the analysis of these anions within a reasonable time. Carbonate, sulfate and oxalate eluents were used at a concentration of 0.9 m*M* at pH 9. Citrate having the highest elution power at the selected pH could be employed at a concentration of 0.45 m*M*, thereby still resulting in the lowest retention times and allowing the elution of strongly retained analytes within a reasonable time. The mobile phase contained 10% acetonitrile in each case to increase the sensitivity of the mass spectrometric detection. Because the flow-rate of 1 ml/min was not compatible with the API interface, the flow was split after the column so that only 12% entered the API interface. As can be seen from Fig. 1, citrate promised to be the most suitable eluent for the separation of a number of anions offering short analysis times and high sensitivity.

In another series of experiments the influence of the citrate concentration and the content of acetonitrile was investigated in detail. Fig. 2 shows the dependence of the mass spectrometric abundance of iodate, bromate and bromide on the citrate concentration ranging from 0 to 2 m*M* at a constant acetonitrile content of 50%. The measurements were performed by flow injection using the HPLC system without separation column in order to avoid changes in retention times and peak shapes resulting from different citrate concentrations. It can be seen that the decrease of the citrate concentration results in a more than proportional increase in sensitivity for all analyte ions. Additionally, the increase of the acetonitrile content in the mobile phase led to a noticeable increase of the signal intensities but the influence was by far not as strong as of the citrate concentration. Acetonitrile contents higher than 80% caused precipitation of analyte anions and were not investigated.

Using 0.45 m*M* ammonium citrate at pH 9 with 10% acetonitrile as the mobile phase the sensitivity of the mass spectrometer was optimized by adjustment of different parameters such as the pressure of the spraying gas, the flow-rate and temperature of the drying gas and the voltage at the end of the transfer capillary of the API interface. Especially the latter parameter strongly influences the sensitivity, since a different degree of fragmentation takes place depend-

