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QUANTITATIVE CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF LOW LEVELS OF NITRILOTRIACETIC AND ETHYLENEDIAMINETETRAACETIC ACIDS IN DIETHYLENETRIAMINEPENTAACETIC ACID

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SUMMARY

A quantitative method for determination of low levels (0.05%, w/w) of nitrilotriacetic and ethylenediaminetetraacetic acids in diethylenetriaminepentaacetic acid is described. Palmitic acid is added to the chelator as an internal standard before esterification with methanol containing 2% (v/v) H_2SO_4. The methyl esters of palmitic, nitrilotriacetic, and ethylenediaminetetraacetic acids are first separated from diethylenetriaminepentaacetate by silicic acid column chromatography and are subsequently quantitated by gas–liquid chromatography. The method is both accurate and reproducible with less than 10% relative error. Thin-layer chromatographic separations of the methyl esters, and quantitation at the 1% level, are also described.

INTRODUCTION

Zinc trisodium diethylenetriaminepentaacetate (Zn-DTPA) was recently approved by the Food and Drug Administration as an investigational new drug (IND) for treatment of patients contaminated internally with actinide compounds. The drug is being managed by the Medical and Health Sciences Division of Oak Ridge Associated Universities for the Division of Human Health Studies, Office of Environmental Research, U.S. Department of Energy. Since nitrilotriacetic acid (NTA) is a carcinogen[1] and could be present as a contaminant in preparations of DTPA, a method was required to determine small amounts of NTA in the DTPA used to prepare the Zn-DTPA for clinical use. Ethylenediaminetetraacetic acid (EDTA) is another possible contaminant in DTPA and, although not as significant as NTA from the health aspect, the need for a sensitive assay for its presence in DTPA is also apparent.

Several gas–liquid chromatographic (GLC) methods for the determination of NTA, as well as other chelators, have been described primarily for detection of trace levels of NTA and other chelators in aqueous samples[2–8]. However, the detection of low levels of NTA and EDTA in DTPA presents a somewhat different problem

because of the necessity to detect minute amounts of a minor component in the presence of a large amount of a second component that has similar chromatographic properties. Since most columns used in GLC analyses are subject to being overloaded when submilligram quantities are analyzed, a preliminary separation of the NTA and EDTA from the DTPA is needed to do quantitative analyses by GLC if the levels of NTA or EDTA are 0.1% or less. We selected silicic acid column chromatography to provide this initial separation and included an internal standard (palmitic acid) at the start to correct for small losses of sample that could occur during the procedure.

EXPERIMENTAL

Materials

NTA was purchased from Eastman (Rochester, N.Y., U.S.A.). Palmitic acid, EDTA, and DTPA (a special twice-crystallized preparation) were obtained from Sigma (St. Louis, Mo., U.S.A.). Anhydrous diethyl ether was reagent grade from Mallinckrodt (St. Louis, Mo., U.S.A.). Chloroform, containing 1% (v/v) ethanol as a preservative, and methanol were supplied by Burdick & Jackson Labs. (Muskegon, Mich., U.S.A.). We used activated silicic acid, 100–200 mesh (Unisil; Clarkson Chemical Co., Williamsport, Pa., U.S.A.) for column chromatography and silica gel G (Brinkmann, Westbury, N.Y., U.S.A.) for thin-layer chromatography (TLC).

Thin-layer and gas–liquid chromatography

TLC was carried out on glass plates coated with 250-μm layers of silica gel G using a solvent system of chloroform–methanol (98:2, v/v). GLC was performed using a Victoreen instrument (Model 4000) equipped with a flame ionization detector and fitted with an 18 × 1/8 in. stainless-steel column packed with 3% OV-17 on Anakrom Q, 90–100 mesh (Analabs, North Haven, Conn., U.S.A.). The flash injector, at a temperature of 250 °C, contained a small glass capillary tube into which the sample was injected. Helium, at a flow-rate of 20 ml/min, was used as the carrier gas. The column oven was temperature programmed from 150° to 300° at a rate of 10°/min and the detector was maintained at a temperature of 270°.

