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Journal of Chromatography A, 1085 (2005) 240–246
JOURNAL OF CHROMATOGRAPHY A
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Determination of synthetic chelating agents in surface and waste water by ion chromatography–mass spectrometry

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Received 26 October 2004; received in revised form 31 May 2005; accepted 6 June 2005

Abstract

Coupling of ion chromatography with electrospray mass spectrometry (IC–MS) is a simple, sensitive and quick method for the determination of polar organic traces in water samples without derivatization. Analysis of the chelating agents ethylenediamino tetraacetate (EDTA) and diethylenetriamino pentaacetate (DTPA) in aqueous samples was done by IC–MS on an anion exchange column after simple sample preparation steps. Quantification down to a concentration level of 1 $\mu g\,L^{-1}$ even in wastewater influents and effluents was achieved utilizing ^{13}C marked internal standards and measuring the individual $[M-H^+]^-$ and stable $[M-4H^+ + Fe^{3+}]^-$ cluster ions. The method was validated against certified, but more time consuming routine methods. Applying this method a series of several European water samples were analyzed for EDTA and DTPA indicating their nature as polar persistent pollutants.

Keywords: Chelating agents; Polar organic micropollutants; Ion chromatography–mass spectrometry; IC–MS; analysis; EDTA; DTPA; Aquatic environment

1. Introduction

Synthetic chelating agents, e.g. the investigated ethylenediamino tetraacetate (EDTA) and diethylenetriamino pentaacetate (DTPA) (Fig. 1) are utilized in many industrial applications, e.g. in the textile, photo, and pulp and paper industries as well as in galvanic enterprises [1]. Even if the toxicity of the investigated synthetic complexing agents is low, they still can be classified as environmentally relevant, since they are microbial poorly degradable and exhibit an excellent water solubility. Thus, their removal during drinking water treatment utilizing filtration and biodegradation steps tends to be low or non existent [1–8].

EDTA is present in the $\mu g\,L^{-1}$-range in almost all anthropogenically influenced surface waters in, e.g. Germany [1,2]. Concentration data regarding DTPA are quite scarce [1,9,10]. Reported values of these compounds in surface waters are mostly in the range of the detection limit (LOD) between 1 and 2 $\mu g\,L^{-1}$, whereas maximum concentrations can easily reach values of several 10th of $\mu g\,L^{-1}$. DTPA-concentrations detected in the effluents of paper and pulp mills are in the $mg\,L^{-1}$ range.

The validated and certified analysis of EDTA and DTPA is recommended to be done after enrichment on either anion exchange material or vaporization and derivatization to the tetra-isopropyl- or tetrabutyl-ester. After gas chromatographic (GC) separation either nitrogen–phosphorous detection (NPD) or mass spectrometric (MS) detection can be performed [11]. Problems are often encountered in both the enrichment step and the derivatization part which is quite often incomplete. The determination of EDTA–Fe complexes by high-performance liquid chromatography (HPLC) with UV detection is inadequate for the analysis of low concentrations as it lacks the necessary sensitivity [12].

It was proposed that the development of a sensitive method to analyze the polar and ionic organic acids without derivatization may simplify the existing methods and allow the determination in complex matrices including a high salt content. The most selective and sensitive method for such

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doi:10.1016/j.chroma.2005.06.045

