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Determination of ethylenediamine tetraacetic acid in injection forms by ion-pair chromatography

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Abstract

A high performance liquid chromatographic method was developed for the determination of ethylenediamine tetraacetic acid (EDTA) in injection forms. The method consists of direct extraction of the samples with ethyl acetate; the organic layers were evaporated to dryness and further diluted to a 0.025% (w/v) copper nitrate in order to achive the formation of the EDTA–copper solution complex. The chromatographic separation was performed on a C8 Hypersil column. The mobile phase consisted of a mixture of acetonitrile–0.015 M tetrabutylammonium hydroxide (10:90, v/v), (pH* 7.0) pumped at a flow rate of 1.5 ml min^{-1}. The UV detector was operated at 300 nm. Correlation coefficients of the calibration graphs were better than 0.9995, relative standard deviation was less than 2.5%. Detection limit of EDTA was found to be 1.97 μg ml^{-1}.

Keywords: EDTA; High performance liquid chromatography; Injection forms; Ion-pair

1. Introduction

Ethylenediamine tetraacetic acid (EDTA) is a nontoxic antioxidant ingredient, and is widely used as an additive in several pharmaceutical forms. It is also used as a chelate agent for the inactivation of heavy metals and for the strengthening of the efficacy of conservatives in the pharmaceutical formulations.

Due to its low biodegradability [1,2], it is present in sewage effluents [3–5], fresh water [6,7], and groundwaters [8]. EDTA has often been suspected to remobilize, adsorb or precipitate heavy metals from river sediments or aquifers as it forms very stable complexes with them. Recent methods have shown that the presence of aerobial microorganisms [1,9] and hydrogen peroxide [10] lead to diminish of the chelation properties of EDTA complexes with heavy metal ions. Additionally, the presence of titanium dioxide [11] enforces the degradation of EDTA. However, the injection form in which the proposed method applied, fulfills the sterile standards. Moreover, there is no presence of titanium dioxide or hydrogen peroxide, therefore, there is no $[Cu–EDTA]^{-2}$ degradation related with the developed method in the injection form.

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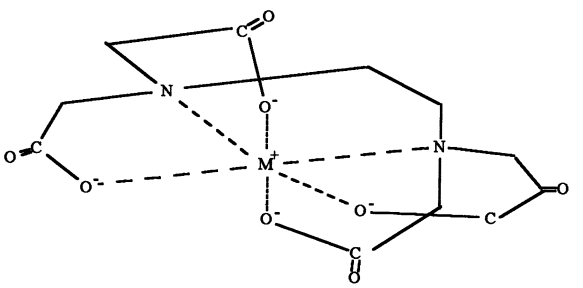
EDTA possesses six binding sites, thus it can form chelates with nearly all metal ions. This chelation is the basis for several titrimetric procedures that have been developed for the quantitation of metal ions. Structure of the chelate complex is presented in Scheme 1.

Several HPLC methods have been reported for the determination of EDTA in nonpharmaceutical formulations [12–28]. To our knowledge, few only reports have been mentioned in literature for the quantitative determination of EDTA in drugs [29–31]. The proposed method has been applied specifically for the quantitative determination of EDTA in injection form comparing to the previously mentioned HPLC methodology. Additionally, other methods include the use of gas chromatography [32–34], thin layer chromatography [35–37] and spectrophotometry [38–42].



Scheme 1. Structure of EDTA complex with a metal ion, M^+.

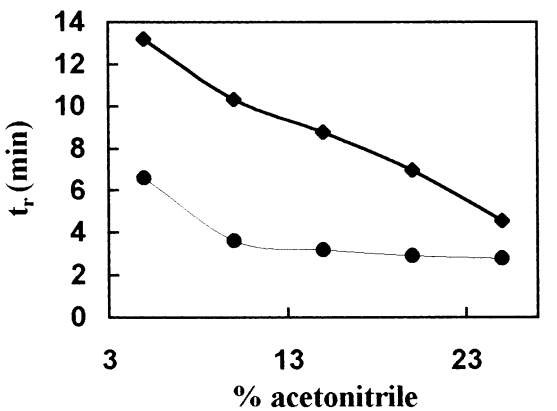


Fig. 1. Plots of the retention time, t_r, of $[EDTA–Cu]^{-2}$ (◆), and paracetamol (●) versus acetonitrile concentration in the mobile phase.

