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Chromatographic separations of metal chélates present in commercial fertilisers

II. Development of an ion-pair chromatographic separation for the simultaneous determination of the Fe(III) chelates of EDTA, DTPA, HEEDTA, EDDHA and EDDHMA and the Cu(II), Zn(II) and Mn(II) chelates of EDTA

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ABSTRACT

The 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 by ion-pair chromatography has been investigated. Addition of the ion-pairing reagent tetrabutylammonium hydroxide to the mobile phase gave rise to a separation of all iron(III) chelates on a Chromspher C_{18} column. A solvent-switching system was used to achieve this separation. The switching sequence was 5 min mobile phase A (0.05 *M* TBAOH, pH 7.5), 20 min mobile phase B (0.05 *M* TBAOH, pH 7.5, 30% acetonitrile) and 25 min mobile phase A. For the iron(III) chelates, both limit of determination and linear range studies showed that the method is capable of analysing the concentration ranges found in commercial fertilisers. The Cu(II), Zn(II), and Mn(II) chelates of EDTA were separated using 0.01 *M* tetradecyltrimethylammonium bromide, 0.01 *M* KH_2PO_4, pH 7.5.

INTRODUCTION

The micronutrients iron, copper, zinc and manganese, necessary for the growth of plants, are usually added to fertilisers in a chelate form. The 76/116/EC directive of the European Communities has recommended the use of the chelating agents listed in Table I. The chelates most commonly found in fertilisers are the Fe(III) chelates of EDTA, DTPA, HEEDTA, EDDHA and EDDHMA and the Cu(II), Zn(II) and Mn(II) chelates of EDTA. It can be seen that iron is by far the most common element used in fertilisers. This directive dictates that there are two categories of fertiliser which may be marketed: fertilisers containing only one of the trace elements listed, and fertilisers containing at least two different trace elements. To evaluate the

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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*-hydroxyphenylacetic) acid	EDDHA
Ethylenediaminedi(*o*-hydroxy-*p*-methylphenyl)acetic acid	EDDHMA

trace element content of the fertiliser, 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 quantity of the trace element which is chelated be determined. This paper, and the previous paper in this series [1], addresses the determination of the chelated form in which the trace element is present and the quantity of the trace element which is chelated.

However, as discussed previously [1], due to the very nature of the metal chelate equilibrium and the pH, buffer and solvent conditions imposed on this metal chelate equilibrium during analysis by a chromatographic method, chromatographic methods can only be used for the determination of the quantity of chelated metal in a sample containing a single metal in combination with a single chelating agent. This paper aimed therefore to develop a separation of the iron(III) chelates of EDTA, DTPA, HEEDTA, EDDHA and EDDHMA and the copper(II), zinc(II) and manganese(II) chelates of EDTA which may be used for the analysis of specific chelate content of a fertiliser containing one of these chelates. The results of such an analysis reflect the content of the fertiliser as manufactured but do not necessarily give the value as a fertiliser in the field. This is due to the fact that the bio-availability of the micronutrients derived from a fertiliser depends upon, among other factors, the soil solution pH, the type of soil, and the presence of other metals and ligands in the soil. The analysis of micronutrient availability will therefore require a holistic approach including all of these factors. A meaningful analysis would involve the determination of the total quantity of chelating agent and the total quantity of micronutrients. These results could then be used, together with a model of the behaviour of metal chelates in soils, to predict the effectiveness of the fertiliser under various conditions of usage.

In the first paper of this series, a gel permeation technique was described which was an improvement on previously described methods [1]. However, the analysis time was still too long for samples containing FeEDDHA and FeEDDHMA. The chelates Fe(III)-DTPA and Cu(II)-, Zn(II)- and Mn(II)-EDTA were found to co-elute, and identification of the metal ion present must be confirmed by atomic absorption spectrometry (AAS) or inductively coupled plasma (ICP)-MS for positive identification of these chelates. Thus, the capabilities of ion-pair chromatography to resolve these species was investigated. To date, this technique has been used for the determination of the chelating agents EDTA, DTPA and HEEDTA by the addition of a metal ion to aid detection, or alternatively for the determination of metal ions by the addition of a chelating agent such as EDTA. The method has never been used for the determination of the chelates themselves. Buchberger *et al.* [2] used EDTA to extract Cu(II), Fe(III), Pb(II), Cd(II), Co(II), Ni(II), Pb(II) and Zn(II) from soil sediments with subsequent determination by ion-pair chromatography using the ion-pairing reagent hexadecyltrimethylammonium bromide. Detection of Cu(II), Fe(III) and Pb(II) was obtained at 250 nm and for Cd(II), Co(II), Ni(II), Pb(II) and Zn(II) also at 250 nm after postcolumn reaction with copper sulphate at low pH. In studies of the determination of the chelates the tetrabutylammonium ion (TBA^+) has been the most popular ion-pairing reagent

