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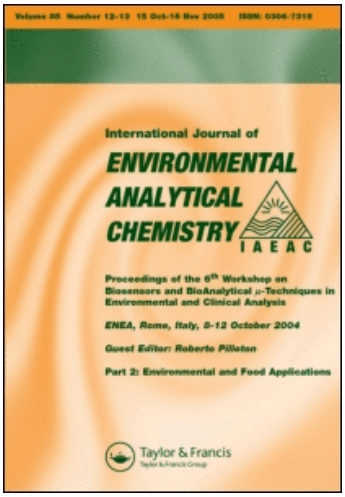

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DETERMINATION OF NITRILOTRIACETIC ACID IN WASTE AND NATURAL WATERS

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An analytical method and the method validation for the determination of NTA in waste waters and in the aquatic environment is presented. An aqueous sample, after evaporation to dryness, was treated with an ethyl esterification reagent. The resulting ester was identified by MS and determined by GC-NPD apparatus. The response was linear within realistic scale (0.006 mg/l-50 mg/l) for lake and waste waters. The recoveries were found to vary between 105 and 123 % for lake water and between 113 and 124 % for waste water. The run-to-run repeatabilities and reproducibilities were all below 2.1 %, revealing the high precision of the method. The detection limit was 6 μg/l and quantitation limit 13 μg/l. No interferences were observed.

Keywords: Nitrilotriacetic acid determination; aminocarboxylic acid determination; gas chromatography; waste water; natural water

INTRODUCTION

The widespread usage of ethylenediaminetetraacedic acid (EDTA) and other persistant, synthetic ligands, such as diethylenediaminetetraacedic acid (DTPA), has led to the concern of the environmental fate of these compounds[1–7]. Their occurrence in receiving waters is reported[8–10]. In addition to being relatively recalcitrant to microbial attack and/or non-adsordable during activated sludge treatment, EDTA and DTPA are known to cause environmentally adverse effects, e.g., remobilising trace metals from sediments[11–15]. Thus, it would be favourable to substitute EDTA and DTPA by compounds not affecting the aquatic environment in the long term.

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Nitrilotriacetic acid is one of the most potential alternatives to EDTA and DTPA. In contrast to EDTA and DTPA, it degradates well in normal waste water treatment[16]. Actually, basing on extensive, long-term study, Kari concluded that NTA is an ideal substitute for EDTA from the environmental point of view[17]. NTA has traditionally analysed by spectrofotometry or using different electrochemical methods. Chromatography has also been applied. Kaiser has reviewed these earlier procedures[18]. The limit of quantitation has however remained high and selectivity has been a main drawback. For the trace determination, chromatography using esterification of NTA is practically the only option[18–20]. No sensitive LC methods have been presented.

The aim of this study was to provide a procedure for the analysis of NTA in waste water and in natural water applications for practical purposes. The methods presented in the literature are most commonly based on established BF_3-CH_3OH method. In this study, several alcohols were tested for the esterification and ethylation was found more suitable for the trace analysis. To our knowledge, the procedure presented here is the first analytical method for the NTA determination as ethyl ester. For our earlier studies, the method for the analysis of EDTA and DTPA was developed[21], and later, it turned out that there is a need for analyzing alternative chelates. Thus, we present here a promising method for NTA analysis at trace concentrations. Importantly, this procedure makes possible the simultaneous detection of trace amounts of β-alaninediacetic acid (β-ADA), EDTA, DTPA and NTA within one run.

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EXPERIMENTAL

Instrumental analysis

The gas chromatographic analyses were done with a Hewlett-Packard (Avondale, PA, USA) Model 5890 Series II Plus chromatograph equipped with a nitrogen phosphorous detector (NPD), a HP 7673 automatic sampler and a 10 µl syringe. The column used was a HP-5 capillary column (30 m×0.25 mm×0.25 µm). Nitrogen was used as carrier gas with constant flow rate of 1.2 ml/min. The chromatograph oven temperature program was: setpoint 100°C, initial time 1 min, increased at 60°C/min to 200°C, where held for 3 min, increased at 50°C/min to 250°C, where held for 8.5 min and increased 20°C/min to 290°C where held for 5 minutes.

