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ORIGINAL PAPER

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A new method for the determination of complexing agents in river water using HPLC

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Abstract An HPLC-method is described for the rapid and sensitive determination of NTA, EDTA and DTPA in surface and ground water at the sub-µg/L level. The analytes are enriched on anion-exchange cartridges, eluted with formic acid and analyzed on RP 8 columns within 6 min. Recoveries for EDTA and NTA were 110% resp. 94% at an interlaboratory test. They are significantly lowered when higher amounts of sulfate are present in the sample. Reproducibility and comparability with other methods are investigated. The new technique is faster than the well known GC-analysis.

1 Introduction

Analyzing complexing agents as nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) in the environment has become an important part in monitoring programs for surface and ground water, e.g., concentrations above 100 µg/L of NTA may remobilize the toxic heavy metals nickel and copper in Rhine water from sediments [1]. Since the ban of phosphates in detergents in several countries the use of NTA and similar complexing agents as substitute for phosphate has increased. Furthermore they are widely used in industrial processes for complexing metal ions. Mainly EDTA is used widespreadly. All complexing agents are very soluble in water due to their amphoteric character. EDTA and most of its heavy metal complexes are not degradable in wastewater treatment plants and therefore EDTA is found ubiquitously in the environment.

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Analyzing complexing agents at low µg/L concentrations in the environment is not easy. The mostly practiced GC method consists of the enrichment of the analytes either by anion-exchange [2] or by evaporating the acidified water sample to dryness and derivatize the analytes to the corresponding esters. This procedure was developed in the early eighties [3, 4] and was published as a Swiss standard method for the determination of NTA and EDTA in drinking water [5].

Although there were some investigations with HPLC, a reliable robust method for analyzing complexing agents at trace levels does not exist. Some authors described HPLC methods by analyzing complexing agents as iron(III)-complexes in rubber stoppers or waste waster [6, 7]. But normaly the working range is at the high µg/L or mg/L level [8–10]. This paper describes a practical method for the routine analysis of complexing agents down to a 0.2 µg/L concentration.

2 Experimental

2.1 Reagents and materials. Nitrilotriacetic acid (NTA, Titriplex I), ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), N-(2-hydroxyethyl) ethylenediamine-N,N′,N′-triacetic acid (Titriplex VII) and cyclohexylene-(1,2)-dinitrilotetraacetic acid (Titriplex IV), all of p.a. quality, were from E. Merck (D). Formic acid (puriss. p.a. 98%) and tetrabutylammonium hydroxide solution (TBAOH, puriss. for ion chromatography, 40% in water) and 1,2-diaminopropane-N,N,N′N′-tetraacetic acid (DATA, min. 99%) were purchased from Fluka (Buchs, Switzerland). Sodium chloride (p.a.), sulfuric acid (95–98% p.a.), ammonia-iron-(III)-sulfate (p.a.), potassium hydroxide (pellets, p.a.), ethylene glycol (p.a.), methanol (suprasolv for organic trace analysis), formaldehyde solution (p.a., min. 37%) and acetonitrile (LiChrosolv, for chromatography) were all purchased from E. Merck (Darmstadt, D).

2.2 Conditioning of the anion-exchange cartridges. The anion-exchange cartridges we used are comercially available from Baker (3 mL Nr. 7991-03, 500 mg of SAX in the chloride form).

These were prepared carefully before the enrichment step as follows: each cartridge is rinsed 3 times with 2 mL portions of

methanol, then washed 3 times with 2 mL portions of pure water. The cartridge is now ready for the enrichment of the sample. Each cartridge was used only once.

