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ORIGINAL ARTICLE

Determination of Exemestane in bulk and pharmaceutical dosage form by HPTLC

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Abstract An HPTLC method for analysis of Exemestane in bulk and pharmaceutical formulation has been established and validated. The analyte was separated on aluminium plates precoated with silica gel 60 F_{254}. The mobile phase was chloroform:methanol 9.2:0.8 (v/v). Quantification was done by densitometric scanning at 247 nm. Response was a linear function of Exemestane concentration in the range of 100–500 μg mL^{-1}. The limit of detection and quantification for Exemestane were 5.8 and 17.58 μg mL^{-1}, respectively. Average recovery of Exemestane was 100.1, which shows that the method was free from interference from excipients present in the formulation. The established method enabled accurate, precise, and rapid analysis of Exemestane in bulk as well as pharmaceutical formulation.

1. Introduction

Exemestane is an orally active irreversible steroidal aromatase inhibitor used for the therapy of metastatic postmenopausal breast cancer, with estrogen-dependent pathological conditions (Johannessen et al., 1997; Evans et al., 1992; Geisler et al., 1998). The chemical name of Exemestane is 10,13-dimethyl-6-methylidene-7,8,9,10,11,12,13,14,15,16-decahydrocyclopenta[a] phenanthrene-3,17-dione (Fig. 1).

Literature survey reveals that several methods were reported for the estimation of Exemestane. The determinations of Exemestane in biological samples using HPLC (Persiani et al., 1996), mass (Allievi et al., 1995) and liquid chromatography–mass spectrometry (Cenacchi et al., 2000) is also reported. To the best of our knowledge no official report for the determination of Exemestane in bulk material and tablets has ever been published by densitometry. Thus, we thought that it would be of particular interest to develop and validate an HPTLC method for the quality control of Exemestane in the pharmaceutical preparations that would also reduce the sample throughput. The proposed method can be applicable for routine analysis and content uniformity test of Exemestane in tablets and complies well with the validation requirements in the pharmaceutical industry. The developed method is validated according to ICH guidelines (see Fig. 2).

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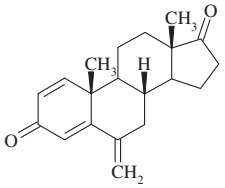

Figure 1 The structure of Exemestane.

2. Experimental

2.1. Materials

The tablet formulation was procured from local pharmacy (Sun Pharmaceuticals Ltd., India). Pure Exemestane were obtained from (Cipla Ltd., Mumbai, India). All chemicals were of analytical grade. All dilutions were performed in standard volumetric flasks.

2.2. Instrumentation

HPTLC system—Desaga HPTLC system with densitometer CD 60 and auto sampler model AS 30 (Desaga, Wiesloch, Germany). Twin tough developing chamber (Desaga) 200×100 mm, Mettler analytical balance (Mettler-Toledo, Columbus, Ohio), and model AE 240 (accuracy ± 0.01 mg) Ultrasound cleaning bath.

2.3. Stock and working standard solution

Exemestane (25 mg) was accurately weighed into a 25 mL volumetric flask, and dissolved in a minimum volume of water, and diluted to required volume with water to furnish a solution of concentration 1000 μg mL^{-1}. This was used as stock solution.

Calibration standards were prepared over the concentration range 100–500 μg mL^{-1} for Exemestane by appropriate dilutions of the above mentioned standard stock solution in 10 mL volumetric flask with methanol.

2.4. Sample preparation

Twenty tablets were weighed and the average weight was calculated. The tablets were then powdered and amount equivalent to 25 mg of pure Exemestane tablet was dissolved in a minimum volume of methanol. This was then filtered through Whatman filter paper number 41 and filtrate was collected in a 10 mL volumetric flask and diluted to volume with methanol to get stock solution of concentration of 100 μg mL^{-1}. Similarly various required strength dilutions of the tablet sample solution is done and analyzed by proposed method and concentration was calculated by using the calibration curve.

2.5. Calibration curve

Separate stock standard solutions of Exemestane were used for the preparation of calibration standard solutions. All calibration standards were prepared freshly every day and found to be stable during the analysis time. The plate was developed, dried, and scanned as described above. After densitometric scanning the peak area was recorded for each concentration and a calibration plot was obtained by plotting average peak area against concentration of Exemestane (ng $spot^{-1}$). The slope and correlation coefficient were also determined.

