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Gradient ion-pair chromatographic method for the determination of iron *N*,*N*′-ethylenediamine-di-(2-hydroxy-5-sulfophenylacetate) by high performance liquid chromatography–atmospheric pressure ionization electrospray mass spectrometry☆

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Abstract

The most effective remedy for iron deficiency is the use of synthetic iron chelates, specifically chelates derived from polyamine-carboxylic acids as EDDHSA (*N*,*N*′-ethylenediamine-di-(2-hidroxy-5-sulfophenylacetic) acid). A gradient ion-pair chromatographic method was developed to quantify the total amount of chelated iron in EDDHSA/Fe^{3+} fertilizers. Two mobile phases were used containing, respectively, 35 and 75% acetonitrile in a 0.005 M tetrabutylammonium hydroxide aqueous solution at pH 6.0. The stationary phase was a reverse phase C-18 column (150 mm × 3.9 mm i.d., d_p = 5 μm). Two chromatographic peaks appeared and were identified by Electrospray Mass Spectrometry. The first peak corresponds to the monomer of EDDHSA/Fe^{3+} and the second peak has been assigned to condensation molecules. Quality parameters indicate that the method is suitable for the quantification of iron chelate by EDDHSA fertilizers.

Keywords: EDDHSA/Fe^{3+}; *N*,*N*′-Ethylenediamine-di-(2-hydroxy-5-sulfophenylacetic acid); Chelated iron; Fertilizers; HPLC/APIES-MS

1. Introduction

Iron chlorosis is a nutritional disorder that decreases the yield of susceptible crops, such as fruit trees, growing in calcareous soils. The most effective remedy for iron deficiency is the use of synthetic iron chelates, specifically chelates derived from polyamine-carboxylic acids with a structure analogous to EDDHA [1]. These are complex organic molecules, in which a chelating agent co-ordinates the Fe^{3+} ion, and yields an anion of relatively low reactivity in soils and high stability in either neutral or alkaline solutions [2,3], thus allowing the iron to be maintained in solution and transported to the plant root. Since these fertilizers were prepared for the first time their use has dramatically increased not only in agriculture but also in other fields [1,4,5].

Abbreviations: HPLC, high-performance liquid chromatography; APIES-MS, Atmosphere pressure ionization electrospray mass spectrometry; EDDHSA, *N*,*N*′-ethylenediamine-di-(2-hidroxy-5-sulfophenylacetic) acid; EDDHA, *N*,*N*′-ethylenediamine-di-(*o*-hydroxyphenylacetic) acid; EDDHMA, *N*,*N*′-ethylenediamine-di-(*o*-hydroxy-*p*-methylphenylacetic) acid; EDTA, ethylenediaminetetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; HEDTA, 2-hydroxyethylethylenediaminetriacetic acid; CDTA, trans-1,2-cyclohexanediaminetetraacetic acid; HBEP, N,*N*′-bis(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-dipropionic) acid; HBED, *N*,*N*′-bis(2-hydroxybenzyl)ethylenediamine-*N*,*N*′-diacetic) acid; TBAOH, tetrabutylammonium hydroxide

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The present European Regulation on fertilizers (EC Regulation No. 2003/2003 of the European Parliament and of the Council of 13 October 2003) allows 11 chelating agents to be used in agriculture, all of them being polyamine-carboxylic acids. *N*,*N*′-Ethylenediamine-di-(2-hydroxy-5-sulfophenylacetic) acid (EDDHSA) was first used in the US [6]. EDDHSA has been present in the Spanish market since 1987. In an analysis of the Spanish market of EDDHA analogues, Álvarez-Fernández [7] has shown that 28% of commercial products sold between 1998 and 1999 contained EDDHSA/Fe^{3+}.

Several synthetic methods have been developed to obtain EDDHSA [6,8–13]. Its typical synthesis follows a Mannich reaction whereby 2 mol of *p*-hydroxybenzenesulfonic acid react with 2 mol of glyoxylic acid and 1 mol of ethylenediamine in alkaline solution [6,8–10]. In this synthesis, the *ortho*-hydroxy isomer is produced exclusively, owing to the well known directing ability of the hydroxy group in the presence of the *para* position occupied by sulfonic group (Fig. 1A). The presence of two sulfonic groups makes EDDHSA/Fe^{3+} much more soluble than the other polyamine-carboxylic acids [7]. Further, the greater acidity of the phenolic groups results in an increased iron affinity of this ligand, as compared to the parent molecule EDDHA [14].

