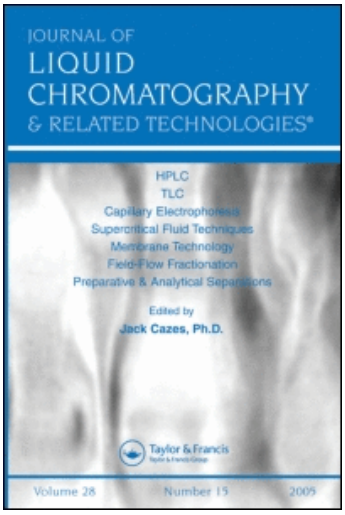

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HPLC METHOD FOR THE DETERMINATION OF EDTA IN AN OPHTHALMIC CLEANSER

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ABSTRACT

A reversed-phase high-performance liquid chromatographic (HPLC) procedure using ultra-violet (UV) detection for the analysis of edetate disodium (EDTA) via complexation with iron, in an ophthalmic cleanser, is reported. The method is selective, accurate, and reproducible. The peak area versus EDTA concentration is linear over the range of 50-150% of its label claim of 0.30 mg/mL, with a detection limit of 200 ng/mL. The mean absolute recovery of EDTA, using the described method, is 102.7 ± 0.4% (mean ± SD, n = 10).

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A stress study with heat, acid, base and UV radiation indicates that the method is stability-indicating with no interference from excipients or degradation products.

INTRODUCTION

An HPLC method for the determination of EDTA in a sterile ophthalmic cleanser that contains no dyes or perfumes, is reported. This cleanser is ideal for conditions that require daily eyelid hygeine and can also help in removing accumulated oily debris.

EDTA is a chelating agent that forms stable water soluble complexes with metal ions. EDTA is used commercially as a water softener, antioxidant, antibacterial, and anticoagulant.[1] Several methods exist for the determination of EDTA, including polarography,[2] amperometry,[3] catayltic-fluorometry,[4] spectrophotometry,[5] and potentiometry.[6,7] HPLC methods have been developed for EDTA, based on absorbance ratioing[8] and complexation with either iron or copper.[9-13]

To our knowledge, only two HPLC methods exist for the determination of EDTA in ophthalmic products.[9,13] Neither method examined the effect of stressing the ophthalmic product with heat, acid, base, or UV radiation. The degradation resulting from stressed samples could pose a selectivity problem. Also, the method described by Hall and Takahasi[9] is based on the method described by Bauer et al.,[13] whereby EDTA was complexed with copper.

Yamaguchi et al.[12] reported that the $Fe(EDTA)^-$ complex exhibited greater stability than the $Cu(EDTA)^{2-}$ complex. Consequently, iron complexation was selected for this current work.

The method described herein for the quantitation of EDTA is stability-indicating and satisfies the USP XXII guidelines under Assay Category I.[14] According to the USP XXII guidelines, analytical methods for the quantitation of major components of bulk drug substance or preservatives in finished pharmaceutical products fall under Assay Category I.[14] Data elements required for Assay Category I include precision, accuracy, selectivity, range, linearity, and ruggedness.

EXPERIMENTAL

Chemicals and Reagents

The ophthalmic cleanser was formulated at IOLAB Corporation (Claremont, CA, USA), which was subsequently acquired by CIBA Vision Ophthalmics (Duluth, CA). Edetate disodium was a USP reference standard. Ferric chloride hexahydrate and tetrabutylammonium hydrogen sulfate were purchased from Aldrich (Milwaukee, WI, USA). The water was deionized and then distilled.

All reagents were used without further purification.

Apparatus

The chromatographic system consisted of a Waters Model 600E system controller and pump, a WISP 712 autosampler, and a 486 variable-wavelength UV detector set at 254 nm (Waters Associates, Milford, MA, USA). A stainless-steel Adsorbosphere HS C_{18} column (4.6 x 150 mm, 3 μm, Alltech Associates, Inc., Deerfield, IL, USA) was maintained at ambient temperature.

Mobile Phase

The mobile phase simply consisted of 50 mM tetrabutyl ammonium hydrogen sulfate in water. The flow rate was 1.0 mL/minute with a typical operating pressure of *ca.* 130 bar.

Sample Preparation

An EDTA Stock solution was prepared at 1.50 mg/mL in water. A Standard solution was prepared by diluting the Stock solution 1:125 (v/v) with 0.1 mM ferric chloride hexahydrate prepared in water.

The Test solution was prepared by diluting the ophthalmic cleanser (label claim 0.30 mg/mL EDTA) 1:25 (v/v) with 0.1 mM ferric chloride hexahydrate prepared in water.

