Stability-indicating methods for the determination of doxazosin mezylate and celecoxib

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Abstract

Two stability-indicating methods were developed for the determination of doxazosin mesylate (**I**) and celecoxib (**II**) in the presence of their degradation products. The first method depends on the use of first derivative spectrophotometry (D_1) at 256, 269 nm for (**I**) and (**II**), respectively. This method determines (**I**) and (**II**) in concentration ranges of 0.8–12 and 1–20 µg ml^{-1} with mean percentage accuracies of 99.21 ± 0.88 and $99.59 \pm 1.67\%$ for (**I**) and (**II**), respectively. The second method depends on the quantitative densitometric evaluation of thin-layer chromatography of of (**I**) and (**II**) in the presence of their degradation products without any interference. Methylisobutyl ketone–glacial acetic acid–water (20:10:10) was used as a mobile phase for (**I**) and cyclohexane–dichloromethane–diethyleamine (50:40:10) for (**II**). The chromatograms were scanned at 248 and 253 nm for (**I**) and (**II**), respectively. This method determines (**I**) and (**II**) in concentration ranges of 1–4 µg per spot for both drugs with mean percentage accuracies of 100.19 ± 0.95 and $99.91 \pm 1.95\%$ for (**I**) and (**II**), respectively. The suggested methods were used to determine doxazosin mesylate and celecoxib in bulk powder, laboratory-prepared mixtures and pharmaceutical dosage forms (cardura tablet and celebrex capsule). The results obtained by applying the proposed methods were statistically analysed and compared with those obtained by the reported methods. © 2002 Published by Elsevier Science B.V.

Keywords: Doxazosin mesylate; Celecoxib; Derivative spectrophotometry; Thin layer chromatography-UV densitometry; Pharmaceutical formulation

1. Introduction

Doxazosin mesylate, l-(4-Amino-6,7-dimethoxy-2-quinazolinyl)-4- [(2,3-dihydro-l,4-benzodioxin-2-yl)carbonyl] piperazine, is selective α_1-adrenergic blocker related to prozosin [1]. Its chemical structure and degradation products are shown in Fig. 1. Several methods have been reported for the determination of doxazosin mesylate including HPLC for its determination in plasma and pharmaceutical formulations [2–6], differential-puls-polarography [7–9], cathodic-stripping voltametry [10], adsorptive stripping voltametry [11], UVspectrophotometry and square-wave voltametry [12], and HPTLC [13].

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Celecoxib,4-[5-(4-methylphenyl)-3-(triflurome-thyl)-lH-prazol-l-yl] benzen sulfonamid, is a nons-teroidal anti-inflammatory drug that exhibits anti-inflammatory, analgesic and antipyretic activities [14]. Its chemical structure and degradation product are shown in Fig. 1. In literature survey there is no method for determination of celecox-ibe. Only one HPLC method [14] for its determi-nation in pharmaceutical formulations.

The aim of this work was to develop spec-trophotometric and densitometric stability-indi-cating methods for analysis of doxazosin mesylate and celecoxib in the presence of their degradation products. The methods developed were compared with reference methods, having satisfactory sensi-tivities, accuracy and precision, as well as simplic-ity and rapid.
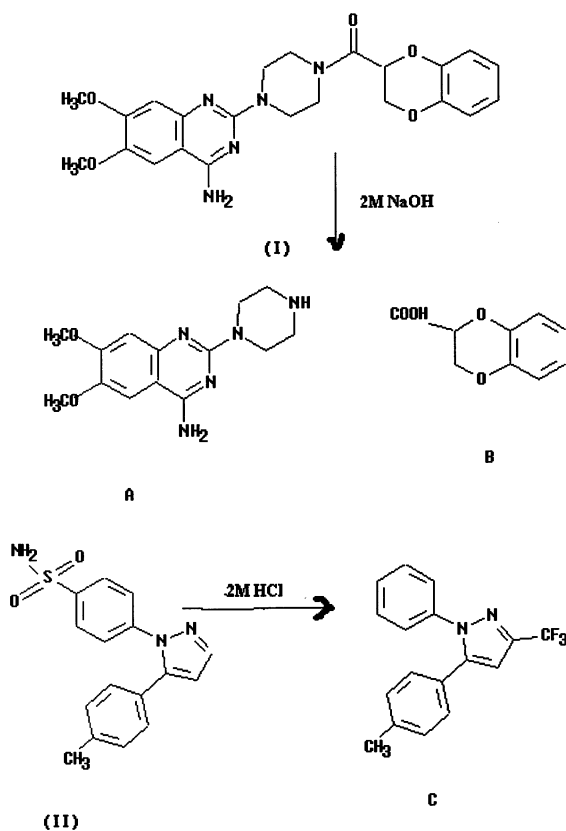

Fig. 1. Chemical structures of doxazosin mezylate (**I**), its degradation products (A and B), celecoxib (**II**) and its degra-dation products (C).

2. Experimental

2.1. Apparatus

1. UV/VIS Spectrophotometer (SHIMADZU 1601/PC). The derivative curves of the spectra were obtained with the following parameters: scane speed 120 nm mm^{-1}; chart speed 60 nm; response time 10 s.
2. Densitometer-dual wavelength SHIMADZU flying CS-9301.
3. UV lamp-short wave length 254 nm.
4. Thin-layer chromatographic plates precoated with silica gel GF, 20×20 cm, 0.25 mm thick-ness, fluorescent at 254 nm (E. Merck Darm-stadt Germany).

2.2. Materials

2.2.1. Pure samples

1. Doxazosin mesylate, working standard,was kindly supplied by Pfizer Co, Cairo, Egypt. The purity of the sample was found to be $100.08 \pm 1.16\%$ according to reported method [2].
2. Celecoxib working standard, was kindly sup-plied by Pfizer Co, Cairo, Egypt. The purity of the sample was found to be $99.01 \pm 1.11\%$ according to the reported method [14].

2.2.2. Market samples

- Cardura tablets (Pfizer Co); batch No. 9122 and 0203, labeled to contain 1 and 4 mg doxa-zosin mesylate per tablet, respectively.
- Celebrex capsules (Pfizer Co); batch No. 101 and 002, labeled to contain 100 and 200 mg celecoxib per capsule, repectively.

2.3. Reagents

All chemicals were of analytical grade and all solvents were of spectroscopic grade.
1. Mobile phase for (**I**): Methyl isobutyl ketone–glacial acetic acid–water (20:10:10).

2. Mobile phase for (**II**): Cyclohexane–dichloromethane–diethylamine (50:40:10).
3. Sodium hydroxide − 2 M, aqueous solution.
4. Hydrochloric acid − 2 M, aqueous solution.

