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A Systematic Review on Analytical Method Development and Validation for Relugolix

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Abstract:

Relugolix is an orally active, non-peptide gonadotropin-releasing hormone (GnRH) receptor antagonist used in the treatment of advanced prostate cancer. It produces rapid suppression of testosterone levels by directly blocking pituitary GnRH receptors, without causing an initial hormonal flare. The oral administration of relugolix offers improved convenience compared to injectable androgen-deprivation therapies. This review article summarizes the pharmacological profile of relugolix, including its mechanism of action, pharmacokinetics, pharmacodynamics, adverse effects, and drug interactions. In addition, various reported analytical methods such as UV spectrophotometry, HPLC, UPLC, LC-MS/MS, and GC-MS for the estimation and quality control of relugolix in bulk drug and pharmaceutical dosage forms are reviewed.

Keywords: Relugolix, GnRH receptor antagonist, Prostate cancer, UV, HPLC, UPLC,LC-MS/MS, GC-MS

1. INTRODUCTION

Introduction of Prostate Cancer

Prostate cancer arises from uncontrolled growth of cells in the prostate gland, a small organ located below the bladder that is essential for male reproductive function. Many prostate cancers grow slowly and can be managed through active surveillance. The disease is androgen-dependent, meaning its growth increases when testosterone levels are high. Testosterone production is regulated by gonadotropin-releasing hormone (GnRH) receptors in the pituitary gland, blocking these receptors lowers testosterone levels and helps to slow prostate cancer progression, representing a key therapeutic target in prostate cancer management [1].

Prostate cancer mainly affects men between 45-60 years of age and is one of the most common cancers in men worldwide. Risk factors include increasing age, family history, ethnicity, obesity, and environmental exposure, with genetic predisposition playing a major role. The disease usually begins as prostatic intraepithelial neoplasia (PIN) and can progress to invasive cancer and metastasis if not treated. Globally, cases are expected to rise to 1.7 million with nearly 500,000 deaths by 2030. These rising numbers highlight the importance of early diagnosis, PSA screening, and increased awareness to reduce prostate cancer-related deaths [2,3].

STAGES OF PROSTATE CANCER

The staging of prostate cancer is most commonly performed using the TNM classification system established by the American Joint Committee on Cancer (AJCC). This system evaluates three key parameters[4]:

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T (Tumor): Describes the size of the primary tumor, its location, and the extent of growth into surrounding prostate tissue.

N (Nodes): Indicates whether cancer cells have spread to nearby (regional) lymph nodes.

M (Metastasis): Refers to the spread of cancer to distant organs or tissues beyond the prostate region.

Based on the TNM classification, prostate cancer is categorized into four clinical stages:

Stage I: Cancer limited to the prostate.

Stage II: Localized cancer with higher risk of progression.

Stage III: Locally advanced disease beyond the prostate.

Stage IV: Metastatic disease to lymph nodes or distant organs.

INTRODUCTION OF GnRH RECEPTOR ANTAGONIST

GnRH receptor antagonists act by directly inhibiting pituitary GnRH receptors, resulting in rapid suppression of LH and FSH secretion and consequent reduction in testosterone levels without an initial flare. This mechanism makes them effective agents for androgen-deprivation therapy in prostate cancer, with additional applications in other hormone-responsive diseases, including breast cancer and gynaecological disorders[5].

INTRODUCTION OF RELUGOLIX

Relugolix is the first orally active, non-peptide gonadotropin-releasing hormone (GnRH) receptor antagonist approved for androgen-deprivation therapy in advanced prostate cancer. It acts by directly blocking pituitary GnRH receptors, leading to rapid suppression of testosterone levels and effective control of hormone-dependent tumour growth. Androgen-deprivation therapy is a central component of prostate cancer management, as reducing testosterone slows disease progression. Unlike injectable GnRH antagonists such as deguelin, relugolix is administered as an oral tablet, offering greater convenience by avoiding injections, clinic visits, and injection-related discomfort. Clinical studies have demonstrated that relugolix achieves effective and sustained testosterone suppression and shows superior testosterone reduction compared with leuprolide, a widely used androgen-deprivation therapy, making it a valuable and patient-friendly treatment option[6-8]

