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Determination of ethylenediaminetetraacetic acid at very low concentrations by high-performance liquid chromatography coupled with electrospray mass spectrometry

A. Dodi*, V. Monnier

Département d'Etudes des Déchets, Direction de l'Energie Nucléaire, C.E.A. Cadarache, 13108 Saint Paul lez Durance, France

Abstract

Ethylenediaminetetraacetic acid (EDTA) is a metal complexing agent which is commonly used for removing metallic surface contaminations. But its presence in aqueous industrial effluents or in wastes, by example those which are generated from nuclear power facilities, is forbidden or strictly regulated by the legislation. The implementation of high-performance liquid chromatography coupled with mass spectrometry (MS) via an electrospray interface has exhibited a powerful capacity in the measurement of this complexing agent, in the form of its iron complex. The complex is eluted through a reversed stationary phase under slightly acidic conditions and the detection is performed by mass spectrometry in the single ion monitoring mode. This technique has allowed us to reach a detection limit of about 1 μg/l in EDTA for only 20 μl injected without any previous preconcentration, and to solve some analytical interferences observed in the case of industrial effluents with other analytical techniques such as ion chromatography.
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1. Introduction

Within the framework of the research of organic complexants in aqueous effluents, it is necessary to know how to measure ethylenediaminetetraacetic acid (EDTA), with the lowest detection limit as possible. Indeed, the presence of this powerful organic complexant in effluents is submitted to a regulation by the legislation.

Ethylenediaminetetraacetic acid is an organic complexant whose formula is $C_{10}H_{16}N_2O_8$ and molecular weight 292.2 g/mol. It is usually symbolized by H_4Y, because of its four acidic functions. The values of the four corresponding pK_a are: $pK_{a1} = 2$; $pK_{a2} = 2.8$; $pK_{a3} = 6.2$; $pK_{a4} = 10.3$.

This hexadentate ligand is a very powerful chelating agent which forms, with a large number of metal ions, a stable complex in a 1/1 proportion. The values of the complexation constants of this tetra acid with some usual metal ions are reported in the literature [1,2].

With the ferric ions Fe^{3+}, the complexation constant is particularly high ($pK_C = 25.1$) and this results in the formation of an octahedral complex $[FeY]^-$ exhibiting a negative charge and whose molecular mass is m/z 344.1. EDTA and iron are linked by four Fe–O bonds and two Fe–N bonds.

The implementation of high-performance liquid chromatography associated with UV detection was initially employed by a certain number of authors in order to measure a metallic complex formed by pre-column derivatization. However, this technique is sometimes subjected to problems of chromatographic interferences, which results in the increase of the detection limit of the complex.

In the literature, various work deals with the measurement of EDTA in aqueous media. In all these studies, metal cations are added to the samples prior to the detection of the complex. Two principal techniques of detection are used: ultraviolet absorbance and mass spectrometry (MS).

Owens et al. [3] studied EDTA and nitrilotriacetic acid (NTA) mixtures by capillary electrophoresis. Various metal ions (Ca^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+} and Fe^{3+}) were added to the samples and an electrolyte composed by Na_2HPO_4 25 mM (pH 6.8) was used. The authors reached a detection limit (UV detection at 185 nm) ranging between 2 and 50 μM according to the nature of metal complexed (ex.: detection limit of 4 mg/l for the $[FeY]^-$ complex).

* Corresponding author. Tel: +33-4422-54096; fax: +33-4422-53284.
E-mail address: alain.dodi@cea.fr (A. Dodi).

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Padarauskas and Schwedt [4] have separated the complexes formed between various metal ions (Co^{3+}, Bi^{3+}, Fe^{3+}, Cr^{3+}, V^{4+}, Pb^{2+}, Hg^{2+}, Co^{2+}, Cu^{2+} and Ni^{2+}) and aminopolycarboxylic acids such as diethylenetriaminepentaacetic acid (DTPA). The best resolution was obtained with an electrolyte composed by DTPA 5 mM (pH 8.5). The detection limit (UV detection at 241 nm) is in the range of 2–8 μM according to the observed complex. These authors have also separated the ferric complexes of EDTA, DTPA, cyclohexane-1,2-diaminetetraacetic acid (CDTA) and NTA with an electrolyte composed by 20 mmol/l sodium acetate, 0.5 mmol/l TTAOH, pH 4.0 with acetic acid with an UV direct detection at 254 nm.

