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DETERMINATION OF GLUCOSAMINE IN DRUG FORMULATION

BY

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DECLARATION

I hereby declare that this thesis is my own work towards the Msc and that, it contains no material previously submitted by any other person nor material which has been accepted for the award of any other degree of the University.

The various journals, books, periodicals and the other sources I consulted for information have been duly acknowledged by reference to the authors.

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Student Name & ID Signature Date

Certified by:

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Supervisor's Name. Signature Date

Certified by:

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Head of Dept's Name. Signature Date

DEDICATION

This work is dedicated to my Parents Kwadwo Asamoah and Josephine Gadzanaki. I am forever grateful for your support and appreciate the sacrifices you made for me to pursue this endeavour.

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ABSTRACT

The purpose of this work is to evaluate and present the results of analysis of actual content of several products in the marketplace containing glucosamine salts and to determine if they significantly deviate from label claim.

A total of nine products containing glucosamine sulphate and glucosamine hydrochloride were evaluated using two methods, non-aqueous titration and HPLC- UV method. Both salts are sparingly soluble in most organic solvents including glacial acetic acid and so the sulphate of the glucosamine was displaced using mercury (II) acetate, just as the chloride was treated prior to titration. From the titration results, the average percentage recoveries for the glucosamine hydrochloride standard and products **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9**, are 100.8, 102.8, 103.5, 100.4, 84.3, 102.7, 99.3, 98.8, 102.9, and 102.7% respectively. Product 4 failed the test. The products that passed the test range between 98.8 and 103.5%.

With regard to the HPLC, a pre-column derivatization was carried out by addition of phenylisothiocyanate (PITC). Analysis was completed by injection of 5.3µg/ml (5.292 µg/ml) of sample solution into an isocratic HPLC column made up of C18 column, a mobile phase of methanol: water: phosphoric acid (12: 88: 0.1), a flow rate of 1.5ml/min, and UV detector set at 240nm. The glucosamine anomer peak 1 eluted at 5.6 minutes while glucosamine anomer peak 2 eluted at 6.9 minutes post injection.

The average percentage recoveries for products **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9** are 104.4, 102.2, 99.9, 83.9, 102.3, 97.4, 98.2, 103.1 and 101.9% respectively. The products that passed the test range between 97.4 and 104.4%.

Dissolution tests were carried out on the nine products. Aliquots of the each sample were taken from the dissolution medium at 15min time interval for 1hour. A portion of filtrate sample was derivatized by addition of PITC. Analysis was completed by injection of 5.6µg/ml of sample solution into an isocratic HPLC column using the chromatographic conditions mentioned above. Nothing was detected for products **3**, **5** and **6** at the end of 1 hour. The average percentage release of the active ingredients for product **1** at 15min, 30min, 45min, and 60min is 84.2, 90.0, 92.6, and 98.4% respectively. With regard to product **2**, the average percentage release is 49.6, 52.2, 74.7 and 85.8% respectively. For product **4**, the average percentage release is 52.3, 61.5, 76.5 and 91.7% respectively. For product **7**, the average percentage release is 36.4, 47.5, 58.6 and 67.3% respectively. For product **8**, the average percentage release is 49.6, 56.9, 82.0 and 89.0% respectively. For product **9**, the average percentage release is 54.5, 62.6, 86.1 and 91.3% respectively. Both assay methods gave reproducible results.

TLC analysis was carried out on the nine products; the results are shown in appendix 3.0. The R_f values of phenylthiourea derivatives glucosamine anomer spot 1 and spot 2 are respectively 0.19 and 0.30.

Lastly, weight uniformity tests were carried out on the nine products. 20 tablets, capsules, and caplets were selected at random individually and their weights measured and recorded. The average weights were also measured and recorded. The average weights of products **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9** are 1.7076, 0.5375, 1.0247, 0.7691, 1.0416, 1.0149, 1.0297, 0.5356 and 0.5421g respectively. Product **7** failed the weight uniformity test. Three capsules deviated from average weight by the following percentages -8.86%, -8.20% and 8.70%.

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CHAPTER ONE

ARTHRITIS AND ITS TREATMENT (OR MEDICATION).

1.0.0 Introduction

Literally, the term arthritis[1, 2] means joint inflammation, in a broad term, it really refers to a group of more than hundred rheumatic diseases and conditions that can cause pain, stiffness and swelling in the joints. Thus, it destroys the workings of a normal joint.

Arthritis may occur in your back, neck, hips, knees, shoulders or hands, but it also occurs in your feet and ankles. Almost half of people in their 60s and 70s have arthritis of the foot and, or ankle. It should, however, be noted that arthritis is not just an old person's disease. Nearly two-thirds of people with arthritis are younger than 65. Although arthritis affects children and people of all racial and ethnic groups, it is more common among women and older adults.

Arthritis is a public health problem. In 2002, 43 million American adults (about 1 in 5) reported doctor-diagnosed arthritis, making arthritis one of the nation's most common health problems. Arthritis is the nation's leading cause of disability, limiting everyday activities for 16 million Americans in 2002. Work limitations attributable to arthritis affect more than 5% of the general population and nearly 30% of people with arthritis[4, 5, 6].

In 2003, the prevalence of doctor-diagnosed arthritis in adults in United States and territories, ranged from 17.9% to 37.2% and that of arthritis-attributable activity limitation ranged from 6.3% to 16.7%, with particularly high rates in southern states. Arthritis and arthritis-attributable activity limitation are common problems in all states and territories and likely to increase in the future.

Each year, arthritis results in 750,000 hospitalizations and 36 million outpatient visits. Direct medical costs for arthritis were more than $51 billion in 1997. Figure 1.1.1 shows a projected number of adults with arthritis and arthritis-attributable activity Limitations, 2005 – 2030 in US. As the U.S. population ages, these numbers are likely to increase dramatically. For example, the number of US adults who have doctor-diagnosed arthritis is projected to increase to 67 million (25%) by 2030. In addition, adults with arthritis-attributable activity limitation are projected to increase from 16.9 million (7.9%) to 25 million (9.3% of the US adult population) by 2030. These projections herald an increasing societal and health care system burden.

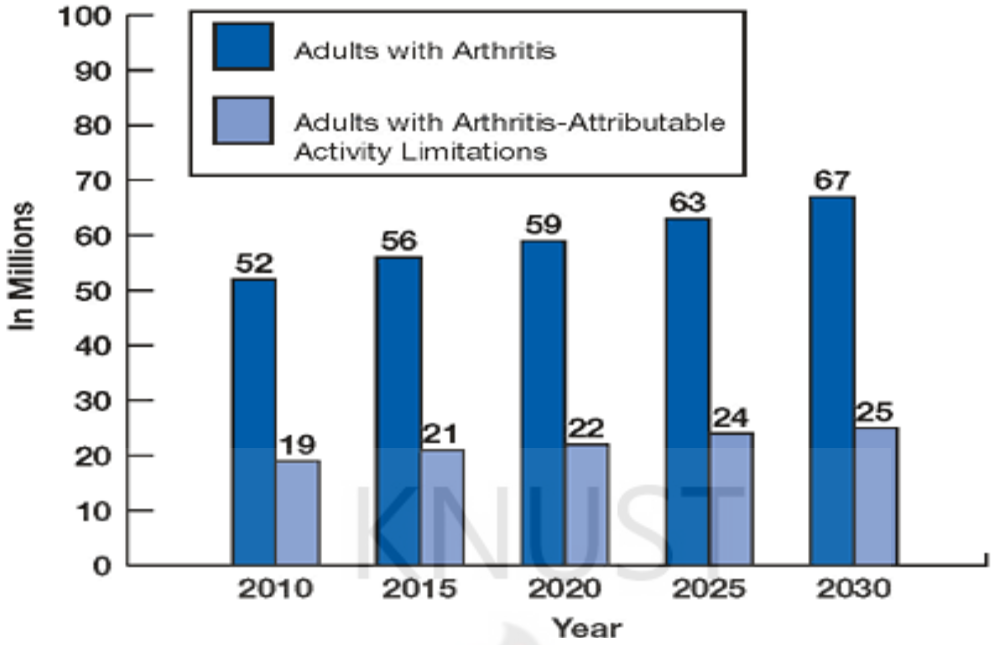


Figure 1.1.1: Projections of U.S. Prevalence of Arthritis and Associated Activity Limitations. *Arthritis and Rheumatism* 2006; 54(1):226–9. (Source: Hootman JM, Helmick CG.)

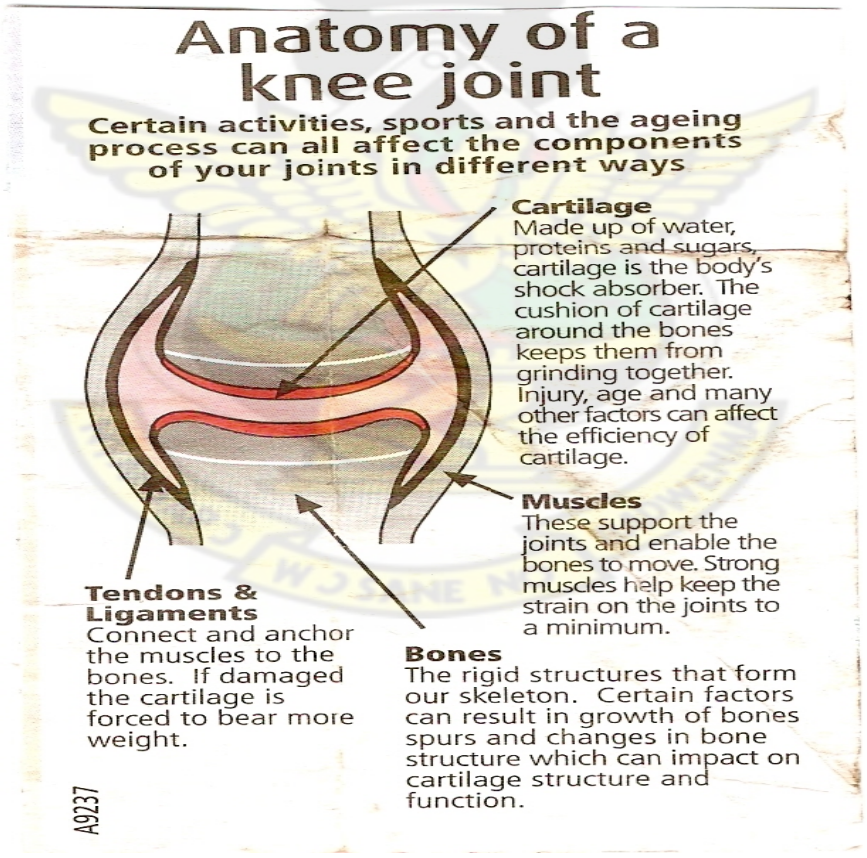


Figure 1.1.2: **Anatomy of a Knee Joint (** Source: Seven Seas JointCare**)**

1.1.0 General Treatment of Arthritis

According to the national Centres for Disease Control (CDC) and Prevention, arthritis disables more Americans than heart diseases and stroke. If left untreated, this nagging pain can grow

worse, eventually becoming so excruciating that one can no longer walk even short distances. Severe arthritis can restrict one's mobility and limit his quality of life, but with awareness, early diagnoses and an aggressive or proper treatment, one can slow the development of arthritis and lead a more productive life.

Treatment involves orthodox methods, nutritional or dietary supplements, traditional or herbal treatment[4]. Treatment requires initial diagnosis of the disease by a health care professional. For example orthopaedic surgeons (medical doctors who specialize in the nonsurgical and surgical care of foot and ankle problems), rheumatologist (medical arthritis specialist), physiatrist (rehabilitation specialist), pedorthist (footwear specialist), physical therapist, orthotist (brace specialist), occupational therapist, nurse and clinical social worker, can diagnose and treat arthritis.

To diagnose the disease, a complete medical history and physical examination of the patient should be carried out. X-rays and laboratory tests often can confirm the type and extent of the arthritis. Other tests such as a bone scan, computed tomography (CT) scan, or magnetic resonance imaging (MRI) may be used to evaluate the condition.

1.1.1 Orthodox Treatment

Once the doctor confirms that one has arthritis, he or she will recommend a treatment regimen, which may include medications by:

(a)Mouth (Anti-inflammatory).

(b)Injections(steroids).

(c)Physical therapy or exercise.

(d)Occupational therapy or orthotics such as pads in your shoes, shoe inserts, additions to the insoles or heels of your shoes, or custom-made braces.

(e)Surgery, which involves the cleaning arthritic joint, eliminating the painful motion of the joint, replacing the joint with an artificial joint or a combination of all these.

After surgery, the patient will require a period of rehabilitation when his/her foot might have to be in a cast and he/she might have to wear special shoes or braces for a while.

In addition, the patient should take medications as directed, exercise, control his or her weight, and participate in all aspects of treatment programs.

1.1.2 Physical Treatment (Exercise) of Arthritis

According to the Arthritis Foundation, proper exercises performed on a regular basis are an important part of arthritis treatment. Twenty years ago, doctors advised exactly the opposite, fearing that activity would cause more damage and inflammation. Not exercising causes weak

muscles, stiff joints, reduced mobility, and lost vitality, say rheumatologists, who now routinely advise a balance of physical activity and rest.

According to the 1996 Surgeon General's Report on Physical Activity and Health, regular, moderate physical activity is beneficial in decreasing fatigue, strengthening muscles and bones, increasing flexibility and stamina, and improving the general sense of well-being. The National Institutes of Health (NIH) advises that the amount and form of exercise should depend on which joints are involved, the amount of inflammation, how stable the joints are, and whether a joint replacement procedure has been done. A skilled physician who is knowledgeable about the medical and rehabilitation needs of people with arthritis, working with a physical therapist, can design an exercise plan for each patient.

Three main types of exercises are recommended:

- Range-of-motion-moving a joint as far as it will comfortably go and then stretching it a little further to increase and maintain joint mobility, decrease pain, and improve joint function. These can be done daily, or at least every other day.
- Strengthening-using muscles without moving joints to help increase muscle strength and stabilize weak joints. These can be done daily, or at least every other day, unless there is severe pain or swelling.
- Endurance-aerobic exercises such as walking, swimming and bicycling to strengthen the heart and lungs and increase stamina. These should be done for 20 to 30 minutes, three times a week, unless there is severe pain or swelling.

1.1.3 Herbal (Traditional) Treatment

Of late more and more people are seeking an alternative treatment to combat the pain of arthritis. Herbs and spices are very valuable sources of antioxidants. At least 30 food spices and herbs have been shown to possess antioxidant properties.

One such treatment that may hold promise is the use of the herb Gotu Kola (*Centella asiatica*). Gotu Kola also called Indian pennywort, marsh penny and water pennywort, is a creeping Perennial herb, native to tropical and sub-Tropical Asia, some South Pacific islands, coastal and central Africa. It has been used for centuries in both Ayurveda and Chinese medicine and was first accepted as a drug in France in the 1880's. In Australia, the fresh leaves of Gotu Kola (commonly known as Pennywort, 'The Arthritis Herb'), are used to treat arthritic conditions and has become extremely popular. Following the frequent reports of its efficacy, the Herbal Medicines research and Education Centre has begun a research project to investigate the popular use of Pennywort (*Centella asiatica*) for the treatment of arthritis.

Numerous reports have emerged from the general public that eating two(2) leaves each day is said to relieve the pain of arthritis and even plant nurseries in Australia sell the plant as 'the Arthritis Herb'

Pregnant women, breast-feeding mothers or people taking certain medications should not take Gotu Kola.

Another herb is the Sea Cucumber, which is a good source of chondroitin sulphate and several other joint friendly nutrients. For thousands of years sea cucumbers have been used successfully in China for treating arthritis. Eugenol is the active antioxidant compound in garlic and flavenoids are found in rosemary.

1.2.0 TYPES OF ARTHRITIS

- Osteoarthritis
- Rheumatoid arthritis
- Inflammatory arthritis or other forms of arthritis related conditions.

Osteoarthritis and rheumatoid arthritis are the two most common forms of the disease.

1.2.1. OSTEOARTHRITIS

This is the most common type of arthritis, especially among older people. Sometimes it is called degenerative joint disease or osteoarthrosis. Osteoarthritis is a joint disease that mostly affects the cartilage[8, 9, 10]. Cartilage is the slippery tissue that covers the ends of bones in a joint. Healthy cartilage allows bones to glide over one another. It also absorbs energy from the shock of physical movement. In osteoarthritis, the surface layer of cartilage breaks down and wears away. This allows bones under the cartilage to rub together, causing pain, swelling, and loss of motion of the joint. The joints most often affected are the knee, hip and hand.

Also, a sudden and traumatic injury such as a broken bone, torn ligament, or moderate ankle sprain can cause the injured joint to become arthritic in the future. Sometimes a traumatic injury will result in arthritis in the injured joint even though the joint received proper medical care at the time of injury. It affects approximately 12% of the population in the United States[5, 6]. Also, the incidence is higher in women than men, with approximate ratio of two to one.

Other risk factors include joint trauma, obesity, and repetitive joint use.

1.2.1.1 Signs and Symptoms of Osteoarthritis:

- Early morning joint stiffness and pain.
- Loss or restriction of joint mobility.
- Pain that is worse after use.
- Stiffness after periods of rest.

- Creaking/cracking of joints after movement (also known as crepitus).
- Tenderness and swelling in certain areas.
- Restricted mobility.
- Pain in the joint before or during changes in the weather.
- Deformity of the joints.

1.2.1.2 Treatment of osteoarthritis.

Treatment involves the use of drugs, dietary supplements and exercise

(**a**)(**i**). ***Use of Non- Steroidal Anti-Inflammatory Drugs (Analgesics).***

Regular intakes of traditional nonsteroidal anti-inflammatory drugs (NSAIDs) can relief pain. But high doses can cause gastrointestinal (GI) bleeding or ulcers. Because of this problem, a new type of NSAID, cyclo-oxygenase-2 inhibitors, better known as COX-2 inhibitors, has joined the old standbys. These drugs help suppress arthritis with less stomach irritation.

Cyclo-oxygenases are enzymes needed for the synthesis of hormone-like substances called prostaglandins, which promote many inflammatory processes, including pain and fever.

There are two types of cyclo-oxygenases; namely, the COX-2 enzyme that *mediates inflammation and pain*, and the COX-1 enzyme that *helps maintain other physiological functions in the body.*

Traditional NSAIDs inhibit both enzymes. The new NSAIDs, however, block mostly the COX-2 enzyme, offering a new treatment option for people who have had difficulty tolerating the old NSAIDs. A medical officer, Maria Villalba, M.D., with the Food and Drug Administration's Center for Drug Evaluation and Research, says *"COX-2 inhibitors are just as effective in treating osteoarthritis as other NSAIDs"*

The FDA approved the first COX-2 inhibitor, Celebrex (celecoxib), in 1998 to treat rheumatoid arthritis and osteoarthritis. Vioxx (refecoxib) became the second COX-2 inhibitor to receive approval, in 1999, for the treatment of osteoarthritis, dysmenorrhea (pain with menstrual periods), and the relief of acute pain in adults, such as that caused by dental surgery.

Another COX-2 inhibitor, Bextra (generic name, Valdecoxib), a NSAIDs, is used in the treatment of osteoarthritis, rheumatoid arthritis, painful menstruation and other types of acute pain. These drugs, taken orally, were found to substantially lower the risk of stomach and upper intestinal ulcers detected by endoscopy in clinical trials, compared with some of the other NSAIDs tested.

It must be noted, however, that, although these drugs have dangerous side effects, two recently completed large clinical studies of approximately one-year duration did not support removal of

the standard NSAID warning of the risk of serious gastrointestinal events from the Celebrex, Bextra and Vioxx labels. These large studies did not show an advantage in overall safety (as measured by the total number of deaths, serious adverse events, discontinuations and hospitalizations due to adverse events) favoring the selective COX-2 inhibitors compared to the other NSAIDs tested.

(**ii**). ***Use of Non-Drug Alternatives.***

Two non-drug alternatives for the treatment of pain in osteoarthritis of the knee were approved by the FDA's Center for Devices and Radiological Health in 1997 for patients who have failed to respond adequately to simple analgesics, such as acetaminophen, and to conservative nonpharmacologic therapy. Hyalgan and Synvisc are viscous solutions composed of hyaluronan (hyaluronic acid, a lubricant found naturally in the joints), and are injected directly into the knee joint. Both are believed to increase the quality of synovial fluid, although the mechanism of action for these products is not well understood.

The most common side effects reported from these treatments are injection site pain and knee pain and, or swelling-were found to be temporary. These treatments may be an ideal option for patients who cannot tolerate oral medications and who are not candidates for surgical knee replacement.

(**iii**). ***Use of Glucosamine Salts (Non- Analgesic)***

In addition, synergistic nutrition[10] ensures the proper building blocks for a good foundation. Glucosamine suphate (GS), the only one of the building blocks of healthy cartilage, is effective for soft tissue healing, and so used for treating osteoarthritis. Although it has become popular in this respect as it has been used for over thirty years to treat arthritis, it is not the only building block for healthy cartilage. In fact, glucosamine is used as a precursor for another important building block, N-acetyl glucosamine (NAG), which comprises part of the "cellular cement" around bone tissue that contributes to the "total structure" of connective tissue. Giving NAG directly not only saves the body conversion energy, it bypasses the acetylation step that is impaired in many disorders, including inflammatory bowel disease, and is also impaired by alcohol and non-steroidal anti-inflammatory drugs. Also, many glycosaminoglycan patterns (bigger building blocks, of which NAG is an ingredient), are impaired in connective tissue diseases. Providing a synergistic nutrient profile assures sufficient building blocks for complete repair, eliminates the need for high dosing, and guards against mild side effects that are possible from high dosing, including: stomach upset, heartburn, diarrhoea, nausea, and indigestion. Glucosamine sulphate has an excellent safety record, and in fact, is considered the treatment of

choice, "for prolonged oral treatment of rheumatic disorders". However, even mild side effects are a signal that high-dosing is not the best approach. Joseph Pizzorno Jr., ND, cautions that glucosamine sulphate is not an analgesic and takes several weeks before symptomatic relief in osteoarthritis can be obtained.

Many clinical trials have emphasized the therapeutic value of GS in degenerative joint disease such as osteoarthritis, noting that unlike the drugs, which relieve pain but destroy cartilage, GS, "with its chondrometabolic, antireactive and antiarthritic properties, represents the pharmacological rationale for the use of GS as a disease-modifying agent in osteoarthritis" [13]

Recently, it is found that GS has good multifunction to absorb free radical, to loss weight and to regulate the internal secretion. Also, it may have other therapeutic effects such as antiviral, anti-cancer, anti-aging, immune boosting or cholesterol lowering activity.

As important as GS is to healthy cartilage, N-acetyl glucosamine (NAG) is just as important to the extracellular matrix that also helps to comprise our, "cellular cement". NAG is needed for synthesis of the chondroitin proteoglycans (bigger building blocks). When exogenous GS is given and some is converted to NAG, there are scientists who believe that it is the NAG, "which finally determines the antitoxic and antioxidative properties in this amino sugar" . NAG has the ability to reduce joint pain, swelling, and restricted motion by itself; in fact the Merck Index puts NAG in an antiarthritic, therapeutic category of its own [14]. N-acetyl glucosamine forms a web-like, highly viscous layer over the intestinal mucosa, which protects underlying tissues from bacterial and toxic assault. The effect NAG has on closing a "leaky gut" can go a long way in inhibiting the cause of joint pain - that is, preventing entry of microorganisms which aggravate and even inhabit joint tissue. There are several ways in which NAG acts to eliminate the origin of joint problems:

- It decreases bacterial growth by strengthening intestinal mucosa, promoting growth of bifidobacteria and reducing adherence of organisms such as *Candida albicans* and *E. coli* to the gut wall .
- It prevents cell damage
- It inhibits lectin interactions(lectins can cause inflammation and intestinal permeability
- It blocks the release of the leukocyte elastase enzyme (elastase is an enzyme which breaks down elastin connective tissue) [15]
- It eliminates parasites.
- It acts as a helmintic (dewormer) [16]
- It increases protective mucous production [16].

- It helps fight inflammatory free radicals and lipid peroxidation.

NAG has a strong effect on the liver, which detoxifies many of the substances which can aggravate/cause joint pain. NAG is instrumental in preventing liver peroxidation, and has been credited with contributing to the significant regression (up to 84%) of a patient's liver tumor [17]

(**iv**). ***Use of Anti-oxidants and Accessory Nutrients.***

Antioxidants[9,11,18], also, referred as 'free-radical scavengers', are the body's natural defence against free radical damage. Free radicals are produced as a result of normal body processes, physical injury, stress, exposure to certain environmental substances such as pollutants, UV light, radiation or allergens. Most of the time, they are unable to cause lots of damage as the body has its own defence against them, in the form of antioxidants. However, there are times when these free radicals overwhelm the defence mechanisms and cause damage. This is known as oxidative stress and has effects in the in ageing and disease processes.

Diets rich in polyunsaturated oils increase the requirement for antioxidants, especially vitamin E. Some nutrients and dietary components have antioxidant properties which may offer a protective role against a number of diseases.

Another accessory nutrient, **N-acetyl cysteine (NAC)**, protects against cell death, and is noted by Life Sciences as a "reference compound" for, "inhibition of liver injury and lipid peroxidation" [18]. **Silymarin and milk thistle** support the cleansing activities of the liver.

Soluble trachea is a natural source of chondroitin sulphate, and **green-lipped mussel** is not only a natural source of mucopolysaccharides (bigger building blocks), it is a natural source of superoxide dismutase (the enzyme that scavenges the inflammatory superoxide radical), and was shown to be beneficial for severe osteoarthritis patients who did not respond to medication [19].

Pantothenic acid is a nutrient that supports the adrenal glands, which produce natural, anti-inflammatory corticosteroids such as cortisol.

Glutathione is an antioxidant, and also the major conjugating agent in the liver which pulls toxins out of the body. **Cysteine, glycine, taurine, and glutamic acid** all contribute to efficient removal of toxins from the liver.

Controlled trials have shown that 400 - 600 IU's of **vitamin E** can be helpful in the treatment of osteoarthritis [20]. Vitamin E at 600 mg/d has also been recommended by J. Pizzorno, ND, who notes its ability to inhibit the activities of the lysosomal enzymes and stimulate increased deposition of proteoglycan. [21]

Current report from epidemiological study on arthritis and rheumatism shows that antioxidants are able to reduce the progression of osteoarthritis in the knees of 640 patients, independent of diet/food allergies[22]

Vitamin C, had the highest correlation (70%); followed by **beta carotene** and **vitamin E.** **Vitamin C,** a water soluble antioxidants in extra-cellular fluids is necessary for manufacture of collagen and so, associated with reduced cartilage loss, protects body against oxidative damage, neutralizes harmful reactions in blood and fluid inside and surrounding cells, protects the heart and other tissues.

Vitamin E, a major antioxidant, it protects cells against free radical damage, may help prevent onset of cardiovascular disease and cancer.

Dr. Steve Austin observes that doctors of nutritional medicine almost universally prescribe glucosamine sulphate and allergy elimination, and that, "antioxidants may soon be added to this list" [23]

(b). Dietary Help for Osteoarthritis.

Detoxification, **stress reduction**, and **proper diet** are all important [10].

The following dietary sources have been recommended for the treatment of Osteoarthritis:

(i)It is a good idea to increase complex carbohydrates, dietary fiber, fruits, vegetables and nuts.

