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New Spectrophotometric Methods for Determination of Albendazole in Presence of Cerium as Oxidant and Both Indigo Carmine and Alizarin Red Dyes in Bulk and Dosage Forms

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ABSTRACT

Novel spectrophotometric methods have been developed for the determination of albendazole in bulk, tablet and suspension dosage forms. Solution of albendazole in methanol was oxidized using known excess ceric ammonium nitrate (Ce4+) then the un-reacted amount of Ce4+ was determined using two different dyes, indigo carmine and alizarin red using universal buffer. The reaction showed maximum absorbance at 610 nm for indigo carmine and at 401 nm for alizarin red. Different factors were studied such as type of buffer, dye volume, oxidant volume, time, temperature, organic solvents, and sequence addition. It was found that Beer's law is obeyed in the range of 1.32-7.95 µg mL-1 for albendazole with both dyes with molar absorptivity of 4.69× 103 L.mol-1.cm-1 for indigo carmine and 97.53× 103 L.mol-1.cm-1 for alizarin red. Also the methods showed a high degree of sensitivity in respect of limit of detection which was reported to be as low as 0.24 and 0.35 µg mL-1 in case of indigo carmine and alizarin red, respectively. By applying statistical treatment, it was found that the methods were accurate and precise with low standard deviation values. The methods were then applied for the determination of albendazole in tablet and suspensions with satisfactory results.

Keywords: *spectrophotometric; albendazole; ceric ammonium nitrate; indigo carmine; alizarin red.*

INTRODUCTION

Albendazole (ABZ), chemically known as methyl-5-(propylthio)-2-benzimidazole carbamate **[1]** (Fig. 1), is widely used as an anthelmintic with a broad spectrum of activity **[2]**. ABZ is an inactive moiety but it readily gets metabolized to an active metabolite, albendazole sulfoxide (ABZSO) which is then further metabolized to an inactive metabolite, albendazole sulfone ($ABZSO_2$) **[3]**. As ABZ is metabolized extensively, therefore, the plasma concentration of ABZ is also very low, as a result, the pharmacokinetic studies were done using ABZSO as an active metabolite and $ABZSO_2$ as an inactive metabolite **[4-8]**. As such, the therapeutic importance of this compound justifies research to develop analytical methods for its determination in bulk form, pharmaceutical formulations and in biological samples. Several techniques can be found in the literature for monitoring ABZ therapy such as UV-vis spectrophotometry **[9-12]**, spectroflourimetry **[13]**, HPLC **[14]**, HPTLC **[15]**, LC/MS **[16]**, capillary electrophoresis **[17]** and voltammetry **[18].**

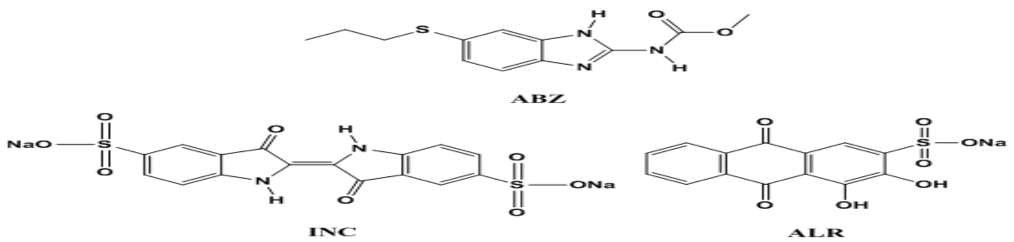

Figure1. *Chemical structures of albendazole (ABZ), indigo carmine (INC) and alizarin red (ALR)*

To our knowledge, no spectrophotometric method for the determination of ABZ using cerium as an oxidant has yet been reported despite the versatility, simplicity and reliability of the technique in chemical analysis. The requirements of pharmaceutical quality control are more severe than in other fields; drug control requires excelled accuracy, specificity and precision. Further, because active components are often present in low amounts in pharmaceutical formulations, the methods must be very sensitive. These requirements are fulfilled by the two methods presented in this paper which are based on an oxidation reaction of ABZ with a known excess of ceric ammonium nitrate (Ce^{4+}) followed by a subsequent determination of the residual oxidant by reacting it with a measured amount of the dyes, indigo carmine (INC) at 610 nm or alizarin red (ALR) at 401 nm.

