COMPARATIVE EVALUATION OF THE ASCORBIC ACID CONTENT OF MINERAL ASCORBATE AND ASCORBIC ACID TABLETS MARKETED IN ZARIA

BY

CHUKWU SYLVIA DORIS

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NIGERIA

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DECLARATION

I declare that the work in this thesis entitiled '**COMPARATIVE EVALUATION OF THE ASCORBIC ACID CONTENT OF MINERAL ASCORBATE AND ASCORBIC ACID TABLETS MARKETED IN ZARIA** has been performed by me in the department of pharmaceutical and Medicinal chemistry. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No partof this thesis was previously presented for another degree or diploma at any university.

Chukwu Sylvia Doris……. Signature. …… Date…….

Name of Student

CERTIFICATION

This thesis entitled 'COMPARATIVE EVALUATION OF THE ASCORBIC ACID CONTENT OF MINERAL ASCORBATE AND BRANDS OF ASCORBIC ACID TABLETS MARKETED IN ZARIA' meets the regulations governing the award of Masters Degree in Pharmaceutical and medicinal Chemistry of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

Dr M.A. Usman	_____	_____
Chiarman, Supervisory Committee	Signature	Date.
Dr. Aminu Musa	_____	_____
Member, Supervisory Committee	Signature	Date.
Dr. Aliyu Musa	_____	_____
Head of Department	Signature	Date.
Prof Hassan Zoaka	_____	_____
Dean Schoool of Postgraduate studies	Signature	Date.

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ABSTRACT.

The ascorbic acid content of mineral ascorbate and six brands of ascorbic acid tablets marketed in Zaria with brand names Calcium ascorbate, Ascorbion, Emvit C, Ascorvite, Topcee, Bioracee and Sofa C respectively were comparatively evaluated using four methods. These include titrimetric method of British pharmacopoiea 2009, sodium thiosulphate titrimetric method, Ultra violet spectrophotometric method employing stabilizers and a newly developed 2,4-dinitrophenyl hydrazine method. The developed method was validated and the analyte showed an absorption maxima at 527nm and a percentage recovery of 97.4%. Beer's law was obeyed in the concentration range of 5ug to 25ug/ml, giving a regression equation of y= 0.041x + 0.032 and a correlation coefficient of 0.9910. The percentage content of ascorbic acid in Calcium ascorbate, Ascorbion, EmvitC, Ascorvite, Topcee, Bioracee, and SofaC in the Ultra violet spectroscopy method using stabilizers were 108.2%, 29.7%, 26.3%, 33%, 66.7%, 46.7%, 27.948% respectively. The values obtained from the sodium thiosulphate titrimetric method were 95.6%, 98.30%, 101.90%, 94.86%, 94.18%, 98.89% and 100.02% respectively. The results obtained from the B.P 2009 titrimetric method were 100.58%, 102.49%, 108.51%, 100.12%, 79.29%, 102.05% and 104.84% respectively, while that of the modified 2,4-dinitrophenyl hydrazine method were 100.47%, 101.99%, 107.898%, 99.83%%, 78.02%, 101.97%, 104.96% respectively. These results obtained showed that the UV spectrophotometric method gave values below the normal range. The values from the sodium thiosulphate titrimetric method was within the normal range but does not compare favourably with the official method, whereas the values for the modified 2,4 dinitrophenylhydrazine method and that of the B.P 2009 titrimetric method were both within the normal range and compared favourably with each other. Both results subjected to Student's T-testing showed no significant difference at P value 95% confidence interval (p=0.05). The B.P 2009 titrimetric method showed that there is one substandard product, which is Sofa C on the basis that it has a percent label claim not within 90±5%. The findings of this work further showed that the newly modified method can be used as a substitute for the official method.

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CHAPTER ONE

INTRODUCTION

1.1 ASCORBIC ACID

Vitamin C otherwise known as ascorbic acid is a water soluble vitamin that is naturally present in some foods, added to others and taken as a dietary supplement. Though it is derived from glucose, humans and some other primates like guinea pigs, teleost, fishes, bats and birds cannot synthesize it invivo due to the genetic mutation that prevents them from synthesizing L-gluconolactone oxidase enzyme, which converts glucose to ascorbic acid. Vitamin C can be synthesized by some other animals like dogs, goats, sheep, etc. (Storn, 1972).

Vitamin C is an important component of a healthy diet. Its history revolves around that of the human scurvy disease; a severe form of vitamin C deficiency, probably the first human sickness to be recognized as a deficiency disease. Its symptoms includes exhaustion, massive haemorrhaging of flesh and gums, general weakness and diarrhoea. Presently, it is rare to be seriously deficient of vitamin C, although evidence suggests that many people may have low levels of Vitamin C. Smoking cigarettes lowers the amount of vitamin C in the body. Smokers are therefore at a higher risk of its deficiency. (retrievded from national institute of health, United States). Vitamin C is needed for the growth and repair of tissues in all parts of the body. It helps the body make collagen; an important protein used to make skin, cartilage, tendons, ligaments and blood vessels. It is also needed for healing of wounds and maintenance of bones and teeth.(Briggs, 1981).

In recent years, the determination of ascorbic acid has become an important subject. This is because of the important role it plays in maintaining human health. (Chen and Sato, 1995).

One of the most intensely studied areas of vitamin C benefits is in the area of cardiovascular health. Researchers are finding that vitamin C impacts several aspects of cardiac health, ranging from blood pressure to endothelia health. As the relationship between oxidative damage, inflammation and atherosclerosis becomes increasingly investigated by science, vitamin C is seen as a key protective element against the aspects of cardiovascular disease. (Forther et al, 2000).

Laboratory studies have confirmed that high dosing with vitamin C is cytotoxic to wide range of cancer cell lines and that it boots the anti-cancer activities of several common chemotherapy drugs. In animal models (rats), intra-venous vitamin C has been shown to significantly decrease growth rates of liver, ovarian, pancreatic and globlastoma tumors with dosages easily achievable in humans.(Riordan et al, 2005).

Vitamin C has also been shown to have an effect against the HIV virus. A study carried out by Linus Pauling in 1990 clearly showed that the vitamin is very effective in killing HIV virus by inhibiting its replication invitro. (Jeffery, 1995). Also in a report published by Harakeh and Jariwalla in December, 1991, calcium ascorbate and two thiol based reducing agents (glutathione and N-acetyl-L-cysteine) has the ability of reducing extracellular HIV reverse transcriptase an vitamin C does. Mega dosing with vitamin C is known to be able to strengthen the immune system, reduce anxiety and oxidative stress, it is also important for treating opportunistic infections like viral pneumonia and candidiasis. (Cathcart, 1981)

For ascorbic acid tablets to be effective, it must contain active ingredient in conformity to the specifications in official compendia. There has been a lot of report on fake drugs worldwide. (WHO, 1999). Drugs play an important role in improving human health and promoting well-being, however, to produce the desired effect, they have to be safe, efficacious and of

acceptable quality. The use of ineffective and poor quality drugs will not only endanger therapeutic treatment but also erode public confidence in a country's health program. (WHO,1999). In the past few decades, many pharmaceutical industries and distribution channels have flourished throughout the world leading to an increase in the number of products circulating in national and international markets. At the same time, the presence of counterfeit and substandard drugs in those markets has increased substantially as a result of ineffective regulation of the manufacturing and trading of pharmaceutical products by both exporting and importing countries. (WHO 1999). Though in pharmacovigilance, it is often assumed that drug quality and efficacy are assured therefore safety monitoring does not take into consideration the quality of drugs being monitored. For many countries where counterfeit drugs have been reported, it is no longer safe to make this assumption while monitoring safety of medicines, (Akunyili, 2005). Also, empirical observations have shown in Nigeria that there may be more fake than genuine drugs in circulation (Osibo, 1998). According to Adenika, (1998) vitamins are among the most used drugs in Nigeria that have been found to have 70% less of the active ingredient, hence the need for the quantitative evaluation of drugs, particularly vitamins to ascertain their authenticity.

Due to the importance of ascorbic acid in humans, qualitative and quantitative evaluation of ascorbic acid has gained a significant increase in several areas of analytical chemistry such as pharmaceutical analysis. Several analytical methods have been developed. Most of these methods are laborious and some require much reagents that may be either not readily available or are expensive. In a report by Pourmorad et al, (2003), some of the common methods for ascorbic acid analysis have some failures regarding manipulative steps, therefore the need to develop a simple, sensitive, reliable and accurate UV spectrophotometric method for the

estimation of ascorbic acid in tablet dosage forms that is comparable with existing official method.

1.2. STATEMENT OF RESEARCH PROBLEM.

Several methods for ascorbic acid analysis have been developed, most of these methods are laborious and some require much reagents that may be either not readily available or are expensive. In a report by Pourmorad *et al*, (2003), some of the common methods for ascorbic acid analysis have some failures regarding manipulative steps; therefore the neeed to develop a simple sensitive and accurate UV spectrophotometric method for the estimation of ascorbic acid in tablet dosage forms that is comparable with existing official method.

1.3 JUSTIFICATION OF THE STUDY

Vitamins are among the most used drugs in Nigeria (Adenika, 1998). Vitamin C has been reported to be of great value in tackling global health issues of HIV/AIDS and cancer (Cathcart,1981). In view of the widespread use of vitamin C, several methods were developed for the determination of vitamin C in pharmaceutical preparation. According to a report by Pourmorad *et al* (2003) titrimetric and colorimetric methods commonly used to assay ascorbic acid have some failures regarding manipulative steps.

1.4 RESEARCH HYPOTHESIS

1.4.1 Null

Mineral ascorbates and ascorbic acid tablets marketed in Zaria conform to specifications in official compendia

1.4.2 Alternative hypothesis

Mineral ascorbates and ascorbic acid tablets marketed in Zaria do not conform to specifications in official compendia

1.5 AIMS AND OBJECTIVES.

1.5.1 Aim

To evaluate the ascorbic acid content of mineral ascorbate and six brands of ascorbic acid tablets

1.5.2 Specific objectives

- To develop a new spectrophotometric method for determination of ascorbic acid
- To apply the developed method along with official methods in the determination of percentage content of ascorbic acid in mineral ascorbate and six brands of tablet.
- To compare the values obtained from the different methods.

CHAPTER TWO

LITERATURE REVIEW

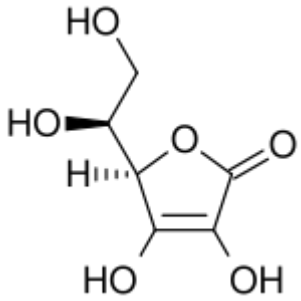


FIG. 2.0. STRUCTURE OF ASCORBIC ACID

2.1 CHEMISTRY OF ASCORBIC ACID.

Ascorbic acid has chemical names as; 2-oxo-l-threo-hexono-1, 4-lactone-2, 3-enediol(R)-3, 4-dihydroxy-5-((s)-1,2-dihydroxyethyl)furan-2(5H)-one;

L-ascorbic acid, L-xyloascorbic acid; 3-oxo-1-gulofuranolactone (enol form)

Molecular formula**:** $C_6H_8O_6$

Molecular mass: 176.1

Melting point: 190 to 192

2.2 DRUG INDICATIONS OF ASCORBIC ACID

It is used for the prevention and treatment of scurvy. It has been used as urinary acidifier and correcting tyrosinemia in premature infants on high-protein diets. The drug may be useful in treating idiopathic methaemoglobinemia. It can also be used as prophylaxis for common cold and cancer. (Linus, 1978).

2.3 CAUTION

Ascorbic acid is contraindicated in patients with hyperroxaluria (Dollery, 1991) and glucose-6-phosphate dehydrogenase deficiency. Ascorbic acid; a strong reducing agent also interferes with laboratory tests involving oxidation and reducing reactions. Ascorbic acid crosses the placenta and is distributed into breast milk of lactating mothers. (Dollery, 1991).

2.4 PHARMACOKINETICS OF ASCORBIC ACID

Ascorbic acid is readily absorbed from the gastrointestinal tract and is widely distributed in the body tissues. Plasma concentrations of ascorbic acid rises as the dose ingested increases until a plateau is reached with doses of about 90 to 150mg/day. The body store of ascorbic acid in healthy individuals is about 1.5mg. Although more may be stored at intakes above 200mg daily. The concentration is higher in leucocytes and platelets than in erythrocytes and plasma. In deficiency state, the concentration in leucocytes declines and at a slower rate. This has been considered a better criterion for the evaluation of deficiency of ascorbic acid. Ascorbic acid is reversibly oxidized to dehydroascorbic acid, some to ascorbate-2-sulfate; which is inactive and oxalic acid which are excreted in the urine. Ascorbic acid in excess of the body's need is rapidly eliminated unchanged in the urine. In Lactating mothers, ascorbic acid crosses the placenta and is distributed in breast milk. This can be removed by hemodialysis. (Martindale, 2007)

2.5 MODE OF ACTION OF ASCORBIC ACID

One of the most important properties of vitamin C is its antioxidant property.

$$RO\bullet + C_6H_7O_6^- \rightarrow ROH + C_6H_6O_6^{\bullet -}$$

Nevertheless, it has a wide range of antioxidant properties outside the body and can quench most biologically active radicals. It scavenges superoxide, nitroxide, hydroxide, hydrogen peroxide and will reduce vitamin E (Hickey and Roberts, 2004). It is also found to be a strong antioxidant as it helps to neutralize harmful free radicals. (Izuagie and Izuagie, 2007). Vitamin C can help to

prevent and treat common cold, mental illness, infertility, scurvy, cancer and acquired immune deficiency syndrome (AIDS),(Yusuf and Gurel, 2005). It is reported to lower cancer risk and also said to have important interactions with other vitamins. For example, excessive intake of vitamin A is less toxic to the body when vitamin C is readily available. (Izuagie and Izuagie, 2007). Vitamin C is a co-factor in many biological processes; including the conversion of dopamine to noradrenaline and in the hydroxylation step of adrenal steroid hormone synthesis. It is also required in tyrosine metabolism, conversion of folic acid to folinic acid, carbohydrates metabolism, synthesis of lipids and proteins, iron metabolism, just to mention, but a few. (Dollery, 1991).

