Innovare Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 7, Issue 3, 2015

Original Article

NEW FLUORESCENCE QUENCHING BASED METHOD FOR THE DETERMINATION OF TRANDOLAPRIL IN BULK AND CAPSULES

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Received: 10 Nov 2014 Revised and Accepted: 01 Dec 2014

ABSTRACT

Objective: The objective of the method was to develop a new, simple, rapid and accurate spectrofluorimetric method for the determination of trandolapril in bulk and capsules.

Methods: Trandolapril reacts with fluorescein to form a charge transfer complex which results in fluorescence quenching of the fluorescein dye. The fluorescence quenching intensity was measured at 515 nm after excitation at 470 nm.

Results: Under the optimum conditions, the quenched fluorescence intensity was linear with the concentration of trandolapril in the range of 1.80 – 9.60 µg/mL (4.18×10^{-6} – 22.30×10^{-6} M) (R^2 = 0.9983) with a detection limit of 0.345 µg/mL. In order to validate the method the results were compared with those obtained by a high performance liquid chromatography method. The proposed method was successfully applied to the analysis of trandolapril in pure form and capsules with good precision and accuracy compared to the reported method as revealed by *t*- and *F*- tests.

Conclusion: The developed method was simple, fast, accurate and precise. It could be applied for routine quality control analysis of trandolapril in its pure form and in capsules.

Keywords: Fluorescence quenching, Fluorescein, Trandolapril, Charge transfer complex.

INTRODUCTION

Trandolapril is the ethyl ester pro drug of the angiotensin converting enzyme (ACE) inhibitor, trandolaprilat. Trandolapril is chemically described as (2S, 3aR, 7aS)-1-[(S)-N-[(S)-1-Carboxy-3phenylpropyl] alanyl] hexahydro-2-indolinecarboxylic acid, 1-ethyl ester, fig. 1. Its empirical formula is $C_{24}H_{34}N_2O_5$ and its structural formula is:

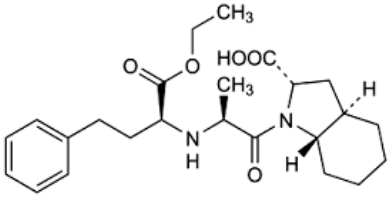


Fig. 1: Structural formula of trandolapril

Trandolapril belongs to the category of Angiotensin converting enzyme inhibitors (ACE inhibitors) that inhibit the conversion of angiotensin I to angiotensin II. Trandolapril is indicated for the treatment of hypertension. This effect appears to result primarily from the inhibition of circulating and tissue ACE activity thereby reducing angiotensin II formation, and decreasing vasoconstriction. Trandolapril is also indicated for patients with congestive heart failure. Administration of trandolapril to Caucasian patients has been shown to decrease the risk of death (principally cardiovascular death) [1].

Literature reported only few chromatographic methods for the determination of trandolapril in its bulk, dosage forms and human plasma, such as high performance liquid chromatography [2-9], HPLC-MS [10], and high performance thin layer chromatography [11, 12], plus one spectrophotometric method [13]. However, the reported methods were found to have certain drawbacks. The chromatographic methods require expensive equipment and are complicated in operation. On the other hand, spectrophotometric method is not such sensitive. Therefore it is still significant to develop new simple and economic method for determination of trandolapril.

There seems to be no reports on the determination of such important drug, trandolapril, using spectrofluorimetry. 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.

In the present work, a novel spectrofluorimetric method has been developed for the determination of trandolapril using fluorescein as a fluorescence probe. Fluorescein is a fluorophore commonly used as labeling compound in microscopy, and as a diagnostic tool in the field of ophthalmology [14], and was used as a fluorogenic reagent in the determination of arsenic III [15]. To the best of our knowledge, fluorescein has not been used before for the determination of drugs. The proposed method has been validated and applied for the determination of trandolapril in its pure forms and in capsules.

MATERIALS AND METHODS

Apparatus

Fluorescence spectra and measurements were obtained using fluorescence spectrophotometer (Hitachi F-2700, Japan) equipped with a xenon lamp. Excitation and emission wavelengths were set at 470 and 515 nm, respectively. The slit widths for excitation and emission monochromators were fixed at 5 nm. All measurements were performed in 1 cm quartz cell at room temperature.

