

Spectrophotometric determination of some pharmaceutical piperazine derivatives through charge-transfer and ion-pair complexation reactions

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Abstract

Simple and sensitive spectrophotometric methods are described for the assay of three piperazine derivatives; ketoconazole, piribedil and prazosin hydrochloride based on charge-transfer and ion-pair complexation reactions. The first method is based on the reaction of the basic drug with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in acetonitrile. The orange-red colour formed due to the formation of charge-transfer complex showed maximum absorbance at 460 nm. The second method is based upon the interaction of the basic drug in dry chloroform with bromophenol blue (BPB) in the same solvent to produce a stable yellow ion-pair complex which absorbs at 410 nm. Beer's law was obeyed for both methods and the relative standard deviations were found to be less than 1%. The two methods can be applied to the analysis of tablets, with no evidence of interference from excipients. A more detailed investigation of the complex was made with respect to its composition, association constant and free energy change. © 1997 Elsevier Science B.V.

Keywords: Pharmaceutical piperazine derivatives; Spectrophotometry; Charge-transfer and ion-pair complexes; Tablets

1. Introduction

Ketoconazole, *cis*-1-acetyl-4-{4-[2-(2,4-dichlorophenyl)-2-imidazol-1-ylmethyl-1,3-dioxolan-4-ylmethoxy] phenyl} piperazine has activity against a great number of fungus and some gram-positive microorganisms [1]. Some methods have been reported for its determination including potentiometry [2,3], spectrophotometry [4–6], polarography [7] and HPLC [8–10].

Piribedil, is an alkoxybenzyl-4-(2-pyrimidinyl)piperazine derivative with vasodilatory activity [11]. Few methods for the analysis of piribedil or its basic metabolites in biological specimens have been used, including gas chromatography using a nitrogen-sensitive detector [12] or combined with mass spectrometry [13] and HPLC [14].

Prazosin hydrochloride, 1-(4-amino-6,7-dimethoxy-2-quinazoliny)-4-(2-furoyl) piperazine

monohydrochloride, is antihypertensive agent [15]. Some methods that have been reported for the determination of prazosin include potentiometry [16], spectrophotometry [17,18], coulometry [19], thin-layer chromatography [16,20] and HPLC [21–24].

This paper introduces two spectrophotometric methods for the determination of three pharmaceutical piperazine derivatives, using 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in acetonitrile and bromophenol blue (BPB) in chloroform as chromogenic reagents. The proposed methods were applied successfully to the determination of ketoconazole, piribedil and prazosin hydrochloride either pure or in dosage forms, with good accuracy and precision. The results were compared with those given by the official methods [2,16].

2. Experimental

2.1. Apparatus

A Camspec M 301, UV-vis spectrophotometer, with matched quartz cells of 1-cm optical path length was used.

2.2. Materials

Ketoconazole (Janssen, Beerse, Belgium), piribedil (Eutherapia, France) and prazosin hydrochloride (Industry Chimiche Farm, S.R.L., Italy) were used as working standards.

3. Reagents

All the reagents and solvents used were of analytical-reagent grade. DDQ (Merck) as a 2×10^{-3} M solution in acetonitrile and BPB (Merck) as a 2×10^{-3} M solution in dry chloroform were used as chromogenic reagents. Dry chloroform was prepared according to BP [16].

3.1. Preparation of standard solution

Ketoconazole or piribedil stock solution, was prepared by dissolving 40 mg of ketoconazole or 25 mg of piribedil in acetonitrile (DDQ method) or in dry chloroform (BPB method) in a 100-ml standard flask and diluting to volume with the same solvent. Whenever required dilute solutions were obtained by further dilution with the appropriate solvent.

Prazosin stock solution, was prepared by mixing an accurately weighed amount of the drug salt, equivalent to 20 mg of the base, with about 20 ml of 0.1 M potassium hydroxide and shaking with five 20 ml portions of chloroform. The combined extracts were dried with anhydrous sodium sulphate for 5 min and filtered through dry filter-paper into a 100-ml standard flask and diluted to volume with dry chloroform to provide a standard $200 \mu\text{g ml}^{-1}$ solution of the drug base. Working standard solution of $100 \mu\text{g ml}^{-1}$ was prepared by making further dilution with dry chloroform.

