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Development and validation of a RP-HPLC method for the determination of anastrazole in rat plasma by liquid-liquid extraction

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ABSTRACT

Anastrazole is non-steroidal aromatase-inhibiting drug used for the treatment of breast cancer after surgery and metastasis in both pre and post-menopausal women. It acts by binding reversibly to the aromatase enzyme, inhibits the conversion of androgens to estrogens in peripheral tissues. Since HPLC methods were more accurate, precise and sensitive, the present work describes a simple, precise and accurate analytical HPLC method for the estimation of anastrazole in pure and tablet dosage form. The separation was carried out using phenomenex C18 (250 x 4.6 mm, 5 µm particle size) column, with a mobile phase consisting of acetonitrile and phosphate buffer (7.2) in the ratio of 80:20%, v/v. The flow rate was set at 0.9 ml/min and detection was monitored at 214 nm. The retention time of anastrazole is 3.3 min with linearity coefficient of 0.9999 and percentage recovery of 99.79. The linearity was found in the concentration range of 100-400 µg/ml for anasrazole. The proposed HPLC method was extended for the estimation of anastrazole in rat plasma. Anastrazole in spiked rat plasma was extracted with Ethyl acetate by liquid- liquid extraction procedure.The liquid chromatography method was extensively validated for linearity, accuracy, precision, and robustness. All these analytical validation parameters were observed and the %RSD was determined which indicates the usefulness of method for determination of anastrazole in formulation.

Key words: Anastrazole, validation, RP-HPLC, formulations, acetonitrile and buffer (pH7.2).

INTRODUCTION

Anastrazole is chemically known as 2, 2'-[5-(1*H*-1,2,4-triazol-1-ylmethyl)-1,3-phenylene]bis(2-methyl propanenitrile), its empirical formula is $C_{17}H_{19}N_5$ and molecular weight is 293.366 Literature survey of the drug revealed that there are methods for the determination of anastrazole by LC and spectrophotometry[1-8]. The growth of many cancers of the breast is stimulated or maintained by estrogens. In postmenopausal women, estrogens are mainly derived from the action of the aromatase enzyme, which converts adrenal androgens (primarily androstenedione and testosterone) to estrone and estradiol. The suppression of estrogen biosynthesis in peripheral tissues and in the cancer tissue itself can therefore be achieved by specifically inhibiting the aromatase enzyme. Anastrozole is a potent and selective non-steroidal aromatase inhibitor. It significantly lowers serum estradiol concentrations and has no detectable effect on formation of adrenal corticosteroids or aldosterone. The purpose of this work was to develop a method in economic point of view and for regular analysis. It was decided to develop a novel, simple, rapid, economic, precise, efficient RP-HPLC method for quantitative analysis of anastrozole with shorter runtime, simple mobile phase preparation and to validate the method accordingly with ICH guidelines.

MATERIALS AND METHODS

Equipment
SHIMADZU LC-20AD system with SPD-20A Prominence UV-Vis detector equipped with Sphinchrom software was used for method development, double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer was used for spectral measurements and ELICO pH meter was used for pH measurements.

Reagents and standards
Water and acetonitrile of HPLC grade (Merck India Ltd.), 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide were used for the experimental work.

Chromatographic conditions
The separation was achieved on a phenomenex C18 (250 mm x 4.6 mm, 5 µm) column, with a mobile phase of acetonitrile: buffer (pH 7.2) in the ratio of 80:20%, v/v and at a flow rate of 0.9 mL/min. The absorbances were monitored at 214 nm at ambient temperature.

Preparation of mobile phase
Preparation of pH (7.2) buffer
To 125 ml of 0.1 M potassium dihydrogen orthophosphate in 250 ml volumetric flask 86.75 ml of 0.1 M sodium hydroxide was added and the volume was made up to 250 ml with water. The pH was adjusted to 7.2 with 0.2 M sodium hydroxide. Acetronitrile and phosphate buffer were filtered through 0.45 µm membrane filter and sonicated before use.

