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A New Method to Determine the New C-Met Inhibitor "Cabozantinib" In Dosage Form and Human Plasma via Micelle-Enhanced Spectrofluorimetry

Hany W. Darwish[a,b*], Ali S. Abdelhameed[a], Ahmed H. Bakheit[a], Amer M. Alanazi[a]

[a] Department of Pharmaceutical Chemistry, College of Pharmacy, King Saud University, P.O. Box 2457 Riyadh, 11451, Kingdom of Saudi Arabia

[b] Analytical Chemistry Department, Faculty of Pharmacy, Cairo University, Kasr El-Aini St., Cairo 11562, Egypt

[*]Correspondence to: E-mail: hdarwish75@yahoo.com ; Fax: +966 1146 76 220

Abstract

A highly sensitive and simple micelle-enhanced spectrofluorimetric method was developed for the determination of cabozantinib (CBZ) in its pharmaceutical formulation and spiked human plasma without any derivatization. The proposed method is based on the investigation of the fluorescence spectral behavior of CBZ in a Cremophor RH 40 (Cr RH 40) micellar system. In aqueous solution, the fluorescence intensity of CBZ was greatly enhanced (nine folds) in the presence of Cr RH 40. The fluorescence intensity was measured at 343 nm after excitation at 244 nm. The fluorescence –concentration plot was linear over the range 25–800 $ngmL^{-1}$, with lower detection limit of 13.34 $ngmL^{-1}$. The proposed method was successfully applied to the determination of CBZ in laboratory-prepared dosage form and spiked human plasma. Recovery values of CBZ with the current method were 99.68±0.88, 100.53±0.51 and 100.44±3.91 % for pure powder, lab-prepared dosage form and spiked human plasma, respectively. The results were statistically compared with those obtained by reported chromatographic method and were found to be in good agreement.

Keywords: Cabozantinib, Spectrofluorimetry, Human plasma, Validation

1. Introduction

Cabozantinib (CBZ), chemically N-{4-[(6,7-dimethoxyquinolin-4-yl)oxy]phenyl}-N_-(4-fluorophenyl)-cyclopropane-1,1-dicarboxamide, (2S)-hydroxybutanedioate (Fig. 1), is a new small molecule multitargeted tyrosine kinase inhibitor (TKI) with potent activity toward mesenchymal–epithelial transition factor (MET) and vascular endothelial growth factor receptor types 1 (VEGFR-1), 2 (VEGFR-2) and 3 (VEGFR-3) [1, 2]. In both preclinical and clinical studies CBZ has been shown to inhibit the tumor angiogenesis, invasiveness and metastases [3]. CBZ (COMETRIQ^{TM}; Exelixis Inc.) was approved in 2011 by U.S. FDA for the treatment of progressive, metastatic medullary thyroid cancer and is under evaluation by the European Medicines Agency (EMA). The efficacy of CBZ against castration-resistant prostate cancer and other solid tumors has also been observed [3, 4].

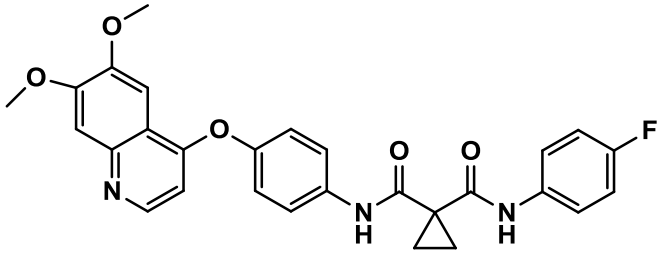

Fig. 1. Chemical structure of Cabozantinib (CBZ)

Several micelle-enhanced spectrofluorimetric studies have been reported for determination of various drugs [5-7]. This in fact is due to the ability of micelle formation to increase the fluorescence intensity of the ultimately weak fluorescent compounds. Moreover, these methods introduced sensitive and eco-friendly methodology, since no organic solvents were used. Micellar enhanced analytical methods has always relied on surfactants such as sodium dodecyl sulphate (SDS) [7, 8], Tween [9, 10] and cyclodextrin [11, 12], *etc*. However, the use of the non-ionic surfactant "Cremophor RH 40" has never been reported for any analytical determination of drugs. Cremophor RH 40 is manufactured by reacting 40 mol of ethylene oxide with hydrogenated castor oil. It contains mainly the tri-ricinoleate ester of ethoxylated glycerol, with smaller amounts of polyethylene glycol ricinoleate and the corresponding free glycols [13].

