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Demetalation of Fe, Mn, and Cu Chelates and Complexes: Application to the NMR Analysis of Micronutrient Fertilizers

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Supporting Information

ABSTRACT: The application of nuclear magnetic resonance (NMR) for the quality control of fertilizers based on Fe^{3+}, Mn^{2+}, and Cu^{2+} chelates and complexes is precluded by the strong paramagnetism of metals. Recently, a method based on the use of ferrocyanide has been described to remove iron from commercial iron chelates based on the *o*,*o*-EDDHA [ethylenediamine-*N*,*N*′bis(2-hydroxyphenylacetic)acid] chelating agent for their analysis and quantification by NMR. The present work extended that procedure to other paramagnetic ions, manganese and copper, and other chelating, EDTA (ethylenediaminetetraacetic acid), IDHA [*N*-(1,2-dicarboxyethyl)-D,L-aspartic acid], and complexing agents, gluconate and heptagluconate. Results showed that the removal of the paramagnetic ions was complete, allowing us to obtain ^{1}H NMR spectra characterized by narrow peaks. The quantification of the ligands by NMR and high-performance liquid chromatography showed that their complete recovery was granted. The NMR analysis enabled detection and quantification of unknown impurities without the need of pure compounds as internal standards.

KEYWORDS: NMR, chelates, fertilizers, manganese, copper, iron, zinc, HPLC

INTRODUCTION

The correction of metal micronutrient deficiencies in soils is a problem still not fully solved in agriculture. The low solubility of the Fe^{3+}, Mn^{2+}, Cu^{2+}, and Zn^{2+} compounds in the pH range of calcareous soils contributes, among other factors, to the low availability of these nutrients to plants, so chelates and complexes are used as fertilizers to increase the solubility of the metal cations.[1]

The most effective and widely used iron fertilizers available on the market are based on *o*,*o*-EDDHA [ethylenediaminedi-(2-hydroxyphenylacetic) acid; see Figure 1A].[1−3] Fertilizers based on other polyaminocarboxylic acids are commercially available, less effective, but cheaper than EDDHA. Mn, Zn, and Cu deficiencies are generally treated with products based on their salts, mainly sulfates, complexes, or chelates of EDTA (ethylenediaminetetraacetic acid; see Figure 1D) or analogous. Regulation (EC) No. 2003/2003[4] and its subsequent modifications[5−7] regulate these chelating agents and require that each commercial formulation must be labeled with the amount and kind of metal chelate on which it is based. Quality control of fertilizers containing metal chelates is typically carried out by means of ion chromatography.[8−13] At present, there are not specific official methods to identify and quantify the complexing agent in fertilizers based on micronutrient complexes, as most of them have an unknown chemical structure due to their natural source. However, a method is available to determine the complexed metal contained in the fertilizer through precipitation of the free metal at pH 9.[14,15] Proton nuclear magnetic resonance (^{1}H NMR) has in principle the power of detecting signals from all of the organic compounds contained in a mixture, provided that they dissolve in a suitable solvent and their molecular weight does not exceed a threshold that depends on the operating frequency of the spectrometer. Unfortunately, its application to a commercial formulation based on Fe^{3+}, Mn^{2+}, and Cu^{2+} requires the prior removal of the metal ions. Their paramagnetism leads to spectra characterized by large, and uninformative, peaks. Recently, a method has been developed[16] to remove Fe^{3+} from commercial formulation of *o*,*o*-EDDHA, based on potassium ferrocyanide as a reactant, which forms with Fe^{3+} the insoluble compound Prussian Blue, $Fe_4[Fe(CN)_6]_3$, with a $K_{sol} = 2 \times 10^{-37}$.[17] The method was shown not to modify the composition of the fertilizing mixture for the analysis and quantification by NMR.

Among the micronutrients typically added to the soils through fertilizers, *o*,*o*-EDDHA/Fe^{3+} shows the highest stability constant. On the other hand, manganese and copper form with ferrocyanide quite insoluble ferrocyanide compounds, having $Mn_2[Fe(CN)_6]$ and $Cu_2[Fe(CN)_6]$ and a K_{sol} of 8×10^{-13} and 1.3×10^{-16}, respectively,[18,19] releasing the ligand in the solution for the NMR analysis. Because of the low solubility constants of these ferrocyanide compounds, this demetalation method seems also to be adequate to remove Mn and Cu from fertilizer samples.

