Development and Validation of New Analytical Methods for the Determination of Some Steroidal Drugs

A Thesis Presented by

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Cairo University
2015
تطوير وتقديم طرق تحليلية جديدة لتقدير بعض العقاقير الستيروئيدية

رسالة مقدمة من

إيناس حامد محمد طلبة

بكالوريوس العلوم الصيدلية- خلية الصيدلة- جامعة القاهرة

2009

للإستيفاء الحصول على درجة الماجستير في العلوم الصيدلية

(الكيمياء الصيدلية)

تتم إشرافه

الأستاذ الدكتور / رمزي إسماعيل البقري

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الدكتور / مروة أحمد فؤاد

2015
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUP</td>
<td>Area under the peak</td>
</tr>
<tr>
<td>BP</td>
<td>British Pharmacopoeia</td>
</tr>
<tr>
<td>C</td>
<td>Concentration</td>
</tr>
<tr>
<td>EuP</td>
<td>European Pharmacopoeia</td>
</tr>
<tr>
<td>F-test</td>
<td>Variance ratio test</td>
</tr>
<tr>
<td>$^1$H-NMR</td>
<td>Proton nuclear magnetic resonance</td>
</tr>
<tr>
<td>HPTLC</td>
<td>High performance thin layer chromatography</td>
</tr>
<tr>
<td>K’</td>
<td>Capacity factor</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>mM</td>
<td>Millimolar concentration</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectroscopy</td>
</tr>
<tr>
<td>N</td>
<td>Number of theoretical plates</td>
</tr>
<tr>
<td>n</td>
<td>Number of experiments</td>
</tr>
<tr>
<td>P=0.05</td>
<td>The probability of results in 95%</td>
</tr>
<tr>
<td>$r^2$</td>
<td>Regression coefficient</td>
</tr>
<tr>
<td>RP-HPLC</td>
<td>Reversed phase- high performance liquid chromatography</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>%RSD</td>
<td>Relative standard deviation</td>
</tr>
<tr>
<td>$S_a$</td>
<td>Standard deviation of intercept</td>
</tr>
<tr>
<td>$S_b$</td>
<td>Standard deviation of slope</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>T</td>
<td>Tailing factor</td>
</tr>
<tr>
<td>(t_R)</td>
<td>Retention time</td>
</tr>
<tr>
<td>t-test</td>
<td>Student’s “t” test</td>
</tr>
<tr>
<td>(\mu l)</td>
<td>Microliter (10^{-6} liter)</td>
</tr>
<tr>
<td>USP/NF</td>
<td>United States Pharmacopoeia/ National Formulary</td>
</tr>
<tr>
<td>(\lambda)</td>
<td>Wavelength in nm</td>
</tr>
<tr>
<td>(\lambda_{\text{max}})</td>
<td>Wavelength of maximum absorption</td>
</tr>
<tr>
<td>(\Delta \lambda)</td>
<td>Wavelength difference</td>
</tr>
</tbody>
</table>
Steroidal Drugs

Steroids consist of four fused rings (A, B, C and D). Chemically, these hydrocarbons are cyclopentanoperhydrophenanthrenes; they contain a five membered cyclopentane (D) ring plus the three rings of phenanthrene [1]. The steroids vary by the functional groups attached to this four-ring core and by the oxidation state of the rings.

Figure (1): Skeletal core of steroids

Steroids term cover two classes: adrenocorticoids and sex hormones.

I. Adrenocorticoids:

The adrenal cortex synthesizes both corticosteroids, based on a 21-carbon nucleus, and some sex hormones, primarily androgens, based on a 19-carbon nucleus. The corticosteroids are traditionally divided into those with mainly glucocorticoid actions, of which cortisol (hydrocortisone) is the most important endogenous example, and those that are primarily mineralocorticoid, of which aldosterone is the more important [2].
**Classification of corticosteroids:**

Corticosteroids are classified into different groups according to biological activity, chemical structure and route of administration.

1. **According to biological activity:**

1. a. **Glucocorticoids:**

Glucocorticoids are potent anti-inflammatories, regardless of the inflammation's cause; they inhibit many inflammation-associated molecules such as cytokines, chemokines, arachidonic acid metabolites, and adhesion molecules. In contrast, anti-inflammatory mediators often are up-regulated by glucocorticoids [3].

