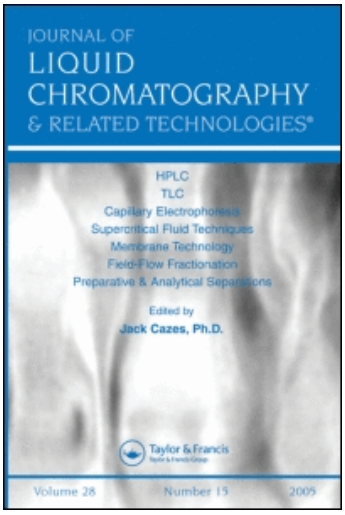

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC METHOD FOR THE ASSAY OF UNDECYLENIC ACID AND UNDECYLENATES IN PHARMACEUTICAL PRODUCTS AFTER CONVERSION TO 4'-NITROPHENACYL ESTERS

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ABSTRACT

A high performance liquid chromatographic (HPLC) method with spectrophotometric detection is presented for the assay of undecylenic acid, undecylenates (calcium, zinc), and the combination undecylenic acid-zinc undecylenate in commercial antifungal products. To enhance detection, an acetone solution of the sample preparation and sodium octanoate, the internal standard, was derivatized with 4'-nitrophenacyl bromide in the presence of triethylamine at 50°C for 30 min. An aliquot of the reaction mixture was directly analyzed on a Microsorb-MV C_8 column, with methanol-acetonitrile-water (50:30:20) as the mobile phase, and detection at 265 nm. At a flow rate of 1.6 mL/min, baseline resolution of the mixture of 4'-nitrophenacyl esters was attained in less than 7 minutes.

Detector responses were linearly related to concentrations of reacted undecylenic acid, or its equivalent in an undecylenate, in the range 12.5-300 μg. The recovery of undecylenic acid or an undecylenate from spiked commercial products ranged from 99.3 to 102.3% (n = 2). The proposed method was found suitable for the analysis of both liquid and solid pharmaceutical products.

INTRODUCTION

Undecylenic acid and its salts (undecylenates) comprise a group of drugs that are applied to the skin for the control and prevention of surface infections designated as tineas or ringworms.[1-4] Singly, in the form of the free acid or the calcium salt, or as a combinations of the free acid and the zinc salt, these topical antifungal agents are particularly effective to treat tinea pedis (athlete's foot) and tinea cruris (the jock itch).[1-4] Relative to the parent compound, undecylenates are considered to offer advantages such as astringency,[1] nonvolatility,[5] more selective toxicity,[6] and greater potency.[6,7]

Commercial formulations of undecylenic acid and its salts include dusting powders, tinctures, solutions, creams, ointments, and various spray products.[2-4] In addition, a hydrophilic ointment of compound undecylenic acid (i.e., a mixture of 5 parts of undecylenic acid and 20 parts of zinc undecylenate)[7-9] is prepared extemporaneously when required.

Analytical techniques for the assay of undecylenic acid and its salts in pharmaceutical samples have varied depending on the type and composition of the test sample.[8-17] For instance, an acidimetric titration with colorimetric,[8-12] potentiometric,[13] or radiometric[14] end-point detection and high performance thin-layer chromatography with densitometric scanning[15] have been used to measure undecylenic acid drug substance; and a complexometric titration of the zinc content has served as the basis for quantifying zinc undecylenate.[16] The amount of undecylenic acid in pharmaceutical products, on the other hand, has been determined by a direct HPLC method with spectrophotometric detection;[17] and that of the components of compound undecylenic acid ointment has necessitated two separate and lengthy steps, one for the zinc portion by gravimetry[8] or atomic absorption spectroscopy;[9] and the other for the total undecylenate using an acidimetric titration[8] or gas-chromatography.[9]

The purpose of the present report is to describe the development of a simple, straightforward and accurate HPLC method for the assay of undecylenic acid and its salts after their conversion to photometrically-active 4'-nitrophenacyl ester derivatives. With this method the quantitative analysis of

liquid and solid pharmaceutical products containing undecylenic acid, calcium undecylenate or mixtures of undecylenic acid with its zinc salt can be performed under the same experimental conditions.

