Pergamon

0731-7085(95)01266-4

Journal of Pharmaceutical & Biomedical Analysis, Vol. 13, No. 6, pp. 777-784, 1995
Elsevier Science Ltd
Printed in Great Britain
0731-7085/95 $9.50 + 0.00

Liquid chromatographic determination of flunarizine dihydrochloride in the presence of its degradation product

ABDEL-AZIZ M. WAHBI,*† ABDEL-FATTAH M. EL-WALILY,† EKRAM M. HASSAN,† FARID G. SALIMAN‡ and ALAA EL-GENDI§

† *Pharmaceutical Analytical Chemistry Department, University of Alexandria, Alexandria 21521, Egypt*
‡ *Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Alexandria, Alexandria 21521, Egypt*
§ *Alexandria Co. for Pharmaceutical and Chemical Industries, Egypt*

Abstract: A simple, stability-indicating liquid chromatographic method has been developed for the assay of flunarizine dihydrochloride in the presence of its acid-induced degradation product. A Bondapak-C_{18} column was used with a mobile phase consisting of methanol–water (75:25, v/v) containing 0.5% w/v sodium chloride and 0.2% v/v triethanolamine adjusted to pH 6.6 with 30% hydrochloric acid at a flow rate 2 ml min^{-1}. Quantitation was achieved with UV detection at 254 nm based on peak area or peak height ratios. The proposed method was successfully applied to the determination of the drug in laboratory-prepared mixtures in the presence of its degradation product and in capsules. Moreover, the method was utilized to investigate the kinetics of the degradation process at different temperatures and the apparent first-order rate constant, half-life and activation energy calculated.

Keywords: *Flunarizine; liquid chromatography; stability; pharmaceutical capsule; kinetics of degradation.*

Introduction

Flunarizine is the difluorinated derivative of cinnarizine. It has vasodilator properties, and is used for the treatment of peripheral vascular disorders. A survey of the literature revealed that there have been several studies concerned with the determination of flunarizine in biological fluids. In brain tissues and in plasma of epileptic patients flunarizine has been determined using HPLC [1, 2]. Other HPLC [3–6] and GC methods [7–10] have been described for the assay of flunarizine in biological samples, together with biopharmaceutical and clinical applications for the assay of flunarizine in plasma [11] and serum [12]. However, no methods have been reported for the assay of flunarizine in commercial preparations or in presence of its degradation product.

The aim of the present work was to establish a stability-indicating liquid chromatographic method for the determination of flunarizine dihydrochloride in the presence of its degradation product. Furthermore, the developed method has been used to investigate the kinetics of the drug degradation at different temperatures.

Experimental

Instrumentation

The HPLC system comprises a Waters Associates Model 501 pump (Milford, MA, USA), with automated gradient controller Model 680 equipped with U6K universal injector and a Model 481 variable wavelength UV-detector. The instrument was also equipped with a Waters 740 data module. Separation and quantitation were made on a 30 cm × 3.9 mm i.d. column of Waters Bondapak-C_{18} (10 μm). Detection was made at 254 nm at a sensitivity of 0.1 a.u.f.s. The samples were injected (20 μl) with a 25 μl Waters analytical syringe (Scientific Glass Engineering, Australia). The chart speed was 2 cm min^{-1}.

Glass TLC plates (20 × 20 cm) were covered with 0.2 mm layer of Silica Gel 60 GF 254 (E. Merck, Darmstadt, Germany) and activated at 100–105°C for at least 1 h before use. The

* Author to whom correspondence should be addressed.

mobile phase used consisted of methanol–ammonia (100:1, v/v). The spots were examined at 254 nm using a UV source for TLC (Desaga, Heidelberg — Germany).

The infra-red spectrophotometry used was the Shimadzu IR-408.

PMR spectra were recorded on a Varian — Gemini 200, using tetramethylsilane as the internal standard.

A Hewlett–Packard mass spectrometer Model 5988 was used at 70 eV.

