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طريقة قياس طيفي محققة لتعيين بيفونازول في المستحضرات الصيدلانية بواسطة معقدات انتقال الشحنة

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الملخص:

لقد تم تطوير طريقتين طيفيتين بسيطتين لتعيين الدواء بيفونازول في شكليه النقي ومستحضره الصيدلاني. هذه الطرق الطيفية المطورة تعتمد على تفاعلات انتقال الشحنة لمعقدات البيفونازول مع 5,2-ثنائي كلورو-6,3-ثنائي هيدروكسي-4,1-بنزوكوينون (CAA) للطريقة أ و 3,2-ثنائي كلورو-6,5-ثنائي سيانو-4,1-بنزوكوينون (DDQ) للطريقة ب، نتج عنها تكون معقد ملون. تم تعيين كمية نواتج التفاعل الملونة طيفيا عند 517 نانوميتر و457 نانوميتر لكل من معقدات بيفونازول-CAA و بيفونازول-DDQ على التوالي. المعقدات اتبعت قانون بيير عند المدى التراكيزي من 5.0-50.00 ميكروغرام/ملتر و 50.00-400.00 ميكروغرام/ملتر وامتصاصية مولارية عند 0.0956×10^4 ليتر/مول-سم وعند 0.6953×10^4 ليتر/مول سم للطريقة أ وللطريقة ب على التوالي. لقد تم تطبيق هذا النهج في التعيين الكمي للبيفونازول في شكل مستحضراته الصيدلانية. وقد تم كذلك تخليق معقدات انتقال شحنة البيفونازول مع كل من كشاف مستقبل-π وتم تشخيصها بواسطة الأشعة تحت الحمراء باستخدام مطيافية تحويلات فوريير.

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ORIGINAL ARTICLE

Validated spectrophotometric methods for the determination of bifonazole in pharmaceuticals by charge transfer complexation

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KEYWORDS

Bifonazole;
Charge transfer complexation;
2,5-Dichloro-3,6-dihydroxy-1,4-benzoquinone;
2,3-Dichloro-5,6-dicyano-1,4-benzoquinone;
Spectrophotometry;
FT-IR characterization

Abstract Two simple and selective visible spectrophotometric methods were developed for assay of bifonazole in pure drug and in its pharmaceutical formulation. The developed methods were based on the charge transfer complexation reaction of bifonazole with 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (CAA) for method A and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) for method B, resulting in the formation of colored complex. The colored reaction products were quantitated spectrophotometrically at 517 nm and 457 nm for bifonazole-CAA and bifonazole-DDQ complexes, respectively. The complexes obeyed Beer's law in the concentration range of 50.00–400.00 and 5.00–50.00 μg mL^{-1} with molar absorptivities at 0.0956×10^4 and 0.6953×10^4 L mol^{-1} cm^{-1} for method A and method B, respectively. The proposed procedures were successfully applied for the quantitative determination of bifonazole in its pharmaceutical formulation. The solid charge transfer complexes of bifonazole with each of the π-acceptor reagent were also synthesized and characterized by FT-IR spectroscopy.

1. Introduction

Bifonazole is a substituted imidazole analogue (Lackner and Clissold, 1989), chemically known as 1-[(1,1′-biphenyl)-4-ylphenylmethyl]-1*H*-imidazole (Fig. 1). It is a potent antifungal agent which suppresses the proliferation of dermatophytes, yeasts and fungi affecting the skin and nails (Lindsay et al., 2010). It exerts its action by blocking the fungal ergosterol biosynthetic pathway and also possesses additional inhibition of terpenoid biosynthesis (Betty et al., 2011). Due to its high efficacy and the selective interference with metabolism pathway of fungi and yeasts, it has become one of the therapeutic choices for the treatment of invasive mucosal infections. Bifonazole is available as 1% topical cream, powder and lotion (Adriana, 2010).

