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Determination of ethylenediaminetetra-acetic acid in aqueous rinses of detergent-washed rubber stoppers of pharmaceutical vials using solid-phase extraction and capillary gas chromatography

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Abstract: A fused silica capillary gas chromatographic method is presented for the determination of traces of ethylenediaminetetra-acetic acid (EDTA) in aqueous rinses of rubber stoppers of pharmaceutical vials after treatment with detergents containing EDTA. Isolation and enrichment of EDTA from the aqueous medium is achieved using a commercially available strong anion-exchange solid-phase extraction cartridge, transformed to the formate form. A 2.0-ml volume of methanolic HCl is used for both elution of EDTA from the extraction column and formation of the tetramethyl ester derivative. With the incorporation of a methanolic wash to eliminate interfering components prior to elution with methanolic HCl, a limit of detection of 25 ng EDTA per ml water with a non-selective flame ionization detector is possible.

Keywords: *Ethylenediaminetetra-acetic acid; capillary GC; rubber stoppers of vials; solid-phase extraction; anion exchange.*

Introduction

The tetrasodium salt of ethylenediaminetetra-acetic acid (EDTA) is incorporated into detergents used in washing rubber stoppers used for bottling parenteral pharmaceutical preparations. The final cycle of the washing procedure includes thorough rinsing of the stoppers with water to remove traces of EDTA. A reliable and sensitive method was required to monitor the levels of EDTA in the water rinses.

A variety of analytical methods has been developed to determine EDTA in aqueous solutions, which depend upon spectrophotometry as the Co (II) or Cu (II) EDTA complexes [1, 2], potentiometry [3], liquid chromatography [4–6] and gas chromatography [7–11].

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Gas chromatography (GC) is generally used for determining trace quantities of EDTA in water samples because it provides the necessary sensitivity and specificity for differentiating EDTA from other aminocarboxylic acids. GC-based methods generally rely on filtration or extraction of interfering components and pre-concentration of the EDTA from the water matrix prior to derivatization of the acid as tetramethyl [9, 10, 12, 13], tetraethyl [11], tetrapropyl [14], tetrabutyl [7], and tetra-trimethylsilyl [8] esters. The derivatives are then analysed on packed columns with flame ionization detection (FID).

EDTA esterifies readily with straight-chain alcohols, which are normally selected on the basis of which ester can be chromatographed with the least interference on the packed column used. Methylation reagents, the easiest to remove prior to chromatography, can only be used with comparatively unadulterated water samples. Esterification of EDTA by silylation is difficult, especially where salts are present [7].

Most pre-concentration steps require evaporation of water, a cumbersomely slow process that limits the sample throughput especially where large volumes are necessary for trace analysis. Extraction into organic solvents is difficult because of the amphoteric nature of EDTA. Column extraction pre-concentration was reported using a quaternary ammonium chloride anion-exchange resin [11]. The method, although rapid, simple and selective, yielded low and variable recoveries.

The method developed for the determination of EDTA in aqueous rinses of detergent-washed rubber stoppers includes a purification–concentration step using a modified anion-exchange solid-phase extraction cartridge, a simple derivatization step to form the tetramethyl ester and finally high-resolution GC using a fused silica capillary column. The method is capable of detecting 25 parts per billion (25 ng/ml) EDTA in the water samples using a non-selective flame ionization detector.

Experimental

Reagents

Ethylenediaminetetra-acetic acid tetrasodium salt (anhydrous) was from MCB (S. Plainfield, NJ, USA). 1,6-Hexamethylenediaminetetra-acetic (HDTA) was purchased from Aldrich (Milwaukee, WI, USA). Baker analyzed HPLC water, methanol, *n*-hexane, and chloroform (J. T. Baker Chemical Co., Phillipsburg, NJ, USA) were used. Formic acid (about 88% m/m) was from Merck (Rahway, NJ, USA). 2.0 M formic acid was made by dilution of formic acid with HPLC grade water. Methylation reagent was purchased from Alltech-Applied Science (Deerfield, IL, USA); it comprised a bottle of anhydrous methanol and 5-ml ampoules of anhydrous acetyl chloride. The reagent (3% m/v HCl in methanol) was prepared by slowly reacting 5 ml of anhydrous acetyl chloride with about 75 ml of the methanol and diluting to 100 ml after cooling to room temperature. The reagent was kept in a refrigerator and a fresh batch was prepared weekly or as needed. Sodium hydroxide was A.C.S. grade from Fisher (Fair Lawn, NJ, USA). 0.5 M sodium hydroxide was made in HPLC water.

