Resolution and Quantitation of Triamcinolone Acetonide and Its Coformulated Drug in the Presence of Its Impurities and Degradation Products by HPTLC and HPLC

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Two specific, sensitive, and precise stability-indicating chromatographic methods have been developed for the determination of triamcinolone acetonide (TMC) and its coformulated drug, econazole nitrate (ECZ), in the presence of TMC impurities and degradation products. The first method was based on HPTLC-spectrodensitometry in which resolution and quantitation was achieved by using silica gel 60 F254 HPTLC plates and an ethyl acetate–tetrahydrofuran–ammonia mobile phase (10.0 + 7.0 + 0.1, v/v/v). The second method was a reversed-phase HPLC method in which separation was achieved using an acetonitrile–methanol–0.05 M potassium dihydrogen phosphate mobile phase, pH 3.0 (25.0 + 15.0 + 60.0, v/v/v). In both methods, the separated components were detected at 225 nm. Validation of both methods was conducted in compliance with International Conference on Harmonization (ICH) guidelines, and system suitability was confirmed. The linearity ranges were 0.20–28.00 and 0.50–55.00 µg/band for TMC and ECZ by HPTLC, whereas for HPLC, the range was 0.05–30.00 and 1.00–40.00 µg/mL for both drugs, respectively. The methods were successfully applied for the analysis of a pharmaceutical formulation and were compared with the reported method with no significant difference.

Triamcinolone acetonide (TMC) is chemically designated as pregna-1,4-diene-3,20-dione, 9-fluoro-11,21-dihydroxy-16,17-[1-methylethyldiene] bis(oxyl), (11β,16α) (1; Figure 1). It is a topical corticosteroid belongs to the synthetic steroid class of drugs and is used as an anti-inflammatory and antipruritic agent either alone or in combination with other drugs (2). Econazole nitrate (ECZ) is 1H-imidazole,1-[2-[(4-chlorophenyl) methoxy]-2-(2,4-dichlorophenyl)[ethyl]–mononitrate, [(±)] (1) and is used as an antifungal agent (2; Figure 1). A combination of TMC and ECZ is topically administered for the treatment of inflammatory dermatoses, superficial pyodermia, paronychia, diaper dermatitis, dry subchronic dermatoses, and other inflammatory diseases accompanied by fungal infection (3). Two impurities were stated in the United States Pharmacopeia (USP), British Pharmacopoeia (BP), and European Pharmacopoeia (EP; 1, 4, 5): triamcinolone (TMC Imp I) and TMC 21-acetate (TMC Imp II). TMC shows marked instability and undergoes degradation under oxidative, alkali, acid, and photostress conditions, whereas ECZ is considerably stable (6).

TMC monographs describe HPLC assays (1, 4, 5). Other methods were reported for its determination by micellar electrokinetic chromatography (7) and spectrofluorimetry (8). TMC was also determined with coformulated drugs by HPLC (9–13) and GC (14). TMC along with salicylic acid were determined by HPLC (15, 16) and by capillary electrophoresis (17). On the other hand, several HPLC methods were reported for ECZ determination, either officially (1, 5) or nonofficially (18, 19). It was also determined among other azole antifungal drugs by UV-spectrophotometry (20). ECZ and estradiol were determined in human plasma by near-IR spectrometry (21). Two HPLC methods were found for the determination of TMC and ECZ in binary mixtures (22) and in the presence of their degradation products (23) without any isolation or identification of the degradation products. However, no method was found for the determination of the studied compounds in the presence of TMC impurities. A literature survey revealed that there is no stability-indicating TLC method for TMC and ECZ.

So, our work aimed primarily to prepare, isolate, and identify oxidative, alkali, acid, and photoinduced degradation products of TMC and, secondarily, to develop and validate stability-indicating methods for the determination of TMC and ECZ in the presence of TMC impurities and degradation products.

Experimental

Apparatus

(a) Densitometer.—Camag dual-wavelength lamp flying TLC scanner 3 densitometer (Muttenz, Switzerland) controlled by winCATS software (Version 3.15; Camag) and run in...
absorbance mode, with a deuterium lamp as a source of radiation; the slit dimension was 3.00 × 0.45 mm and the scanning speed 20 mm/s.

