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RP-HPLC method development and validation of RELUGOLIX

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Abstract

Zobrax stationary conditions are used in chromatography (160mm x5.5 mm, 5m). , portable stage ACN:Ammonium was used in a 55:45 ratio, with a detection wavelength of 310 nm, a column temperature of 30OC, and mobile phase as the diluent. A 2.79-minute retention time was discovered. Between 25% and 150% levels, a linearity research was conducted, and an R2 value was discovered.0.999 is to be. The results showed that the method precision was 0.5 and the intermediate precision was 0.2. The corresponding LOD and LOQ values are 0.4 g/ml and 1.2 g/ml.

Keywords: RP-HPLC, Relugolix, Method development ICH Guidelines.

Full length article **Corresponding Author*, e-mail: sathishmeruva85@gmail.com

1. Introduction

It is a 1-(4-{1-[(2,6-difluorophenyl)methyl]-5-[(dimethylamino)methyl]-3-(6-methoxypyridazin-3-yl)-2,4-dioxo-1H,2H,3H,4H-thieno[2,3-d]pyrimidin-6-yl}phenyl)-3-methoxyurea The actions of testosterone seem to be at least partially responsible for the aetiology and development of prostate cancer. Androgen deprivation therapy (ADT) has become a standard in the treatment of prostate cancer, particularly in advanced illness, since it has been shown to cause cell death and tumour shrinkage in several well-differentiated prostate cancer cell lines. Relugolix is used in men to treat advanced prostate cancer. Relugolix is a type of medicine called a gonadotropin-releasing hormone (GnRH) antagonist. It helps treat prostate cancer by lowering the amount of testosterone hormone in the blood. In some patients, testosterone will cause prostate cancer to grow large.

2. Materials and Methods

Pure medication of Relugolix (fig.1) were delivered by spectrum labs. A local pharmacy provided Relugolix tablet. All of the chemicals and buffers used in this method were given by Rankem in India.

2.1 Characteristics of the study region

a) Instrumentation

WATERS HPLC, model: 2696 SYSTEMwith Photo diode array detector was used for the development and method validation, with an automated sample injector with software Empower 2.

b) Chromatographic conditions:

Flow rate: 1.0 ml/min
Column :Zobrax (160mm x5.5 mm, 5□).
Mobile phase: Acetonitrile: Ammonium (55:45)
Detector:PDA 310nm Temperature: 30°C Injection volume :10.0□L Run time :6 min

c) Preparation of 0.1N Ammonium Formate:

Weigh accurately 0.63gm of Ammonium Formate and add to 1000-mL graduated cylinder to measure 900 mL of filtered HPLC-grade water and mix well,Check the pH of the solution. the reservoir bottle and mix well and sonicate for 20 minutes.

