Bull. Chem. Soc. Ethiop. **2016**, 30(3), 333-346.
Printed in Ethiopia
DOI: http://dx.doi.org/10.4314/bcse.v30i3.2

ISSN 1011-3924

SPECTROPHOTOMETRIC METHODS BASED ON CHARGE TRANSFER COMPLEXATION REACTION FOR THE DETERMINATION OF SOME ANTIFUNGAL DRUGS IN PURE AND DOSAGE FORMS

Ayman A. Gouda[1*], Ragaa El Sheikh[1], Alaa S. Amin[2] and Sara H. Ibrahim[1]

[1] Chemistry Department, Faculty of Science, Zagazig University, Zagazig, Egypt
[2] Chemistry Department, Faculty of Science, Benha University, Benha, Egypt

(Received November 23, 2015; revised November 16, 2016)

ABSTRACT. Charge transfer complexes of some antifungal drugs fluconazole (FLU), sertaconazole nitrate (SER) and miconazole nitrate (MCO) as electron donor with the σ-acceptor iodine (I_2) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), *p*-chloranilic acid (*p*-CLA) and 7,7,8,8-tetracyanoquinodimethane (TCNQ) as π-acceptors in acetonitrile were investigated. The formation of the colored charge-transfer complexes were utilized in the development of simple, rapid and accurate spectrophotometric methods for the analysis of the cited drugs in pure forms and pharmaceutical formulations at 365 nm for MCO and SER using I_2 method, at 460 nm for FLU and SER using DDQ method, at 535 and 530 nm for FLU and SER, respectively, using *p*-CLA method and at 842 nm for MCO and SER using TCNQ method. The optimum experimental conditions have been studied carefully and optimized. Beer's law was obeyed over the concentration ranges of 2.0–28, 10–240 and 2.0–160 μg mL^{-1} for MCO, FLU and SER, respectively, with good correlation coefficients (0.9996–0.9999). Molar absorpitivity, Sandell sensitivity, relative standard deviation, limit of detection and quantification were calculated. The obtained data refer to high accuracy and precision of the proposed method. The obtained results were confirmed by inter and intra-day accuracy and precision with percent recovery of 99.1–100%, 99.3–101% and 99.1–101% for MCO, FLU and SER, respectively. These data were compared with those obtained using official methods for the determination of the cited drugs. The proposed methods were applied successfully for simultaneous determination of the cited drugs in their pharmaceutical formulations with good accuracy and precision and without interferences from common additives.

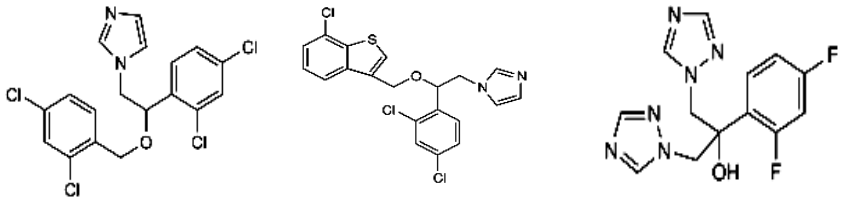
KEY WORDS: Fluconazole, Sertaconazole nitrate, Miconazole nitrate, Charge transfer complexes, Spectrophotometry, Pharmaceutical formulations

INTRODUCTION

Azoles have a common mode of action; they inhibit ergosterol synthesis, the main sterol constituent of fungal membranes, through blocking cytochrome P450-dependent enzyme: lanosterol 14-α-demethylase. Lack of ergosterol and accumulation of 14-α-methylated precursors result in dysfunction of membrane fluidity and activity of several enzymes located in the membrane (e.g. chitin synthase). Consequently fungal growth and replication of its DNA are inhibited. Moreover azoles decrease the adhesion potential of pathogen cells to host tissues and morphogenetic transformation of yeasts to mycelial form [1, 2]. The chemical structure of the studied azole antifungal drugs; fluconazole (FLU), 2-(2,4-Difluorophenyl)-1,3-bis(1*H*-1,2,4-triazol-1-yl)propan-2-ol;sertaconazole (SER), (±)-1-{2,4-Dichloro-β-[(7-chlorobenzo[*b*] thien-3-yl)methoxy] phenethyl}imidazole nitrate; and miconazole (MCO), 1-[2,4-Dichloro-β-(2,4-dichlorobenzyloxy) phenethyl] imidazole nitrate are shown in Figure 1.

The literature survey revealed that a few analytical methods have been reported for the determination of the studied drugs in pure drug, pharmaceutical dosage forms and biological samples using liquid chromatography either in single or in combined forms [3-5], electrochemical methods [6, 7], spectroflourimetric methods [3, 8] and spectrophotometric methods [9-14].

*Corresponding author. E-mail: aymangouda77@gmail.com,



Miconazole Nitrate (MCO) | Sertaconazole Nitrate (SER) | Fluconazole (FLU)

Figure 1. The chemical structure of the studied antifungal drugs.

Visible spectrophotometry, because of simplicity and cost effectiveness, sensitivity and selectivity, fair accuracy, precision and available in most quality control laboratories, have remained competitive in an area of chromatography techniques for pharmaceutical analysis. Furthermore, they do not need costly instrumentation required for the published HPLC methods. The molecular interactions between electron donors and acceptors are generally associated with the formation of intensely colored charge transfer complexes, which absorb radiation in the visible region [15]. A variety of electron donating compounds have been reported to yield charge-transfer complexes with various acceptors. The rapid formation of these complexes leads to their utility in the development of simple and convenient spectrophotometric methods for these compounds [16-18].

The studied drugs are good n-electron donors and form charge transfer complexes with σ-acceptor like iodine (I_2) and π-acceptors such as 7,7,8,8-tetracyanoquinodimethane (TCNQ); 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and *p*-chloranilic acid (*p*-CLA). Therefore the aim of the present study was directed to investigate simple, direct, sensitive and precise spectrophotometric methods for simultaneous determination of some antifungal drugs (FLU, SER and MCO) via complexation with σ- and π -acceptors in pure and dosage forms.

