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BROMATOMETRIC ESTIMATION OF IRBESARTAN, LOSARTAN, AND BISOPROLOL IN BULK AND TABLET FORMS: STATISTICAL ANALYSIS

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Abstract

A new spectrophotometric method is described for determination of irbesartan, losartan and bisoprolol in bulk and pharmaceutical dosage forms using insitu generated bromine as oxidizing agent and rhodamine B as chromogenic agent. Drugs are treated with known excess of bromine and residual unreacted bromine is determined by treating with fixed amount of rhodamine B then measuring absorbance at 557nm. The amount of bromine reacted corresponds to the amount of each drug. Effect of acidity, bromate-bromide volume and reaction time, on the absorption were studied. Calibration curves were linear over ranges of 4-14μg/mL, 1-5μg/mL, and 5-25μg/mL for irbesartan,

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losartan, and bisoprolol, respectively. The method was satisfactory applied for the determination of drugs in both bulk and pharmaceutical forms and results were compared statistically with reference methods.

1. Introduction

Irbesartan (IRB), is chemically 2-butyl-3-({4-[2- (2H-1, 2, 3, 4-tetrazol-5-yl) phenyl] phenyl} methyl)-1, 3-diazaspiro [4,4] non-1-en-4-one [1] as seen in Figure 1. It is an angiotensin II receptor antagonist that affect rennin angiotensin system, and is used in treatment of hypertension [2]. Several methods have been developed for determination of IRB including UV spectrophorometric methods [3-6], extractive and non-extractive spectrophotometry [7, 8], spectrofluorimetry [9], novel 96-microwell spectrofluorometry [10], HPLC [11, 12], and micro-emulsion liquid chromatography [13].

Losartan (LOS), is chemically (2-butyl-4-chloro-1-{[2'-(1H-tetrazol-5-yl)biphenyl-4-yl]methyl}-1H-imidazol-5-yl)methanol (Figure 1) [14]. It is non-peptide angiotensin II receptor antagonist used for the treatment of hypertension [15]. Several methods have been developed for determination of LOS including UV spectrophotometry [16-18], kinetic spectrophotometry [19], HPLC [20-23], HPTLC [24], and analytical study for the charge-transfer complexes of LOS [25].

Bisoprolol (BIS) is chemically (±)-1-[4-[[2-(1-methylethoxy) ethoxy] methyl] phenoxy]-3-[(1-methylethyl) amino]-2-propanol (Figure 1) [26]. It's a beta blocker used in treatment of mild to moderate hypertension [27]. Several methods had been reported for determination of BIS such as spectrophotometric methods [28-30], HPLC [31, 32], HPTLC [33], and direct compression techniques [34].

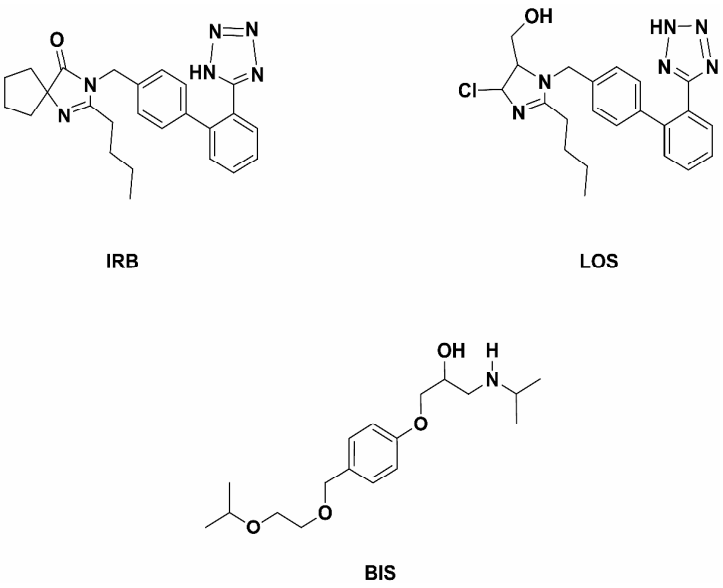

Figure 1. Chemical structures of irbesartan (IRB), losartan (LOS), and bisoprolol (BIS).

