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HPLC analysis of imidazole antimycotic drugs in pharmaceutical formulations*

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Abstract: Reversed-phase HPLC on different column packing materials (Hypersil C-18, Spherisorb-CN, Chromspher-B) is used to obtain selective separations of imidazole antimycotic drugs, such as ketoconazole, clotrimazole, tioconazole, bifonazole, isoconazole, econazole, miconazole and fenticonazole. The use of a post-column on-line photochemical reactor is shown to be useful for the enhancement of the sensitivity of the HPLC analysis with UV detection. The proposed HPLC methods are applied to the analysis of commercial dosage forms (creams) with solid-phase extraction (SPE) procedure, using a diol sorbent, being found to be a convenient technique for the sample preparation giving quantitative drug recovery.

Keywords: *Reversed-phase chromatography (HPLC); imidazole antimycotic drugs; post-column photochemical reaction; solid-phase extraction; pharmaceutical formulations.*

Introduction

Imidazole antimycotic drugs constitute an important class of drugs that continues to expand [1] (Scheme 1). These antimycotics are currently used in a variety of pharmaceutical formulations (tablets, creams, lotions, etc.) at relatively low concentrations (1–2%). The poor detectability (weak UV absorptivity) of these drugs makes it difficult to identify and determine them in formulations of complex composition. A search of the literature suggests [2–13] that the chromatographic techniques (HPLC, GC, HPTLC) represent the favourite approach for the quality control of clotrimazole [2, 7, 10, 11], miconazole [2–5, 7], econazole [2, 5, 6], bifonazole [8], ketoconazole [7, 9] and tioconazole [7, 12, 13] dosage forms. However, a need exists for further enhancement of the selectivity and sensitivity of the methods and for improvements in the sample preparation.

The present study, concerned with the development of HPLC methods suitable for the reliable analysis of imidazole antimycotic dosage forms (primarily creams), had the following objectives: (a) the provision of a selective chromatographic separation of all the cited antimycotic drugs; (b) the enhancement of the method sensitivity by means of a post-column on-line photochemical reaction; (c) the improvement in drug identification by the combination on-line photoreaction–diode array detection; and (d) the development of a practical sample preparation routine based on solid-phase extraction (SPE). The resulting improved HPLC methods were then applied to the analysis of commercial formulations (creams) of clotrimazole, ketoconazole, isoconazole and bifonazole.

Experimental

Materials

Clotrimazole (Bayer, Italy), ketoconazole and miconazole nitrate (Janssen, Belgium), econazole nitrate (Cilag, Italy), isoconazole nitrate (Shering, Italy), fenticonazole nitrate (Recordati, Italy), bifonazole (Menarini, Italy) and tioconazole (Pfizer, Italy) were supplied by their manufacturers. The solutions of clotrimazole (200 μg ml^{-1}), econazole (100 μg ml^{-1}) and ketoconazole (100 μg ml^{-1}) were used as internal standards. All the stock solutions of the drugs were prepared in methanol. For chromatographic separations methanol, acetonitrile and tetrahydrofuran (THF) were of HPLC grade from Farmitalia C. Erba (Italy); water was de-ionized and double distilled. Triethylammonium (TEA) phosphate