Literature survey reveals that several analytical techniques have been developed for the quantitative analysis of bifonazole. These techniques include high performance liquid chromatography (Ferreyra and Ortiz, 2005; Imran and Zahid, 2013; Di Pietra et al., 1992; Čudina et al., 2005), derivative spectrophotometry (Popović et al., 2003; Sayad and Imran, 2013; Ekiert and Krzek, 2009; Bonazzi et al., 1998) and extractive

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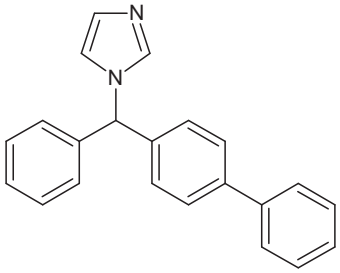

Figure 1 Chemical structure of bifonazole.

visible spectrophotometry (Vladimirov et al., 1993) for the assay of bifonazole.

Reported visible spectrophotometric method involves determination of bifonazole using bromophenol blue in the presence of citrate buffer at 414 nm. Unfortunately, this method suffers a major setback such as involving extraction procedures of ion-pair associates with an organic solvent. In the present study, an attempt is made to develop a simple and sensitive visible spectrophotometric method using charge transfer reagents. The suggested charge transfer complexation methods are highly advantageous over the other reported analytical methods as the complexes are formed instantaneously which permits the rapid quantification of bifonazole and moreover, these methods do not involve any critical experimental conditions. Thus, the proposed methods are successfully validated according to ICH guidelines and applied for the determination of bifonazole in bulk drug and in their formulation. The spectral characteristics of the charge transfer complexes are also included in the present investigation.

2. Experimental

2.1. Instrumentation

Spectrophotometric measurements were carried out using SHIMADZU UV-2550 double beam spectrophotometer (Shimadzu Corporation, Japan) with 1 cm matched quartz cells. The infrared spectrum of the complexes was recorded using KBr discs on SHIMADZU FT-IR-Prestige-21 spectrometer.

2.2. Materials

Pharmaceutical grade bifonazole drug was provided by CAD Pharma Inc., Bangalore, India. Mycospor® gel (product of Bayer Pharmaceuticals Pvt. Ltd., India) labeled to contain 10 mg of bifonazole per gram of the gel was purchased from commercial source. Reagents and solvents such as acetonitrile, methanol, acetone and 1,4-dioxane were purchased from Spectrochem Pvt. Ltd., India.

2.3. Reagents and standards

All the reagents used in the present investigation were of analytical grade. Solution of 0.1% (w/v) CAA was prepared by dissolving 0.1 g in 100 mL 1,4-dioxane. Solution of 0.1% (w/v) DDQ was prepared by dissolving 0.1 g in 100 mL acetonitrile.

A standard stock solution equivalent to 1000 μg mL^{-1} was prepared by accurately weighing 100 mg of the drug and dissolving in 100 mL of 1,4-dioxane and acetonitrile for method A and method B, respectively. The solutions were diluted approximately to get working concentrations. All the reagents were freshly prepared in their respective solvents.

2.4. Procedure for the determination of bifonazole

2.4.1. Method A

Accurately measured aliquots (50.00–400.00 μg mL^{-1}) were transferred into 10 mL calibrated volumetric flasks. Then, 2 mL of 0.1% CAA was added to each flask. The reaction was allowed to proceed at room temperature for 5 min after which it was diluted to 10 mL with the same solvent. The absorbance of the resulting solution was measured at 517 nm against the corresponding reagent blank.

2.4.2. Method B

Accurately measured aliquots (5.00–50.00 μg mL^{-1}) were transferred into 10 mL calibrated volumetric flasks. Then, 1 mL of 0.1% DDQ was added to each flask and kept aside for 10 min and the volume was made up to the mark with same solvent. The absorbance of the resulting solution was measured at 457 nm against the corresponding reagent blank.

