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Spectroscopic Studies and Applications of the Reactions of Some Anti-Diabetic and Anti-Hypertensive Drugs with Rose Bengal

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Abstract

Simple, rapid and sensitive spectrophotometric methods were developed and validated for the microdetermination of terazosin HCl, doxazosin mesylate and pioglitazone HCl drugs in pure and pharmaceutical dosage forms. These methods are based on ion-pair formation reaction between these drugs and a chromogenic reagent Rose Bengal (RBeng). These reactions were studied under various conditions and the optimum parameters were selected. The spectrophotometric microdeterminations have been done at λ_{max}=570 nm for terazosin HCl and pioglitazone HCl and at λ_{max}=575 nm for doxazosin mesylate. Under proper conditions the suggested procedures were successfully applied for microdetermination of these drugs in pure and in pharmaceutical dosage forms. The values of SD, RSD, recovery %, LOD, LOQ and Sandell sensitivity refer to the high accuracy and precession of the applied procedures. The results obtained were compared with the data obtained by the official methods, referring to confidence and agreement with rose bengal procedure results. The solid drugs-reagent ion-pairs were prepared, separated and their structures were investigated using elemental analysis, FT-IR, ^{1}H-NMR and thermal analyses and the results confirm the structures proposed by the stoichoimetric ratio in the solution work. The biological activities of the drugs and their solid ion-pairs against some types of (G-) and (G+) bacteria and fungai were studied and compared with each other. It is found that terazosin-RBeng and pioglitazone-RBeng reaction products have antibacterial effect higher than the parent drugs, but doxazosin-RBeng reaction product has almost the same antibacterial effect of the parent drug.

Keywords: Terazosin HCl; Doxazosin mesylate; Pioglitazone HCl; Spectrophotometric microdetermination; Rose Bengal Reagent; Spectroscopic study

Introduction

Terazosin (TRZ) HCl dihydrate has an IUPAC name (1-(4-amino-6, 7- dimethoxyquinazoline-2-yl)-4-[[(2RS)-tetrahydrofuran-2-yl] carbonyl] piperazine hydrochloride dihydrate [1]) and structure given in Figure 1. It has a general formula $C_{19}H_{25}N_5O_4.HCl.2H_2O$, and mole mass=459.9 g.mol^{-1}.

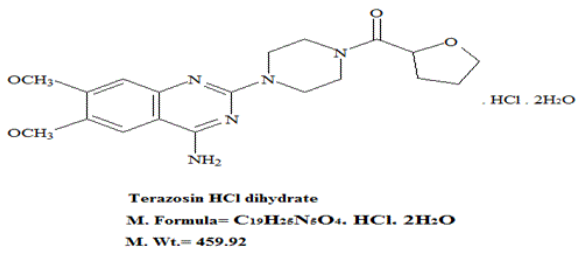


Figure 1: Structure of TRZ HCl dehydrate.

Doxazosin (DOX) mesylate has an IUPAC name (1-(4-amino-6, 7-dimethoxy-2- quinazolinyl)-4-[(2, 3-dihydro-1, 4-dioxin-2-yl) carbonyl] piperazine methanesulphonate) [2] and structure given by Figure 2. It has a general formula $C_{23}H_{25}N_5O_5.CH_3SO_3H$, and mol mass=547.6 g mol^{-1}.

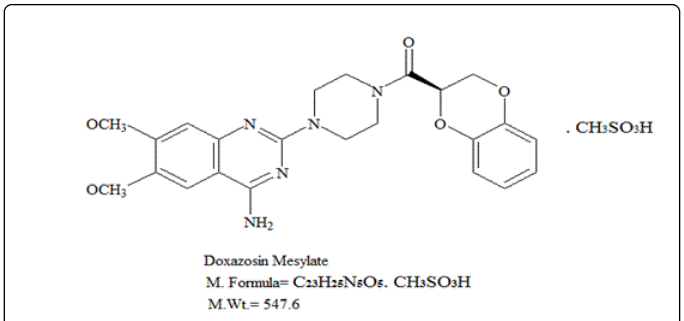


Figure 2: Structure of DOX Mesylate.

TRZ HCl dihydrate and DOX mesylate both are highly selective potent alpha-1 adrenoreceptor antagonists used in the treatment of hypertension [3] and benign prostatic hyperplasia [4,5]. The methods available for the determination of TRZ in pure, pharmaceutical and biological samples included titrimetric method [1], high performance liquid chromatography (HPLC) [3], spectrophotometric methods [6], potentiometric sensors [7] and stripping voltammetry [8].

A number of studies were described for the determination of DOX in pure, pharmaceutical and biological samples. These methods include reversed phase (RP)-HPLC [9], liquid chromatography (LC)-mass spectrometry (MS) [10], spectrophotometric methods [11] and voltammetry [12].

Pioglitazone (PIOG) HCl has an IUPAC name (5-[[4-[2-(5-Ethyl-2 pyridyl) ethoxy] phenyl] methyl]-2, 4-thiazolidinedione

hydrochloride) and structure given in Figure 3; it has a general formula C19H20N2O3S.HCl, and mol mass=392.9 g mol^{-1} [13].

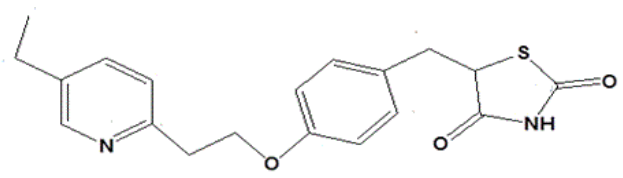


Figure 3: Structure of PIOG HCl.

PIOG is indicated as an adjunct to diet and exercise to improve glycemic control in adults with type 2 diabetes mellitus in multiple clinical settings [14]. It can also be used as a stopper in horseracing [15].

PIOG was determined in pure, pharmaceutical and biological samples using various methods alone or in a combination with other drug substances. These methods include HPLC [16], LC/MS/MS [17], spectrophotometric methods [18], Potentiometric Sensors [19], and voltammetry [20].

