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ORIGINAL ARTICLE

Spectrofluorimetric method for determination of some angiotensin II receptor antagonists

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Abstract A simple, rapid, accurate and highly sensitive spectrofluorimetric method has been developed for determination of some angiotensin II receptor antagonists (AIIRA's), namely Losartan potassium (Los-K), Irbesartan (Irb), Valsartan (Val) and Candesartan cilexetil (Cand) in pure forms as well as in their pharmaceutical dosage forms. All the variables affecting the relative fluorescence intensity (RFI) were studied and optimized. Under the optimum conditions, linear relationships with good correlation coefficients (0.9982–0.9991) were obtained over the concentration range from 0.006 μg/mL to 1.7 μg/mL. Good accuracy and precision were successfully obtained for the analysis of tablets containing each drug alone or combined with hydrochlorothiazide (HCTZ) without interferences from the co-formulated HCTZ or the additives commonly present in tablets.

1. Introduction

Angiotensin II receptor antagonists (AIIRA's) are orally effective potent antihypertensive drugs that selectively blocking AT_1 receptors in many tissues leading to marked decrease in angiotensin II synthesis which is potent vasoconstrictor and then lowering the blood pressure [1]. The investigated drugs were Losartan potassium (Los-K), Irbesartan (Irb), Valsartan (Val) and Candesartan cilexetil (Cand) (Fig. 1).

The studied drugs have been approved by FDA since the end of 1990s and are prescribed as the first line treatment of hypertension, left ventricular hypertrophy, nephropathy in type 2 diabetic patients and heart failure [2]. Recently they became official in the USP 2007 [3], only Losartan potassium is official in the BP 2010 [4] while Cand is not official in any pharmacopeia.

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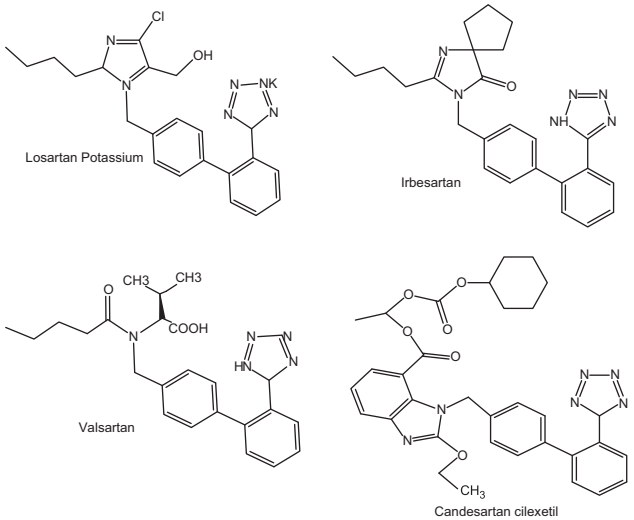

Figure 1 Chemical structure of the studied drugs.

Several methods have been reported for the analysis of the investigated AIIRA's, including high performance liquid chromatography [5–9], high pressure thin layer chromatography [10–12], capillary electrophoresis [13–15], voltammetric methods [16–19] and spectrophotometric methods [20–25].

The presence of different fluorescent functional groups (fluorophores) such as biphenyl, tetrazole and/or imidazole in the chemical structures of the investigated drugs allowed their determination by measurements of its native fluorescence. Limited spectrofluorimetric methods were found in the literatures regarding the analysis of Los-K [26], Irb [27] and Val [28] depending on their native fluorescence. Also, spectrofluorimetry was reported for determination of the pK_a value of all the investigated drugs [29]. Unfortunately, all these previously reported methods did not establish complete study for determination of the fluorescence of these drugs as well as lack of sensitivity was noticed in all cases. So it is deemed necessary to enhance the sensitivity of the native fluorescence of the investigated drugs.

It is well known that, the relative fluorescence intensity of any chemical compound is greatly affected by different solvents, acids, bases, pH or even temperature. The presence of tetrazole ring (pK_a of tetrazole ~5) is responsible for the acidic properties of the cited drugs as well as, its pK_a values, which are 3.15, 4.70, 4.90 and 6.00 for Los-K, Irb, Val and Cand, respectively [29] so it is clear that, for maximum determination of the native fluorescence of these drugs with the highest sensitivity, the RFI should be measured in acids or acidic medium. All these aforementioned factors drew our attention to use different acids, bases and buffers to develop a highly sensitive, simple, rapid and reproducible spectrofluorimetric method for determination of Los-K, Irb, Val and Cand based on measurement of their native fluorescence in pure forms and pharmaceutical formulations.