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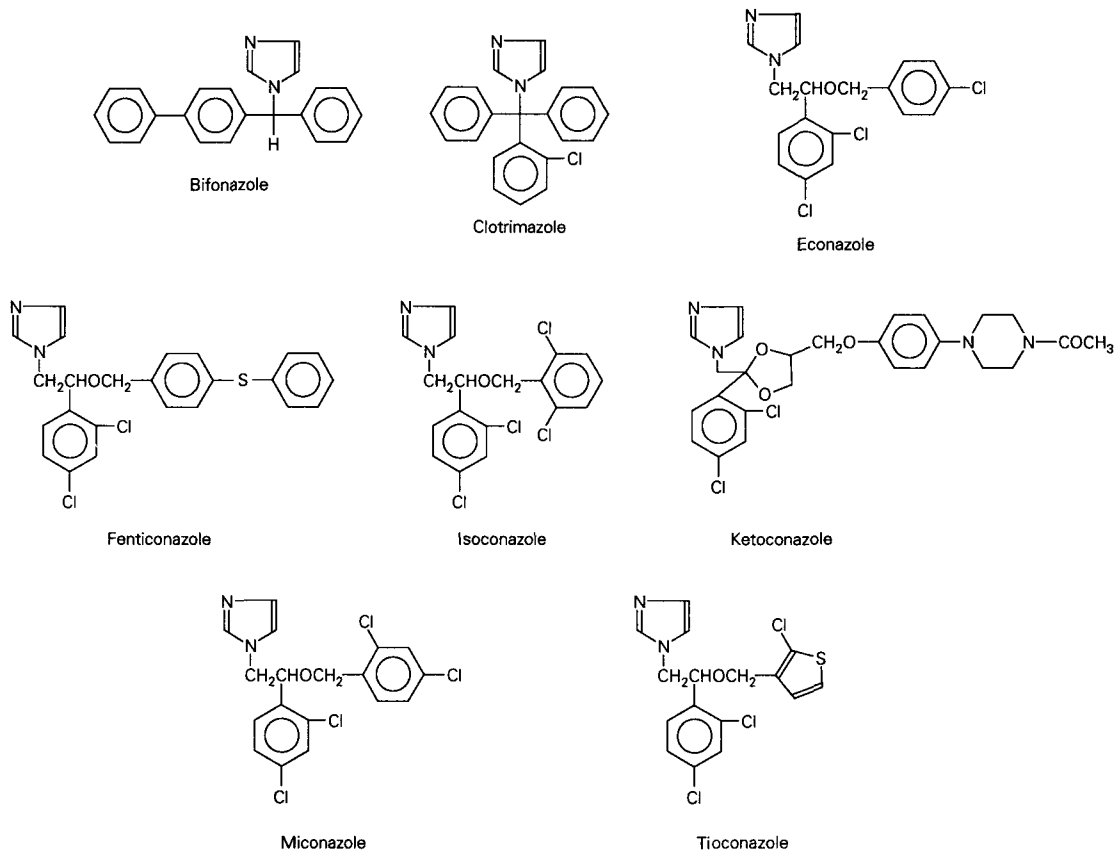

Scheme 1
Structural formulae of imidazole antimycotics.

buffers were prepared by adding diluted phosphoric acid to triethylamine solutions of appropriate concentration (0.03–0.08 M) to adjust the pH to the desired value.

Solid-phase extraction was performed on Bond–Elut cartridges (Diol sorbent; 500 mg) using the Baker 10 SPE system connected to a water aspirator. The SPE columns were conditioned by rinsing with 6 ml of methylene chloride.

Apparatus

The HPLC system consisted of a Varian 5020 chromatograph and a photometric diode array detector (HP 1040 A) connected to a HP 79994A work station. A photoreactor Beam Boost Mod. C6808 (I.C.T., Frankfurt) was arranged on-line between analytical column and detector (reaction coil 10 m × 0.3 mm i.d.). Manual injections were made using a Rheodyne model 7125 injector with a 20 μl sample loop.

Chromatographic separations were carried out at ambient temperature on three different bonded stationary phases: 5 μm Hypersil C-18 column (250 × 4.6 mm i.d.), 5 μm Spherisorb-CN column (250 × 4.6 mm i.d.) and 5 μm Chromspher-B (150 × 4.6 mm i.d.).

Routine analyses of ketoconazole and isoconazole were performed on the Hypersil column using methanol–0.05 M TEA phosphate buffer (pH 7.0) (85:15, v/v) as the mobile phase at a flow rate of 1 ml min^{-1}, whilst the analysis of bifonazole was performed on the nitrile column using acetonitrile–THF–0.05 M TEA phosphate (pH 3.0) (17:17:66, v/v/v) at a flow rate of 1 ml min^{-1}. The analytes were detected by measurement at 230 nm. Hypersil column conditions also were used for the analysis of clotrimazole, but post-column photochemical derivatization was applied and the UV detection was at 270 nm.

