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VALIDATED HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR THE DETERMINATION OF FULVESTRANT IN PHARMACEUTICAL DOSAGE FORMS

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ABSTRACT

A simple and rapid normal phase high performance liquid chromatography (HPLC) method was developed and validated for quantitative determination of fulvestrant in bulk drug samples and formulations. Fulvestrant was analyzed by using normal phase cyano column (4.6 mm x 25 cm, 5 µm) at ambient temperature, with gradient elution of n-hexane and isopropyl alcohol as a mobile phase (70 : 30 v/v). The flow rate was set 1.5 mL/min and the analysis was performed at a wavelength of 220 nm using Photo Diode Array (PDA) detector. Two peaks were obtained (FST-A and FST-B) for the corresponding enantiomers. The retention time (RT) for FST- A was around 30.5 ± 1 minutes and retention time for FST-B was 30.0 ± 1 min. The calibration curves were linear over a concentration range from 2.5 mg to 7.5 mg/mL. Limit of detection (LOD) for FST-A was 0.0011 mg/mL and LOD for FST-B was 0.0010 mg/mL. Limit of quantitation (LOQ) FST-A was 0.0033 mg/mL and for FST-B was 0.0030 mg/mL respectively. The developed method was successfully applied to estimate the amount of fulvestrant in formulations.

Key words: Fulvestrant, High performance liquid chromatography, Normal phase liquid chromatography, Validation.

INTRODUCTION

Fulvestrant is primarily used in the treatment of hormone receptor positive metastatic breast cancer in post-menopausal women with disease progression following anti-

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estrogen therapy. It is chemically 13-methyl-7-[9-(4,4,5,5,5-pentafluropentyl sulfinyl)nonyl]-7,8,9,11,12,13,14,15,16,17-decahydro-6H-cyxlopenta [a] phenanthrene-3,17- diol. The drug is official in Martindale: The Complete Drug Reference[1]. Its chemical structure is shown in Fig. 1.

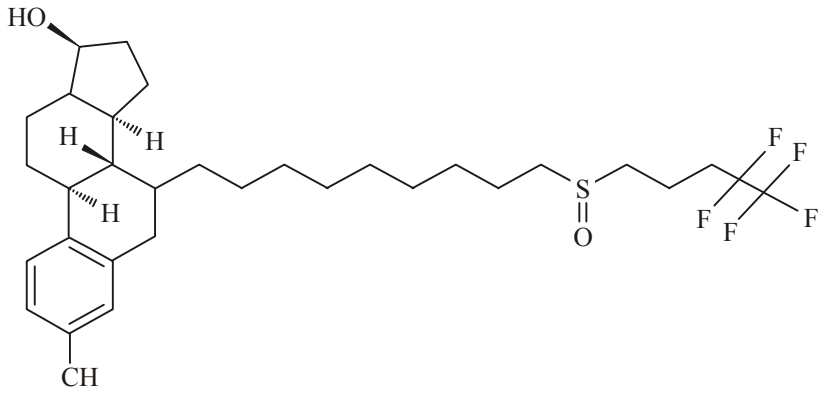

Fig. 1: Chemical structure of fulvestrant

It is an estrogen receptor antagonist with no agonist effects, which works both by down-regulating and by degrading the estrogen receptor. Estrogen is a female hormone that produces growth stimulatory effects on a significant portion of breast cancer cells. By binding to estrogen receptors, fulvestrant inhibits the growth stimulatory effects that estrogen would normally produce. In addition, fulvestrant causes the estrogen receptors to degrade, leaving fewer receptors for estrogen to bind [1].

No HPLC method for quantitative determination of fulvestrant in formulations was reported in the literature. Some of the reports in the literature included the biological activity of fulvestrant[2-6]. The objective of this research was to develop and validate a rapid, economical and sensitive HPLC method for quantitative determination of fulvestrant in bulk drug samples and injectable preparations. In order to minimize batch-to-batch variation, there is an immense need for developing a rapid, sensitive and validated analytical method for day-to-day analysis of the drug in pharmaceutical dosage forms.

