Journal of Pharmaceutical & Biomedical Analysis
Vol. 10, No. 2/3, pp. 205–211, 1992
Printed in Great Britain

0731–7085/92 $5.00 + 0.00

Determination of sertaconazole nitrate, a new imidazole antifungal, by high-performance liquid chromatography

C. ALBET,* J.M. FERNANDEZ, E. ROZMAN, J.A. PEREZ, A. SACRISTAN and J.A. ORTIZ

Department of Analysis and Pharmacokinetics, Ferrer Group Research Centre, Juan de Sada 32, 08028 Barcelona, Spain

Abstract: A high-performance liquid chromatographic method is developed for the determination of bulk sertaconazole nitrate and related compounds (potential impurities and degradation products) as well as a sertaconazole nitrate cream formulation. A 10-μm Spherisorb CN column is used along with a mobile phase consisting of acetonitrile and aqueous 0.01 M sodium phosphate (37:63, v/v). The sertaconazole nitrate peak is monitored at a wavelength of 260 nm, the retention time being 19.3 min. The detector response for sertaconazole nitrate is linear over the concentration range from 64 to 96 μg ml^{-1}. The method is found to be sufficiently selective for the reliable determination of related compounds, FI-7001, FI-7009 and FI-7011, as indicated by same-day and between-day relative standard deviations (RSD) for replicate assays of 1.72% (n = 9) and 2.17% (n = 24), respectively. The application of this method to a cream formulation of sertaconazole nitrate is found to give a mean percentage recovery of 99.4% with RSD of 1.14% (n = 9); none of the cream vehicle peaks are found to interfere with the determination of sertaconazole nitrate.

Keywords: *Sertaconazole nitrate; high-performance liquid chromatography; determination in presence of related compounds; determination in a cream formulation.*

Introduction

Sertaconazole nitrate, a new imidazole antifungal, has been shown to have a wide spectrum activity against dermatophyte fungi (*Trichophyton microsporum*, *Epidermophyton*), pathogen yeasts (*Candida albicans*, *C. tropicalis*, C. spp., *Malassezia furfur*) and Aspergillus. Also, it is effective against such fungal species as *Trichophyton rubrum* (100%), *T. mentagrophytes* (94.1%) *Epidermophyton floccosum* (100%) and *Microsporum canis* (100%) which are resistant to other imidazoles. Clinical pharmacology studies of sertaconazole dermal cream made revealed clinical, microscopic (KOH) and microbiological (culture) healing rates of 86, 98.7 and 99.6%, respectively, with low relapse rates.

In order to assure a rapid, reproducible and, above all, selective analytical procedure for the determination of sertaconazole nitrate in bulk drug and in a cream formulation, a HPLC-method has been developed to determine intact drug in the presence of related compounds and from the cream formulation excipients. Other antifungal agents such as miconazole, econazole, clotrimazole and tioconazole, also have been determined by HPLC, though under different chromatographic conditions [1–14].

The proposed chromatographic method has been used, by modifying the sample preparation procedure only, for the determination of sertaconazole nitrate in other dosage forms which are still under investigation. Some changes in the proposed chromatographic procedure were found to be necessary for pharmacokinetic studies in humans following topical and vaginal administrations, as will be described in subsequent publications.

Experimental

Material and reagents

Sertaconazole nitrate (FI-7056), 1-[2-[(7-chlorobenzo [*b*] thien-3-yl)methoxy]-2-(2,4-dichlorophenyl)ethyl]-1H-imidazole nitrate, related compounds FI-7001, α-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol, FI-7009, 3-(bromomethyl)-7-chlorobenzo [*b*] thiophene, FI-7011, 7-chlorobenzo [*b*] thiophene-3-methanol (Fig. 1) were synthesized in the Organic Synthesis Department of Ferrer

* Author to whom correspondence should be addressed.