Calibration graphs

Standard solutions of ketoconazole (10–40 μg ml^{-1}) containing a fixed concentration (40 μg ml^{-1}) of clotrimazole (the internal standard) were prepared in methanol. Tripli-

cate injections (20 μl) were made for each solution and the peak-height ratio (drug to internal standard) was plotted against the corresponding concentration ratio to obtain the calibration graphs. Similarly, the calibration graphs for isoconazole, bifonazole and clotrimazole were constructed (concentrations and the internal standards were as shown in Table 1).

Sample preparation

Tablets. An amount of powdered tablets, equivalent to about 30 mg of ketoconazole, was extracted with 100 ml of methanol under sonication for 5 min. The extract was filtered, a 2-ml aliquot was added to 5 ml of the internal standard solution and then made up to 25 ml with methanol.

Creams. A sample equivalent to about 5 mg of the drug, was treated with 30 ml of methylene chloride and, after sonication for 3 min, was diluted to 100 ml with the same solvent. When bifonazole was analysed, the cream sample was first treated with 4 ml of methanol and then with methylene chloride as above described. The resulting opalescent solution was filtered and a 2-ml aliquot was applied to the conditioned SPE diol column. After sample application the column was washed with 2 ml of methylene chloride–methanol (4:1, v/v) and 1 ml of methanol (ketoconazole, isoconazole and bifonazole) or with 2 ml of methylene chloride for clotrimazole. The elution step was then performed as follows:

Ketoconazole and isoconazole were eluted with 3 × 1 ml of the mobile phase (pH 7.0) for HPLC analysis, the eluates were added to 1.0 ml of the internal standard solution (clotrimazole for ketoconazole, econazole for isoconazole) and the volume was adjusted to 5 ml with methanol.

Bifonazole was eluted with 3 × 1 ml of the mobile phase for HPLC analysis (pH 3.0), the eluates were added to 0.5 ml of the internal standard (ketoconazole) solution diluting to 5 ml with the same mobile phase.

Clotrimazole was eluted as ketoconazole and isoconazole and the eluates were added to a 0.5 ml of the internal standard (ketoconazole) solution diluting to 5 ml with the same mobile phase.

Assay procedure

The sample solutions obtained from the SPE procedure were analysed by the HPLC method with UV detection at 230 nm (photo-reactor OFF) for ketoconazole, isoconazole and bifonazole and at 270 nm (photo-reactor ON) for clotrimazole. The drug content in each sample analysed was determined by comparison with an appropriate standard solution.

Results and Discussion

Chromatography

All the antimycotic drugs examined are structurally characterized by the presence of an imidazole moiety. Chromatographic separations of these basic drugs were investigated on three different column packing materials under reversed-phase mode conditions. Using C-18 Hypersil column, according to literature data [12, 14–17] and previous experience [5], triethylamine (TEA) was used as a mobile phase additive to reduce the adverse silanol interactions. In effect, TEA proved to be an effective amine modifier providing symmetric peaks for all the imidazole antimycotics at pH 7.0 (Fig. 1). The resolution obtained, however, was partial, clotrimazole coeluting with bifonazole and econazole with tioconazole. In order to improve the selectivity of the reversed-phase system, tetrahydrofuran (THF) was introduced as additional organic modifier in the mobile phase. Thus, using a ternary mixture methanol–THF–0.05 M TEA phosphate, pH 7.0 (34:22:44, v/v/v) the complete resolution of clotrimazole (t_r = 8.0), bifonazole (t_r = 9.8), tioconazole (t_r = 17.0) and

Table 1
Data for the calibration graphs (n = 6) for the HPLC determination of selected imidazole antimycotics