To the best of our knowledge, there is no method that has been reported for the spectrophotometric determination of the three drugs using bromatometric technique. As such, the present work introduces a simple, rapid, reproducible and sensitive method that has been established and validated for the determination of the antihypertensive drugs in their pure forms and in their tablet dosage form according to ICH guidelines [35].

2. Materials and Method

2.1. Apparatus

Labomed® Spectro UV-VIS Double Beam (UVD-2950) Spectrophotometer with matched 1cm quartz cells connected to Windows compatible computer using UV Win 5Software v5.0.5 was used for experiments.

2.2. Materials and reagents

All chemicals used are of analytical grade. IRB was provided by Sigma Company, Egypt, LOS was provided by Eipico Company, Egypt, while BIS was provided by Global Nabi Pharmaceuticals, 6 October City, Egypt. Standard stock solutions of IRB, LOS, and BIS equivalent to 1mg/mL were prepared by dissolving 100mg of each pure drug in 100mL calibrated flask with methanol. Working solutions of IRB and BIS equivalent to 100µg/mL were prepared by diluting 10mL from each standard stock solution with methanol in 100mL calibrated flasks. On the other hand, LOS working solution equivalent to 50µg/mL was prepared by diluting 5mL from standard stock solution with methanol in 100mL calibrated flask. Bromate-bromide stock solution was prepared by dissolving 0.10gm of potassium bromate and 1.00gm of potassium bromide in 100mL distilled water. Working solution was freshly prepared daily by diluting 5mL of stock solution to 100mL with distilled water. 5M HCl was prepared by diluting 225mL of concentrated HCl (34%) to 500mL with distilled water. Rhodamine B dye (50µg/mL) was prepared by dissolving 50mg in 1000mL distilled water (stable for 2 weeks at least).

2.3. Pharmaceutical tablets

The following available tablet preparations were analyzed: Kansartan® tablets, labelled to contain 150mg of IRB, batch No. 25368 (Chemipharm, Egypt), Cozaar® tablets, labelled to contain 50mg of LOS, batch No.19460 (Merk sharp Dohme), and Concor® tablets, labelled to contain 10mg of BIS, batch No. 162697 (Amoun Pharmaceutical Company, El-Obour City, Egypt).

2.4. General spectrophotometric procedure and construction of calibration curves

Accurately measured aliquots of standard solutions containing 4-14µg/mL of IRB, 1-5µg/mL of LOS, and 5-25µg/mL of BIS were transferred into a series of 10mL volumetric flasks. In case of IRB to each

flask, 0.6mL of bromate-bromide working solution was added, followed by 0.4mL of 5M HCl, flasks closed and stood for 15 minutes, then 1mL of rhodamine B dye was added then stood for 5 minutes. In case of LOS to each flask, 0.8mL of bromate-bromide working solution was added, followed by 0.8mL of 5M HCl, flasks closed and stood for 5 minutes, then 1mL of rhodamine B dye was added then stood for 3 minutes. In case of BIS to each flask, 0.4mL of bromate-bromide working solution was added, followed by 0.6mL of 5M HCl, flasks closed and stood for 15 minutes, then 1mL of rhodamine B dye was added then stood for 3 minutes, finally the volume was brought up to mark with distilled water for all drugs. The resulting solutions were measured at λ_{max} 557nm (Figure 2) for all drugs against the blank. All these analytical parameters are mentioned in Table 1.