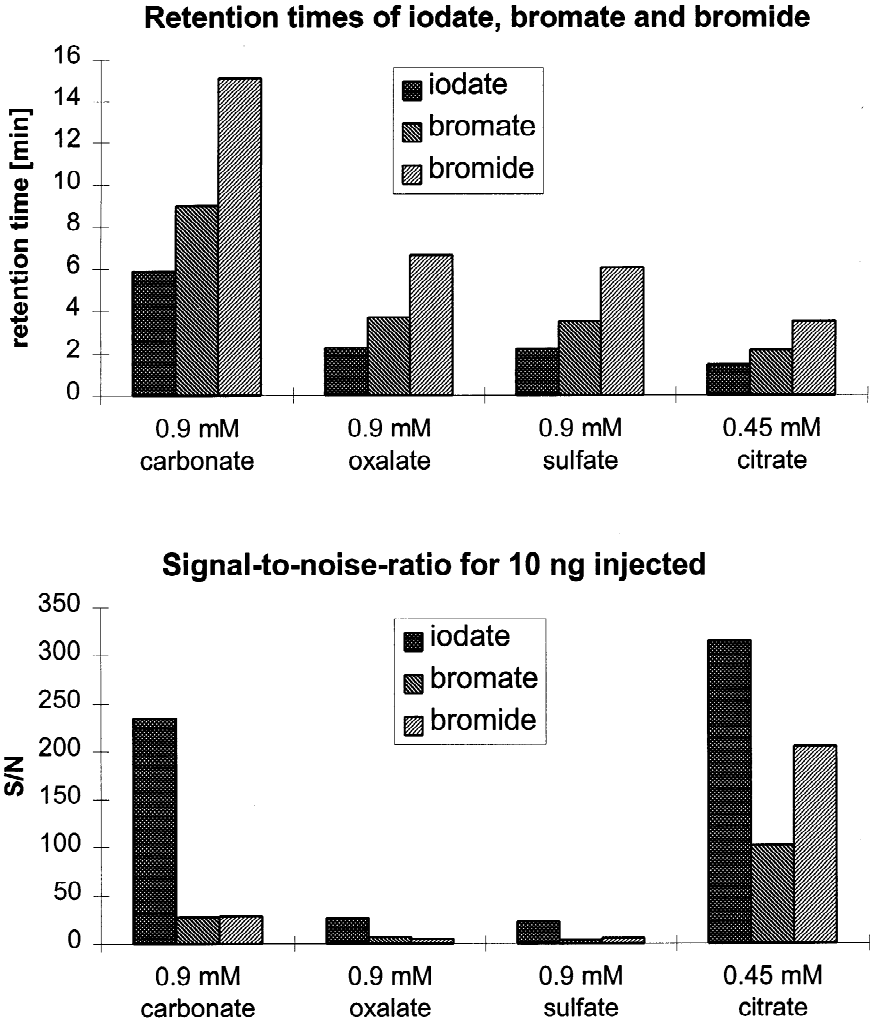

Fig. 1. Comparison of four different anions as the eluting species in non-suppressed IC with respect to retention times and signal-to-noise ratios (10 ng absolute amount) of iodate, bromate and bromide. Injection volume: 10 μl. Content of acetonitrile in the mobile phase: 10% (v/v). Flow rate: 1 ml/min. Split: 120 μl/min to the MS interface.

ing on the voltage at the capillary exit. The experiments revealed that there are two different aspects which must be considered when optimizing the capillary exit voltage: on the one hand the fragmentation, which occurs in the region of collisionally induced dissociation (CID), normally increases with increasing the absolute value of the capillary exit voltage; on the other hand the capillary exit voltage affects the acceleration and focusing of the ions depending on their molecular mass. The combination of these two mechanisms results in curves with minima and maxima as shown in Fig. 3. Iodate and bromate loose gradually oxygen resulting in fragments X^-, XO^- and XO_2^-, where X stands for either iodine or bromine. Especially bromate shows how the increase of the absolute capillary exit voltage favors the fragmentation; at a voltage of approximately −100 V the BrO_3^- is the most abundant ion, at −180 V the BrO_2^- fragment becomes the most intense ion, at −210 V the BrO^- is the predominant fragment and finally at voltages above −250 V only the Br^- fragment is stable. As a result from these

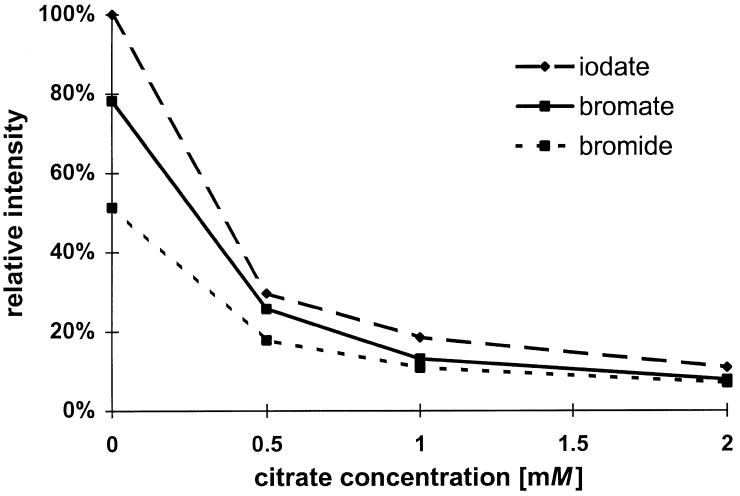

Fig. 2. Dependence of the mass spectrometric signal intensities of iodate, bromate and bromide on the citrate concentration at an acetonitrile content of 50% obtained from flow injection experiments. Injection volume: 10 μl. Concentration of anions: 10 mg/l each.

investigations it can be said that the proper adjustment of the voltage helps to achieve highest sensitivity in mass spectrometric detection. Iodate for example can be detected most sensitively either by the molecular ion (capillary exit voltage of about −150 V) or by the iodide-fragment (voltage of −300 V). The molecular ion of bromate shows the highest signal at a voltage of −90 V but the background noise is rather high. Therefore it is preferable to detect a fragment ion at a higher voltage, in this case the two bromide isotopes at a capillary exit voltage of about −200 V yielding the best signal-to-noise ratio. The optimum parameters for all analyte ions are summarized in Table 1.

