Pharmacokinetics of Naproxen in Bangladeshi Type II diabetic Patients treated with Gliclazide

A dissertation submitted to the University of Dhaka, as a partial fulfillment of the requirement for the Degree of Master of Philosophy

SUBMITTED BY

Registration No.: 312/2008-2009

Department of Pharmaceutical Technology
Faculty of Pharmacy
University Of Dhaka
Dhaka, Bangladesh

Dedicated to.....
My Wife & Beloved Children

Acknowledgement

All praise is for God who has given me the ability to complete my M. Phil research under the department of Pharmaceutical Technology , Faculty of Pharmacy , University of Dhaka.

It is my pleasure and proud privilege to express my heartiest regards and gratitude to my respected teacher and supervisor Dr. Sitesh C. Bachar, Professor, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka; for his mastermind direction, constant supervision and support, optimistic counseling and continuous backup to carry out the research work as well as to prepare this dissertation.

I feel proud to express my heartiest gratitude to my revered teacher, Prof. Dr. Abu Sara Shamsur Rouf, and Prof. Dr. Md. Selim Reza, Chairman, Department of Pharmaceutical Technology, Faculty of Pharmacy, University of Dhaka, for extending his helping hands & affectionate attitude whenever I needed.

Finally I would like to extend my thanks to the office staffs of the Faculty of Pharmacy who have cooperated with me and all the research students in the lab for their help and assistance, friendly behavior and earnest cooperation, which enabled me to work in a very congenial and comfortable atmosphere.

I remember here the inspiring words of my family members and to all my well wishers. I say many thanks to them for their wholehearted inspiration during my thesis work.

University of Dhaka **Date: 26.11.2014**

DECLARATION BY THE CANDIDATE

*I hereby declare that the matter embodied in the dissertation entitled "**Pharmacokinetics of Naproxen in Bangladeshi Type II diabetic Patients treated with Gliclazide**." is a bonafide and genuine research work carried out by me under the guidance of Prof. Dr. Sitesh Chandra Bachar, Chairman, Department of Pharmacy, University of Dhaka. The work embodied in this thesis is original and has not been submitted as the basis for the award of degree, diploma, associate ship (or) fellowship of any other university (or) institution.*

Tushar Kanti Pal
Place: Department of Pharmaceutical Technology,
University of Dhaka

University of Dhaka

Date: 26.11.2014

Certificate by Guide

*This is to certify that, the dissertation entitled "**Pharmacokinetics of Naproxen in Bangladeshi Type II diabetic Patients treated with Gliclazide**." is a bonafide and genuine research work carried out by Tushar Kanti Pal submitted as the partial fulfillment for the award of the degree of M.Phil from the department of Pharmaceutical Technology, University of Dhaka. The work embodied in this thesis is original and has been carried out under my supervision.*

Prof. Dr. Sitesh Chandra Bachar
Chairman, Department of Pharmacy
University of Dhaka
Dhaka-1000.Bangladesh

Table of Content

SL No.		Page No.
	Abstract	
	Chapter-1: Introduction	**01**
	Chapter-2: Literature Review	**05**
2.1.	Disease profile	05
2.1.1.	Diabetic Mellitus	05
2.1.2.	Type I diabetic mellitus	05
2.1.3.	Type II diabetic mellitus	06
2.1.4.	Prevalence of Type II diabetic mellitus	06
2.2.	Drug Profile	09
2.2.1.	Anti Diabetic Drug market Analysis	09
2.2.2.	Chemistry of Glicalzide	10
2.2.3.	Mode of Action of Gliclazide	11
2.2.4.	Metabolism of Gliclazide	12
2.2.5.	Drug Interaction of Gliclazide	12
2.3.1.	Naproxen	13
2.3.2.	Mode of Action of Naproxen	14
2.3.3.	Metabolism of Naproxen	15
2.4.	Pharmacokinetic consideration	16
	Chapter-3: Method and Materials	17
3.1.	Study Design	17
3.2.	Ethics	17
3.2.1.	Standard of Practice	17
3.2.2.	Ethical and Regulatory authority approval	18
3.3.	Data collection form	18
3.4.	Consent Form	23
3.5.	Subjects	24
3.6.	Inclusion and Exclusion Criteria	24
3.7.	Dosage and Drug Administration	27
3.8.	Blood Sample collection	27

3.9.	Methodology	27
3.9.1.	Sample preparation and HPLC condition	48
3.9.2.	HPLC method of analysis	50
3.9.3.	Chromatographic condition	50
3.9.4.	Method Validation	51
3.9.5.	Pharmacokinetic Parameter	52
3.9.6.	Reagents and chemicals	53
	Chapter-4: Result and Discussion	54
4.	Result and Discussion	54
4.1.	Volunteer demography and safety	54
4.2.	Method Validation	57
4.2.1.	Linearity	57
4.2.2.	Limit of Detection and Quantification	58
4.2.3.	Recovery	59
4.2.4.	Precision and accuracy	59
4.3.1.	UV analysis	61
4.3.2.	IR analysis	62
4.4.	Pharmacokinetics of Naproxen	64
4.5.	Pharmacokinetic of Naproxen in presence of Gliclazide	65
4.6.	Alteration of naproxen pharmacokinetics in presence of Gliclazide	66
4.7.	Mean Plasma level Time curve	67
4.8.	Statistical analytical report	68
4.9.	Pharmacokinetic parameter	68
4.10.	Alteration of Gliclazide Pharmacokinetics in Presence of Naproxen	76
4.11	Statistical Analysis Report	78
4.12	Discussions	79
	Chapter-5: Conclusion	**82**
	Chapter-6: References	**83**

List of Tables

1	List of countries with the highest numbers of estimated cases of diabetes for 2000 and 2030.	08
2	Enzyme responsible for the metabolism of Gliclazide	12
3	Demographic features of healthy subject	55
4	Demographic features of diabetic subject	56
5	Linearity test	57
6	Precision and accuracy of HPLC assay (In Plasma)	59
7	Absolute Recovery of Analyte from Plasma samples Analyte	60
8	Pharamacokinetics of Naproxen	64
9	Pharamacokinetics of Naproxen in Presence of Gliclazide in Diabetic Patient	65
10	Pharamacokinetics parameter of blood sample for both groups	69

List of Figures

1	Structure of Naproxen	3
2	Structure of sodium salt of Naproxen	3
3	Structure of Gliclazide	4
4	Chemical structure of Gliclazide	10
5	Mode of action of Gliclazide	11
6	Mode of action of Naproxen	14
7	Metabolism of Naproxen	15
8	Representative Chromatogram of Gliclazide as standard solution	37
9	Representative Chromatogram of Gliclazide as sample solution	37
10	Flow chart of sample preparation for HPLC column.	49
11	Standard curve of Gliclazide for in vivo analysis	58
12	UV analysis of Gliclazide	61
13	UV analysis of Naproxen	61
14	IR analysis for Gliclazide	62
15	IR analysis of Naproxen	62
16	Pharamacokinetic of Naproxen in presence of Gliclazide	66
17	Plasma level time curve of N (Naproxen) and NG (Naproxen in presence of Gliclazide) group	67

18	Plasma concentration and Time curve	70
19	Plasma concentration and Time curve	70
20	Plasma concentration and Time curve	70
21	Plasma concentration and Time curve	71
22	Plasma concentration and Time curve	71
23	Plasma concentration and Time curve	72
24	Plasma concentration and Time curve	72
25	Plasma concentration and Time curve	73
26	Plasma concentration and Time curve	73
27	Plasma concentration and Time curve	74
28	Plasma concentration and Time curve	74
29	Plasma concentration and Time curve	75
30	Mean Plasma concentration and Time curve	75
31	Alteration of Gliclazide pharmacokinetics in presence of Naproxen	76
32	Alteration of Gliclazide pharmacokinetics in presence of Naproxen	77

Abstract

This study was aimed to investigate pharmacokinetics of naproxen in Bangladeshi type II diabetic subjects treated with gliclazide. All the subjects were divided into three groups; group G, diabetic subjects only took gliclazide in the first session, GN/NG group diabetic subjects took both gliclazide and naproxen in the second session and N group, healthy subjects only took naproxen in the 3rd session.Glucose solution with breakfast was served at 2.0 h, a standard lunch was served at 5.0 h and snacks was served at 8h after dosing in every session. Blood samples were collected for 24.0 h after drug administration. Drug plasma concentrations were determined by HPLC with a UV detector. Analysis of pharmacokinetic characteristics was based on a non-compartmental model. Pharmacokinetic parameters for single oral dose naproxen are Cmax, $AUC_{0-\infty}$, AUC_{0-24}, T_{max}, $T_{1/2}$ and K_{el} were 65.33µg./mL^{-1}, 926.68 µg.h.mL^{-1}, 856.25 µg.h.mL^{-1}, 0.93h, 10.07h and 0.07 respectively. In case of concurrent administration of naproxen and gliclazide, Cmax, $AUC_{0-\infty}$, AUC_{0-24}, T_{max}, $T_{1/2}$ and K_{el} values for naproxen were 58.37 µg./mL-1, 889.57µg.h.mL^{-1}, 819.76µg.h.mL^{-1}, 1.04 h, 10.61h and 0.069 respectively. Likewise, for single dose oral gliclazide tablet, Cmax, $AUC_{0-\infty}$, AUC_{0-24}, T_{max}, $T_{1/2}$ and K_{el} values were 4.07µg./mL-1, 64.39 µg.h.mL^{-1}, 57.59µg.h.mL^{-1}, 3.58 h, 7.073h and 0.099 respectively. However, in presence of naproxen Cmax, $AUC_{0-\infty}$, AUC_{0-24}, T_{max}, $T_{1/2}$ and K_{el} values of gliclazide were 3.58µg./mL^{-1}, 79.94µg.h.mL^{-1}, 70.38µg.h.mL^{-1}, 4.02 h, 8.64h and 0.078 respectively.

Over all the pharmacokinetic parameters, naproxen shows no significant change in its pharmacokinetics in presence of gliclazide. Although, AUC change was to some extent considerable. Presumably, it is due to healthy vs diabetic subjects comparison. However, Gliclazide pharmacokinetics alteration was surprisingly significant. AUC ($p<0.056$*, $p<0.048$*), C_{max} ($p<0.$**007****) and $T_{1/2}$ ($p<$ **0.054***) have been considerably changed in presence of naproxen.

Introduction

Bioavailability and bioequivalence of drug products have emerged as critical issues in pharmacy and medicine during the last three decades. Bioavailability is a pharmacokinetic term that describes the rate and extent to which the drug ingredient is absorbed from a drug product and becomes available in the systemic circulation. The area under the concentration versus time curve (AUC) serves as the extent of absorption, the time to reach the peak concentration ($t_{max)}$ reflects the rate of absorption, while the peak concentration (C_{max}) reflects both the extent and the rate of absorption.

Type II diabetes mellitus has become a global public health problem. Bangladesh is no exception. All over the world the numbers of people suffering from this disease are increasing due to certain factors including aging, urbanization, decrease in the physical activities and increase in obesity. In global scenario for all age groups, the occurrence of diabetes was predicted to be 2.8% in 2000 and 4.4% in 2030. The total number of people with diabetes is expected to rise from 171 million in 2000 to 366 million in 2030. Moreover this incidence of diabetes is higher in men than women (Wild *et al*., 2004). In Bangladesh diabetes mellitus patients numbered 3.2 million in 2000 and it is projected that in 2030 it will soar to 11.1 million**.** [Diabetic association of Bangladesh, Ibrahim memorial Diabetes Centre, Annual report July 2005-June 2006; abridged version, pp22.]There is a sharp rise in the prevalence of type 2 diabetes mellitus in recent years in both the urban 8.5% [Rahman*et al* 2007] and the rural 6.8% [Rahim *et al*; 2007]**.**So type II diabetes mellitus has become a global public health problem including Bangladesh. Understanding of the pathogenesis, finding better therapeutic options and to ensure their dose profile, clinical safety and efficacy is crying need of the hour.

In more commonly, anti-diabetic drugs are the classes of drugs interact more with other classes drugs. Even some of the NSAIDs do same. Gliclazide and naproxen are preferably sometimes may be prescribed by doctors in Bangladesh for type-II diabetic patient. But we don't have any investigation regarding their pharmacokinetic interactions among Bangladeshi patients.

Establishing firmly therapeutic endpoints include the anticipated and desired clinical effects from drug therapy that are expected, ultimately, to achieve the desired outcome(s). Pharmacokinetic interactions are crucial and key determinant of expected therapeutic response as well.

Naproxen

Naproxen is a non steroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. It is indicated for the relief of the signs and symptoms of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and juvenile arthritis. Naproxen is also indicated for the relief of the signs and symptoms of tendonitis, bursitis and acute gout; for the management of pain and primary dysmenorrhoea.

Naproxen is a propionic acid derivative related to the arylacetic acid group of non steroidal anti inflammatory drugs. Naproxen (CAS 22204-53-1) and naproxen sodium (CAS-26159-34-2) are known chemically as (S)-6-methoxya- methyl-2-naphthaleneacetic acid and (S)-6-methoxy-α- methyl-2-naphthaleneacetic acid, sodium salt, respectively. Naproxen has a molecular weight of 230.26 and a molecular formula of $C_{14}H_{14}O_3$. Naproxen sodium has a molecular weight of 252.23 and a molecular formula of $C_{14}H_{13}NaO_3$. Naproxen is an odorless, white to off-white crystalline substance. It is lipid soluble, practically insoluble in water at low pH and freely soluble in water at high pH. The octanol/ water partition coefficient of naproxen at pH 7.4 is 1.6 to 1.8. Naproxen sodium is an odorless crystalline powder, white to creamy in color. It is soluble in methanol and water at neutral pH.

Chemical structures of naproxen and sodium salt of naproxen are given below.

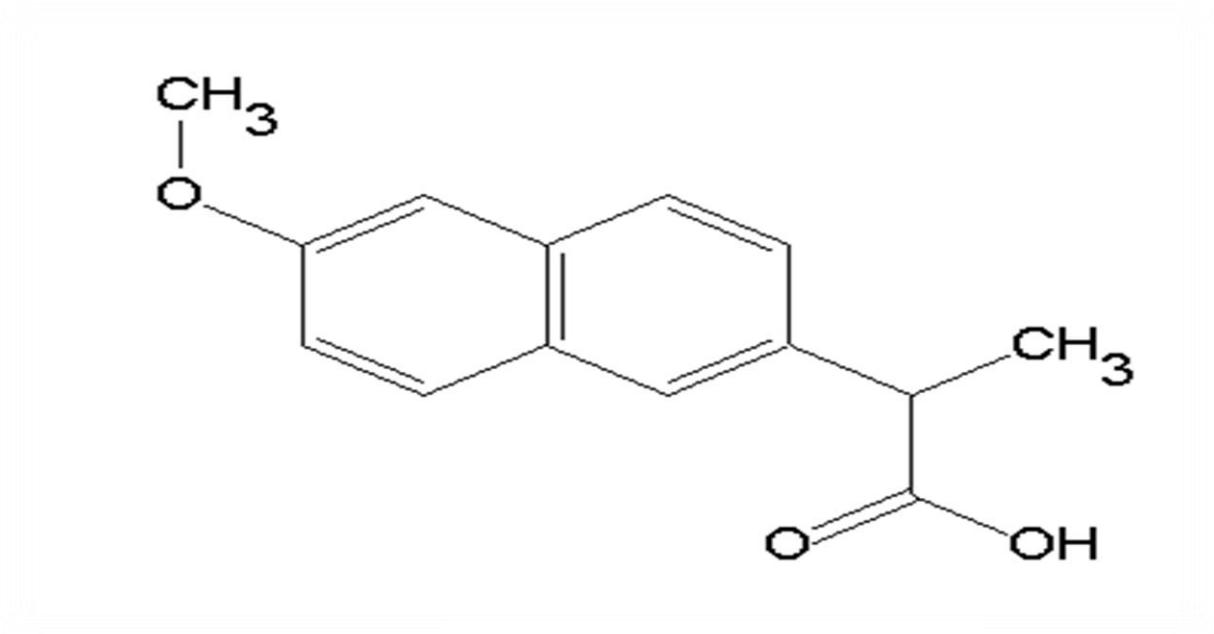


Figure-1: Structure of Naproxen

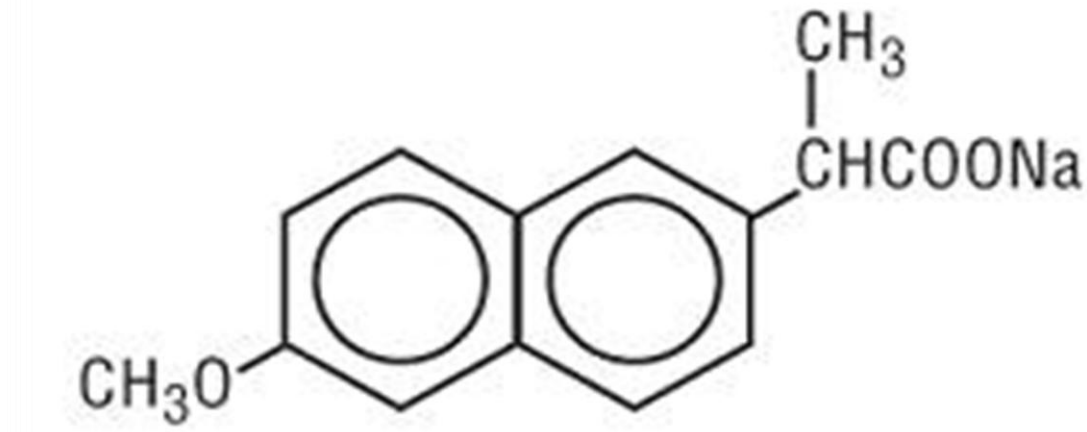


Figure-2: Structure of sodium slat of Naproxen

Gliclazide

Gliclazide is a second-generation sulfonylurea, an oral hypoglycemic agent used in the treatment of noninsulin dependent diabetes mellitus (NIDDM). It decreases blood glucose level by stimulating insulin secretion from pancreatic β-cells and increasing sensitivity of the β-cells to a glucose stimulus. Moreover, long-term treatment of gliclazide improves insulin-mediated glucose utilization and potentiates the post-receptor insulin sensitive pathway. It may have advantages in reducing platelet aggregation and slowing the progression of diabetic retinopathy.

Gliclazide is a weak acid compound with high lipophilicity and has pH-dependent solubility. The drug is insoluble under acidic condition in the stomach and its solubility increases with increasing pH in the intestinal region. Due to its lipophilicity, drug absorption is limited by the ability of the drug to be released from the dosage form. Immediate release preparations of gliclazide have been marketed for a long time with the general administration of twice daily taken with the main meals.

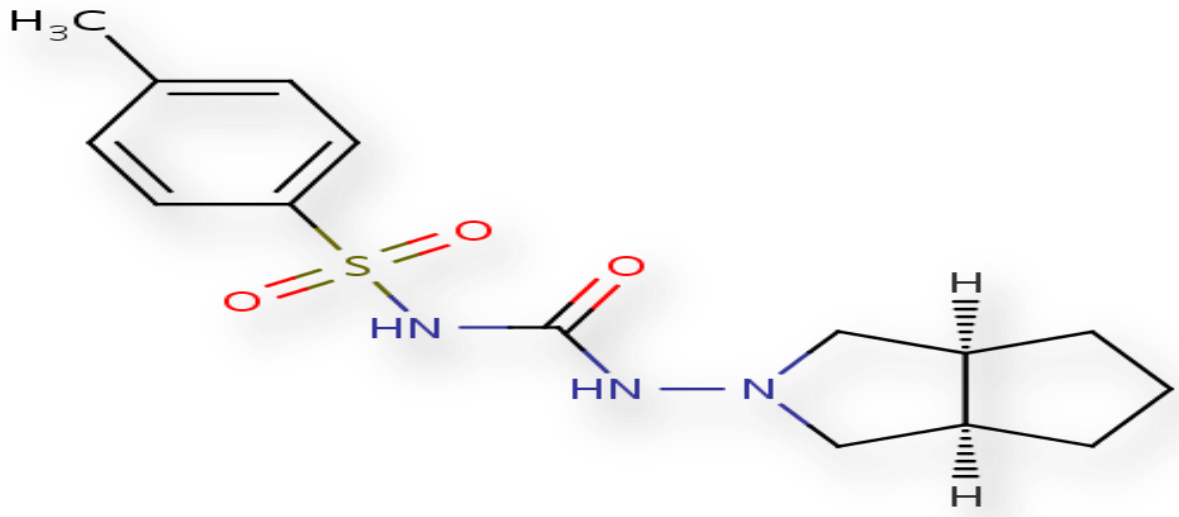


Figure-3: Structure of Gliclazide

2. Literature Review

2.1. Disease Profile

2.1.1. Diabetes Mellitus

Diabetes Mellitus commonly referred to as "diabetes", is a group of metabolic diseases characterized by defects at multiple organ sites. These defects include insulin secretion, insulin action or both (Lilliojaet *al.*, 1993; Ferrannini, 1998; Barnett, 2007). These cases rise due to defects in function or loss of the pancreatic beta-cell (LeRoith, 2002; Kahn, 2003; ADA, 2009) and due to decreased insulin motivated glucose uptake in skeletal muscle (Shulman, 2000). These abnormalities varies between individuals and it is estimated that due to insulin resistant more than 92% of patients progressing to type II diabetes (Haffneret *al.*, 1999). Inspite of carbohydrate metabolism, lipid and protein metabolism also play an important role in the development of this disease (Uusitupaet *al.*, 1993).

2.1.2. Type I Diabetes Mellitus

Type 1 diabetes mellitus is also known as insulin dependent diabetes mellitus (IDDM). This occurs as a result of cellular-mediated autoimmune destruction of the beta cells of the pancreas. Generally this demolition of the beta cells leads to absolute insulin deficiency (Atkinson and Maclaren, 1994; ADA, 2009 a). As the beta cells destruction is quite rapid in infants and children therefore prevalence of type 1diabetes is greater in children and young as compared to others (Zimmetet *al.*, 1994). It also developed in many type II diabetic patients with severe insulin resistance (Suehiroet *al.*, 2005). This form accounts for only 5 - 10% of those with diabetes (ADA, 2009 b).