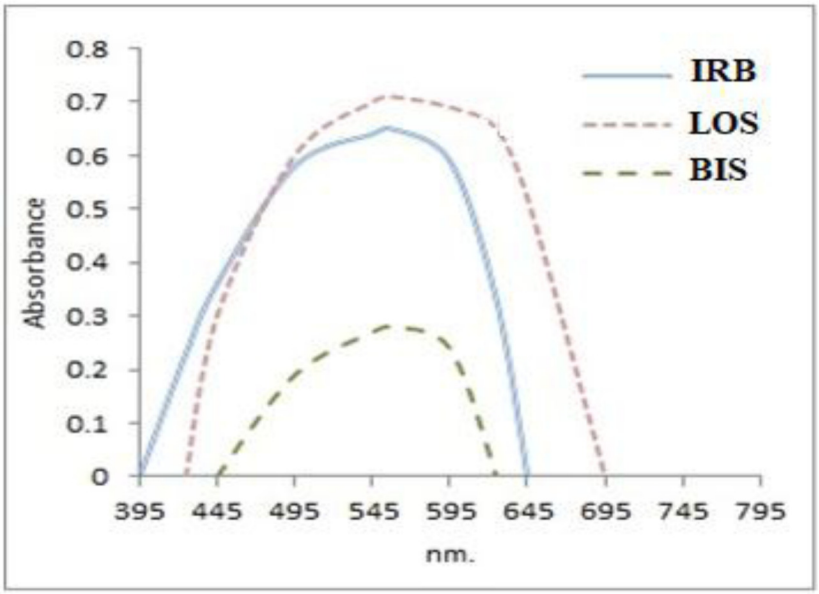

Figure 2. Absorption spectra for rhodamine B in presence of IRB (10µg/mL), LOS (5µg/mL), and BIS (10µg/mL) at λ_{max} 557nm.

Table 1. Analytical parameters for the determination IRB, LOS, and BIS using rhodamine B method

Parameters	Rhodamine B (50µg/mL)		
	IRB	LOS	BIS
λ_{max}, nm	557		
Volume of 50µg/mL dye, mL	1		
Volume of 50µg/mL bromate-bromide mixture, mL	0.6	0.8	0.4
Volume of 5M HCl, mL	0.4	0.8	0.6
Time required to oxidize the drug before dye addition, min	15	5	15
Time required to oxidize the dye, min	5	3	3
Diluting solvent	distilled water		

2.5. Procedure for pharmaceutical preparations

Twenty tablets of Kansartan®, Cozaar®, and Concor® were weighed and finely powdered. An accurately amount of the powder equivalent to the concentration of each drug in the proposed method was dissolved in 20mL methanol in beaker, stirred for about 5-10 min, and filtered through Whatman® filter papers to remove the insoluble matter. The residue was washed with 10mL of methanol three times, then the filtrate and washing volumes were collected and completed with methanol to 100mL in volumetric flasks. Aliquots from these solutions equivalent to those in authentic samples were used for the application of the proposed method applying standard addition technique.

3. Results and Discussion

The proposed bromatometric method is based on the determination of the residual bromine after allowing the reaction between each drug and a measured amount of bromine to be complete. The insitu generation of bromine is carried out using a mixture of potassium bromate and

potassium bromide in presence of 5M HCl. The surplus bromine was determined through its reaction with a fixed amount of rhodamine B dye. The method relies on the bleaching action of bromine on the dye due to oxidative destruction of rhodamine B dye [36] as seen in Figure 3. When a drug is added in increasing amounts to a fixed amount of insitu generated bromine, it consumes the latter proportionately with a concomitant fall in the concentration of bromine. When a fixed amount of dye is added to the decreasing amounts of bromine, a concomitant increase in the concentration of dye results. Consequently, a proportional increase in the absorbance at the respective λ_{max} is observed with increasing concentration of the drug.

