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A Novel RP-HPLC Method for the Quantification of Fulvestrant in Formulations

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ABSTRACT
A simple, precise and accurate RP-HPLC method was developed and validated for rapid assay of Fulvestrant in tablet dosage form. Isocratic elution at a flow rate of 1 ml/min was employed on a symmetry Chromosil C18 (250x4.6mm, 5µm in particle size) at ambient temperature. The mobile phase consisted of Methanol: 1% OPA: 85:15 % (V/V). The UV detection wavelength was 243 nm and 20µl sample was injected. The retention time for Fulvestrant was 5.3 min. The percentage RSD for precision and accuracy of the method was found to be less than 2%. The method was validated as per the ICH guidelines. The method was successfully applied for routine analysis of Fulvestrant in tablet dosage form and bulk drug.

Keywords: Fulvestrant, RP-HPLC, UV detection, recovery, precise, 243 nm

1. INTRODUCTION
Fulvestrant is used in treatment of hormone receptor-positive metastatic breast cancer in postmenopausal women with disease progression following anti-estrogen therapy. It is an estrogen receptor antagonist with no agonist effects, which works both by down-regulating and by degrading the estrogen receptor[1]. It is administered as a once-monthly injection. Fulvestrant is indicated for the treatment of hormone receptor positive metastatic breast cancer in postmenopausal women with disease progression following anti-estrogen therapy. The dosing schedule for fulvestrant remains under investigation in an attempt to optimize its effectiveness[2]. Headache, back pain, nausea, constipation, diarrhea, vomiting are side effects.

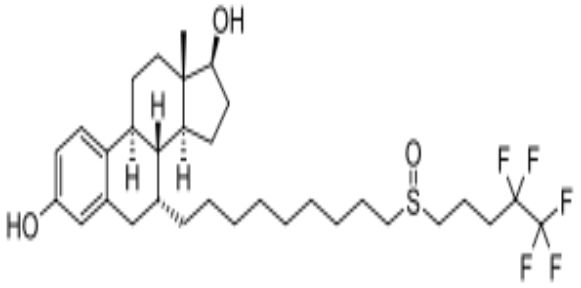

Fig-1

2. EXPERIMENTAL
2.1 *Materials*
Working standard of Fulvestrant was obtained from well reputed research laboratories. HPLC grade methanol was purchased from E. Merck (Mumbai, India).

2.2 *Apparatus*
A Series HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil C18. 250×4.6mm, Electronic balance-DENVER (SI234), a manual Rheodyne injector with a 20 µl loop was used for the injection of sample. PEAK LC software was used. UV 2301 SPECOPHOTOMETER was used to determine the wavelength of maximum absorbance

2.3 *Determination of wavelength of maximum absorbance*
The standard solutions of Fulvestrant were scanned in the range of 200-400 nm against mobile phase as a blank. Fulvestrant showed maximum absorbance at 243 nm. So the wavelength selected for the determination of Fulvestrant was 243 nm.

2.4 *Chromatographic equipment and conditions*
The development and validation of the assay was performed on A Series 200 HPLC system PEAK LC7000 isocratic HPLC with PEAK 7000 delivery system. Rheodyne manual sample injector with switch (77251), Analytical column Chromosil 100-5 C18. 250×4.6mm, manual injector rheodyne valve) with 20µL fixed loop, PEAK LC software was used.

The mobile phase consisted of Methanol: 1% OPA: 85:15 (v/v). Injections were carried out using a 20 µl loop at room temperature (20+2°C) and the flow rate was 1 ml/min. Detection was performed at 243 nm with 8 min runtime.

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2.5 *Standard and sample solutions*

A 10 mg amount of Fulvestrant reference substance was accurately weighed and dissolved in 10 ml mobile phase in a 10 ml volumetric flask to obtain 1000 ppm concentrated solution. From standard solution by the serial dilution we prepared required concentrations of 100 ppm. From this 2 ml was taken and made upto 10 ml using mobile phase. A composite of 20 tablets was prepared by grinding them to a fine, uniform size powder. 10 mg of Fulvestrant was accurately weighted and quantitatively transferred into a 100 ml volumetric flask. Approximately 27 ml mobile phase were added and the solution was sonicated for 15 min. The flask was filled to volume with mobile phase, and mixed. After filtration, an amount of the solution was diluted with mobile phase to a concentration of 20 µg/ml.

2.6 *Method validation*

Method validation was performed following ICH specifications for specificity, range of linearity, accuracy, precision and robustness.

3. RESULTS AND DISCUSSION

3.1 *System Suitability*

Having optimized the efficiency of a chromatographic separation the quality of the chromatography was monitored by applying the following system suitability tests: capacity factor, tailing factor and theoretical plates.

