Determination of nitrilotriacetic acid and ethylenediaminetetraacetic acid in environmental samples as their methyl ester derivatives by gas chromatography–mass spectrometry

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Abstract

A method for the determination of nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) in environmental samples such as river water and sediment is reported. An aqueous sample, after evaporation to dryness, was treated with a boron trifluoride–methanol mixture. The resulting methyl ester derivatives were determined by capillary GC–MS with selected-ion monitoring. NTA and EDTA could be determined in the ranges 4.1–12.2 and 3.9–11.8 ng/ml in water with relative standard deviations (R.S.D.s) of 3.1–7.8% and 11.0–19.7%, respectively. Their recoveries from river water and sediment were 74–92% with R.S.D. 1.5–6.8% and 51–60% with R.S.D. 14–16%, respectively.

1. Introduction

Nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) have received much attention as chelating agents in analytical chemistry. As they form water-soluble and stable chelates with many metals, NTA and EDTA have been used as detergents and in a variety of other products, and their use results in their ultimate release to the environment. These chelating agents may affect the distribution of metals within aquatic ecosystems.

Early analytical procedures for NTA and EDTA [1] involved mainly three techniques,

GC, polarography and spectrophotometry. GC methods mostly involve converting the analyte into a methyl or other volatile ester and determining the ester using a flame ionization [2–5] or a nitrogen-specific detector [6–8]. Polarographic and spectrophotometric techniques are critical with respect to selectivity. To increase the selectivity, ion-pair liquid chromatographic (LC) combined with electrochemical [9–10] or UV–Vis [11–13] detection has been used. Although the application of ion-pair LC to environmental samples has been investigated, considerable difficulties are encountered owing to the sample matrix effects.

The more recent GC–MS technique with selected-ion monitoring (SIM) provides excellent

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sensitivity and selectivity. In this paper, a convenient method is presented for determining NTA and EDTA in environmental sample at ng/ml levels by capillary GC–MS-SIM via methylation with BF_3–CH_3OH.

2. Experimental

2.1. Reagents and apparatus

NTA disodium salt and EDTA disodium salt dihydrate, used as standards because their free acids are slightly soluble in water, trans-1,2-cyclohexanediamine-N,N,N',N'-tetraacetic acid (CDTA) monohydrate, which was dissolved in 1 M NaOH and used as surrogate standard, and Fe(III)–EDTA were of special grade from Aldrich (Milwaukee, WI, USA) and Dotite (Kumamoto, Japan). [$^2H_{10}$]Acenaphthene (acenaphthene-d_{10}), [$^2H_{10}$]phenanthrene (phenanthrene-d_{10}) and [$^2H_{10}$]fluoranthene (fluoranthene-d_{10}), used as internal standards, were obtained from MSD Isotopes (Montreal, Canada). Boron trifluoride–methanol complex (BF_3–CH_3OH), containing ca. 14% BF_3 in gas chromatographic grade methanol, and other reagents of special grade were purchased from

Wako (Osaka, Japan) and Tokyo Kasei (Tokyo, Japan).

A Yamato (Tokyo, Japan) RE-46 rotary evaporator was used for concentration of sample solutions. A Branson (Shelton, CT, USA) B-220 ultrasonic extractor and a Poly Toron PT10-30 homogenizer were used for extraction from sediment and fish samples, respectively. A Tomy Seiko (Tokyo, Japan) LC06-SP centrifuge was employed for phase separation of sediment or fish samples.