The commercial preparations of EDDHSA/Fe^{3+} chelates are usually obtained by adding inorganic iron salts to an unpurified solution of the ligand [11]. Unfortunately, due to its high solubility in water, the non-reacted *p*-hydroxybenzenesulfonic acid cannot be easily removed from the reaction mixture and it is often detected in these products as an impurity [15]. The synthesised iron chelate is very stable, it is not affected by factors such as pH and the interaction with calcareous and alkaline soils and soil components [16] and has a lower production cost than the other chelates of the same family. All these facts indicate that EDDHSA/Fe^{3+} can be used competitively for the treatment of iron chlorosis.

The European Commission has asked for the development of an analytical method for the determination of de Fe chelated by EDDHSA (CAS# 57368-07-7) including their condensation products (CAS# 642045-40-7) in order to be included in the list of authorised chelating agents. Many chromatographic techniques have been applied for the analysis of this kind of compounds, for example, paper chromatography [17], thin layer chromatography [18], glass column preparative chromatography [19,20] and high-performance liquid chromatography (HPLC) [21–24]. The latter has proven to be the most useful technique in this field [25]. Lucena et al. [23] proposed a fast ion-pair HPLC method to determine the percentage of the Fe chelated in iron fertilizers in 15 min. EDTA/Fe^{3+}, DTPA/Fe^{3+}, CDTA/Fe^{3+}, EDDHA/Fe^{3+}, EDDHMA/Fe^{3+}, HBEP/Fe^{3+} and HBED/Fe^{3+} were well separated with good resolution and selectivity, including the separation of their stereoisomers when present. Hernández-Apaolaza et al. [24] applied this method to several EDDHA/Fe^{3+} and EDTA/Fe^{3+} commercial products available on the Spanish market and found that none of the tested EDDHA/Fe^{3+} formulations reached the chelated iron percentage declared on the label by the manufacturer. Hernández-Apaolaza et al. [25] and Álvarez-Fernández et al. [15] used this method to quantify the chelated iron in fertilizers containing EDDHSA/Fe^{3+} and were able to separate for the first time their stereoisomers. The chromatographic separation showed two peaks (assigned to the EDDHSA/Fe^{3+} stereoisomers, racemic and meso), having typical UV–vis spectra of this type of products. However, that method [23] does not account for the Fe chelated by the condensation products (CAS# 642045-40-7) included in the EC Mandate. In fact, previous work in our laboratories showed discrepancies between the amount of chelated iron determined by the chromatographic method described by Lucena et al. [23] and other methodologies such as the photometric titration of the chelating agent, that are reviewed in this paper (see Section 3.1 in Section 3).

In this paper, we show an improvement of the HPLC method for the quantification of chelated iron in EDDHSA/Fe^{3+} fertilizers based on gradient chromatography. We also characterise each chromatographic peak with the help of mass spectrometry coupled to an HPLC system.

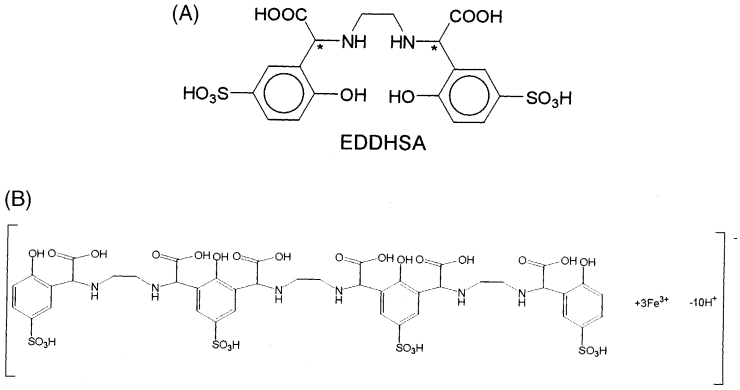

Fig. 1. Molecular structure of the EDDHSA/Fe^{3+} chelate (A) and the presumibly condensation products (B) used as fertilizers to control Fe chlorosis in plants ('*' denotes chiral carbons).

