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Review on Determination of EDTA in Various Products and Water by Different Analytical Methods

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Patel Ankita*, Patel Alisha

Department of Pharmaceutical Quality Assurance
ROFEL Shri G.M. Bilakhia College of pharmacy, Vapi, Gujarat, India- 396191.

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ABSTRACT

EDTA is a chelating agent which is used in industry for the (clean in place) CIP process and is also used in pharmaceutical formulation and personal care and hygiene products (shampoos, liquid soaps, creams, and lotions). EDTA was determined by different analytical methods like RP-HPLC, UV Spectroscopy, Ion Chromatography and Gas Chromatography. In the RP-HPLC method determination of EDTA in dairy wastewater and surface water was done using the C18 column as stationary phase and methanol as mobile phase, at a flow rate of 0.9 mL min^{-1} with a UV detection at 265 nm. A simple, sensitive, cost-effective and reproducible UV-Spectroscopy method has been developed and validated for the estimation of Disodium edetate in topical gel formulations showing EDTA absorbed at 270nm. The ion chromatographic method was applied for the assay of EDTA in contact lens care solutions anion exchange column (Dionex AS-14, 4 mm × 250 mm eluents are 10 mM carbonate buffer and flow rate of 1.0 ml min^{-1} with the conductometric detector. The gas chromatography method was applied for analysing three different river glasses of water from regions of Japan to determine the concentrations of EDTA, water samples were 115 µg/l, and the concentrations ranged from 18.8 to 44.3 µg/l. The highest concentration of EDTA (44.3 µg/l) was observed in the Tsurumi River.

INTRODUCTION

Ethylene diamine tetraacetic acid (EDTA) is a chelating agent that can bind to metal ion Structure shown in Fig no.1. It is an amino polycarboxylic acid, white, odourless, nonhygroscopic crystalline powder. It is widely used in medicine, chemical industry, food technology, agriculture and pharmaceutical technology. (1)

EDTA use in a cleaning solution is to prevent the precipitation of calcium, magnesium and heavy metal salts, which can cause deposits to appear on both the cleaning equipment and the plant to be cleaned. EDTA is the cheapest and most suitable complexing compound for many technical purposes and is used in large quantities. In the dairy industry, EDTA has been used as a cleaning additive to improve the cleansing efficiency during the clean–in–place (CIP) procedure.

The chemical characteristics of ETDA, particularly its polarity, relatively strong water solubility, and chelating capabilities, make it difficult to remove EDTA from typical water treatment plants. As a result of the interaction of these factors, EDTA appears in surface water as an anthropogenic chemical at high concentration levels.

Toxic Effects of Chemical Substance In pharmaceutical formulations, it enhances the action of preservatives and antibacterial and stabilizes the action of antioxidants. In foods, EDTA is added to prevent deteriorative changes, and to preserve colour, odour and flavour (e.g., canned or pickled vegetables, canned mushrooms, mayonnaise and salad dressings).

EDTA is unsafe to use in human health for more than 3grams per day or to take longer than 5 to 7 days. It can cause abdominal cramps, nausea, vomiting, skin problem, fever, and diarrhoea too much can cause kidney damage and dangerously low calcium levels in the blood. (2)

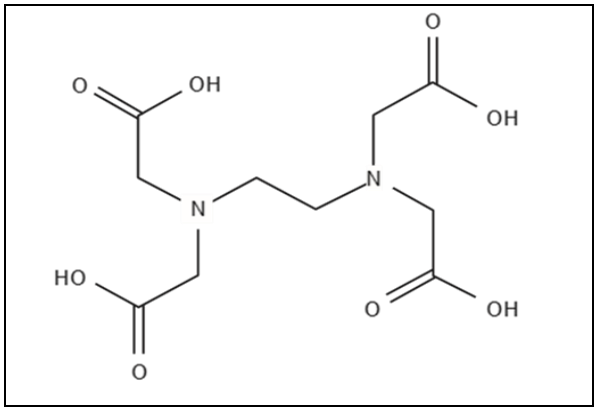

Fig. No. 1: Structure of EDTA

EDTA had been used extensively in medicine as a chelating agent for the removal of toxic heavy metals. It is also used in several personal cares and hygiene products (shampoos, liquid soaps, creams, and lotions). In food additives in a range of products, including canned shrimp and prawns, canned mushrooms, and frozen French fries. It is also used in many industrial processes, agriculture, photochemical, pharmaceuticals, textiles, galvanizing and paper manufacturing.

