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Research Article

SIMULTANEOUS DETERMINATION OF MICONAZOLE NITRATE AND HYDROCORTISONE IN BULK POWDER AND INTOPICAL CREAM USING FOUR CHEMOMETRIC METHODS

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ABSTRACT

Four methods are developed for simultaneous determination of Miconazole Nitrate and Hydrocortisone without previous separation. The first method depends on first derivative of the ratios spectra by measurements of the amplitudes at 233, 285 nm for Miconazole nitrate and 240,256 nm for Hydrocortisone. The second method depends on measuring the absorbance at the isosbestic point at 231.5 nm for the total concentration of both drugs while the concentration of Hydrocortisone is determined by direct spectrophotometric method at λ_{max} 241.5 nm in the presence of Miconazole nitrate, the concentration of Miconazole nitrate is calculated by subtraction. The third method is factorized absorptivity method at which both drugs are determined at more than one isosbestic point 217.5, 224nm.The fourth method involved application of the bivariate calibration algorithm for spectrophotometric simultaneous determination of the mixture. The suggested procedures are validated using laboratory prepared mixtures and are successfully applied for the analysis of pharmaceutical preparations. The methods retained their accuracy and precision when the standard addition technique is applied. The results obtained are statistically analyzed and compared with those obtained by the manufacturer method.

Keywords: Miconazole Nitrate and Hydrocortisone, First derivative of the ratios spectra.

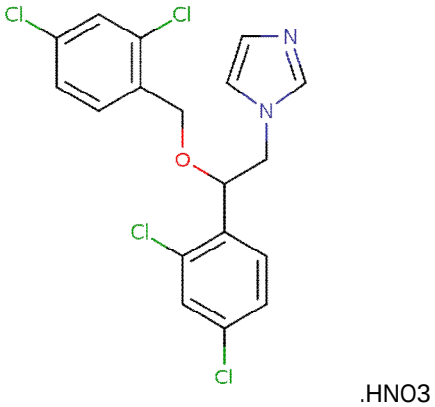
1. INTRODUCTION

Miconazole Nitrate; 1- [2,4- dichloro -β-(2,4-dichlorobenzyloxy) phenethyl] -imidazole (Figure 1)[1] is an anti-fungal medication related to fluconazole (Diflucan), ketoconazole (Nizoral), itraconazole (Sporanox), and clotrimazole (Lotrimin, Mycelex). It is used either on the skin or in the vagina for fungal infections. Miconazole was approved by the FDA in 1974. Miconazole prevents fungal organisms from producing vital substances required for growth and function. This medication is effective only for infections caused by fungal organisms. It will not work for bacterial or viral infections.

Miconazole interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents[3].

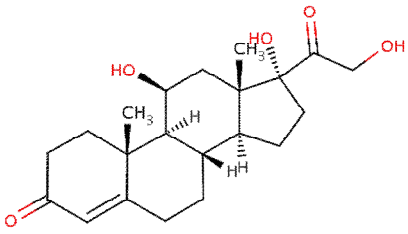
Hydrocortisone; 11β, 17α,21-trihydroxypregn-4-ene-3,20-dione (Figure 1) is a principal glucocorticoid hormone[1,2]. It is produced by the adrenal cortex[2] and has been used clinically to treat skin problems such as rashes, eczema and also used to treat endocrine (hormonal) disorders (adrenal insufficiency, Addisons disease). It is also used to treat many immune and allergic disorders, such as arthritis,

lupus, severe psoriasis, severe asthma, ulcerative colitis, and Crohn's disease. Hydrocortisone is the most important human glucocorticoid. It is essential for life and regulates or supports a variety of important cardiovascular, metabolic, immunologic and homeostatic functions[3]. Several analytical methods have been reported for the determination of Miconazole Nitrate (MIC) in pharmaceuticals and biological samples includes Spectrophotometric[4-5], HPLC[6-10], GasChromatograhy[11-12], HPTLC[13] Voltammetric determination[14]. Besides, several analytical methods have been reported for the determination of hydrocortisone (HYD) in combination with other drugs using HPLC technique[15-18]. This work can develop quality control of miconazole and hydrocortisone in its semisolid dosage form by using simpler, available and less tedious methods. Thus, the aim of this work is to develop simple and validated chemometric methods.



.HNO3

a. Miconazole Nitrate, MIC



b. Hydrocortisone

Fig. 1: Miconazole Nitrate, MIC Hydrocortisone, HYD

2. EXPERIMENTAL

2.1. Apparatus

Spectrophotometer: SHIMADZU UV-1800 PC, dual beam UV–visible spectrophotometer with two matched 1 cm quartz cells, connected to an IBM compatible personal computer (PC) and an HP-600 inkjet printer. Bundled UV-PC personal spectroscopy software version (3.7) was used to process the absorption and the derivative spectra. The spectral band width was 0.2 nm with wavelength scanning speed of 2800 nm min−1,Japan.

