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Stability indicating HPTLC method for the estimation of estradiol☆

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Abstract

Estradiol (ESD) is widely used in post climacteric replacement therapy. Most of the methods used for quantitation are expensive and time consuming. A rapid, selective and precise stability indicating high performance thin layer chromatography method was developed and validated for the estimation of ESD in bulk and pharmaceutical dosage forms. The method employed TLC aluminium plate precoated with silica gel $60F_{254}$ as the stationary phase. The solvent system employed consisted of chloroform–acetone–isopropyl alcohol–glacial acetic acid (9:1:0.4:0.1, v/v/v/v). Such a complex system was essential to obtain a dense and compact spot of the drug at an *Rf* value of 0.40 ± 0.02. The drug on intentional degradation gave two products with *Rf* values of 0.52 ± 0.01 and 0.58 ± 0.01 respectively. Spectrodensitometric scanning-integration was performed on a Camag system using a wavelength of 286 nm. The polynomial regression data for the calibration plots exhibited good linear relationship ($r = 0.9947$) over a concentration range of 1–8 μg. Recovery studies were also performed at three experimental levels. The recovery data reveals that the RSD for intra-day and inter-day analysis was found to be 1.27% and 1.75%, respectively. The proposed method was found to be stability indicating. Statistical analysis proves that the method is precise, accurate and reproducible, hence can be employed for the routine analysis of the drug.

Keywords: Estradiol; HPTLC; Stability indicating

1. Introduction

Estradiol (ESD), a potent estrogenic hormone, finds wide utility in post-climacteric replacement therapy [1]. It is administered alone or in combination with other hormones by the oral, transdermal, subcutaneous and intravaginal routes in a variety of dosage forms. Literature reveals a variety of analytical methods for its estimation in pharmaceutical dosage forms as well as biological fluids. The powerful combination of gas or liquid chromatography and mass spectrometry is used to detect and assay ESD in biological fluids [2]. Also, several liquid chromatographic systems [3–5], gas

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chromatography [6–8], radioimmunoassay [9–11] and chemiluminescence immunoassays [12] have been developed. Although these methods have their own advantages, they are often time-consuming, expensive and cumbersome.

Over the past decade, high performance thin layer chromatography (HPTLC) has been successfully used in the analysis of pharmaceuticals, plant constituents and biomacromolecules [13–16]. The advantage of this method is that a large number of samples can be simultaneously analysed. The proposed method utilises less quantities of solvents and the time taken for analysis is considerably reduced in comparison to the other documented methods. The primary objective was to develop and validate a stability indicating HPTLC method for ESD which could also be employed for the routine analysis of drug in pharmaceutical dosage forms.

2. Experimental

2.1. Materials

ESD was received as a gift sample from Cipla, India. All chemicals and reagents employed were of analytical grade and were purchased from Ranbaxy Chemicals, India.

2.2. Instrumentation

Narrow bands of 3 mm in width were spotted using a Camag microlitre syringe on precoated silicagel aluminium plate 60 F-254 (10×10 cm with 250 micron thickness; E. Merck, Germany) using a Camag Linomat IV (Switzerland). A constant application rate of 15 s/μl was employed. The solvent system consisted of chloroform–acetone–isopropyl alcohol–glacial acetic acid (9:1:0.4:0.1, v/v/v/v). The chromatogram was developed in a Camag twin trough glass chamber using a linear ascending technique. The chamber saturation time for mobile phase was optimized to 30 min. The length of chromatogram run was set to 9 cm. Subsequent to the development, the TLC plates were dried in a current of air with the help of an air dryer.

The densitometric analysis was performed on a Camag TLC scanner II using 286 nm. Densitograms were obtained by integration performed using a Perkin Elmer integrator system LCI-100.

2.3. Calibration plots

A series of standard curves were prepared over a concentration range of 1–8 μg from a stock solution of ESD (1 mg/ml) in methanol. The procedure for the same is discussed in Section 2.2. The standard curves were evaluated for intra-day and inter-day reproducibility. Each experiment was repeated in triplicate.

2.4. Analysis of marketed formulation

The transdermal patch area corresponding to 1 cm^2 containing 0.4545 mg of ESD, was subjected to extraction in methanol. To ensure complete extraction of the drug it was sonicated for 15 min and the solution made up to 10 ml. The resulting solution was filtered and the filtrate was analysed for the drug content. One hundred microliters of the solution was spotted onto the plate followed by development and scanning as described in Section 2.2. The analysis was repeated in triplicate. The marketed formulation excipients included co-polymer ethylene vinyl acetate, dipropylene glycol, octyldodecanol and ethyl cellulose. A placebo patch was subjected to the same extraction process as discussed above and spotted. The possibility of excipient interference in the analysis was studied.