EXPERIMENTAL

Apparatus

All absorption spectra were made using double beam Unikon 930 spectrophotometer (Kontron Instruments, Munchen, Germany) with a scanning speed of 200 nm/min and a band width of 2.0 nm, equipped with 10 mm matched quartz cells.

Materials

Fluconazole (FLU) working standard was kindly supplied by Pfizer Pharmaceuticals Co. Ltd., USA. Diflucan capsules labeled to contain 150 mg FLU/capsule was obtained from Pfizer PGM, 37530 Poce sur Cisse, France, under authority of Pfizer Inc., New York, USA. Sertaconazolenitrate (SER) was provided by October Phrama. Co., Egypt. Dermofix cream (Ferrer International, SA-Spain) labeled to contain (2.0 mg SER/g) was obtained from October Phrama. Co., Egypt. Miconazol nitrate (MCO) working standard was supplied by Amriya pharmaceutical Industries Co. Egypt. Miconaz cream, labeled to contain (2.0% MCO/15 g) was obtained from Medical Union Pharmaceuticals, Abu- Sultan, Ismailia, Egypt. Micoban cream, labeled to contain 2.0% MCO/15 g was obtained from Amriya pharmaceutical Industries Co. Egypt.

Preparation of stock standard solutions

Stock standard solutions of FLU, MCO and SER (500 μg mL^{-1}) were prepared by accurately weighting 50 mg of pure drug and dissolving in the suitable solvent 1,2-dichloroethane (I_2 method), methanol (DDQ method) and acetonitrile (TCNQ and *p*-CLA methods) and the volume was diluted to the mark in a 100 mL calibrated flask with the same solvent. These stock solutions were diluted with the respective solvents to obtain suitable concentrations that lie in the linear range of each particular assay method. In the same manner, another set of stock solutions of the drugs (1.0 x 10^{-3} mol L^{-1}) were also prepared for the stoichiometric study. The stock solutions of drugs are stable for a period of at least one week when kept in the refrigerator (at about 4 ºC).

Reagents

All reagents and solvents used were of analytical-reagent grade. Iodine, resublimed (Riedel-De-Haen AG, Germany) (1.0 x 10^{-3} mol L^{-1}) in 1,2-dichloroethane was prepared. 7,7,8,8-Tetracyanoquinodimethane (TCNQ) (Aldrich Chem. Co., Milwaukee, USA); *p*-chloranilic acid (*p*-CLA), (Fluka, Switzerland), (1.0 x 10^{-3} mol L^{-1}) and 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ), (Merck-Schuchardt, Munich, Germany), (1.0 x 10^{-3} mol L^{-1}) solutions were prepared in acetonitrile. All the reagent solutions were prepared fresh daily and were stable for at least one week at 4 ºC.

General procedures

Iodine method. Into 10 mL volumetric flasks (0.2-2.8 mL) and (0.2-2.0 mL) of 100 μg mL^{-1} MCO and SER solutions, respectively, were placed and 2.0 mL of (1.0 x 10^{-3} mol L^{-1}) iodine in 1,2-dichloroethane was added. The reaction was achieved instantaneously and allowed to stand in the dark at 25±2 ^{o}C, then the solution was diluted to volume with 1,2-dichloroethane. The absorbance was measured at 365 nm for both drugs against a reagent blank similarly prepared.

DDQ method. Into 10 mL volumetric flasks (0.2-3.2 mL) and (0.2-4.0 mL) of 500 μg mL^{-1} SER and FLU solutions, respectively, were transferred and 2.0 mL of (1.0 x 10^{-3} mol L^{-1}) DDQ solution was added. The reaction mixture was mixed and allowed to stand for 10 min at (60±2 ^{o}C). The volume was made up to 10 mL with acetonitrile and the absorbance was measured at 460 nm for both drugs against a reagent blank prepared similarly.

Chloranilic acid (p-CLA) method. Into 10 mL calibrated flasks (0.2-3.0 mL) and (0.2-4.8 mL) of 500 μg mL^{-1} SER and FLU solutions were placed and 2.0 mL of (1.0 x 10^{-3} mol L^{-1}) *p*-CLA solution was added. The reaction mixture was mixed and allowed to stand for 10 min at (60±5.0 ^{o}C). The volume was made up to 10 mL with acetonitrile and the absorbance was measured at 530 and 535 nm for SER and FLU, respectively, against a reagent blank prepared similarly.

TCNQ method. Into 10 mL calibrated flasks (0.1-1.4 mL) and (0.1-1.2 mL) of 200 μg mL^{-1} SER and MCO solutions were placed and 2.0 ml of (1.0 x 10^{-3} mol L^{-1}) TCNQ was added. The reaction mixture was heated in a water-bath at (60±2 ºC) for 15 min (MCO or SER). The reaction mixture was cooled and then diluted up to 10 mL with acetonitrile and the absorbance was measured at 842 nm for both drugs against a reagent blank prepared in the same manner.

Applications to pharmaceutical formulations

Capsules. The contents of ten capsules (Diflucan, 150 mg FLU/capsule) were crushed, finely powdered, weighed out and the average weight of one tablet was determined for each drug. An accurate weight equivalent to 50 mg FLU was transferred into a 100 mL calibrated flask,

dissolved in less volume of methanol with shaking for 5.0 min and filtered through a sintered glass crucible (G_4). The first portion of the filtrate was rejected. Then the filtrate was diluted quantitatively with a suitable solvent to yield concentrations within the linear ranges of each particular assay method. An aliquot of the cited solutions was taken and analyzed as described under the above recommended procedures for construction of calibration curves. The method of standard addition was used for the accurate determination of FLU content.

Cream sample solution. A quantity 2.5 g of pharmaceutical cream (Dermofix, 20 mg SER/g and Miconaz or Micoban, 20 mg MCO/g) was weighed into a 25 mL beaker and dispersed with methanol using a stirring rod for 10 min. Each solution was transferred quantitatively to a 50 mL measuring flask to obtain a concentration of 1.0 mg mL^{-1}. Each flask was sonicated for 15 min and diluted to the mark with 1,2-dichloroethane (I_2 method) or acetonitrile (DDQ, *p*-CLA and TCNQ methods). The solution was filtered for the powdered form, while an aliquot was centrifuged for 15 min, for cream. Appropriate solutions were prepared to obtain 500 µg mL^{-1} in methanol. An aliquot of the cited solutions was taken and analyzed as described under the above recommended procedures for construction of calibration curves. The method of standard addition was used for the accurate determination of MCO and SER contents.