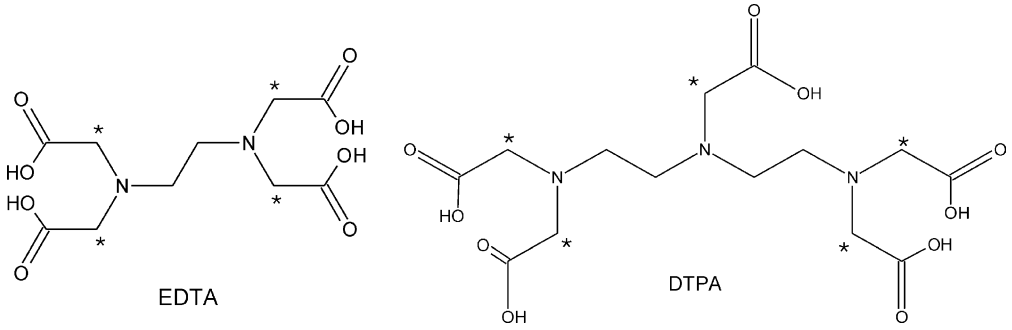

Fig. 1. Chemical structures of the investigated chelating agents. (*) Position of ^{13}C in the ^{13}C-labelled standards.

an application could be ion chromatography–electrospray ionization mass spectrometry (IC–ESI-MS) with subsequent removal of the salts used as eluent [13]. In the recent literature the application of IC–ESI-MS for the analysis of oxyhalides as well as polar organic pollutants including chelating agents has already been described using a suppressor between IC and MS resulting in a cation exchange of the effluent as well as from the sample against H^+ [13,14].

The usefulness of this new method for the determination of inorganic compounds, such as perchlorate has also been shown [15]. EDTA in concentrations down to 1 μg L^{-1} could also be analyzed by reversed-phase LC–MS, but no data applying this method have been published so far [16].

The aim of our study was not only the development of a reliable quantitative method for very polar organic polyacids, but also the application to samples with a high matrix content and high salt concentrations.

2. Experimental

2.1. Chemicals

All chemicals used were of analytical grade. EDTA, formic acid, sodium hydroxide, sodium carbonate and sodium hydrogen carbonate were obtained from Merck (Darmstadt, Germany), DTPA from Riedel-de Haen (Seelze, Germany). Milli-Q water was used in all experiments. ^{13}C-Labelled standards of EDTA and DTPA (Fig. 1) were obtained from the ESWE Institute (Wiesbaden, Germany) [17]. The SAX material used was obtained from ICT (part no. 9502-0100, mean particle size: 40–70 μm; Bad Homburg, Germany).

2.2. Sample preparation

Water samples (100 ml each) were adjusted to pH 3 by means of 16 M formic acid. After spiking the samples with 5 μg of [^{13}C]EDTA and [^{13}C]DTPA each, enrichment was performed by solid phase extraction. Glass cartridges containing 1 g of SAX anion-exchange material were conditioned with 3 × 2 ml methanol and 3 × 2 ml distilled water. After enrichment the SAX material was rinsed with 3 × 2 ml distilled water and eluted with 6 × 1 ml of 16 M formic acid. The combined eluates were concentrated in a nitrogen stream at 90 °C to dryness and redissolved with 1 ml of LC-Eluent A. After filtration the samples (200 μl) were injected via an autosampler into the IC system.

The ESI-MS system used consisted of a PE series 200 LC pump, a PE series 200 autosampler and a PE Sciex API 150 single quadrupole mass spectrometer equipped with an atmospheric pressure ionization (API) source, via a Turbo ionspray interface. The instrument was run in negative ion mode at an ionization voltage of −3000 V, an orifice voltage of −15 V and a ring voltage of −120 V. The interface temperature was held at 450 °C (the recommended temperature for daily use is 400 °C). As Turbo ion spray and curtain gas in the API source 5.0 purity nitrogen was used at a flow rate of 7 L min^{-1}, and oxygen was used as nebulizing gas, at a flow rate of 1.48 L min^{-1}.

Optimization of the ESI-MS ionisation was performed in order to obtain the most abundant ions for identification and quantification. At the optimum voltage of −32 V for EDTA in addition to the $[M - H^+]^-$-ion (m/z = 291 (295 for ^{13}C); see Figs. 1 and 2) an intense ion corresponding to the Fe^{3+}–EDTA complex $[M - 4H^+ + Fe^{3+}]^-$ (m/z 344 (348 for ^{13}C); see Figs. 1 and 2) was also obtained. This was also found to be the optimum voltage for the $[M - H^+]^-$ ion of DTPA leading to the formation of m/z 392 (397 for ^{13}C); and at a lesser intensity the Fe^{3+}–DTPA complex at m/z 445 (450 for ^{13}C); $[M - 4H^+ - Fe^{3+}]^-$.