Fig. 4 shows a typical separation of anions using the conditions described in Table 1 except for the injection volume which was 10 μl in this case. Iodate was detected at m/z values of 174.9 and 126.9, bromate and bromide at 78.9 and 80.9, sulfate at 97.0, thiosulfate at 112.9 and iodide at 126.9. Using the S/N-criterion, absolute detection limits were calculated for a S/N ratio of 3 and found to be 0.36 ng for iodate, 0.48 ng for bromate, 0.6 ng for sulfate and thiosulfate and 0.72 ng for bromide and iodide. Taking into account that the flow of the mobile phase was split (12% to the MS interface) and a injection volume of 100 μl was applied, these values correspond to analyte concentrations of 0.03 mg/l for iodate, 0.04 mg/l for bromate, 0.05 mg/l for sulfate and thiosulfate and 0.06 mg/l for bromide and iodide. Linearity of the calibration curve was observed from the detection limit up to 10 mg/l for each analyte anion except for thiosulfate which only showed a concentration of 1 mg/l as the upper limit of linearity. Correlation coefficients were obtained with standards of seven different concentration levels (each injected twice) and found to lie between 0.9993 (thiosulfate) and 0.9999 (bromide).

Among a number of possible applications, the method was applied to the determination of the ratio of phosphate to ^{18}O-labeled phosphate in a sample of a lyophilized culture medium which seemed to be suitable to demonstrate the power and usefulness of IC–MS of low-molecular-mass anions. In this special case the citrate concentration had to be lowered to 0.2 mM in order to separate phosphate from bromide. This separation was necessary because the detection of the phosphate species was performed at the PO_3^--fragment ions with m/z values of 79 for the non-labeled and 81 for the labeled phosphate respectively, which would have caused interference with the bromide isotopes. As a result the ratio of phosphate to ^{18}O-labeled phosphate was found to be 95.6% to 4.4% (m/m) for this culture medium.

The detection limits for some anions being achieved with the commercial IC-Pak Anion HR column may be sufficiently low for certain applications but trace analysis of anions down to the low

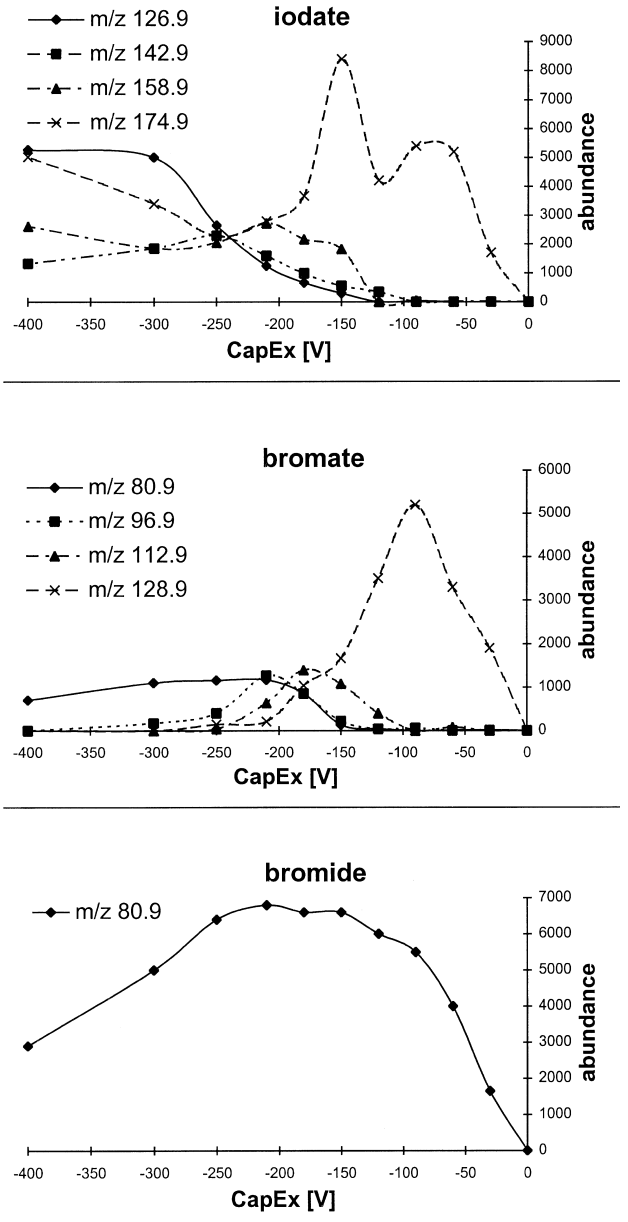

Fig. 3. Dependence of the fragmentation of anions on the voltage at the capillary exit (CapEx) in the API interface.