Stoichiometric relationship

Job's method of continuous variation [19] was employed to establish the stoichiometry of the colored products. A 1.0 x 10^{-3} mol L^{-1} standard solution of drugs and a 1.0 x10^{-3} mol L^{-1} solution of I_2, DDQ, *p*-CLA and TCNQ were used. A series of solutions was prepared in which the total volume of drug and reagent was constant (2.0 mL). The drugs and reagents were mixed in various proportions and diluted in a 10 mL calibrated flask with 1,2-dichloroethane (I_2 method) or acetonitrile (DDQ, *p*-CLA and TCNQ methods). The absorbance was measured at the optimum wavelengths after treating each reagent at the best time and temperature against a reagent blank following the above mentioned procedure.

Procedures for method validation

All assay validation procedures were carried out according to current ICH guidelines [20], which include linearity, limits of detection (LOD) and quantification (LOQ), intra-day and inter-day precision and accuracy, robustness and ruggedness, selectivity and recovery.

Linearity, LOD and LOQ

Linearity was assessed by analysing a set of six calibration standards. Calibration curves were prepared as described above in the "general procedures". The linearity of the methods was determined by plotting the absorbance (*Y*) against the theoretical concentration (*X*) of the studied drugs. Calibration measurements were subjected to least square regression analysis to obtain information related to the slope, the Y-intercept, the correlation coefficient (*r*) and the back-calculated concentrations. The coefficient of correlation should be 0.9996 or greater. The limit of detection (LOD) is defined as the minimum level at which the analyte can be reliably detected was calculated using the following equation [20, 21]: LOD = *3s/k*. Where *s* is the standard deviation of replicate (n = 6) blank absorbance's under the same conditions as for the sample analysis in the absence of the analyte and *k* is the slope of the calibration graph. The limits of quantization (LOQ) is defined as the lowest concentration that can be measured with acceptable accuracy and precision [20, 21]: LOQ = *10s/k*.

Accuracy and precision

The accuracy and precision of the methods were evaluated by performing six replicate analyses on a pure drug solution at three different concentration levels (within the working range) to

same day (intra-day) analysis as well as on six consecutive days (inter-day analysis) by preparing all solutions a fresh each day. The mean and standard deviations (SD) were obtained by back-calculated drug concentration at each level. Percentage relative standard deviation (RSD %) as precision and percentage relative error (RE %) as accuracy of the proposed spectrophotometric methods were calculated. The relative standard deviation (RSD) values were less than 2.0% in all cases, indicating good repeatability of the suggested methods. This level of precision of the proposed methods was adequate for the quality control analysis of the studied drugs. The percentage relative error calculated using the following equation: RE % = [(found – added)/added] x 100.

Robustness and ruggedness

Robustness of the procedures was assessed by evaluating the influence of small variations in experimental variables, including the amounts of analytical reagents and the reaction time, on the performance of the proposed methods. In these experiments, one experimental parameter was changed while the others parameters were kept constant and the changes had negligible influence on the results as revealed by small intermediate precision values expressed as RSD (≤ 3.0%). Ruggedness was also tested by applying the proposed methods to determine the studied drugs using the same experimental conditions by three analysts using the same instrument (inter-personnel) and also by a single analyst using three different instruments (inter-instrument). The robustness and the ruggedness were checked at three different drug levels.

RESULTS AND DISCUSSION

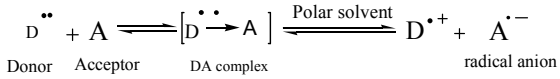
Spectral characteristics of the reaction

Reaction with σ-acceptor (iodine). The color of iodine in 1,2-dichloroethane is violet showing absorption maximum (λ_{max}) at 520 nm. This color was immediately changed into lemon yellow, and the absorption spectrum of drug-I_2 reaction product showed absorption peaks at 292 and 365 nm. This change in color, and the appearance of these two peaks were attributed to the formation of charge-transfer complex between the drugs and I_2, having an ionized structure $DI^+ \ldots I_3^-$, taking into account that the absorption spectrum of I_3^- in 1,2-dichloroethane showed the two absorption maxima at 292 and 365 nm. This complex should originate from an early intermediated outer complex $D \ldots I_2$, according to the following scheme:

$$\underset{\text{Outer complex}}{D + I_2} \rightleftharpoons \underset{\text{inner complex}}{D\text{-}I^+ I^-} \rightleftharpoons \underset{\text{tri-iodide ion pair}}{[D\text{-}I^+] + I^-} \overset{I_2}{\rightleftharpoons} I_3^-$$

For further confirmation of the charge-transfer nature of the reaction, the studied drugs were extracted from the complex by shaking with aqueous mineral acids. The color of iodine in 1,2-dichloroethane layer was restored to violet, confirming the charge-transfer nature of the reaction. Measurements were carried out at 365 nm due to the interference from the native UV absorption of MCO or SER at 292 nm.

Reaction with π-acceptors. The interaction of the studied drugs with selective polyhaloquinone and polycyanoquinone π-acceptors in non-polar solvents such as 1,2-dichloroethane was found to produce colored charge-transfer complexes with low molar absorptivity values [17]. In polar solvents such as methanol or acetonitrile, complete electron transfer from the studied drugs (D), as an electron donor, to the acceptor moiety (A) takes place with the formation of intensely colored radical ions with high molar absorptivity values, according to the following scheme:



The dissociation of the (D–A) complex was promoted by the high ionizing power of the polar solvent and the resulting peaks in the absorption spectra of D-acceptor reaction mixtures were similar to the maxima of the radical anions of the acceptors obtained by the iodide reduction method [22].

DDQ method

The reaction of the studied drugs SER or FLU with DDQ results in the formation of an intense orange-red color, which exhibits two maxima at 530 and 460 nm. The 460 nm band for (SER or FLU), having the highest absorption intensity, was selected for construction of Beer's plot. The predominant color with DDQ is from the reddish brown radical anion DDQ^{-}, which was probably formed by the dissociation of an original donor-acceptor (DA) complex with the studied drugs (Figure 2 A and B) [23].