2.6 INTERACTIONS OF ASCORBIC ACID

Ascorbic acid, just like other drugs, has been found to interact with some drugs. This interaction is either positive or negative. Ascorbic acid has been reported to decrease the excretion of aspirin when administered concurrently (Mcevoy, 1993). It decreases the half-life of paracetamol, (Dollery, 1991), in concomitant administration with Desferrioxamine, the activity of the drug is improved, though when there is excess of iron in the tissues, co-administration with ascorbic acid worsens the disease state (Martindale, 2005). Large supplements of vitamin C has been reported to increase serum ethinylestradiol concentrations in women taking oral contraceptives and it also decrease serum level of fluphenazine in patients with bi-polar disorder.(Briggs et al, 1981).

2.7 OTHER IMPORTANCE OF VITAMIN C

Vitamin C participates in the growth and repair of tissues in all parts of the body (Kleszczewski and Kleszczewska, 2002). Vitamin C plays crucial roles in electron transport, hydroxylation reactions and oxidative catabolism of aromatic compounds in animal metabolism. (Gazdik *et al*,

2008). Vitamin C is a natural antioxidant that mostly found in fruits and vegetables. The main sources of vitamin C are citrus fruits, strawberries, peppers, tomatoes, cabbage, and spinach.

In the food industry, vitamin C is used as food additive (Mai and Mohammed, 2004). It preserves and protects food from any color changes and act as an important component of our nutrition as well. Vitamin C helps to prevent the degradation of soft drinks and juice which helps to retain their flavors. Hence, it increases the quality of food and nutritional value as well. (Burdurlu *et al*, 2005). Vitamin C is also used as an index of the nutrient quality for fruit and vegetable products. This is because; it is much more sensitive to various modes of degradation in food processing and subsequent storage (Ozkan *et al*, 2004). It is well known that vitamin C is easily oxidized to dehydroascorbic acid in alkaline solutions, while it is relatively stable in acidic solution. Vitamin C of fruit juices is readily oxidized and lost during storage of the juices (Kabasakalis *et al*, 2000). As stated earlier, ascorbic acid can be taken in tablet forms as supplements. The tablets are normally formulated with starch and sometimes glucose. It can also be formulated combined with minerals called mineral ascorbates. Some of them are; sodium ascorbate, potassium ascorbate, magnesium ascorbate and calcium ascorbate; which is the commonest found in Nigeria and which is involved in this experiment

2.8 SYNTHESIS OF ASCORBIC ACID

Vitamin C otherwise known as L-ascorbic acid is a water soluble vitamin that is naturally present in some foods, added to others and taken as dietary supplement. Though derived from glucose, man and some other primates cannot synthesize vitamin C, due to the genetic mutation that prevents them from synthesizing L-gluconolactone; an oxidase enzyme that converts glucose to ascorbic acid. But some other animals that possess this enzyme can synthesize ascorbic acid invivo. Some examples are: goats, dogs, castles, etc. (Storn, 1972). Chemically, there exists D-

ascorbic acid which does not occur in nature but can be synthesized with its antioxidant properties far less than that of L-ascorbic acid (retrieved from Kascst.edu.sci, 2012).

Ascorbic acid is a six carbon compound chemically related to glucose. It was first isolated in 1928 by Szent-Gyorgyi and structurally characterized by Haworth in 1933. In 1934 Reichstein worked out a simple, inexpensive five step process for synthesizing ascorbic acid from glucose. In the first of a five-step process, glucose is catalytically hydrogenated to sorbitol, which is then oxidized by the microorganism *Acetobacter suboxydans* to sorbose. Only one of the six hydroxyl groups is oxidized by this enzymatic reaction. From this point, two routes are available. Treatment of the product with acetone in the presence of an acid catalyst converts four of the remaining hydroxyl groups to acetals. The unprotected hydroxyl group is oxidized to the carboxylic acid by reacting with the catalytic oxidant TEMPO ((2,2,6,6-Teramethyl-piperidin-1-yl)oxyl) regenerated by sodium hypochlorite — bleaching solution. Acid-catalyzed hydrolysis of this product performs the dual function of removing the two acetal groups and ring-closing lactonization, this step yields ascorbic acid. Each of the five steps has yield larger than 90 %. (Eggersdorfer *et al*, 2007).

A more biotechnological process, first developed in China in the 1960s but further developed in the 1990s, bypasses the use of acetone protecting groups. A second genetically-modified microbe species such as mutant *Erwinia*, among others oxidises sorbose into 2-ketogluconic acid (2-KGA), which can then undergo ring-closing lactonization via dehydration. This method is used in the predominant process by the ascorbic acid industry in China, which supplies 80% of world's ascorbic acid. (csmonitor.com (2007-07-20)). American and Chinese researchers are competing to engineer a mutant which can carry out a one-point fermentation directly from glucose to 2-KGA, bypassing both the need for a second fermentation and the need to reduce

glucose to sorbitol. (BASF's description of vitamin C—developments in production methods. Competition-commission.org.uk).

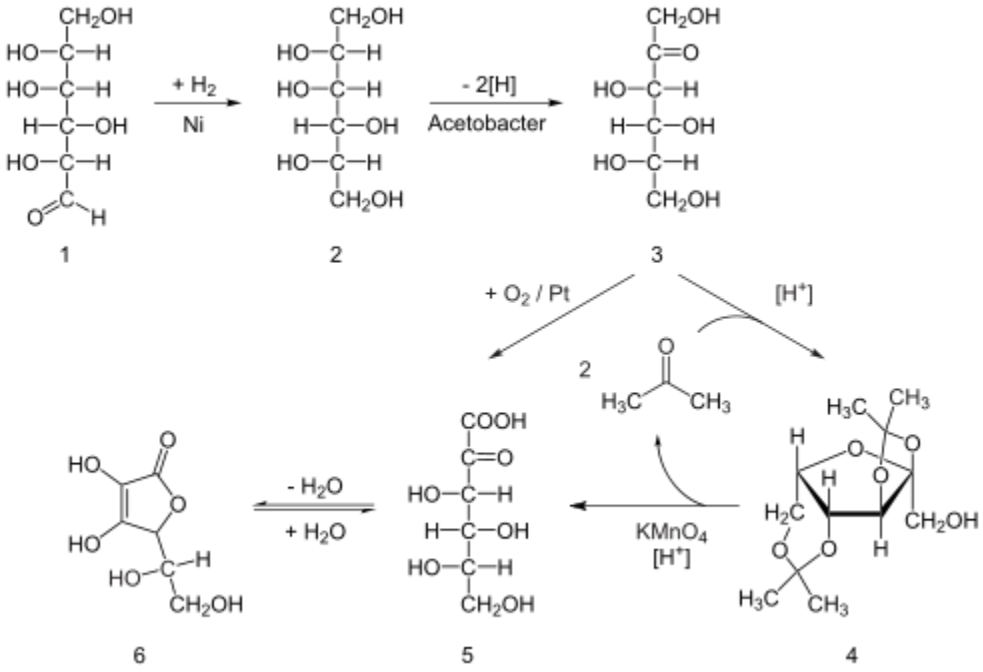


FIG.2.1. REICHSTEIN SIX STEPS PROCESS OF ASCORBIC ACID SYNTHESIS

The term vitamin C is used as generic term for all compounds exhibiting qualitatively the biological activity of ascorbic acid. The molecular structure of vitamin C is $C_6H_8O_6$ and the molecular weight is 176.1 (Ball, 2006). Vitamin C is highly polar and readily soluble in aqueous solution and insoluble in non-polar solvents (Fennema, 1996). It is an acidic compound due to the facile ionization of hydroxyl group on carbon 3 (pk1 = 4.17) while the hydroxyl group on carbon 2 is much more resistant to ionization (pk2 = 11.79).

2.9 OXIDATION OF ASCORBIC ACID

Ascorbic acid is easily and reversibly oxidized to dehydroascorbic acid, forming the ascorbyl radical anion which is also known as semi-dehydroascorbate as an intermediate. (Ball,2006).

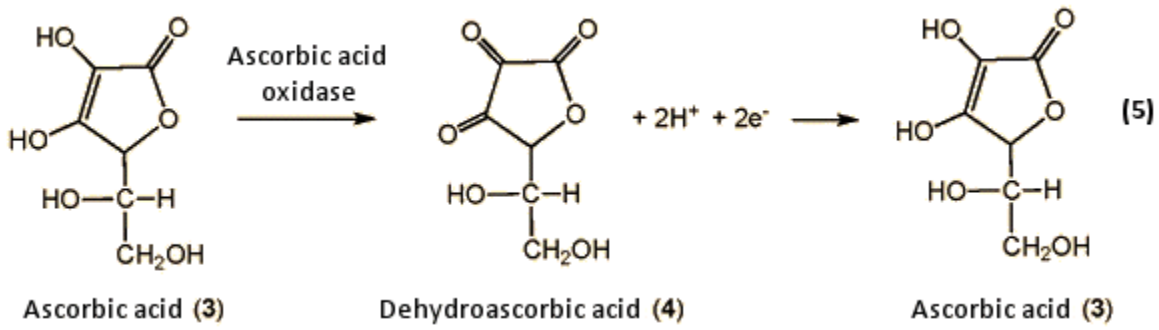


FIG.2.2. OXIDATION OF ASCORBIC ACID.

Dehydroascorbic acid possesses full vitamin C activity because it is readily reduced to ascorbic acid invivo. However, dehydroascorbic acid is not an acid in the chemical sense, as it does not have the dissociable protons that ascorbic acid has at carbon 2 and carbon 3 positions.

Vitamin C is an almost odorless white or pale yellow crystalline powder with a pleasant sharp taste and melting point of about 190ºc. (Izuagie and Izuagie, 2007).

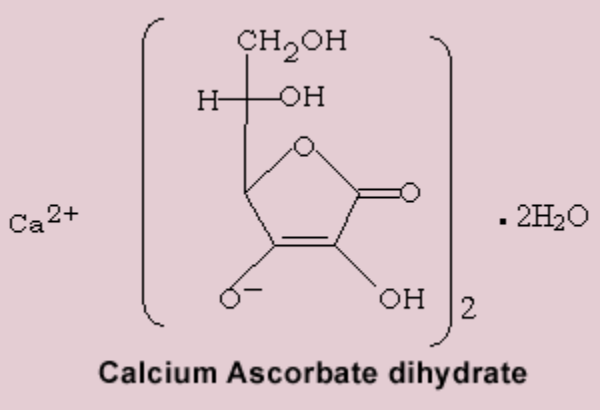
Vitamin C is highly susceptible to oxidation, especially when catalyzed by metal ions such as copper (II) ion and iron (III) ion. The functions and activities of vitamin C are based on its properties as a reversible biological reductant. (Hickey and Roberts, 2004).

$RO \bullet + C_6H_7O_6^- \rightarrow ROH + C_6H_6O_6^{\bullet -}$

Ascorbic acid is a white crystalline powder which becomes discolored on exposure to air and moisture, though slight discoloration does not impair the therapeutic activity of ascorbic acid. In

the dried state, it is reasonably stable in air, but rapidly oxidizes in solution. It is freely soluble in water (1 in 3 volumes), less soluble in alcohol (1 in 40), insoluble in chloroform, ether and benzene.

2.10 CALCIUM ASCORBATE.



Calcium Ascorbate dihydrate

FIG. 2.3

Calcium ascorbate is a calcium salt of ascorbic acid. It is a source of both ascorbic acid and calcium. Commercially it is synthesized by bacterial fermentation of glucose followed by chemical oxidation with calcium. It is used as an antioxidant, preservative and a source of vitamin C supplement. It is well absorbed in the gut, though it cannot supply a mega dose of vitamin C. Calcium ascorbate aids in fighting bacterial infections and also possess anti-tumor and anti-carcinogenic properties. It also aids in tooth and bone formation. (Sirah, 2011).

