International Journal of Pharmacy
Journal Homepage: http://www.pharmascholars.com

Research Article **CODEN: IJPNL6**

DEVELOPMENT AND VALIDATION OF UV SPECTROSCOPIC METHOD FOR DETERMINATION OF KETOCONAZOLE IN PHARMACEUTICAL FORMULATIONS

Olga Popovska[1*], Zoran Kavrakovski[2], Vesna Rafajlovska[1]

[1]Ss. Cyril and Methodius University in Skopje, Faculty of Technology and Metallurgy, Rudjer Boskovic 16, 1000 Skopje, Republic of Macedonia
[2]Ss. Cyril and Methodius University in Skopje, Faculty of Pharmacy, Vodnjanska 17, 1000 Skopje, Republic of Macedonia

***Corresponding author e-mail:** olga_popovska@yahoo.com

ABSTRACT

A UV spectroscopic method which is simple, accurate and rapid is developed for the determination of ketoconazole in pharmaceutical formulations as active substance in tablets, shampoos and cream. The absorption maxima of ketoconazole solutions in methanol are recorded at 244 nm and 296 nm. The linearity for ketoconazole in methanol with correlation coefficient values higher than 0.999 is found in the range of 0.005–0.025 mg/ml at 244 nm, R^2 = 0.9996 and 0.05–0.25 mg/ml at 296 nm, R^2 = 0.9996. The limit of detection (LOD) and the limit of quantification (LOQ) accounts to 0.000597 mg/ml and 0.00181 mg/ml at 244 nm and 0.00647 mg/ml and 0.01963 mg/ml at 296 nm, respectively. The intra– and inter–day assay is within 2% relative standard deviation. The obtained results for tablets, cream and shampoos are in good agreement with their respective product, label claims. The developed method can be successfully applied for the purpose routine analysis of ketoconazole in pharmaceutical formulations.

Keywords: Antimycotic drug, Formulations, Ketoconazole, UV spectroscopy

INTRODUCTION

Ketoconazole (*cis*-1-acetyl-4-[4-[[2-(2,4-dichloro phenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine) is a lipophilic chiral imidazole antimycotic drug, administered mainly as a racemic (1:1) mixture of enantiomers of *cis* configuration (Figure 1). The pharmaceutical dosage formulations of ketoconazole: cream, shampoo and tablet are applied in the treatment of human systemic fungal infections[1-3].

It is prudent to note that several different analytical methods have been described for the determination of ketoconazole in pharmaceutical formulations, including spectrophotometry[4–9], spectro fluorimetry[10], thin–layer chromatography[11], high–performance liquid chromatography[8,12–17], capillary zone electrophoresis[18], voltammetric and polarographic methods[19]. The quantitative determination of ketoconazole with UV–Vis spectrophotometric methods utilizes the formulation of coloured complexes with the use of reagents such as iron(III) chloride[7], bromothymol blue and picric acid[4], triodide ion in addition to alizarin red S[5], copper(II) and cobalt(II)[6]. As depicted in the literature, spectrophotometric methods for the determination of ketoconazole in pharmaceutical dosage forms are achieved by one of two possible means: by amplification reactions[20] or by stabilizing diprotonated form of ketoconazole in HCL[9]. The analytical methods which have already been published have a tendency to demand sample preparation and/or are time consuming[4,15,20]. In order to overcome these obstacles, a new UV spectroscopic method is developed which in comparison to other methods, utilizes less consumption of solvent and time for analysis. Although there are few spectrophotometric methods for the determination of

ketoconazole in pharmaceutical formulations[4,7], there is unfortunately no validated UV spectroscopic method reported for the direct quantitative determination of ketoconazole in three pharmaceutical formulations (tablets, cream, shampoo). The aim of such research is to develop a simple, accurate and less laborious UV spectroscopic method for the quantitative analysis of ketoconazole in available commercial dosage forms.

