



Derivative, derivative of the ratio spectrophotometric and stability-indicating RP-HPLC methods for the determination of mometasone furoate and miconazole nitrate in cream

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ABSTRACT

Three simple, selective and precise methods, namely, derivative, derivative of the ratio spectrophotometric and high performance liquid chromatographic (HPLC) methods were developed and validated for the simultaneous determination of mometasone furoate and miconazole nitrate in mixture. The first method involves the use of derivative spectrophotometry with the zero-crossing technique where mometasone furoate was determined using its ¹D ($\lambda= 8$) amplitude at 270.5 nm and miconazole nitrate was determined using its ³D ($\lambda= 4$) amplitude at 282.1 nm. The second method involves the application of the second derivative of the ratio spectrophotometric method where mometasone furoate and miconazole nitrate were determined using $\Delta\lambda= 4$ at 267.2 nm and 281.2 nm, respectively. The third method is based on gradient elution of mometasone furoate and its alkaline degradation products along with miconazole nitrate on reversed phase C18 column, (3.9 × 300 mm, 10 μ m) - Waters, using a mobile phase consisting of 1.5% w/v aqueous ammonium acetate buffer, pH 7.6 and acetonitrile, at a flow rate of 2 mL/min. Quantitation was achieved applying dual wavelength detection where mometasone furoate and its alkaline degradation products were detected at 240 nm and miconazole nitrate was detected at 230 nm at ambient temperature. In addition, products from alkaline degradation of mometasone furoate were verified by LC-MS. The methods were validated according to the International Conference on Harmonization (ICH) guidelines. The selectivity of the proposed methods was tested using laboratory-prepared mixtures. The developed methods were successfully applied for the determination of mometasone furoate and miconazole nitrate in bulk and in its pharmaceutical preparation.

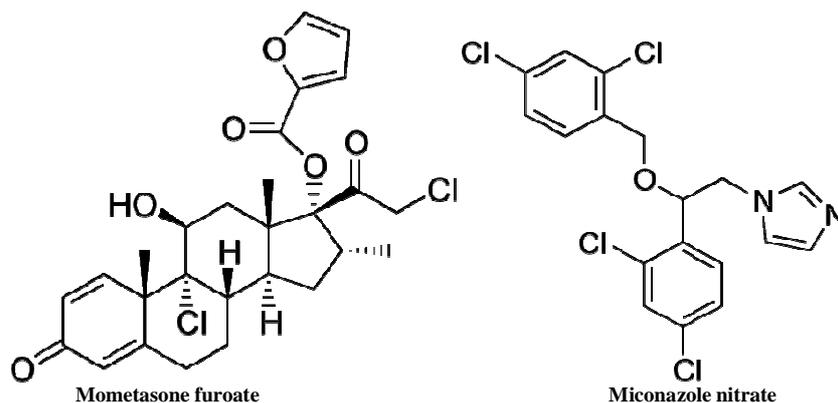
Keywords: Mometasone furoate; Miconazole nitrate; Derivative spectrophotometry; Derivative of the ratio spectrophotometry; Stability-Indicating HPLC.

INTRODUCTION

Mometasone furoate (MF), (9 α , 21-dichloro-11 β , 17-dihydroxy-16 α - methylpregna-1, 4-diene-3, 20-dione 17-(2-furoate)) (Fig.1 a) is a topical corticosteroid. It has anti-inflammatory, anti-pruritic, and vasoconstrictive properties. It is indicated for a number of conditions such as allergic reactions, eczema and psoriasis. Corticosteroids act by the induction of phospholipase A2 inhibitory proteins, collectively called lipocortins. It is postulated that these proteins control the biosynthesis of potent mediators of inflammation such as prostaglandins and leukotrienes by inhibiting the release of their common precursor arachidonic acid [1].

Miconazole nitrate (MZ), (RS)-1-(2-(2,4-Dichlorobenzoyloxy)-2-(2,4-dichlorophenyl)ethyl)-1H-imidazole (Fig. 1b) is an antimycotic imidazole derivative with a wide activity spectrum. It is endowed with a powerful activity against dermatophytes and *Candida albicans*, as well as against several Gram-positive germs. The generally accepted mode of action of azole antifungals is the inhibition of 14- α -lanosterol demethylase, a key enzyme in ergosterol biosynthesis, resulting in depletion of ergosterol and accumulation of toxic 14- α -methylated sterols in the membrane of susceptible yeast species [2].

Figure (1): Chemical structure of (a) mometasone furoate , (b) miconazole nitrate



Literature survey revealed the determination of mometasone furoate (MF) in plasma by LC-tandem mass spectrometry [3], and by HPLC with stability studies [4]. Also, MF was determined in its dosage form by HPLC [5]. Kinetic study of MF in aqueous system [6] and supercritical fluid chromatographic impurity analysis [7] were carried out. Moreover, determination of MF with fucidic acid, tazarotene, oxymetazoline and ketoconazole using HPLC [1,8-10] and with nadifloxacin using HPTLC [11] were reported.

Several methods are available for the determination of miconazole nitrate (MZ) either alone or in combination with various drugs in bulk, pharmaceutical preparations and in biological fluids using HPLC [2, 12-14], UV spectrophotometry [13,15] and NMR spectroscopy [16].

No spectrophotometric method has been reported for the simultaneous determination of MF and MZ in mixture. Besides, only one HPLC method was reported for the determination of these two drugs in combination but in absence of MF alkaline degradation product [17]. So, the aim of the present work was to develop simple, sensitive and validated two spectrophotometric and Stability indicating HPLC methods for the simultaneous determination of MF and MZ in their pure powdered forms, laboratory prepared mixtures and in their pharmaceutical preparations.

