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High-performance thin-layer chromatographic determination of ketoconazole in pharmaceutical formulations

SUWANNA SAYSIN, BOONSOM LIAWRUANGRATH, and SAISUNEE LIAWRUANGRATH, *Department of Pharmaceutical Science, Faculty of Pharmacy (S.S., B.L.); and Department of Chemistry, Center for Innovation in Chemistry, and Institute for Science and Technology Research and Development, Faculty of Science (S.L.), Chiang Mai University, Chiang Mai 50200, Thailand.*

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Synopsis

A high-performance thin-layer chromatographic method was developed for the determination of ketoconazole. The sample was separated on a silica gel 60 F_{254} plate and developed in ethanol-acetone-1.0 mol l^{-1} H_2SO_4 by means of an automatic multiple-development system. The area of the spot was quantified by a TLC scanner at a wavelength of 298 nm. A linear calibration curve was established over the range of 3–20 μg/ml of ketoconazole, with a correlation coefficient of 0.9992. The relative standard deviations for intraday and interday precisions, for three replicate determinations, were found to be 1.72% and 0.69% for 5 μg/ml and 2.18% and 0.94% for 10 μg/ml of ketoconazole, respectively. The average percentage recoveries of ketoconazole shampoos (Nora, Kenalyn, and Nizoral) and ketoconazole creams (Nizoral, Fungasin, and Ketazon) were found to be 96.10, 97.06, and 99.58, and 96.77, 97.26, and 95.74, respectively. This method has been applied to the determination of ketoconazole in various pharmaceutical dosage forms. Common excipients in formulations do not interfere. This method is simple, precise, accurate, and inexpensive. It should be used for routine analysis.

INTRODUCTION

Ketoconazole (cis-1-acetyl-4-[4-2-(2,4-di-chlorophenyl)-2-(1H-imidazole-1-yl methyl-1,3-dioxolan-4-yl] methoxy piperazine) is an imidazole antifungal agent (Figure 1) (1). As with other imidazoles, it has a five-membered ring structure containing two nitrogen atoms. Ketoconazole is available in many drug dosage treatment forms: cream, shampoo, solution, gel/jelly, and foam. The side effects of ketoconazole treatment products are rash, itching, nausea, vomiting, abdominal pain, headache, dizziness, fatigue, impotence, and blood count abnormalities (1). Rarely do the treatment products of ketoconazole have serious allergic reactions (anaphylaxis). Ketoconazole 2% shampoo is used to treat dandruff and it is used to treat "sun fungus" (4–5). Ketoconazole shampoos may cause abnormal hair texture, scalp pustules (pimples), dry skin, and itching. There may also be oiliness and dryness of the hair and scalp. Rarely, there may be some

Address all correspondence to Boonsom Liawruangrath.

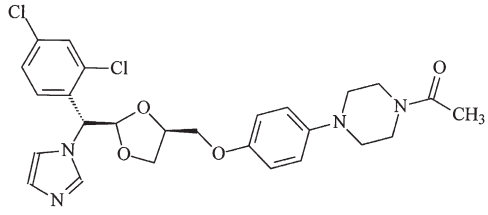

Figure 1. Structure of ketoconazole.

hair loss. Ketoconazole is the only member of the immidazole class that is currently used for treatment of systemic infections. Ketoconazole was more widely used before the development of newer, less toxic, and more effective triazole compounds, fluconazole and itraconazole, and its use has now been limited. It now appears as an alternative drug for specific indications. Ketoconazole works principally by inhibition of cytochrome P450 14a-demethylase (P45014DM). This enzyme is in the sterol biosynthesis pathway that leads from lanosterol to ergosterol (2). The affinity of ketoconazole for fungal cell membranes is less than that of fluconazole and itraconazole. Ketoconazole has thus more potential to affect mammalian cell membranes and to induce toxicity (3).

The official method normally involves titration in non-aqueous solvent (4–5). Various analytical methods have been reported for the determination of ketoconazole. They are spectrophotometric methods (6–11,13,14), spectrofluorimetric methods (12), high-performance liquid chromatography (15–28), the stripping voltammetric and polarographic method (24), capillary zone electrophoresis (30), and high-performance thin-layer chromatography (31,32). Very few high-performance thin-layer chromatographic methods (HPTLC) have been reported in the literature and there is no report on the determination of this drug in shampoo formulations. It has the advantages of being sensitive, selective, rapid, accurate and reproducible. The HPTLC method can be used for identification and for control of batch-to-batch consistency in the stability testing of drugs and for purposes of control throughout the entire manufacturing process of drugs, as well as for quality control of the finished product.