Extraction cartridges

Bond Elut® anion-exchange (SAX) cartridges from Analytichem International (Harbor City, CA, USA) were used for extraction. Each cartridge contained 500 mg of anion-exchange resin; the total reservoir volume was 3 ml. The SAX resin, a quaternary amine in the chloride form, was transformed to the formate form by percolating

successively through the column 2 × 3 ml (equivalent to 20–30 bed volumes) of 0.5 M sodium hydroxide, 2 × 3 ml of HPLC grade water, 2 × 3 ml of 2.0 M formic acid, and finally 2 × 3 ml of water. Cartridges were processed on a Vac-Elut® processing station (including a vacuum manifold) obtained from Analytichem International. To accommodate large volumes, the cartridges were fitted with 75-ml reservoirs.

Stock standard solution

The stock solution containing 0.50 mg/ml EDTA (0.65 mg/ml of the tetrasodium salt) in HPLC water was prepared in a glass-stoppered volumetric flask, which was then sealed with Parafilm and stored in a refrigerator.

Stock internal standard

The stock solution containing 0.50 mg/ml of HDTA was prepared in a glass-stoppered volumetric flask. 0.5 M sodium hydroxide was added dropwise to facilitate dissolution of the acid. The final pH of the solution was adjusted to 8–9 with 0.1 M HCl. The solution was stored in a refrigerator.

Preparation of spiked controls

Ordinary tap water or HPLC grade water in volumes of 25–200 ml were spiked with stock EDTA solution at various concentrations, typically 200 ng/ml.

Unextracted standards

The required μl-volume aliquots of the stock standards, typically 40 μl of EDTA stock solution and 40 μl of HDTA stock solution, were transferred to 2-ml vials and evaporated under nitrogen at 60°C. Chloroform was then added to remove residual water azeotropically by evaporation under nitrogen. The dried residue was kept in a sealed vial and stored in a refrigerator until derivatization.

Sample preparation

Samples were prepared by autoclaving rubber stoppers in a 1% (m/v) solution of rubber DET detergent. After autoclaving, the container was drained and the stoppers rinsed for 1 min with distilled water by the overflow method. After draining, more distilled water was added and the stoppers were autoclaved for 30 min to extract adsorbed EDTA. A second extraction was performed where high concentrations were found in the first rinse and for selected rubber stoppers that retain significant amounts of EDTA.

Enrichment and cleanup

To 25–100 ml samples and controls, according to the expected EDTA level, HDTA internal standard was added, typically 20 μg, and extracted with 5 ml of *n*-hexane to remove neutral contaminants; the hexane extract was discarded. The solutions were then adjusted to pH 3.0 with concentrated formic acid (about 1 μl of acid for each 2 ml of sample). The solutions were then passed through the prepared ion-exchange cartridges fitted with 75-ml reservoirs, at flow rates up to 5 ml/min, by applying a slight vacuum. The cartridges were then sequentially rinsed with 3 ml of water and 1 ml of methanol, with vacuum drying after each rinse. The cartridges were removed from the processing station, suspended in small test tubes and centrifuged for 5 min at 500 **g** to remove residual water from the cartridge packing. Then 2.0 ml of methanolic HCl (3% m/v) was

transferred by pipette into a cartridge and, with a syringe fitted to a cartridge adapter, passed through the cartridge into a 2-ml receiving vial. The contents were sealed in the vials with Teflon-lined aluminum crimp caps.

Derivatization

The extracts in the sealed vials were heated in a block for 60 min at 80°C. After cooling to room temperature, the reaction mixture was evaporated to complete dryness, under nitrogen, and then reconstituted with 50–1000 μl of chloroform, depending on the anticipated concentration. The same procedure was applied to the unextracted standard dissolved in 200 μl of derivatizing reagent.

GC

GC was performed with a Hewlett–Packard model 5890A GC (Hewlett–Packard, Avondale, PA, USA) equipped with a split/splitless capillary injector and FID. A fused silica capillary column (25 × 0.318 mm i.d.) coated with a bonded immobilized stationary phase (DB-17, film thickness about 0.25 μm) was employed (J & W Scientific, Harbor City, CA, USA). GC conditions were: helium carrier, 0.7 atm (10 psi); injector temperature, 275°C; oven temperature, 1 min at 150°C then increased at 25°C/min to 300°C and held for 5 min; detector temperature, 325°C; detector make-up gas, helium at 25 ml/min. Samples (1–3-μl aliquots) were injected using the splitless technique [15]. The carrier gas split vent (about 50 ml/min) was reopened 45 s after injection. The tetramethyl EDTA and tetramethyl HDTA were eluted at the maximum temperature with retention times of 7.7 and 9.7 min, respectively.