Rose Bengal (RBeng) dye has an IUPAC name (4, 5, 6, 7-tetrachloro-2′, 4′, 5′, 7′-tetra iodo fluorescein disodium salt) and possible structure given in Figure 4. It is an anionic water soluble xanthene dye [21].

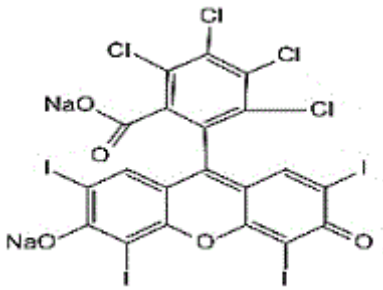


Figure 4: Structure of RBeng disodium salt.

Various spectrophotometric methods were reported; where RBeng was used as a reagent for the determination of different drugs [22,23].

This present work is concerned with the spectrophotometric study of the reaction between TRZ, DOX and PIOG drugs with RBeng reagent, and how to use these reactions as new, simple and sensitive methods for the microdetermination of these drugs. It also contains spectroscopic studies on the solid reaction products for their structures investigation and a study of their biological activities.

Experimental

Materials and reagents

All chemicals used were of analytical reagent grade (AR), and of highest purity available. They included PIOG HCl, an authentic sample was kindly supplied by Elrazy Pharmaceutical Co, Ismailia (Egypt), TRZ HCl dihydrate and DOX mesylate, and authentic samples were kindly supplied by national organization for drug and control research (NODCAR), Cairo (Egypt). Itrin tablets (Kahira Pharm. & Chem. IND. CO, Egypt, under license from ABBOTT Laboratories) labeled to contain 2 mg TRZ per tablet, Dosin (EIPICO, Egypt) labeled to contain 4 mg DOX per tablet and Glustin (Takeda Pharmaceutical Company Limited, Japan) labeled to contain 30 mg PIOG per tablet, were collected from local market in Cairo, Egypt. RBeng disodium salt reagent was supplied from BDH Chemicals Ltd, Poole, England.

Absolute ethanol (99.8%, Sigma Aldrich, Germany), phosphoric acid (88%, BDH, England), acetic acid (El Salam for chemical industries, Egypt), boric acid (ADWIC), sodium hydroxide (Merck, Germany) and distilled water, obtained from all glass equipment, were used.

Instruments

The spectrophotometric measurements in solutions were carried out using Spectrophotometer, Thermo fisher scientific, model Evolution 60 v2 recording spectrophotometer, USA, UV-Vis ranged from 190 to 1100 nm, with matched quartz cell of 1 cm optical length. Elemental microanalysis of the separated solid ion-pairs, for C, H and N were performed in the Microanalytical Centre, Cairo University using Elementar CHNS analyzer, model Vario EL III. Infrared Spectra were recorded on FTIR 4100, Jasco spectrophotometer in wavenumber region 4000-400 cm^{-1}. The spectra were recorded as KBr pellets. The ^{1}H-NMR spectra were recorded with a varian-300 MHz in DMSO-d6 as solvent, where the chemical shifts were determined relative to the solvent peaks. The thermal analyses (TGA, DTG and DTA) were carried out in dynamic nitrogen atmosphere (20 mL min^{-1}) with a heating rate of 10°C min^{-1} using Shimadzu system of DTG-60H thermal analyzers.

Solutions

Stock solution of (1×10^{-3} M) of the three drugs were prepared by dissolving the accurately weighed amount of the pure drugs (0.0393 g, 0.0459 g, 0.0548 g for PIOG, TRZ and DOX, respectively) in the appropriate volume of absolute ethanol for PIOG, distilled water for TRZ and (ethanol: distilled water) mixture (1:3) for DOX and the volume was completed to 100 mL volumetric flask. Diluted solutions were prepared by accurate dilution from the stock solutions to get the desired concentrations. Solution of 1×10^{-3} M RBeng disodium salt was prepared by dissolving the accurately weighed amount in the appropriate volume of distilled water and the volume completed to 250 mL volumetric flask.

The universal buffer (0.04 M acid mixture of acetic, boric and phosphoric acids) solutions of different pH values (2.00 to 11.05) were prepared as recommended by Britton and Robinson [24].

Sample Solutions

Ten tablets of Itrin (2 mg/tablet) and Glustin (30 mg/tablet) were powdered well separately. Equivalent amount of powder to two tablets of Itrin and one tablet of Glustin were weighed and dissolved in sufficient amount of distilled water and absolute ethanol, respectively, with gentle warming. The resulting solutions were filtered. The solutions were transferred to 100 mL volumetric flask after cooling and the volume completed to the mark with the appropriate solvent.

For dosing (4 mg/tablet), 20 tablets were powdered well. Equivalent amount of powder to 2 tablets were weighed and dissolved in about 60

mL ethanol with gentle warming for 30 min. The resulting solutions were filtered. Ethanol was evaporated to volume equal to 5 mL. The solution was transferred to 100 mL volumetric flask after cooling and the volume completed to the mark with distilled water and filtered if necessary.

Procedures

Selection of suitable wavelength procedure: The spectra of 1×10^{-4} M of the three drugs and 0.25×10^{-4} M of RBeng were measured separately at 200-400 nm, and 450-650 nm, respectively in order to determine the λ_{max} of each of them. On the other hand 1 mL of 10^{-3} M solution of RBeng was added to 1 mL of 10^{-3} M solution of the standard drugs solution in 10 mL measuring flask. The mixture obtained was scanned in the wavelength range 450-650 nm using the reagent as a blank in order to determine the λ_{max} of the formed products.

Selection of suitable pH, time and temperature procedures: To select the optimum pH value; the spectra of mixture of 1×10^{-4} M of the standard drugs and 1×10^{-4} M of RBeng were measured at 450-650 nm using the reagent as a blank at different pH values using buffer solutions. A suitable amount of ethanol was added to the solutions to dissolve any formed precipitates. To select the optimum time and temperature; these mixtures were measured also at different time intervals and different temperature values using the reagent as a blank.