Due to the great importance of vitamin C in human beings, the quantitative analysis of vitamin C has gained increased significance in several areas of analytical chemistry such as pharmaceutical and food applications. (Yusuf and Gurel, 2005). Therefore, ingestion of fake and adulterated forms do not only expose the patient to the risk of resistance development by the diseased cells but also deterioration of the disease condition. Hence the need for the evaluation of the different brands sold in pharmaceutical shops and outlets.

2.11 GENERAL APPROACH TO PHARMACEUTICAL ANALYSIS

Pharmaceutical analysis is the use of analytical methods in systematic tests and procedures using physical, physicochemical and or chemical technique to analyze and validate chemical identities, either in pure or pharmaceutical formulations. It involves investigation and quantification to ascertain those formulated biopharmaceutical characteristics inbuilt in a given dosage form which confers safety and therapeutic efficacy. (Olaniyi and Ogungbamila, 1991)

The science of pharmaceutical analysis is an extremely active one in terms of research on newer and more reliable or more sensitive methods of analysis. The various analytical methods that have been developed in pharmaceutical preparations are:

2.11.1 Physicochemical Methods

These comprises of Titrimetric and gravimetricmethods, Electro analytical techniques, Chromatography, Spectrophotometry (UV visible, IR, NMR, Spectrofluorimetry), Mass spectra, Atomic absorption spectra and flame photometry, Reflectrometry and polarimetry.

2.11.1.1 *Titrimetric Method.*

Titration is an analytical technique which allows the quantitative determination of a specific substance (analyte) dissolved in a sample. It is based on a complete chemical reaction between the analyte and a reagent (titrant) of known concentration which is added to the sample. The titrant is added until the reaction is complete. In order to be suitable for determination of the end of the titration reaction which should be easily observable. This means that the reaction has to be monitored (indicated) by appropriate techniques, e.g. Potentiometry (potential measurement with a sensor) or with colour indicators. The measurement of the dispensed titrant volume allows the calculation of the analyte content based on the stoichiometry of the chemical reaction. The reaction involved in a titration must be fast, complete, unambiguous and observable.

Titrimetric methods are the most common methods for ascorbic analysis. The procedure are easy to follow and understand and the equipment and reagents are common and readily available. Titrimetric method can be either acid- base or redox, therefore it can be used to determine the acid content or basic content of a substance. (Helmistine, 2011)

2.11.1.2 *Gravimetric Method*.

All Gravimetric analyses rely on some final determination of weight as a means of quantifying an analyte. Since weight can be measured with greater accuracy than almost any other fundamental property, gravimetric analysis is potentially one of the most accurate classes of analytical methods available. These methods are among the oldest of analytical techniques, and they may be lengthy and tedious. Samples may have to be extensively treated to remove interfering substances. As a result, only a very few gravimetric methods are currently used in analysis.

There are four fundamental types of gravimetric analysis: physical gravimetry, thermo gravimetry, precipitative gravimetric analysis, and electrodeposition. These differ in the procedure of sample preparation before the weighing of the analyte. Physical gravimetry is the most common type used in environmental engineering. It involves the physical separation and classification of matter in environmental samples based on volatility and particle size (e.g., total suspended solids). With thermogravimetry, samples are heated and changes in sample mass are recorded. Volatile solids analysis is an important example of this type of gravimetric analysis. As the name implies, precipitative gravimetry relies on the chemical precipitation of an analyte. Its most important application is in the environmental field in the analysis of sulfite. Electrodeposition involves the electrochemical reduction of metal ions at a cathode, and

simultaneous deposition of the ions on the anode (electroplaiting). A basic procedure in Gravimetric analysis include drying to constant weight. Generally, the sample is dried in a 103^0C to 110^0C oven for about 1 hour and allowed to cool to room temperature in a desiccator. It is then weighed, and heated again for about 30 minutes. The sample is cooled and weighed a second time. The procedure is repeated until successive weighings agree to within 0.3 mg. Gravimetric methods have been developed for most inorganic anions and cations, as well as for such neutral species as water, sulphudioxide, carbon dioxide, and iodine. A variety of organic substances can also be easily determined gravimetrically. Examples include lactose in milk products, salkylates in drug preparations, phenolphthalein in laxatives, nicotine in pesticides, cholesterol in cereals, benzaldehyde in almond extracts and ascorbic acid in fruit and vegetable extracts. Indeed, gravimetric methods are among the most widely applicable of all analytical procedures.(Rubison, 1987).

2.11.1.3 *Chromatographic Method*

Chromatography is a technique by which a mixture sample is separated into components. Although originally intended to separate and recover (isolate and purify) the components of a sample, today, complete chromatography systems are often used to both separate and quantify sample components. It is a physical method of separation that distributes components to separate between two phases moving in a definite direction. Substances are separated based on their differential distribution between two phases. Substances will move with the mobile phase at different rate depending upon their partition or distribution coefficients. There are various types of chromatography; thin layer, paper, column, gas, liquid, affinity etc. The term, “chromatography" was coined by the Russian botanist, Tswett, who demonstrated that, when a

plant extract was carried by petroleum ether through a column consisting of a glass tube packed with calcium carbonate powder, a number of dyes were separated. He named this analysis method "Chromatographie" after "chroma" and "graphos", which are Greek words meaning "color" and “to draw," respectively. "Chromatography" represents a separation technique; whereas a "chromatograph" is a system for performing chromatography. The chart displaying the time-dependent change in signal intensity as a result of the separation is called a "chromatogram”. This analytical technique is needed in a variety of situations that range from an assay of the new chemical entity (NCE) in the presence of related compounds, including optical isomers to complex determination of trace or ultratrace level of various related or transformation products. Chromatography plays a major role in pharmaceutical analysis in Purity/Impurity Analysis, also in Separations of Isomers, support of Biotechnology Products, Support of Toxicology Studies, Biopharmaceutic/Pharmacokinetic Studies, Metabolic Studies, Clinical Studies, Forensic Studies, Diagnostic Studies, Animal-derived Food Analyses, Post-mortem Toxicology, to mention but a few.(www.nkpatel.co.in/semester/chromatography).

2.11.1.4 *Uv- Spectrophotometric Method*

Spectrophotometry in chemistry is the quantitative measurement of the reflection properties of a material as a function of wave length (Blauch, 2009). It involves the use of a spectrophotometer. This is a photo meter that can measure intensity as a function of the light source wave length. Spectrophotometer is able to determine depending on the control, what substances are present in a target and exactly how much, through calculations of observed absorbances. The sequence of events in a modern spectrophotometer is as follows;

1. The light source is imaged upon the sample

2. A fraction of the light is transmitted or reflected from the sample

3. The light from the sample is imaged upon the entrance slit of the monochomator

4. The monochromator separates the wave lengths of light and focuses each of them onto the photo detector sequentially.

Older spectrophotometers are calibrated by a procedure known as zeroing. The absorbency of a control or reference substance is set as a baseline value, so the absorbances of all other substances are recorded relative to the initial zeroed substance. The spectrophotometer then displays percentage absorbency, i.e (the amount of light absorbed relative to the initial substance or control). In a nut shell, the spectrophotometer measures the amount of light the sample absorbs. The beam of light from the light source consists of a stream of photons. When a photon encounters an analyte molecule (which normally should contain a chromophore), there is a chance that the analyte will absorb some photons. This absorption reduces the number of photons in the beam of light, thereby reducing the intensity of the light beam. Therefore the intensity of the light reaching the detector is less than the intensity emitted by the light source.

During an experimental measurement, the intensity (number of photons per second) of light passing through the blank is measured. The blank is identical to the sample solution except that the blank does not contain the solute or analyte that absorbs light. Secondly, the intensity of light passing through the sample solution is measured. This is what is used to calculate the transmittance and absorbance as follows:

(T) and absorbance(A).

$T = I/Io$

$A = -\log 10t$

I-T = the fraction of the light absorbed by the sample.

In relating the amount of light absorbed to the concentration of the absorbing material or specie, the absorbance is the most useful other than the transmittance. If no light is absorbed, the absorbance is zero (100% transmittance).

Each unit in absorbance corresponds in order of magnitude in the fraction of light transmitted. For A = 1, 10% of the light is transmitted (t = 0.10) and 90% of the light is absorbed by the sample. (Blauch, 2009).

The quantitative aspect of spectrophotometry is based on two laws;

Beer's law and Lambert's law

Beer's law states that; the decrease in intensity of light due to its absorption by a sample is related exponentially to the absorptive substance in the solution. It is represented mathematically thus;

$A = \log Io/I = K$

Where A=absorbance

Io = incident light intensity

I = transmitted light intensity

K = proportionality constant

Lambert's law states that; the absorption is directly proportional to the path of length thickness (thickness of the absorption layer). Expressed mathematically as

$\text{Log } Io/I = ECL$

Where E = molar absorptivity; a constant that is dependent upon the wave length of the incident beam of light and nature of the absorbing material.

L = path length (cm)

C = concentration.

A combination of the two laws known as Beer Lambert's law states that; the absorbance of a solution is related directly to the path length of light passing through the solution as well as its concentration. It is expressed mathematically as follows;

Log Io/I = ecl or A=ecl. UV Spectrometry is used extensively in chemical and pharmaceutical analysis. (Blauch, 2009)

2.11.1.5 *Infrared Spectrometric Method.*

Infrared Spectroscopy is the analysis of infrared light interacting with a molecule. This can be analyzed in three ways by measuring absorption, emission and reflection. The main use of this technique is in organic and inorganic chemistry. It is used by chemists to determine functional groups in molecules. IR Spectroscopy measures the vibrations of atoms, and based on this it is possible to determine the functional groups. Generally, stronger bonds and light atoms will vibrate at a high streching frequency (wavenumber). The use of infrared spectroscopy began in the 1950's by Wilbur Kaye. He had designed a machine that tested the near-infrared spectrum and provided the theory to describe the results. Karl Norris started using IR Spectroscopy in the analytical world in the 1960's and as a result IR Spectoscopy became an accepted technique. There have been many advances in the field of IR Spectroscopy, the most notable was the application of Fourier Transformations to this technique thus creating an IR method that had higher resolution and a decrease in noise. The year this method became accepted in the field was in the late 1960's. (Mustafa *et al*, 2000). Infrared light imposed on a molecule will not create electronic transitions but it does contain enough energy to interact with a molecule causing vibrational and rotational changes. For example, the molecule can absorb the energy contained in

the incident light and the result is a faster rotation or a more pronounced vibration. The possible rotations are around the axis of symmetry for a given molecule or either of the two perpendicular axis. Vibrations can be in the form of a bend or a stretch for each bond.

The components of an IR machine are the IR source, beam splitter, monochromator, a transducer, an analog to digital converter and a digital machine to quantify the readout. The IR light exits the source and becomes split into to beams, one to be directed to the sample the other to a reference. The intensity of the beam is measured by the intensity emitted divided by the intensity observed, also known as the Transmittance. All frequencies are measured in wavenumber, cm^{-1}. To make a sample with a liquid, the liquid is placed between two pure salt sheets of NaCl and for a solid it is pressure pressed with KBr to incorporate both into one sheet. The reason for using salt to suspend the molecule is because the salt structures form a lattice that is strongly ionically bonded and will not absorb IR light because it lacks the vibrational capability. The Background scan or reference tends to be air. Below 1500 cm^{-1} the spectra have very high sensitivity and this region is known as the fingerprint region where carbon-carbon bond stretching and bending motions overlap, making it difficult to predict functional group. Infra-red spectroscopy is used mainly in structural elucidation and qualitative analysis of carbonyl compounds among others. (Mustafa *et al*, 2000)

2.11.2 Biological Methods

Which include:Bioassay, sterility tests, microbiological methods and radio immunoassay.

2.11.2.1 *Bioassay Method*

Bioasssay is defined as estimation or determination of concentration or potency of a physical, chemical or biological substance by means of measuring and comparing the magnitude of the

response of test with that of standard over a suitable biological system under standard set of conditions. In bioanalysis, the response produced by the test compound with that of standard sample are way similar to analytical methods, but here, the biological system is involved in the determination. In principle, the bioassay compares the test sample with same internationally applicable standard substance. It determines the quantity of test sample required to produce an equivalent biological response to that of standard substance. Standard samples are accepted by expert committee at international level and they represent fixed units of activity. (Ramesh and Goyal, 2008)

2.11.2.2 *Microbiological Method*

Microbiological assay may be defined as a qualitative or quantitative determination of any chemical compound from a simple or even complex material with the use of microorganism. It is used mainly for antibiotics. It is necessary to assay antimicrobial agents for determination of potency, determining the pharmacokinetics of the drug in animals or man and for monitoring and controlling antimicrobial chemotherapy. Vitamins and amino acids can be standardized by microbiological assay. Microbiological assays are relatively as accurate as chemical methods. It is a simple, specific, inexpensive and convenient method. Compared with biological assay methods using animals, microbiological techniques posses the advantages of minimal requirement of space, labor, materials, and time. Microbiological assay are very useful for detecting changes in potency of antibiotics and their preparations and vitamins. (Kavanagh and Ragheb,1979)

2.11.2.3 *Radio Immunoassy Method*

Radioimmunoassay (RIA) was developed by Berson and Yalow for the measurement of insulin in human plasma. Specifically RIA measures the actual effect change in concentrations of a

particular substance present in a biological fluid based on invitro system consisting of radio-active standards of the same substance and a specific antibody.

