

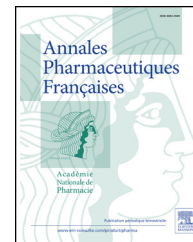


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ORIGINAL ARTICLE

Application of HPTLC, spectrofluorimetry and differential pulse voltammetry for determination of the antifungal drug posaconazole in suspension dosage form



Application de HPTLC, spectrofluorimétrie et voltampérométrie différentielle par impulsions pour la détermination de l'antifongique posaconazole en suspension

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HIGHLIGHTS

- This study introduces three different analytical procedures for determination of the recently introduced antifungal agent posaconazole.
- To the best of our knowledge, there are no published articles describing the determination of posaconazole by HPTLC or differential-pulse cathodic voltammetry.
- Very few fluorescence-based assay methods for posaconazole are available in the literature.
- Applicability of the proposed methods to real life situations was assessed by the successful analysis of posaconazole suspension dosage form.

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KEYWORDS

Posaconazole;
HPTLC;
Spectrofluorimetry;
Differential-pulse
voltammetry;
Suspension dosage
form

Summary This work presents the development, validation and application of three simple and direct analytical methods for determination of posaconazole (PSZ) in its pure form and in suspension dosage form. Method I is based on high performance thin layer chromatography (HPTLC) where effective separation of PSZ and the internal standard (itraconazole) was achieved using Merck HPTLC plates (20 × 10 cm aluminium plates with 250 μm layer thickness precoated with silicagel 60 F₂₅₄) and a mobile phase composed of acetone and chloroform (1:2, by volume), followed by densitometric measurement of the drugs' spots at 262 nm. Method II involves measurement of the native fluorescence of PSZ in 0.1 M H₂SO₄ at excitation and emission wavelengths of 260 and 365 nm, respectively. Method III depends on the voltammetric analysis of PSZ. A well-defined cathodic wave was obtained for PSZ in Britton-Robinson buffer pH 6.5 using the differential-pulse mode at the hanging mercury drop electrode (HMDE). The developed methods were validated according to the International Conference on Harmonization (ICH) guidelines regarding linearity, ranges, accuracy, precision, robustness and limits of detection and quantification. The proposed methods showed good linearity over the concentration ranges 5–50, 0.05–0.3, 0.005–0.05 μg/mL PSZ for methods I, II, and III respectively. Intra and inter-day precision were verified by the RSD% values which were less than 2%. The proposed methods were successfully applied for the quantification of PSZ in suspension dosage form with no observable interferences. Assay methods were favorably compared with those obtained by previously reported HPLC method.

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MOTS CLÉS

Posaconazole ;
HPTLC ;
Spectrofluorimétrie ;
Voltamétrie à
impulsions
différentielles ;
Forme posologique
en suspension

Résumé Ce travail présente le développement, la validation et l'application de trois méthodes analytiques simples et directes pour la détermination du posaconazole (PSZ) sous sa forme pure et sous sa forme posologique en suspension. La méthode I est basée sur HPTLC où la séparation efficace du PSZ et de l'étalon interne (itraconazole) a été réalisée à l'aide de plaques Merck HPTLC (plaques en aluminium de 20 × 10 cm avec une épaisseur de couche de 250 μm préalablement revêtue de silicagel 60 F₂₅₄) et d'une phase mobile composée d'acétone et de chloroforme (1/2, en volume), suivie d'une mesure densitométrique des taches à 262 nm. La méthode II implique la mesure de la fluorescence native du PSZ dans 0,1 M H₂SO₄ à des longueurs d'onde d'excitation et d'émission de 260 et 365 nm, respectivement. La méthode III dépend de l'analyse voltamétrique du PSZ. Une onde cathodique bien définie a été obtenue pour le PSZ dans le tampon Britton — Robinson pH 6,5 en utilisant le mode d'impulsion différentielle au niveau de l'électrode à goutte de mercure suspendue (HMDE). Les méthodes développées ont été validées conformément aux directives de la Conférence internationale sur l'harmonisation (ICH) concernant la linéarité, les intervalles, la précision, la précision, la robustesse et les limites de détection et de quantification. Les méthodes proposées ont montré une bonne linéarité sur les plages de concentration comprises entre 5–50, 0,05–0,3 et 0,005–0,05 μg/mL pour les méthodes I, II et III, respectivement. La précision intra- et inter-jours a été vérifiée par les valeurs RSD % inférieures à 2 %. Les méthodes proposées ont été appliquées avec succès pour la quantification du PSZ sous forme posologique en suspension, sans interférences observables. Les méthodes de dosage ont été comparées favorablement à celles obtenues par la méthode HPLC précédemment décrite.

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Introduction

Posaconazole (PSZ) is a broad-spectrum triazole antifungal used for treatment and prophylactic purposes in invasive fungal infections. PSZ has been recently recognized by the Food and Drug Administration (FDA) in 2014 and is available in the form of suspension (40 mg/mL). Similar to other azoles, PSZ acts by inhibiting the ergosterol biosynthesis,

a vital component of the fungal cell membrane, by binding and inhibiting the lanosterol-14 α -demethylase enzyme, present in the majority of fungi. This leads to the inhibition of fungal cell growth and conclusively cell death [1]. Chemical structure of PSZ is given in Fig. 1.

The reports found in the literature for PSZ determination mainly concentrated on chromatographic methods, among these, HPLC was a predominant technique. HPLC with