2.5. Determination of stoichiometry of the complexes

The stock solutions of equimolar concentrations of bifonazole, CAA and DDQ were prepared in their respective solvents as mentioned above. Different complimentary proportions (0.5:4.5, 1.5:3.5. . .4.5:0.5) of the drug and each of the π-acceptor were transferred into a series of 10 mL calibrated flasks. The solutions were diluted up to the mark with their respective solvents and analyzed according to the procedure described under Section 2.4. The absorbance of resulting solutions was measured at their wavelength of maximum absorption against a reagent blank treated similarly.

2.6. Analysis of pharmaceutical formulations

An amount of gel equivalent to 10 mg of bifonazole was dissolved in 50 mL 1,4-dioxane and acetonitrile for methods A

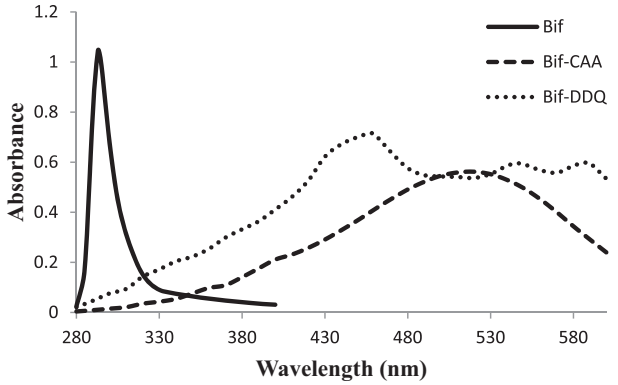

Figure 2 Absorption spectra of bifonazole (1000 μg mL^{-1}), bifonazole-CAA (400 μg mL^{-1}), bifonazole-DDQ (50 μg mL^{-1}).

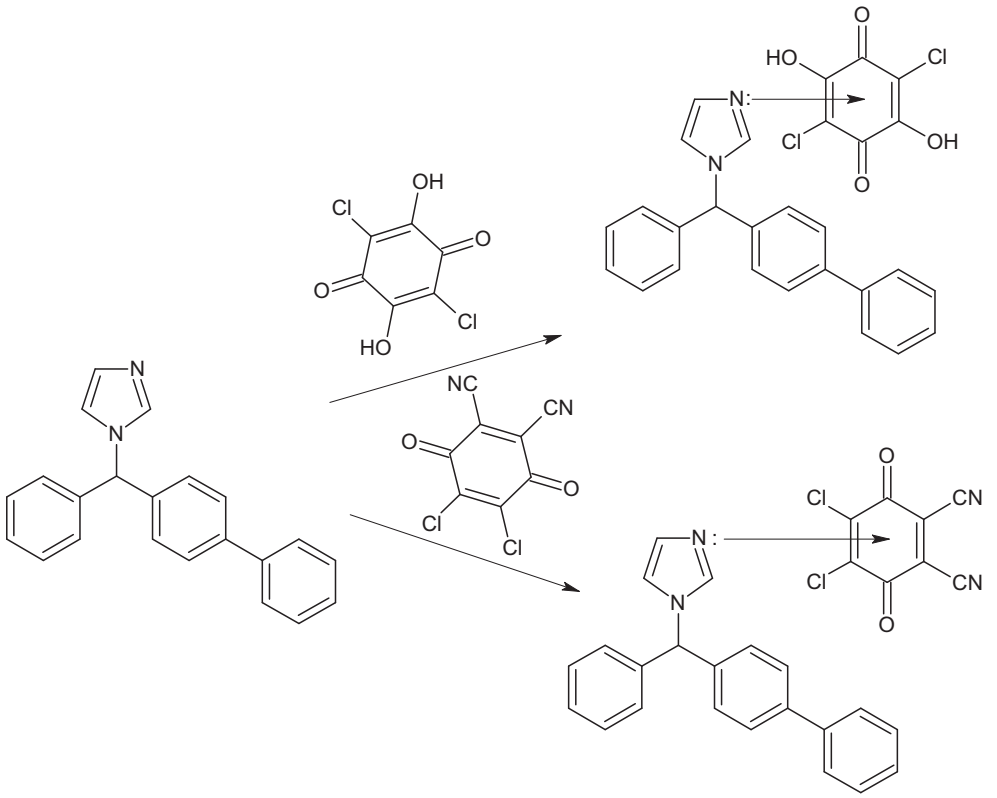

Figure 3 Reaction of bifonazole with CAA and DDQ.

