ADDIS ABABA UNIVERSITY SCHOOL OF GRADUATE STUDIES

EVALUATION OF THE DIURETIC AND ANALGESIC ACTIVITIES OF THE RHIZOMES OF *RUMEX ABYSSINICUS* JACQ IN MICE

BY: TESHALE MEKONNEN SEMRE

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HR: Hager's Reagent

IASP: International Association for the Study of Pain

ISE: Ion Selective Electrode

LRF: Lateral reticular formation

M10: Morphine (10 mg/kg)

MR: Mayer's Reagent

NHE3: Na^+/H^+ exchanger

NKCC2: $Na^+/K^+/2Cl^-$ cotransporter

NMDA: N-Methyl-D-Aspartate

NOS: Nitric oxide synthetase

NSAIDs: Nonsteroidal anti-inflammatory drugs

PAG: Periaqueductal Gray

PCT: Proximal convoluted tuble

PG: Prostaglandin

PGD_2: Prostaglandin D_2

PGE_2: Prostaglandin E_2

$PGF_{2\alpha}$: Prostaglandin $F_{2\alpha}$

RA500: Aqueous extract of *Rumex abyssinicus Jacq* (500 mg/kg)

RA750: Aqueous extract of *Rumex abyssinicus Jacq* (750 mg/kg)

RA1000: Aqueous extract of *Rumex abyssinicus Jacq* (1000 mg/kg)

RM250: 80% methanolic extract of *Rumex abyssinicus Jacq* (250 mg/kg)

RM500: 80% methanolic extract of *Rumex abyssinicus Jacq* (500 mg/kg)

RM750: 80% methanolic extract of *Rumex abyssinicus Jacq* (750 mg/kg)

TGF: Tubuloglomerular feedback

TW80: Tween 80 (4%)

Abstract

Rumex abyssinicus Jacq (Polygonaceae) is a widely spread medicinal plant used traditionally for treatment of several ailments, including hypertension, inflammatory and painful conditions. The present study aimed to examine the diuretic and analgesic activities of aqueous and 80% methanol extracts of the rhizomes of the plant at different doses in mice.

To this effect, negative controls were orally treated with distilled water (DW) or Tween 80(4%) (TW80), solvents used for reconstitution of the extracts. Positive controls were treated with furosemide (10 mg/kg) (Fr10) for diuretic test or aspirin (100 mg/kg) (ASA100) and morphine (10 mg/kg) (M10) for acetic acid-induced writhing and hot-plate analgesic studies, respectively. For the diuretic study, treatment groups received an oral dose of 500 mg/kg (RA500), 750 mg/kg (RA750) or 1000 mg/kg (RA1000) of the aqueous extract or 250 mg/kg (RM250), 500 mg/kg (RM500) or 750 mg/kg (RM750) of 80% methanolic extract. Urine volume was then measured at different time (1, 2, 3, 4, and 5 h) and the urinary Na^+, K^+ and Cl^- also measured at 5 h. For both analgesic tests, 250 mg/kg (RM250), 500 mg/kg (RM500) or 1000 mg/kg (RM1000) of 80% methanolic extract doses were used. Whereas the number of writhes was counted for 20 min just 5 min after intraperitonial injection of 0.6% acetic acid (0.15 mL/10g) for the writhing test, the reaction time of each mouse was evaluated at 30, 45, 60, and 90 min after treatment for the hot-plate test. For the acute toxicity study, 5000 mg/kg of aqueous or 80% methanolic extract was administered orally and observed for the following 15 days.

Both extracts displayed a clear dose-dependent diuretic and analgesic effect as compared to controls. RA1000 and RM750 were able to increase diuresis significantly *($P<0.001$)* compared to controls. Both extracts also increased urinary excretion of electrolytes, with RA1000 producing an increase by 56.9, 54.9 and 93.6% *($P<0.001$)* and RM750 increasing by 78.9, 99.5 and 76.3% ($P<0.001$) of Na^+, K^+ and Cl^-, respectively, compared to controls. The diuretic and saluretic effects of the extracts were also found to be qualitatively similar to that of Fr10. RM1000 was noted to reduce the number of acetic acid-induced writhing by 67.6% (*$P<0.001$*) compared to controls and this effect was comparable to that of ASA100 (72.36%, *$P<0.001$*). RM1000 also conferred more than 70% protection against thermally-induced pain stimuli after 45 min of treatment compared to controls. The extracts did not show overt toxicity in mice in the study period.

In conclusion, the extracts had been shown to exert diuretic and analgesic activities comparable to that of the standard drugs and to be relatively safe. Hence, they could be considered as a potential alternative diuretic and analgesic agent. Moreover, the study lends support to the ethnobotenical use of the plant as diuretic and analgesic in different parts of Ethiopia.

Keywords: *Rumex abyssinicus* Jacq, Diuretic effect, Saluretic effect, Analgesic activity, Acetic acid-induced writhing test, Hot-plate test.

1. INTRODUCTION

1.1. Diuresis and Diuretics

A diuretic is any drug or herb that elevates the rate of urine flow and sodium excretion. Technically, a "diuretic" is an agent that increases urine volume, while a "natriuretic" causes an increase in renal sodium excretion. Because natriuretics almost always also increase water excretion, they are usually called diuretics *(Harlan, 2007)*.

Diuretics not only alter the excretion of Na^+ but also may modify renal handling of other cations (*e.g.*, K^+, H^+, Ca^{2+}, and Mg^{2+}), anions (*e.g.*, Cl^-, HCO_3^-, and $H_2PO_4^-$) and uric acid *(Jackson, 2006; Harlan, 2007)*.

Looking the historical background of diuretics, they have been available since the 16th century for the treatment of edema. Mercurous chloride was known to be diuretic by Paracelsus (1493-1543). In 1930, Swartz discovered that the antimicrobial sulfonamide could be used to treat edema in patients with heart failure due to an increase in renal excretion of Na^+ *(Blumental et al., 2007)*.

Although various agents that increase urine volume have been described since antiquity, it was not until 1957 that a practical and powerful diuretic agent (chlorothiazide) became available for widespread use *(Harlan, 2007)*.

1.1.1. Renal Anatomy and Physiology

To understand the action of diuretics, it is first necessary to review how the kidney filters fluid and forms urine. As blood flows through the kidney, it passes into glomerular capillaries located within the cortex. These glomerular capillaries are highly permeable to water and electrolytes. Glomerular capillary hydrostatic pressure drives (filters) water and electrolytes into Bowman's space and into the proximal convoluting tubule (PCT) *(Rang et al., 2003; Jackson, 2006; Harlan, 2007)*. About 20% of the plasma that enters the glomerular capillaries is filtered (termed filtration fraction) *(Miriam, 1998)*. The PCT, which lies within the cortex, is the site of sodium, water and bicarbonate transport from the filtrate, across the tubule wall, and into the interstitium of the cortex. About 65-70% of the filtered sodium is removed from the tubular fluid found within the PCT. This sodium is reabsorbed isosmotically, meaning that every molecule of sodium that is reabsorbed is accompanied by a molecule of water *(Richard, 2007)*.

As the tubule dives into the medulla, or middle zone of the kidney, the tubule becomes narrower and forms a loop (Loop of Henle) that reenters the cortex as the thick ascending limb (TAL) that travels back to near the glomerulus. Because the interstitium of the medulla is very hyperosmotic and the Loop of Henle is permeable to water, water is reabsorbed from the Loop of Henle and into the medullary interstitium *(Jackson, 2006)*. This loss of water concentrates the urine within the Loop of Henle. The TAL, which is impermeable to water *(Miriam, 1998)*, has a cotransport system ($Na^+/K^+/2Cl^-$ cotransporter) that reabsorbs sodium, potassium and chloride at a ratio of 1:1:2. Approximately 25% of the sodium load of the original filtrate is reabsorbed at the TAL *(Richard, 2007)*. From the TAL, the urine flows into the distal convoluting tubule (DCT), which is another site of sodium transport (~5% via Na^+/Cl^- cotransporter) into the cortical interstitium (the DCT is also impermeable to water) *(Miriam, 1998)*.

Finally, the tubule dives back into the medulla as the collecting duct and then into the renal pelvis where it joins with other collecting ducts to exit the kidney as the ureter. The distal segment of the DCT and the upper collecting duct has a transporter that reabsorbs sodium (about 1-2% of filtered load) in exchange for potassium and hydrogen ion, which are excreted into the urine. It is important to note two things about this transporter. First, its activity is dependent on the tubular concentration of sodium, so that when sodium is high, more sodium is reabsorbed and more potassium and hydrogen ion are excreted. Second, this transporter is regulated by aldosterone, which is a mineralocorticoid hormone secreted by the adrenal cortex. Increased aldosterone stimulates the reabsorption of sodium, which also increases the loss of potassium and hydrogen ion to the urine *(Harlan, 2007)*. Water is reabsorbed in the collecting duct through special pores that are regulated by antidiuretic hormone (ADH), which is released by the posterior pituitary. ADH increases the permeability of the collecting duct to water, which leads to increased water reabsorption, a more concentrated urine and reduced urine outflow. Nearly all of the sodium originally filtered is reabsorbed by the kidney, so that less than 1% of originally filtered sodium remains in the final urine *(Miriam, 1998; Richard, 2007)*.

1.1.2. Mechanisms and Sites of Actions of Diuretics

Most diuretics produce diuresis by inhibiting the reabsorption of sodium at different segments of the renal tubular system. Sometimes a combination of two diuretics is given because this can be significantly more effective than either compound alone (synergistic effect). The reason for this is that one nephron segment can compensate for altered sodium reabsorption at another nephron segment; therefore, blocking multiple nephron sites significantly enhances efficacy *(Richard, 2007)*. Tubule transport system and sites of action of common diuretics is shown in Fig. 1.1.

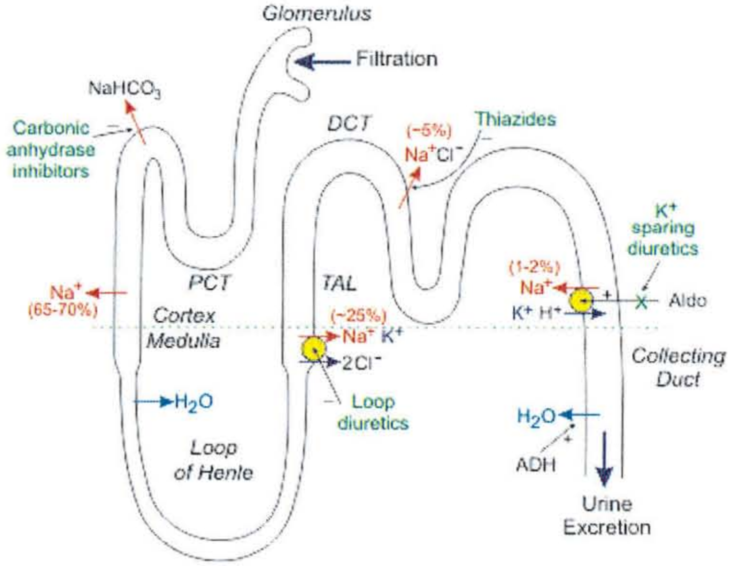

Fig.1.1. Tubule transport systems and sites of action of diuretics (Copied from *Richard, 2007*)

Loop diuretics (e.g. furosemide, bumetanide, ethacrynic acid, torsemide) inhibit the sodium-potassium-chloride co-transporter in the TAL. By acting on the TAL, which handles a significant fraction of sodium reabsorption, loop diuretics are known to be very powerful diuretics (high ceiling diuretics) *(Chennavasin, 1979; Puscas et al., 1999).*

Thiazide diuretics (e.g. bendroflumethiazide, chlorthiazide, hydrochlorthiazide, methylclothiazide, chlorthalidone, indapamide), which are the most commonly used diuretics; inhibit the sodium-chloride transporter in the distal tubule. They are sufficiently powerful to satisfy most therapeutic needs requiring a diuretic *(O'Brien and Shorten, 2001).*

