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A Novel RP – HPLC Analytical Method Development And Validation Of Fulvestrant Injection As PER ICH Guidelines

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Abstract

In this present project, a novel, Reverse Phase - High Performance Liquid Chromatographic analytical method was developed for determination of Fulvestrant Injection, which is fast & economical too. Retention time of Fulvestrant was at 21 minutes, which quite fast by using the Zorbax XDB C18; 150 × 4.6 mm, 3.5, column as stationary phase with mobile phase consisted of a mixture of Mobile phase –A: Water, Acetonitrile and Methanol (410:320:270), Mobile phase-B: Acetonitrile, Methanol and Water (490:410:100) in a gradient elution and at a flow rate of 2 ml/min. Detection was carried out at 225 nm in HPLC. Newly developed method shows linearity in the range of 80-120 µg/ml & correlation co-efficient for this method was found to be 0.999. The accuracy studies showed % recovery of Fulvestrant injection, was in the range 99.7-102 % in the newly developed method. Validation parameters were within the permitted limits so this method was found to be precise, accurate and specific. Present method is better in terms of economical aspect, easy to perform & is very much specific towards the targeted drug, which is evidenced from the validation parameter. So this unique method can be efficiently employed for determination of Fulvestrant in commercial products, economically.

Keywords: RP HPLC, Fulvestrant injection, Validation, ICH guidelines, Method Validation

Introduction

Any drug analysis requires a sensitive and precise pro cess that gives a confirmed result. Analytical chemistry [1-4] is an essential chemistry chapter that offers comprehensive details about the method development process. [5-9] Fulvestrant (7-alpha-[9(4,4,5,5,5-penta fluoropentylsulphinyl) nonyl] estra-1,3,5-(10)-trine-3,17-beta-diol) [10-12] is a new Estrogen receptor antagonist that is globally available in postmenopausal women for the treatment of hormone receptor-positive metastatic breast cancer. For the past few years, tamoxifen has been used in the treatment of breast cancer, but due to some of its side effects, it has not been used as before. The novel oestrogen receptor antagonist Fulvestrant has proved to be a safe and effective treatment for advanced breast cancer in women. Breast cancer in women all over the world is a very common form of cancer. Different clinical trials have been published in cancer treatment journals to date. Fulvestrant is a poorly water soluble molecule, so it is available in extended release form to address this problem. There are a large number of research papers that provide a clear idea of this drug's pharmacology & pharmacokinetics, but very little information is available about its analytical methodology. Few analytical methods [13-18] using RP-HPLC were extracted from a comprehensive literature review. [19-24] Table No 1, which demonstrates the overall aspects of the previously established approaches, offers a comparative analysis.

In various ways, this approach is better than the previously existing methods, which are explained in detail below.

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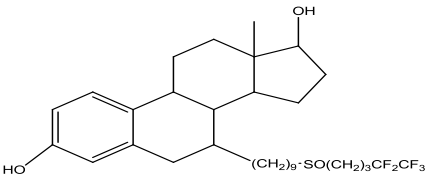

fulvestrant

Fig 1. Chemical structure of the Fulvestrant.

As compared to other methods, the column length of the newly developed system is smaller, so the solvent consumption would be smaller than that of the other methods, resulting in lower research costs. The column size of the particles used in this process is 3.5 µm, which is less than the previous methods, i.e. 5 µm, so the chromatogram quality would be better than the other methods. Mobile phase is very easy to prepare, more ever there are two parts of mobile phase i.e. Phase A & Phase B which is used in a gradient elution, gradient scheme is mentioned in the Table no 2, whereas the other methods are isocratic. Because of this method's gradient elution, it is very specific & no of USP plate count obtained is 9647, which is more than the number needed as per ICH guideline & other method established, so the method is superior to previous methods. This method 's overall chromatographic condition such as peak area, height, USP telling, resolution, etc. is within the permitted limit, so it is convenient and easy with regard to other established methods.

Colum length (150 mm) of the newly developed method is lesser, as compared to other methods (250 mm), so the solvent consumption will be less than the other methods, as a result, cost of analysis will be less. Particle size of the column used in this method is 3.5 µm, which is lesser than the previous methods i.e. 5 µm, so the quality of Chromatogram will be better than the other methods. Mobile phase is very easy to prepare, more ever there are two parts of mobile phase i.e. Phase A & Phase B which is used in a gradient elution with a total run time of approx..67 min, gradient scheme is mentioned in the Table no 2, whereas the other methods are isocratic. Due to gradient elution of this method, it is very much specific & no of USP plate count obtained is 9647, which is more than the required number as per ICH guideline & other developed method, so the method is superior than previous methods. Overall Chromatographic condition of this method like, peak area, height, USP telling, resolution etc. is within the permitted limit so it is convenient & simple in respect to other developed methods [25-28]. (ICH, Harmonized Tripartite Guideline. Validation of Analytical Procedures, 2005).