This communication describes the development of the HPTLC method and its applications to the quantitative analysis of ketoconazole in pharmaceutical treatment products such as anti-dandruff shampoos and creams.

EXPERIMENTAL

REAGENTS AND CHEMICALS

Ketoconazole (reference standard assay ≥ 98%, Sigma, Switzerland), ethanol, acetone, and ethyl acetate (BDH Laboratory Supplies, England), glacial acetic acid, hydrochloric acid, and sulfuric acid (Farmitalia Carlo Erba, Italy) were used. Ethanol-acetone-1.0 mol/l H_2SO_4 (80:10:10, v/v/v) was the solvent used for TLC development. The preparation of standard and sample solutions was as follows:

Preparation of standard solutions. The required quantities of the ketoconazole standard were accurately weighed and dissolved in ethanol to a final concentration of 1000 μg/ml.

Working standard solutions (3–20 μg/ml) were obtained by appropriate dilution of the ketoconazole stock solution in ethanol.

As samples, three ketoconazole shampoos, including Nora, Kenalyn, and Nizoral, were taken for analysis. Each sample contained 2% of ketoconazole in an aqueous suspension consisting of coconut fatty acid diethanolamide, disodium monolauryl ether sulfosuccinate, FD & C Red No. 40, hydrochloric acid, imidurea, laurdimonium hydrolyzed animal collagen, macrogol 120 methyl glucose dioleate, perfume bouquet, sodium chloride, sodium hydroxide, sodium lauryl ether sulfate, and purified water.

Three ketoconazole creams, including Nizoral, Fungasin, and Ketazon, were taken for analysis. Each gram of white, odorless cream contained ketoconazole 20 mg (2%). Nonmedicinal ingredients were cetyl alcohol, isopropyl myristate, polysorbate, propylene glycol, purified water, sodium bisulfite, sorbitan monostearate, and stearyl alcohol. All samples were purchased from drugstores in Chiang Mai Province, Thailand.

Preparation of sample solutions. Shampoo and ketoconazole cream samples containing ketoconazole were transferred to a tarred 50-ml screw-capped centrifuge tube and accurately weighed. A 50-ml sample of ethanol was added (ca. 2%). The tube was agitated for 10 min and centrifuged at 7,600 rpm for 10 min. The clear supernatant was collected in a stoppered test tube and used for spotting on the TLC plate. Commercially available precoated silica gel 60 F_{254} plates (Merck; 10×20 cm, 0.25-mm thickness) were employed. The plates were activated for 30 min at 120°C prior to use.

APPARATUS

A Linomat IV (CAMAG) was used for sample application. An automated multiple development (AMD system) of CAMAG was used for chromatographic development. A TLC Scanner III (CAMAG, Switzerland), computer-aided testing software (CATS) for evaluation, a deuterium lamp, and scanning by absorbance at 298 nm (evaluation via peak area) were also used.

PROCEDURES

The plate first was cleaned up by development with acetone in a saturated tank. Later, the plate was dried at room temperature. Standard ketoconazole in ethanol solutions containing 3, 5, 7, 10, and 20 μg/ml of ketoconazole and the sample solutions (shampoo or ketoconazole cream samples) were applied, respectively, on a precoated silica gel 60 F_{254} aluminum plate (Merck; 10×20 cm, 0.25-mm thickness). A Linomat IV was employed with a constant rate of 3 s/μl, a 3-mm band width was applied, and the space between two bands was 5 mm. The plate was inserted into an automated multiple development (AMD) system containing ethanol-acetone-1.0 mol/l H_2SO_4 (80:10:10, v/v/v) as the mobile phase, which was saturated with solvent vapor for 15 min prior to use. The plate was developed to a height of 9 cm. The chromatogram was dried automatically in the chamber and then viewed under short-wavelength UV light (254 nm) to mark the scanning area. The peak area of each band was quantitatively measured by means of a TLC scanner III at a wavelength of 298 nm, under the following parameters: Photo mode: reflection; scan mode: zigzag. Other parameters were set according to the Camag TLC scanner's

instruction manual. A calibration curve of ketoconazole was constructed by plotting peak areas versus various concentrations of ketoconazole.

RESULTS AND DISCUSSION

In order to achieve optimum separation, during the development of the high-performance thin-layer chromatographic method for determining ketoconazole, various experimental conditions were investigated.

