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Analysis of miconazole and econazole in pharmaceutical formulations by derivative UV spectroscopy and liquid chromatography (HPLC)*

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Abstract: Methods based on derivative UV spectrophotometry and high-performance liquid chromatography (HPLC) have been developed for the selective determination of miconazole and econazole in pharmaceutical dosage forms. A solid-phase extraction (SPE) procedure using a diol column gave quantitative drug extraction from formulated creams and provided purified sample solutions suitable for assay by the derivative UV spectrophotometric and HPLC methods. The proposed methods gave comparable accurate results, whereas a conventional UV spectrophotometric method was found to be seriously affected by excipients.

Keywords: *Miconazole and econazole determination; derivative UV spectrophotometry; HPLC; solid-phase extraction; pharmaceutical formulations.*

Introduction

Miconazole and econazole are structurally related imidazoles that are widely used for the treatment of topical fungal infections [1]. The drugs are used as nitrate salts in a variety of pharmaceutical formulations (powders, creams, lotions) at a relatively low concentration (1–2%). The weak UV absorptivity [2] of these drugs results in difficulty in their determination in complex formulations. Spectrophotometric [3, 4] and HPLC [5–7] methods have been proposed for the quality control of various pharmaceutical dosage forms containing miconazole [3–5] or econazole [5–7].

Pharmacopoeial methods for the determination of miconazole nitrate [8, 9] and econazole nitrate [9] in creams are based on a gas chromatographic assay, but these methods involve preliminary extraction procedures that are laborious and time-consuming. Thus it was considered desirable to develop a simpler and more rapid procedure that would serve as an alternative to the official methods. To this end, a method based on a preliminary quantitative SPE of the drugs from the cream excipients was devised; extraction was followed by a selective derivative UV spectrophotometric or HPLC assay procedure.

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Derivative spectrophotometry is a well-established technique for resolution enhancement; it allows selective discrimination of sharp bands over broad bands in UV spectra, offering an effective approach to the suppression of broad background matrix absorption [10–12]. Thus, in the present study, derivative UV spectroscopy was applied to the determination of miconazole and econazole in commercial dosage forms (creams and powders) and the results were compared with those obtained by a reference HPLC method.

Experimental

Materials

Miconazole nitrate (Janssen, Belgium) and econazole nitrate (Cilag, Italy) were kindly supplied by their manufacturers. Cartridges Bond–Elut containing 500 mg of diol sorbent were from Analytichem International (Harbor City, USA). Methanol (C. Erba, Milan, Italy) and tetrahydrofuran (Merk, FRG) were HPLC grade; water was de-ionized and double distilled. All other chemicals were obtained from C. Erba (Milan, Italy).

0.1 M triethylammonium acetate (pH 7.0) was prepared by adding acetic acid to 0.1 M triethylamine.

Apparatus

Spectrophotometric analyses were performed on a Jasco Uvidec-610 double beam spectrophotometer using 1-cm cells. Suitable settings were: slit width 1 nm, scan speed 100 nm min^{-1} and chart speed 20 nm min^{-1}. For the derivative mode $\Delta\lambda = 3$ nm was selected and absorbance scale expansions ×10 (miconazole in powder), ×20 (miconazole in creams) and ×15 (econazole) were used.

HPLC separations were performed on a Varian 5020 liquid chromatograph equipped with a Rheodyne model 7125 injector with a 10-μl sample loop. The measurements were made at ambient temperature using a variable wavelength UV detector (Varian UV-50) connected to a Varian 4270 integrator. The detector wavelength was set at 230 nm. Routine analyses were carried out isocratically on a 5-μm reversed-phase Nova–Pak RP-18 column (150 × 3.9 mm, i.d.) using a mobile phase of methanol–tetrahydrofuran–0.1 M triethylammonium acetate (pH 7.0) (70:12:18, v/v/v) at a flow-rate of 0.8 ml min^{-1}.

Solid-phase extraction was carried out using the Baker-10 SPE system connected to a water aspirator.

Calibration curves

Derivative spectrophotometry. Standard solutions of miconazole nitrate and econazole nitrate were prepared in methanol (80–240 μg ml^{-1}) for the drug assay in powders; for their determination in creams, standard solutions (40–140 μg ml^{-1}) were prepared in methanol–triethylammonium acetate (0.1 M; pH 7.0) (22:3, v/v). Second- and third-order derivative spectra of the standard solutions were recorded and the appropriate amplitudes (Table 1) were measured and plotted against the corresponding concentrations to obtain calibration curves.