Another working prazosin standard solution in acetonitrile was prepared by evaporating 37.5 ml ($200 \mu\text{g ml}^{-1}$) of prazosin in chloroform to dryness, using a stream of nitrogen. The residue was dissolved in acetonitrile in a 25-ml standard flask and diluted to volume with the same solvent to obtain a standard $300 \mu\text{g ml}^{-1}$ solution of prazosin.

3.2. Construction of calibration curves

Aliquots of a solution of the drug in 2 ml of acetonitrile or dry chloroform (in the concentration range cited in Table 1 and Table 2) were transferred into separate 10-ml standard flasks. To each flask, 2 ml of DDQ or 1 ml of BPB solution was added, mixed well and allowed to stand at room temperature ($25 \pm 0.5^\circ\text{C}$) for 10 min. The solution was diluted to volume with acetonitrile (DDQ method) or dry chloroform (BPB method). The absorbance of the resultant complex measured at 460 nm (DDQ method) or 410 nm (BPB method) against a reagent blank similarly prepared.

Table 1
Analytical data for the piperazine-DDQ complexes in acetonitrile ($\lambda_{\max} = 460$ nm)

Drug	Conc. range ($\mu\text{g ml}^{-1}$)	Linear regression					R.S.D. (%)	ϵ ($\text{l}\cdot\text{mol}^{-1}\text{cm}^{-1}$)
		Intercept	\pm S.E. ^a	Slope	\pm S.E. ^b	Corr. Coeff. (r)		
Ketoconazole	10–80	0.003	0.000025	0.0090	0.00008	0.9999	0.32	4.82×10^3
Piribedil	5–50	0.005	0.000041	0.0145	0.00011	0.9998	0.30	4.36×10^3
Prazosin	5–60	0.004	0.000023	0.0129	0.00014	0.9997	0.70	4.97×10^3

R.S.D., relative standard deviation ($n = 6$).

ϵ , apparent molar absorptivity.

^aStandard error of the intercept ($n = 6$).

^bStandard error of the slope ($n = 6$).

3.3. Analysis of Nizoral (Ketoconazole) and Trivastal (piribedil) tablets

An accurately weighed amount of powdered tablets equivalent to about 40 mg of the active drug of ketoconazole or 25 mg of piribedil was transferred into a 100-ml conical flask and extracted with five 20 ml portions of acetonitrile (DDQ method) or dry chloroform (BPB method). The combined extracts were filtered into a 100-ml standard flask and diluted to volume with the same solvent. An aliquot portion of acetonitrile solution (1–2 ml) was subjected to the DDQ method.

Then 5 ml of the chloroform filtrate was diluted to volume in a 50-ml standard flask. The BPB method was applied to 1–2 ml of this solution.

3.4. Analysis of Minipress (prazosin hydrochloride) tablets

An accurately weighed amount of the finely powdered tablets equivalent to 20 mg of the drug base was transferred into a 100-ml separatory funnel and mixed with 20 ml of 0.1 M potassium hydroxide. The drug base was extracted as described under 'prazosin stock solution' and proceeded as described under 'construction of calibration curves'.

3.5. Stoichiometric relationship

Job's method of continuous variations [25] of equimolar solutions was employed: a 1×10^{-3} M or 1×10^{-4} M standard solution of drug base and 1×10^{-3} M or 1×10^{-4} M solution of DDQ or BPB, respectively, were used. A series of solutions was prepared in which the total volume of drug and reagent was kept at 4 or 5 ml for DDQ or BPB, respectively. The reagents were mixed in various proportions, allowed to stand at $25 \pm 0.5^\circ\text{C}$ for 10 min and then diluted to volume in 10-ml standard flask with the appropriate solvent. The absorbance was measured at 460 and 410 nm for DDQ and BPB reagents, respectively.

3.6. Association constant and free energy

Serial volumes of 1–5 ml of 10^{-3} M piribedil solution (in 1.0 ml steps) in acetonitrile were transferred to 10 ml standard flasks. To each flask, 2 ml of DDQ in acetonitrile (4×10^{-4} M) was added and continued as directed under construction of calibration curves.

Another serial volumes of 1–7 ml of 10^{-4} M piribedil solution (in 1.0 ml steps) in dry chloroform were transferred to 10-ml standard flasks, and continued as above by using 1 ml of BPB solution in chloroform (0.9×10^{-4} M).