μg/l level requires further improvements of detection limits. The analysis of oxyhalides in drinking water seemed to be a challenging problem for IC–MS. An attempt to lower the detection limits was the employment of a separation column with a smaller inner diameter together with the application of a suppressed IC mode. For this purpose a stainless steel column with an I.D. of 2 mm and a length of 130 mm was prepared and tested for the separation of oxyhalides using the resin from an IC-Pak Anion HR Column as the stationary phase. Sodium carbonate containing 10% acetonitrile was chosen as the mobile phase at a flow-rate of 150 μl/min without split. The considerably lower elution strength of the

Table 1
Optimized MS operating conditions for the separation of inorganic anions in the non-suppressed IC mode using a pneumatically assisted electrospray interface in the negative SIM mode (column: Waters IC-Pak Anion HR, 75×4.6 mm; mobile phase: 0.45 m*M* ammonium citrate pH 9.0, 10% (v/v) acetonitrile; flow rate: 1 ml/min; split: 12% to the API interface; injection volume: 100 μl)

Nebulizing gas pressure:	60 p.s.i. (N_2) (1 p.s.i.=6894.76 Pa)	
Drying gas flow:	7 l/min (N_2)	
Drying gas temperature:	300°C	
Quadrupole temperature:	100°C	
Capillary voltage:	4000 V	
End plate voltage:	3500 V	
Cylinder voltage:	4000 V	
Dwell time:	300 ms (iodate, bromate, bromide)	
	600 ms (sulfate, thiosulfate, iodide)	
m/*z* values monitored:	Iodate:	174.9, 126.9
	Bromate	78.9, 80.9
	Bromide	78.9, 80.9
	Sulfate:	97.0
	Thiosulfate:	112.9
	Iodide:	126.9

carbonate anion compared to the citrate anion only allowed the separation of early eluting anions when applied at low concentrations (below 10 m*M*). Higher carbonate concentrations could not be employed satisfactorily because the ion-exchange capacity of the suppressor column would have been too low. A carbonate concentration of 5 m*M* was sufficient high to elute iodate, bromate and chlorate within a reasonable time (12 min) and allowed the operation of the suppressor column for about 5 h without regeneration.

The MS operating conditions were the same as in

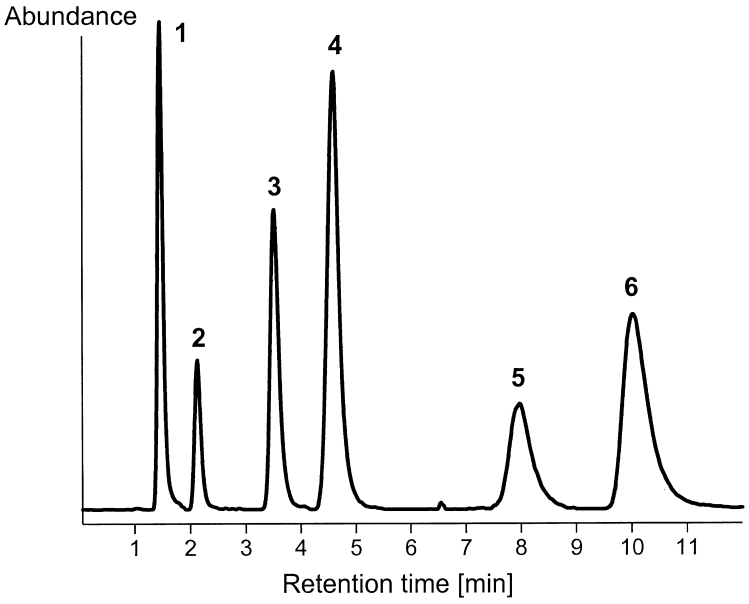

Fig. 4. Separation of a standard mixture of anions by ion-exchange chromatography with API-MS detection. Column: Waters IC-Pak Anion HR, 75×4.6 mm. Mobile phase: 0.45 m*M* ammonium citrate pH 9 containing 10% acetonitrile. Flow rate: 1 ml/min. Injection volume: 10 μl. The eluent was split so that only 120 μl/min entered the MS interface. 1=Iodate, 2=bromate, 3=bromide, 4=sulfate, 5=thiosulfate, 6=iodide. Concentrations: 10 mg/l each.

