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UV Spectrophotometric method for determination of posaconazole: comparison to HPLC

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ABSTRACT

The aim of this work was to develop a simple, fast and reproducible spectrophotometric method for the analysis of posaconazole in raw material. The established conditions were: methanol as extracting solvent, detection wavelength of 260 nm, Shimadzu double beam spectrophotometer 1800 model with 1 cm quartz cells. Linearity was demonstrated in the concentration range of 5.0 a 25.0 µg/mL (r = 0.9999). Reproducibility and intermediate precision were confirmed by low RSD values (0.49 to 0.82%). Accuracy, evaluated through recovery test, was adequate, with 98.20% of mean recovery. Specificity and robustness were also demonstrated. The mean amount found for samples was 100.82%. The proposed method was considered suitable for the intended purpose, mainly in routine analysis of quality control laboratories. When compared to the previously developed HPLC, no statistical difference was observed, what made the UV spectrophotometric method a reliable alternative.

Keywords: Posaconazole. UV spectrophotometry. Quality control.

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INTRODUCTION

Posaconazole, chemically 4-{4-[4-(4-{[(3R,5R)-5-(2,4-difluorophenyl)-5-(1H-1,2,4-triazol-1-ylmethyl)-tetrahydrofuran-3-yl]methoxy}phenyl) piperazin-1-yl]phenyl}-2-[(1S,2S)-1-ethyl-2-hydroxy-propyl]-2,4-dihydro-3H-1,2,4-triazol-3-one, is an antifungal drug belonging to triazole class, approved by Food and Drug Administration (FDA) in September of 2006 (Figure 1) (Thompson *et al*., 2009).

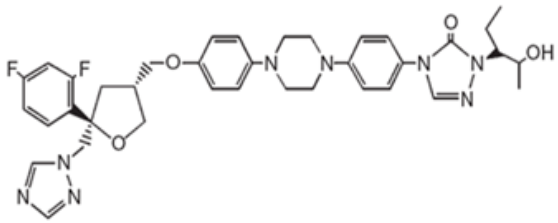

Figure 1. Chemical structure of posaconazole.

It is characterized by a wide spectrum of action and few drug interactions, being indicated for the prophylaxis of invasive infections for *Aspergillus spp*. and *Candida spp*., mainly in patients above thirteen years old, who has high risk to develop these infections when they are being immune suppressed such as in cancer therapy, transplants or HIV infections (Keating, 2005; Frampton & Scott, 2008).

Concerning its analytical determination, there are some works in literature describing the analysis in human plasma, alone or in the presence of other azoles, and in dog serum applying liquid chromatography with UV or mass spectrometry detectors (Kim *et al*., 2000, Kim *et al*., 2003; Shen *et al*., 2007; Chhun *et al*., 2007; Cunliffe *et al*., 2009; Alffenaar at al., 2010; Ekiert *et al*., 2010; Reddy *et al*., 2011; Beste *et al*., 2012). Recently, a capillary electrophoresis method was developed for patients' plasma assay using a field-amplified sample stacking (Liao *et al*., 2012). For raw material analysis, there is only a liquid

chromatographic-PDA detection method, which is stability-indicating and was developed by our group (Garcia *et al*., 2012). Considering impurities elucidation, Zhong *et al*. (2012) have characterized a thermal degradant, found in the product, by mass spectrometry (MS) and nuclear magnetic resonance (NMR).

Once posaconazole is still not cited in any pharmacopoeia and there is the necessity of alternative methods which can be easily adopted and applied in quality control laboratories and research centers, the aim of this manuscript was to develop a simple and fast method to determine posaconazole in raw material. Besides, it will be compared to the previously published HPLC-PDA one (Garcia *et al*., 2012). In order to certify the appropriateness, a validation will be performed according to international guidelines (ICH, 2005; USP 37, 2014).

MATERIALS AND METHODS

Chemicals

Posaconazole reference standard (99.5%) and raw material were purchased from ACC Corp. (San Diego, CA, USA). Methanol analytical grade was provided by Tedia (Fairfield, OH, USA) and Vetec (São Paulo, Brazil).

Equipment and conditions

A Shimadzu (Kyoto, Japan) double beam spectrophotometer, 1800 model with 1 cm quartz cells was used to perform the absorbance measurements. Prior to use, background correction was carried out using the matrix solvent (methanol). For the analysis, the wavelength was set at 260 nm.