Table 2

Analytical data for the piperazine-BPB complexes in chloroform ($\lambda_{\max} = 410$ nm)

Drug	Conc. range ($\mu\text{g ml}^{-1}$)	Linear regression					R.S.D. (%)	ϵ ($\text{l}\cdot\text{mol}^{-1}\text{cm}^{-1}$)
		Intercept	\pm S.E. ^a	Slope	\pm S.E. ^b	Corr. Coeff. (r)		
Ketoconazole	2–18	–0.001	0.000021	0.0485	0.00043	0.9996	0.56	2.58×10^4
Piribedil	1–10	–0.004	0.000062	0.0810	0.00073	0.9994	0.70	2.42×10^{-4}
Prazosin	2–18	–0.003	0.000053	0.0490	0.00060	0.9998	0.35	1.87×10^4

R.S.D., relative standard deviation ($n = 6$). ϵ , apparent molar absorptivity.^aStandard error of the intercept ($n = 6$).^bStandard error of the slope ($n = 6$).

4. Results and discussion

The reaction of DDQ with basic nitrogenated drugs results in the formation of an intense orange-red product which exhibits absorption maxima at 460, 550 and 590 nm (Fig. 1). These bands may be attributed to the formation of the DDQ radical anion [26]. The radical anion results from the dissociation of an original charge-transfer complex formed by the interaction of the investigated drug bases as n -electron donors (D) and DDQ as π -acceptor (A). The dissociation is promoted by the high dielectric constant of the solvent used (acetonitrile, $D = 37.5$) [27]. The absorption spectrum of the reaction product with piribedil as a model example in the range 360–640 nm is shown in Fig. 1.

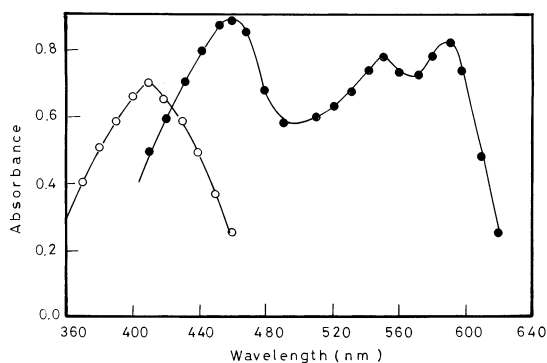


Fig. 1. Absorption spectrum of: ●, Piribedil-DDQ reaction product ($60 \mu\text{g ml}^{-1}$); ○, Piribedil-BPB reaction product ($9 \mu\text{g ml}^{-1}$) vs. reagent blank.

The spectrophotometric properties of the coloured species as well as the different parameters affecting the colour development were extensively studied to determine the optimal conditions for the assay procedure. The reaction was studied as a function of the volume of the reagent, nature of the solvent, reaction time and stability. The maximum absorbance is attained using 2 ml of 2×10^{-3} M DDQ solution for all drugs studied (Fig. 2). Acetonitrile was considered to be an ideal solvent for the colour reaction as it offers excellent solvent capacity for DDQ and gives the highest yield of the radical.

In chloroform, ketoconazole, piribedil and prazosin react instantaneously with bromophenol blue (BPB) to give yellow chromogen, which ex-

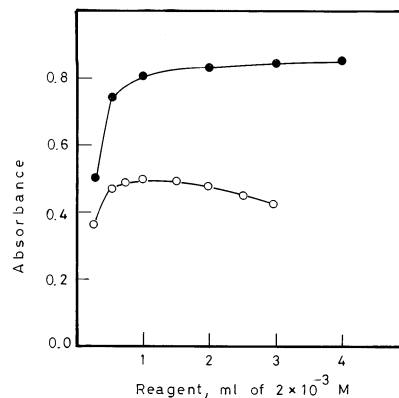


Fig. 2. Effect of the volume of reagent (2×10^{-3} M) on the absorbance of: ●, Piribedil-DDQ complex ($60 \mu\text{g ml}^{-1}$); $\lambda = 460$ nm. ○, Piribedil-BPB complex ($6 \mu\text{g ml}^{-1}$); $\lambda = 410$ nm.