Glucocorticoids have also potent immunosuppressive effect and they also have profound metabolic effects. Glucocorticoids facilitate the action of many active endogenous substances as adrenal catecholamine [4], and affect the function of cardiovascular system, kidneys, skeletal muscle and CNS [2]. However, the use of glucocorticoids as therapeutics is often restrained due to two major drawbacks. First, long-term treatment with glucocorticoids is often accompanied by severe side effects, such as diabetes, osteoporosis, hypertension, and muscle atrophy. Second, the occurrence of glucocorticoid resistance limits the success of many glucocorticoid-based therapies [5]. Hydrocortisone, mometasone furoate, betamethasone and fluorometholone are examples of glucocorticoids.

1. b. **Mineralocorticoids:**

The main mineralocorticoid actions are on fluid and electrolyte balance. They enhance sodium reabsorption in the kidney and hence expand the extracellular fluid volume, and they enhance renal excretion of potassium.
and H⁺ [2]. The primary mineralocorticoid is aldosterone, there are a number of disease states: hypoadrenalism and Addison’s disease where the adrenal glands fail to produce aldosterone and replacement therapy is required [6]. Other example is deoxycorticosterone.

**Table (1): Natural mineralocorticoids:**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldosterone</td>
<td><img src="image1" alt="Aldosterone Structure" /></td>
</tr>
<tr>
<td>Deoxycorticosterone</td>
<td><img src="image2" alt="Deoxycorticosterone Structure" /></td>
</tr>
</tbody>
</table>

2. **According to chemical structure:**

In general, corticosteroids are grouped into four classes, based on chemical structure. Allergic reactions to one member of a class typically indicate an intolerance of all members of the class. This is known as the "Coopman classification", after S. Coopman, who defined this classification in 1989 [7, 8].

2. a. **Group A- (Hydrocortisone type):**

Hydrocortisone is a corticosteroid with both glucocorticoid and to a lesser extent mineralocorticoid activity. As cortisol, it is the most important of the
predominantly glucocorticoid steroids secreted by the adrenal cortex. Hydrocortisone is used, usually with a more potent mineralocorticoid, for replacement therapy in adrenocortical insufficiency [2]. Other examples are hydrocortisone acetate, cortisone acetate, tixocortol pivalate, prednisolone, methylprednisolone and prednisone.

*Table (2): Most commonly (Hydrocortisone type) of steroids:*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone</td>
<td><img src="image1" alt="Structure" /></td>
<td>Aveeno®</td>
</tr>
<tr>
<td>Prednisone</td>
<td><img src="image2" alt="Structure" /></td>
<td>Prednis Tab®</td>
</tr>
<tr>
<td>Tixocortol pivalate</td>
<td><img src="image3" alt="Structure" /></td>
<td>Pivalone®</td>
</tr>
</tbody>
</table>

2. b. Group B - (Acetonide type):

Corticosteroid acetonides are used pharmaceutically, especially in dermatology, because their increased lipophilicity leads to better penetration into the skin [9]. Triamcinolone acetonide, budesonide and fluocinolone acetonide are examples of acetonide type.
### Table (3): Most commonly (Acetonide type) of steroids:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triamcinolone acetonide</td>
<td><img src="image" alt="Structure" /></td>
<td>Kenalog®</td>
</tr>
<tr>
<td>Fluocinolone acetonide</td>
<td><img src="image" alt="Structure" /></td>
<td>Flucort®</td>
</tr>
<tr>
<td>Budesonide</td>
<td><img src="image" alt="Structure" /></td>
<td>Budecort®</td>
</tr>
</tbody>
</table>

### 2. c. Group C - (Betamethasone type):

Betamethasone is a corticosteroid used as a topical cream to relieve skin irritation, such as itching and flaking from eczema. It is used as a treatment for local psoriasis. Other examples are dexamethasone, dexamethasone sodium phosphate and fluocortolone.
Table (4): Most commonly (Betamethasone type) of steroids:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betamethasone</td>
<td><img src="image" alt="Structural formula of Betamethasone" /></td>
<td>Diprosone®</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td><img src="image" alt="Structural formula of Dexamethasone" /></td>
<td>Sonexa®</td>
</tr>
</tbody>
</table>

2. d. Group D - (Ester type):

2. d. 1. Group D₁ - Halogenated (less labile esters):

Alclometasone dipropionate, betamethasone valerate, betamethasone dipropionate, clobetasol-17-propionate and mometasone furoate are examples of this group.

