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Determination of ethylenediaminetetraacetic acid (EDTA) levels in surface waters by high performance liquid chromatography (HPLC)-Ultraviolet/Visible (UV/VIS) detector

Oltan Canlı[1]*, Barış Güzel[1], Kartal Çetintürk[2]

[1] Water Management and Treatment Technologies Research Group, Climate Change and Sustainability Vice Presidency, TUBITAK Marmara Research Center, 41470, Kocaeli, Türkiye
[2] İstanbul University, Institute of Marine Science and Management, 34470, İstanbul, Türkiye

Abstract

Ethylenediaminetetraacetic acid (EDTA), a chemical harmful to human health with its high solubility in water, is used as a metal chelating agent in various sectors. Thus, it is necessary to be monitored in surface waters taken from dams supplying drinking and utility water. This work presents the applicability of the HPLC-UV/VIS system for the quantification of EDTA in surface waters based on the limit values of national and international legislations. The applicability of EDTA quantification in surface water was checked with validation study. The method validation consisted of selectivity, calibration curve linearity, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery), and precision. The linearity of EDTA was obtained ranging from 10 µg/L to 200 µg/L concentrations with the correlation coefficient of 0.9985 and the calibration curve equation of $y = 4659.4x - 50223$. The LOD and LOQ values of EDTA were 2.85 µg/L and 9.51 µg/L with the RSD of 5.36. In accuracy, the mean recovery of EDTA in surface water has been determined as 87.51 percent with an RSD of 6.11. The repeatability (RSD, %) varied from 5.44% to 7.02% with concentrations of 35.19 ± 1.91 µg/L and 17.11 ± 1.20 µg/L, whereas the reproducibility (RSD, %) was obtained at 3.45% with the concentration of 34.13 ± 1.18 µg/L. In this study, the presence of EDTA was investigated in approximately 300 surface water samples and EDTA was found as positive in the concentration range of 11.17 µg/L to 52.14 µg/L in eleven real samples.

Keywords: EDTA, HPLC, pollution, water

1. Introduction

Ethylenediaminetetraacetic acid (EDTA) is an aminopolycarboxylic acid with multiple bonded –COOH groups and $-NH_2$ bonded in its structure (Fig. 1). It is a white, water-soluble solid commonly used to bind acid, iron, and calcium ions. Most of the EDTA available in general is in the form of the free acid and disodium salt. The free acid form is insoluble in water. Depending on the pH value, metals form stable complexes with EDTA. It is in the form of chelating agents in the pharmaceutical, food, personal care product, agricultural industries, and household [1,2]. The polarity, chelating ability towards metal ions, high solubility, and low biodegradability properties of EDTA in water allow it to be identified as a persistent organic pollutant in the aquatic environment [3,4]. The use and sustainable management of land and water resources have been made important with increasing environmental awareness recently. The widespread use and diversification of EDTA usage in industrial areas along with the developing technology have brought about an increase in environmental concerns. Therefore, the monitoring of EDTA presence especially in the environment is critical for human health and the environment [5].

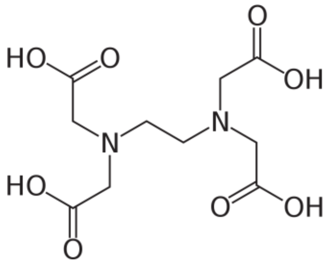

Figure 1. The chemical structure of EDTA

Its physical and chemical properties can lead to spread easily on the environment and may have carcinogenic,

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***Author of correspondence:** oltan.canli@tubitak.gov.tr
Tel: +90 (262) 677 2922
Fax +90 (262) 641 2309

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mutagenic, and toxic effects on humans and aquatic organisms [2]. Thus, the release of EDTA has been monitored and controlled in the world continuously with national and international lists and regulations. In this context, there is national and international legislation such as the Turkish Regulation on the Management of Surface Water Quality [6] and the EU Water Framework Directive (2008/105/EC on Environmental Quality Standards) [7] for monitoring EDTA concentration in surface waters. According to Table 4 of the Annexes of the Surface Water Quality Regulation in Turkey, the environmental quality standard limit for EDTA in lakes, rivers, and coastal and transitional waters is 39 μg/L.

From the past to the present, there are many studies that reported the determination of EDTA in water samples in different analytical methods and systems such as HPLC [8], ion chromatography/mass spectrometry (IC/MS) [4,9], gas chromatography/mass spectrometry (GC/MS) [1,2,10], and liquid chromatography/tandem spectrometry (LC-MS/MS) [11]. The aim of the current work was to investigate the applicability of the HPLC-UV/VIS system for the quantification of EDTA in surface waters based on the limit values of national and international legislation such as the Turkish Regulation on the Management of Surface Water Quality. In the literature, studies conducted on samples prepared with the use of solvents in GC/MS are contrary to the "Green Chemistry Approach", which is based on the protection of human and environmental health. In this regard, this study is important in terms of obtaining fast and precise EDTA results and savings on time and solvent cost.

