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Development and validation of HPLC-UV method for the estimation of fulvestrant in bulk drug

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ABSTRACT

Aim: The present work is aimed to develop a simple, rapid, selective, sensitive and validated HPLC method for the determination of fulvestrant in bulk drug. **Method:** Fulvestrant was measured using a validated HPLC method with UV detector at 225nm chromatographic peaks were separated on Phenomix, C18 column (250 mm x 4.6 mm x3.5µ) using 50% aetonitrile in channel A and acetonitlile in channel B as mobile phase with gradient elution at a flow rate of 1 ml/min. **Results & Discussions:** The chromatograms showed good peak shapes and no interference of solvent peaks. The retention time of fulvestrant was found to be 13.6 min. LOD and LOQ were found to be 0.0226µg/ml (0.001%) and 0.0712µg/ml,(0.004%) respectively. The percentage recovery of fulvestrant was found to be 99.1% - 100.75% which are within the limit. The method was linear over the concentration range of 0.08to 2µg/ml with coefficient of correlation (r^2) 0.99997. Both intraday and inter day accuracy and precision data showed good reproducibility. **Conclusions:** The low % RSD values (= 2) indicated that the method was precise and accurate. this method was successfully applied to forced degradation studies and can be adopted for routine estimation of fulvestrant in quality control laboratories.

KEY WORDS: Fulvestrant, HPLC Method Development, Validation.

INTRODUCTION:

Fulvestrant chemically 7-alpha-[9(4,4,5,5,5pentafluoropentylsulphinyl) nonyl]estra-1,3,5-(10)- triene-3,17beta-diol, 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]. Sunandamma Yet.al., were developed a validated RP-HPLC method using Methanol: 1% OPA: 85:15 % (V/V) with isocratic elution on a symmetry Chromosil C18 column[2]. BalaramVM et.al., were developed and validated a HPLC and electrospray tandem mass spectrometry method for estimation of fulvestrant in rabbit plasma[3]. Mehmet Gumustas et.al., were developed HPLC method using Waters X-Terra RP18 column (250×4.6 mm.×5 ìm) and a mobile phase, consisting of a mixture of acetonitrile–water(65:35;v/v) containing phosphoric acid (0.1%)[4].TULASAMMA. et.al., worked on Development and Validation of Spectrophotometric method for the determination of fulvastrant in its dosage forms[5]. Gedela Srinubabuet.al., worked on Development and Validation of Liquid Chromatographic and UV Derivative Spectrophotometric Methods for the Determination of fulvastrant in Pharmaceutical Dosage Forms[6]. Vishnukulaka et.al., worked on Development and Validation of LC Method for the Determination of fulvastrant in Pharmaceutical Formulation Using methanol and phosphate buffer (50:50;v/v) as mobile phase[7]. The devised method was found to be selective and reliable, and faster and more straightforward than other reported methods.

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MATERIALS AND METHODS:

Fulvestrant working Standard & Reference standards were obtained as gift samples from CIPLA Pharmaceuticals, Mumbai. HPLC grade acetonitrile and methanol were purchased from SD fine chemicals, Mumbai, India. Analytical Grad ortho phosphoric acid was purchased from SD fine chemicals, Mumbai, India.

Chromatographic Conditions:

The HPLC system consisted of Shimadzu UV –Visible Detector – SPD 10A vp Pump – LC- AT vp. The wavelength of detection as set at 225nm. Separation was carried out on Phenomix, C18 column (250 mm x 4.6 mm x3.5µ) using 50% aetonitrile in channel A and acetonitlile in channel B as mobile phase with gradient elution at a flow rate of 1 ml/min. The mobile phase filtered through nylon milli pore(0.2µm) membrane filter, purchased from pall life sciences, Mumbai and degassed

with Ultrasonicator prior to use. Chromatography was carried out at room temperature 25^{0}C and maintains the column temperature at 32^{0}C.

Standard & Sample Preparation:.

Standard and Test solutions were prepared in separate 20 ml volumetric flasks by dissolving 20mg in 20ml of acetonitrile to give 1mg/ml solutions.

Preparation of Linear Standard Solutions:

Stock solutions of fulvestrant working (1mg/ml) and reference (1mg/ml) standards were prepared in acetonitrile. Further dilutions were carried out in 60% acetonitrile. Calibration standards were prepared freshly with fulvestrant stock solution to give the concentrations of 0.08, 0.5, 1, 1.5 and 2 µg/ml.

Injection sequence:

Separately injected 20 µL each of one diluent sample as blank, five standard solutions and two test solutions into the liquid chromatography, recorded the chromatograms and measured the responses for all peaks excluding the peaks of blank. The formula for calculating test concentration is given below

Calculate the amount of drug by using the followings

$$\text{\% Assay} = \frac{\text{Avg. area of Test solun.}}{\text{Avg. area of Standard solun}} \times \frac{\text{Standard Conc.}}{\text{Sample Conc.}} \times \text{\% Assay of Standard}$$

VALIDATION:

System precision:

A series of one blank (ACN) and five fulvestrant standards of 10µg/ml were injected and % RSD of retention time and area were calculated.