EXPERIMENTAL

Chemicals and reagents

Fulvestrant bulk drug (99.70 % purity) and formulations were kind gifts from TherDose Pharma Pvt. Ltd., Hyderabad, India. Isopropyl alcohol and n-hexane (HPLC grade) were obtained from Rankem, India.

Instrumentation

The HPLC system consisted of a LC – 2010 C_{HT}, Shimadzu with PDA Detector. Data acquisition was performed by LC solutions software operated on a Pentium® IV microprocessor. Analysis was carried out at 220 nm with Alltech Platinum Cyano column, (4.6 mm x 25 cm, 5 µm) at ambient temperature, with gradient elution of n-hexane and isopropyl alcohol as a mobile phase (70 : 30 v/v) and the flow rate was set at 1.5 mL/min. The mobile phase was degassed and filtered through 0.2 µm membrane filter before pumping into HPLC system.

Preparation of solutions

Preparation of drug stock solution

The stock solution of fulvestrant was prepared by dissolving accurately weighed quantity of 25.07 mg of the drug in 5 mL of mobile phase (concentration, 5.014 mg/mL).

Calibration standards and quality control samples

Different calibration standards ranging from 50 %, 76 %, 100 %, 126 % and 150 % of the target concentration of 5.0 mg/mL were prepared using stock solution by transferring accurately 2.5 mL, 3.8 mL, 5.0 mL, 6.3 mL and 7.5 mL of stock solution into the 10 mL volumetric flasks and brought to volume with the mobile phase as diluent. An aliquot of 10 µL of above prepared solutions in duplicate were injected onto the chromatographic system connected to Alltech Platinum Cyano Column and the average area in each case was calculated.

Method validation

System suitability

The system suitability was assessed by replicate analysis of six injections of the drug at a concentration of 5 mg/mL. The acceptance criterion was not more than 2% for the percentage relative standard deviation (% RSD) for the peak area and 1.5% for retention time of fulvestrant peaks. The number of theoretical plates should not be less than 2500 and resolution between FST-B and FST-A should be more than 1.0.

Determination of limit of detection and limit of quantitation (sensitivity)

Standard stock solution: Stock solution was prepared by weighing 25.06 mg of fulvestrant and dissolving in 100 mL mobile phase. (Concentration: 0.2506 mg/mL).

A series of solutions were prepared in 3 concentrations of 0.0025 mg/mL, 0.005 mg/mL and 0.0075 mg/mL using the standard stock solution by transferring accurately 1.0 mL, 2.0 mL and 3.0 mL of stock solution into the 100 mL volumetric flasks and brought to volume with the mobile phase as diluent. An aliquot of 10 µL of above prepared solutions was injected into the chromatographic system for 6 times and the mean, standard deviation and relative standard deviation were calculated. Based on the data obtained, the standard deviation at zero concentration was calculated and this value was used for the calculation of the limit of detection and limit of quantitation. The limits of detection (LOD) and quantitation (LOQ) were calculated using the following formulae:

$$LOD = (3.3\ \sigma/S) \quad ...(1)$$

and

$$LOQ = (10\ \sigma/S) \quad ...(2)$$

Where, σ is the standard deviation of the response and S is the slope of the regression line.

Linearity (Calibration curve)

Different calibration standards ranging from 50 %, 76 %, 100 %, 126 % and 150% of the target concentration of 5.0 mg/mL were prepared using stock solution (10.002 mg/mL) by transferring accurately 2.5 mL, 3.8 mL, 5.0 mL, 6.3 mL and 7.5 ml of stock solution into the 10 mL volumetric flasks and brought to volume with the mobile phase diluent. An aliquot of 10 µL of above prepared solutions in duplicate were injected into the chromatographic system and the average area in each case was calculated.

The peak area ratio of the drug was considered for plotting the linearity graph. The linearity was evaluated by linear regression analysis, which was calculated by the method of least squares.

Accuracy and precision

Accuracy of the method was carried out by recovery experiments. Quality control sample solutions of three concentrations 70%, 100% and 130% of the actual concentration of 5.0 mg/mL of fulvestrant containing the excipients used in the inventor formulation were tested and the recovery was calculated in each of the case using the regression line equation.