Standard and sample preparation

Posaconazole reference standard was accurately weighed and dissolved in a 200 mL volumetric flask using methanol to yield the concentration of 25.0 µg/mL. An aliquot of this solution was diluted to 10.0 µg/mL using the same solvent.

The raw material was also accurately weighed and transferred to a 100 mL volumetric flask. It was completed with methanol until reach the concentration of 50.0 µg/mL. From this solution, it was prepared a 10.0 µg/mL one using the same solvent.

Method validation

Specificity

Specificity was demonstrated through the analysis of the spectra obtained between 200-400 nm using the sample matrix (methanol), since no excipient is present in raw material.

Linearity

It was determined by preparing standard solutions in the concentrations of 5.0; 7.5; 10.0; 12.5; 15.0; 20.0 and 25.0 µg/mL, in triplicate. From the obtained data, standard plots (concentration versus absorbance) were constructed, which were statistically evaluated by linear regression analysis through least square method and Analysis of Variance (ANOVA).

Precision

Repeatability and intermediate precision were evaluated by preparing six sample solutions (10.0 µg/mL) which were analyzed in the same day and three different days, respectively. New solutions were prepared in each day and the measurements were performed in triplicate. In the third day, a different analyst conducted the assay. Values of relative standard deviation (RSD) for sample amount were observed.

Accuracy

Accuracy was determined by the recovery of known amounts of posaconazole added to sample solutions at 10.0 µg/mL, reaching the final concentrations of 15.0; 20.0 and 25.0 µg/mL, named R1, R2 and R3, respectively. Each solution was prepared in triplicate.

Robustness

It was demonstrated through small modifications in the established analytical conditions: wavelengths of 258 and 262 nm and methanol brand (usually Vetec™ and now Tedia™). Eventual alterations in the amount results were observed.

HPLC method

Posaconazole was analyzed in a Shimadzu Liquid Chromatograph, equipped with PDA detector and controlled by LC Solution V. 1.24 software (Shimadzu, Kyoto, Japan). Mobile phase consisted of methanol-water (75:25; v/v), at flow rate of 1.0 mL/min, which was daily prepared and filtered through 0.45 µm membrane filter (Millipore, Bedford, USA). The wavelength of the PDA detector was set at 260 nm. A Shim-pack C8 column (250 x 4.6 mm; 5 µm – Shimadzu) was used. The HPLC system was operated at 25 ± 1 °C. The injection volume was 20 µL.

Comparison between UV and HPLC methods

Eighteen samples were analyzed by both methods. The comparison of the results was done by Student's t- test.

RESULTS

A representative UV spectrum of posaconazole is presented in Figure 2, where is possible to observe a well defined maximum at 260 nm, wavelength selected for the analysis. Under the established conditions, sample matrix (methanol) did not interfere with the determinations, since it does not absorb in that wavelength, demonstrating the method is specific.

Linearity was confirmed in concentration range of 5.0 to 25.0 µg/mL. The regression equation obtained was $y = 0.0462 x + 0.0191$ and the correlation coefficient was

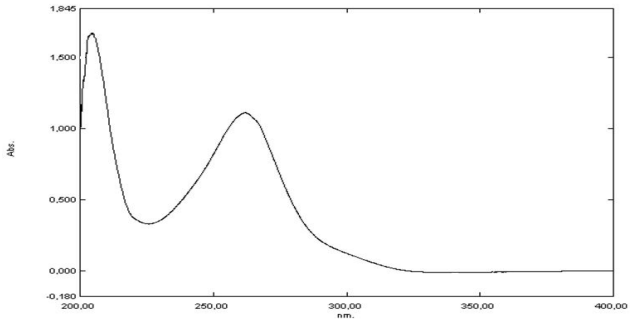

Figure 2. UV spectrum of posaconazole reference substance in methanol, 10.0 µg/mL.

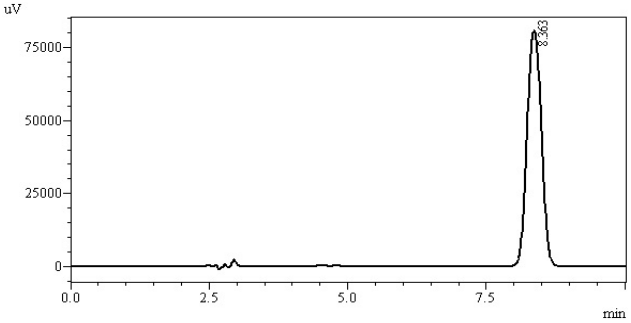

Figure 3. Representative chromatogram of posaconazole reference substance.

r = 0.9999. ANOVA showed the proposed method has significative regression (F_{calc} = 4572 > $F_{critical}$= 4.60, p = 0.05) and no linearity deviation (F_{calc} = 0.94 < $F_{critical}$= 2.96, p = 0.05).