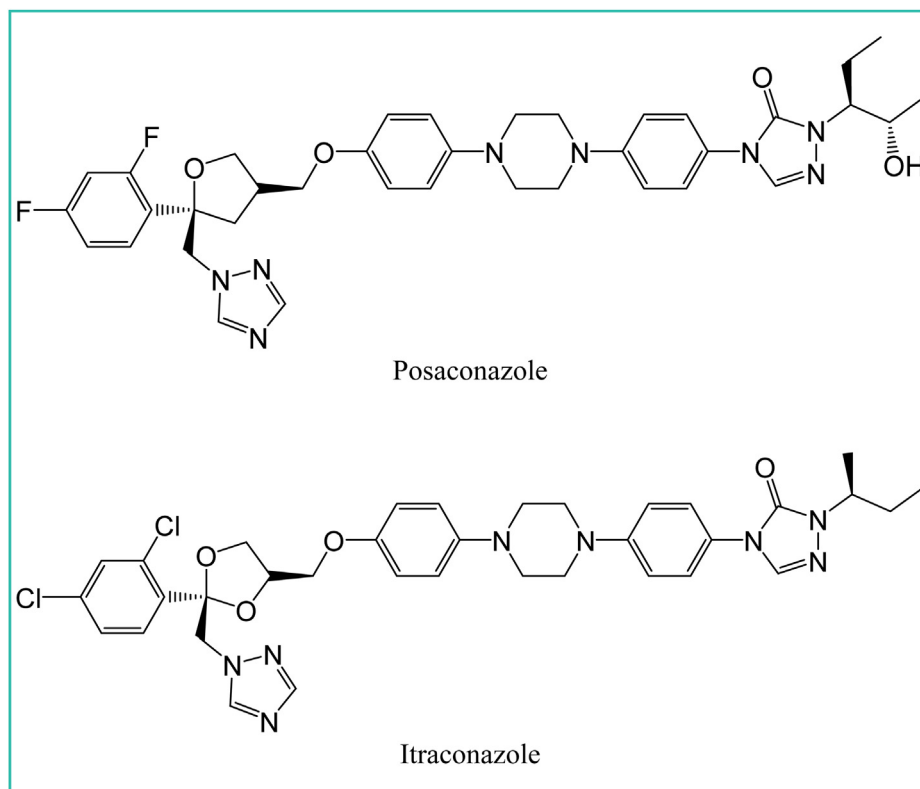


Figure 1. Chemical structures of posaconazole (PSZ) and itraconazole (ITZ).
Structures chimiques du posaconazole (PSZ) et de l'itraconazole (ITZ).

ultraviolet (UV) detection procedures were described for the determination of PSZ solely in human plasma [2–4] and its determination simultaneously with other antifungals [5–7]. A validated stability-indicating HPLC method for PSZ bulk assay was developed [8]. HPLC with fluorescence detection [9,10] and HPLC-tandem mass spectrometry (HPLC-MS-MS) methods for the determination of PSZ either individually [11–14] or in mixtures with other antifungals were also developed [15–18].

Additionally, PSZ was determined in human plasma samples using capillary electrophoresis [19]. Moreover, simultaneous determination of PSZ and similar triazole antifungal drugs in human plasma was achieved using sweeping-micellar electrokinetic chromatography [20]. On the other hand, a photostability study of triazole antifungal drugs including PSZ in the solid state was performed using high performance thin layer chromatography (HPTLC) [21]. As an example, for the few non-chromatographic analysis methods for PSZ, a disposable carbon nanotubes-screen printed electrode was recently exploited for determination of the drug in biological samples [22]. Only a single report could be found in the published literature describing the spectrophotometric and fluorimetric determination of PSZ based on native absorbance and fluorescence measurement. [23]. Finally, we reported HPLC-DAD methods for assay of PSZ in its dosage form and for study of its pharmacokinetics, hepatic and pulmonary uptake in rat [24,25].

This study introduces three different analytical procedures for the determination of PSZ in bulk powder and suspension dosage form. An HPTLC, spectrofluorimetric,

and electrochemical analysis methods were developed. The structurally related antifungal drug itraconazole (ITZ) (Fig. 1) was included in the HPTLC method as an internal standard. All the investigated procedures were thoroughly validated and successfully applied to the assay of PSZ in commercial oral suspension.

Experimental

Instrumentation

HPTLC

The solutions were applied onto silica gel plates (precoated TLC silica gel aluminum Plates 60 F₂₅₄ 20 × 20 cm, 200 μm thickness with fluorescent indicator at 254 nm, Merck, Darmstadt, Germany) using a Cammag microliter syringe (100 μL) under nitrogen stream using a Cammag Linomat IV sample applicator (Switzerland). A Cammag twin trough glass chamber (20 × 20 cm) saturated with the mobile phase was used to perform the development step. Densitometric scanning was performed on Cammag TLC scanner III operated by CATS software (V 3.15 CAMAG). A deuterium lamp emitting a continuous UV spectrum between 190 and 400 nm was utilized as the source of radiation.

Spectrofluorimetry

Measurements were executed on a Perkin-Elmer fluorescence spectrometer LS 55 (Waltham, MA, USA) equipped

with xenon flash lamp using a 10-mm quartz cell. Excitation and emission monochromators were set at a slit width of 10 nm and a scanning speed of 500 nm/min was employed. Data were collected by a computer loaded with FL WinLab software v. 4.00.03.

Voltammetry

Measurements were made using a computer controlled 797 VA Computrace analyzer (Metrohm, Herisau, Switzerland) with a multimode electrode (MME). Hanging mercury drop electrode (HMDE) as working electrode, an auxiliary platinum electrode and an Ag/AgCl reference electrode (saturated with a 3.0M KCl solution) completed the three electrode cells.

Materials and reagents

Posaconazole was procured from Selleckchem (Houston, TX, USA). Itraconazole was generously donated by (Nifty Labs PVT LTD., Hyderabad, India). HPLC grade Methanol (Fisher Scientific UK Limited, Loughborough, Leicestershire, UK), analytical grade sulfuric acid, glacial acetic acid, boric acid, ortho-phosphoric acid, sodium hydroxide, chloroform and acetone (El-Nasr Pharmaceutical Chemicals Co., Egypt) and high purity distilled water were used. Noxafil[®] oral suspension (Patheon Inc., Ontario, Canada, BN 3005A) labeled to contain PSZ 40 mg/mL was purchased from Schering-Plough S.A.

Preparation of stock and working solution

Method I: HPTLC

PSZ 100 µg/mL and ITZ internal standard 100 µg/mL stock solutions were prepared in HPLC grade methanol. Stock solutions were kept refrigerated at 4°C and protected from light. The final working solutions were prepared by diluting appropriate aliquots of the stock solutions with HPLC grade methanol into different sets of 10-mL volumetric flasks to reach the concentration range of 5–50 µg/mL PSZ. A volume of 50 µL of a 10 µg/mL internal standard (ITZ) solution was added to all PSZ working solutions.

Method II: spectrofluorimetry

PSZ stock solution (100 µg/mL) was prepared in methanol. Working standard solution of 0.5 µg/mL PSZ was prepared by dilution from the aforementioned stock solution with methanol. Stock and working standard solutions were protected from light and kept at 4°C in a refrigerator. An aqueous solution of sulfuric acid (0.1M) was prepared and used in the study. For development of the calibration curve, aliquots of the working standard solution of PSZ were transferred into a set of 10-mL volumetric flasks in order to prepare working solutions in the concentration range of 0.05–0.3 µg/mL PSZ. The solutions in all flasks were completed to volume with 0.1M H₂SO₄.

