Stability Indicating Methods for the Determination of some Anti-fungal Agents Using Densitometric and RP-HPLC Methods

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Two chromatographic methods were developed for the determination of some anti-fungal drugs in the presence of either their degradation products or cortisone derivatives. The densitometric method determined mixtures of each of ketoconazole (KT), clotrimazole (CL), miconazole nitrate (MN) and econazole nitrate (EN) with the degradation products of each one. Mixtures of MN with hydrocortisone (HC) and of EN with triamcinolone acetonide (TA) were also successfully separated and determined by this technique. For KT and CL, a mixture of methanol : water : triethylamine (70 : 28 : 2 v/v) was used as a developing system and the spots were scanned at 243 nm and 220 nm for KT and CL, respectively. For MN and EN, a mixture of hexane : isopropyl alcohol : triethylamine (80 : 17 : 3 v/v) was used as a developing system and the spots were scanned at 225 nm for both drugs. The HPLC method determined mixtures of CL or EN with their degradation products which were separated and quantified on a Zorbax C8 column. Elution was carried out using methanol : phosphate buffer pH 2.5 (65 : 35 v/v) as a mobile phase at a flow rate of 1.5 ml/min and UV detection at 220 nm for CL. For EN, a mixture of methanol : water containing 0.06 ml triethylamine pH 10 (75 : 25 v/v) was used as a mobile phase at a flow rate of 1.5 ml/min and UV detection at 225 nm. The methods were also used to separate mixtures of CL with betamethasone dipropionate (BD) and EN with TA in a laboratory prepared mixture and in pharmaceutical preparations. The methods were sensitive, precise and applicable for determination of the drugs in pharmaceutical dosage forms.

Key words densitometric; HPLC; ketoconazole; clotrimazole; miconazole nitrate; econazole nitrate

There are more than 100000 different species of fungi, many of which are beneficial to food and fermentation industries; however, hundreds are pathogenic to plants and a few hundreds have been shown to cause infection in humans.[1)]

Treatment of fungal infections is considerably more difficult than that of a bacterial infection as many fungal infections occur in poorly vascularised or avascular tissues.[2)]

Ketoconazole (KT), clotrimazole (CL), miconazole nitrate (MN) and econazole nitrate (EN) (Fig. 1) are imidazole derivatives which represent a large and emerging group of synthetic antifungal agents.

Several methods for the determination of ketoconazole, clotrimazole, miconazole nitrate and econazole nitrate, have been described in the literature. They include volumetric,[3)] spectrophotometric,[4—18)] spectrofluorimetric methods,[19—21)] capillary electrophoresis method,[22,23)] thin layer chromatography,[24,25)] gas chromatography[26,27)] and high performance liquid chromatographic methods.[28—40)]

However, the imidazole antifungal derivatives are susceptible for the degradation due to the effect of temperature, oxygen, light and pH and the degraded products may be pharmacologically inactive. In addition, there are many antifungal pharmaceutical preparations containing some corticosteroid hormones and the pharmacopeial methods don't report any method for the determination of these drugs either in presence of their degradation products or in presence of corticosteroid hormones.

In this study simple, rapid precise and selective densitometric and HPLC method for the determination of some antifungal drugs KT, CL, MN, and EN in pure form or in the presence of their degradation products were developed. The methods were successfully applied to determine these drugs in pharmaceutical dosage forms and in presence of some corticosteroid hormones.

Experimental

Materials and Reagents Ketoconazole (KT) Sedico Pharmaceutical Co., 6 October City–Egypt. The purity of the sample was found to be 99.82±1.157 applying a non-aqueous titration pharmacopoeial method.[3)]

Clotrimazole (CL) Pharcopharmaceuticals, Alexandria–Egypt. The purity of the sample was found to be 99.87±1.856 applying a non-aqueous titration pharmacopoeial method.[3)]

Miconazole nitrate (MN) Minapharm–Egypt. The purity of the sample was found to be 99.776±1.695 applying a non-aqueous titration pharmacopoeial method.[3)]