EXPERIMENTAL

Apparatus

All absorbance measurements were made with a Systronics (model 6800 UV / VIS digital spectrophotometer JENWAY) equipped with 1 cm matched quartz cells.

Materials

Name	Formula	Mol.Wt	Supplier
Albendazole	$C_{12}H_{15}N_3O_2S$	265.34mg/mol	EIPICO
ceric ammonium nitrate	$(NH_4)_2Ce(NO_3)_6$	548.26	GLOBAL chemie
Indigo carmine	$C_{16}H_8N_2Na_2O_8S_2$	466.34	SDFCL
Alizarin red	$C_{14}H_8O_4$	240.21	SDFCL
Methanol	CH_3OH	32.04	SIGMA
Ethanol	CH_3CH_2OH	46.06	SIGMA
Propanol	$CH_3CH_2CH_2OH$	60.096	SIGMA
DMF	$HCON(CH_3)_2$	73.095	SIGMA
Acetone	C_3H_6O	58.08	SIGMA
Formaldehyde	CH_2O	30.026	SIGMA
Ethylene glycol	$C_2H_6O_2$	62.068	SIGMA

- A stock solution of 1×10^{-3} M ABZ was prepared by dissolving an exact weight in 100 mL measuring flask and completed to the mark by diluted methanol**.**
- Stock solutions of 1×10^{-3} M INC dye, ALR dye, and Ce^{4+} were prepared by dissolving an exact weight in 100 mL measuring flasks and completing to the mark by distilled water.

Buffers

Universal Buffer Solutions

A series of universal buffer solutions, covering the pH range from (2 to 10) were prepared as recommended by Britton **[19].**

Borate Buffer Solutions

A series of borate buffer solutions, covering the pH range from (7 to 9) were prepared as recommended by Bower and Bates **[20].**

Acetate Buffer Solution

A series of acetate buffer solutions, covering the pH range from (3 to 6) were prepared.

Phosphate Buffer Solution

A series of universal buffer solutions, covering the pH range from (7 to 10) were prepared as recommended by Lurie **[21].**

Pharmaceutical Preparations

Drug	Trade Name	company	Formulation
ABZ	Alzental® 200mg	EIPICO	Tablets
	Alzental® 20mg/mL	EIPICO	suspension
	Bendax® 20mg/mL	SIGMA	suspension

General Procedure

In 10 mL volumetric flask, accurate volume of ABZ ($1{\times}10^{-3}$ M) and 2 mL Ce^{4+} were mixed for 10 minutes in presence of universal buffer (pH 9 for INC dye or pH 10 for ALR dye). 1.25 mL of INC dye followed by 1 mL ethanol or 1 mL of ALR dye followed by 1 mL propanol was then added with shaking for 1 minute before completing to the mark with distilled water. Using spectrophotometer, the un-reacted INC dye or ALR dye was measured at 610 nm, and 401 nm, respectively, using a reagent blank similarly prepared without ABZ. The concentration of ABZ was then determined form calibration curve previously constructed under the optimum condition.

Pharmaceutical Applications

ABZ was determined in different drug samples of different forms and companies such as Alzental® tablet, Alzental® and Bendax ® suspensions. A suitable weight or volume of each form was dissolved and diluted in methanol in 100 mL volumetric flasks. 1.00 – 3.00 mL sample of each was mixed with 2 mL Ce^{4+} and universal buffer (pH 9 for INC dye or pH 10 for ALR dye) and the methods were completed according to the above mentioned procedure.

