Determination of [*S,S*']-Ethylenediaminedisuccinic Acid by High-Performance Liquid Chromatography

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Abstract

A new high-performance liquid chromatography (HPLC) method for the determination of ethylenediaminedisuccinic acid (EDDS) is presented. Free $EDDS^{4-}$ and EDDS complexes with divalent metals undergo conversion to the Fe(III) complex in the presence of $Fe(III)Cl_3$. Fe(III)EDDS is separated by HPLC on an ion exchange column using $(NH_4)_2SO_4$ eluent with detection at 258 nm. The detection limit is 0.01µM. The method is applied to natural waters and soil solution samples. A background of natural water results in a reduction in EDDS peak area. The method is suited for EDDS analysis in samples with well-defined, simple matrices such as those used in laboratory experiments or biodegradation studies.

Introduction

The biodegradable chelating agent *S,S*'-ethylenediaminedisuccinic acid (EDDS) has received some attention in the last few years as a potential replacement for ethylenediaminetetraacetic acid (EDTA) and other recalcitrant chelating agents (1) in, for example, laundry detergents (2). The *S,S*-isomer of EDDS is produced by some bacteria (3,4) and fungi (4) and is easily biodegradable, while the *R,R*- and *S,R*-isomers are not (5,6). There is some interest in EDDS for its use in the remediation of metal-contaminated land, both by soil washing and by chelant enhanced phytoremediation (7–10).

Very few methods for the analysis of EDDS can be found in the literature, probably because of the fact that it is a new compound and only recently became commonly used. Ammann (11) described an ion chromatography (IC) inductively coupled plasma (ICP) mass spectrometry (MS) method for the detection of metal-EDDS complexes. This method is not suitable for routine analysis in most laboratories, however, because of the advanced instrumentation that is needed. Other methods based on the colorimetric principle have very high detection limits (0.1mM) (5) compared to the IC-ICP-MS method. Knepper (12) mentions that the ISO method for complexing agents (a gas chromatography-based method) (13) may be used for the analysis of EDDS, but no such use has been documented.

Three papers mentioned that high-performance liquid chromatography (HPLC) methods have been used for EDDS analysis, but the details given are not comprehensive, and although the detection limits are not given, it seems from their data that they would be relatively high (10–50µM) (3,6,14). The methods rely on the same basic principle, a separation using ion-pairing reagents with pre- or post-column conversion to the Fe(III) or Cu(II) complex followed by UV detection. Similar methods have been used extensively for the analysis of EDTA (15–20), nitrilotriacetate (NTA) (15), diethylenetriaminepentacetic acid (15), and phosphonates (21). Some methods measure directly UV-active complexes, giving a partial speciation of the sample, and others use conversion of all complexes to the Fe(III) or Cu(II) form before measurement.

Another method has been developed by the same authors, which is based on the derivatization of EDDS followed by HPLC separation and fluorescence detection (22). The detection limit for this method is low (0.01µM), and it can be used in complex matrices (natural water, soil solution, and plant extracts). However, it is time consuming because of the derivatization step prior to analysis.

The aim of this work was to develop a simple HPLC-based method for the analysis of EDDS at sub-micromolar concentrations. However, the aim was not to separate the chiral isomers *S,S*'-, *R,R*'-, and *S,R*-EDDS. The method is based on the pre-column conversion of all EDDS complexes to Fe(III)-EDDS (15,18) and separation of the iron complex by ion chromatography (11,23) and subsequent detection by UV.

Experimental

Reagents and chemicals

All chemicals were obtained from Merck (Darmstadt, Germany) unless otherwise stated. Pure water (Milli-Q system, Billerica, MA) was used in all preparations. A 0.5M stock solution of $(NH_4)_2SO_4$ (Fluka, Buchs, Switzerland) was prepared in pure water and used to prepare the working eluent. $Fe(III)Cl_3$ (1mM)

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was prepared from the anhydrous chemical and dissolved in 1mM HCl to prevent the precipitation of iron. A 1mM stock standard of Fe(III)-[*S*,*S*']-EDDS was prepared from Na_3-[*S*,*S*']-EDDS (Procter and Gamble, Brussels, Belgium) and anhydrous Fe(III)Cl_3 in 1mM HCl. All stock standards were stored at approximately 4°C in the dark to prevent photodegradation and biodegradation. All working standards were prepared from the stock standards in 1mM HCl. They were also stored at approximately 4°C and in the dark to prevent deterioration.

