Spectrophotometric determination of some pharmaceutical piperazine
derivatives by charge-transfer and ion-pair
complexation methods

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Abstract

Simple and sensitive spectrophotometric methods are described for the
assay of three piperazine derivatives ketoconazole, trimetazidine
hydrochloride and piribedil based on charge-transfer and ion-pair
complexation reactions. The first method is based on the reaction of the basic
drug with iodine as σ-acceptor in dry 1,2-dichloroethane to form a yellow
colour due to the formation of charge-transfer complex showing maximum
absorbence at 363, 364 and 359 nm for ketoconazole, trimetazidine
hydrochloride and piribedil, respectively. The second method is based on the
reaction of basic drug with bromocresol green (BCG) in dry 1,2-
dichloroethane to form a stable yellow coloured complex with maximum
absorbance at 407, 408 and 410 nm for ketoconazole, trimetazidine
hydrochloride and piribedil, respectively. 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 for the analysis of tablets and cream, 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.

Keywords: Ketoconazole, Trimetazidine hydrochloride, Piribedil, Spectrophoto-
metry, Charge-transfer and ion-pair complexes.

1. Introduction

Ketoconazole (KC), cis-1-acetyl-4-[4] [2-(2,4-dichlorophenyl)-2-(1H-
imidazole-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl] piperazine is a highly
effective broad spectrum antifungal agent [1]. It is used to treat a wide variety of
superficial and systemic mycoses [2] and has the advantage over other imidazole
derivatives of producing adequate sustained blood levels following oral
administration [3]. Some methods have been reported for its determination
including potentiometry [4,5], spectrophotometry [6-13], polarography [14] and
chromatography [15-23].

Trimetazidine hydrochloride (TMH), 1-[(2,3,4-trimethoxyphenyl)
methyl] piperazine dihydrochloride regulates ionic and extracellular exchanges,
correcting the abnormal flow of ions across the cell membrane caused by ischaemia and preventing cellular oedema caused by anoxia [24]. Few methods for the estimation of trimetazidine in biological fluids were reported, TLC [25], HPLC with fluorescence [26] or electrochemical detection [27] and GC-MC [28]. Trimetazidine is also determined in tablets using HPTLC [29]. These methods are often time-consuming, expensive and cumbersome.

Piribedil (PD), is an alkoxybenzyl-4-(2-pyrimidinyl) piperazine derivative with vasodilatory activity [30]. Piribedil has proved active in patients with Parkinson’s disease, particularly in the control of tremors [31]. The drug is not cited in any pharmacopoeia. Methods for the analysis of piribedil or its basic metabolites in biological specimens have used gas chromatography with a nitrogen-sensitive detector [32] or combined with mass spectrometry [33], spectrophotometry [13, 34], HPLC [35] and ion-selective electrodes [36].

This paper introduces two spectrophotometric methods for the determination of three pharmaceutical piperazine derivatives using iodine (I₂) as σ-acceptor and bromocresol green (BCG) as chromogenic reagent in 1,2-dichloroethane. The proposed methods were applied successfully to the determination of ketoconazole, trimetazidine hydrochloride and piribedil either in pure or dosage forms with good accuracy and precision. The results were compared with those given by the official methods (USP and JP).

2. Experimental

2.1. Apparatus

A Shimadzu UV-1601 spectrophotometer with quartz cells of 1-cm optical path length was used.

2.2. Materials

Ketoconazole, piribedil and trimetazidine hydrochloride were of pharmaceutical grade, ketoconazole (Janssen, Beerse, Belgium), piribedil (Eutherapia, France) and trimetazidine hydrochloride (Servier, France). Pharmaceutical preparations of ketoconazole, piribedil and trimetazidine hydrochloride were obtained from commercial sources.