Unlike loop and thiazide diuretics, some of potassium-sparing diuretics do not act directly on sodium transport. Some drugs in this class antagonize the actions of aldosterone (aldosterone receptor antagonists e.g. spironolactone, canrenone, eplerenone) at the distal segment of the distal tubule *(Harlan, 2007).* This causes more sodium (and water) to pass into the collecting duct and be excreted in the urine. They are called K^+-sparing diuretics because they do not produce hypokalemia like the loop and thiazide diuretics. The reason for this is that by inhibiting aldosterone-sensitive sodium reabsorption, less potassium and hydrogen ion are exchanged for sodium by this transporter and therefore less potassium and hydrogen are lost to the urine *(Rang et al., 2003).* Other potassium-sparing diuretics (e.g. triamterene, amiloride) directly inhibit sodium channels associated with the aldosterone-sensitive sodium pump, and therefore have similar effects on potassium and hydrogen ion as the aldosterone antagonists. Because this class of diuretics has relatively weak effects on overall sodium balance, they are often used in conjunction with thiazide or loop diuretics to help prevent hypokalemia *(Kau, 1978; Richard, 2007).*

Carbonic anhydrase inhibitors (CAIs) (e.g. acetazolamide, dichlorphenamide, methazolamide) inhibit the transport of bicarbonate out of the PCT into the interstitium by inhibiting the carbonic anhydrase (CA) enzyme, which leads to less sodium reabsorption at this site and therefore greater sodium, bicarbonate and water loss in the urine. In view of the large quantity of NaCl absorbed in this segment, a drug that specifically blocked proximal tubular absorption of NaCl would be a particularly powerful diuretic. No such drug is currently available *(O'Brien and Shorten, 2001; Harlan, 2007).* CAIs are the weakest of the diuretics and seldom used in cardiovascular disease. Their main use is in the treatment of glaucoma *(Harlan, 2007).*

Osmotic diuretics (e.g. mannitol, urea) are relatively biologically inert molecules which are filtered by the glomerulus and not reabsorbed by the nephron. Their administration increases the osmolality of the tubular fluid, decreasing water reabsorption, decreasing Na^+ concentration of the fluid and hence its reabsorption *(O'Brien and Shorten, 2001; Harlan, 2007).*

1.1.3. Therapeutic Uses of Diuretics

Most patients with hypertension, of which 90-95% have hypertension of unknown origin (primary or essential hypertension), are effectively treated with diuretics *(Shah et al., 2004; Richard, 2007).* The vast majority of hypertensive patients are treated with thiazide diuretics. Potassium-sparing diuretics (e.g., spironolactone) are used in secondary hypertension caused by hyperaldosteronism, and sometimes as an adjunct to thiazide treatment in primary hypertension to prevent hypokalemia *(Richard, 2007).* The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT) study *(The ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group, 2002)* provides strong evidence that thiazide diuretics are the best initial therapy for uncomplicated hypertension, a conclusion endorsed by the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure *(Chobanian et al., 2003).*

The primary use for diuretics in heart failure is to reduce pulmonary and/or systemic congestion and edema, and associated clinical symptoms (e.g., shortness of breath - dyspnea). Long-term treatment with diuretics may also reduce the afterload on the heart by promoting systemic vasodilation, which can lead to improved ventricular ejection *(Richard, 2007).* The available data from several small trials show that in patients with chronic heart failure, conventional diuretics appear to reduce the risk of death and worsening heart failure compared to placebo. Compared to active control e.g. Angiotensine converting enzyme (ACE) inhibitors, digoxin, diuretics appear to improve exercise capacity *(Faris et al., 2006).*

In addition, doctors prescribe certain diuretics to prevent, treat or improve variety of conditions, such as the nephrotic syndrome *(Inoue et al., 1987)*, cirrhosis *(Ochs, 1978)*, chronic venous insufficiency & edema *(James and Shobha, 2005)*, diabetes insipidus & osteoporosis *(Gronbeck et al., 1998)*, glaucoma (*Jackson, 2006; Harlan, 2007)*, epilepsy *(Jackson, 2006)*, acute mountain sickness *(Harlan, 2007)* and as an alternate treatments in asthma *(Alexander et al., 2003)*.

1.1.4. Adverse Effects of Diuretics

Thiazide and loop diuretics may cause skin reactions and interstitial nephritis *(Brater, 1998)*. Loop diuretics may cause ototoxicity, usually in patients receiving both very high doses and other ototoxic drugs, particularly aminoglycoside antibiotics *(Sheffield and Turner, 1971)*. Ototoxicity is usually transient. Large doses of spironolactone can cause gynecomastia *(Rose et al., 1977)*.

The most serious adverse effects of diuretics are abnormalities in fluid and electrolyte homeostasis. Both loop and thiazide diuretics cause loss of potassium and magnesium in the urine, and when administered in combination, they may result in substantial depletion of these cations (*Brater, 1996)*. Other classes of diuretics are also associated with various specific adverse effects (For review see *Richard, 2007*).

1.1.5. Diuretic Tolerance

There are two forms of diuretic tolerance. Short-term tolerance, so-called braking, refers to a decrease in the response to a diuretic after the first dose has been administered. The mechanism by which short-term tolerance occurs is unclear. It may be mediated by activation of angiotensin II or the sympathetic nervous system, but neither the inhibition of ACE nor adrenergic blockade, separately or together, consistently prevents it *(Kelly, 1983)*.

With long-term administration of a loop diuretic, the solute that escapes from the loop of Henle floods more distal regions of the nephron. By unknown mechanisms, increased exposure to solute causes hypertrophy of distal nephron segments, with concomitant increases in the reabsorption of sodium *(Kaissling and Stanton, 1988)*.

Sodium that escapes from the loop of Henle is therefore reabsorbed at more distal sites, decreasing overall diuresis. The result is long-term tolerance of the loop diuretic. Thiazide diuretics block the nephron sites at which hypertrophy occurs, accounting for the synergistic response to the combination of a thiazide and a loop diuretic. This phenomenon reinforces the logic of using combinations of loop and thiazide diuretics in patients who do not have adequate responses to optimal doses of a loop diuretic *(Ellison, 1991)*.

1.1.6. Future Diuretics

All currently available diuretics perturb K^+ homeostasis. However, studies in animals have established that there are some agents which induce a brisk natriuresis without significantly increasing urinary K^+ excretion *(Kuan et al., 1993)*.

Adenosine A_1 receptor antagonists

Clinical studies with highly selective A_1-receptor antagonists FK453 *(Balakrishnan et al., 1993; van Buren et al., 1993)*, KW-3902 *(Yao et al., 1994)*, CVT-124 *(Gellai et al., 1998)* and FK838 *(Christine et al., 2003)* confirm that blockade of A_1 receptors induces natriuresis with minimal effects on K^+ excretion.

The natriuretic mechanism of this novel class of diuretics has been partially elucidated *(Takeda et al., 1993)*. Elevated intracellular cyclic AMP (cAMP) reduces basolateral Na^+-HCO_3^- symport in proximal tubular cells. Endogenous adenosine normally acts on A_1 receptors in these cells to inhibit adenylyl cyclase and reduce cAMP accumulation. Blockade of A_1 receptors removes this inhibition, permits cellular cAMP to rise, and results in reduced activity of the Na^+-HCO_3^- symporter. Because A_1 receptors are involved in tubero glomerular feedback (TGF), A_1-receptor antagonists uncouple increased distal delivery of Na^+ from activation of TGF *(Wilcox et al., 1999)*. Other mechanisms, including an effect in the collecting tubules, contribute to the natriuretic response to A_1-receptor antagonists; however, it is not known why this class of diuretics has little effect on K^+ excretion. In some patients, loop diuretics may compromise renal hemodynamics and actually reduce glomerular filtration rate (GFR), a phenomenon known as *diuretic intolerance*. Importantly, A_1-

FLAME ATOMIC ABSORPTION SPECTROPHOTOMETRIC DETERMINATION OF SOME SELECTED MINERALS IN MULTIVITAMINS WITH MULTIMINERALS PHAREMACEUTICAL DOSAGE FORMS MARKETED IN ETHIOPIA

By

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Flame atomic absorption spectrophotometric determination of some selected minerals in multivitamin pharmaceutical dosage forms marketed in Ethiopia

BY

Mequanent Mengistu Feleke

DEPARTMENT OF PHARMACEUTICAL CHEMISTRY
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ABBREVIATIONS

AAS	Atomic absorption spectroscopy
ADD	Attention deficit disorder
ATP	Adenosine triphosphate
BCAA	Branched chain amino acid
CV	Coefficient of variation
DNA	Deoxy ribonucleic acid
EDTA	Ethylene diamine tetra acetic acid
FAAS	Flame atomic absorption spectrometry
FW	Formula weight
GF-AAS	Graphite furnace atomic absorption spectrometer
ICP-MS	Inductively coupled plasma mass spectrometer
ICP-OES	Inductively coupled plasma optical emission spectrometer
LMW_{Cr}	Low molecular weight chromium
LOD	Limit of detection
LOQ	Limit of quantification
PCOS	Polycystic ovarian syndrome
PMT	Photomultiplier tube
ppb	Parts per billion
ppm	Parts per million
PTH	Parathyroid hormone
RSD	Relative standard deviation
RSE	Relative standard error
SD	Standard deviation

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Abstract

Six commercial brands of multivitamins with multimineral marketed in Ethiopia were analyzed for their macro Ca (II), Mg (II) and K (I)) and micro Cr (III and VI)) elemental quantitation , prior to analysis samples were digested in Kjeldahl apparatus with 10 ml analytical grade of concentrated (67-70) HNO_3 % w/v and 5 ml of 30% H_2O_2 w/v heated at a temperature of 150 ^oC in order to remove all the organic matters of multivitamin until a clear solution was obtained. Each sample solution was quantitatively transferred to 100 ml volumetric flask and adjusted to the mark with deionized water. The calibration curve was linear in the range of 0.001 μg/mL to 0.0225 μg/mL for magnesium (II), 0.01 μg/mL to 3.00 μg/mL of calcium (II), 0.01 μg/mL to 1.5 μg/ml of potassium (I) and 0.05μg/ml to 3.00 μg/mL of total chromium (III and VI).The correlation coefficients for all standard metallic solutions of Ca (II), Mg (II), K (I) and total Cr (III and VI) in the linear range of $r > 0.998$. The precision were evaluated in an inter-day and intera-day determination of magnesium (II) and calcium (II) in Maxamine forte tablets, the RSD and RSE were lower than 2% and the recoveries were carried out and found to be between 98% and 101.01% that ascertains good precision of the method. Thus, FAAS was found to be simple to operate, sensitive, precise and suitable for quantitative determination of metal ions in multivitamin with multimineral preparations and could be useful in a routine work.

Key words: FAAS, Wet digestion, Elemental analysis, Multivitamins with multiminerals dosage forms

1. INTRODUCTION

1.1. Minerals

It has long been known that minerals are inorganic substance essential for many metabolic and physiologic processes in the human body ranging from maintaining the integrity of skin to the, participation in the structural composition of the skeletal system, maintaining body fluid composition. Minerals are essential participants in many protein and enzymatic reactions, nerve impulse conduction, oxygen transport, oxidative phosphorylation, immune functions, antioxidant activity, bone health, and acid-base balance of the blood [1, 2].

Minerals as pharmaceutical composition help to prevent reduction-oxidation, improving the stability of oxidizable vitamins and stabilizing the disintegration time of a multi-vitamin. This combination vitamin/mineral product is used to prevent or treat nutritional deficiency caused by certain stressful health conditions [3]. It is evident that the relationship of essential minerals and human health that keeping minerals balance in every organ of the human body helps in maintaining human health [4, 5].

Table 1: Sources, percent by mass and daily recommended amount in the human body of potassium, calcium, magnesium and chromium

Metal ions	**sources**	**RDA (Recommended Daily Allowance)**	**Percent by mass in human body**
Mg (II)	Dried peas and beans, whole grains and soy products.	350 mg	0.05
Ca (II)	Cow milk, Meat, Almonds and Sunflower seeds.	1200 mg	1.5
K (I)	Bananas, oranges, watermelon, potato, milk, lean meats.	1600 mg	0.2
Cr (III and VI)	Liver, beef, apples, eggs, bananas, chicken and green peppers.	(50-200) μg	Not known

1.1.1. Calcium

Calcium is a soft grey alkaline earth metal, the fifth most abundant element by mass in the earth's crust, estimated to be about 3.64 percent, the fifth most abundant element in the human body and the fifth most abundant dissolved ion in seawater by both molarity and mass [6]. About 99% of the calcium in the body is found in bones and teeth, while the other 1% is found in the blood and soft tissues. Forms of calcium salts in multivitamin preparation which calcium pantothenate- ($C_{18}H_{32}CaN_2O_{10}$), dibasic calcium phosphate - ($CaHPO_4$) and calcium gluconate - ($C_{12}H_{22}CaO_{14}$) [7].

