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Short Communication

High-performance liquid chromatographic determination of tioconazole in pharmaceutical formulations

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Introduction

Ticonazole, 1-[2-{(2-chloro-3-thienyl)methoxy}-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole, is a new imidazole derivative used in the treatment of fungal infections [1, 2]. Several dosage forms have been developed including creams, soft gelatin vaginal capsules, vaginal tablets and a single-dose vaginal ointment. This paper describes a high-performance liquid chromatographic (HPLC) method for the determination of tioconazole in dosage forms and for assessment of the stability of the bulk drug substance. The method is rapid and is specific for tioconazole in the presence of its degradation products.

Experimental

Apparatus

The liquid chromatograph comprised a Constametric II G pump and a Spectromonitor III variable wavelength detector (Laboratory Data Control, Stone, Staffs., UK) set at 225 nm. Samples were injected by a Model 725 automatic sampler equipped with a 20-μl loop (Micromeritics, Norcross, Georgia) and chromatograms were recorded and integrated by a Model 301 computing integrator (Laboratory Data Control). Separations were carried out using a 100 × 5 mm i.d. column, slurry packed with 5-μm Hypersil ODS (Shandon Southern, Cheshire, UK).

Chemicals and reagents

Water was glass-distilled; other solvents were HPLC grade (Rathburn Chemicals, Peebles, UK). Potassium dihydrogen phosphate was Analar grade (BDH, Poole,

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Dorset, UK). The mobile phase was methanol–0.02 M aqueous potassium dihydrogen phosphate (85:15, v/v) vacuum filtered before use through a 0.2-μm membrane filter (Nuclepore). The flow rate was 1.5 ml/min.

Sample preparation

Vaginal tablets (100 mg). Ten tablets were selected randomly, weighed and crushed to a fine powder. An accurately weighed sample of the powder (1 g equivalent to 100 mg of tioconazole) was transferred to a 100-ml volumetric flask and 10 ml of 0.01 M hydrochloric acid was added. The mixture was shaken to disperse the solids, diluted to volume with methanol and stirred for 30 min with a magnetic stirrer. The suspension was filtered through a Whatman No. 1 filter paper and a 10-ml aliquot of the filtrate diluted to 100 ml with methanol to provide a test solution containing tioconazole 0.1 mg/ml.

Dermal creams (1% m/m and 2% m/m). A 2-g portion of the cream was accurately weighed into a 250-ml volumetric flask and extracted with 25 ml of a mixture of hexane, dichloromethane, methanol and propan-2-ol (1:1:1:2, v/v). The extract was diluted to volume with the mobile phase and the suspension filtered through a Whatman No. 42 filter paper to provide a test solution containing tioconazole 0.16 mg/ml (2% cream) or 0.08 mg/ml (1% cream).

Soft gelatin vaginal capsules (100 mg and 300 mg). The contents of one capsule were transferred quantitatively into a 250-ml volumetric flask using 1,4-dioxan to rinse the capsule shell (50 ml for a 100 mg capsule, 75 ml for a 300 mg capsule); 1.0 g of sodium chloride was added and the sample diluted to volume with the mobile phase. The suspension was filtered through a Whatman No. 1 filter paper and, for the 100 mg capsule, the filtrate used for assay without further dilution (0.4 mg tioconazole per ml). For the 300 mg capsule 10-ml aliquot of the filtrate was further diluted to 250 ml with the mobile phase to provide a test solution containing tioconazole 0.048 mg/ml.

Single dose vaginal ointment (6.5% m/m). A 1-g portion of the ointment was accurately weighed into a 250-ml volumetric flask. The ointment was dissolved in 15 ml of warm dichloromethane and the solution diluted to volume with the mobile phase. The resultant suspension was subjected to ultrasonic agitation for 15 min and then filtered through a Whatman No. 42 filter paper to provide a filtrate containing tioconazole 0.26 mg/ml.

Bulk drug substance. A 100-mg portion of the sample was accurately weighed into a 100-ml volumetric flask and diluted to volume with methanol. A 10-ml aliquot was further diluted to 100 ml with methanol to provide a test solution containing tioconazole 0.1 mg/ml.

External standard solutions

For each dosage form a standard solution was prepared from an amount of tioconazole equivalent to that expected in the test sample by a procedure identical to that used for the dosage form.