Materials and reagents

Flunarizine dihydrochloride USP, Dolder Ltd, Basle, Switzerland and clotrimazole, Bayer Leverkusen, Germany, were kindly supplied by Alexandria Pharmaceutical Company and their purity was certified and analysed to be 100%. HPLC grades of methanol and chloroform (Romil Chemicals Ltd, UK) were used. The water was double-distilled and filtered through a 0.45-μm membrane filter. Triethanolamine and sodium chloride were of analytical grade.

Flunarizine capsules (Alexandria Pharmaceutical Co.) labelled to contain 5 mg flunarizine dihydrochloride were used. Sibelium capsules (Advanced Biochem. Industries, A.R.E., under licence from Janssen Pharmaceutica, Beerse, Belgium) labelled to contain flunarizine dihydrochloride equivalent to 5 mg flunarizine were used.

The optimized mobile phase consisted of methanol–water (78:22, v/v) containing 0.5% w/v sodium chloride and 0.2% v/v triethanolamine; the pH of the aqueous components was adjusted to pH 6.6 with 30% hydrochloric acid before mixing. The mobile phase was filtered and degassed before use at a flow rate of 2 ml min^{-1}.

Preparation of degradation product

An accurately weighed 100-mg aliquot of fluanarizine dihydrochloride (I) was dissolved in 100 ml 0.1 N hydrochloric acid and refluxed on a water bath at 90°C for 70 h. The solution was rendered alkaline with 1 N sodium hydroxide and extracted with 4 × 25 ml portions of chloroform. The chloroformic extract was evaporated to dryness under nitrogen without heat. The residue was dissolved in ether and HCl gas was passed into the solution to precipitate the salt. The separated dihydrochloride salt was filtered and dissolved in hot methanol. The methanolic solution was filtered and crystallized using ether. The deposited crystalline product was filtered, washed with ether and dried under vacuum. The material was tested for complete degradation using the TLC system discussed above. A single spot at $R_f = 0.28$ was observed; the melting point was 233°C with decomposition. The analysis was calculated for: C_{13} H_{20} Cl_2 N_2: C = 56.73%; H = 7.32%; N = 10.18%; the percentage of elements found were: C = 56.68%; H = 7.17%; N = 10.21%.

Stock solutions

Flunarizine dihydrochloride solution was prepared by dissolving 100 mg flunarizine dihydrochloride (I) in 250 ml methanol. The solution of degradation product was prepared by dissolving 10 mg of the degradation product (II) prepared as above in 100 ml methanol. The internal standard solution (IS) was prepared by dissolving 400 mg clotrimazole in 100 ml methanol.

Preparation of calibration graph. A quantity 2–8 ml of flunarizine dihydrochloride solution was transferred into a series of 50-ml volumetric flasks. Five ml of internal standard were added to each flask and completed to volume with methanol. Aliquots of 20 μl of these solutions were chromatographed. Peak-area ratios and peak-height ratios of flunarizine dihydrochloride vs internal standard were used for calibration.

Analysis of capsules

An accurately weighed portion of the powder (mixed contents of 20 capsules) equivalent to about 40 mg of flunarizine dihydrochloride (or 34 mg of flunarizine base) was transferred into a 100-ml conical flask. Fifty millilitres of methanol were added and the flask was shaken for 10 min. The resulting suspension was filtered by washing through a filter paper into a 100-ml calibrated flask, then diluted to volume with methanol. A 5-ml aliquot of this solution was added to 5 ml of internal standard solution and the volume was adjusted to 50.0 ml with methanol. A 20-μl aliquot of the final solution was chromatographed. Quantitation was based on comparing peak-area or peak-height ratios of the sample with a standard, taking the average of three runs. The formula used was

