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RESEARCH PAPER

Stability-Indicating Spectrophotometric and Densitometric Methods for Determination of Aceclofenac

Y. S. El-Saharty, M. Refaat, and S. Z. El-Khateeb

Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, El-Kasr El-Aini St., ET-11562 Cairo, Egypt

ABSTRACT

Three methods were developed for the determination of aceclofenac in the presence of its degradation product, diclofenac. In the first method, third-derivative spectrophotometry (D_3) is used. The D_3 absorbance is measured at 283 nm where its hydrolytic degradation product diclofenac does not interfere. The suggested method shows a linear relationship in the range of 4–24 $\mu g\, mL^{-1}$ with mean percentage accuracy of 100.05±0.88. This method determines the intact drug in the presence of up to 70% degradation product with mean percentage recovery of 100.42±0.94. The second method depends on ratio-spectra first-derivative (RSD_1) spectrophotometry at 252 nm for aceclofenac and at 248 nm for determination of degradation product over concentration ranges of 4–32 $\mu g\, mL^{-1}$ for both aceclofenac and diclofenac with mean percentage accuracy of 99.81±0.84 and 100.19±0.72 for pure drugs and 100.17±0.94 and 99.73±0.74 for laboratory-prepared mixtures, respectively. The third method depends on the quantitative evaluation of thin-layer chromatography of aceclofenac using chloroform:methanol: ammonia (48:11.5:0.5 v/v/v) as a mobile phase. Chromatograms were scanned at 274 and 283 nm for aceclofenac and diclofenac, respectively. The method determined aceclofenac and diclofenac in concentration ranges of 2–10 and 1–9 $\mu g\, spot^{-1}$ with mean percentage accuracy of 100.20±1.03 and 100.14±0.98 for pure drugs and 99.77±0.74 and 100.07±0.78 for laboratory-prepared mixtures, respectively. This method retains its accuracy in the presence of up to 80% degradation product for the studied drug.

The suggested procedures were checked using laboratory-prepared mixtures and were successfully applied for the analysis of their pharmaceutical preparation. The validity of the proposed methods was further assessed by applying a standard addition technique. The obtained results agreed statistically with those obtained by the reported method.

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Key Words: *Aceclofenac; Determination; Diclofenac sodium; Spectrodensitometry; Spectrophotometry; Stability indicating*

INTRODUCTION

Aceclofenac, 2({2-[(2,6-dichlorophenyl)amino]-phenyl}acetyl)oxy acetic acid (Fig. 1), is a new non-steroidal anti-inflammatory, antirheumatic, and analgesic drug of the phenyl acetic acid group (1). It is used for the treatment of inflammatory disorders and acute pain with better gastric tolerance (2–5).

Diclofenac, 2-[(2,6-dichlorophenyl)amino]phenyl acetic acid (Fig. 1), is a non-steroidal anti-inflammatory drug used in the treatment of rheumatic diseases (2–4). 4′-Hydroxy aceclofenac, diclofenac, and 4-hydroxy diclofenac are the metabolites of aceclofenac in rat and human (6–8).

High-performance liquid chromatography (HPLC) methods are reported for the simultaneous determination of aceclofenac, diclofenac, and their metabolites in biological samples (6,7,9). A few techniques are described for the determination of aceclofenac in preparations, including spectrophotometric and spectrofluorimetric methods (10) and stripping voltammetry (11). Several analytical methods have been reported for the determination of diclofenac alone or with its metabolites in biological fluids and pharmaceutical preparations including gas chromatography (12,13), gas chromatography with mass spectrometry (14–16), HPLC (17–20), spectrophotometric methods (21–24), and thin-layer chromatography (25,26).

One of the classical analytical problems is the simultaneous determination of two or more compounds in the same sample, without previous chemical separation. Derivative spectrophotometry has been applied extensively to the simultaneous determination of substances with overlapping spectra, which is frequently made on the basis of zero-crossing measurements. Recently, a new spectrophotometric method for resolving binary mixtures was developed (27). The method is based on the use of first derivatives of the ratio spectra. The absorption spectrum of the mixture is obtained and divided (amplitudes at each wavelength) by the absorption spectrum of a standard solution of one of the components, and the first derivative of the ratio spectrum is obtained. The only method available for determination of the two drugs is HPLC (9), so there was a need to develop simple and accurate alternative methods that can be used for the determination of the two analytes. The purpose of this study was to determine aceclofenac in the presence of its degradation product, diclofenac, by simple, rapid, and selective stability-indicating derivative spectrophotometric and densitometric assays for quality control and routine analysis.

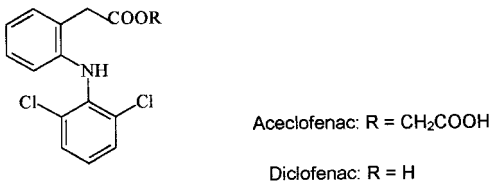

Figure 1. Chemical structures of aceclofenac and diclofenac.