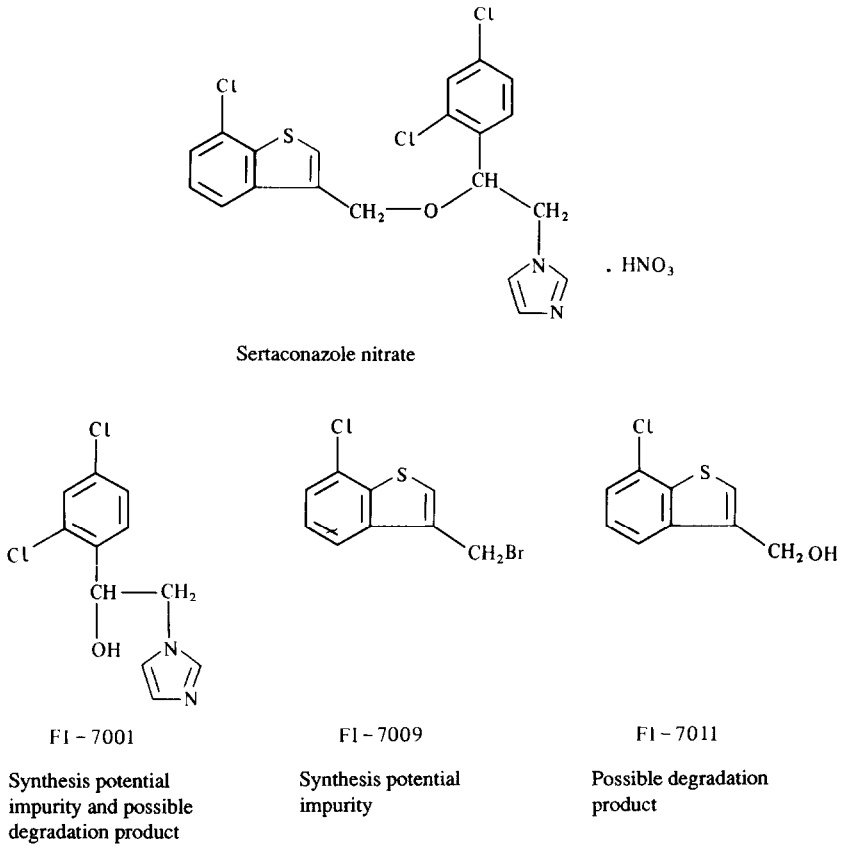

Figure 1
Structures of sertaconazole nitrate and related compounds FI-7001, α-(2,4-dichlorophenyl)-1H-imidazole-1-ethanol; FI-7009, 3-(bromomethyl)-7-chlorobenzo [*b*] thiophene; FI-7011, 7-chlorobenzo [*b*] thiophene-3-methanol.

Group Research Centre (Barcelona, Spain). Acetonitrile and methanol were HPLC grade and were obtained from Merck (Darmstadt, Germany). Monobasic sodium phosphate was analytical grade and was obtained from Montplet–Esteban, S.A. (Barcelona, Spain).

Apparatus

The chromatographic system consisted of two Waters 510 solvent delivery pumps with a Wisp 710B automatic injector (Waters Associates, Milford, MA, USA). The analytical column used was a 10-μm Spherisorb column (25 cm × 4 mm i.d.) (Tracer Analitica, Barcelona, Spain) thermostatted in a column oven equipped with a TCM temperature controller (Waters Associates, Milford, MA, USA). A Waters 480 variable wavelength detector was used along with a Merck–Hitachi D-2500 parallel integrator (Hitachi Ltd, Tokyo, Japan) with a Baseline 810 Chromatography Workstation (Waters Associates, Milford, MA, USA) and a Seiko Epson IQ-500 printer (Nagano, Japan). An ultrasonic water bath from Selecta (Barcelona, Spain) was used to degas the mobile phase. For all volumetric transfers, an Eppendorf pipette from Eppendorf–Netheden–Hinz GmbH (Hamburg, Germany) was used.

Chromatographic conditions

The mobile phase consisted of acetonitrile–0.01 M monobasic sodium phosphate (37:63, v/v). Each solvent was delivered by an individual pump and mixed in a high-pressure solvent-mixing valve. The mobile phase was operated at a flow rate of 1.6 ml min^{-1} at a constant temperature of 35°C. Both the solvent volume and the flow rate were monitored with a computer using the Baseline 810 software.

