Stability Indicating HPTLC Method Development for Determination of Betamethasone and Dexchlorpheniramine Maleate in a Tablet Dosage Form

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Citation

Abstract
A simple, specific, precise, accurate, robust and stability-indicating HPTLC method for determination of the two drugs in tablet dosage form was developed and validated. The method employed HPTLC aluminum plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of ethyl acetate: methanol: ammonia (2 : 13 : 1 v/v/v). This system was found to give compact spots for betamethasone ($R_f = 0.32 ± 0.04$) and for dexchlorpheniramine maleate ($R_f = 0.76 ± 0.05$). Densitometric analysis of the drugs was carried out in the absorbance mode at 226 nm. Linearity was found over the concentration range of 25 - 137.5 ng/ul with $r^2 = 0.997 ± 0.00102$ for betamethasone and 100 - 800 ng/ul for dexchlorpheniramine maleate with $r^2 = 0.999 ± 0.141$, respectively. LOD for the method was found to be 2.49ng and 19.7ng for betamethasone and dexchlorpheniramine maleate, respectively. LOQ was found to be 7.54 ng for betamethasone and 59.70 ng for dexchlorpheniramine maleate. Extra peaks were observed for betamethasone treated with 30% $H_2O_2$ ($R_f = 0.36$), 1N HCl ($R_f = 0.19, 0.25$ and $0.36$), 1N NaOH ($R_f = 0.36$) and thermal condition ($R_f = 0.26$ and $0.36$). Dexchlorpheniramine maleate degraded with 30% $H_2O_2$ showed additional peak at $R_f$ value of 0.67. Statistical analysis proved that the method is reproducible and selective for the simultaneous estimation of betamethasone and dexchlorpheniramine maleate. As the method could effectively separate the drugs from their degradation products, it can be employed as a stability indicating.

1. Introduction
Betamethasone 9 α-fluoro-16 β-methyl-11 β, 17 α 21-trihydroxy-1, 4-pregnadiene- 3, 20-dione (Figure 1 a) is a synthetic glucocorticoid that suppress the activity of endogenous mediators of inflammation including prostaglandins, kinins, and histamine [1]. Dexchlorpheniramine maleate (3S)-3-(4-chlorophenyl)-N, N-dimethyl-3-(pyridin-2-yl) propan-1-amine (Z)-butanedioate (Figure 1 b) is a potent antihistamine used for the
treatment of several allergies and skin irritation. It readily crosses the blood brain barrier and one of its collateral effects is the induction of sedation [2].

Various methods have been reported for the determination of betamethasone in a single dosage form and in combination with other drugs. Quantification of betamethasone in human plasma by liquid chromatography–tandem mass spectrometry using atmospheric pressure photo ionization in negative mode has been reported [3]. Betamethasone and betamethasone 17-monopropionate determinations were reported in human plasma by liquid chromatography–positive/negative electrospray ionization tandem mass spectrometry [4]. Analysis of corticosteroids including betamethasone in hair has been reported using liquid chromatography–electrospray ionization mass spectrometry [5]. Coupled-column liquid chromatographic–tandem mass spectrometric method has been reported for the determination of betamethasone in urine sample [6]. HPLC method has been reported for determinations of betamethasone in combination with cyanocobalamin and diclofenac sodium in pharmaceutical formulations [7] and betamethasone dipropionate and salicylic acid in pharmaceutical preparations [8]. Ion-paired RP-HPLC assay for the concurrent assay of betamethasone dipropionate and tolnaftate in combined semisolid formulation [10]. Determination of betamethasone and dexamethasone in plasma has been reported using fluorogenic derivatization and liquid chromatography [11]. Simultaneous analysis of betamethasone valerate and miconazole nitrate in cream [12] and determination of betamethasone in tablet dosage form [13] by densitometric method has also been reported.

Thermal degradation of betamethasone sodium phosphate under solid state and determination of the degradation products using LC–MS–NMR methods has been described [14]. A stability indicating RP-HPLC method has been described for simultaneous determination of salicylic acid, betamethasone dipropionate, and their related compounds in Diprosalic Lotion® [15], for assay of betamethasone and estimation of its related compounds [16] and separation of betamethasone from low level dexamethasone and other related compounds [17].

