Spectrophotometric Determination of Albendazole Drug in Tablets: Spectroscopic Characterization of the Charge-transfer Solid Complexes

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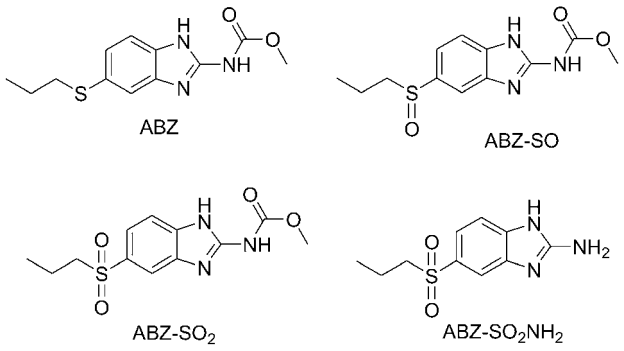
Simple, rapid and reliable method for the determination of albendazole (ABZ) was described. This includes the utility of some π-acceptors such as 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) and 3,6-dichloro-2,5-dihydroxy-*p*-benzoquinone (*p*-CLA) for estimation of ABZ drug (act as donor). The experimental conditions were optimized and the system obeys Beer's law for 7.50—80 and 10.00—85.00 μg•mL^{-1} of ABZ using DDQ and *p*-CLA, respectively. The molar absorptivity and Sandell sensitivity were calculated to be 1.83×10^3 and 1.12×10^3 L•mol^{-1}•cm^{-1}, and 2.60 and 3.40 ng•cm^{-2} using DDQ and *p*-CLA, respectively. The limits of detection and quantification were calculated to be (7.42 and 6.73) and (9.94 and 4.13) μg•mL^{-1} using DDQ and *p*-CLA, respectively. The proposed methods were successfully applied to the determination of ABZ in commercially available dosage forms. The reliability of the assays was established by parallel determination by the official method and recovery studies. The chemical structures of the solid charge-transfer (CT) complexes formed via reaction between ABZ under study and π-acceptors, have been elucidated using elemental analyses (C, H and N), IR, ^{1}H NMR and mass spectra.

Keywords albendazole, DDQ, *p*-CLA, spectrophotometry, charge-transfer complexes

Introduction

Albendazole (ABZ, Scheme 1), methyl-[(5-propylthio)-1*H*-benzimidazol-2-yl] carbamate, is a broad-spectrum anthelmintic agent active against the most common helminth parasites and is widely used for treatment of veterinary and human helminthiasis.[1] ABZ is a pro-drug and requires biotransformation via both cytochrome P450s (CYP) and flavin-containing monoxygenases (FMO) in order to exert its cytotoxic activity.[2] After administration, ABZ undergoes extensive metabolism to its therapeutically active metabolite, ABZ sulphoxide (ABZ-SO).[3,4] Further ABZ-SO is oxidized to ABZ sulphone (ABZ-SO_2),[3,5] in a process catalyzed by cytochrome P450 and, finally, to ABZ 2-aminosulphone (ABZ-SO_2NH_2), the *N*-deacetylation product of ABZ sulphone.[6] Of all three metabolites, pharmacokinetics studies indicate that ABZ-SO exhibit anthelmintic activity[7] and toxic effects,[8] whereas ABZ-SO_2 and ABZ-SO_2NH_2 are considered biologically inactive.[8,9] Chemical structures of ABZ and its metabolites are displayed in Scheme 1. ABZ-SO has a stereogenic center at the sulphur atom existing as two enantiomers, (+)-ABZ-SO and (−)-ABZSO. Clinical studies have demonstrated that (+)-sulphoxide is the predominant form in plasma of human and animal species.[10]

Scheme 1 Chemical structures of ABZ, ABZ-SO, ABZ-SO_2 and ABZ-SO_2NH_2



Thus, it is important that clinical studies include an enantioselective determination of the disposition of (+)- and (−)-ABZ-SO, as well as quantification of ABZ-SO_2 and ABZ-SO_2NH_2, which may provide additional information about the overall metabolism. Furthermore, pharmacokinetic profiles of benzimidazoles in biological fluids have become increasingly important in order to evaluate the maximum efficacy of existing anthelmintic drugs. One example of this trend is the

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direct administration of racemic ABZ-SO to animals, marketed as ricobendazole (RBZ). The slightly greater solubility in water of (±)-ABZ-SO than that of the parent drug has allowed the development of an injectable aqueous solution for animals. In Brazil, Ricobendazole from OuroFino Saú de Animal and Ricover from VetBrands Saú de Animal are examples of injectable products for cattle. A number of liquid chromatography methods for analyzing benzimidazoles in human[11,12] and sheep plasma,[13,14] human serum,[15] spermatozoa and seminal plasma,[16] animal tissue,[17] parasite animal[18] and bovine milk[19] have been developed using a chiral or chiral columns[10,14,20-23] and different compositions of mobile phases. Prior to chromatographic analysis, most of these methods used liquid-liquid extraction as a common approach to sample clean-up, usually with aqueous extraction at high pH with partitioning into an immiscible organic solvent.[15,16] Some sample pre-treatments were based on matrix solid-phase dispersion (MSPD),[24] supercritical fluid extraction[25] and multiple stages of liquid-liquid extraction and/or solid-phase extraction clean-ups.[10,11,13,18,26] In addition to these labour-intensive and time-consuming sample treatments, in the enantioselective assays chiral analysis of (±)-ABZ-SO was performed by an indirect method, where the (±)-ABZ-SO fractions were collected, evaporated to dryness and re-chromatographed using a chiral stationary phase, except for studies assayed on Chiralpak AD® column in normal elution mode.[27-30]

