Spectrophotometric methods for the determination of letrozole in bulk and pharmaceutical dosage forms

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INTRODUCTION

Letrozole, 4-[(4-cyanophenyl)-(1,2,4-triazol-1-yl)methyl] benzonitrile, is a selective nonsteroidal inhibitor of the aromatase (oestrogen synthetase) system, which is used for the treatment of estrogen-dependent breast cancers. Letrozole is readily and completely absorbed from the gastrointestinal tract. It is slowly metabolized in the liver to an inactive carbinol metabolite, which is then excreted as glucoronide in the urine.

On a detailed literature survey, it was found that letrozole could be estimated by spectrophotometry,[3-4] High-performance liquid chromatography (HPLC),[5-10] the microarray approach,[11] capillary gas chromatographic method with flame ionization detector,[12] and by gas chromatography – mass spectrometry (GC / MS)[13] methods.

Experimental

Chemicals and reagents

The Letrozole working standard was kindly provided by Alembic Ltd., (Vadodara, India) and was used as received. A commercial tablet formulation was purchased from the local market. Analytical reagent grade methanol was used for the preparation of solutions.

Instrumentation

A double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) connected to a computer loaded with a spectra manager software UV Probe, with 1.0 cm quartz cells, was used. The spectra were obtained with the
instrumental parameters as follows: wavelength range: 200 – 400 nm; scan speed: medium; sampling interval: 0.2 nm; derivative mode: 1D (first order derivative, dA / dλ) and 2D (second order derivative, d²A / dλ²); band width (Δλ): for 1D and 2D, 10.0 nm; spectral slit width: 1 nm. All weights were taken on an electronic balance (Denver, Germany).

Preparation of standard stock solution
The standard solution of letrozole was prepared by dissolving 10 mg of the drug (accurately weighed) in methanol and diluted to 100 ml with methanol to obtain a final concentration of 100 µg ml⁻¹. This stock solution was used to prepare further dilutions of standard solutions.

Method I
UV-spectrophotometry
Series dilutions of the stock solution were made by pipetting out 0.025, 0.05, 0.1, 0.25, 0.5, 1.0, and 2.0 ml stock solution into separate 10 ml volumetric flasks and diluting to volume with methanol to produce concentrations ranging from 0.25 – 20.0 µg ml⁻¹. The above-mentioned solutions were scanned over the range of 400 nm to 200 nm against the blank. The λ_max was found to be at 240.0 nm. The calibration curve was constructed by plotting the concentration (0.25 – 20.0 µg ml⁻¹) versus absorbance, at 240.0 nm.

Method II
First-derivative spectrophotometry
The spectra obtained in method I were derivatized to get first-order derivative spectra and the response (dA / dλ) of the spectra were measured at 224.0 nm, and then the calibration curve was constructed by plotting the concentration (0.25 – 20.0 µg ml⁻¹) versus response (dA / dλ), at 224.0 nm.

Method III
Second-derivative spectrometry
The spectra obtained in method I were derivatized to get second-order derivative spectra and the response (d²A / dλ²) of the spectra were measured at 241.0 nm, and then the calibration curve was constructed by plotting the concentration (0.5 – 20.0 µg ml⁻¹) versus response (d²A / dλ²), at 241.0 nm.

Method IV
Area under curve
The AUC (area under curve) method is applicable where there is no sharp peak or when broad spectra are obtained. It involves calculation of the integrated value of absorbance with respect to the wavelength between the two selected wavelengths λ₁ and λ₂. The area calculation processing item calculates the area bound by the curve and the horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. This wavelength range is selected on the basis of repeated observations, so as to get the linearity between the area under curve and concentration. The spectra obtained in method I were used to calculate the AUC. The calibration curve was constructed by plotting the concentration (0.25 – 20.0 µg ml⁻¹) versus AUC.

Estimation of Letrozole in Tablets
For the analysis of the pharmaceutical dosage form, a total of 20 tablets were weighed and finely powdered. A portion of the powder, equivalent to about 10 mg letrozole was weighed accurately and transferred into a 100 ml volumetric flask and 50 ml methanol was added. After ultrasonic vibration for 30 minutes, the mixture was diluted to volume with methanol and filtered through Whatman filter paper (No. 41). Appropriate dilution was made into 5.0 µg ml⁻¹ with methanol from the stock solution for all the methods, and the amounts of letrozole were determined. The percent labeled claim and Standard Deviation (S.D) were calculated.

Validation of Methods
Linearity
For all the methods, six-point calibration curves were prepared on three different days. The results obtained were used to calculate the equation of the line by using linear regression by the least-squares regression method.

Precision
The intra-day and inter-day precisions of the proposed spectrophotometric methods were determined by estimating the corresponding response thrice on the same day and on three different days, over a period of one week, for three different concentrations of letrozole (2.5, 5.0, and 10.0 µg ml⁻¹) and the results were reported in terms of relative standard deviation.