Table-1: System suitability parameters

Mobile phase	Methanol: 1% OPA : 85:15%(v/v)
Pump mode	Isocratic
pH	4.2
Diluents	Mobile phase
Column	Zodiac C18 column (250 X 4.6 mm, 5µ)
Column Temp	Ambient
Wavelength	243nm
Injection Volume	20 µl
Flow rate	1 ml/min
Run time	8 minutes
Retention Time	5.3 minutes

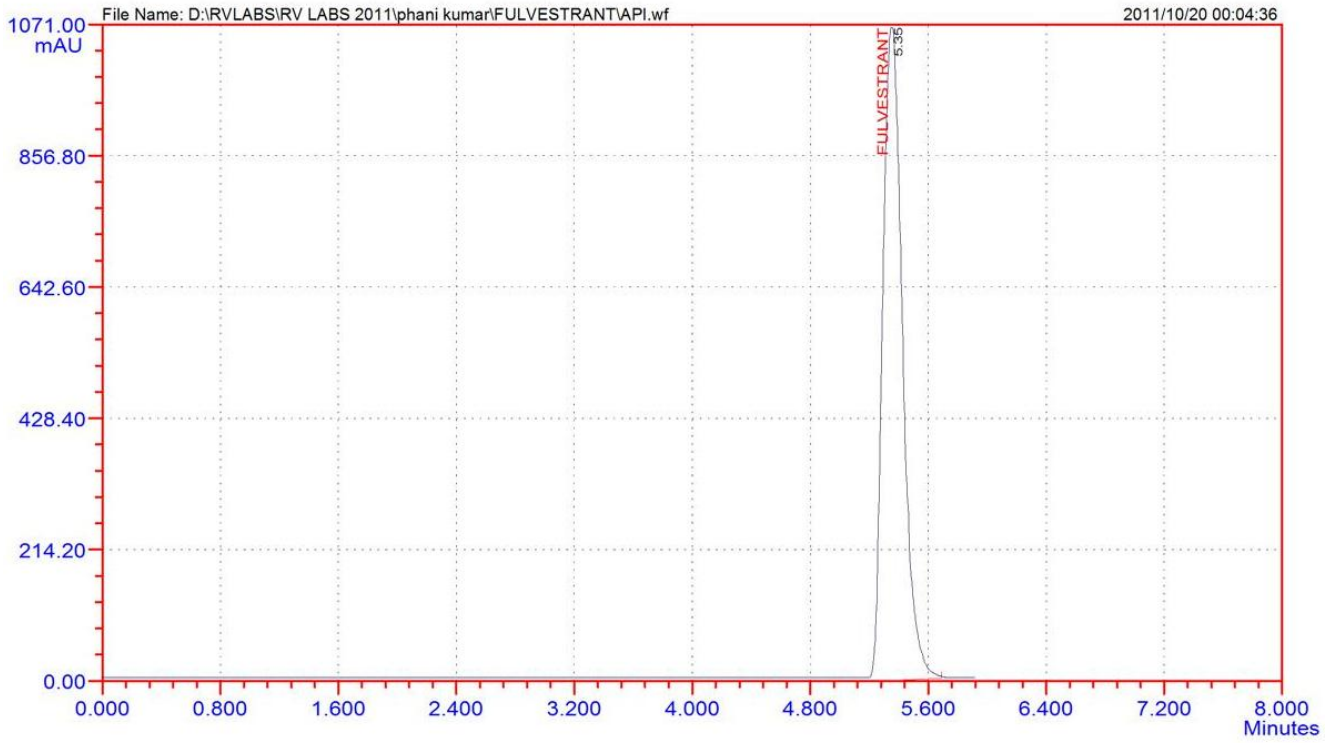


Fig-2

The system suitability method acceptance criteria set in each validation run were: capacity factor >2.0, tailing factor ≤2.0 and theoretical plates >2000 13. In all cases, the relative standard deviation (R.S.D) for the analytic peak area for