2. Experimental

2.1. Apparatus

Spectrofluorophotometer RF-5301 PC (Shimadzu, Tokyo, Japan) was used with the excitation and emission slit control set at slit width 5 mm equipped with 1 cm quartz cell.

Sartorius handy balance-H51 was obtained from GmbH Gottingen, Germany. Milwaukee SM 101 pH meter and MLW type thermostatically controlled water bath, which was obtained from Memmert GmbH, Schwabach, Germany.

2.2. Materials and reagents

All solvents and reagents used were of analytical grade. Losartan potassium (Los-K) (Amriya Pharmaceutical Industries, Alexandria, Egypt), Irbesartan (Irb) (Memphis Pharm. Co, Cairo, Egypt), Valsartan (Val) (G.N.P. Pharm. Co, 6th of October, Egypt) and Candesartan cilexetil (Cand) (Pharoania Pharmaceutical Corroboration) were obtained as a gift and used as supplied.

Hydrochloric acid (0.1 M and 0.5 M), phosphoric acid (0.1 M), citric acid (0.1 M), NaOH (0.1 M) and Teorell and Stenhagen buffer (pH 2.0–7.0) [30] were prepared using double distilled water. Pharmaceutical formulations (Tablets) containing the studied drugs were purchased from a local market. Losarmepha® (Sigma Pharm.Co., Cairo, Egypt) and Hysartan® (Amriya Pharm. Ind. Co., Alexanderia, Egypt), which are labeled to contain 50 mg Los-K or 50 mg Los-K and 12.5 mg hydrochlorothiazide (HCTZ), respectively, Aprovel® and Co-Aprovel® (Sanofi Winthrop, France), which are labeled to contain 300 mg Irb or 300 mg Irb and 12.5 mg HCTZ, respectively, Tareg® and Co-Tareg® (Novartis Pharm., Cairo, Egypt), which are labeled to contain 80 mg Val or 160 mg Val and 12.5 mg HCTZ, respectively, Atacand® and Atacand plus® (Astra-Zenica, 6th October, Egypt), which are labeled to contain 8 mg Cand or 8 mg Cand and 12.5 mg HCTZ, respectively.

2.3. Preparation of standard solutions

An accurately weighed amount (10 mg) of Los-K, Irb, Val or Cand was transferred into 25 mL volumetric flasks, dissolved and completed to the volume with methanol to obtain the stock standard solutions for each drug. The working standard solutions of the studied drugs were prepared by further dilutions of suitable volumes of the stock solution with methanol to obtain the concentrations ranged from 0.25 to

10 μg/mL for either Los-K or Irb, 0.06 to 6.50 μg/mL for Val and 0.5 to 17 μg/mL for Cand.

2.4. Preparation of sample solutions

Ten tablets of each formulation containing Los-K, Irb, Val or Cand alone or combined with HCTZ were accurately weighed and finely powdered. An accurately weighed quantity of the powdered tablets equivalent to 10 mg of each studied drug was transferred into different 25 mL volumetric flasks, dissolved in about 10 mL methanol, sonicated for 15 min and then completed to the mark with methanol. The resulting solutions were filtered and the first portion of the filtrate was rejected. A suitable aliquot of the obtained solutions was quantitatively diluted with methanol to obtain concentrations within the linear range of each studied drug as mentioned under preparation of standard solutions.

2.5. General procedure

Accurately measured 1.0 mL of the working solutions of each drug was transferred into separate 10 mL volumetric flasks. The solutions were completed to the volume with Teorell and Stenhagen buffer at pH 2.3 for Los-K, pH 3.5 for Cand or 0.5 M HCl for both Irb and Val. The relative fluorescence intensity (RFI) was measured at $\lambda_{Ex}/\lambda_{Em}$ equal to 260/390 nm, 262/410 nm, 258/430 nm and 260/389 nm for Los-K, Irb, Val and Cand, respectively, against blank treated similarly.

3. Results and discussion

Initial investigations have been made to show the excitation and emission wavelengths of each studied drug using different solvents with different polarities such as water, acetonitrile, ethanol, methanol and propan-2-ol. As can be seen from Table 1 each studied drug is being absorbed in the interfering UV area showing excitation wavelengths ranged from 231 to 262 nm and relatively considerable fluorescence with emission wavelengths in the range of 377–430 nm. It has been found that, the fluorescence of all studied drugs was increased upon using water as solvent and decreased by decreasing the solvent polarity. Furthermore, it was observed that, the position of the excitation wavelengths showed hypsochromic shift for all investigated drugs in all studied solvents accompanied by either hypsochromic or bathochromic shifts of their emission wavelengths.