2.4. Preparation of degradation products

2.4.1. Doxazosin mesylate degradation products

Fifty milligrams of Doxazosin mesylate was dissolved in 10 ml methanol in a conical flask. 50 ml of 2 M sodium hydroxide was added and put on a water bath at 80 °C for 24 h. The solution was neutralized with 2 M hydrochloric acid and evaporated on a water bath to a few milliliters. 10 ml of methanol was added and filtered if necessary. The methanolic solution was applied in band form to TLC plates. 20 μl of standard solution in methanol (1 mg ml^{-1}) was also spotted as a reference. The plates were developed to 16 cm using meyhyl isobutyl ketone–glacial acetic acid–water (20:10:10) as a mobile phase previously saturated for 15 min, and then air dried. The two bands corresponding to degradation products were visualized under UV light at 254 nm, each band was scrapped and extracted three times each with 10 ml portions of methanol. The extracts were filtered and evaporated just to dryness on boiling water bath. The residue left after evaporation were used for the preparation of laboratory prepared mixtures.

2.4.2. Celecoxib degradation products

Using 2 M hydrochloric acid instead of 2 M sodium hydroxide, the procedure described in Section 2.4.1. was followed. Then it was neutralised with 2 M of sodium hydroxide.

2.5. Stock standard solutions

1. Doxaxosin mesylate 40 μg ml^{-1} in methanol for first derivative spectrophotometric method.
2. Doxazosin mesylate 1mg ml^{-1} in methanol for densitometric method.
3. Doxazosin mesylate degradation products 40 μg ml^{-1} and 1 mg ml^{-1} in methanol for first derivative spectrophotometric method and densitometric method, respectively, to prepare the laboratory-prepared mixtures.
4. Celecoxib 1 mg ml^{-1} in methanol for densitometric method.
5. About 10 ml of methanolic solution (1 mg ml^{-1}) was diluted to 100 ml with 0.1 M sodium hydroxide (100 μg ml^{-1}) for first derivative method.
6. Celecoxib degradation products (100 μg ml^{-1}) in 0.1 M sodium hydroxide and (1 mg ml^{-1}) in methanol for first derivative spectrophotometric and densitometric methods, respectively, to prepare the laboratory-prepared mixtures.

2.6. Laboratory-prepared mixtures

2.6.1. For doxazosin mesylate

2.6.1.1. Derivative spectrophotometric method.
Aliquot portions equivalents to 8–120 μg of doxazosin mesylate from its stock standard solution (40 μg ml^{-1}) were transferred into a series of 10 ml volumetric flasks. About 10–90% of degradation products (40 μg ml^{-1}) solution were added to the same flasks.

2.6.1.2. Densitometric method.
Aliquot portions equivalent to 1–4 mg of doxazosin mesylate from its stock standard solution (1 mg ml^{-1}) were transferred into a series of 5 ml volumetric flasks. 10–90% of degradation products (1 mg ml^{-1}) solution were added to the same flasks.

2.6.2. For celecoxib

2.6.2.1. Derivative spectrophotometric method.
Aliquot portions equivalent to 10–200 μg of celecoxib from its stock standard solution (100 μg ml^{-1}) were transferred into a series of 10 ml volumertic flasks. About 10–90% of degradation products (100 μg ml^{-1}) solution were added to the same flasks.

2.6.2.2. Densitometric method.
Proceeded as described under Section 2.6.1.2.

2.7. Procedures

2.7.1. Construction of calibration curves for derivative spectrophotometry of doxazosin mesylate

Aliquots of stock standard solution (40 µg ml^{-1}) equivalent to 8–120 µg of doxazosin mesylate were transferred into a series of 10 ml volumetric flasks and completed to the mark with methanol. The first derivative curves were recorded for each solution using methanol as a blank and the absorbance (D_1) values were measured at 256 nm. The calibration curve was constructed and the regression equation was calculated.

2.7.2. Construction of calibration curves for densitometric method of doxazosin mesylate

Aliquots of stock standard solution (1 mg ml^{-1}) equivalent to 1–4 mg of doxazosin mezylate were transferred into a series of 5 ml volumetric flasks and diluted to volume with methanol. Five micrograms of each solution was applied to TLC plate and the plate was developed to 16 cm. using the developing mobile phase, methyl isobutyl ketone–glacial acetic acid–water (20:10:10). The spots were determined densitometrically at 248 nm. The calibration curve representing the relationship between the recorded area under the peak and the corresponding concentration was plotted and the regression equation was calculated.

2.7.3. Assay of pharmaceutical formulations (tablets)

Twenty tablets of doxazosin mesylate were weighed and grounded to fine powder. An amount of the powder equivalent to 10 mg of the drug was weighed, dissolved three times each with 20 ml methanol by shaking in ultrasonic bath for about 30 min. The solution was filtered each time, evaporated to small volume and transferred quantitatively into 10 ml volumetric flask. The volume was completed to the mark with methanol (1 mg ml^{-1}).

2.7.3.1. For derivative spectrophotometric method.
One millilitre of tablet solution was transferred to 25 ml volumetric flask and diluted to the mark with methanol (40 µg ml^{-1}), then the procedure described in Section 2.7.1 was followed.

2.7.3.2. For densitometric method..
The procedure described in Section 2.7.2 was repeated.

2.7.4. Construction of calibration curve for derivative spectrophotometry of celecoxib

An aliquot portions equivalent to 10–200 µg of celecoxib from its stock standard solution (l00 µg ml^{-1}) in 0.1 M sodium hydroxide were transferred into a series of 10 ml volumetric flasks and completed to the mark with 0.1 M sodium hydroxide. The first derivative curves of each solution was recorded using 0.1 M sodium hydroxide as a blank. The absorbance (D_1) values were measured at 269 nm. The calibration curve was constructed and the regression equation was calculated.

2.7.5. Construction of calibration curve for densitometric method of celecoxib

Proceed as under construction of calibration curve of doxazosin mesylate, using cyclohexane–dichloromethane–diethylamine (50:40:10) as a mobile phase and the spots were scanned at 253 nm. The calibration curve was constructed and the regression equation was calculated.

2.7.6. Assay of pharmaceutical formulations (Capsules)

The contents of 10 capsules were evacuated and weighed. An amount equivalent to 50 mg celecoxib, was weighed and dissolved in 30 ml methanol using ultrasonic bath for about 15 min. The solution was filtered in 50 ml volumetric flask, washed and completed to volume with methanol (1 mg ml^{-1}).

2.7.6.1. For derivative spectrophotometric method.
One millilitre of test solution was transferred to 10 ml volumetric flask and diluted to its mark line with 0.1 M sodium hydroxide (100 µg ml^{-1}) then the procedure described in Section 2.7.4 was followed.