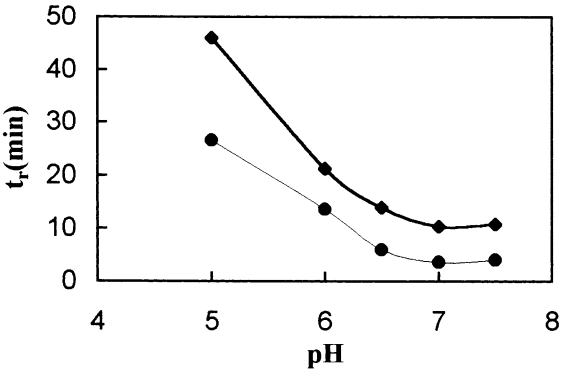


Fig. 2. Plots of the retention time, t_r, of $[EDTA–Cu]^{-2}$ (◆), and paracetamol (●) versus phosphate concentration in the mobile phase.

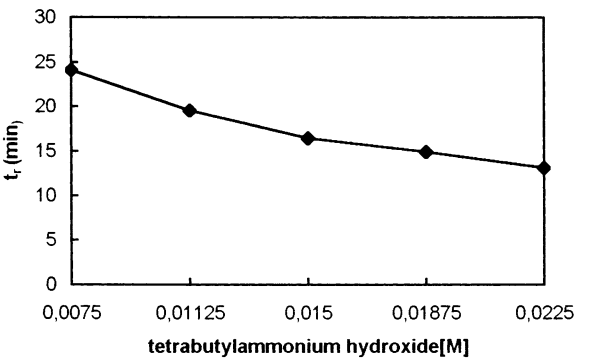


Fig. 3. Plots of the retention time, t_r, of $[EDTA–Cu]^{-2}$ versus tetrabutylammonium hydrohide concentration in the mobile phase.

The purpose of this work, was to develop a sensitive, specific and inexpensive HPLC method for the quantitative determination of EDTA in injection forms, which contain paracetamol as the active ingredient in a concentration of 150 mg ml^{-1}. A primary consideration was the simplicity of the assay, so that it could be used for the quality control, using minimal instrumentation, such as an isocratic HPLC system coupled with a UV detector, which are available in most laboratories.

2. Experimental

2.1. Materials and reagents

Solvents were of HPLC grade and were purchased from Lab-Scan Analytical Sciences Ltd. Ireland. Disodium salt of EDTA ($C_{10}H_{14}N_2Na_2O_8$) $M_r = 336.21$ was purchased from E. Merck Ltd., (Darmstadt, Germany). Tetrabutylammonium hydroxide and copper nitrate