2.2. MATERIALS

2.2.1. Pure samples

Miconazole Nitrate and Hydrocortisone were kindly supplied by Sigma, Egypt.

2.2.2. Market samples

Dactacort cream (Sigma), Egypt. It is labeled to contain 20 Miconazole Nitrate and 10 mg Hydrocortisone for each 1 gram of cream were provided and manufactured by Sigma Pharmaceutical Industries Company. (Cairo, Egypt).

2.3. Chemicals and reagents

All chemicals are of analytical grade and the solvents are of spectroscopic grade.

2.4. Standard solutions

Stock solutions

Miconazole Nitrate and Hydrocortisone stock solutions (200µg ml−1) are prepared by weighing accurately 0.01gm of each powder into two separate 50 ml volumetric flasks. Methanol (25 ml) is added, shaken for a few minutes and completed to volume with the same solvent.

3. Determination of linearity range

3.1. First derivative of the ratio spectrophotometric method (DD1)

For determination of Miconazole Nitrate, the spectra of (MIC) of different concentrations range(5-30µg ml−1) are divided on the spectra of different concentrations of (HYD) range(5-30µg ml−1) then the first derivative of the ratio spectra are obtained taking Δλ=10 nm. The calibration curves of (MIC) first derivative ratio are obtained at the peak at 233,285 nm. After comparing the results, the spectrum of (HYD) 30µg ml−1 is chosen as adivisor as giving the least intercept and best correlation coefficient.

For determination of Hydrocortisone, the spectra of (HYD) of different concentrations range (5-30µg ml−1) are divided on the spectra of different concentrations of (MIC) range(5-30 µg ml−1) then the first derivative of the ratio spectra are obtained taking Δλ=10 nm. The

calibration curves of first derivative ratio of (HYD) are obtained at the peaks at 240,256 nm, after comparing the results the spectrum of (MIC) 15μg ml−1 is chosen as adivisor as giving the least intercept and best correlation coefficient.

3.2. Isosbestic spectrophotometric method

The zero order absorption spectra of 25 μgml-1 of each MIC and HYD were recorded and the spectrum of 12.5 μgml-1 of MIC/HYD in a mixture was recorded. Aliquots from stock solutions (MIC) and (HYD) equivalent to final concentration 5-30μg ml−1of (MIC) and 5-30μg ml−1of (HYD) are transferred into two separate sets of 10 ml volumetric flasks and completed to the mark with methanol. The zero order absorption spectra are recorded for both drugs using methanol as a blank; then the absorbance is measured at 231.5nm (isosbestic point) for (MIC) and (HYD) and at 241.5nm for (HYD). Two calibration curves are constructed for each wavelength relating the absorbance to the corresponding drug concentrations and the regression equations are computed.

3.3. Factorized absorptivity method

The zero order absorption spectra of 6.25 μgml-1 of MIC and 12.5 μgml-1 of HYD were recorded. Aliquots from (MIC) stock solution equivalent to final concentration 3-25μgml−1are transferred into10 ml volumetric flasks and completed to the mark with methanol. The zero order absorption spectra are recorded using methanol as a blank; then the absorbance measured at 217.5,224 nm (two isosbestic points). Two calibration curves are constructed for each wavelength relating the absorbance to the corresponding drug concentrations and the regression equations are computed. Aliquots from (HYD) stock solution equivalent to final concentration 5-25μg ml−1 are transferred into10 ml volumetric flasks and completed to the mark with methanol. The zero order absorption spectra are recorded using methanol as a blank; then the absorbance measured at 217.5,224 nm (two isosbestic points) and at 241.5nm(λmax of HYD). Three calibration curves are constructed for each wavelength relating the to the corresponding drug concentrations and the regression equations are computed.

3.4. Bivariate method

Aliquots of standard (MIC) and (HYD) equivalentto final concentration 5-35μg ml−1 of both drugs are transferred separately into two sets of 10 ml volumetric flasks then diluted to volume with methanol. Calibration curves at differentwavelengths 220,223,228,231.5,234 and 237nm are constructedand the regression equation at each wavelength is calculated. From both sets ofregression equations, the sensitivity matrices K is calculated, the optimum pair of wavelengths chosen is (223 and 237 nm) to carry out the determination and theregression equations used in the bivariate algorithm are deduced.

4. Laboratory prepared mixtures

Accurate aliquots equivalent to final concentration (5-25 μg/mL) of (MIC) are transferred from its stock solution into a series of 10 ml volumetric flasks and portions equivalent to the same final concentration are transferred from (HYD) stock solution then added tothe same flasks and volumes are completed to mark with methanol and mixed well to make different ratios.

5. Procedures

5.1. First derivative of the ratio spectrophotometricmethod (DD1)

According to the theory of the ratio spectra derivative method. The stored UV absorptionspectra of standard solutions of (MIC)are divided by a standard spectrum of (HYD) (30μg/mL) wavelength-by-wavelength. The first derivativecalculated for the obtained spectra with Δλ = 10 nm.The amplitudes at 233,285 nm are measured and foundto be linear to the concentrations of (MIC). For (HYD), the stored UV absorption spectra of standardsolutions of it are divided by a standard spectrum of (MIC) (30μg/mL) wavelength-by-wavelength. The first derivative calculated for the obtained spectra with Δλ = 10 nm. The amplitudes at 240, 256 nm are measured and found to be linear to the concentration of (HYD).