Thorough survey of the literature revealed that only few very recent reports were published for the analysis of CBZ. The first one was devoted for studying its degradation kinetics using HPLC-

UV for quantitation and LC/TOF-MS and LC–MS/MS for structure elucidation of degradation products [14]. Another one was developed using UPLC-MS/MS to study CBZ Pharmacokinetics and tissue distribution in rats [15]. The last one reported until now, was developed for CBZ quantitation in rat plasma using LC-MS/MS[16]. However, these methods are time-consuming, complex, and expensive. Ambient mass spectrometry has greatly reduced the complexity associated with LC-MS/MS for the analysis of drug molecules from body-fluids. However, there is no such report of analysis of CBZ using ambient mass spectrometry [17-19]. Additionally, there is no reported method for the analysis of CBZ in bulk powder or in dosage form. Thus, development of simple analytical method for determination of CBZ in its bulk drug, pharmaceutical capsules and human plasma is essential. This paper presents a new sensitive spectrofluorimetric method for the determination of CBZ in capsules. Depending on the high sensitivity of the proposed method it can be applied for the determination of CBZ in human plasma. The method allows a quick determination of CBZ in different matrices with high accuracy and precision. The proposed method can be considered as a simple and fast alternative to the already existing LC/MS procedures for the determination of CBZ in human plasma. Fluorescence spectrometry is a very simple, rapid, efficient, selective, and highly sensitive technique for determination of drug in plasma [20-22]. Therefore, the aim of the current study is to develop and validate a simple and rapid spectrofluorimetric method for the in vitro determination of CBZ in spiked human plasma in addition to pharmaceutical capsules. This method does not require derivatization of the drug due to the intrinsic fluorescent activity of CBZ.

2. Experiments

2.1. Apparatus

Fluorescence measurements were carried out on a Jasco FP-8200 Fluorescence Spectrometer (Jasco Corporation, Japan) equipped with a 150 W xenon lamp and 1 cm quartz cells. The slit widths for both the excitation and emission monochromators were set at 5.0 nm. The calibration and linearity of the instrument were frequently checked with standard quinine sulphate (0.01 μg/mL). Wavelength calibration was performed by measuring λ_{ex} at 275 nm and λ_{em} at 430 nm; no variation in the wavelength was observed. All recorded spectra converted to ASCII format by SpectraManager® software. A Hanna pH-Meter (Romania) was used for pH adjustments.

2.2. Reagents and Materials

All the chemicals used were of Analytical Reagents grade, and the solvents were of HPLC grade.

- Cabozantinib reference standard (purity ~ 99.6%) was purchased from Weihua Pharma Co. Ltd (Zhejiang, China).
- Polyoxyl 40 hydrogenated castor oil (Cremophor RH40), Polyoxyl 35 hydrogenated castor oil (Cremophor EL) were purchased from BASF (Ludwigshafen, Germany) and used as 1 % w/v aqueous solution for Cremophor RH 40 and 1 % *v/v* aqueous solution for Cremophor EL
- Sodium dodecyl sulphate (SDS; 95 %) was purchased from Winlab (UK) and used as 1 % w/v aqueous solution.
- β–cyclodextrin (β–CD) and carboxymethylcellulose (CMC) were obtained from Merck (Germany) and used as 1 % *w/v* aqueous solution.
- Tween–20, tween–80 and tween–85 (Techno Pharmchem Haryana Company (INDIA)), used as 1 % *v/v* aqueous solution.
- HPLC grade methanol, ethanol (Prolabo, France) and acetonitrile (Sigma-Aldrich Chemie GmbH, Germany).
- Boric acid, sodium hydroxide, phosphoric acid, potassium chloride, potassium dihydrogen phosphate, disodium hydrogen phosphate, and triethanolamine were all of spectroscopic grade
- phosphate buffer (0.1 M, pH 2–7), and borate buffer (0.1 M, pH 8–10) solutions were freshly prepared.
- Ultrapure water of 18 $\mu\Omega$ was obtained from a Millipore Milli-Q® UF Plus purification system (Millipore, Bedford, MA, USA) was used throughout the study.
- Human plasma was kindly provided by King Khaled University Hospital (King Saud University, Riyadh, KSA). After informed consent was obtained, fasting blood samples were taken and plasma was separated and stored at −70°C.