In the present investigation, DDQ and *p*-CLA reagents are utilized as π-acceptors for the spectrophotometric determination of ABZ drug. The main task of this study is to find fast, cheep, accurate and sensitive spectrophotometric method for the determination of the drug under investigation in raw materials and in some commercial pharmaceutical preparations. Different experimental conditions are carried out in order to select the optimum conditions suitable for CT complexes formation and hence quantitative determination of ABZ drug. Statistical treatment of the data obtained, like SD, RSD, Sandell sensitivity, ε, relative error, t- and F-tests are also made. Also the solid CT complexes were separated and characterized using elemental analyses, IR and ^{1}H NMR spectra.

Experimental

Materials

All chemicals and reagents were of analytical reagent grade and all of them were used as such without any further purification. They included ABZ provided by USP 23 India. Reagents used included 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) supplied from Arcos-USA. While 2,5-dichloro-3,6-dihydroxy-1,4-benzoquinone (*p*-CLA) was supplied from BDH chemicals UK. Absolute ethanol and sodium hydroxide were supplied from ADWIC Company, while acetonitrile (AR) was supplied from Fisher chemicals and methanol was supplied from Sigma. Chloroform, acetone, 1,4-dioxane, methylene chloride, 1,2-dichloroethane and dimethyl formamide were supplied from El-Nasr Company.

The ABZ pharmaceutical preparations were bought from Alzental capsules with 200 mg/cap from EIPICO and Vermizole capsules with 200 mg/cap from Amoun Co.

Solutions

1.9×10^{-3} mol•L^{-1} ABZ solution was prepared by dissolving the accurately weighed amounts of the drug in wormed methanol. 1.0 g/L 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and 1.0 g/L 2,3-dichloro-5,6-dihydroxy-1,4-benzoquinone (*p*-CLA) reagents were prepared by dissolving 100 mg of each reagent in 100 mL of acetonitrile. All solutions must be protected from light by keeping them in a dark colored quickfit bottles during the whole work. 0.1 mol•L^{-1} NaOH solution was prepared by dissolving 400 mg of NaOH in 100 mL of methanol.

The water was always twice distilled from all glass equipments. Redistillation was carried out from alkaline permanganate solution.

Ten tablets of ABZ were accurately weighed and the average weight of one tablet was calculated. The tablets were crushed well to a fine powder. A portion of the powder equivalent to 50 mg of ABZ was dissolved in 75 mL of methanol, and then filtered on a dry filter paper in 100 mL volumetric flask. The volume was completed to the mark with methanol.

Equipments

All the absorption spectral measurements were made using the PerkinElmer automated spectrophotometer ranged from 200—900 nm with scanning speed 400 nm/min and band width 2.0 nm, equipped with 1 cm matched quartz cells.

Elemental analysis (C, H and N) was determined at the Microanalytical Center of Cairo University using CHNS-932 (LECO) Vario Elemental analyzers. Infrared measurements (KBr discs) of the isolated CT complexes were carried out on a PerkinElmer 1430 ratio recording Infrared spectrometer (400—4000 cm^{-1}). ^{1}H NMR spectra in DMSO-d_6 (200 MHz) were recorded on a Varian spectrophotometers Gemini 200 using solvent signals as a reference. The mass spectra of the CT complexes were carried out at 70 eV by using an EI-MS 30 mass spectrometer.

Procedures

General procedure 1 mL of 1.0 g/L DDQ or *p*-CLA was added. The mixtures were completed up to 10 mL with acetonitrile. The absorbances of the colored CT complexes were measured at the specific wavelengths against reagents blank prepared similarly without drugs.

Day-by-day measurements In order to prove the

validity and the applicability of the proposed method and the reproducibility of the results obtained, four replicates experiments at different concentrations of ABZ were carried out. Using the above mentioned procedures, the absorbances of the four samples were measured daily for 4 d and the results were recorded to make statistical calculations.

General procedure for pharmaceutical preparations Different concentrations of ABZ drug (10—70 μg•mL^{-1}) was added to 1 mL of 1.0 g/L DDQ or *p*-CLA reagents. The volumes were made up to the mark with acetonitrile in 10 mL of calibrated measuring flask. The absorbance was measured at λ_{max}=455 nm using DDQ reagent and at λ_{max}=500 nm using *p*-CLA reagent, against reagents blank.

Synthesis of the charge transfer complexes The solid CT complexes of ABZ drug with DDQ and *p*-CLA reagents were prepared by mixing saturated solution of the drug in chloroform (10 mL) with continuous stirring for about 1 h at room temperature. The colored complexes developed and the solution was allowed to evaporate slowly at room temperature. Colored solid complexes were formed, filtered, washed several times with little amounts of methanol, and dried under vacuum over anhydrous calcium chloride.