Accuracy
This parameter was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120%, which consisted of adding a known amount of letrozole reference material to a prequantified sample solution. An aliquot of the sample solution containing letrozole at 5.0 µg ml⁻¹ was transferred to three 10 ml volumetric flasks containing, 4.0, 5.0, and 6.0 µg ml⁻¹ letrozole reference solutions, respectively. The contents were mixed and diluted to volume, in order to obtain the final concentrations of 9.0, 10.0, and 11.0 µg ml⁻¹ letrozole. The recoveries were verified by the estimation of drugs in triplicate preparations, at each specified concentration level. The spectra were recorded in the UV range and then analyzed. The results were reported in terms of % recovery.

Specificity
The results of the tablet solution showed that there was no interference of excipients when compared with the working standard solution. Thus, the methods were said to be specific.
Robustness

The robustness of the proposed methods was tested by changing parameters such as wavelength range and slit width. None of these variables significantly affected the absorbance of the drugs, indicating that the proposed methods could be considered as robust.

Ruggedness

Ruggedness of the proposed methods was determined by analyzing aliquots from a homogenous slot (5.0 \( \mu g/ml \)) in different laboratories, by different analysts, using similar operational and environmental conditions. The results are reported in terms of % RSD.

RESULTS AND DISCUSSION

Figures 1, 2, and 3 show overlaid UV-spectrophotometric (0.25 – 20.0 \( \mu g/ml \)), first-derivative (0.25 – 20.0 \( \mu g/ml \)), and second-derivative (0.5 – 20.0 \( \mu g/ml \)) absorption spectra of letrozole, respectively, and the spectra are found to be similar in nature and overlapping. Figure 4 shows the absorption spectrum of letrozole (5.0 \( \mu g/ml \)) in methanol for method IV. The optical characteristics of letrozole have been calculated by the proposed methods and are presented in Table 1.

Table 1: Optical characteristics of letrozole

| Parameters | Method I | Method II | Method III | Method IV |
|------------|----------|-----------|------------|-----------|
| Beer-Lambert’s range (\( \mu g \text{ ml}^{-1} \)) | 0.25–20.0 | 0.25–20.0 | 0.5–20.0 | 0.25–20.0 |
| \( \lambda_{\text{max}} \) (nm)/wave length range (nm) | 240.0 | 224.0 | 241.0 | 235.0–245.0 |
| Molar absorptivity (l/mol.cm) | 35976.71 | 1169.7423 | -485.015 | 339253.8 |
| Sandell sensitivity (\( \mu g \text{ cm}^{-2}/0.001 \text{ A} \)) | 0.00793 | – | – | – |
| Slope | 0.119333 | 0.003897 | -0.00157 | 1.132 |
| Standard deviation of slope | 0.001155 | 2.08X10^{-5} | 2.65 X10^{-5} | 0.002 |
| %RSD of slope | 0.967626 | 0.534217 | -1.68519 | 0.176678 |
| Intercept | 0.002233 | 0.000185 | 0.00463 | 0.214333 |
| Standard deviation of intercept | 1.527X10^{-5} | 1.527X10^{-6} | 3.055X10^{-6} | 1.155X10^{-3} |
| %RSD of intercept | 0.683967 | 0.9987313 | 0.99849 | 0.99937 |
| Correlation coefficient | 0.999373 | 0.9987313 | 0.99849 | 0.99937 |
| %RSD of correlation coefficient | 0.004729 | 0.003897 | -0.00157 | 0.009171 |
| Limit of detection | 0.000432 | 0.001294 | -0.00642 | 0.003366 |
| Limit of quantitation | 0.000432 | 0.001294 | -0.00642 | 0.003366 |

Stock solution of the drug was prepared in methanol and further dilutions were carried out with methanol, 0.1N HCl, 0.1N NaOH, and with water separately, to produce concentrations ranging from 0.25 – 20.0 \( \mu g/ml \). However, in the present study, dilutions carried out with methanol were selected because the drug

Table 2: Comparison of absorbance of letrozole in different solvents

| Conc. (\( \mu g/ml \)) | Dilution with methanol (Abs at 240.0 nm) | Dilution with 0.1N HCl (Abs at 240.0 nm) | Dilution with 0.1N NaOH (Abs at 241.0 nm) | Dilution with water (Abs at 241.0 nm) |
|----------------------|----------------------------------|-----------------------------|-------------------------------|-----------------------------------|
| 0.25                 | 0.035                            | 0.017                       | 0.023                        | 0.081                            |
| 0.5                  | 0.069                            | 0.024                       | 0.055                        | 0.127                            |
| 1                    | 0.142                            | 0.053                       | 0.176                        | 0.185                            |
| 2.5                  | 0.320                            | 0.121                       | 0.325                        | 0.369                            |
| 5                    | 0.641                            | 0.251                       | 0.575                        | 0.662                            |
| 10                   | 1.261                            | 0.502                       | 1.143                        | 1.249                            |
| 20                   | 2.402                            | 0.994                       | 2.160                        | 2.379                            |
| slope                | 0.120                            | 0.049                       | 0.107                        | 0.116                            |
| intercept            | 0.022                            | 0.001                       | 0.035                        | 0.071                            |
| r                    | 0.999                            | 0.999                       | 0.998                        | 0.999                            |
solutions were stable and the Beer-Lambert’s law was followed properly [Table 2]. From the calibration curve [Figure 5], it was observed that with an increase in letrozole concentration, the responses increased. In Method I, the $\lambda_{\text{max}}$ was found to be at 240.0 [Figure 1]. Hence, the study was carried out at 240.0 nm, where the Beer-Lambert’s law was followed properly.