When EDTA enters the aquatic environment, its speciation is measured by the characteristics of the water and the presence of trace metals with which it can combine. The fate and behaviour of the various complexes may differ significantly. Because it is photoactive, the Fe3+ EDTA complex is the most labile. For the photolysis of Fe3+ EDTA dissolved in water and exposed to sunlight compared to the yearly maximum intensity, a half-life of 11 minutes was estimated.

After discharge to the environment, the Fe3+ EDTA will gradually exchange with other trace metals, a process that will be affected by the pH of the water, as each trace metal has an optimum pH for chelation. Other EDTA metal complexes are much more persistent and do not biodegrade quickly in the aquatic environment. Deterioration has been reported in soil–water systems, however, the extent of degradation varies with soil type and exposure time. Biodegradation in sewage treatment plants can only remove a small amount of EDTA from municipal wastewater. Adsorption on sludge allows for only a limited removal. Similarly, EDTA removal by various drinking-water treatment technologies, such as activated charcoal filtration, is low. The most effective elimination is by ozonation. Heavy metals are transported by EDTA, which has been a source of worry in the environment. However, based on stoichiometry, 40 g of EDTA per litre (the greatest concentration reported in the Rhine and

Meuse rivers) would only complex 4–15 g of metals per litre, and thus would result in a metal concentration of 4–15 g per litre. Only in terms of cadmium is it likely to cause difficulties for drinking water. Another modifying element is that the influence on cadmium leaching will be limited since at these concentrations, EDTA is largely linked to other metals. If EDTA is not present in stoichiometric excess, it will be linked mostly with calcium in the majority of fresh waters. Competition from hydrogen ions for available ligands becomes more important in fluids with a pH of less than 6.0. Most EDTA's uses will result in its release into the aquatic environment. It has been estimated that concentrations of 50–500 µg/litter are present in wastewater.

Annual average concentrations of EDTA in European surface waters ranged between >1 and >60 µg/litter, and a concentration of 900 µg/litre was found in the Zerkaa River in Jordan (van Dijk-Looyard *et al.*, 1990). Measured concentrations in natural waters were also reported to range from 10 to70µg/litre, with a median value of 23µg/litre (Frank & Rau, 1990). Mean EDTA concentrations at 45 different sampling points on 29 different rivers of Germany in 1993 ranged between almost 50 µg/litre (Lippe River at Wesel) and a few µg/litters, with most annual mean values being between 5 and 15 µg/litre (EFA-Germany, 1995). EDTA has also been detected in surface waters and drinking water prepared from surface waters at concentrations of 10–30 µg/litre (van Dijk-Looyard *et al*., 1990). [3]

- Determination Of EDTA By Different Analytical Methods:

1. RP-HPLC (Reverse -phase high-performance liquid chromatography)
2. UV- Spectroscopy
3. Ion chromatography
4. Gas chromatography

1. RP- HPLC (Reverse- phase high performance liquid chromatography): [4,5,6,7]

RP-HPLC method using determination of levels of EDTA in dairy effluent water. In this method, a C18 reversed-phase (RP) column and UV detector has been developed. The chromatographic separation was optimized by compositions of the mobile phase and flow rate. The interfering compounds of calcium/magnesium and nitrates at levels occurring in dairy wastewaters were investigated, and the accuracy and precision of the method were determined. The analysing of dairy wastewater and surface water samples. Analysed results

demonstrate levels of EDTA in dairy effluents from a large dairy site. Contents of EDTA in the adjacent river showed the change in EDTA concentrations due to the dairy effluent discharge.

Instrumental condition:

The analytical separation was carried out on HPLC (Shimadzu LC 10 -AT VP with UV detector at 265 nm. the analytical column Hypersil C18 RP column of length 200mm, a diameter of 4.6mm and a particle size of 5µm. The HPLC recording and integration software was Power Chrom attached to a Power lab/8sp.