Assay

Duplicate 20-μl aliquots of the test solution and external standard solution were chromatographed. Peak heights were either measured to the nearest mm with a ruler or

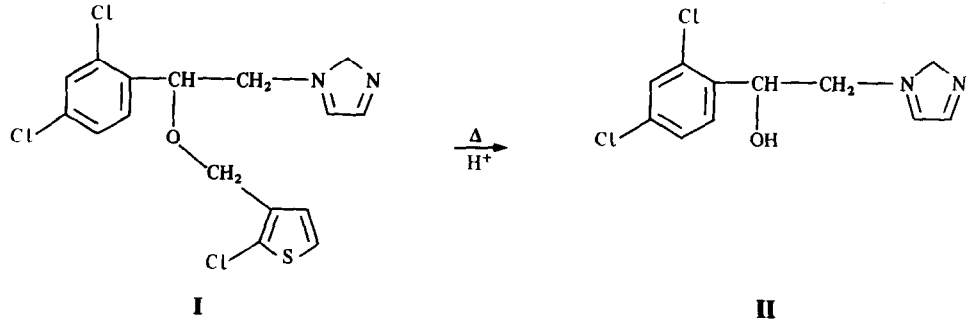
computed by the integrator; the mean peak heights of the replicate injections were calculated. The tioconazole concentration was calculated by the usual procedure with reference to the external standard.

Results and Discussion

The essential criteria that the developed method was required to meet were that it should be suitable for use with a wide variety of dosage forms, it should be free from interference from excipients and that it should be stability indicating.

No major interference from any excipients or extraction solvents was observed. Peak shapes and heights were not influenced by the various proportions of the organic solvents used for extraction. Excipients carried through the extraction procedure were not detected or gave peaks at or near the solvent front and so did not interfere with the analysis.

The major degradation product of tioconazole is also poorly retained. Tioconazole has been shown to degrade in acid solution at high temperature to yield 1-(2,4-dichlorophenyl)-2-(1H-imidazol-l-yl)ethanol (**II**) as the major degradation product (Scheme 1).



I **II**

Scheme 1
Degradation pathway of tioconazole (**I**).

The other minor degradation products associated with the thiophen ring system have not been isolated or identified. The degradation product **II** has been observed in formulations subjected to accelerated degradation studies. Figure 1 shows an example of a chromatogram of a deliberately degraded cream formulation compared with a chromatogram of the original sample. The capacity factors for tioconazole (**I**) and its major degradation product (**II**) are 2.1 and 0.5, respectively.

The response of the chromatographic system was shown to be linear over the concentration range 0.02–0.6 mg/ml tioconazole in the injected solution ($r = 0.999$, $n = 6$).

Recovery experiments were performed on samples prepared by adding known amounts of tioconazole to the appropriate placebos, where applicable. The percentage recovery was independent of the amount of tioconazole; recoveries were in the range 98–103% with relative standard deviations of 0.8–1.7%. The reproducibility of the assay has proved to be excellent for the large numbers of batches analysed; relative standard deviations are typically less than 2% (Table 1).

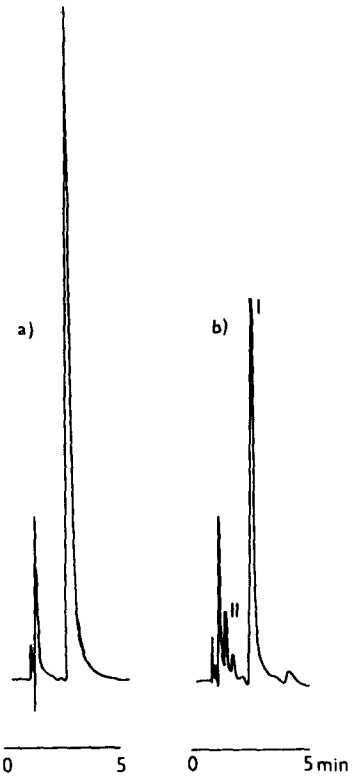


Figure 1
Chromatograms of tioconazole cream. (a) Original sample; (b) degraded sample. For HPLC conditions, see text.

Table 1
Typical assay results for tioconazole dosage forms

Dosage form	Found* (as % of declared amount) or concentration	Relative standard deviation
Tablet (100 mg)	100.5	1.2
Cream (1%)	102.0	2.0
Cream (2%)	99.5	0.5
Soft gelatin capsule (100 mg)	98.8	1.7
Soft gelatin capsule (300 mg)	99.4	5.0
Vaginal ointment (6.5%)	99.1	3.0
Bulk drug	99.8	0.1

* Based upon three determinations for each dosage form.

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References

[1] S. Jevons, G. E. Gymer, K. W. Brammer, D. A. Cox and M. R. G. Leeming, *Antimicrob. Agents Chemother.* **15**, 597–602 (1979).
[2] M. S. Marriott, J. R. C. Baird, K. W. Brammer, J. K. Faulkner, G. Halliwell, S. Jevons and M. H. Tarbit, *Dermatologica* **166**, Suppl. 1, 1–7 (1983).

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