Effect of ethanol volume: Effect of ethanol volume was studied on DOX reaction product with RBeng; where different volumes of ethanol (0.5-5 mL) were added to mixture of 1×10^{-4} M of the standard drug and 1×10^{-4} M of RBeng. The spectrophotometric measurements were recorded using the reagent as a blank to select the optimum ethanol volume.

The stoichiometric ratio of reaction: The stoichiometry of these reactions was also studied applying Job's continuous variation method (CVM) [25] and molar ratio method (MRM) [26].

Calibration curve: RBeng solution (1×10^{-3} M) was added to variable concentrations of the standard drugs (4.599-41.39 μg mL^{-1}, 10.95-54.76 μg mL^{-1} and 11.79-31.43 μg mL^{-1} for TRZ, DOX and PIOG, respectively) under proper selected conditions and the volume was completed to 10 mL H_2O. The absorbance was plotted against drugs concentrations at selected λ_{max}=570 nm for TRZ and PIOG and 575 nm for DOX.

Within- and In-between-day measurements: The effect of long time on the spectra of the standard drugs was carried out on five replicate experiments, at different concentrations of the standard drugs under the proper selected conditions.

Application of suggested procedures: The suggested procedures were applied for microdetermination of TRZ, DOX and PIOG in pharmaceutical Itrin, Dosin and Glustin Tablets, respectively using RBeng reagent in comparison with the official methods [6,27,28] respectively. To variable concentrations of the drugs in their pharmaceutical forms (3.334-10.00, 14.55-33.96 and 13.84-24.91 μg mL^{-1} for TRZ, DOX and PIOG, respectively) RBeng was added under previously mentioned proper selected conditions. The spectra of the obtained mixtures were measured applying the suggested procedures.

Preparation of the solid drugs-reagent ion-pairs: The solid ion-pairs of TRZ, DOX and PIOG drugs with RBeng were prepared, by addition of a warm solution of appropriate weight of RBeng of 0.5088 g (0.5 mmol), dissolved in least amount of water; to warm solutions of 0.2300 g (0.5 mmol) TRZ, 0.2738 g (0.5 mmol) DOX, dissolved in least amount of water and 0.1965 g (0.5 mmol) PIOG, dissolved in least amount of ethanol. The resulted solid ion-pairs were appeared as precipitates. These precipitates leaved with gentle warming for 10 min, filtered, dried and recrystallized from ethanol. The melting points of these ion-pairs were measured.

Results and Discussion

Spectrophotometric studies on the reaction of RBeng with the selected standard drugs

Figure 5 shows the spectra of: RBeng 0.25×10^{-4} M and TRZ (10^{-4} M) - RBeng (10^{-4} M), PIOG (10^{-4} M) - RBeng (10^{-4} M), DOX (10^{-4} M) - RBeng (10^{-4} M) reaction products using reagent as a blank.

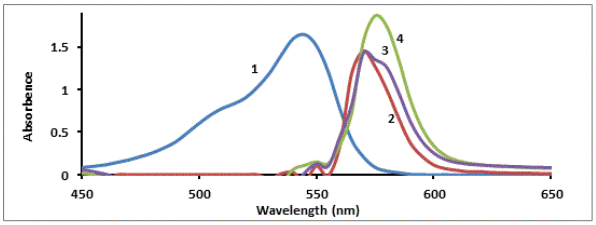


Figure 5: Vis. absorption spectra of: (1) RBeng 0.25×10^{-4} M, (2) TRZ (10^{-4} M) - RBeng (10^{-4} M) reaction product, (3) PIOG (10^{-4} M) - RBeng (10^{-4} M) reaction product, (4) DOX (10^{-4} M) - RBeng (10^{-4} M) reaction product.

From Figure 5, λ_{max}=570 nm is selected as a suitable wavelength for microdetermination of TRZ and PIOG with RBeng reagent and λ_{max}=575 nm is selected as a suitable wavelength for microdetermination of DOX with RBeng reagent, which are away from that of RBeng reagent (λ_{max}=545 nm).

Effect of pH (2.00-11.05) on the spectrum of TRZ-RBeng and PIOG-RBeng products was studied spectrophotometrically at λ_{max}=570 nm. The results show that the maximum absorbance attained at pH 5 for both of them with the highest molar absorptivity, ε=1.442 x 104 and 1.488 x 10^4 L mol^{-1} cm^{-1} for TRZ and PIOG, respectively.

Effect of temperature was studied in the temperature range 25-70°C on absorption spectrum of TRZ-RBeng and PIOG-RBeng reaction products at λ_{max}=570 nm, pH 5 and in the range 26-45°C on absorption spectrum of DOX-RBeng reaction product at λ_{max}=575 nm. These results show that the optimum temperatures are found to be; room temperature (25 ± 2 °C), 35 ± 1°C and 30-35 °C, for TRZ, PIOG and DOX, respectively with molar absorptivity ε =1.198×10^4, 1.640×10^4 and 2.070×10^4 L mol^{-1} cm^{-1}, respectively.

Studying the effect of time (0-60 min.) on the formation of the three products at the selected conditions shows that the three reaction products are stable over one hour and the time has no significant effect on their stabilities.

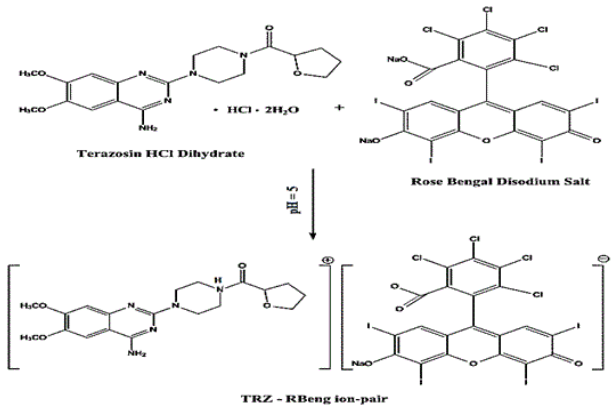
For DOX-RBeng reaction product an extra ethanol had to be added to prevent any formation of precipitates, but this addition affects the absorbance, so ethanol volume effect was studied and the results show that the absorbance remains constant at 1.5 and 2 mL of ethanol, so ethanol volume of 2 mL is chosen as the optimum volume for the microdetermination.

