Quantitative Quenching of Fluorescein-based Method for Determination of Valsartan in Some Pharmaceutical Product

Aryan F. Qader, Musher I. Salih and Tara F. Tahir

Department of Chemistry, Koya University,

Daniel Mitterrand Boulevard, Koya KOY45 AB64, Kurdistan Region – Iraq

***Abstract*—*Simple,* inexpensive, rapid, and sensitive determination of valsartan (VAL) spectrofluorometrically was suggested using fluorescein dye as a reagent. The method based on the quantitative quenching effect of VAL on the native fluorescence of fluorescein dye due to the reaction of VAL with fluorescein dye to form a charge transfer complex which results in fluorescence quenching of the fluorescein. The fluorescence quenching intensity was measured at 515 nm after excitation at 470 nm. Fluorescence concentration plot was rectilinear over the range of 1.0-8.0 µg/ mL with correlation coefficient 0.9965 and detection limit 0.06 µg/mL. No interference was observed from the excipients that are commonly present in pharmaceutical formulations. The proposed method was for the determination of VAL in some commercial tablets, and their results were compared with that of high-performance liquid chromatographic method revealed with good agreements and no significant differences in the accuracy and precision.**

***Index Terms*—Charge transfer complex, Fluorescein dye, Fluorescence quenching, Valsartan.**

I. Introduction

Valsartan (VAL) is N-[-P-(0-1 H-Tetrazole-5-yl phenyl) Benzyl]-N-Valeryl-L-Valine (Fig. 1) [1]. It is an angiotensin receptor blocker (ARB) that is used to treat a variety of cardiac conditions including hypertension, diabetic nephropathy, and heart failure [2]. VAL lowers blood pressure by antagonizing the renin-angiotensin-aldosterone system. It competes with angiotensin II (AT1) for binding to the type-1 AT1 receptor subtype and prevents the blood pressure increasing effects of angiotensin II [3]. Unlike angiotensin-converting enzyme inhibitors, ARBs do not have the adverse effect of dry cough. VAL is used to treat hypertension, isolated systolic hypertension, left ventricular hypertrophy, and diabetic nephropathy. It may also be used as an alternative agent for the treatment of heart failure, systolic dysfunction, myocardial infarction, and coronary artery disease [3]. Several analytical methods such as liquid chromatography-tandem mass spectrometry [4], micellar electrokinetic chromatographic method [5], capillary zone electrophoresis [6], and high-performance liquid

chromatographic (HPLC) [7] were reported for the determination of VAL and other pharmaceutical products. The reported methods require expensive equipment and are complicated in operation. Although spectrophotometric method is not such sensitive, VAL determination was reported using UV spectrophotometry [8].

Fluorescence spectroscopy is one of the most powerful methods that require a small amount of material and has a high signal-to-noise ratio. This technique has been widely used to estimate pharmaceuticals due to its simplicity, high sensitivity, low cost, and less time consumption comparing with other analytical techniques [9].

In the present work, spectrofluorimetric method has been developed for the determination of VAL based on quenching the fluorescence of fluorescein by VAL due to the formation of charge transfer complex.

II. Materials and Methods

A. Apparatus

Agilent cary eclipse fluorescence spectrophotometer with slit width 5 nm and a quartz cell 1.0 cm was used for fluorescence spectra measurements.

B. Reagent and Samples

All chemicals used in the study were of analytical grade. All the solvents were purchased from Scharlau, Spain, except methanol and ethanol were from GCC, Kazakhstan. A standard solution of 4×10^{-5} M fluorescein (Merck, Germany) was prepared in methanol. This solution was stable at refrigerator for several weeks.

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Corresponding author's e-mail: tara.fuad@koyauniversity.org

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Pharmaceutical formulations of VAL such as VAL (JOSWE medical) containing 160 mg, VAL Awa (Awamedica) containing 160 mg, and VAL TAD (TAD Pharma) containing 80 mg were purchased from local medical store.

C. Standard Stock Solution

Standard stock solution (1000 µg/mL) of VAL provided from Awamedica Company of drugs in Erbil- Kurdistan Region of Iraq, was prepared by dissolving 0.05 g of standard VAL powder from Awamedica, in 40 mL methanol with carefully stir, then completed to 50 mL with the same solvent and kept in refrigerator. Working standard solutions were prepared daily by suitable dilution of the stock standard solution with the same solvent.

