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Determination of ethylenediaminetetraacetic acid in foods by reversed-phase high-performance liquid chromatography

Tomoko Kemmei [a,*], Shuji Kodama [a], Atsushi Yamamoto [b], Yoshinori Inoue [c], Kazuichi Hayakawa [d]

[a] Toyama Institute of Health, 17-1 Nakataikoyama, Imizu 939-0363, Japan
[b] Department of Biological Chemistry, College of Bioscience and Biotechnology, Chubu University, 1200 Matsumoto-cho, Kasugai 487-8501, Japan
[c] Products Planning & Development Dep. R&D Center, Nippon Filcon Co., LTD., 2220 Ohmaru, Inagi 206-8577, Japan
[d] Institute of Medical, Pharmaceutical and Health Sciences, Kanazawa University, Kakuma-machi, Kanazawa 920-1192, Japan

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ABSTRACT

A convenient HPLC method for the quantitative determination of EDTA in foods was developed. EDTA in food samples was easily extracted with water by ultrasonication. After converting to Fe(III) complex in the presence of Fe(III) ions, EDTA was separated on a reversed-phase C30 column and detected with ultraviolet detection (260 nm). Citrate and malate, which are present in many foods, also formed Fe(III) complexes but they did not interfere the chromatographic detection of EDTA. The method allowed determination of EDTA in foods at concentrations as low as 0.01 mmol/kg. Good recoveries (95.2–101%) were obtained by the standard addition method on four samples with high repeatability (RSD, 0.8–3.4%). The method was successfully applied to the analysis of EDTA in carbonated drinks, jellies, canned beans, canned corn and food supplements.

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1. Introduction

Because of its ability to form stable complexes with most metal ions, ethylenediaminetetraacetic acid (EDTA) is used as a synthetic chelating agent in various fields and applications (Frimmel, 1997; Nowack & VanBriesen, 2005). In foodstuffs, EDTA is frequently added as an antioxidant or a stabilizer of color and flavor (McCord & Kilara, 1983). Oxidation is the major cause of deterioration of foods. EDTA chelates the metal ions to prevent them from catalyzing oxidation reactions.

Various chromatographic methods are used to determine EDTA in different sample matrices (Sillanpaa & Sihvonen, 1997). In the case of foodstuffs, EDTA has been analyzed by gas chromatography (GC) after derivatization to its ester form (Retho & Diep, 1989; Williams, 1974). However, derivatization is time-consuming and often incomplete (Retho & Diep, 1989). EDTA has also been analyzed by reversed-phase high-performance liquid chromatography (HPLC) combined with ultraviolet detection (Jong, Polanen, & Driessen, 1991; Perfetti & Warner, 1979; Retho & Diep, 1989; Yabe, Tan, Ninomiya, & Okada, 1983). Sample preparation is simpler for HPLC than it is for GC, but for some foods, good recovery requires sample clean-up (Retho & Diep, 1989). Recently, ion chromatography with suppressed conductimetric detection (Krokidis, Megoulas, & Koupparis, 2005) and HPLC-post-column chemiluminescence detection (Perez-Ruiz, Martinez-Lozano, & Garcia, 2007) have been used to determine EDTA in food samples.

We previously reported an HPLC method for the simultaneously determination of several aminopolycarboxylic acids (APCAs) including EDTA in cosmetics and synthetic detergents (Kemmei, Kodama, Yamamoto, Inoue, & Hayakawa, 2007). APCAs in samples containing surface-active agents and many other matrices were successfully analyzed by using C30 columns and UV detection. In this study, we extended our previous method to determine EDTA in food samples, which have many potential interfering substances, including natural ingredients and additives. We investigated the effects of potential interfering substances such as organic acids and optimum analytical conditions. The method was subsequently used to determine EDTA in food samples and food supplements.

2. Experimental

2.1. Chemicals

Calcium disodium EDTA dihydrate (Ca-EDTA·$2H_2O$) was obtained from Dojindo (Kumamoto, Japan). Water was purified with an Milli-Q Direct 8 (Millipore SAS, Molsheim, France). Other chemicals (analytical grade) were purchased from Kanto (Tokyo, Japan).

* Corresponding author. Tel.: +81 766 56 8145; fax: +81 766 56 7326.
E-mail address: tomoko.kenmei@pref.toyama.lg.jp (T. Kemmei).

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2.2. Standard preparation and calibration

A stock standard solution of 1 mM Ca-EDTA was prepared by dissolving Ca-EDTA·$2H_2O$ in purified water. The stock solutions were stored at 4 °C and diluted before use.

EDTA was quantified by calibration curve ranging from 0.1 to 100 μM. A Fe(III) solution containing 10 mM Fe(III) chloride and 0.5 M sulfuric acid was prepared by dissolving 135 mg of Fe(III) chloride hexahydrate in 25 ml of 1 M sulfuric acid and making up to 50 ml with purified water. Unless stated otherwise, 50 μl of the Fe(III) solution was added to 5 ml of diluted standard solutions prior to injection. By adding Fe(III) solution, the concentrations of Fe(III) chloride and sulfuric acid in the injection solution were the same as those in the mobile phase and good resolution was achieved from system peaks.

2.3. Food sample pretreatment

Eight food samples (two carbonated drinks, two jellies, three canned beans and a canned corn), on whose containers EDTA was indicated as ingredients, were purchased from a local market. Three food samples (a carbonated drink, a jelly, canned beans), on whose containers EDTA was not indicated as ingredients, were also purchased.

