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Research Article

Simultaneous Determination of EDTA, Sorbic Acid, and Diclofenac Sodium in Pharmaceutical Preparations Using High-Performance Liquid Chromatography

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Abstract. A simple high-performance liquid chromatographic method for simultaneous determination of ethylenediaminetetraacetic acid (EDTA), sorbic acid, and diclofenac sodium was developed and validated. Separation was achieved on a C_{18} column (10 cm×4.6 mm) using gradient elution. The mobile phase consisted of acetonitrile–ammonium dihydrogen phosphate buffer solution (0.01 M, pH=2.5, containing 0.8% tetra-*n*-butyl ammonium hydroxide). The detector wavelength was set at 254 nm. Under these conditions, separation of three compounds was achieved in less than 10 min. The effect of two metal salts and metal concentration on peak area of EDTA was investigated. The pH effect on retention of EDTA and sorbic acid was studied. The method showed linearity for EDTA, sorbic acid, and diclofenac in the ranges of 2.5–100.0, 5.0–200.0, and 20.0–120.0 μg/mL, respectively. The within- and between-day relative standard deviations ranged from 0.52 to 1.94%, 0.50 to 1.34%, and 0.78 to 1.67% for EDTA, sorbic acid, and diclofenac, respectively. The recovery of EDTA, sorbic acid, and diclofenac from pharmaceutical preparation ranged from 96.0–102.0%, 99.7–101.5%, to 97.0–102.5%, respectively. To the best of our knowledge, this is the first report about simultaneous determination of EDTA, sorbic acid, and diclofenac.

KEY WORDS: diclofenac sodium; EDTA; high-performance liquid chromatography; pharmaceutical preparations; sorbic acid.

INTRODUCTION

Ethylenediaminetetraacetic acid (EDTA) and edetate salts are used in pharmaceutical formulation, cosmetics, and foods as chelating agents; that is, they form stable water-soluble complexes with alkaline earth and heavy metal ions. The stability of the metal–edetate complex depends on the metal ion involved and on the pH of solution. The calcium chelate is relatively weak and will preferentially chelate heavy metals, such as iron, copper, and lead, with the release of calcium ions. For this reason, edetate calcium disodium (calcium EDTA) is used therapeutically in cases of lead poisoning. Calcium is displaced by heavy metals, such as lead, to form stable EDTA complexes that are excreted in urine.

EDTA and disodium edetate possess some antimicrobial activity but are most frequently used in combination with other antimicrobial preservatives owing to their synergistic effects. Disodium edetate is also used as an anticoagulant since it will chelate calcium and prevent the coagulation of blood *in vitro* (1).

Sorbic acid is an antimicrobial preservative (2) with antibacterial and antifungal properties used in pharmaceuticals, foods, enteral preparations, and cosmetics. Generally, it is used at concentrations of 0.05–0.2% in oral and topical pharmaceutical formulations, especially those containing nonionic surfactants. Sorbic acid has limited stability and activity against bacteria and thus is frequently used in combination with other antimicrobial preservatives or glycols, where synergistic effects appear to occur.

Diclofenac sodium [sodium (*o*-{(2,6-dichlorophenyl) amino}phenyl)acetate] is a synthetic nonsteroidal anti-inflammatory drug widely used in clinical medicine for the treatment of inflammatory conditions such as rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis (3,4).

The various analytical methods have been proposed for the determination of EDTA (5–9), sorbic acid (10–14), and diclofenac sodium (15–31). To the best of our knowledge, this study is the first report describing simultaneous determination of EDTA, sorbic acid, and diclofenac sodium in pharmaceutical preparations.

Based on this study, a simple chromatographic method for the simultaneous determination of EDTA, sorbic acid, and diclofenac was developed and validated. The method was subsequently used for the determination of EDTA, sorbic acid, and diclofenac sodium in ophthalmic solutions.