2.1.3. Type II Diabetes Mellitus

It is also known as the non-insulin dependent diabetes mellitus (NIDDM) and occurs as a result of progressive insulin secretory defect along with insulin resistance (Turner *et al*., 1979; Olefsky*et al.*, 1982; ADA, 2009 a). Its different forms are characterized by a variable degree of insulin resistance and beta cells dysfunction (ADA, 2001). Type II DM is the most common form of diabetes. About 90 to 95% of people with diabetes are suffering from type II DM (DeFronzo, 1997; ADA, 2009). This form of diabetes is related with family history, age, ethnicity, previous history of gestational diabetes and physical inactivity (Bogardus, 1985; Zimmet, 1992; Harris *et al.*, 1995).

2.1.4. Prevalence of Type II Diabetes Mellitus

It is well established that the most common form of diabetes is type II diabetes. The global rise in diabetes [Bagust A*et al*; 2002, Motala*et al*; 2003] occurs because of population growth and ageing, and be-cause of increasing trends towards an unhealthy diet, obesity, and sedentary lifestyles [Harris *et al*; 1998]. Type II diabetes represents about 85% to 95% of the people with diabetes in developed countries and an even higher percentage in developing countries [International Diabetes Federation [IDF; 2005. Amos *et al*.]estimated that there were 124 million persons with diabetes in the world in 1997 and predicted this number would grow to 221 million in 2010 [Amos *et al*; 2010]. Another study group estimated that the number of persons with diabetes was 150 million in 2000 and this number is expected to double by 2025 [King *et al*; 1998]. In 2003, it was estimated that approximately 194 million people worldwide, or 5.1% in the age group 20-79, have diabetes.

The largest increase in the prevalence numbers is thought likely to appear in India, China and other developing countries. This estimate is expected to increase to 6.3% in the adult population, by 2025. In the United States, the National Health and Nutrition Examination Surveys (NHANES) I and II showed that the prevalence of DM between 1976 and 1994 among American adults increased from 6.6% to 7.8% [Harris MI *et al*; 1998]. Similar pictures have been observed in Europe, in which DM affects about 8.5% of the adult

population [The Diabetes Epidemiology; 1998]. The European Region with 48 million and Western Pacific Region with 43 million currently have the highest number of people with diabetes. How-ever, the prevalence rate of 3.1% for the Western Pacific Region is significantly lower than 7.9% in the North American Region and 7.8% in the European Region. By 2025, the region with the greatest number of persons with diabetes is expected to change to the South-East Asian Region with about 82 million.

The region's prevalence of 7.5% will however continue to be lower than that of North America, estimated at 9.7% and Europe at 9.1%. [Sicree*et al*; 2003] .Gu*et al*. conducted a national study to investigate the prevalence of diabetes in 15,540 adults from 31 provinces in China. In Bangladesh diabetes mellitus patients numbered 3.2 million in 2000 and it is projected that in 2030 it will soar to 11.1 million**.** [Diabetic association of Bangladesh, Ibrahim memorial Diabetes Centre, Annual report July 2005-June 2006; abridged version, pp22.]There is a sharp rise in the prevalence of type II diabetes mellitus in recent years in both the urban 8.5% [Rahman*et al* 2007] and the rural 6.8% [Rahim *et al*;2007]**.** Table 1 showed the 10 countries estimated to have the highest numbers of people with diabetes in 2000 and 2030 [Wild, Roglic, Green, *et al*., 2004].The 40-59 age groups currently have the greatest number of persons with diabetes. By 2025, because of the aging of the world's population, there will be 146 million aged 40-59 and 147 million aged 60 or older with diabetes.

Table 1: List of countries with the highest numbers of estimated cases of diabetes for 2000 and 2030.

Ranking	2000		2030	
	Country	People with diabetes (millions)	Country	People with diabetes (millions)
1	India	31.7	India	79.4
2	China	20.8	China	42.3
3	U.S.	17.7	U.S.	30.3
4	Indonesia	8.4	Indonesia	21.3
5	Japan	6.8	Pakistan	13.9
6	Pakistan	5.2	Brazil	11.3
7	Russia	4.6	**Bangladesh**	**11.1**
8	Brazil	4.6	Japan	8.9
9	Italy	4.3	Philippines	7.8
10	**Bangladesh**	**3.2**	Egypt	6.7

Reference:[Wild, Roglicand Green, *et al*.2004]

2.2. Drug Profile

2.2.1. Anti-Diabetic Drug- Market Analysis

Diabetes is one of the major health concerns of the entire world. It doesn't leave people of any age irrespective of gender. Sometimes it results in multiple complications for example, diabetes with coma, gestational diabetes. As the day goes by, the population affected by diabetes is increasing at a proportional rate. When, there is a major health concern the first two things that come into the mind how to find a cure for the mitigation of the concern and how to control the population affected by the disease which doesn't depend on one factor only rather multiple groups of factors. In order to work with the first burning issue, the choice and development of anti-diabetic drugs comes into the light. Depending on the patientspatho physiology, different types of anti-diabetic drugs are medicated to the patients. The drugs that are used as anti-diabetic drugs are: sulfonyl urea derivatives, biguanide derivatives, Thiazoldione derivatives etc. According to a statistics by the International Diabetes Federation, among 346 million affected by diabetes, a majority are taking anti-diabetes drugs as medication. The most popular drug dominated in the global market is Glicazide. The most popular glicazide available in the world is prepared by Servier, France under the brand named of Diamicron, MR (Modified Release Tablet).

In Bangladesh, around 10 companies produce gliclazide formulations, 3 among the top 10 pharmaceutical companies produce and supply dominantly in the market.Among 61% of the patients suffering from diabetes are taking anti-diabetes drugs as medication

2.2.2. Chemistry of gliclazide

Gliclazide is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). It belongs to the sulfonylurea class of insulin secretagogues, which act by stimulating β cells of the pancreas to release insulin. Chemically it is known as N-(4-Methylbenzenesulfonyl)-N'-(3-azabicyclo(3.3.0)oct-3-yl)urea. Chemical formula is $C_{15}H_{21}N_3O_3S$.

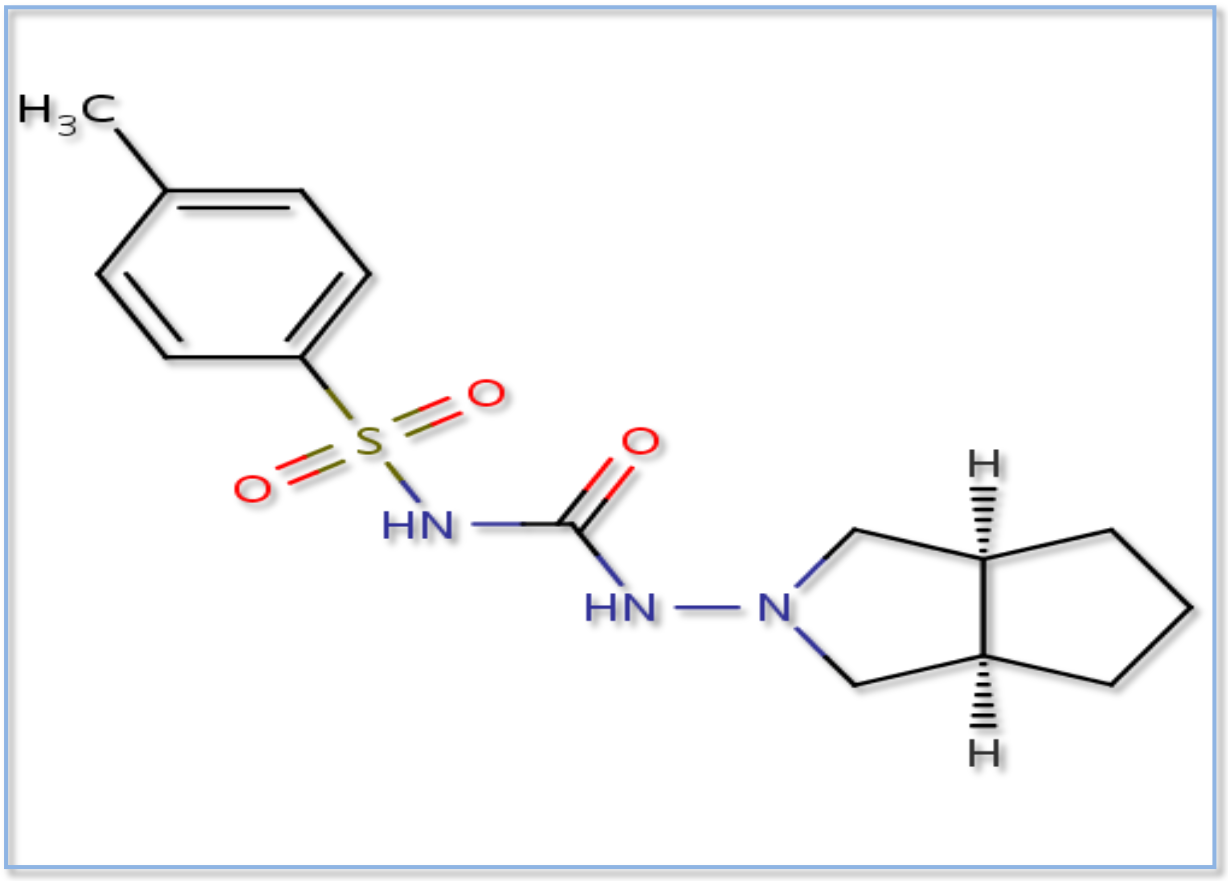


Figure 4: Chemical Structure of Gliclazide

2.2.3.Mode of action of gliclazide:

Gliclazide selectively binds to sulfonylurea receptors (SUR-1) on the surface of the pancreatic beta-cells. It was shown to provide cardiovascular protection as it does not bind to sulfonylurea receptors (SUR-2A) in the heart.[5] This binding effectively closes the K+ ion channels. This decreases the efflux of potassium from the cell which leads to the depolarization of the cell. This causes voltage dependent Ca++ ion channels to open increasing the Ca++ influx. The calcium can then bind to and activate calmodulin which in turn leads to exocystosis of insulin vesicles leading to insulin release.

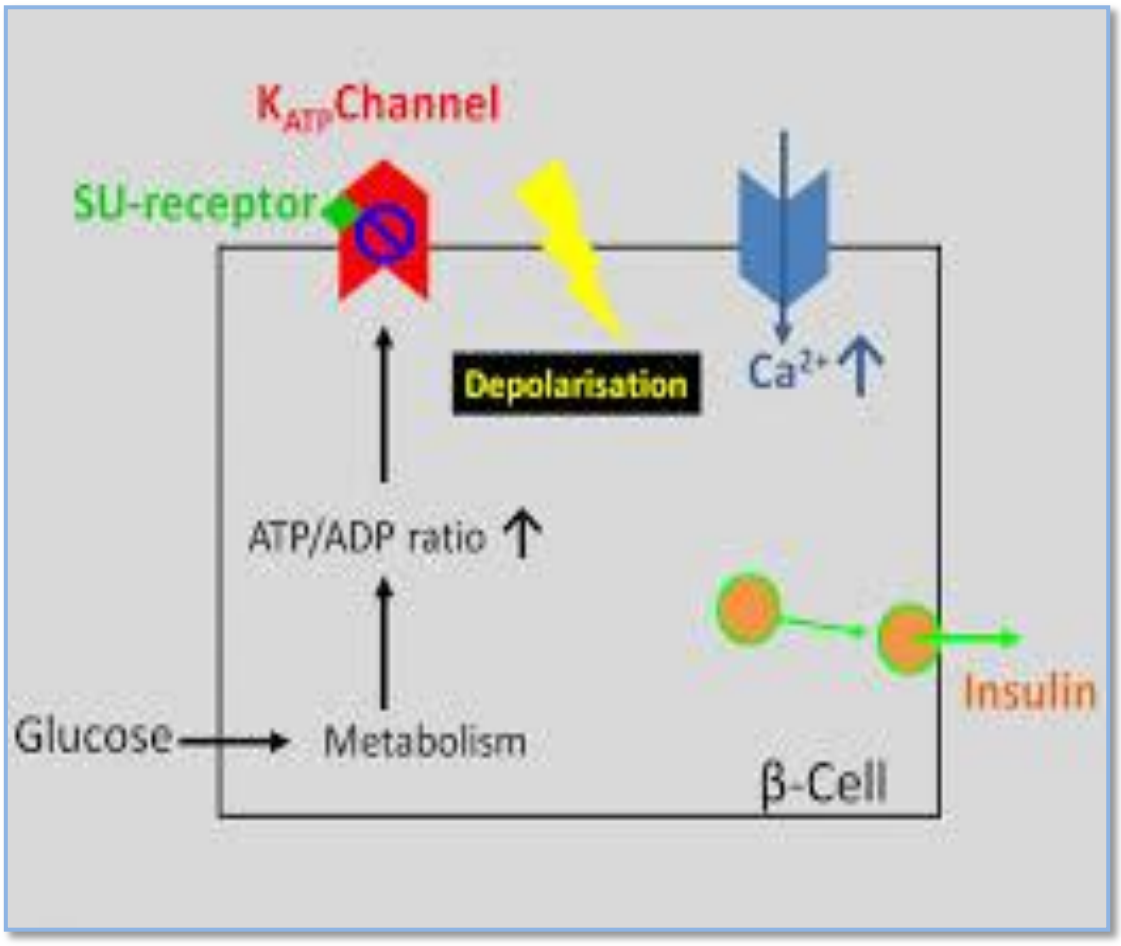


Figure 5: Mode of action of Gliclazide

2.2.4. Metabolism of Gliclazide:

Gliclazide is extensively metabolized in the liver. Less than 1% of the orally administered dose appears unchanged in the urine.Metabolites include oxidized and hydroxylatedderivates, as well as glucuronic acid conjugates.

Table-2: Enzyme responsible for the metabolism of Gliclazide

Enzyme	Metabolites	Reaction
1.Cytochrome P45O 2C9	7-Hydroxygliclazide, 6-Hydroxygliclazide	hydroxylation
2. Cytochrome P4502C19	Methylhydroxygliclazide	

2.2.5. Drug interaction of Gliclazide:

There have been several evidence of drug- drug interaction of gliclazide. Some of these drug interactions are given below:

ACE inhibitors (e.g., enalapril)	Nonsteroidal anti-inflammatory drugs(e.g., ibuprofen, naproxen)
Anticoagulants (e.g., warfarin, heparin)	acetylsalicylic acid· Salicylates
Barbiturates (e.g., phenobarbital, thiopental)	Other antidiabetic drugs (e.g., insulin, metformin)
Beta-blockers (e.g., metoprolol, propranolol)	Tuberculosis medications (e.g., isoniazid, ethambutol)
Clarithromycin	· Corticosteroids (e.g., prednisone)
Danazol	Disopyramide
Diuretics (e.g., thiazides, furosemide)	Fibrates (e.g., fenofibrate)
Monoamine oxidase inhibitors(e.g., selegiline, phenelzine)	H_2 receptor antagonists(e.g., ranitidine, famotidine, cimetidine)

2.3.1. Naproxen

Naproxen is a nonsteroidal anti-inflammatory drug (NSAID) with analgesic and antipyretic properties. This drug is indicated for the relief of the signs and symptoms of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and juvenile arthritis. Naproxen is also indicated for the relief of the signs and symptoms of tendonitis, bursitis and acute gout; for the management of pain and primary dysmenorrhoea.

Naproxen is rapidly and completely absorbed from the gastrointestinal tract with an in vivo bioavailability of 95%.The plasma half-life of naproxen anion in human ranges from 12 to 17 hours (mean:14 hours). Steady-state levels of naproxen are reached in 4 to 5 days, and the degree of naproxen accumulation is consistent with this half-life. The corresponding half-lives of both naproxen's metabolites and conjugates are shorter than 12 hours, and their rates of excretion have been found to coincide closely with the rate of naproxen disappearance from plasma. Small amounts, 3% or less of the administered dose, are excreted in feces. In patients with renal failure, metabolites may accumulate.

Naproxen is a propionic acid derivative related to the arylacetic acid group of nonsteroidal anti-inflammatory drugs. Naproxen (CAS 22204-53-1) and naproxen sodium (CAS-26159-34-2) are known chemically as (S)-6-methoxya- methyl-2-naphthaleneacetic acid and (S)-6-methoxy-α- methyl-2-naphthaleneacetic acid, sodium salt, respectively. Naproxen has a molecular weight of 230.26 and a molecular formula of $C_{14}H_{14}O_3$. Naproxen sodium has a molecular weight of 252.23 and a molecular formula of $C_{14}H_{13}NaO_3$. Naproxen is an odorless, white to off-white crystalline substance. It is lipid soluble, practically insoluble in water at low pH and freely soluble in water at high pH. The octanol/ water partition coefficient of naproxen at pH 7.4 is 1.6 to 1.8. Naproxen sodium is an odorless crystalline powder, white to creamy in color. It is soluble in methanol and water at neutral pH.

3.3.2. Mode of action of Naproxen

The mechanism of action of the naproxen anion, like that of other NSAIDs, is not completely understood but may be related to prostaglandin synthesis inhibition. NSAIDs inhibit both cyclooxygenase-1 (COX-1) and cyclooxygenase- 2 (COX-2) activities, and thereby inhibit synthesis of prostaglandins and thromboxane. The inhibition of COX-2 is thought to mediate the antipyretics, analgesic, and anti-inflammatory action of NSAIDs. Naproxen is rapidly and completely absorbed from the gastrointestinal tract with an in vivo bioavailability of 95%. The rapidity, but not the extent, of absorption is influenced by the presence of food in the stomach. After administration of naproxen and naproxen sodium tablets, peak plasma levels are attained in2 to 4 hours and 1 to 2 hours, respectively.

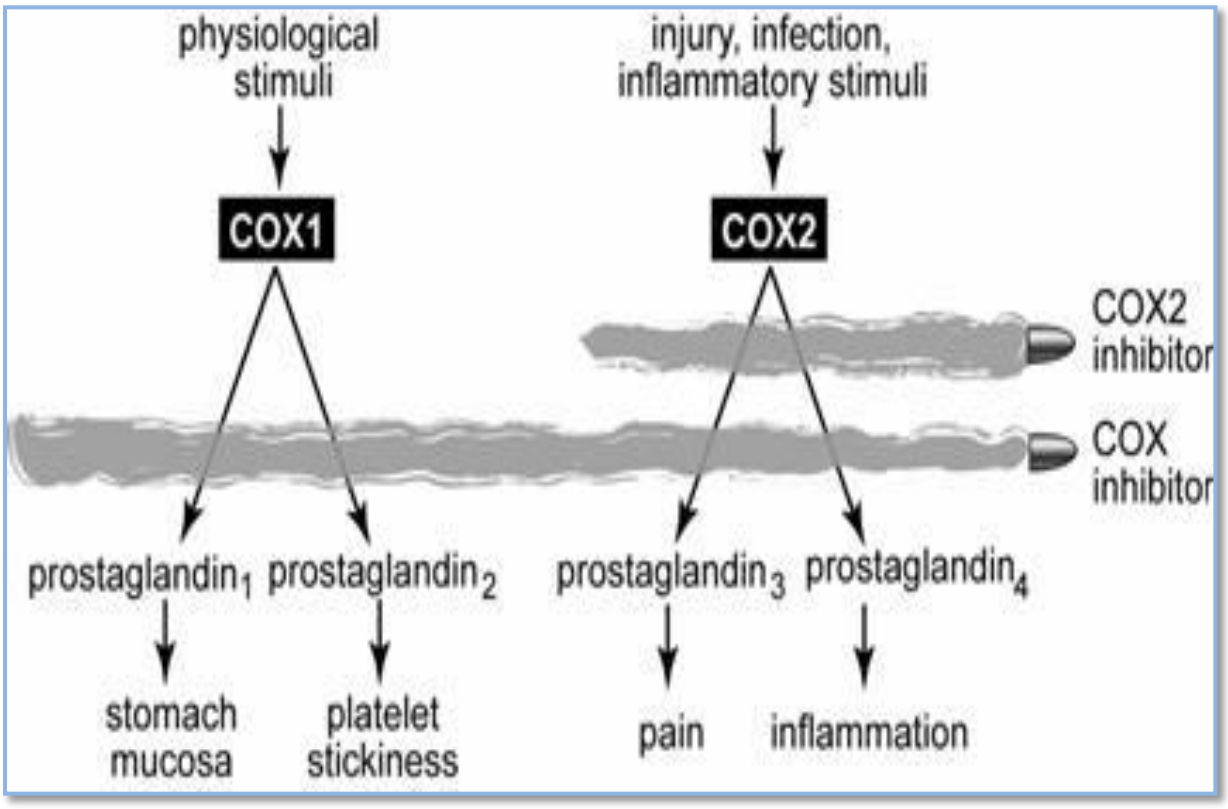


Figure 6: Mode of action of Naproxen

2.3.3. Metabolism of Naproxen

(*S*)-Naproxen is metabolized by CYP2C9, CYP2C19 CYP1A2, UGT2B7, and SULT1A1 (Rodrigues et al., 1996; Bowalgaha et al., 2005; Falany et al., 2005). (*S*)-Naproxen is metabolized in humans by acyl glucuronidation, O-demethylation, and further sulfation and glucuronidation.Proposed metabolic pathways of (*S*)-naproxen in humans. This figure was drawn from the data of Sugawara et al. (1978).

