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DEVELOPMENT AND VALIDATION OF HPTLC METHOD FOR THE DETERMINATION OF LETROZOLE IN BULK AND TABLET DOSAGE FORM

N. Tamilselvi[1*], R. Arivukkarasu[2], Ancymol Antony[1], Anitha T. J,[1], Boominathan A.[1], Chandrasekar R.[1], Deepthi Visakh[1]

[1]Department of Pharmaceutical Analysis, KMCH College of Pharmacy, Coimbatore, Tamil Nadu.

[2]Department of Pharmacognosy, KMCH College of Pharmacy, Coimbatore, Tamil Nadu.

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***Correspondence for Author**
N. Tamilselvi
Department of Pharmaceutical Analysis, KMCH College of Pharmacy, Coimbatore, Tamil Nadu.

ABSTRACT

Letrozole is a potent and selective non – steroidal Aromatase inhibitor approved for the use in post - menopausal woman who have breast cancer that has progressed after antiestrogen therapy.The present work describes a simple, precise and accurate HPTLC method for its estimation as bulk and in tablet dosage form. The chromatographic separation was carried out on precoated silica gel $60F_{254}$ aluminium plates using mixture of chloroform : methanol (9:1 v/v) as mobile phase and densitometric evaluation of spots was carried out at 254 nm using Camag TLC Scanner-3 with win CAT 1.3.4 version software. The experimental parameters like band size of the spot applied, chamber saturation time, solvent front migration, slit width etc. were critically studied and optimum conditions were evolved. The drug was satisfactorily resolved with Rf value 0.11±0.01. The accuracy and reliability of the proposed method was ascertained by evaluating various validation parameters like linearity (500-5500 ng/spot), precision (intra-day RSD 0.17-1.54%, inter-day RSD 0.30-1.43%), accuracy (99.3 to 99.8 %) and specificity according to ICH guidelines. The proposed method can analyze eight or more formulation units simultaneously on a single plate and provide a faster and cost-effective quality control tool for routine analysis of letrozole as bulk drug and in tablet formulation.

KEYWORDS: Letrozole, HPTLC, densitometric estimation, method development and validation.

INTRODUCTION

Letrozole (4, 4'-((1H-1, 2, 4-triazol-1- yl) methylene) dibenzonitrle) is a potent and selective non – steroidal aromatase inhibitor approved for the use in post - menopausal woman who have breast cancer that has progressed after antiestrogen therapy.[1] Few methods of analysis of Letrozole have been reported like RP- HPLC[2-4],UV-spectrophotometric method.[5] However there is no High Performance Thin Layer Chromatographic (HPTLC) method reported for Letrozole in pharmaceutical dosage forms. The present study describes the determination of Letrozole in tablet dosage form by using a HPTLC method. Unlike HPLC, consumption of mobile phase per sample basis is quite low. This saves cost per analysis and analysis time as well. HPTLC facilitates repeated detection (scanning) of the chromatogram with the same or different parameters. HPTLC technique is most suited for impurity profile of drug substances and content uniformity test as per compendia specifications.[6] The aim of this study is to develop a simple, precise, rapid, accurate and repeatable HPTLC method for the estimation of letrozole in tablet dosage form as per ICH guidelines.[7-10]

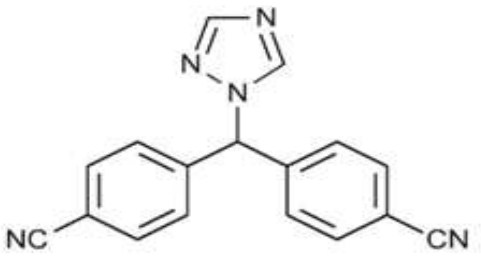

Fig 1: Chemical structure of Letrozole

MATERIALS AND METHODS

Chemicals and reagents

Letrozole was supplied as a gift sample from Sris Pharmaceuticals (Hyderabad,India). Letrosoul was procured from private Pharmacy, Coimbatore, India and reagents used were of analytical grade (MERCK Chem. Ltd., Mumbai). Methanol was selected as the solvent for sample preparation.

