A STUDY OF THE QUALITY OF SELECTED MULTIVITAMIN SUPPLEMENTS IN NAIROBI CITY, KENYA

A thesis submitted in partial fulfillment of the requirements for the award of the degree of Master of Pharmacy in Pharmaceutical Analysis of the University of Nairobi

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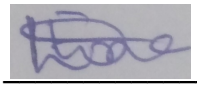
Signature 

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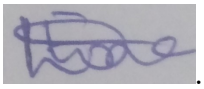
Sign……  …… Date ……22 November 2020……..

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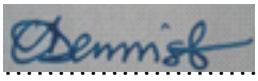
Sign……  …….. Date..22 November 2020..

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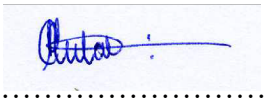
Sign…….  Date……22 November 2020..

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DEDICATION

This work is dedicated to my family

To my parents Cosmus Mutua and Jaqueiline Mutua, thank you for having Faith in me and convincing me that I can make it and importance of hard work in life.

To my siblings, Muthio, Tony, Diana and Makosi thank you for the moral support

To my late grandfather, John N Mbiti, you left us before the work was complete, thank you for your wise counsel and advices. Till we meet again.

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LIST OF ABBREVIATIONS AND ACRONYMS

`Abbreviation/Symbol	Full name(s)
ACP	Acyl carrier protein
BP	British Pharmacopeia
˚C	Degree Celsius
CBD	Central Business District
CE	Capillary Electrophoresis
CFU	Colony Forming Unit
CoA	Coenzyme A
DAD	Photo Diode Array Detector
FDA	The US Food and Drug Administration
FLD	Fluorescence Detector
FSA	Food Standards Authority
HPLC	High Performance Liquid Chromatography
ICH	International Conference on Harmonization.
IU	International unit
LIF	Laser-Induced Fluorescence
MEKC	Micellar Electro kinetic Chromatography
MEEKC	Micro Emulsion Electro kinetic Chromatography
CEC	Capillary Electrophoresis Chromatography
MVMS	Multivitamin Mineral Supplements
MVS	Multivitamin Supplements

NA	Nutrient Agar
NQCL	National Quality Control Laboratory
PPB	Pharmacy and Poisons Board
RSD	Relative Standard Deviation
SDA	Sabouraud Dextrose Agar
TAMC	Total Aerobic Microbial Count
TMP	Thiamine Monophosphate
TPP	Thiamine Pyro-phosphate
TTP	Thiamine Triphosphate
TYMC	Total yeast/ molds count
USP	United States Pharmacopeia
UV-VIS	Ultraviolet - Visible Spectroscopy
WHO	World Health Organization

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ABSTRACT

Vitamins are invaluable nutrients that play integral roles in human bodies such as maintenance of cell functions. Vitamins are broadly classified into two groups: fat-soluble and water-soluble vitamins. Humans do not synthesize vitamins but obtain them from food and therefore it is important to maintain a balanced diet for continuous supply. Vitamin deficiencies mainly arise from malabsorption and malnutrition. In such instances, vitamin supplements become important to augment the diet or act as replacement. Vitamin supplements will contain either one category of vitamins or both, with or without minerals. Data on quality studies and literature reviews on regulation of multivitamin supplements in the Kenya is not available.

The main objective of this study was to determine the quality of multivitamin supplements. Quality was assessed for packaging and labelling, assay of content and microbial contamination.

Purposive random sampling was applied whereby samples were collected randomly from pharmacy outlets in Nairobi County Central Business District area. Samples collected consisted of liquid and solid dosage forms. A total of 40 samples were collected, consisting of solid dosage forms (14 tablet formulated products and 6 hard gelatin capsules) and liquid dosages forms (19 syrups and 1 oral drop).On conformity with labeling and packaging specifications, manufacturing/expiry dates, batch numbers, patient information leaflets, storage conditions and country of origin were inspected. High Performance Liquid Chromatography adopted methods were used for the assay test, on which one was applied for simultaneous determination of water-soluble vitamins (B_1, B_3, B_6 and folic acid) while the other was used in fat-soluble vitamins (A, D and E) analysis. The water- soluble method was verified for accuracy, linearity, range and repeatability as per the ICH guidelines. Microbial Load determination was carried out by plate count method by 10 fold serial dilutions.

For the compliance with packaging and labelling, 21% of the samples did not contain patient information leaflets but complied with the Pharmacy and Poisons Board (PPB) packaging and labeling specifications on all other aspects (Presence of manufacturing/expiry dates, batch numbers and storage conditions). The linearity range of the analyzed vitamins spanned from (50-200%) with a regression coefficient (R^2) being > 0.99; accuracy was demonstrated by recoveries

that were in the range of 98-102% while precision was measured using the RSD of six injections which was < 2percentage in all instances. Samples collected originated from six countries: India (37.5%), Kenya (27.5%), England (25%), Bangladesh (5%), Pakistan (2.5%), and Egypt (2.5%). In determination on the label claim compliance, 14.3% of the samples met the pharmacopeial limits. For water- soluble vitamins, two (7.1%) samples complied with the pharmacopeial specifications while none complied with the assay limits for fat-soluble vitamins. Further review of the results for the water - soluble vitamins, 51% failed to meet the pharmacopeia specifications (40.9% below the limits, 31.8% above and 27.3% within the specified range), 21% did not comply on pyridoxine (20% were below and above the specifications, & 60% within the range) , 15% on folic acid (50% below the limits, 8.5% above and 41.7% within) and 13% were outside nicotinamide limits (11.1% below, 16.7% above and 72.2% within the limits). All the samples complied with Total Aerobic Microbial Count (TAMC) as per the BP 2018 specifications while two (10.5%) samples failed to comply with the Total Yeast and Molds Count (TYMC).

From the study, all the samples complied with TAMC specifications, while 89% of the samples complied with the BP 2018 specifications for TYMC in non-sterile products as well as labelling and packaging specifications as per the PPB guidelines. On the other hand, 85.7% failed to comply with the assay specifications for both water- and fat- soluble vitamins. These results indicate that there is a gap in the regulation of multivitamin supplements in the country. Therefore, the regulator should adopt a risk-based approach to assure the quality of multivitamin products are in the market. In addition, the Pharmacy and Poisons Board should have an updated database for all multivitamin supplements in the Kenyan market. The university should encourage more research on multivitamin in the Kenyan context to generate more data on the quality of multivitamins

CHAPTER ONE: GENERAL INTRODUCTION

1.1 Background

Vitamins are minor constituents of food but play an integral part in body functioning and also play a part in growth and development, body self-repair, maintenance and normal functioning of cells and organs in both humans and animals. Demand for vitamins markedly rises during growth periods, pregnancy, and lactation (1). Vitamins are categorized as nutritional supplements and can further be defined as products apart from tobacco intended for supplementing diet and they do not elicit medicinal effects (2). Multivitamin supplements may refer to either a brand with different quantities and doses of vitamins and minerals while others have vitamins only (3). Accuracy of label claim is important in order to ensure the daily consumption is per the recommended daily allowance. In pharmaceutical formulations, vitamin loss during manufacturing can occur due to; technology employed during manufacturing, the type of formulation been manufactured and storage conditions (3). Deficiency arises from insufficient amounts in food, which is the major sources of vitamins. In case of deficiency or increased body requirements, the need of supplementing arises.

Balanced diet is important to maintain the required levels of vitamins in the body since humans do not synthesize them. High concentrations of water-soluble vitamins in the body are harmless because they readily dissolve then are excreted in urine. On the other hand, liver acts as a reservoir of excess fat-soluble vitamins and can hold reserves for vitamin A for many months. Deficiency may be due to malnutrition and malabsorption. To rectify these deficiencies, dietary supplementation become important. Different multivitamins formulations, which include, hard gelatin capsules, soft gelatin capsules, suspensions and tablets are available in the market.

The world health organization (WHO) and the American Medical Association (AMA) both recommended changes in regulation of the dietary supplements. The changes will require dietary supplements to have the food and drugs authority (FDA) approval about evidence of efficacy and safety to meet USP specifications for quality, identity, strength packaging, labeling and the post marketing rules on reporting of adverse effects (5). FDA is encouraging manufacturers' of dietary supplements and foods to make accurate and research-based evidence on the benefits of this products in order to eliminate falsified claims (6).

1.2 History of vitamins

Casimir Funk a polish biochemist devised the word *vitamine* ("vital amines") in 1912 (7). It was a term, which denoted essential factors in a diet with the assumption that all these factors were amines. However later, it was discovered they were distinct chemically and only some were amines. 150 years prior, James Lind's, a British naval physician had discovered that citrus juice, was a good source of an important factor that prevented sailors from scurvy and later after two centuries it was discovered out to be vitamin C (8). The first vitamin, thiamin, was isolated in 1913, and its deficiency causes beriberi (9). Subsequently thirteen vitamins have been identified as important factors in nutrition (10). At first when vitamins were discovered, alphabetical letters were used for identification. Afterwards they were isolated to pure forms and their chemical structures elucidated, chemical names were allocated. Nomenclature by chemical names is the right correct way even though the original form of letter numbering which is more familiar is still used (11). Food fortification was started 1924 in the United States where to prevent goiter, iodine was added to table salt in Switzerland and US in 1933 (12), vitamin D to milk for rickets prevention, margarine fortified with vitamin A in Denmark and in 1941, thiamine, riboflavin, niacin, and iron to flour (13). Pharmacies as well as grocery stores began stocking multivitamin products in the mid-1930s as people appreciated the importance vitamins.

In the United States vitamin deficiency has been eliminated (14). The elimination of deficiencies is attributed to increased food fortification and uptake of vitamin supplements. Rise in supplement intake can be attributed to increased exposure and marketing (10).

1.3 Regulation of Multivitamin supplement

The regulation of multivitamin supplements differs across different jurisdictions internationally. In some countries they are classified as foods, while in others regulate them as health products or medicines.

The World Health organization (WH O) Food and Agricultural Organization (FAO) have established Codex Alimentarius Commission (CAC). CAC is compilation of standards, guidelines and codes of practice to enhance consumer protection, harmonize food standards and promote fair

practices in food trade (15).The organization in 2005 adopted guidelines for vitamins and mineral supplements to guide on the composition, safety, purity and bioavailability of supplements.

In the USA, the congress ratified the Dietary Supplement Health and Education Act (DSHEA) that placed dietary supplement regulation under the FDA and through this change (16). However, the FDA regulates supplements less rigorously than conventional and non-conventional medicines (17). This has left consumers to depend on the industries to ensure they produce quality products (18).

Multivitamin supplements in the European Union are regulated under directive 2002/46/EC (European Commission 2002a). The European Food Safety Authority determines the safe maximum and minimum levels of vitamins and minerals(19).

Japan in Asia, multivitamin supplements are regulated under the ministry of health labour and welfare where they are classified as foods with health claims (20). The food health claims are categorized into three sub-groups; foods with nutrition function, foods for specific health uses and those with non-function claims. Therefore, multivitamin supplements are regulated under the foods with health claims.

There was no information on how the African Union or the regional bodies such as the East Africa Community regulate multivitamin supplement. However, in Kenya, the regulator (Pharmacy and Poisons Board) has provided guidelines for listing of dietary supplements. In the guidelines, multivitamin supplements are classified as dietary supplements. In this categorization, they are not required to undergo the rigorous registration process as conventional medicines (2). In addition, no data, reviews or survey on the quality of multivitamins/dietary supplements in the Kenyan market.

1.4 Classification of vitamins

Vitamins fall into two classes: fat-soluble vitamins or water - soluble vitamins depending on their physical characteristics to dissolve in either aqueous solutions or fats. Vitamin A (retinol), D (calciferous), E (tocopherols), K (phylloquinone and menaquinones) are fat-soluble while vitamins C (ascorbic acid) and B_1 (thiamine), B_2 (riboflavin), B_3 (niacin), B_5 (pantothenic acid), B_6

(pyridoxine), B_7 (biotin), B_9 (folate), and B_{12} (cobalamin) are water–soluble (21). Table 1.1 gives as summary of some of the physicochemical characteristics of selected vitamins.

Table 1.1 Physicochemical characteristics of vitamins

Vitamin	Solubility	Stability
B_1	Dissolves in water, slightly soluble in ethanol, and insoluble in ether and benzene	Stable in acids, sensitive to heat and unstable on heat.
B_2	Dissolves in bases, slightly soluble in ethanol and water, insoluble in chloroform and ether.	Not stable on exposure to light and heat, slightly unstable in basic solutions.
B_3	Completely soluble in water, ethanol and glycerin	Stable in both acids and bases and on air.
B_5	Dissolves completely in water, ethanol, carbonates, hydroxide solutions and insoluble in ether.	Unstable in bases and acids, heat but the Calcium salts are stable.
B_6	Dissolves in water, ethanol, methanol, and acetone. Does not dissolve in ether and chloroform	Stable in acids but unstable in basic solutions.
B_9	Soluble in alkalis, sparingly soluble in methanol, insoluble in water and ethanol	Stable on exposure to air, unstable in light conditions.
C	Soluble in water and ethanol, unsolvable in ether, acetone, and chloroform.	Decomposes upon exposure to air.
Retinol	Soluble in primary alcohols, chloroform, ether and oils. Does not dissolve in water and glycerin.	Easily oxidized, highly hygroscopic.
D	Soluble in alcohol, ether, acetone, chloroform, and vegetable oil, insoluble in water.	More stable than ergocalciferol. Stable at 4^oC in dark conditions.
α-Tocopherol	Dissolves in alcohol, ether, acetone, chloroform, oil .Insoluble in water	Stable in basic solutions and on heat, slightly oxidized in air, unstable in Ultra violet light.

Table adopted from (22).

1.4.1 Water- soluble vitamins

Niacin

Niacin comprises of nicotinamide and nicotinic acid. In the body, niacin is used in the synthesis of nicotinamide-adenine dinucleotide [NAD (H)] and nicotinamide–adenine dinucleotide phosphate NADP (H). NAD (H) and NADP (H) are pyridine nucleotides. These nucleotides are

coenzymes which transport protons and electrons in reduction-oxidation reactions in the body (23). Deficiency of niacin causes pellagra, normally linked with cereal diet with low amounts of niacin, tryptophan and nutrients required in synthesis of tryptophan and niacin. In the body, niacin is synthesized from tryptophan.

Niacin (Figure 1.1) is a pyridine-3-carboxylic acid and has a MW of 123.1. It forms colourless, spikes that are non-hygroscopic and stable in air. At temperature of 234-237 $^{\circ}$C, it sublimes without decomposition. It is amphiprotic with pKa values of 4.9 and 2.07, the pH of the concentrated solution is 2.7, it dissolves in hot water, alcohol, hydroxides of alkali metals, carbonates, propylene glycol, and it is insoluble in diethyl ether. It has a molar extinction coefficient of 2300 M^{-1} cm^{-1} in 0.005 M KH_2PO_4 buffer pH 7.0 at 260 nm.

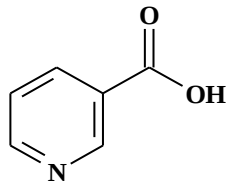

Figure 1.1 Chemical structure niacin

Nicotinamide

Nicotinamide (Figure 1.2) is an amide whose chemical name is pyridine-3-carboxamide ($C_6H_6ON_2$ MW 122.12) and a melting point of 129-131 ^{o}C. It exists in form of a colourless, crystalline compound that on crystallization forms benzene like needles. It is completely soluble in acetone, chloroform and butanol and soluble in ether and benzene. When heated in acids and alkali at a temperature of 100 $^{\circ}$C it changes to nicotinic acid without disintegration. Its molar extinction coefficient is 3300 M^{-1} in water at 260 nm. The food are as listed in Table 1.2.

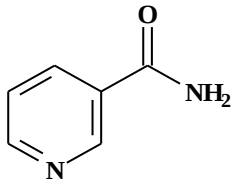

Figure 1.2 Chemical structure of nicotinamide

Nicotinic acid in large doses lowers serum cholesterol in hypercholesterolemia and this further reduces triglycerides levels. Reduced triglycerides levels prevent the risk of atherosclerosis and coronary heart disease. It also acts as a vasodilator (23) as well as reducing urinary excretion of amino acid tryptophan; which in turn increases the amounts of serotonin synthesized. High concentrations of serotonin enhances anti-depressive effects (24). In addition, nicotinamide prevents destruction of NAD dependent islets of Langerhans β-cells (23) and NAD^+ repairs damaged skin DNA.

Folate (vitamin B_9)

The backbone structure of folate (Figure 1.3) is Pteroyl-L-glutamic acid (folic acid). It is highly stable and this property allows it to be used in food fortification (23).

Folate is used as a coenzyme in the biosynthesis of pyrimidine and purine and in amino acid interconversions. Its role in red blood cell synthesis is connected to cyanocobalamin thus a severe deficiency of both folic acid and cyanocobalamin leads to megaloblastic anemia. Shortage of folic acid during pregnancy increases chances of neural tube teratogenic defects in infants while in adults it may cause cardiovascular diseases, diminished cognitive ability and cancer (25).

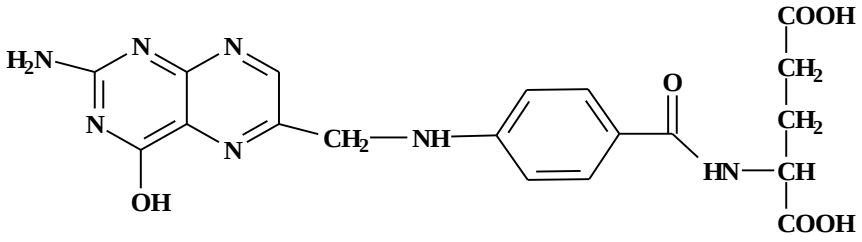

Figure 1.3 Chemical structure of folic acid

Thiamine (vitamin B_1)

The chemical structure consists of a pyrimidine group and a thiazole group joined by a methylene group. The three thiamine esters found in the body are thiamine monophosphate (TMP), thiamine pyrophosphate (TPP) and thiamine triphosphate (TTP). TPP is the active coenzyme form of thiamine and it represents about three quarters of the entire content of thiamine in cells, however, thiamine triphosphate lacks any coenzyme activity. Dietary deficiency causes beriberi that is found in two forms: the cardiac and neurological forms. The Cardiac form presents with heart failure and is prevalent in neonates whereas the neurological form is connected with chronic peripheral neuritis (26). Thiamine deficiency is rare and occurs in famine and drought stricken areas. Major causes of deficiency are thiamine metabolism disorder and chronic alcoholism. Other factors are intake of high amounts of processed cereals.

The active form of thiamine is coenzyme thiamine pyrophosphate (TPP). It is used carbohydrates and amino acids metabolism. The enzymes include pyruvate dehydrogenase, pyruvate decarboxylase, transketolase, oxoglutarate dehydrogenase, carboxylate carboligase and branched chain α-acid dehydrogenase. In the pyruvate and oxoglutarate oxidative decarboxylation, TPP carries aldehyde to form hydroxyethyl-TPP and α-hydroxyl-β-carboxypropyl-TPP. Hydroxyethyl-TPP is relocated to coenzyme A (CoA) to make α-hydroxyl-β-carboxypropyl-TPP while acetyl-CoA forms succinyl-CoA. It also preserves the nerve functions by acting as an antipolyneuritis factor (23).

The chloride – hydrochloride double salt of thiamine ($C_{12}H_{17}N_4OSCl^-HCl$) dissolves completely in water, slightly in methanol and glycerol and insoluble in ether. In water, thiamine is stable at pH 2 and 4 while it is unstable at alkaline pH and on heating. Factors affecting decomposition include pH and heat exposure time.

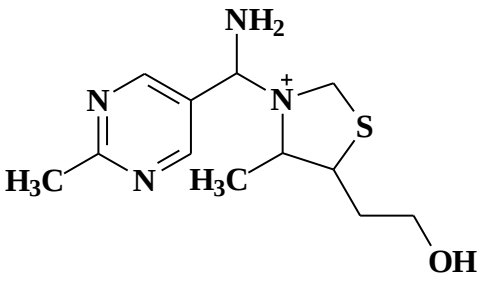

Figure 1.4 Chemical structure of thiamine

Pyridoxine (Vitamin B_6)

Pyridoxine was discovered in 1932 as a component of the B complex which acted as an anti-pallegra factor (28). In the body, pyridoxine exists in several forms: pyridoxamine (an amine), pyridoxine and the 5' phosphates. Notably, Pyridoxal-5'-phosphate coenzyme is the most prevalent and is required in heme synthesis and amino acid metabolism (29).. Severe deficiency results from chronic alcoholism and low consumption of dairy products. Deficiency causes dermatitis, convulsion, peripheral neuritis, brain dysfunction, depression and microcytic anemia

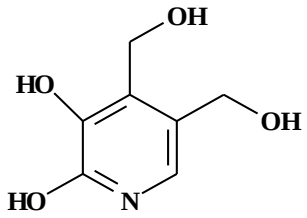

Figure 1.5 Chemical structure of pyridoxine

1.4.2 Fat-soluble vitamins

Vitamin A

Vitamin A was discovered in 1911 as a component of diet, which was essential for growth (30). The term *vitamin A* (Figure 1.6) is a general term for compounds with a β-ionone structure and exhibit retinol like biological activity. Carotenoids include β-carotene, α-carotene and cryptoxanthin. Cryptoxanthin are mainly in yellow vegetables and fruit. Carotenoids are precursor forms of vitamin A and are converted to retinol after first-pass metabolism to make them

biologically active. On the other hand, retinoid are vitamers of vitamin A that can be either synthetic or natural. Carotenoids are not classified as retinoid (31). Vitamin A maintains optimal functioning of visual system, cells, maintaining integrity of epithelial cells, reproduction and maintaining body immunity. Deficiency causes night blindness, keratinization of the mucus ciliated epithelium amongst other epithelial changes that affect skin, trachea, testis and salivary gland. In the eyes, it manifest with destruction of epithelial layers of the cornea, foamy bitot's spot and exophthalmia. These changes in epithelium differentiation eventually causes blindness. Impaired cell division and differentiation may adversely affect the hematopoiesis process (17,18,19). Deficiency also affects the natural immune system by interfering with the rejuvenation of mucosal barriers broken during infections, the functioning of neutrophils, macrophages and natural killer cells thus affecting innate immunity. In addition, vitamin A maintains proper functioning of adaptive immunity, aids in growth of T-helper (Th) cells and B- lymphocytes. Decreased antibiotic – mediated response guided by the T-helper type 2 cells may reduce due to deficiency. Vitamin A deficiency further compromise Th-1-mediated immunity by inhibiting regeneration of mucosal epithelial cells. These decreases immunity especially in infants, children and pregnant women, with vitamin A deficiency (34).