Results and Discussion

The extraction procedure uses a strong anion-exchanger, commercially available as a packed cartridge, after transforming it from the chloride form to the formate form. The elution behaviour of a transformed anion-exchange resin with a series of organic acids has been reported [16]. Unlike the methods reported for the determination of nitrilotriacetic acid (NTA) in environmental waters [17–19], where a large volume (10–50 ml) of formic acid was used, the present method utilizes a small volume of the derivatizing solution, methanolic HCl, for elution of the analyte and the internal standard. The volatile eluting solvent contributes to faster analysis with less chance of mechanical evaporative losses of the analyte. Potentially interfering neutral contaminants were eliminated by extraction into *n*-hexane prior to passing the solution through the anion-exchange column. The majority of the polar contaminants were removed from the column by the methanol wash prior to elution of EDTA with methanolic HCl. The components which were co-eluted with the analyte were resolved with high-resolution gas chromatography.

The degree to which the methanol pre-rinse reduced the interference is illustrated by a comparison of Figs 1 and 2; these chromatograms represent about 25 ng of EDTA injected, from a 50-ml water sample containing 100 ng/ml of EDTA, reconstituted with 200 μl of chloroform after extraction and derivatization. The particular water sample had a murky, grey appearance and yielded a black oily extract, the majority of which was eliminated with the methanol rinse. With the reduction of the interference, base line resolution of all compounds was achieved, resulting in a more sensitive assay. Without the methanol rinse, injections of severely contaminated extracts caused deterioration of

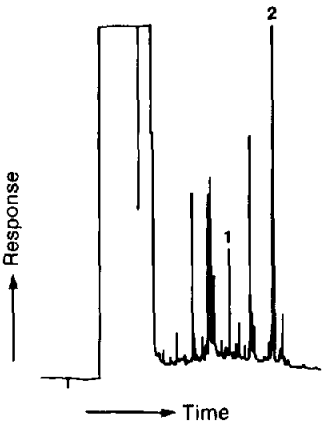

Figure 1
Chromatogram of sample extract, with methanolic HCl elution of the analyte and the internal standard from the extraction column performed prior to methanolic rinse of the column. Peak 1: tetramethyl EDTA, 7.7 min; Peak 2: tetramethyl HDTA, 9.7 min.

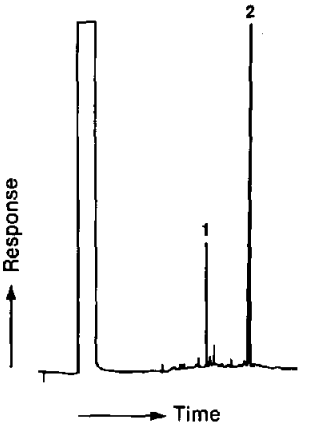

Figure 2
Chromatogram of sample extract, with methanolic HCl elution of the analyte and the internal standard from the extraction column performed after methanolic rinse of the column. Peaks 1 and 2: identified in Fig. 1.

the chromatographic response, as shown by drastic attenuation of the HDTA response and, to a lesser degree, the EDTA response. Remedial measures required to restore the chromatographic performance involved changing the injector insert, removing about 5 cm from the front portion of the capillary column and rinsing the capillary column with methanol or chloroform.

HDTA was selected as the internal standard primarily on the basis of its proximate retention time, 9.70 min, and its similar extraction and derivatization characteristics to those of EDTA. HDTA is not usually found in DET detergents or in process waters and it is free of EDTA. Another candidate, 1,2-propylenediaminetetra-acetic acid (PDTA), was not chromatographically resolved from EDTA. 1,2-Cyclohexylenediaminetetra-acetic acid (CDTA) and diethylenetriaminepenta-acetic acid (DTPA) exhibited poor recoveries and had long retention times relative to EDTA. DTPA also was prone to discrimination in the GC injector resulting in relative standard deviations of about 20% for multiple injections of a standard solution.

Normalizing EDTA to HDTA compensates for variations in sample volume and extraction or in derivatization efficiency. The use of an internal standard also performs a second function, which is to normalize the injection volume and hence reduce the

imprecision inherent in splitless injections. Because of the slow-flash vaporization technique, sample decomposition and pre-volitalization of the chloroform solvent from the needle cause irreproducible loss of the high boiling solutes, especially at the high injection temperature of this method. The relative standard deviations for five injections of a standard were 6.7% and 5.5% for EDTA and HDTA, respectively, but only 1.5% for the area ratio (EDTA/HDTA). When the GC system deteriorates, the absolute sensitivity of the internal standard is adversely affected and becomes unreliable for normalization. Rejuvenation of the chromatographic system, previously described, is then required. The HDTA response remained stable for approximately thirty injections before maintenance was required.

