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RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF RELUGOLIX IN BULK AND TABLET DOSAGE FORM

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ABSTRACT

A new simple, precise, accurate, sensitive and rapid chromatographic method based on RP-HPLC was developed and validated for the estimation of Relugolix in bulk and tablet dosage form. Methanol: Water (70:30 v/v)) was used as mobile phase. A isocratic programing has been done, on a reverse phase Qualisil C18, (250 mm× 4.6mm, 5 µm) with rate 0.8 mL/min, monitored at 290 nm. The mean retention times of Relugolix were found to be 4.10 min respectively. Linearity of Relugolix was found to be 2-12 µg/mL, R^2= 0.9993 respectively. The developed methods have shown the best findings in terms of linearity, accuracy, precision, LOD and LOQ for API and in tablet. The depicted method can routinely be used for the determination of Relugolix in bulk and tablet formulation.

KEYWORDS: Relugolix, Methanol, RP-HPLC, Antineoplastic, validation, Isocratic mode, pharmaceutical tablet.

1. INTRODUCTION

The FDA authorized Relugolix on December 18, 2020, and CDSCO approved it on October 16, 2023, for the treatment of advanced prostate cancer in adults. In the United States and the European Union, relugolix is authorized for the treatment of advanced hormone-sensitive prostate cancer and advanced prostate cancer, respectively. Relugolix is a chemical molecule with a molecular weight of 623.63 g/mol and the molecular formula C29H27F2N7O5S. The FDA authorized Relugolix for advanced prostate cancer in 2020 after it was approved in Japan for uterine fibroids in 2019. Another way to identify this substance is by its scientific name, relugolix. First, 4-(1-(2,6-difluorobenzyl) (dimethylamino)methyl -5- Methoxypyridazin-3-yl, or -3- [2,3-d] -2,4-dioxo -1,2,3,4-tetrahydrothieno 6-yl)phenylpyrimidin 3-methoxyurea. Orgovyx was its brand name, and it was used to treat advanced prostate cancer. [1-3] Relugolix is a small non-peptide anti-androgen drug which binds to gonadotropin releasing hormone (GNRH)

receptors in the anterior pituitary gland, which reduces the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[1]

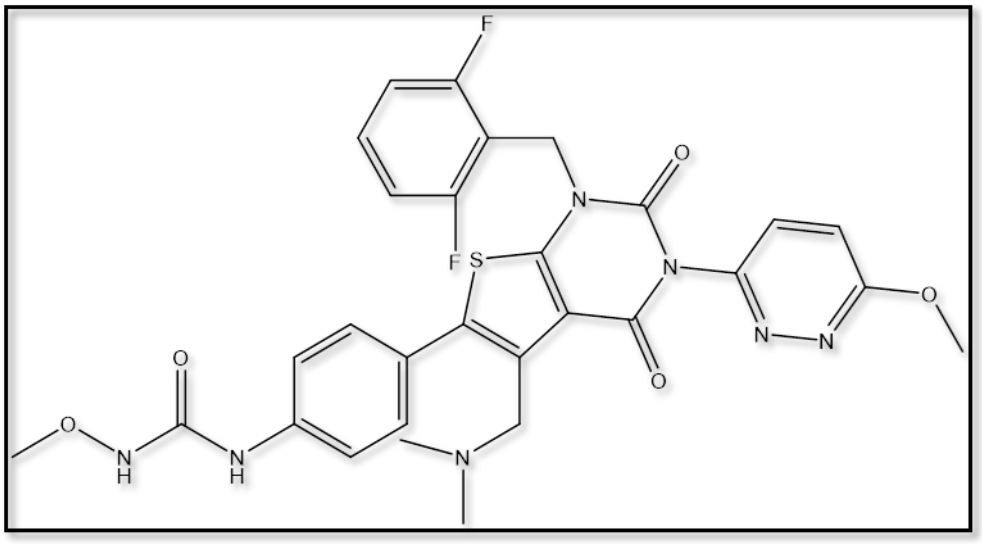

Fig. 1: Structure of Relugolix.