The present work aimed thus at verifying the possibility to extend the demetalation method to fertilizers based on other micronutrient than Fe^{3+} and to other chelating or complexing agents than EDDHA for the NMR identification and quantification of fertilizers samples.

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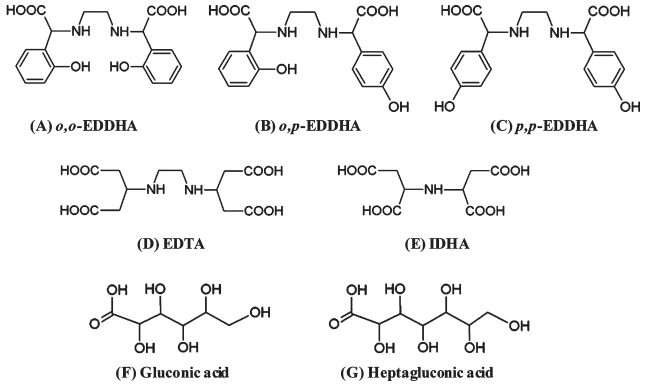

Figure 1. Structure of the chelating agents studied for their NMR analysis in micronutrient fertilizers.

Table 1. HPLC and NMR Results for Isomers of EDDHA Ligand[a]

	o,o-EDDHA	*o,p*-EDDHA	*p,p*-EDDHA
HPLC	21.6 ± 0.1	14.2 ± 0.2	
NMR	22.7 ± 0.7	14.2 ± 1.1	3.3 ± 0.7

[a] The results have been expressed as % (g ligand 100 g^{-1} product).

Thus, an alternative, or additional, method to the chromatography can be used to a better characterization of those samples. In detail, commonly used products based on *o,o*-EDDHA, EDTA, and the novel biodegradable IDHA [*N*-(1,2-dicarboxyethyl)-D,L-aspartic acid; see Figure 1E] chelating Fe^{3+}, Mn^{2+}, Cu^{2+}, Zn^{2+}, and their mixtures have been considered. Fertilizers based on gluconate and heptagluconate (see Figure 1F,G) complexes also have been studied. For each of the above molecules, standard samples were analyzed too, to better focus on the ability to completely recover the ligand after the metal removal procedure. To obtain quantitative information about the organic ligands recovery of the method, their quantification was performed also through high-performance liquid chromatography (HPLC) methods.

MATERIALS AND METHODS

Standard Ligand Samples. The standard chelating agents used were H_4*o,o*-EDDHA (Promochem, United Kingdom), H_4*o,p*-EDDHA [ethylenediamine-*N*-(2-hydroxyphenylacetic acid)-*N*′-(4-hydroxyphenylacetic acid)] (Syngenta, Spain), Na_2EDTA (Tritriplex III, 99%, Merck), Na_4IDHA solution (78.1%, Adob, Poland), and sodium gluconate (PRS, 98%, Panreac). The titrimetric purity of H_4*o,o*-EDDHA and H_4*o,p*-EDDHA were determined as in Yunta et al.[20] by a photometric titration with Fe^{3+} solution and corresponded to 93.9 and 93.1%, respectively.

EDDHA Samples. EDDHA/Mn^{2+}, EDDHA/Zn^{2+}, and EDDHA/Cu^{2+} chelates were prepared by adding to a water solution of EDDHA (21% w/w) an amount of $Mn(NO_3)_2 \cdot 4H_2O$, $Zn(NO_3)_2 \cdot 6H_2O$, and $Cu(NO_3)_2 \cdot 3H_2O$ (PA, Panreac), calculated to be 5% in excess of the molar amount of ligand. During the chelation process, the pH was maintained between 5.0 and 7.0 and adjusted to 6.0 at the end. Solutions were left to stand overnight to allow excess metal to precipitate as oxides. Final solutions were filtered through 0.45 μm pore size and 25 mm diameter Millex Millipore cellulose membrane. The EDDHA sample used as a ligand was provided by Syngenta. This sample contains a mixture of *o,o*-EDDHA, *o,p*-EDDHA, *p,p*-EDDHA (see Figure 1A−C), and other byproducts, and it is employed for the synthesis of the commercial product sold under the name of Sequestrene 138 Fe G 100. Light exposure was avoided during the preparation and storage of the chelate solutions because of the potential photodecomposition of chelates.[21,22] Water was removed from all samples by means of rotary evaporation.