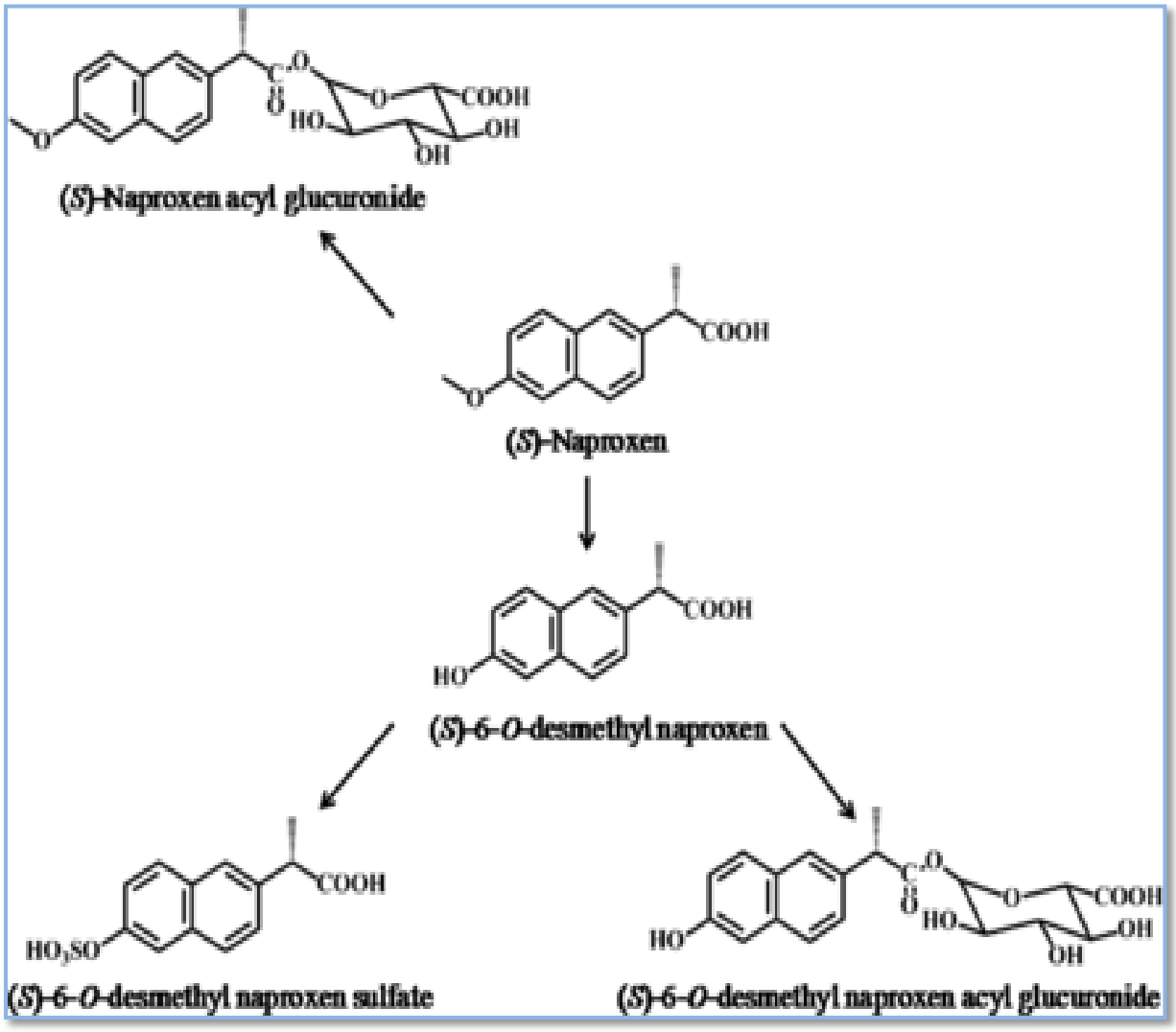


Figure 7: Metabolism of Naproxen

2.4. Pharmacokinetic Consideration

Pharmacokinetic interaction is one of the most important factor that can have a decisive moment in the efficacy of the treatment of diabetes. From the choice of drugs we have selected in terms of the drugs commonly prescribed to the diabetic patient for treatment, the properties of the drug have shown that they are highly protein bound, gliclazide 94% and that of naproxen is 90%. On the other hand, the half-life of both drug is low, gliclazide is lowest among them. We also come to know from the lethal dose concentration that naproxen is highly toxic in comparison to gliclazide. The hypothesis suggest us that since both of them are highly protein bound drug, there will be high competition to replace one another from the site of action, the common being the displacement of gliclazide by naproxen. Since naproxen has a few hours more elimination half life than gliclazide it can have more pharmacological effect than gliclazide to show its effect.

Basic knowledge about the side effect of NSAIDs suggest that in the long run they can cause serious damage like gastric wall perforation, and naproxen is not recommended in acute gout under 16 years of age, for the pharmacokinetic interaction the efficacy of the drug would be less.

3. Method and materials

3.1 Study Design

All the subjects were divided into three different groups. Each group containing 12 volunteers.

Group-G: To determine plasma drug gliclazide concentration at different time period after oral administration of gliclazide in a group comprising of 12 Diabetic subjects.

Group- GN/NG: To determine plasma drug concentrations of gliclazide and naproxen at different time periods after oral administration of naproxen and gliclazide tablets simultaneously in the same12 Diabetetic subjects.

Group- N : To determine plasma drug, naproxen concentration at different time period after oral administration of naproxen in a group comprising of 12 healthy subjects to consider as a normal or population pharmacokinetic of it and comparison

3.2 Ethics

3.2.1 Standards of Practice

The studies were conducted in accordance with the International Conference of Harmonization (ICH) for Good Clinical Practice (GCP) and in compliance with the Declaration of Helsinki and its further amendments .Ethical Permission was taken to approve the protocol. Each volunteer signed an informed consent document before entering the study and was free to withdraw from the study at any time without any obligation.

3.2.2 Ethical and Regulatory Authority Approval

All the national legal requirements were fulfilled which are relevant to the study. Before conducting the study, the volunteers were given both verbal and written information about the study in easy language and terminologies. Only those were registered who provided the written consent.

3.3. Data Collection Form

Title of Research: Pharmacokinetics of Naproxen in Bangladeshi Type II diabetic Patients treated with Gliclazide

1. Identification:

1.1 ID code:

1.2 Name:

1.3 Father's name:

1.4 Sex

1.5 Marital status:

1.6 Date of Birth

1.7 Age:

1.8 Mailing address:

1.9 Permanent address:

1.10Telephone No:

1.11Religion:

2. Personal history

2.1 Area of residence:

	Rural	**Urban**
Where have you spent your boyhood (1-15Y)?		
Where have you spent at least 1/3rd or more of your life time?		

2.2Educational level

Illiterate can read	
Only can write	
SSC or Equivalent	
HSC or Equivalent	
Graduate or Higher	

2.3 Occupation

Student		Unemployed	
Business		Housewife	
Technical		Others	

2.4 Impression about social class:

Rich Upper		Lower middle	
Middle		poor	

2.5 Smoking habit:

Never	
Ex-smoker	
Current smoker	

3. Biophysical characteristics

Height		Temperature	
Weigh		BP	
Pulse/min		BMI	

4. Medical history:

Previous history of hypersensitivity reaction to any drug	**Yes**		**No**	
Previous history of renal dysfunction	**Yes**		**No**	
Previous history of hepatic dysfunction	**Yes**		**No**	
Previous history of GIT problem	**Yes**		**No**	

3.4 Consent form

Consent Form

Department of Pharmaceutical Technology

University of Dhaka

Dhaka.

Person agreement to the study

(Approved by BIRDEM)

Person Details

Name of Person……..

Date of Birth: ……..

Responsible health professional……

Job title…….

Identification No……..

Contact address……..

Phone No…….

Special requirement……

……. **…….**

Signature of the volunteers **Signature of the Supervisor**

3.5 Subjects

Twenty four Bangladeshi male volunteers, divided into three groups were used in this study.

12 Type II Diabetic subjects (G and GN/NG group) Mean age 43.5Y old and BMI; 21.23-23.56 kg/m^2, Blood pressure; 120-130 (mmHg for systolic)/80-85 (mmHg for diastolic) were participated in the study.

12 Healthy subjects (N group) Mean age 22.5 Y old , BMI= 18.13 – 22.97 kg/m^2 , Blood pressure (100 – 125 mmHg for systolic, and 60 – 80 mmHg for diastolic). Pulse rate between 60 and 90 bpm.

All volunteers were in a good health condition on the basis of medical history, physical examination, routine blood test, and possessing negative test for hepatitis B surface antigen (HBs-Ag), anti-hepatitis-C antibody (anti-HCV), and anti-HIV. Volunteers with known contraindication or hypersensitivity to gliclazide were excluded as well as those with known history of drug abuse, alcohol consumer, or cigarette smokers. No drug was allowed 1 month before the study period to avoid the effect of inducting or inhibiting hepatic metabolizing enzyme and the risk of drug interaction. The method and condition of the study were clearly informed to all volunteers, and the signed informed consent was obtained from all volunteers before entering the study.

3.6. Inclusion and Exclusion Criteria

Inclusion Criteria:

1. Age of healthy patients in the range of 20 to 30 years and average weight is 60 kg.

2. Age of diabetic patients in range 40 to 45 years and average weight is 80 kg.

3. Physical examination, medical history and screening tests found to be normal.

4. Unless any abnormality was considered to be clinically irrelevant to study. Some subjects were diabetic without hypertension.

5. Volunteers who gave their written consent to participate and follow up the study.

6. Average BMI in the range of 22-23kg/m^2.

Exclusion Criteria:

1. Volunteers who suffered with mental or physical illness within the past year were rejected.

2. Whose mental capacity was limited to the extent that he would not be able to provide information regarding side effects of the drug were also excluded.

3. Who had any major systemic disease which could be a source of study results biasness were excluded.

4. User of any known hepatic or renal clearance altering agents for a period of 30 days prior to study initiation were excluded.

5. Person joining in other drug studies within 3 months prior to study were excluded.

6. Drinkers of alcohol or wine were also excluded.

7. Smokers and those who had started smoking recently (3 months) prior to the study were also eliminated.

8. Volunteers with a history of cancer.

9. Habitual blood donors were also excluded.

10. A known history of Gliclazide and Naproxen hypersensitivity prohibited inclusion, as did a history of significant or active allergic reactions.

11. Positive hepatitis B and C surface antigens.

12. Person having any positive symptoms of gastrointestinal diseases which may interfere with absorption, distribution, metabolism or elimination of the studied drug were excluded.

UNIVERSITY OF DHAKA
DEPARTMENT OF PHARMACEUTICAL TECHNOLOGY
DHAKA-1000
VOLUNTEER CONSENT FORM

1.
I .....theundersigned voluntarily agree to take part in, the study: titled as: '**Pharmacokinetics of Naproxen in Bangladeshi Type 2 diabetic Patients treated with Gliclazide,** I understand that the investigation will involve the administration ..... X..... on.....study days in.....dosing sessions at the interval of .....days.

2. I have been given a full explanation by the member of the study team, of the nature, purpose and likely duration of the study and what 1 will be expected to do and I have been advised about my discomfort and possible ill effects on any health or well being which he believes may result.

3. I have been given the opportunity to question the study team, on aspects of the study and have understood the advice and information given as a result.

4. I agree to comply with any instruction given during the study and to cooperate faithfully with the study learn, and to tell immediately, if 1 suffer from any deterioration of any kind in my health or well being or any unexpected or unusual symptoms, however they may have arisen.

5. I agree that I will not seek to restrict the use to which the results of the study may be put in particular, I accept that they may disclose to regulatory authorities for medicines.

6. I understand that I am free to withdraw from the study at any time without needing tojustify my decision,

Signature of the Volunteer Address: .....
Date: .....
.....

Signature of Investigator:
Date: ……

Signature of the Witness:
Date: …….

3.7. Dosage and Drug Administration

From 12 Type II diabetic patients 6 patients were given only Gliclazide and other 6 patients were given both Gliclazide and Naproxen concurrently. Other 12 healthy volunteers were given only Naproxen tablet.

- 1st session: Diabetic subjects only took gliclazide → (G group)
- 2nd Session: Diabetic subjects took both gliclazide and naproxen → (GN/NG group)
- 3rd session: Healthy subjects only took naproxen → (N group)

3.8. Blood Samples Collection

A 5-mL blood sample was collected at predose (0h) and at 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 24h after dose for all three sessions of blood collection. Glucose solution with breakfast was served at 2h, a standard lunch was served at 5h and snacks was served at 8h after dosing in every session.

3.9. Methodology

Test Methodology of Gliclazide and Naproxen:

For Gliclazide:

Description:

Spread the sample over a piece of white paper and examine visually the colour, nature, odor, presence of any foreign particle and compare with the specifications.

Solubility :

Take required amount of sample with specified amount of solvent. Then shake for about 5 minutes or sonicate if required.

Descriptive term	**Volume of solvent required for 1 g of sample**
Practicaly insoluble in water	More than 10,000 ml
Freely soluble in methylene chloride	From 1 ml to 10 ml
Sparingly soluble in acetone,	From 30 ml to 100 ml
Slightly soluble in ethanol (96 per cent).	From 100 ml to 1000 ml

3.0 Identification

Take about 2.0 mg of sample and about 200 mg of potassium bromide (previously dried at 105^0 C for one hour) in a dry mortar and triturate to a fine powder. Prepare a disc using the resulting powder and scan for IR spectrum. The IR spectrum of sample should correspond to that of similarly prepared standard of Gliclazide.

4.0 related substances (by HPLC)

Chromatographic conditions:

Column **:** C8, 0.25 m x 4 mm, 5-μm

Mobile phase **:** Buffer: Acetonitrile = 55:45

Flow rate **:** 0.9 ml/min

Detector **:** UV

Wave length **:** 235 nm

Injection volume **:** 20 µl.

Column oven temperature **:** Ambient

Run time **:** Twice the retention time of Gliclazide.

Buffer solution preparation:

Add 2 ml of triethylamine and 2 ml of trifluoroacetic acid in 996 ml of water and mix well. Filter through 0.2-µm filter paper and collect the filtrate.

Solvent mixture: Acetonitrile, water (45:55 V/V).

Sample solution:

Dissolve 50 mg of sample in 23 ml of acetonitrile and dilute to 50 ml with water. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

Reference solution (a):

Dilute 1 ml of the sample solution to 100 ml with the solvent mixture. Dilute 10 ml of this solution to 100 ml with the solvent mixture. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

Reference solution (b):

Dissolve 5 mg of sample and 15 mg of gliclazide impurity F CRS in 23 ml of acetonitrile and dilute to 50 ml with water. Dilute 1 ml of this solution to 20 ml with the solvent mixture. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

Reference solution (c):

Dissolve 10 mg of gliclazide impurity F CRS in 45 ml of acetonitrile and dilute to 100 ml with water. Dilute 1 ml of this solution to 100 ml with the solvent mixture. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

System suitability Solution:

Reference solution (b).

Resolution: Minimum 1.8 between the peaks due to Gliclazide and Impurity F.

Relative retention:

With reference to gliclazide (retention time = about 16 min): impurity F = about 0.9.

Procedure:

Equilibrate the column with the mobile phase. Inject the reference solution (a), reference solution (b), reference solution (c) and the sample solution .Calculate the related substance / substances by using following equation. Disregard limit: 0.02 per cent {0.2 times the area of the principal peak in the chromatogram obtained with reference solution (a)}.

Calculation:

% of impurity F:

$$\frac{\text{Peak area of impurity F in sample solution}}{\text{Peak area of impurity F in reference solution (c)}} \times 0.1$$

% of any single impurity:

$$\frac{\text{Peak area of individual single impurity in sample solution}}{\text{Peak area of principle peak in reference solution (a)}} \times 0.1$$

% of sum of impurities other than F:

$$\frac{\text{Sum of the all peaks area except impurity F in sample solution}}{\text{Peak area of principle peak in reference solution (a)}} \times 0.1$$

Impurity b (by hplc)

Chromatographic Conditions:

Column	**:** C8, 0.25 m x 4 mm, 5-μm
Mobile phase	**:** Buffer: Acetonitrile = 55:45
Flow rate	**:** 0.9 ml/min
Detector	**:** UV
Wave length	**:** 235 nm
Injection volume	**:** 50 µl
Column oven temperature	**:** Ambient
Run time	**:** About 16 minutes

Buffer solution preparation:

Add 2 ml of triethylamine and 2 ml of trifluoroacetic acid in 996 ml of water and mix well. Filter through 0.2-µm filter paper and collect the filtrate.

Sample solution:

Dissolve 0.400 g of sample in 2.5 ml of dimethyl sulfoxide and dilute to 10 ml with water. Stir for 10 min, store at 4 °C for 30 min and filter this solution through 0.45- µm disc filter and collect the filtrate.

Reference solution:

Dissolve 20 mg of gliclazide impurity B CRS in dimethylsulfoxide and dilute to 100 ml with the same solvent. To 1 ml of the solution, add 12 ml of dimethyl sulfoxide and dilute to 50 ml with water. To 1 ml of this solution, add 12 ml of dimethyl sulfoxide and dilute to 50 ml with water. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

Relative retention time:

Impurity B = about 8 min.

Procedure:

Equilibrate the column with the mobile phase. Inject the reference solution and the sample solution. Calculate the ppm of impurity B by using following equation.

Calculation:

ppm of impurity B:

$$\frac{\text{Peak area of impurity B in sample solution}}{\text{Peak area of impurity B in reference solution}} \times 0.0002 \times 10{,}000$$

Heavy metals:

pH 3.5 Acetate Buffer:

Dissolve 25.0 g of ammonium acetate in 25 ml of water, and add 38.0 ml of 6 N hydrochloric acid. Adjust if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 ml, and mix.

Thioacetamide Reagent:

Thioacetamide Solution: 4% w/v solution of thioacetamide.

Add 1 ml of a mixture of 15 ml of 1M sodium hydroxide , 5 ml of water and 20 ml of glycerol (85%) to 0.2 ml of thioacetamide solution , heat in a water bath for 20 seconds, cool and use immediately.

Lead Standard Solution (10 ppm Pb):

Dissolve 0.400 g of lead (II) nitrate in sufficient water to produce 250.0 ml (0.1% Pb). Dilute 1 volume of lead standard solution (0.1% Pb) to 10 volumes with water immediately before use(100 ppm Pb). Dilute 1 volume of lead standard solution (100 ppm Pb) to 10 volumes with water immediately before use (10 ppm Pb).

Test solution:

Place 1.5 g of the sample in a clean, dry, 100 ml long-necked combustion flask (a 300 ml flask may be used if the reaction foams excessively). Clamp the flask at an angle of 45°. Add a sufficient volume of a mixture of 8 ml of sulfuric acid and 10 ml of nitric acid to moisten the substance thoroughly. Warm gently until the reaction commences, allow the reaction to subside and add additional portions of the same acid mixture, heating after each addition, until a total of 18 ml of the acid mixture has been added. Increase the amount of heat and boil gently until the solution darkens. Cool, add 2 ml of nitric acid and heat again until the solution darkens. Continue the heating, followed by the addition of nitric acid until no further darkening occurs, then heat strongly until dense, white fumes are produced. Cool, cautiously add 5 ml of water, boil gently until dense, white fumes are produced and continue heating to reduce to 2-3 ml. Cool, cautiously add 5 ml of water and examine the colour of the solution. If the colour is yellow, cautiously add 1 ml of strong hydrogen peroxide solution and again evaporate until dense, white fumes are produced and reduce to a volume of 2-3 ml. If the solution is still yellow in colour, repeat the addition of 5 ml of water and 1 ml of strong hydrogen peroxide solution until the solution is colourless. Cool, dilute cautiously with water and rinse into a 50 ml colour comparison tube, ensuring that the total volume does not exceed 25 ml. Adjust the solution to pH 3.0-4.0, using short range pH indicator paper as external indicator, with concentrated ammonia R1 (dilute ammonia R1 may be used, if desired, as the specified range is approached), dilute with water to 40 ml and mix. Add 2 ml of buffer solution pH 3.5. Mix and add to 1.2 ml of thioacetamide reagent. Mix immediately. Dilute to 50 ml with water and mix.

Reference solution:

Prepare at the same time and in the same manner as the test solution, using 1.5 ml of lead standard solution (10 ppm Pb).

Monitor solution:

Prepare as described for the test solution, adding to the sample (1.5 g) and 1.5 ml of lead standard solution (10 ppm Pb).

Blank solution:

Prepare as described for the test solution, omitting the sample adding.

Procedure:

Examine the solutions vertically against a white background after 2 min.

System suitability:

The reference solution shows a brown colour compared to the blank solution,

The monitor solution is at least as intense as the reference solution.

Result:

Any brown colour in the test solution is not more intense than that in the reference solution.

If the result is difficult to judge, filter the solutions through a suitable membrane filter (nominal pore size 45 µm). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston. Compare the spots on the filters obtained with the different solutions.

Loss on drying:

Dry a crucible at 105 ^{o}C for 15 minutes in a oven. Then remove the crucible from oven and cool to room temperature in desiccator for about 15 minutes. Accurately weigh the empty crucible (W_1). Take about 1.0 g of sample in the crucible and accurately weigh (W_2). Spread the sample by shaking. Place the loaded crucible in the drying oven at 105°C ± 5 °C for 2 hours. Remove the crucible from oven. Cool to room temperature for 15 minutes in desiccator. Weigh the crucible and record the weight (W_3). Calculate the loss on drying as below.

Calculation:

$$\% \text{ of Loss on drying} = \frac{(W_2 - W_3) \times 100}{(W_2 - W_1)}$$

Sulfated ash

Ignite a silica or platinum crucible to redness for 30 minutes allow to cool in a dessicator and weigh the crucible (W_1). Take about 1.0 g of sample in the crucible and accurately weigh (W_2). Heat gently at first, until the substance is thoroughly charred, cool and moisten the residue with 1ml of sulphuric acid, heat gently until white fumes no longer are evolved and ignite at 600 $\pm$ 50^{o}C until the black particles disappear(for about 4 hours). Cool the crucible in a dessicator and weigh. If the weight of the residue is more than 0.5 mg again moisten the residue with 1 ml of Sulphuric acid, heat and ignite as before. Allow to cool and weigh (W_3). Repeat the operation to constant weight.

Calculation:

$$\% \text{ of Sulphated ash} = \frac{(W_3 - W_1) \times 100}{(W_2 - W_1)} \%$$

Assay:

Transfer about 250 mg of sample into a 250 ml conical flask. Dissolve in 50 ml of anhydrous acetic acid. Titrate with 0.1 M perchloric acid, determine the end point potentiometrically. Perform a blank determination, and make any necessary correction.

Each ml of 0.1 M perchloric acid is equivalent to 32.34 mg of Gliclazide.

Calculation:

Content of Gliclazide (on dried basis):

$$\frac{V \times F \times 32.34 \times 100 \times 100}{WT \times (100\text{-}X)} \%$$

Where, V = Volume of 0.1 M perchloric acid required in ml for sample titration

F = Molarity factor of 0.1 M perchloric acid

WT = Weight of the sample taken in mg.

X = Loss on drying

Alternative Assay Method-I (By HPLC):

Chromatographic conditions, buffer solution preparation, solvent mixture and sample solution preparation as like as related substances.

Standard solution:

Dissolve 50 mg of standard Gliclazide in 23 ml of acetonitrile and dilute to 50 ml with water. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

Procedure:

Equilibrate the column with the mobile phase for about 30 minutes. Inject standard solution and sample solution into the chromatograph, record the chromatograms and measure the responses for the major peaks. The relative standard deviation of standard solution for replicate injections is not more than 2.0%. Calculate the quantity in % of Gliclazide using following equation.