2.3. Reagents

All the reagents and solvents used were of analytical grade and of highest purity available, bromocresol green (BCG) and iodine obtained from Merck, 1,2-Dichloroethane, dichloromethane, ethanol, methanol, 1,4-dioxane, chloroform, dimethylformamide (DMF), acetone and benzene were of spectroscopy grade.
Bromocresol green (BCG) solution (1x10^{-3} M) was prepared by dissolving in dry 1,2-dichloroethane and iodine solution (2x10^{-3} M) was prepared in 1,2-dichloroethane (stable for 1 week at 4°C). The solutions were stored at 4°C in PVC containers.

2.4. Preparation of standard solutions

Ketoconazole or piribedil stock solution was prepared by dissolving 50 mg of ketoconazole or piribedil in dry 1,2-dichloroethane in a 100-ml standard flask and diluting to volume with the same solvent. Whenever required diluted solutions were obtained by further dilution with the same solvent.

Trimetazidine stock solution was prepared by dissolving 50 mg of the drug salt in about 20 ml of distilled water. The solution was made alkaline (pH ≥ 11) with 2 M KOH solution, then quantitatively transferred into a 100-ml separatory funnel and extracted successively with five 20-ml portions of 1,2-dichloroethane. 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 1,2-dichloroethane to provide a standard 500 μg ml^{-1} solution of the drug. The diluted solution (100 μg ml^{−1}) was freshly prepared. Such drug solutions are stable for a period of 3 days when refrigerated.

2.5. Construction of calibration curves

Aliquots of a solution of the three drug bases in 2 ml of 1,2-dichloroethane in the concentration range 20-200 μg were transferred into separate 10-ml standard flasks. To each flask, 3 ml of BCG (10^{-3} M) solution in 1,2-dichloroethane was added, mixed well and allowed to stand at room temperature (25 ± 1°C) for 15 min. The solution was diluted to volume with 1,2-dichloroethane. The absorbances of the resultant complexes were measured at 408, 410 and 407 nm for ketoconazole, piribedil and trimetazidine, respectively, against a reagent blank similarly prepared.

In case of using iodine, into 10 ml calibrated flasks were placed 0.2-2.0 ml of 100 μg ml^{−1} of drug or sample solution in 1,2-dichloroethane and 3 ml of 2x 10^{-3} M iodine in the same solvent was added. The reaction mixture was well mixed and allowed to stand in the dark at 25 ±1 °C for 20 min in case of ketoconazole and trimetazidine and for 50 min in case of piribedil. The solution was diluted to volume with the same solvent and the absorbance was measured at 364, 359 and 363 nm for ketoconazole, piribedil and trimetazidine, respectively, against a reagent blank prepared and treated similarly.
2.6. Analysis of Nizoral tablets or cream (Ketoconazole) and Trivastal tablets (Piribedil)

An accurately weighed amount of cream or powdered tablets equivalent to 50 mg of drug was transferred to 100 ml conical flask and extracted with five 20 ml portions of 1,2-dichloroethane. The combined extracts were filtered into a 100-ml calibrated flask and diluted to volume with 1,2-dichloroethane. A volume of the later solution was diluted with the same solvent to obtain a solution equivalent to 100 µg ml⁻¹ of drug and then subjected to analysis as directed under ‘construction of calibration curves’.

2.7. Analysis of Vastarel tablets (Trimetazidine hydrochloride)

An accurately weighed amount of the finely powdered tablets equivalent to 50 mg of drug salt was dissolved in about 20 ml distilled water. The solution was made alkaline (pH ~ 11) with 2 M KOH solution. The drug base was extracted as described under ‘trimetazidine stock solution’ and proceeded as described under ‘construction of calibration curves’.

2.8 Stoichiometric relationship

Job’s method of continuous variations [37] of equimolar solutions was employed: a 5 x 10⁻⁴ M standard solution of drug base and 5 x 10⁻⁴ M solution of BCG or I₂, were used. A series of solutions was prepared in which the total volume of drug and reagent was kept at 2 ml for BCG or I₂. The reagents were mixed in various proportions in a 10-ml standard flask and continued as directed under ‘construction of calibration curves’ for BCG or I₂ method. The total molar concentration was 1 x 10⁻⁴ M in the final solution.

