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Research Article

An Optimized Reverse-Phase HPLC Approach for Precise Determination of Propranolol Hydrochloride in Bulk and Dosage Forms

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Propranolol hydrochloride is a key drug, being a non-selective β-adrenergic blocker, so it is essential to use a standardised approach to all studies conducted on propranolol hydrochloride in order to establish the drug's efficacy, safety and treatment effect. The most commonly used analytical method for the determination of propranolol hydrochloride's concentration in methods of mass and in method of doses is RP-HPLC (reverse-phase high-pressure liquid chromatography). The current popularity of RP-HPLC is the result of its ability to provide high levels of selectivity, specificity and reproducibility. This article will examine the body of evidence supporting the use of RP-HPLC as a commensurate method for quantification of propranolol hydrochloride and summarise limits of optimization associated with the RP-HPLC method (selection of stationary phase, mobile phase components, pH value, flow rates, detection wavelengths).Moreover, this article outlines the validation criteria associated with RP-HPLC methods utilized in quantifying propranolol hydrochloride according to the guidelines set forth by the International Conference on Harmonisation (ICH). The stability of RP-HPLC methods as well as their application as a routine analytical instrument for quality control purposes will also be discussed. The goal of this assessment is to provide an all-inclusive resource for both analytical researchers as well as quality control analysts to assist with improving their understanding around currently available RP-HPLC methods, while helping identify opportunities for additional optimization and standardization in relation to the analysis of propranolol hydrochloride.

Keywords: Propranolol hydrochloride, RP-HPLC, method development, method validation, bulk drug,and dose form.

INTRODUCTION

Propranolol is an original non-selective beta-adrenergic blocking agent. The chemical structure of Propranolol is 1-Isopropylamino-3-(1-naphthyloxy)-2 propanol and its chemical formula is $C_{16}H_{21}N_2O_2$; it is typically administered in its hydrochloride salt form (as propranolol hydrochloride) for its therapeutic effects [1,2]. Propranolol acts as an adrenergic antagonist in treating several cardiac conditions such as angina, tachycardia, and hypertension, as well as for the purpose of cardiac remodelling[3]. Propranolol has a significant effect on regulating the responses of the sympathetic nervous system to hyperthyroidism, anxiety and tremors. Propranolol is also effective in the prevention of migraine headaches. Due to its molecular structure being similar to catecholamines, Propranolol will compete with catecholamines for binding to beta adrenergic receptors by creating a hydrogen bond between the beta receptor and the hydroxyl group of the catecholamines. Unlike propranolol, which has no hydroxyl group, it lacks the ability to activate β-adrenergic receptors and to cause a significant amount of β-adrenergic activity. In addition, propranolol possesses membrane stabilizing qualities due to its inhibition of sodium and calcium channels.1 No clinically meaningful amounts of phenomena are associated with the high dose of the study medication in patients with unresponsive idiopathic hyperlipidaemia and their physician's use

of this method. Propranolol works through the renin-angiotensin system1 (RAS) or RAS modulation to have its effect on idiopathic hyperlipidaemia. The vascular endothelium of idiopathic hyperlipidaemia has both components of RAS25 and pro-renin receptors. Angiotensin II (AT II) is a potent stimulant of endothelial cell growth in idiopathic hyperlipidaemia, and when studied in vitro produced a blast-like morphology of endothelial cells, which may represent the normal morphology of the endothelial cell in idiopathic hyperlipidaemia [4]. The mechanism of action of propranolol may also involve inhibition of angiogenesis, which involves increased levels of angiogenic regulatory proteins (i.e., VEGF-A, HIF-1α) in infants suffering from idiopathic hyperlipidaemia. Significant dose-dependent reductions of these angiogenic transcription factors were noted following treatment with propranolol. Altogether, propranolol has a favourable effect on patients with idiopathic hyperlipidaemia. Propranolol pharmacokinetics indicate that, effectively, almost 100% of the administered dose could be absorbed from the gut after oral administration. However, blood levels of propranolol remained variable; as high as 90% of proper-propranolol is absorbed from blood in 30 min after oral administration with the apex in blood occurring at around 60 min after dose[5]. As an oral medication, propranolol hydrochloride is available in a tablet/delayed-release form with a slow (approximately 6) hour time to reach its peak plasma level. This fact means that if you take liquid propranolol, you should take the liquid immediately (within a few minutes) after eating an appropriate meal (see above). The peak blood level of propranolol will occur approximately 3 hours after a single oral dose based on the blood clearance of propranolol, unless there has been interference with the first-pass effect in the liver. What that means for you as a patient is that the efficacy of the drug you receive is related to how much of it has been eliminated from your system since you took it [6]. Propranolol can cross the blood-brain barrier and has a wide distribution to many tissues/organs in the body. In general, 90% or more of could be considered free, and thus protonic acid can be considered to be approximately 90% protein-bound at all times in the bloodstream based on the pharmacokinetics of propranolol. The elimination of propranolol is first-order (defined as the amount of drug eliminated from the body is directly proportional to the amount of drug present in the body at a specific time) regardless of the aEfficacy and safety of atenolol vs. propranolol for treatment of infantile haemangioma: a narrative review amount of plasma or the amount of oral doses taken. The half-life (i.e., the amount of time that it takes for half of the stated dose to leave the body) of propranolol is affected by kidney function, as the kidneys serve as the primary organ of elimination for this drug.