Calculation:

Content of Gliclazide (in % on dried basis):

$$\frac{PT}{PS} \times \frac{WS}{50} \times \frac{50}{WT} \times \frac{P}{(100-x)} \times 100$$

PT = Peak area of Gliclazide in the sample solution

PS = Average peak area of Gliclazide in the standard solution.

WS = Weight of the Gliclazide in mg.

WT = Weight of the sample in mg.

P = Potency of the standard Gliclazide in % on as is basis

x = % of Loss on drying

Alternative Assay Method-II (By UV):

Standard preparation:

Take about 30 mg of standard Gliclazide in a 100 ml volumetric flask. Add 70 ml of mixture of 2 volumes acetonitrile and 3 volumes water and shake mechanically to dissolve. Make volume up to the mark with same solvent and mix. Dilute 5 ml of this solution to 100 ml with same solvent and mix.

Sample preparation:
Take about 30 mg of sample in a 100 ml volumetric flask. Add 70 ml of mixture of 2 volumes acetonitrile and 3 volumes water and shake mechanically to dissolve. Make volume up to the mark with same solvent and mix. Dilute 5 ml of this solution to 100 ml with same solvent and mix.

Blank Solution: A mixture of 2 volumes acetonitrile and 3 volumes water**.**

Procedure:

Measure the absorbance of the resulting standard and sample solutions in 1 cm cell at about 229 nm against the blank solution. Calculate the quantity in % of Gliclazide using following equation.

Calculation:

Content of Gliclazide (in % on dried basis):

$$\frac{AT}{AS} \times \frac{WS}{100} \times \frac{5}{100} \times \frac{100}{WT} \times \frac{P}{5} \times \frac{100}{(100-x)}$$

AT = Absorbance of the sample solution

AS = Absorbance of the standard solution.

WS = Weight of the standard in mg.

WT = Weight of the sample in mg.

P = Potency of the standard Gliclazide expressed in % on as is basis

x = % of Loss on drying

5% dispersion in water

Transfer about 5 g sample into a 250 ml conical flask. Add 100 ml of purified water, shake for 5 minutes and then observe the suspension for any foreign particle.

Chromatogram:

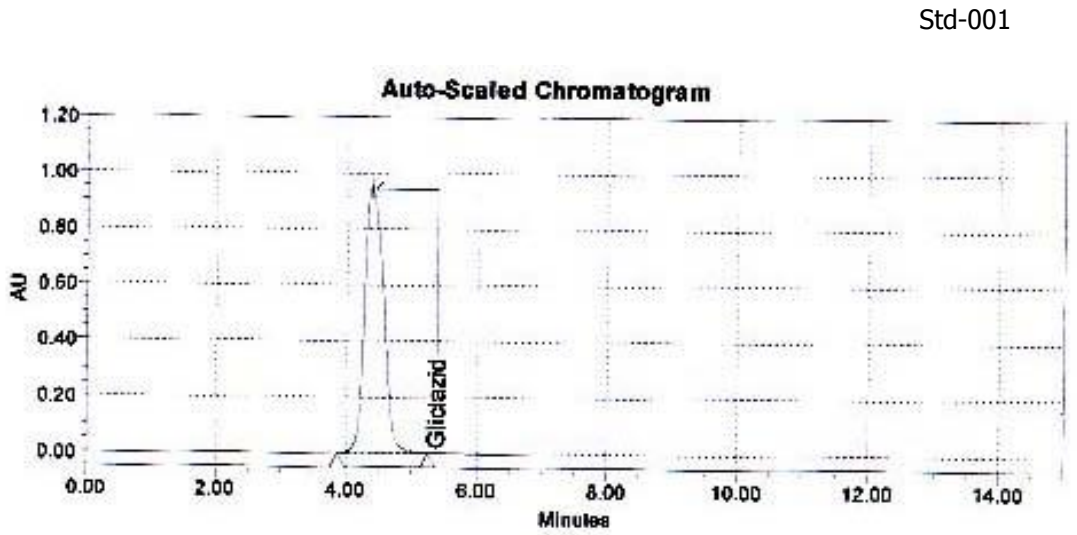


Figure 8: Representative Chromatogram of Gliclazide as standard solution (Alternative assay method-I)

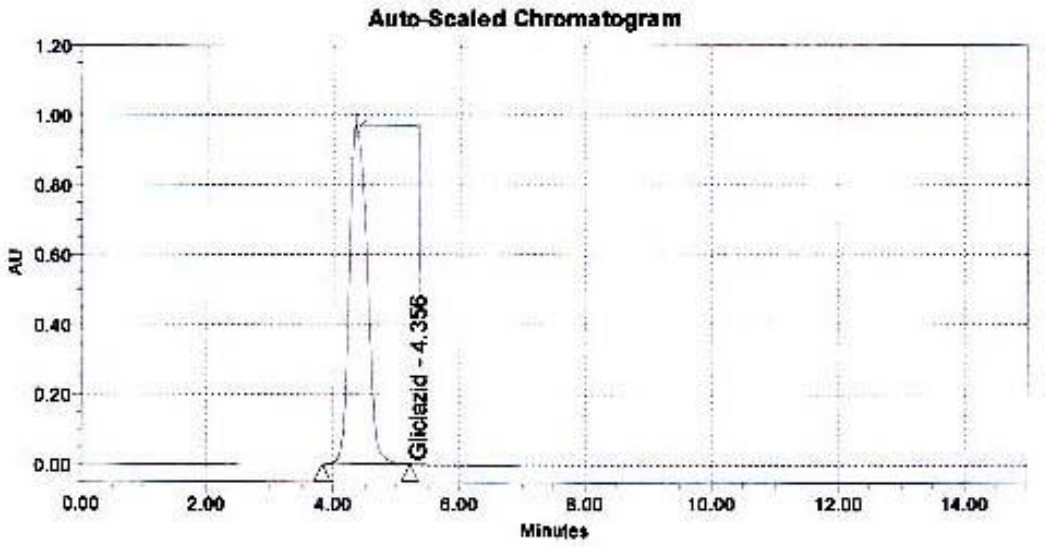


Figure 9: Representative Chromatogram of Gliclazide as sample solution (Alternative assay methodI)

For Naproxen:

Description:

Spread the sample over a piece of white paper and examine visually the colour, nature, odor, presence of any foreign particle and compare with the specifications.

Solubility:

Take required amount of sample with specified amount of solvent. Then shake for about 5 minutes or sonicate if required.

Descriptive term	Volume of solvent required for 1 g of sample
Practicaly insoluble in water	More than 10,000 ml
Soluble in ethanol (96%) and in methanol.	From 10 ml to 30 ml

Identification:

By IR:

Take about 2.0 mg of sample and about 200 mg of potassium bromide (previously dried at 105^0 C for one hour) in a dry mortar and triturate to a fine powder. Prepare a disc using the resulting powder and scan IR spectrum. The IR spectrum of sample should correspond to that of similarly prepared standard of Naproxen.

Specific optical rotation:

Dissolve 0.50 g of the sample into a 25 ml volumetric flask with ethanol (96%). Dilute to volume with same solvent. Measure the optical rotation with a suitable, calibrated polarimeter against ethanol (96%) as blank at 20°c.

Calculation:

$$[\alpha]^{\circ} = \frac{\alpha \times 100 \times 100}{l \times c \times (100 - X)}$$

Where, α = Angle of rotation in degree
l = Path length in decimeter of the polarimeter cell
c = Concentration in g/Dl; X = Loss on drying

Melting point:

Place 1 to 2 mg of sample in a dried capillary tube (with one end closed) and pack in a layer of 4 to 6 mm height by dropping it the closed end down repeatedly. Raise the temperature of the bath to about 10ºC below the presumed melting point and then adjust the rate of heating to about 1 ºC per min. When the temperature is 5 ºC below the presumed melting point, correctly introduce the capillary tube in the melting point apparatus and continue the heating up to melt the sample. Record the temperature at which the last particle passes into the liquid phase.

Appearance of solution:

Sample solution:

Dissolve 1.25 g in methanol and dilute to 25 ml with the same solvent.

Observation:

Solution is clear and not more intensely coloured than reference solution BY_7 , when compare as per following procedure.

Preparation of primary solution:

Yellow Solution:

Dissolve 46 g of ferric chloride R in about 900 ml of a mixture of 25 ml of hydrochloric acid R and 975 ml of water R and dilute to 1000.0 ml with the same mixture. Titrate and adjust the solution to contain 45.0 mg of $FeCl_3,6H_2O$ per ml by adding the same acidic mixture. Protect the solution from light.

Titration: Place in a 250 ml conical flask fitted with a ground-glass stopper, 10.0 ml of the solution, 15 ml of water R, 5 ml of hydrochloric acid and 4 g of potassium iodide R, close the flask, allow to stand in the dark for 15 min and add 100 ml of water. Titrate the liberated iodine with 0.1 M sodium thiosulphate, using 0.5 ml of starch solution , added towards the end of the titration, as indicator.

1 ml of 0.1 M sodium thiosulphate is equivalent to 27.03 mg of $FeCl_3,6H_2O$.

Red Solution:

Dissolve 60 g of cobalt chloride R in about 900 ml of a mixture of 25 ml of hydrochloric acid R and 975 ml of water R and dilute to 1000.0 ml with the same mixture. Titrate and adjust the solution to contain 59.5 mg of $CoCl_2$, $6H_2O$ per milliliter by adding the same acidic mixture.

Titration: Place in a 250 ml conical flask fitted with a ground-glass stopper, 5.0 ml of the solution, 5 ml of dilute hydrogen peroxide solution R and 10 ml of a 300 g/l solution of sodium hydroxide R. Boil gently for 10 min, allow to cool and add 60 ml of dilute sulphuric acid R and 2 g of potassium iodide R. Close the flask and dissolve the precipitate by shaking gently. Titrate the liberated iodine with 0.1 M sodium thiosulphate, using 0.5 ml of starch solution R, added towards the end of the titration, as indicator. The end-point is reached when the solution turns pink.

1 ml of 0.1 M sodium thiosulphate is equivalent to 23.79 mg of $CoCl_2,6H_2O$.

Blue Solution:

Dissolve 63 g of copper sulphate in about 900 ml of a mixture of 25 ml of hydrochloric acid and 975 ml of water R and dilute to 1000.0 ml with the same mixture. Titrate and adjust the solution to contain 62.4 mg of $CuSO_4$, $5H_2O$ per ml by adding the same acidic mixture.

Titration: Place in a 250 ml conical flask fitted with a ground-glass stopper, 10.0 ml of the solution, 50 ml of water R, 12 ml of dilute acetic acid R and 3 g of potassium iodide R. Titrate the liberated iodine with 0.1 M

sodium thiosulphate, using 0.5 ml of starch solution R, added towards the end of the titration, as indicator. The end-point is reached when the solution shows a slight pale brown colour.

1 ml of 0.1 M sodium thiosulphate is equivalent to 24.97 mg of $CuSO_4,5H_2O$.

Preparation of Standard solution BY:

Prepare a mixture of 2.4 ml of yellow solution, 1 ml of red solution, 0.4 ml of blue solution and 6.2 ml of hydrochloric acid(10 g/L).

Preparation of Reference solution BY_7:

Prepare a mixture of 2.5 ml of standard solution BY and 97.5 ml of hydrochloric acid(10 g/L). Prepare the reference solutions immediately before use from the standard solutions.

Procedure:

Using identical tubes of colourless, transparent, neutral glass with a flat base and an internal diameter of 15 mm to 25 mm, compare the sample solution with the reference solution BY_7 the depth of the layer being 40 mm. Compare the colours in diffused daylight, viewing vertically against a white background.

Enantiomeric purity (by HPLC)
impurity g:

Chromatographic Conditions:

Column	**:** 0.25 m x 4.6 mm, Silica gel p-acceptor/p-donor for chiral separations (5 µm) (S,S);
Mobile phase	**:** Glacial acetic acid**:** Acetonitrile**:**2-propanol**:**Hexane(5:50:100:845 V/V/V/V)
Flow rate	**:** 2 ml/min
Detector	**:** UV
Wave length	**:** 263 nm
Injection volume	**:** 20 µl.
Column oven temperature	**:** 25 °C
Run time	**:** 1.5 times the retention time of naproxen (retention time = about 5 min).

Sample solution:

Dissolve 25.0 mg of sample in tetrahydrofuran and dilute to 50 ml with the same solvent. Dilute 2 ml of this solution to 20 ml with the mobile phase. Then filter this solution through 0.45-µm disc filter and collect the filtrate.

Reference solution (a):

Dilute 2.5 ml of the sample solution to 100 ml with the mobile phase. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

Reference solution (b):

Dissolve 5 mg of racemic naproxen CRS in 10 ml of tetrahydrofuran and dilute to 100 ml with the mobile phase. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

System suitability Solution:

Reference solution (b).

Resolution: Minimum 3 between the peaks due to Naproxen and Impurity G.

Procedure:

Equilibrate the column with the mobile phase. Inject the reference solution (a), reference solution (b) and the sample solution. Calculate the % of impurity G in the taken portion of Naproxen.

Calculation:

% of impurity G:

$$\frac{\text{Peak area of impurity G in sample solution}}{\text{Peak area of principle peak in reference solution (a)}} \times 2.5$$

Related substances (by HPLC):

Chromatographic Conditions:

Column	**:** C_{18}, 0.10 m x 4 mm ,3-µm
Mobile phase	**:** Buffer: Acetonitrile = 58:42
Flow rate	**:** 1.5 ml/min
Detector	**:** UV
Wave length	**:** 230 nm
Injection volume	**:** 20 µl.
Column oven temperature	**:** 50 °C
Run time	**:** 1.5 times the retention time of impurity N.

Buffer solution preparation:

1.36 g/L solution of potassium dihydrogen phosphate previously adjusted to pH 2.0 with phosphoric acid. Filter through 0.2-µm filter paper and collect the filtrate.

Sample solution:

Dissolve 12 mg of sample in the mobile phase and dilute to 20 ml with the mobile phase. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

Reference solution (a):

Dilute 1.0 ml of the sample solution to 50.0 ml with the mobile phase. Dilute 1 ml of this solution to 20 ml with the mobile phase. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

Reference solution (b):

Dissolve 6 mg of bromomethoxynaphthalene (impurity N), 6 mg of 1-(6-methoxynaphthalen-2-yl)ethanone (impurity L) and 6 mg of (1RS)-1-(6-methoxynaphthalen-2-yl)ethanol(impurity K) in acetonitrile and dilute to 10 ml with the same solvent. To 1 ml of this solution add 1 ml of the sample solution and dilute to 50 ml with the mobile phase. Dilute 1 ml of this solution to 20 ml with the mobile phase. Then filter this solution through 0.45- µm disc filter and collect the filtrate.

System suitability Solution:

Reference solution (b).

Resolution: Minimum 2.2 between the peaks due to Naproxen and Impurity K.

Relative retention:

With reference to naproxen (retention time = about 2.5 min): impurity K = about 0.9; impurity L = about 1.4; impurity N = about 5.3.

Procedure:

Equilibrate the column with the mobile phase. Inject the reference solution (a), reference solution (b) and the sample solution .Calculate the related substance / substances by using following equation. Disregard limit: 0.05 per cent.

Calculation:

% of impurity L:

$$\frac{\text{Peak area of impurity L in sample solution}}{\text{Peak area of impurity L in reference solution (b)}} \times 0.1$$

% of any single impurity:

$$\frac{\text{Peak area of any single impurity in sample solution}}{\text{Peak area of principle peak in reference solution (a)}} \times 0.1$$

% of Total impurities:

$$\frac{\text{Sum of the all peaks area in sample solution}}{\text{Peak area of principle peak in reference solution (a)}} \times 0.1$$

Heavy metals:

pH 3.5 Acetate Buffer:

Dissolve 25.0 g of ammonium acetate in 25 ml of water, and add 38.0 ml of 6 N hydrochloric acid. Adjust if necessary, with 6 N ammonium hydroxide or 6 N hydrochloric acid to a pH of 3.5, dilute with water to 100 ml, and mix.

Thioacetamide Reagent:

Thioacetamide Solution: 4% w/v solution of thioacetamide.

Add 1 ml of a mixture of 15 ml of 1M sodium hydroxide , 5 ml of water and 20 ml of glycerol (85%) to 0.2 ml of thioacetamide solution , heat in a water bath for 20 seconds, cool and use immediately.

Lead Standard Solution (10 ppm Pb):

Dissolve 0.400 g of lead (II) nitrate in sufficient water to produce 250.0 ml (0.1% Pb). Dilute 1 volume of lead standard solution (0.1% Pb) to 10 volumes with water immediately before use(100 ppm Pb). Dilute 1 volume of lead standard solution (100 ppm Pb) to 10 volumes with water immediately before use (10 ppm Pb).

Sample solution:

Place 2 (g) in a silica crucible with 4 ml of a 250 g/l solution of magnesium sulphate in dilute sulphuric acid R. Mix using a fine glass rod. Heat cautiously. If the mixture is liquid, evaporate gently to dryness on a water-bath. Progressively heat to ignition and continue heating until an almost white or at most greyish residue is obtained. Carry out the ignition at a temperature not exceeding 800 °C. Allow to cool. Moisten the residue with a few drops

of dilute sulphuric acid. Evaporate, ignite again and allow to cool. The total period of ignition must not exceed 2 h. Take up the residue in 2 quantities, each of 5 ml, of dilute hydrochloric acid. Add 0.1 ml of phenolphthalein solution R, then concentrated ammonia until a pink colour is obtained. Cool, add glacial acetic acid R until the solution is decolorised and add 0.5 ml in excess. Filter if necessary and wash the filter. Dilute to 20 ml with water.

Standard solution:

Prepare as described for the sample solution, using 2 ml of lead standard solution (10 ppm Pb) instead of sample solution. To 10 ml of the solution obtained add 2 ml of the sample solution.

Monitor solution:

Prepare as described for the sample solution, adding to the sample the volume of lead standard solution (10 ppm Pb) (2 ml) for preparation of the standard solution. To 10 ml of the solution obtained add 2 ml of the sample solution.

Blank solution:

A mixture of 10 ml of water and 2 ml of the sample solution.

Procedure:

To 12 ml of each solution, add 2 ml of buffer solution pH 3.5 R. Mix and add to 1.2 ml of thioacetamide reagent R. Mix immediately. Examine the solutions after 2 min. The test is invalid if the reference solution does not show a slight brown colour compared to the blank solution or if the monitor solution is not at least as intense as the standard solution. The material complies with the test if any brown colour in the test solution is not more intense than that in the standard solution.

If the result is difficult to judge, filter the solutions through a membrane filter (pore size 3 µm; see Figure 2.4.8.-1, without the prefilter). Carry out the filtration slowly and uniformly, applying moderate and constant pressure to the piston. Compare the spots on the filters obtained with the different solutions.

Calculation:

$$\% \text{ of Loss on drying} = \frac{(W_2 - W_3) \times 100}{(W_2 - W_1)}$$

Sulfated ash:

Ignite a silica or platinum crucible to redness for 30 minutes allow to cool in a dessicator and weigh the crucible (W_1). Take about 1.0 g of sample in the crucible and accurately weigh (W_2). Heat gently at first, until the substance is thoroughly charred, cool and moisten the residue with 1ml of sulphuric acid, heat gently until white fumes no longer are evolved and ignite at 600 $\pm$ 50^{o}C until the black particles disappear(for about 4 hours). Cool the crucible in a dessicator and weigh. If the weight of the residue is more than 0.5 mg again moisten the residue with 1 ml of Sulphuric acid, heat and ignite as before. Allow to cool and weigh (W_3). Repeat the operation to constant weight.

Calculation:

$$\% \text{ of Sulphated ash} = \frac{(W_3 - W_1) \times 100}{(W_2 - W_1)} \%$$

Assay:

Transfer about 200 mg of sample into a 250 ml conical flask. Dissolve in a mixture of 25 ml water and 75 ml of methanol. Add 1 ml of phenolphthalein solution and mix. Titrate with 0.1 M sodium hydroxide. Perform a blank determination, and make any necessary correction.

Each ml of 0.1 M sodium hydroxide is equivalent to 23.03 mg of Naproxen.

Calculation:

Content of Naproxen (on dried basis):

$$\frac{V \times F \times 23.03 \times 100 \times 100}{WT \times (100\text{-}X)} \%$$

Where, V = Volume of 0.1 M Sodium Hydroxide required in ml for sample titration

F = Molarity factor of 0.1 M Sodium Hydroxide

WT = Weight of the sample taken in mg.

X = Loss on drying

Alternative Assay Method (By UV):

Standard preparation:

Transfer about 40 mg of standard Naproxen into a 100 ml volumetric flask. Add 70 ml of methanol. Shake to dissolve and dilute up to volume with same solvent. Dilute 10 ml of this solution to 50 ml with the same solvent.

Sample preparation:

Transfer about 40 mg of sample into a 100 ml volumetric flask. Add 70 ml of methanol. Shake to dissolve and dilute up to volume with same solvent. Dilute 10 ml of this solution to 50 ml with the same solvent.

Blank solution: Methanol.

Procedure:

Measure the absorbance of the resulting standard and sample solutions in 1 cm cell at about 331 nm against the blank solution. Calculate the quantity of Naproxen in sample solution by using following equation.

Calculation:

$$\textbf{Content of Naproxen (\%) (On dried basis)} = \frac{AT}{AS} \times \frac{WS}{100} \times \frac{10}{50} \times \frac{100}{WT} \times \frac{50}{10} \times \frac{P}{(100-X)} \times 100$$

Where,

AT = Absorbance of the sample solution

AS = Absorbance of the standard solution

WS = Weight of the standard in mg

WT = Weight of the sample in mg

X = % of Loss on drying

P = Potency of the standard in % on as is basis

5% dispersion in water

Transfer about 5 g sample into a 250 ml conical flask. Add 100 ml of purified water, shake for 5 minutes and then observe the suspension for any foreign particle.