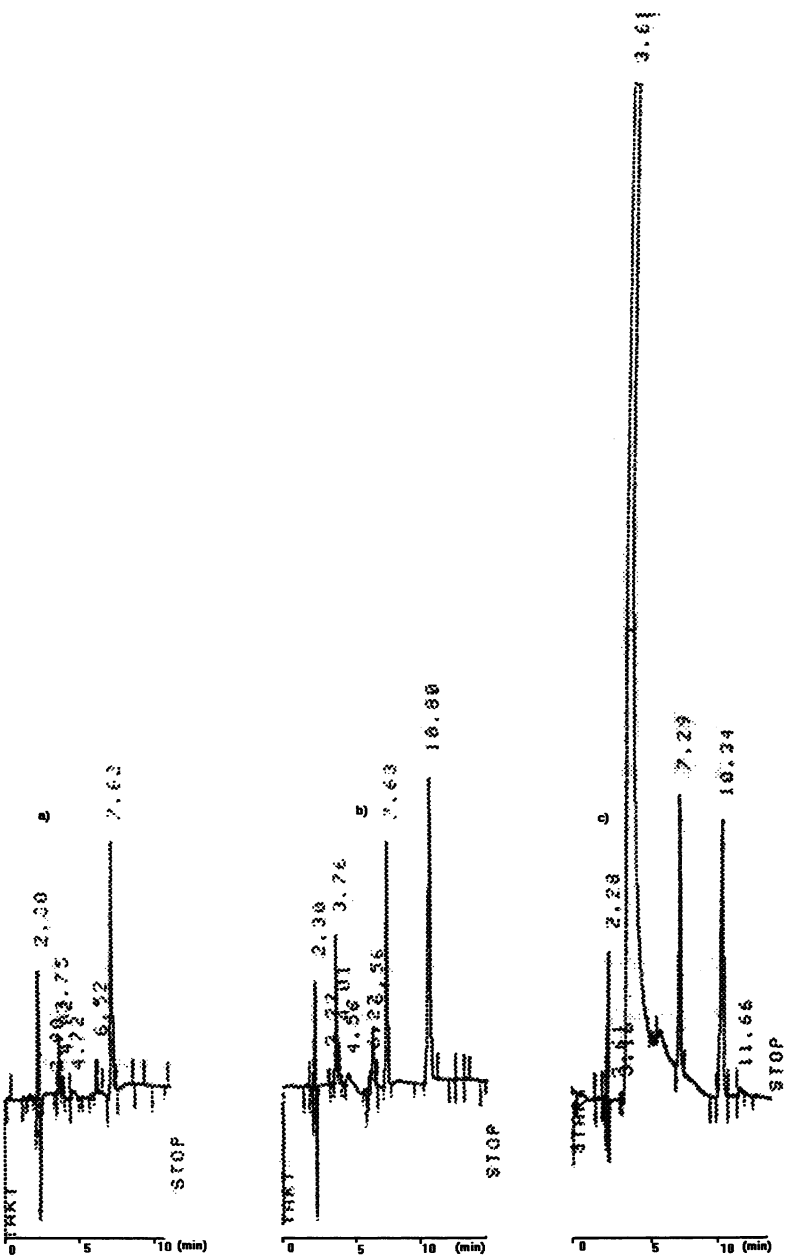


Fig. 4. Representative chromatograms of injection samples of Apotel: (a) blank sample of injection matrix; (b) injection matrix in presence of EDTA; (c) injection matrix in presence of both EDTA and paracetamol.

Table 1
Calibration equation for the determination of EDTA by HPLC in aqueous and injection solutions, respectively

Fluid	C_{EDTA} (μg ml^{-1})	Regression equations[a]	r[b]	S.D.[c]		S_r[d]
				Slope	Intercept	
Water	5.00–40.00	$S_{EDTA} = 1.4 + 11.5\ C_{EDTA}$	0.99995	0.07	1.6	1.87
Injection matrix	5.00–50.00	$S_{EDTA} = 7.2 + 12.9\ C_{EDTA}$	0.9995	0.23	7.7	7.36

[a] Peak area $\times 10^3$ of EDTA versus concentration, C_{EDTA} in μg ml^{-1}; five standards.
[b] Correlation coefficient.
[c] Standard deviation of slope and intercept.
[d] Standard error of estimate.

were purchased from Lab-Scan Analytical Sciences Ltd., Ireland. Phosphoric acid (analytical reagent grade) was purchased from Panreac Quimica SA, Spain. Water was deionised and further purified by means of a Milli-Q Plus water purification system (Millipore Co., USA). Paracetamol and lidocaine hydrochloride of pharmaceutical purity grade were kindly provided by Uni-Pharma Hellas A.E. Athens Greece and were used without any further purification. Apotel® is a 4 ml injectable solution containing 150 mg ml^{-1} of paracetamol, lidocaine hydrochloride, different excipients and suitable mixture solvents. EDTA is used as an excipient in this injection form in a 0.10 μg ml^{-1} concentration.

2.2. Instrumental and chromatographic conditions

The HPLC system consisted of a Waters Model 501 solvent-delivery system with a Waters Model 486 variable-wavelength UV–Vis detector (flow cell 8 μl). The recorder used was a Hewlett Packard model HP 3394A. A Shandon Hypersil C8 column (250 × 4.6 mm i.d., 5 μm particle size) was used. The injection system was Rheodyne Model 7125 and the syringe used was a 100 μl Hamilton–Bonaduz-Schweiz. The volume of the injection loop was 5 μl and the effluent was monitored at 300 nm.