2.6. Standard chromatographic HPTLC conditions

Analysis was performed on silica gel 60 F_{254} plates of size 200×100 mm. Different volumes of the samples (experimental sample solution and reference standard solution) were applied on the plate separately or together with standard drug solution. Samples (5.00 μL) were applied as 5 mm bands by means of Desaga auto sampler automatic applicator (model AS30) equipped with 10 μL syringe at different positions (lane) on the HPTLC plate under N_2 gas stream. Application parameters were the length of the band is 5 mm, distance between bands was 10 mm, the application position along Y axis was 15 mm, and the start position along X axis was 15.0 mm. Each spot was given by a 1 μL/cycle with application rate of 0.1 μL/s. Linear ascending development is carried out in twin trough glass chamber (200×100 mm) covered with stainless steel lid. The optimized chamber saturation time for mobile phase was 25 min at room temperature and the plates were developed up to 80 mm from the bottom of the plate using the solvent systems chloroform:methanol 9.2:0.8 (v/v) as a mobile phase. The average development time was 20 min. After development

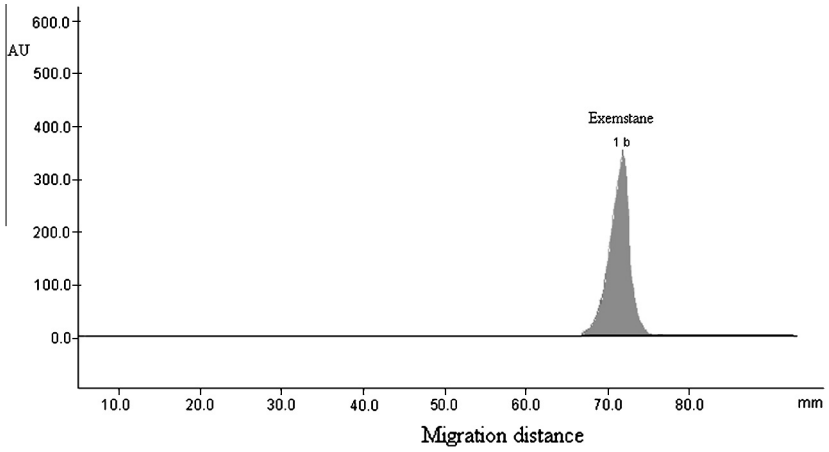

Figure 2 HPTLC densitogram obtained from Exemestane.

the plate was air-dried for 15 min. Optical Densitometric scanning at $\lambda = 247$ nm was performed. After densitometric scanning, chromatograms were evaluated via peak area. Scanned peak areas were recorded for each sample at each concentration level. In assay experiment six samples were weighed separately and analyzed. For each sample, each concentration was applied six times to the plate at each concentration level. These developed plates are scanned by performing six replicate measurements of peak areas of the six bands of same concentration. The average peak areas, variations in peak area obtained were expressed as a coefficient of variation (% CV) and concentration of the drug present were automatically calculated by comparing the peak area values of the sample with that of the reference standard. The plot was obtained by plotting average peak area at each concentration against the corresponding band concentrations of Exemestane (ng spot^{-1}). From the constructed calibration curve slope, correlation coefficient was calculated. The % CV of six independent assays values is calculated. From amount of drug present in sample, % assay of Exemestane was calculated.

2.7. Validation

The proposed method was validated according to the ICH guidelines by carrying out analysis of six replicate samples of the tablets. The method was validated for accuracy, precision, robustness, linearity and range, selectivity, limit of detection, limit of quantification and robustness.

2.7.1. Linearity

The linearity of the method was evaluated by analysis of the five standard solutions at five different concentrations from 100 to 500 μg mL^{-1}, prepared from the stock solution. At each concentration level these solutions (1 μL) were applied to plates and then these plates were developed, scanned as described above. Peak area was recorded for Exemestane. Areas under the curve and drug concentration were subjected to regression analysis to calculate the regression equations and correlation coefficients.

2.7.2. Limit of detection and limit of quantification

The limits of detection (LOD) and quantification (LOQ) were estimated experimentally and mathematically using formulae:

$$\text{LOD} = 3:3 \times (\text{standard deviation of intercept/slope of the calibration plot})$$

$$\text{LOQ} = 10 \times (\text{standard deviation of intercept/slope of the calibration plot})$$

LOD and LOQ values were experimentally verified by diluting known concentrations of a standard solution of Exemestane until the average responses were approximately 3/10 times the standard deviations of the responses for the six replicate determinations. The LOD and LOQ were determined from the graph of the lowest part of the calibration plot and found to be 5.8 and 17.58 ng spot^{-1}.