2.3. Standard Solutions

CBZ stock solution ($mgmL^{-1}$) was prepared by dissolving accurately measured amounts of CBZ reference standard material in acetonitrile. During the course of the study, this solution was

found to be stable for several weeks when kept in the refrigerator. A working standard of 20 μgmL^{-1} was prepared daily by dilution of stock standard solution with acetonitrile.

2.4. Construction of the Calibration Graph

Aliquots of CBZ standard solution were transferred into a series of 5 mL volumetric flasks to give final concentrations of 25-800 $ngmL^{-1}$. A volume of 0.4 mL of Cremophor RH40 (Cr RH 40) was added followed by 1 mL of phosphate buffer and completed the volume with distilled water, the contents of the flasks were mixed well and the relative fluorescence intensity (RFI) was measured at 343 nm after excitation at 244 nm. RFI was plotted *vs.* nominal drug concentration ($ngmL^{-1}$) to obtain the calibration graphs. The linear regression equation for the data was computed.

2.5. Assay of laboratory-made capsule samples

Laboratory-made capsules of CBZ (equivalent to COMETRIQ™ 20 mg capsules) were prepared. An accurately weighed portion of the powder equivalent to 20 mg of CBZ was transferred to a 100 mL volumetric flask. A volume of 50 mL of acetonitrile was added, the contents were mechanically shaken for 10 min, ultrasonicated for 5 min, and the volume was diluted to 100 ml with acetonitrile. This solution (0.2 $mgmL^{-1}$) was further diluted as required for analysis.

2.6. Assay of human plasma samples

Plasma samples were stored at -20 ^{o}C and allowed to thaw at room temperature before processing. An appropriate amount of standard solutions of CBZ were spiked into 1 mL of human plasma and mixed for 60 seconds. 5 mL of diethyl ether was added and the solution was vortexed for 3 minutes and left to stand till complete separation of the two layers. Then 4 mL of the upper organic layer was transferred into glass vials and dried under a gentle stream of nitrogen. Finally, reconstitution of the residue occurred in acetonitrile and the procedures described under “Construction of the calibration graph” were then performed. A blank plasma sample was treated similarly. RFI was measured at 343 nm after excitation at 244 nm and the concentration of the drug was determined from the regression equation.

3. Results and Discussion

Spectrofluorimetry was adopted in this study because of its inherent high sensitivity, improved selectivity, practical simplicity, and wide availability in quality control laboratories. Different experimental parameters affecting the relative fluorescence intensity (RFI) of CBZ were carefully studied and optimized. Such factors were changed individually, where others kept constant.

3.1. Fluorescence spectra and characteristics of CBZ

The conventional fluorescence spectrum of a molecule consists of two spectra: (i) one related to the excitation process and (ii) other related to the emission of the absorbed radiation. The excitation spectrum can be obtained by varying the wavelength of the excitation monochromator (λ_{ex}) in a given range and keeping the emission monochromator at a fixed wavelength (λ_{em}). The emission spectrum can be obtained using the same strategy, but scanning the emission monochromator and fixing the excitation monochromator at a particular wavelength. For a fluorescent molecule, a pair of wavelengths is observed where maximum intensity appears in the respective bands of emission and excitation spectra (λ_{em} and λ_{ex}). The first requirement to a compound generates a fluorescence spectrum is to absorb electromagnetic radiation. CBZ exhibits an excitation wavelength of 244 nm. CBZ being also able to give a fluorescent spectrum. Its fluorescent spectrum was recorded (using a 1 µg/mL) fixing the excitation monochromator at 244 nm and scanning the emission monochromator in the range of 300– 450 nm. An intense emission band with maximum at 343 nm appeared in the spectrum, evidencing the fluorescence of the CBZ. Afterwards, the excitation spectrum was recorded, this was followed by fixing the emission monochromator at 343 nm and scanning the excitation monochromator to ensure the excitation wavelength which appeared at 244 nm.

