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Spectrophotometric determination of flunarizine dihydrochloride through the formation of charge-transfer complex with iodine

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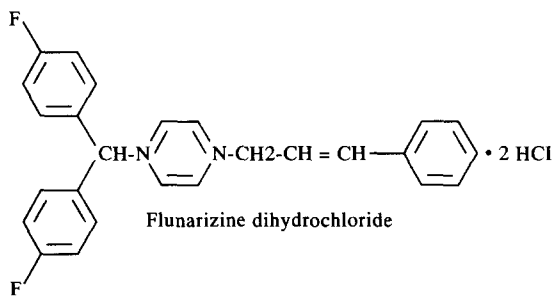
Abstract: A spectrophotometric method is described for the assay of flunarizine dihydrochloride. The method is based on the molecular interaction between the drug and iodine, to form a charge-transfer complex in which the drug acts as n-donor and iodine as σ-acceptor. The iodine was found to form charge-transfer complex in a 1:1 stoichiometry with absorption bands at 295 and 355 nm. The concentrations were linear over 8–13 μg ml^{-1} at both 295 and 355 nm, respectively. A complete, detailed investigation of the formed complex was made with respect to its composition, associated constant and free energy change. The method has been applied successfully to the analysis of commercially available flunarizine dihydrochloride capsules without interference from the capsules excipient. To validate the proposed method, its accuracy and precision, the results were statistically compared with a newly developed reversed-phase HPLC procedure using Student-*t* and *F*-ratio tests.

Keywords: *Flunarizine dihydrochloride; iodine; charge-transfer; spectrophotometric determination.*

Introduction

Flunarizine dihydrochloride (FL), [trans-1-cinnamyl-4-(4,4-difluorobenzenhydryl) piperazine dihydrochloride], is the difluorinated derivative of cinnarizine [1]. (See Scheme I.) It has antihistaminic and CNS depressant effect, but it is mainly used as an inhibitor of central and peripheral vasoconstriction [2]. The drug is not officially available in any pharmacopoeia. Chromatographic procedures for the determination of (FL) have been described, but these GLC [3–6] and HPLC [7–10] methods were all used for the analysis of the drug and its metabolites in biological fluids. Only one direct spectrophotometric method is available for the determination of (FL) in its injection form [11]. No colorimetric or other spectrophotometric methods are available for the analysis of (FL) in its pharmaceutical dosage forms.



Flunarizine dihydrochloride

Scheme I

Charge-transfer complex-forming reactions have been used in the determination of electron-donating basic compounds through the interaction with σ-acceptors [12–15] or π-acceptors [16–18]. As (FL) contains a tertiary amino group in its molecular structure, it represents a basic centre with the availability of non-bonding electron as donors. The complex formed between iodine and n-donors (basic centre) compound exhibited high values of association constant and molar absorptivity [19].

The described facts encouraged attempts to use the formation of a complex between iodine and (FL) for the determination of the drug in its capsules dosage form. At the same time, the spectroscopic features, such as the association constant, the molar ratio of reaction and the free energy change ($\blacktriangle G°$), were determined.

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The results obtained by the above spectrophotometric method were compared statistically with a simple newly developed reversed-phase HPLC procedure.

Experimental

Instrumentation

A Perkin–Elmer (Norwalk, CT, USA) Model 550S UV-VIS spectrophotometer, with a fixed slit width of 2 nm, 1-cm quartz cuvette and Hitachi Model 561 recorder, were used. For chromatographic procedure the system consisted of a Waters (Milford, MA, USA) high performance liquid chromatograph (Model 640) with a solvent delivery system (Model 501) equipped with U6K universal injector and connected to a multiple wavelength detector (Model 481). The peak area integrations were performed using a Waters 740 chromatographic data module. The column used was a Bondapak C_{18} (250 × 4.6 mm, i.d.). The samples were injected with a 25-μl WL-Waters analytical syringe.

