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SPECTROPHOTOMETRIC AND SPECTROFLUORIMETRIC DETERMINATION OF CERTAIN ANGIOTENSIN RECEPTOR BLOCKERS THROUGH COMPLEX FORMATION

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Abstract:
This paper describes simple, rapid, sensitive, and non extractive spectrophotometric and spectrofluorimetric methods for determination of certain angiotensin receptor blockers (ARBs) namely Losartan (Los.), Irbesartan (Irb.), Telmisartan (Telm.) and Valsartan (Vals.) in their pure forms and pharmaceutical tablets. The spectrophotometric method based on the interaction of Los., Irb. and Telm. with sulphophthalein dyes to form a stable , yellow colored, ion pair complex peaking at 413-419nm. The dyes used were bromothymol blue (BTB), bromophenol bue (BPB) and bromocresol green (BCG). The spectrofluorimetric method was based on the formation of non extractive binary complex between eosin and Los., Irb., Telm. and Vals. without using any surfactant. The proposed methods have been successfully applied for determination of these drugs in their tablets and in presence of their degradation products with good recoveries. They are recommended for quality control and routine analysis where time and cost effectiveness are of great importance.

Keywords; Angiotensin receptor blockers, Spectrophotometry, Spectroflourimetry, Complex formation, Pharmaceutical analysis.

1. Introduction:

Los., 2-Butyl-4-chloro-1-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1H-imidazole-5-methanol. Irb., 2-Butyl-3-[[2′-(1H-tetrazol-5-yl)[1,1′-biphenyl]-4-yl]methyl]-1,3-diazaspiro[4.4]non-1-en-4-one. Telm., 4′-[(1,4′-Dimethyl-2′-propyl[2,6′-bi-1H-benzimidazol]-1′-yl)methyl]-[1,1′-biphenyl]-2-carboxylic acid and vals., N-[p-(o-1H-Tetrazol-5-ylphenyl)benzyl]-N-valeryl-L-valine (Scheme 1) , are angiotensin II receptor antagonist that affect renin-angiotensin system, used mainly for the treatment of hypertension [1], The various side effect profiles of the older antihypertensive therapies can limit compliance. Clinical trials indicate that angiotensin II antagonists are effective and safe as antihypertensive agents. Moreover, their specific blocking action has led to the inhibition of the breakdown of bradykinin and other kinins, enhancing prostaglandin synthesis and thus do not appear to cause the persistent dry cough which commonly accompanies ACE inhibitors therapy [2]. The reported methods for determination of ARBs are UV spectrophotometric [3-10], Spectrofluorimetric [11, 12], HPLC [13-26] , capillary electrophoresis [27-29] and electrochemical [30-32] methods. To our best knowledge, very few colorimetric methods have been reported [33, 34] and the literatures are still poor in such analytical procedures. Pharmacopoeial methods have been reported for the analysis of Los and Irb [35]. The acid dye technique is a general procedure for the quantitative analysis of a variety of pharmaceutical amines. In practice, a buffered aqueous solution containing the amine and a suitable indicator dye is shaken with an organic solvent. The concentration of the resulting ion-pair in the organic phase is then determined spectrophotometrically. The ion-pair extraction technique [36, 37] had some difficulties in accuracy and reproducibility arising from incomplete extraction using manual technique and it was considered time consuming, and sometimes lead to emulsion formation. The extraction-free technique was reported by few other studies for the analysis of pharmaceutical compounds through formation of ion-pair, without extraction or preliminary adjustment of pH, followed by spectrophotometric

determination [38, 39]. So the present work describes a simple, highly sensitive and non extractive colorimetric and spectrofluorimetric methods for determination of certain ARBs in pure form and pharmaceutical tablets.

2. Experimental:

2.1. Apparatus

- Spectronic™ genesys™ ultraviolet-visible spectrophotometer (Milton Roy Co., USA) with matched 1 cm quartz cell was used connected to an IBM computer loaded with the Winspec™ application software.
- Jennway® 6505, Ultraviolet/Visible spectrophotometer(London, U.K.)
- A Perkin- Elmer UK model LS 45 Luminescence Spectrometer, equipped with a 150 Watt Xenon arc lamp, gratting excitation and emission monochromators for all measurments. Slit width for both monochromators were set at 10 nm. A 1 cm quartz cell was used, connected to an IBM computer loaded with the FL WinLabTM application software.
- Milwakee SM 101 pH meter, (Portugal).
- Ultrasonic sonicator (Cole-Parmer, Chicago, U.S.A.).

2.2 Materials and reagents

All chemicals were of analytical grade and were used as such without further purification:

- Losartan potassium was kindly provided by El-Hikma Pharmacuticals , Amman, Jordan as a working standard 99.2%. **Cozaar®** 50 mg (B.N. LT209L05) labeled to contain 50 mg of losartan potassium per tablet, manufactured by Global Napi Pharmaceuticals- Egypt under license from: Merck & Co.Inc., Whitehouse station, N.J., USA.
- Irbesartan was kindly provided by Medical Union Pharmaceuticals, Abu-sultan, Ismailia, Egypt as a working standard 99.8%. **Approvel®** 150 mg (B.N. 608) labeled to contain 150 mg of irbesartan per tablet ,manufactured by Sanofi Aventis Egypt, s.a.e, under license of sanofi- aventis France.
- Telmisartan was kindly provided by Boehringer – Ingelheim Pharmaceuticals, Ingelheim, Germany as a reference standard. **Micardis®** 40 mg (B.N.702009) labeled to contain 40 mg of Telmisartan per tablet, manufactured by Boehringer - Ingelheim Pharma, GmbH and Co.KG for Boehringer-Ingelheim international, Rhien, Germany.
- Valsartan was kindly provided by Novartis pharma S.A.E. Cairo as a working standard 99.7%. **Tareg®** 160 (B.N. Y0016) labeled to contain 160 mg valsartan per tablet, manufactured by Novartis Pharma S.A.E., cairo under license from Novartis Pharma AG., Basle, Switzerland.
- Bromothymol blue, bromophenol blue and bromocresol green (Merck, Darmstadt, Germany) were prepared as 0.1 % w/v (1mg mL^{-1}) in chloroform.
- Eosin Y. (Merck, Darmstadt, Germany) was prepared as 2×10^{-5} M in aqueous solution.
- Acetate buffer, 0.4 M, were prepared by mixing various volumes of 0.4 M acetic acid and 0.4 M sodium acetate solutions to get the required pH values (2.7-4.7).

2.3. Standard solutions

2.3.1. Spectrophotometric method

Stock solutions containing (500 µg mL^{-1}) of each of the studied drugs were prepared. 50 mg of each drug was accurately weighed, carefully transferred into 100 mL volumetric flasks. Then 1.0 mL methanol was

added and the resultant mixture was sonicated for about 5 minutes till complete dissolution of the drug. The resultant solution was completed to volume with chloroform .This solution was further diluted quantitatively with the same solvent to obtain working standards covering the range of 30.0 – 240.0 μg mL^{-1}.

2.3.2. Spectrofluorimetric method

Stock solutions containing (500 μg mL^{-1}) of each of the studied drugs were prepared. 50 mg of each drug were accurately weighed, carefully transferred into 100 mL volumetric flasks, followed by addition of 1.0 mL methanol, sonicated for about 5 min till complete dissolution. Finally the resultant solution was completed to the volume with distilled water. Serial dilutions were then prepared to obtain working standards covering the range of 8.0 to 80 μg mL^{-1}.

2.4. General procedures and Calibration graphs

2.4.1. Spectrophotometric method

Aliquot volumes of the working standard solutions of the Los, Irb and Telm were accurately transferred into a series of 10 mL volumetric flasks, so that the final concentration was in the range of 3.0–24.0 μg mL^{-1}. Then 1.0 mL of BTB, BPB or BCG solution (0.1% w/v) was added. The contents were mixed well and completed to volume with chloroform. A blank experiment was performed simultaneously for each case. The absorbance of the resulting chlolroformic solutions was measured against a reagent blank treated similarly at 413, 416, 419 nm for drug-BTB, drug-BPB and drug-BCG respectively.

2.4.2. Spectrofluorimetric method

Accurately measured aliquots of Los, Irb, Telm and Vals working standard solutions were transferred into a series of 10 mL volumetric flasks. Eosin Y, 1.0 mL of 2×10^{-5} M solution was added to each flask and the resultant solution was mixed well followed by addition of 2.0 mL of acetate buffer (pH 3.0). The mixture was diluted with distilled water to the mark and mixed well. The difference in the relative fluorescence intensities between blank, treated similarly, and the product at a 546 nm emission wavelength with excitation at 310 nm was measured.

2.5. Procedure for assay of tablets

Ten tablets were weighed accurately, finely powdered and mixed thoroughly. An accurate amount from the powdered tablets equivalent to 50 mg of each studied drug was then transferred quantitatively into 100 mL volumetric flask. The mixture was shacked thoroughly, sonicated with about 1.0 mL methanol for about 10 minutes.

2.5.1. Spectrophotometric method

The resultant mixture was completed to volume with chloroform. The solution was filtered into 100 mL measuring flask and the first portion of the filtrate was rejected. Further dilution with chloroform was made to obtain working solutions have a final concentration (3.0–24.0 μg mL^{-1}), and then general procedures were followed.

2.5.2. Spectrofluorimetric method

The resultant mixture was completed to volume with distilled water. The solution was filtered into 100 mL measuring flask and the first portion of the filtrate was rejected. Then a portion of the filtrate was diluted quantitatively by distilled water to the required final concentration 0.8 to 8.0 μg mL^{-1}, and then general procedures were followed.

2.6. Stability Studies

[The stability- indicating capability] of the spectrofluorimetric method was demonstrated by accelerated degradation of ARBs. Samples of the studied drugs were exposed for acidic and basic conditions by heating (6 μg mL^{-1} of Los) with 5 mL of 2M HCl and 2M NaOH in a boiling water bath.

Samples were taken at increasing time intervals and the drug concentration was derived from previously plotted calibration or according to the regression equation.

3. Results and discussion:

3.1. Spectrophotometric method

3.1.1. Absorption spectra

The studied drugs are soluble in methanol and weakly absorbing light in the UV region in the area of 240-300 nm. Consequently, poor sensitivity will be achieved by conventional UV Spectrophotometric method. These drugs possessing imidazole ring, the aromatic amino group is protonated in a chloroformic solution of acidic sulphophthalein dyes as chromogenic reagent. Upon interaction of the cited drugs with theses dyes in chloroform a highly yellow colored ion-pair complex was formed non extractive with chloroform. The ion-pair complex shows maximum absorbance at 413,416 and 419 nm for BTB, BPB and BCG respectively against a reagent blank treated similarly. This is due to the conversion of the dye into an open quinonoidal anionic derivative [40], (Fig 1).