System suitability

A series of one blank (ACN), five fulvestrant standards of 10µg/ml and two control samples of 10µg/ml were injected and evaluated the system suitability parameter.

Limit of Detection and Limit of Quantification(LOD & LOQ):

The steyx/slope method was employed to determine LOD and LOQ. A series of linearity standard solutions of Fulvestrant from the expected minimum level of detection 0.0001% (0.001µg/ml) to 0.1% (1µg/ml) with respect to the test concentration will be prepared and injected. Formulas for the calculation of LOD & LOQ are given below.

$$LOD = \frac{3.3 \times S_a}{b} \qquad LOQ = \frac{10 \times S_a}{b}$$

Accuracy/Recovery and Precision of the test method

The accuracy/recovery study for unknown impurity will be performed with the fulvestrant API at concentrations spanning from 50% to 200 % of the proposed specification limit for single maximum unknown impurityof NMT 0.05 % with respect to the test concentration of 2.0mg/mL of Azacitidine. Six preparations will be made at 100% & 200% levels and three preparations will be made at 50% & 150% levels. Each solution will be injected once and analyzed.

Linearity of the test method:

The mean at each level will be plotted as average amount added (µg/mL) versus average amount found (µg/mL) from LOQ to 200% levels and a linear regression analysis without forcing through the origin will be performed. The correlation coefficient (r), slope and Y –intercept will be calculated and reported.

RESULTS & DISCUSSION:

Under the chromatographic conditions employed, the sample showed sharp peak of Fulvestrant. The retention time of the drug was found to be 13.9 min shown in figure-1.

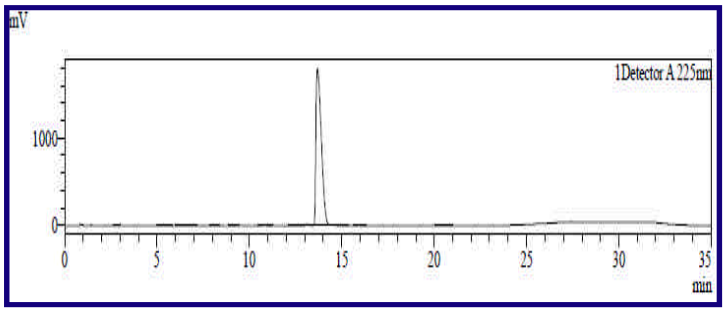

Figure: 1 Chromatogram showing sharp peak of Fulvestrant retained at 13.9min

VALIDATION RESULTS:

The method developed was validated for specificity, accuracy & precision, linearity and stability as per ICH guidelines [8]. The results of validating parameters are given below.

System Suitability:

Table: 1 Showing results for system suitability of Fulvastrant

S.No	Retention time	Peak area	Theoretical Plate count	Tailing Factor
1	13.934	4205075	3573	1.26
2	13.932	4213570	3572	1.26
3	13.931	4214751	3571	1.26
4	14.034	4218102	3572	1.25
5	14.032	4211038	3573	1.26
6	13.931	4217385	3572	1.25
MEAN	13.968	4213320	3572.167	1.26
SD	0.052	4789.354	-	-
%RSD	0.372279	0.149047	-	-

Specificity:

Table: 2 Showing Results of Specificity

No.of Injections	GDAHPL22 Retention time	Area
Inj-1	13.989	4535605
Inj-2	13.968	4456197
Inj-3	13.973	4536504
Inj-4	13.969	4535818
Inj-5	13.986	4536037
Inj-6	13.928	4536021
AVERAGE	**13.962**	**4536030**
SD	**0.0087**	**309.2777**
% RSD	**0.1**	**0.9**

Detection Limit & Quantification Limit:

Table 3: Showing Results of LOD & LOQ:

% test Conc.	Conc.(µg/mL)	Area
0.00010	0.0020	330
0.00025	0.0050	408
0.00050	0.0100	686
0.00075	0.0150	790
0.00100	0.0200	1230
0.00250	0.0500	2586
0.00500	0.1000	4542
0.00750	0.1500	6231
0.01000	0.2000	8101
0.02500	0.5001	20108
0.05000	1.0002	39142
0.07500	1.5003	59742
0.1000	2.0004	78660
Styex		**280.7821**
Slope		**38230.9813**
LOD (µg/mL)		**0.0226**
LOQ (µg/mL)		**0.0712**
LOD(%)		**0.001**
LOQ (%)		**0.003**

Results of Accuracy, Recovery, Precision and Linearity:

