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

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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.

Keywords:
EDTA
Reversed-phase HPLC
Food additives
UV detection

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 & Van Briesen, 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·2H2O) 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).

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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·2H₂O 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 carbonateddrink 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 to the volume of 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 5000 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 to the volume of 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 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 CHEMI-CAL, 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 ACPAs 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.](image-url)
Usage of EDTA is strictly regulated by the US Food and Drug Administration and related agencies. Although EDTA is used as a food additive in both calcium disodium salt and disodium salt forms, the levels prescribed are calculated as anhydrous calcium disodium EDTA. Most disodium EDTA added to foods reacts with a large amount of Ca ions that are present in foods to form Ca-EDTA complexes (Yamaguchi, Yamaguchi, Shiroishi, Shimizu, & Takasugi, 1985). So a stock standard solution was prepared by dissolving Ca-EDTA/C$_2$H$_2$O in this work. A linear calibration curve ($r^2 > 0.999$) was obtained using ten dilutions of stock standard solution from 0.1 to 100 μM. Three consecutive determinations for 0.1 μM standard solution gave a high repeatability (RSD 5.7%). Based on a signal-to-noise ratio of 10, the limit of quantification (LOQ) of EDTA was determined to be 0.1 μM (Kemmei et al., 2007).

3.2. Analysis of EDTA in canned foods and supplements

EDTA must be extracted from food samples prior to chromatographic analysis. Previous studies used the solvent extraction method coupled with heating and/or agitation (Jong et al., 1991; Krokidis et al., 2005; Perez-Ruiz et al., 2007; Perfetti & Warner, 1979; Retho & Diep, 1989; Williams, 1974; Yabe et al., 1983). Ultrasonic extraction works well for analytes in solid matrices (Palma & Barroso, 2002; Zuo, Zhang, Wu, Fritz, Medeiros, & Rego, 2004). In the present study, solid food samples were sonicated in 30 ml purified water for 20 min and liquid food samples were sonicated for 5 min. The sonication was evaluated by measuring the recovery of EDTA from four spiked samples. A carbonated drink, a jelly, and canned beans, whose EDTA contents were <0.01 mmol/kg, were spiked with 0.1 mmol/kg and the supplement, whose EDTA content was <0.001 mmol/g, was spiked with 0.01 mmol/g. The mean recoveries (n = 5) ranged from 95.2% to 101%, and the repeatabilities of the measurements were high (RSD, 0.8–3.4%) (Table 1).

In solvent extraction, when the food sample is large compared to the volume of water, the aqueous extract is too thick to directly use for chromatographic analysis (Retho & Diep, 1989). In the present study, food samples were diluted 100 times with water, so that the aqueous extract could be subjected to HPLC analysis immediately after filtration. The canned beans and canned corn suspensions were centrifuged before filtration because of their high solids content.

### Table 1
Recoveries of EDTA from samples (n = 5).

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Fortification level</th>
<th>Recovery (%)</th>
<th>RSD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonated drink</td>
<td>0.1 mmol/kg</td>
<td>95.4</td>
<td>2.0</td>
</tr>
<tr>
<td>Jelly</td>
<td>0.1 mmol/kg</td>
<td>95.2</td>
<td>3.4</td>
</tr>
<tr>
<td>Canned beans</td>
<td>0.1 mmol/kg</td>
<td>101</td>
<td>1.0</td>
</tr>
<tr>
<td>Supplement</td>
<td>0.01 mmol/g</td>
<td>99.4</td>
<td>0.8</td>
</tr>
</tbody>
</table>

### Table 2
Concentrations of EDTA in food samples.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Product</th>
<th>EDTA mmol/kg</th>
<th>Citrate mmol/kg</th>
<th>Malate mmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonated</td>
<td>Soda</td>
<td>0.075</td>
<td>0.028</td>
<td>6.6</td>
</tr>
<tr>
<td>drink</td>
<td>Ginger ale</td>
<td>0.052</td>
<td>0.019</td>
<td>8.1</td>
</tr>
<tr>
<td>Jelly</td>
<td>Ai yu jelly</td>
<td>0.44</td>
<td>0.16</td>
<td>1.5</td>
</tr>
<tr>
<td>Canned beans</td>
<td>Garbanzo beans</td>
<td>0.32</td>
<td>0.12</td>
<td>5.0</td>
</tr>
<tr>
<td>Kidney beans</td>
<td>Navy beans</td>
<td>0.18</td>
<td>0.067</td>
<td>9.4</td>
</tr>
<tr>
<td>Canned corn</td>
<td>Young corn</td>
<td>0.067</td>
<td>0.025</td>
<td>1.7</td>
</tr>
</tbody>
</table>

* Calculated as CaNa$_2$EDTA.

* Not determined (<0.02 mmol/kg).

### Table 3
Concentrations of EDTA in supplements.

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>EDTA mmol/g</th>
<th>Orotate mg/g</th>
<th>Malate mmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6</td>
<td>601</td>
<td>n.d.</td>
</tr>
<tr>
<td>2</td>
<td>2.2</td>
<td>835</td>
<td>n.d.</td>
</tr>
<tr>
<td>3</td>
<td>0.48</td>
<td>179</td>
<td>0.68</td>
</tr>
<tr>
<td>4</td>
<td>0.44</td>
<td>165</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

* Calculated as CaNa$_2$EDTA.

* Not determined (<0.02 mmol/g).

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Fig. 2. Chromatograms of (a) a carbonated drink (ginger ale) and (b) canned beans (kidney beans). The concentrations of the EDTA, citrate and malate are shown in Table 2.

Fig. 3. Chromatogram of supplement no. 3. The concentrations of the EDTA and orotate are shown in Table 3.
EDTA was determined in eight food samples, with values between 0.052 and 0.44 mmol/kg (Table 2). These levels were below the maximum concentrations allowed in soft drinks and canned foods in Japan (0.035 and 0.2 g Ca-EDTA/kg, respectively). Citrate was detected in all food samples and malate was detected in one carbonated drink. Representative chromatograms for a carbonated drink (ginger ale) and canned beans (kidney beans) are shown in Fig. 2(a) and (b), respectively.

Of the four supplements examined (Table 3), EDTA was determined in the range from 0.44 to 2.2 mmol/g. For supplement no. 3, which contained orotate according to the label, orotate concentration was found to be 0.68 mmol/g (Fig. 3).

4. Conclusions

A simple and reliable HPLC method for the determination of EDTA in foods was developed. The use of EDTA as a food additive is regulated in many countries, so a method to measure its concentration is needed. After ultrasonic extraction with water, EDTA contents in foods were satisfactorily determined by using a conventional isocratic HPLC system with a UV detector. Commonly coexisting substances in foods, such as citrate and malate, were not only separated from EDTA but also quantified simultaneously. The proposed method gave excellent results in the analysis of EDTA in various types of foods and food supplements.

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References