2.7.3. Precision

Repeatability studies were performed by the analysis of three different concentrations (200, 300 and 500 ng per band). Repeatability was determined by running minimum of six analyses per sample and evaluating the coefficient of variation (CV [%]) for each sample. Each concentration was applied six times to the HPTLC plate on the same day. The precision of the method as repeatability intra-day assay precision [CV %] was assessed by performing six independent analyses of sample and qualified reference standards together at 100% of the test concentration. Inter-day precision was determined by repeating the analysis of the same Exemestane tablet by repeating studies by three different analysts on three different days over a period of 1 week also expressed in terms of [%] CV.

2.7.4. Repeatability and reproducibility

Repeatability can be defined as the precision of the procedure when repeated by same analyst under the same operating conditions (same reagents, equipments, settings and laboratory) over a short interval of time. Repeatability was further confirmed by determination of CV [%] for standard addition at three concentrations levels. In assay experiment six samples were weighed separately analyzed and the results of independent assay of the 200, 300 and 500 ng per band given in table. Reproducibility means the precision of the procedure when it is carried out under different conditions.

2.7.5. Accuracy by recovery

Accuracy of the method was ascertained by performing recovery studies by the standard addition method at different levels, i.e., 0%, 80%, 100% and 120% by adding pure standard drug of Exemestane to previously analyzed tablet powder sample and mixtures were reanalyzed by the proposed method. From the amount of drug found percentage recovery was calculated. The experiment was conducted in triplicate.

2.7.6. Selectivity and specificity

The selectivity of an analytical method is its ability to measure accurately and specifically the analyte of interest in the presence of components that may be expected to be present in the sample matrix for example impurities, degradation products, and matrix components. A UV spectrum of Exemestane was initially obtained by scanning a developed plate in screening mode to select the detection wavelength. For each sample, UV spectra taken at the edges and maxima of the Exemestane peaks were compared automatically to verify peak purity. Specificity of the method was ascertained by analyzing standard drug and samples of equivalent concentration (25 mg). The specificity of the method was established by analyzing marketed tablet as an experimental sample together with the reference standard using proposed method. The spot for Exemestane in sample was confirmed by comparing the R_F and spectra of the spot in samples with those of standard. The peak purity of sample was judged by comparing the spectra at peak start, peak apex and peak end positions of the spot.

2.7.7. Robustness

The robustness of an analytical method is a measure of its capacity to remain unaffected by small but deliberate variation in method parameters and provides an indication of its reliability during normal usage by introducing small changes in the mobile phase composition; the effects on the results were examined. Mobile phases having diverse composition of chloroform:methanol was tried at two different concentration levels of 100 and 200 ng spot^{-1} and % CV of peak area was calculated.

2.7.8. Ruggedness

The ruggedness of an analytical method is the degree of reproducibility of test results obtained by the analysis of the same samples under a variety of normal test conditions such as different laboratories, different analysts, using operational and environmental conditions that may differ but are still within the specified parameters of the assay. The ruggedness of the method was assessed by analysis of 100 ng Exemestane by two different analysts under the same experimental conditions.

2.8. Analysis of marketed formulation

In assay experiment five samples weighed separately were analyzed. The standard solution of Exemestane of strength (100 μg mL^{-1}) and the experimental solution (extracted from marketed tablet) of strength (100 μg mL^{-1}) were prepared by the above described procedure. For tablet assay linearity study the solutions were prepared containing 100–500 μg mL^{-1}. The standard solution of Exemestane of strength (100 μg mL^{-1}) and the experimental solution of strength (100 μg mL^{-1}) was applied on HPTLC plate together with reference standard by using the proposed method. Then it was developed and scanned under the optimized conditions by the proposed method.

Table 1 Summary of calibration data.

Sr. No.	Parameters	Mean value
1	Linear range	100–500
2	Correlation coefficient (r)	0.9944
3	Slope of regression	26.12
4	Limit of detection	5.8
5	Limit of quantification	17.57

Each linear range is a mean of six observations.

3. Results and discussion

HPTLC of Exemestane using the conditions described above enabled good separation of Exemestane (R_F 0.60). Results from calibration showed there was excellent correlation between area under curve and drug concentration in the range of 100–500 μg mL^{-1}. The regression coefficient was 0.9969 for Exemestane. The limit of detection and quantification was found to be 1.068–3.237 μg mL^{-1} (Table 1).

Average recovery of Exemestane from the formulation was 100.097%, which shows the method is accurate and free from interference of excipients present in the formulation (Table 2).