used [3–7]. Parkes *et al.* [7] achieved a separation of EDTA, HEEDTA and NTA in the copper form using TBA^+. EDTA, DTPA, HEEDTA, DHEG and NTA have also been separated as their copper chelates using benzyltrimethylammonium chloride (BTAC) [8]. The determination of the total chelating agent by conversion to the copper form cannot be used in this particular application as, unlike the applications mentioned [7,8], a fertiliser sample may already contain a mixture of metals. On examination of the conditional formation constants available for the chelates of iron and copper it is evident that a method converting the chelates to the copper form will suffer from interference from iron. Therefore a separation of the chelating agents in the iron form would be required for bioavailability studies.

In this paper the ion-pairing reagents TBA^+, BTAC and tetradecyltrimethylammonium bromide (TDTMABr) were therefore investigated for the separation of the Fe(III) chelates of EDTA, DTPA, HEEDTA, EDDHA and EDDHMA and the Cu(II), Zn(II) and Mn(II) chelates of EDTA. A possible future application of this work would be to convert the chelates present in a fertiliser or soil sample to the iron(III) form and use the separation achieved for the determination of the total amount of chelating agent present.

EXPERIMENTAL

Reagents

Deionised water was obtained by passing distilled water through a Waters Milli-Q water purification system. Sodium hydroxide (40%) solution was obtained from BDH. Potassium dihydrogen phosphate was obtained from Merck. Sodium chloride was analytical grade. Benzyltrimethylammonium chloride, potassium dihydrogenphosphate and acetonitrile (analytical-reagent grade) were obtained from Merck. Tetrabutylammonium hydroxide (40% solution in water; 1.5 *M*) was obtained from Fluka. FeEDTA was obtained from Koch-Light, and FeDTPA was obtained from Aldrich. CuEDTA was obtained from Sigma. ZnEDTA was obtained from Koch-Light. FeHEEDTA, FeEDDHA and FeEDDHMA standards could not be found commercially or within the network of experts working on this analysis. However, they are manufactured industrially but not to high purification levels. Samples of these chelating agents were obtained from various industrial sources. All standards and samples for analysis were made up in the mobile phase and filtered with a 0.45-μm filter prior to analysis.

Preparation of mobile phase for ion-pair chromatography

Mobile phase A: 32.5 ml of 1.5 *M* solution of tetrabutylammonium hydroxide was added to 200 ml of water. The pH was adjusted to 7.5 with NaOH and made up to 1 l in a volumetric flask. Final mobile phase B: 32.5 ml of 1.5 *M* tetrabutylammonium hydroxide solution was added to 200 ml of water. This was made up to approximately 650 ml, the pH adjusted to 7.5 with NaOH and the solution filtered with a 0.45-μm filter. 300 ml of acetonitrile was added and made up with filtered water to 1 l in a volumetric flask. All mobile phases were degassed in an ultrasonic bath.

Ion-pair chromatography system

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. A Chromspher C_{18} column 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.