Table (5): Most commonly halogenated ester type of steroids:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mometasone furoate</td>
<td><img src="image" alt="Structural formula of Mometasone furoate" /></td>
<td>Elocon®</td>
</tr>
</tbody>
</table>
Betamethasone dipropionate

Diprosone®

2. d. 2. Group D2 - Labile prodrug esters:

Hydrocortisone-17-butyrate, hydrocortisone-17-aceponate, hydrocortisone-17-buteprate, ciclesonide are labile prodrug esters.

Table (6): Most commonly (labile prodrug esters) of steroids:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrocortisone-17-butyrate</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Locoid®</td>
</tr>
<tr>
<td>Ciclesonide</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>Alvesco®</td>
</tr>
</tbody>
</table>
3. **According to the route of administration:**

3. a. **Topical steroids:**

Topical steroids have anti-inflammatory properties. They are the most commonly prescribed medications for the treatment of rash, eczema, and dermatitis. Betamethasone dipropionate and mometasone furoate are examples of topical steroids, Table (5).

3. b. **Inhaled steroids:**

Inhaled corticosteroid therapy in combination with long-acting beta-adrenergic agonists represents the most important treatment for chronic airways diseases such as asthma and chronic obstructive pulmonary disease (COPD) [10]. This group includes fluticasone propionate, Beclomethasone dipropionate, mometasone furoate, ciclesonide and budesonide.

*Table (7): Most commonly inhaled steroids:*

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluticasone propionate</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Flixonase®</td>
</tr>
<tr>
<td>Beclomethasone dipropionate</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>Beclo®</td>
</tr>
</tbody>
</table>
3. c. Oral forms:

Oral corticosteroids are used to treat conditions such as arthritis, blood disorders, breathing problems, severe allergies, skin diseases, cancer, eye problems, and immune system disorders. Examples of this group are prednisone, methylprednisolone and hydrocortisone, Table (2).

3. d. Systemic forms:

Intravenous steroids are indicated in acute asthma if lung function is < 30% of predicted and if there is no significant improvement with a nebulised β2 agonist [11].

**Structure activity relationship of corticosteroids:**

Various structure-activity relationships are understood for the corticosteroids and have been made use of in the development of new compounds. The presence of a hydroxyl group at position 11 seems to be essential for glucocorticoid activity, while a hydroxyl group at position 21 is required for mineralocorticoid activity. Fluorination at position 9 enhances both mineralocorticoid and glucocorticoid activity. Substitution at carbon 16 (as in betamethasone, dexamethasone, or triamcinolone) virtually eliminates mineralocorticoid activity. Esterification of corticosteroids at the 17 or 21 positions with fatty acids generally increases the topical activity. The formation of cyclic acetonides at the 16 and 17 positions further increases topical anti-inflammatory activity, usually without increasing systemic glucocorticoid activity [2].

II- Sex hormones:

The main classes of sex steroids are androgens, estrogens and progestins.
II-1- Androgens:

II-1-a- Natural androgens:

Testosterone is a potent androgen that is found in the blood at higher concentrations than other androgens. Other examples are: Dehydroepiandrosterone, Androstenedione and Androstenediol,

II-1-b- Synthetic androgens:

Examples are Methyltestosterone, fluoxymesterone and mesterolone.

II-1-c- Exogenous anabolic androgenic steroids:

Examples are: nandrolone, danazol, boldenone.

II-2- Estrogens:

II-2-a- Natural estrogens:

The active endogenous estrogens are estradiol, estrone and estriol.

II-2-b- Oral etrogens (Semisynthetic estrogens):

Synthetic estrogens have a greater bioavailability than natural ones [12]. Examples are estradiol dipropionate and ethinyl estradiol.

