High-Performance Liquid Chromatographic Method for the Analysis of Fluconazole in Pharmaceutical Preparations

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ABSTRACT

High-performance liquid chromatographic method was developed for the analysis of fluconazole in pharmaceutical preparations. The analyzed drug was separated on a reversed-phase column [Hypersil ODS column (120 x 46 mm, 5 μm particle size)], using a mobile phase containing methanol : 10 mM pH 7.0 phosphate buffer (50:50) with UV detection at 260 nm. The proposed method is specific, sensitive, reproducible and reliable. It can be successfully applied as a stability-indicating method for the determination of fluconazole in pharmaceutical preparations. With a run time of less than 2 minutes, the method is rapid and easy to use for routine analysis of fluconazole in pure form and dosage form as well as for its dissolution testing.

Key words: Fluconazole, HPLC, Pharmaceutical preparations, Dissolution

INTRODUCTION

Fluconazole[α-(2-4-difluorophenyl]-α-[1H-1,2,4-triazole-1-ylmethyl-1H-1,2,4-triazole-1-ethanol] is a synthetic triazole antifungal drug (Fig.1) (Budavaris, 2001). It is available as tablets, capsules, oral suspension and injection for treatment of oral, esophageal and vaginal candidiasis (Kalant and Walter, 1998 ; Bennett, 2001).

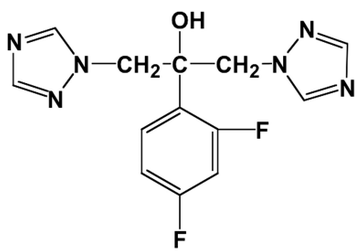

Figure 1. Chemical structure of flueonazole.

Literature reviews showed that various methods had been used to determine the fluconazole content in formulations. Those methods were high-performance liquid chromatography (Guo and Wen, 2000; Abdel-Moety et al., 2002), gas liquid chromatography (Harris et al., 1989) and spectrophotometry (El-Bayoumi et al., 1997; Kelani and Bebawy,1997). At

present, no official analytical method exists for fluconazole determination in pharmaceutical preparations since this drug has not yet been made official in Pharmacopeias although it has already been marketed and prescribed.

The purpose of this study was to develop a rapid, specific, precise and accurate method for the determination of fluconazole in pharmaceutical preparations. The proposed method was high-performance liquid chromatography (HPLC) which was validated according to the International Conference on Harmonisation guideline for validation of analytical procedures (ICH Q2B,1996). In addition, the stability-indicating capabilities of the method were also demonstrated by an accelerated degradation study.

MATERIALS AND METHODS

Materials

Fluconazole reference standard was obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. Methanol and acetonitrile were of HPLC grade (Merck, Germany). Ten mM phosphate buffer was prepared from 0.02 M monobasic potassium phosphate solution and adjusted to pH 7.0 with 0.2 M sodium hydroxide solution. Ten mM acetate buffer was prepared from sodium acetate solution adjusted to pH 5.0 with glacial acetic acid. Chemicals used for preparing buffer solutions were all of analytical grade. Samples of fluconazole capsules (Diflucan®, Biozole®, Flucozole®, Funa®, Flunco200®, Stalene200®) labeled to contain 200 mg fluconazole per capsule and intravenous injection labeled to contain 2 mg fluconazole per ml (Diflucan®) were purchased from drugstores.

Apparatus

High-performance liquid chromatograph (HP1100 Modular LC system, Agilent Technologies) consisted of a solvent delivery system (G1311A quarternary pump), a UV detector (G1314A variable wavelength UV detector), an auto-injector (G1313A Autosampler) and a data analysis system (HP Chemstation, Hewlett Peckard, USA). Dissolution Tester : USP Apparatus 1 (SR2 Dissolution Station and Validata, Hanson Research Corp.), UV Spectrophotometer (Model7800, Jasco , Japan Spectroscopic Co. Ltd.)

