ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
28 (2002) 711–719

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

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LC and TLC determination of cinnarizine in pharmaceutical preparations and serum

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Received 6 July 2001; received in revised form 18 October 2001; accepted 26 October 2001

Abstract

New high performance liquid chromatography (HPLC) and thin layer densitometry (TLC) methods are developed for quantification of cinnarizine in dosage forms in the presence of its photo-degradation products and related substances and in the presence of its metabolites in serum. Mobile phases consisting of benzene–methanol–formic acid (80:17:3) and methanol–acetate buffer of pH 4 (70:30) are satisfactorily used for resolution of cinnarizine from associated substances by TLC and HPLC techniques, respectively. The lower detection limits are 16 and 10 ng μl^{-1} of cinnarizine with standard deviations of 1.3 and 1.1% with TLC and HPLC, respectively. The methods are used for assessment of drug purity, stability, bioavailability, bioequivalency and tablet dissolution rate. Four cinnarizine related substances and six drug degradation products are isolated and identified by infrared and mass spectrometry. The results obtained by both techniques are in good agreement and offer the advantages of reproducibility and accuracy.

Keywords: High performance liquid chromatography; Thin layer densitometry; Cinnarizine assay; Cinnarizine photo- and acid-degradation products; Tablet dissolution rate; Bioequivalency; Bioavailability

1. Introduction

Cinnarizine [1-(diphenylmethyl)-4-(3-phenyl-2-prop-2-enyl)-piperazine] is used as an antihistaminic drug, calcium entry blocker, and for the treatment of cerebral and peripheral vascular insufficiency [1]. Cinnarizine has also been prescribed for motion sickness, and vestibular symptoms of different origins, and gives accurate efficiency for different syndromes such as Meniere's disease or cerebrovascular vertigo [2].

Available methods in the literature for quantification of cinnarizine in pharmaceutical preparations and biological materials include spectrophotometry [3–6], polarography [7], and potentiometry [8–10]. Apart from the non-specific non-aqueous visual titrimetric procedures introduced in the British Pharmacopoeia [11,12] for the determination of cinnarizine, no procedures have been described, so far, in all other international

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0731-7085/02/$ - see front matter
PII: S0731-7085(01)00662-8

pharmacopoeias. These methods, however, are not suitable for detecting and quantifying drug metabolites, degradation products and related substances. Chromatographic examination offers the possibility of testing the drug in the presence of related substances in various matrices. Little is known about the use of gas chromatography [13–15], high performance liquid chromatography (HPLC) [16–22] and thin layer chromatography (TLC) [23,24] for quantification of cinnarizine.

The TLC methods available in the literature are either applicable only for qualitative detection [11,12] or used for the assay of the free and conjugated cinnarizine in urine by prior enzymatic hydrolysis and spectrophotometric evaluation of the isolated spots [23]. HPLC methods previously described suffer from interferences caused by related substances, not accurate enough for accurate quantification and used insensitive UV detection at 280 nm [16–20].

The present TLC and HPLC methods offer many advantages over all those previously described: These are: (i) high sensitivity and accuracy for quantitative measurements of cinnarizine in dosage forms and serum, (ii) detection and evaluation of all cinnarizine related and degradation substances, (iii) application to the study of tablet dissolution rate and bioequivalency, and (iv) biomedical and pharmacokinetic study of the fate of the drug. Four associated photodecomposition and related impurities and six degradation products of cinnarizine are identified by mass spectrometry and quantified by the proposed techniques for the first time.

2. Experimental

2.1. Equipment

A HPLC system (Waters 510) equipped with Phenomenex loop injector (20 μl) and a variable wavelength UV detector (Waters, Lambda Max Model 481) was used. The chromatograms were recorded with Waters data station (model 730) with system controller programmable (model 721). All measurements were carried out at 50 °C with solvent delivery pump of isocratic system (Waters M 600), and Bondapack C_{18} column (250×4.6 mm id) with a particle size of 5 μm. The chart speed was 0.5 inch/min, sensitivity 0.05, run time 15 min, pump pressure 1750 psi and flow rate 1.5 ml/min. The mobile phase consisted of acetate buffer (20 ml glacial acetic acid, adjusted to pH 4):methanol (30:70).