HPLC. Standard solutions of miconazole nitrate (20–70 μg ml^{-1}) containing a fixed concentration (40 μg ml^{-1}) of econazole nitrate (the internal standard) were prepared in methanol. Triplicate injections (10 μl) were made for each solution and the peak-height ratio of the drug to internal standard was plotted against the corresponding concen-

Table 1
Data for the calibration curves ($n = 6$) for the determination of miconazole nitrate and econazole nitrate by derivative UV spectrophotometry and HPLC

Drug	Methods*	Slope	Intercept	Correlation coefficient	Working range ($\mu g\ ml^{-1}$)
Miconazole	$^2D_{285.2,280.8}$†	1.3435	−0.00025	0.9999	80–240
	$^2D_{280.8}$	2.1450	0.00122	0.9997	40–140
	$^3D_{282.8}$	2.1110	0.00365	0.9990	40–140
	$^3D_{274}$	1.5960	0.00007	0.9992	40–140
	HPLC	0.8726	0.00047	0.9998	20–70
Econazole	$^2D_{285.2,281.2}$†	1.1134	0.00400	0.9986	120–240
	$^3D_{282.8}$†	1.0073	0.01433	0.9994	120–240
	$^3D_{282.8}$	0.8101	0.00158	0.9990	40–140
	$^3D_{279.6}$	0.8600	0.00310	0.9993	40–140
	HPLC	1.1245	0.00210	0.9990	20–70

*The notation for amplitude measurements in the derivative domain was made according to ref. [17].
†From methanolic standard solutions for the powder analysis.

tration ratio to obtain the calibration curve. Similarly, the calibration curve for econazole was constructed using miconazole as the internal standard.

Sample preparation

Powders. A sample equivalent to about 10 mg of the antimycotic drug was extracted with two 20-ml portions of methanol with magnetic stirring. The extracts were filtered, combined in a 50-ml volumetric flask and diluted to volume with methanol. The resulting solution was subjected directly to spectrophotometric analysis using methanol as reference; for the HPLC determinations, a 2.5-ml aliquot was added to 2.0 ml of the internal standard solution (200 $\mu g\ ml^{-1}$) and the volume was adjusted to 10 ml with methanol.

Creams.

Procedure A: A sample equivalent to about 10 mg of the drug was treated with 30 ml of methylene chloride and, after sonication for 2 min, the volume was adjusted to 50 ml with the same solvent. The resulting opalescent solution was filtered and a 2-ml aliquot of the clarified solution was applied to a diol extraction column, previously conditioned by rinsing with 6 ml of methylene chloride. After application of the sample the column was washed with two 3-ml portions of *n*-hexane–methylene chloride (4:1, v/v) and aspirated to dryness. The retained drug was then eluted with three 1-ml portions of methanol–0.1 M triethylammonium acetate (pH 7.0) (4:1, v/v) under aspiration and the combined eluates were diluted to 5 ml with methanol. The resulting solution was used for the spectrophotometric determinations; for HPLC analyses a 2.5-ml aliquot was added to 1.0 ml of internal standard solution (200 $\mu g\ ml^{-1}$) and diluted to 5 ml with methanol.

Procedure B: A sample equivalent to about 10 mg of the drug was added to 20 ml of 1 M sodium hydroxide and extracted with three 10-ml portions of methylene chloride. The extracts were combined into a 50-ml volumetric flask and diluted to volume with *n*-hexane. A 2-ml aliquot of the resulting opalescent solution was then subjected to the SPE procedure as described above.

Assay procedure

The sample solutions were analysed by the derivative spectrophotometric and HPLC methods and the drug content in each sample was evaluated by comparison with an appropriate standard solution. The samples were also analysed by a conventional (zero-order) UV spectrophotometric procedure at 272 nm.

Results and Discussion

Derivative spectrophotometry

The absorption (zero-order) UV spectra of miconazole nitrate (Fig. 1) and econazole nitrate (Fig. 2) exhibit similar benzenoid profiles with weak absorptivity (miconazole: λ_{max} = 272 nm with $A_{1\%,1cm}$ = 17 in methanol; econazole: λ_{max} 271 with $A_{1\%,1cm}$ = 12.5 in acidic methanol) [2]. When derivative UV spectra are recorded, sharp bands of great amplitude (Figs 1 and 2) are produced which may permit more selective identification and determination of the drugs in their pharmaceutical formulations.