3.9.1. Sample Preparation and HPLC Condition

Blood samples were centrifuged for 10 min at 3000 rpm and the plasma was taken and immediately kept at −20°C until assay. The drug was extracted from the plasma by solid phase extraction using a 5-µm C-18 reversed phase extraction column (Strata, Phenomenex, US.). The drug content was determined by HPLC with a UV detector (Shimadzu, Japan). Determination method was modified from the assay validation employed by Noguchi et al. Chromatographic system consisted of a 5-μm C-18 reversed phase column equipped with a guard column of the same material (Inertsil ODS-3, GL Sciences, Japan) and the UV detector set at 230 nm. Mobile phase was a mixture of 10 mMperchloric acid (pH 3.5)/acetonitrile/2-propranolol/triethylamine (500/230/230/0.5 v/v).

The system was an isocratic run with a flow rate of 1.0 mL/min at 55°C using glibenclamide as an internal standard. A linear regression was performed to determine the drug concentration in the range of 10–2000 ng/mL. The lower limit of quantitation was 10.0 ng/mL with the accuracy (%) and precision (CV %) of 114.26 and 14.99, respectively. The accuracy and precision for intra-day assay validation were 104.79 and 8.56, respectively, and for inter-day assay validation were 99.85 and 8.88, respectively. The phase extraction validation was presented by average accuracy and precision of 96.86% and 6.27% for a fasted unknown sample and 95.69% and 10.39% for a fed unknown sample, respectively. Less than 67% (4 out of 6) of OC samples were within 15% deviated of their respective nominal value.

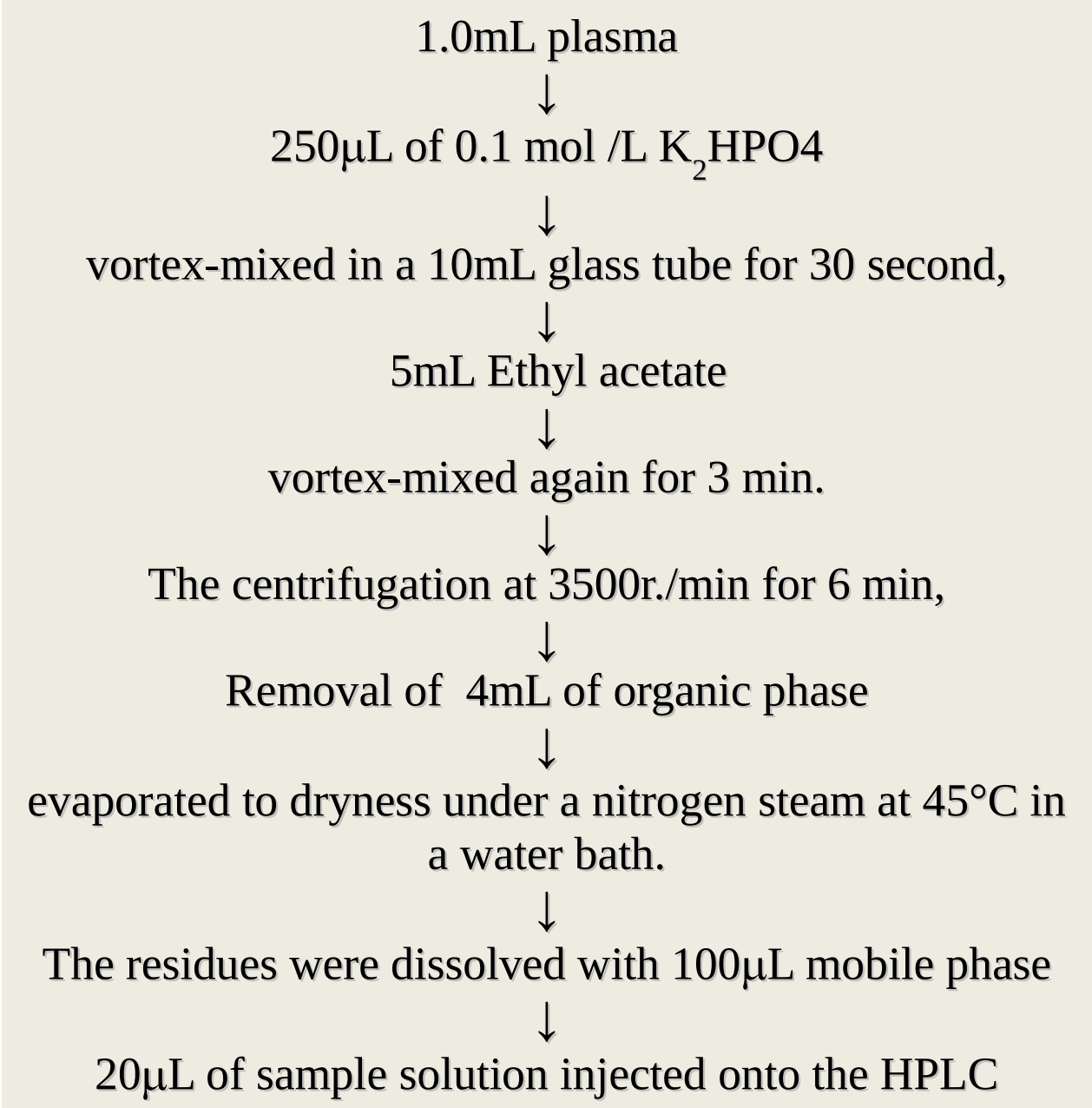


Figure 10: Flow chart of sample preparation for HPLC column.

3.9.2 HPLC Method of Analysis

The drug content was determined by HPLC with a UV detector (Shimadzu, Japan). Determination method was modified from the assay validation employed by Noguchi et al. for gliclazide and Setiawati E et al. for naproxen. Chromatographic system consisted of a 5-µm C-18 reversed phase column equipped with a guard column of the same material (Inertsil ODS-3, GL Sciences, Japan) and the UV detector set at 230 nm for gliclazide and at 240 nm for naproxen.

3.9.3. Chromatographic condition:

Parts	Condition
Column	Luna C_{18} (250x4.6mm,5u)
Mobile phase for naproxen	Acetonitrile: KH_2PO4 25 mM pH 3.00 (45 : 55, v/v).
Mobile phase for gliclazide	45:55 v/v acetonitrile and water.
The flow rate	1.0ml /min.
Effluent monitored	at 230 nm and 240 nm for gliclazide and naproxen respectedly

3.9.4 Method Validation

The method was validated according to Good Laboratory Practice (GLP) standards to achieve the unbiased results that could be properly interpreted. The linearity, specificity, system suitability, accuracy and precision (Intra- and inter-day) of this method indicate that the method is quite suitable to get the reliable results as stated in guidelines (ICH Guidelines, 1996; FDA Guidance for industry, 1998).Plasma calibration curve was drawn from concentration versus area of Gliclazide and Naproxen. Least squared regression analysis was then used to calculate the concentration of Gliclazide and Naproxen.

Limits of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) were estimated from the signal-to-noise ratio. A signal larger than two to three times of the base line noise is defined as limit of detection (LOD). For LOQ a signal at least five times greater than the base line noise detected with a sufficient precision (< 20 %) and accuracy (> 80 %).

Linearity

Working standard solutions of Gliclazide and Naproxen were used for the evaluation of linearity. The calibration curve was constructed by plotting concentration versus area in plasma. Linear regression analysis was performed to investigate the linearity.

Recovery

The recovery was examined from quality control samples (QCs) for low, medium and high concentration ranges in plasma samples. Concentration from extracted quality control samples was compared with those obtained after injection of known volumes of stock solutions which were prepared with the same process as the QCs.

Precision and Accuracy

The accuracy was mentioned as the percentage of analytes recovered by the assay. System precision was calculated from results from six replicate injections of the QCs. Analysis of the QCs was used to determine the precision (Intra-day and inter-day) and accuracy of the assay. For this purpose analysis of QCs at each concentration was performed on three consecutive days. After this, in plasma intra- and inter-day means, coefficients of variation and standard deviations were calculated.

3.9.5 Pharmacokinetic Parameters

Different pharmacokinetic parameters were calculated on the basis of plasma concentration of the Gliclazide and Naproxen obtained from each volunteer at different time intervals. For this purpose PC Computer Program, APO, MWPHARMversion3.02, a MEDIWARE product, Holland, was used. The following pharmacokinetic parameters were evaluated:

AUC_{0-t} = The area under the plasma concentration-time curve from time 0 to the last time point.

$AUC_{0-\infty}$ = The area under the plasma concentration-time curve from time 0 to infinity.

C_{max} = Maximum plasma concentration

T_{max} = Time at which Cmax occurred

K_{el} = Elimination rate constant

$T_{1/2}$ = Elimination half-life

3.9.6. Reagents and Chemicals

Reagents and chemicals used in the present study experiment were collected from the following sources

	Reagents	Sources
HPLC analysis Reagent		
1.	Reference standard naproxen and gliclazide	Dr. Reddy's lab. Ltd. India.
2.	HPLC grade Acetonitrile.	Active Fine Chemicals
3.	Analytical grade Ammonium acetate.	Merck , Germany
4.	Analytical grade Glacial Acetic Acid.	Scharlau, Spain
5.	Analytical grade Dipotassium Hydrogen Phosphate.	Scharlau , Spain
6.	Analytical grade Ethyl acetate	RCI Labscan , Thailand
7.	Analytical grade Dimethyl sulfoxide (DMSO)	Scharlau, Spain

4. Result and Discussion

4.1. Volunteer Demography and Safety

Twelve healthy Bangladeshi male volunteers participated in the study. Their mean values of age, weight and height were 22.5± 2.75 yr, 60.46 ± 4.60 kg and 162.92 ± 6.3cm respectively. Diabetic subjects were of age, weight and height: 43.5 Yr, 81kg and 162.92 cm. Demographic features of the volunteers categorized into group no. 1 and group no. 2 are summarized in Table-3, Table-4. The minimal pre-dose blood glucose level was 75 mg/dL. The dose regimen was well tolerated in all volunteers. There was no complaint on the therapy or presence of sign and symptom of hypoglycemia. Three volunteers had fever, sore throat, and running nose on the third visit of the study while one volunteer had stuffy nose and running nose on the forth visit. Necessary treatment was provided.

Table-3: Demographic features of healthy subject

SL No.	**Age (Yrs)**	**Weight (Kg)**	**Height (cm)**	**Sequence of administration**	
				First session	Second session
1.	23	64	157.48	G	GN
2.	22	50	160.02	G	GN
3.	18	55	162.56	G	GN
4.	20	52	165.10	G	GN
5.	24	58	152.40	G	GN
6.	22	60	157.48	G	GN
7.	2	63	165.10	G	GN
8.	23	55	167.64	G	GN
9.	19	58	160.02	G	GN
10.	21	56	162.56	G	GN
11.	23	63	152.40	G	GN
12.	23	65	152.40	G	GN
Mean	**22.5**	**60.46**	**162.92**		
±SD	**2.75**	**4.60**	**6.30**		

Table-4: Demographic Features of Diabetes Subjects

SL No.	**Age (Yrs)**	**Weight (Kg)**	**Height (cm)**	**Sequence of administration**	
				First session	Second session
1.	43	79	157.48	G	GN
2.	44	84	160.02	G	GN
3.	48	81	162.56	G	GN
4.	40	76	165.10	G	GN
5.	44	83	152.40	G	GN
6.	44	81	157.48	G	GN
7.	40	78	165.10	G	GN
8.	43	84	167.64	G	GN
9.	49	81	160.02	G	GN
10.	41	79	162.56	G	GN
11.	43	79	152.40	G	GN
12.	43	83	152.40	G	GN
Mean	**43.5**	**81**	**162.92**		
±SD	**2.35**	**2.85**	**7.30**		

4.2. Method Validation

4.2.1. Linearity

The linearity was evaluated by linear regression analysis, which was calculated by the least square regression method. The calibration curve for plasma was constructed by plotting concentration versus area. The linearity of the method was determined over a concentration range from 0.050 to 2.0 µg/mL in plasma .The calibration curves of gliclazide was described by the following equations: y= 64510 x -1128.6, r2 = 0.9995. Standard curves of gliclazide in plasma is shown in Figs.**11**

Table 5: linearity Test

Linearity of Test				
X (ppm)	Y(area)	x^2	Y^2	XY
1.000	41968.0	1.0000	1.8E+09	4.197E+04
2.000	81337.0	4.0000	6.6E+09	1.627E+05
4.000	165909.0	16.0000	2.8E+10	6.636E+05
8.000	334186.0	64.0000	1.1E+11	2.673E+06
20.000	853684.0	400.0000	7.3E+11	1.707E+07
Sx	Sy	Sx^2	Sy^2	Sxy
35.000	1477084	485.000	8.8E+11	2.06E+07
$(Sx)^2$	$(Sy)^2$			
1225	2.2E+12			
n	6			
Coefficient, r	0.9997			

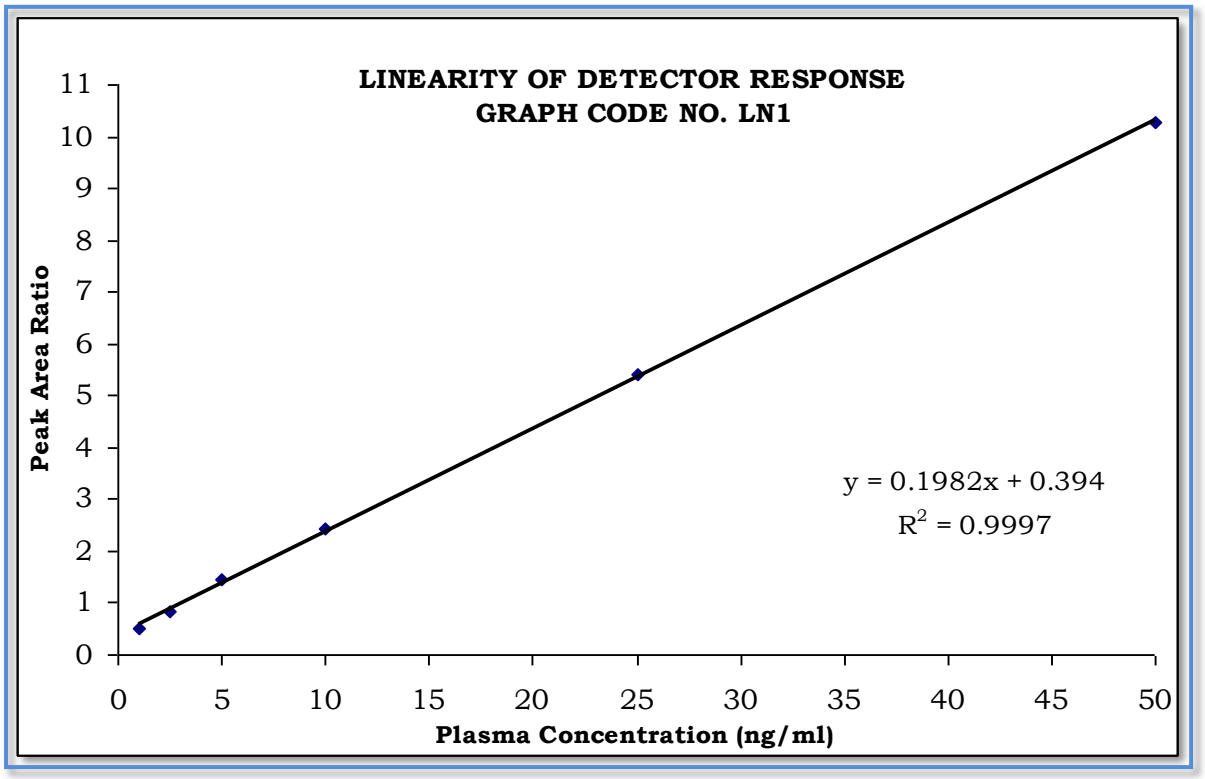


Figure-11: Standard curve of gliclazide for in vivo analysis.

4.2.2 Limit of Detection and Quantification

The limit of detection (LOD) and limit of quantification (LOQ) of the described method were calculated by injecting progressively low concentrations of the standard solutions. Limits of detection (LOD) and limits of quantification (LOQ) were evaluated from the signal-to-noise ratio. A signal larger than two to three times (3:1) of the base line noise was considered as limit of detection and a signal at least larger than five times (5:1) of the base line noise detected with a sufficient precision (< 20%) and accuracy (> 80%) was considered as limit of quantification. For plasma samples LOD obtained was 25 ng/mL and LOQ was 50 ng/mL.

4.2.3. Recovery

The recovery was examined from quality control samples (QCs) for low, medium and high concentration ranges in plasma. The recovery was expressed as the percentage of analytes recovered by the assay (ICH, 1996; Eksteen and Schoemakers, 1998). In plasma the average recovery of the assay was more than 80% .The high recovery confirms the suitability of the method for analysis of given samples.

4.2.4. Precision and Accuracy

LINEARITY CODE	QCL 3 ng/ml	QCM 30 ng/ml	QCH 40 ng/ml
LIN 1	2.936	29.539	38.948
LIN 1	2.864	28.613	38.780
LIN 1	2.835	26.440	37.928
LIN 1	2.781	25.927	37.582
LIN 1	2.768	25.551	36.326
LIN 2	2.675	25.221	35.813
LIN 2	2.522	26.748	35.467
LIN 2	2.494	26.682	34.993
LIN 2	2.424	26.476	34.475
LIN 3	2.356	25.634	34.260
LIN 3	2.315	25.428	33.832
LIN 3	2.284	24.951	32.530
LIN 3	2.131	24.874	31.895
Mean	2.568	26.314	35.602
S.D.	0.258	1.389	2.254
C.V.%	10.057	5.279	6.331
Absolute percent bias (%)	85.60	87.71	89.01

Table- 6: Precision and accuracy of HPLC assay (In Plasma)

Table 7: Absolute Recovery of Analyte from Plasma samples Analyte:

INJ No.	**Aqueous**			**In Plasma**		
	QCL 3 ng/ml	**QCM 30 ng/ml**	**QCH 40 ng/ml**	**QCL 3 ng/ml**	**QCM 30 ng/ml**	**QCH 40 ng/ml**
1	2.839	27.359	39.864	2.697	25.554	34.229
2	2.708	28.588	36.604	2.194	24.300	35.440
3	2.912	26.936	39.779	1.976	21.961	31.615
4	2.707	29.444	37.242	2.170	23.752	32.536
5	2.970	28.742	38.523	2.529	22.490	30.878
Mean	**2.827**	**28.214**	**38.403**	**2.313**	**23.612**	**32.940**
	% Recovery			**81.82**	**83.69**	**85.77**