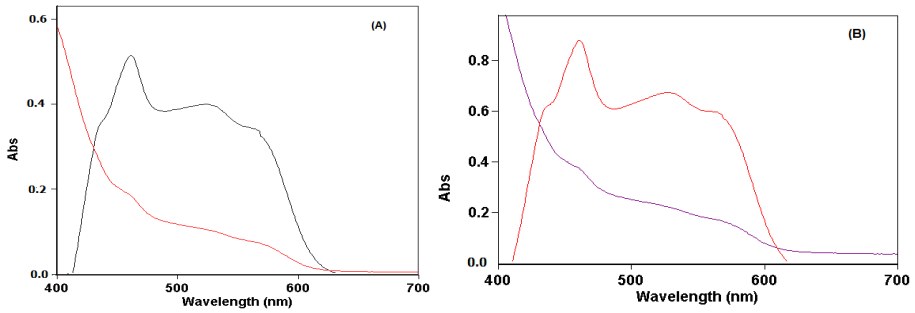


Figure 2. Absorption spectra of the reaction product of DDQ (1.0 x 10^{-3} mol L^{-1}) with (A) 160 µg mL^{-1} (SER) and (B) 200 µg mL^{-1} (FLU) in acetonitrile.

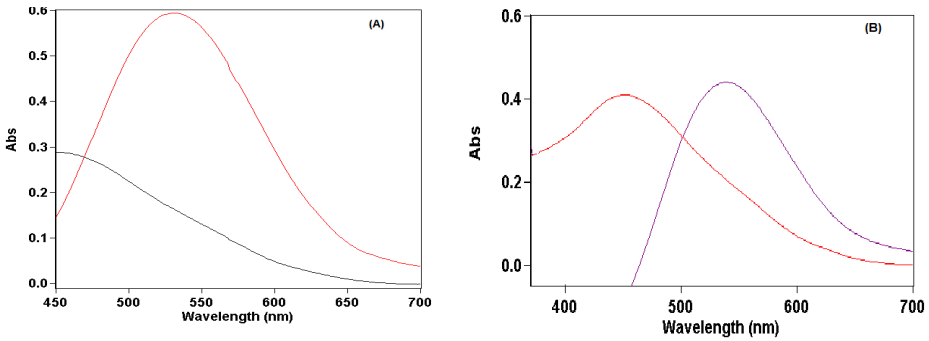


Figure 3. Absorption spectra of the reaction product of *p*-CLA (1.0 x 10^{-3} mol L^{-1}) with (A) 150 µg mL^{-1} (SER) and (B) 240 µg mL^{-1} (FLU) in acetonitrile.

Chloranilic acid (p-CLA) method

Chloranilic acid (*p*-CLA) exists in three ionic forms, the neutral yellow-orange H_2A at very low pH, the dark purple HA^{-} which is stable at pH 3.0 and a colorless A^{2-}, which is stable at high pH; these transformations are illustrated in the following scheme:

$$H_2A \rightleftharpoons H^+ + HA^- \text{ (violet)},$$

$$HA^- \rightleftharpoons H^+ + A^{2-} \text{ (colorless)}.$$

Since the interaction of SER and FLU with *p*-CLA in acetonitrile gave a violet product, it might be concluded that HA^- was the form of *p*-CLA involved in the reaction described herein [24, 25]. The *p*-CLA radical anion absorbs at 530 and 535 nm for SER and FLU, respectively (Figure 3 A and B).

TCNQ method

SER and MCO yields intense colours with TCNQ in acetonitrile, absorbing maximally at 842 nm (Figure 4) most probably due to the formation of charge-transfer complexes between the drug acting as n-donor (D) or Lewis base, and TCNQ, as π-acceptors(A) or Lewis acids [26,27].

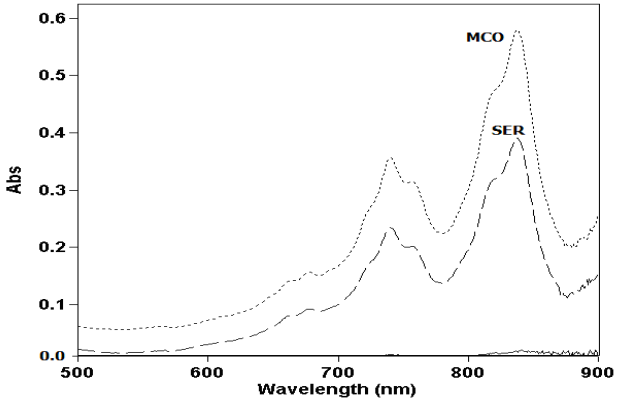


Figure 4. Absorption spectra of the reaction product of TCNQ (1.0×10^{-3}mol L^{-1}) with 28 and 24 µg mL^{-1} of SER and MCO, respectively in acetonitrile.

Optimization of reaction conditions

Effect of acceptors concentration. The results for variation of reagent concentration indicated that 2.0 mL of (1.0×10^{-3} mol L^{-1}) each reagent was suitable. The highest concentrations of the reagents may, on the other hand, be useful for rapidly reaching equilibrium and complete color development. This minimizes the time required to attain the maximum absorbance at the corresponding wavelength of the charge transfer complexes.

Effect of solvent. 1,2-Dichloroethane was found to be an ideal solvent in case of iodine, because it is favorable for the formation of a tri-iodide ion pair (inner complex). With iodine, the studied drugs showed a major charge-transfer band at 365 nm. Dichloromethane and chloroform produced lower absorbance readings. Polar solvents such as acetonitrile and alcohols were found to be unsuitable as their blanks with iodine gave high absorbance. It is obvious that, the rate of transformation of the outer complex to inner complex is in the order of 1,2-dichloroethane > dichloromethane > chloroform [22]. There is actually a considerable decrease in the energy of activation along with an increased dielectric constant ε_r of the medium; in 1,2-dichloroehane (ε_r = 10.2) the transformation of inner complex proceeds much faster than that in dichloromethane (ε_r = 9.1) and chloroform (ε_r = 4.8). This is in support of the proposed three-step mechanism. In fact, the resulting charged transition states in going from the outer

complexes to the inner ones (as the rate determining step of the mechanism) are expected to be more stabilized in 1,2-dichloroethane because of higher solvating ability and relative permittivity than dichloromethane and chloroform [28].