2. Experimental

2.1. Reagents, standards and samples

Analytical-reagent grade NaOH, $FeCl_3 \cdot 6H_2O$ were obtained from Merck Chemical Co., hydrochloric acid from Panreac, acetonitrile of HPLC grade from Riedel-de Haën and tetrabutylammonium hydroxide (40% solution in water) from Aldrich.

EDDHA, acid form, was obtained from Sigma (98% pure, Lot No. 117F5022l) and different EDDHSA ligands were used:

ST-1 (EDDHSA, potassium salt, provided by NAC Química S.L.) was obtained following a similar method than that previously described in [6] but adding and excess of glyoxylic acid to increase the amount of condensation products.

ST-3 (EDDHSA, diammonium salt, provided by S.A. DABEER) was synthesised following the method previously described in [6]. The reaction solution was purified, by passing the product through a strong cationic resin (Lewatit S-100) in the H^+ form.

ST-2 (EDDHSA, acid form, provided by S.A. DABEER) was obtained following the same reaction pathway as ST-3, but with a 10-fold excess of *p*-hydroxybenzenesulphonic acid sodium salt. In the reaction product, most of the non-reacted sodium salt of this acid was recovered following fractionated crystallization. The product was purified using exchange resins in a similar way to ST-3 product. Its complete characterization (protonation and stability constants for Ca^{2+}, Mg^{2+}, Cu^{2+} and Fe^{3+}, pM values and species distribution) is shown in [14].

The purity of the four chelating agents was determined following the methodology described in [14]. In brief, chelating agent solutions buffered at pH 6 were titrated with a Fe^{3+} standard solution. The formation of the complex was followed measuring the increase of absorbance at 480 nm with a white light spectrode connected to a Metrohm 721 potentiometer. A Metrohm 719 potentiometer, fitted with combined pH glass electrode, was used to maintain pH 6 during the titration with 0.200 M NaOH. The end-point of the titration was calculated from the intersection of the two linear segments. Also the purity of the ST-3 was checked by measuring the areas of the HPLC peaks using the gradient method developed in this paper after adding increasingly amounts of a Fe^{3+} standard on a solution of the ST-3. The result was the same than with the photometric titration.

One solid commercial EDDHSA/Fe^{3+} fertilizer (6% nominal chelated iron) was also analyzed. All aqueous solutions were prepared with type I water (electrical conductivity max: 0.056 $\mu S\,cm^{-1}$ at 25 °C; electrical resistivity min: 18.0 MΩ cm at 25 °C; total organic carbon max: 100 $\mu g\,l^{-1}$; sodium max: 1 $\mu g\,l^{-1}$; chlorides max: 1 $\mu g\,l^{-1}$; total silica max: 3 $\mu g\,l^{-1}$) [26].

2.2. Preparation of standards and sample solutions

To prepare the EDDHA/Fe^{3+} and EDDHSA/Fe^{3+} standard solutions, the ligands were dissolved in NaOH (ligand:NaOH, 1:3 molar ratio). Then an amount of $FeCl_3 \cdot 6H_2O$, calculated to be 5% in excess of the molar amount of the ligand, was added while keeping the solution pH in the range 6–8 with NaOH or HCl. The solution pH was adjusted at 7.0 at the end of the iron addiction. The solution was left to stand overnight to allow excess Fe to precipitate as oxides. The final solution, with an iron concentration of 1.79 mmol l^{-1} (100 mg l^{-1}) assessed by Atomic Absortion Spectrophotometry, was filtered through 0.45 μm Millipore cellulose membrane and made up to volume with water. These solutions were diluted as required.

A solution of the commercial product containing 1.79 mmol l^{-1} (100 mg l^{-1}) of Fe, based on the Fe content indicated in the product's label, was prepared by dissolving the formulation in water and filtered through 0.45 μm Millipore cellulose membrane prior to HPLC analysis.

Light exposure of all chelate solutions was avoided during their preparation and storage because of the potential photodecomposition of chelates [27].

