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VALIDATED SPECTROPHOTOMETRIC DETERMINATION OF ZAFIRLUKAST IN TABLET DOSAGE FORM VIA CHARGE TRANSFER COMPLEX FORMATION

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ABSTRACT

Two simple and selective visible spectrophotometric methods were developed for assay of Zafirlukast (ZAF) in pure drug and in its pharmaceutical formulation. Studies were carried out to use the charge-transfer reactions of Zafirlukast (ZAF), extracted from neutralized Zafirlukast, as n-electron donor with the π-acceptor, dinitrophenol (DNP) for method A and σ-acceptor, and iodine (I_2) For method B, resulting in the formation of colored complex. The colored reaction products were quantities spectrophotometrically at 502 nm and 417 nm for ZAF-CAA and ZAF –I_2 complexes, respectively. Beer's law is obeyed over the concentration ranges of 2.0–40.0 and 5.0–60.0 μg mL^{-1} for DNP and I_2, respectively, with correlation coefficients (r) of 0.9991 and 0.9985, and molar absorptive 0.2409×10^4 and 0.2631×10^4 L mol^{-1} cm^{-1} for method A and method B, respectively The analytical parameters such as apparent limits of detection (LOD) and quantification (LOQ) are also reported for two methods. The described methods were successfully applied to the determination of ZAF in tablets. No interference was observed from the common excipients present in tablets. The reaction stoichiometry in two methods was evaluated by Job's method of continuous variations and was found to be 1: 1 (donor: acceptor).

KEYWORDS

Spectrophotometry, Charge-transfer complexes, Zafirlukast and Pharmaceutical.

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INTRODUCTION

Anti-leukotrienes drugs have recently become available for the clinical management of asthma and they functioning either by blocking the interaction of leukotrienes with receptors or by inhibiting leukotriene synthesis[1,2]. Zafirlukast (ZAF), 4-(5-cyclopentyloxycarbonylamino-1-methylindol-3-ylmethyl) -3-methoxy-o-oylsulphonyl benzamide (Figure No.1) is a cysteinyl leukotriene which used in the prophylaxis and treatment mild to moderate persistent and chronic asthma[3,4]. ZAF has shown effective in the inhibition of allergen, exercise, sculpture dioxide and aspirin induced asthma[5,6].

ZAF effectively improved symptoms and benefited lung function with asthmatic patients[7]. Because ZAF is a novel drug, only a few analytical methods for its determination in pharmaceutical formulations and biological fluids have been described in the literature, including high performance liquid chromatography (HPLC)[8-11]. Ultra-performance Liquid Chromatography[12]. RP-HPLC[13], HPTLC[14], derivative spectrophotometry[9], capillary zone electrophoresis (CZE)[15] and electrochemical methods such as square-wave voltammetry (OSWV)[16], and differential adsorptive stripping voltammetry (DPAdSV)[17]. There are few UV spectrophotometric methods for the analysis of ZAF in pharmaceutical formulations has been reported in literature[9, 18].

This paper, for first time, describes the application of these three reagents for the rapid, selective and sensitive spectrophotometric assay of ZAF in bulk drug and in its dosage forms. The methods involve the charge-transfer (C-T) complex formation reaction of the drug with DNP (method A), and I_2 (method B) in dichloromethane to form intensely colored radical anions measurable at 502 nm in methods A and at 417 nm in method B. The proposed methods are determined to be simple and rapid employing low cost reagents and instruments.

MATERIALS AND METHOD

Apparatus

A Genesys 10S UV-Vis double beam spectrophotometer (Thermo Spectronic, USA) with a fixed slit width (1.8nm) connected to an IBM computer loaded with Thermo Spectronic VISION Lite version 4 software and 1-cm quartz cell were used for the registration and treatment of absorption spectra.

Materials and Reagents

All Chemicals used were of analytical reagent grad unless otherwise is mentioned, Zafirlukast (ZAF) standard powder (purity 99.8%) were kindly supplied by SPIMACO ADDWAEIH, Al-Qassim, Saudi Arabia.

Dinitrophenol (DNP) (0.05%) Prepared by dissolving 0.05 g of dinitrophenol (Sigma-Aldrich Co) in 100 mL of dichloromethane and used for the assay in method A.

Iodine solution (0.5%) Prepared by dissolving 0.5 g of the pure resublimed iodine (Sigma-Aldrich Co) in 100 mL of dichloromethane and used after 30 min for the assay in method B.

Sodium hydroxide (1.0 M) accurately weighed 1 g of the pure NaOH (Merck) was dissolved in water; the solution was made up to 100 mL with water.

Preparation of Standard Stock Solution

The stock standard solution of ZAF was prepared in methanol to a concentration of 100 μgmL^{-1} and kept stored at -20 °C in dark glass flasks. Working standard solutions were prepared from the stock standard solutions.