Different solvents such as acetone, methanol, ethanol, methylene chloride, 1,2-dichloroethane, 1,4-dioxane, acetonitrile and chloroform were examined. Acetonitrile was found to be the best solvent for all the reagents, because it has a high relative permittivity and its high dielectric constant (37.5) [29] which ensures the maximum yield of $DDQ^{\cdot-}$, $TCNQ^{\cdot-}$ and *p*-$CLA^{\cdot-}$ species (Figure 5) when compared with all other solvents (benzene, chloroform, ethylene chloride and methanol), a property which is known to promote the dissociation of the original charge transfer complexes to the radical anions in addition to the high solvating power of the reagent and drug. The formation of $DDQ^{\cdot-}$, $TCNQ^{\cdot-}$ and *p*-$CLA^{\cdot-}$ radicals were possible in methanol or ethanol, however, the color intensity was lower than in acetonitrile.

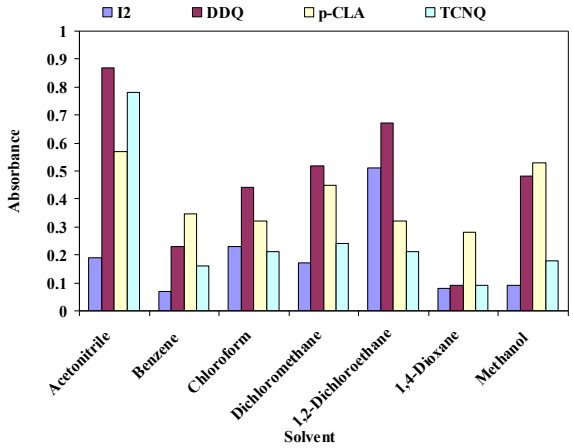


Figure 5. Effect of solvent on the absorbance of charge transfer complex of FLU with DDQ, I_2, *p*-CLA, and TCNQ acceptors.

Effect of reaction time and temperature

Complete color development, was attained instantaneously using iodine for all drugs. Whereas for DDQ and *p*-CLA, complete color development was attained after 10 min at (60 ± 2 °C) for SER or FLU. On using TCNQ complete color development was attained after 90 min. To consume the time required for complete color development heating in a water bath at 60 ± 2 °C for 15 min for (MCO or SER). The color remained stable for 3.0 and 4.0 h using DDQ and (TCNQ or *p*-CLA), respectively. In case of iodine, the yellow color remained stable at least a further 1.0 h in the dark.

Molar ratio of the reaction

Job's continuous variation graph [19] for the reaction between the studied drugs and different reagents showed that the interaction occurs on an equimolar basis via the formation of a charge transfer complex (1:1). The absorbance of the complex was used to calculate the association constant using the Benesi-Hildebrand equation [30].

$$\frac{[A^{\circ}]}{A_{\lambda}^{AD}} = \frac{1}{\varepsilon_{\lambda}^{AD} K_c^{AD} \varepsilon_{\lambda}^{AD}} + \frac{1}{[D^{\circ}]} \times \frac{1}{\varepsilon_{\lambda}^{AD}}$$

Where $[A_o]$ and $[D_o]$ are the total concentrations of the interacting species, A_λ^{AD} and $\varepsilon_\lambda^{AD}$are the absorbance and molar absorptivity of the complex at their λ_{max}, and K_c^{AD} is the association constant of the complex. On plotting the values of $[A_o]/A_\lambda^{AD}$ versus $1/[D_o]$, a line was obtained with slope equals $(\varepsilon_\lambda^{AD}\ K_c^{AD})^{-1}$ and intercept of this line with the ordinate is $(\varepsilon_\lambda^{AD})^{-1}$. The calculated association constants are recorded in Tables 1 and 2, whereas the molar absorptivities were comparable with those obtained from the regression line equation of Beer's law. The lower values obtained for the association constants are common in these complexes due to the dissociation of the original donor–acceptor complex to the radical anion.

Method validation

Linearity and sensitivity. Following the proposed experimental conditions, linear relationship was found between the absorbance at λ_{max} and drug concentration in the concentration ranges given in Tables 1 and 2. The regression equations were derived using the least-squares method [29]: A = *a*+*b*C. Where A = absorbance, a = intercept, b = slope and C = concentration in µg mL^{-1}. Beer's law ranges, correlation coefficient (r), molar absorptivity (ε), and Sandell's sensitivity values are summarized in Tables1 and 2. The validity of the proposed methods was evaluated by statistical analysis [29], between the results achieved from the proposed methods and that of the official methods. Regarding the calculated Student's *t*-test and variance ratio *F*-test (Tables 1 and 2), there is no significant difference between the proposed and official methods regarding accuracy and precision. The calculated values of limits of detection (LOD) and quantification (LOQ) are also included in Tables 1 and 2, high-lighting the high sensitivity of these methods.

Table 1. Statistical Analysis for determination of MCO and FLU using the proposed methods.

Parameters	MCO		FLU	
	I_2	TCNQ	p-CLA	DDQ
Wavelengths, λ_{max} (nm)	365	842	535	460
Solvent	1,2-Dichloroethane	Acetonitrile	Acetonitrile	Acetonitrile
Color stability (h)	1.0	4.0	4.0	3.0
Beer's law limits (µg mL^{-1})	2.0-28	2.0-28	10-240	10-200
Ringbom optimum concentration range (µg mL^{-1})	5.0-25	5.0-25	15-225	15-185
Molar absorptivity ε, ($Lmol^{-1}cm^{-1}$) x 10^3	6.800	1.041	1.285	0.638
Sandell's sensitivity (ng cm^{-2})	70.46	46.02	23.84	480
Regression equation [a]				
Slope (b)	0.0137	0.0207	0.0020	0.0043
Intercept (a)	0.0017	0.0041	0.0017	0.0015
Correlation coefficient (*r*)	0.9997	0.9998	0.9998	0.9998
Mean recovery ± SD[b]	99.8 ± 0.9	99.7 ± 1.1	99.6 ± 0.7	99.8 ± 0.6
RSD[b]	0.94	1.07	0.71	0.56
RE[b]	0.99	1.12	0.74	0.59
LOD, (µg mL^{-1})[c]	0.38	0.46	2.27	2.61
LOQ, (µg mL^{-1})[c]	1.27	1.53	7.57	8.70
Calculated *t*-value[d]	0.54	0.66	0.29	0.20
Calculated *F*-value[d]	1.49	1.15	1.55	1.04

[a] $A = a + bC$, where C is the concentration in µg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope. [b]SD, standard deviation; RSD, relative standard deviation, RE, relative error. [c]LOD, limit of detection; LOQ, limit of quantification. [d]The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom (p = 0.05).