EXPERIMENTAL

Apparatus

- All absorption spectra and derivatives were recorded with a Shimadzu UV-1601 PC (UV–visible double-beam spectrophotometer with 1-cm quartz cuvettes, Shimadzu Corporation, Kyoto, Japan).
- Densitometer: dual wavelength Shimadzu flying CS-9000 with video display and high-speed, high-quality, parallel-head printer/plotter.
- Hamilton micro-syringe, 25 μL, calibrated at 0.2 μL per unit.
- Thin-layer chromatography (TLC) plates: precoated with silica gel GF, 0.25 mm thickness, fluorescent at 254 nm (E. Merck, Darmstadt, Germany).

Materials

Samples

- Aceclofenac was kindly supplied by Bristol-Myers Squibb (Egypt). Its purity was found to be 100.29±0.50 according to the British Pharmacopoeia method (1).

- Diclofenac sodium was kindly supplied by GlaxoWellcome Egypt (El-Salam City, Cairo, Egypt). Its purity was found to be 99.98±0.55 according to the British Pharmacopoeia method (1).
- Bristaflam® tablets (Bristol-Myers Squibb, Egypt). Batch No. c00557; labeled to contain 100 mg aceclofenac per tablet.
- Rheumafen® tablets (Amoun Pharmaceutical Industries, El-Obour City, Cairo, Egypt). Batch No. 143; claimed to contain 25 mg diclofenac sodium per tablet.
- Rheumafen injection, 75 mg diclofenac sodium per ampoule. Rheumafen SR capsules, 100 mg diclofenac sodium per capsule. Rheumafen Acti gel, 1 g diclofenac sodium per 100 g gel. All from GlaxoWellcome Egypt (El-Salam City, Cairo, Egypt). Batch No. 001688A, 001326B, and 000074A, respectively.

Reagents and Solvents

All chemicals and reagents were of pure analytical grade. Methanol, chloroform, concentrated ammonia (25%), and sodium hydroxide all obtained from El-Nasr Pharmaceutical Chemicals (Abu Zabaal, Cairo, Egypt).

Methods

Preparation of Degradation Product of Aceclofenac

Weigh accurately 50 mg of pure authentic aceclofenac in a 250 mL flask. Dissolve in 10 mL methanol, add 25 mL 2 M sodium hydroxide, and make reflux for 18–20 hr. Neutralize the solution with 2 M hydrochloric acid. Filter the formed precipitate and dissolve in 10 mL methanol. Apply the methanol solution in band form to a TLC plate side-by-side with 20 μL of stock solution of aceclofenac and diclofenac sodium in methanol.

The plates were developed using chloroform:-methanol:ammonia (25%) (48:11.5:0.5) as a mobile phase in chromatographic tanks previously saturated with the developing mobile phase for 30 min. The degradation product band corresponding to diclofenac sodium spot was visualized under ultraviolet (UV) light at 254 nm, scraped, and extracted with 3×10 mL portions of methanol. The extract was filtered and evaporated just to dryness in a boiling water bath. The residue left after evaporation was used as the degradation product of aceclofenac, diclofenac.

Preparation of Standard Solutions

Stock Solutions

Aceclofenac and diclofenac sodium stock solutions (1 mg mL^{-1}): weigh accurately 100 mg each of aceclofenac and diclofenac sodium powder in two separate 100-mL measuring flasks. Add 50 mL methanol, shake for a few minutes, and complete to volumes with the same solvent.

Working Solutions

Transfer 1 mL each of the stock solutions of aceclofenac and diclofenac sodium in two separate 25 mL measuring flasks and dilute to the mark with methanol to get a final concentration of 40 μg mL^{-1} of both drugs for D_3 and RSD_1 spectrophotometric methods. Meanwhile use the stock solutions of aceclofenac and diclofenac sodium, (1 mg mL^{-1} in methanol) for the spectrodensitometric method.

Laboratory-Prepared Mixtures

For the D_3 and RSD_1 spectrophotometric methods, transfer aliquot portions $1, 2, \ldots, 6$ mL and $1, 2, \ldots, 8$ mL of aceclofenac from its working solution (40 μg mL^{-1}) into a series of 10-mL measuring flasks. Add 10–90% of diclofenac sodium (degradation product) using its prepared working solution (40 μg mL^{-1}) to the same flasks.

For the densitometric method transfer aliquot portions equivalent to $2, 4, \ldots, 10$ μg of aceclofenac from its stock solution 1 mg mL^{-1} to 5 mL measuring flasks. Add 10–90% of diclofenac sodium, using the prepared stock solution (1 mg mL^{-1}) to the same flasks.

Procedures

D_3 Spectrophotometric Method

Construction of Calibration Curve

Transfer accurately measured volumes $(0.5, 1, \ldots, 3$ mL) of aceclofenac working solution (40 μg mL^{-1}) into 5-mL measuring flasks, and dilute to volume with methanol to get a final concentration ranging from 4 to 24 μg mL^{-1}. Plot peak amplitudes at 283 nm vs. the corresponding

concentrations to obtain the calibration curve, and compute the regression equation.