Relugolix is a N-phenyl urea derivative, non-peptide, small molecule compound structurally different from gnrh analogues.[4] The most common symptoms are abnormal uterine bleeding, abdominal protrusion, pelvic pressure, urinary urgency, frequency or incontinence; constipation, tenesmus, etc., and reproductive dysfunctions.[5] By blocking this receptor, it inhibits the release of FSH and LH hormones, resulting in reduced production of testosterone in men and estrogen in women. This effect can help slow or halt the progression of prostate cancer in men and is also beneficial in treating endometriosis in women.[6] Relugolix is used in the treatment of uterine fibroids and advanced prostate cancer 3-6 in adult men.[7] Some literature of Relugolix is also exist in Merck index and Martindale. In literature, few uplc procedures have been reported for the analysis of Relugolix-. Although Chromatographic and Spectroscopic methods are reported for the quantitative estimations of Relugolix, but these methods are few. Therefore, looking at the current scenario of quantitative estimation of the said drug in bulk and tablet formulation, it was thought worthwhile to set an objective to developed and validate RP-HPLC.The method has been satisfactorily applied to the estimation of Relugolix in bulk and tablet dosage form. The current works emphasize simple, precise, sensitive and effective RP-HPLC method for estimation of relugolix in bulk and in-tablet dosage form. The method was validated as per ICH guidelines.

2. EXPERIMENTAL

2.1 Chemicals and Reagent

The working standard of Relugolix was obtained as a gift a sample from BDR Pharmaceuticals International Pvt Ltd, Vadodara. Methanol (HPLC grade) was obtained from Merck Ltd Mumbai. MilliQ water was used.

2.2 Instrument used

Analysis was performed on LC- 20 AD (Shimadzu Corporation, Japan) consisting of LC -20 AD solvent delivery system (pump), UV-Visible detector and CTO – 10 AS vp; column oven, a Rheodyne injector with 20 µl loop and a Hamilton syringe (100 µl). Separations were achieved on a Qualisil BDS C18 column (250mm× 4.6mm×5µ). Data

collection and analysis were performed with LC-solution (Shimadzu Corporation, Japan). All weighing operations for the present analysis were carried out with the help of SHIMADZU AUX-120 analytical balance. Ultra sonication of samples was performed using Ultrasonicator; ENERTECH Electronics Pvt. Ltd., India.

2.3 Preparation of Mobile Phase

The HPLC grade methanol and water in the ratio of (70:30 v/v) was filtered through 0.4 µm membrane filter paper and mobile phase was prepared and sonicated for 15 min.

2.4 Preparation of Standard Stock Solution and Study of Calibration Curve

Stock solution of Relugolix was prepared with a concentration of 100 µg/mL in mixture of methanol and water (70:30 v/v). Determination of linearity involved analysis of six working solution having concentration range 2-12 µg/mL. Appropriate dilutions were prepared separately and 20 µl of each was injected into the HPLC system and their chromatograms were recorded under the same chromatographic condition. Peak areas were recorded for all the peaks and a standard calibration curve was plotted.

2.5 Preparation of sample solution

The sample solution was prepared from formulated Relugolix tablets. Twenty Relugolix tablets were accurately weighed, average weighed determined and finely powered. A quantity of powered drug equivalent to 10 mg Relugolix transferred into 100 ml of volumetric flask containing 50 ml methanol, sonicated for 5 min and volume was adjusted using the same solvent. Then it was filtered through Whatman filtered paper. The above prepared solution was filtered through 0.4µ membrane filter paper and was used as standard stock solution. Appropriate aliquot was pipetted out from the standard stock solution and was further diluted with the mobile phase. A 20 µl volume of each Relugolix sample was injected into sample rheodyne injector of HPLC system and the chromatograms were recorded under the chromatographic condition i.e. temperature, flow rate, mobile Phase etc. The area of each peak was determined at 290 nm and the amount of drug present in the sample mixture was determined.