MATERIAL AND METHODS

Chemicals

Disodium EDTA dihydrate, sorbic acid, ammonium dihydrogen phosphate, orthophosphoric acid, tetra-*n*-butyl

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Table I. Gradient Elution Program

Time (min)	Acetonitrile (%)	Buffer (%)
0.0	20	80
5.0	20	80
8.0	100	0
10.0	20	80
12.0	20	80

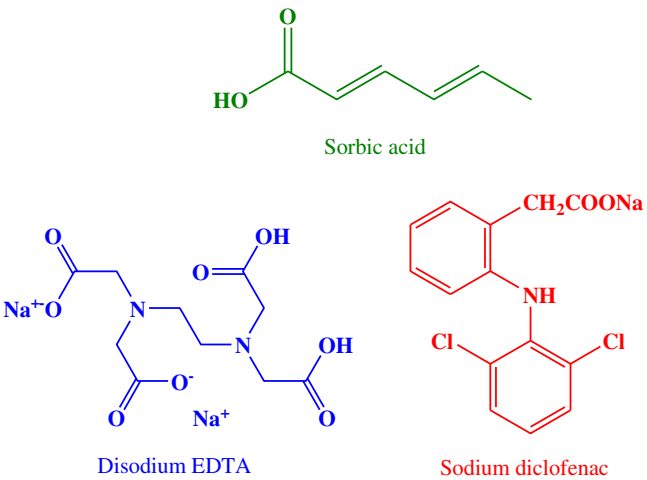

Fig. 1. Chemical structure of disodium EDTA, sorbic acid, and sodium diclofenac

ammonium hydroxide (20% solution in water), acetonitrile, $FeCl_3 \cdot 6H_2O$, and $Cu(NO_3)_2 \cdot 3H_2O$ were obtained from Merck (Darmstadt, Germany). Diclofenac sodium was supplied by EXCELLA (Germany). Deionized water from a Milli-Q system (Millipore, USA) was used for preparation of the buffer and sample solutions. Biofenac Ophthalmic solution (containing 0.1% *w/v* of diclofenac sodium, 0.1% *w/v* of disodium EDTA, and 0.2% *w/v* of sorbic acid) was obtained from the Bakhtar Bioshimi Pharmaceuticals Company (Kermanshah, Iran). Voltaren Ophthalmic (Novartis Pharmaceuticals Corporation, Canada) solution (containing 0.1% *w/v* of diclofenac sodium, 0.1% *w/v* of disodium EDTA, and 0.2% *w/v* of sorbic acid) was obtained from the market.

Apparatus and Conditions

The HPLC system (Waters, USA) which consisted of a binary pump (model 1525) with a Waters UV–vis detector (model 2487) was used. The Chromolith Speed Rod column (RP-C18, 100×4.6 mm) was purchased from Merck (Germany). The mobile phase was acetonitrile–ammonium dihydrogen phosphate buffer solution (0.01 M, pH=2.5, containing 0.8% tetra-*n*-butyl ammonium hydroxide (Bu_4NOH) as ion-pairing reagent) with gradient elution (Table I). The mobile phase flow rate was kept constant at 1.0 mL/min. The solutes were detected at 254 nm. The injection volume was 20 µL.

Preparation of Stock and Working Standard Solutions

Working standard solutions containing disodium EDTA and sorbic acid were prepared by dilution of individual aliquots of stock solutions with the diluent (solution of 250 µg/mL $FeCl_3 \cdot 6H_2O$). Two stock solutions were made by dissolving 100 mg of disodium EDTA in 100 mL water and 200 mg of sorbic acid in 100 mL water/methanol (1:1), separately. From these solutions, serial dilutions were made to obtain mixed standard solution with different concentration levels: 2.5, 5.0,