(ii)Increasing cold water fish for an **essential fatty acid source** (Omega-3), including salmon, mackerel, sardines and herring is a good idea.

(iii)Avoid saturated fats and trans fatty acids.

(iv)Follow a low-fat diet, but one that is proportionately rich in good fatty acids, such as the omega 3's mentioned above, and also gamma linolenic acid (found in black currant seed oil, borage oil and evening primrose oil).

(v)The Rheumatoid Disease Foundation suggests the use of boron to treat osteoarthritis, rheumatoid arthritis, and osteoporosis. Boron – rich foods include alfalfa, lettuce, peas, cabbage, apples, dates, prunes, raisins, almonds and hazelnuts.

(vi)Boron apparently plays a role in the retention of **calcium** and also positively stimulates hormonal factors for both men and women, contributing to healthy bones[24]. Since boron can raise estradiol levels, some premenopausal women with high amounts of circulating estrogen may not choose to supplement with boron.

(vii)Sulfur and methionine-containing foods such as legumes, cabbage, brussel sprouts, garlic and onions, are also beneficial [25].

(viii)An elimination/rotation diet is recommended, since allergy foods are implicated in osteoarthritis. The most common offenders include dairy products, refined foods, meat, citrus fruits and nightshade foods (tomatoes, white potatoes, eggplant, and peppers) and margarine. The nightshade foods are alkaloids that can increase inflammation and inhibit collagen repair; they also contain a toxic substance called solanine that triggers reactions in people and should be avoided. Caffeine, alcohol and tobacco are also no-no's.

(ix)Regular chiropractic care is a good idea to address structural or postural problems and prevent arthritic tendencies.

1.2.2. RHEUMATOID ARTHRITIS.

It is a chronic inflammatory illness. It is an autoimmune disease that occurs when the bodies own immune system mistakenly attacks the synovium (cell lining inside the joint) as if they were invading pathogens. This chronic, potentially disabling disease causes pain, stiffness, swelling and loss of function in the joints.

People with rheumatoid arthritis for at least 10 years almost always develop arthritis in some part of the foot or ankle. This illness affects about one percent of the world's population. Although the inflammation associated with rheumatoid arthritis primarily attacks the linings of the joints, the membranes lining the blood vessels, heart, and lungs may also become inflamed. The hands and feet are most often affected, but any joint lined by a membrane may be involved. The inflammation can be controlled by medication. If the inflammation is not controlled the joints may become deformed.

Rheumatoid arthritis usually manifests itself over a period of a few months. However for some, the disease may appear over night. Rapid onset does not mean the individual is at greater \risk of disease progression.

Rheumatoid Arthritis may have different effects on different people. Some individuals may experience extreme pain while others may not. Patients often suffer cycles of severe and light symptoms. While the cause remains elusive, doctors suspect that genetic factors are important in rheumatoid arthritis. Recent studies have begun to tease out the genetic characteristics that can be passed from generation to generation. However, the inherited trait alone does not cause the illness. Researchers think this trait, along with some other unknown factor-probably in the environment-triggers the disease. But rheumatoid arthritis can be difficult to diagnose early because it may begin gradually with subtle symptoms. According to the CDC, this form of arthritis affects more than 2 million people in the United States; and two to three times more women are affected than men.

1.2.2.1. The Symptoms of Rheumatoid Arthritis

The most common symptoms of rheumatoid arthritis are:

* Joint swelling, especially in the small joints of the hands and feet.
* Joint tenderness, stiffness, and pain, especially in the morning.

1.2.2.2. Effects of Rheumatoid Arthritis

(a) **Hardened Lumps.**

About twenty five percent of rheumatoid arthritis patients develop hardened lumps under the skin. These hardened lumps are called rheumatoid nodules. Development of hardened lumps usually develops in the later course of the disease. Most often, the nodules are found on bony sites such elbows, hips, heels, and back of the head. However, they can also form under the skin in the finger, toe or heel pads, or in tendons.

(b) **Cartilage and Bone Destruction**.

If joint inflammation persists, cartilage and bone destruction can occur. When cartilage and bone destruction occurs, the joint becomes deformed and immobile. Inflammation and deformity are most often seen in the hands and feet. Nonetheless, the knees, hips, and shoulders may also be affected. In addition to joint deterioration, people more severely affected may also experience weight loss, low-grade fever, and malaise because of the disease's effects on the whole body.

1.2.2.3. Risk factors for developing Rheumatoid Arthritis

- Women suffer from it two to three times more than men.
- Relatives of people with rheumatoid arthritis have an increased risk of developing the disease.
- The siblings of severely affected rheumatoid arthritis patients are at highest risk.

1.2.2.4. Diagnoses of Rheumatoid Arthritis.

Most patients suffering from rheumatoid arthritis have antibodies called rheumatoid factors in their bloodstream that are part of the inflammatory process of the disease. The presence of rheumatoid factor is used by doctors to help confirm a diagnosis of rheumatoid arthritis. However, rheumatoid factor may not be a definitive test for rheumatoid arthritis. Rheumatoid factor is also found in cases of chronic infection and in some other types of autoimmune disease. High levels of rheumatoid factor are often seen in severe cases of rheumatoid arthritis. The diagnostic criteria of the American College of Rheumatology state that four out of seven signs and symptoms must be present for the diagnosis of rheumatoid arthritis to be made. Symptoms such as morning stiffness and swelling should be present for at least six weeks before the diagnosis is considered certain.

1.2.2.5. Treatment of Rheumatoid Arthritis.

For years, the pain and inflammation of arthritis have been treated with varying success, using medications, local steroid injections, and joint replacement. Seldom did the therapies make the pain go away completely or for very long, nor did they affect the underlying joint damage. There should be medication to control pain and stiffness and reduce the risk of joint deformity.

(a). ***Use of NSAIDs and Disease-Modifying Anti-Rheumatic Drugs (DMARDs).***

Typical treatments for rheumatoid arthritis have relied on a combination of NSAIDs, such as ibuprofen or aspirin (which reduce swelling and alleviate pain but do not change the course of the disease), and disease-modifying anti-rheumatic drugs (DMARDs).

DMARDs such as methotrexate and sulphasalazine, also called slow-acting drugs. DMARDs work to slow inflammation and can, in many cases, alter the course of the disease. Until recently, most doctors reserved the use of DMARDs for patients who failed to respond to other therapies. Now, most physicians use DMARDs early and aggressively in the hope of slowing disease progression and damage to joints and internal organs.

Also, as mentioned earlier, Celebrex and Bextra (both NSAIDs) can be used to treat rheumatoid arthritis.

(b)***Use of Biological Response Modifiers***

The most recently approved treatment regimen for rheumatoid arthritis is one that combines the genetically engineered biological drug **Remicade** (infliximab) with the drug **methotrexate.** (Not all patients with rheumatoid arthritis can tolerate or respond to methotrexate alone, a standard treatment for the disease.) **Remicade** is the second in a new class of drugs known as biologic response modifiers, which bind to and block the action of a naturally occurring protein called tumor necrosis factor (TNF), believed to play a role in joint inflammation and damage. Elevated levels of TNF are found in the synovial fluid of rheumatoid arthritis patients. Remicade, which is administered intravenously by a health-care professional in a two-hour outpatient procedure, was approved by the FDA in 1999 to reduce the signs and symptoms in patients who have not experienced significant relief from methotrexate alone.

Also, the drug, **Enbrel** (etanercept), approved in 1996, is the first biologic response modifier to receive FDA approval for patients with moderate to severe rheumatoid arthritis. Taken twice weekly by injection, Enbrel was shown to decrease pain and morning stiffness and improve joint swelling and tenderness. In 2000, the drug's uses were expanded to include delaying structural damage. Jeffrey N. Siegel, M.D., a medical officer with FDA's Center for Biologics Evaluation and Research, says that **Enbrel** is an exciting breakthrough because it helps a majority of

patients who have not responded to any of the other commonly used therapies. Although it is injected, the treatment can be administered at home. In addition, Enbrel has been shown to be effective for children with the juvenile form of rheumatoid arthritis. In clinical trials, Enbrel was generally well tolerated, and one of the most common side effects was an injection site reaction. Both **Remicade** and **Enbrel** show promise in treating rheumatoid arthritis, although the long-term risks and benefits of these agents are unknown. In post-marketing reports, serious infections, including fatalities, have been reported with these agents. Caution should be used in patients with a history of recurring infections or with underling conditions that may predispose patients to infections.

(c) ***Use of Immunomodulators (Antirheumatics)***

Arava (leflunomide) is the first oral treatment approved for slowing the progression of rheumatoid arthritis. Although its effects are similar to those of methotrexate, this drug works by a different chemical mechanism that blocks at least one enzyme in certain immune cells called lymphocytes (a type of white blood cell that is part of the immune system), and thereby retards the progression of the disease. However, Arava is not a cure for rheumatoid arthritis. It may cause birth defects, and the label carries a special warning for pregnant women and those planning to become pregnant. Also, liver damage, including deaths, has been reported. The drug is not recommended for patients with severe immunodeficiency, bone marrow dysplasia, or severe, uncontrolled infections.

(d). ***Use of Non-drug Alternatives.***

The first non-drug alternative for adult patients with moderate to severe rheumatoid arthritis and longstanding disease was approved by the FDA in 1999. The Prosorba column, which was initially approved in 1987 to treat an immune blood disorder, is a single-use medical device, about the size of a coffee mug, containing a material that binds antibodies and antigen-antibody complexes.

In a two-hour process performed in a hospital or specialized treatment center, a patient's blood is removed and passed through a machine that separates the blood cells from the plasma (the liquid portion of the blood). The plasma is then passed through the Prosorba column, recombined with the blood cells, and returned to the patient. Although this filtering process is believed to remove proteins that may inadvertently attack the joint cells, the mechanism of action of the Prosorba column is not well understood. The treatment is given once a week for 12 weeks. The most common side effects include joint pain and/or swelling, fatigue, hypotension (low blood pressure), and anaemia.

"For those patients who have failed or are intolerant to DMARDs, including Arava and the anti-TNF agents," says Sahar M. Dawisha, M.D., a medical officer in the FDA's Center for Devices and Radiological Health, "the Prosorba column may be an additional treatment option." Occasionally, particularly painful joints may be injected with steroid medications. Psoriasis treatment is usually continued or started.

(e) ***Rest, Splinting and Exercise*.**

Rest, splinting of affected joints and exercise programs are recommended.

(f) ***Nutrient/Dietary Supplements***

Good nutrition is very important. This is because patients with a more advanced disease often experience anaemia and weight loss.

1.2.3. Types of ***inflammatory arthritis*** or other ***arthritis related conditions*** include:

1.2.3.1 Gout Arthritis

This is a disease that causes sudden, severe attacks of pain, tenderness, redness, warmth, and swelling in some joints. It usually affects one joint at a time, especially the joint of the big toe. The pain and swelling associated with gout are caused by uric acid crystals that precipitate out of the blood and are deposited in the joint. Factors leading to increased levels of uric acid and then gout include excessive alcohol intake, hypertension, kidney disease, and certain drugs.

1.2.3.2. Lupus Arthritis

Systemic lupus erythematosus is an autoimmune disease that can involve the skin, kidneys, blood vessels, joints, nervous system, heart, and other internal organs. Symptoms vary among those affected, but may include a skin rash, arthritis, fever, anaemia, hair loss, ulcers in the mouth, and kidney damage. In most cases, the symptoms first appear in women of childbearing age; however, lupus can occur in young children or older people. Studies suggest that there is an inherited tendency to get lupus. Lupus affects women about 9 to 10 times as often as men. It is also more common in African-American women.

1.2.3.3 Ankylosing Spondylitis.

Ankylosing spondylitis is a chronic inflammatory disease of the spine that can fuse the vertebrae to produce a rigid spine. Spondylitis is a result of inflammation that usually starts in tissue outside the joint. The most common early symptoms of spondylitis are low back pain and stiffness that continues for months. Although the cause of spondylitis is unknown, scientists have discovered a strong genetic or family link, according to the Arthritis Foundation. Most people with spondylitis have a genetic marker known as HLA-B27. Genetic markers are protein

molecules located on the surface of white blood cells that act as a type of “name tag.” Having this genetic marker does not mean a person will develop spondylitis, but people with the marker are more likely to develop the disease than those without it. Ankylosing spondylitis usually affects men between the ages of 16 and 35, but it also affects women. Other joints besides the spine may be involved.

1.2.3.4 Psoriatic Arthritis

This is a type of arthritis that is associated with psoriasis. Psoriasis a chronic skin condition characterized by inflamed, red, raised areas that develop silvery scales. It is generally mild and involves only a few joints. However, in a few people, Psoriatic arthritis is severe and affects the fingers and the spine. When the spine is affected, the symptoms are very much like those of ankylosing spondylitis. It is similar to rheumatic arthritis, but fewer joints may be involved, and there is no rheumatoid factor in the blood.

The exact cause of psoriatic arthritis is unknown. However, people with psoriatic arthritis have other family members with psoriatic arthritis or with psoriasis.

Symptoms may vary from patient to patient. The most common symptoms of psoriatic arthritis are:

- Nail abnormalities
- Skin lesions of psoriasis
- Joint swelling and joint pain
- Hip pain, elbow pain, ankle pain
- Pain and swelling at the site of attachment of tendons to bone

1.2.3.5 Infectious Arthritis

This is a form of joint inflammation that is caused by bacteria, viruses or fungi. The diagnosis is made by culturing the organism from the joint. Most infectious arthritis can be cured by antibiotic medications.

1.2.3.6 Juvenile Arthritis.

This is a general term for all types of arthritis that occur in children. Juvenile rheumatoid arthritis is the most prevalent form in children, and there are three major types:

(i) ***polyarticular*** (affecting many joints),

(ii) ***pauciarticular*** (pertaining to only a few joints), and

(iii) ***systemic*** (affecting the entire body).

The signs and symptoms of juvenile rheumatoid arthritis vary from child to child. There is no single test that establishes conclusively a diagnosis of juvenile arthritis, and the condition must

be present consistently for six or more consecutive weeks before a correct diagnosis can be made. Heredity is thought to play some part in the development of juvenile arthritis. However, the inherited trait alone does not cause the illness. Researchers think this trait, along with some other unknown factor (probably in the environment), triggers the disease. The Arthritis Foundation says that juvenile arthritis is even more prevalent than juvenile diabetes and cerebral palsy.

1.2.3.7 Reiter's Syndrome (*Reactive Arthritis*).

It involves inflammation in the joints, and sometimes where ligaments and tendons attach to bones. This form of arthritis usually develops following an intestinal or a genital/urinary tract infection. People with Reiter's syndrome have arthritis and one or more of the following conditions: urethritis, prostatitis, cervicitis, cystitis, eye problems, or skin sores.

1.2.3.8 Bursitis, Tendinitis and Myofascial Pain.

These are localized, nonsystemic (not affecting the whole body) painful conditions.

(i) Bursitis is inflammation of the sac surrounding any joint that contains a lubricating fluid.

(i) Tendinitis is inflammation of a tendon, and

(iii)Myofascial pain is a problem that results from the strain or improper use of a muscle. These conditions may start suddenly, and usually stop within a matter of days or weeks.

1.3.0 Preventive Measures

There are effective ways to prevent arthritis and to reduce the symptoms, lessen the disability and improve the quality of life for the people with arthritis. Both Centres for Disease Control (CDC) and the American College of Rheumatology recommend maintaining ideal weight, taking precautions to reduce repetitive joint use and injury on the job, avoiding sports injuries by performing warm-ups and strengthening exercises using weights, and by choosing appropriate sports equipment.

There should be early diagnosis and appropriate management, including self-management activities such as weight control and physical activity.

Self-management, education programs can reduce pain and costs. For example, the Arthritis Foundation Self-Help Program teaches people how to manage arthritis and lessen its effects.

Lyme arthritis may develop after a bacterial infection is transmitted to humans through tick bites. To prevent this type of arthritis, health experts advise people to use insect repellents, wear long-sleeved shirts and pants while walking near wooded areas, and check for and remove ticks to help reduce the risk of getting the disease. CDC, also, recommends the prompt use of

antibiotics for Lyme disease symptoms. In December 1998, FDA approved the first vaccine, **Lymerix,** to help prevent Lyme disease[26].

In an efficacy and safety trial, the vaccine's effectiveness in preventing Lyme disease was 49 percent after two injections and 76 percent after three. Vaccination should be considered by people 15 to 70 years old who live in or visit high-risk areas and have frequent or prolonged exposure to ticks. The vaccine has not yet been approved for use in children.

1.3.1. Role of Centres for Disease Control (CDC) and Future Directions

With funded states and other partners, CDC hopes to:

- Create a nationwide program to improve the quality of life for people affected by arthritis.
- Help state arthritis programs reach more people.
- Fund evaluation efforts to discover how best to deliver arthritis programs.
- Develop and evaluate
  - culturally appropriate programs to better serve diverse groups;
  - health communications programs to increase physical activity among people with arthritis, especially minorities, older adults, and people of low socioeconomic status.

1.4.0 GLUCOSAMINE.

1.4.1 Rationale for the Use of Glucosamine.

Much attention is given to glucosamine in my work because of the following reasons:

- Clinical trials have repeatedly shown that although glucosamine sulphate has no direct analgesic effect, it is far superior in relieving pain and inflammation of osteoarthritis than NSAIDs.
- Danger of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). Aspirin and other NSAIDs may suppress the symptoms but actually accelerate the progression of osteoarthritis. NSAIDS actually inhibit cartilage repair and accelerate cartilage destruction. Since osteoarthritis itself is caused by cartilage degeneration, NSAIDS worsen the condition. In addition, while aspirin very effectively relieves the pain and inflammation associated with osteoarthritis, large doses (2 - 4 grams/day) are needed. These amounts of aspirin almost certainly result in toxicities such as tinnitus and gastric irritation (GI). Other NSAIDS may be more tolerable, but are also associated with GI upset, headaches, and dizziness.

- Glucosamine addresses the underlying problem of osteoarthritis (cartilage degeneration). It is the most fundamental building block required for biosynthesis of the classes of compounds including glycolipids, glycoproteins, glycosaminoglycans (important components of the cartilage needed for healthy joints), hyaluronate, and proteoglycans. As a component of these macromolecules, glucosamine has a role in the synthesis of cell membrane lining, collagen, osteoid, and bone matrix. Also, it is required for the formation of lubricants and protective agents such as mucin and mucous secretions.
- Glucosamine's effects are not seen immediately, but when they do appear, the effects are much better and last longer than NSAIDs.

1.4.2 Alternative Forms of Glucosamine

Pure glucosamine is very "hygroscopic" and degrades (breaks down) rapidly when exposed to moisture or air. To avoid this, glucosamine needs to be bound to a stabilizer to be sold commercially. Commercially, glucosamine is available in a variety of forms - that is, *glucosamine hydrochloride, glucosamine hydro-iodide, glucosamine sulphate, and N- acetyl glucosamine*, and may be taken as tablets, caplets, capsules, powder or liquid. Although all contain glucosamine, they do not work in the same manner nor are they equally effective. Glucosamine sulphate is the best and the only form or source of glucosamine to be used because it stimulates the manufacture of glycosaminoglycans (components of cartilage) and promotes incorporation of sulphur into cartilage. Hence scientific literature does not support the use of glucosamine hydrochloride. As the sulphur component of glucosamine sulphate is crucial to its mechanism of action.

Glucosamine hydrochloride is more stable than glucosamine sulphate. Glucosamine HCl uses hydrochloride acid as a stabilizer. This product is especially good for those with high blood pressure, on salt or potassium restricted diets or those having adverse reactions to sulphate. Glucosamine hydrochloride ($C_6H_{13}NO_5 \cdot HCl$) provides more glucosamine than glucosamine sulphate($(C_6H_{11}O_5NH_2)_2.H_2SO_4$). So, Glucosamine hydrochloride is the most cost effective form of glucosamine available. 1g of glucosamine sulphate material only contains 0.7851g of glucosamine free base (GFB), but 1g of glucosamine HCl contains 0.8307g.

Glucosamine sulphate is made from glucosamine HCl by adding either sodium or potassium sulphate and co-crystallizing the resulting mixture. It is relatively unstable to moisture and air. This necessitates the use of stabilizers (most often, sodium chloride and potassium chloride stabilizers) in the preparation of glucosamine sulphate salt. Both forms appear to be effective in

stabilizing GS, but KCl is the preferred source since the Western diet is much higher in sodium and does not contain enough potassium.

1.4.3. Monograph of Glucosamine, Glucosamine Hydrochloride and Glucosamine Sulphate

1.4.3.1 D-Glucosamine

Molecular Formula** and **weight:	$C_6H_{13}NO_5$; M_r =179.17
***Chemical Abstract Service Registry number*(CAS)**:	3416-24-8.
***Systematic chemical name*:**	*(3R,4R,5S,6R)-3-Amino-6-(hyroxymethyl)oxane-2,4,5-triol. OR* *2-Amino 2-deoxy-D-glucopyranose*
Proposed quantitative evaluation of purity	Determination of active ingredients in the drug by:
	(1) Perchloric Acid Titration.
	(2) HPLC with Pulse Amperometric Detection
	(3) HPLC with Pre-column Derivatization using (a) Phenyl Isothiocyanate (PITC) as derivatization reagent with detection by UV at 240nm (b) 9-fluorenylmethyl chloroformate (c) N-(9-fluorenylmethoxy carbonyloxy) succinimide (FMOC-Su) reagent with detection by UV at 265nm
	(4) HPLC using Evaporative Light Scattering Detection(ELSD)
	(5). Spectrophotometric method using PITC as derivatization reagent with detection by UV at 240nm.

1.4.3.2. Glucosamine Hydrochloride.

Molecular Formula and weight:	$C_6H_{13}NO_5$.HCl; M_r = 215.63.
CAS:	66-84-2.
Physico-Chemical Analysis	
Appearance	White crystalline powder
Loss on drying	< 0.1%
Assay	99.9%
Solubility in water	Colourless clear solution
Specific Rotation	+70
pH value	4.0
Melting point	> 300^{o}C
Heavy metal Analysis	
Lead	< 3 ppm
Iron	< 6 ppm
Arsenic	< 2 ppm
Microbiological Analysis	
Total plate count	< 100 cfu/gm
Bacteria of coli group	< 10 cgu/gm
Mould & Yeast	< 10 cfu/gm
Test for Specific Pathogens	
E. coli(1g)	Absent in 1g
Salmonella typhi	Absent in 25g
S aureus(1g)	Absent
Particle size	100% through 80mesh
Packing	Paper Barrels 30/50kgs
Shelf life	Two years from date of Mfg

1.4.3.3 Glucosamine Sulphate

Molecular Formula and weight:	$((C_6H_{11}O_5NH_2)_2.H_2SO_4)$; $M_r = 456.418$
CAS:	29031-19-4.
Physico-Chemical Analysis	
Appearance	White crystalline powder
Loss on drying	< 0.5%
Assay	99.0% - 104.0%
Solubility in water	Colourless clear solution
Specific Rotation	+48.0 - +53.0
pH value	3.5 - 4.5
Residue on ignition	23.8% - 31.0%
Heavy metal Analysis	
Lead	Max 0.5mg/kg
Cadmium	Max 0.01mg/kg
Mercury	Max 0.02 mg/kg
Arsenic	Max 0.2mg/kg
Zinc	Max 50mg/kg
Copper	Max 20mg/kg
Microbiological Analysis	
Total plate count	Max 500/g
Bacteria of coli group	Max 10/g
Mould & Yeast	Max 100/g
Test for Specific Pathogens	
E. coli	Absent in 1g
Bacillus cereus	Max 100/g
Salmonella	Absent in 25g
Particle size	100% through 80mesh
Packing	Paper Barrels 30/50kgs
Shelf life	Two years from date of Mfg

1.4.4 Dietary Supplements

Commercially, glucosamine is combined with one or more of the following complementary ingredients chondroitin sulphate, methylsulphonylmethane(MSM) or dimethyl sulphone, Omega- 3 Fatty Acids, Manganese Ascorbate, collagen, cod liver oil enriched with vitamin Vitamin A, D and E, Vitamin C, Boswellia (Boswellin) Serrata, Bromelaine, Yucca, Niacinamide, and Aloe Vera Gel, Pro joint formula, containing vitamins, calcium, amino acids, shark or bovine cartilage and minerals.

Collagen Type II is a powerful anti-inflammatory, rebuilds cartilage in arthritic joints; 60% of cartilage is Collage Type II, reduces cholesterol in blood, cardio-protective agent, and lowers risk of heart attacks.

Chondroitin, found naturally in the body, is a major component of cartilage. It works to protect the cartilage from premature degeneration by blocking enzymes that seek to break down cartilage and by absorbing excess fluid in the connecting tissue. It also assists in cartilage repair. Chondroitin sulphate is proven anti-inflammatory; it is more effective than NSAID's, and works with glucosamine to stimulate cartilage production.

Methylsulphonylmethane (MSM) is a natural sulphur compound found in all living things. It is essential dietary sulphur and unlike the bad sulphurs (e.g. sulphur, sulphate, sulphite and sulphide), the sulphur in MSM, called sulphonyl, is as safe and as important in our diet as Vitamin C. MSM ensures healthy connective tissue, healthy joint function, proper enzyme activity and hormone balance; proper function of the immune system, and increases athletic stamina and elimination of muscle soreness.

1.4.5 Chemistry of Glucosamine.

Glucosamine, called chitosamine, is a naturally occurring amino sugar synthesized in the body from L-glutamine and glucose. Although glucosamine molecule contains glucose, it is not an energy source for the body tissues. Instead, glucosamine sulphate serves to incorporate sulphur into the cartilage and other body tissues. Glucosamine is involved in constructing nails, skin, eyes, bones, ligaments, tendons, heart valves, discharging mucous from the respiratory system, digestive system, and urinary tract[27].

Glucosamine has simple structure of glucose, and an amino group at carbon number 2, so an amino sugar, or an aminomonosaccharide naturally occurring in the human body. Glucosamine is produced from the shells of chitin (shellfish e.g. lobster, crab, and shrimp), and a key component of cartilage. Glucosamine is also available in synthetic forms. Although, both the α- and β- forms of glucosamine are possible, the beta form predominates in nature.

Glucosamine (*polyhydroxylated primary amine*) is a small molecule (M_r =179.17, m.p = 423), with polar properties, very soluble in water and soluble in hydrophilic organic solvents such as methanol. At 37^{o}C, glucosamine has a pK_a of 6.91. This means that at pH 7.4, e.g. in the blood, 25% of glucosamine is ionized, and 75% is not ionized. At pH 6.8, e.g. in the small intestine, 46% is ionized, and 54% is not ionized. At pH 1 - 3, e.g. in the stomach glucosamine is completely ionized. Therefore, the pK_a of glucosamine is very favourable for absorption from the small intestine and in general for crossing of biological barriers in the body. All these properties candidate glucosamine as an easily absorbable and easily diffusible substance, as in fact it was experimentally found. Like the free base form, the salt form is soluble in water but insoluble in methanol.

Figure 1.1.3: Chemical structure of **(A)** 2-Amino-2-deoxy-D-glucopyranose;
(B) N–Acetyl-Glucosamine.