RESULTS AND DISCUSSIONS

Investigation of the ion-pairing reagent benzyltrimethylammonium chloride

Fig. 1 shows the influence of pH on the separation of the Fe(III) chelates of EDTA, DTPA and HEEDTA using the ion-pairing reagent benzyltrimethylammonium chloride. This figure shows both the change in retention time and the increase in resolution. As the pH increased to pH 7.5 the retention time increased and the resolution between FeDTPA and

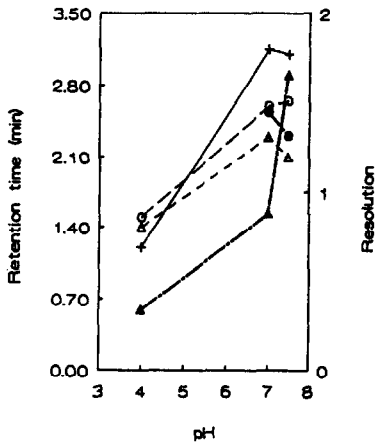

Fig. 1. The influence of pH on the retention time (t_R) and resolution (R_S) of some iron(III) chelates. Column: Chromspher C_{18}. Eluent pH 4: 0.05 *M* BTAC, 0.2 *M* NH_4Cl; eluent pH 7, 7.5: 0.05 *M* BTAC, 0.05 *M* KH_2PO_4, pH variable. Flow-rate: 1 ml/min. Injection loop: 20 μl. Detection at 300 nm. Symbols: + = t_R FeEDTA; △ = t_R FeDTPA; ○ = t_R FeHEEDTA; ▲ = R_S FeDTPA and FeHEEDTA; ● = R_S FeHEEDTA and FeEDTA.

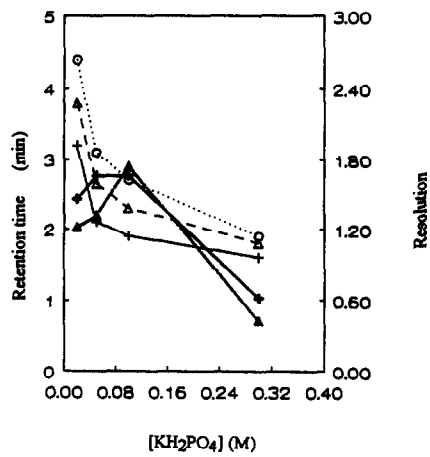

Fig. 2. The influence of buffer concentration on the retention time (t_R) and resolution (R_S) of iron(III) chelates. Column: Chromspher C_{18}. Eluent: 0.05 *M* BTAC, pH 7.5, [KH_2PO_4] variable. Flow-rate: 1 ml/min. Injection loop: 20 μl. Detection at 300 nm. Symbols: + = t_R FeDTPA; △ = t_R FeHEEDTA; ○ = t_R FeEDTA; + = R_S FeDTPA and FeHEEDTA; ▲ = R_S FeHEEDTA and FeEDTA.

FeHEEDTA increased. This may be explained by the formation of mono- and dihydroxo-forms of the three chelates and thus an increased negative charge. A more detailed study might reflect the exact pH values for the different chelates at which the mono- and dihydroxo-complexes form and the effect this has on the retention. Good resolution of the three chelates was obtained at pH 7.5. A higher pH was not investigated as hydrolysis of Fe(III)-EDTA occurs above pH 8.

The influence of phosphate buffer concentration was then investigated (Fig. 2). It was found that as the concentration of the buffer was increased from 0.02 *M* to 0.3 *M*, the retention time of the chelates decreased. Bidlingmeyer and Warren [9] also found this behaviour for the retention of alkylsulphonates with the ion-interaction reagent cetylpyridinium chloride above 10^{-3} *M* KH_2PO_4. With addition of phosphate there is an increase in the amount of reagent adsorbed as the charge repulsion between the reagent ions on the stationary phase is reduced. However, the increase in ionic strength of the eluent also results in a decrease in electrostatic attraction between the analyte and the ion-interaction reagent such that retention of the sample by adsorptive forces increases. The overall result in this investigation, as was that of Bidlingmeyer and Warren [9], is a decrease in retention of the analyte. In the present investigation this decrease in electrostatic attraction and increase in adsorptive forces was accompanied by a decrease in peak width and increased resolution up to a buffer concentration of 0.1 *M*. Beyond this concentration the decrease in peak width was not enough to compensate for the decrease in retention time, so that the solution started again to decrease. Therefore at this concentration the combination of adsorptive and electrostatic forces is optimal. To improve resolution further, especially between FeHEEDTA and FeEDTA, the concentration of BTAC was increased, as shown in Fig. 3. It was expected that as the concentration of BTAC was increased, the sample would have more interaction with the ion-pairing reagent, and therefore have a longer retention time. It was found that there was very little change in the retention time, but that the resolution increased.