Absorption spectra were recorded using UV-Visible spectrophotometer (Jasco V750, Japan).

Chromatographic analysis was performed on HPLC apparatus (Agilent, Germany) equipped with UV detector, auto sampler, and column oven. Chromatographic separation was achieved on a C18 column (5 µm, 100 mm × 4.6 mm).

Reagents and solutions

Trandolapril (Aurobindo Pharma - India) working standard solution of 1×10^{-4} M (0.043 mg/mL) was prepared in methanol. This solution

was stable for several weeks at refrigerator. Fluorescein (Merck, Germany) the standard solution of 4×10^{-5} M was prepared in methanol. This solution was stable at refrigerator for several weeks. Methanol (Extra pure grade) and acetonitrile (HPLC grade) were purchased from Merck, Germany. All other solvents were of analytical grade.

Trandolapril capsules, (Gopten®, Abbott) containing 2 mg were purchased from local medical store.

General procedure

Aliquots of trandolapril working standard solution were transferred into 5 ml volumetric flask that contains 1 ml of fluorescein standard solution. Volume was completed to mark with methanol and mixed before the fluorescence intensity was measured at 515 nm after excitation at 470 nm. The fluorescence intensity for complex product (F) and reagent blank (F_0) was measured and the quenched fluorescence intensity was calculated $\Delta F = F_0 - F$

Procedure for trandolapril capsules

The contents of 10 capsules were pulverized carefully. An accurately weighed amount of the powder equivalent to one capsule was transferred to a centrifuge tube, 1 ml of acetone was added and content was mixed by vortex for 5 min. the solution was then centrifuged at 5000 rpm for 10 min. 0.5 ml of the supernatant was diluted to 5 ml with methanol. Suitable volume of the last solution was then transferred into 5 ml volumetric flask that contains 1 ml of fluorescein standard solution. Volume was made up to 5 ml with methanol and its fluorescence signal was measured as mentioned above.

RESULTS AND DISCUSSION

Fluorescence spectra

Fluorescein has strong native fluorescence in methanol, whereas trandolapril doesn't have any fluorescence. It was observed that addition of drug to fluorescein solution cause a decrease in the fluorescence intensity of fluorescein without any shift (Fig. 2).

This fluorescence quenching of fluorescein by the drug was found to be directly proportional to drug concentration in a certain range. The excitation and emission wavelengths selected for fluorescein were 470 and 515 nm, respectively.

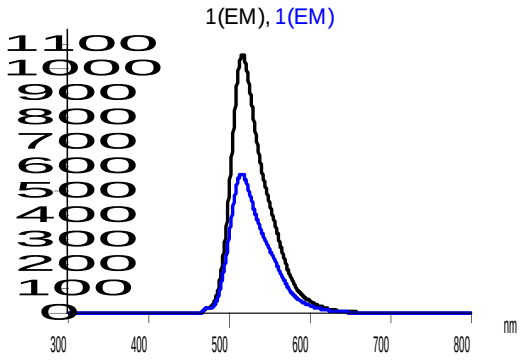


Fig. 2: Emission spectra of fluorescein (8×10^{-6}M) in methanol: without drug (black), and with 7.25 µg/mL of drug (blue), after excitation at 470 nm

Absorption spectra

Fluorescein methanolic solution has a maximum absorption wavelength at 495 nm. On contrast, trandolapril methanolic solution is transparent and doesn't show any absorbance in the visible light. Once a charge transfer complex has formed between fluorescein and trandolapril, the absorbance intensity at 495 nm drops significantly as shown from fig. 3.