Chemical and reagent:

- Formic acid buffer solution (pH 3.3): dissolved 0.17 gm sodium formate and 0.33ml 90% formic acid in 1L of H_2O.
- Ion pair reagent solution: dissolved 4.836 gm of tetra n-butyl ammonium bromide in 1L OF pH = 3.3 buffer solution.
- EDTA standard solution: dissolved 0.1462 gm ethylene diamine tetraacetic acid iron sodium salt in 1L of water.
- Fe3+ solution: dissolved 2.4203gm $FeCl_3.6H_2O$ and 0.144ml HCl in 500ml H_2O.

Sample collection: Dairy wastewater was collected at various sites. 24-hour composite flow-proportion. The sample includes Plant processing wastewater, wastewater treatment processing samples and dairy effluents. Sample preparation: Sample of dairy effluent taking 1-5 ml equal volume of Fe3+ solution and leaving them overnight to allow complexing of Fe (III) EDTA than filter through 0.45µm cellulose nitrate filters (Phenomenex), and injecting 50 µL sample into the HPLC or to perform derivatization process was carried out by transferring 10 mL of the standard stock solution into 100 mL volumetric flask. Add 5 mL of Ferric chloride solution Shake well and keep in a water bath at 70 °C for 20 minutes, finally make-up the volume with diluent after it attains room temperature. Dilute the solution with diluent to achieve the final concentration of 0.0002 mg/mL.

RESULTS AND DISCUSSION:

In reverse phase separation, the sample retention can be controlled by varying the solvent strength of a mobile phase. % MeOH shortened the retention time buffer solution with 2%

MeOH selected for the experiment. Methanol rather than ACN was chosen because of its low toxicity and low cost.

The addition of the ion pair reagent to the mobile phase can improve peck shape and large changes in separation. Ion pair reagent tetrabutylammonium (TAB), tetrabutylammonium bromide (TBABr), and tetrabutylammonium hydrogen sulphate are used as TBA+ positively charged on its nitrogen and completed with anions. The various concentration of TBABr in the mobile phase was studied and the result was observed.

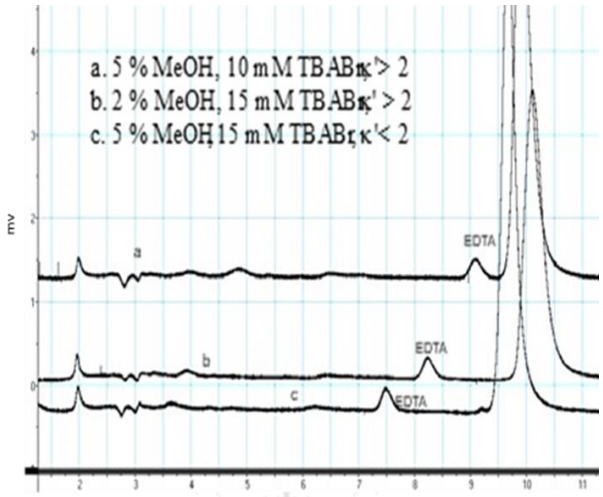

Fig. No. 2: Optimization of chromatographic separation of dairy wastewater

Method Validation:

The developed analytical method for the determination of EDTA in dairy wastewater was validated for its performance characteristics such as Accuracy, Precision, Recovery, and Repeatability.

Accuracy:

The accuracy is determined by recovery studies. Accuracy was evaluated by spiking the samples. 100µg L-1 of EDTA was added dairy wastewater sample. The recovery found 98% to 102 %.

Precision:

The precision of the method was determined to analyze each sample 12 times. The repeatability given as the relative standard deviation was less than 1.5%.