Linear relationships between selected amplitudes from the second- and third-order derivative spectra and drug concentration were observed (Table 1). To assess the precision of the procedure, replicate (n = 6) recordings of the zero-order UV spectrum of a single drug solution (100 μg ml^{-1}) were made and the corresponding derivative spectra were obtained. For all the amplitudes examined the relative standard deviation (RSD) was 0.56–1.30%, indicating good precision.

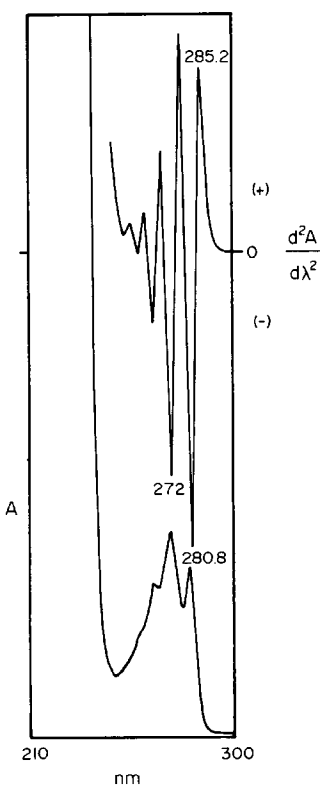

Figure 1
Conventional zero-order and second-order derivative spectra of miconazole nitrate (100 μg ml^{-1} in methanol). The spectra in methanol–triethylammonium acetate (22:3, v/v) were essentially identical.

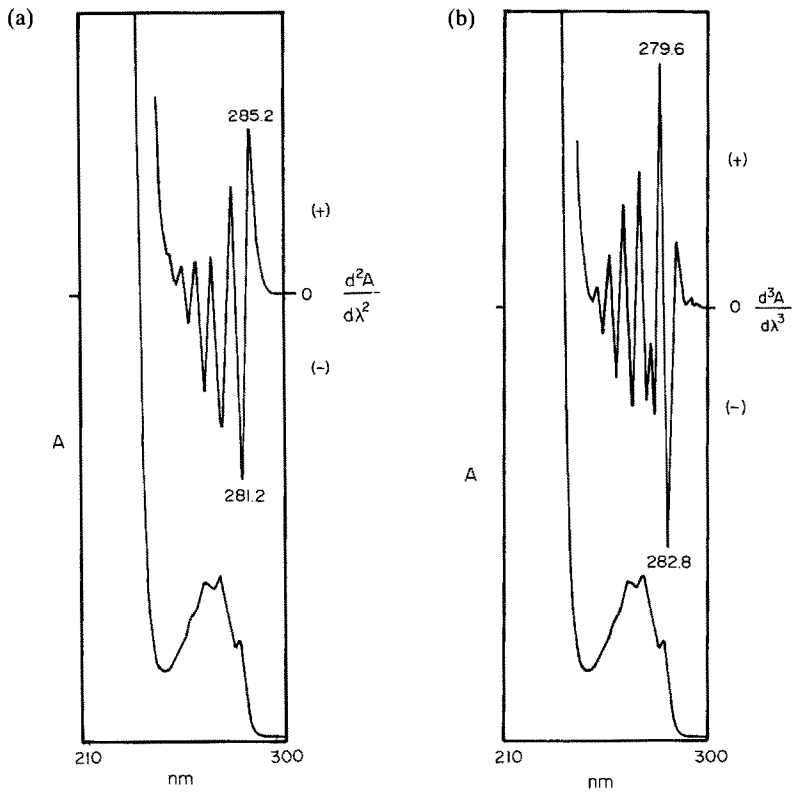

Figure 2
UV spectra of econazole nitrate: (a) conventional zero-order and second-order derivative spectra; (b) conventional zero-order and third-order derivative spectra. Experimental conditions as in Fig. 1.

Chromatography

A reversed-phase HPLC procedure was developed to provide a suitable reference method for the determination of miconazole and econazole in dosage forms by derivative spectrophotometry. From recent studies on HPLC separations of basic drugs [13–15] and from previous experience [16], a reversed-phase mode was chosen and triethylamine was used as an amine modifier.