All TLC densitometry measurements were carried out at 250 nm using Desaga densitometer CD-60 chromatogram (Desaga, Heidelberg) with a slit width of 1 mm and slit height of 10 mm. Ascending technique, one dimension in through chamber at normal saturation, a development time of 40 min, and a run distance of 15 cm were utilized while sample application was carried out using TLC plate 20×20 cm (Whatman K6 F silica gel) with a mobile phase consists of benzene:methanol:formic acid (80:17:3). The detection was made using both UV and Xenon lamps.

Tablet dissolution rate measurements were made according to 1 USP XX1 method using Pharma Test (PTW 1) dissolution measurement instrument (Hainburg, Germany) at a rate of 100 rpm.

The infrared spectrometric measurements were carried out using Shimadzu IR-470 using KBr pellets from 400 to 4000 cm^{-1}. The ultraviolet measurements were carried out with Perkin Elmer Lambda 15 UV/Vis spectrophotometer using 1.0-cm quartz cuvettes.

Mass spectrometric measurements were carried out using GC MS-QP 1000 Ex-Shimadzu under the conditions: ionization mode, EI positive; electron voltage, 70 eV; source temperature, 290 °C; scan time, 35 min. The gas chromatography temperature rate was programmed at 50–170 °C with a rate of 25 °C/min; 170–250°C with a rate of 4 °C/min and at 250 °C for 10 min.

2.2. Reagents and materials

All chemicals used were of pharmaceutical purity. Methanol and glacial acetic acid were spectroscopic grade (Merck, Dermstadt, Germany). Carbon tetrachloride, chloroform, formic acid, benzene, sulfanilamide and sodium acetate were obtained from BDH (Poole, UK).

Cinnarizine powder was obtained from El-Nasr Pharmaceutical Chemicals Company (Abu-Zaable, Egypt). A stock solution of cinnarizine was freshly prepared by dissolving 0.368 g of the pure powder in 15 ml 0.05 M hydrochloric acid; the solution was made up to 100 ml in a measuring flask with 0.05 M ethanol, stored in brown bottles, and kept in a refrigerator when not in use. Under these conditions, the drug solution is stable for at least 3 weeks.

The pharmaceutical preparations of cinnarizine used were: cinnarizine (ADWIC, Cairo, Egypt), Stugron (Glaxo, England), Sureptil (Memphis/Delgland), Stuval (Cid, Egypt), and Cerebal (Alex. Pharm. Comp., Egypt). Twenty tablets were weighed and powdered. A quantity equivalent to 25 mg of cinnarizine was transferred to a 250 ml volumetric flask, followed by 15 ml 0.05 M hydrochloric acid, sonicated and completed to volume with ethyl alcohol.

The TLC mobile phase used throughout was a mixture consisting of benzene, methanol and formic acid in the ratio of 80:17:3, respectively. For the ascending technique, one dimension in a heavy borosilicate glass chamber (20 cm long × 10 cm wide × 40 cm high) at normal saturation for 1 h, development time of 40 min, and a run distance of 13 cm. Separation was carried out under a well ventilated fume hood. The HPLC mobile phase was a mixture of methanol and acetate buffer of pH 4 in the ratio of 70:30, respectively. Before use the eluent was degassed by sonication for 15 min.

Experimental rats of the same family (Sprague–Dawley), sex male, age $\sim$6 months, and weight 180–200 g were used. They were housed in stainless steel cages under optimum conditions for 1 week before drug administration to assure that they were healthy. They were supplied with a conventional diet and drinking water.