(b) Applicator.—Linomat V, using a 100 µL syringe.
(c) UV lamp.—Short wavelength, 254 nm.
(d) HPTLC plates.—Nano silica gel 60 F254 plates, glass card, 10 × 10 cm, 0.2 mm thickness (Fluka, Sigma-Aldrich, Buchs, Switzerland).
(e) Precoated TLC glass plates.—Silica F254 plates, G-50 UV254, 20 × 20 cm, 0.2 mm (Machery-Nagel, Darmstadt, Germany).
(f) HPLC system.—Agilent 1100 series control module, equipped with a quaternary pump and Rheodyne injector (Rohnert Park, CA), with a 20 µL loop and a UV-variable wavelength detector (Model No. G1316 A) set at 225 nm.
(g) Column.—Hypersil BDS C18 column (California); 250 × 4 mm, 5 µm particle size.
(h) IR spectrometer.—Vector 8201 PC (Darmstadt, Germany).
(i) LC-MS.—Agilent Technologies QQQ 6420 Triple Quad LC-tandem MS (MS/MS), equipped with TCC 1290 quaternary pump and 1290 autosampler.

Samples

(a) Pure samples.—TMC, ECZ, and TMC Imps I and II were kindly supplied by Sigma-Aldrich Chemie GmbH (Taufkirchen, Germany). Their purities were confirmed to be 99.87, 99.92, 99.76, and 99.90%, respectively, according to the USP (1).
(b) Market samples.—Pevisone Cream, manufactured by MINAPHARMA Egypt under license of Janssen CILAG-Switzerland (Batch No. EEE 1787), was labeled to contain 0.1 g TMC and 1.0 g ECZ per 100 g cream and was obtained from a local market.

Chemicals and Solvents

All chemicals used were of analytical grade and solvents were of HPLC grade. Methanol, acetonitrile, ethyl acetate, tetrahydrofuran, formic acid, and H2O2 (30%) were obtained from Lab-Scan Analytical Science (Dublin, Ireland). Potassium dihydrogen phosphate was obtained from Riedel-de Haen (Seelze, Germany). Sodium hydroxide (NaOH), hydrochloric acid (HCl), ammonia solution, and phosphoric acid were obtained from El-Nasr Pharmaceutical and Chemical Co. (Abu-Zaabal, Cairo, Egypt).

(a) Preparation, isolation, and structural elucidation of degradation products.—(1) Preparation of degradation product stock solutions.—(i) Preparation of the oxidative degradation product of TMC.—To accurately weighed 50.00 mg pure TMC, 5 mL 30% H2O2 was added, mixed, and diluted to a 50 mL volume with methanol and refluxed at 90°C for 6 h. The solution was evaporated to near-dryness in a water bath; the residue was dissolved in methanol, cooled, transferred to a 50 mL volumetric flask, and diluted to volume with methanol.
(ii) Preparation of the alkali-induced degradation product of TMC.—Accurately weighed 50.00 mg pure TMC was dissolved in 50 mL NaOH (1 M methanolic solution) and refluxed for 4 h in water bath. The solution was cooled, neutralized, and filtered. The filtrate was evaporated to near-dryness; the residue was dissolved in methanol, transferred to a 50 mL volumetric flask, and diluted to volume with methanol.
(iii) Preparation of the acid-induced degradation product of TMC.—Accurately weighed 50.00 mg pure TMC was dissolved in 50 mL HCl (1 M methanolic solution), refluxed for 8 h, and then neutralized, filtered, and evaporated to dryness. The residue was dissolved in methanol, transferred to a 50 mL volumetric flask, and diluted to volume with methanol.
(iv) Preparation of the photodegradation product of TMC.—Accurately weighed 50.00 mg pure TMC was diluted in 50 mL methanol solution, transferred to a 50 mL volumetric flask, and diluted to volume with methanol. The solution was subjected to a UV light source at 254 nm for 24 h.
(2) Isolation of the prepared degradation product.—Prepared stock solutions under different stress conditions (equivalent to 1.00 mg/mL TMC) were applied in the form of bands onto precoated TLC glass plates and allowed to develop in a chromatographic tank previously saturated with a
developing system of ethyl acetate–tetrahydrofuran–ammonia (10.0 + 7.0 + 0.1, v/v/v) at ambient temperature. The developed bands were air-dried, scratched, and mixed with methanol; sonicated for 30 min; filtered; and then air-dried.

(3) Identification and structural elucidation of the prepared degradation product.—The dried pure degradation products were dissolved in methanol and subjected to identification and structural elucidation by an LC-MS/MS method at 25°C using an Acquity UPLC BEH shield RP18 column (2.1 × 150 mm, 1.7 µm) and an acetonitrile–0.1% formic acid mobile phase (35 + 65, v/v) delivered at a flow rate of 0.3 mL/min with an injection volume of 2.0 µL. The following MS/MS conditions were applied: positive-ion electrospray ionization, ion spray voltage of 4500 V, gas temperature of 325°C, 8 L/min flow rate, entrance potential of 7 V, and nebulizer operated under 50 psi.