Method III: differential pulse (DP) voltammetry

The previously mentioned PSZ stock and working standard solutions in method II were used for setting up the calibration curve. Britton-Robinson buffer (0.04M in each of acetic,

o-phosphoric and boric acids) was used to carry out the study and was adjusted to the required pH with 1M sodium hydroxide solution. The calibration curve was attained using aliquots of the working standard solution of PSZ that were transferred into a set of 10-mL volumetric flasks in order to prepare working solutions in the concentration range of 0.005–0.05 µg/mL PSZ. The working solutions were completed to volume with Britton-Robinson buffer pH 6.5.

General procedures

Method I: HPTLC

Sample loading

A TLC CAMAG linomat syringe was utilized to apply the solutions to the marked start edge of the TLC plate at a height of 15 mm from the lower edge of the plate. The spotted volume for all solutions was 10 µL and spotting was performed in the form of bands of 6-mm width and the spots were kept at a constant distance of 4 mm from each other. Each solution was applied in triplicates and allowed to air-dry for 5 min.

Chromatogram development and scanning

The mobile phase consisted of chloroform and acetone in the volume ratio 2:1. The TLC chamber was first rinsed with the mobile phase, and then 24 mL of the mobile phase was poured in the chamber. The chamber was covered with a lid and saturated for 30 min at room temperature (25 ± 2 °C). The sample-loaded TLC plates were transferred to the chamber. Linear ascending development of plates was carried out and the run was kept up to 90 mm. Spectrodensitometric analysis of the separated spots was implemented using Camag TLC Scanner using deuterium lamp set at 262 nm. The slit dimension used was 5 × 0.45 mm and the scanning speed was 20 mm/s. Integration of the chromatogram was achieved using Planar chromatography manager-win CATS (CAMAG).

Method II: spectrofluorimetry

The relative fluorescence intensities of the prepared standard solutions were recorded at 260 nm and 365 nm as the excitation and emission wavelengths respectively, against similarly prepared blank solutions.

Method III: differential pulse voltammetry

The content of each flask was transferred into the measuring vessel and purged with pure nitrogen for 5 min then the DP voltammograms were recorded using the HMDE as working electrode. The differential-pulse voltammetric measurements were performed with 0.12 V pulse amplitude and maximum drop size 9 (0.6 mm² drop area). The voltammograms were recorded from 0 to –1.8V at a scan rate of 25 mV/s versus Ag/AgCl reference electrode. The peak current was measured at peak potential of –1.462 V.

Measured responses were plotted against corresponding PSZ concentrations to construct the calibration curves for the three methods.

Assay of Noxafil[®] oral suspension

Noxafil[®] oral suspension was vortexed for 2 min to assure the homogeneity at the time to take the aliquot. An aliquot

of 250 μL of the suspension was quantitatively transferred to a 100-mL volumetric flask in order to prepare the stock sample solution. The volume was completed with methanol to obtain a final concentration of 100 $\mu\text{g}/\text{mL}$.

Method I (HPTLC)

Aliquots of the stock sample solution were transferred into 10-mL volumetric flasks and diluted with methanol to obtain final concentrations within the specified range then analyzed using the preceding chromatographic conditions. A volume of 50 μL of a 10 $\mu\text{g}/\text{mL}$ internal standard (ITZ) was added to all sample solutions.

Method II (spectrofluorimetry)

A working sample solution of concentration 10 $\mu\text{g}/\text{mL}$ was prepared by transferring 1 mL from the stock sample solution to a 10-mL volumetric flask and completed to volume with methanol. Aliquots of the working sample solution were transferred into 10-mL volumetric flasks and completed to volume with 0.1 M H_2SO_4 to obtain final concentrations within the specified range and then analyzed using the previously described spectrofluorimetric procedure.

Method III (differential pulse voltammetry)

The previous working sample solution (10 $\mu\text{g}/\text{mL}$) was used. An Aliquot of 1 mL was then transferred to a 10-mL volumetric flask to prepare the final working sample solution of concentration 1 $\mu\text{g}/\text{mL}$. The solution was then completed to volume with methanol. Aliquots of the final working solution were transferred to 10-mL volumetric flasks and completed

to volume with Britton-Robinson buffer pH 6.5 to obtain final concentrations within the specified range and then analyzed using the previously described voltammetric procedure.

Results and discussion

Development and optimization of the methods

Method I: HPTLC

Preliminary trials were focused on the separation of PSZ and the internal standard (ITZ) on 10 cm TLC plates. Mobile phases composed of: chloroform and methanol; acetone and methanol, resulted in poor separation of the drugs whereas, the use of: toluene, ethyl acetate and methanol has produced overlapped spots. Then a trial was done using chloroform and acetone and it was clear that this system produced the best results in terms of resolution, peak shape and symmetry. The chamber was saturated with the mobile phase 30 min prior to development at room temperature ($25 \pm 2^\circ\text{C}$). The optimized procedure resulted in well-defined spots with reproducible R_f values. Due to the structural similarity between PSZ and ITZ (Fig. 1), they share similar absorption characteristics, where they considerably absorb UV light at 262 nm. Finally, the developed spots of PSZ and ITZ were observed at R_f values equivalent to 0.15 ± 0.02 and 0.55 ± 0.05 respectively. A typical densitogram obtained from the analysis of PSZ using the proposed method is shown in Fig. 2. Overlapped purity spectra of PSZ obtained from standard and sample solutions is shown in Fig. 3.