Econazole nitrate (EN) Minapharm–Egypt. The purity of the sample was

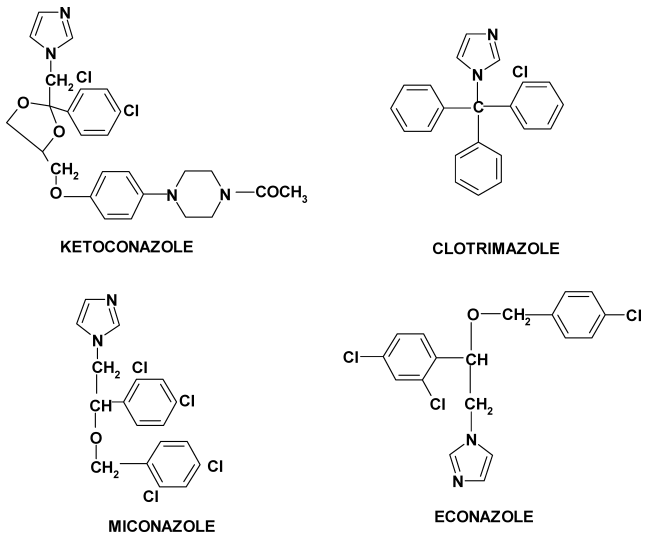

Fig. 1. Chemical Structure of Ketoconazole, Clotrimazole, Miconazole Nitrate and Econazole Nitrate

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found to be 100.02±1.96 applying a non-aqueous titration pharmacopoeial method.[3)]

Betamethasone dipropionate (BD) Pharcopharmaceuticals, Alexandria–Egypt. The purity of the sample was found to be 99.579±1.147 applying a spectrophotometric pharmacopoeial method.[3)]

Hydrocortisone (HC) Minapharm–Egypt. The purity of the sample was found to be 100.25±1.551 applying a spectrophotometric pharmacopoeial method.[3)]

Triamcinolone acetonide (TA) Minapharm–Egypt. The purity of the sample was found to be 100.548±2.017 applying a spectrophotometric pharmacopoeial method.[3)]

Nizoral tablets, labeled to contain 200 mg of ketoconazole per tablet, B.N. 961031610, manufactured by Janssen Pharmaceutica, Beerse–Belgium.

Nizoral cream, labeled to contain 20 mg of ketoconazole per one gram cream. B.N. 96k281090, manufactured by Janssen Pharmaceutica, Beerse–Belgium.

Dermatin cream, labeled to contain 10 mg clotrimazole per one gram cream B.N. 761. Produced by Pharcopharmaceuticals, Alexandria–Egypt.

Lotriderm cream, labeled to contain 10 mg clotrimazole and 0.64 mg betamethasone dipropionate per one gram cream, B.N. 011292. Manufactured by MUP. Co. Abu-Sultan Ismailia–Egypt under license of Schering Plough Co.–U.S.A.

Triderm cream, labeled to contain 10 mg clotrimazole, 0.64 mg betamethasone dipropionate and 1 mg gentamycin base per one gram cream, B.N. 011876. Manufactured by MUP, Co. Abu-Sultan Ismailia–Egypt under license of Schering Plough Co.–U.S.A.

Daktarin cream, labeled to contain 20 mg miconazole nitrate per one gram cream, B.N. 010625. Manufactured by Minapharm–Egypt under license of Janssen Pharmaceutica–Belgium.

Daktacort cream, labeled to contain 20 mg miconazole nitrate and 10 mg hydrocortisone per one gram cream, B.N. 011092. Produced by Minapharm–Egypt under license of Janssen Pharmaceutica–Belgium.

Pevaryl cream, labeled to contain 1 g econazole nitrate per 100 g cream, B.N. 11974. Manufactured by Minapharm–Egypt under license of Cilag–Switzerland.

Pevisone cream, labeled to contain 1 g econazole nitrate and 0.1 g triamcinolone acetonide per 100 g cream, B.N. 010953. Produced by Minapharm–Egypt under license of Cilag–Switzerland.

Hydrochloric acid (Riedel-dehaen–Germany), methanol (Analar), methanol (HPLC grade), hexane (BDH), triethylamine (BDH), isopropyl alcohol (Analar), phosphate buffer pH 2.5.[41)]

Developing System: A mixture of methanol : distilled water : triethylamine (70 : 28 : 2) was prepared for each of KT or CL and their degradation products.

A mixture of hexane : isopropyl alcohol : triethylamine (80 : 17 : 3) was prepared for each of MN or EN and their degradation products.

The Mobile Phase: A mixture of methanol : phosphate buffer pH 2.5 (65 : 35) was prepared for CL and BD.

A mixture of methanol : double distilled water containing 0.06 ml triethylamine pH 10 (75 : 25) was prepared for EN and TA.

