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Research

Method Validation For Estimation Of Atenolol and Indapamide in Tablet Formulation

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Abstract

This study focuses on the method validation for the estimation of Atenolol and Indapamide in tablet formulation using High-Performance Liquid Chromatography (HPLC). The method involves using a mobile phase of methanol:water (55:45) with 0.1% v/v Ammonium Hydroxide, a Phenomenex C18 analytical column, and UV detection at 260nm. The method was validated for parameters such as selectivity, specificity, linearity, precision, limit of detection, and limit of quantification. The results showed that the method is reproducible, accurate, and precise for the estimation of Atenolol and Indapamide in tablet formulation.

Keywords: Atenolo, Indapamide, High Performance Liquid Chromatography (HPLC), UV Detection, Methanol, Ammonium Hydroxide, Pharmacokinetics, Pharmacodynamics, Bioavailability, Standardization, Adverse Effects, Contraindications.

INTRODUCTION

A growing proportion of Indian pharmaceutical firms are pursuing regulatory approval for novel molecules. In this advancing environment, unwavering commitment to stringent quality control standards and validation procedures is imperative and pertinent throughout the entire product registration process, both in India and globally. The notion of quality concerning drugs and their products includes all factors that affect, whether directly or indirectly, the purity, safety, efficacy, and dependability of pharmaceutical provisions.
Analytical chemistry encompasses the discipline of accurate measurement. Drug analysis centers on the identification of substances, the comprehension of their molecular structures, and the quantitative evaluation of their compositions. The examination of an analyte within a complex sample frequently evolves into a challenge-solving endeavor. To perform their roles effectively, analytical chemists are required to possess proficiency in the utilization of diverse tools

to tackle an array of challenges. Generally, a signal is produced that encapsulates the chemical and physical attributes of the drugs involved. These signals may be employed in their original form or transformed into alternative representations, enhanced in strength, and exhibited on a device. The attributes of the initial system can subsequently be deduced from the final measurement. This relationship between the ultimate signal and the original system acts as the cornerstone for instrumental analysis.

The importance of contemporary analytical methodologies:
With the growing demand for potable water, enhanced food safety, and more pristine environments, analytical chemists are emerging as increasingly essential contributors to modern society. Qualitative and quantitative chemical analyses are employed in manufacturing to verify that raw materials meet predetermined specifications and to evaluate the quality of the ultimate products. Raw materials are subjected to scrutiny to ascertain the absence of anomalous compounds, as such substances may constitute hazardous contaminants in the final products. The quantity of novel pharmaceuticals entering the market is rapidly increasing, including both entirely new medications and modified formulations of existing ones. Innovative approaches are developed for these medications and their combinations due to various factors:
The drug or combination of drugs may not be included in any official pharmacopeia.
A thorough examination of existing literature may not reveal an established analytical method for the drug or its formulation.
Analytical techniques may not be available for the specific drug combination. In contrast, the prevailing techniques may:
Require expensive instruments, reagents, or solvents.
Demand extensive extraction or separation procedures that may prove to be notably time-consuming.
Deficient in terms of velocity, dependability, or sensitivity. The recently established analytical techniques are vital across a wide range of sectors:
Research institutions.
- Quality control departments in various industries.
Accredited testing facilities.
Biopharmaceutics and bioequivalence investigations, in conjunction with clinical pharmacokinetics.

Estimation of drugs in dosage forms
For various medical purposes, it is essential to mix two or more drugs, and certain combinations have shown effectiveness due to their shared actions in the body. The process of estimating these combined doses can be intricate. It is vital to ensure that one drug does not disrupt the estimation of another.

Classification of analytical method
Analytical techniques are typically grouped into two categories: classical or instrumental. This categorization is primarily based on historical context, with classical techniques being established significantly earlier than instrumental ones.

Classical methods
Historically, most analyses involved isolating the target components in a sample through methods like precipitation, extraction, or distillation. To quantify the analyte, its amount was measured through gravimetric or titrimetric methods. These traditional techniques for isolating and measuring analytes are still utilized in many labs today. However, their widespread use is diminishing over time, especially with the introduction of instrumental techniques that enhance these methods.