The effect of pH of control water on extraction recovery was negligible through the pH range of 3–7. Most metal–EDTA complexes are effectively decomposed at pH 3, liberating EDTA [2]. NTA, complexed with Ca, Mg, Fe, Zn, Cu, and Cd ions, was shown to exhibit higher recoveries by lowering the pH of water to 3–4 before column extraction on an anion-exchange resin [17]. Sample adjustment to pH 3 with formic acid was chosen to liberate EDTA from metal chelates that may have formed during the washing cycle of the rubber stoppers.

The effect of sample volume on EDTA recovery was studied to determine the breakthrough volume for the extraction column. The data in Table 1 represent the recoveries when volumes of 25–200 ml of HPLC grade water, spiked with two levels of EDTA, were extracted. The absolute recovery was determined by comparing the sample area of EDTA to that in an unextracted control. The final chloroform volumes were 50–400 μl to normalize all extracts to the same concentration of injection solution. The relative recoveries were determined by comparing EDTA/HDTA response in the sample to that in an unextracted control. As shown in Table 1, good relative recoveries were obtained with volumes up to 200 ml.

Table 1
Recovery of 25 and 500 ng/ml of EDTA from spiked HPLC water

Extracted (ml)	Recovery (%) EDTA = 25 ng/ml*		EDTA = 500 ng/ml†	
	Absolute	Relative	Absolute	Relative
25	65.5	87.5	98.8	93.6
50	70.1	92.5	96.1	96.8
100	72.5	94.2	90.1	96.2
150	50.8	84.2	70.6	90.1
200	32.3	90.0	49.0	87.3

*HDTA = 100 ng/ml.
†HDTA = 1000 ng/ml.

Table 2 shows the results of three recovery experiments. Tap water fortified with EDTA (25–500 ng/ml) showed mean recoveries of 92–101% with standard deviations of 6–9%. The tap water samples showed no interfering constituents and yielded satisfactory accuracy and precision.

Figure 3 shows a calibration graph obtained from 50 ml tap water controls spiked with 0, 25, 50, 100 and 200 ng/ml EDTA; each solution contained 200 ng/ml HDTA. The results of the linear regression analysis of area ratio versus amount ratio yielded an

Table 2
Recovery of EDTA from tap water

Sample	EDTA (ng/ml)* Added	Found†	Recovery (%)‡
High control	500	484.0 ± 29.5	97 ± 6
Medium control	100	100.8 ± 8.2	101 ± 8
Low control	25	23.1 ± 2.0	92 ± 9

*50-ml aliquots of tap water were spiked for extraction.
†Mean ± standard deviation; $n = 3$.
‡Mean ± standard deviation; $n = 3$.

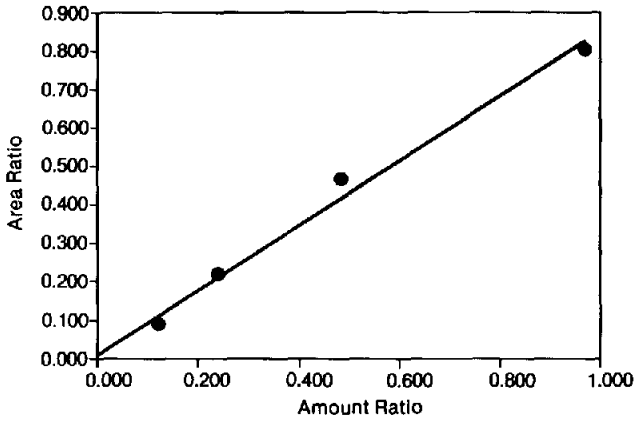

Figure 3
Graph of area ratio (area of EDTA divided by area of HDTA) versus amount ratio (ng of EDTA divided by ng of HDTA).

equation $y = 0.836x + 0.009$ ($r = 0.996$). Thus a high degree of linearity was attained between peak area ratio and amount ratio.

A practical limit of quantitation is determined by the sample complexity, extraction volume, and volume of methylated extract. A quantitation limit for EDTA of 25 ng/ml for a 50-ml sample was established for tap water, with a relative standard deviation of 9%.

Stopper-rinse samples obtained from the parenteral bottling facility were found to contain 0.0–3.4 μg/ml EDTA. On average, each 50-ml sample resulted from rinsing 10 stoppers, each weighing 2.38 g, and gave 0–17 μg EDTA per stopper or 0–7 μg EDTA per g of stopper.

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