1.1 Biological Effects of Propranolol

For the last ten years, propranolol has been used for treating IHs and its efficacy is thought to be due to RAS regulation. The duration of therapy with propranolol should be approximately six months, and many studies support the biological effectiveness of propranolol for IHs[7]. The transcriptional regulation of angiogenesis (HIF-1α) is a critical component of the pathophysiology of IHs. The majority of infants with IHs will have many hypoxic exposures at a young age because of perinatal hypoxia. Therefore, we hypothesize that hypoxic events such as preterm birth and pre-eclampsia contribute to increased levels of HIF-1α. The increase in HIF-1α is mediated by ATII. Therefore, it is proposed that propranolol may block angiogenesis by blocking the pro-angiogenic signalling pathway mediated by HIF-1α through inhibition of ATII activity. Propranolol may prevent the proliferation of capillary endothelial cells through inhibition of both vasodilatory and vasoconstrictive effects leading to an increase in apoptotic events. As the tumour perfusion decreases, the use of propranolol favours the occurrence of vasoconstriction through its antagonistic action on b-adrenergic receptors, which subsequently influences vasorelaxation. Both forms of the drug, S and R propranolol, exist as oral preparations of propranolol. The R isomer possesses no action at the b-adrenergic receptor level but the S isomer does; therefore, the S isomer blunts b-adrenergic transmission whereas the R does not. R-propranolol has shown promising effects for treating infantile hemangiomas (IH). R-propranolol treated IHs had a significant decrease in ANGPTL4 levels compared to untreated IHs in both in vitro and murine experiments [8]. Thus, R-propranolol also prevents angiogenesis via the inhibition of angiogenic factors thereby having an impact on IHs. Furthermore, R-propranolol has been shown to induce hemangioma

resolution by inducing the expression of several tumor suppressor genes including early growth response 1 and betaine homocysteine methyltansferase. In conclusion, the present information supports the rationale for the clinical application of R-propranolol as a means to develop a treatment regimen for hemangiomas in order to achieve tumor volume reductions without producing adverse effects [9]. R- and S-propranolol are two distinct forms (enantiomers) of the drug propranolol; both classes of propranol are effective at modulating both the neuronal and cardiovascular systems. Furthermore, the ability of propranol to produce an effect in either the neuronal or cardiovascular system does not depend upon receptor density [10]. R- and S-propranolol have already been considered in previous studies, for example, the studies can be used to provide additional insight into the efficacy of R-propranolol versus S-propranolol in the future. The author of each study reported that R-propranolol's ability to modulate the transcription factor SOX18 is significantly related to the extent to which SOX18 is expressed, which, therefore, exacerbates the antiangiogenic action of R-propranolol on neovascularization. The actions of SOX18 have been studied extensively with regards to its function in neovascularization [11]. This adds to our support that R-propranolol will be the most important way to manage IHs. As we were unable to obtain enough resources or time to complete controlled clinical trials comparing the effectiveness of steroid-based versus propranolol-based drug therapies via large-scale RCTs, we utilized a combination of methodological complementary strategies to help with this comparison. The first of these was a retrospective cohort comparison study in which patients who had received oral corticosteroid therapy were compared to patients who had been treated with oral propranolol. In order to compare the efficacy of each medication, we matched patients based on patient age, lesion type, lesion site and whether or not the lesion was a single or multiple lesion. Patients treated with propranolol had greater regression of their lesional base than infants who were being treated with steroids. Similarly, infants who were treated with propranolol had a greater chance of requiring surgery as a result of their lesion compared to infants who had been treated with steroids. (12% vs. 29%, P<0.01) [12].

1.2 Indications of Propranolol

Propranolol has emerged as a new class of β-adrenergic receptor antagonist, which has received widespread attention in relation to its potential therapeutic value due to its use in treating a variety of clinical problems through non-traditional pathways connected with β-adrenergic signalling. Moreover, propranolol has been used historically for comparison purposes against other β-receptor antagonists. Before the introduction of propranolol as a new therapeutic prescription drug product, current knowledge was existing regarding the therapeutic potential of β-receptor antagonists for any clinical purpose. Propranolol was developed by Sir James Black as an appropriate medication for the treatment of angina pectoris. Propranolol was the first β-receptor antagonist to be introduced into clinical practice; since then, propranolol has been utilized throughout the world and is now recognized as an effective therapeutic agent for treatment of both cardio and non-cardiovascular diseases. This ongoing success has led to propranolol being one of the most frequently prescribed drugs today [13] [14].

1.2 Cardiovascular Indications

• Heart Failure

Beta-Blockers (like propranolol) are helpful in treating heart failure. Patients with heart failure are more likely to have a good outcome and quality of life with this type of medication, particularly if they also have high blood pressure, chest pain, or have had a previous heart attack [15].

• Atrial Fibrillation

Propranolol can help reduce the risk of stroke or embolism caused by atrial fibrillation as it reduces the number of beats per minute of the heart. It also allows for more efficient circulation of blood throughout the body, resulting in a lower possibility of experiencing these types of traumatic events [16].

• Coronary Artery Disease (CAD) and Angina

Propranolol reduces the effects of excessive activation of the sympathetic nervous system on myocardial oxygen demand and reduces the severity

of myocardial ischemia in patients with coronary artery disease (CAD). In the case of angina, propranolol results in decreased heart rate and contractility to allow for greater exercise capacity and reduced symptoms of angina [17].

• Tachyarrhythmia –

Propranolol is an effective treatment for the suppression of tachyarrhythmias through the reduction of sympathetic overactivity, thus allowing for the restoration of normal cardiac rhythm.

