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Different spectrophotometric methods for determination of miconazole nitrate and hydrocortisone in bulk and in topical pharmaceutical preparation without prior separation

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Abstract Three simple, accurate, sensitive, selective and cost effective spectrophotometric methods were developed and validated for quantitative determination of miconazole nitrate (MIC) and hydrocortisone (HDC) in pure form, laboratory prepared mixtures and topical pharmaceutical preparation without any preliminary separation step. HDC was determined directly at λ_{max} 242.6 nm in the presence of MIC, while MIC was determined by three spectrophotometric methods namely; isoabsorptive point, ratio subtraction and ratio difference. In isoabsorptive point spectrophotometric method, the isoabsorptive point (A_{iso}) at 231 nm was chosen for determination of MIC. The second method was ratio subtraction at which MIC was determined at its λ_{max}220 nm after subtraction of interference exerted by HDC using 12 µg mL^{-1} of HDC as a divisor. While in ratio difference method, MIC was determined by dividing the recorded spectra by a 12 µg$mL^{-1}$of HDC as a divisor then measuring the difference in peak amplitude values between 211 and 230 nm for determination of MIC. The developed methods were found to be linear in the range of (2 – 24 µg mL^{-1}) with correlation coefficient 0.9999, and found to be linear in the range of (4 – 24 µg mL^{-1}) with correlation coefficient 0.9999 for MIC and HDC respectively. The developed methods were validated according to ICH guidelines. The results of the three methods were statistically compared to those obtained by the official method for determination of the cited drugs and showed that the proposed methods were accurate, reliable and precise as the reported one.

Keywords Miconazole nitrate, Hydrocortisone, isoabsorptive point, Ratio subtraction, Ratio difference, Topical preparation

1. Introduction

Miconazole nitrate (MIC); is chemically known as 1-[(2*RS*)-2-[(2,4-Dichlorobenzyl)oxy]-2-(2,4-dichlorophenyl)ethyl]-1*H*-imidazole nitrate [1]. Miconazole is a broad-spectrum imidazole antifungal agent used in the topical treatment of cutaneous dermatophyte infections and mucous membrane *Candida* infections, such as vaginitis [2]. Hydrocortisone (HDC); is chemically known as11β,17,21-Trihydroxypregn-4-ene-3,20-dione[1]. In humans, cortisol (hydrocortisone) is the main glucocorticoid and aldosterone is the main mineralocorticoid. Glucocorticoids can prevent or suppress inflammation in response to multiple inciting events, including radiant, mechanical, chemical, infectious, and immunological stimuli. Glucocorticoids are remarkably efficacious in the

treatment of a wide variety of inflammatory dermatoses. As a result, a large number of different preparations and concentrations of topical glucocorticoids of varying potencies are available. A typical regimen for an eczematous eruption is 1% hydrocortisone ointment applied locally twice daily [3]. The chemical structures are shown in **Fig. 1a** and **1b** for MIC and HDC, respectively. The literature survey for simultaneous determination of MIC and HDC as binary mixture or with other drugs revealed different methods for their determination such as reversed phase high performance liquid chromatographic method (RP-HPLC)[4-5], thin layer chromatographic (TLC)- densitometric method [4] and chemometric methods [6]. In this work, three spectrophotometric methods are applied successfully for determination of MIC and HDC in their binary mixture and in topical pharmaceutical formulations containing this mixture. The developed methods are simple, rapid, selective and don't need any special program, hence they can be easily applied as alternative method to reported LC method which requires time, expensive instruments, experience, and solvents.