Preparation of the Degradation Product Solutions a) For Ketoconazole: Accurate weight of KT (250 mg) was introduced into a 50 ml round bottom flask, 3 ml of hydrochloric acid was added and the solution was refluxed for 5 h. The solution was evaporated under reduced pressure to dryness to a constant weight and the residue was dissolved in 30 ml methanol. The degradation product solution was applied on TLC plates in bands and the plates were developed in chromatographic tanks previously saturated with the developing system, methanol : distilled water : triethylamine (70 : 28 : 2). The plates were air dried and visualized under UV light at 254 nm. The bands of the degradation products were scraped extracted with methanol three times each with 30 ml portion and filtered. The combined methanolic extract was evaporated to dryness to a constant weight. The residue was weighed and dissolved in methanol to produce a concentration of 2 mg/ml of degradation products.

b) For Clotrimazole, Miconazole Nitrate and Econazole Nitrate: Accurate weight of each of CL, MN or EN (250 mg) was introduced into a 50 ml round bottom flask, 3 ml of hydrochloric acid was added and the mixture was refluxed for 5 h. The solution was evaporated under pressure to dryness to a constant weight. The residue was weighed, and then dissolved in methanol to get a concentration of 2 mg/ml of degradation products.

Preparation of Laboratory Prepared Mixture for Selectivity Indicating Characteristics of the Methods a) Preparation of Miconazole Nitrate and Hydrocortisone Mixture: A laboratory prepared mixture containing 2 mg/ml and 1 mg/ml of MN and HC, respectively, in methanol was prepared.

b) Preparation of Econazole Nitrate and Triamcinolone Acetonide Mixture: A laboratory prepared mixture of 2 mg/ml and 0.2 mg/ml of EN and TA, respectively, in methanol was prepared.

Preparation of Pharmaceutical Dosage Form Solutions a) For Nizoral Tablets: An accurate weight of the powdered Nizoral tablets equivalent to 40 mg of KT was transferred into a 150 ml conical flask and 30 ml methanol was added. The mixture was shaken for 15 min using an ultrasonic shaker and filtered. The filter paper and the residue were washed three times each with 10 ml methanol. The combined filtrate and washings were collected into a 100 ml volumetric flask and the volume was completed with the same solvent to produce a solution of 0.4 mg/ml of KT.

b) For Nizoral, Dermatin, Daktarin, Daktacort, Lotriderm, Triderm, Pevaryl and Pevisone Creams: Two grams of the specified cream were weighed into a 100 ml conical flask and 30 ml of methanol was added, the mixture was warmed to 50 °C with continuous shaking, cooled to 10 °C and filtered. Warming, cooling and filtration steps were repeated three times to assure the complete extraction of the drug. The combined filtrates were diluted to the specified concentration.

Apparatus Dual wavelength, flying scanning densitometer, Shimadzu CS-9301PC, Japan.

HPLC system, Hewlett Packard series, 1100. Equipped with quaternary pump, diode array detector and manual 20 μl loop, Germany.

Procedure. A) Stability Indicating Methods for the Determination of Ketoconazole, Clotrimazole, Miconazole Nitrate and Econazole Nitrate Using the Densitometric Method General Procedure and Linearity: Different aliquots (0.8—9 ml) equivalent to (2—22.5 mg) of each of KT, CL, MN or EN stock solution (2.5 mg/ml) were transferred into a series of 10 ml volumetric flasks and the volumes were completed using methanol. Aliquots (20 μl) of each solution were applied on thin layer chromatographic plates 20×20 cm using a micropipette. The spots were spaced 2 cm apart and from the bottom edge of the plate. The plates were developed in chromatographic tanks by ascending chromatography for a distance of 16 cm. The tanks were previously saturated with the specified developing system for 1 h. Plates were air dried and the spots were detected under UV lamp at 254 nm. The drugs were scanned under the following parameters: Photomode: reflection, lane: AutoScan mode: zigzag, swing width: 12 cm, beam width: 0.4×0.4 cm. Wavelength: 243 nm for KT, 220 nm for CL, 225 nm for MN and EN.

Resulting outputs, chromatograms and area under peaks (*AUP*) were recorded. A calibration curve for each drug representing the relationship between $AUP\times10^{-1}$ and its corresponding concentration in μg/20 μl was plotted. The regression Eqs. 1—4 were computed to calculate the concentration of KT, CL, MN and EN respectively, and were found to be:

$$C(\mu g/20\,\mu l)=0.0212A-0.0912,\quad r=0.9994\quad \text{for KT} \tag{1}$$

$$A=47.17C(\mu g/20\,\mu l)+4.302$$

$$C(\mu g/20\,\mu l)=0.013A-0.4055,\quad r=0.9955\quad \text{for CL} \tag{2}$$

$$A=76.92C(\mu g/20\,\mu l)+31.19$$

$$C(\mu g/20\,\mu l)=0.0213A+0.1098,\quad r=0.9960\quad \text{for MN} \tag{3}$$

$$A=46.95C(\mu g/20\,\mu l)-5.16$$

$$C(\mu g/20\,\mu l)=0.0113A+0.1420,\quad r=0.9991\quad \text{for EN} \tag{4}$$

$$A=88.5C(\mu g/20\,\mu l)-12.66$$

Where C is the concentration in μg/20 μl, A is the $AUP\times10^{-1}$ and r is the correlation coefficient.

