Doctor of Philosophy (Ph.D.)

Pharmacokinetic Drug Interactions of

Multivitamins and Proton-pump Inhibitors

Thesis submitted
in partial fulfillment of the requirements
for the Degree of Doctor of Philosophy

Submitted by
Sherejad Sanam
Reg no: 82/2013-14 and 49/2018-19 (Re Reg.)

Department of Clinical Pharmacy and Pharmacology
Faculty of Pharmacy
University of Dhaka -1000
September, 2023

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DECLARATION

I do at this moment declare that the project report entitled "**Pharmacokinetic Drug Interactions of Multivitamins and Proton-pump Inhibitors"** is submitted as a requirement for the fulfillment of the Doctor of Philosophy (Ph.D.) degree in the Department of Clinical Pharmacy and Pharmacology, University of Dhaka, is original research works of mine and have not been previously submitted elsewhere for the award of any Degree or Diploma.

Sherejad Sanam

Reg no. 82/2013-14, and 49/2018-19 (Re. Reg.)

APPROVAL

This thesis entitled "**Pharmacokinetic Drug Interactions of Multivitamins and Proton-pump Inhibitors,"** submitted by Sherejad Sanam, in fulfillment of the degree of Doctor of Philosophy, Clinical Pharmacy and Pharmacology, Faculty of Pharmacy, is a record of unique work conducted under my guidance. The thesis contains no material previously published or written by another person for any other degree or diploma.

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ABSTRACT

Background: To recognize and comprehend how various medications can interact with one another when taken concurrently or quickly after one another is the goal of investigating drug-drug interactions. It's crucial to know this because drug interactions might harm the security and efficiency of the medications involved, possibly resulting in hazardous drug responses, unsuccessful treatment attempts, or other undesirable effects. Healthcare practitioners can take action to reduce the risk of patient injury and maximize the advantages of their prescription regimens by recognizing and analyzing potential drug interactions. Due to the increased rapid use of various drugs co-administration, either complementary or alternative medicine, the possibility of drug-drug interactions increased. This may cause severe organ damage or toxic effects in our bodies.

Aims: To find potential interactions between proton pump inhibitors (PPIs) and multivitamins, researchers study the drug-drug interactions of these two drugs. Proton pump inhibitors function by lessening the quantity of acid produced in the stomach. They are frequently used to treat illnesses, including gastroesophageal reflux disease (GERD) and peptic ulcer disease. Multivitamins are designed to provide a convenient way for people to obtain the recommended daily intake of essential vitamins and minerals necessary for normal bodily functions. This type of research still needs to be observed based on the pharmacokinetics interactions between proton pump inhibitors and multivitamins in vivo and in vitro studies; the current research was carried out to investigate such potential interactions. Pantoprazole (PNT) and a vitamin B (VTB) complex were given to the participants in this trial. The vitamin B complex consisted

of VTB1, VTB6, and VTB12 in this investigation. This study aimed to determine the effect of the combination of these two drugs on the pharmacokinetics of pantoprazole (PNT).

Methods: First, based on prescription analysis in the local area, considering government and private hospitals, age, and disease pattern. Based on the prescription survey, pantoprazole with vitamins B1, B6, and B12 were observed both in vitro and in vivo. Pantoprazole and vitamin B complex were investigated in single and combined form under XRPD, DSC, and FT-IR. Further study validated all components under High Performance Liquid Chromatography (HPLC). Additionally, pharmacokinetics parameters were investigated in healthy volunteers after 0 hours, 0.5 hours, 1 hour, 2 hours, 3 hours, 5 hours, and 6 hours after administration. In this research, sensitive and effective procedures for simultaneous determination in human plasma using HPLC were developed in line with the bioanalytical standards established by the US Food and Drug Administration.

Results: PPI and multivitamins were only included in 200 of the total 500 prescriptions. According to the findings of this study, those between the ages of 30 and 50 received the highest frequency of PPI and multivitamin prescriptions. According to the results of a prescription survey, PNT, VTB1, VTB6, and VTB12 should be investigated in both *in vitro* and *in vivo* studies to determine any possible drug interactions. The linearity of the PNT, VTB1, VTB6, and VTB12 validated parameters was evaluated, and the results showed that the plasma PNT, VTB1, VTB6, and VTB12 retention durations, throughout the range of 1–100 µg/mL, were 6.8± 0.2, 2.7± 0.4, 4.5±0.5, and 3.8± 0.1 min; respectively. This information was discovered when the linearity of these validated parameters was evaluated. For every analyte, the intra-assay and inter-assay biases were within 15% and 13.5%, respectively, for the lower limit of quantification and all other values. This study investigated the pharmacokinetic properties of PNT, VTB1, VTB6, and VTB12 when the medications were taken individually or combined

with other vitamins. We could not assess the pharmacokinetic profile of VTB12 in an *in vivo* trial despite an in vitro examination revealing that both interactions were minor. After analyzing the AUC curve, we found that the PNT, VTB1, and VTB6 single-dose concentrations were, respectively, 3.88 ± 1.239, 8.44 ± 0.514 and 62.91 ± 3.046 μg/mL*h. Following the combination, the AUC curves exhibited respective values of 3.56 ± 0.356, 7.90 ± 0.130 and 56.52 ± 6.816 μg/mL*h. In every instance, the p-value indicated that the deal was less than 0.99. When the PNT and VTB samples were evaluated in vitro in various physical combinations, there were scarcely any interactions between the two types of models. In the pharmacokinetics investigation, the administration of VTB did not significantly alter the pharmacokinetic parameters of PNT. An approach to analyzing drug-drug interactions was devised as a result of the outcomes of the experimental investigation that was carried out. Investigations into bioequivalence and therapeutic medication monitoring are two possible applications for this approach.

Conclusion: When PNT was administered with VTB1, VTB6, and VTB12, it showed no interactive properties and did not reduce any of their activity. It also maintained average AUC profiles, which may represent a stable C_{max} and t_{max} both in single and combined form. Hence, this combination therapy may be a cost-effective, less toxic, and potential remedy for general uses.

Keywords: Polypharmacy, Drug-Drug interactions, Multivitamins, Proton pump inhibitors, Plasma, Pantoprazole, Vitamin B1, Vitamin B6, Vitamin B12.

Table of Contents

CHAPTER 1: INTRODUCTION

CHAPTER 2: MATERIALS AND METHOD

CHAPTER 3 RESULTS AND DISCUSSION

List of Figures

List of Figures

List of Tables

List of Tables

Topic		**Page**
Table 2.1	Flow pattern of HPLC gradient mobile phase	63
Table 2.2	Demographic and baseline characteristics of study participants	70
Table 3.1	Existence of PPIs and multivitamins in prescriptions survey	77
Table 3.2	Percentage of individual Proton-pump Inhibitors and others in the prescription	78
Table 3.3	Combination of intake Proton-pump Inhibitors	79
Table 3.4	Prescription of Proton pump inhibitors drug and Multivitamin in various department	81
Table 3.5	Prescription containing proton pump inhibitors of different companies	82
Table 3.6	Multivitamins prescribed by physicians	84
Table 3.7.	Distribution of Proton pump inhibitors and Multivitamin drug on different age group of patients	86
Table 3.8	Some important peaks observed in FT-IR spectra of PNT, VTB1, VTB6, and VTB12 and their composite with their possible assignment	95
Table 3.9	Analytical parameters for robustness of HPLC method	100
Table 3.10	Analytical parameters for the robustness of the HPLC method	101
Table 3.11.	Intra-day and inter-day accuracy, precision, and recovery matrix effects for PNT, VTB1, VTB6, and VTB12 determination in plasma	107
Table 3.12	Percentage of recovery with different methods of deproteinization	110
Table 3.13	Pharmacokinetic profile data of PNT, VTB1, VTB6 and VTB12	112

List of Elaborations

ADRS:	Advance drug responses	PK:	Pharmacokinetics
ATC:	Anatomical Therapeutic Chemical	PM:	Physical mixture
AST:	Aspartate aminotransferase	PNT:	Pantoprazole
AUC:	Area under the concentration curve	PPI:	Proton pump inhibitor
CYP:	Cytochrome	QC:	Quality control
C_{max}:	Maximum plasma concentration	RT:	Room temperature
DDI:	Drug-drug interactions	SD:	Standard deviation
DSC:	Differential scanning calorimetry	SSRI:	serotonin reuptake inhibitor
ESM:	Esomeprazole	T_{max}:	Time to reach maximum plasma concentration
FT-IR:	Fourier transform infrared spectroscopy	UV:	Ultraviolet
HPLC:	High-performance liquid chromatography	VTB:	Vitamin B
IM:	Intramuscular	VTB1:	Vitamin B1
IS:	Internal standard	VTB6:	Vitamin B6
LAN:	Lansoprazole	VTB12:	Vitamin B12
LOD:	Limit of detection	XRPD:	X-ray powder diffraction
LOQ:	Limit of quantification	ZES:	Zollinger- Ellison syndrome
OMP:	Omeprazole	μg/mL:	Microgram per milliliter
PD:	Pharmacodynamics	μ:	Micron

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Medicines are necessary for guiding the course of illness and increasing life expectancy in humans and animals alike, but inappropriate or excessive use of pharmaceuticals frequently results in unintended consequences. Most medical practitioners can detect patients taking more drugs than can be reasonably and securely taken. The term "polypharmacy" has been used to describe this issue. People over 18 taking three to five drugs simultaneously are said to engage in polypharmacy [1]. When applied to individuals with several chronic conditions, disease-specific recommendations that aim to achieve disease-specific outcomes might lead to polypharmacy or the chronic co-prescribing of many medicines. Polypharmacy, the use of numerous drugs to address a variety of ailments, is frequent among older people [2]. Death falls, lousy medication responses, extended hospital stays, and hospital readmission shortly after release are some adverse outcomes associated with polypharmacy [3][4]. The risk of side effects and disaster rises proportionately to the medications used [5]. Drug-disease interactions (DDIs) and DDIs between drugs also contribute to the risk of adverse effects [6]. Lower functional capacity, greater prevalence of geriatric syndromes, and higher healthcare costs are only some of the negative consequences associated with polypharmacy [6,7]. Drug-drug interactions, in which one medicine alters how another works, can significantly negatively impact patients who take many medications at once [8]. When a patient has many conditions and takes multiple medications, the risk of adverse drug reactions rises [9]. The index guideline, however, failed to account for several potentially fatal drug-drug interactions. Target patients might have many diseases, each requiring a separate medication class, yet there are only a handful of disorders for which recommendations exist. Chronic obstructive pulmonary disease (COPD), acute or chronic renal disease, heart failure, and diabetes mellitus are among the conditions that, according to updated recommendations, have a high risk of probable comorbidities. Unfortunately, these guidelines primarily focus on one comorbid disorder at a

time, and they don't offer many particular suggestions for dealing with patients who have several. More studies have recently been uncovered into the possible connections between comorbidities and drug-drug interactions [10]. Interactions between medications are a worldwide epidemic. Interactions between drugs occur when they are exposed to other substances, including those found in food, beverages, herbs, juices, medical conditions, and dietary supplements. The interaction may improve the drug's effectiveness, absorption, or activity.

Conversely, it might be harmful, resulting in a loss of therapeutic efficacy. Furthermore, it may result in potentially fatal toxicities that necessitate medical attention. Because so many seniors use medications for long periods to manage their chronic conditions, there is a growing need for well-designed investigations of drug-herb interactions. When multiple doses of drugs are co-administered, they may cause drug–drug interactions (DDIs) [11].

1.1 Drug Interactions

A DDI develops when a drug's pharmacokinetics (PKs) and pharmacodynamics (PDs) are affected by one or more other medicines. DDIs are often created based on each drug's knowledge and are determined by monitoring changes in plasma drug concentrations and the patient's clinical analysis [12]. Also, drug-drug interaction (DDI) can be defined as the interplay between drugs taken during joint administration. When compared to treatment with the medication alone, DDIs almost always result in either an increase or a decrease in the effects of the medicine.

Although PK interactions can take place at any point in the four stages of absorption, distribution, metabolism, and elimination, the stage of drug metabolism is the one in which

they are most frequently seen. The cytochrome (CYP) P450 enzyme system found in the liver is the principal site of drug metabolism and is responsible for 86 percent of drug-drug interactions [13,14]. If a physician can better understand how drug interactions occur, they may be better able to prevent them. It is determined to be a clinically relevant interaction either when there is a reduction in the therapeutic effectiveness of one drug that interacts with another or when there is an adverse reaction [15]. One of the most common types of adverse drug reactions, or ADRs, is drug-drug interaction (DDI), which occurs most frequently in patients who take multiple medications [16]. DDIs are one of the most prevalent causes of medication mistakes [17]. They have a common effect of 20–40 percent in diverse nations and are mainly noted in older people as a result of polytherapy. In particular, polytherapy raises the bar for therapeutic management and, as a result, the risk of clinically significant DDIs, both of which can result in the development of adverse drug reactions and a reduction in clinical effectiveness [18,19].

1.2 Classification of drug interactions

Multiple drug interactions can be categorized externally **(Figure 1.1)** or internally **(Figure 1.2)**. Pharmacological interactions occur inside the body, although the vast majority of interactions between pharmaceutical drugs arise outside the body (also known as incompatibilities). Medication interactions are typically classified if they occur after administration [20]. Drug interactions are categorized into Pharmaceutical and Pharmacological effects. In Pharmaceutical development, interactions mainly happen outside the body by chemical or physical change. Another common interaction is the Pharmacological effect, where pharmacokinetics and pharmacodynamics are involved inside the body. Therefore, the concurrent administration of pharmaceuticals may result in changes to the

pharmacokinetics and pharmacodynamics of co-administered treatments, which can either reduce the therapeutic efficacy of the medications or increase the drug's toxicity [17,21].

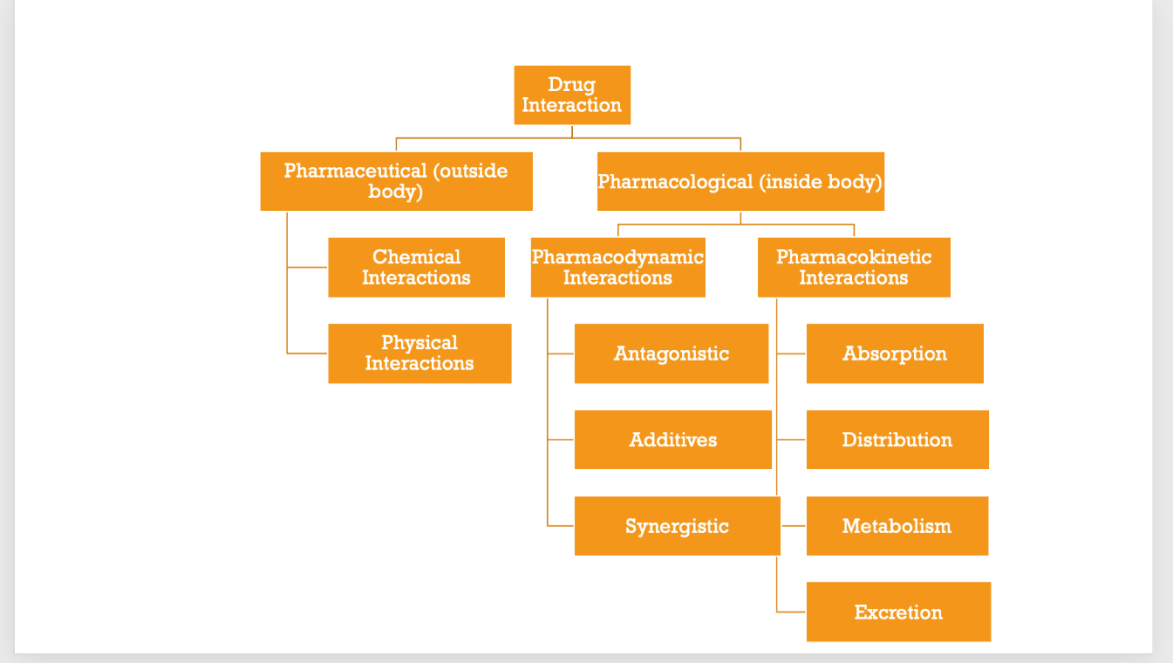


Figure 1.1 Classification of drug interactions

1.2.1 Pharmaceutical Drug Interactions

The term "pharmaceutical interaction" refers to the interaction between two chemicals when those compounds are physically or chemically incompatible [22,23].

1.2.2 Pharmacological Drug Interactions

Pharmacological drug interactions are those when one drug alters the pharmacological effects of another drug following concurrent administration of two or more drugs.

Pharmacodynamic and pharmacokinetic interactions are two types of pharmacological interactions [24].

1.2.2.1. Pharmacokinetic Drug Interactions

Pharmacokinetic drug interactions mean the alternation of pharmacokinetic features such as absorption, distribution, metabolism, and excretion (ADME) of a drug by co-administered agents or drugs. A drug may increase or decrease the above pharmacokinetic features of another drug administered simultaneously.

1.2.2.1.1. Absorption

The first phase in pharmacokinetics is absorption. Medicines must first be absorbed to have a pharmacologic effect **(Figure 1.2)**. When taken orally, medications must pass via the stomach or the intestines to reach the bloodstream and the site of action. Pharmaceuticals given by nasal, sublingual (under the tongue), or other non-oral methods, as well as intramuscular (IM) or subcutaneous injection, must also be absorbed from the delivery site.

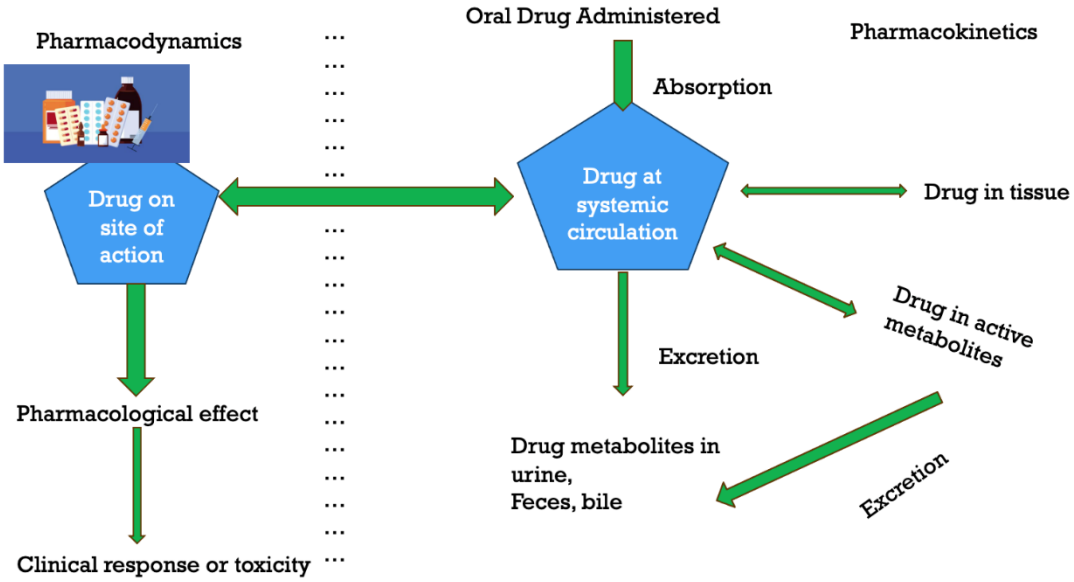


Figure 1.2 Pharmacokinetic and Pharmacodynamic Drug Activity

Without an absorption system, intravenous medications are directly administered into the systemic circulation and have an immediate effect. IV treatment has a faster pharmacological effect than Subcutaneous, intramuscular, and extraoral administration. Several variables can affect how well the gastrointestinal mucosa absorbs a medicine. Most drugs cannot be digested and absorbed orally unless the stomach's pH changes to between 2.5 and 3. As a result, medications, including antacids, anticholinergics, PPIs, and H2-antagonists, that may change or elevate the pH of the stomach can affect how other drugs are absorbed when taken concurrently. Co-administration of medicines like antifungal agents (such as ketoconazole or itraconazole) can elevate stomach pH, and thereby it may reduce both solubility and absorption of the antifungal drugs because an acidic environment is required for their optimal disintegration [25].

1.2.2.1.2. Distribution

The second most crucial component is distribution. It is the method by which drugs are circulated systematically. DDIs can typically happen at any time during the distribution phase. The medication's effect on lipid solubility or partition co-efficient is one element that affects drug distribution. Organs that receive more blood, like the liver, heart, and kidney, receive their medicine more rapidly than organs that receive less blood, such as muscle, fat, and peripheral tissues. The extent to which a medication binds to proteins in plasma and tissues determines how that drug is distributed. Drugs have varying degrees of affinity for albumin and other plasma proteins. Protein-bound drugs are inactive from a pharmacological aspect.

1.2.2.1.3. Metabolism

DDIs can occur when a drug that the CYP metabolizes is taken with a medication that alters the production or activity of the CYP enzyme. The CYP enzyme is essential for the metabolism of drugs. Some medications operate as CYP enzyme inhibitors, lowering their activity, while others enhance the effects of substrate medications. Some medicines are inducers that increase enzyme production and lessen the treatment results for the substrate. While drug absorption and distribution continue, the body detoxifies to eliminate the medicine. (Clearance is another term for elimination, whereas metabolism is often called biotransformation.) It is easiest to eliminate drugs from the body if they are water-soluble (hydrophilic, ionized). Drugs are removed mainly by the liver's enzymatic metabolism and the kidney's excretory system. Positive and negative outcomes can result from DDIs that hasten or slow down these procedures. The liver is responsible for Phase I and II drug metabolism and enzyme production. The liver generates enzymes during phase I metabolism to make medications more water-soluble and more uncomplicated to remove. A metabolite is a byproduct of drug metabolism that an enzyme has broken down. Metabolites' pharmacological action may be the same as,

different from, or absent from the parent chemical (the original medicine). Several different metabolic pathways exist for a given drug. During phase I metabolism, a metabolite is formed that is different from the parent glucuronide; the kidneys then flush out the inactive glucuronide metabolite. Phase I metabolism slows with age, whereas phase II metabolism does not. Phase I metabolism is a significant contributor to DDIs.

1.2.2.1.4. Excretion

Excretory systems collaborate with metabolic processes to eliminate harmful substances, such as drugs, from the body. The kidney is the primary site of drug elimination in the body. Minor excretion channels include breath (which is how a breathalyzer detects alcohol), breast milk (which may affect a nursing newborn if the mother has taken drugs), feces, and sweat. The kidneys use three pathways to eliminate drugs from the body: glomerular filtration, tubular secretion, and tubular reabsorption. The kidneys filter medications out of the blood, and the tubules secrete them. The process of tubular reabsorption prevents a drug from being excreted in the urine. Drug elimination and absorption have standard techniques. The glomerular filtrate can reabsorb lipophilic, non-ionized medications back into circulation. However, hydrophilic or ionized drugs are not reabsorbed and are eliminated. Drug absorption can be affected by both urine pH and cellular transporters. Medication excretion can be modified by altering urine pH. Medicines that are only slightly acidic get more ionized in alkaline urine. Ionized drugs are not reabsorbed into the circulation but are flushed out in the urine. The kidneys and intestines both play a role in eliminating antifungal drugs. Changes in drug levels in the blood can result from alterations in drug clearance due to age, illness, iatrogenic unpleasant events, or medication interactions. Medication monitoring may need to be increased in renal-impaired patients [26].

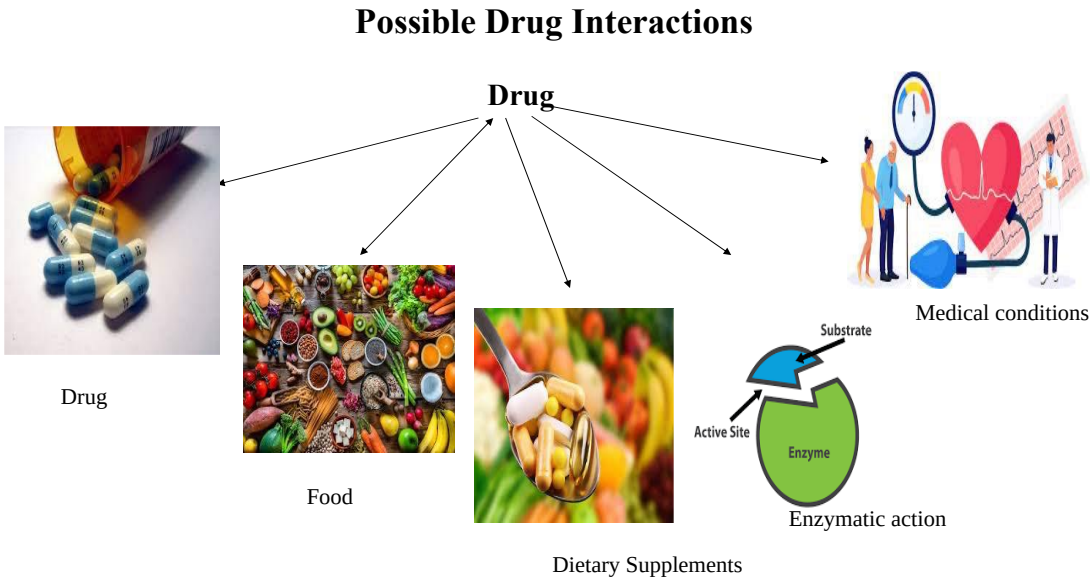


Figure 1.3 Possible Drug Interactions can affect the activity of the drug.

1.2.2.2. Pharmacodynamic drug-drug interactions

When two or more drugs interact at the site of action within the body, this is known as a pharmacodynamic drug-drug interaction and can modify the pharmacological impact of one or both drugs. This may happen if the medications have complementary or antagonistic actions on the same receptor or pathway, increasing or decreasing the overall effect. The combined effect of two medications, for instance, that both have a sedative effect when taken together, may be more potent than either drug alone, resulting in excessive sedation or other adverse effects. Conversely, the overall impact may be reduced or inhibited if two medicines have antagonistic actions on the same receptor. Pharmacodynamic interactions can be particularly significant when a medicine has a narrow therapeutic index or when there is little difference between a therapeutic dose and a hazardous dose. Even a slight modification in a drug's pharmacological action can have a significant therapeutic impact in certain situations. This

method considers interactions between medications with various mechanisms of action, such as an anticholinergic and a benzodiazepine [27].

1.3. Causes of Drug Interactions

There are several potential root causes of drug interactions. The concurrent use of another drug may alter the pharmacokinetics of a given drug. Due to competition for a single receptor or signaling pathway, drug interactions can also arise. The following are the main reasons that contribute to medication interactions:

i. Combination therapies

Since one medication has the potential to interact with others, the risk of drug interactions increases when many medications are used at once. Pharmacodynamic and pharmacokinetic interactions are two types of medication interactions that can occur when many medicines are taken at once.

ii. Multiple prescribers

For various reasons, prescribing multiple medications can raise the risk of drug interactions. First off, it's more likely that two or more prescribed medications would have overlapping pharmacological effects, which could result in potential pharmacodynamic interactions. Second, pharmacokinetic interactions may result from the prescription of numerous drugs. This may happen if one drug interferes with another's metabolism or excretion, changing the levels of each drug in the body and perhaps producing harmful or inefficient effects. Third, prescribing several medications might complicate a patient's medication schedule and raise the risk of administration or adherence mistakes [25].

iii. Multiple pharmacologic consequences of a drug

The many pharmacologic effects that a drug may have regarding interactions are the possible outcomes that may occur when a drug interacts with another chemical or medicine. These repercussions may have both favorable and unfavorable effects. Drug interactions may result in improved therapeutic outcomes or diminished adverse effects. For instance, some medications may work better when combined with another medicine that increases their activity or lessens their breakdown in the body. Alternatively, some medications may have fewer side effects when taken alongside a medication that mitigates the negative effects [25]. Drug interactions might result in decreased therapeutic effectiveness or increased harmful effects. For instance, two medicines with comparable pharmacological effects could fight for the same receptor.

iv. Multiple diseases/illnesses

Drug interactions can manifest themselves in a wide range of illnesses and conditions. To begin, the requirement for many medications to alleviate symptoms among those with diverse diseases increases the likelihood of drug interactions. Patients with many chronic conditions, such as diabetes, hypertension, and arthritis, are at greater risk of experiencing unwanted drug interactions [28]. Second, how a patient's body processes their medication may change if a medical condition affects their metabolism or drug elimination. Higher medication levels in the body and an increased risk of adverse effects may occur in patients with impaired drug metabolism or elimination due to, for example, liver or renal illness. Third, there is an increased risk of pharmacodynamic interactions between medications in patients with several disorders whose pathophysiology overlaps. For instance, patients with heart failure and hypertension may be prescribed many cardiovascular-system medicines, raising the risk of drug interactions and adverse consequences.

Fourth, there is a higher risk of medication errors or non-adherence if patients with multiple diseases have to follow complex prescription regimens. This may increase the likelihood of undesirable side effects or lead to less-than-desirable therapeutic outcomes. Reducing the risk of drug interactions in patients with multiple disorders requires healthcare professionals to weigh the benefits and risks of each prescription before writing them and to monitor patients for signs of adverse effects or changes in their condition.

v. Poor patient compliance

Poor patient compliance can be related to drug interactions in several ways. When patients do not adhere to their medication regimen as prescribed, they may inadvertently increase the risk of drug interactions by taking medications at the wrong time or in the wrong dose. This may cause undesirable shifts in drug levels in the body, raising the possibility of side effects or decreasing the medication's efficacy.

vi. Patient advancing age

There are several ways in which an aging patient might affect a drug interaction. Several medical problems, each requiring a unique treatment, may become more common as a patient ages. There is a greater chance of adverse drug reactions when a patient takes multiple medications, and although people over the age of 65 only account for about 14% of the population, they account for more than one-third of all outpatient prescription drug costs in the United States. A recent population report projected an increase from 46 million people over 65 to at least 98 million by 2060 [29]. Drug metabolism is another bodily function that might be affected by becoming older. For example, age-related liver and renal function declines can affect how drugs are metabolized and eliminated from the body. This might lead to dangerously high blood levels of the drug and increase the likelihood of adverse effects.

vii. Drug associated elements

Drug association factors affect the incidence and seriousness of medication interactions. These components include medicine dosage, therapy duration, administration route, and patient characteristics. Because higher doses of a medicine may result in higher drug levels in the body and a greater likelihood of pharmacodynamic and pharmacokinetic interactions, higher doses of a drug are more likely to produce drug interactions. The length of treatment might influence the risk of drug interactions. More extended therapy periods generally increase the risk of medication interactions because they may result in changes in drug metabolism or elimination over time that may impact the body's drug levels. The methods by which drugs are absorbed, distributed, metabolized, and removed can vary depending on the route of administration, which can impact how medications are processed in the body and their potential for drug interactions.