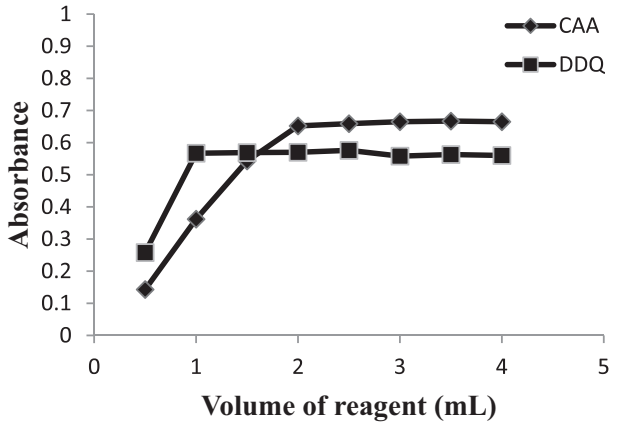

Figure 4 Effect of volumes of the reagent on the reaction of bifonazole (400 μg mL^{-1} and 50 μg mL^{-1}) with CAA and DDQ, respectively.

and B, respectively. The solutions were then filtered through Whatmann No. 40 filter paper. The filtrates were transferred into two separate 100 mL volumetric flasks and diluted up to the mark with respective solvents. A suitable aliquot from the resulting solution was then subjected to analysis by the proposed methods.

2.7. Synthesis of solid charge transfer complexes

The solid charge transfer complexes (1:1) were synthesized by mixing a saturated solution of bifonazole (0.5 mmol, 0.1551 g) and saturated solution of CAA (0.5 mmol, 0.1044 g) or DDQ (0.5 mmol, 0.1335 g) in two separate conical flasks. The mixtures were stirred for about 45 min and then filtered to avoid further contamination with residual reagents. The solutions were kept aside for few hours to obtain the solid reaction products. The products were then isolated and washed with minimal amounts of acetonitrile and dried.

3. Results and discussion

The interaction of basic drugs with charge transfer agents in particular solvent, gives colored product and color formation

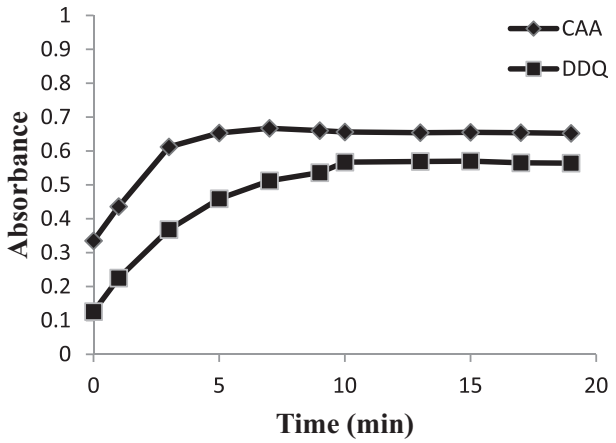

Figure 5 Effect of time on the reaction of bifonazole (400 μg mL^{-1} and 50 μg mL^{-1}) with CAA and DDQ, respectively.

