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High-pressure liquid chromatographic (HPLC) analysis of imidazole antifungals in commercial dosage forms

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Summary

A high-pressure liquid chromatographic (HPLC) method is presented for determining clotrimazole, miconazole nitrate and econazole nitrate in various commercial formulations. The analysis was performed on a reversed-phase column (RP 18) under isocratic conditions using a UV detector (230 nm). The described procedure proved to be more specific and versatile than the pharmacopoeia methods.

Introduction

Clotrimazole, 1-[(2-chlorophenyl)diphenylmethyl]imidazole (I), miconazole nitrate, 1-[2,4-dichloro-β-(2,4-dichlorobenzyloxy)phenethyl]imidazole mononitrate (II) and econazole nitrate, 1-[2,4-dichloro-β-(*p*-chlorobenzyloxy)phenethyl]imidazole mononitrate (III), are 3 chlorinated imidazole derivatives widely used in the treatment of fungal infections. While clotrimazole is useful mainly for topical infections, miconazole and econazole have recently become available also for the treatment of systemic mycoses (D'Arcy and Scott, 1978; Heel et al., 1980).

These drugs are a relatively recent addition to the range of clinically useful antifungals and, at present, little information pertaining to their analysis in pharmaceutical dosage forms is available. The pharmacopoeia method (USP XX-NF XV, 1980; Addendum a to 1S USP-NF, 1980) for quantitative clotrimazole determination in creams, topical solutions and tablets is based on a non-specific semimicrotitrimetric procedure using sodium lauryl-sulphate. A sensitive colorimetric method for the estimation of miconazole nitrate in commercial dosage forms was

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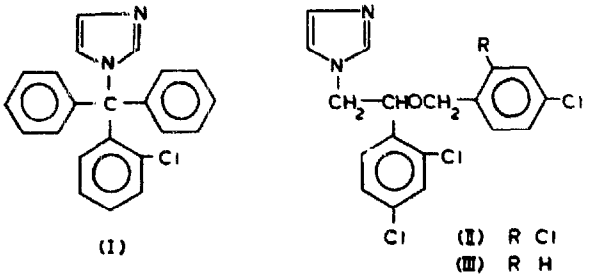

Fig. 1. Chemical structures of clotrimazole (I), miconazole (II) and econazole (III).

developed recently (Cavrini et al., 1981), based on the yellow complex formation between the drug and bromocresol green.

The present study was undertaken to provide a more specific and versatile high-pressure liquid chromatographic (HPLC) procedure, suitable for assay as well as identity test of these antifungals. The HPLC method reported (Brodie et al., 1978) for quantitative plasma econazole determination was chosen for initial investigations but the chromatographic conditions were found to be too retentive. The reversed-phase HPLC method described in this paper is rapid, specific for each imidazole antifungal investigated and suitable for its analysis using the pharmaceutical dosage forms commercially available.

Materials and Methods

Apparatus and reagents

A Varian liquid chromatograph, model 5000, equipped with a Valco (Field Instruments, Richmond, U.K.) high-pressure injection valve fitted with a 10-μl sample loop was used. All measurements were made at ambient temperature using a variable wavelength detector UV-50 (Varian), connected with y/t recorder with integrator (Bryans Southern Instruments). The detector wavelength was adjusted to 230 nm with a sensitivity of 0.05 a.u.f.s. The methanol used was HPLC grade and the water was double-distilled in glass. Ammonium dihydrogen phosphate and sodium lauryl-sulphate were C. Erba RPE grade.

Analytical column and mobile phase

The chromatography was performed on a reversed-phase MicroPak MCH-10 (monomeric C_{18} bonded onto 10 μm silica gel) column, 30 cm × 4 mm i.d.

The mobile phase was methanol–aqueous 0.05 M ammonium dihydrogen phosphate (85 + 15), filtered through a 0.2 μm mini-capsule filter (Gelman Sciences) and degassed before use. The column flow-rate was set at 2 ml/min.

Standard solutions and calibration curves

Clotrimazole (Bayer Italia), miconazole nitrate (Italfarmaco) and econazole nitrate (Cilag-Chemie Italiana) were used as received. Stock solutions for each drug were

prepared by dissolving about 20 mg, accurately weighed, of the compound in methanol and diluting to 100 ml with methanol.

Aliquots of 2, 3, 4 and 5 ml of miconazole nitrate stock solution were transferred into separate 10-ml volumetric flasks. To each were added 3 ml of econazole nitrate (as the internal standard) stock solution and the contents diluted to volume with methanol. Duplicate preparations were made for each standard solution. A 10-μl injection volume was used and all injections were carried out in triplicate. A calibration curve was constructed by plotting the peak height ratios of miconazole to the internal standard versus weight ratios of miconazole to the internal standard. The calibration curves for econazole nitrate and clotrimazole were prepared in the same manner, using as the internal standard, clotrimazole and econazole, respectively.