2.5. Method validation

Reproducibility and recovery studies were performed in a view to justify the accuracy, suitability as well as precision of the proposed method. A concentration within the linearity range (4 μg) was selected and analysed six times. This assay was repeated six times. For recovery, known quantities of previously analysed reference standard corresponding to 50, 100 and 150% of the label claim, were added during the extraction procedure. The extraction solvent employed was methanol. Samples were analysed in the same way

as described in Section 2.2. Each level was repeated in triplicate. The intra-day and inter-day variation was studied and the percent recovery was computed.

In order to estimate the limit of detection and limit of quantitation, blank methanol was spotted six times following the same method explained in Section 2.2. The noise level was determined. The limit of detection was calculated to be three times the S.D. and ten times the S.D. value gave the limit of quantitation. The ruggedness of the proposed method was studied using reagents from different lots and different manufacturers.

The stability of ESD in solution state was also assessed. Accurately weighed quantity of the pure drug was dissolved in methanol and suitably diluted to get a final concentration of 1 mg/ml. The solution was subjected to HPTLC analysis immediately and after a period of 6, 12, 24, 48 and 72 h.

2.6. Stability indicating method

The drug was subjected to forced degradation under acidic conditions (1 M methanolic HCl) and basic conditions (1 M methanolic NaOH) by heating at 70°C for 2 h. The resultant solutions were further neutralised and spotted after suitable dilution. The chromatogram was run as described in Section 2.2. The photochemical stability of the drug was also studied by exposing ESD to UV radiations (wavelength 254 nm) for 24 and 48 h. Furthermore, a stock solution of the drug was prepared, suitably diluted and spotted.

3. Results and discussion

3.1. Optimization of chromatogram

Initial trial experiments were conducted, in a view to select a suitable solvent system for the accurate estimation of the drug. Chloroform–acetone in varying ratios (1:1, 6:4, 7:3, 8:2 and 9:1, v/v) were tried. However a diffused spot was obtained in all the cases. To improve the compactness of the spot, glacial acetic acid was incorporated in varying concentrations (0.1–0.5% v/v). The ratio of chloroform–acetone was kept constant at 9:1, v/v. Addition of the acid gave a less diffused spot and had no appreciable influence on the *Rf* value. Isopropyl alcohol in the range of 0.1–0.5% v/v was further tried to improve spot characteristics. A minimum concentration of 0.4% v/v was essential to obtain a compact spot. Hence, a solvent system of chloroform–acetone–isopropyl alcohol–glacial acetic acid (9:1:0.4:0.1, v/v/v/v) was selected as the optimum mobile phase for the development of the chromatogram. The solvent system gave a dense and compact spot with an *Rf* value of 0.40 ± 0.02 ($n = 6$). It was observed that well defined spots of the drug under study were obtained when the chamber was equilibrated with the mobile phase for a period of 30 min.

3.2. Calibration plots

The peak area versus drug concentration was plotted to construct a standard curve of ESD. The polynomial regression for the calibration plots showed good linear relationship with coefficient of correlation $r = 0.9947 \pm 0.00056$; slope $= 149372.03 \pm 418.49$ and intercept $= 241279.46 \pm 9062.41$ ($n = 6$) over the concentration range studied. The range of reliable quantification was set at 1–8 μg as no significant difference (ANOVA; $P > 0.05$) was observed in the slopes of the standard curves in this range. The RSD for within day and day-to-day analysis was found to be $< 2\%$ in all the cases.

3.3. Marketed product analysis

Table 1 depicts the results of marketed product analysis. The RSD for the studies was found to be 1.61% for intra-day and 1.38% for inter-day analysis. This indicated the suitability of the method for routine analysis in pharmaceutical dosage forms. The transdermal excipient components gave a diffuse spot near the point of spotting. The spot was well separated from the that of the drug. Thus, excipient interference was not observed.

3.4. Validation

The precision of the method was expressed in terms of RSD. Excellent method precision was evident with a low RSD of 1.75%. The results of the recovery studies are shown in Table 2. The recovery data reveals that the RSD for intra-day and inter-day variation was found to be 1.27% and 1.75%, respectively.