EXPERIMENTAL SECTION

2.1. Instrumentation

A double-beam Shimadzu (Japan) 2450PC UV visible spectrophotometer connected to a computer fitted with UV-PC personal spectroscopy software version 3.7 (Shimadzu) used. The spectral bandwidth was 2 nm and the wavelength scanning speed was 2800 nm min⁻¹.

The absorbance spectra of test and reference solutions were recorded in 1-cm quartz cells over the range 200–400 nm.

The chromatographic system consisting of Agilent 1200 series (CA, USA); interface equipped with an Agilent quaternary pump G1311A, Agilent UV-visible detector G1314B, an Agilent manual injector G1328B equipped with (20 μ L) injector loop and Agilent degasser G1322A. Separation and quantitation were made on C18 column (300 mm x 3.9 mm, 10 μ m) - Waters. 125 A, Bondapak™, (Ireland).

2.2. Reagents and reference samples

Mometasone furoate and miconazole nitrate were kindly supplied from Sigma-Aldrich and Pharco Pharmaceutical and were certified to contain 99.6% and 99.8% respectively. Methanol (for Spectroscopy) was supplied by Sigma-Aldrich, Germany. HPLC grade acetonitrile and methanol were supplied by Scharlau, Spain. Ammonium acetate was supplied by Adwic Company. Methylene chloride was supplied by Fisher Scientific, Ireland. Bi-distilled water was produced in-house (Aquatron Water Still, A4000D, UK). Membrane filters of 0.45 μm were purchased from Teknokroma (Barcelona, Spain). All the chemicals were of analytical grade.

Elica-M cream, nominally containing 0.1% w/w Mometasone furoate and 2% w/w Miconazole nitrate (Batch No. MK 014), was supplied by Jamjoom Pharmaceuticals Company, KSA.

2.3. Solutions

2.3.1. Stock standard solutions

2.3.1.1. For derivative and derivative of the ratio spectrophotometric method

Stock standard solutions of MF (0.5 mg mL^{-1}) and MZ (2 mg mL^{-1}) were prepared in methanol. Appropriate dilutions of this stock solution were prepared using the same solvent to be used for linearity studies and assay purposes.

2.3.1.2. For HPLC method

Stock standard solutions of MF (0.5 mg mL^{-1}) and MZ (2 mg mL^{-1}) were prepared in acetonitrile. Appropriate dilutions of this stock solution were prepared using the same solvent to be used for linearity studies and assay purposes

2.3.2. Laboratory-prepared mixtures

2.3.2.1. For derivative spectrophotometric method

Solutions containing different ratios of MF (5-25 $\mu\text{g mL}^{-1}$) and MZ (100-500 $\mu\text{g mL}^{-1}$) were prepared by transferring aliquots from their stock solutions into a series of 10-ml volumetric flasks and the volume of each was completed to the mark with methanol.

2.3.2.2. For derivative of the ratio spectrophotometric method

Solutions containing different ratios of MF (5-30 $\mu\text{g mL}^{-1}$) and MZ (100-600 $\mu\text{g mL}^{-1}$) were prepared by transferring aliquots from their stock solutions into a series of 10-ml volumetric flasks and the volume of each was completed to the mark with methanol.

2.3.2.3. For HPLC method

Solutions containing different ratios of MF (10-40 $\mu\text{g mL}^{-1}$) and MZ (200-800 $\mu\text{g mL}^{-1}$) were prepared by transferring aliquots from their stock solutions into a series of 10-ml volumetric flasks and the volume of each was completed to the mark with acetonitrile.

2.3.3. Sample preparation

2.3.3.1. For derivative and derivative of the ratio spectrophotometric method

Five grams of Elica-M® cream were transferred to a conical flask, taking care to avoid sticking cream to the flask wall. Twenty five mL of methanol was added and the mixture was heated in a water bath at 80°C for 5 min or until the cream melted completely; in between, the flask was occasionally swirled. The conical flask content was quantitatively transferred to a 50 mL volumetric flask. The flask was washed with 2X10 mL methanol. Volume was made up to the mark with methanol and mixed (Solution A). The solution was centrifuged at 3500 rpm for 6 min. Aliquots were transferred from the prepared stock solution to 10-mL volumetric flasks and diluted with methanol for the determination of both drugs.

2.3.3.2. For HPLC method

Solution B was prepared in the same manner of solution A preparation using acetonitrile instead of methanol. Supernatant solution was filtered through a 0.45- μm membrane filter.

2.4. Procedures

2.4.1. Construction of the calibration curves

2.4.1.1. For derivative spectrophotometric method

Aliquots equivalent to 5–80 $\mu\text{g mL}^{-1}$ of MF and 50–500 $\mu\text{g mL}^{-1}$ of MZ were accurately transferred into two series of 10-ml volumetric flasks and the volumes were completed to the mark with methanol. The zero crossing first derivative spectra were recorded for MF with trough amplitude measurement at 270.5 nm (Fig. 3a). The third derivative zero crossing spectra were recorded for MZ with peak amplitude measurement at 282.1 nm (Fig. 3 b). Two calibration curves were constructed by plotting the amplitudes against the corresponding concentrations of each drug in micrograms per milliliter.

