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QUANTITATIVE GAS CHROMATOGRAPHIC DETERMINATION OF NITRILOTRIACETIC ACID IN THE PRESENCE OF OTHER CARBOXYLIC ACIDS

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SUMMARY

Nitrilotriacetic acid was separated (as its tri-*n*-butyl ester) from twenty-four other fatty, phenolic and polycarboxylic acids, using prepacked commercial OV-210 columns. Quantitative analysis down to 0.010 mg nitrilotriacetic acid per liter was demonstrated. The molar responses and retention times of the acids, relative to phthalic acid, were determined.

INTRODUCTION

The synthetic complexing agent nitrilotriacetic acid (NTA, Fig. 1), in the form of its trisodium salt, is used in various industrial and agricultural processes and is a potential candidate for addition to detergent formulations (as a phosphate-replacing builder) in the United States. A maximum concentration of 6% trisodium nitrilotriacetate in such formulations is legal in Canada; it is also used in other countries.

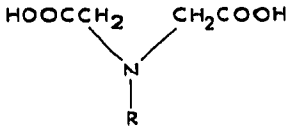

Fig. 1. NTA (R = CH_2COOH) and some of its possible degradation products, IDA (R = H) and MIDA (R = CH_3).

An early matter of concern regarding NTA was that the compound could present a hazard to human health by virtue of its chelating properties for toxic metal ions, or through formation of carcinogenic N-nitroso derivatives of its products of breakdown. Recently, potential ecological dangers of widespread domestic use of NTA have become controversial. Resolution of such problems requires study of the effects of known concentrations of NTA on natural aquatic and terrestrial ecosystems. If NTA is approved for domestic use, it will be necessary to monitor its presence in soils, sediments, drinking water, surface waters and sewage treatment plants.

Reliable methods of determination of NTA have only recently become available. Most published methods are subject to interference from inorganic and organic substances, particularly at low NTA concentrations. Estimation of NTA by colorimetric or fluorimetric methods which measure total chelating ability, such as the copper solubilization method of Kunkel and Manahan[1], the zinc–Zincon procedure[2] or the gallium–oxine method[3], is subject to several sources of error. Chelating agents such as citric acid or natural polyphenolic polycarboxylic acids are present in many waters, and some of the high-molecular-weight acids are themselves colored or fluorescent. In addition, some of the known degradation products of NTA, *e.g.* iminodiacetic acid (IDA, Fig. 1), are rather good chelating agents. On a molar basis, the ability of IDA to retain copper in solution is 70 percent of that of NTA[4]. Polarographic methods, although sensitive, are subject to interferences under certain conditions from metal ions[5], sulfur compounds, polyphosphates and natural organic chelating agents[6]. Also, possible degradation products of NTA cannot be determined.

Gas chromatographic (GC) methods are potentially very sensitive, but there have been no thorough studies of the interference of organic substances in NTA analysis. Taylor *et al.*[7] showed that the trimethylsilyl ester of NTA eluted between those of lauric and myristic acids, but did not report studies with other interfering substances. Citric acid tributyl ester has been reported[8] to co-elute with the tributyl ester of NTA on Carbowax 20M columns. One GC method[9] has been particularly successful in affording rapid reproducible analysis at concentrations in the μg/l range. In particular, citric acid is well resolved from NTA. However, it requires the use of a special column consisting of Carbowax 20M on acid-washed Chromosorb W. The column must first be strongly heated and then continuously extracted for prolonged periods, leaving a thin film of unextractable polymer on the support[7].

The difficulties in this approach prompted us to investigate the capabilities of more common, commercially available, GC liquid phases, especially those similar to phases already used for analysis of esters. The liquid phase would have to permit reproducible analyses in the sub-milligram-per-liter NTA concentration range, and also be free from interference by other acid derivatives which could conceivably be present in the environment. We report a method, using a commercial 3% OV-210 column, which gives complete separation of NTA from its potential degradation products and twenty-two other fatty, phenolic and polycarboxylic acids, and which affords reproducible analyses of solutions containing as little as 10 μg of NTA per liter.

EXPERIMENTAL

General

Procedures for the processing of water samples and the derivatization of acids closely followed those of Aue *et al.*[9]. Unfiltered 50-ml samples of NTA in natural stream water (White Clay Creek, Chester County, Pa., U.S.A.) were acidified, cleaned by anion exchange (Bio-Rad AG 1-X2), eluted (10 ml of 16 *M* formic acid) into a screw-cap test-tube, and evaporated to dryness in a Kontes tube concentrator (85°).