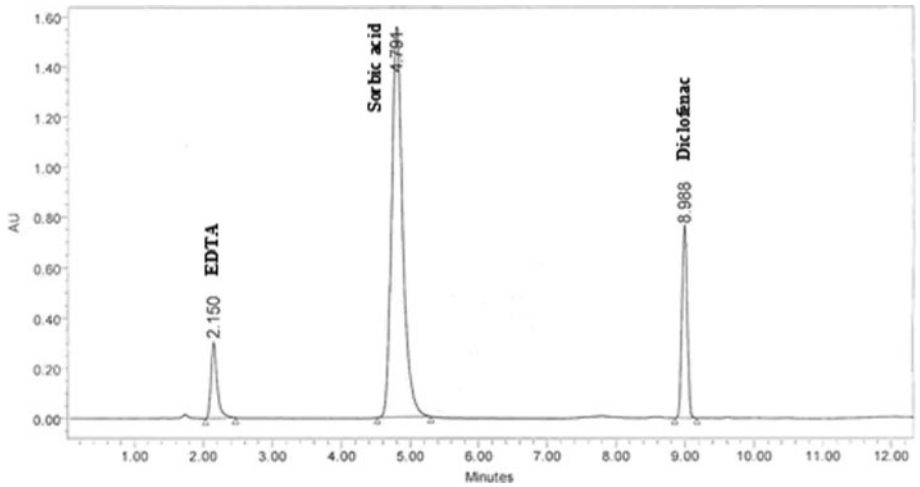

Fig. 2. A typical chromatogram for the mixture of disodium EDTA, sorbic acid, and diclofenac. Conditions: flow rate, 1.0 mL/min; mobile phase, acetonitrile–ammonium dihydrogen phosphate buffer solution (0.01 M, pH=2.5, containing 0.8% tetra-*n*-butyl ammonium hydroxide); $FeCl_3 \cdot 6H_2O$ concentration, 250 µg/mL

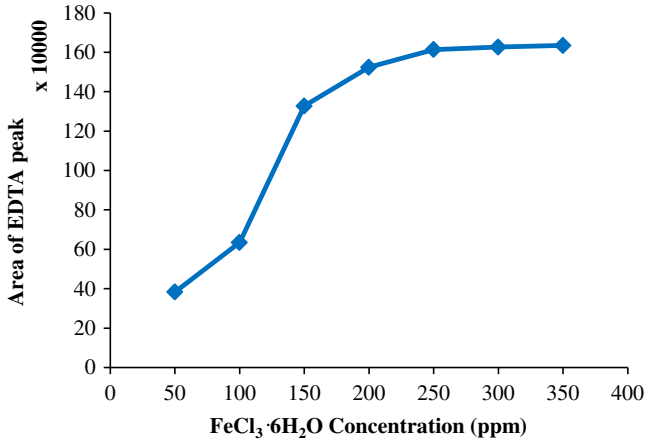

Fig. 3. Effect of $FeCl_3 \cdot 6H_2O$ concentration in diluent on peak area of the Fe–EDTA complex. Conditions: flow rate, 1.0 mL/min; mobile phase, acetonitrile–ammonium dihydrogen phosphate buffer solution (0.01 M, pH=2.5, containing 0.8% tetra-*n*-butyl ammonium hydroxide)

10.0, 20.0, 40.0, 60.0, 80.0, and 100.0 μg/mL, for disodium EDTA, and 5.0, 10.0, 20.0, 40.0, 80.0, 120.0, 160.0, and 200.0 μg/mL, for sorbic acid. Each solution was injected three times. The average peak area of each compound was plotted *vs*. concentration, and calibration curves were constructed using a least square regression equation.

The calibration curve for diclofenac sodium was constructed using standard addition method. The standard solution of diclofenac sodium was prepared by dissolving 100 mg of diclofenac sodium in 100 mL water. From this solution, different volumes (0.0, 0.1, 0.2, 0.4, 0.6, 0.8, and 1.0 mL) were transferred to a 10-mL volumetric flask containing 0.2 mL of sample (ophthalmic solution) and diluted with the diluent to obtain the standard solutions with different concentration levels (20.0, 30.0, 40.0, 60.0, 80.0, 100.0, and 120.0 μg/mL). The peak area of diclofenac was plotted *vs*. concentration of standard solution.