2.3. Ion suppression center

The IC 828 Dual Suppressor (METROHM, Herisau, Switzerland) consisted of a suppressor cell, a direct current source and a degassing unit. To assure a flow of 0.3 mL min^{-1} into the ESI-MS interface the IC effluent flow (0.6 mL min^{-1}) was split (1:1) behind the suppressor. An additional conductivity detector (IC Detector 732) was used to follow-up the separation of the inorganic anions, such as chloride, nitrate and sulfate.

2.4. Chromatographic conditions

Separation was performed on a Metrohm Metrosep A Supp5 anion-exchange column (column dimensions

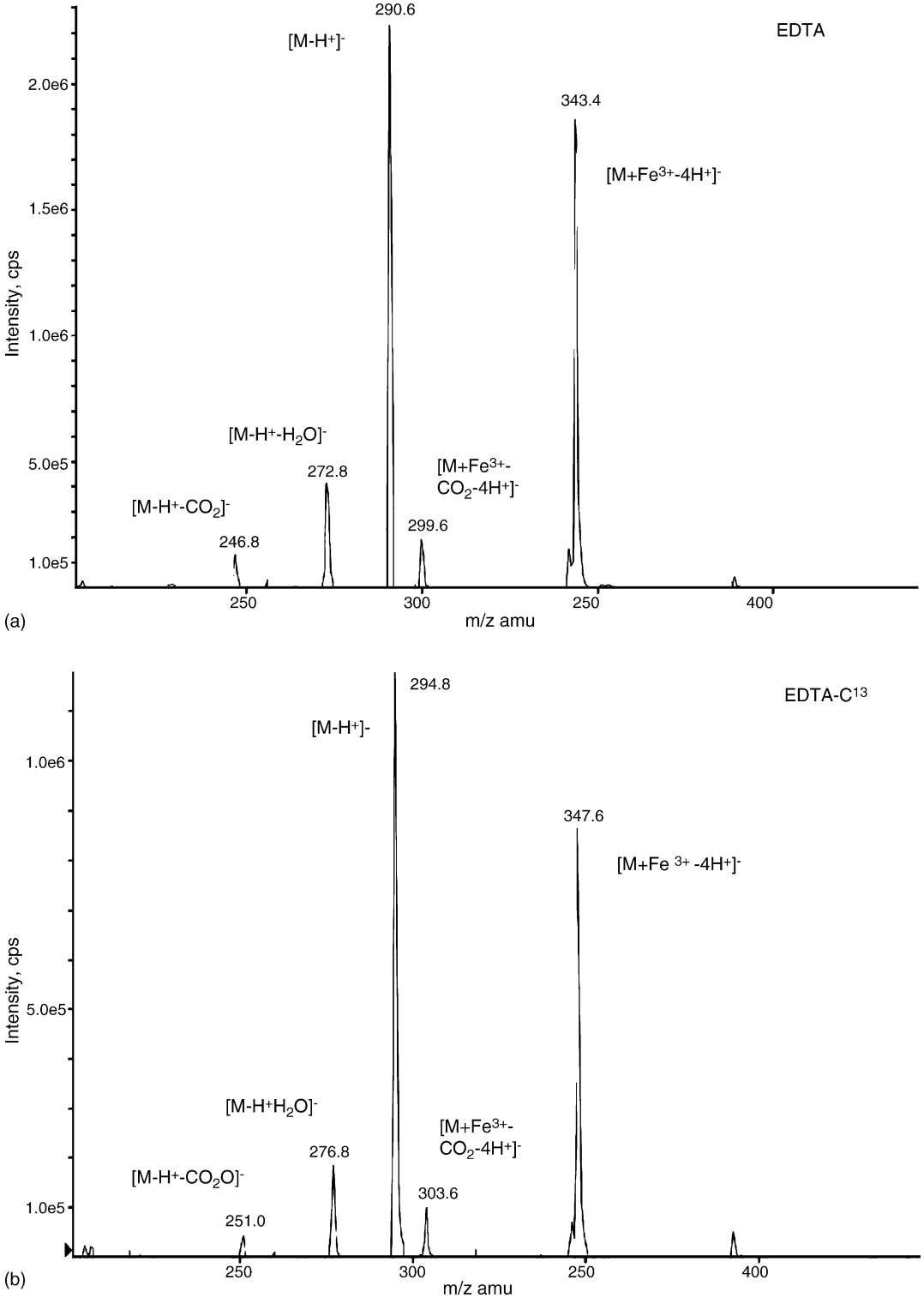

Fig. 2. (−) IC–ESI-MS spectra of (a) EDTA and (b) [^{13}C]EDTA.

150 mm × 4.0 mm, particle size 5 μm, part no. 6.1006.520) under gradient conditions. Mobile phase A was Na_2CO_3 (3.2 mmol L^{-1}) and $NaHCO_3$ (2.0 mmol L^{-1}) and eluent B was Na_2CO_3 (1.2 mmol L^{-1}) and NaOH (40 mmol L^{-1}). The initial conditions of the gradient program were 100% A and 0% B, held for 12 min. In the next 2 min eluent B was increased to 100%, held for 9 min and after this in 4 min adapted again to the starting conditions. The stationary phase was equilibrated for 5 min before starting the next run.

3. Results and discussion

3.1. Method development

From the few published IC–MS applications for the analyses of organic analytes it is known, that the presence of an organic modifier in the eluent is a prerequisite [13]. The organic modifier, such as acetone or methanol serves two requirements: (i) the cleaning of the suppressor resin from both, organic analytes and organic matrix compounds and (ii) the enhancement of the ionization process during ESI.