Calcium is an element that a human body needs for numerous functions, such as building and maintaining the bones and teeth, blood clotting, transmitting of the nerve impulses lowers cholesterol levels, reduces the risk of colon cancer by neutralizing the toxic effects of cancer promoting fats and prevents leg cramps. Any reduction of this element in the dermal conjunctive tissue causes more or less quick early aging of the skin [8].

Calcium concentrations in the blood and fluid that surrounds cells are tightly controlled in order to preserve normal physiological functioning. When blood calcium decreases, calcium-sensing proteins in the parathyroid glands send signals resulting in the secretion of parathyroid hormone (PTH) [9]. PTH stimulates the conversion of vitamin D to its active form, calcitriol, in the kidneys. Calcitriol increases the absorption of calcium from the small intestine. Together with PTH, calcitriol stimulates the release of calcium from bone by activating osteoclasts (bone resorbing cells), and decreases the urinary excretion of calcium by increasing its reabsorption in the kidneys [10].

Excitable cells, such as skeletal muscle and nerve cells, contain voltage-dependent calcium channels in their cell membranes that allow for rapid changes in calcium concentrations. Thus, when a muscle fiber receives a nerve impulse that stimulates it to contract, calcium channels in the cell membrane open to allow a few calcium ions into the muscle cell [11].

These calcium ions bind to activator proteins within the cell that release a flood of calcium ions from storage vesicles inside the cell. The binding of calcium to the protein, troponin-c, initiates a series of steps that lead to muscle contraction. The binding of calcium to the protein, calmodulin, activates enzymes that breakdown muscle glycogen to provide energy for muscle contraction [12].

Calcium is necessary to stabilize or allow for optimal activity of a number of proteins and enzymes. The binding of calcium ions is required for the activation of the seven "vitamin K-dependent" clotting factors in the coagulation cascade. The term, "coagulation cascade," refers to a series of events, each dependent on the other that stops bleeding through clot formation [13].

A low blood calcium level usually implies abnormal parathyroid function, and is rarely due to low dietary calcium intake since the skeleton provides a large reserve of calcium for maintaining normal blood levels [14]. Calcium in the form of chloride used is an intravenous therapy for the treatment of hypocalcaemia (low serum calcium). Parenteral calcium can be used when epinephrine has failed to improve weak or ineffective myocardial contractions [15].

The aqueous form of calcium chloride is used in genetic transformation of cells by increasing the cell membrane permeability, inducing competence for DNA uptake (allowing DNA fragments to enter the cell more readily) [16]. Calcium in the form of gluconate reduce the capillary permeability, increase density and maintain normal muscle and nerve excitability, strengthen myocardial contractility and help with bone formation, used as a treatment for allergic diseases, such as urticaria, itching of the skin, contact dermatitis, as well as serum disease, vascular nerve edema as adjuvant therapy[17].

1.1.2. Magnesium

Magnesium is a fairly strong, silvery-white, light-weight alkaline earth metal and the ninth most abundant element in the universe by mass. Its abundance in the earth's crust is estimated to be about 2.1 percent [18].

The element is not found free naturally on earth. It is highly reactive and the free magnesium burns with a characteristic brilliant white light and reacts slowly with cold water and more rapidly with hot water. Magnesium is known as the anti-stress mineral, in multivitamin pharmaceutical preparations it exists as, magnesium carbonate-$MgCO_3$, magnesium oxide-MgO, magnesium citrate-$C_{12}H_{14}MgO_{14}$, magnesium gluconate-$C_{12}H_{22}MgO_{14}$ and $MgSO_4$ [19].

Magnesium has been identified as a cofactor in over 300 enzymatic reactions involving energy metabolism and protein and nucleic acid synthesis. Approximately half of the total magnesium in the body is present in soft tissue, and the other half in bone. Less than 1% of the total body magnesium is present in blood [20].

Virtually, all chemical reactions in the body require an enzyme system to help the biochemical reaction to take place. An enzyme system generally consists of three parts. They are a specific protein molecule, another smaller organic compound, which is often a vitamin, such as pyridoxine or vitamin B6, and finally a charged mineral, such as calcium, potassium, chromium or magnesium [21].

It controls cellular metabolism and maintains electrical potentials of nerve and muscle membranes for the transmission of impulses across junctions. Furthermore, magnesium deficiency reduces the tissues sensitivity to insulin which is common in diabetes. It results from both insufficient magnesium intakes and increase magnesium losses, particularly in the urine [22].

The antioxidant glutathione also requires magnesium for its synthesis, and it is vital to the conduction of nerve impulses, the contraction of muscles, and normal heart rhythm [23]. Magnesium is an allosteric activator of many enzyme systems and plays an important role in oxidative phosphorylation and glycolysis. Within the cell, it is bound primarily to proteins, negatively charged molecules and 80% of cytosolic magnesium is bound to ATP. Most ATP is used for maintenance of intracellular homeostasis and ATP-driven pumps for stabilization of transmembrane concentration gradients of magnesium [24].

Magnesium regulates the neuromuscular activity of the heart, and improves energy production within the heart; dilates the coronary arteries. It helps to maintain normal heart rhythm; used in treating migraine and tension headaches, premenstrual syndrome and high blood pressure [25].

It also aids in bone growth and strengthens tooth enamel; controls bad breath and body odor and aids in fighting depression and prevents calcium deposits. When magnesium combined with calcium acts as a natural tranquilizer; promotes relaxation of the bronchial muscles, thus opening airways and making breathing easier; reduces hyperactivity in children, and may help with ADD (attention deficit disorder) and eliminates mental confusion [26].

Magnesium is very involved with ATP production in the Kreb's cycle and Glycolysis, adenylcyclase and other reactions involving nerve impulse transmission. It regulates the absorption of calcium and is involved in the structural integrity of bones and teeth, regulates the contractility of the heart muscle. It is concentrated 18x greater in the heart muscle than in the blood stream [27]. It has a relaxing effect on smooth muscle of the bronchioles and the arterioles (lowering blood pressure) [28]. It may relax uterine tissue (decreasing the cramping of dysmenorrhea), and also useful in the treatment of angina and decreases coagulation and acts as a calcium channel blocker. Thus, it is one of the cofactors for delta 6 desaturase which is involved in the production of PGE1, activates vitamin B-complex, and helps in protein synthesis and in neuromuscular transmitters [29].

1.1.3. Chromium

Chromium is the 21st most abundant element in earth's crust which has a steely-gray, lustrous, hard metal that takes a high polish and has a high melting point. Chromium is a fairly active metal, does not react with water, but reacts with most acids and combines with oxygen at room temperature to form chromium oxide (Cr_2O_3) [30]. Chromium is remarkable by its magnetic properties: it is the only elemental solid which shows antiferromagnetic ordering at room temperature [31].

Chromium (Cr) can exist in various chemical valence states ranging from Cr^{3+} to Cr^{6+}, among which the more stable chemical form are Cr^{3+} and Cr^{6+}. Cr^{3+} is relatively non-toxic and is an essential nutrient in the human diet to maintain effective glucose, lipid and protein metabolism. However, Cr^{6+} is primarily anthropogenic and as CrO_4^{2-} or $HCrO^{4-}$ can diffuse through the cell membranes and oxidize biological molecules with toxic results [32]. Chromium is one of the essential trace elements in multivitamins with minerals pharmaceutical formulations that contains Cr^{3+} either in the forms of chromium chloride (inorganic source) or chromium picloinate and chromium polynicotinate (organic source) [33].

A biologically active form of chromium participates in glucose metabolism by enhancing the effects of insulin. First, the inactive form of the insulin receptor is converted to the active form by binding insulin [34]. The binding of insulin by the insulin receptor stimulates the movement of chromium into the cell and results in binding of chromium to LMWCr. Once it binds chromium the LMWCr binds to the insulin receptor and enhances its activity. The ability of the LMWCr to activate the insulin receptor is dependent on its chromium content. When insulin levels drop due to normalization of blood glucose levels, the LMWCr may be released from the cell in order to terminate its effects [35].

Chromium is an insulin cofactor, and its theorized ergogenic effect is based on the role of insulin to facilitate BCAA transport into the muscle. Chromium appears to enhance the action of insulin, its deficiency results in impaired glucose tolerance and insufficiency hypothesized to be a contributing factor to the development of type 2 diabetes [36]. Chromium is used as an effective treatment for polycystic ovarian syndrome (PCOS), a hormonal condition. The condition can lead to infertility if untreated, and is associated with insulin resistance and type 2 diabetes. It plays an indirect role in lowering blood lipids, reduce the risk of cardiovascular disease in men, decrease total cholesterol and triglyceride levels and reduce hypertension [37, 38].

1.1.4. Potassium

Potassium is the eighth most abundant element in the Earth's crust and is estimated to be about 2.0 to 2.5 percent. Elemental potassium is a soft silvery-white alkali metal that oxidizes rapidly in air and is very reactive with water [39]. Potassium salts in multivitamin preparations exists as, potassium acetate-$KOOCH_3$, potassium citrate effervescent-$KO_7H_7C_6$ and potassium iodide/chloride. Potassium is of great physiological importance, an electrolyte which is essential for a healthy nervous system and a regular heart rhythm. Potassium aids in maintaining stable blood pressure and in transmitting electrochemical impulse and regulates the transfer of nutrients through cell membranes [40].

Together with magnesium, it helps to prevent kidney stones, promotes healthy adrenal glands, helps stop the buildup of cholesterol-laden plaque, aids in clear thinking by sending oxygen to the brain. Moreover, the macro nutrient potassium, stimulates the kidneys to eliminate poisonous body wastes; very effective in reducing high blood pressure, keeps the heart muscle strong and prevents leg cramps [41].

Potassium is also important in allowing muscle contraction and the sending of all nerve impulses through action potentials. By the nature of their electrostatic and chemical properties, K^+ ions are larger than Na^+ ions, and ion channels and pumps in cell membranes can distinguish between the two types of ions, actively pumping or passively allowing one of the two ions to pass, while blocking the other. A shortage of potassium in body fluids may cause a potentially fatal condition known as hypokalemia, a condition where there is not enough potassium in the blood [42].

The reverse of this condition is hyperkalemia (too much potassium in the blood). Hyperkalemia is due to decreased kidney function or renal failure and it is a serious problem, resulting in life threatening heart rhythm abnormalities (arrythmias). Pharmaceutical dosage forms of multivitamin containing potassium helps in dilating blood vessels, prevents blood clotting and in lowering the formation of free radicals [43, 44].

The body holds most potassium within cells over 95% whereas most sodium is outside of cells within the body's blood and other body fluids. The body actually has a mechanism known as the sodium-potassium pump in order to do this; cell membranes of all cells of the body actively pump sodium out and potassium in as an important function to prevent cellular swelling (and bursting) and to maintain the proper electrical charge of the cell [45].

Additionally, potassium is required for the conversion of blood sugar into glycogen within the muscles and liver. Without adequate potassium, low levels of storage glycogen causes muscle weakness, fatigue and other symptoms of potassium deficiency [46]. It is an important intracellular cation and needed for all cellular functions, maintains the alkalinity of the bile and blood, it plays in the regulation of acid-base balance in the cell, increases maximal activity of glycolytic enzyme pyruvate kinase and helps in the digestion of food and in the proper function of the eyes [47]. Potassium supplements can interact with a number of medicaments including digitalis-based heart medications, potassium-sparing diuretics and blood pressure lowering drugs of the angiotensin-converting enzyme inhibitor type [48].

1.2. Atomic Absorption Spectrometer

Atomic absorption spectrometry (AAS) is one of the most extensively used techniques that provide means for quantitative determination of metals at trace levels in solution. In atomic absorption, there are two methods of adding thermal energy to a sample [49]. A graphite furnace AAS uses a graphite tube with a strong electric current to heat the sample and in flame AAS, a sample aspirate into a flame using a nebulizer [50]. Over sixty two elements can be determined in almost any matrix by atomic absorption like metal analysis include various forms of industrial manufacturing, geology, medicine, agriculture, heavy metals in body fluids, environmental samples including, food stuffs, soft drinks and beer, the analysis of metallurgical, crude oils, petroleum products and plastics [51].

The principles of quantitation in AAS rely on the adherence to the Beer-Lambert law, the increase in absorbance has a linear relationship to the concentration of gas-phase atoms [52]. The instrumentation required for AAS is similar to that required by other high-resolution spectroscopic techniques. AAS differs from other spectroscopic techniques predominantly in the nature of the radiation source that is used and the use of heat to produce the absorbing species [53].

