IDENTIFICATION OF Fe-CONTAINING IMPURITIES IN COMMERCIAL FERTILIZERS BY COLLISION INDUCED DISSOCIATION TANDEM MASS SPECTROMETRY

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INTRODUCTION

The synthetic ferric chelate of ethylenediamine-N,N'-bis( o-hydroxyphenylacetic) acid, commonly known as Fe(III)-o,o,oEDDHA, is one of the most used and efficient fertilizers to control Fe-chlorosis of crops grown in calcareous soils. A common characteristic of this fertilizer and others based on synthetic ferric chelates is the occurrence in the commercial product of a significant water-soluble Fe fraction non-bound to the EU-authorized chelating agents [1]. The occurrence of impurities in commercial fertilizers was first suggested by HPLC-UV-Vis [2] and later confirmed with 1- and 2-dimensional nuclear magnetic resonance [3-4] and electrospray mass spectrometry (ESI-MS) [5].

The collision-induced dissociation tandem mass spectrometry (CID MS-MS) is a good analytical technique to obtain structural information or identity since it provides molecular fragmentation patterns. The aim of this study is to identify Fe-containing impurities of a Fe(III)-EDDHA commercial fertilizer by CID MS-MS after separation by high performance liquid chromatography (HPLC). Identification is carried out by comparison of the CID mass spectra of unknown Fe-containing compounds with those obtained for pure Fe(III)-o,o,oEDDHA.

MATERIALS AND METHODS

MATERIALS: Pure Fe(III)-o,o,oEDDHA was prepared as indicated in [2]. A commercial Fe(III)-EDDHA fertilizer (6% of soluble Fe being only a 3% Fe(III)-o,oEDDHA) was dissolved and filtered obtaining a final solution of 41 mg/L of product.

HPLC-ESI-CID-MS-MS ANALYSIS: the instrumentation used was a quadrupole time of flight tandem mass spectrometer (QTOF; Bruker Daltonics), equipped with an ESI source and coupled to a 1100 HPLC system (Agilent). Firstly, the CID fragmentation pattern of Fe(III)-o,o,oEDDHA was obtained by direct injection of the standard solution into the QTOF instrument setting at 15 eV of collision energy. Daughter ions were identified using the Smart Formula and Smart Formula 3D™ tools of the Data Analysis software using the criteria of mass accuracy and SigmaFit™ value that provides a numerical comparison of theoretical and measured isotopic patterns. Secondly, the fertilizer solution was HPLC separated by the method [6] and then analyzed by ESI-MS. Iron-containing compounds were detected using the Isotope Cluster Analysis Chromatogram tool. After that, the fertilizer was HPLC separated once again, this time followed by an ESI-CID MS-MS analysis of the Fe-containing ions previously found. The CID daughter ions were identified as it describes above. The structure elucidation of impurities was based on the comparison of the CID-MS-MS spectra with that obtained for pure Fe(III)-o,o,oEDDHA.

RESULTS

In the commercial fertilizer, two groups of Fe-containing impurities were detected at m/z 453.1 and 484.0 (Fig 1) with retention times and CID fragmentation patterns (insets Fig 1) close to those of pure Fe(III)-o,o,oEDDHA (Figure 2). The most accurate molecular formula for the impurities were FeC_{20}H_{17}N_{2}O_{9} (M.W. 454.070726 g/mol, Fig 1A) and FeC_{20}H_{20}N_{3}O_{6} (M.W. 453.128970 g/mol, Fig 1B).

Impurity 1 CID-MS-MS spectra matched well with the structure of Fe(III)-o,o,oEDDHA including a dehydrogenated diethylenetriamine group instead of ethylenediamine (Fig 1A). The presence of 2 asymmetric chiral C in such structure result in 2 d,l-racemic mixtures which explain the elution of this impurity in two chromatographic peaks.

Impurity 2 CID-MS-MS spectra agreed with the structure of Fe(III)-o,o,oEDDHA plus a glyoxyl group (Fig 1B). Such structure has 3 chiral centers (2C and 1N) resulting in 8 possible isomers, which explain the elution of this impurity in a group of bad-resolved chromatographic peaks.

The HPLC-ESI-CID-MS-MS technique has allowed for the identification of Fe-containing impurities in EDDHA-based commercial fertilizers. This methodology is set as a powerful tool to study agricultural (fertilizer quality and efficiency, etc.) and environmental (persistence, mobility, metal mobilization, etc.) issues related to Fe fertilization in crops.

REFERENCES


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