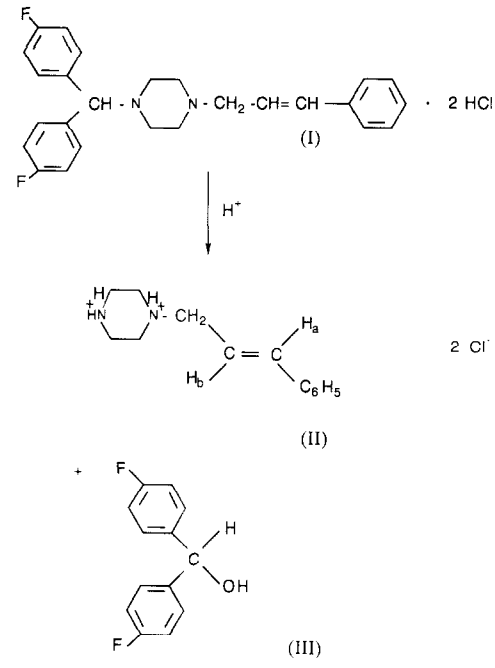
mg found per capsule =

$$\left(\frac{R_t}{R_s}\right) \times C_s \times \frac{1}{2} \times \frac{100}{5} \times \frac{\text{Average weight per capsule}}{\text{Weight taken}},$$

where R_t and R_s are the ratios of peak heights or peak areas of the test and standard solutions, respectively; C_s is the concentration of the final standard flunarizine dihydrochloride solution in mg/100 ml; and W is the weight of the sample. The results obtained can be converted to the free base by multiplying by 404.51/477.4, the ratio of the molecular weights of base and salt, respectively.

Kinetic investigation

An accurately weighed aliquot of 250 mg flunarizine dihydrochloride (I) was transferred into a 250-ml volumetric flask, dissolved and diluted to volume with 0.1 N hydrochloride acid for each temperature investigation. Separate 25-ml aliquots of this solution were transferred into separate stoppered conical flasks. The flasks were placed in a thermostatic oven at different temperatures (90, 80, 70, 65, 60°C) for different time intervals (1–10 h). At the specified time interval the contents of the flasks were neutralized to pH 7 using predetermined volumes of 1 N sodium hydroxide solution (a precipitate of the free base was obtained). The contents of the flask were transferred into a 100-ml volumetric flask and diluted to volume with methanol (a clear solution was obtained). Appropriate volumes of the resulting solutions were mixed with 5 ml of internal standard solution and diluted to 50 ml with methanol, aliquots of 20 µl of each solution were chromatographed and the concentration of the remaining flunarizine dihyrochloride (I) was calculated at each temperature and at time interval.



Scheme 1.

Results and Discussion

Identification of the degradation product

The suggested pathway for degradation of flunarizine dihydrochloride (I) in acid medium is presented in Scheme 1. The ultraviolet absorption characteristics of (I) and (II) were found to be greatly overlapping, with a maximum absorption at 253 nm in methanol. Such absorption characteristics are mainly due to absorption by the cinnamyl group. Using the above-mentioned TLC system, the R_f values were found to be 0.74 and 0.28 for (I) and (II), respectively.

The assignment of the degradation product (II), as 1-*trans*-cinnamylpiperazine dihydrochloride was based on its elemental analysis and comparison of the IR and PMR spectral data of purified specimens, separated from the degradation reaction, with those of the intact compound. The IR spectrum of (II) (KBr) revealed a broad band in the 3700–3000 cm^{-1} region which can be attributed to NH_2^+ and NH^+ absorptions of the mineral acid salts of the secondary and tertiary amines, respectively (Table 1). The spectrum lacked the characteristic C–F absorption band of I around 1000 cm^{-1}. By contrast the IR spectrum of (I) showed the tertiary amine salt (NH^+) absorption as a broad band at 3400 cm^{-1} and the C–F absorption as a sharp band of medium intensity at 1010 cm^{-1}. The PMR spectra of (II) in trichloroacetic acid (Fig. 1a) was characterized by the appearance of only five aromatic protons as multiplet between 7.1–7.3 ppm; eight N-monosubstituted piperazine protons as multiplet at δ 3.55–3.9 ppm; a CH_2-doublet at δ 3.98 ppm (J = 7.0 Hz); and two olefinic protons as a multiplet at δ 6.0–6.19 ppm for H_b and as a doublet at δ 6.8 ppm (J = 16.3 Hz) for