A method has been described for the determination of dexchlorpheniramine maleate [18] in human plasma by HPLC-MS. RP-HPLC with aqueous-organic and micellar-organic mobile phases has been reported to determine the correlation between hydrophobicity and retention data of several antihistamines including dexchlorpheniramine [19]. HPLC method has been reported for determination of optical purity of dexchlorpheniramine maleate with circular dichroism (CD) detector [20]. The use of the first and second derivatives of the ratio of the emission spectra with a zero-crossing technique has been reported for determination of mixture of dexamethasone, dexchlorpheniramine maleate and fluphenazine hydrochloride [21]. There is no reported method for the simultaneous determination of this combination of drugs.

According to ICH guidelines, stress testing is necessary to elucidate the inherent stability of the active substance. Susceptibility of the active substance to oxidation, hydrolysis, and photolysis has to be tested [22]. The hydrolytic degradation of a new drug in acidic and alkaline conditions can be studied by exposing the drug in 0.1 N HCl / NaOH for 1-7 days [23, 24]. If reasonable degradation is seen, testing can be stopped at this point. However, in case no degradation is seen under these conditions, the drug should be exposed to acid/alkali of higher strengths and for longer duration. Alternatively, if total degradation is seen after subjecting the drug to initial conditions, acid/alkali strength can be decreased along with decrease in the reaction temperature. To test for oxidation, it is suggested to use hydrogen peroxide in the concentration range of 3–30% [23]. The photochemical degradation should be carried out by exposure to direct sunlight for 24 h [24]. Thermal degradation can be study by exposing the drug substance in solid state to temperature of ≥ 70 °C [24].

Nowadays, HPTLC has become a routine analytical technique due to its advantages of reliability in quantification of analytes at micro and even in nanogram levels and cost

Figure 1. Structure of betamethasone [A]; dexchlorpheniramine maleate [B].
effectiveness. Due to the lower sample capacity of the HPTLC layer, the amount of sample applied to the layer is reduced. A further advantage is that the compact starting spots allow an increase in the number of samples which may be applied to the HPTLC plate. Simultaneous assay of several components in a multicomponent formulation is possible [25, 26]. As there is no official or reported analytical method for the simultaneous determination of the two drugs, the objective of this study was to develop and validate a stability indicating HPTLC-densitometry method for simultaneous determination of betamethasone and dexchlorpheniramine maleate combination in commercial tablet dosage form.

2. Experimental

2.1. Materials

2.1.1. Chemicals

Analytical grade methanol (Sigma- Aldrich, Germany) and ethyl acetate (BDH, England) were purchased from Pharmaceuticals Fund and Supply Agency, Ethiopia. Celestamine® tablet (0.25mg betamethasone/2mg dexchlorpheniramine maleate, Sharing-Plough, France) was purchased from pharmacy retail outlet in Addis Ababa. Working standards of betamethasone (98.7% purity) and dexchlorpheniramine maleate (99.7% purity) (Sharing-Plough, France) were obtained from Food, Medicine and Health Care Control Authority FMHACA, Ethiopia.

2.1.2. Instruments

Micro syringe (Linomat syringe 659.004), linomat 5 applicator, twin trough chamber 20x10cm, UV chamber, TLC scanner III, winCATS version 1.4.0 software (all from Camag, Muttenz, Switzerland), precoated aluminum backed HPTLC plates (silica gel 60 F-254, 20 x 20 cm with 200 μm, thickness, Merck, Germany) and hair dryer (Philip lady 1000, Type HP 4312, Hong Kong) were used in the study.

2.2. Method

2.2.1. Preparation of Standard Solutions

(i) Stock Standard Solution

10mg of betamethasone and 80mg dexchlorpheniramine maleate were weighed and transferred to 100 ml volumetric flask and dissolved in 50 ml methanol. Then, it was diluted to the volume with methanol to obtain a concentration of 0.1 mg/ml and 0.8 mg/ml of betamethasone and dexchlorpheniramine maleate respectively. The solution was kept at room temperature until used.

(ii) Calibration Standard Solutions

Fifteen different concentration levels of calibration standard solutions were freshly prepared by diluting 1-15 ml of the stock standard solution with 1ml interval to 25 ml in appropriate volumetric flasks using methanol.

2.2.2. Preparation of Test Solutions

30 Celestamine® tablets whose mean weight was determined were finely powdered. Powder equivalent to 6.25 mg of betamethasone and 50 mg of dexchlorpheniramine maleate was transferred into a 50 ml volumetric flask containing 25 ml methanol, sonicated for 30 min and diluted to volume with methanol. The resulting solution was filtered using Whatman No 42 filter paper. Supernatant containing 1mg/ml of dexchlorpheniramine maleate and 0.125 mg/ml of betamethasone was used as stock solution.