4.3.1. UV Analysis

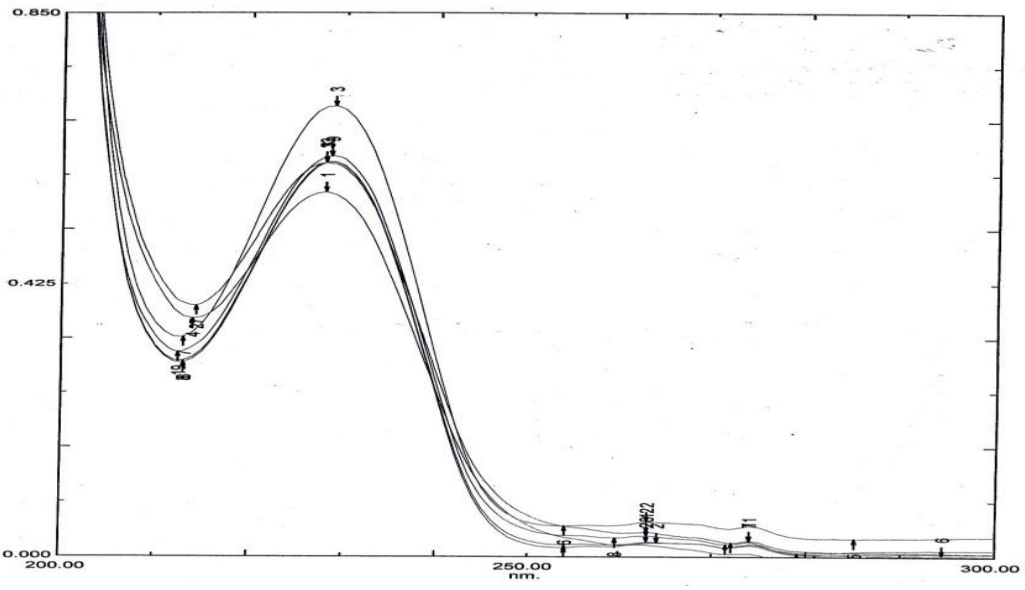


Figure-12: UV Analysis of Gliclazide

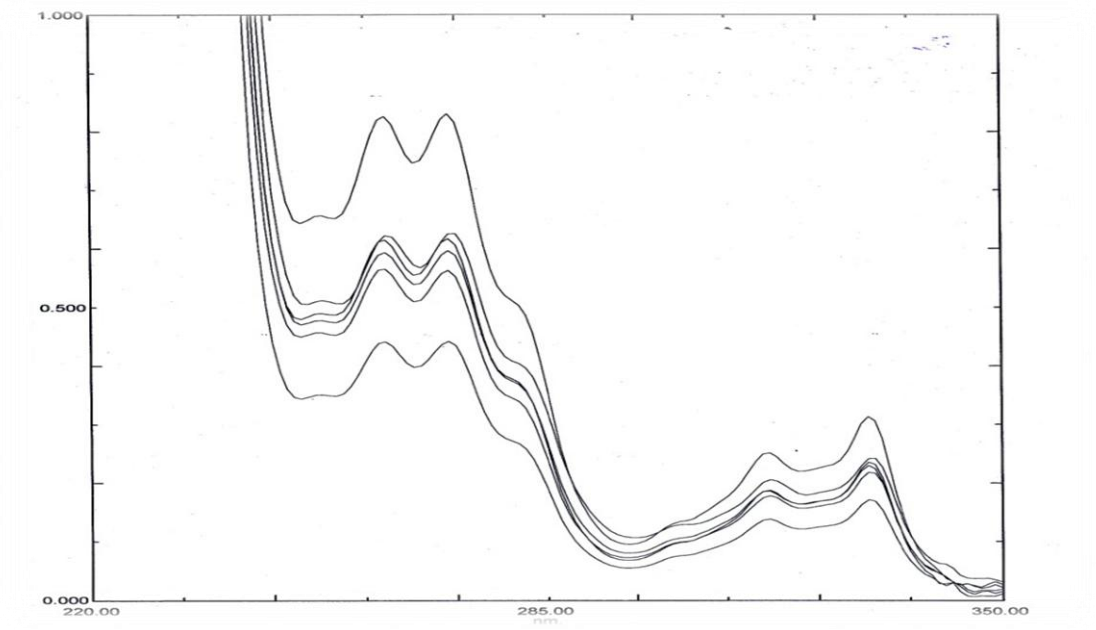


Figure 13: UV analysis of Naproxen

4.3.2. IR Analysis

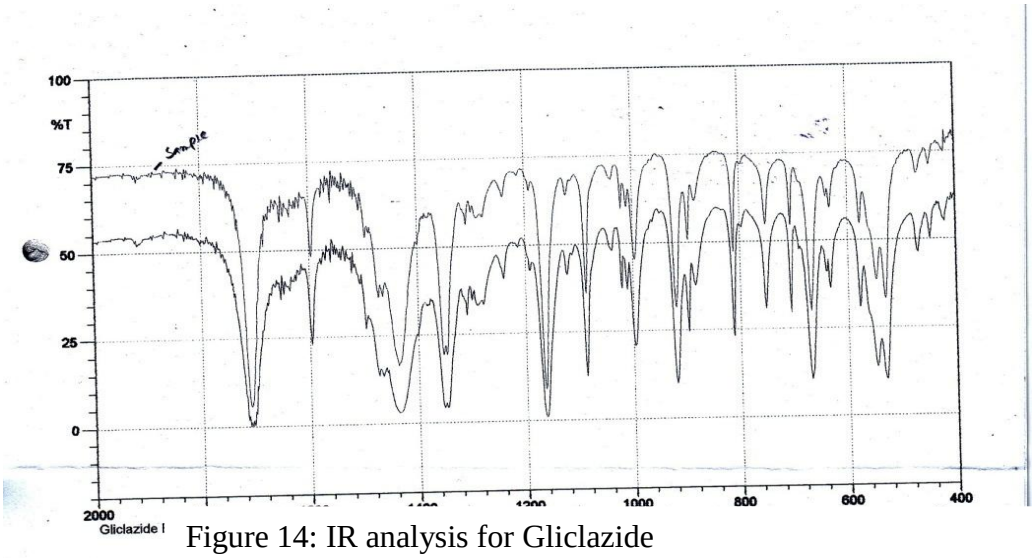


Figure 14: IR analysis for Gliclazide

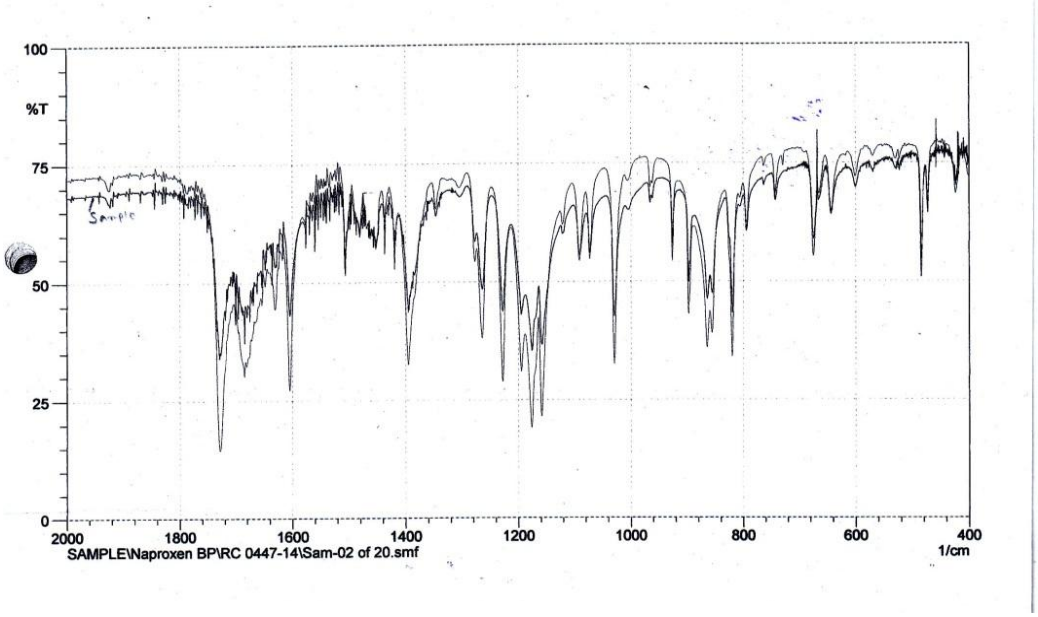
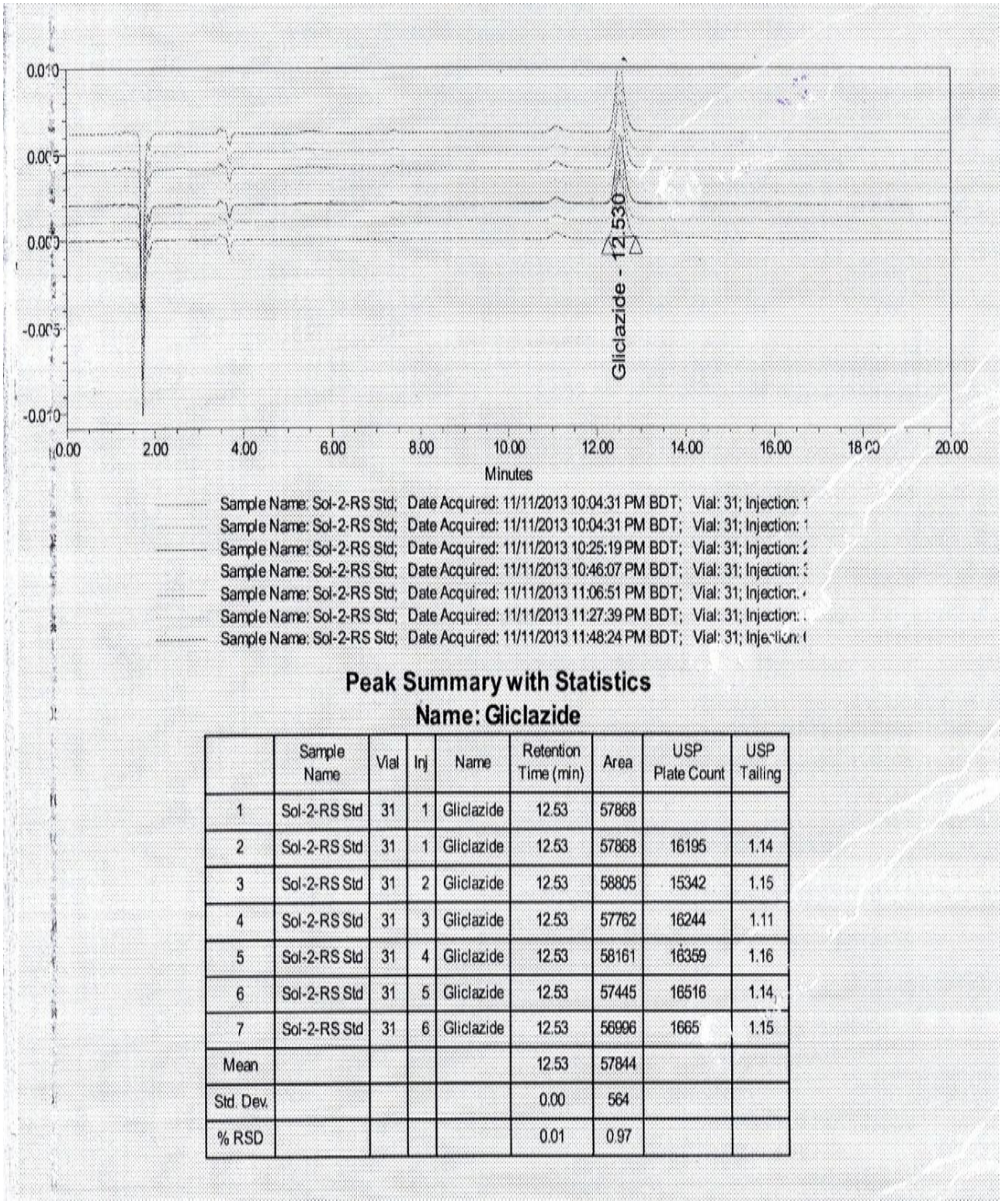


Figure 15: IR analysis for Naproxen

Peak summary with statistics of gliclazide



Peak Summary with Statistics
Name: Gliclazide

	Sample Name	Vial	Inj	Name	Retention Time (min)	Area	USP Plate Count	USP Tailing
1	Sol-2-RS Std	31	1	Gliclazide	12.53	57868		
2	Sol-2-RS Std	31	1	Gliclazide	12.53	57868	16195	1.14
3	Sol-2-RS Std	31	2	Gliclazide	12.53	58805	15342	1.15
4	Sol-2-RS Std	31	3	Gliclazide	12.53	57762	16244	1.11
5	Sol-2-RS Std	31	4	Gliclazide	12.53	58161	16359	1.16
6	Sol-2-RS Std	31	5	Gliclazide	12.53	57445	16516	1.14
7	Sol-2-RS Std	31	6	Gliclazide	12.53	56996	1665	1.15
Mean					12.53	57844		
Std. Dev.					0.00	564		
% RSD					0.01	0.97		

4.4. Pharmacokinetics of naproxen

Table-8: Pharmacokinetics of naproxen 500mg tablet after oral administration in 12 healthy subjects

	Pharmacokinetics Parameters					
Sub No.	**AUC_{inf} µg.hr/ml**	**AUC_t µg.hr/ml**	**C_{max} µg**	**T_{max} hr**	**$T_{1/2}$ hr**	**K_{el} hr^{-1}**
1	925.24	910.38	68.99	0.5	11.54	**0.06**
2	835.46	777.77	65.33	1	10.75	0.064
3	784.38	705.4	70.96	0.5	9.56	0.077
4	1054.35	945.26	74.25	1	12.42	0.055
5	945.32	836.37	72.43	2	10.56	0.065
6	865.74	785.36	63.54	1	9.38	0.075
7	859.46	798.35	56.86	0.5	9.56	0.073
8	968.35	902.22	54.92	0.5	8.44	0.096
9	977.63	905.39	70.23	1	9.84	0.086
10	1056.38	985.11	83.28	2	10.88	0.073
11	899.47	821.52	52.65	0.5	9.33	0.084
12	948.49	901.88	58.46	0.5	8.11	0.085
Mean	**926.68**	**856.25**	**65.33**	**0.93**	**10.07**	**0.07**
±SD	**83.02**	**81.44**	**9.79**	**0.38**	**1.29**	**0.01**

4.5. Pharmacokinetics of naproxen in presence of gliclazide:

Table-9: Pharmacokinetics of Naproxen in presence of Gliclazide in Diabetic Patients (After oral administration of Naproxen 500gm tablet with Gliclazide 80gm tablet.

	Pharmacokinetics Parameters					
Sub No.	**AUC_{inf} µg.hr/ml**	**AUC_t µg.hr/ml**	**C_{max} µg**	**T_{max} hr**	**$T_{1/2}$ hr**	**K_{el} hr^{-1}**
1	910.53	868.53	56.96	1	13.54	0.05
2	825.77	715.77	62.53	1	11.75	0.06
3	794.38	715.4	68.96	0.5	9.15	0.077
4	894.35	805.26	54.25	1	12.28	0.055
5	915.32	826.37	62.43	2	11.56	0.065
6	825.74	795.36	55.54	1	10.38	0.076
7	899.46	733.35	53.86	1	9.96	0.072
8	918.35	802.22	64.92	0.5	9.44	0.076
9	917.63	896.39	52.23	1	9.83	0.086
10	898.38	915.11	53.28	2	10.08	0.076
11	999.47	951.52	58.65	0.5	10.33	0.064
12	875.49	811.88	52.46	1	9.11	0.081
Mean	**889.57**	**819.76**	**58.37**	**1.04**	**10.61**	**0.069**
±SD	**54.27**	**76.92**	**10.02**	**0.33**	**1.37**	**0.01**

4.6. Alteration of naproxen pharmacokinetics in presence of gliclazide

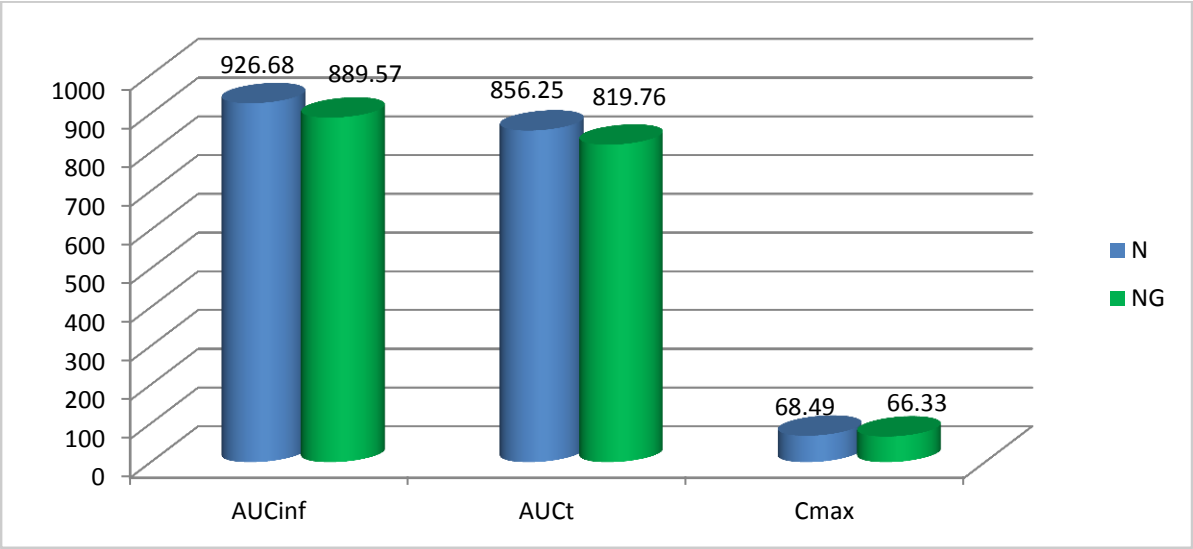


Figure-16: Pharmacokinetics of naproxen in presence of gliclazide N(Naproxen), NG(naproxen in presence of gliclazide)

4.7. Mean Plasma level Time curve

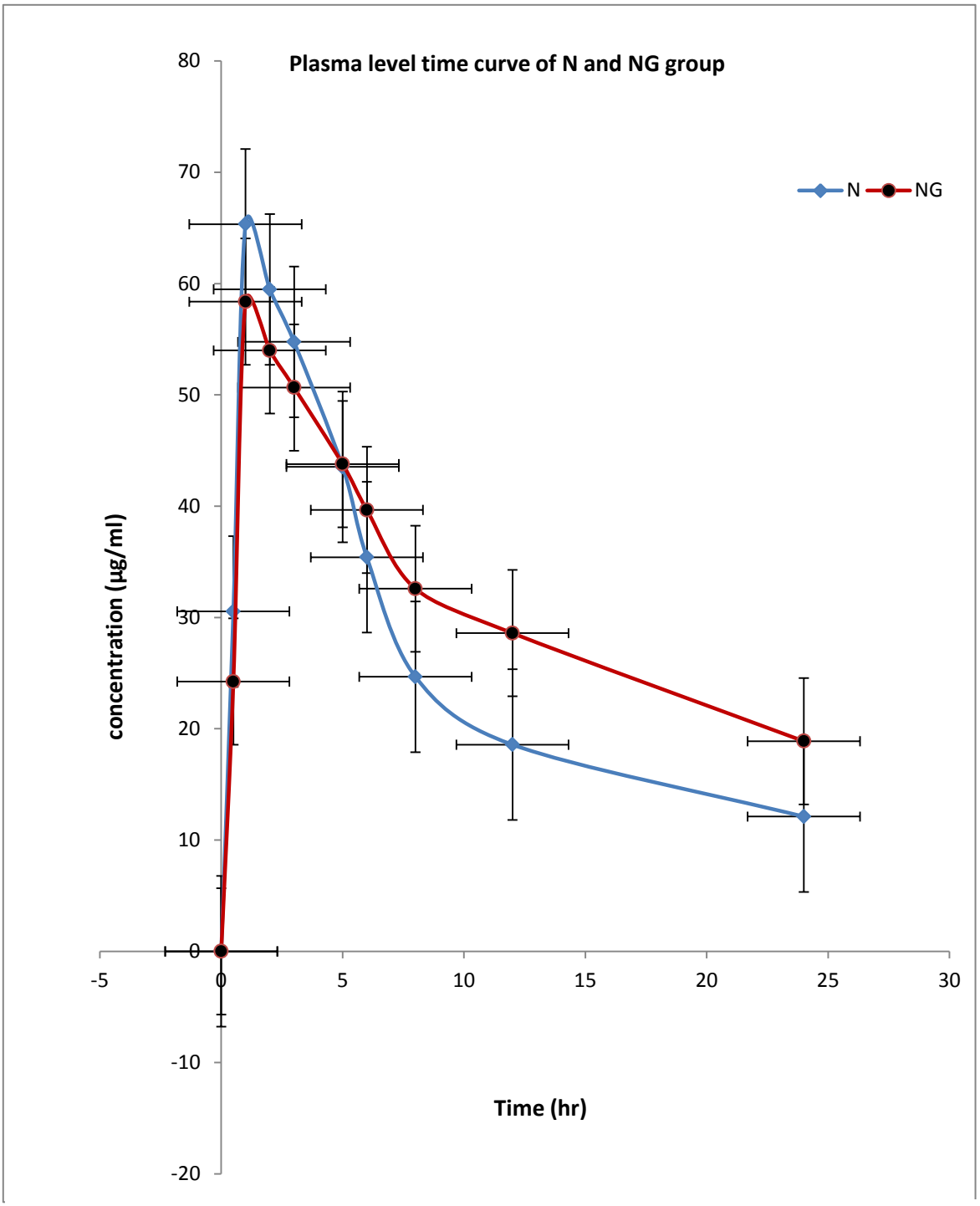


Figure-17: Plasma level time curve of N(naproxen) and NG (naproxen in presence of gliclazide) group

4.8. Statistical Analytical Report

Statistical Analysis

T test for equality of means **Independent sample test**

	$AUC_{0-\infty}$ $\mu g.h.mL^{-1}$	AUC_{0-24} $\mu g.h.mL^{-1}$	C_{max} $\mu g./mL^{-1}$	T_{max} h	$T_{1/2}$ h	K_{el}
N	926.68	856.25	65.33	0.93	10.07	0.07
NG	889.57	819.76	58.37	1.04	10.61	0.069
P value	**0.084**	**0.096**	**0.127**	**0.538**	**0.964**	**0.185**

P value: at 95% confidence interval

4.9. Pharmacokinetic Parameters

The pharmacokinetic parameters of Gliclazide and Naproxen after oral dose of 80mg Gliclazide tablet and 500mg Naproxen tablet were determined by PC-Computer Program, APO, MWPHARM version 3.02. The pharmacokinetic parameters of each volunteer were determined. Important parameters include Maximum plasma concentration (C_{max}), elimination half-life (T1/2), time to reach maximum concentration (T_{max}), area under the curve $(AUC)_{0-t}$, area under the curve $(AUC)_{0-\infty}$, and Elimination constant (k_{el})

Table-10: Pharmacokinetics Parameters of blood sample for both groups.

