Research Article Available online www.ijsrr.org ISSN: 2279–0543

International Journal of Scientific Research and Reviews

Development and Validation of RP-HPLC method for determination of Ethylenediaminetetraacetic acid (EDTA) in Cosmetics with UV detection using precolumn derivatization technique

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ABSTRACT

Analytical method for the quantitative determination of EDTA in Cosmetics has been developed and validated by high-performance liquid chromatography (HPLC) with UV detection at wavelength 280 nm. The analysis was performed in gradient mode on a reversed phase C18 column, 4.6mm x 250mm using mobile phase consisted of a 25mM tetrabutyl ammonium hydrogen sulphate, in water as mobile phase A and acetonitrile as mobile phase B. The developed method was validated for various parameters such as Specificity, Linearity, Precision, Recovery, Repeatability and Ruggedness by employing HPLC. A linear calibration curve was observed in the range of 0.5-10.0 mg/kg with r^2 values ≥ 0.99. The limits of detection and quantification were 0.25 mg/kg and 0.5 mg/kg respectively. Cosmetics samples were spiked at 0.5, 1.0 and 2.0mg/kg fortification levels. Better recoveries between 70% to 120% were obtained with the acceptable relative standard deviation (% RSD) i.e. <20%. The validated method has been satisfactorily applied to the analysis of EDTA in Cosmetics samples due to its high sensitivity and selectivity.

KEYWORDS: Cosmetics, EDTA, Method Validation, Pre-column Derivatization, HPLC

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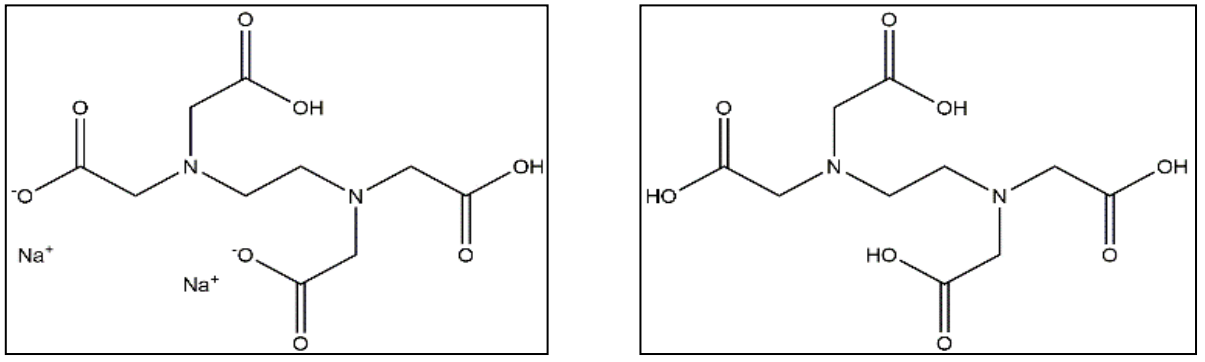
1. INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA) (Figure No. 1) and its salts (collectively known as Edetates) are substituted diamines. EDTA is a powerful chelating agent, forming stable complexes with most metal ions[1].EDTA is less stable than its salts. Disodium edetate is disodium salt of EDTA and is commonly used in pharmaceutical formulations, cosmetics, and foods as chelating agents. These ingredients function as chelating agents in cosmetic formulations by combining with polyvalent metal cations in solutions to form soluble ring structures[2].

Cosmetics, detergents, and pharmaceutical preparations require protection against microbial growth to ensure the safety of their use and to extend the length of their shelf life[3]. EDTA increases the permeability of cell membranes and make them more sensitive to antimicrobial agents. In addition, chelating agents block the iron required for metabolism and microbial growth, and can enhance the antimicrobial efficacy of the used preservatives[4; 5].The binding of metal ions helps prevent deterioration of cosmetics and personal care products. It also protects fragrance compounds and prevents rancidity. It form complexes with calcium, magnesium and iron which allow for better foaming and cleaning performance of cosmetics and personal care products. By binding with metal ions, these ingredients prevent metals from being deposited onto the hair, scalp and skin[6].



EDTA **Disodium EDTA**

Figure No. 1: Structure of EDTA and Disodium EDTA

Disodium EDTA (Figure No. 1) primarily works as a preservative, chelator and stabilizer, but has also been shown to enhance the foaming and cleaning capabilities of a cosmetic solution[7]. It stabilizes or helps preserve all kinds of cosmetics products like creams, lotions, shampoos, conditioners, make-up products, sunscreen products. Disodium EDTA, as a stabilizer in cosmetics is

used to prevent ingredients in a given formula from binding with trace elements that can be present in water. It stabilizes emulsions, surfactants and foam builders. In shampoos, cleaners and other personal care products. EDTA acts as co-preservative that enhances efficacy of preservatives and other anti-bacterial agents. EDTA does not contain any chromophoric group. Hence, it is very difficult to determine EDTA by direct UV detection[8]. The quantification of the residual EDTA is essential as per regulatory requirements.

