Application of ion interaction chromatography to the determination of metal ions in natural water samples

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Abstract

The retention behaviour of Fe(III), Cu, Pb, Zn, Ni, Co, and Mn in ion interaction chromatography was investigated to optimise metal ion separation and detection in water samples. A solution of an alkanesulfonate, the ion interaction reagent (IIR), tartaric acid, and acetonitrile (or methanol) was used as eluent. Retention times were found to be accelerated by increasing the following: organic modifier concentration, tartaric acid concentration, and the pH of the eluent solution. In contrast, increasing the length of the IIR carbon chain was found effective in enhancing the retention times of metal ions. An analytical method for the determination of metals at the μg/l level was developed and applied to the analysis of natural water samples.

1. Introduction

During the last decade, increasing attention has been paid to the application of HPLC techniques for the determination of metal ions. Robards et al. [1] have summarised a large number of HPLC procedures applied to the determination of heavy metals, emphasising the rapid evolution of such techniques in metal analysis.

The chromatographic determination of metals with RP-HPLC can be grouped in two main approaches. The first is based on the preliminary addition of a chelating agent to the water sample and the extraction of the metal complexes with a convenient organic solvent. After separation from the aqueous layer, the organic phase is injected on a reversed-phase column where the metal complexes are separated with a aqueous–organic eluent [2-5]. This "pre-column" analytical strategy has several advantages, some of them related to the extraction step, such as the low limit of detection achievable and the reduction of matrix effects. Others advantages are connected to the use of conventional reversed-phase chromatography. The main disadvantages are the use of organic solvents–in the extraction and/or chromatographic steps–and the time-consuming sample manipulation.

The second approach is based on the dynamic modification of a conventional C_{18} reversed-phase into a low-capacity ion-exchange phase, accomplished by using an eluent containing an

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anionic surfactant agent. After separation in the column, the metals are detected using post-column reactions that provide for a rapid formation of a coloured compound. This approach allows for less sample treatment and more complete speciation of metals, given that, theoretically, no sample treatment–i.e. no matrix modification–should be required. The techniques which use an ion interaction reagent (IIR) to accomplish the partitioning of chemical species between a stationary and a mobile phase, have been named in different ways, e.g. dynamic ion-exchange chromatography [6,7], ion-pair chromatography [8], soap chromatography [9]. We prefer to name the technique ion interaction chromatography (IIC) [10,11] and we refer to this definition throughout the paper. IIC has already been extended to the determination of metal ions in various types of samples. Cassidy and Elchuk [6] were the first to propose the dynamic derivatization of a reversed-phase column with sodium octanesulfonate for the separation of metals. More recently such a method was applied by Schuster and Hampel to determine metals in fermentation broth [12], by Sturaro and co-workers to analyse human hair [13,14], and by Saraswati to analyse Nd-Fe-B magnetic material [15]. Litvina et al. [16], used a C_{18} phase, modified with dodecylbenzenesulphonic acid and cetyltrimethylammonium bromide, to separate alkaline earth and transition metal cations. Voloschik et al. [17] have recently reported the separation and determination of some transition metals on a dynamically coated C_{18} column with conductimetric detection.

For the analysis of natural waters, IIC seems particularly attractive for its low cost compared to other instrumental analysis (e.g. atomic absorption spectroscopy) and for the ability to provide, in a relatively short time, a complete screening of the possible species present in the sample. The purpose of this paper is to investigate the chromatographic behaviour of metal ions in IIC when the stationary phase is modified with an alkanesulfonate and to assess a method for the determination of transition metals at low concentration level in natural water by IIC.

2. Experimental

2.1. Apparatus

The HPLC system consisted of an isocratic pump (Waters Model 501) and an injection valve (Rheodyne Model 7125, metal free). The detector signal was monitored with a variable-wavelength absorbance detector (Waters Model 484) and the raw data were acquired and analyzed with a computer program (Maxima 820, Dynamic Solution) and an integrator (Shimadzu Model CR34A). The eluted metal ions were detected with a post-column derivatization system (Water delivery system module). The post-column reagent was a solution of 4-(2-pyrydilazo)resorcinol (PAR) $(2 \cdot 10^{-4}\ M)$ in 2 M ammonia and 1 M ammonium acetate [6]. To obtain the maximum absorbance signal of the PAR–metal complexes it was necessary to maintain the flow of both the PAR reagent and the eluent at the same value (1 ml/min) and to read the absorbance at 510 nm. The chromatographic column was kept at constant temperature with a glass water jacket connected to a thermostat (Haake). All the experiments were performed at 30°C.