2.3. Preparation of the mobile phase

Two mobile phases (A and B) were used containing respectively, 35 and 75% acetonitrile in 0.005 M TBAOH aqueous solution at pH 6.0. The solutions were filtered through 0.20 μm Whatman nylon membranes.

The gradient used was: 5 min 100% mobile phase A; 1 min change mobile phase A to mobile phase B; 5 min 100% mobile phase B; 1 min change mobile phase B to mobile phase A and 4 min 100% mobile phase A. The flow rate and the injection volume used were 1.5 ml min^{-1} and 20 μl, respectively.

The pH of the mobile phases (6.0) was chosen such so as to maintain the EDDHSA/Fe^{3+} in the ferrated ligand form (FeL) [14]. Higher pH could lead to decomplexing (Eq. (1)) or hydroxylation (Eq. (2)) of the iron chelate according to the following equilibria:

$$3\,OH^- + FeL^{3-n} \leftrightarrows Fe(OH)_3 + L^{n-} \quad (1)$$

$$FeL^{3-n} + yOH^- \leftrightarrows FeL(OH)_y{}^{3-n-y} \quad (2)$$

Lower pH could lead to the protonation and decomplexing of the chelate (Eq. (3)) or the presence of the protonated iron (III) form (Eq. (4)):

$$nH^+ + FeL^{3-n} \leftrightarrows Fe^{3+} + LH_n \quad (3)$$

$$FeL^{3-n} + xH^+ \leftrightarrows FeH_xL^{3+x-n} \quad (4)$$

2.4. Ion-pair chromatographic system

A Waters 2695 Separation Module (Alliance) and a Waters 996 Photodiode Array Detector were used. A Symmetry

C-18 (150 mm × 3.9 mm and d_p = 5 μm column) was used. The chromatograms and spectra were collected and processed using Empower chromatography data system.

2.5. Chromatographic peaks characterization

The chromatographic peaks were separated and characterized by HPLC/MS using a Agilent model 1100 system with a Photodiode Array Detector and electrospray as ionization source operated in negative ion mode at atmospheric pressure. The column was a Symmetry C-18, i.d. 150 mm × 3.9 mm and d_p = 5 μm; the same gradient conditions described in Section 2.3 were used, except for the fact that flow rate was 0.8 ml min^{-1} and injection volume was 10 μl. Mass spectra were acquired by scanning the quadrupole mass analyzer in the range 500–1500 *m*/*z*. All data were processed with a Chemstation data system.

3. Results and discussion

3.1. Purity of the standards

The purity of EDDHSA ligands (ST-1, ST-2 and ST-3) was determined by two methods: (1) a spectrophotometric titration with Fe^{3+} [14], and (2) the chromatographic method described in [23]. In order to quantify EDDHSA/Fe^{3+} by HPLC, pure EDDHA/Fe^{3+} was used as a standard, and a correction for the difference in the absorptivity at 480 nm between them was applied [25].

The values of EDDHSA standard purities determined by the two methods are presented in Table 1. The tritrimetric purity is higher than the HPLC purity in those samples where the ligand has been synthesized using a stoichiometric ratio of the components (ST-3) or an excess of glyoxylic acid (ST-1), according to the industrial procedures [6,8–10]. On the contrary, the two results are identical in ST-2, whose synthesis required a 10-fold excess of *p*-hydroxybenzenesulfonic acid.

Differences between results obtained with both methods should be attributed to the presence of chelate not observed in the chromatographic determination [23]. The experimental data (corrected absorbance in regard to Fe concentration) obtained in the photometric titrations before the end point fit well to a straight line. This indicates either the existence of only one species or the presence of several species with the same absorptivity. As the titrimetric purity does not agree with the one calculated by HPLC, the second hypothesis seems more likely. Then the titrimetric purity could be higher than the HPLC purity because of the presence of other compounds that chelate iron through iron-phenolate bonds and have thus similar absorptions to EDDHSA/Fe^{3+}. This hypothesis is in agreement with the fact that ST1 and ST3, both synthesized to include the condensate products, are the standards that present the higher differences, while ST2, without the condensates does not present differences.