Solutions

(a) Triamcinolone acetonide standard solutions.—(1) Stock standard solution of TMC (1.00 mg/mL).—Prepared in methanol.
(2) Working standard solution I of TMC (0.10 mg/mL).—Prepared by the appropriate dilution of the stock standard solution of TMC in methanol or mobile phase for the HPTLC and HPLC methods, respectively.
(3) Working standard solution II of TMC (0.01 mg/mL).—Used for HPLC only and prepared by the appropriate dilution of TMC working standard solution I in mobile phase.

(b) Triamcinolone acetonide impurity solutions.—(1) Stock standard solutions of TMC impurities (1.00 mg/mL).—Accurately weighed 25.00 mg each of TMC impurities, Imps I and II, were separately transferred into two separate 25 mL volumetric flasks, dissolved, and diluted with methanol.
(2) Working standard solution I of TMC impurities (0.10 mg/mL).—Prepared by the appropriate and separate dilution of TMC impurities stock standard solutions (equivalent to 1.00 mg/mL TMC).
(3) Working standard solution II of TMC impurities (0.01 mg/mL).—Prepared by the appropriate and separate dilution of the degradation product stock solutions of TMC in methanol or mobile phase for the HPTLC and HPLC methods, respectively.

(c) Triamcinolone acetonide degradation product solutions.—(1) Stock solutions of degradation products (equivalent to 1.00 mg/mL TMC).—Prepared in methanol.
(2) Working solution I of TMC oxidative, alkali, acid, or photodegradation products (equivalent to 0.10 mg/mL TMC).—Prepared by the appropriate and separate dilution of the degradation product stock solutions of TMC in methanol or mobile phase for the HPTLC and HPLC methods, respectively.
(3) Working solution II of TMC oxidative, alkali, acid, or photodegradation products (equivalent to 0.01 mg/mL TMC).—Used for HPLC and prepared by the appropriate and separate dilution of working solution I of TMC degradation products.

(d) Econazole nitrate standard solutions.—(1) Stock standard solution of ECZ (1.00 mg/mL).—Prepared in methanol.
(2) Working standard solution of ECZ (0.10 mg/mL).—Prepared by the appropriate dilution of stock standard solution of ECZ in methanol or mobile phase for the HPTLC and HPLC methods.

(e) Laboratory-prepared mixture solutions.—(1) HPTLC method.—Aliquots of TMC and ECZ stock solutions (1.00 mg/mL), working standard solution I of TMC (0.10 mg/mL), and working standard solution of ECZ (0.10 mg/mL) were accurately transferred into a series of 10 mL volumetric flasks. To these solutions aliquots of TMC impurity stock and working solutions were added in order to prepare mixtures containing from 10 to 90% impurities; each flask was diluted to volume with methanol. The laboratory-prepared mixtures of the degradation products were prepared in the same manner.
(2) HPLC method.—Aliquots of working standard solution I of TMC (0.10 mg/mL), working standard solution II of TMC (0.01 mg/mL), and working standard solution of ECZ (0.10 mg/mL) were transferred into a series of 10 mL volumetric flasks to which aliquots of TMC impurity sample solutions were added to prepare different mixtures of impurities containing 10–90% impurities. Each solution was diluted to volume with mobile phase. The laboratory-prepared mixtures of the degradation products were prepared in the same manner.

(f) Pharmaceutical formulation solution.—Ten grams of Pevisone Cream (labeled to contain 0.1 g TMC and 1 g ECZ per 100 g cream) were accurately weighed, warmed in a water bath, and sonicated for 30 min after addition of 80 mL methanol. The solution was filtered and transferred into a 100 mL volumetric flask and diluted to volume with methanol to reach a final concentration of 0.10 and 1.00 mg/mL TMC and ECZ, respectively.

Procedures

(a) Chromatographic conditions.—(1) HPTLC method.—The HPTLC method was performed on 10 × 10 cm HPTLC silica gel
plates (60 F254, 0.20 mm thickness), which were prewashed with methanol and activated at 100°C for 5 min before use. The samples were applied in bands (6 mm in length, 4 mm away from each other, and 15 mm away from the lower edge of the plate). Linear ascending development was allowed in a chromatographic tank previously saturated with an ethyl acetate–tetrahydrofuran–ammonia developing system (10.0 + 7.0 + 0.1, v/v/v) at ambient temperature. The developed plates were then air-dried and scanned at 225 nm.