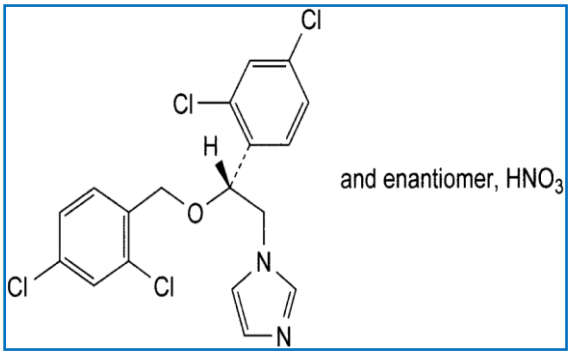

Figure 1a: Chemical structure of Miconazole Nitrate

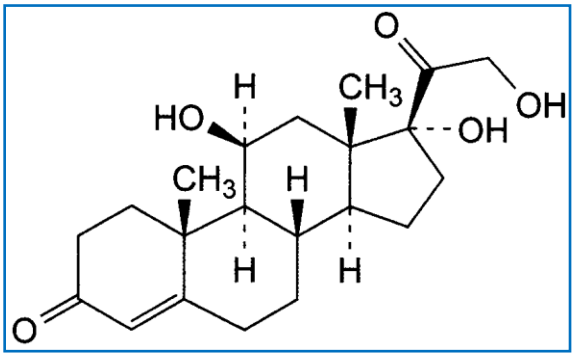

Figure 1b: Chemical structure of Hydrocortisone

2. Experimental

2.1. Instruments

A double beam UV-visible spectrophotometer (SHIMADZU, Japan) model UV-1800PC with quartz cell of 1 cm and UV-Probe personal software version 2.3 was used. The spectral band width is 2 nm and wavelength-scanning speed 2800 nm/min.

2.2. Materials

2.2.1. Authentic samples

Standard MIC and HDC was kindly supplied by Pharco Pharmaceuticals Industries (Alexandria, Egypt) with certified purities of 99.7 % and 99.8 %, respectively according to the official method [1].

2.2.2. Pharmaceutical preparation

Daktacort® cream (batch No. DIE3690) was manufactured by Minapharm (Egypt) under licence of Janssen pharmaceutica (Belgium). Each 1 gram contains 20 mg miconazole nitrate and 10 mg hydrocortisone.

2.2.3. Solvents

Methanol HPLC grade (Sigma –Aldrich Chemie GmbH, Germany).

2.2.4. Standard solutions

a. Standard stock solution of MIC and HDC were prepared in methanol in the concentration of 2 mg mL^{-1}.

b. Standard working solutions of MIC and HDC were prepared in methanol in the concentration of 0.1 mg mL^{-1}

2.2.5. Laboratory-prepared mixtures

Different mixtures containing different ratios of MIC and HDC were prepared using their respective working solutions (0.1 mg mL^{-1}).

2.3. Procedures

2.3.1. Isoabsorptive spectrophotometric method

Linearity: Aliquots equivalent to 40 - 240 µg and 20 - 240 µg of HDC and MIC, respectively were transferred separately from their respective standard working solutions (0.1 mg mL^{-1}) into two separate series of 10-mL volumetric flasks and the volume was completed using methanol. The zero order absorption spectra were recorded for MIC and HDC using methanol as a blank, then the absorbance was measured at 242.6 nm for HDC and 231 nm (A_{iso}) for MIC. Two calibration curves were constructed for each drug relating the absorbance at the selected wavelength to the corresponding concentrations of the drug and the regression equations were computed.

Assay of laboratory-prepared mixtures: Absorbance of the spectra of laboratory-prepared mixtures containing MIC and HDC at different ratios were measured at 242.6 nm corresponding to the contents of HDC only, and at 231 nm (A_{iso}) corresponding to the total content of MIC and HCD in the mixture. The total concentration of the two drugs and the concentration of HDC alone were calculated from their corresponding regression equations; then the concentration of MIC in the mixture was calculated by subtraction of HDC concentration from the total mixture concentration, yielding the actual concentration of MIC in the mixture.

2.3.2. Ratio subtraction spectrophotometric method

Linearity: Aliquots equivalent to 40 - 240 µg and 20 - 240 µg of HDC and MIC, respectively were transferred separately from their respective standard working solutions (0.1 mg mL^{-1}) into two separate series of 10-mL volumetric flasks and the volume was completed using methanol. The zero order absorption spectra were recorded for both drugs using methanol as blank, then the absorbance was measured at λ_{max} 242.6 and 220 nm for HDC and MIC, respectively. Two calibration curves were constructed for each drug relating the absorbance at the selected wavelength to the corresponding drug concentrations and the regression equations were computed.