OPTIMIZATION OF THE EXPERIMENTAL CONDITIONS

Selection of mobile phase. During the development of the HPTLC method three different compositions of the mobile phase, including ethanol-acetone (90:10), ethanol-ethyl acetate (50:50), and ethanol-acetone-1.0 mol/l H_2SO_4 (80:10:10), were tested. It was found that the mobile phases consisting of ethanol-acetone (90:10) and ethanol-ethyl acetate (50:50) provided bad constituent separation and that tailing occurred. The mobile phase, ethanol-acetone-1.0 mol/l H_2SO_4 (80:10:10, v/v/v) proved to be the best (Figure 2) because its resolution was good and also gave well-defined and reproducible spots of ketoconazole with an R_f value of 0.70, as shown in Table I.

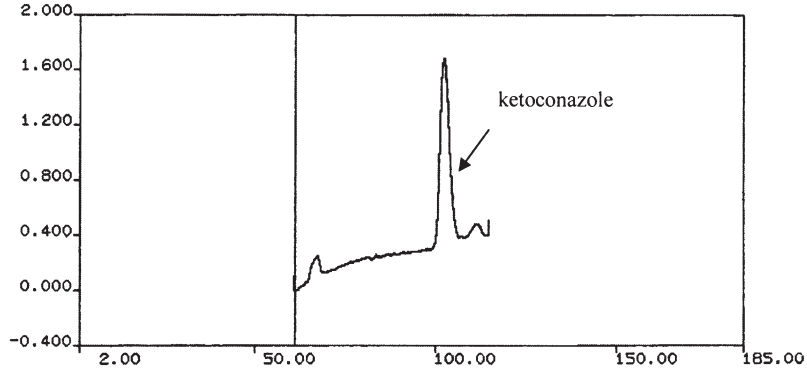

Figure 2. Densitogram of ketoconazole on silica gel 60 F_{254} in the solvent system, ethanol-acetone-1.0 mol/l H_2SO_4 (80:10:10) with an R_f of 0.70.

Table I
The R_f Values of Ketoconazole on a Silica Gel 60 F_{254} Plate in Various Mobile Phases

Mobile phase	R_f
Ethanol-acetone (90:10)	0.57
Ethanol-ethyl acetate (50:50)	0.56
Ethanol-acetone-1.0 mol/l H_2SO_4 (80:10:10)	0.70

The optimum wavelength. The optimum absorption wavelength of ketoconazole was also investigated. Ketoconazole standard (5 μg/ml) was applied to a silica gel 60 F_{254} plate, and then we carried out the procedure as mentioned above. It was found that the drug gave a maximum peak area at 298 nm, which was chosen as the optimum wavelength.

METHOD VALIDATION

The following parameters have been used to validate the developed HPTLC method for the estimation of ketoconazole in shampoo samples:

Sensitivity and linearity. The sensitivity of the assay was determined in terms of limit of detection (LOD), limit of quantitation (LOQ), linearity range, and correlation coefficient (33,34). The limit of detection (LOD) is defined as the compound concentration that produced a signal-to-noise ratio greater than three (S/N = 3), measured from peak to peak. The limit of quantitation (LOQ) is defined as that concentration of the analyte producing the signal that is at least ten times the baseline noise (S/N = 10). The detection limit of the method was investigated by applying various concentrations of ketoconazole standard solution to the precoated HPTLC plate. After development, the spot areas were quantified by TLC scanner. The limits of detection and quantitation were calculated from the standard deviation (SD) of the densitometric response and the slope of the curve (S) using the following equations:

$$\mathrm{LOD} = 3.3(\mathrm{SD}/\mathrm{S})$$
$$\mathrm{LOQ} = 10(\mathrm{SD}/\mathrm{S})$$

After the densitometric analysis of ketoconazole at 298 nm, the lowest amounts of the drug that could be detected and quantified were found to be 0.72 μg/ml and 2.18 μg/ml, respectively.

Robustness of the method. A test of the robustness of the proposed method was carried out by making small changes in the composition of the mobile phase or developing solvent. The changes in the R_f of the spot, the difference in the area of the peak, was observed with respect to mobile phase composition, and we calculated the %RSD. A low value of %RSD indicates that the method is valid for small changes, and so the method is robust, as shown in Table II.