(2) HPLC method.—A Thermo Hypersil BDS C18 column (250 × 4 mm, 5.0 µm) was used at ambient temperature with an acetonitrile–methanol–0.05 M potassium dihydrogen phosphate mobile phase adjusted to pH 3.0 with orthophosphoric acid (25 + 15 + 60, v/v/v). The mobile phase was filtered through a 0.45 µm Millipore membrane filter (Billerica, MA) and delivered at a flow rate of 1.5 mL/min. The injection volume was 20.0 µL and detection was at 225 nm.

Figure 3. MS spectra of (a) triamcinolone acetonide, (b) econazole nitrate, (c) triamcinolone acetonide Imp I, (d) triamcinolone acetonide Imp II, (e) triamcinolone acetonide Deg. I, (f) triamcinolone acetonide Deg. II, (g) triamcinolone acetonide Deg. III, (h) triamcinolone acetonide Deg. IV, (i) triamcinolone acetonide Deg. V, and (j) triamcinolone acetonide Deg. VI.
Construction of calibration curves.—(1) HPTLC method.—(i) TMC.—Aliquots of 10.00–28.00 and 2.00–50.00 µL TMC stock standard solution (1.00 mg/mL) and working standard solution I (0.10 mg/mL), respectively, were accurately and separately transferred into 10 mL measuring flasks.

(ii) ECZ.—Aliquots of 5.00–55.00 and 10.00–50.00 µL stock standard solution of ECZ (1.00 mg/mL) and working standard solution of ECZ (0.10 mg/mL), respectively, were separately and accurately transferred into a series of 10 mL measuring flasks.

Both series were diluted to volume with methanol and applied in triplicate onto the HPTLC plates and subsequently chromatographed and scanned. The calibration curves relating the integrated peak area to the corresponding concentrations of TMC and ECZ as µg/band were respectively constructed.

(2) HPLC method.—(i) TMC.—Accurately measured aliquots of TMC working standard solutions I (0.10 mg/mL) and II (0.01 mg/mL) were further diluted to 10 mL with mobile phase to obtain solutions in the range of 0.05–30.00 µg/mL.

(ii) ECZ.—Accurately measured aliquots of ECZ working standard solution (0.10 mg/mL) were further diluted to 10 mL with mobile phase to obtain dilutions in the range of 1.00–40.00 µg/mL.

Each solution was injected in triplicate and chromatographed. The integrated peak areas were plotted against the corresponding concentrations, and the curves were constructed.

(c) Application to laboratory-prepared mixtures and the pharmaceutical formulation.—The laboratory-prepared mixture solutions previously mentioned in the Solutions section were analyzed using the chromatographic conditions mentioned above.

Results and Discussion

An ideal stability-indicating method is the one that quantifies the intact drug and also resolves its possible degradation products (24). TMC and ECZ are coformulated drugs; for their QC, an efficient method has to be developed and applied not only to quantify the drugs, but also to detect and identify any impurities or degradation products that may be present. Official impurities have to be tested in both raw materials and finished product samples. Moreover, for successful stability studies, assay methods for the determination of drug in the presence of its possible degradation products are needed. The routine HPLC assay of expired batches of a TMC and ECZ formulation just after 1 week of the expiry...
date reveals the presence of unknown compounds. So, the importance of developing a stability-indicating assay method for their determination and the identification of new products emerged.

TMC has two reported impurities, triamcinolone (Imp I) and TMC 21-acetate (Imp II), which should not exceed certain limits due to variable pharmacological effect strengths (25, 26). TMC Imps I and II are considered process impurities according to the USP (1), BP (4), and EP (5). Moreover, TMC was found to be unstable under different conditions, yielding a TMC oxidative degradation product (TMC Deg. I), TMC alkali degradation product (TMC Deg. II), and TMC acid degradation products (TMC Degs. III, IV, V, and VI; Figure 2; 27). However, TMC undergoes partial photodegradation, also producing TMC Degs. II, III, and IV.

ECZ was found to be stable under the previously mentioned stress conditions (6). Benzoic acid and butylated hydroxyanisole were found in the dosage form and showed marked stability (28). One HPLC method was reported for the stability study of TMC and ECZ along with benzoic acid and butylated hydroxyanisole under different stress conditions using gradient elution and changing the wavelength of analysis (23).