Precision results are described in Table 1. Intraday and interdays evaluation were performed. Posaconazole mean amount was 100.82%. The recovery test showed the proposed method has great accuracy, as can be seen in (Table 2). The mean recovery of posaconazole reference standard was 98.20% (RSD = 0.41).

Analyzing the robustness of the method, it was observed that the small deliberate modifications done did not interfere with the results, demonstrating the method is robust (Table 3). Statistical evaluation, performed by ANOVA, confirm the similarity of the amounts found (F_{calc} = 0.40 < $F_{critical}$= 4.06, p = 0.05).

Comparison between UV and HPLC methods

The comparison of the methods, done by Student's t- test, confirmed that both methods are statistically equivalent (t_{calc} = 0.64 < $t_{critical}$ = 2.13, p = 0.05) (Table 1). A representative chromatogram is presented in (Figure 3). The retention time was about 8.3 minutes.

DISCUSSION

UV spectrophotometry is a very useful technique, already employed for other azole antifungal drugs, such as fluconazole, bifonazole, clotrimazole, econazole, itraconazole, miconazole and ketoconazole in the zero-order or derivative modes, but there is no description for posaconazole (Ekiert & Krzek, 2009).

Specificity and selectivity are the most common problems found when this technique is applied, since drugs and excipients can absorb UV radiation in the same wavelength (Oliveira *et al*., 2010; Watson, 2005). However, in the proposed method, it was not found any interference, what allowed a simple sample preparation and analysis.

The concentration range tested for linearity is in accordance to method's sensitivity and statistical evaluation confirmed its significance.

Table 1. Precision results for posaconazole raw material determination by UV spectrophotometry and comparison to HPLC.

	Day 1	**Day 2**	**Day 3**
Amount (%)	100.30	101.67	99.80
	100.06	101.88	101.92
	99.46	100.62	100.01
	100.26	101.46	101.07
	99.88	100.41	100.86
	99.06	101.04	101.28
Mean amount (%)	99.85	101.18	100.82
RSD	0.49	0.57	0.80
Interdays amount* (%)		100.82	
Interdays RSD		0.82	
HPLC mean amount* (%)		100.01	
RSD		1.21	

*mean of eighteen determinations

Table 2. Recovery test for UV spectrophotometric method applied to posaconazole raw material determination

	Concentration added (µg/mL)	**Concentration found* (µg/mL)**	**Recovery (%)**
R1	15.0	14.85	97.59
R2	20.0	19.75	98.09
R3	25.0	24.67	98.39

*mean of three determinations

Table 3. Robustness evaluation of UV spectrophotometric method applied to posaconazole raw material

Parameter	**Amount (%)**
λ258nm	100.70
λ262nm	100.40
Methanol TediaTM	100.80
Normal condition	100.82

Intraday and interdays precisions could be demonstrated by low RSD values obtained in the performed determinations. It must be emphasized that in the third day, the analyst was different from the first two days, which means the method has intermediate precision and could be easily performed by trained professionals.

HPLC is an important separation technique and it was applied to posaconazole intending to obtain a stability-indicating method, in which was possible to observe drug's sensitivity to oxidative degradation (Garcia *et al*., 2012). When the focus is the assay of drugs, UV spectrophotometry can be very useful and cheaper, since there is no need to columns and filters, besides of fewer disposals of residual solvents. Besides, UV techniques can be easily adapted to dissolution studies, in which there are a great number of samples to be analyzed.

CONCLUSION

The UV spectrophotometric method developed demonstrated to be fast, precise and suitable for posaconazole determination in raw material, representing a suitable alternative to HPLC in routine quality control, since no statistical difference was observed between them.

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RESUMO

O objetivo deste trabalho foi desenvolver um método espectrofotométrico simples, rápido e reprodutível para a análise de posaconazol na matéria-prima. As condições estabelecidas foram: metanol como solvente extrator, comprimento de onda de 260 nm e espectrofotômetro de duplo feixe Shimadzu, modelo 1800, com cubetas de quartzo de 1 cm. A linearidade foi demonstrada na faixa de concentração de 5,0 a 25,0 µg/mL (r = 0,9999). A reprodutibilidade e a precisão intermediária foram confirmadas pelos baixos valores de desvio padrão relativo (0,49 a 0,82%). A exatidão, avaliada pelo teste de recuperação, foi adequada, com recuperação média de 98,20%. A especificidade e a robustez também foram demonstradas. O teor médio encontrado nas amostras foi de 100,82%. O método proposto foi considerado adequado, principalmente para a análise de rotina em laboratórios de controle de qualidade. Quando comparado com o método por HPLC, não houve diferença estatística, o que torna o método por espectrofotometria UV uma alternativa segura.