2.7.6.2. For densitometric method.
The procedure given in Section 2.7.2 was repeated using cyclohexane–dichloromethane–diethylamine (50:40:10) as a mobile phase and the spots were scanned at 253 nm.
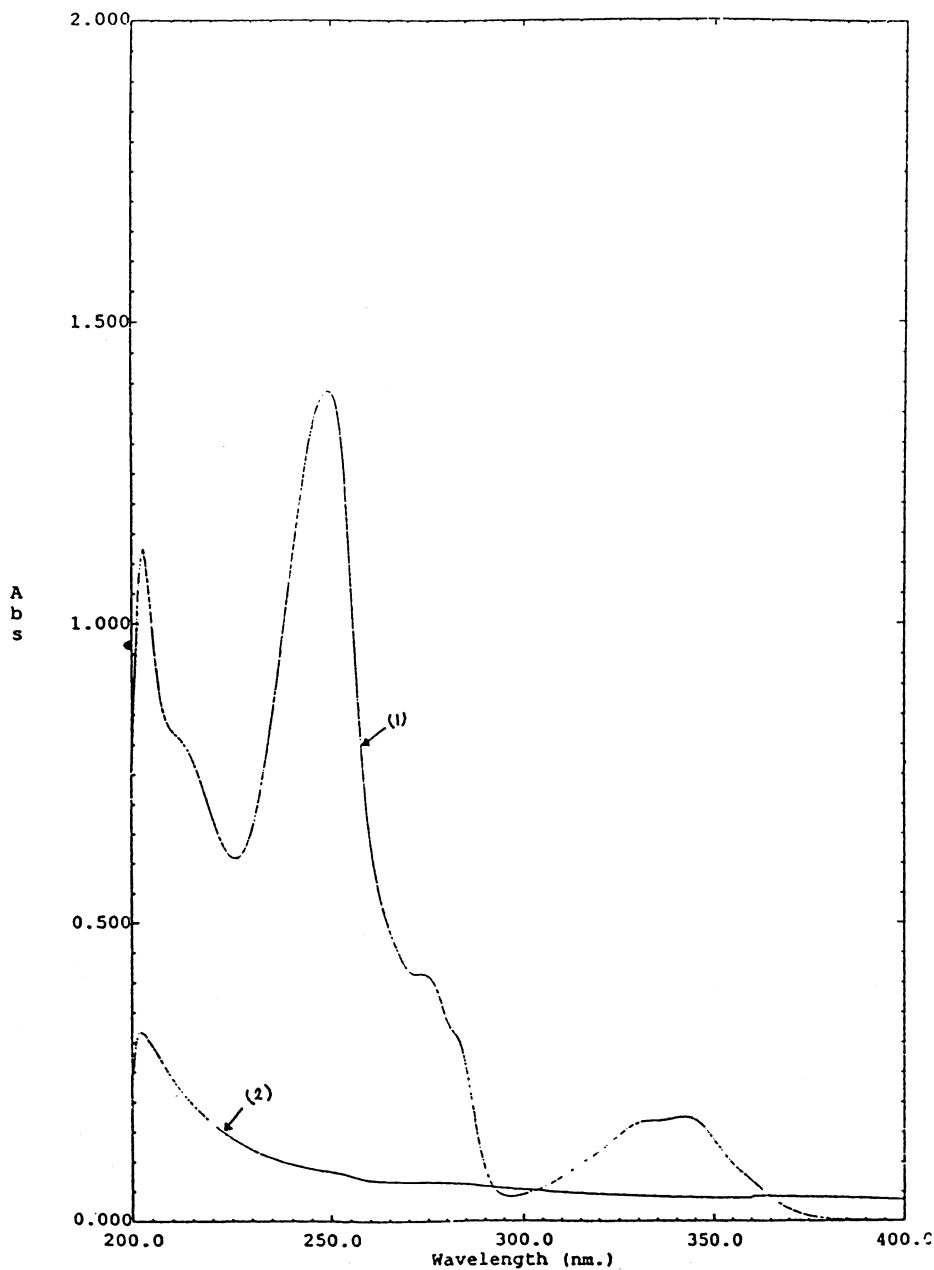
Fig. 2. Zero order absorption spectra of doxazosin mezylate (1), and degradation products (2) 10 μg ml^{-1} in methanol.

3. Results and discussion

3.1. Derivative spectrophotometric method

Zero-order absorption spectra (Do) of both (**I**) and (**II**) and their degradation products show certain overlapping that interfere with the direct determination of pure (**I**) and (**II**) (Figs. 2 and 3).

In the present work the first derivative technique (D_1) was used to resolve the spectral over

lapping and show zero-crossing point for the degradation products at 256, 269 nm for (**I**) and (**II**), respectively. (Figs. 4 and 5).