D. Sample Preparations

Three different brands of pharmaceutical products were used. 10 tablets were weighed and grounded into a fine powder, then mixed thoroughly. An accurately weighed amount of the powder was mixed with about 40 mL of methanol. The solution was stirred for 10 min to increase solubility. A Whatman No. 41 filter paper was used for filtration the insoluble mass, which was washed with methanol. The filtrate and these washings were diluted in a volumetric flask to 50 mL with methanol.

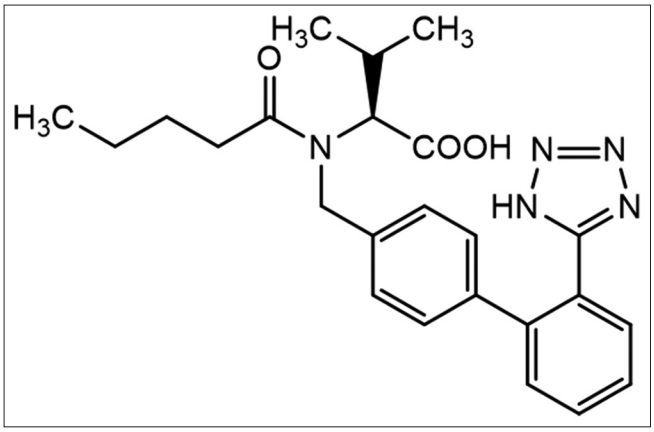

Fig. 1. Chemical structure of valsartan.

E. Preliminary Test

A 1.0 mL of fluorescein solution (4.0×10^{-5} M) was added into 10 mL volumetric flask that containing an aliquot of 5.0 µg/mL VAL. The solution was shacked thoroughly and diluted to the mark with methanol. The difference in fluorescence intensity between the reagent blank and each experiment was measured at λem = 515 nm after excitation at λex = 470 nm using 1.0 cm quartz cell.

F. Recommended Procedure

An adequate volume of 1.2 mL of fluorescein solution (4.0 µ 10^{-5} M) was added into 10 mL volumetric flask, followed by the addition of an aliquot from each sample solution in the range of calibration curve. The mixture was mixed

well and adjusted to the volume with methanol. The difference in fluorescence intensity between the reagent blank and each experiment was measured at λem = 515 nm after excitation at λex = 470 nm.

III. Results and Discussion

A. Optimization of Experimental Parameters

All chemical conditions of the reaction have been studied to obtain maximum sensitivity. Different variable conditions were investigated to obtain optimum conditions of the proposed method.

B. Emission Spectra

The native fluorescence of fluorescein dye is strongly observed in methanol at λem = 515 nm after excitation at λex = 470 nm, and it was found that addition of drug to fluorescein solution causes a decrease in the fluorescence

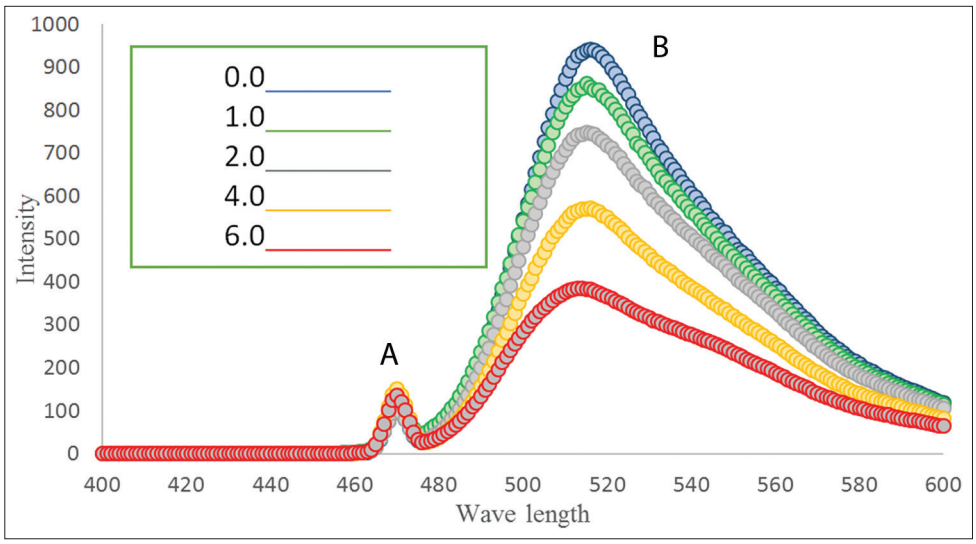

Fig. 2. (a) Excitation and (b) emission spectra of the reaction product of 0.0, 1.0, 2.0, 4.0, and 6.0 µg/mL valsartan with fluorescein.