2.9. Association constant and free energy change

Serial volumes of 1-5 ml of 10⁻⁴ M drug bases solution (in 1.0 ml steps) in 1,2-dichloroethane were transferred to 10-ml standard flasks. To each flask, 1 ml of BCG or I₂ in 1,2-dichloroethane (0.5 x 10⁻⁴ M) was added and continued as directed under ‘construction of calibration curves’.

3. Results and Discussion

Charge-transfer complex forming reactions have been used in the determination of electron-donating basic compounds through the interaction with σ-acceptors [38-42]. Ketoconazole (KC), trimetazidine hydrochloride (TMH) or piribedil (PD) contains one or more tertiary amino groups in its molecular structure, these represent basic centres with the availability of non-bonding electron pair as donor. The complex
formed between iodine and n-donor (basic centre) compound exhibited high values of association constant and molar absorptivity [43]. The complex formed between iodine and KC, TMH or PD was used for the determination of these drugs in their dosage forms. At the same time the association constant, the molar ratio of reactants and the free energy change (ΔG⁰), were determined.

Charge-transfer complex formation is distinguished from other slow oxidation or substitution reactions of iodine with amines by being practically instantaneous, analogous to ionic reactions. Upon the reaction of the drugs with iodine, the violet colour of iodine is changed immediately to yellowish purple or yellow, depending on the amount of drug present. Another confirmation of the charge-transfer nature of the complex was obtained by extracting the drug from the complex by shaking with aqueous hydrochloric acid, whereby the violet colour of iodine was restored in the solvent layer. The regeneration of the drug from the charge-transfer complex is easy, since the binding energy of charge-transfer complexes is usually small; addition of a solvent of relatively high polarity causes complete decomposition [44].

Therefore, the 1,2-dichloroethane must be anhydrous to prevent the lack of absorbance in the wavelength region studied, but a low water content of 0.02% was permitted for the drug-iodine complex formation [45].

3.1. Selection of the suitable wavelength

The absorption spectrum of iodine showed only one absorption maximum at 520 nm. Whereby the absorption spectrum of the cited drugs with iodine exhibited bands at 364, 363 and 359 nm, besides the strong bands at 295, 294 and 292 nm for KC, TMH and PD, respectively. Because all the studied drugs showed an absorption band nearly at 295 nm in 1,2-dichloroethane, then the wavelengths at 364, 363 and 359 nm were used for carrying out the procedure of iodine charge-transfer complexes. The new absorption bands corresponding to the iodine complex showed an isosbestic point at 460 nm (Fig. 1), this indicates the formation of 1:1 stoichiometry [46]. As described in the literature [47], the formation of I⁻ ions, which are the measured 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 the drug (R-N) 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:

\[ R - N + I_2 \rightarrow R - N_I_2 \rightarrow [R - N^+ - I^-] I^- + I_2 \rightarrow [R - N^+ - I^-] I^-_3 \]

Outer complex   Inner complex   Tri-iodide ion pair

Fig. 1: Absorption spectra of pribedil-iodine complex showing the isosbestic point. The iodine concentration was fixed at 6 x 10^-4 M. The concentration of pribedil (µg ml^-1) were: (1) 2; (2) 4; (3) 6; (4) 8; (5) 10; (6) 14 and (7) 18.

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.

3.2. Optimum conditions for drug determination

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 3 ml of 2 x10^-3 M iodine solution in the total volume of 10 ml
for all drugs studied (20 µg ml\(^{-1}\)). 1,2-Dichloroethane was considered to be an ideal solvent for the colour reaction as it offers excellent solvent capacity for iodine and gives the highest yield of complex. Although the complex is formed rapidly, constant absorbance readings are obtained only after the solution was left for 20 min (50 min for PD) of standing in the dark at 25 ± 1 °C and remained constant for at least 80 additional min.