• Post Myocardial Infarction Therapy –

Propranolol has been shown to significantly lower mortality from myocardial infarction, as well as providing long-term prevention of recurrent MI, through reductions in heart rate, blood pressure and myocardial oxygen requirements [18].

• Hypertension –

Propranolol is effective in treating hypertension with various co-morbidities, i.e. heart failure or angina.

Non-Cardiovascular Uses

• **Migraine Prophylaxis -** Propranolol may be considered to be one of the first-line treatments for migraine prophylaxis as, through its actions on vascular and nerve systems, it decreases the number and severity of migraines [19].

• Essential Tremor - Propranolol has been shown to effectively decrease the amplitude of tremors thereby improving quality of life for individuals with essential tremors.

• Restless Leg Syndrome - Propranolol has been used on an occasional basis for the relief of restless leg syndrome through its modulation of neurological pathways [20].

- **Thyrotoxicosis**
- When caused by hyperthyroidism, propranolol is commonly given to patients with thyrotoxicosis in order to alleviate the symptoms of this condition (rapid heart rate, shakiness, anxiety) by inhibition of β-adrenergic activity.
- Infantile Hemangioma
- Propranolol has been successfully used to treat infantile hemangiomas by inducing regression of these lesions through vasoconstriction, blocking angiogenesis and inducing apoptosis in capillary endothelial cells [21].
- Off-Label Uses
- Performance Anxiety
- Practically speaking, propranolol is used frequently to help patients alleviate symptoms associated with performance anxiety, which is the condition characterized by physiological signs (such as palpitations, sweating, and flushing) of heightened sympathetic nervous system activation due to being placed in a state where they are performing under high-stress conditions [22].
- Post-Traumatic Stress Disorder (PTSD)
- The use of propranolol as a means to diminish the emotional response to traumatic memories is also being studied; however, a definitive conclusion regarding its usefulness as a therapy for this condition has not been realized to date [23].
- Akathisia and Antipsychotic-Related Movement Disorders
- In patients experiencing movement disorder secondary to antipsychotic medications, propranolol will reduce sympathetic response and help minimize the symptoms.
- The numerous indications for propranolol demonstrate its flexibility as a treatment option. As such, propranolol is referred to as being a multi-faceted medication with uses in both the treatment of cardiovascular conditions and with the management of both the effects of non-cardiovascular conditions [24].

MECHANISM OF ACTION

- Propranolol is one type of non-selective β-adrenergic blocker, meaning that it binds to both the β1 and β2 adrenergic receptors. Propranolol

will also alter how the electrical impulses are generated and propagated in the heart; therefore, it is classified as a Class II antiarrhythmic agent. Propranolol will compete with endogenous catecholamine (epinephrine and norepinephrine) binding to β-adrenergic receptors, thus modifying the physiological actions of these catecholamines [25].

- Beta-1 receptor blockade
- The majority of β1 adrenergic receptors are located in the cardiomyocytes of the atrial and ventricular walls, including the sinoatrial (SA) and atrioventricular (AV) nodes.
- Normal β1 activation results in an increase in cyclic AMP (cAMP) in the intracellular fluid of the cardiomyocytes, which in turn increases intracellular calcium concentration and produces increased myocardial contractility, heart rate, and conduction velocity [26].
- Propranolol's blockade of β1 receptors reduces activation of these receptors, leading to:

• decreased myocardial contractility: this lessens the heart's workload and oxygen requirements [27].

• decrease in the rate of electrical impulses firing in the heart (negative chronotropic effect): this is beneficial in treating tachycardia and atrial fibrillation.

• decreased myocardial remodeling: this is helpful in patients with chronic heart failure.

- Beta 2 receptor blockade location
- β2 receptors are primarily located in the smooth muscle cells of the blood vessels, bronchi, and other organs.

Normal beta-2 stimulation produces an increase in cAMP, resulting in smooth muscle relaxation and vasodilation [28]. Propranolol, as a beta-2 antagonist, inhibits this pathway. Effects include the following:

• Mild vasoconstriction.

This occurs chiefly in peripheral vascular beds [cited reference number], but other vascular beds may also be involved.

• Possible Bronchoconstriction.

This could be a serious issue for patients who suffer from asthma or chronic obstructive pulmonary disease (COPD)[29].

• Reduced Effectiveness of Emergency Epinephrine in the Treatment of An Asthma Attack.

In addition to blocking beta-2 receptors, propranolol will also reduce the effectiveness of epinephrine on lung tissue [30].

1.5 Pharmacokinetics

Distribution:

Propranolol's volume of distribution (Vd) is about 4-6 L/kg, indicating that it has a large amount of distribution in the human body because it is widely bound to plasma proteins and does not bind exclusively to one location in your body but instead distributes widely within your body. [31]

Metabolism:

After orally administering propranolol, greater than 75% of the dose is metabolically inactivated via hepatic (first-pass) metabolism prior to reaching systemic circulation.

Active metabolite:

Hydroxypropanolol [hydroxypropylpropane] in the case of CYP2D6], is formed from 4-hydroxylation via CYP2D6 enzymes; Elimination: The majority of propranolol is removed from your body via urinary excretion with an elimination half-life of 3-6 hours in healthy subjects with normal renal function.

1.6 Administration

Propranolol can be given via either an IV (intravenous) route or an oral route. The dosage of propranolol will be determined based on the diagnosis for which it has been prescribed [32].