2.2. Reagents and materials

Standard metal solutions were prepared from an atomic absorption standard solution of 1000 ppm (Titrisol, Merck). All other chemicals were of analytical grade from Aldrich (Milwaukee, WI, USA). All solutions were prepared with water purified with a Milli-Q apparatus (Waters). Eluents were prepared by dissolving the appropriate amount of reagents in water, adjusting the pH with NaOH, filtering and degassing through a 0.22-μm filter. We used two different reversed-phases: 10 μm silica-based Bondclone C_{18} (Phenomenex), and 5 μm silica-based Hypersyl C_{18} (HPLC Technology). To remove metal traces, all glassware was cleaned for 10 h on a Tolg apparatus, refluxing with a 20% solution of HCl [18]. Nylon on-line filter (Phenomenex) and C_{18} solid-phase extraction (SPE) micro-columns

(HPLC Technology) were used for filtering and clean-up of natural water samples.

3. Results

3.1. Effect of ion interaction reagent

The main role of the anionic surfactant (IIR) is to make available, via its adsorption on the stationary phase, exchangeable sites for the adsorption of charged species. Given that the number of exchangeable sites depends on the amount of surfactant adsorbed, the concentration of IIR in the mobile phase and the kind of IIR used strongly affects retention time and chromatographic selectivity.

Fig. 1 shows the effect of the concentration of various alkanesulfonates–from five to ten carbon atoms in the alkyl chain–on the metal's capacity-
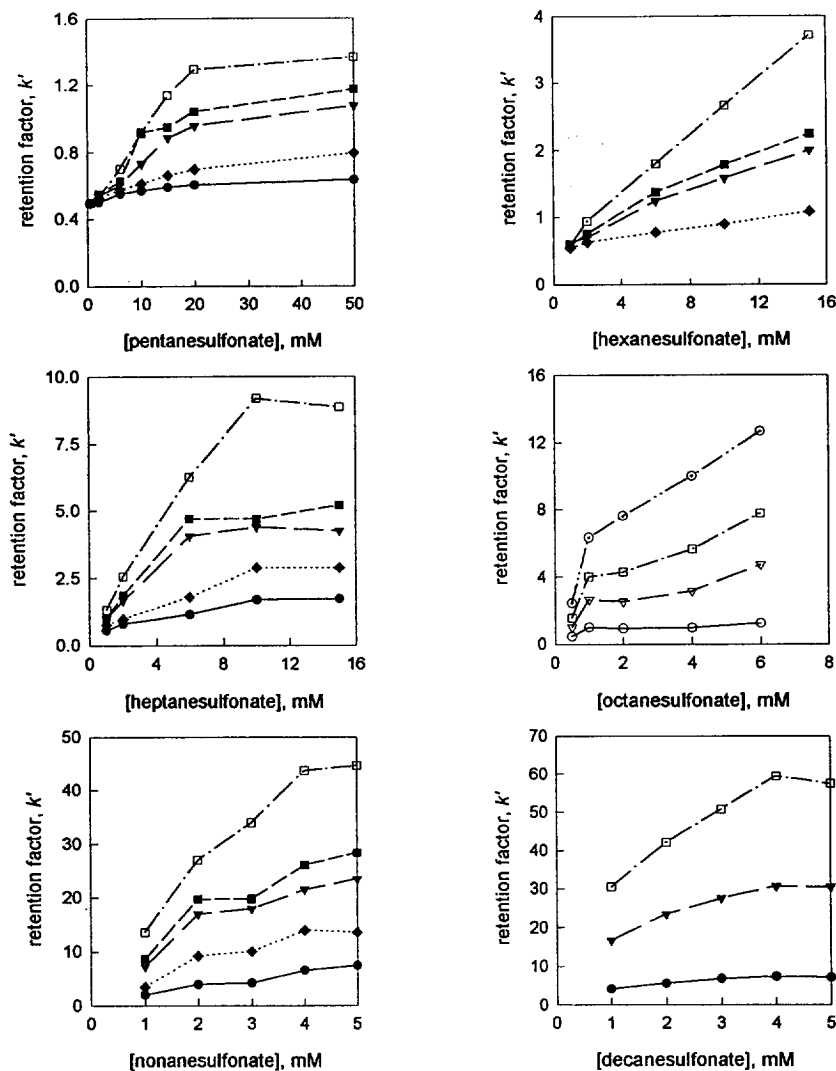