Table 2. Statistical Analysis for determination of SER using the proposed methods.

Parameters	I_2	DDQ	p-CLA	TCNQ
Wavelengths, λ_{max} (nm)	365	460	530	842
Solvent	1,2-Dichloroethane	Acetonitrile	Acetonitrile	Acetonitrile
Color stability (h)	1.0	4.0	4.0	4.0
Beer's law limits (μg mL^{-1})	2.0-20	10-160	10-150	2.0-24
Ringbom optimum concentration range (μg mL^{-1})	5.0-18	15-150	15-140	5.0-20
Molar absorptivity ε, ($Lmol^{-1}cm^{-1}$) x 10^4	1.2354	0.1735	0.1796	0.8744
Sandell's sensitivity (ng cm^{-2})	40.54	288.65	278.84	32.13
Regression equation [a]				
Slope (b)	0.024	0.0035	0.0038	0.0165
Intercept (a)	0.0033	- 0.0017	- 0.0053	0.005
Correlation coefficient (*r*)	0.9997	0.9998	0.9996	0.9999
Mean recovery ± SD[b]	100 ± 1	99.9 ± 1.6	100 ± 1.4	99.9 ± 1.2
RSD[b]	1.18	1.56	1.35	1.24
RE[b]	1.24	1.64	1.42	1.30
LOD, (μg mL^{-1})[c]	0.35	2.73	2.34	0.47
LOQ, (μg mL^{-1})[c]	1.17	9.10	7.80	1.57
Calculated *t*-value[d]	0.34	0.67	0.43	1.0
Calculated *F*-value[d]	1.59	1.10	1.22	1.44

[a] $A = a + bC$, where C is the concentration in μg mL^{-1}, A is the absorbance units, a is the intercept, b is the slope. [b]SD, standard deviation; RSD, relative standard deviation; RE, relative error. [c]LOD, limit of detection; LOQ, limit of quantification. [d]The theoretical values of t and F are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom ($p = 0.05$).

Accuracy and precision

The results of intra-day and inter-day precision and accuracy for the proposed methods are presented in Tables 3 and 4. The precision is expressed as the %RSD for the intra-day (reproducibility) and the inter-day (inter-mediate precision) assays. The precision for the intra-day was in the ranges 0.49-1.36%, 0.57-1.45% and 0.53-1.50% for MCO, FLU and SER, respectively and the inter-day assays were in the ranges 0.62-1.40%, 0.48-1.32% and 0.47-1.50% for MCO, FLU and SER, respectively. The accuracy, expressed as the percentage relative error (RE %) for the intra-day and the inter-day assays, was in the ranges of -0.90-0.20%, -0.70-0.30% and -0.70-0.50% for MCO, FLU and SER, respectively and -0.70-0.40%, -0.70-0.60% and -0.90-0.50% for MCO, FLU and SER, respectively, for inter-day assays. The intra-day and inter-day precision and accuracy results show that the proposed methods have good repeatability and reproducibility.

Robustness and ruggedness

The results of this study, the intermediate precision expressed as (%RSD), was in the range 1.25–2.80%, which is a measure of robustness and ruggedness. Small alterations in method variables did not significantly affect the results, as shown by low values of % RSD (<3.0%). The results for person-to-person and instrument-to-instrument variations were also reproducible, with %RSD values of approximately 2.5%. This provided an indication of the reliability of the proposed methods during routine work.

Recovery studies

The accuracy and validity of the proposed methods were further ascertained by performing recovery studies. Pre-analyzed tablet powder was spiked with pure drug at different levels and

the total was determined by the proposed methods using the standard addition technique. The percent recovery of pure drug added was in the range 99.3–100% with relative standard deviation of 0.41–0.76% (Tables 5, 6 and 7) indicating that the recoveries were good, and that the co-formulated substance and common excipients did not interfere with the determination.

Table 3. Evaluation of intra-day and inter-day precision and accuracy for MCO and FLU obtained by the proposed methods.

Methods	Added (μg mL^{-1})	Intra-day				Inter-day			
		Recovery %	Precision RSD %[a]	Accuracy RE %	Confidence Limit[b]	Recovery %	Precision RSD %[a]	Accuracy RE %	Confidence Limit[b]
MCO									
I_2	5.0	99.20	0.49	-0.80	4.96±0.03	99.60	0.70	-0.40	4.98±0.04
	15	99.70	0.80	-0.30	14.96±0.13	100.40	0.91	0.40	15.06±0.14
	25	99.10	1.20	-0.90	24.78±0.31	99.30	1.14	-0.70	24.83±0.30
TCNQ	5.0	99.90	0.50	-0.10	4.99 ± 0.03	99.70	0.62	-0.30	4.99 ± 0.03
	15	100.20	0.94	0.20	15.03±0.15	99.50	0.81	-0.50	14.93 ± 0.13
	25	99.40	1.36	-0.60	24.85±0.36	100.10	1.40	0.10	25.03 ± 0.37
FLU									
DDQ	40	99.60	0.57	-0.40	39.84±0.24	99.70	0.48	-0.30	39.88±0.20
	80	99.90	0.91	-0.10	79.92±0.76	100.10	0.85	0.10	80.08±0.71
	160	99.30	1.08	-0.70	158.88±1.80	100.60	0.99	0.60	160.96±1.67
p-CLA	50	100.30	0.75	0.30	50.15±0.40	99.70	0.67	-0.30	49.85±0.35
	100	100.20	1.16	0.20	100.20±1.22	99.30	0.87	-0.70	99.30±0.92
	200	99.50	1.45	-0.50	199.00±3.02	100.30	1.32	0.30	200.60±2.78

[a]Mean of six determinations, RSD%, percentage relative standard deviation; RE%, percentage relative error. [b]Mean ± standard error.