3.1. Optimization of the variables

The effects of acids, bases, buffers, temperature and time were optimized to achieve maximum native fluorescence for all investigated drugs.

3.1.1. Effect of acids and bases

Different acids such as HCl, citric acid, acetic acid, sulfuric acid, nitric acid, oxalic acid and phosphoric acid (0.1 M) in addition to bases such as NaOH and KOH (0.1 M) were used to study their effects on the fluorescence intensity for each studied drug. Experiments showed substantial decrease in the fluorescence of all investigated drugs upon using NaOH or KOH (0.1 M), thus all alkalis were excluded from this study.

In the case of acids, the highest fluorescence intensity was obtained on using 0.1 M citric acid for both Los-K and Cand. On the other hand, Irb and Val gave maximum fluorescence intensity when 0.1 M HCl was used as a milieu for their determination (Table 2). When other acids were used, the relative fluorescence intensity of all studied drugs was obviously decreased rather than citric acid or HCl and so, citric acid was chosen as the best acid for measurement of the fluorescence intensity for both Los-K and Cand while HCl was selected for determination of Irb and Val.

3.1.2. Effect of acid concentration

Different concentrations of HCl and citric acid (0.1–1 M) were tried in order to select the proper acid concentration to give maximum fluorescence intensity for determination of these drugs. It was observed that, no effect on the intensity of fluorescence for both Los-K and Cand upon using different concentrations of citric acid (0.1–1 M). On the other hand, the fluorescence intensity of Irb and Val slightly increased by increasing concentration of HCl till 0.3 M and then remained constant up to 1 M. Accordingly, the best selected concentration of HCl for determination of either Irb or Val was achieved using 0.5 M HCl.

3.1.3. Effect of pH

Trials have been made to enhance the native fluorescence intensity for the studied drugs by proper choice of the pH medium instead of acids. Different buffer systems with various pH values and different constitution in the acidic pH range were used to study the effect of buffer systems on the fluorescence intensity.

Table 1 Effect of different solvents on the relative fluorescence intensity (RFI) of the investigated AIIRA's.

Solvent	Solvent polarity	Los-K (0.5 μg/mL)		Cand (0.8 μg/mL)		Irb (0.5 μg/mL)		Val (0.2 μg/mL)	
		$\lambda_{Ex}/\lambda_{Em}$	RFI[a]	$\lambda_{Ex}/\lambda_{Em}$	RFI[a]	$\lambda_{Ex}/\lambda_{Em}$	RFI[a]	$\lambda_{Ex}/\lambda_{Em}$	RFI[a]
Water	9.0	260/390	91	258/389	193	262/410	123	258/430	125
Acetonitrile	5.8	237/394	85	245/377	190	247/422	105	249/399	98
Ethanol	5.2	239/392	80	247/382	188	246/411	92	256/402	91
Methanol	5.1	241/394	79	241/387	187	243/413	90	252/400	90
Propan-2-ol	4.0	231/412	62	238/381	173	233/391	62	232/388	83

[a]Average of three determinations.

Table 2 Effect of different acids (0.1 M) on the relative fluorescence intensity (RFI) of the studied drugs.

Acid (0.1 M)	RFI[a]			
	Los-K (0.5 μg/mL)	Cand (0.8 μg/mL)	Irb (0.5 μg/mL)	Val (0.2 μg/mL)
HCl	389	167	412	295
Citric acid	408	280	252	88
Acetic acid	183	200	111	224
Sulfuric acid	305	193	376	200
Nitric acid	288	131	357	265
Oxalic acid	89	183	97	114
Phosphoric acid	383	149	232	211

[a]Average of three determinations.

Since Los-K and Cand in citric acid (0.1–1 M) and Irb and Val in HCl (0.5 M) showed high native fluorescence intensities, it was anticipated that, buffer systems with lower pH value in the neighborhood to the pH values of the aforementioned acids would give the same results.

Teorell and Stenhagen buffer (0.1 M citric acid, 0.1 M phosphoric acid, 0.1 M HCl and 0.1 M NaOH) (pH 2.0–7.0) [30] with various pH values was tried in order to enhance the fluorescence intensity for all studied drugs.

It was observed that, the fluorescence intensity of Los-K at the pH range 2–2.5 was increased about 10% in comparison to the results obtained with citric acid. After that, gradual decrease in the intensity occurred till pH 7. Consequently, pH 2.3 of this buffer system was chosen as the optimum milieu for determination of Los-K.