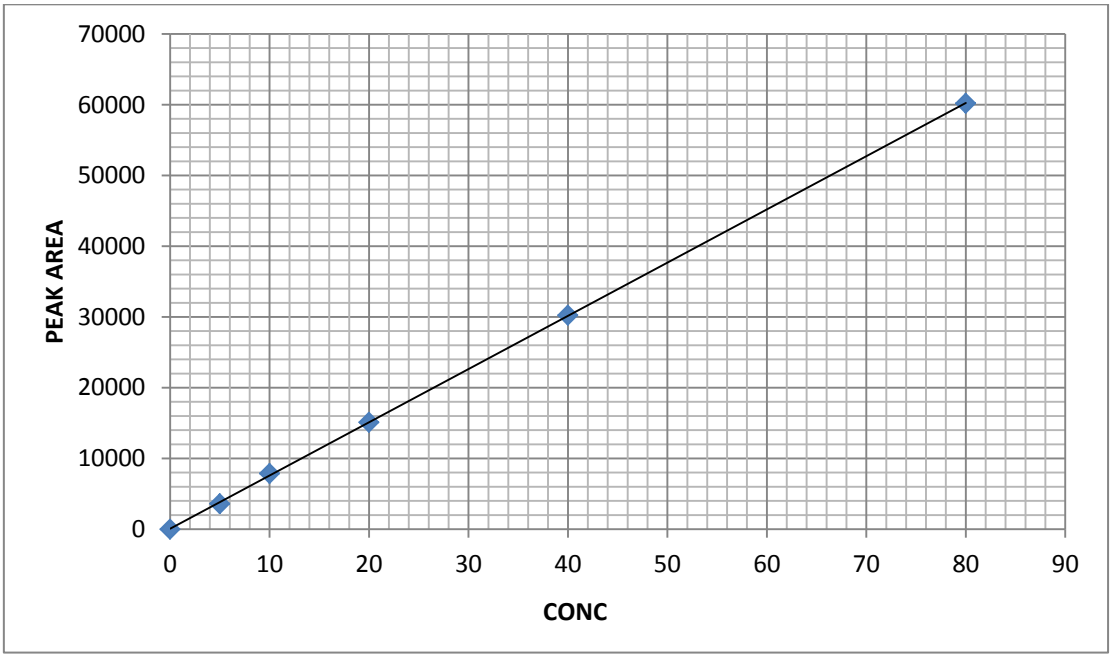
two consecutive injections was < 2.0%. A chromatogram obtained from reference substance solution is presented. System suitability parameters were shown in Table-1. Standard chromatogram was given in Figure.2

3.2 *Range of linearity*

Standard curves were constructed daily, for three consecutive days, using five standard concentrations in a range of 5, 10, 20, 40, 80 µg/ml for Fulvestrant. The linearity of peak area responses versus concentrations was demonstrated by linear least square regression analysis. The linear regression equation was y = 42598 + 6031x (r= 0.997). Linearity values can shown in Table-2.

Table-2

Level	Concentration of Fulvestrant in PPM	Peak Area
Level 1	5	3592
Level 2	10	7856
Level 3	20	15098
Level 4	40	30247
Level 5	80	60187
Range 5 ppm to 80 ppm	SLOPE INTERCEPT CORREALATION COEFFICIENT	752.3 60.45 0.999



Graph-1: Linearity Curve

3.3 *Precision*

To study precision, six replicate standard solutions of Fulvestrant (20 ppm) were prepared and analyzed using the proposed method. The percent relative standard deviation (% RSD) for peak responses was calculated and it was found to be which is well within the acceptance criteria of not more than 2.0%. Results of system precision studies are shown in Table-3 and Table-4.

3.4 *Precision Results for Fulvestrant*

Table-3

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTER DAY RSD (Acceptance criteria ≤ 2.0%)
Fulvestrant	20	1	15426	0.21
		2	15349	
		3	15427	
		4	15365	
		5	15418	
		6	15411	

Table-4

Sample	Conc. (in ppm)	Injection No.	Peak Areas	INTRA DAY RSD (Acceptance criteria ≤ 2.0%)
Fulvestrant	20	1	15489	0.42
		2	15326	
		3	15319	
		4	15425	
		5	15420	
		6	15417	

3.5 *Limit of Detection and Limit of Quantification*

To determine the Limit of Detection (LOD) sample was dissolved by using Mobile phase and injected until peak was disappeared. After 0.02 ppm dilution Peak was not clearly observed, based on which 0.02 ppm is considered as Limit of Detection and Limit of Quantification is 0.05 ppm.

Table-5

Parameter	Measured Value
Limit of Quantification	0.05 ppm
Limit of Detection	0.02 ppm

3.6 *Robustness*

Typical variations in liquid chromatography conditions were used to evaluate the robustness of the assay method. In this study, the chromatographic parameters monitored were retention time, area, capacity factor, tailing factor and theoretical plates. The robustness acceptance criteria set in the validation were the same established on system suitability test describe above.

Table-6

S.no	Parameter	Condition	Area	% of Change
1	Standard	Standard conditions	15098	100%
2	Mobile phase	Methanol: 1% OPA : 80:20%	15211	100.7%
3	Mobile phase pH	4.4	15340	101.6%
4	Wavelength	241 nm	15229	100.8%

3.7 *Recovery*

Recovery test was performed at 3 different concentrations i.e. 20ppm, 40ppm, 80ppm. Results are given in table.7

Table-7

Recovery	Conc. of sample(ppm)	Recovery(ppm)	% of recovery
50%	20	19.64	98.2%
100%	40	39.92	99.8%
150%	80	79.96	99.95%

Table-8: Formulation Analysis

S.NO	Tablet	Dosage	Sample conc	Sample estimated	% of Drug Estimated in Tablet
1	FASLODEX	500 mg	50 ppm	49.96 ppm	99.9%

4. CONCLUSION

The proposed method for the assay of Fulvestrant in tablets or capsules is very simple and rapid. It should be emphasized it is isocratic and the mobile phase do not contain any buffer. The method was validated for specificity, linearity, precision, accuracy and robustness. Although the method could effectively separate the drug from its products, further studies should be performed in order to use it to evaluate the stability of pharmaceutical formulations.

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