MATERIALS AND METHODS

Chemicals: The standard formulation of ketoconazole was received as a gift for the purpose of this research from the Pharmaceutical Company, Replek Farm DOOEL in Skopje, the Republic of Macedonia. Merck (Germany) supplied analytical grade methanol. The commercial ketoconazole formulations produced in the Republic of Macedonia derive from couple of pharmaceutical companies: Alkaloid AD, Republic of Macedonia (shampoo "1") and Replekfarm AD (tablets, shampoo "2" and cream) were purchased from the local market.

Instruments: A double beam UV-Visible spectrophotometer (Varian Cary Scan 50, Switzerland) with 10 mm quartz cuvette is utilized for the purpose of acquiring spectrophotometric measurements. Sample mixtures are homogenized on Vortex, EV–102 *Tehnika Železniki* (Slovenia). In addition, an analytical balance *Mettler Toledo* (Switzerland) is applied for sample weighting with 0.1 mg accuracy.

Standard solutions: The stock solution of the ketoconazole standard with the concentration of 1.0 mg/ml is prepared in methanol. The working solutions with the concentration of 0.005–0.25 mg/ml are in fact prepared daily by serial dilutions from stock solution with methanol.

Preparation of sample solutions

Tablets: Ten tablets finely crushed to powder are used for analysis of ketoconazole. The tablet samples of 0.04 g weighted to 0.0001 g accuracy are extracted with 8 ml methanol by vortexing at 25 °C for duration of half an hour (30 minutes). The obtained filtrate through a fine porosity fritted glass Büchner funnel is transferred to a 25 ml volumetric flask and diluted to the calibration mark with methanol.

Shampoo: Accurately weighted shampoo samples (0.03 g ± 0.0001 g) are transferred with 1 ml methanol in volumetric flask (5 ml). Following the homogenization process by vortexing for an interval of half an hour (30 minutes), at 25 ºC, the samples are diluted to 5 ml with methanol.

Cream: The samples of 0.1 g ketoconazole cream, weighted to 0.0001 g accuracy, are extracted with methanol by vortexing at 25 °C, for the duration of half an hour (30 minutes) time of extraction and a solid/liquid ratio of 1:30 *w/v*. The extracted samples are kept at -18 ^{o}C, for the extent of 10 minutes and filtrated through a fine porosity fritted glass Büchner funnel. The filtrate is diluted with methanol in 10 ml volumetric flask.

Method validation: The method is validated taking into consideration linearity and range, precision and accuracy, reproducibility, limit of detection (LOD), limit of quantification (LOQ) and robustness according to the recommendations prescribed by International Conference on Harmonization (ICH) guidelines[21,22].

Linearity range: The linearity of the method is determined by preparing three individual series of ketoconazole solutions in methanol in the range of 0.005 mg/ml–0.025 mg/ml at 244 nm and 0.05 mg/ml–0.25 mg/ml at 296 nm. The least square regression analysis and the ANOVA test used for statistical data processing were applied.

The absorption maxima of ketoconazole solutions in methanol, 0.015 mg/ml and 0.15 mg/ml are recorded at 244 nm and at 296 nm, respectively. As a consequence of the obtained results, the molar absorption coefficients (ε) of the drug are calculated[14,22]. The UV spectrum of ketoconazole solution in methanol (0.02 mg/mL) is shown in Figure 2.

Precision: The intra–day and inter–day precision and accuracy of the method is calculated by analyzing the repeatability of the values of three independent sets of working standard solutions of ketoconazole in methanol (0.005 mg/ml–0.25 mg/ml) in the period of one solar day (24 hours) as well as in the interval of three consecutive days.

Accuracy: Accuracy is tested by the standard addition method at three different levels 80, 100 and 120%. The percentage recoveries are analyzed of the obtained amount of ketoconazole in pharmaceutical formulations.

Limit of detection (LOD) and limit of quantification (LOQ): The calculation of LOD and LOQ is based on the standard deviation of response and the slope of calibration curve determined as average value. It is performed by taking 3 individual series of ketoconazole in methanol, where the absorption

maxima at 244 nm and 296 nm are recorded during the span of three consecutive days. This is done by utilizing the following equations of LOD = $3.3\sigma/S$ and LOQ = $10\sigma/S$, where S is slope of the calibration curve and σ is the standard deviation of response.