Fig. 1. Effect of the concentration of some alkanesulfonates on the retention factor of some metal ions: Cu = ●; Pb = ◆; Zn = ▼; Ni = ■; Co = □; Mn = ○. Eluent: 50 mM tartaric acid, 5% (v/v) acetonitrile, pH 3.4. Flow-rate, 1 ml/min. Stationary phase, Hypersil C_{18}.

factor. It can be observed that retention time increases with the concentration of surfactant in the eluent. In the lower concentration range the increase of k' is more rapid, corresponding to a more efficient and rapid coverage of the stationary phase with the surfactant. Above a certain surfactant concentration, which depends on the surfactant chain-length, the slope of the curve decreases, indicating an almost complete saturation of the phase with the IIR. This behaviour closely follows the trend of the adsorption isotherms of the IIR, which are Langmuir-type curves [19]. The reaching of a plateau is clearly evident with pentanesulfonate (above a concentration of 20 mM), which allows to reach a relatively high concentration in the eluent without exceeding the critical micelle concentration (CMC). For the other surfactants this behaviour is less evident. In any case the reduction of the slope of the k' vs. [IIR] curve can be used as an indication of stationary phase saturation.

For each surfactant it is possible to determine the concentration which gives good separation of the metals. The longer the chain, the lower the concentration of surfactant can be. For analytical purposes, octane-, nonane-, and decanesulfonate
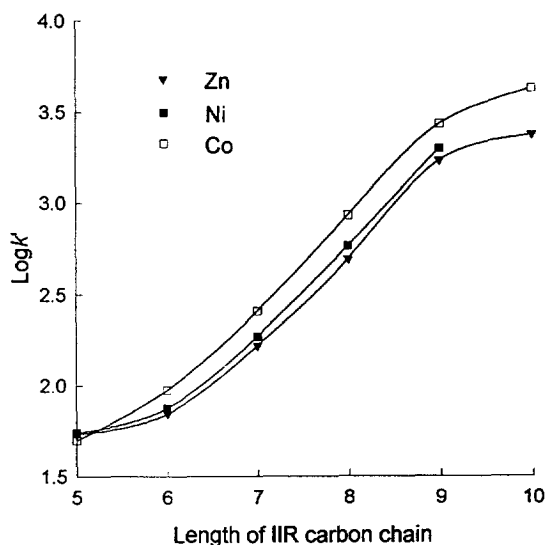

Fig. 2. Effect of surfactant carbon number on the retention factor of some metals. Eluent: 2 mM alkanesulfonate, 50 mM tartaric acid, 5% (v/v) acetonitrile, pH 3.4. Flow-rate, 1 ml/min. Stationary phase, Hypersil C_{18}.

are the IIRs of choice. To avoid fluctuation in retention time, it is important to select an IIR concentration far from the zone where the slope of k' vs. [IIR] is at its maximum. Under the experimental used, octanesulfonate was found to offer both good metal separation and an adequate analysis time.

It is interesting to underline the role of chain length on retention factor. Fig. 2 shows the increase of the retention factor of metals with increasing alkyl chain length of the IIR. Actually, for equal surfactant concentrations the dimension of the chain is the factor governing the metal retention. This behaviour is certainly due to the higher hydrophobicity of longer-chain surfactants, which results in a more efficient adsorption on the stationary phase. A plot of the logarithm of the retention factor versus the carbon number of the surfactant alkyl chain is clearly linear in the range from 6 to 9. A similar log-linear dependence has been observed, from 8 to 13 carbon atom, by Tomlinson et al. [20] for the retention of large anionic solutes with alkylbenzyldimethylammonium chlorides at $1 \cdot 10^{-4}$ M.