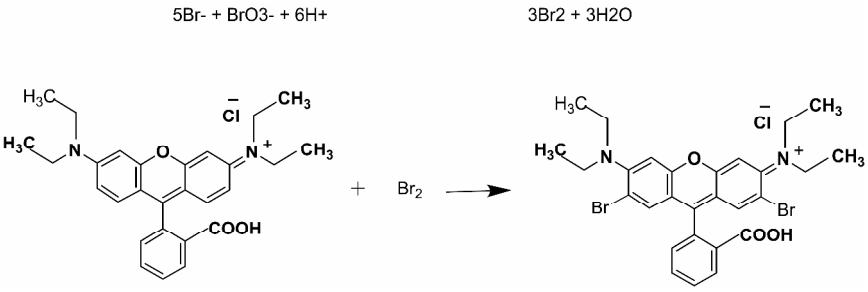

Figure 3. Proposed reaction mechanism between rhodamine B and Br_2.

3.1. Method optimization

3.1.1. Effect of bromate-bromide volume

Bromate-bromide volume was studied by varying the reagent volume while other factors were held constant. It was found that 0.6mL (in case of IRB), 0.8mL (in case of LOS), and 0.4mL (in case of BIS) of bromine are sufficient for its bleaching action using these stated concentrations (Figure 4).

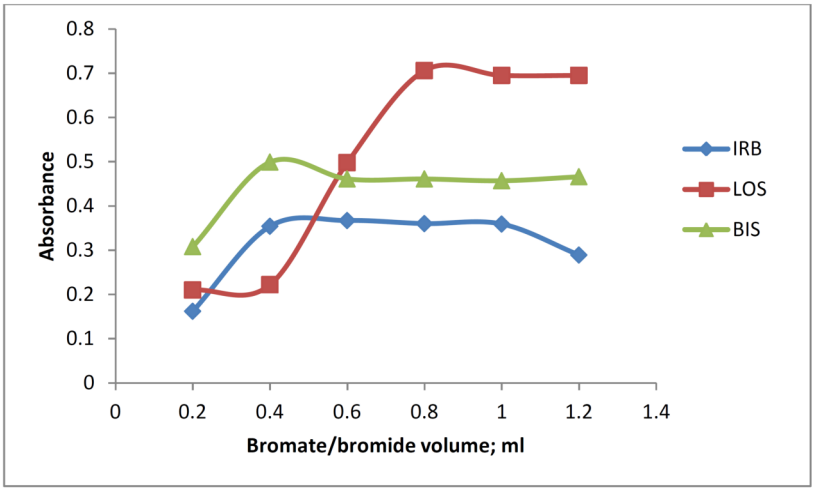

Figure 4. Effect of volume of bromate-bromide mixture on absorbance of rhodamine B in presence of IRB (10µg/mL), LOS (5µg/mL), and BIS (10µg/mL) at λ_{max}557nm.

3.1.2. Effect of acidity

5M HCl was used throughout experiments and it was found that 0.4mL (in case of IRB), 0.8mL (in case of LOS) or 0.6mL (in case of BIS) are the appropriate acid volumes while increasing HCl volume results in a decrease in absorption as seen in Figure 5.