Robustness: Robustness of the proposed method is determined by the analysis of sample and standard ketoconazole solution in methanol at different wavelengths (± 3 nm).

RESULTS AND DISCUSSION

Selection of the solvent: The criterion for selection methanol as solvent is the high solubility of ketoconazole in methanol and the fact that methanol does not have absorption in the region of the absorption of ketoconazole.

Linearity range: The linearity range is in the interval of 0.005 mg/ml–0.25 mg/ml at 244 nm and 0.05 mg/ml–0.25 mg/ml at 296 nm (Table 1). The overlay spectra of ketoconazole in methanol at 244 nm and 296 nm are shown in Figure 3. The optical characteristics, as well as the validation data for ketoconazole are calculated (Table 2). The correlation coefficient is 0.9996 for both wavelengths, indicating excellent linearity (R^2 > 0.999). The least square regression analysis is utilized to calculate linear equations at 244 nm and 296 nm. The significant linear regression is validated by ANOVA test within the F value. It is evident that F calculated value is greater than F critical at 244 nm and 296 nm.

Precision: The repeatability results indicate the precision over a short interval of time, as well as during inter–day assessment. The intra– and inter–day relative standard deviation (RSD) values obtained by the proposed method are within 2% relative standard deviation (Table 3).

Accuracy: The UV spectra of solutions of ketoconazole pharmaceutical formulations are shown in Figure 4. The recovered quantities of ketoconazole in: tablets, two different types of shampoos and cream are presented in Table 4 and 5. The results of the recovery studies undoubtedly demonstrate accuracy of the proposed method.

Limit of detection (LOD) and limit of quantification (LOQ): The determined values of LOD and LOQ of ketoconazole are 0.000597 mg/ml and 0.001810 mg/ml, respectively at 244 nm and 0.00647 mg/ml and 0.01963 mg/ml, respectively at 296 nm.

Robustness: Robustness of the proposed method demonstrated a non-significant alteration of the absorption level through analysis of the sample and standard ketoconazole solution in methanol at different wavelengths (± 3 nm) (Table 6).

CONCLUSION

The analytical method is simple and rapid, as well as sensitive as result of the low values for LOD and LOQ at 244 nm and 296 nm. The statistical data of the developed method are reproducible. In addition, the reliability, validity and usability of the method is sound and could be replicated with ease for future research practices and endeavors.

ACKNOWLEDGEMENT

Replek Farm DOOL in Skopje, R. Macedonia is acknowledged for the gift of standard Ketoconazole.