Instrumental methods
In the early 20th century, chemists started to utilize different phenomena beyond what classical methods offered for tackling analytical challenges. Consequently, measurements related to the physical properties of analytes, including conductivity, light absorption, fluorescence, and electrode potential, began to be employed for quantitative analysis of various inorganic, organic, and biochemical substances. These modern approaches for isolating and measuring chemical entities are collectively referred to as instrumental analysis methods.

Types of instrumental methods1-8
Spectroscopic Techniques
Electrochemical Techniques

Chromatographic techniques
Miscellaneous Techniques
Hyphenated Techniques

Selection of methods
The requirement of a satisfactory method for analysis is many and hence the chemist needs to take into account several parameters such as complexity of the materials to be analyzed. However, the most important parameters are selectivity and sensitivity of the analytical method. Some methods that are sensitive lack the inherent selectivity to allow straifht forward application to highly complex materials. Speed and simplicity are also considered and hence direct approaches for analysis are more desirable. When the sample is present in milligram concentration, volumetric or gravimetric methods can be used. However when the component is in low concentrations then optical methods or spectroscopic methods like UV-Visible, IR spectroscopy etc are used.
Selected methods should possess the following parameters
¬ As simple as possible
¬ Specific and selective
¬ Productive, economical and convenient
¬ As accurate and precise required
¬ Multiple sources of key components should be avoided

Stages of analytical methods9-11
Quantitative information may include the required accuracy and precision, range of expected analyte.
♣ Sampling
♣ Select the method of standardization
♣ Evaluate the results of analyses
♣ Presentation of results
UV- VISIBLE ABSORPTION SPECTROSCOPY 6-15:
High performance liquid chromatography (HPLC)

Research objectives
In today's world, creating analytical methods is crucial. The pharmaceutical sector depends on accurate chemical analysis to confirm that both the raw ingredients and the end products fulfill necessary standards. The ongoing and extensive use of these medications, alongside reports of new side effects and patient resistance, indicates that existing guidelines and analytical techniques may not be readily found in pharmacopoeias. Consequently, the innovation of new analytical processes becomes vital. With these considerations in mind, this study focuses on the drug diac and its formulations from various therapeutic areas that are currently available in the market. A thorough review of existing literature shows that some spectrophotometric and HPLC methods have been documented for measuring these medications in their separate formulations and biological samples. However, there is a lack of reports concerning their assessment via HPLC and spectrophotometric methods within the formulation. Therefore, it is crucial to create faster, modern analytical techniques like HPLC.

Drug profile
Atenolol
Chemical profile25-30, 36:
Chemical Name:
2-(4-{2-hydroxy-3-[(propan-2-yl) amino] propoxy} phenyl) acetamide
Molecular Formula : $C_{14}H_{22}N_2O_3$
Molecular Weight : 266.33608 [g/mol]
Appearance : White or almost white, crystalline powder.
Solubility: Sparingly soluble in water, freely soluble in alcohol, Freely soluble in acetonitrile, practically insoluble in ether.
Melting point: 146-148℃
Log P= 0.57 Log S= - 2.73
Pka= 15.95
Uses35 : Antihypertensive Agents, Adrenergic Agents, Adrenergic beta-Antagonists, Sympatholytics, Antiarrhythmic Agents, Anti-Arrhythmia Agents

Pharmacologic profile29,35:
Dosage:
If the dosage is greater than 100 mg, it is often split up and administered twice daily. The daily dose should be decreased in patients with compromised kidney function based on each patient's clinical response. Typically, 50 mg are given after each dialysis procedure for a patient with end-stage renal failure receiving regular dialysis.

Mechanism of Action 27,29,35:
By competing with sympathomimetic neurotransmitters such as catecholamines for binding at (1)-adrenergic receptors in the heart and vascular smooth muscle, atenolol blocks sympathetic activation. Higher doses of atenolol also competitively block (2)-adrenergic reactions in the bronchial and vascular smooth muscle, which causes a reduction in resting heart rate, cardiac output, systolic and diastolic blood pressure, and reflex orthostatic hypotension.