Table 4. Evaluation of intra-day and inter-day precision and accuracy for SER obtained by the proposed methods.

Methods	Added (μg mL^{-1})	Intra-day				Inter-day			
		Recovery %	Precision RSD %[a]	Accuracy RE %	Confidence Limit[b]	Recovery %	Precision RSD %[a]	Accuracy RE %	Confidence Limit[b]
I_2	5.0	99.55	0.53	-0.45	4.98±0.03	99.75	0.80	-0.25	4.99±0.04
	10	100.30	0.90	0.30	10.03±0.10	99.40	1.15	-0.60	9.94±0.12
	20	99.80	1.05	-0.20	19.96±0.22	99.60	1.40	-0.40	19.92±0.29
DDQ	40	100.10	0.74	0.10	40.04±0.31	100.40	0.79	0.40	40.16±0.33
	80	99.30	0.97	-0.70	79.44±0.81	99.10	0.81	-0.90	79.28±0.67
	160	99.70	1.30	-0.30	159.52±2.18	99.80	0.63	-0.20	159.68±1.06
p-CLA	30	99.50	0.85	-0.50	29.85 ± 0.27	99.90	0.59	-0.10	29.97 ± 0.19
	90	100.50	0.98	0.50	90.45 ± 0.93	99.60	0.69	-0.40	89.64 ± 0.65
	150	99.60	1.20	-0.40	149.4 ± 1.88	99.30	0.92	-0.70	148.95±1.44
TCNQ	5.0	99.80	0.56	-0.20	4.99 ± 0.03	100.20	0.47	0.20	5.01 ± 0.024
	10	100.30	0.90	0.30	10.03 ± 0.10	100.50	0.70	0.50	10.05 ± 0.07
	20	99.50	1.50	-0.50	19.9 ± 0.31	99.40	1.50	-0.60	19.88 ± 0.31

[a]Mean of six determinations, RSD%, percentage relative standard deviation; RE%, percentage relative error. [b]Mean ± standard error.

Specificity and interference

The proposed spectrophotometric methods have the advantages that the measurements in all of these methods are performed in the visible region, away from the UV-absorbing interfering substances that might be co-extracted from drug containing dosage forms. Regarding the interference of the excipients and additives usually presented in pharmaceutical formulation

(lactose monohydrate, starch, croscarmellose sodium, povidone, magnesium stearate and a coating containing hypromellose, titanium dioxide, lactose monohydrate and triacetin), the energy of the charge transfer (ECT) depends on the ionization potential (IP) of the donor and the electron affinity of the acceptor (EA), hence the λ_{max} values of the other π-donors mostly differ from that of the investigated compounds if they are able to form CT complexes. Preliminary experiments showed that all additives and excipients did not form CT complexes with the studied acceptors indicating the high selectivity of the proposed methods and applicability to use for routine determination in pure and in dosage forms.

Table 5. Application of the standard addition technique for the determination of MCO in dosage forms using the proposed methods.

Sample		Miconaz cream				Official method [2]	Micoban cream				Official method [2]
	Taken (µg mL^{-1})	I_2		TCNQ			I_2		TCNQ		
		Added (µg mL^{-1})	Recovery (%)[a]	Added (µg mL^{-1})	Recovery (%)[a]		Added (µg mL^{-1})	Recovery (%)[a]	Added (µg mL^{-1})	Recovery (%)[a]	
	2.0	-	100	-	99.2		-	99.3	-	98.7	
		4.0	99.4	4.0	99.8		4.0	101	4.0	99.2	
		8.0	98.9	8.0	99.5		8.0	101	8.0	99.9	
		16	98.7	16	99.7		16	99.5	16	99.7	
		20	99.9	20	100		20	101	20	101	
		24	99.8	24	99.1		24	99.1	24	101	
Mean±SD			99.5± 0.6		99.6±0.4	99.6±0.5		100±0.8		99.8±0.7	99.1±0.7
RSD%			0.57		0.44			0.75		0.74	
V			0.32		0.192			0.539		0.543	
SE			0.23		0.18			0.31		0.30	
t-value[b]			0.28		0.14			1.98		1.55	
F-value[b]			1.54		1.09			1.25		1.22	

[a]Average of six determinations. [b]The theoretical values of *t* and *F* are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom ($p = 0.05$).

Table 6. Application of the standard addition technique for the determination of FLU in Diflucan capsules using the proposed methods.

	Taken (µg mL^{-1})	DDQ		p-CLA		Official method [2]
		Added (µg mL^{-1})	Recovery[a] (%)	Added (µg mL^{-1})	Recovery[a] (%)	
	10	-	99.80	-	99.50	
		20	99.10	40	99.30	
		60	98.90	90	99.80	
		100	98.80	140	99.70	
		140	99.60	190	100.40	
		180	100.30	230	98.50	
Mean ± SD			99.41±0.58		99.53±0.63	99.30± 0.91
RSD%			0.58		0.63	
V			0.342		0.395	
SE			0.239		0.246	
t-value[b]			0.23		0.465	
F-value[b]			2.43		2.01	

[a]Average of six determinations. [b]The theoretical values of *t* and *F* are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom ($p = 0.05$).

Table 7. Application of the standard addition technique for the determination of SER in Dermofix cream using the proposed methods.

	Taken (μg mL^{-1})	Iodine		DDQ		Taken (μg mL^{-1})	p-CLA		TCNQ		Official method [2]
		Added (μg mL^{-1})	Recovery[a] (%)	Added (μg mL^{-1})	Recovery[a] (%)		Added (μg mL^{-1})	Recovery[a] (%)	Added (μg mL^{-1})	Recovery[a] (%)	
	2.0	-	99.0	-	99.3	10	-	100	-	100	
		4.0	99.5	4.0	99.8		20	98.9	20	98.7	
		8.0	98.7	8.0	98.8		50	99.0	50	101	
		12	101	12	101		80	99.2	80	99.3	
		16	99.9	16	100		110	101	110	99.4	
		18	99.5	20	101		140	99.4	140	99.3	
Mean±SD			99.5±0.7		99.9± 0.7			99.6±0.8		99.6±0.7	99.5±0.7
RSD%			0.67		0.72			0.76		0.674	
V			0.451		0.52			0.58		0.455	
SE			0.274		0.274			0.311		0.275	
t-value[b]			0.07		0.83			0.21		0.16	
F-value[b]			1.19		1.03			1.08		1.18	

[a]Average of six determinations. [b]The theoretical values of *t* and *F* are 2.57 and 5.05, respectively at confidence limit at 95% confidence level and five degrees of freedom ($p = 0.05$).