In 1,2-dichloroethane, KC, TMH and PD react instantaneously with BCG to give yellow chromogen, which exhibits a broad absorption maximum at 408, 407 or 410 nm for KC, TMH or PD, respectively. BCG belongs to the family of sulphonephthalein dyes. These dyes have the following ionic forms, the yellow species HL\(^-\) are present in aqueous solutions. On acidification, they turn purple (H\(_2\)L as an ampholyte), this colour change corresponds to protonation of the quinoid oxygen. The second symmetric resonance structure (L\(^2\)) arises by splitting of a proton from the hydroxy group, accompanied by a bathochromic shift [47].

Since the reaction product in 1,2-dichloroethane is yellow, so we may consider that HL\(^-\) is the form of the BCG involved in the reaction with the chosen drugs. This may lead to an assumption that, by the interaction of BCG with the drug base, a proton transfer from BCG to the basic center of the drug takes place. The obtained ion-pair salt dissociates to the yellow HL\(^-\) anion.

The effect of solvent on the formation of the BCG complex was studied using acetonitrile, 1,2-dichloroethane, dichloromethane and chloroform. 1,2-Dichloroethane was preferred because of the higher molar absorbivities and stabilities of the complexes formed. The effect of BCG concentration has been studied, 3 ml of 1 x 10\(^{-3}\) M BCG in the total volume of 10 ml was required for maximum complex formation, using 20 µg ml\(^{-1}\) of the drug.

Reaction time is determined by following the colour development at different time intervals at room temperature (25± 1°C). Maximum absorption is attained after 10 min and the colour remains stable for at least 2 h, thus permitting quantitative determination to be carried out with good reproducibility.

The intensity of the ion-pair colour was stable within the temperature range 20-40°C, then room temperature 25°C, was used.

No interferences (< 2 is considered non interferent) were observed in the determination of KC, TMH and PD using iodine or BCG reagent in the presence of the common excipients of the tablets or cream; e.g. talc powder, magnesium stearate, starch, lactose, glucose and sucrose (100 fold excess was the maximum molar ratio tested).
3.3. Stoichiometry of the complexes

The molar ratio of the reactants (drug : iodine or BCG) in the charge-transfer or ion-pair complex was determined by the method of continuous variations (Job’s method) [37] and found to be about 1:1 (Fig. 2). This finding is in conformity with the presence of one basic center or electron-donating center (piperazine ring) in the drugs studied.

Fig. 2: Continuous variation plots for (1) ketoconazole- iodine complex (364 nm) and (2) ketoconazole- BCG complex (408 nm).

A more detailed examination was made for drug-iodine or drug-BCG complex. The absorbance of the complex was used to calculate the association constant using Benesi-Hildebrand equation [48].

\[
\frac{[A_0]}{A_{\lambda}} = \frac{1}{\varepsilon_{\lambda}} + \frac{1}{K_{\lambda} \varepsilon_{\lambda}} \times \frac{1}{[D_0]}
\]

Where \([A_0]\) and \([D_0]\) are the total concentration of \(\sigma\)-acceptor (iodine) and \(n\)-donor (drug), respectively. \(A_{\lambda}^{AD}\) and \(\varepsilon_{\lambda}^{AD}\) are the absorbance and molar absorptivity of the complex (AD) at its absorption maximum and \(K_{\lambda}^{AD}\) is the association constant of the complex.
On plotting the values of \([A_0] / \lambda^{AD} \) versus \(1/[D_0] \), a line was obtained (Fig. 3). The intercept of this line with the ordinate is \((E_{\lambda}^{AD})^{-1}\) and the slope equals \((E_{\lambda}^{AD} \cdot K_c^{AD})^{-1}\), from which the association constant, the free energy change and the molar absorptivity were obtained (Table 1).