5.2. Isosbestic spectrophotometric method

Absorbance of the spectra of laboratory prepared mixtures containing different ratios of (MIC) and (HYD) are measured at 241.5nm corresponding to the contents of (HYD) only, and at231.5 nm, corresponding to the total content of (MIC) and (HYD) in the mixture. The concentration of (HYD) alone and the total concentration of the two drugs are calculated from their corresponding regression equations; then by subtraction of (HYD) concentration from the total mixture concentration, the actual concentration of (MIC) in the mixture obtained.

5.3. Factorized absorptivity method

Absorbance of the spectra of laboratory prepared mixtures containing different ratios of (MIC) and (HYD) are measured at 241.5 nm

corresponding to the contents of (HYD) only and at217.5 and at 224 nm, corresponding to concentration of (MIC) and (HYD) in the mixture. The concentration of (HYD) alone and the total concentration of the two drugs are calculated from their corresponding regression equations; then by subtraction of (HYD) concentration from the total mixture concentration then dividing the result by 2, the actual concentration of (MIC) in the mixture obtained.

5.4. Bivariate method

Different volumes 0.25-1.25ml of (MIC) 200µg ml–1 aretransferred and mixed with 0.25-1.25mlof(HYD) 200µg ml–1 in a set of 10ml volumetric flaks and completed to mark with methanol. The absorbance ofeach mixture is recorded at 223 and 237 nm. The concentrations of the two drugs arecalculated using Kaiser method[19].

6. Assay of pharmaceutical formulations

Daktacort® cream: 1gm of cream accurately weighed and transferred into a 100 ml beaker, sonicated in 20 ml methanol for 10 min and filtered into a 100 ml volumetric flask. The residue washed three times using 20 ml methanol each time and the volume completed to the mark with methanol forming tablet stock solution (100 ml) that contain 20 mg (MIC) and 10 mg (HYD).

For first derivative of ratio method: aliquots (0.25,0.4,0.5 ml) equivalent to final concentration(5,8,10µg/mL) are separately transferred from both authentic drugs(200µg/mL) to 10 ml volumetric flasks and 1mlfrom cream stock solution equivalent to 30µg/mL (20µg/mL MIC,10µg/mL HYD) final concentration were added to each flask and diluted with methanol.

For isosbestic point method : aliquots (0.25,0.4,0.5 ml) equivalent to final concentration (5,8,10µg/mL) are separately transferred from both authentic drugs (200µg/mL) to 10ml volumetric flasks and 1ml from cream stock solution equivalent to 30µg/mL (20µg/mL MIC,10µg/mL HYD) final concentration were added to each flask and diluted with methanol.

Factorized absorptivity method: aliquots (0.25,0.5,0.75 ml) equivalent to final concentration (5,10,15µg/mL) are separately transferred from both authentic drugs (200µg/mL) to 10ml volumetric flasks and 1ml from cream stock solution equivalent to 30µg/mL (20µg/mL MIC,10µg/mL HYD) final concentration were added to each flask and diluted with methanol.

For bivariate method: aliquots (0.25,0.4,0.5ml) from both authentic drugs (200µg/mL) equivalent to final concentration (5,8,10µg/mL) are separately transferred to 10 ml volumetric flasks and1ml from cream stock solution equivalent to final concentration 30µg/mL were added to each flask and diluted with methanol .

The general procedures under linearity are followed.The validity of the methods assessed by applying the standard addition technique.

7. RESULTS AND DISCUSSION

Analytical methods for the determination of binary mixture without previous separation are of interest.

As shown in Fig. 2, the zero-order spectra of standard drugs are found to be overlapped making their simultaneous determination difficult.

7.1. DD1 method

The main parameters that affect the shape of the derivative ratio spectra are the concentration of the standard solution used as a divisor and the wavelength intervals over which the derivative is obtained ($\Delta\lambda$). These parameters need to be optimized to give a well resolved large peak with good selectivity and higher sensitivity. The obtained ratio spectra (Fig. 3, 4) were differentiated with respect to wavelength to afford the first derivative ratio spectra. Good measurements could be obtained at 233, 285 nm for MIC and at 240, 256 nm for HYD (Fig. 5, 6). Effect of the wavelength intervals revealed that $\Delta\lambda$=10 nm was the most suitable interval for measurement of both drugs with scaling factor of 1. Increasing that interval led to a less sensitive peak.

7.2. Isosbestic spectrophotometric method

Hydrocortisone can be determined by direct measurement of absorbance at 241.5 nm since MIC show neglected absorbance while the absorption spectra of MIC and HYD showed severe overlap, which makes the determination of MIC concentration in the mixture more difficult figure (2). By applying the proposed method to the spectral data of the mixture, both MIC and HYD concentrations could be determined without any interference figure (7).