Partial degradation of TMC was observed upon using 0.1 M HCl, 0.1 M NaOH, 10% H2O2, or photodegradation for 30 min without heating. For our intent of identifying and separating the degradation products, our experimental trials were seeking their complete degradation. Refluxing for 6 h with

![Figure 5. HPLC chromatogram of (a) triamcinolone acetonide (30 µg/mL), econazole nitrate (40 µg/mL), triamcinolone acetonide impurities (30 µg/mL), triamcinolone acetonide degradation products (30 µg/mL), and benzoic acid (40 µg/mL). (b) Pevisone Cream showing triamcinolone acetonide (4 µg/mL), econazole nitrate (40 µg/mL), and benzoic acid (80 µg/mL). (c) Expired batch of Pevisone Cream: triamcinolone acetonide (4 µg/mL), econazole nitrate (40 µg/mL), and benzoic acid (80 µg/mL) after 1 month, using acetonitrile–methanol–0.05 M potassium dihydrogen phosphate (25 + 15 + 60, v/v/v) pH 3 as the mobile phase and UV detection at 225 nm.](image-url)
30% H₂O₂ was capable of the complete formation of one oxidative degradation product, TMC Deg. I (an olefinic double bond was oxidized to epoxide; 29). Complete degradation under alkali stress conditions was achieved using 1 M methanolic NaOH and heating for 4 h, yielding TMC Deg. II (alkyl fluoride, as a tertiary halogenoalkane, reacts with NaOH with the elimination of HF from the TMC compound, and NaF was produced; 30). On the other hand, upon using 1 M methanolic HCl and heating for 8 h, complete degradation of TMC was achieved by yielding TMC Deg. III (addition reaction of the alkene double bond with H₂O to form alcohol; 27, 31), Degs. IV and V (produced through elimination reaction of alcohol to form alkene; 27, 32), and Deg. VI (produced by addition reaction of the alkene double bond with CH₃OH; 27). TMC undergoes photodegradation with the formation of Degs. II, III, and IV (Figure 2). First, identification of the degradation products was tried using IR-spectrometry, but it failed to differentiate between TMC and its impurities and degradation products, which was attributed to the presence of similar functional groups. To be more specific, MS was used, and the MS spectrum of TMC showed a molecular ion peak at m/z 435. The mass ion peaks at m/z 394 and 477 for TMC Imps I and II, respectively, were identified. The mass ion peaks at m/z 451, 421, 457, 413, 419, and 441 for TMC Degs. I, II, III, IV, V, and VI, respectively, were found, and, accordingly, the suggested structures for the degradation products are shown in Figure 3a–j.

For separation of TMC and ECZ along with degradation products and impurities by TLC method, an efficient stationary phase–mobile phase combination is needed. HPTLC plates were chosen as the stationary phase as they are characterized by smaller particles of stationary phase (<10 µm diameters) and have more advantages over TLC, such as higher resolving power per unit distance, faster development times, and reduced solvent consumption. The excipients did not interfere in the analysis of the two due to their high polarity. Numerous developing systems were tried to obtain moderately good resolution between the 10 components. Toluene–chloroform–methanol–ammonia (5.0 + 6.0 + 2.0 + 0.05, v/v/v/v) was found suitable for isolation, but it was preferable to use less toxic and more environmentally friendly (green) solvents and avoid the use of chloroform and toluene for their severe toxicity (33, 34). The 10 components were completely and efficiently resolved using ethyl acetate–tetrahydrofuran–ammonia (10.0 + 7.0 + 0.1, v/v/ v). Compact bands and sharp symmetrical peaks were obtained with good resolution (Figure 4). In order to minimize band diffusion, the optimum bandwidth was chosen to be 6 mm and the interspaces between bands 4 mm. Different scanning wavelengths were tested in which scanning at 225 nm was suitable, providing good sensitivity for both TMC and ECZ with a single plate scan; also, the selected wavelength did not accommodate the excipient, butylated hydroxyanisole (λₘₐₓ = 290 nm). The Rₚ values were 0.77 for TMC; 0.64 and 0.83 for the TMC impurities; 0.05, 0.36, 0.42, 0.46, 0.61, and 0.80 for the TMC degradation products; and 0.56 for ECZ (Figure 4). The system suitability parameters of the proposed HPTLC method comply with the specifications of the USP (1; Table 1).