Sillanpää et al. [5] were interested in EDTA and DTPA mixtures that they have complexed prior to analysis with ferric ions. The stationary phase used was an Hypersil ODS 5 μm column and the mobile phase a buffer solution composed by KH_2PO_4 containing an ion pairing reagent (0.1 mol/l of cetrimide, pH 4.5). The detection limit (UV detection at 260 nm) was in the range of 0.5 mg/l for 20 μl injected.

Yamaguchi et al. [6], performed high-performance liquid chromatography with a reverse phase (Zorbax ODS) in order to measure the EDTA and NTA complexes formed with ferric ions. The mobile phase was a buffer solution $NH_4H_2PO_4$ containing tetrabutylammonium bromide (TBABr) used as an ion pairing reagent. These compounds were detected by molecular absorption and the lower limit of measurement was of about 0.7 mg/l for the $[FeY]^-$ complex for a 10 μl injection loop.

By modifying somewhat the preparation of the samples (addition of a very great excess of ferric ions and particularly by performing a preconcentration by evaporation), Bergers and de Groot [7] have obtained a detection limit (UV detection at 254 nm) of 0.8 μg/l for the $[FeY]^-$ complex and for 100 μl injected. They used a reversed phase (Nucleosil 5-C_{18}) and a mobile phase consisting of a buffer solution acetic acid/sodium acetate and ammonium hydroxide.

Few works relating to studies of measuring EDTA by mass spectrometry has been reported.

In order to measure EDTA in human plasma and urines, Sheppard and Henion [8] developed the analysis by capillary electrophoresis/mass spectrometry in negative mode. They used an electrolyte buffer composed by ammonium formate 30 mM at pH 3. The authors reached a detection limit of 7 μg/l in free EDTA.

Sharp et al. [9], without using any preliminary chromatographic separation, were interested from a qualitative point of view, with $[NiY]^-$ complexes formed in aqueous and methanolic mediums. They used a mass spectrometer equiped with an ion-spray source.

Lastly, Baron and Hering [10] studied complexes of EDTA with various metal ions (Cu^{2+}, Pd^{2+}, Cd^{2+}, Al^{3+} et Fe^{3+}) by mass spectrometry on line with an electrospray source in positive mode. The mobile phase used was a CH_3OH–water (50:50) mixture. However, the detection limits remained relatively high: 0.3 mg/l for the $[CuY]^-$ complex and 0.5 mg/l for the $[PbY]^-$ complex in continuous injection. The authors were not interested by the $[FeY]^-$ complex.

Ion chromatography has also been used with a nitric acid mobile phase [11,12]. The stationary phase is an anion exchanging resin (AG7/AS7 from Dionex). The implementation of an acidic mobile phase composed by nitric acid limits the ionisation of EDTA. This allows the formation of monocharged EDTA which is poorly retained by the stationary phase, and thus may be eluted by nitrate ions. A complexation of the ions with a post-column reagent $[Fe(NO_3)_3{\cdot}9H_2O]$ allows ultraviolet detection at 330 nm. The detection limit observed is then equal to 50 μg/l (injection loops of 500 μl).

The aim of this paper, is to describe a new method which uses a reversed phase separation associated with the very selective detection allowed by mass spectrometry. More precisely, an electrospray interface used in negative mode was implemented.

2. Experimental

2.1. Materials

For all eluents and standards preparations, deionised (DI) water was provided by a point-of-use water purification system (Milli-Q system; Millipore).

All chemicals used were analytical grade reagents. Ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2H_2-EDTA·$2H_2O$) and iron chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) were purchased from Merck.

Stock solution (1 g/l) of iron species was prepared from $FeCl_3{\cdot}6H_2O$ (Merck). Stock solution (1 g/l) of EDTA was prepared by dissolving the disodium reagent in deionized water.

The solution used for tuning the mass spectrometer (mass and intensity calibration) was purchased from Agilent Technologies (Tuning Mix reference G2421A).