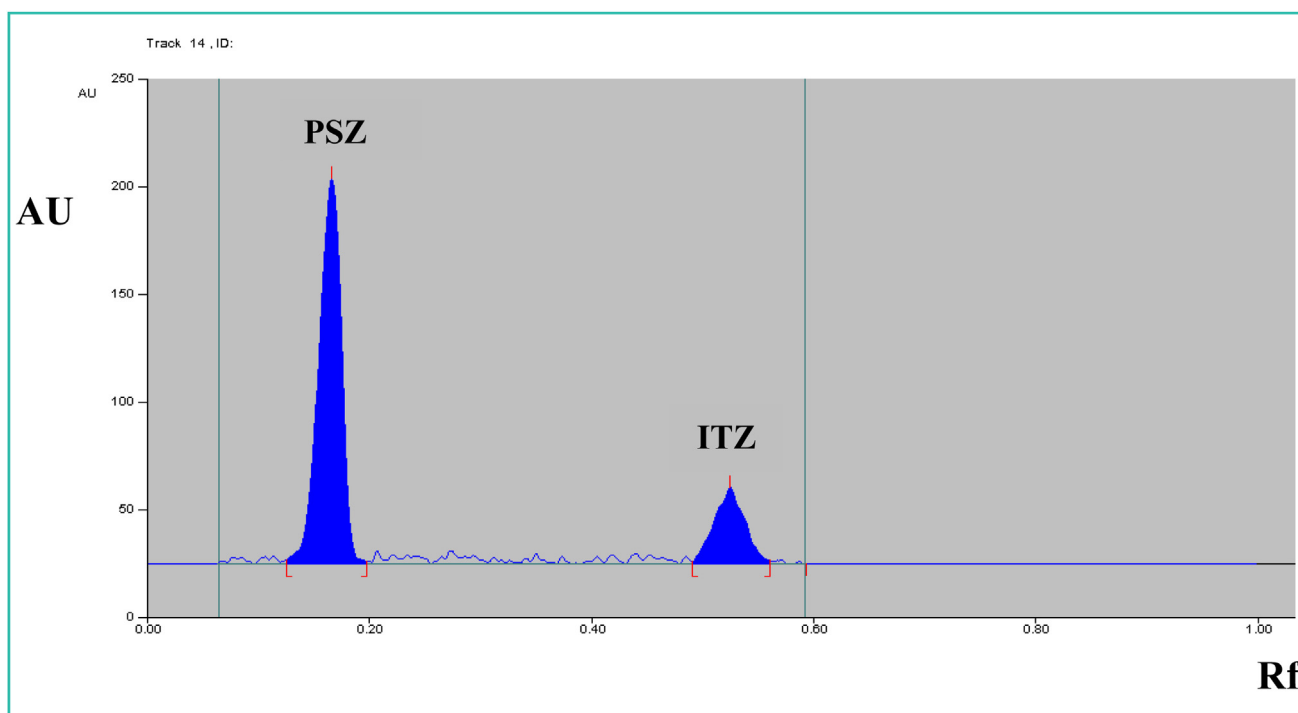


Figure 2. Densitogram of 50 $\mu\text{g}/\text{mL}$ PSZ and 10 $\mu\text{g}/\text{mL}$ ITZ at 262 nm.
Densitogramme de PSZ à 50 $\mu\text{g}/\text{mL}$ et de ITZ à 10 $\mu\text{g}/\text{mL}$ à 262 nm.

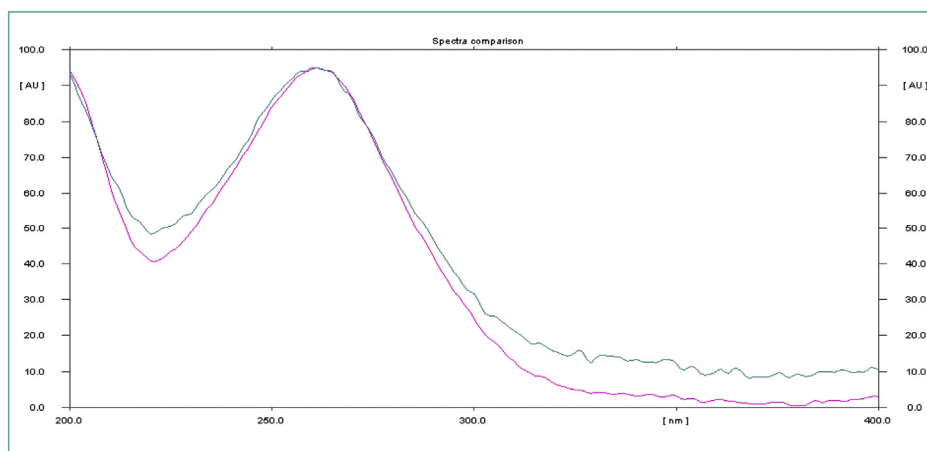


Figure 3. Overlapped purity spectra of PSZ between 200–400 nm obtained from standard and sample solutions.
Spectres de pureté superposés de PSZ entre 200–400 nm obtenus à partir de solutions standard et d'échantillons.

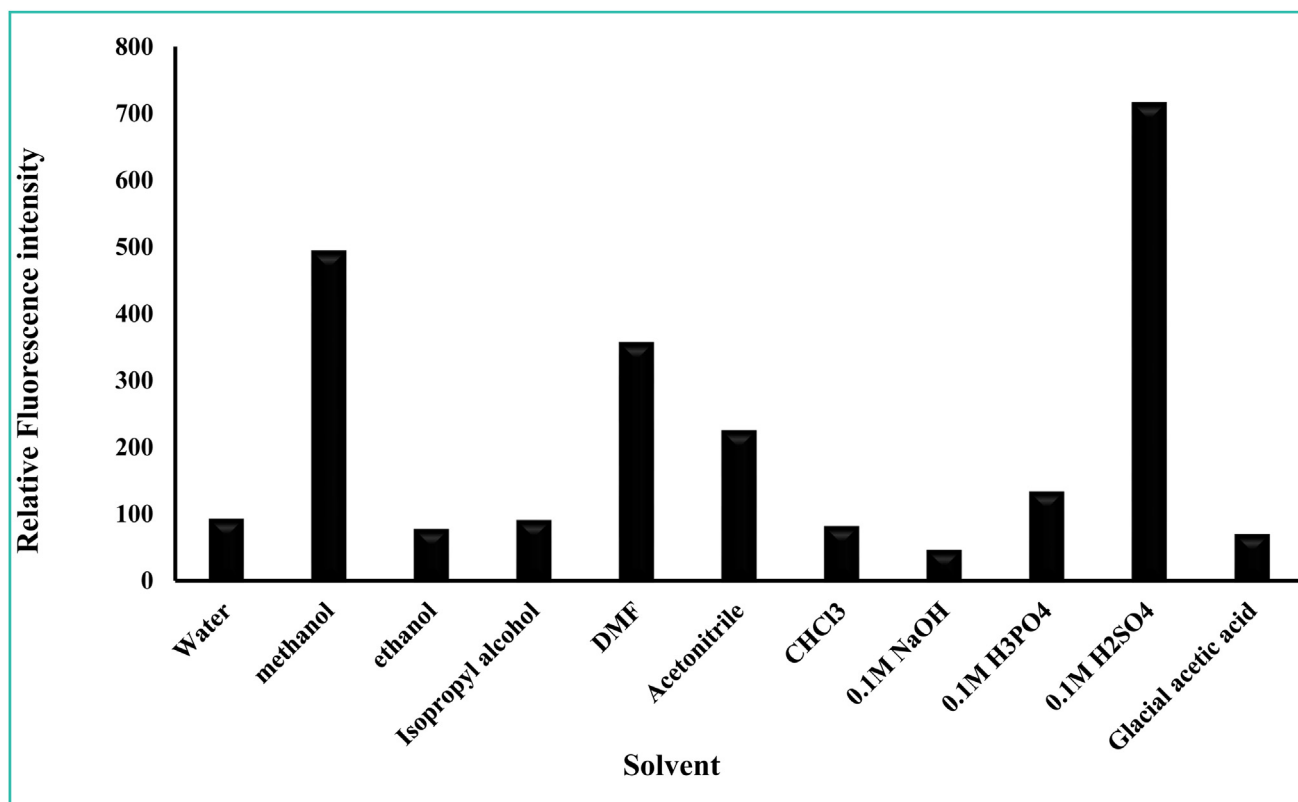


Figure 4. Effect of different solvents on the relative fluorescence intensity of 0.3 µg/mL PSZ.
Effet de différents solvants sur l'intensité de fluorescence relative de 0,3 µg/mL de PSZ.