The mobile phase consisted of tetrabutylammonium hydroxide 0.015 M–acetonitrile (90:10, v/v). The pH* value of the mobile phase was adjusted to 7.0 using phosphoric acid 10% (v/v). The mobile phase was filtered through a nylon membrane filter (0.45 μm, Millipore) and pumped at a flow-rate of 1.5 ml min^{-1}. All separations were achieved at room temperature. A pH* meter (Metrohm, model 654 Herisau) was used for all pH* measurements.

2.3. Stock and working standard solutions

The following stock standard solutions in injection matrix were kindly provided by Uni-Pharma: (i) stock solution of paracetamol (150 mg ml^{-1}) and lidocaine hydrochloride (unknown concentration); (ii) stock solution of EDTA (0.10 mg

Table 2
Accuracy and precision of the proposed HPLC method for the determination of EDTA

EDTA			
Nominal concentration (μg ml^{-1})	Assayed concentration (μg ml^{-1})		
	Mean ± S.D.	R.S.D.(%)[a]	E_r(%)[b]
Inter-day ($n = 5$)			
8	8.16 ± 0.18	2.2	2.08
10	10.16 ± 0.15	1.5	1.76
12	12.18 ± 0.26	2.1	1.48
Intra-day ($n = 5$)			
8	8.05 ± 0.15	1.9	0.63
10	10.08 ± 0.16	1.6	0.8
12	11.94 ± 0.23	1.9	−0.5

[a] Percentage relative standard deviation.
[b] Relative percentage error.

Table 3
Determination of EDTA in laboratory synthetic mixtures

Drug	Amount added (mg)	Amount found (mg)	m[a]	Recovery[b] (%)
EDTA	80	74.78	0.82	82
	100	91.6		
	120	107.37		

[a] m is the slope of the linear regression analysis of the amount found versus the amount added.
[b] Recovery (%) $= m \times 100$.

ml^{-1}); (iii) injection matrix containing none of the three main ingredients (EDTA, paracetamol and lidocaine·HCl). Stock standard solution of EDTA (0.10 mg ml^{-1}) was prepared by dissolving the compound in water.

Stock standard solutions of $Cu(NO_3)_2$ (0.025%, w/v) was prepared by dissolving 25 mg $Cu(NO_3)_2$ with water to a final volume of 100 ml. These solutions were stored in the dark under refrigeration and were found to be stable for several weeks.

A series of mixed standard solutions were prepared by the appropriate dilution of the above mentioned stock standard solutions in water to reach in a concentration range of 8–12 μg ml^{-1} for EDTA. Working standard solutions of EDTA (10 μg ml^{-1}) were prepared by the appropriate dilution of EDTA in water.

2.4. Sample preparation

A 1.0 ml aliquot of the injection solution was transferred into a 10 ml volumetric flask and diluted to volume with water. In a 1.0 ml aliquot of this solution 1.0 ml $Cu(NO_3)_2$ solution 0.025% (w/v) was added. The resulting solution was vortex mixed for 1 min and 7.0 ml of pure ethyl acetate were added. The mixture was vortex mixed for another 1 min and the aqueous and organic phases were separated by centrifugation at 3000 rpm ($2890 \times g$) for 10 min. The organic layer was rejected and 5 μl of the aqueous phase were injected into the column. Peak area ratios of each compound were then measured for the determination.

2.5. Calibration procedure

Two calibration curves were constructed, in water and injection matrix in order to show that the EDTA concentration was linear over the concentration range studied. The concentration range covered was 5.0–40.0 μg ml^{-1} EDTA in aqueous solutions and 5.0–50.0 μg ml^{-1} EDTA in injection matrix. Triplicate 5-μl injections of each solution were carried out and the peak area ratio of each drug was plotted against the corresponding concentration in order to obtain the calibration graph.

The overall precision and accuracy of the assay was evaluated by analysing three series of standard solutions of EDTA at concentrations of 8.0, 10.0 and 12.0 μg ml^{-1}. The relative standard deviation (%R.S.D.) was determined in order to assess the precision of the method, while the accuracy was expressed by the relative percentage error (E_r%).