Results and discussion

Molecular charge-transfer complexes (CT) are of particular interest in pharmaceutical science. They can be applied as useful means in the qualitative and quantitative analysis of different pharmaceutical compounds.[31] A charge transfer complex is the name given to a stable molecular system formed in solution between an electron donating molecule, having sufficiently low ionization potential, and an electron accepting molecule having high electron affinity.

The principal feature of this type of complex formation is the appearance of a new and intense absorption bands in ultra-violet or visible region of spectrum. Absorption bands of this type are known as charge transfer bands, since they involve electronic transitions from orbital on the donor to the vacant orbital on the acceptor. Many explanations were given to the phenomenon based on quantum mechanical theory of Mülliken. The formation of molecular complexes from two aromatic molecules could arise from the transfer of an electron from a π-molecular orbital of the donor (Lewis base) to a vacant π-molecular orbital of the acceptor (Lewis acid) *i.e.* π-π* electronic interaction.[32,33]

Absorption spectra

The absorption spectra of DDQ and *p*-CLA reagents in acetonitrile solvent (Figure 1) showed no absorption bands. While, three and one maxima were found in the absorption spectra of ABZ-DDQ and ABZ-*p*-CLA CT complexes, respectively at λ=455 nm (ε^1=1.83×10^3 L•mol^{-1}•cm^{-1}), 540 nm (ε^2=1.55×10^3 L•mol^{-1}•cm^{-1}) and 580 nm (ε^3=1.54×10^3 L•mol^{-1}•cm^{-1}) and λ=500 (ε=1.12×10^3 L•mol^{-1}•cm^{-1}). The peak at λ=455 nm was selected for ABZ-DDQ CT complex because it gives the highest absorption intensity as indicated from the ε values. The polar solvents such as acetonitrile and methanol were reported to promote complete transfer of electron from a donor (D) to the π-acceptor (A), resulting in complete formation of DDQ radical anion (A^-) as a predominant chromogen.

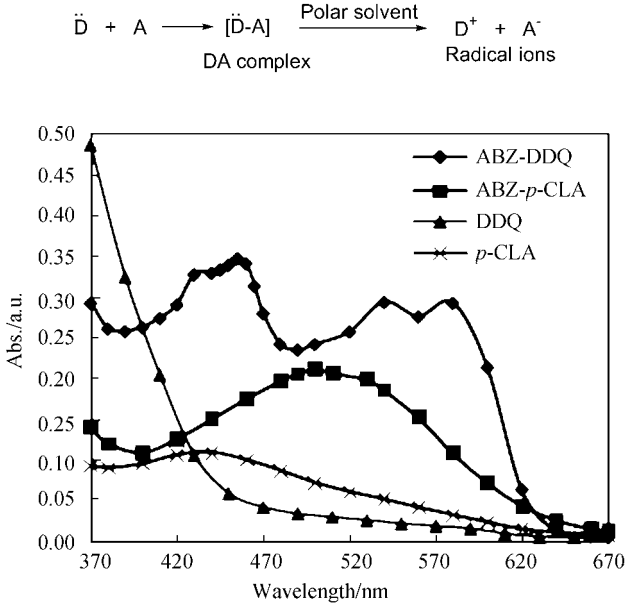

Figure 1 Absorption spectra of DDQ, *p*-CLA, ABZ-DDQ and ABZ-*p*-CLA in acetonitrile.

Effect of solvents

In order to select the suitable solvent for CT complex formation, the reaction of DDQ and *p*-CLA reagents with ABZ drug is made in different solvents. These solvents include acetonitrile, chloroform, ethanol, methanol, acetone, 1,4-dioxane, dichloromethane, 1,2-dichloroethane and dimethyl formamide. The results obtained are shown in Table 1. It is clear from these results that, dimethyl formamide, acetone or methanol is found to have the high molar absorptivity than acetonitrile. From these results it is clear that acetonitrile is considered to be an ideal solvent for the colour reaction as it offers solvent capacity and gives the highest yield of the radical anions as indicated by high ε values. This is because that it possesses the high dielectric constant of all solvents examined; a property which is known to promote the dissociation of the original CT complex to radical ions *i.e.* the dissociation of donor-acceptor complex is promoted by the high ionizing power of the solvent.

Effect of reagents concentration

Figure 2 shows the effect of DDQ and *p*-CLA (1.0 g/L) reagents concentrations on the quantitativeness of their reactions with ABZ drug. It is found that, when

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Table 1 Absorbance and molar absorptivity values of ABZ-DDQ and ABZ-*p*-CLA CT complexes in different solvents

Solvent	DDA[a]		*p*-CLA[b]	
	Abs./a.u.	$\varepsilon^c/10^3$	Abs./a.u.	$\varepsilon^c/10^3$
Acetonitrile	0.398	2.10	0.227	1.20
Methanol	0.276	1.45	0.234	1.23
Ethanol	0.337	1.77	0.221	1.16
Acetone	0.415	2.18	0.258	1.36
Dichloromethane	0.158	0.83	0.152	0.80
1,2-Dichloroethane	0.185	0.97	0.167	0.88
DMF	0.553	2.91	0.369	1.94
Chloroform	0.116	0.61	0.136	0.72
1,4-Dioxane	0.107	0.56	0.086	0.45

[a]λ=455 nm; [b]λ=500 nm, [c] The unit of ε is L•mol^{-1}•cm^{-1}.

various concentrations of DDQ or *p*-CLA solutions were added to a constant concentration of ABZ, it is obvious that 1000 μg•mL^{-1} of DDQ or *p*-CLA solutions are found to be sufficient for quantitative determination of the drugs under study as mentioned. It also means that, maximum and reproducible color intensities are obtained and higher concentration of reagents does not affect the color intensity.

