IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Review Article
August 2023 Vol.:28, Issue:1

Analytical Method Development of 5-α-Reductase Inhibitors

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Manisha Subba[1], Dev Prakash[2], Abigel Gurung[3], Gautam Rai[4], Pallavi Sharma[5]

[1]Research Scholar, Department Of Pharmaceutical Chemistry, Abhilashi University, Chailchowk, Mandi (H,P) India.

[2]Department Of Pharmaceutical Chemistry, Abhilashi University Chailchowk, Mandi (H.P) India.

[3]Research Scholar, Department Of Pharmaceutical Chemistry, Abhilashi University, Chailchowk, Mandi (H,P) India.

[4]Research Scholar, Dep artment Of Pharmaceutical Chemistry, Abhilashi University, Chailchowk, Mandi (H,P) India.

[5]Research Scholar, Department Of Pharmaceutical Chemistry, Abhilashi University, Chailcowk, Mandi (H,P) India.

Submitted: 25 July 2023
Accepted: 18 August 2023
Published: 30 August 2023

Keywords: Validation, New Drug Application (NDA), Analytical Method Development, Benign Prostatic Hyperplasia (BPH), α-reductase inhibitor, drug development

ABSTRACT

This review paper delves into the development and validation of analytical methods for assessing the efficacy and safety of these inhibitors. The paper explores the process of new drug approval in India and the FDA's New Drug Application (NDA) procedure for market permission. Analytical method development, validation, and transfer are crucial components of pharmaceutical development, optimizing resources, and enhancing drug testing procedures. Challenges in method development and validation are addressed, emphasizing their impact on drug development timelines and cost efficiency. The review also provides insights into the pathophysiology of BPH, which is primarily caused by the enlargement of the prostate, leading to lower urinary tract symptoms. Various treatment approaches, including non-pharmacological and pharmacological options, are discussed, with a focus on α-reductase inhibitors. In conclusion, this review paper outlines the advancements made in 5α-reductase inhibitor research and its potential impact on medical interventions. The ongoing efforts in understanding the mechanisms of action and optimizing the efficacy of these inhibitors hold promise for improving patients' quality of life and contributing valuable knowledge to the medical community. Future research endeavors should focus on addressing existing knowledge gaps, refining treatment strategies, and advancing drug development in this field.

INTRODUCTION

Approval of New Drug in India

When a company in India wants to manufacture/import a new drug; it has to apply to seek permission from the licensing authority (DCGI) by filing Form 44 and also submitting the data as given in Schedule Y of the Drugs and Cosmetics Act 1940 and Rules 1945. In order to prove its efficacy and safety in the Indian population it has to conduct clinical trials in accordance with the guidelines specified in Schedule Y and submit there port of such clinical trials in a specified format (1)**.**

New Drug Application (NDA)

NDA is an application submitted to the FDA for permission to market a new drug. To obtain this permission a sponsor submits preclinical and clinical test data to the NDA for analyzing the drug information, and description of manufacturing procedures. After NDA is received by the agency, it undergoes a technical screening. This evaluation ensures that sufficient data and information have been submitted in each area to justify "filing" the application that is FDA formal review (2)**.**

At the conclusion of FDA review of an NDA, there are 3 possible actions that can be sent to sponsor:

i. **Not approvable-** In this letter list of deficiencies and explain the reason.

ii. **Approvable-** It means that the drug can be approved but minor deficiencies that can be corrected like-labelling changes and possibly request commitment to do post-approval studies.

iii. **Approval-** it state that the drug is approved. If the action taken is either approvable or not approvable, then FDA provides applicant with an opportunity to meet with the agency and discuss the deficiencies (2)**.**

Analytical Method Development

Analytical method development, validation, and transfer are key elements of any pharmaceutical development program. This technical brief will focus on development and validation activities as applied to drug products. Often considered routine, too little attention

is paid to them with regard for their potential to contribute to overall developmental time and cost efficiency (3).

