Determination of EDTA in Water Samples by SPE-Gas Chromatography/Mass Spectrometry

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ABSTRACT

Japan's recommended method of EDTA determination is complex and time-consuming. In this study, a new method to prepare the solution to determine EDTA in water by solid-phase extraction-GC/MS was developed. Recovery yields were excellent with values ranging from 98.1 to 100.5%. Due to this method's ease and simplicity, it is suggested that this approach be adopted as Japan's recommended method for EDTA analysis. The method was applied to assess the concentrations of EDTA in river water from three regions of Japan. Median concentration of EDTA in river water samples was 115 µg/L, and the concentrations ranged from 18.8 to 443 µg/L. The highest concentration of EDTA (443 µg/L) was observed in Tsurumi River. Sewage treatment plant (STP) effluent significantly contributed to high EDTA levels.

Keywords: EDTA, GC/MS, river water, solid-phase extraction.

INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA) is one of the most widely employed aminopolycarboxylic acids with uses in the pharmaceutical, food, personal care product, and agricultural industries. Because of its widespread use, high water solubility, and low biodegradability in the environment, EDTA has emerged as a persistent organic pollutant in the aquatic environment. EDTA has been detected at µg/L level in various anthropogenically influenced waters (including surface water and sewage water) in many studies worldwide (Kari and Giger, 1995; Knepper *et al.*, 2005). Thus, control of EDTA in drinking water is critical.

In Japan's drinking water quality control, standards are categorized into three groups. The first group is composed of legally binding standards (Drinking Water Quality Standards; 50 items). The second group consists of non-legally binding standards (Complementary parameters to set the target quality management; 27 items (128 compounds)). The third group contains 44 items which require further studies for risk assessment; EDTA is presently categorized into the third group and the standard value is 0.5mg/L. The standard analytical methods for these items are legally bound and the Japanese Standard Methods for the Examination of Water are established for the analysis of second and third group items, including EDTA analysis. In these standard methods, EDTA concentration is determined by the evaporation of water, methyl ester derivatization, dichloromethane extraction, and then gas chromatography/mass spectrometry. However, sample preparation is complex and time-consuming, because this method includes evaporation of water sample from 100 mL to ca. 2 mL using rotary evaporator and derivatization processes (1 h heat treatment), and these processes take a long time. In this study, we investigated how to improve the analytical procedure. Various analytical methods have been reported using high performance liquid

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chromatography (Nowack *et al.*, 1996; Kemmei *et al.*, 2009), ion chromatography/mass spectrometry (Bauer *et al.*, 1999; Knepper *et al.*, 2005), gas chromatography/mass spectrometry (Nishikawa and Okumura, 1995), and liquid chromatography/tandem spectrometry (Quintana and Reemtsma, 2007). To simplify Japan's recommended method of EDTA determination by using gas chromatography-mass spectrometry, we focused on the pretreatment of EDTA by using solid-phase extraction in the present study. In comparison with evaporation using rotary evaporator, solid-phase extraction is simple, rapid, and efficient.

MATERIALS AND METHODS

Chemicals

Ethylenediaminetetraacetic acid, disodium salt, dehydrate and trans-1, 2-cyclohexanediaminetetraacetic acid monohydrate (CyDTA) were obtained from Dojindo (Kumamoto, Japan) and Strem Chemicals, Inc. (Newburyport, MA, USA), respectively. Boron trifluoride methanol complex methanol solution, formic acid, potassium dihydrogenphosphate, sodium sulfate, sodium hydroxide, L(+)-ascorbic acid, methanol and dichloromethane were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). All chemicals and solutions were of analytical grade. Milli-Q water was used in all experiments.

Gas chromatography/mass spectrometry analysis

Gas chromatography was carried out by using HP6890 series gas chromatography system (Hewlett Packard, Wilmington, DE, USA) with an HP6890 series auto sampler and split/splitless injector. The analytical column was a DB-5 fused-silica capillary column, 30 m × 0.25 mm i.d., 0.25 µm film thickness (J & W Scientific, Folsom, CA, USA). The temperature program for the column oven was 70°C as initial temperature for 2 min; ramped at 15°C/min to 300°C then held at 300°C for 3 min. The carrier gas (helium) flow rate was set at 1.2 mL/min.