In pregnancy, administration of vitamin A is restricted because high doses are teratogenic. In this regard, the World Health Organization (WHO) has recommended the daily maximum dose of 10,000 IU or alternatively 25,000 IU every week (22,23).

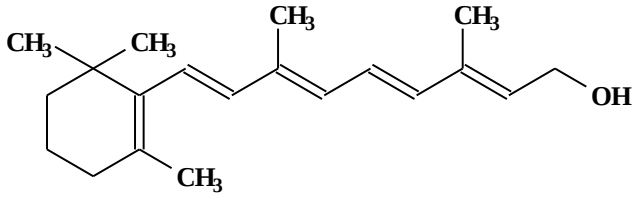

Figure 1.6 Chemical structure of vitamin A

All-*trans* retinol is an isoprenoid consisting of 5 conjugated double bonds is the vitamin A parent compound. It has absorbance maxima at 325 nm in hexane or ethanol.in acidic condition retinol degrades fast to anhydrous retinol while in light, retinol and its derivatives isomerizes.

Vitamin D

The backbone structure of vitamin D and its metabolites (ergocalciferol and cholecalciferol) consists of 9, 10-secosteroids. Vitamin D_2 (ergocalciferol) is majorly obtained from plant sources and D_3 (cholecalciferol) from animal sources. The two are hydroxylated to form the active forms (25-hydroxy (calcidol) and 1, 25-hydroxyvitamin D (calcitriol). Active forms is useful in maintaining calcium hemostasis. Deficiency symptoms are muscle weakness, soreness and bone pain. Among children, rickets are main manifestation while in adults osteomalacia is common. The risk factors for deficiency are old age, obesity and fat malabsorption. It is used in milk fortification to prevent rickets in children and reduce the osteoporosis in advanced age.

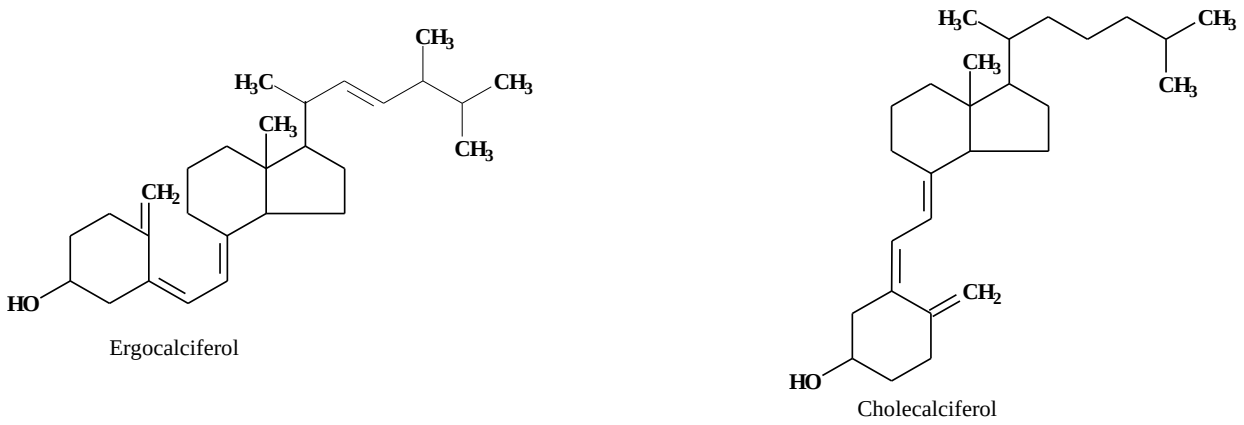

Figure 1.7 Chemical structure of vitamin D

Vitamin E

Vitamin E (Figure 1.7) denotes the naturally existing antioxidants tocotrienols and tocopherols. The antioxidant activity of is due to δ-, α-, β-, γ-tocopherol and δ-, α-, β-, γ- tocotrienols (23). Alpha tocopherol is the natural predominant type and has the highest biological activity. Tocopherols (Figure 1.7) appear as yellowish viscous oils. They are insoluble in water but readily dissolve in fats, alcohols, non-polar solvents.

Vitamin E maintains the structural integrity of cell membranes and neutralizes free lipid peroxide radicals thus preventing disease-causing microorganisms from penetrating into cells. In men, it maintains continuous spermatogenesis thus preventing infertility. Vitamin E is also involved in

cellular signaling (37), embryogenesis and cellular differentiation (38). Vitamin E is found in many varieties of foods, therefore deficiency is rare. Diseases such as cystic fibrosis or those due to fat malabsorption may also cause deficiency. Severe deficiency may cause nerve damage, muscle damage, motor nerve paralysis, retinitis pigmentosa and anemia (39).

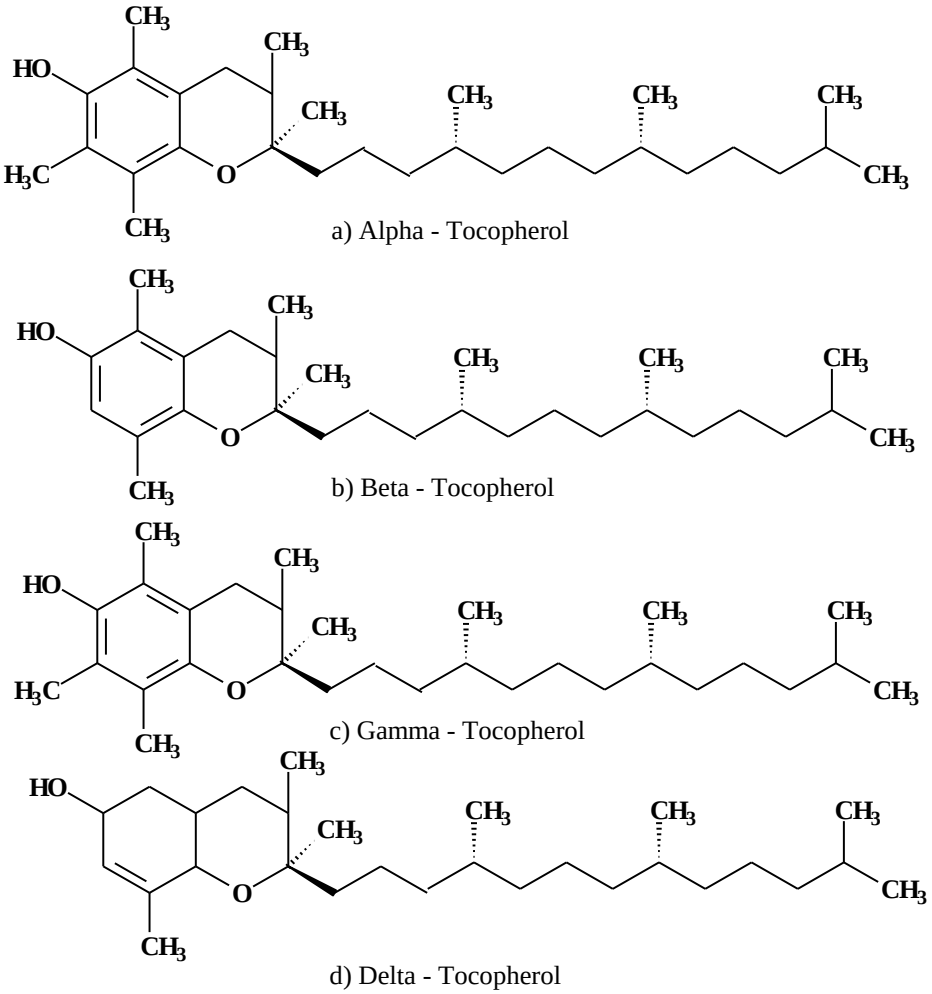

Figure 1.8 Chemical structures of tocopherols

1.4.3 Sources of vitamins

Human beings do not synthesize vitamins but obtain them majorly from food. The food sources ranges from plants to animal products. Table 1.2 below indicates the different food sources of various vitamins.

Table 1.2 Sources and roles of vitamins

Vitamin	Food source	Roles in the Body
Vitamin A	Animal products: liver, egg yolk, fish, whole milk, butter, and cheese. From plants as provitamin A (carrots, yellow and dark green leafy vegetables, pumpkin, apricots, melon and red palm oil).	Maintaining healthy mucous membranes that prevents infections, important for normal vision and teeth development.
Vitamin D	Fish-liver oils, eggs, meat and milk.	Useful in bone and teeth development, increases calcium absorption
Vitamin E	Vegetable oils (peanut, soya, palm, corn and sunflower), nuts, seeds, whole grains, leafy green vegetables.	Prevents damage of cell membranes. Antioxidant
Vitamin K	Green vegetables, soybeans, beef, liver, green tea, egg yolks, potatoes, oats, asparagus, cheese	Important in maintaining healthy bones and blood coagulation.
Vitamin C	Citrus fruits, peppers, parsley, cauliflower, potatoes, broccoli, mango, Brussels sprouts.	Useful in collagen formation, wound healing; maintaining structural integrity of vascular vessels, bones and teeth, Ensuring proper iron, calcium, and folate absorption, brain hormones, immune factors production, acts as an antioxidant.
Thiamine (B_1)	Dried brewer's yeast, animal products (beef, liver, dried milk) whole grains, nuts, dried legumes	A coenzyme in carbohydrate and amino acids metabolism. Important in the functioning of the nervous system.
Folate	Liver, dark green leafy vegetables, beans, wheat germ and yeast. Egg yolk, beet, orange juice, whole wheat bread.	Assists in protein metabolism, important in hematopoiesis, prevents spine, brain birth defects, Lowers blood homocysteine levels, and this reduces risks of coronary heart disease.
Cobalamins (B_{12})	Liver, kidneys, heart, and brain, fish, eggs, and dairy products.	Aids building of DNA, hematopoiesis and maintenance of nervous system.
Pyridoxine (B_6)	Chicken, liver of beef, pork, fish (tuna, trout, salmon, herring), peanuts and walnuts, bread, whole-grain cereals.	A coenzyme in amino acids glycogen and sphingoid bases metabolism, which are involved in protein metabolism, helps body to utilize fats and it is important in hematopoiesis.
Biotin	Yeast, liver, kidney, egg yolk, soybeans, nuts and cereals.	Important in fat synthesis and aids in energy release from carbohydrates.
Pantothenic acid	Yeast, liver, heart, brain, kidney, eggs, milk, vegetables, acid legumes, whole grain cereals.	Useful in production of energy and helps in hormone production.

Vitamin	Food source	Roles in the Body
Niacin	Yeast, liver, chicken, lean meats, nuts and legumes. Found in small quantities in milk and green leafy vegetables.	Involved in energy production and helps in digestion.
Riboflavin (B_2)	Yeast, liver, milk and milk products, meat, eggs, and green (B_2) leafy vegetables.	A coenzyme in many redox reactions. Helps to keep healthy skin and mucous membranes and plays a key role in cataracts prevention

Table adopted from (40)**.**

1.5 Uses of and benefits of multivitamin supplements.

In women during antenatal period, folic acid is important mostly in the first trimester in preventing neural tube defects (41). In some instances especially with no balanced diet, lack of multivitamin supplements has led to pre-term births and low weight babies. These negative outcomes of vitamin deficiencies are major contributors of infant mortality and morbidity (42).

Vitamin A reduces lower respiratory tract infection and severe diarrhea in HIV (Human Immunodeficiency Virus) -infected children thus reducing their mortality and morbidity. Supplements containing vitamins B, C, E and K reduce incidence of premature births and under weight babies, they also reduce vertical HIV transmission in infected women. Multivitamins prolong the pre-ART phase (Anti-Retroviral Therapy) in patients at early stages of AIDS (43).

Vitamin A and mineral zinc play a key role in maintenance of immune system. They also help in reducing malaria incidence especially malaria due to *P. falciparum* in children (9,10). Vitamin C supplement lowers of pneumonia infections (46) and common colds in people with vitamin C deficiency (47).

Vitamin and mineral supplements containing β-carotene and vitamin E reduces incidences of esophageal and stomach cancers (48). Daily multivitamin supplementation in men reduce total cancer risk as shown in randomized controlled trials (49). Vitamins also act as biomarkers of some diseases such as, cystic fibrosis in children, which is an indicator of fat-soluble vitamins deficiency. Folate containing multivitamins before and during pregnancy may offer the fetus protection against childhood cancers (50).

Vitamin D is important in maintaining bone strength. The Food Standard Agency (FSA) of the United Kingdom has recommended a daily limit of (1.5 mg) to reduce osteoporosis risk. Intake of more than 3000 IU of vitamin A (51) increases risks of hip fracture. Ascorbic acid reduces sympathetic dystrophy in elderly patients (52).

Deficiency of cyanocobalamin and folic acid may lead to dementia (53). Vitamin E increases the levels of super oxide dismutase (SOD) and the increased levels minimizes risk of tardative dyskinesia. In addition, it prevents tissue injury due to oxidation in people on antipsychotic drugs

(54). In addition, Lutein, a carotenoid, prevents age-related macular degeneration, slows the advancement of age-related macular degeneration and improves oxyopia (55).

Vitamin D decreases diabetic risk in diabetes prone populations. The β - cells of the pancreas possess vitamin D receptors where the vitamin binds and aid in transcription of both the insulin gene and the gene promoter (56).A daily administration of 0.1 g of vitamin B_1 and Vitamin B_6 aids in relieving menstrual cramps pain (57)

The oxidation of low-density lipoproteins cholesterol (LDL - C) is associated with artherogenesis, generation of fatty streaks, macrophage migration and affects coagulation pathways dynamics. It also causes impaired production of endothelium derived relaxation factors that mediates blood vessels relaxation. The susceptibility of LDL - C to oxidation aggravates atherosclerosis. Vitamin C enhances vitamin E effects of preventing LDL - C oxidation.(12). Pyridoxal- 5'- phosphate, cyanocobalamin and folate reduce levels of homocysteine concentration in blood. High blood concentrations of homocysteine is a risk factor for vascular disease and atherosclerosis (58).

1.6 Drug – vitamin interactions and adverse effects

1.6.1 Drug – vitamin interactions

Concurrent administration of vitamins with drugs may lead to a number of interactions as summarized in Table 1.3, below

Table 1.3 Drug – vitamin interaction

Drug	Vitamin	Mechanism of interaction	Treatment
Antiepileptic's	Vitamin D	Phenobarbitone and phenytoin increases the expression of CYP24 A (Cytochrome P24), which results in decreased elimination of vitamin D metabolites and lower serum levels of 25-hydroxyl vitamin D.	Monitor Vitamin D levels. Give supplements in cases of low levels (59).
Bisphosphonates'	Vitamin D	Demasks deficiency of vitamin D_3 deficiency leading to hypocalcemia (60).	Supplement daily with 1500 mg of elemental calcium and 800 - 1000 IU of Vitamin D.
Colchicine	Vitamin B_{12}	Colchicine interferes with vitamin B_{12} receptor development of the mucosal cells.	Monitoring of vitamin B_{12} concentrations in patients on high doses of colchicine for long periods and supplement appropriately (61).
Corticosteroids	Vitamin D	Prevent vitamin D – induced calcium absorption. This interferes with in the deposition of Vitamin D in bones (62).	For patients on 7.5 mg or higher doses of prednisolone and those on long-term steroids. Supplement daily

Drug	Vitamin	Mechanism of interaction	Treatment
			with 800 IU vitamin D
DMPA (Depot Medroxyprogesterone Acetate)	Vitamin D	Reduces the bone density of lumbar spine and femoral neck in women on DMPA for long periods and this raises the risk of osteoporosis.	Supplement daily with 400 IU Vitamin D.
Biguanides e.g. Metformin.	Vitamin B_{12}	They compete with calcium for the intrinsic factor B_{12} receptor at the ileum (63).	Administration of 1.2g calcium daily improves cyanocobalamin absorption (64).
Nitrous oxide anesthesia	Vitamin B_{12}	Nitrous oxide is a strong oxidizing agent while cobalamins are easily oxidized, therefore NO oxidizes cyanocobalamin making it unstable (65).	Levels of cyanocobalamin should be check Cyanocobalamin levels pre and post-surgery then supplement where necessary.
Orlistat	Vitamins A, D, E, K and β-carotene.	Orlistat inhibits lipase enzyme and prevents, absorption of fat-soluble vitamins in the gut.	Daily supplementation with vitamins. Orlistat should be taken two hours either before or after food.

Drug	Vitamin	Mechanism of interaction	Treatment
Proton pump inhibitors (PPIs)	Vitamin B_{12}	PPIs inhibit production of gastric acid. Which inhibits breakdown of protein bound vitamin B_{12} (66).	Deficiency occurs after 4 years of PPI. Supplement to cater for decreased dietary absorption (67).
Rifampicin	Vitamin D	interferes with CYP 450 hence increasing the hepatic metabolism of vitamin D(68).	Isoniazid is administered to counter the negative effect of rifampin, this maintains the required levels of Vitamin D (69).
Sulfasalazine	Folic acid	Sulfasalazine interferes with folate absorption and prevents breakdown of folate in the diet to absorbable forms (70).	Administer Folic acid daily (1 mg when formulated as sole formulation or 0.4 mg in multivitamin (71).

1.6.2 Adverse effects

Multivitamins are viewed as harmless and nontoxic since they are classified as natural substances although currently most are organic but in excess amounts, they can be harmful products. Because of these assumptions, there are chances of overdose and undetermined adverse effects.

Vitamin A

Exceeding the limits of vitamin A liver storage capacity leads to hypervitaminosis A. Serum concentration of more than 1.0 mg/L (72) is an indicator of hypervitaminosis. Hypervitaminosis A can be either acute or chronic. Acute hypervitaminosis arises due to consumption of huge quantities of vitamin. on the other hand, chronic hypervitaminosis A occurs due to a continuous modest uptake of vitamin A (36). Acute poisoning symptoms include malaise, dizziness, severe headache, nausea; sit phobia, increased intracranial pressure, and hepatosplenomegaly and skin changes. Chronic poisoning is characterized by; diplopia, sleep disturbances, nystagmus, gum bleeding lip cracking, hair loss, skin ulceration, muscular coordination disturbances, chronic fatigue, irritability and renal dysfunction which leads to hematuria and optic nerve edema. In addition, chronic hypervitaminosis A affects the auditory system (15,16), as well as causing aplastic anaemia by interfering with the hematopoietic system. Aplastic anaemia is characterized by accelerated erythrocyte sedimedantion rate and prolonged prothrombin time. It also leads to weak immune system and causes leukopenia (74). Hypervitaminosis A interferes with absorption of mineral salts and β-carotene from food. In addition, it may lead to decalcification of bones leading to high levels of calcium in the blood, rendering bones fragile thus increasing the chances of osteoporosis and femoral neck fractures (75), reduced bone mineral density (76) and ossification of long bones epiphyses, which causes retarded growth in children (77). Consumption of alcohol exacerbates the adverse effects (78). However, adverse effects reduce upon complete withdrawal of vitamin A or reducing the amounts of vitamin A administered.

Vitamin A is contraindicated in conditions such as liver cirrhosis and bile duct obstruction. It is also not safe in high risk groups like pregnant and breast feeding women because of its teratogenic effects (21,23).

Vitamin E

At high doses, vitamin E inhibits 5-lipoxygenase in blood platelets and leucocytes. This leads to decreased production of thromboxane and leukotrienes, reduced coagulation platelet aggregation and interruption of both granulocyte and phagocyte functions which rely on oxygen radicals (24,25). High plasma concentrations of depletes systemic reserves of vitamin A. Administration of pure α-tocopherol or the acetate form, reduces absorption of γ-tocopherol in the gut, food being its only source (26, 27).

The anticoagulation effects of vitamin E interferes with vitamin K coagulation functions. Patients on anticoagulants (e.g. warfarin) or on estrogens and are vitamin K deficient may have unexpected bleeding. Therefore, these two groups of patients should not exceed 40 mg/d of vitamin E (80).