The limit of detection was found to be 300 ng where the drug could be detected without any noise. The limit of quantitation was 900 ng. In the ruggedness study, the RSD for system precision and recovery studies was found to be 1.22% and 1.88% (for different lots of reagents) and 1.44% and 1.10% (for different manufacturers) respectively. The RSD for the samples analysed at different elapsed assay times (6, 12, 24, 48 and 72 h) was found to be $< 2\%$. Thus, the drug is stable in solution state.

Table 1
Marketed product: ESD content analysis[a]

Label claim (μg)	Experimental content (μg)	% Recovery avg ± S.D.
0.4545 mg/sq.cm	Within day 0.4545 mg/cm^2	99.3 ± 1.6
	Day-to-day 0.4545 mg/cm^2	100.2 ± 1.3

[a] $n = 6$

Table 2
Recovery studies of marketed transdermal formulation of ESD[a]

Experimental level	Theoretical content (mg)	% Recovery (avg ± S.D.)
Level 1	Within day 2.5 mg	100.9 ± 1.2
	Inter day 2.5 mg	99.4 ± 1.7
Level 2	Within day 5.0 mg	98.1 ± 1.1
	Inter day 5.0 mg	101.2 ± 1.5
Level 3	Within day 7.5 mg	99.0 ± 1.2
	Inter day 7.5 mg	100.1 ± 1.6

[a] $n = 6$

3.5. Stability indicating method

Steroidal molecules are stable in basic conditions. Treatment with 1 M methanolic NaOH, results in the formation of a phenoxide ion which has a tendency to revert back to the original moiety when neutralised, hence spot of the original drug was obtained at a *R*f of 0.40 ± 0.02. However, steroids are prone to elimination (dehydration) in acidic conditions [17]. In the presence of an acid, protonation of the 17-β-hydroxyl group occurs, followed by the loss of a water molecule. This would further result in elimination of a proton at C-16 position leading to the formation of a double bond at C_{16-17} position (degradation product I). Furthermore this product could undergo a possible rearrangement to give a double bond at C_{15-16} position (degradation product II). Thus, the two spots corresponding to the degraded components obtained after acidic degradation, can be attributed to these two alkenes (degradation product I and II) with not much significant difference in the *Rf* values (0.52 ± 0.01 ($n = 6$) and 0.58 ± 0.01 ($n = 6$)). These compounds being more non polar in nature have a *Rf* value higher as compared to the pure drug. The products may also undergo racemization in acidic conditions. The separation of the pure drug from it's degradation counterparts is depicted in Fig. 1. The structures of the proposed degradation products are given in Fig. 2.

In case of photochemical stability, no spot other than the pure drug was obtained, indicating the stability of drug to UV light.

As the proposed method could effectively separate the drug from its degradation products, it can be employed as a stability indicating one.

4. Conclusion

The proposed HPTLC method of analysis is selective. Statistical data analysis (ANOVA)

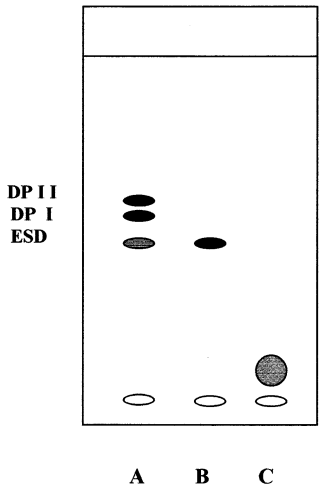
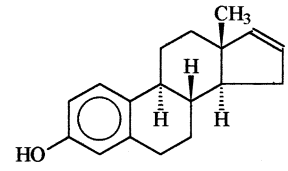
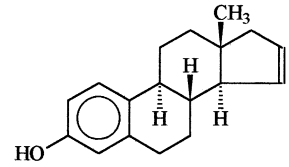


Fig. 1. Schematic representation of separation of ESD from corresponding degradation products and formulation excipients on TLC plate (after development). A, Drug + degraded; B, pure drug (ESD); and C, placebo formulation. Drug (ESD) ($Rf = 0.40 \pm 0.02$); Degradation Product I (DP I) ($Rf = 0.52 \pm 0.01$); and Degradation Product II (DP II) ($Rf = 0.58 \pm 0.01$).

proves that the method is reproducible and precise. The method is economical and can be employed for the routine analysis of the drug in pharmaceutical formulations as well as estimation in bulk drug.



Degraded product I



Degraded product II

Fig. 2. Proposed degradation products of ESD after forced degradation in acidic medium.

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