EXPERIMENTAL

Samples and Materials

Dosage forms

Dusting powders (10% calcium undecylenate, 19% or 25% total undecylenate), ointments (25% total undecylenate) and a topical solution (10% undecylenic acid) were obtained from commercial sources.

Chemicals

4'-Nitrophenacyl bromide (2-bromo-4'-nitroacetophenone) was from Aldrich Chemical Co., Milwaukee, WI. Analytical reagent grade triethylamine and sodium carbonate tetrahydrate were from Fischer Scientific, Pittsburgh, PA. Calcium chloride dihydrate was obtained from Mallinckrodt, Inc., St. Louis, MO. The methanol, acetonitrile, acetone, and water were of HPLC grade and from EM Science, Gibbstown, NJ.

Standards

Undecylenic acid, sodium octanoate, and zinc undecylenate were obtained from Aldrich Chemical Co., Milwaukee, WI. Calcium undecylenate was prepared by treating a solution of sodium undecylenate (Aldrich) with an excess of calcium chloride solution, collecting the product by filtration, rinsing the solid thoroughly with water, and drying it in an oven to a constant weight.

Solutions

Derivatizing (NPB) solution

The NBP solution was prepared by dissolving 4'-nitrophenacyl bromide in acetone to a concentration of about 15 mg/mL. This solution was stable for at least 2 weeks when stored in the refrigerator.

Basic (TEA) solution

A TEA solution was prepared by diluting triethylamine in acetone to a concentration of about 2.8 mg/dL. This solution was stable for at least 2 weeks when stored in the refrigerator.

Sodium carbonate solution

Sodium carbonate solution was prepared by dissolving $Na_2CO_3.10H_2O$ in water, to a final concentration of about 14 g/dL.

Internal standard solution

The internal standard solution was prepared by transferring about 30 mg of sodium octanoate to a 100 mL volumetric flask, adding 30 mL of water, swirling until dissolved, bringing with acetone to volume, and mixing. The concentration of this solution was about 0.3 mg/mL.

Undecylenic acid standard solution

An accurately weighed amount of undecylenic acid, equivalent to about 250 mg, was transferred to a 100 mL volumetric flask, diluted with acetone to volume, and mixed. A 5.0 mL portion of the solution was transferred to a 25 mL volumetric flask, diluted with acetone to volume, and mixed. This solution was about 0.5 mg/mL in concentration.

Sample Preparations for the Assay of Undecylenic Acid

Ointments

An amount of ointment, equivalent to about 125 mg of total undecylenate, was accurately weighed in a 250 mL beaker, mixed with about 50 mL of acetone, and sonicated until completely dissolved. The solution was quantitatively transferred to a 100 mL volumetric flask with the aid of various portions of acetone, diluted with the same solvent to volume, and mixed.

A portion of the solution was transferred to a screw-capped test tube, and centrifuged at 900 x g for 10 min. A 10.0 mL portion of the clear supernatant was transferred to a 25 mL volumetric flask, diluted with acetone to volume, and mixed.

Dusting powders

An accurately weighed amount of powder, equivalent to about 250 mg of total undecylenate (or about 100 mg of calcium undecylenate), was transferred to a 100 mL volumetric flask, mixed with about 50 mL of acetone, sonicated for 5 min, diluted with acetone to volume, and mixed. A portion of the solution was transferred to a screw-capped test tube, and centrifuged at 900 x g for 10 min. A 5.0 mL portion of the clear supernatant was transferred to a 25 mL volumetric flask, diluted with acetone to volume, and mixed.