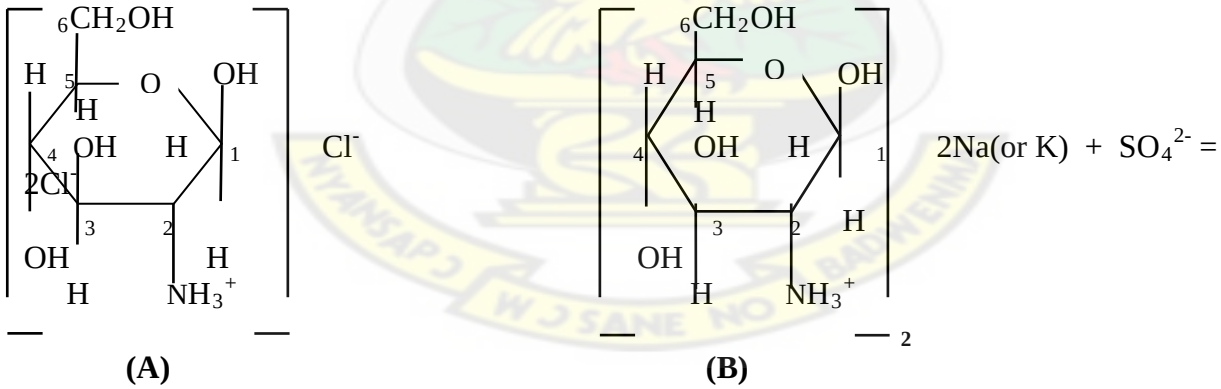
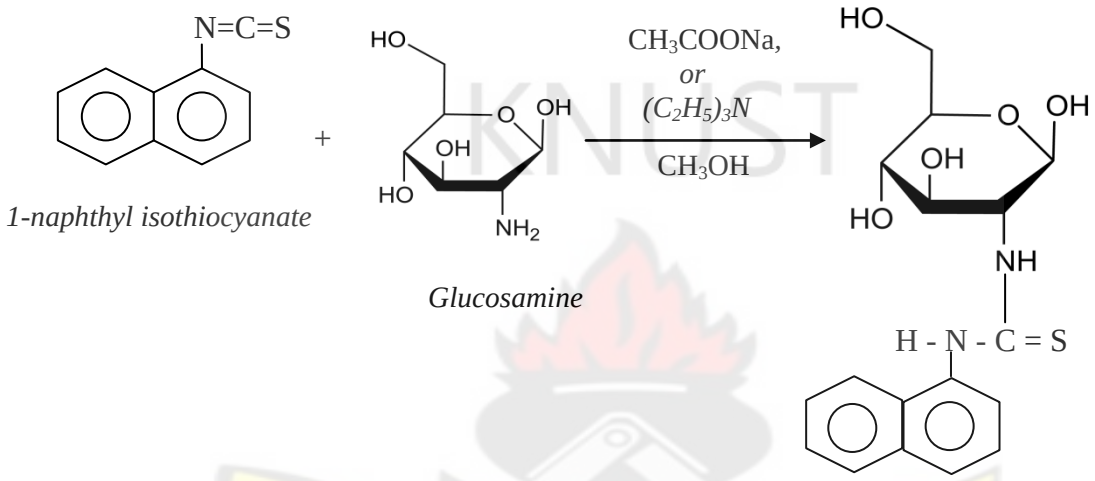


Figure 1.1.4: Chemical structure of: **(A)** 2-Amino-2-deoxy-D-glucose hydrochloride;
(B) 2-Amino-2-deoxy-D-glucose sulphate.

Glucosamine does not have adequate UV chromophore. Extraction and detectability can be improved by appropriate derivatization of the amino functional group. Various derivatization reagents have been used for quantitative HPLC determination of glucosamine in standard aqueous solutions. For example, pre-column derivatization of amines with 9-fluorenylmethyl chloroformate under alkaline (borate buffer 0.02M, pH 9.6) or neutral conditions has been

attempted successfully. In addition, pre-column derivatization of the amino group with N-(9-fluorenylmethoxy carbonyloxy) succinimide under alkaline conditions (using triethylamine) has been applied to determine glucosamine in raw materials and dietary supplements containing glucosamine sulphate and glucosamine hydrochloride by HPLC. Also, reaction of glucosamine with naphthyl(or phenyl) isothiocyanate, using standard aqueous solution produces a derivative that exhibit favourable UV absorbing properties. This method has been applied to the determination of glucosamine in dosage forms and in plasma using ion-exchange cartridges.



(*Phenylthiourea derivative*).

The hydrogen atoms in the glucosamine at position 1, 2, 3, 4 and 5, not shown are implied.

Figure 1.1.5: *Derivatization of Glucosamine with 1-naphthyl isothiocyanate*

Since Glucosamine has two natural stereoisomers or diastereomeric forms (alpha and beta), and interconversion of these two in aqueous solution is not preventable, two peaks result in the chromatogram. Their relative ratios may vary according to the equilibrium times. The sum of the peak areas is used for the quantification of the glucosamine free base. Stability tests indicated that this derivative is sufficiently stable to permit overnight automatic sample injection onto the HPLC system. Much attention will be given to Glucosamine sulphate (GS). GS provides the joints with the building blocks they need to repair damage to the cartilage. Cartilage that was synthesized without GS contained few normal-sized chondroitin sulphate chains and only about half of the normal serine residues. Subsequent addition of GS resulted in a time-dependent recovery of sulphate incorporation after 2 hours. However, the chondroitin sulphate chains were restored to normal size within 15 minutes. Glucosamine sulphate is, "required for optimal glycoconjugate synthesis"[28] Dietary sources of glucosamine sulphate are not found in most diets, since GS is mainly derived from seashells.

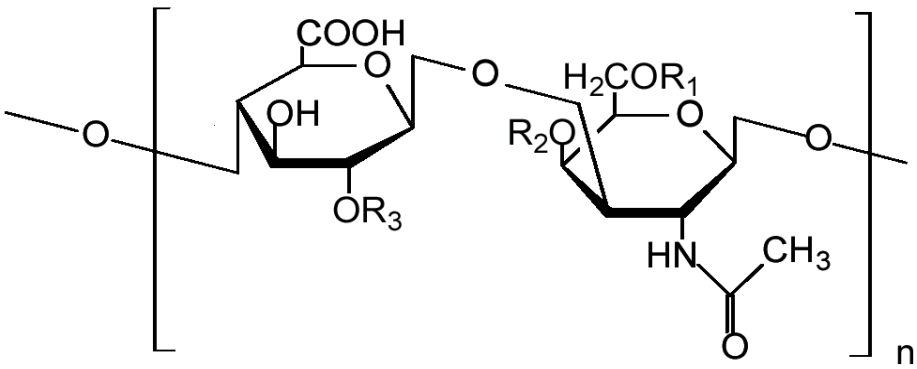


Figure 1.1.6: Chemical structure of one unit in a chondroitin sulphate chain

Chondroitin -4-sulphate: R_1 = H; R_2 = SO_3H; R_3 = H

Chondroitin -6-sulphate: R_1 = SO_3H; R_2, R_3 = H

The hydrogen atoms at position 1, 2, 3, 4 and 5, not shown are implied.

1.4.6 Mechanism of Action

Glucosamine works to stimulate joint function and repair. Glucosamine sulphate is capable of stimulating proteoglycan (found in the structural matrix of joints) synthesis, inhibiting the degradation of proteoglycans, and stimulating the regeneration of experimentally-induced cartilage damage [29, 30].

It has been proven effective in numerous scientific trials(biochemical and pharmacological data combined with animal and human studies) for easing osteoarthritis pain, aiding in the rehabilitation of cartilage, renewing synovial fluid, and repairing joints that have been damaged from osteoarthritis. Glucosamine exhibits anti-inflammatory properties via the inhibition of proteolytic enzymes. Having enough glucosamine in your body is essential to producing the nutrients required to stimulate the production of synovial fluid, which lubricates cartilage and keeps joints healthy. Without ample glucosamine, the cartilage in the hips, knees and hands deteriorates, a condition known as osteoarthritis. Naturally, each person produces a certain amount of glucosamine within his or her body, but the amount might not be sufficient for healthy joint maintenance, especially as age increases. This has led to the worldwide consumption of large amounts of a great variety of over-the-counter glucosamine preparations. Therefore, the establishment of official glucosamine or reliable analytical methods is needed to test the product quality and regulation**.**

Products containing glucosamine are sold in Europe as standardized prescription drugs [31] In the United States, these products are sold as dietary supplements. As such, no prescription is required.

1.4.7 Pharmacokinetics/ Bioavailability

Absorption

In humans, about 90 percent of glucosamine, administered as an oral dose of glucosamine sulphate, is absorbed from the digestive tract [32].

Distribution

Glucosamine is transported bound to plasma proteins. When it is administered orally, it rapidly accumulates in the liver where it can be incorporated into newly synthesized proteins such as plasma proteins, many of which are glycoproteins. Excess glucosamine accumulate at articular space of the joints. Glucosamine is transported into cells. Insulin enhances the uptake of glucosamine into cells by ten folds.

Metabolism

After an oral dose, glucosamine concentrates in the liver, where it is either incorporated into plasma proteins, degraded into smaller molecules, or utilized for other biosynthetic processes.[32]

Excretion

A small amount of glucosamine or glucosamine derivative is eliminated in the faeces. A major route of excretion is in the urine as metabolites. Elimination is delayed in people with reduced kidney function.

1.4.8 Toxicity

No LD50 is established for glucosamine sulphate, since even at very high levels (5000 mg/kg oral, 3000 mg/kg i.m., and 1500 mg/kg i.v.), there is no mortality in mice or rats.[33]

The incidence of mild side-effects secondary to oral administration of glucosamine sulphate is reported to be 6 -12 percent. The most commonly reported side-effects include gastrointestinal disturbances (such as epigastric pain/tenderness, heartburn, diarrhoea, nausea, dyspepsia, vomiting, and constipation), drowsiness, headaches, and skin reactions. These complaints are generally mild in character and are reversed when treatment with glucosamine sulphate is discontinued. [34, 35]

Glucosamine sulphate has been administered safely to patients with a variety of disease conditions, including circulatory disease, liver disorders, diabetes, lung disorders, and depression, with no observed interference with either the course of the illness or pharmacological treatment for the conditions[36]. However, some concern exists regarding the use of glucosamine sulphate by individuals with type II diabetes, since evidence is suggestive of glucosamine sulphate contributing to insulin resistance, possibly contributing to glucose toxicity

in insulin-sensitive tissues[37]. Evidence also indicates that individuals with active peptic ulcers and individuals taking diuretics tend to have an increased incidence of side effects from glucosamine sulphate [35].

1.4.9 Dosage

The advised oral dosage routine for glucosamine sulphate is 500mg three times daily for a minimum of six weeks. Since obesity has been associated with a below average response to glucosamine sulphate [35], a higher dose might be required by these individuals.

1.4.10 Glucosamine Drug Interactions

Glucosamine might enhance the effects of tetracycline, a common antibiotic, and decrease the effects of penicillin and chloramphenicol. Glucosamine sulphate may interact with potassium chloride, a medication used to treat high blood pressure. So people should take this into account when using blood pressure regulating medication. Always, seek advice from a health care professional before starting the use of glucosamine, especially if you are currently taking any medication.

1.5.0 THE AIMS OF THE PROJECT

The aims of this project are:

- To separate and quantify actual content of several products in the marketplace containing glucosamine sulphate and/or glucosamine hydrochloride and to determine if they significantly deviate from label claim by:

  HPLC Using Pre-column Derivatization of the Amino Group with Phenyl Isothiocyanate (PITC;

  Non-aqueous Titration using Potentiometric End Point Detection.

- To compare the results of both methods to find out if they are sensitive, specific and reproducible.

CHAPTER TWO

2.0.0 EXPERIMENTAL WORK

2.1.0 MATERIALS

2.1.1 Drugs

Table: 2.1.1: Profile of Drug Products

Drug	Product 1 Caplet	Product 2 Tablet	Product 3 Capsule	Product 4 (Tablet)
Name	Glucosamine Sulphate (1000mg)	Glucosamine Sulphate (100mg)	Glucosamine Sulphate (100mg)	Glucosamine Sulphate (500mg)
Supplements	-		Cod liver oil + Omega-3	
Manufacturer:	Holland & Barrett Ltd., USA	Premier Health Products, England	Seven Seas Health Care Ltd, Hull, England	BR Pharmaceuticals Ltd., UK
Manufacturing Date	April, 2005	-	-	February, 2005
Expiry Date	April, 2007	September, 2008	April, 2009	February, 2008
Batch/Lot No.	0501849	D3107	362287	L04417

Continuation of Table 2.1.1

Drug	Product 5 Capsule	Product 6 Capsule	Product 7 Capsule	Product 8 Tablet
Name	Glucosamine Sulphate (100mg)	Glucosamine Sulphate (250mg)	Glucosamine Sulphate (500mg)	Glucosamine Sulphate (100mg)
Supplements	Cod liver oil + Omega-3	Chondroitin sulphate (200mg) + fish oil (250mg) + Omega-3(150mg)	Manganese Ascorbate (2mg)	-
Manufacturer:	Seven Seas Health Care Ltd, Hull, England	Seven Seas Health Care Ltd, Hull, England	Tablets (India) Ltd.	Premier Health Products, England.
Manufacturing Date	-	-	May, 2006	-
Expiry Date	September, 2009	September, 2009	April, 2008	September, 2008
Batch / Lot No.	370743	361999	615120	D3107

Drug	Product 9 Tablet
Name	Glucosamine Sulphate (100mg)
Supplements	-
Manufacturer:	Premier Health Products, England.
Manufacturing Date	-
Expiry Date	September, 2008
Batch/Lot No.	D3107

2.1.2. Chemicals /Reagents:

Perchloric acid, 72%, 1.7g/ml(BDH)); Potassium hydrogen Phthalate, Not < 99.9%)(BDH); Glacial acetic acid(Not < 99.6% (M &B); Acetic anhydride; Mercury (II) Acetate; Distilled Water; Methanol; Methanol(BDH); Sodium Acetate, anhydrous; Phosphoric acid, Silica gel powder(BDH), 85%; Phenyl Isothiocyanate, 99%, Sigma; Hexane; D-(+)-glucosamine HCl standard, ChromaDex, PN 07256-1, ≥ 98%, $C_6H_{14}ClNO_5$, MW 215.63;

2.1.3 Glassware

Transfer pipettes(Class B), Burette(Class B), Conical flasks, Volumetric flasks(Class B), Filter funnel, Sintered glass, Beakers, Glass rods, Syringe, HPLC injection vials, Reaction vial with caps, 5ml or centrifuge tubes with caps.

2.1.4 Special Equipment

pH meter (Mettler Delta 350), Combination electrode (glass/SCE reference), Magnetic stirrer motor(HI 190M), Analytical balance(Shimadzu), Spatula, Grinder(mortar with pestle), Water bath B-480,(BUCHI), Programmable oven(810 series), HPLC system, Column, Phenomenex Luna C18, 4.6 x 250mm.

2.2.0 PREPARATION OF SOLUTIONS.

2.2.1 Preparation of 0.1M Potassium Hydrogen Phthalate ($C_8H_5O_4K$)

204.22g. ≡ 1000ml. ≡ 1.0M $C_8H_5O_4K$
5.1055g. ≡ 25ml. ≡ 1.0M $C_8H_5O_4K$
0.51055g. ≡ 25ml. ≡ 0.1M $C_8H_5O_4K$

0.5g of potassium hydrogen phthalate was accurately into a 100ml conical flask. 25ml of glacial acetic acid was added and warmed until the salt dissolved. The solution was allowed to cool and then titrated with 0.1M perchloric acid.

2.2.2 Preparation of 0.1M Perchloric Acid ($HClO_4$)

100.46g ≡ 1000ml. ≡ 1.0 M $HClO_4$
10.046g. ≡ 100ml. ≡ 1.0 M $HClO_4$

Density = mass/volume

Volume = $\frac{10.046g}{1.7g/ml}$ = 5.9094 ml

72% $HClO_4$ means 72ml of the acid is present in 100ml solution.

If 72% of $HClO_4$ contains 5.9094ml

$\therefore 100\% = \frac{5.9094ml \times 100\%}{72\%}$ = 8.21ml

900ml of glacial acetic acid was placed in 1L (1000ml) volumetric flask. 8.2ml of 72% perchloric acid was added slowly and mixed thoroughly. 30ml of acetic anhydride was added.

More glacial acetic acid was added to the 1L mark. The flask was corked, shaken for solution to mix and allowed to stand for 24 hours before use.

Glucosamine HCl (*M_r 215.63*).

Glucosamine HCl ionizes as shown: $C_6H_{13}O_5N.HCl \longrightarrow C_6H_{13}O_5.N^+H + Cl^-$.

The base is weak, since it is in the form of a chloride (Cl^-), so the Cl^- must be removed before titration. This is achieved by the addition of mercury (II) acetate this assay. Thus,

$2Cl^- + Hg(OAc)_2 \longrightarrow HgCl_2 + 2OAc^-$; where $Ac = CH_3CO^-$.

The liberated acetate(OAc^-) is then titrated with acetous perchloric acid. Thus,

$OAc^- + HClO_4 \longrightarrow HOAc + ClO_4^-$

$C_6H_{13}O_5N.HCl = Cl^- = OAc^- = HClO_4$,

215.63g $C_6H_{13}O_5N.HCl$ = 1000ml 1M $HClO_4$.

$\therefore$ 0.021563g $C_6H_{13}O_5N.HCl$ = 1ml 0.1M $HClO_4$.

Glucosamine Sulphate (M_r = 456.418): $(C_6H_{11}O_5NH_3)_2SO_4$, or $(C_6H_{12}O_5N)_2 . H_2SO_4$

Glucosamine sulphate ionizes as shown:

$(C_6H_{11}O_5NH_3)_2SO_4 \longrightarrow (C_6H_{11}O_5NH_3)^+ + SO_4^{2-}$

1mol of $C_6H_{11}O_5NH_3)_2SO_4$ = 1mole of $HClO_4$.

(456.418)g $(C_6H_{11}O_5NH_3)_2SO_4$) = 1000ml 1M $HClO_4$.

$\therefore$ 0.0456418g $(C_6H_{11}O_5NH_3)_2SO_4$ = 1ml 0.1M $HClO_4$.

2.2.3 Preparation of 0.1M Sodium Acetate solution (NaOAc).

4.1g of sodium acetate was accurately weighed into a beaker. Some distilled water was added and stirred to dissolve. The solution was quantitatively transferred into a 500ml volumetric flask. The solution was diluted to 500ml mark with distilled water. The flask was corked, shaken and labelled 0.1M sodium acetate.

2.2.4 Preparation of 0.756 mg/ml Standard Glucosamine Hydrochloride.

37.8mg (or 0.0378g) of glucosamine HCl reference standard was accurately weighed into a beaker. 40ml of 0.1M NaOAc solution was added and stirred to dissolve. The solution was quantitatively transferred into a 50ml volumetric flask. The solution was diluted to the 50ml mark with 0.1M NaOAc solution.

2.2.5 Preparation of 0.5M Alcoholic Potassium hydroxide solution.

3g of potassium hydroxide is dissolved in 5ml of water and sufficient aldehyde free ethanol (96%) is added to produce 100ml. The solution was allowed to stand for 24hours and the clear solution decanted.

2.2.6 Preparation of Test Sample for Identification Test (Qualitative analysis)

0.1g of glucosamine hydrochloride or glucosamine sulphate was dissolved in 10ml distilled water.

2.3.0 METHODOLOGY

2.3.1 Weight Uniformity

2.3.1.1.1 Tablets:

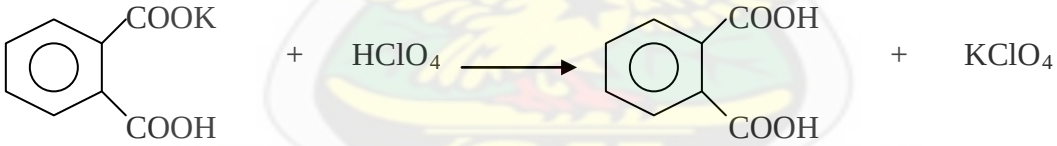
Twenty (20) tablets were selected at random. One tablet was weighed by difference and its average weight was then calculated. The procedure was repeated for the remaining nineteen tablets selected at random. The average weight of contents was then calculated.

2.3.1.1.2 Capsules

Twenty (20) capsules were selected at random. One intact capsule was weighed by difference, and then opened without loss of any part of the shell. The contents were completely removed. The shell was then washed in ether to remove the oil, and allowed to dry completely. The shell material was weighed and the difference in weight equals the weight of contents. The procedure was repeated for the remaining nineteen capsules selected at random. The average weight of contents was then calculated.

2.3.2 Titration

2.3.2.1 Standardisation of 0.1M Perchloric acid Using Potassium Hydrogen Phthalate



Mole ratio: 1mol $C_8H_5O_4K$: 1mol $HClO_4$ ≡ 1000ml

204.22g $C_8H_5O_4K$ ≡ 1000ml. ≡ 1.0 M $HClO_4$

20.422g. $C_8H_5O_4K$ ≡ 1000ml. ≡ 1.0 M $HClO_4$

0.020422g. $C_8H_5O_4K$ ≡ 1ml. ≡ 0.1M $HClO_4$

0.5g of Potassium Hydrogen Phthalate (dried at a temperature of 120^oC for 2hrs and allowed to cool in a desiccator) was accurately weighed into a 100ml conical flask. 25ml glacial acetic acid was added and warmed until the salt dissolved. The solution was cooled and then titrated with 0.1M perchloric acid using potentiometric titration.

2.3.2.2.1 Procedure for assay of tablet.

An equivalent of 0.25g of glucosamine sulphate was weighed by difference into a 150ml beaker. 8ml of mercury (II) acetate solution was added and then 20ml of anhydrous glacial acetic acid. The mixture was heated in a water bath till the solution turns yellow (between

60 - 65^{o}C for the drug to dissolve). It was then cooled to room temperature, filtered through sintered glass, and washed with additional 20ml of anhydrous glacial acetic acid. The filtrate was titrated with 0.1M perchloric acid, VS. The end point was determined potentiometrically. The procedure was repeated until two consistent results are obtained.

A blank determination was performed and any necessary corrections were made.

2.3.2.3 Procedure for assay of capsule containing cod liver oil.

An equivalent of 0.25g of glucosamine sulphate was weighed by difference into a 150cm^3 beaker. 20ml of acetone (or preferably hexane) was added, stirred, allowed to standard for the solute to settle. The content was decanted carefully through a sintered glass to remove the oil. The residue was washed with additional 20ml of acetone (or hexane) and again carefully decanted through the sintered glass. The sintered glass was tilted and rinsed with 10ml of acetone (or hexane) into the beaker containing the residue. The mixture was heated (at 60^{o}C) to evaporate much of the acetone (or hexane). 20ml of anhydrous glacial acetic acid was added to the residue and heated (between 60 - 65^{o}C for the drug to dissolve) in a water bath till the solution turns yellow. It was then cooled to room temperature, filtered through sintered glass, and washed with additional 20ml of anhydrous glacial acetic acid.

The filtrate was titrated with 0.1M perchloric acid, VS. The end point was determined potentiometrically. The procedure was repeated until two consistent results are obtained.

A blank determination was performed and any necessary corrections were made.

The equation for the reaction during the titration is:

$$OAc^- + HClO_4 \longrightarrow HOAc + ClO_4^- \quad \text{or}$$

$$CH_3COO^- + HClO_4 \longrightarrow HOOCH_3 + ClO_4^-$$

2.3.3 HPLC Pre-column Derivatization of Glucosamine with Phenyl Isothiocyanate

2.3.3.1 Chromatographic Conditions

Column: Phenomenex Luna C18, 4.6 x 250mm

Mobile phase: Isocratic MeOH/H_2O/H_3PO_4 (12/88/0.1).

Flow rate: 1.5ml/min

Detector: UV at 240nm

Injection Volume: 10µl (or 0.01ml)

Run Time: 10 min.

Relative Retention Times: Glucosamine anomer peak 1: 5.62 min

Glucosamine anomer peak 2: 6.93 min

2.3.3.2 Derivatization of Stock Standard

5ml of the stock standard was pipetted into a 50ml volumetric flask. 400µl (or 0.4ml) of PITC was added. 15ml of methanol was added and shaken vigorously until all the PIT dissolved. The solution was diluted to volume with MeOH/H_2O (60:40) and mixed.

Approximately 5ml of the solution was transferred to a reaction vial and sealed tightly. The solution was heated in a water bath or dry block heater at 80^oC for 15 minutes and cooled to room temperature. Approximately 5ml of heptane (or hexane) was added to the reaction vessel. The mixture was shaken mechanically or by hand for 1 minute to extract unreacted PITC. 0.7ml aliquot was removed from the methanolic layer and placed in 10ml volumetric flask and diluted to the mark with MeOH/H_2O (60:40), shaken and then transferred into an HPLC vial for analysis.

2.3.3.3 Preparation of Test (Sample) Solutions.

40mg (0.04g) of glucosamine sulphate (powdered sample for tablets; ground content for capsules) was accurately weighed into a beaker, 40ml of 0.1M NaOAc solution was added and stirred to dissolve. The solution was quantitatively transferred into a 50ml volumetric flask. The solution was diluted to the 50ml mark with 0.1M NaOAc solution.

2.3.3.4 Derivatization of Test (sample) Solutions.

5ml of the sample was pipetted into a 50ml volumetric flask. 400µl (or **0.4ml**) of PITC was added. 15ml of methanol was added and shaken vigorously until all the PITC dissolved. The solution was diluted to volume with MeOH/H_2O (60:40) and mixed. Approximately 5ml of the solution was transferred to a reaction vial and sealed tightly. The solution was heated in a water bath or dry block heater at 80^oC for 15 minutes and cooled to room temperature.

Approximately 5ml of heptane was added to the reaction vessel. The mixture was shaken mechanically or by hand for 1 minute to extract unreacted PITC. 0.7ml aliquot was removed from the methanolic layer and placed in 10ml volumetric flask and diluted to the mark with MeOH/H_2O (60:40), shaken and then transferred into an HPLC vial for analysis.

After 10 - 12 injections, the column is flushed for 10 minutes with 100% acetonitrile or methanol to remove unreacted PITC build up on the column.

2.3.4 Dissolution Test

Two clean round-bottomed beakers were each filled with 0.1M sodium ethanoate to 900ml marks. The dissolution apparatus was switched on and the temperature of the water bath stabilised at 37^oC. Two glucosamine sulphate tablets, caplets or capsules of known masses were

each placed in the beaker. The paddle stirrer was rotated at 50 revolutions per minute and lowered into the dissolution medium. A stopwatch was started at the same time and 10ml of the sample were withdrawn from the dissolution medium at 15min time intervals for 1 hour and filtered using cotton wool. The volume of the solution in the beaker was maintained constant by adding 10ml of the 0.1M sodium ethanoate after each removal of sample.

2.3.4.1 Derivatization of Filtrate Sample

0.5ml aliquot of the filtrate sample was placed in 10ml volumetric flask. 0.04ml of PITC was added. 1.5ml of methanol was added and shaken vigorously until all the PITC dissolved. The solution was diluted to volume with MeOH/H_2O (60:40) and mixed. Approximately 5ml of the solution was transferred to a reaction vial and sealed tightly. The solution was heated in a water bath or dry block heater at 80^oC for 15 minutes and cooled to room temperature. Approximately 5ml of heptane was added to the reaction vessel. The mixture was shaken mechanically or by hand for 1 minute to extract unreacted PITC. 1.0ml aliquot was removed from the methanolic layer and placed in 10ml volumetric flask and diluted to the mark with MeOH/H_2O (60:40), shaken and then transferred into an HPLC vial for analysis.