To perform radioimmunoassay, a known quantity of an antigen is made radioactive, frequently by labeling it with gamma-radioactive isotopes of iodine attached to tyrosine. This radio-labeled antigen is then mixed with a known amount of antibody for that antigen, and as a result, the two specifically bind to one another. Then, a sample of serum from a patient containing an unknown quantity of that same antigen is added. This causes the unlabeled (or "cold") antigen from the serum to compete with the radio-labeled antigen ("hot") for antibody binding sites. As the concentration of "cold" antigen is increased, more of it binds to the antibody, displacing the radio-labeled variant, and reducing the ratio of antibody-bound radio-labeled antigen to free radio-labeled antigen. The bound antigens are then separated from the unbound ones, and the radioactivity of the free antigen remaining in the supernatant is measured using a gamma counter. Using known standards, a binding curve can then be generated which allows the amount of antigen in the patient's serum to be derived. (Werner *et al*, 1974)

RIA involves the separation of the drug using the specificity of antibody - antigen binding and quantization using radioactivity. The principle of radio immuno assay is based on an immune reaction [antigen(Ag)-antibody(Ab) reaction] to estimate a ligand.

Ag+Ag*+Ab→ Ag-Ab+Ag*Ab+Ag+Ab*

- Unbound Ag* and Ag washed out
- Radio activity of bound residue measured.
- Ligand concentration is inversely related to the radio activity.
- [Ag: ligand to be measured; Ag*: radiolabelled ligand].

In drug analysis, the sample is mixed with fixed quantity of labeled drug and antibody which is allowed to equilibrate – incubate. Separate drug bound to antibody from unbound drug, Charcoal adsorption of antibody (and bound drug). Antibody - antibody binding precipitates bound drug, Antibody bonded to container. Measure radioactivity associated with bound labelled drug. Low drug concentration means more bound radioactivity and higher measurement while high drug concentration means less bound radioactivity and lower measurement. Determine standard curve Non-linear plot of radioactivity versus concentration Log-concentration plot is linear. RIA principles have found application in the field of drug analysis, pharmacokinetic studies, drug therapy monitoring. (Werner *et al*, 1974)

2.11.2.4 *Sterility Test.*

The quality attributes of manufactured pharmaceutical product include the physical, chemical, and microbiological characteristics of the raw materials, excipients, active pharmaceutical ingredient (API) as well as the final drug product .Absence of microbiological contamination is considered a critical quality attribute due to its potential to dramatically impact, directly or indirectly, the safety and/or the efficacy of the drug product. "Sterile", is a medieval word derived from the Latin word *sterilis* (unfruitful), meaning, in modern terms, free from living germs or microorganisms. In pharmaceutical manufacturing, it is critical to assure sterility of "sterile" products in order to release aseptic and safe medicines to patients. Therefore, the importance of adequate and effective microbiological controls cannot be overstated enough. Sterility testing is performed to evaluate a finished pharmaceutical product as a batch release quality control test by following the requirements delineated in the compendia (USP <71>, 2011a; EP Section 2.1.6, 2010a; JP, 2006). The test is used to determine the Presence or absence of viable, multiplying microorganisms (bacteria, yeast and fungi) under standardized growth

conditions. As the sterility test is a very exacting procedure, it is performed by qualified personnel under tightly controlled environmental conditions where strict asepsis is ensured, maintained and monitored .(Claudio and Reyes, 2010)

2.11.3 Biopharmaceutical Methods

These include; particle size analysis, disintegration, dissolution rate and friability determinations as well as bioavailability assessment.

2.11.3.1 *Particle Size Analysis*

Particle size analysis or particle measurement or simply particle sizing is the collective name of the technical procedures, or laboratory techniques which determines the size range and or the average or mean size of the particles in a powder or liquid sample. Particle size analysis is part of particle science and its determination is carried out generally in particle technology laboratories. It has importance in chemical, mining, forestry, agriculture and aggregate industries. Some of the methods for particle size analysis are; sieving, sedimentation, elutriation, microscope counting, dynamic light scattering and imaging particle analysis. (Howard, et al, 1987)

2.11.3.2 *Disintegration*

Disintegration is a measure of the quality of the oral dosage form like tablets and capsules. The disintegration test is performed to find out the time it takes for a solid oral dosage form like a tablet to completely disintegrate. The time of disintegration is a measure of quality. This is because, for example, if disintegration time is too high, it means the tablet is too highly

compressed or the capsule shell gelatin is not of pharmacopoeial quality or it may imply several other reasons. And also if disintegration time is not uniform in a set of samples being analyzed, it indicates batch inconsistency and lack of uniformity. The test is carried out by a disintegration apparatus. The disintegration test for each dosage form is given in the pharmacopeia. It has its application in the pre formulational stage to the formulator. It helps in the optimization of manufacturing variables such as compressional force and dwell time. It also helps to control uniformity from batch to batch among different tablets and a quality control tool for tablets and hard gelatin capsules.(Ratna, 2002)

2.11.3.3 *Dissolution Rate*

Tablet dissolution is the standard method for measuring the rate of drug release from dosage form and hence, its quality. The rate of dissolution of a drug is used to optimize its therapeutic effectiveness, routine assessment of product quality to ensure uniformity between product lots, prediction of invivo availability and assessment of bioequivalence of several drugs. (Kaunisto *et al,* 2010)

2.11.3.4 *Friability Determination*

Friability is the tendency for a tablet to chip, crumble or break following compression. The friability test is closely related to tablet hardness and is designed to evaluate the ability of the tablet to withstand abrasion in packaging, handling and shipping. It is usually measured by the use of the Roche friabilator. A number of tablets are weighed and placed in the apparatus where they are exposed to rolling and repeated shocks as they fall 6 inches in each turn within the apparatus. After four minutes of this treatment or 100 revolutions, the tablets are weighed and the weight compared with the initial weight. The loss due to abrasion is a measure of the tablet friability. The value is expressed as a percentage. A maximum weight loss of not more than 1%

of the weight of the tablets being tested during the friability test is considered generally acceptable and any broken or smashed tablets are not picked up. Normally, when capping occurs, friability values are not calculated. A thick tablet may have less tendency to cap whereas thin tablets of large diameter often show extensive capping, thus indicating that tablets with greater thickness have reduced internal stress. (Rippie, 1990)

In deciding on which analytical method to use in order to solve an analytical problem, the analyst must take into account the complexity of materials to be analyzed, concentration of the species of interest, the number of samples to be analyzed, the accuracy required and the urgency of the analytical result. (Davis et al., 2010), bearing in mind the following criteria;

2.12 Selectivity

The chosen method must be selective or specific for the active ingredient, it should be capable enough of discriminating between the analyte and other impurities that might be present.

2.13 Sensitivity

The sensitivity of an instrument is the ratio of the change in response to the change of the quantity (concentration) which is measured, i.e., the instrument must be sensitive enough to measure the analyte in the range of concentrations present in the drug.

2.14 Reliabiblity

The method must give accurate and reproducible results with high precision.

2.15 METHODS OF ASCORBIC ACID ANALYSIS

Chemical methods for determining vitamin C are based upon the reducing properties of the vitamin and include titration procedures with various oxidizing agents. In 1937, Roe introduced a color reaction with 2, 4-dinitrophenylhydrazine to determine vitamin C content. In 1943, Roe and Kuether further developed the method and applied it to analyses of blood, plasma, and urine. There are many analytical methods used to determine the concentration of vitamin C in the pharmaceutical samples, natural samples, biological fluids and pharmaceutical formulations. Which are colorimetric method, titration, enzymatic method, flow injection analysis (FIA) and high- performance liquid chromatography (HPLC). (Arya and Mahajan, 1997). These methods for the determination of vitamin C can be categorized into spectrophotometric methods and non-spectrophotometric methods (Arya and Mahajan, 1997). Non-spectrophotometric methods are methods such as high-performance liquid chromatography (HPLC), titration, enzymatic method and fluorometry (Arya *et al*, 2000). Direct spectrophotometry use spectrophotometer. This has been applied to determine the vitamin C content in soft drinks, fruit juices, and cordials.

2.15.1 HPLC

In a method developed and reported by Margherita, *et al* (2012,) for detection and quantization of water soluble vitamins in biological samples, separation was done at 30^0C on a reversed-phase C18-A column using combined isocratic and linear gradient elution with a mobile phase consisting of 0.01% TFA aqueous and 100% methanol. Total run time was 35 minutes. Detection was performed with diode array set at 280nm. Each vitamin was quantitatively determined at its maximum wavelength. Spectra comparism was used for peal identification in the biological samples and percentage recovery for all vitamins ranges from 93% to 100%. Also Castro *et al*, (2001) reported another method for determination of ascorbic acid in honey samples by HPLC,

of which the percentage recovery was over 90%. In another development, Sanchez,*(*2000) reported a method which compared HPLC and UV spec method for the analysis of vitamin C content of green beans. Of which he concluded that the HPLC analysis shows acceptable linearity, precision and accuracy in vitamin c determination.

2.15.2 Enzymatic Method

A wide variety of methods involving different chromophores detection systems and enzymes has been developed, for the determination of ascorbic acid and dehydroascorbic acid in biological samples. In a recent development, Winnie and Roberts, (1997) developed and reported an automated enzymatic method for ascorbic acid analysis using Cobas Fara centrifugal analyzer for determination of ascorbic acid in biological samples. In this method, the biological sample is deprotonated with metaphosphoric acid and the ascorbic acid in the sample is oxidized to dehydroascorbic acid by ascorbic acid oxidase. The product is then coupled to o-phenylenediamine to produce a chromophore for which the absorbance is measured at 340nm. In comparing their method with the spectrophotometric methods and HPLC, as well as chromatographic methods, they stated that the spectrophotometric method has significant advantages over the other methods and that its less laborious, less cost intensive, faster and yields results that correlate well and provide improved precision. The linearity extends beyond the reference range of 26.1-84.6 umol/l. Also Tulley reported another enzymatic method for ascorbic acid analysis which is based on the measurement of an enzymatic reaction of ascorbic acid which involves a Beckman synchron CX5 analyser.

In another report by Danielczuk *et al*, (2004), in which they compared the enzymatic method described by Boehringer Mannheim R-Biopharm company using three groups of fruits and vegetable juices. The methods were compared by analysis of regression function of results obtained in parallel experiments. In conclusion the agreement of the results obtained from both

methods were presented. In 2006, Shekhoytsovant et al; also reported an enzymatic method for ascorbic acid analysis in foods. The method is based on the action of ascorbic acid on a second substrate; horseradish and peanuts peroxidases in the reaction of 0-dianisidine and 3,3',5,5'-tetramethyl benzidine oxidation with hydrogen peroxide. The rate of the reaction was monitored spectropphotometrically and the procedure very sensitive (c (L) =0.1microm), simple and rapid.

2.15.3 Fluorometric Methods

Fluorometric analysis has been used for ascorbic acid assay in pharmaceutical preparations, beverages, and special dietary foods and even for human serum (Arya, *et al*, 2000). This method had been reported to have successful application to a wide range of foodstuffs, including liver, milk, fresh and canned fruit, raw and cooked vegetables, and potato powder (Ball, 2006). Previously, fluorometric determinations of vitamin C have been developed based on condensation reactions of vitamin C with o-phenylenediamine and on the oxidation of vitamin C with mercury (II) to form quinoxaline derivative. The reaction products of these methods exhibit fluorescensce which can be measured. (Yusuf and Gurel, 2005). Dehydroascorbic acid also reacts with 1,2-phenylenediamine dihydrochloride to form fluorescent quinoxaline derivative 3(1,2dihydroxyethyl) furol[3,4-b]quinoxaline-1-one. The blank can be prepared by complexing the oxidized vitamin with boric acid to prevent the formation of the quinoxaline derivative. It is used to reveal any fluorescence due to interfering substances (Ball, 2006).

Yusuf and Gurel have described a method by using Methylene Blue (MB) for the determination of vitamin C. The experiment was run by using a spectrofluorimeter to record the spectra and carry out fluorescence measurements. This method was used to determine the amount of vitamin C in the purified materials, specifically vitamin C tablets. MB is a member of thiazine dye group which is widely used in many different areas. For example, a photo sensitizer is used to produce

singlet oxygen in photodynamic therapy for the treatment of cancer. The highly colored oxidized form of MB can be reduced to the colorless leuco form; Leuco-Methylene Blue (LMB). (Yusuf and Gurel, 2005). This is shown in the figure bellow.