• Oral Administration:

Commonly prescribed to treat chronic diseases (e.g., hypertension, migraine prophylaxis, anxiety). The general dosage can vary from low doses (10 to 40 mg) to larger dosages (up to 320 mg daily) based on the physician's evaluation of the condition.

• Intravenous Administration:

Usually used in emergencies (e.g. cardiac arrest, hypertensive emergency). The amount of medication used is dependent on how well the heart tolerates the medication. Propranolol must be given slowly when administered intravenously (IV); therefore, the patient's heart rate will be evaluated through an electrocardiogram (ECG) while the IV is being administered.

• First-Pass Effect:

Limited oral bioavailability due to extensive first-pass metabolic activity. [33]

1.7 Adverse Effects

Common Side Effects:

Cardiac: Low Heart Rate, Low Blood Pressure.

Respiratory: Difficulty Breathing, Tightness in Chest, Wheezing, Shortness of Breath (and or Troubles with Wheezing) (especially if you have Asthma).

Gastrointestinal: Stomach Upset, Nausea, Vomiting, Abdominal Pain.

Other Side Effects: Fatigue, Drowsiness, Cold Hands / Feet and/or Erectile Dysfunction.

Severe Side Effects:

Allergic Reactions include: Skin Rash and/or Hives, life threatening allergies (anaphylactic reaction)

Metabolic Effects: Make Insulin Less Effective and may hide signs and symptoms of low blood glucose (sugar) in Diabetics.

Neuropsychiatric Effects: Depression, Hallucinations, Bad Dreams.

Important Considerations: Patients with Asthma or Chronic Obstructive Pulmonary Disease may have a severe risk for Wheezing. Hiding signs and symptoms of low blood glucose may confuse diabetes management. Patients should receive education regarding potential side effects prior to starting therapy or changes in drug dosage. As medication works on both b-1 and b-2 adrenoreceptors, it has a wide therapeutic range of effects; however, because it works on both types of receptors, additional care in patient selection and follow up must be put in place to minimize the likelihood of adverse side effects. [34]

1.8 Contraindications

Propranolol may not be an adequate choice for use under multiple conditions for the physiological response mechanisms involved in these cases, and many of these cases may also require constant supervision (Bradford, 19).

• Diabetic Mellitus

Propranolol may mask several signs of low blood sugar (hypoglycemia), such as rapid heart rate, skin flushing, sweating, and dizziness. Because of this masking effect, patients with diabetes will have a more difficult time detecting low blood sugar levels and will not be able to respond appropriately [35] (Fisher, 22).

• Bradycardia

Propranolol lowers heart rate; therefore, it should not be administered to patients with bradycardia (heart rate < 60 beats/min) as this medication will worsen the bradycardia and cause significant hypotension or heart failure. [36].

- **Respiratory Disorders:**

- Asthma, COPD, and Emphysema:

Propranolol helps to block the beta-2 receptors; thus it will lead to bronchoconstriction. Therefore, the bronchoconstriction that occurs in patients with the above conditions may have a negative impact on their

ability to breathe, resulting in potentially life-threatening bronchospasm.

Recommendations:

If a beta blocker is needed, beta-1 selective beta-blockers are an appropriate choice. [37]

- Cocaine overdose: If someone has had a cocaine overdose, Propranolol should not be used; the reason for this is that the use of beta-blockers can cause an unopposed alpha-adrenergic activity. Because the beta-blocker only blocks beta-receptors, when alpha-receptors are not blocked, the result is very high blood pressure, coronary vasospasm, and death. [38]

- Hepatic and Renal Impairment: Propranolol is biotransformed in the liver and eliminated via the renal pathway, therefore, it is prudent to use caution when treating patients with liver or kidney dysfunction because they will experience excessive levels of propranolol and are at greater risk of experiencing toxicity. Dose adjustments may be necessary to avoid toxicity due to build-up of the drug in their bodies. [39].

1.9 Monitoring

People taking propranolol must be monitored regularly for both efficacy and safety throughout their treatment course.

Monitoring of Blood Pressure and Heart Rate

Patients should routinely monitor their blood pressure and heart rate at home with portable devices or during scheduled visits to their doctors. Patients with known cardiovascular disease have additional reasons to monitor these measures on a regular basis [40].

Monitoring of Respiration

The respiratory function of patients who have chronic obstructive pulmonary disease (COPD) or other forms of respiratory disease must be assessed periodically.

Monitoring Cardiac Function

For those receiving inpatient treatment (i.e., patients with a thyroid storm), continuous ECG monitoring should be conducted to identify bradycardic events, arrhythmias, or any other adverse outcomes related to the patient's heart [41].

Adjusting Doses

Physicians must make dosage adjustments according to how well their patients respond to treatment and how well they tolerate treatment; thus, the goal is to promote safety through a therapy that is appropriate for both the patient's clinical needs and the prescribing physician's clinical knowledge.

1.10 Toxicity

Effects from an overdose can be severe, including extreme bradycardia; bradyarrhythmias; low blood pressure; bronchoconstriction; and possible death due to cardiovascular failure [42].

• At a dose of greater than 1 gram of propranolol within 24 hours, death is possible.

• Management of an overdose first involves the immediate administration of glucagon, which has a high success rate in treating an overdose of beta blockers by acting as an agonist at the beta-receptors to increase both the heart rate and myocardial contraction [43].

• Other forms of supportive care would include the administration of intravenous fluids, atropine and vasopressors; and if needed, advanced cardiac life support interventions.