Method II: spectrofluorimetry

The cited drug exhibited intrinsic fluorescence in a wide variety of solvents, as seen from Fig. 4. In view of the sensitivity and reproducibility of measurements, as well as the background (blank) readings, the optimum solvent was investigated and selected. It was found that acetone resulted in a complete quenching of PSZ fluorescence. Moreover, the relative fluorescence intensity of PSZ decreased noticeably when using other organic solvents such as ethanol

and isopropanol, however intense fluorescence was produced with methanol. Acidic (0.1M solutions of acetic, o-phosphoric) and basic (0.1M sodium hydroxide) solutions were also tested as possible diluting solvents and showed inferior results. On the other hand, using 0.1M sulfuric acid resulted in a remarkable increase in the sensitivity of PSZ determination, and among all the tested solvents, it resulted in the highest readings. Consequently, PSZ measurement was achieved in 0.1M H₂SO₄ at excitation and emission

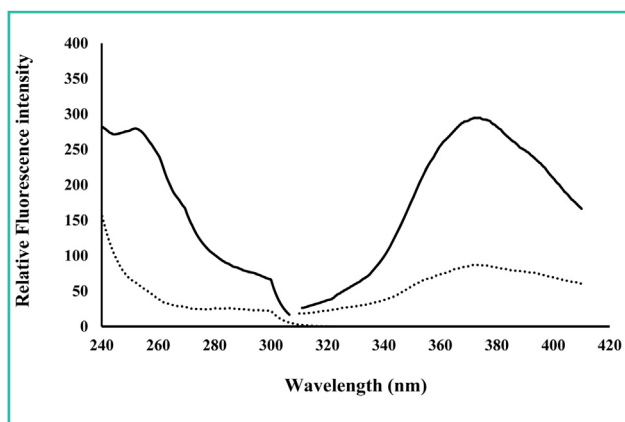


Figure 5. Excitation and emission spectra of 0.1 µg/mL PSZ against the solvent blank.
Spectres d'excitation et d'émission de 0,1 µg/mL de PSZ par rapport au blanc de solvant.

wavelengths of 260 and 365 nm, respectively (Fig. 5). A study was carried out to investigate the effect of different surfactants including cationic (cetyl pyridinium bromide, CPB), anionic (sodium lauryl sulfate, SLS) and non-ionic (Triton X-100 and Tween) surfactants on the fluorescence intensity of the drug. Two sets of solutions were prepared for the drug where increasing volumes of 0.025 M CPB, 0.1 M SLS, 2% v/v Triton X-100 and 0.5% w/v Tween aqueous solutions were added to the drug aliquots and solutions were completed to volume with either water or 0.1 M H₂SO₄. Unfortunately, quenching of fluorescence signal was observed in most solutions and sometimes this quenching was dramatic. Thus, no surfactant was used in this work.

Method III: differential pulse (DP) voltammetry

Factors affecting the peak current were studied and optimized. Britton-Robinson buffer was chosen as a supporting electrolyte for the DP voltammetric determination of the drug. The effect of pH on the peak current was studied over the pH range 3–9. With a pH of 3.0 to 5.5 it was impossible to evaluate the voltammograms as the peaks were deformed and irregular in shape. On the contrary, PSZ exhibited a well-defined differential pulse cathodic peak in the pH range 6–9 (Fig. 6). The PSZ peak appeared in the potential range of –1.3 to –1.5 V throughout the studied pH range. The maximum peak current was obtained using B-R buffer pH 6.5 which can be successfully used to determine PSZ by applying a differential-pulse voltammetric method and measuring the peak current at peak potential of –1.462 using a scan rate of 25 mV/s (Fig. 7).

Instrumental conditions affecting the peak current were also investigated. The effect of pulse amplitude on the peak current was tested. As the pulse amplitude was increased, the response increased reaching a maximum at –120 mV above which it declined (Fig. 8). In addition, it was found that increasing the drop size had a positive influence on the peak current values and hence, drop size 9 (0.6 mm² drop area) was chosen for the analytical measurement of PSZ.

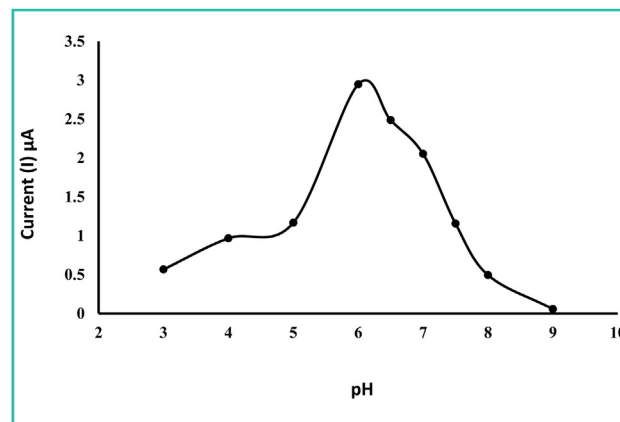


Figure 6. Effect of pH on the current produced by the differential-pulse voltammetry of 0.05 µg/mL PSZ.
Effet du pH sur le courant produit par la voltamétrie à impulsions différentielles de 0,05 µg/mL de PSZ.

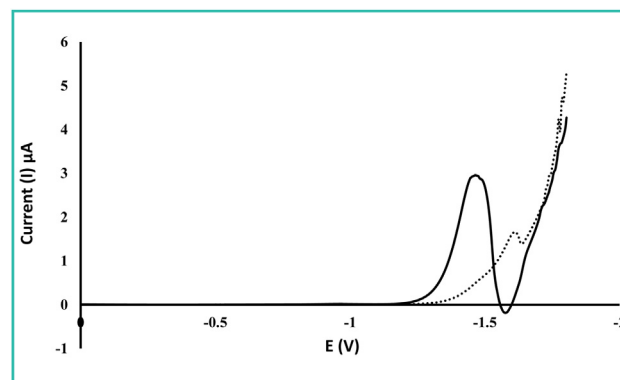


Figure 7. Differential-pulse voltammogram of 0.0375 µg/mL PSZ (—) and B-R buffer pH 6.5 (....) versus Ag/AgCl reference electrode.
Voltammogramme par impulsions différentielles de 0,0375 µg/mL de PSZ (—) et tampon B–R pH 6,5 (....) par rapport à une électrode de référence Ag/AgCl.