Pharmaceutical Formulations

Twenty tablets were weighed and pulverized. The amount of tablet powder equivalent to 100 mg of ZAF was transferred into a 100 mL volumetric flask. The content was shaken well with about 50 mL of water for 20 min and diluted to the mark with water. It was filtered using Whitman No. 42 filter paper. First 10 mL portion of the filtrate was discarded. Twenty milliliters of the tablet extract (100 μgmL^{-1}) was quantitatively transferred to a separating funnel, pH was raised by adding 5.0 mL of 1.0 M NaOH and the content was mixed well. The ZAF base was extracted with three 20 mL portions of dichloromethane, the extract was passed over anhydrous sodium sulphate and collected in 100 mL volumetric flask, the volume was made up to mark with dichloromethane and the resulting solution (10 $\mu g\ mL^{-1}$ ZAF) was used in methods.

CONSTRUCTION OF CALIBRATION CURVES

Method a (Using DNP)

ZAF solution in the concentration range of 2-40 μgmL^{-1} was transferred in to 10 mL volumetric flaks using a micro burette. One milliliter of 0.05% DNP solution was added to each flask and diluted to volume with dichloromethane. The content was mixed well and the absorbance was measured at 502 nm against a reagent blank.

Method B (Using I_2)

Aliquots of stock standard solution of ZAF in the concentration range of 5-60 μgmL^{-1} was transferred into a series of 10 mL volumetric flasks and 1 mL of 0.5% iodine solution was added to each flask, the content was mixed well and the flasks were allowed to stand at room temperature for 15 min. The volume was brought up to the mark with dichloromethane and the absorbance was measured at 417 nm against a reagent blank similarly prepared without adding ZAF base solution. Standard graph was prepared by plotting the absorbance versus drug concentration, and the concentration of the unknown was read from the calibration graph or computed from the respective regression equation.

Procedure for the Analysis of Placebo Blank and Synthetic Mixture

A placebo blank containing starch (40 mg), lactose (35 mg), sodium citrate (35 mg), hydroxyl cellulose (35 mg), magnesium stearate (35 mg), talc (35 mg) and sodium chloride (35 mg) was prepared by mixing all the components into a homogeneous mixture. A 10 mg of the placebo blank was accurately weighed and its solution was prepared as described under 'tablets', and then subjected to analysis by following the general procedures. An accurately weighed quantity of ZAF was added to200 mg of placebo blank and homogenized. An amount of synthetic mixture equivalent containing 10.0 mg ZAF was accurately weighed and transferred into a 50 mL volumetric flask and the extract equivalent to 100 $\mu g\ mL^{-1}$ZAF was prepared as described under the general procedure for pure drug and further diluted to the required concentration and used for the assay in all the two methods.

RESULTS AND DISCUSSION

Absorption Spectra

The reaction of Zafirlukast base (ZAF) as n-electron donor and the p-acceptors such as DNP, and r-acceptor, I_2 result in the formation of charge-transfer complexes. The absorption spectra of ZAF-DNP charge-transfer complex resulted in the formation of intense read products which exhibit absorption maxima at 502 nm (Figure No.2). Similarly, the reaction of ZAF with I_2 results in the formation of a yellow product which exhibits an absorption maximum 417 nm (Figure No.3).

Reaction Scheme

Charge-transfer complex is a complex formed between an electron donor and an electron-acceptor, characterized by electronic transition(s) to an excited state in which there is a partial transfer of electronic charge from the donor to the acceptor moiety. As a result, the excitation energy of this resonance occurs very frequently in the visible region of the electro-magnetic spectrum. This produces the usually intense color characteristic of these complexes. Therefore, ZAF, a nitrogenous base is an n-donor, was made to react with dinitrophenol, (p-acceptors) and iodine (r-acceptor) to produce a colored charge transfer complex in dichloromethane.

Reaction with p-Acceptors

Dinitrophenol (Method A):

Dinitrophenol react with electron donor molecule to form charge-transfer and proton transfer complexes[19, 20]. It was used for the determination of some amine derivatives through the formation of intense yellow colored complex. When an amine is combined with a polynitrophenol, one type of force field produces an acid-base interaction, and the other, an electron donor-acceptor interaction. The former interaction leads to the formation of true phenol ate by proton- transfer, and the latter, to a true molecular compound by charge-transfer [21]. Based on this, the mechanism for method A can be discussed in terms of transfer of electronic charge from the benzene ring of ZAF, an electron-rich molecule (A Lewis-base donor), to the ring of DNP, an electron-deficient molecule (a Lewis- acid acceptor), and at the same time the proton of the hydroxyl group of DNP will transfer to the tertiary amine of ZAF. The explanation for the produced color in method A lies in the formation of complex between the pairs of molecules ZAF-DNP, and this complex formation leads to the production of two new molecular orbitals and, consequently, to a new electronic transition. Because ZAF has two tertiary amino groups in their molecular structure with the availability of non-bonding electron donors, it reacts with dinitrophenol in dichloromethane to yield a

read colored charge-transfer complex peaking at 502 nm (Figure No.2). The interaction between ZAF (D), an n-donor and dinitrophenols (A), p-acceptors, is a charge transfer complexation reaction followed by the formation of radical ions[22] according to the Scheme No. 1.