Linear correlations were obtained between (D_1) value at 256, 269 nm for the concentration ranges 0.8–12, 1–20 $\mu g \, ml^{-1}$, for (**I**) and (**II**), respectively.
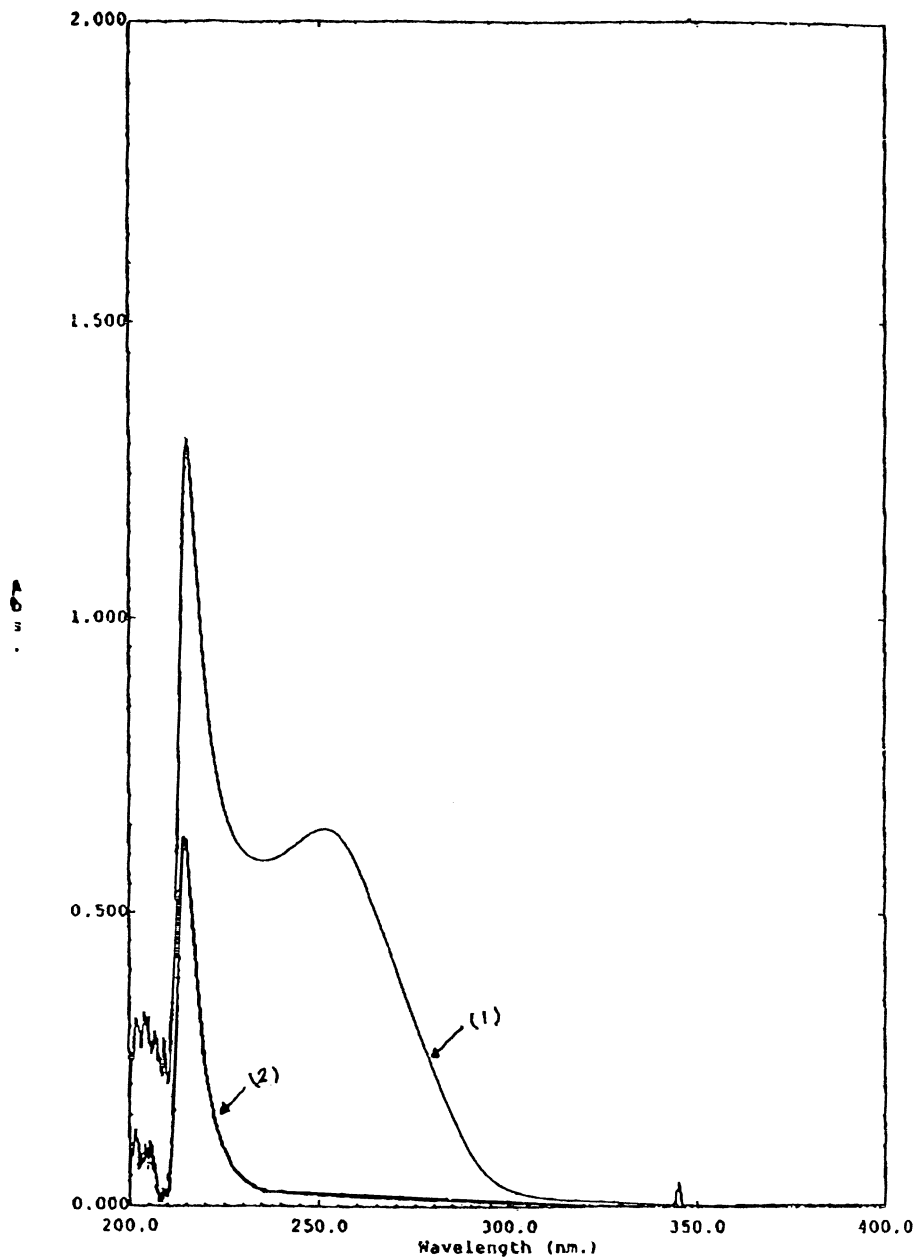

Fig. 3. Zero order absorption spectra of celecoxib (1) and degradation product (2) 10 $\mu g \, ml^{-1}$ in 0.1 M sodium hydroxide.
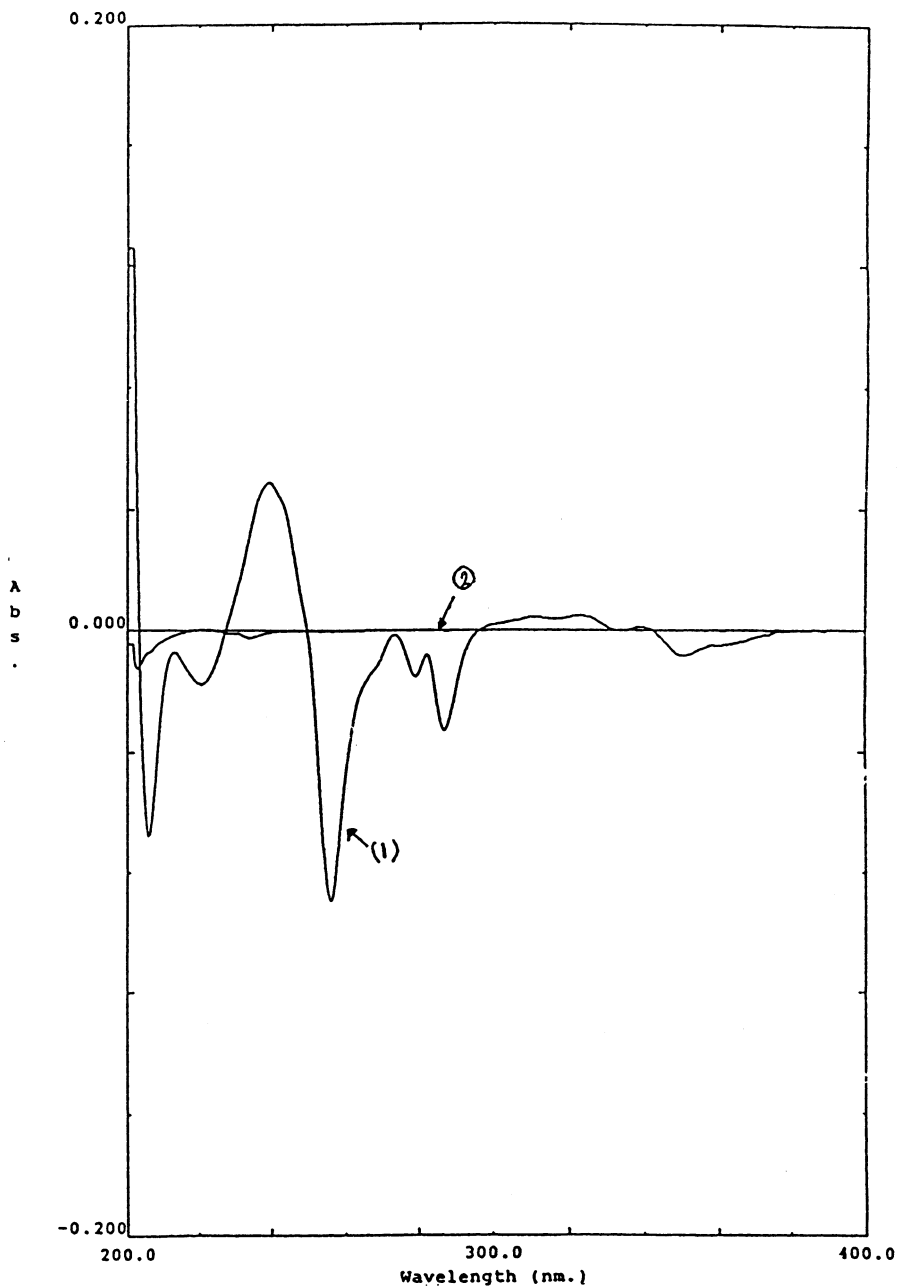
Fig. 4. First derivative spectra of doxazosin mesylate (1) and degradation products (2) 10 µg ml^{-1} in methanol.

$D_1 = 0.0018 + 0.0092C \quad r = 0.9999 \quad$ for (**I**).

$D_1 = 0.00016 + 0.0019C \quad r = 0.9980 \quad$ for (**II**).

Where D_1 is the first derivative value, C is the concentration in µg ml^{-1} and r is the correlation coefficient.

By applying the regression equation, it was possible to determine (**I**) and (**II**) with mean percentage accuracy of 99.21 ± 0.88 and $99.59 \pm 1.67\%$, respectively.