In our case the log-linear dependence is not fully respected for pentane- and decanesulfonate. In the case of pentanesulfonate this could be ascribed to the fact that the short retention time observed at the concentration of 2 mM did not allow for a correct evaluation of k'. With decanesulfonate shorter than expected retention times of the eluted metals are observed. This can, however, not be explained by the formation of micelles in the mobile phase, because the concentration of 2 mM is far from the value of about 40 mM corresponding to the CMC of decanesulfonate [21].

3.2. Effect of organic modifier

Fig. 3 shows the effect of the concentration of acetonitrile and methanol on the retention factors of metals. The retention factors of all metals are lowered by reducing the dielectric constant of the mobile phase. This effect is stronger with acetonitrile, due to its lower polarity.
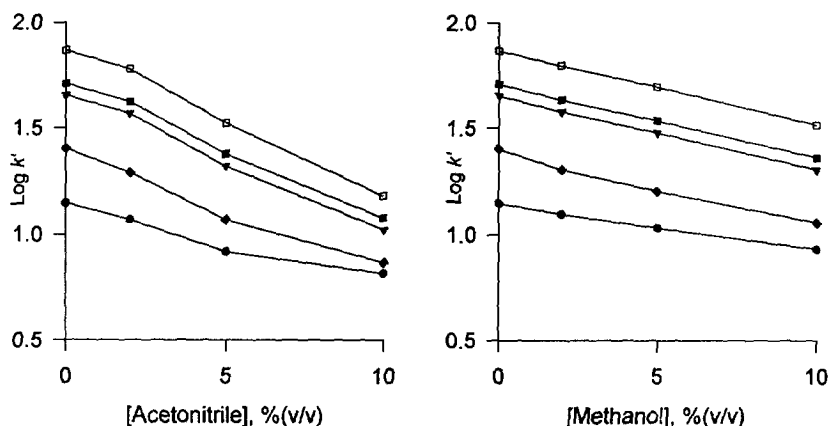
Fig. 3. Dependence of the retention factor of metals on the concentration of the organic modifier in the eluent. (left) Acetonitrile, (right) methanol. Eluent: 2 mM octanesulfonate, 50 mM tartaric acid, pH 3.4. Flow-rate, 1 ml/min. Stationary phase, Hypersil C_{18}. Cu = ●; Pb = ◆; Zn = ▼; Ni = ■; Co = □.

Both organic modifiers follow closely the relationship characterising ion-pair elution:

$$log\ k' = log\ k - S\varphi \qquad (1)$$

where φ is the volume fraction of the solvent, k is the retention factor in pure water, and S is a parameter dependent on the polarity of the mobile phase and on the kind of stationary phase [22]. This correlation suggests that the eluted analyte is a neutral compound.

When acetonitrile is used as organic modifier, the linearity predicted by Eq. 1 is less evident. This lack of linearity can be explained by considering that the addition of an organic modifier to the mobile phase induces two different phenomena. The first, which is accounted for in Eq. 1, is the enhancement of analyte partitioning in the mobile phase. The second phenomenon is the reduction of IIR adsorption, caused by the reduction of mobile-phase polarity. The result of this lowered adsorption is the reduction of the number of exchangeable sites on the stationary phase (i.e. of the exchange capacity), and the consequent increase of the elution rate. This second phenomenon is not predicted by Eq. 1 and caused the observed deviation from experimental data. To obtain an acceptable time of analysis with good peak resolution, the water samples were analysed using a mobile phase with 5% of acetonitrile.

3.3. Effect of organic ligand and pH

Fig. 4 shows the reduction of retention factors and of chromatographic selectivity with increasing pH of the mobile phase. In Fig. 4 the species distribution of the ligand as a function of pH is also shown. Tartaric acid concentration and pH have similar effects on retention and selectivity [11] because both variables act on the species distribution of the ligand. Increase of the tartaric acid concentration shifts the reaction of metal complex formation to the right. On the other hand, raising the pH increases the fraction of the completely dissociated tartrate (L^{2-})–i.e. the strongest complexing species–resulting in an acceleration of the elution rate.