Table 1. Comparative study of Literature of Fulvestrant

SL No.	Method	Parameters
Method - 1	RP-HPLC	Column: Chromosil (250 mm × 4.6 mm, 5µm) Mobile phase: Methanol: 1 % OPA (85:15) Flow rate: 1 ml/min. Wavelength: 243 nm.
Method – 2	LC MS	Column: ODS-AM Column, (250 mm × 4.6 mm, 5µm) Mobile phase: Ammonium acetate in Water: Methanol (10: 90) Flow rate: 1 mL/min. Wavelength: 254 nm.
Method – 3	HPLC	Column: Chromosil C8 column (250 mm×4.6 mm,5 µm), Mobile phase: Methanol: Acetonitrile: Water) (70: 7.5: 22.5) Flow rate: 1.2 mL/min. Wave length: 285 nm
Method – 4	RP- HPLC	Column: Phenomix, C18 column (250 mm x 4.6 mm x 3.5µ) Mobile phase: 50% Aetonitrile in channel A and Acetonitlile in channel B Flow rate: 1 ml/min. Wavelength: 225 nm.
Method – 5	RP- HPLC	Column: Waters X- Terra RP_{18} column (250 × 4.6 mm i.d. × 5 µm) Mobile phase: Acetonitrile: Water (65:35; v/v) Wavelength: 215 nm.

Table 2. Shows gradient elution scheme of mobile phase

Time(min.)	%A	%B	Elution
0-25	100	0	Isocratic
25-55	100 0	0 100	Linear gradient
55-65	0	100	Isocratic
65-66	0 100	100 0	Linear gradient
66-70	100	0	Equilibration

EXPERIMENTAL:

Chemicals & reagents:
Fulvestrant bulk drug (99.70 % purity) and formulations were kind gifts from, Natco Pharma Ltd., Hyderabad, India. Acetonitrile (HPLC grade), Methanol (HPLC grade, Water for injection were obtained from Rankem, India. It was kept over molecular sieves (3Å, Merck), to remove moisture after purification.

Chromatographic condition:

The HPLC system consisted of Shimadzu LC-2010 with UV/PDA Detector. Pump – LC- AT vp. The wavelength of detection as set at 225 nm. Separation was carried out on Zorbax XDB C18; 150×4.6mm, 3.5 µ or equivalent using mobile phase 'A' i.e., Water, Acetonitrile and Methanol in the ratio of 410:320:270, and Mobile phase 'B' i.e., Acetonitrile, Methanol and Water in the ratio of 490:410:100 respectively in a gradient elution at a flow rate of 2 ml/ min. The mobile phase filtered through nylon milli pore (0.2µm) membrane filter.

Preparation of solutions:

Mobile phase preparations:

Mobile Phase - A: A mixture of water, acetonitrile and methanol in the ratio of 410:320:270 was prepared respectively. Filter through 0.22 µ membrane filter and the mixture was degassed.

Mobile Phase - B: A mixture of Acetonitrile, methanol and water in the ratio of 490:410:100 was prepared respectively. Filter through 0. 22 µ membranes filter and the mixture was degassed.

Standard solution: 100 mg of Fulvestrant working standard or reference standard was weighed accurately & was dissolved in to 10 ml volumetric flask and dilute to volume with diluent mix well.

Test solution: About 2 ml of sample was transferred in to a 10 ml volumetric flask, was dissolved and diluted to volume with diluent mix well.

Mobile Phase Optimization: The mobile phase was tried with gradient elution technique of mobile phase A i.e., Water, Acetonitrile and Methanol in the ratio of 410:320:270 respectively, and mobile Phase B i.e., Acetonitrile, Methanol and Water in the ratio of 490:410:100 respectively and then it was optimized.

Optimization of Column: The method was performed with various columns like Hypersil ODS column and Zorbax XDB C18. Zorbax XDB C18 Column was found to be ideal as it gave good peak shape at 2 ml/min flow.

RESULTS AND DISCUSSION:

Method Development:

During the development of analytical method, different solvents were used with varying Chromatographic condition. After different trials the final Mobile phase was selected as –A: Water, Acetonitrile and Methanol (410:320:270), Mobile phase-B: Acetonitrile, Methanol and Water (490:410:100) and at a flow rate of 2 ml/min. Optimized Chromatograms from standard & sample are shown in the Figure no 2 & 3 respectively.