RESULTS AND DISCUSSION

Absorption Spectra

Ce^{4+} has been used as an effective oxidizing agent for determination of different materials like ABZ giving a number of oxidized products. The un-reacted Ce^{4+} oxidizes known amount of the dye while the remaining dye is measured spectrophotometrically at corresponding maximum wavelengths. The absorption spectra of the remaining dye showed characteristic λ_{max} values as shown in Fig. 2 at 610 for INC and at 401 nm for ALR.

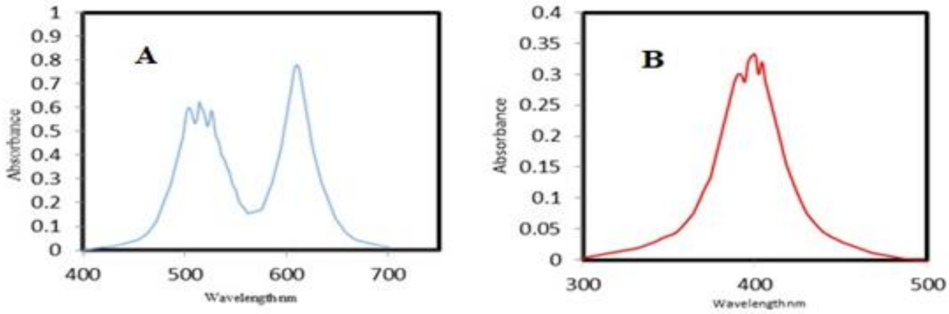

Figure2. *Absorption spectra of ABZ with (A) INC dye at 610 nm and (B) ALR dye at 401 nm.*

Factors Affecting the Absorbance

Effect of Buffer Type

The effect of buffer on the oxidation of ABZ with Ce^{4+} was studied. Different Buffers were used such as universal buffer (pH 2-10), borate buffer (pH 7-9), acetate buffer (pH 3-6), and of phosphate buffer solution (pH 7-10). The absorbance was measured with 1 mL ABZ (1×10^{-3} M) against a blank solution prepared by the same way without ABZ. Universal buffer (pH 9) was chosen as the optimum buffer in case of INC while universal buffer (pH 10) was the optimum media for ALR as shown in Fig. 3.

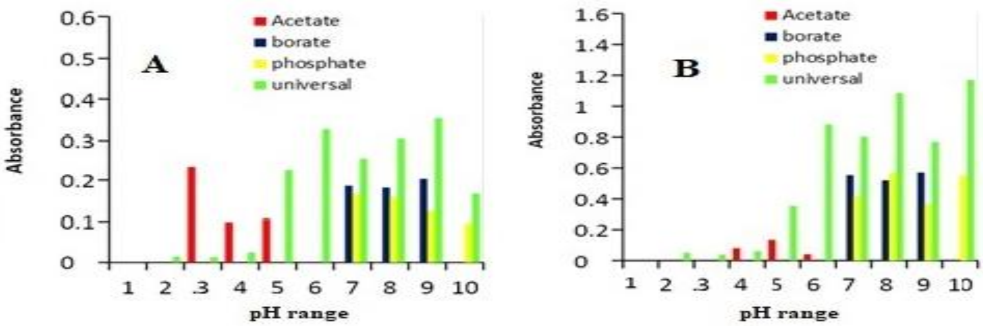

Figure3. *Effect of buffer pH on the absorption of ABZ with (A) INC dye at 610 nm and (B) ALR dye at 401 nm.*

Effect of Oxidant Volume

The effect of different volumes of Ce^{4+} in presence of dye on the oxidation process was investigated using 1 mL ABZ (1×10^{-3} M) and it was found that the suitable volume of Ce^{4+} was 2 mL in case of both dyes.

Effect of Dye Concentration

The effect of dye concentration on the oxidation process was investigated by adding different concentrations of dye and it was recorded that 1.25 mL is the best volume for INC while 1 mL is the best one for ALR.