Pharmacokinetics29,35
Following oral administration, atenolol is well absorbed but undergoes significant hepatic (first pass) metabolism. It has a bioavailability of 40%. Before being eliminated via urine, it is less fully metabolized and has a wide distribution volume. Because of hepatic metabolism, a portion of the oral dosage may not reach the peripheral circulation, hence the plasma half-life does not correspond to the therapeutic effects, which are rather prolonged. This is due to the fact that the plasma half-life declines exponentially, following first-order kinetics, while the impact diminishes linearly, following zero-order kinetics. The medication has a half-life of 6 to 7 hours.

Negative impacts29,35:
Allergies: a response to the beta-blocker that causes an allergic reaction.
Pregnancy: The use of beta-blockers during pregnancy has been linked to hypoglycemia, dyspnea, bradycardia, and low blood pressure in the neonate.
Breastfeeding: Atenolol may transfer to breast milk. Bradycardia, hypotension, and dyspnea are some of the issues that have been observed in nursing infants.
Older adults: Older people, who are generally more susceptible to the effects of beta-blockers, are more prone to experiencing some side effects. Furthermore, older patients may have a lower tolerance for cold temperatures when taking beta-blockers.
Contraindication28,29:
Its major contraindications are cardiogenic shock, untreated heart failure, heart block of higher than second degree, and sinus bradycardia.

Indapamide25-36
Chemical profile31-34, 36:
Chemical Name: 4-chloro-N-(2-methyl-2,3-dihydro-1H-indol-1-yl)-3-sulfamoylbenzamide
Molecular Formula : c16h16cln3o3s
Molecular Weight : 365.83454 [g/mol]
Appearance : White or almost white, crystalline powder.
Solubility : Insoluble in water, freely soluble in alcohol, acetonitrile.
Melting point : 160℃
Log P : 2.52 Log S : - 4.03
Pka : 11.69
Uses : Hypertension and edema due to congestive heart failure.

Pharmacological profile31,35 :
Dosage: The recommended adult dosage ranges from 1.25 to 5 mg administered orally once daily.
Mode of Action33,35:
Indapamide inhibits the slow component of the delayed rectifier potassium current (iks) while leaving the rapid component (ikr) and the inward rectifier current unchanged. Specifically, it inhibits or opposes the function of the proteins KCNQ1 and KCNE1. It is also hypothesized that Indapamide enhances the production of the vasodilatory and hypotensive prostaglandin PGE2.

Pharmacokinetics33,35:

It is absorbed rapidly and completely from the gastrointestinal tract, achieving maximal blood concentrations in about 2.3 hours. The concurrent administration of indapamide with meals or antacids does not diminish its bioavailability. The extent of binding to plasma proteins is roughly 76%. The clearance of indapamide from the bloodstream exhibits a biphasic pattern, characterized by a terminal half-life of approximately 16 hours.

Adverse effects33-35:

Hypokalemia, characterized by insufficient potassium levels,
Tiredness
Orthostatic hypotension presents with allergic manifestations.

Contraindication33-35:

Indapamide is contraindicated in individuals with a known hypersensitivity to sulfonamides, severe renal insufficiency, hepatic encephalopathy, or severe hepatic failure, as well as hypokalemia (low serum potassium levels). Due to a lack of sufficient safety data, the use of indapamide during pregnancy or breastfeeding is not recommended

MATERIAL AND METHODS

Working Standard: The drug "Atenolol and Indapamide" was procured from industry.
Drug Sample: ATEN-D tablet was purchased from market.
♣ **Batch No. :** ZHK-1601
♣ **Date of MFG. :** 05/20
♣ **Date of EXP :** 09/22
♣ **Label Claim :**
♣ **Atenolol :** 50 mg.
♣ **Indapamide :** 2.5 mg.
Chemicals and Solvents Used:
♣ Methanol HPLC grade (Merck Pvt. Ltd., Mumbai)
♣ Water HPLC grade (Merck Pvt. Ltd., Mumbai)

Instruments Used
♣ Shimadzu LC 20AD HPLC System consisting of
O Pump- LC 20AD
O Detector- UV-SPD-M20A
O Autoinjector- SIL-20AC
Chromatographic Condition:
♣ **Column :** Phenomenex C18 Analytical Column (25×0.46 cm, i.d, 5 µm)
♣ **FR:** 1.0ml/min
♣ **Inj.** Vol. : 20µl
♣ **Wavelength:** 260nm
♣ **MP: Methanol:**Water (55:45) with 0.1%v/v Ammonium Hydroxide.