Drug	Internal standard	Slope	Intercept	Correlation coefficient	Working range (μg ml^{-1})	RSD (%)
Ketoconazole	Clotrimazole	1.2876	0.0042	0.9999	10–40	0.65
Isoconazole	Econazole	0.6789	0.0130	0.9997	20–70	0.82
Bifonazole	Ketoconazole	0.5864	0.0427	0.9998	20–70	0.73
Clotrimazole	Ketoconazole	0.8854	0.0175	0.9998	10–60	0.85

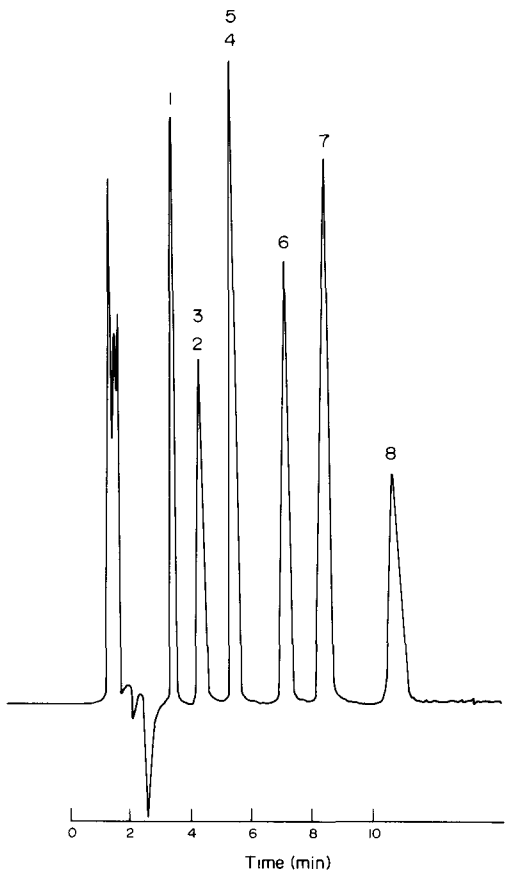

Figure 1
HPLC separation of imidazole antimycotics: ketoconazole (1), clotrimazole (2); bifonazole (3); tioconazole (4); econazole (5); isoconazole (6); miconazole (7); and fenticonazole (8). Column: Hypersil C-18 (5 μm); mobile phase: methanol–aqueous 0.05 M TEA phosphate, pH 7.0 (85:15, v/v) at a flow rate of 1 ml min^{-1}. Detection at 230 nm.

econazole (t_r = 22.5) was achieved. The pH of the 0.05 M TEA phosphate solution in the mobile phase was of importance; lower pH values (4.5 and 3.0) were responsible for peak tailing and broadening. In contrast, when a cyano-column (Spherisorb-CN) was used in reversed-phase mode, symmetrical peaks were obtained by simply selecting low pH values for the TEA phosphate solution. A cyano-column already proposed for reversed-phase separation of basic drugs [12, 18–20], proved to be suitable for the separation of the imidazole antimycotic drugs and THF confirmed its good selectivity. As shown in Fig. 2, full separation of all the examined antimycotics was accomplished with a simple binary mixture THF–0.05 M TEA phosphate, pH 3.0 (30:70, v/v). The partial substitution of THF with methanol or acetonitrile led to inferior resolution between the pair bifonazole–tioconazole but the conditions were adequate and convenient for the analysis of pharamceutical formulations (Fig. 3).

