

A NEW NON-EXTRACTIVE ION-PAIR SPECTROPHOTOMETRIC METHOD FOR THE ASSAY OF ALBENDAZOLE

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Abstract

The paper presents a new non-extractive spectrophotometric method for the assay of albendazole (ABZ) as ion pair with Congo red (CR). A soluble and stable ion pair (ABZ-CR) with an absorption maximum at 294 nm was obtained using a $2 \cdot 10^{-8}$ M CR concentration and Britton Robinson (BR) buffer with pH = 2.5. The method is linear in the range 2.5 - 4.7 $\mu\text{g/mL}$, with limits of detection and quantification of 0.09 $\mu\text{g/mL}$ and 0.28 $\mu\text{g/mL}$, respectively. The proposed method was applied to the assay of ABZ in bulk pharmaceutical substance. Because CR is a dye with pharmacological applications used as model in biochemical studies, the research also focused on the mechanism of interaction with ABZ. Spectral changes and physico-chemical constants (conductivity, dielectric constant) indicated that ABZ-CR ion pairing takes place at the surface of CR aggregates by electrostatic and charge transfer interactions.

Rezumat

Lucrarea prezintă o nouă metodă spectrofotometrică non-extractivă de dozare a albendazolului (ABZ) ca pereche de ioni cu roșu de Congo (CR). Perechea de ioni (ABZ-CR), solubilă și stabilă, cu maxim de absorbție la 294 nm s-a obținut folosind o concentrație de CR de $2 \cdot 10^{-8}$ M și soluție tampon Britton-Robinson (BR) cu pH = 2,5. Metoda este liniară pe domeniul de concentrații 2,5 - 4,7 $\mu\text{g/mL}$, cu limita de detecție de 0,09 $\mu\text{g/mL}$ și limita de cuantificare de 0,28 $\mu\text{g/mL}$. Metoda a fost aplicată la dozarea substanței farmaceutice ABZ. Deoarece CR este un indicator cu proprietăți farmacologice și folosit ca model în studiile de biochimie, studiul a avut în vedere și stabilirea mecanismului de interacțiune cu ABZ. Modificările spectrale și constantele fizico-chimice (conductivitate, constantă dielectrică) au aratat că perechea de ioni ABZ-CR se formează la suprafața agregatelor de CR prin interacțiuni electrostatice și transfer de sarcină.

Keywords: albendazole, Congo red, non-extractive, assay, resonance light scattering

Introduction

ABZ is a benzimidazole derivative, chemicaly: (methyl N-[5-(propylsulfanyl-1-H-benzimidazol-2-yl)] carbamate), used as antihelmintic agent [2].

Recent studies indicated it as having antitumor effect in various forms of cancer [18] and Alzheimer disease [29]. Thus, elucidation of action mechanism, obtaining pharmaceutical forms with controlled release [15] and development of new rapid and sensitive assay methods are studies of great interest [12]. The completion of the analytical profile of pharmaceutical substances with the ability to form ion pairs with various counterions is useful mainly for drug control but also for pharmaceutical technology, by obtaining new formulations with predictable biodisponibility, and in pharmacological studies, ion pairing being one of the mechanisms of the absorption [6]. We previously reported ion-pair based determination methods of different pharmaceutical substances [3, 5, 6]. Ion pairs are

also known as ionic associations or ionic association complexes. Mechanisms underlying the formation of ion pairs depend on the structure of the interacting ions and reaction media; spectrophotometric techniques are the most used in this regard. Ion pairs formation is based on the electrostatic or hydrophobic interactions, charge transfer and proton transfer [5, 25]. For the drugs assay, widely used are the extractive ion-pair spectrophotometric methods. Extraction is a laborious procedure and the trends are to develop non-extractive (extraction-free) methods. For the determination of ABZ two non-extractive ion pair spectrophotometric methods are reported [23].

The objective of this work was to investigate the ability of ABZ to form ion pairs, in aqueous media, in order to develop a non-extractive spectrophotometric assay method. The dye used as counterion was Congo red (CR), a sulfonated azo dye (3,3'-[[1,1'-biphenyl]-4,4'-diylbis(azo)]bis[4-amino-1-naphthalene-sulfonic acid] di-sodium salt).

CR has a fully symmetric structure, only one pK_a value (4.93 - 5.95) being reported for the two aromatic amino groups [13]. Recent applications of CR in analytical chemistry are ion pair based light scattering methods used for the drug assay [7, 28]. Interesting and studied applications are the pharmacological ones, being considered to have neuroprotective effect in a variety of models of neurodegenerative diseases [29] and it has the ability to selectively interact with antigen-complexed antibodies, aspect that indicate the possibilities for its use in the targeted transport of drugs [22].