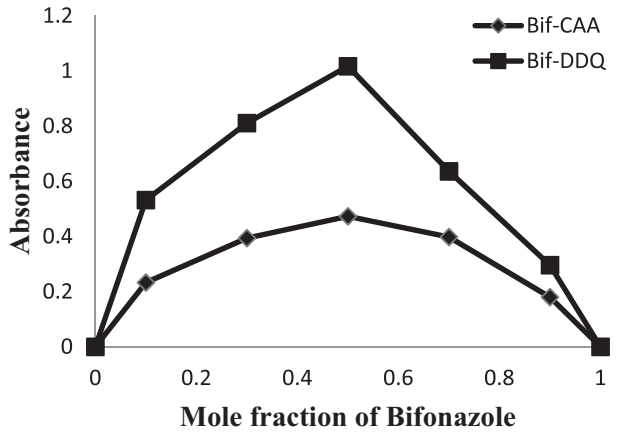

Figure 6 Continuous variation plots for bifonazole-CAA and bifonazole-DDQ complexes.

Table 1 Validation parameters for the determination of bifonazole.

Parameters	Method A	Method B
λ_{max} (nm)	517	457
Beer's law limits (μg mL^{-1})	50–400	5–50
Molar absorptivity (L mol^{-1} cm^{-1})	0.0956×10^4	0.6953×10^4
Sandell sensitivity (μg cm^{-2})	0.3246	0.0446
Limit of Detection[a] (μg mL^{-1})	0.9428	0.8839
Limit of Quantification[a] (μg mL^{-1})	2.8571	2.6785
Regression Equation[b]	Y = a + bX	Y = a + bX
Slope (b)	0.0014	0.0112
Intercept (a)	0.0696	0.0664
Correlation coefficient (r)	0.9973	0.9988

[a] Limit of detection and quantification calculated according to ICH guidelines.
[b] Y is the absorbance and X concentration in μg mL^{-1}.

is attributed to the dissociation of ion-pair salt into radical anions (Mahrous et al., 1986). The absorption spectrum of bifonazole is recorded at 293 nm which shows a bathochromic shift when it reacts with charge transfer reagents (Fig. 2). The nitrogen atom present in the imidazole ring of the bifonazole drug serves as n-electron donor, donating electrons to π-acceptors like CAA and DDQ, resulting in the formation of charge transfer complexes. The dissociation of these charge transfer complexes results in intensely colored radical ions, intense violet for bifonazole-CAA complex and deep red for bifonazole-DDQ complex which gives maximum absorbance at 517 nm and 457 nm for method A and method B, respectively. The proposed mechanism for the formation of complex is illustrated in Fig. 3.

3.1. Optimization of methods

3.1.1. Effect of solvent

Different solvents like ethanol, methanol, acetone, acetonitrile, 1,4-dioxane have been tried for the charge transfer reaction in the present study. For method A, 1,4-dioxane is best suited as it leads to the formation of stable complex and also gives lower experimental error. For method B, maximum color intensity is obtained when acetonitrile is used as the solvent. Thus, the same solvents 1,4-dioxane and acetonitrile are considered to be the ideal diluting solvents for method A and method B, respectively.

3.1.2. Effect of reagent volume

It is found that increasing the reagent concentration increases the absorbance of the colored complex. However, 2 mL of 0.1% CAA for method A and 1 mL of 0.1% DDQ for method B are found to be sufficient for attainment of maximum and reproducible color intensity (Fig. 4).

3.1.3. Effect of reaction time

The optimum reaction time is determined by following the absorbance of the color developed with respect to different time intervals (Fig. 5). In method A, complete color develop-

Table 3 Recovery of bifonazole in the presence of excipients.

Excipient	Mean recovery (%)	
	Bifonazole-CAA	Bifonazole-DDQ
Benzyl alcohol	99.45	99.99
Methyl paraben	100.77	99.92
Isopropyl alcohol	99.57	100.18
Polyethylene glycol	100.84	100.26
Glycerin	101.03	100.25

Table 2 Evaluation of accuracy and precision of the proposed methods.