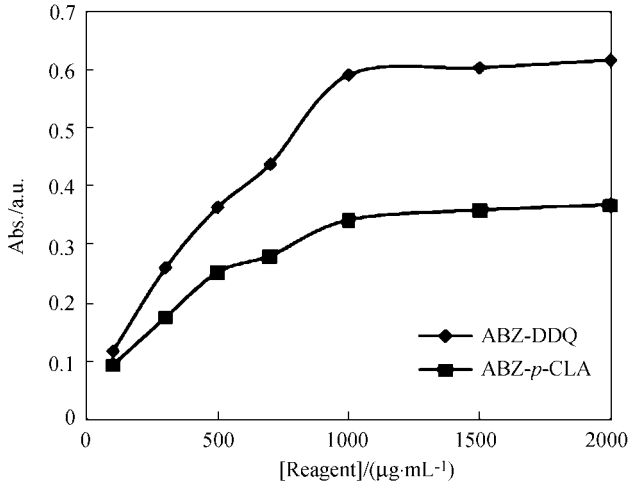

Figure 2 Effect of DDQ and *p*-CLA concentrations on the formation of ABZ CT complexes in acetonitrile.

Effect of time and temperature

The optimum reaction time and temperature are determined spectrophotometrically at different time intervals or in the temperature range from 5 to 60 ℃ and at λ_{max}=455 and 500 nm for ABZ-DDQ and ABZ-*p*-CLA CT complexes, respectively, using 50 μg•mL^{-1} drug solution. It is found that complete color development is attained after 20 and 30 min for ABZ-DDQ and ABZ-*p*-CLA CT complexes, respectively. Also the color remains stable for 1 d at least using these reagents. The effect of time on the reaction of this drug under study with DDQ and *p*-CLA reagents was performed in acetonitrile. It is seen from these figures that although acetonitrile has the low absorbance values as observed in studying solvent effect, the absorbance readings are more stable and reproducible, a fact which encourages us to complete our work in acetonitrile rather than dimethyl formamide, acetone or ethanol.

It is shown from the given results that the absorbance attains a maximum color at temperature (25±1) ℃ for ABZ-DDQ and ABZ-*p*-CLA CT complexes, respectively. The colors of the CT complexes are remained constant for at least 24 h.

Stoichiometry of the CT complexes

Molar ratio and Job's continuous variation methods[34.35] are applied in order to determine the suitable ratio between ABZ drug and DDQ or *p*-CLA reagents. Figures 3 and 4 show that the interactions between this drug and reagents occur in equimolar basis, *i.e*. the two straight lines are intersected at [Drug]∶[Reagents]=1∶1. It takes place through the transfer of electron from a donor (drug) to the π-acceptor reagent (DDQ or *p*-CLA).[34]

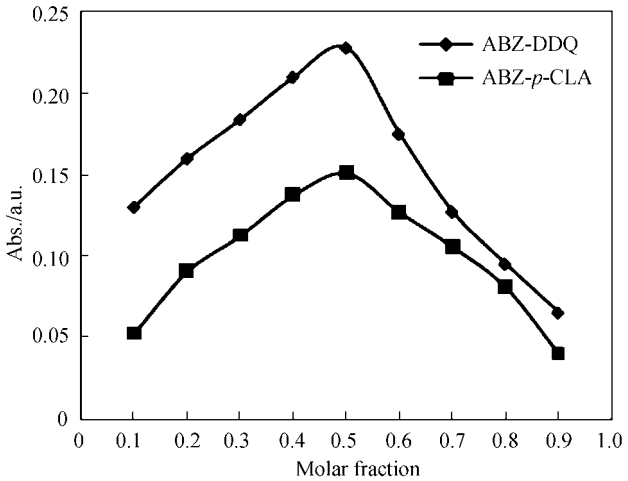

Figure 3 Job's method for ABZ CT complexes with DDQ and *p*-CLA in acetonitrile.

Validity of Beer's law

After the selection of suitable solvents, reagent concentrations, reaction time, temperature, and ratio of reactants, it is also important to know the concentration limits of ABZ drug at which these reactions are quantitative. Consequently, it is easy to apply this spectrophotometric method to determine this drug under investigation quantitatively in pharmaceutical formulations via its reaction with electron acceptor reagents like DDQ or *p*-CLA.