Chromatographic conditions

The mobile phase was prepared by mixing methanol and water in ratio of 75:25, to which 0.5% w/v sodium chloride and 0.2% v/v of triethanolamine were added. The pH was adjusted with hydrochloric acid to be 6.6. The injection volume was 20 μl with a flow rate of 2 ml min^{-1}. All the determinations were performed at ambient temperature. The column effluent was monitored at 254 nm and the sensitivity set at 0.1 AUFS. The chart speed of the integrator was set at 2 cm min^{-1} with an attenuation of 64.

Materials

Pharmaceutical grade (FL) (Dolder Ltd, Basle, Switzerland) and clotrimazole (Bayer, Leverkusen, Germany) were kindly supplied by Alexandria Pharm. Co., certified to contain 98% and 99%, respectively, and used without further purification. Iodine (BDH, Poole, UK) solution (10^{-3} M) was prepared in chloroform; this solution was stable for 1 week at 4°C. The HPLC solvents (methanol and water) were purchased from Romil Chemicals Ltd (UK). All other reagents and solvents were of analytical grade.

Standard solutions

Pre-spectrophotometric procedure. A solution was prepared by dissolving and transferring an accurately weighed 25 mg of (FL) dihydrochloride salt into 250-ml volumetric flask. Fifty millilitres of the solution was quantitatively transferred to a separating funnel, made alkaline with 10 ml of 1 M sodium hydroxide and shaken with four 20 ml portions of chloroform. The combined extracts were passed through 2 g of anhydrous sodium sulphate supported by glass wool in a small funnel into a 100-ml volumetric flask and completed to volume with chloroform to provide a standard of (FL) base equivalent to 5 μg ml^{-1} of the (FL) salt.

HPLC procedure. Stock standard solutions for (FL) and clotrimazole were prepared in methanol, to contain 0.4 and 4 mg ml^{-1}, respectively. Different aliquot from (FL) solution were transferred quantitatively into 50-ml volumetric flasks. To each flask, 5 ml of the internal standard solution was added followed by addition of methanol to complete the volume to the mark. Samples (20 μl) of each solution were repeatedly injected into the column, until the ratio of the peak area of the drug to the internal standard peak area was constant.

Spectrophotometric procedure

Five millilitres of the working standard or sample solution were transferred by pipette into a 25-ml volumetric flask; 5 ml of iodine solution was added, the solution was well mixed and allowed to stand at 25 ± 2°C for 20 min. The solution was diluted to volume with chloroform. The absorbance was measured at 295 and 355 nm against blank prepared similarly with chloroform replacing the standard or the sample solution.

Assay of pharmaceutical capsules

The contents of 10 capsules were emptied, as completely as possible, and weighed. For the spectrophotometric procedure, an accurately weighed portion of the powder equivalent to 5 mg was mixed and transferred into a 100-ml beaker with the aid of 60 ml water and stirred mechanically for 30 min. The aqueous solution was transferred quantitatively to a separating funnel and proceed exactly as mentioned above in the preparation of the standard solution.

For the HPLC procedure, an accurately weighed portion of the powder equivalent to

5 mg (FL) was mixed and transferred in to a 25-ml volumetric flask with the aid of 25 ml methanol. The contents of the flask were mixed, for 10 min, with the aid of magnetic stirrer. The mixture was filtered and the first 2 ml of the filtrate was discarded. A 10 ml amount of the clear filtrate was transferred into a 25-ml volumetric flask, 2.5 ml of the internal standard were added, and the volume was adjusted with methanol. The samples were filtered through a 0.45-μm millipore filter. A 20-μl volume of the sample was injected into the chromatograph in triplicate.

The samples were chromatographed concurrently with the appropriate standard solution and peak area ratios (FL to internal standard) were measured for the determination of (FL) in every sample.