Topical solutions

A volume of solution, equivalent to about 250 mg of undecylenic acid, was accurately transferred to a 100 mL volumetric flask, diluted with acetone to volume, and mixed. A 5.0 mL portion of this solution was transferred to a 25 mL volumetric flask, diluted with acetone to volume, and mixed.

Sample Preparations for the Assay of Total Undecylenate

Ointments

An amount of ointment, equivalent to about 125 mg of total undecylenate, was accurately weighed in a 250 mL beaker, mixed with about 50 mL of acetone, and sonicated until completely dissolved. The solution was quantitatively transferred to a 100 mL volumetric flask with the aid of several portions of acetone, acidified with 6N HCl (0.1 mL/0.1 g of sample), mixed well, made basic with 14% sodium carbonate (0.15 mL/0.1 g of sample), and sonicated for about 15 min. After diluting with acetone to volume, and mixing, a portion of the solution was transferred to a screw-capped test tube, and centrifuged at 900 x g for 10 min. A 10.0 mL portion of the clear supernatant was transferred to a 25 mL volumetric flask, diluted with acetone to volume, and mixed.

Dusting powders

An accurately weighed amount of powder, equivalent to about 250 mg of total undecylenate, was transferred to a 100 mL volumetric flask, mixed with about 50 mL of acetone, and sonicated for about 5 min. After acidification with 6N HCl (0.1 mL/0.1 g of sample), and mixing, the suspension was made basic with 14% sodium carbonate (0.15 mL/0.1 g of sample), and sonicated for about 15 min.

After diluting with acetone to volume, and mixing, a portion of the solution was transferred to a screw-capped test tube and centrifuged at 900 x g for 10 min. A 5.0 mL portion of the clear supernatant was transferred to a 25 mL volumetric flask, diluted with acetone to volume, and mixed.

Derivatization Reaction

In a screw-capped test tube, 0.5 mL of sample preparation or undecylenic acid standard solution, 0.5 mL of internal standard solution, 0.2 mL of NPB solution, and 0.2 mL of TEA solution were added in succession. After capping tightly, the test tube was swirled gently to mix its contents, and then incubated at 50°C for 30 min on a preheated block heater. After cooling to room temperature, a 20 μL aliquot of the reaction mixture was injected into the liquid chromatograph.

HPLC Method

Apparatus

An isocratic system consisting of Series 10 liquid chromatograph, LC 90 UV spectrophotometric detector (Perkin-Elmer Corporation, Norwalk, CT), and Model SP4400 (ChromJet) integrator (Spectra-Physics, San Jose, CA), was used. Samples were introduced through a Model 7125 injection valve fitted with a 20 μL sample loop (Rheodyne, Cotati, CA). Separations were carried out on a Microsorb-MV C_8, 25 cm x 4.6 mm i.d., 5 μm column (Rainin, Woburn, MA), and monitored at 265 nm and 0.5 AUFS.

Mobile phase

A mixture of methanol-acetonitrile-water (50:30:20), filtered and degassed prior to use. The flow rate was 1.6 mL/min.

Calculations

Percentage of undecylenic acid (UA) in the sample preparation

Solution, % UA = (Hu/Hs) x (S/V) x 100
Powder, % UA = (Hu/Hs) x (S/M) x 100
Ointment, % UA = (Hu/Hs) x (S/M) x 50

Table 1

Retention Times (k' Values) on RP-C_8 and C_{18} Columns of Phenacyl-Type Ester Derivatives of Undecylenic Acid and Sodium Octanoate, the Internal Standard[a]