Reaction With r-Acceptor (Iodine) (Method B)

ZAF, an n-donor (D), in dichloromethane forms a lemon yellow colored charge-transfer complex with iodine (I_2) (r-acceptor) and the resulting colored species was found to absorb maximally at 417 nm (Figure No.3). The color of iodine in dichloromethane is violet showing absorption maximum (λ_{max}) at 500 nm. This color was changed into lemon yellow when mixed with drug. This change in color and the appearance of the peak were attributed to the formation of charge-transfer complex between ZAF and iodine. The interaction between the donor and acceptor occurs according to the Scheme No.2.

Effect of Reagent Concentration

The effect of the reagent concentration on the intensity of the color at the selected wavelengths was ascertained by adding different amounts of the reagents NDP and I_2 to fixed concentrations of 15 and 20 μgmL^{-1} ZAF in NDP-method and I_2-method, respectively. It was found that 1.0mL each of 0.1% NDP and 0.5% I_2 solutions were sufficient for the production of maximum and reproducible color intensity, and the highest absorbance remained unaffected by further addition of these reagents (Figure No.4).

In order to select a suitable solvent for preparation of the reagent solutions used in the study, the reagents were prepared separately in different solvents such as 1,4-dioxane, chloroform, acetonitrile, acetone, t-butanol,2-propanol and dichloromethane. The reaction of ZAF with DNP or I_2 was followed; dichloromethane was best suited for the preparation of DNP and I_2 solution. Similarly, the effect of the diluting solvent was studied for all the methods and the results showed that the ideal diluting solvent to achieve maximum sensitivity and stability of the colored species was dichloromethane in all the tow methods.

Effect of Reaction Time and Temperature

The optimum reaction time was determined by monitoring the color developed at room temperature (25 ± 5 C^o). Complete color development was attained instantaneously with DNP and after 15 min for iodine. The developed colors remained stable at room temperature for at least for 3h except for iodine 50 min where the color decreased dramatically after 30 min resulting in higher imprecision of the reading.

Stoichiometry of the Reaction

The composition of the charge-transfer complex was established by Job's method of continuous variations [23] using equimolar concentrations of the drug (base form) and reagents (2.15×10^{-4} mol L^{-1} in method A and 2.25×10^{-4} mol L^{-1} in method B). The results indicated that 1:1 (drug/reagent) complex is formed in all the methods. Five solutions containing ZAF and the reagent DNP or I_2 in various molar ratios, with a total volume of 10 mL in all the methods were prepared. The absorbance of solutions was subsequently measured at 502 nm in method. A and 417 nm in method B. The graphs of the results obtained (Figure No.4) gave a maximum at a molar ratio of X_{max} =0.5 in all the methods which indicated the formation of a 1:1 charge-transfer complex between ZAF and reagent DNP or I_2. This finding was anticipated by the presence of more basic or electron donating center (–NH) in the ZAF.

Method Validation

The proposed methods were validated for linearity, sensitivity, selectivity, accuracy, precision, robustness, ruggedness and recovery according to the current ICH guidelines[24].

Linearity and Sensitivity

At the established experimental conditions, standard calibration curves for ZAF with DNP and I_2 were constructed by plotting absorbance verses concentration. The linear regression curves were obtained in the Beer's law range of 2.0-40 and 5.0-60 μgmL^{-1} with correlation coefficient 0.9991 and 0.9985 in each case respectively. Regression characteristics including slope, intercept, correlation coefficient and also the molar absorptivity values for each proposed method are given in Table No.1. The detection limit (LOD) and quantification limit

(LOQ) were calculated by using the following equations

$$LOD = \frac{3.3 \times \sigma}{S} \& LOQ = \frac{10 \times \sigma}{S}$$

Where, σ is the standard deviation of seven replicate determinations under the same conditions as for the sample in the absence of the analyte and S is the slope of the calibration graph. The LOD values were calculated to be 0.677 and 1.430 μgmL^{-1}respectively (Table No.1).

Accuracy and Precision

In order to determine the accuracy and precision of the proposed methods, pure drug ZAF solution at three different concentration levels (within the working range) were prepared and analyzed in seven replicates during the same day (intra-day precision) and on five consecutive days (inter-day precision) and the results are presented in Table No.2. The percentage relative error (RE %) was ≤ 1.50 which indicates that the accuracy of the methods is satisfactory. Percentage relative standard deviation (RSD %) for intra-day was ≤ 1.36 and for inter-day it was ≤ 1.33 indicating repeatability and usefulness of the proposed methods in the routine analysis.

The assay results were in good agreement with the label claim. Also, the effect of commonly found excipients was determined by scanning the blank solution of ZAF and the placebo solutions. The percent recovery values given in Table No.3 indicate that excipients of tablet did not found to interfere during the assay.

Robustness and Ruggedness

To evaluate the robustness of the methods, two important experimental variables volume of reagent and reaction time, were slightly altered and the effect of this change on the absorbance of the charge transfer complexes was studied. The results of this study are presented in Table No.4 and indicated that the proposed methods are robust. Method ruggedness was evaluated by performing the analysis following the recommended procedures by three different analysts and on three different spectrophotometers by the same analyst. From the %RSD values presented in Table No.4, one can conclude that the proposed methods are rugged.