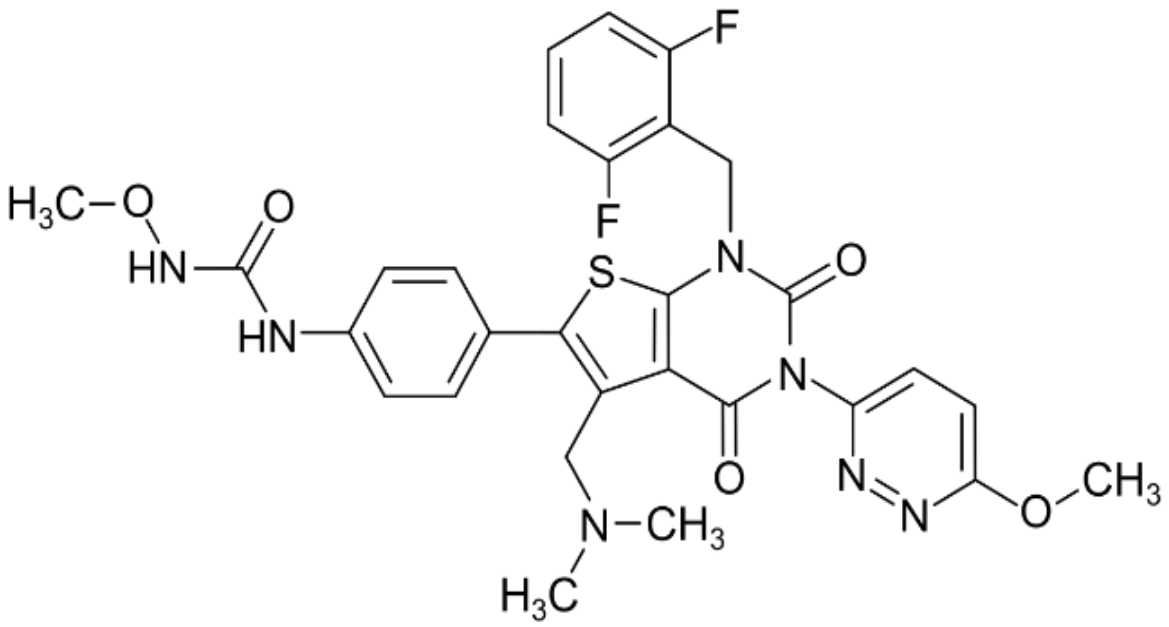

Figure-1: Structures of Relugolix

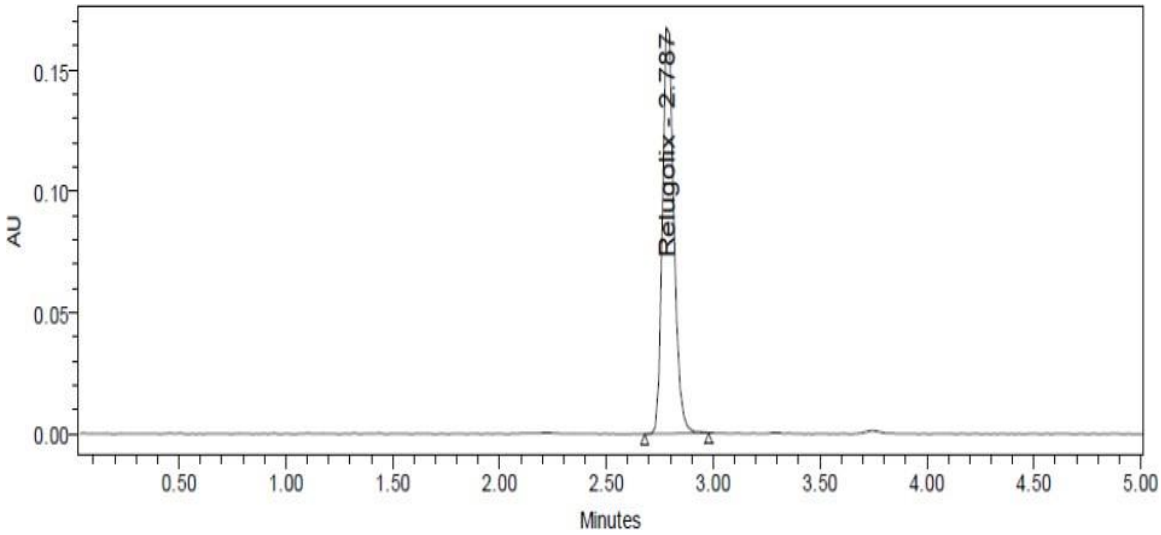

Figure 2: System suitability Chromatogram of Relugolix

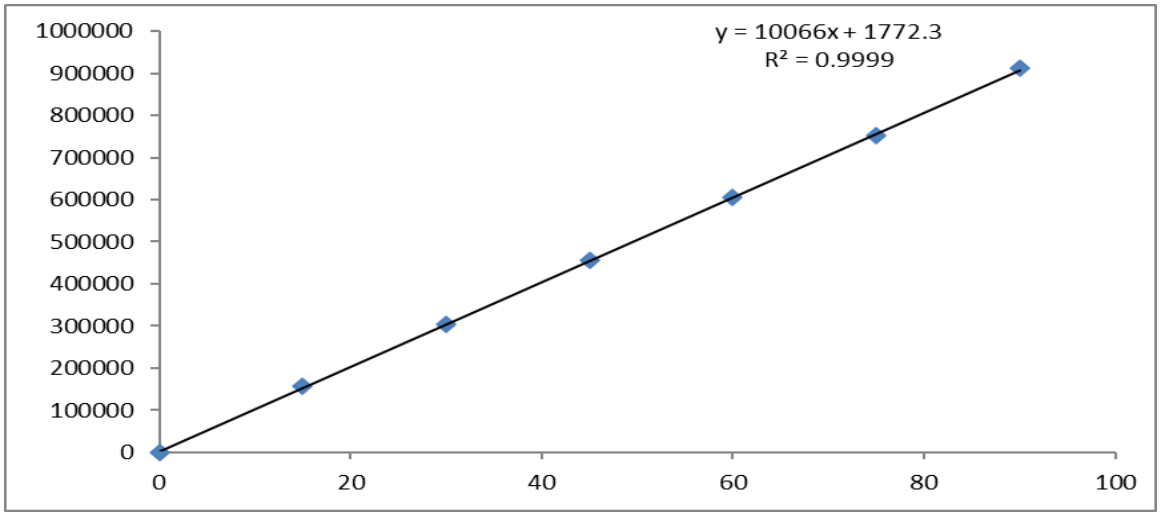

Figure 3: Relugolix Calibration curve

Table 1: LINEARITY DATA

Linearity Level (%)	Concentration (ppm)	Area
0	0	0
25	15	155988
50	30	303455
75	45	454789
100	60	605780
125	75	752267
150	90	910967

Table 2: SYSTEM SUITABILITY

Relugolix		
RT(min)	USP	Tailing
2.784	13459	1.2
2.783	13472	1.2
2.792	13241	1.18
2.792	13398	1.12
2.791	13335	1.10
2.794	13612	1.10

Table 3: PRECISION

Method precision: %RSD was found to be 0.5 and chromatograms was shown in fig 6.10.

Peak Area
615300
616879
617488
610320
611663
617487
614868
3130.1
0.5

Table 4: ACCURACY

Accuracy: Three Concentrations of 50%, 100%, 150% are Injected in a triplicate manner & %Recovery was calculated as100 %.

% Level	**Amount Spiked (µg/mL)**	**Amount recovered (µg/mL)**	**% Recovery**	**Mean %Recovery**
50%	30	30.27	100.90	100%
	30	29.82	99.40	
	30	30.25	100.85	
100%	60	59.80	99.67	
	60	60.14	100.23	
	60	60.11	100.19	
150%	90	89.88	99.87	
	90	89.24	99.15	
	90	90.38	100.20	

Table 4: ASSAY

Standard	Sample	%Assay
617044	615427	100.04
610075	616882	100.28
614225	617455	100.38
612371	610306	99.21
617980	611663	99.43
611766	617486	100.38
613911	614868	99.96
3104.7	3130.1	0.51
0.5	0.5	0.5

Table 5: SUMMARY AND CONCLUSION

Parameters	Relugolix
Linearity**:**Range(µg/ml)	15-90µg/ml
Regression coefficient	0.999
Slope(m)	55112
Intercept(c)	756.1
Regression equation(Y=mx+c)	y = 10066x + 1772.3
Assay(% mean assay)	99.96%
Specificity	Specific
System precision %RSD	0.5
Method precision %RSD	0.5
Accuracy %recovery	100%
LOD	0.4
LOQ	1.2

d) Preparation of Standard stock solutions:
Accurately weighed 30mgof Relugolix transferred 50ml and volumetric flasks, 3/4 th of diluents was added and sonicated for 10 minutes. Flasks were made up with diluents and labeled as Standard stock solution (600µg/ml of Relugolix).