HPTLC instrumentation

Chromatography was performed on 20 cm × 10 cm aluminum-backed TLC plates coated with 200 µm layers of silica gel $60F_{254}$ (E. Merck, Darmstadt, Germany; supplied by Merck India, Mumbai, India). The plates were prewashed by methanol and activated at 100 – 110 °C for 10

min prior to chromatography. The samples were applied on the plates as 6 mm wide bands, by means of a CAMAG (Muttenz, Switzerland) Linomat-5 sample applicator fitted with a 100 µL sample syringe (Hamilton, Bonaduz, Switzerland). Plate was developed to a distance of 8 cm using chloroform: methanol (9:1) as mobile phase in a Camag twin-trough glass chamber previously saturated with mobile phase vapors for 10 min at ambient temperature. Densitometric scanning was performed at 254 nm using Camag TLC Scanner 3 equipped with win CATS software version 1.3.0.

Preparation of standard solution

Standard stock solution containing 10 mg /ml of Letrozole was prepared in methanol by dissolving 100 mg of Letrozole in 10 ml methanol. Standard solution was further diluted with methanol to obtain working standard solutions in a concentration range of 500-5500 ng/spot for Letrozole.

Preparation of sample solution

For analysis of tablet dosage form, twenty tablets, each containing 2.5 mg Letrozole, was weighed and their average weight was calculated. The tablets were finely powdered and powder equivalent to 100mg of Letrozole was accurately weighed and transferred into 10 ml of volumetric flask containing 5 ml of methanol, Sonicated for 30 min and make up to the mark with methanol to give 10mg/ml of Letrozole. The solution was centrifuged for 15 min at 600 rpm, filtered through what mann No 41 filter paper and the residue was washed with methanol. The volume of the filtrate was adjusted to 10 ml with the same solvent. This above solution was further diluted with methanol to get the concentrations of 2500 and 4500 ng/spot for Letrozole. The procedure was repeated as per the analysis of formulation. The amount of drug recovered was calculated by using slope and intercept values from the calibration graph.

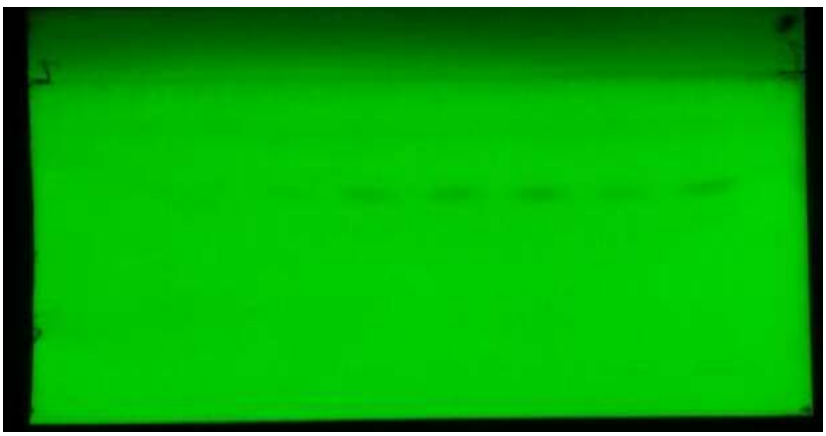

Fig 2. Image of developed plate

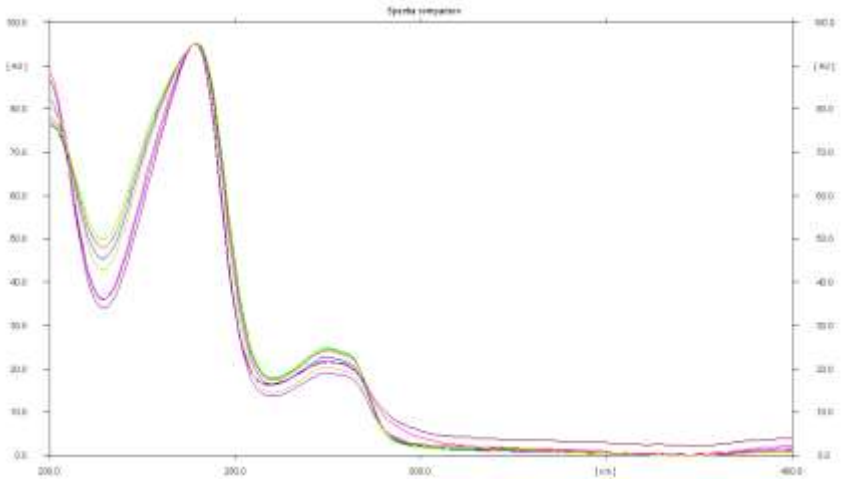

Fig 3. Overlay spectrum of Letrozole standard and sample

METHOD VALIDATION

The method was validated as per ICH guide lines[7-10] for precision, accuracy, specificity, linearity, reproducibility, LOD and LOQ.

