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Short communication

Separation and quantitation of *Z*-isomer in lanoconazole by normal phase HPLC

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ABSTRACT

An isocratic normal phase high-performance liquid chromatographic (NP-HPLC) method has been developed and validated for the quantitation of *Z*-isomer in lanoconazole. Separation was achieved with a Thermo Hypersil Silica column. The ratio of 2-propanol, n-hexane and triethylamine in the mobile phase were optimized to obtain the best separation. UV detection was performed at 296 nm. The described method is linear over a range of LOQ – 15.0 μg/ml of *Z*-isomer. The mean recovery of *Z*-isomer was found to be in the range of 97–99%. The method is simple, rapid, selective, accurate and precise, useful in the quality control of bulk manufacturing.

1. Introduction

Lanoconazole (NND-318), (±)-(*E*)-2-[4-(2-chlorophenyl)-1,3-dithiolan-2-ylidene]-1-imidazol-1-ylacetonitrile (Fig. 1), invented by Nihon Nohyaku Co. Ltd. and Tsumura Juntendo (Japan) is a potent new antifungal imidazole that is active against a wide range of pathogenic fungi, including dermatophytes [1–3]. It is available mostly in the Japanese market as a cream formulation of 1% lanoconazole (ASTAT) and an ointment of 1% lanoconazole with 10% urea (PASTARON) for the treatment of superficial skin fungal infections. Of the two enantiomers of the racemic drug the (*R*)-enantiomer is more active [2,3].

An obvious impurity of lanoconazole (*E*-isomer) can be the *Z*-isomer. No analytical method for the determination of impurities is reported in literature. The aim of this study is to develop and validate as per the ICH guideline [4] a liquid chromatographic (HPLC) procedure for this purpose with special respect to the *Z*-isomer in lanoconazole.

2. Experimental

2.1. Materials

Samples of lanoconazole and its *Z*-isomer were synthesized at SMS Pharma Research Centre (Hyderabad, India). HPLC grade 2-propanol and n-hexane were obtained from Merck (India). Analytical grade triethylamine was purchased from SD Fine Chemicals (India). LC grade water was deionized with Milli-Q Elix and then filtered using Milli-Q gradient, Millipore water purification system (Milford, MA, USA).

2.2. Instrument

The HPLC system consisted of quaternary gradient pump, auto sampler, column oven and a variable wavelength detector. The output signal was monitored and integrated using EZ-Chrom Elite Chromatography Data Software (1200 series HPLC, Agilent, USA).

2.3. Chromatographic conditions

A Thermo Hypersil Silica analytical column (250 mm × 4.6 mm, 5 μm packing) was used. A mixture of 2-propanol, n-hexane and triethylamine in the ratio of 50:50:0.05 (v/v/v) was used as mobile phase. It was filtered through a 0.45 μm-nylon membrane using a Millipore vacuum filtration system. The mobile phase was pumped through the column at a flow rate of 1.0 ml/min. The sample injection volume was 20 μl. The detector was set to 296 nm.

2.4. System suitability

Accurately weighed quantities of lanoconazole and *Z*-isomer working standards are dissolved in mobile phase to obtain a solution having known concentration of about 0.01 mg/ml and injected into the system. The resolution between lanoconazole and *Z*-isomer is not less than 1.5. The system suitability chromatogram is shown in Fig. 2.

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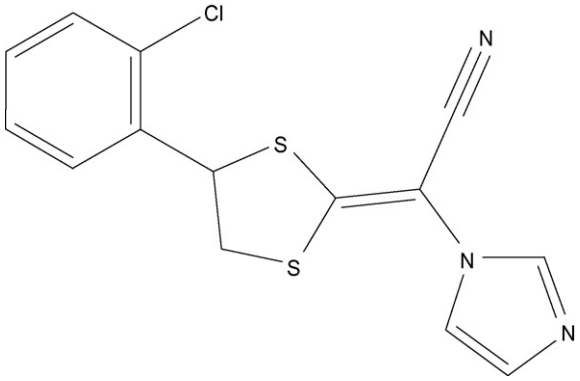

Fig. 1. Structure of Lanoconazole.

2.5. Solutions

An accurately weighed quantity of *Z*-isomer working standard was dissolved in mobile phase to obtain the standard solution having known concentration of about 0.01 mg/ml and injected into the system. The freshly prepared lanoconazole sample solution (1.0 mg/ml) in mobile phase was injected into the system.

3. Results and discussion

3.1. Method development

3.1.1. Separation of isomers

In order to develop a suitable and robust LC method for the quantitation of *Z*-isomer, different mobile phase compositions were employed to achieve the best separation. During the development of the method, reversed phase stationary phase (C-18) with water and acetonitrile in the ratio of 50:50 (v/v) as a mobile phase was studied and found not selective enough compared to normal phase separation. Finally, the mobile phase consisting of 2-propanol, n-hexane and triethylamine in the ratio of 50:50:0.05 (v/v/v) at a flow rate of 1.0 ml/min, Hypersil Silica, 250 mm × 4.6 mm 5 μm column was found to be appropriate, allowing good separation (resolution greater than 2.5) of lanoconazole and the *Z*-isomer with symmetrical peaks. The chromatograms showing the separation of *Z*-isomer in real sample and the real sample spiked with 1.0%, m/m of *Z*-isomer are shown in Fig. 3.