e) Preparation of Sample stock solutions

10 Tablets were weighed and the average weight of each capsule was calculated, then the weight equivalent to 1 capsulewas transferred into a 10 ml volumetric flask, 5ml of diluents was added and sonicated for 25 min, further the volume was made up with diluents.

f) Method validation

The validation of the HPLC method was carried out in accordance with the ICH recommendations

e) System suitability:

The system suitability parameters were determined by preparing standard solutions of Relugolix (60ppm) and the solutions were injected six times and the parameters like peak tailing, resolution and USP plate count were determined. The % RSD for the area of six standard injections results should not be more than 2%. (fig.2)

Specificity (Selectivity): We should not find interfering peaks in blank and placebo at retention times of these drugs in this method. So this method was said to be specific.

Linearity: This study was carried out between 25% to150 % levels, R2 value was found to be as 0.999. (fig.3) (Table-1).

Accuracy: The percentage mean recovery was found to be 100.12%.

The corresponding LOD and LOQ values are 0.4 g/ml and 1.2 g/ml.

Degradation Studies: From the results, no degradation was observed when the samples were exposed to acid, base, hydrolysis, thermal, light and water. According to the stress study, none of the degradant co-eluted with the active drug peaks formed.(Table-3).

4. Conclusions

Linearity study was carried out between 25% to150% levels, R2 value was found to be as 0.999. Precision was found to be 0.5 for Method precision and 0.2 for intermediate precision. LOD and LOQ are 0.40µg/ml and 1.20µg/ml respectively. By using above method assay of marketed formulation was carried out 100% was present.

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