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Note

High-performance liquid chromatographic, stability indicating assay for disodium EDTA in ophthalmic preparations

JOHN BAUER*, DOUGLAS HEATHCOTE and SUZANNE KROGH
Abbott Laboratories, North Chicago, IL 60064 (U.S.A.)
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The use of the chelating agent ethylenediaminetetraacetic acid (EDTA) is widespread in the pharmaceutical industry. It is used as a treatment of heavy metal poisoning[1] as well as an antioxidant in many pharmaceutical preparations. EDTA is generally added as the disodium salt and functions by chelating heavy metals which often catalyze oxidations. The current analytical methodology for EDTA in formulations is titrimetric. It involves titration of a preparation of chelometric standard calcium carbonate using the EDTA formulation as titrant with hydroxy naphthol blue indicator (for sterile EDTA solution for injection)[2] or titration of the EDTA solution with standard magnesium solution using Arsenazo I as the indicator and tris(hydroxymethyl)aminomethane as a buffer[3]. Both of these methods are time-consuming and non-specific. Other methods for determining EDTA are reported in the literature including thin-layer chromatography, gas chromatography[4] and high-performance liquid chromatography (HPLC)[5], however, in these methods EDTA has to be either derivatized or converted to a photochemically unstable iron complex prior to analysis. In 1981 Parkes *et al.*[6] developed an HPLC method for the determination of the impurity nitrilotriacetic acid in bulk EDTA. This system has been modified and adapted in our laboratories to the analysis of EDTA in ophthalmic preparations and has provided a direct stability indicating assay for EDTA in a variety of media. The method has increased sample throughout by approximately 300%. Although the method has only been fully validated for ophthalmic preparations it has also been successfully applied to both serum and feces samples.

EXPERIMENTAL

The nitrilotriacetic acid disodium salt used in these studies was obtained from Aldrich. The EDTA was a USP reference standard.

The HPLC system comprised a Waters Model 6000A chromatographic pump, a DuPont variable-wavelength detector with UV lamp operated at 254 nm and a Waters μBondapak C_{18} column, 30 cm × 4 mm I.D. Data were collected and chromatograms displayed on a Hewlett-Packard 3388 recording integrator. The injection volume was 30 μl.

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Mobile phase

Ten ml of 1 *M* tetra-*n*-butylammonium hydroxide solution was added to 910 ml of water and the pH of the solution was adjusted to 7.5 with phosphoric acid. An 80-ml volume of methanol was added, the solution mixed thoroughly, filtered through a 0.45-μm filter and deaerated for approximately 10 min.

Sample solvent (0.2% in copper sulfate)

Cupric sulfate pentahydrate (3 g) is dissolved was 1 l water.

Nitrilotriacetic acid internal standard solution

A solution containing approximately 200 mg of nitrilotriacetic acid disodium salt was prepared by dissolving and diluting to volume with water to 100 ml (approximately 2 mg/ml).

EDTA standard

Approximately 200 mg of disodium EDTA was weighed accurately into a 100-ml volumetric flask and dissolved and diluted to volume with distilled water. A 5.0-ml volume of this solution was pipetted into a 25-ml volumetric flask and 5.0 ml of internal standard solution was added. The mixture was then diluted to volume with 0.2% copper sulfate solution (approximately 0.4 mg/ml).

Sample preparation

A 5.0-ml volume of sample was pipetted into a 25-ml volumetric flask. A 5.0-ml volume of internal standard solution was added and the mixture was diluted to volume with 0.2% cupric sulfate solution.

RESULTS AND DISCUSSION

The comparative precision data presented (Table I) demonstrate that the HPLC method is more reproducible than the titrimetric method. A typical chromatogram is shown in Figure 1. The EDTA response is linear and gave a correlation coefficient of 0.99999 in the range of 0.2 to 2.1 mg/ml. The analysis is applicable to

TABLE I

COMPARISON OF PRECISION FOR EDTA ANALYSES

	Precision for EDTA assay by HPLC (%)	*Precision for EDTA titrimetic assay (arsenazo I method) (%)*
	100.05	97.90
	98.75	98.00
	99.58	99.80
	99.62	98.90
	100.58	101.00
	99.65	100.20
Mean	99.71	99.3
S.D.	0.603	1.24
R.S.D.	0.605	1.25

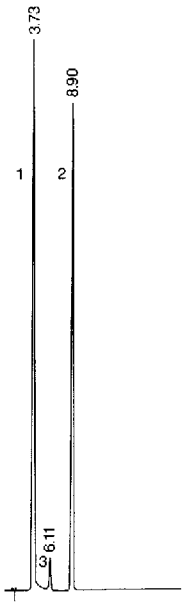

Fig. 1. Typical chromatogram of EDTA in opthalmic preparation. Peaks: 1 = EDTA; 2 = nitrilotriacetic acid.

a variety of ophthalmic solutions (Table II) and has been successfully used to quantitate EDTA in recalcified blood serum, EDTA solution and human feces.

The HPLC assay presented constitutes a direct, stability indicating analysis for

TABLE II

OPTHALMIC PREPARATIONS ASSAYED BY HPLC METHOD

All samples were purchased retail.

Product	*Label claim (mg/ml)*	*Found (mg/ml)*
Decongestants		
Clear Eyes	1.0	1.008
Murine	0.5	0.504
Murine Plus	1.0	0.988
Visine	1.0	1.000
Moisture Drops	*	1.082
Vaso Clear A	*	0.262
Collyrium	1.0	0.991
Tear Gard	1.0	1.021
Degest 2	*	0.196
Vasocon	*	0.291
Lens care		
Lensineyes	1.0	1.002
Sensitive eyes	*	0.248
Soft-mate.	2.0	1.923

* No label claim available.

a very common antioxidant used to chelate metals in topical occular decongestants. The technique is more reproducible than the present titrimetric assay and does not suffer from the interferences possible in the titrimetric assay. The HPLC method presented can be easily automated and allows analysis of a variety of EDTA containing solutions on a single system.

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