The UV detector was operated at 260 nm for sertaconazole nitrate, FI-7009 and FI-7011, and at 220 nm for FI-7001. The volume of the solution injected into the column was 25 μl. The retention times of sertaconazole nitrate and the related compounds FI-7011, FI-7009 and FI-7001 were 19.3, 2.3, 3.3 and 6.2 min, respectively.

Preparation of the standard solutions

Sertaconazole nitrate solutions were prepared by weighing 16, 18, 20, 22 and 24 mg of sertaconazole nitrate and dissolving in acetonitrile to make 25-ml solutions. Then, 1 ml of each solution was transferred to a 10-ml flask and the required amount of acetonitrile was added. In this way, five standard solutions of sertaconazole nitrate with concentrations of 64, 72, 80, 88 and 96 μg ml^{-1} were prepared. An aliquot of each solution was transferred to a tube and 25 μl were injected into the chromatographic column to 1.6, 1.8, 2.0, 2.2 and 2.4 μg of sertaconazole nitrate on-column.

Preparation of standard solutions of the related compounds

The solutions containing the related compounds FI-7001, FI-7009 and FI-7011 were prepared by weighing 10 mg of each compound and dissolving in acetonitrile to make 10-ml solutions. Then, 1 ml of each solution was transferred to a 50-ml flask and the required amount of acetonitrile was added. A final solution was prepared from 1 ml of the preceding solution which was transferred to a 10-ml flask and the required amount of acetonitrile was added. In this way, the solutions containing the impurities with a concentration of 2 μg ml^{-1} each were prepared. An aliquot of 25 μl was injected to give 50 ng on-column of FI-7001, FI-7009 and FI-7011, respectively.

Preparation of the cream formulation solution

A 2 g mass of cream was weighed into a 25-ml beaker. A 15 ml volume of acetonitrile–methanol (80:20, v/v) was added and dispersed manually using a spatula. The resulting solution was transferred to a 50-ml flask, the beaker was washed three times with the same solvent and the washings added to the flask. The flask was agitated for 10 min by introducing it directly into an ultrasonic water bath, and the required amount of acetonitrile was added. Then, an aliquot was centrifuged at 4500 rpm for 10 min, 1 ml of the supernatant was transferred to a 10-ml flask and the required amount of acetonitrile was then added. An aliquot of this latter solution was transferred to a vial and 25 μl were injected into the chromatographic column.

Results and Discussion

Analysis of bulk drug

Calibration curve. The calibration curve was constructed from the standard solutions of sertaconazole nitrate in a concentration range from 80 to 120% of the analytical nominal concentration. Each sample was injected in triplicate and the mean value of peak areas was plotted over their respective concentrations. The calculation of column efficiency (plate count) and tailing factor (peak asymmetry) resulted in a mean value of $n = 14.371$ and $T = 1.18$, respectively. The standard solutions were repeatedly injected over a 72-h period and the integration values, retention times and peak asymmetry compared with data obtained for freshly prepared solutions. This revealed sertaconazole nitrate to be highly stable at least over a 72-h period. The calibration curve was linear passing through the coordinate origin. The statistical data for the calibration curve are shown in Table 1.

Precision. Same-day relative standard deviation (RSD) (several samples at the same concentration) and between-day RSD (several samples at different concentrations) of both the chromatographic system and the analytical method were studied. Same-day RSD for the chromatographic system was 0.61% ($n = 8$) at the concentration of 80 μg ml^{-1} (2 μg injection) and for the analytical method was 1.72% ($n = 9$) at the same concentration. Overall between-day RSD value was 2.17% ($n = 24$) for a concentration range between 64 and 96 μg ml^{-1} (1.6 and 2.4 μg injection).

Table 1
Linear regression analysis for the assay of sertaconazole nitrate

Regression coefficient (r)	Slope ± S.E. ($\times 10^4$)	Intercept ± S.E. ($\times 10^4$)	Identity of slope* t_{exp}
0.9968	38.3938 ± 1.0887	0.8047 ± 0.2865	0.2689

$y = mx + n$, where y is the chromatographic peak areas, m is the slope value tailed during validation, x is the injected amount in μg and n is the intercept expressed as nominal analytical response rate.