Preparation of stock solution of anastrazole
Stock solutions of anastrazole is prepared by dissolving 25 mg of each drug taken in a separate 25 ml volumetric flask in phosphate buffer of pH 7.2 and completed to volume, sonicated for about 15 min. From the individual stock solutions, working standard solutions were prepared in a concentration range of 100-400 µg/ml for anastrazole.

Procedure for Calibration curve
Different volumes of stock solutions were accurately transferred to 10 ml volumetric flasks to bring to 100-400 µg/ml of anastrazole. Six replicate solutions in the above range were prepared for each concentration. The calibration curve was constructed by plotting the analyte peak area against concentration.

Procedure of calibration standards by extraction from rat plasma :
The collected rat blood was mixed with EDTA to precipitate plasma proteins and centrifuged for 5 min. The supernatant was used further for preparing calibration standards and as blank. Aliquots of anastrazole was added to 0.9 ml of plasma into a series of 2 ml eppendroffs so that the volume was maintained to 1ml and concentrations to 0.1- 0.4 mg/ml, shaken for 5 to 10 min and then 1 ml of ethyl acetate was added, vortexed for 10 min and centrifuged at 2,500 rpm for 5 min. Ethyl acetate layer was separated and evaporated to dryness at room temperature using a steam of nitrogen. The residue was reconstituted with 1 ml of mobile phase to produce 100-400 µg/ml of anastrazole.

RESULTS AND DISCUSSION

Method optimization
The suitable parameters were chosen after several trails with buffers of pH 6.8, 7.2, 7.4 and 7.8 with various compositions of methanol/acetronitrile with buffer; the composition of acetonitrile and buffer of pH 7.2 was found to be satisfactory. The trails revealed that with the decrease in acetronitrile concentration, the peak obtained was broad and showed severe tailing. The peak obtained with a composition of acetronitrile and buffer in the ratio of 80:20%, v/v was proved to be most suitable of all the combinations since the obtained chromatograms were better defined, with good symmetry and free from tailing. To determine the effect of flow rate, the method was performed at different flow rates 0.5 ml/min, 0.7 ml/min, 0.9 ml/min, 1 ml/min and 1.2 ml/min. The optimum flow rate was found to be 0.9 ml/min with the retention time of 3.33 min. The absorbances measured at 214 nm had given better linear responses. For extraction of drug spiked in rat plasma, several solvents were tried and found ethyl acetate as a suitable extraction medium, as it showed better recovery from rat plasma. This permits the determination of anastrazole with reasonable response for the well defined peaks with reliable results.

The chromatogram of anastrazole obtained by the optimized method was shown in fig-1.

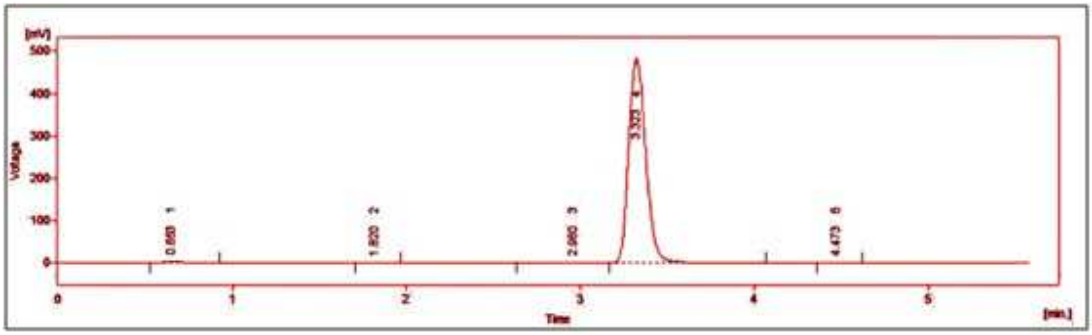

Fig-1 Chromatogram of anastrazole standard solution

Method validation

After development of HPLC method for the estimation of anastrazole, validation of the method was carried out with respect to several parameters like precision, accuracy, linearity, robustness, ruggedness to ensure that the developed method copes with all the requirements for the intended purpose. A series of solutions were prepared using anastrazole stock solution and the linearity responses were evaluated across a concentration range of 100-400 µg/ml; shown in fig. 2. The linear regression data was provided in table -1.