2.7 Chromatographic Conditions

HPLC System	Shimadzu – LC 20 AD
Detector	UV-Visible
Column	Qualisil BDS C18
Dimensions	(250mm×4.6mm×5µ)
Mobile- Phase	Methanol: Water (70:30 V/V)
Mode	Isocratic
Flow Rate	0.8 ml/min
Temperature	Ambient temperature
Detection wavelength	290 nm
Injection Volume	20 µl

3. RESULT AND DISCUSSION

3.1 Optimization of chromatographic conditions

In method development and validation process of Relugolix, various mobile phase compositions were tested. The optimized mobile composition was found to be methanol: water (70:30 v/v) with run time 20 min. and the peak is optimized in 4.10 min. Before analysis, mobile phase and sample solutions were filtered through 0.45 µ membrane filter and ultra-sonicated for 15 min. Chromatographic studies were performed at ambient temperature, with flow rate 0.8 mL/min and injection volume of 20 µl followed by detection wavelength at 290 nm. Shown in fig 2.

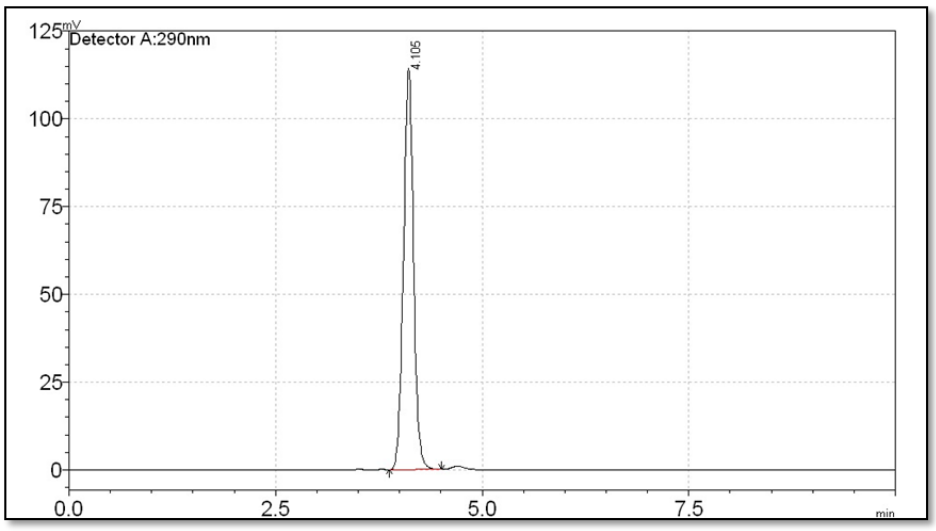

Fig. 2: Optimizations of Relugolix in Methanol: Water in (70:30 v/v).

3.2 Validation of method

The analytical method was validated by various parameter such as Linearity, Precision, Limit of quantification (LOQ), Limit of Detection (LOD), Accuracy, Recovery studies, Robustness and Bulk Assay as per International Conference on Harmonization (ICH) guidelines.[9]

3.2.1 Linearity Studies

Different concentration for Relugolix of the drug was prepared for linearity studies. A typical HPLC chromatogram obtained during the determination of Relugolix. The calibration curve obtained by plotting the peak area versus concentration showed linear relationship over a concentration range of 2-12 µg/mL respectively. The linear regression equation for Relugolix was found to be **y = 88860x + 33605** and the regression coefficient value **(R^2) = 0.9993** for drug indicating high degree of linearity. Characteristic parameter of the HPLC method are seen in Table 1 and Calibration Curve are represented in fig 3.