Preparation of methyl esters

Samples subjected to the esterification procedures included: NTA, EDTA, DTPA, palmitic acid, blanks, and several mixtures of either NTA or EDTA and palmitic acid (internal standard). Palmitic acid was chosen as the internal standard since no evidence of it was found in this special preparation of DTPA. Esterifications were done in sealed, 8-ml culture tubes, containing the samples dissolved in 2 ml of methanol with 2% (v/v) H_2SO_4, by heating at 100° for 1 h. The tubes were then cooled to room temperature and NH_3 fumes bubbled through the solution until no more precipitation of ammonium sulfate could be seen (basic pH). A 5-ml volume of diethyl ether was added to the tubes, mixed, and centrifuged to remove the precipitate. After evaporation of the supernatants with nitrogen at 40° to 50°, the residues were suspended in 3 ml of chloroform and centrifuged again to remove the remaining ammonium sulfate. Supernatants were evaporated to dryness and dissolved in 2 ml of chloroform for separation by column chromatography.

Column chromatography

Glass columns, 20 × 0.85 cm, were packed to a height of 11 cm with a chloroform slurry of Unisil and washed with 50 ml of chloroform before the samples were applied to the column. The methyl esters of NTA, EDTA, and palmitic acid (internal standard) were eluted with 100 ml of chloroform. After evaporation of the chloroform at reduced pressure, the samples were transferred with chloroform to small, 1-ml conical vials. After removal of the chloroform with a stream of nitrogen, the samples were dissolved in 25 μl of methanol for analysis by GLC.

RESULTS AND DISCUSSION

Resolution of NTA, EDTA, and DTPA by TLC

The TLC R_F values of the methyl esters of NTA, EDTA, and DTPA were 0.6, 0.4 and 0.1, respectively. Using narrow outside lanes of an 8 × 8 in. TLC plate for standards, the sample is applied as a band between them, developed in the solvent mixture described under Experimental and the NTA esters located by staining the compounds on the outside lanes with iodine vapor. The trimethyl-NTA is then eluted from the silica gel on a sintered glass funnel using chloroform–methanol (1:1, v/v). After evaporation of the filtrate, it is dissolved in an appropriate volume of methanol for quantitation by GLC using an external standard method. We found, however, that if the level of NTA being determined was so low that excessive amounts of DTPA (>10 mg) were required for TLC, significant amounts of the DTPA esters streaked up the plate and overlapped the NTA ester band. This TLC technique was satisfactory down to about 1% NTA, but it was not sufficiently quantitative for the range (0.05–0.2% NTA) required for analysis of purity when DTPA is used clinically. The problem of plate overloading was even more severe in the separation of EDTA esters from large amounts of DTPA esters.

Resolution of NTA, EDTA, and DTPA by column chromatography

Initial separations of a mixture of methyl palmitate, trimethyl-NTA, tetramethyl-EDTA, and pentamethyl-DTPA by silicic acid column chromatography revealed that the esters of palmitic acid, NTA, and EDTA were eluted with the first 100 ml of chloroform. Esters of DTPA were usually not eluted from the column with 100 ml of chloroform; occasionally, however, small amounts of pentamethyl-DTPA (<0.1 mg) were present in this fraction. The methyl esters of DTPA can be eluted by using 50 ml of 10% methanol in chloroform.

Quantitation by GLC

After GLC analyses of the chloroform fraction from column chromatography of the methyl esters from 20 mg of DTPA, no peaks were observed at the retention times of standards of methyl palmitate, tetramethyl-EDTA, or trimethyl-NTA. Analyses of several mixtures of methyl palmitate and trimethyl-NTA by GLC indicated the NTA peak areas (peak height × peak width at half-height) needed to be multiplied by 2.82 (±0.21 S.D.) to equate its peak area to its weight relative to methyl palmitate. The response factor correction for tetramethyl-EDTA was 1.51 (±0.07 S.D.).

