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Chromatographic separations of metal chelates present in commercial fertilizers

I. Development of a gel permeation chromatographic separation method for the identification of metal chelates in commercial fertilizers

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ABSTRACT

The separation of iron(III) chelates of ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), hydroxy-2-ethylenediaminetriacetic acid (HEEDTA), ethylenediaminedi(*o*-hydroxyphenyl)acetic acid (EDDHA) and ethylenediaminedi(*o*-hydroxy-*p*-methylphenyl)acetic acid (EDDHMA) and the Cu(II), Zn(II) and Mn(II) chelates of EDTA by gel permeation chromatography on Bio-Gel P2 and Fractogel HW-40(S) was investigated. The chelates FeDTPA, FeHEEDTA, FeEDDHA and FeEDDHMA were separated on a 35 × 1 cm I.D. Fractogel column using 0.05 *M* NaCl–0.005 *M* KH_2PO_4 (pH 8.0) with a total analysis time of 3 h. FeEDTA was not completely resolved from FeDTPA, and FeHEEDTA, CuEDTA, ZnEDTA and MnEDTA were found to co-elute with FeDTPA. The method is applicable to the qualitative analysis of fertilizers containing a single trace element in combination with a single chelating agent. Positive identification of a chelate is achieved by comparison of retention time data and identification of the metal present by atomic absorption spectrometry or inductively coupled plasma mass spectrometry. Quantification can be achieved by comparison of peak height or area with that of standards.

INTRODUCTION

Synthetic chelates are added to commercial fertilizers for the supply of micronutrients to plants. These fertilizers have two main areas of application, *viz.*, hydroponics (the growth of plants in water culture) and the correction of micronutrient deficiencies in soils. The availability of micronutrients to a plant is largely dependent on their solubility at the pH of the soil/hydroponic solution. A pH range of 4–10 is found in hydroponic solutions, whereas soil, in which micronutrient deficiencies are found, is usually alkaline. The chelates supply micronutrients in a soluble form which is accessible to the plant. The chelate form in which the micronutrient is present in the fertilizer will depend on the conditions of use, *e.g.*, pH of the growing media, the stability of the chelate and the cost of

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the chelating agent. The elements most commonly added to fertilizers are iron, copper, zinc and manganese. The 76/116/EC Directive of the European Communities has recommended the use of the chelating agents listed in Table I. These fertilisers may be manufactured in solid or liquid form. This Directive dictates that there are two categories of fertilizer which may be marketed: fertilizers containing only one of the trace elements listed, and those containing at least two different trace elements. To evaluate the trace element content of the fertilizer, the Directive recommends that the total content in respect of each nutrient, the water-soluble content, the chelated form in which the trace element is present and the amount of the trace element which is chelated must be determined. Where a trace element is present in a chelated form, the pH range guaranteeing acceptable stability of the chelated fraction must be stated.

Currently, four methods of analysis have been developed and considered at EC level for the quality control of trace elements in fertilizers. Method 1 details a gel permeation technique and is based on a method developed by Boxma [1]. This method, however, cannot distinguish the chelating agents present and has a very long analysis time. Method 2 identifies the chelating agents present using TLC. Although the amount of chelated metal can be determined using this method, it does not quantify each chelating agent present, and the method may contain metal chelated by any negatively charged chelating agent. Method 3 only applies to the analysis of iron chelates. The method does not identify or determine the chelating agents present. Method 4 identifies the chelating agent present by TLC and determines the amount of chelating agent. The method can only be used, however, when one chelating agent is present, and the quantitative method used cannot distinguish between the different chelating agents.