1.4. Clinical Resources for Drug Interactions

Drug interactions might enhance the drug's efficacy, absorption, or activity. Conversely, it might have negative effects and lead to decreased drug use. In addition, it could cause toxicities that are dangerous enough to require medical attention in a hospital. Particularly among the elderly, who are often prescribed long-term drugs to address a wide range of age-related conditions, there is an increasing demand for rational study of drug-herb interactions [30,31]. These clinical resources with medication interactions are listed below:

1.4.1 Proper Documentation

As of March 2021, an accurate search of the PubMed database was conducted to determine how one medicine affected another. Drug-food or drug-gene interaction literature, as well as other unrelated sources, were excluded. Then, with duplicates omitted, all the prescription guidelines that the US FDA had deemed necessary for preventing significant adverse effects. Finally, the document library for interaction annotation used a total of 9460 scholarly publications and pharmaceutical product labels [32].

1.4.2. Potential Metabolism Interactions

Many metabolic DDIs include cytochrome P450 (CYP) enzymes, which are believed to be responsible for the maximal metabolism of (about) two-thirds of medicinal medications [34]. The CYPs-mediated metabolic profile is valuable background information for elucidating and making sense of possible metabolic interactions. Over 80% of the oxidative metabolism and roughly 50% of the total elimination of typical therapeutic drugs in humans can be attributed to one or more of the numerous CYPs [35]. Metabolic effects for elimination are not the only kind of metabolism that CYPs may affect; they can also affect medication action, safety, bioavailability, and drug resistance in metabolic organs and at local sites of action.

1.4.3. Online Database Implementation

DDI may have many adverse effects on patients. We should gather knowledge and information about clinically significant DDIS to create awareness about DDI. There are few open-access information systems specifically for DDIs. However, there are several, including DDInter (a

curated DDI database with thorough data, helpful pharmaceutical advice, and potent visualization for the scientific community). There are currently 1833 approved medications and 1972 entities in the database, which has a capacity of around 0.24 million associations. Each drug is provided with basic chemical and pharmacological information and an assortment of interactions [32]. Besides, Alternative medications were recommended based on the ATC (Anatomical Therapeutic Chemical) code [33], a commonly used drug classification system in academic and clinical practice, to obtain an acceptable facilitated clinical prescription. DDI identified potential replacement medications by examining this ATC code corresponding to a pharmacological subgroup.

1.5. Challenges to Overcome Drug Interactions

The vast distance between theoretical clinical analysis and actual clinical practice is the primary barrier to progress when addressing the issue of drug-drug interactions. Even though there are precise regulatory standards for drug production and marketing, we cannot investigate the effectiveness of medicinal product intervention against DDI. After marketing monitoring, only the adverse effects and concerns are discussed; these outcomes that potentially put patients in danger are disclosed. Patients older than 65 have been shown to have higher levels of sensitivity. Because of physiological changes that alter medication pharmacokinetics and pharmacodynamics, the likelihood of drug-drug interactions increases with age in people who take multiple medications. Finally, older people are underrepresented in the standards and the research used to develop the guidelines. This is a problem because older people have valuable insights to offer. Because adverse events caused by interactions between drugs are rarely recorded, we do not know the frequency of adverse events caused by drug interactions in any given group. Previous statements by the European Medicines Agency indicate that

"spontaneous reports of adverse events can be used to identify patterns of drug-disease and drug-drug interactions that were not apparent prior to drug use." [36]. It is of the utmost importance to educate patients as well as clinicians in advance about the necessity of reporting circumstances like these. Although we have only skimmed the surface of the potential additive effect that can arise from interactions between different drugs and genetic issues, it is important to note that drugs can also interact with genetic issues. Consider the medication clopidogrel, which has a therapeutic effect that shifts depending on the individual's genetic makeup. Vascular restriction occurs in an unknown manner when a proton pump inhibitor, which may impair clopidogrel's therapeutic impact, is paired with a genetically poor metabolizer (with decreased active drug bioavailability) [37]. This combination may reduce the amount of clopidogrel that is available in the body for therapeutic use.

-To address these concerns, it is necessary to perform a comprehensive evaluation on each patient to collaborate on the formulation of a therapeutic plan that strikes a balance between the potential benefits and drawbacks of the treatment while also taking into account the patient's goals and preferences.

-In older people, these factors may include cognitive and functional decline, in addition to a deficiency in social support.

-The relevant evaluation and fully integrated technologies should be made available to medical professionals. There are too many potential drug-drug interactions for medical professionals to be able to remember all of them or provide advice for every possible combination of chronic illnesses.

- A thoroughly computerized and up-to-date prescription can efficiently inform practitioners of possible concerns, enhancing prescribing and decreasing interactions.

-Policies and guidelines may also investigate adopting electronic documents that are interactively searchable, adaptive, and hosted online.

- The development of online tools for generating interactive, documented rules that are compatible with decision support systems, are accessible on mobile devices such as tablets and smartphones, and are kept up to date mechanically. Because of the new format, medical professionals can now view many guidelines and query them using a predetermined list of questions.

1.6. Explanation of Present Research Topic

Recent research has shown that taking PPI simultaneously as VTB12 may reduce the amount of VTB12 absorbed [38]. In addition, according to the difficulty of DDI, senior patients have a higher risk of DDI. In this scenario, older patients taking PPI for longer than a year may have trouble absorbing nutrients [39,40]. We decided to conduct research on PPIs and multivitamins to establish a novel approach that could be used simultaneously to evaluate possible DDIs. In this particular instance, the study was carried out in three stages. The first thing that we did was conduct a prescription survey to find out what kinds of PPI and vitamins the patients were taking and how often they took them. Following acquiring adequate data, the subsequent stage addressed the possibility of in vitro DDI. The primary goal of observing interactions in vitro is to determine whether or not there was a possibility that chemical interactions took place. And in the third stage, we wanted to investigate pharmacological drug interactions.

1.6.1. Definition and Concept of Proton Pump Inhibitors

PPIs, or proton pump inhibitors, are a class of medications that considerably reduce gastric acid production by blocking the operation of proton pumps in the parietal cells of the stomach. For example, credible facts do not support the claim that taking PPI is the best method for reducing stomach acid production. This class of medications has succeeded and has entirely displaced an earlier class of therapy with the same effects because they are the most potent acid secretion inhibitors now on the market. The first proton pump inhibitor, omeprazole, is one of the most widely prescribed drugs in the world and is included on the World Health Organization's (WHO) Model List of Essential Medicines. In terms of global consumption, proton pump inhibitors (PPIs) have surpassed all others [41]. By 2020, PPIs will have become the most widely used category of medications in the United States. They are universally acknowledged as the safest and most palatable pharmaceutical options. The H+/K+ pump **(Figure 1.4)** is comprised of PPIs, a family of medicines. The basolateral membrane of the parietal cell is where you can find this pump. They gather on the acidic surface of the secretory canalicular organelles of the parietal cell and activate. Around 70% of active pumps have this component bound irreversibly to H+ /K adenosine triphosphatase, preventing acid production in the connected parietal cell. **Figure 1.4** depicts this. Irreversible disulfide bonds are formed between cysteine residues in the proton pump during the protonation process. The two most essential cysteine residues are CYS813 and CYS822. To obtain acid exposure in the parietal cell while preventing exposure in the stomach, PPIs should be administered 30 minutes before eating breakfast. The elimination pathways, peak plasma concentrations, and elimination half-lives of PPIs are what set them apart from one another. Their half-lives are brief, at around an hour, but because acid secretion requires constant new pump synthesis, they can be effective for up to 24 hours. All PPIs are cleared from the body via hepatic CYP2C19, with help from CYP3A4. Among proton pump inhibitors, lansoprazole, pantoprazole, and dexlansoprazole have the

highest bioavailability and titers in the blood. The most significant therapeutic promise belongs to rabeprazole, the PPI with the highest acid-lability. The PPI with the least reactive potential is pantoprazole. Whether or not these differences are clinically significant and, if so, whether they would justify choosing one PPI over another has been the subject of several research. No randomized controlled trial has shown that any PPI is superior to the others. The benefits and risks of PPIs, as well as their misuse, are discussed in this article.

1.6.2. Historical background of Proton-pump Inhibitors

Proton pump inhibitors are a family of drugs used to lower the amount of acid produced by the stomach. Since their inception in the late 1980s, they have seen widespread use in the medical community.

In the 19th century, researchers concluded that the stomach generates acid to help the digestive process. However, it wasn't until the middle of the 20th century that scientists began to better understand the mechanics behind the production of stomach acid. Histamine H2 receptor antagonists, more often referred to as H2 blockers because they are the first successful treatment to control stomach acid, were initially released in the 1970s [40]. The management of acid-related illnesses was radically altered with the introduction of medications such as cimetidine, ranitidine, and famotidine, which were extensively recommended. The decade of the 1980s saw tremendous leaps forward in terms of researchers' knowledge of the mechanisms behind acid secretion. They discovered the proton pump, which is an enzyme known as H+/K+ ATPase. This enzyme is responsible for the final stage in acid generation in the stomach.

The first proton pump inhibitor, omeprazole, was discovered by scientists working for the Swedish pharmaceutical company Astra in the late 1970s and early 1980s [40]. Proton pump inhibitors are medications that reduce the amount of acid the stomach produces. In 1988, the

Swedish Medical Products Agency approved the therapeutic use of omeprazole. Subsequently, this approval was granted in additional countries.

Following the achievements of omeprazole, further proton pump inhibitors were created and made available on the market. These proton pump inhibitors include lansoprazole, pantoprazole, rabeprazole, and esomeprazole, among others. Each PPI has a somewhat different chemical structure and a unique pharmacokinetic profile. In recent years, proton pump inhibitors have emerged as the therapy of choice for various acid-related conditions. These conditions include gastroesophageal reflux disease (GERD), peptic ulcers, Zollinger-Ellison syndrome, and the prevention of ulcers generated by nonsteroidal anti-inflammatory drugs (NSAID). They are also used in conjunction with antibiotics to eradicate the Helicobacter pylori bacterium, which is a significant contributing factor in the development of stomach ulcers. The use of proton pump inhibitors has skyrocketed in recent years, and they are now among the treatments that are prescribed the most commonly throughout the world. Concerns have been raised, however, concerning the abuse of these medications and the long-term negative effects they may cause, including an increased risk of infections, fractures, renal disease, and vitamin deficiencies. Generic copies of the original proton pump inhibitors (PPIs) were accessible once the patents protecting those PPIs ran out, which resulted in a significant price drop for those treatments. Several proton pump inhibitors, such as omeprazole and lansoprazole, can also be purchased without a doctor's prescription over-the-counter (OTC) and used for a shorter period.

1.6.2.1. Omeprazole

The first proton pump inhibitor (PPI) available to the public was omeprazole in 1988 [42]. It's a drug with the same molecular framework as timoprazole but with two methoxy and two methyl groups swapped for the timoprazole atoms, making it a 1:1 racemate. Methyl groups

occupy positions 3 and 5 on the pyridine, whereas positions 6 and 4 on the benzimidazole and 4 on the pyridine are occupied by methoxy groups, respectively. Omeprazole comes in various dosage forms, including enteric-coated tablets, capsules, chewable tablets, powder for oral solutions, and powder for intravenous injection.

1.6.2.2. Pantoprazole

The development of pantoprazole serves as a notable illustration of the progressive evolution in research pertaining to proton pump inhibitors (PPIs). The central emphasis of the structural alteration of timoprazole mostly revolved on the benzimidazole moiety. The introduction of trifluoromethyl group to the benzimidazole moiety resulted in the synthesis of a diverse range of compounds that exhibited significant levels of activity, while also displaying different levels of solubility [43]. It has been demonstrated that the presence of fluoro substituents typically leads to a reduction in metabolic activity at the places where they are attached. The lipophilic and highly electron-withdrawing substituent trifluoromethyl was ultimately substituted with the comparatively neutral fluoroalkoxy. It was anticipated that the compounds obtained would exhibit significant activity, along with enhanced half-lives and solution stability.

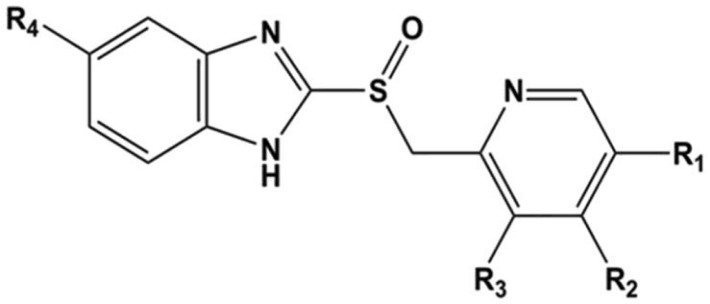
1.6.2.3. Esomeprazole

Due to the drug's inter-individual variability, many individuals with acid-related diseases needed larger or more doses of omeprazole to get symptom alleviation and recovery. In 1987, Astra initiated a new study to find an omeprazole alternative with lower interpatient variability [44]. The magnesium salt of the (S)-(O)-isomer of omeprazole, esomeprazole, was the only chemical that proved more effective than omeprazole. The first version of esomeprazole magnesium, sold under the trade name Nexium, was approved in 2000. It was shown to control acid secretion more effectively than omeprazole and to cause less fluctuation in response from

patient to patient. In 2004, more than 200 million people had been helped by Nexium (brand name).

1.6.2.4. Rabeprazole

Compared to currently available proton pump inhibitors, rabeprazole sodium, a novel substituted benzimidazole, has numerous advantages. Recent research has shown that compared to omeprazole, rabeprazole is a more effective inhibitor of H+, K+-ATPase, and acid secretion and a faster inhibitor of proton pumps. This is likely due to the quicker activation of rabeprazole in the canaliculus of parietal cells. Human investigations have shown that once-daily dosages of rabeprazole in the 5-40 mg range significantly reduce stomach acid output [45].



Drug	R_1	R_2	R_3	R_4
ESO	Me	OMe	Me	OMe
LAN	H	OCH_2CF_3	Me	H
PAN	H	OMe	OMe	OCF_2H
RAB	H	$O(CH_2)_3OMe$	Me	H

Figure 1.4 List of Widely accepted Proton Pump Inhibitors

1.6.3. Adverse Effects of Proton Pump Inhibitors

The most often seen major adverse effects of the treatment are headache, constipation, abdominal pain, feeling dizzy, and rash, with a prevalence ranging from around 1% to 5% [46]. Adverse effects may manifest as a result of extended utilization of proton pump inhibitors (PPIs). The prolonged utilization of this substance is associated with additional hazards such as osteoporosis, heightened gastric acid output, heightened vulnerability to enteric infections, and modified drug metabolism. Extensive research has been conducted on the possible interaction between clopidogrel and proton pump inhibitors due to the fact that clopidogrel's activation relies on biotransformation by CYP2C19, which happens to be the primary metabolic pathway for proton pump inhibitors [47]. Some investigations have yielded conflicting findings on the relationship between the two. In the first of these trials [48], researchers found that taking both a PPI and clopidogrel increased the risk of future cardiac events by a factor of 1.29. Some of the negative outcomes associated with PPI usage include:

-Recent studies have discovered various complicating factors in iron-vitamin B12 interactions. People on proton pump inhibitors have lower magnesium levels.

-PPIs at high doses or for extended periods may increase bone fracture risk.

-Researchers found a relationship between proton pump inhibitors and Clostridium difficile infections. Clostridium difficile causes large intestine infections.

-A proton pump inhibitor is usually prescribed to a patient taking aspirin for cardiovascular disease due to its antiplatelet effects. It is known that proton pump inhibitors (PPIs) can slow the metabolism of clopidogrel, a platelet inhibitor advised for heart disease patients.

-Proton pump inhibitors enhance pneumonia risk, especially in the first 30 days after initiating a new medication, when the risk was 50% higher in community settings. This is especially true in the first 30 days of a new medicine. A primary research study using pharmacoepidemiologic claims data analysis indicated a 1.38 hazard ratio between PPI use and dementia in persons over 75 years [49].

1.6.4. Clinical Pharmacology of Proton Pump Inhibitors

Despite the fact that the drugs omeprazole, lansoprazole, pantoprazole, and rabeprazole all have a similar structure and manner of action, each of these medications has a distinct clinical pharmacology that differentiates it from the others in the group. There is a significant amount of variation in the physical and chemical composition of the features as a result of the fact that the pyridine and benzimidazole substituents are distinct from one another. When pantoprazole sodium was directly compared to other anti-secretory medications, the results suggested that it was much more effective than H2-receptor antagonists and either equivalent to or superior to other PPIs used in clinical practice. This was the case regardless of whether the comparison between pantoprazole sodium and other PPIs was made. Rabeprazole operates throughout a larger pH range than omeprazole, lansoprazole, and pantoprazole, as the findings of another study demonstrated, and it converts to the sulphenamide form more quickly than any of these three medications. Most oral PPI formulations include an enteric coating to protect them from the rapid disintegration that occurs when the drug is exposed to the stomach's acidic environment. For example, the omeprazole half-life in acid is around two minutes when the pH is between 1 and 3, but it is significantly longer when the pH is 7 (almost twenty hours). The acid protective layer prevents the lumen of the stomach from converting into the active principle, which would then react with any available sulfhydryl group in food and would not

penetrate the lumen of the secretory canaliculus. The acid protective barrier also prevents the active principle from escaping the lumen of the stomach.

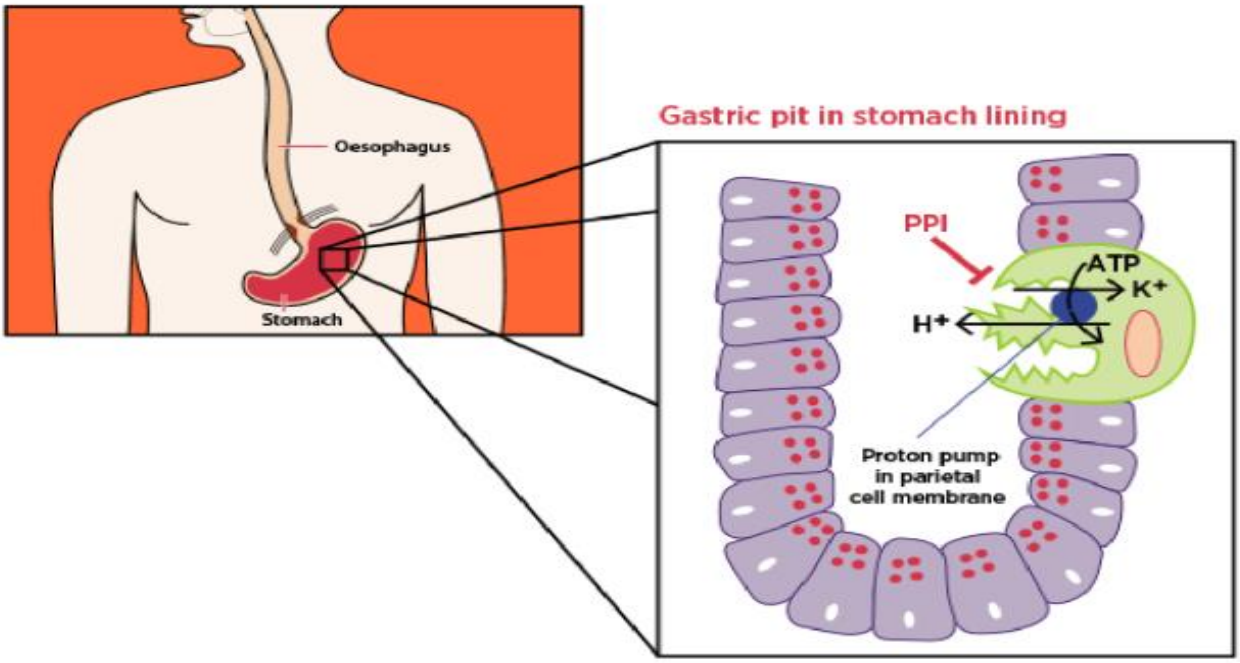


Figure 1.5 FDA-Approved Mechanism of PPI

The oral bioavailability of proton pump inhibitors is high. For example, the bioavailability of pantoprazole is 77%, the bioavailability of lansoprazole is 80–90%, and the bioavailability of esomeprazole is 89%. The accelerated metabolism of all proton pump inhibitors in the liver, except tenatoprazole, is due to an enzyme known as cytochrome P450 2C19, abbreviated as CYP2C19. While metabolizing xenobiotic compounds, this protein, which is an integral part of the cytochrome P450 mixed-function oxidase system, plays an important role. These xenobiotics include many proton pump inhibitors and antiepileptic drugs, most of which are classified as CYP3A4. PPIs are vulnerable to the activity of CYP enzymes and display a diversity of pharmacokinetic properties. This is because of their structure. Esomeprazole and tenatoprazole have a more robust acid suppression and maintain a higher intragastric pH for a

more extended period (pH > 4), as indicated by research comparing and contrasting the efficacy of proton pump inhibitors.

1.6.5. FDA-Approved Indications of Proton Pump Inhibitors Drug

PPI is used for various indications, some depicted in Figure 1.6. The FDA has authorized the following uses for PPI:

- Amoxicillin, the maintenance of healed duodenal ulcers, active benign gastric ulcers, the healing of and risk reduction for NSAID-associated gastric ulcers, gastroesophageal reflux disease (GERD), the maintenance and healing of erosive esophagitis, and hypersecretory disorders.
- GERD- erosive esophagitis related to gastroesophageal reflux disease (GERD), maintenance healing of erosive esophagitis, and hypersecretory disorders.
- Healing or maintenance of erosive or ulcerative GERD, as well as GERD, duodenal ulcers, H. pylori (when paired with amoxicillin and clarithromycin), and hypersecretory disorders.
- Containing CYP2C19 enzymatic activity, it has the ability to influence the effects of diazepam and alpha-phenytoin.

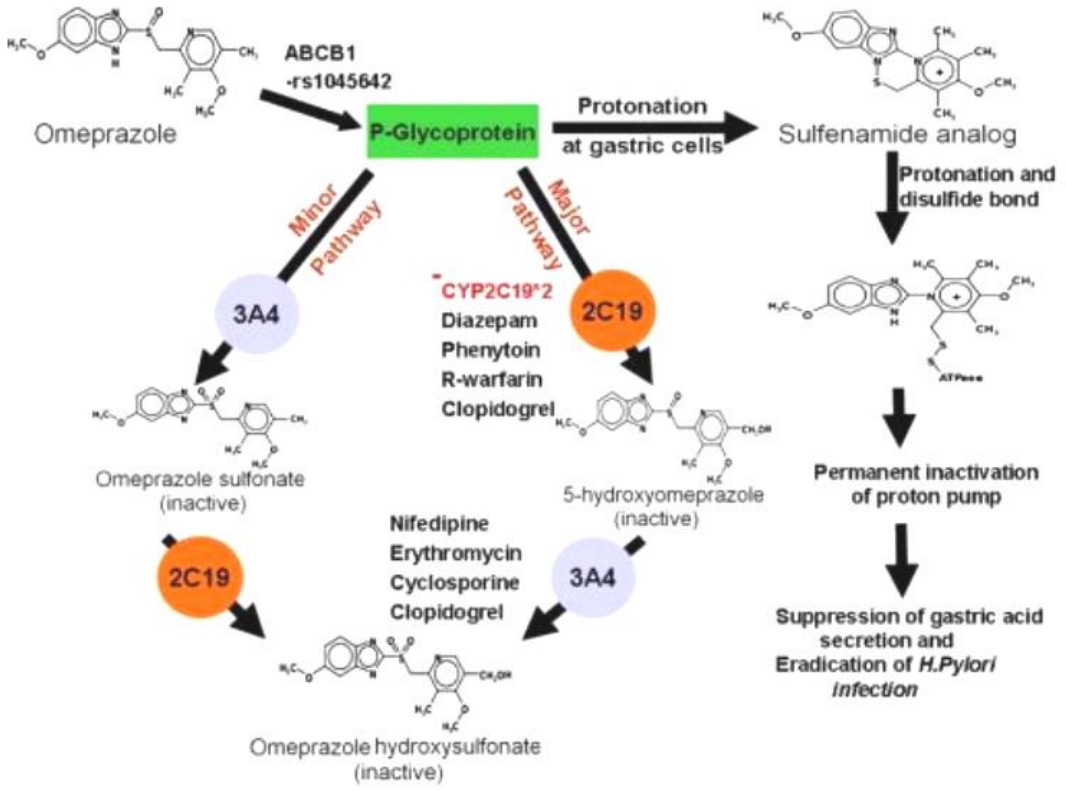


Figure 1.6 FDA-Approved Indications Mechanism of action of Proton Pump Inhibitors with glycoprotein

1.6.6. Multivitamin

Although multivitamins and minerals are often used, there is no consensus over what they are in the scientific, regulatory, or commercial communities. Therefore, multivitamins and minerals relate to a wide range of different products, each of which has a unique composition and set of qualities. Databases that track the composition of multivitamin-multimineral supplements employ label values as a stand-in for analytical values. On the other hand, the levels of vitamins and minerals that are mentioned on product labels may not always

correspond to the amounts that are actually ingested. When it comes to the vitamin and mineral bioavailability of dietary supplements, there needs to be a consistent scientific and regulatory definition, as well as validated in vitro and animal models that accurately represent human bioavailability. In addition, there is a lack of a unified scientific and regulatory definition. There is a shortage of comprehensive data on the bioavailability and bioequivalence of vitamins and minerals in goods on the market for purchase, in addition to the potential for drug interactions. Because there is a lack of information on the characteristics of the products themselves, it is challenging for us to make direct comparisons between the findings of various pieces of research, evaluate how consumption or usage patterns have changed over time, and extrapolate from results that have been published to products that are currently available for purchase.

1.6.7. Multivitamin Conceptual Background and Definition:

The term "multivitamin" refers to a formulation that is intended to be consumed in the manner of a dietary supplement and that contains several vitamins, dietary minerals, and other nutritional components. Tablets, capsules, pastilles, powders, liquids, injectable formulations, and injectable formulations are some of the numerous forms these kinds of preparations might take. Other possible forms include injectable formulations. Except for injectable formulations, which are not available to the general public and must be given under the supervision of a medical professional, the Codex Alimentarius Commission, which is the authority on food standards for the United Nations, has recognized multivitamins as a category of food. This is with the exception of injectable formulations, which must be given under the supervision of a medical professional. Because of their significance to maintaining a balanced diet, minerals are usually contained inside multivitamin supplements. A supplement is considered a multivitamin and mineral supplement in the United States if it includes three or more distinct types of vitamins and minerals but does not include any herbs, hormones, or pharmaceuticals in its

composition. In addition, the dose of each vitamin and mineral included in the supplement is lower than the maximum amount considered safe by the Food and Drug Administration. This means that the supplement does not in any way represent a risk to the user's health. "multivitamin" and "multimineral" are often used synonymously in everyday conversation. This is a widespread practice. The scientific literature does not define either of these terms. People who are otherwise healthy and taking multivitamin supplements regularly are not necessary to do so, according to the great majority of the research that has been conducted on the subject in recent years. This research also implies that multivitamin supplements cannot prevent cancer or heart disease. On the other hand, specific subsets of the population may benefit from taking multivitamin supplements. For example, those who have a poor diet or who are at a high risk of developing macular degeneration may find that taking multivitamin supplements is beneficial for them.

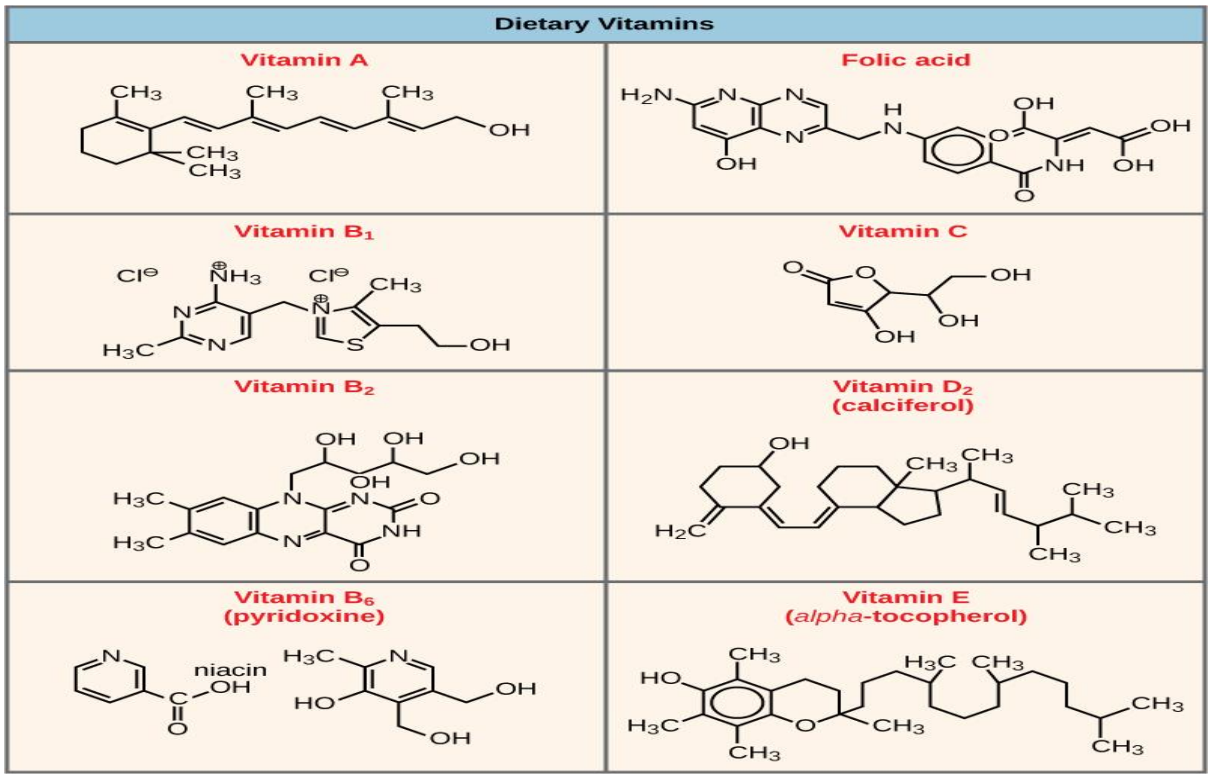


Figure 1.7 List of Multivitamins available as a dietary supplement

1.6.8. Historical Background of Multivitamin

Early in the 20th century, researchers began discovering and isolating several types of vitamins, which are vital nutrients. Casimir Funk, a Polish biologist, is credited with first proposing the name "vitamin" in 1912. This revelation provided the impetus for further investigation into these chemical molecules' role in the upkeep of one's health. As medical researchers gained more knowledge about vitamins, they also began to recognize disorders linked to inadequate vitamin intake. For instance, a shortage of vitamin C was discovered to cause scurvy, whereas a lack of vitamin D was shown to cause rickets. These findings provide light on the critical function that vitamins play in warding against a variety of illnesses. At the beginning of the 20th century, there was an attempt made to treat vitamin deficiencies by changes in dietary practices. To combat the widespread prevalence of nutritional deficiencies, governments and health organizations added additional nutrients to particular foods, such as vitamin D to milk and iodine to salt.