Application of the proposed methods to pharmaceutical formulations

The obtained satisfactory validation results made the proposed procedures suitable for the routine quality control analysis of the studied drugs and its pharmaceutical formulations. The results obtained by the proposed methods were statistically compared with those obtained by the official methodsfor FLU, SER and MCO [2] (Tables 5, 6 and 7). On the *t*- and F-tests, no significant differences were found between the calculated and theoretical values of both the proposed and the official methods at 95% confidence level [21]. This indicated similar precision and accuracy in the analysis of the studied drugs in their formulations. It is evident from these results that all the proposed methods are applicable to the analysis of the studied drugs in its tablets with comparable analytical performance.

CONCLUSION

The present study described the successful evaluation of some σ and π-acceptors (I_2, DDQ, *p*-CLA and TCNQ) as analytical reagents in the development of simple, rapid and accurate charge transfer spectrophotometric methods for the determination of some antifungal drugs (FLU, SER and MCO) in pure form and pharmaceutical formulations. The methods described herein have many advantages: they do not need expensive sophisticated apparatus, are direct, simple, rapid, precise and sensitive. The proposed methods used inexpensive reagents with excellent shelf life, which complied with the validation scheme of the ICH and can therefore be used for quality control and routine analysis.

REFERENCES

1. Ekiert, R.J.; Krzek, J.; Talik, P. *Talanta* **2010**, 82, 1090.
2. British Pharmacopoeia *Monographs: Medicinal and Pharmaceutical Substances*, Vol. I and II, electronic version, British Pharmacopoeia Commission Office: London, UK; **2012**.
3. Salama, N.N.E.A.; Mohamed, A.O. *Bull. Chem. Soc. Ethiop*. **2009**, 23**,** 167.
4. Ashour, S.; Kattan, N. *Int. J. Biomed. Sci*. **2010**, 6, 13.

5. De Zan, M.M.; Cámara, M.S.; Robles, J.C.; Kergaravat, S.V.; Goicoechea, H.C.; *Talanta* **2009**, 79, 762.
6. Knoth, H.; Petry, T.; Gärtner, P. *Pharmazie* **2012**, 67, 987.
7. Gil, É.S.; Cordeiro, D.D.; Matias, A.E.B.; Serrano, S.H.P. *J. Braz. Chem. Soc.* **2011**, 22, 767.
8. El-Bayoumi, A.; El-Shanawany, A.A.; El-Sadek, M.E.; Abd El-Sattar, A. *Spectrosc. Lett*. **1997**, 30, 25.
9. Alizadeh, N.; Ansari, R.; Rezaee, N.K. *J. Pharm. Health Sci*. **2012**, 1, 45.
10. Reddy, C.M.B. *Int. J. Sci. Eng. Res*. **2012**, 3, 1.
11. Pandey, S.; Pandey, P.; Dubey, S.; Chaturvedi, U.; Rai, A.K. *Thai J. Pharm. Sci.* **2012**, 36, 55.
12. Singh, A.; Sharma, P.K.; Majumdar, D.K. *Indian J. Chem. Techn*. **2012**, 18, 357.
13. Alizadeh, N.; Rezakhani, Z. *J. Chil. Chem. Soc*. **2012**, 57, 1104.
14. Kadam, S.R.; Janjale, M.V.; Akole, S.B.; Bhosale, S.S. *Int. J. Pharm. Biolog. Arch*. **2012**, 3, 383.
15. Foster, R. *Organic Charge-Transfer Complexes*, Academic Press: London; **1969**; p 470.
16. Gouda, A.A.; EL-Sheikh, R.; Amin, A.S. *Chem. Pharm. Bull*. **2008**, 56, 34.
17. Gouda, A.A. *Talanta* **2009**, 80, 151.
18. Amin, A.S.; Gouda, A.A.; El Sheikh, R.; Youssef, E.H. *Bull. Chem. Soc. Ethiop.* **2015**, 29, 187.
19. Job, P. *Advanced Physicochemical Experiments*, 2nd ed., Oliner and Boyd: Edinburgh; **1964**; p 54.
20. International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology, Q2(R1), *Complementary Guideline on Methodology*, London; November **2005**.
21. Miller, J.N.; Miller, J.C. *Statistics and Chemometrics for Analytical Chemistry*, 5th ed., Prentice Prentice Hall: New York; **2005**; p 202.
22. Taha, A.; Rücker, G. *Arch. Pharm. (Weinheim)* **1977**, 310, 485.
23. Refat, M.S.; Saad, H.A.; Adam, A.A. *Spectochim. Acta. A* **2015**, 141, 202.
24. Adam A.A., Refat M.S., Saad H.A., *J. Mol. Struct.***2013**, 1037, 376.
25. Refat, M.S.; Adam, A.A.; Saad, H.A. *J. Mol. Struct.* **2015**, 1085, 178.
26. Refat, M.S.; Ibrahim, O.B.; Saad, H.A.; Adam, A.A. *J. Mol. Struct.* **2014**, 1064, 58.
27. Refat, M.S.; Ismail, L.A.; Adam, A.A. *Spectochim. Acta. A* **2015**, 134, 288.
28. Gutmann, V. *The Donor-Acceptor Concepts of Molecular Interactions*, Plenum: New York; **1978**.
29. Cannors, K.A.A. *Textbook of Pharmaceutical Analysis*, 3rd ed., John Wiley and Sons: New York; **1982**; p 50.
30. Benesi, H.A.; Hildebrand, J.H. *J. Amer. Chem. Soc.***1949,** 71, 2703.