Stoichiometric ratios of drugs-RBeng reaction products

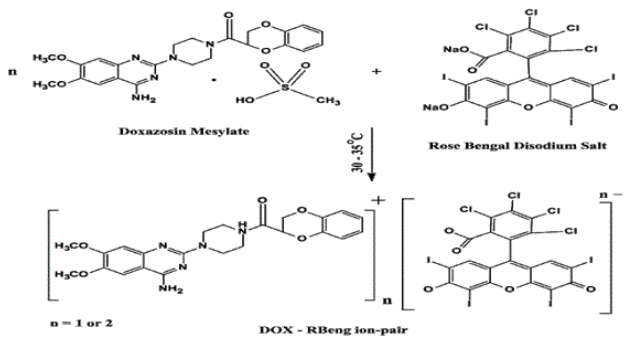
The Stoichiometric ratio of RBeng reaction with the selected drugs, which depends on ion-pair formation, was studied by the CVM [25] and MRM [26]. The results obtained from the MRM of the reaction of RBeng with variable [TRZ] refer to stoichiometric ratio between RBeng reagent and TRZ drug, (R:D), equal to (1:1). For DOX, the results obtained from CVM and MRM of the reaction of DOX with variable [RBeng] refer to stoichiometric ratio (1:1) and the results obtained from the MRM of the reaction of RBeng with variable [DOX] refer to stoichiometric ratio, equal to (1:2).

For PIOG, the results obtained from CVM refer to stoichiometric ratio, equal to (1:1) and the results obtained from the MRM of the reaction of RBeng with variable [PIOG] refer to stoichiometric ratios, (1:1) and (1:2).

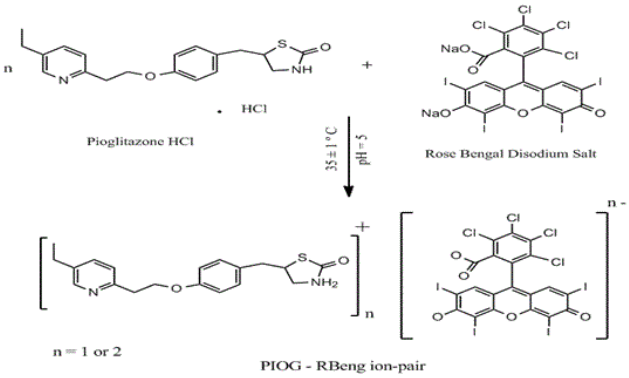
Depending upon these results; the proposed reaction for ion-pair formation between TRZ, DOX and PIOG drugs and RBeng reagent may be given by the schemes 1, 2 and 3, respectively.



Scheme (1): Proposed reaction for TRZ-RBeng ion-pair formation



Scheme (2): Proposed reaction for DOX-RBeng ion-pair formation.



Scheme (3): Proposed reaction for PIOG-RBeng ion-pair formation.

Calibration curve

The calibration curves of TRZ, DOX and PIOG drugs and RBeng reagent were constructed by plotting the absorbance against variable concentrations of the drugs under the selected proper conditions. These calibration curves were constructed at λ_{max}=570 nm for TRZ and PIOG and at 575 nm for DOX. The results show that the three calibration curves are rectilinear in the concentration range of 4.599-41.39 μg mL^{-1}, 10.95-54.76 μg mL^{-1} and 11.79-31.43 μg mL^{-1}, for TRZ, DOX and PIOG, respectively (Table 1).

Parameters	Drug		
	TRZ	DOX	PIOG
Reagent	RBeng		
Temperature (°C)	Room Temperature (25 ± 2)	30-35	35 ± 1°C
λmax (nm)	570	575	570
pH	5	-	5
Linearity (μg mL^{-1})	4.599-41.39	10.95-54.76	11.79-31.43
LOD (μg mL^{-1})	0.7731	1.303	1.051
LoQ (μg mL^{-1})	2.343	3.947	3.184
R^2	0.9998	0.9994	0.9982
Regression equation	Y=0.0398x + 0.0044	Y=0.0334x + 0.175	Y=0.0391x + 0.0861
Molar absorptivity (L mol^{-1} cm^{-1})	1.831×104	1.826×10^4	1.534×10^4
SD	0.0503-0.1501	0.0650-0.4604	0.1030-0.2798
RSD %	0.1414-1.767	0.2651-1.099	0.4344-1.082
Sandell sensitivity (μg cm^{-2})	0.0054	0.0055	0.0065

Recovery %	98.57-101.14	97.87-101.6	98.52-101.4

Table 1: Analytical parameters for spectrophotometric determination of standard TRZ, DOX and PIOG drugs by proposed RBeng method.

From Table 1, the high values of the molar absorptivities (1.831×10^4, 1.826×10^4 and 1.534×10^4 L mol^{-1} cm^{-1}) indicate the sensitivity of the proposed methods. The correlation coefficient values are found to be 0.9998, 0.9994 and 0.9982; which supports the linearity of the curves. Also the mean recovery values obtained are in the ranges of 98.57-101.14%, 97.87-101.6% and 98.52-101.4%; which indicate the high accuracy of the applied procedures in determination of standard TRZ, DPX and PIOG drugs, respectively. The low values of standard deviation (SD) (0.0503-0.1501, 0.0650-0.4604 and 0.1030-0.2798) and relative standard deviation (RSD %) (0.1414-1.767%, 0.2651-1.099% and 0.4344-1.082%), for n=5, indicate the high precision of the applied procedures under the selected proper conditions. The values of the limit of detection (LOD) are found to be 0.7731, 1.303 and 1.051 µg mL-1 for TRZ, DOX and PIOG, respectively, and limit of quantification (LoQ) are found to be 2.343, 3.947 and 3.184 µg mL^{-1} for TRZ, DOX and PIOG, respectively. The low values of Sandell sensitivity (S.S=0.0054, 0.0055 and 0.0065 µg cm^{-2}) refer to the high sensitivity of the proposed methods. From these parameters it is concluded that, the proposed spectrophotometric methods can be applied successfully for the determination of TRZ, DOX and PIOG drugs, respectively, in the concentration range mentioned above with a high accuracy, precision and sensitivity.