Table 1
Liquid chromatographic determination of flunarizine dihydrochloride (I) in laboratory-prepared mixtures with its corresponding degradation product (II)

	Mixture composition mg/100 ml		% Recovery of (I)	
Mixture no.	I	II	PA*	PH*
1	1.6	1.2	99.9	99.2
2	2.4	1.6	100.3	99.9
3	3.2	2.8	100.0	99.8
4	4.0	2.0	100.1	100.0
5	4.8	3.2	100.0	100.4
6	5.6	2.4	100.0	100.2
7	6.4	3.0	100.1	100.6
Mean			100.1	100.0
RSD (%)			0.13	0.46

*PA and PH are the peak area and peak height ratios, respectively.

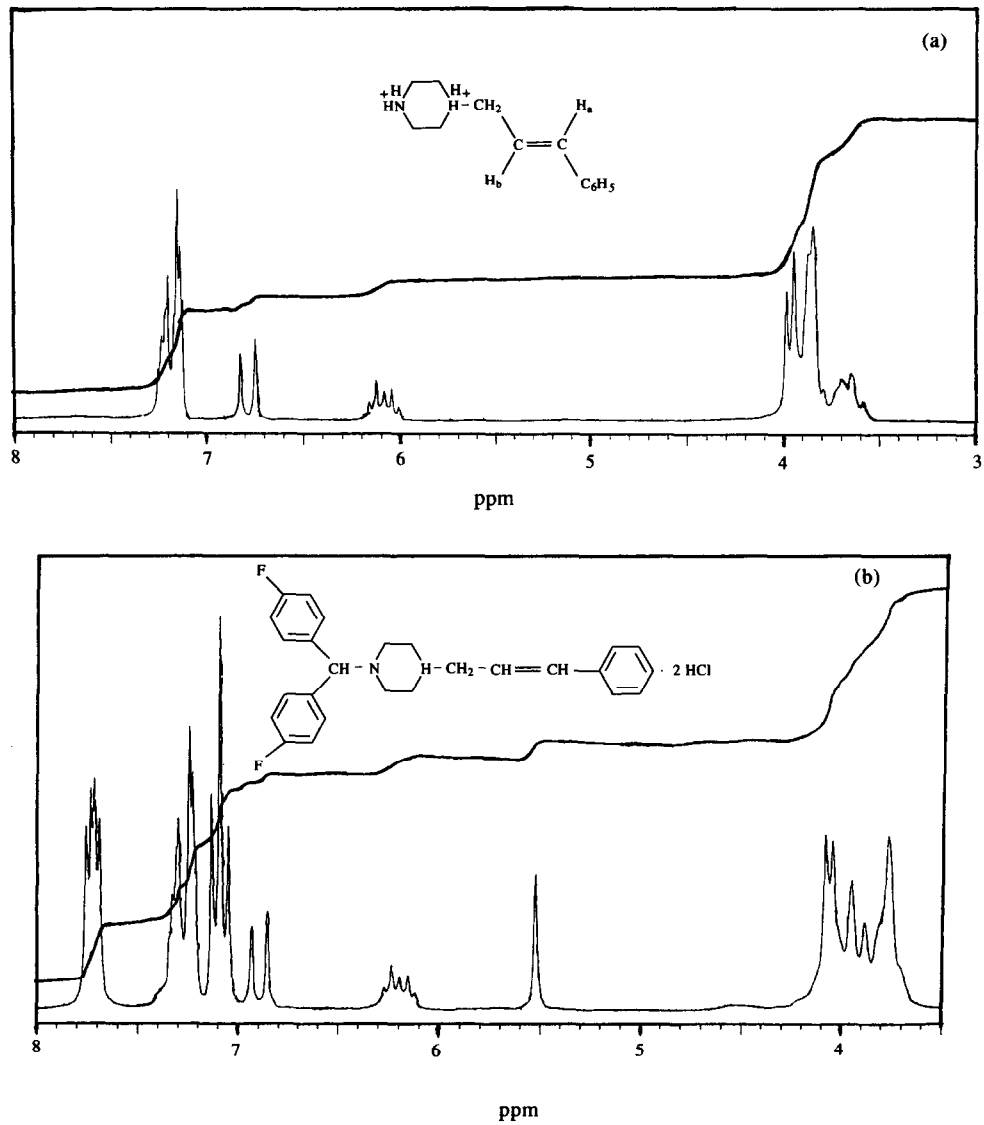


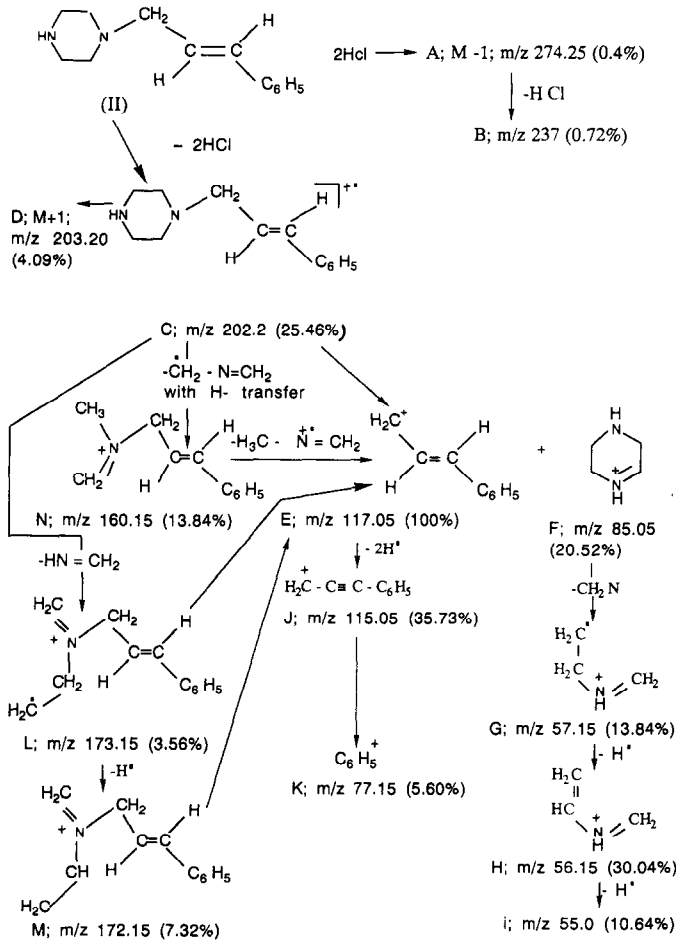
Figure 1
(a) PMR spectrum of degradation product (II) [1-cinnamylpiperazine dihydrochloride] in CF_3COOH; (b): PMR spectrum of flunarizine dihydrochloride (I) in CF_3COOH.

H_a. The calculated coupling constants indicated that the olefinic hydrogen atoms of the styryl residue exist in the *trans*-configuration. The spectrum lacked a methine signal, whereas, the PMR spectrum of flunarizine (I) in the same solvent (Fig. 1b) showed the eight N^1, N^4-disubstituted piperazine protons as a multiplet at δ 3.65–4.0 ppm. In addition there were: the CH_2 doublet at δ 4.05 ppm (J = 7.0 Hz); the N–CH proton singlet at δ 5.51 ppm; the two olefinic protons appearing as a multiplet at δ 6.1–6.3 ppm for H_b and as a doublet at δ 6.9 ppm (J = 16.3 Hz) for H_a. The spectrum also showed the eight di (4-fluorophenyl) protons as a multiplet at δ 7.0–7.35 ppm and the five styryl aromatic protons as a multiplet at δ 7.67–7.8 ppm.