2.3. Construction of HPLC and TLC calibration graphs

Calibration curves were made using standard cinnarizine solutions (10^{-3}–10^{-6} M). For HPLC measurements, a 10 μl aliquot of each concentration was injected and the peak area at R_t of 4.5 ± 0.2 was measured and plotted against concentration. For TLC measurements, 20 μl aliquots of each standard cinnarizine solution were applied on silica gel plate and slid into the tank which contained the mobile phase. The tank was covered and a run for 40 min was performed. The plates were removed and R_f values were detected under a UV or Xenon lamp. The peak area of cinnarizine spot R_f 0.4 was measured using Desaga densitometer and a calibration graph was plotted. The calibration graph was used for determination of unknown cinnarizine samples. Secondary spots in TLC of cinnarizine were scratched, extracted in chloroform, filtered, evaporated to dryness and identified using infrared, ultraviolet, and mass spectrometry.

2.4. HPLC and TLC determination of cinnarizine in dosage forms

Ten tablets of the drug were finely powdered and an accurate weight equivalent to one tablet (25 mg) was transferred into a 250 ml standard measuring flask, dissolved in 15 ml of 0.05 M HCl and 20 ml ethanol. The solution was shaken for 5 min, completed up to volume with ethanol, sonicated for 3 min and filtered. Aliquots (10 and 20 μl) of the solution were applied to HPLC and TLC for separation, respectively. Standard cinnarizine solutions ($\equiv$0.5, 5, and 50 μg) were applied in parallel. In case of TLC, the standard was applied in an increasing concentration order from right to left across the TLC (20 × 20 cm) plate and five identical aliquots (20 μl) of the pharmaceutical test samples were applied to the starting line in the central region of the plate at a distance of 1 cm above the lower edge. After separation, the concentration was measured using Desaga densitometer at 250 nm.

2.5. HPLC and TLC determination of cinnarizine in blood serum (pharmacokinetics)

The pharmacokinetics study was carried out by giving a 5 ml portion of cinnarizine solution (10 mg ml^{-1}) in 1% carboxy methyl cellulose (CMC) orally by means of curved canula to 10 experimental rats of the same sex, age, and family. Blood samples were collected from each rat from

the tail vein at time intervals of 30-min for 5 h. The serum was stored at 4 °C or frozen until taken for analysis. To a 1.0 ml aliquot of the serum in a B24 ground glass centrifuge tube, 0.15 ml of 0.5 M HCl was added and thoroughly mixed. The solution was extracted with 1 ml of chloroform. After shaking for 1 min, and centrifugation for 15 min at $1500 \times g$, the organic layer was aspirated off and transferred to a second tube by means of disposable pasture pipette. The collected organic layer was evaporated to dryness under nitrogen gas flow in a water bath at 50 °C and the residue was dissolved in 100 μl methanol. Aliquots (50 and 20 μl) were applied for TLC and HPLC, respectively.

2.6. Identification of cinnarizine acid-degradation products

A portion of cinnarizine (500 mg) was dissolved in 100 ml of 0.5 M HCl and refluxed on a water bath at 90 °C for 60 h. After cooling, white crystals were formed, separated by filtration on glass crucible G-2 and collected for analysis. The mother liquor was made alkaline with 1 M NaOH and extracted with 2×25 ml of chloroform. The chloroform extract was evaporated to dryness under nitrogen gas stream and the residue was isolated. TLC, HPLC, infrared, mass spectrometry and elemental analyses of the residues were made.

2.7. HPLC determination of cinnarizine tablets dissolution rate (Vitro bioequivalency)

Vitro bioequivalency test of cinnarizine was made using two products from El-Nasr Pharmaceutical Chemicals Company (local company, Egypt) and Glaxo Pharmaceutical Company (international company, England). One tablet of each drug (25-mg dosage cinnarizine) was allowed to dissolve in 500 ml of 0.1 M HCl at a stirring rate of 100-rpm using the standard dissolution apparatus 1. The amount of cinnarizine dissolved was determined by the developed HPLC method and by spectrophotometric method for comparison. For spectrophotometric measurement, 1.0 ml of the test solution was taken every 10 min, filtered, diluted with 0.1 M HCl up to 10 ml in a measuring flask and the absorbance was measured at 251 ± 2 nm against a blank of 0.1 M HCl in a 1.0 cm quartz cuvette. The absorbance was compared with a calibration graph made using standard cinnarizine. For HPLC measurements, a 1.0-ml aliquot of the test solution was taken every 10 min, filtered, diluted to 10 ml with methanol, and a 10 μl aliquot of the test solution was chromatogrammed. The peak area was determined and the results were compared with a standard calibration graph.