2.4.1.2. For derivative of the ratio spectrophotometric method

Stored UV absorption spectra of different concentrations of standard solutions of MF (5–50 $\mu\text{g mL}^{-1}$) were divided wavelength-by-wavelength by a standard spectrum of MZ (20 $\mu\text{g mL}^{-1}$). The second derivative was calculated for the obtained spectra with $\Delta\lambda=4$. The trough amplitudes at 267.2 nm were measured (Fig. 4a). For MZ, the stored UV absorption spectra of different concentrations of standard solutions of MZ (30-600 $\mu\text{g mL}^{-1}$) were divided wavelength-by-wavelength by a standard spectrum of MF (5 $\mu\text{g mL}^{-1}$). The second derivative was calculated for the obtained spectra with $\Delta\lambda=4$. The trough amplitudes at 281.2nm were measured (Fig. 4b).

2.4.1.3. For HPLC method

Aliquots equivalent to 5–320 $\mu\text{g mL}^{-1}$ of MF and 25–800 $\mu\text{g mL}^{-1}$ of MZ were accurately transferred into two series of 10-mL volumetric flasks and the volumes were completed to the mark with acetonitrile. Twenty μL aliquot of each solution was injected onto a Waters column (3.9 x 300 mm, 10 μm), using the mobile phase consisting of 1.5% w/v aqueous ammonium acetate buffer, pH 7.6 (A) and Acetonitrile (B), at a flow rate 2.0 mL min^{-1} . The separation was achieved with a gradient elution consisting of 0–3 min 45% (A), 3-10 min gradient down to 40% (A) and 10-14 min gradient up to 45% (A). The mobile phase was filtered through 0.45 μm membrane filter. The column temperature was 25 °C. UV detector was operated at 240 nm for detection of MF and its alkaline degradation products, and 230 nm for MZ. Two calibration curves were constructed by plotting the peak areas against the corresponding concentrations of each drug in micrograms per milliliter.

2.4.2. Assay of laboratory-prepared mixtures

2.4.2.1. For derivative spectrophotometric method

The absorption spectra of the laboratory-prepared mixtures prepared in section 2.3.2.1. were scanned, processed as mentioned in section 2.4.1.1. using concentrations equivalent to (5-25 $\mu\text{g mL}^{-1}$) of MF and (100- 500 $\mu\text{g mL}^{-1}$) of MZ, respectively. The concentration of each drug was calculated using the specified regression equation.

2.4.2.2. For derivative of the ratio spectrophotometric method

The absorption spectra of the laboratory-prepared mixtures prepared in section 2.3.2.2. were scanned, processed as mentioned in section 2.4.1.2. using concentrations equivalent to (5-30 $\mu\text{g mL}^{-1}$) of MF and (100- 600 $\mu\text{g mL}^{-1}$) of MZ, respectively. The concentration of each drug was calculated using the specified regression equation.

2.4.2.3. For HPLC method

The procedure mentioned in Section 2.4.1.3. was repeated using concentrations equivalent to (10 - 40 $\mu\text{g mL}^{-1}$) of MF and (200- 800 $\mu\text{g mL}^{-1}$) of MZ, respectively. The concentration of each drug was calculated using the specified regression equation.

2.4.3. Assay of Elica-M Cream

2.4.3.1. For derivative and derivative of the ratio spectrophotometric methods

For derivative spectrophotometric method, solution (A) prepared in Section 2.3.3.1. was serially diluted to get concentrations equivalent to 5-15 $\mu\text{g mL}^{-1}$ and 100-300 $\mu\text{g mL}^{-1}$ of MF and MZ, respectively and equivalent to 8-20 $\mu\text{g mL}^{-1}$ and 160-400 $\mu\text{g mL}^{-1}$ of MF and MZ, respectively for derivative of the ratio spectrophotometric method. The peak amplitudes were measured and the concentrations of MF and MZ were calculated using calibration equations.

2.4.3.2. For HPLC method

Solution B prepared in Section 2.3.3.2. was serially diluted to get concentrations equivalent to 10-30 $\mu\text{g ml}^{-1}$ and 200-600 $\mu\text{g ml}^{-1}$ of MF and MZ, respectively. Samples then were injected in triplicates. The concentrations of MF and MZ were calculated using calibration equations.

2.4.4. Preparation of alkaline degradation of mometasone furoate

Mometasone furoate (200 mg) was dissolved in a mixture of methylene chloride: methanol (9:1) containing sodium hydroxide with a final molarity of 0.5 M NaOH [18]. The solution was stirred and followed up by injection onto the column using the same chromatographic conditions till complete disappearance of mometasone furoate peak (1hour).

RESULTS AND DISCUSSION

3.1. Method development

3.1.1. For derivative spectrophotometric method

The zero order absorption spectra of MF and MZ show severe overlapping that prevents the use of direct spectrophotometry for their analysis without preliminary separation, (Fig. 2). Thus, the derivative spectroscopy was applied to solve the problem of the overlapped absorption spectra of the cited drugs. For the determination of MF, the first derivative spectra were calculated using $\Delta\lambda=8$ nm and a scaling factor of 10. The trough amplitudes of the obtained first derivative spectra were measured at 270.5 nm for MF where MZ showed zero crossing, (Fig. 3a). For the determination of MZ, the third derivative spectra were recorded using $\Delta\lambda=4$ nm and a scaling factor of 50. The peak amplitudes were measured at 282.1 nm for MZ where MF displayed zero value.(Fig. 3b).

3.1.2. For derivative of the ratio spectrophotometric 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 [19]. The obtained ratio spectra were differentiated with respect to wavelength to afford the second derivative ratio spectra. Good measurements could be obtained at the trough 267.2 nm and 281.2 nm amplitudes for MF and MZ, respectively (Fig. 4a, 4b). Effect of the wavelength intervals revealed that $\Delta\lambda=4$ was the most suitable interval for measurement of both drugs with scaling factor of 1. Increasing that interval led to a less sensitive peak.