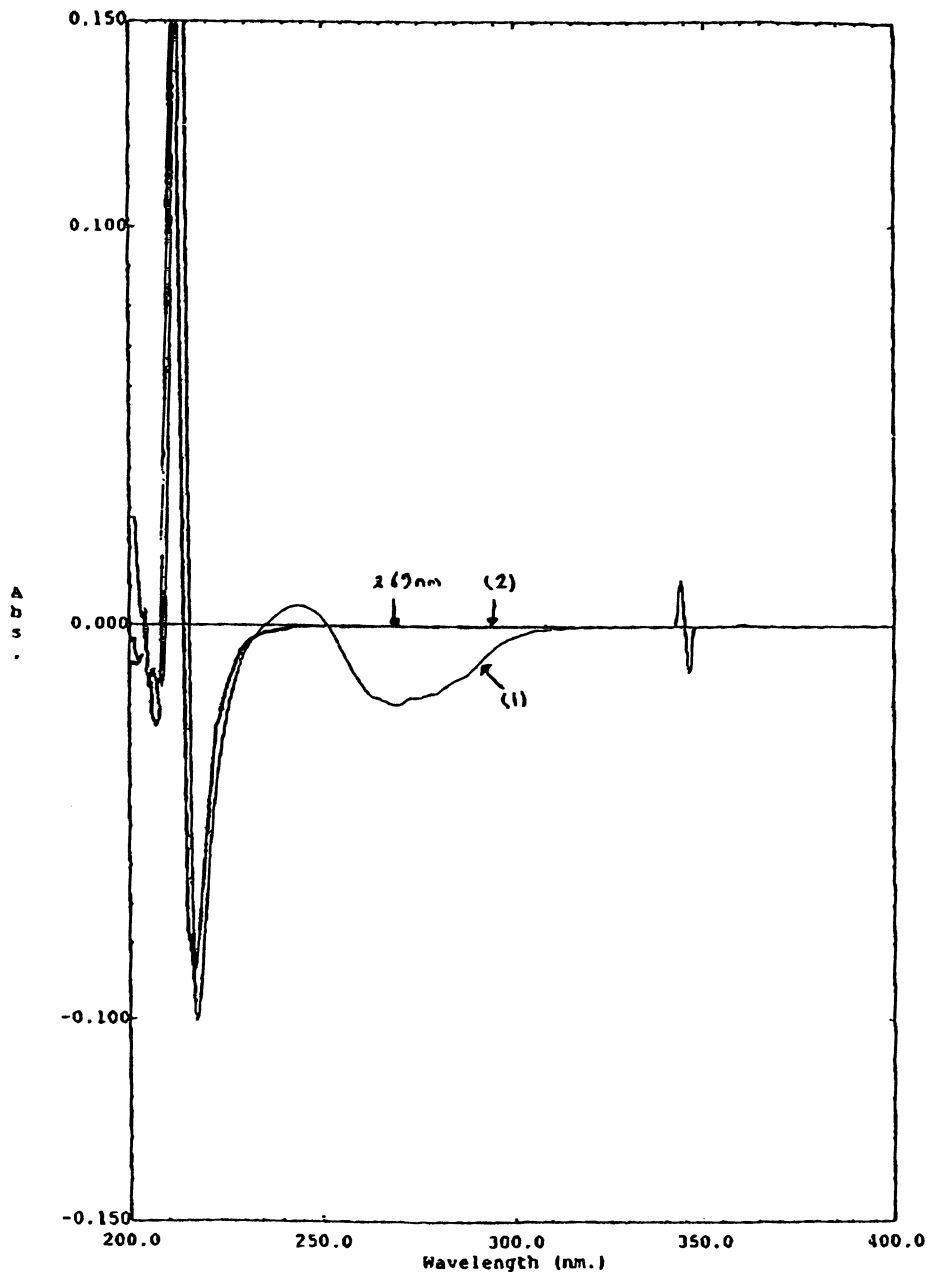The laboratory-prepared mixtures were analysed by first derivative technique at the men-

Fig. 5. First derivative spectra of celecoxib (1) and degradation product (2) 10 µg ml^{-1} in 0.1 M sodium hydroxide.

Table 1
Analytical data and statistical comparison of results for the determination of doxazosin mesylate and celecoxib by proposed and the reported methods

	Doxazosin mesylate			Celecoxib		
	First derivative method	Densitometric method	Reported method [2]	First derivative method	Densitometric method	Reported method [14]
Range of concentration	0.8–12 µg ml^{-1}	1–4 µg per spot	2–10 µg ml^{-1}	1–20 µg ml^{-1}	1–4 µg per spot	200–800 µg ml^{-1}
Mean ± C.V.%	99.21 ± 0.88	100.19 ± 0.95	100.08 ± 1.16	99.59 ± 1.67	99.90 ± 1.95	99.01 ± 1.11
C.V.	0.88	0.95	1.16	1.67	1.95	1.11
Correlation coefficient	0.999	0.9999		0.998	0.999	
Variance	0.77	0.90	1.35	2.79	3.80	1.23
N	6	6	6	6	6	6
F	1.75 (5.05)[a]	1.50 (5.05)[a]		2.27 (5.05)[a]	3.09 (5.05)[a]	
t	1.45 (2.23)[a]	0.18 (2.23)[a]		0.71 (2.23)[a]	0.97 (2.23)[a]	

[a] The values between parenthesis are corresponding to the theoretical values of F and t at the 95% confidence level.

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Table 2
Comparison of the proposed method and the reported method for the determination of doxazosin mesylate and celecoxib in the presence of their degradation products

Sample number	% of degradation product[b]	Doxazosin mesylate			Celecoxib		
		Densitometric method	First derivative method	Reported method	Densitometric method	First derivative method	Reported method
		Found,%[a]	Found,%[a]	Found,%	Found,%[a]	Found,%[a]	Found,%
1	10	100.53	99.53	98.12	101.06	99.98	99.51
2	30	99.40	100.50	99.56	100.69	100.00	100.56
3	50	101.20	97.56	97.91	98.38	99.50	98.91
4	80	100.37	100.00	99.75	99.64	100.00	99.79
5	90	100.48	100.00	100.12	100.98	100.00	100.95
	Mean	100.60	99.52	99.09	100.15	99.90	99.94
	C.V.	0.82%	1.15%	1.01%	1.14%	0.22%	0.81%

[a] Found % of pure sample.
[b] Calculated with respect to the total weight (drug-degradation mixture).