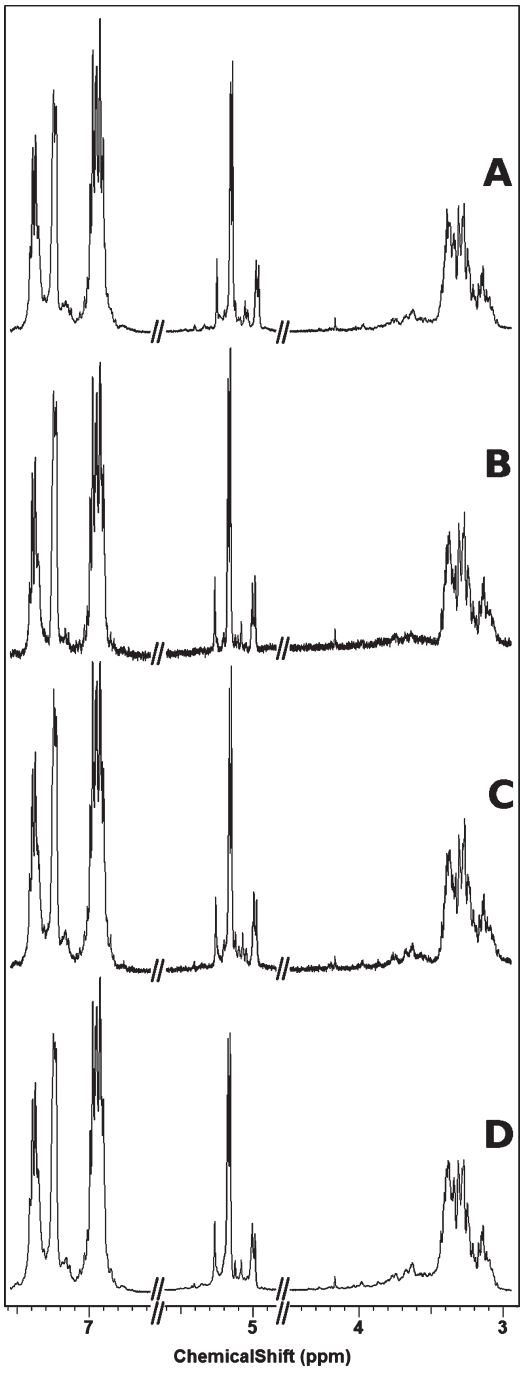

Figure 2. ^{1}H NMR spectra of standard EDDHA (A) and of three commercial samples based on EDDHA/Cu^{2+} (B), EDDHA/Mn^{2+} (C) from which the metal was removed with the method under investigation, and EDDHA/Zn^{2+} (D). The spectra were suitably scaled to ease their visual comparison.

EDTA Samples. Solid samples of EDTA/Fe^{3+}, EDTA/Mn^{2+}, EDTA/Zn^{2+}, and EDTA/Cu^{2+} (Tradecorp AZ) were provided by Tradecorp, Spain.

IDHA Samples. Solid samples containing as the main components IDHA/Fe^{3+}, IDHA/Mn^{2+}, IDHA/Zn^{2+}, and IDHA/Cu^{2+} and one sample

containing a mix of Fe^{3+}, Mn^{2+}, Zn^{2+}, and Cu^{2+} chelated by IDHA were provided by Adob, Poland.

Gluconate and Heptagluconate Samples. Manganese gluconate solution (Fertimix Mn) was provided by Arvensis Agro S.A. A solid sample of heptagluconate with Mn and Zn (Welgro Mn+Zn) was from C.Q. Massó, and a commercial solution of iron heptagluconate (Proferfol) was from Productos Foliares S.L. Water was removed from liquid samples by means of rotary evaporation to reduce water interference in the NMR spectra.