Assay of Laboratory-Prepared Mixtures

Record the third derivative spectrum of laboratory-prepared mixtures containing different ratios (10–90%) of aceclofenac and its degradation product, diclofenac sodium. Measure D_3 values at 283 nm, then calculate the concentration of aceclofenac from the corresponding regression equation.

Application to Pharmaceutical Preparation (Bristaflam Tablets)

Weigh and finely powder not less than 20 tablets. Transfer a portion of powdered tablets equivalent to one tablet (100 mg of aceclofenac) into a 100-mL measuring flask, shake, and dissolve in methanol ($1\,mg\,mL^{-1}$). Transfer 1 mL of extract into a 25-mL volumetric flask and dilute to the mark with methanol to get a final concentration of $40\,\mu g\,mL^{-1}$, then proceed as for construction of the calibration curve.

RSD_1 Spectrophotometric Method

Construction of Calibration Curve

For aceclofenac The absorption spectra of aceclofenac in the range of $4–32\,\mu g\,mL^{-1}$ were divided by the absorption spectra of diclofenac sodium ($25\,\mu g\,mL^{-1}$), where the obtained ratio spectra were differentiated with respect to wavelength. First-derivative values at 252 nm were plotted vs. the corresponding concentration, and the regression equation was computed.

For diclofenac sodium The absorption spectra of diclofenac sodium in the range of $4–32\,\mu g\,mL^{-1}$ were divided by the absorption spectra of aceclofenac ($25\,\mu g\,mL^{-1}$), where the obtained ratio spectra were differentiated with respect to wavelength. First-derivative values at 248 nm were recorded, plotted vs. the corresponding concentration, and the regression equation was computed.

Assay of Laboratory-Prepared Mixtures

The absorption spectra of different laboratory-prepared mixtures were divided by either the absorption spectra of diclofenac sodium ($25\,\mu g\,mL^{-1}$) for the determination of aceclofenac or the absorption spectra of aceclofenac ($25\,\mu g\,mL^{-1}$) for the determination of diclofenac sodium. The RSD_1 values were recorded at 252 and 248 nm for aceclofenac and diclofenac sodium, respectively. The concentration of each one was calculated from the corresponding regression equation.

Application to Pharmaceutical Preparations

For aceclofenac (*Bristaflam tablets*) Proceed exactly as mentioned for the D_3 spectrophotometric method (application to pharmaceutical preparations) up to "into a 25-mL volumetric flask... to get a final concentration of $40\,\mu g\,mL^{-1}$." Then follow the procedure detailed for construction of the calibration curve for aceclofenac by RSD_1 spectrophotometric method.

Ten milliliters of the methanol extract of the tablets, 1 mg of aceclofenac per milliliter, were subjected to degradation appling the ICH (International Conference on Harmonization) guideline for degradation of tablets (28). Then 10 mL of 0.1 N sodium hydroxide was added to the 10 mL tablets extract, refluxed for 30 min, and neutralized with 0.1 N hydrochloric acid. The formed precipitate was filtered and dissolved in 10-mL methanol. The whole solution was quantitatively transferred to a 100-mL volumetric flask and completed to the mark with the same solvent. Ten milliliters of the degraded tablets extract were transferred into a 25-mL volumetric flask and diluted to the mark with methanol to get a final concentration of $40\,\mu g\,mL^{-1}$. We then proceeded as for construction of the calibration curve. The degraded extract was also analyzed by HPLC (9).

For diclofenac sodium (a) Rheumafen tablets. Weigh and finely powder not less than 20 tablets. Transfer a portion of powdered tablets equivalent to one tablet (25 mg diclofenac sodium) into a 25-mL measuring flask and dissolve in methanol ($1\,mg\,mL^{-1}$). Transfer 1 mL of extract into a 25-mL measuring flask and complete to the mark with methanol to get a final concentration of $40\,\mu g\,mL^{-1}$, then proceed as for construction of the calibration curve for diclofenac sodium by RSD_1 spectrophotometric method.

(b) Rheumafen injection. Transfer accurately 4 mL from the content of two Rheumafen injections, equivalent to 100 mg diclofenac sodium, to a 100-mL measuring flask and complete to the volume with methanol ($1\,mg\,mL^{-1}$). Transfer 1 mL of extract into a 25-mL measuring flask and dilute to the mark with methanol to get a final concentration of $40\,\mu g\,mL^{-1}$.

Then follow the procedure for construction of the calibration curve for diclofenac sodium by RSD_1 spectrophotometric method.

(c) Rheumafen SR capsules. Weigh accurately the content of 10 Rheumafen SR capsules and mix well. Transfer a portion of powder equivalent to one capsule (100 mg diclofenac sodium) into a 100-mL measuring flask and dissolve in methanol ($1\,mg\,mL^{-1}$). Transfer 1 mL of extract into a 25 mL measuring flask and complete to the mark with methanol to get a final concentration of $40\,\mu g\,mL^{-1}$, then proceed as for construction of the calibration curve for diclofenac sodium by RSD_1 spectrophotometric method.