Demonstration of precision was done under two categories. The injection reproducibility was assessed by injecting six replicate injections of the standard solution of fulvestrant and the relative standard deviation of the replicate injections was calculated.

Six individual preparations of fulvestrant were prepared with target concentration of about 5 mg/mL for method precision.

An aliquot of 10 µL of above prepared solutions was injected in duplicate into the chromatographic system, the chromatograms were recorded and the peak areas of fulvestrant peaks was calculated. Also the mean, standard deviation and the relative standard deviation of six replicate injections were calculated.

Specificity

The specificity of the method shall be demonstrated by interference check by injecting the diluent blank and placebo solution to determine, whether any peaks in the diluent and placebo solution are co-eluting with fulvestrant peaks.

RESULTS AND DISCUSSION

Method development and optimization

Fulvestrant is freely soluble in isopropyl alcohol. The drug can be separated on a cyano column as it is slightly polar with unique selectivity for polar compounds in normal phase mode. The optimization of the method development was done by changing mobile composition by gradient elution. The peak shape and symmetry were good and FST-B and FST-A peaks were resolved with greater than 1.0 resolution at a flow rate of 1.5 mL/min.

Method validation

System suitability

Resolution between FST–B and FST–A was not less than 1.0, number of theoretical plates was not less than 2500, and percentage relative standard deviation (% RSD) for RT was not more than 1.5% and peak area was not more than 2.0 % for fulvestrant peaks. (Both FST–A and FST–B).

The % RSD of peak area and RT for the drug are within 2% indicating the suitability of the system (Table 1). The efficiency of the column as expressed by number of theoretical

plates for the 6 replicate injections was 15, 3915 for FST-B and 18, 3113 for FST-A and mean resolution between FST-B and FST-A is 1.51.

Table 1: System suitability study of fulvestrant

System suitability parameter	FST-B *	FST-A *
Retention time	30.17 + 0.45	30.62 + 0.39
Number of theoretical plates	153915 + 7.12	183113 + 5.45
Peak area	10129563 + 0.52	11546767 + 0.87
Resolution	1.51 +1.22	1.51 +1.22

* Mean ± Standard deviation (n = 6)

Determination of limit of detection and limit of quantitation (Sensitivity)

Limit of detection (LOD) for FST-A was 0.0011 mg/mL and LOD for FST-B was 0.0010 mg/mL. Limit of quantitation (LOQ) for FST-A was 0.0033 mg/mL and for FST-B was 0.0030 mg/mL, respectively.

Linearity

The calibration curve constructed was evaluated by its correlation coefficient. The peak area of the drug was linear in the range of 2.5 to 7.5 mg/mL.

The average areas for each of the concentration obtained were plotted against the concentration of the analyte. The correlation coefficient for the data was calculated as 0.9988 for FST-B and 0.9988 for FST-A indicating a strong correlation between the concentration and the area under the curve.

A linear regression graph was drawn between the concentration of the analyte versus area. The regression line was determined to be y = 2E + 06x –55879 for FST-B and y=2E + 06x – 30930 for FST-A. These experiments indicated that there was a linear relationship between the amounts of analyte and the areas within the range studied (2.5 mg/mL to 7.5 mg/mL). The chromatogram of fulvestrant extracted from the formulation and pure fulvestrant can be observed in Figs. 2 and 3.