The mass spectrometric data of authentic 1-cinnamylpiperazine dihydrochloride (II) was recorded. The possible major fragmentation pathways are illustrated in Scheme 2. The spectrum (Fig. 2) was characterized by the M-1 ion peak at *m/z* 274.25 and a relatively more intense ion at *m/z* 202.2, which corresponds to the free base radical ion (C). The latter (C) was cleaved to its components, the cinnamyl cation (E) at *m/z* 117.05 which constitutes the base peak of the spectrum and the piperazine cation (F) at *m/z* 85.05. Ion (E) may also result through fragmentation originating in the piperazine ring of the parent base (C) in a manner analogous to that reported for N-methylpiperazine [13]. In addition, the spectrum showed the species G–i which has been attributed to the fragmentation of the piperazine cation [13].

Assay parameters

To optimize the assay parameters, the effect of methanol composition and pH of the mobile phase on the capacity factor (k') were studied. A satisfactory separation was obtained with a mobile phase consisting of methanol–H_2O mixture (75:25, v/v). Increasing methanol



Scheme 2.

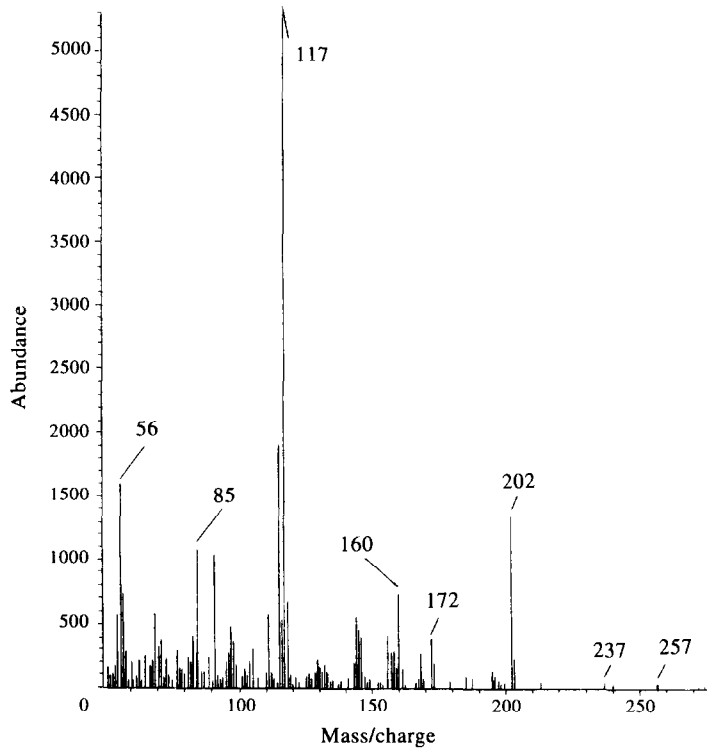


Figure 2
Mass spectrum of 1-cinnamylpiperazine dihydrochloride.

composition to more than 75% led to inadequate separation. At lower methanol composition, separation occurred but with excessive tailing and increased retention time. Variation of pH of the mobile phase resulted in maximum k' values at pH 7.5 with loss of peak symmetry. At lower pH values (3–4.5) bad resolution was observed. At pH 4.5–6.6 improved resolution was observed; however, at pH 6.6 optimum resolution with reasonable retention time was affected. Sodium chloride 0.5% and triethanolamine 0.2% were added to improve the sharpness of the peaks and to decrease the retention time.

For method validation, calibration graphs were prepared with seven different concentrations. Linear correlations were obtained between peak-area or peak-height ratios vs concentration, C, over the range 1.6–6.4 mg/100 ml. Each measurement represented the average of three replicates. The least squares regression equations were

$$Y = 0.004886 + 0.6840C,\ r = 0.9999$$
(for peak area ratios)
$$Y = 0.0106 + 0.4265C,\ r = 0.9999$$
(for peak height ratios).

The average retention time ± SD for intact flunarizine dihydrochloride (I), clotrimazole (IS) and the degradation product II (Fig. 3) were: 6.799 ± 0.016, 5.104 ± 0.007, 3.363 ± 0.004 min, for 13 replicates, respectively. The lower limit of detection was found to be 0.1 mg/100 ml.