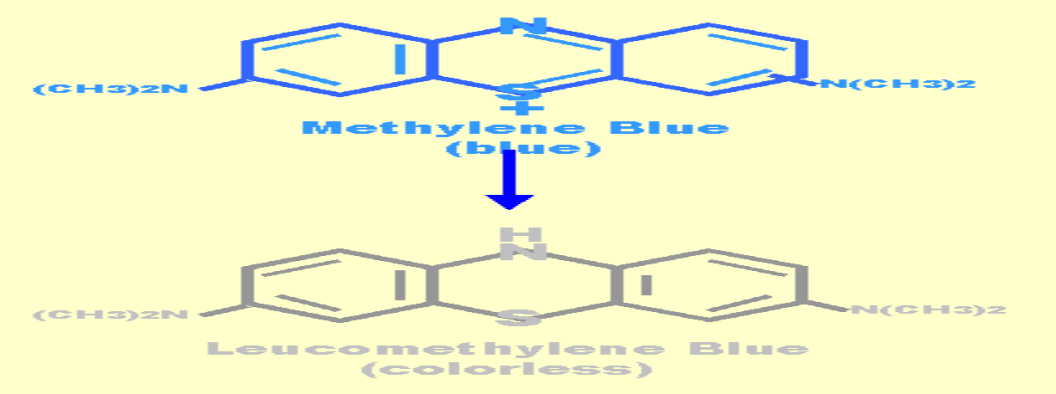


FIG.2.4. REDUCTION OF METHYLENE BLUE TO LEUCO-MB.

According to Yusuf and Gurel, the fluorescence bands of MB were obtained at 664nm for excitation state and 682nm for emission peaks. This was proven by the other researchers who also examined the emission bands at 682nm for MB and 452nm for LMB.The emission peak of MB at 682nm increased due to the increase of its concentration. A linear relationship between MB concentration and intensity was obtained over a concentration range of mol L-1 MB (y= 49.082x + 94.46, r2=0.9969). The excitation peak of MB at 664 nm also linearly increased depending on the increase of its concentration. (Yusuf and Gurel, 2005).

Mori K, et al, also reported a simple flourometric method for ascorbic acid analysis and dehydroascorbicacid using 4, 5-dimethyl -o-phenylenediamine. It was reported that the DMPD is a good fluorescent agent and its reaction product with dehydroascorbic acid showed strong and stable fluorescence.

2.15.4 Titrimetric Method

Helmienstine (2000), reported a trimetric method for ascorbic acid analysis using iodine. This is a redox reaction in which ascorbic is oxidized by iodine. Iodine is relatively insoluble, but this can be improved by complexing the iodine with iodide to form triiodide

: $I_2 + I^- <--> I_3^-$

Triiodide oxidizes vitamin C to form dehydroascorbic acid:

$C_6H_8O_6 + I_3^- + H_2O --> C_6H_6O_6 + 3I^- + 2H^+$

As long as vitamin C is present in the solution, the triiodide is converted to the iodide ion very quickly. However, when all the vitamin C present is oxidized, excess iodine and triiodide will react with starch indicator to form a blue-black complex, indicating the end point of the titration.

Another method involves the use of bromine in the redox titration instead of iodine. The bromine is generated by adding excess of potassium bromide (KBr) to an acidified solution of the sample. In comparing the acid base method and the redox titration, Helmienstine reported that the latter is better than the former. The reason is, since there could be additional acids in the sample (especially juices), few of them will interfere with the oxidation of ascorbic acid by iodine, which is not the case in acid redox titration. Titrimetric method has also been reported in the British pharmacopeia as a method for analysis of ascorbic acid tablets.

2.15.5 Spectrophotometric Methods

Direct spectrophotometric methods for ascorbic acid analysis has been widely reported. Farajzadeh and Nagizadeh in 2003, developed and reported a spectrophotometric method based on the reaction between ascorbic acid and copper(II)-ammonia complex. During the reaction, ascorbic acid is oxidized and the copper(II)-ammonia is reduced to copper(I) -ammonia complex, and absorbance at 600nm is measured for cu(II) NH3). In another report by Shrivas and Kumar,

(2005), a new simple and sensitive method for the spectrophotometric indirect determination of ascorbic acid in fruits, beverages, and pharmaceuticals was described. In this method, the ascorbic acid reduces copper II ion (Cu^{2+}) to coppper I ion (Cu^{+}) and reacts with 2, 9-dimethyl-1,10-phenanothroline (neucoproine) to form Copper-neucoproine complex, which was extracted with N-phenylbenzimidoylthiourea (PBITU) in chloroform. The apparent value of molar absorptivity of the complex in terms of ascorbic acid is (3.52) 104L mole -1cm-1 at l max 460. Selimović *et al*, **(**2011) reported yet another direct spectrophotometric method for ascorbic acid analysis in pharmaceutical preparations using a stabilizer; sodium oxalate. In this method, Sodium oxalate (0.0056 mol/dm 3) was used to stabilize L- ascorbic acid in aqueous medium. The molar absorptivity of the proposed method, which does not require an extraction procedure was 1.42x104dm3 mol-1cm-1, at 266 nm. Beer's law was obeyed in the concentration range of 0.857 – 12.0 μg ascorbic acid/cm3. Salkić *et al;* (2008) developed a back ground correction method for the determination of ascorbic acid in pharmaceutical preparations using direct UV spectrophotometry. In this method, ascorbic acid is oxidized by nitrite in an acid medium containing a stabilizer. The absorbance is measured at 256nm against the blank which is a solution of the stabilizer in the acidic medium. Also, Kwakye (2000) developed a direct UV spectrophotometric method for ascorbic acid analysis using stabilizers. Sodiumthiosulphate stabilizer was used to stabilize the ascorbic acid in solution and its absorbance taken at 256nm.

Reo and Kuether, 1943 developed a spectrophotometric method for ascorbic acid analysis which is based upon treatment with 85% H_2SO_4 of the chromogen formed by the coupling of 2,4-dinitrophenylhydrazine with oxidized ascorbic acid incubated at 37 degrees for 3hours, to produce a red color complex. The absorbance was spectrophotometrically measured at 521 nm. In a bid to modify this method, Shaffert and Kinsley in 1955, increased the temperature of the incubation to 100 degrees for 10minutes. According to a report submitted by Joseph Reo, the

modification was not successful due to interference from glucose, fructose and other simple sugars which have similar structure as ascorbic acid and which are present in the samples of fruits and vegetables. In this present study, the method of Sharffert and Kinsley (1955) was adopted and modified for analysis of ascorbic acid content of pure ascorbic acid tablets. The 2, 4-dinitrophenylhydrazine colorimetric method has been widely used for determination of ascorbic acid level in biological fluids. It has been used successfully by researchers in determining the ascorbic acid content of fruits and vegetables. Khan *et al;* (2006) used this method to determine the Total vitamin C (ascorbic acid + dehydroascorbic acid) in various fruits and vegetables. Also, Opara *et al*; (2007) reported the ascorbic acid content of fruits and vegetables using the same method. Other reports for the determination of ascorbic acid content of leaves and vegetables using the 2, 4-dinitrophenylhydrazine method include; Mohammed and Mai (2004), Kapur *et al;* (2012). To mention but a few.

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Equipment.

Electronic weighing balance, water bath, spectrophotometer, retort stand, clamp,

Glass Wares (Pyrex),beakers(25ml to 500ml), pipettes (0.1ml-10ml), storage bottles, measuring cylinder(10mls-1litre), measuring scoop, spatula, , stirring rod, test tubes, conical flasks(50ml-500ml) burette, volumetric flasks(5ml-1litre)

3.1.2 Reagents

Solid iodine, sodiumthiosulphate, potassium iodide, potassium iodate, concentrated H_2SO_4, bromine water, distilled water, deionized water, thiourea, 2,4-dinitrophenylhyrazine, starch powder.

3.1.3 Test Samples

1. CALCIUM ASCORBATE

Manufacturing date: November, 2012

Expiry date: November 2015.

Nafdac number: 04-4053

2. ASCORBION

Manufacturing date: February 2013

Expiry date: February 2016

Nafdac number: 04-3928

3. EMVIT C

Manufacturing Date: October 2012

Expiry date: January 2016

Nafdac number: 04-5918

4. ASCORVITE

Manufacturing date: March, 2013

Expiry date: March 2016.

Nafdac number: 04-9085

5. TOPCEE

Manufacturing date: October 2010

Expiry date: September 2013.

Nafdac number: 04-2333.

6. BIORACEE

Manufacturing date: march 2013

Expiry date: March 2016.

Nafdac number: 04-3987

7. SOFA C

Manufacturing date: November, 2012.

Expiry date: October, 2015.

Nafdac Number:

3.2 METHODOLOGY

The methods that were compared include

B.P 2009 official method, Kwakye, 2000 UV spectrophotometric method, sodium thiosulphate titrimetric method and a newly developed2, 4-dinitrophenylhyrazine spectrophotometric method.

3.2.1 Preparation of Reagents

3.2.1.1 *0.05 M iodine solution*

0.05M iodine solution was prepared by adding 12.7g iodine to 40g of potassium iodide in a 100ml beaker, to it is added 10ml of distilled water and stirred. An additional 20ml of water was added and stirred, until the entire solid was dissolved. Then the bulk of the liquid was carefully decanted into a storage bottle containing 1 liter of distilled water. (Mcnevin and Kriege1953).

3.2.1.2 *standardization of iodine solution*

The iodine solution was standardized using sodiumthiosulphate (0.1M). 25ml aliquots of the iodine solution was transferred to a 250ml conical flask and diluted to 50ml. 1ml of 3M suphuric acid was introduced into it and titrated immediately with 0.1M standard sodium thiosulphate until the solution becomes faint yellow. Then 5ml of starch solution was added and the titration completed; a color change from blue to colorless indicates the end point of titration and the volume of thiosulphate used recorded.

3.2.1.3 *preparation of 0.1 M thiosulphate solution*

1 Liter of distilled water was boiled for 20 minutes and allowed to cool to room temperature. Then 25g of sodiumthiosulphate and 0.1g of sodium carbonate crystals was added to it and stirred until the solid was dissolved completely. The solution was poured in a plastic container and stored in a dark place.

3.2.1.4 *standardization of 0.1 M thiosulphate solutions*

0.1M thiosulphate solution was standardized with 0.01M potassium iodate. 50ml of 0.01M potassium iodate into a 250ml conical flask, to it 2g of solid potassium iodide and 10ml 0.5M sulphuric acid was added. This was titrated with 0.1M thiosulphate solution until a pale yellow color is obtained. 2ml of starch indicator was added and the titration continued until a dark blue color is obtained indicating the end point. The end point volume of the thiosulphate solution is recorded form further calculations.

3.2.1.5 *preparation of 0.01M potassium iodate*

2.1g of dried potassium iodate was transferred to 1000ml volumetric flask, dissolve in 200ml of distilled water and dilute to the mark and mix thoroughly.

3.2.1.6 *preparation of starch solution (indicator)*

1g of starch powder was rubbed with 15ml of distilled water into a paste and diluted to 500ml with boiling water and heated until the mixture is clear. It is allowed to cool and stored in a tightly stoppered bottle.

3.2.1.7 *preparation of 0.04% sodiumthiuosulphate*

0.04g of sodiumthiosulphate crystals was weighed and transferred to a 100ml volumetric flask, to it 50ml of distilled water was added to dissolve, it is then diluted to the mark.

3.2.18 *preparation of 2, 4-dinitrophenylhydarzine solution*

2g of 2,4-dinitrophenylhydrazine and 4g thiourea were dissolved in 100ml 4.5M sulphuric acid. The resulting solution was stored in container.

3.2.1.9 *preparation of 3% bromine water*

3% bromine water was prepared from 99.8% bromine by transferring 3ml of 99.8% bromine to a 100ml volumetric flask in a fume cupboard and made up to the mark with distilled water. This was transferred into a beaker and left for few minutes well covered with a glass lid. The solution was decanted into a storage bottle and stored in a dark place.

3.2.1.10 *preparation of 10% thiourea*

10g of thiourea was weighed and transferred to a 100ml volumetric flask; to it 50ml of distilled water was added, mixed thoroughly and made up to the mark. Then it is transferred to a storage bottle and kept in a dark place.

3.2.1.11 *preparation of 4.5 m sulphuric acids (100ml)*

This was prepared from 96.98% concentrated sulphuric acid. To 75.3ml of distilled water in a measuring cylinder, 24.7ml of 96.8% sulphuric acid was added.

3.2.1.12 *preparation of 85% sulphuric acid (50ml)*

To 6.2ml of distilled water in a measuring cylinder, 43.8ml of 96.98% sulphuric acid was added. The resulting solution was transferred to a storage container and stored in a refrigerator.

3.2.2 B.P 2009 Official Method

20 tablets of the drug sample was powdered and weighed. Weight equivalent to 0.15g ascorbic acid was measured and dissolve in 10ml of dilute sulphuric acid and 50ml of deionized water. 1ml of starch solution was added. The resulting solution was titrated with 0.05M iodine solution until a persistent violet-blue color is obtained. 1ml of 0.05M iodine solution used is equivalent to 8.81mg ascorbic acid.