Table No.1: Repeatability test result of Dairy wastewater analysis:

Tests	Sample 1	Sample 2
1	107.2	1792.4
2	107.9	1759.3
3	107.1	1758.9
4	107.5	1780.3
5	106.2	1756.3
6	107.7	1737.5
7	104.7	180.1
8	107.0	1757.0
9	103.0	1753.6
10	106.7	1765.7
11	107.0	1766.8
12	103.0	1759.3
Average	106.7	1765.2
STDEV	1.43	17.97
%RSD	1.34	1.01

2. UV- SPECTROSCOPY METHOD: [8,9,10,11]

The UV-Spectroscopy method is simple sensitive, cost-effective reproducible it has been developed and validated for the estimation of disodium edetate in topical gel formulations. Solution of disodium edetate reacts with ferric chloride to complex form and absorbed at 270nm. Spectrophotometry has been well accepted for quantitative analysis (Itabashi *et al*., 1992; Hamano *et al*., 1993; Cacace *et al*., 2007). It is a fast, easy and low-cost process with high precision and sensitivity, though rarely used for EDTA determination. Itabashi *et al.* (1992) conducted redox reactions of Cu2+ and Fe2+ in the presence of EDTA and diethylene triamine pentaacetic acid concentration of EDTA was indirectly determined through spectrophotometric absorbance of the complex formed between Cu+ and the chelating agent. Hamano *et al.* (1993) also reported a protocol for EDTA determination, in which EDTA was first complexed with excessive Fe3+ followed by removal of Fe3+ in excess by a chelating extraction using N-benzoyl-N-phenylhydroxylamine and chloroform.

The ferric ions in Fe3+-EDTA were reduced to ferrous ions using ascorbic acid as the reducer and subsequently chelated with 4,7-diphenyl-l, l0-phenanthroline-disulfonic acid, leading to the formation of a chromophore. A novel and sensitive spectrophotometric protocol for EDTA determination method.

EDTA was first chelated with excessive Fe3+; Fe3+ ions in excess were reduced to Fe2+ using sodium sulphate. The resulting ferrous ions were immediately chelated in situ with l, l0-phenanthroline monohydrate (PTM) without the need for any extraction or separation, leading to the formation of a chromophore, ferroin, a colour developing reagent. Spectrophotometric absorbance of the chromophore was determined. EDTA concentration was obtained therefrom. The reliability and precision of the method were checked through a process of EDTA oxidation degradation by ozone.

Instrument:

UV-VLS Spectrophotometric

Shimadzu model 1601

Detector – (DAD) diode array detector (190-1100 nm)

Material and method:

Ethylene diamine tetraacetic acid (EDTA), sodium sulphate (Na_2SO_3), ammonium ferric sulphate (AFS, $(NH_4)_2SO_4Fe_2(SO_4)_324H_2O$) and hydroxylamine hydrochloride ($HONH_3Cl$), l, l0-Phenanthroline monohydrate (PTM), Hydrochloric acid (36%) and sodium acetate (NaAc).

RESULTS AND DISCUSSION:

Accuracy:

Accuracy is determined by ٪ recovery studies. The accuracy was evaluated by preparing samples at different levels of drug concentrations of 20, 25,30 µg/ml. ٪ recovery studies were found in the range of 98.48- 100.16. The excellent mean ٪ recovery values are close to 100٪.

Table No. 2: Accuracy data of disodium EDTA per cent in pharmaceutical product

Level(µg/ml)	Range (µg/ml)	Mean (±S. D)	R. S. D (%)	Mean % Recovery (±S. D)	Accuracy (%)
20	19.78-20.34	19.97(±0.23)	1.15	99.89(±1.15)	-0.11
25	24.36-24.92	24.62(±0.21)	0.85	98.48(±1.04)	-1.15
30	29.83-30.29	30.05(±0.17)	0.56	100.16(±0.84)	0.16

Table No. 3: Accuracy data of Standard addition of disodium edetate in the formulation

The drug in formulation (µg/ml)	Pure drug added (µg/ml)	Total drug found (µg/ml) (±S. D)	Mean % Recovery (±S. D)	Accuracy (%)
25	0	24.65(±0.17)	-	-
25	12.5	37.15(±0.26)	99.06(±1.26)	-0.93
25	25	49.65(±0.23)	99.3(±1.15)	-0.7
25	37.5	62.15(±0.20)	99.44(±0.99)	-0.56

Precision:

Precision was determined by studying the repeatability and intermediate precision. Repeatability was found in a range of 19.98 – 29.92 µg/ml. In an intermediate precision study %, the RSD value was found to be less than 2%. The RSD values were found to be within the acceptable range indicating that the proposed method has excellent repeatabilities and intermediate precision.