The study also focused on the mechanism of interaction between ABZ and CR. The ionic association equilibrium between ABZ and CR was studied using spectrophotometric methods, namely UV-VIS absorption and resonance light scattering (RLS), and physico-chemical constants (conductivity, dielectric constant). The influence of the experimental conditions on the interaction with CR was studied starting from the physicochemical characteristics (lipophilicity, acidity) of ABZ. A new non-extractive ion-pair spectrophotometric method for the determination of ABZ was developed.

Materials and Methods

Reagents and solutions

A 2 mM stock solution of ABZ (Sigma - Aldrich) was prepared by dissolving the appropriate amount of substance in acetic acid (analytical grade – 99.8%, 1.05 g/cm³). Working solutions were prepared by diluting 5 mL of stock solutions to 50 mL using the same solvent.

CR, solution A, was prepared by dissolving 0.1000 g substance (Scharlau) in water using a 250 mL volumetric flask. Solution B was prepared by diluting 8.8 mL solution A in water, using a 25 mL volumetric flask.

Britton-Robinson (BR) buffer was prepared by mixing 0.04 M phosphoric acid, 0.04 M acetic acid and 0.04 M boric acid in 1 L of water. The pH of the buffer was adjusted to 2 - 3.5 pH values with 0.2 M NaOH solution.

All reagents were of analytical grade and double distilled water was used throughout.

Apparatus

UV-Vis spectra were recorded on a Jasco V-730 spectrophotometer, in the range 300 - 700 nm.

For the RLS spectra, a dual monochromator spectrofluorimeter (LS50B from Perkin Elmer, Inc., USA) was used, in the synchronous scanning mode, at the same excitation and emission wavelengths ($\lambda_{excitation} = \lambda_{emission}$), using a 1 cm path length quartz cell and 1% emission attenuation filter in the emission beam.

Conductivity measurements were performed at a Conductivity Meter, DK 60GK and dielectric constant

was determined on a Radelkis Dielectric Constant Meter.

General procedure

In a 10 mL volumetric flask appropriate amounts of ABZ working solutions, acetic acid to 2 mL, 6 mL of water, 1 mL of BR buffer solution (pH = 2.5) and 1 mL of CR, solution B, were successively added; the mixture was thoroughly shaken. The reagent blank was prepared using the same procedure, without ABZ.

Validation procedure

Validation of the method was performed according to ICH Q2(R1) [8] instructions for linearity, detection and quantification limits, precision (repeatability, intermediate precision) and accuracy.

Assay of ABZ in bulk pharmaceutical substance

ABZ pharmaceutical substance was a gift from Arena Group SA, Romania. Determinations were performed according to the procedure described at *General procedure* section.

Results and Discussion

Solvent selection

ABZ is a hydrophobic compound, highly soluble in dimethylsulphoxide, acetic acid and chloroform [10]. In order to develop a non-extractive ion-pair spectrophotometric method, a water miscible solvent was selected. Acetic acid is a protogenic solvent with levelling effect of the basic function of dissolved substances. ABZ is an amphiprotic compound. In water, pK_a values reported corresponds to deprotonation of amino group at position 1 (pK_{a1} : 9.79 - 10.4) and to deprotonation of protonated nitrogen at position 3 (pK_{a2} : 2.90 - 3.37) [10]. Thus in acidic media ABZ exists in cationic form, $ABZH^+$. In the same solvent, due to the two $-SO_3^-$ groups, CR exists in anionic form. The protonated amino groups can form ion pairs with CH_3COO^- (Figure 1).

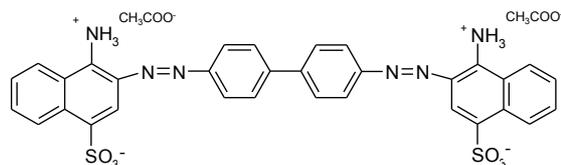
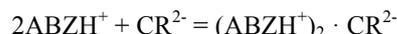


Figure 1.

CR as ionized form in CH_3COOH

Thus, ABZ-CR ion pairing can take place due to the electrostatic attraction between $ABZH^+$ and CR^{2-} :



Dielectric constant of CH_3COOH/H_2O mixtures

According to the Bjerrum model, ion pairs were considered only in solvents with relative permittivity between 10 and 40 [27]. Solvent mixtures have characteristic dielectric constants. In order to evaluate the mechanism of the ion pairing between ABZ and

CR, dielectric constants for different mixtures of $\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ were determined. The obtained results are presented in the Table I.