For the development and optimization of the HPLC method, several trials were carried out to obtain symmetrical resolved peaks. These trials involved the use

| Table 2. System suitability of the developed HPLC method for the determination of triamcinolone acetonide and econazole nitrate in the presence of triamcinolone acetonide impurities and degradation products |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Parameters      | ECZ             | TMC Imp I       | TMC Deg. I      | TMC Deg. II     | TMC Deg. III    | TMC Deg. IV     | TMC Deg. V      | TMC Deg. VI     | Reference value |
| Resolution (Rₛ) | 5.60            | 4.00            | 4.67            | 5.00            | 3.50            | 8.50            | 3.50            | 11.20           | R > 1.50         |
| Selectivity (α) | 1.36            | 1.16            | 1.23            | 1.27            | 1.10            | 1.27            | 1.12            | 1.00            | >1.00           |
| Tailing factor (T) | 1.00             | 1.00            | 1.00            | 1.00            | 1.00            | 1.00            | 0.97            | 1.00            | 0.97            |
| Column capacity (K) | 1.00             | 1.88            | 2.33            | 3.11            | 4.23            | 7.22            | 9.10            | 8.03            | 11.13           |
| Column efficiency (N) | 2460            | 4327            | 2460            | 9216            | 9216            | 2460            | 9216            | 8534            | 7292            |
| High equivalent to theoretical plate, cm/plate | 1.4 × 10⁻³ | 2.3 × 10⁻³ | 1.4 × 10⁻³ | 3.2 × 10⁻³ | 3.2 × 10⁻³ | 3.2 × 10⁻³ | 3.2 × 10⁻³ | 3.2 × 10⁻³ |
| Retention time, min | 1.90 | 3.00 | 4.71 | 7.40 | 8.13 | 11.03 | 11.78 | 12.70 |
of different mobile phases with different ratios, pH, and flow rates. The best resolution with sharp and symmetrical peaks was obtained upon using an acetonitrile–methanol–0.05 M potassium dihydrogen phosphate mobile phase (25 + 15 + 60, v/v/v) pH 3.0 adjusted with orthophosphoric. The flow rate was adjusted to 1.5 mL/min. Retention times were 7.40, 2.59, and 12.70 min for intact TMC and TMC Imps I and II, respectively. Those for the six degradation

Table 3. Results of the validation parameters of the proposed methods for the determination of the binary mixture of triamcinolone acetonide and econazole nitrate by the proposed HPTLC and HPLC methods

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Proposed HPTLC method for the binary mixture, µg/band</th>
<th>Proposed HPLC method for the binary mixture, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>Triamcinolone acetonide 0.20–28.00</td>
<td>Econazole nitrate 0.50–55.00</td>
</tr>
<tr>
<td></td>
<td>Triamcinolone acetonide 0.05–30.00</td>
<td>Econazole nitrate 1.00–40.00</td>
</tr>
<tr>
<td>Linearity</td>
<td>Slope 25.784</td>
<td>Intercept 5.626</td>
</tr>
<tr>
<td></td>
<td>0.9939°</td>
<td>-51.433°</td>
</tr>
<tr>
<td></td>
<td>5.4833°</td>
<td>-5.833°</td>
</tr>
<tr>
<td>Interception</td>
<td>67.55</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>73.222</td>
<td>0.9999</td>
</tr>
<tr>
<td></td>
<td>0.9999</td>
<td>0.9999</td>
</tr>
<tr>
<td>Correlation coefficient (r)</td>
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<td></td>
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<tr>
<td>Accuracy</td>
<td>Mean ± SD 99.50 ± 1.45</td>
<td>99.33 ± 1.75</td>
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<tr>
<td></td>
<td>100.09 ± 0.76</td>
<td>100.73 ± 1.58</td>
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<tr>
<td>Precision: RSD, %</td>
<td>1.46</td>
<td>0.76</td>
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<td></td>
<td>1.76</td>
<td>1.57</td>
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<tr>
<td>Repeatability&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.95</td>
<td>1.25</td>
</tr>
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<td></td>
<td>1.29</td>
<td>0.59</td>
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<tr>
<td>Intermediate precision&lt;sup&gt;d&lt;/sup&gt;</td>
<td>1.33</td>
<td>1.67</td>
</tr>
<tr>
<td></td>
<td>1.31</td>
<td>1.42</td>
</tr>
<tr>
<td>Specificity and selectivity</td>
<td>100.29 ± 0.95</td>
<td>100.15 ± 1.46</td>
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<tr>
<td></td>
<td>100.42 ± 1.46</td>
<td>100.63 ± 1.55</td>
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<tr>
<td>Mean ± SD</td>
<td>100.38 ± 1.41</td>
<td>100.31 ± 1.15</td>
</tr>
<tr>
<td></td>
<td>100.61 ± 1.41</td>
<td>100.04 ± 134</td>
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<tr>
<td>Robustness&lt;sup&gt;e&lt;/sup&gt;</td>
<td>101.25 ± 1.25</td>
<td>98.75 ± 1.50</td>
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<tr>
<td></td>
<td>98.73 ± 0.50</td>
<td>100.20 ± 1.65</td>
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<tr>
<td>LOD&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.056</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td>0.015</td>
<td>0.317</td>
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<tr>
<td>LOQ&lt;sup&gt;g&lt;/sup&gt;</td>
<td>0.17</td>
<td>0.48</td>
</tr>
<tr>
<td></td>
<td>0.044</td>
<td>0.96</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of three separate determinations.