II-3- Progestins:

Progestins are classified by generation [13] and examples are shown in table (8).
**Table (8): Most commonly sex steroids:**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Structure</th>
<th>Trade name</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Androgens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Natural androgens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testosterone</td>
<td><img src="https://via.placeholder.com/150" alt="" /></td>
<td>Androderm®</td>
</tr>
<tr>
<td>-Synthetic androgens:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mesterolone</td>
<td><img src="https://via.placeholder.com/150" alt="" /></td>
<td>Proviron®</td>
</tr>
<tr>
<td>-Exogenous anabolic androgenic steroids:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Danazol</td>
<td><img src="https://via.placeholder.com/150" alt="" /></td>
<td>Danol®</td>
</tr>
<tr>
<td>Boldenone</td>
<td><img src="https://via.placeholder.com/150" alt="" /></td>
<td>Bolde®</td>
</tr>
<tr>
<td><strong>Estrogens</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-Natural estrogens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estradiol</td>
<td><img src="https://via.placeholder.com/150" alt="" /></td>
<td></td>
</tr>
</tbody>
</table>

13
### Steroidal Drugs

#### Introduction

- **Semisythetic estrogens**
  - **Ethinyl estradiol**
    - Diane® (in combination with cyproterone acetate)

#### Progestins

- **Natural progesterone**
  - **Progesterone**
    - Cyclogest®

- **17-Hydroxyprogesterone derivatives (pregnanes)**
  - **Medroxyprogesterone acetate**
    - Depo Provera®

- **19-Nortestosterone derivatives (estranes)**
  - **Norethisterone**
    - Primolut-N®
Mometasone Furoate [2,14,15]

Structure:

Chemical name:
9α, 21-dichloro-11β, 17-dihydroxy-16α- methylpregna-1, 4-diene-3, 20-dione 17- (2-furoate).

Molecular formula:
C_{27}H_{30}O_{6}Cl_{2}

Molecular weight:
521.43 g/mol.

Properties:
A white to off white powder, insoluble in water, moderately soluble in ethyl alcohol, soluble in acetone and in dichloromethane.

Melting point:
218-220 °C.

Action and uses:
Anti-inflammatory, prophylaxis of asthma and various skin disorders.
**Salicylic Acid [16]**

**Structure:**

![Salicylic Acid Structure](image)

**Chemical name:**

2- Hydroxy benzene carboxylic acid.

**Molecular formula:**

C₇H₆O₃

**Molecular weight:**

138.12 g/mol.

**Properties:**

A white or almost white, crystalline powder or white or colourless, acicular crystals. Slightly soluble in water, freely soluble in ethanol (96%), sparingly soluble in methylene chloride.

**Melting point:**

158-161 °C.

**Action and uses:**

Keratolytic.
Formoterol Fumarate [14-16]

**Structure:**

![Structure Diagram]

**Chemical name:**

(±)-2'-Hydroxy-5'-[(R*)-1-hydroxy-2-[[[(R*)-p-methoxy-α-
methylphenethyl]amino]ethyl]formanilide fumarate (2:1) (salt), dihydrate

**Molecular formula:**

\[(C_{19}H_{24}N_{2}O_{4})_2 \cdot C_4H_4O_4 \cdot 2H_2O\]

**Molecular weight:**

840.91 g/mol.

**Properties:**

White or almost white or slightly yellow powder. Freely soluble in glacial acetic acid, soluble in methanol, sparingly soluble in ethanol, isopropanol, slightly soluble in water. Practically insoluble in acetone, ethyl acetate and diethyl ether.

**Melting point:**

138-140 °C.

**Action and uses:**

Antiasthmatic, bronchodilator.
Miconazole Nitrate [14-16]

**Structure:**

![Chemical structure of Miconazole Nitrate](image)

**Chemical name:**

1H-Imidazole, 1-[2-(2,4-Dichlorophenyl)-2-[(2,4-dichlorophenyl) methoxy]ethyl]-, mononitrate; 1-[2,4-Dichloro- β-[(2,4- dichlorobenzyl)oxy] phenethyl] imidazole mononitrate.

**Molecular formula:**

C\(_{18}\)H\(_{14}\)Cl\(_4\)N\(_2\)O.HNO\(_3\)

**Molecular weight:**

479.14 g/mol.

**Properties:**

White or almost white powder. Very slightly soluble in water, sparingly soluble in methanol, slightly soluble in ethanol (96%).

**Melting point:**

184-185 °C.

**Action and uses:**

Antifungal azole.
Fluorometholone [2, 15, 16 ]

**Structure:**

![Chemical structure of Fluorometholone](image)

**Chemical name:**

9α-fluoro-11β,17α-dihydroxy-6α-methylpregna-1,4-diene-3,20-dione.

**Molecular formula:**

C\textsubscript{22}H\textsubscript{29}FO\textsubscript{4}

**Molecular weight:**

376.462 g/mol.

**Properties:**

A white to yellowish white, crystalline powder. Practically insoluble in water, slightly soluble in absolute ethanol and in ether.