3.2.3 Uv Spectrophotometric Method Using Stabilizers of Kwakye (2000)

20 tablets of the drug sample was Powdered and Weighed, weight equivalent to 0.15g ascorbic acid was measured and dissolved in 0.04% sodiumthiosulphate in distilled water, the resulting solution was filtered and made up to 100ml. 10ml of the this was further diluted to 100ml with 0.04% sodiumthiosulphate solution and the absorbance at 264nm was determined using 0.04% sodium thiosulphate solution as blank.

3.2.4 Sodium Thiosulphate Tirimetric Method

20 tablets of the ascorbic acid tablets were counted and weighed and powdered. The ground powder was to a clean container. A quantity equivalent to 0.25g was weighed and transferred to a 250 conical flask. This was dissolved in 0.5M sulphuric acid. 2g of potassium iodide and 50ml of standard potassium iodate was added with the aid of a pipette. This was immediately titrated with standardized 0.1M thiosulphate solution with starch as indicator, until a sharp persistent blue black color is seen. The volume of the thiosulphate used was recorded and used to calculate the quantity of ascorbic acid present.

3.2.5 The Developed 2, 4-Dinitrophenylhydrazine Method

In this method; Ascorbic acid is oxidized to dehydroascorbic acid by bromine water, thiourea was added to remove excess of the bromine. After that, the dehydroascorbic acid reacts with 2,4-dinitrophenylhydrazine and produces an osazone which when treated with 85% sulphuric acid forms a red colored solution.

3.2.5.1 *Method development*

To 4ml of pure ascorbic acid solution, 0.23ml of 3%bromine water was added and 0.13ml of 10% thiourea, then 1ml of 2,4-dinitrophenylhydrazine solution was added to form osazone, the same was done in a separate test tube containing 4ml of water as blank. All standards and blank

were kept in a water bath at 100^0C for 10minutes, after which all were treated with 5ml chilled 85% suphuric acid with constant stirring and the absorbance of the resultant red solution was taken at 527nm after zeroing the machine with the blank which contains all the reagent except the analyte (ascorbic acid).

3.2.5.2 *Reaction*

Ascorbic acid is oxidized to dehydroascorbic acid by bromine water; the thiourea added was to remove excess of the bromine. After that, the dehydroascorbic acid reacts with 2,4-dinitrophenylhydrazine and produces an osazone which when treated with 85% sulphuric acid forms a red colored solution.

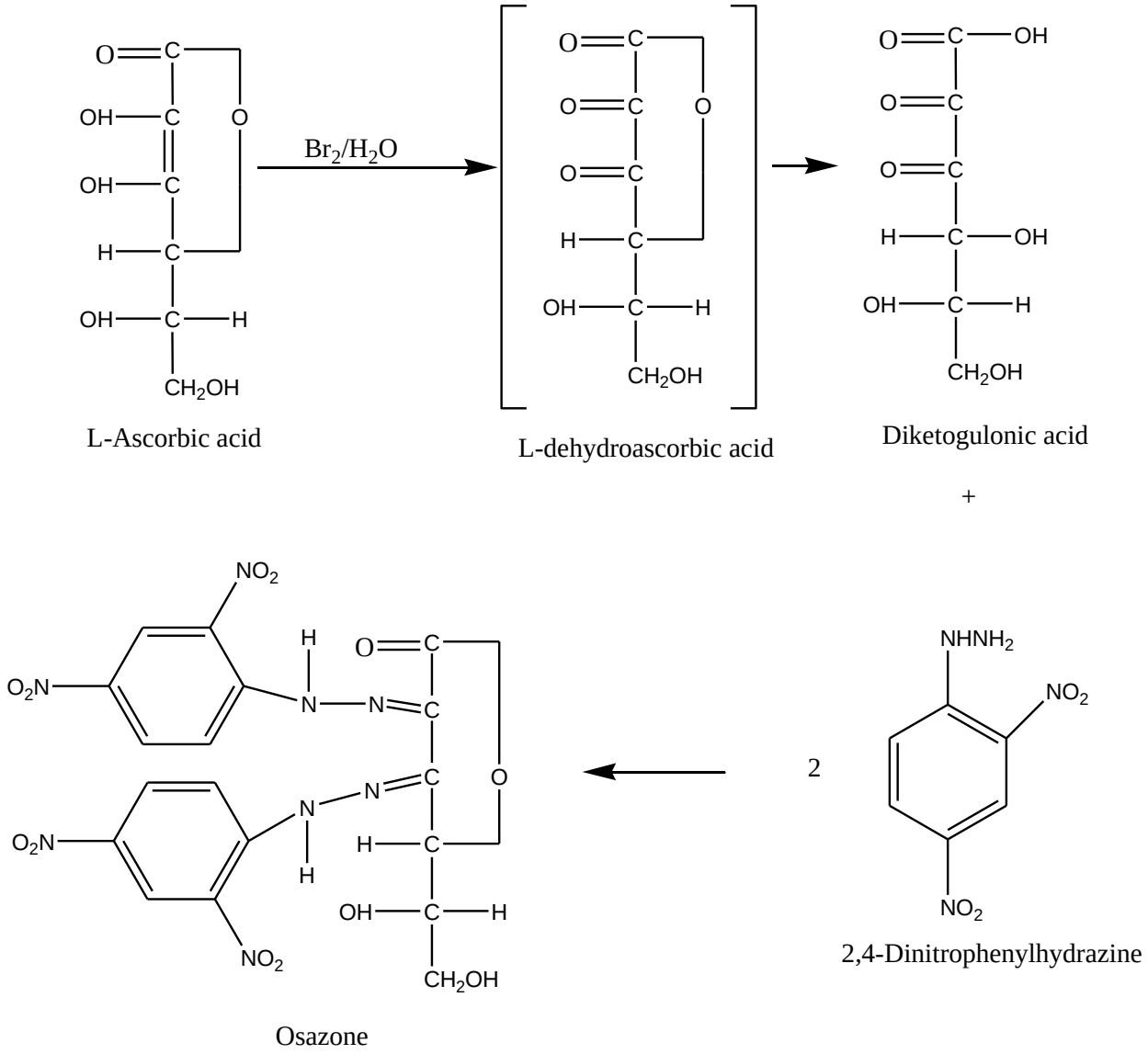


FIG 3.0 REACTION OF ASCORBIC ACID WITH 2,4-DINITROPHENYLHYDRAZINE.

3.2.5.3 *Determination Of Wavelenght Of Maximum Absorption*

The resulting red solution was scanned and a single peak was recorded at 527nm. This was repeated the next day and the same value was obtained. Standard solutions of different concentrations were prepared and their absorbance reading taken at 521nm and 527nm, (table1.0). The absorbance reading at 527 of the various samples gave higher values compared to those of 521 even after increasing the wave length.

The method was validated both in the time of reaction, temperature of the reaction and the wavelength of absorbance reading.

3.2.5.4 *Method Application*

Twenty tablets of ascorbic acid was powdered and weighed. A weight equivalent of 100mg was weighed and transferred to a conical flask containing 1000ml of deionized water and mixed thoroughly until all the ascorbic acids is dissolved. This is filtered using 450 Watman filter paper into a 100ml volumetric flask. 4ml of the solution (100µg/ml ascorbic acid) was transferred to a 50ml beaker, 0.23ml of 3% bromine water was added to the solution and mixed, to it 0.13ml of 10% thiourea was added. Then 1ml of 2, 4-dinitrophenylhydrazine solution was added to form osazone. This was heated for 10minutes at a temperature of 100 degrees. Then 5ml of chilled 85% sulphuric acid was added with constant stirring resulting in a red colored solution whose absorbance was taken at 527nm using a spectrophotometer. A 1/10 dilution was made for each of the samples.

CHAPTER FOUR

RESULTS

The results for all analysis carried out in this research work are stated in this chapter.

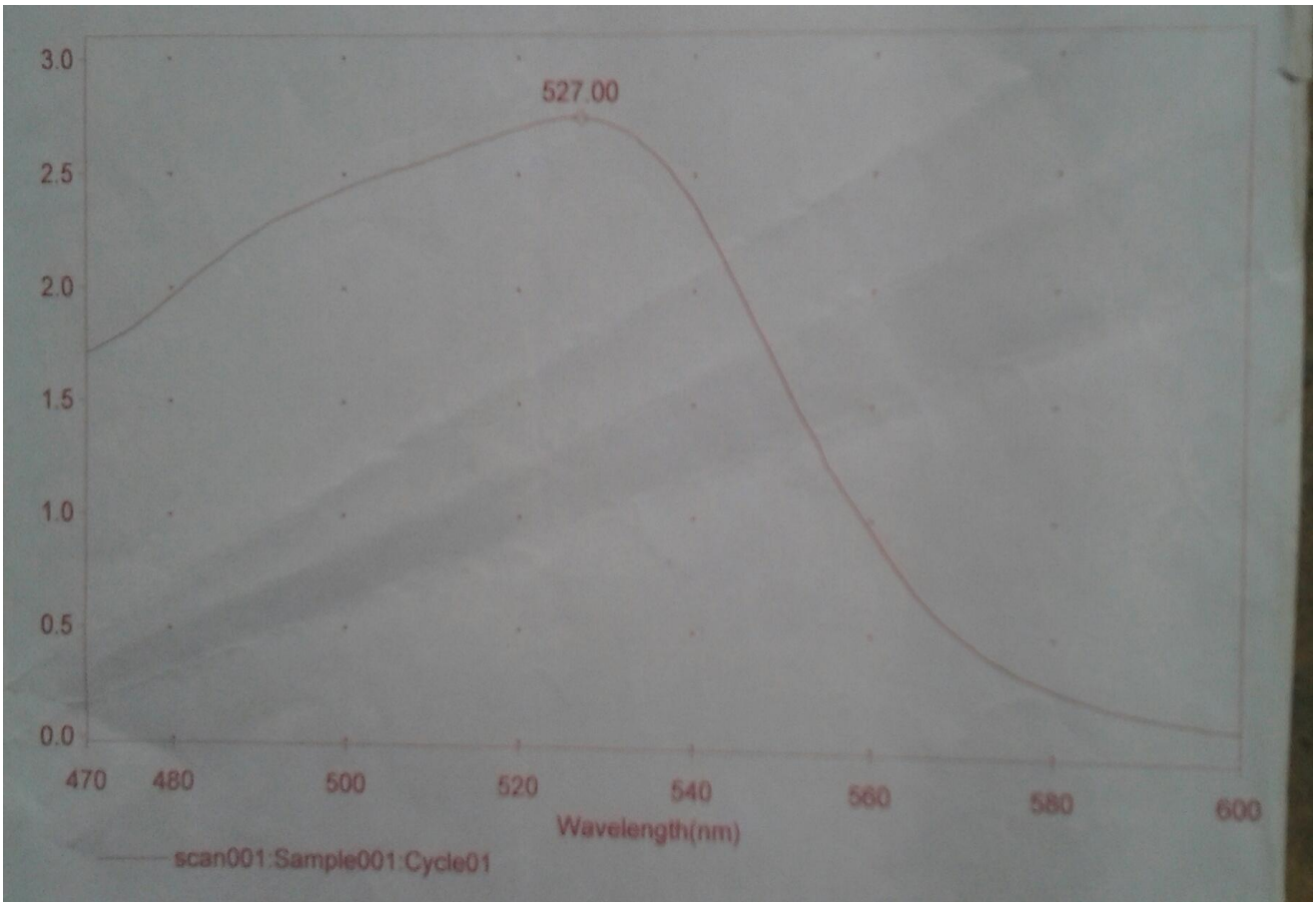


FIG.4.0 GRAPH OF WAVELENGHT OF MAXIMUM ABSORPTION.

TABLE 4.0

TABLE OF ABSORBANCE READINGS FOR THE DETERMINATION OF MAXIXMUM WAVE LENGTH.

S/n	Sample(µg/ml)	Absorbanc(521nm)	Absorbance(527nm)
1	5	0.300	0.311
2	10	0.394	0.408
3	15	0.526	0.542
4	20	0.732	0.747
5	25	0.902	0.908
6	30	1.149	1.155
7	40	1.577	1.581

4.1. CALIBRATION CURVE

4.1.1 Linearity

Six points calibration graph was constructed, covering a concentration range of 5-25ug/ml. Five different readings were taken for each concentration. Linear relationships between absorbance and concentration were observed.The standard deviations of the slope and intercept were low. The correlation coefficient (r^2) is 0.991.

The repeatability study was carried out at (n=5) and it showed a relative standard deviation of 0.15057, implying that the equipment used for the study worked correctly for the development of analytical method and is highly repetitive.