Table II
Robustness of the Method (mobile phase composition (ethanol-acetone-1.0 mol/l H_2SO_4))

Original (v/v)	Used (v/v), area		
80:10:10 (R_f = 0.70)	89:10:1 (R_f = 0.57)	85:10:5 (R_f = 0.66)	80:10:10 (R_f = 0.70)
	76321.65	87321.48	87849.16
	76464.85	93741.12	90644.85
	76475.60	90592.44	87913.44
%RSD of area	0.09	2.89	1.46

Linearity. The linear range of the standard curve was also studied. The plate was developed in acetone for elimination of impurity. Then the standard ketoconazole solutions containing 3, 5, 7, 10, and 20 μg/ml of ketoconazole were applied to a silica gel 60 F_{254} plate and determined by using the procedure mentioned in the Experimental section. A linear relationship between peak areas and various concentrations of ketoconazole was established over the range of 3–20 μg/ml (Figure 3), with a correlation coefficient of 0.9992.

Precision and accuracy. The precision of the method was established by using solutions of two different concentrations, 5 μg/ml and 10 μg/ml, of ketoconazole standard solution. Each was analyzed three times (n=3) on the same day, and the relative standard deviation (RSD) was calculated to ascertain intraday precision. The studies were also repeated on three different days to establish interday precision. The results are presented in Table III.

The accuracy of the proposed HPTLC method was also investigated. Known quantities of ketoconazole were added to previously analyzed samples of ketoconazole and analyzed by the proposed method. The average percentage recovery of shampoo A, shampoo B, shampoo C, cream A, and cream B were found to be 96.10, 97.06, 99.58, 96.77, 97.26 and 95.74, respectively. The mean recoveries at each level for all the components, given in Table IV, ranged between 91.68% and 100.59% (acceptance criteria: 90–110%), and the %RSD was between 1.15% and 7.93% (acceptance criteria: NMT 10.0%), establishing that the method was accurate for the quantitative determination of all the known and unknown impurities (33,34).

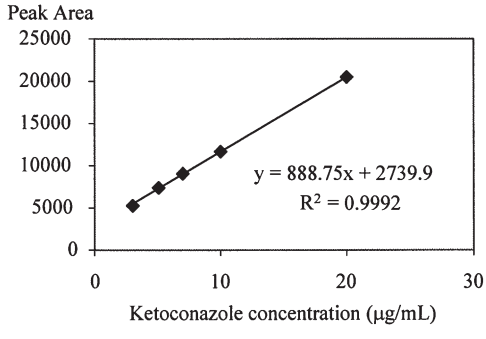

Figure 3. Calibration graph for ketoconazole obtained by the HPTLC method (concentration range 3–20 μg/ml of ketoconazole).

Table III
Precision Study for Ketoconazole

Ketoconazole (μg/ml)	Intraday precision (n=3)		Interday precision (n=3)	
	Concentration (μg/ml)	%RSD	Concentration (μg/ml)	%RSD
5	5.02	1.72	5.11	0.69
10	10.07	2.18	10.06	0.94

Table IV
Recovery Studies on Ketoconazole

Ketoconazole in sample	Concentration in sample (μg/ml)*		% Recovery* (mean ± SD)
	Added	Found	
Shampoos:			
Nora	5	4.91 ± 0.13	98.27 ± 2.52
	10	9.86 ± 0.35	98.58 ± 3.47
Kenalyn	5	4.71 ± 0.26	98.09 ± 1.73
	10	9.21 ± 0.23	96.04 ± 1.15
Nizoral	5	5.03 ± 0.40	100.59 ± 7.93
	10	9.86 ± 0.35	98.58 ± 3.47
Creams:			
Nizoral	5	4.54 ± 0.43	96.91 ± 2.86
	10	9.33 ± 0.31	99.63 ± 1.57
Fungasin	5	4.95 ± 0.18	99.03 ± 3.67
	10	9.55 ± 0.34	95.49 ± 3.45
Ketazon	5	4.97 ± 0.49	99.80 ± 3.27
	10	9.33 ± 0.31	96.63 ± 1.57

* Mean of three determinations.

METHOD APPLICATION TO SAMPLE

The proposed HPTLC method was successfully applied to the determination of ketoconazole in the three commercially available shampoos and ketoconazole creams. The densitograms of the sample solutions are shown in Figure 4. The contents of ketoconazole in shampoo A, shampoo B, and ketoconazole cream A were analyzed using the procedure described in the Experimental section. The results generated by the HPTLC method and the published spectrophotometric method (12) were compared with those expected by the label claims (Table V).