3. Results and discussion

3.1. Cromatographic characteristics

Cromatographic separations were carried out on a C8 Hypersil column. The effect of composition and pH* of the mobile phase on the retention time (t_r) of $[Cu–EDTA]^{-2}$ were investigated. Satisfactory separations were obtained with a mobile phase consisted of tetrabutylammonium hydroxide 0.015 M–acetonitrile (90:10, v/v). Increasing acetonitrile concentration more than 10% led to

inadequate separation of the compounds (Fig. 1). At lower acetonitrile concentration, separation occurred but with excessive tailing and increased retention time for $[Cu–EDTA]^{-2}$ peak. pH* values ranging from 5.0 to 6.5 led to increasing retention time and excessive tailing where at pH* value 7.5 the separation did not occur. Thus, a pH* value of 7.0 was found to be the optimum, as at this pH* value the analyte peaks were good resolved and almost free of tailing (Fig. 2).

The effect of the concentration of counterion on the t_r values of $[Cu–EDTA]^{-2}$ was examined as well. A plot of t_r versus counterion concentration is illustrated in Fig. 3. The use of tetrabutylammonium hydroxide as a counterion leads to a creation of a primary cation layer which is countered by an hydroxylic and phosphoric secondary anion layer on the surface of the static phase. As the concentration of counterion increases a corresponding charge is observed on the secondary anion layer, leading to complex carboxylic anions destruction. At the same time phosphoric anions due to their selectivity to TBA^+ favor ion pairing governed to rapid complex elution.

The specificity of the HPLC method is illustrated in Fig. 4, where complete separation of the compounds (Par and EDTA) was observed. Typical chromatograms of: (a) blank sample of injection matrix; (b) EDTA alone in injection matrix; and (c) Par and EDTA in injection matrix.

Paracetamol was eluted at 3.61 min while $[Cu–EDTA]^{-2}$ appeared at 10.34 min. It is obvious that a very good separation of $[Cu–EDTA]^{-2}$ complex and the other ingredients of the injection form was accomplished. This implies that using the proposed HPLC method, EDTA could be determined accurately without any interference.

3.2. Statistical analysis of chromatographic data

Under the experimental conditions described above, good correlation was observed between the peak area ratio of EDTA and the corresponding concentration as shown by the equations presented in Table 1. The correlation coefficient (r) and the standard error of estimate (Sr) of the calibration curves are also given, along with the standard deviation (S.D.) of the slopes and intercepts.

The average regression equations for EDTA in standard solutions and in injections forms were:

$$S_{EDTA} = 1.4\ (\pm 1.6) + 11.5\ (\pm 0.07)\ C,$$

$$S_{EDTA} = 7.2\ (\pm 7.7) + 12.9\ (\pm 0.23)\ C.$$

The correlation coefficient of each calibration curve constructed invariably exceeded 0.9995.

The limit of detection attained, as defined by IUPAC [43] $LOD_{(k=3)} = kS_a/b$, where b is the slope of the calibration graph and S_a is the S.D. of the blank signal, was found to be 1.97 μg ml^{-1} for EDTA. The limit of quantitation LOQ was also attained according to the IUPAC definition $LOQ_{(k=10)} = kS_b/b$, and was found to be 5.96 μg ml^{-1} for EDTA.

Inter-day data for the precission and accuracy of the method given in Table 2, indicate for EDTA R.S.D.% = 1.5–2.2 and E_r% = 1.48–2.08. Moreover, the intra-day R.S.D.% and E_r% values (Table 2) for the determination of EDTA were ranged from 1.6 to 1.9 and −0.5 to 0.8, respectively.

The accuracy of the method was also assessed by analysing placebo solutions spiked with known amounts of EDTA corresponding to 80, 100 and 120% of the theoretical value. These experiments were performed in tripicate. The recovery results for EDTA in the specific injection forms are shown in Table 3.

The statistical evaluation of EDTA reveals its good linearity and reproducibility and led us to the conclusion that it could be applied for determination of EDTA in pharmaceutical forms.

In conclusion, a simple high-performance liquid chromatographic method was developed for the analysis of EDTA in injection forms. The method proved to be selective, accurate, precise and suitable for the quantitative determination of EDTA in this complex matrix. Furthermore, a new approach for the detection of EDTA, based on the complexation of this compound with Cu^{+2} ions, was used and proved to be successfull. This approach could also be applied for the detection of EDTA in a more complex matrix. The method was also applied successfully to the analysis of commercial injection forms containing EDTA as an additive.

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