Assay of laboratory-prepared mixtures: The absorption spectra of the laboratory-prepared mixtures containing MIC and HDC at different ratios were scanned and recorded. For direct determination of HDC in mixtures, measure the absorbance at (λ_{max} 242.6 nm), the concentration was calculated from their corresponding regression equation. Then the spectra were divided by the standard spectrum of 12 µg ml^{-1} of HDC as suitable divisor to obtain ratio spectra and then subtract the absorbance in the plateau region (the constant). By multiplication of the obtained spectra by the spectrum of the previously selected divisor the original curves for direct determination of MIC at 220 nm were obtained and the concentration was calculated from the corresponding regression equation.

2.3.3. Ratio difference spectrophotometric method

Linearity: Aliquots equivalent to 40–240 µg from HDC working solution (0.1 mg mL^{-1}) were transferred into a series of 10 ml volumetric flasks then the volume was completed with methanol. To obtain ratio spectra the zero order absorption spectra of each solution were recorded then divided by the standard spectrum of 12 µg ml^{-1} of HDC as suitable divisor. Calibration curve was constructed relating the difference in absorbance of the resultant ratio spectra at 211 and 230 nm ($\Delta A_{211-230\ nm}$) to the corresponding concentrations of MIC and the regression equation was computed.

Assay of Laboratory-prepared mixtures: The laboratory prepared mixtures were assayed by applying the previously mentioned procedure under linearity.

2.3.4. Analysis of pharmaceutical preparation

2.5 g Daktacort® cream was accurately weighed in a 100 ml beaker and ultrasonicated in 20 mL methanol for 45 min, then it was allowed to cool slightly, then the solution was filtered into 50 ml volumetric flask and the volume was completed to the mark using methanol. Appropriate dilutions of the prepared solution were made to prepare its working solution (0.1 mg mL^{-1}) and the procedures previously mentioned under laboratory prepared mixtures of each method were followed.

Validity of the methods was assessed by spiking the pharmaceutical formulation by known amounts of standard drug powders (standard addition technique). The recovery of the added standards was then calculated after applying the proposed method.

3. Results and Discussion

This work concerns with the development of simple, sensitive, economic and rapid methods for determination of the studied drugs in raw materials and in topical pharmaceutical formulation without previous separation. HDC could be determined directly at (λ_{max}= 242.6 nm) while MIC cannot be measured in the presence of HDC as shown in **Fig. 2**. MIC could be determined by three simple, rapid, sensitive and selective spectrophotometric methods; namely isoabsorptive point, ratio subtraction and ratio difference which have the advantage of no need to any derivatization or sophisticated manipulation steps like other spectrophotometric methods. They can be applied as alternative method to the published chromatographic methods which require time, expensive instruments, experience, and solvents.