2.2. Gas chromatography–mass spectrometry

A Hewlett-Packard (Avondale, PA, USA) HP 5790 gas chromatograph and a Nihondenshi (Tokyo, Japan) JEOL-DX303 mass spectrometer with a DA-5000 data processing system were employed. The analytical column used was Ultra-2 cross-linked with 5% phenyl–methylsilicone (25 m × 0.32 mm I.D., 0.52 μm film thickness). The GC temperature programme was as follows: initial temperature, 70°C held for 4 min, then increased at 15°C/min to 300°C and held there for 10 min. The temperatures of the injector, transfer line and ion source were 250°C. The carrier gas was helium at 7.5 psi (61 cm/s). Samples were injected in the splitless mode with a 2-min purge off. The mass spectrometer was
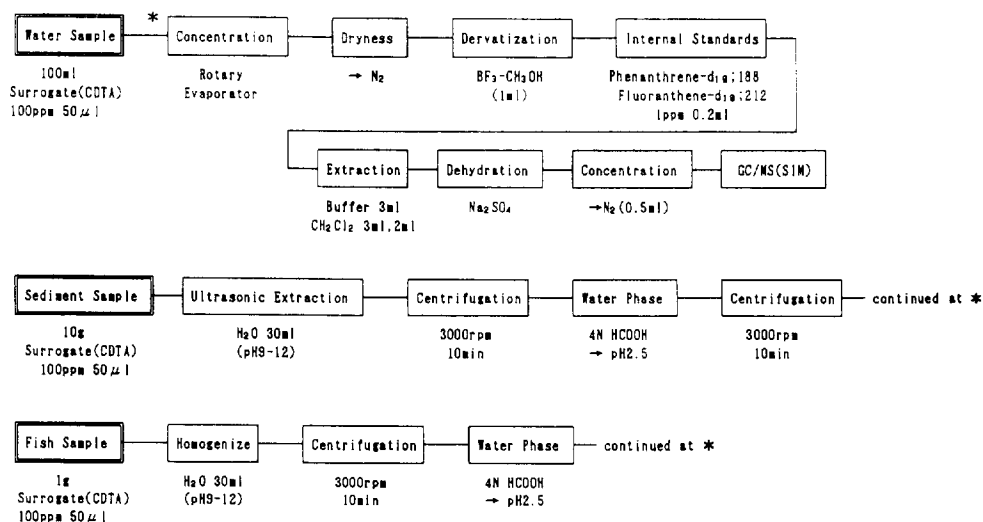

Fig. 1. Flow scheme for the determination of NTA and EDTA in environmental samples.

operated at 70 eV and 300 μA with electron impact ionization using the scan or SIM mode. Between retention times of 8 and 20 min, ions at m/z 174 (NTA and EDTA), 188 (phenanthrene-d_{10}), 212 (fluoranthene-d_{10}), 233 (NTA), 289 (EDTA), 348 (EDTA) and 402 (CDTA) were monitored.

2.3. Analytical procedure

The procedure for the determination of NTA and EDTA in environmental samples is outlined in Fig. 1. A 100-ml volume of the water sample was spiked with the surrogate standard and adjusted to pH 2.5 with 4 M formic acid. The sample was reduced to about 2–3 ml in a rotary evaporator, transferred into a 10-ml test-tube and evaporated completely to dryness under nitrogen. A 1-ml volume of BF_3–CH_3OH solution was added to the dry sample and the tube was stoppered tightly with a clamp and allowed to stand for 1 h at 60°C or 30 min at 80°C. To this reactant, 0.2 ml of the internal standard solution, 3 ml of buffer solution (1 M KH_2PO_4 adjusted to pH 7 with 10 M NaOH) and 3 ml of methylene chloride were added, followed by shaking in a separating funnel. The extracted organic phase was dehydrated by passing it through anhydrous Na_2SO_4. The aqueous phase was re-extracted with 2 ml of methylene chloride in the same way. The organic phase was combined with the first extract in a 10-ml test-tube. The organic phase was evaporated to 0.5 ml under a stream of nitrogen, then a 1-μl aliquot was analysed by GC–MS-SIM.

For sediment, 10 g of sample spiked with the surrogate were added to 30 ml of pure water with stirring and the pH was adjusted to 9–12. The muddy sample was extracted in an ultrasonic extractor for 10 min and then centrifuged at 3000 rpm (1600 g) for 10 min. The aqueous phase was adjusted to pH 2.5 with 4 M formic acid. As a small amount of precipitate was occasionally observed at this stage, the turbid solution was re-centrifuged and then the supernatant solution was subjected to the same procedure as for water samples.

For fish, 1 g of sample was homogenized and centrifuged in a similar way.