	C_8 Column[b]		C_{18} Column[b]	
Ester Derivative	**Na Octanoate**	**Undecylenic Acid**	**Na Octanoate**	**Undecylenic Acid**
4'-Bromophenacyl	6.20 (5.20)	9.92 (8.92)	7.39 (8.24)	15.44 (18.30)
4'-Methoxyphenacyl	4.46 (3.46)	6.80 (5.80)	5.10 (5.38)	9.07 (10.34)
4'-Methylnaphthacyl	6.47 (5.47)	10.43 (9.43)	9.13 (10.41)	6.67 (7.34)
4'-Methylphenacyl	4.50 (3.50)	6.83 (5.83)	ND[d]	Nd[d]
4'-Nitrophenacyl	4.39 (3.39)	6.63 (5.63)	5.14 (5.43)	9.07 (10.34)
Phenacyl	4.52 (3.52)	6.94 (6.94)	5.00 (5.25)	9.57 (10.96)
4'-Phenylphenacyl	7.89 (6.89)	13.00 (12.00)	11.42 (13.28)	21.90 (26.38)

[a] Column: Microsorb-MV C_8 or C_{18}, 25 cm x 4.6 mm i.d.; mobile phase: methanol-acetonitrile-water (0:30:20; detection wavelength 265 nm at 0.5 AUFS.
[b] Flow rate: 1.6 mL/min; t_0 (methanol) =1.0 min.
[c] Flow rate: 2 mL/min; t_0 (methanol) = 0.8 mi.n.
[d] Not done.

where Hu and Hs = the peak height ratios of the sample preparation and standard solution, respectively; S = the weight of undecylenic acid taken to prepare the standard solution, g; V = the volume of liquid product taken for the analysis, mL; and M = the weight of solid product taken for the analysis, g.

Percentage of zinc undecylenate (ZnU) or calcium undecylenate (CaU) in the sample preparation

ZnU (or CaU), % = [% Total UA - % Free UA] x [FW ZnU (or CaU)/FW UA x 2]

where FW (formula weight) of ZnU = 431.92, FW of CaU =406.54, and FW of UA = 184.28.

RESULTS AND DISCUSSION

Undecylenic acid is an unsaturated fatty acid whose detection during HPLC analysis is hampered by the lack of a strong chromophore or fluorophore functional group. To overcome this problem, this compound was reacted with a halogenated alkylating agent in the presence of a tertiary amine to form a photometrically detectable ester derivative. Using the experimental conditions reported by Borch[18] for the phenacylation of long fatty acids, seven α-

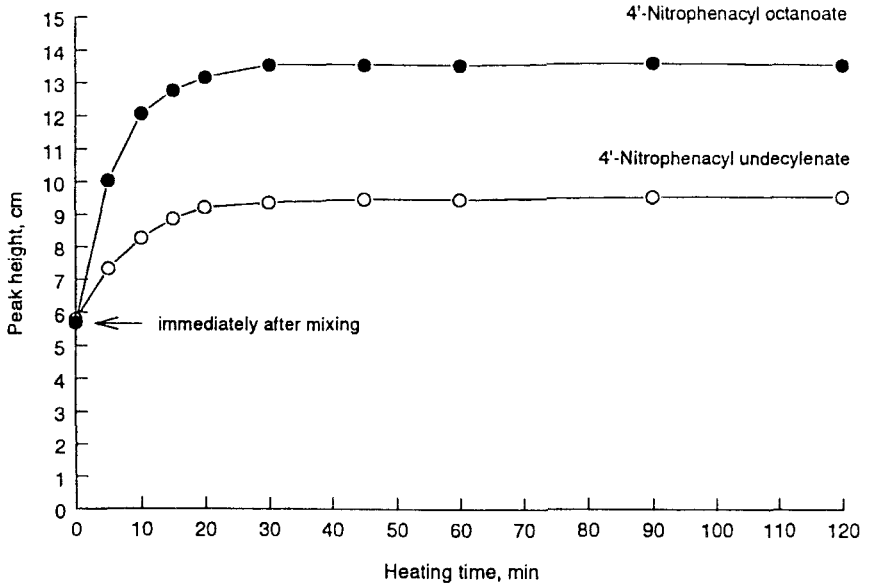

Figure 1. Time course of the derivatization reaction of undecylenic acid and sodium octanoate, the internal standard, to 4'-nitrophenacyl derivatives. The reaction mixture was incubated at 50°C