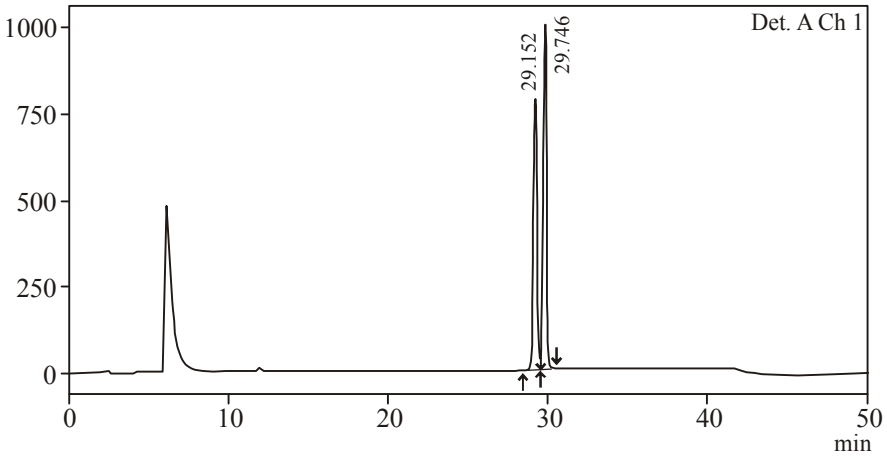

Fig. 2: QC sample chromatogram of fulvestrant extracted from formulation (50 mg/mL)

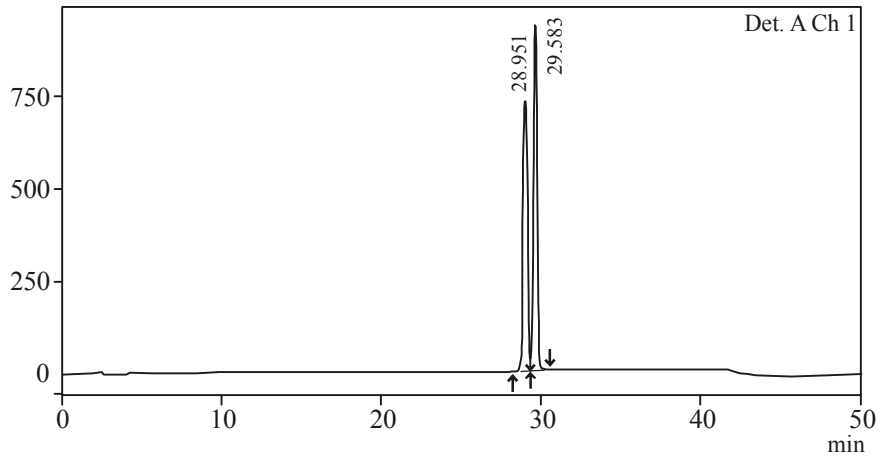

Fig. 2: Chromatogram of fulvestrant extracted from standard solution (5 mg/mL)

Accuracy and precision

Accuracy of the method was determined by recovery experiments. Quality control sample solutions of 3 concentrations 70%, 100% and 130% of the actual concentration of 5.0 mg/mL of fulvestrant containing the excipients used in the inventor formulation were tested and the recovery was calculated in each case using the regression line equation. A regression line graph was drawn using the amount added on the x-axis and the amount found on the y-axis. The slope and intercept were calculated for the regression line (Method of least squares). The results revealed that there was a strong correlation between the amount added and amount found for both; FST-A and FST-B (Tables 2 and 3). The results obtained

from precision experiments also indicated a good method precision (Table 4). The injection reproducibility was assessed by using 6 injections of the standard solution and the relative standard deviation of the replicate injection was calculated (Table 4). Six individual preparations of fulvestrant were prepared with target concentration of about 5 mg/mL for method precision.

Table 2: Accuracy data for FST – B

Amount added	Amount found	% Recovery
3.5000	3.5010	100.0
3.5000	3.4888	99.7
3.5000	3.5088	100.3
5.0000	4.9500	99.0
5.0000	5.0147	100.3
5.0000	5.0381	100.8
6.5000	6.5076	100.1
6.5000	6.4876	99.8
6.5000	6.5034	100.1
Mean ± Standard deviation for % recovery was 100 ± 0.49		

Table 3: Accuracy data for FST –A

Amount added	Amount found	% Recovery
3.5000	3.5005	100.0
3.5000	3.4772	99.3
3.5000	3.5129	100.4
5.0000	4.9682	99.4
5.0000	5.0213	100.4
5.0000	5.0295	100.6
6.5000	6.5033	100.1
6.5000	6.4840	99.8
6.5000	6.5033	100.1
Mean ± Standard deviation for % recovery was 100 ± 0.44		