Since the resin of the here firstly applied continuous regeneration-free solid phase suppressor does not allow the utilization of any organic modifier in the eluent, there was a need to adjust and develop all methods based on solely buffer-solutions. It is known from other studies that memory effects, either resulting from the organic matrix or high analyte concentrations, may be enhanced under such eluent conditions. Therefore, before optimizing the chromatographic process, at first, 30 injections of EDTA at a concentration of 100 μg L^{-1} each followed by a blank injection with Milli-Q water were analyzed in the negative scan modus at *m/z* 200–450 u without any chromatographic separation. A memory effect, especially resulting from adsorption of EDTA in the suppressor could be ruled out. The matrix of, e.g. WWTP did also not show any adverse impact under such conditions. The most prominent ions of both, EDTA and DTPA were the individual $[M - H^+]^-$-ions and $[M - 4H^+ + Fe^{3+}]^-$ adduct ions (Fig. 2). Additional losses of H_2O and CO_2 were observed. The formation of Fe^{3+} complexes, which are by far the most stable of both, EDTA and DTPA, could not be suppressed by any method, since, additionally to their presence in the sample, they are formed during the analytical method, e.g. stemming from either the IC or the MS [1].

During the further validation of the method different inorganic salts, such as Ca^{2+}, Cu^{2+} and Mg^{2+} were added in concentrations from 0.1 to 1.0 mg mL^{-1} to samples containing 100 μg L^{-1} EDTA. An influence in the formation of other complexes other than the described Fe^{3+} complex was not observed. Resulting from that, the MS parameters were optimized for the SIM ions of EDTA, DTPA and the ^{13}C standards as listed in Section 2. For quantitation of the individual compounds both, the $[M - H]^-$-ion as well as the corresponding Fe^{3+} adduct were taken into account.