Vitamin C

Generally vitamin C is not toxic (84) but doses higher than 500 mg/d are associated with gut disturbances which are characterized by nausea, heartburns diarrhea, painful urination, erythrocyte lysis in glucose-6-phosphate dehydrogenase (G-6-PD) and vitamin B_{12} deficiency (85). It impairs elimination of weak acids and bases by inducing urine acidification which may cause renal calculi (86). Doses greater than 1 g/d increases oxalic acid concentrations in in blood and urine and this cause formation of calcium oxalate salt. Concentration levels should be monitored in chronic kidney patients and those at risk of gout, urate and oxalate calculosis (87).

In therapeutic uses, vitamin C causes hypernatremia and hypokalemia and thus patients on it should be monitored for sodium and potassium concentration levels (88).

Vitamin C interacts with drugs either by limiting their excretion or enhancing metabolism. Vitamin C destroys vitamin B_{12} when used together. It also increases elimination of tricyclic antidepressants and derivatives of amphetamine by blocking their reabsorption (77). Vitamin C is reacts oxidizing drugs (89) and by urine acidification it leads to crystallization of *p*-amino salicylic acid and sulphonamides (29). During measurement of glucose, bilirubin, creatinine and glucose concentration in the body using redox based methods, high levels of vitamin C may show false results (88) .

In addition, high doses prevent copper absorption thus inhibiting the functions of enzyme ceruloplasmin and super oxide dismutase (Cu-Zn-SOD). On the other hand, it increases absorption of iron to toxic levels by increasing reabsorption and by triggering iron release from tissue. The unbound excess iron piles up in the skin causing irritation of the gut mucosa. Thus causes vitamin C is contra indicated in people with sideroblastic anaemia, thalassemia conditions and hemochromatosis (34,35).

Vitamin B_6

Abnormal change on the MTHHER gene, which is responsible for activating vitamin B6 into the active form, causes toxicity even in low doses. Higher doses than 10 mg/day causes peripheral sensory neuritis, which presents with hyperesthesia, weakness of the muscles, partial paralysis and loss of sense of equilibrium and vibration (91).

Vitamin D

Hypercalcemia and hypercalciuria results from high doses of vitamin D (92). The two occur due to excess calcium, which may be caused by excessive absorption from the gastrointestinal tract and resorption of bones. The excess calcium may be deposited in soft tissues and it is associated with loss of minerals in the bones (93) and permanent renal and cardiovascular systems toxicity. Because vitamin D is lipophilic, it can accumulate in adipose tissues and toxic effects can last for a longer period after the causant has ceased. Toxicity signs are nausea, vomiting, anorexia, torpor, untraceable body aches, thirst, constipation cardiac dysrhythmias and dysuria.

1.7 Recommended daily dose limits of vitamins

Arising from the potential toxicities of vitamins, upper daily limits have been established as shown in table 1.4.

Table 1.4 Recommended daily dose of selected vitamins

Vitamin	Recommended daily dose	Coenzyme or Active form/function
Vitamin A	0.8-1.0 mg	11-cis-Retinal
Vitamin D3	0.08 mg	1,25-Dihydroxycholecalciferol
Vitamin E	8-10 mg	
Niacin (vitamin B3)	15-20 mg	Nicotinamide adenine nucleotide (NAD, NADP)
Pyridoxine (vitamin B6)	2.0 mg	
Folic acid	0.4 mg	Tetrahydrofolic acid
Vitamin C	60 mg	
Thiamine (vitamin B1)	For pregnant 1.5 mg, lactating women should take 1.9 mg	
Vitamin B2	1.6 mg	

The table was generated from: (60, 61,109)

1.8 Problem statement

Currently in Kenya, the analysis of multivitamins is not a requirement before the Pharmacy and Poisons Board (PPB) list them. Despite their increased use, there is no documented data or publications on the quality of the multivitamin supplements in our Kenya.

1.9 Study justification

In Kenya, the Pharmacy and Poisons board has classified multivitamins as food/nutritional or nutraceuticals and they are only listed and not registered as conventional medicines (2). Multivitamin supplements do not undergo the rigorous tests required for drugs thus leaving them up to manufacturers to ensure the label claim is accurate. Any under dose or overdose may have serious health implications such as chelosis, beriberi, teratogenic effects and neurological complications. In the USA, the FDA regulates supplements in a less rigorous manner than conventional prescription and non-prescription products. Therefore, consumers have to rely on self-regulation by dietary supplement manufactures on the quality, consistency, potency and purity

of the products and since consumers are accustomed to high quality products on the conventional prescription and non-prescription conventional products, they have little reason to doubt the package label claims on conventional medications (98). This assurance cannot be guaranteed in Kenya where cases of substandard/counterfeit conventional drugs is common (99). In addition, there is market liberalization in Kenya and hence many multivitamins are imported and their label claim has not been verified.

1.10 Study objectives

1.10.1 General objective

The general objective of this study was to assess the quality of multivitamin supplements in Central Business District area, Nairobi County.

1.10.2 Specific objectives

The specific objectives of this study will be:

i. To asses compliance of the sampled products with the labelling and packaging specifications.
ii. To carry out the assay of fat- soluble vitamins (A, D, E) and water-soluble vitamins (B_1, B_3, $B_{5)}$ and folic acid in multivitamin formulations in Nairobi city.
iii. To determine the microbial bioburden on multivitamin suspensions.

CHAPTER TWO: LITERATURE REVIEW OF QUALITY CONTROL OF MULTIVITAMIN PRODUCTS.

2.1 Analytical methods

2.1.1 Analytical methods for vitamins

A sensitive and reliable method is required in the analysis of vitamin supplements since they are formulated in complex matrices. Several analytical methods for analyzing multivitamins simultaneously or individually in different matrices for each class of vitamins have been developed. The methods have been based on different detection methods based on UV-Vis (Ultraviolet-Visible) spectrophotometry, fluorimetry, chemiluminescence and chemical reactions. Other methods developed include; capillary electrophoresis microbial load assay, and immunoassay (100). The disadvantages with most these methods are that they are laborious and tedious (101). High-performance liquid chromatography (HPLC) is the common analytical technique for separating vitamins in many formulations because of its selectivity and accuracy (35,36).

There are official compendial monographs for analysis of vitamins, however, they have a major challenge that each vitamin is assayed separately and this becomes laborious, expensive and time consuming in analyzing multicomponent samples. To solve this problem, simultaneous analytical methods have been developed for the assay of multicomponent samples.

In the assay of fortified powdered drinks and food samples for both water and fat-soluble vitamins, Kledjus B developed a simultaneous HPLC method to quantify riboflavin, nicotinamide, pantothenic acid, pyridoxine hydrochloride, pyridoxal, folic acid, ascorbic acid, *trans*-retinol and α-tocopherol. The samples analyzed included B-komplex tablets. Food samples included soja milk. For the B-komplex sample the average recoveries for all the components was 99 – 100% while for soja milk and nestle BEBA was 103% and 101% respectively. The results obtained with this method agreed with the declared values (104).

A reverse phase ion – pair liquid chromatography was developed by Amin & Reusch to simultaneously separate and analyze thiamine, riboflavin, nicotinamide, pyridoxine,

cyanocobalamin, ascorbic acid and folic acid in capsule formulations. They used a vertex Lichrosrb RP-C18 (250 × 4 mm, 10 μm) at flow rate 1.5 mL/min, column temperature 22-25 ˚C, and detection at 254 nm. Methanol, water, 85 % phosphoric acid (55:45:1) with ion pairing reagent (octane sulphonic acid ,65 mg) as the mobile phase (105). This method was sensitive and could detect low vitamin concentration of 5 – 10 ng.

Amdizic *et al* used RP-HPLC with a UV-Vis detector to analyse thiamine, nicotinamide, pyridoxine hydrochloride, folic acid and cyanocobalamin in Pentovit® multivitamin tablets. Because cyanocobalamin was in low concentration, it was analyzed separately. For analysis of thiamine, nicotinamide, pyridoxine and folic acid, a superclosil, a C_{18} ABZ^{+} analytical column was used. The mobile phase was composed of methanol: 0.005M heptane sulfonic sodium salt/0.1 % triethylamine (25:75), adjusted to a pH of 2.8 using phosphoric acid. Wavelength of detection was 290 nm. For cyanocobalamin, mobile phase was water - methanol in a ratio of 22:78. A Suplex pkb-100 analytical column was used and detection carried out at 550 nm The average percentage recoveries of B_1, B_3, B_6, folic and B_{12} were 90.4%, 107.2%, 105.2%, 108.5% and 91.7% respectively. The results obtained were within the label claim limits (106).

In order to perform a comparative analysis on three different pods of okra (*Abelmoschus esculentus*), a vegetable found in Egypt and Asia, Sami *et al* used a HPLC coupled with a UV-Vis and DAD to quantify riboflavin, nicotinamide, pyridoxine, ascorbic acid, A, D, E K, and β-carotene .The three cluster of varieties analyzed were Dakhalia (D), kafr El-sheikh (K), Suez (S) and Mansoura (M). Riboflavin, nicotinamide and pyridoxine were analyzed simultaneously while ascorbic acid and the fat-soluble vitamins were each analyzed separately. The results showed that the S pod contained the highest amount of B_6 and α-tocopherol, while it had the lowest amounts of B_3 and B_{12}. The K pod had the lowest amount of vitamin C, the highest amount of vitamin B_3, B_{12} and C were in pod M. Pod D had the lowest amounts of vitamin E, B_{12} and B_6 (107).

In Syria, Saad *et al* developed an RP-HPLC ion-pair to quantify riboflavin, nicotinamide, pyridoxine, cyanocobalamin and ascorbic acid in milk bebelac food infant and Squeeze orange juice powder. The HPLC system was coupled to UV-DAD/FLD detectors. A BDS C18 (100mm × 4.6 mm, 3 μm) column was used with two mobile phases. Mobile phase A was composed of

(0.00584 M hexane-1-sulfonic sodium salt: ACN 95:5/0.1% trimethylamine adjusted to pH 2.5 using 1 M phosphoric acid) and B (0.00584 M hexane 1- sulfonic acid sodium salt: ACN 1:1 / 0.1% trimethylamine at pH 2.5 adjusted using 1 M phosphoric acid). Analysis was carried out a temperature of 40 °C with a 1.6 mL/min flow rate and a 20 μL injection volume and detected using UV-DAD detector at 246 nm for vitamins C and B_1, 267 nm for thiamine, 260 nm for nicotinamide, 290 nm for vitamin pyridoxine, 282 nm, folic acid and 361 nm cyanocobalamin (108).

Marco *et al* developed a HPLC method coupled with UV-Vis detector to simultaneous analyze 5 water-soluble vitamins (B_2, B_3, B_5, B_9 and C) in honey collected from 12 different plants (Sardinia, Italy). Even though the amounts of analytes were low, the concentrations were dependent on the botanical origin. Altima C-18 column (250×4.6 mm, 5 μm) analytical column using mobile phases A (Triflouroacetic acid solution 0.025% v/v) and B (ACN). Detection was carried at 254 nm for nicotinamide and ascorbic acid while for thiamine , pantothenic acid and folic detection was at 210 nm (109). For the assay of vitamins A, D_3 and E, Gabriela *et al* developed a precise and accurate simultaneous method to determine vitamin A, D_3 and E in pharmaceutical preparations(110)

In addition to HPLC methods, capillary electrophoresis has been employed in the in the assay vitamins in a multicomponent sample (26, 91). Compared to the other methods it is fast, requires minimal reagent hence cost effective and utilizes less sample, however, it is less sensitive because of small light path length. UV/Vis detector, laser-induced fluorescence (LIF) detector, electrochemical and Mass spectroscopy detector have been linked to capillary electrophoresis making it a robust technique (112). Fotsing *et al* developed a capillary zone electrophoresis to analyse vitamin B and C which are ampholytes (113). Ong CP *et al* used micellar electro kinetic chromatography (MEKC) which enhanced selectivity for simultaneous separation of water-soluble vitamins. The analyzed vitamins and their recoveries were thiamine (108.5%), nicotinamide (100.8%), riboflavin (93.6%) pyridoxine (106.5%), ascorbic acid (104.3%) and pantothenic acid (99.6%) (114). The results were within the limits hence making capillary electrophoresis in assay of vitamins in pharmaceutical preparations. Wang *et al* used a micro emulsion electro kinetic chromatography/ Capillary electrophoresis (MEEKC/CEC) for separation of fat- soluble vitamins (101). MEEKC/CEC was applicable to fat - soluble vitamins because they are neutral, and are insoluble in water.

From the review-reversed ion - pair HPLC has been used extensively in the identification and assay of vitamin in food, plants and pharmaceutical products. No method, which was able to simultaneously analyze both fat and water soluble vitamins due to their difference in properties, therefore different mobile phases are required. Amdizic et al and Gabriela et al methods were applicable to analyze water and fat-soluble vitamins respectively because the reagents, column and equipment available. In addition, the samples analyzed were pharmaceutical products similar to those under study.

2.2 Method verification

Method verification was important to ensure that the method can be applied locally to this study and give assurance to the results obtained. The parameters investigated included linearity, range, accuracy and precision.

2.2.1 Linearity and Range

Linearity of a method has been defined as "its ability to generate test results that have direct proportional to the concentration of the analyte in the sample". Linearity is presented by linear regression method. Demonstration of linearity is carried out examining the regression coefficient (R^2), y- intercept, and SS (residual sum of squares. A plot of the data should be included (97).

2.2.2 Accuracy

Accuracy is defined as "the closeness of agreement between values accepted conventionally as a true value or as an accepted reference value and results obtained". It is presented as percentage nominal. The ICH Q2 guidelines has recommended that accuracy be evaluated using at least nine determinations over a minimum of three concentration levels over the specified range (97).

2.2.3 Precision

Precision "illustrates the closeness of agreement among a series of values obtained from multiple sampling of the same homogenous sample under the set conditions" (96). It is assessed in 3 levels repeatability, intermediate precision and reproducibility. Same analyst using same equipment over a short time in the same laboratory presents repeatability precision as RSD of the results. Data is recorded from at least 6 replica measurements carried out at 100% of the test target concentration.

The RSD should not be more than 2%. Intermediate precision is defined by ICH as the long-term variability of the measurement process.

Intermediate precision is obtained by results from within the laboratory due to different events such as different day, analysts, analytical columns and equipment. Intermediate precision verifies that in the same laboratory the method will give similar results.

Reproducibility is determined by analyzing homogeneous samples in various laboratories as an inter-laboratory study. The purpose of reproducibility is to ascertain that the method can produce same results in different laboratories (97).

2.3 Microbiological analysis

Microbial load determines the absence or the minimum presence of specified microorganisms detected under the prescribed conditions. It ascertains whether a sample complies with a specified microbiological quality. For non-sterile pharmaceutical preparations, bioburden should lie within the set acceptable limits. The presence of some microorganisms may affect quality of product which may in turn adversely affect the patients' health (115). *Escherichia Coli* is controlled in aqueous non-sterile pharmaceutical preparations.

A microbial analysis study on brands of multivitamin syrups in Maiduguri, Nigeria showed compliance with microbiological requirement for syrups as only one out of the seven samples collected showed microbial contamination greater than the accepted level. The major contaminant was found to be *salmonella spp* (115).

Abdullah *et al*, carried out a microbial load in multivitamin and cough syrups in Dhaka city revealed that 91 % of multivitamin syrup complied with the official requirement of microbiological quality (116).

Therefore, manufacturers should ensure finished products meet the specified limits on bioburden. This can be achieved by applying guidelines on Good Manufacturing Practices during the manufacture, storage and Good distribution Practices during the distribution of pharmaceutical preparations.

The acceptance criteria for non-sterile pharmaceutical products relies on total aerobic microbial count (TAMC) and total combined yeasts/molds count (TYMC). During the assessment of results, average replicate counts or individual results is considered. Finally results obtained are evaluated against the specified pharmacopeia set limits (117).

CHAPTER THREE: EXPERIMENTAL

3.1 Study design

The study was carried out in Nairobi Central Business District. Nairobi is Kenya's capital city, home to the largest airport in East Africa, thus a commercial hub and the most populous county in the country contributing to 8.1% of the Kenyan population (118). In addition, it is the headquarters of most pharmaceutical distributors and wholesalers in the country (119). These factors make it the ideal sampling location for the study.

Laboratory testing was carried out at the National Quality Control Laboratory (NQCL), located in Kenyatta National Hospital (KNH) complex within the School of Pharmacy, University of Nairobi building.

3.2 Sample collection

Inclusion criteria for the samples collected was multi - component samples with or without minerals. Samples were collected randomly from selected facilities in Nairobi Central District area (NCBD). Mystery shoppers were employed in the sample collection. The facilities from which the samples collected were 10 retail pharmacies, 2 supermarkets and 3 nutrition shops. The sampling period was from July 2018 to February 2019. 40 samples were collected which consisted of; solid dosage forms (14 tablet formulation products and 6 hard gelatin capsules) and liquid dosages forms (19 suspensions and 1 oral drop). The sample size was determined from the available brands in the market, whereby 40 samples met the sampling criteria. Samples were collected according to their brand names. The collected samples were categorized into two; Antenatal supplements which contained folic acid, thiamine, nicotinamide, cyanocobalamine, pyridoxine and nervous system supplements, which contained nicotinamide, thiamine, and pyridoxine with no minerals. Before analysis was carried out, the samples were coded to avoid bias.

3.3 Labelling and packaging

For conformity to labelling specifications, the manufacturing and expiry dates, batch numbers, presence of patient information leaflets, sample storage conditions, country of origin and any advertisement were inspected on all the samples

3.4 Reagents and solvents and Equipment

HPLC grade methanol (Rankem, Guajarat, India), n-hexane (Scharlau, Gato Perez, Spain), Ethanol (scharlau, Gatoperez, Spain), Triethylamine (Fischer Scientific, Leicestershire, UK), Heptane-1-sulphonic acid salt sodium (Merck, Damstdart, Germany), orthophosphoric acid (Merck, Damstadart, Germany) and analytical grade anhydrous sodium sulphate (RFCL, Guajarat, India). All aqueous solutions were prepared using purified water from Arium water system (Sartorius AG, Göttigen, Germany) consisting of a reverse osmosis module and an ultrafiltration module with a UV irradiation component. Nutrient agar (Himedia Laboratories pvt. Ltd, India), Sabouraud dextrose agar (Himedia Laboratories pvt. Ltd, India) and buffered peptone water (Himedia Laboratories pvt. Ltd, India) were used for microbial load tests.

An Agilent 1260 (Agilent Technologies, Carlifonia, USA) HPLC system was used throughout this study consisted of a quaternary pump (DEAB818623), an auto sampler (DEAAC37011), a column oven (DEACN39585) with a block heating type thermostatic chamber and a Diode Array Detector (DEAAX07949). Evaluation and quantification were achieved on Open LAB CD (EZ chrom edition) Version A.04.07 chemstation, which controlled the HPLC system. All mobile phase preparations were degassed using a MRC DC 200H ultrasonic bath (MRC Ltd., Holon, Israel). Waters Xterra RP18, 5 μm, 250 × 4.6 mm chromatographic column was used as the stationary phase.

In pH adjustment, a Jenway 3540 (Jenway, Staffordshire, UK) pH and conductivity meter (32954) which consisted of electrode B16 and a temperature probe (32959) while for accurate weighing a Shimadzu AUW220D (Shimadzu Corporation, Kyoto, Japan) semi - micro analytical electronic weighing balance (D450012073) with a sensitivity of ± 0.1 mg was used.

3.5 Chemical reference substances

The reference standards used in this study were donated by NQCL (Hospital Road, Nairobi, Kenya), Regal Pharmaceuticals Limited and some bought from Sigma Aldrich Taufkirchen Germany. Table 3.1 shows the particulars of the reference standards used in the study.

Table 3.1 Details of reference standards.

Compound	Type	Batch No	% Potency (as is)
Niacinamide (USP)	Primary	NIH378	99.9
Thiamine Hydrochloride (USP)	Primary	POK366	99.7
Pyridoxine Hydrochloride (USP)	Working	Q0Q409	99.8
Folic acid	Working	81/WRS 370	90.6
Cholecalciferol	Working	LRAB2929	99.7
α-Tocopherol	Working	FN09201703	99.1
Retinyl Acetate	Working	MKCH7456	99.0
Retinyl Palmitate	Working	LRAB6688	98.7

Verification of Analytical methods.

The methods used for analysis in this study for both water-and fat-soluble vitamins were obtained from literature and method verification carried out (106,118). Verification was only carried out for the water – soluble vitamin. The fat-soluble vitamin method was used without any verification due to limited quantities of the fat-soluble reference standards.

3.5.1 Linearity

Linearity was performed for each of the four vitamins under analysis (Thiamine, Nicotinamide, Pyridoxine and Folic acid).

Preparation of 200% standard stock solution

For thiamine, 12.42 mg was accurately weighed into 20 mL volumetric flask, diluted to volume and then 4 mL was transferred into a 50 mL volumetric flask and diluted to volume. For nicotinamide, 11.63 mg was accurately weighed into a 20 mL volumetric flask, diluted to volume and then 8 mL was transferred into a 50 mL volumetric flask and diluted to volume. For pyridoxine, 10.78 mg was accurately weighed into a 20 mL volumetric flask diluted with to volume then 2 mL was further transferred into a 50 mL volumetric flask and diluted to volume. For folic acid 11.54 mg was accurately weighed into a 10 mL volumetric flask, diluted to volume with diluent where 2 mL was further transferred into a 100 mL volumetric flask diluted to volume and from this solution, 4 mL was transferred into 50 mL volumetric and diluted to volume.