Development of a solvent-switching system

The retention times of EDDHA and EDDHMA were extremely long using an eluent of 0.15

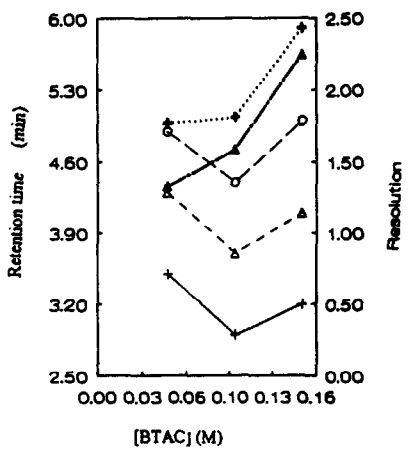

Fig. 3. The influence of the concentration of BTAC on the resolution of chelates. Column: Chromospher C_{18}. Eluent: 0.1 *M* KH_2PO_4, pH 7.5, [BTAC] variable. Flow-rate: 0.5 ml/min. Injection loop 20 μl. Detection at 300 nm. Symbols as in Fig. 2.

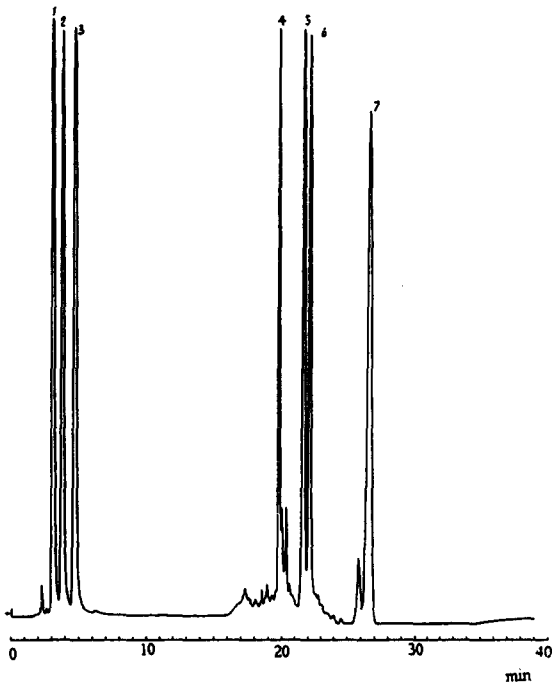

Fig. 4. Separation of (1) 24 μg FeDTPA, (2) 45 μg FeHEEDTA, (3) 18 μg FeEDTA, (4), (5) 80 μg FeEDDHMA, (6), (7) 74.5 μg FeEDDHA. Eluent: mobile phase A: 0.15 *M* BTAC, 0.1 *M* KH_2PO_4, pH 7.5, mobile phase B: 0.15 *M* BTAC, 0.1 *M* KH_2PO_4, pH 7.5, 25% acetonitrile. Switching sequence: 5 min mobile phase A, 15 min mobile phase B, 25 min mobile phase A. Flow-rate: 0.5 ml/min. Injection loop 20 μl. Detection at 283 nm.

M BTAC, 0.1 *M* KH_2PO_4, pH 7.5, (2.5 h for EDDHA). Addition of an organic modifier to decrease the retention time of Fe(III)-EDDHA and Fe(III)-EDDHMA was achieved using a solvent switching system. A switching valve was placed between the solvent reservoir and the pump, which was activated by a pulsed signal (given in this case by the Gilson auto-injector). Using this system the column was firstly equilibrated with mobile phase A (0.15 *M* BTAC, 0.1 *M* KH_2PO_4, pH 7.5) and the Fe(III)-EDTA, Fe(III)-DTPA and Fe(III)-HEEDTA chelates were allowed to elute. The solvent was then switched to mobile phase B (0.15 *M* BTAC, 0.1 *M* KH_2PO_4, pH 7.5 with variable acetonitrile concentration). Investigation of the addition of 10%, 20%, 25% and 30% acetonitrile showed 25% acetonitrile to be the optimum. Elution with 25% acetonitrile for 15 min was necessary to elute FeEDDHA and FeEDDHMA. A further 25 min was needed to re-equilibrate the system with mobile phase A. The switching sequence was thus as follows: 5 min mobile phase A, 15 min mobile phase B followed by 25 min mobile phase A. Fig. 4 shows the separation of the iron chelates achieved. Two peaks were obtained for both FeEDDHA and FeEDDHMA, representing the separation of the respective diastereoisomers. This is important as for FeEDDHA it has been shown that only one of the isomers is effective as a fertiliser. The optimum wavelength for determination of the iron chelates was found to be 283 nm. However, it was found that the Zn(II)-EDTA and Mn(II)-EDTA chelates only exhibit an absorption below 220 nm. However, as BTAC also has a high background absorbance at 220 nm, determination of Zn(II)-EDTA and Mn(II)-EDTA was not possible using this system.