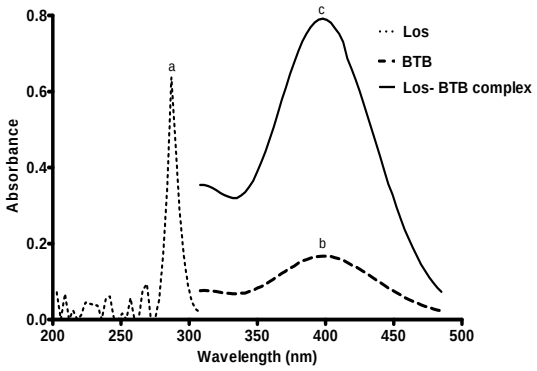


Figure 1: Absorption spectra of (a) Los (10 µg ml $^{-1}$) (.....) (b) BTB (0.1mg ml $^{-1}$) (-----) (C) Los-BTB complex (10 µg ml^{-1}) (——) all in chloroform.

It is important to explain that no positive results were observed with Vals; who can be explained based on the fact that it is considered as an amide derivative, in addition the steric hindrance and formation of hydrogen bonding with tetrazolium ring prevent its reaction.

3.1.2. Optimum reaction conditions for complex formation

The effects of the reagents were studied by measuring the absorbance of solutions containing a fixed concentration of each drug and different amounts of reagent; maximum color intensity of the complex was achieved immediately. The highest (λ_{max}) intensities were obtained with 2.0 mL in case of Los, Irb and 1.0 mL in case of Telm in 0.1 % w/v chloroformic solution of BTB, BPB and BCG measured at 413,416 and 419 respectively. The absorbance of the complex was found to be stable for at least 1 h, also different solvents were studied, and it was found that chloroform is the best solvent that gave high intensity.

3.1.3. Stoichiometric relationship

The stoichiometry of the reaction was determined using Job's method of continuous variation [41]. Master equimolar solutions (1×10^{-3} M) of BTB, BPB and BCG with each drug were prepared. The method revealed 1:1 ratio in case of Los, Irb while Telm showed a ratio 2:1 (dye:drug). The results obtained from molar ratio studies were in agreement with the suggested reaction mechanism (scheme 2), (Fig 2).

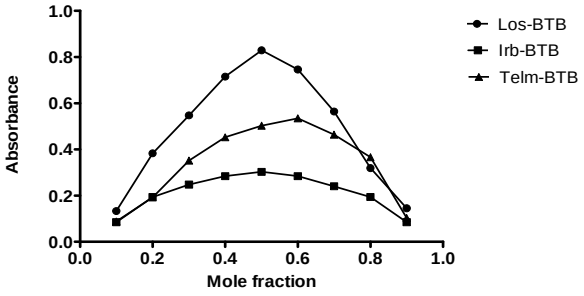


Figure 2: Plot of Job's method for determination of molar ratio of the formed Los, Irb and Telm -BTB ion pair complex.

3.1.4. Conditional formation constants (K_f) of the ion-pair complex

The conditional formation constants (K_f) of the ion-pair complex for the studied drugs were calculated from the continuous variation data using the following equation [42].

$$k_f = \frac{\frac{A}{A_m}}{\left(\frac{1 - \frac{A}{A_m}}{}\right)^{n+1} C^n n^n}$$

where A= maximum absorbance, A_m=Absorbance corresponding to intersection of the two tangets of the curve in molar ratio, C =Molar concentration of drug corresponding to maximum absorbance and n=the stoichiometry with which dye ion associates with drugs. (Table 1) shows the calculated formation constants (K_f) for the formed complex between the studied drugs and each one of the used dyes. The calculated formation constants are in good correlation with the measured absorption intensities of the studied chelates and so they were

Table 1: Statistical analysis of calibration graphs and analytical data in the determination of the studied drugs using the proposed methods.

Parameter	BTB			BPB			BCG			Eosin Y.			
	Los	Irb	Telm	Los	Irb	Telm	Los	Irb	Telm	Los	Irb	Telm	Vals
λ_{max}	413	413	413	416	416	416	419	419	419	λ_{ex} = 310		λ_{em} = 546	
Linear range (µg ml^{-1})	5-18	8-24	3-10	5-15	8-24	3-9	5-15	8-24	3-10	0.8 – 8	0.8 – 7	0.9 – 4	1.0 – 8
Intercept (a)	0.0327	0.0508	0.0455	0.0457	0.0106	0.0156	0.0206	0.0124	0.0177	87.28	35.86	199.53	10.78
SE of intercept(S_a)	0.0070	0.0059	0.0075	0.0088	0.0083	0.0158	0.0092	0.0071	0.0039	0.622	0.666	0.839	0.871
Slope (b)	0.0399	0.0295	0.0784	0.0566	0.0458	0.1067	0.0623	0.0371	0.0818	10.11	19.89	24.67	21.81
SE of slope (S_b)	0.0006	0.0004	0.0011	0.0008	0.0006	0.0023	0.0008	0.0004	0.0005	0.127	0.144	0.313	0.177
Correlation Coefficient (r)	0.9997	0.9998	0.9997	0.9997	0.9997	0.9996	0.9997	0.9998	0.9998	0.9998	0.9999	0.9998	0.9998
SD of residuals($S_{y.x}$)	0.0059	0.0044	0.0064	0.0064	0.0071	0.0112	0.0070	0.0053	0.0030	0.739	0.756	0.720	1.015
ε (L^{-1} mol^{-1} cm^{-1})×10^4	1.67	1.29	1.87	2.39	1.78	2.48	2.33	1.40	2.15				
LOD (µg ml^{-1})	0.581	0.661	0.317	0.517	0.599	0.488	0.972	0.631	0.157	0.203	0.110	0.112	0.132
LOQ (µg ml^{-1})	2.230	2.161	1.235	1.68	1.82	1.48	1.49	1.93	0.59	0.617	0.335	0.340	0.399
Formation constant (K_f)	5.1×10^5	4.3×10^4	1.8×10^4	4.8×10^5	1.3×10^4	3.9×10^7	5.2×10^5	1.7×10^4	9.3×10^5	--------	-------	----------	----------
Gibbs free energy (ΔG)	-9.52×10^3	-7.30×10^3	-2.06×10^4	-1.36×10^4	-8.04×10^3	-2.61×10^4	-1.32×10^4	-7.98×10^3	-2.37×10^4	--------	--------	----------	---------