System Suitability

The system suitability results are calculated according to Chromatography <621> of the USP XXII from typical chromatograms.[14] The instrument precision, as determined by six successive injections of an EDTA Standard solution, should provide a relative standard deviation (RSD) not greater than 1.0%. The column efficiency should be greater than 4500 theoretical plates. The tailing factor should not exceed 2.0 at 5% peak height.

The Test solution (ophthalmic cleanser with sample work-up) is used to verify that the method meets all suitability limits, with exception of the instrument precision.

Stress Study

The selectivity of the method was studied through the analysis of stressed Test and Placebo (Test solution without EDTA) solutions. The stressed samples were subjected to heat, acidic, basic, and UV light environments.

Three mL aliquots of the Test and Placebo solutions were sealed in transparent containers and exposed to a UV radiation source (200-400 nm, 35 mWatt/cm^2) for 40 hours. Other 3.0 mL aliquots were adjusted to either pH 2 with concentrated HCl or pH 12 with 50% NaOH and sealed in glass containers with equal headspace and stored at 85°C for 40 hours.

Data Acquisition

The peak area of EDTA was measured using a PE Nelson 900 series interface and down-loaded to a PE Nelson Turbochrom 3 workstation (Perkin-Elmer Corporation, Cupertino, CA, USA). The chromatographic data was automatically processed for peak area, followed by an unweighted linear regression analysis.

Calculations

The response factor, **RF**, of the Fe-EDTA complex is determined by:

$$RF = \frac{W_S \times F}{V \times PA_S} \qquad (1)$$

where $\mathbf{W_s}$ is the weight (mg) of the edetate disodium standard used, **F** is the purity factor (mg/mg) of edetate disodium in the standard, **V** is the dilution volume, and $\mathbf{PA_S}$ is the peak area of Fe-EDTA complex for the Standard solution. Edetate disodium content of the Test sample, $\mathbf{C_T}$, is:

$$C_T(\text{mg / mL}) = \text{RF} \times \text{PA}_T \qquad (2)$$

where $\mathbf{PA_T}$ is the peak area of Fe-EDTA complex for the Test solution.

RESULTS AND DISCUSSION

Chromatography

Typical chromatograms obtained from a 20 μL injection of a Standard, Test and Placebo solution are illustrated in Figure 1 (a-c). The retention time of the Fe-EDTA complex is 5.2 minutes, with an overall chromatographic run time of 20 minutes.

System Suitability

The column efficiency for EDTA was 5723 theoretical plates. The tailing factor of EDTA was 1.2. The instrument precision, determined by 6 replicate injections of the Standard solution, exhibited a RSD of 0.5%.

Precision and Accuracy

The precision (RSD) and accuracy (relative error, RE) was determined by analyzing EDTA standards ranging from 50-150% (0.15 - 0.44 mg/mL), in replicates of six (Table 1).

Linearity

A linear response in peak area for EDTA over the range of 50-150% its label claim was observed. The correlation coefficients were 0.999 or better (n=6).