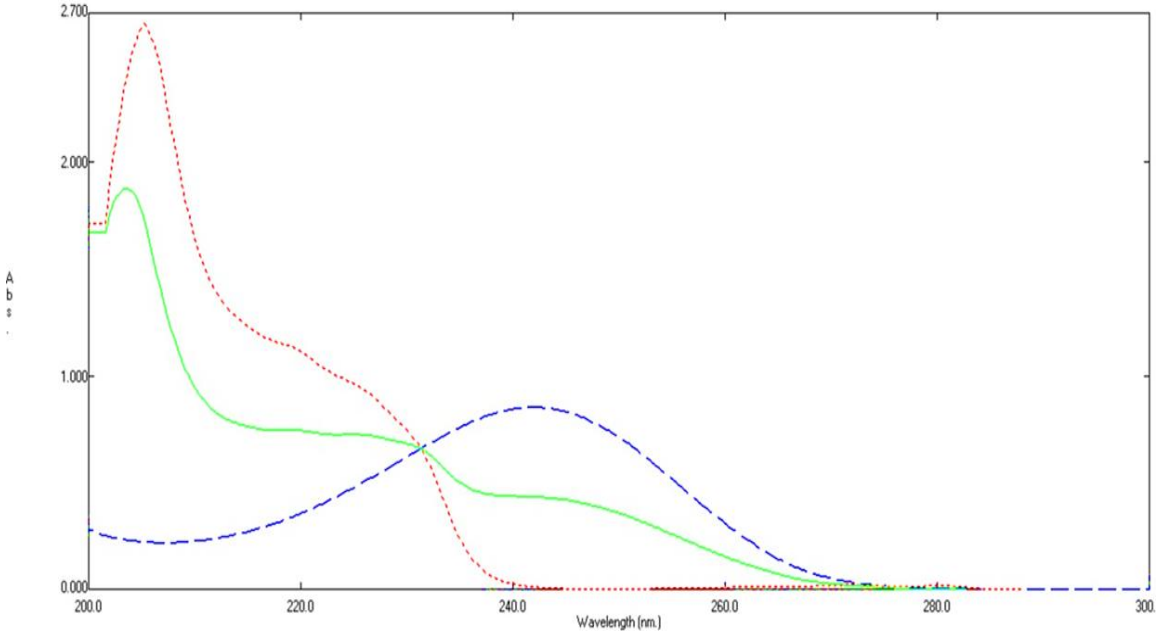

Figure 2: Zero order absorption spectra of 20 µg ml^{-1} of MIC (……..), 20 µg ml^{-1} of HDC (- - - - - - -) and (1:1) mixture containing 10 µg ml^{-1} of each (----------) using methanol as blank.

3.1. Isoabsorptive spectrophotometric method

The proposed method was developed by *Erram and Tipnis* [7] and is used for determination of MIC in presence of HDC in the presented work. At the isoabsorptive point the mixture of drugs act as a single component and gives the same absorbance value as pure drug. The best results regarding selectivity and sensitivity were obtained by using the isoabsorptive point at 231 nm (A_{iso}). The total concentration of both drugs could be calculated at this isoabsorptive point, while the concentration of HDC in the mixture could be calculated, without any interference, at 242.6 nm. Accordingly, the concentration of MIC could be calculated by subtraction.

A linear correlation was obtained between the absorbance values and the corresponding concentrations of both drugs at their corresponding wavelengths. The regression equations were:

$A_{iso} = 0.0332\ C + 0.0072$ r = 0.9999 at 231 nm.

$A = 0.0422\ C + 0.0205$ r = 0.9999 at 242.6 nm.

Where A_{iso} and A are the absorbances at 231 and 242.6 nm, respectively, C is the concentration of the drug in µg ml^{-1} and r is the correlation coefficient.

The proposed method was applied for the determination of MIC and HDC in bulk powder, where satisfactory results were obtained (**Table 1**).

3.2. Ratio subtraction spectrophotometric method

Following the theory of ratio subtraction [8]; The method was applied for determination of mixture of MIC and HDC as the spectrum of HDC is extended than the spectrum of MIC as shown in **Fig. 2**. The determination of MIC could be achieved by scanning the zero order spectra of the laboratory prepared mixtures containing MIC and HDC in methanol, then dividing the mixtures spectra by suitable divisor of HDC (12 μgml^{-1}) to produce a new ratio spectra as in (**Fig. 3.a**.), then subtraction of the absorbance values of these constants in plateau as in (**Fig. 3.b**.), followed by multiplication of the obtained spectra by the divisor as shown in (**Fig. 3.c**.), then finally the original spectra of MIC was obtained which are used for direct determination of MIC at (λ_{max}= 220 nm) and the concentration could be calculated from the linear regression equation. A linear correlation was obtained between the absorbance and the corresponding concentration of MIC at its corresponding wavelength, and the regression equation was:

$A = 0.0536\ C + 0.0237$ $r = 0.9999$ at 220 nm.

Where A is the peak amplitude, C is the concentration of the drug in μg ml^{-1} and r is the correlation coefficient.