TABLE I

CHELATING AGENTS RECOMMENDED BY DIRECTIVE 76/116/EC OF THE EUROPEAN COMMUNITIES

Chelating agent	Abbreviation
Ethylenediaminetetraacetic acid	EDTA
Diethylenetriaminepentaacetic acid	DTPA
Hydroxy-2-ethylenediaminetriacetic acid	HEEDTA
Ethylenediaminedi(*o*-hydroxyphenyl) acetic acid	EDDHA
Ethylenediaminedi(*o*-hydroxy-*p*-methyl phenyl)acetic acid	EDDHMA

In this work, the capabilities of gel permeation chromatography (GPC) for the identification and determination of the iron(III) chelates of EDTA, DTPA, HEEDTA, EDDHA and EDDHMA and the Cu(II), Zn(II) and Mn(II) chelates of EDTA was investigated, as these are the chelates most commonly found in commercial fertilizers. As GPC is generally associated with biological applications, very little investigation into its use as a method for the determination of chelates has been carried out. However, GPC provides an alternative mechanism of separation, *i.e.*, separation on the basis of size. Yoza *et al.* [2] showed that a mixture of Mg(II) and Mg(II)–EDTA could be separated on Sephadex G-15 when eluted with sodium chloride solution. Deguchi [3] studied the behaviour of Co(II)–, Ni(II)–, Cu(II)–, Cr(III)–, Fe(III)–Co(III)– and Bi(III)–EDTA on Sephadex columns. It was found that the complexes with an oxidation state of two were separated from those with an oxidation state of three on a Sephadex G-15 column when eluting with sodium chloride. The separation of free and complexed metal developed by Boxma [1], which is the basis of method 1, is currently used for the evaluation of the chelated fraction of metal in fertilizers in the Netherlands. In this method, however, FeDTA co-elutes with FeDTPA and FeEDDHA co-elutes with FeEDDHMA, making it impossible to analyse a mixture of these chelates. Also, FeEDDHA and FeEDDHMA have a very long retention time of 4 h. The present investigation set out to improve both the selectivity of this method and to reduce the time required for separation.

EXPERIMENTAL

Chemicals and Reagents

Deionized water was obtained by passing distilled water through a Waters Milli-Q water-

purification system. Sodium hydroxide solution (40%) was obtained from BDH. Potassium dihydrogenphosphate was obtained from Merck. Sodium chloride was of analytical-reagent grade. FeEDTA was obtained from Koch-Light, FeDTPA from Aldrich, and FeHEEDTA, FeEDDHA and FeEDDHMA from the Instituut voor Bodemvruchtbaarheid (Haren, Netherlands). All standards and samples for analysis were made up in the mobile phase and filtered with a 0.45-μm filter prior to analysis.

Apparatus

A Waters M45 pump, a Waters solvent-select valve and a Gilson Model 231 injector with a 20-μl injection loop were used in conjunction with a Merck–Hitachi L-4000 UV detector was used. The system was interfaced to a Nelson Analytical 900 series interface. Data were processed using a Nelson Analytical 3000 Series chromatography data system. A Corning 450 × 10 mm I.D. adjustable column casing was used. Fractogel TSK HW-40(S) was obtained from Merck and Bio-Gel P2 from Bio-Rad.

Methods

Pretreatment of sample. A sample mass approximately equivalent to 3 mg of the metal present in the chelate is taken. The mass of the sample in grams is estimated from $1/(U/0.3)$, where U is an estimate of the percentage of chelated metal in the sample. The sample is dissolved in approximately 20 ml of deionized water and placed in an ultrasonic bath for 30 min. The solution is diluted to 25 ml in a volumetric flask and filtered with a 0.45-μm filter.

Analysis of sample. A 20-μl volume of the pretreated sample is injected onto a Fractogel column (35 × 1 cm I.D.) in a mobile phase consisting of 0.05 *M* NaCl–0.005 *M* KH_2PO_4 (pH 8) at a flow-rate of 0.5 ml/min. The chromatogram is recorded by measuring the absorbance at 210 nm. The chelate fraction is collected, 1 ml of 5 *M* HCl is added and the sample is diluted to 10 ml with the mobile phase. Analysis of the fraction by atomic absorption spectrometry (AAS) or inductively coupled plasma mass spectrometry (ICP-MS) leads to identification of the metal present. This information, in combination with the retention time of the fraction, may be used to identify the chelate present. Quantification can be achieved by comparison of peak height or area with that of standards.