The total run time was 22.17 min for wastewater samples, but for the other matrixes 15.2 minutes, as the last heating section could be omitted. The injector was set at 250°C and the detector at 300°C. Injected sample size was 1µl and inlet purge off-time 1 min. The chromatographic data was processed with a HP

3365A ChemStation. Mass spectrometry was used to identify the resulting ester. The equipment used was Jeol DX 303/DA 5000 mass spectrometer using electron ionization and 70 eV voltage.

Reagents and solutions

Disodium-NTA was purchased from Sigma-Aldrich (Steinheim, Germany). Dried CH_3OH, concentrated H_2SO_4, anhydrous Na_2SO_4, $FeCl_2 \cdot 4H_2O$, $CuCl_2 \cdot 2H_2O$ and $MnCl_2$ were obtained from Merck (Darmstadt, Germany), $KHCO_3$, HCOOH and $C_6H_5CH_3$ from Riedel-de-Haën (Seelze, Germany). C_2H_5OH was from Primalco (Rajamäki, Finland), C_3H_7OH from BDH (Poole, UK), CH_3COOH was from JT Baker (Deventer, Netherlands) and $C_{16}H_{33}CN$ and $CaNa_3$-DTPA from Fluka. β-ADA was produced by BASF and Na_4-EDTA by Sigma (St. Louis, MO, USA). All the chemicals except $MnCl_2$ (>98%) were at least of analytical reagent grade.

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Standard solutions

A stock standard solution containing 1000 mg/l NTA was made by weighting and diluting to the volume with distilled water. An internal stock standard solution of 1000 mg/l was prepared by dissolving 100 mg $C_{16}H_{33}CN$ in 100 ml of ethanol. A 1000 mg/l Fe^{2+}, Cu^{2+} and Mn^{2+} stock standard solutions were prepared by dissolving 0.08908 g $FeCl_2 \cdot 4H_2O$ in 25 ml, 0.13417 g $CuCl_2 \cdot 2H_2O$ in 50 ml and 0.11687 g $MnCl_2$ in 50 ml of distilled water. The standard solutions containing organic compounds were stored in the dark to avoid photolysis at room temperature for no more than 1 month.

Esterification reagent

The esterification reagent was prepared in a 100 ml calibrated flask by mixing 5.0 ml of H_2SO_4, 50 µl of CH_3COOH and about 60 ml of ethanol, adding 5.0 ml of 1000 mg/l internal standard solution and diluting to the volume with the alcohol. Some esterification studies were done without the acetic acid to study the catalytical effect of this compound.

Sample preparation

A sample amount of 10 ml in a 20-ml glass vial was used. The samples were evaporated to dryness in an oven set at 100°C. After cooling to room tempera-

ture, 3.0 ml of esterification reagent was added. Vials were closed with screw cap and shaken. The esterification took place in an oven set at 78°C for 6 hours. After cooling, 1.5 ml of $C_6H_5CH_3$ was added and the sample was transferred into a 50 ml separating funnel. Sample vial was flushed thoroughly with small portions of $KHCO_3$ (totally about 20 ml) which were then poured into the funnel. The separating funnel was shaken for 1 minute and the organic extract was transferred back to the glass vial, where Na_2SO_4 was added to remove water. The organic phase was transferred into 2-ml GC sample vial. The samples were analysed within 24 hours. The procedure for the NTA determination is outlined in Figure 1.