Figure (2): Zero order spectra of mometasone furoate ($10\mu\text{g mL}^{-1}$) and miconazole nitrate ($100\mu\text{g mL}^{-1}$)

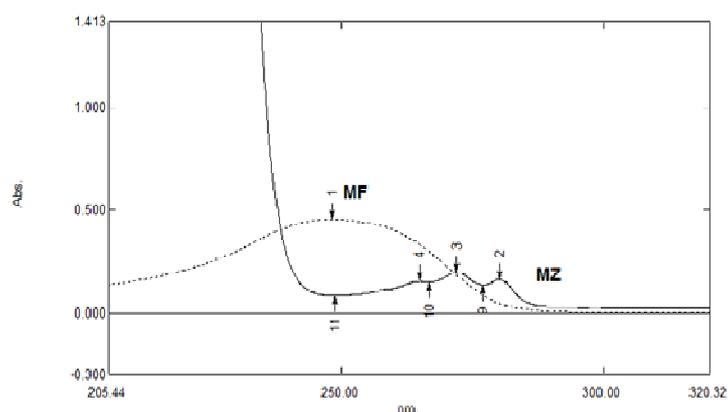


Figure (3): (a) First derivative spectra of mometasone furoate ($10\mu\text{g mL}^{-1}$) and miconazole nitrate ($100\mu\text{g mL}^{-1}$) using $\Delta\lambda=8$ and Scaling factor=10 (b) Third derivative spectra of mometasone ($10\mu\text{g mL}^{-1}$) and miconazole ($100\mu\text{g mL}^{-1}$) using $\Delta\lambda=4$ and scaling factor= 50

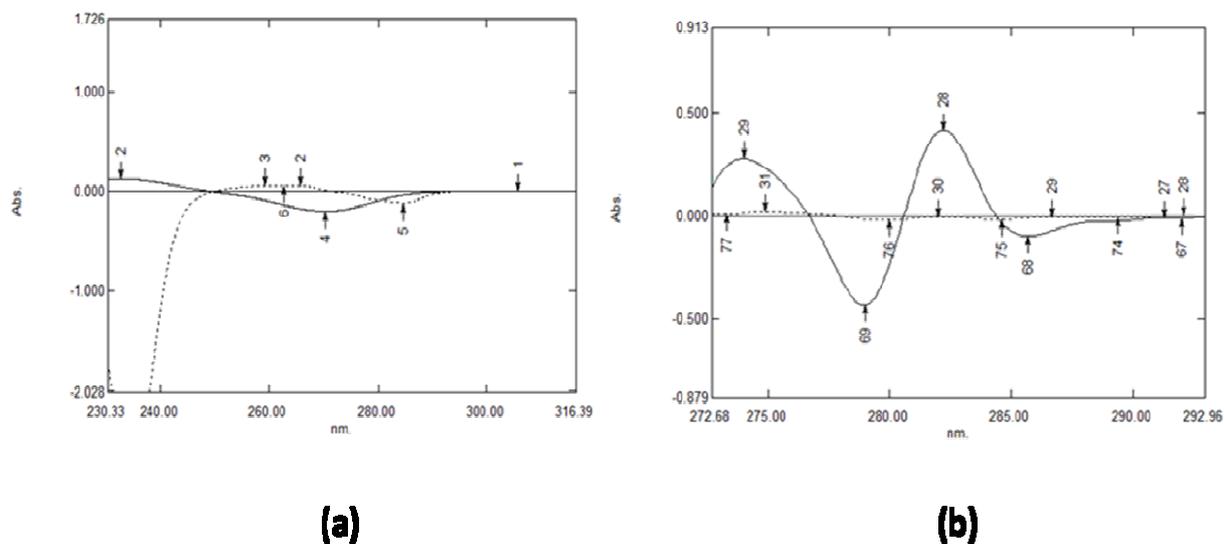
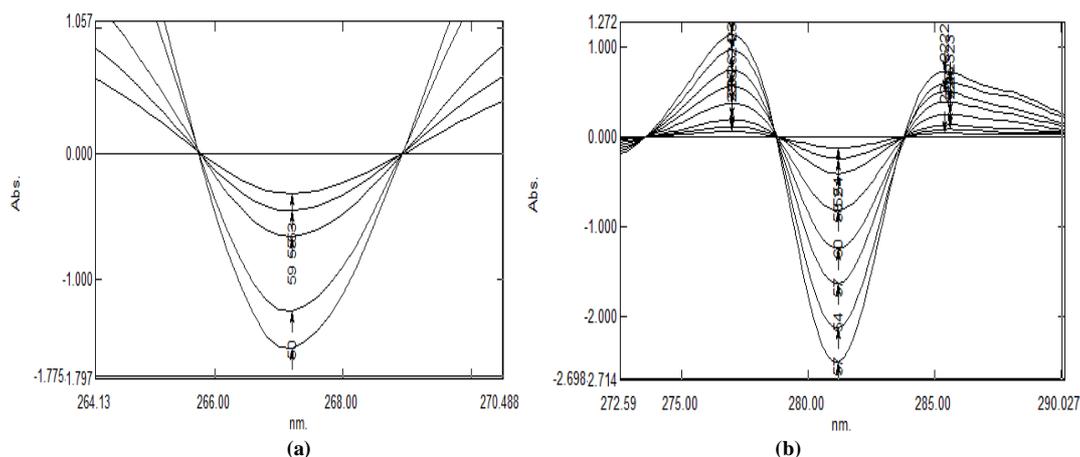