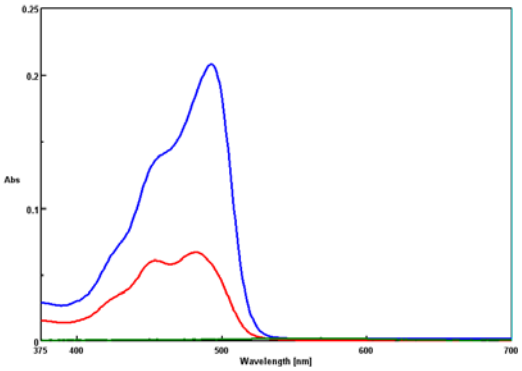


Fig. 3: Absorption spectra of trandolapril (7.25 µg/mL) in methanol (green), fluorescein (8×10^{-6}M) in methanol without drug (blue), and with drug (red).

Optimum reaction conditions

Effect of solvent

Effect of solvent polarity on the fluorescence intensity of fluorescein was investigated using fixed concentration of trandolapril (4.3 µg/mL). Eight different organic solvents with different polarities were tested (methanol, ethanol, isopropanol, acetone, dimethylsulfoxide, dichloromethane, chloroform, and n-hexan).

Interestingly, the fluorescence intensity was never changed in the nonpolar solvents: n-hexane, dichloromethane, and chloroform, nor in the polar aprotic solvents: acetone and dimethylsulfoxide. In contrast, the fluorescence intensity was found to be quenched increasingly when changing from isopropanol to the more polar solvent methanol. Thus, methanol was chosen for continuing the study (Fig. 4).

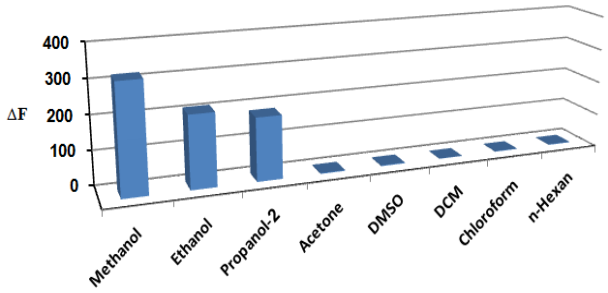


Fig. 4: Solvent effect on fluorescence quenching of fluorescein (8×10^{-6}M) in presence of trandolapril (4.3 µg/mL)

Effect of adding β-Cyclodextrin

Cyclodextrins play a major role in fluorescent systems. It can enhance the fluorescence intensity of some fluorescent moieties [16]. Effect of β-cyclodextrin on the fluorescence intensity of fluorescein was investigated using fixed concentration of trandolapril and fluorescein of 10×10^{-6} M (4.3 µg/mL) and 4×10^{-6} M, respectively, with increasing moles of β-cyclodextrin ranging from $4\times10^{-6}$to 30×10^{-6} M which covered molar ratios of cyclodextrin: drug correspond to 1:1 and 2:1, and molar ratios of cyclodextrin: fluorescein correspond to 1:2, 1:1, and 2:1. It was found that quenched fluorescence intensity of fluorescein suffered a little decrease after adding β-cyclodextrin, (Fig. 5). This might be due to the retention of free fluorescein molecules inside the cyclodextrin's cavity which have prevented the complex formation with trandolapril or caused a fluorescence enhancement and thus decreasing in the net quenched fluorescence intensity. Given that result, β-cyclodextrin was not used in the proposed method.

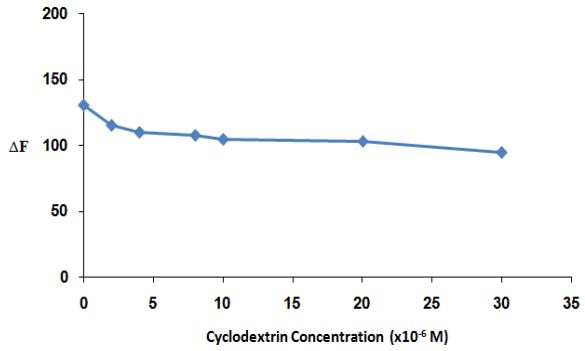


Fig. 5: Effect of β-cyclodextrin on fluorescence quenching of fluorescein (4×10⁻⁶M) in presence of trandolapril (4.3 µg/mL)

Effect of temperature

The effect of temperature on complex formation and ΔF value was also investigated. For that, reaction was carried out at lab temperature (about 25°C), in addition to four different temperatures of 30°C, 40°C, 50°C, and 60°C. Solutions were cooled to the lab temperature before measuring the fluorescence intensity. It was observed that ΔF was slightly decreased at high temperatures (Fig. 6). Therefore further experiments were carried out at lab temperature (25±2 °C.).