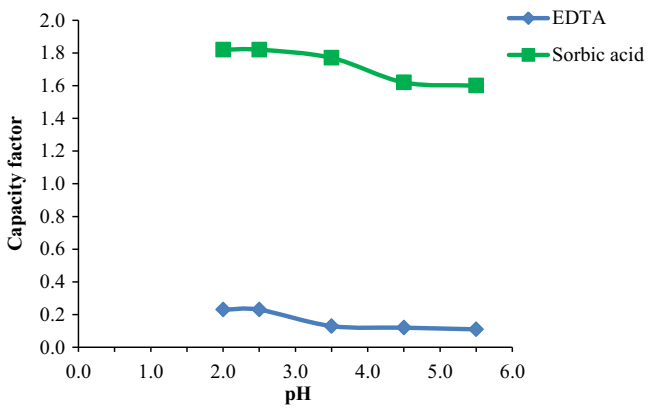

Fig. 4. The effect of pH on retention of the Fe–EDTA complex and sorbic acid. Conditions: flow rate, 1.0 mL/min; mobile phase, acetonitrile–ammonium dihydrogen phosphate buffer solution (0.01 M, containing 0.8% tetra-*n*-butyl ammonium hydroxide); $FeCl_3 \cdot 6H_2O$ concentration, 250 μg/mL

Table II. System Suitability Data

Parameters	EDTA	Sorbic acid	Diclofenac
Resolution factor[a]	–	8.5	12.0
Tailing factor[b]	1.25	1.34	1.18
%RSD for six injections	0.98	0.55	0.76

[a] Resolution factor is calculated between each peak and its nearest preceding neighbor ($R = 2\Delta t_r / w_1 + w_2$)

[b] Tailing factor is calculated at 5% of peak height according to the USP method ($T = \frac{a+b}{2a}$; b=distance from the point at peak midpoint to the trailing edge, a=distance from the leading edge of the peak to the midpoint)

Preparation of Sample Solutions

Sample solutions were prepared by transferring about 0.5 mL of the ophthalmic solutions (Biofenac and Voltaren Ophthalmic solutions) to a 10-mL volumetric flask and dilution with diluent to obtain a solution containing 50.0 μg/mL diclofenac sodium, 50.0 μg/mL disodium EDTA, and 100.0 μg/mL sorbic acid.

RESULTS AND DISCUSSION

The goal of this study was to develop a HPLC method for the quantification of EDTA, sorbic acid, and diclofenac (Fig. 1) in pharmaceutical formulations. To save time, simultaneous determination of the three compounds was developed. A typical chromatogram for these compounds is shown in Fig. 2. It is clear that the proposed method has a superior resolution between the three compounds.

The commonly used methods for EDTA determination by HPLC are based upon the complex formation of EDTA with copper or iron salts. The complexation between EDTA with Fe^{3+} leads to the formation of the Fe–EDTA complex that absorbs at 257 nm (32).

Analysis was performed with pH adjusted to 2.5, with the addition of tetra-*n*-butyl ammonium hydroxide as ion pair reagent to the mobile phase. Under these conditions, sorbic acid and diclofenac are electrically neutral. The Fe–EDTA complex forms an ion pair with tetra-*n*-butyl ammonium hydroxide in the mobile phase to become electrically neutral. The use of ion-pairing reagents as mobile phase additives allows the separation of ionic and polar substances on reversed-phase HPLC columns. The increase in hydrophobic character of the ion pair results in a greater

Table III. Linear Analytical Response Statistical Summary for Compounds Using Peak Area

Compound	Calibration range (μg/mL)	Slope	Intercept	R^2
EDTA	2.5–100.0	43,178	29,133	0.999
Sorbic acid	5.0–200.0	28,763	2×10^6	0.992
Diclofenac	20.0–120.0	28,179	93,347	0.996

Table IV. Precision of Within and Between Run Analysis (n=9; Three Sets for 3 Days)

Compound	Concentration (μg/mL)	RSD (%)[a]
Within day (n=3)		
EDTA	20.0	0.87
	40.0	0.72
	60.0	0.52
Sorbic acid	40.0	0.68
	80.0	0.53
	100.0	0.50
Diclofenac	20.0	0.99
	40.0	0.82
Between day (n=9)	60	0.78
EDTA	20.0	1.94
	40.0	1.67
	60.0	1.15
Sorbic acid	40.0	1.34
	80.0	1.08
	100.0	0.97
Diclofenac	20.0	1.67
	40.0	1.43
	60	1.11