Chromatographic conditions

Reversed-phase HPLC with isocratic elution was performed at ambient temperature on a Hypersil ODS column (120 x 46 mm, 5 μm particle size). The mobile phases were (1) methanol : 10 mM pH 7.0 phosphate buffer 50:50, (2) methanol : 10 mM pH 5.0 acetate buffer 30:70, (3) methanol : 10 mM pH 5.0 acetate buffer : acetonitrile 20:70:10 and (4) acetonitrile : water 26:74. All were filtered through 0.45 μm membrane filter and degassed prior to being operated at a flow rate of 1 ml per minute. The standard and sample solutions were all filtered through 0.45 μm membrane filter and 20 μl of each was injected in replicates and detected at 260 nm.

HPLC system screening

Fluconazole standard solutions were prepared at the concentration of 200 μg per ml in each of the mobile phase previously mentioned in the chromatographic conditions. A 20 μl of the filtered solution was injected in replicates to obtain the chromatogram of fluconazole

from the four HPLC solvent systems. The best mobile phase was selected for further study.

Validation

1. Specificity

To evaluate the specificity of the method, interferences from sample matrix, degradation products and capsule shells were studied. Common excipients used in pharmaceutical formulations, i.e., lactose, starch, talcum, microcrystalline cellulose, magnesium stearate, sodium lauryl sulfate, sodium chloride and dextrose were dissolved in the mobile phase at a suitable concentration and filtered. Forced degradation of fluconazole solutions was carried out in acidic condition (1N HCl, 100°C 30 minutes and 80°C 72 hours), alkaline condition (1N NaOH, 100°C 30 minutes and 80°C 72 hours) and oxidation condition (3% hydrogen peroxide, 80°C 72 hours). The acidic and alkaline solutions were then neutralized and diluted with the mobile phase before being analyzed by the same chromatographic conditions. Observations were made if any interfering peaks from sample matrix and degradation products were present. Capsule shells of five brands of fluconazole capsules were taken and dissolved in three solvents, commonly used as dissolution medium, i.e., 0.1N HCl, pH 6.8 phosphate buffer and pH 4.5 phosphate buffer, and detected for interfering peaks.

2. Linearity and range

Standard solutions of fluconazole in the concentration ranging from 20 to 500 μg per ml were accurately prepared by diluting 1 mg per ml fluconazole stock solution with the mobile phase. A 20 μl of each solution was injected and the detector response of fluconazole peak (peak area or peak height) was plotted against its concentration to construct the calibration curve. Solvents for dissolution medium were also used to prepare standard solutions of fluconazole in the same concentration range and calibration curves were constructed in the same manner.

3. Accuracy and precision

Synthetic mixtures of fluconazole capsules and injection at three concentration levels (50%, 100% and 150% of the active ingredient) were prepared. Each was determined in three replicates. The percent recovery of fluconazole and the relative standard deviation (RSD) of the nine determinations were calculated.

Assay of fluconazole capsules and injection

For fluconazole capsules: accurately weighed 20 fluconazole capsules and homogeneously mixed the total capsule contents. A portion of the capsule contents equivalent to about 50 mg of fluconazole was accurately weighed and transferred to a 50-ml volumetric flask. Thirty milliliters of the mobile phase was added and the mixture was sonicated for 20 minutes and adjusted to volume with the same solvent. The sample solution was then filtered and diluted with the mobile phase to obtain the concentration of the assay preparation about 200 μg per ml.

For fluconazole injection : diluted a suitable volume of the injection with the mobile phase to obtain the final concentration of the assay preparation about 200 μg per ml. The injection volume of all assay preparations was 20 μl and the amounts of fluconazole in the sample capsules and injection were determined by comparing the peak area of the assay

preparations with the standard preparations at the same concentration.

The same batch of fluconazole capsules and fluconazole injection were assayed by direct spectrophotometric method and the assay results from both methods were compared.

Dissolution testing of fluconazole capsules

The dissolution testing of fluconazole capsules was carried out according to the US FDA method for immediate-release solid dosage forms (US FDA, 1997). The dissolution samples were taken at 45 minutes, filtered and measured for fluconazole dissolved by the same chromatographic conditions. The results were compared to those measured by direct spectrophotometric method of the same dissolution samples.