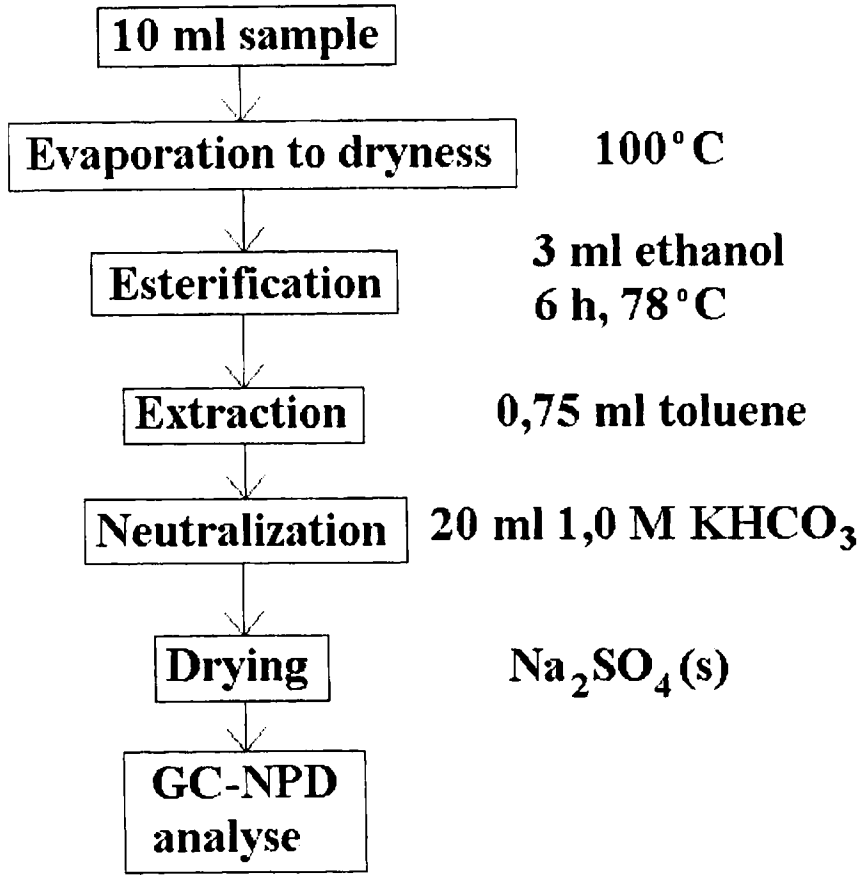

FIGURE 1 Flow scheme for the NTA analysis

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Treatment of the results

Results were calculated by means of the area ratio of the peaks of the internal standard and of the nitrilotriacetic acid ester. The limit of detection is based on signal-to-noise ratio of 3 and the limit of quantification to that of 6.

RESULTS AND DISCUSSION

Preliminary tests

Influence of esterification time and temperature

A test plan was created with Modde 4.0 (Umetri AB, Sweden) computer program to study the impact of different esterification parameters, that is to say, pH, esterification temperature and time. The test plan is shown in Table I. Distilled water solutions containing 0, 0.5 and 1.0 mg/l of NTA (n=9) were esterified under diverse conditions suggested by test plan. The screening method, having interactive process model was used in Modde. Eight runs were done in edge points and 1 in centre point. Esterification with CH_3OH, C_2H_5OH or C_3H_7OH was examined. Tests were performed using H_2SO_4 and CH_3COOH as catalysts with the exception of ethyl esterification where H_2SO_4 was also used alone to observe the effect of CH_3COOH. The esterification time was varied between 0.5 and 6 hours and the esterification temperature 70°C and 120°C. The pH was adjusted with HCOOH and $KHCO_3$ to pH 2–7 before drying. When H_2SO_4 was used alone, the peaks were broader and its area smaller than using both H_2SO_4 and CH_3COOH as catalyst, illustrating the importance of small amount of CH_3COOH added to the esterification reagent. Also the impact of the liquid volume of sample before esterification reagent addition was studied.

A model was created with Umetri Ab's Simca computer program. According to principal component analysis (PCA), esterification with methanol gave the lowest recovery. Propanol was the most sensitive, but, unfortunately, there was an interfering compound eluted simultaneously with the propyl ester analyte (Figure 2). This compound could not be separated from propyl ester. Thus, ethanol was chosen as an esterification reagent.

At the next step, a more accurate partial least square (PLS) model was performed by using data from ethanol esterification tests. In addition separate models were created for ethyl ester and internal standard peak areas because the peak area of ISTD was affected by the esterification conditions. Goodness of fit (R2 = 0.953) and goodness of predict (Q2 = 0.866) were very promising for the model of ethyl ester. The pH had negligible influence on response. On the other

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hand, the model showed strong interaction between esterification time, volume and response. The highest response was achieved using a long time and minimising the sample volume before esterification, that is evaporating samples to dryness. Esterification temperature was found to have lesser impact. These results were confirmed with additional tests.