Table I summarizes the analyses of several samples of the DTPA containing

TABLE I

QUANTITATIVE ANALYSIS OF NTA AND EDTA IN DTPA BY GAS–LIQUID CHROMATOGRAPHY

μg NTA or EDTA added to 20 mg DTPA		*μg NTA or EDTA found**
NTA	10	8.9, 9.1
	20	21.0 ± 1.0 (5)
	40	40.2 ± 2.5 (3)
EDTA	10	10.3 ± 1.2 (3)
	20	20.0 ± 1.0 (3)

* Values represent the mean of separate samples ± S.D. except for the 10-μg level of NTA where only two samples were analyzed; numbers in parentheses indicate the number of samples analyzed. All samples contained 10 μg of palmitic acid as an internal standard. GLC analyses were performed on the chloroform fraction eluted from silicic acid columns as described in the text.

three different concentrations of NTA, which represent levels of 0.05 to 0.20%, and two different concentrations of EDTA, 0.05 and 0.10%. The data show the method to be linear with an accuracy and reproducibility of less than 10% relative error at the level of 0.1% EDTA and NTA. Fig. 1 demonstrates a typical GLC tracing obtained with NTA and EDTA at concentrations of 0.10 and 0.05%, respectively, in the DTPA standard. All of the extraneous peaks were also found when GLC analysis was performed on blank samples that were run through the entire procedure. These extraneous peaks, therefore, are contaminants present in the solvents, glassware, and/or silicic acid used in the procedure and are not related to the DTPA sample analyzed.

Our results demonstrate that the procedure we developed for the determination of small amounts of EDTA and NTA (0.05%) in preparations of DTPA is

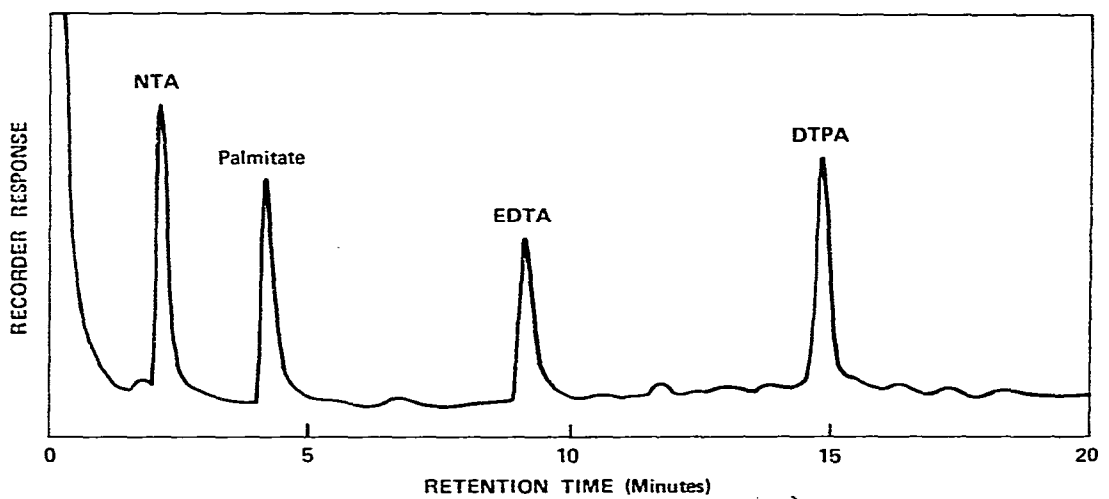

Fig. 1. A typical GLC separation using an aliquot of the chloroform fraction eluted by silicic acid column chromatography of the methyl esters from a sample containing 10 μg palmitic acid, 10 μg of EDTA, and 20 μg of NTA in 20 mg of DTPA. This aliquot had approximately 2 μg of pentamethyl-DTPA added to it before GLC for comparison of relative retention times.

quantitative, accurate, and reproducible. It would be possible, by scaling up our procedure, to determine even lower concentrations of NTA in DTPA if necessary, providing that the reagents used for silicic acid chromatography are of very high purity.

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