Table: 4 Showing Results of Accuracy, Recovery, Precision, Linearity

Level (%)	Preparations	Amount found (µg/mL)	Amount added (µg/mL)	% Recovery	Average	% RSD
50%	Prep-1	0.4854	0.5002	99.0	**98.5**	**0.5**
	Prep-2	0.4920	0.5002	98.4		
	Prep-3	0.4812	0.5002	98.2		
100%	Prep-1	0.9584	1.0004	95.8	**96.2**	**0.3**
	Prep-2	0.9626	1.0004	96.2		
	Prep-3	0.9662	1.0004	96.7		
	Prep-4	0.9615	1.0004	96.1		
	Prep-5	0.9643	1.0004	96.3		
	Prep-6	0.9634	1.0004	96.2		
150%	Prep-1	1.4414	1.5005	96.3	**96.1**	**0.3**
	Prep-2	1.4439	1.5005	96.2		
	Prep-3	1.4362	1.5005	95.8		
200%	Prep-1	1.9296	2.0007	95.9	**96.4**	**0.7**
	Prep-2	1.8932	2.0007	97.2		
	Prep-3	1.9141	2.0007	97.2		
	Prep-4	1.9184	2.0007	96.4		
	Prep-5	1.9226	2.0007	96.1		
	Prep-6	1.9137	2.0007	95.8		

Table 4: Results of Accuracy at LOQ

Amount found (µg/mL)	Amount added (µg/mL)	% Recovery	Mean Recovery (%)	%RSD
0.0774	0.0800	96.5		
0.0763	0.0800	97.0		
0.0768	0.0800	96.0	**96.6**	**1.06**
0.0756	0.0800	96.2		
0.0759	0.0800	97.1		
0.0767	0.0800	96.9		

Linearity:

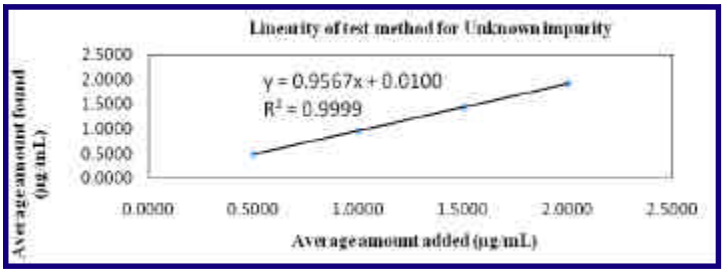

Figure 2: Showing Calibration Curve of Linear Concentrations

Table 5: Showing Results of Linearity

Level%	Average amount added (µg/mL)	Average amount found (µg/mL)
LOQ	0.0800	0.0763
50	0.5002	0.4928
100	1.0004	0.9626
150	1.5005	1.4416
200	2.0007	1.9283
	Correlation coefficient	**0.999973**
	Slope	**0.9608**
	Y-intercept	**0.0038**

Table: 6 Showing Results for linearity of detector response

Level (%)	Concentration (µg/mL)	Area
LOQ	0.0800	3608
50	0.5001	20545
75	0.7501	30443
100	1.0001	41243
125	1.2501	50397
150	1.5002	60110
200	2.0002	80655
Correlation coefficient		**0.999906**
Slope		**39998.7465**
Y-Intercept		**540.0523**
% Y-Intercept		**1.3**

Table: 7 Showing Acceptance Criteria for Validation Parameters

Characteristics	Acceptable range
Accuracy	Recovery (98%-102%) %RSD for =2%
Precision	Assay validation-RSD =2% FPP=1% API RS validation =2%
Robustness	RSD < 2%
Specificity	No Interference
LOD	S/N = 3
LOQ	S/N = 10
Linearity	Correlation Coefficient(r)>0.999 %y intercept – should be + 2.0

DISCUSSION:

The solution of 10µg/ml of fulvestrant in diluent (ACN) was prepared and the solution was scanned in the range of 200-400nm, at 225nm the drug shows maximum absorbance spectrum and better detector response. The mobile phase proportion was optimized to Acetonitrile : Water and ACN run in gradient mode and flow rate 1.0ml/min was selected. The retention time of fulvestrant was found to be 13.985min. The calibration was linear in concentration range of 0.08to 2µg/ml, with regression 0.999, for Fulvestrant and obeyed the Beer – Lamberts law. LOD & LOQ of were determined by styex-slope method. LOD and LOQ were found to be 0.0226µg/ml (0.001%) and 0.0712µg/ml,(0.004%) respectively. The percentage recovery of fulvestrant was found to be 99.1% - 100.75% which are within the limit. The low % RSD values (= 2) indicated that the method was precise and accurate. Accuracy was confirmed by recovery studies by proposed method.

CONCLUSION:

The developed HPLC method was found to be rapid, simple, precise, accurate and economical and can be adopted for routine estimation of fulvestrant in quality control laboratories.

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