Investigation of the ion-pairing reagent tetrabutylammonium hydroxide

The ion-pairing reagent TBAOH was then investigated. Good resolution of the chelates was obtained. As with BTAC, the Fe(III) chelates of EDTA, DTPA and HEEDTA eluted very quick-

ly, while the EDDHA and EDDHMA chelates had a longer retention time and were well separated. At an equivalent concentration of TBAOH the retention time of all the chelates was longer with respect to BTAC.

The dependence of the separation on the TBAOH concentration in shown in Fig. 5. Unlike the behaviour of BTAC, where the retention remained almost constant, it was found that as the concentration of TBAOH was decreased, the retention time and resolution of the chelates increased. This is contrary to what would normally be expected and cannot be explained by such theories as ion-pair, ion-exchange or the ion-interaction mechanism proposed by Bidlingmeyer *et al.* [10], which would all predict that the retention time should increase as ion-pair reagent concentration increases. It has, however, been shown by Hung and Taylor [11], that over wide ranges in mobile phase ion-pairing concentration, the dependence of capacity factors on ion-pairing concentration is complex. It may reach a plateau or even pass through a maximum. It was predicted that the mechanism occurring was a combination of ion-exchange and desolvation. As the concentration of ion-pairing reagent increases, the C_{18} surface area available for desolvation subsequent to ion-exchange will decrease. Also, the capacity factor will decrease as the counter ion concentration increases as a result of added pairing reagent. This mechanism was modelled for aqueous systems and over concentration ranges similar to that used in this study. It is possible that the behaviour predicted by Bidlingmeyer *et al.*, who carried out research over the narrower concentration range of 0–20 m*M* and in the presence of an organic modifier, would have been seen if we had also employed the same conditions. The optimum separation achieved in this study was at 0.05 *M* TBAOH. Below this concentration the retention time continued to increase but resolution decreased. This may be explained by increased hydrophobic interaction of the chelates with the stationary phase due to increased area available for desolvation and less competition from the ion-pair reagent. However, there are not enough ion-exchange sites to maintain selectivity.

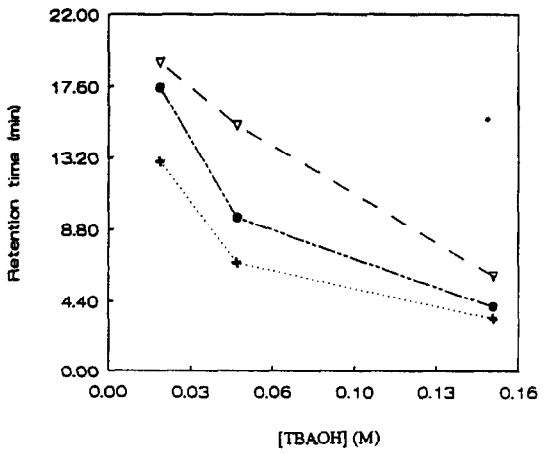

Fig. 5. The influence of TBAOH concentration on retention time of iron(III) chelates. Column: Chromspher C_{18}. Eluent: mobile phase A: [TBAOH] variable, pH 7.5, mobile phase B: [TBAOH] variable, pH 7.5, 25% acetonitrile. Flow-rate 0.5 ml/min. Injection loop 20 μl. Detection at 300 nm. Symbols: + = FeHEEDTA; ● = FeDTPA; ▽ = FeEDTA.