Table 2 shows the results of studying quantitativeness of the reaction between ABZ and DDQ or *p*-CLA reagents under selected optimum conditions. It is found that, Beer's law is valid over the concentration ranges

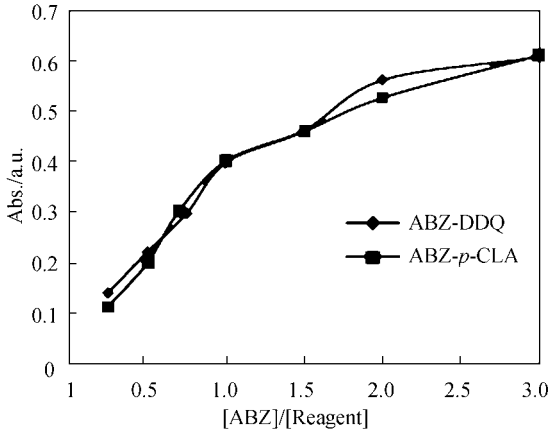

Figure 4 Molar ratio of ABZ CT complexes with DDQ and *p*-CLA in acetonitrile.

of 7.5—80 and 10—85 μg•mL^{-1} of ABZ using DDQ and *p*-CLA reagents, respectively. Table 2 shows the slope, intercept, correlation coefficient, Sandell sensitivities, molar absorptivity (ε), range of error, standard deviation, relative standard deviation, limits of detection (LOD) and quantification (LOQ). The small values of Sandell sensitivity indicate the high sensitivity of the proposed method in the determination of the drugs under investigation.

Table 2 Spectral characteristics of ABZ CT colored reaction products and the analytical characteristics (accuracy and precision) of these reactions

Parameter	DDQ method	*p*-CLA method
λ_{max}/nm	455	500
Molar absorptivity/ (L•mol^{-1}•cm^{-1})	1.83×10^3	1.12×10^3
Sandell sensitivity/ (μg•cm^{-2})	0.0026	0.0034
Beer's law limit/ (μg•mL^{-1})	7.50—80.00	10.00—85.00
Percentage recovery/%	99.07—101.2	99.40—100.4
Range of error/%	0.02—0.93	0.02—0.60
Standard deviation (SD)	0.12—0.53	0.15—0.56
Relative standard deviation (RSD)/%	0.18—0.96	0.18—0.79
Regression equation[a], slope (*b*)	0.0041	0.0023
Intercept (*a*)	0.0439	0.0277
Correlation coefficient (r^2)	0.9929	0.9868
LOD/(μg•mL^{-1})	7.42	9.94
LOQ/(μg•mL^{-1})	6.73	4.13

[a] $A=a+bC$, where C is the concentration in μg•mL^{-1}.

Four to six replicates measurements are performed at different concentrations of ABZ drug using DDQ and *p*-CLA reagents. The relative standard deviation and the range of error values are calculated and found that the small values of them indicate the high accuracy and high precision of the proposed spectrophotometric method. The low values of limits of detection (LOD) and quantification (LOQ) indicate the possibility of applying DDQ and *p*-CLA reagents in routine analysis of the drugs under investigation.

Between-day precision

In order to prove the validity and applicability of the proposed method and reproducibility of the results obtained, four replicates experiments at four concentrations of ABZ are carried out. Table 3 shows the values of the between-day relative standard deviations for different concentration of the drug, obtained from experiments carried out over 4 d. It is found that, the within day relative standard deviations are less than 1%, which indicates that the proposed method is highly reproducible, and DDQ and *p*-CLA reagents are successfully applied to determine ABZ via the charge transfer reaction.

Table 3 Between-day precision of the determination of ABZ drug using DDQ and *p*-CLA reagents under optimum conditions

Drug	[Drug]/(μg•mL^{-1})		Percentage recovery/%	SD	RSD/%
	Taken	Found[a]			
Using DDQ	15.00	14.92	99.45	0.42	2.80
	25.00	25.06	100.2	0.26	1.05
	35.00	34.76	99.32	0.28	0.80
	75.00	75.80	99.73	0.53	0.70
Using *p*-CLA	15.00	14.91	99.42	0.28	1.89
	35.00	35.00	100.0	0.21	0.60
	50.00	49.87	99.74	0.40	0.79
	75.00	75.04	100.1	0.36	0.49

[a] The average of four replicates.

Spectrophotometric micro determination of ABZ drug in different pharmaceutical preparations

The spectrophotometric micro determinations of ABZ drug via its reaction with DDQ and *p*-CLA reagents, coming from EPICO, GlaxoSmithKline, EVA Pharma and AMOUN Company are carried out. The results obtained are given in Table 4. These data show that, the determined concentrations of ABZ drug by the proposed methods are close to that obtained from the applied standard method.[36,37] In order to check the confidence and correlation between the suggested spectrophotometric procedures and the official method[36,37] for micro determination of ABZ drug, it is better to do the F- and t-tests (Table 4). The calculated F- and t-tests at the 95% confidence level do not exceed the theoretical values indicating that there is no significant difference between the proposed and official methods. The small values of SD and RSD indicate the reliability, accuracy and precision of the suggested procedures.