Within- and In-between-day measurements

The results obtained from within- and in-between-day measurements are shown in Tables 2 and 3, respectively.

From Tables 2 and 3, the within- and in-between-day recovery percentage, SD and RSD % values for the three standard drugs indicate that, the proposed methods are reproducible and RBeng can be successfully applied for determination of standard TRZ, DOX and PIOG drugs via the proposed ion-pair formation reaction.

Drug	[wt.] taken (µg mL^{-1})	[wt.] found (µg mL^{-1}) ± SD	Recovery (%)	SD[a]	RSD (%)[a]
TRZ	29.18	28.97 ± 0.2058	98.67-100.4	0.2058	0.7107
	34.05	33.44 ± 0.3384	97.55-99.59	0.3384	1.014
DOX	32.85	33.09 ± 1.085	100.4-101.3	1.085	3.279
	38.33	37.88 ± 0.9191	98.21-99.46	0.9191	2.425
	41.99	42.92 ± 0.8022	101.5-103.1	0.8022	1.869
	47.31	46.56 ± 0.9286	97.50-99.33	0.9286	1.998
	52.56	52.37 ± 1.470	99.13-100.5	1.47	2.806
PIOG	25.54	25.69 ± 0.3228	98.99-102.7	0.3228	1.254
	27.5	28.05 ± 0.1877	101.2-102.7	0.1877	0.6694
	31.48	31.37 ± 0.3707	98.19-100.6	0.3707	1.18

Table 2: Within-day spectrophotometric microdetermination of standard TRZ, DOX and PIOG drugs by the proposed RBeng method. [a]Mean values for six determinations for TRZ, four determinations for DOX and five determinations for PIOG, within 5 h.

Drug	[wt.] taken (µg mL^{-1})	[wt.] found (µg mL^{-1}) ± SD	Recovery (%)	SD[a]	RSD (%)[a]
TRZ	29.18	30.46 ± 0.3421	103.0-106.9	0.3421	1.123
	34.05	35.12 ± 0.3524	101.9-104.7	0.3524	1.002
DOX	32.85	32.59 ± 1.293	96.68-101.6	1.293	3.279
PIOG	25.54	25.36 ± 0.3285	98.13-100.4	0.3285	1.297
	27.5	28.25 ± 0.2658	101.6-105.0	0.2658	0.9368
	31.48	31.41 ± 0.6141	98.19-100.6	0.6141	1.95

Table 3: In-between-day spectrophotometric microdetermination of standard TRZ, DOX and PIOG drugs by the proposed RBeng method. [a]Mean values of four determinations for five days for TRZ and DOX and of four determinations for six days for PIOG.

Application of the applied procedures in comparison with the official methods

The RBeng reagent was successfully applied for the microdetermination of TRZ in Itrin, 2 mg/tablet, DOX in Dosin, 4 mg/tablet and PIOG in Glustin, 30 mg/tablet under proper conditions at λ_{max}=570 nm for TRZ and PIOG and λ_{max}=575 nm for DOX. The results obtained are compared with the official methods [6,27,28], for TRZ, DOX and PIOG, respectively and are presented in Table 4.

From Table 4, the values of the recovery and the low values of SD of the proposed methods indicate the high accuracy and precision of the proposed methods for the microdetermination of TRZ, DOX and PIOG drugs in the pharmaceutical dosage forms. The accuracy and the precision of the proposed methods are compared with those obtained from the official methods by student's t-test and F-test, respectively, at confidence limit 95% and P=0.05 [29]. The obtained values of F-test and t-test indicate that there is no significant difference between the accuracy and the precision of the proposed and the official methods and hence the reliability of the proposed methods for the routine analysis of TRZ, DOX and PIOG drugs in pure and in their pharmaceutical formulations.

Sample	Proposed method					Official methods					F-test	t-test
	[Drug] µg mL^{-1}					[Drug] µg mL^{-1}						
	Taken	Found	Recovery	SD[a]	Mean ± SD	Taken	Found	Recovery (%)[b]	SD[b]	Mean ± SD		

			(%)[a]									
TRZ in Itrin Tablet (2mg/ Tablet)	3.334	3.332	99.93	0.0208	100.52 ± 0.0521	4	3.986	99.65	0.0367	99.71 ± 0.0652	1.566 (6.256)*	22.45 (2.26)*
	5.002	4.791	98.04	0.0417		8	7.978	99.73	0.0824			
	6.669	6.783	101.7	0.0136		12	11.99	99.95	0.0696			
	8.336	8.551	102.6	0.1348		14	13.93	99.5	0.0714			
	10	10.04	100.3	0.0498								
DOX in Dosin Tablet (4mg/ Tablet)	14.55	14.66	100.7	0.1349	99.56 ± 0.3463	4	3.994	99.84		99.46 ± 0.3900	1.268 (6.944)*	0.3788 (2.45)*
	19.41	19.59	100.9	0.2691		6	5.938	98.96				
	24.26	23.78	98.03	0.3912		8	7.974	99.68				
	29.11	28.84	99.07	0.3712		10	9.934	99.34				
	33.96	33.63	99.03	0.5652								
PIOG in Glustin Tablet (30 mg Tablet)	13.84	13.53	97.8	0.0968	100.0 ± 0.1772	20	19.92	99.58		99.79 ± 0.1976	1.243 (6.256)*	1.837 (2.26)*
	16.6	16.65	100.3	0.2005		30	29.95	99.83				
	19.37	19.6	101.2	0.1825		40	39.99	99.97				
	22.14	22.29	100.7	0.2222								
	24.91	24.92	100.1	0.1841								

Table 4: Spectrophotometric microdetermination of TRZ, DOX and PIOG drugs in pharmaceutical formulations by proposed RBeng method. [a]Average of five determinations. [b]Average of six determinations for TRZ, of three determinations for DOX, *The values between brackets are the tabulated F- and t-values at P=0.05 and confidence limit 95% [29].