HPLC

A Jasco (Easton, MD) HPLC system (PU-980) equipped with an 851-AS autosampler, 200-µL sample loop, and UV spectrophotometric detector (UV 970) set at 258 nm was used. A pre-instrument degassing unit was also installed (Gastorr GT-102, Lab-Quatec, Tokyo, Japan). The HPLC separations were carried out using a Dionex (Sunnyvale, CA) Ion Pac AS11 column (230 × 4 mm) at room temperature. Eluent A for the separation of EDDS was 1mM HCl (pH 3.0), and eluent B was 5mM $(NH_4)_2SO_4$ (pH 3.3). The following gradient at a flow rate of 1 mL/min was used: 0–100% B in 12 min; 1 min at 100% B; then back to 0% B in 1 min.

Sample preparation

All samples were filtered through 0.45-µm membrane filters. The samples were adjusted to pH 3 with HCl. For most samples, it was sufficient to add HCl to give a final sample concentration of 1mM. Once at pH 3, Fe(III)Cl_3 in 1mM HCl was added to make the Fe(III) concentration in excess of the chelating agent concentration. The sample was then thoroughly mixed and stored in the dark at approximately 4°C until analyzed to prevent photo-degradation of the Fe complexes.

The effect of ionic strength on EDDS analysis was tested using NaCl to adjust the ionic strength of pure water standards, which were later prepared for analysis as described previously.

The effect of different metals on the analysis of EDDS was tested by spiking 1µM EDDS standards with 1µM Cu, Zn, Ni, Pb, or 1mM Ca. The standards were also analyzed without metals in the same matrix (nitrate concentration) as the spiked standards. The analysis was carried out with two types of standards prepared using Fe(III)EDDS and Na_3EDDS prior to metal spiking.

Results and Discussion

Chromatography

Figure 1 shows a chromatogram for Fe(III)EDDS (10µM) in 1mM HCl using a 100-µL sample injection. The analyte peak was well separated from the reagent peak. Plots of peak area versus the concentration of EDDS were linear from 0.01 to 1µM ($r^2 = 0.9994$, $n = 7$) and from 1 to 10µM ($r^2 = 1$, $n = 5$). The detection limit was 0.01µM (signal-to-noise ratio = 3)

Concentration of Fe(III)

When the uncomplexed Fe(III) concentration was increased, peak height decreased, although peak area remained constant. However, this only started to occur when the free (uncomplexed) Fe(III) concentration was greater than 50–100µM. This can be seen in Figure 2, which shows the effect on peak height of excess Fe(III) for a 10µM EDDS standard. Fortunately, the peak area was not greatly affected. Excess Fe(III) concentrations may occur in real samples where the EDDS concentration is unknown. For such samples, enough Fe(III) has to be added so that all EDDS can be converted into the Fe(III) complex even in the presence of other Fe-binding ligands. However, a 10 times excess of Fe does not affect peak area and only slightly affects the peak height.

Other chelating agents such as EDTA and NTA do not interfere with the analysis of EDDS as they elute at different retention times.

Influence of matrix ions

The ionic strength of the sample also seems to affect the peak

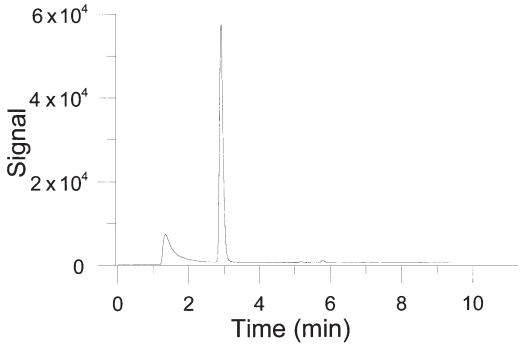

Figure 1. Chromatogram of 10mM EDDS after complexation with Fe(III).