Characterization of charge-transfer (CT) complexes

Charge-transfer (CT) complexes formed between ABZ drug as donors with DDQ and *p*-CLA as acceptors have been isolated in solid form. The synthesis and

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Table 4 Spectrophotometric determination of ABZ drug in different pharmaceutical preparations using DDQ and official methods[a]

Sample	Taken/(μg•mL^{-1})	[Drug]/(μg•mL^{-1})		Percentage recovery/%		SD[b]	SD[c]	F-test	t-test
		p-CLA method	Official method	*p*-CLA method	Official method				
Using DDQ:ABZ	30.00	30.01	30.08	100.0	100.3	0.09	0.16	0.32	1.55
	50.00	50.05	49.96	100.1	99.92	0.11	0.14	0.62	1.63
	70.00	69.99	69.93	99.99	99.90	0.14	0. 819	0.54	0.86
D1	30.00	29.97	28.39	99.90	94.63	0.52	1.74	0.09	0.61
	50.00	50.87	53.36	101.7	106.7	0.69	1.83	0.14	0.72
	70.00	70.23	68.45	100.3	97.79	0.57	0.67	0.72	0.62
D2	30.00	29.60	29.95	98.67	99.83	0.43	1.48	0.08	1.63
	50.00	50.21	50.32	100.4	100.6	0.65	1.02	0.41	0.34
	70.00	68.39	69.14	97.70	98.77	0.95	1.29	0.54	1.58
Using *p*-CLA:ABZ	30.00	29.83	30.08	99.43	100.3	0.21	0.16	1.72	2.38
	50.00	49.86	49.96	99.72	99.92	0.37	0.14	6.01	0.54
	70.00	70.43	69.93	100.6	99.90	0.63	0.19	1.09	1.58
D1	30.00	31.19	28.39	103.9	94.63	0.82	1.74	0.22	0.68
	50.00	50.27	53.36	100.5	106.7	0.72	1.83	0.15	0.86
	70.00	70.82	68.45	101.2	97.79	0.91	0.67	1.84	0.52
D2	30.00	29.64	29.25	98.80	97.50	0.34	1.41	0.06	2.29
	50.00	49.52	51.17	99.04	102.3	0.87	1.22	0.51	0.38
	70.00	70.24	69.13	100.3	98.76	1.03	1.29	0.64	2.16

[a] No. of replicates (n)＝4; D1, Alzental tablets (200 mg/ cap.), EPICO, Cairo, Egypt; D2, Vermizole tablets (200 mg/cap.), AMOUN Co., Cairo, Egypt; Standard F-values at 95% confidence level＝6.39; Standard t-values at 95% confidence level＝2.77. [b] Standard deviation values using proposed method. [c] Standard deviation values using official method.

characterization of ABZ CT-complexes of DDQ and *p*-CLA, ABZ-DDQ and ABZ-*p*-CLA, were described. These complexes are readily prepared from the reaction of ABZ with DDQ and *p*-CLA within chloroform and/or methanol solvents. IR, ^{1}H NMR, mass spectra and elemental analyses (C, H, N) were performed to characterize the charge-transfer complexes.

Compositions and solubility of the ABZ CT-complexes Results of elemental analyses for all the ABZ, APN and SILC CT complexes are listed in Table 5. From Table 5, it can be seen that values found are in a good agreement with the calculated ones, and the composition of the CT-complexes is matched with the stoichiometry (1∶1; drug∶reagents) of the charge transfer complexes, which is examined by applying continuous variation and molar ratio methods. All the CT-complexes are insoluble in cold and hot water, but easily soluble in dimethyl formamide and dimethyl sulfoxide.

IR spectral studies The IR spectra of 1∶1 CT-complexes formed from the interaction of the donor and the respected acceptors with the general formula, ABZ-acceptor, together with the corresponding free acceptors (DDQ and *p*-CLA) and ABZ donor. Full assignments concerning all infrared bands located in the spectra are listed in Table 6.

A comparison of the relevant IR spectral bands of the free donor; ABZ, and acceptors; DDQ and *p*-CLA, with their corresponding isolated solid CT-complexes clearly indicate that the characteristic bands of ABZ show some shift in the frequencies (Table 6), as well as some change in bands intensities. This could be attributed to the expected symmetry and electronic configuration changes upon the formation of the CT-complex. The infrared explanation will take separately for each CT-complex to give an idea about the position of com-

Table 5 Elemental analysis (C, H and N) and physical parameters data of the CT-complexes formed from the reaction of the ABZ drug with DDQ and *p*-CLA reagents

Complex (Molecular formula)	M_r, found (calcd)	Elemental analysis, found (calcd)			Physical data	
		C	H	N	Color	m.p./℃
ABZ-DDQ ($C_{20}H_{15}N_5O_4SCl_2$)	477.0 (492.3)	48.55 (48.75)	2.98 (3.05)	14.15 (14.22)	Yellowish brown	202
ABZ-*p*-CLA ($C_{18}H_{17}N_3O_6SCl_2$)	519.0 (473.3)	45.43 (45.63)	3.45 (3.59)	8.75 (8.87)	Red	210

Table 6 Infrared frequencies[a] (cm^{-1}) and tentative assignments for DDQ, *p*-CLA, ABZ, ABZ-DDQ and ABZ-*p*-CLA CT-complexes