(d) Rheumafen Acti gel. Weigh accurately and mix well the content of three Rheumafen Acti gel tubes. Transfer a portion of gel equivalent to a 100 mg diclofenac sodium into a 100-mL measuring flask and dissolve in methanol ($1\,mg\,mL^{-1}$). Transfer 1 mL of extract into a 25-mL measuring flask and dilute to the mark with methanol to get a final concentration of $40\,\mu g\,mL^{-1}$, then follow the procedure for construction of the calibration curve for diclofenac sodium by RSD_1 spectrophotometric method.

Spectrodensitometric Method

Construction of Calibration Curves

Apply separately 2–10 μL of aceclofenac working solution ($1\,mg\,mL^{-1}$) and 1–9 μL of diclofenac sodium working solution ($1\,mg\,mL^{-1}$) to a TLC plate ($20 \times 20\,cm^2$) using a 10-μL Hamilton syringe. Leave a space of 1 cm between each spot and 2 cm from the bottom edge of the plate. Develop the plate in a chromatographic tank previously saturated for at least half an hour with the developing mobile phase, chloroform:methanol:ammonia (48:11.5:0.5 v/v/v) by ascending chromatography at room temperature. Scan the spots of aceclofenac and diclofenac sodium at 274 and 283 nm, respectively. Construct calibration curves by plotting the area under the peak vs. the corresponding concentration, and calculate the corresponding regression equations.

Assay of Laboratory-Prepared Mixtures

Apply 5 μL of prepared mixtures to a silica gel plate as for construction of the calibration curves. Record the area under the peaks at 274 nm for aceclofenac and 283 nm for diclofenac sodium. Calculate the concentration of aceclofenac and diclofenac sodium from the corresponding regression equations.

Application to Pharmaceutical Preparations

For aceclofenac (*Bristaflam tablets*) Follow the procedure of the D_3 spectrophotometric method (application to pharmaceutical preparations) up to "into a 100-mL volumetric flask and complete to the volume with methanol ($1\,mg\,mL^{-1}$)." Then follow the procedure for construction of the calibration curve for aceclofenac by spectrodensitometric method.

For diclofenac sodium (a) Rheumafen tablets. Follow the procedure of the RSD_1 spectrophotometric method (for Rheumafen tablets) up to "complete to volume with methanol ($1\,mg\,mL^{-1}$)." Then follow the procedure for construction of the calibration curve for diclofenac sodium by spectrodensitometric method.

(b) Rheumafen injection. Follow the procedure of the RSD_1 spectrophotometric method (for Rheumafen injection) up to "a 100-mL measuring flask and complete to the volume with methanol ($1\,mg\,mL^{-1}$)." Then proceed as for construction of the calibration curve for diclofenac sodium by spectrodensitometric method.

(c) Rheumafen SR capsules. Follow the procedure of the RSD_1 spectrophotometric method (for Rheumafen SR capsules) up to "into a 100-mL measuring flask and complete to the mark with methanol ($1\,mg\,mL^{-1}$)." Then proceed as for construction of the calibration curve for diclofenac sodium by spectrodensitometric method.

(d) Rheumafen Acti gel. Follow the procedure of the RSD_1 spectrophotometric method (for Rheumafen Acti gel) up to "into a 100-mL measuring flask and complete to the mark with methanol ($1\,mg\,mL^{-1}$)." Then follow the procedure for construction of the calibration curve for diclofenac sodium by spectrodensitometric method.

RESULTS AND DISCUSSION

New methods for simultaneous determination of two or more compounds in the same sample, without previous chemical separation, are always of interest. This work is devoted to the analysis of aceclofenac alone or in the presence of diclofenac sodium, its degradation product.

D_3 Spectrophotometric Method

Derivative spectrophotometry offers greater selectivity than normal spectrophotometry, because it decreases spectral overlap and allows better resolution (29).

Zero-, first-, and second-order absorption spectra of aceclofenac and diclofenac sodium showed severe spectral overlapping, Figs. 2–4. Trials to solve the overlap of aceclofenac and diclofenac sodium absorption spectra by using different buffers with pH ranging between 5 and 8 failed.

Third-derivative (D_3) spectrophotometry was used to resolve spectral overlapping of the absorption spectra of aceclofenac and diclofenac sodium, Fig. 5. By applying the D_3 technique, the zero crossing point for diclofenac sodium with aceclofenac was shown at 283 nm. A linear correlation was obtained between the peak amplitude and the corresponding concentration in the range of 4–24 $\mu g\,mL^{-1}$ for aceclofenac, from which the linear regression equation was computed and found to be:

$$Y = 0.6386X + 0.0012 \qquad r = 0.9991$$

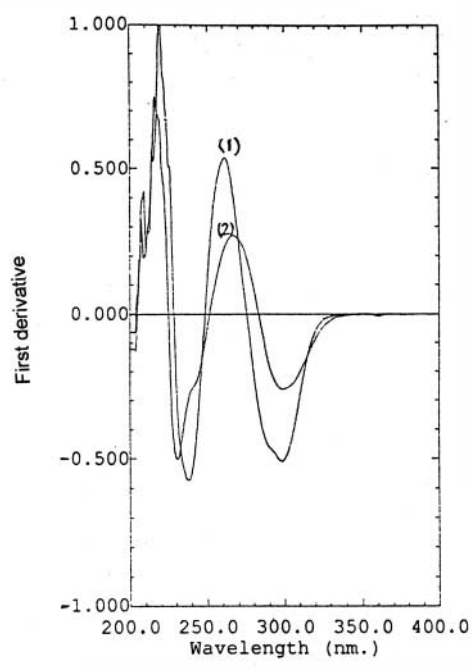

Figure 3. First-derivative absorption spectra of (1) aceclofenac 50 $\mu g\,mL^{-1}$ in methanol and (2) diclofenac 25 $\mu g\,mL^{-1}$ in methanol.

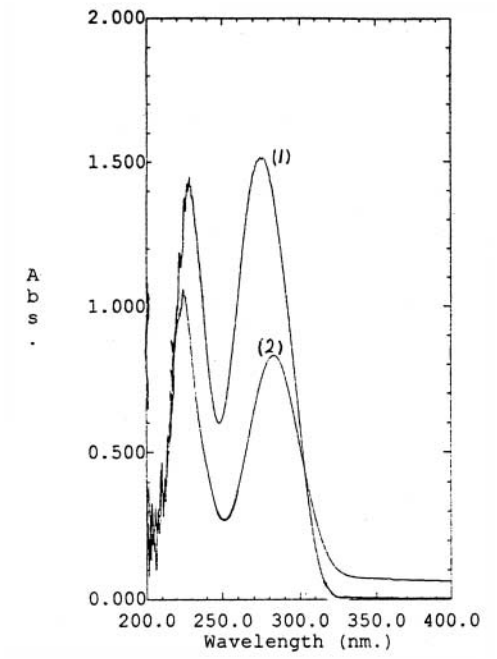

Figure 2. Absorption spectra of (1) aceclofenac 50 $\mu g\,mL^{-1}$ in methanol and (2) diclofenac 25 $\mu g\,mL^{-1}$ in methanol.

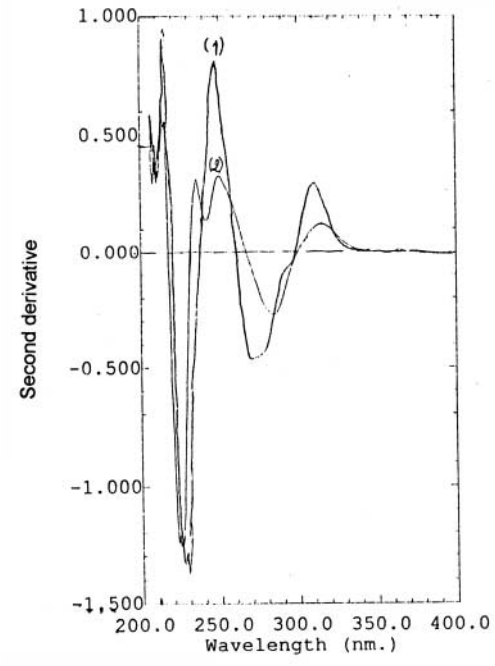

Figure 4. Second-derivative absorption spectra of (1) aceclofenac 50 $\mu g\,mL^{-1}$ in methanol and (2) diclofenac 25 $\mu g\,mL^{-1}$ in methanol.

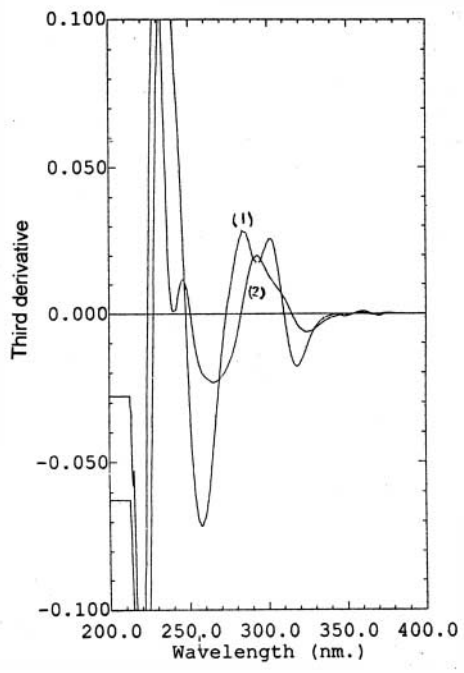

Figure 5. Third-derivative absorption spectra of (1) aceclofenac $50\,\mu g\,mL^{-1}$ in methanol and (2) diclofenac $25\,\mu g\,mL^{-1}$ in methanol.

where Y is the peak amplitude at 283 nm, X is the concentration ($\mu g\,mL^{-1}$), and r is the correlation coefficient.