RESULTS AND DISCUSSION

HPLC system screening

The separation of fluconazole was performed on four HPLC solvent systems. The other chromatographic conditions, i.e., flow rate, column, temperature and detector used were the same. The approximate retention times and peak heights of fluconazole in 4 different mobile phases are shown in Fig. 2-5. Methanol : 10 mM pH 7.0 phosphate buffer 50:50 was selected for method validation and applications in pharmaceutical quality control since it gave the most rapid separation (retention time 1.8 min.) and highest sensitivity (peak height 110 mAU).

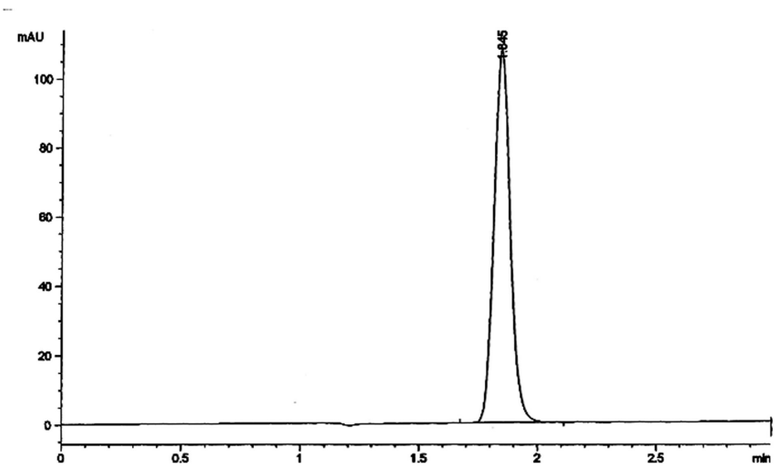

Figure 2. Chromatogram of fluconazole (200 μg/ml) eluted with methanol : 10 mM pH 7.0 phosphate buffer 50:50 (retention time 1.8 min., peak height 110 mAU).

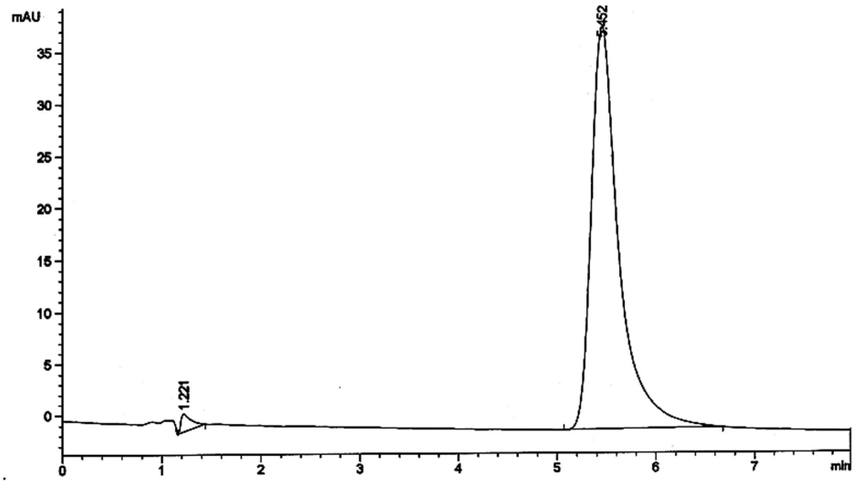

Figure 3. Chromatogram of fluconazole (200 μg/ml) eluted with methanol : 10 MM pH 5.0 acetate buffer 30 : 70 (retention time 5.4 min., peak height 39 mAU).