2.2. Apparatus and columns

A Hewlett-Packard 1100 high-performance liquid chromatograph coupled with a mass spectrometer, controlled by the Chemstation software (Agilent) was used for this work. The HPLC system is equipped with an on-line degasing system, a quaternary pump, an automatic sampler, an injection loop with adjustable volume, and a quadrupole analysing mass spectrometer equipped with an electrospray ionisation (ESI) interface working in the negative ionisation mode.

Because of the low flow rates used, the exit flow from the column can be sent directly into the ionisation chamber of the mass spectrometer without having to be split. The MS module of the instrument comprised the ionisation chamber, a silica capillary driving the ions (eventually

solvated) into the quadrupole selector and the ion detector to generate the spectrum (total ionic current mode) or a signal (SIM mode). The HP MSD 1100 is capable of two types of ionisation: electrospray and atmospheric pressure chemical ionisation (APCI), although in this work, we used only ESI, samples are subjected to "gentle" ionisation such that in-source fragmentation generally does not occur to any great extent. Neutral compounds will not be detected unless they acquire a charge, usually a proton, sodium or potassium.

The electrospray interface consists in an atmospheric pressure ionisation spray chamber for ion generation and a low pressure ion transport and focusing region with a serie of lenses and skimmers that lead the ions into the mass spectrometer. These two regions are connected by a capillary that serves as a pathway for the transport of the ions generated in the spray chamber into the low pressure region. The sample led by mobile phase out of the chromatographic column is introduced into the spray chamber by a nebulization procedure. Nitrogen was used as a drying and nebulizing gas.

A fine spray is thus generated by the nebulizing gas then is introduced at high pressure around the needle combined with the strong electrostatic field in the spray chamber. Droplet desolvatation is aided by heated drying gas introduced into the spray chamber around the capillary. In this work, the electrospray interface was operated in the negative ion mode (which produces negatively charged gas phase ions). A voltage usually set in the range 1–5 kV is applied at the capillary entrance.

A reversed ODS stationary phase (EC 250/4.6 Nucleosil 100-5-C_{18}, purchased from Macherey–Nagel) was used to perform chromatographic separations upstream the ionization chamber.

2.3. Standards preparation

Biohit Proline pipettes were used to prepare standards solutions and to sample every solution.

Stock solutions (1 g/l) were prepared from $FeCl_3 \cdot 6H_2O$ ($M_r = 270.3\,g\,mol^{-1}$) and $C_{10}H_{14}O_8N_2Na_2 \cdot 2H_2O$ (Tritriplex III) ($M_r = 372.2\,g\,mol^{-1}$) obtained from Merck. Solutions for HPLC-ESI-MS analysis were prepared freshly the day of the analysis.

From these reagents, various solutions of $[FeY]^-$ complex formed with 1.5 equivalents of ferric ions, were prepared:

S1: 1 g/l EDTA (H_4Y) solution in water (64 mg of Tritriplex III in 50 ml water).
S2: 1 g/l Fe^{3+} ions solution in water (241 mg of $FeCl_3$, $6H_2O$ in 50 ml water).
S3: stock solution of the $[FeY]^-$ complex at the concentration of 10 mg/l, prepared from the solutions S1 and S2 (84 μl of S1 + 24 μl of S2, adjusted to 10 ml with deionized water, which corresponds to a molar excess of 50% in iron).

Lower concentration solutions were prepared from S3.

3. Results and discussion

The measurement of an organic complexant may be carried out in two ways: either by completely decomplexing the ligand, or on the contrary by forming a very stable complex. It is this second method, widely inspired by Yamagushi et al.'s works [6], that we have chosen to use.

The development of this method of measurement was carried out in several steps. First, the various operating conditions (preparation of the samples, instrumental parameters) were optimized from standard solutions of $[FeY]^-$ complex; then real samples were analyzed. The observation of unstability of the signal led us to implement a chromatographic separation. This last step have necessitated the modification of the mobile phase.

3.1. Optimization of the electrospray signal

The $[FeY]^-$ complex exhibiting a negative charge, electrospray in negative mode was chosen as ionisation mode. Preliminary experiments have been carried out with a 5 mg/l solution in order to optimize the spectrometric and spray chamber parameters.

