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Determination of imidazole antimycotics in creams by supercritical fluid extraction and derivative UV spectroscopy[1]

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Abstract

A supercritical fluid extraction (SFE) method was developed for the isolation of imidazole antimycotic drugs (miconazole, econazole, clotrimazole and bifonazole) from cream preparations. The SFE process involved static (1 min) and dynamic (4 min) extraction steps using pure and 10% methanol modified carbon dioxide. The SFE step was then followed by derivative UV spectrophotometric analysis. The method proved to be suitable for quality control assays of the examined antimycotics in commercial cream formulations.

Keywords: Supercritical fluid extraction; Imidazole antimycotics; Pharmaceutical creams; Derivative UV spectroscopy; Sample preparation

1. Introduction

Sample preparation is an important and critical part of any analytical procedure. When pharmaceutical formulations of complex composition have to be analyzed, considerable efforts are required to develop rapid, selective and quantitative extraction methods. Over the past few years, extraction with supercritical fluids (SFE) has attracted great interest [1–3], because this technique offers a promising approach to the selective isolation of drugs from various matrices [4–9]. Super critical fluid extraction (SFE) combines liquid-like solvating capabilities with almost gas-like transport properties; moreover, using carbon dioxide or carbon dioxide-rich mixture as extraction media, the use of hazardous organic solvent can be eliminated or kept to a minimum.

The aim of the present study was to evaluate the feasibility of SFE as a sample preparation step in the analysis of complex formulations (creams) containing imidazole antymicotics (miconazole, econazole, clotrimazole and bifonazole). The ef-

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Table 1
Supercritical fluid extraction (SFE) conditions

SFE parameters	Drugs			
	Miconazole	Clotrimazole	Econazole	Bifonazole
Carbon dioxide density (g ml^{-1})	0.88	0.88	0.91	0.91
Carbon dioxide pressure (bar)	380	380	370	370
Extraction temperature (°C)	60	60	50	50
CO_2 flow rate (ml min^{-1})	4	4	4	4
Modifier[a]	10% MeOH	10% MeOH	10% MeOH	10% MeOH
Equilibration time (min)	1	1	1	1
Extraction time (min)	4	4	4	4
Elution solvent	MeOH	CH_2Cl_2	CH_2Cl_2	CH_2Cl_2
Elution volume (ml)	1.7	1.7	1.5	1.5
Elution number	3	3	4	4
Elution rate (ml min^{-1})	2	2	2	2
Restrictor temp. (°C)				
Extraction	45	45 and 70	45 and 70	45 and 70
Elution	45	35	35	35
Trap temp. (°C)				
Extraction	40	40 and 66	40 and 66	40
Elution	40	30	30	30

[a] Used only in the third and fourth extraction step.

fect of the extraction parameters (temperature, pressure, supercritical fluid composition and extraction time) on the drug recovery were investigated and the drug content was then determined by derivative UV spectroscopy. The results were compared with those obtained by a solid phase extraction (SPE) procedure.

2. Experimental

2.1. Materials

Miconazole nitrate (Janssen, Belgium), bifonazole, econazole and clotrimazole (Sigma chimica, Italy) were used as received without further purification. All the other chemicals and solvents were obtained from C. Erba reagents (Milan, Italy).

Phosphate buffer solutions were prepared by standard methods. Instrumental-grade liquid carbon dioxide was supplied in cylinders with a dip tube from SIAD (Bologna, Italy). Bond-Elut 2-OH (500 mg/2.8 ml) cartridges from Analytichem (Varian, USA) were used for solid-phase extractions. Before use, the SPE columns were conditioned by rinsing with 6 ml of dichloromethane and then 1 ml of *n*-hexane.

2.2. Apparatus

UV spectra were recorded on a Jasco Uvidec 610 double beam spectrophotometer using 1 cm quartz cells with a slit width of 1 nm. Suitable settings were: scan speed 100 nm min^{-1}, absorbance scale 0–2.0 and $\Delta\lambda = 3$ for the derivative method.

Supercritical fluid extractions were performed with a computer-controlled HP 7680 T SF3 system (Hewlett Packard), equipped with a variable restrictor and an SPE trap.

The HPLC system consisted of a Waters 504 pump and a Jasco Uvidec 100 V variable wavelength UV detector connected to a HP 3396 series integrator.

2.3. SFE extraction

A cream sample, equivalent to ~ 10 mg of antimycotic drug, was mixed with celite and anhydrous sodium sulfate (1: 4: 2, w/w/w), and placed

in the thimble into the extraction chamber. The general extraction program included four static (1 min equilibration) steps followed by a dynamic (1 min) extraction step. The supercritical fluid was pure carbon dioxide (first and second extraction steps) and then 10% methanol-modified carbon dioxide; it was delivered through the restrictor to an internal trap packed with ODS (hypersil) material. The sudden depressurization at the restrictor caused the supercritical fluid to evaporate, depositing the extracted analytes on the trap. The analytes were then eluted from the trap into vials with an appropriate solvent and the collected extracts, after making to volume, were subjected to derivative UV spectrophotometric analysis.