Analytical methods are intended to establish the identity, purity, physical characteristics and potency of the drugs that we use. Methods are developed to support drug testing against specifications during manufacturing and quality release operations, as well as during long-term stability studies. Methods may also support safety and characterization studies or evaluations of drug performance (3).

Challenges in Method Development

Effective method development ensures that laboratory resources are optimized, while methods meet the objectives required at each stage of development. Method transfer is the formal process of assessing the suitability of methods in another laboratory. Each of these processes contributes to continual improvement of the methods and results in more efficient drug development (4).

Method Development Process

The steps involved in method development and method validation depend upon the type of method being developed (4). The process employed for method development shown in the figure 1.1.

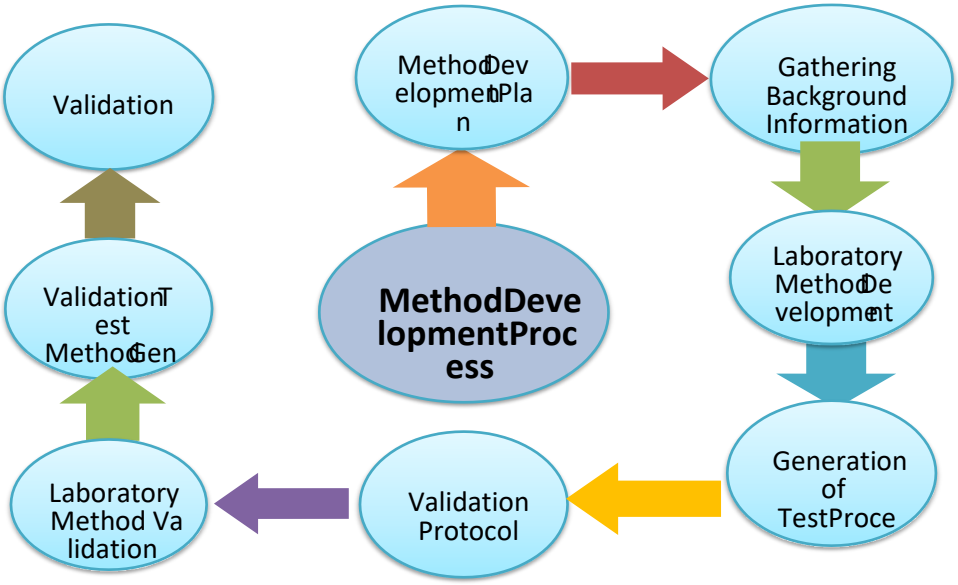

Fig.1.1. Schematic diagram showing Method Development Process

When should methods be validated?

A method should be validated when it is necessary to verify that its performance parameters are adequate for use for a particular analytical problem. For example:

- Method just developed
- Revised method or established method adapted to a new problem;
- When a review of quality control indicates an established method is changing with time;
- When an established method is used in a different laboratory, with different analysts or with different equipment.
- Demonstration of the equivalence between two methods, e.g. a new method and a standard. Certain areas of analytical practices, such as in clinical chemistry will specify validation requirements relevant to the method. This ensures that particular validation terminology together with the statistics used is interpreted in a manner consistent within the relevant sector. Official recognition of a method may require characterization using a collaborative study.

Validation of developed analytical method

Method validation is the process of demonstrating that analytical procedures are suitable for their intended use and that they support the identity, strength, quality, purity and potency of the drug substances and drug products. ICH and USFDA guidelines are used worldwide for harmonized analytical method validation. Various parameters are used to validate the methods:

- Linearity
- Accuracy
- Precision
- Limit of Quantification
- Limit of Detection

- Ruggedness and Robustness

Benign Prostatic Hyperplasia (BPH)

Benign prostatic hyperplasia (BPH) is the non-malignant enlargement of the prostate and clinically occurs predominantly in men aged over 60 years. When the prostate increases in size it can exert pressure on the urethra as it emerges from the bladder, resulting in gradual obstruction to urine flow. Although the manifestations and severity of the disease vary, the symptoms that bring the patient to the doctor before complications develop are usually frequency of urination and poor flow. However, the increased awareness that there are multi-factorial causes, including bladder dysfunction, of such lower urinary tract symptoms (LUTS) has led to the discovery that although many men with LUTS have true prostatic enlargement, many do not. Furthermore, size appears not to matter: a study has shown that there exists no correlation between prostate volume, urinary symptom score, or urinary flow (5)**.**