RESULTS AND DISCUSSION

General considerations

The bioavailability of a nutrient (*i.e.*, the percentage of it that is complexed and thus in soluble form and available to the plant) is dependent on the growing media conditions, *e.g.*, the soil solution pH and the presence of other metal ions and ligands. The analytical method used to evaluate the fertilizer must reflect this. Therefore, while the content of the fertilizer as manufactured is of some practical value in terms of quality control, it must be appreciated that this content must not be used as the absolute "nutrient value" of the fertilizer. A more correct evaluation of the value of the fertilizer in the field would involve both the identification and determination of the chelating agents and the trace elements present. Using this information, together with studies of the behaviour of metal chelates in soil as described, for instance, by Linsay *et al.* [4], the composition of the fertilizer under changing conditions of usage may be predicted. To this end the identification and determination of the chelating agent content of fertilizers should be investigated in addition to the specific chelate composition of the fertilizer as stated by the manufacturer.

The effect of the analytical method on the equilibrium condition of a metal chelate must also be considered. The analytical method imposes its own demands with regard to pH, buffer and solvent conditions on the metal chelate. Within this environment the metal chelate will further equilibrate. For a solid fertilizer, which contains a single trace element in the presence of a single chelating agent, this effect may be accounted for by the use of standards. A standard of FeEDTA of 95% purity, for example, may dissociate on equilibration in the analytical system, such that 80% of the metal remains chelated. The detector response achieved, how-

ever, may be taken as that corresponding to a sample of 95% purity as manufactured. This example illustrates how previous methods, in which an analytical system was used to isolate a chelate, followed by analysis of the fraction for metal content, did not reflect the true chelate content of the sample, but rather the chelate content under the conditions of analysis. However, where a fertilizer contains a mixture of trace elements and chelating agents, the competing reactions of the metal ions and ligands must be considered. Again the mixture of metal ions and chelating agents will achieve an equilibrium dependent upon the conditions employed in the analytical method. However, the analytical conditions not only have an influence on the extent of association/dissociation of the chelate but also on the extent of replacement reactions of metal chelates. Thus, a chelate which is very stable in an alkaline environment, such as FeEDDHA, if analysed at an acidic pH where, owing to the high pK_a values of the free acid the conditional stability constant relative to that of FeEDTA will be considerably less than at pH 11, the iron may be replaced to some extent by EDTA, thus decreasing the FeEDDHA concentration. Hence an incorrect evaluation of the fertilizer will be obtained should this fertilizer be used under alkaline conditions. Another example is the analysis of a mixture of FeEDTA and ZnEDTA. The results of such an analysis will differ if the analysis is carried out at pH 6 or 8, owing to the change in conditional constants as the stability of ZnEDTA increases from pH 6 to 8 whereas that of FeEDTA decreases. Therefore, the analysis of a mixture of metal ions and chelating agents is impossible using a chromatographic method of fixed pH, as the analysis will reflect the equilibrium achieved by the metals under the conditions of analysis and not the composition as manufactured or the composition under the conditions of usage. This paper therefore details the development of a method for the determination of the chelate content of a fertilizer containing one chelating agent in combination with one trace element.

Hydroponic, ionic and structural interactions all play a part in the behaviour observed by Boxma [1] and Deguchi [3]. If the Boxma separation is studied in detail, it is evident that it does not exhibit gel permeation behaviour, *i.e.*, separation on the basis of size. It can be seen that, contrary to GPC where the larger components of a sample have a shorter elution time than the smaller components, in the Boxma separation the smaller chelates FeEDTA and FeDTPA elute before the larger chelates FeEDDHA and FeEDDHMA. There is evidence that FeEDTA and FeDTPA are excluded from the gel owing to the presence of a small number of carboxylic acid groups in the gel matrix. These repel anions from the gel phase when they are applied to the gel in small amounts and eluted with ion-free water. This effect can be removed for inorganic anions by the addition of other electrolytes to the eluent [5]. This is possibly why Boxma and Deguchi both used calcium chloride and sodium chloride. However, this behaviour, or the investigation of it, was not mentioned in either paper. Therefore, this behaviour was investigated in a gel other than Sephadex to see if this phenomenon can be used to help achieve a separation.