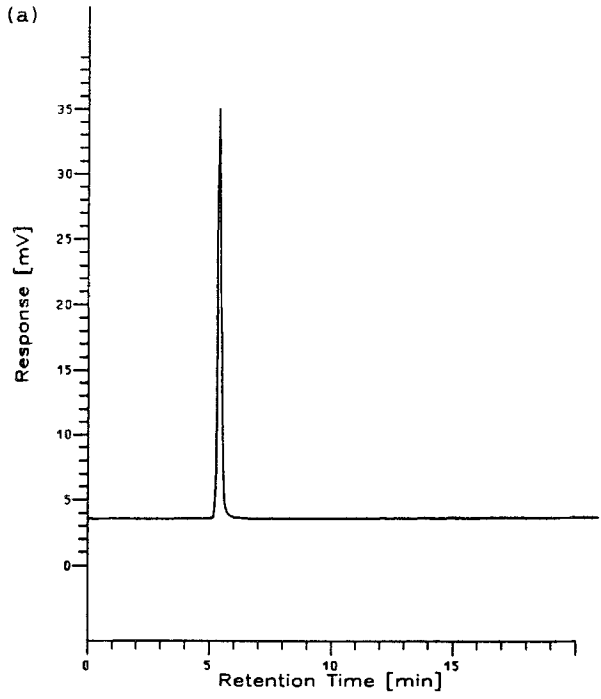
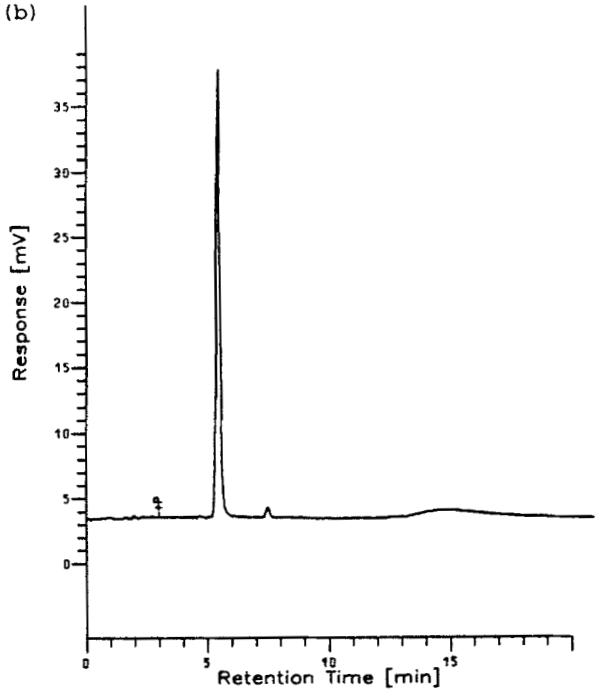
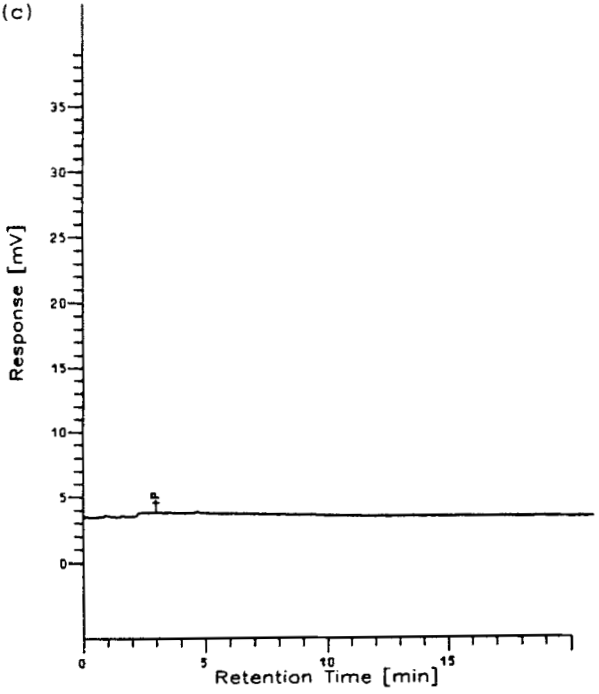


Figure 1. Typical chromatograms of (a) a Standard solution, (b) a Test solution (ophthalmic cleanser containing EDTA). (c) Typical chromatogram of a Placebo (ophthalmic cleanser not containing EDTA).

(b)



(c)



Selectivity

The ophthalmic cleanser was stressed with heat, acid, base, and UV radiation. The heat-stressed ophthalmic cleanser at 40°C for 40 hours did not result in any degradation. The acid-stressed samples were adjusted to pH 2 with concentrated HCl and heated at 85°C for 40 hours. EDTA degradation of 8% was observed for the acid-stressed samples under the described conditions.

The base-stressed samples were adjusted to pH 12 with 50% NaOH and heated at 85°C for 40 hours. EDTA degradation of 2% was observed for the base-stressed samples under the described conditions. Ultraviolet light-stressed samples were placed in the path of a UV lamp at 40 mWatt/cm^2 for 40 hours. EDTA degradation of 32% was observed for the UV stressed samples under the described conditions.

Despite the observed degradation, no interfering peaks at the retention time of the Fe-EDTA complex were observed in any of the stressed samples.

Table 1

Precision and Accuracy of EDTA in Ophthalmic Cleanser

Nominal Conc. (mg/mL)	n	Mean Found Conc. (mg/mL)	%RSD	%RE
0.152	6	0.157	0.3	3.2
0.223	6	0.230	0.4	2.9
0.294	6	0.302	0.5	2.8
0.364	6	0.372	0.2	2.2
0.436	6	0.448	0.2	2.6

CONCLUSION

The precision of the method is below 0.5%, while the accuracy is within 0.8%. The method is rapid and requires minimal sample pretreatment, resulting in *ca.* 100 samples being analyzed daily.

The described assay for the analysis of EDTA is selective, sensitive, and robust. Furthermore, the method is stability-indicating with no interference

from degradation products or excipients under the described stress conditions. Consequently, it is anticipated that this method could be used for the routine analysis of EDTA in ophthalmic preparations.

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