Due to the longer retention time of the chelates, the chelates of EDDHA and EDDHMA again needed addition of acetonitrile. Acetonitrile was added to a second mobile phase and a solvent-switching system used. The amount of acetonitrile added was 30%. The final conditions were thus: mobile phase A: 0.05 *M* TBAOH, pH 7.5, mobile phase B: 0.05 *M* TBAOH, pH 7.5, 30% acetonitrile, using a switching sequence of 5 min mobile phase A, 20 min mobile phase B, 25 min mobile phase A. The separation achieved is shown in Fig. 6.

Investigation of the separation of Mn(II)-EDTA, Cu(II)-EDTA and Zn(II)-EDTA with TBAOH showed that these species coeluted.

Investigation of the ion-pairing reagent tetradecyltrimethylammonium bromide

To separate Mn(II)-EDTA, Cu(II)-EDTA and Zn(II)-EDTA, an alternative ion-pairing reagent was needed. It has been shown that the larger the ion-interaction reagent the greater the amount adsorbed on the stationary phase for a given concentration [11]. Therefore, at equivalent concentrations, TDTMABr will provide more ion-exchange sites than TBAOH. From the behaviour of TBAOH, it was predicted that as

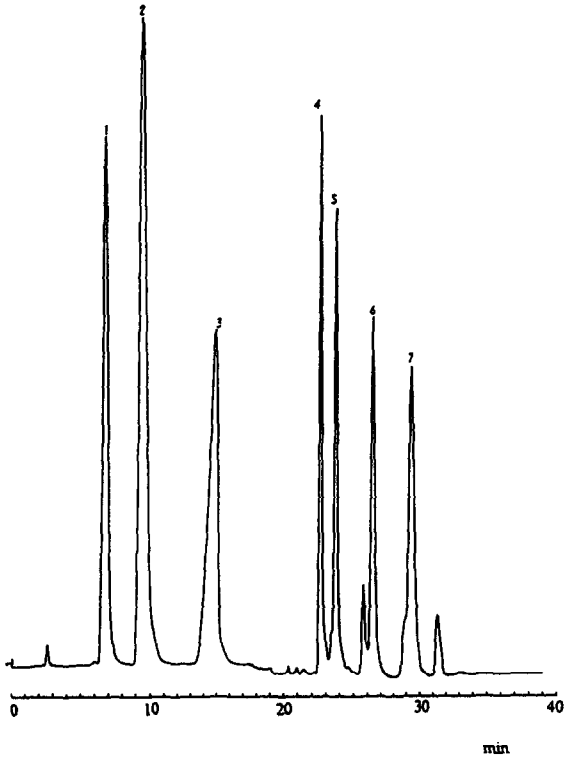

Fig. 6. Separation of (1) 67 μg FeHEEDTA, (2) 61 μg FeDTPA, (3) 47 μg FeEDTA, (4), (5) 94 μg FeEDDHA, (6), (7) 120 μg FeEDDHMA. Column: Chromspher C_{18}. Eluent: mobile phase A: 0.05 *M* TBAOH, pH 7.5, mobile phase B: 0.05 *M* TBAOH, pH 7.5, 30% acetonitrile. Switching sequence: 5 min A, 20 min B, 25 min A. Flow-rate: 0.5 ml/min. Injection loop: 20 μl. Detection at 300 nm.

the concentration decreased the increased area available for desolvation improved the separation. However, below 0.05 *M*, there was a lack of ion-exchange sites. Therefore, an ion-pairing reagent which allows sufficient interaction with the reversed phase and gave sufficient ion-exchange sites was needed. Thus TDTMABr was chosen, as at low concentration it would coat the column more efficiently. Fig. 7 shows how the retention of Mn(II)-EDTA, Zn(II)-EDTA, Cu(II)-EDTA and Fe(III)-HEEDTA increases as the concentration of TDTMABr decreases. Fig. 8 shows the separation achieved. It can be seen that the detection of ZnEDTA is very insensitive; this could possibly be improved by the development of a post-column reaction with copper [2].