2.2.3. Sample Application and Chromatogram Development

The sample was applied in the form of band with a length of 6 mm using Camag Linomat 5 sample applicator and 100 µl Camag syringe, 10 mm from the bottom and 10 mm from the side edges of the plate. The band was dried with the aid of an online nitrogen gas. 20 x10 cm twin trough Camag chamber containing 16 ml of the selected solvent was used for the development. Ascending mode of development was employed throughout the course of the experiment. The optimized chamber saturation time for mobile phase was 15 min at room temperature (25°C ± 2) at relative humidity of 60 % ± 5. The length of chromatogram run was 8 cm that was achieved in about 30 min. Subsequent to development; chromatograms were dried in a current of warm air with the help of hair dryer for 20 min.

2.2.4. Selection of Mobile Phase

The mobile phase composition is generally selected by a controlled process of trial and error. The mobile phase should be chosen taking into consideration the chemical properties of analytes and the sorbent layer. After these experiments the solvents giving the most appropriate separations are chosen for further optimization of the mobile phase. At the optimization level mixtures of solvents from different selectivity groups are investigated by adjusting strength, if required. At this level addition of small amounts of acidic or basic modifier can be considered and tested whenever this appears promising.

2.3. Validation of the Developed Method

2.3.1. Determination of Linearity

Linearity relationship between peak area of the spots and concentration of the drugs were evaluated over a range of concentrations (ng/band). Fifteen levels of standard solutions were prepared as described in section 2.2.1.2. From these serial dilutions, solutions with concentrations of 0.004 mg/ml to 0.056 mg/ml with a variation of 0.004 mg/ml for betamethasone and between 0.032 mg/ml to 0.48 mg/ml with a variation of 0.032 mg/ml for dexchlorpheniramine maleate were obtained. 3 µl of these solutions were applied to the plates to get 12.5-187.5 ng/band of betamethasone and 100-1500 ng/band of dexchlorpheniramine maleate.

2.3.2. Precision Studies

Repeatability and intermediate precision were studied by taking a concentration within the linearity range at three
by applying the method on drug sample to which known
dexchlorpheniramine maleate was assessed by comparing the
standard. The peak purity of betamethasone and
end (E) positions of the spot.
spectra at three different levels, i.e., peak start (S), peak apex
and peak end (E) positions of the spot.
five minutes and from chromatography to scanning was
varied from 20 ± 5 minutes. Robustness of the method was
checked by analyst variation. For all robustness studies, three
corresponding to 80, 100 and 120 % of label claim had been
added.

2.3.4. Robustness
By introducing small changes in the mobile phase
composition, the effects on the results were examined.
Mobile phases having different composition like ethyl
acetate–methanol–ammonia (1.8: 13.0: 1.0 ± 0.1 v/v/v); (2.2:
13.0: 1.0 ± 0.1 v/v/v); (2.0: 11.7: 1.0 ± 0.1 v/v/v); (2.0: 14.3:
1.0 ± 0.1 v/v/v); (2.0: 13.0: 0.9 ± 0.1 v/v/v) and (2.0: 13.0:
1.1 ± 0.1 v/v/v) were tried and chromatograms were run. The
volume of mobile phase (16ml) was varied in the range of ±
5%. Time from spotting to chromatography was varied from
10 ± 5 minutes and from chromatography to scanning was
varied from 20 ± 5 minutes. Robustness of the method was
checked by analyst variation. For all robustness studies, three
congestion levels; 50, 75, 100 ng/spot and 400,
600, 800 ng/spot for betamethasone and dexchlorpheniramine maleate, respectively were used.

2.3.5. Detection and Quantitation Limits
The detection and quantitation limits of the developed
method were calculated using the following formulae LOD =
3.3 × SD/S and LOQ = 10 × SD/S where ‘SD’ is the standard
declaration of the y-intercepts of the regression lines
constructed as described in section 2.3.1. The blank
response and ‘S’ is the slope of the calibration plot.

2.3.6. Specificity
Specificity of the method was ascertained by analyzing
standard drug and sample. The spot for betamethasone and
dexchlorpheniramine maleate in sample was confirmed by
comparing the Rf and the UV spectra of the spot with that of
standard. The peak purity of betamethasone and dexchlorpheniramine maleate was assessed by comparing the
spectra at three different levels, i.e., peak start (S), peak apex
(M) and peak end (E) positions of the spot.