Stability Indicating Characteristics of the TLC Densitometric Method: Twenty microliters of the laboratory prepared mixture solutions for each drug and its degradation products (prepared for stability indicating characteristics) were applied on TLC plates and the method was completed [as mentioned under linearity starting from "The plates were developed…."]. Concentrations of the intact drugs in the prepared mixtures were calculated using the regression Eqs. 1—4.

Selectivity of the Proposed Densitometric Method: Into two series of 10 ml volumetric flasks (2—8 ml) of the laboratory prepared mixture for selectivity of each of MN and HC or EN and TA were transferred. The volumes were adjusted with methanol and 20 μl of each solution was spotted on TLC plates. The method was completed [as under linearity starting from "The plates were developed…."]. The concentrations of both MN and EN in the prepared mixtures were calculated using the regression Eqs. 3 and 4, re-

spectively.

Application of the TLC Densitometric Method to Determine the Investigated Drugs in Their Pharmaceutical Formulations: Different aliquots (2—7ml) of the prepared pharmaceutical dosage form solutions (0.4 mg/ml in methanol) for each drug were transferred into a series of 10 ml volumetric flasks and the volumes were completed with methanol. A twenty microliter aliquot was applied to TLC plates, and the method was completed [as mentioned under linearity starting from "The plates were developed…."]. The same experiment was repeated applying the standard addition technique. Concentrations of the added standards of each of KT, CL, MN or EN were calculated using the regression Eqs. 1, 2, 3 or 4, respectively.

B) Stability Indicating Methods for the Determination of Clotrimazole and Econazole Nitrate Using HPLC Technique Preparation of Internal Standard Solutions: Diclofenac sodium solution (internal standard). A solution of 1 mg/ml of diclofenac sodium (DS) in methanol was prepared.

Zopiclone solution (internal standard). A solution of 3 mg/ml of zopiclone in methanol was prepared.

General Procedure and Linearity: a) For clotrimazole and betamethasone dipropionate. Different aliquots (1—8 ml) of clotrimazole (CL) standard solution (250 μg/ml in methanol) were transferred into a series of 10 ml volumetric flasks. One milliliter from the DS internal standard solution was added to each flask and the volumes were adjusted with methanol.

Different aliquots (1—6.5 ml) of betamethasone (BD) standard solution (19.2 μg/ml in methanol) were transferred into a series of 10 ml volumetric flasks, and then 1 ml of DS standard solution (1 mg/ml) was added to each flask as an internal standard. The volume was completed using methanol.

b) For econazole nitrate and triamcinolone acetonide. Different aliquots (1—9 ml) of econazole nitrate (EN) standard solution (300 μg/ml in methanol) were transferred into a series of 10 ml volumetric flasks. To each flask 1 ml of zopiclone standard solution (3 mg/ml) was added as an internal standard and the volume was adjusted using methanol.

Different aliquots (1—9 ml) of triamcinolone acetonide (TA) standard solution (30 μg/ml in methanol) were transferred into another series of 10 ml volumetric flasks, and then 1 ml of zopiclone standard solution was added to each flask (IS). The volumes were adjusted using methanol.

From each of CL, BD, EN and TA prepared solutions, 20 μl was injected into a zorbax XDB-CB 15 cm×4.6 mm column using a Hamilton syringe and the chromatogram was recorded using the following instrumental parameters: Flow rate: 1.5 ml/min, wavelength: 220 nm for CL and BD, 225 nm for EN and TA.

The areas under peaks (*AUP*) were recorded, ratios of the recorded *AUP* of each drug/*AUP* of the internal standard were used for construction of the calibration curves of CL, BD, EN and TA.

The following regression Eqs. 5, 6, 7, and 8 were computed:

$$C(\mu g/ml) = 32.482A - 1.4583, \quad r = 0.9995 \quad \text{for CL} \tag{5}$$

$$A = 0.031C(\mu g/ml) + 0.045$$

$$C(\mu g/ml) = 1.6601A + 0.0471, \quad r = 0.9985 \quad \text{for BD} \tag{6}$$

$$A = 0.602C(\mu g/ml) - 0.0284$$

$$C(\mu g/ml) = 111.73A + 0.0659, \quad r = 0.9983 \quad \text{for EN} \tag{7}$$

$$A = 0.009C(\mu g/ml) - 0.0006$$

$$C(\mu g/ml) = 16.464A + 0.0018, \quad r = 0.9956 \quad \text{for TA} \tag{8}$$

$$A = 0.061C(\mu g/ml) - 0.0001$$

where C is the concentration in μg/ml, A is the ratio of *AUP* of drug/*AUP* of IS and r is the correlation coefficient.