**Melting point:**

292-303 °C.

**Action and uses:**

Anti-inflammatory glucocorticoid for allergic and inflammatory conditions of the eye, various skin disorders.
Investigated Drugs

Sodium Cromoglycate [15, 16]

Structure:

![Structure of Sodium Cromoglycate](image)

Chemical name:
Disodium 5,5'-(2-hydroxypropane-1,3-diyl) dioxy] bis (4-oxo-4H-1-benzopyran-2-carboxylate).

Molecular formula:
C_{23}H_{14}Na_{2}O_{11}

Molecular weight:
512.33 g/mol.

Properties:
White or almost white, hygroscopic, crystalline powder. Freely soluble in water, practically insoluble in chloroform and alcohol.

Melting point:
241-242 °C.

Action and uses:
Prophylaxis of allergic conditions.
Tetrahydrozoline Hydrochloride [14-16]

Structure:

Chemical name:
1H- Imidazole, 4,5- dihydro-2-(1,2,3,4-tetrahydro-1-naphthalenyl)-,monohydrochloride.

Molecular formula:
C_{13}H_{16}N_{2}.Hcl

Molecular weight:
236.74 g/mol.

Properties:
White or almost white, crystalline powder. Freely soluble in water, in anhydrous ethanol and in ethanol (96%), practically insoluble in acetone.

Melting point:
256-257 °C.

Action and uses:
Adrenoceptor agonist, decongestant.
Different methods for the determination of some steroidal drugs, in pure and dosage forms have been introduced in this thesis. These drugs are: mometasone furoate and fluorometholone.

The thesis consists of the following sections:

**Section 1: Aim and Basis of the work**

In this section, the aim of this work and the basis on which the proposed methods were chosen, have been clarified.

**Section 2: Introduction**

It includes:

I. A review about the activity of steroidal drugs, their structure activity relationship and classification.

II. A review about drugs under investigation.

III. Literature review about the official and reported methods for the quantitative determination of the drugs under investigation.

**Section 3: Experimental and Discussion**

This section was further divided into two parts:
Part I- High Performance Liquid Chromatographic Methods

I-1- Forced degradation study of mometasone furoate by RP-LC method

In this method, mometasone furoate was subjected to acid and alkali hydrolysis, oxidation, thermal and photo-degradation. The degradation products were well separated from the pure drug. The method was based on isocratic elution of mometasone furoate and its degradation products on reversed phase Phenomenex® C8 column (250 mm x 4.6 mm, 7 µm) - using a mobile phase consisting of acetonitrile: water: methanol: glacial acetic acid (60:30:10:0.1, v/v/v/v) at a flow rate of 2 ml min\(^{-1}\). Quantitation was achieved with UV detection at 240 nm. In addition, mometasone furoate aqueous alkaline degradation products were verified using LC-MS.

I-2- Stability-indicating RP-LC method for the simultaneous determination of mometasone furoate and salicylic acid in the presence of mometasone furoate alkaline degradation products in mixture and pharmaceutical preparation

A reversed-phase liquid chromatographic method was proposed for the simultaneous determination of mometasone furoate and salicylic acid in the presence of mometasone furoate aqueous alkaline degradation products using the same chromatographic conditions of the forced degradation study.

The method was successfully applied for the determination of mometasone furoate and salicylic acid in laboratory prepared mixture with mean percentage recoveries of 99.65 ± 0.950 and 100.08 ± 0.988, respectively.
The method was successfully applied for the determination of **mometasone furoate** and **salicylic acid** in “Elicasal” ointment with mean percentage recoveries of 100.57 ± 0.757 and 99.66 ± 0.437, respectively.

**I-3- Stability-indicating Ion pair-LC method for the simultaneous determination of Mometasone Furoate and Formoterol Fumarate in the presence of their degradation products in mixture and pharmaceutical preparation**

In this method, ion pair liquid chromatographic method was proposed for the simultaneous determination of **mometasone furoate** and **formoterol fumarate** in the presence of their alkaline, acidic and oxidative degradation products. Chromatographic separation was achieved on XTerra® C18 column (250 x 4.6 mm, 5 µm) applying isocratic elution based on a mobile phase consisting of acetonitrile : 3mM sodium lauryl sulphate) (60:40, v/v) at a flow rate of 1ml min⁻¹. The column temperature was set at 30 °C. Detection was carried out at 214 nm for **formoterol fumarate** and its acidic degradation product then at 247 nm for **mometasone furoate** and its alkaline degradation products.