3. Results and discussion

3.1. Derivatization reaction

As aminopolycarboxylic acids such as NTA and EDTA are non-volatile, it is preferable that their carboxyl groups are derivatized to volatile ester groups. First, dimethylformamide dimethylacetal was used as a methylation reagent, but some difficulties were encountered, i.e., masking reagents and thorough prewashing of glassware with acid were required, and the
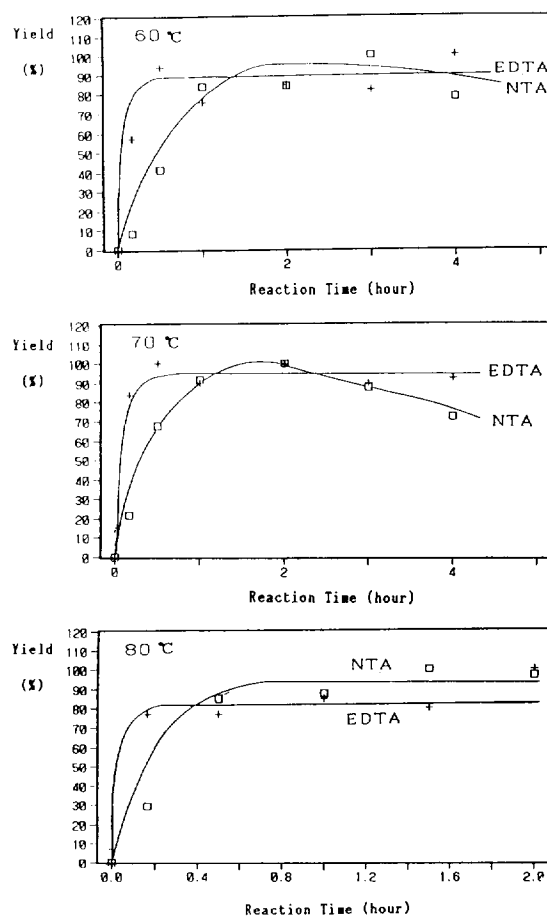

Fig. 2. Effects of reaction temperature and time on NTA and EDTA methyl esterfication yields.

results obtained varied widely, particularly at
low μg/ml levels in the esterification reaction.
The difficulties were overcome by using BF_3–
CH_3OH instead of dimethylformamide di-
methylacetal.

The reaction temperature and time were in-
vestigated using BF_3–CH_3OH. Fig. 2 shows the
yields for NTA and EDTA at different tempera-
tures and times. The optimum reaction condi-
tions were 30 min at 80°C or 60 min at 60°C.

3.2. Internal standard and surrogate standard

It is preferable that deuterium-substituted sub-
stances with retention times near to those of the
analytes are used as internal standards. The
retention time of acenaphthene-d_{10} was near to
that of NTA, but interference on the chromato-
gram was observed with real samples. The inter-
nal standards selected were phenanthrene-d_{10} for
NTA and fluoranthene-d_{10} for EDTA. CDTA,
which is a similar aminopolycarboxylic acid that

is not present in the environmental samples, was
used as a surrogate standard. When CDTA was
added to an environmental sample just before
processing, the reaction yield and losses due to
manipulation compensated each other. The re-
covery of EDTA improved, but the precision
was bad owing to the large retention time gap for
NTA.

3.3. Mass spectra and GC–MS-SIM traces of standards

Fig. 3 shows the mass spectra of methyl esters
of NTA, EDTA and CDTA. The base peak ions
were at m/z 174, 174 and 168 and the molecular
peak ions at 233, 348 and 402, respectively.
The base peak ions of NTA and EDTA at
m/z 174 are assumed to be ions of
$[CH_2N(CH_2COOCH_3)_2]^+$. The base peaks
showed lower selectivity than the molecular ions,
so the latter ions were used for the determi-
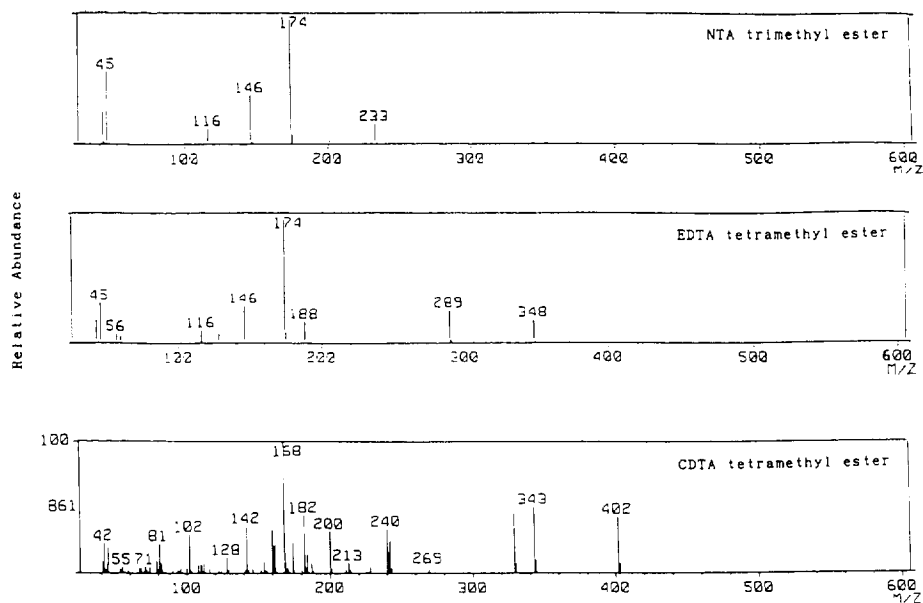nation. Fig. 4 shows typical GC–MS-SIM traces