<sup>b</sup> Confidence limit.

<sup>c</sup> Intraday (n = 9): average of three different concentrations repeated three times within the same day.

<sup>d</sup> Intraday (n = 9): average of three different concentrations repeated three times on 3 successive days.

<sup>e</sup> Average change in pH (±0.20), flow rate (±0.10 mL/min), and ratio of mobile phase (±2.00%).

<sup>f</sup> The LOD was calculated by (SD of the response/slope) × 3.30.

<sup>g</sup> The LOQ was calculated by (SD of the response/slope) × 10.

Table 4. Determination of triamcinolone acetonide and econazole nitrate in the presence of TMC impurities or degradation products in the laboratory-prepared mixtures by the proposed HPTLC method

<table>
<thead>
<tr>
<th>Impurities or degradation product, %</th>
<th>In the presence of impurities or degradation products</th>
<th>Recovery of the drug, %&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>TMC:ECZ ratio</td>
<td>TMC:TMC Imps or Degr.ECZ</td>
<td>Triamcinolone acetonide</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Impurities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Degradation products</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Econazole nitrate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Impurities</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Degradation products</td>
</tr>
<tr>
<td>10</td>
<td>1:10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4.50:0.50:50.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>1:1</td>
<td>20.00:5.00:25.00</td>
</tr>
<tr>
<td>30</td>
<td>2:1</td>
<td>14.00:6.00:10.00</td>
</tr>
<tr>
<td>40</td>
<td>1:2</td>
<td>12.00:8.00:40.00</td>
</tr>
<tr>
<td>50</td>
<td>1:10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.50:2.50:50.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>60</td>
<td>5:1</td>
<td>10.00:15.00:5.00</td>
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<td>70</td>
<td>1:3</td>
<td>3.00:7.00:30.00</td>
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<td>80</td>
<td>1:1</td>
<td>5.00:20:00.25:00</td>
</tr>
<tr>
<td>90</td>
<td>1:10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.50:4.50:50.00&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>100.29 ± 0.95</td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td>100.38 ± 1.41</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.15 ± 1.46</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100.31 ± 1.15</td>
</tr>
</tbody>
</table>

<sup>a</sup> Average of three separate determinations.

<sup>b</sup> The ratio of TMC–ECZ in the cream.
products were 3.00, 4.71, 8.13, 9.81, 11.03, and 11.78 min, whereas for ECZ, it was 1.90 min. The method succeeded in isolating the 10 components from benzoic acid with suitable resolution (Figure 5). The optimum detection wavelength was 225 nm. System suitability parameters of the proposed HPLC method were calculated, showing good resolution and selectivity and symmetrical peaks (Table 2).

Validation of the proposed methods was performed according to International Conference on Harmonization guidelines (24). Table 3 shows the results of accuracy, repeatability, and intermediate precision of the methods. The HPTLC method was valid and applicable for the determination of TMC and ECZ, with mean recoveries of 99.50 ± 1.45 and 99.33 ± 1.75%, respectively, in their pure form. TMC and ECZ were accurately determined by the proposed HPLC method, achieving mean recoveries of 100.09 ± 0.76 and 100.73 ± 1.58% in their pure form, respectively. The specificity of the proposed methods was checked by the analysis of the laboratory-prepared mixtures of TMC, ECZ, and TMC impurities or degradation products in different ratios, as presented in Tables 4 and 5, respectively. The developed methods were applied for the determination of TMC and ECZ in the pharmaceutical formulation. Satisfactory results were obtained, in good agreement with the label claims (Table 6).

The validity of the proposed methods was assessed by applying the standard addition technique, which showed accurate results, and there was no interference from excipients, as shown in Table 7. Statistical comparison of the results of the TMC and ECZ analysis obtained by the proposed methods and the reported HPLC method (23) was also done using Student’s t-test and an F-value at a 95% confidence level (Table 8), and it was clear that there was no significant difference between the proposed methods with regard to accuracy and precision.