On the other hand, these metrics were only applicable to certain nutrients. Around the middle of the 20th century, someone came up with the idea of multivitamins, which are supplements that contain many vital vitamins and minerals combined into a single pill. To guarantee that the nutritional requirements of the armed forces were satisfied during World War II, the United States military produced multivitamin supplements. The popularity of these dietary supplements contributed to their increased availability in commercial settings following the war's end. The widespread use of multivitamins began to increase in the decades after the end of World War II. They were advertised as a handy means to guarantee appropriate intake of nutrients, particularly for persons whose diets were unbalanced or who had limited access to fresh vegetables. Research conducted by scientists throughout the years has investigated the usefulness of multivitamins and their safety. Fortification of diets on a large scale began in the

United States in 1924 when iodine was added to table salt to prevent goiter. This was followed by adding vitamin D to milk in 1933 to prevent rickets, and in 1941, thiamin, riboflavin, niacin, and iron were added to wheat [50]. In the middle of the 1930s, pharmacists and grocery shops began stocking multivitamin/multimineral products that contained more nutrients than just vitamins A and D. The initial MVM tablet was marketed to the public for the first time in the early 1940s [51]. Studies have shown conflicting findings regarding their overall influence on health outcomes in people who are generally in good health, despite the fact that they may be helpful for those who are deficient in particular vitamins or who suffer from certain illnesses.

1.6.9. Uses of Multivitamins

Supplementing the diet with additional vitamins and minerals may have a positive influence on the health of some individuals, notably older people, but the vast majority of people will not benefit from this. Dietary imbalances can affect those who are following restricted diets as well as those who are unable or unwilling to eat a diet that is high in nutrients. A physician may recommend a multivitamin for pregnant women and older individuals since these populations have unique dietary requirements that differ from those of other adults. Unless otherwise directed by a qualified medical practitioner, pregnant women are strongly discouraged from taking multivitamins, particularly ones that include vitamin A, throughout their pregnancy. On the other hand, the National Health Service (NHS) suggests taking ten micrograms of vitamin D each day throughout pregnancy and during breastfeeding and 400 micrograms of folic acid during the first trimester (the first 12 weeks of pregnancy). Iron, vitamin C, or calcium supplements may be necessary for certain women during pregnancy; however, this should only be done on a physician's recommendation. 52% of individuals in the United States reported using at least one dietary supplement in the previous month, and 35% reported using

multivitamin-multimineral supplements regularly, according to the National Health and Nutrition Examination Survey conducted in 1999–2000 [52]. Women were more likely to take multivitamins than males, older persons were more likely to take them than younger adults, non-Hispanic whites were more likely to take them than non-Hispanic blacks, and those with higher education levels were more likely to take them than those with lower education levels (among other groups). People who use dietary supplements, such as multivitamins, typically have greater dietary nutrient intakes and better diets than those who do not take such supplements. Furthermore, those with a history of prostate or breast cancer were more likely to utilize dietary and multivitamin supplements than adults without.

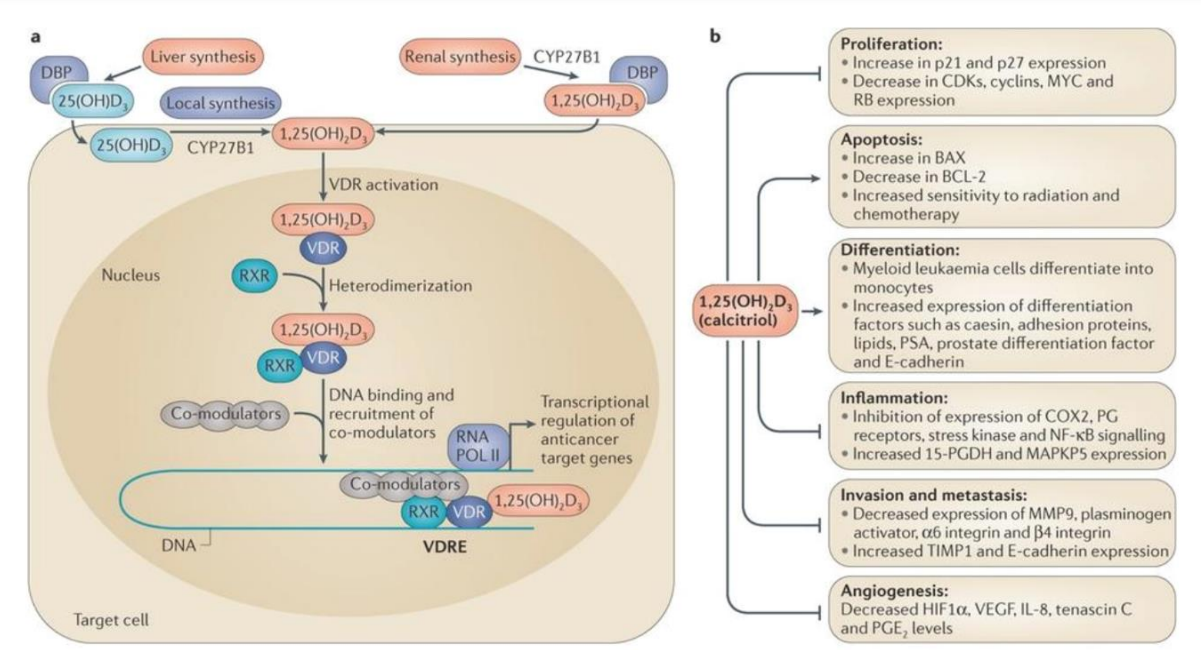


Figure 1.8 Mechanism of action of multivitamin

1.6.9.1. The Effect of Multivitamins

As shown in Figure 1.8, one strategy for lowering one's chance of developing age-related cataracts is to take antioxidant vitamin supplements as a dietary supplement. This strategy is highly recommended. Nevertheless, there has been data contradicting the antioxidant vitamins A, C, and E and the development of cataracts in observational studies and clinical trials. Researchers seek to determine how effective multivitamins and mineral supplements are in lowering the risk of developing age-related cataracts by conducting a systematic review and meta-analysis of the relevant data. In September 2013, we examined a large number of databases to locate relevant papers. These studies included both cohort studies and randomized controlled trials (RCTs). A random-effects model was utilized to compute the pooled relative risks (RR) along with a confidence interval (CI) that was set at 95%. There was a total of twelve prospective cohort studies and two randomized controlled trials included. The results of the cohort studies that were pooled showed that multivitamin and mineral supplements have a substantial favorable impact in lowering the risk of cataracts overall (RR: 0.66; 95% CI: 0.39–0.93), nuclear cataracts (RR: 0.73; 95% CI: 0.64–0.82), and cortical cataracts (RR: 0.81; 95% CI: 0.68–0.94).

1.6.9.2. Defects and Other Unfavorable Outcomes of Pregnancy and Childbirth

Numerous studies have been conducted to investigate several additional kinds of birth defects and adverse pregnancy outcomes. Taking a multivitamin throughout the periconceptional phase, defined as the three months before conception and the first trimester of pregnancy, may reduce the chance of having omphalocele, a malformation of the abdominal wall. This research was conducted using a population-based case-control methodology. However, the precision of this analysis was hindered by the fact that the case-infant sample size only comprised 72 occurrences. The Atlantic Birth Abnormalities Case-Control research was a population-based

study that explored the links between periconceptional multivitamin consumption and respiratory tract abnormalities, pyloric stenosis, and anal atresia. However, none of the correlations were determined to have a significance level that could be considered statistical. The connection between taking many vitamins and minerals and premature delivery is another topic of investigation. Case-control studies showed that taking a multivitamin supplement while pregnant might lower the risk by 1.6 to 1.8 times. Case-control research came to an unexpected conclusion when it showed a connection between taking multivitamins and an increased risk of premature delivery during the third trimester of pregnancy. However, due to the lack of clarity on what led to this result, any interpretation must be approached with extreme caution.

1.6.9.3. The Frequency of Taking Multivitamins During Pregnancy at Any Time

There is no set pattern for how often women should take multivitamins when pregnant. According to the findings of the National Maternal and Infant Health Survey conducted in the United States in 1998, 43.8% of pregnant women utilized multivitamins and minerals three times per week after giving birth. Since the mandatory addition of folic acid to food caused a rise in the incidence rate, it currently varies from 68.8 to 78% in 2015.

It was revealed that the prevalence of multivitamin consumption during pregnancy in India was 76.2%, the highest compared to other nations in the Asia Pacific area in Bangladesh. Because the data on the prevalence of multivitamin usage were combined with the data on the use of minerals, the comparability of these two data sets was compromised. Furthermore, two further investigations conducted an estimation of the prevalence of the illness by combining a variety of vitamins and minerals. According to research, a notable proportion of participants, namely 30.8%, reported the utilization of multivitamins and minerals throughout the duration of their pregnancies. However, additional research conducted a detailed analysis of the prevalence rate.

The data presented in the study indicates that multivitamin-mineral supplement usage among women was distributed throughout three trimester periods. Specifically, 23.3% of women reported using such supplements during the first trimester, 14.1% during the second trimester, and 18.6% during the third trimester.

1.6.9.4. The Use of Multivitamins During the First Trimester of Pregnancy and in the Elderly

The utilization of multivitamins may be evaluated in a few different ways. First, women are often considered to be multivitamin users if they use multivitamins at the time of the evaluation or if they reported having taken multivitamins while pregnant. This is because women are more likely to use multivitamins during pregnancy. However, this evaluation does not consider the frequency or duration of multivitamin usage, which may lead to an overestimation of the number of individuals who regularly use multivitamins. Women who had just used a multivitamin once were likely counted as users of multivitamins. This scenario is feasible. It was observed that the prevalence of utilizing multivitamins when pregnant in the United States was between 78.0 and 82.5% of women. One study reported that 23.8% of women said using multivitamins during the periconceptional period, while another study suggested that 21.0% of women reported regularly using multivitamins (3 days a week) during the periconceptional period [53]. These findings pertain to the prevalence of periconceptional multivitamin use in the United States.

1.6.10. Multivitamin vs. Single Vitamin

The consumption of single vitamins (such as vitamins A, B, C, D, E, and folic acid) in addition to multivitamins was investigated in this study. It would appear that only a tiny percentage of pregnant women who took multivitamins also took extra single vitamins. According to the

findings of a population-based survey conducted in the UK, which evaluated the frequency and amount of all single vitamins taken during pregnancy, including vitamins A, C, and E, none of the amounts were over 5%. Furthermore, 68 It was revealed that the prevalence of taking folic acid was between 71.4 and 78.7% of pregnant women, much higher than the prevalence of taking other single vitamins. Unlike other single vitamins, many pregnant women who used multivitamins also took extra folic acid supplements.

1.6.11. Multivitamin Exposure

Several distinctions were made between the time frames for multivitamin exposure during pregnancy and the periconceptional phase. In the case of pregnancy, multivitamin exposure was often monitored after the woman had determined that she was pregnant or utilized the beginning of the most recent menstrual cycle as the starting time point for pregnancy. A period of time passes between a woman's last menstrual period and when she recognizes she is pregnant. Because of this time gap, women can be unaware that they are pregnant even while the fetus is developing. These two-time points were merged in population-based research that asked women about their usage of multivitamins since their last menstrual cycle or when they recognized that they were pregnant. Because illnesses such as atherosclerosis, diabetes, and malignant tumors have cumulative causes and grow more common with age, there is a significant correlation between aging and increased drug usage. Analgesics are typically used to treat arthritic and rheumatic conditions and the discomfort associated with tumors. Involutional depression can be treated while reducing the need for analgesic medication. As people get older, they are more likely to suffer from insomnia as a result of diminished brain circulation. It will often respond similarly to coffee, somnifacients, and tranquilizers. On the other hand, there is no correlation between age and the use of anti-asthmatics or antibiotics. The more prescribed medications, the higher the risk of adverse effects and interactions

between medications, as well as a rise in the complexity of determining the appropriate dosage. High drug usage rates also place a significant financial burden on public health insurance systems. Despite this, it would be immoral to withhold necessary medical assistance from the elderly to enhance the condition of vitamin repletion, even if this course of action succeeded in improving the state of vitamin repletion. As a result, the only practical solution is to identify any issues brought on by drug-vitamin interactions and work toward preventing them by developing an adequate dose schedule.

1.7. Goal and Objective of this Study

Based on the prescription survey, both in vivo and in vitro drug interactions were observed on Pantoprazole and vitamin B- complex, as previous journals showed that omeprazole and vitamin B12 might cause interactions in the absorption site. Our research on any potential DDI between PNT and VTB in the healthy Bangladeshi population was prompted by an adverse drug report (ADR) regarding the development of VTB deficiency while being treated with PNT [54]. Although VTB and PNT are prescribed concurrently in a significant percentage of cases in Bangladesh, there is still little information on potential pharmacokinetic interactions or their potential effects. In primary, secondary, and ambulatory care settings, the overuse of PPI often results from failing to review the required ongoing medication or underusing on-demand and step-down therapy as alternative treatment choices. Ambulatory care settings may also be responsible for the misuse of PPI because of a failure to reassess the requirement of ongoing medication. According to data about the use of costs from 2010, brand-name PPIs earned close to $7.2 billion in sales, whereas generic PPIs generated close to $2.5 billion in sales. Pantoprazole and omeprazole, which rank in the top five in generic prescription expenditure, are the two generic PPIs with the biggest prescription expenditures [54]. The potential risks associated with the incorrect and irrational short- and long-term use of PPIs in hospital and

ambulatory care practices have been brought to light in landmark publications over the past ten years in various specialties, including primary care, managed care, gastroenterology, and pharmacology. These publications have been published in various fields, including primary care, gastroenterology, and pharmacology. These papers have concentrated their attention on the application of PPIs in ambulatory care practices as well as hospitals. PPIs have been linked to several adverse effects, including an increased risk of enteric infections such as diarrhea caused by Clostridium difficile [55,56], community-acquired pneumonia [57–59], altered metabolism of antiplatelet medications (such as clopidogrel) [60–62], osteoporotic-related fractures, and disruption of vitamin and mineral metabolism.

The current study aimed to identify potential drug-drug interactions and their potential pharmacokinetic effects when PNT and VTBs (**Figure 1.9**) were administered concurrently to healthy Bangladeshi volunteers. To the best of our knowledge, no analytical technique can simultaneously determine PNT and VTB in biological fluids and pharmaceutical formulations. Therefore, new analytical approaches for pharmacokinetics, DDI, and bioequivalence investigations are believed to be essential to better understand the pharmacodynamic and combinatorial effects of the drugs that are the focus of this research. As a result, the purpose of this research was to develop and validate a technique for determining the amount of PNT and VTBs that were given simultaneously, as well as to evaluate the pharmacokinetic characteristics of both substances to identify any potential DDIs.

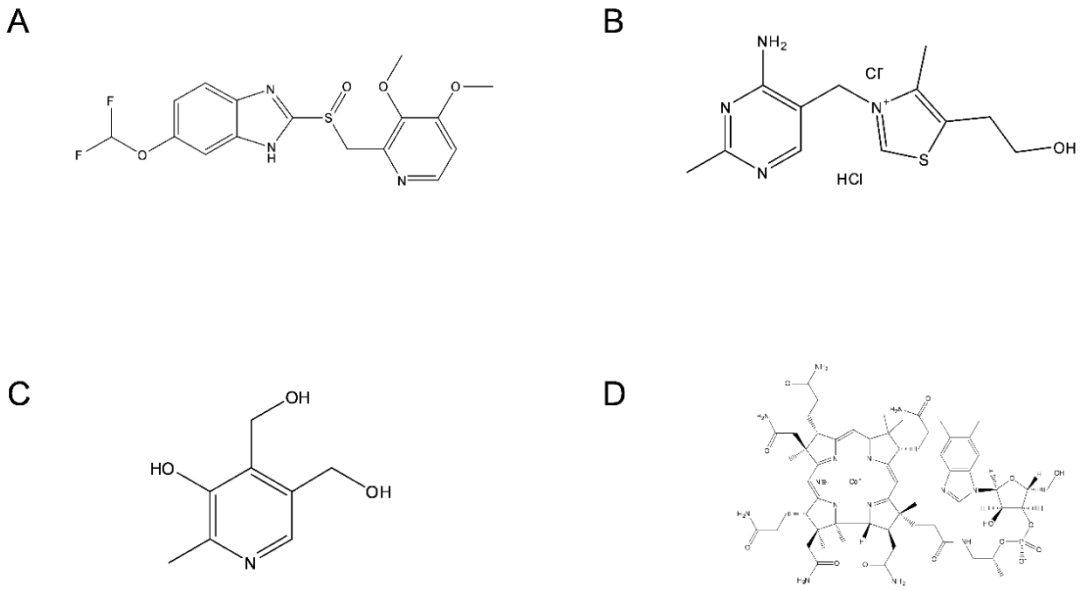


Figure 1.9 Structure of (A) PNT, (B) VTB1, (C) VTB6, and (D) VTB12

Objective of the study:

1. We are monitoring the prescription pattern to establish the rate of concurrent use of the drugs.
2. On the basis of the prescription survey, one of the proton pump inhibitors was selected to observe interactions with multivitamins (especially Vitamin B1, B6, and B12).
3. Method development for the quantification of individual drugs in a mixture by HPLC.
4. Determination of concentration of individual drugs in plasma after oral administration.
5. Determination of Pharmacokinetic parameters such as AUC, $C_{max,}$ Tmax, etc.
6. Data analysis and determining the drug interaction.

1.8. Possible Drug Interactions of Multivitamins and Proton pump Inhibitors; A short review

The general public did not pay much attention to the second concern until the Food and Drug Administration (FDA) of the United States issued warnings in 2010 and 2011 suggesting a relationship between long-term PPI consumption and osteoporosis-related fracture and hypomagnesemia, respectively [63]. It is considered that there is a big functional reserve for the absorption of vitamin B12 since the usual human diet already includes a significant quantity of vitamin B12 that is more than what is necessary [64]. The acidity level in the stomach affects the ability of vitamin B12 to break its bindings with the proteins in the food being consumed and bind to R-proteins, which in turn prevent vitamin B12 from being digested by the pancreas [65]. Acid suppression has been postulated as a potential cause of malabsorption, which, in turn, may result in vitamin B12 insufficiency due to atrophic gastritis and achlorhydria [65,66]. Pernicious anemia and diseases that induce poor gastrointestinal absorption have been connected to vitamin B12 deficiency [67]. Ito and Jensen have only recently made public the findings of more recent research that looked at the risk of vitamin B12 deficiency due to using a PPI medication [68]. These results were derived from a recent in-depth research project carried out worldwide. One case-control research [69] compared 125 long-term (more than three years) PPI users from general practices who were 65 years of age or older to the same number of controls. The participants had all used PPIs for at least three years. Every single participant had been using PPIs for at least three years. Measurements of the mean corpuscular volume of red blood cells, as well as the levels of vitamin B12 and homocysteine in the serum, were obtained to ascertain the severity of a deficit in vitamin B12. In a research study with a duration of up to 18 years [70], 61 acid hypersecretors who were all receiving long-term treatment with PPIs and 46 of them were diagnosed with ZES (Zollinger-Ellison syndrome)

were observed to determine the likelihood of vitamin B12 malabsorption. Everyone who took part in the study was receiving PPI treatment over a prolonged period. 10% of the 61 patients had low amounts of vitamin B12 in their blood, and additional analysis revealed that 31% of patients had vitamin B12 deficiencies while having acceptable serum levels of vitamin B12.

Mowat and his colleagues investigated the effect of consuming 40 milligrams of omeprazole for four weeks on the quantity of vitamin C in healthy adults' stomach juice. The concentrations of vitamin C dropped from 5 µm/L before treatment with omeprazole to 3 µm/l while the individuals were receiving omeprazole. This change reflects a significant reduction in the amount of ascorbic acid that is in its physiologically active form. The intragastric pH of the participants increased from 1.4 (before omeprazole therapy) to 7.2 (range: 3.5–8.5) when they were taking omeprazole. The content for this rise was from 3.5 to 8.5. Before treatment with omeprazole, the median pH level in the intragastric region was 1.4. The relevance of vitamin C deficiency as a therapeutic factor stems from the hypothesis that it may protect against the conversion of nitrite to N-nitroso compounds by bacteria that may conceivably populate the achlorhydric stomach environment. This is where the belief originates. This idea was conceived in the 1960s, and several investigations have provided evidence in favor of its validity.

PPI medication and calcium metabolism are inextricably linked, and a review that Insogna authored provides the groundwork upon which to comprehend the connection between the two [71]. It has been postulated that to extract ingested calcium from a food bolus and make it available for absorption, both the stomach acid and the moderately acidic environment of the proximal duodenum are necessary. This is because the stomach acid breaks down the food bolus into smaller pieces. If this environment did not exist, there would be no absorption of elemental calcium, which might lead to compensatory physiologic changes such as secondary hyperparathyroidism. There is conflicting information about the role that intragastric

hydrochloric acid plays in calcium absorption; however, one study revealed that gastric acid secretion and gastric acidity do not generally play a role in the absorption of dietary calcium [72]. PPIs are known to disrupt this pathway, and there is also conflicting information about intragastric hydrochloric acid's role in calcium absorption. PPIs are thought to have the potential to directly influence the activity of osteoclasts [73], which is supported by the fact that osteoclasts also possess proton pumps. The resorption of calcium from bone is slowed down due to the usage of PPIs.

The study of patients with renal failure on hemodialysis or those with hypo- or achlorhydria, two chronic conditions known to influence calcium metabolism adversely, is a limitation of many studies assessing PPIs' impact on intestinal calcium absorption [72].

The use of proton pump inhibitors (PPIs) concurrently has been related to an increased incidence of bone fracture; however, no long-term, prospective, randomized, or controlled studies have been carried out to evaluate this potential relationship. This is because all of the published data comes from retrospective, case-controlled, or cross-sectional studies. This is the reason behind this. PPI medication has been connected to an increased risk of osteoporotic fracture due to several studies. This has prompted a critical evaluation of the likely linkage between the two, revealing a causal relationship [73]. The review was driven by the fact that PPI treatment has been associated with an increased osteoporotic fracture risk. However, the researchers who carried out the investigation concluded that using PPIs was not associated with a shift in bone mineral density [74]. This shows that research participants using PPI medication may be at an increased risk of osteoporotic fracture compared to a person at an average risk of developing osteoporosis. On the other hand, more recent study has shown data that don't fit in with this idea.

The presence of stomach acid helps substantially with the absorption of nonheme iron. It is easier for food sources that include nonheme iron to dissociate and become soluble in iron salts when gastric acid is present. Because of this, complexes can be formed with sugars and amines, which makes it easier for the substance to be absorbed [68].

There is some evidence to support the hypothesis that there is a connection between using proton pump inhibitors (PPIs) and developing iron deficiency anemia. In these case studies, the participants did not react to iron replacement while simultaneously getting therapy with PPIs; nevertheless, they did react favorably once the PPI medication was withdrawn [75]. This was the case even though the individuals did respond favorably once the PPI therapy was terminated.

A retrospective cohort study was undertaken with adult patients in an academic outpatient environment who had received PPI medication for at least one year to examine any possible change in hematologic indices vs. matched controls [76]. This research was done to determine whether there was a correlation between the PPI medication and the potential change in hematologic indices. As was the case with the findings of earlier trials carried out in ambulatory care settings [77], thirty-five percent of the people who participated in this experiment and used PPIs did not have any documented or clinically significant reason to be taking such medicine. In spite of these findings, there is no recommendation given at this time to monitor patients who are on PPI medication for an extended period for iron deficiency anemia.

It is now a well-researched condition, although prolonged use of proton pump inhibitor (PPI) medication can still only produce a very small number of cases of hypomagnesemia. Even though no mechanism is universally acknowledged as capable of explaining such a relationship [78]. All proton pump inhibitors (PPIs), which are biochemically substituted pyridyl methyl sulphonyl benzimidazole derivatives (in order of potency: rabeprazole, esomeprazole,

omeprazole, and lansoprazole, and pantoprazole), have been linked to hypomagnesemia. This occurrence is assumed to be the consequence of a class effect [79], in spite of the fact that a number of studies have shown that hypomagnesemia may reoccur after switching from one PPI to another.

Since 2006, there have been less than thirty cases of hypomagnesemia associated with the use of PPI medication. These cases were published in the literature. 61% of these cases involved patients who had been receiving PPI medication for at least five years, and 29% of these cases had been receiving PPI therapy for at least ten years [80]. The impairment was reported more frequently in females than in males, and the median age at diagnosis was 70 years old (the range was 51–82 years old). The content of ages affected by the deficit was 51–82 years. The majority of patients with hypomagnesemia that was considered to be caused by PPIs came with concomitant hypokalemia and hypocalcemia, in addition to severe ataxia, paresthesia's, seizures, disorientation, and gastrointestinal symptoms that necessitated hospitalization. In addition, PPIs were assumed to be the cause of hypomagnesemia. There was no sign of magnesium malabsorption, renal wasting, or histological abnormalities on either the small intestine or the colonic biopsies that were done on these individuals. According to the research findings, individuals with the lowest concentrations of magnesium and calcium in their plasma experienced the most severe symptoms [79].

The Food and Drug Administration (FDA) issued a warning in 2011, which was based on multiple published case reports [63], stating that prolonged use of proton pump inhibitors (PPIs) may result in hypomagnesemia if the medicine is used for more than one year. Because magnesium supplementation was insufficient to raise blood magnesium levels, the PPI prescription had to be stopped in around 25 percent of the evaluated individuals. Patients undergoing continuous PPI therapy who have a history of cardiac arrhythmias or are currently

receiving medication that treats cardiac arrhythmias should be screened for low blood magnesium levels. This is a practice that is considered to be clinically reasonable.

An experiment with a cross-sectional design was carried out by Gau and colleagues [81] to assess the potential consequences that using PPI medicine may have in connection to the onset of hypomagnesemia.

PPIs have been associated with an increased risk of vitamin and mineral deficiencies, which can affect the metabolism of vitamin B12, vitamin C, calcium, iron, and magnesium. This association was made after PPIs were found to be connected to an increased risk of these conditions. Although these risks are believed to be relatively low in the general population, they may be considerable in patients who are old, malnourished, or taking PPI medication in addition to undergoing chronic hemodialysis at the same time. There is inadequate evidence to recommend routine vitamin and mineral testing or supplementation for patients undergoing either short-term or long-term PPI medication. This is true for both short-term and long-term PPI therapy. Reducing the number of needless prescriptions for PPIs can lower the risk of vitamin and mineral deficiencies [82].

According to statistics about cost usage from 2010, brand-name PPIs generated around $7.2 billion in sales, whereas generic PPIs generated approximately $2.5 billion in sales. Nexium® (esomeprazole; Astra Zeneca), which ranks first in brand-name prescription expenditure, and pantoprazole and omeprazole, which both rank in the top five in generic prescription expenditure, are the two generic PPIs that have the most significant prescription expenditures [83]. Nexium® (esomeprazole; Astra Zeneca) ranks first in brand-name prescription expenditure. Nearly eighty percent of all purchases of proton pump inhibitors (PPIs) in the United States are made without a prescription or a physician's evaluation of upper gastrointestinal symptoms [84].

The potential risks associated with the erroneous and irrational short- and long-term use of PPIs in both hospital and ambulatory care practices have been brought to light in landmark publications over the past ten years in a variety of specialties, including primary care, managed care, gastroenterology, and pharmacology [85]. These publications have focused on using PPIs in hospitals and ambulatory care practices. An increased risk of enteric infections, such as diarrhea caused by Clostridium difficile [56,86,87], community-acquired pneumonia [57,58,88], altered metabolism of antiplatelet medications (such as clopidogrel) [60,61,89], osteoporotic-related fracture, and disruption of vitamin and mineral metabolism are some of the reported side effects of PPI.

CHAPTER 2

MATERIALS AND METHODS

2. MATERIALS AND METHOD

Methods of DDI have three consecutive parts:

- Prescription survey: A total of 500 prescriptions issued by physicians were utilized for the purpose of conducting a survey study aimed at identifying random instances of Proton Pump Inhibitor (PPI) and multivitamin usage.
- *In vitro* study: Based on the survey, the most frequently used proton pump inhibitors and vitamins were set for in vitro tests, including XRPD, FT-IR, and DSC.
- Pharmacokinetic drug-drug interaction: Finally, to conduct *in vivo* study, volunteers were selected, and a 3X3 crossover study was performed with an RP-HPLC-validated method.

2.1. Observe the prescription Pattern in Local Area

2.1.1 Study Settings

The research survey was carried out by personally evaluating the prescriptions that doctors had written for their patients between the years 2015 and 2018 in a variety of hospitals and private clinics located in two of the most important districts in Bangladesh. This survey was carried out in the cities of Dhaka and Barisal in Bangladesh. This prescription survey was more focused on fieldwork than previous ones. A patient's age, gender, illness, prescribed generic and brand names, place of practice, and specialty are among the pieces of information that may be gleaned from their prescriptions.

2.1.2. Study Design

The purpose of this study was to conduct a statistical analysis of the prescription survey between PPIs and multivitamins simultaneously consumption with patients' age, gender, and the kind of hospital visited (region-based), as well as doctors' specialty, in the major divisions of Bangladesh. A recent study looked at the contents of 500 different prescriptions written between 2015 and 2018 and found that proton pump inhibitors (PPI) and multivitamins were common—every one of the chosen prescriptions called for a proton pump inhibitor and a multivitamin.

2.1.3. Statistical and Analytical Considerations

Using Microsoft Office Excel 2016, the data that was gathered from the survey was evaluated, and displayed graphically.

2.2. Study of Drug-Drug Interactions *in vitro* and *in vivo*

2.2.1. Chemicals and Reagents

Samples of the reference standards PNT, VTB1, VTB6, and VTB12 were purchased from Sigma-Aldrich in the United States. Aristopharma Ltd. of Bangladesh was kind enough to present us with several working samples as a present. The commercial dose forms of PNT and VTBs were procured from the many pharmacies located across the city. All other chemicals and reagents were obtained from commercial sources and were of an analytical or reagent grade. These included the following: potassium dihydrogen phosphate (Daejung Chemicals & Metal Co., Korea); dipotassium hydrogen phosphate (Scharlau, Spain); o-phosphoric acid (Merck, Germany); potassium hydroxide (Merck, Germany); HPLC grade acetonitrile (RCI

Labscan, Thailand); HPLC grade water (Evoqua Water Technologies, USA), Distilled water (Evoqua Water Technologies, USA), Acetone (Merck, Germany), and Methanol (Daejung Chemicals and Metals Co.).