a) Accuracy

Accuracy of the method was determined by recovery experiments. The reference standards of the respective drug were added to the sample solution at the level of 50%, 100% and 150%. These were further diluted by procedure as followed in the estimation of formulation. The concentrations of the drugs present in the resulting sample solution were determined by using assay method.

b) Linearity and range

From the standard stock solutions, a suitable standard solution was prepared. Letrozole was found to be linear in the range of 500 to 5500 ng/spot. .The solutions were examined by the assay procedure. The calibration curve was plotted using peak area Vs concentration of the standard solution. From the calibration curve, the slope and intercept were calculated.

c) Precision

Precision of the method was determined by

Intra-day precision

Inter-day precision

Repeatability

a) Intra-day Precision

Intra-day precision was found out by carrying out the analysis of the standard drug solutions at a concentration of 1500-3500 ng/spot of Letrozole for three times on the same day. The Percentage RSD was calculated.

b) Inter-day precision

Inter-day precision was found out by carrying out the analysis of the drug solution at a concentration of 1500-3500 ng/spot of Letrozole for three different days and the Percentage RSD was calculated.

c) Repeatability

Repeatability of measurement of the peak area was determined by spotting 2500 ng/spot of Letrozole of drug solution on a pre-coated TLC plate. The separated spots were scanned six times without changing the position of the plate and the percentage RSD was calculated.

d) Limit of Detection (LOD) and Limit of Quantification (LOQ)

The detection limit of an individual analytical procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantitation limit of an individual analytical procedure is the lowest amount of analyte in a standard which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were experimentally verified by the known concentration of a standard solution of Letrozole until the average response approximately 3 or 10 times the standard deviation of the responses for the 6 replicate determinations.

e) Specificity

It was observed that other constituents' presents in the formulation did not interfere either with the peak of Letrozole. Therefore the method was specific. The overlay spectrum of the standard Letrozole spots present in the samples was found to be similar or overlap. The peak purity of the Letrozole was assessed by comparing the spectra at three different levels, viz. peak start, and peak apex and peak end positions of the spot.

f) Robustness of the method

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small changes in the mobile phase composition, mobile phase volume and duration of mobile phase saturation and the effects on the results were examined.

g) Ruggedness

It expresses the precision within laboratory variations like different days, different analyst, and different equipments. Ruggedness of the method was assessed by spiking the standard concentrations of Letrozole 2500 ng/spot, 6 times in two different days with different analyst.

RESULTS AND DISCUSSION

A HPTLC method was developed for the estimation of Letrozole in tablet dosage forms, which can be conveniently employed for routine quality control in pharmaceutical dosage forms.

1. Analysis of Formulation

The percentage of drug in formulation, mean and relative standard deviation were calculated. The result of analysis showed that the amount of drug present in the formulation is in good correlation with the label claim of the formulation

Table 1. Assay of Letrozole in tablet Dosage Form.

Formulation (Letrozole)	Labeled amount (mg)	Amount found (mg)	Percentage Assay (%w/w)
	2.5	2.48	99.53

*- each value is the mean of six observations

2. Linearity

Letrozole was found to be linear in the range of 500 to 5500 ng/spot. The correlation coefficient of Letrozole was found to be 0.9699. The linearity range of Letrozole was shown in **Table 2 .** The calibration curves was plotted between peak area and concentration of the standard solutions (**Fig: 4)**.

Table 2. Linearity range of Letrozole

Concentration (ng/spot)	LETROZOLE	
	R_f value*	Peak area*
500	0.64	2848
1500	0.63	2181
2500	0.63	5343
3500	0.62	8299
4500	0.62	10486
5500	0.62	11631

*- each value is the mean of six observations

The calibration graph shows that linear response was obtained over the range of concentrations used in the assay procedure. The range demonstrates that the method is linear.