*$t_{table} = 2.145$ ($P = 0.95$).

Selectivity. In order to check that the proposed method was sufficiently selective, standard solutions of sertaconazole nitrate at the concentration of 80 μg ml^{-1} (2 μg injection), and solutions containing related compounds (possible synthesis impurities and degradation products) FI-7001, FI-7009 and FI-7011 at the concentration of 2 μg ml^{-1} each (50 μg injection) were examined. For the synthesis impurities and degradation products chromatograms were obtained with detector wavelengths of 220 and 260 nm. Representative chromatograms (Figs 2, 3 and 4) revealed that sertaconazole nitrate was well separated from the other compounds.

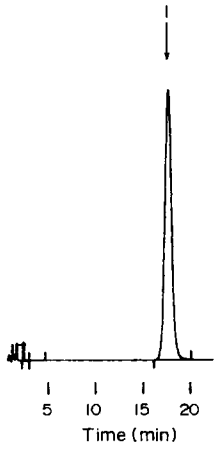

Figure 2
A typical chromatogram of sertaconazole nitrate; (1) sertaconazole nitrate peak (80 μg ml^{-1}); detector sensitivity: 0.001 AUFS at 260 nm.

Figure 3
Chromatogram of related compounds: (1) sertaconazole nitrate peak; (2) FI-7001 peak; (3) FI-7009 peak and (4) FI-7011 peak at the concentration of 2 μg ml^{-1} (50 ng injection); detector sensitivity: 0.001 AUFS at 260 nm. At the sertaconazole nitrate retention time, no other interfering peak was found.

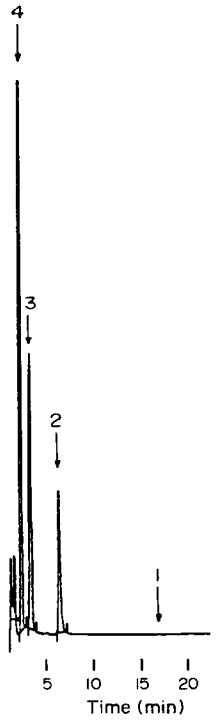

Figure 4
Chromatogram of related compounds: (1) sertaconazole nitrate peak; (2) FI-7001 peak; (3) FI-7009 peak and (4) FI-7011 peak at the concentration of 2 μg ml^{-1} (50 ng injection); detector sensitivity: 0.001 AUFS at 220 nm. At the sertaconazole nitrate retention time, no other interfering peak was found.

The calculation of the resolution between sertaconazole nitrate and the nearest peak corresponding to degradation product FI-7001 resulted in $R > 17.0$. This high value of R in addition to the long retention time of sertaconazole nitrate suggested the possibility of changing the proportions of solvents in the mobile phase so that the major peak could be eluted more rapidly. However, this is not possible if it is desirable to achieve at the same time a good separation for related compounds, and avoid FI-7011 being eluted at the dead time together with the response of the diluent.

Table 2 shows the statistical comparison between the area means of sertaconazole nitrate and sertaconazole nitrate plus related compounds. No significant differences were found.

Cream formulation

Recovery. A 4 g mass of cream was weighed into three glass mortars, 72, 80 and 88 mg of sertaconazole nitrate added and then the sample was homogeneized manually. Recovery determination was carried out by weighing 2 g

Table 2
Evaluation of the selectivity of the assay for sertaconazole nitrate

Sample statistics	Sertaconazole nitrate	Sertaconazole nitrate + related compounds	t_{exp}*	F_{exp}**
No. of observations	7	7	0.470	1.541
Area unit mean	728398	725482		
Variance	1.06×10^8	1.64×10^8		
RSD	1.42%	1.76%		

*$t_{table} = 2.179$ ($P = 0.95$).
**$F_{table} = 3{,}79$ ($P = 0.95$).