the non-suppressed mode except for the m/z values in the SIM acquisition. In the suppressed mode it was favorable to detect iodate, bromate and chlorate at their molecular ions with m/z values of 174.9, 126.9 and 83.0, respectively. For the application of the described method to the determination of oxyhalides in drinking water pretreatment of water samples was inevitable. The high content of chloride, sulfate and hydrogen carbonate, eluting near the oxyhalides caused interferences with the analyte anions resulting in assymmetric peak shapes and low sensitivity of the MS detection. To overcome these problems, sulfate, chloride and hydrogen carbonate were removed by passing the water samples through three cartridges filled with a strong cation-exchange material which had been brought into the Ba^{2+}, Ag^{+} and H^{+} form respectively.

This pretreatment allowed the following separation of iodate, bromate and chlorate with detection limits of 0.5 μg/l (50 pg absolute) applying an injection volume of 100 μl. Fig. 5 shows a chromatogram of a fortified drinking water sample (10 μg/l of each oxyhalide) after pretreatment. Although sulfate, eluting after chlorate, could not be removed completely the sulfate content could be lowered to an extent that did not interfere with the detection of chlorate.

In a series of further experiments recoveries and linearity data were investigated. The recoveries of the oxyhalides were determined using a drinking water sample spiked with 10 μg/l of each oxyhalide. Mean recoveries after removal of sulfate, chloride and hydrogen carbonate were found to be 96.1% for iodate, 95.6% for bromate and 98.7% for chlorate; relative standard deviations were 8.7% for iodate, 6.5% for bromate and 7.3% for chlorate respectively (four replicates). The repeatability was only checked for the determination of bromate in real water samples and was between 1.8 and 5.1% (expressed as relative standard deviation for four different samples and six replicates each). Accuracy will also be checked from the results of a currently running interlaboratory trial for trace determination of bromate.

Linearity of the calibration curves was given from the detection limit up to 100 μg/l with correlation coefficients of 0.9997 (iodate), 0.9979 (bromate) and 0.9998 (chlorate).

The method was applied to the determination of

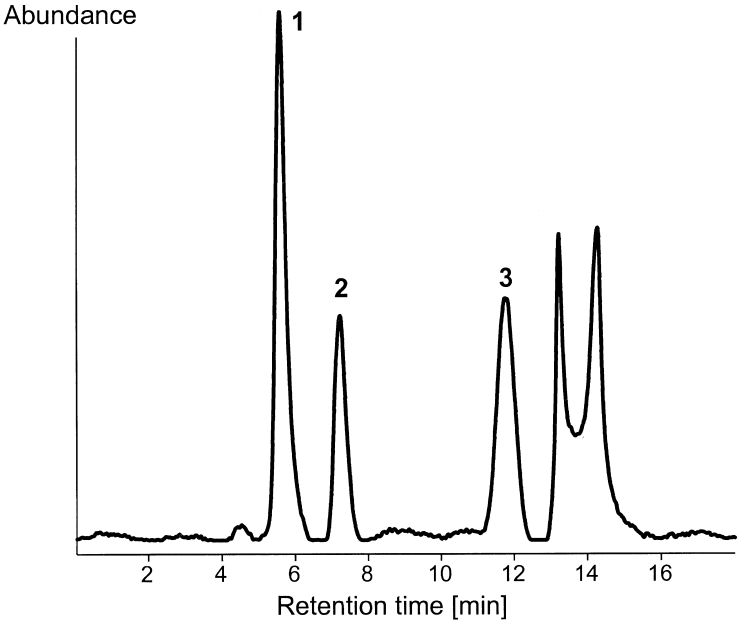

Fig. 5. Chromatogram of a drinking water sample spiked with 10 μg/l of each oxyhalide after removal of sulfate, chloride and hydrogen carbonate. Column: Waters IC-Pak Anion HR, 130×2 mm. Mobile phase: 5 m*M* sodium carbonate, 10% acetonitrile. Flow rate: 150 μl/min. Injection volume: 100 μl. MS detection. Other conditions see text. 1=Iodate, 2=bromate, 3=chlorate.

oxyhalides in Austrian drinking water samples. Typical water samples contained up to 30 mg/l nitrate, 40 mg/l chloride and 100 mg/l sulfate with a water hardness up to 3.5 mmol/l. In three of five samples chlorate was found at concentrations between 2.7 and 6.2 μg/l. Iodate and bromate were not detected in any sample. Additionally, bromate was determined in five samples during an interlaboratory trial at concentrations between 2.3 and 8.8 μg/l.