Table 2: Effect of extreme pH (2M NaOH and 2M HCl) on the Kinetic parameters of ***Los*** (6 µg mL^{-1}).

Media of degradation	**K (min^{-1})**	**$t_{1/2}$ (min)**
2M NaOH	0.089	7.71
2M HCl	0.111	6.27

Table 3: The intra-day precision and accuracy data for the studied drugs obtained by the proposed methods

	Added concentration (µg ml^{-1})				Found concentration (µg ml^{-1})				Recovery (%)				R.S.D			
	Los	Irb	Telm	Vals	Los	Irb	Telm	Vals	Los	Irb	Telm	Vals	Los	Irb	Telm	Vals
BTB	5	8	3	--------	4.90	8.12	3.06	--------	98.2	101.5	102.0	--------	0.662	1.019	0.817	--------
	7.5	12	6	--------	7.53	12.27	6.06	--------	100.4	102.3	101.1	--------	0.880	0.802	0.765	--------
	10	16	8	--------	10.16	15.92	7.96	--------	101.6	99.5	99.5	--------	1.417	0.595	0.406	--------
	12.5	20	10	--------	12.87	20.20	9.80	--------	102.9	101.0	98.0	--------	0.780	0.519	1.003	--------
	15	24	12	--------	14.94	24.33	11.96	--------	99.6	101.4	99.7	--------	1.827	0.153	0.282	--------
BPB	5	8	3	--------	5.01	7.98	2.96	--------	100.2	99.7	98.7	--------	0.626	1.021	1.016	--------
	8	10	5	--------	7.97	9.89	4.93	--------	99.6	98.9	98.6	--------	1.026	0.949	0.652	--------
	10	12	7	--------	9.83	11.9	6.89	--------	98.3	99.2	98.4	--------	0.348	0.957	0.712	--------
	12	16	8	--------	12.10	16.16	8.09	--------	100.8	101	101.1	--------	0.456	0.754	0.450	--------
	15	20	9	--------	14.95	20.22	8.96	--------	99.7	100.2	99.6	--------	0.405	0.864	0.821	--------
BCG	5	10	3	--------	4.93	9.95	2.99	--------	98.6	99.5	99.6	--------	0.814	0.408	1.170	--------
	7	12	5	--------	7.13	11.89	4.92	--------	101.8	99.0	98.4	--------	0.779	0.454	1.374	--------
	10	16	7	--------	9.94	16.21	7.12	--------	99.4	101.3	101.7	--------	0.604	0.363	0.569	--------
	12	20	8	--------	11.96	19.92	7.97	--------	99.6	99.6	99.6	--------	0.657	0.753	1.223	--------
	15	24	10	--------	15.22	23.94	9.96	--------	101.4	99.7	99.6	--------	0.250	0.702	0.561	--------
Eosin Y.	0.8	0.8	0.9	0.8	0.81	0.78	0.91	0.79	101.2	98.71	101.1	98.8	0.619	0.311	0.307	0.390
	2	2	2	2	2.02	1.98	1.98	1.99	101.1	99.18	99.2	99.7	0.682	0.364	0.430	0.435
	4	4	2.5	4	4.01	3.99	2.48	3.99	100.2	99.78	99.5	99.8	0.557	0.390	0.429	0.354
	6	6	3	6	5.98	5.97	3.01	6.08	99.7	99.50	100.3	101.3	0.347	0.336	0.484	0.406
	8	7	4	8	7.96	6.97	3.98	7.94	99.5	99.58	99.6	99.2	0.423	0.572	0.427	0.510

in good correlation with the calculated molar absorptivity values of each one of them.

The standard free energy changes of complexation (ΔG) were calculated from the association constants (Table 1) by the following equation [43];

$\Delta G = -2.303\ R\ T \log K_f$,

where ΔG = Gibbs free energy change of the reaction (kJ mol^{-1}), R = Universal gas constant (1.987cal mol^{-1} degree^{-1}), T = Absolute temperature (298) and K_f = formation constant of the reaction.

3.2. Spectrofluorimetric method

3.2.1. Excitation and Emission spectra

A non extractive binary complex between the studied drugs and Eosin Y. in acidic medium was formed based on ion-pair associates.