3.4. Analysis of real samples

Fig. 5 shows the chromatogram of eight metals [Fe(III), Cu, Pb, Zn, Ni, Co, Cd, and Mn]. The chromatogram was obtained by using an eluent with the following composition: 2 mM octanesulfonate, 50 mM tartaric acid, 5% acetonitrile, adjusted to pH 3.0 with NaOH. Under these conditions a very good resolution can be achieved; even if one operates in the ion-interaction mode, the number of theoretical plates of the column is lower than that obtained in the reversed-phase mode (Table 1).
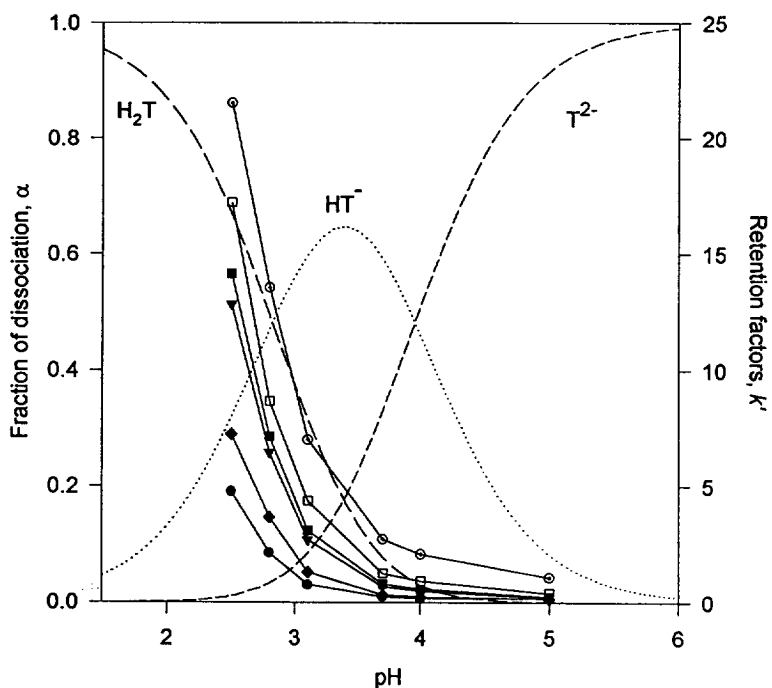
Fig. 4. Effect of pH on the retention factor of metals and on tartaric acid (H_2T) dissociation fraction (α). Eluent: 2 mM octanesulfonate, 60 mM tartaric acid. Flow-rate, 1 ml/min. Stationary phase, Hypersil C_{18}. Cu = ●; Pb = ◆; Zn = ▼; Ni = ■; Co = □; Mn = ○.
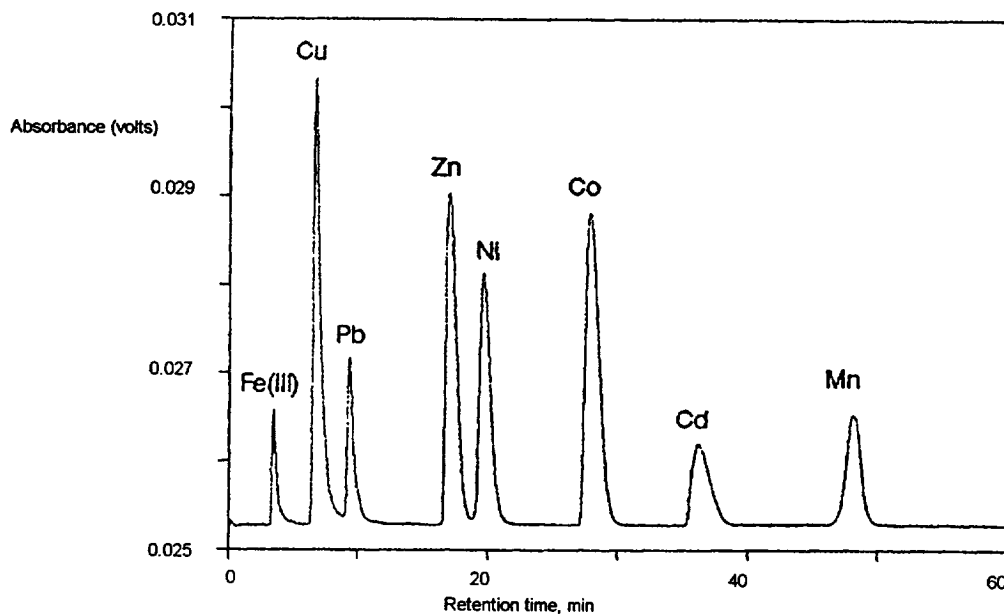