TABLE I Test plan for the esterification reagent and optimization of esterification parameters

ExpNo	*ExpName*	*RunOrder*	*InOut*	*Reagent*	*pH*	*Temp*	*Time*
17	N17	1	In	PrOH	7	80	0,5
16	N16	2	In	EtOH	7	120	6
19	N19	6	In	PrOH	2	80	6
11	N11	3	In	EtOH	7	80	6
7	N7	5	In	MeOH	7	80	6
12	N12	4	In	EtOH	2	120	6
22	N22	7	In	PrOH	7	120	0,5
8	N8	9	In	MeOH	2	120	6
9	N9	8	In	EtOH	2	80	0,5
13	N13	13	In	EtOH	7	80	0,5
21	N21	18	In	PrOH	2	80	0,5
1	N1	19	In	MeOH	7	80	0,5
5	N5	24	In	MeOH	2	80	0,5
10	N10	14	In	EtOH	7	120	0,5
18	N18	16	In	PrOH	2	120	0,5
14	N14	20	In	EtOH	2	120	0,5
6	N6	21	In	MeOH	7	120	0,5
2	N2	27	In	MeOH	2	120	0,5
26	N26	11	In	EtOH	4,5	100	3,25
23	N23	10	In	PrOH	7	80	6
3	N3	23	In	MeOH	2	80	6
15	N15	25	In	EtOH	2	80	6
25	N25	22	In	PrOH	4,5	100	3,25
20	N20	12	In	PrOH	7	120	6
4	N4	15	In	MeOH	7	120	6
24	N24	17	In	PrOH	2	120	6
27	N27	26	In	MeOH	4,5	100	3,25

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Goodness of fit (R2 = 0.976) and goodness of predict (Q2 = 0.948) were also excellent for the model of ISTD. Most important factors were esterification temperature and time, which both were suggested to be low. Thus long esterification time and low temperature as well as evaporating the samples to dryness before the addition of esterification reagent were adopted in further experiments to obtain optimal conditions for the analyte.