From the stock solutions dilutions of 75%, 100%, 125% and 150% were prepared for each of the compounds. The 150% concentrations were prepared by pipetting 15 mL from the stock solution

into 20 mL volumetric flasks then diluted to volume. The 125% concentrations were prepared by pipetting 12.5 mL from the stock solution into 20 mL volumetric flasks then diluted to volume the 100% concentrations were prepared by pipetting 10 mL from the stock solution into 20 mL volumetric flasks then diluted to volume. Similarly, the 75% concentrations were prepared by pipetting 15 mL from the 100% solution into 20 mL volumetric flasks and diluting to volume. The corresponding concentrations of each dilutions are as in Table 3.2. The solutions of each of the concentrations were filtered using 0.45 μm filter, put into the vials then analyzed in duplicate and injected using the assay procedure (3.6.1) by HPLC.

Table 3.2. Concentrations Levels (mg/mL).

Compound	**Concentration level (mg/ml)**			
	50%	**100%**	**150%**	**200%**
Thiamine (B_1)	0.015	0.02	0.03	0.04
Nicotinamide (B_3)	0.03	0.04	0.06	0.08
Pyridoxine (B_6)	0.0075	0.01	0.015	0.02
Folic acid	0.0006	0.0008	0.0012	0.0016

3.5.2 Accuracy

Accuracy was determined by spiking one of the samples under test with a known amount of the standard. The standards were added to the samples at three different concentrations corresponding to 80%, 100% and 120%. The determination was carried out at 80%, 100% and 120% standard concentration levels. The spiking standards were prepared by making concentrations equivalent to 10 % of the sample label claim for each active ingredient. The spiking standard concentrations of the various compounds were thiamine 0.3 mg/mL prepared by weighing 10 mg of the standard into 10 mL volumetric flask and diluted to volume then diluting 3 mL was put into 10 mL volumetric flask and diluted to volume. Pyridoxine 1 mg/mL prepared by weighing 10.19 mg of the standard into 10 mL volumetric flask and diluted to volume. Nicotinamide concentration of 2 mg/mL was prepared by weighing 19.69 mg of the standard into 10 mL volumetric flask and diluted to volume. Folic acid 0.04 mg/ml prepared by weighing 19.53 mg of the standard into 20 mL volumetric flask diluted to volume then further 4 mL was Pipetted into 100 mL volumetric flask and diluted to volume. Sample MVS 017 was randomly chosen for the verification and was spiked with the prepared standard solutions. Sample stock solutions were prepared by taking weight equivalent to 80%, 100% and 120% average tablet weight respectively into a 50 mL

volumetric flask, then spiked by adding 1 mL of each of the the various standards into the sample and diluted to volume. From the spiked solution, subsequent dilutions for the compounds were prepared. For nicotinamide, 2 mL was put in a 20 mL volumetric flask and diluted to obtain the final concentration. For Pyridoxine, 3mL was diluted into a 50 mL volumetric flask to obtain final concentration. For Thiamine 3 mL was diluted in a 10 mL volumetric flask to get final concentrations. For folic acid, 2 mL was pipetted into a 20 mL volumetric flask, diluted to volume then 5 mL was further diluted to volume in a 50 mL volumetric flask. After obtaining the final concentration of 0.04 mg/mL nicotinamide, 0.012 mg/mL pyridoxine, 0.018 mg/mL thiamine and 0.0008 mg/mL folic acid. The samples were then analyzed triplicates using HPLC.

3.5.3 Precision

In this study, repeatability (intraday precision) and intermediate (inter-day) precision were determined. Standards were prepared at 100 % concentration as per the method (120). Pyridoxine (0.02 mg/mL) was prepared by weighing 12.59 mg of the standard into a 20 ml volumetric flask diluted to volume and 4 mL diluted to volume in 100 ml volumetric flask. Nicotinamide 0.04 mg/mL was prepared by weighing 9.56 mg of the standard into 20 mL volumetric flask diluted to volume then 10 mL was Pipetted into 100 mL volumetric flask and diluted to volume. Folic acid 0.0008 mg/mL prepared by weighing 5.79 mg of the standard into 10 mL volumetric flask diluted to volume further 1 mL was Pipetted into 50 mL volumetric diluted to volume and then 8 ml was Pipetted into 100 mL volumetric flask and diluted to volume. The solutions were injected six times in triplicate at the interval of 3 hours. The standard solutions were prepared as indicated in the (section 3.6) assay procedure. The same procedure was repeated during inter-day testing. The standards were added at amounts that would increase the concentration of each compound in the sample by 20% and the percentage recovery of each added working standard was regarded as the accuracy.

3.5.4 Detection

Maximum absorption wavelengths for individual vitamins was extracted using a Photo Diode Array Detector (PDA). Nicotinamide and thiamine absorbed maximally at 260 nm while pyridoxine and folic acid maxima absorption was at 290 nm. Therefore, 290 nm and 260 nm were

the wavelengths of analysis for the water-soluble vitamins. Maxima absorption wavelength for fat – soluble was 265 nm.

3.6 Analytical Procedures

3.6.1 Uniformity of weight

Twenty units (tablets/ capsules) were weighed individually with an analytical balance by tare weight method. For capsules, the weight of the capsule and powder were weighed then the powder was emptied into a container and the weight of empty capsule weighed. The weight of the powder was the difference of the weight of intact capsule and the weight of empty capsule shells. The average weights of tablets and capsules for each brand and the percentage deviation from the mean value were calculated. Not more than two of the individual masses should deviate from the average mass as per the British Pharmacopeia (BP) compendia limits of weight variation and none should deviate by more than twice that percentage (121).

3.6.2 Assay

Preparation of mobile phases

The mobile phase for the analysis of water soluble vitamins (B_1, B_3 & B_6) and folic acid was consisted of methanol –5 mM heptane-1-sulphonic acid sodium salt /0.1% TEA (25:75 V/V). The pH was adjusted to 2.8 with orthophosphoric acid. The 5 mM heptane-1-sulphonic acid sodium salt /0.1 % TEA solution was prepared by dissolving 1 gm of heptane -1- sulphonic sodium acid salt and 1 mL of TEA in 1000 mL HPLC grade water.

The mobile phase was prepared by mixing 250 mL HPLC grade methanol with 750 mL of the heptane-1-sulphonic acid/ TEA solution (106).

For the fat-soluble vitamins, 100% methanol was used as the mobile phase. Mobile phases were filtered through 0.45 μm filter and degassed for 30 minutes in an ultrasonic bath (110).

Diluent preparation

Diluent for fat-soluble vitamins was 15 % methanol, which was prepared by mixing 850 mL of de-ionized HPLC grade water with 150 mL HPLC grade methanol. For fat-soluble vitamins 100% methanol was used as the diluent.

Water-soluble vitamins

Preparation of stock and working standards solutions

Standard stock solutions of thiamine, nicotinamide and pyridoxine were prepared by dissolving 10 mg of each standard powder in 20 mL volumetric flask and diluted to the mark with diluent. Folic acid stock solution was prepared by dissolving 10 mg of folic acid in 50 mL volumetric flask and diluted to volume then 1 mL of the standard stock was pipetted into a 50 mL volumetric flask and made to volume with the diluent.

The working standard solution of thiamine, nicotinamide, pyridoxine and folic acid was achieved by pipetting 1 mL, 10 mL, 4 mL and 10 mL respectively of the stock solutions to one 100 mL volumetric flask with diluent and then put in vials and analyzed through the HPLC system.

Fresh standard solutions were prepared for all assays. Concentration of the standards were based on the concentration of folic acid, which had the lowest concentration in the samples. The concentration was maintained within the linearity range.

Sample preparation

All the 20 tablets were crushed to fine powder and for capsules all; the powder was emptied into a clean bottle and mixed uniformly. Powder weight equivalent to one tablet/ capsule (0.4 mg folic acid) was transferred into 100 mL volumetric flasks and deionized water added. The mixture was sonicated (30 minutes) with intermediate shaking and diluted with the diluent to mark. An aliquot of 2 mL was pipetted into a 20 mL volumetric flask and diluted to volume with diluent. The solution was filtered through 0.45 μm filters into 2 mL HPLC vials and then analyzed.

For the suspensions, relative density of each sample was measured and a weight equivalent to 5 ml was transferred into 100 mL volumetric flask, dissolved in distilled HPLC grade water. The mixture was sonicated (30 minutes) with intermediate shaking then diluted to volume. An aliquot of 2 mL was pipetted into a 10 mL volumetric flask and diluted with the same solvent, filtered through 0.45-μm filter into 2 mL HPLC vials and subjected to chromatographic analysis.

Chromatographic conditions

The mobile phase for the analysis of water soluble vitamins (B_1, B_3 & B_6) and folic acid was composed of methanol – 5 mM heptane-1-sulphonic acid sodium salt /0.1 % TEA (25:75 V/V) at pH 2.8. Flow rate of 1 mL min^{-1} with an injection volume of 10 µL through an Xterra reversed phase C_{18} 5 µm (250 mm × 4.6 mm ID) column maintained at a temperature of 35°C were used. Detection wavelength was 260 nm for vitamin B_3 and B_1 and 290 nm for vitamin B_6 and folic acid.

Fat - soluble vitamins

Standard preparation

Reference standard final concentrations were prepared to match sample concentrations as per the method. In the preparation of standard stock solution, 32 mg of vitamin A was weighed in a 20 mL volumetric flask and dissolved in methanol to volume to obtain a concentration of 1.6 mg/mL. For vitamin E, 1 mL (15 mg) was weighed in a 10 mL volumetric flask, dissolved in methanol to volume to a final concentration of 1.5 mg/mL, while 10 mg of vitamin D was weighed in a 100 mL volumetric flask, and dissolved in methanol to volume to attain a concentration of 0.1 mg/mL. Working standard concentrations were obtained through serial diluting of the respective standards to match concentration of the samples.

Sample preparation

Powder weight equivalent to one tablet was transferred into a 50 mL volumetric flask, equal volumes of ethanol (25 mL) and orthophosphoric acid 0.2% (25 ml) were added. The samples were sonicated (30 minutes) to ensure the powder completely dissolved. The mixture was transferred into a separating funnel for extraction whereby 50 mL of n-hexane was added and shaken vigorously for 30 mins, then the layers were left to separate. The n-hexane layer was collected and dried over anhydrous sodium sulphate. To ensure complete extraction, n-hexane was added three times and the layers collated. The combined n-hexane layers were evaporated using rotary evaporator and the residue was dissolved in 10 ml methanol. The samples were then filtered through 0.45 µm filters into HPLC vials.

Chromatographic conditions

Methanol (100%) was used as the mobile phase at a flow rate of 1.5 mL min^{-1}. The mobile phases were filtered through 0.45 μm filter and degassed using an ultrasonic bath. Sample was injected at a volume of 20 μL through an Xterra reversed phase C_{18} 5 μm (250 mm × 4.6 mm ID) column maintained at a temperature of 40 °C. Detection was carried out using a DAD detector at 265 nm.

3.7 Microbial load Determination

3.7.1 Preparation of nutrient media

Nutrient media used in the study were Nutrient agar (NA) and Sabouraud Dextrose Agar (SDA). Buffered peptone water (20 g) was weighed into a conical flask dissolved in 1000 mL and mixed by stirring, while in separate conical flasks 28 g of NA and 65 g of SDA were dissolved in 1000 mL. Each of the dissolved media was dispensed in aliquots of 90 mL in separate culture media bottles and sterilized by autoclaving at a pressure of 15 lbs. at 121 °C for 15 minutes. The media aliquots were left to cool to 50 °C before use.

3.7.2 Sample preparation

Nineteen multivitamin syrups were tested for microbial load. Pour plate method by 10 fold serial dilution was used for sample preparation (117). In the preparation of the first dilution, about 10 mL of the sample was added into the 90 mL buffered peptone water and the mixture agitated to homogeneity then 2 mL of the solution was put into two separate Petri dishes. About 25 mL of SDA was put into one of the Petri dishes' and NA media were added to the other. A further 10 fold dilution was carried out whereby 10 mL was Pipetted from the first dilution solution into a 90 mL buffered peptone water and mixed to obtain a homogeneous mixture. The same procedure for preparing Petri dishes for first dilution was repeated. The procedure was repeated for all the samples. In order to allow the media to solidify before incubation, Petri dishes were left stand for 2 hours. The Petri dishes were incubated in inverted position at 25 °C for fungi and 35 °C for bacteria. After 5 days, the Petri dishes were retrieved and analyzed for microbial growth.

CHAPTER FOUR: RESULTS AND DISCUSSION

4.1 RESULTS

4.1.1 Method verification

Method verification before use was important to ensure the method would be ideal for this study. The results obtained informed on the quality, reliability and consistency of analytical results. Linearity range, precision and accuracy were determined according to the ICH guidelines. Method verification was only carried out for the water – soluble method. There was a challenge of obtaining fat-soluble vitamin standards therefore the method was not verified. Results reported were only for the water- soluble vitamins method.

Linearity

Linearity was carried out at 75%, 100%, 125%, 150% and 200% sample concentrations and results were as presented in Table 4.1. According to the ICH guidelines, the coefficient of determination ($r^{2)}$) should be equal or greater than 0.99 (97). Figures 4.1, 4.2, 4.3 and 4.4 are the linearity plots for nicotinamide, thiamine, pyridoxine and folic acid respectively.

Table 4.1 Results of Linear regression analysis.

Compound	**Coefficient of determination (R^2)**	**Y-Intercept**	**Slope of regression line**	**Residual sum of squares (10^4)**
Nicotinamide	0.9994	-3.8896	4.076	143655
Thiamine	0.9995	-2.5622	3.9022	44651
Pyridoxine	0.998	+6.7752	4.3596	10421
Folic acid	0.9935	+0.0129	6.188	134