The proposed method is valid for determination of aceclofenac in the presence of diclofenac sodium in different laboratory-prepared mixtures with mean percentage recoveries of 100.42±0.94, respectively (Table 1).

The suggested method has been applied to assay aceclofenac in Bristaflam tablets, and its validity was further assessed by applying the standard addition technique (Table 2).

RSD_1 Spectrophotometric Method

This takes into consideration the theory of derivative ratio spectrophotometry (27) to solve the problem of overlapping absorption spectra of aceclofenac and diclofenac sodium.

For determination of aceclofenac in the presence of diclofenac sodium, the absorption spectra of different concentrations of aceclofenac in the range of 4–32 $\mu g\,mL^{-1}$ were divided by the absorption spectra of $25\,\mu g\,mL^{-1}$ diclofenac. The obtained ratio spectra were differentiated with respect to wavelength, Fig. 6. The RSD_1 values showed good linearity and accuracy at 252 nm. The linear regression equation was computed and found to be:

$$Y = 11.333X + 0.0865 \qquad r = 0.9991$$

where Y is the RSD_1 value at 252 nm, X is the concentration ($\mu g\,mL^{-1}$), and r is the correlation coefficient.

Determination of aceclofenac in the presence of diclofenac sodium can also be made by RSD_1 at 309.5 nm. The new wavelength showed good linearity and accuracy, but less than at 252 nm.

Determination of diclofenac sodium in the presence of aceclofenac was achieved by dividing the spectra of different concentrations of diclofenac sodium in the range of 4–32 $\mu g\,mL^{-1}$ with the spectrum of $25\,\mu g\,mL^{-1}$ aceclofenac. The obtained ratio spectra were then differentiated with respect to

Table 1

Determination of Aceclofenac in the Presence of Diclofenac Sodium in Different Laboratory-Prepared Mixtures by D_3 Method

Mixture No.	Degradation Product (Diclofenac) Added (%)[a]	Aceclofenac Found (%)[b]
1	10	100.00
2	30	101.12
3	50	101.25
4	70	99.29
Mean±SD		100.42±0.94

[a]Calculated with respect to the total weights (drug–degradation mixtures).
[b]Average of four determinations.

Table 2

Application of Standard Addition Technique to the Analysis of Aceclofenac in Bristaflam Tablets by D_3 Method

Product	Found (%)	Pure Added ($\mu g\,mL^{-1}$)	Found[a] ($\mu g\,mL^{-1}$)	Recovery (%)
Bristaflam (Batch No. c00557)	99.63±0.21	5	5.00	100.00
		10	10.05	100.50
		15	14.83	98.87
Mean±SD				99.79±0.84

[a]Average of four determinations.

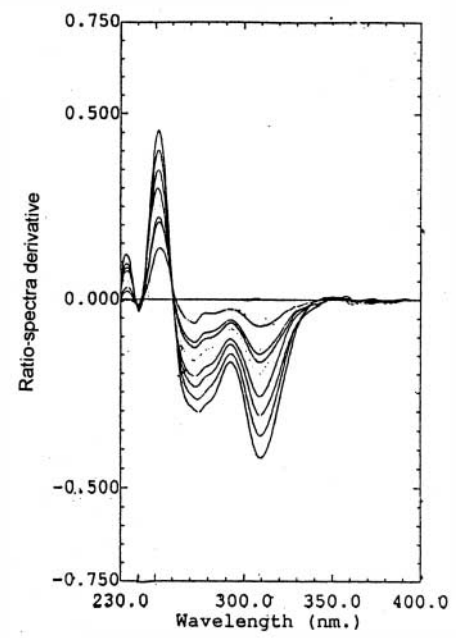

Figure 6. Calibration curve of ratio-spectra first derivative of 4–32 $\mu g\,mL^{-1}$ of aceclofenac/25 $\mu g\,mL^{-1}$ of diclofenac.

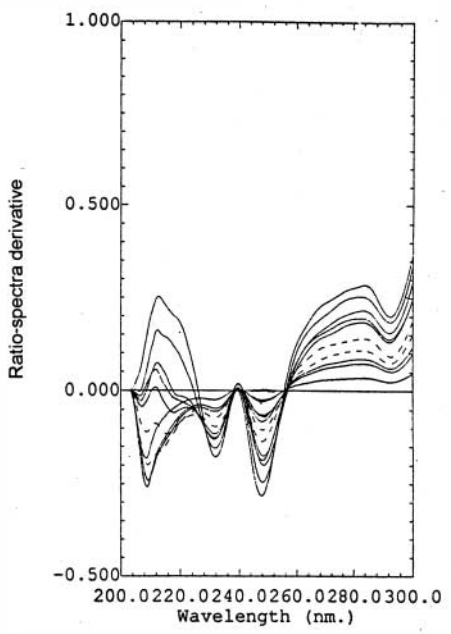

Figure 7. Calibration curve of ratio-spectra first derivative of 4–32 $\mu g\,mL^{-1}$ of diclofenac/25 $\mu g\,mL^{-1}$ of aceclofenac.

wavelength, Fig. 7. The RSD_1 values showed good linearity and accuracy at 248 nm. The linear regression equation was computed and found to be:

$$Y = 8.9392X - 0.0042 \qquad r = 0.9996$$

where Y is the RSD_1 value at 248 nm, X is the concentration ($\mu g\,mL^{-1}$), and r is the correlation coefficient.