2.2.2. Instruments and Other Types of Equipment

2.2.2.1 X-ray Powder Diffraction (XRPD)

X-ray powder diffraction (XRPD) is one of the techniques often employed for the characterization of crystalline solids. Crystalline forms are the focus of this approach. The diffraction pattern of crystalline material is displayed as a series of peaks that are collectively referred to as Bragg's peaks [90]. An XRPD sample is what's known as a "polycrystalline" sample, which means it consists of several microscopic crystallites that are orientated in a random pattern (as on the banner above). Because of this, it cannot be compared to a sample that was, for instance, utilized in monocrystal X-ray diffraction. In general, polycrystalline samples can be found in various forms, including solid form (such as metals or ceramics), as a loose powder, in the shape of a film, or as a liquid suspension **(Figure 2.1)**. It is recommended that the term XRPD be used [91] to examine materials that exhibit a polycrystalline nature. Recording the XRPD patterns of PNT, VTB1, VTB6, and VTB12 samples that emit Cu-K radiation at 30 mA and 40 kV was accomplished with the assistance of a smart lab studio X-ray diffractometer that was manufactured by Rigaku and located in Tokyo, Japan. All Samples were scanned at two angles of short range, ranging from five degrees to thirty-five degrees, with a step size of 0.2 degrees and a scanning speed of four degrees per minute.

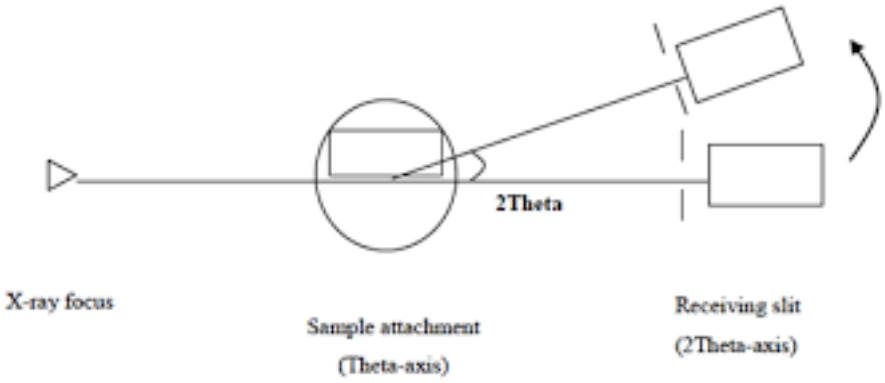


Figure 2.1 A schematic steps of PXRD.

This Rigaku multifunctional X-ray diffraction (XRD) device can probe from 2-theta angles as small as 2 degrees to 90 degrees without limitations. This allows the system to represent many materials, such as powder and granular samples, thin-layer films, and solid membranes. Phase identification and quantitative and qualitative analysis are two of the most popular uses of X-ray diffraction. Other applications include:

- Quantitative and qualitative analysis
- Observe the ratio or percentage of crystal product
- Crystallite size/strain analysis
- Precise powder parameter determination [92].

However, given that we do not yet own the auto sample changer, we can only mount and test a single sample at a time in the apparatus being used. The time it takes to complete a single measurement is determined by the fundamental parameters used for the measures, which include the scan range, scan speed, and step size.

2.2.2.2. Differential Scanning Calorimetry (DSC)

The thermos-analytical method, known as differential scanning calorimetry, or DSC **(Figure 2.2),** detects the change in sample temperature that occurs as a direct result of a change in the temperature applied to the sample. Throughout the experiment, the temperature of the samples is maintained at a stable level. In most cases, the DSC study's temperature program will cause the sample holder's temperature to increase linearly with time. In some situations, it is necessary for the samples to demonstrate a stable capacity for heat absorption throughout the whole temperature range that is of interest. The quantity of heat that is introduced into a sample during a process that involves the use of heat to bring about a physical change, such as a phase transition, is dependent on whether or not the process is endothermic or exothermic. The thermal behavior of 3 mg samples of PNT, VTB1, VTB6, and VTB12 was assessed by heating the samples at a rate of 5°C/min in covered aluminum pans using a DSC (Netzsch, Germany) and a nitrogen gas purge (50 mL/min). The samples were heated in a nitrogen gas purge chamber. As a standard for the calibration of the system, indium, which was 99.999 percent pure and had an onset temperature of 156.6 degrees Celsius, was used.

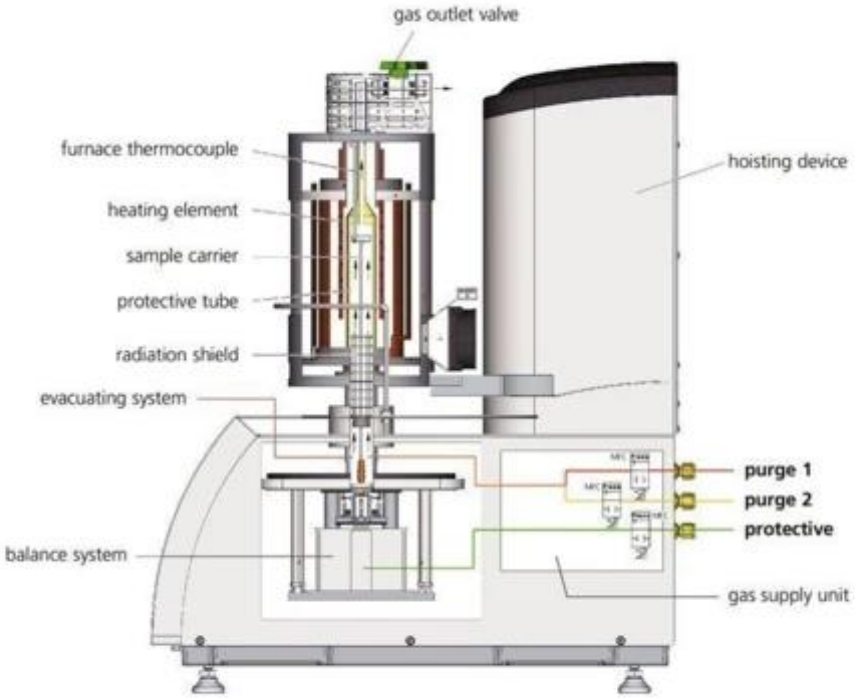


Figure 2.2 Netzsch DSC basic instrumentation

A three-dimensional symmetrical construction with heating distinguishes NETZSCH DSC instruments, and they function in accordance with the idea of heat flow. The operation of these devices is based on the application of this fundamental concept. High detection sensitivity and stable, reproducible baselines over the entire life cycle of a calorimeter are guaranteed by sensors with high calorimetric sensitivity, short time constants, and a condensation-free sample chamber in the calorimeter cell. These are the kinds of qualifications that are suitable for effective use in research and academics, as well as in materials development and quality control [93].

2.2.2.3. Fourier Transform Infrared Spectroscopy (FT-IR)

The term "Fourier-transform infrared spectroscopy," abbreviated "FTIR," refers to a method that may be used to determine the infrared spectrum of absorption or emission of a solid, liquid, or gas. A Fourier transform infrared (FTIR) spectrometer may acquire high-resolution spectral data concurrently over a broad spectral range. A dispersive spectrometer, which measures intensity over a limited range of wavelengths at once, has a substantial disadvantage in comparison to this method, which measures power over a broader range of wavelengths simultaneously. The purpose of the several methods that fall under the category of absorption spectroscopy **(Figure 2.3)** (FTIR, ultraviolet-visible ("UV-vis") spectroscopy, etc.) is to determine how much light a sample absorbs at each specific wavelength. The "dispersive spectroscopy" method is the most straightforward approach to accomplish this goal. It involves directing a beam of monochromatic light at a sample, determining how much of the light is absorbed by the sample, and then repeating this process for each of the other wavelengths [94]. An investigation using Fourier transform infrared spectroscopy (FT-IR) was carried out with the purpose of determining the potential for hydrophobic interactions between the drug and the polymers. Spectrum 10 was used to gather infrared spectra in the range of 4000–600 cm^{-1} from each of the samples after they were individually put on the sample platform of the instrument (a Perkin Elmer L160000A from the United States). During the process of analysis, each sample's baseline was adjusted as necessary and then normalized. A smoothing function consisting of 9 points was used on the collected spectra in order to give them a smooth appearance. *In vitro* testing takes place in a laboratory and typically involves research conducted on clinical trials. This is the most significant aspect. Researchers are able to analyze any possible interactions between proton pump inhibitors and multivitamins by using this technique. The testing that is done in vitro is a basic analytical procedure that is used to confirm the many sorts of interactions that might take place.

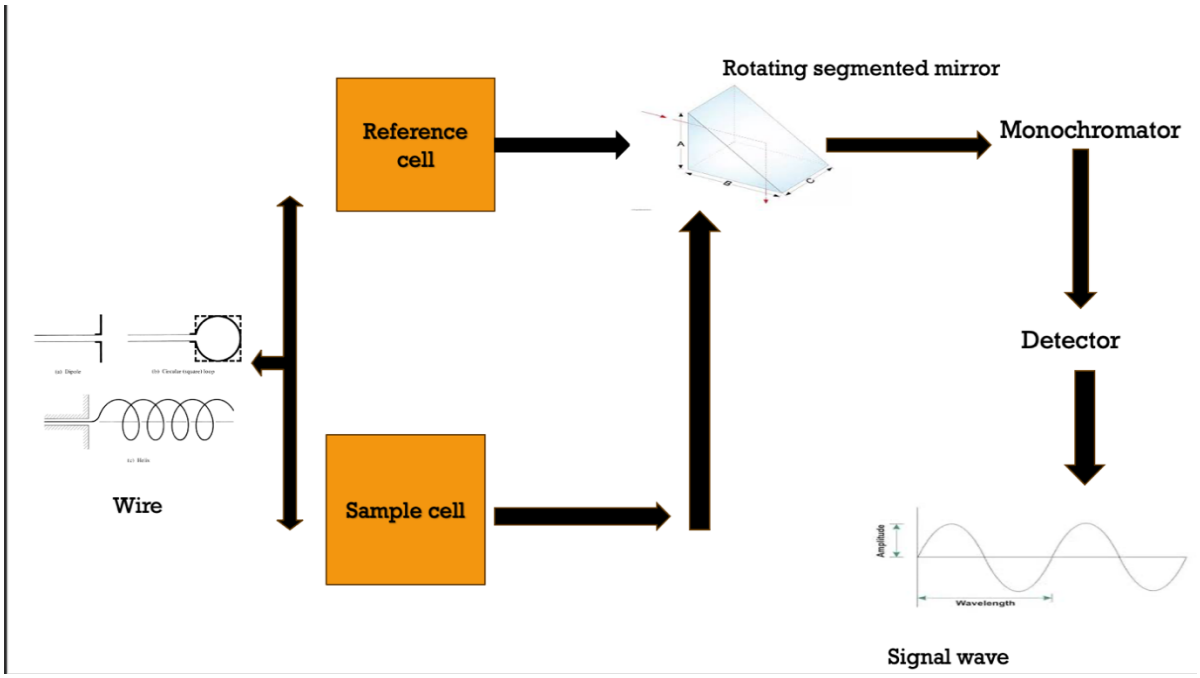


Figure 2.3 Schematic diagram of Perkin-Elmer FTIR spectrophotometer

In the present investigation, infrared spectra of polymer electrolyte films were recorded on Perkin-Elmer FTIR spectrophotometer [Model 1605] in the range of 400-4000 cm-^{1}.

2.2.2.4. High Pressure Liquid Chromatography (HPLC)

The abbreviation "HPLC" stands for "High Performance Liquid Chromatography." The term "chromatography" refers to a process of separation. Chromatographs require a number of critical components, the most important of which are high-performance pumps for delivering solvent at a consistent flow rate and columns, which are devices that have been purpose-built for the separation of molecules.

In point of fact, at this point, research was conducted on the "Basic Overview of the HPLC Process" **(as shown in Fig. 2.4**) and its Mechanisms. A few examples of the components that

go into the construction of an HPLC system are a solvent supply pump, a degassing device, a sample injector, a column oven, a detector, and a data processor. **Figure 2.4** depicts the flow diagram for HPLC as well as the role of each individual component. HPLC is able to differentiate between the components as well as identify them due to the fact that each component goes through the column at a distinct pace. In high-performance liquid chromatography (HPLC), there are two phases: the mobile phase and the stationary phase. The liquid that is used to dissolve the target component is referred to as the mobile phase. The portion of a column known as the stationary phase is the component that is responsible for interacting with the chemical of interest.

If there is a high affinity (for example, a van der Waals force) between the component and the mobile phase, then the component will travel through the column at the same rate as the mobile phase. On the other hand, the item's movement through the column will be slowed down according to the strength of its affinity for the stationary phase.

A "chromatogram" is the name given to the plot that is created by the chromatography method. The chromatogram is a two-dimensional figure with the analysis time represented along the horizontal axis and the concentration, as determined by the detector signal strength, represented along the vertical axis. When a line is drawn on the horizontal axis, when there are no chemicals being eluted from the column, the horizontal axis will be parallel. This is what we mean when we talk about the baseline. The reaction of the detector is determined by the amount of the target molecule that is present in the elution band. The blueprint that was drawn out seems more like a bell than it does a triangle. This shape is referred to as a "peak" in the industry.

The retention time (tR) is defined as the amount of time that elapses between the point at which the sample is injected and the highest point of the peak. The amount of time that must pass

from the injector to the detector before non-retained compounds (compounds that do not interact with the stationary phase) may be detected is referred to as the dead time, or t_0.

The amount of PNT, VTB1, VTB6, and VTB12 was determined with the help of an HPLC system with UV detection. This particular system was a Shimadzu HPLC system (LC-20A VP, Japan), and it consisted of a SCL-10Avp system controller, an LC-20ADvp solvent delivery pump, a DGU-14A degasser, a CTO-20Avp column oven, and an SPD-20Avp UV-vis detector. The analysis was carried out at a wavelength of 280 nm using an ODS reversed-phase C18 column that measured 150 mm by 4.6 mm and had a 5 µ particle size [95,96].

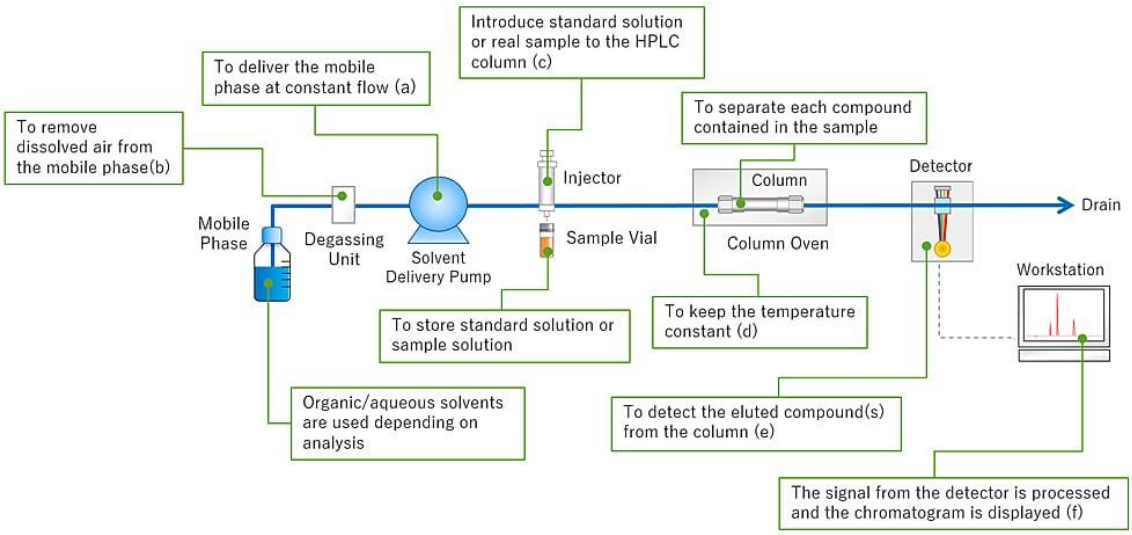


Figure 2.4 Overview of High-performance liquid chromatography (HPLC) mechanism

2.2.2.5. Centrifugal Machine

A centrifuge is a piece of scientific equipment used to separate fluids, gases, or liquids depending on their relative densities. It is possible to separate the material by quickly rotating the container containing it. The centrifugal force causes heavier materials to migrate to the exterior of the container. This apparatus may be found in most labs, whether academic, clinical, or research-based, and it is used to purify cells, subcellular organelles, viruses, proteins, and nucleic acids. In addition, it is widely available. There are many different kinds of centrifuges, and they may be classed according to the rotor's design or the function they are designed to perform. The researcher has access to a wide variety of centrifuges, ranging from gigantic floor models all the way down to little centrifuges. The Eppendorf, known as the removal of liquid solvent for the concentration or desiccation of samples by means of vacuum, centrifugal force, temperature, and gas, can be accomplished using vacuum centrifuges. This apparatus is ideal for the purification or preparation of a wide variety of materials, such as nucleic acids, proteins, peptides, and other compounds, and may be used for a wide range of scientific applications. Most vacuum centrifuges are equipped with their own built-in heating systems, which are used for the evaporation of solvents. This page features a selection of vacuum concentrators and centrifuges for your perusal. We utilized a Japanese-made Mikro 200 centrifuge for this operation, which had a high-capacity drum rotor with 60 positions and an angle rotor with 48 positions. The lower the RCF values, the faster the output. In addition to rotors for conical tubes, blood tubes, pediatric tubes, urine tubes, hematocrit capillaries, a 24-place swing-out rotor for microliter tubes, a 6-place PCR strip rotor, and rotors for other tubes with optional temperature control (-20 to +40 °C), the MIKRO 200 R can cool down to +4 °C in as little as ten to fifteen minutes when using the Fast-Cool function.

2.2.2.6. Vortex

To agitate the contents of individual vials of liquid, laboratories commonly make use of a straightforward device known as a vortex mixer. It is composed of an electric motor that is arranged in a manner that is considerably offset from the center, and the driving shaft is vertically attached to a section of cupped rubber. As the motor rotates, the rubber component performs a quick oscillation pattern in a circular motion. When a test tube or another appropriate container is pushed into it (or touched to its edge), the motion is transmitted to the liquid that is contained within the rubber cup, and a vortex is generated. The vast majority of vortex mixers feature either two or four plates, are capable of being made to operate continuously or only when a specific amount of downward pressure is applied to the rubber piece, and have variable speed settings that range from 100 to 10,000 revolutions per minute (rpm).

2.2.2.7. Ultrasonic bath

Ultra-sonic bath was used to mix well components by creating vibration. For laboratory purpose, Human Lab Instruments Co. (origin Korea) are used for the purpose.

2.2.2.8. Analytical balance

Shimadzu Corporation –TX 323L (origin Japan) was used for analytical balance.

2.2.2.9. Filtration Membrane

For HPLC preparation, the mobile phase must be filtered with 0.45 μm filter paper (Restek, USA).

2.2.2.10. Vacuum Pump

Vacuum pumps are used for clearing unwanted particles from mobile phases, specifically buffer or inorganic salt solutions with machine specification Cole-Parmer, origin UK.

2.3. Method Development

2.3.1. Preparation of samples for use in *in vitro* experiments

Solid state characterization was carried out in the form of X-ray powder diffraction, differential scanning calorimetry, and Fourier transform infrared spectroscopic experiments in order to examine the in vitro interaction between PNT and VTB. In order to adequately characterize the physicochemical condition of the solid, a 1:1 ratio of PNT, VTB1, VTB6, and VTB12 was taken in a mortar and combined with a pestle until it was uniform.

2.3.2. Drug Sample

Commercially available brands of Pantoprazole (PNT), Vitamin B1 (VTB1), Vitamin B6 (VTB6), and Vitamin B12 (VTB12) tablets, each with a label claim 20 mg, 100 mg, 200 mg, and 200ug, respectively, were purchased from the various retail pharmacies of Dhaka city in Bangladesh. Innovator products of these three APIs, Pantonix and Solbion, were used in this study for comparison.

2.3.3. Preparation of Stock and Working Standard Solutions

A stock solution is a concentrated solution (like a developer) that must typically be diluted before use with a particular solvent. Internal standard (IS) (diclofenac sodium at a fixed concentration of 10 µg/mL) and stock solutions of PNT (100 µg/mL), VTB1 (100 µg/mL), VTB6 (100 µg/mL), and VTB12 (100 µg/mL) were produced separately in methanol and kept shaded from light at -20°C. The stock solutions were serially diluted with methanol to create the working solutions.

2.4. Method Validation for HPLC

Evaluation of the method's specificity and selectivity, sensitivity, accuracy, precision, and recovery were all part of the HPLC method's validation process. For the purpose of defining sensitivity, limits of detection (LOD) and limits of quantitation (LOQ) were utilized. LOD and LOQ are abbreviations for "limits of detection" and "limits of quantitation," and they refer to concentrations that must have a signal-to-noise ratio of at least 3 and 10, respectively. The linearity of the data was evaluated using something called the correlation coefficient (r), which was determined through the use of a least-squares regression line. As was noted earlier, in order to test the intra- and inter-day precision and accuracy, three quality control (QC) samples were employed at low, medium, and high concentrations of 10, 20, and 30 µg/mL, respectively. These concentrations represented the low, middle, and high ranges of the assay. The intra-day precision was determined by calculating the % recovery for the analysis of the QC samples in triplicates, and the inter-day precision was estimated by performing the analysis of the QC samples on three separate days. The accuracy was computed and then reported as a relative error percentage after being determined through a comparison of the averaged values to the

nominal values. For the purpose of determining the level of recovery, the peak area ratios of the analytes in the plasma at the QC concentrations were compared to those in the mobile phase at the corresponding concentrations. These ratios were then reported as a percentage. The analytes of interest in the plasma were tested for their stability for short periods of time (up to 24 hours) at room temperature and 4 degrees Celsius, for longer periods of time (up to two weeks) at -80 degrees Celsius, and after three cycles of freezing and thawing.

2.4.1. Chromatographic Separation

The separation of PNT, VTB1, VTB6, and VTB12 was carried out in a linear gradient elution mode (A:B) utilizing HPLC grade water as mobile phase A and acetonitrile as mobile phase B. The column used was a Phenomenex C18 column that measured 150 by 4.6 millimeters and had a particle size of 5µm. The detector used was a Shimadzu UV detector 20A. The following descriptions apply to the gradient conditions of the mobile phase: 0 to 15.00 minutes worth of time. The temperature of the column was maintained at 35 degrees Celsius, and the flow rate was held constant at 0.5 milliliters per minute. As was discussed in the previous section, an internal standard approach was utilized in order to do the examination of the filtrate utilizing a Shimadzu HPLC system. At the same time, an analysis of the PNT, VTB1, VTB6, and VTB12 samples was carried out using the wavelength of 280 nm [95,96]. The internal standard analysis was carried out under identical conditions as before. There was a list of HPLC flow patterns provided in **table 2.1.**

Table 2.1 Flow pattern of HPLC gradient mobile phase

Time	Mobile Phase B% (Acetonitrile)
0-4.00 minute	40
4.01-10.00 minute	98
10.01-15.00 minute	85

2.4.2. Method for the Estimation

Under the most favourable chromatographic conditions, a stable baseline was established. Following the injection of the standard solution in the appropriate aliquots at the appropriate concentration, the chromatogram was recorded until the peak areas were determined to be satisfactory. This process was carried out with the sample solution on a second occasion in order to guarantee that a second injection of the sample solution was followed by an injection of the standard solution. This procedure was repeated a total of six times.

2.4.3. Verification and Authentication of the Procedure

The new technique was validated in accordance with the criteria provided by ICH Q2 (R1) for a number of different characteristics, including accuracy, precision, linearity, robustness, limit of detection (LOD), limit of quantitation (LOQ), and stability [97]. Lower quantities of the standard solutions were injected into the RP-HPLC column while the chromatographic conditions were tuned in accordance with 3.3 s/n and 10 s/n requirements, respectively. In this context, s/n refers to the signal-to-noise ratio. The LOD and LOQ values for PNT, VTB6, and VTB12 were determined.

2.4.4. Accuracy and Precision

The precision of the RP-HPLC technique was evaluated by selecting three distinct doses to test: the lower quantitation limit concentration (LQC), the medium quantitation limit concentration (MQC), and the higher quantitation limit (HQC). Through analysis of both intraday and inter-day data, a mechanism for increasing accuracy was developed. Intraday variations were investigated by injecting the standard and sample solutions three times in rapid succession on the same day. Inter-day variations were investigated by taking the measurements of the medications that were contained inside the multicomponent dosage forms on three separate days. Six injections of the reference solution and the sample solution were performed each and every day [98].

2.4.5. Linearity and Range

In this case, 10 different series of standard solutions were chosen to be used for the linearity range determination. Plotting peak area vs concentration of the standard solution was used to generate the calibration curve, and then the regression equations were determined using that data. In order to determine the linearity range, six replicates of the standard solution were chosen. Plotting the response factor vs. the concentration of the standard solution allowed for the creation of the calibration curve. The slope and intercept were determined by using the calibration curve as a source [99].

2.4.6. Stability and Robustness

The variables that were changed were the mobile phase, the flow rate, the temperature of the column, and the wavelength. Variations were also made. It was possible to determine the capacity factor, the retention period, and the peak heights.

2.5. Pharmacokinetic and Drug-Drug Interaction Studies

2.5.1. Ethical Consideration and Consent Form

Before extracting the necessary information from these patients' prescriptions, we first got the agreement of both the patients and their physicians. They were informed of all of the ethical implications, and they were given guarantees that the confidentiality of their data would be maintained throughout the procedure. Because we did not utilize names, addresses, or any other personally identifying information during the research, we were free from the need for participants to provide written informed permission. In addition to that, the information that was gleaned from the prescriptions was re-identified. In addition, an ethical consent form **(Table 2.2)** from each participant who volunteered their time was gathered as a document.

Study title: Pharmacokinetic Drug interactions of multivitamins and proton-pump inhibitors.

Ethical consent form of volunteers (PhD Registration number: 82/2013-14 and 49/20117-18)

Use tick sign for the left box if agreed:

Certificate of consent

Study title: A study on Pharmacokinetic Drug -Drug interaction in between Proton pump inhibitors and multivitamis.

PhD Registration number: 82/2013-14

Use tick sign for left box if agreed:

I confirm that I have read and understood the information share for the above study and have had an opportunity to ask questions:	
I understand that my participation is voluntary and that I am free to withdraw at any time without having to justify my withdrawal and without my medical care or rights as a patient being affected	
I understand that certain sections of my medical notes may be looked at by certain individuals or authorities for the purpose of this research. I give permission for these individuals to access my medical records.	
I have been given an opportunity to ask questions and my questions have been answered satisfactory.	
I agree to take part in this research.	
I understand that I will be given a copy of the signed consent documents.	

Research Participant Name: ..... Signature : ..... Date and Time:	Researcher Name :..... Signature : ..... Date and Time : .....

Figure 2.5. Ethical consent form of volunteers (PhD Registration number: 82/2013-14 and 49/20117-18)

2.5.2. Study Design for Selected PNT & VTB

This open-label 3X3 crossover experiment was carried out to investigate any conceivable pharmacokinetic interactions that may exist between PNT and VTB. In this crossover experiment, each patient received the same number of PNT and VTB treatments and participated in the same number of periods. In contrast to the previous experiment, subjects for

this inquiry were split up into three groups: Group 1 got the combined dosage of PNT and VTB, Group 2 was given the single-dose pharmacokinetics of VTB, and Group 3 was given the single dose of PNT. After fasting the previous night and again for the next six hours after receiving their doses, these groups underwent pharmacokinetic sampling. Blood samples were taken from all of the different groups so that the results could be analyzed. Following the 10-day washing durations, the same methods were carried out again for each group, using a new alternative. Following the washing intervals of ten days, identical procedures were performed on each group, but this time with a different alternative dosage. The participants in the research spent the entirety of their time at the location where samples were collected. The number of participants was determined using a t-test with a single tail and no paired data [100].

2.5.3. Study Population

The one-tailed unpaired t-test will determine whether there is a statistically significant difference between the means of the two populations. The groups will be assumed to be the same size (n1 = n2) and have the same standard deviations (σ1 = σ2 = σ) to keep things simple. One-two is chosen to represent the mean difference between the two populations. Typically, 0.8 or 80% is given for the power and significance level. Let n = n1 + n2. Finding the ideal total sample size, n is the goal.

$$N \approx \left[\frac{2(Zpower+Z1-\alpha)}{2(\mu1-\mu2)/\alpha}\right]^2$$

where $z_{1-\alpha}$ denotes the 1-α quantile of the standard normal distribution and the value of which may be obtained from statistics tables, where $z_{1-\alpha}$ indicates the standard normal distribution. Simply substituting this equation with α/2 will allow us to calculate the appropriate sample size for the unpaired t-test; otherwise, the technique will remain the same. The standard deviation is denoted by the symbol here.

This unpaired t-test example serves as an example of a frequently used method for calculating sample size. First, the essential parameters, including means and standard deviations, must be estimated, and the threshold of significance must be determined. Following that, the sample size is determined using various test power assumptions. In general, the larger the sample size required for the investigation, the more confident one can be that a significant result will be found. To achieve the required power, the smallest sample size is chosen [101].

2.6. Deproteinization Procedure

Deproteinization is to be the easiest and most effective method for extraction of the analyzed samples[102].

Serum along with spiked drug was vortexed first then mixed for 1-2 minutes, then added deproteinizing agent with different ratios (1:1, 1:4, 1:10). Finally full samples are centrifuged at 10000X g for ten minutes, supernatant was collected and filtered again with 0.25 micron paper, injected onto the column. To clarify the result, apparent recovery was observed where first only peak height of drug with deproteinizing agents and later plasma spiked with 10 ug/ml solution that had been made for method validation. Here we used three replicas for each process.