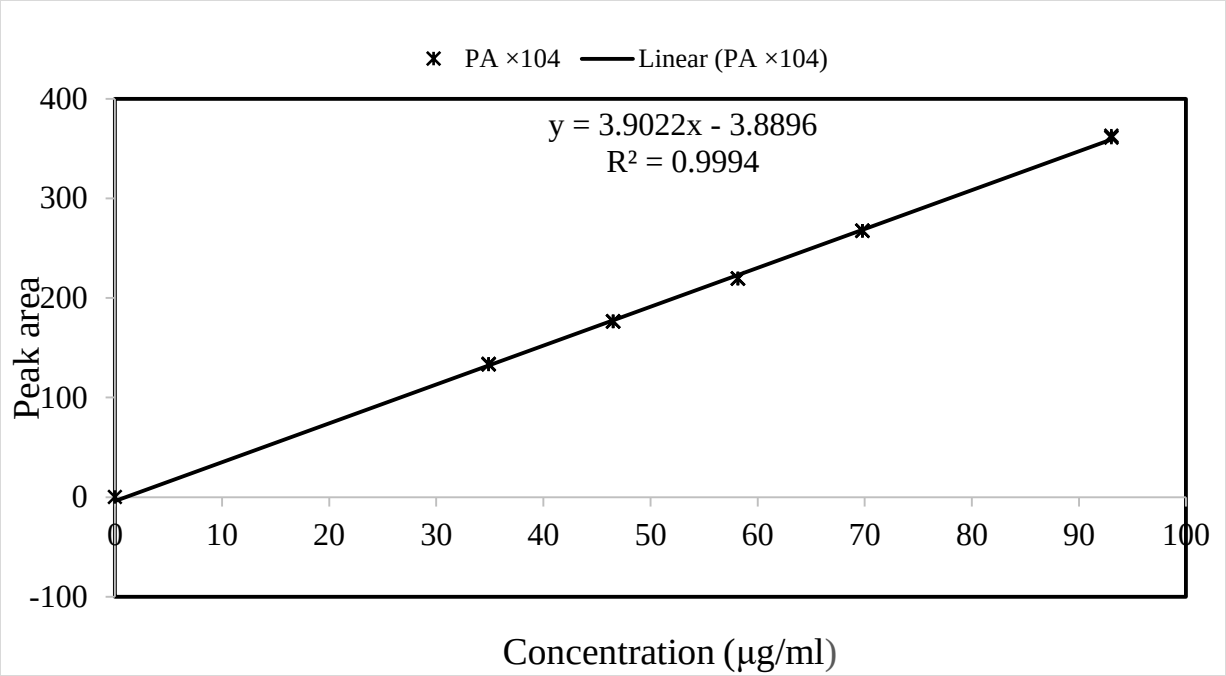

Figure 4.1 Linearity curve for Nicotinamide

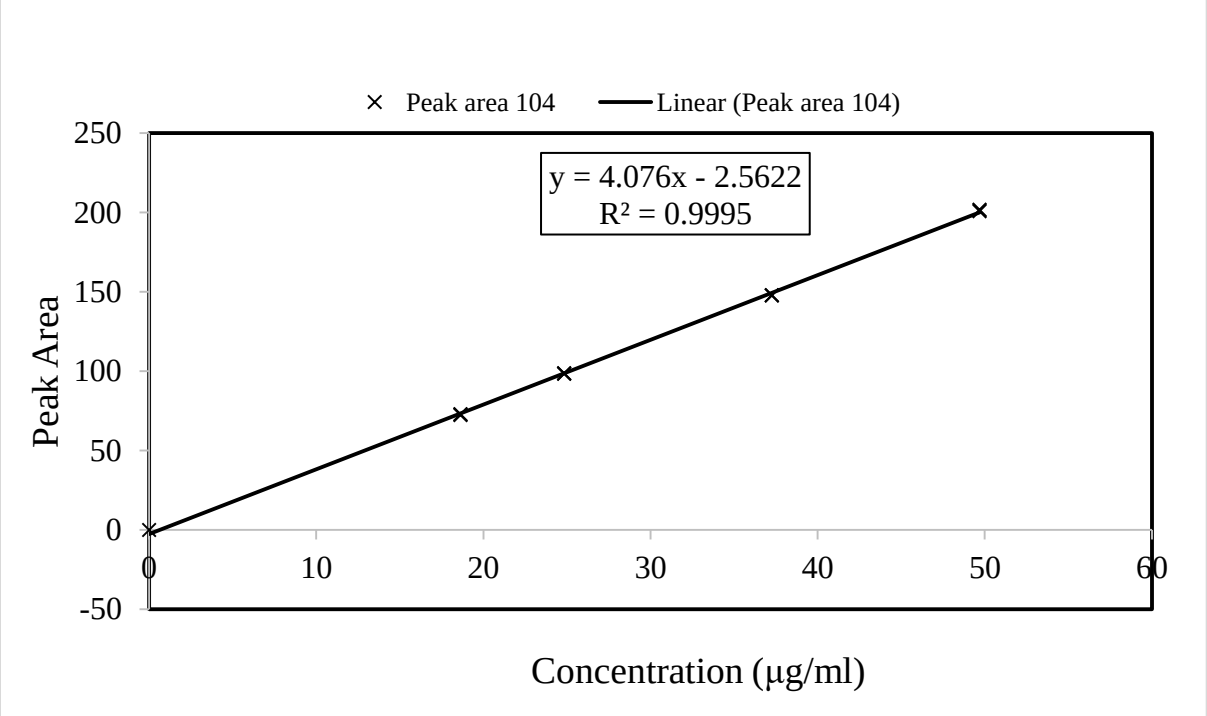

Figure 4.2 Linearity curve Thiamine

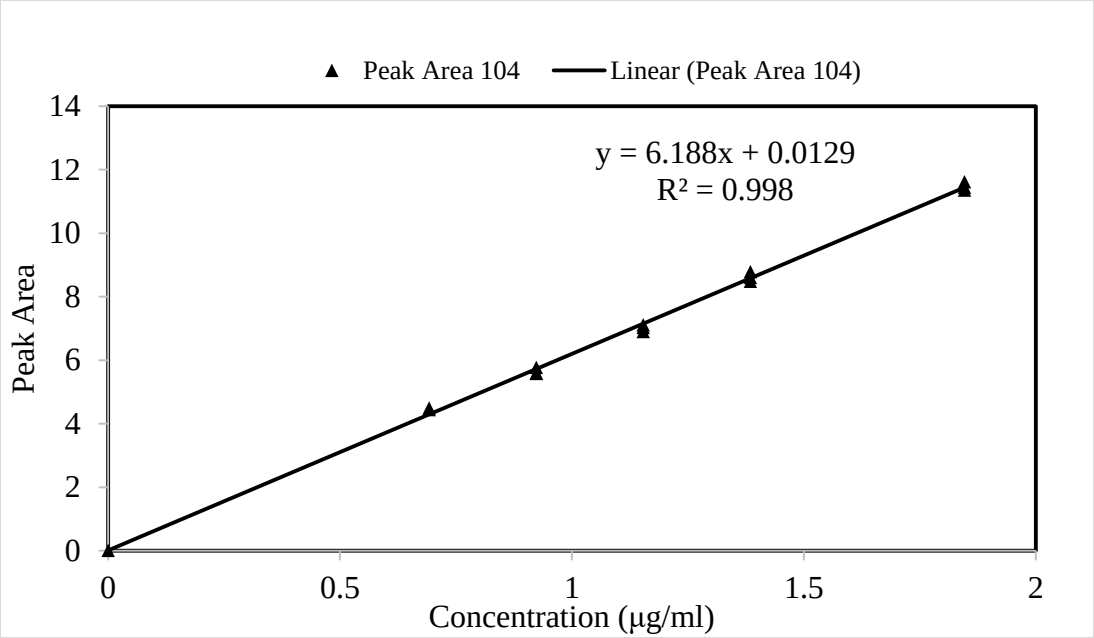

Figure 4.3 Linearity curve for folic acid

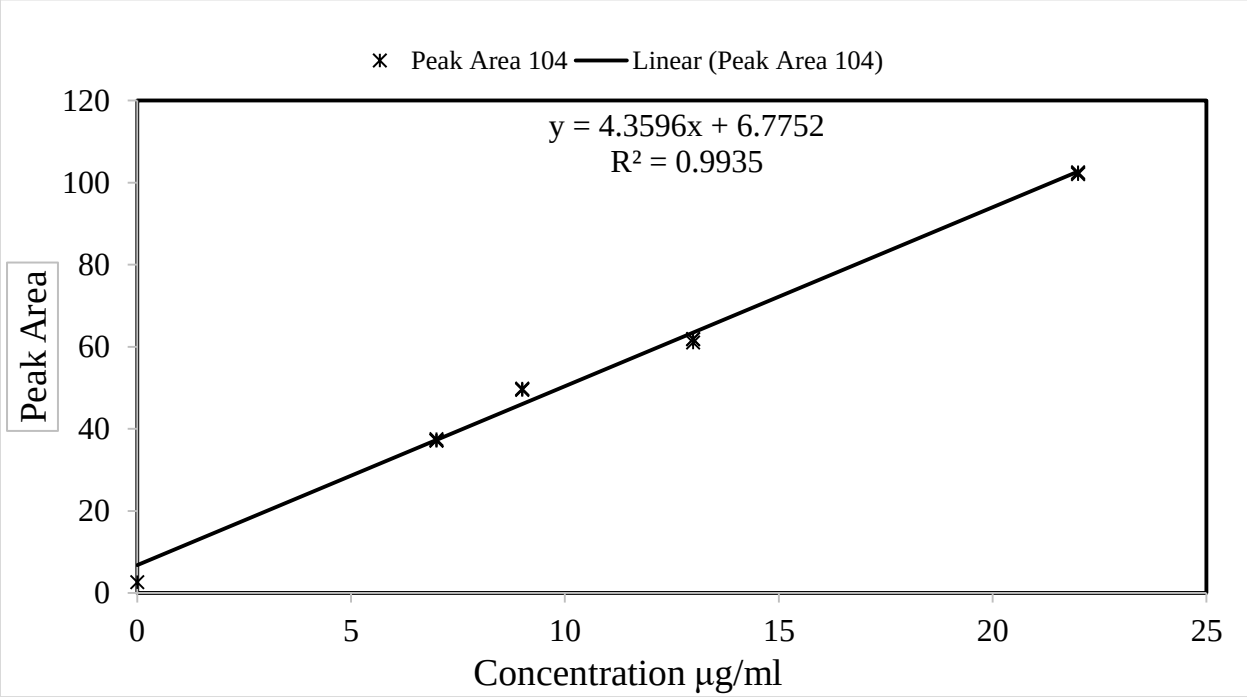

Figure 4.4 Linearity curve of Pyridoxine Hcl

Accuracy

Accuracy was demonstrated by recoveries that was carried out at concentrations corresponding to 80% 100% and 120% of the assay concentration. The percentage recovery of each added working standard was regarded as the accuracy. Table 4.2 shows the percentage recovery of the compounds under study.

Table 4.2 Percentage recoveries

Compound	80%	100%	120%
Nicotinamide	97.93	98.46	100.95
Pyridoxine	98.08	98.89	101.6
Thiamine Hcl	105.01	100.65	100.29
Folic acid	97.15	103.71	98.56

Precision

Precision is expressed as standard deviation. For assay methods, the Relative standard deviation should be less than 2% for six replica injections. All the RSD values for the compounds were within the specified limits. Table 4.3 indicates both the repeatability results and intermediate precision results.

Table 4.3 Repeatability and intermediate precision results.

Compound	Repeatability peak areas RSD (n=6)	Intermediate precision peak areas RSD (n=18)
Nicotinamide	0.13	0.26
Pyridoxine	0.43	0.55
Thiamine Hcl	0.79	1.72
Folic acid	0.21	0.21

4.1.2 Labelling and packaging

Label claim, manufacturing/ expiry dates, batch numbers, storage conditions and country of origin were inspected to check whether they conformed to the PPB guidelines. All the samples were labelled and the required information indicated .on the sample-packaging while on the other hand, 21 % of the samples did not contain the patient information leaflet. The results were as indicated in appendices 5.

4.1.3 Country of origin of the products

Each of the sample was inspected for the details of the country of origin and the data obtained was presented in a bar graph as indicated in Figure 4.5.

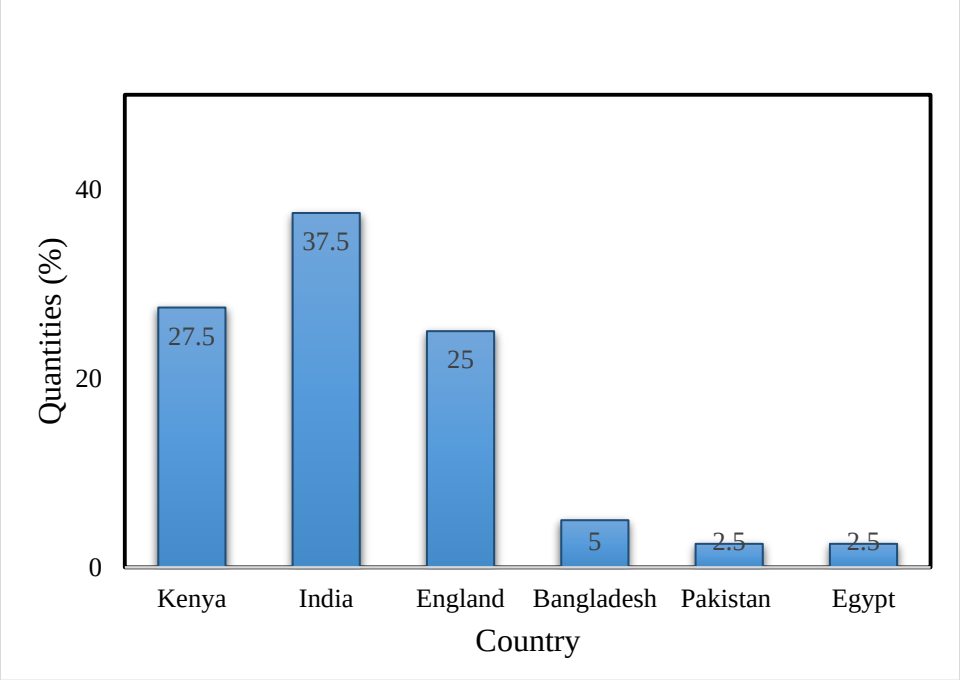

Figure 4.5 Country of origin of products

4.1.4 Assay results

4.1.4.1 Water -soluble vitamins results

Results for the tested samples are as in Tables 4.4 and 4.5. The USP 40 NF 35 pharmacopeia assay specifications for tablets and capsules are 90-165% while those for oral solutions/suspensions are 90-200%).

Table 4.4 Percentage label claim of water- soluble vitamins in antenatal care products.

	Sample code	Nicotinamide	Thiamine	Pyridoxine	Folic acid
1	MVS 004	102.5 (1.2)	132.66 (1.2)	113.2 (3.5)	85.3 (3.26)
2	MVS 013	108.8 (6.22)	142.47 (9.03)	113.55 (1.13)	122.35 (3.78)
3	MVS 016	108.41 (0.4)	84.6 (3.13)	97.76 (1.54)	159.1 (5.27)

4	MVS 016 (1)	117.4 (1.17)	84.2 (2.13)	114.4 (1.21)	147.2 (4.55)
5	MVS 017 (1)	109.5 (3.99)	42.4 (5.07)	175.3 (0.72)	41.1 (23.56)
6	MVS 017 (2)	114.8 (1.75)	46.4 (2.37)	176.2 (2.18)	121.2 (7.13))
7	MVS 020 (1)	97.9 (0.39)	77.47 (3.23)	85.97 (0.46)	17.1 (25.67)
8	MVS 020 (2)	97.4 (0.42)	61.7 (3.98)	81.9 (1.84)	127.82 (1.76)
9	MVS 029	96.6 (0.12)	56.84 (10.63)	92.31 (7.32)	81.7 (8.09)
10	MVS 029	95.5 (1.85)	70.9 (1.82)	74.1 (3.62)	60.4 (4.89)
11	MVS 030	199.18 (2.56)	111.2 (3.42)	90.07 (4.63)	105.4 (6.71)
12	MVS 030 (2)	172.7 (16.9)	61.05 (5.1)	1101.5 (8.69)	102.6 (4.97)
13	MVS 033	85.8 (0.76)	79.71 (6.13)	161.47 (1.90)	135.33 (0.32)
14	MVS 033	65.02 (6.8)	315 (2.63)	181.7 (1.26)	196.9 (1.07)

Key. Values in parenthesis represent the RSD.

Table 4.5 Percentage Label claim of multivitamins for nervous system

	Sample code	Nicotinamide	Thiamine	Pyridoxine
1	MVS 002 Batch 1	115 (1.81)	300 (3.05)	99.8 (5.79)
2	MVS 002 Batch 2	116 (0.87)	302.6 (1.30)	117.1 (0.49)
3	MVS 006 Batch 1	105.5 (0.7)	247.2 (2.43)	100.1 (2.64)
4	MVS 006 Batch 2	96.5 (1.11)	249 (0.49)	95.6 (5.13)
5	MVS 007	N/A	151.4 (0.59)	113.5 (1.06)
6	MVS 008	N/A	145 (1.27)	109.7 (0.42)
7	MVS 015	92.6 (0.59)	338 (2.73)	46.8 (0.74)
8	MVS 018 Batch 1	N/A	191.7 (0.28)	156.7 (0.70)
9	MVS 018 Batch 2	N/A	301.3 (0.78)	151.4 (0.35)
11	MVS 019	N/A	193 (0.56)	146.5 (1.27)
12	MVS 021	100.3 (2.18)	65.0 (1.94)	N/A
13	MVS 023 Batch 1	96.5 (4.31)	120 (2.61)	96.5 (4.31)
14	MVS 023 Batch 2	234 (0.39)	199 (2.24)	110 (3.67)

Key. N/A -The particular vitamin was not in the sample.

Values in parenthesis represent RSD. Samples in this category did not have folic acid

The water-soluble multivitamin, which did not comply with the specified limits, were further analyzed to check sample compliance per component. The results obtained were presented in a pie-chart figure 4.6 below.

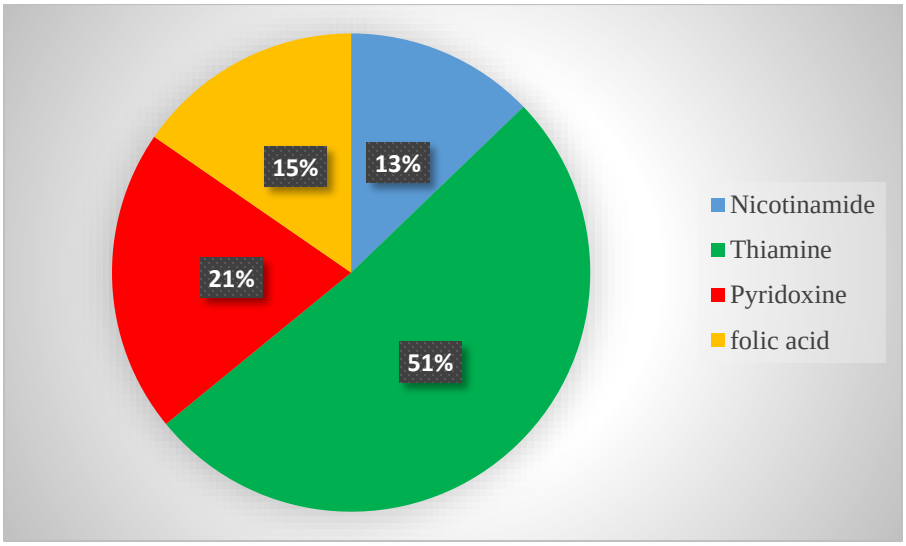

Figure 4.6 Percentage of samples that did not comply with the limits per component

4.1.4.2 Fat soluble vitamins

The results of the fat-soluble vitamins are as indicated in Table 4.6**.** The USP 40 NF 35 pharmacopeia assay specifications for tablets and capsules are 90-165% while those for oral solutions/suspensions are 90-200%).

Table 4.6 Percentage label claim of fat-soluble vitamins.

	Sample code	**Retinylacetate**	**Cholecalciferol**	**Alpha tocopherol**
1.	MVS 016	N/A	88.4 (2.68)	132.1 (1.66)
2.	MVS 017	N/A	88.4 (2.68)	132.1 (1.66)

	Sample code	Retinylacetate	Cholecalciferol	Alpha tocopherol
3.	MVS 017 Batch2	N/A	164.9	N/A
4.	MVS 020	N/A	85.99 (1.44)	100 (1.37)
5.	MVS 023	26.4 (4.09)	110.4 (5.35)	99.6 (2.04)
6.	MVS 023 Batch 2	60.6 (12.28)	67.6 (5.68)	105.5 (1.51)
7.	MVS 029	0.55	52.1 (6.11)	132 (1.66)
8.	MVS 033	43.03 (1.21)	64.9 (11.05)	134.7 (15.32)
9.	MVS 012	4.7	85.1 (10.35)	N/A
10.	MVS 011	104 (0.98)	173.04 (17.17)	91.0 (0.99)
11.	MVS 004 (Suspension)	93.6 (0.98)	154.3 (13.51)	95 (5.11)

Key N/A – The particular vitamin was not in the sample.
Values in parenthesis represent RSD.

Eleven samples were analyzed with regard to fat-soluble vitamins; tablets (5), capsules (3) and syrups (3). Only one sample complied with the USP limits for fat-soluble vitamins. The content of

retinyl acetate was consistently low on all the samples tested, while that of cholecalciferol was high in one of the samples and low on the rest.

4.1.5 Microbial load results

Microbial load analysis was carried out on 19 suspensions. The microbial load results were indicated in Table 4.7. All the samples complied with the British pharmacopeia (2017) specifications for TAMC while 17 samples (89 %) complied with the limits for TYMC. Two of the samples failed to comply with the limits for TYMC.

Table 4.7 Microbial load results

Sample code	TAMC (CFU/ml)	TYMC (CFU/ml)	Inference
MVS 003	<10	<10	Complies
MVS 004	<10	<10	Complies
MVS 009	<10	<10	Complies
MVS 010	<10	<10	Complies
MVS 011	<10	<10	Complies
MVS 022	<10	<10	Complies
MVS 025	<10	<10	Complies
MVS 026 Batch 1	<10	4×10^2	Does not comply
MVS 026 Batch 2	<10	<10	Complies
MVS 027	<10	<10	Complies
MVS 028	<10	<10	Complies
MVS 034	<10	<10	Complies
MVS 035 Batch 1	<10	<10	Complies
MVS 035 Batch 2	<10	<10	Complies

Sample code	TAMC (CFU/ml)	TYMC (CFU/ml)	Inference
MVS 036	<10	<10	Complies
MVS 037	<10	3×10^1	Does not comply
MVS 038	<10	<10	Complies
MVS 039	<10	<10	Complies
MVS 040	<10	<10	Complies

4.2 DISCUSSION

4.2.1 Conformity to labeling Guidelines.

The Pharmacy and Poisons Board has provided guidelines for registration of dietary supplements and borderline products, which specify the labelling requirements. For instance, it states that every product should be labelled clearly with indelible letters in English or in Kiswahili. In instances where material is not originally in English or Kiswahili, a copy of the original language and a full translation should be provided. The guidelines require that the label should include the brand name, generic name, quantity of active ingredient or percentage RDA of each ingredient per dosage unit, total packed quantity in a unit pack, manufacturing date, address expiry date, batch number, storage conditions, warning and precautions and directions for use (2).

All the collected samples had the manufacturer's details, manufacturing and expiry dates, batch numbers, storage conditions and quantity per packet clearly indicated on the packets. However, 10 % of the samples did not contain the patient information leaflet.

The guidelines require manufacturers to include scientific package inserts. Inserts provide information on safe and effective use of supplements (122). The precautions and warnings are written according to the regulator specifications. All the samples had instructions on their use. These guidelines prohibit promotional statements and making of comparison about the superiority of a product to other products (2). All the products from UK had a promotional statement indicating they are the number one UK's supplementary brands against the regulations in Kenya. Most of the products did not indicate the specific forms of vitamins E and A in the products. Sample MVS 017 had colour variation even though it was the same batch.

4.2.2 Country of origin

Upon analysis of the samples, 37.5% of the samples were from India, 27.5% from Kenya, 25% from England while Egypt and Pakistan had 2.5% each. This is consistent with a previous survey conducted by Health Action International which indicated that most of the imported drugs and supplements in the Kenya market are from India (123).

4.2.3 Uniformity of weight

All the samples collected complied with the British pharmacopeia specifications for uniformity of weight (121).

4.2.4 Content determination (Assay)

The analyzed samples were fixed dose multicomponent vitamin supplements, some with and others without minerals. Multivitamin supplements used in antenatal care contained four vitamins (folic, pyridoxine, thiamine and nicotinamide) with minerals while multivitamins used in treatment of nervous system contained three vitamins (thiamine, folic acid and pyridoxine) without minerals. From the sample tested, 14.3% complied with the USP 40 NF 35 pharmacopeia specifications on assay of multivitamin/ multimineral, while 85.7% did not comply with the specified limits. 51% of the analyzed samples did not comply with the specified limits of thiamine, 21% on pyridoxine, 15% on folic acid and 13% on nicotinamide. On the samples that did not meet the pharmacopeial limits for thiamine, 40.9 % of the samples were below the limits, 31.8% above the limits and 27.3 % within the given range. A similar trend was also observed in folic acid where 50% of the samples containing folic acid were below the pharmacopeia limits, 8.3% above and 41.7% within the limit range. In pyridoxine, 60% of the samples were within the limits for those with below and above limits had same rate of 20%. Nicotinamide had 11.1% of the samples below the limit range, 16.7% of the samples were above the limits and 72.2% were within the given pharmacopeial range.

In the assay of fat – soluble vitamins, 66.7% of the samples assayed for retinylacetate were below the pharmacopeial limits while 33.3% were above the limits, none of the samples had retinylacetate within the limits. For cholecalciferol, 63.6% of the samples were above the limits, 9.1% above the limits and 27.3% complied with the given limits. All the samples had alpha tocopherol within the pharmacopeial limits.

High concentration of vitamins may be toxic especially when consumed over a long period. In the United Kingdom, nutritionist and other healthcare professionals are have been concerned about the increase on the availability and use of high-dose vitamins and minerals in both the retail and internet(124). General symptoms of multivitamin toxicity are fatigue, headaches, amenorrhea, joint and bone pain, dry flaking skin, alopecia, gastrointestinal disturbances, vomiting and weight loss. Vitamin A may cause hepatotoxic effects, visual changes and teratogenic effects. Beta-carotene may cause risk of lung cancer among smokers and people with asbestosis. At high intake, vitamin D may cause soft-tissue calcification or hypercalcemia. Pyridoxine may cause sensory neuropathy and ataxia while niacin may lead to vasodilation, gastrointestinal upset and hyperglycemia. Vitamin E may cause nausea, vomiting, diarrhea and anticlotting effect (125). The products with less label claim may not supplement the body requirement and may lead to deficiencies. From the assay results, the inconsistencies of the contents is a pointer that the intended end purpose of supplementation may not be achieved.

The inconsistency on the RSD values can be attributed to the extracting process which may not have been complete due to varying solubility in the extracting solvents (126). However, the results were sufficient for the scope of this study.

The PPB guidelines on the listing of multivitamin supplements do not require compendia testing of the products before registration neither does the post market surveillance framework incorporate multivitamin supplements. This has left the market authorization holders with the sole responsibility of ensuring quality of multivitamin supplements. Furthermore, there are no published reports on the quality of multivitamins in Kenya, their consumption data and popularity among the population. This study is important and significant in giving an overview on the quality of multivitamin products in the market but more needs to be undertaken. According to the persistence market research report of January 2018, vitamins are projected to dominate the dietary supplements in the USA. This trend is most likely to repeat in the rest of the world at large and in Kenya and this makes it important to have a robust regulation framework for multivitamins.

The findings are also invaluable to policy makers, Pharmacy, and Poisons Board as a stimulant to strengthen the regulation of dietary supplements and enhance post market surveillance.

4.2.5 Microbial load determination

Nineteen syrups were tested for microbial contamination and the results are as in Table 4.7. The acceptance criteria for aqueous preparation for oral use is 10^2 (TAMC) and 10^1 (TYMC). All the samples analyzed for TAMC had CFU/mL of <10, thus they complied with the British pharmacopeia (BP) 2017 volume IV limits of total aerobic microbial enumeration.