Stability Indicating Characteristics of the Method: Different aliquots (1—3 ml) of the laboratory prepared mixture solutions for each drug and its degradation products (prepared for stability indicating characteristics) were transferred into 10 ml volumetric flasks. To the flasks of CL, 1 ml of DS standard solution (1 mg/ml) was added and to those containing EN, 1 ml of zopiclone solution (3 mg/ml) was added, then the volumes were adjusted with methanol. Each solution was chromatographed applying the procedure mentioned [under linearity starting from "20μl was injected…."]. The concentrations of the intact CL or EN were calculated using the regression Eq. 5 or 7, respectively.

Simultaneous Determination of CL–BD or EN–TA in Laboratory Prepared Mixture Using the HPLC Method: Different aliquots (1—6.5 ml) of CL–BD mixture solution (300 μg/ml CL and 19.2 μg/ml BD) or (1—9 ml) of EN–TA mixture solution (300 μg/ml EN and 30 μg/ml TA) were transferred into two separate series of 10 ml volumetric flasks. To the series containing CL–BD mixture, 1 ml of DS solution (1 mg/ml) was added and to the other series containing EN–TA mixture, 1 ml of zopiclone solution (3 mg/ml) was added as an internal standards.

Each solution was determined using the HPLC method applying the procedure mentioned [under linearity starting from "20 μl was injected…"].

The concentrations of each drug in the prepared mixtures were calculated using the regression Eqs. 5—8.

Application of the HPLC Method to Determine the Investigated Drugs in Their Pharmaceutical Formulations: Different aliquots (1—6 ml) of the prepared pharmaceutical dosage forms solutions (300 μg/ml in methanol) for each drug were transferred into a series of 10 ml volumetric flasks and 1 ml of the IS was added. The HPLC method mentioned under linearity was applied for the determination of each solution [starting from "20 μl was injected⋯"]. The same procedure was repeated applying the standard addition technique.

Results and Discussion

Densitometric Method The method was based on the different *Rf* values of the drug of choice and its acid degradation products. The acid degradation products of KT, CL, MN and EN may be imidazole derivatives. For example, the acid degradation products of CL are imidazole and (2-chlorophenyl)-diphen methanol (Fig. 2).[42)]

Complete degradation of CL, MN and EN was achieved by refluxing with hydrochloric acid for 5 h. On the other hand, the degradation products of KT were isolated using preparative TLC plates after refluxing for at least 5 h with hydrochloric acid.

Different developing systems were used to obtain well defined spots and better separation. The use of triethylamine for KT and CL prevented tailing and provided better separation. For MN and EN the use of isopropyl alcohol and triethylamine provided better separation and prevented tailing, while the use of methanol or ethyl alcohol resulted in overlapping of spots (Table 1).

Linear relationships between $AUP \times 10^{-1}$ and concentration were obtained and the regression equations were computed (Eqs. 1—4). The method was tested for stability by analyzing laboratory prepared mixtures containing different percentages of the drug with its degradation products. The method was found suitable for determination of the drug in the presence of 10% up to 90% of its degradation products. The mean percentage recoveries of the intact drugs in laboratory prepared mixtures were 99.89±1.163, 100.27±1.114, 100.00±1.097 and 100.21±1.084 for KT, CL, MN and EN, respectively.

The methods were tested for selectivity and found able to

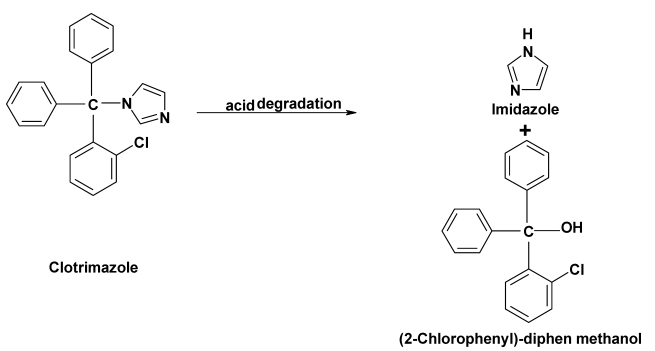

Fig. 2. The Suggested Acid Degradation Products of Clotrimazole

Table 1. Parameters of the Determination of the Studied Drugs by Densitometric Method