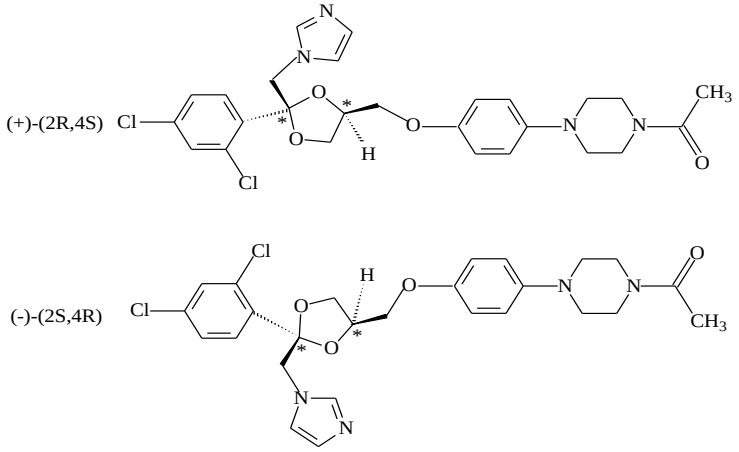


Figure 1: Chemical structure of ketoconazole

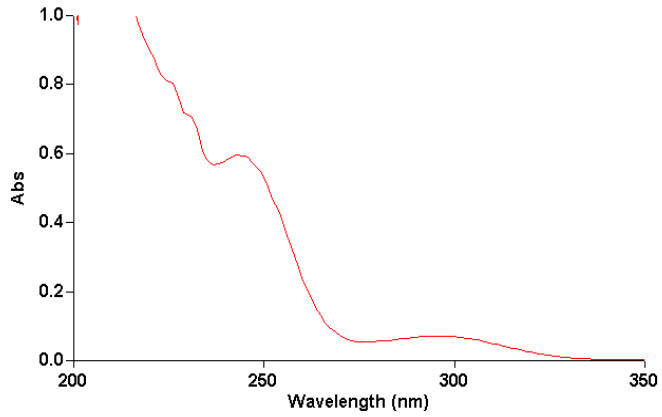


Figure 2: UV spectrum of ketoconazole solution in methanol (0.02 mg/ml)

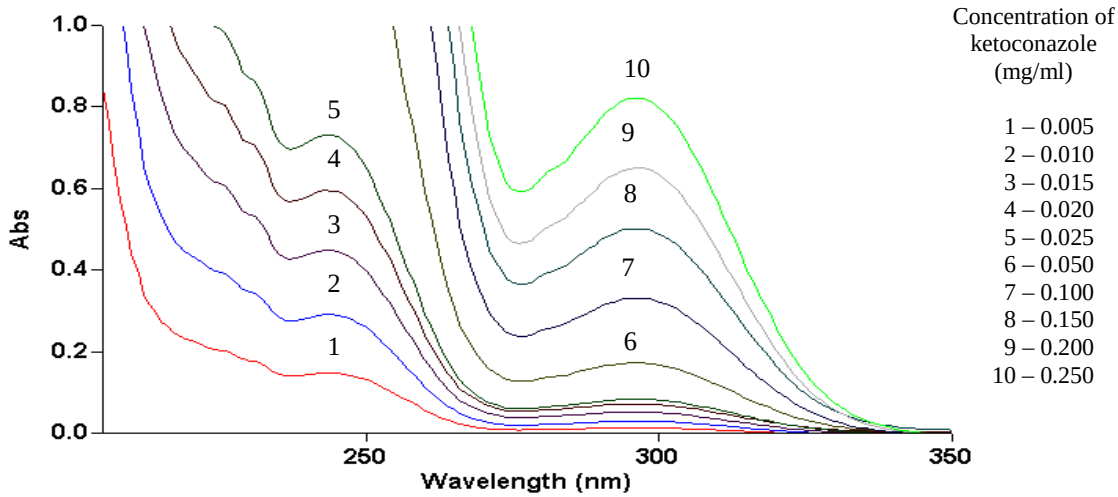
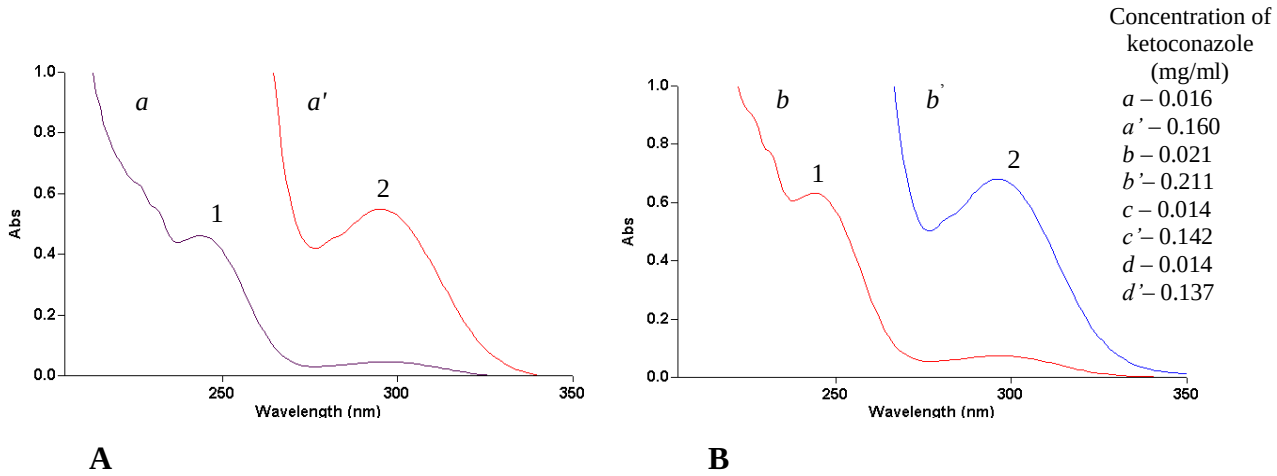