Two samples (11 %) analyzed for did not comply with BP limits on total yeast and mold count. Sample MVS 026 (1) had 4×10^1 cfu/ml and Mvs 037 had 3×10^1 that were above the specified limits and they were both manufactured from the local pharmaceutical industries.

From the study, 89 % of the samples complied with the microbial load specifications. This was consistent with a study carried out by Abdullah *et al* on the microbial contamination of multivitamins. The study was carried out in Dhaka city on different brands of multivitamins

suspensions sold locally. From the results 91% of tested samples complied with the microbiological requirements for multivitamins (116).

4.3 STUDY LIMITATIONS

There was no comprehensive list from the Pharmacy and Poisons board on the listed multivitamin supplements in the market and this made it difficult for sample size determination and planning of field.

The brands and batches of the available products in the market were limited and this reduced the number of sampled products.

Fat – soluble reference standards had to be sourced from Germany and this impacted on the cost and time of the study, in addition, the solid phase extraction (SPE) for the separation and extraction of fat- soluble vitamins was too expensive to obtain and use in the study.

The method employed could not be utilized on some formulations in the market such as the soft gel capsules and analysis of minerals in the products selected.

CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.1 Conclusion

From this study, it was established that all the multivitamin supplements met the packaging and labelling requirements as per the PPB guidelines. However, 21% samples did not contain the patient information leaflet.

Samples on which microbial load analysis was carried out (89 %) were within the British Pharmacopeia limits. All the samples complied with TAMC specifications while 11 % did not comply with TYMC specifications.

Only, four samples (14.3%) complied with the USP pharmacopeia specifications on assay of multivitamin/ multimineral, while 24 samples (85.7%) did not comply with the specified limits. Upon further analysis the samples which did not comply with the limits, 51% of the of the samples did not meet the pharmacopeia limits on thiamine, 21% on pyridoxine, 15% on folic acid and 13% on nicotinamide.

5.2 Recommendations

The Pharmacy and Poisons Board should keep an updated list of all the listed multivitamin supplements in Kenya and formulate a risk-based approach to assure the quality of multivitamin supplements in the market.

More research on quality of multivitamin supplements in Kenya to be carried out in order to build upon the local database on the quality of multivitamin supplements in the market. The studies should further be extended to other dietary supplements including those containing minerals.

Research on the microbial load determination studies should be extended to incorporate isolation and identification of specific microbes.

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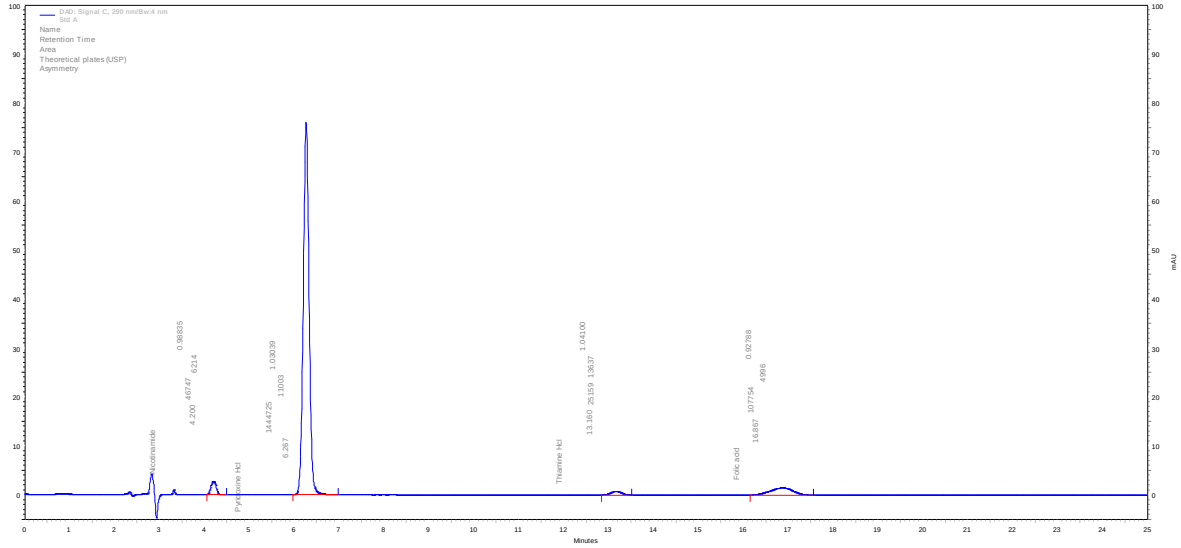
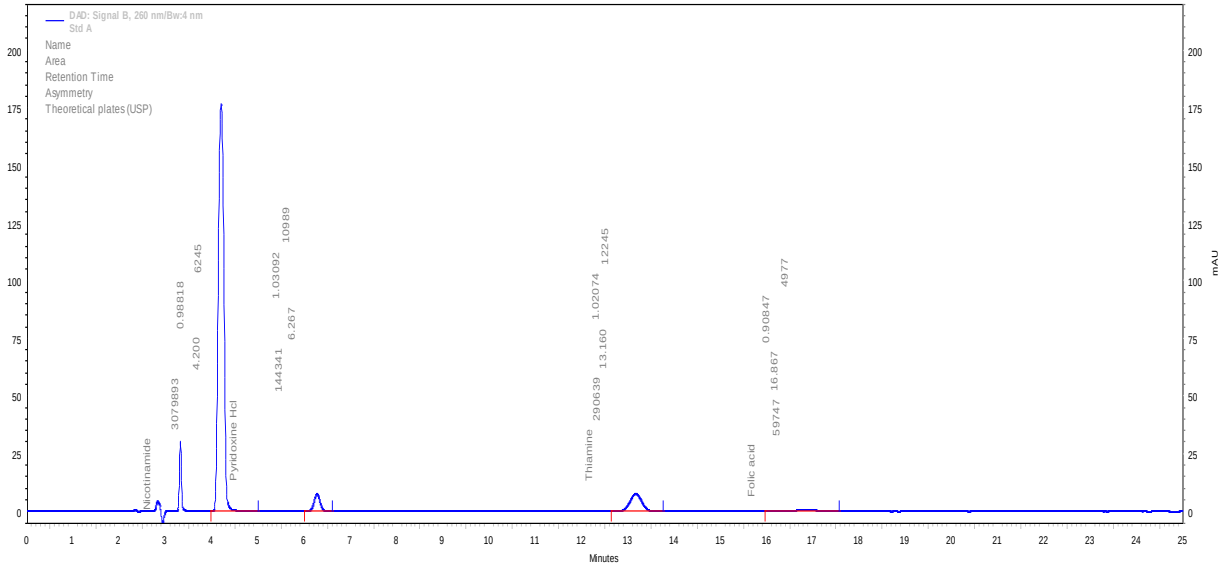
APPENDICES

Chromatogram at 290 nm

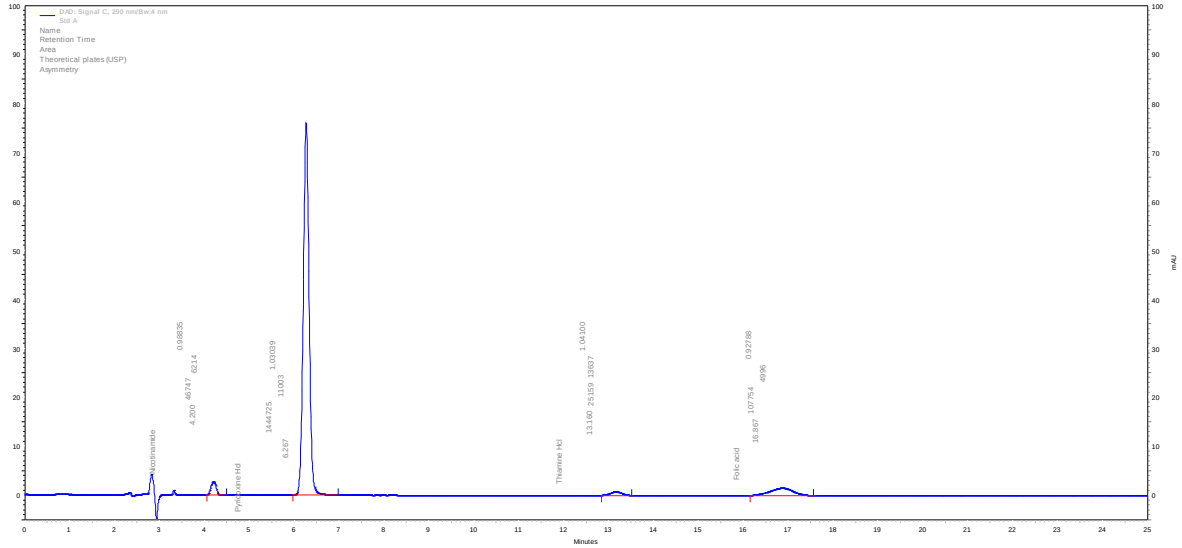


Chromatogram 260 nm

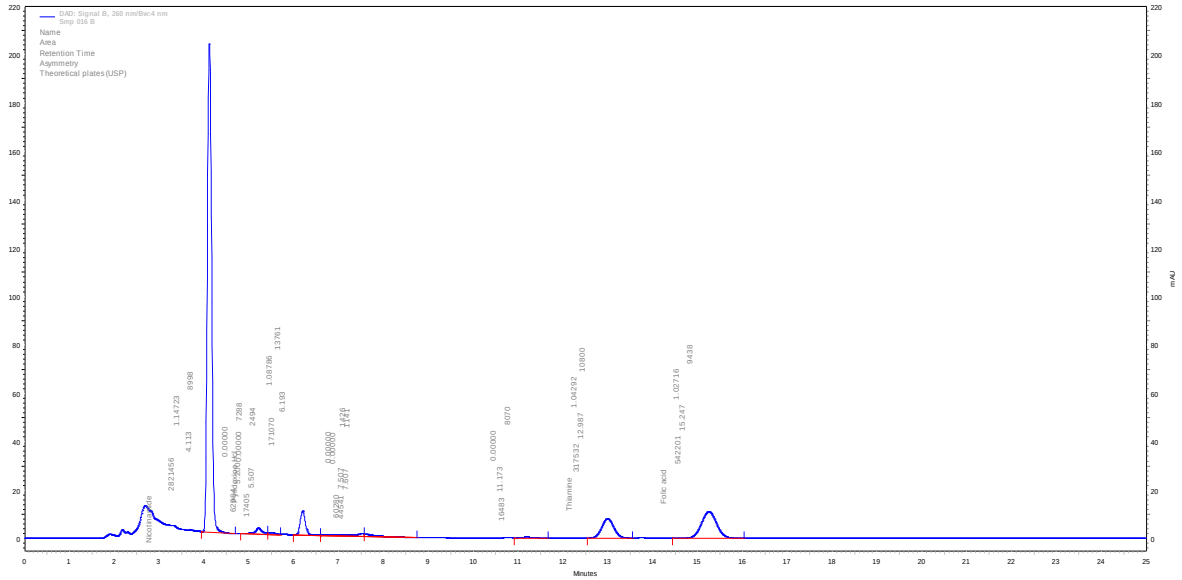


Appendix 1. Water-soluble standard chromatogram at 260 nm and 290 nm

Chromatogram at 290 nm

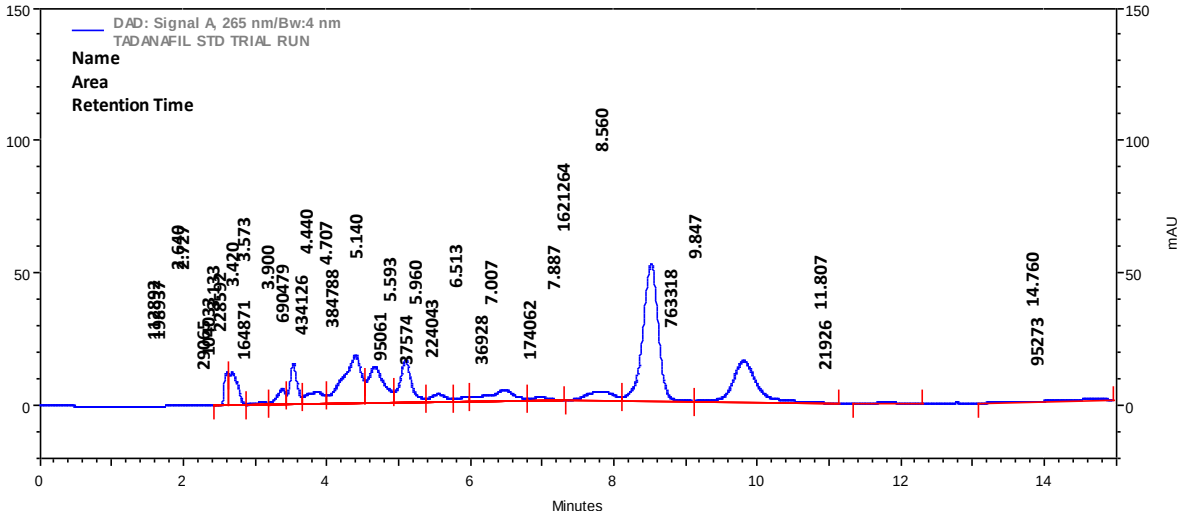


Chromatogram at 260 nm

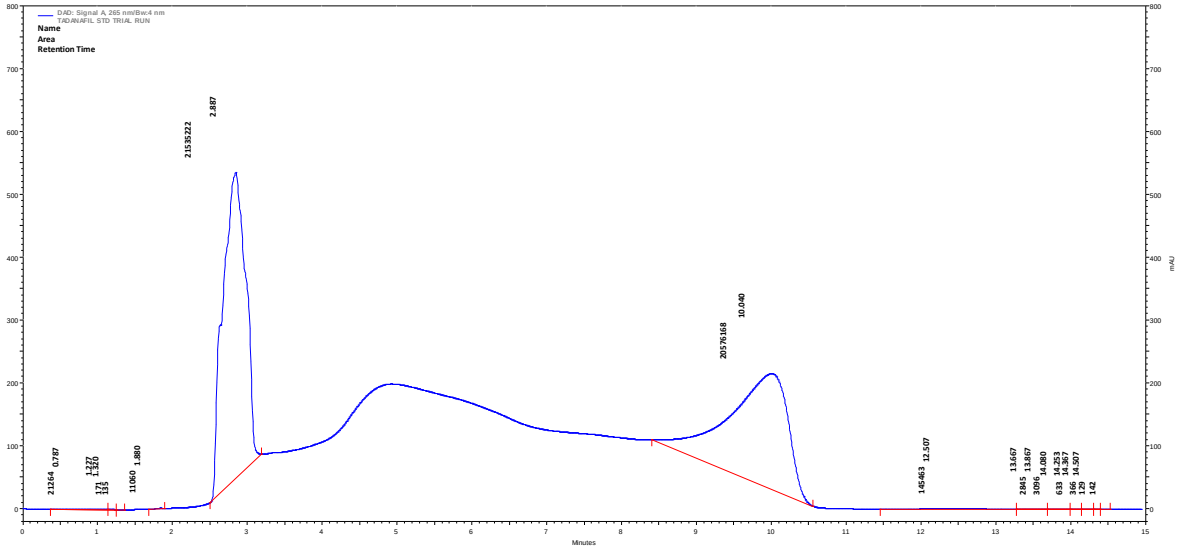


Appendix 2. Water –soluble sample chromatogram at 260 nm and 290 nm

Extracted Sample

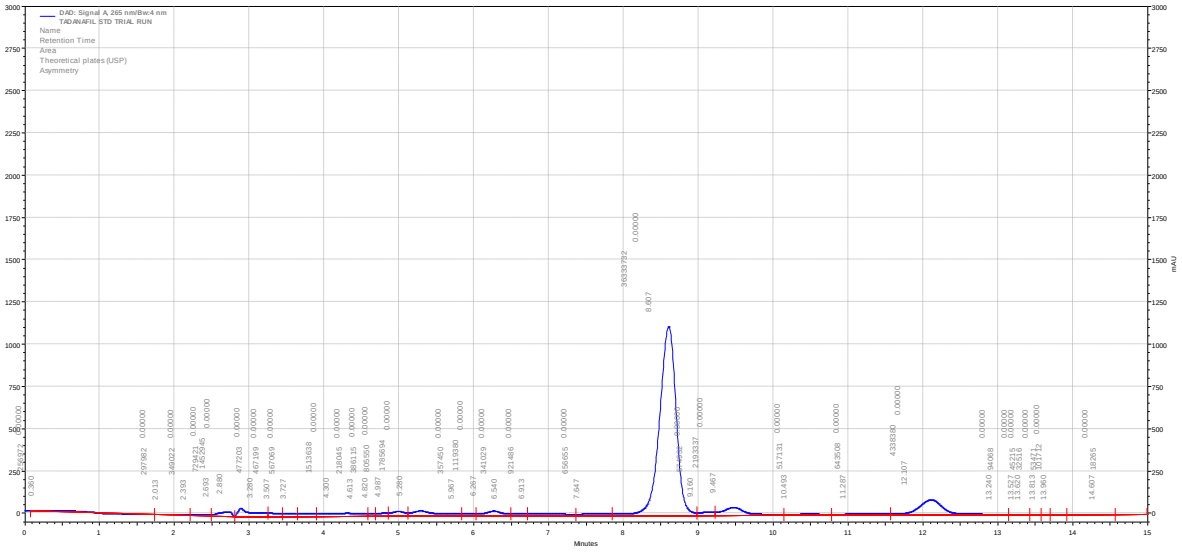


unextracted sample

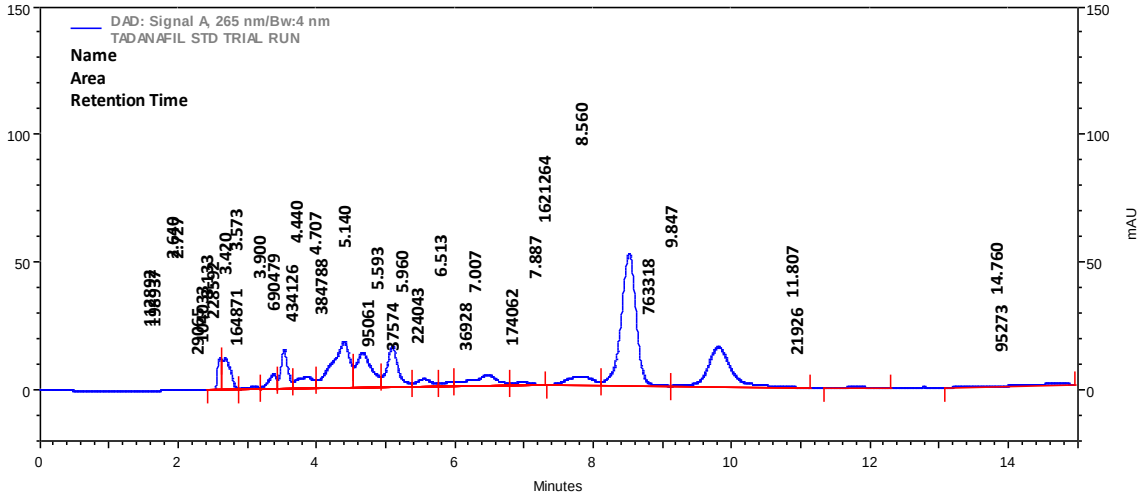


Appendix 3. Chromatogram for extracted and unextracted fat-soluble vitamin samples

Standard chromatogram



Sample chromatogram



Appendix 4**.** Chromatograms for fat-soluble vitamins standard and sample. Vitamins.