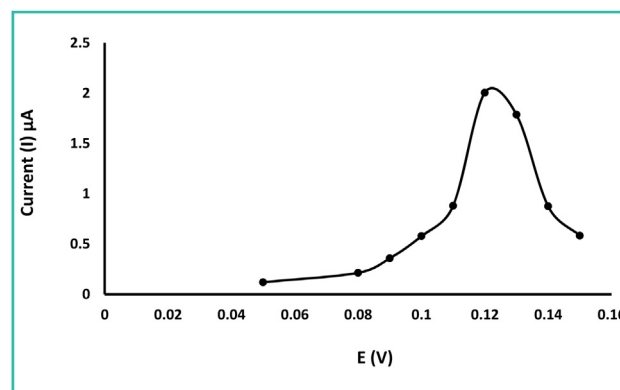


Figure 8. Effect of pulse amplitude on the current produced by the differential-pulse voltammetry of 0.0375 µg/mL PSZ.
Effet de l'amplitude des impulsions sur le courant produit par la voltampérométrie différentielle par impulsions de 0,0375 µg/mL PSZ.

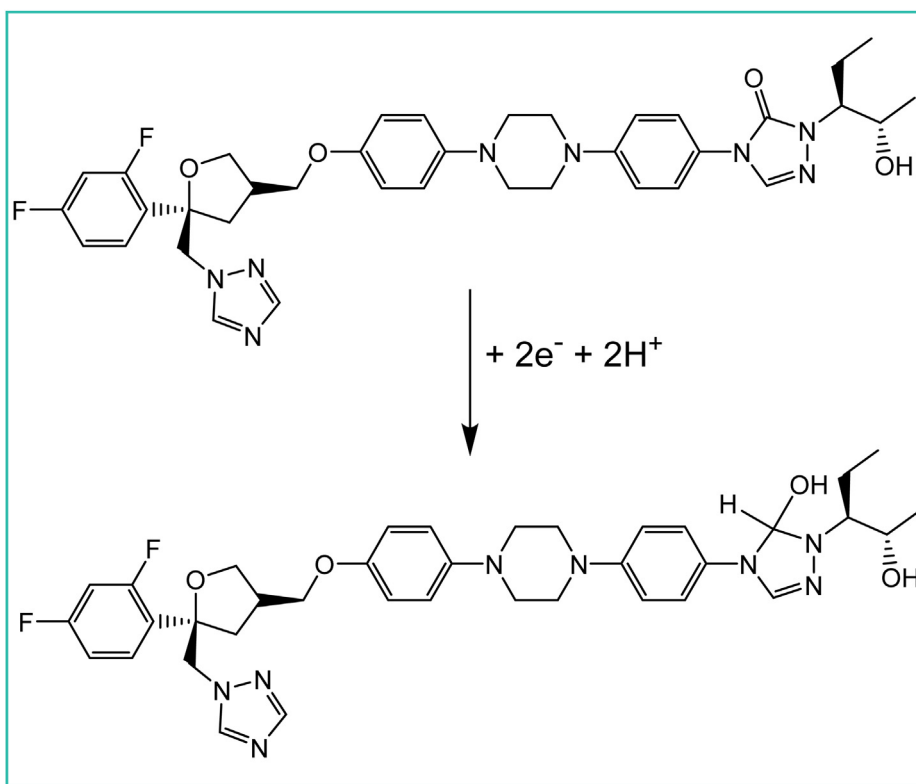


Figure 9. Proposed mechanism for the reduction of PSZ at the HMDE.
Mécanisme proposé pour la réduction de PSZ au de la HMDE.

The electrode reaction

PSZ exhibited a voltammetric peak at -1.462 V using the HMDE versus Ag/AgCl electrode in Britton-Robinson buffer pH 6.5. A previous study reported the mechanism of reduction of the structurally related antifungal drug itraconazole (ITZ) [26] using differential pulse polarography (DPP) at a dropping mercury electrode (DME) in Britton-Robinson buffer at pH values 6–8. ITZ and PSZ both comprise a triazolone structure. Owing to the similarity in their structure and in the experimental conditions, it is proposed that the same mechanism will be followed by PSZ. The carbonyl group of the triazolone structure is assumed to undergo an irreversible reduction to the equivalent alcohol through the uptake of two electrons and two protons. The proposed mechanism for PSZ is shown in Fig. 9.

The reversibility of the electrode reaction can be investigated through the application of cyclic voltammetry technique. This is achieved by scanning the potential to more negative values till complete reduction of the electroactive analyte, then back to the original value to cause oxidation of the reduced analyte. Appearance of both cathodic and anodic waves in the cyclic voltammogram provides an indication that the electrode reaction is reversible. On the other hand, irreversible reactions demonstrate either cathodic or anodic waves on the voltammogram. The reduction reaction of PSZ was found to be irreversible as illustrated by its cyclic voltammogram (Fig. 10). It demonstrates a single cathodic wave at -1.58 V without the appearance of any waves in the anodic pathway.

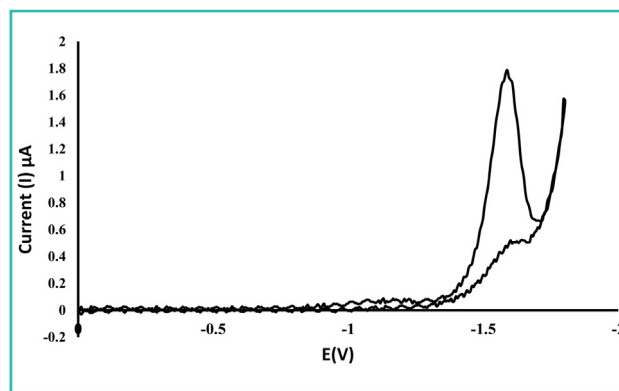


Figure 10. Cyclic voltammogram of $0.025 \mu\text{g/mL}$ PSZ in B-R buffer pH 6.5.
Voltammogramme cyclique de $0,025 \mu\text{g/mL}$ de PSZ dans un tampon B-R pH 6,5.

