

CHROM. 17,007

DETERMINATION OF NITRILOTRIACETIC ACID IN WATER BY HIGH-RESOLUTION GAS CHROMATOGRAPHY

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(Received June 30th, 1984)

SUMMARY

Nitrilotriacetic acid was determined in waters and waste-waters by high-resolution gas chromatography of the butylated acids isolated on an anion-exchange resin. The presented method is applicable to heavily loaded waste-waters and natural waters. A quantitation limit of 0.2 µg of the acid per litre of water was attained.

INTRODUCTION

The trisodium salt of nitrilotriacetic acid (NTA) can be used as a replacement for the detergent-builder trisodium tripolyphosphate. The environmental aspects of the use of NTA have been assessed¹⁻⁴. As a consequence of the lowering of the permitted limits of phosphates in detergent formulations, increasing amounts of NTA are presently employed in The Netherlands and in Switzerland. In order to help to improve the eutrophied Swiss lakes, the authorities in Switzerland are considering an almost complete ban on the usage of phosphates in laundry detergents. In Germany it has been suggested that the use of up to 25,000 metric tons per year should be permitted^{1,2}. As a consequence of these developments, it is intended to monitor the levels of NTA in waste-waters and particularly in natural and drinking waters of these European countries.

A prerequisite for such investigations is the availability of a reliable, sensitive and rapid method to determine trace quantities of NTA in rivers, lakes, ground-waters, drinking waters as well as in treated and untreated waste-waters. The available analytical techniques have recently been reviewed^{5,6}: gas chromatography (GC), polarography and colorimetry have been employed. For trace determinations of NTA in natural and drinking waters, only the GC methods can provide the necessary specificity and sensitivity. Most frequently, these techniques are based on the method developed by Aue *et al.*⁷ who used an enrichment of NTA on an anion-exchange resin followed by an esterification to convert NTA into its tris-*n*-butyl ester. Williams *et al.*⁸ and Malaiyandi *et al.*⁹ applied nitrogen-selective detectors to determine trace concentrations of NTA. Games *et al.*¹⁰ modified the original procedure of Aue *et al.* in order to determine NTA in highly complex matrices such as influents and effluents

of municipal waste-water treatment plants. They suggested the use of radioactively labelled NTA to overcome problems with interfering substances and variable recoveries. Kirk *et al.*¹¹ compared various methods for NTA determination and observed interferences in the analyses of waste-water samples.

In light of the reported difficulties with interferences and trace concentrations, it is surprising that the advantages of high-resolution gas chromatography (HRGC) have not yet been exploited for NTA determinations. HRGC has been highly successful for the qualitative and quantitative determinations of many other environmental pollutants such as polycyclic aromatic hydrocarbons¹², polychlorinated phenols¹³, polychlorinated dioxins¹⁴ and alkylphenol ethoxylates¹⁵. The books edited by Keith^{16,17} and Bjørseth and Angeletti^{18,19} contain many references to the application of HRGC to the determination of organic water pollutants. The major advantage of HRGC is its capability to cope with the high compositional complexity of mixtures of organic compounds encountered in environmental samples. When used with selective detectors and with directly coupled mass spectrometry, high sensitivities and reliable identifications can be achieved.

In this paper we describe a method for the determination of NTA using HRGC to analyze the butylated acids isolated from waste-waters and other waters. The isolation and derivatization procedures of Aue *et al.*⁷ and Games *et al.*¹⁰ were modified in order to improve the reliability and practicality of the method. The precision, accuracy and sensitivity of the described analytical technique are reported.

EXPERIMENTAL

Reagents and materials

Nitrilotriacetic acid (Titriplex I), *n*-butanol (analytical grade) and concentrated formic acid (analytical grade) were purchased from Merck (Darmstadt, F.R.G.). *n*-Pentane and *n*-hexane were Nanograde quality from Mallinckrodt. Octadecanoic acid nitrile [stearonitrile, CH₃(CH₂)₁₆CN, purum] and acetyl chloride (puriss.) were obtained from Fluka (Buchs, Switzerland). All chemicals were used as obtained from the supplier without any further purification.

The anion-exchange resin (Dowex 1X-2, 50–100 mesh, analytical grade) was applied after transforming it from the chloride to the formate form. This was achieved by percolating a larger batch of the resin with twenty bed volumes of 1 *M* sodium hydroxide solution in a glass chromatographic column. The resin was then washed with twice distilled water until the effluent was neutral and subsequently rinsed with two bed volumes of 1 *M* formic acid, again followed by twice distilled water until the effluent was neutral. The anion-exchange resin was then packed to a height of *ca.* 5 cm into a glass column (150 × 5 mm I.D.) equipped with a solvent reservoir (100 ml) and a PTFE tap. After use, the anion-exchange resin was regenerated with 10 ml of 16 *M* formic acid and washed with twice distilled water until the effluent was neutral.