At the isosbestic point the mixture of drugs acts as a single component and gives the same absorbance value as pure drug. Thus, by measuring the absorbance value at the chosen isosbestic point 231.5 nm (Aiso) (Fig. 7), the total concentration of both (MIC) and (HYD) could be calculated, while the concentration of (HYD) in the mixture could be calculated, without any interference, at 241.5 nm. Thus the

concentration of (MIC)could be calculated by subtraction.

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

A(iso) =0.004C – 0.035 r = 0.9999 at 283.5 nm

A =0.0096C – 0.0297 r = 0.9999 at 250 nm

Where A is the absorbance, C is the concentration of the drug in μg ml−1 and r is the correlation coefficient.

The proposed method is applied for the determination of (MIC) and (HYD) in cream, applying standard addition technique.

7.3. Factorized absorptivity method

This method is used to improve the isosbestic point in this mixture so if we draw the spectra of certain concentration of MIC and HYD concentration double that of MIC we will have new isosbestic point like shown in figure(8).

7.4. Bivariate method

Bivariate calibration spectrophotometric method is a direct method which is proposed for the resolution of mixtures. The principle of bivariate calibration is the measurement of two components (A and B) at two selected wavelengths ($\lambda 1, \lambda 2$) to obtain two equations[19]

$AAB1=mA1 \cdot CA+mB1 \cdot CB+eAB1$

$AAB2=mA2 \cdot CA+mB2 \cdot CB+eAB2$

The resolution of each equation set allows the evaluation of $C_A$and C_B values:

$$C_B=m_{A2}(A_{AB1}-e_{AB1})+m_{A1}(e_{AB2}-A_{AB2}) / m_{A2}m_{B1}-m_{A1}m_{B2}$$

$$C_A=(A_{AB1}-e_{AB1}-m_{B1}C_B) / m_{A1}$$

Where *CA* and *CB*are the concentration of MIC and HYD ,respectively *mA1,mA2* are the slope values of MICat $\lambda 1, \lambda 2$; *mB1, mB2* are the slope values of HYDat $\lambda 1, \lambda 2$;A_{AB1},A_{AB}2are the absorbance of the binary mixture at $\lambda 1, \lambda 2$; *eAB1, eAB2* are the sum of the intercepts of the two drugs at $\lambda 1, \lambda 2$, respectively.This simple mathematic algorithm allows the resolution of the two components by measuring the absorbance of their mixture at the two selected wavelengths and using the parameters ofthe linear regression functions evaluated individually for each component at the same wavelengths.In order to apply the bivariate method in the resolution ofMIC and HYD, the absorbance of the two components at nine different selected wavelengths is recorded in the region of overlapping; 220,223,228,231.5,234 and 237 nm. Fig.2.

The calibration curve equations and their respective linear regression coefficients are obtained directly with the aim of ensuring that there isa linear relationship between the absorbance and the corresponding concentration. All of the calibration curves at the selected wavelengths showed a satisfactory linear regression coefficient (r > 0.9985).The method of Kaiser[19] is used for the selection of the optimum wavelengths set, which assured the best sensitivity and selectivity of the determination. A series of sensitivity matrices *K* are created for each binary mixture and for every pair of pre-selected wavelengths:

$$K=[m_{A1} \quad m_{B1}] [m_{A2} \quad m_{B2}]$$

Wherem_{A1}, m_{A2} are the slopes, which are consideredas the sensitivity parameters of the component*A* at two selected wavelengths (1, 2) andm_{B1}, m_{B2} are the parameters for the component*B*. The resolution of these matrices is calculated:

$$K=(m_{A1}*m_{B2})-(m_{A2}*m_{B1})$$

The values of *K* obtained and the values of the absolute selectivity ofKaiser's Determinant $(K\times10^5)$ are obtained and used as the optimization criterion; the wavelengths set selected isthat with the highest absolute matrix determinantvalue.For the bivariate determination of MIC and HYD, 223 and237 nm are found to give the maximum value of *K* and thus can be used for the analysis (Table2).

7. Quantification, accuracy and precision

The characteristic parameters and the linear regression equations together with correlation coefficients, slope, intercept, R.S.D. of slope and intercept, repeatability (within day) and reproducibility (between-day) obtained for each drug are collected in (Table 1). There are no significant difference for the assay, which is tested within-day (repeatability) and between-days (Reproducibility). In order to demonstrate the validity and applicability of the proposed DD1,Isosbestic, Factorized absorpitivityand Bivariate methods, recovery studies were performed by analyzing laboratory prepared mixtures of Miconazole Nitrate and Hydrocortisone with different composition ratio (Table 3). Results obtained are compared with the reference methods[20] by student's t-test and variance ratio F-test (Table 4). The calculated values did not exceed the tabulated ones.