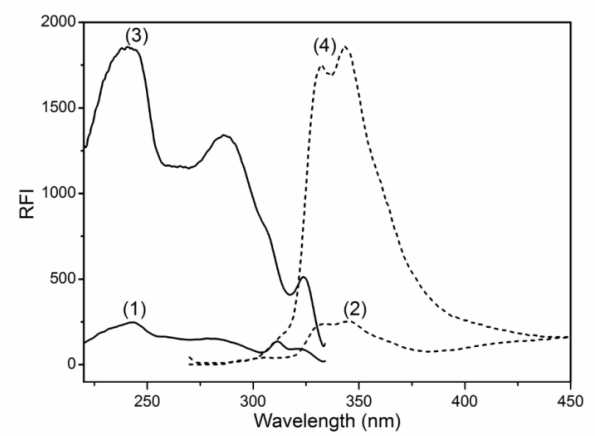

Fig. 2. Excitation (1) and emission (2) spectra of CBZ in acetonitrile (1 μgmL^{-1}); Excitation (3) and emission (4) spectra of CBZ (1 μgmL^{-1}) in Cr RH 40 (1%, *w/v*).

The fluorescence spectra of CBZ in both aqueous and Cr RH 40 systems were studied (Figure 2). CBZ showed native fluorescence in aqueous solution measured at 343 nm after an excitation at 244 nm. In the presence of Cr RH 40, the fluorescence intensity of CBZ was enhanced nearly nine folds in comparison with its native fluorescence intensity in aqueous medium. This enhancement reflects that the microenvironment around CBZ is quite different from that in aqueous solution. This can be attributed to restrictions imposed on the free rotational motions which are competitive with luminescent emission [23].

3.2. Optimization of the Experimental Conditions

3.2.1. Effect of Organized Media

The effect of different organized media on the RFI of CBZ was studied by adding 0.5 mL of an aqueous solution of each one of them to the drug solution. Different surfactants, like sodium dodecyl sulfate (SDS) [anionic surfactant], Cremophor El, Cr RH 40, carboxymethylcellulose (CMC), tween 80 [nonionic surfactants] and macromolecules such as β-cyclodextrin were tried. Nonionic surfactants usually are better solubilizing agents than ionic surfactants for hydrophobic drugs, because of their lower critical micelle concentration (*cmc*) values [24]. More specifically, non-ionic surfactants with a relatively high hydrophilic lipophilic balance (HLB) values are the most widely recommended surfactants. Cr RH 40 with an HLB of 14-16 and a pH value of 6-7 [25] gave the highest RFI compared to the other surfactants investigated as shown in

figure 3, thus it was used throughout the study. Chemically Cr RH 40 is a glycerol polyethyleneglycol oxystearate, which forms the hydrophobic part of the product. The hydrophilic part consists of polyethylene glycols and glycerol ethoxylated. Due to this non-ionic hydrophilic property and a favorable pH value, both of which attribute to CBZ solubilization.