Since the ion-pair chromatographic method [23] does not determine the Fe chelated by the condensation products, it does not meet the requirements of the EC. Then, adequate chromatographic conditions permitting the identification of the total iron chelated present in these products, including the condensation products, were studied. Based on the chromatographic method elaborated by Lucena et al. [23], the influence of the different components from the eluent (concentration of ion pair reagent, percentage of acetonitrile, pH) was investigated.

Table 1
Titrimetric and HPLC purities (%), using the Lucena et al. [23] method, of EDDHSA-H_6 products (ST-1, ST-2 and ST-3)

Sample references	Titrimetric purity	Lucena et al. [23] method	Gradient method		
			Peak 1	Peak 2	P1 + P2
ST-1	54	19	23	25	48
ST-2	81	81	78	4	82
ST-3	74	35	37	27	64

3.2. Gradient ion-pair HPLC method: development and quality parameters

First, the influence of the TBAOH concentration in the mobile phase was investigated. TBAOH concentrations of 0.005, 0.01, 0.03 and 0.05 M were tested. Fig. 2 shows the retention time of the EDDHSA/Fe^{3+} chelate with respect to the concentration of the added TBAOH. When the concentration of TBAOH in the mobile phase increased from 0.005 to 0.05 M the retention time decreased. This apparently counter-intuitive result agrees with previous findings for the EDDHA/Fe^{3+} and EDDHMA/Fe^{3+} chelates with the same ion-pair reagent [22,23]. In fact, it has been shown by Hung and Taylor [28] that the mechanism occurring is a combination of ion exchange and desolvatation. As the concentration of ion-pairing reagent increases, both the C-18 surface area available for desolvatation and the counter ion (in this case chloride) behaviour as an anion-exchange competing ion which is more effective at higher concentrations, will cause a decrease in the retention time of the solute. The separation of the meso and racemic stereoisomers of EDDHSA/Fe^{3+} as two partially overlapped peaks is possible when the TBAOH

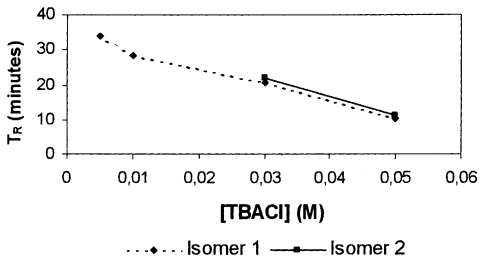

Fig. 2. Influence of the TBAOH concentration on the retention time of the EDDHSA/Fe^{3+} chelate studied. Column, Symmetry Waters C-18; eluent, variable concentration of TBACl-30% acetonitrile (pH 6.0); flow-rate, 1.5 ml min^{-1}; injection volume, 20 μl; detection wavelength, 480 nm.

concentration is kept above 0.03 M. However, in order to minimize mass spectra complications we used a TBAOH concentration of 0.005 M.

Due to the long retention time of the solute using an eluent consisting of 0.005 M TBAOH, 30% acetonitrile and pH 6, larger concentrations of acetonitrile were tested, and then a gradient separation was considered. The gradient has been presented in Section 2.3. The detection was performed at 480 nm and no interferences were detected. EDDHA/Fe^{3+}, EDDHMA/Fe^{3+}, EDTA/Fe^{3+}, DTPA/Fe^{3+}, CDTA/Fe^{3+}, HEDTA/Fe^{3+} as well as byproducts normally found in the commercial products containing these chelates do not interfere, since they are well separated from chelated iron in EDDHSA/Fe^{3+} fertilizers.

The gradient ion-pair chromatographic method described gave rise to a linear calibration for the determination of the iron chelated in the range of 0.090–1.79 mmol l^{-1} of Fe (5.0–100 mg Fe l^{-1}). The concentrations used were 0.090, 0.179, 0.448, 0.895, 1.07 and 1.79 mmol l^{-1} of chelated Fe (5.0, 10, 25, 50, 60 and 100 mg Fe l^{-1}) and the sum of both peak areas were measured. The calibration line was Area = 62522 × (mg Fe l^{-1}) − 3967. For the slope S_b = 134 and for the intercept S_a = 7124. The correlation coefficient obtained was r = 0.999990. The detection limit ($3.3S_B$) was 0.01 mmol l^{-1} (0.6 mg Fe l^{-1}) and the quantification limit ($10S_B$) was 0.03 mmol l^{-1}. This implies that the determination could be successfully done in a commercial sample containing as low as =0.1% of iron as EDDHSA/Fe^{3+} when prepared as indicated in Section 2. Repeatability ($2.8S_r$) for all the procedure determined for 14 commercial samples (three replicates per solid sample) was 0.09% Fe (as EDDHSA/Fe^{3+}). Since the tolerance for commercial samples containing more than 2% of Fe as EDDHSA/Fe^{3+} is 0.4% (EC Regulation No. 2003/2003) the method has enough repeatability for the determination.