Pure acids were weighed directly into a test tube. The pure acid (or the dry residue from evaporation of a water sample) was suspended in 3 *M* HCl in *n*-butanol (2.0 ml), heated at 85° for 30–35 min and stirred intermittently with a vortex mixer

("Maxi-mix", Thermolyne Corp.). The solution was then evaporated to dryness with a stream of dry air at room temperature. Just before analysis, dry acetone (0.800 ml) was added and the solution was transferred to a dry sampling vial having a PTFE-faced seal. Dibutyl phthalate (10 μl of a 10% solution) was added to each vial as an internal standard.

Apparatus

The gas chromatograph was a Hewlett-Packard Model 7620 equipped with dual flame detectors, automatic injector, electronic integrator and 10-in. recorder. The glass GC columns (1.83 m × 6.3 mm) (Applied Science Labs., Cleveland, Ohio, U.S.A.) contained 3% OV-1, OV-210 or OV-225 silicone liquid phases, prepacked on 60–80 mesh acid-washed Chromosorb W.

All glassware was cleaned in 6 *M* HCl, rinsed with double-distilled water and dried before use. Test tubes used for derivatization had PTFE-lined screw-caps. Anion-exchange resin was Bio-Rad AG 1-X2 (150 mesh).

Reagents

NTA was obtained from Aldrich (Milwaukee, Wisc., U.S.A.) as its "Gold Label" 99+% disodium salt monohydrate. Its tributyl ester showed no detectable impurities upon GC analysis. Other acids were purchased from Sigma (St. Louis, Mo., U.S.A.), Aldrich, Nutritional Biochemicals, (Cleveland, Ohio, U.S.A.), J. T. Baker (Phillipsburgh, N.J., U.S.A.) and Matheson, Coleman & Bell (East Rutherford, N.J., U.S.A.), and were of the highest available quality. Acetone and *n*-butanol were reagent grade (J. T. Baker), redistilled from $MgSO_4$ in an all-glass apparatus. Acetone was stored over Linde 4A molecular sieves. Hydrogen chloride, generated by adding concentrated H_2SO_4 to solid NaCl, was dried by passage through H_2SO_4 in an oven-dry apparatus before dissolving in *n*-butanol. The HCl concentration was determined by titration with standard NaOH solution to a phenolphthalein end-point. [1-^{14}C]NTA was supplied by Dr. R. L. Downey (Procter and Gamble, Cincinnati, Ohio, U.S.A.) as a 10^{-3} *M* solution of the trisodium salt containing 3.7×10^6 dpm/ml.

Gas chromatographic conditions

The gas flow-rates to the flame detector were 300 ml/min of air and 40 ml/min of hydrogen. The carrier gas (helium) flow-rate was 55 ml/min. Injection-port and flame-detector temperatures were held at 250°. Temperature program I, which was used to separate all the acids as their *n*-butyl esters, was isothermal at 145° for 8 min and then increased 6°/min to 240°. Program II, used for rapid analysis of NTA, was isothermal at 185°. The retention time of NTA was *ca.* 19 min on program I and 9–10 min on program II. The automatic sampler was set to inject 4.5 μl amounts of test solution.

RESULTS AND DISCUSSION

Initially, weighed amounts of each of thirty-six pure acids were separately derivatized and injected into the gas chromatograph. Most of the acids chosen are naturally occurring substances. Eleven acids (oxalic, capric, caproic, caprylic, lauric,

oleic, protocatechuic, salicylic, chlorogenic, caffeic and gallic) gave no detectable peaks and were not further studied. Some of these acids may not have formed an ester under the reaction conditions. Others, particularly oxalic acid and the lower fatty acids, may have formed esters which were too volatile to be detected and still others may have formed undetectable involatile esters. Peak areas for the remaining twenty-five acids were electronically integrated and their molar responses were determined relative to dibutyl phthalate. Retention times were also corrected to that of dibutyl phthalate. Table I summarizes this data.

TABLE I

RELATIVE RETENTION TIMES (*RRT*) AND RELATIVE MOLAR RESPONSES (*RMR*) OBTAINED ON GC ANALYSIS OF *n*-BUTYL ESTERS OF TWENTY-FIVE CARBOXYLIC ACIDS ON 3% OV-210

Column as in Experimental; carrier gas (helium) flow-rate, 55 ml/min.