Mass spectrometry was carried out using a 5973 Mass Selective Detector (Hewlett Packard, Wilmington, DE, USA) in electron-ionization mode with an ionization voltage of 70 eV and ion source temperature of 230°C. The instrument was operated in selected-ion monitoring (SIM) mode. The monitor ion of EDTA and CyDTA were m/z of 174 (for identification : m/z = 289, 348) and 402, respectively. CyDTA was used as an internal standard.

Sample collection

River water samples used in this study were collected from six rivers located in the Shikoku region (Kochi and Tokushima prefectures), Kansai region (Hyogo and Kyoto prefectures), and Kanto region (Kanagawa Prefecture and Tokyo Metropolis) (Fig. 1). River water sampling was conducted in January (for Shikoku region and Hyogo Prefecture) and April (Kyoto Prefecture and Kanto region) 2010. River water samples were collected in 300 mL glass bottles and stored in the dark at 4°C until analysis.

Standard and sample preparation

A standard stock solution of EDTA was prepared by dissolving 0.127 g of ethylenediaminetetraacetic acid, disodium salt, dehydrate in 1 L Milli-Q water. On the

Fig. 1 - River water sampling locations

other hand, a standard stock solution of CyDTA was prepared by dissolving 0.01 g of trans-1, 2-cyclohexanediaminetetraacetic in 100 mL of 1 M sodium hydroxide.

Extraction of EDTA from water samples was performed according to the method of Knepper *et al.* (2005) with modifications. Water samples (100 mL each) were adjusted to pH 3 by 16 M formic acid and filtered with glass fiber filter (0.7 μm , Millipore, Billerica, MA, USA). Bond Elut Jr. -SAX (500 mg, Varian, Inc., Palo Alto, CA, USA) cartridges were conditioned with 3 mL methanol and 3 mL of Milli-Q water, respectively. Extraction of water samples was carried out at a flow rate of 10 mL/min. After extraction, cartridges were rinsed with 3 mL of Milli-Q water and eluted into stoppered glass test tubes with 3 mL of 16 M formic acid. The eluates combined with internal standard (CyDTA) were concentrated in a nitrogen stream at 80°C to complete dryness. After exsiccation, 1 mL of boron trifluoride-methanol-complex solution was added to the glass test tubes. Derivatization was carried out by 1 h heat treatment at 80°C using a water bath. After derivatization, 3 mL of 1 M phosphate buffer (pH 7) and 1 mL of dichloromethane were added to the test tubes and rigorously shaken. Thereafter, test tubes were centrifuged for 5 min at 900 g. After centrifugation, the layer of dichloromethane was collected in a glass tube and dehydrated by sodium sulfate. The dehydrated dichloromethane solution was used for analysis. The limit of quantification (LOQ) was determined by analyzing the lowest level standard at least 5 times. The LOQ was calculated as 10-fold the standard deviation of these determinations. The LOQ value was 0.1 $\mu\text{g/L}$ in sample water.

RESULTS AND DISCUSSION

Recovery experiments

To evaluate the efficiency, solid-phase extraction was performed as described below.

The EDTA standard solution was added to 100 mL Milli-Q water and tap water at concentrations of 0.1 mg/L (1/5 of EDTA standard value in drinking water) and 0.01 mg/L (1/50 of EDTA standard value in drinking water) and subsequent extraction and derivatization were carried out as described previously. Tap water samples were dechlorinated by L(+)-ascorbic acid before use. Control samples for recovery test by using Milli-Q water were prepared by adding the same amount of EDTA standard solutions to 3 mL Milli-Q water. In the case of using tap water for the recovery test, control samples were prepared by adding the same amount of EDTA standard solution to the eluate from tap water extraction. All controls, samples, and blanks were determined in triplicate. Recovery percentages of EDTA from Milli-Q and tap water samples are shown in Table 1. In the case of Milli-Q water samples, excellent recovery percentages at each concentration were obtained, and the values were 100.5% (concentration: 0.01 mg/L) and 100.2% (concentration: 0.1 mg/L). Moreover, the relative standard deviation (RSD) of the ratio of EDTA to CyDTA in each sample was within 5% (ranging from 0.3 to 4.2%) and the variability among samples was small. No influence of matrix from the SPE cartridge was observed in blank samples.