2.3.5 Thin Layer Chromatography (TLC) of Derivatized Glucosamine Standard.

The chromatographic tank was first lined with filter paper on three sides. The mobile phase (methanol : water: phosphoric acid of composition 12:88:0.1) was then added to a depth of 1cm. The tank was covered and allowed to equilibrate with its vapour for not less than 30 minutes. By means of a pencil, a line was drawn parallel to, and 2cm from the bottom of a pre-coated glass plate. Samples of derivatized glucosamine(75.6µg/ml) and the blank were spotted on the line using melting point tubes. The space between the spots was at least 1.0cm apart. The samples were allowed to dry. After equilibration, the TLC plate was placed in the airtight chamber such that the base line was above the level of the mobile phase. The solvent allowed to rise to a depth of about 10 to 15cm up the plate. After development the TLC plate was removed from the tank; the position of the solvent front was quickly marked, and then allowed to dry for 10 minutes. The spots were located by placing the plate under UV light (254nm and 365nm), and then in iodine vapour to produce brown/yellowish brown zones of glucosamine derivative in a white background.

2.3.6 Qualitative Tests

Table 2.1.2: Identification Tests.

Test	Observation	Inference
Test for Reducing Sugar		
2ml of the test solution + 1ml Benedict's solution + heat.	Brick red precipitate	Presence of reducing sugar
Carbylamine Test.		
1ml of 0.5M alcoholic KOH solution + Two drops of the test solution + Three drops of chloroform + warm	Evolution of characteristic foul smell of isocyanide	Presence of primary amine
Test for Chloride ion		
2ml of the test solution + 1ml of Dilute HNO_3 + 0.5ml of $AgNO_3$ solution. Precipitate + 3ml of Dilute NH_3	White curdled precipitate The precipitate dissolved.	Chloride ion (Cl^-) confirmed
Test for Sulphate (vi) ion.		
2ml of the test solution + 1ml of Dilute HCl + 1ml of $BaCl_{2(aq)}$ solution.	White curdled precipitate	Sulphate (vi) ion(SO_4^{2-}) confirmed

CHAPTER THREE

3.0.0 RESULTS AND CALCULATIONS

3.1.0 Weight Uniformity

Table 3.1.1: Weight Uniformity Test Product **1** (Caplet)**.**

Tablet No.	Weight/g	Deviation/g	% Deviation
1	1.7021	0.0055	0.3221
2	1.6754	0.0322	1.8857
3	1.6960	0.0116	0.6793
4	1.7586	-0.0510	-2.9866
5	1.6980	0.0096	0.5622
6	1.7380	-0.0304	-1.7803
7	1.6874	0.0202	1.1829
8	1.7014	0.0062	0.3631
9	1.6824	0.0252	1.4758
10	1.6741	0.0335	1.9618
11	1.6870	0.0206	1.2064
12	1.6905	0.0171	1.0014
13	1.7288	-0.0212	-1.2415
14	1.7108	-0.0032	-0.1874
15	1.6930	0.0146	0.8550
16	1.7504	0.0428	2.5064
17	1.6983	0.0093	0.5446
18	1.7356	-0.0280	-1.6397
19	1.7161	-0.0085	-0.4978
20	1.7275	-0.0199	-1.1654

Total weight of 20 tablets = 34.1513g

Average weight of tablet = $\frac{34.1513g}{20}$ = 1.707565g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 1.7076 – 1.7021 = 0.0055g

% Deviation = $\frac{0.0055}{1.7076}$ x 100% = 0.3221.

Table 3.1.2: Weight Uniformity Test Product **2**:

Tablet No.	Weight/g	Deviation/g	% Deviation
1	0.5416	-0.0041	-0.7628
2	0.5258	0.0117	2.1767
3	0.5355	0.0020	0.3721
4	0.5413	0.0038	0.7070
5	0.5281	0.0094	1.7488
6	0.5360	0.0015	0.2791
7	0.5356	0.0019	0.3535
8	0.5378	0.0003	0.0558
9	0.5384	-0.0009	-0.1674
10	0.5414	0.0039	0.7256
11	0.5344	0.0031	-0.5767
12	0.5432	-0.0057	-1.0605
13	0.5362	0.0013	0.2419
14	0.5427	-0.0052	-0.9674
15	0.5529	0.0154	-2.8651
16	0.5360	0.0015	0.2791
17	0.5212	0.0163	3.0326
18	0.5282	0.0093	1.7302
19	0.5489	-0.0114	-2.1209
20	0.5445	-0.0070	1.3023

Total weight of 20 tablets = 10.7497g

Average weight of tablet = $\frac{10.7497}{20}$ = 0.5375g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 0.5375 – 0.5416 = - 0.0041g

% Deviation = $\frac{0.0041g \times 100\%}{0.5375}$ = 0.7628%

Table 3.1.3: Weight Uniformity Test Product **3**.

Capsule No.	Weight of intact capsule / g	Weight of shell/g	Weight of content/g	Deviation /g	% Deviation
1	1.5673	0.5263	1.0410	-0.0163	-1.5907
2	1.5604	0.5181	1.0423	-0.0176	-1.7176
3	1.5742	0.5422	1.0320	-0.0073	-0.7124
4	1.5529	0.4996	1.0533	-0.0286	-2.7911
5	1.5879	0.5597	1.0302	-0.0055	-0.5367
6	1.6219	0.5422	1.0797	-0.0550	-5.3674
7	1.6301	0.5886	1.0415	-0.0168	-1.6395
8	1.5763	0.5358	1.0405	-0.0158	-1.5419
9	1.5309	0.5014	1.0295	-0.0048	-0.4684
10	1.5548	0.5348	1.0200	0.0047	0.4587
11	1.5387	0.4887	1.0500	-0.0253	-2.4690
12	1.5461	0.5368	1.0093	0.0154	1.5029
13	1.5360	0.5566	0.9794	0.0453	4.4208
14	1.5850	0.5524	1.0326	-0.0079	-0.7710
15	1.5702	0.5400	1.0302	-0.0055	-0.5367
16	1.5539	0.5319	1.0220	0.0027	0.2635
17	1.5768	0.6056	0.9712	0.0535	5.2210
18	1.5191	0.5264	0.9927	0.0320	3.1229
19	1.5196	0.5634	0.9562	0.0685	6.6849
20	1.5558	0.5157	1.0401	-0.0154	-1.5029

Total weight of 20 tablets = 20.4937g

Average weight of tablet = $\frac{20.4937g}{20}$ = 1.0247g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 1.0247 – 1.0410 = -0.0163 g

% Deviation = $-\frac{0.0163}{1.0247}$ x 100% = -1.5907.

Table 3.1.4: Weight Uniformity Test Product **4**.

Tablet No.	Weight/g	Deviation/g	% Deviation
1	0.7710	-0.0019	-0.2470
2	0.7691	0.0000	0.0000
3	0.7693	-0.0002	-0.0260
4	0.7695	-0.0004	-0.0520
5	0.7736	-0.0045	-0.5851
6	0.7645	0.0046	0.5981
7	0.7722	-0.0031	-0.4031
8	0.7686	0.0005	0.0650
9	0.7671	0.0030	0.2600
10	0.7731	-0.0040	-0.5200
11	0.7705	-0.0014	-0.1820
12	0.7670	0.0021	0.2731
13	0.7689	0.0002	0.0260
14	0.7690	0.0001	0.0130
15	0.7773	-0.0082	-1.0662
16	0.7679	0.0012	0.1560
17	0.7638	0.0053	0.6891
18	0.7656	0.0035	0.4551
19	0.7627	0.0064	0.8321
20	0.7708	-0.0017	-0.2210

Total weight of 20 tablets = 15.3815g

Average weight of tablet = $\frac{15.3815g}{20}$ = 0.7691g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 0.7691 – 0.7710 = -0.0019g

% Deviation = $\frac{-0.0019}{0.7691}$ x 100% = -0.2470.

Table 3.1.5: Weight Uniformity Test Product **5**.

Capsule No.	Weight of shell + Content / g	Weight of Shell/g	Weight of Content/g	Deviation /g	% Deviation
1	1.5208	0.4740	1.0468	-0.0052	-0.4992
2	1.5233	0.4849	1.0384	0.0032	0.3072
3	1.5383	0.4995	1.0388	0.0028	0.2688
4	1.5153	0.4793	1.0360	0.0056	0.5376
5	1.5161	0.4800	1.0361	0.0055	0.5280
6	1.5421	0.5027	1.0394	0.0022	0.2112
7	1.5153	0.4800	1.0353	0.0063	0.6048
8	1.5416	0.4057	1.1359	-0.0943	-9.0534
9	1.5333	0.4010	1.1323	-0.0907	-8.7078
10	1.5089	0.4784	1.0305	0.0111	1.0657
11	1.5211	0.4932	1.0279	0.0137	1.3153
12	1.5374	0.4975	1.0399	0.0017	0.1632
13	1.5206	0.4883	1.0323	0.0093	0.8929
14	1.5144	0.5100	1.0044	0.0372	3.5714
15	1.5189	0.4873	1.0316	0.0100	0.9601
16	1.5228	0.5164	1.0064	0.0352	3.3794
17	1.5413	0.5122	1.0291	0.0125	1.2001
18	1.5408	0.4877	1.0536	-0.0120	-1.1521
19	1.5366	0.5060	1.0306	0.0110	1.0561
20	1.5194	0.5132	1.0062	0.0354	3.3986

Total weight of 20 tablets = 20.8315g

Average weight of tablet = $\frac{20.8315g}{20}$ = 1.0416g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 1.0416g – 1.0468 = -0.0052 g

% Deviation = $\frac{-0.0052}{1.0416g}$ x 100% = -0.4992 .

Table 3.1.6: Weight Uniformity Test Product **6**.

Capsule No.	Weight of intact capsule / g	Weight of Shell/g	Weight of Content/g	Deviation /g	% Deviation
1	1.3944	0.3909	1.0035	0.0114	1.1233
2	1.4230	0.3972	1.0258	-0.0109	-1.0740
3	1.4115	0.3784	1.0331	-0.0182	-1.7933
4	1.3967	0.3857	1.0110	0.0039	0.3843
5	1.4012	0.3808	1.0204	-0.0055	-0.5419
6	1.3955	0.3862	1.0093	0.0056	0.5518
7	1.4014	0.3879	1.0135	0.0014	0.1379
8	1.3994	0.3895	1.0099	0.0050	0.4927
9	1.3973	0.3848	1.0125	0.0024	0.2365
10	1.3988	0.3808	1.0180	-0.0031	-0.3055
11	1.4228	0.3872	1.0356	-0.0207	-2.0396
12	1.3984	0.3850	1.0134	0.0015	0.1478
13	1.3998	0.3899	1.0099	0.0050	0.4927
14	1.4206	0.3816	1.0390	-0.0241	-0.8846
15	1.4020	0.3820	1.0200	-0.0051	-0.5025
16	1.3996	0.3834	1.0162	-0.0013	-0.1281
17	1.3969	0.3906	1.0063	0.0086	0.8474
18	1.3940	0.3881	1.0059	0.0090	0.8868
19	1.3950	0.3970	0.9980	0.0169	1.6652
20	1.3979	0.4019	0.9960	0.0189	1.8623

Weight of 20 tablets = 20.2973g

Average weight per tablet = $\frac{20.2973}{2}$ = 1.0149g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 1.0149g – 1.0035 = 0.0114g

% Deviation = $\frac{0.0114g}{1.0149g}$ x 100% = 1.1233.

Table 3.1.7: Weight Uniformity Test Product **7**.

Capsule No.	Weight of intact capsule / g	Weight of Shell/g	Weight of Content/g	Deviation /g	% Deviation
1	1.5391	0.4855	1.0536	-0.0239	-2.3106
2	1.4622	0.4834	0.9788	0.0509	4.9432
3	1.5404	0.4509	1.0895	-0.0598	-5.8075
4	1.4794	0.5208	0.9586	0.0711	6.9049
5	1.5626	0.4417	1.1209	-0.0912	-8.8569
6	1.5525	0.4384	1.1141	-0.0844	-8.1965
7	1.5448	0.5226	1.0222	0.0075	0.7284
8	1.5214	0.5185	1.0029	0.0268	2.6027
9	1.4386	0.5280	0.9706	0.0591	5.7395
10	1.4991	0.4744	0.9647	0.0650	6.3125
11	1.5493	0.4563	1.0930	-0.0633	6.1474
12	1.5255	0.5854	0.9401	0.0896	8.7016
13	1.5502	0.5441	1.0061	0.0236	2.2919
14	1.5992	0.5651	1.0341	-0.0044	-0.4273
15	1.5360	0.5075	1.0285	0.0012	0.1165
16	1.4396	0.4918	0.9478	-0.0440	4.2725
17	1.4788	0.4944	0.9844	0.0453	4.3993
18	1.5380	0.4576	1.0804	-0.0507	-4.9238
19	1.5218	0.4239	1.0979	-0.0682	-6.6233
20	1.5350	0.4293	1.1057	-0.0760	7.3808

Total weight of 20 tablets = 20.5939 g

Average weight of tablet = $\frac{20.5939}{20}$ = 1.0297g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 1.0297 – 1.0536 = -0.0239g

% Deviation = $-\frac{0.0239}{1.0297}$ x 100% = -2.3106.

Table 3.1.8: Weight Uniformity Test Product **8**.

Tablet No.	Weight/g	Deviation/g	% Deviation
1	0.5484	-0.0128	-2.3898
2	0.5330	0.0026	0.4854
3	0.5203	0.0153	2.8566
4	0.5421	-0.0065	-1.2136
5	0.5336	0.0020	0.3734
6	0.5406	-0.0050	-0.9335
7	0.5531	-0.0175	-3.2674
8	0.5221	0.0135	2.5205
9	0.5415	-0.0059	-1.1016
10	0.5238	0.0118	2.2031
11	0.5445	-0.0089	-1.6617
12	0.5211	0.0145	2.7072
13	0.5346	0.0010	0.1867
14	0.5478	-0.0122	-2.2778
15	0.5274	0.0082	1.5310
16	0.5461	-0.0105	-1.9604
17	0.5323	0.0033	0.6161
18	0.5302	0.0054	1.0082
19	0.5381	-0.0025	-0.4668
20	0.5305	0.0051	0.9522

Total weight of 20 tablets = 10.7111g

Average weight of tablet = $\frac{10.7111}{20}$ = 0.5356g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 0.5356 – 0.5484 = -0.0128 g

% Deviation = $\frac{-0.0128}{0.5356}$ x 100% = -2.3898.

Table 3.1.9. Weight Uniformity Test Product **9**.

Tablet No.	Weight/g	Deviation/g	% Deviation
1	0.5371	0.0050	0.9223
2	0.5528	-0.0107	-1.9738
3	0.5533	-0.0112	-2.0660
4	0.5401	0.0020	0.3689
5	0.5356	0.0065	1.1990
6	0.5373	0.0048	0.8854
7	0.5451	-0.0030	-0.5534
8	0.5281	0.0140	2.5826
9	0.5391	0.0030	0.5534
10	0.5518	-0.0097	-1.7893
11	0.5500	-0.0079	-1.4573
12	0.5327	0.0094	1.7340
13	0.5460	-0.0039	-0.7194
14	0.5453	-0.0032	-0.5903
15	0.5483	-0.0062	-1.1437
16	0.5332	0.0089	1.6418
17	0.5458	-0.0037	0.6825
18	0.5413	0.0008	0.1476
19	0.5472	-0.0051	-0.9408
20	0.5312	0.0109	2.0107

Total weight of 20 tablets = 10.8413g

Average weight of tablet = $\frac{10.8413}{20}$ = 0.5421 g

Deviation = Average weight of tablet – weight of tablet

For tablet No.1, deviation = 0.5421 – 0.5371 = 0.0050g

% Deviation = $\frac{0.0050g}{0.5421}$ x 100% = 0.9223.

3.2.0 Titration Data

3.2.1. Weighing:

Table 3.2.1: Weight of Pure Glucosamine Hydrochloride Standard used for analysis.

Weights	Sample(HCl)1 /g	Sample (HCl)2 /g	Sample (HCl)3/g
Weight of dish + sample	82.0254	82.3967	82.5890
Weight of dish	81.7713	82.1423	82.3347
Weight of sample.	0.2541	0.2544g	0.2543g

Table 3.2.2: Weight of Potassium Hydrogen Phthalate (KHP) used for analysis

Weights	Sample(KHP)1 /g	Sample (KHP)2 /g	Sample (KHP)3/g
Weight of dish + sample	54.7384	82.2793	82.0151
Weight of dish	54.2360	81.7712	81.5105
Weight of sample.	0.5024	0.5081	0.5046

Table 3.2.3: Weight of Product **1** used for analysis.

Weights	Sample A_1/g	Sample A_2/g	Sample A_3/g
Weight of dish + sample	82.7618	82.5698.	82.1983
Weight of dish	82.3345	82.1424.	81.7713
Weight of sample.	0.4273	0.4274.	0.4270

Table 3.2.4: Weight of Product **2** used for analysis**.**

Weights	Sample B_1/g	Sample B_2/g	Sample B_3/g
Weight of dish + sample	83.4865	83.6784	83.1173
Weight of dish	82.1423	82.3345	81.7712
Weight of sample.	1.3442	1.3439	1.3461

Table 3.2.5: Weight of Product **3** used for analysis.

Weights	Sample C_1/g	Sample C_2/g	Sample C_3/g
Weight of dish + sample	84.3325	84.8965	84.0724
Weight of dish	81.7712	82.3345	81.5105
Weight of sample.	2.5613	2.5620	2.5619

Table 3.2.6: Weight of Product **4** used for analysis.

Weights	Sample D_1/g	Sample D_2/g	Sample D_3/g
Weight of dish + sample	81.8955	82.5275	82.1561
Weight of dish	81.5105	82.1424.	81.7712
Weight of sample.	0.3850	0.3851	0.3849

Table 3.2.7: Weight of Product **5** used for analysis.

Weights	Sample E_1/g	Sample E_2/g	Sample E_3/g
Weight of dish + sample	84.9405	84.3776	84.3779
Weight of dish	82.3345	81.7713	81.7714
Weight of sample.	2.6060	2.6063	2.6065

Table 3.2.8: Weight of Product **6** used for analysis.

Weights	Sample F_1/g	Sample F_2/g	Sample F_3/g
Weight of dish + sample	82.7867	82.5259	82.526
Weight of dish	81.7713	81.5107	81.5107
Weight of sample.	1.0154	1.0152	1.0153

Table 3.2.9: Weight of Product **7** used for analysis.

Weights	Sample G_1/g	Sample G_2/g	Sample G_3/g
Weight of dish + sample	82.0258	82.6575	82.2867
Weight of dish	81.5106	82.1424.	81.7714
Weight of sample.	0.5152	0.5151	0.5153

Table 3.2.10: Weight of Product **8** used for analysis.

Weights	Sample H_1/g	Sample H_2/g	Sample H_3/g
Weight of dish + sample	83.1122	83.6739	82.8512
Weight of dish	81.7714	82.3345	81.5107
Weight of sample.	1.3408	1.3394	1.3405

Table 3.2.11. Weight of Product **9** used for analysis.

Weights	Sample I_1/g	Sample I_2/g	Sample I_3/g
Weight of dish + sample	83.6898	83.127	82.8667
Weight of dish	82.3344	81.7714	81.5112
Weight of sample.	1.3554	1.3556	1.3555

3.2.2 Standardization of $HClO_4$ against Potassium Hydrogen Phthalate (KHP)

Table 3.2.12: Titration of $HClO_4$ against Potassium Hydrogen Phthalate$(KHP)_1$

Volume of $(KHP)_1$ used = *25ml;*

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /°C**	**ΔV/*ml***	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = \frac{(V_2 + V_1)}{2}$ /*ml***
0.00	282.3	26.2	0.00	0.0	0.00	10.00
20.00	290.2	26.1	20.00	7.9	0.40	20.50
21.00	296.5	26.1	1.00	6.3	6.30	21.50
22.00	310.2	26.3	1.00	13.7	13.70	22.50
23.00	312.2	26.4	1.00	2.0	2.00	23.20
23.40	318.6	26.5	0.40	6.4	16.00	23.50
23.60	326.7	26.7	0.20	8.1	40.50	23.70
23.80	328.9	27.0	0.20	2.2	11.00	23.90
24.00	332.4	27.1	0.20	3.5	17.50	24.10
24.20	436.8	**27.3**	0.20	104.4	**552.00**	**24.30**
24.40	495.2	27.5	0.20	58.4	292.00	24.50
24.60	527.5	27.4	0.20	32.3	161.50	24.70
24.80	546.5	27.4	0.20	19.0	95.00	24.90
25.00	554.5	27.5	0.20	8.0	40.00	25.10
25.20	563.3	27.6	0.20	8.8	44.00	25.30
25.40	567.7	27.2	0.20	4.4	22.00	25.50
25.60	572.6	27.4	0.20	4.9	24.50	25.70
25.80	577.4	27.6	0.20	4.8	24.00	-

Table 3.2.13: Titration of $HClO_4$ against Potassium Hydrogen Phthalate $(KHP)_2$

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/ml	**Potential E^o / mV**	**T_2 /^oC**	**ΔV/ml**	**ΔE^o/ mV**	**$\Delta E^o/\Delta V$ mV/ml**	**$V' = \frac{(V_2 + V_1)}{2}$ /ml**
0.00	279.2	29.0	0.00	0.0	0.00	10.00
20.00	285.6	29.30	20.00	6.4	0.32	20.50
21.00	294.5	29.50	1.00	8.9	8.90	21.50
22.00	300.0	29.40	1.00	5.5	5.50	22.50
23.00	311.2	29.70	1.00	11.2	11.20	23.20
23.40	322.8	29.70	0.40	11.6	29.00	23.50
23.60	324.4	29.80	0.20	1.6	8.00	23.70
23.80	334.5	30.0	0.20	10.1	50.50	23.90
24.00	389.5	30.3	0.20	55.0	275.00	24.10
24.20	447.0	30.4	0.20	57.5	287.50	24.30
24.40	545.0	**30.50**	0.20	98.0	**490.00**	**24.50**
24.60	553.0	30.70	0.20	8.0	40.00	24.70
24.80	561.0	30.80	0.20	8.0	40.00	24.90
25.00	568.0	30.9	0.20	7.0	35.00	25.10
25.20	576.0	31.1	0.20	8.0	40.00	25.30
25.40	578.0	31.3	0.20	2.0	10.00	25.50
25.60	579.0	31.3	0.20	1.0	5.00	25.70
25.80	581.0	31.2	0.20	2.0	10.00	-

Table 3.2.14: Titration of $HClO_4$ against Potassium Hydrogen Phthalate $(KHP)_3$

Temperature of the titrant during standardization, $T_1 = 26.1^oC$

Volume of $HClO_4$ added/ml	**Potential E^o / mV**	**T_2 /^oC**	**ΔV/ml**	**ΔE^o/ mV**	**$\Delta E^o/\Delta V$ mV/ml**	**$V' = (\underline{V_2 + V_1})$ /ml 2**
0.00	280.5	26.9	0.00	0	0.00	10.00
20.00	288.7	26.7	20.00	8.2	0.41	20.50
21.00	294.5	26.7	1.00	5.8	5.80	21.50
22.00	306.8	26.6	1.00	12.3	12.30	22.50
23.00	309.2	26.5	1.00	2.4	2.40	23.20
23.40	313.0	26.4	0.40	3.8	9.50	23.50
23.60	319.7	26.4	0.20	6.7	33.50	23.70
23.80	327.6	26.3	0.20	7.9	39.50	23.90
24.00	334.3	26.2	0.20	6.7	33.50	24.10
24.20	349.4	26.2	0.20	15.1	75.50	24.30
24.40	**449.0**	**26.1**	0.20	**99.6**	498.00	**24.50**
24.60	497.0	26.1	0.20	48.0	240.00	24.70
24.80	539.0	26.2	0.20	42.0	210.00	24.90
25.00	546.0	26.2	0.20	7.0	35.00	25.10
25.20	549.0	26.1	0.20	3.0	15.00	25.30
25.40	553.0	26.2	0.20	4.0	20.00	25.50
25.60	555.0	26.1	0.20	2.0	10.00	25.70
25.80	558.0	26.0	0.20	3.0		-

3.2.3 Titration of $HClO_4$ against Pure Glucosamine Hydrochloride Standard ($S_{G.HCl}$)

Table 3.2.15: Titration of $HClO_4$ against Standard Glucosamine Hydrochloride($S_{G.HCl})_1$.