These ion pair associates of the studied drugs with the anion of the studied halofluorescein dye were slightly soluble in water, but under the optimized experimental conditions, they become freely soluble and neither needed an extraction into organic solvents nor addition of non-ionic surfactants. It was found that keeping the sample concentrations at maximum dilution before addition of the dye solution at acidic pH, and mixing well before the addition of acidic buffer had greatly increased stability of the product with complete prevention of precipitate formation. The difference in relative fluorescence intensity between the blank, treated similarly, and binary complex was measured at $\lambda_{ex.}$/ $\lambda_{em.}$; 310 / 546 nm.(Fig.3).

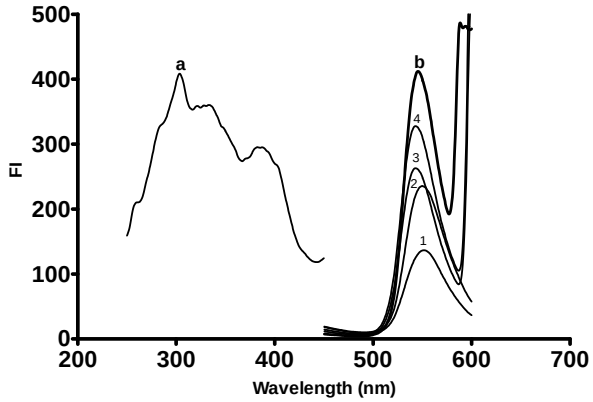


Figure (3): The fluorescence spectra of a: excitation of blank Eosin Y.(2×10^{-5} M)
b: Emission of blank Eosin Y. (2×10^{-5} M) 1: Emission spectra of **Telm** (3 µg mL^{-1})

2: Emission spectra of ***Irb*** (7 µg mL^{-1}) 3: Emission spectra of ***Los*** (6 µg mL^{-1})
4: Emission spectra of ***Vals*** (6 µg mL^{-1})

3.2.2. Optimization of experimental parameters

The effect of the reagent was studied by measuring the ΔRFI of solutions containing a fixed concentration of each drug and different amounts of Eosin Y (2×10^{-5} M), it was found that the maximum ΔRFI was attained using 1.0 mL in case of Los and Irb, and 0.5 mL in case of Telm and Vals; also the use of 2.0 mL of acetate buffer (pH=3.0) without using any surfactant was optimum for all studied drugs.

3.2.3. Investigation of the drug-dye ion-pair complex

The composition of the ion pair associates for the studied drugs was established by the Limiting Logarithmic method [44], ΔRFI of the reaction product was alternatively measured in the presence of excess Eosin Y.

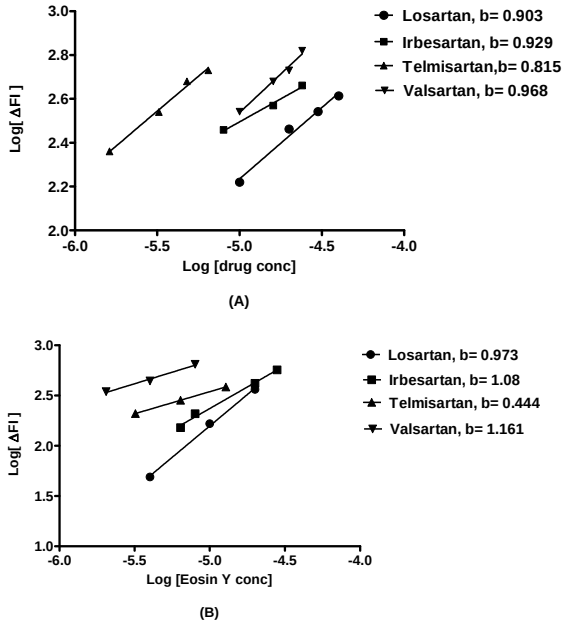


Figure 4: Limiting Logarithmic plots for molar ratio: (A) Log ΔRFI Vs. Log [drug concentration] ; at λ546 nm, (B) Log ΔRFI Vs. Log [Eosin Y concentration] ; at at λ 546 nm.

Plots of log ΔRFI versus log Eosin concentration [Eosin Y] and log ΔRFI versus log drug concentration [drug] for the studied drugs gave straight lines, the value of their slopes (b) are shown in Fig. (4 A, B).

Hence, it was concluded that the reaction proceeds in the ratio 1:1 in case Los, Irb, and Vals while Telm the ratio was 2:1 [dye:drug] respectively. A detailed reaction mechanism was explained in (scheme 3).

3.2.4. Stability indicating for the proposed spectrofluorimetric method

Stability studies of the ARBs had been previously investigated [16, 45]. The stability indicating capability of the proposed spectrofluorimetric method was tested after accelerated degradation study of losartan, as representative example of the studied drugs, using acidic and basic conditions at boiling temperature. In all cases, degradation products did not interfere with the intact complex peak (drug-Eosin Y.). These results demonstrated the ability of the proposed method to be used as stability indicating method for the analysis of the studied ARBs. In addition, it was observed that the ΔRFI decreased exponentially by increasing the boiling time with NaOH or HCl. Drug concentrations were determined from previously plotted calibration graph or by applying the corresponding regression equation. (Fig.5) revealed that the drug concentration decreased with increasing boiling time intervals in alkaline or acidic medium. Plotting the logarithms of drug concentrations against time resulted in straight line as shown in (Fig. 6) for alkaline and acidic degradation. From the slopes of the lines, the apparent first order degradation rate constants and also the half-life times were calculated and listed in (Table 2)