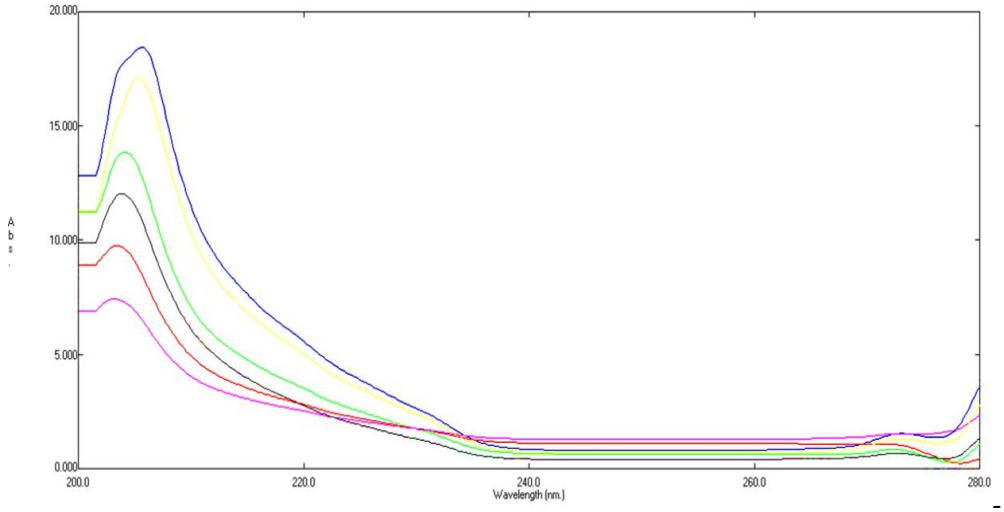

Figure 3a: Ratio spectra of laboratory prepared mixtures of MIC and HDC using 12 μg ml^{-1} of HDC as a divisor and methanol as a blank

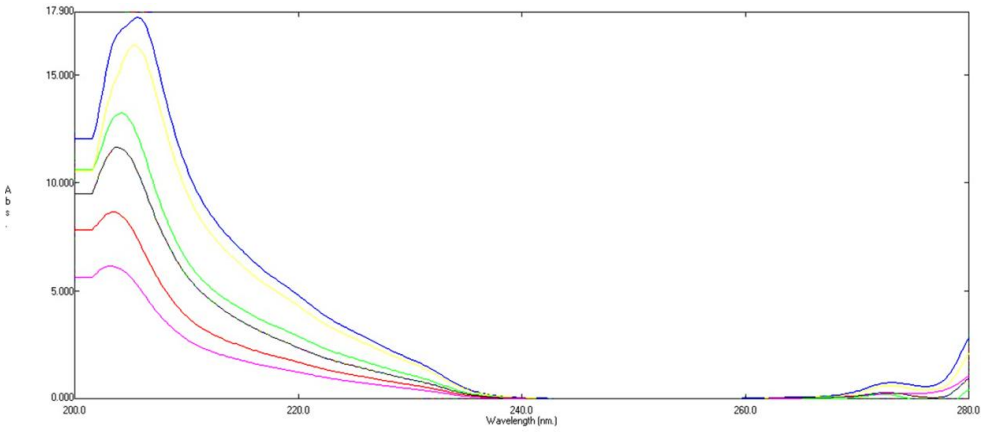

Figure 3b: Ratio spectra of laboratory prepared mixtures of MIC and HDC using 12 μg ml^{-1} of HDC as a divisor and methanol as a blank after subtraction of the constant.