Table No. 4: Precision data for the development method

Concentration (µg/ml)	Intermediate precision (µg/ml) (% RSD)			Repeatability (µg/ml) (% RSD)
	Day 1	Day 2	Day 3	
20	19.93(0.96)	19.86(1.12)	20.05(1.07)	19.96 (1.18)
25	24.19(0.83)	24.83(0.88)	24.95(0.79)	24.65 (0.84)
30	29.89(0.56)	30.14(0.63	29.74(0.55)	29.92 0.61)

Linearity:

The solution was prepared with different concentrations of disodium edetate solution 5 to 50 µg/ml from the stock solution. The linearity evaluated by plotting the calibration curve was concentration versus absorbance. The regression equation was originated y = 0.0191x – 0.0013. The Linear calibration curve with correlation coefficient (r2) >0.9997 was obtained and proved the linearity of the method.

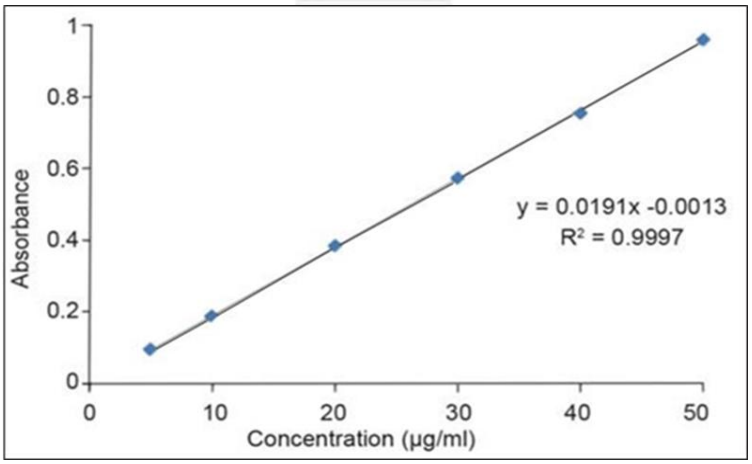

Fig. No. 3: Calibration curve of disodium EDTA

Limit of detection (LOD) and Limit of quantification (LOQ):

The limit of detection and limit of quantification of the calibration curve was calculated which was based on the standard deviation of the y-intercept of the regression line and the slope of the calibration curve at the levels approximating the LOD and LOQ was found to 1.190 and 3.608µg/ml respectively from topical gel sample.

3. ION CHROMATOGRAPHY [12,13,14,15]

The ion chromatography method effect EDTA and various substances, such as inorganic and organic ions (Fe3+, Cu2+, Ca2+, Cl−, S2O52−, ascorbate, citrate), similar amino polycarboxylic acids with complexing properties (EGTA, NTA, DTPA) and pharmaceutical substances. The ion chromatographic method with suppressed conductimetric detection for the direct (without derivatization step) determination of EDTA was developed and validated. The method was used for the determination of EDTA in pharmaceutical formulations such as contact lens care solutions and synthetic injections and canned food (mushrooms and mayonnaise).

METHOD AND MATERIAL:

Instrumentation: DX-100 high pressure one piston pump, a sample injector equipped with a 25 µl loop, an Ion Pac AG14 guard (4 mm × 50 mm) and an AS-14 analytical column (4 mm × 250 mm) an ASRS-I micro-membrane suppressor operating in the auto suppression recycle mode (selectable current intensity 50–500 mA), and a conductimetric detector equipped with a thermistor for the compensation of temperature variations. The chromatographic peaks were electronically integrated and recorded by the recorder.

Reagent: Injection drug solution, water, EDTA, NaCl.

Preparation of standard solutions and stock solutions:

The stock solution was prepared by dissolving the appropriate amount of EDTA disodium salt in water and stored in a plastic bottle and refrigerated condition (at 40C). Working standard solutions (in the range of 2.7–100 µg ml−1) were daily prepared by appropriate dilution with the mobile phase.

Sample preparations:

Liquid samples such as contact lens care solutions, injection drug solutions and surrounding liquid of canned mushrooms were diluted with the eluent, filtered and injected into the chromatographic system. Mayonnaise samples for removal of the fatty substance were mixed with water, mildly heated, filtered and extracted with benzene.