Figure 3: Overlay spectra of ketoconazole solutions in methanol within the linearity range at 244 nm (0.005–0.025 mg/ml) and at 296 nm (0.050–0.250 mg/ml)



A **B**

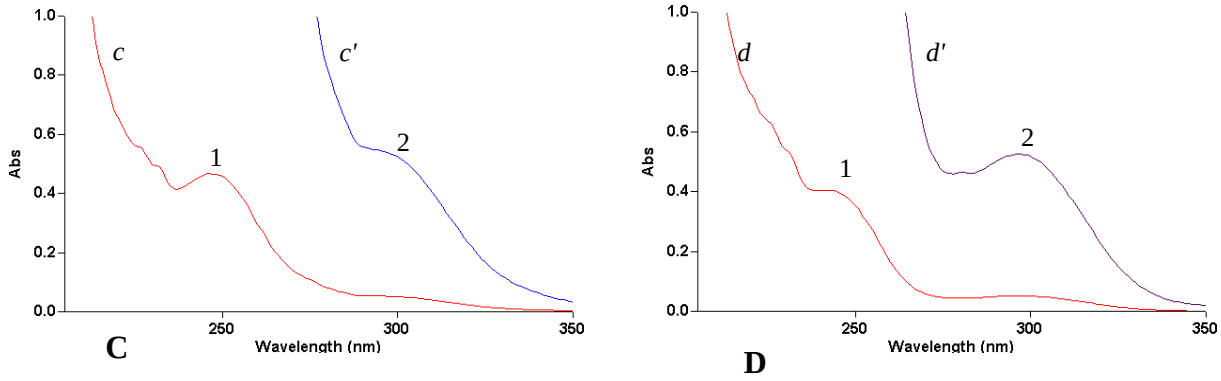


Figure 4: UV spectra solutions of tablets (A), cream (B), shampoo "1" (C) and shampoo "2" (D) in methanol within the linearity range at 244 nm (1) and 296 nm (2)

Table 1: Linearity of ketoconazole in methanol

Wavelength (nm)					
244			296		
Concentration (mg/ml)	Absorbance[1] ± SD	RSD (%)	Concentration (mg/ml)	Absorbance[1] ± SD	RSD (%)
0.005	0.1510 ± 0.0006	0.38	0.05	0.1720 ± 0.0018	1.07
0.010	0.3002 ± 0.0049	1.63	0.10	0.3419 ± 0.0049	1.43
0.015	0.4444 ± 0.0019	0.42	0.15	0.5064 ± 0.0064	1.27
0.020	0.5983 ± 0.0049	0.82	0.20	0.6656 ± 0.0018	0.28
0.250	0.7585 ± 0.0019	0.24	0.25	0.8483 ± 0.0037	0.44

[1] *Mean ± standard deviation (n = 3). SD – standard deviation. RSD – relative standard deviation.*

Table 2: Optical characteristics, statistical data of the regression analysis and validation of the method[1]

Optical characteristic		
Wavelength (nm)	244	296
Molar absorption coefficient (L mol^{-1} cm^{-1})	15746.32	1794.15
	Method wavelength (nm)	
	244	296
Regression analysis		
Slope (SE)[a]	30.2643 (0.3448)	3.3526 (0.0407)
95% confidence limit of slope	29.1668; 31.3616	3.2229; 3.4823
Intercept (SE)[a]	- 0.0035 (0.00571)	0.0039 (0.0068)
95% confidence limit of intercept	- 0.02167; 0.0147	- 0.0176; 0.0254
Correlation coefficient (R^2)	0.9996	0.9996
Standard error of estimate	5.55 x 10^{-3}	6.24 x 10^{-3}
Calculated *F*-value (critical *F*-value)[b]	16.5403 (5.3176)	8.3189 (5.3176)
Validation parameters		
Linearity (mg/ml)	0.005–0.025	0.05–0.25
Limit of detection–LOD (mg/ml)	0.000597	0.00647
Limit of quantification–LOQ (mg/ml)	0.001810	0.01963