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intensity of fluorescein without any shift (Fig. 2). It is observed in Fig. 2 that fluorescence quenching of fluorescein by VAL is directly proportional to the concentration of the drug (VAL) in a certain range.

C. Effect of Solvent

Fluorescence intensity of fluorescein was investigated in different solvents using fixed concentration of VAL (5.0 µg/ mL). Different polarities of organic solvents were tested (methanol, ethanol, acetone, dimethyl sulfoxide, chloroform, and n-hexane).

Remarkably, the fluorescence intensity was never changed in the non-polar solvents: N-hexane and chloroform, nor in the polar aprotic solvents: Acetone and dimethyl sulfoxide. In a different way, quenching the intensity of the fluorescence was increased when changing from acetone to the more polar solvent methanol. Increasing solvent polarity is largely red-shifted fluorescence band. This is due to the increase in dipole moment of excited state compared to ground state [10]. In general, the polarity of a solvent will influence the emission spectra of fluorophores by changes in quantum yields and spectral shifts. Thus, methanol was chosen for continuing the studies (Fig. 3).

D. Effect of Temperature

The reaction of VAL with fluorescein dye was carried out at laboratory temperature (about 25°C), in addition to four different temperatures of 30, 40, 50, and 60°C. The difference in fluorescence intensity ΔF of the solutions was measured after cooling the solutions to the laboratory temperature. The investigation of the effect of temperature on complex formation and ΔF value indicated that ΔF was slightly decreased at high temperatures [11] as shown in Fig. 4. Accordingly, further, experiments were accomplished at laboratory temperature (25 ± 2°C) [10].

Reaction between fluorescein and VAL to form a complex is resulted in fluorescence quenching of fluorescein reagent. Three mechanisms of fluorescence quenching are available; static quenching, dynamic quenching, and resonance energy transfer [12]. The dynamic quenching is included enhancing collisions between molecules with increasing the temperature and results increasing the value of ΔF at high temperatures. On the other hand, the difference in fluorescence intensity ΔF is decreased with increasing temperature in static quenching due to complex dissociation [12]. Depending on the results of Fig. 4 where five different degrees (25, 30, 40, 50, and 60°C) were investigated, the value of ΔF is dropped with increasing the temperature from 25 to 60°C. Therefore, fluorescence quenching mechanism is considered as static quenching.

E. Effect of Time

It was found from the investigation of the time effect of reaction on fluorescein - VAL system that the complex got stabilized immediately after mixing. The difference in fluorescence intensity ΔF value was remained stable for at least 24 h. Therefore, the fluorescence measurements could be taken directly after addition of fluorescein reagent.

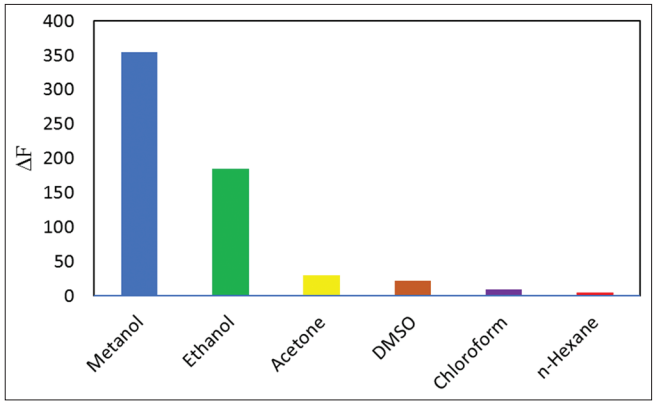

Fig. 3. Solvent effect on fluorescence quenching of fluorescein (4.0×10^{-5} M) in the presence of valsartan (5.0 µg/mL).

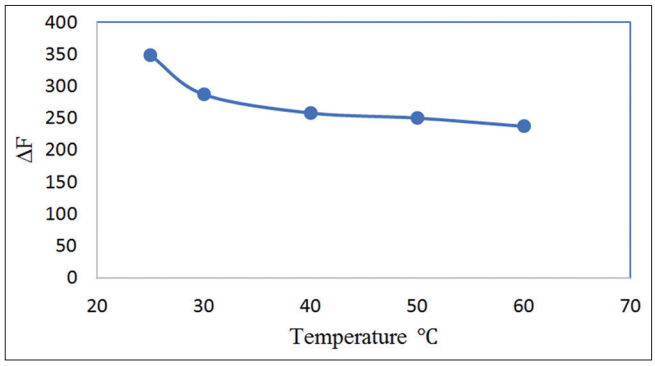

Fig. 4. Effect of temperature on fluorescence quenching of fluorescein (4.0×10^{-5} M) in the presence of valsartan (5.0 µg/mL)