The detector was adjusted on the quasi-molecular ion (*m/z* 344.1) corresponding to the $[FeY]^-$ complex. Direct infusion of samples at low flow rates facilitated on-line optimisation of the MS conditions.

The mobile phase used was a mixture CH_3OH–water (60:40). The flow rate was 0.48 ml/min.

Let us note that additions of 0.05% formic acid or 0.05% ammonia in the aqueous phase did not provide a better sensitivity.

Various parameters were changed until the cleanest spectra with the highest intensity peaks were obtained. At all times, due consideration was taken on the fact that the method developed would obviously have to be compatible with electrospray chamber and mass spectrometer but also with a further chromatographic separation. The optimized parameters are: nebulization pressure: 50 psig (1 psi = 6894.76 Pa); fragmentor voltage: 90 V; drying gas flow: 11 l/min; drying gas temperature: 350 °C; capillary voltage: 3000 V.

Under these conditions, we have obtained the mass spectrum of the $[FeY]^-$ complex which is represented in Fig. 1.

This mass spectrum presents three majority ions. The peak *m/z* 344.1 is the quasi-molecular ion. The ions *m/z* 300.1 and *m/z* 256.1 correspond to successive losses of carboxylate groupings ($–COO^-$) starting from the *m/z* 344.1 ion. These fragmentations can be confirmed by analyses carried out by increasing the voltage of the fragmentor. Indeed, in this case, the abundance of the 344.1 ion decreases with the increasing formation of the ions *m/z* 300.1 and *m/z* 256.1.

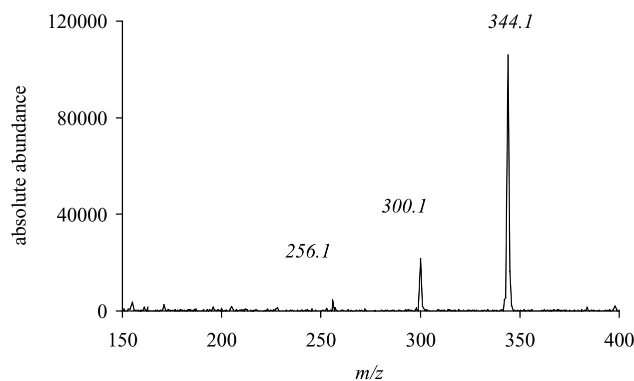

Fig. 1. Mass spectrum of the Fe–EDTA complex.

3.2. Implementation of a chromatographic column

In order to measure EDTA in reducing the reproducibility problems and the possible interferences with the matrices encountered with real samples, a reversed-phase column (RP C_{18}) has been implemented.

The mass detector always being regulated on the quasi-molecular ion (*m/z* 344.1), the chromatographic parameters were optimized. The new mobile phase conditions are: methanol–water + 0.1% formic acid (40:60); flow rate: 0.3 ml/min.

Formic acid was added to the aqueous phase in order to protonate the ungrafted hydroxides on the support. Furthermore, the protonation of hydroxyls groups enhanced the retention of the negatively charged complex. Under these new conditions, the retention time of the complex was of about 8 min, which corresponds to a retention volume of 2.4 ml, the dead volume of the column being around 1.5 ml.

The method was implemented with standards solutions then applied to real samples for which we had to practice the standard addition method in order to solve interference problems due to the particularly complex matrices encountered with the samples from industrial facilities.

In real samples, EDTA may be present in form of free and/or complexed with the various cations present in the matrices. It was thus necessary to add to these samples a large excess of iron(III) in order to completely shift the equilibriums towards the formation of the single $[FeY]^-$ complex. In order to calculate the quantity of ferric ions to be added, the concentration EDTA in the real samples was estimated at 0.2 mg/l (two times the required specification), and a large excess of ferric ions (25 equivalents) was added. The real samples were filtered through cellulose filter of 0.45 μm porosity (Millipore type filter) so that particles do not obstruct the various parts (capillaries) of the apparatus. The various samples (E1–E5) for the standard addition method were prepared according to Table 1.