Various analytical methods have been proposed for the determination of EDTA in a wide variety of sample matrices[9]. These include titrimetry[10], spectrophotometry[11], electrochemistry polarography[12], differential pulse olorography[13], catalytic potential tirtrimetry[14], differential pulse anodic stripping voltammetry[15], amperometry[16], capillary electrophoresis[17], and chromatography. Gas chromatography and HPLC (reverse phase) are the prevailing techniques. The gas chromatographic methods always include a time consuming derivatization steps, in which EDTA is converted into methyl, ethyl, propyl and butyl esters to obtainvolatility[18, 19].

Up to date, different analytical methods have been reported for determination of Edetates based on high-performance liquid chromatography (HPLC) in commercial pharmaceutical products. To our knowledge, there is not much report about determination of Edetates in Cosmetic products which is particularly important for both quality assurance and consumer protection.

The aim of this work was to develop fast, simple, selective, and easy-to-use method for control of EDTA in Cosmetics. The present study deals with the development and validation of HPLC derivatized method for quantitative determination of EDTA in Cosmetics products utilizing trivalent iron for complex formation with ferric chloride solution and its subsequent determination by reversed phase HPLC.

2. EXPERIMENTAL

2.1. Chemicals and Reagents

Cosmetics samples were purchased from market. Di-sodium salt of Ethylenediaminetetraacetic acid dihydrate (Disodium EDTA) and Ferric chloride anhydrous (AR grade) were purchased from Qualigens. Tetra butyl ammonium hydrogen sulphate (AR grade) was procured from Spectrochem, Acetonitrile (HPLC grade) was purchased from Rankem. Deionized water (Millipore-Advantage A10) was used for the preparation of standard and sample solutions.

2.2. Preparation of Stock and Working Standard Solution

Diluent: 25mM Tetrabutyl Ammonium Hydrogen Sulphate Solution. Standard and sample solutions were prepared in the diluent.

A **standard stock solution** of 1000mg/Kg was prepared by dissolving accurately weighed 63.5mg of disodium salt of EDTA dihydrate (equivalent to 50 mg of EDTA)[2] in 2.5ml of acetonitrile followed by derivatization process carried out by adding 25ml of 1mM ferric chloride solution and final volume was made up to 50 mL with diluent.

Working standard solutions were prepared by serial dilution of the standard stock solution into 100mL volumetric flasks containing 5mL of acetonitrile and 50mL of 1mM Ferric chloride solution to achieve the desired linearity range of 0.5-10.0 mg/Kg of EDTA. Finally the volume was made up to the mark with diluent, shaken well and allowed to stand for 15 minutes at room temperature. The solution was filtered through 0.45 µm syringe filter prior to injection on HPLC.

2.3. Extractionprocedure

About 1g of the cosmetic sample was weighed into 100mL capacity volumetric flask. Derivatization was carried out by adding 5mL of acetonitrile and 50ml of 1mM Ferric chloride solution into the flask. The final volume was made up with the diluent, shaken well and allowed to stand for 15minutes at room temperature. The solution was centrifuged at 5000rpm for 5 minutes and then filtered through 0.45 µm syringe filter prior to injection on HPLC.

2.4. Instrumentation and Conditions

The analytical separations were carried out on HPLC-Agilent 1200 Technologies, equipped with UV detector and a Prominence autosampler. The analytical column, Agilent C18column (250mm × 4.6 mm), 5 µm was used for chromatographic analysis. The mobile phase consisted of 25mM tetra butyl ammonium hydrogen sulphate, in water as mobile phase A and acetonitrile as mobile phase B. The mobile phase was degassed and filtered through a 0.45 µm membrane filter. The gradient profile was 0-7 minutes 2% B; 7-15 minutes 80% B; 15-20 minutes 80% B, 20-25minutes 2% B and 25-30 minutes2% B. The flow rate was 1 mL/min and total runtime was 30 minutes. Column temperature was maintained at 25°C. UV detection was measured at 280 nm and the volume of sample injected on to the column was 25 µL.

3. RESULTS AND DISCUSSION

3.1. Method Validation

The developed analytical method for the determination of EDTA was validated for their performance characteristics such as Specificity, Linearity, Precision, Recovery, Repeatability and Ruggedness.

3.2. Specificity

Chromatograms of blank and spiked samples were analyzed to examine interference, if any. No peak from the blank was observed at the retention time of EDTA peak ensuring that the peak is pure and there were no interferences in the retention time of the target analyte. Hence it can be said that the proposed analytical method is specific and selective for the determination of EDTA in cosmetic products.