2.3 Samples. Spiked samples were prepared by adding NTA, EDTA and DTPA from aqueous stock solutions containing 1 mg/mL of the analytes each of basic pure water. Real samples from the rivers of the region (Rhine, Birs, Wiese) were collected either in glass bottles and analyzed within 24 h or conservated with 1.4% aqueous formaldehyde (20 mL of 37% formaldehyde solution per 500 mL of sample). The samples were stable for four weeks when stored in darkness and below 8 °C [5]

2.4 Enrichment and cleanup. 200 mL of the sample were prefiltered through a 0.45 µm membrane (Supor-450, 47 mm diameter). After adjusting the pH with 16 mol/L formic acid to about 3 the samples were drawn with vacuum via a precleaned viton plastic tube directly from the sample bottle through a prepared SAX-cartridge (see 2.2) using a vac eluate manifold from Baker. After the extraction the cartridge was rinsed 3 times with 2 mL portions of pure water avoiding drying out the adsorbens. Then the analytes were eluted from the solid phase with five 2 mL portions of 16 mol/L formic acid and collected in a 10 mL conical vial. The eluate was dried at 90 °C in a drying-oven over night or as long until no odour of formic acid was present anymore. The residue was carefully resolved in a conical autosampler vial with 0.5 mL of the HPLC eluent.

2.5 HPLC-conditions. The eluent for the HPLC was prepared as follows: 1 mL of concentrated sulfuric acid, 100 µL of 0.4 mol/L ammonia iron(III)-sulfate solution, 0.5 mL ethylene glycol, 1.5 mL of 10 mol/L KOH and 1 mL of TBAOH were solved in 1 L of pure water. The pH of the eluent was between 1.8 and 2.0.

The analysis were performed on a Merck-Hitachi-system L-6200A intelligent pump. The system consisted of a D-6000 interface, diode array detector (DAD L-4500), column thermostat (L-5025) and autosampler (AS-2000A). Acquisition and calculations were made with the D-6500 DAD system manager evaluation method software from Merck. The analytes were separated on a 250 mm × 4 mm Supersphere 100 RP8 endcaped column (Lichrocart 250–4) with a Lichrosphere 100 (Lichrocart 4–4) as precolumn both from E. Merck (Darmstadt, D). The column flow was kept isocratically at 1 mL/min, the column temperature was thermostated at 40 °C. For detection and quantification a diode array detector was used at 258 nm for quantification. 30 µL of the final solution were injected on to the column via an autosampler. The peaks of the three analytes were well separated within 6 min. The NTA eluted just after the system peak at 3.4 min. EDTA and DTPA elute at 4.3 and 5.8 min, respectively (Fig. 1).

2.6 Quantification. The analytes were quantified by 5 point calibration curves ranging from 0.2 to 10 µg/L. The calibration solutions were prepared by spiking an aqueous salt solution (6.2 mg/L of chloride, 22.7 mg/L of sulfate and 0.22 mg/L of N-nitrate to simulate real river water) with the analytes. The calibration solutions were treated analogously to the samples. In this way the actual recoveries were included in the calibration curve (calibration over the whole analysis).

3 Results and discussion

3.1 Enrichment

A problem may occur during the drying process of the extract in the drying board (losses by evaporation). The recoveries were tested with several extracts by drying them for 2 h or over night. No influence of an extended drying time on the recoveries were seen. But when the drying time was too short and formic acid was still present bad recoveries were obtained. Poor recoveries resulted also when air bubbles were transferred onto the cartridges during the extraction.