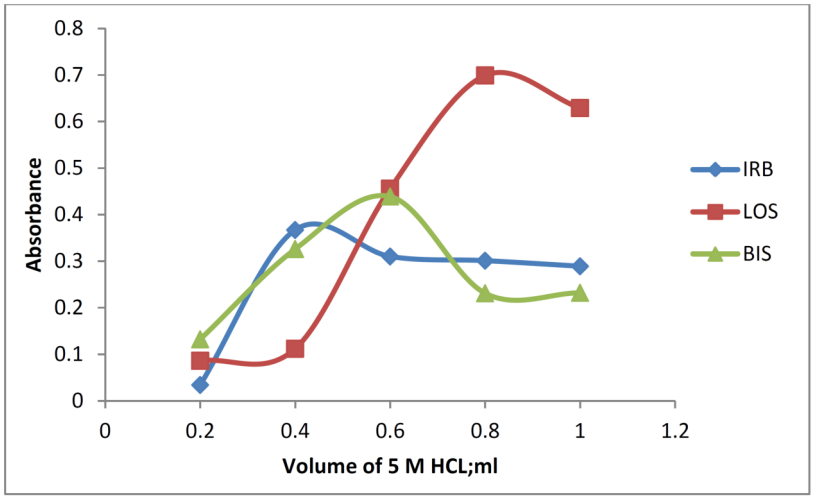

Figure 5. Effect of volume of 5M HCl on absorbance of rhodamine B in presence of IRB (10µg/mL), LOS (5µg/mL), and BIS (10µg/mL) at λ_{max} 557nm.

3.1.3. Effect of time

Time required for bromination and subsequent oxidation of the drug before addition of dye and time required to irreversibly oxidize the dye after its addition was studied. The bromination reaction was found to be complete within 15 minutes (in case of IRB and BIS) and 5 minutes (in case of LOS) as depicted in Figure 6. On the other hand, contact times of 3 minutes (in case of LOS and BIS) and 5 minutes (in case of IRB) were necessary for the bleaching of the dye colour by the residual bromine (Figure 7) and the colour of residual dye remains stable for at least two hours after mixing with the reaction mixture.

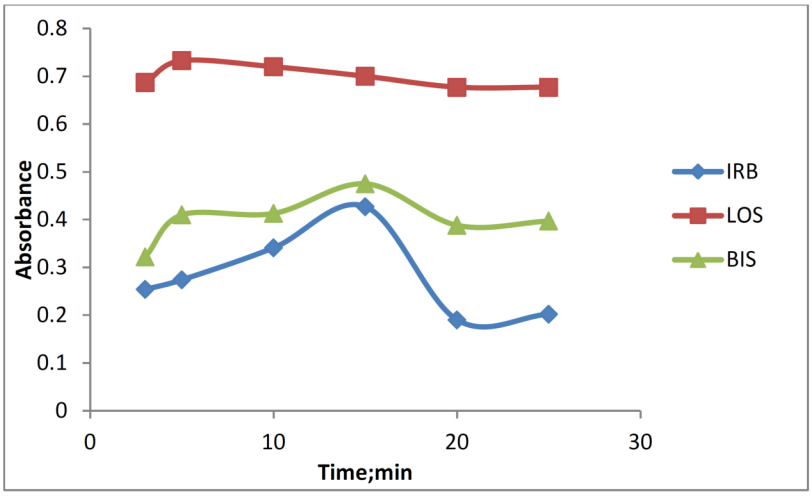

Figure 6. Effect of time before addition of rhodamine B in presence of IRB (10µg/mL), LOS (5µg/mL), and BIS (10µg/mL) at λ_{max} 557nm.

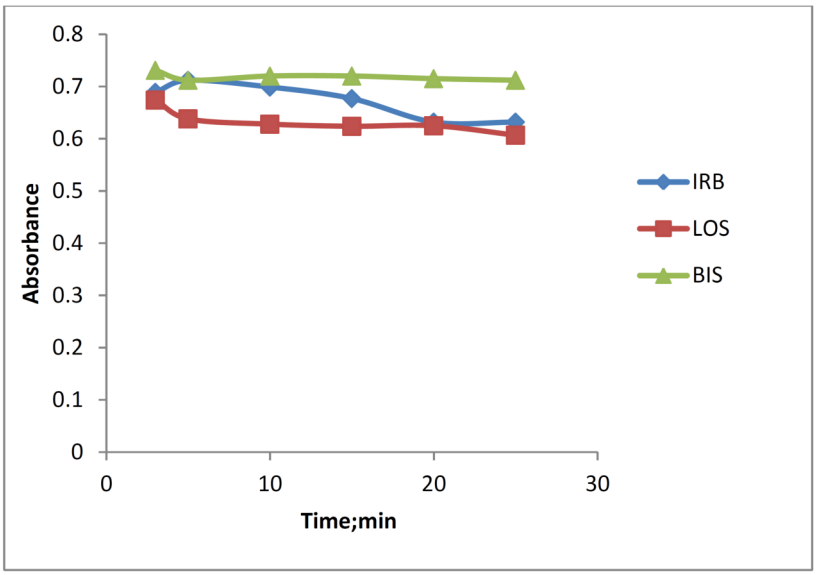

Figure 7. Effect of time after addition of rhodamine B in presence of IRB (10µg/mL), LOS (5µg/mL), and BIS (10µg/mL) at λ_{max} 557nm.