8. CONCLUSION

For routine analytical purposes, it is always of interest to establish methods capable of

analyzing a large number of samples in a short time period with good accuracy and precision, either in laboratory prepared mixtures or in commercial pharmaceutical dosage form. Spectrophotometric techniques can generate large amounts of data within a short period of analysis. The proposed methods provide a clear example of the high resolving power and low cost while HPLC method is more specific, it needs expensive equipment and materials.

Table1: Spectral data for determination of Miconazole Nitrate and Hydrocortisone by DD1, Isosbestic point, Factorized absorptivity and bivariate methods

Methods / Validation Parameter	DD1		Isosbestic point	Factorized absorptivity		Bivariate		DD1		Isosbestic point	Factorized absorptivity	Bivariate	
Linearity range (µg/ml)	5-30	5-30	5-30	3-15	3-25	10-30	20-35	5-30	5-30	5-30	5-25	10-35	5-30
Wavelength (nm)	233	285	231.5	217.5	224	223	237	240	256	241.5	241.5	223	237
Slope	0.0034	0.0013	0.0359	0.048	0.0573	0.0508	0.0053	0.2165	0.1592	0.0496	0.0444	0.0237	0.0429
Intercept	0.0037	0.0057	-0.0435	0.0323	-0.0052	0.0713	-0.0054	0.14-	0.2912	-0.0633	0.0205	-0.0232	-0.0273
Correlation coefficient	1	1	0.9999	0.9999	1	0.9996	0.9999	0.9998	0.9998	0.9999	0.9999	0.9998	0.9999
LOD (µg/ml)	0.6	0.5	1.3	0.81	0.79	1.5	0.3	1.2	1.4	0.81	0.87	0.8	1.1
LOQ (µg/ml)	2	1.7	4.2	2.7	2.64	5	1	3.9	4.7	2.7	2.9	2.7	3.5
S.E.	0.2	0.17	0.42	0.27	0.26	0.51	0.10	0.39	0.43	0.27	0.27	0.27	0.35
Repeatability (R.S.D. %)	1.02	0.46	0.56	0.25	0.35	0.12		0.31	0.28	1.59	1.13	0.23	
Reproducibility (R.S.D. %)	0.8	0.51	0.35	0.81	0.91	0.12		0.54	0.63	0.52	1.3	0.23	

Table 2: Application of the method of Kaiser for the selection of the wavelengths set for MIC. And HYD. the absolute values of determinants of sensitivity matrices (K×10⁵)

λ1, λ2	220	223	228	231.5	234	237
220	0	8.762	-	-	-	-
223	-	0	-	-	-	-
228	25.1	46.09	0	-	-	-
231.5	69.07	104.27	64.6	0	-	-
234	109.978	157.05	128.6	70.45	0	-
237	147.238	205.61	185.5	131.39	58.39	0

Table 3: Inter-day and intra-day accuracy and precision determination of Miconazole Nitrate and Hydrocortisone by the proposed methods

	Drug	Miconazole Nitrate						Hydrocortisone				
	Method	DD1		Isosbestic point	Factorized absorptivity		Bivariate	DD1		Isosbestic point	Factorized absorptivity	Bivariate
	Wavelength (nm)	233	285	231.5	217.5	224	223,237	240	256	241.5	241.5	223,237
	Weight taken(µg)	20	20	20	10		20	10	10	10	10	10
	Validation Parameters / %Recovery / Experiment											
Inter-day	1	102	102	100				100	99	102		
	2	103	101	101	100	102	101	101	99	99	99	101
	3	100	101	100	101	101	101	101	99	103	100	101
	4	102	102		100	100	101	101	99		101	102
	5	102	102					100	99			
	Mean	102	102	100	100	101	101	101	99	101	100	101
	S.D.	1.04	0.58	0.56	0.25	0.35	0.12	0.32	0.28	1.59	1.13	0.23
	R.S.D.	1.02	0.57	0.55	0.25	0.34	0.12	0.31	0.28	1.58	1.13	0.23
	%Recovery / Experiment											
Intra-day	1	100	101					100	100			
	2	102	101	100	102	103	101	100	99	103	99	102
	3	102	101	101	101	103	101	100	99	102	99	101
	4	100	101	101	102	101	101	99	99	102	101	102
	5	100	102					99	100			
	Mean	101	101	101	102	102	101	100	100	102	100	102
	S.D.	0.81	0.52	0.35	0.81	0.91	0.12	0.54	0.63	0.52	1.3	0.23
	R.S.D.	0.8	0.51	0.34	0.79	0.89	0.11	0.55	0.63	0.51	1.3	0.23

Table 4: Statistical data for determination of Miconazole Nitrate and Hydrocortisone in authentic, laboratory prepared mixture and pharmaceutical dosage form using DD1, Isosbestic point, Factorized absorptivity, Bivariate and Official methods