Applications to Analysis of Tablets

The proposed methods were applied to the determination of ZAF in tablets and capsules (Table No.5). The results obtained were statistically compared with those of the official method[14] by applying the Students t-test for accuracy and F-test for precision. The official method describes a potentiometric titration of ethanolic solution of ZAF with sodium hydroxide. As can be seen from the Table No.5, the calculated t-test and F-value at 95 % confidence level did not exceed the tabulated values of 2.78 and 6.39, respectively, for four degrees of freedom. The results indicated that there is no difference between the proposed methods and the official method with respect to accuracy and precision.

Recovery Study

To further ascertain the accuracy of the proposed methods, recovery experiment was performed Via standard addition technique. To a fixed and known amount of ZAF in tablet powder (preanalyzed), pure ZAF was added at three concentration levels (50, 100, and 150% of the level present in the tablet), and the total was measured by the proposed methods. The determination with each concentration was repeated three times, and the results of this study presented in Table No.6 indicated that the various excipients present in the formulations did not interfere in the assay, thereby further confirming the accuracy of the methods.

Table No.1: Optimum Conditions and Analytical Parameters

S.No	Parameters	ZAF-DNP complexes	ZAF-I_2 complexes
1	λmax (nm)	502	417
2	Linearity range μgmL^{-1}	2.0 - 40	5.0 - 60
3	Molar absorptivity $LMol^{-1}cm^{-1}$	2409.30	2631.11
4	Slope (b)	0.0302	0.0023
5	Intercept (a)	0.0449	-0.0050
6	Correlation coefficient	0.9991	0.9985
7	LOD μgmL^{-1}	0.677	1.430
8	LOQ μgmL^{-1}	1.921	4.347

Table No.2: Evaluation of Intra-day and Inter-day Precision and Accuracy

S.No	Method	ZAF taken μgmL^{-1}	Intra-day (n=5)			Inter-day (n=5)		
			Found [a] μgmL^{-1}	% RSD [b]	% RE [c]	Found [a] μgmL^{-1}	% RSD	% RE
1	Method A	5	4.96	0.46	0.80	4.92	0.75	1.2
		10	9.93	0.34	0.70	9.96	0.90	0.90
		15	15.19	1.36	1.27	15.08	0.82	0.53
2	Method B	10	9.92	0.41	0.80	10.01	1.33	0.10
		20	19.97	0.67	1.50	20.04	0.83	0.19
		30	30.08	0.50	0.27	30.15	0.61	0.49

[a]Mean value of five determinations; [b]Relative standard deviation (%); [c]Relative error (%)

Table No.3: Percent Recovery for 20 μgmL^{-1} of ZAF in the Presence of 250 $\mu gmL^{-1}$of Excipients

S.No	Excipients	Recovery% ± RSD *	
		Method A	Method B
1	Lactose	99.25±0.36	99.91±0.90
2	Hydroxyl cellulose	100.91±0.47	101.23±0.52
3	Talc	99.49±1.02	100.62±0.18
4	Starch	100.51±0.82	99.67±0.74
5	Magnesium stearate	99.31±0.16	101.58±0.37
6	Sodium Citrate	101.25±0.61	99.01±0.83
7	Sodium chloride	98.99±1.03	101.67±1.24

***Average of three determinations**

Table No.4: Robustness and Ruggedness

S.No	Method	ZAF taken μgmL^{-1}	Method robustness		Method ruggedness	
			Parameters altered			
			Reagent volume[a], mL RSD% (n=3)	Reaction time[b] RSD%, (n=3)	Inter-analysts RSD%, (n=3)	Inter-cuvettes RSD%, (n=3)
1	BTB Method	5	1.13	0.83	1.49	1.20
		10	0.79	1.05	1.21	1.37
		30	1.01	1.39	1.67	1.40
2	DDQ Method	5	0.92	0.75	1.30	1.19
		10	1.08	1.22	1.53	0.99
		20	1.30	1.18	1.65	0.93

[A] In both methods, the volume of reagent was 0.8, 1.0 and 1.2 mL. [B] the reaction time was 4, 5 and 6 min.

Table No.5: Results of Analysis of Tablets by the Proposed Methods

S.No	Tablet brand name	Labeled amount mg/tablet	Method	Amount found* (in mg)	% Recovery ±RSD*	T-test**	F-test***
1	Zafirlukast	10	Method A	10.12	102.1± 1.09	1.53	1.62
			Method B	9.91	99.11± 1.35	2.61	2.53
			Official method	10.11	101.10± 0.83	-	-
2	Zafirlukast	20	Method A	20.26	101.3± 1.80	1.91	2.84
			Method B	20.18	100.9± 1.67	1.57	2.46
			Official method	19.88	99.43± 1.05	-	-

*Mean value of five determinations.