Table I

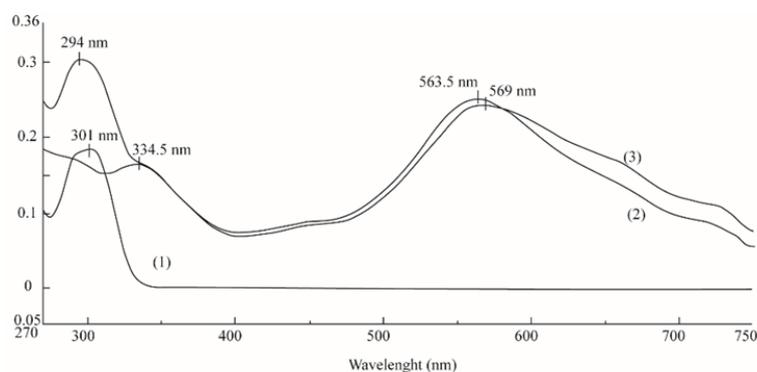
Dielectric constant values for $\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ mixtures

$\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ (v/v)	CH_3COOH (%)	ϵ
2/8	21	20.3
1.5/8.5	15.75	20.5
1/9	10.50	22.8
0.5/9.5	5.25	23.5
0/10	0	72

Values of the determined parameter confirm that in $\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ mixture the main driving force for ABZ-CR ion pairing is electrostatic interaction.

The influence of pH

Further, ABZ-CR ion pairing was studied without and with buffer system added. The optimum pH of the buffer system was selected by analysing the changes in the UV-Vis absorption spectra registered for solutions of ABZ 2.86 $\mu\text{g}/\text{mL}$, CR $2 \cdot 10^{-8}$ M and ABZ-CR ion pair, using as solvent a $\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ 2/8 (v/v) mixture and BR buffer with pH values between 2 and 3.5. For the buffer with pH = 2.5, changes in the absorption spectra indicated ABZ-CR formation (Figure 2).

**Figure 2.**

Absorption spectra for (1) ABZ 2.86 $\mu\text{g}/\text{mL}$, (2) CR $2 \cdot 10^{-8}$ M and (3) ABZ-CR ion-pair

In the CR absorption spectrum, two maximum appeared at 334 and 563 nm. After the interaction with ABZ, maximum at 563 nm was red-shifted with 6 nm, at 569 nm. ABZ has a maximum at 301 nm, blue and hyperchromically shifted at 294 nm, when the ABZ-CR ion pair is formed, indicating that the charge transfer takes place between the interacting ions [5]. For the further determinations, the maximum at 294 nm was considered.

Conductivity measurements

Electrical conductivity measurements provide experimental evidence for the existence of ion pairs in solution. A decrease of the observed conductivity can be attributed to ion pairing [4]. Conductivity values determined for the CR $2 \cdot 10^{-8}$ M and ABZ-CR, solutions in $\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ 2/8 (v/v), decrease as ABZ concentration increase confirming thus the ion pair formation by electrostatic interaction (Table II).

Table II

Conductivity values for CR and ABZ-CR ion pair solutions

$\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ 2/8 (v/v)	Conductivity ($\mu\text{s}/\text{cm}$)
CR	1940
ABZ-CR (2.38 $\mu\text{g}/\text{mL}$)	1920
ABZ-CR (3.34 $\mu\text{g}/\text{mL}$)	1909

The influence of reagents addition order

Experiments showed that the reagents addition order has an important influence on the solubility of the ionic association ABZ-CR [7]. The optimum reagents addition order is (1) ABZ solution, (2) H_2O , (3) BR buffer and (4) CR solution.

The influence of concentration of CR

The influence of CR concentration was studied in the range 0.5 - $3 \cdot 10^{-8}$ M. Soluble and stable ion pair was obtained for the concentration $2 \cdot 10^{-8}$ M.

Stability of the ABZ-CR ion pair

The stability of the ABZ-CR ion pair was studied within an hour by determining the absorbance at 294 nm from 10 to 10 minutes. The absorbance values did not undergo significant changes indicating the stability of the ion-pair.