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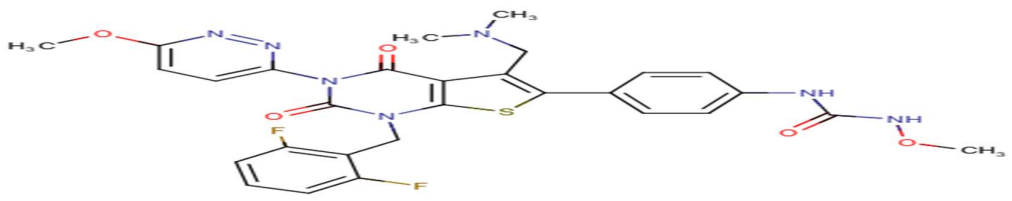


Fig 1: Chemical Structure of Relugolix

2. MECHANISM OF ACTION OF RELUGOLIX

Relugolix functions as an orally administered GnRH receptor antagonist that directly targets receptors in the anterior pituitary. Its antagonistic action suppresses the secretion of luteinizing hormone and follicle-stimulating hormone, resulting in a rapid decrease in testosterone levels in men and estrogen levels in women. This hormonal suppression is effective in managing advanced prostate cancer as well as hormone-dependent gynecological conditions such as uterine fibroids and endometriosis (Figure 1.2). In contrast to GnRH agonists, relugolix avoids an initial hormone flare, allowing a quicker and more controlled hormonal suppression[9].

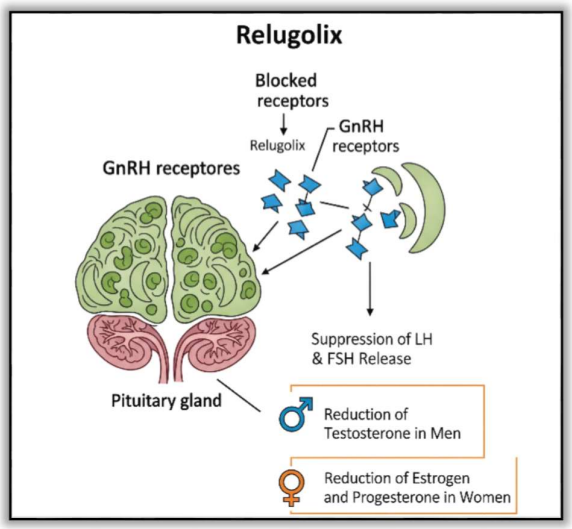


Fig 2 : Mechanism of Action Relugolix

HISTORY OF RELUGOLIX Relugolix was originally developed by Takeda Pharmaceuticals as TAK-385 in the early 2000s and was licensed to Roivant Sciences in April 2016. Following this agreement, Myovant Sciences was established to further develop and commercialize relugolix. Phase 3 clinical trials for uterine fibroids began in 2017, leading to the drug's first approval in Japan in January 2019 for the treatment of symptomatic uterine fibroids. In December 2020, relugolix received U.S. FDA approval for androgen-deprivation therapy in advanced prostate cancer. Subsequent approvals included a combination product for uterine fibroids in 2021 and approval for prostate cancer treatment in the European Union in 2022[10-12]

CHEMISTRY OF RELUGOLIX

Relugolix is an orally active, non-peptide gonadotropin-releasing hormone (GnRH) receptor antagonist. It's molecular formula is $C_{29}H_{27}F_2N_7O_5S$ and molecular weight is 623.63 g/mol. IUPAC name of relugolix is 1-[4-[1-[(2,6-difluorophenyl)methyl]-5-[(dimethylamino)methyl]-3-(6-methoxypyridazin-3-yl)-2,4-dioxo-1,2,3,4-tetrahydrothi-eno[2,3-d]pyrimidin-6-yl]phenyl]-3-methoxyurea. Relugolix appears as a white to off-white solid and it's melting point is 228–232 °C. Relugolix is soluble in methanol, acetonitrile, 0.1 N HCl and insoluble in water, 0.1 N NaOH[13,14].

3. PHARMACOKINETICS OF RELUGOLIX

Absorption:

Relugolix has low oral bioavailability of about 12%, primarily due to P-glycoprotein–mediated intestinal efflux. After a 120 mg once-daily dose, it is rapidly absorbed with a median Tmax of approximately 2.25 hours. Food reduces drug exposure by about 20%, but this does not affect treatment, so relugolix can be taken with or without food[15].