[a] Percentage relative standard deviation

Table V. Accuracy Data for EDTA, Sorbic Acid, and Diclofenac Spiked in Pharmaceutical Formulations

Compound	Concentration added (μg/mL)	Concentration found (μg/mL)	Recovery (%)	Percent error
EDTA	20.0	19.2	96.0	−4.0
	40.0	39.3	98.2	−1.75
	60.0	61.2	102.0	2.0
Sorbic acid	40.0	40.2	100.5	0.5
	80.0	79.8	99.7	−0.25
	100.0	101.5	101.5	1.5
Diclofenac	20.0	19.4	97.0	−3.0
	40.0	41.0	102.5	2.5
	60	60.5	100.8	0.83

Table VI. Assay Data

Real sample	Compound	Label amount (μg/mL)	Determined amount (μg/mL)	Assay
Voltaren	EDTA	1,000.0	1,010.8	101.1
	Sorbic acid	2,000.0	2,012.0	100.6
	Diclofenac	1,000.0	996.8	99.7
Biofenac	EDTA	1,000.0	1,014.2	101.4
	Sorbic acid	2,000.0	1,986.5	99.3
	Diclofenac	1,000.0	989.2	98.9

affinity for the reverse stationary phase and leads to sample resolution.

The Effect of Metal Type and Concentration of Metal Ion

Most metal ions complex with a stoichiometric amount of EDTA at pH 10, but only a few, such as Fe^{3+} and Hg^{2+}, also complex at acidic pH. The effect of the two metal salt diluents ($FeCl_3{\cdot}6H_2O$ and $Cu(NO_3)_2{\cdot}3H_2O$) as diluent on the peak area of the EDTA complex was investigated. By using the $FeCl_3$ salt as the diluent, the peak area of Fe–EDTA was greater than when $Cu(NO_3)_2{\cdot}3H_2O$ was used to form the Cu–EDTA complex. Also, the K_f (formation constant) for the Fe–EDTA complex ($K_{Fe}=1.3\times10^{25}$) was greater than the K_f of Cu–EDTA ($K_{Cu}=6.3\times10^{18}$). Therefore, Fe salt was used as the diluent for further studies. The following complexation takes place when solutions of Fe^{3+} and $EDTA^{4-}$ are mixed:

$$Fe^{3+}(aq) + EDTA^{4-}(aq) \rightarrow [Fe(EDTA)]^{-}(aq)$$

In order to investigate the effect of $FeCl_3{\cdot}6H_2O$ on the response, 1.0 mL of the EDTA stock solution (1,000 μg/mL) was transferred to a 10-mL volumetric flask and diluted with various concentrations of diluent. The effect of concentration of $FeCl_3{\cdot}6H_2O$ salt on the peak area of the Fe–EDTA complex in the range of 50.0–350.0 μg/mL is