Table 3
Determination of doxazosin mesylate by the proposed and the reported methods [2]

Preparation	First derivative method		Densitometric method		Reported method [2]
	Found ± R.S.D. (%)	Recovery ± R.S.D. (%)[a]	Found ± R.S.D. (%)	Recovery ± R.S.D. (%)[a]	Found ± R.S.D. (%)
Cardura tablets 1 mg per tablet B.N. 9122	99.66 ± 0.52	99.49 ± 0.60	101.81 ± 0.51	98.26 ± 1.15	100.61 ± 1.10
	$F = 4.48\ (9.28)^b$ $t = 1.34\ (2.77)^b$		$F = 4.65\ (9.28)^b$ $t = 1.69\ (2.77)^b$		
Cardura tablets 4 mg per tablet B.N. 0203	98.99 ± 1.59	99.88 ± 1.67	99.27 ± 1.35	100.06 ± 0.79	100.49 ± 1.38
	$F = 1.33\ (9.28)^b$ $t = 1.52\ (2.77)^b$		$F = 1.50\ (9.28)^b$ $t = 1.14\ (2.77)^b$		

[a] Average of six analytes.
[b] The values between parenthesis are corresponding to the theoretical values of t and F at the 95% confidence level.

Table 4
Determination of celecoxib in pharmaceutical formulations by the proposed and the reported methods [14]

Preparation	First derivative method		Densitometric method		Reported method [14]
	Found ± R.S.D. (%)	Recovery ± R.S.D. (%)[a]	Found ± R.S.D. (%)	Recovery ± R.S.D. (%)[a]	Found ± R.S.D. (%)
Celebrex tablets 100 mg per tablet B.N. 101	99.47 ± 0.91	100.62 ± 0.89	98.59 ± 0.76	98.44 ± 1.21	98.56 ± 0.99
	$F = 1.18\ (9.28)^b$ $t = 1.17\ (2.77)^b$		$F = 1.69\ (9.28)^b$ $t = 0.04\ (2.77)^b$		
Celebrex tablets 200 mg per tablet B.N. 002	99.51 ± 1.87	100.35 ± 0.75	99.56 ± 1.69	100.39 ± 1.37	100.07 ± 1.72
	$F = 1.33\ (9.28)^b$ $t = 0.42\ (2.77)^b$		$F = 1.66\ (9.28)^b$ $t = 0.41\ (2.77)^b$		

[a] Average of six analytes.
[b] The values between parenthesis are corresponding to the theoretical values of t and F at the 95% confidence level.

tioned wavelengths. The method is valid for determining (**I**) and (**II**) in laboratory-prepared mixtures containing up to 90% degradation products.

3.2. Densitometric method

Complete separation of (**I**) and (**II**) from their degradation products were obtained using meth-ylisobutyl ketone–glacial acetic acid–water (20:10:10) and cyclohexane–dichloromethane–di-ethylamine (50:40:10) as a mobile phase, respec-tively. The R_f values for (**I**) and its degra-dation products were 0.6, 0.12 and 0.31, respectively, whereas the R_f values for (**II**) and its degradation product were 0.23 and 0, respectively.

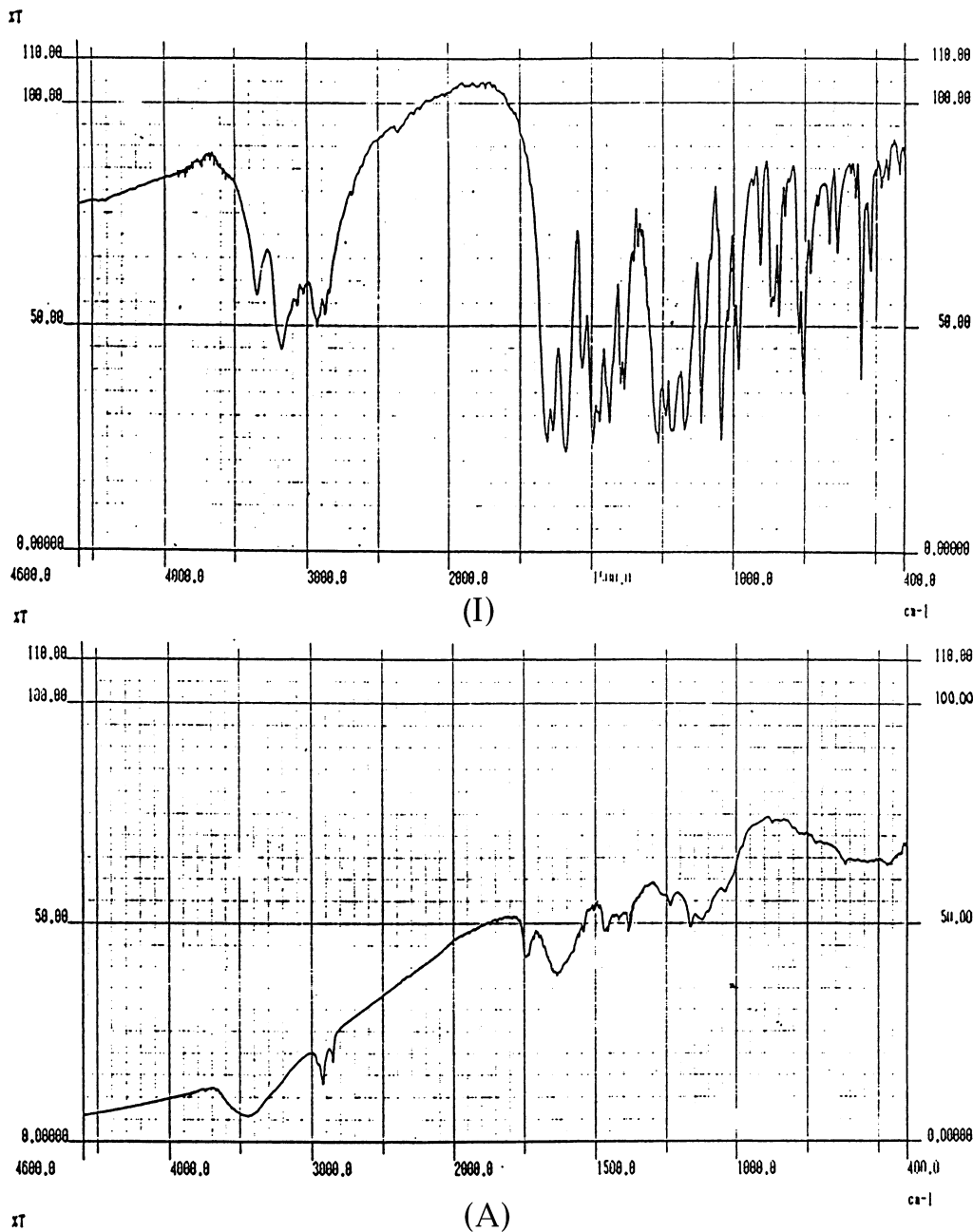Linear correlations were obtained between the area under the peak and the concentration of (**I**)

Fig. 6. IR spectrum of doxazosin mesylate (I) and its degredation products (A and B).

and (**II**) in the range of 1–4 μg per spot for both, from which the linear regression equations were calculated.

$$A = 11.835 + 10.83C \quad r = 0.9990 \quad \text{for (I)}$$
$$A = 0.3696 + 17.962C \quad r = 0.9990 \quad \text{for (II)}$$

Where A is the area under the peak, C is the concentration in μg and r is the correlation coefficient.