Finally, a derivatizated silica material, designed specifically for basic compounds, (Chromspher-B) was evaluated. This material (silica support coated with a monomolecular layer of the polymeric C18 stationary phase) was found to be appropriate and versatile, giving symmetrical peaks for all the compounds over the pH range of 3.0–7.0. In addition, good band shape also was maintained when the amino modifier (TEA) in the mobile phase was omitted. A representative chromatogram is reported in Fig. 4. The effect of pH on the separation was evaluated. On increasing the pH (3.0–7.0) of the aqueous buffer, with and without TEA, a general increase of k' was observed for all the drugs, the effect being marked for pH values greater than 4.5, with slight alteration of the system selectivity. This behaviour suggests a retention

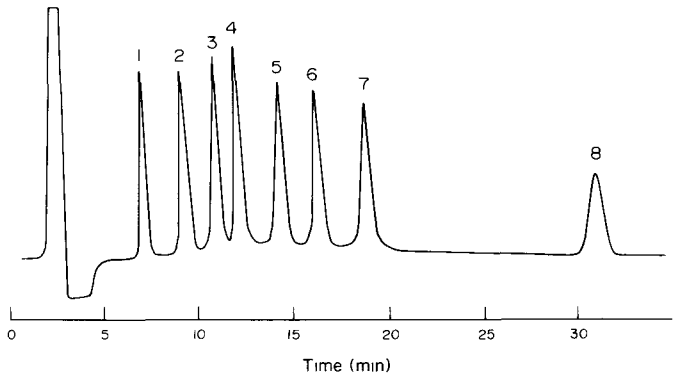

Figure 2
HPLC separation of imidazole antimycotics (compounds as in Fig. 1) on Spherisorb-CN column using THF–aqueous 0.05 M TEA phosphate, pH 3.0 (30:70, v/v) as the mobile phase at a flow rate of 1 ml min^{-1}. Detection at 230 nm.

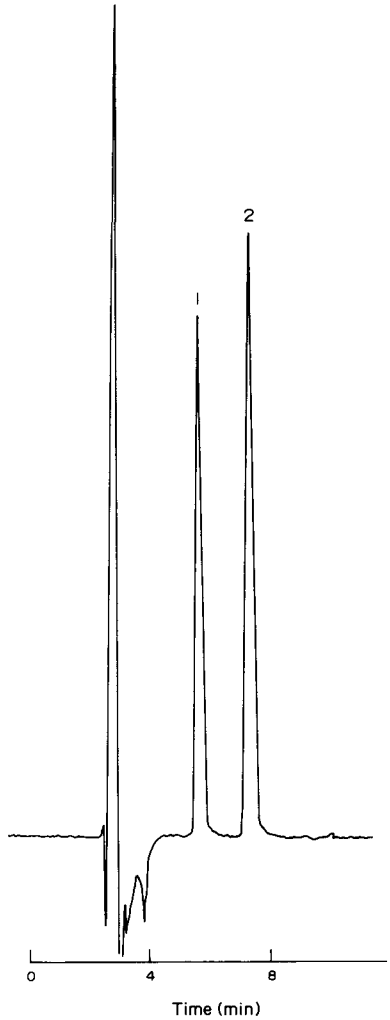

Figure 3
HPLC chromatogram obtained from a commercial bifonazole cream: ketoconazole (1; the internal standard), bifonazole (2). Column: Spherisorb-CN; mobile phase: acetonitrile–THF–aqueous 0.05 M TEA phosphate, pH 3.0 (17:17:66, v/v/v) at a flow rate of 1 ml min^{-1}. Detection at 230 nm.

mechanism predominantly governed by solvophobic interactions (reduced silanol effects). This packing material can offer a wider variety of chromatographic conditions suitable for the HPLC analysis of imidazole antimycotics.