A ternary mixture of methanol–tetrahydrofuran–triethylammonium acetate (0.1 M, pH 7.0) (70:12:18, v/v/v) at a flow-rate of 0.8 ml min^{-1} was found to be an appropriate mobile phase allowing for adequate and rapid separation (R_s = 2.0) between econazole (k' = 1.7) and miconazole (k' = 2.8) (Fig. 3).

The addition of triethylamine to the mobile phase resulted in symmetric peaks, while the partial substitution of methanol by tetrahydrofuran reduced the retention times of the hydrophobic drugs examined. The pH of the buffer solution was adjusted to 7.0, because at lower values (3.0 and 4.5) slight reduction of the peak symmetry was observed for both drugs (pK_a = 6.65).

For quantitative applications, linear calibration curves were obtained (Table 1) between the peak height ratio of analyte to internal standard (I.S.) and the corresponding concentration ratio. The good precision of the HPLC procedure was

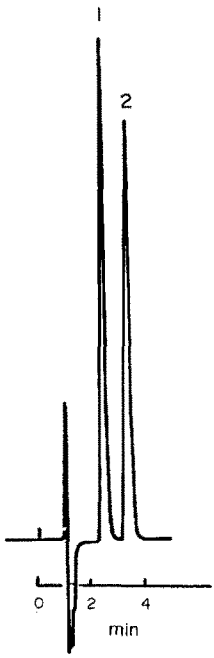

Figure 3
HPLC separation of econazole (1) and miconazole (2). Column: Nova–Pak RP-18 (5 μm); mobile phase: methanol–THF–0.1 M triethylammonium acetate (pH 7.0) (70:12:18, v/v/v) at a flow-rate of 0.8 ml min^{-1}. Detection: 230 nm.

indicated by the RSD (0.17–0.45%) of the peak height ratio (analyte to I.S.) obtained from replicate (n = 6) analyses of a single drug solution (50 μg ml^{-1}).

Analysis of pharmaceutical formulations

Quantitative extraction of the drugs from the powder samples was easily accomplished using methanol as the solvent, whereas the analysis of cream samples required detailed studies on the extractive conditions. A convenient, simple procedure (Procedure A) involved dissolution of a sample of the cream in methylene chloride to obtain a slightly opalescent solution which was clarified by filtration through paper and then applied (2 ml) to a diol column for SPE. Drug retention was found to be quantitative by analysing the filtrate and the subsequent washings by HPLC. The drug retained was then quantitatively eluted (monitoring by HPLC) with methanol–triethylammonium acetate (0.1 M; pH 7.0) (4:1, v/v). The resulting eluate was used for HPLC and spectrophotometric (conventional and derivative mode) determinations.

The SPE was unable to eliminate benzoic acid, present as a preservative in the analysed creams at the level of 10% of the antimycotic drug. Accordingly, it was verified that the zero-order UV spectrum of the miconazole eluate from SPE corresponded to the addition of the spectra of the miconazole standard and benzoic acid in the methanol–triethylammonium acetate (4:1, v/v) solvent system. However, when the derivative mode was applied, the different spectral characteristics of benzoic acid and miconazole in the final solvent system (methanol–triethylammonium acetate (22:3, v/v)) allowed for the selective determination of the drug in the presence of the preservative. As shown in Fig. 4, the selected peak-to-zero amplitudes $^2D_{280.8}$ (second-derivative) and $^3D_{282.8}$, as well as $^3D_{274}$ (third-derivative) were not affected by the benzoic acid peaks and were useful for miconazole quantitation giving data in close agreement with those from the HPLC procedure (Table 2).