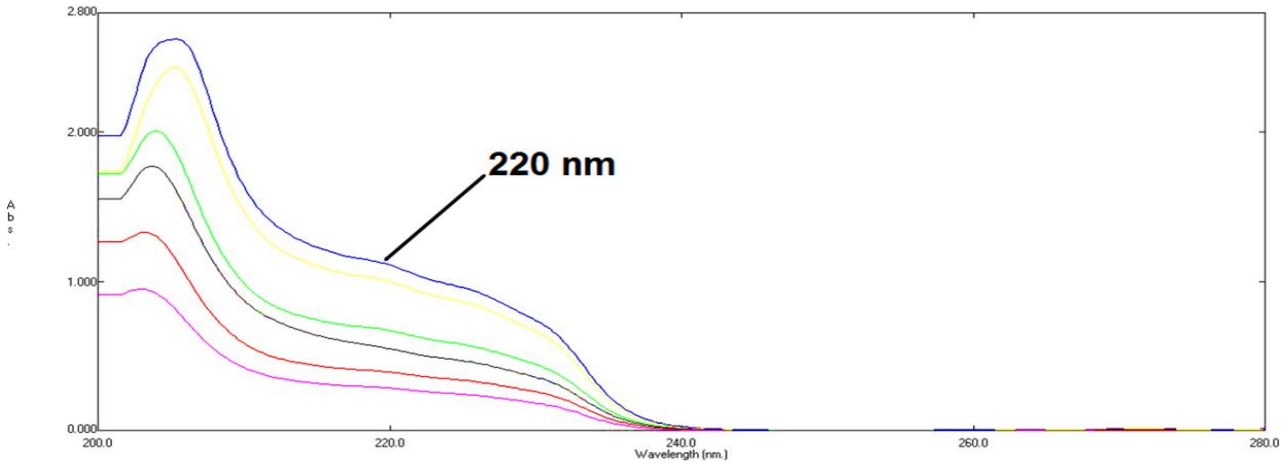

Figure 3c: The zero order absorption spectra of MIC obtained by the proposed ratio subtraction method for the analysis of laboratory prepared mixtures after multiplication by 12 µg ml^{-1} of HDC.

3.3. Ratio difference spectrophotometric method

Following the theory and mathematical explanation of ratio difference [9]; the amplitude difference between two points on the ratio spectra of a mixture is directly proportional to the concentration of the component of interest. It is affected by two critical steps; the first is the choice of the suitable divisor where the selected divisor should be compromise between minimal noise and maximum sensitivity. The second one is the choice of the wavelengths at which measurements are recorded. Any two wavelengths can be chosen provided that they exhibit different absorbance in the ratio spectrum and a good linearity is present at each wavelength individually [10]. Accordingly, to optimize the method, different concentrations of HDC as divisors and wavelengths were tested, but the best results were obtained when using 12 µg ml^{-1} of HDC as a divisor and measuring absorbance difference between 211 and 230nm ($\Delta A_{211-230nm}$) (**Fig.** 4). The linear regression equation was calculated:

$A = 0.3531C + 0.5286$. $r = 0.9999$.

Where A is the absorbance, C is the concentration of the drug in µg ml^{-1} and r is the correlation coefficient.