- **Apparent recovery calculation:** $\frac{\textbf{peak height of drug with deprotenized plasma}}{\textbf{peak height of drug with deprotenized agent}}$ **X100**

2.6.1. Volunteers

Thirty healthy young adults, 18 to 35 years of age (inclusive) with a body weight average of 40-75 kg were included in the study. Among them, 22 were male and 08 were female volunteers. Individuals with previous disease history (diabetes, hypertension, genetic disorder, pregnancy, psychological disorder etc.) were excluded.

a) Criteria for participation

1. Volunteers in good health
2. Have reached the age of twenty or older
3. Participants in the research consisted of both males and females.
4. The research population was used to determine the demographic characteristics of the controls, including gender, age, and socioeconomic status.

b) The criterion for exclusion

1. a lack of cooperation
2. A serious problem with the body's overall health
3. Children younger than 18 years old
5. Must be less than sixty years of age

Table 2.3 contains information on the individuals' demographics as well as their baseline characteristics. There were 10 participants in each group, for a total of 30 among the three groups. They were given a balanced meal plan in order to ensure that they were maintaining optimal bodily function prior to the sample. In group 1, the individuals received a combined

dosage of 20 milligrams of PNT as well as 100 milligrams of VTB1, 200 milligrams of VTB6, and 200 micrograms of VTB12. Subjects in group 2 were only given VTB dosages consisting of 100 mg of VTB1, 200 mg of VTB6, and 200 g mg of VTB12. Participants in group 3 got a total of only 20 mg of PNT.

Table 2.2 Demographic and baseline characteristics of study participants

		Group 1	Group 2	Group 3
Age (years)		23.00 ± 2.66	22.63 ± 3.61	21.66 ± 0.96
Weight (kg)		64.90 ± 9.51	65.63 ± 12.11	66.50 ± 14.02
Height (cm)		165.20 ± 9.29	165.18 ± 11.35	166.30 ± 1.07
BMI, (kg/m2)		23.64 ± 1.62	23.67 ± 2.56	23.78 ± 2.95
Gender	Male	8	6	8
	Female	3	3	2

2.7. Declaration of Ethical Principles

The laws of the United States Food and Drug Administration, the Declaration of Helsinki in its most recent updated form, and the guidelines established by the International Conference on Harmonization were all adhered to in order to carry out this study [103,104]. The research protocol and the informed consent form were reviewed by the ethical committee of the University of Dhaka's Faculty of Biological Science, and both were given the green light (approved number: 111). All of the individuals participated in the study only after signing a written permission form, and they were free to drop out at any point during the process.

2.7.1. Concerns of an Ethical Nature

When selecting volunteers, it will always be vital to ask questions and have a conversation about some delicate problems that deal to family and personal affairs. Nevertheless, while working on the thesis, every effort would be taken to preserve ethical norms and prevent any physical, psychological, social, or spiritual harm to the participants. This would be done so that the thesis could be successfully defended. Several considerations on the ethical issues are as follows:

1. No human organs or foetal tissues were used in this research project in any way.
2. measures were made to ensure that the privacy of every individual was preserved.
3. The participant or relevant relatives were provided with complete information on the purpose of the study as well as its limitations.
4. A verbal or written consent was obtained from the subject and/or from the subject's key relatives if the subject was unable to supply accurate information (and a consent form was provided for this purpose).
5. The participants who were not interested in taking part in the study were not considered for inclusion.
6. Measures were made to protect the privacy of individuals' personal information and to ensure that they remain anonymous.
7. The customer and the respondents were not solicited to participate financially in the study in any way.
8. The fundamental human rights to refuse or accept anything were not violated.
9. During the course of the experiment, the participant in the study did not get any further medicine.

10. During the course of this study, Precautions were taken not to produce any environmental hazards or breaches.

2.8. Safety Parameters

Throughout the entire trial, the safety and tolerability of PNT and VTB were assessed by looking at adverse events, clinical laboratory results, vital sign assessments, skin evaluations, and concurrent drug use.

2.9. Collection of Plasma Sample

When collecting plasma samples from volunteers, there are often a number of essential criteria and protocols that must be adhered to protect both the donors and the quality of the samples that are taken. There is a possibility that particular procedures will change based on the organization or institution that is in charge of the collection; nonetheless, the following are some basic requirements that are often adhered to:

1. In order to guarantee that volunteers are suitable candidates for plasma donation, some eligibility requirements must be met. Age limits (usually ranging from 20 to 55 years old), overall excellent health, and the absence of specific medical disorders or drugs that may be considered as potentially disqualified.

2. Before being allowed to donate plasma, volunteers are required to give their informed permission. They should be provided with unambiguous information related on the types drug that they might insert the body and details procedure of the collection, and the possible dangers and benefits, they should be given the chance to inquire and get their questions answered about the procedure.

3. Examination of the volunteer's health was performed prior to the drug intake. In some instances, it may also be necessary to do a physical examination, which would include taking measurements of the patient's vital signs. The blood of volunteers was tested for a variety of variables, including blood type, infectious illnesses (such as HIV, hepatitis B and C).

4. Before donating plasma, volunteers were asked to abstain from food and drink for a certain amount of time, however this will vary depending on the goal of the collection. This is often done either to get particular components of plasma or to reduce the amount of interference caused by recent meal intake.

5. Adequate hydration was suggested before collecting to ensure that they were well-hydrated, which can expedite the donation process and lessen the likelihood of any harmful effects. Hydration comes in the form of drinking water or sports drinks.

6. The actual collection of plasma was normally carried out in chemical biology and DNA research lab, which ensured the safety of the volunteer and the plasma.

It is essential to keep in mind that these requirements may be different based on the particular regulations that are adhered to by the company that is in charge of the plasma collection.
Samples were collected intravenously with specific time interval and transferred into a EDTA tube which precipitated blood cells randomly. After that all collected samples were centrifuged and transferred supernatant with discarding red cells. Finally new EDTA plasma samples were stored with label until further investigation started.

Blood collection apparatus:

1. Disposable syringe
2. Centrifugal tube for collecting samples
3. Cotton pad
4. Alcohol pad
5. Banded tape for stopping blood
6. Pipette for withdrawal of blood
7. Refrigerator (-80 ^{o}C) for sample blood preservation

2.9.1. Plasma Sample Preparation

Before processing, plasma samples that had been kept at -80 degrees Celsius were allowed to thaw at room temperature and then were vortexed for thirty seconds. An aliquot of plasma measuring 100 μL was placed into a fresh Eppendorf tube measuring 1.5 mL, and then 10 μL of IS (internal standard) was added after it. After giving the samples a 30-second spin in a vortex, an excess of acetonitrile was added to them in order to deproteinize them. The samples were centrifuged at 4 °C for ten minutes at a rpm of 10000. After being separated and transferred to a new tube, the supernatant was stored in the refrigerator in preparation for analysis.

2.10. Determination of Pharmacokinetic Parameters

The pharmacokinetic parameters were calculated by means of noncompartmental methods using the PKSolver (a freely available menu-driven add-in program for Microsoft Excel) [105].

2.11. Statistical Analysis

The data are all shown as the mean along with the standard deviation (SD). Graphpad Software, located in LaJolla, California, was utilized to produce the graphs using their Prism 8.0 program. In order to make statistical comparisons, a one-way analysis of variance was performed with pairwise comparisons while employing Fisher's method for finding the least significant difference. In each of the studies, significance was determined to be achieved when the p-value was less than 0.05.

CHAPTER 3

RESULTS AND DISCUSSION

3. RESULTS AND DISCUSSION

3.1. Assessment of prescriptions

Prescription pattern in Bangladesh is based on types of diseases and patient criteria. In the present study, 500 prescriptions (male = 369, female = 131) were used under the survey, whereas 200 prescriptions showed that patients related to intake of both Proton Pump Inhibitors and multivitamin drugs in 40.00% usage. Among the prescriptions, **(Table 3.1),** only PPI with or without multivitamins was 75.20%, multivitamin with or without PPI was 57.60%, and combined PPI and multivitamins were 40.00%, respectively.

Table 3 .1 Existence of PPIs and multivitamins in prescriptions survey

Types of Drugs	Number of Prescriptions	Percentage of Total Prescription
PPIs	376	75.20%
Multivitamins	288	57.60%
Both PPI and Multivitamin	200	40.00%

According to this survey, in **Table 3.1**, we found that 200 prescriptions in which both PPI and multivitamins were prescribed.

From **Table 3.2**, OMP, ESOM, PNT, RABE & LAN are given in a percentage of 27.4%, 4.60%, 11.40%, 9.6%, and 2.40%, respectively. From this survey, it was assumed that physician had still chosen OMP most of the cases of gastritis problems. Considering 200 prescriptions, Multivitamin with minerals combinations used most frequently. Here, in

combination with PPI, percentage of multivitamins was 27.40%, Minerals 10.60% and the combination of B1, B2 & B12 was 2.00%. Multivitamins are widely used as a dietary supplement. Analyzing prescription, multivitamins may be acceptable in all ranges and areas. There was also an analysis on what area physicians are prescribed PPI and multivitamins. According to this **(Table 3.4),** the medicine department prescribed PPI and multivitamins most, at 44.52%, whereas ENT had a percentage of 02.91%. Surprisingly, the gastro-liver department had a percentage of 14.59%, lower than that of the medicine department. The Gynecology Department had a percentage rate of 13.86%.

Table 3.2 Percentage of individual Proton-pump Inhibitors and others in the prescription

Generic Name	**Percentage (%) of Generic PPI used**
Omeprazole	27.40
Esomeprazole	4.60
Rabeprazole	11.40
Pantoprazole	9.60
Lansoprazole	2.40

The prescription survey was only based on PPI and multivitamins given simultaneously. **Table 3.3** describes only physicians of different arenas who prescribe both PPIs. The percentages indicate the number of persons out of a total population of 500 who use particular generic proton pump inhibitors (PPIs) as a regular component of their prescription regimen. An explanation of what these percentages signify may be as follows:

Omeprazole is the PPI drug that around 27.40% of the overall group, or approximately 137 people, had taken. This complete group consists of 500 persons. The PPI medicine esomeprazole was taken by around 23 persons, which corresponds to roughly 4.60% of the total group size of 500 individuals. Rabeprazole was the PPI drug that accounted for about 11.40%, or approximately 57 persons, of the group's total population of 500 individuals. Approximately 48 patients were prescribed pantoprazole as their PPI prescription. This represents 9.60% of the group. Lansoprazole was present in 2.40% of the group or about 12 individuals. PPIs are frequently administered to patients in order to help heal acid-related diseases, including GERD, peptic ulcers, and other stomach ailments. Acid production in the stomach is what PPIs aim to reduce. Factors such as the patient's present health state, response to medication, and the advice of their healthcare provider may all play a role in determining which PPI will be most effective.

Table 3.3 Combination of intake Proton-pump Inhibitors

Generic Name	**Percentage (%) of Multivatimins were used**
Multivitamin	27.40
Minerals only	10.60
Combination B1, B6 & B12	2.00

According to **Table 3.3**, out of a total population of 500 people, the proportion of persons who take particular kinds of supplements is indicated. An interpretation of the following data is as follows: There are around 137 persons who had taken a multivitamin supplement out of a total population of 500 people. This accounts for 27.40% of the entire population. A multivitamin

is a type of dietary supplement that often includes a number of different vitamins and minerals in a single dosage form.

10.60% of the individuals, or around 53 persons, utilized simply a mineral supplement. Mineral supplements often did not contain any additional vitamins but contained vital minerals such as calcium, iron, zinc, and magnesium, amongst others. 2.0% of all persons

2.00% of the overall population, used dietary supplements that include all three of the vitamins B1, B6, and B12 in combination. Because of the synergistic effects that they have and the potential advantages that they offer for energy generation, nerve function, and brain health, these B vitamins are frequently combined as dietary supplements.

The statistics, taken as a whole, show the percentage of persons within the population who make the conscious decision to consume these particular kinds of dietary supplements. It is essential to keep in mind that the data that has been presented is fictitious and that the actual usage patterns may differ in populations that actually exist.

Table 3.4 Prescription of Proton pump inhibitors drug and Multivitamin in various department

Department	Percentage of prescription of PPI and Multivitamin
Medicine	44.52%
Gynecology	13.86%
ENT	02.91%
Dermatology	03.64%
Gastro-Liver	14.59%
Orthopedics	10.94%
Geriatrics	09.48%

Based on **Table 3.4**, below is the proportion of PPI and multivitamin prescriptions in different departments from a 500-prescription survey:

In the Medicine section, 44.52% (223 prescriptions) comprised PPI and multivitamin. Medical professionals may prescribe PPIs to treat GERD, peptic ulcers, and stomach acid production. Multivitamins may be advised for dietary deficits or general wellness. PPI and multivitamin are prescribed in 13.86% of gynecology prescriptions (around 69). Patients with acid reflux or gastrointestinal issues may be offered PPIs. Multivitamins may improve general health, reproductive health, or women's frequent nutritional shortages. PPI and multivitamin are prescribed in 2.91% of ENT prescriptions. ENT specialists suggested PPIs for laryngopharyngeal reflux (LPR) or acid reflux in the throat and voice box. Multivitamins may boost immunity and health. In dermatology, 3.64% of prescriptions (around 18) include PPI and multivitamin. In the Gastro-Liver section, 14.59% (73 prescriptions) include PPI and

multivitamin. The Gastro-Liver section treats liver and gastrointestinal problems. PPIs are often recommended for GERD, gastric ulcers, and gastrointestinal bleeding prevention. GI and liver problems may require multivitamins to prevent nutritional deficits. In orthopedics, 10.94% (55 prescriptions) contain PPI and multivitamin. In the geriatrics category, 9.48% (47 prescriptions) include PPI and multivitamin. These percentages show how many PPI and multivitamin prescriptions each department writes. PPI and multivitamin doses may be recommended many times depending on patient needs, medical conditions, and departmental healthcare providers. Note that the data is hypothetical and may not reflect real-world prescription trends. Healthcare experts should also consider an individual's medical history, existing medicines, and treatment plan before deciding whether to take several doses.

Table 3.5 Prescription containing proton pump inhibitors of different companies

Name of companies providing PPI	**Percentages of drug used**	**Name of companies providing PPI**	**Percentages of drug used**
Incepta Pharmaceutical Ltd.	15.95	Labid Pharma Limited	05.01
Square Pharmaceutical Ltd	12.76	IBN SINA pharmaceuticals	05.01
Eskayef Bangladesh Ltd	08.51	Healthcare Pharmaceutical	05.01
Beximco Pharmaceuticals Limited	08.45	Opsonin Pharma Limited	04.52
Aristopharma ltd	06.38	Acme Laboratories Ltd.	04.23
Popular Pharmceuticals Ltd.	06.66	Radiant Pharmaceutical Ltd.	03.98
Orion Pharma	05.05	Others	03.05

Prescription analysis for multivitamin had contained almost some common pharma companies like PPIs'. **In Table 3.5**, displayed the proportions of medication utilization across various pharmaceutical businesses that offer Proton Pump Inhibitors (PPIs). The following is a comprehensive depiction of the table: Incepta Pharmaceutical Ltd. had a medicine use rate of 15.95%. It plays a substantial role in the use of proton pump inhibitors (PPIs). Labid Pharma Limited represented a significant proportion of medication utilization, accounting for 5.01% of the overall drug consumption. Square Pharmaceutical Ltd was a company that has a medication consumption percentage of 12.76%. This company holds a major position in the realm of PPI consumption. IBN SINA Pharmaceuticals, a prominent pharmaceutical company, made a significant contribution of 5.01% to the overall use of drugs. Eskayef Bangladesh Ltd, a pharmaceutical company, exhibited a medicine use rate of 8.51%. The healthcare pharmaceutical represented a proportion of 5.01% in terms of medication utilization. Beximco Pharmaceuticals Limited had a medicine use rate of 8.45%. Opsonin Pharma Limited made a significant contribution of 4.52% to the total use of pharmaceutical products. Aristopharma Ltd, a pharmaceutical company, had a medicine use rate of 6.38%. Acme Laboratories Ltd represented a significant proportion, namely 4.23%, of the overall medication utilization. Popular Pharmaceuticals Ltd: The medicine usage percentage of Popular Pharmaceuticals Ltd was 6.66%. Radiant Pharmaceutical Ltd was responsible for a significant proportion, specifically 3.98%, of the total medication utilization. Orion Pharma had a medication use rate of 5.05%. Other firms, who did not expressly identified, make up 3.05% of the medication consumption in this category.

It should be noted that the table presented data pertaining to the proportions of medicine utilization among various pharmaceutical businesses that offer Proton Pump Inhibitors (PPIs). The overall drug used by individuals is not explicitly specified. In order to ascertain the aggregate medication use among individuals, other data, such as the total populace or the

cumulative count of prescriptions dispensed for PPIs or proton pump inhibitors, would be required.

Table 3.6 Multivitamins prescribed by physicians

Name of companies	Percentages of Multivitamin drug used	Name of companies	Percentages of Multivitamin drug used
Square Pharmaceutical Ltd	30.20	Reneta Limited	11.08
Beximco Pharmaceuticals Ltd.	16.66	Popular Pharmaceuticals Ltd.	09.54
Incepta Pharmaceutical Ltd.	15.97	Eskayef Bangladesh Ltd.	06.54
Opsonin Pharma Limited	12.84	General Pharmaceuticals Ltd.	05.42
Aristopharma Limited	11.11	Others	03.28

Table 3.6 indicated the proportions of multivitamin pharmaceutical utilization across various industries. The revised depiction of the table is as follows: Square Pharmaceutical Ltd hold the greatest proportion of multivitamin medicine utilization, accounting for 30.20% of the total. Multivitamin ingestion was significantly influenced by this factor. Renata Limited represented 11.08% of the overall consumption of multivitamin drugs. Beximco Pharmaceuticals Ltd was reported to have a medicine use rate of 16.66% for multivitamins. Popular Pharmaceuticals Ltd was responsible for a significant proportion, specifically 9.54%, of the total utilization of multivitamin drugs. Incepta Pharmaceutical Ltd: The medicine use rate for multivitamins by

Incepta Pharmaceutical Ltd demontrated at 15.97%. Eskayef Bangladesh Ltd was responsible for a significant proportion, specifically 6.54%, of the use of multivitamin drugs in the market. Opsonin Pharma Limited had a medicine use rate of 12.84% for multivitamins.

General Pharmaceuticals Ltd was responsible for a significant proportion of the entire utilization of multivitamin drugs, specifically accounting for 5.42% of the total consumption. Aristopharma Limited, a pharmaceutical company, exhibited a medicine use rate of 11.11% specifically for multivitamins. The "Others" group include firms that are not specifically mentioned, constituting around 3.28% of the overall consumption of multivitamin drugs. It was important to acknowledge that the table presents data pertaining to the proportions of multivitamin medicine utilization across various firms. The overall drug use by individuals was not explicitly stated.

In Table 3.7, there was a chart of different ages having both PPI and multivitamins. Age range of the prescription survey was calculated for a ten-years gap. The percentage of the combined drug consumption was found to be 9.12% for the 15-24 age group. The highest percentage (27.16%) was found for the age group 35-44.

Table 3.7 Distribution of Proton pump inhibitors and Multivitamin drug on different age group of patients.

Age	Percentage according to age of intaking both PPI and multivitamins
15-24	09.01 %
25-34	20.00 %
35-44	27.16 %
45-54	26.02 %
55-64	10.00%
65-74	06.44%
75-84	02.12%

Here, the distribution pattern of different ages is listed in **Figure 3.1** where average age of 35 to 54 years were taking most of the combinations. According to this survey, for obtaining better pharmacological data age distributions were selected in a range of 24-54 years.

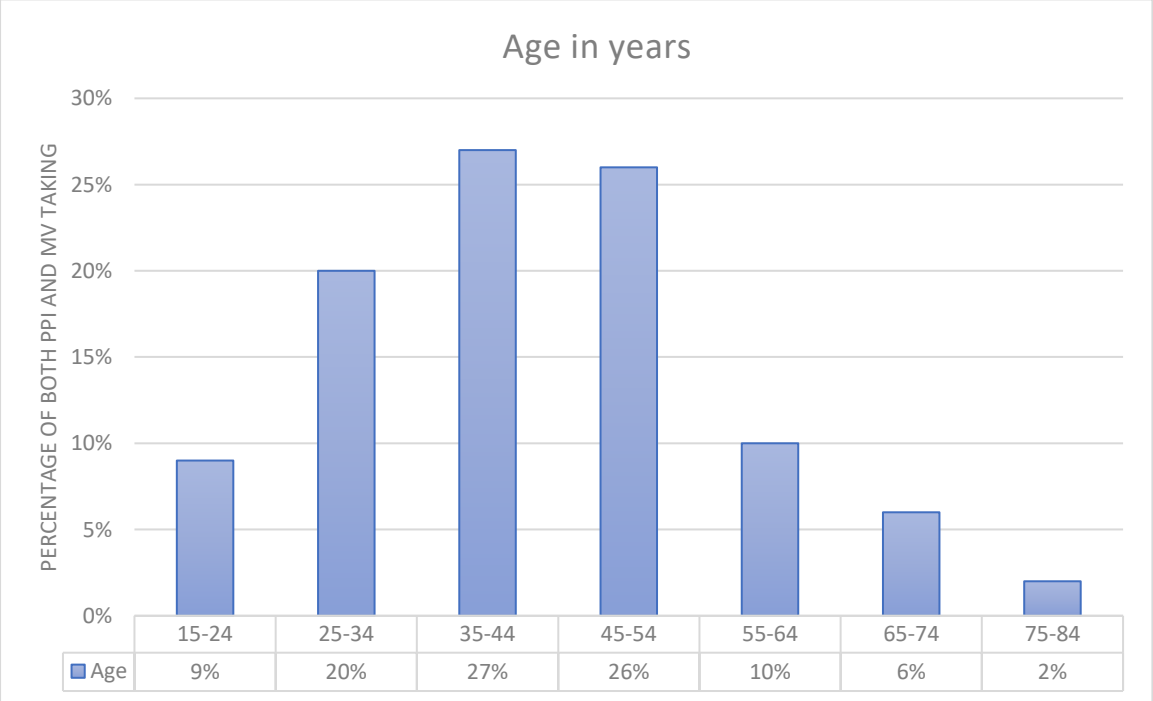


Figure 3.1 Percentage of Prescription Containing PPI and Multivitamins

3.1.1. Reason for Observing Interactions of PNT and VTB

In this survey, the number of patients was 500 where the maximum patient prescribed of omeprazole and Esomeprazole but in case of Gynaecology department, the uses of Pantoprazole are most. Besides recent survey mentioned that uses of Rabeprazole is increasing in Geriatric patient most. Pantoprazole is safe and well-tolerated when used as recommended and under medical supervision. It is used to treat GERD, peptic ulcers, and other stomach and esophageal diseases. This interaction between pantoprazole and vitamin B12 is likely due to the inhibitory impact of PPIs on B12 absorption. Vitamin B12 is digested and absorbed in the stomach and upper small intestine. The absorption process involves stomach acid and intrinsic factor. Vitamin B12 is absorbed through intrinsic factor. PPIs can lower stomach acid, which can impair Vitamin B12 solubility and intrinsic factor binding, reducing absorption. This may cause Vitamin B12 insufficiency. Vitamin B12 is needed for red blood cell creation, neuron function, and DNA synthesis. Vitamin B12 deficiency can cause tiredness, anaemia, neuropathy, and other health concerns. Thus, long-term PPI users, including pantoprazole, should check Vitamin B12 levels. If a deficit is suspected, doctors may prescribe Vitamin B12 supplements or other treatments to resolve the pantoprazole-Vitamin B12 absorption interaction. PPIs and Vitamin B12 may interact differently in different people and to different degrees. Individual susceptibility, PPI dose and duration, and Vitamin B12 consumption might affect absorption.

3.2. Physicochemical Interaction of the Solid Samples of PNT, VTB1, VTB6, and VTB12

3.2.1. Solid State Characterization

To clarify any transitions in chemical structures of PNT and VTB, solid state physicochemical characterization of physical mixture (PM) of the samples were characterized using XRPD and DSC studies (**Figure 3.2**). From the results, it indicated that PNT exhibited as crystalline state with several intense peaks in XRPD analysis, whereas when mixture with VTBs, negligible changes were observed in intensities indicating absence of significant interactions. In DSC analysis, no shift in endothermic peak of PNT when mixed with VTBs indicating negligible transitions on melting endotherm of PNT as well as VTBs. Results from XRPD and DSC analyses revealed that particles in PNT and VTB samples were still in a stable condition, with little evidence of recrystallization or phase transition.

3.2.1.1. Drug- Drug Interaction Using PXRD

In powder X-ray diffraction (PXRD), a sample of powder is irradiated with an X-ray beam, and the scattered X-rays are then detected and studied. In PXRD analysis, the angle of scattering is a crucial parameter that is commonly calculated in terms of 2θ, which stands for "two theta or 2θ." The angle of 2θ depicts the angle that exists between the X-ray beam that is incident on the sample and the detector, with the sample situated in the middle. The scattering angle is related to the interplanar spacing of crystal lattice planes inside the sample, and the 2θ angle is used to calculate the connection described by Bragg's equation, which connects the scattering angle to the interplanar spacing. In accordance with Bragg's rule, when X-rays

contact with the lattice of a crystal, the resulting interaction will be one of constructive interference if the following condition is satisfied:

$2d \sin(\theta) = n\lambda$

Where d is the separation distance between the lattice planes, is the scattering angle (half of 2θ), n is the diffraction order (1, 2, 3, etc.), and is the X-ray's wavelength.

A diffraction pattern may be created by adjusting the angle of incidence of the dispersed X-rays, also known as the 2θ angle, and measuring the strength of the X-ray scattering at a variety of angles. The diffraction pattern consists of a sequence of peaks that correspond to the constructive interference of X-rays from distinct lattice planes inside the crystal. These peaks may be seen when the crystal is subjected to x-ray diffraction. In the 2θ scan, the locations and intensities of the diffraction peaks offer extremely useful information on the crystal structure of the material. By applying Bragg's law, one is able to determine the interplanar spacing (d-values) based on the locations of the peaks. The intensities of the peaks give information on the crystallinity of the sample as well as the relative abundances of the various phases that are present in the specimen. **Figure 3.2** displays the results of an investigation into the X-ray powder diffractograms (XRPD) produced by various powder mixes including PNT, VTB1, VTB6, and VTB12. In contrast to the PNT and VTB1 diffractograms, as PNT had no potential peaks at 2 degree, but prominent peaks of VTB1 and PNT-VTB1 mixtures were observed in the diffractogram at 14.30, 16.50; 18.24; 20.20; 24.60; 26.50; 28.60; and 14.00; 16.50; 22.40; 26.00; 28.00; and 30.00 respectively at 2θ. Similar pattern can be observed in the diffractogram of the VTB6 and PNT-VTB6 mixture having peak 16.00; 22.00; 25.00; 35.00; and 12.00; 20.00 respectively per 2θ, although the peak intensities were somewhat lower. Another observation including PNT, VTB12, and a blend of PNT and VTB12 revealed that there was no possible peak at every 2θ. Based on the findings, it is safe to believe that neither the relative peak intensities nor the crystal sizes were considerably affected by the experiment. According to the

published research, any changes in the PXRD patterns, such as relative intensities and crystallite size, revealed not only a change in the crystal shape but also provided evidence of a polymorphic transition that caused a drug-drug interaction. The peaks that match the pattern, the purity of which is 95%, are represented by the diffractogram that is shown below **3.2**. As was the case with the previous sample, the analysis that corresponded to the powder formula did not find any evidence of a change in the crystalline arrangement of the previous sample, nor did it find any evidence of a change in the excipients that were under investigation, as can be seen in **Figure 3.2**. It would appear, on the basis of the information that has been presented, that the examination of the PNT, VTB1, VTB6, and VTB12 by means of X-ray diffraction (XRD) did not indicate any changes in comparison to what has been established in the literature.

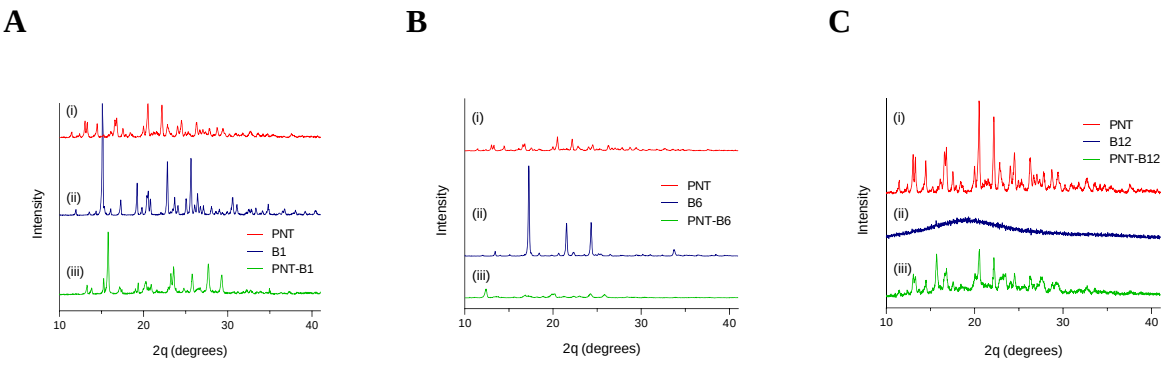


Figure 3.2 Diagram of PXRD for A- i. PNT, ii. VTB1, iii. Combination of PNT-VTB1, B- i. PNT, ii. VTB6, iii. Combination of PNT-VTB6, C- i. PNT, ii. VTB12, iii. Combination of PNT-VTB12.

3.2.1.2. Drug-Drug Interaction Using DSC

Thermo-analytical equipment like differential scanning calorimetry allows us to track variations in the amount of heat needed to bring the samples up to the desired temperature. Throughout the experiment, the temperature of all the samples was kept quite consistent. Pantoprazole has a melting temperature of around 150°C, while vitamins B1 and B6 melt at around 160°C and B12 at around 230°. DSC was used to examine the thermal stability of pharmaceuticals and pharmaceutical mixtures.

The heat flow that is related to variations in temperature in a material may be measured via DSC analysis. It assists in the identification of various thermal phenomena like melting, decomposition, and the loss of water. In the case of PNT, the thermogram reveals an endothermic event at 220 degrees Celsius, which coincides with the substance's melting point. Following the melting process, there occurs a breakdown of PNT, which is indicated by a breakdown of PNT at a temperature of above 210 ºC. The temperature range in which these thermal events take place is quite restricted. After doing a DSC study on the PNT, VTB1 was observed at 200ºC showed an endothermic reaction, and also showed the perfect melting point of pure compound. But in the case of observing the combination, there were no potential changes. Since the melting point of the VTB1 was quite near to that of the material in its purest form, that suggests the mixture did not have a substantial impact on the temperature at which the VTB1 melts. The thermograms of the samples did not reveal any new exothermic peaks, which indicates that there are not any unanticipated reactions or contaminants present.

The differential scanning calorimetry (DSC) study of the VTB6, taken as a whole, revealed a consistent pattern with values of 200ºC. It went through a certain exothermic process in terms of enthalpy (heat content). With the combination of the PNT-VTB6 mixture, no potential exo- or endothermic action was observed. In **Figure 3.3**, the potential degradation of VTB12 was

above 300ºC, and besides, the mixture of PNT-VTB12 was not monitored with any degradation situations.