Validation of the proposed methods

The three proposed analytical methods were validated according to the guidelines of the International Conference on Harmonization (ICH) [27]. Validation experiments were performed on standard PSZ solutions.

Linearity and concentration ranges

Under the optimal conditions, a linear relationship exists between the analytical responses (Peak area ratio, relative fluorescence intensity and current amplitude for methods

Table 1 Analytical parameters for determination of PSZ using the proposed methods.
Paramètres analytiques pour la détermination de PSZ en utilisant les méthodes proposées.

Parameter	HPTLC	Spectrofluorimetry	DP voltammetry
Concentration range, ($\mu\text{g/mL}$)	5–50	0.05–0.3	0.005–0.05
Intercept, (a)	–0.0170	–52.3	–0.052
S_a	0.0276	6.30	0.0246
Slope, (b)	0.0880	2666	54.76
S_b	0.0009	33.4	0.86
RSD% of the slope, ($S_b\%$)	1.02	1.25	1.57
Correlation coefficient, (r)	0.9998	0.9998	0.9997
$S_{y/x}$	0.0355	6.41	0.0293
LOD, ($\mu\text{g/mL}$)	1.04	0.0078	0.0015
LOQ, ($\mu\text{g/mL}$)	3.14	0.0236	0.0045

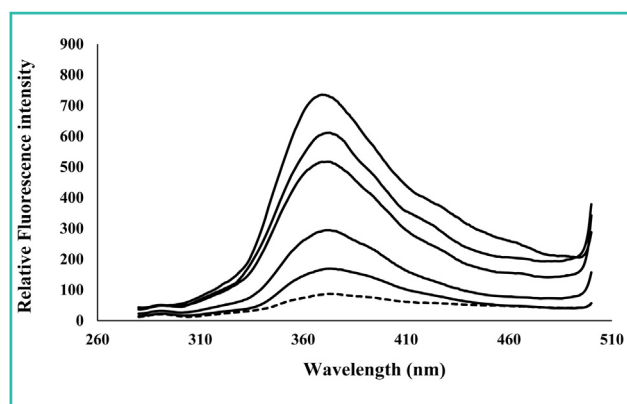


Figure 11. Emission spectra of PSZ standard concentrations of 0.05, 0.1, 0.2, 0.25, 0.3 $\mu\text{g/mL}$ (—) and the solvent blank (---).
Spectre d'émission des concentrations standard de PSZ de 0,05, 0,1, 0,2, 0,25, 0,3 $\mu\text{g/mL}$ (—) et du blanc de solvant (---).

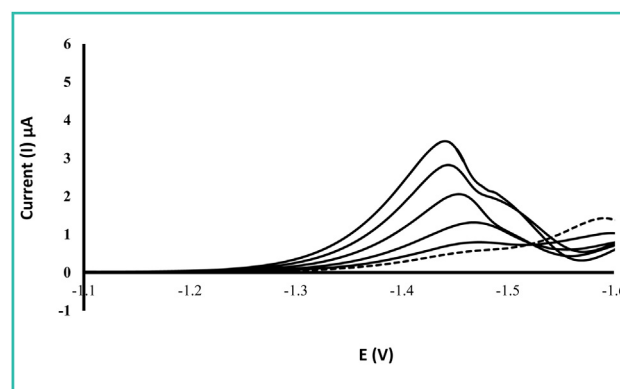


Figure 12. Differential-pulse voltammograms of PSZ standard concentrations of 0.005, 0.0125, 0.025, 0.0375, 0.05 $\mu\text{g/mL}$ (—) and B-R buffer pH 6.5 (---) versus Ag/AgCl reference electrode.
Voltammogrammes à impulsions différentielles de concentrations standard PSZ de 0,005, 0,0125, 0,025, 0,0375, 0,05 $\mu\text{g/mL}$ (—) et tampon B–R pH 6,5 (---) par rapport à une électrode de référence Ag/AgCl.

I, II and III respectively) and the corresponding concentrations of PSZ. The linearity data and statistical parameters for the studied methods including linear regression equations, concentration ranges, correlation coefficient (r) values, standard deviation of the intercept (S_a), the slope (S_b) and standard deviation of residuals ($S_{y/x}$) are illustrated in Table 1. Figs. 11 and 12 show emission spectra and DP voltammograms for serial concentrations of PSZ, respectively. By employing regression analysis, good linearity of the developed methods was confirmed as revealed from the value of correlation coefficient ($r > 0.9997$) and RSD% of the slope ($S_b\% < 1.57\%$).

Detection and quantification limits

The standard deviation of the intercept (S_a) and the slope (b) method were used to determine the limit of detection (LOD) and limit of quantification (LOQ). LOD is expressed as $3.3 S_a/b$, whereas the LOQ is calculated by the formula $10 S_a/b$. The minimum amount detected under the described conditions used was estimated and listed in Table 1. The developed methods demonstrated sufficient sensitivity as indicated by the low LOD and LOQ values especially for the fluorimetric and voltammetric methods.

Accuracy and precision

Investigation of the accuracy and within-day precision (repeatability) of measurement of analyte responses was performed at three concentration levels of PSZ using three replicate determinations for each concentration within one day. In the same manner, the accuracy and between-day precision were tested by analyzing the same three concentrations using three replicate determinations repeated on three days. The recovered concentrations were calculated using the corresponding regression equations and were found to be satisfactory. Analytical results obtained from these investigations are summarized in Table 2. The low values of percentage relative standard deviation (RSD %) and percentage relative error (E_r %) (less than 2%) indicate the high precision and good accuracy of the developed methods for estimation of PSZ in bulk form.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small but intended variations in method parameters and illustrates its reliability during normal usage. For HPTLC method, variations

Table 2 Precision and accuracy for determination of PSZ in bulk form using the proposed methods. *Précision et exactitude pour la détermination de PSZ en vrac en utilisant les méthodes proposées.*

Method	Nominal value (µg/mL)	Within-day			Between-day		
		Found ± SD ^a (µg/mL)	RSD(%) ^b	E _r (%) ^c	Found ± SD ^a (µg/mL)	RSD(%) ^b	E _r (%) ^c
HPTLC	5	5.043 ± 0.042	0.83	0.86	5.094 ± 0.063	1.24	1.88
	20	20.01 ± 0.293	1.46	0.05	20.33 ± 0.348	1.71	1.65
	50	50.15 ± 0.202	0.40	0.30	50.17 ± 0.269	0.54	0.34
Spectrofluorimetry	0.05	0.0502 ± 0.00082	1.63	0.40	0.0503 ± 0.00092	1.83	0.60
	0.1	0.0996 ± 0.00043	0.43	-0.40	0.1012 ± 0.00055	0.54	1.20
	0.3	0.303 ± 0.00513	1.69	1.00	0.304 ± 0.00517	1.70	1.33
Differential Pulse Voltammetry	0.005	0.00504 ± 0.00006	1.19	0.80	0.00501 ± 0.00008	1.60	0.20
	0.025	0.0253 ± 0.00019	0.75	1.20	0.0251 ± 0.00048	1.90	0.40
	0.05	0.0509 ± 0.00081	1.59	1.80	0.0507 ± 0.00091	1.80	1.40

^a Mean ± standard deviation for 3 determinations.
^b Percentage relative standard deviation.
^c Percentage relative error.