**Tabulated t-value at the 95% confidence level is 2.78.

***Tabulated F-value at the 95% confidence level is 6.39.

Table No.6: Results of Recovery Study by Standard Addition Method

S.No	Tablets studied	Method A				Method B			
		ZAF in tablets $\mu g.mL^{-1}$	Pure ZAF added $\mu g.mL^{-1}$	Total found $\mu g.mL^{-1}$	% Recovery* ±SD	ZAF in tablets $\mu g.mL^{-1}$	Pure ZAF added $\mu g.mL^{-1}$	Total found $\mu g.mL^{-1}$	% Recovery* ±SD
1	Zafirlukast-10	10	5	15.24	101.6 ± 1.22	10	5	14.88	99.22±0.87
			10	19.80	98.99±1.64		10	20.48	102.4±0.56
			15	25.55	102.2 ± 0.78		15	25.45	101.8±0.48
2	Zafirlukast-20	16	8	24.33	101.4±1.56	20	10	30.57	101.9±1.52
			16	31.54	98.56±1.03		20	4.32	100.8±0.97
			24	39.64	99.10±0.98		30	51.10	102.2±0.96

*Mean value of three determinations.

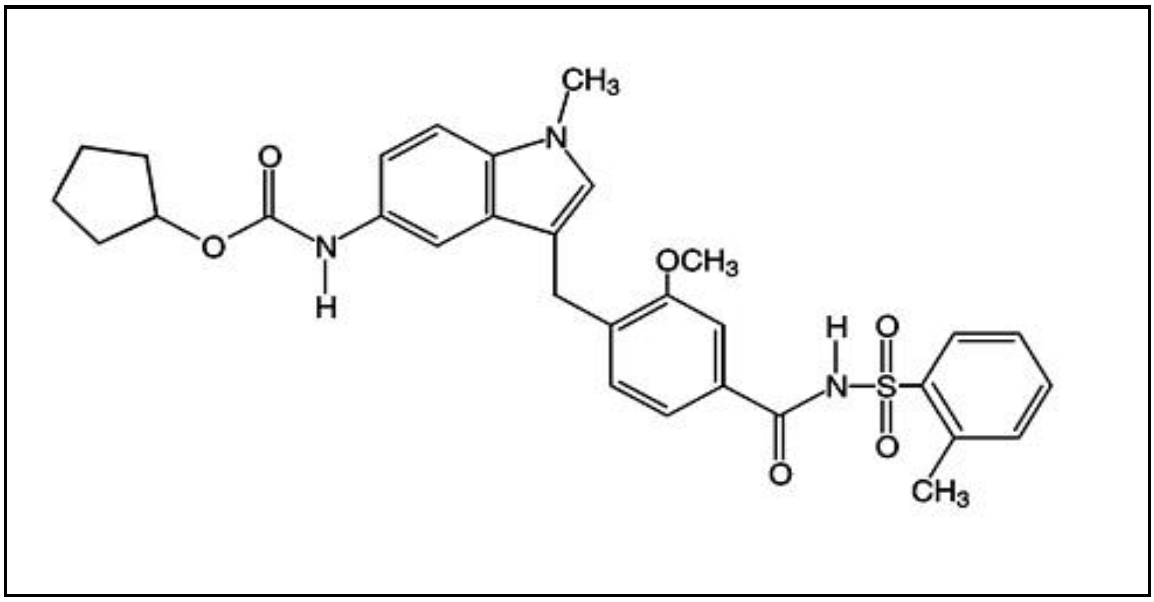


Figure No.1: Structure of Zafirlukast (ZAF)