Results obtained in Table 3 showed that the proposed method is valid and applicable for determination of aceclofenac and diclofenac sodium simultaneously in different laboratory-prepared mixtures with mean percentage recoveries of 100.17±1.04 and 99.73±0.74, respectively. Moreover, the intra- and inter-day precision and accuracy of the assay were studied.

The validity of RSD_1 spectrophotometry was further assessed by applying a standard addition technique for the analysis of Bristaflam tablets, Rheumafen tablets, Rheumafen injection, Rheumafen SR capsules, and Rheumafen Acti gel (Table 4). Furthermore, the methanol extract of aceclofenac tablets, subjected to stress conditions by applying the ICH guideline for degradation of tablets (28), revealed that no degradation occurred, the results obtained conformed with those in Table 4.

Spectrodensitometric Method

Thin-layer chromatography is used for both qualitative and quantitative analysis. Quantitative methods depend on both measuring spot size and intensity or determination after elution of compounds from TLC plates. Densitometry offers a simple way of quantifying directly on a TLC plate by measuring the optical density of the separated spots. The amount of compounds is determined by comparing them to a standard curve from reference materials chromatographed simultaneously under the same conditions. Quantitative evaluations of thin-layer chromatograms by densitometry are based on differential measurements of a beam emerging from the sample-free and sample-containing zones of the plate (30).

This method is concerned with the application of spectrodensitometry for the determination of aceclofenac in the presence of diclofenac sodium and in their pharmaceutical preparations. The proposed technique is based on the difference in R_f values of aceclofenac ($R_f = 0.32$) and diclofenac sodium ($R_f = 0.4$).

Different developing systems were tried, but complete separation of the two drugs was achieved

Table 3

Intra-day and Inter-day Assay Precision for Aceclofenac and Diclofenac Sodium in Laboratory-Prepared Mixtures by RSD_1 Spectrophotometric Method

Mixture No.	Aceclofenac Claimed Taken ($\mu g\,mL^{-1}$)	Aceclofenac Found[a] ($\mu g\,mL^{-1}$)	Aceclofenac Found (%)	Diclofenac Claimed Taken ($\mu g\,mL^{-1}$)	Diclofenac Found[a] ($\mu g\,mL^{-1}$)	Diclofenac Found (%)	CV (%) Intra-day Aceclofenac	CV (%) Intra-day Diclofenac	CV (%) Inter-day Aceclofenac	CV (%) Inter-day Diclofenac
1	32	32.352	101.10	8	7.943	99.29	1.58	1.83	2.25	2.77
2	28	27.799	99.28	12	11.949	99.58	2.68	2.60	2.85	2.76
3	20	19.818	99.09	20	19.796	98.98	1.69	2.13	2.08	2.07
4	12	12.000	100.00	28	27.966	99.88	2.59	1.45	2.59	1.85
5	8	8.112	101.40	32	32.295	100.92	2.61	1.58	2.72	2.15
Mean±SD			100.17±1.04			99.73±0.74				

[a]Average of four determinations.

Table 4

Application of Standard Addition Technique to the Analysis of Aceclofenac and Diclofenac Sodium in Pharmaceutical Preparations by RSD_1 Spectrophotometric Method

Product	Found (%)	Pure Added ($\mu g\,mL^{-1}$)	Found[a] ($\mu g\,mL^{-1}$)	Recovery (%)
Bristaflam tablet	100.42±0.465	5	5.025	100.50
(Batch No. c00557)		10	9.960	99.60
		15	15.165	101.10
Mean±SD				100.40±0.75
Rheumafen tablet	100.63±0.718	5	5.000	100.00
(Batch No. 143)		10	10.130	101.30
		15	14.895	99.30
Mean±SD				100.20±1.01
Rheumafen injection	99.25±0.835	5	5.026	100.52
(Batch No. 001688A)		10	9.885	98.85
		15	14.925	99.50
Mean±SD				99.62±0.84
Rheumafen SR capsule	100.50±0.286	5	4.963	99.26
(Batch No. 0013262B)		10	9.932	99.32
		15	15.000	100.00
Mean±SD				99.53±0.41
Rheumafen Acti gel	100.49±0.538	5	5.075	101.50
(Batch No. 000074A)		10	10.035	100.35
		15	14.956	99.71
Mean±SD				100.52±0.91

[a]Average of four determinations.

using chloroform:methanol:ammonia (48:19.5:0.5 v/v/v). The separated spots of both aceclofenac and diclofenac sodium can be scanned on the same plate at 274 and 283 nm, respectively. A linear relationship was obtained between the peak area and the concentration in the range of 2–10 $\mu g\,spot^{-1}$ for aceclofenac and 1–9 $\mu g\,spot^{-1}$ for diclofenac sodium. The linear regression equation was computed and found