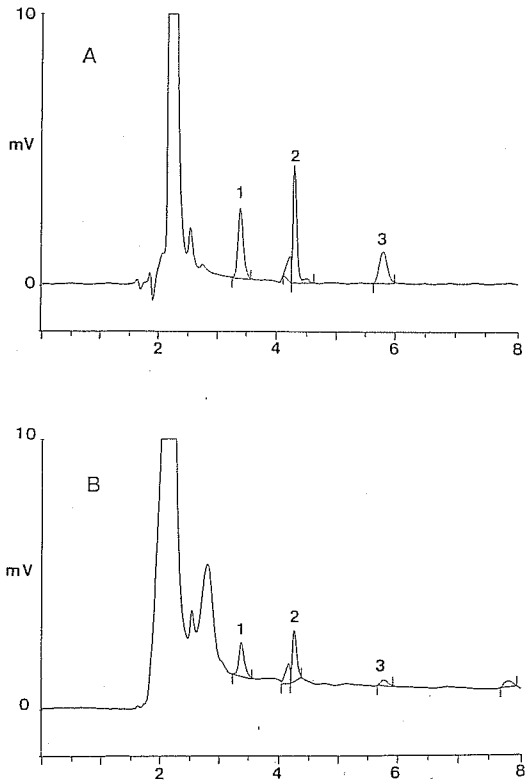

Fig. 1A,B Retention time in minutes. *1* = NTA, *2* = EDTA, *3* = DTPA. **A** Chromatogram of a standard solution (2.5 µg/L each component). **B** Chromatogram of a real sample (Rhine water, near Basel). The concentrations were 1.1 µg/L of NTA, 1.8 µg/L of EDTA and 0.3 µg/L of DTPA

3.2 Chromatography

Basic parameters in HPLC, pH and the polarity of the eluent were tested.

Influence of the pH on chromatography. At a pH below 2 the NTA peak was not baseline separated from the system peak. At pH = 2 all three analytes were well separated, NTA eluted just after the water peak. At pH = 2.5 NTA and EDTA began to shift towards each other, being no more baseline separated. At pH = 3 EDTA eluted earlier than NTA. The response of all three analytes was quite poor (broad peaks). At the optimal pH, finally set at pH = 2, all three analytes are well resolved with high response.

Concentration of the ion pair reagent. The eluent was spiked with TBAOH at several concentrations (0, 2, 6

and 18 mmol/L). The pH was adjusted with KOH to pH = 2, if necessary. The optimal concentration was at 2 mmol/L TBAOH. Higher concentrations of the ion pair reagent increased the retention time of the NTA peak. At a concentration of 18 mmol/L TBAOH NTA eluted 2 min after EDTA. Also the chromatograms showed relatively small and broad peaks. With no TBAOH in the eluent NTA coeluted with the system peak.

Best conditions were obtained with the eluent at pH = 2 containing 2 mmol/L of TBAOH. The retention times were for NTA, EDTA and DTPA 3.4, 4.3 and 5.8 min, respectively.

3.3 Calibration

To test the working range of our new method calibrations of each substance were carried out as follows. Ten calibration solutions were prepared with concentrations ranging from 0.2 to 9.2 µg/L by steps of 1 µg/L. The solutions were prepared from an aqueous salt solution as described in 2.7. For EDTA, NTA and DTPA data of linear regression were: slope (mV/µg/L) 8907; 8086 and 6361. Intercept (mV) : 8086; 4114 and − 423. Regression coefficients: 0.9984; 0.9926 and 0.9956. Standard error of the fit (%) : 3.9; 8.3 and 6.5. The limits of detection on the basis of 5σ (N = 5) are for EDTA and NTA 0.020 resp. 0.019 µg/L. For DTPA the area of the 0.2 µg/L concentration was considered as detection limit because there was no signal at the retention time of DTPA. Determination limits on the basis of 95% confidence level [11] were for EDTA, NTA and DTPA 0.57, 1.22 and 0.96 µg/L, respectively.

For routine analysis daily new calibrations were prepared with the following five concentrations: 0.2, 0.5, 1.0, 2.5 and 10 µg/L.

3.4 Blank

Problems may occur with blank solutions when glass ware and viton plastic tubes were not rinsed carefully. The whole equipment was tested for the three analytes. Neither new SPE cartridges nor our pure water (milli Q from Waters) were contaminated. Memory effects occurred from the used viton plastic tubes connected to the cartridges. Special precleaning of the glass ware and the viton plastic tubes is recommended as follows: first rinsing the dishes and tubes with 2 mol/L KOH, then pure water, 2 mol/L HCl and at last pure water until a neutral pH is achieved.