In order to decrease the background conductivity of the eluent and increase the sensitivity of the organic analytes in the mass spectrometer, it is not only necessary to eliminate the cations from the eluent buffer utilizing a suppressor, but also to separate the inorganic from the organic ions chromatographically in order to minimize suppression effects [13]. For following up the IC separation of inorganic and organic anions a conductivity detector and the MS detector were switched in-line. Separation was optimized in such a way, that the prominent inorganic anions such as chloride and nitrate were eluting at 5.8 and 8.4 min. These retention times are much shorter than those of the organic analytes. Sulfate was eluting at 10.9 min, but did not effect the ionization of the partly coeluting EDTA (Fig. 3).

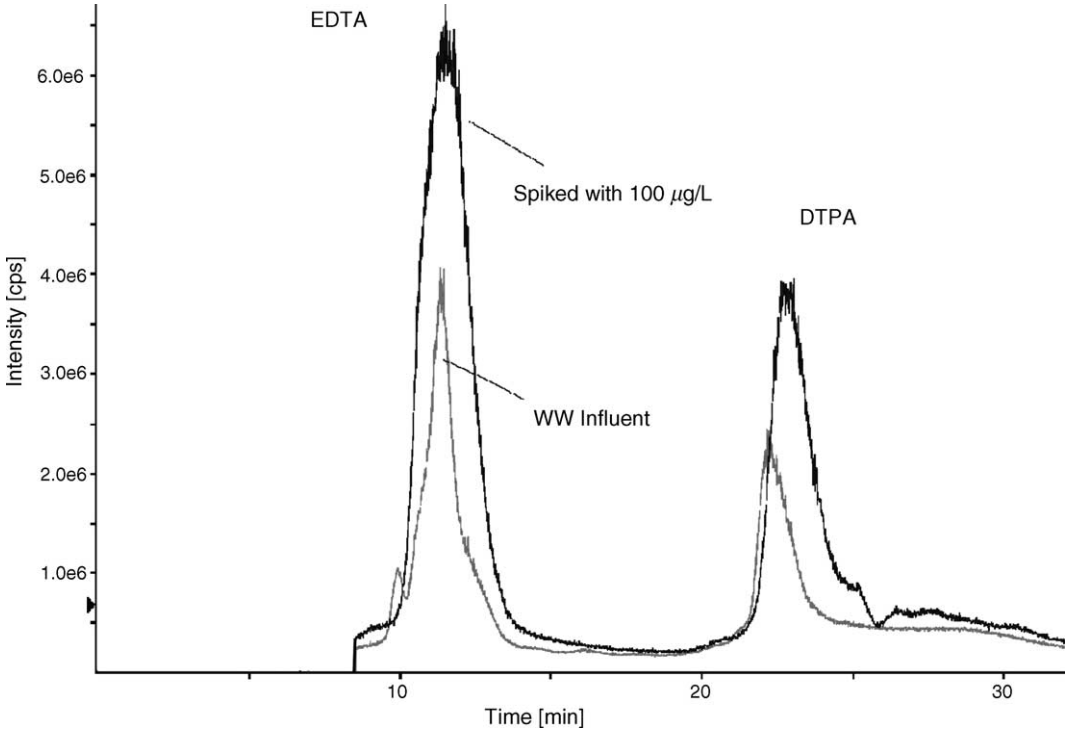

Fig. 3. (−) IC–MS Chromatogram of EDTA and DTPA in wastewater influent (WWTP-K 05/2004; unspiked and spiked with 100 μg/l EDTA and DTPA each); detection with the MS detector in the SIM mode.

This allowed, until complete elution of the inorganic anions chloride and nitrate after 10 min, to direct the eluent prior to separation of the chelating agents to the waste. Subsequent to that, the eluent was introduced into the ESI source and analyzed for the chelating agents (Fig. 3). Under these conditions EDTA was eluting after 11 min and DTPA after 22 min.