Amount taken (μg mL^{-1})	Amount found[a] (μg mL^{-1})	RE[b] (%)	SD[b] (μg mL^{-1})	RSD[b] (%)	Recovery (%)
Method A					
50	50.33	0.64	0.60	1.21	100.64
150	150.32	0.21	0.99	0.65	100.21
200	200.48	0.24	1.49	0.74	100.24
Method B					
15	15.02	0.16	0.09	0.61	100.16
25	25.23	0.90	0.31	1.26	100.90
35	35.19	0.56	0.15	0.44	100.56

[a] Mean value of five determinations.
[b] RE – relative error; SD – standard deviation; RSD – relative standard deviation.

Table 4 Statistical results of the assay of formulation by proposed methods.

Brand name	Labeled amount (mg)	Amount found[a] ± SD	
		Method A	Method B
Mycospor	10	10.09 ± 1.98 t test[b] = 0.35 % Rec[c] = 100.90	10.07 ± 0.38 t test[b] = 0.41 % Rec[c] = 100.70

[a] Mean value of five determinations.
[b] Theoretical *t* value at 95% confidence level is 2.7.
[c] Recovery.

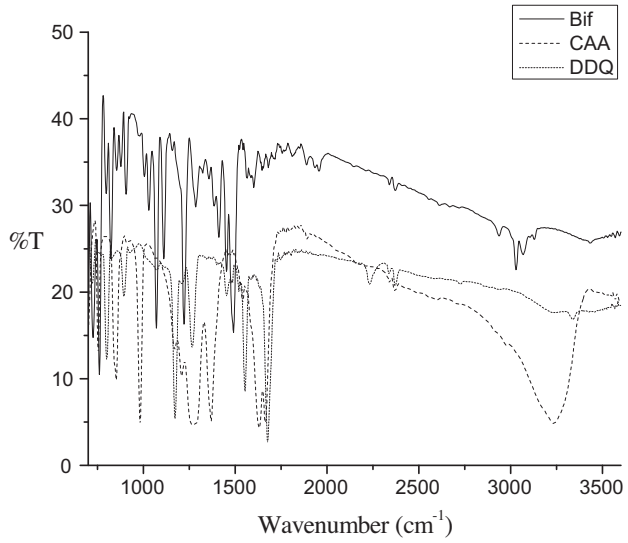

Figure 7 Infrared spectra of bifonazole, CAA and DDQ.

ment is attained after 5 min and the complex remains stable for 5 h at room temperature. In method B, it is found that complete color development is attained after 10 min and complex remains stable for 2 h at room temperature.

3.2. Stoichiometry of the reaction

The composition of the complexes formed, is determined by employing Job's method of continuous variation (James, 2013). A molar ratio of 1:1 is obtained from the reaction of equimolar solutions of bifonazole and each of the π-acceptor reagents. The stoichiometric ratio of drug: reagent is illustrated in Fig. 6.

Table 5 The characteristic peaks of bifonazole and the acceptors.

Name of compound	Characteristic peaks (cm^{-1})	Corresponding functional groups
Bifonazole	3028	Ar–H
	1490	–C═N–
CAA	754	C–Cl
	1664	1,4-quinone (C═O)
	3234	–OH
DDQ	721	C–Cl
	2231	–C≡N–
	1676	1,4-quinone (C═O)

3.3. Method validation

3.3.1. Linearity

Under the described experimental conditions for both the methods, calibration graphs are obtained by plotting the measured absorbance versus varying concentration of the drug and least regression analysis was carried out for getting the values of correlation coefficients. The proposed methods adhere to Beer's law and linear plots with low intercept and good correlation coefficients are obtained in the concentration ranges of 50.00–400.00 and 5.00–50.00 $\mu g\,mL^{-1}$ for determination of bifonazole with CAA and DDQ, respectively. Other statistical parameters, namely intercept (b), slope (a), molar absorptivity and sandell's sensitivity values are calculated as well and given in Table 1.