Table 2 also gives some other characteristics of the method proposed to determine Fe chelated in EDDHSA/Fe^{3+} fertilizers.

Table 2
Retention times (t_R, min), number of theoretical plates (N), capacity factor (k') and sensitivity (S) based on the peak area (absorbance/mM) obtained for the EDDHSA/Fe^{3+} with the method described

Number	Parameter	EDDHSA/Fe^{3+}
1	t_R	6.90 ± 0.01
	N	8462
	k'	5.90
	S	50.09
2	t_R	7.04 ± 0.01
	N	4664
	k'	6.04
	S	20.20

Mobile phases, 35 and 75% acetonitrile in a 0.005 M tetrabutylammonium hydroxide aqueous solution at pH 6.0; flow rate 1.5 ml min^{-1}; injection volume 20 μl. Numbers 1 and 2 correspond with the sum of the stereoisomers and the condensation molecules, respectively. Each retention time value represents the t_R (± S.D.) (n = 3).

3.3. Nature of compounds separated by the HPLC analysis of EDDHSA/Fe^{3+} standards

The separation achieved with the gradient method is shown in Fig. 3. The chromatogram shows two chromatographic peaks whose UV–vis spectra are similar: both display peaks at about 207 (aromatic ring), 246 (–HSO_3 groups) and 285 nm (–OH groups). Also a broad band of absorption in the visible range typical of the iron–phenol complex at about 480 nm is observed [15]. Overall both spectra are typical of iron chelates of polyaminocarboxylic acids [21,29].

Table 1 shows the purities calculated by this gradient method. It appears that the purities of ST-1 and ST-3 obtained from the gradient method only match (within experimental errors) the titrimetric purity if the *sum* of the two chromatographic peaks is considered. Further, the purities obtained by considering the first chromatographic peak alone are similar to that obtained from the method in ref. [23]. As the first chromatographic peak corresponds to the coelution of the two stereoisomers of EDDHSA/Fe^{3+} monomer, the

