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Short Communication

Determination of ethylenediaminetetraacetic acid (EDTA) in pharmaceutical dosage forms using flame atomic absorption spectroscopy

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Abstract

In this study, flame atomic absorption spectroscopy coupled with the ion-exchange technique was adopted for the determination of ethylenediaminetetraacetic acid (EDTA) in certain dosage forms. The method is based on complexation of EDTA with an excess of calcium(II) or magnesium(II) at pH 10. The remaining metal ions were fixed on a cationic ion-exchange resin, while Ca–EDTA or Mg–EDTA sequestrates were eluted. The eluted sequestrates were determined using flame atomic absorption spectroscopy. The experimental conditions were carefully studied, and the method was applied for the determination of EDTA over the concentration ranges of 4–160 and 2–32 μg ml^{-1} upon using Ca(II) and Mg(II), respectively. The proposed method was successfully applied for the determination of EDTA in pharmaceutical dosage forms. The proposed method is characterized by high sensitivity, satisfactory accuracy and precision.

Keywords: Ethylenediaminetetraacetic acid (EDTA); Flame atomic absorption spectroscopy; Dosage forms; Ion-exchange resin; Pharmaceutical analysis

1. Introduction

Ethylenediaminetetraacetic acid (EDTA) is widely used in the pharmaceutical industry as an antioxidant, chelating agent for heavy metals and preservative in many pharmaceutical formulations. EDTA is added to eye wash and ophthalmic solutions having bactericidal properties [1]. The British and US pharmacopoeias [2,3] recommend classical titrimetric methods for the determination of EDTA. Various methods have been reported for the determination of EDTA in pharmaceutical preparations, including colorimetry [4,5], high-performance liquid chromatography (HPLC) [6,7] and polarography [8,9]. A good guide to the work published for EDTA is found in the excellent reviews written by Sillanpää and Sihvonen [10] and Huber [11]. As for atomic absorption spectroscopy (AAS), EDTA was determined through its masking effect on the extraction of copper oxinate into methylisobutylketone at pH 6.5. The decrease in the AAS signal for Cu(II) was linearly proportional to the EDTA concentration [12]. Another method based on the addition of an excess of Ni(II) was described; the unused Ni(II) was removed as its dimethyl glyoximate and the nickel content of Ni–EDTA complex was determined by AAS [13]. The last method, however, is limited by the oxidative property of Cu(II) ions, thus it cannot be used for drugs susceptible to oxidation, such as ascorbic acid, phenylephrine or drugs that may react with Cu(II) or Ni(II), e.g. penicillins, etc., in addition to the higher affinity of Cu(II) and Ni(II) to form stable complexes with a large number of pharmaceutical compounds. This led us to study the use of Ca(II) and Mg(II) for the AAS determination of EDTA.

2. Experimental

2.1. Materials

- Sodium edetate dihydrate (Aldrich). 0.1% aqueous solution was prepared. Pharmaceutical preparations containing EDTA as a preservative were obtained from commercial sources in the Egyptian market.

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2.2. Reagents

- Calcium atomic absorption standard solution (100 ppm) and magnesium atomic absorption standard solution (100 ppm) were obtained from Aldrich.
- Ammonia buffer pH 10.
- Cation exchanger (Amberlite IR-120).

2.3. Apparatus

An atomic absorption spectrometer (Perkin-Elmer, model 2380) was used, with Pye Unicam hollow cathode lamps for calcium and magnesium, and a conventional 10 cm slit burner head for an air–acetylene flame; absorbance values were taken after averaging one second integration time. The optimum experimental parameters are listed in Table 1.

2.4. Procedures

2.4.1. Construction of empirical calibration curve

The calibration curves for Ca(II) and Mg(II) were constructed by aspiration of a suitable concentration of standard solutions of Ca(II) and Mg(II) ions into the flame atomic spectrometer. The absorbance values at 422.8 and 285.5 nm for Ca(II) and Mg(II) ions, respectively, were found to be linear functions over the concentration ranges 4–160 and 2–32 μg ml^{-1} for calcium and magnesium ions, respectively.