3.1.2. Quantification of Z-isomer

Known concentration of standard solution (0.01 mg/ml) was used for the quantification of *Z*-isomer in lanoconazole sample (1.0 mg/ml). Not more than 0.50% m/m of *Z*-isomer was found in lanoconazole API.

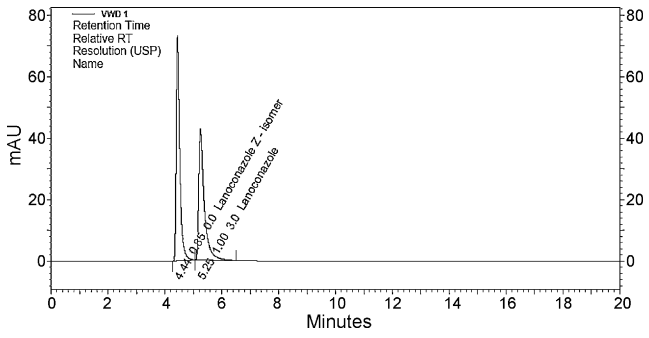

Fig. 2. Typical system suitability chromatogram.

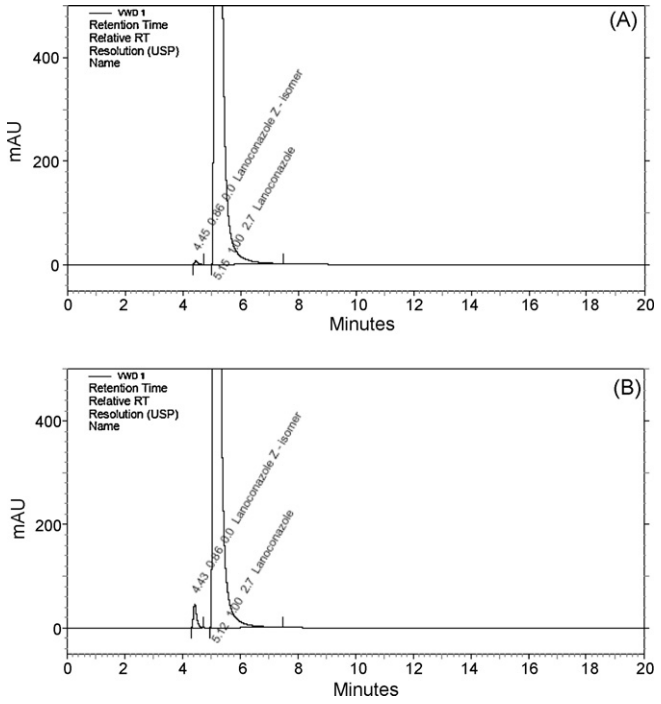

Fig. 3. Typical chromatogram for Lanoconazole (1.0 mg/ml) in real sample (A) and real sample spiked with *Z*-isomer (1.0%, m/m) (B).

3.2. Method validation

The LC method developed has been validated for the quantitation of *Z*-isomer in lanoconazole using the following parameters.

3.2.1. Specificity

Lanoconazole and *Z*-isomer were injected separately to confirm the retention times. System suitability solution was then injected. *Z*-isomer and lanoconazole peaks were eluted at 4.44 and 5.25 min, respectively (relative retention 0.85). The resolution between the peaks was found to be 3.0. The asymmetry for *Z*-isomer and lanoconazole peaks was 1.7 and 2.2, respectively.

3.2.2. Linearity

Standard solutions at ten different concentration levels ranging from 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 10.0, 12.5 and 15.0 μg/ml were prepared (0.05–1.5% of analyte concentration of 1.0 mg/ml.). Each sample solution was injected in triplicate. The mean responses recorded were plotted against concentration. The correlation coefficient for *Z*-isomer was found to be 0.999, which indicated good linearity. The calibration equation for *Z*-isomer was found to be $y = 624600x - 6063$.

3.2.3. Accuracy

Lanoconazole sample was spiked with *Z*-isomer at 0.25, 0.50 and 1.0% of analyte concentration of 1.0 mg/ml. Each spiked solution was prepared in triplicate and injected. The mean recoveries, recovery percentage and %RSD were calculated. The mean recoveries of *Z*-isomer at each spike solution with 95% confidence level were found to be 98.9 ± 0.91%, 97.8 ± 1.13% and 97.9 ± 1.53%, respectively. Accuracy results are shown in Table 1. The acceptance criteria for recovery at each level were between 80 and 120% as per in-house validation protocol.

3.2.4. Precision

Repeatability was demonstrated by analyzing lanoconazole sample 6 times. Intermediate precision was demonstrated by analyzing same sample of lanoconazole by two different analysts on

Table 1
Accuracy results for *Z*-isomer.