3. Results and discussion

The British Pharmacopoiea [11,12] described a TLC method for identification of cinnarizine and related substances using toluene–methanol or methanol solvent as a mobile phase and silica gel as a stationary phase. A trail was made to use this method for identification of some drug related substances and for quantitative determination of cinnarizine by densitometry. Upon testing an impure cinnarizine sample containing four related substances, only two spots were displayed.

Different mobile phases (e.g. chloroform, methanol:chloroform, hexane:methanol, dichloroethane:methanol:acetic acid, toluene:methanol: acetic acid, benzene:methanol:acetic acid, benzene:methanol:chloroform, benzene:methanol: formic acid with various ratios), and some commonly used stationary phases (e.g. Kieselgel, silica gel, alumina) were examined. With alumina and Kieselgel, the rate of solvent migration was significantly slow while silica gel provided the best compromise for fast rate of solvent migration, good resolution and regular spots. Examination of an impure cinnarizine sample with the developed TLC densitometric method gave sharp resolved five spots compared with only two spots obtained by the British Pharmacopoeia method.

HPLC techniques were also used for determination of cinnarizine and its related substances in different matrices. Examination of cinnarizine test solution containing cinnamyl piperazine, benzahydral piperazine, cinnamyl chloride and piperazine, as related substances and impurities, using the

mobile phases previously recommended [16–20] showed severe interferences due to peak overlapping. Although methanol:sodium acetate–HCl buffer at pH 5.2 as a mobile phase in the ratio (85:15) was suitable for identification of cinnarizine in the presence of its related substances [17], quantification of these substances was not accurate. Trials to optimize the separation conditions by using methanol:acetate acetic acid buffer of different ratios and different pHs indicated that reasonable resolution of cinnarizine related substances takes place using a mobile phase with the optimum composition 70:30 methanol:acetate acetic acid buffer of pH 4.

On the other hand, two approaches have been mentioned in the literature about chromatographic detection of cinnarizine. These are ultraviolet measurements at either 230–250 or 285 nm and fluorimetric measurement at 310 nm (excitation at 245 nm). When a cinnarizine sample containing 12% benzahydral piperazine was subjected to TLC separation using the proposed mobile phase and ultraviolet or fluorescence detector, quantitative recovery of benzahydral piperazine was obtained with UV detection and only 2.8% was recovered upon using the fluorescence detection. However, ultraviolet detection at 285 nm as previously suggested [16] showed only about 7% of the absorbance at 250 nm. All subsequent measurements were made at 250 nm.

3.1. TLC and HPLC determination of cinnarizine

Validation of the proposed TLC and HPLC methods was made by measuring the lower limit of detection (LOD), range, accuracy (recovery), precision, trueness, repeatability (CV_w) and between day variability (CV_b). Measurements were conducted according to the quality assurance standards [25] using six batches (six determinations each). The results obtained are shown in Table 1. A statistical analysis of the results indicated that at the 95% confidence level, TLC and HPLC methods show no statistical difference ($t = 0.78$).

The purity of some cinnarizine powders and dosage forms from different companies were assessed by TLC densitometric measurement at 250 nm of the main spot at R_f 0.4. These formulations contain various percentages of lactose, maize starch, talc powder, poly vidon, hydrogenated vegetable oil and magnesium stearate as diluents and excipients. The results obtained (Table 2) showed an average recovery of 98.6% with a standard deviation of 1.3% ($n = 5$). Cinnarizine was also determined using HPLC. The cinnarizine peak of each dosage forms at $R_t = 4.5$ was evaluated at 250 nm, and compared with the calibration graph. The mean standard deviation was 1.1% and the average recovery was 99.6% (Table 2). Results obtained using the standard non-aqueous titrimetric methods of the British pharmacopoeia [11] are included in Table 2 for comparison.