In conclusion, the differential scanning calorimetry (DSC) study offers useful insights into the thermal behavior of the substances that were investigated. These insights assist in understanding the substances' melting points, heat absorbed or released, and other in vitro interactions. Here, any potent changes of drugs were indicating the possibilities of drug-drug interactions. DSC was monitored by endothermic situation vs temperature changes.

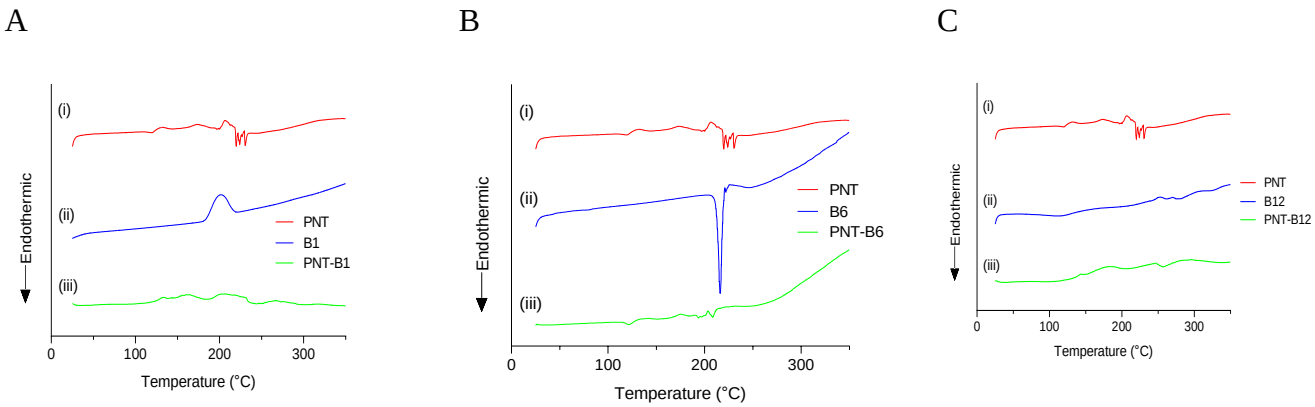


Figure 3.3 Solid state characterization of physically mixed samples using DSC. (I) PNT-VTB1, (II) PNT-VTB6, and (III) PNT-VTB12.

3.2.1.3. Drug-Drug Interaction Using FT-IR

FT-IR analysis was also performed to evaluate the molecular status of PNT, VTBs and PMs.

A B C

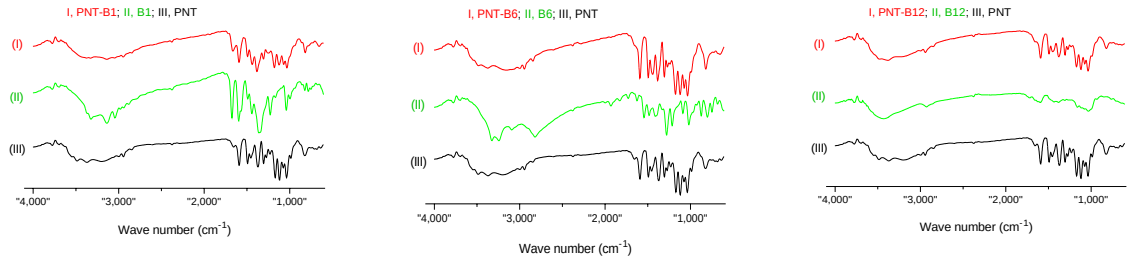


Figure 3.4 Baseline-corrected and normalized FT-IR spectrum. AI-PNT-VTB1, A-II-VTB1; A-III- PNT; B-I, PNT-VTB6, B-II VTB6, B-III-PNT; and C-I-PNT-VTB12, C-II-VTB12, C-III-PNT.

Figure 3.4 displayed the comparable FT-IR spectra and **Table 3.8**, shows FT-IR analysis data of all samples. In **table 3.8**, the N-H stretching vibration bond is found at PNT: 3485.37 cm^{-1}, VTB1: 3321.42 cm^{-1}, and VTB6: 3323.35 cm^{-1}. The peak positions may vary slightly depending on the specific chemical environment of the N-H group, although VTB12 and PNT-VTB12 mixture was not visualize N-H bond. In O-H (Oxygen-Hydrogen) Peaks, VTB1: 3136.25 cm^{-1}, VTB6: 3776.62 cm^{-1}, VTB12: 3136.36 cm^{-1}, and all three types of mixtures exhibited same bond indicating the stretching vibration of the O-H bond. There was no O-H peak listed for PNT, for this visual data, no new group was formed for observing in vitro interaction. Besides, C-H bond was very important because that can indicate there would be an aromatic or cyclic ring in structure. Here, PNT: 3196.05 cm^{-1}, VTB1: 2881.65 cm^{-1}, VTB6: 3091.89 cm^{-1}, VTB12: 2926.01 cm^{-1} and also all mixtures contained-H bond which represent the stretching vibration of C-H bonds in the molecules. These positions of the peaks can vary depending on the specific types of C-H bonds present (e.g., aromatic, aliphatic). For C=O, PNT: 1589.34 cm^{-1}, VTB6: 1543.05 cm^{-1}, VTB12: 1593.20 cm^{-1}, and all pattern of mixtures except VTB1 had carbonyl group. These peaks correspond to the stretching vibration of the

C=O bond in carbonyl groups. The peak positions may slightly differ depending on the specific chemical environment of the carbonyl group. For C-N Peaks, PNT: 1371.39 cm^{-1}, VTB1: 1359.82 cm^{-1}, VTB12: 1159.22 cm^{-1} and mixtures except VTB6 represent the stretching vibration of the C-N bond in the molecules. The peak positions can vary depending on the nature of the carbon and nitrogen atoms involved. Same as, S=O Peaks: VTB1: 1035.77 cm^{-1}, VTB6: 1037.70 cm^{-1}, PNT: 1031.92 cm^{-1} including mixing samples monitored the stretching vibration of the S=O bond in the molecules. It's important to note that the interpretation of FT-IR data requires additional information about the specific compounds being analysed, their chemical structures, and the sample preparation. The provided explanation gives a general understanding of the vibrational modes associated with the observed peaks.

In contrast to FT-IR spectral analysis, the results revealed that the addition of VTBs might not alter the chemical structure of PNT because the spectra curves were same. The aforementioned findings showed that medication interactions were barely detectable in vitro. This is beneficial theoretically because drug-drug interactions can slow dissolution in some cases, and when there are few or none, the dissolving rate is driven more by thermodynamics [106,107].

Table 3.8 Some important peaks observed in FT-IR spectra of PNT, VTB1, VTB6, and VTB12 and their composite with their possible assignment.

Peak assignment	Peak position (cm^{-1})						
	PNT	VTB1	VTB6	VTB12	PM of PNT and VTB1	PM of PNT and VTB6	PM of PNT and VTB12
N-H	3485.37	3321.42	3323.35		3373.50	3373.50	
O-H		3136.25		3776.62 3431.36	3136.25	3155.54	3772.76
N-H	3369.64	3041.74	3242.34			2943.37	3379.29
C-H	3196.05	2881.65	3091.89	2926.01	2945.30	2845.00	2941.44
C=O	1589.34		1543.05	1593.20	1591.27	1589.34	1591.27
C-N	1371.39	1359.82		1159.22	1381.03	1382.96	1377.17
C-O	1118.71		1276.88	1029.99	1118.51	1118.71	
S=O	1035.77	1037.70			1031.92	1031.92	1033.85

3.3. Method validation and simultaneous quantification of PNT, VTB1, VTB6 and VTB 12

Although several different chromatographic techniques have been established for the in vitro and in vivo determination of PNT, VTB1, VTB6, and VTB12, no simultaneous approach has yet been discovered. As a result, we established an RP-HPLC technique for their plasma measurement and confirmed its accuracy. The following are the settings that were tuned to achieve great sensitivity and selectivity.

3.3.1. Optimization of Separation Conditions

A number of different reversed-phase columns and a number of different gradient mobile phases were put through a series of tests in order to find the chromatographic conditions that should be used for the separation of the target analytes in a mixture. This was done in order to determine the optimal chromatographic conditions that should be used for the separation of the target analytes. The conclusion reached as a consequence of these testing was that the best possible circumstance should be chosen. **Table 3.9** displays the analytical parameters that need to be determined in order to evaluate how reliable the HPLC method is for performing simultaneous measurements of PNT and VTBs. In order to obtain an appropriate run time, symmetric peak shape, and high resolution, reversed phase column C18 columns were employed. This was necessary due to the hydrophilic nature of both PNT and VTBs. We were able to acquire a decent resolution of both drugs by using a Phenomenex C18 column that had a particle size of 5 microns and measured 4.6 millimeters by 150 millimeters. This type of column was often utilized for the separation of PNT and VTB. Isocratic elution was at first utilized to separate the analytes; however, this approach had a limited ability to separate the target analytes concurrently. As a result, gradient elution was chosen in order to obtain the maximum possible amount of separation while still keeping a high level of resolution. In order to separate PNT, VTB1, VTB6, and VTB12, a Phenomenex C18 column with dimensions of 150 millimeters by 4.6 millimeters and a particle size of 5um (Shimadzu, UV detector 20A) was utilized in the separation process. The elution mode that was utilized was a linear gradient, and the mobile phases that were utilized were HPLC grade water (A) and acetonitrile (B). The linear gradient was employed for the elution. The conditions of the gradient for the mobile phase are as follows: 85% B for 0–15:00 minutes. Both the column's temperature, which was

maintained at 35 ºC, and its flow rate, which was maintained at 0.5 mL/min, were kept constant. The PNT analysis, along with the VTB1, VTB6, and VTB12 sample analyses, were all done simultaneously using the internal standard technique with a wavelength of 280 nm. The evaluation of the internal standard was carried out using the exact identical conditions, including the wavelength (280 nm), and the operations that were being performed.

3.3.2. Validation of the Method

The current technique was validated by referring to the United States Guidance of Industry on Bioanalytical Technique validation and the criteria that were given in the section on Experimental Procedures 2.4. A simple straight-line equation was used to establish a linear connection under ideal experimental conditions, and the relationship was shown to be linear. The calibration curve was constructed by plotting the peak area against the concentration. PNT, VTB1, VTB6, and VTB12 all exhibited linear behavior across the concentration range of 1–100 g mL^{-1}. Selectivity, linearity, and the LOQs are all important considerations. This approach was assessed by analyzing blank plasma, blank plasma that had been spiked with the analytes, and plasma that had been collected at 0.5, 5.1, 2, 3, 5, and 6 hours after the combination had been administered.

3.3.3 Linearity and Range

Ten different concentration series were utilized for the purpose of determining the linearity range. A graph of the calibration curve utilizing the area of the standard solution as a function of its concentration may be found in **Figure 3.5.** The slope and intercept were computed by

using the calibration curve as their source. The linearity range provided the source for the data, which was Y= 18907x+99796, R^2= 0.98, Y=24681x + 300000, R^2 = 0.97 and Y= 15735x + 90661, R^2=0.97 for PNT, VTB6 and VTB12 respectively.

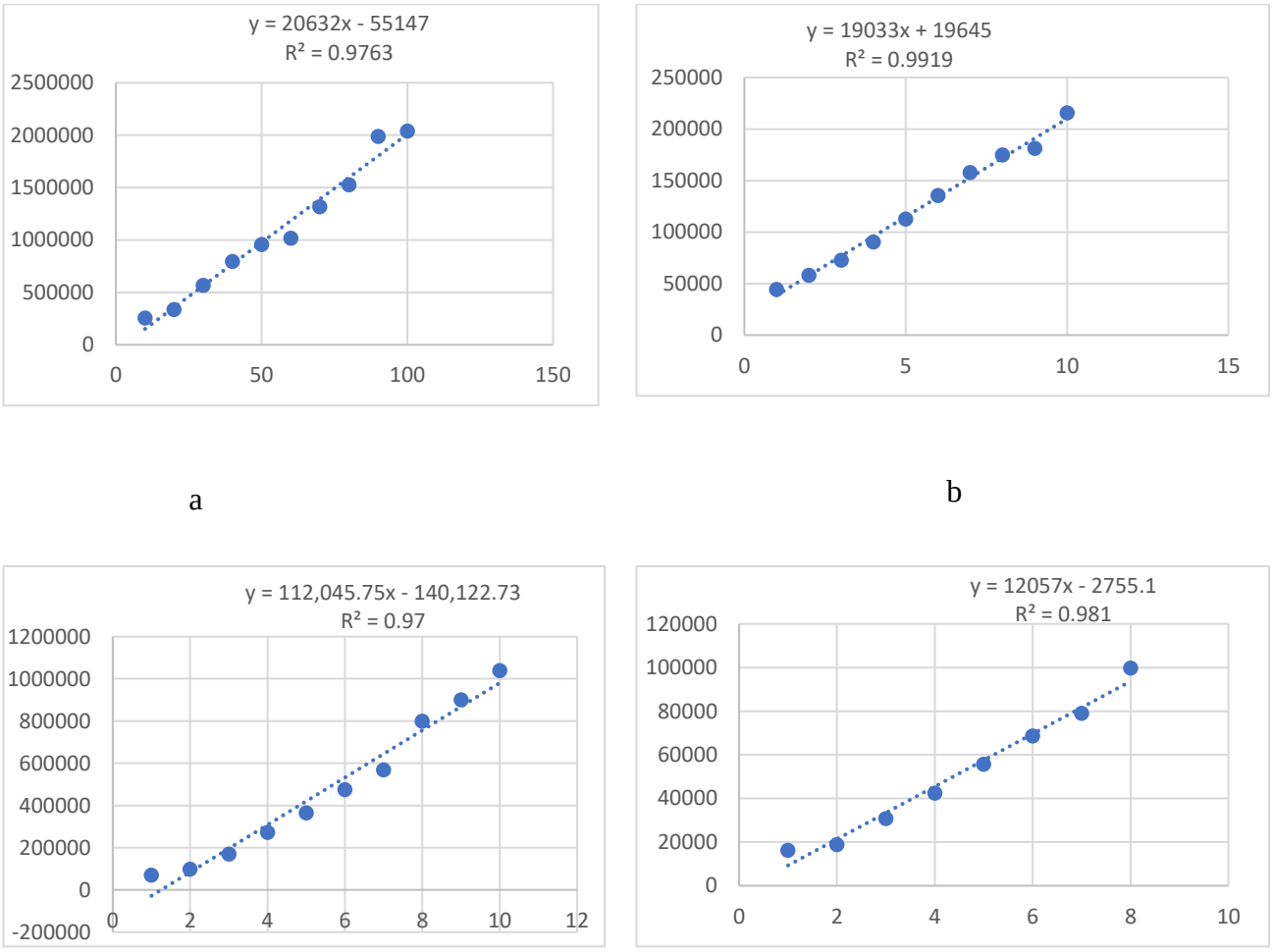


Figure 3.5 Standard calibration curve of a) PNT, b) VTB1, c) VTB6, d) VTB12.

3.3.4. Limit of Detection (LOD) and Limit of Quantification (LOQ)

In order to estimate the LOD and LOQ of the compounds, gradually decreasing concentrations of the standard solutions were injected into the HPLC column under the optimum

chromatographic conditions. This allowed for the determination of LOD and LOQ. The LOD values that were determined to be appropriate were as follows: 0.5 ng/mL for PNT; 0.59 ng/mL for VTB1; 15.34 ng/mL for VTB6; and 0.04 ng/mL for VTB12. Lower limits of quantification (LOQs) values were determined to be 1.50 ng/ml for PNT, 1.78 ng/ml for VTB1, 69.34 ng/ml for VTB6, and 0.10 ng/ml for VTB12, respectively.

3.3.5. Robustness

In order to assess the resilience, we made several minor adjustments to the optimal settings, such as changing the flow rate, the pH of the mobile phase, the temperature of the column, the wavelength, and the amount of organic solvent. Three separate injections of the mixed standard solution were performed, after which the % RSD of the test was determined for each of the conditions. As a flow rate of 0.5 mL/min, the findings that were obtained **(Table 3.9)** showed that the retention time was ideal along with the potency, and they also had beautiful resolution. In addition to using a variety of columns, we were able to successfully isolate each analyte by utilizing the Phenomenex C18 (150 X 4.6 nm) column. In this case, temperature does not affect the retention duration; thus, 35 degrees Celsius was used for the study. Wavelength was selected at 280 nm for perfect separation with mobile phase A composed of water (HPLC grade).

Table 3.9 Analytical parameters for system suitability test of HPLC method

Parameter	PNT	VTB1	VTB6	VTB12
Retention time (min)	6.8 ± 0.2	2.7 ± 0.4	4.5 ± 0.5	3.8 ± 0.1
Assay (%)	104.13 ± 2.30	102.99 ± 2.56	114.36 ± 1.94	90.01 ± 1.56
Peak height	3,539 ± 175.68	7,308 ± 956.72	51,846 ± 1,749.48	3,993.33 ± 633.33
No of theoretical plates	547.66 ± 72.23	1,214 ± 61.02	4,360 ± 1,480.81	1,058 ± 249.52
USP Tailing Factor	0.79 ± 0.06	1.47 ± 0.27	0.81 ± 0.3	1.81 ± 0.07
Capacity factor	2.5 ± 1.18	0.71 ± 0.07	1.48 ± 0.50	0.79 ± 0.21
LOD (ng/mL)	0.50	0.59	15.34	0.04
LOQ (ng/mL)	1.50	1.78	69.34	0.10

System suitability test indicates suitable validated method for pharmacological analysis. Here, Peak height for PNT, VTB1, VTB6, and VTB12 was monitored 3,539 ± 175.68, 7,308 ± 956.72, 51,846 ± 1,749.48, 3,993.33 ± 633.33.

3.3.6. Precision

This method's precision, accuracy, recovery, and absolute matrix effect were analyzed and compared in **Table 3.10**. Both the intra-day and the inter-day precision and accuracy were represented as a percentage of the potency, and the relative standard deviation (RSD) did not surpass 2.3%. The computed recoveries and absolute matrix effect values were within the bounds for their respective categories. All of the data pointed to the fact that the assay was accurate and repeatable for the purpose of determining PNT, VTB6, and VTB12 levels in human plasma.

Table 3.10 Analytical parameters for the robustness of the HPLC method

Parameters	Variables	PNT		VTB1		VTB6		VTB12	
		RT	% Recovery	RT	% Recovery	RT	% Recovery	RT	% Recovery
Flow rate (mL/min)	0.3	13.2	83.2 ± 5.34	5.1	113.25 ± 2.87	9.2	112.8 ± 1.98	6.5	68.05 ± 2.67
	0.5	6.6	123.70 ± 8.87	2.5	83.45 ± 3.25	4.5	98.73 ± 2.30	4.3	107.44 ± 1.34
Mobile Phase	Acetonitrile	7.5	123.70 ± 8.87	3.5	83.45 ± 3.25	3.5	98.73 ± 2.30	4.3	107.44 ± 1.34
	Methanol	ND	ND	ND	ND	ND	ND	ND	ND
% Mobile Phase	50	ND	ND	ND	ND	ND	ND	4.6	434.34 ± 8.76
	85	6.7	123.70 ± 8.87	3.2	83.45 ± 3.25	5.5	98.73 ± 2.30	4.3	107.44 ± 1.34
Column (µm)	250X4.6	9.2	102.22 ± 3.43	3.2	77.45 ± 2.39	ND	ND	ND	ND
	150X4.6	7.5	123.70 ± 8.87	3.5	83.45 ± 3.25	5.2	98.73 ± 2.30	4.2	107.44 ± 1.34
Wavelength (nm)	270	ND	ND	ND	ND	ND	ND	4.2	65.44 ± 4.90
	280	7.6	123.70 ± 8.87	3.2	83.45 ± 3.25	4.5	124.36 ± 2.30	4.5	107.44 ± 1.34
	305	ND	ND	ND	ND	ND	ND	ND	ND
	505	ND	ND	ND	ND	ND	ND	4.4	125.67 ± 7.32
Column Tem. (°C)	30	6.5	123.70 ± 8.87	3.2	98.37 ± 2.49	6.1	112.23 ± 5.65	4.2	107.44 ± 1.34
	35	7.5	123.70 ± 8.87	3.4	83.45 ± 3.25	5.5	98.73 ± 2.30	4.1	107.44 ± 1.34

ND, not detected; RT, retention time; Data represent the mean ± SD of experiments.

3.3.7. Point of Specificity

Figure 3.6-3.10 illustrates the specificity of the present technique of analysis by HPLC; the complete and clear separation of PNT, VTB6, and VTB12 was observed without any interference in retention time. This was observed even though there was no interference in retention time.

3.3.8. Accuracy & Recovery

Figures 3.6 through 3.12 display chromatograms of these samples that are representative of the norm. When using an HPLC equipped with a UV detector, the detection of PNT, VTB1, VTB6, VTB12, and IS was extremely selective and did not suffer from any interference from the endogenous compounds or from any of the other components. Within a runtime of 15.00 minutes, the retention times for PNT, VTB1, VTB6, and VTB12 were 6.8 ± 0.2, 2.7 ± 0.4, 4.5 ± 0.5, and 3.8 ± 0.1 min, respectively, in a runtime of 15.00 min. With correlation coefficient (r) values that were more than 0.96, each of the calibration curves demonstrated strong linearity within the limits that were set for them. To estimate LOD and LOQ, smaller quantities of the standard solutions were injected into the HPLC column under the optimum chromatographic conditions **(Table 3.9).**

It evaluated this method's precision, accuracy, recovery, and absolute matrix effect, and the results are listed in Table 12. The intra and inter assay bias precision and accuracy were both given as standard deviation (SD) and relative error (RE), respectively, and were within a range of 15%. The intra assay bias did not exceed 5%, and the inter assay bias did

not exceed 5%. The recoveries that were estimated and the total matrix effect values were found to be in the range of 85.0-115%. The determination of PNT, VTB1, VTB6, and VTB12 in human plasma was shown to be repeatable and reliable by all of the findings, which showed that the assay was accurate.

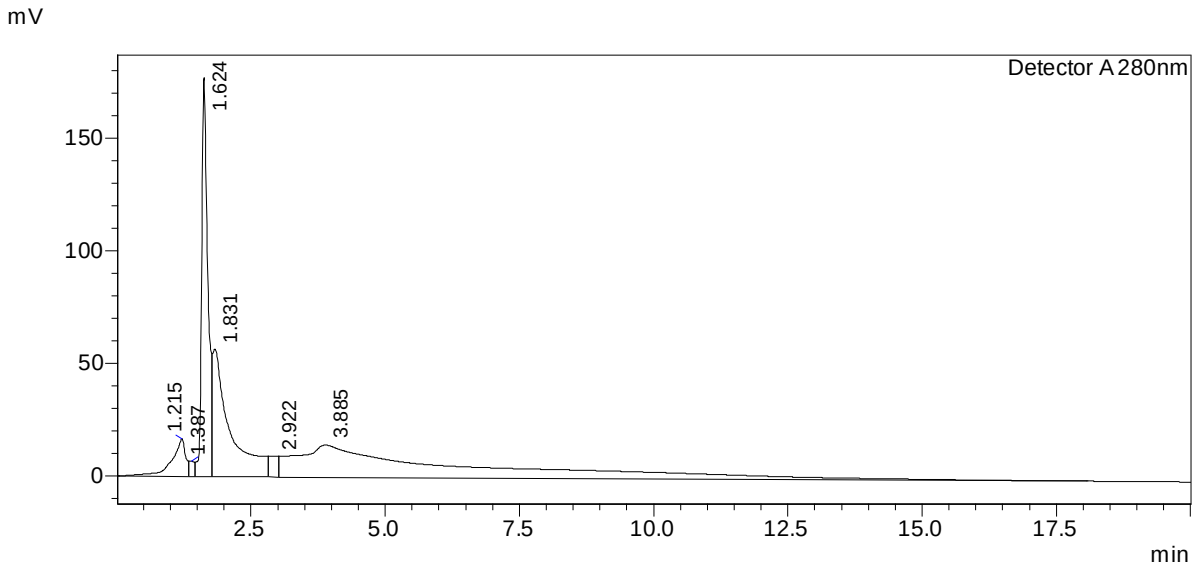


Figure 3.6 Chromatogram of Blank Plasma

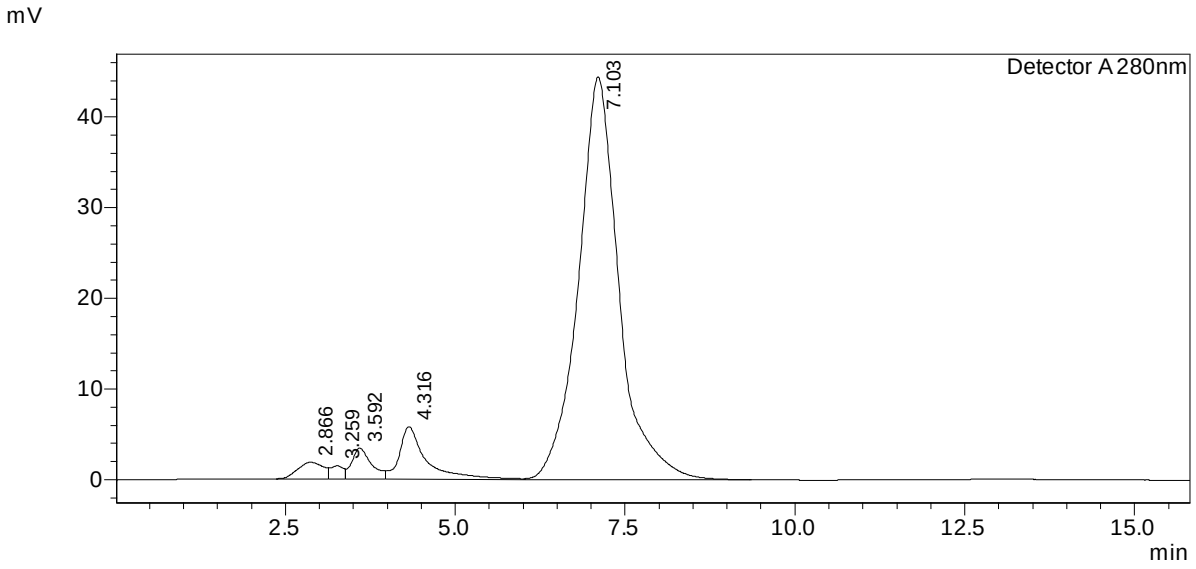


Figure 3.7 Chromatogram Pantoprazole in plasma (RT = 7.103)

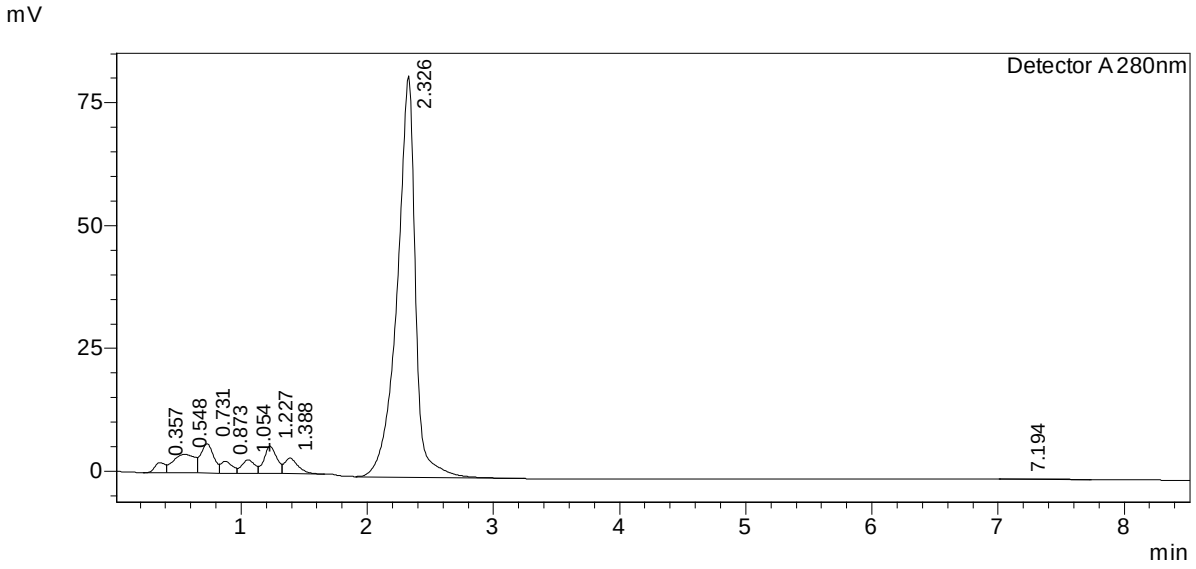


Figure 3.8 Chromatogram of VTB1 in plasma (RT=2.33)

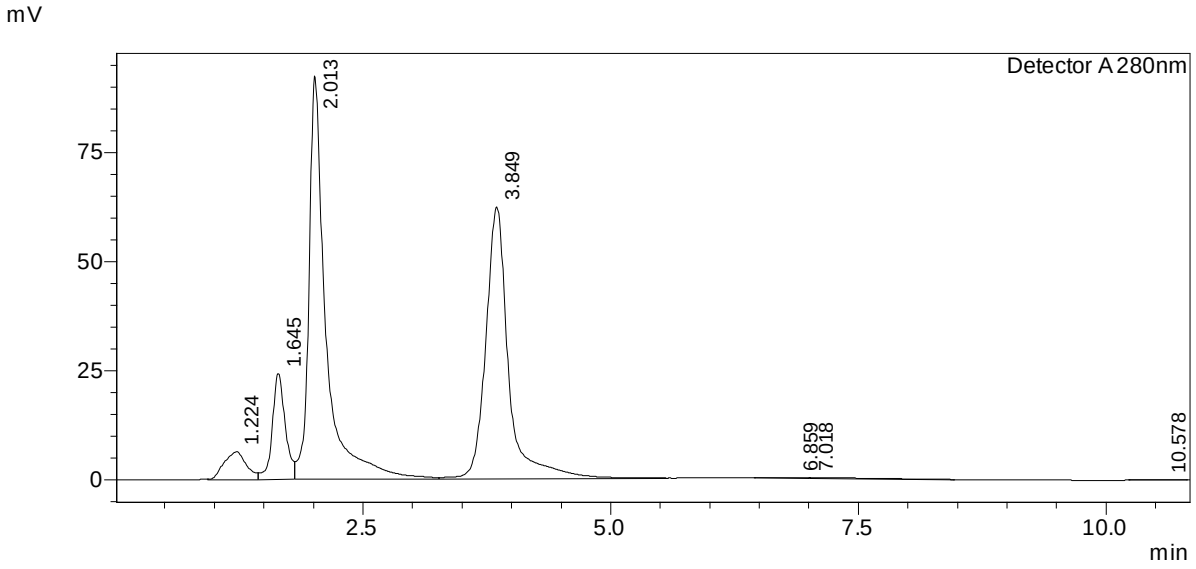


Figure 3.9 Chromatogram of VTB12 in plasma (RT= 3.8)