In the case of the coexistence of organic drugs that are adsorbed on the polymeric column (e.g., paracetamol), removal of the drug molecules by using liquid-liquid extraction with ethyl acetate is required before injection of the chromatograph a periodical washing of the

analytical column with acetonitrile is required in case coexistence of surfactants (contact lens care solutions) are present.

Mobile phase phases:

The selected mobile was prepared by dissolving sodium hydrogen carbonate in water and adjusting the pH with a concentrated sodium hydroxide solution. They were stored in the refrigerator in a plastic bottle and renewed every 2 weeks.

Other examined mobile phases were: (i) 20–32mm borate aqueous solutions ($Na_2B_4O_7 \cdot 10H_2O$) without adjustment pH, (ii) 28–150 mM borate buffers of pH 8.5–9.5(H_3BO_3 + NaOH), and (iii) 7–20 mM carbonate buffers of pH 9.5 to 11.

Table No. 5: Selection of Mobile phase

Mobile phase	**t_R (min)**	**Correlation coefficient**	**% RSD**
Carbonate buffer			
8mm, pH10.5	9.4	-	-
10mm, pH10.5	6.3	0.998	3.0
10mm, pH11.0	5.5	0.998	1.5
Borate buffer H_3BO_3 + NaOH, pH8.5			
112Mm	17.0	0.997	1.5
120Mm	16.2	0.993	1.5
140Mm	12.5	0.999	0.7
150Mm	11.8	0.996	2.6

RESULTS AND DISCUSSION:

Selection of mobile phase:

EDTA (H4Y) is a weak tetraprotic carboxylic acid. Five EDTA species (H4Y, H3Y−, H2Y2−, HY3− and Y4−) dissociation constants: K1 = 1.02 × 10−2, K2 = 2.14 × 10−3, K3 = 6.92 × 10−7 and K4 = 5.50 × 10−11) coexist in aqueous solution and their concentration distribution depend on the pH of their mobile phase. The electric charge of EDTA species strongly influences its selectivity coefficient (retention) but not the conductimetric detector response factor, since electrochemical suppression occurred.

Separation from potential interfering substances:

Interferences from substances usually coexisting with EDTA in foods and formulations, using the carbonate eluent. It was examined, whether ascorbate and citrate, common preservatives for canned foods, affect the chromatographic behaviour of EDTA. The applied mobile phase was the10 mM carbonate buffer (pH = 10.5). No interference was observed for both molecules, since the retention time of citrate was 52.1 min, while EDTA resolution from ascorbate was Rs = 1.2.

Table No. 6: Recovery of EDTA from canned food and pharmaceutical formulation

Sample type	**EDTA found (%w/v) (n=5)**	**Concentration (µg/ml^{-1})**	**% Recovery**	**% Mean recovery (± S.D)**
Contact lens care solution (Allergan)	0.56±0.007	10	105	99(±4.3)
		30	95	
		50	99	
		70	97	
Contact lens care solution (Bausch Lomb)	0.087±0.002	20	96	99(±4.7)
		30	102	
		50	98	
		70	101	
Canned mushrooms	0.095±0.002	10	74	79(±4.7)
		30	77	
		50	85	
		70	80	
Synthetic drug injection (containing Nacl 0.9% and EDTA 100µg ml^{-1})	-	-	-	97-108

4. Gas chromatography: [16,17,18,19]

Gas chromatography-mass spectrometric (GC-MS) method for EDTA and other low molecular weight organic compounds in the waste water. A multistage extraction procedure was used to isolate a wide range of compounds from municipal wastewater. Gas chromatography requires the volatility of the compound to be determined, so EDTA is most commonly converted into its methyl, ethyl, propyl, or butyl ester. Methyl esterification is the most commonly used method of derivatization. Sialylation has also been reported, but salts can interfere in seawater. sample preparation is complex and time-consuming, because this method includes evaporation of water samples from 100 mL to ca. 2 mL using rotary evaporator and derivatization processes (1 h heat treatment), and these processes take a long time.