Table 1
Association constant \(K_c^{AD}\), molar absorptivity \((E_{\lambda}^{AD})\) and correlation coefficient \((r)\) obtained from Benesi-Hildebrand equation and free energy change \((\Delta G^o)\) of drug-iodine or drug-BCG complex

| Drug    | \(K_c^{AD} \times 10^{-4}\) 1.mole\(^{-1}\) | \(\Delta G^o\), Kcal | \(E_{\lambda}^{AD} \times 10^{-4}\) 1.mole\(^{-1}\) \(\text{cm}^{-1}\) | Correlation coefficient \((r)\) |
|---------|------------------------------------------|----------------------|-------------------------------------------------|-------------------------------|
| Iodine method          |                                          |                      |                                                 |                               |
| KC (364 nm)            | 2.20                                     | - 5.957              | 2.38                                            | 0.9995                        |
| TMH (363 nm)           | 1.93                                     | - 5.875              | 1.79                                            | 0.9993                        |
| PD (359 nm)            | 1.33                                     | - 5.655              | 1.25                                            | 0.9996                        |
| BCG method             |                                          |                      |                                                 |                               |
| KC (408 nm)            | 1.21                                     | - 5.604              | 3.17                                            | 0.9994                        |
| TMH (407 nm)           | 1.11                                     | - 5.552              | 2.56                                            | 0.9995                        |
| PD (410 nm)            | 0.98                                     | - 5.478              | 2.32                                            | 0.9993                        |

The standard free energy change of complexation \((\Delta G^o)\), is related to the association constant and the negative sign indicated exothermic reaction. The high value of the association constants is common to \(n\)-electron donors where the intermolecular overlap may be considered [43]. The molar absorptivities are comparable with those obtained from the regression line equation of Beer’s law (Tables 2 and 3).
Table 2
Analytical parameters for iodine complexes of KC, TMH and PD

| Parameters                               | Drug  |   |   |
|------------------------------------------|-------|---|---|
|                                          | KC    | TMH | PD |
| λ_max (nm)                               | 364   | 363 | 359 |
| Beer's law limits (μg ml⁻¹)              | 2-20  | 2-18 | 2-20 |
| Detection limit (μg ml⁻¹)                | 0.23  | 0.07 | 0.26 |
| Molar absorptivity (l. mol⁻¹ cm⁻¹)       | 2.38 x 10⁴ | 1.86 x 10⁴ | 1.26 x 10⁴ |
| Sandell sensitivity (μg cm²)             | 0.022 | 0.018 | 0.024 |
| Regression line equation                 |       |     |     |
| Slope*                                   | 0.045 | 0.054 | 0.043 |
| Intercept*                               | -0.002 | 0.006 | -0.007 |
| Correlation coefficient (r)              | 0.9998 | 0.9997 | 0.9996 |
| Relative standard deviation (%) (n = 6)  | 0.52  | 0.20  | 0.55  |
| Ringbom optimum concentration range (μg ml⁻¹) | 2.9 – 20.0 | 2.7 – 18.0 | 3.5 – 19.1 |

* Absorbance – concentration (μg ml⁻¹) relation.
## Table 3
Analytical parameters for ion pairs of KC, TMH and PD with BCG

| Parameters                              | Drug | KC   | TMH  | PD   |
|-----------------------------------------|------|------|------|------|
| $$\lambda_{\text{max}}$$ (nm)           |      | 408  | 407  | 410  |
| Beer's law limits ($\mu$g ml\(^{-1}\))  |      | 2-16 | 1.4-12.6 | 1.2-12.0 |
| Detection limit ($\mu$g ml\(^{-1}\))    |      | 0.17 | 0.14 | 0.15 |
| Molar absorptivity ($1$ mol\(^{-1}\) cm\(^{-1}\)) |      | $3.17 \times 10^4$ | $2.55 \times 10^4$ | $2.35 \times 10^4$ |
| Sandell sensitivity ($\mu$g cm\(^{-2}\)) |      | 0.017 | 0.013 | 0.013 |
| Regression line equation                |      |      |      |      |
| Slope *                                 |      | 0.059 | 0.075 | 0.079 |
| Intercept *                             |      | 0.007 | 0.001 | -0.003 |
| Correlation coefficient ($r$)           |      | 0.9998 | 0.9999 | 0.9997 |
| Relative standard deviation (%)         |      | 0.50 | 0.54 | 0.60 |
| Ringbom optimum concentration range ($\mu$g ml\(^{-1}\)) |      | 2.2-16.0 | 2.0-11.8 | 2.0-12.0 |

* Absorbance – concentration ($\mu$g ml\(^{-1}\)) relation.
Fig. 3: Benesi-Hildebrand plots for (a) iodine complexes and (b) BCG associates with KC, TMH and PD.