Figure (4): (a) Second derivative ratio spectra of mometasone furoate ($5\text{-}50\mu\text{g mL}^{-1}$) using miconazole nitrate ($20\mu\text{g mL}^{-1}$) as a divisor. (b) Second derivative ratio spectra of miconazole nitrate ($30\text{-}600\mu\text{g mL}^{-1}$) using mometasone furoate ($5\mu\text{g mL}^{-1}$) as a divisor



3.1.3. For HPLC method

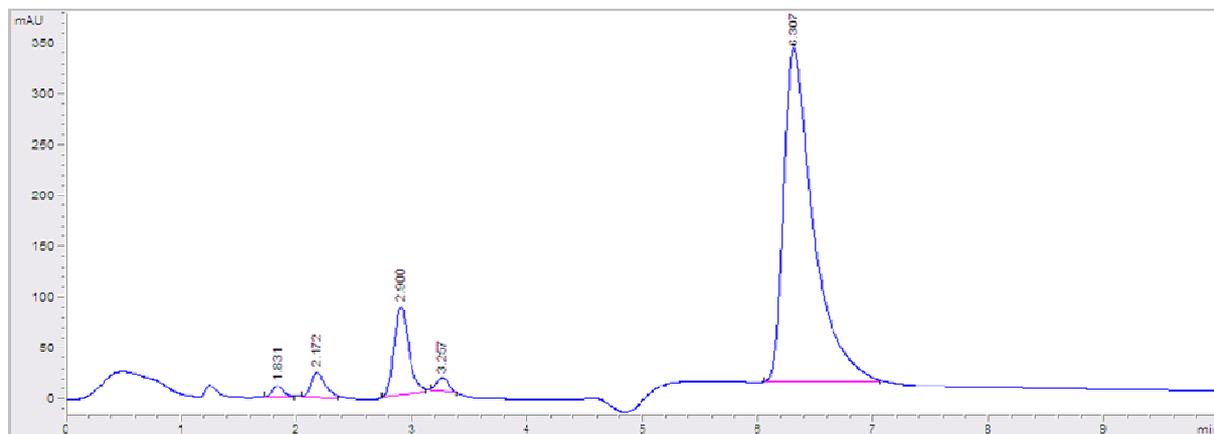
3.1.3.1. Peak Optimization

HPLC has become a widely used tool for the routine analysis and separation of drugs either alone in pure form [20], or in admixture with other drugs [21,22] or degradation products [22-24] and in pharmaceutical formulations [20-24]. Several chromatographic conditions were attempted using C18 and C8 column. Various mobile phases compositions like (methanol: H_2O) and (methanol: H_2O : glacial acetic acid) in different ratios were tried in an isocratic mode. Using the second composition with C8 column was applicable for separation of MF and MZ but with excessive peak tailing of MF. Introduction of phosphate buffer (pH from 3 - 6.5) in the mobile phase together with methanol and acetonitrile prevents elution of MZ.

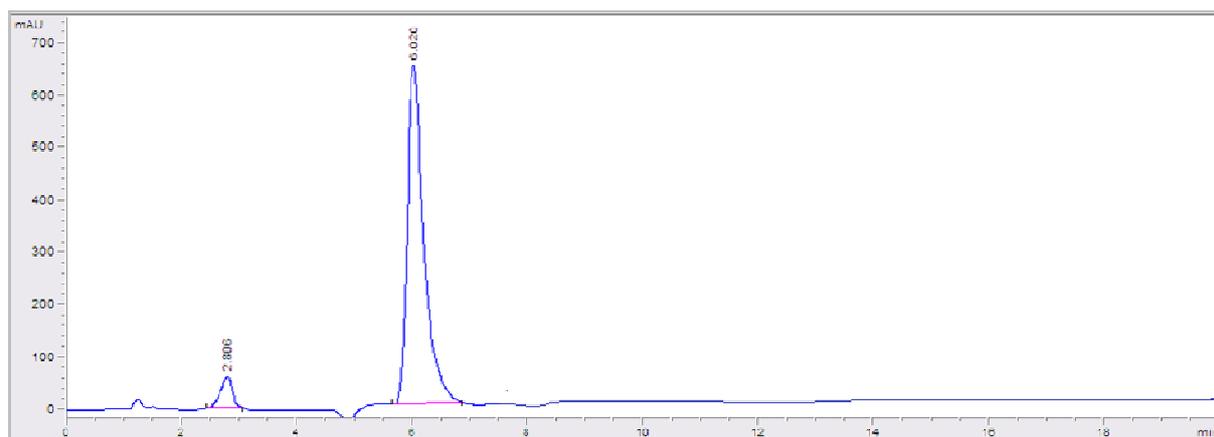
Also, a mobile phase consisting of 1.5% Ammonium acetate buffer : acetonitrile (45:55, v/v) , pH 4 adjusted with orthophosphoric acid at a flow rate 2 ml/min, UV detector operated on 240 nm for detection of MF and it's alkaline degradation product and 230 nm for detection of MZ, in an isocratic mode gave good separation of MF and MZ but MZ peak was broadened showing low number of theoretical plates. Increasing the percentage of organic modifier (acetonitrile) to 60% optimizes shape and symmetry of MZ peak but it creates a problem of peak overlapping with bad resolution between MF and its degradation products. To obtain a good chromatogram and to avoid all the above

drawbacks, two elution systems were applied: isocratic elution with mobile phase composition of buffer: acetonitrile (45:55, v/v) for the first 3 min in order to resolve MF and its degradation products followed by gradient elution through increasing acetonitrile percentage to 60%, (Fig. 5a,5b).