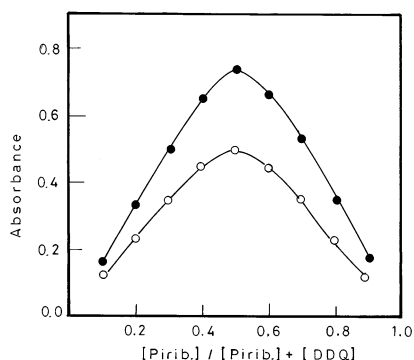


Fig. 3. Continuous variation plot for: ●, Piribedil-DDQ complex (4×10^{-4} M); $\lambda = 460$ nm. ○, Piribedil-BPB complex (5×10^{-5} M); $\lambda = 410$ nm.

hibits a broad absorption maximum at 410 nm (Fig. 1). BPB has been reported for the determination of pharmaceutical basic compounds, it reacts as an acidic ion-pairing reagent [28,29]. BPB belongs to the family of sulphonphthalein dyes. These dyes have following ionic forms, the yellow species HL^- are present in aqueous solutions. On acidification, they can turn purple (H_2L as an ampholyte); this colour change corresponds to protonation of the quinoid oxygen. The second symmetric resonance structure (L^{2-}) arises by spitting of a proton from the hydroxy group, accompanied by a bathochromic shift [30].

Since the reaction product in chloroform is yellow, so, It may be considered that HL^- is the form of the BPB involved in the reaction with the chosen drugs. This may lead to an assumption that, by the interaction of BPB with the drug base, a proton transfer from BPB to the basic center of the drug takes place. The obtained ion-pair salt is dissociated to the yellow HL^- anion.

The effect of solvent on the formation of the BPB complex was studied using acetonitrile, 1,2-dichloroethane, dichloromethane and chloroform. Chloroform was preferred because of the higher molar absorptivities and stabilities of the complexes formed in it. The effect of the BPB concentration has been studied, 1 ml of 2×10^{-3} M BPB in the total volume of 10 ml was required for maximum complex formation (Fig. 2).

Reaction time is determined by following the colour development at different time intervals at

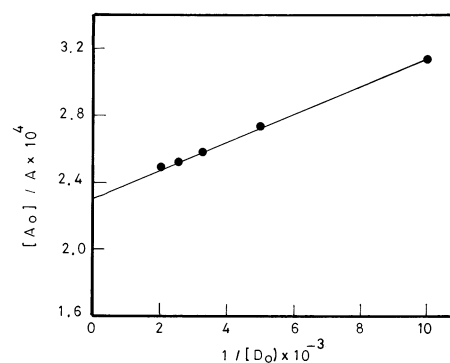


Fig. 4. Benesi-Hildebrand for piribedil-DDQ complex; $\lambda = 460$ nm.

room temperature ($25 \pm 0.5^\circ\text{C}$). Maximum absorption is attained after 10 min (for two methods) and the colour remains stable for at least 2 h (DDQ method) or 8 h (BPB method), thus permitting quantitative to be carried out with good reproducibility.

The molar ratio of the reactants (drug: DDQ or BPB) in the charge-transfer or ion-pair complex was determined by the method of continuous variations (Job's method), and found to be about 1:1 (Fig. 3). This finding was anticipated by the presence of one basic or electron-donating center (piperazine ring) in the drugs studied.

A more detailed examination was made for piribedil-DDQ or piribedil-BPB complex. The absorbance of the complex was used to calculate the association constant using the Benesi-Hildebrand equation [31].

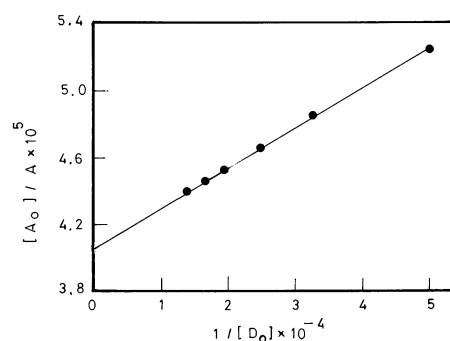


Fig. 5. Benesi-Hildebrand plot for piribedil-BPB complex; $\lambda = 410$ nm.