3.2. Separation of organic acids

In contrast to the liquid chromatographic (LC) analysis of inorganic anions which is generally done by ion-exchange chromatography, the separation of organic acids may utilize different chromatographic techniques such as reversed-phase, ion-pair, ion-exclusion, or ion-exchange chromatography. For the combination of these techniques with API-MS, system optimization seems to be more complex than in the case of inorganic anions. Similar to the analysis of inorganic anions, the operating conditions have to be optimized in terms of both LC separation and MS detection; additionally, the degree of dissociation of organic acid may be of significant importance for the mass spectrometric sensitivity.

3.2.1. Reversed-phase and ion-pair chromatography

Reversed-phase chromatography was performed on a YMC ODS-column using either diluted trifluoroacetic (TFA) or formic acid as the mobile phase. A concentration of 1 m*M* TFA (pH 3.1) and a flow-rate of 100 μl/min resulted in baseline-separation of lactic, succinic, propionic, glutaric and butyric acid but the mass spectrometric sensitivity was very poor. In a series of experiments the influence of the TFA concentration on the mass spectrometric sensitivity was investigated. As can be seen in Fig. 6A, the sensitivity decreased drastically when TFA was added to the mobile phase even at very low TFA concentrations. Measurements were performed by flow injection analysis without separation column in order to avoid changes of peak areas and peak shapes due to different retention times depending on the TFA concentration.

To overcome the obvious unsuitability of TFA, it was replaced by formic acid. A comparable pH of the eluent as well as comparable retention times could be achieved with a formic acid concentration of 5 m*M*. By flow injection experiments the dependence of the MS sensitivity on the concentration of formic acid was investigated and the results are given in Fig. 6 B. It can be seen that the decrease of intensity with increasing formic acid concentration is much lower for lactic, succinic and glutaric acid compared to the strong decrease with TFA. Propionic and butyric acid showed a rather low intensity even with formic acid as eluent.

Although the signal intensities of some organic acids were much higher using diluted formic acid instead of TFA as the mobile phase, the method suffered from low resolution and a tailing peak shape of some acids and therefore seemed to be not fully appropriate for practical purposes.

The attempt to improve resolution by addition of ion-pairing reagents (octylamine at pH 5.4) to the mobile phase resulted in a low MS sensitivity that is not really surprising. Obviously the organic acids were eluted as electrically uncharged species consisting of the organic anion and octylammonium as the counter-ion, so that decreasing transfer efficiencies from the atmospheric part of the interface into the vacuum region caused a decrease in MS signal intensity.

3.2.2. Ion exclusion chromatography

The alternative to reversed-phase and ion-pair chromatography for the separation and sensitive detection of organic acids might be ion exclusion chromatography. Using a Bio-Rad Fast Acid Analysis Column HPAH and 3 m*M* formic acid as the eluent several organic acids such as citric, malic, fumaric, propionic, butyric and gallic acid could be separated within 35 min. Although this column is usually employed with flow-rates of about 1 ml/min, a flow-rate of only 0.2 ml/min was chosen which caused higher retention times but also prevented the loss of sensitivity due to splitting of the eluent. Further tests showed that the low flow-rate did not decrease the theoretical plate number, on the contrary the highest numbers were achieved between 0.15 and 0.2 ml/min.