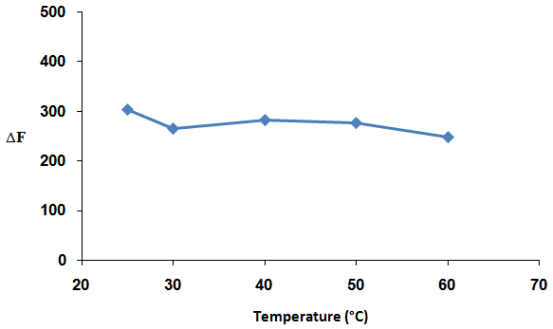


Fig. 6: Effect of temperature on fluorescence quenching of fluorescein (8×10⁻⁶M) in presence of trandolapril (4.3 µg/mL)

Effect of time

The effect of reaction time on fluorescein-trandolapril system was studied. It was observed that the complex got stabilized immediately after mixing and ΔF value remained stable for at least 24 hours. So the fluorescence measurements could be taken directly after the addition of reagent, or it can be kept until the next day for any reason.

Stoichiometric relationship

The composition ratio of the complex was determined using the molar ratio method (Fig. 7). From the obtained plot, it was concluded that fluorescein: trandolapril ratio is 1:1. This confirms that only one site of interaction was involved in the formation of the complex. The quenching effect is most probably due to the formation of non-fluorescent charge transfer complex between the drug acting as n-donor (D) and fluorescein, as π-acceptors (A):

$$D^{\bullet\bullet} + A \leftrightarrow [D^{\bullet\bullet} \rightarrow A] \leftrightarrow D^{\bullet+} + A^{\bullet-}$$

DA complex, A radical anion

The dissociation of DA complex is promoted by the high dielectric constant of the solvent, methanol, (32.7). This radical anion absorbs light and immediately returns to the ground state without emission of photon, as a result only the un-complexed fluorophore gives the fluorescence emission. A schematic proposal of the reaction pathway is given in scheme 1.

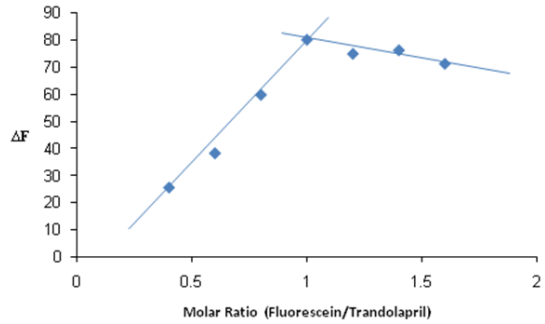
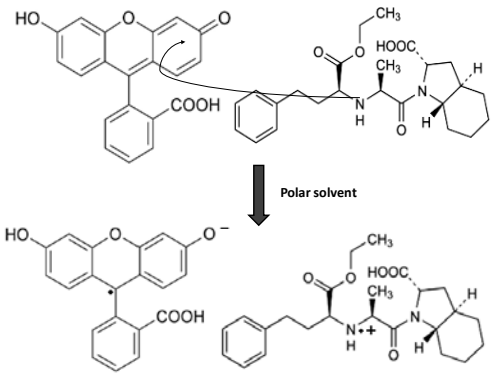


Fig. 7: Molar ratio plot for the stoichiometry of reaction between trandolapril and fluorescein



Scheme 1: Schematic illustration of reaction between trandolapril and fluorescein

Mechanism of quenching

The complex formation between fluorescein and trandolapril resulted in fluorescence quenching of fluorescein. There are three mechanisms of fluorescence quenching include: static quenching, dynamic quenching, and resonance energy transfer [16]. In dynamic quenching, collisions between molecules are enhanced with increasing temperature. This results in an increased ΔF value at high temperatures. On contrast, increasing temperature decreases ΔF in static quenching due to complex dissociation [16]. Based on this fact, the temperature effect on ΔF was investigated at six different degrees (2, 22, 30, 40, 50, and 60 °C). The fluorescence intensities were carefully measured immediately without losing of heat. As shown in fig. 8, the value of ΔF drops significantly as temperature increases from 2 to 60 °C and thus, fluorescence quenching is considered to be static. Another evidence is the change in absorption spectra and decrease of fluoresce in absorbance at λ_{max} 495 nm (fig. 3).