Drug	Developing system	Wavelength (nm)	*Rf*	
			Intact drug	Degradation products
Ketoconazole	Methanol : distilled water : triethylamine (70 : 28 : 2)	243	0.71	0.40, 0.53, 0.81, 0.89
Clotrimazole	Methanol : distilled water : triethylamine (70 : 28 : 2)	220	0.66	0.28, 0.49
Miconazole nitrate	Hexane : isopropyl alcohol : triethylamine (80 : 17 : 3)	225	0.90	0.36, 0.49
Econazole nitrate	Hexane : isopropyl alcohol : triethylamine (80 : 17 : 3)	225	0.68	0.36, 0.53

Table 2. Statistical Comparison between the Results of Analysis of Pure Samples Applying Densitometric and Official Methods[3)]

Statistical item	Densitometric methods				Official methods			
	KT	CL	MN	EN	KT	CL	MN	EN
Mean±S.D.	100.49±1.646	100.08±1.212	100.38±2.058	100.55±1.486	99.82±1.157	99.87±1.856	99.78±1.695	100.02±1.96
RSD	1.638	1.211	2.050	1.478	1.159	1.858	1.699	1.960
n	6	6	6	6	5	5	5	5
Variance	2.709	1.469	4.235	2.208	1.339	3.445	2.873	3.842
Student's *t* test	0.794 (1.83)[a)]	0.219 (1.83)[a)]	0.536 (1.83)[a)]	0.494 (1.83)[a)]	—	—	—	—
F ratio	2.023 (6.26)[a)]	2.345 (6.26)[a)]	1.474 (6.26)[a)]	1.740 (6.26)[a)]				
Confidence intervals	100.493±0.534	100.078±0.108	100.382±0.450	100.547±0.300				
Low limit of detection	1.3 μg/20 μl	0.66 μg/20 μl	1.34 μg/20 μl	1.39 μg/20 μl				
Low limit of quantification	4.37 μg/20 μl	2.20 μg/20 μl	4.47 μg/20 μl	4.63 μg/20 μl				
Standard deviation of the slop (s_b)	0.55	0.56	0.43	0.70				
Confidence limit of the slop	47.17±1.35	76.92±1.44	46.95±1.05	88.50±1.72				
Standard deviation of the intercept (s_a)	13.86	13.91	10.94	13.28				
Confidence limit of the intercept	4.30±33.96	31.19±35.75	−5.15±26.80	−12.57±32.54				

a) Figures in parentheses are the theoretical *F* and *t* values at confidence limit 95% and *n* is the number of experiments.

determine MN or EN in the presence of HC or TA, respectively. The mean percentage recoveries were 101.107±1.067 and 99.917±1.494 for MN and EN, respectively. The methods were successfully applied to determine of the investigated drugs in their pharmaceutical dosage forms. Validity of the methods was checked by applying the standard addition technique and the resulting percentage recoveries of the added authentic were: 100.44±1.678 and 100.06±0.804 for KT in Nizoral tablets and cream, respectively; 101.67±1.941 for CL in Dermatin cream; 100.42±2.273 and 101.06±1.77; for MN in Daktarin cream and Daktacort cream, respectively; 99.65±1.444 and 99.13±1.734 for EN in Pevaryl cream and Pevisone cream, respectively.

Statistical analysis of the results obtained by the suggested densitometric procedures was compared with the official method[3)] of analysis of the cited drugs and no significant difference between them was indicated, as shown in Table 2.

HPLC Method Various mobile phase systems were employed for use in HPLC separation and solvent polarity optimization. The mobile phase methanol : phosphate buffer pH 2.5 (65 : 35 v/v) was used at a flow rate of 1.5 ml/min for the determination of CL in the presence of either its acidic degradation products or betamethasone dipropionate. Decreasing the pH to 2 gave a bad resolution, while increasing the pH to 3 gave a tailing of peaks. The elution order was t_R (retention time)=2.022 min for CL, t_R=9.15 min and t_R=0.963 min for CL degradation products, t_R=5.247 min for diclofenac sodium (IS), t_R=6.143 min for BD as shown in Figs. 3, 4.