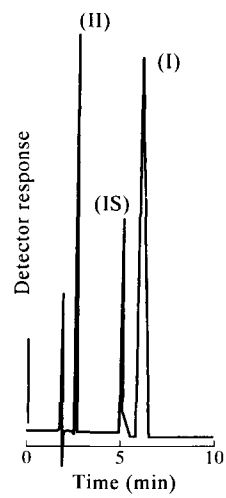


Figure 3
A typical chromatogram of a 20-μl injection of synthetic mixture of: 4 mg/100 ml intact flunarizine dihydrochloride (I); 2 mg/100 ml degradation product (II); and 40 mg/100 ml clotrimazole (IS).

The accuracy of the proposed method was checked by analysing seven laboratory-prepared mixtures of the drug (I) and different ratios of its degradation product (II), using a constant amount of the internal standard (Table 1). Satisfactory recoveries with small relative standard deviations (RSD) were obtained, which indicated the high repeatability and accuracy of the method [14].

Capsule analysis

The proposed method was applied to the determination of the drug in commercial capsules. The mean percentage recoveries ± SD (seven replicates), calculated as peak area and peak height ratios, respectively, were found to be 100.0 ± 0.14, 100.1 ± 0.45 for flunarizine capsules and 100.3 ± 0.42, 100.0 ± 0.46 for sibelium capsules. Moreover, to check the validity of the proposed method, the standard addition method was applied by adding different amounts of intact and degraded flunarizine to the previously analysed capsules. The results obtained are shown in Table 2 to support validation of the method.

Kinetic investigation

To assess the method specificity and selectivity for the assay of intact drug without interference from its degradation product, stability studies were conducted. In this connection acid degradation of flunarizine dihydrochloride was carried out under the previously described experimental conditions. A regular decrease in the concentration of intact drug with increasing time intervals was observed. The influence of temperature on the degradation process in 0.1 N HCl is shown in (Fig. 4). At the selected temperatures (90, 80, 70, 65, 60°C), the degradation process followed pseudo-first-order kinetics. From the slopes of the straight lines it was possible to calculate the apparent first-order degradation rate constant and the half-life at each temperature (Table 3). Plotting log K_{obs} values vs $1/T$, the Arrhenius plot (Fig. 5) was obtained, which was found to be linear at the temperature range 60–90°C.

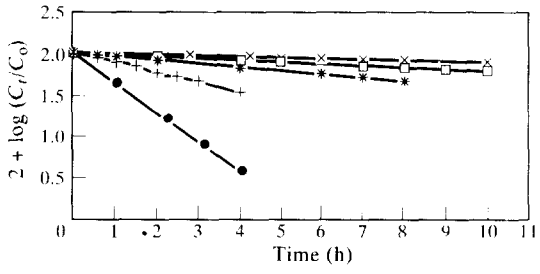


Figure 4
First-order plots for the degradation of (I) in 0.1 N hydrochloric acid at various temperatures using peak-area measurements. Key: 90°C (-●-); 80°C (——); 70°C (-*-); 65°C (—□—); and 60°C (—×—); C_t = concentrations at time t, and C_0 = concentration at time zero.

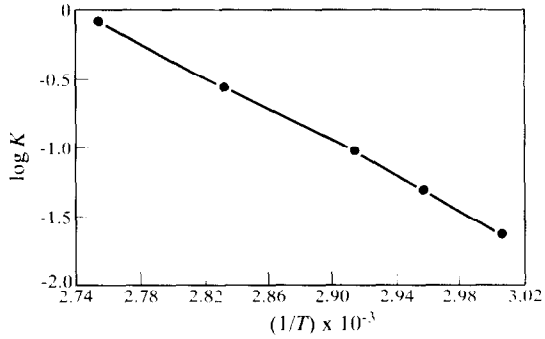


Figure 5
Arrhenius plot for the degradation of (I) in 0.1 N hydrochloric acid using peak area measurements.