2.4.2. General procedures for the determination of EDTA in pure form

2.4.2.1. Using calcium(II)

Aliquots containing suitable quantities of EDTA over the concentration range cited in Table 1 were transferred into separate 25 ml standard flasks. 10 ml of 100 μg ml^{-1} calcium atomic absorption standard solution and 5 ml of ammonia buffer of pH 10 were added to each flask. The reaction mixture was diluted to the 25 ml mark with double distilled water. The whole contents of the flask was subjected to a cation exchanger (Amberlite IR-120) column 10 cm in length and 1 cm^2 in area. The Ca–EDTA sequestrate was aspirated into the spectrometer and the absorbance was measured at 422.8 nm.

2.4.2.2. Using magnesium(II)

Aliquots containing suitable quantities of EDTA over the concentration range cited in Table 1 were transferred into separate 25 ml standard flasks. 1.5 ml of 100 μg ml^{-1} magnesium atomic absorption standard solution and 5 ml of ammonia buffer pH 10 were added to each flask. The method was repeated as described in the previous section starting from the words "The reaction mixture was diluted ...". The absorbance was measured at 285.5 nm.

2.4.3. Procedure for pharmaceutical dosage forms

2.4.3.1. Ophthalmic preparations

Mix the contents of 10 eye drop bottles, centrifuge if necessary. Transfer aliquots of the clear mixed solutions equivalent to 25 mg of EDTA into a 50 ml volumetric flask. Complete to the 50 ml mark with double distilled water. Transfer 1 ml of solution into a 25 ml standard flask and proceed as described in Section 2.4.2 upon using calcium or magnesium. The edetate content of Phenylephrine eye drops (phenylephrine 10%), Prefrin eye drops (phenylephrine 1.2 mg), FML eye drops (neomycin 5 mg), FML–NEO eye drops (neomycin 5 mg and fluorometholone, 1 mg) and Visine eye drops (tetrahydrozoline hydrochloride, 0.05%) was determined from calibration graphs upon using Ca(II) and Mg(II) or from the corresponding regression equations.

2.4.3.2. Procedure for ampoules

Mix the contents of 10 Cevarol ampoules (containing 20% ascorbic acid solution). Transfer 2.5 ml of the mixed solution into a 50 ml volumetric flask and complete to the 50 ml mark with double distilled water. Transfer 2 ml of this solution into a 25 ml standard flask and proceed as described under in Section 2.4.2 upon using Ca(II) or Mg(II). The edetate content in Cevarol ampoules was calculated from the previously plotted calibration graphs or from a comparison with the absorbance values obtained from a standard solution prepared simultaneously.

3. Results and discussion

EDTA reacts with calcium(II) and magnesium(II) salts at pH 10 to form sequestrates with stability constants of 10.7 and 8.7, respectively [14]. The calcium and magnesium sequestrates were separated from the residual reagent using cationic-exchange resin (Amberlite IR). The calcium and magnesium sequestrates were then aspirated on the AAS. The signals of calcium and magnesium ions were linearly proportional to EDTA concentration over the ranges 4–160 and 2–32 μg ml^{-1} upon using calcium and magnesium ions,

Table 1
Instrumental parameters used for atomic absorption spectrometric determination of calcium(II) and magnesium(II)

Cation	Working range (μg ml^{-1})	Wavelength (nm)	Slit width (nm)	Burner type	Lamp current (mA)	Air flow rate (l min^{-1})	Acetylene flow rate (l min^{-1})
Calcium	4–160	422.8	0.2	air/acetylene	10	4.5–5.5	1.1–1.5
Magnesium	2–32	285.5	0.2	air/acetylene	4	4.5–5.4	1.1–1.5

Table 2
Application of the proposed AAS method and official methods to the determination of EDTA in pure form

Calcium(II) method			Magnesium(II) method			Official method [2]
Amount taken ($\mu g\ ml^{-1}$)	Amount found ($\mu g\ ml^{-1}$)	EDTA recovery (%)	Amount taken ($\mu g\ ml^{-1}$)	Amount found ($\mu g\ ml^{-1}$)	EDTA recovery (%)	
4	3.99	99.75	2	2.02	101.00	
8	8.20	102.50	4	3.95	98.75	
12	12.18	101.50	8	7.95	99.38	
16	16.09	100.56	16	15.96	99.75	
18	18.19	101.06	24	24.30	101.25	
40	39.93	99.83	32	31.80	99.38	
80	79.56	99.45				
120	118.49	98.74				
160	161.27	100.79				
$\bar{X} \pm$ S.D.		100.46 ± 1.151			99.92 ± 0.992	99.72 ± 0.71
t		1.174 (2.201)			0.345 (2.306)	
F		2.629 (4.07)			1.952 (5.41)	

Each result is the average of three separate determinations.
The figures in brackets are the tabulated values of t and F.