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/*ml*	Potential E^o / *mV*	T_2 /^oC	Vc /*ml*	ΔV/*ml*	ΔE^o/ *mV*	$\Delta E^o/\Delta V$ *mV/ml*	$V' = (\underline{V_2 + V_1})$ /*ml* 2
0.00	360.8	27.1	0.0000	0.00	0	0.00	5.00
10.00	416.5	27.1	10.0022	10.0022	55.7	5.57	10.30
10.60	422.7	27.3	10.6000	0.5978	6.2	10.37	10.70
10.80	430.3	27.6	10.7964	0.1964	7.6	38.70	10.90
11.00	438.7	27.6	10.9964	0.2000	8.4	42.00	11.05
11.10	444.1	27.8	11.0939	0.0975	5.4	55.39	11.14
11.20	455.8	28.1	11.1901	0.0962	11.7	121.62	11.24
11.30	469.5	28.2	11.2888	0.0987	13.7	138.81	11.34
11.40	488.6	28.4	11.3862	0.0974	19.1	196.10	11.43
11.50	508.4	28.7	11.4823	0.0961	19.8	206.04	11.53
11.60	531.1	28.5	11.5847	0.1024	22.7	**221.68**	**11.63**
11.70	546.1	28.7	11.6820	0.0973	15.0	154.16	11.73
11.80	554.7	28.7	11.7818	0.0998	8.6	86.17	11.83
11.90	563.1	28.8	11.8804	0.0986	8.4	85.19	11.93
12.00	569.3	29.1	11.9762	0.0958	6.2	64.72	12.02
12.10	574.4	29.6	12.0694	0.0932	5.1	54.72	12.12
12.20	579.0	29.7	12.1678	0.0984	4.6	46.74	12.22
12.30	683.6	29.9	12.2648	0.0970	4.6	47.42	12.32
12.40	588.0	29.9	12.3645	0.0997	4.4	44.13	12.41
12.50	592.2	30.0	12.4629	0.0984	4.2	42.68	12.51
12.60	596.2	30.3	12.5584	0.0955	4.0	41.88	12.66
12.80	602.5	30.4	12.7564	0.1980	6.3	31.82	

Table 3.2.16: Titration of $HClO_4$ against Standard Glucosamine Hydrochloride $(SG.HCl)_2$

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2} + \underline{V_1})/ml$ 2**
0.00	353.4	26.4	0	0.0000	0.0	0.00	5.01
10.00	403.5	26.4	10.0099	10.0099	50.1	5.01	10.31
10.60	424.7	26.6	10.6082	0.5983	21.2	35.43	10.71
10.80	430.3	26.8	10.8059	0.1977	5.6	28.33	10.91
11.00	442.3	25.8	11.0182	0.2123	12.0	56.52	11.07
11.10	448.5	25.6	11.1208	0.1026	6.2	60.43	11.17
11.20	456.6	25.2	11.2259	0.1051	8.1	77.07	11.27
11.30	466.4	26.6	11.3087	0.0828	9.8	118.36	11.36
11.40	478.3	26.7	11.4075	0.0988	11.9	120.45	11.46
11.50	495.4	26.9	11.5051	0.0976	17.1	175.21	11.55
11.60	517.5	27.1	11.6026	0.0975	22.1	**226.67**	**11.65**
11.70	536.5	27.1	11.7026	0.1000	19.0	190.00	11.75
11.80	557.7	27.2	11.8013	0.0987	21.2	214.79	11.85
11.90	567.9	27.6	11.8961	0.0948	10.2	107.59	11.95
12.00	576.0	27.8	11.9934	0.0973	8.1	83.25	12.04
12.10	583.9	27.9	12.0920	0.0986	7.9	80.12	12.14
12.20	590.1	28.1	12.1893	0.0973	6.2	63.72	12.24
12.30	596.1	28.3	12.2865	0.0972	6.0	61.73	12.34
12.40	601.9	28.3	12.3864	0.0999	5.8	58.06	12.44
12.50	607.2	28.4	12.4849	0.0985	5.3	53.81	12.53
12.60	611.9	28.6	12.5820	0.0971	4.7	48.40	

Table 3.2.17: Titration of $HClO_4$ against Standard Glucosamine Hydrochloride$(S_{G.HCl})_3$

Temperature of the titrant during standardization, $T_1 = 26.1^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2} + \underline{V_1})/ml$ 2**
0.00	342.2	26.3	0.0000	0.0000	0.0	0.00	5.00
10.00	407.0	26.8	9.9923	9.9923	64.8	6.48	10.29
10.60	431.0	27.6	10.5825	0.5902	24.0	40.66	10.68
10.80	434.0	27.8	10.7798	0.1973	3.0	15.21	10.88
11.00	437.0	27.9	10.9782	0.1984	3.0	15.12	11.03
11.10	441.0	28.2	11.0744	0.0962	4.0	41.58	11.12
11.20	447.0	28.3	11.1729	0.0985	6.0	60.91	11.22
11.30	451.0	28.5	11.2702	0.0973	5.0	51.39	11.32
11.40	456.0	28.2	11.3737	0.1035	5.0	48.31	11.42
11.50	467.0	28.2	11.4734	0.0997	11.0	110.33	11.52
11.60	478.0	28.4	11.5707	0.0973	11.0	**113.05**	**11.62**
11.70	483.0	28.9	11.6640	0.0933	5.0	53.59	11.71
11.80	487.0	29.2	11.7598	0.0958	4.0	41.75	11.81
11.90	490.0	29.4	11.8568	0.0970	3.0	30.93	11.91
12.00	492.0	29.5	11.9551	0.0983	2.0	20.35	12.00
12.10	494.0	29.7	12.0521	0.0970	2.0	20.62	12.10
12.20	495.0	29.9	12.1490	0.0969	1.0	10.32	12.20
12.30	496.0	30.0	12.2472	0.0982	1.0	10.18	12.30
12.40	497.0	30.1	12.3454	0.0982	1.0	10.18	12.40
12.50	498.0	30.2	12.4436	0.0982	1.0	10.18	12.49
12.60	500.0	30.2	12.5432	0.0996	2.0	20.08	12.64
12.80	502.0	30.2	12.7423	0.1991	2.0	10.05	

3.2.4 Titration of $HClO_4$ against Glucosamine Sulphate Drug.

Table 3.2.18: Titration of $HClO_4$ against Sample A_1

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/ml	Potential E^o / mV	T_2 /°C	Vc /ml	ΔV/ml	ΔE^o/ mV	$\Delta E^o/\Delta V$ mV/ml	$V' = (\underline{V_2} + \underline{V_1})/ml$ 2
0.00	352.7	26.8	0.0000	0.0000	0.0	0.00	2.10
4.2	381.4	26.9	4.2023	4.2023	28.7	6.83	4.25
4.3	382.9	26.9	4.3019	0.0996	1.5	15.06	4.38
4.40	384.6	27.0	4.4015	0.0996	1.7	17.07	4.45
4.50	386.4	27.4	4.4995	0.0980	1.8	18.37	4.55
4.60	388.5	27.8	4.5975	0.0980	2.1	21.43	4.65
4.70	393.3	27.9	4.6969	0.0994	5.3	53.32	4.75
4.80	398.8	28.0	4.7963	0.0994	5.5	55.33	4.84
4.90	405.0	29.0	4.8908	0.0945	6.2	65.61	4.94
5.00	408.6	29.8	4.9863	0.0955	3.6	37.69	5.04
5.10	416.4	30.1	5.0843	0.0980	7.8	79.59	5.13
5.20	425.4	30.1	5.1840	0.0997	9.0	90.27	5.23
5.30	436.1	30.2	5.2831	0.0991	10.7	107.97	5.33
5.40	448.7	30.3	5.3822	0.0991	12.6	127.14	5.43
5.50	462.6	30.3	5.4819	0.0997	13.9	**139.42**	**5.53**
5.60	472.2	30.4	5.5809	0.0990	9.6	96.97	5.63
5.70	479.5	30.5	5.6799	0.0990	7.3	73.74	5.73
5.80	486.4	30.5	5.7796	0.0997	6.9	69.21	5.83
5.90	488.0	30.6	5.8786	0.0990	1.6	16.16	5.93
6.00	489.3	30.4	5.9795	0.1009	1.3	12.88	6.03
6.10	492.7	30.2	6.0805	0.1010	3.4	33.66	6.13
6.20	498.5	30.2	6.1802	0.0997	5.8	58.18	6.23
6.30	500.6	30.3	6.2792	0.0990	2.1	21.21	6.33
6.40	502.6	30.5	6.3775	0.0983	2.0	20.35	6.43
6.50	504.0	30.7	6.4757	0.0983	1.4	14.24	6.53

6.60	505.5	30.8	6.5746	0.0989	1.5	15.17	6.67
6.80	509.1	30.9	6.7738	0.1992	3.6	18.07	-

Table 3.2.19: Titration of $HClO_4$ against Sample A_2

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/ml	**Potential E^o / mV**	**T_2 /°C**	**Vc /ml**	**ΔV/ml**	**ΔE^o/ mV**	**$\Delta E^o/\Delta V$ mV/ml**	**$V' = (\underline{V_2} + \underline{V_1})/ml$ 2**
0.00	330.3	27.8	0.0000	0.0000	0.0	0.00	2.10
4.2	370.3	27.8	4.1977	4.1977	40.0	9.53	4.25
4.3	372.3	27.8	4.2976	0.0999	2.0	20.02	4.35
4.40	374.8	27.8	4.3976	0.1000	2.5	25.00	4.45
4.50	377.7	27.8	4.4975	0.0895	2.9	32.40	4.55
4.60	383.3	27.8	4.5975	0.1000	5.6	56.0	4.65
4.70	389.2	27.8	4.6974	0.0999	5.9	59.06	4.75
4.80	395.9	28.9	4.7916	0.0942	6.7	71.13	4.84
4.90	403.2	29.0	4.8908	0.0992	7.3	73.58	4.94
5.00	409.6	29.4	4.9885	0.0977	6.4	65.51	5.04
5.10	416.1	29.8	5.0860	0.0975	6.5	66.67	5.14
5.20	424.2	30.1	5.1840	0.0980	8.1	82.65	5.23
5.30	434.0	30.1	5.2837	0.0997	9.8	98.29	5.33
5.40	444.7	30.2	5.3828	0.0991	10.7	107.97	5.43
5.50	455.5	30.3	5.4819	0.0991	10.8	**108.98**	**5.53**
5.60	463.4	30.3	5.5815	0.0996	7.9	79.32	5.63
5.70	469.3	30.3	5.6812	0.0997	5.9	59.18	5.73
5.80	477.2	30.3	5.7809	0.0997	7.9	79.24	5.83
5.90	481.5	30.4	5.8799	0.0990	4.3	43.43	5.93
6.00	482.3	30.5	5.9789	0.0990	0.8	8.08	6.03
6.10	484.4	30.5	6.0785	0.0996	2.1	21.08	6.13
6.20	486.1	30.5	6.1782	0.0997	1.7	17.05	6.23
6.30	487.7	30.6	6.2771	0.0989	1.6	16.18	6.33
6.40	488.9	30.5	6.3775	0.1004	1.2	11.95	6.43
6.50	489.9	30.6	6.4764	0.0989	1.0	10.11	6.53

6.60	491.4	30.6	6.5760	0.0996	1.5	15.06	6.68
6.80	493.6	30.8	6.7738	0.1978	2.2	11.12	-

Table 3.2.20: Titration of $HClO_4$ against Sample A_3

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/ml	**Potential E^o / mV**	**T_2 /°C**	**Vc /ml**	**ΔV/ml**	**ΔE^o/ mV**	**$\Delta E^o/\Delta V$ mV/ml**	**$V' = (\underline{V_2} + \underline{V_1})$/ml 2**
0.00	358.8	27.1	0.0000	0.0000	0.0	0.0	2.10
4.2	397.0	27.1	4.2009	4.2009	38.2	9.09	4.25
4.3	398.0	27.1	4.3010	0.1001	1.0	9.99	4.35
4.40	400.0	27.1	4.4010	0.100	2.0	20.00	4.45
4.50	402.0	27.3	4.5000	0.0990	2.0	20.20	4.55
4.60	404.0	27.3	4.6000	0.1000	2.0	20.00	4.65
4.70	405.0	27.4	4.6995	0.0995	1.0	10.05	4.75
4.80	406.0	27.3	4.8000	0.1005	1.0	9.95	4.85
4.90	408.0	27.5	4.8989	0.0989	2.0	20.22	4.95
5.00	409.0	27.5	4.9989	0.1000	1.0	10.00	5.05
5.10	412.0	27.6	5.0983	0.0994	3.0	30.18	5.15
5.20	415.0	27.7	5.1977	0.0994	3.0	30.18	5.25
5.30	419.0	27.9	5.2965	0.0988	4.0	40.49	5.35
5.40	423.0	28.1	5.4012	0.1047	4.0	38.20	5.45
5.50	431.0	28.0	5.4958	0.0946	8.0	**84.57**	**5.55**
5.60	436.0	28.0	5.5957	0.0999	5.0	50.05	5.65
5.70	440.0	28.1	5.6950	0.0993	4.0	40.28	5.75
5.80	444.0	28.2	5.7943	0.0993	4.0	40.28	5.84
5.90	447.0	28.3	5.8935	0.0992	3.0	30.24	5.94
6.00	450.0	28.2	5.9941	0.1006	3.0	29.82	6.04
6.10	453.0	28.3	6.0933	0.0992	3.0	30.24	6.14
6.20	456.0	28.5	6.1918	0.0980	3.0	30.61	6.24
6.30	458.0	28.5	6.2917	0.0999	2.0	20.02	6.34
6.40	460.0	28.7	6.3901	0.0984	2.0	20.33	6.44

6.50	463.0	28.9	6.4886	0.0985	3.0	30.45	6.54
6.60	465.0	28.9	6.5884	0.0998	2.0	20.04	6.69
6.80	469.0	28.4	6.7918	0.2023	4.0	19.77	-

Table 3.2.21: Titration of $HClO_4$ against Sample B_1

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/*ml***	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml*** **2**
0.00	320.1	26.1	0.0000	0.0000	0.0	0.00	2.10
4.2	349.3	26.3	4.2055	4.2055	29.2	6.94	4.25
4.3	351.7	26.5	4.3038	0.0983	2.4	24.42	4.35
4.40	354.7	26.8	4.4024	0.0986	3.0	30.43	4.45
4.50	358.0	27.6	4.4985	0.0961	3.3	34.34	4.55
4.60	361.0	27.8	4.5975	0.0990	3.0	30.30	4.65
4.70	363.7	27.9	4.6969	0.0994	2.7	27.16	4.75
4.80	365.7	28.2	4.7953	0.0984	2.0	20.33	4.85
4.90	368.8	28.3	4.8946	0.0993	3.1	31.22	4.94
5.00	371.7	28.5	4.9934	0.0988	2.9	29.35	5.04
5.10	375.7	28.6	5.0927	0.0993	4.0	40.28	5.14
5.20	379.2	28.2	5.1949	0.1022	3.5	34.25	5.25
5.30	382.2	28.2	5.2948	0.0999	3.0	30.03	5.34
5.40	387.7	28.4	5.3935	0.0987	5.5	55.72	5.44
5.50	395.6	28.9	5.4903	0.0968	**7.9**	**81.61**	**5.54**
5.60	400.0	29.1	5.5889	0.0986	4.4	44.62	5.64
5.70	404.0	29.2	5.6881	0.0992	4.0	40.32	5.74
5.80	407.0	29.4	5.7866	0.0985	3.0	30.45	5.84
5.90	409.0	29.4	5.8864	0.0998	2.0	20.04	5.94
6.00	411.0	29.5	5.9855	0.0991	2.0	20.18	6.04
6.10	413.0	29.7	6.0839	0.0984	2.0	20.32	6.13
6.20	414.0	29.9	6.1823	0.0984	1.0	10.16	6.23
6.30	415.0	29.8	6.2827	0.1004	1.0	9.96	6.33

6.40	416.0	30.0	6.3810	0.0983	1.0	10.17	6.43
6.50	417.0	30.1	6.4800	0.0990	1.0	10.10	6.53
6.60	418.0	30.1	6.5797	0.0997	1.0	10.03	6.68
6.80	421.0	30.2	6.7783	0.1986	3.0	15.11	-

Table 3.2.22: Titration of $HClO_4$ against Sample B_2

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/*ml***	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml* 2**
0.00	367.8	30.0	0.0000	0.0000	0.0	0.00	2.09
4.2	401.1	30.1	4.1871	4.1871	33.3	7.95	4.24
4.3	403.3	30.1	4.2868	0.0997	2.2	22.07	4.34
4.40	406.1	30.2	4.3860	0.0992	2.8	28.23	4.44
4.50	409.5	30.1	4.4861	0.1001	3.5	34.96	4.54
4.60	412.3	29.6	4.5884	0.1023	2.8	27.37	4.64
4.70	413.8	29.9	4.6866	0.0982	1.5	15.27	4.74
4.80	414.6	30.0	4.7857	0.0991	0.8	8.07	4.84
4.90	415.8	30.1	4.8849	0.0992	1.2	12.10	4.94
5.00	416.8	30.2	4.9841	0.0992	1.0	10.08	5.03
5.10	418.2	30.2	5.0837	0.0996	1.4	14.06	5.13
5.20	419.6	30.3	5.1828	0.0991	1.4	14.13	5.23
5.30	421.5	30.4	5.2819	0.0991	1.9	19.17	5.33
5.40	426.0	29.7	5.3857	0.1038	4.5	43.35	5.44
5.50	433.3	29.6	5.4861	0.1004	**7.3**	**72.71**	**5.54**
5.60	436.4	29.6	5.5858	0.0997	3.1	31.09	5.64
5.70	438.7	29.7	5.6850	0.0992	2.3	23.19	5.74
5.80	439.6	29.4	5.7866	0.1016	0.9	8.86	5.84
5.90	440.4	29.2	5.8877	0.1011	0.8	7.91	5.94
6.00	441.0	29.3	5.9868	0.0991	0.6	6.05	6.04
6.10	441.7	29.2	6.0873	0.1005	0.7	6.97	6.14
6.20	442.3	29.2	6.1870	0.0997	0.6	6.02	6.24

6.30	443.2	29.2	6.2868	0.0998	0.9	9.0	6.34
6.40	444.1	29.3	6.3859	0.0991	0.9	9.02	6.44
6.50	444.9	29.4	6.4850	0.0991	0.8	8.07	6.54
6.60	445.7	29.4	6.5848	0.0998	0.8	8.02	6.68
6.80	447.8	29.5	6.7835	0.1987	2.1	10.57	-

Table 3.2.23: Titration of $HClO_4$ against Sample B_3

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/*ml***	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = \frac{(V_2 + V_1)}{2}$ /*ml***
0.00	302.4	27.1	0.0000	0.0000	0.0	0.00	2.10
4.2	332.8	27.1	4.2009	4.2009	30.4	7.17	4.25
4.3	333.8	27.2	4.3005	0.0996	1.0	10.04	4.35
4.40	335.0	27.2	4.4005	0.1000	1.2	12.00	4.45
4.50	336.4	27.0	4.5015	0.1010	1.4	13.86	4.55
4.60	338.0	26.9	4.6020	0.1005	1.6	15.92	4.65
4.70	339.5	27.0	4.7016	0.0996	1.5	15.06	4.75
4.80	341.3	27.1	4.8011	0.0995	1.8	18.09	4.85
4.90	343.3	27.5	4.8989	0.0978	2.0	20.45	4.95
5.00	345.4	27.7	4.9978	0.0989	2.1	21.23	5.05
5.10	348.0	27.9	5.0966	0.0988	2.6	26.32	5.15
5.20	350.4	28.1	5.1954	0.0988	2.4	24.29	5.25
5.30	355.2	28.2	5.2948	0.0994	4.8	48.29	5.35
5.40	360.5	28.3	5.3941	0.0993	5.3	53.73	5.44
5.50	365.9	28.4	5.4934	0.0993	5.4	54.38	5.54
5.60	371.8	28.4	5.5932	0.0998	**5.9**	**59.12**	**5.64**
5.70	376.8	28.6	5.6919	0.0987	5.0	50.66	5.74
5.80	381.3	28.7	5.7911	0.0992	4.5	45.36	5.84
5.90	385.7	28.7	5.8909	0.0998	4.4	44.09	5.94
6.00	389.9	28.8	5.9901	0.0992	4.2	42.33	6.04
6.10	392.9	28.9	6.0893	0.0992	3.0	30.24	6.14

6.20	395.7	29.0	6.1884	0.0991	2.8	28.23	6.24
6.30	398.0	29.2	6.2868	0.0984	2.3	23.37	6.34
6.40	399.0	29.3	6.3859	0.0991	1.0	10.09	6.44
6.50	400.0	29.1	6.4871	0.1012	1.0	9.88	6.54
6.60	401.0	29.2	6.5862	0.0991	1.0	10.09	6.69
6.80	403.0	29.2	6.7858	0.1996	2.0	10.02	-

Table 3.2.24: Titration of $HClO_4$ against Sample C_1

Temperature of the titrant during standardization, T_1 = 27.3°C

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/*ml***	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml* 2**
0.00	386.9	27.1	0.0000	0.0000	0.0	0.00	2.20
4.2	443.0	27.1	4.2009	4.2009	56.1	13.35	4.25
4.3	444.0	27.1	4.3010	0.1001	1.0	9.99	4.35
4.40	445.0	27.1	4.4010	0.1000	1.0	10.00	4.45
4.50	446.0	27.0	4.5015	0.1005	1.0	9.95	4.55
4.60	447.0	26.9	4.6020	0.1005	1.0	9.95	4.65
4.70	448.0	27.0	4.7016	0.0996	1.0	10.04	4.75
4.80	449.0	27.1	4.8011	0.0995	1.0	10.05	4.85
4.90	451.0	27.5	4.8989	0.0978	2.0	20.45	4.95
5.00	453.0	27.7	4.9978	0.0989	2.0	20.22	5.05
5.10	456.0	27.9	5.0966	0.0988	3.0	30.36	5.15
5.20	460.0	28.1	5.1954	0.0988	4.0	40.49	5.25
5.30	468.0	28.2	5.2948	0.0994	**8.0**	**80.48**	**5.34**
5.40	473.0	28.4	5.3935	0.0987	5.0	50.66	5.44
5.50	477.0	28.4	5.4935	0.1000	4.0	40.00	5.54
5.60	479.0	28.6	5.5920	0.0985	2.0	20.30	5.64
5.70	481.0	28.6	5.6919	0.0999	2.0	20.02	5.74
5.80	482.0	28.7	5.7911	0.0992	1.0	10.08	5.84
5.90	483.0	28.8	5.8903	0.0992	1.0	10.08	5.94
6.00	484.0	28.9	5.9894	0.0991	1.0	10.09	6.04

6.10	485.0	29.0	6.0886	0.0992	1.0	10.08	6.14
6.20	486.0	29.0	6.1884	0.0998	1.0	10.02	6.24
6.30	487.0	29.1	6.2875	0.0991	1.0	10.09	6.34
6.40	488.0	29.2	6.3866	0.0991	1.0	10.09	6.44
6.50	489.0	29.2	6.4864	0.0998	1.0	10.02	6.54
6.60	490.0	29.6	6.5833	0.0969	1.0	10.32	6.68
6.80	493.0	29.7	6.7821	0.1988	3.0	15.09	-

Table 3.2.25: Titration of $HClO_4$ against Sample C_2

Temperature of the titrant during standardization, $T_1 = 27.3^oC$

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = \frac{(V_2 + V_1)}{2}$ /*ml***
0.00	346.6	27.1	0.0000	0.0000	0.0	0.00	2.10
4.2	379.4	27.4	4.1995	4.1995	32.8	7.81	4.25
4.3	380.5	27.9	4.2972	0.0977	1.1	11.26	4.35
4.40	381.8	28.2	4.3956	0.0984	1.3	13.21	4.45
4.50	383.3	28.5	4.4941	0.0985	1.5	15.23	4.54
4.60	385.0	28.7	4.5929	0.0988	1.7	17.21	4.64
4.70	386.2	28.6	4.6933	0.1004	1.2	11.95	4.74
4.80	387.6	28.4	4.7942	0.1009	1.4	13.88	4.84
4.90	389.2	28.4	4.8941	0.0999	1.6	16.02	4.94
5.00	391.0	28.6	4.9929	0.0988	1.8	18.21	5.04
5.10	394.4	28.5	5.0933	0.1004	3.4	33.87	5.14
5.20	398.5	28.7	5.1920	0.0987	4.1	41.54	5.24
5.30	405.0	28.7	5.2918	0.0998	6.5	65.13	5.34
5.40	413.0	28.6	5.3923	0.1005	**8.0**	**79.60**	**5.44**
5.50	419.0	28.8	5.4909	0.0986	6.0	60.85	5.54
5.60	424.0	28.6	5.5920	0.1011	5.0	49.46	5.64
5.70	427.0	28.6	5.6919	0.0999	3.0	30.03	5.74
5.80	430.0	28.40	5.7930	0.1011	3.0	29.67	5.84
5.90	432.0	28.30	5.8935	0.1005	2.0	19.90	5.94
6.00	434.0	28.40	5.9927	0.0992	2.0	20.16	6.04
6.10	436.0	28.40	6.0926	0.0999	2.0	20.02	6.14
6.20	437.0	28.60	6.1911	0.0985	1.0	10.15	6.24
6.30	438.0	28.50	6.2917	0.1006	1.0	9.94	6.34
6.40	439.0	28.50	6.3916	0.0999	1.0	10.01	6.44
6.50	440.0	28.40	6.4921	0.1005	1.0	9.95	6.54
6.60	441.0	28.40	6.5920	0.0999	1.0	10.01	6.69
6.80	444.0	28.30	6.7925	0.2005	3.0	14.96	-

Table 3.2.26: Titration of $HClO_4$ against Sample C_3

Temperature of the titrant during standardization, T_1 = 26.1°C.

Volume of $HClO_4$ added/*ml*	Potential E^o / *mV*	T_2 /^oC	Vc /*ml*	ΔV/*ml*	ΔE^o/ *mV*	$\Delta E^o/\Delta V$ *mV/ml*	$V' = (\underline{V_2 + V_1})$/*ml* 2
0.00	351.8	27.8	0.0000	0.0000	0.0	0.00	2.10
4.2	383.6	27.9	4.1922	4.1922	31.8	7.59	4.24
4.3	384.8	28.0	4.2910	0.0988	1.2	12.15	4.34
4.40	386.0	28.1	4.3903	0.0993	1.2	12.08	4.44
4.50	387.4	28.1	4.4901	0.0998	1.4	14.03	4.54
4.60	389.3	28.0	4.5904	0.1003	1.9	18.94	4.64
4.70	390.6	28.0	4.6902	0.0998	1.3	13.03	4.74
4.80	392.0	27.9	4.7905	0.1003	1.4	13.96	4.84
4.90	394.0	27.9	4.8903	0.0998	2.0	20.04	4.94
5.00	396.2	27.8	4.9907	0.1004	2.2	21.91	5.04
5.10	400.0	28.2	5.0882	0.0975	3.8	38.97	5.14
5.20	404.0	28.2	5.1880	0.0998	4.0	40.08	5.24
5.30	409.0	28.1	5.2883	0.1003	5.0	49.85	5.34
5.40	414.0	28.3	5.3869	0.0986	**7.0**	**70.99**	**5.44**
5.50	419.0	38.6	5.4849	0.0980	5.0	51.02	5.54
5.60	423.0	28.6	5.5846	0.0997	4.0	40.12	5.64
5.70	426.0	28.5	5.6850	0.1004	3.0	29.88	5.74
5.80	429.0	28.4	5.7853	0.1003	3.0	29.91	5.84
5.90	432.0	28.4	5.8851	0.0998	3.0	30.06	5.94
6.00	434.0	28.4	5.9848	0.0997	2.0	20.06	6.04
6.10	436.0	28.3	6.0852	0.1004	2.0	19.92	6.14
6.20	438.0	28.2	6.1857	0.1005	2.0	19.90	6.24
6.30	439.0	28.4	6.2841	0.0984	1.0	10.16	6.33
6.40	440.0	28.3	6.3845	0.1004	1.0	9.96	6.43
6.50	441.0	28.3	6.4843	0.0998	1.0	10.02	6.53
6.60	442.0	28.4	6.5833	0.0990	1.0	10.10	6.68
6.80	445.0	28.5	6.7821	0.1988	3.0	15.09	-

Table 3.2.27: Titration of $HClO_4$ against Sample D_1

Temperature of the titrant during standardization, $T_1 = 26.1^oC$

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/*ml***	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml* 2**
0.00	381.0	27.5	0.0000	0.0000	0.0	0.00	2.10
4.2	407.0	28.0	4.1912	4.1912	26.0	6.20	4.24
4.3	409.0	28.1	4.2905	0.0993	2.0	20.14	4.34
4.40	411.0	28.1	4.3903	0.0998	2.0	20.04	4.44
4.50	417.0	28.6	4.4876	0.0973	**6.0**	**61.67**	**4.54**
4.60	420.0	28.7	4.5868	0.0992	3.0	30.83	4.64
4.70	424.0	28.8	4.6860	0.0992	4.0	40.32	4.74
4.80	428.0	28.8	4.7857	0.0997	4.0	40.12	4.83
4.90	432.0	29.9	4.8795	0.0938	4.0	42.64	4.93
5.00	435.0	29.0	4.9841	0.1046	3.0	28.68	5.03
5.10	438.0	29.1	5.0832	0.0991	3.0	30.27	5.13
5.20	440.0	29.4	5.1811	0.0979	2.0	20.43	5.23
5.30	442.0	29.5	5.2802	0.0991	2.0	20.18	5.33
5.40	443.0	29.6	5.3792	0.0990	1.0	10.10	5.43
5.50	444.0	29.8	5.4776	0.0984	1.0	10.16	5.53
5.60	445.0	29.9	5.5766	0.0990	1.0	10.10	5.63
5.70	446.0	29.9	5.6762	0.0996	1.0	10.04	5.73
5.80	447.0	29.8	5.7764	0.1002	1.0	9.98	5.83
5.90	448.0	29.6	5.8773	0.1009	1.0	9.91	5.93
6.00	449.0	29.4	5.9782	0.1009	1.0	9.91	6.03
6.10	450.0	29.4	6.0779	0.0997	1.0	10.03	6.13
6.20	451.0	29.3	6.1782	0.1003	1.0	9.97	6.23
6.30	452.0	29.1	6.2792	0.1010	1.0	9.90	6.33
6.40	453.0	29.0	6.3796	0.1004	1.0	9.96	6.43
6.50	454.0	28.9	6.4800	0.1004	1.0	9.96	6.53
6.60	455.0	28.7	6.5811	0.1011	1.0	9.89	6.68
6.80	458.0	28.5	6.7821	0.2010	3.0	14.93	