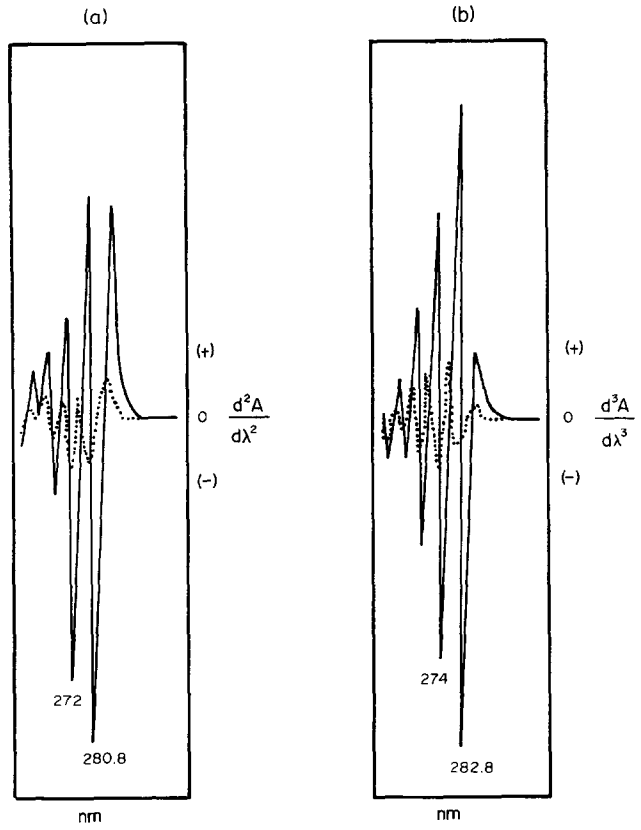

Figure 4
(a) Second-derivative UV spectra of miconazole (solid line) and benzoic acid (broken line); (b) third-derivative UV spectra of miconazole (solid line) and benzoic acid (broken line).

As expected, a conventional UV spectrophotometric assay (λ = 272 nm) was seriously affected by the absorption contributions from the excipients. In order to eliminate benzoic acid, a preliminary liquid–liquid extraction step was introduced before the SPE (Procedure B). This procedure was applied to the analysis of miconazole and econazole creams (Table 2), providing results comparable with those obtained by Procedure A and a slightly improved precision. The conventional UV method again was found to be inapplicable, giving inflated drug contents. The accuracy of the HPLC and derivative UV determinations was verified by analysing synthetic mixtures which reproduced the composition of the commercial formulations (miconazole powder) or samples (creams) spiked with known quantities of drug. In each instance, a quantitative recovery (99.40–100.12%) with good precision (RSD = 0.73–1.15%) was obtained.

The selectivity of the proposed methods for the intact drugs in the presence of their degradation products has not been investigated because the compounds were not available. It is likely, however, that the HPLC approach may offer advantages over the derivative UV mode as the known degradation products contain the phenyl moiety [6].

The importance of the SPE step in the cream extraction procedure was also examined. This is illustrated in Fig. 5, where the zero-order UV spectra of (a) a chloromethylenic extract (liquid–liquid extraction) from econazole cream, (b) the subsequent SPE eluate

Table 2
Assay results for the analysis of miconazole and econazole in commercial formulations by derivative spectrophotometric and HPLC methods

	Miconazole nitrate*†				Econazole nitrate*‡			
	Powder		Cream		Powder		Cream	
Method	Found (%)	RSD (%)	Found (%)	RSD (%)	Found (%)	RSD (%)	Found (%)	RSD (%)
UV — 272 nm	107.00	2.03	160.00	3.10	170.60	2.80	142.00	3.50
			201.00§	2.60§				
$^2D_{285.2,280.8}$	101.30	1.27						
$^2D_{280.8}$			98.70	0.57				
			99.45§	1.40§				
$^3D_{282.8}$			98.13	0.68	99.50	1.70	100.10	1.64
			98.80§	1.55§			99.60§	2.20§
$^3D_{274}$			98.43	0.96				
			98.10§	1.30§				
$^2D_{285.2,281.2}$					98.95	1.60		
$^3D_{279.6}$							99.00	2.40
HPLC	100.40	0.44	98.60	0.70	98.40	0.62	100.92	0.67
			98.70§	0.78§				

*Mean of five determinations and expressed as percentage of the claimed content.
†Other ingredients. Powder: zinc oxide, colloidal silicon dioxide, talc. Cream: tefose 63, labrafil M 1944 CS, mineral oil, butylated hydroxyanisole, benzoic acid.
‡Other ingredients. Powder: colloidal silicon dioxide, perfume No. 4074, zinc oxide, talc. Cream: mixture of esters of stearic acid with glycols, mixture of fatty acids and polyethylene glycol, petrolatum, butylated hydroxyanisole, perfume No. 4074, benzoic acid.
§Procedure A was followed for the drug extraction.

and (c) an equimolar econazole standard solution, relative to the appropriate solvent blank, are compared. As can be seen, the SPE procedure enables the sample to be purified considerably and results in a correct spectrum for the drug. Under these conditions the application of derivative UV spectrophotometry allows suppression of the residual matrix absorption to give accurate analyses. This effect was not achieved when the derivative technique was directly applied to the initial chloromethylenic extract omitting the SPE. Preliminary experiments indicated also the applicability of the described SPE procedure to the analysis of other imidazole antimycotic drugs, such as ketoconazole and isoconazole, in commercial creams that did not contain benzoic acid.