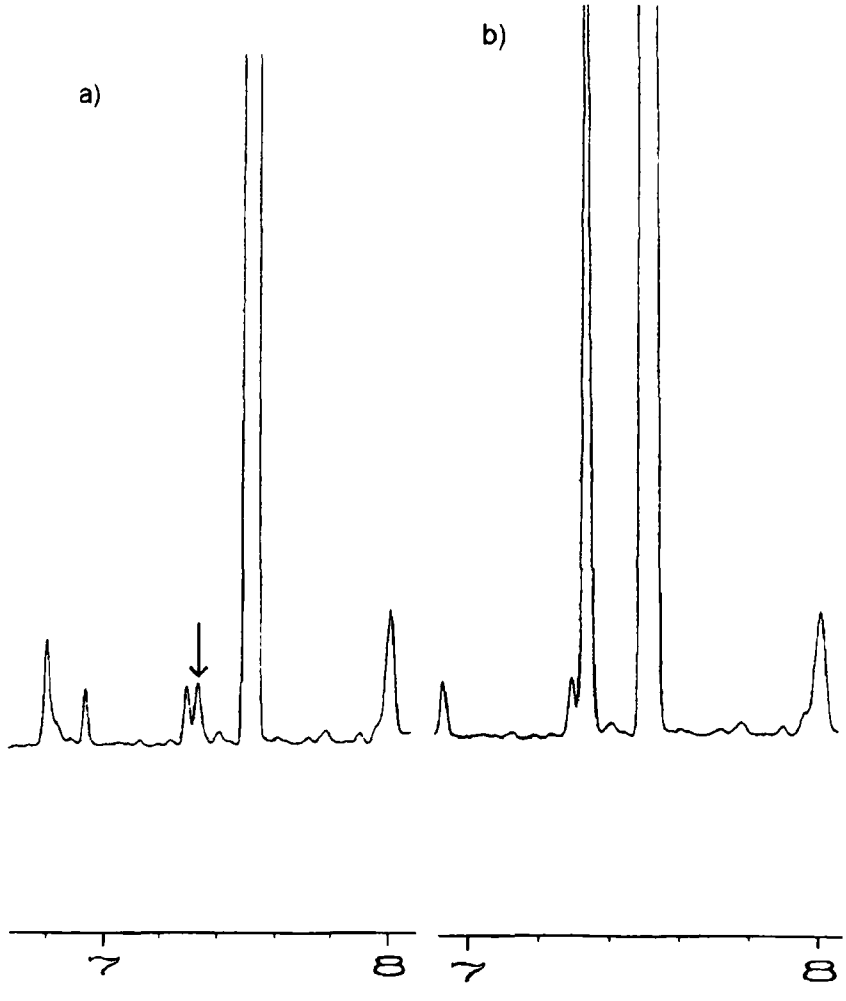

FIGURE 2 Interfering peak eluting simultaneously with NTA propyl ester: a) blank experiment containing no propyl ester of NTA, b) 1 mg/l of NTA propyl ester. Experimental conditions are given above

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Studies of the Ethyl Esterification Method

The identification of the ester

The retention time for nitrilotriacetic acid ethyl ester was about 5.7 min. The ester was identified with mass spectroscopy. A very strong NTA solution was esterified without internal standard. This sample was extracted to a small volume of toluene, which was evaporated. The residual was analysed with mass spectrometer. The mass spectrum is shown in Figure 3.

FIGURE 3 Mass spectrum of NTA ethyl ester

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The molecule peak at m/z 275 is triethylester. The peak at m/z 229 represents the fragmentation of one OCH_2CH_3 group and m/z 202 represents the fragmentation of $COOCH_2CH_3$ group, while m/z 103 is obtained removing $COOCH_2CH_3$ and $COOCH_2CH_2$ groups from the original triethylester.

The validation of the method

For the method quantitation studies temperature of 78°C and long esterification time (6h) were used. The pH was seven and the sample size 10 ml. The amount of internal standard in esterification reagent was 5.0 mg/l.

In preliminary tests the sample was extracted with 1.5 ml of toluene. For the analysis only a few microliters are needed. Thus, tests were performed to minimize the use of the solvent using 1.0 ml, 0.75 ml and 0.5 ml toluene in extraction step. The lowest volume was found to be not enough for the autosampler, but 0.75 ml toluene was adequate and thus adopted.

The repeatability between runs was tested with 0.1 mg/l and 10.0 mg/l samples of NTA (n=5). The samples were extracted with 1.5 ml of toluene, because 0.75 ml was too small amount for five successive measurements. The relative standard deviation (RSD) was 1.9 % at the lower concentration and 0.65 % at the higher. The reproducibility was examined with 0.1 mg/l and 5.0 mg/l NTA samples (n=5). The RSDs were 1.7 % and 2.1 %, respectively. All these variations are excellent, revealing the minimal effect of sample preparation procedure to the result. At lower concentrations the variation in measurements is only due to GC repeatability, while at higher analyte concentrations sample treatment has a slight

influence on the variation. The day-to-day repeatability was tested measuring same samples in successive days. 0.05 mg/l and 10.0 mg/l samples were stored in daylight to reveal the possible decomposition due to photolysis. The RSDs were around 8 % and 3 %, respectively within one week period (n=7). This shows the stability of the ethyl ester of NTA for analytical purposes.

The linearity of the method was tested in distilled water (n=3) up NTA concentration of 20 mg/l and in lake (n=3) and waste waters (n=3) up to 50 mg/l. The method was found to be linear in that range. The correlation coefficients were 1.0000 for distilled water, 0.9995 for lake water and 1.0000 for waste water. The corresponding calibration line equations were $y = 0.7783x+0.0126$, $y = 0.7741x + 0.1413$ and $y=0.8502x + 0.0647$. Limit of detection in distilled water was 6 µg/l and limit of quantitation 13 µg/l. The recoveries varied between 105 and 123 % in lake water and between 113 and 124 % in waste water. The detailed data is given in Table II.