Fig. 5. Chromatogram showing the separation of eight metals obtained in ion interaction chromatography. Eluent: 2 mM octanesulfonate, 50 mM tartaric acid, pH 3.4, 5% (v/v) acetonitrile. Flow-rate, 1 ml/min. Stationary phase, Hypersil C_{18}, 5 μm. Concentration of metals: 3 ppm for Fe, 1 ppm for the other metals. Injection volume, 50 μl.

Table 1
Comparison of the number of theoretical plates of columns used in reversed-phase mode and in ion-interaction mode with two different alkanesulfonates

Phase	Particle size (μm)	Length (mm)	Width (mm)	N/m			%	
				RP	C_8	C_{10}	C_8	C_{10}
Bondclone	10	300	3.9	17 000	14 000	15 500	83	91
Hypersyl	5	250	4.6	44 000	35 000	39 500	80	90

Legend: RP = reversed-phase; C_8 = ion-interaction mode with sodium octanesulfonate; C_{10} = ion-interaction mode with sodium decanesulfonate.

The regression parameters and the detection limits for the investigated metals are reported in Table 2. The linearity ranged from 25 ng (50 ppb) to 500 μg (1 ppm) with an injection volume of 500 μl. The absolute detection limits, reported in Table 2, were calculated as the amount injected that gave a signal that was three times the background noise.

Matrix effects on metal recovery in real samples are mainly due to physical and chemical phenomena such as hydroxide formation, side reactions with natural and anthropic ligands, and adsorption on colloids and particles. A common practice in natural water analysis is to add nitric acid to the sample to prevent hydroxide and complex formation. Unfortunately, injecting a large amount (500 μl) of a sample acidified with strong mineral acids resulted in poor repeatabili-

Table 2
Limits of detection for the metals

Metal	Limit of detection (ng ml^{-1})	a	b	R
Fe	100	− 57	5	0.9999
Cu	20	− 560	230	0.9997
Pb	20	− 280	40	0.9998
Zn	2.5	1600	280	0.9999
Ni	2.5	90	190	0.9999
Co	2.0	2200	280	0.9996
Mn	2.0	− 50	60	0.9994

Calibration equation $y = a + b \cdot x$, where y is the metal area and x is the amount of metal injected in ng.

ty of retention times and in a lack of resolution for the peaks eluting near the solvent peak. Thus, we took advantage of the intrinsic acidity of the eluent using tartaric acid to "acidify" the sample. A low amount of 1 M tartaric acid (about 1% v/v) was normally sufficient to reach a pH near 3. Moreover, because of its complexing ability, tartaric acid can facilitate the mobilisation of metals and make the composition of the sample closer to that of the eluent.

To evaluate the effect of matrix complexity on analytical performance, we investigated the recovery of heavy metals from three different water samples: tap water, rain water (wet deposition), and fog water. The parameters tested were: the effectiveness of using tartaric acid in stead of any strong mineral acid for sample acidification; the effect of filtering on 0.22-μm nylon filter to remove particulates; and the effect of elution of the sample on a C_{18} SPE column to reduce dissolved organic matter.

Before HPLC analysis each sample was divided into four portions that were treated in the following way:

Treatment A: 50 ml of sample were filtered on a 0.22-μm Nylon filter;

Treatment B: 50 ml of sample were spiked with Cu, Pb and Co, till a final concentration of 100 ppb each, then filtered.

Treatment C: 50 ml of sample were treated as in B and then eluted through a C_{18} SPE micro-column.