After optimization of the chromatographic and mass spectrometric parameters, a calibration was performed in both, ground water and wastewater. The analytes were spiked together with the ^{13}C-labelled compounds (5 μg L^{-1}each) to the water matrices in concentrations between 1 and 200 μg L^{-1} (EDTA) and 2 and 200 μg L^{-1} (DTPA).

The enrichment according to the German DIN-method by evaporating the water phase at 110 °C [11] resulted in too low recoveries, especially in the wastewater samples. Thus, a method based on anion exchange enrichment was used, additionally resulting in a clean-up. Applying this method calibration curves were obtained with six points and a linearity for EDTA of $R^2 = 0.998$ ($y = 0.0179x + 0.0291$) and for DTPA of $R^2 = 0.993$ ($y = 0.0144x + 0.0346$). As LOD the lowest values analyzed were defined, being 1 μg L^{-1} for EDTA and 2 μg L^{-1} for DTPA.

Table 1
Recoveries of EDTA and DTPA in different wastewater samples

Samples	EDTA (μg L^{-1})	Recovery (%)	DTPA (μg L^{-1})	Recovery (%)
A: WW influent[a]	16	104	2.0	108
A + 100 μg/L	121		110	
B: WW influent[b]	84	103	3.4	112
B + 100 μg/L	189		116	
C: WW effluent[c]	56	110	3.4	109
C + 100 μg/L	171		113	

[a] Wastewater influent WWTP K.
[b] Wastewater after primary settlement WWTP W.
[c] Wastewater effluent WWTP W.

With this calibration three different wastewater influent and effluent samples were analyzed upon EDTA and DTPA (Table 1). EDTA could be quantified in these three samples in concentrations between 16 and 84 μg L^{-1} and DTPA in concentrations between 2.0 and 3.4 μg L^{-1}. Standard addition of 100 μg L^{-1} EDTA and DTPA each gave recovery rates