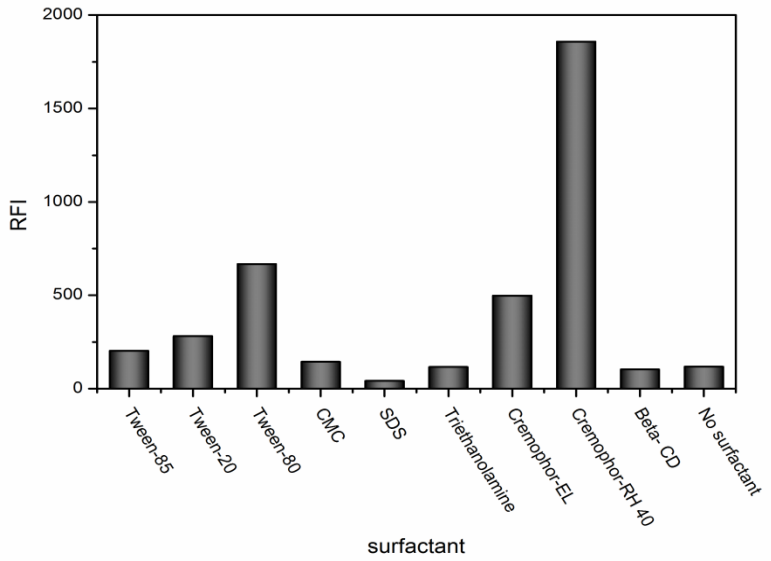

Fig. 3. Effect of the type of organized media (0.5 mL, 1% *w/v* solution of each) on RFI of CBZ (800 ngmL^{-1})

3.2.2. Effect of the concentration of Cr RH 40

The influence of Cr RH 40 on the RFI was studied using increasing concentrations of Cr RH 40 (as % *w/v*) with and without CBZ. Based on the results shown in figures 4 and 5, it is suggested that upon increasing of the Cr RH 40 concentration, the fluorescence intensity of both CBZ-Cr RH 40 and Cr RH 40 alone increased gradually. However, beyond 0.06% w/v the corrected RFI of CBZ in the micellar solution (RFI of tested samples-RFI of the blank "Cr RH 40") appeared to attain a constant value. Additionally, the enhancement of the fluorescence intensity of CBZ on increasing concentrations of the surfactant may also be attributed to the increase in the quantum efficiency of fluorescence [26]. Therefore 0.08 % *w/v* Cr RH 40 solution was chosen as the optimum concentration for CBZ (Fig. 4).

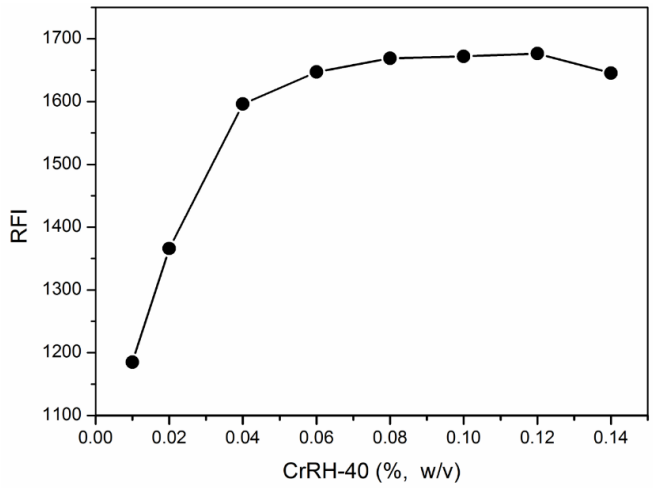

Fig. 4. Effect of Cr RH 40 concentration on RFI of CZB (800 ngmL^{-1})

3.2.3. Effect of pH

The pH of micellar solutions can show significant influence on the extent of solubilization of drugs, since it may change the equilibrium between ionized and molecular forms of some drugs [24]. The effect of pH on the CBZ, Cr RH 40 and CBZ-Cr RH 40 RFI was investigated using different types of buffers covering the whole pH range, such as 0.1 M phosphate buffer over the pH range 2–7 and 0.1 M borate buffer over the pH range 8–10. Results represented in figure 5 show that the RFI of CBZ without Cr RH 40 increased gradually at pH values higher than 3.0 until reaching its maximum value at pH 10.0, which can be attributed to altering the ionization of CBZ. Additionally, for Cr RH 40 alone, the RFI had a maximum value at pH 7.0 ± 0.2 which may be due to breaking of tri-ricinoleate ester of Cr RH 40 at pH ranges 2-5 and 8-11, it can also be attributed to the significant changes in micellar properties of nonionic surfactants with pH [27]. Moreover, in the presence of Cr RH 40, the RFI of CBZ reached its maxima at pH 7, then upon increasing the pH (*i.e.* >7.0), the RFI was reduced and nearly remained constant until pH 10.0, which is consistent with the overall observations (Fig 5).