Table 3
Determination of the piperazine derivative drugs in commercial tablets

Tablet preparation	Label claim (mg)	% Found \pm S.D. ($n = 6$)		
		Proposed methods		Official method [2,16]
		DDQ	BPB	
Nizoral ^a (Ketoconazole)	200	99.5 \pm 0.43	99.8 \pm 0.72	99.7 \pm 0.75
Trivastal ^b (Piribedil)	20	99.8 \pm 0.56	100.2 \pm 0.81	—
Minipress ^c (Prazosin hydrochloride)	2	99.9 \pm 0.61	100.3 \pm 0.76	99.8 \pm 0.67

^aNizoral tablets (Janssen, Beerse, Belgium).

^bTrivastal tablets (Servier Egypt Industries, under licence of les laboratoires Servier, France).

^cMinipress tablets (Pfizer, Egypt).

$$\frac{[A_0]}{A_{\lambda}^{\text{AD}}} = \frac{1}{\epsilon_{\lambda}^{\text{AD}}} + \frac{1}{K_c^{\text{AD}} \epsilon_{\lambda}^{\text{AD}}} - \frac{1}{[D_0]} \quad (1)$$

where $[A_0]$ and $[D_0]$ are the total concentration of the interacting species, A_{λ}^{AD} and $\epsilon_{\lambda}^{\text{AD}}$ are the absorbance and molar absorptivity of the complex at 460 or 410 nm for DDQ or BPB reagent, respectively, and K_c^{AD} is the association constant of the complex. On plotting the values of $[A_0]/A_{\lambda}^{\text{AD}}$ vs. $1/[D_0]$, a line was obtained (Fig. 4 and Fig. 5) that is described by the following equation:

$$\frac{[A_0]}{A_{\lambda}^{\text{AD}}} = 2.270 \times 10^{-4} + \frac{1}{[D_0]} (0.95 \times 10^{-8}) \quad (\text{DDQ complex}) \quad (2)$$

or

$$\frac{[A_0]}{A_{\lambda}^{\text{AD}}} = 4.065 \times 10^{-5} + \frac{1}{[D_0]} (0.238 \times 10^{-9}) \quad (\text{BPB complex}) \quad (3)$$

The S.E. of the slope = 0.108×10^{-9} or 0.11×10^{-10} , $n = 5$ or 7 for Eq. (2) or Eq. (3), respectively. The intercept of this line with the ordinate is $(\epsilon_{\lambda}^{\text{AD}})^{-1}$, the slope equals $(\epsilon_{\lambda}^{\text{AD}} \cdot K_c^{\text{AD}})^{-1}$. From Eq. (2) and Eq. (3), the association constants are 2.389×10^4 and $1.708 \times 10^5 \text{ l} \cdot \text{mol}^{-1}$, and the standard free energies of complexation, ΔG° are -6.009 and -7.181 kcal for DDQ and BPB complexes, respectively.

From the above, the molar absorptivities are equal to 4.40×10^3 and $2.46 \times 10^4 \text{ l mol}^{-1} \text{ cm}^{-1}$

for DDQ- and BPB-piribedil complexes, respectively, which are comparable with those obtained from regression line equation of Beer's law (Table 1 and Table 2).

4.1. Analytical parameters

Under the experimental conditions described, standard calibration curves for ketoconazole, piribedil and prazosin were constructed by plotting absorbance versus concentration. Conformity with Beer's law was evident in the concentration range of the final dilution cited in Table 1 and Table 2. The molar absorptivities and the regression line equations for each drug are tabulated in Table 1 and Table 2. The correlation coefficients were between 0.9994 and 0.9999 indicating good linearity.

The slopes of the calibration curves reflect the degree of formation of the radical anion. The higher slope for piribedil is probably due to its greatest basicity with compared to ketoconazole ($\text{p}K_a = 2.9$) and prazosin ($\text{p}K_a = 6.5$) [32].

Six replicate determinations at different concentration levels were carried out to test the precision of the methods. The relative standard deviations were found to be less than 1%, indicating reasonable repeatability of the selected methods. The results obtained for each drug using the two proposed methods show that the BPB method is more sensitive and stable than the DDQ method (Table 1 and Table 2).

The proposed methods were applied to the analysis of commercial tablets of ketoconazole

(Nizoral), piribedil (Trivastal) and prazosin hydrochloride (Minipress) (Table 3). These results were compared with those obtained by the official methods [2,16]. There was no evidence of interference from excipients in the commercial tablets analysed. The proposed methods offer the advantages of accuracy and time saving as well as simplicity of reagents and apparatus.

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