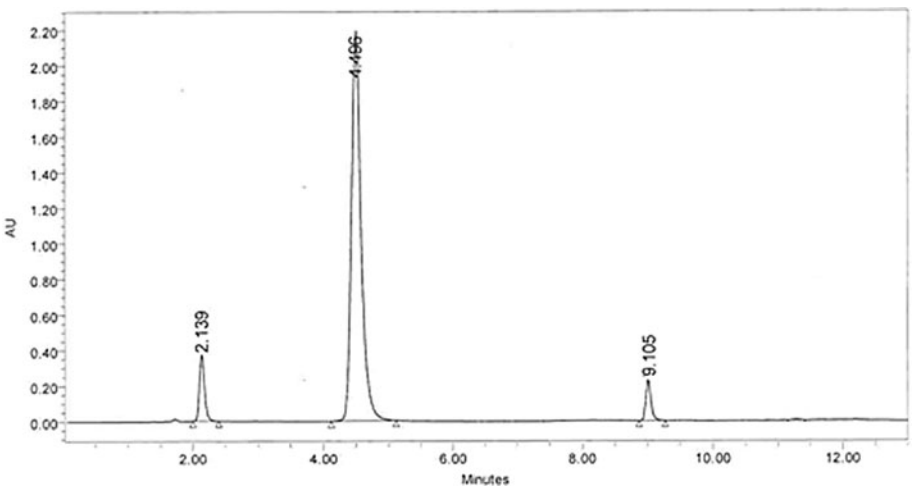

Fig. 5. HPLC chromatogram of Biofenac ophthalmic solution. Conditions: flow rate, 1.0 mL/min; mobile phase, acetonitrile–ammonium dihydrogen phosphate buffer solution (0.01 M, pH=2.5, containing 0.8% tetra-n-butyl ammonium hydroxide); $FeCl_3{\cdot}6H_2O$ concentration, 250 μg/mL

shown in Fig. 3. As is readily seen from Fig. 3, the peak area is considerably increased with the increasing of $FeCl_3 \cdot 6H_2O$ concentration from 50.0 to 250 μg/mL and remained constant with further increase in $FeCl_3 \cdot 6H_2O$ concentration. Thus, the diluent was prepared in 250 μg/mL concentration of $FeCl_3 \cdot 6 \times H_2O$.

The Effect of pH on Retention of the Fe–EDTA Complex and Sorbic Acid

The influence of mobile phase pH on retention (capacity factor) of the Fe–EDTA complex and sorbic acid on C_{18} phase was studied by changing the pH of the mobile phase from 2.0 to 5.5. Figure 4 shows the effect of pH on the capacity factors of the Fe–EDTA complex and sorbic acid. As is obvious, an increase in pH from 2.0 to 5.5 resulted in no large change in the capacity factors of the Fe–EDTA complex and sorbic acid. However, in low pH, the capacity factors of the Fe–EDTA complex and sorbic acid were increased slowly. Therefore, pH 2.5 was selected as the optimum value for further studies.

System Suitability

The purpose of the system suitability test is to ensure that the complete testing system (including instrument, reagents, column, and analyst) is suitable for the intended application. The system suitability was determined by making six replicate injections and analyzing each solute for its peak area, resolution, and peak tailing factor. The system suitability requirements for these compounds were a relative standard deviation (RSD) of less than 1.0, a peak tailing factor of less than 2.0, and a resolution (R_s) greater than 8.0 between adjacent peaks for all analytes. The results are shown in Table II.

Linearity

Linearity is the ability of the test method to provide results that are directly proportional to analyte concentration within a given range. Linearity range using this method for three compounds was wide. Standard curves were plotted using peak area *vs*. solute concentration. The method was linear from 2.5–100.0, 5.0–200.0, to 20.0–120.0 μg/mL for the Fe–EDTA complex, sorbic acid, and diclofenac, respectively. Each compound had R^2 values of 0.992 or greater. Statistical summaries of linear response for all analytes are shown in Table III.

Precision

Instrumental precision was determined by analyzing test samples by six replicate determinations. RSDs for these determinations were shown in Table II. Within-day precision was calculated by analyzing three standard solutions with different concentration levels for each analyte in the mixture. Between-day precision was performed by analyzing standard solutions in three different days. The results are shown in Table IV.

Accuracy

In order to investigate the presence of matrix effects on the proposed method, a recovery study was carried out. Percent error and recovery of the method were evaluated using spiked samples containing each analyte in different concentration levels. The results shown in Table V indicate that the procedure gives acceptable accuracy and recovery for all analytes in the pharmaceutical preparation.

Assay

The method developed in the present study was applied for the determination of EDTA, sorbic acid, and diclofenac in Biofenac Ophthalmic solution from the Bakhtar Bioshimi Pharmaceutical Company and Voltaren Ophthalmic solution. Figure 5 shows an HPLC chromatogram of the Fe–EDTA complex, sorbic acid, and diclofenac in a pharmaceutical preparation. Results of the assay experiment are exhibited in Table VI.

CONCLUSION

An accurate, sensitive, and reproducible HPLC method for the simultaneous separation and quantitation of EDTA, sorbic acid, and diclofenac in ophthalmic solutions has been developed. This method can be used for routine analysis and quality control of pharmaceutical preparations containing any of these pharmaceutical ingredients.

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