Demetalation of Chelates. Approximately 2 mg of samples based on gluconate and heptagluconate and 5 mg of the other samples were dissolved in 500 μL of D_2O in an Eppendorf tube. About 300 μL of a 40 mM solution of potassium ferrocyanide $[K_4[Fe(CN)_6]_3 \cdot 3H_2O]$ in D_2O was then added so that ferrocyanide exceeded the metal concentration estimated from the soluble element indicated on the product label adjusted for its stoichiometry in the complex to be formed. The pH of the solution was then lowered to 0.7 with HCl(g) (HCl fuming, PA, Panreac, 37%), to form the metal−ferrocyanide complex and to completely dissolve the organic ligands.[23] When EDTA chelates were considered, the pH was lowered to 1.0 to avoid EDTA precipitation. Ferrocyanide precipitates were removed by centrifugation at 18000*g* for 10 min in a Beckman Coulter Eppendorf centrifuge model Microfuge 18. Although residual low-spin ferrocyanide ion $[Fe(CN)_6]_4^-$ is diamagnetic and would not alter the spectrum, it is readily oxidized by the dissolved oxygen to paramagnetic ferricyanide $[Fe(CN)_6]_3^-$, the presence of which would broaden the NMR spectral lines. Therefore, 700 μL of the centrifugate was taken, and the excess of ferrocyanide was removed by the addition of a D_2O solution of $ZnSO_4$ until precipitation of $K_2Zn_3[Fe(CN)_6]_2$ was complete ($K_{sol} = 10^{-95}$).[24] Typically, an aliquot of 5−10 μL of a 1 M solution of $ZnSO_4$ was sufficient for this purpose. Finally, $K_2Zn_3[Fe(CN)_6]_2$ was removed by centrifugation at 18000*g* for 10 min, leaving a clear solution that contained the ligand with Zn, which then was analyzed by NMR. EDDHA ligand, IDHA, EDTA, and gluconate samples were measured in triplicate. Because a good reproducibility was obtained on such replicates, heptagluconate samples was measured in only one replicate.

NMR Analysis. ^{1}H NMR spectra were acquired at 400 MHz with a Mercury-Plus spectrometer (Varian, Palo Alto, CA), by minimizing the residual water peak through a PRESAT sequence, which saturates the

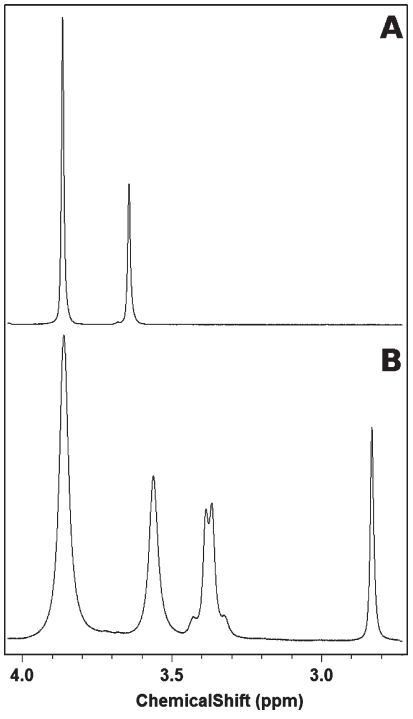

Figure 3. (A) ^{1}H NMR spectrum of standard EDTA. (B) ^{1}H NMR spectrum of a commercial sample based on EDTA/Mn^{2+} on which the metal removal procedure was applied, with an intentionally high amount of $ZnSO_4$, so as to highlight the presence of two sets of peaks.

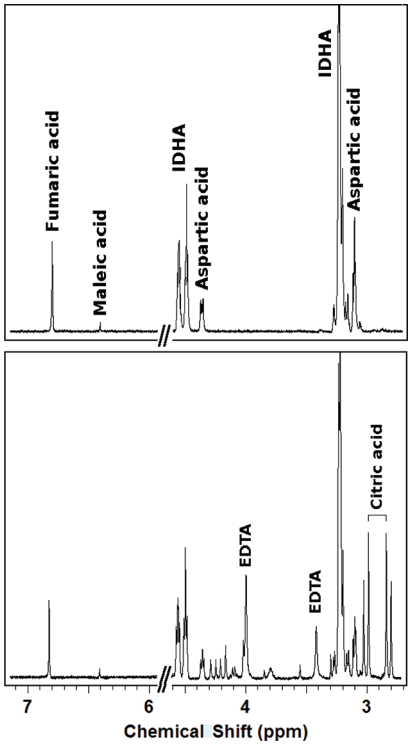

Figure 4. Upper part: ^{1}H NMR spectrum of standard IDHA. Lower part: ^{1}H NMR spectrum of a commercial IDHA/Fe^{3+} sample. Assignments are given only for the peaks that cannot be found in the standard sample.