The specific SFE conditions used for the various imidazole antimycotic drugs are summarised in Table 1.

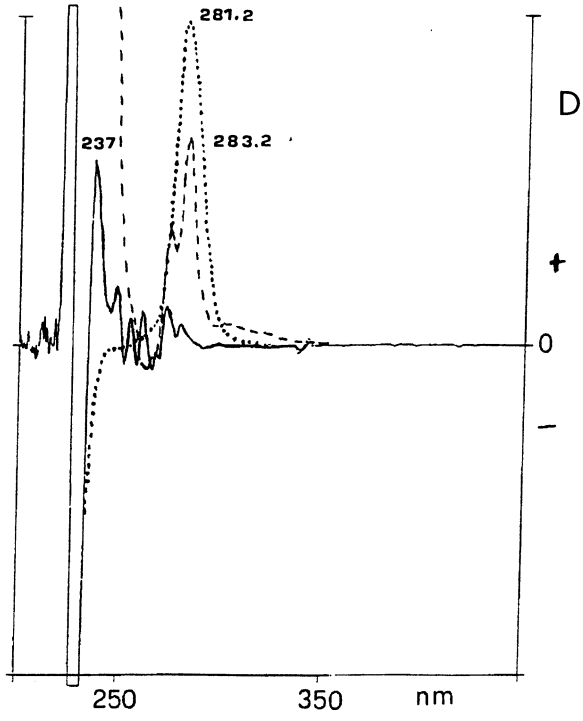

Fig. 1. Second-order derivative UV spectrum of clotrimazole (—) and first-order derivative UV spectra of econazole (---) and bifonazole (· · · ·).

2.4. SPE extraction

A previously described procedure [10–13] was applied. Briefly, a 2 ml aliquot of the sample solution in dichloromethane was applied to an SPE diol column; after washings with dichloromethane-*n*-hexane of appropriate composition, the retained drugs were eluted with methanol (econazole and bifonazole) or methanol–phosphate buffer (pH 7.4) 4: 1 (v/v).

3. Results and discussion

Antimycotic imidazole drugs are widely used as cream formulations. The low drug content (1–2%) and the poor spectroscopic properties (weak molar absorptivity) constitute an analytical problem for their determination in complex matrices. Therefore, there is a real need for an appropriate sample preparation and to this end solid phase extraction (SPE) proved to be a suitable technique for both HPLC [10,12] and spectrophotometric [13] determinations. As an alternative approach, supercritical fluid extraction (SPE) was evaluated in this study, followed by a derivative UV spectrophotometric analysis.

3.1. SFE extraction

The cream sample was mixed with celite and anhydrous sodium sulfate in the ratios 1: 4: 2 (w/w/w). Celite was used to disrupt the sample continuity and anhydrous sodium sulfate as a drying agent. For the method development, the effects of the various SFE parameters were studied. In particular, the effects of supercritical fluid density, the extraction temperature and pressure, the flow rate and the organic modifier concentration on the extraction performance (first step of the over-all process) were evaluated. The second step, including depressurization and evaporation of the supercritical fluid, analyte deposition on C-18 trap and subsequently their recovery, involved the appropriate choice of restrictor and trap temperature, and of the nature and volume of the elution solvent.

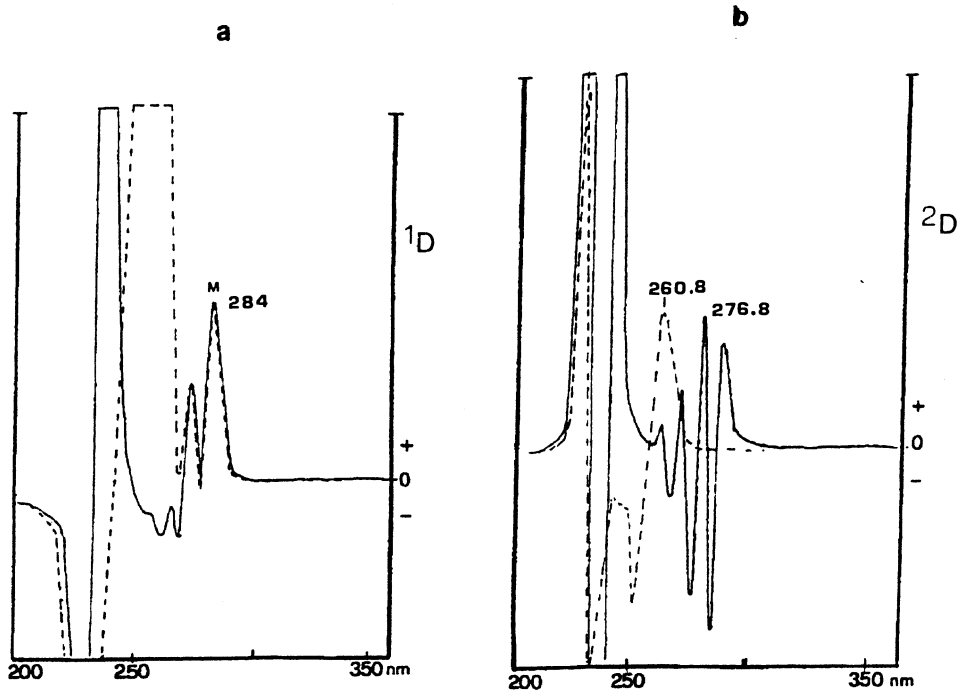

Fig. 2. (a) First-order dervative UV spectra of miconazole (—) and a standard solution (---) containing miconazole and hydrocortisone in the same molar ratio as in the commerical formulation. (b) Second-order derivative UV spectra of miconazole (—) and hydrocortisone (---).