Hence, BPH is now discussed in terms of benign prostatic enlargement and LUTS, and there remains considerable uncertainty surrounding the diagnosis and treatment of BPH, with no clear definition of symptoms. Development of BPH has both static and dynamic components relating to the prostate: the static component is related to increased prostatic tissue mass whereby progressive proliferation leads to increased prostatic size and consequent bladder outlet obstruction (BOO).

Indeed, the ratio of stroma to epithelium can increase from 2:1 in the healthy prostate to 5:1. In contrast, the dynamic component of BPH is related to the tone of prostatic smooth muscle which is supplied by sympathetic nerve fibers (6)**.**

To help diagnose BPH and rule out other diagnoses, every patient requires a detailed medical history, including a symptom assessment questionnaire from which a “symptom score“ can be calculated, plus a physical examination and laboratory tests. The maximum flow rate is usually the most informative measurement, but the results are non-specific: reduced flow may because by impaired detrus or contract ion as well as urethra structure (6)**.**

Pathophysiology of BPH

The pathophysiology of bladder outlet obstruction in men with BPH has been attributed to both static and dynamic factors. The static obstruction is due to the bulk enlargement of the prostate encroaching upon the prostatic urethra and bladder outlet, whereas the dynamic obstruction is related to the tension of the prostate smooth muscle. The medical therapies widely used today for the treatment of BPH are targeted to diminishing bladder outlet obstruction in order to reduce prostate volume and relax prostate smooth muscle tension (7)**.**

In summary, there is no clinically significant relationship between prostatic enlargement and LUTS in men with clinical BPH. There is only a weak relationship between LUTS and bladder outlet obstruction. Therefore, factors other than prostatic enlargement and bladder outlet obstruction must contribute to the development and severity of LUTS (7)**.**

Treatments of BPH

The aim of the therapy is to improve LUTS and quality of life and to prevent disease progression or the need for surgery. Patients with BPH who have complications such as recurrent urinary tract infections, haematuria, bladder stones or renal failure should be treated surgically. If the assessment reveals mild symptoms (IPSS<8), then nonpharmacological treatments such as watchful waiting or lifestyle modifications may be adopted (8)**.**

The various approaches for the management of BPH are-

Non-Pharmacological Approaches-

Watchful waiting

Watchful Waiting is a viable option to many men as the risk of progression (acute urinary retention, need for surgery, renal insufficiency, bladder stones) is small. It is customary for this type of management to include the following components: education, reassurance, periodic monitoring and lifestyle advice**.** Optimization of WW can be achieved with lifestyle modifications including reduction of fluid intake, avoidance or moderation of caffeine/ alcohol, distraction techniques (penile squeezing, breathing exercises), bladder retraining, and reviewing medication. WW is recommended for patients with minimal symptoms or

moderate/severe symptoms with little impairment of quality of life. Reassurance, periodic monitoring and lifestyle modifications are advisable (9)**.**

Lifestyle modification

Bothersome symptoms can sometimes be sufficiently reduced with minor lifestyle modifications and is particularly useful for men who choose to avoid surgery or drug, therapy. Some simple suggested changes to lifestyle include.

- Avoid drinks that are high in caffeine.
- A diet low in fat and red meat and high in protein and vegetables, as well as regular alcohol consumption, may reduce the risk of symptomatic BPH.
- Moderate consumption of alcohol may be beneficial but heavy alcohol consumption may increase LUTS.
- Drinking green tea which contains flavonoids may, however, benefit prostatitis.
- Increase the amount of fiber and fruit avoid constipation and lower the risk of BPH. A higher risk of BPH is associated with high intake of butter and margarine.
- Low zinc status has been observed in cancer patients, suggesting a possible link between zinc and cancer development. So zinc may serve an important role in regulating cell growth and apoptosis in prostate cancer and hyperplasia cells.
- Genistein, a chemical found in soy, reduces the growth of BPH tissue in laboratory.
- Avoiding medications that can increase obstructive urinary symptoms like tricyclic anti-depressants and anticholinergic drugs, diuretics, narcotics and first generation antihistamines and decongestants (9).