3.2. HPLC measurement of the dissolution rate of cinnarizine tablets

Tablet uniformity and dissolution profile of cinnarizine were measured at 250 nm using the proposed HPLC method. The content uniformity test indicated 96–101% ($n = 15$) of the label amount with a standard deviation of less than 1.2%. A dissolution rate of cinnarizine at 100 rpm in 0.1 M HCl (stimulated gastric fluid) was examined using two different formulations; one from local company (El-Nasr) and the other from an international company (Glaxo). The results obtained indicated that the rate of cinnarizine disso-

Table 1
Validation parameters of TLC and HPLC methods used for cinnarizine quantification

Parameter	TLC	HPLC
Limit of detection (LOD) (ng ml^{-1})	16	10
Accuracy (%)	98.6	99.6
Trueness (%)	98.1	98.8
Repeatability (CV_w) (%)	0.8	0.9
Between-day-variability (CV_b) (%)	1.1	1.2
Calibration range (μg ml^{-1})	4–30	0.3–10
Slope (peak area μg^{-1})	11.38 ×	7 ×
Intercept (peak area)	23.09	−1.2
Correlation coefficient (r)	0.9997	0.9996
Standard deviation (%)	1.3	1.1

Table 2
TLC densitometry and HPLC determination of cinnarizine in dosage forms

Drug trade name (source)	Cinnarizine (mg/tablet)	Recovery* (%)[a]		
		TLC	HPLC	BP [12]
Sureptil (Memphis)[b]	20	98.0 ± 1.2	99.0 ± 0.9	99.0 ± 0.9
Stugeron (Glaxo)[c]	25	99.0 ± 0.9	100.0 ± 0.9	100.0 ± 1.2
Cinnarizine (El Nasr)[c]	25	98.9 ± 1.1	98.9 ± 1.0	99.5 ± 1.2
Cerebal (Alexandria)[c]	25	98.8 ± 1.1	98.8 ± 1.1	98.1 ± 0.9
Stuval (Cid-Pharm.)[c]	25	98.7 ± 1.3	98.7 ± 1.2	99.2 ± 1.1

[a] Average of five measurements.
[b] Each tablet contains heptaminol acefyllinate (200 mg) + cinnarizine (20 mg).
[c] Contains lactose, maize starch, talc, polyvidon, hydrogenated vegetable oil and magnesium stearate.

lution in both samples was almost equivalent. Over 90% tablet dissolution was detected after 2 min. Since the area under the average dissolution rate plot is used as an index for comparing bioequivalency [26], the present results revealed a relative higher bioequivalency of Glaxo drug (Stugron) compared to that of El-Nasr drug (Cinnarizine) by a factor of 1.1. Stability indicating assays are similarly conducted by following the concentration of cinnarizine with time.

3.3. TLC and HPLC determination of cinnarizine in blood serum (pharmacokinetics)

Cinnarizine level in blood serum was measured after a prior extraction. Recovery of cinnarizine by extraction from blood serum and spectrophotometric detection at 250 nm was investigated using alkaline and acidic blood sera with different organic solvents. The study showed that the recovery of extraction of cinnarizine at pH 2–3.5 is more favorable due to high solubility and stability of cinnarizine. Extraction over this pH range showed an average recovery of 85%, while extraction at pH 6.8 showed less than 50% recovery. These results are in good agreement with data obtained by other authors [17,18]. In general, the recovery of cinnarizine extracted from alkaline blood serum was 50% less than that extracted from acidified blood serum. The recoveries of cinnarizine extracted from acidified blood serum with different solvents such as chloroform, methylene chloride, and carbon tetrachloride were 97, 86 and 70%, respectively. This indicated maximal extraction of cinnarizine with acidified blood serum (pH 2–3.5) using chloroform.