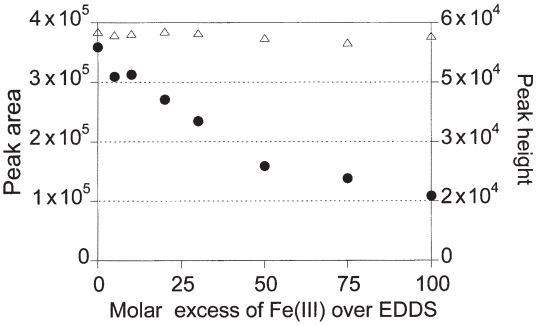

Figure 2. The effect of excess Fe(III) [ratio of Fe(III)–EDDS] on the peak area (△) and peak height (●) of 10µM EDDS.

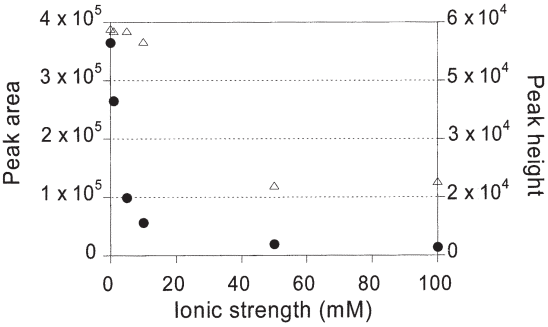

Figure 3. Effect of ionic strength of the sample (adjusted with NaCl) on peak area (triangles) and peak height (circles) of 10µM EDDS after complexation with Fe(III).

response. As can be seen in Figure 3, increasing the sample ionic strength (using NaCl) reduced the peak area and height and also broadened the peak. A reduction in peak area was observed beyond 5mM ionic strength. At 50mM ionic strength the peak area was only 30% of the original value. At higher ionic strengths peak area remained constant. Peak height, however, started to decrease immediately as ionic strength was increased and also came to a constant low at 50mM. $NaNO_3$ was also used to check the EDDS peak response to increasing strength. The effect on peak height was exactly the same as for NaCl, but the effect on peak area deviated from that of NaCl. Up to 50mM, the response of peak area with NaCl and $NaNO_3$ was about the same, but at 100mM ionic strength, $NaNO_3$ reduced the peak area even more than NaCl.

Complexation of EDDS with Fe(III) is only slightly dependant on ionic strength. The decrease in peak area was therefore not caused by a lower complexation efficiency. The added effect of $NaNO_3$ was probably because of $NaNO_3$ increasing the retention time of the peak and making it coelute with a very low broad peak, which appears at high $NaNO_3$ concentrations. The phenomena of reduced peak heights are probably also connected with the decreased sample focusing at the top of the column during injection because of the increased ionic strength (or to a strong elution strength of the sample matrix).

The effect of phosphate on EDDS peak area and height were also investigated. Peak area was unaffected at phosphate concentrations of 10mM but showed a nearly 50% reduction at 100mM. Peak height, however, showed a steady increase between 0 and 10mM phosphate, then reached a plateau of 160% of the original peak height. As peak area is usually the measured parameter, only high concentrations of phosphate would effect the measurement of EDDS. It is essential, given these findings, that standards be prepared in the same matrix as the samples so as to avoid any matrix effects.

Effect of metals on formation of Fe(III)EDDS

By the addition of 1µM Cu, Zn, Ni, or Pb to 1µM Na_3EDDS or 1µM Fe(III)EDDS prior to converting it to Fe(III)EDDS, it is proven that none of these metals affect the peak area of EDDS (Table I). This is different from the related compound, EDTA, where the transformation of NiEDTA to Fe(III)EDTA required heating at 90°C for 3 h (18).