Distribution:

Relugolix binds about 70% to plasma proteins, mainly albumin, and shows a very large volume of distribution 3900 L, indicating that it spreads widely throughout body tissues. The blood-to-plasma ratio of 0.78 suggests limited entry of the drug into red blood cells[15].

Metabolism:

Relugolix is metabolized mainly by CYP3A4/5 (45%) and CYP2C8 (37%), with additional metabolism by intestinal microflora[15].

Elimination:

Relugolix is primarily excreted in faces (81%, with 4.2% unchanged) and to a lesser extent in urine (4.1%, with 2.2% unchanged), indicating that most of the drug undergoes metabolism before elimination[15].

Half-life:

The average effective half-life of relugolix is 25 hours, while the average terminal elimination half-life is 61.5 hours.

PHARMACODYNAMICS OF RELUGOLIX

Relugolix rapidly reduces testosterone levels in men. About 56% of patients achieve castrate testosterone levels (<50 ng/dL) within 4 days, and nearly 97% of patients maintain these levels for up to 48 weeks with once-daily oral dosing. Like other androgen-deprivation therapies, relugolix may prolong the QTc interval and should be used carefully in patients with heart disease, electrolyte imbalance, or those taking medicines that affect QT prolongation. Due to its hormonal effects, relugolix may cause fatal harm. therefore, male patients with female partners are advised to use effective contraception during treatment and for 2 weeks after stopping therapy[16].

ADVERSE EFFECTS OF RELUGOLIX Relugolix may cause some side effects mainly due to hormone suppression. Commonly reported adverse effects include hot flushes, night sweats, fatigue, and a general lack of strength. Gastrointestinal effects such as constipation and diarrhoea may also occur. Metabolic changes, including increased blood glucose and triglyceride levels have been observed in some patients. Musculoskeletal complaints such as bone pain and myalgia have also been reported. In addition, patients may experience pollakiuria (frequent urination) and redness of the face, neck, and arms[17].

DRUG INTERACTION

Relugolix exposure is affected by drug interactions. P-glycoprotein or CYP3A inhibitors (e.g., erythromycin) can increase relugolix levels and the risk of side effects, while CYP3A and P-glycoprotein inducers (e.g., rifampicin) can reduce drug levels and decrease effectiveness; therefore, such combinations should generally be avoided[18].

4. LITERATURE REVIEW

Table 2.1 Reported Methods for Assessment of Relugolix

Sr. no.	Title	Description	Ref. no.
UV-Spectrophotometric Method			
1.	Analytical Method Development for the Estimation of **Relugolix** API Using **UV-Spectrophotometry**	**Solvent:** Methanol **Wavelength:** 287 nm **Linearity:** 4-24 µg/mL	19
HPLC (High Performance Liquid Chromatographic) Method			
1.	**RP-HPLC Method** Development and Validation of **Relugolix**	**Mobile Phase:** Acetonitrile : Ammonium Formate (55 : 45 %v/v) **Stationary Phase:** Zobrax C_{18} Column (160 mm x 5.5 mm, 5 µm) **Wavelength:** 310 nm **Flow Rate:** 1 mL/min **Injection Volume:** 10 µL **Retention Time:** 2.79 min **Run Time:** 6 min	20
2.	Development and Validation of Stability Indicating **RP-HPLC Method** for Estimation of **Relugolix** in Tablet Dosage Form	**Mobile Phase:** Buffer (pH 2.4) : Acetonitrile (70 : 30 %v/v) **Stationary Phase:** Inertsil ODS-3 Column (150 mm x 4.6 mm, 5 µm) **Wavelength:** 220 nm **Flow Rate:** 1 mL/min	21