The mobile phase methanol: double distilled water containing 0.06 ml triethyl amine for the adjustment of the pH to 10 (75 : 25 v/v) was used at a flow rate 1.5 ml/min to determine EN in the presence of either its acidic degradation products or triamcinolone acetonide. No resolution occurred except with the addition of triethyamine and at lower pH tailing of peaks was observed. In case of EN mixture the elution order was EN at (t_R=5.983 min), EN degradation products at (t_R=1.724, 2.026 min), TA at (t_R=1.529 min) and zopiclone IS at (t_R=1.375 min) as shown in Figs. 5, 6.

For determination of the optimum HPLC-UV detector wavelength, the method was repeated using the same chromatographic conditions at different wavelengths and the best wavelength which gave optimal sensitivity and low noise of CL and BD was 220 nm and that of EN and TA was 225 nm.

The proposed method was sensitive and accurate as it could be applied to determine the investigated drugs over concentration ranges of: (25—200 μg/ml) for CL, (1.92—12.48 μg/ml) for BD, (30—270 μg/ml) for EN and (3—

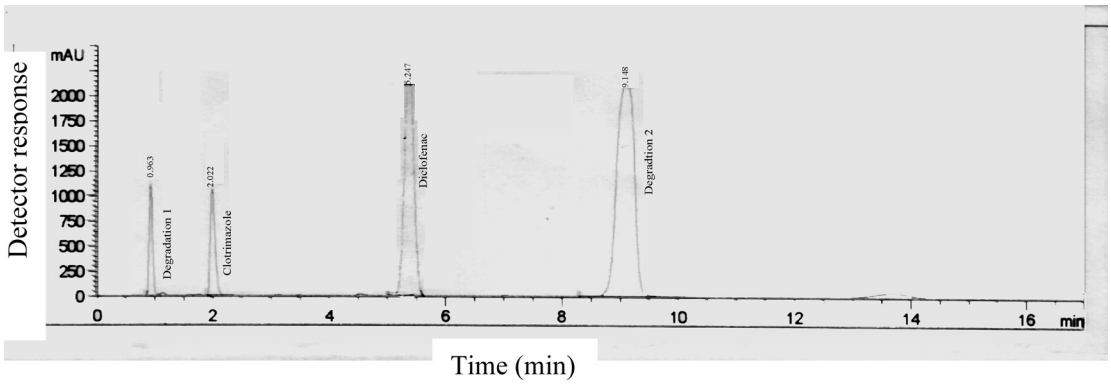

Fig. 3. Separation of Clotrimazole (50 μg/ml) and Its Degradation Products (25 μg/ml) in the Presence of Diclofenac Sodium (1 mg/ml) as an Internal Standard by HPLC Method

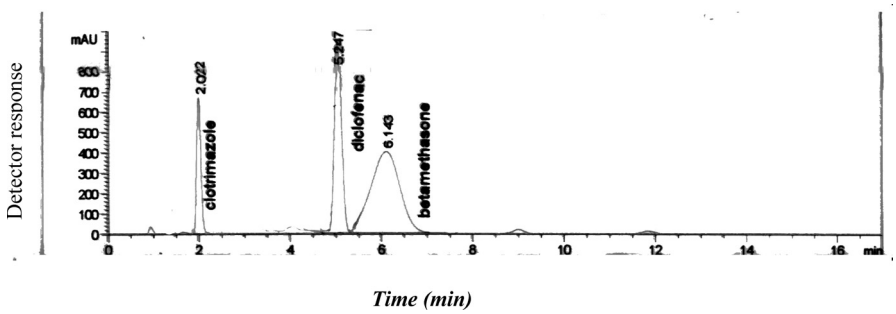

Fig. 4. Separation of Clotrimazole (50 μg/ml) and Betamethasone Dipropionate (3.2 μg/ml) in the Presence of Diclofenac Sodium (1 mg/ml) as an Internal Standard by HPLC Method

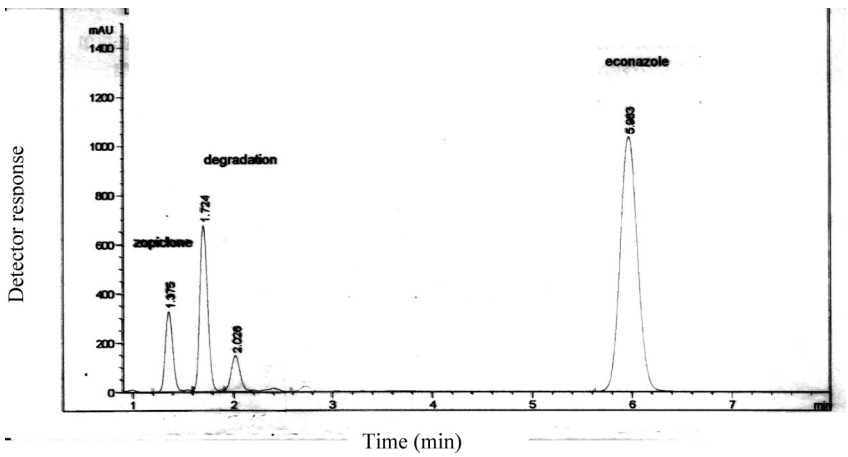

Fig. 5. Separation of Econazole Nitrate (200 μg/ml) and Its Degradation Products (100 μg/ml) in the Presence of Zopiclone (3 mg/ml) as an Internal Standard by HPLC

27 μg/ml) for TA.