Table 1: Characteristics parameter of the HPLC method for determination of Relugolix

Parameter	Relugolix
Linearity range µg/mL	2-12 µg/mL
Slope	88860
Intercept	33605
Correlation Coefficient	0.9993

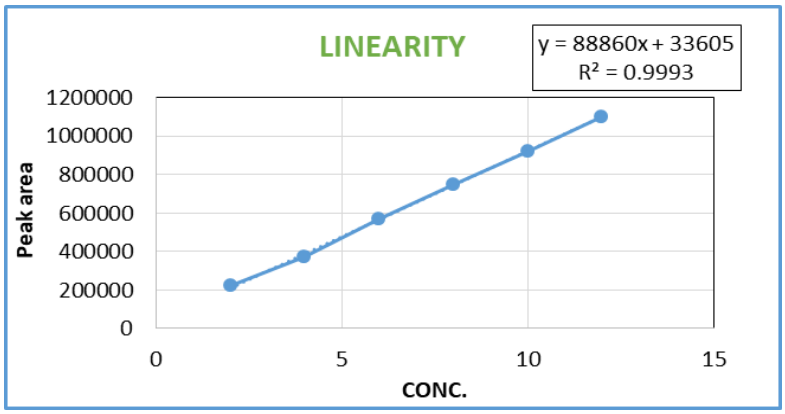

Fig. 2: Calibration Curve for Relugolix.

3.2.2 Accuracy Studies

Accuracy study of developed method was evaluated in terms of % recovery studies, recovery experiments were performed at three different level, 80%, 100%, and 120% percentage drug recovered, when known amount of standard drug was added to pre-analysed sample and subjected to proposed HPLC method. Recovery studies of Relugolix were carried out by spiking three different amounts of Relugolix standard 4.8, 6.0, and 7.2 µg/mL to the developed tablet formulation 6 µg/mL standard addition method. The recovery values for Relugolix in ranged from 101.30 to 100.21 are shown in Table 2.

Table 2: Accuracy Studies.

Drug	Initial Amount [µg/mL]	Amount of drug added to the analyte [%]	Total amount found± S.D. [µg/mL]	Recovery [%] [n=3]	%RSD [n = 3]
Relugolix	6	4.8	4.81 ± 0.04	101.30	0.83
	6	6.0	6.02 ± 0.03	100.40	0.44
	6	7.2	7.21 ± 0.04	100.21	0.52

n- number of determination

3.2.3 Precision

The precision was evaluated at three levels, repeatability, reproducibility, and intermediate precision each level of precision was investigated by six replicate injections of concentrations 4, 8, and 10 µg/mL respectively. The result of precision was expressed as % RSD are shown in Table 3.

Table 3: Precision Studies [Intra and Inter-day].

Standard Concentration [µg/mL]	Amount Found [µg/mL]	% Amount found [µg/mL] [n=3]	% RSD
Intra-day Precision			
4	3.96	99.15	0.75
8	8.05	100.74	0.74
10	9.97	99.75	0.60
Inter-day Precision			
4	3.96	98.3	0.67
8	8.01	100.23	0.23
10	9.93	99.32	0.09

n - number of determinations

3.2.4 Assay of Tablet formulation

Assay of Relugolix tablet containing 10 mg of Relugolix performed at concentration of 6 µg/mL. % amount found value for Relugolix ranged from 99.72 to 101.35 and % RSD value 1.19 are shown in Table 4.

Table 4: Analysis of Tablet Formulation.

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
Relugolix	6	5.95	99.72
	6	6.06	101.01
	6	5.98	99.72
	6	6.08	101.38
	6	6.06	101.01
	6	6.08	101.35
	Mean ± SD	6.06 ± 0.07	101.05 ± 1.20
	% RSD	1.19	1.19

n -number of determinations

3.2.5 Analysis of Bulk

An accurately weighed 10 mg of Relugolix was transferred into 100 mL volumetric flask; dissolved in 50 mL methanol and the volume was made with the same solvent to give 100 µg/mL. From the filtrate, measured volume was taken and diluted with mobile phase to get the final aliquots of 6 µg/mL were prepared and injected; the area determined for selected peak. The concentrations of the drug were determined from linear regression equations. % amount found value for bulk assay ranged from 99.72 to 101.35 and average value 100.40, % RSD 1.08 are shown in Table 5.

Table 5: Analysis of Bulk Material.

Drug	Amount taken [µg/mL]	Amount found [µg/mL]	% Amount found
Relugolix	6	5.98	99.72
	6	5.97	99.57
	6	5.91	98.56
	6	6.06	101.01
	6	6.05	100.99
	6	6.08	101.35
	Mean ± SD	6.01 ± 0.06	100.40 ± 0.80
	% RSD	1.08	1.08

n- number of determinations

3.2.6 Robustness studies

Robustness is an analytical method to remain unchanged, but deliberate changes in parameter.