Z-isomer spike level (%, *m*/*m*)	Added (μg) (*n* = 3)	Recovered (μg)	% Recovery	Mean Recovery	%RSD
0.25	117.81	116.61	99.0	98.9	0.8
		115.58	98.1		
		117.48	99.7		
0.50	235.63	227.85	96.7	97.8	1.0
		232.23	98.6		
		231.49	98.2		
0.75	353.44	350.64	99.2	97.9	1.4
		345.98	97.9		
		341.17	96.5		

Table 2
Precision results for *Z*-isomer.

Repeatability	
Mean of *Z*-isomer content (%, *m*/*m*) (*n* = 6)	0.09
Standard deviation (SD)	0.0055
%RSD	6.1
Intermediate Precision	
Analyst-1/Day-1	
Mean of *Z*-isomer content (%, *m*/*m*) (*n* = 6)	0.09
Standard deviation (SD)	0.0055
%RSD	6.1
Analyst-2	
Mean of *Z*-isomer content (%, *m*/*m*) (*n* = 6)	0.10
Standard deviation (SD)	0.0050
%RSD	5.0
Overall %RSD (*n* = 12)	8.8
Day-2	
Mean of *Z*-isomer content (%, *m*/*m*) (*n* = 6)	0.09
Standard deviation (SD)	0.0041
%RSD	4.6
Overall %RSD (*n* = 12)	5.4

Table 3
Robustness results for *Z*-isomer.

	Effect of change in flow rate				
Flow rate (ml/min)	Compound	RT	RR	Resolution	Asymmetry
0.9	*Z*-isomer	4.95	0.84	–	1.8
	Lanoconazole	5.89	1.00	3.0	2.2
1.0	*Z*-isomer	4.45	0.84	–	1.7
	Lanoconazole	5.31	1.00	2.9	2.2
1.1	*Z*-isomer	4.05	0.84	–	1.7
	Lanoconazole	4.81	1.00	2.8	2.2
	Effect of change in mobile phase composition				
Composition (v/v/v)	Compound	RT	RR	Resolution	Asymmetry
52: 48: 0.05	*Z*-isomer	4.38	0.85	–	1.7
	Lanoconazole	5.17	1.00	2.9	2.1
50: 50: 0.05	*Z*-isomer	4.45	0.84	–	1.7
	Lanoconazole	5.31	1.00	2.9	2.2
48: 52: 0.05	*Z*-isomer	4.48	0.84	–	1.6
	Lanoconazole	5.32	1.00	3.1	2.1

RT: Retention time, RR: Relative retention with respect to lanoconazole.

two different days. Intra-day variations of *Z*-isomer content in lanoconazole were expressed in terms of %RSD values. The values calculated were found to be 6.1 for repeatability, 5.0 and 4.6% for intermediate precision. Repeatability and intermediate precision results are shown in Table 2.

3.2.5. Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection and limit of quantification for *Z*-isomer was calculated from the linearity data using residual standard deviation of the response and slope of the calibration curve. A typical S/N ratio of 2–3 and 9–10 are generally considered to be acceptable for LOD and LOQ, respectively. LOD and LOQ values were found to be 0.005 and 0.017 μg/ml respectively.

3.2.6. Robustness

In order to demonstrate the robustness of the method, system suitability parameters were verified by making deliberate changes in the chromatographic conditions, viz. change in flow rate by ±0.1 ml/min and change in the ratio of mobile phase (±2% absolute). The method was found to be robust over an acceptable working range of its HPLC operational parameters. The results are shown in Table 3.

3.2.7. Batch analysis

The *Z*-isomer content in three different batch samples of lanoconazole was determined. The results obtained are presented in Table 4. Other related substances were evaluated by a reversed phase HPLC method, any single impurity was less than 0.10% and total impurities were less than 0.50% (excluding *Z*-isomer content by normal phase HPLC).

4. Conclusion

The present paper describes the development of a new HPLC method for the quantitation of *Z*-isomer in lanoconazole and its validation. The method was found to be selective, sensitive, precise and accurate for the quantitation of *Z*-isomer. This method can be used for the routine analysis as well as for stability studies to evaluate $Z \rightarrow E$, $E \rightarrow Z$ isomerisation in pharmaceutical quality control.

References

[1] H. Tanuma, M. Tanuma, M. Abe, H. Kume, Mycoses 44 (2001) 181–190.
[2] Y. Niwano, T. Ohmi, A. Seo, H. Kodama, H. Koga, A. Sakai, Curr. Med. Chem.: Anti-Infective Agents 2 (2003) 147–160.
[3] Y. Niwano, T. Ohmi, A. Seo, H. Kodama, K. Kanai, Recent Res. Develop. Antimicrob. Ag. Chemother. 4 (2000) 81–102.
[4] ICH Q2(R1), Guidelines on Validation of Analytical Procedures: Text and methodology, November 2005.