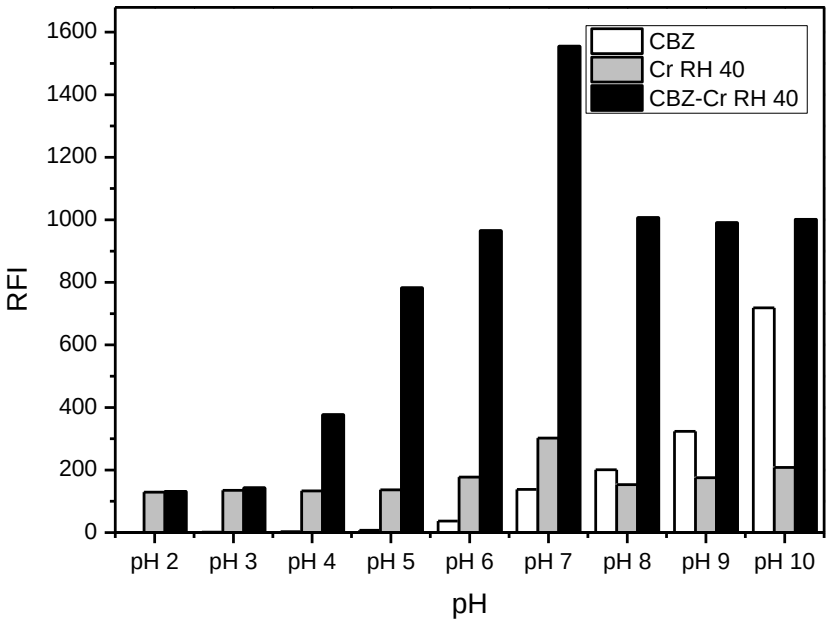

Fig. 5. Effect of pH on the RFI of 800 ngmL^{-1} CBZ in 0.08%, *w/v* Cr RH 40 solution

3.2.4. Effect of diluting solvent

Dilution with different solvents including water, methanol, ethanol and acetonitrile was employed. Water showed the highest RFI compared to the other solvents studied, this can be attributed to the change in medium polarity which may result in a physical interaction between those other solvents and the excited singlet state of the drug molecules. Thus, water was chosen as the diluting solvent throughout the study. Sharp decrease in the RFI was observed in the micellar system using methanol, acetonitrile or ethanol. This effect may be due to the denaturating effect of short-chain alcohols (methanol and ethanol) on the micelles, where they are solubilized mostly in the aqueous phase and affect the micelle formation by altering the solvent properties. Additionally these alcohols may decrease the size of the micelles, and at high concentration may breakdown the surfactant aggregate [28].

3.2.5. Effect of time

The effect of time on the stability of the RFI of CBZ in micellar system was also studied. It was found that the RFI developed instanteously and remained stable for at least one hour.

3.3. Validation of the Method

The proposed methods were validated according to the ICH-guidelines for validation of the analytical procedures [29] in terms of the linearity, sensitivity, accuracy, specificity, repeatability and reproducibility

3.3.1. Calibration and sensitivity

Under the optimized conditions (Table 1), calibration curve for the quantitation of CBZ was constructed by plotting the RFI versus the corresponding CBZ concentration. The regression equation for the results was: RFI = $a + bC$, where RFI is the relative fluorescence intensity, C is the concentration of CBZ in $ngmL^{-1}$. Linear relationship with small intercept and excellent correlation coefficient (r = 0.9991) was obtained in the range of 25–800 $ngmL^{-1}$. The limit of detection (LOD) and limit of quantification (LOQ) were determined according to ICH guidelines for validation of analytical procedures [29].The LOD and LOQ values were found to be 13.34 and 20 $ngmL^{-1}$, respectively. The parameters for the analytical performance of the proposed fluorimetric method are showed in Table 1.