3.2. Method validation

The developed method was validated according to international conference on harmonization (ICH) guidelines [35].

3.2.1. Linearity

Five to six different concentrations of the three drugs were prepared for linearity studies. The calibration curves obtained by plotting absorbance against concentration showed linearity in the concentration range of 4-14μg/mL, 1-5μg/mL, and 5-25μg/mL for IRB, LOS, and BIS, respectively. Linear regression equations of IRB, LOS, and BIS were found to be $y = 0.0697 \times - 0.056$, $y = 01419 \times - 0.0051$, and $y = 0.0172 \times + 0.022$, respectively and the regression coefficient values (r) were found to be 0.9999 for the three drugs indicating a high degree of linearity for all drugs as depicted in Figure 8. However, all analytical merits for the calibration data are summarized in Table 2.

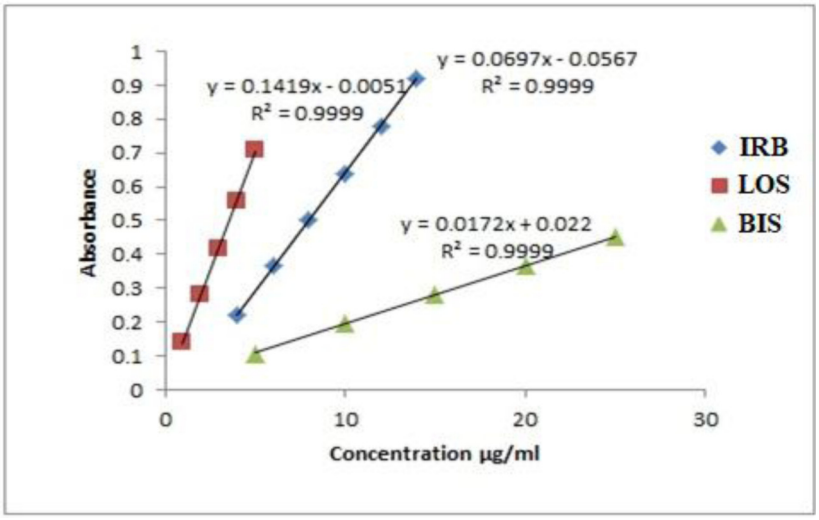

Figure 8. Calibration curves for IRB, LOS and BIS using rhodamine B method.

Table 2. Results of the analysis for determination of IRB, LOS and BIS

Parameters	Rhodamine B					
	IRB			LOS		
	Taken µg/mL	Found µg/mL	Recovery %	Taken µg/mL	Found µg/mL	Recovery %
	4	3.945	98.637	**1**	1.014	101.418
	6	6.054	100.908	**2**	2.014	100.709
	8	8.005	100.071	**3**	3.007	100.236
	10	9.971	99.713	**4**	4.007	100.177
	12	11.979	99.833	**5**	5.049	100.992
	14	14.002	100.020			
Mean*			**99.863**			**100.706**
SD			0.733			0.522
RSD			0.734			0.518
SE			0.299			0.233
Variance			0.537			0.272
Slope			0.069			0.142
Intercept			0.056			0.0051
Correlation Coefficient			0.9999			0.9999
LOD			1.194			0.298
LOQ			3.983			0.997
Apparent Molar bsorbitivity $Mol^{-1}.cm^{-1}$**			26642.18			59065.89