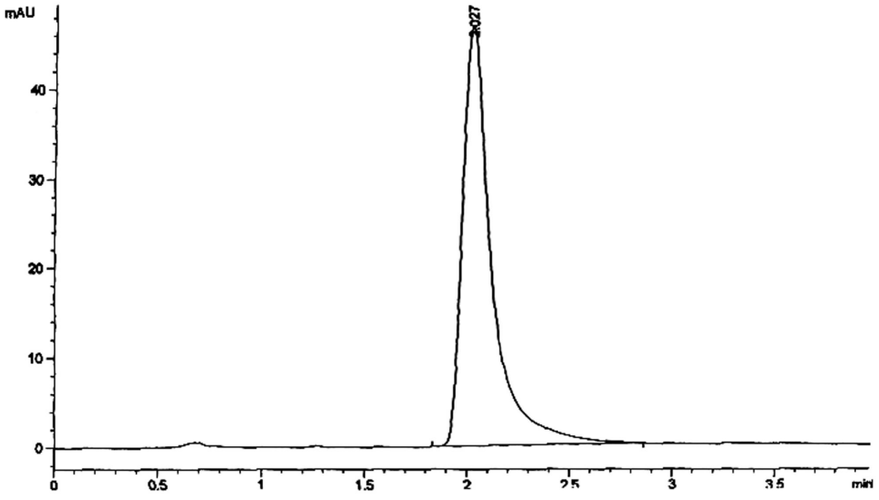

Figure 4. Chromatogram of fluconazole (200 μg/ml) eluted with acetonitrile : water 26: 74 (retention time 2.0 min., peak height 47 mAU).

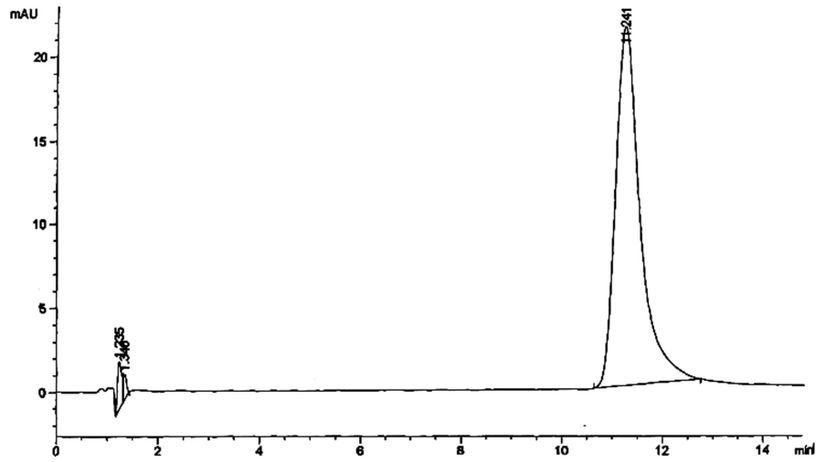

Figure 5. Chromatogram of fluconazole (200 μg/ml) eluted with methanol : 10 mM pH 5.0 acetate buffer : acetonitrile 20 : 70 : 10 (retention time 11.2 min, peak height 22 mAU).

Specificity

No interfering peaks were observed from the chromatograms of excipients. This chromatographic condition was therefore suitable for the determination of fluconazole in pharmaceutical preparations such as capsules and injection since no separation step was required in the assay method. Besides, capsule shells from every manufacturer did not show any interfering peaks either, so it is recommended to be used as the measurement technique in the dissolution testing of fluconazole capsules. Also, no interfering peaks from fluconazole degradation studies were observed, therefore the suggested method could be applied as stability-indicating method for the determination of fluconazole.

Linearity and range

A linear response (r^2= 0.9999) was observed over the concentration range 20 to 500 μg per ml (Fig.6). Either peak height or peak area could be used to construct the calibration curve since sharp peaks were eluted.