Pharmacokinetics parameters													
	Group G (Pharmacokinetic parameter of Gliclazide)							Group GN (Pharmacokinetic parameter of Gliclazide in presence of naproxen)					
Sub	AUC 0-∞ μg.h.mL^{-1}	AUC 0-24 μg.h.mL^{-1}	C_{max} μg./mL^{-1}	T_{max} h	$T_{1/2}$ h	K_{el}		AUC 0-∞ μg.h.mL^{-1}	AUC 0-24 μg.h.mL^{-1}	C_{max} μg./mL^{-1}	T_{max} h	$T_{1/2}$ h	K_{el}
1	63.7	57.49	3.10	3.5	7.71	0.078		85.35	74.986	2.865	4	8.54	0.081
2	53.1	44.41	2.49	4	7.34	0.067		74.26	68.046	2.36	3.5	10.5	0.066
3	69.92	66.59	2.827	3.5	5.014	0.241		85.65	58.867	2.827	6	6.35	0.1
4	68.94	66.95	3.88	3.5	9.072	0.148		65.76	57.043	3.21	4	10.4	0.066
5	67.9	55.99	4.47	2.5	8.373	0.505		77.39	73.456	3.26	3.5	9.22	0.075
6	68.13	57.24	2.67	3.5	7.03	0.258		76.48	68.867	2.677	4	10.9	0.063
7	59.6	54.938	5.54	3.5	8.87	0.197		87.49	86.467	3.85	4	9.17	0.075
8	73.43	66.814	3.64	4	3.515	0.328		85.64	75.234	3.534	4	5.36	0.129
9	56.39	49.621	4.69	3	5.737	0.525		84.55	74.123	4.69	3.5	10.4	0.066
10	68.85	60.9	5.025	3.5	9.007	0.247		76.48	66.111	4.85	4	5.35	0.129
11	57.10	55.491	6.321	2.5	5.45	1.197		75.76	67.867	4.69	3.5	5.965	0.116
12	65.92	54.72	4.131	3.5	7.761	0.394		84.51	73.547	4.131	3.5	11.45	0.0605
Mean	64.39	57.59	4.07	3.58	7.073	0.099		79.94	70.38	3.58	4.02	8.64	0.078
±SD	**18.1**	**16.96**	**1.19**	**0.42**	**1.78**	**0.30**		**19.05**	**17.9**	**0.85**	**0.69**	**2.28**	**1.12**