3.3.2. Limits of detection and quantification

The limits of detection (LOD) and the limits of quantification (LOQ) for the proposed methods are calculated according to International Conference on Harmonization (ICH) recommendations (ICH, 1996) and the values are given in Table 1. The slope of the calibration curve and standard deviation of five reagent blank responses are used for the calculation of detection and quantification limits.

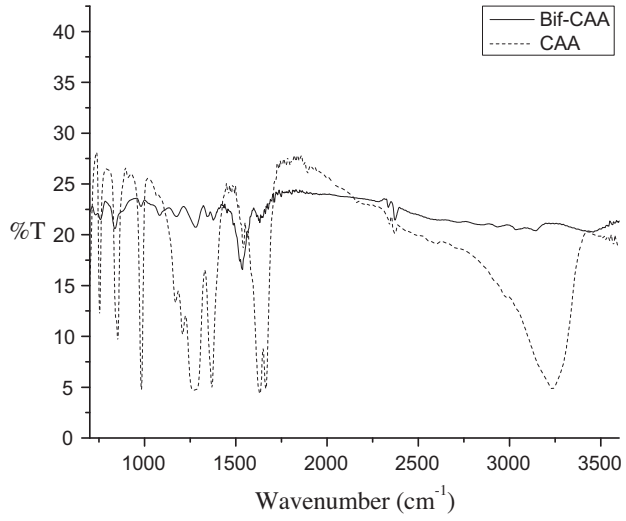

Figure 8 Infrared spectra of CAA and bifonazole-CAA complex.

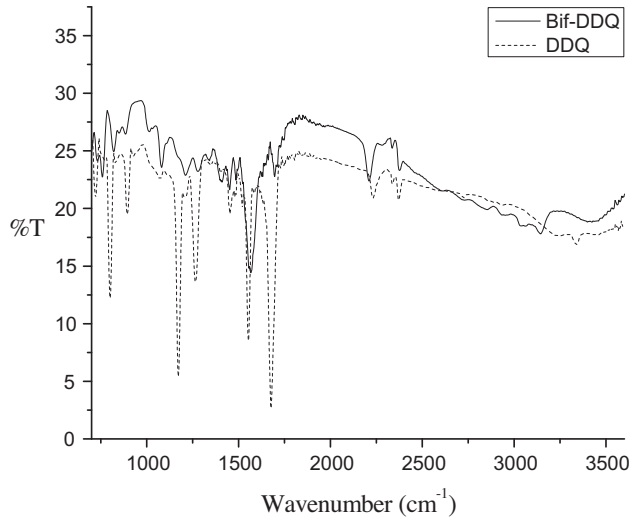

Figure 9 Infrared spectra of DDQ and bifonazole-DDQ complex.

3.3.3. Accuracy and precision

The accuracy and precision of the methods are determined by preparing solutions of different concentrations of bifonazole and analyzing them in five replicates. The precision of the proposed methods are evaluated as percentage relative standard deviation (RSD%) and accuracy as percentage relative error (RE%) and the values are given in Table 2. The RSD values are in the range of 0.65–1.21% and 0.44–1.26% and RE values are in the range of 0.21–0.64% and 0.16–0.90% for method A and method B, respectively. The results obtained in the present study are satisfactory which suggests that the proposed methods are accurate and precise.

3.3.4. Specificity

The effect of common excipients added as additives in the formulation is experimentally studied. In this approach, a known concentration of bifonazole is prepared and it is spiked with three different concentrations of additives such as benzyl alcohol, methyl paraben, isopropyl alcohol, polyethylene glycol and glycerin and the absorbance of the resulting solutions is recorded. The mean percentage recovery values given in Table 3 indicate no potential interference from the excipients, which confirms the specificity of the proposed methods.

3.4. Application to analysis of formulation

The proposed methods are applied to the determination of bifonazole in pharmaceutical dosage forms and the results obtained are given in Table 4. The statistical values of the t-test obtained at 95% confidence level, indicate that the suggested charge transfer complexation methods are reliable and accurate. The high percentage recovery value shows that these methods have the advantage of being potentially free from excipients.