Table 4: Determination of the studied drugs in pharmaceutical tablets

Drug	Pharmaceutical tablets	% Recovery ± S.D							
		Reported methods [11, 33, 49, 50] (n=5)				Proposed methods (n=5)			
		BTB	BPB	BCG	Eosin Y.	BTB	BPB	BCG	Eosin Y.
Los	Cozaar® tablets	99.96 ± 0.42	100.31 ± 0.87	100.12 ± 0.63	99.86 ± 0.544	100.08 ± 0.72 *t* = 0.315 *F* = 2.88	100.54 ± 0.34 *t* = 0.54 *F* = 3.94	100.53 ± 0.29 *t* = 1.31 *F* = 4.61	100.21 ± 0.62 *t* = 0.65 *F* = 1.31
Irb	Aprovel® tablets	99.50 ± 0.85	100.05 0.62	100.23 ± 1.11	100.72 ± 0.572	98.95 ± 0.42 *t* = 1.28 *F* = 4.01	99.38 ± 0.91 *t* = 1.37 *F* = 2.14	99.56 ± 0.51 *t* = 0.98 *F* = 4.90	100.13 ± 0.973 *t* = 1.16 *F* = 2.89
Telm	Micardis® tablets	99.53 ± 0.49	99.72 1.15	100.02 ± 1.21	100.11 ± 0.619	99.28 ± .0.28 *t* = 0.97 *F* = 3.16	100.2 ± 0.93 *t* = 0.36 *F* = 1.55	99.38 ± 0.67 *t* = 1.10 *F* = 2.73	99.69 0.442 *t* = 1.22 *F* = 3.49
Vals	Co-Diovan® tablets	---------	---------	---------	99.82 ± 1.02	---------	---------	---------	99.94 ± 1.56 *t* = 0.72 *F* = 3.02

Each value is the mean of 5 determinations. The tabulated values at the 95 % confidence limits are t = 2.78 and F = 6.39, respectively.

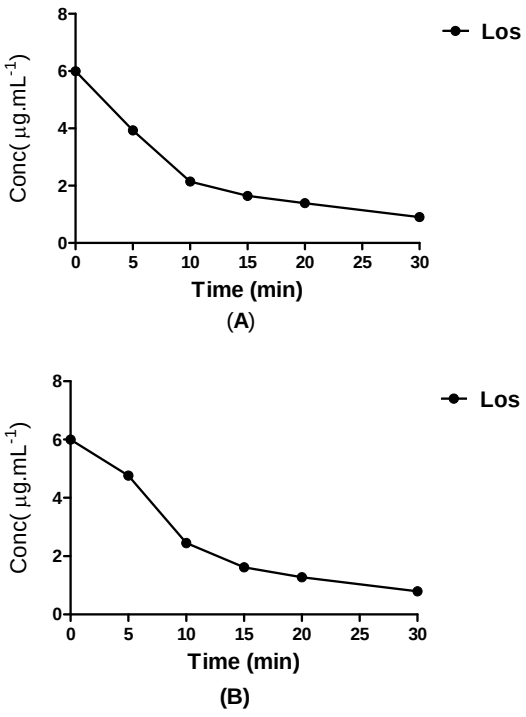


Figure 5: Plot of *Los* concentration (calculated from calibration graph preconstructed by the proposed spectrofluorimetric method) *Vs* different times of boiling with: (A) 2M NaOH and (B) 2M HCl