Structure identification of drugs-RBeng products by different physicochemical methods of analyses

The structures of drugs-RBeng solid ion-pairs have been identified by different physicochemical tools which are elemental analyses (C, H, and N); IR, 1H-NMR and thermal analyses (TGA, DTGA and DTA). The products spectra are compared with those of the drugs and RBeng aiming chiefly to shed light on the mechanism of the reaction between the three drugs and RBeng in solution.

Elemental Analyses (EA) of the solid ion-pairs

The elemental analyses results, analytical and physical data of RBeng-drugs ion-pairs are given in Table 5. From these data; the general formulae of the formed solid ion-pairs are determined and their mole masses are calculated.

Ion-pair	R:D	m.p (°C)	Elemental analysis Found (calcd %)		
			C	H	N
TRZ-RBeng	1:1	248	33.08	3.14	5.94
($C_{39}H_{27}Cl_4I_4N_5O_9$)			-34.36	-2.2	-5.14
Mol Mass=1359.06					
DOX-RBeng	1:2	232	41.4	3.12	6.8
($C_{66}H_{52}Cl_4I_4N_{10}O_{15}$)			-42.28	-2.793	-7.473
Mol Mass=1874.62					
PIOG-RBeng	1:2	180	41.8	2.15	3.09
($C_{58}H_{42}Cl_4I_4N_4O_{11}S_2$)			-42.32	-2.509	-3.325
Mol Mass=1685.46					

Table 5: Analytical and physical data of RBeng-drug ion-pairs.

FT-IR analysis

The FT-IR of RBeng refers to the bands of ν (C=O) stretching of the carboxylate at 1550.49 and 1492.63 cm^{-1}, these bands are shifted to lower values of wavenumbers, (1540.85-1511.92 cm^{-1} and 1490.7-1447.31 cm^{-1}), for the three drugs ion-pairs [30].

The FT-IR of TRZ refers to the bands of ν (C=O) stretching of the amide at 1633.41 cm^{-1}, and ν (C-O) stretching of the hydro-furan ring at 1111.79 and 1078.98 cm^{-1} [30]. These bands are shifted to lower values of wavenumber in the corresponding TRZ-RBeng ion-pair, ν (C=O) stretching of the amide at 1627.63 cm^{-1} and ν (C-O) stretching of the hydro-furan ring at 955.55 cm^{-1}. The FT-IR of DOX refers to the bands of, ν (C=O) stretching of the amide at 1635.34 cm^{-1}, ν (C-O) of the six membered ring at 1214.93 and 1171.54 cm^{-1} and ν (sulfoxide) stretching at 1042.34 cm^{-1} [30]. These bands are shifted to lower values of wavenumber in the corresponding DOX-RBeng ion-pair, ν (C= O) stretching of the amide at 1631.48 cm^{-1}, ν (C-O) stretching of the six membered ring at 1108.87 and 1034.62 cm^{-1} and ν (sulfoxide) stretching is disappeared. The FT-IR of PIOG refers to the bands of ν

(C-H) stretching of the aliphatic alkane at 2742.28 and 2615.97 cm^{-1}, ν (C= O) stretching of the amide at 1742.37 and 1690.3 cm^{-1}. These bands are shifted to lower values of wavenumber in the corresponding PIOG-RBeng ion-pair, ν (C-H) stretching of the aliphatic alkane are disappeared, ν (C=O) stretching of the amide at 1760.69 and 1701.87 cm^{-1}. The data of the IR spectra of RBeng, the three drugs and their reaction products are listed in Table 6.

The shift of the bands frequencies of some groups of the reagent and the drugs into lower and higher wavenumbers may be attributed to the electrostatic attraction between the cationic drugs and the anionic form of RBeng reagent.

These data confirm the proposed structures of drugs-RBeng ion-pair in schemes (1-3).

^{1}H-NMR analysis

The ^{1}H-NMR spectra of RBeng; show two peaks with chemical shifts 7.410 and 7.912 ppm. These peaks appear in the ^{1}H-NMR spectra of TRZ-RBeng ion-pair at chemical shifts 7.373 and 7.913 ppm; DOX-RBeng ion-pair at chemical shifts 7.395 and 7.918 ppm and PIOG-RBeng ion-pair with a chemical shift range 7.394-7.713 ppm. This refers to the presence of RBeng in these ion-pairs.

The ^{1}H-NMR spectra of TRZ; show peak with chemical shift 12.451 ppm; which corresponds to the proton of NH_2 group and peak with chemical shift 8.620 ppm; which corresponds to the proton of NH+ group. These peaks are shifted to 6.961 ppm (H of NH_2 group) or disappeared (H of NH+ group) in the 1H-NMR spectra of TRZ-RBeng ion-pair. The ^{1}H-NMR spectra of DOX show peak with chemical shift 11.725 ppm; which corresponds to the proton of NH_2 group, two peaks with chemical shifts 8.702 and 8.812 ppm; which corresponds to the proton of NH+ group and peak with chemical shift 2.328 ppm; which corresponds to H of the CH_3 of the mesylate group. These peaks are shifted to 6.704 ppm (H of NH_2 group) or disappeared (H of NH+ group and H of CH_3 of the mesylate group) in the ^{1}H-NMR spectra of DOX-RBeng ion-pair. The ^{1}H-NMR spectra of PIOG; show peaks in the chemical shift range 7.929-8.698 ppm; which corresponds to the protons of pyridine ring. These peaks are shifted to 8.420 ppm in the ^{1}H-NMR spectrum of PIOG-RBeng ion-pair.