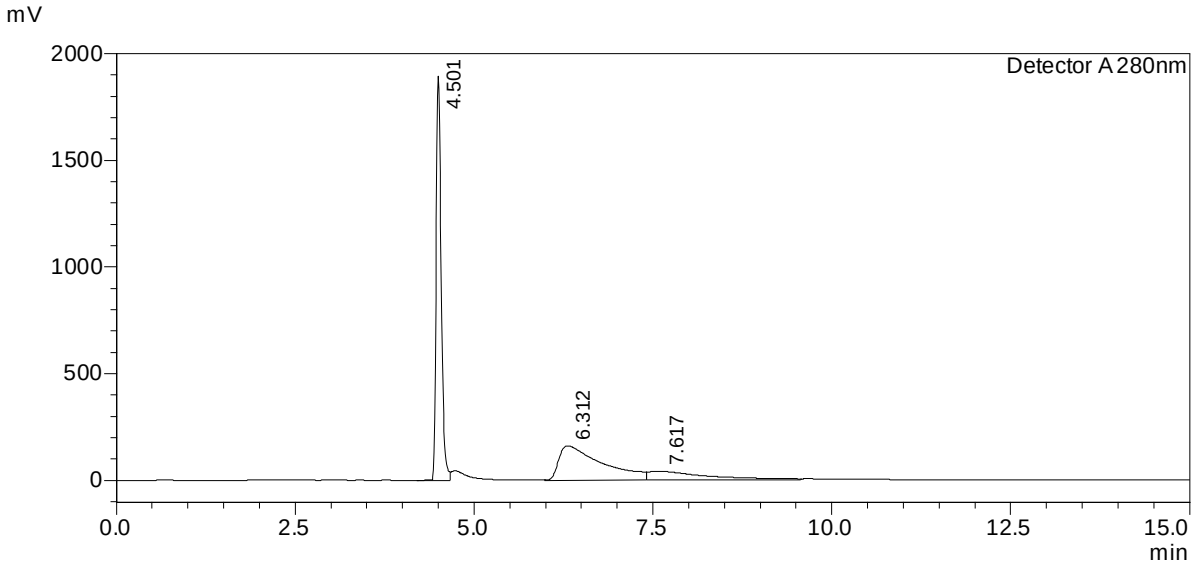


Figure 3.10 Individual chromatogram of VTB6 in plasma (RT=4.5)

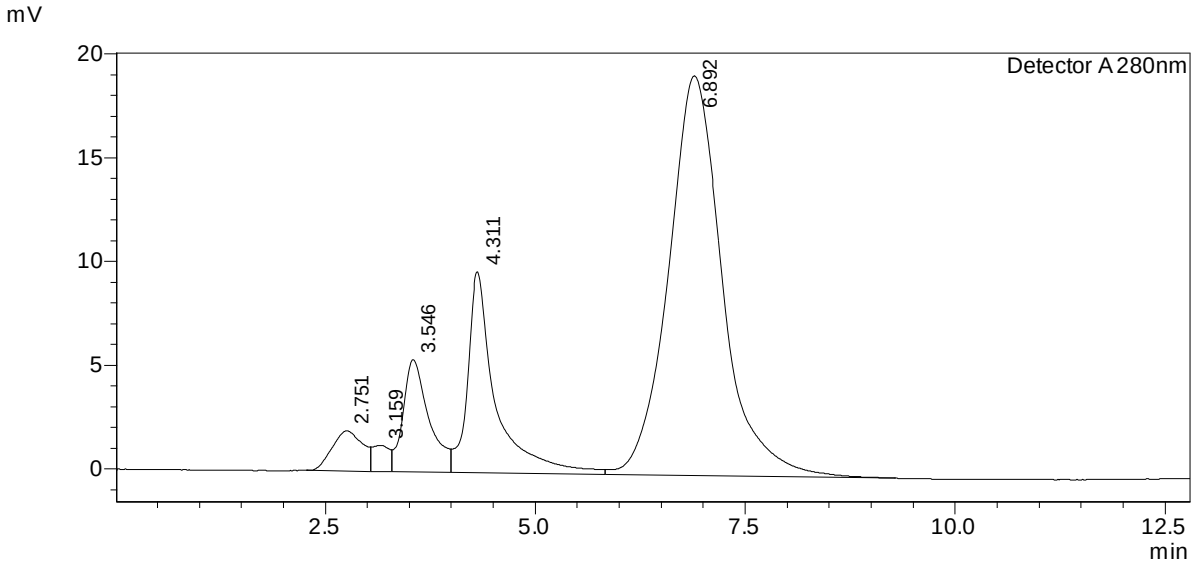


Figure 3.11 Chromatogram of VTB1 (RT- 2.7), VTB12 (RT-3.6), VTB6 (RT- 4.3) and PNT (RT - 6.9) in plasma deproteinization

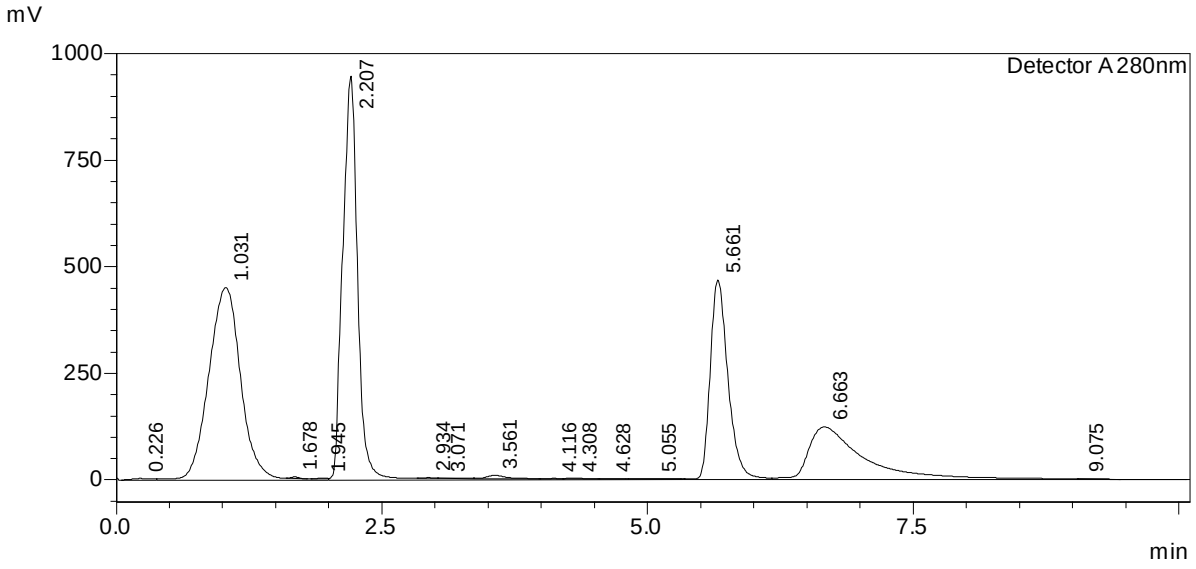


Figure 3.12 Chromatogram of VTB1, VTB6 and PNT after oral administration (without VTB12)

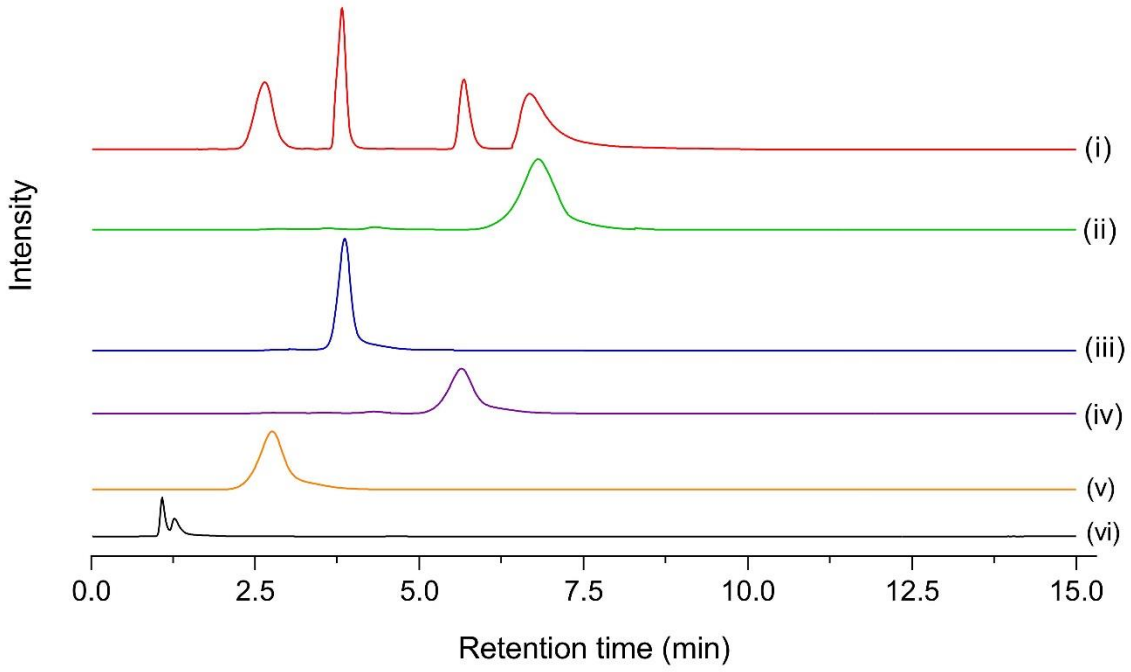


Figure 3.13 Comparison of typical RP-HPLC chromatograms plasma. (vi) Blank Plasma; (ii) PNT; (iii) VTB12; (iv) VTB6; (v) VTB1; and (i) representative chromatogram of PNT, VTB1, VTB6, and VTB12 in spiked human plasma.

Table 3.11 Intra-day and inter-day accuracy, precision, and recovery matrix effects for PNT, VTB1, VTB6, and VTB12 determination in plasma

Sample	Spiked analyte (µg/mL)	Intra day				Inter day			
		Mean ± S.D	CV%	Accuracy (RE %)	Recovery (%)	Mean ± S.D	CV%	Accuracy (RE %)	Recovery (%)
PNT	10	13.07±0.98	7.53	-14.78	114.70	13.12±1.34	10.22	-31.22	113.83
	20	19.65±0.72	3.62	3.67	98.45	17.93±3.17	17.72	10.23	89.65
	30	27.36±1.44	5.26	8.77	91.20	28.34±0.99	3.49	5.53	94.56
VTB1	10	10.89±1.48	13.69	-8.92	108.90	9.53±0.78	8.24	4.73	95.30
	20	17.37±0.78	4.50	-2.45	86.85	15.51±3.45	22.21	22.43	77.55
	30	25.73±1.56	6.07	14.20	85.76	26.16±1.96	7.38	11.28	87.20
VTB6	10	10.23±1.34	2.33	-2.33	102.30	11.27±0.23	2.34	3.45	112.70
	20	19.23±.024	1.44	6.22	96.15	18.54±0.34	6.34	2.34	92.70
	30	29.34±0.03	2.44	8.22	97.8	26.97±4.76	18.54	7.23	89.90
VTB12	10	7.31±0.50	6.84	26.80	73.15	8.28±0.27	3.36	17.12	82.8
	20	21.23±3.56	6.09	-14.18	106.15	26.19±7.10	27.12	-30.95	87.3
	30	30.45±3.58	11.77	-1.57	101.50	38.08±3.16	9.38	-30.24	129.33

Data represent the mean ± SD of 3 experiments.

3.4. Deproteinization of plasma sample

The removal of proteins and other compounds that behave similarly to proteins is the goal of the process known as deproteinization [108]. When there is protein activity as well as activity from other enzymes, it might be challenging to examine the minute molecules that are present in biological samples. Before doing bioassays on samples, it is usually necessary to first eliminate any proteins present in the samples. In the instance of TCA, which is a derivative of weak acetic acid, it cannot hydrolyze the peptide bonds; nonetheless, it has an acidic behavior when dissolved in water. When TCA solution is added to protein molecules, this disrupts the hydrogen bonds that are present between the surrounding water molecules and the protein. Following that, these protein molecules do not continue to exist in a soluble state.

In contrast, when TCA disrupts the hydrogen bonds between protein molecules, the proteins not only lose their current structure but also become denatured [109]. Zinc sulfate, one of the inorganic components commonly utilized, can potentially remove up to 85 percent of plasma proteins [110]. For the purpose of analyzing various medicines and compounds in plasma, acetonitrile is frequently utilized as a deproteinization agent. According to the findings published by Romitelli et al. [111], treating the bioanalytical sample with acetonitrile is the most effective approach for precipitating proteins in the Griess test. In spite of this, Sakuma et al. [112] have questioned the evaporation of acetonitrile, which results in a loss of volume and potentially contributes to a more significant recovery when determining the amount of uric acid in serum.

According to **Table 3.12, the** ACN 1:10 ratio gave highest percentage of recovery, 99.01±4.37 compared with 1:1 and 1:4 ratios of deproteinization. Unlike PNT, VTB1, VTB6, and VTB12 had highest recovery of 109.23±2.45 and 109.76±0.34, respectively at a 1:4 ratio. With TCA, PNT, VTB6 and VTB12 they observed highest recovery at 1:10 ratio, including 105.83±0.24,

92.28±1.39 and 98.99±3.76, respectively. As zinc sulfate is an inorganic substance, but it showed a potential recovery procedure having a 1:4 ratio of PNT and VTB6 101.98±3.23, 112.34±4.34 and 123.15±7.83 respectively, VTB12 with 1:10 ratio, observed highest recovery of 123.15±7.83.

From this study, only methanol may be less effective than others for accuracy recovery, so a combination of TCA and zinc-sulphate was observed. Others have the minimal level to regain the sample perfectly. Among them, the variety of ACN, TCA, and zinc sulfate combination have the maximum (almost above 100%) recovery at 1:4 and 1:10 ratios. Basically, deproteinization is an easy and rapid method for obtaining results more sharply, but those who having trace amount of sample to determine from HPLC may use some solid or liquid phase extraction methods instead of deproteinization agents**.**

Table 3.12 Percentage of recovery with different methods of deproteinization.

Analysis	ACN (% of recovery) ±RSD			TCA-MEOH (% of recovery) ±RSD			$ZnSO_4$-MEOH (% of recovery) ±RSD		
	1:1	1:4	1:10	1:1	1:4	1:10	1:1	1:4	1:10
PNT	77.90±3.57	83.56±5.65	99.01±4.37	101.01±2.61	95.21±3.21	105.83±0.24	70.67±5.71	101.98±3.23	101.23±2.45
VTB1	72.85±1.87	95.76±0.41	83.65±1.54	84.65±1.29	48.76±3.18	90.43±2.98	67.76±2.89	106.54±0.54	94.54±1.89
VTB6	89.34±2.30	109.23±2.45	67.23±3.54	56.45±6.45	73.23±8.43	92.28±1.39	92.47±3.50	112.34±4.34	89.23±2.21
VTB12	103±3.52	109.76±0.34	93.46±4.66	88.94±4.34	90.26±3.04	98.99±3.76	126.12±3.20	116.23±10.82	123.15±7.83

Data represent the mean ± SD of 3 experiments.

3.5. Pharmacokinetic and DDI Studies in Healthy Adults

When determining the efficacy and safety of medication combinations, drug-drug interactions (DDIs) are an extremely important element to consider. The pharmacokinetics (PK) of interactions may frequently be explained by the effect that each drug has on a specific enzyme, membrane drug transporter, or plasma transport protein. The cytochrome P450 (CYP450) enzyme superfamily is principally responsible for the generation of such DDIs [113], which accounts for the majority of the DDIs that are produced throughout the metabolic process. Medications that do not contain CYP metabolic enzymes or drug transporters can also contribute to drug-induced diarrhea, however this depends on the mix of medications being taken [114].

Table 3.13 Pharmacokinetic profile data of PNT, VTB1, VTB6 and VTB12.

Parameters	Unit	**PNT (20 mg/kg, *p.o.*)**			**VTB1 (100 mg/kg, *p.o.*)**			**VTB6 (200 mg/kg, *p.o.*)**		
		Alone	Combination	*P*	Alone	Combination	*P*	Alone	Combination	*P*
C_{max}	µg/mL	0.95 ± 0.347	0.98 ± 0.295	*0.949*	1.63 ± 0.154	1.58 ± 0.053	*0.742*	20.66 ± 1.969	24.09 ± 3.179	*0.410*
T_{max}	h	2.67 ± 0.333	2.67 ± 0.333	*>0.99*	1.67 ± 0.333	2.33 ± 0.333	*0.230*	2.00 ± 0.00	2.67 ± 0.333	*0.116*
AUC0–6	µg/mL*h	3.88 ± 1.239	3.56 ± 0.356	*0.816*	8.44 ± 0.514	7.90 ± 0.130	*0.366*	62.91 ± 3.046	56.52 ± 6.816	*0.440*
AUC 0–inf	µg/mL*h	5.03 ± 0.950	4.73 ± 0.344	*0.782*	39.72 ± 10.417	51.37 ± 18.469	*0.612*	95.03 ± 29.349	60.61 ± 6.732	*0.317*
MRT	h	4.95 ± 0.993	4.71 ± 0.334	*0.832*	24.93 ± 8.890	34.83 ± 14.736	*0.596*	5.13 ± 1.882	3.36 ± 0.165	*0.402*
CL	Lh^{-1}	4.33 ± 0.731	4.27 ± 0.291	*0.936*	2.78 ± 0.601	2.44 ± 0.697	*0.725*	2.18 ± 0.914	3.16 ± 0.370	*0.376*
V_d	L	17.94±6.516	16.78 ± 1.605	*0.969*	59.06 ± 6.071	63.60 ± 4.763	*0.588*	7.58 ± 3.021	9.71 ± 6.258	*0.774*
$t_{1/2}$	h	2.74 ± 0.827	2.72 ± 0.155	*0.988*	17.01 ± 6.177	23.85 ± 10.302	*0.600*	2.38 ± 1.200	1.18 ± 0.442	*0.401*
K_e	h^{-1}	0.32 ± 0.120	0.26 ± 0.014	*0.609*	0.05 ± 0.016	0.04 ± 0.014	*0.610*	0.45 ± 0.169	0.75 ± 0.229	*0.354*

Data Represents pharmacokinetic parameters for three drugs: PNT, VTB1, and VTB6. The AUC values are reported for each drug alone (Alone) and in combination (Combination) with another drug. The AUC values are expressed in µg/mL*h (micrograms per milliliter-hour).

In the **Table 3.13**, the parameters represent the pharmacokinetic properties of three drugs: PNT, VTB1, and VTB6. The drugs were administered orally at specific doses (mg/kg).

Cmax: This parameter indicates the maximum observed concentration of the drug in the plasma after administration. It is measured in μg/mL (micrograms per milliliter). The values reported in the table represent the mean ± standard deviation for each drug, both when administered alone and in combination with another drug.

Tmax: This parameter represents the time it takes for the drug to reach its maximum concentration in the plasma after administration. It is measured in hours (h). The values provided in the table represent the mean ± standard deviation for each drug, both alone and in combination.

AUC_{0-6}: This measure refers to the area under the concentration-time curve from time zero to time six hours after the medication has been administered. AUC stands for area under the concentration-time curve. It offers a rough estimation of how much of the medication was taken in during the early phase. The values reported in the table are expressed in μg/mL*h (micrograms per milliliter-hour) and represent the mean ± standard deviation for each drug, both alone and in combination.

MRT: MRT measures how long a medicine stays in the body on average. The unit of time used is the hour (h). The values provided in the table represent the mean ± standard deviation for each drug, both alone and in combination.

CL: The clearance (CL) of a medicine is its rate of elimination from the body. It is measured in L/h (liters per hour). The values reported in the table represent the mean ± standard deviation for each drug, both alone and in combination.

Vd: Volume of Distribution, is a pharmacokinetic metric that measures the volume of the fluid in which medication is really circulating in the body. It is measured in liters (L). The values provided in the table represent the mean ± standard deviation for each drug, both alone and in combination.

$t_{1/2}$: The half-life (t1/2) of a medication is the amount of time it takes for the drug's concentration in the body to decline by 50%. The unit of time used is the hour (h). Also, values reported in the table represent the mean ± standard deviation for each drug, both alone and in combination.

Ke: A pharmacokinetic parameter, elimination rate constant (Ke) describes how quickly a substance leaves the body. It is measured in h^{-1} (per hour).

The column labeled "P" in the table is the p-value, a statistical metric employed to assess the significance of observed disparities between the medication alone and combination groups for each parameter. Typically, a p-value below 0.05 is indicative of a statistically significant disparity.

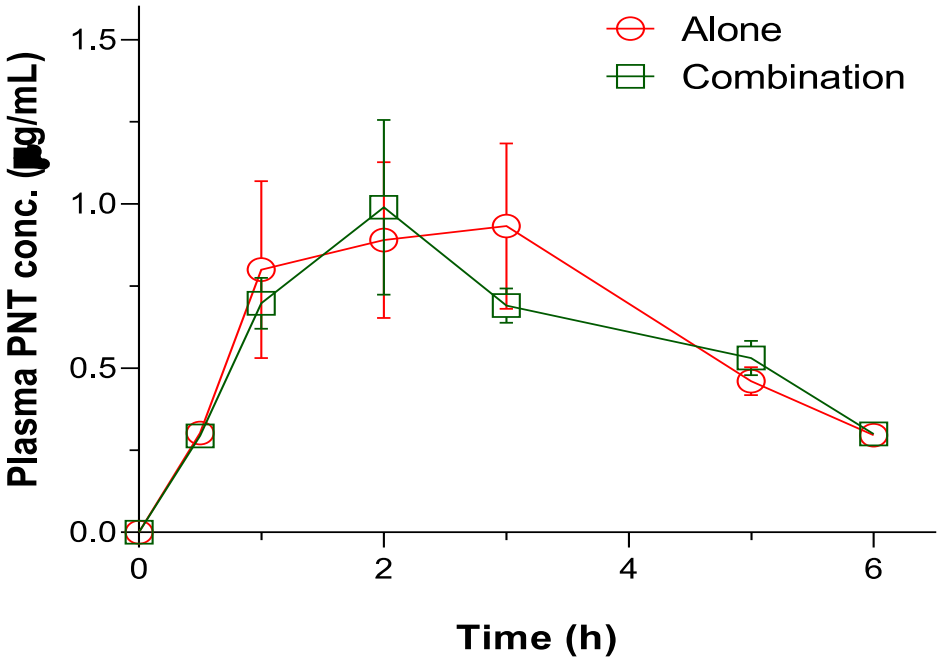


Figure 3.14 Pharmacokinetic study after single and combined dose administration in healthy adults, PNT (20 mg-PNT/kg, *p.o.*). Data represent the mean ± SE of 21–25 experiments.

High-performance liquid chromatography (HPLC) technique was effectively utilized to ascertain the pharmacokinetic characteristics of PNT and VTBs in human plasma following a single oral dosage, whether administered alone or in combination. **Figure 3.14** displays the plasma concentration-time curves, while **Table 3.13** summarized the primary pharmacokinetic parameters, which were estimated using PKSolver. The absorption of PNT occurred rapidly, with a mean time to reach maximum plasma concentration (T_{max}, 2.67 ± 0.333h). The peak plasma concentration (Cmax) was observed to be 0.95 ± 0.347 µg/mL^{-1}. Furthermore, PNT exhibited a high area under the curve (AUC0-6) of 3.88 ± 1.239 µg*mL^{-1}h^{-1}. The compound PNT demonstrated a prolonged terminal half-life (t1/2) of 2.74 ± 0.827 hours and a mean residence time of 4.95 ± 0.993 hours, which may be attributed to its extensive distribution volume of 4.95 ± 0.993 liters and low plasma clearance of 4.33 ± 0.731 Lh^{-1}.

PNT had the elimination rate constant of 0.32 ± 0.120 h^{-1}. In combination with VTBs, PNT had a maximum concentration of 0.98 ± 0.295 µg/mL ($p < 0.949$) and T_{max} 2.67 ± 0.333 h, showing no specific change in both T_{max} and C_{max}. In combination with VTs, results of AUC_{0-6}, AUC 0–inf, MRT, CL, V_D, $t_{1/2}$, and Ke was 3.56 ± 0.356 µg/mL*h ($p < 0.949$), 4.73 ± 0.344 µg/mL*h ($p < 0.949$), 4.71 ± 0.334 h ($p < 0.949$), 4.27 ± 0.291 Lh^{-1} ($p < 0.949$), 16.78 ± 1.605 L ($p < 0.949$), 2.72 ± 0.155 h ($p < 0.949$), 0.26 ± 0.014 h^{-1} ($p < 0.949$) respectively for PNT.

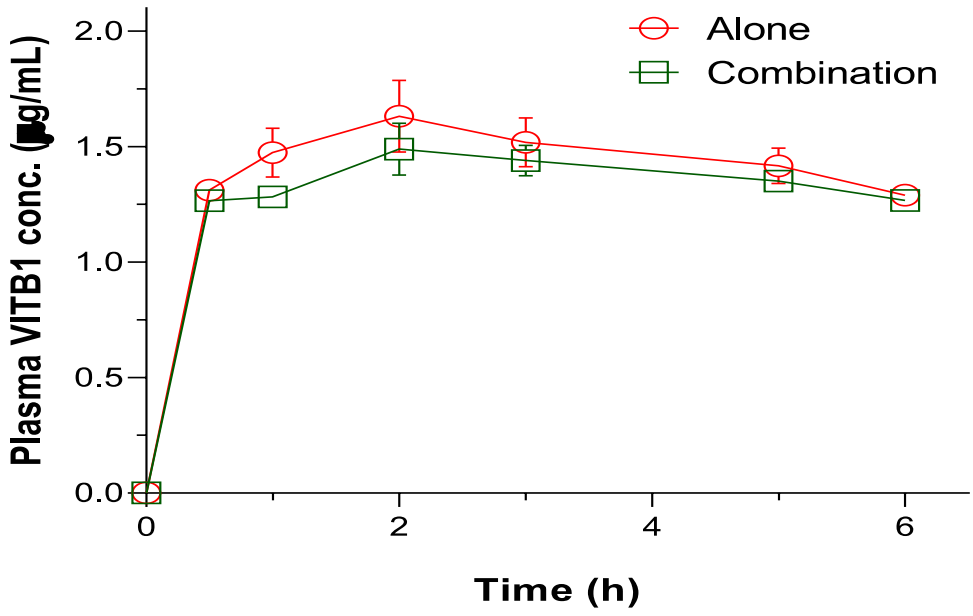


Figure 3.15 Pharmacokinetic study after single and combined dose administration in healthy adults, VTB1 (100 mg-VTB1/kg, *p.o.*). Data represent the mean ± SE of 21–25 experiments.

On the other hand, **Figure 3.15**, VTB1 and VTB6 showed excellent pharmacokinetic profiles, which are unique among VTBs. VTB1 is well absorbed with AUC_{0-6} of VTB1 was 8.44 ±

0.514 µg*mL^{-1}h^{-1} and C_{max} was 1.63 ± 0.154 µg*mL^{-1}, which was attained in a very short time (T_{max}, 1.67 ± 0.333 h). VTB1 has a prolonged half-life (17.01 ± 6.17 h) with a moderate volume of distribution (59.06 ± 6.071 L) and plasma clearance of 2.78 ± 0.601 Lh^{-1}. VTB1 had an elimination rate constant of 0.05±0.016 h^{-1} which was very short compared to others, indicating the drug will be cleared from the body relatively quickly, resulting in a shorter duration of action. This means that the therapeutic effects of the drug may not last for an extended period of time, and the drug may need to be administered more frequently to maintain its desired outcomes compared with others. Also, drug accumulation in the body is less likely with repeated dosing. Accumulation can occur when the elimination rate is slower than the rate of drug administration, leading to higher drug levels in the body over time. This risk is reduced in the case of a drug with a short elimination rate constant. In addition, a combination of VTB1 with PNT, $C_{max,}$ and t_{max} was observed at 1.58± 0.053 µg*mL^{-1} ($p \approx 0.73$) and 2.33± 0.333 h, respectively. For t_{max}, the combination part is slightly higher than the single dose. Other values for VTB1 in combined dosage were $AUC_{0–6}$ and $AUC_{0–inf}$ 8.44± 0.514 µg/mL*h and 39.72± 10.417 µg/mL*h respectively, higher Area under curve with infinity level indicated prolonged exposure of VTB1, which may result in higher drug levels, which can potentially lead to increased therapeutic effects or increased risk of adverse effects. Other values such as MRT, Cl, V_d, $t_{1/2}$ and k_e was observed at 34.83± 14.736 h, 2.44± 0.697 Lh^{-1}, 63.60 ± 4.763 L, 23.85 ± 10.302 h, and 0.04± 0.014 h^{-1} respectively. Here, higher V_d and $t_{1/2}$ were observed than in a single administration, possibly introducing the potential for drug accumulation with repeated dosing. If the drug is administered at regular intervals, it may not be completely eliminated from the body between doses, leading to drug accumulation over time·

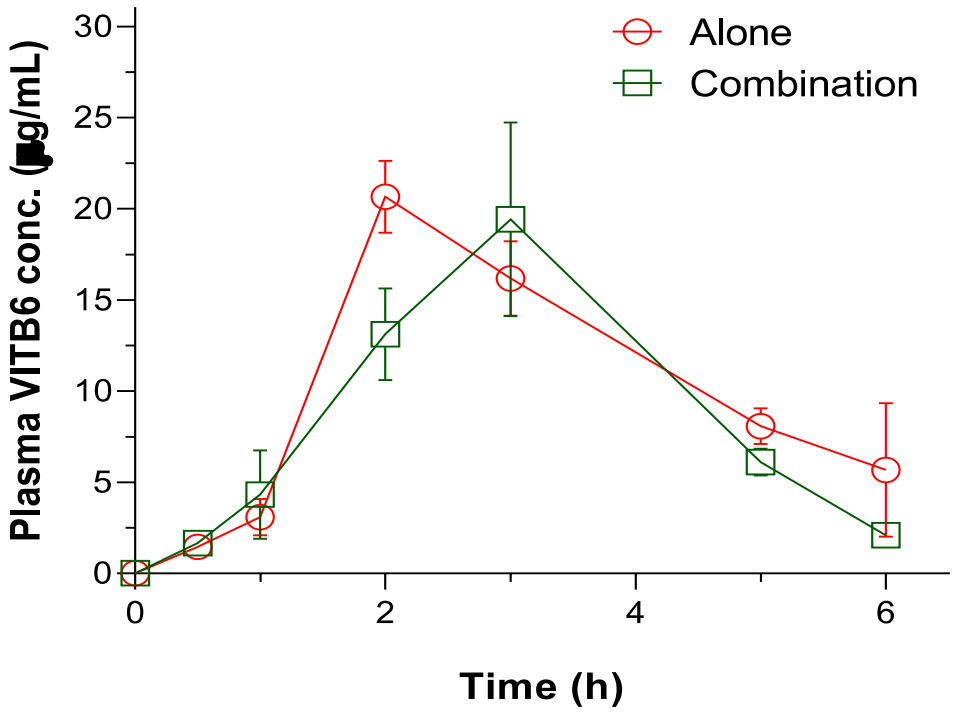


Figure 3.16 Pharmacokinetic study after single and combined dose administration in healthy adults, VTB6 (200 mg-VTB6/kg, *p.o.*). Data represent the mean ± SE of 21–25 experiments.

