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Development and Validation of UV Spectrophotometric method for *in-vitro* studies of Anastrozole Invasomes

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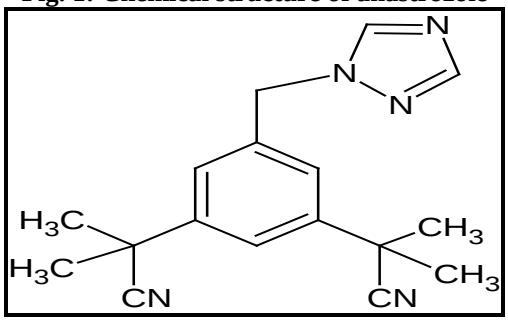
Abstract:
Anastrozole is a nitrile and triazole derivative, act as a selective non-steroidal aromatase inhibitor. It used in the treatment of estrogen nuclear receptor breast cancer in postmenopausal women. A new spectrophotometric method was developed in the UV region for determination of anastrozole in dissolution samples. In water, its solubility is very poor, (0.5 mg/mL at 25 deg C) and solubility is dependent of pH in the physiological range. Absorption characteristics of pure drug was checked in solvents like water, ethanol, phosphate buffer pH 6.8 and pH 7.4 phosphate buffer saline and in pH 7.4 phosphate buffer saline it has good absorption characteristics. Absorption maximum was found at 210 nm and the method was optimized and validated using this wavelength. All validation parameters were found to be in the acceptance range. The developed UV spectrophotometric method was successfully applied for the *In Vitro* studies of anastrozole in its invasomal formulation.
Keywords: Anastrozole, Aromatase inhibitor, Invasomal formulation, UV spectroscopic method.

INTRODUCTION

Anastrozole is a nonsteroidal inhibitor of estrogen synthesis that resembles paclitaxel in chemical structure. As a fourth-generation aromatase inhibitor, anastrozole selectively binds to and reversibly inhibits aromatase, a cytochrome P-450 enzyme complex found in many tissues including those of the premenopausal ovary, liver, and breast; aromatase catalyzes the aromatization of androstenedione and testosterone into estrone and estradiol, the final step in estrogen biosynthesis [1]. In estrogen-dependent breast cancers, anastrozole may inhibit tumor growth. Anastrozole is chemically 2-[3-(2-cyanopropan-2-yl)-5-(1,2,4-triazol-1-ylmethyl) phenyl]-2-methylpropanenitrile and molecular formula $C_{17}H_{19}N_5$. The structure is given in Fig. 1. It is freely soluble in methanol, ethanol, acetone, tetrahydrofuran and very soluble in acetonitrile. In water, its solubility is very poor, (0.5 mg/mL at 25 deg C) and solubility is dependent of pH in the physiological range.

A detailed literature survey reveals few RP-HPLC methods reported for the determination of assay of anastrozole in tablet dosage form [2] and for its anticancer activity [3]. There exists only one UV spectrophotometric method reported for the determination of anastrozole [3]. Hence, we have developed a new, simple and fast analytical method by UV-Visible spectroscopy to quantify anastrozole in the prepared invasomes and its marketed formulations.

Fig. 1: Chemical structure of anastrozole



MATERIAL AND METHODS

Chemicals and Reagents

Analytically pure sample of anastrozole with purity greater than 99% was obtained as gift sample from Suven life sciences Ltd, Hyderabad, India and invasomal formulation of anastrozole was prepared in the pharmaceutics lab at G. Pulla Reddy College of pharmacy, Hyderabad, India.

Instruments

A double beam UV-visible spectrophotometer (Shimadzu, model 1800) having two matching quartz cells with 1 cm light path and loaded with UV probe software (version 2.41). It was used for recording of spectra and measuring absorbance. An electronic analytical weighing balance (0.1mg sensitivity, Shimadzu AY 220), digital pH meter (DELUX model 101) and a sonicator (Sonica, model 2200 MH) were used in this study.

Selection of solvent

Solvent selection is the first step involved in the method development. Solvent is selected based on the solubility of the drugs [4]. Four solvents were selected, and the trials were done using water, ethanol, phosphate buffer pH 6.8 and phosphate buffer saline 7.4 (PBS). Trials were performed by preparing solutions of concentration of 100µg/mL of drug separately using selected solvents and the absorption spectra were recorded and the suitable solvent was selected based on the absorption characteristics. The absorption spectra of all the trials in different solvents are shown in the fig. 2.

Preparation of standard solutions

Standard stock solutions of anastrozole (1000 µg/mL) were prepared separately in pH 7.4 PBS Working standard solutions of the drug (100 µg/mL) was obtained by dilution of the respective stock solutions in PBS [5].