Table 3
Application of the proposed AAS method to the determination of EDTA in pharmaceutical preparations

Pharmaceutical preparation	Found and S.D. (%)	
	Ca(II) method	Mg(II) method
1. Cevarol ampoules [a] (EDTA 0.5%)	98.42 ± 0.655	97.83 ± 0.343
2. Phenylephrine [b] eye drops (EDTA 0.05%)	98.61 ± 0.508	97.89 ± 0.404
3. Prefrin [c] eye drops (EDTA 0.05%)	98.46 ± 0.506	97.58 ± 0.419
4. FML eye drops [d] (EDTA 0.1%)	98.53 ± 0.317	97.93 ± 0.394
5. FML–Neo eye drops (EDTA 0.1%)	98.70 ± 0.437	97.96 ± 0.444
6. Visine eye drops [d] (EDTA 0.1%)	98.74 ± 0.474	98.11 ± 0.405

Each result is the average of three separate determinations.
[a] Memphis, Cairo, Egypt.
[b] Misr, Cairo, Egypt.
[c] E.I.P.I., Ramadan City, Egypt.
[d] Pfizer, Cairo, Egypt.

respectively. Regression analysis of the results gave the following equations:

– upon using calcium(II),

$$C = 0.663 + 350.68A, \quad R = 0.9999$$

– upon using magnesium (II),

$$C = 1.381 + 33.35A, \quad R = 0.9999$$

where C is the concentration of EDTA in $\mu g\ ml^{-1}$, A is the absorbance at the corresponding wavelength for either ions, and R is the correlation coefficient.

Calcium and magnesium ions were found to be the metals of choice for the present study. They were preferred to Cu(II) and Ni(II) which were frequently utilized in such cases. They have lower affinity to form complexes because of their large atomic size and consequently lower charge density. In addition, they have no oxidation/reduction properties, thus avoiding the possible reaction with the active ingredients which are amenable to oxidation by Cu(II), e.g. ascorbic acid [15] and phenylephrine [16]. Similarly, aminoglycosides, penicillins and cephalosporins are reported to react with Cu(II), forming stable complexes [17].

A cationic-exchange resin (Amberlite IR-120) was found to be the most suitable one for the present study. Upon using anionic exchange resin, poor resolution was obtained; this might be due to the competitive effect of other interfering anions present in the medium which would occupy some sites of the resin.

Under the described experimental conditions, calibration curves of EDTA were constructed by plotting the absorbance values versus the concentrations. The proposed method was applied for the determination of EDTA in pure form. The results obtained were compared statistically with those given by the official method [2] using the Student t test and the variance ratio F test [18]. The results in Table 2 show that there is no significant difference between the proposed and official method regarding accuracy and precision.

The proposed method was further applied for the determination of EDTA in certain pharmaceutical preparations (Table 3). It was found that, during the analysis of certain

ophthalmic preparations (Prefrin, FML and FML–Neo), low results were obtained. This was found to be due to the presence of phosphates in their formulations which are frequently added to eye drops.

Phosphates were reported to interfere seriously in the determination of calcium and magnesium [19–22]. This is due to the formation of calcium phosphate that has low volatility, thus reducing the rate at which the compound is atomized [19]. In addition, phosphate will precipitate magnesium ions at pH 10 as magnesium ammonium phosphate ($K_{sp}=2.5\times10^{-13}$). Therefore, elimination of phosphates ought to be carried out before the analysis of EDTA in such preparations. It was reported that addition of an excess of strontium(II) or lanthanum(II) minimizes the interference of phosphate during the determination of calcium or magnesium [19]. In this study lanthanum chloride was successfully utilized for the elimination of phosphate interference.

Standard-addition recovery experiments were performed to assess the validity of the proposed method and to ensure the lack of interference in the procedure. Different concentrations of EDTA were added to the pharmaceutical preparations and the proposed methods were applied. The results obtained in Table 3 were found to be satisfactorily accurate.

4. Conclusions

A simple and sensitive method is described for the micro determination of EDTA in pharmaceutical preparations. Interference likely to be produced by the major components in the preparations could be overcome by the use of Ca(II) or Mg(II). Interference coming from other additives could be overcome by modifying the procedure or adopting the standard-addition technique.

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