2.4. Sample Solution Stability Study
Solutions having three different concentrations (50, 75
and 100 ng/spot for betamethasone) and (400, 600 and 800 ng/spot for dexchlorpheniramine maleate) were prepared from
reference solution as described in section 2.2.1.2 and stored at
room temperature for 2.0, 4.0, 6.0, 12.0 and 24 hrs.

2.5. Analysis of Dosage Form
For determination of betamethasone and
dexchlorpheniramine maleate in commercial tablet dosage
form, sample solution prepared as described in section 2.2.2
were used. From this solution, 1 ml was transferred to a 10ml
volumetric flask and diluted to volume with methanol. 6 μl
from the diluted solution was spotted in triplicate.
Betamethasone and dexchlorpheniramine maleate content
was determined from peak area of densitogram. The
 calibration curves, constructed as described in section 2.3.1
were used for determination of the actual contents of the two
substances in the dosage form.

2.6. Stress Degradation Studies
All degradation studies were carried out at a drug
concentration of 1mg/ml. Acid (1N) and alkaline (1N)
hydrolysis and oxidation by 3 and 30 % H2O2 was done by
refluxing the drug solutions in water bath at 70°C for 6 hrs.
Thermal degradation studies were carried out by exposing the
powdered drugs to dry heat at 105°C for 24 hrs. Photo
degradation studies were performed by exposing the
powdered drugs to direct sun light for 3 days. Samples for
each treatment were subjected to HPTLC analysis at a
concentration of 1000 ng/spot.

3. Results and Discussion
3.1. Method Development
Well resolved and sharp peaks for betamethasone (Rf =
0.32 ± 0.04) and dexchlorpheniramine maleate (Rf = 0.76 ±
0.05) were obtained [Figure 2], when ethyl acetate: methanol:
ammonia (2: 13: 1 v/v/v) was used as a mobile phase. The
solvent system composed of dichloromethane: methanol:
ammonia (3: 13: 1 v/v/v) showed good resolution for the two
spots but the spot for dexchlorpheniramine maleate appeared
near the solvent front while propanol: methanol: ammonia (6:
14: 1 v/v/v) showed small migration distance for
betamethasone from the application point. Good separation
was observed in the solvent system methanol: ethanol:
ammonia (12: 4: 1 v/v/v) but a tailed spot was observed for
betamethasone. Quantitative determinations of
betamethasone and dexchlorpheniramine maleate were
performed by scanning the spots of the two substances at 226
nm [Figure 3]. The selected and optimized mobile phase was
let to saturate the chamber for 20minutes. The plate was then
allowed to dry for 20minutes using hair dryer.

3.2. Linearity and Calibration Curves

Linearity was found over the concentration range of 25 -
137.5 ng/spot with r² ± RSD of 0.997 ± 0.00102 for
betamethasone and 100-800 ng/spot with r² ± RSD of 0.999 ±
0.141 for dexchlorpheniramine maleate. Peak area and
concentration were subjected to least square linear regression
analysis to obtain the calibration equation and correlation coefficients [Table 1].

**Table 1.** Linear regression data for the calibration curves (n = 5)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Betamethasone</th>
<th>Dexchlorpheniramine maleate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linearity range</td>
<td>25 - 137.5 ng/spot</td>
<td>100-800 ng/spot</td>
</tr>
<tr>
<td>$r \pm$ RSD</td>
<td>0.998 ± 0.00102</td>
<td>0.999 ± 0.0141</td>
</tr>
<tr>
<td>Slope ± RSD</td>
<td>15.17 ± 0.14</td>
<td>2.303 ± 0.836</td>
</tr>
<tr>
<td>Intercept ± RSD</td>
<td>271.0 ± 2.5</td>
<td>309.7 ± 4.44</td>
</tr>
<tr>
<td>Confidence limit of slope a</td>
<td>15.17 ± 0.133</td>
<td>2.303 ± 0.037</td>
</tr>
<tr>
<td>Confidence limit of intercept a</td>
<td>271.0 ± 0.21</td>
<td>309.7 ± 26.45</td>
</tr>
</tbody>
</table>

*a* 95% confidence limit.