The results obtained were compared for agreement with those obtained using the spectrophotometric method. Statistical analysis of the results by using a *t*-test showed that the calculated *t*-values were less than the table list *t*-value at the 95% confidence limit (p=0.057). Therefore, these two methods are not significantly different in any of the samples.

CONCLUSION

The proposed HPTLC procedure can be used for the determination of ketoconazole in commercial shampoos and pharmaceuticals. The detection limit of this method was reasonably accepted. Sample pretreatment is not necessary. This method is simple, fast, relatively inexpensive, precise, accurate, sensitive, and uses a minimum number of reagents and chemicals. Therefore, the speed of analysis and the precision make this method suitable for quality control of ketoconazole in commercial shampoo and pharmaceutical formulations. It is therefore suitable for quality control in drug industries.

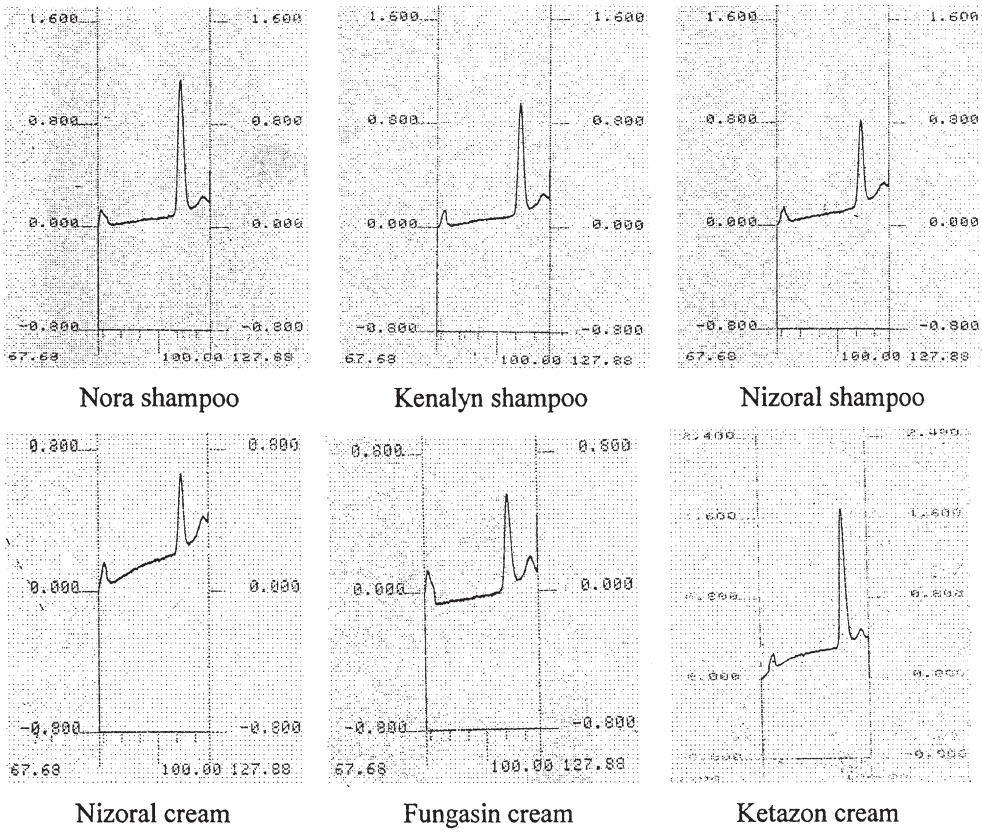

Figure 4. Densitogram of ketoconazole sample on silica gel 60 F_{254} in the solvent system, ethanol-acetone-1.0 mol/l H_2SO_4 (80:10:10).

Table V
Comparison of HPTLC and Spectrophotometric Methods for the Determination of Ketoconazole in Shampoo and Pharmaceutical Formulations

		Amounts of ketoconazole found; % found ± SD* (% labeled amount)	
Sample	Labeled content (% w/w)	HPTLC method	Spectrophotometric method (12)
Nora	2	2.23 ± 0.13 (111.50%)	2.35 ± 0.45 (117.50%)
Kenalyn	2	2.27 ± 0.17 (113.50%)	2.42 ± 0.28 (121.00%)
Ketazon	2	2.24 ± 0.27 (112.00%)	2.37 ± 0.39 (118.50%)
Paired Student's *t*-test		*P* value = 0.057	

* Mean of three determinations.

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