Table 1: Statistical parameters for the determination of CBZ by the proposed spectrofluorimetric method

Parameter	Value
Linear range ($ngmL^{-1}$)	25-800
Intercept	23.575
SD of intercept	7.404
Slope	1.832
SD of slope	0.028
Correlation coefficient (*r*)	0.9991
LOD ($ngmL^{-1}$)	13.34
LOQ ($ngmL^{-1}$)	20

3.3.2. Accuracy and precision

The accuracy and precision of the proposed fluorimetric method was determined by replicate analysis of three different concentrations of the working standard. The recovery percentages was

99.68 ± 0.88 (Table 2) indicating the accuracy of the proposed method. Intra-assay precision was assessed by analyzing varying concentrations of CBZ (100, 300, 500 and 700 $ngmL^{-1}$) in triplicate in one assay batch. Furthermore the inter-assay precision was assessed by analyzing the same concentrations in triplicate on consecutive days. The average recovery percentages were around 100% and the low relative standard deviations (RSD) indicated the high accuracy and precision of the proposed method, respectively (Table 2). The accuracy of the proposed method was further assessed by comparing the results of the assay of the drug in pure form with those obtained by reported LC-MS/MS method [16]. Statistical analysis of the results obtained by the proposed and reference methods using Student's t-test and variance ratio F-test showed no significant difference between them regarding accuracy and precision, respectively (Tables 3).

Table 2 Intra-assay and inter-assay precision and accuracy for determination of CBZ by the proposed spectrofluorimetric method

Nominal conc. ($ngmL^{-1}$)	Intra-assay		Inter-assay	
	Measured conc. ($ngmL^{-1}$)	Recovery (% ± RSD) [a]	Measured conc. ($ngmL^{-1}$)	Recovery (% ± RSD) [a]
100	97.22	97.22± 1.33	100.61	100.61± 1.21
300	298.29	99.43± 1.63	300.43	100.14± 1.64
500	502.45	100.49± 0.71	495.45	99.09± 1.01
700	702.77	100.40± 0.62	697.61	99.66± 0.88

[a] Mean of three determinations

Table 3 Statistical comparison between analysis results of CBZ in pure powder form by proposed spectrofluorimetric method and the reported LC-MS/MS method [16]

Value	Proposed method	Reported method[16]
Mean	99.24	99.70
SD	2.313	2.108
RSD%	2.331	2.115
N	8	7
Variance	5.351	4.446
F value	1.204 (4.207) *	
T test	0.402 (2.160) *	

* tabulated value at p= 0.05

3.3.3. Robustness.

It was estimated by testing the susceptibility of measurements to deliberate changes of the analytical conditions. It was found that small variations that may take place during the experimental operation did not affect the RFI of the proposed method. The results for the proposed method are summarized in Table 4.

Table 4: Robustness of the proposed spectrofluorimetric method

Experimental parameter variation	Recovery (%) ± SD [a]
No variation [b]	99.68 ± 0.88
Cr RH40 volume (µL)	
380	100.34 ±1.06
420	99.04 ±2.29
pH	
6.8	98.29 ±1.38
7.2	97.88 ±1.71
Buffer volume (µL)	
0.95	99.09± 1.47
1.05	99.48± 2.95
Temperature (C °)	
20	99.88 ± 1.23
30	100.77± 3.15

[a] Mean of three determinations

[b] following the general calibration procedures

3.3.4. Specificity

The specificity of the method was investigated by observing any interference encountered from common capsule excipients. It was shown that these compounds did not interfere with the results of the proposed method (Table 5).

Table 5 Results of the determination of CBZ in pure form, lab-made capsules and plasma samples

Parameter	Pure form			Capsules			Plasma samples		
	Amount taken ($ngmL^{-1}$)	Amount found ($ngmL^{-1}$)	% Found	Amount taken ($ngmL^{-1}$)	Amount found ($ngmL^{-1}$)	% Found	Amount added ($ngmL^{-1}$)	Amount found ($ngmL^{-1}$)	% Found
	400	400.28	100.07	400	403.54	100.88	400	413.59	103.4
	600	601.80	100.3	600	604.64	100.77	600	611.53	101.92
	800	789.44	98.68	800	799.54	99.94	800	768.1	96.01
Mean			99.68			100.53			100.44
± S.D.			0.88			0.51			3.91