The method was successfully applied for the determination of **mometasone furoate** and **formoterol fumarate** in laboratory prepared mixture with mean percentage recoveries of 99.48 ± 0.444 and 100.64 ± 0.594, respectively.

The method was successfully applied for the determination of **mometasone furoate** and **formoterol fumarate** in “Dulera” inhaler with mean percentage recoveries of 97.81 ± 0.785 and 99.19 ± 0.963, respectively.

**I-4-Stability-indicating RP-LC method for the simultaneous determination of Mometasone Furoate and Miconazole Nitrate in the presence of**
**Summary**

**Mometasone Furoate alkaline degradation products in mixture and pharmaceutical preparation**

In this method, *mometasone furoate* and *miconazole nitrate* were simultaneously separated and quantified in the presence of *mometasone furoate* non aqueous alkaline degradation products on Waters® C18 column (3.9 x 300 mm, 10μm) applying gradient elution based on a mobile phase consisting of 1.5% w/v aqueous ammonium acetate buffer, pH 7.6 (A) and Acetonitrile (B), at a flow rate of 2.0 ml min⁻¹. The gradient program of was consisting of 0–3 min 45% (A) and 55% (B), 3-10 min gradient down to 40% (A) and 60% (B). Additional five minutes was left for conditioning the column using 45% (A) and 55% (B). Analyses were performed at ambient column temperature and detection was programmed to be at 240 nm from 0-5 min then at 230 nm from 5-10 min.

The method was successfully applied for the determination of *mometasone furoate* and *miconazole nitrate* in laboratory prepared mixture with mean percentage recoveries of 99.73 ± 1.008 and 100.70 ± 0.620, respectively.

The method was successfully applied for the determination of *mometasone furoate* and *miconazole nitrate* in “Elica-M” cream with mean percentage recoveries of 99.53 ± 1.046 and 103.98 ± 0.233, respectively.

In addition, non aqueous alkaline degradation products of *mometasone furoate* were verified by LC-MS.

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**I-5- Stability-indicating RP-LC method for the simultaneous determination of Fluorometholone and Sodium Cromoglycate in the presence of their alkaline degradation products in mixture and pharmaceutical preparation**
In this method, *Flurometholone* and *sodium cromoglycate* were simultaneously separated and quantified in the presence of their alkaline degradation products on ACE Generix ® C18 column (250 mm x 4.6 mm, 5 µm) - applying isocratic elution based on a mobile phase consisting of methanol: water (70:30, v/v). The mobile phase was pumped through the column at a flow rate of 1.2 ml min$^{-1}$. Analyses were performed at ambient temperature and detection was carried out at 240 nm.

The method was successfully applied for the determination of *fluorometholone* and *sodium cromoglycate* in laboratory prepared mixture with mean percentage recoveries of 100.73 ± 0.716 and 100.87 ± 0.857, respectively.

The method was successfully applied for the determination of *fluorometholone* and *sodium cromoglycate* in “Fluca” eye drop with mean percentage recoveries of 101.22 ± 0.558 and 101.98 ± 0.495, respectively.

*I-6- Stability-indicating RP-LC method for the simultaneous determination of Flurometholone and Tetrahydrozoline hydrochloride in the presence of Flurometholone alkaline degradation product in mixture and pharmaceutical preparation*

In this method, *fluorometholone* and *tetrahydrozoline hydrochloride* were simultaneously separated and quantified in the presence of *fluorometholone* alkaline degradation product on ACE Generix ® C8 column (250 mm x 4.6 mm, 5 µm) applying isocratic elution based on a mobile phase consisting of acetonitrile : 50 mM potassium dihydrogen orthophosphate (40:60, v/v) at a flow rate of 2 ml min$^{-1}$. Analyses were performed at ambient temperature and detection was carried out at 240 nm for *fluorometholone* and its alkaline degradation product and at 215 nm for *tetrahydrozoline hydrochloride*. 
The method was successfully applied for the determination of fluorometholone and tetrahydrozoline hydrochloride in laboratory prepared mixture with mean percentage recoveries of 100.72 ± 0.702 and 100.95 ± 0.406, respectively.