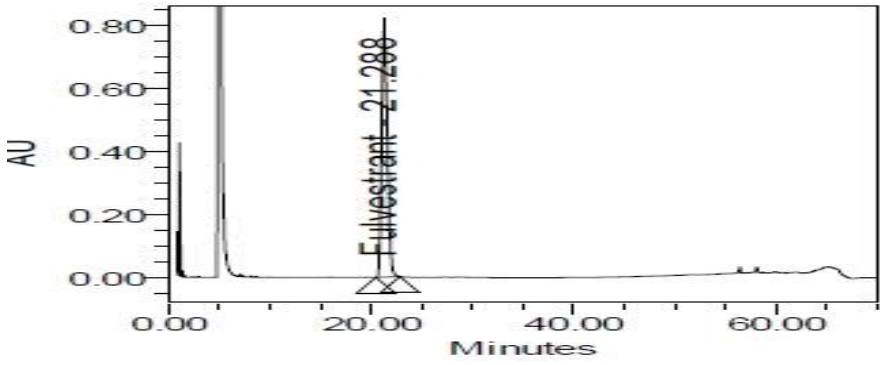

Fig 2. Shows optimized Chromatogram for Standard

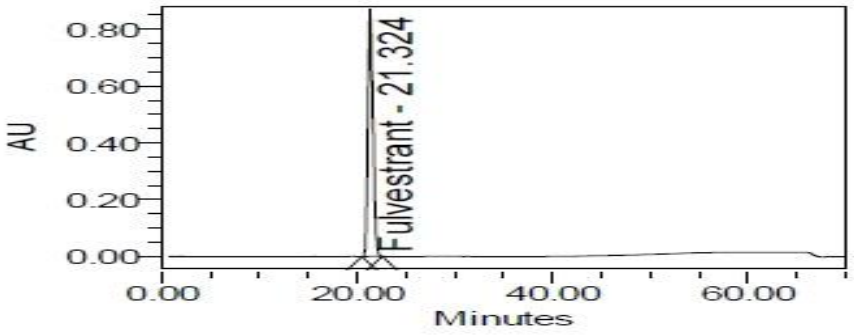

Fig 3. Shows optimized Chromatogram for Sample

Table 3. Shows Recovery studies for Fulvestrant inj.

S. No	Accuracy %	Amount added (mg)	Area obtained	Amount found	% Recovery
1.	**80%**	7.92	22257411	7.85	99.12
2.		7.94	22372016	7.89	99.37
3.		7.91	22231672	7.84	99.12
1.	**100%**	9.92	28044036	9.89	99.69
2.		9.91	28347717	9.99	100.81
3.		9.92	27990355	9.87	99.49
1.	**120%**	11.89	33413030	11.78	99.07
2.		11.87	33609946	11.87	100.0
3.		11.90	33809046	11.92	100.2

Table 4. Shows Average % Recovery for Fulvestrant injection

Accuracy%	Avg.% recovery
80%	99.20
100%	99.99
120%	99.76

Table 5. Shows Results of Standard solution summary Precision

S. No.	RT of Fulvestrant inj.	Peak area of Fulvestrant inj.
Injection-1	19.70	28527587
Injection-2	19.56	28878505
Injection-3	19.44	28489747
Injection-4	19.37	28881694
Injection-5	19.36	28625915
Injection-6	19.35	28559384
MEAN	**19.46**	**28660472**
STANDARD DEVIATION	**0.00**	**175895.4128**
% RSD	**0.00**	**0.61**

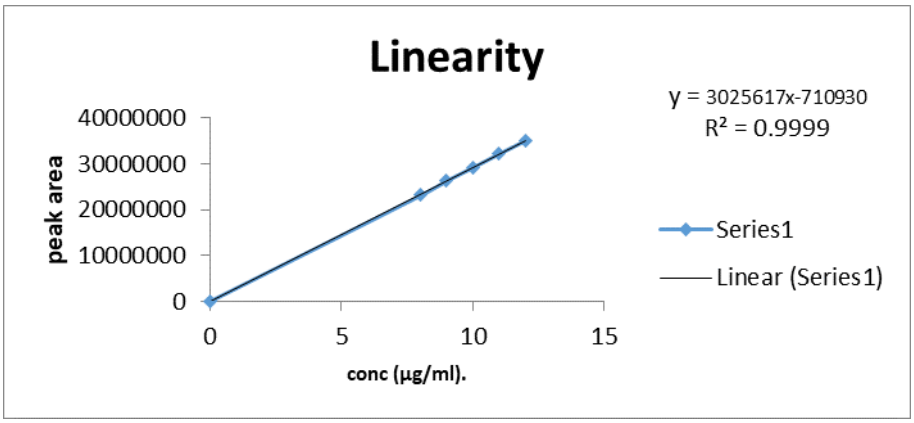

Fig 4. Shows Calibration graph for linearity of Fulvestrant inj.