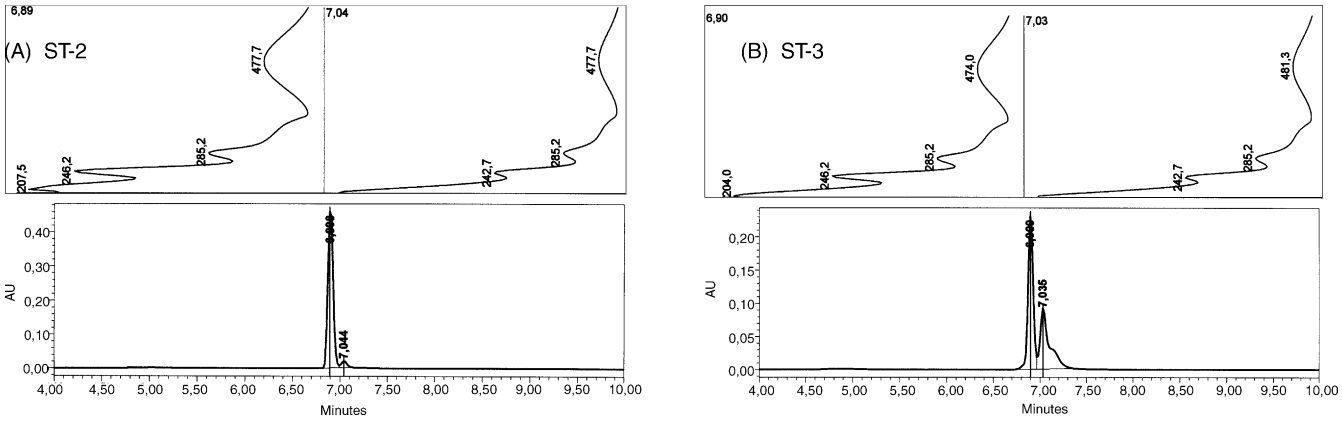

Fig. 3. Chromatograms and UV–vis spectra for (A) ST-2 and (B) ST-3 chelated iron. Column, Symmetry Waters C-18; eluent: mobile phase A: 0.005 M TBAOH–35% acetonitrile (pH 6.0) and mobile phase B: 0.005 M TBAOH–75% acetonitrile (pH 6.0); flow-rate, 1.5 ml min^{-1}; injection volume, 20 μl; detection wavelength, 480 nm.