Sample preparation

Methanol was used as the extracting solvent as the imidazole antifungals analyzed were readily soluble in it.

Tablets. Ten tablets were selected randomly, weighed and triturated to a fine powder. An accurately weighed sample of powder, equivalent to approximately 40 mg of clotrimazole, was extracted twice with 20-ml aliquots of methanol in a 50-ml screw-capped centrifuge tube by vigorous agitation for 10 min. The methanolic extracts were then filtered and combined in a 50-ml volumetric flask and brought to volume with methanol. The resulting solution was diluted further, 5.0 ml to 50.0 ml with methanol. A 5-ml aliquot of this solution was transferred into a 10-ml volumetric flask containing exactly 3 ml of the internal standard (econazole nitrate) solution. It then was diluted to volume with methanol and shaken.

Creams, lotions and powders. An amount equivalent to approximately 10 mg of the active ingredient was extracted twice with 20-ml aliquots of methanol in a 50-ml screw-capped centrifuge tube by vigorous agitation for 10 min. The methanol extracts then were filtered and combined in a 50-ml volumetric flask and brought to volume with methanol. A 3-ml aliquot of the resulting solution was transferred into 10-ml volumetric flask containing exactly 3 ml of the internal standard solution. It then was diluted to volume with methanol and shaken.

Assay procedure

Prior to introdution into high-pressure liquid chromatograph the analytical solutions were filtered through a 0.45-μm teflon membrane filter (Gelman Sciences). All formulations were first chromatographed without internal standard to ensure that interfering peaks were not present. A 10-μl aliquot of the analytical solutions was injected and triplicate injections were made for each solution. The standard solutions were chromatographed both at the beginning and at the end of a day's run and these results were averaged. The peak height ratio of the drug to internal standard was used for further calculations.

Results and Discussion

The composition of the mobile phase was adjusted after several trials with mixtures of acetonitrile–water and methanol–water of varying compositions. The mobile phase chosen brings about an optimum separation of the 3 imidazole antifungals in a reasonable time and with peaks well formed (Fig. 2). The order of elution was: clotrimazole ($t_r = 3.3$), econazole nitrate ($t_r = 4.7$) and miconazole nitrate ($t_r = 7.4$), according to a reversed-phase mode of separation. The k′-values for I, II and III were 2.00, 3.27 and 5.73, respectively. The very adequate separation

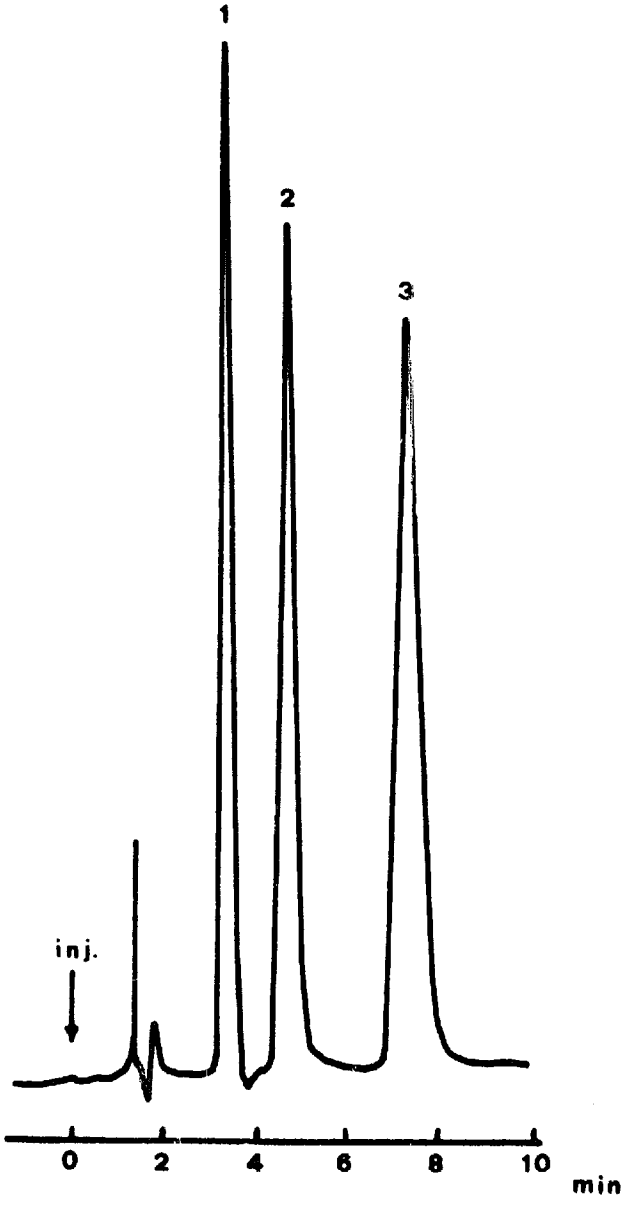

Fig. 2. Separation of imidazole antifungals on a RP_{18} column; mobile phase: methanol–aqueous 0.05 M $NH_4H_2PO_4$ (85 + 15); flow rate: 2 ml/min. 1, clotrimazole; 2, econazole nitrate; 3, miconazole nitrate.