Derivative spectrophotometry is an analytical technique for the enhancement of sensitivity and specificity in qualitative and quantitative analysis of various compounds, including pharmaceuticals. Hence, methods II and III have been carried out for letrozole. For Method II [Figure 2], 224.0 nm is selected because at 208.0 nm the peaks are distorted and the maximum wavelength of the peaks as well as the zero
### Table 3: Assay results of letrozole in pharmaceutical dosage form (Tablet-2.5 mg) by using the proposed spectrophotometric methods

| Label claim (mg/tab) | % Label claimed± SD (n=5) | %RSD |
|---------------------|---------------------------|------|
|                     | Method I | Method II | Method III | Method IV | Method I | Method II | Method III | Method IV |
| 2.5                 | 99.98±0.1353 | 99.85±0.889 | 100.64±0.942 | 100.43±0.859 | 0.1354 | 0.8913 | 0.9360 | 0.8555 |

### Table 4: Results for accuracy studies of letrozole by proposed spectrophotometric methods

| Methods | Accuracy (% recovery±SD) |
|---------|--------------------------|
|         | 80% 5.0 + 4.0 µg ml⁻¹ | 100% 5.0 + 5.0 µg ml⁻¹ | 120% 5.0 + 6.0 µg ml⁻¹ |
| I       | 99.85±0.28              | 100.33±0.21              | 99.73±0.14              |
| II      | 99.88±0.08              | 100.22±0.10              | 99.95±0.29              |
| III     | 99.61±0.56              | 100.16±0.17              | 100.46±0.66              |
| IV      | 100.28±0.51             | 100.10±0.12              | 100.19±0.89              |

*Mean of three determinations

### Table 5: Results for precision studies of letrozole by proposed spectrophotometric methods

| Method | Intraday (n=3); | Interday (n=3); |
|--------|----------------|----------------|
|        | Drug conc. taken (µg ml⁻¹) | RSD, % | Drug conc. taken (µg ml⁻¹) | RSD, % |
|        | 2.5 | 5.0 | 10.0 | 2.5 | 5.0 | 10.0 |
| I      | 0.34 | 0.44 | 0.57 | 0.36 | 0.46 | 0.59 |
| II     | 0.42 | 0.42 | 0.35 | 0.59 | 0.91 | 0.46 |
| III    | 0.67 | 0.76 | 0.54 | 0.38 | 0.67 | 0.74 |
| IV     | 0.54 | 0.52 | 0.38 | 0.87 | 0.68 | 0.76 |

### Table 6: Ruggedness data of letrozole (5.0 µg ml⁻¹) by proposed methods

| Analyst I, %RSD | Analyst II, %RSD |
|-----------------|------------------|
| Method I | Method II | Method III | Method IV | Method I | Method II | Method III | Method IV |
| 0.44 | 0.54 | 0.38 | 0.57 | 0.46 | 0.52 | 0.41 | 0.61 |

crossing point do not remain constant, and at 247.0 nm, the Beer-Lambert’s law is not followed properly. For Method III [Figure 3], the wavelength 241.0 nm is selected, because the zero crossing point and maximum wavelength do not remain constant for each concentration at 213.0 nm, and at 251.0 nm, the Beer- Lambert’s law is not followed properly. In Method IV [Figure 4], the study has been carried out at two wavelength ranges, that is, 235.0 – 245.0 nm and 230.0 – 250.0 nm, but good linearity range was obtained at the wavelength range of e 235.0 – 245.0 nm.

Tablets were analyzed and the amount of the drug determined by the proposed methods; it was in good agreement with the label claim [Table 3]. It was also observed that there was no significant difference in the content of letrozole obtained by using the different proposed spectrophotometric methods.

The recoveries of letrozole, which was evaluated by the percent recovery studies at concentration levels of 80, 100, and 120% were found to be in the acceptable range [Table 4]. Excipients used in the formulation did not interfere with the response of the drug at its analytical wavelengths. Also, no significant change in response to letrozole was observed by changing parameters, such as, wavelength range and slit width. The intra-day and inter-day precision values (%RSD) were calculated [Table 5] and were lying in the acceptable range for letrozole. Ruggedness of the proposed methods were determined with the help of two different analysts and the results were evaluated by calculating the %RSD value, and were found to be within the range [Table 6]. Hence, the proposed methods were precise, specific, accurate, rugged, and robust for the estimation of letrozole in bulk and pharmaceutical formulations.

### CONCLUSIONS

The four methods that were developed for the determination of letrozole were based on different analytical techniques, zero-derivative, first-derivative, and second-derivative spectrophotometry, and the AUC method. All the methods were validated and found to be simple, sensitive, accurate, and precise. Hence, all the methods could be used successfully for the routine analysis of the pharmaceutical dosage forms of letrozole.

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