The method is valid for determining (**I**) and (**II**) in the laboratory-prepared mixtures containing up to 90% of their degradation products.
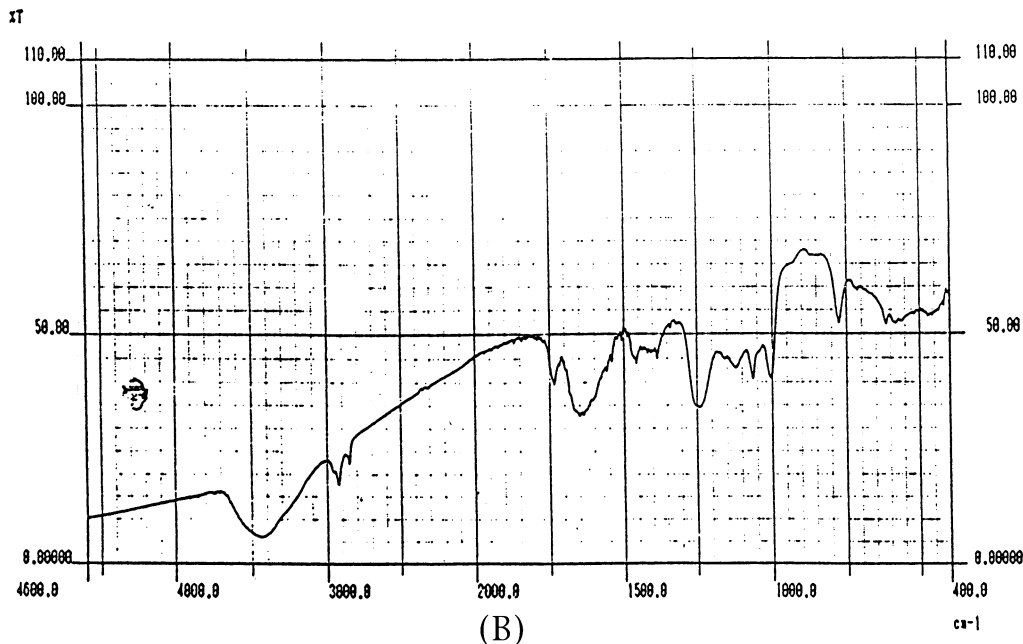
Fig. 6. (*Continued*)

Table 1 shows the analytical data and statistical comparison of analytical results for pure samples by the proposed method and reference methods [2,14]. Calculated t and F values are less than the theoretical ones, indicating with 95% confidence limit that there is no significant difference between the proposed and the reference methods with respect to accuracy and precision.

To assess the stability-indicating efficiency of the proposed methods, the degradation products of doxazosin mesylate and celecoxib were mixed separately with authentic sample in different ratios and analysed. Results are shown in Table 2.

Results of analysis of doxazosin mesylate and celecoxib in dosage forms are shown in Tables 3 and 4.

The validity of the proposed methods was assessed by applying the standard addition technique. Results are shown also in Tables 3 and 4.

The degradation products of doxazosin mesylate were prepared in the laboratory by hydrolysis with 2 M sodium hydroxide, and separated by TLC using methylisobutyl ketone–glacial acetic acid–water (20:10:10) as a developing sys-

tem. Two spots appeared for the degradation products (complete degradation), one spot at $R_f = 0.12$ for 4-amino-2-piperazin-1-yl-6,7-dimethoxy quinazoline (A) and the other at $R_f = 0.31$ for 1,4-benzodioxan-2-carboxylic acid (B). This is indicated by spraying the plate with Marshall reagent (N,N,1-naphthyl ethylene diamine-dihydrochloride), the amine compound gives a purple colour. The suggested degradation products was confirmed by IR spectrum which showed that the degradation of doxazosin mesylate may occur at the amide linkage to give A and B. The IR spectrum of A indicates the disappearance of band due to C=O group and the appearance of single broad band at 3400 cm^{-1} which attributed to N–H of secondary amine, in addition to C–H stretching at 2960–2850 cm^{-1}. The IR spectrum of B showed broad band at 3400 cm^{-1} due to –OH association and broad band at 1650 cm^{-1} due to C=O acid, in addition to C–H stretching at 2960–2850 cm^{-1} as shown in Fig. 6.