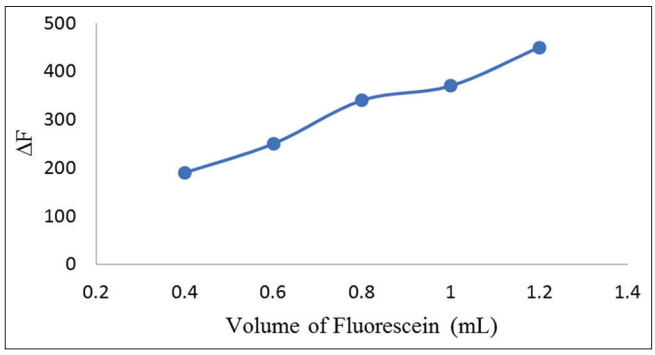

Fig. 5. Effect of volumes of 4.0×10^{-5} M fluorescein on the quantitative quenching intensity.

F. Effect of Fluorescein Concentration

Effect of different volumes of 0.4–1.2 mL of 4.0×10^{-5} M fluorescein solution was examined on the maximum quantitative quenching product. Fig. 5 shows increasing the quantitative quenching with the increase of volume of fluorescein reagent. The figure shows that 1.2 mL of fluorescein solution is the optimum, while adding more than 1.2 mL volume of fluorescein reagent will cause over range reading of ΔF value.

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G. Validation of the Method

Calibration curve

A standard calibration curve was obtained under the optimum experimental conditions, by plotting the difference in fluorescence intensity ΔF versus VAL concentration. The calibration curve was linear in the concentration range of 1.0– 8.0 µg/mL with detection limit 0.06 µg/mL of VAL (Fig. 6). The limit of detection (LOD) and limit of quantification (LOQ) were determined using the formulas: LOD = 3.3 σ/S and LOQ = 10 σ/S, respectively. Where, σ is the standard deviation of five reagent blank determinations and S is the slope of the calibration curve. The optical and characteristics of the method are shown in Table I.

Accuracy and precision

The precision and accuracy of the proposed method were checked according to the recommended procedure and under the optimum conditions by calculating the values of relative standard deviation (RSD %) and relative error percentage (error %), for five replicate aliquots of three standard sample solutions (1.0, 5.0, and 8.0 µg/mL). Table II summarizes the precision and accuracy data where accuracy at high concentration of VAL is lower than at low concentration of VAL due to the high effect of interferences on lower concentration. Overall, the accuracy data at low and high concentrations of VAL are <5%.

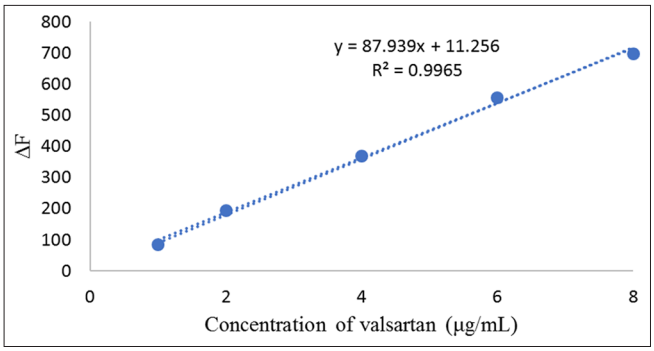


Fig. 6. Calibration curve of spectrofluorometric determination of valsartan using fluorescein as a reagent.

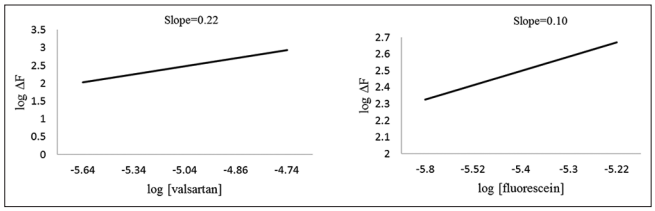
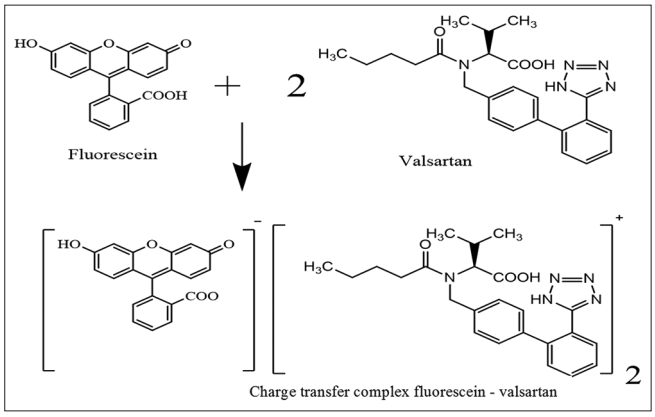