In order to apply the proposed method to actual tap water samples, a recovery test was performed using tap water (Table 1). Satisfactory results were obtained from recovery tests using tap water samples as well as those of Milli-Q water. Variability between samples was small and the pretreatment process of our method was simple and took a relatively short time compared with the existing Japanese Standard Methods for the Examination of Water. We therefore suggest that the SPE-derivatization-GC/MS method should be considered for the Japanese Standard Methods for the Examination of Water as an EDTA analytical method. The next stage to add this new SPE-derivatization-GC/MS method to the Japanese Standard Methods for the Examination of Water would be to perform an inter-laboratory validation study of the proposed method.

Determination of EDTA in river water samples

For the application of this analytical method to environmental water samples, concentrations of EDTA in river water samples from urban and rural areas of Japan were investigated using SPE-derivatization-GC/MS. River water samples were taken from three regions of Japan (Fig 1). As shown in Fig 2, EDTA was detected in ten of thirteen river water samples. Although EDTA was not detected from river water samples of Kagami, Yoshino, and Kamo rivers (concentration: < 0.1 µg/L), EDTA was detected at comparatively high concentration in other river water samples. The median concentration of EDTA in river water samples was 115 µg/L and the concentrations detected ranged from 18.8 to 443 µg/L.

In the Kanto region, the highest concentration of EDTA (443 µg/L) was observed in a

Table 1 – Recovery of EDTA from Milli-Q water and tap water samples

Analyte	Vehicle	Concentration (mg/L)	Recovery of triplicate samples (%)			Mean	SD
			A	B	C		
EDTA	Milli-Q water	0.01	100.1	100.9	100.6	100.5	0.4
	Milli-Q water	0.1	100.0	99.8	100.8	100.2	0.5
	Tap water	0.01	102.0	98.7	99.8	100.1	1.7
	Tap water	0.1	98.3	98.9	97.2	98.1	0.9

Fig.2 - Concentration of EDTA in river water samples from three regions of Japan

river water sample collected at Ochiai Bridge of Tsurumi River. It is generally considered that effluent from sewage treatment plants (STP) is one of the major sources of EDTA (Kari and Giger, 1996). This sampling site is located at about 150 m downstream from the effluent output of an STP and the contribution of effluent to the EDTA concentration is high. In Tsurumi River, there are three STPs near the sampling sites. Two STPs are located upstream of Ochiai Bridge and between Kamenokou Bridge and Nippa Bridge, respectively. Moreover an STP is located at Yagami River (tributary of Tsurumi River) between Otsuna Bridge and Takano-ohhashi Bridge. The river water sample taken from Nippa Bridge contained high levels of EDTA. The EDTA concentration of the river water sample taken from Takano-ohhashi bridge was lower than those of other river water samples collected downstream of STPs. Because the STP is located on a tributary of Tsurumi River, the EDTA concentration might be affected by dilution with the influent of Yagami River at this sampling site. The low EDTA concentration in the river water sample from Tsurumi Bridge (downstream of Tsurumi River) might also be caused by dilution. EDTA concentrations of Tama River samples ranged from 18.8 to 56.8 µg/L and the values were comparatively lower than those from Tsurumi River. These sampling sites were located at lower-middle and lower portions of Tama River while the STPs are located at the upper-middle portion of Tama River. Therefore, it was concluded that concentrations of EDTA decreased going downstream due to dilution. In Kansai region, EDTA was only detected in the river water sample of Muko River and the value (196 µg/L) was comparable to that of Tsurumi River. There is an STP in the upper part of Muko River and EDTA may originate from that source. However, because there is no STP upstream of the sampling site of Kamo River, EDTA was not detected in Kamo River sample. On the other hand, in Shikoku region, EDTA was not observed in the river water samples. The concentration of EDTA might be low due to these samples being collected in estuarine regions. In further studies, it will be necessary to survey the differences in EDTA contamination levels between urban and rural areas.