Table 3
Recovery and precision of the assay of sertaconazole nitrate in a cream formulation

Theoretical concentration ($\mu g\ ml^{-1}$)	Found (mean) concentration	Recovered (mean) percentage	S.D.	RSD (%)
	71.42	99.2	1.27	1.78
72	70.25	97.6	1.26	1.79
	71.62	99.5	0.47	0.65
	79.89	99.9	1.01	1.27
80	78.40	98.0	0.84	1.07
	81.07	101.3	1.16	1.43
	87.47	99.4	0.30	0.34
88	88.09	100.1	0.70	0.79
	87.38	99.3	1.00	1.14

of cream from each mortar equivalent to 36, 40 and 44 mg of sertaconazole nitrate respectively, and then analysing as described previously. This operation was repeated for 3 consecutive days. Final concentrations were 72, 80 and 88 $\mu g\ ml^{-1}$. An aliquot of these solutions was transferred to respective vials and 25 μl (equivalent to 1.8, 2 and 2.2 μg of sertaconazole nitrate on-column) were injected into the chromatographic column. Recovery was calculated by comparing the chromatographic peak areas with the calibration plots (Table 3). The between-day mean percentage recovery and RSD at the three tested concentrations were 99.4 and 1.17% ($n = 9$), respectively.

Selectivity. Data from accelerated stability studies on a commercial cream formulation of sertaconazole nitrate packed in aluminium tubes were used to validate the selectivity of the method. As it was a cream formulation, the maximal exposure temperature was adjusted to 40°C in order to protect it against an excessive increase of fluidity. After 6 months, a sample of the cream formulation and a freshly prepared placebo cream were simultaneously analysed according to the above procedure.

As shown in chromatograms for placebo cream (Figs 5 and 6), there are no peaks with a retention time close to that of sertaconazole

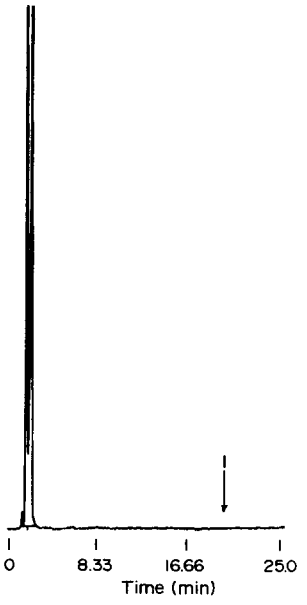

Figure 5
Chromatogram of a placebo cream. (1) Sertaconazole nitrate peak. Detector sensitivity: 0.001 AUFS at 260 nm. At the sertaconazole nitrate retention time, no other interfering peak was found.

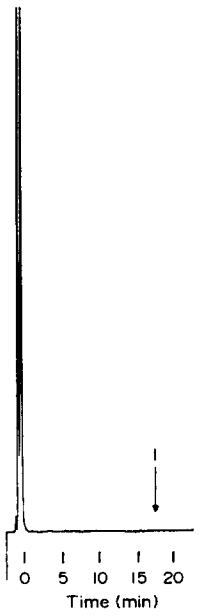

Figure 6
Chromatogram of a placebo cream. (1) Sertaconazole nitrate peak. Detector sensitivity: 0.001 AUFS at 220 nm. At the sertaconazole nitrate retention time, no other interfering peak was found.

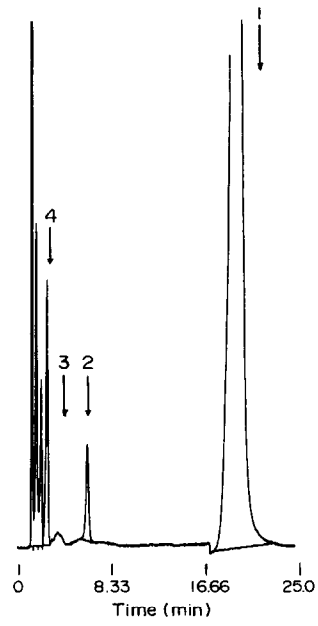

Figure 8
Chromatogram illustrating the stability of sertaconazole nitrate cream stored for 6 months at 40°C. (1) Sertaconazole nitrate peak (80 μg ml^{-1}); (2) FI-7001 peak (degradation product); (3) FI-7009 peak (impurity); (4) FI-7011 peak (degradation product). Detector sensitivity: 0.001 AUFS at 220 nm.