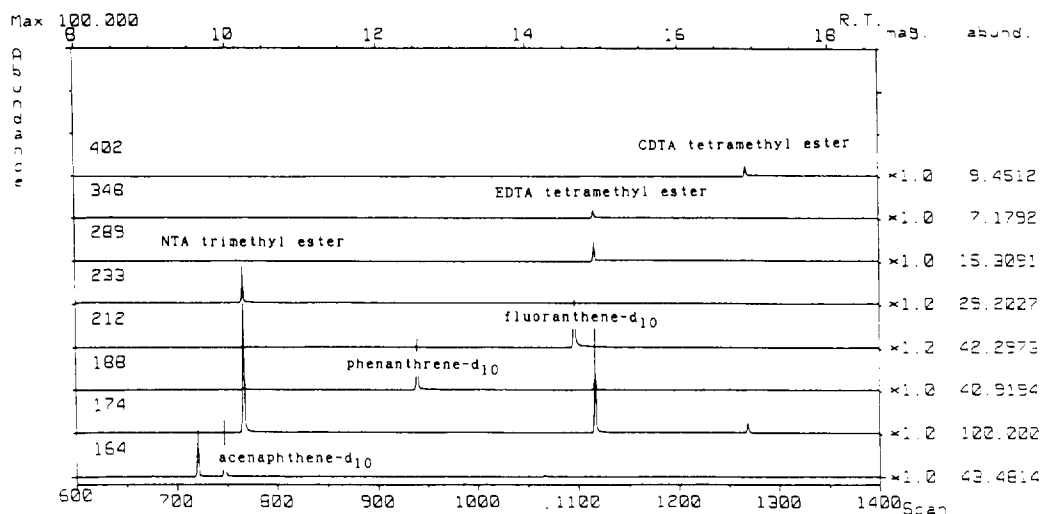Fig. 3. GC–MS of methyl ester derivatives of NTA, EDTA and CDTA.

Fig. 4. Typical GC–MS-SIM traces of methyl ester derivatives of NTA, EDTA, CDTA and internal standards. R.T. = Retention time in min; mag. = magnitude; abund. = abundance.

for the standards. The retention times of the methyl esters of NTA, EDTA and CDTA were 10.2, 14.8 and 16.9 min, respectively.

3.4. Calibration

Calibration graphs for NTA and EDTA were obtained by plotting the amount injected against the peak-area ratio of the analyte to the internal or surrogate standard. Examples of calibration graphs are shown in Fig. 5. The concentration of NTA or EDTA in environmental sample was calculated as follows:

Concentration(ng/ml or ng/g) = F (ng)

·FSV (ml)/I (ml) · SV (ml or g) × γ

γ (NTA) = NTA/NTA disodium = 0.79

γ (EDTA) = EDTA/EDTA disodium

dihydrate = 0.81

where F is the amount found by using the calibration graph, FSV the final sample volume, SV the sample volume, I the amount injected and γ the conversion coefficient to each free acid.