Figure (5): (a) HPLC chromatogram of mometasone ($30\mu\text{g mL}^{-1}$), degradation products ($30\mu\text{g mL}^{-1}$) and miconazole ($375\mu\text{g mL}^{-1}$) using UV detection at 240 nm from 0-3 min and at 230 nm from 3-10 min. (b) HPLC chromatogram of Elica-M cream containing $40\mu\text{g mL}^{-1}$ MF and $800\mu\text{g mL}^{-1}$ MZ



(a)



(b)

3.1.3.2. Selection of wavelength

In order to reach sufficient intensity peaks for both drugs, UV detector was operated at 240, 264, 270 and 282 nm. Maximum MZ peak intensity was reached at 230 nm while it was found that MF and its degradation products showed the best peak intensities at 240 nm. Therefore, UV detector was adjusted to measure peak intensities at 240 nm from zero to three min. and at 230 nm from three to ten min. Fig. 5.

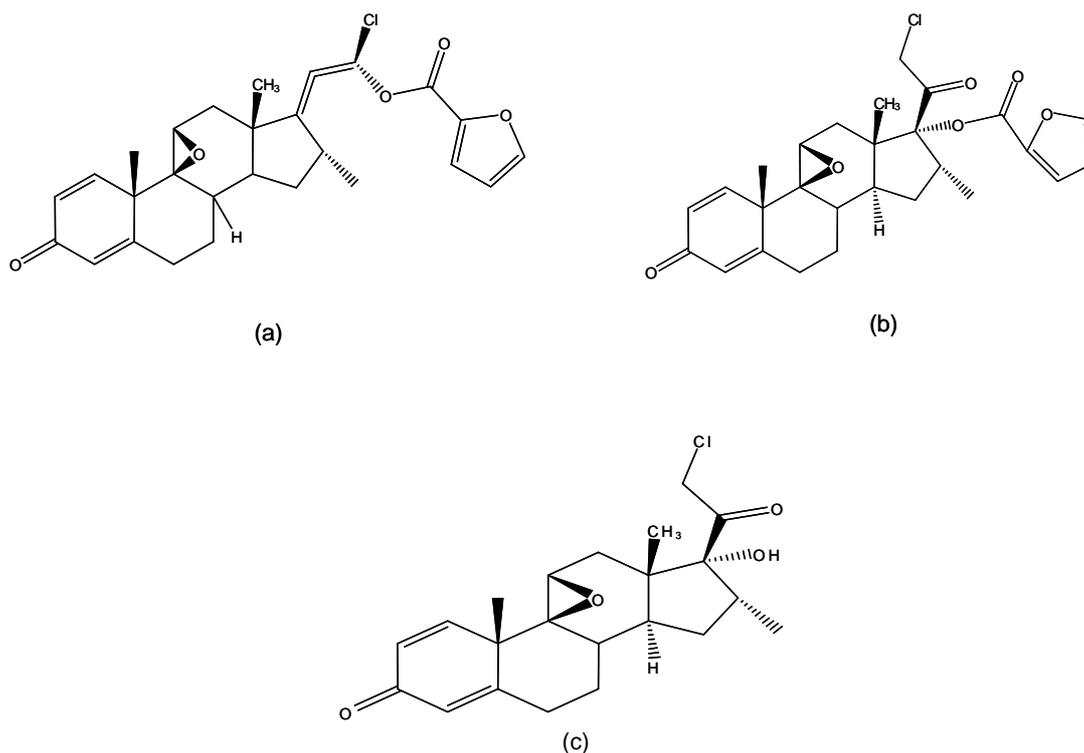
3.1.3.3. pH adjustment

At acidic pH, good separation of MF and MZ peaks was achieved but on the other hand, when applying this method in the presence of MF degradation products, an overlap between MF and its degradation product (C) was obtained. Changing mobile phase composition by using different ratios of organic modifier or changing the gradient program did not resolve the two peaks. Finally, good resolution was obtained by changing the pH from 4 to 7.6.

Alkaline degradation products of MF were prepared by a very mild and rapid procedure [14] reported for the efficient alkaline hydrolysis of esters in non-aqueous conditions. This procedure resulted in complete disappearance of the intact drug within one hour and three different degradation products were obtained. Verification of the

products was carried out using LC-MS. The major peak (Retention time 2.1 min, Fig. 5a) has a molecular ion peak 466.99 (M⁺), which may correspond to structure (a) in Fig. 6. The mechanism of the reaction has been proposed by Foe *et al.* [25]. It involves a stereospecific nucleophilic attack of the 11 β -hydroxyl on C-9 on departure of the 9-halogen, leading to the formation of 9 β ,11 β -epoxide derivative in addition to a rearrangement of the furoate moiety and dehydration on the C-17 side-chains of MF. The two other peaks (Retention time 1.86 and 3.2 min, Fig. 5a) have the molecular ion peaks of 484.9 (M⁺) and 388 (M⁻) which may correspond to structures (b) & (c) in Fig 6, respectively. Epoxide formation without the rearrangement of the furoate moiety and the dehydration resulted in the formation of structure (b) while in structure (c), epoxide formation was accompanied by ester hydrolysis.

Figure (6): Structures of mometasone furoate alkaline degradation products



3.2. System suitability tests

System suitability tests are an integral part of liquid chromatographic methods in the course of optimizing the conditions of the proposed method. They are used to verify that the resolution and reproducibility were adequate for the analysis performed. The parameters of these tests are column efficiency (number of theoretical plates), tailing of chromatographic peak, repeatability as %R.S.D of peak area for six injections of a solution of 160 $\mu\text{g mL}^{-1}$ and 400 $\mu\text{g mL}^{-1}$ of MF and MZ respectively and reproducibility of retention as %R.S.D of retention time. The results of these tests for the proposed method are listed in Table 1.