Method / Parameters (Mean±S.D., N, VAR, t-test, F-test)	DD1 (233,285) (240,256)	Isosbestic point(231.5, 241.5)	Factorized absorptivity (217.5,224,241.5)	Bivariate (223,237)	Reference method[20]
Authentic Miconazole Nitrate (single)	100±0.5 N=6 V=0.25 t-test=1.98(2.365)* F-test=4.6(6.79)*	100.19±0.94 N=5 V=0.89 t-test=1.64(2.447)* F-test=1.29(6.94)*	99.98±0.66 N=6 V=0.44 t-test=1.7(2.365)* F-test=2.6(6.79)*	99.9±1.02 N=4 V=1.04 t-test=1.13(2.571)* F-test=1.11(9.66)*	99±1.07 N=3 V=1.15
	102.03±0.41 N=6 V=0.21 t-test=2.4(2.365)* F-test=6.2(19)*		99.88±0.70 N=7 V=0.49 t-test=1.58(2.306)* F-test=2.35(6.14)*	99.25±0.21 N=4 V=0.04 t-test=0.47(2.571)* F-test=29(19.16)***	
Authentic Hydrocortisone (single)	100.19±0.89 N=6 V=0.80 t-test= 6.44(2.306)** F-test= 1.6(6.41)*	100±0.66 N=6 V=0.43 t-test=7.5(2.306)** F-test=1.14(6.41)*	99.95±0.77 N=7 V=0.59 t-test=6.8(2.202)** F-test=1.2(4.76)*	99.76±0.66 N=6 V=0.44 t-test=6.89(2.306)** F-test=1.1(6.41)*	96.75±0.81 N=4 V=0.49
	100.23±1.05 N=5 V=1.11 t-test=5.65(2.306)** F-test=2.27(6.69)*			99.86±0.86 N=6 V=0.74 t-test=5.99(2.306)** F-test=1.5(6.41)*	
Laboratory prepared mixture for Miconazole Nitrate		100±0.77	101±0.79 101.68±0.88	100±0.96	
Laboratory prepared mixture for Hydrocortisone		102±0.96	101±0.72	100.66±0.76	
Standard addition technique for Miconazole Nitrate		101±0.82	100±0.88 101±0.79	100±0.66	
Standard addition technique for HYD.		100±0.65	101±0.54	99±0.10	

*Tabulated values of t and F at p = 0.05
**There is significance difference between the calculated and the tabulated values indicating that the proposed method is more accurate than the reference one.
***There is significance difference between the calculated and the tabulated values indicating that the proposed methods are more precise than the reference one, since they have the smallest variance values.

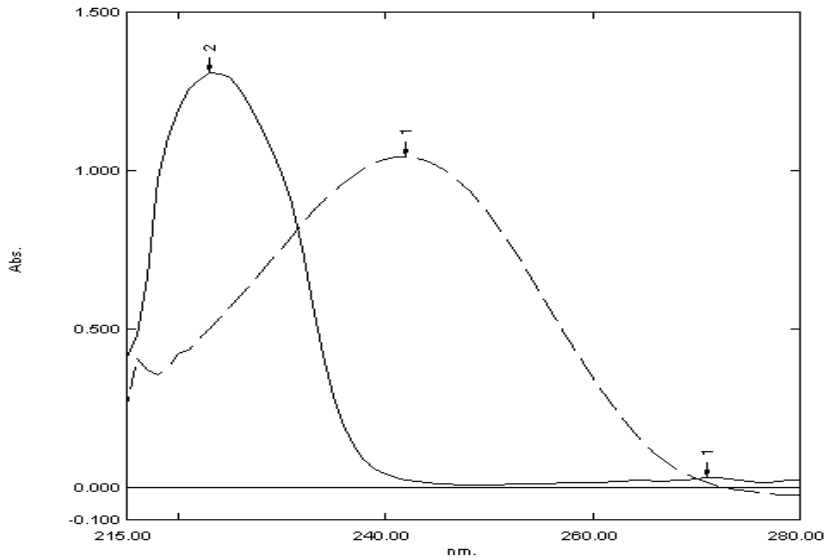


Fig. 2: Zero-order absorption spectra of Miconazole nitrate (___), 25 µg/mL and Hydrocortisone (__ __) 25µg/mL

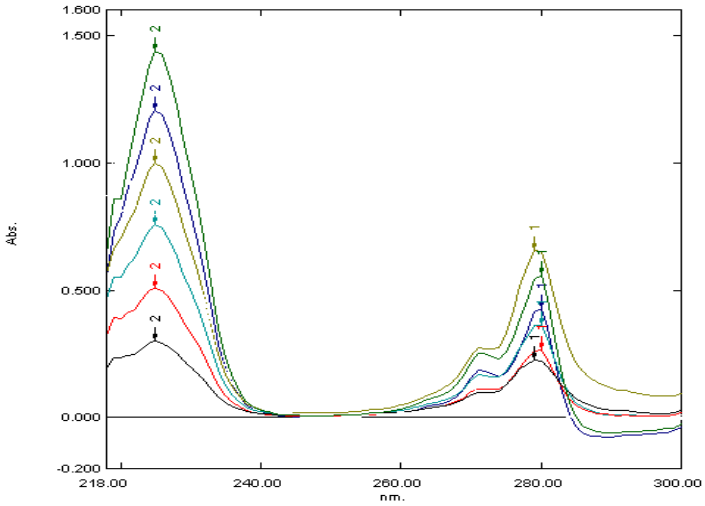


Fig. 3: Ratio spectra of Miconazole Nitrate (5-30µg/ml), using the spectrum of Hydrocortisone 15 µg/ml as divisor