3.2.3. Ion-exchange chromatography

Best results for the analysis of organic acids with

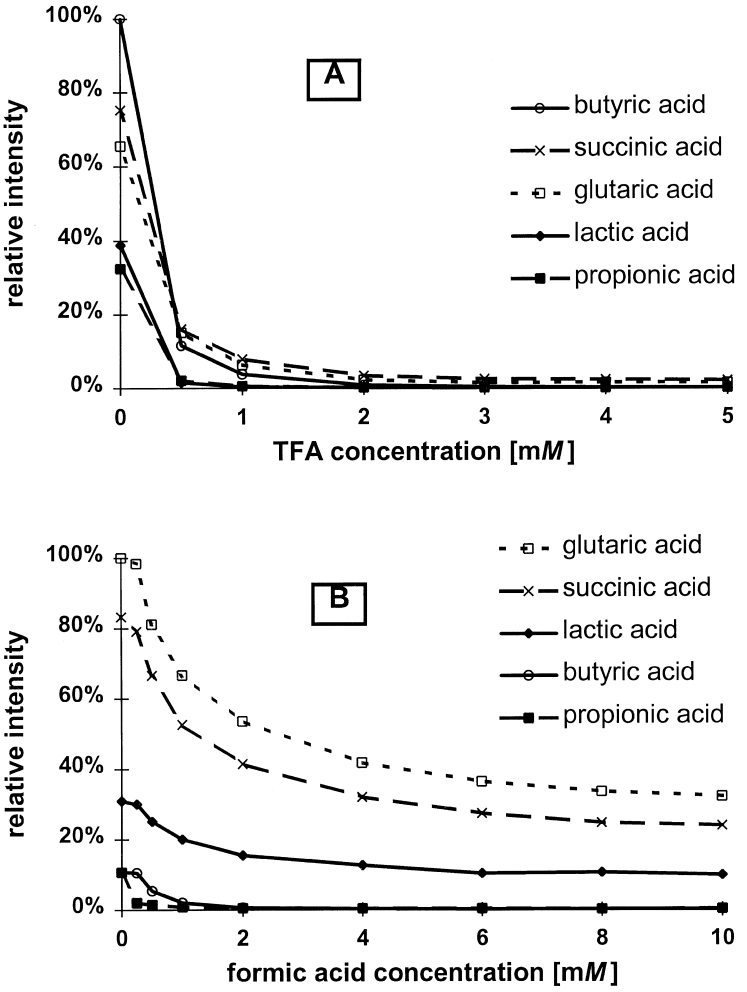

Fig. 6. Dependence of the mass spectrometric signal intensities of organic acids on the concentration of trifluoroacetic acid (A) and formic acid (B) in ion exclusion chromatography with MS detection obtained by flow injection experiments. Flow rate: 100 μl/min. Injection volume: 1 μl. Concentrations: 125 mg/l each.

respect to detection limits were obtained with a 200×1 mm micro column packed with dimethylamino-modified Nucleosil particles. Under acidic conditions an anion-exchange mechanism can be generated with the exchange capacity being dependent on the pH of the mobile phase. The addition of acetonitrile positively influenced sensitivity of the MS detection but had only little effect on retention times within a range between 20 and 80%. Therefore it could be varied without restrictions for achievement of maximum signal-to-noise ratios of the analytes. A mixture of 50% of a 1 m*M* ammonium citrate pH 4.8 and 50% acetonitrile as the mobile phase at a flow-rate of 100 μl/min proved to be suitable for both the separation of a number of organic anions and their sensitive detection by API-MS. Fig. 7 shows a typical separation of propionate, gallate, malonate, succinate, glutarate, malate, tartrate and fumarate. Except for propionate and gallate all analytes showed baseline separation; the background noise could be reduced to a minimum except for glutarate and fumarate. The latter two anions are also produced in the API-interface by degradation of citrate and therefore led to a higher background noise