Pharmacological Treatment

α-Reductase Inhibitors (5ARIs)

Steroid 5α reductase inhibitors (5ARIs) have been approved for use clinically in treatment of benign prostate hyperplasia (BPH) and accompanying lower urinary tract symptoms (LUTS)

and have also been evaluated in clinical trials for the prevention and treatment of prostate cancer. (10)**.**

Currently two 5ARIs, one selectively inhibiting 5a-reductase type II (finasteride) and one inhibiting 5α-reductase type I and II (dutasteride) are available. Finasteride leads to a reduction of serum dihydro testosterone (DHT) by 70–75%, the remaining DHT is the result of 5α-reductase type I. Dutasteride induces a more profound reduction of serum DHT in the range of 90–95%. Both drugs have been extensively tested in several RCTs, for dutasteride two-year RCT data, for finasteride up to 5.5-year RCT data are available (10).

5ARIs are more effective in men with BPE (>30–40 ml). Clinical efficacy is seen delayed, usually after 3–6 months. Although no published data of a head-to-head comparison are yet available, the clinical efficacy of finasteride and dutasteride seems to be comparable. Both drugs lower serum PSA by approximately 50%, and both do not mask early detection of PC. Side effects mainly relate to sexual function with a decreased libido in 6%, erectile dysfunction in 8% and decreased ejaculation in 4%.

Both drugs reduce the risk of acute urinary retention and the need for surgery by approximately 50% as compared to placebo**.**

5ARIs are an acceptable treatment option for patients with moderate/severe LUTS and an enlarged prostate (>30–40 ml). 5ARIs may also be offered to patients with BPE to prevent the progression of disease; potential disadvantages of this approach (long-term medication, side effects, costs, PC) have to be carefully discussed with the patient (11)**.**

α-Blockers

α-blockers therapy is based on the hypothesis that LUTS are caused by α1-adrenergic mediated contraction of smooth muscle cells within the prostate, prostate capsule and bladder neck resulting in BPO. Within the past 10 years, market shares of α-blockers increased substantially and are currently the preferred first-line medical therapy for men with moderate/ severe LUTS. Alfuzosin, doxazosin, tamsulosin and terazosin have been extensively studied in RCTs with duration of up to 12 month. Symptom scores improve by 4–6 points, and Q_{max} by 2–3 ml/s. Clinical efficacy are observed within 48 hrs and data of single-arm studies over several years are available. α1-blockers have no effect on prostate volume and PSA and do not prevent further prostate growth. The most commonly reported

side effects are headaches, dizziness, postural hypotension, asthenia, nasal congestion and retrograde ejaculation (12).

α1-blockers are an acceptable treatment option for patients with moderate/ severe LUTS. All four α1-blockers (tamsulosin, terazosin, alfuzosin, doxazosin) have a similar clinical efficacy, although side-effect profiles for some drugs are reported to be more favourable. (12).

Steroidal and Non-Steroidal 5α-Reductase Inhibitors

As the only information available about the 5α-reductase isozymes is their primary sequence estimated from c-DNAs the design of novel inhibitors is affected. Due to the unstable nature of enzyme during purification its crystal structure is not known. The first inhibitors have been therefore, designed by modifying the structure of natural substrates, including the substitution of one carbon atom of the rings of the steroids by a heteroatom such as nitrogen thereby forming azasteroids. Singh and as well as other groups 8 have published comprehensive reviews on co-workers biological activity of azasteroids. Azasteroidal compounds having nitrogens at various positions have also been covered in this review. However, their 5a-reductase inhibitory activity has either not been done or they are devoid of activity. Some azasteroids have been found to be 5αreductase inhibitors. In the following section aza steroidal inhibitors have been discussed depending upon the position of nitrogen in the steroidal nucleus i.e. nuclear azasteroids (13).