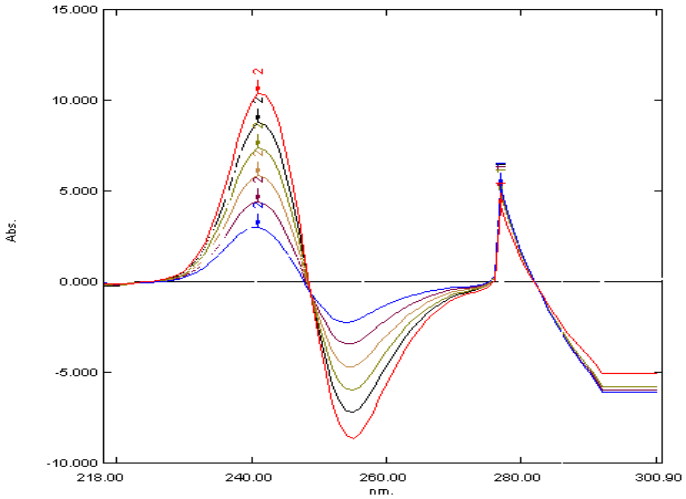


Fig. 4: Ratio spectra of Hydrocortisone (5-30µg/ml), using the spectrum of Miconazole Nitrate 15 µg/ml as divisor

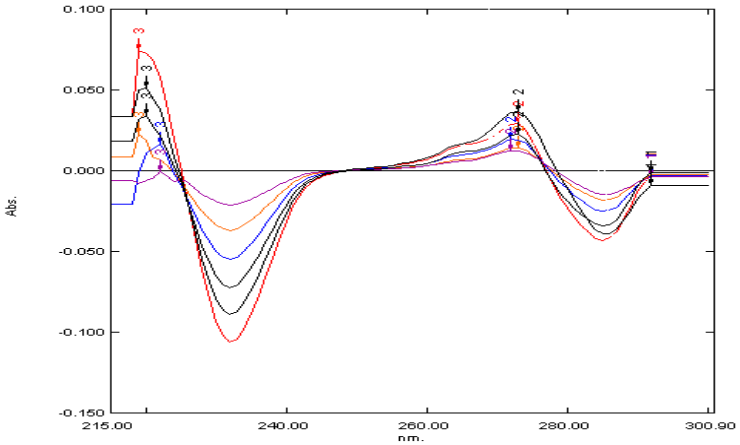


Fig. 5: First derivative of the ratio spectra of Miconazole Nitrate (5-30 µg/mL). Divisor is 15 µg /ml Hydrocortisone

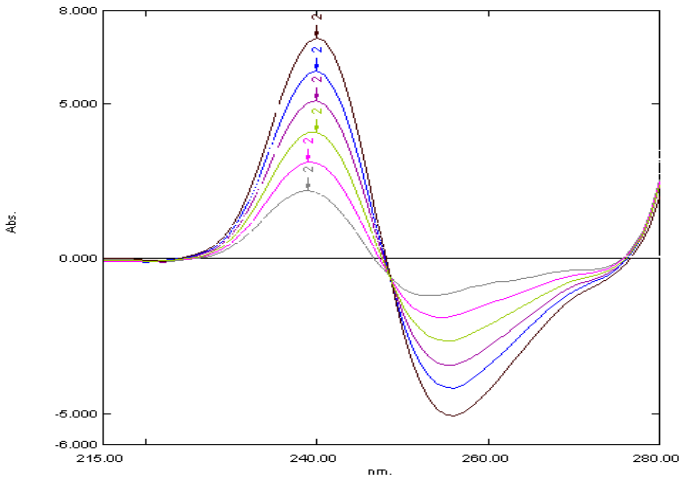


Fig. 6: First derivative of the ratio spectra of Hydrocortisone (5-30 µg/mL). Divisor is 15 µg /ml Miconazole Nitrate