3.5. Esterfication reaction of Fe(III)–EDTA

NTA and EDTA form stable chelates with various metals. Fe(III)–EDTA is one of the

Table 1
Detection limits and precision for NTA and EDTA

Compound	DL (ng/ml)	Precision		
		Concentration (ng/ml)	Found[a] (ng/ml)	R.S.D. (%)
NTA	2.1	4.1	4.3	7.8
		8.1	7.7	5.6
		12.2	11.3	3.1
EDTA	6.2	3.9	3.5	11.0
		7.9	7.5	19.7
		11.8	9.7	11.8

Detection limits (DL) were calculated from the sensitivity of response estimating standard deviation as follows:

$D = t(n - 1, 0.05)\sigma/\sqrt{n}(dC/dR)$;   DL $= 3\bar{D}$,

where D is the detection power, $\bar{D}$ is the average value of D calculated from different concentrations (detection limits are defined as three times the detection power), $t(n - 1, 0.05)$ is the t-distribution at 95% reliability, σ is the standard deviation of the response, n is the number of replicates, c is the concentration of NTA or EDTA and R is the peak-area ratio of analyte to internal standard.
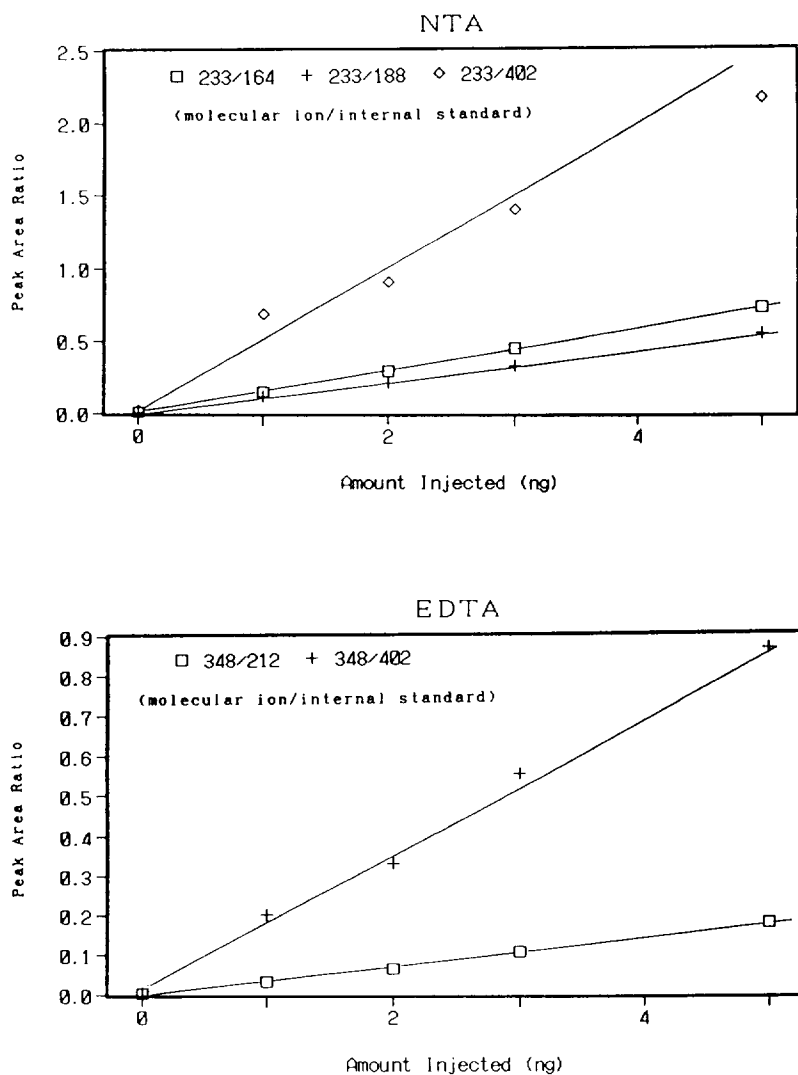[a] Average of four experiments.

Fig. 5. Typical calibration graphs for NTA and EDTA with different internal and surrogate standards.

most stable chelate compounds and one of probable forms that exist in the environment. Its esterification yield with the proposed method was therefore investigated. The results show that for 23.7 nmol of Fe(III)–EDTA the amount determined was at 18.3 nmol (yield 77%) and 23.3 nmol (yield 98%) as EDTA when calibrating with fluoranthene-d_{10} internal standard and CDTA surrogate standard, respectively. This result indicates that a quantitative reaction proceed even in the metal chelate form.