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TABLE II Recoveries of NTA in waste and lake water

C (mg/l)	*Waste water*	*Lake water*
0.01	113 ± 16	123 ± 22
0.05	107 ± 11	118 ± 9
0.1	104 ± 6	105 ± 5
1	108 ± 4	112 ± 3
10	105 ± 1	109 ± 2

Influence of iron(II), copper(II) and manganese(II)

The interferences of iron, copper and manganese were studied. For example, in pulp and paper industry, copper is typically present at concentrations less than 1 mg/l and iron at those of some dozens of mg/l [22]. Manganese has been measured at concentrations over 200 mg/l, but the concentrations in Finnish pulps are usually less than 100 mg/l [23, 24]. The sensitivity of the procedure towards these ions was tested at NTA concentrations of 0.02 mg/l and 10.0 mg/l. The spiked metal concentrations were 0, 0.1, 1.0, 10.0 and 100.0 mg/l. Results are shown in Table III. Iron affects slightly the analysis when present at concentrations exceeding 10 mg/l. Neither copper nor manganese has any observable effect even at high concentrations.

TABLE III The impact of potential interferences on the analytical recovery of ethyl ester NTA: a) NTA 0.02 mg/l, b) NTA 1.00 mg/l

C(mg/l)	*Fe*	*Cu*	*Mn*
0	100	100	100
0.1	96	120	100
1	100	95	99
10	75	99	104
100	86	100	102

C(mg/l)	*Fe*	*Cu*	*Mn*
0	100	100	100
0.1	98	99	101
1	97	98	102
10	87	98	103
100	84	97	102

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Simultaneous determination of complexing agents

A sample containing 1 mg/l of β-ADA, EDTA, DTPA and NTA was esterified as described above. It was measured with the GC method described here. The chromatogram is shown in Figure 4. It can be seen that each of the analytes has distinct peaks, which do not overlap. The simultaneous determination of these aminocarboxylic acids is therefore possible with the suggested procedure.

CONCLUSIONS

There is a need to develop analytical methods for the trace determination of complexing agents, which might substitute EDTA in the future. Here we propose a procedure for low-level determination of NTA – a potential, biodegrading alternative chelate. The excellent repeatability and reproducibility values as well as high recoveries suggest that the suggested procedure for the determination of NTA is applicable for routine analysis industrial and municipal waste waters as well as for the natural aquatic environment. The low detection limit at the sample amount of 10 ml shows the applicability of the method for trace analysis. The limit of quantitation can be further lowered by using higher sample volume. In

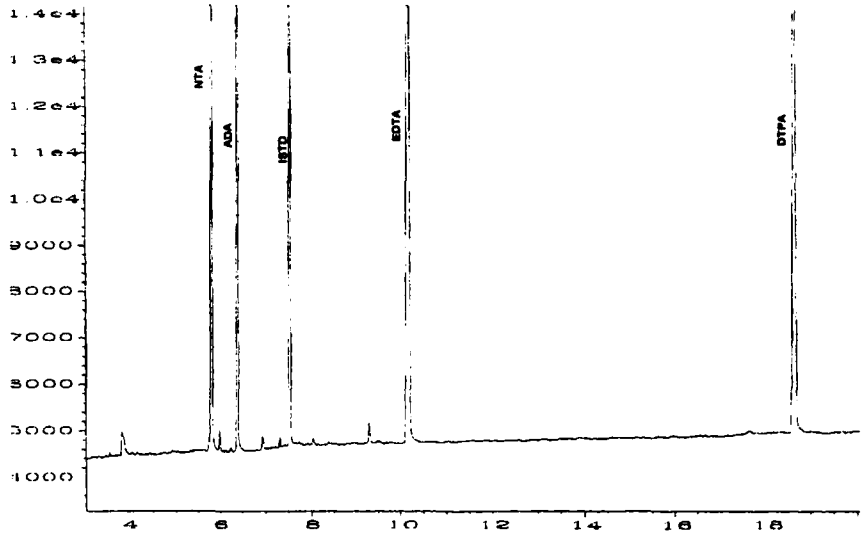

FIGURE 4 Chromatograms of β-ADA, EDTA, DTPA and NTA ethyl esters. Separation conditions are given in the Experimental section

addition, no interferences affecting the analysis were observed at any realistic level.

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Acknowledgements

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