3.5. Characterization of reaction products

The infrared spectrum of bifonazole and the π-acceptors (Fig. 7) with their characteristic peaks corresponding to various functional groups (Williams and Fleming, 2011) are given in Table 5. The formation of complexes is ascertained by disappearance or reduction in the intensity of characteristic peaks of π-acceptors and also by observing the considerable shifts in the frequency of infrared bands of the donor and the acceptor (Sharma et al., 2012). The infrared spectrum of bifonazole charge transfer complexes is depicted in Figs. 8 and 9 and the FT-IR data with their band assignments are given in Table 6. The infrared spectrum of charge transfer complexes closely resembles to the spectra of both drug and reagent but reveals shift in the wave numbers compared to those of the free reactants. These shifts in the values can be attributed to changes in the electronic structures and molecular symmetries of the reactants upon complex formation (Refat et al., 2010). The infrared spectrum of bifonazole exhibits characteristic peak of C═N at 1490 cm^{-1} which shows a significant shift to 1535 cm^{-1} and 1566 cm^{-1} in the infrared spectrum of bifonazole-CAA and bifonazole-DDQ complex respectively, confirming that the charge transfer interaction has occurred at –C═N– site of bifonazole.

The infrared spectra of CAA report to give peaks at 3234 cm^{-1},1664 cm^{-1},1631 cm^{-1}, 1369 cm^{-1} and 754 cm^{-1} corresponding to –OH, C═O, C═C, C–O and C–Cl stretching frequencies, respectively which correlates with the previously reported IR of CAA (Refat et al., 2010; Pawlukojć et al., 2003). In the IR spectrum of bifonazole-CAA complex, the –OH peak of CAA shifts to 3458 cm^{-1} followed by the shift in C═O, C═C, C–O and C–Cl to 1645 cm^{-1}, 1627 cm^{-1}, 1379 cm^{-1} and 759 cm^{-1} respectively.

Table 6 Infrared frequencies[a] (cm^{-1}) and their assignments[b] for CAA, DDQ, bifonazole-CAA and bifonazole-DDQ complex.

Bifonazole	CAA	DDQ	Bifonazole-CAA	Bifonazole-DDQ	Assignments
	3234 s,br		3458 w		ν (–OH)
3034 ms			3143 ms	3143 ms	ν (Ar–H)
		2231 ms		2210 ms	ν (C≡N)
	1664 vs	1676 vs	1645 ms	1697w	ν (C═O)
	1631 vs		1627 ms		ν (C═C)
1490 s			1535 s	1564 s	ν (C═N)
	1369 s		1379 w		ν (C–O)
	1265 vs		1278 ms		∂ (O–H)
	981 vs		985 w		∂ (C–H)
	754 ms	721 ms	759 ms	759 ms	ν (C–Cl)

[a] ν – stretching; ∂ – bending.
[b] s – strong, w – weak, v – very, br – broad.

The infrared spectra of DDQ report to give peaks at 2231 cm^{-1}, 1676 cm^{-1} and 721 cm^{-1} corresponding to –C≡N–, C=O, and C–Cl stretching frequencies respectively which coincides with the previously reported IR of DDQ (El-Habeeb et al., 2013; Shahada et al., 2009). Upon complexation, there appears to be a significant shift of –C≡N–, C=O and C–Cl to 2210 cm^{-1}, 1696 cm^{-1} and 759 cm^{-1} respectively.

4. Conclusion

The proposed methods are simple, rapid, accurate and precise and also employ inexpensive reagents that are available in any laboratory. The reliable validation data obtained indicate that the proposed procedures could be successfully applied for the determination of bifonazole in bulk and formulation. Hence, the suggested charge transfer spectrophotometric methods are economical and practical for routine analysis of bifonazole in quality control laboratories. In addition, the spectroscopic characterizations of the synthesized bifonazole charge transfer complexes have also been described.

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