The esterifying reagent (1 *M* hydrogen chloride in *n*-butanol) was prepared by bubbling dry hydrogen chloride gas through preweighed *n*-butanol. The hydrogen chloride gas was produced by slowly adding concentrated sulphuric acid to sodium chloride. The hydrogen chloride dissolved in *n*-butanol was determined by weighing and adjusted by dilution to 3.6 g of hydrogen chloride per 100 ml of *n*-butanol. The

reagent was kept in a refrigerator and a fresh batch prepared every 2 weeks. A 10% solution of acetyl chloride in *n*-butanol can also be used as an esterifying reagent which is easier to prepare²⁰. The dissolution has to be performed carefully because it is exothermic.

Two internal standard solutions (stearonitrile) were prepared in *n*-hexane at concentrations of 0.1 and 1 $\mu\text{g}/\mu\text{l}$, respectively.

Samples and sample preservation

Spiked NTA samples were prepared by adding NTA from an aqueous stock solution containing 1 μg of the acid per μl of water. Environmental samples were collected as grab samples and analyzed within 24 h or were preserved with 1% aqueous formaldehyde [10 ml of min. 37% formaldehyde solution (p.a., Merck) per 1 l of sample]. For composite sampling a similar preservation is recommended.

Isolation and derivatization

Water and waste-water samples of 25–100 ml (according to the expected NTA levels) were acidified to pH 2.3 with 8 *M* formic acid. Nitrogen gas was used to purge the carbon dioxide from the samples (20 min at 80°C in a water-bath). After cooling, the pH was checked again and, if necessary, readjusted. The water samples were then percolated through the anion-exchange resin columns by gravity flow or by applying a slight pressure of nitrogen gas (flow-rate, *ca.* 2 ml/min). The resin columns were then eluted with 10 ml of 16 *M* formic acid into 25-ml pear-shaped flasks. The eluents were cautiously evaporated to dryness in a rotary evaporator. A 2-ml volume of 1 *M* HCl in *n*-butanol or acetyl chloride in *n*-butanol was added to the completely dry samples and the closed flasks were kept for 30 min in a water-bath at 80°C. The reaction mixture was then transferred with a glass pipette to a 50-ml volumetric flask which had been filled with twice distilled water up to the beginning of the neck. The flask was closed with a glass stopper and shaken until the *n*-butanol was completely dissolved in the water. After adding *ca.* 1 ml of *n*-pentane, the internal standard (2–10 μl) was added directly into the *n*-pentane phase with a microsyringe. The closed volumetric flask was then shaken vigorously for *ca.* 1 min. After phase separation the pentane layer was transferred with a pipette into a PTFE-lined screw-cap vial. The reaction solution was again extracted using 1 ml of *n*-pentane. The combined extracts were dried with sodium sulphate and, if necessary, concentrated by evaporation under a nitrogen stream.

Gas chromatography

Gas chromatographic determinations were performed with Carlo Erba instruments equipped with a split/splitless injection device and a nitrogen-specific detector (NPD-40). Persilylated glass capillary columns (20 m \times 0.32 mm I.D.) coated with immobilized stationary phase²¹ (PS-255, film thickness *ca.* 0.15 μm) were employed. The GC conditions were as follows: hydrogen carrier gas, 0.4 atm; injector temperature, 300°C; oven temperature, 1 min at 40°C increased rapidly up to 160°C then at 5°/min to 240°C; detector temperature, 300°C; make-up gases, hydrogen 1.1 atm, air 1.3 atm, helium 1.4 atm. Aliquots of the samples (1–2 μl) were introduced using the splitless injection technique²². The carrier-gas split was reopened after *ca.* 30 sec. The elution temperature of NTA under these conditions was *ca.* 180°C. The peak areas

were electronically integrated with a Spectra-Physics (SP 4270) or a Hewlett-Packard (3390A) integrator.

Determination of the response factor and quantitation

From a standard solution of NTA in water (pH *ca.* 10) a known amount of NTA (1–5 μg) was transferred with a microlitre syringe into a 25-ml pear-shaped flask. After evaporation to dryness in a rotary evaporator, NTA was butylated and extracted into *n*-pentane together with the internal standard (IS) as already described. The same GC procedure was used to obtain the response factor, RF

$$\text{RF} = \frac{C_{\text{NTA}}}{C_{\text{IS}}} \cdot \frac{F_{\text{IS}}}{F_{\text{NTA}}} \quad (1)$$

where C_{NTA} , C_{IS} are the amounts (μg) of NTA and IS and F_{NTA} , F_{IS} are the corresponding peak areas.