The optimal conditions found for the extraction of the examined imidazole antimycotics from their commercial formulations are summarized in Table 1.

3.2. Analysis of commercial formulations

According to previous experiences [11,13], the analysis of the drugs in the sample extracts was accomplished by derivative UV spectroscopy.

This simple technique offers the opportunity to achieve useful qualitative information on the analyte identity, to suppress interference from residual excipients and to enhance the sensitivity in the determination of benzenoid drugs, such as the examined imidazole antimycotics.

First-order derivative spectrum was used for the analysis of econazole and bifonazole (Fig. 1), while second-order was used for clotrimazole. For the determination of miconazole in the presence of hydrocortisone first- and second-order were both applied (Fig. 2).

Linear relationships between the selected amplitudes and the drug concentration have been found for each drug (Table 2). Using the derivative mode, the selective determination of miconazole in the presence of hydrocortisone can be performed, because the $^1D_{284}$ amplitude (Fig. 2a) and $^2D_{276.8}$ amplitude (Fig. 2b) are specific for miconazole and not affected by the derivative UV spectrum of hydrocortisone. The simultaneous determination of hydrocortisone, based on its characteristic $^2D_{260.8}$ amplitude, was attempted but significant interference from miconazole was observed; for this application, a previously developed HPLC method [12] was used.

Commercial pharmaceutical creams were then analysed by the SFE-derivative spectrophotometric methods and the obtained results are reported in Table 3. As shown, the drug content was found to be in close agreement with the label claim, the recoveries being essentially quantitatives ($>97\%$) with a satisfactory infra-assay reproducibility. For

Table 2
Data for the calibration graphs ($n = 6$)

Drug	Method	Slope	Intercept	Correlation coefficient	Concentration range (mg ml^{-1})
Miconazole	$^{1}D_{284\ nm}$	5.060	−0.012	0.999	0.120–0.320
	$^{2}D_{276.8\ nm}$	12.43	−0.031	0.999	0.120–0.320
Clotrimazole	$^{2}D_{237.2\ nm}$	1.981	−0.009	0.995	0.018–0.140
Bifonazole	$^{1}D_{281.2\ nm}$	4.490	−0.049	0.997	0.062–0.017
Econazole	$^{1}D_{283.2\ nm}$	3.040	−0.020	0.998	0.060–0.161

Table 3
Results for the assay of imidazole antimycotic drugs in commercial cream formulations

Formulation	Drug	SFE % Found (RSD%)		SPE % Found (RSD%)
		50°C	60°C	
Daktacort	Miconazole		97.3 (2.15)	98.50 (1.80)
Canesten	Clotrimazole		97.1 (2.10)	97.60 (1.20)
Desamix F	Clotrimazole		98.8 (2.25)	100.50 (1.30)
Bifazol	Bifonazole	100.0 (2.50)	98.6 (2.80)	100.50 (1.35)
Pevaryl	Econazole	102.0 (2.10)		100.70 (1.80)

The data are the average of five determinations.

a validation of the method developed, the SFE recoveries were compared with those obtained for the same formulations by an independent and established method based on solid-phase extraction and liquid chromatography (HPLC) [10,12]. No significant differences were observed between the two techniques ($P > 0.05$).

Sample processing by the SFE method provided purified sample solutions giving corrected derivative UV spectra profiles useful for both identification and quantitation. In this preliminary study, however, the simultaneous quantitative extraction of miconazole and hydrocortisone from a commercial association failed, because under the SFE conditions suitable for miconazole (Table 1) a reduced extraction yield (< 50%) was obtained for hydrocortisone. Higher extraction temperatures (80°C) resulted in the degradation of the steroidal drug as confirmed by HPLC analysis. This analytical problem was easily overcome using a solid phase extraction (SFE) procedure using a diol sorbent [12]. These exploratory experiments suggest that difficulties can arise to optimize the SFE process when the simultaneous extraction of drug with different structural characteristics has to be obtained.

4. Conclusion

The SFE method developed proved to be suitable for control assays of imidazole antimycotic drugs in commercial cream formulations. In particular, the SFE process yielded essentially quantitative recoveries of the imidazole antimycotics, giving sample solutions suitable for the subsequent analysis by derivative UV spectroscopy. In preliminary investigations, however, difficulties were found in the simultaneous extraction of the miconazole–hydrocortisone association.

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