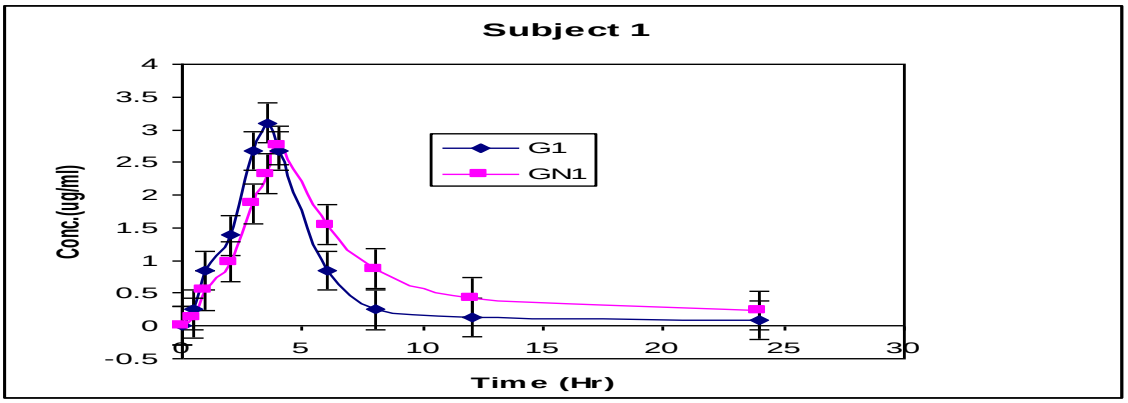


Figure 18: Plasma concentration and Time curve

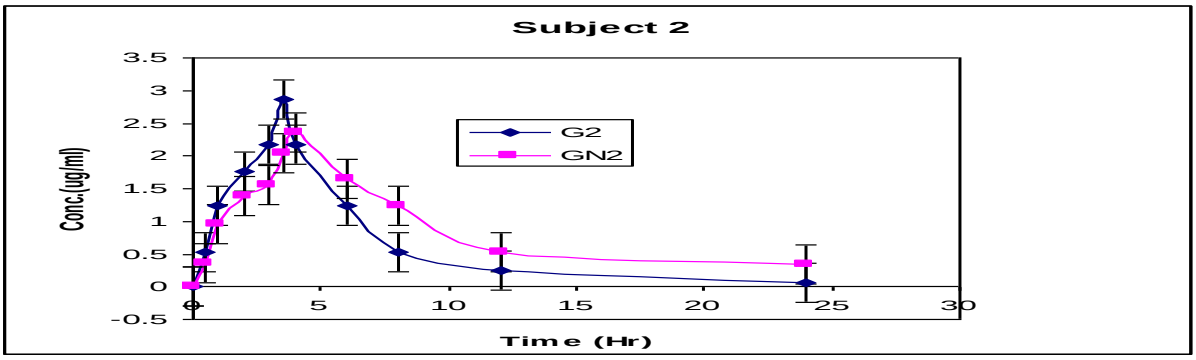


Figure 19: Plasma concentration and Time curve

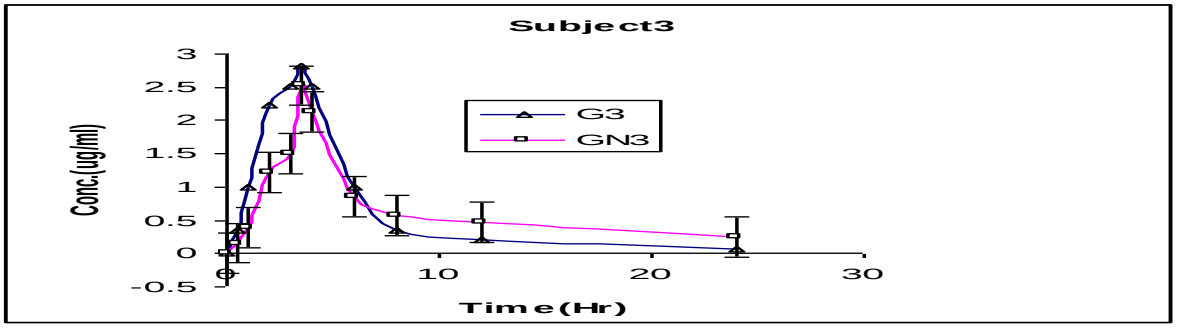


Figure 20: Plasma concentration and Time curve

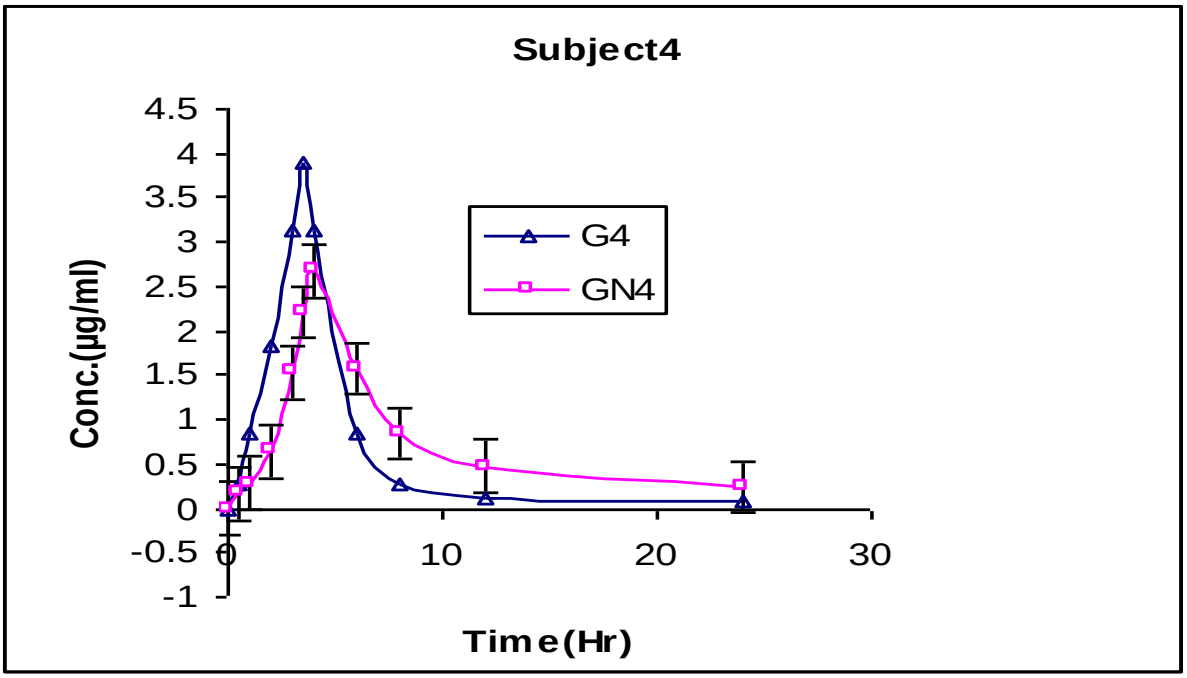


Figure 21: Plasma concentration and Time curve

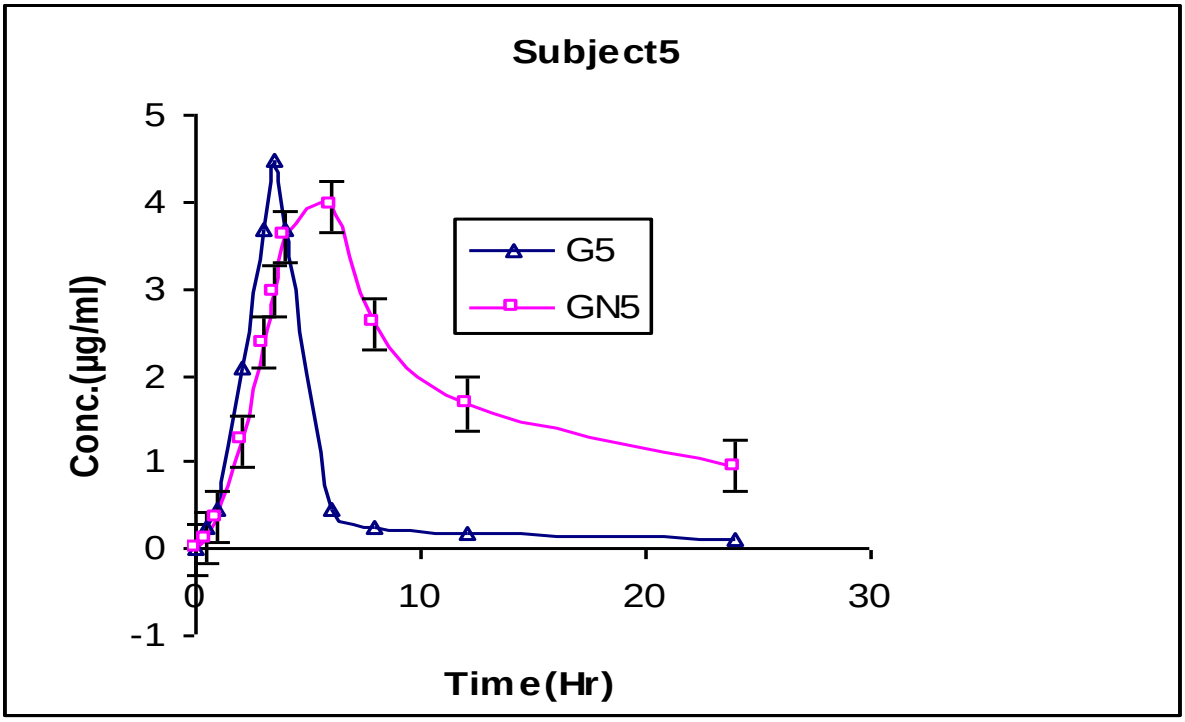


Figure 22: Plasma concentration and Time curve

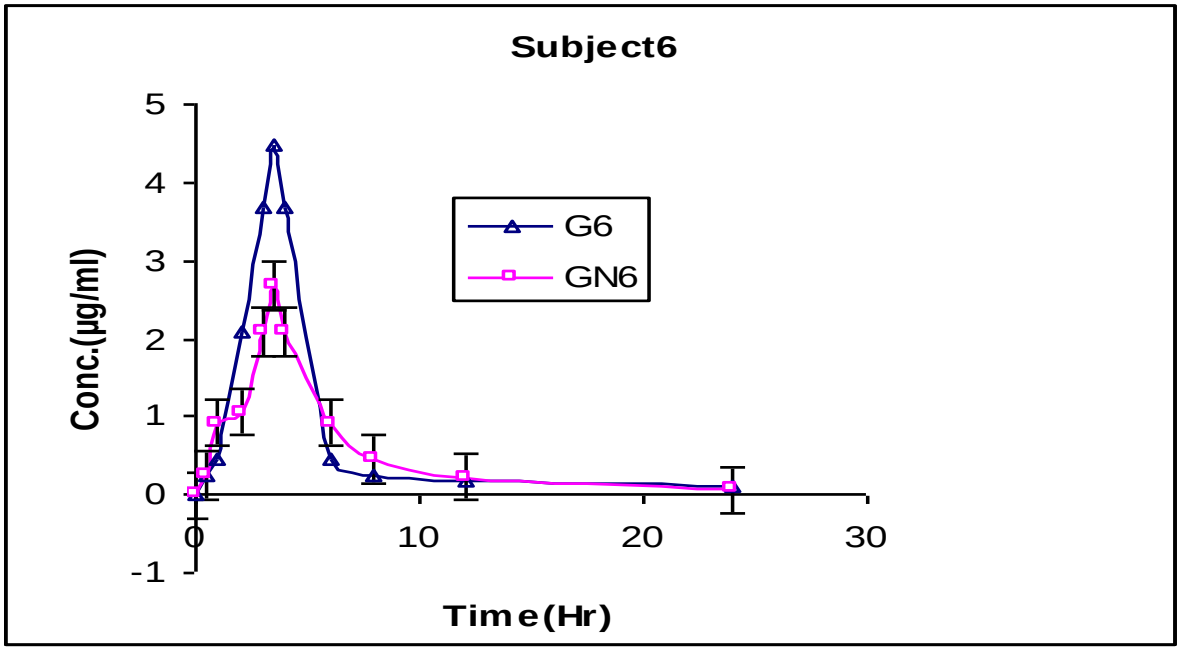


Figure 23: Plasma concentration and Time curve

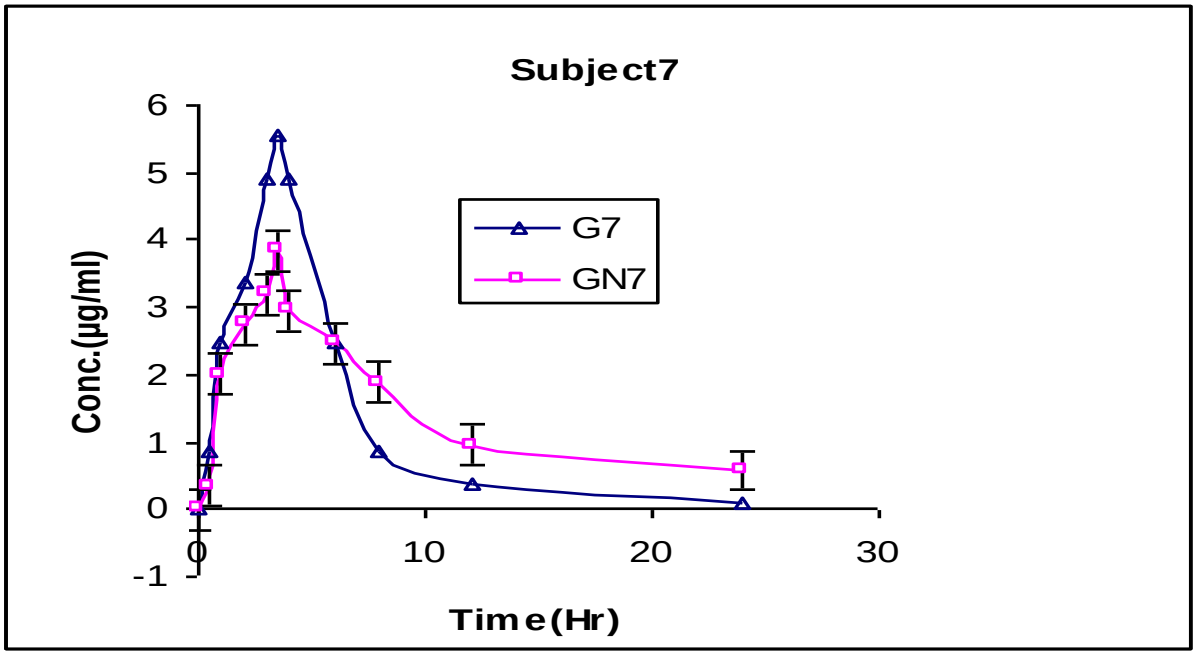


Figure 24: Plasma concentration and Time curve

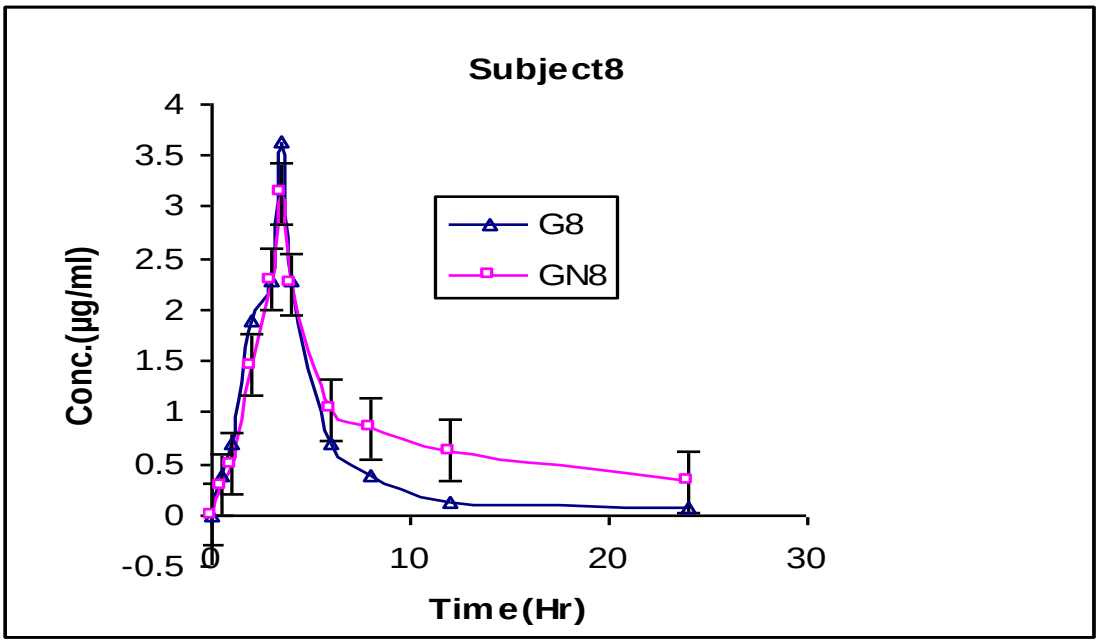


Figure 25: Plasma concentration and Time curve

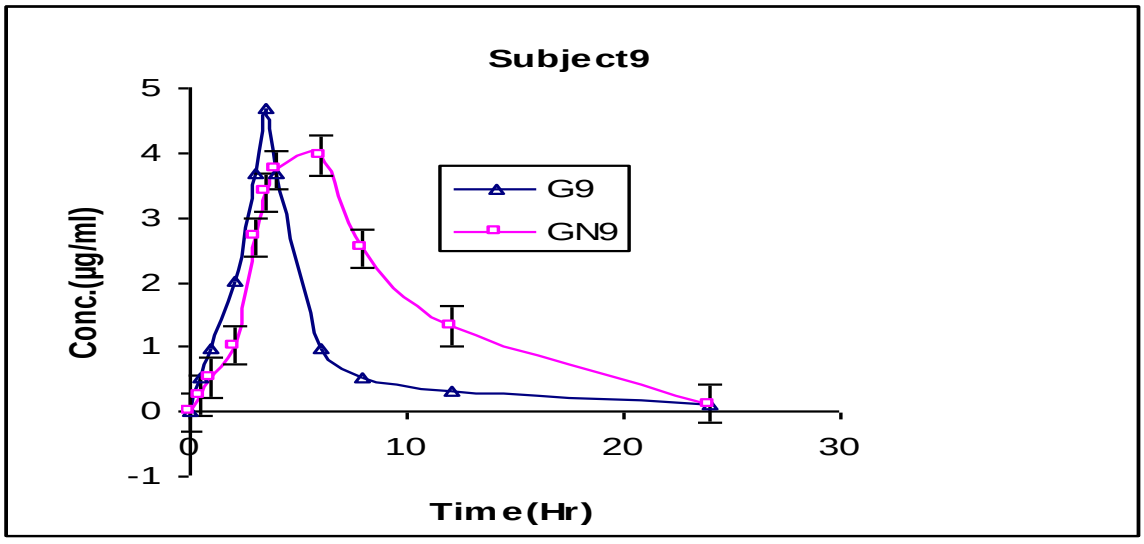


Figure 26: Plasma concentration and Time curve

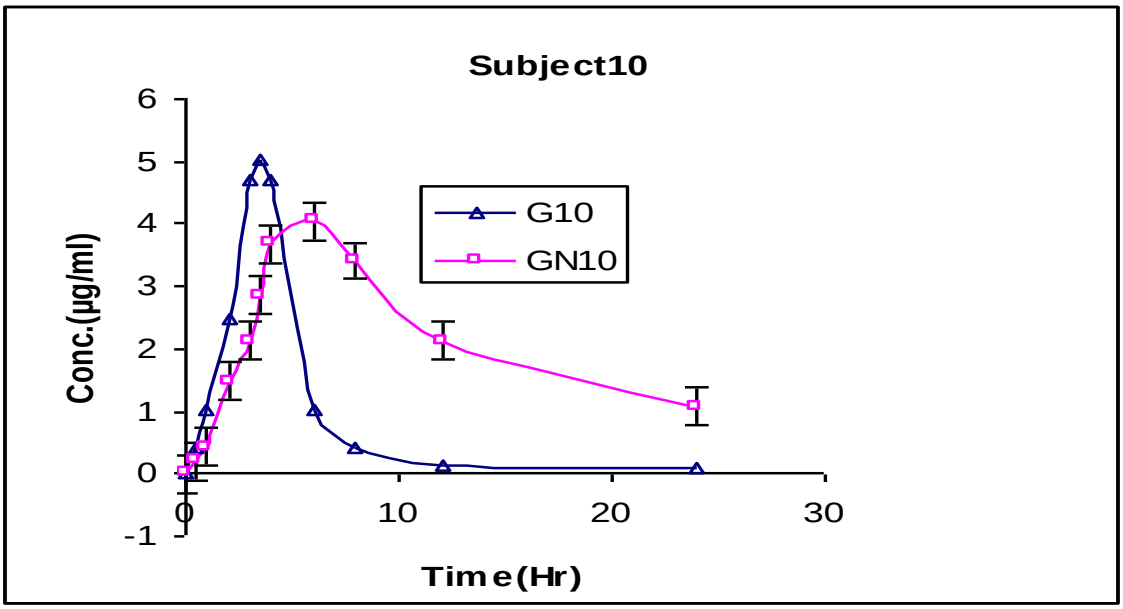


Figure 27: Plasma concentration and Time curve

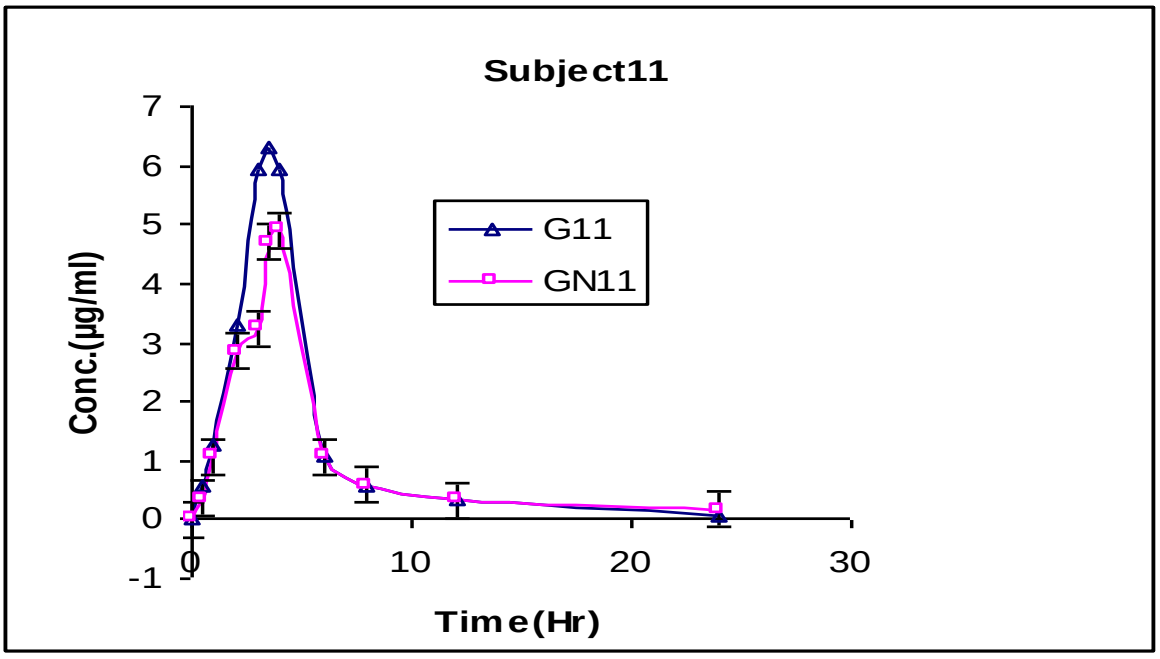


Figure 28: Plasma concentration and Time curve

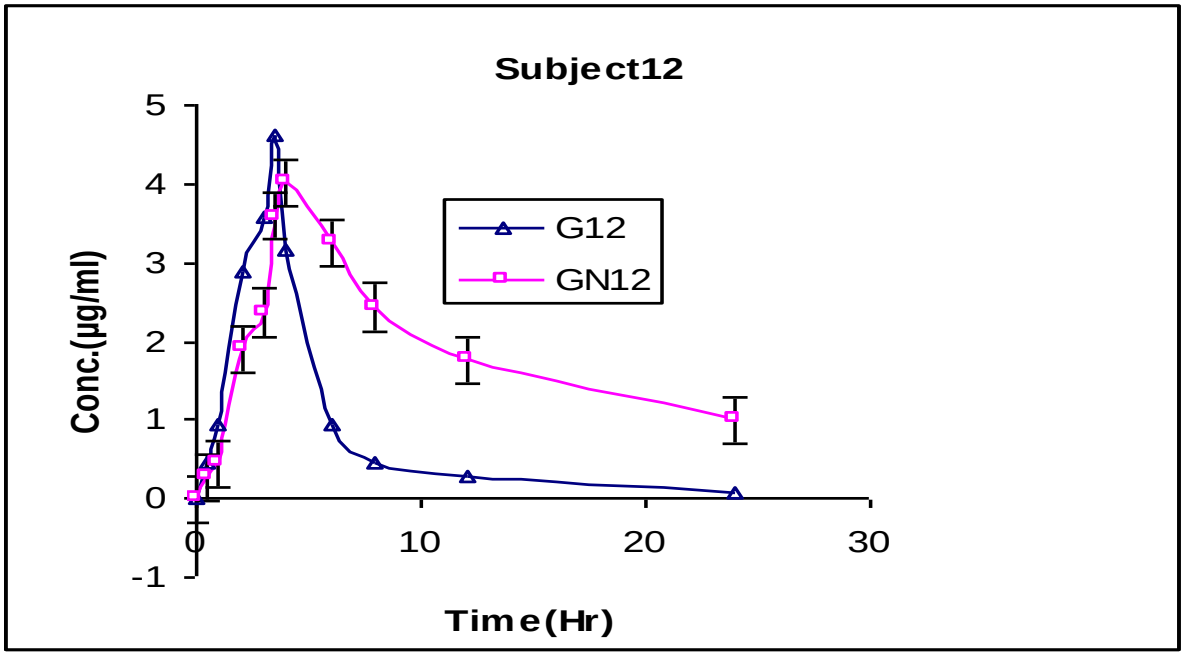


Figure 29: Plasma concentration and Time curve

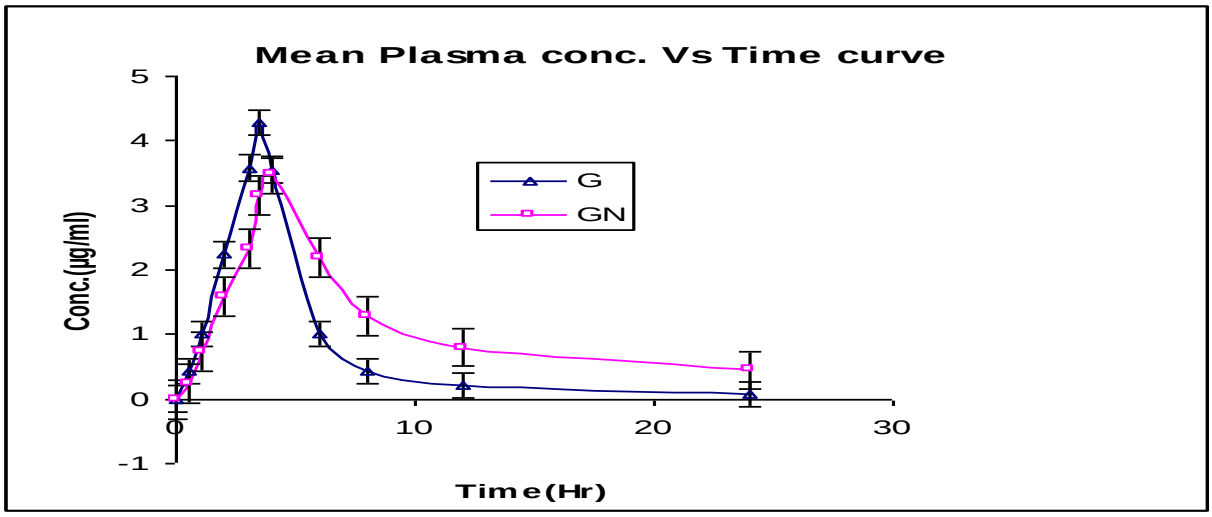


Figure 30: Mean Plasma concentration and Time curve

4.10. Alteration of gliclazide pharmacokinetics in presence of naproxen

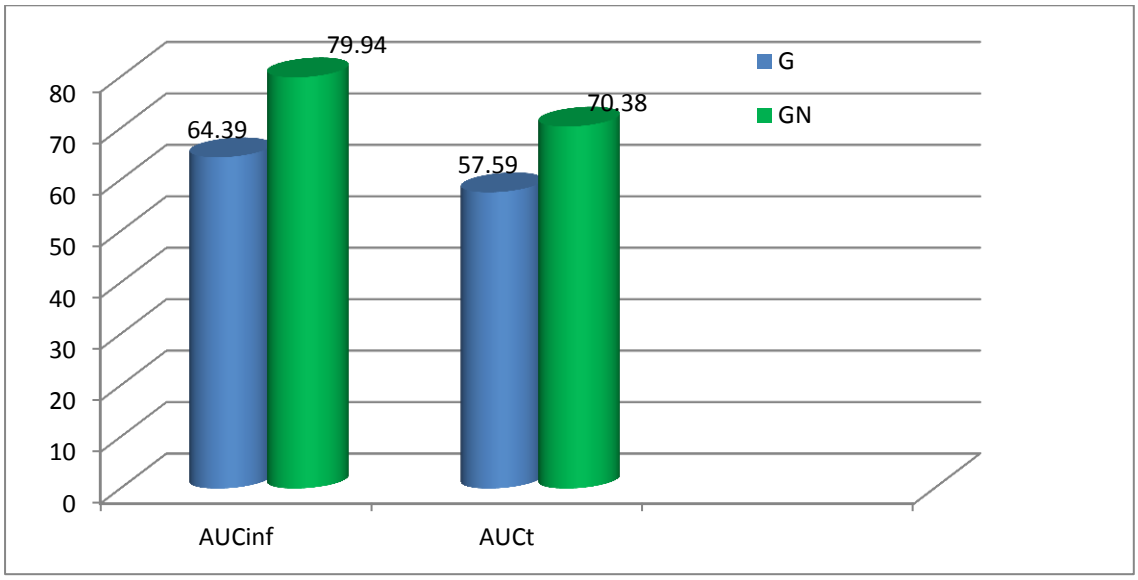


Figure 31: Alteration of gliclazide pharmacokinetics in presence of naproxen

$AUC_{0-\infty}$: In presence of naproxen this value of gliclazide was 79.94µg.h.mL^{-1} which is higher and significance (P value =0.056 *).

AUC_{0-24}: However, in case of gliclazide$AUC_{0-24}$was 57.59µg.h.mL^{-1} whereas in presence of naproxen this value of gliclazide was 70.38µg.h.mL^{-1}. Which is higher and significance (P value =0.048 *).

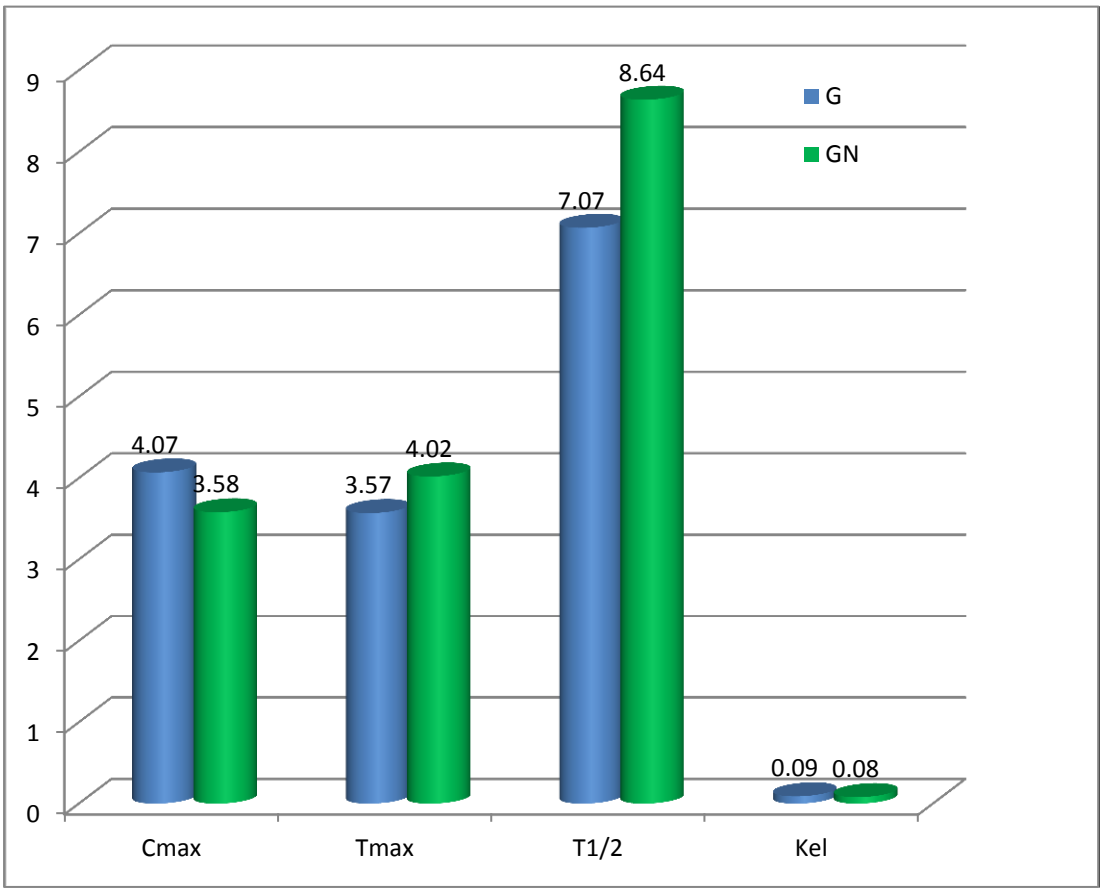


Figure 32: Alteration of gliclazide pharmacokinetics in presence of naproxen

C_{max}: In case of gliclazide C_{max} was 4.07 µg./mL-^{1} whereas in presence of Naproxen this value was 3.58 µg./mL-^{1} which is lower and significant (P<0.007*)

T_{max}: In case of gliclazide$T_{max}$was 3.58 h whereas in presence of naproxen this value of gliclazide was 4.02 h which is higher but not significance (P value =0.12).

$T_{1/2}$: In case of gliclazide$T_{1/2}$ was 7.073h whereas in presence of naproxen this value of gliclazide was 8.64h which is higher and significance (P value =0.054 *).

K_{el}: In case of gliclazide K_{el} was 0.099 whereas in presence of naproxen, this value of gliclazide was 0.078 which is higher but not significance (P value =0.068).

4.11. Statistical analysis Report

Statistical Analysis **T test for equality of means** **Independent sample test**						
	$AUC_{0-\infty}$ µg.h.mL^{-1}	**AUC_{0-24} µg.h.mL^{-1}**	**C_{max} µg./mL^{-1}**	**T_{max} h**	**$T_{1/2}$ h**	**K_{el}**
G	**64.39**	**57.59**	**4.07**	**3.58**	**7.073**	**0.099**
GN	**79.94**	**70.38**	**3.58**	**4.02**	**8.64**	**0.078**
P value	**0.056 ***	**.048 ***	**0.007****	**0.12**	**0.054 ***	**0.068**

P value at 95% confidence interval

4.12. Discussion:

By the year 2030, diabetes mellitus is expected to affect almost 5% of the world's population—an estimated 366 million people (Wild S, Roglic G, Green A, et al.,2004). The vast majority of these cases will be type II diabetes, with current diagnosis currently accounting for approximately 90%–95% of diabetes cases. Diabetes prevalence is currently about 3% in the general population, with increased prevalence in some ethnic minority groups (such as South Asians, Africans, Afro- Caribbeans, and Chinese), as well as in less affluent populations, including those that are less physically active, have central obesity, and have a high body mass index (BMI; weight(kg)/height (m)2). Some populations, such as Pima Indians, Australian Aboriginal communities, and Pacific and Indian Ocean Islanders, have a far higher prevalence, at up to 40% (Zimmet P et al., 2001). The presence of diabetes mellitus is associated with a range of vascular complications (such as myocardial infarction, stroke, heart failure, renal failure, angina, and retinopathy) leading to a reduced life expectancy and a reduced quality of life (UK Prospective Diabetes Study (UKPDS) Group.,1998). The large and increasing burden of type II diabetes and the potential for modifying risk through adequate treatments and lifestyle alterations make the identification of methods for the early detection of persons at greater risk an important public health challenge.

Now type II diabetes mellitus (T II DM) has become a global public health problem including Bangladesh. Moreover, diabetic subjects are more prone to develop multi-various micro-vascular and macro-vascular diseases. They are therefore, suitable candidate to be prescribed multiple drugs, even they may be recommended to take these drug at a time. As a consequence, the pharmacokinetic interactions possibility is higher although which depends on whether those drugs are recommended precisely investigating their pharmacokinetics profile.

In more commonly, anti-diabetic drugs are the classes of drugs interact more with other classesdrugs.Even some of the NSAIDs do it. Gliclazide and naproxen are preferably sometimes may be prescribed by doctors in Bangladesh for type-II diabetic patient. But we don't have any investigation regarding their pharmacokinetic interactions among Bangladeshi patients.Establishing firmly therapeutic endpoints include the anticipated and desired clinical effects from drug therapy that are expected, ultimately, to achieve the desired outcome(s). Pharmacokinetic interactions, crucial and key determinant of expected therapeutic response as well.

C_{max}: Among pharmacokinetic parameters, C_{max} value of naproxen was 65.33µg./mL-^{1} whereas in presence of gliclazide this maximum concentration of naproxen was 58.37µg./mL^{1}, which is lower but not significant (P value = 0.127).However, in case of gliclazide ,C_{max} was 4.07µg./mL-1 but in presence of naproxen this maximum concentration of gliclazide plummeted to 3.58µg./mL-1. Which is lower and significant (P value =0.007**).

$AUC_{0-\infty}$: For this , $AUC_{0-\infty}$of naproxen was 926.68 µg.h.mL$^{-1,}$in presence of gliclazide this value was 889.57µg.h.mL^{-1} which is lower but not significant (P value=0.084).However, in case of gliclazide$AUC_{0-\infty}$was 64.39µg.h.mL^{-1} whereas in presence of naproxen this value of gliclazide was 79.94µg.h.mL^{-1} . Which is higher and significant (P value =0.056 *).

AUC_{0-24}: While, $AUC_{0-24}$of naproxen was 856.25µg.h.mL^{-1}whereas in presence of gliclazide this value was 819.76µg.h.mL^{-1} which is lower but not significant (P value=0.096). However, in case of gliclazide AUC_{0-24} was 57.59µg.h.mL^{-1} whereas in presence of naproxen this value of gliclazide was 70.38µg.h.mL^{-1}. Which is higher and significance (P value =0.048 *).

T_{max}: $T_{max}$of naproxen was 0.93hwhereas in presence of gliclazide this value was 1.04 h which is higher but not significant (P value=0.538).However, in case of gliclazide$T_{max}$was 3.58 h whereas in presence of naproxen this value of gliclazide was 4.02 h. Which is higher but not significance (P value =0.12).

$T_{1/2}$: Among the pharmacokinetic parameters, $T_{1/2}$ of naproxen was 10.07h whereas in presence of gliclazide this value was 10.61h which is higher but not significant (P value=0.964). However, in case of gliclazide$T_{1/2}$ was 7.073h whereas in presence of naproxen this value of gliclazide was 8.64h. Which is higher and significant (P value =0.054 *).

K_{el}: $K_{el}$of naproxen was 0.07whereas in presence of gliclazide this value was 0.069 which is higher but not significant (P value=0.185). However, in case of gliclazide$K_{el}$was 0.099whereas in presence of naproxen this value of gliclazide was 0.078. Which is higher but not significance (P value =0.068).

Conclusion

- Over all the pharmacokinetic parameters, naproxen shows no significant change in its pharmacokinetics in presence of gliclazide. Although, AUC change was to some extent considerable. Presumably, it is due to healthy vs diabetic subjects comparison.

- However, Gliclazide pharmacokinetics alteration was surprisingly significant. AUC ($p<0.056$*, $p<0.048$*) C_{max}($p<$**.007****) and $T_{1/2}$($p<$**0.054***) has been considerably changed in presence of naproxen.

- It is therefore, demonstrated that naproxen causes pharmacokinetic interaction with gliclazide in Bangladeshi Type II Diabetic patients.

- Further study may be entailed on metabolites and metabolising genes as well to rationally stabilize dose regimen of these two drugs.

References

- A. Harrower, "Gliclazide modified release: from oncedaily administration to 24-hour blood glucose control," *Metabolism*, vol. 49, no. 10, supplement 2, pp. 7–11, 2000.
- Carrasco-Portugal MDC, Herrera JE, Reyes-Garcia G, Medina-Santillan R, Flores-Murrieta FJ (2006) Comparative bioavailability of two oral suspensions of
- Center for Drug Evaluation and Research (CDER), *Guidance for Industry, Food-Effect Bioavailability and Fed Bioequivalence Studies*, US FDA, 2002.
- Center for Drug Evaluation and Research (CDER), *Guidance for Industry, Bioavailability and Bioequivalence Studies for Orally Administered Drug Products-General Considerations*, US FDA, 2003.
- Center for Drug Evaluation and Research. Bioequivalence reviews: Naproxen delayed release
- D. B. Campbell, R. Lavielle, and C. Nathan, "The mode of action and clinical pharmacology of gliclazide: A review,"
- *Diabetes Research and Clinical Practice*, vol. 14, supplement 2, pp. S21–S36, 1991.
- F. Ishibashi and S. Takashina, "The effect of timing on gliclazide absorption and action," *Hiroshima Journal of Medical Sciences*, vol. 39, no. 1, pp. 7–9, 1990.
- G. Schernthaner, "Gliclazide modified release: a critical review of pharmacodynamic, metabolic, and vasoprotective effects," *Metabolism*, vol. 52, no. 8, supplement 1, pp. 29–34, 2003.
- Guideline for Bioequivalence Studies. National Agency for Drug and Food Control, Jakarta, December 2004.
- H. Noguchi, N. Tomita, S. Naruto, and S. Nakano, "Determination of gliclazide in serum by high-performance liquid chromatography using solid-phase extraction," *Journal of Chromatography*, vol. 583, no. 2, pp. 266–269, 1992.
- Hardman JG, Lee EL, Alfred GG (2001) Goodman & Gilman's the Pharmacological Basis of Therapeutics. 10th ed. New York: McGraw-Hill Companies Inc 712: 1987.

- J. F. Yang, G. L. Wei, R. Lu, C. X. Liu, B. Z. Zheng, and P. Feng, "Bioavailability of gliclazide sustained release tablet in healthy volunteers," *Asian Journal of Pharmacodynamics and Pharmacokinetics*, vol. 6, no. 2, pp. 153–160, 2006.

- J. K. McGavin, C. M. Perry, and K. L. Goa, "Gliclazide modified release," *Drugs*, vol. 62, no. 9, pp. 1357–1364, 2002.

- K. G. Alberti, "Gliclazide: review of metabolic and vascular action," *Diab`ete & M´etabolisme*, vol. 20, no. 3, part 2, pp. 341– 348, 1994 (French).

- K. J. Palmer and R. N. Brogden, "Gliclazide—an update of its pharmacological properties and therapeutic efficacy in noninsulin- dependent diabetes mellitus," *Drugs*, vol. 46, no. 1, pp. 92–125, 1993.

- Medina L, Garcia L, Medina J, Diaz-Oliveros J, Jung H (1998) Implementation of a system for evaluating and ensuring quality of bioequivalence studies. Proc West Pharmacol Soc 41: 1757.

- Naprosyn Online. Description, chemistry, ingredients, pharmacology, pharmacokinetics, studies and metabolism of naproxen: monograph.

- Niazi SK, Alam SM, Ahmad SI (1996) Dose dependent pharmacokinetics of naproxen in man. Biopharm Drug Dispos 17: 355-61.

- P. Delrat, M. Paraire, and R. Jochemsen, "Complete bioavailability and lack of food-effect on pharmacokinetics of gliclazide 30mgmodified release in healthy volunteers," *Biopharmaceutics and Drug Disposition*, vol. 23, no. 4, pp. 151–157,

- P. Drouin, "Diamicron_MR once daily is effective and well tolerated in type 2 diabetes: a double-blind, randomized, multinational study," *Journal of Diabetes and its Complications*, vol. 14, no. 4, pp. 185–191, 2000.

- P. E. Jennings, "From hemobiology to vascular disease: a review of the potential of gliclazide to influence the pathogenesis of diabetic vascular disease," *Journal of Diabetes and* 6 ISRN Pharmacology *its Complications*, vol. 8, no. 4, pp. 226–230, 1994.

- PDR® Physicians' Desk Reference. 59th ed. Thomson PDR; Montvale, NJ: 2005. p. 2874-6. » CrossRef » Pubmed » Google Scholar tablets.