The mechanism of ABZ-CR ion pair formation

The interaction mechanism underlying the formation of ABZ-CR ion pair was established by correlating the data obtained from absorption spectrometry with those obtained from RLS technique and physico-chemical constants (dielectrical constant, conductivity). The structure of CR suggests that it can react through a combination of hydrophobic and electrostatic interactions [11]. In addition, it has the property to self-associate forming supramolecular assemblies of a polyanionic nature [16] or rod-like micelles due to parallel stacking of the aromatic groups [19]. In water

solutions this property is manifested at concentrations higher than $5 \cdot 10^{-6}$ M, low pH values or high ionic strength. In order to establish the mechanism of interaction with ABZ we have investigated the influence of the used reaction media on the aggregation of CR. For the purpose, RLS spectrometry was used. RLS is a useful spectroscopic technique for the study of the conformational and volume changes of the molecules, especially because of the aggregation phenomena. It was first predicted by Placzek in the mid-thirties. Later it was studied as resonance enhanced Rayleigh scattering (RERS) for diphenylpolyenes [1], for a series of coumarin dyes [21], for aggregates containing porphyrins [17]. When chemical interactions lead to an increase of the molecular volume, changes appeared in the steady-state elastic scattering (Rayleigh) spectrum and correlate with the absorption and scattering spectra

of the parent molecule as follows: (a) if the volume remains within the Rayleigh range (i.e. for larger molecules), then the light scattering spectrum has the same shape as the parent molecule, the emission intensity increases, and there are no correlations with the absorption spectra [26] and, (b) if the volume is considerably greater than that associated to Rayleigh scattering (i.e., for aggregates or ionic associations), then new bands appear in the scattering spectrum, largely correlated to the absorption UV-VIS bands of the molecules alone. In this case, the intensity of the scattered light is greatly enhanced due to a resonance effect and no longer obeys the Rayleigh law ($I \sim \lambda^{-4}$, where λ is the excitation wavelength) [17].

RLS spectra registered for solutions containing CR $2 \cdot 10^{-8}$ M and 1 mL BR buffer pH = 2.5 and increasing amounts of acetic acid are presented in Figure 3.

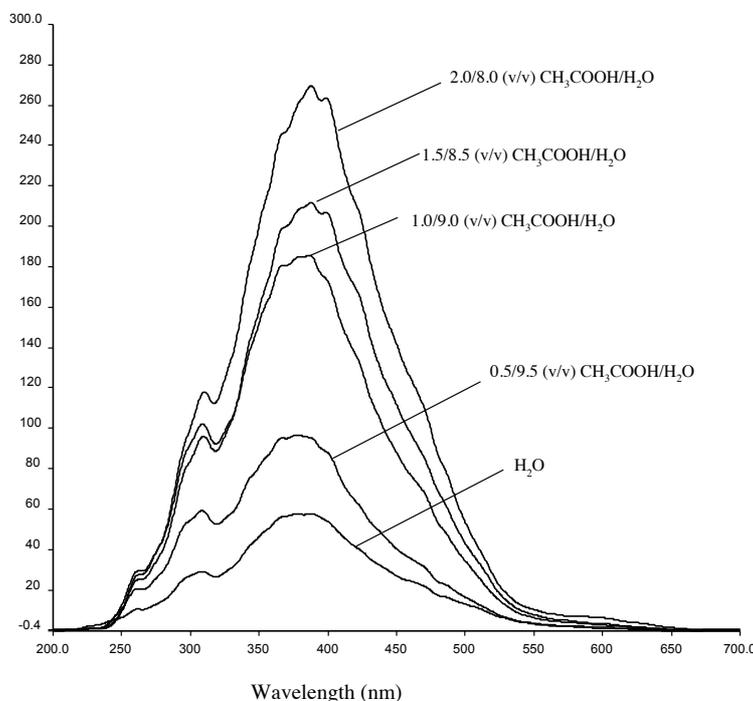


Figure 3.

RLS spectra for CR solutions ($2 \cdot 10^{-8}$ M) in $\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ solvent mixtures

It can be noticed a great intensity enhancement of the scattered light for the CR solution prepared using as solvent $\text{CH}_3\text{COOH}/\text{H}_2\text{O}$ mixture 2/8 (v/v), with a maximum at 375 nm, maximum that does not correlate with the absorption spectra. These changes indicate the aggregation of CR molecules [26].

UV-Vis spectra were registered for solutions with constant concentration of CR ($2 \cdot 10^{-8}$ M) and increasing concentration of ABZ. It was observed an increase of the absorbance measured at 294 nm with the increasing ABZ concentrations until it reached a plateau (above 4.3 $\mu\text{g}/\text{mL}$ ABZ) (Figure 4). This can

be an indication that the ABZ-CR ion pair is formed at the surface of CR aggregates.