Table 4: Method precision

Solution ID	Conc. (mg/mL)	Assay % of FST – A and FST – B
Standard solution	5.0140	100.0
Preparation – 1	5.0020	99.2
Preparation – 2	5.0780	101.6
Preparation – 3	5.0100	101.5
Preparation – 4	5.0060	99.9
Preparation – 5	5.0200	101.4
Preparation – 6	5.0040	100.6
Mean ± Standard deviation for the assay % was 100.7 ± 0.98		

Application of the method to dosage forms

The HPLC method developed is sensitive and specific for the quantitative determination of fulvestrant. The method is validated for different parameters and hence, it has been applied for the estimation of drug in pharmaceutical dosage forms. Injections of inventor formulation from Ther Dose Pharma Pvt. Ltd, India, were evaluated for the amount of fulvestrant present in the formulation. Each sample was analyzed in triplicate and the amount of fulvestrant in the formulation was 100 %. None of the injection excipients interfered with the analyte peak as seen in the Figs. 2 and 3.

Specificity

The specificity of the method was demonstrated by checking the interference of any other peaks with drug peaks. This was performed by injecting the diluent blank and placebo solution to determine whether any impurity peaks in the diluent and placebo solution peaks are co-eluting with fulvestrant peaks. No interference of peaks eluted in the blank and placebo solution with fulvestrant peaks was observed (Fig. 3).

A rapid and specific, gradient HPLC method was developed for the determination of fulvestrant using PDA detector. The method was validated for accuracy, precision, linearity, specificity, limit of detection and limit of quantitation. The method used a simple mobile phase composition with gradient elution. Efficient UV detection at 220 nm was found to be suitable without any interference from injectable solution excipients or solvents. Two peaks

were obtained (FST-A and FST-B) for the corresponding enantiomers. The retention time for FST- A was around 30.58 minutes and retention time for FST-B was 30.12 minutes. The calibration curves were linear ($r \geq 0.9988$ and 0.9988) over a concentration range from 2.5 mg to 7.5 mg/mL. Limit of detection (LOD) for FST-A was 0.0011 mg/mL and LOD for FST-B was 0.0010 mg/mL. Limit of quantitation (LOQ) for FST-A was 0.0033 mg/mL and for FST-B was 0.0030 mg/mL, respectively. The developed method was successfully applied to estimate the amount of fulvestrant in injection formulations. The proposed HPLC method is precise, accurate, sensitive, specific and efficient and can be used in routine analysis in quality control laboratories.

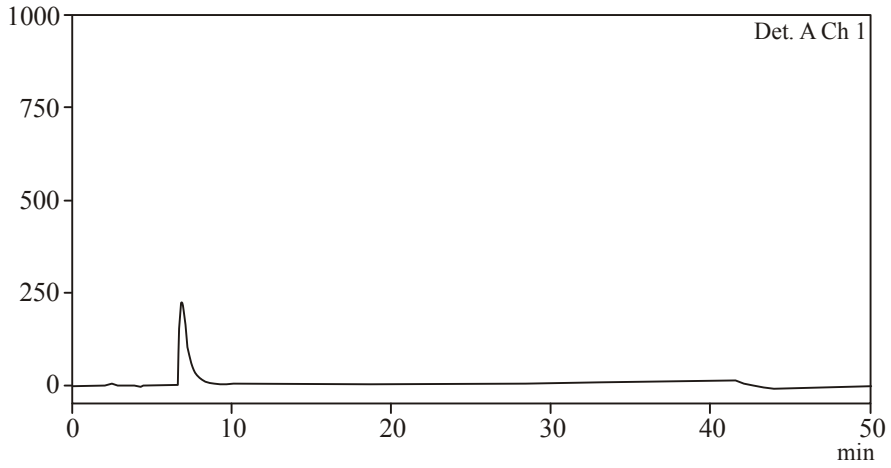

Fig 3: Chromatogram depicting the specificity of fulvestrant; Chromatogram obtained by injecting placebo solution

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