brominated arylacetophenone- (phenacyl-) type of compounds (Table 1) were tested for their suitability as a derivatizing reagent. Based on this study, 4'-nitrophenacyl bromide was the reagent eventually selected because it reacted with the analytes almost immediately to yield ester derivatives that were chromatographically well resolved and which eluted in a relatively short time. Furthermore, the gradual appearance and intensification of a yellow color in the reaction mixture when using a nitrated arylacetophenone afforded a means of checking the occurrence and time course of the derivatization reaction.

The 4'-nitrophenacylation of undecylenic acid was rapid. As seen in Figure 1, it started soon after the addition of the derivatizing reagents, was accelerated by incubation at 50°C, and reached near completion in about 30 min. In contrast, neither zinc undecylenate nor calcium undecylenate were amenable to direct esterification owing to their insolubility in acetone, the derivatizing medium. This problem was circumvented by converting these two undecylenates to the more soluble and reactive sodium salt by a preliminary acidification with dilute mineral acid to generate the free acid, followed by alkalinization with sodium carbonate solution. The recommended volumes of

Table 2

Regression Equations and Correlation Coefficients (r) of Calibration Curves for Undecylenic Acid and Undecylenates

Analyte	Regression Equation[a,b]	r
Calcium undecylenate	y = 0.005122x + 0.006514	0.9999
Sodium undecylenate	y = 0.005195x - 0.005768	0.9999
Zinc undecylenate	y = 0.005461x - 0.002118	0.9998
Undecylenic acid	y = 0.005192x + 0.008977	0.9996

[a] y = peak height; x = amount of undecylenic acid, or its equivalent in undecylenate, reacted.
[b] All calibration curves were obtained using duplicate samples.

aqueous acid and alkali were those experimentally found to effect the solubilization of a measured amount of zinc or calcium undecylenate in acetone. Furthermore, potentially troublesome acetone-insolubles such as calcium or zinc carbonate and certain formulation excipients were easily removed by centrifugation. As reported in the literature,[19] the presence of more than 3% of water in the reaction mixture was found to adversely affect the esterification reaction. For this reason, all sample and standard preparations were kept as anhydrous as possible by preparing or diluting them with acetone.

An evaluation of detector peak height responses, as a function of detection wavelength, demonstrated the responses to be highest in the wavelength range 254-275 nm, and maximal at about 265 nm. The linearity of the proposed HPLC method was tested by serially diluting a stock solution of undecylenic acid (or of sodium undecylenate in acetone-water 7:3) with acetone, mixing an aliquot of each dilution with the internal standard solution, and subjecting the mixtures to derivatization. Peak height ratios were found to be rectilinearly related to quantities of reacted undecylenic acid in the approximate range 12.5-300 μg, with the line passing through the origin. The regression line equations and corresponding correlation coefficients are given in Table 2. Calibration curves for calcium and zinc undecylenates were prepared in identical manner from serial dilutions of these salts that would yield concentrations of free undecylenic acid equivalent to those used for the undecylenic acid curve, after converting them to the sodium salt form. Evidence of a stoichiometric conversion was gained from the calculated slopes for the line regression data presented in Table 2, which were virtually identical to those of the parent acid and sodium salt.