Effect f Sequence of Addition

Under the above mentioned conditions, the effect of sequence of additions on the oxidation process of ABZ was investigated by measuring the absorbance of solution prepared by different sequences of addition against a blank solution prepared by the same way without the studied material. The most favorable sequence was (ABZ - Ce^{4+} - buffer universal - dye) which showed the best results for determination of ABZ while other sequences gave lower absorbance values.

Effect of Time

The time required to complete the oxidation of ABZ by Ce^{4+} was studied at different time intervals (5-50 min). The proper time for completing the oxidation process is 10 minutes for both dyes. On the other hand, the effect of time after dye addition was studied by measuring the absorbance at different time intervals. The results indicated that shaking for one minute was sufficient to give reliable and stable values.

Effect of Temperature

The effect of temperature on the oxidation process was studied by measuring the absorbance of a solution containing ABZ and Ce^{4+} against blank solution at different temperatures (30 – 60˚C). The optimum temperature is almost at room temperature (30 ± 1 C˚) for both dyes as shown in Fig. 4.

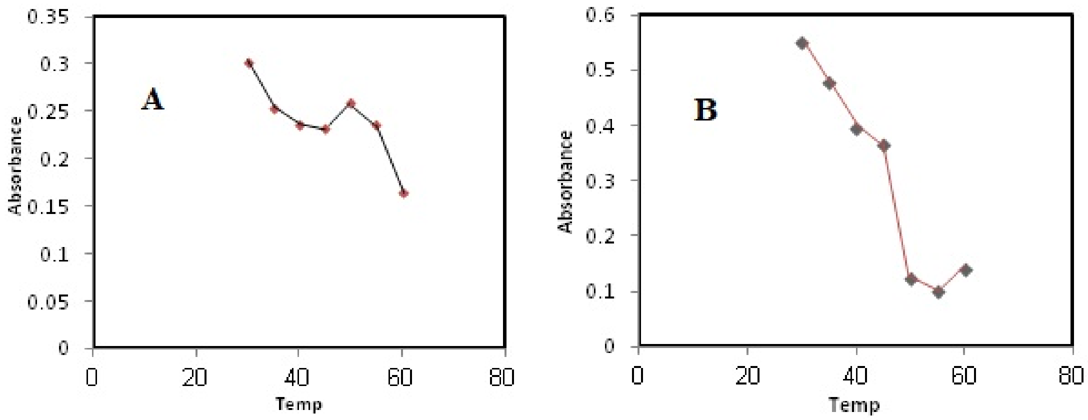

Figure4. *Effect of temperature (0C) on the absorption of ABZ and Ce4+ with (A) INC dye at 610 nm and (B) ALR dye at 401 nm*

Effect of Organic Solvents

The effect of organic solvents on the absorption spectra was studied in presence of different organic solvents such as propanol, ethanol, DMF, acetone, formaldehyde, and ethylene glycol and the absorbance was measured against the blank solution. The results illustrated that the value of absorbance was improved by using ethanol as a solvent in case of INC while it increased in presence of propanol in case of ALR as shown in Fig. 5.