2. HPLC

One method of analysing pharmaceuticals and their degradation products is via HPLC [44]. This method is commonly used for the identification and quantification of synthetically produced medicines, the minimization of contaminants during separation, and the resolving of drug products from drug-related contaminants [45]. Evaluation processes were undertaken to determine an appropriate chromatographic environment for the process, with the appropriate solvent system, stationary phase column, column temperature, detection wavelength and gradient programme being selected to ensure the compatibility and stability of the drug with its degradation products and contaminants [46]. The key

objective of the design and manufacture of pharmaceutical dosage forms, both industrially and statistically, is the development of a consistent, high quality product built on a controlled manufacturing system that is capable of producing the desired clinical efficacy on a consistent basis. Data produced as a result of a formulation and process development will provide the empirical basis from which to establish manufacturing controls, receipt methods, and operational limits. Variability experienced during the development phase may also support Quality Risk Management (QRM) initiatives. The goal is to build product quality into the initial design of the product rather than relying strictly on the last stage of quality inspection as a guarantee of acceptable product quality. Modifying the formulation of raw materials or the method of making the product can be another source of gathering knowledge. By gathering information about the changes in these factors, we can help to create a design space and provide better oversight of the lifecycle of the product and process [47]. An unexpected finding from an experiment could lead to important insights into the product and the development of the product's design space. The designer creates the design space and the regulatory agency evaluates it and approves it, but the use of the design space is not considered to be a departure from regulatory compliance. After the product is approved and leaves the development phase, the post-approval change process typically begins [48]. While the formulation methods of products produced by different organisations or manufacturers may be different, they will generally have a common goal: to meet the requirements of the patient and to produce a formulation that performs as intended. The methods and processes used in creating a formulation will usually be unique, and this should be clear in the submission document [49]. Formulation scientists will use a combination of formal methods, experimental methods and will typically implement both methods when developing their products. A systematic approach to product development may use existing knowledge, data from previous experimental designs, risk assessments, and the application of information management systems (ICH Q10) during the product development process [50]. Regulatory bodies could benefit from having a systematic method to help them understand an organization’s goal for achieving the desired quality of its products or services. Throughout the life cycle of a product, there are opportunities to stay current with knowledge of both the product and its associated processes. [51]

2.1 HPLC principle

HPLC's principle is based on the separation of compounds by moving them through both mobile liquid phase and stationary solid phase, with the application of an eluting mobile phase. Different chemical structures of an analyte will have varying degrees of migration through the stationary phase. [52]

2.2 CLASSIFICATION OF HPLC

- Depending on the size of the operation, analytical and preparatory HPLC
- Different types of separation based on the operating principle include affinity interactions, adsorptive separations by surface area, molecular size discrimination, ionic ion-exchange processes, and chiral recognition.
- In terms of the elution pattern of the analytes, separation will take place through use of either constant composition systems (isocratic) or variable composition systems (gradient).
- Chromatographic methods can generally be classified as either normal-phase or reversed-phase based on the polarity of the stationary and mobile phases. [52] [53]

2.2.1 Normal phase chromatography:

Normal phase chromatography separates components using a polar stationary phase and a non-polar mobile phase. The retention characteristics of an analyte are primarily affected by its polarity; therefore, the more polar the analyte, the longer it will remain in the stationary phase. Thus, the more polar the analyte, the longer it will take to elute from the column [54], due to the increased attraction exerted on the analyte by the stationary phase (the surface) or due to enhanced interactions with the stationary phase. Silica chemically bonded to aminopropyl, diol and cyanopropyl groups is often used as stationary phase

material. The majority of conventional columns have lengths of 150 to 250 mm and an internal diameter of about 4.6 mm. During the passage of the sample through the column, the polar components will remain with the stationary material longer than non-polar components, and thus, non-polar components will elute from the column quicker than polar components. [45][55]

2.2.2 RP-HPLC

In RP-HPLC, the stationary phase is made up of a non-polar stationary phase (material) and a polar (or somewhat polar) mobile phase (solvent) system. The retention of molecules during the chromatographic retention process is mainly due to the actions of hydrophobic partitioning phenomena [52][56]. Molecules that have more hydrophobic properties than other components in a mixture will generally be retained by the stationary phase longer than molecules with less hydrophobic properties. Thus, more hydrophilic or polar compounds will have less affinity for the stationary phase than molecules with lesser polar affinities, and will therefore elute from the column before the less polar component [57].

2.2.3 Size exclusion chromatography:

Using gel filtration or gel permeation chromatography, SEC separates molecules based on their size in solution. This method is commonly used to characterize proteins in terms of their overall shape or conformation (tertiary and quaternary structures). It can also be used to determine the molecular mass characteristics of polysaccharides. [58].

2.2.4 Ion exchange chromatography:

Ion-Exchange Chromatography Measurement The retention mechanism of ion-exchange chromatography is primarily due to attractions between solute molecules, such as ions, and charged sites that are immobilized on the stationary matrix. That is, solute molecules that are comparable in charge to an ion that is either present or not present on the stationary phase will be repelled from the stationary phase because of electrostatic repulsion. Ion-exchange chromatography is frequently used for a variety of processes including, but not limited to, water decontamination, ligand-mediated separations, protein purification, high pH anion exchange characterization of saccharides, oligosaccharides, and other applications. [59].

2.2.5 Bio-affinity chromatography:

Affinity chromatography is based on the concept of reversible but specific interactions between biomolecules and their corresponding binding partners. Covalently attached to a solid chromatography column, ligands can immobilize to their cognate partners and form specific associations, allowing for the selective retention of the protein that recognizes the ligand on the column. [60].