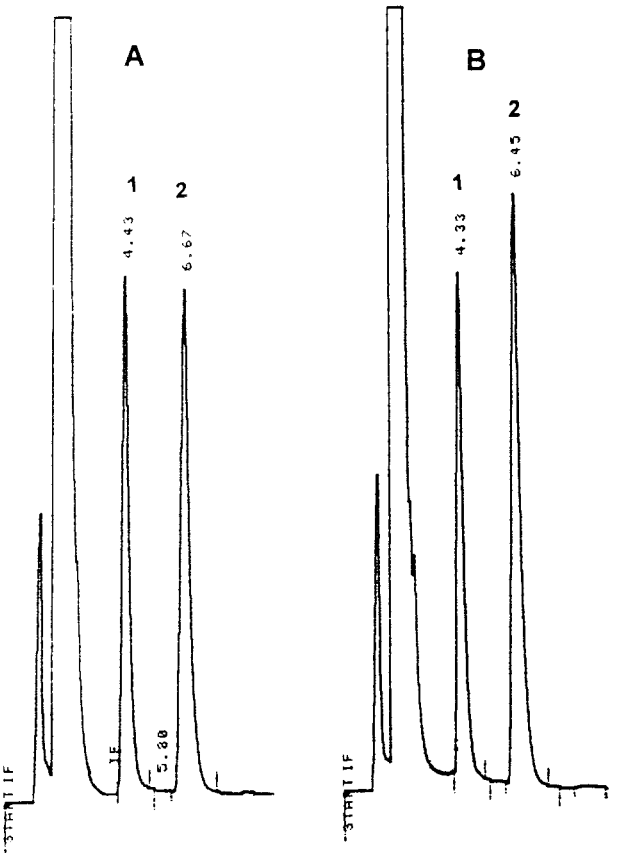

Figure 2. High-performance liquid chromatograms of the 4'-nitrophenacyl ester derivatives of 1, sodium octanoate and 2, undecylenic acid in (A) a standard preparation and (B) a commercial dusting powder.

Figure 2A shows the chromatogram of a mixture of the 4'-nitrophenacyl esters of undecylenic acid and the internal standard in a standard preparation. The ester derivative of the internal standard eluted ahead of that of undecylenic acid or an undecylenate, with the resolution factor, R, being greater than 2.0. As inferred from the retention times (k' values) presented in Table 1, compounds such as 2-methyl octanoic acid, valproic acid, and nonanoic acid will also be suitable as an internal standard. Likewise, a reversed phase C_{18} column was found to be an appropriate alternative to a C_8 column but, under identical experiment conditions, retention times with this column were longer,

Table 3

Results of Recovery of Undecylenic Acid (UA), Calcium Undecylenate (CaU) and Zinc Undecylenate (ZnU) from Spiked Dosage Forms by Proposed HPLC Method

Matrix	Drug Added	Amount of UA Found, % of Added			
		Run 1	Run 2	Mean	SD
Ointment A, 25%	ZnU[a]	102.3	101.7	102.0	0.42
Ointment B, 25%	ZnU[a]	99.5	99.6	99.6	0.07
Powder, 10%	CaU[b]	99.5	99.0	99.3	0.35
Powder, 25%	ZnU[a]	102.8	101.7	102.3	0.77
Solution, 25%	UA[b]	101.5	100.6	101.5	0.64

[a] Spiked at the 50% level of declared using the standard additions method.
[b] Spiked at the 100% level of declared using the standard additions method.

even at higher flow rates (Table 1). A faster elution was possible by raising the concentration of acetonitrile in the mobile phase to 40 parts while proportionally decreasing that of water. With this modification, baseline resolution of a mixture of the 4'-nitrophenacyl esters of undecylenic acid and the internal standard was attained in less than 5 minutes. Neither the excess of derivatizing reagents nor the solvent of the reaction mixture interfered with the chromatographic analysis.

The precision of the proposed HPLC method was tested by measuring the detector response ratios for a set of replicate injections of a derivatized standard preparation containing 250 μg of undecylenic acid and 150 μg of internal standard. The mean values for peak height and peak area ratios were 0.71 and 1.09, respectively; the SD values were in both cases <0.02, and the corresponding RSD values were 0.56% and 1.1%, respectively (n = 6). Method accuracy was assessed using the method of standard additions. To this effect, samples of commercial products of known drug content were spiked with known amounts of zinc undecylenate (ointment, powder), calcium undecylenate (powder), or undecylenic acid (solution), representing 50 or 100% of the amount declared, and next analyzed by the proposed HPLC method. As indicated by the data given in Table 3, mean recoveries for duplicate samples were in all cases greater than 99% (range 99.3-102.3%) of the amount added.