Table 3.2.28: Titration of $HClO_4$ against Sample D_2

Temperature of the titrant during standardization, $T_1 = 26.1^oC$

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/*ml***	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml*** **2**
0.00	383.0	26.8	0.000	0.0000	0.0	0.00	2.10
4.2	410.0	26.8	4.1968	4.1968	27.0	6.43	4.25
4.3	412.0	26.8	4.2967	0.0999	2.0	20.02	4.35
4.40	417.0	26.9	4.3961	0.0994	5.0	50.30	4.45
4.50	425.0	26.9	4.4960	0.0999	**8.0**	**80.08**	**4.55**
4.60	432.0	26.9	4.5960	0.1000	7.0	70.00	4.65
4.70	438.0	26.9	4.6959	0.0999	6.0	60.06	4.75
4.80	445.0	26.9	4.7958	0.0999	7.0	70.07	4.85
4.90	452.0	26.8	4.8962	0.1004	7.0	69.72	4.95
5.00	458.0	26.8	4.9962	0.1000	6.0	60.00	5.05
5.10	462.0	26.7	5.0966	0.1004	4.0	39.84	5.15
5.20	466.0	26.7	5.1966	0.1000	4.0	40.00	5.25
5.30	469.0	26.7	5.2965	0.0999	3.0	30.03	5.35
5.40	472.0	26.8	5.3958	0.0993	3.0	30.21	5.45
5.50	475.0	26.8	5.4958	0.1000	3.0	30.00	5.55
5.60	478.0	26.8	5.5957	0.0999	3.0	30.03	5.65
5.70	480.0	26.9	5.6950	0.0993	2.0	20.14	5.74
5.80	482.0	27.1	5.7936	0.0986	2.0	20.28	5.84
5.90	484.0	27.1	5.8935	0.0999	2.0	20.02	5.94
6.00	486.0	27.3	5.9921	0.0986	2.0	20.28	6.04
6.10	488.0	27.3	6.0920	0.0999	2.0	20.02	6.14
6.20	489.0	27.3	6.1918	0.0998	1.0	10.02	6.24
6.30	490.0	27.2	6.2924	0.1006	1.0	9.94	6.34
6.40	491.0	27.2	6.3923	0.0999	1.0	10.01	6.44
6.50	492.0	27.2	6.4921	0.0998	1.0	10.02	6.54
6.60	493.0	27.2	6.5920	0.0999	1.0	10.01	6.69
6.80	496.0	27.2	6.7918	0.1998	3.0	15.02	

Table 3.2.29: Titration of $HClO_4$ against Sample D_3

Temperature of the titrant during standardization, $T_1 = 26.1^oC$

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/*ml***	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml* 2**
0.00	384.5	28.0	0.0000	0.000	0.0	0.00	2.10
4.2	413.0	28.1	4.1908	4.1908	28.5	6.80	4.24
4.3	415.0	28.1	4.2905	0.0997	2.0	20.06	4.34
4.40	418.0	28.1	4.3903	0.0998	3.0	30.06	4.44
4.50	426.0	28.2	4.4896	0.0993	**8.0**	**80.56**	**4.54**
4.60	430.0	28.1	4.5899	0.1003	4.0	39.88	4.64
4.70	433.0	18.1	4.6897	0.0998	3.0	30.21	4.74
4.80	438.0	28.2	4.7889	0.0992	5.0	50.40	4.84
4.90	442.0	28.1	4.8892	0.1003	4.0	39.88	4.94
5.00	445.0	28.1	4.9890	0.0998	3.0	30.06	5.04
5.10	448.0	28.2	5.0882	0.0992	3.0	30.24	5.14
5.20	451.0	28.2	5.1880	0.0998	3.0	30.06	5.24
5.30	453.0	28.2	5.2878	0.0998	2.0	20.04	5.34
5.40	455.0	28.3	5.3869	0.0991	2.0	20.18	5.44
5.50	457.0	28.3	5.4868	0.0999	2.0	20.02	5.54
5.60	458.0	28.2	5.5877	0.1009	1.0	9.91	5.64
5.70	459.0	28.2	5.6868	0.0991	1.0	10.09	5.74
5.80	460.0	28.2	5.7866	0.0998	1.0	10.02	5.84
5.90	461.0	28.2	5.8864	0.0998	1.0	10.02	5.94
6.00	462.0	28.2	5.9861	0.0997	1.0	10.03	6.04
6.10	463.0	28.2	6.0859	0.0998	1.0	10.02	6.14
6.20	464.0	28.3	6.1850	0.0991	1.0	10.09	6.24
6.30	465.0	28.3	6.2848	0.0998	1.0	10.02	6.34
6.40	466.0	28.3	6.3845	0.0997	1.0	10.03	6.43
6.50	467.0	28.3	6.4843	0.0998	1.0	10.02	6.53
6.60	468.0	28.3	6.5840	0.0997	1.0	10.03	6.68
6.80	470.0	28.4	6.7835	0.1995	3.0	15.04	

Table 3.2.30: Titration of $HClO_4$ against Sample E_1

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/ml	Potential E^o / mV	Temp /^oC	Vc /ml	ΔV/ml	ΔE^o/ mV	$\Delta E^o/\Delta V$ mV/ml	$V' = (\underline{V_2 + V_1})$/ml 2
0.00	375.7	29.1	0.0000	0.0000	0.0	0.00	2.10
4.2	417.3	29.2	4.2060	4.2060	41.6	9.89	4.26
4.3	418.8	29.2	4.3062	0.1002	1.5	14.97	4.36
4.40	420.0	29.3	4.4058	0.0996	1.2	12.05	4.46
4.50	421.0	29.3	4.5059	0.1001	1.0	9.99	4.56
4.60	422.0	29.3	4.6061	0.1002	1.0	9.98	4.66
4.70	423.0	29.3	4.7062	0.1001	1.0	9.99	4.76
4.80	424.0	29.2	4.8069	0.1007	1.0	9.93	4.86
4.90	425.0	29.2	4.9070	0.1001	1.0	9.99	4.96
5.00	427.0	29.2	5.0072	0.1002	2.0	19.96	5.06
5.10	429.0	29.1	5.1079	0.1007	2.0	19.86	5.16
5.20	432.0	29.1	5.2080	0.1001	3.0	29.97	5.26
5.30	435.0	29.1	5.3082	0.1002	2.0	19.96	5.36
5.40	441.0	29.0	5.4089	0.1007	**6.0**	**59.58**	**5.46**
5.50	444.0	29.0	5.5091	0.1002	3.0	29.94	5.56
5.60	446.0	29.0	5.6092	0.1001	2.0	19.98	5.66
5.70	448.0	29.0	5.7094	0.1002	2.0	19.96	5.76
5.80	449.0	28.9	5.8102	0.1008	1.0	9.92	5.86
5.90	450.0	28.9	5.9104	0.1002	1.0	9.98	5.96
6.00	451.0	28.9	6.0106	0.1002	1.0	9.98	6.06
6.10	452.0	28.9	6.1107	0.1001	1.0	9.99	6.16
6.20	453.0	28.9	6.2109	0.1002	1.0	9.98	6.26
6.30	454.0	29.0	6.3104	0.0995	1.0	10.05	6.36
6.40	455.0	28.8	6.4120	0.1016	1.0	9.84	6.46
6.50	456.0	28.9	6.5114	0.0994	1.0	10.06	6.56
6.60	457.0	28.8	6.6123	0.1009	1.0	9.91	6.71
6.80	460.0	28.8	6.8127	0.2004	3.0	14.97	-

Table 3.2.31: Titration of $HClO_4$ against Sample E_2

Temperature of the titrant during standardization, T_1 = 26.1 ^oC

Volume of $HClO_4$ added/ml	Potential E^o / mV	Temp /^oC	Vc /ml	ΔV/ml	ΔE^o/ mV	$\Delta E^o/\Delta V$ mV/ml	$V' = (\underline{V_2} + \underline{V_1})/ml$ 2
0.00	369.6	27.0	0.0000	0.0000	0.0	0.00	2.10
4.2	398.0	27.3	4.1945	4.1945	28.4	6.77	4.24
4.3	399.0	27.8	4.2920	0.0975	1.0	10.26	4.34
4.40	400.0	28.7	4.3874	0.0954	1.0	10.48	4.46
4.50	401.0	28.6	4.4876	0.1001	1.0	9.98	4.56
4.60	402.0	28.6	4.5874	0.0998	1.0	10.02	4.66
4.70	403.0	28.5	4.6876	0.1002	1.0	9.98	4.76
4.80	404.0	28.4	4.7879	0.1003	1.0	9.97	4.86
4.90	405.0	28.3	4.8881	0.1002	1.0	9.98	4.96
5.00	407.0	28.3	4.9879	0.0998	2.0	20.04	5.06
5.10	409.0	28.4	5.0871	0.0992	2.0	20.16	5.16
5.20	412.0	28.4	5.1868	0.0997	3.0	30.09	5.26
5.30	417.0	28.3	5.2872	0.1004	5.0	49.80	5.36
5.40	425.0	28.3	5.3869	0.0997	**8.0**	**80.24**	**5.46**
5.50	432.0	28.3	5.4867	0.0998	7.0	70.14	5.56
5.60	438.0	28.4	5.5858	0.0991	6.0	60.55	5.66
5.70	442.0	28.4	5.6856	0.0998	4.0	40.08	5.76
5.80	444.0	28.5	5.7847	0.0991	2.0	20.18	5.86
5.90	445.0	28.5	5.8844	0.0997	1.0	10.03	5.96
6.00	446.0	28.6	5.9835	0.0991	1.0	10.09	6.06
6.10	447.0	28.5	6.0840	0.1005	1.0	9.95	6.16
6.20	448.0	28.6	6.1830	0.0990	1.0	10.10	6.26
6.30	449.0	28.6	6.2827	0.0997	1.0	10.03	6.36
6.40	450.0	28.7	6.3817	0.0990	1.0	10.10	6.46
6.50	451.0	28.8	6.4807	0.0990	1.0	10.10	6.56
6.60	452.0	28.8	6.5804	0.0997	1.0	10.03	6.71
6.80	455.0	28.9	6.7791	0.1987	3.0	15.10	-

Table 3.2.32: Titration of $HClO_4$ against Sample E_3

Temperature of the titrant during standardization, T_1 = 26.1 ^oC.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})/ml$ 2**
0.00	372.1	27.7	0.0000	0.0000	0.0	0.00	2.10
4.2	411.0	27.7	4.1926	4.1926	38.9	9.28	4.24
4.3	412.0	27.8	4.2920	0.0994	1.0	10.05	4.34
4.40	413.0	27.8	4.3918	0.0998	1.0	10.02	4.44
4.50	414.0	28.3	4.4891	0.0973	1.0	10.28	4.54
4.60	415.0	28.3	4.5889	0.0998	1.0	10.02	4.64
4.70	416.0	28.2	4.6891	0.1002	1.0	9.98	4.74
4.80	417.0	28.2	4.7889	0.0998	1.0	10.02	4.84
4.90	418.0	28.2	4.8887	0.0998	1.0	10.02	4.94
5.00	419.0	28.3	4.9879	0.0992	1.0	10.08	5.04
5.10	420.0	28.4	5.0871	0.0992	1.0	10.08	5.14
5.20	422.0	28.5	5.1863	0.0992	2.0	20.16	5.24
5.30	426.0	28.7	5.2848	0.0985	4.0	40.61	5.33
5.40	431.0	28.8	5.3840	0.0992	5.0	50.40	5.43
5.50	438.0	28.9	5.4831	0.0991	**7.0**	**70.64**	**5.53**
5.60	443.0	28.9	5.5828	0.0997	5.0	50.15	5.63
5.70	446.0	28.6	5.6843	0.1015	3.0	29.56	5.73
5.80	449.0	28.6	5.7841	0.0998	3.0	30.06	5.83
5.90	452.0	28.5	5.8844	0.1003	3.0	29.91	5.94
6.00	454.0	28.4	5.9848	0.1004	2.0	19.92	6.04
6.10	455.0	28.2	6.0859	0.1011	1.0	9.89	6.14
6.20	456.0	28.2	6.1857	0.0998	1.0	10.02	6.24
6.30	457.0	28.1	6.2861	0.1004	1.0	9.96	6.34
6.40	458.0	28.2	6.3852	0.0991	1.0	10.09	6.44
6.50	459.0	28.1	6.4857	0.1005	1.0	9.95	6.54
6.60	460.0	28.1	6.5855	0.0998	1.0	10.02	6.69
6.80	463.0	28.1	6.7850	0.1995	3.0	15.04	-

Table 3.2.33: Titration of $HClO_4$ against Sample F_1

Temperature of the titrant during standardization, $T_1 = 26.1^oC$

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = \frac{(V_2 + V_1)}{2}$ /*ml***
0.00	392.0	27.8	0.0000	0.0000	0.0	0.00	2.10
4.2	428.0	28.0	4.1912	4.1912	36.0	8.59	4.24
4.3	430.0	28.0	4.2910	0.0998	2.0	20.04	4.34
4.40	432.0	28.0	4.3908	0.0998	2.0	20.04	4.44
4.50	436.0	28.1	4.4901	0.0993	4.0	40.28	4.54
4.60	440.0	28.6	4.5874	0.0973	4.0	40.11	4.64
4.70	443.0	28.8	4.6860	0.0986	3.0	30.43	4.74
4.80	449.0	28.9	4.7852	0.0992	6.0	60.48	4.84
4.90	456.0	29.1	4.8838	0.0986	7.0	70.99	4.93
5.00	465.0	29.2	4.9830	0.0992	9.0	90.73	5.03
5.10	474.0	29.4	5.0815	0.0985	9.0	91.37	5.13
5.20	486.0	29.7	5.1794	0.0979	12.0	122.57	5.23
5.30	501.0	30.1	5.2767	0.0973	15.0	**154.16**	**5.33**
5.40	513.0	30.3	5.3751	0.0984	12.0	121.95	5.42
5.50	520.0	30.5	5.4734	0.0983	7.0	71.21	5.52
5.60	525.0	30.9	5.5704	0.0970	5.0	51.55	5.62
5.70	529.0	31.2	5.6680	0.0976	4.0	40.98	5.72
5.80	533.0	31.8	5.7636	0.0956	4.0	41.84	5.81
5.90	537.0	31.9	5.8624	0.0988	4.0	40.49	5.91
6.00	541.0	31.9	5.9617	0.0993	4.0	40.28	6.01
6.10	545.0	31.2	6.0658	0.1041	4.0	38.43	6.12
6.20	548.0	31.0	6.1666	0.1008	3.0	29.76	6.22
6.30	551.0	30.0	6.2730	0.1064	3.0	28.20	6.32
6.40	553.0	29.8	6.3740	0.1010	2.0	19.80	6.43
6.50	555.0	29.4	6.4764	0.1024	2.0	19.53	6.53
6.60	557.0	29.4	6.5760	0.0996	2.0	20.08	6.68
6.80	561.0	29.1	6.7776	0.2016	4.0	19.84	-

Table 3.2.34: Titration of $HClO_4$ against Sample F_2

Temperature of the titrant during standardization, $T_1 = 26.1^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = \frac{(V_2 + V_1)}{2}$/*ml***
0.00	352.6	26.2	0.0000	0.0000	0.0	0.00	2.10
4.2	394.4	26.2	4.1995	4.1995	41.8	9.95	4.25
4.3	396.3	26.2	4.2995	0.1000	1.9	19.0	4.35
4.40	398.5	26.2	4.3995	0.1000	2.2	22.00	4.45
4.50	401.0	26.2	4.4995	0.1000	2.5	25.00	4.55
4.60	404.0	26.4	4.5985	0.0990	3.0	30.30	4.65
4.70	407.0	26.3	4.6990	0.1005	3.0	29.85	4.75
4.80	409.0	26.4	4.7984	0.0994	2.0	20.12	4.85
4.90	412.0	26.5	4.8978	0.0994	3.0	30.18	4.95
5.00	415.0	26.6	4.9973	0.0995	3.0	30.15	5.05
5.10	419.0	26.7	5.0966	0.0993	4.0	40.28	5.15
5.20	424.0	26.8	5.1960	0.0994	5.0	50.30	5.25
5.30	434.0	26.8	5.2959	0.0999	**10.0**	**100.10**	**5.35**
5.40	443.0	26.9	5.3952	0.0993	9.0	90.63	5.45
5.50	448.0	26.9	5.4952	0.1000	5.0	50.0	5.55
5.60	453.0	27.0	5.5945	0.0993	5.0	50.35	5.65
5.70	457.0	27.0	5.6944	0.0999	4.0	40.04	5.74
5.80	460.0	27.2	5.7930	0.0986	3.0	30.43	5.85
5.90	462.0	27.2	5.8993	0.1063	2.0	18.82	5.95
6.00	465.0	27.2	5.9927	0.0934	3.0	32.12	6.04
6.10	468.0	27.3	6.0920	0.0993	3.0	30.21	6.14
6.20	471.0	27.3	6.1918	0.0998	3.0	30.06	6.24
6.30	473.0	27.4	6.2910	0.0992	2.0	20.16	6.34
6.40	475.0	27.4	6.3909	0.0999	2.0	20.02	6.44
6.50	478.0	27.4	6.4907	0.0998	3.0	30.06	6.54
6.60	480.0	27.7	6.5884	0.0977	2.0	20.47	6.69

6.80	484.0	27.9	6.7865	0.1981	4.0	20.19	-

Table 3.2.35: Titration of $HClO_4$ against Sample F_3

Temperature of the titrant during standardization, $T_1 = 26.1^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})/ml$ 2**
0.00	343.8	27.0	0.0000	0.0000	0.0	0.00	2.10
4.2	368.1	27.1	4.1954	4.1954	24.3	5.79	4.25
4.3	370.1	27.1	4.2953	0.0999	2.0	20.02	4.35
4.40	373.5	27.2	4.3947	0.0994	3.4	34.21	4.45
4.50	377.7	27.2	4.4946	0.0999	4.2	42.04	4.54
4.60	382.3	27.5	4.5929	0.0983	4.6	46.80	4.64
4.70	386.6	27.6	4.6923	0.0994	4.3	43.26	4.74
4.80	391.0	27.6	4.7921	0.0998	4.4	44.09	4.84
4.90	395.6	27.8	4.8908	0.0987	4.6	46.61	4.94
5.00	401.0	27.9	4.9901	0.0993	5.4	54.38	5.04
5.10	407.0	28.1	5.0888	0.0987	6.0	60.79	5.14
5.20	414.0	28.2	5.1880	0.0992	7.0	70.56	5.24
5.30	422.0	28.3	5.2872	0.0992	**8.0**	**80.65**	**5.34**
5.40	429.0	28.4	5.3863	0.0991	7.0	70.65	5.44
5.50	435.0	28.6	5.4849	0.0986	6.0	60.85	5.54
5.60	440.0	28.6	5.5846	0.0997	5.0	50.15	5.64
5.70	444.0	28.5	5.6850	0.1004	4.0	39.84	5.74
5.80	448.0	28.4	5.7853	0.1003	4.0	39.88	5.84
5.90	451.0	28.3	5.8857	0.1004	3.0	29.88	5.94
6.00	455.0	38.3	5.9855	0.0998	4.0	40.08	6.04
6.10	458.0	28.4	6.0846	0.0991	3.0	30.27	6.14
6.20	461.0	28.3	6.1850	0.1004	3.0	29.88	6.24
6.30	464.0	28.2	6.2855	0.1005	3.0	29.85	6.34
6.40	466.0	28.2	6.3852	0.0997	2.0	20.06	6.44
6.50	468.0	28.1	6.4857	0.1005	2.0	19.90	6.54

6.60	470.0	28.1	6.5855	0.0998	2.0	20.04	6.69
6.80	474.0	28.3	6.7835	0.1980	4.0	20.20	-

Table 3.2.36: Titration of $HClO_4$ against Sample G_1

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml*** **2**
0.00	355.4	29.2	0.0000	0.0000	0.0	0.00	2.10
4.2	377.6	29.8	4.2032	4.2032	22.2	5.28	4.25
4.3	379.0	29.9	4.3028	0.0996	1.4	14.06	4.35
4.40	380.6	30.1	4.4019	0.0991	1.6	16.15	4.45
4.50	382.3	30.2	4.5015	0.0996	1.7	17.07	4.55
4.60	384.4	30.0	4.6025	0.1010	2.1	20.79	4.65
4.70	386.4	29.9	4.7031	0.1006	2.0	19.88	4.75
4.80	388.7	29.9	4.8032	0.1001	2.3	22.98	4.85
4.90	390.9	30.0	4.9027	0.0995	2.2	22.11	4.95
5.00	393.3	30.1	5.0022	0.0995	2.4	24.12	5.05
5.10	397.0	30.3	5.1011	0.0989	3.7	37.41	5.15
5.20	403.0	30.2	5.2017	0.1006	**6.0**	**59.64**	**5.25**
5.30	407.0	30.4	5.3006	0.0989	4.0	40.45	5.35
5.40	411.0	30.5	5.4000	0.0994	4.0	40.24	5.45
5.50	414.0	30.5	5.5000	0.1000	3.0	30.00	5.55
5.60	416.0	30.6	5.5994	0.0994	2.0	20.12	5.65
5.70	418.0	30.7	5.6988	0.0994	2.0	20.12	5.75
5.80	420.0	30.8	5.7981	0.0993	2.0	20.14	5.85
5.90	421.0	30.8	5.8981	0.1000	1.0	10.00	5.95
6.00	422.0	30.9	5.9974	0.0993	1.0	10.07	6.05
6.10	423.0	30.8	6.0980	0.1006	1.0	9.94	6.15
6.20	424.0	30.8	6.1980	0.1000	1.0	10.00	6.25
6.30	425.0	30.8	6.2979	0.0999	1.0	10.01	6.35
6.40	426.0	30.9	6.3972	0.0993	1.0	10.07	6.45

6.50	427.0	30.9	6.4971	0.0999	1.0	10.01	6.55
6.60	428.0	30.9	6.5971	0.1000	1.0	10.00	6.70
6.80	432.0	30.9	6.7970	0.1999	4.0	20.01	

Table 3.2.37: Titration of $HClO_4$ against Sample G_2

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = \frac{(V_2 + V_1)}{2}$ /*ml***
0.00	354.2	27.7	0.0000	0.0000	0.0	0.00	2.11
4.2	375.2	28.0	4.2116	4.2116	21.0	4.99	4.26
4.3	376.7	28.2	4.3109	0.0993	1.5	15.11	4.36
4.40	378.5	28.5	4.4097	0.0988	1.8	18.22	4.46
4.50	380.4	28.7	4.5089	0.0992	1.9	19.15	4.56
4.60	382.6	29.1	4.6071	0.0982	2.2	22.40	4.66
4.70	384.9	29.3	4.7062	0.0991	2.3	23.21	4.76
4.80	387.3	29.0	4.8079	0.1017	2.4	23.60	4.86
4.90	389.6	29.0	4.9081	0.1002	2.3	22.95	4.96
5.00	392.1	29.4	5.0061	0.0980	2.5	25.51	5.06
5.10	396.2	29.7	5.1045	0.0984	4.1	41.67	5.15
5.20	402.0	29.9	5.2034	0.0989	**5.8**	**58.65**	**5.25**
5.30	406.0	30.0	5.3029	0.0995	4.0	40.20	5.35
5.40	409.0	29.9	5.4036	0.1007	3.0	29.79	5.45
5.50	412.0	29.8	5.5042	0.1006	3.0	29.82	5.55
5.60	415.0	29.8	5.6043	0.1001	3.0	29.97	5.66
5.70	417.0	29.7	5.7050	0.1007	2.0	19.86	5.76
5.80	419.0	29.7	5.8051	0.1001	2.0	19.98	5.86
5.90	421.0	29.8	5.9045	0.0994	2.0	20.12	5.95
6.00	422.0	29.9	6.0040	0.0995	1.0	10.05	6.05
6.10	423.0	29.9	6.1040	0.1000	1.0	10.00	6.15
6.20	424.0	30.0	6.2034	0.0994	1.0	10.06	6.25
6.30	425.0	30.1	6.3028	0.0994	1.0	10.06	6.35

6.40	426.0	30.1	6.4028	0.1000	1.0	10.00	6.45
6.50	427.0	30.2	6.5022	0.0994	1.0	10.06	6.55
6.60	428.0	30.2	6.6022	0.1000	1.0	10.00	6.70
6.80	431.0	30.3	6.8015	0.1993	3.0	15.05	

Table 3.2.38: Titration of $HClO_4$ against Sample G_3

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = \frac{(V_2 + V_1)}{2}$/*ml***
0.00	356.7	28.1	0.0000	0.0000	0.0	0.00	2.10
4.2	380.1	28.8	4.2079	4.2079	23.4	5.56	4.26
4.3	381.4	28.9	4.3076	0.0997	1.3	13.04	4.36
4.40	382.9	28.9	4.4077	0.1001	1.5	14.99	4.46
4.50	384.4	29.1	4.5069	0.0992	1.5	15.12	4.56
4.60	386.3	29.0	4.6076	0.1007	1.9	18.88	4.66
4.70	388.3	28.7	4.7093	0.1017	2.0	19.67	4.76
4.80	390.3	29.0	4.8079	0.0986	2.0	20.28	4.86
4.90	392.4	28.8	4.9092	0.1013	2.1	20.73	4.96
5.00	394.7	28.9	5.0088	0.0996	2.3	23.09	5.06
5.10	398.0	29.0	5.1084	0.0996	3.3	33.13	5.16
5.20	404.0	29.1	5.2080	0.0996	**6.0**	**60.24**	**5.26**
5.30	408.0	29.3	5.3070	0.0990	4.0	40.40	5.36
5.40	411.0	29.4	5.4065	0.0995	3.0	30.15	5.46
5.50	414.0	29.6	5.5055	0.0990	3.0	30.30	5.56
5.60	416.0	29.7	5.6049	0.0994	2.0	20.12	5.66
5.70	418.0	29.6	5.7056	0.1007	2.0	19.86	5.76
5.80	420.0	29.6	5.8057	0.1001	2.0	19.98	5.86
5.90	421.0	29.7	5.9052	0.0995	1.0	10.05	5.96
6.00	422.0	29.8	6.0046	0.0994	1.0	10.06	6.06
6.10	423.0	29.8	6.1047	0.1001	1.0	9.99	6.16
6.20	424.0	29.8	6.2048	0.1001	1.0	9.99	6.26

6.30	425.0	29.9	6.3042	0.0994	1.0	10.06	6.36
6.40	426.0	29.8	6.4049	0.1007	1.0	9.93	6.46
6.50	427.0	29.9	6.5043	0.0994	1.0	10.06	6.55
6.60	428.0	29.9	6.6044	0.1001	1.0	9.99	6.71
6.80	431.0	29.9	6.8045	0.2001	3.0	14.99	