Palavras-chave: Posaconazol. Espectrofotometria UV. Controle de qualidade.

REFERENCES

Alffenaar JWC, Wessels AMA, van Hateren K, Greijdanus B, Kosterink JG, Uges DRA. Method for therapeutic drug monitoring of azole antifungal drugs in human serum using LC/MS/MS. J Chromatogr. B 2010; 878:39-44.

Beste KY, Burkhard, O, Kaever V. Rapid HPLC–MS/MS method for simultaneous quantitation of four routinely administered triazole antifungals in human plasma. Clin Chim Acta. 2012; 413:240-5.

Chhun S, Rey E, Tran A, Lortholar O, Pon G, Jullien V. Simultaneous quantification of voriconazole and posaconazole in human plasma by high-performance liquid chromatography with ultra-violet detection. J Chromatogr. B 2007; 852:223-8.

Cunliffe J, Noren C, Hayes R, Clement R, Shen J, Shen J. A high-throughput LC–MS/MS method for the quantitation of posaconazole in human plasma: Implementing fused core silica liquid chromatography. J Pharm Biom Anal. 2009; 50:46-52.

Ekiert RJ, Krzek J, Talik P. Chromatographic and electrophoretic techniques used in the analysis of triazole antifungal agents—a review. Talanta. 2010; 82:1090-1100.

Ekiert RJ, Krzek J. Determination of azole antifungal medicines using zero-order and derivative UV spectrophotometry. Acta Pol Pharm. 2009; 66:19-24.

Frampton JE, Scott L. Posaconazole. A review of its use in the prophylaxis of invasive fungal infections. Drugs. 2008; 68:993-1016.

Garcia CV, Costa GR, Mendez ASL. Stability-Indicating HPLC Method for Posaconazole Bulk Assay. Sci Pharm. 2012; 80:317-27.

International Conference on Harmonisation (ICH). Commission of the European Communities. Validation of analytical procedures: text and methodology Q2 (R1). Geneva: ICH; 2005.

Keating GM. Posaconazole. Drugs. 2005; 65:1553-67.

Kim H, Kumari P, Laughlin M, Hilbert M, Indelicato S, Lim J, Lin C, Nomeir A. U se of high-performance liquid chromatographic and microbiological analyses for evaluating the presence or absence of active metabolites of the antifungal posaconazole in human plasma. J Chromatogr. A 2003; 987:243-8.

Kim H, Likhari P, Lin C, Nomeir A. High-performance liquid chromatographic analysis of the anti-fungal agent SCH 56592 in dog serum. J Chromatogr. B 2000; 738:93-8.

Liao H, Lin S, Wu U, Kuo C. Rapid and sensitive determination of posaconazole in patient plasma by capillary electrophoresis with field-amplified sample stacking. J Chromatogr. B 2012; 1226:48-54.

Oliveira DM, Markman BEO, Uessugui O, Wu EM, Magnelli RF. Ensaio de dissolução de cápsulas de fluconazol: problemas encontrados na determinação por espectrofotometria na região do UV. Rev Ciênc Farm Básica Apl. 2010; 31(2):211-3.

Reddy TM, Tama CI, Hayes RN. A dried blood spots technique based LC-MS/MS for the analysis of posaconazole in human whole blood samples. J Chromatogr. B 2011; 879:3626-38.

Shen J, Krishna G, Hayes R. A sensitive liquid chromatography and mass spectrometry method for the determination of posaconazole in human plasma. J Pharm Biomed Anal. 2007; 43:228-36.

The United States Pharmacopeia (USP), 37 ed. Rockville: United States Pharmacopeial Convention; 2014.

Thompson GR, Cadena J, Patterson TF. Overview of antifungal agents. Clin Chest Med. 2009; 30:203-15.

Watson DG. Pharmaceutical Analysis. 2nd. ed. Edimburg: Elsevier/Churchill Lingstone, 2005.

Zhong W, Yang X, Tong W, Martin GE. Structural characterization of a novel degradant of the antifungal agent posaconazole. J Pharm Biomed Anal. 2012; 66:40-9.

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