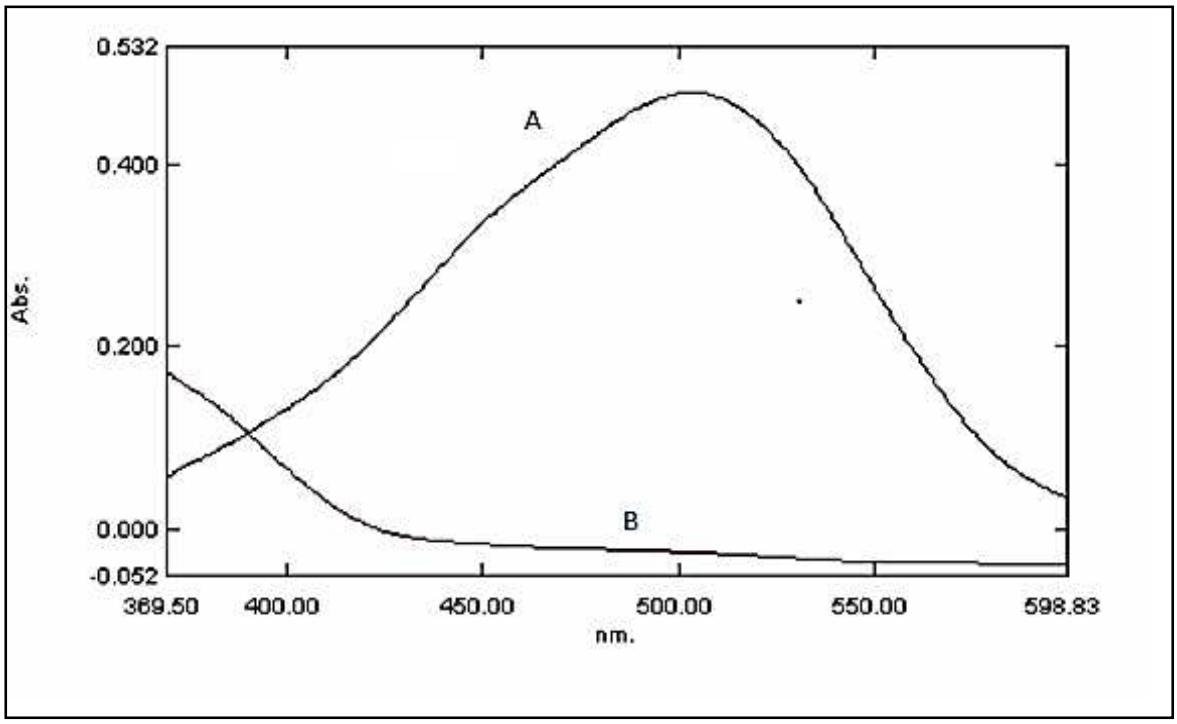


Figure No.2: Absorption Spectra of A: 15 µgmL^{-1} ZAF-DNP Complex against Reagent Blank, B: Reagent Blank Against Chloroform, Under Optimum Conditions

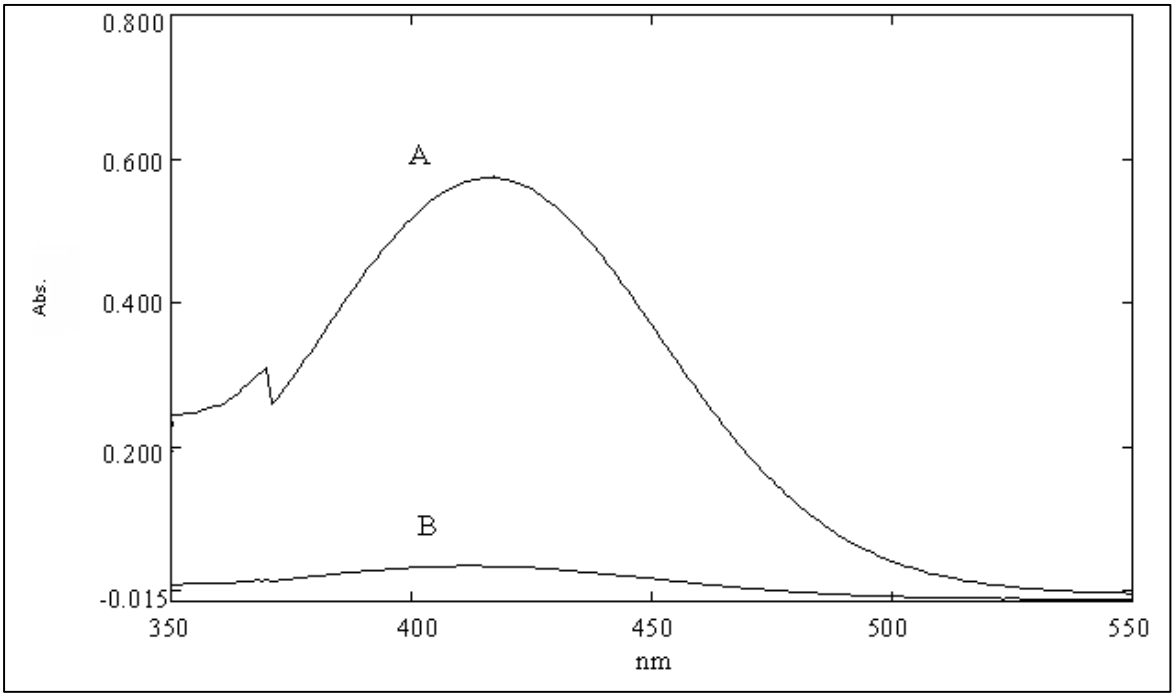
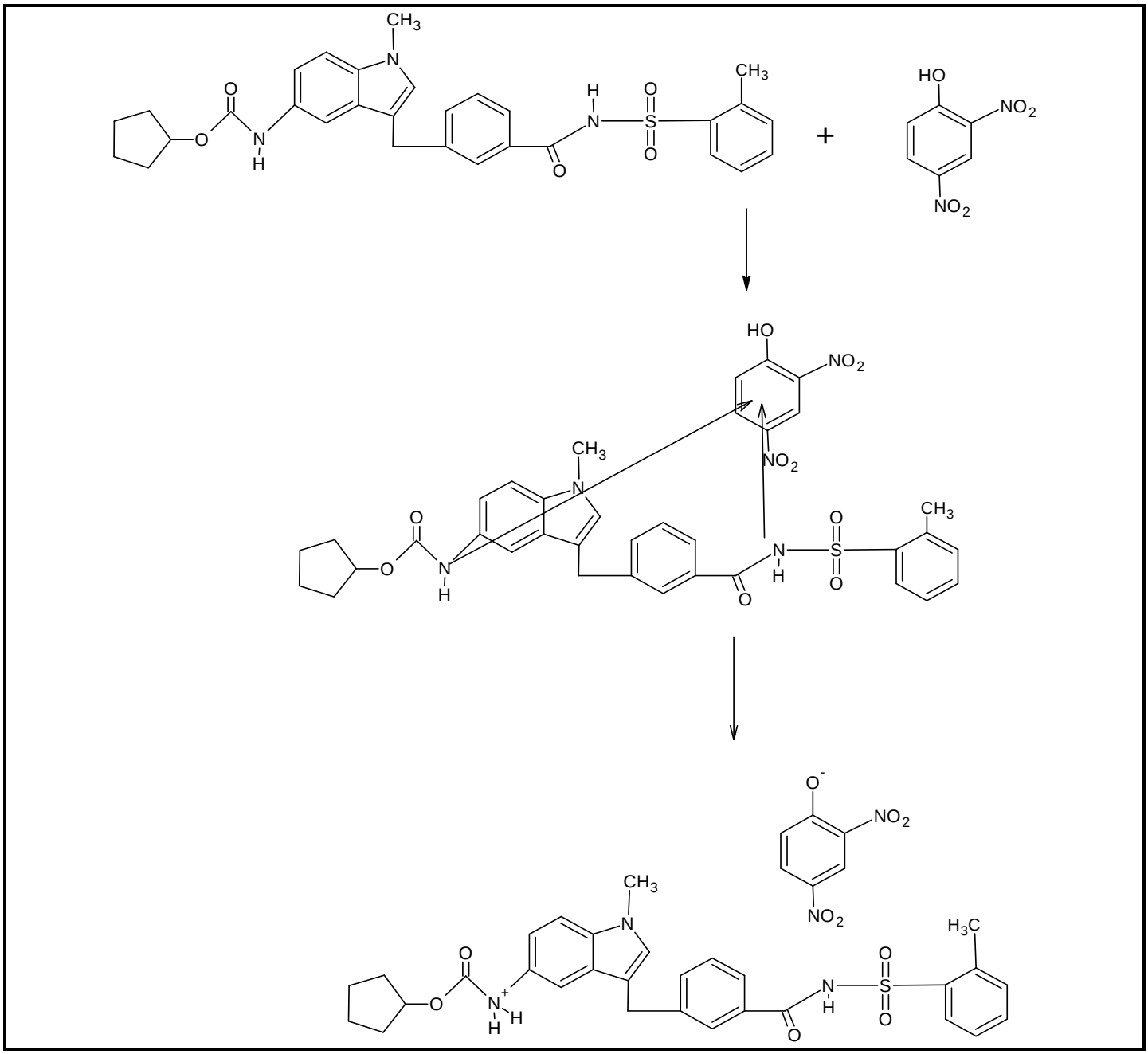