The response factor was regularly monitored because it was quite sensitive to deteriorating chromatographic performance. A RF value of 1.2–1.5 indicated that the GC system was operating well. In the case of elevated RF values (> 1.5), measures were taken to improve the column behaviour, *i.e.*, washing with pentane, methylene chloride and methanol. If no improvement was observed, one loop at the inlet of the column was removed or the column was replaced.

The NTA concentration in the water and waste-water samples was calculated by considering the employed sample volume and using eqn. 2

$$C_{\text{NTA}} = C_{\text{IS}} \cdot \frac{F_{\text{NTA}}}{F_{\text{IS}}} \cdot \text{RF} \quad (2)$$

where C_{NTA} and C_{IS} are the concentrations ($\mu\text{g}/\text{l}$) of NTA and IS. The internal standard was added in such an amount that the $F_{\text{NTA}}/F_{\text{IS}}$ ratio was close to unity (0.1–5).

Gas chromatography–mass spectrometry (GC–MS)

Mass spectrometric analyses were performed with a Finnigan Model 4000 system combined with a Carlo Erba gas chromatograph and an INCOS-2000 data system. The glass capillary column was connected via a quartz capillary column directly to the ion source. The GC conditions and procedures were as used for the NTA determinations with the nitrogen-specific detector, except that helium was the carrier gas at 0.7 atm. Mass spectrometric conditions were: electron impact ionization with ionizing energy 70 eV; ionizer temperature, 250°C; electron multiplier voltage, 1.6 kV; mass range and scanning rate, m/z 45–450 in 1.2 sec.

For nitrilotriacetic acid tri-*n*-butyl ester the following mass spectrum was obtained: m/z 359 (relative intensity 2, M^+), 302 (0.4, $\text{M}^+ - 57$), 285 (1), 259 (15), 258 (100), 202 (6), 158 (27), 146 (5), 144 (9), 112 (5), 102 (6), 88 (28), 57 (39), 56 (10).

RESULTS AND DISCUSSION

Isolation and derivatization

The isolation procedure presented was based on the methods developed by

Aue *et al.*⁷ and Games *et al.*¹⁰. The elution from the anion-exchange resin was performed in a simpler way than by Games *et al.*, *i.e.*, only one eluted fraction, because the applied HRGC provided a complete separation of NTA from palmitic acid (see Fig. 2). The extraction of the tributylated NTA into *n*-pentane helped further to remove interfering compounds. Our procedure included only a complete evaporation step of the free NTA and no evaporation to dryness of the esterified NTA. This modification made the clean-up method more practical and less prone to evaporation losses. Consequently, the reported procedure ensured good and reliable recoveries of NTA and the use of radioactively labelled NTA, as suggested by Games *et al.*¹⁰, was not necessary.

The anion-exchange resin could be re-used many times after sufficient regeneration. It is recommended, however, that the same enrichment column be applied only for samples containing similar levels of NTA. An anion-exchange resin which had been employed for the analysis of a treated municipal waste-water containing 247 μg NTA/l was re-eluted with 10 ml of 16 *M* formic acid. This second eluate contained 2.4 μg NTA per l water (assuming a sample volume of 25 ml).

For low-level samples (< 2 μg NTA/l) special care must be taken to prevent problems caused by NTA contaminations from previously analyzed samples containing higher levels of NTA. In particular, the equipment used for purging with nitrogen must be carefully cleaned. When the necessary precautions were taken, excellent blank results were achieved as demonstrated in Fig. 1C for the analysis of a mineral water. The regular checking of blank samples is highly recommended when samples with low NTA concentrations are studied. In this case, fortified samples containing similarly low NTA concentrations should also be analyzed.

The derivatization with acetyl chloride in *n*-butanol gave equivalent results to those obtained with HCl in *n*-butanol. The preparation of the former reagent, however, is much easier and therefore recommended for routine use.

GC and GC-MS

Fig. 1 shows typical gas chromatograms obtained from the butylated acids isolated from waste-water and water samples according to the described procedure. HRGC in combination with the nitrogen-selective detector allowed dependable determinations of NTA in all samples including the untreated municipal waste-water. An elution time of *ca.* 10 min for NTA made the method suitable for routine applications.