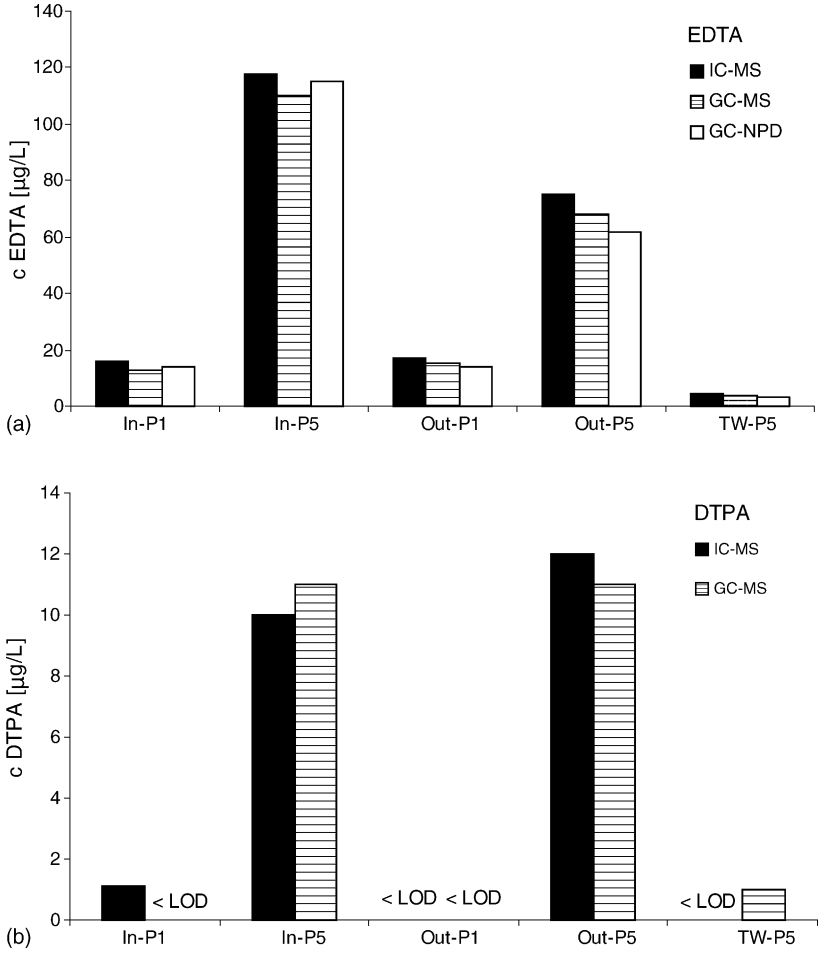

Fig. 4. Comparison of different analytical methods for the analysis of (a) EDTA and (b) DTPA in water samples: WW influent (In-P1 and In-P5); WW effluent (Out-P1 and Out-P5); drinking water (TW-P5). The applied GC–NPD method (derivatization to the butyl ester) was not sensitive enough for analysis of DTPA in these samples.

between 104 and 112%. Thus, the developed method proved to be useful, fast and reliable in quantifying these very polar compounds even in wastewater.

The developed method was compared to the German DIN-ISO method [11] for analyzing the chelating agents. The latter consists of an additional derivatization step after enrichment of the sample followed by gas chromatographic separation. As can be seen in Fig. 4a and b, the data show consistency for all three investigated methods regarding the determined concentrations of EDTA and DTPA in different wastewater and drinking water samples. Compared to the DIN-ISO method the achieved LODs are similar, but due to skipping of the derivatization process, the sample preparation time for the IC–MS method is significantly shorter.

3.2. Monitoring data

Due to the poor biodegradation and the excellent water solubility the entry of complexing agents into surface waters and from there into bankfiltrate water is possible. In this regard there are already many detailed investigations from the aminocarboxylates nitrilo triacetate (NTA), EDTA and DTPA [1,2,7]. EDTA leaches quite easily into the bankfiltrate, whereas NTA is well biodegraded and DTPA seems to adsorb to the soil. The adsorption of amino polycarboxylates on activated charcoal is also poor and it is known from waterworks in practice, that complexing agents such as EDTA are almost completely bypassing activated charcoal filtration [8].

With the developed IC–MS method 10 influent and 18 effluent samples taken from different wastewater treatment plants in Spain, Germany, Austria, The Netherlands and Belgium were analyzed for EDTA and DTPA (Fig. 5). EDTA could be quantified in all investigated wastewater samples in concentrations between 4 and 970 $\mu g\,L^{-1}$ whereas DTPA was not present in all the samples. The analyzed concentrations of DTPA were also significantly lower with values between 1 and 155 $\mu g\,L^{-1}$. The concentrations of EDTA in the investigated surface water samples in the five different