Table 2. NMR and HPLC Results for EDTA Commercial Samples Expressed as % EDTA and % Metal Chelated by EDTA[a]

	EDTA (g/100 g)		metal chelated (g/100 g)		
	NMR	HPLC	NMR	HPLC	metal soluble on the label (g/100 g)
EDTA/Fe^{3+}	70.1 ± 2.2	70.4 ± 0.2	13.4 ± 0.4	13.45 ± 0.03	13
EDTA/Zn^{2+}	67.7 ± 1.6	67.5 ± 0.2	15.1 ± 0.3	15.12 ± 0.05	14
EDTA/Mn^{2+}	70.0 ± 1.6	72.9 ± 0.4	13.2 ± 0.3	13.70 ± 0.08	13
EDTA/Cu^{2+}	70.9 ± 1.2	68.4 ± 0.4	15.4 ± 0.3	14.87 ± 0.08	14.5

[a] Also, the soluble metal declared in the label of commercial samples is presented. The results have been expressed as % EDTA (g EDTA 100 g^{-1} product) and as % metal chelated by EDTA (g metal 100 g^{-1} product), taking into account a 1:1 metal:ligand ratio.

Table 3. NMR and HPLC Results for IDHA Standard and Commercial Micronutrient Samples

	NMR						HPLC	
	% IDHA	% aspartic	% fumaric	% maleic	% EDTA	% citric	% IDHA	% EDTA
IDHA std	59.6 ± 1.5	11.7 ± 0.3						
IDHA/Fe^{3+}	27.1 ± 0.9	4.1 ± 0.2	2.3 ± 0.2		5.2 ± 0.1	8.8 ± 0.5	27.5 ± 0.2	4.8 ± 0.0
IDHA/Mn^{2+}	36.1 ± 0.1	6.0 ± 0.1	3.0 ± 0.3	0.7 ± 0.0			34.9 ± 0.1	
IDHA/Cu^{2+}	38.8 ± 1.0	6.5 ± 0.2	3.1 ± 0.2	0.7 ± 0.1			38.3 ± 0.2	
IDHA/Zn^{2+}	39.6 ± 0.9	7.2 ± 0.2	3.4 ± 0.2	0.7 ± 0.0			39.8 ± 0.0	
IDHA mix	28.2 ± 0.2	6.7 ± 0.1	1.7 ± 0.1	0.3 ± 0.0	2.8 ± 0.0	0.9 ± 0.0	28.3 ± 0.0	2.5 ± 0.0

residual H_2O signal.[25] Care was taken to avoid using too intense a B1 field for saturation. We found that in our conditions, a B1 field of 70 Hz applied at the frequency of the residual water signal for 1.5 s was sufficient to saturate the water line without affecting the nearby signals. A recycle delay of 10 s was chosen based on a preliminary measure of the T_1 characterizing the protons of the substances under investigation. A known amount of pure acetic acid (50 μL of about 116.7 mM solution in D_2O) was added as an internal reference for concentration and chemical shift (methyl ^1H signal, 2.04 ppm; ^{13}C signal, 20.0 ppm). A temperature of 35 °C for EDDHA and EDTA and 25 °C for IDHA, gluconate, and heptagluconate samples were chosen to minimize the overlap between the residual water peak and the nearby chelate peaks. The spectra were processed by means of VNMRJ 1.1D software from Varian. EDDHA 1D spectra were submitted to reference deconvolution[26] with the routine "fiddle" of VNMRJ 1.1D, using the methyl line of the internal standard (acetic acid) as line shape template. In this way, a sufficient resolution was achieved in the benzylic region of *o,o*-, *o,p*-, and ethylenediaminedi-(4-hydroxyphenylacetic) acid (*p,p*-EDDHA) to perform line fitting and quantification through the "fitspec" routine of VNMRJ 1.1D. Fe, Mn, Cu, and mixed samples were measured after their demetalation while standard and Zn samples were measured directly after dissolving.