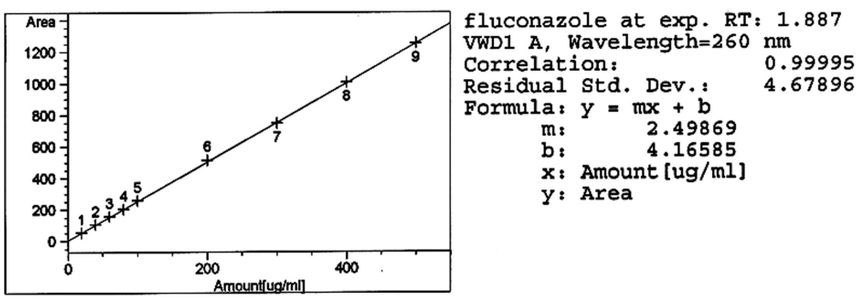

Figure 6. Calibration curve of fluconazole.

Accuracy and precision

The percent recovery of fluconazole from synthetic mixtures of capsules and injection was found to be satisfactorily high, mean recoveries being 100.93 ± 1.07 (n=9) and 98.81 ± 1.00 (n=6) respectively, as shown in Tables 1 and 2. Furthermore, the results were precise at all concentration levels.

Table 1. % Recovery of fluconzole from synthetic mixture of fluconazole capsules.

Amount added(mg)	Amount found(mg)	% Recovery
25.0	25.4	101.60
25.4	25.8	101.60
26.3	26.1	99.24
50.1	50.5	100.80
50.3	49.9	99.20
50.6	51.4	101.58
75.0	75.4	100.53
75.7	77.2	101.98
75.0	76.4	101.87
Mean		100.93
RSD		1.07

Table 2. % Recovery of fluconzole from synthetic mixture of fluconazole infections.

Amount added(mg)	Amount found(mg)	% Recovery
50.1	46.8	97.00
50.4	49.3	97.82
50.4	50.1	99.40
50.5	50.2	99.40
50.3	49.9	99.20
50.3	49.4	98.21
Mean		98.81
RSD		1.00

Application to formulation assay and dissolution testing

The method was applied to the determination of fluconazole content in two available formulations in Thailand, capsules and injection. The assay results obtained by direct spectrophotometric method were higher than those obtained by the proposed HPLC method as shown in Table 3 since there were interferences from diluents. The method was also applied to the determination of fluconazole dissolved from dissolution testing as shown in Table 4. It was shown that the proposed HPLC method was suitable for measurement of fluconazole from dissolution testing since there were no interferences from sample matrix and capsule shells.

Table 3. Assay results of fluconazole capsules and injection.

Sample	% Labeled amount (Mean ± RSD)*	
	HPLC method	Spectrophotometric method
Diflucan®capsules	99.3 ± 0.91	102.7 ± 2.00
Biozole® capsules	104.5 ± 0.90	110.5 ± 0.82
Flucozole® capsules	99.9 ± 1.00	107.1 ± 0.66
Funa® capsules	100.9 ± 0.30	102.1 ± 0.63
Flunco200® capsules	98.7 ± 0.89	105.8 ± 0.80
Stalene200® capsules	106.8 ± 0.70	111.3 ± 0.42
Diflucan® injection	96.4 ± 0.61	98.8 ± 1.00

* Mean of 6 determinations

Table 4. Dissolution testing of fluconazole capsules.

Sample	% Fluconazole dissolved at 45 minutes (Mean ± RSD)*	
	measured by the proposed HPLC method	measured by direct spectrophotometric method
Diflucan® capsules	98.95 ± 0.84	110.44 ± 2.09
Biozole® capsules	101.48 ± 1.16	118.74 ± 1.95
Flucozole® capsules	100.09 ± 2.21	113.70 ± 1.57
Funa® capsules	94.50 ± 3.77	111.71 ± 3.14
Flunco200® capsules	95.96 ± 1.08	112.36 ± 1.98
Stalene200® capsules	105.56 ± 2.06	134.19 ± 2.84

* Mean of 6 capsules tested

CONCLUSION

The proposed high-performance liquid chromatographic method is a rapid and specific method for the analysis of fluconazole contents in pharmaceutical preparations as well as for the measurement of fluconazole dissolved from dissolution testing. It gives precise and accurate results and suitable for the routine analytical work in the quality control laboratory.

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