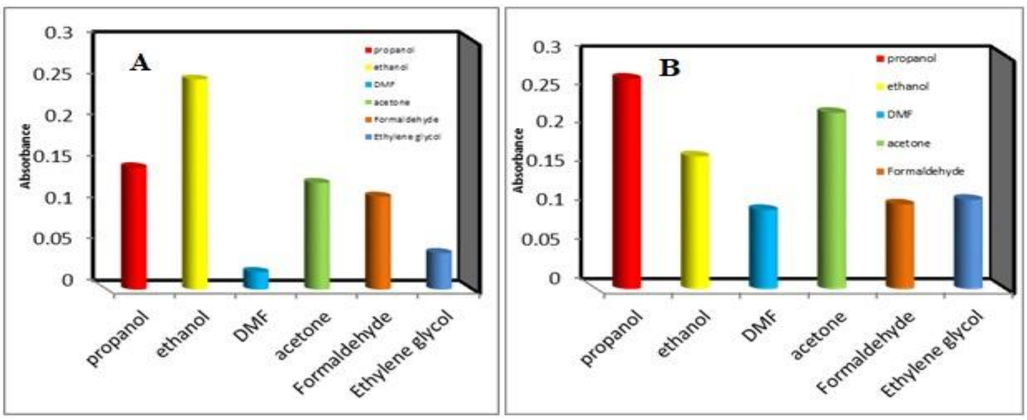

Figure5. *Effect of organic solvents on the absorption of ABZ with (A) INC dye at 610 nm and (B) ALR dye at 401 nm.*

Linearity

Seven different concentrations of ABZ were specified for linearity studies. The calibration curves obtained by plotting absorbance against concentration showed linearity in the concentration range of 1.23-7.95 µg mL^{-1} for both dyes. Also the methods showed a high degree of sensitivity in respect of limit of detection which was reported to be as low as 0.24 and 0.35 µg mL^{-1} in case of INC and ALR, respectively. Other parameters are reported in Table 1 indicating a high degree of linearity, accuracy and precision.

Table1. *Analytical merits for determination of ABZ in pure sample using the proposed spectrophotometric methods.*

Parameters	INC			ALR		
	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery %	Taken µg mL^{-1}	Found µg mL^{-1}	Recovery %
	1.32	1.30	98.48	**1.23**	1.22	99.18
	1.85	1.84	99.45	**1.85**	1.83	98.91
	2.65	2.64	99.62	**3.97**	3.95	99.49
	3.44	3.42	99.41	**4.50**	4.49	99.77
	3.97	3.96	99.74	**5.30**	5.20	98.11
	4.50	4.48	99.55	**6.62**	6.61	99.84
	7.95	7.93	99.74	**7.95**	7.94	99.89
Mean			**99.42**			**99.31**
±SD			0.43			0.64
±RSD			0.44			0.65
±SE			0.13			0.19
Variance			0.19			0.41
LOD (µg mL^{-1})			0.24			0.35
LOQ (µg mL^{-1})			0.72			1.07
Molar absorptivity (L.mol^{-1}.cm^{-1})			4.69× 10^{3}			97.53× 10^{3}

Analytical Applications of Pharmaceutical Formulations

Alzental® tablet, Alzental® and Bendax® suspensions containing ABZ had been successfully analyzed by the proposed methods. Excipients and impurities did not show interference indicating a high degree of specificity regarding the percentage recovery and RSD % for the methods as recorded in Table 2.

Table2. *Application of the spectrophotometric methods for the determination of Alzental® tablet, Alzental® and Bendax® suspensions dosage forms.*

Type	RSD %	Recovery %	Recommended (µg mL^{-1})	Found (µg mL^{-1})	Taken (µg mL^{-1})	Dye
Alzental® Tablet	**INC**	7.50	92.50	1.83	1.85	2
Alzental® suspension		3.00	98.80	9.74	9.88	10
Bendax® suspension		2.80	97.20	4.85	4.86	5
Alzental® Tablet	**ALR**	3.00	97.00	1.97	1.94	2
Alzental® suspension		2.30	97.70	9.73	9.77	10
Bendax® suspension		0.26	98.20	4.90	4.91	5

Conclusion

The proposed methods are reported to be simple, sensitive, precise and accurate based on oxidation of albendazole with ceric ammonium nitrate in presence of indigo carmine or alizarin red dyes. Beer's law is obeyed in the range of 1.23-7.95 µg mL^{-1} for albendazole with both dyes with good molar absorptivities. The methods were then applied for the determination of albendazole in different dosage forms with good recoveries indicating that excepients have no interference effect on the proposed methods.

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