HPLC Analysis. The EDDHA ligand sample was measured by the ion-pair HPLC method proposed by Lucena et al.,[8] which was adopted as European Standard by CEN[11] for the determination of Fe chelated by *o,o*-EDDHA and *o,o*-EDDHMA. A Waters 2695 Separation Module, a Waters 996 Photodiode Array Detector, and a Symmetry C-18 (150 mm × 3.9 mm and dp = 5 μm) column were used. The mobile phase consisted of 30% acetonitrile in 0.03 M tetrabutylammonium hydroxide (TBAOH) aqueous solution at pH 6.0 at a flow rate of 1.5 mL/min. The injection volume was 20 μL. Spectra were recorded between 200 and 600 nm. Quantification was done at 280 nm. That samples were also analyzed by an HPLC method adopted by CEN[12] for the quantification of the *o,p*-EDDHA isomer. The column used was a Waters Spherisorb C-18 (250 mm × 4.6 mm) and a mobile phase of 1.0 g/L of sodium formate and 8.5% acetonitrile aqueous solution at pH 3.0 at a flow rate of 1.0 mL/min. The injection volume was 20 μL. Spectra were recorded between 200 and 600 nm. Quantification was done at 277 nm.

IDHA samples were measured by the recently described ion-pair HPLC method.[13] The column consist of a C-18 (250 mm × 4.6 mm and dp = 5 μm), while the mobile phase is made up of 2.5 g/L of tetra *N*-butylammonium-hydrogensulphate (TBAHS) and 0.17% TBAOH aqueous solution at pH 2.5 at a flow rate of 0.5 mL/min. The injection volume was 20 μL. Spectra were recorded between 200 and 600 nm. Quantification was done at 260 nm. The same method was used for the determination of EDTA in both EDTA and IDHA samples where traces of EDTA were detected by NMR. All of the HPLC analysis were performed in duplicate.

RESULTS AND DISCUSSION

General Remarks about Metal Removal Procedure. The procedure described by Laghi et al.[16] to remove Fe from commercial fertilizers based on *o,o*-EDDHA was extended in this work to other metals, namely, Mn^{2+} and Cu^{2+}, and to other ligands used in straight and mixed fertilizers without the need of major modifications to the original method. Similarly to EDDHA/Fe^{3+}, the chelate EDDHA/Cu^{2+}, which forms a very stable chelate (log $K_{0.1}$ *o,o*-EDDHA/Cu^{2+} = 25.13),[20] intensely blue colored, was quantitatively dissociated only when the solution pH was lowered below 1, as highlighted by the formation of an intense red color. Manganese forms a weaker chelate with *o,o*-EDDHA (unpublished data). For this reason, the creamy color typical of $Mn_2[Fe(CN)_6]$ could be noticed as far as ferrocyanide was added to the samples based on *o,o*-EDDHA/Mn^{2+} without pH modification.

The pH needed to quantitatively remove the metal was found to depend also on the binding ability of the ligand. While EDDHA and EDTA form very stable chelates with Fe and Cu[20], and thus required a pH close to 1, the formation of metal ferrocyanide solids was possible for IDHA,[27] gluconate, and heptagluconate[28] and all Mn chelates even above pH 3. Finally, while the pH of each ligand was set to 0.7 for NMR analysis to minimize chemical shift differences among the samples,[29] the pH of the samples based on EDTA was set to 1, as below that value precipitation of solids was evident.

To add the proper amount of ferrocyanide to the samples under investigation, the metal concentration was estimated from the product label. To extend the same procedure to samples with unknown metal concentration, a preliminary quantification of the soluble metal would be necessary.[4,14] In most practical cases, this could be performed based on the strong and characteristic colors of the original samples and ferrocyanide complexes.

Effectiveness of the Procedure Evaluated through NMR and HPLC. *EDDHA Samples.* To study the ability of the proposed method to remove Mn and Cu from fertilizers based on *o,o*-EDDHA, a sample of ligand was characterized by NMR and HPLC (Table 1). Its NMR spectrum was then compared with one from EDDHA/Zn^{2+} and one from EDDHA/Mn^{2+} and EDDHA/Cu^{2+} after complete removal of the metal (Figure 2). The peaks assignment was performed according to the literature.[16,23] The spectra showed peaks with similar width, leading to the conclusion that both Mn and Cu removal could be considered as complete. Besides, changes in the ratio between the signals due to the various regioisomers were not found, suggesting that the ligand could be quantitatively recovered. Such a finding was confirmed by a similar quantification performed with HPLC (Table 1).