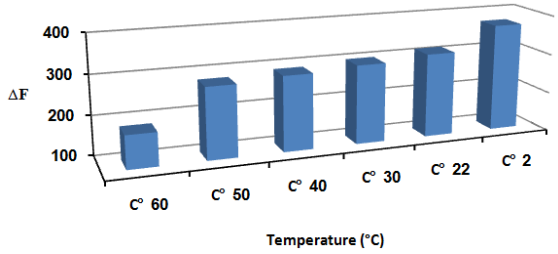


Fig. 8: Effect of temperature as an indicator of quenching mechanism
(Fluoresce in: 8×10⁻⁶M with trandolapril: 4.3 µg/ml)

Validation of the proposed method

Linearity

Under the optimum experimental conditions, standard calibration curve was constructed by plotting fluorescence intensity versus trandolapril concentration (fig. 9). The correlation coefficient was 0.9983 indicating good linearity, in the concentration range of 1.818 – 9.696 µg/mL. The intercept, slope, limit of detection (LOD), and limit of quantitation (LOQ) are summarized in table 1. LOD and LOQ values were calculated according to ICH Q2B [17] using the following equations:

$$LOQ = 10\ \sigma/S$$

$$LOD = 3.3\ \sigma/S$$

Where σ is the standard deviation of intercept of the regression line and S is the slope of the calibration curve (table 1).

Table 1: Statistics and analytical parameters for the developed method

Parameter	Result
$\lambda_{ex}/\lambda_{em}$ (nm)	470/515
Linearity range (µg/mL)	1.818 – 9.696
Slope	-75.397
Standard deviation in the slope	1.285
Intercept	1055.5
Standard deviation in the intercept	7.887
Correlation coefficient	0.9983
Limit of detection (µg/mL)	0.345
Limit of quantification (µg/mL)	1.046

Specificity

The effects of some common excipients used in pharmaceutical preparations were examined by analyzing solutions containing suggested amounts of each excipient. Frequently encountered excipients or additives were studied such as lactose, microcrystalline cellulose (Avicel), soluble starch, polyvinylpyrrolidone (PVP k30), talc, and magnesium stearate. Except for PVP, none of the studied excipients has native fluorescence, or quenching activity. However, to avoid the quenching effect of PVP, a screening of PVP solubility has been made. It was found that PVP is not soluble in dimethyl ether, ethyl acetate, acetone, toluene, xylene and carbon tetrachloride. Among those solvents, acetone was an ideal one, that it can dissolve trandolapil without dissolving PVP. Thus, the proposed method can be considered suitable for the routine analysis of trandolapril in its dosage forms and applicable in quality control laboratories.

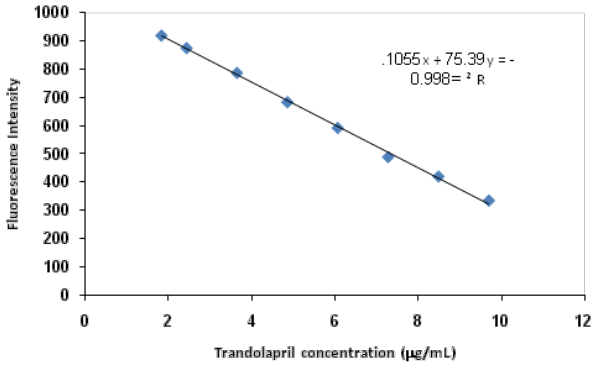


Fig. 9: Plot of trandolapril concentration versus fluorescence intensity.

Precision

Repeatability of the proposed method was estimated by measuring five replicate samples of each concentration of trandolapril prepared in one laboratory on the same day. The relative standard deviations (RSD%) were less than 4.74% for the smallest concentration, which indicating good precision (table 2).