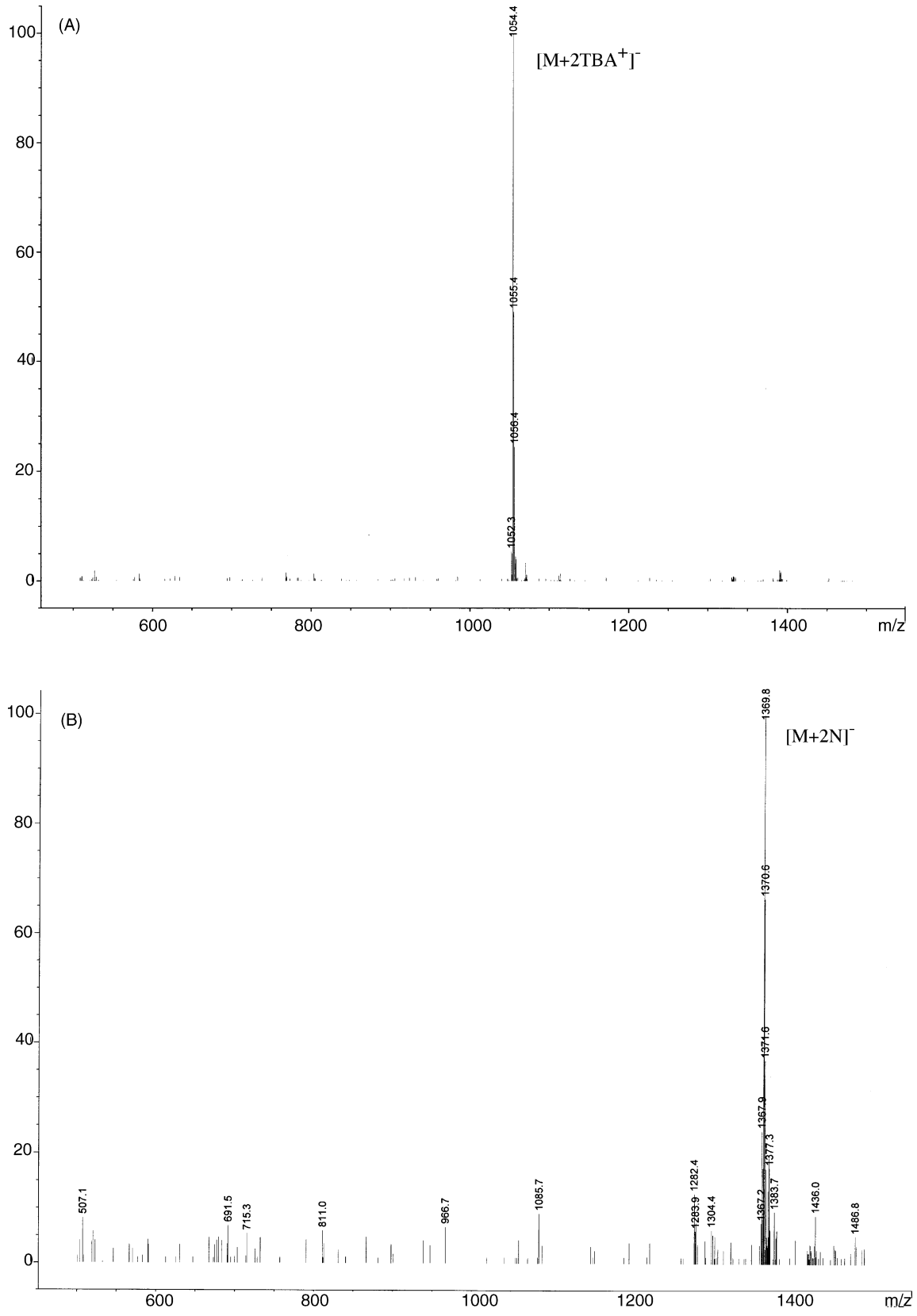

Fig. 4. Electrospray mass spectra of the two fractions separated by HPLC of a solution of ST-3 corresponding to the ion $[M + 2TBA^+]^-$ (A) and ion $[M + 2N]^-$ (B). M is a EDDHSA/Fe^{3+} molecule, TBA^+ is the ion-pair reagent and N is a EDDHSA/Fe^{3+} molecule without one of the benzene rings.

above findings prove that the second peak is due to one or more unknown compounds able to chelate iron through iron-phenolate bonds in a similar manner to EDDHSA. In addition, as the amount of these unknown impurities is different in ST-1 and ST-3, their presence should depend on the conditions chosen for the synthesis of the EDDHSA ligand. Interestingly, ST-2, which was synthesized in the presence of a 10-fold excess of *p*-hydroxysulfonic acid, contains a very small amount of the iron-chelating impurities. It is thus reasonable to infer that the impurities may derive from an oligomerization caused by a repeated Mannich reaction taking place at the two *ortho* positions to the hydroxy group and comprising several *p*-hydroxybenzenesulfonic acid molecules; the yield of the condensation compounds would obviously be reduced using a great excess of *p*-hydroxybenzenesulfonic acid. These compounds would still contain the EDDHSA scaffold, thus retaining a similar iron chelating ability and, yet, would be invisible under the conditions of [23] because of the presence of many ionizable groups that make them strongly retained by the stationary phase.

The above arguments are nicely confirmed by HPLC/API-ES experiments. The electrospray mass spectrum obtained from the first chromatografic peak (Fig. 4A) showed an intense ion signal at *m*/*z* 1054 corresponding to the ion $[M + 2TBA^+]^-$ (where *M* is a EDDHSA/Fe^{3+} molecule and TBA^+ the ion-pair reagent used in the chromatographic method), and confirmed the assignment of the first peak to the EDDHSA/Fe^{3+} chelate. The electrospray mass spectrum of the second peak (Fig. 4B) showed ion signals at *m*/*z* 1370 compatible with a condensation compound shown in Fig. 1B.

3.4. Analysis of commercial EDDHSA/Fe^{3+} fertilizer

The analysis of a commercial fertilizer containing the EDDHSA/Fe^{3+} chelate yielded a similar chromatogram to that of ST-3. Two peaks corresponding to EDDHSA/Fe^{3+} and to the condensation compounds were detected. No interferences were encountered in the analysis and the retention times agreed with those previously obtained from the EDDHSA/Fe^{3+}standard solution.

Using ST-3 as standard, the amount of chelated iron in the commercial product was 3.51%. This amount is larger than the 1.53% that was obtained for the EDDHSA/Fe^{3+} monomer using the isocratic chromatographic method [23]. Since the soluble iron in this product was 6.05% the chelated fraction (chelated iron respect soluble iron) was 58%. These data is in good agreement with the findings of Cantera et al. [30]. They measured the soluble iron and the EDDHSA/Fe^{3+} monomer after 22 days of interaction of five EDDHSA commercial products with a calcareous soil and they found that it remains in solution around 77% more Fe than the one explained by the monomer, indicating the presence of other high stable chelates in the commercial products. These chelates are undoubtedly the condensation products that are also included in the HPLC method here presented.

4. Conclusions

A gradient ion-pair chromatographic method has been developed to determine the iron chelated in commercial fertilizers containing EDDHSA as chelating agent used as remedy for the iron chlorosis in calcareous soils. Two chromatographic peaks appeared, assigned to the different forms of chelated iron: stereoisomers of EDDHSA/Fe^{3+} monomer and the additional compounds (condensation products). This method could be used to quantify commercial chelates according to the directives regulating this type of products. Also the method will be an important tool to help the research on the behaviour of these condensation products in plant and soil systems.

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