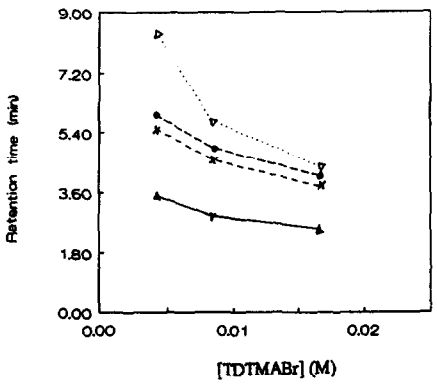

Fig. 7. Influence of TDTMABr concentration on the retention time of various chelates. Column: Chromspher C_{18} Eluent: [TDTMABr] variable, 0.1 *M* KH_2PO_4, pH 7.5. Flow-rate: 0.5 ml/min. Injection loop: 20 μl. Detection at 220 nm. Symbols: ▲ = MnEDTA; × = ZnEDTA; ● = CuEDTA; ▽ = FeHEEDTA.

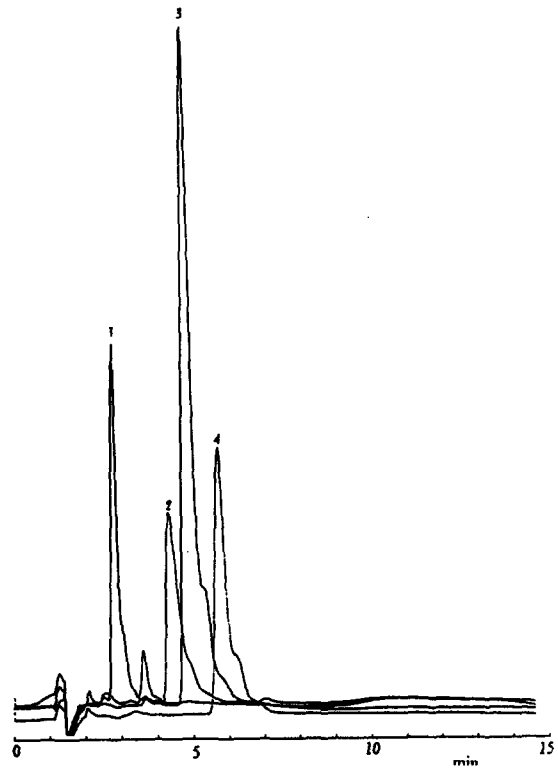

Fig. 8. Separation of (1) 46 μg MnEDTA, (2) 330 μg ZnEDTA, (3) 41 μg CuEDTA, (4) 67 μg FeHEEDTA. Column: Chromospher C_{18}. Eluent: 0.01 *M* TDTMABr, 0.1 *M* KH_2PO_4, pH 7.5. Flow-rate: 0.5 ml/min. Injection loop: 20 μl. Detection at 220 nm.

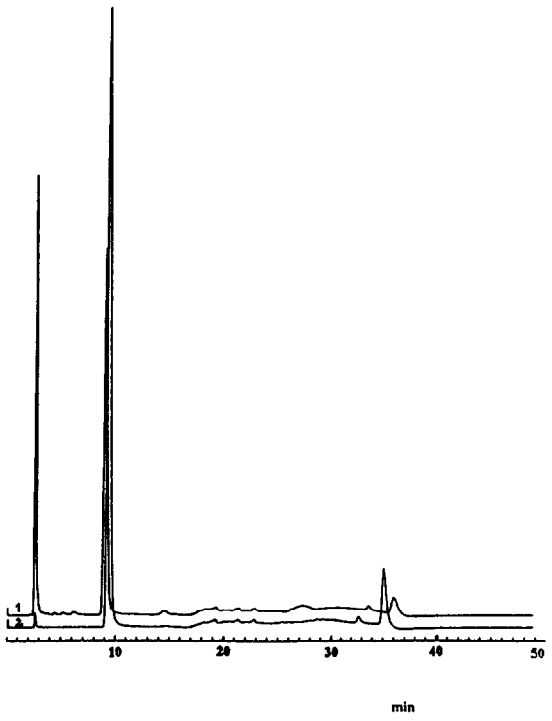

Fig. 9. Overlay of (1) fertiliser sample containing 3.2% iron in the form of FeDTPA with (2) FeDTPA standard.