Several advantages were attained by the selection of a relatively non-polar, nitrogen-containing compound as an internal standard which need not be derivatized. First, problems with different behaviours of the internal standard in the derivatization step were avoided. Second, the response factor (see Experimental) could be used to control the quality of the gas chromatography.

Fig. 2 depicts the HRGC-MS analysis of the butylated acids extracted from a mechanically treated municipal waste-water. The wide separation between the palmitic acid butyl ester and the tributylated NTA contrasts sharply with the incomplete separation of these two compounds using packed column GC, *e.g.*, ref. 10. Thus, one can reliably prevent the interference of palmitic acid in NTA determinations by using HRGC. As shown in Fig. 2, a highly specific and sensitive NTA determination was obtained from the mass chromatogram of *m/z* 258 which is the base peak in the mass

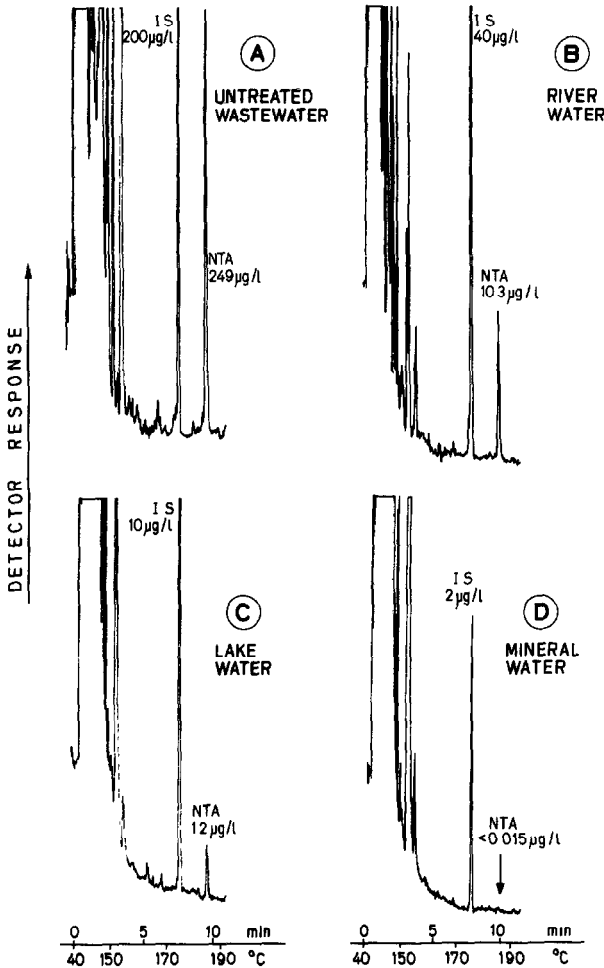


Fig. 1. Gas chromatograms of NTA determinations by HRGC. For experimental details, see text.

spectrum of tributyl-NTA. Such single-ion monitoring techniques could greatly improve the detection limits of NTA determinations.

Precision, accuracy and sensitivity

Table I shows the reproducibilities of the NTA determinations which were measured in three different types of water samples. Excellent standard deviations were achieved for the primary sewage effluent, from the mechanical treatment of municipal waste-water, and for the river-water. An acceptable precision (relative standard deviation $\pm 10\%$) was found for the analysis of a lake-water with a NTA concentration of $0.79 \mu\text{g/l}$.

In Table II the results of eight recovery experiments are reported. Fortified drinking water solutions with NTA concentrations from 10 to $1000 \mu\text{g/l}$ showed mean recoveries from 96 to 99% with standard deviations between 2 and 5%. At the lower concentrations of 1 and $0.2 \mu\text{g NTA/l}$ recoveries of 89, 90 and 108% with