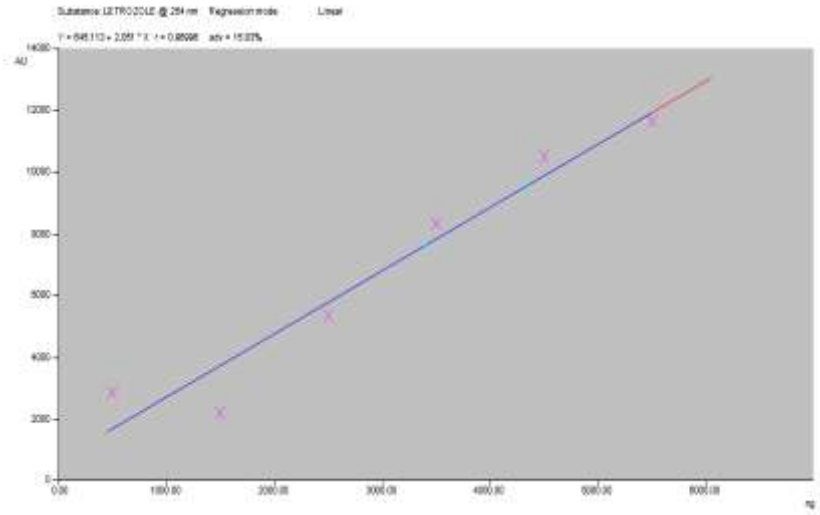

Fig 4. Calibration curve for Letrozole

3. Accuracy (Recovery studies)

The accuracy of the method was determined by recovery experiments. A known quantity of the pure drug was added to the pre-analyzed sample formulations at 50%, 100% and 150% levels. The recovery studies were carried out 6 times of each level and the percentage recovery and percentage relative standard deviation were calculated and given in **Table 3.** The percentage recovery of Letrozole was found to be in the range of 99.3-99.8%

Table 3. Recovery studies of Letrozole

Drug	Label Claim mg/tab	Spike Level (%)	Amount of drug added (ng/spot)	Amount of drug recovered (ng/spot)	Percentage Recovery (%)	%RSD*
Letrozole	2.5	50	250	248.3	99.3	0.36
		100	500	498.5	99.7	0.31
		150	750	748.6	99.8	0.27

*-Each value is a mean of six observations.

4. Precision

The precision of the method was determined by studying reproducibility and repeatability. The area of drug peaks and percentage relative standard deviation of intraday and inter day were calculated and presented in **Table 4.** The results revealed that the developed method was found to be reproducible in nature.

Table 4. Intra-day and inter-day precision of the developed method

Concentration (ng/spot)	Intraday			Interday		
	Peak area	SD	%RSD *	Peak area	SD	%RSD *
LETROZOLE						
1500	2156	33.37	1.54	2154	31.69	1.46
2500	5432	31.19	0.40	5496	25.17	0.42
3500	8398	14.95	0.17	8351	23.11	0.30

*- each value is the mean of six observations

5. Repeatability

Repeatability of measurement of the peak area was determined by spotting 2500 ng/spot of Letrozole of drug solution on a pre-coated TLC plate.

6. LOD and LOQ of letrozole

Table 5. LOD and LOQ

Parameter	LETROZOLE (ng/spot)
LOD	42.81
LOQ	129.74

7. Ruggedness

The sample was analyzed by a different chemist and same instruments on a different day have been performed. The deviation among the results obtained by two chemists on a different day is well within the limits. Hence the method is rugged.

Table 6.Ruggedness

Drug	Concentration (ng/spot)	Mean Peak area	% R.S.D*
Day I, Analyst I			
LETROZOLE	2500	5410	0.42
Day II, Analyst II			
LETROZOLE	2500	5730	0.24

*- each value is the mean of six observations

8. ROBUSTNESS

The Robustness studies were performed for the standard solutions and were presented in **Table 7.** The assay values were within the limits that the developed method is robust.

Table 7 .Robustness studies

Parameter	Modification	Letrozole Recovery (%)
Mobile Phase Ratio	8.5-1.5	99.1
	9.5-0.5	99.9
Development Distance	9mm	99.5
Detection Wavelength(nm)	252 nm	99.7
Slit Dimension	5.00 x .30m micro	99.6

CONCLUSION

Simple, accurate, reproducible, robust and cost effective HPTLC chromatographic method requiring simple reagents were developed and statistical analysis proved that method are reproducible and selective for quantitative determination of Letrozole in pharmaceutical dosage form.

It was concluded that developed method offered several advantages such as rapid, cost effective, simple mobile phase and it is in good agreement with the label claim of the drug. The additives present in the pharmaceutical formulation of the assayed sample did not interfere with determination of Letrozole HPTLC method can be used for routine analysis of Letrozole in their dosage form without any interference of excipients.

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