The majority of the real samples thus could be analyzed and the exploitation of the linear regression curves: surface peak (*m/z* 344.1) versus concentration plotted thanks to the external calibration technique or with the standard additions

Table 1
Standard addition method in Section 3.2

	E0 (ml)	S4 (μl)	Water	$[FeY^-]$ added (μg/l)
E1	2	0	8	0
E2	2	100	7.9	10
E3	2	200	7.8	20
E4	2	400	7.6	40
E5	2	600	7.4	60

E0 = 10 ml of filtered sample added with 10 μl of S2 solution ($[Fe^{3+}]$ = 1 g/l), i.e. with 10 μg of ferric ions. S4: synthetic solution of the complex ($[FeY^-]$ = 1 mg/l) prepared from S3 solution.

method led to EDTA concentrations, for the whole analyzed samples, lower than the specification required, thus largely improving the detection limits obtained by ion chromatography (0.1 mg/l for a 500 μl injection loop).

However, for few real samples analyzed, the presence of closely related species (generating isobaric ions at 344.1 u in the electrospray chamber) always generated shoulders on the peak of the target species. Indeed, several chromatographic peaks corresponding to ions *m/z* 344.1 can be observed close to the peak of the complex.

For this reason, new chromatographic conditions were researched. Finally, we find that a mobile phase composed by a mixture of acetonitrile (70%) and water with 0.1% HCOOH (30%) provided better separations of the various peaks. In these new conditions, the retention time of the $[FeY]^-$ complex was of about 7 min, which corresponds to a retention volume of about 2.1 ml.

4. Main characteristics of the final method

4.1. Detection limit

The observed detection limit with the two eluting systems used [CH_3OH–water + 0.1% HCOOH (40:60) and CH_3CN–water + 0.1% HCOOH, (30:70)] is of about 1 μg/l for 20 μl injected (S/N = 3).

In general, the method provides calibration curves with good regression characteristics (correlation coefficient > 0.998). A typical equation for the calibration curve obtained with external standards is $A = 621.1c$ (where A is the area of the peak and c is the concentration of the standard solution in μg/l).

Fig. 2 shows the chromatogram of a 2 μg/l standard solution (20 μl injected) and allows to estimate the sensibility of the method.

The mass spectrum of the complex obtained in these new mobile phase conditions is totally similar to the one showed in Fig. 1.

4.2. Repeatability

We have evaluated the repeatability of the method for two concentrations values. Peak height has been considered.