Details of suspension samples

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
MVS 003	Vitamin A (Retinal/Retinol) 1000 IU Vitamin B_1 (Thiamine) 0.5 mg Vitamin B_2 (Riboflavin) 0.5 mg Vitamin B_3 (Niacin/Niacinamide) 5 mg Vitamin C (Ascorbic acid) 20 mg Vitamin D_3 200 iu	✓	✓	✓	✓	India
MVS 005	Vitamin B_1 (Thiamine) 5 mg Vitamin B_2 (Riboflavin) 3 mg Vitamin B_6 (Pyridoxine) 1.5 mg Vitamin B_3 (Niacin/Niacinamide) 25 mg Calcium Pantothenate 5 mg Vitamin B_{12} (Cyanocobalamin) 2.5 Folic acid 0.75mg	✓	✓	✓	✓	England

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Vitamin C (Ascorbic acid) 40 mg Vitamin A(Retinal/Retinol) 2500iu Vitamin D_3 200 iu Vitamin E (Tocopherol)7.5 iu					
MVS 009	Cyproheptadine Hcl 2 mg Vitamin B_1 (Thiamine) 10 mg Vitamin B_6 (Pyridoxine) 10 mg Vitamin B_{12} (Cyanocobalamin) 100 mcg	✓	✓	✓	✓	India
MVS 010	Cyproheptadine Hydrochloride 2 mg Vitamin B_1 (Thiamine) 1 mg Vitamin B_2 (Riboflavin) 0.5 mg Vitamin B_6 (Pyridoxine) 0.5 mg Vitamin B_3 (Niacin/Niacinamide) 10 mg	✓	✓	✓	✓	India

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Vitamin B_{12} (Cyanocobalamin) 1 µg					
MVS 011	Vitamin A 666.66 IU Vitamin B_1 0.3mg Vitamin B_2 0.4mg Vitamin B_6 0.35mg Vitamin C 25mg Vitamin D 140 IU vitamin E 2 mg Nicotinamide 5 mg Pantothenic acid 1.33mg	✓	✓	✓	✓	India
MVS 012	Vitamin A (Retinal/Retinol) 1000 units Vitamin B_1 (Thiamine)1.5 mg VitaminB_2 (Riboflavin)1.5mg VitaminB3 (Niacin/Niacinamide) 10mg Vitamin B12 (Cyanocobalamin) 10 mg Vitamin C (Ascorbic acid) 40 mg	✓	✓	✓	✓	England

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Vitamin D 200 units					
MVS 022	Vitamin B_1 (Thiamine) 10 mg Vitamin B_6 (Pyridoxine) 10 mg Vitamin B_{12} (Cyanocobalamin)100 µg	✓	✓	✓	✓	India
MVS 024	Vitamin B_1 (Thiamine) 0.8 mg Vitamin B_2 (Riboflavin) 0.6 mg Vitamin B_3 (Niacin/Niacinamide) 8 mg Vitamin B_5 (Pantothenic acid) 1 mg Vitamin B_6 (Pyridoxine) 0.5 mg Vitamin A (Retinal/Retinol) 1000 I U Vitamin D3 200 IU Vitamin E (Tocopherol) 2.5 IU Vitamin (Ascorbic acid)40 mg	✓	✓	✓	✓	England

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Vitamin B_7 (Biotin) 20 µg Zinc 3 µg					
MVS 025	Zinc 34.85 mg Vitamin B3 (Niacin/Niacinamide) 5 mg Vitamin B_1 (Thiamine)1.5 mg Vitamin B_2 (Riboflavin)1.5 mg Vitamin B_{12} (Cyanocobalamin) 1.5 µg Vitamin A (Retinal/Retinol) 1500iu Vitamin E (Tocopherol) 3 mg Vitamin B_6 (Pyridoxine)1 mg Vitamin C (Ascorbic acid)30 mg Iron 7 mg Magnesium 10 mg	✓	✓	✓	✓	England
MVS 026	Vitamin B_1 (Thiamine) 2 mg Vitamin B_2 (Riboflavin) 0.5 mg	✓	✓	✓	✓	India

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Vitamin B_{12} (Cyanocobalamin) 2.5 µg Vitamin B_3 (Niacin/Niacinamide) 5 mg Green iron and Ammonium citrate200 mg					
MVS 027	Cyproheptadine chlorhydrate 2 mg Vitamin B_1 (Thiamine) 1 mg Vitamin B_2 (Riboflavin) 0.5 mg Vitamin B_6 (Pyridoxine) 0.5 mg Vitamin B_3(Niacin/Niacinamide) 10 mg Vitamin B_{12} (Cyanocobalamin) 1 µg	✓	✓	✓	✓	India
MVS 028	Cyproheptadine chlorhydrate 2 mg Vitamin B_1 (Thiamine) 1 mg Vitamin B_2 (Riboflavin) 0.5 mg	✓	✓	✓	✓	Kenya

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Vitamin B_6 (Pyridoxine) 0.5 mg Vitamin B_3 (Niacin/Niacinamide) 10 mg Vitamin B_{12} (Cyanocobalamin) 1 µg					
Mvs 034	Vitamin A 1000 iu Vitamin D3 100 iu Vitamin B1 0.5 mg Vitamin B2 0.5 mg Vitamin B6 0.5 mg Nicotinamide 8.0 mg Vitamin C 20.0 mg Vitamin B12 2.5 mcg	✓	✓	✓	✓	Kenya
MVS 035	Vitamin A palmitate BP 1500 iu Thiamine Hcl 0.5 mg Riboflavin 0.5 mg	✓	✓	✓	✓	India

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Nicotinamide 5.0 mg Ascorbic acid 20 mg Vitamin D3 25 iu					
MVS 036	Vitamin A 1500 iu Vitamin D3 400 iu Vitamin E 5.0 mg Vitamin B1 0.5 mg Vitamin B2 0.6 mg Vitamin B6 0.6 mg Nicotinamide 8.0 mg Vitamin C 40.0 mg	✓	✓	✓	✓	England
MVS 037	L-glutamic acid 100 mg Thiamine Hcl 4.5 mg Pyridoxine HCL 1.5 mg	✓	✓	✓	✓	England

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Cyanocobalamine 5.0 mcg Nicotinamide 30.0 mg					
MVS 038	Vitamin B_1 0.5mg	✓	✓	✓	✓	England
MVS 039	Vitamin B_1 (Thiamine) 1.5 mg Vitamin B_2 (Riboflavin) 1.5 mg Vitamin B_{12} (Cyanocobalamin) 2.5 µg Vitamin B_3 (Niacin/Niacinamide) 20 mg Folic acid 0.75 mg Vitamin E 7.5 iu Cholecalciferol 200 iu	✓	✓	✓	✓	Bangladesh
MVS 040	Vitamin B_1 0.7mg Vitamin B_2 0.8mg Vitamin B_6 0.35mg Vitamin C 12mg Vitamin D 10 µg vitamin E 5 mg Vitamin A 200µg	✓	✓	✓	×	England

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
	Nicotinamide 8 mg Folic acid 100 µg Pantothenic acid 2mg Vitamin B12 1 µg Iron 5 mg Zinc 4 mg Copper 150µg					

Details of tablets and capsules samples collected for this study

Product code	Label claim	Manufacturing & expiry dates	Batch number	Patient information literature	Storage conditions	Country of origin
MVS 002	Cyproheptadine Hcl 4mg Vitamin B_1 2mg Vitamin B_2 0.5mg Vitamin B_6 1mg	✓	✓	✓	✓	Kenya

	Nicotinamide 10mg Vitamin B_{12} mcg Glycine 40mg Elemental Iron8mg Elementa Zinc 6mg					
MVS 004	Thiamin B1 19.5 mg Riboflavin B_2 25 mg Niacin B_3 25 mg vitamin B_6 10 mg Folic Acid 400 µg Vitamin B_{12} 25 µg Biotin 25 µg pantothenic acid 25 µg Vitamin C 500 mg Choline Bitartrate 25 mg Inositol 25 mg Paba 25 mg	✓	✓	✗	✓	Kenya
MVS 006	Cyproheptadine chlorhydrate 2 mg Vitamin B_1 (Thiamine)1 mg Vitamin B_2 (Riboflavin)0.5 mg Vitamin B_6 (Pyridoxine) 0.5 mg VitaminB_3 (Niacin/Niacinamide) 10 mg	✓	✓	✓	✓	Kenya

	Vitamin B_{12} (Cyanocobalamin) 1 mcg					
MVS 007	Thiamine 200 mg Pyridoxine 50 mg Cyanocobalamine 1000 µg	✓	✓	✓	✓	India
MVS 008	Thiamine 200 mg Pyridoxine 50 mg Cyanocobalamine 1000 µg	✓	✓	✓	✓	Bangladesh
MVS 013	Vitamin D_3 5 Vitamin E 17mg vitamin C 120mg Thiamin B_1 2.8mg Riboflavin B_2 3.2mg Niacin B_3 36mg Vitamin B_6 4mg Folic acid 400 µg Vitamin B_{12} 2 µg Biotin 300 µg Pantothenic acid 12mg	✓	✓	×	✓	Kenya
MVS 015	Thiamine mononitrate 10 mg Riboflavine 10mg Pyridoxine Hydrochloride 3 mg Cyanocobalamine 15 µg Nicotinamide 45mg Calcium pantothenate 50mg	✓	✓	×	✓	Pakistan

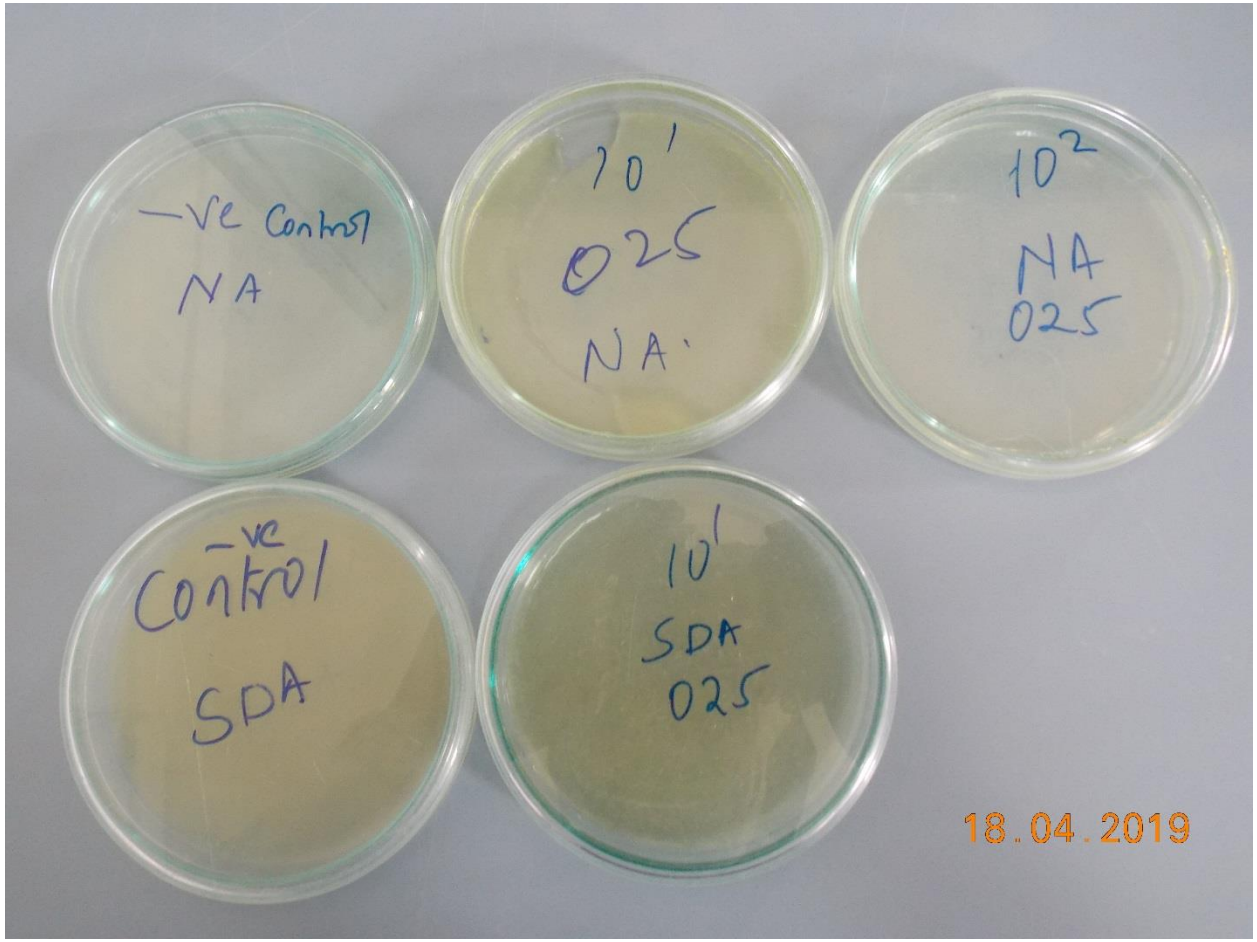
MVS 016	vitamin D_3 10 µg vitamin E 4 mg Vitamin C 70 mg Vitamin B_1 3 mg Vitamin B_2 2 mg Vitamin B_3 20 mg Folic Acid 400 µg Vitamin B_{12} 6 µg Vitamin B_7 150 µg Vitamin B_5 150mg Vitamin K_3 70 Vitamin B_6 10mg Iron 17mg Magnesium 150mg Zinc 15mg iodine 140 copper 1mg selenium 30 µg Natural mixed carotenoids 2mg	✓	✓	✓	✓	India
MVS 017	Vitamin D 2.5 µg Vitamin E 20 mg Vitamin C 70mg Thiamin 3 mg Riboflavin 2 mg Niacin 20mg Vitamin B_6 10 mg Folic acid 0.4mg Vitamin B_{12} 6 Vitamin K 0.2mg Iron 20 mg Zinc 15 mg	✓	✓	✓	✓	India

	copper 1 mg Iodine 140 µg					
MVS 018	L-glutamic acid 250 mg Vitamin B_1 (Thiamine) 10 mg Vitamin B_6 (Pyridoxine) 3 mg Vitamin B_{12} (Cyanocobalamin) 10 µg	✓	✓	×	✓	Kenya
MVS 019	Thiamine Hcl 100 mg Pyridoxine Hcl 50 mg vitamin B_{12} 100 µg L-glutamin 500mg	✓	✓	×	✓	India
MVS 020	Thiamine 3 mg Riboflavin 2mg Pyridoxine 10 mg vitamin B_{12} 6 µg Vitamin C 70mg vitamin D 10 µg Vitamin K 70 µg niacin 20mg Biotin 150 µg Pantothenic acid 6 mg Vitamin E 4 mg Folic acid 400 Magnesium 150mg Iron 17mg zinc 15 mg	✓	✓	✓	✓	India

	selenium 30 µg copper 1 mg					
MVS 021	Thiamine Hcl 1 mg Riboflavin 1 mg Nicotinamide 10 mg	✓	✓	✖	✓	Kenya
MVS 023	Vitamin B_1 10 mg Vitamin B_2 10 mg Vitamin B_3 50 mg Vitamin B_5 10 mg Vitamin B_6 2 mg Vitamin B_{12} 7.5 mg Vitamin A 5000 iu Vitamin D_3 400 iu Vitamin C 75 mg Vitamin E 15 mg	✓	✓	✓	✓	Kenya
MVS 029	Vitamin A 2666iu Vitamin B_1 3mg Vitamin B_2 2 mg vitamin B_3 10 mg Vitamin B_6 1 mg Vitamin B_{12} 2 µg Vitamin C 50 mg Vitamin D_3 400 iu calcium 230 mg copper 0.15mg Folic acid 500 Iodine 0.01mg Iron 20mg Magnesium 0.5 mg manganese 0.05mg Molybdenum 0.025 mg potassium 0.84 mg	✓	✓	✓	✓	Egypt

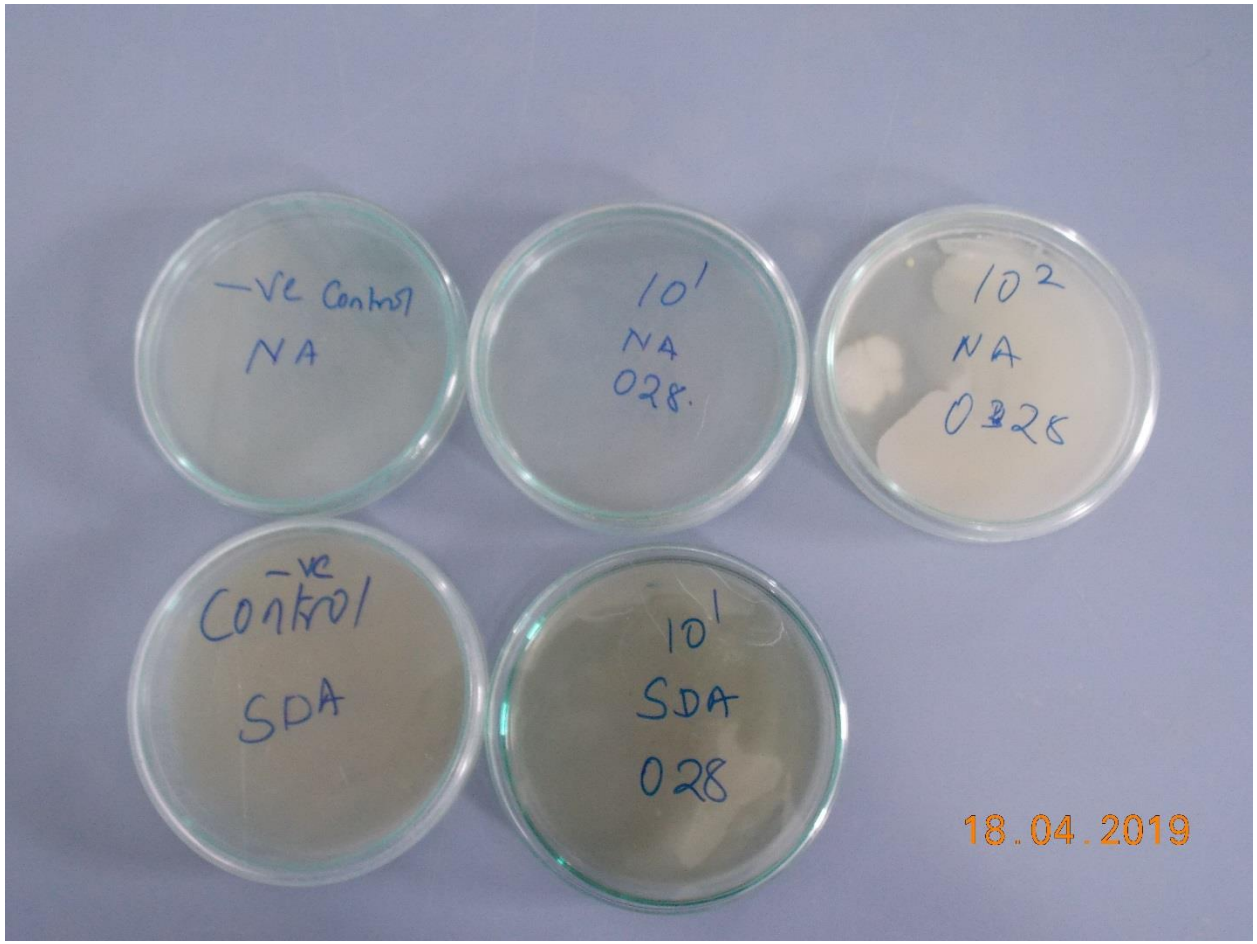
	zinc 0.085mg					
MVS 030	Vitamin D 2.5 Vitamin E 20 mg Vitamin C 70mg Thiamin 3 mg Riboflavin 2 mg Niacin 20mg Vitamin B_6 10 mg Folic acid 0.4mg Vitamin B12 6 Vitamin K 0.2mg Iron 20 mg Zinc 15 mg copper 1 mg iodine 140	✓	✓	✓	✓	India
MVS 031	Thiamine mononitrate 100mg Pyridoxine Hcl 200mg Cyanocobalamine 200mcg	✓	✓	✓	✓	Britain
MVS 032	Thiamine nitrate 200mg Pyridoxine Hcl 50mg Cyanocobalamine 1000 mcg	✓	✓	✓	✓	India
MVS 033	Thiamine mononitrate 100mg Pyridoxine Hcl 200mg Cyanocobalamine 200mcg	✓	✓	×	×	Britain

Appendix 5. Details of the samples analyzed.



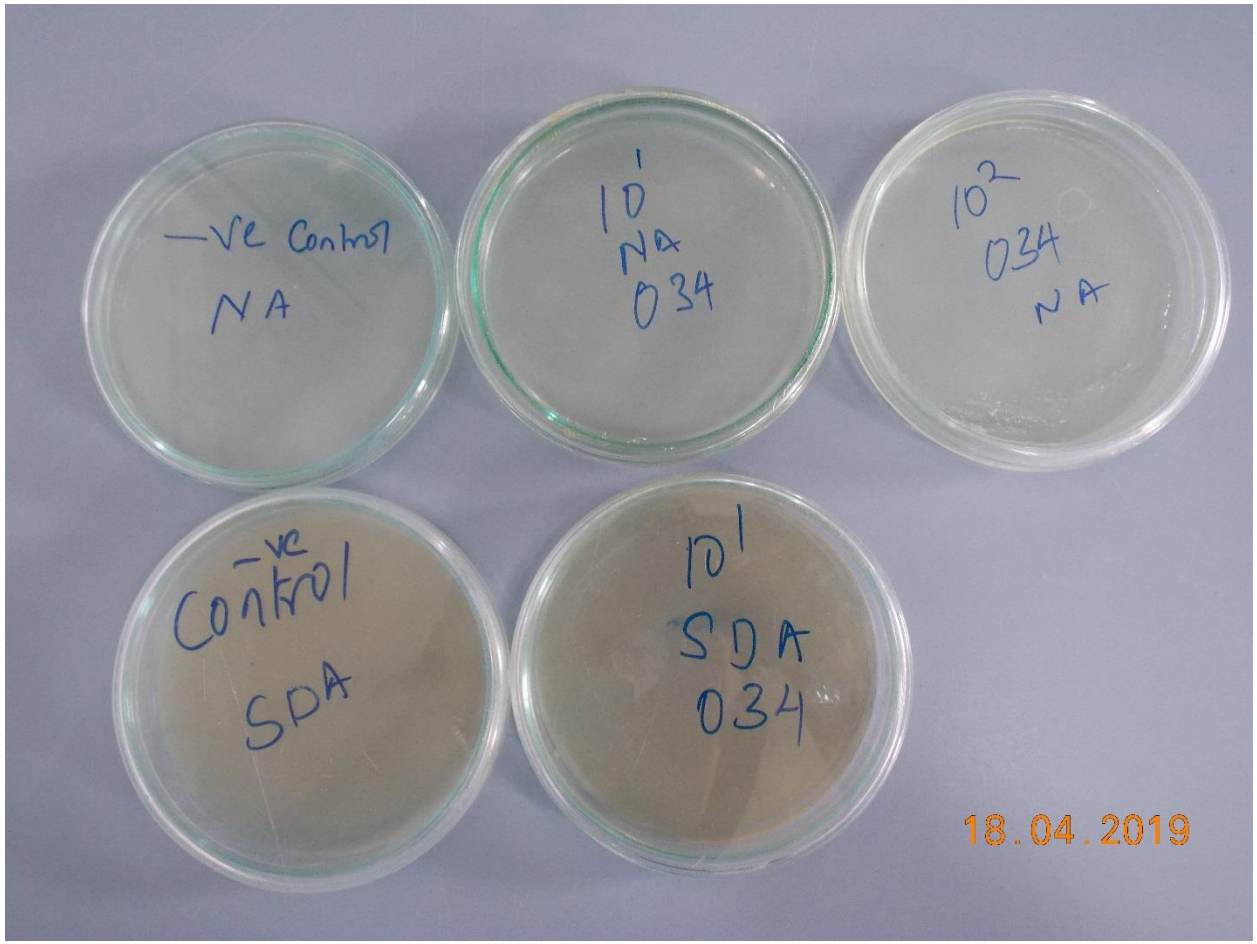
Appendix 6**.** Typical Nutrient agar and Sabourand dextrose agar plate

for sample Mvs 025 showing no growth of bacteria and fungus.