Table 6. showing Linearity results for Fulvestrant Injection

Sl.No	Linearity level	Fulvestrant inj.	
		Conc. (mg/ml)	Peak area
1.	80%	7.91	23126222
2.	90%	8.90	26320694
3.	100%	9.89	29248506
4.	110%	10.88	32246028
5.	120%	11.87	35141021
Linearity range (mg/ml)		7.91-11.87	
Y Intercept		-710930	
Slope		3025617	
Correlation co-efficient (r^2)		0.999	

Table 7. Shows Results of effect of flow rate

SL. No	RT of Fulvestrant inj.	Area of Fulvestrant inj.	RT of Fulvestrant inj.	Area of Fulvestrant inj.
	Low flow rate	Low flow rate	High flow rate	High flow rate
1.	20.23	20748953	20.132	20742441
2.	20.10	20748853	20.120	207424852
Avg.	20.1	2097471	20.1	20742559
SD	0.00	1836.5	0.00	145.66
RSD	0.00	0.08	0.00	0.07

Table 8. Shows Sample solution summary Intermediate precision (Day-1)

Name	RT of Fulvestrant inj.	Peak area of Fulvestrant inj.
Ruggedness-(Day-1)-1	21.29	31043363
Ruggedness-(Day-1)-2	21.20	30972897
Ruggedness-(Day-1)-3	20.90	31037132
Ruggedness-(Day-1)-4	20.79	31132498
Ruggedness-(Day-1)-5	20.75	31125157
Ruggedness-(Day-1)-6	20.69	30877450
MEAN		**31043363**
STANDARD DEVIATION	**0.00**	**96239.65**
% RSD	**0.00**	**0.31**

Table 9. Shows Sample solution summary Intermediate precision (Day-2)

Name	RT of Fulvestrant inj.	Peak area of Fulvestrant inj.
Ruggedness-(Day-2)-1	21.29	31043633
Ruggedness-(Day-2)-2	21.20	30972667
Ruggedness-(Day-2)-3	20.90	31037231
Ruggedness-(Day-2)-4	20.79	31132489
Ruggedness-(Day-2)-5	20.75	31125517
Ruggedness-(Day-2)-6	20.69	30877050
MEAN	**20.9**	31031341
STANDARD DEVIATION	**0.00**	**96471.8**
% RSD	**0.00**	**0.31**

Method validation:

After successful optimization of developed method, it was validated using different validation parameter as per ICH guideline. A details of validation outcomes are described below with proper data & results.

1. Accuracy:

It was determined in three different concentration levels, & by calculating % recovery. Accuracy results are given in the Table no 3 & 4 respectively.

Acceptance Criteria:

- The % Recovery for each level should be between 98.0 to 102.0%.

The results obtained for recovery are within the limits. Hence method is accurate.

2. Precision:

Precision was studied using 6 concentration, % RSD value was obtained as 0.61, which data is mentioned in the Table no 5.

Acceptance criteria:

- % RSD of different analyte should not be more than 2.

The % RSD obtained is within the limit, hence the method is precise.

3. Linearity & Range:

Linearity study was carried out within the concentration range of 7.91 mg/ml & 11.87 mg/ml or in the % range of 80 – 120 %. Coefficient of Correlation (r^2) value was 0.999, which was within the limit. Calibration graph is given in the Figure no. 4 & values are given in the Table no 6.

Acceptance criteria:

Correlation coefficient (R^2) should not be less than 0.999

- The correlation coefficient obtained was 0.999 for Acotiamide which is in the acceptance limit.

4. Robustness:

This study was performed to find, whether the method is susceptible to small changes. Flow rate was changed ± 0.1ml/min, then peak area & retention time was noted as per table no 7. Which show there is no significance change in both the parameter. So, the method was robust as per ICH guideline.

5. Ruggedness: This study is performed to check the change in the Chromatographic parameters with the major change in the method, like change in chemist, day, place etc. In the present study, intermediate precision was performed, in different days. % RSD value was 0.31 % on both the days, which was within the limits. Result is given in the Table no 8 & 9 respectively. So, the method was rugged as per ICH guideline.

CONCLUSION:

In terms of accuracy, linearity, precision, robustness & robustness in HPLC, the developed method was statistically validated. The method developed is compared with the other method developed. The superiority of the developed method was demonstrated in detail with supporting evidence, showing that the new method can be effectively used to test the drug. Calibration curve was obtained by using peak area vs concentration. In the HPLC method development, Fulvestrant Injection shows linearity in the range of 7.91-11.87 mg / ml. Calibration curve was diagrammed and correlation co-efficient for the drug was found to be 0.999. Accuracy, Precision, Ruggedness & Robustness of the newly developed method was carried out as per ICH guideline. The results obtained on the validation parameters was with the statistical specification at per with ICH requirements. The Chromatographic method developed for Fulvestrant inj. was found to be economic, rapid, simple, sensitive, precise, and accurate. This method can be used for analysis of commercial formulation in future also.

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