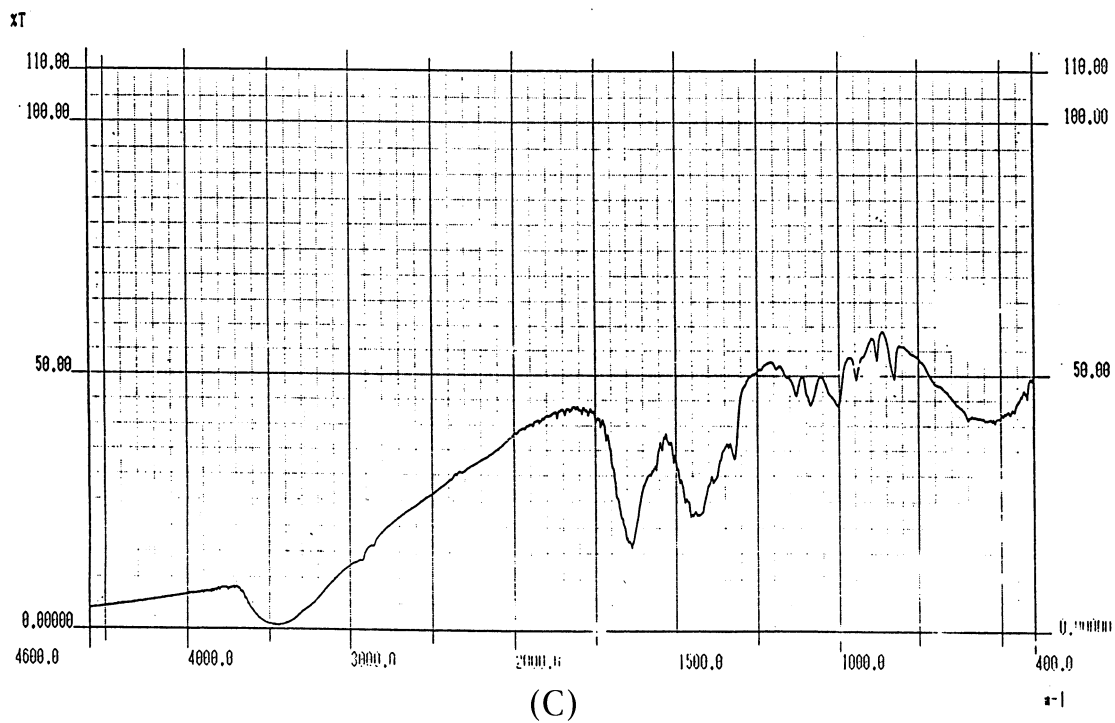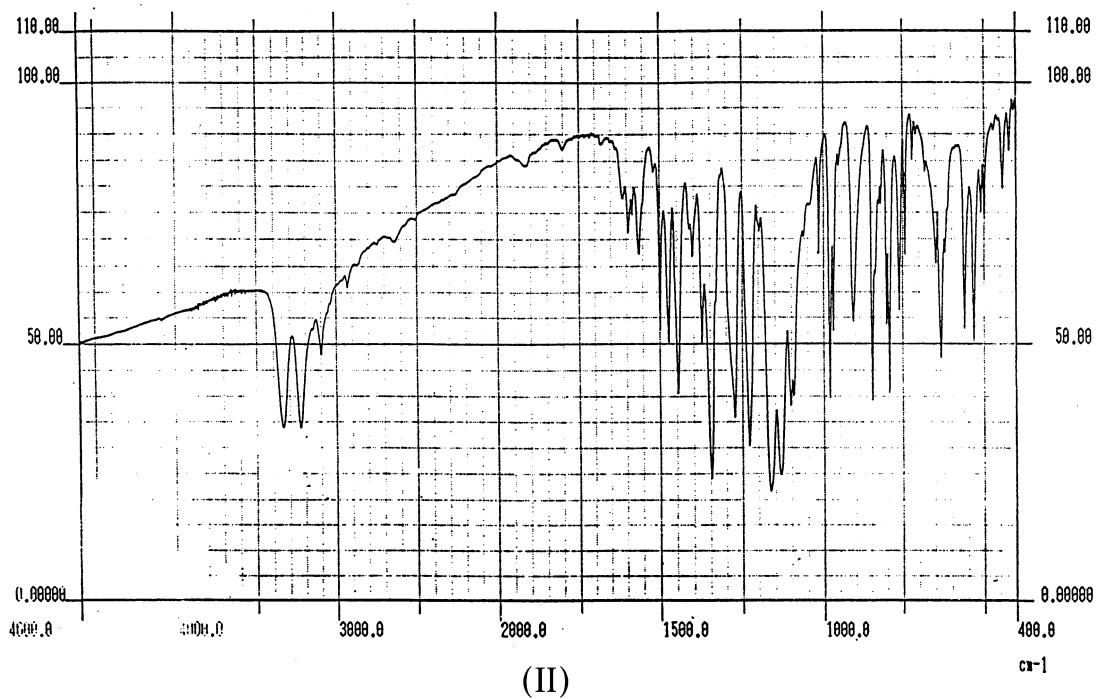The degradation product of celecoxib was prepared in the laboratory by hydrolysis with 2 M

Fig. 7. IR spectrum of celecoxib (II) and its degradation product (C).

hydrochloric acid and separated by TLC using cyclohexane–dichloromethane–diethyleamine (50:40:10) as a developing system. One spot was appeared at $R_f = 0$ for 4-[5-(4-methylphenyl)-3-(trifluoromethyl)–1H-pyrazol-1-yl] (C). This is confirmed by IR spectrum. The IR spectrum of authentic sample revealed the presence of two peaks at 3350 and 3220 cm^{-1} which attributed to NH$_2$ (symmetric and asymmetric), in addition to two strong signals at 1350 and 1180 cm^{-1} due to –SO$_2$–N–. The disappearance of bands due to NH$_2$ and –SO$_2$–N in the IR spectrum of degradation product is indicative to suggest that the degradation may be due to hydrolysis followed by desulfonation of the sulfonylamino group to give structure C, as shown in Fig. 7.

4. Conclusion

The suggested methods can be used as stability-indicating method for the determination of bulk powders or pharmaceutical formulations of doxazosin mesylate and celecoxib without interference from their degradation products or from excipients. In addition to their stability-indicating ability, the proposed methods are more simple, sensitive, accurate, precise, not need to compli-cated instrument and can be used for quality control laboratories.

References

[1] S. Budavari, Merck Index, 12th ed., Merck, Darmstadt, 1996, p. 580.
[2] HPLC manufacturer procedure (Pfizer), personal communication.
[3] M.G. Cowlishaw, J.R. Sharman, J.Chromatogr. Biomed. Appl. 8 (11) (1985) 403–407.
[4] H.G. Fouda, T.M. Twomey, R.P. Schneider, J. Chromatogr. Sci. 26 (11) (1988) 570–573.
[5] G.P. Jckman, F. Colagrande, W.J. Louis, J. Chromatogr. Biomed. Appl. 104 (1991) 234–238.
[6] P.K. Owens, A.F. Fell, M.W. Coleman, J. Chromatogr. 9 (2) (1997) 184–190.
[7] M. Ustun Ozgur, S. Islimyeli, S. Aycan, Pharmazie 52 (7) (1997) 561–562.
[8] G. Altokkia, M. Tuncel, Pharmazie 52 (11) (1997) 879–881.
[9] G. Altiokka, M. Tuncel, J. Pharm. Biomed. Anal. 17 (1) (1998) 169–175.
[10] A. Arranz, S. Fernandez de Betono, J.M. Moreda, A. Cid, J.F. Arranz, Analyst 122 (8) (1997) 849–854.
[11] S. Fernandez de Betono, J.M. Moreda, A. Arranz, J.F. Arranz, Anal. Chim. Acta 329 (1) (1996) 25–31.
[12] S. Fernandez de Betono, A. Arranz Garcia, J.F. Arranz Valentin, J. Pharm. Biomed. Anal. 20 (4) (1999) 621–630.
[13] P.S. Hijli, M.M. Phadke, M.C. Shah, P.P. Deshpande, R.T. Sane, Indian Drugs 35 (10) (1998) 653–657.
[14] HPLC, manufacturer procedure (Pfizer), personal communication.