### Table 5. Determination of triamcinolone acetonide and econazole nitrate in the presence of TMC impurities or degradation products in the laboratory-prepared mixtures by the proposed HPLC method

<table>
<thead>
<tr>
<th>Impurities or degradation products %</th>
<th>TMC:ECZ ratio</th>
<th>TMC:TMC Imps or Degs.:ECZ</th>
<th>Recovery of the drug, %a</th>
<th>TMC:ECZ ratio</th>
<th>TMC:TMC Imps or Degs.:ECZ</th>
<th>Recovery of the drug, %a</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>1:10b</td>
<td>4.60:0.40:40.00b</td>
<td>98.50</td>
<td>99.50</td>
<td>101.60</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>1:1</td>
<td>16.00:4.00:20.00</td>
<td>102.00</td>
<td>101.75</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2:1</td>
<td>21.00:9.00:15.00</td>
<td>99.00</td>
<td>98.33</td>
<td>102.00</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>1:2</td>
<td>12.00:8.00:40.00</td>
<td>98.75</td>
<td>100.65</td>
<td>98.00</td>
<td></td>
</tr>
<tr>
<td>50</td>
<td>1:10b</td>
<td>5.05:0.50:10.00b</td>
<td>101.70</td>
<td>100.50</td>
<td>102.00</td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>5:1</td>
<td>8.00:12.00:4.00</td>
<td>101.00</td>
<td>101.50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>70</td>
<td>1:3</td>
<td>1.50:3.50:15.00</td>
<td>101.60</td>
<td>98.76</td>
<td>99.45</td>
<td></td>
</tr>
<tr>
<td>80</td>
<td>1:1</td>
<td>2.00:8.00:10.00</td>
<td>99.50</td>
<td>101.00</td>
<td>98.75</td>
<td></td>
</tr>
<tr>
<td>90</td>
<td>1:10b</td>
<td>0.10:0.90:10.00b</td>
<td>101.75</td>
<td>102.00</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean ± SD</td>
<td></td>
<td>100.42 ± 1.46</td>
<td></td>
<td>100.61 ± 1.41</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RSD</td>
<td></td>
<td>1.45</td>
<td></td>
<td>1.54</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **a** Average of three separate determinations.
- **b** The ratio of TMC–ECZ in the cream.

### Table 6. Determination of triamcinolone acetonide and econazole nitrate in their pharmaceutical formulation by the proposed HPTLC and HPLC methods

<table>
<thead>
<tr>
<th>Pharmaceutical formulation</th>
<th>Parameter</th>
<th>Triamcinolone acetonide</th>
<th>Econazole nitrate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Proposed HPTLC method</td>
<td>Proposed HPLC method</td>
</tr>
<tr>
<td>Pevisone cream, Batch No.</td>
<td>Mean ± SDa</td>
<td>100.33 ± 0.95</td>
<td>100.03 ± 1.40</td>
</tr>
<tr>
<td>EEE 1787, 0.1 TMC/1 ECZ/g100 g</td>
<td>RSD</td>
<td>0.95</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>Variance</td>
<td>0.90</td>
<td>2.31</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>F-value (5.05)b</td>
<td>2.18</td>
<td>1.18</td>
</tr>
<tr>
<td></td>
<td>Student’s t-test (2.228)b</td>
<td>0.434</td>
<td>0.071</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.046</td>
<td>1.377</td>
</tr>
</tbody>
</table>

- **a** SDs for six determinations.
- **b** The ratio of TMC–ECZ in the cream.
The proposed methods were considered highly efficient in the separation and quantitation of the studied drugs; moreover, the HPLC method is based on isocratic elution, and the reasons given to avoid gradient elution deserve serious reconsideration, especially for those samples that are easily separated isocratically. However, we believe isocratic elution will remain preferable (35); good resolution of the cited active drugs without interference of their excipients was also attained. Identification of the six degradation products of TMC and their resolution, along with the two official impurities of TMC, in a short time of analysis was achieved. Plus, the method is more sensitive than the proposed HPTLC method and the reported HPLC method (23) concerning TMC, which is a very unstable drug.

Conclusions

The proposed methods provided simple, sensitive, selective, and accurate methods for the determination of a TMC and ECZ binary mixture in bulk powder and in their pharmaceutical formulation, without any interference from excipients or TMC official impurities or degradation products. The proposed methods were validated and could be used for routine analysis and stability testing in QC laboratories. HPTLC has the advantages of a short run time, large sample capacity, and the use of a minimal volume of solvents.

References

(1) Authority of the United States Pharmacopeial Convention (2015) The United States Pharmacopeia (USP 38), National Formulary (NF 33), Rockville, MD