EDTA Samples. The NMR spectrum of EDTA was reported by Kula et al.[30] and shows one peak for the methylene protons at 3.42 ppm and one for the CH protons at 4.0 ppm in 1:2 ratio. The same protons give rise to two other lines when zinc:EDTA exceeds a 2:1 ratio.[30,31] In the application to the EDTA sample of

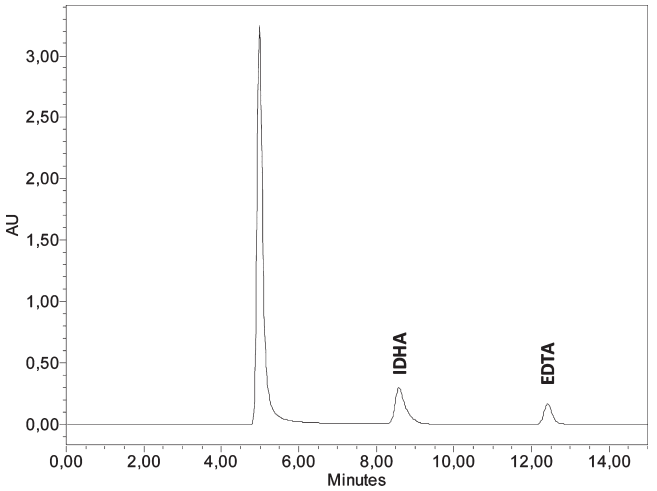

Figure 5. Chromatogram of IDHA/Fe^{3+} commercial sample. Column, Waters Symmetry C18; eluent, 0.17% TBAOH, 2.5 g/L TBAHS (pH 2.5); flow rate, 0.5 mL/min; injection volume, 20 μL; and detection wavelength, 260 nm.

the metal removal method under investigation, a sufficiently accurate addition of zinc in the final step was not always possible, so that 2 or 4 peaks appeared (Figure 3). To circumvent this source of error, the quantification was performed on the sum of all the peaks. The so obtained quantification through NMR paralleled the one with HPLC, as shown in Table 2, and was found to be coherent with the metal soluble percentage declared in the label of each product.

IDHA Samples. The ^{1}H NMR spectra of the IDHA sample under investigation presented six groups of signals (Figure 4, upper part). The triplets with similar intensity at 4.57 and 4.50 ppm were assigned to the CH protons of the meso and racemic forms of IDHA, without the possibility to obtain a specific assignment for the two. The corresponding methylene protons gave a multiplet at 3.24 ppm, again with no possibility to go into deeper detail. The spectrum was characterized by a third triplet signal at 4.37 ppm and another multiplet at 3.10 ppm, suggesting the presence of aspartic acid, and singlets at 6.82 and 6.40 ppm, coherent with the presence of fumaric and maleic acid. Such assignments were confirmed through gHSQC and gHMBC NMR experiments (see the Supporting Information) and the addition of pure substances. The presence of fumaric, maleic, and aspartic acids was not unexpected since they are known impurities from IDHA synthesis.[32] NMR spectra of demetalated IDHA samples were compared with those from IDHA/Zn^{2+} sample and pure ligand, where demetalation process was not applied (see the Supporting Information). The peak width similar for all such spectra showed that a complete removal of the metal was achieved in any case, with no apparent loss of ligand. Interestingly, IDHA/Fe^{3+} and the IDHA mix products presented additional signals at 3.41 and 4.00 ppm and between 2.8 and 3 ppm. A spiking procedure with pure substances enabled the assignment of the first two peaks to EDTA and the latter peaks to citric acid (Figure 4, lower part). These products were prepared with EDTA and citrate as demanded by some distributor. These compounds may enhance the chelating power of IDHA product, so to grant a higher percentage of soluble iron.

The quantification of IDHA samples through NMR was in good agreement with the results from the HPLC, as shown in

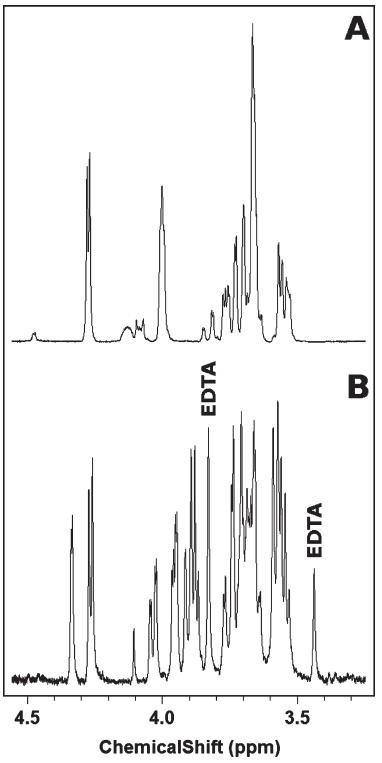

Figure 6. (A) ^{1}H NMR spectrum of standard gluconic acid. (B) ^{1}H NMR spectrum of commercial sample based on the mix MnZn heptagluconate where traces of EDTA were also noticed.