Detection

HPLC determinations of imidazole antimycotics are usually carried out with UV detection at low wavelength (214–230 nm) in order to achieve higher sensitivity. Within this range, UV spectra often do not exhibit definite absorption maxima and the measurements can be affected by the matrix interferences and by baseline noise. On the other hand, at higher

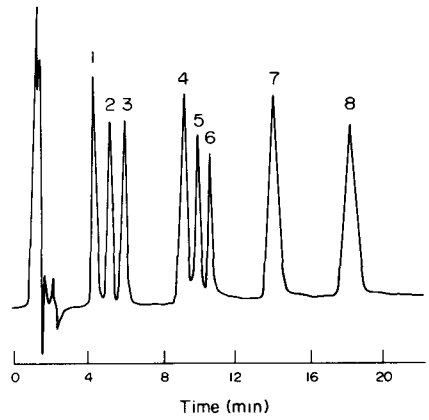

Figure 4
HPLC separation of imidazole antimycotics (Fig. 1) on Chromspher-B using methanol–THF–aqueous 0.02 M KH_2PO_4, pH 4.5 (17:38:45, v/v/v) as the mobile phase at a flow rate of 1 ml min^{-1}. Detection at 230 nm.

wavelengths the molar absorptivity of the compounds is generally weak. Thus, it was considered of interest to evaluate the ability of a photochemical reactor, arranged on-line between column and detector, to modify the spectral properties of the analytes, with the view of obtaining absorption maxima at higher wavelengths. The results obtained can be summarized as follows: (a) marked, useful alterations of the UV spectral profile were obtained for clotrimazole, ketoconazole and tioconazole [Fig. 5(a)–(c)]; (b) slight but significant UV spectral modifications were observed for the other imidazole drugs; (c) the results were comparable using aqueous buffer at pH 7.0 and 4.5 in the mobile phase, while they were less satisfactory at pH 3.0; (d) using a 10 m reaction coil, significant peak broadening was not observed and retention times 1 min longer for all the compounds were obtained.

The possibility of inducing photochemical modifications in the structure of some imidazole antimycotics, resulting in more favourable spectral conditions for UV detection, was exploited for HPLC applications. In particular, the considerable enhancement in the sensitivity obtained for clotrimazole [Fig. 5(a)], led to the development of a HPLC method, involving on-line photochemical reaction and detection at 270 nm, for its determination in cream samples [Fig. 5(d)]. It should be noted that a negative response was obtained for clotrimazole, when amperometric detection was used [20].

These data, moreover, emphasize the potential of the combination photochemical reactor–