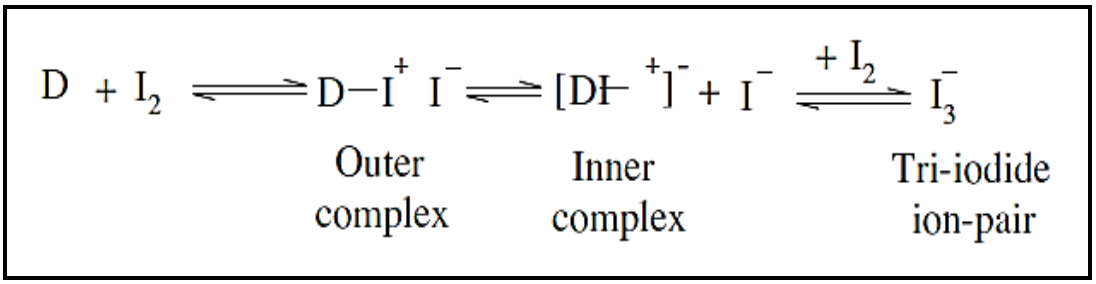
Figure No.3: Absorption Spectra of A: 20 µgmL^{-1} ZAF–I_2 Complex, Against Reagent Blank, B: Reagent Blank against Acetonitrile, Under Optimum Conditions



$$D^{\bullet\bullet} + A \rightarrow [D^{\bullet\bullet} \rightarrow A] \rightarrow D^{\bullet+} + A^{\bullet-}$$

$$[\text{Donor} + \text{Acceptor} \rightarrow \text{Complex} \rightarrow \text{Radical ions}]$$

Scheme No.1: Reaction Pathway for the Formation of Electron Donor - Acceptor Complex and Radical ions Between ZAF and Dinitrophenols



Scheme No.2: Reaction pathway for the Formation of Electron Donor-Acceptor Complex and Radical ion Between ZAF and Iodine