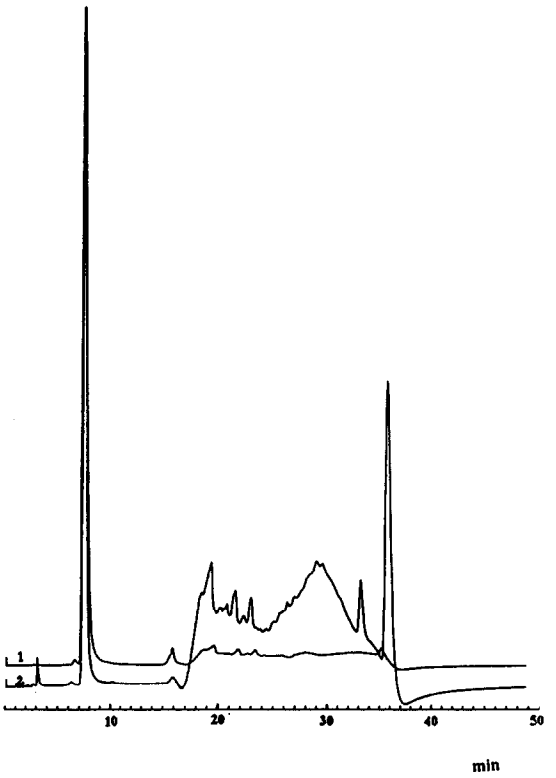

Fig. 10. Overlay of (1) FeHEEDTA "standard" with (2) fertiliser sample containing approximately 5.4% iron in the form of FeHEEDTA.

The ion-pair chromatographic method described using TBAOH as ion-pairing reagent gave rise to a linear calibration for the determination of the iron(III) chelates in the approximate range 1.79–179 mmol Fe in the form of chelate. 1.79 mmol Fe in the form of chelate was the limit of detection for FeEDDHA and FeEDDHMA while for FeEDTA, FeDTPA and FeHEEDTA it was below this. No interferences were encountered in the analysis of real samples and sample retention times agreed very closely with those of standards. Figs. 9 to 11 show examples of three samples analysed. No quantitative results have been quoted, as official standards were not available to carry out such studies.

CONCLUSIONS

An ion-pair chromatographic method has been developed to permit the separation of the chelates FeEDTA, FeDTPA, FeHEEDTA, FeEDDHA, FeEDDHMA, CuEDTA, ZnEDTA and MnEDTA, which may be applied to the analysis of a fertiliser containing one of the above chelates. For FeEDDHA and FeEDDHMA, a separation of the diastereoisomers has been achieved, which is important as research shows only one of these isomers is effective as a fertiliser. This method will be useful for quality control in the manufacture of fertilisers.

Further studies should aim to develop a sample preparation procedure for conversion of all chelating agent to the iron form at low pH, with removal of interfering ions [12] and subsequent determination by the method developed.

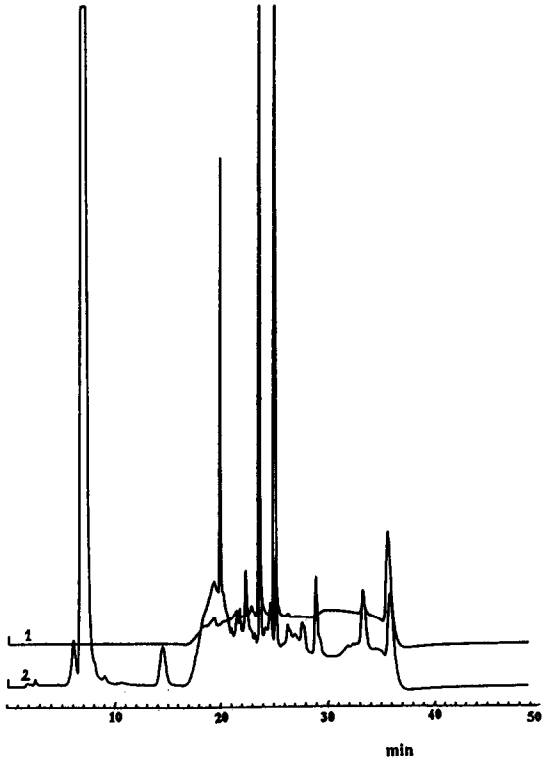

Fig. 11. Overlay of (1) FeEDDHA "standard" with (2) fertiliser sample containing approximately 0.2% iron in the form of FeEDDHA.

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