Results and Discussion

Iodine in solution of chloroform displayed an absorption peak at about 520 nm while the (FL) showed a negligible absorption in the 300–700 nm region. Mixing the chloroformic extract of the (FL) with the chloroformic solution of iodine resulted in a change of the violet colour of the iodine to yellow. As a consequence, the absorption band of the iodine was shifted to a shorter wavelength (hypsochromic shift). The charge-transfer complex between (FL) and iodine exhibited two absorption bands at 295 and 335 nm (Fig. 1). The new absorption bands correspond to the iodinated complex showed an isosbestic point at 460 nm (Fig. 2). The existence of single isosbestic point may conclude that the complex had a 1:1 stoichiometry [20]. As described in the literature [21] the formation of I_3 ions, which is the measurable species, is due to the transformation of an "outer complex" to an "inner complex" liberating I^- ions which react with the free molecular iodine. In other words, the interaction between (FL) and iodine is a charge-transfer complexation reaction between the n-donor (piperazine ring) and the σ-acceptor iodine followed by the formation of a radical ion according to the following scheme.

$$FL + I_2 \text{ ------ } FL\text{-}I_2 \text{ (outer complex)}$$

$$FL\text{-}I_2 \text{ ------ } [FL\text{-}I]^+I^- \text{ (inner complex)}$$

$$[FL\text{-}I]^+I^- + I_2 \text{ ------ } [FL\text{-}I]^+I_3^-$$

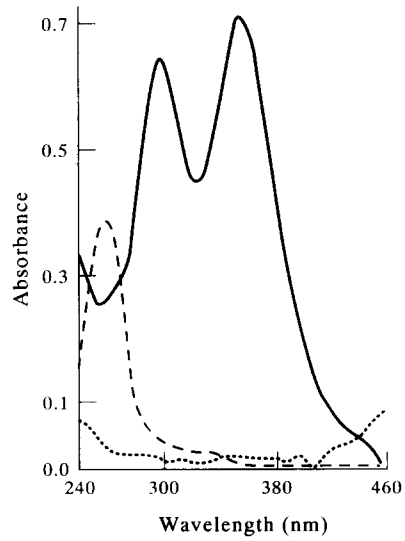

Figure 1
Absorption spectra of flunarizine dihydrochloride, 10 μg ml^{-1} (---); Iodine, 2×10^{-3} (....); and their reaction product in chloroform (——).

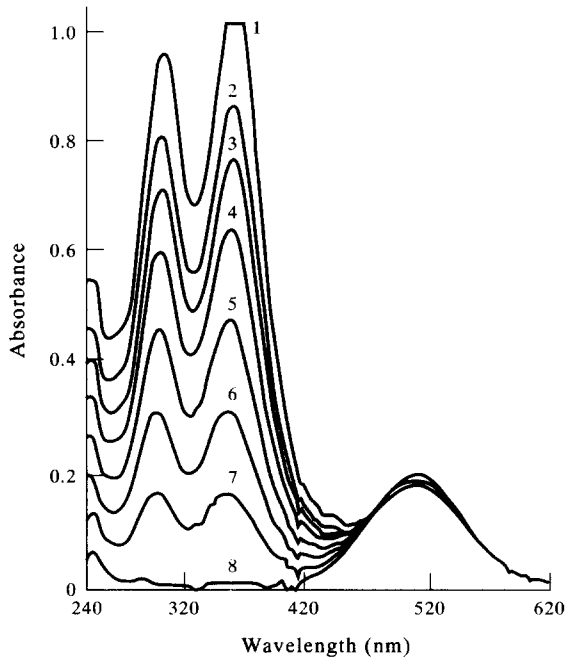

Figure 2
UV–VIS absorption spectra of flunarizine–iodine complex showing the isosbestic point. The iodine concentration was fixed at 2×10^{-4} M. The concentrations of flunarizine (M) were: (1) 2.94×10^{-5}; (2) 2.52×10^{-5}; (3) 2.1×10^{-5}; (4) 1.68×10^{-5}; (5) 1.26×10^{-5}; (6) 8.4×10^{-6}; (7) 4.2×10^{-6}; and (8) 0.