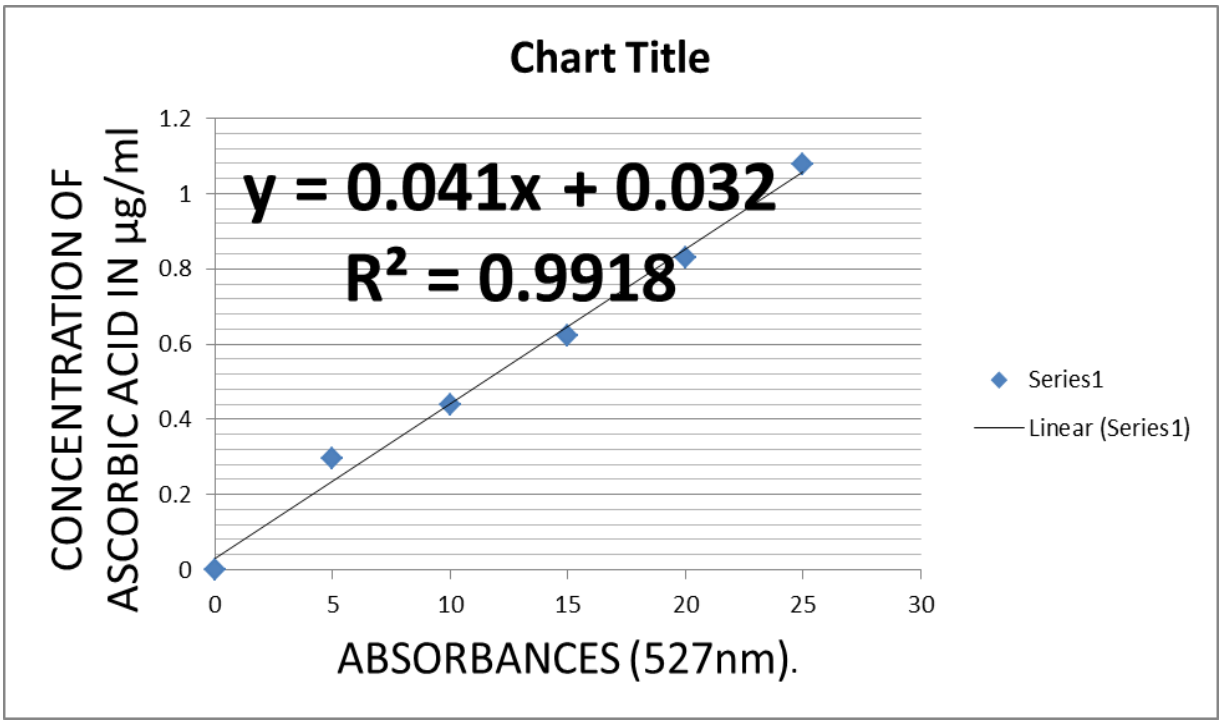


Fig 4.1. GRAPH OF CALIBRATION CURVE OF ASCORBIC ACID IN 2,4-DINITROPHENYL HYDRAZINE.

4.2 PRECISION STUDY

A sample solution of 5ug/ml was prepared and reacted following the procedure stated in the method, and their absorbances taken five times. The values obtained were recorded. This was repeated the next day and the values obtained recorded too. The values from the day before(between day precision) and the present day(within day precision) as recorded in table 2 and 3 were used to calculate for the precision. The value obtained gave 1.5057 percentage coefficient of variation.

TABLE 4.2.0

TABLE FOR WITHIN DAY PRECISION ABSORBANCE READING

S/n	Sample(µg/ml)	Absorbance(527nm)
1	5	0.297
2	5	0.297
3	5	0.297
4	5	0.297
5	5	0.296

TABLE 4.2.1

TABLE FOR BETWEEN DAY PRECISSION ABSORBANCE READING.

S/n	Sample(µg/ml)	Absorbance(527nm)
1	5	0.297
2	5	0.297
3	5	0.297
4	5	0.296
5	5	0.296

4.3 ACCURACY

The data for accuracy were expressed in terms of percentage error. Five samples of same concentration were prepared separately and their absorbances measured. (Table 5) The mean of the value obtained was used to calculate the percentage error (accuracy) and the value obtained was 1.75% relative percentage error.

TABLE 4.3

TABLE FOR ACCURACY ABSORBANCE READING

S/n	Sample(µg/ml)	Absorbance(527nm)
1	5	0.239
2	5	0.241
3	5	0.241
4	5	0.240
5	5	0.242

4.4 PERCENTAGE RECOVERY

3mls of ascorbic acid solution of 2ug/ml were prepared and transferred to each of five different test tubes labelled 1-5. To test tube 1, 1ml of 2ug/ml ascorbic solution was added, to test tube 2, 1ml ascorbic acid solution of 1ug/ml ascorbic acid was added, to test tube 3, 1ml of 2ug/ml was added, to test tube 4, 1ml of 3ug/ml ascorbic acid solution was added, to test tube 5 1ml of 4ug/ml ascorbic acid solution was added. To test tube 6 1ml of 5ug/ml ascorbic acid was added, giving concentrations of 2-7µg/ml. They were all reacted with the reagent following the procedure described in the method and the absorbances recorded. The values obtained (table 7) were used to calculate the percentage recovery.

The average value obtained for the five samples (percentage recovery) was 97.42%.

TABLE 4.4

TABLE FOR PERCENTAGE RECOVERY ABSORBANCE READING

S/n	Sample(µg/ml)	Absorbance(527nm)
1	2	0.134
2	4	0.239
3	5	0.293
4	6	0.324

4.5 LIMIT OF DETECTION

Five samples of 1-5ug/ml were prepared and reacted and their absorbancies were taken and recorded. The values obtained, as stated in table 8; were used to plot a regressional graph from which the value of the limit of detection was calculated to be 0.7778

TABLE 4.5.0

TABLE FOR LIMIT OF DETECTION ABSORBANCE READINGS

S/n	Sample(µg/ml)	Absorbance(527nm)
1	1	0.056
2	2	0.104
3	3	0.151
4	4	0.188
5	5	0.221

4.6 LIMIT OF QUANTITATION.

The value obtained was 2.357µg/ml

4.7 FINAL RESULTS OF PRODUCT ASSESSMENT

4.7.1 Result Table For UV Spec Analysis Absorbance Reading.

TABLE 4.8.1

S/n	Sample	Percentage Quantity
1	Calcium ascorbate	108.2
2	Emvitc	29.7
3	Topcee	26.3
4	Ascorvite	33
5	Bioracee	66.7
6	Ascorbion	46.7
7	Sofac	27.948

TABLE 4.7.2

Result Table For Sodium thiosulphate Titration Analysis.

S/n	Sample	Percentage Quantity
1	Calcium ascorbate	95.60
2	Emvitc	98.30
3	Topcee	101.90
4	Ascorvite	94.86
5	Bioracee	94.18
6	Ascorbion	98.89
7	Sofac	100.02

TABLE 4.7.3

Result Table For 2,4-Dinitrophenylhyrazine Uv Spectrophotometric Analysis.

S/n	Sample	Percentage Quantity
1	Calciun ascorbate	100.47
2	Emvitc	101.99
3	Topcee	107.898
4	Ascorvite	99.83
5	Bioracee	78.02
6	Ascorbion	101.97
7	Sofac	104.96

TABLE 4.7.4

Result Table For British Pharmacopoeia (2009) Analysis.

S/N	Sample	Percentage Quantity
1	Calcium ascorbate	100.58
2	Emvitc	102.489
3	Topcee	108.51
4	Ascorvite	100.12
5	Bioracee	79.29
6	Ascorbion	102.05
7	Sofac	104.84

CHAPTER FIVE

5.0 DISCUSSION OF RESULTS

The present study was undertaken to produce a sensitive and accurate UV spectrophotometric method for the estimation of ascorbic acid in tablet dosage forms, and compare it with other existing assay methods. The maximum absorption was found to be 527nm. Calibration equation for ascorbic acid was constructed by plotting the UV absorbance against the ascorbic acid concentration (analyzed in five cycles each). UV absorbance (y) of ascorbic acid over a concentration (x) range of 05-25µg/ml was linear at y=0.041x+0.032, with a regressional coefficient (r^2) of 0.9910. Fig 5 table 2. This result obtained indicates the linearity of the method.

The percentage recovery of the drug was found to be 97.8%. The limit of detection was found to be 0.7778 and the limit of quantitation was 2.357µg/ml. The accuracy of the method was 1.75%. In comparing the developed method with other existing methods, the result obtained is as follows; The result obtained from assaying ascorbic acid tablets with stabilizers showed great disparity with that of other three methods. That of sodiumthiosulphate titration showed values that are in close range with the BP and developed method. The values obtained from the B.P 2009 titrimetric method were comparable with that of the developed method and when subjected to students T-test for all the brands of ascorbic acid assayed, there was no significant difference.

In a similar study by Pouramorad *et al* (2003) in a comparative evaluation of ascorbic acid content of ascorbic acid tablets using polarography and titrimetric methods, it was reported that the method has comparable precision with the common methods. Also Danielczuk *et al*.,(2004) in comparing enzymatic methods of ascorbic acid analysis with other routine methods (titrimetric and spectrophotometric), it was reported that there was an agreement in the results obtained from the enzymatic and titrimetric methods. Also in another study by Zahoor *et al,* (2010) in which

several methods of ascorbic acid analysis which are n-bromosuccinyl method, glacia acetic acid method and a spectrophotometric method were compared. Results showed that all methods were comparable. Okiei *et al* (2009) also compared voltametric and titrimetric methods with N-bromosuccinyl of ascorbic acid analysis and reported that the results were comparable. In a report by Yew (2004) where he compared titrimetric and spectrophotometric methods for ascorbic acid analysis, he reported that the spectrophotometric method showed higer consistency and accuracy compared to titrimetric method, though both produced satisfactory results. Studies carried out by Nejati-Yazdinejad (2007), using spectrophotometric method for the indirect determination of ascorbic acid in fruits and pharmaceutical products showed a linearity range of 0.6-8.2ppm of which the detection limit was 0.35ppm, the method was said to be simple, inexpensive and rapid. In another work by Fernandes (2000), using potentiometry to determine L- ascorbic acid in pharmaceutical products, the values obtained were between 10^{-3} and 10^{-1} mol/L with the detection limit of 8.5×10^{-4} mol/L. The method was said to be rapid and expensive.

5.1 CONCLUSION

Based on the results obtained, it was found that the results from the developed 2,4 dinitrophenyhydrazine spectrophotometric method, the official B.P 2009 iodometric method and sodium thiosulphate titrimetric methods were within the detection limit, though only the first two were comparable, whereas the result of the UV spetrophotometric method using stabilizers of Kwakye, 2000 were off the detection limit and was in no way comparable with that of the official method. Also the developed method showed a high degree of accuracy, precision, reproducibility and is economical. It can be employed for routine quality control of pure ascorbic acid tablets. This method is also less laborious and does not involve much preparation of reagent and standardization as is the case in the B.P 2009 official method.

5.2 RECOMMENDATION

Further studies need to be done to ascertain the potency of the modified method on other pharmaceutical preparations containing ascorbic acid and (or) with carbonyl groups and compare it with other existing methods. Also the choice of qualitative method for evaluation of tablets should be chosen carefully for accurate results.The newly modified method should be employed in routine ascorbic acid analysis as a substitute for the B.P official method.

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THE APPENDIX

TABLE FOR RAW DATA OBTAINED FROM BP 2009 TITRIMETRIC METHOD

ASCORBION	IST TITRE	2ND TITRE	3RD TITRE	4TH TITRE
INITIAL BURETTE READING	0.00	17.50	0.00	17.50
FINAL BURETTE READING	17.50	35.00	17.42	34.90
AVERAGE VOLUME OF IODINE USED	17.50	17.50	17.42	17.40
% ASCORBIC ACID CONTENT	102.78	102.78	102.25	102.196

EMVIT C	IST TITRE	2ND TITRE	3RD TITRE	4TH TITRE
INITIAL BURETTE READING	0.00	18.50	0.00	18.50
FINAL BURETTE READING	18.50	37.00	18.40	37.00
AVERAGE VOLUME OF IODINE USED	18.50	18.50	18.40	18.50
% ASCORBIC ACID CONTENT	108.66	108.66	108.06	108.66

ASCORVITE	IST TITRE	2ND TITRE	3RD TITRE	4TH TITRE
INITIAL BURETTE READING	0.00	17.00	0.00	17.10
FINAL BURETTE READING	17.00	34.00	17.10	34.20
AVERAGE VOLUME OF IODINE USED	17.00	17.00	17.10	17.10
% ASCORBIC ACID CONTENT	99.84	99.84	100.4	100.4

TOPCEE	IST TITRE	2ND TITRE	3RD TITRE	4TH TITRE
INITIAL BURETTE READING	1.00	18.00	0.00	13.50
FINAL BURETTE READING	14.50	31.50	13.50	27.00

AVERAGE VOLUME OF IODINE USED	13.50	13.50	13.50	13.50
% ASCORBIC ACID CONTENT	79.29	79.29	79.29	79.29

BIORACEE	IST TITRE	2^{ND} TITRE	3^{RD} TITRE	4^{TH} TITRE
INITIAL BURETTE READING	0.00	17.40	0.00	17.40
FINAL BURETTE READING	17.40	34.70	17.40	34.80
AVERAGE VOLUME OF IODINE USED	17.40	17.30	17.40	17.40
% ASCORBIC ACID CONTENT	102.20	101.61	102.20	102.20

CALCIUM ASCORBATE	IST TITRE	2^{ND} TITRE	3^{RD} TITRE	4^{TH} TITRE
INITIAL BURETTE READING	0.00	18.00	0.00	17.1
FINAL BURETTE READING	17.10	35.10	17.10	34.20
AVERAGE VOLUME OF IODINE USED	17.10	17.10	17.10	17.20
% ASCORBIC ACID CONTENT	100.43	100.43	100.43	101.02

SOFA C	IST TITRE	2^{ND} TITRE	3^{RD} TITRE	4^{TH} TITRE
INITIAL BURETTE READING	0.00	17.80	0.00	17.80
FINAL BURETTE READING	17.80	35.60	17.80	35.80
AVERAGE VOLUME OF IODINE USED	17.80	17.80	17.80	18.00
% ASCORBIC ACID CONTENT	104.55	104.55	104.55	105.72

BASIC CALCULATIONS FOR ASCORBIC ACID CONTENT FOR BP 2009 TITRIMETRIC METHOD.