		Injection Volume: 10 µL **Retention Time:** 4.775 min	
3.	Devising a Robust **RP-HPLC Method** for the Determination of **Relugolix's** Related Substances and Characterization of its Degradant using LC-MS/MS	**Mobile Phase:** Trifluoro Acetic Acid Buffer (pH 2.5) : Acetonitrile (50 : 50 %v/v) **Stationary Phase:** Phenyl C_{18} Column (150 mm x 4.6 mm, 3.5 µm) **Wavelength:** 216 nm **Flow Rate:** 1 mL/min **Injection Volume:** 10 µL **Retention Time:** 6.734 min	22
4.	Determination of Hydroxylamine Hydrochloride in **Relugolix** Using Pre-Column Derivatization **HPLC Method**	**Mobile Phase:** Acetonitrile : Phosphate Buffer Solution (pH 2.3) (35 : 65 %v/v) **Stationary Phase:** YMC-Pack ODS-A Column (150 mm x 4.6 mm, 5 µm) **Wavelength:** 254 nm **Flow Rate:** 1 mL/min **Injection Volume:** 10 µL **Run Time:** 30 min	23
5.	Advancing Relugolix Analysis: A Comparative Study and AQbD-Driven Method Optimization with Stability Testing	**Mobile Phase:** Phosphate Buffer (pH 2.5) : Acetonitrile (50 : 50 %v/v) **Stationary Phase:** Kromasil C_{18} Column (250 mm x 4.6 mm, 5 µm) **Wavelength:** 230 nm **Flow Rate:** 1mL/min **Injection Volume:** 10 µL **Retention Time:** 27 min **Run Time:** 60 min	24

UPLC (Ultra Performance Liquid Chromatographic) Method			
1.	A Novel Validated Stability Indicative **UPLC Method** for **Relugolix** for the Determination of Process Related and Degradation Impurities	**Mobile Phase:** 1% Orthophosphoric Acid : Acetonitrile (50 : 50 %v/v) **Stationary Phase:** BEH RP-18 Column (100 mm x 2.1 mm, 1.7 µm) **Wavelength:** 215 nm **Flow Rate:** 0.4 mL/min **Injection Volume:** 5 µL **Run Time:** 10 min	25
2.	An Efficient **UPLC-MS/MS Method** Established to Detect **Relugolix** Concentration in Rat Plasma	**Mobile Phase:** 0.1% Formic Acid : Acetonitrile (90 : 10 %v/v) **Stationary Phase:** BEH C_{18} Column (50 mm x 2.1 mm, 1.7 µm) **Flow Rate:** 0.30 mL/min **Retention Time:** 1.37 min	26
3.	Development and Validation of a Method for Studying **Relugolix** and its Impurities by **UPLC-MS**	**Mobile Phase:** 0.1% Orthophosphate : Acetonitrile (50 : 50 %v/v) **Stationary Phase:** C_{18} Column (100 mm x 2.1 mm, 1.7 µm) **Wavelength:** 247 nm **Flow Rate:** 0.5 mL/min **Injection Volume:** 5 µL **Retention Time:** 4.668 min **Run Time:** 18 min	27
LC-MS/MS (Liquid Chromatography-Tandem Mass Spectrometry) Method			

1.	Bio-Analytical Method Development and Validation for the Quantitation of **Relugolix** in Plasma Samples by **LC-MS/MS**: Application to Bioavailability Study in Rabbits	**Mobile Phase:** Acetonitrile : 0.1% HCOOH (90 : 10 %v/v) **Stationary Phase:** Inertsil C_{18} Column (150 mm x 2.10 mm, 5 µm) **Flow Rate:** 0.8 mL/min	28
GC-MS (Gas Chromatography with Mass Spectroscopy) Method			
1.	A New Analytical Method for Determination and Quantification of Residual Solvents in **Relugolix** Api Bulk Drug by **GC-MS Method**	**Solvents:** 2-Propanol, n-Hexane, Ethyl Acetate, Tetrahydrofuran, Toluene **Film Thickness Column:** DB-5 (30 m, 0.53 mm, 0.25 m) **Carrier Gas:** Helium **Flow Rate:** 1.50 mL/min	29

5. CONCLUSION

Relugolix is an important therapeutic agent for advanced prostate cancer. Accurate analytical evaluation is essential for its quality assessment in bulk drug and pharmaceutical dosage forms. Various spectroscopic and chromatographic methods have been reported in the literature for its estimation. Based on this review, analytical techniques such as UV spectrophotometry, HPLC, UPLC, LC-MS/MS, and GC-MS were found to be reliable, precise, and suitable for routine quality control. This review provides a concise theoretical overview and literature summary supporting future analytical method development.

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