MATERIALS AND METHOD:

Instruments:

Gas chromatography was carried out by using the HP6890 series gas chromatography system (Hewlett Packard, Wilmington, DE, USA) with an HP6890 series autosampler and split/spitless 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 70ºC as the initial temperature for 2 min; ramped at 15ºC/min to 300ºC then held at 300ºC 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 230ºC. 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.

Chemicals:

Ethylene diamine tetraacetic acid, disodium salt, dehydrate and trans-1, 2- cyclohexane diamine tetraacetic 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 dihydrogen phosphate, sodium sulphate, 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.

Standard and sample preparation:

A standard stock solution of EDTA was prepared by dissolving 0.127 g of ethylene diamine tetraacetic acid, and disodium salt, dehydrate in 1 L of water.

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.

Sample pre-treatment:

Activated carbon cartridges were conditioned with 20 mL of 1 M hydrochloric acid. A seawater sample (100 mL) was mixed with 1 mL of 1 mM Fe (III) chloride and 1 mL of 1 M hydrochloric acid. After standing for 15 min, the mixture was passed through the cartridge at a flow rate of 10 mL min−1 using a Sep-Pak® Concentrator Plus (Waters, Milford, MA, USA) water sample preparation system. The cartridge was then sequentially rinsed with 30 mL of purified water and 10 mL of methanol. After the cartridge was dried under suction for 15 min, Fe (III)–EDTA retained on the cartridge was eluted with 30 mL of 0.1 M HCl–methanol. The eluate was evaporated to dryness and the residue was dissolved in 1 ml of the mobile phase.

Derivatization was carried out by 1 h heat treatment at 80ºC using a water bath. After derivatization, 3 mL of 1 M phosphate buffer and 1mL 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 sulphate. The dehydrated dichloromethane solution was used for analysis.

RESULTS AND DISCUSSION:

To evaluate the efficiency, solid-phase extraction was performed. The EDTA standard solution was added to 100 mL Milli-Q water and tap water at concentrations of 0.1 mg/L in drinking water and 0.01 mg/L in drinking water. The extraction and derivatization were performed as described previously. Tap water samples were dechlorinated by L (+)-ascorbic acid before use. Control samples for the recovery test 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 three times. 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). The relative standard deviation (RSD) of the ratio of EDTA to CyDTA in each sample ranged 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.

The application of this analytical method to environmental water samples and 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.

Table No. 7: Recovery of EDTA from milli-Q water and tap water sample

Analyte	**Vehicle**	**Concentration (mg/L)**	**Recovery of sample % (mg/L)**			**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	97.2	97.2	98.1	0.9

CONCLUSION:

Ethylene diamine tetraacetic acid (EDTA) is a chelating agent it can bind to heavy metal ion which is available in the environment. Due to its strong complexing activity, it is very difficult to remove from the environment.

EDTA is the cheapest and most suitable complexing compound for many technical purposes and its use in large quantities. In the dairy industry, EDTA has been used a improve the cleaning efficiency during the Clean-in-place (CIP) procedure. It is also used in personal care, hygiene, and household disinfectants and results in the release of EDTA into the aquatic

environment. EDTA occurred at a higher concentration in surface waters than any other identified anthropogenic organic compounds that cause further environmental issues.

EDTA is determined by different analytical methods RP-HPLC (Reverse- phase high-performance liquid chromatography), UV- Spectroscopy method, ion chromatography, and gas chromatography. In this method, ion chromatography is an easy method as compared to others. RP-HPLC, UV- Spectroscopy, and Gas chromatography method performs the derivatization procedure and it takes more time. In ion chromatography, it separates easily without a derivatization process base on the affinity to the ion exchanger.