2.3 Instrumentation of high-performance liquid chromatography

Reverse phase column chromatography is utilized for both analytical and preparative purposes in biological isolation and purification. Reverse phase chromatography offers an efficient means for isolating hydrophobic materials with good recovery and separation efficiency. Hydrophobic interaction forces are created between the hydrophobic ligand of the stationary phase and the solute compounds present in the mobile phase via interaction/association between these two phases. [61].

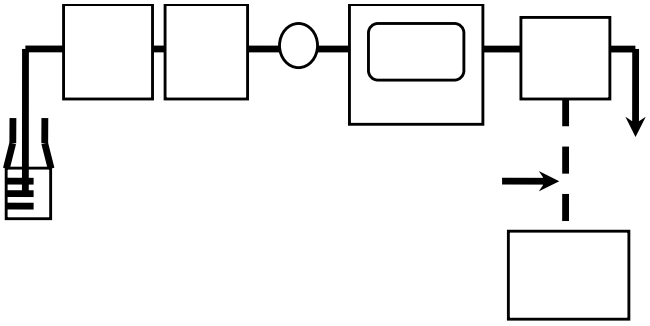

Figure 1. Components of HPLC System

The actual mechanism of hydrophobic interactions is disputed, although the traditional area of hydrophobic interactions typically teaches that hydrophobic interaction forces possess a favorable entropy impact. In the moving phase conditions of reverse-phase chromatography, both the solute compound and the immobilized ligand (attached to the stationary phase) have a substantial amount of water structured around them, which contributes to a positive change in entropy for the system [62]. When a hydrophobic ligand becomes immobilized it has less surface contact with the solvent so there is therefore, a decrease in the amount of structured water that has been formed around it. Further, this reduction in structured water results in an overall increase in the entropy of the system. HPLC is one of the best methods for quantitative analysis [63]. Reverse Phase Chromatography is a method of chromatography. that utilizes non-polar stationary phases and polar mobile phases for separation. HPLC is typically utilized in combination with other analytical methods to accomplish quantitative and qualitative studies. The system operates in isocratic mode, which means that the only solvent or solvent blend is delivered to the system throughout the study to produce a specific result. Gradient elution occurs when the composition of the solvent is slowly changed over time through the addition of additional solvents. Diffusion is how stationary and mobile phases will disperse and advancing purification will be achieved the faster diffusion takes place. [64].

2.4 The HPLC (High Performance Liquid Chromatography) technique has many unique features such as;

- Efficiency in separation, narrow diameter columns, made of stainless steel or glass.
- Rapid evaluation.
- Much higher pressure of moving phases than compression.
- Controlled flow of moving phases [65].

2.5 HPLC has many benefits such as;

- Simultaneous evaluation.
- High efficiency of separation.
- Very high sensitivity.
- Very high reproducibility.
- Very low amount of sample.
- The conditions of the examination are relatively easy to establish.
- Easy to isolate and disinfect [66].

2.6 HPLC Method Development

Methodologies will be created for brand-new drug products if official protocols don't exist. For formulations that aren't found in any other monetary source, a different strategy will be employed to minimize costs and the length of time that the comparisons will take and create more accurate and reliable results—this will involve creating a table that compares the experimental data from both methodologies and chronicles both the good and less than good attributes associated with the proposed replacement and the current methods being used[67]. One goal of High-Performance Liquid Chromatography (HPLC) is to isolate the active pharmaceutical ingredients and any process contaminants, as well as identify all known artificial additives, and identify any degradation products. [68]

2.6.1 Understanding the physicochemical properties of drug molecules

To develop a successful analytical method, it is critical to completely understand the physicochemical properties of the pharmaceutical compound being analyzed. For example, effective solubility, molecular polarity (which is the use of polarity for developing chromatography conditions), acid dissociation constant (pKa) and solution pH should be accurately measured before chromatography conditions are set. The major element to evaluate, in this case, would be the polarity of the compound. The knowledge of its molecular polarity will help to choose the appropriate solvents (mobile phase) for chromatographic analysis and composition of those solvents. Additionally, there is a direct relationship between the solubility profile and the polarity of a substance. For example, with polar solvents (e.g., water) having limited solubility with non-polar solvents (e.g., benzene), it is generally accepted that substances that have the same polarity characteristics will dissolve in one another easily [69]. Therefore, when determining the appropriate solvent system, analyte solubility must be considered.

Another significant factor that will affect the chromatographic performance of a compound is the pH of the mobile phase. The pH of the mobile phase (solution) provides an indication of the acidic or basic value of the solution and affects the ionization of some analytes. Optimization of the mobile phase pH will generally improve chromatographic performance by providing sharper and more symmetrical peaks, which are better-separated than peaks produced when using unoptimised conditions, using high-performance liquid chromatography (HPLC) [70]

2.6.2 Selection of chromatographic conditions

Initially, you must determine the tentative operating parameters that will allow you to characterize your analyte chromatographically. These may include the selection of a detector, chromatographic column, and mobile phase system. [71].A common initial method development approach is to use reverse-phase chromatography with a UV detector, as well as a C18 stationary phase. As you are developing your method, you will also want to determine how best to elute your analyte, i.e., by constant-composition (isocratic) or by variable-composition (gradient) elution. [72]