Table 2: Precision and accuracy study for determination of trandolapril in pure form by the proposed method

Trandolapril concentration (µg/ml)		SD	RSD%	Recovery%	*t*-test[b]
Taken	Found[a]	(µg/ml)			
1.800	1.772 ± 0.104	0.084	4.74	98.45	0.742
2.400	2.354 ± 0.115	0.093	3.95	98.08	1.108
3.600	3.506 ± 0.134	0.108	3.08	97.40	1.938
4.800	4.868 ± 0.159	0.128	2.63	101.43	1.200
6.000	6.068 ± 0.138	0.111	1.83	101.14	1.378
7.200	7.287 ± 0.102	0.082	1.12	101.21	2.375
8.400	8.347 ± 0.079	0.064	0.76	99.37	1.849
9.600	9.537 ± 0.120	0.097	1.01	99.35	1.438

[a]Average of five determination ± Confidence limit, [b]The tabulated *t*- value at 95% confidence limit for 4 degrees of freedom (n =5) is 2.78.

Accuracy

The proposed method was applied on the available commercial capsules, and recoveries are mentioned in table 3. The method's accuracy was judged by (1) determining the average amount of trandolapril in pure form at several levels, and using a significance test to compare it with actual amount µ [18]:

$$t = \frac{\overline{X} - \mu}{SD}\sqrt{n}$$

Where t is an absolute value. As shown in table 2, the calculated *t*-value is less than tabulated *t*(0.05,4) value (2.78), and thus there are no significant differences between the taken and found concentration at 95% confidence level. Accuracy was indicated as well by analyzing the recoveries of known different amounts of trandolapril (Table 2) which varied from 97.40 - 101.43%. (2) comparing results obtained from the presently proposed method, that has been applied on commercial capsules, with those obtained from a reference method such as HPLC [2]. The obtained results were statistically compared with each other (Table 3) using *t*- and *F*-tests. t_{exp} was calculated using the following equation [18]:

$$t_{exp} = \frac{|\overline{X}_A - \overline{X}_B|}{\sqrt{(S_A^2/n_A) + (S_B^2/n_B)}}$$

Where $\overline{X}_A$ and $\overline{X}_B$ are trandolapril mean values in each pharmaceutical product using the proposed and reference methods, respectively. S and n are the standard deviation and the number of replicate trials conducted on samples, respectively.

With respect to *t*- and *F*-tests, no significant differences were found between the calculated values of both the proposed and the reported methods at 95% confidence level.

Table 3: Precision and accuracy for determination of trandolapril in capsules

Capsules	Labeled amount of trandolapril	Average trandolapril found (mg/cap) ± SD[a] (Recovery%) [b]		*t*- and *F*-test[c]
		Proposed method (Spectrofluorimetry)	Reference method (HPLC)[2]	
Gopten®	2 mg	2.025 ± 0.026 (101.25)	2.057 ± 0.012 (102.85)	2.322, 4.885

[a]Average and standard deviation of five determinations for the proposed method, and three determinations for the reference method, [b]Recoveries were calculated considering the labeled amount reported by the manufacturer, [c]The tabulated t value at 95% confidence limit for 4 degrees of freedom (n =5) is 2.78 and the tabulated *F* value at 95% confidence limit for (4, 2) degrees of freedom for the proposed and reference methods, respectively, is 6.944.

Application to capsules

The proposed method was successfully applied to analysis of commercial capsules (Gopten®, Abbott) labeled to contain 2 mg of trandolapril. The mean recovery value was identical to the recovery recorded by the reference method (HPLC) as revealed by *t*- and *F*-test (table 3).

CONCLUSION

The present study describes a successful investment of fluorescein as an analytical reagent in the development of simple, fast and sensitive spectrofluorimetric method for the determination of trandolapril in its pure form and in capsules. The proposed method has the advantages of being simple, accurate, highly sensitive, low cost and do not require sophisticated expensive apparatus. The method developed was fully validated, and therefore, can be applied for the routine analysis of trandolapril in quality control laboratories.

CONFLICT OF INTEREST

Declared None

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