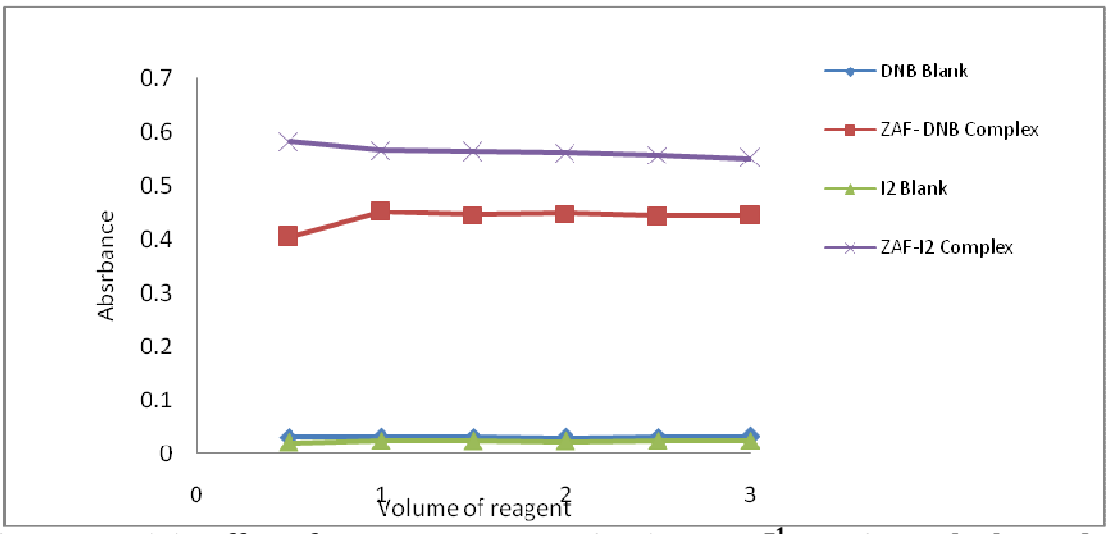


Figure No.4 (A): Effect of Reagent Concentration (15μgmL^{-1} ZAF in Method A and 20 μgmL^{-1} ZAF in Method B)

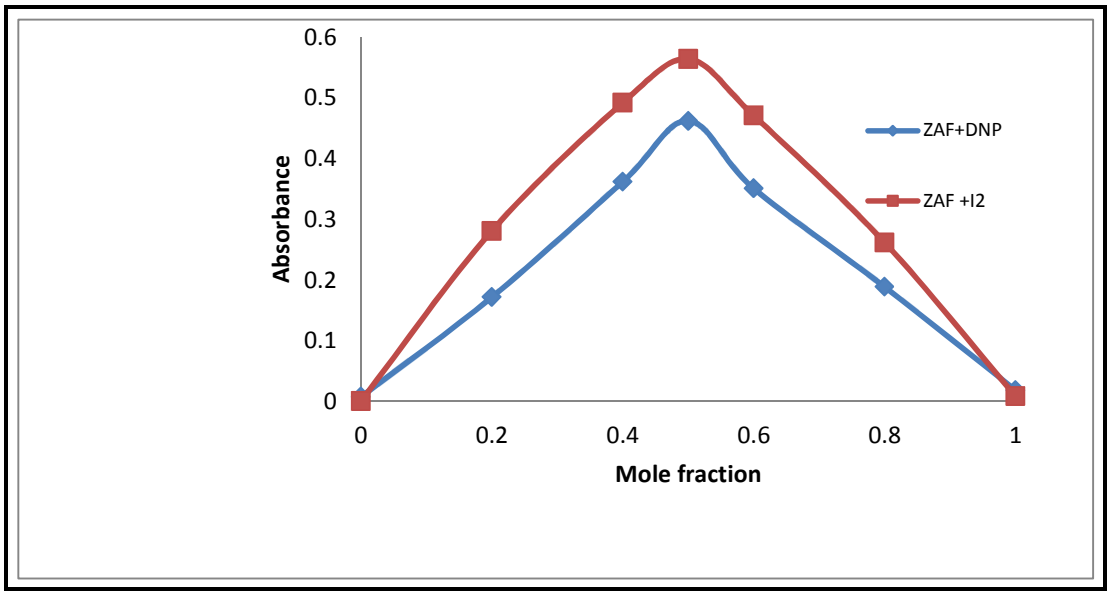


Figure No.4 (B): Job's Continuous-Variations Plots

CONCLUSION

Two simple, sensitive, extraction-free, rapid and cost-effective spectrophotometric methods based on charge transfer complex formation reaction are described for the determination of ZAF. The methods were developed and validated as per the current ICH guidelines. The proposed methods utilize a single step reaction and a single solvent. No substantial differences among the proposed methods arose from analysis of the experimental results. The methods are free from interferences from the common excipients and additives. The statistical parameters and the recovery data reveal good accuracy and precision of the methods. These methods can be used as general methods for the determination of ZAF in bulk powder and tablets, have many advantages over the separation techniques such as HPLC, are of reduced cost, and speed with high accuracy.

Hence, these methods can be used in routine analysis of drug in quality control laboratories.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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