VTB6 is also well absorbed with AUC_{0-6} of VTB6 was 62.91 ± 3.046 µg*$mL^{-1}h^{-1}$ and C_{max} was 20.66 ± 1.969 µg*mL^{-1}, which was attained in a very short time (T_{max}, 2.00 ± 1.20 h). VTB6 has a prolonged $t_{1/2}$ (2.38 ± 1.20 h) with a moderate volume of distribution (7.58 ± 3.021 L) and plasma clearance 2.18 ± 0.914 Lh^{-1}. In combination VTB6 was observed with AUC_{0-6}, C_{max}, T_{max}, $t_{1/2}$, V_d with a value of 24.09± 3.179 µg*$mL^{-1}h^{-1}$, 24.09 ± 3.179 µg*mL^{-1}, 2.76± 0.333 h and 9.71± 6.258 L respectively.

In addition, due to the administration of very low doses, VTB12 could not be detected in human plasma. The technique used to measure vitamin B12 levels may have a limited sensitivity, meaning it may need to be able to accurately detect concentrations below a certain threshold. In such cases, even if there is a low level of vitamin B12 present, the assay may not be able to detect it. Using a more sensitive assay or different analytical method could help in detecting lower levels of vitamin B12.

Recent research has shown that excessive use of acid-inhibiting drugs, particularly PPIs, is linked to a future diagnosis of VTB insufficiency [115]. These investigations were conducted in recent years. The degree of the connection was greater in women and younger age groups who had more severe acid suppression, and it diminished once the usage of the medication was stopped. There was no discernible pattern with increasing length of use, and insufficient evidence supports the hypothesis that the results were influenced by the duration of medical care [116]. PNT has very little of an effect on the absorption of the medication, which was already very low to begin with, which is in line with the pharmacokinetics following combination with PNT reported in earlier trials in healthy patients. For instance, vitamins B1, B6, and others cannot be absorbed very well if the stomach does not produce enough acid, and conversely, increased gastric pH levels might help the process along. Those who require medication with both proton pump inhibitors and vitamin B complex should think about doing so efficiently by assessing each medicine's efficacy individually. The cytochrome P450 (CYP) system is responsible for the metabolism of PNT, and its metabolism can influence the metabolism of other medications processed by CYP enzymes. As a result, it can potentially delay the removal of vitamins, particularly water-soluble ones [117]. However, there is a lack of knowledge of the pharmacokinetic interaction between the two medicines. Based on the present HPLC approach results, it was discovered that the simultaneous administration of PNT

and VTB had a negligible impact on the pharmacokinetic profiles of both drugs. In addition, the absorption of each medication was not affected by the presence of another drug, as was evident by the consistency of the T_{max} and AUC values throughout the experiment. Because administration of VTB results in a minor reduction in the AUC0–6 of PNT, this indicates that the quantity of PNT that was exposed to the body was reduced. Still, the amount that entered the liver, where it is known to exert its pharmacological activity, was increased.

All of the computed p values were more than 0.05, as shown in **Table 3.13**; this indicates no significant changes in the levels of PNT, VTB1, VTB6, and VTB12 between the groups that received combination and single drug. In a nutshell, the study results showed that PNT and VTB did not have a meaningful interaction with one another. As a result, the co-prescribing of PNT and VTB is possible and advantageous to practical applications and would help patients comply with their treatment.

3.6. Safety Parameters

Adverse events were assessed throughout the trial to determine the safety and tolerability of PNT and VTB when administered alone or in combination. For example, there was no convincing evidence of an adverse event following administering a single dosage of PNT or VTB under steady-state conditions with other medications. Volunteers did not report any adverse events throughout the trial. During the study, there were no clinically significant changes in laboratory testing, vital signs, physical examination, or suicidality assessment results.

CHAPTER 4

CONCLUSION

4. CONCLUSION

We are all aware that pharmaceuticals play a significant part in improving human health and maintaining overall well-being. The standard of a drug is one of the most critical determinants of its efficacy and safety. A medicine that is ineffective and has impurities can lower the tolerability of the drug. The same is true if the drug interacts with other medications or meals; this might lower the drug's quality and cause additional negative side effects. In particular, pharmacokinetic interactions can be classified as systematic. It is feasible to anticipate pharmacokinetic interactions if one knows necessary to determine which enzymatic metabolic route is clinically essential to the metabolization of a drug, whether or not the medication is the substrate of a drug transporter, and whether or not the drug inhibits or promotes the activity of these transporters. There is some speculation that inhibitors of particular cytochrome P450 enzymes can affect the way drugs interact with one another. Because of these factors, drugs that are digested more quickly and have a lower bioavailability tend to have a higher potential risk of interactions with other medications. It is generally necessary to better understand the mechanisms of action to predict pharmacodynamic interactions accurately; nonetheless, much as with pharmacokinetic interactions, a particular system can be detected here as well.

The simultaneous measurement of PNT, VTB1, VTB6, and VTB12 in human plasma has been made possible for the first time. This was made possible, in particular, by creating a wholly validated HPLC technique. The validation of this approach is a one-of-a-kind method; previous research has yet to be conducted by this method. The provided strategy provides helpful resources for assessing the pharmacokinetic profiles of the pharmaceuticals that are the study's focus. It is also essential in formulation creation, dosage fixing, and avoiding potential side effects. The strategy that is being used right now is the first method of its kind, and it verifies

DDI between PNT and VTBs while they are being administered concurrently. In addition to that, this study may provide a benchmark for dose monitoring and bioequivalence in healthy individuals. Even though the findings of this study may be of tremendous help for therapeutic drug monitoring and DDI, more in-depth research on the pharmacokinetics parameters of PNT and VTBs is still necessary to get a complete understanding of the dangers and advantages, if any, associated with the simultaneous administration of these drugs. This research was carried out to determine the synergistic effects of PPI and multivitamins since there has been a recent rise in the number of interactions and an accompanying increase in negative consequences. The influence may result in more research being conducted on the design of drugs, the modification of doses, the design of mixed dosages, the development of pharmacokinetics, and the enhancement of patient safety. It would be desirable and necessary to do research of this kind in human participants using a variety of PPIs and multivitamins to evaluate whether or not these results can be correctly extrapolated to assess whether or not any interactions are conceivable. Our research has been constrained by several factors, including a lack of availability of volunteers of various ages, inadequate monitoring of the participants on the sorts of foods they consume, and a deficiency in an adequate quantity of samples for collection.

CHAPTER 5

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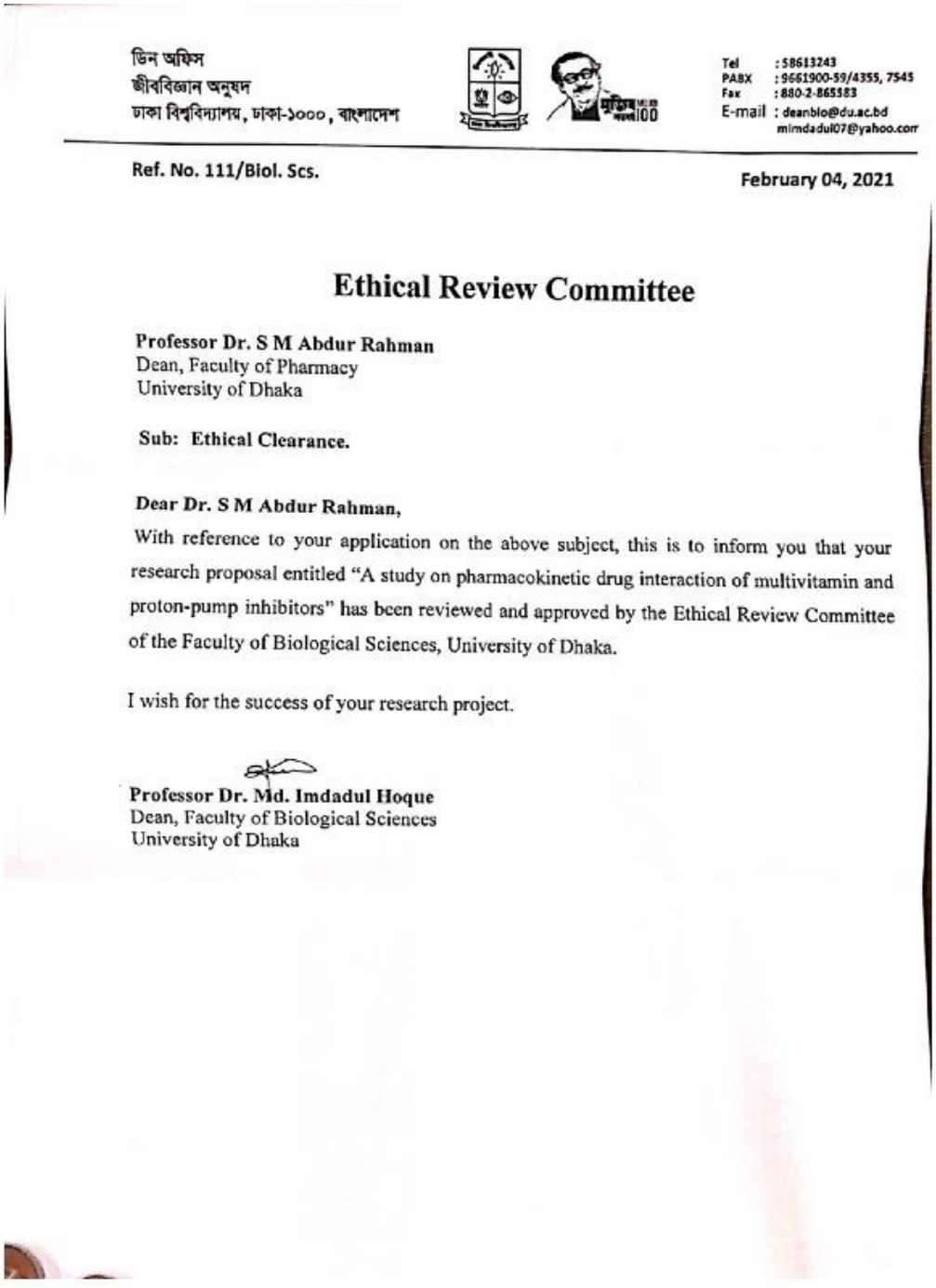
CHAPTER 6

APPENDIX

Appendix

5.1. Ethical Permission

Ethical permission for this study was obtained from Dean's Office, Faculty of Life Science, University of Dhaka.



ডিন অফিস
জীববিজ্ঞান অনুষদ
ঢাকা বিশ্ববিদ্যালয়, ঢাকা-১০০০, বাংলাদেশ

Tel : 58613243
PABX : 9661900-59/4355, 7545
Fax : 880-2-865583
E-mail : deanblo@du.ac.bd
mimdadul07@yahoo.com

Ref. No. 111/Biol. Scs.

February 04, 2021

Ethical Review Committee

Professor Dr. S M Abdur Rahman
Dean, Faculty of Pharmacy
University of Dhaka

Sub: Ethical Clearance.

Dear Dr. S M Abdur Rahman,

With reference to your application on the above subject, this is to inform you that your research proposal entitled "A study on pharmacokinetic drug interaction of multivitamin and proton-pump inhibitors" has been reviewed and approved by the Ethical Review Committee of the Faculty of Biological Sciences, University of Dhaka.

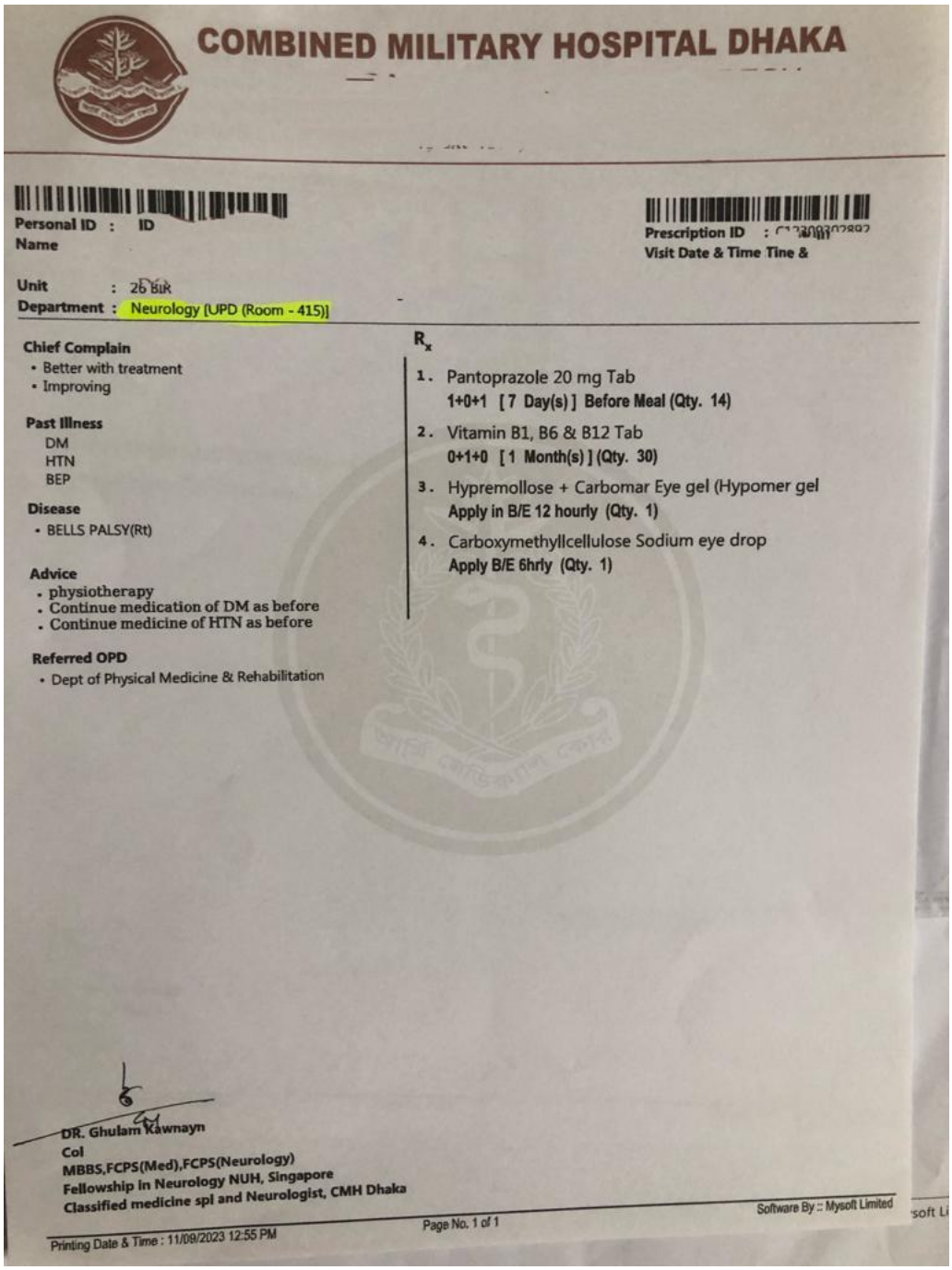
I wish for the success of your research project.

Professor Dr. Md. Imdadul Hoque
Dean, Faculty of Biological Sciences
University of Dhaka

Appendix

Figure 5.1 Ethical Permission from University Of Dhaka

5.2. Some Prescriptions in Dhaka and Barishal District



COMBINED MILITARY HOSPITAL DHAKA

Personal ID : ID
Name

Prescription ID :
Visit Date & Time Tine &

Unit : 26 BIR
Department : Neurology [UPD (Room - 415)]

Chief Complain
- Better with treatment
- Improving

Past Illness
DM
HTN
BEP

Disease
- BELLS PALSY(Rt)

Advice
- physiotherapy
- Continue medication of DM as before
- Continue medicine of HTN as before

Referred OPD
- Dept of Physical Medicine & Rehabilitation

R_x

1. Pantoprazole 20 mg Tab
   1+0+1 [7 Day(s)] Before Meal (Qty. 14)
2. Vitamin B1, B6 & B12 Tab
   0+1+0 [1 Month(s)] (Qty. 30)
3. Hypremollose + Carbomar Eye gel (Hypomer gel
   Apply in B/E 12 hourly (Qty. 1)
4. Carboxymethylcellulose Sodium eye drop
   Apply B/E 6hrly (Qty. 1)

DR. Ghulam Kawnayn
Col
MBBS,FCPS(Med),FCPS(Neurology)
Fellowship in Neurology NUH, Singapore
Classified medicine spl and Neurologist, CMH Dhaka

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Page No. 1 of 1
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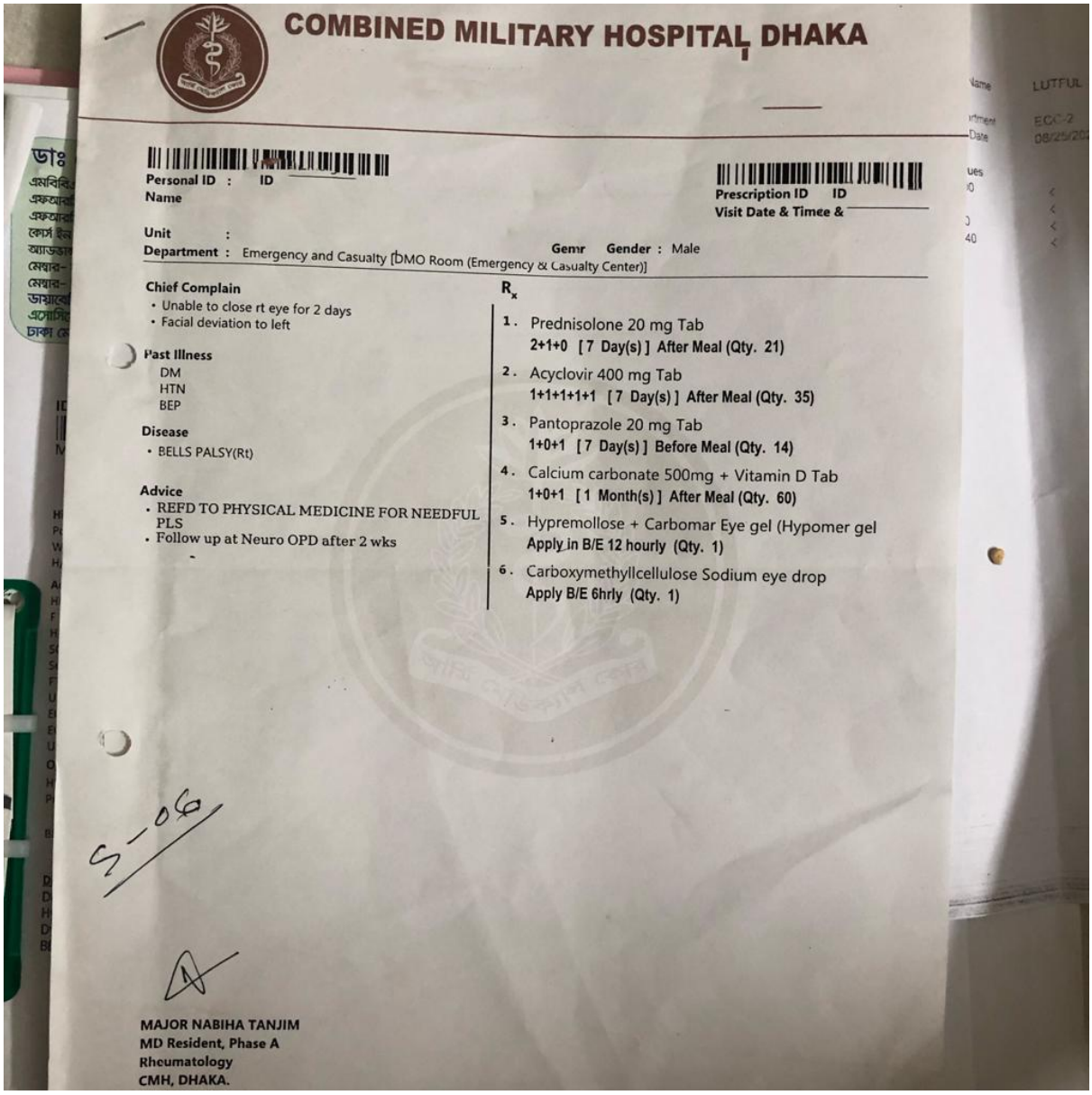
Appendix



COMBINED MILITARY HOSPITAL, DHAKA

Personal ID : ID
Name

Prescription ID ID
Visit Date & Timee &

Unit :
Department : Emergency and Casualty [DMO Room (Emergency & Casualty Center)]

Genr Gender : Male

Chief Complain
- Unable to close rt eye for 2 days
- Facial deviation to left

Past Illness
DM
HTN
BEP

Disease
- BELLS PALSY(Rt)

Advice
- REFD TO PHYSICAL MEDICINE FOR NEEDFUL PLS
- Follow up at Neuro OPD after 2 wks

Rx

1. Prednisolone 20 mg Tab
   2+1+0 [7 Day(s)] After Meal (Qty. 21)
2. Acyclovir 400 mg Tab
   1+1+1+1+1 [7 Day(s)] After Meal (Qty. 35)
3. Pantoprazole 20 mg Tab
   1+0+1 [7 Day(s)] Before Meal (Qty. 14)
4. Calcium carbonate 500mg + Vitamin D Tab
   1+0+1 [1 Month(s)] After Meal (Qty. 60)
5. Hypremollose + Carbomar Eye gel (Hypomer gel
   Apply in B/E 12 hourly (Qty. 1)
6. Carboxymethyllcellulose Sodium eye drop
   Apply B/E 6hrly (Qty. 1)

MAJOR NABIHA TANJIM
MD Resident, Phase A
Rheumatology
CMH, DHAKA.

Figure 5.2 Prescription pattern in Dhaka District

Appendix

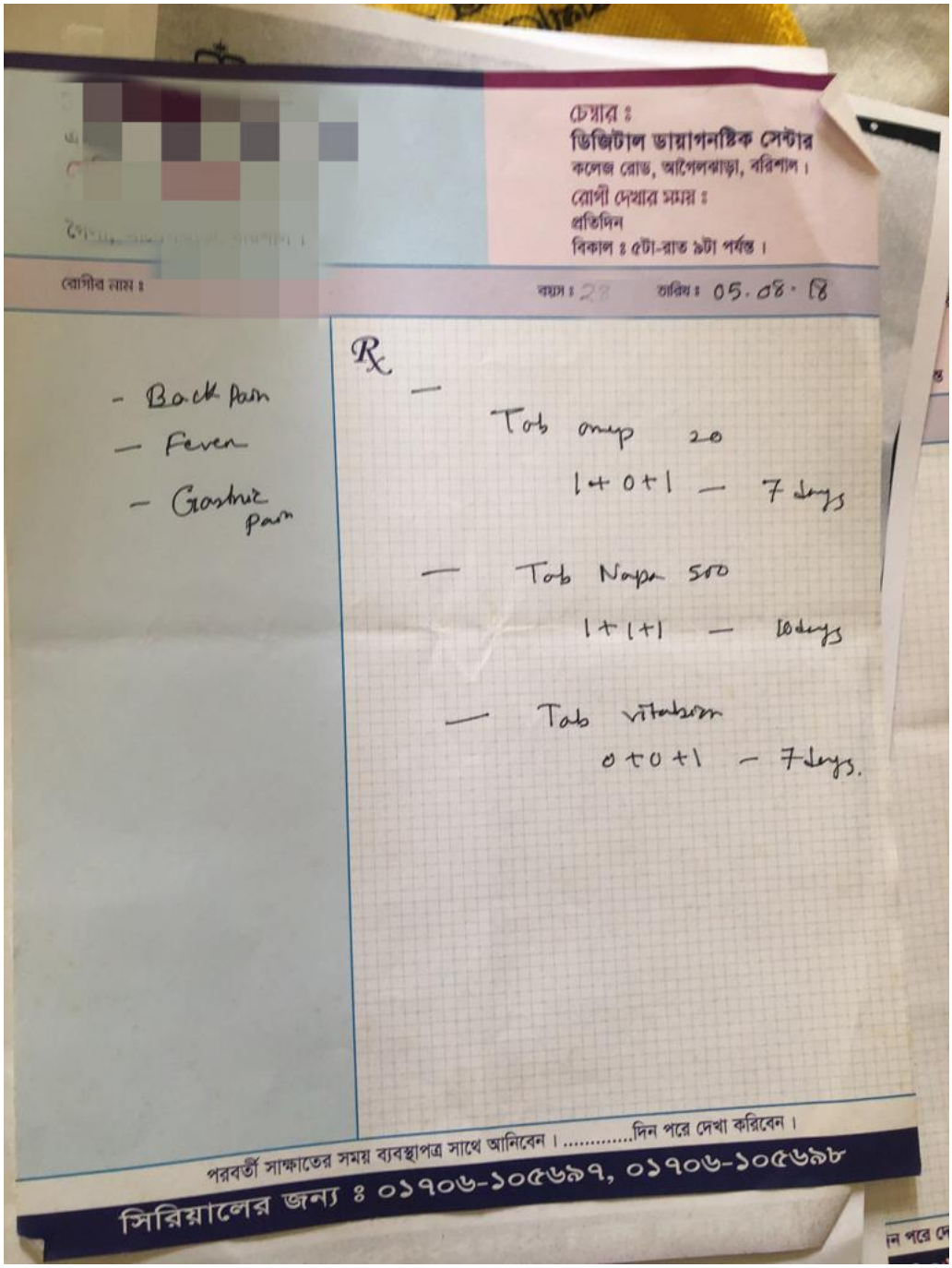


Figure 5.3 Prescription pattern in Barishal District

Appendix

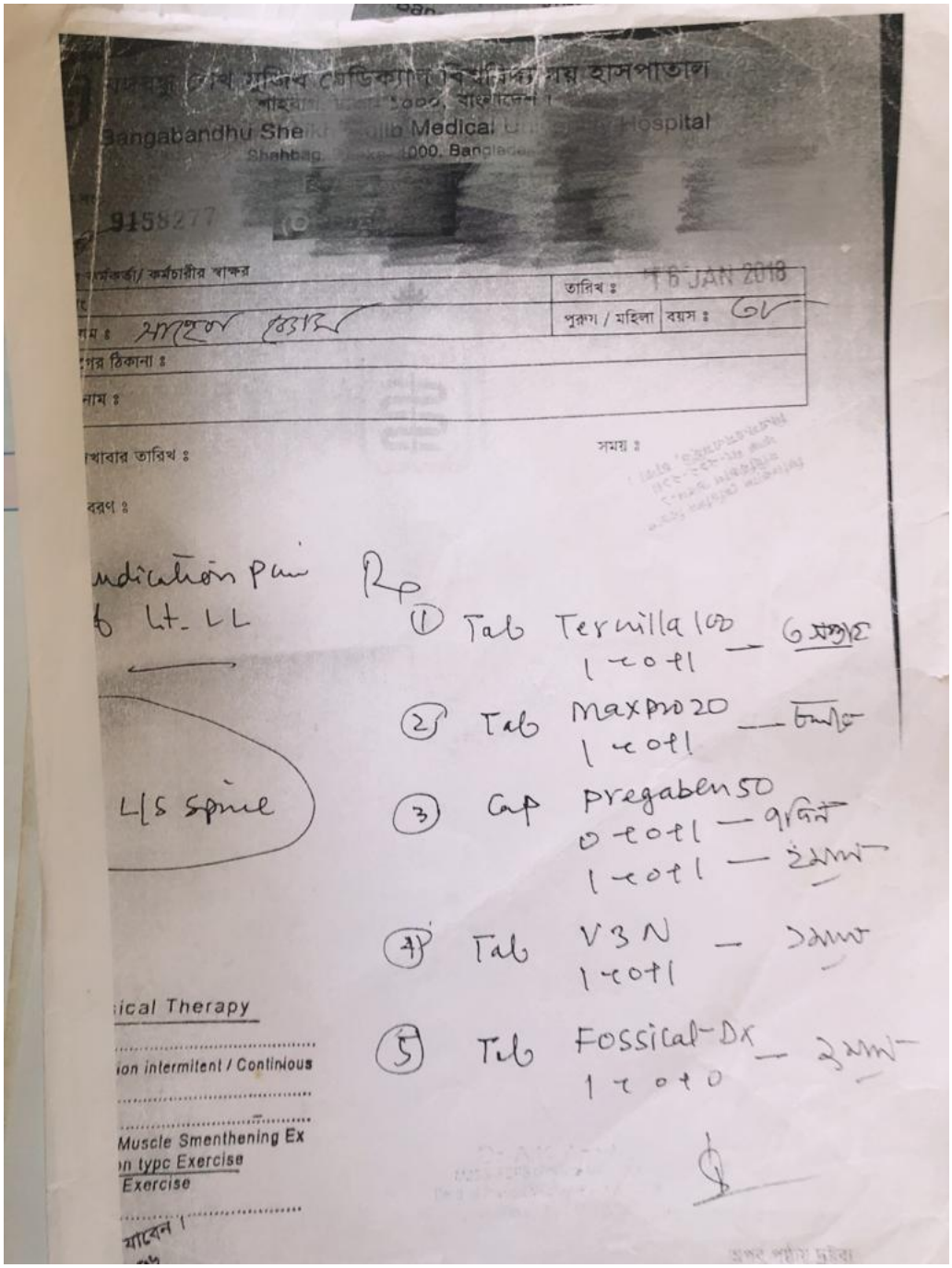


Figure 5.4 Prescription pattern in Dhaka District