Table 5

Determination of Aceclofenac and Diclofenac Sodium in Laboratory-Prepared Mixtures by Spectrodensitometric Method

	Aceclofenac			Diclofenac		
Mixture No.	Claimed Taken ($\mu g\,mL^{-1}$)	Found[a] ($\mu g\,mL^{-1}$)	Found (%)	Claimed Taken ($\mu g\,mL^{-1}$)	Found[a] ($\mu g\,mL^{-1}$)	Found (%)
1	9	8.982	99.80	1	0.996	99.60
2	7	7.001	100.01	3	3.024	100.80
3	5	4.925	98.50	5	4.956	99.12
4	3	3.011	100.37	7	7.065	100.93
5	2	2.003	100.15	8	7.990	99.88
Mean±SD			99.77±0.74			100.07±0.78

[a]Average of four determinations.

Table 6

Application of Standard Addition Technique to the Analysis of Aceclofenac and Diclofenac Sodium in Pharmaceutical Preparations by Spectrodensitometric Method

Product	Found (%)	Pure Added ($\mu g\,mL^{-1}$)	Pure Found[a] ($\mu g\,mL^{-1}$)	Recovery (%)
Bristaflam tablet	99.16±0.86	2	2.010	100.50
(Batch No. c00557)		4	4.033	100.83
		6	5.981	99.68
Mean±SD				100.34±0.59
Rheumafen tablet	99.60±0.58	1	1.005	100.50
(Batch No. 143)		2	1.997	99.85
		3	2.986	99.53
Mean±SD				99.96±0.49
Rheumafen injection	99.91±0.59	1	1.003	100.30
(Batch No. 001688A)		2	2.029	101.45
		3	2.983	99.43
Mean±SD				100.39±1.01
Rheumafen SR capsule	100.68±0.23	1	1.003	100.30
(Batch No. 0013262B)		2	2.002	100.10
		3	3.039	101.30
Mean±SD				100.57±0.64
Rheumafen Acti gel	99.58±0.42	1	0.997	99.70
(Batch No. 000074A)		2	2.002	100.10
		3	3.011	100.37
Mean±SD				100.06±0.34

[a]Average of four determinations.

to be:

$$Y = 0.092X - 0.016 \qquad r = 0.9991$$

$$Y = 0.1069X + 0.0534 \qquad r = 0.9999$$

for aceclofenac and diclofenac sodium, respectively, where Y is the area under the peak, X is the concentration ($\mu g\,spot^{-1}$), and r is the correlation coefficient.

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Table 7

Statistical Analysis of the Results Obtained by Applying the Proposed and Reference Methods (1,9) for the Analysis of Pure Aceclofenac and Diclofenac Sodium

	Proposed Methods					Reference Methods (1)		HPLC Method (9)	
	Third-Derivative (D_3) Method	Ratio-Spectra First-Derivative (RSD_1) Method		Spectrodensitometric Method					
Values	Aceclofenac	Aceclofenac	Diclofenac Sodium	Aceclofenac	Diclofenac Sodium	Aceclofenac	Diclofenac Sodium	Aceclofenac	Diclofenac Sodium
Mean±SD	100.05±0.88	99.81±0.84	100.19±0.72	100.20±1.03	100.14±0.98	100.29±0.50	99.98±0.55	99.62±0.52	100.45±1.23
N	6	6	6	6	6	4	4	5	5
Variance	0.774	0.706	0.518	1.061	0.960	0.250	0.303	0.266	1.501
$t(2.306)$[a]	0.492	1.015	0.491	0.267	0.294	—	—	—	—
$F(9.01)$[a]	3.143	2.898	1.705	4.327	3.148	—	—	—	—

[a]The values in parentheses correspond to the theoretical values of t and F (at $p = .05$).

Results obtained by applying the spectrodensitometric procedure showed that the concentration of aceclofenac and diclofenac sodium can be simultaneously determined in the prepared mixtures with mean percentage accuracy of 99.77±0.74 and 100.07±0.78, respectively (Table 5).

The proposed method has been applied to assay aceclofenac in Bristaflam tablets and diclofenac sodium in Rheumafen tablets, Rheumafen injection, Rheumafen SR capsules, and Rheumafen Acti gel. The validity of the suggested procedures was further assessed by applying a standard addition technique, Table 6.

A statistical comparison of the results obtained by the proposed and reference, non-aqueous and HPLC methods (1,9) for pure drugs separately is shown in Table 7. No significant difference between the proposed methods and reference methods was found with respect to precision and accuracy. In addition, the ruggedness of the derivative spectroscopy method as a function of time, at room temperature, is carried out for different mixtures of the drugs (Table 3). The assay was precise as the coefficient of variation was less than 3.00%.

The results obtained by applying the proposed procedures suggest that they could be applied for the simultaneous determination of aceclofenac and diclofenac sodium. Moreover, the methods are rapid, sensitive, selective, and could safely be used in routine and quality control analysis.

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