Food samples were homogenized before weighing. Carbonated drinks were degassed under ultrasonication, jellies were minced with a kitchen knife and canned beans and canned corn were drained, dried on a paper towel and ground. One gram of the sample was added to 30 ml water. The mixture of carbonated drink and water was ultrasonicated for 5 min and the mixtures of other food samples and water were ultrasonicated for 20 min. Ultrasonic cleaner model US-2R of 80 W and 40 kHz (As one, Osaka, Japan) was employed for all ultrasonications. Then the aqueous extract was adjusted the volume to 100 ml with water and was filtered through a 0.45 μm Minisart RC 15 (Sartorius, Goettingen, Germany). Aqueous extracts of canned beans and canned corn were centrifuged at 3000 rpm for 10 min before filtration. Prior to injection, 50 μl of the Fe(III) solution was added to 5 ml of filtrated solutions.

2.4. Supplement sample pretreatment

Five supplement samples were purchased through the Internet. Four supplements were advertised as chelation products. Another supplement was advertised to take R-lipoic acid.

The contents of five capsules of each supplement were finely ground. One hundred milligrams of the sample was added to 30 ml water. After being ultrasonicated for 20 min, the aqueous extract was adjusted the volume to 100 ml with water and was filtered through a 0.45 μm Minisart RC 15. Prior to injection, 50 or 500 μl of filtrated solution was diluted to 5 ml and to this diluted solution was added 50 μl of the Fe(III) solution.

2.5. Apparatus and chromatographic conditions for analysis of EDTA

The HPLC system consisted of a Tosoh (Tokyo, Japan) CCPD pump, a Rheodyne (Cotati, CA, USA) manual injector, a Shimadzu (Kyoto, Japan) SPD-10AV UV detector, a Shimadzu CTO-10AC column oven, and a Shodex (Tokyo, Japan) DEGAS degasser.

Separations by HPLC were attained with a 4.6 mm i.d. × 250 mm Develosil RPAQUEOUS column (NOMURA CHEMICAL, Seto, Japan) and thermostated at 40 °C. The column was made of silica gel bonded with triacontyl (C30) group and packed with 5 μm particles. The mobile phase, unless stated otherwise, was the mixture of 100 μM Fe(III) chloride and 5 mM sulfuric acid (pH 2.0), and the flow rate was 0.8 ml/min. The injection volume was 10 μl and the detector wavelength was set at 260 nm. Quantification was based on peak areas.

3. Results and discussion

3.1. Separation of EDTA from potential interfering substances

In our previous study (Kemmei et al., 2007), by adding Fe(III) ions to the mobile phase, seven APCAs including EDTA were transformed into Fe(III) complexes during passing through an HPLC system and were successfully separated. In the HPLC analysis, two reversed-phase C30 columns connected in series were used with the mobile phase, 5 mM sulfuric acid containing 100 μM Fe(III) chloride (pH 2.0), and the column oven was set at 50 °C. In this study, EDTA had to be separated from potential interfering substances, such as organic acids, which are commonly coexisting in foods and can be formed Fe(III) complexes. It was examined whether four organic acids (citrate, malate, succinate and tartarate) affected the chromatographic behavior of EDTA. No peak was observed for succinate and tartarate. On the other hand, peaks were observed for citrate and malate. Particularly the peak of malate appeared almost at the same time as EDTA. In order to separate the peaks of EDTA and malate, the effect of column oven temperature was investigated by using a single column (Fig. 1). As the temperature of column oven is lowered, each retention time of EDTA, malate and citrate was delayed. At 40 °C, the peaks of EDTA and malate were fully separated. At 30 °C, the peak shape of citrate was pretty broad. Therefore, unless stated otherwise, one reversed-phase C30 column was used and the column oven was set at 40 °C for further experiments.

When the mobile phase did not contain Fe(III) ions, no peaks were observed for citrate or malate. The chromatographic separation of EDTA by using with the mobile phase containing Fe(III) ions made it possible to quantify citrate and malate together with EDTA. On the other hand, when the mobile phase did not contain Fe(III) ions, the peak of ascorbate was observed near the EDTA peak. However, when the mobile phase contained Fe(III) ions, ascorbate reacted with Fe(III) ions and the ascorbate peak disappeared.

Nutrient supplements contain a variety of vitamins, minerals and amino acids. Aspartate and orotate were listed as ingredients on the product labels of food supplements we purchased. No peak was observed for aspartate but the peak of orotate appeared very sharply behind the peak of citrate.

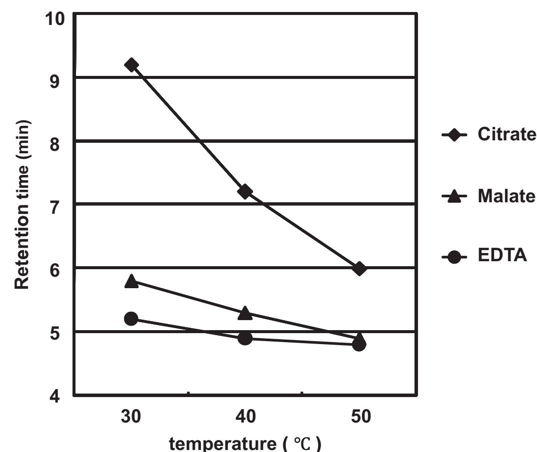

Fig. 1. Relationship between the temperature of column oven and retention time of 0.01 mM EDTA, 1 mM malate and 0.1 mM citrate. A Develosil RPAQUEOUS column was used with 5 mM sulfuric acid containing 100 μM Fe(III) chloride (pH 2.0) as the mobile phase.

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