Conclusions

Derivative UV spectrophotometry and HPLC were suitable techniques for the reliable analyses of commercial formulations (powders and creams) containing miconazole nitrate or econazole nitrate. A SPE procedure using a diol column was found to ensure quantitative extraction of the drugs from the cream excipients and to provide sample solutions suitable for the derivative UV and HPLC determinations. Using the derivative spectrophotometric procedure, the residual background absorption can be suppressed and selective drug identification and estimation can be accomplished. The HPLC method was shown to be a versatile, useful reference method and may serve for the determination of the antimycotic drugs examined in a variety of matrices.

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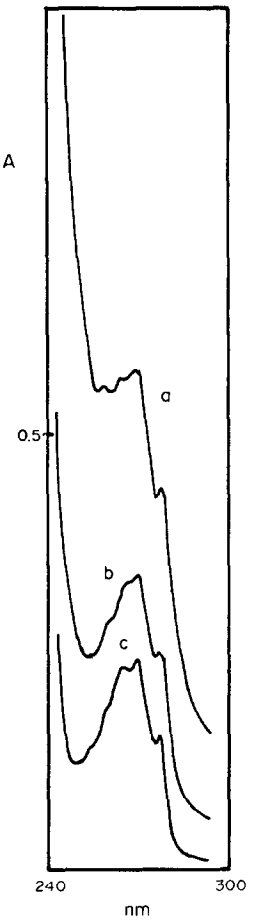

Figure 5
Conventional zero-order UV spectra of (a) extract from liquid–liquid extraction of econazole cream sample; (b) eluate from SPE and (c) equimolar econazole standard solution.

References

[1] Martindale, *The Extra Pharmacopoeia*, 28th edn, pp. 722 and 726. The Pharmaceutical Press, London (1982).
[2] E. C. G. Clarke, *Isolation and Identification of Drugs*, 2nd edn, pp. 579 and 784. The Pharmaceutical Press, London (1986).
[3] V. Cavrini, A. M. Di Pietra and M. A. Raggi, *Pharm. Acta Helv.* **56**, 163–165 (1981).
[4] *The United States Pharmacopeia XXI and the National Formulary XVI.* United States Pharmacopeial Convention, Rockville, MD, USA (1985).
[5] V. Cavrini, A. M. Di Pietra and M. A. Raggi, *Int. J. Pharm.* **10**, 119–124 (1982).
[6] R. Christinat and H. W. Zulliger, *Arzneimittel-Forsch./Drug Res.* **34**, 551–553 (1984).
[7] A. S. Sidhn, J. M. Kennedy and S. Deeble, *J. Chromatogr.* **391**, 233–242 (1987).
[8] *The United States Pharmacopeia XXI and The National Formulary XVI.* United States Pharmacopeial Convention, Rockville, MD, USA, First Supplement (1985).
[9] *The British Pharmacopoeia 1988*, Vol. II. H.M.S.O., London (1988).
[10] G. Talsky, L. Maysing and H. Kreuzer, *Angew. Chem. Int. Ed. Engl.* **17**, 785–799 (1978).
[11] A. F. Fell, *Proc. Anal. Div. Chem. Soc.* **15**, 260–267 (1978).
[12] P. Levillain and D. Fompeydie, *Analasis* **14**, 1–20 (1986).
[13] R. W. Roas and C. A. Lau-Cam, *J. Chromatogr.* **370**, 403–418 (1986) and refs cited therein.
[14] V. Juergens, *J. Liq. Chromatogr.* **11**, 1925–1940 (1988).
[15] R. K. Gilpin, S. S. Yang and G. Werner, *J. Chromatogr. Sci.* **26**, 388–400 (1988) and refs cited therein.
[16] A. M. Di Pietra, V. Cavrini, R. Gatti and M. A. Raggi, *Pharm. Res.* **5**, 709–712 (1988).
[17] A. A. Fasanmade and A. F. Fell, *Analyst* **110**, 1117–1124 (1985).

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