Instrument precision, intra-day and inter-day precision were measured to evaluate the precision of the method (Tables 3 and 4). The low relative standard deviations obtained (< 2%) indicate the method is highly precise. The repeatability of sample application and measurement of peak area were expressed in terms of coefficient of variation and were found to be very low (0.992346939 and 1.020055828 for Exemestane) which, in turn, insured reproducible performance of the instrument.

The results of the specificity studies showed that Exemestane is stable when exposed to different stress conditions. Any degradation byproducts that may be present in Exemestane were not observed in chromatograms studied. Significant no variations of assay values were observed. Robustness was studied during method development by determining effects of small variation of mobile phase composition (±2%), chamber saturation period, development distance, and scanning time. No significant change in R_F or response was observed, indicating the method was robust. Overall method repeatability was determined by calculating the coefficient of variation,

Table 2 Results from the study of the recovery of Exemestane.

Recovery level	Initial amount	Exemestane added	Total amount	Exemestane found	% Recovery	SD	% RSD
0	500	0	500	496.5	99.29	0.056	0.0572
80	500	400	900	895	99.44	1.680	1.69
100	500	500	1000	998.5	99.85	1.532	1.58
120	500	600	1100	1120	101.81	1.466	1.44
Average recovery					100.097	1.184	1.192
SD					1.1659438		
% RSD					1.164808		

Each value is a mean of six observations.

Table 3 Summary of intra-day and inter-day method precision.

Amount [ng $spot^{-1}$]	Intra-day precision by peak area			Inter-day precision by peak area		
	Average peak area	SD of peak area	CV [%]	Average peak area	SD of peak area	CV [%]
200	988.36	16.6	1.68	980.56	16.87	1.72
300	1290.52	24.26	1.88	1287.98	22.15	1.72
500	1951.24	29.85	1.53	1953.12	29.1	1.49
Mean			1.6967			1.6433

Each value is a mean of six observations.

Table 4 System precision studies of the developed method.

	Repeatability of sample application Area of Exemestane 50 ng spot^{-1}	Repeatability of measurement of peak area Area of Exemestane 50 ng spot^{-1}
1	198	988.369
2	199	990.12
3	188	1000.21
4	192	997.158
5	194	992.148
6	196	970.25
Mean area	194.5	989.7091667
SD	4.086563348	10.51022859
CV	0.992346939	1.020055828

Table 5 Summary of method validation parameters.

Parameter	Exemestane
Linearity range (ng spot^{-1})	100–500
Correlation coefficient	0.9944
Limit of detection (ng spot^{-1})	5.8
Limit of quantitation (ng spot^{-1})	17.57
Recovery (mean ± S.D.)	100.0975 ± 1.67
Precision (*CV*)	
Repeatability of application ($n = 6$)	0.99
Repeatability of measurement ($n = 6$)	1.02
Intra-day ($n = 6$)	1.6967
Inter-day ($n = 3$)	1.6433
Robustness	Robust

CV [%], for all the validation experiments and sample analyses; all values were within 5%. The analysis of the Exemestane in tablet formulation was also carried out. The % assay was found to be 103.12.

The summary of all the validation parameters is given in Table 5.

4. Conclusion

The low % CV value obtained indicates that the suitability of this method for routine analysis and quantitative determination of the Exemestane in the fixed dosage form by High-performance thin-layer chromatographic. The statistical analysis of data obtained proves that the method is reproducible and selective, was found to be simple, accurate and precise. The method can, therefore, be used for routine quality-control analysis and quantitative determination of Exemestane in formulations.

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References

Allievi, C., Zugnoni, P., Strolin Benedetti, M., Dostert, P., 1995. J. Mass Spectrom. 30, 693.

Cenacchi, V., Baratte, S., Cicioni, P., Frigerio, E., Long, J., James, C., 2000. J. Pharm. Biomed. Anal. 22, 451.

Evans, T.R., Di Salle, E., Ornati, G., Lassus, M., Benedetti, M.S., Painezzola, E., Coombes, R.C., 1992. Cancer Res. 52, 5933.

Geisler, J., King, N., Anker, G., Ornati, G., Di Salle, E., Lonning, P.E., Dowsett, M., 1998. Clin. Cancer Res. 4, 2089.

Johannessen, D.C., Engan, T., Di Salle, E., Zurlo, M.G., Paolini, J., Ornati, G., Piscitelli, G., Kvinnsland, S., Lonning, P.E., 1997. Clin. Cancer Res. 3, 1101.

Persiani, S., Broutin, F., Cicioni, P., Stefanini, P., Strolin Benedetti, M., 1996. Eur. J. Pharm. Sci. 4, 331.