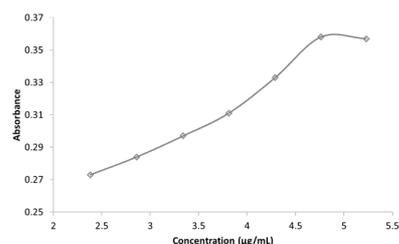


Figure 4.

Absorbance variation at 294 nm from ABZ-CR ion pair spectra depending on ABZ concentration

Based on the presented experimental data, it can be stated that the formation of ABZ-CR ion pair takes place at the surface of the CR micelles because of electrostatic and hydrophobic interactions.

Method validation

Validation parameters were determined by measuring the absorbance at 294 nm for solutions of ABZ-CR ion pair prepared as described in *General procedure* section.

The linearity was investigated between 2 µg/mL and 5.5 µg/mL ABZ and the method was found to be linear between 2.5 µg/mL and 4.7 µg/mL.

The limits of detection (0.09 µg/mL) and quantification (0.28 µg/mL) were evaluated on the basis of the

standard deviation obtained for the standard (measured in six replicates) and the slope.

The precision, expressed as the relative standard deviation of the determined content measured in the same day, by the same analyst on six replicates, was found to be 0.55%. The intermediate precision, expressed as the relative standard deviation of the determined content measured in different days by different analysts, was found to be 0.80%.

Accuracy was evaluated as recovery of ABZ within the range 80% to 120% of the considered concentration. The accuracy of the method was established to be 100.07 ± 0.42 . A summary of the validation parameters is given in Table III.

Table III

Summary of the validation parameters

Validation criterion	Parameter	Results
Linearity	Regression parameters	$y = 0.0291x - 0.1862$
	Correlation coefficient	0.9969
Range		2.5 - 4.7 µg/mL
Detection limit		0.09 µg/mL
Quantification limit		0.28 µg/mL
Accuracy		100.07 ± 0.42
Precision	Repeatability	RSD = 0.55%
	Reproducibility	RSD = 0.80%

The validation parameters indicate that the sensitivity of the method is comparable with the

non-extractive ion pair spectrophotometric published methods (Table IV).

Table IV

Comparison of the performance of the present work with published methods

Counterion	LOQ (µg/mL)	Linearity range (µg/mL)	Reference
Bromophenol blue	0.02	1.5 - 21.0	23
Bromothymol blue	0.49	2.0 - 32.0	23
Congo red	0.28	2.5 - 4.7	Our study

Analytical applications

The aim of any rule governing the production, distribution and use of medicinal products must be the safeguard of pharmaceutical health [24]. In the field of drug analysis, the analytical investigation of bulk drug materials is a very important area of research [14]. In Pharmacopoeias, the official documents that regulate the quality of medicines [20], UV spectroscopic assay methods represent approximately 10% of the assay method of bulk drug materials [14].

The proposed assay method of ABZ as ion pair with CR, was applied for the determination of the drug in bulk pharmaceutical substances. Determinations were performed as described in *General procedure* section. The average recovery obtained was 100.31%.

Reported plasmatic concentration of ABZ was 0.918 µg/mL [9]. The quantification limit of the proposed assay method was 0.25 µg/mL thus being suitable for the identification of ABZ in plasma.

Limitations of the proposed method owe to the solvent used for the solubilisation of ABZ. Acetic acid partially dissolves the excipients from pharmaceutical

formulations, which interferes the determination by modifying the ionic strength in the reaction medium.

Conclusions

A new non-extractive spectrophotometric method for ABZ assay was developed based on the ABZ-CR ion pair with characteristic absorbance at 294 nm. A quantitative ionic association equilibrium was obtained in a binary solvent (CH₃COOH/H₂O 2/8 (v/v) mixture), using a $2 \cdot 10^{-8}$ M concentration of CR and pH = 2.5 BR buffer.

Spectrophotometric data correlated with physico-chemical constants of the reaction medium indicated that CR exists as large aggregates. Consequently, the ABZ-CR ion pair is formed at the surface of "micelles" because of the electrostatic and charge transfer interactions. The main advantage of the study is that completes the analytical profile of ABZ with data regarding the ability to form ion pairs in aqueous solutions with CR, dye used as model in biochemical studies. Experimental data regarding the interaction mechanisms of ABZ with biological active

substances can be used as models for studying the antitumoral mechanism.

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