The results of the present study were compared to those of the previous studies. It was estimated that EDTA concentrations of 50-500 µg/L are present in wastewaters (WHO, 1998). According to Quintana and Reemtsma (2007), mean EDTA concentrations in various water samples from Germany, such as STP influent and effluent, tap water, and surface water, ranged from 1.1 (surface water) to 35 (STP effluent) µg/L. Knepper *et al.* (2005) reported EDTA concentrations ranging from 4 to 970 µg/L in wastewaters and concentrations ranging from 1 to 33 µg/L in surface water of European countries. Our results are comparable to those of wastewater in previous studies. These results suggested that Kanto region rivers, especially Tsurumi River, might be highly affected by STP effluents. It is necessary to further evaluate the contribution of STP effluents to EDTA levels in rivers.

CONCLUSIONS

To improve Japan's recommended method of EDTA determination to a simplified method by using gas chromatography/mass spectrometry, a new solid-phase extraction method of EDTA was developed. Satisfactory results were obtained in recovery tests using both tap water and Milli-Q water samples. Because the evaporation procedure was changed to solid-phase extraction, the analytical method used in this study is simple and rapid compared with Japan's existing standard method and is reasonable to recommend as an EDTA analytical method to the Japanese Standard Methods for the Examination of Water. Occurrence of EDTA in river water samples from three regions of Japan was examined. EDTA was detected in ten of thirteen river water samples. A high level of EDTA was observed in river water samples with significant STP effluents. Further evaluation of the contribution of STP effluents to EDTA levels in rivers is needed.

REFERENCES

- Bauer K. H., Knepper T. P., Maes A., Schatz V. and Voihsel M. (1999). Analysis of polar organic micropollutants in water with ion chromatography - electrospray mass spectrometry, *J. Chromatogr. A*, **837**, 117-128.
- Kari F. G. and Giger W. (1995). Modeling the photochemical degradation of ethylenediaminetetraacetate in the River Glatt, *Environ. Sci. Technol.*, **29**(11), 2814-2827.
- Kari F. G. and Giger W. (1996). Speciation and fate of ethylenediaminetetraacetate (EDTA) in municipal wastewater treatment, *Water Res.*, **30**, 122-134.
- Kemmei T., Kodama S., Muramoto T., Fujishima H., Yamamoto A., Inoue Y. and Hayakawa K. (2009). Study of solidphase extraction for the determination of sequestering agents in river water by high-performance liquid chromatography, *J. Chromatogr. A*, **1216**, 1109-1114.
- Knepper T. P., Werner A. and Bogenschütz G. (2005). Determination of synthetic chelating agents in surface and waste water by ion chromatography-mass spectrometry, *J. Chromatogr. A*, **1085**, 240-246.
- Nishikawa Y. and Okumura T. (1995). Determination of nitrilotriacetic acid and ethylenediaminetetraacetic acid in environmental samples as their methyl ester derivatives by gas chromatography-mass spectrometry, *J. Chromatogr. A*, **690**, 109-118.

- Nowack B., Kari F. G., Hilger S. U. and Sigg L. (1996). Determination of dissolved and adsorbed EDTA species in water and sediments by HPLC, *Anal. Chem.*, **68**, 561-566.
- Quintana J. B. and Reemtsma T. (2007). Rapid and sensitive determination of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid in water samples by ion-pair reversed-phase liquid chromatography-electrospray tandem mass spectrometry, *J. Chromatogr. A*, **1145**, 110-117.
- WHO (1998). Edetic acid (EDTA) in Drinking-water, Background document for development of WHO Guideline for Drinking-water Quality, 2nd ed. Addendum to vol. 2. Health criteria and other supporting information, World Health Organization, Geneva.