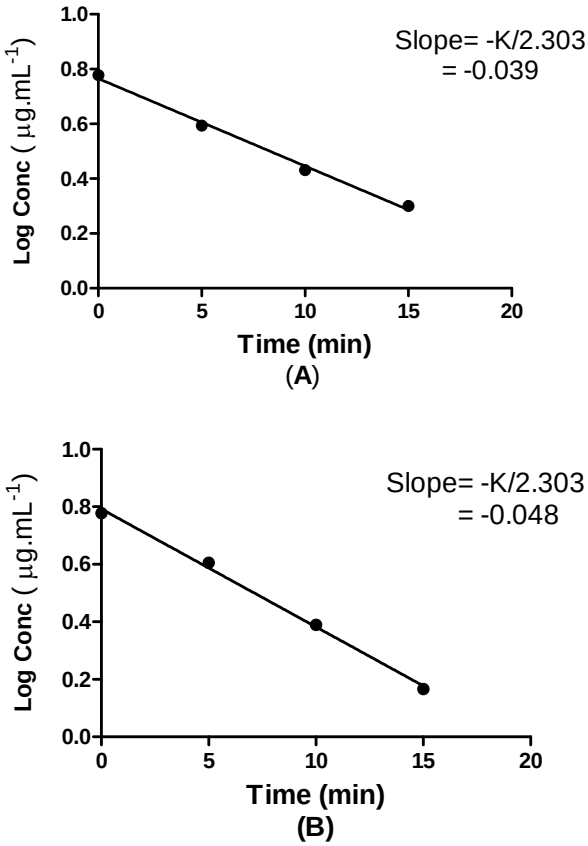
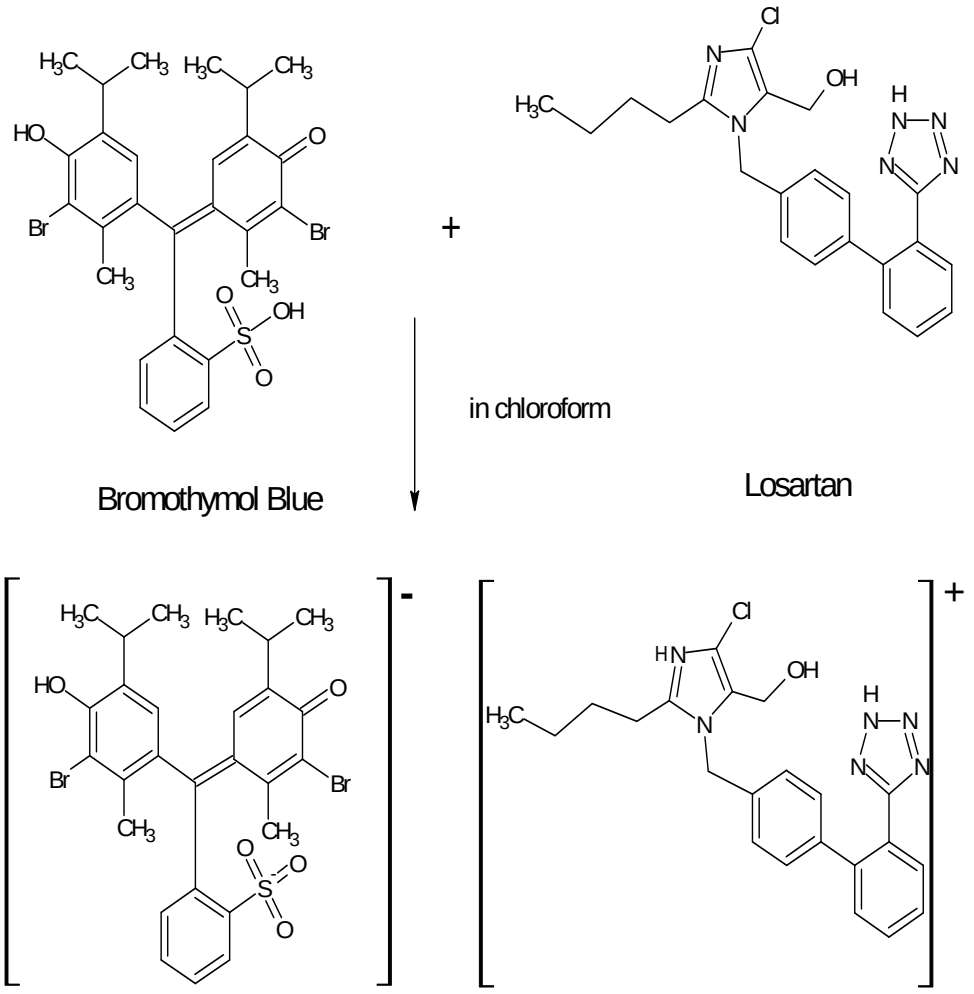


Figure 6: Plot of Log ***Los*** concentration (calculated from calibration graph preconstructed by the proposed spectrofluorimetric method) *Vs* different times of boiling with: (A) 2M NaOH and (B) 2M HCl.

Scheme 1: Proposal of the reaction pathway between **Los** and **BTB**.



4. Validation of the proposed methods:

4.3.1. Linearity of the method

Under the above experimental conditions, The calibration curves were constructed by plotting concentration versus absorbance or ΔRFI for spectrophotometric and spectrofluorimetric methods respectively.

The statistical parameters were calculated from regression equation drived from the calibration graphs, along with the standard deviations of the slope (S_b) and the intercept (S_a) on the ordinate and the standard deviation of residuals ($S_{y/x}$) with good correlation coefficients and small intercepts. The apparent molar absorptivities and detection limits [46] were summarized in (Table 1).

4.3.2. Sensitivity

The limit of detection (LOD) and the limit of quantitation (LOQ) for the proposed methods were calculated using the following equations [47, 48]