Table 4

Results of the Assay of Commercial Dosage Forms Containing Undecylenated Acid (UA) and Undecylenates by Proposed HPLC Method

Dosge Form Amount Declared	Amount Found, %									Mean, % of Declared
	Free UA			Total UA			Undecylenate			
	Run 1	Run 2	Mean	Run 1	Run 2	Mean	Run 1	Run 2	Mean	
Powder, 10%[a]	0.09	0.08	0.90	8.91	8.37	8.64	9.63	9.15	9.39	94.0
Powder, 25%[b]	2.81	2.66	2.74	25.90	24.71	25.30	27.00	26.05	26.53	101.3
Ointment A, 25%[a]	16.30	16.50	16.40	27.00	26.30	26.65	12.40	11.50	12.00	106.4
Ointment B, 25%[b]	20.90	21.20	21.05	24.60	25.10	24.85	4.30	4.60	4.45	99.4
Solution 25%[c]	101.30	101.30	101.30	---	---	---	---	---	---	101.3

Antifungal products of undecylenic acid and its salts are formulated to contain undecylenic alone, calcium undecylenate alone, or a combination of the parent acid with its zinc salt. Experimental conditions for the quantitative analysis of these products were developed based on their differences in solubility in acetone and susceptibility to nitrophenacylation. Thus, whereas the acetone-soluble undecylenic acid was readily derivatized, the acetone-insoluble calcium and zinc salts required a preliminary conversion to the sodium salt. For products containing both undecylenic acid and zinc undecylenate, and bearing a potency declaration in terms of total undecylenate, the content in zinc salt was calculated as the difference between the amount of total undecylenic acid (after conversion of the mixture to the sodium form) and that of free undecylenic acid, measured separately in two aliquots of the same formulation.

The quantitative analysis of commercial products by the proposed method yielded the results presented in Table 4 and Figure 2B. The mean value for total undecylenate in powders and ointments ranged from 94.0 to 106.4% of declared, and that for undecylenic acid in a topical solution averaged 101.3%. For products having their potency declared as total undecylenate, the ratio of undecylenic acid to undecylenate was found to vary according to the type of product analyzed. Thus, ointments contained a greater proportion of free undecylenic acid than of zinc undecylenate, whereas the converse was true for dusting powders. In this regard, none of the products whose potency was declared as total undecylenate bore a declaration of the amounts of undecylenic acid and zinc undecylenate added to them.

Furthermore, the samples of powder formulation of calcium undecylenate and of zinc undecylenate drug substance were found to contain negligible (<0.1%) but detectable levels of free undecylenic acid.

A group of compounds listed as ingredients of commercial formulations were tested for their potential effect as interferences of the proposed HPLC method. The ingredients and levels tested were: chloroxylenol (3%), isopropyl myristate (8%), menthol (2%), methyl paraben (0.025%), phenol (1%), propyl paraben (0.015%), salicylic acid (8%), stearic acid (10%), and thymol (1%).

None of these ingredients interfered with the analysis, even when they reacted with the derivatizing reagents. Indeed, the 4'-nitrophenacyl ester derivative of salicylic acid eluted near the void volume (retention time ca. 2 min), whereas that of stearic acid remained undetected (retention time >10 min), probably because of its low concentration.

In summary, undecylenic acid and its salts were measured in commercial liquid and solid antifungal products in a simple, accurate, and specific manner by HPLC after their conversion to photometrically detectable 4'-nitrophenacyl esters. In this manner, the analysis of these substances was greatly simplified since the same experimental conditions were applicable to the free acid, a salt, or a combination of both.

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