3.6. Preservation in river water

Two sets of 100-ml river water samples spiked with 4.1 μg of NTA and 3.9 μg of EDTA were stored in a refrigerator for 7 days, then analyzed. The amounts determined were 3.5, 4.1 (5.7, 6.1) μg of NTA and 3.7, 3.5 (5.4, 5.7) μg of EDTA, and those for the non-spiked samples were 0.23 (0.06) μg of NTA and 1.6 (1.8) μg of EDTA, where the determined values calibrated with the CDTA surrogate standard are given in parenthe-

Table 2
Recovery of the methyl ester of NTA from environmental samples

Sample	Sample amount	Added (μg)	Found[a] (μg)	Recovery[a] (%)	No. of samples (n)	R.S.D. (%)
Pure water	100 ml	1.22	1.13 (1.90)	92 (156)	4	3.1 (17.2)
River water	100 ml	4.05	3.71 (6.26)	92 (155)	2	1.5 (6.5)
Sea water	10 ml	0.41	0.29 (0.99)	70 (241)	2	11.2 (20.1)
Sediment	10 g	4.05	2.05 (3.60)	51 (89)	7	16.4 (11.2)
Fish	1 g	0.81	0.97 (1.24)	120 (153)	7	24.9 (27.4)

The values in parentheses indicate the determined values calibrated with the CDTA surrogate standard.
[a] The amount found is the difference between the amount detected in spiked sample (S) and that in the non-spiked sample (N); recovery $(\%) = [(S - N)/A] \cdot 100$, where A is the amount added.

ses. Based on the recovery, it appears that NTA and EDTA in river water do not decompose when stored in a cool location for up to 7 days.

3.7. Quantitative response

Table 1 reports the detection limits and precision for NTA and EDTA using the proposed procedure. A blank test was performed using a 100 ml of distilled water and with other chemicals used in analysis. No blank peaks corresponding to NTA and EDTA were observed in the chromatogram. NTA and EDTA were determined in the ranges 4.1–12.3 and 3.9–11.8 ng/ml in water samples with relative standard deviations (R.S.D.s) of 3.1–7.8% and 11.0–19.7%, respectively. The estimated detection limits of NTA and EDTA in water were 2.1 and

6.2 ng/ml, respectively, for a 100-ml water sample.

3.8. Determination in real samples

Analyte recovery was investigated by using 100 ml of pure and river water, 10 ml of sea water, 10 g of sediment and 1 g of fish sample spiked with 0.41–4.05 and 0.39–3.93 μg of NTA and EDTA, respectively. Tables 2 and 3 show that 92% and 74% of NTA and EDTA were recovered from the river water with R.S.D.s of 1.5% and 6.8%, respectively. For sea water, the recovery was lower, especially for EDTA. The low recovery could be due to matrix effects in sea water; in fact, a white, powdery salt was deposited during the analytical procedure. For sediment and fish samples, the recoveries were

Table 3
Recovery of the methyl ester of EDTA from environmental samples

Sample	Sample amount	Added (μg)	Found[a] (μg)	Recovery[a] (%)	No. of samples (n)	R.S.D. (%)
Pure water	100 ml	0.39	0.35 (0.48)	89 (124)	4	11.0 (12.4)
River water	100 ml	3.69	2.72 (4.54)	74 (123)	2	6.8 (3.6)
Sea water	10 ml	0.39	0.07 (0.54)	19 (139)	2	25.8 (34.0)
Sediment	10 g	3.93	2.36 (4.34)	60 (110)	7	14.1 (4.0)
Fish	1 g	0.79	0.50[b] (0.83)	63[b] (105)	7	12.3[b] (8.3)