The validity of the proposed TLC assay method for pharmacokinetics was tested after oral administration of 50-mg cinnarizine to 10 rats of the same weight, age and sex. The cinnarizine concentration in blood serum of the test animals was followed at R_f 0.4 for 5 h using sulphanilamide as internal standard (R_f 0.24) to monitor the efficiency of separation and extraction of cinnarizine metabolites. The HPLC method was also used for determining the concentration level of cinnarizine in blood serum by following up the peak area at R_t 4.3 with respect to internal standard (sulphanilamide) peak at R_t 2.5. The results showed that the concentration level of cinnarizine in the blood serum of rats increased with time to reach a maximum after 4 h then decreased gradually due to its excretion and/or decomposition. Concentration–time profiles of cinnarizine in the blood serum of rats at different times after drug administration as measured by using both HPLC and TLC developed methods were almost identical (Fig. 1).

3.4. Characterization and determination of cinnarizine related substances

Cinnarizine samples with purity of $>98\%$, as determined by the pharmacopoeial non-aqueous titrimetry, showed in the TLC chromatogram five resolved spots at R_f 0.45, 0.4, 0.23, 0.18 and 0.13

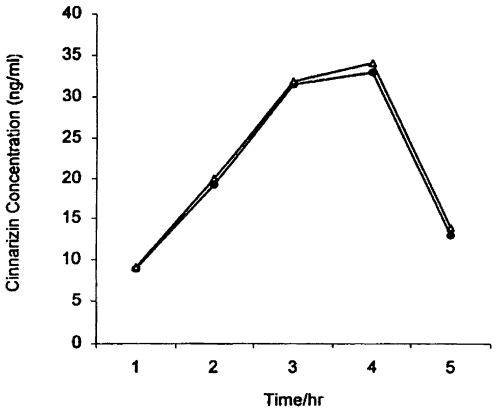

Fig. 1. Cinnarizine concentration–time profile in blood serum of rats after oral administration of 50 mg using the proposed: (○) TLC and (△) HPLC methods.

in the ratio of 4.1, 87.1, 1, 7 and 0.5%, respectively (Fig. 2). These data were confirmed by the HPLC method using 70:30 methanol:acetate buffer of pH 4 as a mobile phase and a detection at 250 nm. Five resolved signals from the same sample at R_t, 2.02, 2.6, 3.09, 4.3 and 5.25; with the ratio of 0.51, 6.37, 4.3, 87.8 and 1.02%, respectively, were obtained which are in good agreement with the TLC data (Fig. 2). This indicates that the sensitivity and selectivity of LC and TLC procedures are sufficient to determine cinnarizine and its related or degradation substances.

In an attempt to identify the nature and composition of the five spots isolated on TLC plates and confirmed by the HPLC method, the spots from several TLC plates were carefully scratched, eluted with chloroform, and spectrophotometrically measured over the range 190–400 nm. The solution of the spots were next evaporated, the residues were mixed with dry KBr for infrared measurements over the range 400–4000 cm^{-1}, and the spectra were examined. Mass spectrometric examination of the spots appearing at R_f 0.45, 0.4, 0.23, 0.18 and 0.13 showed molecular ion peaks at m/e 317, 367, 355, 327 and 251, respectively.

The data revealed that the five separated spots on the TLC plates are due to the presence of the compounds shown in Fig. 3. Spot (1) which appeared at R_f 0.45 (0.1%) has the chemical formula ($C_{22}H_{26}N_2$), with a molecular mass of 318.5 as