2. Materials and methods

2.1. Reagents and chemicals

Ethylenediaminetetraacetic acid disodium salt dehydrate was purchased from Sigma-Aldrich (St. Louis Missouri, USA). HPLC grade methanol was supplied from Merck (Darmstadt, Germany). The purification of water used to prepare the solutions was done with a Milli-Q Plus system (EMD Millipore, Billerica, MA). To draw the calibration graphs, calibration solutions were prepared from 10 mg/L concentrations of stock standard solution, respectively. They were preserved in a freezer (-20 °C) at 1.5 mL vials and were prepared once a month again.

2.2. HPLC-UV/VIS analytical condition

All the measurements were performed on an HPLC-UV/VIS instrument consisting of a binary pump (Shimadzu LC-10AD HPLC Binary Pump model), Shimadzu automatic injector (SIL-10AF model), and a column oven (CTO-10AS model). Shimadzu UV/VIS detector (SPD-10AV model, Light source: D2 deuterium lamp) was used. Column possessing Symmetry C8 150 mm × 3.9 mm id, 5-μm particle size (Waters Technologies, Ireland) as an analytical column was used. The HPLC method used gradient mobile phases containing LC grade distilled water (mobile phase A) and methanol (mobile phase B). The column temperature was set at 25 °C with a flow rate of 0.3 mL/min. The gradient profile was programmed as follows: 0 – 2 min 20% B; 2 – 9 50% B; hold at 20% B 1 min (total run time 10 min). The injection volume was 100 μL. Data acquisition and processing were done with Shimadzu LabSolutions/LCsolution GPC software version 2.1.

2.3. Surface water samples and sample preparation

Surface water samples were collected from five lakes including Naipköy lake (Tekirdağ), Alibey lake (İstanbul), Omerli lake (İstanbul), Elmalı lake (İstanbul) and Sapanca lake (Sakarya) on January 4–18, 2021. They were fully filled in 500 mL glass bottles and were preserved in pursuant to International Organization for Standardization (ISO) standard [12]. The stability of samples was provided by storing below 5 °C. All samples were analyzed by HPLC within 36 hours of reaching the laboratory. Their pH values changed from 7.28 to 7.68.

The preparation of water samples for measurement in HPLC does not contain solvent and consists of a single step. All the samples were taken into a vial by filtering through a 0.22 μm filter.

2.4. Analytical performance of HPLC-UV/VIS method

The applicability of EDTA quantification in surface water was checked with validation study consisting of selectivity, calibration curve linearity, limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery), and precision according to the EURACHEM Guideline [13] and Guidelines for Standard Method Performance Requirements [14].

3. Results and discussions

3.1. Selectivity, linearity and sensitivity (LOD and LOQ)

The selectivity study of EDTA was done with the investigation of blank samples consisting of only ultrapure (deionized organic-free) water. As a result of eight measurements sequentially, no peaks belonging to interfering compounds that would cause false-positive results were found in the chromatograms at the retention time of EDTA (5.773 min.) (Fig. 2).

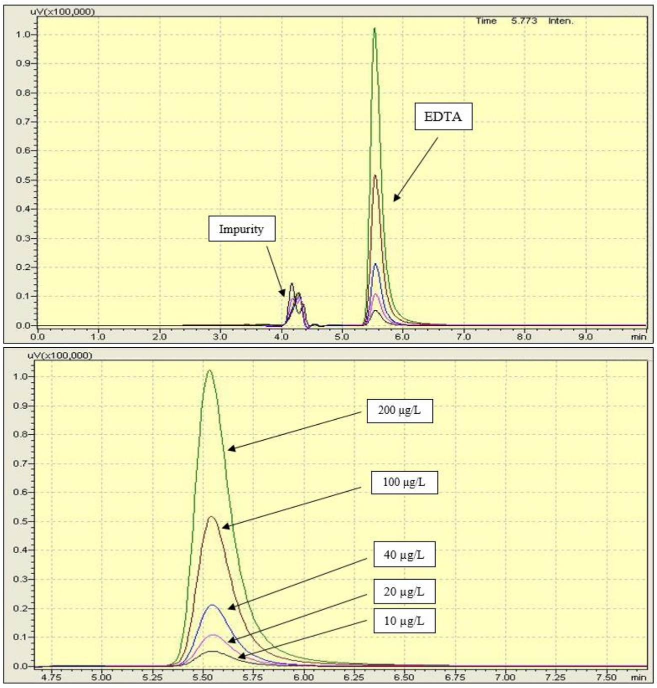

Figure 2. The view of the concentration levels of EDTA in the chromatogram of HPLC-UV/VIS

The linearity of EDTA was obtained ranging from 10 µg/L to 200 µg/L concentrations at five concentration levels prepared by using the necessary amount of stock standard solution in 10 mL of ultra-deionized water (Fig. 2). In the validation study, acceptable linearity of any substance is linear regression with a correlation coefficient better than 0.99 [15–18]. Fig. 3 shows that the correlation coefficient of EDTA with the calibration curve equation of $y = 4659.4x - 50223$ was 0.9985 which defines as excellent in pursuance of the correlation coefficient.