Table 2. (Continued)

Parameters	Rhodamine B		
	BIS		
	Taken µg/mL	**Found µg/mL**	**Recovery %**
	5	4.941	98.823
	10	10.176	101.764
	15	15.235	101.568
	20	20.176	100.882
	25	25.176	100.705
Mean*			**100.749**
SD			1.165
RSD			1.156
SE			0.521
Variance			1.357
Slope			0.017
Intercept			0.022
Correlation Coefficient			0.9999
LOD			1.458
LOQ			4.863
Apparent Molar bsorbitivity $Mol^{-1}.cm^{-1}$**			6227.837

*Average of three different experiments.

**Calculated in the basis of molecular weight of the drug.

3.2.2. Accuracy

The accuracy of the method was determined by investigating the recovery of drugs at 5 concentration levels covering the specified range (three replicates of each concentration). Standard addition method was used for determination of IRB, LOS, and BIS in Kansartan®, Cozaar®, and Concor® tablets and the results showed excellent recoveries (99.873 - 100.244%) as seen in Table 3.

Table 3. Application of standard addition technique for the determination of Kansartan®, Cozaar®, and Concor® tablets using standard addition technique

Items	Kansartan® tablets (IRB)			
	Added pure drug (µg/mL)	**Taken tablet (µg/mL)**	**Conc. found (µg/mL)**	**Recovery %**
	6	**0**	5.941	99.026
	6	**2**	7.994	99.937
	6	**4**	10.065	100.651
	6	**6**	12.051	100.431
	6	**8**	13.904	99.320
Mean*				**99.873**
SD				0.697
RSD				0.697
SE				0.311
Variance				0.520559

Table 3. (Continued)

Items	Cozaar® tablets (LOS)			
	Added pure drug (µg/mL)	**Taken tablet (µg/mL)**	**Conc. found (µg/mL)**	**Recovery %**
	2	**0**	2.000	100.213
	2	**1**	3.000	100.284
	2	**2**	3.962	99.074
	2	**3**	5.031	100.626
	2	**4**	5.992	99.881
Mean*				**100.016**
SD				0.589
RSD				0.589
SE				0.263
Variance				0.455

Items	Concor® tablets (BIS)			
	Added pure drug (µg/mL)	**Taken tablet (µg/mL)**	**Conc. found (µg/mL)**	**Recovery %**
	10	**0**	10.089	100.895
	10	**5**	15.014	100.099
	10	**10**	19.940	99.701
	10	**15**	25.014	100.059
	10	**20**	30.139	100.464
Mean*				**100.244**
SD				0.453
RSD				0.452
SE				0.202
Variance				0.253

*Average of three different experiments.

3.2.3. Precision

Intra-day precision was evaluated by calculating standard deviation (SD) of five replicate determinations using the same solution containing pure drugs. Results in Table 4 show that SD values (0.181–0.738) revealed the high precision of the method. For inter-day reproducibility on a day-to-day basis, a series was run, in which the standard drug solutions were analyzed each for five days where SD values were also in the acceptable range (0.278–0.816) as seen in Table 4.

Table 4. Results of the intra-day and inter-day precision for the determination of IRB, LOS, and BIS with rhodamine B

Drug	Conc. µg/mL	Intra-day		Inter-day	
		Mean ± SD	RSD	Mean ± SD	RSD
IRB	6	101.449 ± 0.483	0.476	101.352 ± 0.775	0.764
	8	100.905 ± 0.181	0.179	101.087 ± 0.424	0.420
	10	100.531 ± 0.221	0.220	100.463 ± 0.278	0.277
LOS	2	100.118 ± 0.738	0.737	99.432 ± 0.816	0.821
	3	99.448 ± 0.722	0.726	99.527 ± 0.647	0.650
	4	100.472 ± 0.369	0.367	100.886 ± 0.396	0.392
BIS	10	101.176 ± 0.588	0.581	101.058 ± 0.766	0.758
	15	101.568 ± 0.392	0.386	101.725 ± 0.447	0.442
	20	100.490 ± 0.449	0.447	100.294 ± 0.465	0.460