Fig. 7. Stoichiometry of the reaction of valsartan (VAL) - fluorescein complex by adopting limiting logarithmic method. (a), log



Scheme 1. Mechanism of charge transfer complex between valsartan and fluorescein.

H. Study of Interference

The effects of some common foreign species which can be found in typical pharmaceutical products were studied using the recommended procedure of spectrofluorometric method under optimum conditions. Various amounts of interfering species were added individually to a standard sample solution containing (5 µg/mL) pure VAL in the final volume of 10 mL. The results obtained are given in Table III. The tolerance limit was found to be as the concentration causing an error of not more than 5% in the determination of the VAL. No interferences were observed from the presence of lactose, silica, magnesium stearate, and stearic acid in the ratios commonly used in pharmaceutical preparations.

TABLE I
Optical and Characteristics for the Proposed Spectrofluorometric Method Using Fluorescein Reagent

Parameter	Characteristic
λex (nm)	470
λem (nm)	515
Beer's law (µg/mL)	1.0–8.0
Linear regression (R^2)	0.9965
Regression equation	y = 87.939x + 11.256
LOD (µg/mL)	0.06
LOQ (µg/mL)	0.19

TABLE II
Precision and Accuracy Data of the Proposed Method

Spiked valsartan (µg/mL)	Found by proposed method (µg/mL)	SD	RSD%	*Error%
1.00	0.97	1.85	2.27	-3.00
5.00	5.14	1.63	0.35	2.80
8.00	7.85	0.53	0.10	−1.90

*Average of three determinations

TABLE III
Study of Interference in the Determination of VAL *by the Proposed Method*

Interfering species	Maximum conc. (µg/mL)	*Error%
Lactose	500	−2.00
Silica	500	1.10
Magnesium stearate	500	1.30
Stearic acid	500	−1.80

*Average of three determinations

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TABLE IV
Results of Analysis of Commercial Drugs Containing Valsartan, by the Proposed Spectrofluorometric Methods Using Fluorescein as a Reagent and Standard Method (HPLC)

Pharmaceutical products	Content (mg) declared	Proposed method	Standard HPLC method	Recovery%	*Error%
JOSWE medical	160	159.29	158.98	100.19	0.19
Awamedica	160	160.90	159.16	101.09	1.04
TAD Pharma	80	80.60	79.90	100.88	0.88

*Average of three determinations

Stoichiometry of the reaction

The stoichiometry of the reaction between the studied drug and fluorescein was studied by adopting the limiting logarithmic method [13]. Fig. 7a and b show plotting of log (VAL) versus log ΔF in the presence of constant concentration of fluorescein and log (fluorescein) versus log ΔF in the presence of constant concentration of VAL, respectively. Both plots gave straight lines and from the slopes of the straight lines, it is concluded that the reaction of VAL - fluorescein complex is of 2:1 ratio (Scheme 1). The quenching mechanism is due to form charge transfer complex [12].

Application of the method

The proposed spectrofluorometric method using fluorescein reagent was successfully applied for the determination of VAL in various pharmaceutical products. The results obtained by the recommended procedure are shown in Table IV. The data of the recommended procedure applied for analysis of the samples were compared with those obtained by standard method (HPLC) from Awamedica Company of drugs in Erbil-Iraqi Kurdistan region which is depending on British pharmacopeia. Table IV summarizes a good agreement between the results obtained by the recommended method for VAL samples and those determined by HPLC technique.

IV. Conclusion

Quantitative quenching method is a sensitive method for the determination of VAL without interferences from common tablet excipients. The proposed method is simple, rapid, low cost, and effective for the determination of pharmaceutical products such as VAL. A wide linear range of calibration curve is obtained by the proposed method with good accuracy and precision. The most important advantage of the method is that the charge transfer complex formed is measured directly without need for pretreatment of the drug such as extraction with organic solvent. Therefore, the method can be used for the quality control of VAL in its dosage forms.

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