Its advantages compared with that of classical (titration and gravimetric methods), spectrophotometric and HPLC technique is due to its relative freedom from interferences by inter-elemental effects. Because of the large dipole changes associated with electronic transitions, the sensitivity of the techniques is high and they are used primarily in the field of trace and minor component elemental analysis [54]. The principal differences in between GFAAS and FAAS are stated in (Table1).

Table 2: Comparison of the flame atomization and electrothermal atomization methods.

Parameters	**Flame atomization**	**Electrothermal atomization**
Sensitivity	ppm level in the solution	ppb level in the solution
Sample Volume	about 1mL for one analysis	(5 – 50)µL for one analysis
Atomizing efficiency	about 10%	More than 90%
Shape of signal	plateau shape	Peak shape
Repeatability	(0.5 - 1.0) % in R.S.D.	(2.0 - 5.0) % in R.S.D.
Matrix effect	Small	large
Time for analysis	(10 – 30) seconds for one sample	(2 - 5)min.for one sample
Relative Cost	low to moderate	moderate to high
Maturity	well established	well established

There are five basic components of an atomic absorption spectrophotometer, the light source (hallow cathode lamp), atomizer (flame and graphite furnace), a monochromator, a detector (PMT) and a signal display. Atomic absorption method for quantitative determination of metals at trace levels in solution requires several considerations of components [55].

1. The hollow cathode lamp

A hollow cylinder made entirely or in part of the element whose spectrum is to be produced and consisting of a cathode and anode enclosed within a glass tube filled with a low pressure of Ne or Ar. When a potential is applied across the electrodes, the filler gas is ionized and the positively charged ions collide with the negatively charged cathode, dislodging, or "sputtering," atoms from the cathode's surface. Some of the sputtered atoms are in the excited state and emit radiation characteristic of the metal from which the cathode was manufactured. By fashioning the cathode from the metallic analyte, a hollow cathode lamp provides emission lines that correspond to the analyte's absorption spectrum [56].

2. Monochromators

Par of the AAS used to select the wavelengths of an atomic line for the element onto a photomultiplier tube of the analyte interest. Different wavelengths may be selected which have various sensitivities and may provide linear calibration curves over different concentration ranges [57].

3. Atomization

The process of converting an analyte in solid or solution form to a free gaseous atom. The two general methods of atomization used are flame atomization and electrothermal atomization. In flame atomization a sample is aspirated into a spray chamber by passing a high-pressure stream consisting of one or more combustion gases passed the end of a capillary tube immersed in the sample [58]. The aerosol mist mixes with the combustion gases in the spray chamber before passing to the burner where the flame's thermal energy desolvates the aerosol mist to a dry aerosol of small, solid particles. Subsequently, thermal energy volatilizes the particles, producing a vapor consisting of molecular species, ionic species, and free atoms [59].

In GFAAS a series of three heating steps are usually then applied to the sample contained in the graphite furnace. The first of these is the drying stage, designed to gently evaporate the solvent from the liquid sample (usually water) without splattering the sample. Following the drying stage, the temperature of the furnace is increased, often in the range of 400-800 ^{O}C for a period of time called the ashing (or charring) stage [60]. During this stage organic components in the matrix can be charred, the composition of the sample can be changed chemically, or high boiling volatile components can be removed. Finally, the atomization stage is applied, in this stage; the temperature of the furnace is very rapidly raised to temperatures often as high as 2700 ^{O} C, effectively volatilizing the remaining components on the rod wall [61]. Many of the volatilized metals will come off of the walls in the atomic form, creating a cloud of atomic vapor, trapped in a very small tube. The actual absorbance measurement that occurs during this stage by using a hollow cathode lamp shined through the furnace, creating the actual transmittance measurement [62].

4. Photomultiplier tube (PMT)

A detector that determines the intensity of photons of the analytical line exiting the monochromator is said to be. When some of the light is absorbed by metal, the beam's intensity is reduced. The detector records that reduction as absorption and shown on output device by the data system [63].

1.2.1. Flame atomic absorption spectrophotometer

Flame atomic absorption spectrometry (FAAS) is one of the most essential techniques for determining various elements with a significant precision and accuracy. In FAAS the molecules are broken down by the flame eliminating any vibrational or rotational spectra, only gaseous atoms remain in the flame, and the spectral peaks are very sharp, since they come from pure electronic transitions [64]. The flame is lined up in a beam of light of the appropriate wavelength and causes the atom to undergo a transition from the ground state to the first excited state. The chemical composition of a flame can have a marked effect on the decomposition process and thus have a considerable effect on the characteristic concentration. It is therefore necessary to optimize the fuel-to-oxidant ratio for each element being analyzed [65]. In flame atomic absorption analysis of some elements, the type and temperature of the flame used is critical; with improper conditions, interferences can occur [66]. The common interferences encountered in flame atomic absorption spectroscopy are:

1. Background or nonspecific absorption interferences

The occur of this forms of interferences produced in the flame that can scatter light and produce an apparent absorption signal. Light scattering may be encountered when solutions of high salt content are being analyzed. They are most severe when measurements are made at shorter wavelengths (for example, below about 250 nm). Background absorption may also occur as the result of the formation of various molecular species that can absorb light. Thus, this can be encountered by using background correction techniques [67].

2. Spectral interferences

This is because when an absorbing wavelength of an element present in the sample, but not being determined, falls within the width of the absorption line of the element of interest. The results of the determination will then be erroneously high, due to the contribution of the interfering element to the atomic absorption signal and such interferences are extremely rare in FAAS [68]. In some cases, multielement hollow cathode lamps may cause a spectral interference by having closely adjacent emission lines from two different elements or from a metal impurity in the lamp cathode, falls with in the band pass of the slit setting when that other metal is present in the sample. This type of interference may sometimes be reduced by narrowing the slit width [69].

3. Ionization interference

This is may be observed when ionized atom is dependent upon the atomic concentration and the presence of other easily ionized atoms. This interference can be controlled by the addition of a high concentration of another easily ionized element that will buffer the electron concentration in the flame. It also occurs when the flame temperature is sufficiently high to generate the removal of an electron from a neutral atom, giving a positively charged ion [70].

4. Chemical interferences

This is because of species present in the sample cause variations in the degree to which atoms are formed in the flame, or when different valence states of a single element have different absorption characteristics. Such interferences may be controlled by adjusting the sample matrix or by the method of standard additions. [71].

5. Physical interferences

This sorts of interferences occurred when the physical properties of the samples vary significantly and thus a change in viscosity and surface tension affect the sample aspiration rate and cause erroneous results. Sample dilution, the method of standard additions, or both, are used to correct such interferences [72].

1.2.2. Analysis of calcium (II), magnesium (II), potassium (I) and total chromium by FAAS

Several techniques have been reported for the elemental determination of calcium (II), magnesium (II), potassium (I) and total chromium at trace levels in pharmaceutical preparations, such as acid-base titration [51], complexometric titration [76], redox reaction [34, 40], UV/Visible spectrophotometer [51], graphite furnace spectrometry (GF-AAS) [78, 81,82] and inductively coupled plasma-mass spectrometry (ICP-MS) [63, 64, 65, 66, 67] or wide extended flame atomic absorption spectrometry (F-AAS) [73, 74, 75]

Moreover, flame atomic absorption spectroscopy is element specific spectroscopic quantization system. It differs from other method of analysis predominantly in the nature of the radiation source and the use of flame to produce the absorbing species. Within the flame, the sample in a solution form undergoes various processes: evaporation of the solvent, volatilization of the metal analyte, dissociation of the analyte to the gaseous metallic element, and excitation of the gaseous metallic element to the excited state [76].

Quantization in FAAS is accomplished by measuring the amount of absorbing species produced at a given analytical wavelength. Since every element has a unique electronic structure, the wavelength of the emitted radiant energy is directly related to the electronic transition which has occurred. In principle, the amount of light absorbed is proportional to the amount of the element that is present which, in turn, is proportional to the amount of the element that is continuously fed into the flame. The principles of quantitation in FAAS rely on the adherence to the Beer-Lambert law, namely that the increase in absorbance has a linear relationship to the concentration of gas-phase atoms [77].

Of all methods used to determine the metallic ions, the greatest advantages of FAAS is its selectivity based on the use of element specific radiation sources that emit the spectrum of the analyt element in the form of very narrow spectral lines. The principle of FAAS is due to absorption of electromagnetic radiation in the visible and ultraviolet regions of the spectrum by atoms resulting in changes in electronic structure, radiation characteristic of a particular element passes through an atomic vapor of the sample [78, 79].

In the determination of metal ions in the multivitamin dosage forms by FAAS the absorbance detected by the detector will be recorded, by plotting the absorption versus the concentrations of the standards [80]. The idealized calibration or standard curve is stated by Beer's law that the absorbance of an absorbing analyte is proportional to its concentration. Unfortunately, deviations from linearity usually occur, especially as the concentration of metallic analytes increases due to various reasons, such as unabsorbed radiation, stray light, or disproportionate decomposition of molecules at high concentrations [81]. When the sample concentration is too high to permit accurate analysis in linearity response range, there are three alternatives that may help to bring the absorbance into the optimum working range i.e. sample dilution, using an alternative wavelength having a lower absorptivity and reducing the path length by rotating the burner head [82].

This method provides both sensitivity and selectivity since other elements in the sample will not generally absorb the chosen wavelength and thus, will not interfere with the measurement. It is thus, simple, inexpensive, rapid, and applicable to wide range of samples because the technique is element selective and it provides analytical sensitivities at the parts-per-million level and less. Normally the sensitivity for an analysis is optimized by aspirating a standard and adjusting operating conditions, such as the fuel-to-oxidant ratio, the nebulizer flow rate, and the height of the burner, to give the greatest absorbance [83, 84].

1.3. Reported (UV-Visible Spectrophotometric method) of determination of metal ions in multivitamin with multimineral pharmaceutical dosage forms

1.3.1. Spectrophotometric determination of total chromium

Spectrophotometers are instruments that measure the absorbance of wavelengths of light in solutions. The absorbance, *A* of a solution is a measure of how much light of a certain wavelength specific to the experiment passes through a solution versus how much is absorbed by the solution. The UV-Vis spectra have broad features that are of limited use for sample identification but are very useful for quantitative measurements. The concentration of an analyte in solution can be determined by measuring the absorbance at some wavelength and applying the Beer-Lambert Law [85].

Since the UV-Vis range spans the range of human visual acuity of approximately 400 - 750 nm, UV-Vis spectroscopy is useful to characterize the absorption, transmission, and reflectivity of a variety of metal ions in pigments, coatings, windows, filters and pharmaceuticals preparations. UV/Vis spectroscopy is routinely used in the quantitative determination of solutions of transition metal ions and highly conjugated organic compounds. Solutions of transition metal ions can be colored (i.e., absorb visible light) because d electrons within the metal atoms can be excited from one electronic state to another. The color of metal ion solutions is strongly affected by the presence of other species, such as certain anions or ligands [86].

It is known that an increase in the content of chromium in pharmaceuticals preparations makes them toxic effect depends on the oxidation state. On the other hand, the introduction of chromium salts pharmaceuticals preparations have been some positive effects due to activation of some bio-chemical processes. In view of such reasons the determination of chromium in pharmaceuticals and biological samples is of great interest. The determination of total chromium from a multivitamin with multimineral dosage forms is based on the conversion of Cr^{+3} to Cr^{+6} by oxidation with nitric acid- perchloric acid mixtures followed by the complexation of Cr^{+6} with 1,5-diphenylcarbazide (DPC) in a mineral acidic solution of pH 1.0 ± 0.5. The diphenylcarbazaide method involves many metal ions and thus requires control of temperature and long reaction time; in addition the stability of the colored products is not satisfactory. The pink –colored dye complex of Cr^{+6}-DPC was estimated at 544nm [87].

2. OBJECTIVES

2.1. General objective

Determination of some selected minerals in multivitamin pharmaceutical dosage forms marketed in Ethiopia by atomic absorption spectrophotometer and to compare its simplicity, sensitivity, accuracy and precision with reported methods.

2.2. Specific objectives

- To determine the concentrations of selected minerals in pure form by FAAS.
- To determine the concentrations of selected minerals in laboratory prepared mixtures by FAAS.
- To determine the concentrations of selected minerals in multivitamin with multimineral pharmaceutical dosage forms by FAAS.
- To compare the FAAS method with other reported methods, with respect to sensitivity, simplicity, accuracy and precision.