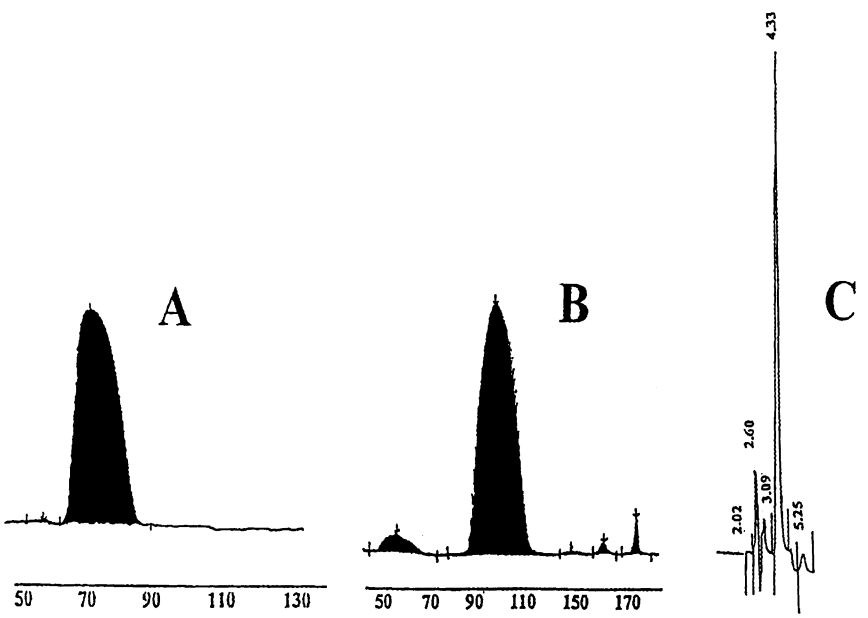

Fig. 2. Chromatograms of impure cinnarizine sample separated by: (A) British pharmacopoeia TLC, (B) proposed TLC, and (C) proposed HPLC methods.

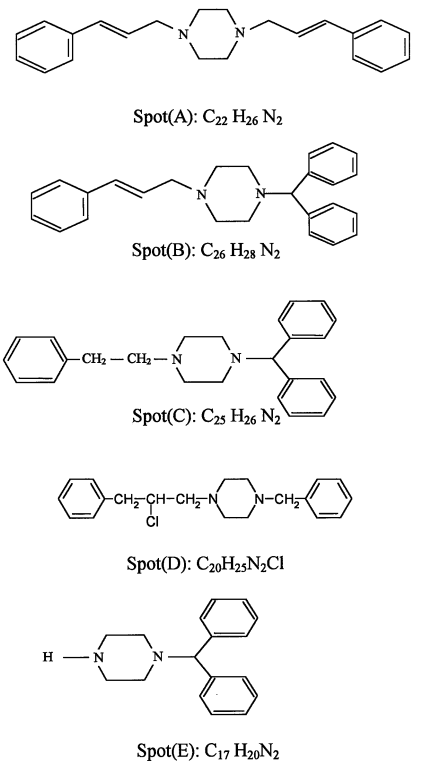

Fig. 3. Cinnarizine related substances isolated by TLC: spots (A) R_f 0.45, (B) R_f 0.4, (C) R_f 0.23, (D) R_f 0.18 and (E) at R_f 0.13.

confirmed by the molecular ion peak signal at m/e 317 due to $[C_{22}H_{25}N_2]^+$. This compound is probably dicinnamyl piperazine ($C_{22}H_{26}N_2$), which may be formed when two molecules of cinnamyl chloride reacted with one molecule of piprazine. The main spot (2) which appeared at R_f 0.4 (87.1%) with a molecular ion peak signal of $[C_{26}H_{27}N_2]^+$ at m/e 367 is due to cinnarizine ($C_{26}H_{28}N_2$). Spot (3) which appears at R_f 0.23 (1%) and displays a signal at m/e 355 in the mass spectrum due to the molecular ion peak $[C_{25}H_{25}N_2]^+$ confirms the presence of a compound with the formula $C_{25}H_{28}N_2$. Spot (4) appears at R_f 0.18 (11%), has a molecular formula of $C_{20}H_{25}N_2Cl$ as verified by the signal appeared at m/e 327 in the mass spectrum, and is probably due to $[C_{20}H_{24}N_2Cl]^+$. Spot (5) appears at R_f 0.13 (0.5%) with a molecular formula of $C_{17}H_{20}N_2$ as confirmed by the signal appeared at m/e 251 in the mass spectrum and is due to $[C_{17}H_{19}N_2]^+$.