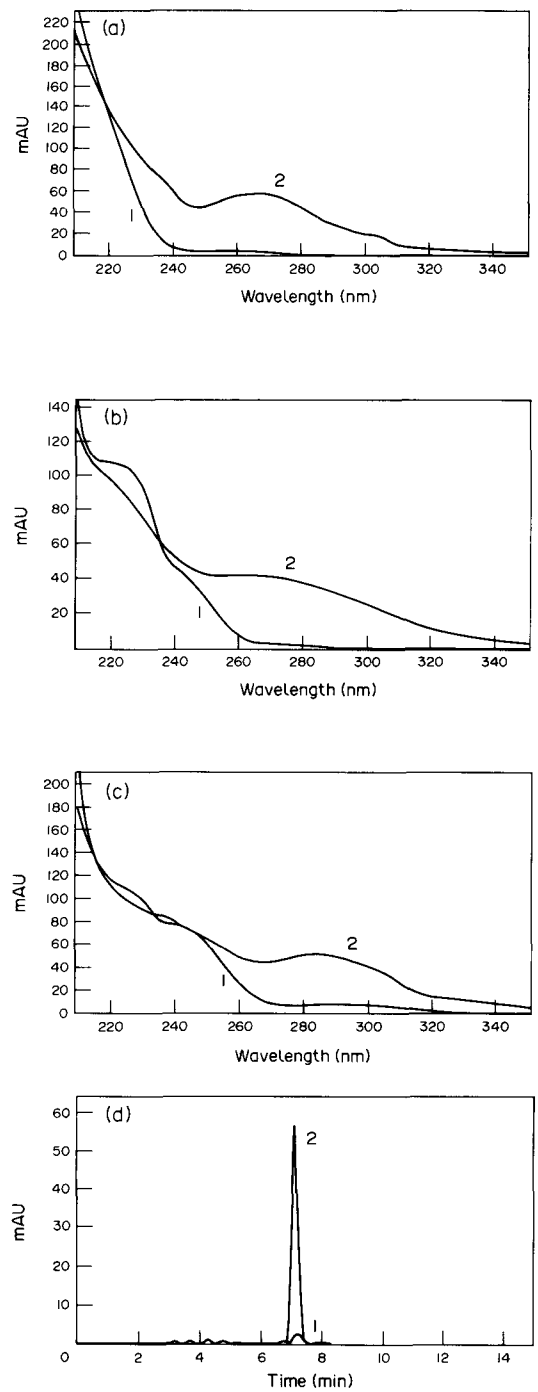

Figure 5
UV spectra of clotrimazole (a), tioconazole (b) and ketoconazole (c) with photoreactor OFF (1) and ON (2), obtained by diode array detector under chromatographic conditions of Fig. 1. (d) HPLC chromatogram of clotrimazole (chromatographic conditions as in Fig. 1) with photoreactor OFF (1) and ON (2) with detection at 270 nm.

diode array detection to achieve spectral information useful for the unambiguous identification of this class of drugs.

Analysis of pharmaceutical formulations

Among the various commercially available formulations containing imidazole antimycotics, interest was primarily directed to the creams because of their wider use and their complex composition. Following previous studies [5], the applicability of the solid-phase extraction (SPE) to the quantitative extraction of imidazole antimycotics from the cream excipients was investigated. The proposed SPE procedure based on Bond–Elut diol sorbent (experimental section), proved to be capable of providing an effective sample clean-up with essentially quantitative analyte recovery for all the drugs examined: namely ketoconazole, isoconazole, clotrimazole and bifonazole. These data suggest that the SPE method, with minor adjustments depending on the sample nature, is of general utility for the analysis of members of this class of drugs. HPLC analyses of the commercial dosage forms were carried out under the chromatographic conditions of Fig. 1 (ketoconazole and isoconazole), Fig. 3 (bifonazole) and Fig. 5(d) (clotrimazole). Linear relationships were found between the peak-height ratios (drug to internal standard) and the corresponding concentration ratios (Table 1). For ketoconazole, isoconazole and bifonazole direct detection at 230 nm was used, whilst for clotrimazole the detection was at 270 nm after on-line photochemical reaction. In each case, the method precision was good as indicated by the relative standard deviation (RSD) of the peak height ratio (analyte to S.I.) obtained from replicate (n = 7) analyses of a single drug solution (30 μg ml^{-1}). The results obtained (Table 2) were in close agreement with the claimed content for each product; the method accuracy was verified by analysing samples spiked with known quantities of drugs.

Conclusion

Selective chromatographic separations of imidazole antimycotic drugs can be accomplished in reversed-phase mode using different packing materials; among these, a material designed for basic compounds (Chromspher-B) proved to be of interest for its versatility. The use of a post-column on-line photochemical reaction was found to be a useful approach that significantly enhances the sensitivity of HPLC methods with UV detection at high wavelength values (270–

Table 2
Assay results for the HPLC analysis of some imidazole antimycotics in commercial formulations

Drug	Formulation	Column	Found (%)*	RSD (%)
Ketoconazole	Cream	Hypersil C-18	102.26	0.90
	Tablets	Hypersil C-18	102.18	0.80
Isoconazole	Cream	Hypersil C-18	101.53	1.0
Bifonazole	Cream	Spherisorb-CN	100.57	1.15
Clotrimazole†	Cream	Hypersil C-18	101.2	1.30

*Average of five determinations and expressed as percentage of the claimed content.
†On-line photochemical reactor was used.

280 nm). This was observed for clotrimazole, ketoconazole and tioconazole, while for the other antimycotic drugs the effect was less marked. In each case, the combination of the post-column photochemical reaction with photodiode array detection constitutes an effective analytical tool for the identification of this class of compounds. Finally solid-phase extraction (SPE), using a diol sorbent, proved to be a convenient method for the sample (creams) clean-up.

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