Adding 1mM Ca, on the other hand, reduced the peak height when added to Na_3EDDS prior to its conversion into Fe(III)EDDS, but not when added to Fe(III)EDDS. This shows that Ca affects the conversion of EDDS from the Na complex into the Fe(III) complex. It is not possible to remove interfering cations from the EDDS-containing solution by cation exchange, as EDDS is also retained on the cation exchange column. However, as Ca only affects the peak height and not peak area, it should only affect the sensitivity and not the actual measurement of EDDS.

Table I. Influence of Metals on Peak Area and Height During of the Conversion of 1µM EDDS Complexes into Fe(III)EDDS

	Peak area	Peak height
Metal free	100%	100%
Cu(II)	96.9%	96.8%
Zn(II)	100.4%	102.4%
Ni(II)	99.1%	100.6%
Pb(II)	100.2%	102.8%
Ca(II)	97.3%	75.0%

EDDS analysis

Drinking water samples spiked with Fe(III)EDDS were analyzed along with pure water standards. The drinking water samples showed peak areas of approximately 50–60% and peak heights of 72–78% of the standards made in pure water (Figure 4). This shows that if drinking water (or natural water with a similar matrix) is to be analyzed, then the standards should be made up in drinking water that is EDDS-free or standard addition has to be used. Soil extract (1mM PO_4^{2-}) was acidified (pH 3) and had Fe(III)Cl_3 added before being spiked with Fe(III)EDDS. These samples showed that in the range of 0.1–100µM EDDS, although the peak area was comparable to that of the pure water standards, the peak height was only half of this, thus increasing the detection limit in this matrix by a factor of two. It is not clear whether this is because of excess Fe in the samples, as the soil extract was made to be 140µM Fe(III) prior to spiking with Fe(III)EDDS or because of the ionic strength of the soil extract. Previously, it was shown that phosphate, at this concentration, increased peak height slightly and did not reduce it; so, phosphate is not responsible for the reduction in peak height in this case.

Conclusion

As demonstrated previously, the conversion of EDDS complexes into Fe(III)EDDS prior to HPLC separation on a Dionex Ion Pac AS11 column makes it possible to detect EDDS to sub-

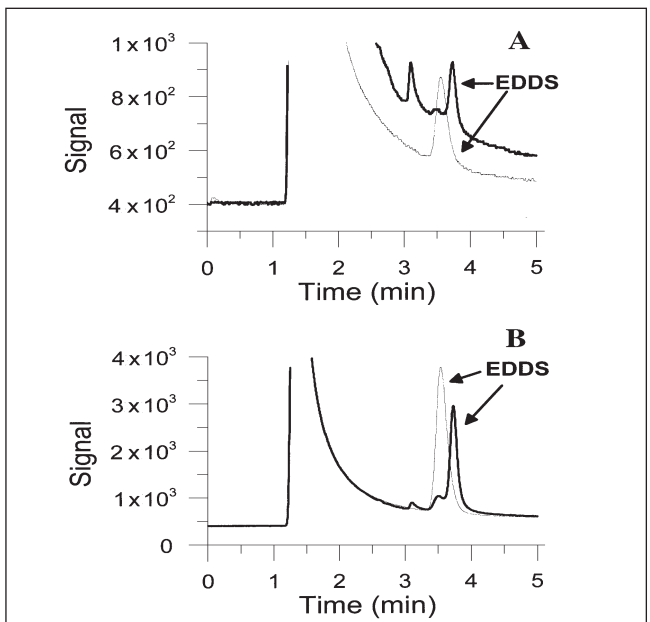

Figure 4. Chromatograms of EDDS 0.1µM (A) 1µM (B) in pure water (thin line) and drinking water (thick line) after complexation with Fe(III).

micromolar levels by UV detection. The method reported here has the same detection limit of 0.01µM EDDS as a new method described using derivatization with a fluorophore (22). Although the derivatization method is better for complex undefined matrices, the Fe-method can be used for samples with well-defined, simple matrices. Such samples may come from laboratory experiments investigating the behavior of EDDS (e.g., adsorption, photodegradation, or biodegradation experiments). The Fe-method is also much quicker to carry out than the derivatization method, which requires heating and solvent extraction steps.

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