The following passages contain a collection of research findings and studies related to the synthesis and development of 5α-reductase inhibitors for the treatment of benign prostatic hyperplasia (BPH). Some of the key points highlighted in these studies are as follows:

- G. M. Clifford and R.D.T. Farmer in 2000 indicated that both classes of drugs licensed for BPH treatment in the UK, α-blockers, and finasteride, offer significant improvement in all criteria used to evaluate LUTS severity (14).

- According to G. Untergasser et al. in 2021, the pathogenesis of BPH is still poorly understood, involving multiple events in biological communication systems during organ aging, rather than a single mechanism (15).

- M. Uemura et al. 2008 found that 5α-steroid reductase enzymes, especially SRD5A2, are strongly associated with BPH and prostate cancer risk (16).

- M. Emberton et al. in 2008 presented that BPH is a complex, progressive disease associated with bothersome LUTS and potential complications. Identifying men at increased risk of BPH progression is crucial for optimal therapy (17).

- In 2010, S. Aggarwal et al. developed predictive 3D-QSAR models for unsaturated 4-azasteroids with flexibility in structure and potency profile against human 5α-reductase. The models indicated electrostatic and shape potential contributions (18).

- According to S. Aggarwal et al. in 2010, over the last two decades, various steroidal and non-steroidal compounds have been evaluated as 5α-reductase inhibitors. Steroidal drugs like finasteride and dutasteride are clinically used, while Epristeride entered clinical trials (18).

- In 2013, M. Malhotra et al. reported that 5α-reductase inhibitors are a preferred treatment for BPH, effectively reducing DHT levels and preventing complications (19).

- In 2017, A. Scaglione et al. discussed the controversial subcellular localization of members of the SRD5A family in humans and mice, particularly SRD5A1 and SRD5A2, which were suggested to be nuclear in the prostate and cytoplasmic in the liver (20).

These findings highlight the complexity of BPH and the importance of developing effective and targeted treatments for this condition. 5α-reductase inhibitors have shown promise in managing BPH symptoms and improving patients' quality of life. However, further research is needed to fully understand the mechanisms of action and optimize their use in clinical practice.

CONCLUSION

The study of steroidal and non-steroidal 5α-reductase inhibitors has provided valuable insights into the potential therapeutic applications of these compounds in various medical conditions. Despite the challenges posed by the lack of crystal structures and limited information on the 5α-reductase isozymes, researchers have made significant strides in designing novel inhibitors.

The discovery of asteroidal compounds and their inhibitory effects on 5α-reductase has been a notable breakthrough. Additionally, non-steroidal inhibitors have been explored as an alternative approach, aiming to reduce potential interactions with other enzymes or receptors and simplify compound synthesis.

Clinical studies evaluating the effectiveness of various inhibitors, such as finasteride and terazosin, have shown encouraging results for treating benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms (LUTS). Despite significant advancements, the pathogenesis of BPH remains incompletely understood, involving multiple biological communication systems during the aging process of the prostate.

Researchers have employed various computational approaches and molecular modeling techniques to predict the inhibitory potential of different compounds against 5α-reductase. These methods hold promise for identifying new lead compounds and optimizing drug candidates.

In conclusion, the research on 5α-reductase inhibitors has opened up new avenues for therapeutic interventions in conditions like BPH and prostate cancer. However, further studies are necessary to fully understand the mechanisms of action and optimize the efficacy of these inhibitors. The ongoing advancements in the field hold the potential to improve the quality of life for patients and provide valuable contributions to the medical community. Future research efforts should focus on addressing the remaining knowledge gaps, advancing drug discovery, and refining treatment strategies for the benefit of patients worldwide.