[1]*Mean value (n = 3).* [a] *Standard error of mean.*
[b]*Theoretical value of F based on one-way ANOVA test at p = 0.05 level of significance.*

Table 3: Results of precision

Concentration (mg/ml)	Intra-day (n = 3)		Inter-day (n = 3 x 3)	
	Experimental concentration[1] ± SD (mg/ml)	RSD (%)	Experimental concentration[2] ± SD (mg/ml)	RSD (%)
0.005	0.00509 ± 0.00058	0.38	0.00513 ± 0.00278	1.83
0.010	0.01002 ± 0.00489	1.63	0.01009 ± 0.00481	1.59
0.015	0.01479 ± 0.00185	0.42	0.01507 ± 0.00863	1.91
0.020	0.01987 ± 0.00490	0.82	0.01995 ± 0.01052	1.75
0.025	0.02517 ± 0.00185	0.24	0.02511 ± 0.01566	2.07
0.050	0.05042 ± 0.00185	1.07	0.05074 ± 0.00358	2.07
0.100	0.10110 ± 0.00490	1.43	0.10131 ± 0.00338	0.98
0.150	0.15018 ± 0.00641	1.27	0.15124 ± 0.00669	1.31
0.200	0.19700 ± 0.00185	0.28	0.19700 ± 0.00940	1.42
0.250	0.25217 ± 0.00370	0.44	0.25280 ± 0.00456	0.54

[1]Mean ± standard deviation (n = 3). [2]mean ± standard deviation (n = 9).
SD – standard deviation. RSD – relative standard deviation.

Table 4: Recovery data

Commercial formulation	Drug declared in formulation	Method wavelength (nm)			
		244		296	
		Drug recovery[1] (%)	RSD (%)	Drug recovery[1] (%)	RSD (%)
Tablets	200 mg/tablet	99.56	1.25	98.06	0.64
Cream	20 mg ketoconazole/1g cream	99.36	0.48	95.03	1.04
Shampoo "1"	20 mg ketoconazole/1ml shampoo	111.00	1.68	112.78	0.62
Shampoo "2"	20 mg ketoconazole/1g shampoo	104.70	0.96	113.93	0.97

[1]Mean value (n = 3). RSD – relative standard deviation.

Table 5: Recovery data for ketoconazole in commercial formulation

Sample	Wavelength (nm)	Level of added ketoconazole (%)	Recovery[1] (%)	RSD (%)
Tablets	244	80	102.15	0.41
		100	103.91	0.10
		120	102.63	0.73
	296	80	99.50	1.25
		100	95.41	0.63
		120	98.80	1.32
Cream	244	80	96.18	0.69
		100	92.90	0.23
		120	92.81	0.75
	296	80	92.53	1.06
		100	92.73	0.41
		120	95.99	0.71
Shampoo "1"	244	80	110.94	1.08
		100	110.91	0.85
		120	112.52	0.19
	296	80	114.11	1.09
		100	111.21	0.24
		120	109.14	1.17
Shampoo "2"	244	80	104.41	1.56
		100	103.60	0.36
		120	103.60	0.59
	296	80	111.38	2.01
		100	111.31	1.57
		120	109.03	1.77

[1]Mean value (n = 3). RSD – relative standard deviation.