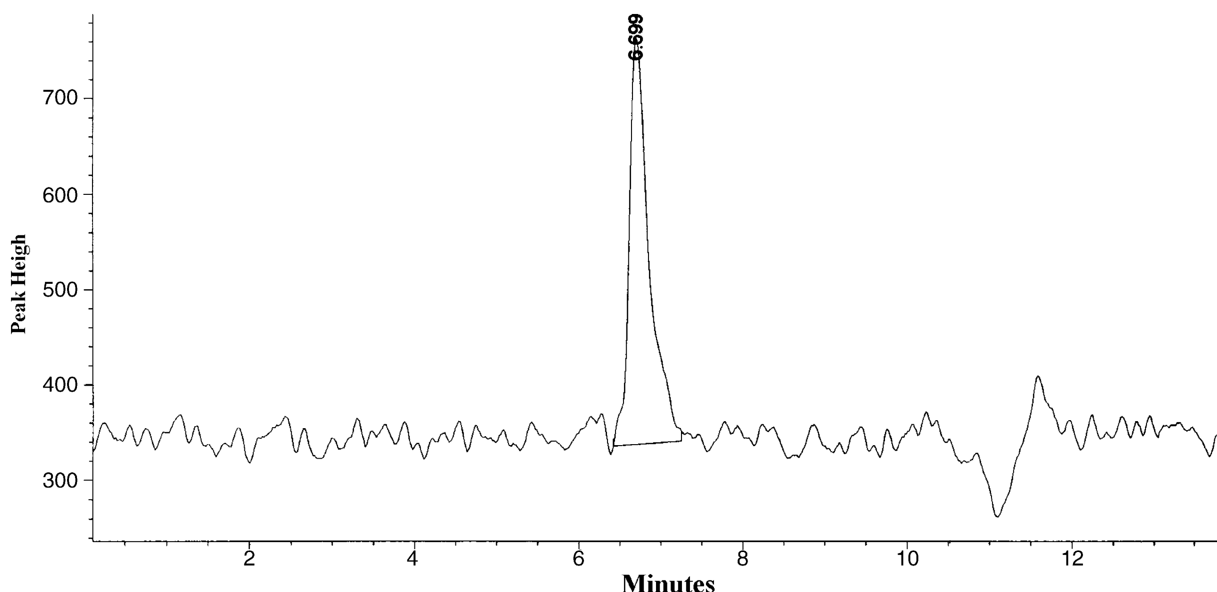

Fig. 2. Chromatogram of a 2 μg/l Fe–EDTA standard solution (20 μl injected).

Ten successive analyses were carried out with two solutions of the [FeY^-] complex. The first one is 10 μg/l and the other corresponding to five times this value. The results are expressed in Table 2.

We may observe that relative uncertainty is around 1% independently of the concentration of the standard solution.

4.3. Accuracy

In order to estimate accuracy, a real sample has been analysed according to the method previously described. In a first time, the standard addition method have been performed, in order to establish the absence of EDTA in this real sample. Then, addition of the Fe-EDTA complex at the concentration of 40 μg/l (corresponding to 34 μg/l of free EDTA) has been realized into this sample. Finally this last sample has been analysed to verify if the complex is recovered in its totality. The equation of the standard addition curve is $y = 1768.4x + 57544$ (y is the peak area of the 344.1 u ion and x is the EDTA concentration in μg/l). The value of EDTA determined by the standard addition method is 32.7 μg/l. The relative difference with the theoretical value is $((34 - 32.7)100)/34 = 3.8\%$.

Table 2
Repeatability

	Peak height of the m/z 344.1 signal	
	[EDTA] = 8.5 (μg/l)	[EDTA] = 42.5 (μg/l)
1	1039	3906
2	1106	3988
3	1095	3913
4	1104	3831
5	1078	3815
6	1086	3946
7	1080	4038
8	1084	3942
9	1058	3851
10	1078	3748
$\bar{X}$	1080.8	3897.8
σ	19.2	82.6
R.S.D. (%)	1.8	2.1
$\frac{(\sigma t)/\sqrt{n}}{\bar{X}}$	0.010 (1.0%)	0.012 (1.2%)

Student's $t = 1.833$ (for $t_{95\%}$ and $n = 10$).

5. Conclusion

The high-performance liquid chromatography technique coupled with mass spectrometry through an electrospray ionization interface made it possible to measure ethylenediaminetetraacetic acid in the form of its iron complex in aqueous effluents at very low concentrations (about 1 μg/l) without any previous preconcentration and for only 20 μl injected. The direct infusion method was not possible because of the unstability of the signal and interferences concerns. The implementation of a separative column (reverse phase C_{18}) was necessary both to improve stability of the signal and to limit interferences. But in the case of certain industrial aqueous effluents, the presence of isobaric ions (m/z 344.1) may involve problems of interferences. A modification in the composition of the mobile phase (use of acetonitrile instead of methanol) was necessary to solve this problem. The technical conditions that we developed are pointed out below: single ion monitoring (344.1 u) in the negative ionization mode; mobile phase: CH_3CN–water + 0.1% HCOOH (30:70) with a flow rate of 0.3 ml/min; stationary phase: RP C_{18}, 5 μm. The spray chamber parameters are: nebulisation pressure: 50 psig; fragmentor voltage: 90 V; drying gas flow:

11 l/min; drying gas temperature: 350 °C; capillary voltage: 3000 V.

Repeatability of the method is of about 1% for a 8.5 μg/l EDTA concentration. Accuracy (determined by the standard addition method on a real sample) is of about 4%. Furthermore, the observed detection limit is of about 1 μg/l for only 20 μl injected without any preconcentration. In order to increase slightly the retention time of the target species, a complementary study involving the use of a less apolar stationary phase (C_8 or C_4 reversed phase), is now envisaged.

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