**Figure 2.** Typical densitogram of betamethasone [1] and dexchlorpheniramine maleate [2].

**Figure 3.** The UV spectral display of dexchlorpheniramine maleate [1] and betamethasone [2].
3.3. Method Validation

3.3.1. Precision

Repeatability and intermediate precision of the developed method were expressed in terms of RSD of the peak area. The results showed that intra- and inter day variations of the peak areas at three different concentration levels of 50, 75, and 100 ng/spot for betamethasone and 400, 600, and 800 ng/spot for dexchlorpheniramine maleate [Table 2] were within the acceptable range. The low RSD values indicate the repeatability of the method.

3.3.2. Recovery Studies

The proposed method when used for extraction and subsequent estimation of betamethasone and dexchlorpheniramine maleate from pharmaceutical dosage forms after spiking with 80, 100 and 120 % of additional drug afforded a recovery rate of 99.70–100.44 % for betamethasone and 99.73-100.49 % for dexchlorpheniramine maleate (Table 3).

3.3.3. Specificity

The results for betamethasone were found to be peak start, peak apex \([r^2 = 0.9999]\), and peak apex and peak end positions \([r^2 = 0.9997]\) while for dexchlorpheniramine maleate the results were found to be peak start, peak apex \([r^2 = 0.9999]\), and peak apex and peak end positions \([r^2 = 0.9997]\). Good correlation i.e. \(r^2 = 0.9996\) and \(r^2 = 0.9997\) was also obtained between standard and sample spectra of betamethasone and dexchlorpheniramine maleate, respectively.

![Figure 4](image-url). *UV spectra comparison of the spots of the standards [2 & 3] and dosage form [1& 4] for betamethasone [*] and dexchlorpheniramine maleate [**].*
3.3.4. Limit of Detection and Quantification

The limit of detection and quantification of the developed method were calculated as in section 3.4.4 and it was found to be 2.49 and 7.54 ng/spot respectively for betamethasone. For dexchlorpheniramine maleate the limit of detection and quantification were found to be 19.51 and 60.70 ng/spot respectively.

3.3.5. Robustness

Standard deviation of peak areas was calculated for each parameter and RSD was found to be less than 2 %. The low RSD values as shown in Table 4 indicate robustness of the method.

Table 4. Results for robustness study of the method (p = 0.05, n = 6; tstat = 4.3)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Betamethasone</th>
<th>Dexchlorpheniramine maleate</th>
</tr>
</thead>
<tbody>
<tr>
<td>SD*</td>
<td>RSD*</td>
<td>t cal</td>
</tr>
<tr>
<td>SD*</td>
<td>RSD*</td>
<td>t cal</td>
</tr>
<tr>
<td>Optimized solvent system</td>
<td>14.5</td>
<td>0.53</td>
</tr>
<tr>
<td>1.8:13.0:1.0</td>
<td>28.7</td>
<td>0.85</td>
</tr>
<tr>
<td>2.2:13.0:1.0</td>
<td>14.1</td>
<td>0.41</td>
</tr>
<tr>
<td>Mobile phase composition (v/v/v)</td>
<td>13.2</td>
<td>0.31</td>
</tr>
<tr>
<td>2.0:13.0:1.0</td>
<td>23.0</td>
<td>0.29</td>
</tr>
<tr>
<td>2.0:13.0:0.9</td>
<td>12.3</td>
<td>0.22</td>
</tr>
<tr>
<td>2.0:13.0:1.1</td>
<td>22.1</td>
<td>0.21</td>
</tr>
<tr>
<td>2.1:13.6:1.1(16.8ml)</td>
<td>22.1</td>
<td>0.21</td>
</tr>
<tr>
<td>Volume of mobile phase (v/v/v)</td>
<td>13.1</td>
<td>0.22</td>
</tr>
<tr>
<td>Optimized time (10min)</td>
<td>12.1</td>
<td>0.41</td>
</tr>
<tr>
<td>15min</td>
<td>23.0</td>
<td>0.22</td>
</tr>
<tr>
<td>20min</td>
<td>24.7</td>
<td>0.34</td>
</tr>
<tr>
<td>Optimized time (20min)</td>
<td>16.5</td>
<td>0.12</td>
</tr>
<tr>
<td>25min</td>
<td>23.2</td>
<td>0.31</td>
</tr>
<tr>
<td>30min</td>
<td>12.3</td>
<td>0.21</td>
</tr>
<tr>
<td>Principal analyst</td>
<td>16.7</td>
<td>0.67</td>
</tr>
<tr>
<td>Analyst one</td>
<td>22.1</td>
<td>0.53</td>
</tr>
<tr>
<td>Analyst two</td>
<td>35.4</td>
<td>0.25</td>
</tr>
</tbody>
</table>

