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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.

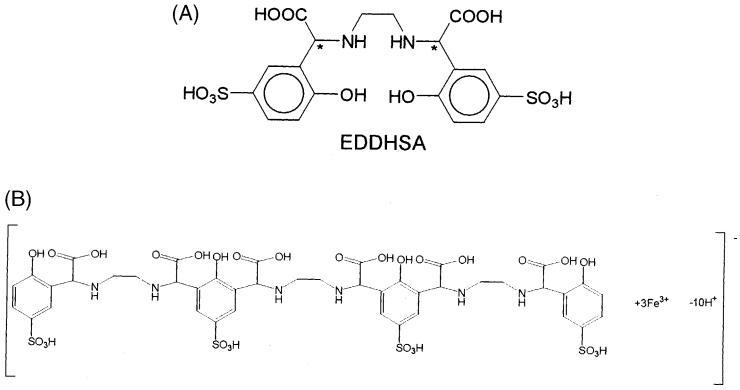

Fig. 1. Molecular structure of the EDDHSA/Fe^{3+} chelate (A) and the presumibly condensation products (B) used as fertilizers to control Fe chlorosis in plants ('*' denotes chiral carbons).