Table 3.2.39: Titration of $HClO_4$ against Sample H_1

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml*** **2**
0.00	351.4	25.6	0.0000	0.0000	0.0	0.00	2.11
4.2	377.9	25.6	4.2226	4.2226	26.5	6.28	4.27
4.3	379.1	25.7	4.3227	0.1001	1.2	11.99	4.37
4.40	380.5	25.8	4.4228	0.1001	1.4	13.99	4.47
4.50	382.0	25.8	4.5233	0.1005	1.5	14.93	4.57
4.60	384.5	25.7	4.6243	0.1010	2.5	24.75	4.68
4.70	386.7	25.7	4.7248	0.1005	2.2	21.89	4.78
4.80	387.6	25.6	4.8259	0.1011	0.9	8.90	4.88
4.90	388.0	25.6	4.9264	0.1005	0.4	3.98	4.98
5.00	389.4	25.4	5.0281	0.1017	1.4	13.77	5.08
5.10	390.6	25.4	5.1286	0.1005	1.2	11.94	5.18
5.20	393.0	25.4	5.2292	0.1006	2.4	23.86	5.28
5.30	394.9	26.2	5.3251	0.0959	1.9	19.81	5.38
5.40	401.0	26.2	5.4255	0.1004	**6.1**	**60.76**	**5.48**
5.50	403.0	26.2	5.5260	0.1005	2.0	19.90	5.58
5.60	405.0	26.4	5.6253	0.0993	2.0	20.14	5.67
5.70	407.0	27.0	5.7220	0.0967	2.0	20.68	5.77
5.80	408.0	27.2	5.8211	0.0991	1.0	10.09	5.87
5.90	409.0	27.5	5.9200	0.0989	1.0	10.11	5.97
6.00	410.0	27.3	6.0211	0.1011	1.0	9.89	6.07
6.10	411.0	27.2	6.1221	0.1010	1.0	9.90	6.17

6.20	412.0	27.5	6.2205	0.0984	1.0	10.16	6.27
6.30	413.0	27.6	6.3201	0.0996	1.0	10.04	6.37
6.40	414.0	27.6	6.4204	0.1003	1.0	9.97	6.47
6.50	415.0	27.4	6.5222	0.1018	1.0	9.82	6.57
6.60	416.0	27.5	6.6218	0.0996	1.0	10.04	6.72
6.80	419.0	27.6	6.8217	0.1999	3.0	15.01	-

Table 3.2.40: Titration of $HClO_4$ against Sample H_2

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml*** **2**
0.00	290.4	27.4	0.0000	0.0000	0.0	0.00	2.11
4.2	321.7	27.4	4.2143	4.2143	31.3	7.43	4.26
4.3	322.8	27.5	4.3142	0.0999	1.1	11.01	4.36
4.40	324.2	27.6	4.4140	0.0998	1.4	14.03	4.46
4.50	325.8	27.7	4.5139	0.0999	1.6	16.01	4.56
4.60	327.6	27.9	4.6132	0.0993	1.8	18.13	4.66
4.70	329.2	27.8	4.7140	0.1008	1.6	15.87	4.77
4.80	331.6	27.6	4.8153	0.1013	2.4	23.69	4.87
4.90	335.1	27.5	4.9162	0.1009	3.5	34.69	4.97
5.00	338.5	27.6	5.0160	0.0998	3.4	34.07	5.07
5.10	342.2	27.7	5.1157	0.0997	3.7	37.11	5.17
5.20	346.4	27.4	5.2177	0.1020	4.2	41.18	5.27
5.30	351.8	27.3	5.3187	0.1010	5.4	53.47	5.37
5.40	358.1	27.2	5.4196	0.1009	6.3	**62.44**	**5.47**
5.50	362.9	27.2	5.5200	0.0997	4.8	48.14	5.57
5.60	367.8	27.3	5.6197	0.0997	4.9	49.15	5.67
5.70	372.1	27.4	5.7194	0.0997	4.3	43.13	5.77
5.80	376.1	27.4	5.8198	0.1004	4.0	39.84	5.87
5.90	380.3	27.3	5.9208	0.1010	4.2	41.58	5.97
6.00	384.2	27.2	6.0218	0.1010	3.9	38.61	6.07

6.10	387.9	27.3	6.1215	0.0997	3.7	37.11	6.17
6.20	391.9	27.3	6.2218	0.1003	4.0	39.88	6.27
6.30	395.5	27.4	6.3215	0.0997	3.6	36.11	6.37
6.40	398.9	27.5	6.4211	0.0996	3.4	34.14	6.47
6.50	402.0	27.6	6.5207	0.0996	3.1	31.12	6.57
6.60	405.0	27.6	6.6211	0.1004	3.0	29.88	6.72
6.80	409.0	27.7	6.8209	0.1998	4.0	20.02	-

Table 3.2.41: Titration of $HClO_4$ against Sample H_3

Temperature of the titrant during standardization, $T_1 = 26.1^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml*** **2**
0.00	308.4	26.8	0.0000	0.000	0.0	0.00	2.10
4.2	334.5	27.0	4.1958	4.1958	26.1	6.22	4.25
4.3	336.4	27.1	4.2953	0.0995	1.9	19.10	**4.35**
4.40	338.6	27.3	4.3942	0.0989	2.2	22.25	4.44
4.50	340.7	27.5	4.4931	0.0989	2.1	21.23	4.54
4.60	342.1	27.7	4.5919	0.0988	1.4	14.17	4.64
4.70	343.4	27.7	4.6917	0.0998	1.3	13.03	4.74
4.80	345.3	27.9	4.7905	0.0988	1.9	19.23	4.84
4.90	348.1	27.8	4.8908	0.1003	2.8	27.92	4.94
5.00	352.0	27.9	4.9901	0.0993	3.9	39.27	5.04
5.10	355.6	28.1	5.0888	0.0987	3.6	36.47	5.14
5.20	360.0	28.2	5.1880	0.0992	4.4	44.36	5.24
5.30	364.7	28.4	5.2866	0.0986	4.7	47.67	5.34
5.40	370.5	28.6	5.3852	0.0986	5.8	58.82	5.44
5.50	377.4	28.6	5.4849	0.0997	**6.9**	**69.21**	**5.53**
5.60	382.6	28.9	5.5828	0.0979	5.2	53.12	5.63
5.70	386.8	28.8	5.6831	0.1003	4.2	41.87	5.73
5.80	390.9	28.8	5.7828	0.0997	4.1	41.12	5.83
5.90	394.9	28.7	5.8831	0.1003	4.0	39.88	5.93

6.00	398.4	28.7	5.9828	0.0997	3.5	35.11	6.03
6.10	402.0	28.6	6.0832	0.1004	3.6	35.86	6.13
6.20	405.0	28.5	6.1836	0.1004	3.0	29.88	6.23
6.30	408.0	28.5	6.2834	0.0998	3.0	30.06	6.33
6.40	411.0	28.2	6.3852	0.1018	3.0	29.47	6.44
6.50	413.0	28.3	6.4843	0.0991	2.0	20.18	6.53
6.60	415.0	28.3	6.5840	0.0997	2.0	20.06	6.68
6.80	418.0	28.3	6.7835	0.1995	3.0	15.04	-

Table 3.2.42: Titration of $HClO_4$ against Sample J_1

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})$/*ml*** **2**
0.00	355.6	27.5	0	0	0	0.00	2.11
4.2	390.0	27.7	4.2129	4.2129	34.4	8.17	4.26
4.3	391.2	28.0	4.3118	0.0989	1.2	12.13	4.36
4.40	392.5	28.2	4.4111	0.0993	1.3	13.09	4.46
4.50	393.7	28.6	4.5094	0.0983	1.2	12.21	4.56
4.60	395.1	28.8	4.6086	0.0992	1.4	14.11	4.66
4.70	396.5	28.8	4.7088	0.1002	1.4	13.97	4.76
4.80	398.0	29.0	4.8079	0.0991	1.5	15.14	4.86
4.90	400.0	29.1	4.9076	0.0997	2.0	20.06	4.96
5.00	402.0	29.5	5.0055	0.0979	2.0	20.43	5.06
5.10	404.0	29.6	5.1051	0.0996	2.0	20.08	5.16
5.20	407.0	29.8	5.2040	0.0989	3.0	30.33	5.25
5.30	412.0	29.9	5.3035	0.0995	5.0	50.25	5.35
5.40	419.0	29.8	5.4042	0.1007	**7.0**	**69.51**	**5.45**
5.50	424.0	30.1	5.5024	0.0982	5.0	50.92	5.55
5.60	427.0	30.2	5.6019	0.0995	3.0	30.15	5.65
5.70	429.0	30.3	5.7013	0.0994	2.0	20.12	5.75
5.80	431.0	30.4	5.8006	0.0993	2.0	20.14	5.85

5.90	432.0	30.5	5.9000	0.0994	1.0	10.06	5.95
6.00	433.0	30.4	6.0007	0.1007	1.0	9.93	6.05
6.10	434.0	30.4	6.1007	0.1000	1.0	10.00	6.15
6.20	435.0	30.5	6.2000	0.0993	1.0	10.07	6.25
6.30	436.0	30.7	6.2986	0.0986	1.0	10.14	6.35
6.40	437.0	30.8	6.3979	0.0993	1.0	10.07	6.45
6.50	438.0	30.9	6.4971	0.0992	1.0	10.08	6.66
6.60	439.0	31.0	6.5964	0.0993	1.0	10.07	6.70
6.80	442.0	31.1	6.7955	0.1991	3.0	15.07	-

Table 3.2.43: Titration of $HClO_4$ against Sample J_2

Temperature at standardization of titrant, T_1 = 30.5°C

Volume of $HClO_4$ added/*ml*	**Potential E^{o} / *mV***	**T_2 /^{o}C**	**Vc /*ml***	**ΔV/ml**	**ΔE^{o}/ *mV***	**$\Delta E^{o}/\Delta V$ *mV/ml***	**$V' = (\underline{V_2 + V_1})/ml$ 2**
0.00	343.9	28.2	0.0000	0.0000	0.0	0.0	2.11
4.2	361.4	28.5	4.2092	4.2092	17.5	4.16	4.26
4.3	362.7	28.8	4.3080	0.0988	1.3	13.16	4.36
4.40	364.2	29.4	4.4053	0.0973	1.5	15.42	4.46
4.50	366.1	29.5	4.5050	0.0997	1.9	19.1	4.56
4.60	367.5	29.6	4.6046	0.0996	1.4	14.1	4.65
4.70	368.6	29.7	4.7041	0.0995	1.1	11.1	4.75
4.80	369.8	29.7	4.8042	0.1001	1.2	12.0	4.86
4.90	371.6	29.6	4.9049	0.1007	1.8	17.9	4.96
5.00	372.9	29.5	5.0055	0.1006	1.3	12.9	5.06
5.10	375.8	29.4	5.1062	0.1007	2.9	28.80	5.16
5.20	378.8	29.4	5.2063	0.1007	3.0	29.79	5.26
5.30	382.9	29.5	5.3058	0.0995	4.1	41.21	5.36
5.40	390.1	29.5	5.4059	0.1001	7.2	**71.93**	**5.46**
5.50	393.1	29.6	5.5055	0.0996	3.0	30.12	5.56
5.6	395.8	29.8	5.6043	0.0988	2.7	27.33	5.65
5.70	397.5	29.8	5.7044	0.1001	1.7	16.98	5.75

5.80	398.8	30.0	5.8032	0.0988	1.3	13.16	5.85
5.90	399.8	30.1	5.9026	0.0994	1.0	10.06	5.95
6.00	401.0	30.2	6.0020	0.0994	1.2	12.1	6.05
6.10	402.0	30.4	6.1007	0.0987	1.0	10.13	6.15
6.20	403.0	30.5	6.2000	0.0993	1.0	10.07	6.25
6.30	404.0	30.6	6.2993	0.0993	1.0	10.07	6.35
6.40	405.0	30.7	6.3986	0.0993	1.0	10.07	6.45
6.50	406.0	30.7	6.4986	0.1000	1.0	10.00	6.55
6.60	407.0	30.7	6.5986	0.1000	1.0	10.00	6.70
6.80	410.0	30.8	6.7978	0.1992	3.0	15.06	-

Table 3.2.44: Titration of $HClO_4$ against Sample J_3

Temperature of the titrant during standardization, $T_1 = 30.5^oC$.

Volume of $HClO_4$ added/*ml*	**Potential E^o / *mV***	**T_2 /^oC**	**Vc /*ml***	**ΔV/ml**	**ΔE^o/ *mV***	**$\Delta E^o/\Delta V$ *mV/ml***	**$V' = \frac{(V_2 + V_1)}{2}$/*ml***
0.00	345.3	29.4	0.0000	0.0000	0.0	0.00	2.10
4.2	361.5	29.6	4.2042	4.2042	16.2	3.85	4.25
4.3	363.4	29.9	4.3028	0.0986	1.9	19.27	4.35
4.40	365.5	30.4	4.4005	0.0977	2.1	21.49	4.45
4.50	367.9	30.2	4.5015	0.1010	2.4	23.76	4.55
4.60	370.7	30.2	4.6015	0.1000	2.8	28.00	4.65
4.70	372.4	30.3	4.7010	0.0995	1.7	17.09	4.75
4.80	374.3	30.4	4.8005	0.0995	1.9	19.10	4.85
4.90	377.2	30.5	4.9000	0.0995	2.9	29.15	4.95
5.00	380.4	30.5	5.0000	0.1000	3.2	32.00	5.05
5.10	382.9	30.5	5.1000	0.1000	2.5	25.00	5.15
5.20	385.7	30.4	5.2006	0.1006	2.8	7.95	5.25
5.30	388.5	30.6	5.2994	0.0988	3.0	30.36	5.35
5.40	396.1	30.7	5.3988	0.0994	**7.6**	**76.46**	**5.45**
5.50	401.0	30.6	5.4994	0.1006	4.9	48.71	5.55
5.60	404.0	30.7	5.5988	0.0994	3.0	30.18	5.65

5.70	407.0	30.6	5.6994	0.1006	3.0	29.82	5.75
5.80	409.0	30.8	5.7981	0.0987	2.0	20.26	5.85
5.90	411.0	30.6	5.8994	0.1013	2.0	19.74	5.95
6.00	413.0	30.7	5.9987	0.0993	2.0	20.14	6.05
6.10	414.0	30.7	6.0987	0.1000	1.0	10.00	6.15
6.20	415.0	30.7	6.1986	0.0999	1.0	10.01	6.25
6.30	416.0	30.6	6.2993	0.1007	1.0	9.93	6.35
6.40	417.0	30.7	6.3986	0.0993	1.0	10.07	6.45
6.50	418.0	30.8	6.4979	0.0993	1.0	10.07	6.55
6.60	419.0	30.8	6.5978	0.0999	1.0	10.01	6.70
6.80	422.0	30.9	6.7970	0.1992	3.0	15.06	-

Table 3.2.45: Percentage Recovery of Glucosamine sulphate/Hydrochloride from Titration data

Product	Sample	Percentage Recovery	Average % Recovery
Glucosamine HCl Standard	S. GHCl)$_1$	100.4%	100.8%
	S. GHCl)$_2$	101.9%	
	S. GHCl)$_3$	100.2%	
Product 1 (Tablet)	A_1	102.6%	102.8%
	A_2	102.5%	
	A_3	103.4%	
Product 2 (Tablet)	B_1	102.9%	103.5%
	B_2	102.9%	
	B_3	104.6%	
Product 3 (Capsule)	C_1	99.2%	100.4%
	C_2	101.0%	
	C_3	101.0%	
Product 4 (Tablet)	D_1	84.2%	84.3%
	D_2	84.4%	
	D_3	84.2%	
Product 5 (Capsule)	E_1	102.8%	102.7%
	E_2	102.8%	
	E_3	102.6%	

Product 6 (Capsule)	F_1	99.3%	99.3%
	F_2	99.3%	
	F_3	99.2%	
Product 7 (Capsule)	G_1	98.5%	98.8%
	G_2	98.8%	
	G_3	99.0%	
Product 8 (Tablet)	H_1	103.1%	102.9%
	H_2	103.0%	
	H_3	102.6%	
Product 9 (Tablet)	J_1	102.7%	102.7%
	J_2	102.8%	
	J_3	102.6%	

3.3.0 Chromatographic (HPLC) Data

3.3.1 Weighings:

Table 3.3.1: Weight of Glucosamine Hydrochloride Standard used for analysis.

Weights	Sample $(GHCl)_1$/g	Sample $(GHCl)_2$/g
Weight of dish + sample	19.4399	19.4398
Weight of dish	19.4021	19.4020
Weight of sample.	0.0378	0.0378

Table 3.3.2: Weight of Product **1** used for analysis.

Weights	Sample A_1/g	Sample A_2/g
Weight of dish + sample	19.4215	19.4748
Weight of dish	19.3531	19.4056
Weight of sample.	0.0684	0.0692

Table 3.3.3: Weight of Product **2** used for analysis.

Weights	Sample B_1/g	Sample B_2/g
Weight of dish + sample	19.6170	19.6710
Weight of dish	19.4020	19.4559
Weight of sample.	0.2150	0.2151

Table 3.3.4: Weight of Product **3** used for analysis.

Weights	Sample C_1/g	Sample C_2/g
Weight of dish + sample	19.8134	19.8025
Weight of dish	19.4035	19.3916
Weight of sample.	0.4099	0.4109

Table 3.3.5: Weight of Product **4** used for analysis.

Weights	Sample D_1/g	Sample D_2/g
Weight of dish + sample	19.4638	19.4441
Weight of dish	19.4020	19.3821
Weight of sample.	0.0618	0.0620

Table 3.3.6: Weight of Product **5**.

Weights	Sample E_1/g	Sample E_2/g
Weight of dish + sample	19.8731	19.814
Weight of dish	19.4555	19.3962
Weight of sample.	0.4176	0.4178

Table 3.3.7: Weight of Product **6** used for analysis.

Weights	Sample F_1/g	Sample F_2/g
Weight of dish + sample	19.6256	19.5591
Weight of dish	19.4598	19.3935
Weight of sample.	0.1658	0.1656

Table 3.3.8: Weight of Product **7** used for analysis.

Weights	Sample G_1/g	Sample G_2/g
Weight of dish + sample	19.4839	19.5689
Weight of dish	19.4012	19.4864
Weight of sample.	0.0827	0.0825

Table 3.3.9: Weight of Product **8** used for analysis.

Weights	Sample H_1/g	Sample H_2/g

Weight of dish + sample	19.5697	19.6642
Weight of dish	19.3550	19.4499
Weight of sample.	0.2147	0.2143

Table 3.3.10: Weight of Product **9** used for analysis.

Weights	Sample J_1/g	Sample J_2/g
Weight of dish + sample	19.6104	19.6725
Weight of dish	19.3935	19.4555
Weight of sample.	0.2169	0.2170

Table 3.3.11: Calculation of Sum of Peak Areas

Product	**Sample**	**Average Area Peak 1**	**Average Area Peak 2**	**Average Sum**
G.HCl Standard		1.505	0.075	1.580
Product 1 (Tablet)	A_1	2.1000	0.1108	2.2108
	A_2	2.1500	0.1200	2.2700
Product 2 (Tablet)	B_1	2.0400	0.1333	2.1733
	B_2	2.0600	0.1200	2.1800
Product 3 (Capsule)	C_1	1.9850	0.1400	2.1250
	C_2	1.9925	0.1400	2.1325
Product 4 (Tablet)	D_1	1.6769	0.1200	1.7969
	D_2	1.7984	0.1200	1.7984
Product 5 (Capsule)	E_1	2.0300	0.1367	2.1667
	E_2	2.0600	0.1400	2.2000

Product 6 (Capsule)	F_1	1.9950	0.1225	2.1175
	F_2	1.7817	0.1200	2.1150
Product 7 (Capsule)	G_1	1.9833	0.1050	2.0883
	G_2	2.0000	0.1017	2.1017
Product 8 (Tablet)	H_1	2.0600	0.1333	2.2033
	H_2	2.0517	0.1400	2.1917
Product 9 (Tablet)	J_1	2.0417	0.1333	2.175
	J_2	2.0450	0.1200	2.1650

Table 3.3.12: Percentage Recovery of Glucosamine sulphate from HPLC Data

Percentage glucosamine free base in the nine products analysed.

Product	Sample	Percentage Recovery	Average % Recovery
Product 1 (Tablet)	A_1	103.7%	104.4%
	A_2	105.2%	
Product 2 (Tablet)	B_1	102.1%	102.2%
	B_2	102.3%	
Product 3 (Capsule)	C_1	99.8%	99.9%
	C_2	99.9%	
Product 4 (Tablet)	D_1	84.0%	83.9%
	D_2	83.8%	
Product 5 (Capsule)	E_1	101.5%	102.3%
	E_2	103.0%	

Product 6 (Capsule)	F_1	97.4%	97.4%
	F_2	97.4%	
Product 7 (Capsule)	G_1	97.7%	98.2%
	G_2	98.7%	
Product 8 (Tablet)	H_1	103.2%	103.1%
	H_2	102.9%	
Product 9 (Tablet)	J_1	102.1%	101.9%
	J_2	101.6%	

Table 3.3.13: Calculation of Sum of Peak Areas from Dissolution Test

Product	**Sample**	**Average Area**	**Time/ minutes**			
			15	30	45	60
Product 1 (Tablet)	A_1	Peak 1	1.5467	1.7200	1.8000	1.8400
		Peak 2	0.0600	0.0767	0.0800	0.0600
		Sum	**1.6067**	**1.7967**	**1.8800**	**1.9000**
	A_2	Peak 1	1.7467	1.7667	1.780	1.9517
		Peak 2	0.0333	0.0567	0.065	0.1100
		Sum	**1.7800**	**1.8234**	**1.845**	**2.0617**
Product 2 (Tablet)	B_1	Peak 1	0.370	0.4333	0.9000	1.0600
		Peak 2	0.025	0.0217	0.0467	0.0400
		Sum	**0.395**	**0.4550**	**0.9467**	**1.1000**
	B_2	Peak 1	0.3750	0.4200	0.8200	1.1133
		Peak 2	0.0250	0.0250	0.0400	0.0400
		Sum	**0.4000**	**0.4450**	**0.8600**	**1.1533**
		Peak 1	NIL	NIL	NIL	NIL

Product 3 (Capsule)	C_1	Peak 2	NIL	NIL	NIL	NIL
		Sum	NIL	NIL	NIL	NIL
	C_2	Peak 1	NIL	NIL	NIL	NIL
		Peak 2	NIL	NIL	NIL	NIL
		Sum	NIL	NIL	NIL	NIL
Product 4 (Tablet)	D_1	Peak 1	0.4950	0.5867	0.7400	0.8820
		Peak 2	0.0250	0.0250	0.0400	0.040
		Sum	**0.5200**	**0.6117**	**0.7800**	**0.9220**
	D_2	Peak 1	0.5070	0.6000	0.7200	0.884
		Peak 2	0.0250	0.0250	0.0400	0.040
		Sum	**0.5320**	**0.6250**	**0.7600**	**0.924**
Product 5 (Capsule)	E_1	Peak 1	NIL	NIL	NIL	NIL
		Peak 2	NIL	NIL	NIL	NIL
		Sum	NIL	NIL	NIL	NIL

Continuation of Table 3.3.13

	Sample	**Average Area**	**Time/minutes**			
			15	**30**	**45**	**60**
Product 5	E_2	Peak 1	NIL	NIL	NIL	NIL
		Peak 2	NIL	NIL	NIL	NIL
		Sum	NIL	NIL	NIL	NIL
Product 6 (Capsule)	F_1	Peak 1	NIL	NIL	NIL	NIL
		Peak 2	NIL	NIL	NIL	NIL
		Sum	NIL	NIL	NIL	NIL
	F_2	Peak 1	NIL	NIL	NIL	NIL
		Peak 2	NIL	NIL	NIL	NIL
		Sum	NIL	NIL	NIL	NIL
Product 7 (Capsule)	G_1	Peak 1	0.3000	0.4150	0.4950	0.6333
		Peak 2	0.0300	0.0450	0.0450	0.0300
		Sum	**0.3300**	**0.460**	**0.540**	**0.6633**
		Peak 1	0.3733	0.4500	0.5950	0.6600

	G_2	Peak 2	0.0300	0.0450	0.0450	0.0300
		Sum	**0.4033**	**0.4950**	**0.6400**	**0.6900**
Product 8 (Tablet)	H_1	Peak 1	0.3600	0.4600	0.9400	1.1200
		Peak 2	0.0250	0.0200	0.0500	0.0400
		Sum	**0.3850**	**0.4800**	**0.9900**	**1.1600**
	H_2	Peak 1	0.3867	0.5900	1.0600	1.1800
		Peak 2	0.0250	0.0200	0.0500	0.0400
		Sum	**0.4117**	**0.6100**	**1.1100**	**1.2200**
Product 9 (Tablet)	J_1	Peak 1	0.3900	0.5900	1.0717	1.1800
		Peak 2	0.0250	0.0250	0.0550	0.0590
		Sum	**0.415**	**0.6150**	**1.1267**	**1.2390**
	J_2	Peak 1	0.5550	0.6700	1.0850	1.1800
		Peak 2	0.0250	0.0350	0.0550	0.0550
		Sum	**0.5800**	**0.7050**	**1.1400**	**1.2350**

Table 3.3.14. Percentage Drug Released from Dissolution Test

Product	**Sample**	Percentage Drug Released			
		15min	**30min**	**45min**	**60min**
Product 1	A_1	79.84%	89.28%	93.42%	94.41%
	A_2	88.45%	90.61%	91.68%	102.45%
		84.2%*	90.0%*	92.6%*	98.4*
Product 2	B_1	49.44%	52.42%	76.86%	84.47%
	B_2	49.69%	51.93%	72.55%	87.12%
		49.6%*	52.2%*	74.7%*	85.8%*
Product 3	C_1	0%	0%	0%	0%
	C_2	0%	0%	0%	0%
Product 4	D_1	51.68%	60.79%	77.52%	91.63%
	D_2	52.87%	62.11%	75.53%	91.83%
		52.3%*	61.5%*	76.5%*	91.7%*

Product 5	E_1	0%	0%	0%	0%
	E_2	0%	0%	0%	0%
Product 6	F_1	0%	0%	0%	0%
	F_2	0%	0%	0%	0%
Product 7	G_1	32.80%	45.72%	53.67%	65.92%
	G_2	40.08%	49.19%	63.60%	68.57%
		36.4%*	47.5%*	58.6%*	67.3%*
Product 8	H_1	48.94%	53.67%	79.01%	87.45%
	H_2	50.27%	60.12%	84.97%	90.44%
		49.6%*	56.9%*	82.0%*	89.0%*
Product 9	J_1	50.44%	60.37%	85.80%	91.38%
	J_2	58.63%	64.85%	86.46%	91.18%
		54.5%*	62.6%*	86.1%*	91.3%*

* = Average percentage recoveries.

CHAPTER FOUR

4.0.0 DISCUSSIONS, CONCLUSIONS AND RECOMMENDATIONS

4.1.0 DISCUSSIONS

A total of nine products containing glucosamine sulphate, and other supplements such as chondroitin sulphate, omega -3 and cod liver oil were analysed using potentiometric titration and HPLC- UV methods.