Fig. 2. Linearity plot of Anastrazole by the RP-HPLC method

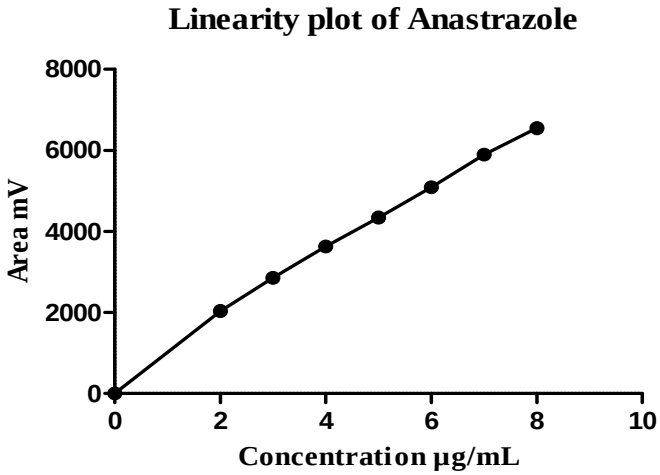

Table -1 Results showing linearity values of Anastrazole

S. No	Conc. (µg/ml)	Peak area
1	100	2036.028
2	150	2354.995
3	200	3628.907
4	250	4046.417
5	300	5092.122
6	350	5316.697
7	400	5782.849

Table-2 Results of validation parameters

Validation parameters	Results
Theoretical plates(N)	27722
Linearity range, µg/ml	100-400
Tailing factor	0.91
R_t (min)	3.33
LOD, µg/ml	8.87
LOQ, µg/ml	26.89

Intraday precision was determined by repeating the analysis of standard solution of anastrazole on the same day and on three consecutive days. Reproducibility was determined by carrying on the above analytical procedures in different laboratories and the obtained results were compared. Table 3 shows the results for precision studies, indicating good precision of the proposed method.

The effect of robustness on retention time and tailing factor was studied and the results indicated that at every condition employed, the chromatographic parameters were within the limits of established values thus proving reliability during normal usage. The results of the ruggedness studies performed by the proposed method validated the method. The LOD and LOQ values of the developed method was determined by analyzing progressively lower concentrations of the standard solution using optimized chromatographic conditions. The minimum concentration of the standard solution, which gave signal to noise ratio of 3 and 10 was taken as the LOD and LOQ values respectively, which was reported in table-1. To assess the accuracy of the aimed method, recovery studies were carried out by standard addition method and assay was performed in triplicate as per test method with equivalent amount of anastrazole into each volumetric flask for each spike level to get the concentration of anastrazole equivalent to 80%, 100% and 120% of the labeled amount as per the test method. The average % recovery was calculated and presented in table -4.

Table 3 Results showing system precision values of Anastrazole

Conc. (µg/ml)	Inter-day	Intra-day
2	2021.995	2037.885
3	2815.057	2638.114
4	3190.503	3095.442
5	4448.833	4321.887
6	5452.224	5531.440
7	5599.772	5645.314
SD	2294.716	2291.898
% RSD	0.15	0.26
Recovery	99.85	99.74

SD, Standard Deviation; RSD, Relative Standard Deviation

Table 4 Results showing accuracy values of anastrazole

Sample	%Recovery	% RSD
80% Sample	99.91	0.7
100%Sample	100.05	0.9
120%Sample1	100.05	0.6

CONCLUSION

The results indicating that the proposed RP-HPLC methods for the determination of anastrazole was precise, accurate, specific and simple. The method was developed and validated according to the ICH guidelines. The extraction of anastrazole by Liquid -liquid extraction procedure from rat plasma was found to be good and economic. The method was validated statistically and the results of recovery studies were in good agreement with the respective label claim of the formulation. There was no interference from the excipients present in the formulation. The retention times of the mentioned drugs were less than 7 min. Thus the method was less time consuming and can be employed for routine analysis in laboratories.

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