Preparation of 7.4 PBS: 8 g of sodium chloride, 200 mg of potassium chloride, 1.44 g of sodium hydrogen phosphate and 240 mg of potassium dihydrogen phosphate was added to 800 mL of distilled water taken in a suitable container. Volume was adjusted to 1 L with distilled water. pH was adjusted to 7.4.

Determination of absorption maximum

100µg/mL solutions of anastrozole in pH 7.4 phosphate buffer saline was prepared and scanned in the range of 200-400 nm to determine the absorption maximum of the drug [6]. The UV absorption spectrum is shown in fig.3.

Selection of analytical concentration ranges

Calibration standards at five levels were prepared by diluting the standard stock solution in the concentration range of 20-100 µg/ml for anastrozole. The absorbance of these solutions was measured at absorption maximum wavelength.

Preparation of Invasomal anastrozole formulation sample

Liposomal vesicles embodying small amounts of ethanol and terpenes or terpene mixtures are called invasomes. They act as potential carriers with increased skin penetration. Anastrozole invasomes were prepared by film hydration technique [7].

Conventional film method was used to prepare invasomes. Phospholipids in ethanol are dissolved in ethanol: Chloroform (2:1 v/v). This mixture is dried to a thin film by slowly reducing the pressure from 500 to 1 mbar at 50°C using the rotary flash evaporator. The film is kept under vacuum (1 mbar) for 2 h at room temperature. Then, the film deposited is either hydrated for 30 min at lipid phase transition with a mixture of phosphate buffer (pH: 7.4; PBS) containing terpenes or it is hydrated using PBS (pH: 7.4) and after cooling to room temperature, terpene was added to obtain invasomes [8,9]. The obtained vesicles are vortexed and ultrasonicated [10]. The obtained invasomal suspension was then centrifuged at 4^0C for 30 min for 3 cycles and the resultant thick suspension was collected.

From this a quantity of suspension containing 1mg of equivalent drug was taken and transferred to a 100 ml volumetric flask containing 100ml diluent and then stirred for 10 minutes, followed by filtration through 0.45µ nylon membrane filter to get sample stock solution of 1mg/ml. 1 ml of the above stock solution was pipetted out and made up to 100 ml to get working sample solution.

Method validation

The method developed was validated for the following parameters according to the ICH Guidelines Q2 (R1): Validation of Analytical Procedures: Text and Methodology [11].

- Linearity and Range
- LOD (Limit of Detection) and LOQ (Limit of Quantification)
- Precision
- Accuracy

RESULTS AND DISCUSSION

A simple UV method was developed and validated for dissolution studies of anastrozole invasomes.

Method Development

For method development various solvents were explored, including using water, ethanol, phosphate buffer pH 6.8 and phosphate buffer saline 7.4 (PBS). Anastrozole was found to be soluble and stable in 7.4 PBS and hence the buffer was initiated for the determination of suitable detection of wavelength and working concentration of standard. To test the applicability of the developed method to a formulation, (anastrozole invasomes) was studied at working concentration. Absorbance and assay for working concentration of sample at 210 nm was in acceptance limits (98- 102%) with the standard working concentration during extraction of drug in the sample using the solvent (ethanol) for 10 minutes then made up by 7.4 PBS. The procedure affords reproducible quantification of the drug in the sample ranging between 98 and 102%, which is the standard level in any pharmaceutical quality control. Hence the method is optimized.

The method was validated as per ICH guidelines and all the validation parameters were within the acceptance range.

Fig. 2: UV spectrum of anastrozole in water, PBS 7.4, ethanol and 6.8 phosphate buffer