In the case of Cand, the relative fluorescence intensity was increased about 25% over the pH range from 3 to 4.5 of this buffer system than the obtained RFI in citric acid. Before and after this pH range, the intensity of fluorescence was markedly decreased so, pH 3.5 was selected as the best medium for determination of Cand.

The relative fluorescence intensity of Irb and Val measured at pH 2, was decreased about 13% and 22%, respectively, in comparison to using 0.5 M HCl as milieu for their determination, followed by marked decrease till pH 7.

Upon changing the constitution of the buffer system in order to increase the intensity of fluorescence, two other buffer systems such as Clark and Lubs buffer (0.1 M potassium bi-phthalate, 0.1 M HCl and 0.1 M NaOH) (pH range 2.2–6.0) and McIlavine buffer (0.2 M disodium hydrogen phosphate and 0.1 M citric acid) (pH range 2.2–7.0) [30] were investigated.

Complete quenching of the fluorescence intensity for all studied drugs was attained using Clark and Lubs buffer (pH range 2.2–6.0). This was probably owing to potassium bi-phthalate-containing buffer, which may quench the fluorescence of the studied drugs and so this buffer system should be omitted.

When measurements were made using McIlavine buffer in the pH range 2.2–7.0, marked decrease in the fluorescence intensity was obtained for all studied drugs in comparison to Teorell and Stenhagen buffer.

As a result of this study, the fluorescence intensities were performed in Teorell and Stenhagen Buffer at pH 2.3 or 3.5 for either Los-K or Cand, respectively, while 0.5 M HCl was adopted for determination of both Irb and Val.

3.1.4. *Effect of temperature and time*

In order to examine the effect of temperature and heating time on the native fluorescence in the selected media for each studied drug, heating was carried out at different temperatures (40, 60, 80 and 100 °C) in a thermostatically controlled water bath for periods ranging from 1 to 5 min. Sharp decrease in the fluorescence intensity was obtained immediately upon raising the temperature over the room temperature. Therefore, measurements were performed at room temperature, for all studied drugs and the fluorescence was stable for at least 30 min.

3.2. *Spectral characteristics*

Under the optimum experimental conditions, the best selected medium for the highest fluorescence intensity was Teorell and Stenhagen buffer at pH 2.3 and 3.5 for Los-K and Cand, respectively, while Irb and Val were determined in 0.5 M HCl. Maximum $\lambda_{Ex}/\lambda_{Em}$ equal to 260/390 nm, 262/410 nm, 258/430 nm and 260/389 nm for Los-K, Irb, Val and Cand, respectively.

In the context of the foregoing discussions, we can relate these results to the functional groups with acidic–basic properties such as tetrazole and/or carboxyl group present in the molecular structures of the studied drugs. The native fluorescence behavior may be attributed to deprotonation of tetrazole group in all investigated drugs as well as the carboxyl group in the case of Val. Since the pK_a values of the studied drugs are 3.15, 4.70, 4.90 and 6.00 for Los-K, Irb, Val and Cand, respectively [29], it is evident that, the best optimum fluorescence was performed in acids or acidic buffers and that is exactly proved by this work.

3.3. *Calibration curves*

Under the specified conditions, the relationship between the fluorescence intensity and concentration at the selected excitation and emission wavelengths for each drug was quite linear. Table 3 shows the most important characteristics and quantitative parameters for all investigated drugs such as slope, intercept, LOD, LOQ and correlation coefficients.

4. Method validation

The proposed method was fully validated according to USP 30 NF25 validation guidelines [3] and complied with the international conference on harmonization (ICH) guidelines [31].

Table 3 Quantitative parameters for the analysis of the studied drugs by the proposed method.

Drug	Range (μg/mL)	Correlation coefficient (*r*)	Slope (*b*) ± SD[a]	Intercept (*a*) ± SD[a]	LOD[b] (μg/mL)	LOQ[c] (μg/mL)
Los-k	0.025–1.000	0.9991	860.71 ± 6.39	0.25 ± 2.08	0.008	0.024
Cand	0.05–1.70	0.9982	433.90 ± 2.91	5.28 ± 2.54	0.019	0.058
Irb	0.025–1.000	0.9987	855.93 ± 7.84	1.59 ± 1.92	0.007	0.022
Val	0.006–0.650	0.9985	1514.02 ± 13.46	7.64 ± 0.78	0.002	0.005

[a]Average of five determinations.
[b]LOD is the limit of detection.
[c]LOQ is the limit of quantitation.