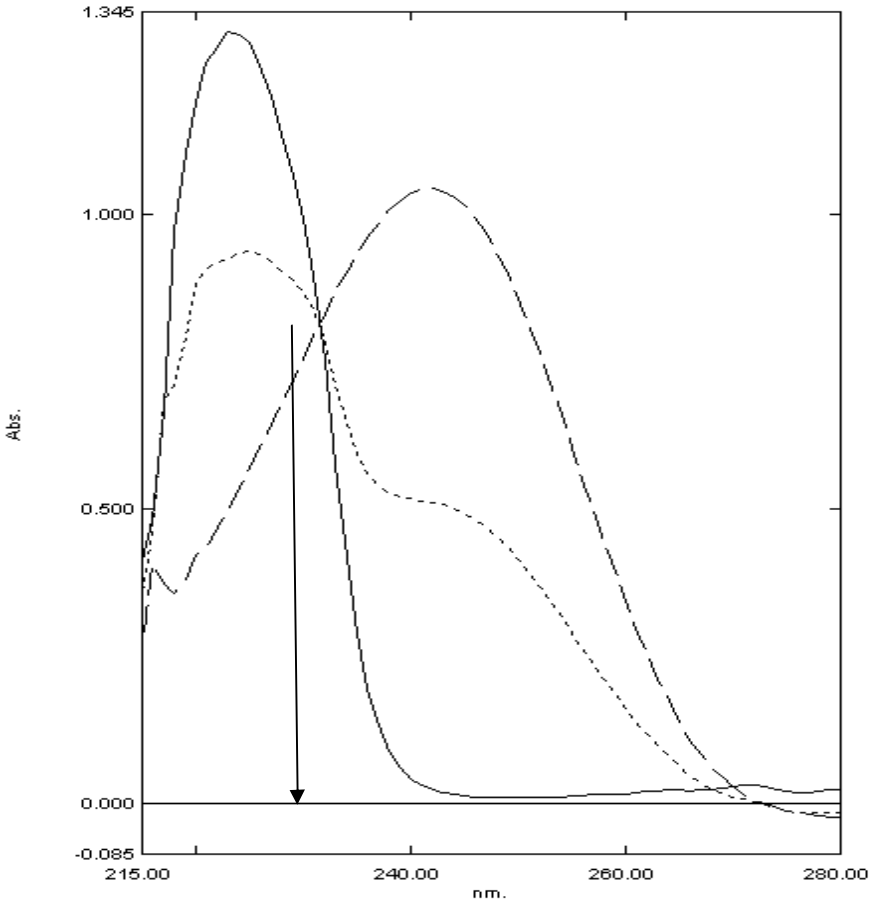


Fig.7: Zero order absorption spectra of 25 µg ml−1 of Miconazole Nitrate (___), 25 µg ml−1 of Hydrocortisone (__ __) and (1:1) mixture containing 12.5 µg ml−1 of each (- - - -) using methanol as a blank

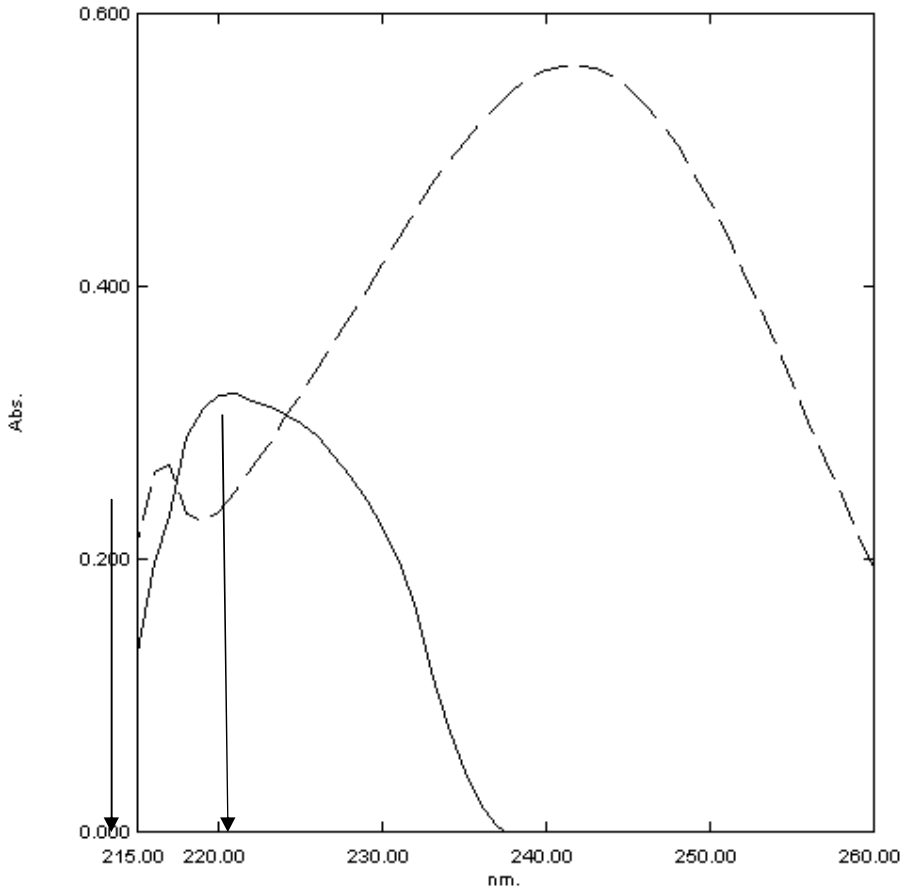


Fig. 8: Absorption spectra of MIC (___), 6.25 µg/mL and HYD (__ __), 12.5 µg/mL

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