REFERENCES

1. Laamanen PL and Mali A. "Simultaneous determination of DTPA, EDTA, and NTA by capillary electrophoresis after complexation with copper". Anal. Bioanal. Chem. 2005, 6, 1264-1271.
2. Sillanpää M and Kokkonen R, ". Determination of EDTA and DTPA as their Fe (III) complexes in pulp and paper mill process and waste waters by liquid chromatography" Anal. Chim. Acta. 1995,10,187-192.
3. Lanigan RS and Yamarik TA." Final report on the safety assessment of EDTA, calcium disodium EDTA, diammonium EDTA, dipotassium EDTA, disodium EDTA, TEA-EDTA, tetrasodium EDTA, tripotassium EDTA, trisodium EDTA, HEDTA, and trisodium HEDTA". Int. J. Toxicol**.** 2002**,** 21, 95-142.
4. World Health Organization. "Edetic Acid (EDTA) in Drinking-Water." Guidelines for Drinking-Water Quality.2003, 1-10.
5. Narola B and Singh AS, "A validated reverse phase HPLC method for the determination of disodium EDTA in meropenem drug substance with UV-detection using precolumn derivatization technique" Anal. Chem. Insights. 2011, 7-14.
6. Xie CZ and Healy T, "Determination of EDTA in dairy wastewater and adjacent surface water". Int. J. Civ. Eng. 2010, 34, 44-48.
7. Loyaux-Lawniczak S., Douch J "Optimisation of the analytical detection of EDTA by HPLC in natural waters" J. Anal. Chem., 1999, 8, 727-731.
8. Tran G and Chen C. "HPLC method for the determination of EDTA in an ophthalmic cleanser." J. Liq. Chromatogr. Relat. Technol. J LIQ CHROMATOGR R T. 1996, 91, 499-508.
9. Kamboj S, Sharma D, "Simple spectrophotometric method for estimation of disodium edetate in topical gel formulations". Pharm. Methods. 2011, 2, 148-151.
10. Laine P, Matilainen R. "Simultaneous determination of DTPA, EDTA, and NTA by UV–visible spectrometry and HPLC". Anal. Bioanal. Chem**.** 2005, 7, 1601-1609.
11. Krokidis AA, Megoulas NC, "EDTA determination in pharmaceutical formulations and canned foods based on ion chromatography with suppressed conductimetric detection". Anal. Chim. Acta. 2005, 11,57-1163.
12. Pistos C, Parissi-Poulou M. "Determination of ethylenediamine tetraacetic acid in injection forms by ion-pair chromatography". J. Pharm. Biomed. Anal. 2002, 6, 1073-1090.
13. Pátzay G, Ramadan Y, Tonkó C." New EDTA determination method based on ion chromatography with suppressed conductimetric detection". J. Radioanal. Nucl. Chem. 2014, 1,161-168.
14. Khan M. Mahmood S "Calibration of shahid's analytical method for adulterated in-EDTA fertilizers by ion chromatography and atomic absorption spectroscopy". J. Chem. Soc. Pak. 2010**,** 6, 613.
15. Desai RV, Shah BN, Patel AP "Microwave-assisted isolation of mucilage from the *Trigonella foenum* graecum seeds" Int J Pharm Sci Rev Res, 2014, 25 (2), 80-82.
16. Kubota R, Tahara M," Determination of EDTA in Water Samples by SPE-Gas Chromatography/Mass Spectrometry". J. Water Environ. Technol.2010, (4), 347-353.
17. Sillanpää M, Sorvari J, ML." Low-level determination of EDTA and DTPA in natural waters by gas chromatography". Chromatographia. 1996**, (**9), 578-582.

18. Heydari R, Shamsipur M, "Simultaneous determination of EDTA, sorbic acid, and diclofenac sodium in pharmaceutical preparations using high-performance liquid chromatography". AAPS J. 2013, (2), 764-769.
19. Kord AS, Tumanova I, "L. A novel HPLC method for determination of EDTA in a cataract inhibiting ophthalmic drug" J. Pharm. Biomed. Anal. 1995, 575-580.

Image *Author -1*	***Author Name – Corresponding Author: Patel Ankita*** *Author Affiliation: Research Scholar, ROFEL Shri G.M. Bilakhia college of pharmacy, Vapi, Gujarat, India.* *Author Address/Institute Address:, ROFEL Shri G.M. Bilakhia college of pharmacy, Vapi, Gujarat, India-396191*
Image *Author -2*	***Author Name: Dr.Alisha patel*** *Author Affiliation: Associate Professor, ROFEL Shri G.M. Bilakhia college of pharmacy, Vapi, Gujarat, India- 396191* *Author Address/Institute Address: ROFEL Shri G.M. Bilakhia college of pharmacy, Vapi, Gujarat, India- 396191*