Table 3 Robustness evaluation for determination of PSZ using the proposed HPTLC method. *Évaluation de la robustesse pour la détermination de PSZ en utilisant la méthode HPTLC proposée.*

Condition variation	PSZ R _f ± SD	ITZ R _f ± SD	% Recovery
Volume of mobile phase: 24 ± 2 mL	0.13 ± 0.015	0.60 ± 0.020	100.4 ± 1.23
Mobile phase composition: chloroform: acetone, (2:1 ± 0.1)	0.15 ± 0.036	0.60 ± 0.015	101.2 ± 0.85
Wavelength: 262 ± 2 nm	0.15 ± 0.010	0.56 ± 0.020	100.2 ± 1.10

were done in mobile phase composition (2:1 ± 0.1), volume of mobile phase (24 ± 2 mL), and wavelength of detection (262 ± 2 nm). Analysis of PSZ solutions was done after the changed parameters and results are presented in Table 3. The method proved to be robust because the obtained recoveries and measured parameters (R_f of the drug and IS) did not change significantly. For the voltammetric method, deliberate variations were done in the buffer pH (6.5 ± 0.05). The recovery value (100.5 ± 1.163) was not significantly affected by this variation and consequently the method can be considered robust.

Stability of solutions

The stability of PSZ working standard solutions in different solvent media: methanol, 0.1 M H₂SO₄ and Britton-Robinson

buffer pH 6.5 at room temperature was examined. No significant changes in the analytical signals were observed within 2 hr. Furthermore, the stock standard solution of PSZ in methanol was stable for at least 1 week when stored in the refrigerator at 4 °C.

Application of the proposed methods on Noxafil® oral suspension

The developed HPTLC, spectrofluorimetric and voltammetric methods were applied for the assay of PSZ in its commercial formulation. PSZ could be directly determined without any pretreatment or interference from the excipients or the components of the suspension (sugar, flavor, preservative, etc.), and this partially demonstrates

Table 4 Assay of PSZ in suspension dosage form using the developed methods and the reference HPLC method. *Analyse du PSZ sous forme posologique en suspension en utilisant les méthodes développées et la méthode de référence HPLC.*

Parameters	HPTLC	Spectrofluorimetry	DP Voltammetry	HPLC [24]
% recovery ± SD ^a	100.24 ± 1.64	100.80 ± 1.46	100.80 ± 1.36	101.57 ± 1.62
RSD, (%) ^b	1.64	1.45	1.35	1.60
Single factor Anova	F = 1.00 (F _{critical} = 3.24)			

^a Mean ± standard deviation for 5 determinations.
^b Percentage relative standard deviation.

specificity of the three methods. Obtained assay results were precise, accurate and in good agreement with the label claim, as revealed by the satisfactory values of percent recoveries, SD and RSD % values (Table 4).

Furthermore, the recoveries obtained from the proposed methods were statistically compared with those obtained from the formerly reported HPLC method [24] using the one-way analysis of variance test (Single factor Anova). The calculated F value did not exceed the critical value, revealing that there were no significant differences between the proposed methods together with the reference method (Table 4).

Conclusions

In this work, simple, direct and sensitive methods for the determination of PSZ in bulk powder and suspension dosage form have been described. An HPTLC, spectrofluorimetric, and electrochemical analysis methods were developed for PSZ estimation. Measurement conditions were optimized and the methods were validated in accordance with ICH recommendations. No pharmacopoeial assay methods could be found for PSZ dosage forms. In addition, few analysis methods are available in the scientific literature for determination of PSZ pharmaceutical preparations. Thin layer chromatography is the simplest separation method in terms of equipment and performance. HPTLC exhibits several advantages over HPLC and consequently it is sometimes favored over it. HPTLC can be regarded as more economic and environment friendly as it consumes by far less amount of solvents. In addition, it is a fast method of analysis allowing the simultaneous processing of large number of samples. Moreover, the elaborate treatment or the sophisticated experimental setup usually associated with HPLC methods of analysis is not required in HPTLC. To the best of our knowledge, there are no published articles describing the determination of PSZ by HPTLC.

Spectrofluorimetry is an analytical tool that allows the estimation of compounds based on either their native fluorescence or their fluorescent products obtained after derivatization. This technique provides a high level of sensitivity and selectivity. To the best of our knowledge, very few HPLC with fluorescence detection methods have been described for quantification of PSZ [9,10]. In addition, only one study reported the fluorimetric determination of the drug based on native fluorescence measurement [23]. The proposed spectrofluorimetric method showed comparable sensitivity of measurement in terms of linearity range and LOD values: 0.05–0.30 and 0.0078 $\mu\text{g}/\text{mL}$ respectively versus 0.022–0.550 and 0.007 $\mu\text{g}/\text{mL}$ for the reported method [23]. On the other hand, the proposed method is advantageous regarding the medium where PSZ was measured in aqueous 0.1 M sulfuric acid solution while an organic solvent (isopropanol) was used in the reported method [23]. This implies that the proposed method herein is more environment friendly and economic.

The electrochemical behavior of PSZ has only been studied through its oxidation on glassy carbon electrode using cyclic voltammetry [28]. According to this previous study, PSZ was measured in concentration range 20–100 $\mu\text{g}/\text{mL}$ [28]. Certainly, PSZ contains some functional groups that

are liable to reduction which can be the basis for a cathodic voltammetric procedure, therefore we developed a simple and reliable method for its determination; based on the differential-pulse cathodic voltammetric measurement of PSZ on the hanging mercury drop electrode (HMDE). Obviously, our current work provides much better sensitivity of measurement, in addition the previous study did not show any results for application to real samples [28]. Finally, the applicability of the proposed methods to real life situations was assessed through the analysis of commercially available suspension and satisfactory results were obtained in comparison to an HPLC reported method.

Disclosure of interest

The authors have not supplied their declaration of competing interest.

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