Table 3. Moreover, HPLC chromatograms registered on IDHA/Fe^{3+} (Figure 5) and IDHA mix confirmed the presence of EDTA. In detail, in the IDHA/Fe^{3+} sample, Fe bound to EDTA and IDHA represented the 0.9 and 5.2% of the total sample weight, respectively. When the IDHA mix was considered, the Fe bound to EDTA and IDHA was found to be 0.4 and 7.1%.

Gluconate and Heptagluconate Samples. ^{1}H NMR spectra were registered on one gluconate standard sample, one gluconate/Mn^{2+}, and three commercial samples based on heptagluconate (Figure 6). The comparable peak width of all such spectra showed that the method under investigation was able to sufficiently remove the metals from both ligands. To compare the results with standard methods, the general method used for the determination of the micronutrient complexed[14,15] was used. Then, this method is not able to distinguish among complexing agents when several of them are present in a fertilizer sample. The quantitification of gluconate obtained through NMR gave results comparable to those obtained through the method described by Villén et al.,[14] suggesting that nearly complete recovery of the ligand was granted (Table 4). Even if the spectra registered on heptagluconate appeared as very crowded, it was still possible to observe that each sample under investigation also contained gluconate and one of them EDTA too impeding the use of the standard method[15] for comparison. The addition of known standards and HPLC analysis confirmed this first identification. Table 4 reports the quantifications performed on the peaks at 4.23, 4.31, and 3.42 ppm for heptagluconate, gluconate and EDTA, respectively.

In conclusion, the procedure originally set up to remove Fe from *o,o*-EDDHA/Fe^{3+} commercial samples was found to be adequate also for other metals commonly added as micronutrients to the soil

Table 4. NMR and Reference Method Results of Gluconate Standard and Gluconate and Heptagluconate Commercial Samples

	gluconic acid (g/100 g)	
sample	NMR	reference method[16a]
gluconate std	92.4	
gluconate/Mn^{2+}	21.6 ± 0.5	21.3 ± 1.0

	heptagluconic acid (g/100 g)	gluconic acid (g/100 g)	EDTA (g/100 g)	
	NMR	NMR	NMR	HPLC
heptagluconate/MnZn	18.1	9.4	3.3	2.1
heptagluconate/Zn^{2+}	6.2	7.6		
heptagluconate/Fe^{3+}	2.5	2.4		

[a] The percentage of gluconic acid obtained by a metal-complexed method[16] determined in gluconate/Mn^{2+}, taking to account a 2:1 metal:ligand ratio, is also shown. The percentage of gluconic acid is obtained after the determination of the Mn complexed in the gluconate/Mn^{2+} and considering a 2:1 metal:ligand ratio.

and for other chelating and complexing agents. The effective removal of the paramagnetic ions afforded ^{1}H NMR spectra characterized by narrow peaks. The quantification of the ligands by NMR and HPLC showed that the recovery was nearly complete. The possibility to obtain sufficiently resolved spectra enabled the study of the impurities eventually present in the fertilizers.

■ ASSOCIATED CONTENT

Supporting Information. Additional NMR spectra and HPLC chromatograms. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

o,o-EDDHA, ethylenediaminedi-(2-hydroxyphenylacetic) acid; *o,p*-EDDHA, ethylenediamine-*N*-(2-hydroxyphenylacetic acid)-*N*′-(4-hydroxyphenylacetic acid); *p,p*-EDDHA, ethylenediaminedi-(4-hydroxyphenylacetic) acid; EDTA, ethylenediaminetetraacetic acid; IDHA, *N*-(1,2-dicarboxyethyl)-D,L-aspartic acid (also known as iminodisuccinic acid); NMR, nuclear magnetic resonance; HPLC, high-performance liquid chromatography; TBAOH, tetrabutylammonium hydroxide; TBAHS, tetra *N*-butylammoniumhydrogensulphate.

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