3.3. Linearity

To establish the linearity of the proposed method, a series of disodium EDTA solution (0.5 to 10.0 mg/Kg) were prepared from the stock solution and analyzed. Linearity was evaluated by plotting calibration curves between peak areas versus concentration at five fortification levels. Linear calibration curves with correlation coefficients (r2) > 0.99 were obtained and proved linearity of the method in defined concentration ranges. The linearity data is shown in Table 1 whereas Linearity graph is shown in Figure 2.

Table No. 1: Linearity Data for EDTA in Cosmetics

S.No.	Concentration (mg/kg)	Area
1	0.5	31825
2	1.0	61006
3	2.0	130215
4	5.0	318370
5	10.0	647021

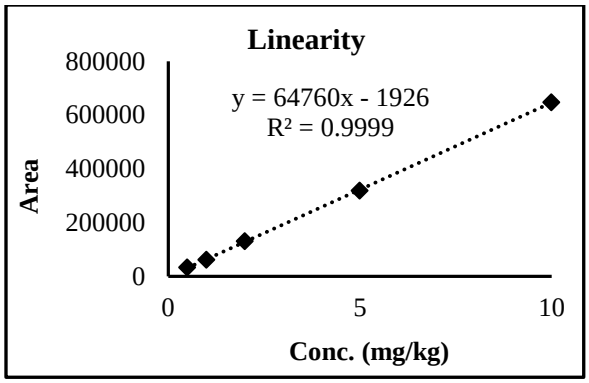


Figure No. 2: Linearity Graph of EDTA

3.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

The sensitivity of the method was expressed as LOD and LOQ. The limits of detection (LOD) and quantification (LOQ) were found by adding decreasing concentrations of standard solution in the samples, and then subjected to extraction and quantification, up to the lowest detectable concentration (LOD) and the lowest quantifiable concentration (LOQ), under suitable conditions of repeatability (n = 5, RSD < 20%). The limits of detection and quantification found were 0.25µg/kg and 0.5µg/kg, respectively, for determination of EDTA in cosmetics.

3.5. Accuracy

Typically, accuracy is represented and determined by recovery studies. The accuracy was evaluated by spiking the samples at three fortification levels i.e. 0.5, 1.0, and 2.5 µg/kg with six replicates at each level. The relative standard deviation (% RSD) for all spiked levels was found lower than 20%. The recovery data shown in Table 2 indicates that the method has an acceptable level of accuracy.

Table No. 2: Recovery Data for EDTA in Cosmetics

Compound	Fortification Level (mg/kg)	Mean Recovery (mg/kg)	% Recovery ± SD
EDTA	0.5	0.58	115.5 ± 1.9
	1.0	1.07	107.3 ± 0.2
	2.0	2.18	108.9 ± 0.4

3.6. Precision

Precision in terms of repeatability (Intra-day precision) and ruggedness (Intermediate precision) was evaluated at three concentration levels i.e. 0.5, 1.0 and 2.5µg/kg with six replicates at each level. The acceptance criterion found was within 20% relative standard deviation (% RSD). The values shown in Table 3were found well within the acceptable range indicating that the proposed method has an excellent repeatability and intermediate precision. These results also suggested that the proposed method may be considered validated in term of precision.

Table No. 3: Repeatability and Ruggedness Data for EDTA in Cosmetics

Compound	Fortification Level (mg/Kg)	Mean Recovery (mg/Kg)		% Recovery ± SD	
		Repeatability	Ruggedness	Repeatability	Ruggedness
EDTA	0.5	0.58	0.57	116.6 ± 1.7	114.8 ± 1.4
	1.0	1.07	1.07	107.2 ± 0.5	106.6 ± 1.1
	2.0	2.18	2.18	108.9 ± 0.4	108.8 ± 0.8

4. CONCLUSION

The reversed-phase RP-HPLC method developed was found to be convenient for the simultaneous determination of EDTA. The validated method is found to be highly sensitive therefore; it could be used for routine analysis of EDTA in cosmetics. Obtained validation parameters proved that the suggested method is convenient enough for routine determination of EDTA in quality control laboratories. This method also has advantages over other techniques as in this method EDTA response is measured by direct UV detection with enhanced sensitivity and method is simpler, highly reproducible, specific and accurate. The proposed method showed good precision and reproducibility with acceptable linearity and accuracy range. Hence, the proposed method can be applied to the analysis of EDTA in commercially available cosmetics.

5. ACKNOWLEDGEMENT

The authors are thankful to the Department of Analytical Science Division, Shriram Institute for Industrial Research, Delhi for providing the facilities for this research work.

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