3. EXPERIMENTAL

3.1. Instruments and equipments

Pharmaceutical dosage form samples: Ginsavit capsules, juiphar- UAE of Batch number-290, manufacturing date 06/2007 and expiry date 10, 2010, V-2 gelatin capsules, PHARCO Pharmaceutical-Egypt of Batch number-535, manufacturing date 09/2007and expiry date 08,2011, Minovit p tablets, CIPLA LTD- India of Batch number-06901PE, manufacturing date 02/2008 and expiry date 2011, Pregenatal tablets, SCANPHARM-Denmark of batch number 0917168B, manufacturing date 07/2008 and expiry date 2012, Viminova tablets, SCANPHARM-Denmark of batch number-0.9211083505B, manufacturing date 11/2007 and expiry date 01/2011 and Maxamine forte, tablets Anglo-French Drugs and Industries LTD of Batch number-06903PE, manufacturing date 03/2008 and expiry date 09/ 2012. Digestions were carried out with a Kjeldahl apparatus equipped with automatic thermostat (Bosch company-Germany). The absorbance measurement was carried out with a Perkin-Elmer 3030 Zeeman Flame Atomic Absorption Spectrophotometer (USA), equipped with appropriate hollow cathode lamps of magnesium, calcium, potassium and chromium, the wave length were set to 285.2, 422.7, 766.9 and 357.9 nm resonance lines respectively. Glassware's, pestle and mortar, analytical balances have been used through out the laboratory work.

3.2. Chemicals and solvents

Analytical grade of FAAS purity (67-70) % HNO_3 w/v and H_2O_2 30 %w/v (Lachema, Laboratory supply, Czech Republic), 70% $HClO_4$ w/v (Germany) single component standards of salt solution of Ca salts as Pantothenate, phosphate and gluconate, Mg salts as, chloride, oxide, gluconate and sulphate, K salts Acetate, citrate effervescent, iodide/chloride and Cr salts as chromium chloride, chromium picolinate and chromium polynicotinate each one with the content of 1000 μg/mL, CPI International, USA) and deionized water were used to prepare all solutions of salt.

3.3. Preparation of reagents and stock solutions

3.3.1. Preparation of standard calcium (II) solution

Standard calcium (II) solution (1000 μg/mL) was prepared by dissolving 2.5 gm of dry primary standard calcium carbonate in 1000 mL volumetric flask where 2 mL of 6 M HCl was added to dissolve it, then dilute to the mark with deionized water and mix thoroughly. 10 μg/mL of calcium standard solution was prepared just before use by taking 1mL from the stock solution and added to 100 mL volumetric flask completed with deionized water to the mark. Serial solutions were prepared by taking 5, 10, 15, 20, 25 and 30 mL from 10 μg/mL standard Ca (II) solution to construct the calibration curve. The standard solution was prepared daily.

3.3.2. Preparation of standard magnesium (II) solution

Standard magnesium (II) solution (1000 μg/mL) was prepared by dissolving 1.67 gm of dry standard magnesium oxide in 1000 ml volumetric flask where analytical grade 2 mL of 6 M HCI was used to dissolve it, then adjusted to the mark with deionized water and mixed thoroughly. The working standard solution (10 μg/mL) was prepared just before use by taking 1 mL and added to 100 mL volumetric flask, the secondary working standard solution (1 μg/mL) was also prepared by taking 10 mL from the working standard solution and then added in to 100 mL flask completed with deionized water to the mark. Serial solutions were prepared by taking 1, 1.25, 1.5, 1.75, 2 and 2.25 mL from the secondary working standard solution added to 100 mL completed to the mark with deionized water to the mark to construct the calibration curve. The standard solution was prepared daily.

3.3.3. Preparation of standard potassium (I) solution

Standard potassium (I) solution (1000 µg/mL) was prepared by dissolving 1.91 gm of dry primary standard potassium chloride in 1000 mL volumetric flask dissolved and dilute to the mark with deionized water and mixed thoroughly .The working standard solution (10 µg/mL) was prepared just before use by taking 1 mL and added to 100 mL volumetric flask and then adjusted to the mark with deionized water. Serial solutions were prepared by taking 2.5, 5, 7.5, 10, 12.5 and 15 mL from the working standard solution to construct the calibration curve. The standard solution was prepared daily.

3.3.4. Preparation of standard ternary mixtures of Ca (II), Mg (II) and K (I) solutions

The laboratory ternary mixtures were prepared by taking 5, 10, 15, 20, 25 and 30 mL of calcium (II), 1, 1.25, 1.50, 1.75, 20, and 2.25 mL of magnesium (II) from the standard working solutions and mixed with 2.5, 5, 7.5, 10, 12.5 and 15 mL of potassium (I) in to 100 mL volumetric flask and adjusted to the mark with deionized water to construct the calibration curves of the metallic ions.

3.3.5. Preparation of total chromium (III and VI) standard solution

Standard total chromium (III and VI) solution (1000 µg/mL) was prepared by dissolving 1.923 gm of dry primary standard chromium trioxide in 1000 mL volumetric flask acidified (to pH = 2) with analytical grade conc. HNO_3 and the volume was completed to the mark with deionized water. The standard working solution (10 µg/mL) was prepared by taking 1 mL from the stock solution and added to 100 mL volumetric flask completed with deionized water to the mark. Serial solutions were prepared by taking 5, 10, 15, 20, 25 and 30 mL from the working solution adding to 100 mL volumetric flask adjusted to the mark with deionized water to construct the calibration curve. The standard solution was prepared daily to prevent dilution effect and oxidation.

3.3.6. Reported (UV-Visible spectrophotometric method) of preparation of total chromium (III and VI) standard solution

Standard chromium solution (2800 μg/ mL) was prepared by dissolving 0.28 gm of dry primary standard potassium dichromate in 100 mL volumetric flask acidified to pH = 1± 0.5 with analytical grade conc. H_2SO_4 and the volume was completed to the mark with deionized water. The standard working solution (100 μg/ mL) was prepared by taking 3.6 mL from the stock solution and added to 100 mL volumetric flask adjusted with deionized water to the mark. Serial solutions were prepared by taking 5, 10, 15, 20, 25 and 30 mL from the working solution added to 100mL volumetric flask adjusted to the mark with deionized water to construct the calibration curve.

3.4. Sample preparation of the studied multivitamins with multi minerals pharmaceutical dosage forms

3.4.1. Maxamine forte, Pregenatal, Minovite p and Viminova tablets

Ten tablets were accurately weighed and crushed separately with pestle and mortar into a fine powder. The average weight equivalent to one tablet powder was taken into four different Kjeldahl apparatus digested with 10 mL analytical grade HNO_3 (67-70%) w/v and 5 mL of H_2O_2 (30%) w/v at a temperature of $150^{O}C$ until a clear solution was obtained. The resulting solutions were transferred quantitatively into four different 100 mL volumetric flasks completed to the mark with deionized water shaked, followed by filtration using filter paper. From the prepared solutions for the determination of each metal ion, in Minovit 1.7 mL Ca (II) and 1 mL Mg (II), in Pregenatal 1mL of Mg (II) and in Maxamine forte 5 mL of Mg (II) were added separately into four different 100 mL volumetric flasks, in Maxamine forte 2.4 mL of Ca (II) in to 50 mL and 15 mL of total chromium in Viminova to 25 mL volumetric flasks were taken and adjusted to the mark with deionized water.

3.4.2. Ginsavit and V-2 capsules

Five different V-2 and Ginsavit gelatin capsules were separately weighed and each of the capsules was quantitavely transferred into the digestion flasks. Analytical grade of 10 mL of HNO_3 (67-70%) w/v and 5 mL of H_2O_2 (30%) w/v were added and digested in the Kjeldahl apparatus at $150^{o}C$ until a clear solution obtained. Each sample solutions were transferred quantitatively into 100 mL volumetric flasks and shaked, followed by filtration using filter paper and adjusted to the mark with deionized water. From the prepared sample solutions in Ginsavit, 2.2 mL of Ca (II) and 1 mL Mg (II), in V-2 capsule 2.3 mL of Ca (II) and 1.7 mL of Mg (II) were taken to 100 mL volumetric flasks, 10 mL of K (I) in Ginsavit and V-2 were taken to 50 mL volumetric flasks completed to the mark with deionized water for the determination of the stated metallic ions in a solution.

3.4.3. Preparation of ternary mixtures of Ca (II), Mg (II) and K (I) from sample solutions

Sample solutions were prepared as indicated in 3.4.1 and 3.4.2, from the minimum concentration of 210 μg/mL Ca (II) in Maxamine forte tablets 20, 25 and 30mL, 60 μg/mL Mg (II) in V-2 gelatin capsules 1.75, 2 and 2.25 mL and 50 μg/mL K (I) in Ginsavit gelatin capsules 10, 12.5 and 15 mL were taken and mixed in 100 mL volumetric flask and completed to the mark with deionized water. From the maximum concentrations of 1210 μg/mL Ca (II) in Minovit P tablets 20, 25 and 30 mL, 1000 μg/mL Mg (II) in Pregenatal tablets 1.75, 2 and 2.25 mL and 50 μg/mL K (I) in Ginsavit gelatin capsules 10, 12.5 and 15 mL were taken, mixed into 100 mL volumetric flasks and diluted to the mark with deionized water for the triplicate determination of each metallic ions .

3.4.4. Preparation of total chromium (III and VI) sample solution by standard addition method

In this approach, 5, 10, 15, 20 and 25 mL concentrations of total chromium were taken from working standard solutions and mixed with 5, 10, 15, 20 and 25 mL concentrations of total chromium from the Viminova tablet solutions as indicated in 3.4.1. The clear solutions of standard and sample was taken into 25 mL volumetric flask and then adjusted to the mark with deionized water.

3.4.5. Reported (UV-Visible spectrophotometric method) of sample preparation of the studied pharmaceutical dosage forms of Viminova tablets

Ten tablets were accurately weighed and crushed with pestle and mortar into a fine powder, the average weight equivalent to one tablet powder was taken in to around bottom flask. A mixture of (5: 5: 20) mL of (67-70 %) HNO_3 w/v, 70% $HClO_4$ w/v and deionized water were added respectively and heated until the fuming of HNO_3 and $HClO_4$ were completely removed. The resulting solution was cooled, filtered using a what man no 4, lowered the pH to (1± 0.5) with 2 mLof conc.H_2SO_4 and quantitatively transferred to 100 mL volumetric. A 0.25gm of 1, 5-diphenyl carbazaide were taken into 50 mLvolumetric flask, dissolved and completed to the mark with acetone and stored in amber glass. From the prepared solution of 1, 5-diphenyl carbazaide, 1 mL was taken and added into 100 mL sample solution, allowed to stand for 5 minutes until a brick red complex solution was appeared and adjusted to the mark with deionized water. The absorbance was measured at 544 nm.

3.4.6. Procedures for determination of metal ions in each sample solutions by FAAS

Triplicate absorbance measurements for each sample solutions prepared as indicated in 3.4.1 and 3.4.2, measurements were carried out in each sample with the specified parameters cited in Table 2, under the same conditions and with appropriate hollow cathode lamp of the elements interest.

4. RESULTS AND DISCUSSION

Quantitative determination in atomic absorption spectrophotometer relies, like any other spectrophotometric method, on the applicability of Beer's Law. The determinations were based on linear range of calibration curve that was obeyed Beer's law, absorbance versus concentration of standard solutions were plotted by dilution from the standard stock solution of each metal ions. The calibration curves were constructed under the optimum conditions from the standard solutions of Ca (II), Mg (II), K (I) and Cr (III and VI) measured at the 422.7, 285.2, 766.5 and 357.9 nm respectively based on the manufacturer recommendation presented in Table 2 . Moreover, the linear regression equation parameters in the determination of calcium (II), magnesium (II), potassium (I) and total chromium (III and VI) by FAAS technique, a: intercept; b: slope; r: correlation coefficient; r^2: coefficient of determination; LOD: limit of detection = 3 σ / S; LOQ: limit of quantitation = 10 σ / S (where σ is the standard deviation of the intercept and S is the sensitivity) and SD: standard deviation of the slope and intercept obtained by the linest formula from the calibration curves.

Table 3: Manufacturer instrumental settings.