These changes in chemical shifts may be due to the electrostatic attraction between the cationic drugs and the bulky anionic reagent (small charge/unit volume); which is different from the electrostatic attraction between the cationic drugs and the small anionic part, chloride in case of TRZ and PIOG and mesylate in case of DOX, (has bigger charge/unit volume). These data confirm the proposed structure of drugs-RBeng ion-pair in schemes (1-3).

Compound	C-H streching of aliphatic alkane	C=O streching of the amide	C=O streching of carboxylate	C-N streching	C-O of six membered ring	C-O streching of hydrofuran ring	Sulfoxide streching	C-O Streching of carboxylate
RBeng	-	-	1550.49 and 1492.63	-	-	-	-	950.734
TRZ	2963.09 and 2682.5	1633.41	-	1283.39 and 1244.83	-	1111.79 and 1078.98	-	-
TRZ-RBeng Prod.	2929.34 and 2364.3	1627.63	1540.85 and 1448.28	1234.22	-	955.55	-	1107.9
DOX	2943.8	1635.34	-	1266.04, 1236.15	1214.93 and 1171.54	-	1042.34	-
DOX-RBeng Prod.	2928.38	1631.48	1541.81 and 1490.7	1261.22 - 1233.25	1108.87 and 1034.62	-	disappeared	985.447
PIOG	2742.28 and 2615.97	1742.37 and 1690.3	-	-	-	-	-	-
PIOG-RBeng Prod.	disappeared	1760 69 and 1701.87	1511.92 and 1447.31	-	-	-	-	1041.37

Table 6: FT-IR characteristic peaks of the drugs (TRZ, DOX and PIOG), RBeng reagent and their ion-pairs.

Thermal analyses

The TGA curve of RBeng shows that it decomposes in three steps. The first step may be related to the loss of 3Cl radicals. The second step may be related to the loss of Na_2CO_3, $2I_2$ and $0.5Cl_2$. The third step may be related to the loss of $C_{18}H_2O$ leaving CO as a remaining part.

The TGA of TRZ refers to the decomposition of this drug in three steps. The first step may be related to the loss of two water molecules. The second step may be attributed to the loss of $C_7H_{13}NO_2$ radical. The third step may be attributed to the loss of $C_{10}H_{11}N_3O_2$.HCl molecule. It appears as strong exothermic peak in DTA; which may refers to chemical rearrangement and/or chemical recombination of the fragments to give the final formula. The TGA of TRZ-RBeng shows three steps of the thermal decomposition. The first step may be due to the loss of Cl_2. The second step may be attributed to the loss of $C_{14}H_{18}N_4O_2$ (from TRZ molecule). The third step is accompanied by two peaks at the DTA, endothermic peak and followed by exothermic one, so this step may occur at two stages. The first stage may be the loss of Cl_2 and $2I_2$ and the second one may be the breaking of the ion pair

which leads to the loss of $C_{20}H_3O_5$ and formation of $C_5H_9NO_2$ as a final residue.

The TGA of DOX shows that it decomposes in three main steps. The first step may be related to the loss of $C_{11}H_{11}NO_3$ radical. The second step may be attributed to the loss of $C_2H_7N.CH_3O_3S$. The third step may be attributed to the loss of $C_{10}H_{10}N_2O_2$ molecule. It appears as strong exothermic peak in DTA; which may refers to chemical rearrangement and/or chemical recombination of the fragments to give the final Chemical formula. The TGA of DOX-RBeng refers to thermal decomposition in four steps. The first step may be due to the loss of Cl_2. The second step may be attributed to the loss of $C_{14}H_{18}N_4O_2$ (from one of DOX molecules). The third step may be attributed to the loss of $C_{14}H_{18}N_4O_2$ (from the other DOX molecule) and the loss of Cl_2 (from RBeng molecule). The fourth step is accompanied by two peaks at the DTA, endothermic peak and followed by exothermic one, so this step may occur at two stages. The first stage may be the loss of $2I_2$ and the second one may be the breaking of the ion pair which leads to the loss of $C_{20}H_3O_5$ and $2C_9H_{10}NO_3$.

The TGA of PIOG shows that it decomposes in two main steps. The first step may be related to the loss of CH_3 and $C_{12}H_{12}NO_3S.HCl$ radicals. The second step may be attributed to the loss of pyridine molecule C_5H_5N leaving CH_2 as a final residual. The TGA of PIOG-RBeng ion-pair refers to thermal decomposition in two steps. The first step may be due to the breaking of the ion-pair and the loss of 2PIOG molecules. The second step is accompanied by two peaks at the DTA, small endothermic peak followed by strong exothermic one, so this step may occurs at two stages. The first stage may be the loss of $2I_2$ and $2Cl_2$ and the second one may be the loss of $C_{19}H_{12}O_2$ and CO_2 leaving H_2O as a final part. Thermal Analyses results of the drugs (TRZ, DOX and PIOG), RBeng reagent and their ion-pairs are given in Table 7.

Biological activity

RBeng has a bacteriostatic action where it prevents the growth of some bacteria. The drugs under study are anti-hypertensive (TRZ and DOX) or anti-diabetic (PIOG) drugs, but they may also have some biological activity and adding RBeng to their structure may affects this activity. The biological activity of TRZ HCl, DOX mesylate and PIOG HCl drugs and their solid ion-pairs with RBeng were determined using a modified Kirby-Bauer disc diffusion method towards two types of bacteria (*Escherichia coli* (G-) and *Staphylococcus aureus* (G+)) and two types of fungus (*Aspergillus flavus* and *Candida albicans*). Ampicillin was used as a reference compound for antibacterial activities and Amphotericin B was used as a reference compound for antifungal activities. Both the antibacterial and antifungal activities were evaluated by measuring the inhibition zone (mm/mg sample) (Table 8).