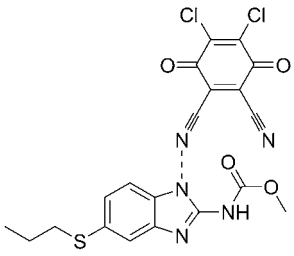
DDQ	*p*-CLA	ABZ	ABZ-DDQ	ABZ-*p*-CLA	Assignments[b]
3325 (w), 3218 (br)	3237 (s, br)	3330 (vs)	3154 (vs, br)	3130 (w, br), 3029 (br)	ν(O—H), ν(N—H), NH_2^+
		2958 (w), 2918 (vw) 2864 (vw), 2806 (vw)	2965 (w), 2872 (w)	2847 (mw)	ν_s(C—H)+ν_{as}(C—H); CH_3 +CH_2
			2600—2700 (w, sh)		Hydrogen bonding
2250 (vw), 2231 (ms)			2221 (vs)		ν(C≡N)
1673 (vs)	1664 (vs)	1709 (mw)	1753 (s), 1686 (ms) 1645 (m)	1738 (sh)	νC=O)
		1625 (vs)	1599 (m)	1620 (sh)	δ_{def}(N—H) Ring breathing bands
1552 (vs), 1451 (s)	1630 (vs)	1586 (vs), 1524 (sh)	1561 (w), 1452 (ms)	1517 (br), 1444 (vw)	ν(C=C)+ν(C=N)+ COO C—H deformation+ NH_2^+ Ring breathing bands
1358 (w), 1267 (s) 1172 (vs), 1072 (w)	1366 (s), 1267 (s, br)	1445 (vs), 1324 (s) 1269 (vs)	1393 (vw), 1350 (vw) 1326 (w), 1296 (vw)	1373 (vw), 1268 (ms)	ν(C—C)+ν(C—N)+ ν(C—O)+ν(C—S)
1010 (vw), 893 (s)	980 (vs), 847 (vs)	1226 (w), 1191 (s) 1097 (vs), 1007 (mw) 957 (ms)	1254 (s), 1197 (w) 1094 (ms)	1193 (vw), 1097 (ms) 985 (s), 921 (m)	δ_{rock}; NH CH, in-plane bend CH-deformation ν(C—Cl)
800 (vs), 720 (s) 615 (ms), 527 (vw) 457 (ms), 432 (mw)	753 (s), 688 (s) 566 (s)	921 (ms), 880 (ms) 789 (ms), 762 (w) 732 (vw), 694 (m) 594 (ms), 516 (ms) 444 (mw)	885 (w), 818 (m) 729 (w), 599 (w) 488 (w), 426 (w)	834 (vs), 694 (w) 574 (vs), 499 (vw) 425 (s), 406 (s)	skeletal vibration CH bend CH out-of-plane bend Skeletal vibration ν(C—S), CNC def. NH_2 rock

[a] s=strong, w=weak, m=medium, sh=shoulder, v=very, br=broad. [b] ν, stretching; δ, bending.

plexation as follows:

In case of ABZ-DDQ CT-complex, the IR spectra of the molecular complex of DDQ with ABZ indicate that ν(C≡N) and ν(C—Cl) of the free acceptor are shifted to lower wavenumber values on complexation. Since DDQ is derived from any acidic centers, thus we may conclude that the molecular complexes are formed through π-π* and/or n-π* charge migration from HOMO of the donor to the LUMO of the acceptor. IR spectrum of the molecular complex of DDQ with ABZ indicates that the single ν(C≡N) of the free acceptor molecule which exhibited at 2250 cm^{-1} is shifted to a lower wavenumber value 2221 cm^{-1}, while the ν(C=O) absorption band of the free DDQ at 1673 cm^{-1} is shifted to lower value 1645 cm^{-1}. Careful interpretation of IR spectra strongly supported that the CT-interaction in case of ABZ-DDQ complex occurs through n-π* transition via deprotonation of NH group of ABZ to only one of the CN groups from DDQ acceptor by forming intermolecular hydrogen bonding (Scheme 2). This suggestion is confirmed by the absence of the characteristic band of NH of free ligand which exists at 3330 cm^{-1}. In addition, the characteristic bands of the hydrogen bonding appear in the IR spectrum of the studied complex at 2600—2700 cm^{-1}, and this group of bands do not exist in both spectra of the free donor and acceptor.

Scheme 2 Structure of the ABZ-DDQ CT-complex



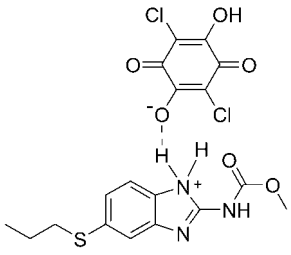
ABZ occurs by accepting a proton from *p*-CLA. The IR spectrum of the ABZ-*p*-CLA CT complex is characterized by the absence of bands due to ν(O—H) and ν(N—H) groups of *p*-CLA and ABZ, respectively, and presence of new bands at 3130 and 3029 cm^{-1} which are not appearing in the spectra of the free donor and acceptor. These bands are attributed to the stretching vibration of a proton attached to the donation site of the donor.[38] These results led to the protonation of the $^+$NH group of the donor through one proton transfer from the one side of the acidic center (OH) for the *p*-CLA ac-

CHINESE JOURNAL OF CHEMISTRY

ceptor to the basic center on the donor $^{+}$NH group. Such assumption is strongly supported by the appearance of a broadening absorbance band at 1517 cm^{-1} due to $^{+}NH_2$ deformation and the absorbance bands near 800 cm^{-1} are assigned to NH_2 rock. This is further supported by disappearance or decrease in the stretching of OH group of *p*-CLA (acceptor) due to intermolecular hydrogen bond forming. The shift of the IR bands concerning the acceptor part to lower wavenumbers and those of the donor part to higher values reflects a donor to acceptor charge transfer of π-π* interaction, $D_{HOMO} \rightarrow D_{LUMO}$ transition.[39]

Accordingly, the hydrogen bonding between the donor and the acceptor can be formulated as shown in Scheme 3.