3.2.4. LOD and LOQ

The calculation of limits of detection and quantification was based on the following equations: LOD = 3.3 S/K and LOQ = 10 S/K, respectively, where S is the standard deviation of the seven replicate values under the same conditions as for the sample analysis in the absence of analyte and K is the sensitivity, namely, the slope of calibration graph. The limit of detection for IRB, LOS, and BIS was reported to be 1.194µg/mL, 0.298µg/mL, and 1.458µg/mL while the limit of quantification was 3.983µg/mL, 0.997µg/mL, and 4.863µg/mL, respectively (Table 2).

3.2.5. Robustness

The robustness of the method was evaluated by making small changes (±0.05) in one parameter keeping the other spectrophotometric conditions constant such as volume of acid, bromate-bromide mixture and dye solution where the effect of the changes was studied on the percent recovery of drugs. The changes had negligible influence on the results as revealed by small SD (≤ 1.813) as shown in Table 5.

Table 5. Results of the robustness for the determination of IRB, LOS, and BIS using rhodamine B method

Parameters	Rhodamine B		
	% of recovery ± SD		
	IRB	LOS	BIS
HCl + 0.05	100.770 ± 0.839	100.792 ± 0.607	100.043 ± 1.434
HCl – 0.05	101.183 ± 1.118	100.508 ± 0.572	100.171 ± 1.271
Br_2 + 0.05	100.797 ± 0.830	100.593 ± 0.505	100.396 ± 1.041
Br_2 – 0.05	101.207 ± 1.155	100.536 ± 0.543	100.171 ± 1.271
Dye + 0.05	100.990 ± 0.885	100.508 ± 0.572	100.043 ± 1.434
Dye – 0.05	100.652 ± 0.930	101.075 ± 1.102	99.730 ± 1.813

3.3. Analysis of pharmaceutical formulations

The validated spectrophotometric method was applied for the determination of IRB, LOS, and BIS in their pharmaceutical preparations. Results obtained were compared to those obtained by applying reported reference methods [30, 37, 38] where Student's t-test and F-test were performed for comparison. It was found that the calculated t and F values were less than tabulated ones for the cited drugs (Table 6) which in turn indicate that there is no significant difference between proposed method and reference ones relative to precision and accuracy.

Table 6. Statistical analysis of results obtained by the proposed method applied on IRB, LOS, and BIS compared with reference methods

Parameters	IRB		LOS	
	Proposed method	**Reference method [37]**	**Proposed method**	**Reference method [38]**
N	6	6	5	5
Mean Recovery*	99.863	99.989	100.706	100.004
SD	0.733	0.699	0.521	0.657
RSD	0.734	0.699	0.518	0.657
SE	0.299	0.285	0.233	0.294
Variance	0.537	0.579	0.272	0.432
t-test**	0.304 **(2.228)**[a]		1.872 **(2.306)**[a]	
F-test**	1.099 **(4.283)**[b]		1.590 **(5.05)**[b]	

Parameters	BIS	
	Proposed method	**Reference method [30]**
N	5	5
Mean Recovery*	100.749	101.216
SD	1.165	0.735
RSD	1.156	0.727
SE	0.521	0.300
Variance	1.357	0.541
t-test**	0.758 **(2.306)**[a]	
F-test**	2.512 **(5.05)**[b]	

*Average of three experiments

**a and b theoretical Student t-values and F-ratio at $p = 0.05$.

4. Conclusion

The proposed indirect spectrophotometric method is simple, fast, accurate, adequately sensitive and inexpensive. It is suitable for routine quality control analysis. The present method is superior to the reference methods with respect to both sensitivity and selectivity. The method has been successfully applied for the analysis of marketed tablets.

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