4.1. Sensitivity

The limit of detection (LOD) and the limit of quantitation (LOQ) for all the studied drugs were calculated. The small values of LOD (0.002–0.019 μg/mL) and LOQ (0.005–0.058 μg/mL) indicate good sensitivity and low background effect of the proposed method (Table 3). Also the using of buffer or acid medium in the developed method led to enhancement in both sensitivity, detection and quantitation limits at concentration level 20, 13 and 1.6 times better than the previously reported methods for fluorimetric determination of Los-K, Irb and Val, respectively [26–28].

4.2. Precision and accuracy

Five determinations at three different concentration levels of each drug covering low, medium and high concentration of the linear range were performed. The small value of relative standard deviation (RSD), indicates high precision of the method for quantitative determination of the fluorescence intensity for all studied AIIRA's. Moreover, the good percentage of recoveries of each standard drug confirms excellent accuracy (Table 4).

4.3. Selectivity

The selectivity of the proposed method for analysis of the investigated AIIRA's was carried out to evaluate the effect of the co-formulated HCTZ and commonly encountered excipients. The results showed that no interferences could be observed from the co-formulated HCTZ as well as other excipients.

4.4. Robustness and ruggedness

Robustness of the presented study was evaluated by changing one experimental parameter of the experimental procedure while the other parameters were kept constant; the recovery percentage was calculated each time. The small variations in any of the variables did not significantly affect the results. This indicates the good reliability of the proposed method.

Ruggedness of the proposed method was assessed by applying the procedure at different elapsed time. The obtained results of the proposed method express good reproducibility of the presented method.

Table 4 Precision and accuracy of the proposed method for the analysis of the studied drugs.

Drug	Conc. (μg/mL)	Recovery (%) ± SD[a]	RSD[a] (%)
Los-K	0.3	101.36 ± 1.23	1.32
	0.5	98.83 ± 0.15	0.39
	0.8	100.83 ± 1.08	0.98
Irb	0.3	101.58 ± 1.25	0.41
	0.5	99.86 ± 1.02	1.20
	0.8	101.47 ± 0.72	1.47
Val	0.1	100.54 ± 0.90	0.94
	0.3	98.75 ± 1.03	0.49
	0.5	99.45 ± 0.78	0.85
Cand	0.5	99.32 ± 0.88	0.17
	1.0	99.47 ± 0.97	0.93
	1.5	99.85 ± 1.66	1.50

SD: Standard deviation.
RSD: Relative standard deviation.
[a]Average of five determinations.

5. Applications

The developed method was applied successfully for the determination of the studied drugs in their pharmaceutical dosage forms. Five replicate measurements were performed for each drug either alone or in combination with other active ingredient HCTZ. The results were validated by comparison to previously reported methods [22–25]. No significant difference was found by applying *t*-test and *F*-test at 95% confidence level, indicating good accuracy and precision of the proposed method for the analysis of the studied drugs in their pharmaceutical dosage forms (Table 5).

6. Conclusion

The proposed method is simple, precise, accurate spectrofluorimetric method for determination of Losartan potassium, Irbesartan, Valsartan and Candesartan cilexetil. The method depended on measurements of the relative fluorescence intensity for the studied drugs using acids or buffers. In comparison to previously reported methods, the sensitivity was enhanced about 20, 13 or 1.6 times more than these methods.

Table 5 Determination of the studied drug in their pharmaceutical dosage forms (Tablets).

Drug	Dosage form (Tablets)	Recovery (%) ± SD[a]		*t*-value[b]	*F*-value[b]	Reference
		Proposed method	Reported method			
Los-K	Losarmepha®	101.32 ± 0.75	98.84 ± 0.38	1.97	5.05	[22]
	Hysartan®	98.25 ± 0.34	98.84 ± 0.44	1.30	3.47	[22]
Irb	Aprovel®	98.85 ± 0.98	100.13 ± 0.51	2.44	2.11	[23]
	Co-Aprovel®	101.12 ± 0.48	98.74 ± 0.21	1.48	3.90	[23]
Val	Tareg®	97.45 ± 0.37	98.62 ± 0.48	2.60	1.62	[24]
	Co-Tareg®	98.30 ± 0.44	99.70 ± 0.41	1.10	1.26	[24]
Cand	Atacand®	100.12 ± 0.11	102.20 ± 0.59	2.18	4.93	[25]
	Atacand Plus®	98.54 ± 0.81	100.04 ± 0.38	1.92	5.45	[25]

[a]Average of five determinations.
[b]The tabulated values of t and F at 95% confidence level are 2.78 and 6.39, respectively.

The method was applied for the determination of the investigated drugs in their dosage forms in the presence of hydrochlorothiazide without interference. Also the statistical data represent the suitability and reproducibility of the proposed method for routine analysis in the quality control laboratories.

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