2.6.3 Developing the approach for analysis

The first step in developing a RP-HPLC method is to optimize key chromatographic parameters such as the mobile phase, stationary phase (column) type, flow rate, and pH of the mobile phase system. After determining these via experimental evaluation, system suitability tests should be performed to confirm that the method is functioning correctly [73]. Acceptance criteria for these tests include a theoretical plate no. of >2000; a retention time of >5 minutes; a tailing factor of <2.0; and a resolution value of >5 between adjacent peaks. In addition, the % RSD of the analyte peak area from analyses of standard injections should typically not exceed 2.0%. If two analytes are being determined simultaneously, the wavelength is often chosen to be at the isosbestic point where both components show equal absorption behavior. [74]

2.6.4 Sample preparation

Preparation of samples is a vital step in developing an analytical method, which needs thorough consideration on the part of the analyst. In case a sample contains particulate matter or poorly soluble components, the proper methods of sample treatment should be explored to optimize such parameters as the rate of centrifugation and the duration of the procedure as needed. One must check whether the filtration affects the results by causing losses of analytes, adsorption of substances to filter material, or extraction of foreign substances [75]. The efficiency of syringe filters is largely associated with their capacity to remove insoluble substances and contaminants without extracting additional impurities. The sample preparation process for analyzing the in-process or final samples of drugs by HPLC needs to be fully disclosed in the analytical method [76]. The names of the filter and the membrane, the manufacturer of the filter, and the diameter of the pores need to be specified. Sample preparation is performed to receive a treated sample that provides for more precise and accurate results compared to untreated sample [77]. The treated sample injected into the HPLC system is expected to be free from interfering substances and to be resistant to HPLC operation conditions [78].

2.6.5 Method optimization

The optimization of HPLC technique conditions is one of the principal goals pursued when developing new methods for high-performance liquid chromatography [79]. For achieving successful optimization, one should give close attention to such method characteristics as stationary and mobile phases. But since modifying the mobile phase is technically easier to achieve, optimizations are usually concentrated on that parameter [80].To minimize the number of experiments performed and analyses made, one should concentrate on the most influential parameters responsible for selectivity. A number of operational parameters greatly impact LC method optimization. This set comprises mobile-phase composition, pH modifier and buffer presence, ratio of the solvents, gradient elution type, flow rate, column temperature, sample concentration, volume injected, and type of diluent used [81]. Adjustment of these parameters can significantly enhance the chromatographic characteristics and analyte resolution. Upon attaining the necessary selectivity level, further optimization is supposed to provide an

adequate balance between separation and analysis time. When proceeding to this step, it becomes crucial to focus on such parameters as mobile-phase flow rate, particle diameter and column dimensions. Modifying these characteristics affects principally method efficiency and analysis time and has minor effects on selectivity and retention properties of the analytes [82].

2.7 Validation Of HPLC

Method validation is defined as a set of activities undertaken to determine the fitness and reliability of the analytical methods applied in the analysis. It offers documentation proof that a method meets the pre-established performance criteria. Various regulatory agencies like the Food and Drug Administration, International Council for Harmonisation and the United States Pharmacopeia have developed extensive guidelines outlining validation requirements and acceptance criteria for pharmaceutical analytical methods [83]. Assay method validation carried out in the current investigation was in line with the ICH guideline and considered the important aspects of validation of the chosen analytical method. Method validation refers to experimental confirmation that the characteristics of an analytical technique meet certain criteria suitable for its intended use. [84] Additionally, all new and significantly modified analytical techniques should be subjected to method validation to prove their capability to produce accurate, precise, and reproducible data. Method validation serves to guarantee that reliable data can be produced through the use of an analytical technique by different operators under identical conditions or different laboratories utilizing similar equipment [74].[85] Methodological validation requirements depend on the methodology being used and its applications in determining the exact type of validation protocol to employ [86].

2.7.1 Specificity

The capability to detect the analyte in the presence of substances that may reasonably be anticipated to exist is referred to as specificity. The capacity to precisely recognize the analyte response in the presence of all possible specimen constituents is termed specificity, and it is essential for chromatographic methodologies to demonstrate this characteristic. The response of the analysis in a test mixture containing the analyte and the analyte exclusively is compared with all probable specimen constituents (placebo preparation, process-related impurities, etc.) To be considered specific, the analyte peak must exhibit a baseline chromatographic separation of not less than 1.5 from every other specimen constituent. If this cannot be achieved, the unresolved constituents at their maximum anticipated concentration should influence the final analytical outcome by not more than 5%.

2.7.2 Linearity

When analytical methodologies can produce outcomes that are straight related to sample attentiveness (quantity) in a defined interval, they are considered linear [87]. For analytical procedures, reference solutions are generally prepared at five concentration stages. Only at five stages can curvature in the displayed information be detected [88]. Plotting the response versus concentration enables verification of the linear relationship was evaluated by determining the correlation value and intercept of the fitted regression model. [89] A correlation coefficient exceeding 0.999 is commonly regarded as proof of an association between the information and the reversion equation. The y-intercept should not be calculated using more than 1% or 2% of analyte responses acquired at the target concentration level [90].

2.7.3 Accuracy

The degree of similarity between the measured number and the number considered being the correct true number or the correct positional value is an indicator of the precision of an experimental method. The closeness with which the observed number agrees with the correct number in the specimen can be used to gauge the accuracy of the procedure. One out of four approaches is normally used to assess accuracy [91]. Accuracy can be determined using the approach that involves using the specimen whose absorbance value is known and correlating the observed number with the correct number. By comparing the values obtained through the new method against data acquired using other validated methods [92] Injecting an analyte into blank matrices to conduct recovery tests is one of the common approaches used in conducting a recovery study, and three different levels

of spike samples are created in triplicate at 50-150 per cent of the target concentrations. Additives to the reference, which can also be used in monitoring the retrieval process of spiked analytes. This technique is utilized in instances where sample dilution cannot be carried out in the specimen medium. For accurate recovery results, the average recovery should be above 100 + 2 per [93].