3.3.6. Application of the Method for Marketed Formulation

Analysis of the marketed formulations was performed in triplicate and results are presented in Table 5. The drug content was found to be 98.20 % of the claimed value (RSD = 0.228) and 101.60 % (RSD = 0.164) for betamethasone and dexchlorpheniramine maleate, respectively. The low % RSD values indicated suitability of the developed method for routine simultaneous determination of betamethasone and dexchlorpheniramine maleate in pharmaceutical dosage forms.

Table 5. Data for analysis of the marketed dosage form (Celestamine®) using the developed method

<table>
<thead>
<tr>
<th>Substance</th>
<th>Average peak area ± SD</th>
<th>% RSD</th>
<th>Amount recovered ± SD</th>
<th>% Recovery ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betamethasone a</td>
<td>1382.17 ± 3.15</td>
<td>0.23</td>
<td>73.69ng ± 0.21</td>
<td>98.20% ± 0.21</td>
</tr>
<tr>
<td>Dexchlorpheniramine maleate b</td>
<td>1684.13 ± 2.77</td>
<td>0.16</td>
<td>609.78ng ± 1.26</td>
<td>101.60% ±1.26</td>
</tr>
</tbody>
</table>

a = 75 ng/band, b = 600 ng/band

3.4. Forced Degradation Studies

Densitograms obtained for the drugs treated with acid, base, hydrogen peroxide, and heat contained well resolved spots for the pure drugs and the degradation products. The Rf values of the parent drugs (betamethasone and dexchlorpheniramine maleate) were not significantly changed from the original position in the presence of the degradation products, which showed the stability-indicating nature of the method.

3.4.1. Degradation Under Acidic Conditions

Betamethasone was degraded under acidic condition and showed three degradation products at Rf of 0.19, 0.25 and 0.36 in addition to the one for the original compound at Rf = 0.32 as shown in Figure 5. No degradation product was observed under acidic condition for dexchlorpheniramine maleate.
3.4.2. Degradation Under Alkaline Conditions
Betamethasone was degraded in alkaline condition and showed an additional spot at $R_f = 0.36$ as shown in Figure 6.

![Figure 6. Densitogram for betamethasone [A] and synthetic mixture [B] under alkaline induced degradation, mobile phase, ethyl acetate: methanol: ammonia 2: 13: 1, v/v/v, scanned at 226 nm.](image)

There was no degradation product for dexchlorpheniramine maleate under the alkaline conditions.

3.4.3. Degradation Studies Under Oxidative Condition
Betamethasone and dexchlorpheniramine maleate when exposed to 3 % $H_2O_2$ showed no degradation product. When the drug solutions were exposed to 30 % $H_2O_2$, betamethasone showed one degradation product at $R_f = 0.36$ and dexchlorpheniramine maleate had one additional peak that appeared at $R_f = 0.67$ [Figure 7].
3.4.4. Thermal Degradation

Betamethasone was degraded when subjected to heat for 24 hrs and degradation products appeared at $R_f = 0.26$ and $R_f = 0.36$ as shown in Figure 8. No degradation was observed under thermal stress for dexchlorpheniramine maleate.

Figure 7. Densitogram for betamethasone [A], dexchlorpheniramine maleate [B] and synthetic mixture [C] under oxidative degradation condition by 30% $H_2O_2$, mobile phase, ethyl acetate: methanol: ammonia 2:13:1, v/v/v, scanned at 226 nm

Figure 8. Densitogram for betamethasone under thermal induced degradation. (Mobile phase, ethyl acetate: methanol: ammonia 2:13:1, v/v/v, scanned at 226nm)
3.4.5. Photolytic Conditions

The photo degradation study conducted showed no degradation product for the two drug substances. The drugs were found to be stable on exposure to day light continuously for three days.

4. Conclusion

The developed HPTLC technique is rapid, precise, specific, robust, accurate and stability-indicating. Statistical analysis proved that the method is reproducible and selective for simultaneous determination of betamethasone and dexchlorpheniramine maleate in pharmaceutical formulations. As the method could effectively separate the drugs from their degradation products it can be employed as a stability indicating one.

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References