No.	*Acid*	*No. of runs*	*RRT* ± *S.D.*	*RMR* ± *S.D.*	*RMR standard error*
1	Malonic	7	0.17 ± 0.02	0.40 ± 0.16	±0.05
2	Succinic	5	0.26 ± 0.02	0.46 ± 0.06	±0.03
3	Fumaric	5	0.27 ± 0.02	0.41 ± 0.05	±0.02
4	Cinnamic	4	0.32 ± 0.01	0.69 ± 0.07	±0.03
5	Malic	5	0.41 ± 0.01	0.41 ± 0.03	±0.01
6	*p*-Hydroxybenzoic	4	0.44 ± 0.02	0.43 ± 0.02	±0.01
7	N-Methyliminodiacetic	20	0.50 ± 0.04	0.76 ± 0.16	±0.04
8	Iminodiacetic	4	0.53 ± 0.01	0.41 ± 0.07	±0.03
9	Tartaric	5	0.55 ± 0.04	0.30 ± 0.05	±0.02
10	Vanillic	5	0.60 ± 0.04	0.66 ± 0.13	±0.06
11	Myristic	10	0.70 ± 0.09	1.26 ± 0.50	±0.16
12	Shikimic	9	0.83 ± 0.04	0.59 ± 0.20	±0.07
13	Palmitic	5	0.94 ± 0.00	1.13 ± 0.10	±0.04
14	Phthalic		1.00	1.00	
15	Coumaric	5	1.07 ± 0.00	0.61 ± 0.06	±0.03
16	Syringic	4	1.12 ± 0.00	0.73 ± 0.10	±0.05
17	Linoleic	10	1.14 ± 0.02	1.16 ± 0.29	±0.09
18	Linolenic	4	1.16 ± 0.02	1.29 ± 0.27	±0.13
19	Stearic	9	1.17 ± 0.02	1.50 ± 0.38	±0.12
20	Ferulic	10	1.23 ± 0.03	0.75 ± 0.16	±0.05
21	Citric	10	1.29 ± 0.04	0.93 ± 0.11	±0.04
22	Arachidic	4	1.31 ± 0.06	1.78 ± 0.07	±0.04
23	Nitrilotriacetic	7	1.36 ± 0.05	0.81 ± 0.11	±0.04
24	Sinapic	10	1.46 ± 0.09	0.73 ± 0.22	±0.08
25	Behenic	10	1.50 ± 0.08	2.09 ± 0.46	±0.15

An equimolar mixture of these acids was dissolved in aqueous acetone. An aliquot of this mixture, containing 2 μmoles of each acid, was evaporated to dryness, derivatized and analyzed by temperature program I (145° for 8 min, then 6°/min to 240°). Fig. 2 shows the resulting GC trace.

The analysis of NTA was studied in detail. First, a standard curve was constructed from measurements on samples of water from White Clay Creek to which NTA had been added, giving concentrations ranging from 0.01 to 200 mg NTA per liter. Samples (50 ml) were subjected to the ion-exchange and derivatization procedure

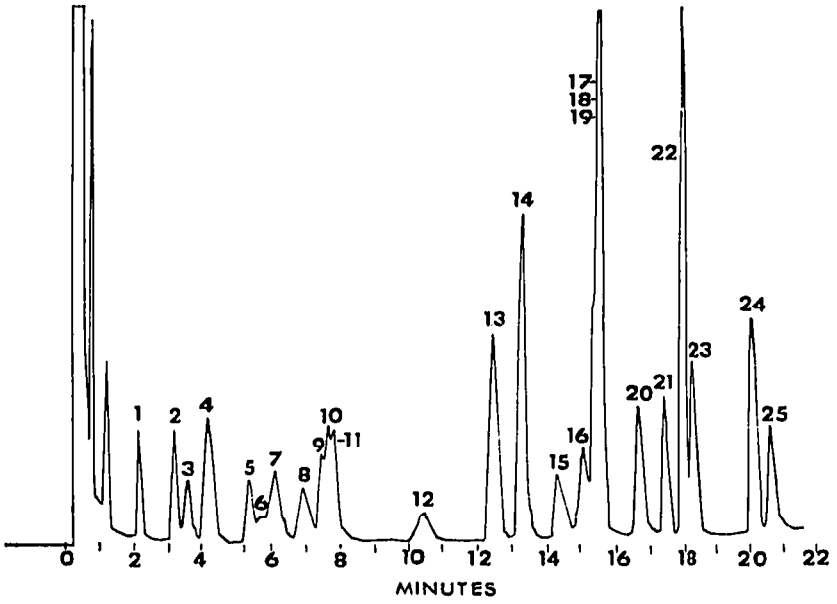

Fig. 2. GC separation of butyl esters of twenty-five carboxylic acids. Support, Chromosorb W AW (60–80 mesh); stationary phase, 3% OV-210. Carrier gas, helium; flow-rate, 55 ml/min. Temperature, 145° for 8 min followed by 6°/min to 240°. Detector, flame ionization. Sample size, 4.5 μl. The numbers refer to the acids in Table I. Internal standard, dibutyl phthalate (peak 14).