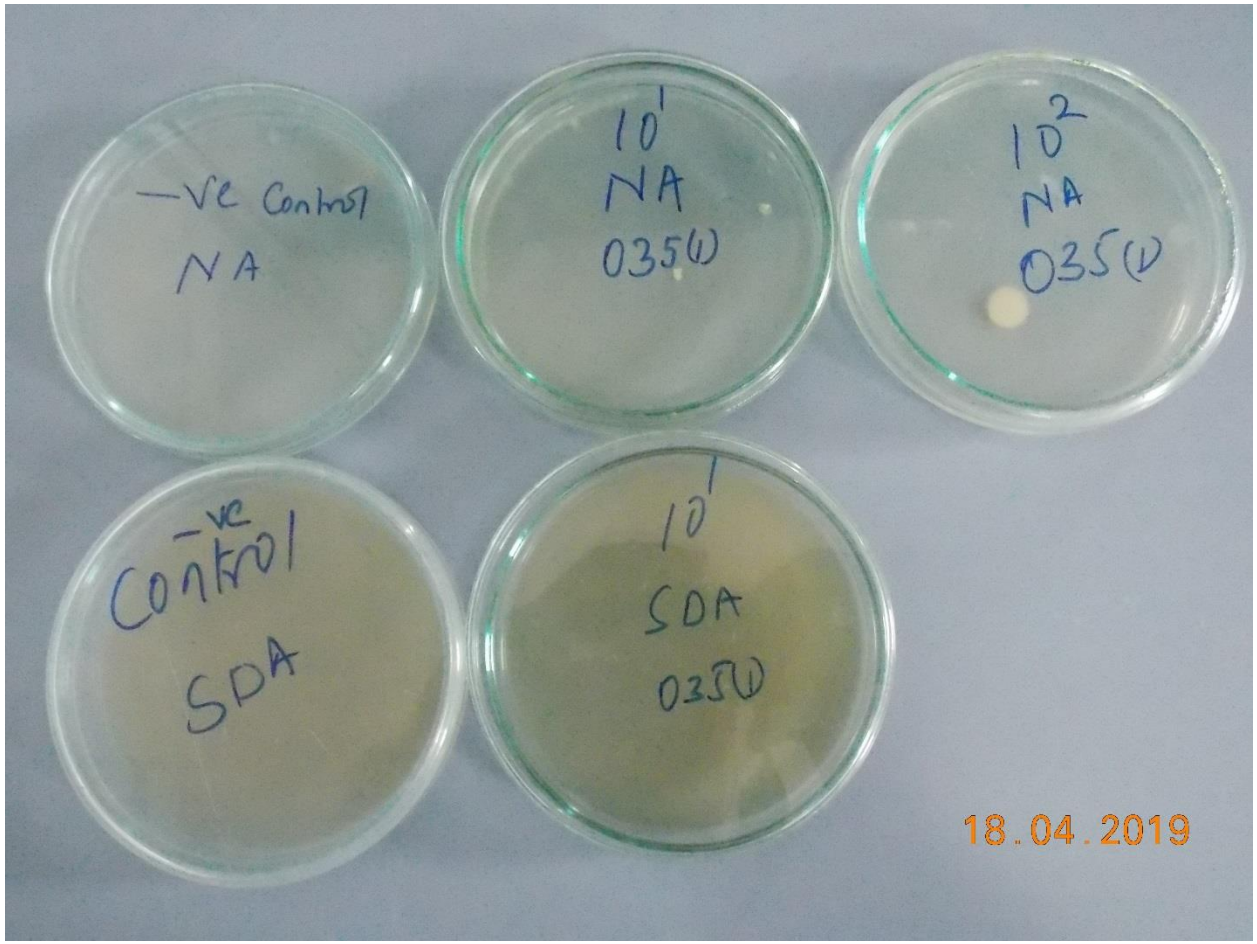
Appendix 7**.** Typical Nutrient agar and sabourand dextrose agar plate

for sample Mvs 028 showing no growth of bacteria and fungus.

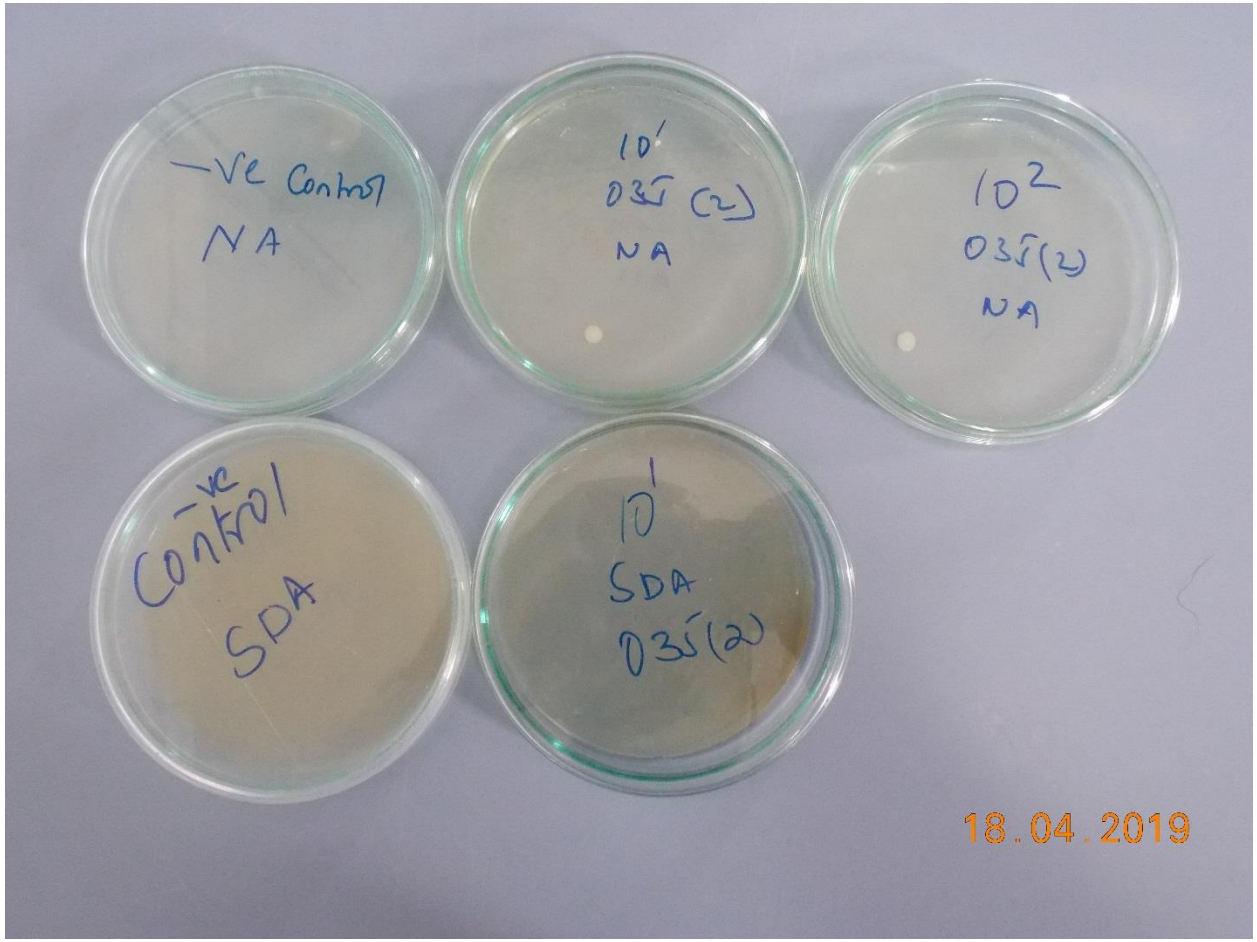


Appendix 8. Typical Nutrient agar and sabourand dextrose agar plate

for sample Mvs 034 showing no growth of bacteria and fungus.

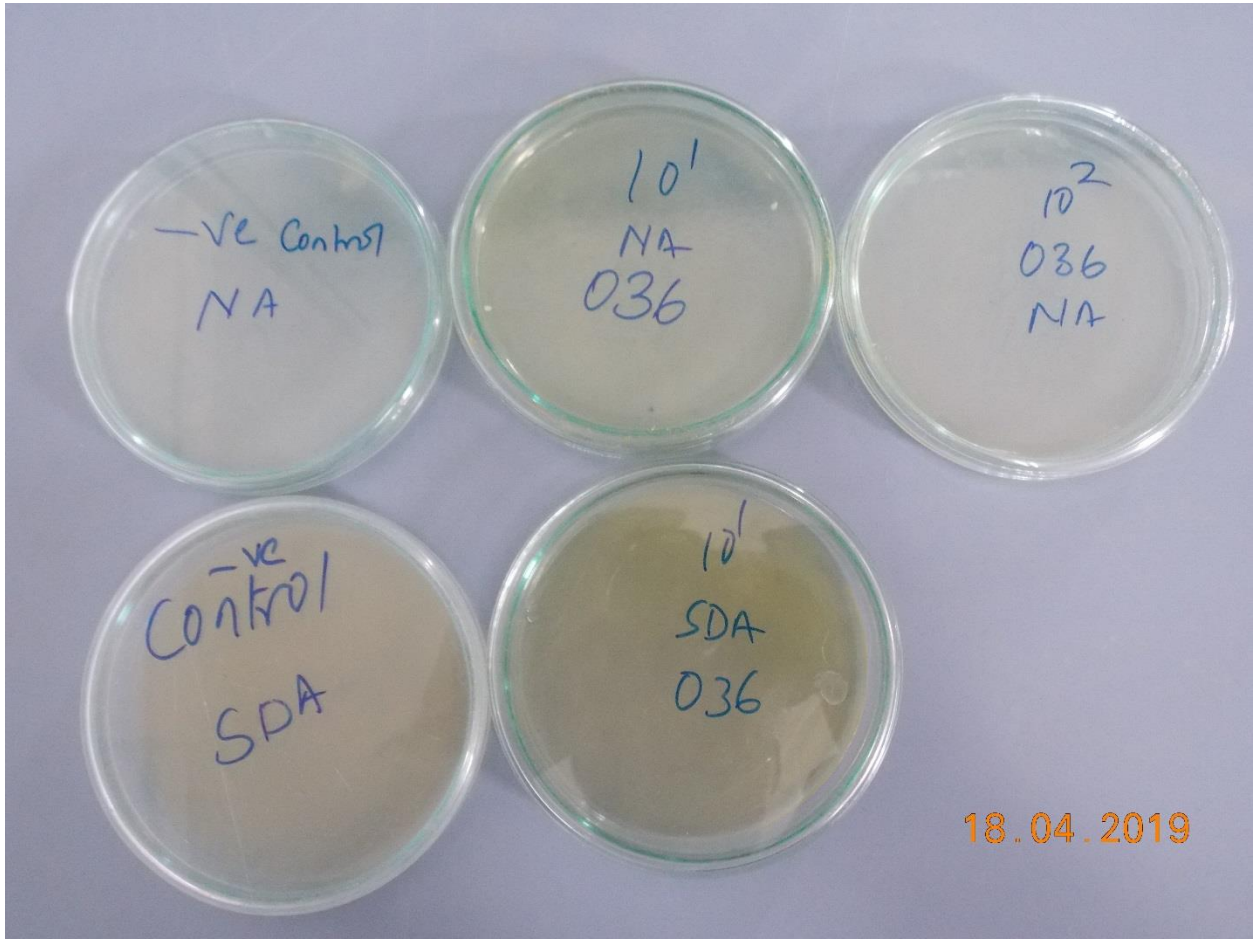


Appendix 9**.** Typical Nutrient agar and sabourand dextrose agar plate for sample Mvs 035 (1) showing no growth of bacteria and fungus**.**



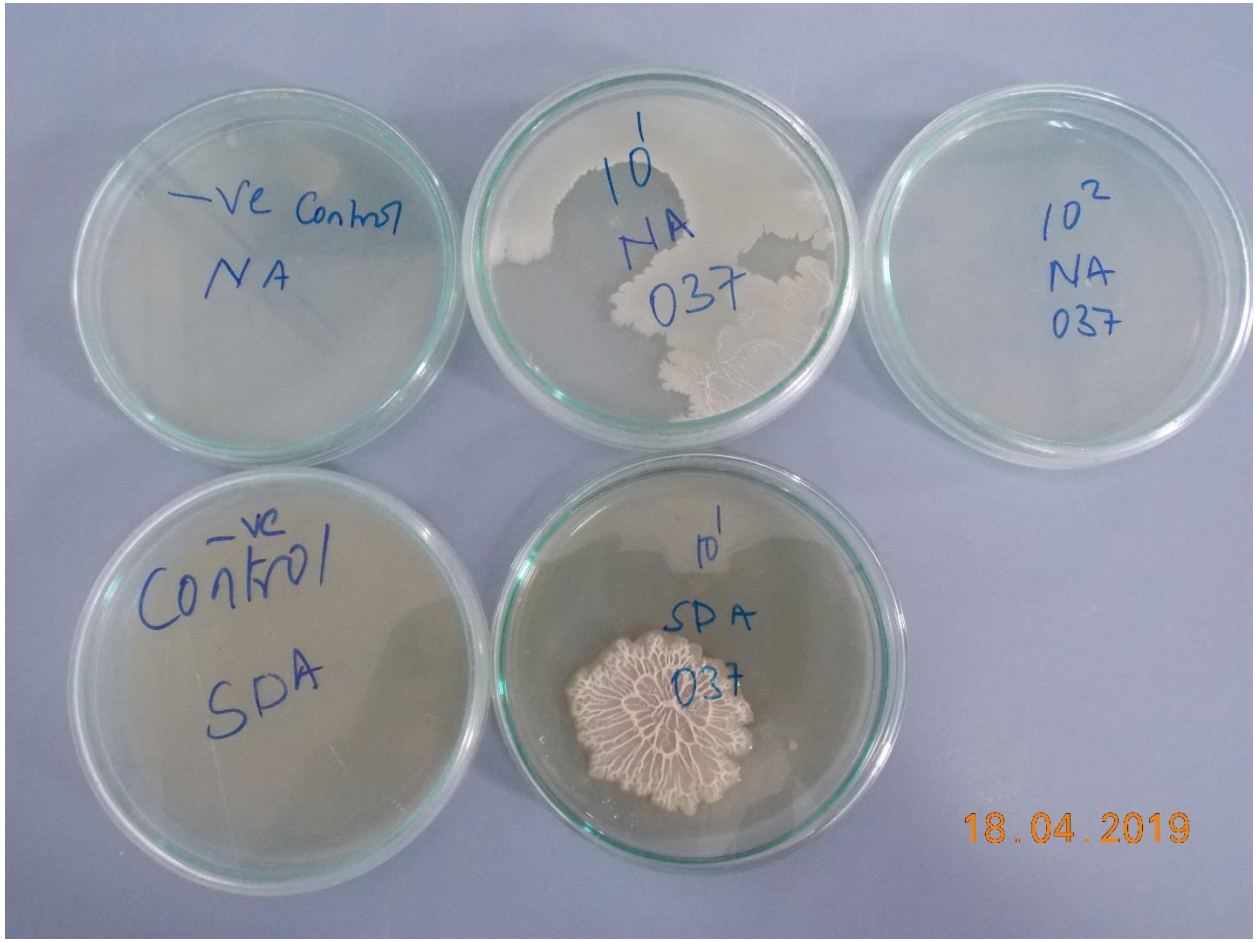
Appendix 10**.** Typical Nutrient agar and sabourand dextrose agar plate

for sample Mvs 035 6 28 showing no growth of bacteria and fungus**.**

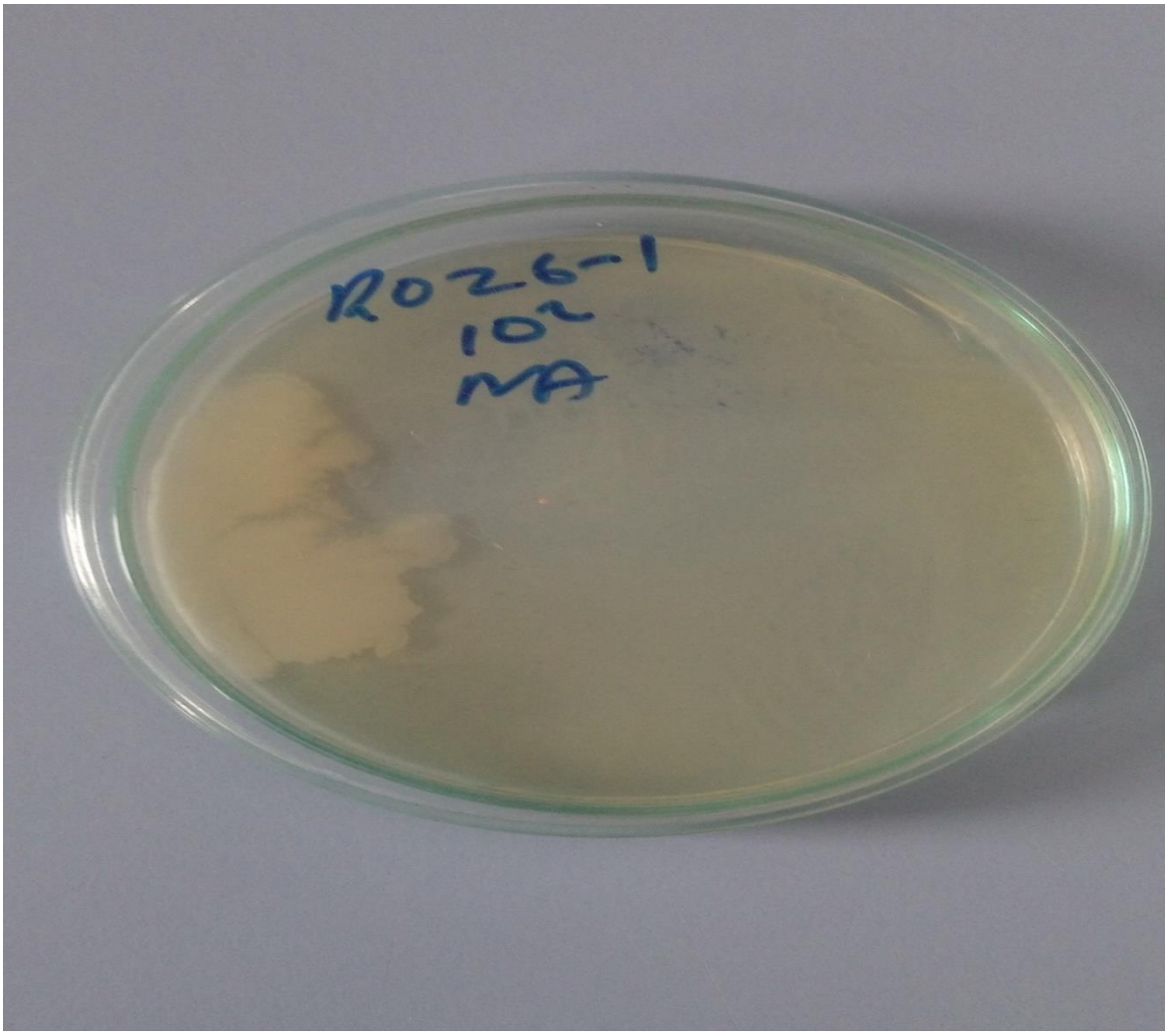


Appendix 11. Typical Nutrient agar and sabourand dextrose agar plate

for sample Mvs 036 showing no growth of bacteria and fungus.



Appendix 12 **.** Typical Nutrient agar for sample Mvs 037 showing growth of fungus.



Appendix 13**.** Typical Nutrient agar Mvs 026 showing fungal growth.