Regarding the third step in the above scheme, iodine alone does not absorb at the wavelength of maximum absorption, hence the stoichiometry will show only the iodide ion released in the second step as a result of one

mole of iodine being consumed in the third step. Although, the complex was formed rapidly, constant absorbance readings were obtained after 20 min of standing in the dark at 20–25°C and remained constant for at least 40 min (Fig. 3). At 295 and 355 nm, linear relationships were obtained between the absorbance and the concentrations over the 8–13 μg ml^{-1} range. The regression equations of the lines were

$$A_{295} = 4.66 \times 10^{-3} + 7.057 \times 10^{-2}\, C$$

$$A_{355} = 7.73 \times 10^{-3} + 7.048 \times 10^{-2}\, C$$

where A is the absorbance at 295 and 355 nm, C is the concentration of (FL) in μg ml^{-1} and r is the correlation coefficient. The slopes of the calibration curves reflect the sensitivity of the procedure.

Application of Job's method of continuous variation [22] indicated 1:1 complexation ratio (Fig. 4). This finding was expected because of the existence of a single isosbestic point (Fig. 2). The molar absorptivity and association constant for the (FL)-iodine reaction product were evaluated by using Benesi–Hildebrand equation, whereby the concentration of the donor is higher than that of the acceptor [23]. From Fig. 4, the intercept of the formed straight line with the ordinate is molar absorptivity $(\epsilon)^{-1}$, and the slope equal the association constant $(cK)^{-1}$. From the above the association constant was equal 3.29×10^{-3} l $mole^{-1}$ and the molar absorptivity equal to 4.4×10^{-4}.

The high value of k is common to complexes between the σ-acceptor iodine and n-electron donors where the intermolecular overlap may be considerable [19]. At the same time, the standard free energy of complexation [24], ▲G° is related to the association constant which was calculated and found to be −4.80 kcal.

To confirm that only one complex was formed, a matrix analysis was carried out as described by Lipty [25]. Table 1 shows the results of the matrix analysis at 410, 415, 420 and 425 nm. The absorbance of a series of solutions of the complexes were recorded. The calculated absorbance of non-complexed iodine was subtracted from each value. Since the donor does not absorb in this region, only absorbance due to free iodine needs to be taken into account. The corrected absorbance values for the different solutions at different wavelengths are arranged in a matrix. The values are then referred to a single wavelength (410 nm, Table 1). For a single complex all values should be identical (within the limits of experimental error).

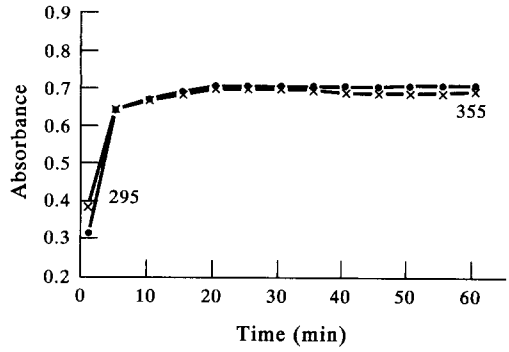

Figure 3
Rate of flunarizine–iodine complex formation measured at both 295 (xxx) and 355 (●●●) nm (10 μg ml^{-1}).

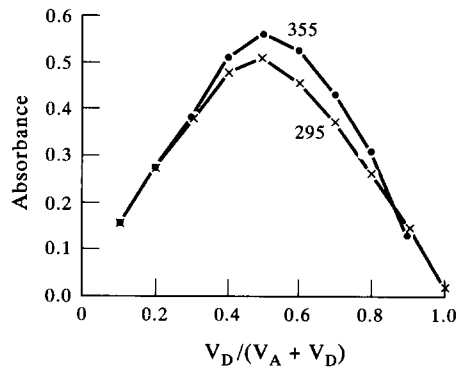

Figure 4
Continuous variation plot for flunarizine–iodine complex (5×10^{-5} M), measured at both 295 and 355 nm.