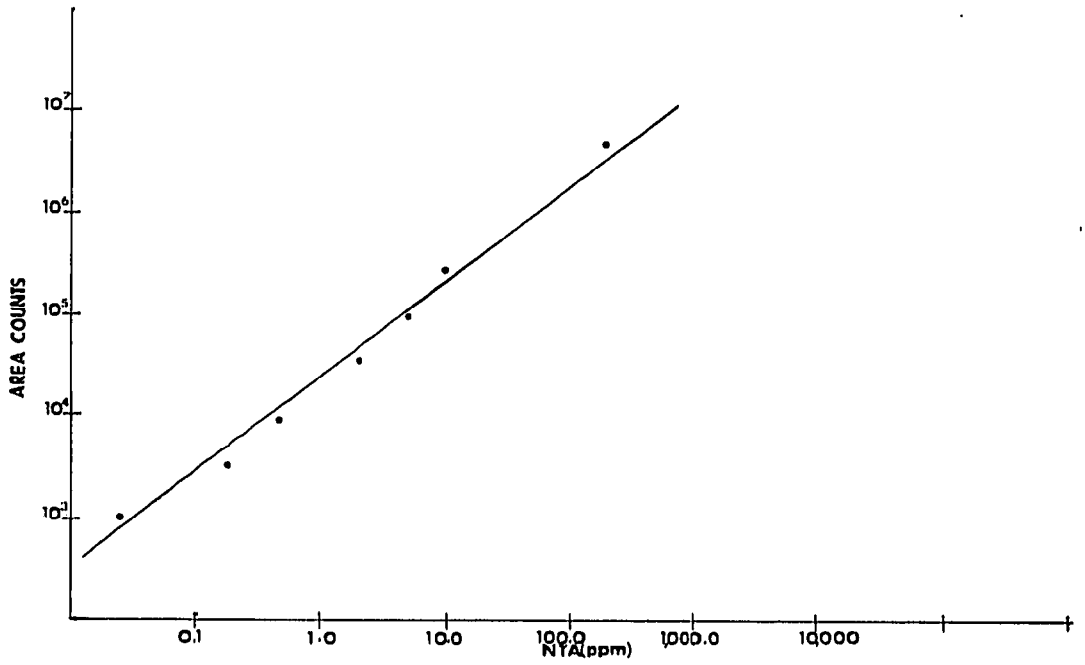

Fig. 3. Standard graph of integrated area *versus* concentration for NTA analysis.

and injected in triplicate into the 3% OV-210 column. Fig. 3 shows the graph of integrated area *versus* NTA concentration. Recovery of NTA by the procedure was studied by use of [1-^{14}C]NTA. Five replicate samples of a 0.20 mg/l solution were taken through the ion-exchange and derivatization steps and examined periodically for radioactivity. The results showed that 98% of the starting NTA adhered to the ion-exchange column, 86% of the total was recovered after elution with 16 *M* formic acid, and 75% remained after conversion into the *n*-butyl ester. Thin-layer chromatography (silica gel/diethyl ether) on the radioactive esterification product showed a single spot containing 95% of the total activity; the remainder was mainly at the origin.

As can be seen from Fig. 2, the NTA tributyl ester peak appeared between those of the butyl esters of arachidic and sinapic acids, using temperature program I. Separation from citric acid tributyl ester was excellent (*ca.* 1.0 min) using this program, and also (*ca.* 0.8 min) using the isothermal program II. Although separation from arachidic acid butyl ester was incomplete using program II, this program is now being used routinely in our laboratory for analysis of NTA in stream water. Ion exchange completely separates the two acids, and, furthermore, it is unlikely that detectable amounts of arachidic acid will occur in our stream water.

In screening experiments, the OV-210 column was superior to columns packed with equivalent amounts of OV-1 or OV-225. On OV-1, separation from citric acid butyl ester was good, but the NTA peak was close to those of the syringic and palmitic acid esters. On OV-225, the NTA and citric acid ester peaks were close together. We have repeated the separation on different OV-210 columns from Applied Science Labs., obtained at different times, with practically identical results.

A few acids were not resolved by program I. Linolenic, linoleic and stearic acid ester peaks overlap almost completely and several others are not resolved to baseline levels. However, NTA and its likely degradation products, IDA and N-methyliminodiacetic acid (MIDA, Fig. 1), are well separated from many potentially interfering acids; only arachidic acid, a relatively uncommon fatty acid, could cause difficulties if present in large excess. Additional studies will be required in situations (such as certain industrial effluents) where acids of synthetic origin may be present, to determine the applicability of this procedure.

CONCLUSION

We conclude that NTA and its possible degradation products can be analyzed on commercially packed GC columns in the presence of some common naturally occurring acids. The method is being used in our laboratory for routine daily analysis of NTA concentrations ranging from 0.02 to 200 mg/l in artificial stream microcosms containing communities of natural aquatic organisms.

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