The method was successfully applied for the determination of fluorometholone and tetrahydrozoline hydrochloride in “Flumetol” eye drop with mean percentage recoveries of 100.56 ± 0.594 and 100.37 ± 0.650, respectively.

**Part II- Spectroscopic Methods**

**II-1- Derivative Spectrophotometric Determination of Mometasone Furoate and Miconazole nitrate in mixture and pharmaceutical preparation**

A spectrophotometric method is developed for the evaluation of mometasone furoate and miconazole nitrate in mixture. A first derivative spectrophotometric method is proposed for the determination of mometasone furoate by measuring the amplitude at 270.5 nm where miconazole nitrate displays zero reading. A third derivative spectrophotometric method is proposed for the determination of miconazole nitrate by measuring the amplitude at 282.1 nm where mometasone furoate displays zero reading.

The method was successfully applied for the determination of mometasone furoate and miconazole nitrate in laboratory prepared mixture with mean percentage recoveries of 100.49 ± 0.543 and 99.02 ± 0.570, respectively.

The method was successfully applied for the determination of mometasone furoate and miconazole nitrate in “Elica-M” cream with mean percentage recoveries of 98.44 ± 0.996 and 105.16 ± 0.434, respectively.
II-2- Derivative Ratio Spectrophotometric Determination of Mometasone Furoate and Miconazole nitrate or Salicylic acid in mixtures and pharmaceutical preparations

Second derivative ratio spectrophotometric methods were proposed for the determination of *mometasone furoate* and *miconazole nitrate* or *salicylic acid* in mixtures. The ratio spectra of *mometasone furoate* have been obtained by dividing its absorption spectra by the absorption spectrum of 20 μg.ml⁻¹ of *miconazole nitrate* or *salicylic acid* as the chosen divisor. Similarly, the ratio spectra of *miconazole nitrate* and *salicylic acid* were obtained using the absorption spectrum of 5 μg.ml⁻¹ and 10 μg.ml⁻¹ of *mometasone furoate*, respectively as a divisor. *Mometasone furoate* and *miconazole nitrate* could be determined by measuring the amplitudes of the troughs at 267.2 nm and 281.2 nm while *mometasone furoate* and *salicylic acid* could be determined by measuring the amplitudes of the troughs and peaks at 259.5 nm and 293.3 nm, respectively.

Laboratory prepared mixture of *mometasone furoate* and *miconazole nitrate* exhibit percentage recoveries of 100.76 ± 0.525 and 100.46 ± 0.647 while *mometasone furoate* and *salicylic acid* laboratory prepared mixture exhibit mean percentage recoveries of 99.44 ± 0.715 and 100.33 ± 0.884, respectively.

Second derivative ratio methods were successfully applied for the determination of *mometasone furoate* and *miconazole nitrate* in “Elica-M” cream with mean percentage recoveries of 99.14 ± 0.740 and 103.00 ± 0.280 and with mean percentage recoveries of 99.98 ± 0.328 and 99.01 ± 0.336 for *mometasone furoate* and *salicylic acid* in “Elicasal” ointment, respectively.
II-3-Derivative Ratio Spectrophotometric Determination of Fluorometholone and Tetrahydrozoline Hydrochloride in mixture and pharmaceutical preparation

In this method, second derivative ratio spectrophotometric method is proposed for the determination of Fluorometholone and tetrahydrozoline hydrochloride in mixture. The ratio spectra of Fluorometholone have been obtained by dividing its absorption spectra by the absorption spectrum of 10 μg.ml⁻¹ tetrahydrozoline hydrochloride. Similarly, the ratio spectra of tetrahydrozoline hydrochloride were obtained using the absorption spectrum of 10 μg.ml⁻¹ Fluorometholone as the divisor. Fluorometholone and tetrahydrozoline hydrochloride could be determined by measuring the amplitudes of the troughs and peaks at 261.2 nm and 269.6 nm, respectively.

The method was successfully applied for the determination of Fluorometholone and tetrahydrozoline hydrochloride in laboratory prepared mixture with mean percentage recoveries of 100.16 ± 0.881 and 99.09 ± 0.415, respectively.

The method was successfully applied for the determination of Fluorometholone and tetrahydrozoline hydrochloride in “Flumetol” eye drop with mean percentage recoveries of 98.59 ± 0.357 and 102.07 ± 0.199, respectively.

Section 4: Statistical Analysis.

Section 5: Summary.

Section 6: References, this section contains (70) references.