3.5. Characterization and determination of cinnarizine acid-degradation products

Cinnarizine undergoes acid degradation reaction with the formation of insoluble white crystals suspended in the aqueous acidic solution on cooling. Other degradation products were soluble in the aqueous degradation acidic solution and were extracted with chloroform. The suspended white crystals were collected by filtration, washing with distilled water, and drying at 25 °C. Elemental analysis data of the solid reaction product revealed the presence of a nitrogen free substance (carbon 82% and hydrogen 7%). A methanolic solution of this product was subjected to TLC using the proposed method. Four spots with good resolution at R_f 0.87, 0.82, 0.46 and 0.43 were detected. The mass spectrometric examination of the spots appeared at R_f 0.87, 0.82, 0.46, and 0.43 showed molecular ion peaks at m/e 131, 133, 183 and 181, respectively.

Based on the data obtained by elemental analysis, infrared spectrometry and mass spectrometry, it is possible to suggest that the acid hydrolysis of cinnarizine proceeds via cleavage of the C–N bond in both benzahydral and cinnamyl sides to give nitrogen free reaction products, cinnamaldehyde (R_f 0.87), cinnamyl alcohol (R_f 0.82) 1-1 biphenyl methanol (R_f 0.46) and benzophenone (R_f 0.43) as shown in Fig. 4.

TLC densitometric measurements of the water soluble degradation products after extraction showed two main spots at R_f 0.13 and 0.26. Mass spectrometric examination of the two spots which appeared at R_f 0.13 and R_f 0.26 showed molecular ion peaks at m/e 251 and 201, respectively. Based on these data, it is possible to suggest formation of benzahydral piprazine (R_f 0.13) with a molecular formula $C_{17}H_{20}N_2$ (molecular mass 252) and cinnamyl piperazine (R_f 0.26) with a molecular formula $C_{13}H_{18}N_2$ (molecular mass 202) (Fig. 5).

In conclusion, this work demonstrates that the proposed HPLC and TLC densitometry methods are satisfactorily used for quantification of cinnarizine over the concentration levels of 0–30 μg/ml in the presence of its related and degradation substances in dosage forms and serum. Both techniques are suitable for determination of drug

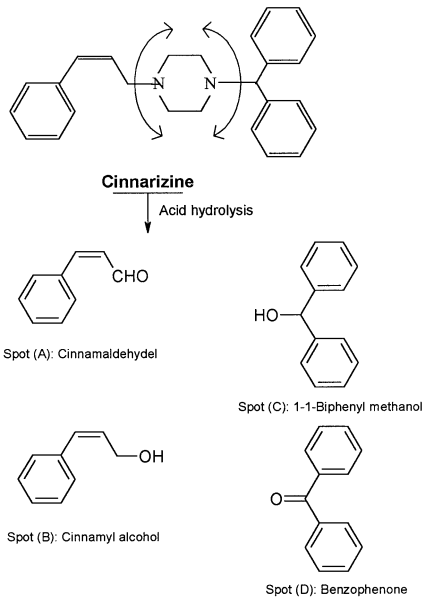

Fig. 4. Water-insoluble acid-degradation products of cinnarizine.

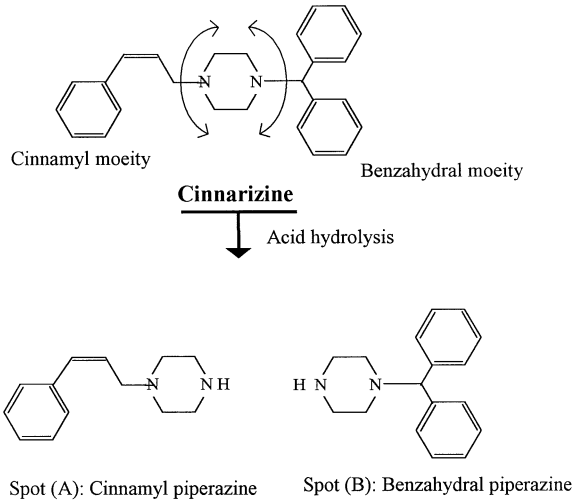

Fig. 5. Water-soluble acid degradation products of cinnarizine.

purity, stability, bioequivalency, bioavailability, and tablet dissolution rate.

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