Parameters	**Calcium**	**Magnesium**	**Potassium**	**Chromium**
Absorption maxima λ_{max} (nm)	422.7	285.2	766.5	357.9
Linearity range (μg/ml)	0.01-3.00	0.001- 0.0225	0.01-1.50	0.05-3.00
Slit width (nm)	0.70	0.070	0.070	0.070
Photomultiplier (v)	306.2	215.90	300.20	312.70
Current of HCL(mA)	2.00	1.00	2.00	2.00
Energy (ev)	3.643	3.480	3.730	3.630
Integration time (sec)	2.50	3.00	3.00	3.00

In this study, magnesium (II), calcium (II), potassium (I) and the total chromium (III and VI) were quantitatively determined in pharmaceutical dosage forms of multivitamins with multimineral (Pregenatal, Minovit P, Maxamine forte, Viminova tablets and Ginsavit and V-2 capsules) by FAAS. The statistical analysis of the results from the experimental data, the regression equations obtained from the calibration graphs, along with standard deviations of the slopes and the intercepts of the ordinate were highly significant.

The higher value of the correlation coefficient indicates the good linearity of the calibration graph, the linearity from the graph of absorbance versus concentration ascertain the method of determination obeys Beer's Law. From the calibration curves depicted below, the determination coefficients (r^2), the sensitivity equal to the slope of the calibration curve were evaluated and the correlation coefficients (r) found to be between 0.9969-0.9996 presented in all tables stated below thus, the higher correlation coefficients for each standard solutions of metallic ion indicates that the results are strongly correlated. The strong correlation of the results shows the suitability of the method for the determination of the metal ions. In an intra-day and inter-day determination of Ca (II) and Mg (II) salt solutions (Table 15, 17 and 19) there is no significant difference among the correlation coefficients which ascertains the precision of the analytical method and indicates the linearity and reproducibility of the method.

4.1. Precision

In order to test the repeatability of FAAS method, separate determinations at different concentration levels were carried out for Ca (II), Mg (II), K (I) and total Cr salt solutions in the presence of certain concentration of the other component. The results obtained in all analysis presented in Table 4, 6, 8-20 shows that, the relative standard deviation were less than 2%, which indicated high degree of precision of the FAAS method.

4.2. Selectivity

The method selectivity was confirmed by preparing different mixtures of the tested Ca (II), Mg (II) and K (I) within the linear range. The mixtures as indicated 3.4.3 contain varying amounts of standard solution of metal ion components and constant amount of the other. The laboratory prepared mixtures were analyzed by passing a beam of light originated from a line-emitting light source which is characteristic resonance line of the element to be measured is directed through the flame and thus the chosen wavelength will not interfere with the measurement due to the narrow width of absorption lines presented in Table 2. Statistical analysis of the data obtained from the experiment shows that the slope of the calibration graph for each metal ion was independent on the concentration of the other component of the mixture. Herewith, the result obtained (Table 4, 6, 8-20) indicates the high selectivity of the FAAS method and its potential for the independent determination of these metal ions in the mixture.

4.3. Linearity

The linearity of FAAS method was evaluated for Ca (II), Mg (II), K (I) and total Cr (III and VI) by analyzing a series of different concentrations of each studied metal ion within the linear range in the absence and presence of a certain concentration of the other component in the mixture. The absorbance measurement for each standard metallic solution were carried out at the specified wavelengths presented in Table 2 and plotted against concentration and straight line was obtained in each cases. The statistical analysis of these graphs using linest formula of Microsoft excel were made for the slope, intercept and correlation coefficients. The results obtained exhibits that the linearity of calibration graphs obeyed with Beer's law, good values of correlation coefficients of the regression equation and the small values of intercepts presented in (Table 3, 5, 7 ...19)

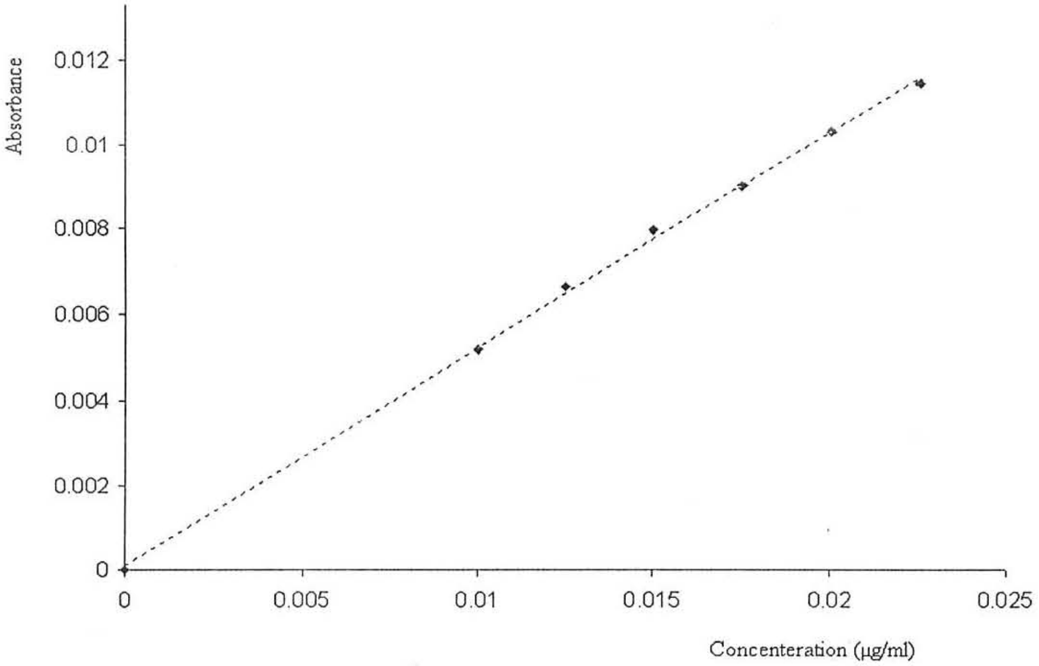

Figure 1: Calibration curve for standard magnesium (II) solution in the determination of minovit p and pregenatal tablets; [Y= 0.507X + 0.0002, $r^2 = 0.9971$, n=6].

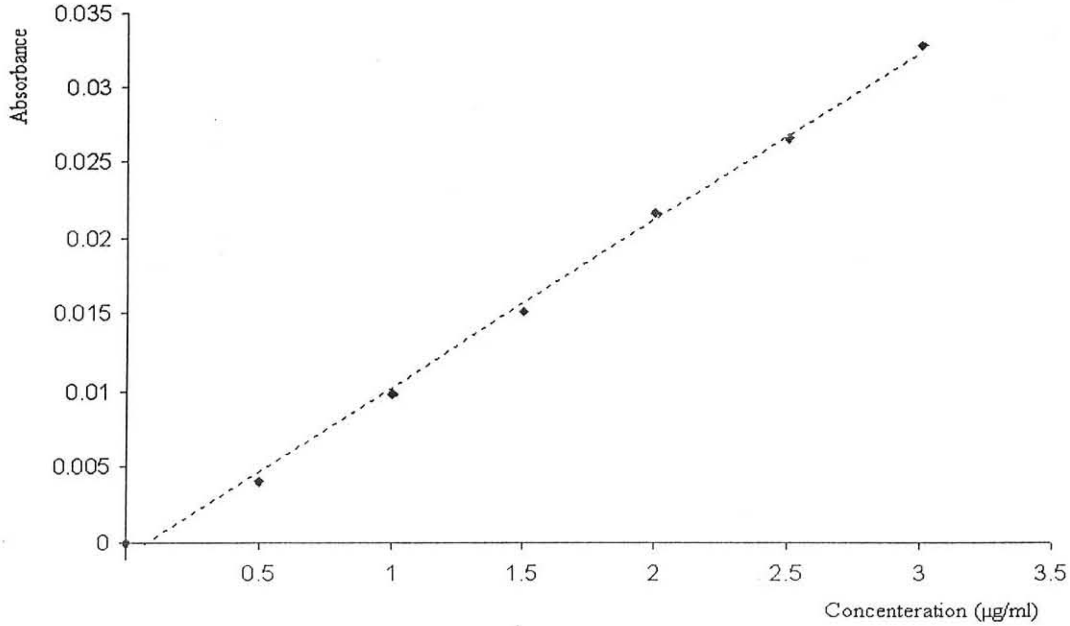

Figure 2: Calibration curve for standard calcium (II) solution in the determination of minovit p tablets; [Y= 0.0115X – 0.0013, $r^2 = 0.9947$, n=6].

Table 4: Analytical parameters for the determination of magnesium (II) and calcium (II) standard solution demined by FAAS technique.

Standard Solution Of	Conc. (μg/ml)	λ (nm)	Linear regression equation parameters					
			a + SD	b ± SD	r	r^2	LOD (μg/mL)	LOQ (μg/mL)
Mg (II)	0.001- 0.0225	285.2	0.0002 ± 0.000308	0.507 ± 0.01833	0.9985	0.9971	0.00182	0.00607
Ca (II)	0.01- 3.00	422.7	-0.0013 ± 0.00032	0.0115 ± 0.000 627	0.9973	0.9947	0.06361	0.54537

Table 5: Actual and predicted amounts of calcium (II) and magnesium (II) in the commercial dosage forms of pregenatal and minovit P tablets sample solutions determined by FAAS.

Metal ions	Real conc. g/ml)	Found (μg/mL) Mean ± SD	RSE	Recovery (%)	RSD (%)
Ca (II) in Minovit p	2.00	1.98765± 0.012702	0.6175	99.38	0.63904
	2.50	2.47150± 0.029050	1.140	98.86	1.1750
	3.00	3.032167± .024585	1.072	101.07	0.8108
Mg (II) in Minovit p	0.0175	0.01736± .0001079	0.800	98.60	0.0232
	0.0200	0.019813± .000189	0.9350	99.06	0.9539
	0.0225	0.022357± .000145	0.6356	99.36	0.6486
Mg (II) in Pregenatal	0.0175	0.017276± .000230	1.280	98.72	0.5017
	0.0200	0.019833± .000168	0.835	99.17	0.7849
	0.0225	0.022471± .000293	0.1289	99.87	0.1780

The results of analysis under the same conditions, after total Kjeldahl digestion of Pregenatal and Minovit P tablets, the quantitative determination of calcium (II) and magnesium (II) were compared with content declared by producer a Mean ± SD of triplicates. The actual and predicted amounts of the concentration of the metal ions determined by FAAS technique was obtained experimentally in the calibration range 0.01-3.00 μg/mL of Ca (II) and 0.001-0.0225 μg/mL of Mg (II) respectively presented in Tables 2. The concentration predicted by this method is considerably close to the real ones in all the samples of calcium (II) and magnesium (II) which actually show the repeatability of the proposed method. Thus, the accuracy and reliability of the results were assessed by analysing the Ca (II) in Minovit p tablets and Mg (II) in Minovit p and in Pregenatal tablets. The analysis results presented in Table 4 shows that the suggested method for the determinations of Ca (II) and Mg (II) in the sample solutions provide quite reliable results of greater than 98.86% recoveries and less than 2% of RSD.

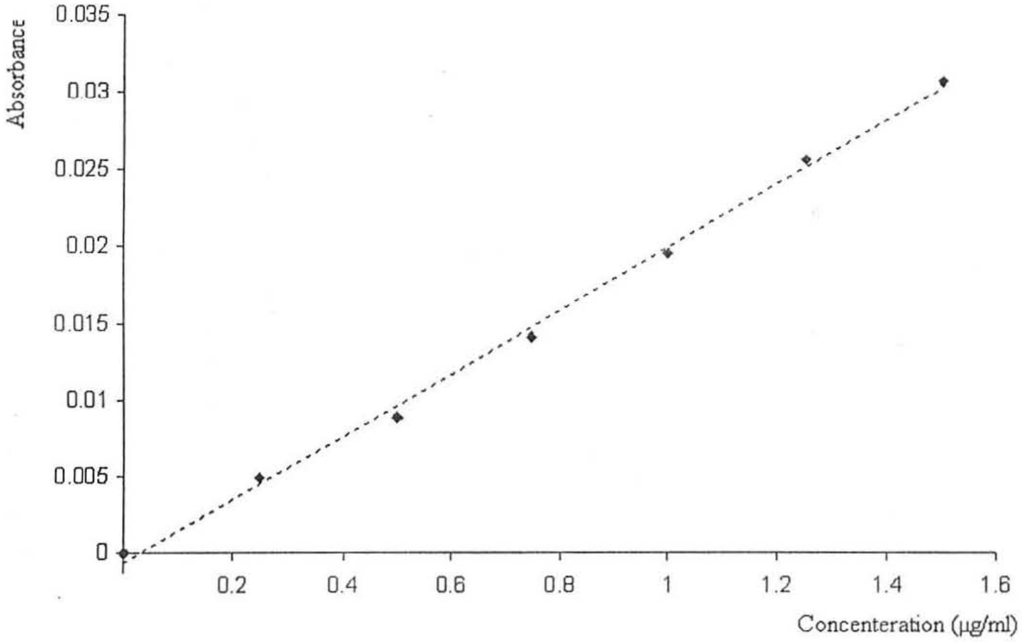

Figure 3: Calibration curve for standard potassium (I) solution in the determination of ginsavit capsules; [Y= 0.0207X -0.000, $r^2 = 0.9962$, n=6].