of I, II and III makes the HPLC procedure a specific and rapid method for the identification of these structurally related antifungal drugs. The official USP method for clotrimazole identification in various formulations involve a time-consuming TLC procedure which was found to be unable to differentiate miconazole from econazole.

For quantitative determination, a linear calibration curve was found for each drug in the range 0.4–1.0 μg of amount injected; regression analysis of the data for I, II and III generates the lines:

(I) $y = 1.1670x + 0.01029$ correlation coefficient $r = 0.9990$

(II) $y = 0.6114x + 0.01320$ correlation coefficient $r = 0.9997$

(III) $y = 0.8443x + 0.00923$ correlation coefficient $r = 0.9999$

where y and x are response ratios and weight ratios, respectively.

The reproducibility of the chromatographic procedure was indicated by replicate injections of the same standard solution; e.g. the repetitive analysis of a single solution of miconazole nitrate gave a relative standard deviation of 0.43% (n = 7).

The described HPLC method was applied to the analysis of different commercial single-component dosage forms of imidazole antifungals. The results demonstrate (Table 1) the general applicability and feasibility of the HPLC procedure as a reliable monitor of drug content in the various types of commercial formulations. The relative standard deviation for the whole analysis (weighing, extraction and chromatography) varied from formulation to formulation (Table 1). The internal standard was selected such that the analysis was carried out in the shortest time. The excipients carried through the extraction (parabens, benzoic acid and other inactive ingredients present in the various formulations) all gave peaks at, or close to, the solvent peak and so did not interfere with the analysis.

For comparison purposes, the clotrimazole in tablets and creams was also assayed

TABLE 1

ASSAY RESULTS FOR COMMERCIAL IMIDAZOLE ANTIFUNGAL DOSAGE FORMS

Active ingredient	Dosage forms	Internal standard	HPLC [a]		USP [a]	
			% of claim	RSD %	% of claim	RSD %
Miconazole	Cream	Econazole	100.70	1.32	98.60	1.67
	Powder		99.20	0.71		
Econazole	Cream	Clotrimazole	99.34	1.30	99.30	0.43
	Lotion		100.60	0.73		
	Powder		98.52	0.85		
Clotrimazole	Cream	Econazole	99.40	0.50	99.13	0.70
	Tablet		99.15	0.65	97.66	0.26

[a] Average of 5 determinations.

by the sodium lauryl-sulphate titrimetric method as described in the USP XX. The same procedure was applied to estimation of miconazole and econazole in creams, using sodium lauryl-sulphate solution standardized against miconazole nitrate and econazole nitrate, respectively. With these formulations, the results from both HPLC and USP methods are comparable (Table 1). The USP method, however, was found to be unsuitable for analyzing the powders, since it is tedious due to a time-consuming two-phase (water–chloroform) separation and it is inaccurate due to difficult end-point visualization.

In summary, the HPLC method is more specific and versatile than the official USP procedures. It could serve as a rapid identity test for the imidazole antifungal drugs and for their quantitative determination in most pharmaceutical dosage forms commercially available.

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References

Brodie, R.R., Chassaud, L.F. and Walmsley, L.M., High-performance liquid chromatographic determination of the antimycotic agent econazole in plasma. J. Chromatogr., 155 (1978) 209–213.

Cavrini, V., Di Pietra, A.M. and Raggi, M.A., Colorimetric determination of miconazole nitrate in pharmaceutical preparations. Pharm. Acta Helv., 56 (1981) 163–165.

D'Arcy, P.F. and Scott, E.M., Antifungal agents. In E. Jucker (Ed.), Progress in Drug Research, Vol. 22, Birkhauser-Verlag, Basel, 1978, pp. 94–147.

Heel, R.C., Brogden, R.N., Pakes, G.E., Speight, T.M. and Avery, G.S., Miconazole: a preliminary review of its therapeutic efficacy in systemic fungal infections. Drugs, 19 (1980) 7–30.

United States Pharmacopeia XX–National Formulary XV (1980), pp. 159–160 and Addendum a to 1 Supplement to USP XX–NF XV (1980), pp. 119–120.