Table 6: Results of the robustness test

Parameters		Low level (–)		Zero level (0)		High level (+)			
A – 244 nm		241		244		247			
B – 296 nm		293		296		299			
	Working standard solutions	Tablets		Cream		Shampoo „1“		Shampoo „2“	
	Concentration of ketoconazole (mg/ml)								
A	0.01	0.016		0.0215		0.0109		0.0112	
B	0.10	0.160		0.2150		0.1050		0.0842	
	Absorbance – Abs; Recovery – Rec								
		Abs	Abs	Rec (%)	Abs	Rec (%)	Abs	Rec (%)	Abs	Rec (%)
A	–	0.2922	0.4380	91.09	0.6576	103.67	0.3358	93.95	0.3352	99.79
	0	0.3017	0.4386	91.21	0.6779	104.85	0.3522	98.50	0.3330	99.14
	+	0.2783	0.4377	90.02	0.6281	101.62	0.3572	99.89	0.3194	95.13
B	–	0.3031	0.4906	90.92	0.6517	90.18	0.4272	110.04	0.3098	108.70
	0	0.3370	0.4912	91.03	0.6591	91.21	0.4217	108.61	0.3152	110.62
	+	0.3081	0.4976	92.22	0.6557	90.74	0.4118	106.06	0.3140	110.19

REFERENCES

1. Kaur IP, Kakkar S. Expert Opin Drug Deliv, 2010; 7(11): 1303-1327.
2. Perez BSH. Med Hypotheses, 2004; 62(1): 112-115.
3. Wang L, Tang X. Int J Pharm, 2008; 350(1-2): 181-187.
4. Alizadeh N, Rezakhani Z. J Chil Chem Soc, 2012; 57(2): 1104-1108.
5. Farhadi K, Maleki R. J Pharm Biomed Anal, 2002; 30(4): 1023-1033.
6. El-Ragehy NA, El-Saharty YS. J AOAC Int, 2001; 84(2): 563-568.
7. Abou-Attia FM, Issa YM, Abdel-Gawad FM, Abdel-Hamid SM. Farmaco, 2003; 58(8): 573-579.
8. Kedor-Hackmann ERM, Santoro MIRM, Singh AK, Peraro AC. Braz J Pharm Sci, 2006; 42(1): 91-98.
9. Vojić MP, Popović GV, Sladić DM, Pfendt LB. J Serb Chem Soc, 2005; 70(1): 67-78.
10. Khashaba PY, El-Shabouri SR, Emara KM, Mohamed AM. J Pharm Biomed Anal, 2000; 22(2): 363-376.
11. Saysin S, Liawruangrath B, Liawruangrath S. J Cosmet Sci, 2010; 61(5): 367-376.
12. De Bruijn P, Kehrer DFS, Verweij J, Sparreboom A. J Chromatogr B, 2001; 753(2): 395-400.
13. Abdel-Moety EM, Khattab FI, Kelani KM, AbouAl-Alamein AM. II Farmaco, 2003; 57(11): 931-938.
14. Dayyih WA, Al saadi N, Hamad M, Mallah E, Matalka K, Arafat T. Int J Pharm Sci Res, 2012; 3(10): 3686-3692.
15. Chou WL, Chang CY, Liu HM, Yang KC, Wu CC. J Food Drug Anal, 2007; 15(1): 25-32.
16. Jat RK, Sharma S, Chhipa RC, Singh R, Alam I. Pharmacophore, 2012; 3(2): 123-129.
17. Staub I, Bergold AM. Acta Farm Bonaerense, 2004; 23(3): 387-390.
18. Velikinac I, Cudina O, Jankovič I, Agbaba D, Vladimirov S. Farmaco, 2004; 59(5): 419-424.
19. Arranz P, Arranz A, Moreda JM, Cid A, Arranz JF. J Pharm Biomed Anal, 2003; 33(4): 589-596.
20. Rane SS, Padmaja P. J Pharm Anal, 2012; 2(1): 43-47.
21. Chan CC. Potency method validation. In: Chan CC, Lam H, Lee YC and Zhang XM (eds.). Analytical Method Validation and Instrument Performance Verification, New Jersey; John Wiley and Sons: 2004, pp. 11-26.
22. Aher KB, Bhavar GB, Joshi HP. J Curr Pharm Res, 2012; 9(1): 49-54.