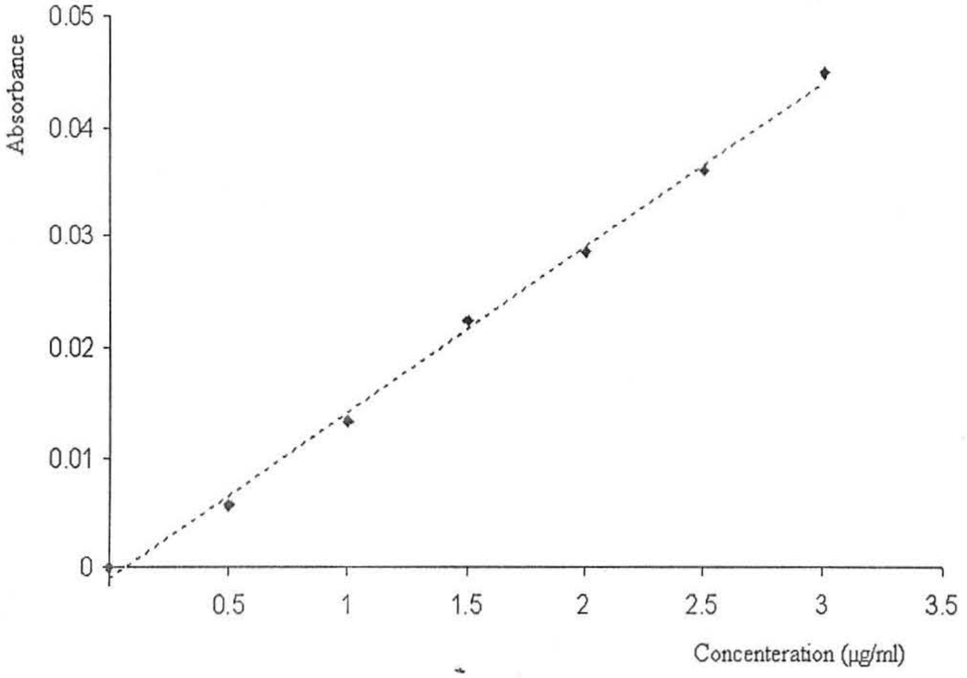

Figure 4: Calibration curve for standard calcium (II) solution in the determination of ginsavit capsules and maxamine forte tablets : [$Y=0.252X-0.0023$, $r^2 = 0.9975$, $n=6$].

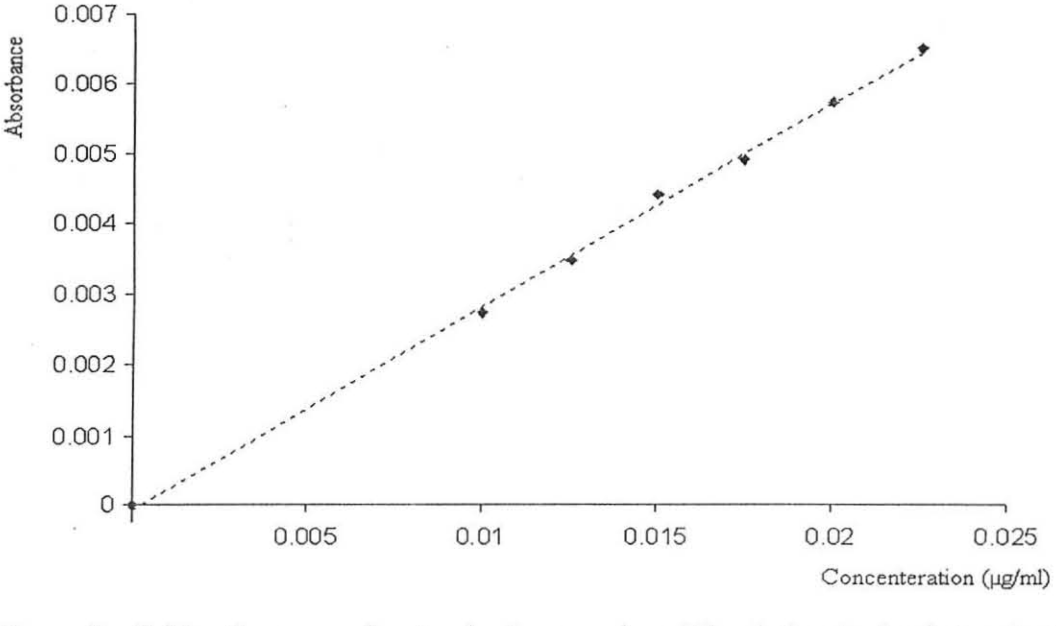

Figure 5: Calibration curve for standard magnesium (II) solution in the determination of ginsavit capsules and maxamine forte tablets; [$Y=0.2882X-0.00004$, $r^2 = 0.9986$, $n=6$].

Table 6: Analytical parameters in the determination of Ca (II), Mg (II) and K (I) standard solution determined by the FAAS technique.

Standard Solution			Linear regression equation parameters					
Of	Conc.(μg/ml)	λ(nm)	a + SD	b ± SD	r	r^2	LOD (μg/mL)	LOQ (μg/mL)
Mg (II)	0.001- 0.0225	285.2	-0.0004 ± 0.000213	0.2882 ± 0.0127	0.9993	0.9986	0.0022	0.0074
Ca (II)	0.01- 3.00	422.7	-0.0023 ± 0.00071	0.252 ± 0.00037	0.9982	0.9975	0.014	0.4671
K (I)	0.05- 1.50	766.5	-0.0007 ± 0.000067	0.0207 ± 0.00069	0.9981	0.9962	0.0969	0.3231

The lower statistical values of the slope and intercept obtained from the calibration curves by the linest formula for Ca (II), Mg (II) and K (I) standard solutions by the proposed technique ascertains the sensitivity of the analytical methods. It is to thus, the sensitivity is equal to the slope of the calibration curve being constant and the higher value of the correlation coefficients indicates the good linearity of the calibration graph. Moreover, the slopes of the calibration graph for each metal ion were independent on the concentration of the other component in the mixture.

Table 7: Actual and predicted amounts of calcium (II) and magnesium (II) in the commercial dosage forms of ginsavit and maxamine forte tablets sample solutions determined by FAAS.

Metal ions	Real conc. (µg/ml)	Found (µg/ml) Mean ± SD	RSE	Recovery (%)	RSD (%)
Ca (II) in Ginsavit	2.00	1.9870 ± 0.01291	0.65	99.35	0.6497
	2.50	2.4695 ± 0.02131	1.220	98.78	0.8629
	3.00	2.968 ± 0.0384	1.067	99.44	0.3918
Mg (II) in Ginsavit	0.0175	0.0174 ± 0.00016	0.5714	98.64	0.8361
	0.0200	0.0199 ± 0.00014	0.500	99.29	0.7252
	0.0225	0.0224 ± 0.00010	0.444	99.63	0.4510
K (I) in Ginsavit	1.000	0.9897 ± 0.01141	1.030	98.97	1.1529
	1.250	1.2349 ± 0.00615	1.208	98.79	0.4979
	1.50	1.4903 ± 0.00972	0.647	99.35	0.6522
Ca (II) in Maxamine fort	2.00	1.9734 ± 0.00771	1.330	98.67	0.3907
	2.50	2.5103 ± 0.01040	0.412	100.41	0.4144
	3.00	2.9872 ± 0.01876	0.427	99.57	0.5892
Mg (II) in Maxamine fort	0.0175	0.01733± 0.00018	0.971	99.02	1.046
	0.020	0.0199 ± 0.00015	0. 500	99.31	0.7557
	0.0225	0.02242 ± 0.0003	0.356	100.32	0.3185

The calibration curves presented in (Figure 3 – 5) obtained under the same conditions shows a linear correlation verified by the strongly correlated coefficients of r > 0.9962 with in the concentration range of 0.001–0.0225 µg/ mL of Mg (II), 0.01-3.00 µg/ mL of Ca (II) and 0.01-1.50 µg/ mL of K (I). The detection limit were 0.0022 µg/ mL of Mg (II) in maxamine forte, 0.0033 µg/mL in ginsavit capsules, 0.014 µg/ mL of Ca (II) in both maxamine forte tablets and Ginsavit capsules and 0.0969 µg/ mL of K (I) presented in Table 5. The values of the relative standard deviations (RSD) exhibit the precision of the proposed method based on the determination of triplicate sample solutions by FAAS were less than 2%. The average recovery was greater than 98.67% that shows the precision of the analytical method presented in Table 6.

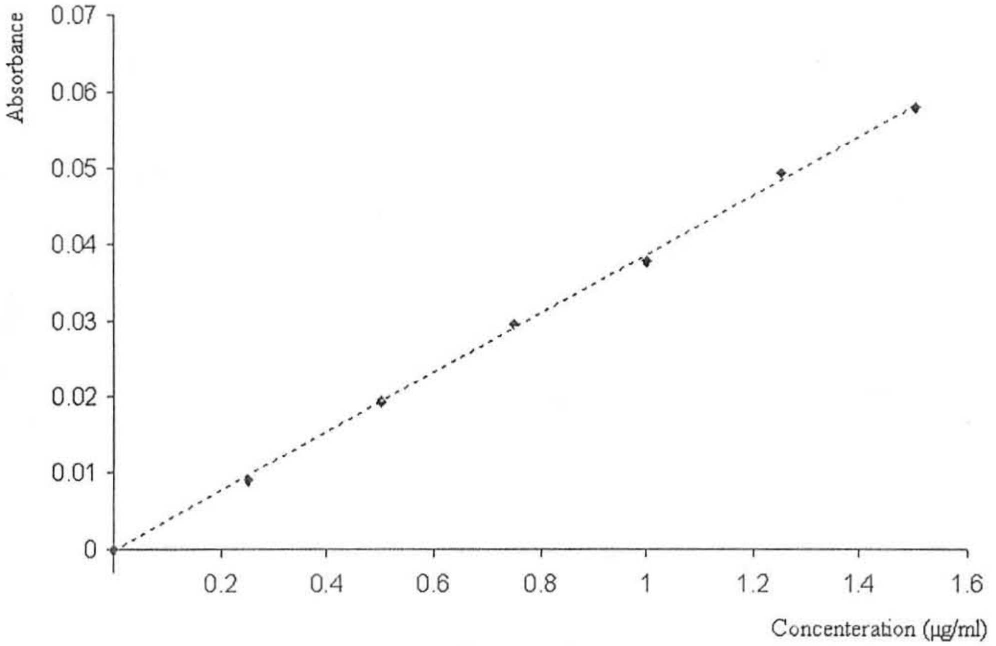

Figure 6: Calibration curve for standard potassium (I) solution in the determination of V-2 capsules; [Y= 0.0391X -0.0003, r^2 = 0.9991, n=6].

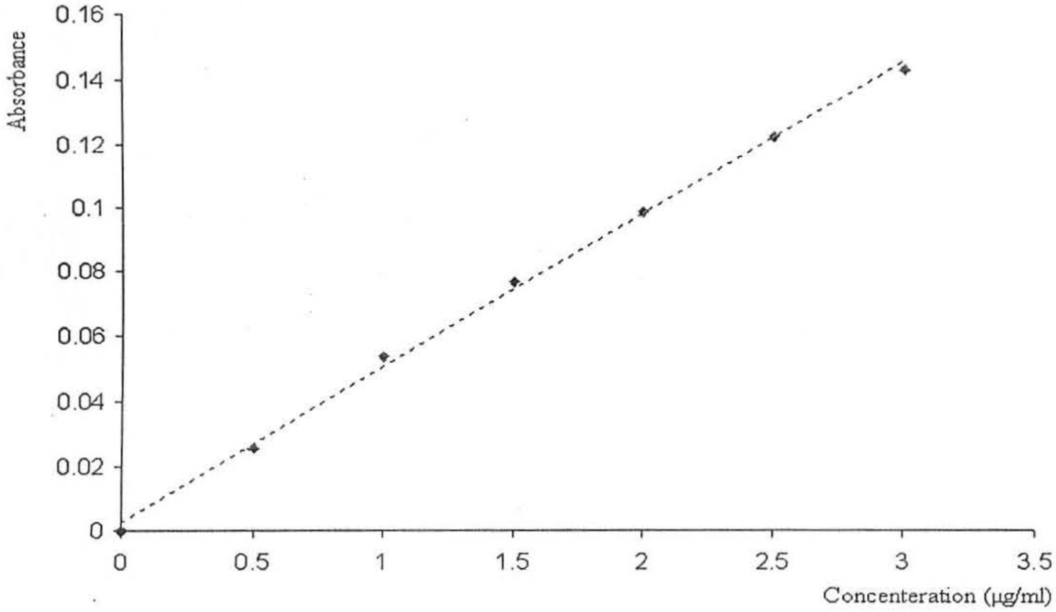

Figure 7: Calibration curve for standard calcium (II) solution in the determination of V-2 capsules; [Y= 0.0468X +0.0039, r^2 = 0.9952, n=6].