REFERENCES

1. Process of approval of new drug in India with emphasis on clinical trials. Gupta , N. Vishal, et al. 2, 20 Mar 2012, International Journal of Pharmaceutical Sciences Review and Research, Vol. 13, pp. 17-23.
2. New Drug Application (NDA). Https://www.fda.gov/drugs/types-applications/new-drug-application-nda. [Online] 21 Jan 2022.
3. Analytical Method Development and Validation. [Online] Lubrizol Life Science, 27 Nov 2019.
4. Strategies And Challenges In Method Development And Validation For The Absolute Quantification Of Endogenous Biomarker Metabolites Using Liquid Chromatography-Tandem Mass Spectrometry. Khamis , Mona M. , Adamko, Darryl J and El-Aneed, Anas. 1, Feb 2021, Mass Spectrometry Reviews, Vol. 40, pp. 31-52.
5. Diseases, Natonal Institute of Diabetes and Digestive and Kidney. Prostate Enlargement (Benign Prostatic Hyperplasia. [Online] U.S. Department of Health and Human Services.

6. Pathophysiology of Benign Prostatic Hyperplasia and Benign Prostatic Enlargement: A Mini-Review. Madersbacher, Stephan , Sampson , Natalie and Culig, Zoran . 5, s.l. : Pub Med, 2019, National Library of Medicine, Vol. 65, pp. 458-464.
7. Pathophysiology of Benign Prostatic Hyperplasia in the Aging Male Population. Lepor, Herbert. 4, s.l. : Pub Med Central, 2005, National Library of Medicine, Vol. 7.
8. Levi A Deters, MD. Benign Prostatic Hyperplasia (BPH) Treatment & Management. Medscape. [Online] 22 Mar 2023.
9. Nordling, Jørgen. Watchful waiting and lifestyle issues associated with LUTS and BPH. [book auth.] Alexander Bachmann and Jean de la Rosette. Benign Prostatic Hyperplasia and Lower Urinary Tract Symptoms in Men. S.l. : Oxford Academics, 2011, pp. 78-84.
10. 5α-Reductase Inhibitors (5aris) and Male Reproduction. Drobnis , Erma Z and Nangia , Ajay K. S.l. : Pubmed, 2017, National Library of Medicine, pp. 59-61.
11. Pharmacology of the lower urinary tract: update on LUTS treatment. Abreu-Mendes, Pedro, Silva, João and Cruz, Francisco. S.l. : Pubmed, 12 Jan 2020, National Library of Medicine, Vol. 12.
12. Lepor , Herbert, Kazzazi, Amir and Djavan, Bob. S.l. : Pubmed, jan 2012, National Library of Medicine, pp. 7-15.
13. An overview on 5α-reductase inhibitors. Aggarwal, Saurabh and Thareja , Suresh. [ed.] Saurabh Aggarwal , Suresh Thareja and Verma Abhilasha . 2, Feb 2010, Elsevier, Vol. 75, pp. 109-153.
14. Medical therapy for benign prostatic hyperplasia: a review of the literature. Clifford, G.M and Farme, R.D. 2002, European Urology, Vol. 38, pp. 2-19.
15. . Benign prostatic hyperplasia: age-related tissue-remodeling. Untergasser , Gerold, Madersbacher, Stephan and Berger, Peter. 3, s.l. : Pubmed, Mar 2005, National Library of Medicine, Vol. 40.
16. Novel 5α-steroid reductase (SRD5A3, type-3) is overexpressed in hormone-refractory prostate cancer. Uemura, M, Tamura , K and Chung , S. 1, 2008, Vol. 9.
17. Management of prostate cancer in older men: recommendations of a working group of the International Society of Geriatric Oncology. Emberton , M, et al. 4, 25 Mar 2010, Vol. 106, pp. 462-469.
18. 3D-QSAR studies on unsaturated 4-azasteroids as human 5α-reductase inhibitors: A self organizing molecular field analysis approach. Aggarwal , Saurabh , Thareja , Suresh and Bhardwa, T.R. 2, Feb 2010, European Journal of Medicinal Chemistry, Vol. 43, pp. 476-481.
19. Targeting 5α-reductase for prostate cancer prevention and treatment. Malhotra, M. S.l. : Pubmed, 2013, National Library of Medicine.
20. Subcellular localization of the five members of the human steroid. Scaglione , Antonella and Parisi, Giacomo. S.l. : Science Direct, 2017, Elsevier, pp. 99-106.