3.5 Recoveries

Recoveries tested at 3 concentration levels. For this purpose solutions of 0.5, 2.5 and 10 µg/L concentration of each analyte, also containing 6.2 mg/L chloride, 0.25 mg/L bromide, 0.22 mg/L N-nitrate and 22.7 mg/L sulfate, were prepared. The results of five separate runs show reproducible recoveries of 82%, 80% and 77% for NTA, EDTA and DTPA, respectively. The relative variation coefficients were 14% for each analyte. These recovery tests were made several times. Sometimes recoveries were poorer and reproducibility worse; these effects were caused by bad anion exchange cartridges. The quality of the cartridges varies from lot to lot of production. Therefore it is very important to control the total recovery at least when changing the lot. We keep recoveries under control by making calibrations for each series over the whole method (including the SPE enrichment).

Influence of real river matrices on the recoveries of analytes. The analytes were added at three different concentrations to samples of the rivers Rhine and Birs (1, 2.5 and 5 µg/L). The recoveries (N = 3) were for NTA, EDTA and DTPA between 92 to 127%, 76 to 92% and 63 to 120%, respectively. The relative standard deviations varied from 1 to 17%. The unspiked river water showed relative standard deviations between 2 and 17% (see Table 1).

3.6 Precision and accuracy

The repeatability (% R.S.D.) as calculated from the differences between five concurrent determinations of a test solution were for EDTA and NTA 3.6 resp. 5.4.

Table 1 Recovery tests with river water; results are mean of three separate determinations

River Birs	Added µg/L	Found µg/L	Recovery %	R.S.D. %	River Rhine	Added µg/L	Found µg/L	Recovery %	R.S.D. %
NTA	2.5	3.2	128	6	NTA	1.0	1.1	110	11
EDTA	2.5	2.1	84	1	EDTA	1.0	0.8	80	8
DTPA	2.5	2.9	116	1	DTPA	1.0	0.6	63	5
NTA	5	5.5	110	6	NTA	2.5	2.3	92	9
EDTA	5	4.6	92	5	EDTA	2.5	2.0	80	5
DTPA	5	6.1	122	1	DTPA	2.5	2.7	108	8

Sample solutions were tested for their stability in time. Three different concentrations were prepared and analyzed immediately, after 24 h and 96 h. The concentrations were 6.2, 7.2 and 8.2 µg/L. The results were calculated with three different, daily prepared calibrations. NTA showed a quite good stability over 96 h. The concentration varied within − 0.3% to + 8% of the original concentrations. EDTA varied from − 0.2% to + 4%, and DTPA from − 0.4% to + 10.5%. The extract solutions were quite stable for at least four days.

3.7 Interferences

Strong competition between ions can occur in ion exchange processes [2]. Mostly sulfate and hydrogencarbonate will influence the enrichment of complexing agents due to double charge and high concentration. Hydrogencarbonate was eliminated quantitatively during the evaporation of the acidic extract solution. Sulfate was added at amounts of 30 and 60 mg/L to solutions containing 2.5 µg/L NTA and EDTA each and analyzed. At a concentration of 30 mg/L of sulfate recoveries were for NTA: 104% ± 13% and for EDTA 100% ± 20%. At 60 mg/L recoveries were significantly lowered: NTA 79% ± 10% and EDTA 55% ± 12%.

3.8 Comparison with GC analysis

Randt et al. showed a good comparison between GC and HPLC analysis for EDTA and NTA at mg/L level [9]. Within the scope of an interlaboratory test we could apply the new method described. In a solution spiked with 20 µg/L EDTA we found 22.0 ± 0.8 µg/L (N = 5), and for 4 µg/L NTA we analyzed 3.7 ± 0.2 µg/L (N = 5). Recoveries were for EDTA and NTA 110% and 94%, respectively.

4 Conclusions and outlook

The usefulness of a routine method depends partly on its total analysis time. In comparison with the official GC method we need less time with the HPLC method developed. For the analysis of ten samples (a five point calibration included) about 9 h are needed. When the eluates were evaporated in the drying board over night analysis time is reduced to about 5 h; this means half an hour per sample.

The described method can be modified for waste water analysis. First experiments show good results and this HPLC method may be adapted for waste water, drainage waters from landfills and other matrices with high organic load. Furthermore the following complexing agents can be analyzed as well: Titriplex IV, Titriplex VII and DATA. We are also testing suitable complexing agents as internal standard.

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