The data in parentheses indicate the determined values calibrated with the CDTA surrogate standard.
[a] See Table 2 for definition of recovery.
[b] For fish samples, $[^2H_{10}]$ = phenanthrene was used as the internal standard instead of $[^2H_{10}]$fluoranthene owing to interference on the chromatogram.
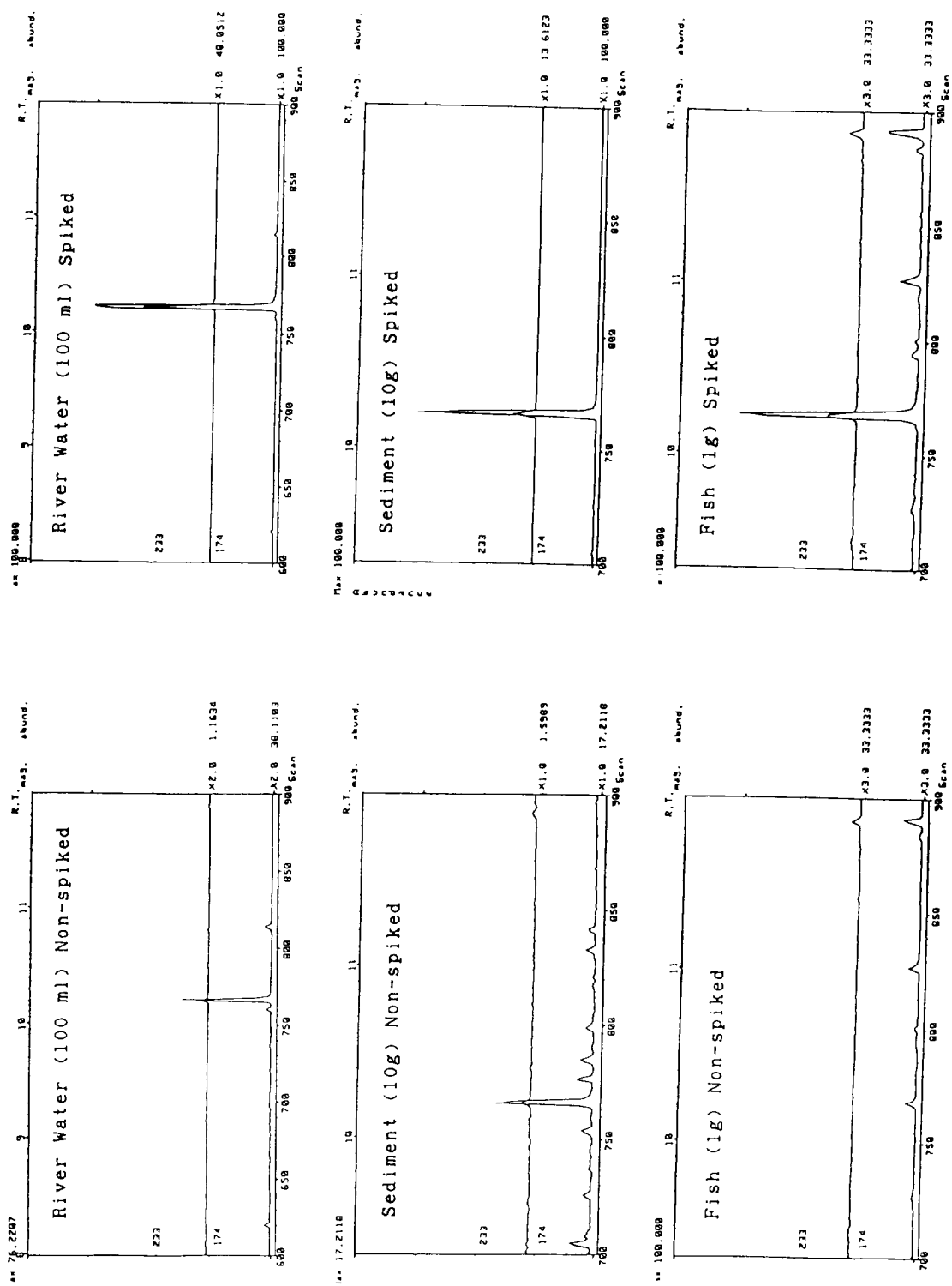
Fig. 6. Determination of NTA in normal and spiked river water, sediment and fish samples.
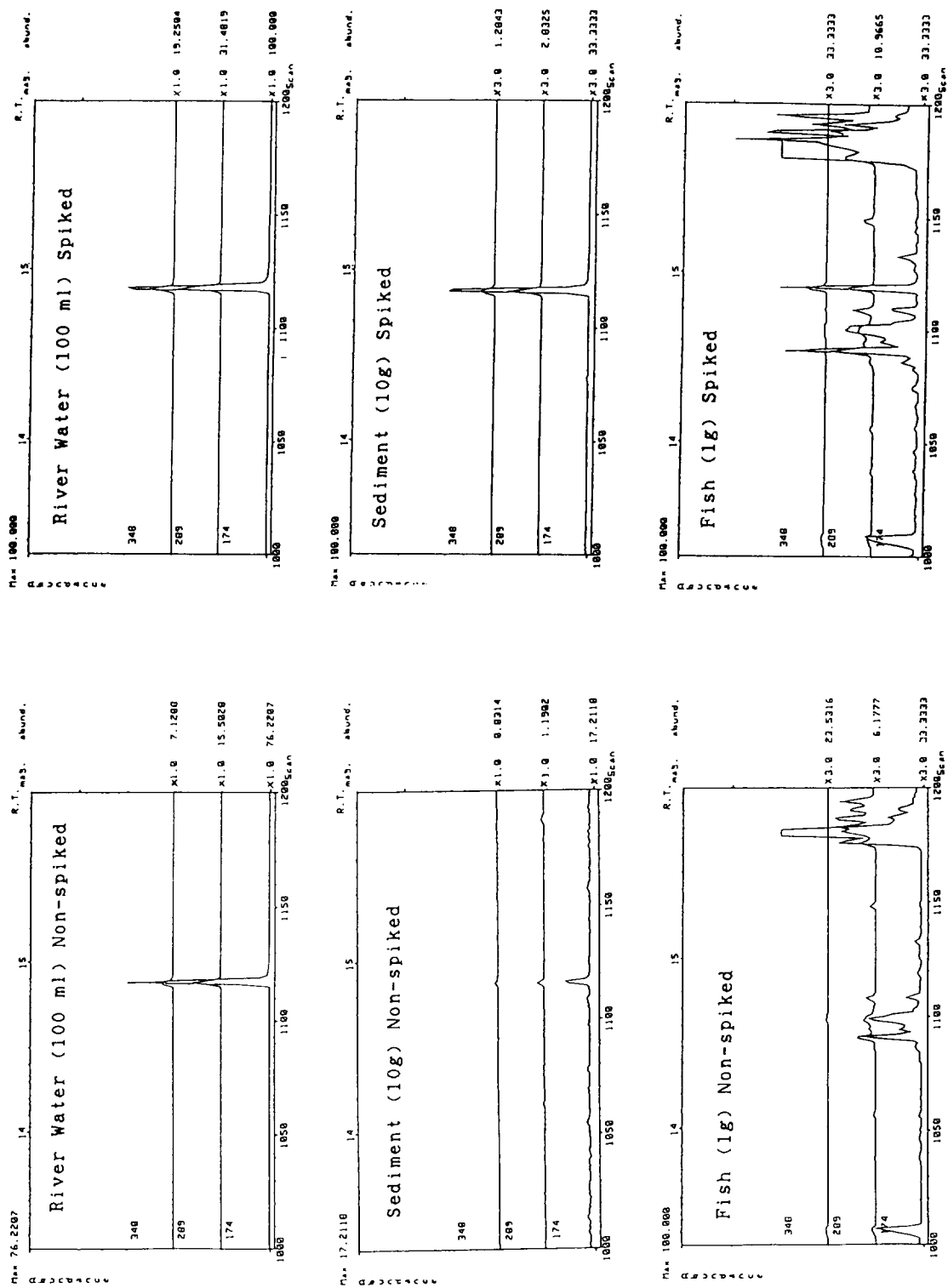
Fig. 7. Determination of EDTA in normal and spiked river water, sediment and fish samples.

51–60% and 63–120% with R.S.D.s of 14–18% and 12–25%, respectively. Whenever the recovery was calculated with the use of the CDTA surrogate standard, it became higher and was more than 100% in many instances. Figs. 6 and 7 show typical chromatograms for non-spiked and spiked samples of river water, sediment and fish. Both NTA (Fig. 6) and EDTA (Fig. 7) were detected in river water and sediment at low ng/ml and ng/g levels, respectively.

4. Conclusions

The proposed GC–MS-SIM method may be useful for the routine analysis of environmental samples at low ng/ml levels. However, it is difficult to analyse sea-water samples owing to the high concentration of salt. Determination of NTA and EDTA by this method could probably be applicable to their chelate forms in environmental samples.

Acknowledgement

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