The long retention times of FeEDDHA and FeEDDHMA are possibly due to the interaction of their aromatic groups with the Sephadex matrix. This behaviour on Sephadex has been reported in the chromatography of compounds containing aromatic functionalities [6]. Williams [7] has suggested that this is due to the involvement of the electron system of the solute with the other oxygens in the glyceryl bridge of the Sephadex. This effect increases as expected as the pore size of the gel decreases. However, for the smallest pore sizes of Sephadex G-10 and G-15, the retention of aromatics increases dramatically, and cannot be explained alone by interaction of the electron system. Determann and Walters [6] have suggested that the added increase in retention was due to structural considerations. However, they were not able to observe this very large increase for the Bio-Gels. In this study, therefore, Bio-Gel P2 was investigated. Fractogel HW-40(S) was also investigated, as it is a small-pore, very rigid gel that can be used at a relatively high linear flow-rate in GPC.

Separation on Bio-Gel P2

Fig. 1 shows the influence of NaCl concen-

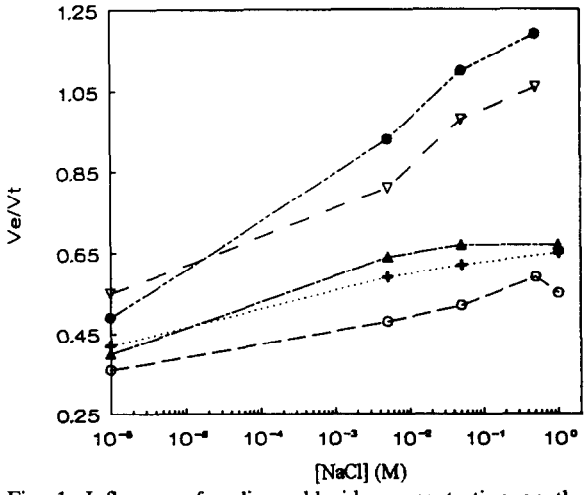

Fig. 1. Influence of sodium chloride concentration on the retention of iron(III) chelates on Bio-Gel P2. Column, 35 × 1 cm. I.D.; flow-rate, 0.25 ml/min; mobile phase sodium chloride solution (pH 7.0). ○ = FeDTPA; + = FeHEEDTA; ▲ = FeEDTA; ● = FeEDDHA; ▽ = FeEDDHMA.

tration on the retention of the chelates on Bio-Gel P2 with an aqueous mobile phase at pH 7 (adjusted with NaOH) at a flow-rate of 0.25 ml/min. The plot of V_e/V_t (elution volume/total common volume) gives a measure of retention independent of column dimension or flow-rate. It can be seen that as the NaCl concentration is increased, the retention of the chelates increased dramatically. This indicates that ionic interactions between the chelates and negatively charged groups in the gel are being overcome and therefore the anions are not being repelled from the column to the same extent. As with the Boxma method, the iron chelates of EDDHA and EDDHMA elute with longer retention times than the chelates of EDTA, DTPA and HEEDTA. However, the retention times of FeEDDHA and FeEDDHMA are significantly shorter than those obtained by us using the Boxma method employing Sephadex G-10 (Table II). The increase in the retention time of both EDDHA and EDDHMA chelates with increased sodium chloride concentration is much more dramatic than that for the EDTA, DTPA and HEEDTA chelates. This is possibly due to the fact that as ionic interactions are overcome, hydrophobic interactions increase and EDDHA and EDDHMA will have a much greater hydrophobic interaction owing to their respective aromatic groups.