Table 3
Recovery of some metals in tap water samples under different sample treatments

Metal	Sample A	Sample B	Rec. B (%)	Sample C	Rec. C (%)	Sample D			
						1% H$_2$T	Rec. (%)	2% H$_2$T	Rec. (%)
Cu	< LOD	93 ± 5	93	94 ± 5	94	97 ± 7	97	105 ± 3	105
Pb	< LOD	104 ± 5	104	102 ± 5	102	102 ± 9	102	110 ± 6	110
Zn	155 ± 5	156 ± 5	–	157 ± 5	–	157 ± 5	–	157 ± 1	–
Co	< LOD	97 ± 5	97	97 ± 5	97	97 ± 4	97	98 ± 3	98

LOD = limit of detection. Standard deviation calculated with four replicated samples. Concentrations are in ppb.

Table 4
Recovery of some metals in rain water samples under different sample treatments

Metal	Sample A	Sample C	Rec. C (%)	Sample D			
				1% H$_2$T	Rec. (%)	2% H$_2$T	Rec. (%)
Cu	< LOD	61.0 ± 0.2	61	73 ± 2	73	102 ± 2	102
Pb	62 ± 2	163 ± 3	101	207 ± 5	130	208 ± 2	130
Zn	22.6 ± 0.6	23 ± 1	–	29 ± 2	–	35.5 ± 0.7	–
Ni	11.1 ± 0.2	11.3 ± 0.5	–	13 ± 1	–	15.6 ± 0.1	–
Co	< LOD	102 ± 2	102	103 ± 2	103	103 ± 1	103
Mn	6.0 ± 0.5	6.0 ± 0.5	–	6.1 ± 0.6	–	6.0 ± 0.4	–

LOD = limit of detection. Standard deviation calculated with four replicated samples. Concentrations are in ppb.

Table 5
Recovery of some metals in a fog water samples under three different sample treatments

Metal	Sample F1	Sample H1	Sample H2
Fe	470 ± 5	484 ± 3	486 ± 2
Cu	9.5 ± 0.5	11 ± 1	15 ± 1
Pb	48 ± 2	65 ± 3	77 ± 1
Zn	128 ± 5	127 ± 3	128 ± 2
Ni	14 ± 1	13 ± 2	13 ± 2
Mn	11 ± 2	12 ± 3	11 ± 1

F1 = filtration and elution on SPE; H1 = acidification with 1% tartaric acid, then the same treatment of F1; H2 = as sample H1 but with 2% of tartaric acid. Standard deviation calculated with four replicated samples. Concentrations are in ppb.

Treatment D: 50 ml of sample were spiked with Cu, Pb and Co, till a final concentration of 100 ppb each. Tartaric acid 1 M (1% and 2%, v/v) was added to the spiked solution, then the sample was filtered on 0.22-μm Nylon filter and eluted through a C$_{18}$ SPE micro-column.

The results of these experiments are reported in Table 3 for tap water and in Table 4 for rain water (wet deposition). One can see that the addition of tartaric acid is effective in enhancing the recovery of the metals. This effect is particularly evident in the case of rain water, where

the matrix effect was more evident. In this sample the complexing action of tartaric acid is probably the main cause for the increased recovery. Eluting the sample through a C_{18} SPE micro-column made it possible to eliminate the interferences due to the dissolved organic matter, which causes severe interference in the chromatographic analysis, such as baseline drift and broad unidentified peaks, without any observable loss of analytes. The fog sample was analysed without spike of metals. Due to a large amount of organic matter, the analysis of this sample (Table 5) was possible only after its elution through a SPE micro-column. The homogeneity of the recoveries obtained with and without tartaric acid addition could be explained by the already low natural pH (4.1) of the sample.

4. Conclusion

Ion interaction chromatography appears to be a valid technique for the determination of metals at low concentration in natural water samples. The intrinsic possibility of controlling the eluent strength, by increasing the concentration of either the organic modifier or the ligand, allows one to achieve the desired separation efficiency and a short analysis time with only little effort from the operator. From the data obtained it is possible to conclude that acidification of the samples with the same acid used as ligand in the eluent (tartaric acid in the present case) is a valid means to obtain good metal recovery. Such precomplexation methods do not affect the subsequent filtration and clean-up procedure to remove particulates and organic matter.

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