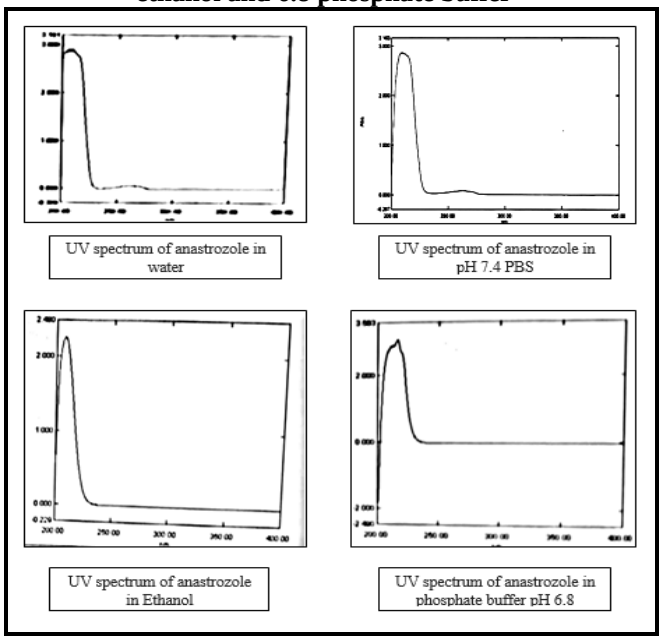


Fig. 3: UV spectrum anastrozole in pH 7.4 PBS

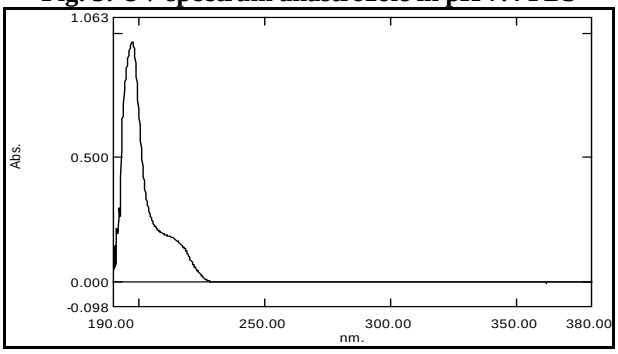


Table 1: Calibration data of anastrozole

Concentration(µg/ml)	Absorbance
20	1.039± 0.8
40	1.596± 0.6
60	2.059± 0.4
80	2.542± 0.2
100	2.906± 0.05
y	0.023x + 0.624
R^2	0.995

Note: All the values are expressed as mean ± SD n=3

Linearity

The results of linearity parameter show an excellent correlation between absorbance and concentration level of drug within the concentration range (20-100 µg/ml) for the drug. The correlation coefficients were greater than 0.995, which meet the method validation acceptance criteria and hence the method is said to be linear in the range of 10-100 µg/ml. The calibration curve data and linearity plot are shown table 1 and fig.4 and fig 5.

Fig. 4: Calibration curve of anastrozole in the conc. range 10-100 µg/ml

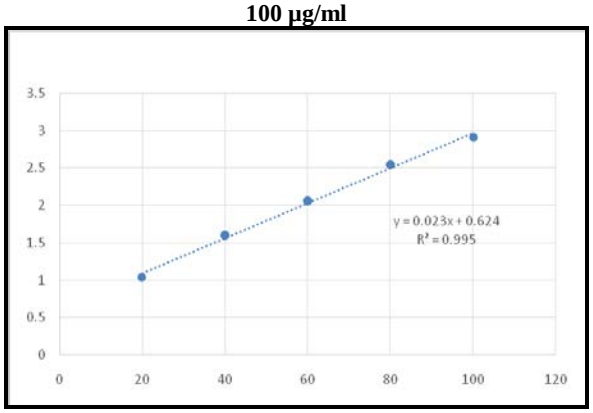
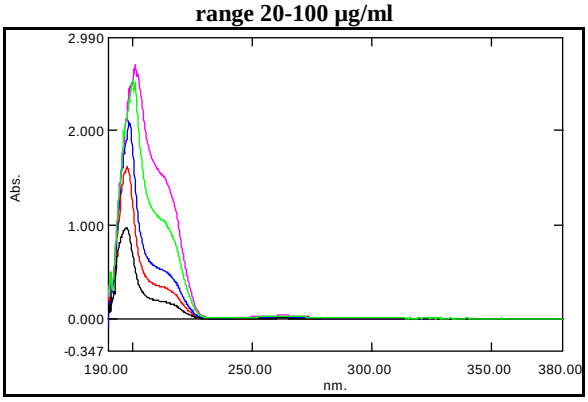


Fig. 5: Overlay absorption spectrum of anastrozole in the con range 20-100 µg/ml



System Precision

Six replicate recording of absorbance at 210nm of standard solution at working concentration showed % RSD (Relative Standard Deviation) less than 2 concerning absorbance for the drug, which shows the adequate reproducibility and thereby the precision of the system. System precision results are tabulated in Table 2.

Table 2: System precision results of anastrozole and its invasomal formulation

n	Absorbance of pure drug	Absorbance of invasomal formulation
1	2.906	1.021
2	2.913	1.018
3	2.943	1.021
4	2.981	1.020
5	2.913	1.017
6	2.987	1.018
average	2.941	1.019
SD	0.036	0.002
%RSD	1.227	0.19

Method precision

Method precision was determined by performing assay of sample under the tests of Intraday precision and Inter day precision, performed during 3 consecutive days by three different analysts, at working concentration. Six consecutive recording of absorbance at 210nm of the sample from the same homogeneous mixture at working concentration (40 µg/ml) showed % RSD less than 2 concerning % assay for the drug which indicate that the method developed is precise by the test of repeatability and hence can be understood that the method gives consistently reproducible results.