Non-Aqueous Titration

Preliminary studies carried out before titration revealed that the salts of glucosamine, the hydrochloride and sulphate, were insoluble most organic solvents including glacial acetic acid but soluble in formic acid. However, formic acid was not available. Since it was possible to displace both the chloride and sulphate, glacial acetic acid in combination with mercury (II) acetate solution was used to dissolve the salt. From the titration results, the average percentage recovery are 100.8, 102.8, 103.5, 100.4, 84.3, 102.7, 99.3, 98.8, 102.9, and 102.7% with respect to the glucosamine hydrochloride standard, products **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9**.

Chromatography

Glucosamine lacks UV chromophore. It suffers low sensitivity from UV detection at 195nm. So, to improve its detectability, the amino functional group was derivatized using phenyl isothiocyanate under alkaline conditions. The purpose of the alkaline conditions is to release the glucosamine free base by displacing the sulphate or the chloride of the salt. This method does not apply to covalently bonded sulphate such as chondroitin sulphate. The reaction is very sensitive to water, so a mixture of methanol and distilled water of ratio 3 : 2 was found to be an excellent solvent for glucosamine standard compound and the test sample. There were slight modifications of the method. Methanol: water: phosphoric acid of mobile phase composition 12 : 88 : 0.1 was used, instead of acetonitrile: water: phosphoric acid of composition 10 : 90 : 0.1, from the literature. The relative retention times between the glucosamine anomer peak 1 and peak 2 was greater than 1.3. Some of the derivatized samples (the standard, product 1, 2, 3, 7 and 9) were stored in HPLC vials for two weeks at ambient temperature, and later analysed. The results were reproducible, showing good stability of the phenylthiourea derivatives.

All the nine products analysed contained glucosamine sulphate as the active ingredients but glucosamine sulphate standard was not available, so they were analysed using glucosamine hydrochloride standard. For this reason, corresponding mass of glucosamine hydrochloride standard weighed was established by calculation.

With regard to the HPLC, the average percentage recoveries for products **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9** are 104.4, 102.2, 99.9, 83.9, 102.3, 97.4, 98.2, 103.1 and 101.9% respectively. Only product **4** failed test. Probably this might be due to formulation errors or the manufacturer is taken an advantage of lack of information on the drug in the BP and USP. By the USP standards, the range usually specified for pharmaceutical dosage forms are within 90 - 110%. As shown in Table 3.2.45 and Table 3.3.12, the amount of product **4** found after analysis by both methods was less than 90%.

The mobile phase(methanol: water: phosphoric acid of composition 12 : 88 : 0.1) worked well for the phenylthiourea derivative, but not for the free glucosamine hydrochloride standard during TLC analysis, shown in appendix 3.0. The R_f values of phenylthiourea derivatives glucosamine anomer spot 1 and spot 2 are respectively 0.19 and 0.30.

Dissolution Test

Dissolution test was carried out on Glucosamine sulphate tablets, caplets and capsules to determine the dissolution rate of the active ingredients. From the results, all the tablets(products **2**, **4**, **8** and **9**) and caplet(product **1**) passed the dissolution test. In other words, the rate at which

the active ingredients were released into solution was appreciable. The average percentage release of the active ingredients for product **1** at 15min, 30min, 45min, and 60min is 84.2, 90.0, 92.6, and 98.4% respectively. With regard to product **2**, the average percentage release of the active ingredients is 49.6, 52.2, 74.7 and 85.8% respectively. For product **4**, the average percentage release of the active ingredients is 52.3, 61.5, 76.5 and 91.7% respectively. For product **7**, the average percentage release of the active ingredients is 36.4, 47.5, 58.6 and 67.3% respectively. For product **8**, the average percentage release of the active ingredients is 49.6, 56.9, 82.0 and 89.0% respectively. For product **9**, the average percentage release of the active ingredients is 54.5, 62.6, 86.1 and 91.3% respectively. Of the capsules, only product **7** passed the test. With the exception of **7**, nothing was detected by the method for product **3**, **5** and **6** even at the end of 60 minutes. Products **3**, **5** and **6** contained oil supplements in the shell. The observation made was that, the active ingredients were trapped in the oil after all the shell had separated from the oil. Product **7** had no oil supplements in the shell and so, the active ingredients were free after the shell had separated from the active ingredients.

Weight Uniformity

20 Glucosamine sulphate tablets, capsules, and caplets were selected at random individually and their weights measured and recorded. The average weights of the 20 tablets, capsules, and caplets were also measured and recorded. The average weights of products **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9** are 1.7076, 0.5375, 1.0247, 0.7691, 1.0416, 1.0149, 1.0297, 0.5356 and 0.5421g respectively.

According to the British Pharmacopoeia(BP) standards, if 20 tablets or caplets are used in the determination of weight uniformity, not more than two tablets or caplets should deviate from the average weight by more than ± 5%, if the tablets or caplets are 250mg or more. From the data, none of tablets and caplet used deviated from the average weight by more than ± 5%. Hence on the basis of weight uniformity test, the Glucosamine sulphate tablets and caplet of 250mg or more used in the experiment conform to the pharmacopoeia standards and thus have passed the test.

Also, by the BP standards, if 20 capsules are used in the determination of weight uniformity, not more than two capsules should deviate from the average weight by more than ± 10%, if the capsules are less than 300mg. In addition, not more than two capsules should deviate from the average weight by more than ±7.5% if the capsules are more than or equal to 300mg. From data, all the capsules passed the weight uniformity test except product **7**. Out of the 20 Glucosamine sulphate capsules used, three deviated from the average weight by the following percentages

-8.8569%, -8.1965% and 8.7016%. So they deviated by more than ±7.5% from the average weight.

4.2.0 CONCLUSION

Both the HPLC and non-aqueous titration methods gave reproducible results. Although glucosamine is not yet accepted as a prescription drug in the United States and United Kingdom, most of the products assayed conform to pharmacopoeia standards. The average percentage recoveries from the titration method are 100.8, 102.8, 103.5, 100.4, 84.3, 102.7, 99.3, 98.8, 102.9, and 102.7% with respect to the glucosamine hydrochloride standard, products **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9**. The average percentage recoveries from the HPLC-UV method, for products **1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, and **9** are 104.4, 102.2, 99.9, 83.9, 102.3, 97.4, 98.2, 103.1 and 101.9% respectively.

In terms of percentage recovery, only one product (product **4**) failed to meet pharmacopoeia requirements.

4.3.0 RECOMMENDATION.

Based on the results of the analysis it is recommended that since the non-aqueous titrimetric method is cheaper than the HPLC method, the former should be utilized in routine analysis of glucosamine salts.

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APPENDICES.

APPENDIX I: CALCULATIONS FROM TITRATION DATA

Calculating Corrected Volume

$Vc = V(1 + 0.0011(T_1 - T_2)$

Where Vc = Corrected volume;

V = Measured volume;

T_1 = temperature of the titrant during standardization;

T_2 = temperature of the titrant at the time of the assay.

From Table 3.2.15: $(SG.HCl)_1$

$T_1 = 27.3^oC$; $T_2 = 27.1^oC$; V = 4.20ml

$Vc = 4.20(1 + 0.0011(27.3^oC - 27.1^oC)$

= **4.2009ml**

Calculating the Factor for Perchloric Acid $HClO_4$.

Factor for KHP = Actual mass / Nominal mass.

$= \frac{0.5024}{0.5000} = \mathbf{1.0048}.$

From the miliequivalent calculation,

204.22g of KHP in 1000ml of solution ≡1M

5.1055g of KHP in 25ml of solution ≡1M

⇒ 5.1055g of KHP in 25ml of solution ≡1M

∴ 0.5024g of KHP in 25ml of solution = $\frac{1M \times 0.5024}{5.1055g}$ = 0.0984M

Now using $f_1V_1C_1 = f_2V_2C_2$.

Where f_2 = factor for $HClO_4$. f_1 = factor for KHP = 1.0048

V_2 = Titre value = 24.30ml. V_1 = Volume of KHP = 25ml.

C_2 = Conc. of $HClO_4$ = 0.1M C_1 = Conc. of KHP = 0.0984M

$f_2 = \frac{f_1V_1C_1}{V_2C_2}$

$f_2 = \frac{1.0048 \times 25ml \times 0.0984M}{24.30 \times 0.1M.} = \mathbf{1.0172}.$

Calculating the Percentage of Glucosamine Hydrochloride in the G.HCl Standard.

For $(SG.HCl)_1$.

Actual volume of $HClO_4$ used = *factor for $HClO_4$* x *titre value*

= 1.0172 x 11.63ml

= 11.83 ml

But, from the miliequivalent calculation,

1ml of 0.1M $HClO_4$ ≡ 0.021563g of glucosamine hydrochloride (SG.HCl).

∴ 11.83 ml of 0.1M $HClO_4$ = $\frac{0.021563g \times 11.83}{1ml}$ **= 0.2551g**

Percentage glucosamine hydrochloride in $(SG.HCl)_1$ = $\frac{0.2551g}{0.2541g} \times 100$ = **100.4%**

The rest were calculated in the same way

Calculating the Percentage of Glucosamine Sulphate

For Product 1 (Sample A_1).

Actual volume of $HClO_4$ used = *factor for $HClO_4$* x *titre value*

= 1.0172 x 5.53ml = 5.6251 ml

But, from the miliequivalent calculation,

1ml of 0.1M $HClO_4 \equiv$ 0.0456418g of glucosamine sulphate ($G.SO_4$).

$\therefore$ 5.6251 ml of 0.1M $HClO_4$ = $\frac{0.0456418g \times 5.6251ml}{1ml}$ = **0.2567g**

Average weight of Sample A = 1.7076g.

Mass of sample A_1 weighed = 0.4283g

If 1.7076g drug contains 1000mg (or 1g) $G.SO_4$

$\therefore$ 0.4283g drug will contain = $\frac{1g \times 0.4273g}{1.7076g}$ = **0.2502g**

Percentage $G.SO_4$ in the sample = $\frac{\textit{Experimental value in grams}}{\textit{Theoretical value in grams}} \times 100$

Percentage $G.SO_4$ in the sample A_1 = $\frac{0.2567g}{0.2502g} \times 100$ = **102.6%**

APPENDIX II: CALCULATIONS FROM CHROMATOGRAPHIC DATA

Calculating Amount of Glucosamine Free Base in the Glucosamine Sulphate Drug.

Amount of glucosamine(in mg), as free base, is calculate by:

Mass (mg) = $\frac{A_u \times C \times 50ml \times \text{dilution factor} \times 0.78511}{A_s}$

Calculating Percentage Label Amount of Glucosamine Sulphate Drug.

% Glucosamine Sulphate Drug = $\frac{A_u \times C \times 50ml \times \text{dilution factor} \times 0.78511 \times Y}{A_s \times W \times \text{mg one Tab(Cap).}} \times 100\%$

Where:

A_u = Sum of peak area for peak 1 and peak 2 in the sample preparation or the unknown.

A_s = Sum of peak area for peak 1 and peak 2 in the standard preparation.

Y is average weight of tablet or capsule; **W** is the sample weight (mg).

C is the concentration of glucosamine free base in the working standard preparation. For example, if using glucosamine HCl, multiply the standard concentration (mg/ml) by (179.2/215.63) or 0.8330; for glucosamine sulphate, multiply the standard concentration (mg/ml) by (2(179.2/456.418) or 0.78511

Calculating the Concentration of Analyte (or Unknown) Injected

37.8mg of GHCl Standard in 50ml MeOH/H_2O

Concentration (mg/ml) = $\frac{37.8mg}{50ml}$ = 0.756.

Calculating Dilution Factor

Dilution

First dilution: 5ml to 50ml.

Dilution factor = 50/5 = 10.

Second dilution: 0.7ml to 10ml.

Dilution factor = 10/0.7 = 14.2857

Total dilution factor = 10 x 14.2857= 142.857

Concentration of Analyte Injected = $\frac{0.756mg/ml}{142.857}$ = 0.005292mg/ml (or 5.292µg/ml)

For Product 1 (Sample A_1)

Sum of Area = 2.21083

% of GSO_4 = $\frac{2.21083 \times 5.292 \times 10^{-3} mg/ml \times 50ml \times 142.857 \times 0.785110 \times 1707.565mg}{1.580 \times 68.4mg \times 1000mg} \times 100$

= **103.7%**

Calculating Percentage Glucosamine Sulphate Drug Released from the Dissolution Test.

Concentration of Drug(mg/ml) = $\frac{A_u \times C \times \text{dilution factor} \times 0.78511}{A_s}$

Calculating Concentration Injected

Concentration of sample **A** in 900ml

$C_i = \frac{1000mg}{900ml}$ = 1.1111mg/ml

Dilution

First dilution: 0.5ml to 10ml.

Dilution factor = 10/0.5 = 20.

Second dilution: 1ml to 10ml.

Dilution factor = 10/1 = 10

Total dilution factor = 20 x 10 = 200.

Dilution factor (D.f) = $\frac{Ci}{C_f}$; => $C_f = C_i/D.f$

Where C_i and C_f denote initial and final concentrations respectively.

Concentration injected, $C_f = \frac{1.1111}{200}$ = 0.0055555mg/ml

For Sample A_1

Time /min	Sum of Peak Areas
15	1.6067

C = 5.5555 x 10^{-3}mg/ml = 5.5555 x 10^{-6}g = 5.5555µg/ml

Mass injected per ml = 5.5555 x 10^{-3} mg = 5.5555 x 10^{-6}g = 5.5555µg

Conc. = $\frac{1.6067 \times 5.5555 \times 10^{-3} \text{ mg/ml} \times 200 \times 0.785110}{1.580}$ = 0.887077mg/ml

If 1ml of solution contains = 0.887077mg glucosamine.

∴900ml will contain = $\frac{0.887077\text{mg} \times 900\text{ml}}{1\text{ml}}$ = 798.3693mg

Percentage Drug Released = $\frac{\text{Assayed amount(mg)}}{\text{Labelled amount(mg)}} \times 100$

= $\frac{798.3693\text{mg} \times 100}{1000\text{mg.}}$ = **79.84%**

Calculating Retardation Factor of Phenylthiourea Derivative.

Distance moved by mobile phase = 8.80cm

Distance moved by peak 1 = 1.70cm

Distance moved by peak 2 = 2.60cm

Distance moved by blank = 1.70cm

R_f value of peak 1 = $\frac{\text{Distance moved by solute}}{\text{Distance moved by solvent.}}$

R_f value of peak 1 = $\frac{1.70}{8.80}$ = 0.1932.

R_f value of peak 2 = $\frac{2.60}{8.80}$ = 0.2955.

R_f value of blank = $\frac{1.70}{8.80}$ = 0.1932.

Calculating Amount of Glucosamine Hydrochloride Standard Weighed for Analysis

M_r (GHCl) = 215.63.

M_r ($G(SO_4)$) = 456.418.

M_r (GFB) = 179.17.

1g of GHCl releases 0.83091g glucosamine free base(GFB)

i.e. $\frac{179.17}{215.63}$ = **0.83091g**

1g of $G(SO_4)$ releases 0.78511g glucosamine free base(GFB).

i.e. $\frac{2(179.17)}{456.418}$ = **0.78511g**

Ratio of $(GFB)_{GHCl}$ to $(GFB)_{(G(SO4),}$ i.e. $\frac{(GFB)_{GHCl}}{(GFB)_{(G(SO4)}} = \frac{0.83091g}{0.78511g} = 1.0583.$

Amount of GHCl Standard Weighed = $\frac{0.04g}{1.0583.}$ = **0.0378g or 37.8mg.**

APPENDIX III: TLC CHROMATOGRAMS OF PHENYLTHIOUREA DERIVATIVES

1.0 Chromatograms of glucosamine products 1, 2, 3, 5; labeled **A, B, C** and **E** respectively; glucosamine hydrochloride standard **(**labeled **P),** and blank sample(labelled BLK).

Mobile phase:- Methanol: Water: Phosphoric acid(12 : 88 : 0.1))

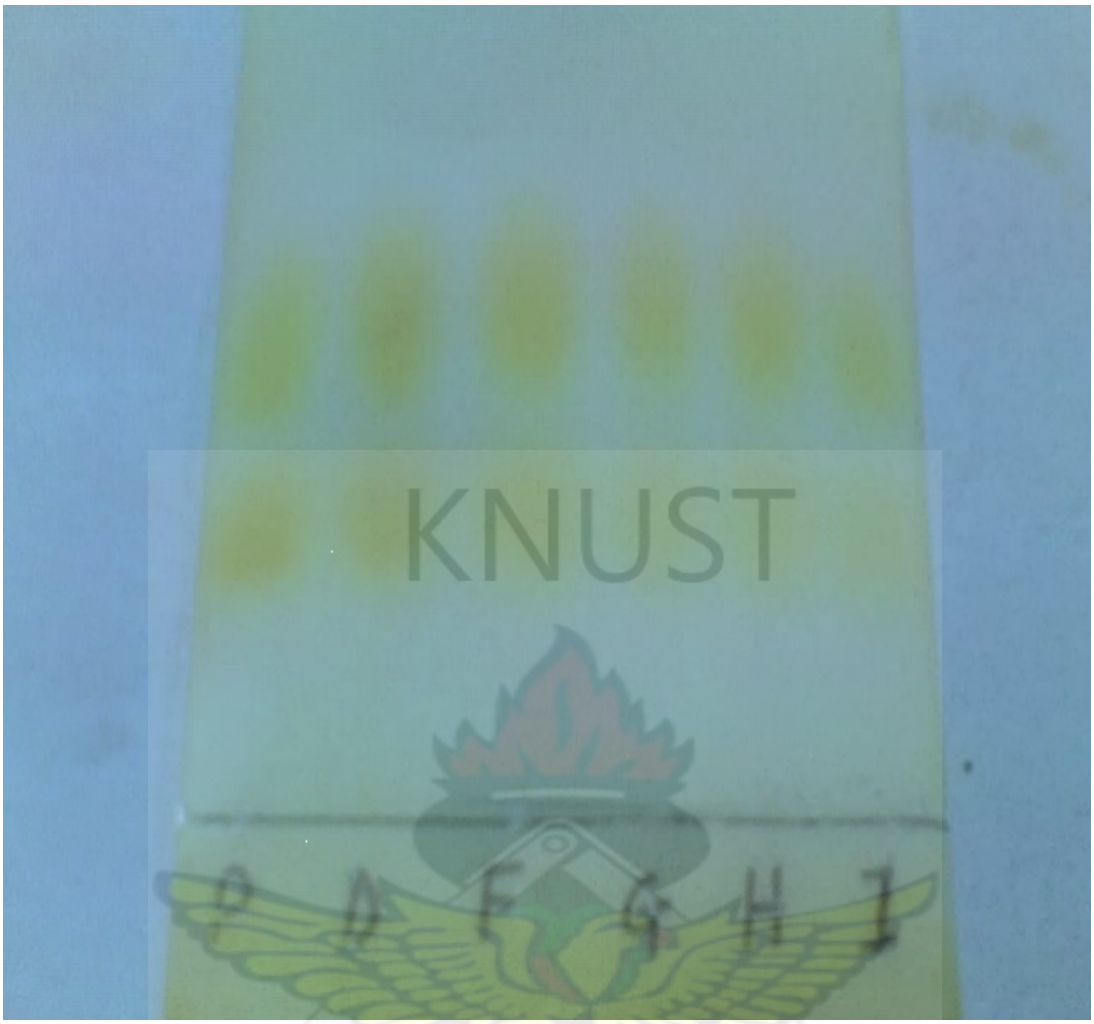
Stationary phase: Silical gel (Precoated plastic plate; size: 5cm x 20cm)

Rf value: Phenylthiourea derivatives:

Anomer 1 = 0.19

Anomer 2 = 0.30.

2.0. Chromatogram of glucosamine products 4, 6, 7, 8, 9; labeled **D, F, G** and **H, J** respectively; glucosamine hydrochloride standard **(** labeled **P)**.



Mobile phase:- Methanol: Water: Phosphoric acid(12 : 88 : 0.1))

Stationary phase: Silical gel (Precoated plastic plate; size: 5cm x 20cm))

Rf value: Phenylthiourea derivatives:

Anomer 1 = 0.19

Anomer 2 = 0.30.

3.0 Chromatograms of Derivatized **(**labeled **P),** Free Glucosamine Hydrochloride Standard **(**labeled **Q)**

Mobile phase:- Methanol: Water: Phosphoric acid(12 : 88 : 0.1))

Stationary phase: Silical gel (Precoated plastic plate; size: 5cm x 20cm)

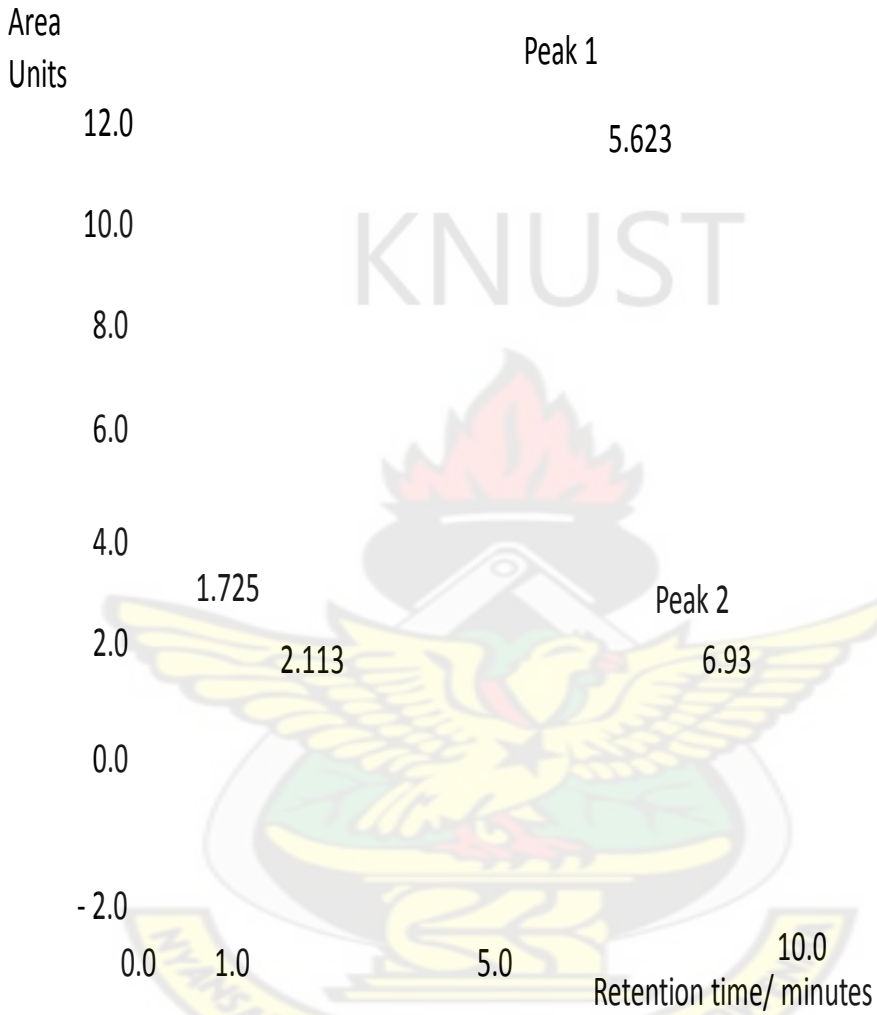
Rf value: Phenylthiourea derivatives:

Anomer 1 = 0.19

Anomer 2 = 0.30.

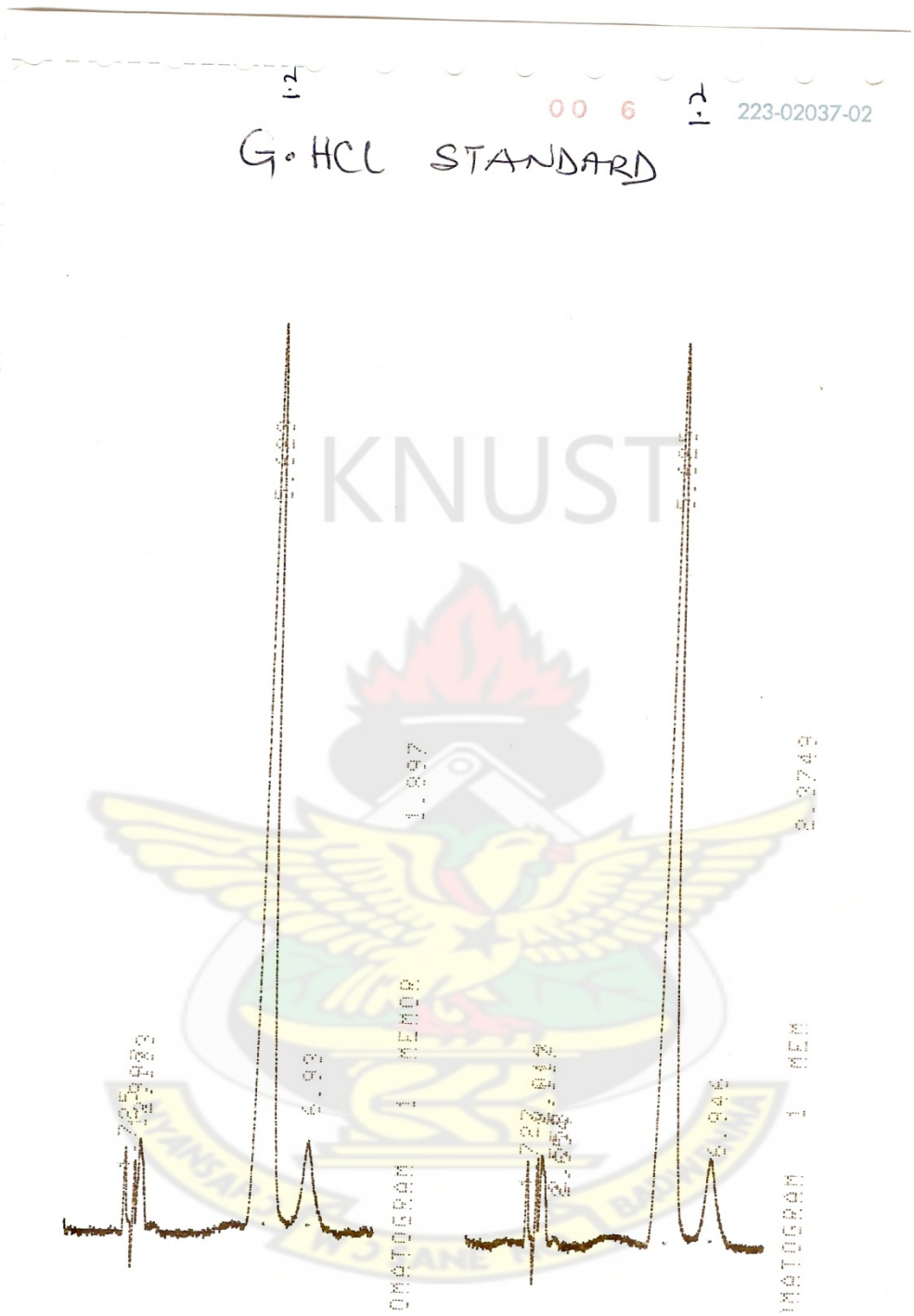
APPENDIX IV: HPLC CHROMATOGRAM OF PHENYLTHIOUREA DERIVATIVES

1.0 Sketch of HPLC Chromatogram, showing glucosamine anomer peaks 1 and 2.



2.0. Chromatogram of Blank (All reagents without glucosamine hydrochloride)

3.0. Chromatogram of Glucosamine Hydrochloride Standard.



4.0. Chromatogram of Glucosamine Sulphate Drug

0.8

00 4 0.8ml 223-02037-02

GS04 SAMPLE