Method validation of aten-d tablet (rp-hplc)
Following parameters were analyzed
♣ Selectivity & Specificity
♣ Linearity
♣ Precision
♣ Limit of detection
♣ Limit of Quantitation or Quantification
♣ Change in Analyst
Specificity and system suitability
Stationary phase : C18 250 mm X 4.6 mm, 5µ, Inertsil ODS 3V.
Mobile phase : Separately, 675 ml of water (HPLC grade) and 825.0 ml of Methanol (HPLC grade) were filtered using membrane nylon filters of size 4.5 and added to the filtered solution. 1.5 ml Ammonium Hydroxide Solution was added, and the mixture was sonicated for 15 minutes before being filtered through membrane nylon filters with a size of 4.5.
Detector parameter : UV at wavelength 260 nm.

Flow rate : 1 ml/min.
Injection volume : 20 µl
Column oven temperature : 250 C.
Mode : Isocratic
Elution order : Atenolol and Indapamide.
Retention time : Atenolol 7.5 min.
Indapamide 8.99 min.
Blank : Methanol.
Run Time : 12 minutes.
Linearity Preparation of Stock Solution A:
In a 10 ml volumetric flask, weigh 50 mg of atenolol and 2.5 milligrammes of indapamide. Add enough methanol, sonicate, chill, and dilute with methanol up to the mark.
Stock Solution B:
Take 1ml of Stock Solution A in a 100ml volumetric flask and dilute it upto 100ml with methanol.
Sample 1:
Using a pipette, dilute 1ml of stock solution A in 10ml of volumetric flask with methanol to the desired concentration.
Sample 2:
Take 1ml of Sample 1 solution in 10 ml of volumetric flask with pipette and dilute it with methanol upto the mark.
Sample 3:
Take 1ml of sample 2 solution in 10 ml of volumetric flask with pippete and dilute it upto mark with methanol.
Sample 4:
Take 9ml of stock solution B in 10ml of volumetric flask with pipette and dilute upto 10ml with methanol.
Precision

Repeatability of Injection
Preparation of solution
From the Stock Solution C 1ml was pippeted out in 10ml of volumetric flask and the volume was made upto 10 ml with methanol.
Limit of detection
(LOD) was determined by:
LOD= 3.3(SD)/S
Limit of quantification
LOQ was determined by:
LOQ= 10 (SD)/S,
Robustness (Change in Analyst)

RESULTS AND DISCUSSION

"The objective of present work was to validate method for estimation of Atenolol and Indapamide in tablet formulation. Validation of method is carried out by using validation parameters viz., Selectivity & Specificity, Linearity, Precision, Limit of detection, Limit of Quantitation or Quantification and Change in Analysis. "The present High Performance liquid chromatographic method is to determine Atenolol and Indapamide from its formulation. Various experiments were carried out to establish the method. The mobile phase was methanol : water (55:45) with 0.1% v/v of Ammonium Hydroxide is found to be ideal for the estimation of Atenolol and Indapamide. The elution order was as followed (Atenolol – 7.5min, Indapamide – 8.9 min). The values of linearity, Precision and standard deviation show that the proposed method was reproducible, accurate and precise."
This information can be considered as part of the results and discussion. Detailed numerical results for parameters like selectivity, specificity, linearity, precision, LOD, LOQ, and robustness are not explicitly provided in the material.

CONCLUSION

The present High Performance liquid chromatographic method is to determine Atenolol and Indapamide from its formulation. Various experiments were carried out to establish the method. The mobile phase was methanol : water (55:45) with 0.1% v/v of Ammonium Hydroxide is found to be ideal for the estimation of Atenolol and Indapamide.

The elution order was as followed (Atenolol – 7.5min, Indapamide – 8.9 min). The values of linearity, Precision and standard deviation show that the proposed method was reproducible, accurate and precise.

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