From the official book,

1ml of iodine = 8.81mg ascorbic acid.

Average volume of iodine × 8.81 = ascorbic acid content(mg)

% ascorbic acid content = $\frac{\text{Average volume of iodine} \times 8.81 = \text{ascorbic acid content(mg)}}{150} \times 100$.

Since weight of powdered ascorbic acid tablet equivalent to 0.15g was used for the analysis.

ABSORBANCE READING FOR MODIFIED 2,4-DINITROPHENYL HYDRAZINE ANALYSIS OF ASCORBIC ACID TABLETS.

ASCORBION	ABSORBANCE READING(527nm)	PERCENTAGE ASCORBIC ACID CONTENT
IST READING	0.448	101.82
2ND READING	0.449	102.05
3RD READING	0.447	101.59
4TH READING	0.451	102.50

EMVIT C	ABSORBANCE READING(527nm)	PERCENTAGE ASCORBIC ACID CONTENT
IST READING	0.483	109.773
2ND READING	0.472	107.273
3RD READING	0.472	107.273
TH READING	0.472	107.273

ASCORVITE	ABSORBANCE READING(527nm)	PERCENTAGE ASCORBIC ACID CONTENT
IST READING	0.439	99.773

2ND READING	0.439	99.773
3RD READING	0.440	100.00
4TH READING	0.439	99.773

TOPCEE	ABSORBANCE READING(527nm)	PERCENTAGE ASCORBIC ACID CONTENT
IST READING	0.344	78.18
2ND READING	0.342	77.72
3RD READING	0.344	78.18
4TH READING	0.343	78.00

BIORACEE	ABSORBANCE READING(527nm)	PERCENTAGE ASCORBIC ACID CONTENT
IST READING	0.450	102.27
2ND READING	0.448	101.80
3RD READING	0.448	101.80
4TH READING	0.449	102.00

SOFA C	ABSORBANCE READING(527nm)	PERCENTAGE ASCORBIC ACID CONTENT
IST READING	0.462	105.00
2ND READING	0.463	105.23
3RD READING	0.461	104.80
4TH READING	0.461	104.80

CALCULATIONS.

The Value Of The Absorbance Reading Is Substituted Into The Equation Of The Calibration Curve;

$Y = 0.041X + 0.032$

Where Y Is the Absorbance

And X The Concentration in µg/ml

The value obtained for the concentration is divided by 10 and multiplied by 100. Since ascorbic acid powder equivalent to 100ug/ml was used and 1/10 dilution was done for each of the samples.

ABSORBANCE READING FOR UV SPEC ANALYSIS USING STABILIZERS.

	ABSORBANCE READING(264nm)	PERCENTAGE ASCORBIC ACID CONTENT
CALCIUM ASCORBATE	3.324	108.02
ASCORBION	0.901	29.28
EMVIT C	0.810	26.32
ASCORVITE	1.085	34.38
TOPCEE	2.019	67.92
BIORACEE	1.407	45.726
SOFA C	0.860	27.948

CALCULATIONS FOR UV- SPEC ANALYSIS.

Calculations

A=ECL

Where A = Absorbance

E = Molecular absorptivity

C = Sample concentration, mol/L

L = Length of sample path, cm

E= 0.1[A (1%1cm) x molecular weight]

Molecular weight of L-Ascorbic Acid = 176.1g/mol

A^{1}_{1}% of L-Ascorbic Acid at 246 wavelength = 579

E=0.1[579x176.1]

E=0.1[101961.9]

E=10196.19

C = A/EL

C = 3.077/10196.19x1

C = 0.00030%$^{W}/_{V}$

Therefore the theoretical concentration = 0.00030%$^{W}/_{V}$

Sample calculation for brand CALCIUM ASCORBATE

Absorbance = 3.324

C = A/EL

C = 3.324/10196.19x1

C = 0.00031%$^{W}/_{V}$

% ascorbic acid content = Experimental value/Theoretical value x 100%

= 0.00031/0.00030 x100%

= 108.02%

BURETTE READINGS FOR SODIUMTHIOSULPHATE TITRATION OF ASCORBATE AND ASCORBIC ACID TABLETs.

CALCIUM ASCORBATE	IST TITRE	2ND TITRE
INITIAL BURETTE READING	0.50	0.00
FINAL BURETTE READING	22.50	22.00
TOTAl VOLUME OF IODINE USED	22.00	22.00
AVERAGE VOLUME OF IODINE USED	22.00	
% ASCORBIC ACID CONTENT	95.60	

ASCORBION	IST TITRE	2ND

		TITRE
INITIAL BURETTE READING	0.01	0.00
FINAL BURETTE READING	21.6	22.00
AVERAGE VOLUME OF IODINE USED	21.80	
% ASCORBIC ACID CONTENT	101.90	

EMVIT C	IST TITRE	2ND TITRE
INITIAL BURETTE READING	0.00	5.00
FINAL BURETTE READING	21.50	26.5
TOTAL VOLUME OF IODINE USED	21.50	21.50
AVERAGE VOLUME OF IODINE USED	21.50	
% ASCORBIC ACID CONTENT	94.18	

ASCORVITE	IST TITRE	2ND TITRE
INITIAL BURETTE READING	0.00	0.00
FINAL BURETTE READING	22.50	21.50
TOTAL VOLUME OF IODINE USED	22.50	21.50
AVERAGE VOLUME OF IODINE USED	22.00	
% ASCORBIC ACID CONTENT	94.86	

TOPCEE	IST TITRE	2ND TITRE

INITIAL BURETTE READING	0.00	0.00
FINAL BURETTE READING	21.00	21.50
TOTAL VOLUME OF IODINE USED	21.00	21.50
AVERAGE VOLUME OF IODINE USED	21.25	
% ASCORBIC ACID CONTENT	98.89	

BIORACEE	IST TITRE	2^{ND} TITRE
INITIAL BURETTE READING	0.50	0.00
FINAL BURETTE READING	21.00	20.00
TOTAL VOLUME OF IODINE USED	21.00	20.00
AVERAGE VOLUME OF IODINE USED	20.05	
% ASCORBIC ACID CONTENT	98.30	

SOFA C	IST TITRE	2^{ND} TITRE
INITIAL BURETTE READING	0.00	0.00
FINAL BURETTE READING	22.28	22.00
TOTAL VOLUME OF IODINE USED	22.28	22.00
AVERAGE VOLUME OF IODINE USED	22.14	
% ASCORBIC ACID CONTENT	100.02	

CALCULATIONS FOR SODIUMTHIOSULHATE TITRATION

MOL_{THIO} = CONC. X VOL. OF THIOSULPHAT USED IN TITRATION.

From The Stoichiometry of the Reaction;

$I_2 + 2S_2O_3^{-2} \rightarrow 2I^- + S_4O_6^{-2}$

1 MOLE OF THIOSULPHATE = 2MOLES IODINE

EXCESS IODINE = 0.5 X MOLE OF THIOSULPHATE

$=0.5 \text{ X } MOL_{THIO}$

NET MOLE OF IODINE = TOTAL MOLE – EXCESS MOLE.

TOTAL MOLE OF IODINE USED (CONC OF IODINE) = 0.01

$= 0.01 – (0.5 \text{ X } MOL_{THIO)}$

NET MOLE OF IODINE USED = MOLES OF ASCORBIC ACID

SINCE THE STOICHIOMETRY OF THE REACTION IS 1 : 1.

$$\% \text{ OF ASCORBIC ACID} = \frac{\text{MOLES OF ASCORBIC ACID X 176.1g}}{0.25} \text{ x } 100.$$

Since ascorbic acid tablet powder of weight equivalence of 0.25g was used in the titration.

Molar mass of ascorbic acid = 176.1

RESULTS OF STUDENT T- TEST ANALYSIS OF PERCENTAGE ASCORBIC ACID CONTENT OF ASCORBATE AND ASCORBIC ACID TABLETS FROM THE BP 2009 OFFICIAL METHOD AND 2,4-DINITROHYDRAZINE METHOD.

CALCIUM ASCORBATE

result table of Analyzed Data 1

Column A(BP 2009) vs Column B (2'4-DINITROPHENYLHYDRAZINE)

100.43	100.90
100.43	101.20
100.43	99.77
101.02	100.00

Unpaired t test

P value 0.3896

P value summary ns

Are means signif. different? (P < 0.05) No

One- or two-tailed P value? One-tailed

t, df t=0.2931 df=6

Mean ± SEM of column A 100.6 ± 0.1475 N=4

Mean ± SEM of column B 100.5 ± 0.3450 N=4

Difference between means 0.1100 ± 0.3752

tguj 95% confidence interval -0.8082 to 1.028

ASCORBION

result table of Analyzed Data 1

Column A(BP 2009) vs Column B (2'4-DINITROPHENYLHYDRAZINE)

102.780	101.82
102.780	102.05
102.200	101.59
102.196	102.50

Unpaired t test

P value	0.0500
P value summary	ns
Are means signif. different? (P < 0.05)	No

One- or two-tailed P value? One-tailed

t, df t=1.943 df=6

How big is the difference?

Mean ± SEM of column A	102.5 ± 0.1680 N=4
Mean ± SEM of column B	102.0 ± 0.1942 N=4
Difference between means	0.4990 ± 0.2568
95% confidence interval	-0.1294 to 1.127
R squared	0.3862

EMVIT C

result table of Analyzed Data 1

Column A(BP 2009) vs Column B (2'4-DINITROPHENYLHYDRAZINE)

108.660	109.773
108.660	107.273
108.060	107.273
108.660	107.273

Unpaired t test

P value	0.1889
P value summary	ns
Are means signif. different? (P < 0.05)	No

One- or two-tailed P value? One-tailed

t, df t=0.9522 df=6

How big is the difference?

Mean ± SEM of column A	108.5 ± 0.1500 N=4
Mean ± SEM of column B	107.9 ± 0.6250 N=4
Difference between means	0.6120 ± 0.6427
95% confidence interval	-0.9608 to 2.185
R squared	0.1313

F test to compare variances

F,DFn, Dfd	17.36, 3, 3
P value	0.0212
P value summary	*
Are variances significantly different?	No

ASCORVITE

result table of Analyzed Data 1

Column A(BP 2009) vs Column B (2'4-DINITROPHENYLHYDRAZINE)

99.840	99.773
99.840	99.773
100.400	100.000
100.400	99.773

Unpaired t test

P value	0.0706
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
t, df	t=1.694 df=6

How big is the difference?

Mean ± SEM of column A	100.1 ± 0.1617 N=4
Mean ± SEM of column B	99.83 ± 0.05675 N=4
Difference between means	0.2902 ± 0.1713
95% confidence interval	-0.1290 to 0.7095
R squared	0.3235

BIORACEE

result table of Analyzed Data 1

Column A(BP 2009) vs Column B (2'4-DINITROPHENYLHYDRAZINE)

102.200 102.270

101.610 101.800

102.200 101.800

102.200 102.000

Unpaired t test

P value	0.3308
P value summary	ns
Are means signif. different? (P < 0.05)	No

One- or two-tailed P value? One-tailed

t, df t=0.4600 df=6

How big is the difference?

Mean ± SEM of column A	102.1 ± 0.1475 N=4
Mean ± SEM of column B	102.0 ± 0.1113 N=4
Difference between means	0.08501 ± 0.1848
95% confidence interval	-0.3672 to 0.5372
R squared	0.03407

F test to compare variances

F,DFn, Dfd	1.756, 3, 3
P value	0.3276
P value summary	ns
Are variances significantly different?	No

SOFA C

result table of Analyzed Data 1

Column A(BP 2009) vs Column B (2'4-DINITROPHENYLHYDRAZINE)

104.550	105.000
104.550	105.230
104.550	104.800
105.720	104.800

Unpaired t test

P value	0.3617
P value summary	ns
Are means signif. different? (P < 0.05)	No

One- or two-tailed P value? One-tailed

t, df t=0.3711 df=6

How big is the difference?

Mean ± SEM of column A	104.8 ± 0.2925 N=4
Mean ± SEM of column B	105.0 ± 0.1023 N=4
Difference between means	-0.1150 ± 0.3099
95% confidence interval	-0.8733 to 0.6433
R squared	0.02244