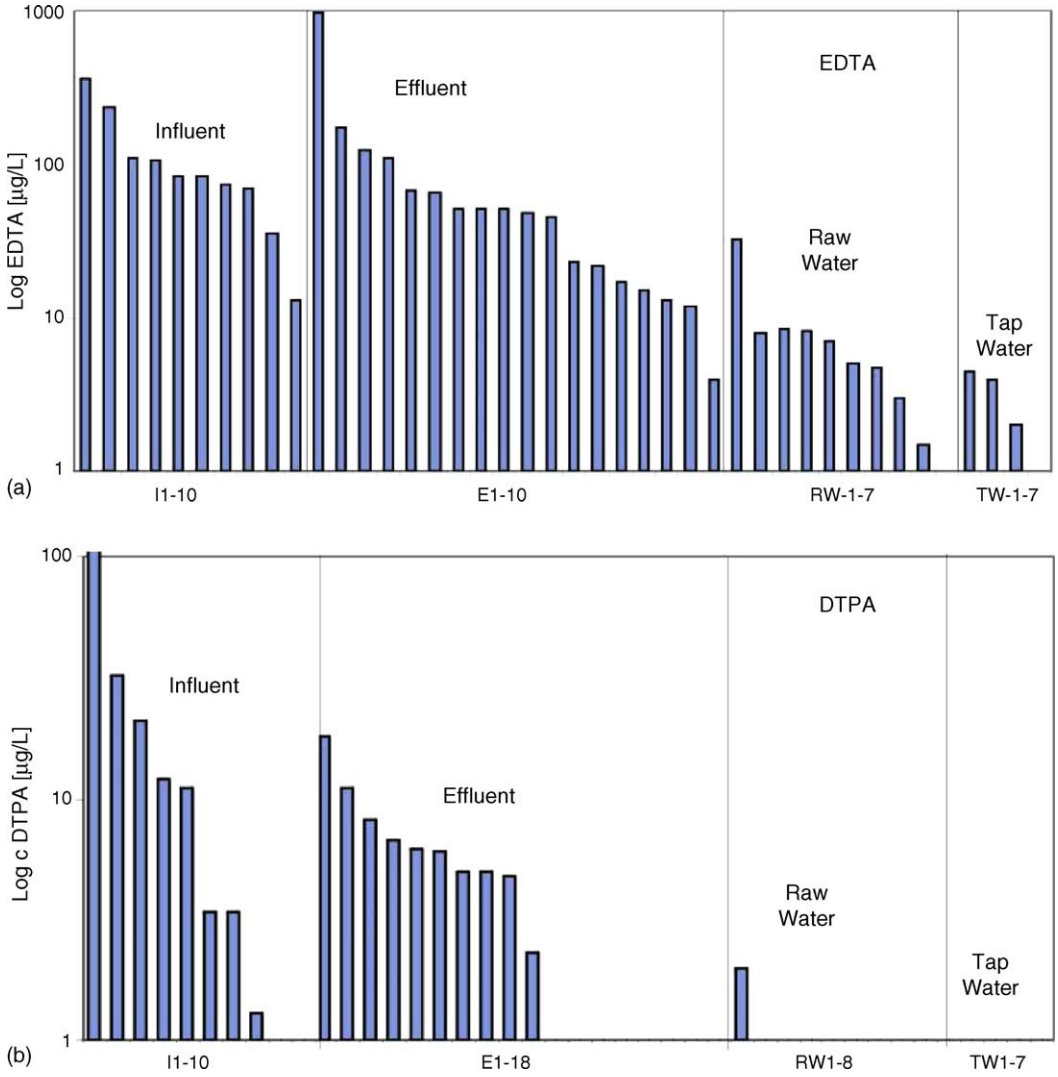

Fig. 5. Monitoring of (a) EDTA and (b) DTPA in water samples taken in the period from March to May 2003 in five different European countries.

European countries gave values between 1 and 33 $\mu g\,L^{-1}$, whereas the EDTA concentrations in tap water were all below 10 $\mu g\,L^{-1}$. DTPA could only be detected in one surface water samples and in none of the seven analyzed drinking water samples.

4. Conclusion

The developed method is ideal for analyzing very polar substances as, e.g. the investigated chelating agents without any further sample preparation other than filtration and, if necessary enrichment. The method is reliable, robust and much less time consuming than the GC methods currently in use, especially when using isotope-labeled standards. Besides the investigated chelating agents other analytical challenging analytes of interest are, among others, for example, organophosphonates and polar degradation products.

Enrichment steps can also be avoided when upgrading the MS detector to a more sensitive MS/MS detector. Regarding structure elucidation of, e.g. polar metabolites coupling of IC with ESI-time-of-flight mass spectrometry might be very suitable.

Since EDTA is a prime example of a drinking water-relevant substance, it is recommended to minimize this kind of pollutants in the water cycle. Wherever possible, substitution with compounds exhibiting better degradability should be sought.

Acknowledgements

This work was financed by the European Commission (5th Framework programme, project P-THREE EVK1-CT-2002-00116). The support of Dr. F. Karrenbrock and O. Rörden from Rheinenergie Köln, Germany during interlab studies of EDTA and DTPA applying GC-NPD analysis is deeply acknowledged.

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