Table (1): System suitability tests for the proposed LC method:

| Parameter | MF | MZ |
|-----------------------------------------|------|------|
| N ^a | 850 | 2013 |
| T ^b | 0.8 | 1.05 |
| %R.S.D. ^c of 6 injections of | | |
| Peak area | 0.16 | 0.43 |
| t _R ^d (min) | 0.29 | 0.12 |

(^a Number of theoretical plates; ^b Tailing factor; ^c %Relative standard deviation; ^d retention time)

3.3. Method Validation

3.3.1. Range and linearity

The linearity of the spectrophotometric and HPLC methods for the determination of MF and MZ were evaluated by analyzing a series of different concentrations of each drug. For derivative spectrophotometric method, different concentrations for MF and MZ were chosen, ranging from 5-80 $\mu\text{g mL}^{-1}$ and 50-500 $\mu\text{g mL}^{-1}$, respectively. For derivative of the ratio spectrophotometric method, different concentrations for MF and MZ were chosen, ranging from 5-50 $\mu\text{g mL}^{-1}$ and 30-600 $\mu\text{g mL}^{-1}$. and from 5-320 $\mu\text{g mL}^{-1}$ MF and 25-800 $\mu\text{g mL}^{-1}$ MZ for HPLC method, respectively. Each concentration was analyzed three times. Good linearity of the calibration curve was verified by the high correlation coefficient. The analytical data of the calibration curve including standard deviations for the slope and intercept (S_b , S_a) are summarized in Tables 2 & 3.

3.3.2. Accuracy

Accuracy of the results was calculated by % recovery of laboratory prepared mixtures of 6 different concentrations of MF and MZ and also by standard addition technique for Elica-m® cream in the three methods. The results obtained including the mean of the recovery and standard deviation are displayed in Table 2&3

Table (2): Beer's law data and statistical analysis for the calibration graphs using the derivative and derivative of the ratio spectrophotometric proposed methods

| Parameter | Derivative | | Derivative of the ratio | |
|----------------------------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|-------------------------------------------------|
| | MF | MZ | MF | MZ |
| Derivative order | 1 | 3 | 2 | 2 |
| Solvent used | Methanol | methanol | Methanol | Methanol |
| λ_{max} of measurements | 270.5 | 282.1 | 267.2 | 281.2 |
| Concentration range | Area = $0.0203 C_{\mu\text{g mL}^{-1}} + 0.0125$ | Area = $0.0032 C_{\mu\text{g mL}^{-1}} + 0.0039$ | Area = $0.0308 C_{\mu\text{g mL}^{-1}} + 0.0159$ | Area = $0.0042 C_{\mu\text{g mL}^{-1}} - 0.012$ |
| Regression equation | 0.9998 | 0.9999 | 0.9998 | 0.9998 |
| S_b | 9.18×10^{-5} | 1.99×10^{-5} | 2.08×10^{-4} | 2.6×10^{-5} |
| S_a | 3.41×10^{-3} | 5.71×10^{-3} | 5.92×10^{-3} | 8.523×10^{-3} |
| LOD | 0.93 | 8.25 | 1.44 | 1.78 |
| LOQ | 2.81 | 25 | 0.48 | 5.95 |
| Confidence limit of the slope | $0.0203 \pm 2.36 \times 10^{-4}$ | $0.0032 \pm 5.11 \times 10^{-5}$ | 0.0308 ± 0.00058 | $0.0042 \pm 6.6 \times 10^{-5}$ |
| Confidence limit of the intercept | $0.0125 \pm 8.76 \times 10^{-3}$ | $0.0039 \pm 1.47 \times 10^{-2}$ | 0.0159 ± 0.0165 | 0.012 ± 0.022 |
| Standard error of the estimation | 5.7×10^{-3} | 8×10^{-3} | 8.3×10^{-3} | 1.4×10^{-2} |
| Inter-day (%R.S.D.) | 0.32-0.58 | 0.39-0.64 | 0.403-0.794 | 0.293-1.06 |
| Intra-day (%R.S.D.) | 0.32-0.55 | 0.38-0.47 | 0.403-0.742 | 0.106-0.423 |
| Drug in bulk | 100.53 ± 0.449 | 99.02 ± 0.57 | 100.83 ± 0.685 | 100.41 ± 0.59 |
| Drug in dosage form | 98.44 ± 0.997 | 105.16 ± 0.433 | 99.14 ± 0.664 | 102.99 ± 0.252 |
| Drug added | 100.47 ± 1.021 | 98.49 ± 0.235 | 98.85 ± 0.576 | 100.85 ± 0.609 |

Table (3): Results obtained by the proposed LC method for the determination of mometasone furoate and miconazole nitrate:

| Parameter | MF | MZ |
|----------------------------------------------|-------------------------------------------------|--------------------------------------------------|
| Retention time (min) | 2.9 | 6.3 |
| Wavelength of detection (nm) | 240 | 230 |
| Range of linearity ($\mu\text{g mL}^{-1}$) | 5-320 | 25-800 |
| Regression equation | Area = $25.784 C_{\mu\text{g mL}^{-1}} + 28.09$ | Area = $15.542 C_{\mu\text{g mL}^{-1}} + 75.593$ |
| Regression coefficient (r^2) | 0.9999 | 0.9996 |
| LOD ($\mu\text{g mL}^{-1}$) | 1.61 | 3.5 |
| LOQ ($\mu\text{g mL}^{-1}$) | 4.88 | 10.86 |
| S_b | 1.13×10^{-1} | 15.56×10^{-2} |
| S_a | 1577×10^{-2} | 5896×10^{-2} |
| Confidence limit of the slope | $25.78 \pm 2.9 \times 10^{-1}$ | $15.54 \pm 4.33 \times 10^{-1}$ |
| Confidence limit of the intercept | $28.09 \pm 405.3 \times 10^{-1}$ | $75.59 \pm 1631.6 \times 10^{-1}$ |
| Standard error of the estimation | 31.72 | 103.2 |
| Inter-day (%R.S.D.) | 0.14-0.24 | 0.29-0.10 |
| Intra-day (%R.S.D.) | 0.11-0.23 | 0.08-0.21 |
| Drug in bulk | 99.73 ± 1.01 | 100.7 ± 0.62 |
| Drug in dosage form | 99.53 ± 0.93 | 103.98 ± 0.21 |
| Standard added | 99.94 ± 0.998 | 98.87 ± 0.53 |