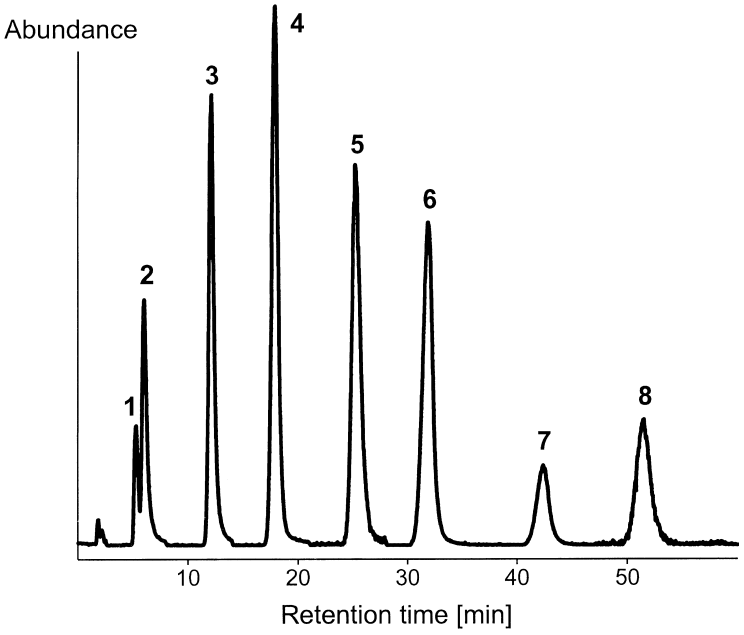

Fig. 7. Separation of a standard mixture of organic acids by ion-exchange chromatography with API-MS detection. Column: Nucleosil 100-5 $N(CH_3)_2$, 200×1 mm. Mobile phase: 0.5 m*M* ammonium citrate pH 4.8, 50% acetonitrile. Flow rate: 100 μl/min. Injection volume: 1 μl. 1=Propionate, 2=gallate, 3=malonate, 4=succinate, 5=glutarate, 6=malate, 7=tartrate, 8=fumarate. Concentrations: 125 mg/l each.

which deteriorated the detection limits especially for fumarate. Detection of all analytes in the SIM-mode was performed at the *m*/*z* values of their singly-deprotonated anions which was 73 for propionate, 169 for gallate, 103 for malonate, 117 for succinate, 131 for glutarate, 133 for malate, 149 for tartrate and 115 for fumarate, the dwell time was adjusted to 600 ms. The temperature of the drying gas was kept at 150°C in order to keep fragmentation (especially decarboxylation) of the analytes low. Other MS conditions were the same as described in Table 1. Under optimized conditions the detection of some analytes down to the low μg/l level was possible with an injection volume of 100 μl. Detection limits and linearity data are summarized in Table 2. The data were calculated from a series of injections of

Table 2
Linearity data and detection limits for eight organic anions separated by ion-exchange chromatography and API-MS detection. Number of data points: 10 (2 replicate each). Values in brackets correspond to the absolute detection limits. For experimental conditions see text.

	Linear range	Correlation coefficient	Detection limit (*S*/*N*=3)
Propionate	0.02–12 mg/l	0.9987	22.7 μg/l (2.27 ng)
Gallate	0.04–12 mg/l	0.9992	39.9 μg/l (3.99 ng)
Malonate	0.006–12 mg/l	0.9988	6.4 μg/l (0.64 ng)
Succinate	0.004–12 mg/l	0.9983	3.7 μg/l (0.37 ng)
Glutarate	0.06–12 mg/l	0.9987	58.0 μg/l (5.80 ng)
Malate	0.006–12 mg/l	0.9988	5.6 μg/l (0.56 ng)
Tartrate	0.04–12 mg/l	0.9992	38.1 μg/l (3.81 ng)
Fumarate	0.07–12 mg/l	0.9991	73.1 μg/l (7.31 ng)

five standard solutions whereby each standard was injected twice. Detection limits were calculated using a S/N criterion of 3.

4. Conclusion

Both non-suppressed and suppressed IC in combination with MS proved to be a versatile and powerful technique for the analysis of low-molecular-mass inorganic anions. In the case of non-suppressed IC citrate was found to be a compatible eluent for IC–MS yielding absolute detection limits below 1 ng. It allowed the determination of strongly retained analytes like sulfate, thiosulfate and iodide beside early eluting species such as iodate, bromate and bromide within a reasonable analysis time. Although it is not volatile, no contamination of the MS system was observed.

Suppressed IC using sodium carbonate as mobile phase resulted in an improvement of the absolute detection limits by approximately one order of magnitude for certain anions like iodate, bromate and chlorate. Using a 100 μl injection these detection limits were low enough to determine these oxyhalides in drinking water at concentrations down to 0.5 μg/l.

Compared to inorganic anions, organic acids partially showed poorer detection limits and long retention times. Different separation techniques such as capillary electrophoresis hyphenated with MS may result in improvements of analysis time and will be the topic of upcoming investigations.

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