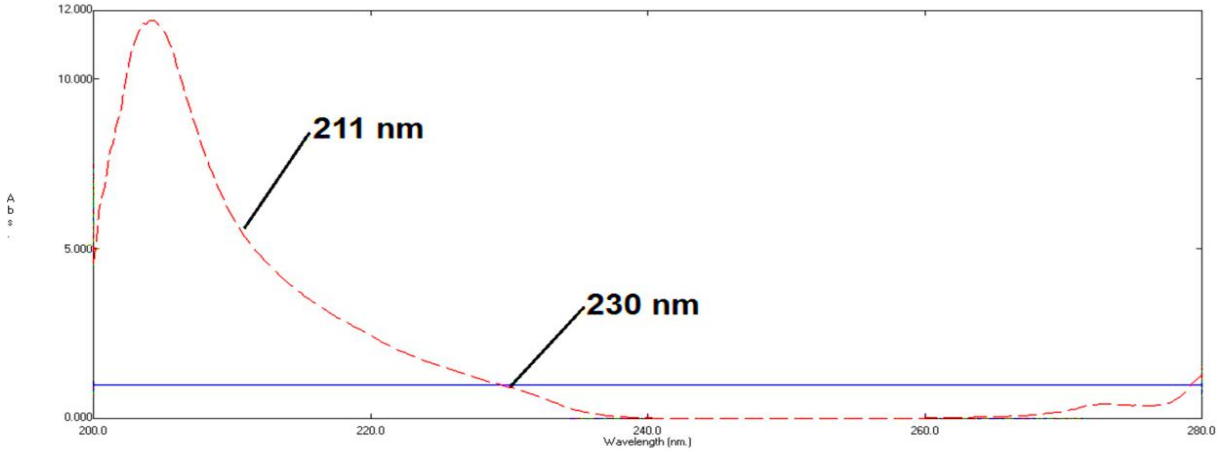

Figure 4: Ratio spectra of 12 µg ml^{-1} HDC (_____) and 10 µg ml^{-1} MIC (- - - - -) using 12 µg ml^{-1} HDC as divisor.

The specificity of the proposed methods was proved by the analysis of different laboratory prepared mixtures containing different ratios of the suggested drugs, where satisfactory results were obtained, (**Table 2**).

The developed spectrophotometric methods were also applied for determination of MIC and HDC in Daktacort® cream without interference from excipients and satisfactory results were obtained. The validity of the methods was

further assessed by applying standard addition technique which also confirmed the accuracy of the proposed methods (**Table 4**). The results obtained by applying the proposed methods were statistically compared to those obtained by applying the official method[1]for determination of Daktacort® cream and no significance differences were obtained between them (**Table 3**). The test ascertains that the proposed methods are as precise and accurate as the official pharmacopeial method [1] and are comparable to one another.

3.4. Method Validation

Method validation of the proposed methods was performed according to ICH guidelines [11].

3.4.1. Linearity and range

The calibration range for MIC and HDC was established through considerations of the practical range necessary according to adherence to Beer-lambert's law to give accurate, precise and linear results. Calibration ranges of MIC and HDC are shown in (**Table. 1**).

3.4.2. Accuracy

Accuracy of the proposed methods was calculated as the percentage recoveries of blind pure samples of the studied drugs. The concentrations were calculated from the corresponding regression equations and the results are shown in (**Table. 1**). Accuracy was further assessed by applying the standard addition technique to Daktacort® cream, where good recoveries were obtained revealing no interference from excipients and good accuracy (**Table. 4**).

3.4.3. Precision

3.4.3.1. Repeatability. Three concentrations (10, 14 and 22 µg ml^{-1}) and (6, 10 and 16 µg ml^{-1}) for both HDC and MIC respectively were analyzed three times intra-daily using the proposed methods. Good results and acceptable relative standard deviations (RSDs) were obtained, (**Table. 1**).

3.4.3.2. Intermediate precision. The previous procedures were repeated inter-daily on three different days for the analysis of the chosen concentrations. Good results and acceptable RSDs were obtained, (**Table 1**).

3.4.4. Specificity

Specificity of the proposed methods was assessed by the analysis of different synthetic laboratory prepared mixtures containing different ratios of MIC and HDC within their calibration ranges. Satisfactory results are shown in (**Table 2**).

3.4.5. LOD and LOQ

ICH recommendations [11] were followed to calculate the LOD and LOQ values of MIC and HDC. Low LOD and LOQ values indicate the high sensitivity of the proposed methods (**Table 1)**.

Table 1: Regression and validation parameters of the proposed methods for determination of MIC in presence of HDC

Parameters	**HDC at 242.6 nm**	**MIC by**		
		Isoabsorptive point method	**Ratio subtraction method**	**Ratio difference method**
Linearity range (µg mL^{-1})	4 - 24	2 – 24		
Slope	0.0422	0.0332	0.0536	0.3531
Intercept	0.0205	0.0072	0.0237	0.5286
Correlation coefficient	0.9999	0.9999	0.9999	0.9999
Accuracy (%)	100.25	100.45	100.46	99.95
Repeatability (RSD %)[a]*	0.004	0.26	0.18	0.39
Intermediate precision (RSD%)[b]*	0.015	0.77	0.25	0.91
LOD (µg mL^{-1})**	0.78	0.60	0.47	0.53
LOQ (µg mL^{-1})**	2.37	1.81	1.42	1.60

*[a]The intraday precision (n=3), average of three different concentrations repeated three times within day.
bThe interday precision (n=3), average of three different concentrations repeated three times in three successive days.
**Limit of detection and quantitation are determined via calculations LOD = (SD of the response/slope) × 3.3; LOQ = (SD of the response/slope) × 10.