3.3.3. Precision

The repeatability of the method was assessed by six determinations for each of the three concentrations representing 80, 100, 120% for each drug. For derivative spectrophotometric method, these concentrations were (32, 40 and 48 $\mu\text{g mL}^{-1}$) and (200, 250 and 300 $\mu\text{g mL}^{-1}$) for MF and MZ, respectively. And for derivative of the ratio spectrophotometric method, (20, 25 and 30 $\mu\text{g mL}^{-1}$) and (240, 300 and 360 $\mu\text{g mL}^{-1}$) for MF and MZ, respectively, were used. For HPLC method, these concentrations used were (128, 160 and 192 $\mu\text{g mL}^{-1}$) and (320, 400 and 480 $\mu\text{g mL}^{-1}$) for MF and MZ, respectively. The repeatability of sample application and measurement of peak area and spectrum absorbance for active compounds were expressed in terms of percentage relative standard deviation (%R.S.D.). All experiments described in repeatability were repeated in three consecutive days by the same analyst to evaluate day to day ruggedness.

3.3.4. Specificity

Specificity is the ability of the analytical method to measure the analyte response in the presence of interferences. Good resolution and absence of interference from any of the MF alkaline degradation products are shown in (Fig. 5a). Besides, the chromatogram of the pharmaceutical formulation samples was checked for the appearance of any extra peaks. No chromatographic interference from any of the excipients was found at the retention time of the examined drug after extraction of the active ingredient (Fig. 5b). In addition, the chromatogram of MF and MZ in the sample solution was found to be identical to the chromatogram obtained by the standard solution. So the proposed methods could be successfully applied for the routine analysis of the studied drugs in their dosage forms without any preliminary separation step. Results for determination of both drugs by the three proposed methods in their dosage forms along with standard addition technique were displayed in Tables 2&3.

3.3.5. Limit of detection and limit of quantitation

According to the International Conference on Harmonization (ICH) recommendations [26], the approach based on the standard deviation (S.D.) of the response and the slope was used for determining the detection and quantitation limits. The theoretical values for spectrophotometric and HPLC methods were assessed practically and given in Tables 2 & 3.

3.3.6. Robustness

Robustness was performed by deliberately changing the chromatographic conditions. Variation of the pH of the 1.5% ammonium acetate buffer of the mobile phase by ± 0.2 units and variation of the flow rate by ± 0.2 units did not have significant effect on chromatographic resolution in HPLC method.

3.3.7. Statistical studies

Statistical comparison between the results of the proposed methods and those of the reference methods, obtained by using the student's t-test and F- ratio showed no significant differences. It can be concluded that the proposed analytical methods are sufficiently accurate and precise and results are given in Table 4.

Table (4): Statistical analysis of the results obtained by the proposed methods and the reference methods

| Statistical term | Derivative | | | | Derivative of the ratio | | | | HPLC | | | |
|-----------------------------|------------|-------------------------------|-------|-------------------------------|-------------------------|-------------------------------|--------|-------------------------------|--------|-------------------------------|-------|-------------------------------|
| | MF | Reference Method ^b | MZ | Reference Method ^b | MF | Reference Method ^b | MZ | Reference Method ^b | MF | Reference Method ^b | MZ | Reference Method ^b |
| Mean | 100.53 | 100.225 | 99.02 | 99.735 | 100.826 | 100.225 | 100.41 | 99.735 | 99.73 | 100.225 | 100.7 | 99.735 |
| S.D. | 0.45 | 0.789 | 0.57 | 0.897 | 0.685 | 0.789 | 0.59 | 0.897 | 1.01 | 0.789 | 0.62 | 0.897 |
| R.S.D. | 0.447 | 0.787 | 0.578 | 0.899 | 0.68 | 0.787 | 0.588 | 0.578 | 1.01 | 0.787 | 0.616 | 0.578 |
| N | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| Variance | 0.202 | 0.623 | 0.325 | 0.805 | 0.469 | 0.623 | 0.348 | 0.325 | 0.1.02 | 0.623 | 0.384 | 0.325 |
| t-value (2.23) ^a | 0.823 | | 1.648 | | 1.606 | | 1.54 | | 0.947 | | 2.167 | |
| F-value (5.05) ^a | 3.08 | | 2.477 | | 1.328 | | 2.31 | | 1.637 | | 2.096 | |

^a Figures in parentheses are the corresponding values for theoretical t- and F-values at $p = 0.05$.

^b Reference method (HPLC method for determination of MF and MZ in mixture) [17].

CONCLUSION

The proposed methods are simple, sensitive, and precise and can be easily applied in quality control laboratories for the simultaneous determination of MF and MZ. Moreover, the HPLC method is a stability-indicating assay which

can be used for the determination of MF and MZ in the presence of MF alkaline degradation products. The three proposed methods could be successfully applied for the routine analysis of the studied drugs either in their pure bulk powders or in their dosage forms without any preliminary separation step.

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