The measurement sensitivity (LOD and LOQ) study of EDTA in HPLC-UV/VIS was done by seven measurements in the solutions prepared by spiking stock standard solution of EDTA to ultra-deionized pure water to have a concentration of 20 µg/L. The calculations of LOD and LOQ were actualized by multiplying the average noise value obtained from the chromatogram by three and ten, respectively. As can be seen in Table 1, the LOD and LOQ values of EDTA were 2.85 µg/L and 9.51 µg/L with the relative standard deviation (RSD) of 5.36. Also, LOD and LOQ values of this method are similar and comparable with the results of Kuran and co-workers [2].

3.2. Accuracy and precision

The reliability of the HPLC-UV/VIS method was checked with accuracy, which is defined as the recovery results of the concentration of 20 µg/L in seven measurements, and precision (repeatability and reproducibility studies). The repeatability was made at two concentrations of 20 µg/L and 40 µg/L and the reproducibility was only done at the concentration of 40 µg/L in six measurements.

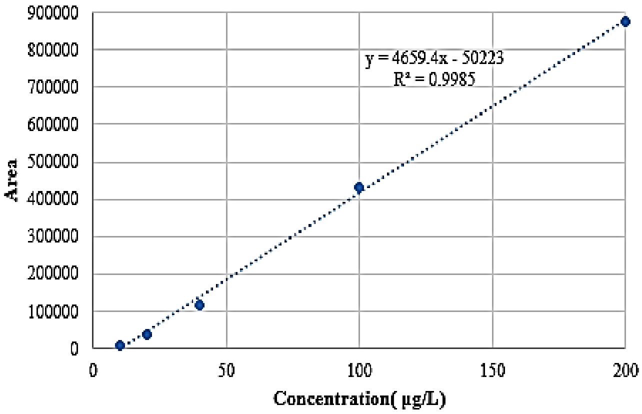

Figure 3. The linearity of EDTA from 10 µg/L to 200 µg/L concentrations

The studied concentrations were prepared by spiking the surface water samples. In Table 1, the mean recovery of EDTA in surface water has been determined as 87.51 percent with an RSD of 6.11%. The repeatability (RSD, %) varied from 5.44% to 7.02% with concentrations of 35.19 ± 1.91 µg/L and 17.11 ± 1.20 µg/L, whereas the reproducibility (RSD, %) was obtained at 3.45% with the concentration of 34.13 ± 1.18 µg/L. As seen in Table 1, the accuracy and precision results of the study show that the method meets the requirements for the rapid and accurate determination of EDTA in surface waters according to the validation guidelines for Standard Method Performance Requirements [14].

Table 1. LOD-LOQ, accuracy, and precision (repeatability and reproducibility) of EDTA

Analytical performance/compound			EDTA
LOD-LOQ	**LOD (µg/L)**		2.85
	LOQ (µg/L)		9.51
	RSD (n=7 %)		5.36
Accuracy	**Recovery (%)**		87.51
	RSD (%)		6.11
Repeatability	**20 µg/L**		17.11 ± 1.20
	RSD (%)		7.02
	40 µg/L		35.19 ± 1.91
	RSD (%)		5.44
Reproducibility	**Day 1**	**Area 1**	211788
		Area 2	217604
		Area 3	218364
		Area 4	209254
		Area 5	197523
		Area 6	195509
		Mean	208340
	Day 2	**Area 1**	196714
		Area 2	200018
		Area 3	201487
		Area 4	198455
		Area 5	201114
		Area 6	209525
		Mean	201219
	40 µg/L		34.13 ± 1.18
	RSD (%)		3.45

3.3. Real samples

In this study, the presence of EDTA was investigated in approximately three hundred surface water samples taken from the dams mentioned in Section 2.3. As a result of the analysis of the relevant samples, the presence and amount of EDTA were determined according to the areas of the standards included in the calibration and the retention time in the chromatogram. EDTA was found as positive in the concentration range of 11.17 ± 0.28 µg/L to 52.14 ± 0.21 µg/L in eleven real samples. In Table 4 of Annexes to the Surface Water Quality Regulation in TURKEY, 39 µg/L EDTA concentration in lakes, rivers, coastal and transitional waters is the environmental quality standard limit. This limit value was exceeded in two of the samples obtained with a positive EDTA result.

4. Conclusions

This paper was presented rapid, simple (solvent-free pre-treatment) and reliable HPLC-UV/VIS analytical method for the determination of presence of EDTA in surface waters. Applicability of the related method in the analysis of EDTA in surface waters was checked with the parameters of selectivity, calibration curve linearity, the limit of detection (LOD) and limit of quantification (LOQ), accuracy (recovery), and precision according to the related documents/guidelines [13,14]. EDTA ranging from 10 µg/L to 200 µg/L concentrations at five concentration levels had excellent linearity with a correlation coefficient of 0.9985. The LOD and LOQ values of EDTA were 2.85 µg/L and 9.51 µg/L with the RSD of 5.36. The related method was applied to approximately three hundred surface water samples and was obtained successful results.

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