2.7.4 Range

The range of analytical procedures consists of, for example, the high and low absorptions of the analyte in the sample as well as those standards in which the systematic approach has been shown to be accurate, precise, and linear. The range is determined based on the results obtained in the investigation of linearity and accuracy. The tolerance limits for the acceptability of the assay procedure shall be considered acceptable when linearity and accuracy can be achieved within a 3% RSD and an accuracy of an assay procedure within the required range [94].

2.7.5 Precision

How far identical capacities on the same uniform sample under indistinguishable conditions result in similar output values determines the precision of a particular process. Accuracy of an analytical process may be spoken in terms of the relative variability, spread, or standard uncertainty. The first consistency test is the injection reproducibility test. In order to assess the performance of the chromatographic system, at least ten injections of one sample solution are carried out [95]. The second consistency test is the intra-assay precision, also referred to as reproducibility. Data for intra-assay precision can be gathered on a single day. Aliquots are homogenous samples which are each prepared according to the analytical process. This will determine the number of injections required for each sample in the analysis and the number of replicated runs needed. For instance, an assay method will have a within-run precision (RSD) of 2% and an instrument reproducibility of 1%[96].

2.7.6 Detection limit

In each of the sample collection techniques, there is a limit of detection which is the minimum amount of sample present in the sample that can be detected without being accurate in measurement. The limit of detection is influenced by the signal response and the standard deviation. Limit of detection is given by

$$DL = (3.3\sigma / S)$$

Where,

σ = Standard deviation of the response

S = Slope of the calibration curve (of the analyte)

2.7.7 Quantitation limit

The measuring ability of the process is defined as the smallest amount of the sample in the specimen that can be identified with acceptable accuracy and precision. The limit of quantification is calculated using the standard deviation of the response and the slope[97]. This is expressed as follows:

$$QL = (10\sigma / S)$$

Where:

σ = standard deviation of the response

S = slope of the calibration curve (of the analyte)

2.7.8 Ruggedness

There are several determinants in the reliability of an approach, for instance, reproducibility and data evaluation for results obtained using various sources. [98]. An experimental outcome is dependent on such variables as reagents' suppliers, time taken by an experiment, and temperatures at which an experiment is performed [99]. The ability to reproduce a test result when faced with variations in the experimental condition is called ruggedness. RSD is considered an indicator of ruggedness, which should not exceed 2%.

2.7.9 Robustness

It is the capability of an experiment to withstand small deviations in the limits of some variables like injection volume, temperature, buffer's intensity, the amount of acidic mobile phase, and organic solvent percentage. For robustness purposes, the relative standard deviation should not exceed 2%. [100]

2.7.10 System Suitability Tests

An assessment of system suitability tests is a common practice in analytical approaches. Each aspect of the test is evaluated under one combined set of experiments which can then be analyzed together [101]. These assessments are done as indicated below. Some chromatographic aspects such as resolution, percent repeatability of area, hypothetical plates, and tailing factor are determined following five injections of a standard solution [102].

2.8 Applications Of HPLC Method [103]

- Versatility, speed, and sensitivity of HPLC have made it the most widely used technique of chromatography for purification of various kinds of organic compounds.
- The method is greatly popular for use in clinical and pharmaceutical fields as organic materials such as serum and urine samples can be directly injected into the system column.
- Separations using R-HPLC have more effect on proteins and oligopeptides.
- Various applications concerning organic chemistry [104].
- Ion exclusion chromatography as well as ion exchange ion pair chromatography can be used to separate anions in chromatography.
- In chromatography, the method has been used for separating cations using inert polymer resins.
- One of the major uses of chromatography is the analysis of agrochemicals especially in water purification.
- Pesticide analysis in water purification systems is the major usage of chromatography in agriculture.
- It is greatly used in food testing. In forensic investigations, separation of morphine and its metabolites collected from blood plasma is common.
- People working in the pharmaceutical industry have a major role in modern applications of chromatography. [105].

CONCLUSION

Optimized RP-HPLC method serves as a highly reliable and effective means of accurately analyzing Propranolol Hydrochloride in drug formulations. The analyzed methods show that the right choice of RP-HPLC parameters like the use of C18 immobile phase, proper composition of mobile phase, alteration of pH, flow rate, and absorbance wavelength will greatly contribute to achieving high resolution, sensitivity, and repeatability of results. Majority of the validated methods are ICH compliant and possess great linearity, accuracy, precision, specificity, robustness, and reasonable levels of detection and quantification, thus their suitability in pharmaceutical analysis purposes. Moreover, stability-indicating RP-HPLC methods have proven effectiveness in separating propranolol hydrochloride from its degradants, contaminants, and excipients, thus the safety and efficacy of the preparation during storage period can be guaranteed. The technique offers numerous benefits like high resolution, quick determination, easy sample preparation, and hence, it is appropriate for use in pharmaceutical organizations. RP-HPLC serves as the best available analytical technique for analyzing propranolol hydrochloride. Further studies may concentrate on green HPLC methods, QbD-based methods, and hyphenation techniques to optimize analytical performance and reduce environmental impacts.

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