Accuracy was determined by means of recovery experiments, by the determination of % mean recovery of sample at three different levels (50-150%). At each level, three determinations were performed. Percent mean recovery was calculated as shown in Table 3. The accepted limits of recovery are 98% - 102% and all observed data are within the required range which indicates good recovery values and hence the accuracy of the method developed.

Sensitivity

The sensitivity of measurement of anastrozole by use of the proposed method was estimated in terms of the limit of quantitation (LOQ), limit of detection (LOD) and was found to be 8.3611 µg/ml and 25.336 µg/ml calculated using standard deviation method.

Application of the method for In vitro studies of Invasomal formulation is shown in table 4. [12]

Table 3: Results of accuracy studies for anastrozole

Drug	Conc. of sample µg/mL	Conc. of standard µg/mL	Recovered concentration µg/mL	% Recovery
Anastrozole	40	10	49.1	98.2
	60	10	68.3	97.5
	80	10	91.7	101.8

Table 4: Dissolution studies data of optimized invasomal anastrozole formulation

Time	Percentage Drug Release
0	0
30	7.88±0.05
60	11.63±0.02
120	16.54±0.01
180	23.94±0.03
240	27.98±0.03
300	35.48±0.08
360	38.56±0.05
420	44.90±0.02
480	52.31±0.07

Note: All the values are expressed as mean ± SD n=3

CONCLUSION

A cost effective and a rapid UV spectrophotometric method was developed and validated for the quantitative estimation of anastrozole as per ICH guidelines. The developed method was applying successfully for in vitro studies of anastrozole formulations during the product development

and optimization. Therefore, it is concluded that the developed UV spectrophotometric method is accurate, precise, linear, rugged and robust and therefore the method can be used for the routine analysis of anastrozole and its formulations.

REFERENCE:

1. Plourde, P. V., Dyroff, M., Dukes, M. Arimidex: a potent and selective fourth-generation aromatase inhibitor. *Breast cancer Res.Treat.*1994, 30, 103.
2. Sathish Kumar, D., Harini, A, Sridhar, D., David Banji, KNV Rao, Guruviah & Yogeswaran. Development and Validation of a HPLC Method for Determination of Anastrozole in Tablet Dosage Form, E-Journal of Chemistry 2011, 8(2), 794-797.
3. Daphal, V.N., Holkar, G., Yadav, R. & Rokade, M. Development and Validation of Simultaneous Determination of Anastrozole and Temozolomide in Pharmaceutical Dosage Forms. *International Journal of Theoretical & Applied Sciences*. 2012, 4(2), 48-55.
4. Sreejith, K.R., Rajagopal, P.L., Premaletha, K. Analytical Method Development and Validation of Anastrozole in Pure and Tablet Dosage Form by UV Spectroscopy *Research J. Pharm. and Tech.* 2017, 10(4), 1015-1019.
5. Mrityunjay Banerjee, Tejaswini Kumari Dash, Ankita Kumari and Swapneswar Khatua. Optimized UV-Vis spectrophotometric method for estimation of anastrozole in pharmaceutical solid dosage form. *Der Pharma Chemica*. 2014, 6 (3), 140-144
6. Srinivasulu, D., Sastry, B. S., Sunil, S.A., Ramana, H. Reverse Phase HPLC Method for The Analysis of Anastrozole In Pharmaceutical dosage Forms. *International Journal of Pharmacy and Pharmaceutical Sciences*. 2010. 2, 75-76.
7. Lakshmi, PK., Kalpana, B., Prasanthi, D. Invasomes novel Vesicular Carriers for Enhanced Skin Permeation. *Syst Rev Pharm.* 2013, 4, 26-30.
8. Haag, SF., Fleige, E., Chen, M., Fahr, A., Teutloff, C., Bittl, R. Skin penetration enhancement of core-multishell nanotransporters and invasomes measured by electron paramagnetic resonance spectroscopy. *Int J Pharm*. 2011, 416, 223-8.
9. Dragicevic-Curic, N., Friedrich, M., Petersen, S., Scheglmann, D., Douroumis, D., Plass W. Assessment of fluidity of different invasomes by electron spin resonance and differential scanning calorimetry. *Int J Pharm*. 2011, 412, 85-94.
10. Kalpana, B and Lakshmi, P.K. Transdermal permeation enhancement of Tolterodine Tartrate through invasomes and iontophoresis, *Der Pharmacia Lettre*. 2013, 5 (6), 119-126.
11. International Conference of Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human use. Validation of analytical procedures: text and methodology ICH Q2 [R1] 2005.
12. Vidya, K., Lakshmi, P.K. Cytotoxic effect of transdermal invasomal anastrozole gel on MCF-7 breast cancer cell line. *J Appl Pharm Sci*, 2019; 9 (03), 050–058.