TABLE II

COMPARISON OF V_e/V_t VALUES OF IRON(III) CHELATES ON VARIOUS GELS

Chelate	Sephadex	Bio-Gel	Fractogel
FeEDTA	0.44	0.67	0.46
FeDTPA	0.44	0.52	0.40
FeHEEDTA	ND[a]	0.62	0.48
FeEDDHA	1.66	1.1	1.77
FeEDDHMA	1.99	0.98	2.93

[a] ND = Not determined.

There was very little change in retention time once the NaCl concentration had increased above 0.05 *M*, and therefore it can be concluded that this is the optimum NaCl concentration for separation as there is no further decrease in ionic repulsion with increased NaCl concentration. FeEDTA and FeDTPA are well resolved using this system. FeDTPA and FeHEEDTA have a resolution factor (R_s) of 1.38. FeHEEDTA and FeEDTA are not resolved, having an R_s value of 0.52. FeEDDHA and FeEDDHMA have an R_s value of 0.8.

Separation on Fractogel HW-40(S)

The chelates behaved similarly on Fractogel, the retention time increasing as the sodium chloride concentration was increased (Fig. 2).

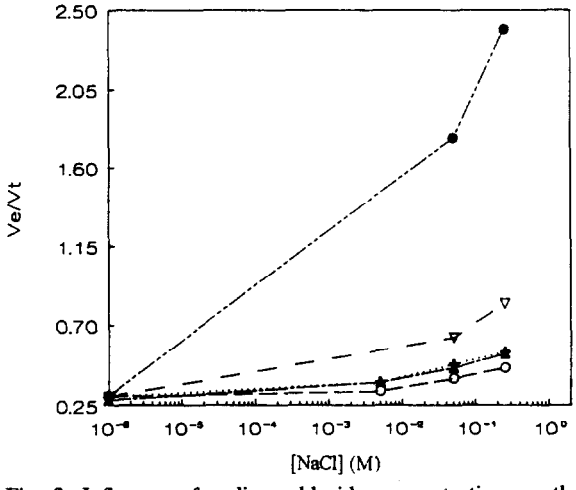

Fig. 2. Influence of sodium chloride concentration on the retention of chelates on Fractogel TSK HW-40(S). Column, 35 × 1 cm I.D.; flow-rate, 0.5 ml/min; mobile phase, sodium chloride solution (pH 7.0). Symbols as in Fig. 1.

The separation increases up to a salt concentration of 0.05 M. A further increase in salt concentration did not bring any improvement in resolution but only caused an increase in retention time; therefore, the optimum salt concentration for separation was found to be 0.05 *M*. Under these conditions, FeDTPA and FeEDTA are resolved, having an R_s value of 2.0. FeEDDHA and FeEDDHMA are also resolved, having an R_s value of 2.1. However, as with Bio-Gel, FeEDTA and FeHEEDTA are not resolved, having an R_s value of 0.3. A comparison of the retentions of the chelates on Fractogel, Bio-Gel and Sephadex is shown in Table II, from which it can be concluded that the behaviour of the chelates on the Fractogel follows more closely that of the Sephadex. The chelates FeEDDHA and FeEDDHMA have a long retention time on Fractogel, suggesting a steric interaction with the gel due to the aromatic functionalities of these chelates, as with Sephadex [6]. However, this interaction is helping to achieve greater resolution than on the Bio-Gel. Owing to the better resolution of FeEDDHA and FeEDDHMA with Fractogel HW-40(S), and the greater rigidity of the gel, which enables higher flow-rates to be employed than with the Bio-Gel, thus decreasing the analysis time, the Fractogel was chosen instead of the Bio-Gel for further study.

As FeEDDHA and FeEDDHMA were well resolved, the improvement of the separation of FeEDTA, FeDTPA and FeHEEDTA was then investigated. As buffer was added to the mobile phase it was found that the order of elution of the chelates changed. It was found that as the buffer concentration decreased, the separation improved for FeDTPA and FeHEEDTA (Fig. 3). The best separation was achieved at a buffer concentration of 0.02 *M*; however, the separation of FeHEEDTA and FeEDTA was still not resolved under these conditions.