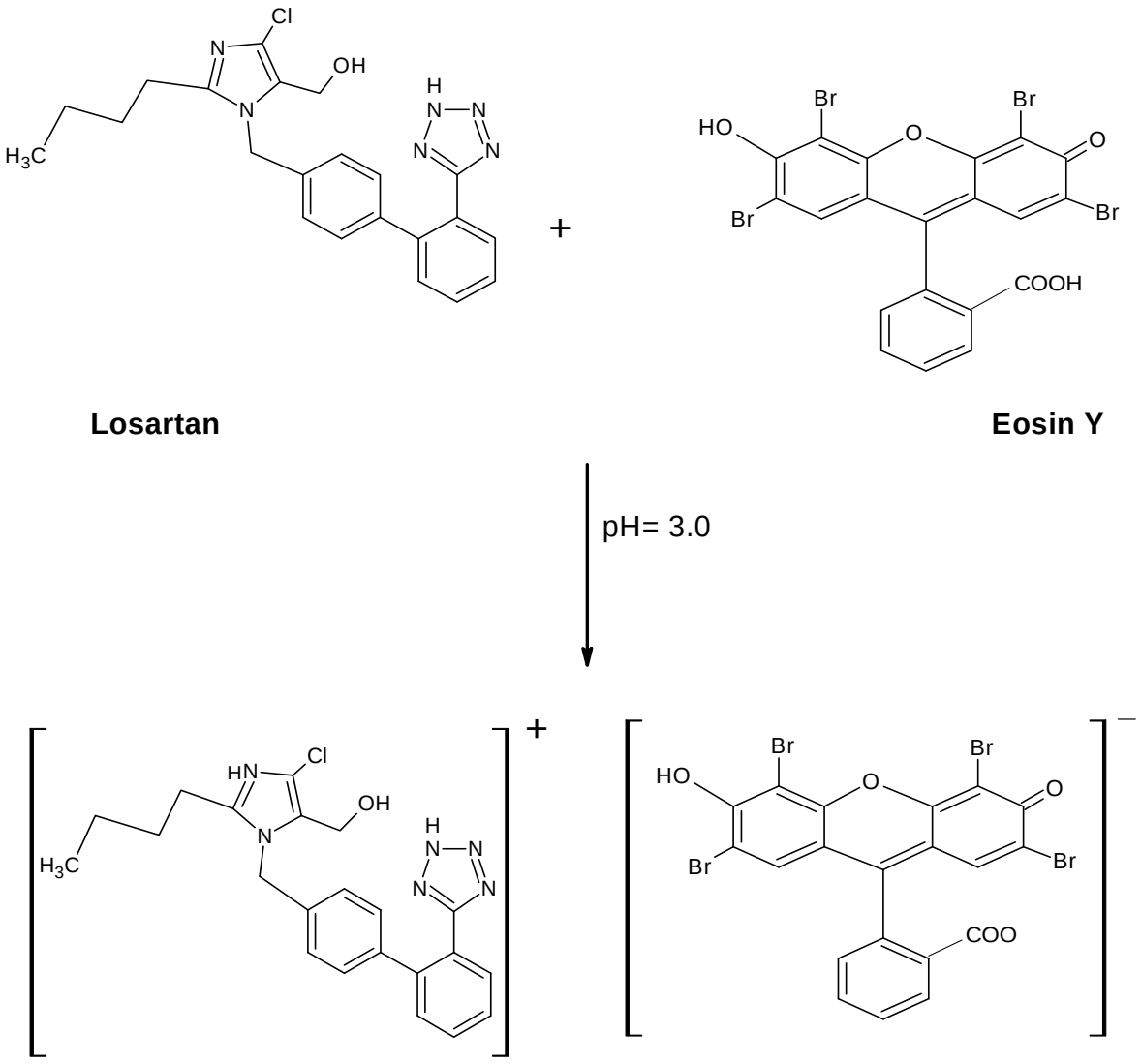
LOD = 3.3 σ/s

LOQ = 10 σ/s

σ is the standard deviation of intercept, ***s*** is the slope of calibration curve.

LOQs and LODs for the studied drugs are listed in (Table 1).

Scheme 2: Suggested reaction mechanism for reaction between **Los** and **Eosin Y**.



4.3.3. Specificity and interference

The specificity of the proposed methods was investigated by observing any interference encountered from common excipients in the pharmaceutical tablets such as starch, magnesium stearate, and Talc. It was found that these excipients did not interfere with the results of the proposed methods. This could be explained on the basis that all these additives are either non extractable by organic solvents or behave as non basic compounds, so they do not contribute in the reaction pathway.

4.3.4. Precision and accuracy

Statistical analysis of the regression equations allowed the calculation of standard deviation of the intercept (S_a), standard deviation of the slope (S_b) and standard deviation of the residuals ($S_{y/x}$). The small values of these parameters indicate the high precision of the proposed methods [46]. In order to determine the accuracy and the precision of the method, the accuracy was checked by

three times analysis for five different concentrations of pure samples. The results obtained in (Table 3) showed the close agreement between the measured and true values indicating good accuracy of the proposed method . Intraday and Interday precision were assessed using three concentration and three replicates of each concentration. The calculated relative standard deviation values were found to be small below 2 % indicating good repeatability and reliability of the proposed methods. The results and their statistical analysis were summarized in (Table 3).

4.3.5. Robustness and ruggedness

For the evaluation of the method robustness, some parameters were interchanged such as dye concentration for the spectrophotometric method, pH and buffer volume for spectrofluorimetric method. The capacity remains unaffected by small deliberate variations. Method ruggedness was expressed as R.S.D. % of the same procedure applied by using two different instruments on different days. The results showed no statistical differences between different instruments suggesting that the developed methods were robust and Spectrophotometric method was rugged.

5. Application to pharmaceutical tablets

The proposed methods have been successfully applied to the determination of the studied drugs in commercial tablets.

The results obtained are shown in Table (3). Six replicate determinations were made. Table (4) shows that satisfactory recovery data were obtained and the assay results were in a good agreement with the label claims. On comparison of the results obtained by the proposed methods with those of the reported methods [11, 33, 49, 50] using the ***t***-test for the accuracy and ***F***-test for the precision assessment, the calculated values did not exceed the corresponding theoretical values (tabulated value of *t*-test and *F*-test is at confidence level 95% = 6.39 and 2.78 for n = 5 degrees of freedom respectively) indicating no significant difference between proposed and reported methods.

6. Conclusion:

The proposed methods are rapid, simple, accurate, and extraction-free for the analysis of certain ARBs in their pure forms and tablets. Compared with the reported methods, the proposed methods are very simple, requiring only one reagent and non expensive instrumentation. The lower quantitation limit of the proposed method is much lower (1.0µg mL^{-1}). These advantages encourage the application of the proposed methods in routine quality control evaluation.

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