3.4. Analytical parameters

Under the experimental conditions described, standard calibration curves for the cited drugs with iodine or BCG were constructed by plotting absorbance versus concentration. Conformity with Beer’s law was evident in the concentration range of the final dilution cited in Tables 2 and 3. The molar absorptivity, Sandell sensitivity and the linear regression equation for each method are listed in Tables 2 and 3. The correlation coefficients (r) were between 0.9996-0.9999 indicating good linearity.

In order to define a suitable concentration range and evaluate the accuracy in photometric analysis, Ringbom plots [49] for optimum
concentration ranges, can be obtained by plotting the photometric data of percent transmittance as ordinate against the logarithm of drug concentration as abscissa. The results are recorded in Tables 2 and 3.

According to ICH recommendation [50], the approach based on the S.D. of the response and the slope of the calibration curve were used for determining the detection limits applying the following equation: D.L. = K x S.D./m, where K = 2, S.D. and m are the standard deviation and the slope of the regression line. The results are shown in Tables 2 and 3.

Five 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 1.0%, indicating reasonable repeatability of the selected methods. The results obtained for each drug using the two proposed methods show that the BCG method is more sensitive and stable than the iodine method (Tables 2 and 3).

Table 4
Determination of the piperazine derivative drugs in commercial dosage forms

| Drug               | Dosage form | % Recovery ± SD<sup>a</sup> | Official methods [4,51] |
|--------------------|-------------|-----------------------------|-------------------------|
|                    |             | Proposed methods            |                         |
|                    |             | Iodine | BCG   |                               |                         |
| Ketoconazole       | Nizoral tablets<sup>b</sup> | 100.09±0.67 | 100.05±0.46 | 99.98±0.76 <sup>[4]</sup> |
|                    |             | t = 0.24 | 0.18   | (2.306)<sup>d</sup>          |                         |
|                    |             | F = 1.29 | 0.73   | (6.390)<sup>d</sup>          |                         |
|                    | Nizoral cream<sup>b</sup> | 100.08±0.53 | 100.03±0.48 |                               |
|                    |             | t = 0.24 | 0.12   |                         |                         |
|                    |             | F = 2.06 | 2.51   |                         |                         |
| Trimetazidine      | Vastarel tablets<sup>c</sup> | 99.93±0.55 | 99.97±0.43 | 99.85±0.68 <sup>[51]</sup> |
|                    |             | t = 0.20 | 0.33   |                         |                         |
|                    |             | F = 1.53 | 2.50   |                         |                         |
| Piribedil          | Trivastal tablets<sup>c</sup> | 100.16±0.72 | 99.9±0.65 |                         |

<sup>a</sup>: Mean ± standard deviation of five determinations
<sup>b</sup>: Nizoral tablets, 20 mg/tab. and Nizoral cream, 20 mg/g (Janssen, Beerse, Belgium).
<sup>c</sup>: Vastarel and Trivastal tablets each contains 20 mg/tab. (Servier Egypt Industries Limited, under licence of les laboratoires, Servier – France).
<sup>d</sup>: Values in parentheses are the theoretical values at p = 0.05
The proposed methods were applied to the analysis of commercial dosage forms of the tested drugs (Table 4). The results were comparable with those obtained by the official methods for ketoconazole [4] and trimetazidine hydrochloride [5]. Student t- and F-ratio tests, show that there is no significance difference between the calculated and theoretical values at $p = 0.05$. This indicates the precision and accuracy of the proposed methods.

The proposed methods offer the advantages of accuracy, precision and time saving as well as simplicity of reagents and apparatus. In addition the proposed methods are suitable for the routine analysis of the tested drugs in their dosage forms and in drug control laboratories.

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