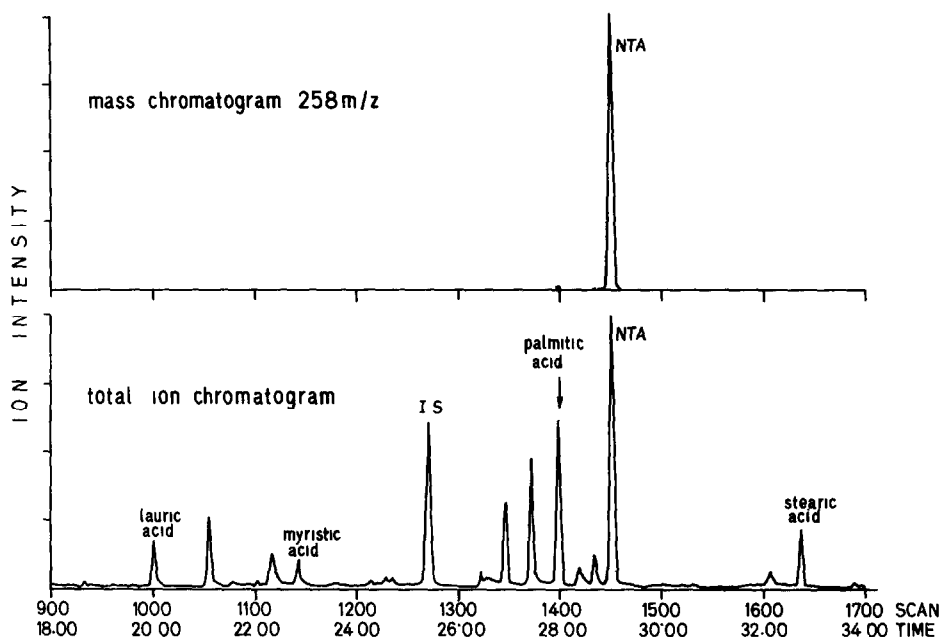


Fig. 2. HRGC-MS analysis of butylated acids from a mechanically treated municipal waste-water. For experimental details, see text.

larger deviations of 12 and 18% were found. The analysis of spiked waste-water samples showed no evidence for interfering constituents and yielded satisfactory accuracies. A practical quantitation limit of $0.2 \mu\text{g NTA/l}$ was achieved for reliable larger deviations of 12 and 18% were found. The analysis of spiked waste-water samples showed no evidence for interfering constituents and yielded satisfactory accuracies. A practical quantitation limit of $0.2 \mu\text{g NTA/l}$ was achieved for reliable determinations. Qualitatively, however, NTA could be detected down to $0.015 \mu\text{g/l}$.

TABLE I

PRECISION OF THE DETERMINATION OF NTA IN WATERS AND WASTE-WATERS

The NTA concentrations are given as μg of the acid per litre of water; this is equivalent to $1.35 \mu\text{g/l}$ of the trisodium salt or 5.24 nmol/l .

Sample	NTA concentration		
	Mean ($\mu\text{g/l}$)	Relative standard deviation*	(%)
Mechanically treated municipal waste-water	198	4	(5)
River-water	12.5	3	(3)
Lake-water	0.79	10	(3)

* Number of replicate determinations in parentheses.

TABLE II
RECOVERY OF NTA FROM WATERS AND WASTE-WATERS

Sample	NTA ($\mu\text{g/l}$)		Recovery* (%)
	Added	Found*	
Drinking water	1000	995 \pm 22 (3)	99 \pm 2
	100	94.7 \pm 5.2 (3)	96 \pm 5
	10	9.6 \pm 0.4 (3)	96 \pm 4
	1	0.90 \pm 0.12 (3)	90 \pm 12
Ground-water	1	0.89 \pm 0.12 (4)	89 \pm 12
	0.2	0.22 \pm 0.04 (4)	108 \pm 18
Raw municipal waste-water	200**	181 \pm 20 (2)	91 \pm 10
Mechanically treated municipal waste-water	160**	164 \pm 17 (2)	103 \pm 11

* Mean \pm standard deviation; number of replicate determinations in parentheses.

** NTA concentrations before spiking: 253 \pm 21 $\mu\text{g/l}$ in the raw waste-water and 198 \pm 7 $\mu\text{g/l}$ in the mechanically treated waste-water.

By using a non-specific flame ionization detector, a quantitation limit of *ca.* 5 μg NTA/l was attained. The application of single-ion monitoring GC-MS techniques (see Fig. 2) would enable the quantitation of much lower concentrations.

CONCLUSIONS

The presented method allows rapid, precise and reliable quantitation of NTA in waters and waste-waters down to 0.2 $\mu\text{g/l}$. Below 2 μg NTA/l it is recommended to carry out duplicate determinations and frequently to analyze blank samples. No interferences could be detected in heavily loaded waste-waters. The method is highly suitable for routine monitoring programs and investigations of dynamic environmental systems, *e.g.*, waste-water treatment plants and rivers, which require the analyses of large numbers of samples.

In view of the very complex composition of organic compounds in waste-waters and waters, we highly recommend the advantages of high-resolution GC. By applying this analytical technique, one can obtain dependable information on the fate of NTA in waste-waters and natural waters.

ACKNOWLEDGEMENT

The authors thank K. and G. Grob for supplying glass capillary columns, and James McEvoy for reviewing the manuscript.

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