Table 1
Lipty matrix for (FL)–iodine complex

Wavelength	Soln 1	Soln 2	Soln 3	Soln 4	Soln 5
	Lipty matrix of corrected absorbance (A_{corr})*				
410 nm	0.921	0.733	0.638	0.450	0.310
415 nm	0.797	0.634	0.552	0.389	0.268
420 nm	0.689	0.549	0.477	0.336	0.232
425 nm	0.597	0.474	0.413	0.291	0.201
	Values referred to 410 nm				
	1	1	1	1	1
	0.865	0.865	0.865	0.864	0.865
	0.748	0.749	0.748	0.747	0.748
	0.648	0.647	0.647	0.647	0.648

Concentration of components of the five solutions: $[I_2]$ fixed at 8×10^{-4} M; soln 1 = 1.2×10^{-4} M; Soln 2 = 9.6×10^{-5} M; Soln 3 = 8×10^{-5} M; Soln 4 = 5.6×10^{-5} M; Soln 5 = 4×10^{-5} M.

Table 2
Assay results of commercial capsules

Capsule† preparation	Recovery (mean ± %SD)* Proposed method 295 nm	355 nm	HPLC method
Flunarizine	99.90 ± 0.61	99.96 ± 0.43	100.06 ± 0.45
t	0.52	0.32	(2.23)
F	1.84	1.09	(5.05)
Recovery‡	100.12 ± 0.62	100.31 ± 0.60	99.78 ± 0.24
Silebum	100.00 ± 0.62	99.89 ± 0.23	100.24 ± 0.39
t	0.80	1.89	—
F	2.53	2.87	—
Recovery	99.95 ± 0.31	100.09 ± 0.52	99.98 ± 0.17

*Mean and standard deviation for six determinations; percentage recovery from the label claim amount.

†Flunarizine capsules are product of Alexandria Company for Pharmaceuticals and Chemicals Industries, Alexandria, Egypt and Sibelium capsules are product of Advanced Biocehmical Industries S.A.E., El Salam City, Cairo, Egypt, under licence of Janssen Pharmaceuticals, Beerse, Belgium, both products are labelled to contain 5 mg Flunarizine.

‡For standard addition method (n = 6).

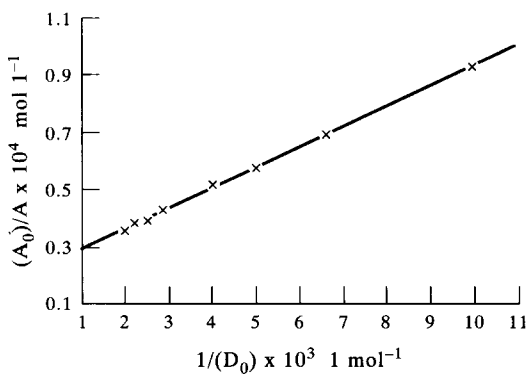

Figure 5
Benesi–Hildebrand plot of flunarizine–iodine complex.

When the proposed method was applied for the analysis of the commercial capsules, labelled to contain 5 mg of (FL), the mean percentage recoveries were around 100% from the labelled claim (n = 6 determinations) (Table 2). Using the standard addition method, recovery experiments were conducted and the results were assembled in Table 2.

Under the described chromatographic conditions, (FL) was adequately separated at 8.57 min retention time, whereas clotrimazole (internal standard) was eluted first at 4.80 min. Peak area ratios were linearly proportional to (FL) concentrations over the range 16–64 μg ml^{-1}. The best-fit line was determined by least-squares regression analysis and found to be $Y = 4.886 + 0.068\ C$ (r = 0.9999); where Y is the peak area ratio, C is the concentration in μg ml^{-1} and r is the correlation coefficient. The mean percentage of drug component found in capsules by the chromatographic method is summarized in Table 2. Statistical comparison of the results of the proposed spectrrophotometric and HPLC methods (Table 2) was performed with regard to accuracy and precision using Student-t and the F-ratio tests at 95% confidence level. From Table 2, it is clear that there is no significant difference between the proposed and the reference HPLC method with regard to accuracy and precision.

The low standard deviation and the statistical comparison presented in Table 2, in addition to its simplicity make the proposed method convenient for routine quality control for the determination of (FL) in its pharmaceutical capsules.

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