Using this buffer concentration, the pH was varied from pH 6.5 to 8.0 to try to improve the separation (Fig. 4). It can be seen that the chelates changed elution order over this pH range. The best separation was found at pH 6.6. However, it was found that if pH 8.0 was used together with a low buffer concentration (Fig. 3), thus encouraging the elution order FeDTPA, FeEDTA, FeHEEDTA, a better separation was achieved (Fig. 5). Fig. 6 shows the separation of FeEDDHA and FeEDDHMA. The double peak for FeEDDHMA shows a partial separation of its diastereomers, but those of FeEDDHA co-elute. The earlier eluting peaks are due to impurities in the standards used. CuEDTA,

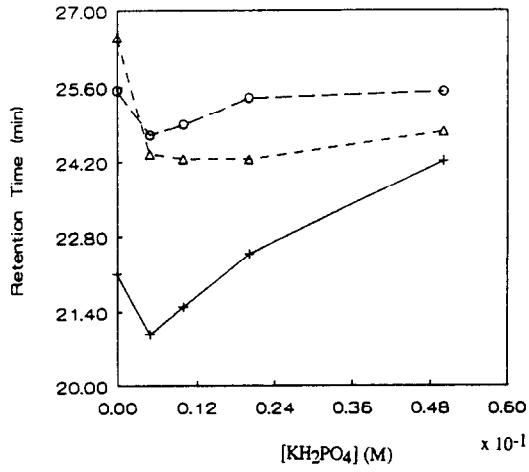

Fig. 3. Influence of phosphate buffer concentration on the retention of iron(III) chelates on Fractogel TSK HW-40(S). Column, 35 × 1 cm I.D.; flow-rate, 0.5 ml/min; mobile phase, 0.05 *M* NaCl (pH 7.0) containing various KH_2PO_4 concentrations. + = FeDTPA; △ = FeHEEDTA; ○ = FeEDTA.

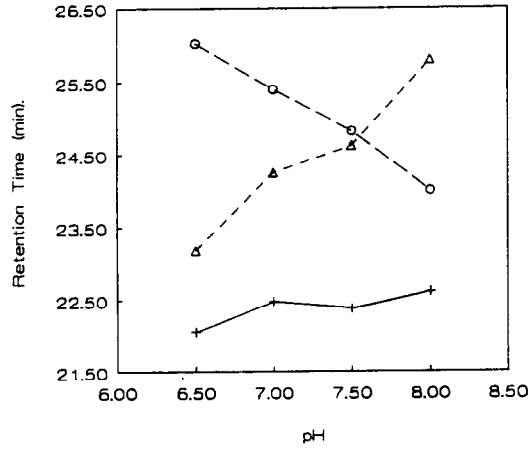

Fig. 4. Influence of pH on the retention of the iron(III) chelates on Fractogel TSK HW-40(S). Column 35 × 1 cm I.D.; flow-rate, 0.5 ml/min; mobile phase, 0.05 *M* NaCl–0.02 *M* KH_2PO_4. Symbols as in Fig. 3.