Table 2: Determination of MIC and HDC in laboratory-prepared mixtures by the proposed methods

Mixture No.	Ratio MIC:HDC	Concentration MIC:HDC	% Recovery**			
			Direct determination of HDC	Isoabsorbtive point	Ratio subtraction	Ratio difference
1	2:1 *	20 : 10	100.12	101.34	101.24	101.79
2	3:2	12 : 8	98.49	101.34	100.48	101.78
3	1:1	15 :15	100.40	99.67	100.91	101.41
4	2:1*	18 : 9	99.53	101.48	100.99	100.64
5	2:1*	12 :6	98.54	100.93	99.08	101.68
6	1:1	10 : 10	99.41	101.01	100.99	101.26
Mean ± SD			**99.42 ± 0.79**	**100.96 ± 0.67**	**100.62 ± 0.79**	**101.43 ± 0.44**

* Indicates the ratio of the cited drugs in the pharmaceutical formulation from the market.
** Average of 3 determinations.

Table 3: Statistical analysis of the proposed methods and reported method for determination of MIC and HDC in their pharmaceutical formulations

Parameters		HDC at 242.6 nm	MIC			Official pharmacopeial method [1]	
			Isoabsorptive method	Ratio subtraction method	Ratio difference method	HDC	MIC
Daktacort® cream	Mean %	99.86	105.99	107.02	106.57	100.08	106.51
	SD	0.48	1.21	1.39	1.23	0.56	1.74
	n	6	6	6	6	6	6
	Student's *t*-test (2.228)*	**0.72**	**0.60**	**0.56**	**0.06**	----	----
	F – value (5.050)*	**1.37**	**2.08**	**1.57**	**2.00**	----	----

*Figures between parentheses represent the corresponding tabulated of *t* and *F* at *P* = 0.05.

Table 4: Quantitative determination of MIC and HDC in Daktacort® cream by the proposed methods and application of standard addition technique

Pharmaceutical formulation	Methods	Taken (µg ml^{-1})	Found[a] (%) ± SD	Added (µg ml^{-1})	% recovery[b]
Daktacort® Cream Claimed to contain 20 mg MIC and 10 mg HDC per 1 gram	HDC at 242.6 nm	10.00	99.86 ± 0.48	6.00	98.74
				7.00	97.83
				9.00	98.47
		Mean (%) ± SD			98.35 ± 0.47
	Isoabsorptive method	10.00	105.99 ± 1.21	6.00	100.05
				8.00	99.25
				9.00	99.75

	Mean (%) ± SD			99.62 ± 0.40
Ratio subtraction method	10.00	107.02 ± 1.39	6.00	98.81
			7.00	98.11
			8.00	97.68
	Mean (%) ± SD			98.20 ± 0.57
Ratio difference method	10.00	106.57± 1.23	6.00	98.62
			7.00	98.48
			8.00	98.30
	Mean (%) ± SD			98.47 ± 0.16

[a]: average of six experiments.
[b]: average of three experiments.

Conclusion

The proposed methods are precise, specific, accurate, sensitive and reproducible, where MIC and HDC can be determined in bulk powder, synthetic mixtures and in topical pharmaceutical formulation without interference from excipients. The advantages of the developed spectrophotometric methods over the published methods is that the spectrophotometric methods are rapid, simple, cost effective, less time consuming, data processing steps are not time consuming and doesn't need application of complex algorithm so it can be used in routine quality control analysis of their pharmaceutical formulation. HDC could be determined directly at (λ_{max}= 242.6 nm) while MIC cannot be measured in the presence of HDC. The developed isoabsorptive, ratio subtraction and ratio difference methods were successfully applied for determination of MIC in presence of HDC in their topical pharmaceutical formulation. Accordingly, they can be used in routine quality control analysis of MIC and HDC in pharmaceutical formulations.

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