Scheme 3 Structure of the ABZ-*p*-CLA CT-complex



^{1}H NMR spectral studies ^{1}H NMR spectra of ABZ drug and its CT-complexes in DMSO are measured and the assignments of spectral data are listed in Table 7. The chemical shifts of proton for the defined peaks are analyzed. Evidently, the results obtained from elemental analyses, infrared spectra, and molar ratio titrations met in the same point with ^{1}H NMR spectra to interpret the mode of interaction between donor and acceptor. It is clear that in ABZ-DDQ and ABZ-*p*-CLA CT complexes, the signal ascribed to proton of OH acceptor at δ 8.90 disappeared due to deprotonation from acceptor to donor, beside that, the downfield shift in H of NH (aromatic ring) from δ 6.94 to δ 7.21 for ABZ-DDQ and to δ 7.11 for ABZ-*p*-CLA, respectively, prove that the CT-interactions in both complexes occur via NH (five aromatic ring) of ABZ donor.

Mass spectra of ABZ, ABZ-DDQ and ABZ-*p*-CLA, CT complexes Mass spectrometry has been applied in order to study the purity and the main fragmentation routes of ABZ charge-transfer complexes. The differentiation in fragmentation was caused by the nature of the attached acceptors through the intermolecular hydrogen bond between donor/acceptor, while the molecular ion peaks characterized to DDQ *m/z* (%) 228 ($M^{+}+1$, 53), *p*-CLA *m/z* (%) 208 (49) and ABZ *m/z* (%) 265 (65) are detected in the fragmentation of their CT-complexes. The corresponding mass spectral data are given in Table 8. The different competitive fragmentation pathways of donors give the peaks at different mass numbers listed in Table 8. The intensities of these peaks reflect the stability and abundance of the ions.[40]

Table 7 ^{1}H NMR spectral data of ABZ, DDQ, *p*-CLA, ABZ-DDQ and ABZ-*p*-CLA CT complexes

Compound	Chemical shift δ	Assignment
ABZ	0.905 (m)	3H, CH_3
	1.534 (m)	2H, CH_2
	2.799 (m)	2H, CH_2S
	3.152 (s)	1H, NH (aliphatic)
	3.868 (s)	3H, OCH_3
	6.942 (m)	1H, NH (aromatic)
	7.048—7.410 (m)	3H, aromatic
DDQ	—	—
p-CLA	8.90 (br)	2H, 2(OH)
ABZ-DDQ	0.930 (m)	3H, CH_3
	1.579 (m)	2H, CH_2
	2.851 (m)	2H, CH_2S
	3.145 (s)	1H, NH (aliphatic)
	3.826 (s)	3H, OCH_3
	7.210 (m)	1H, NH (aromatic)
	7.313—7.507 (m)	3H, aromatic
ABZ-*p*-CLA	0.933 (m)	3H, CH_3
	1.534 (m)	2H, CH_2
	2.868 (m)	2H, CH_2S
	3.146 (s)	1H, NH (aliphatic)
	3.839 (s)	3H, OCH_3
	7.110 (m)	1H, NH (aromatic)
	7.296—7.403 (m)	3H, aromatic

Table 8 Mass fragmentation of ABZ, ABZ-DDQ and ABZ-*p*-CLA CT complexes

Compound	*m/z* (%)
ABZ	265 (65), 233 (50), 191 (100), 122 (22), 59 (47)
DDQ	227 (53), 200 (64), 165 (15), 137 (37), 110 (45), 87 (100), 52 (55)
p-CLA	209 (49), 188 (49), 145 (23), 123(12), 105 (43), 87 (56), 69 (100), 52 (43)
ABZ-DDQ	477 (10), 328 (10), 265 (32), 233 (56), 191 (100), 164 (34), 123 (36), 87 (75), 67 (52), 51 (49)
ABZ-*p*-CLA	519 (75), 461 (75), 333 (75), 292 (75), 204 (75), 173 (75), 147 (62), 138 (62), 111 (37), 88 (75), 58 (100)

Conclusions

Simple, rapid and reliable spectrophotometric method was adopted for the micro determination of ABZ drug via CT complex formation with DDQ or *p*-CLA reagents spectrophotometrically. The effect of different parameters was studied. The results obtained by the suggested procedure were compared with those obtained by the standard method. The data obtained by both procedures were found to be very close to each other and very close to those given by the pharmaceutical companies. The calculated F- and t-tests at the 95%

confidence level do not exceed the theoretical values. The results obtained by the suggested procedure were compared with those obtained by the standard method. The data obtained by both procedures were found to be very close to each other and very close to those given by the pharmaceutical companies. The calculated F- and t-tests at the 95% confidence level do not exceed the theoretical values.

Also, the formed CT complexes were studied using elemental analyses, IR, ^{1}H NMR and mass spectra in order to elucidate the structure of these CT complexes. The results obtained confirm the results of stoichiometry studied before and suggested a 1∶1 reaction between donors and acceptors under study, in addition it helped in elucidation of the site of interaction between donors and acceptor.

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