3.4. Applications

3.4.1. Application of Procedure to Analysis of CBZ in lab-made Capsules

The proposed method was successfully applied to CBZ assay in laboratory made capsules (due to the unavailability of the commercial capsules in the local market). The average percent recoveries of different concentrations were based on the average of three replicate determinations. The mean recovery percentage was 100.53 ± 0.510 (Table 5). These results revealed the excellent accuracy of the proposed method. The results obtained from the analysis of pure powders of the analytes in presence of pharmaceutical excipients added by the manufacturer (silicified microcrystalline cellulose, croscarmellose sodium, sodium starch glycolate, fumed silica, and stearic acid.) are indicated in Table 6. The results obtained were compared for the mean and the standard deviation using the t-test and F-test, respectively. There was no significant difference. In addition, the results found were in good agreement with the data indicated in the formulation given by the manufacturer.

Table 6 Statistical comparison between analysis results of CBZ in presence and absence of pharmaceutical excipients by the proposed spectrofluorimetric method

Sample no.	CBZ Recovery %	
	Absence	Presence
1	100.07	100.88
2	100.30	100.77
3	98.68	99.94
Mean	99.68	100.53
S.D.	0.88	0.51
Variance	0.895	0.974
Degree of freedom	4	
t-test(2.776)*	1.443	
F-test(19)*	2.909	

* tabulated value at p= 0.05

3.4.2. Application of Procedure to Analysis of CBZ in human plasma

The high sensitivity of the proposed method allowed the determination of CBZ in spiked human plasma. CBZ is readily orally bioavailable, reaching peak plasma concentration at 5 hours after administration. The maximum plasma concentration (C_{max}) of CBZ is dose-dependent, ranging from 34.2 to 603 $ngmL^{-1}$ following oral doses of 0.08 $mgkg^{-1}$ to 1.28 $mgkg^{-1}$. *i.e.* lie in the working concentration range of the proposed method. The recoveries of CBZ in plasma were calculated from the regression equation of CBZ in micellar system. The recoveries varied between 96.01 % and 103.40% (Table 5). Accordingly, the results showed the successful applicability of the proposed method for the assay of CBZ in human plasma.

3.5. Mechanism of the micellar-enhancement effect of Cr RH 40

To explore whether the enhancement process of CBZ fluorescence was only due to an rise in quantum yield, or whether it was influenced by an increase in absorption at the wavelength of excitation (λ_{ex}); the molar absorptivity of CBZ in Cr RH 40 was calculated at 243 nm (λ_{ex}). The $\varepsilon_{micellar}$ / $\varepsilon_{acetonitrile}$ ratio was unity which indicates that the increase in sensitivity is not affected by an increase in the absorption of the drug in micellar system at its λ_{ex}. On the other hand, the increase in the quantum yield of CBZ in Cr RH 40 was produced by protection of the lowest excited singlet state from non-radiative processes in the micellar system. The quantum yield of

CBZ was found to be 0.04 in acetonitrile and 0.18 in the presence of Cr RH 40. The quantum yield was calculated according to the following equation (Eq. 1) [30] :

$$\emptyset d = \emptyset q \frac{Fd}{Fq} . \frac{Aq}{Ad} \quad (1)$$

where $\emptyset d$ *and* $\emptyset q$ referred to the fluorescence quantum yields of the drug and quinine, respectively; Fd and Fq represent the integral fluorescence intensities of the drug and quinine, respectively; Ad and Aq referred to the values of absorbance of the drug and quinine at the excitation wavelength, respectively. The concentration was selected so that the absorbance was less than 0.05 to decrease the error arising from the inner effect [31].

4. Conclusion

A simple and sensitive spectrofluorimetric method was developed for the determination of CBZ through enhancement of its native fluorescence. The suggested method is simple, less time consuming and does not require the elaborate treatment associated with chromatographic methods; moreover, it is sensitive, with no need for derivatization reactions. By virtue of its simplicity and rapidity, the proposed method could be applied to the analysis of the CBZ in pharmaceutical dosage form. The method was further extended to the in vitro determination of CBZ in spiked human plasma and so, could be considered as a powerful alternative to the reported chromatographic methods.

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