Table 2
Recovery of flunarizine dihydrochloride (I) added to commercial capsules in presence of its degradation product (II)

				% Recovery of (I)			
	Conc. of (I) in sample solution	mg/100 ml added		Flunarizine cap.		Sibelium cap.	
Exp. no.	mg/100 ml	(I)	(II)	PA*	PH*	PA	PH
1	1.6	4.0	1.8	99.7	100.2	100.1	100.0
2	1.6	2.4	2.0	99.4	100.4	100.0	100.2
3	2.4	1.6	1.4	100.9	100.2	99.6	99.9
4	3.2	1.6	1.8	99.8	100.0	100.0	100.3
5	1.6	3.2	2.4	100.0	99.4	100.1	99.9
6	4.0	1.6	2.8	99.8	100.8	100.1	99.6
7	3.2	3.2	3.0	100.1	100.2	100.1	100.4
Mean				99.95	100.2	100.0	100.0
RSD (%)				0.47	0.42	0.18	0.28

* PA and PH are the peak area and peak height ratios, respectively.

Table 3
Degradation rate constant (K_{obs}) and half-life ($t_{1/2}$) for flunarizine dihydrochloride

Temperature (°C)	K_{obs} (h^{-1})	$t_{1/2}$ (h)
90	0.821	0.84
80	0.276	2.51
70	0.093	7.42
65	0.047	14.66
60	0.024	29.45

The activation energy was calculated to be 28.11 kcal $mole^{-1}$.

Conclusion

The proposed HPLC method provides a simple quantitative analysis for stability studies as well as for the assay of flunarizine dihydrochloride in commercial preparations.

References

[1] H. Waki and S. Ando, *J. Chromatogr. Biomed. Appl.* **86**, 408–412 (1989).
[2] C.D. Torchin, I.M. Kapetanovic, W.D. Yonekawa and H.J. Kupferberg, *J. Chromatogr. Biomed. Appl.* **70**, 444–448 (1988).
[3] S.M. Pradhan, S.M. Samant, R.P. Mehendre, A.D. Bhide and H.P. Tipnis, *Indian Drugs* **28**, 428–429 (1991).
[4] X. Aparicio, J. Gras, A. Campos, E. Fernandez and E. Gelpí, *J. Pharm. Biomed. Anal.* **6**, 167–173 (1988).
[5] S. Kobayashi, K. Takai and A. Inoue, *Yakugaku-Zasshi* **106**, 217–220 (1986).
[6] F. Albani, R. Riva, G. Casucci, M. Contin and A. Baruzzi, *J. Chromatogr. Biomed. Appl.* **47**, 196–199 (1986).
[7] A. Yamaji, K. Kataoka, M. Oishi, N. Kanamori, T. Tagawa and T. Mimaki, *J. Chromatogr. Biomed. Appl.* **6**, 372–376 (1987).
[8] I.M. Kapetanovic, C.D. Torchin, W.D. Yonekawa and H.J. Kupferberg, *J. Chromatogr. Biomed. Appl.* **56**, 223–228 (1986).
[9] S.C. Flor, *J. Chromatogr. Biomed. Appl.* **23**, 315–323 (1983).
[10] R. Woestenborghs, L. Michielsen, W. Lorreyne and J. Heykants, *J. Chromatogr. Biomed. Appl.* **21**, 85–91 (1982).
[11] M. Nieder and H. Jaeger, *J. Chromatogr. Biomed. Appl.* **53**, 443–449 (1986).
[12] K. Kataoka, N. Kanamori, M. Oishi, Y. Arakawa, A. Yamaji, T. Tagawa, T. Mimaki and H. Yabuuchi, *Yakugaku-Zasshi* **108**, 226–231 (1988).
[13] Q.N. Porter and G. Paldas, *Mass Spectrometry of Heterocyclic Compound*, p. 495. Wiley Interscience, New York (1971).
[14] W.R. Orndoff, R.C. Gibbs, S. Alice Mcnulty and C.V. Shapiro, *J. Am. Chem. Soc.* **49**, 1542 (1927).

[*Received for review 3 May 1994*; *revised manuscript received 26 September 1994*]