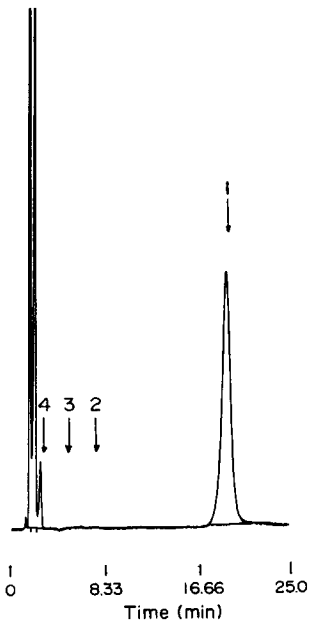

Figure 7
Chromatogram illustrating the stability of sertaconazole cream stored for 6 months at 40°C. (1) Sertaconazole nitrate peak (80 μg ml^{-1}); (2) FI-7001 peak (degradation product not detected at 260 nm); (3) FI-7009 (impurity not detected); (4) FI-7011 (degradation product). Detector sensitivity: 0.001 AUFS at 260 nm.

nitrate. Chromatograms for sertaconazole cream (Figs 7 and 8) stored for 6 months at 40°C show a good resolution in the separation of sertaconazole nitrate, FI-7001 and FI-7011 peaks.

Conclusion

A rapid, precise and selective HPLC-method has been developed for the determination of bulk sertaconazole nitrate. As no interference of the related compounds was observed, the method can be used for quantitative assays of purity. A simple sample preparation enables the use of this method for the determination of the active ingredient in cream formulations with good accuracy, precision and selectivity.

Acknowledgements — The authors wish to thank J. Muñoz of the Analysis Department of Ferrer Group Research Centre for his valuable contribution to the analytical assessment of sertaconazole nitrate.

References

[1] N.R. Badcock, *J. Chromatogr. (Biomed. Appl.)* **306**, 436–440 (1984).

[2] V.L. Pascucci, J. Bennett, P.K. Narang and D.C. Chatterji, *J. Pharm. Sci.* **72**, 1467–1469 (1983).
[3] R. Christinat and H.W. Zulliger, *Arzneim.-Forsch.* **34**, 551–553 (1984).
[4] B. Stuber and K.H. Mueller, *Pharm. Acta Helv.* **59**, 210–217 (1984).
[5] C.M. Riley and M.O. James, *J. Chromatogr. (Biomed. Appl.)* **377**, 287–294 (1986).
[6] C.A. Turner, A. Turner and D.W. Warnock, *J. Antimicrob. Chemother.* **18**, 757–763 (1986).
[7] R.L.H. Lee, *Aust. J. Hosp. Pharm.* **18**, 117–119 (1988).
[8] D.W. Hoffman, K.L. Jones King, C.I. Ravaris and R.D. Edkins, *Anal. Biochem.* **172**, 495–498 (1988).
[9] J.C. Berridge, *J. Chromatogr.* **449**, 317–321 (1988).
[10] A.G. Wright, J.C. Berridge and A.F. Fell, *Analyst* **114**, 53–56 (1989).
[11] K. Selinger, D. Matheou and H.M. Hill, *J. Chromatogr. (Biomed. Appl.)* **434**, 259–264 (1988).
[12] T.A. Tyler and J.A. Genzale, *J. Assoc. Off. Anal. Chem.* **72**, 442–444 (1989).
[13] A.G. Wright, A.F. Fell and J.C. Berridge, *J. Chromatogr.* **464**, 27–38 (1989).
[14] V. Cavrini, A.M. DiPietra and R.J. Gatti, *J. Pharm. Biomed. Anal.* **7**, 1535–1543 (1989).

[Received for review 21 January 1991; revised manuscript received 24 September 1991]