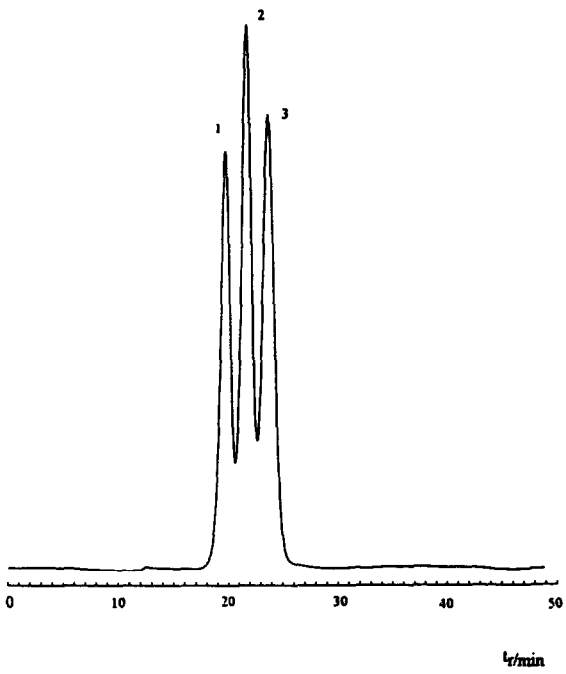

Fig. 5. Separation of (1) 19 μg of FeDTPA, (2) 26 μg of FeEDTA and (3) 28 μg of FeHEEDTA on Fractogel TSK HW-40(S). Column, 35 × 1 cm I.D.; flow-rate, 0.5 ml/min; mobile phase, 0.05 *M* NaCl–0.005 *M* KH_2PO_4 (pH 8.0); injection volume, 20 μl; detection at 210 nm.

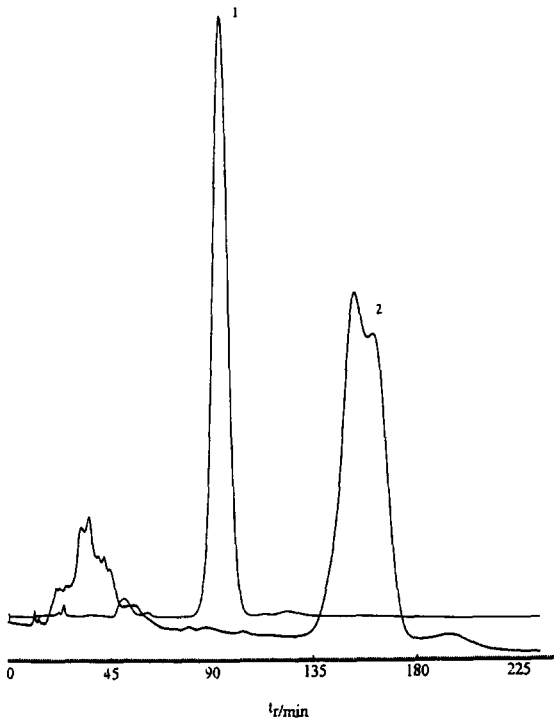

Fig. 6. Separation of (1) 390 μg of FeEDDHA and (2) 44 μg of FeEDDHMA on Fractogel TSK HW-40(S). Column, 35 × 1 cm I.D.; flow-rate, 0.5 ml/min; mobile phase, 0.05 *M* NaCl–0.005 *M* KH_2PO_4 (pH 8.0); injection volume, 20 μl; detection at 210 nm.

ZnEDTA and MnEDTA were found to co-elute with FeDTPA under these conditions.

The relative standard deviation of the retention time based on ten injections of the same standard was less than 1% for the iron(III) chelates of EDTA, DTPA and HEEDTA. The analysis of fertilizer samples showed that matrix interferences were not a problem for the separation as the standard deviation did not increase. However, to avoid matrix interferences, a minimum run time of 50 min is recommended. Quantification using this method should be carried out by a comparison of peak height or area with that of standards as previously discussed, and not by collection and determination of the metal content of chelate fractions.

CONCLUSION

The method developed shows a substantial improvement over the Boxma method. All chelates in a mixture containing FeDTPA, FeHEEDTA, FeEDDHA and FeEDDHMA can be separated. The method is intended for the analysis of fertilizers containing a single micronutrient in combination with a single chelating agent. Therefore, the retention time of the chelate, in combination with identification of the micronutrient present by AAS or ICP-MS, may be used to identify any of the chelates FeEDTA, FeDTPA, FeHEEDTA, FeEDDHA, FeEDDHMA, CuEDTA, ZnEDTA and MnEDTA. Quantification can be achieved by peak height or area analysis. The analysis time of 3 h for a sample containing FeEDDHMA is a disadvantage of the method, however. The most suitable application of the method could be for purification purposes, *i.e.*, the separation of chelated metal from free metal.

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