Limit of detection and limit of quantification were determined and were found to be 5.63 μg/ml and 18.77 μg/ml for CL and 6.18 μg/ml and 20.61 μg/ml for EN, respectively. The method was applied for the determination of CL and EN in pure form (Table 3), it was also applied to determine mixtures of CL–BD and EN–TA and in their mixture with their degradation products, the results were: 101.22±1.141 and 100.15±1.121 for CL in the presence of BD and its degradation products, respectively; 100.76±0.773 and 100.75± 0.482 for EC in the presence of TA and its degradation products, respectively.

The HPLC method was successfully applied for the determination of both single and multi component pharmaceutical preparations containing CL and EN. The validity of this method was assessed by applying the standard addition technique and the resulting percentage recoveries of the added authentic were: 100.58±2.219, 101.56±0.971 and 100.73±1.725 for CL in Dermatin cream, Lotriderm cream and Triderm cream, respectively; 98.89±1.104 and 100.73± 1.437 for EN in Pevaryl cream and Pevisone cream, respectively.

Statistical comparison between the results obtained using the HPLC methods and the official methods[3] showed no sig-

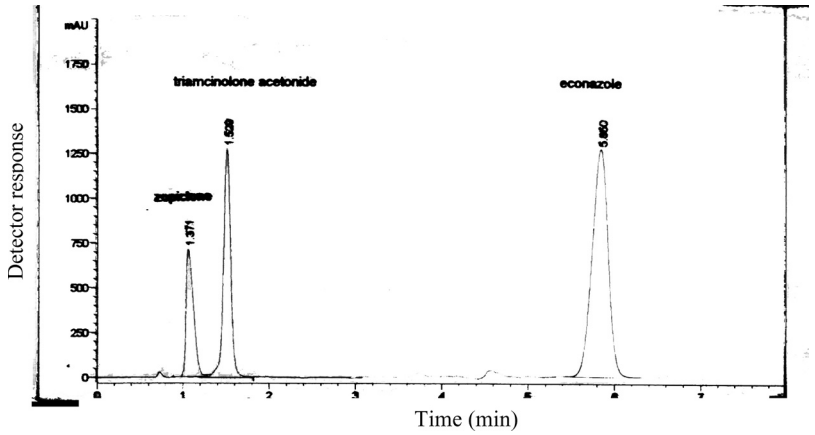

Fig. 6. Separation of Econazole Nitrate (200 μg/ml) and Triamcinolone Acetonide (20 μg/ml) in the Presence of Zopiclone (3 mg/ml) as an Internal Standard by HPLC Method

Table 3. Statistical Comparison between Results of Analysis of Pure Samples of Clotrimazole and Econazole Nitrate Applying HPLC Methods and Official Methods[3)]

Statistical item	HPLC method		Official method	
	Clotrimazole	Econazole nitrate	Clotrimazole	Econazole nitrate
Mean±S.D.	100.68±1.255	100.35±1.019	99.87±1.856	100.02±1.960
RSD	1.247	1.015	1.858	1.960
Variance	1.575	1.038	3.445	3.842
n	7	7	5	5
t test	0.858 (1.812)[a)]	0.341 (1.812)[a)]		
F ratio	2.187 (4.53)[a)]	3.701 (4.53)[a)]		
Confidence intervals	100.681±0.407	100.346±0.131		
LOD	5.630 μg/ml	6.18 μg/ml		
LOQ	18.770μg/ml	20.61 μg/ml		
Standard deviation of the slop (s_b)	2.85×10^{-3}	2.73×10^{-4}		
Confidence limit of the slop	$0.031\pm6.98\times10^{-3}$	$8.95\times10^{-4}\pm6.44\times10^{-4}$		
Standard deviation of the intercept (s_a)	0.607	0.598		
Confidence limit of the intercept	0.045±1.49	−5.90±1.41		

a) Figures in parentheses are the theoretical *F* and *t* values at confidence limit 95%. *n* is the number of experiments.

nificant difference between them (Table 3).

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