The robustness was evaluated by analyzing the sample by varying few parameters like flow rate, pH of water, and mobile phase composition are shown in Table 6.

Table 6: Robustness study for Relugolix.

Parameters	Tailing Factor	Theoretical plates	Retention time
Change in pH of water			
4.2	1.8	4810.8	3.63
4.8	1.3	4181.6	3.86
Change in mobile phase composition			
(Methanol: Water 80 : 20 v/v)	1.39	4011.5	2.90
(Methanol: Water 60 : 40 v/v)	1.51	4120.4	2.58
Change in flow rate			
0.6	1.52	5004.6	4.56
1.0	1.71	5045.9	3.81

3.2.7 Limit of Quantification and Limit of Detection

The LOD and LOQ of validated method was fully depend on the standard deviation of the responses and slope of constructed calibrations curve. the LOD and LOQ value was found to be **0.03** and **0.09** µg respectively.

3.2.8 System Suitability Study

System suitability test are integral part of liquid chromatographic methods. Retention time, capacity factor, number of theoretical plates, and Resolutions were calculated for standard solutions are summarized in Table 7.

Table 7: System suitability studies.

Retention time (tR)	4.10 min
Capacity factor (k0)	0.088
Theoretical plate (N)	5022.028
Resolutions	-

5. CONCLUSION

Proposed study describes a new RP-HPLC method for the estimation of Relugolix in bulk and tablet dosage form using the simple mobile phase. The method gives good resolution with short analysis time. The method was developed and validated and found to be simple, sensitive, accurate, and precise. So, the method can be used rottenly the analysis of Relugolix in bulk and pharmaceutical Tablet dosage form.

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REFERENCES

1. Krina Chaudhary, Vikram Panchal. Relugolix: a gnrh antagonist for the treatment of advanced prostate cancer. Technix International Journal for Engineering Research, 2024; 11(8): a211.
2. Shirley, Matt. "Relugolix: a review in advanced prostate cancer." Targeted Oncology, 2023; 18(2): 295-302.
3. Thrinath, S. R., K. S. Lakshmi, and Manikandan Krishnan. "Development and validation of a method for studying Relugolix and its impurities by UPLC-MS." Acta Chromatographica, 2024.
4. Pulletikurthi, KVK Mohan, et al. "Force degradation studies of relugolix: identification, isolation and structure characterization of stress degradation products by using liquid chromatography-mass spectrometry, auto purification mass mediated preparative-high performance liquid chromatography, high resolution mass spectrometry, nuclear magnetic resonance spectroscopy." Spectroscopy Letters, 2022; 55(2): 128-137.
5. Gummaluri, Ram Kumar, and Ramakrishna Karipeddi. "an in-vitro study of a simultaneous method for determining relugolix, norethindrone acetate, and estradiol by uplc." Rasayan Journal of Chemistry, 2023; 16(3).
6. Li, Song, et al. "A Ligand-and-structure Dual-driven Deep Learning Method for the Discovery of Highly Potent gnrh1r Antagonist to treat Uterine Diseases." arxiv preprint arxiv, 2022; 2207.11547.
7. Alumuri, Thulseedhar, et al. "Devising a Robust Rp-Hplc Method for the Determination of Relugolix's Related Substances and Characterization of its Degradant Using LC-MS/MS."
8. Bharathi, M. Prathibha."RP-HPLC method development and validation of RELUGOLIX.
9. ICH-Guidelines Q2 (R1), Validation of Analytical Procedures: Text and Methodology, 2005.
10. Priyanka Nagar, Arvind Kumar Sharma, Robin Kumar, Chhaya Chauhan, Rini Singha, Minakshi Garg. Advancing relugolix analysis: A comparative study and AQbDdriven method optimization with stability testing. Journal of Applied Pharmaceutical Science, 2025; 15(08): 55-070.