Compound	TG range°C (DTGmax °C)	DTA°C	Calcd (Estim) %		Assignment	Residue Calcd. (Estim) %
			Mass loss	Total mass loss		
RBeng	35-214 (65.27)	114.1 endothermic	10.46 (11.14)	97.27 (98.61)	Loss of $3Cl^-$	CO 2.75 (1.39)
	215-671 (403.9)	522.4 exothermic	63.82 (63.55)		Loss of Na_2CO_3, $2I_2$ and Cl	
	671-954 (911.9)	821.1 endothermic	22.99 (23.92)		Loss of $C_{18}H_2O$	
TRZ	72-139 (114.1)	117.9 endothermic	7.827 (6.765)	90.99 (89.63)	Loss of $2H_2O$	C_2H_7N 9.78 (10.37)
	204-358 (291.6)	345.9 exothermic	30.66 (29.94)		loss of $C_7H_{13}NO_2$	
	358-640 (564.1)	558.7 exothermic	52.51 (54.65)		Loss of $C_{10}H_{11}N_3O_2.HCl$	
TRZ-RBeng ion-pair	47-150 (59.91)	63.64 exothermic	5.22 (4.32)	91.66 (92.41)	Loss of Cl_2	$C_5H_9NO_2$ 8.46 (8.13)
	150-277 (244.4)	239.9 exothermic	20.16 (21.17)		Loss of $C_{14}H_{18}N_4O_2$ (from TRZ molecule)	
	277-632 (585.8)	506.7 endothermic	67.03 (66.17)		Loss of Cl_2, $2I_2$	
		588.2 exothermic			Loss of $C_{20}H_{12}O_5$	
DOX	218-350 (319.3)	347.4 endothermic	37.47 (35.33)	96.90 (94.75)	Loss of $C_{11}H_{11}NO_3$	NH_3 3.10 (5.54)
	350-482 (414.6)	432.8 exothermic	25.57 (24.09)		Loss of $C_2H_7N.CH_3O_3S$	
	482-659 (598.9)	603.6 exothermic	34.68 (35.33)		Loss of $C_{10}H_{10}N_2O_2$	
DOX-RBeng ion-pair	63-174 (75.34)	82.61 exothermic	3.79 (3.19)	100 (99.39)	Loss of Cl_2	-
	174-260 (249.8)	241.7 exothermic	14.62 (14.77)		Loss of $C_{14}H_{18}N_4O_2$ (from one of DOX molecules)	
	260-350 (304.3)	267.8 exothermic	18.40 (17.38)		Loss of $C_{14}H_{18}N_4O_2$ (from the other DOX molecule) and Cl_2 (form RBeng molecule)	
	350-651 (572.39)	547.6 endothermic	63.53 (64.05)		Loss of $2I_2$	

		635.8 exothermic			Loss of $2C_9H_{10}NO_3$ and $C_{20}H_3O_5$	
PIOG	68-334 (291.9)	299.0 endothermic	76.61 (75.53)	96.72 (95.24)	Loss of CH_3 and $C_{12}H_{12}NO_3S.HCl$	CH_2 3.56 (4.76)
	410-600 (537.0)	533.0 exothermic	20.11 (19.71)		Loss of C_5H_5N	
PIOG-RBeng ion-pair	40-309 (284.0)	178.4 exothermic	42.36 (43.36)	99.67 (98.40)	Loss of 2PIOG molecules	H_2O 1.07 (1.31)
	309-584 (483.9)	341.6 endothermic	57.28 (55.04)		$2I_2$ and $2Cl_2$	
		497.7			Loss of CO_2 and $C_{19}H_{12}O_2$	

Table 7: Thermal Analyses results of the drugs (TRZ, DOX and PIOG), RBeng reagent and their ion-pairs.

	Antimicrobial effect (% relative to Ampicillin)		**Antifungal effect (% relative to Amphotericin B)**	
Sample	*Escherichia coli* (G^-)	*Staphylococcus aureus* (G^+)	*Aspergillus flavus*	*Candida albicans*
TRZ	45.45	55.56	0.00	47.37
TRZ-RBeng	72.73	94.44	0.00	0.00
PIOG	0.00	0.00	0.00	0.00
PIOG-RBeng	63.64	83.33	0.00	0.00
DOX	54.55	72.22	0.00	63.16
DOX-RBeng	54.55	66.67	0.00	0.00

Table 8: Comparison between the biological activity of standard drugs and their products with RBeng.

From Table 8; it is found that the antibacterial activities of TRZ and PIOG increased after adding RBeng to their structures. It is found that TRZ-RBeng and PIOG-RBeng ion-pairs are biologically more active than the parent drugs. The antibacterial activity toward *E. coli* and *S. aureus*, in comparison with the standard found in the local market (Ampicillin), are 72.73% and 94.44% (for TRZ-RBeng ion-pair), 63.64% and 83.33% (for PIOG-RBeng ion-pair), respectively. DOX-RBeng ion-pair has almost the same bacterial activity of the parent drug. Increasing the biological activity of the products refers to the biological activity of RBeng itself found in their entities. From studying the antifungal activity of the drugs, it is found that, only TRZ and DOX, have antifungal activity toward *C. albicans*, in comparison with the standard found in the local market (Amphotericin B) (47.37% and 63.16% respectively). It is can be concluded that the drugs under study and their solid ion-pairs with RBeng are more antibacterial agents than antifungal agents.

Conclusion

This manuscript involved fast (not time consuming), cheap and reliable spectrophotometric procedures for the determination of TRZ HCl, DOX mesylate and PIOG HCl drugs depending on color reactions between them and a chromogenic reagent (RBeng). The solid reaction products were prepared, separated and characterized by elemental, spectroscopic (FT-IR, ^1H NMR) and thermal techniques. Antibacterial activity of the drugs and their reaction products with RBeng were tested by diffusion agar method and compared with each other. It is found that TRZ-RBeng and PIOG-RBeng reaction products have antimicrobial effect higher than the parent drugs, but DOX-RBeng reaction product has almost the same antimicrobial effect of the parent drug. Increasing the antimicrobial effect of the products refers to the antimicrobial effect of RBeng itself in their entities. It is also found that the drugs and their solid ion-pairs with RBeng are more antibactrial agents than antifungal agents.

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