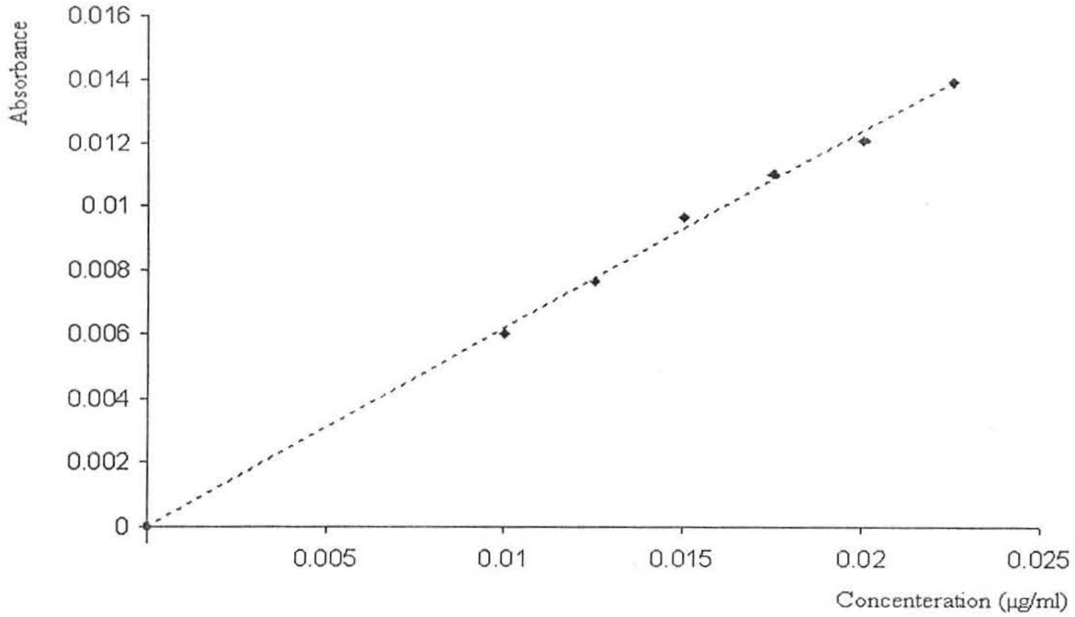

Figure 8: Calibration curve for standard magnesium (II) solution in the determination of V-2 capsules; [Y= 0.06194X -0.000009, $r^2 = 0.9968$, n=6].

Table 8: Analytical parameters for the determination of calcium (II), magnesium (II) and potassium (II) standard solutions determined FAAS technique.

Standard Solution of	Conc. (μg/ml)	λ (nm)	Linear regression equation parameters a ± SD	b ± SD	r	r^2	LOD (μg/mL)	LOQ (μg/mL)
Mg (II)	0.001- 0.0225	285.2	-0.00009 ± 0.00313	0.6194 ± 0.0016	0.9984	0.9968	0.00615	0.0172
Ca (II)	0.010- 3.00	422.7	0.0039 ± 0.00312	0.0468 ± 0.0016	0.997	0.9952	0.0997	0.666
K (I)	0.05- 1.500	766.5	-0.0003 ± 0.00069	0.0391 ± 0.0007	0.9995	0.9991	0.0726	0.1752

Table 9: Actual and predicted amounts of calcium (II), potassium (I) and magnesium (II) sample solution in the commercial dosage form of V-2 capsules determined by FAAS.

Metal ions	Real conc. (µg/ml)	Found (µg/ml) Mean ± SD	RSE	Recovery (%)	RSD (%)
Ca (II)	2.00	1.9786 ± 0.0215	1.070	98093	1.0866
	2.50	2.4812 ± 0.0189	0.752	99.23	0.7617
	3.00	2.9417 ± 0.058	1.943	99.61	0.3911
Mg (II)	0.0175	0.0173 ± 0.00022	1.143	98.17	1.296
	0.020	0.0198 ± 0.00017	1.00	99.20	0.8618
	0.0225	0.02243 ± 0.00029	0.311	98.64	0.7150
K (I)	1.000	0.9913 ± 0.00881	0.870	99.13	0.8887
	1.250	1.2296 ± 0.00388	1.632	98.36	0.3158
	1.500	1.4841 ± 0.01710	1.590	98.94	1.1522

The quantitative determination of the actual concentration of the metal ions Ca (II), Mg (II) and K (I) in V-2 multivitamin with multimineral dosage forms carried out using FAAS technique after digestion by the Kjeldahl apparatus were very close to the claimed amount presented in Table 8 . The regression equations in the calibration curves depicted in (Figure 6- 8) based on standards were strongly correlated. In the determination of the average levels of the elements in the analysed sample correspond with manufacturer labels for Ca (II), Mg (II) and K (I) in each brand of pharmaceutical dosage forms indicating the suitability of the method. To evaluate the experimental precision of the analytical method, the concentrations of magnesium (II), calcium (II) and potassium (I) in multivitamins with multiminerals were determined by FAAS technique. The precision of the method %RSD were obtained for triplicates of the sample solution and thus, typically less than 2%.

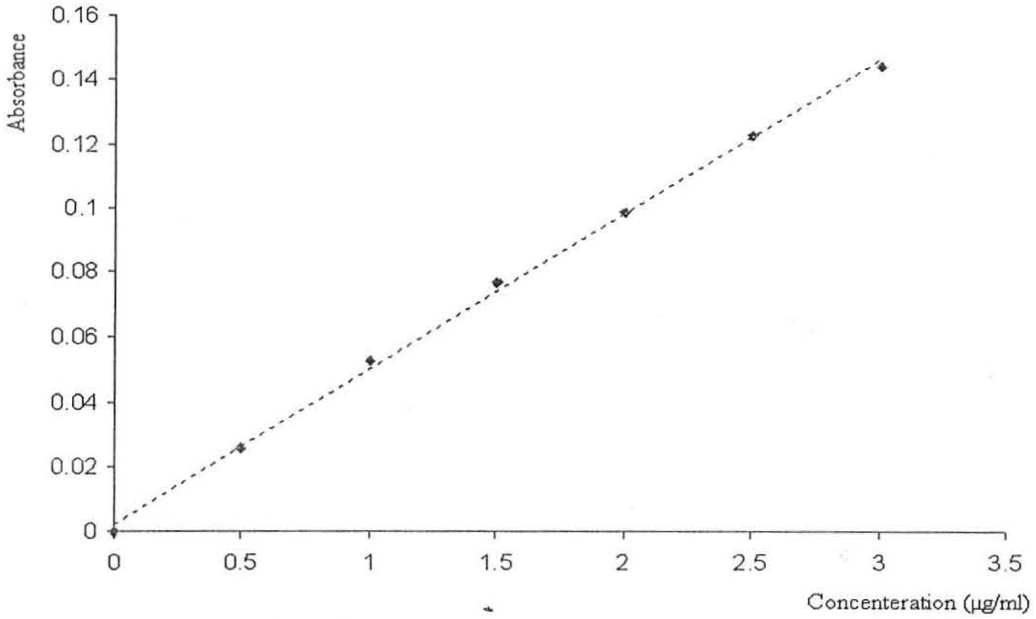

Figure 9: Calibration curve of calcium (II) solution in ternary laboratory prepared standard mixtures; [Y= 0.0468X +0.0039, $r^2 = 0.9995$2, n=6].

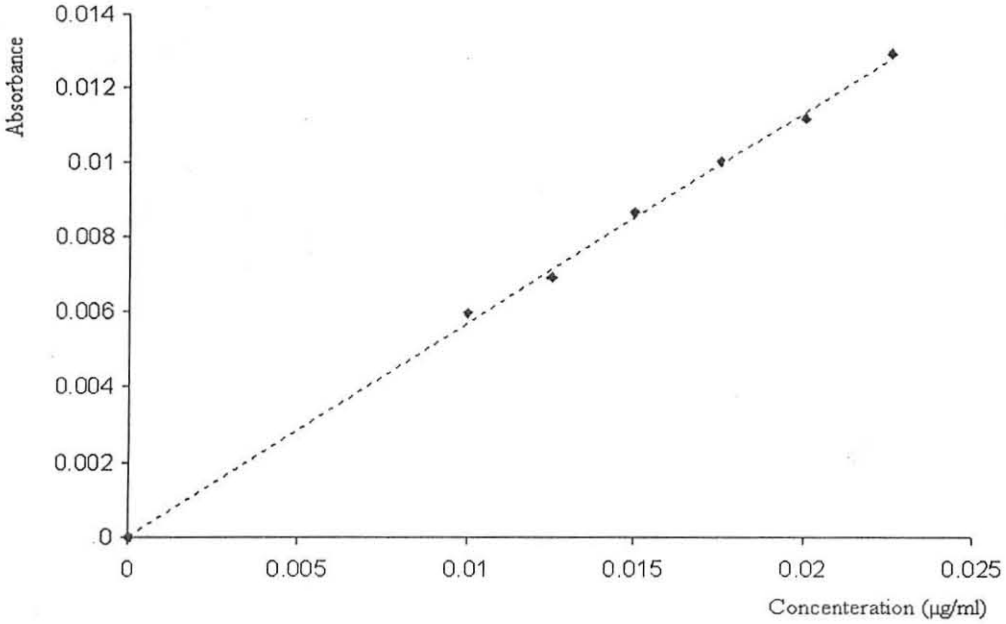

Figure 10: Calibration curve of magnesium (II) solution in the laboratory prepared standard mixtures; [Y= 0.5662X +0.00002, $r^2 = 0.9964$, n=6].

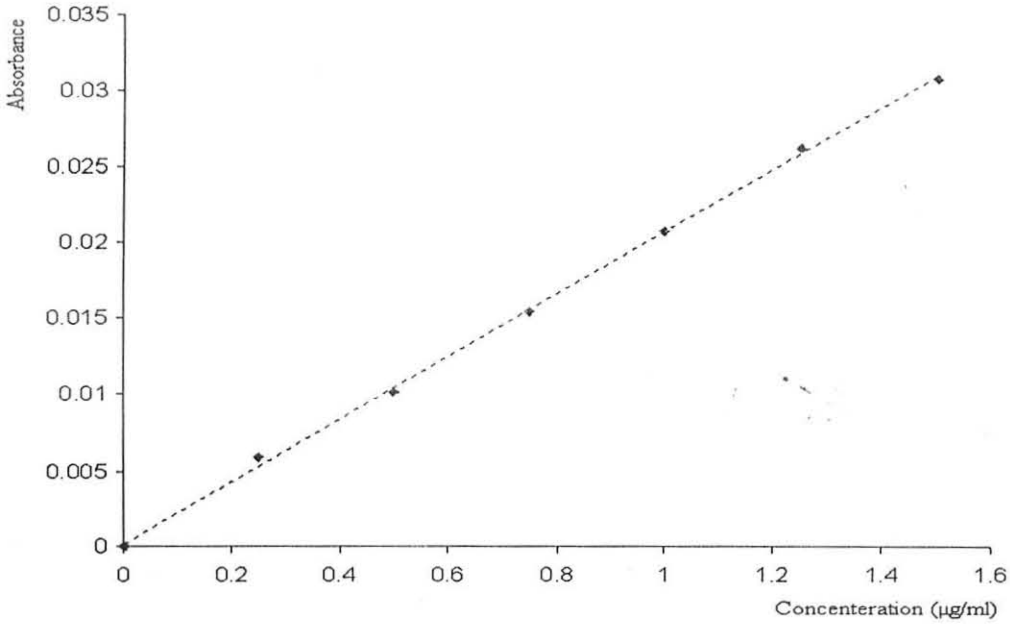

Figure 11: Calibration curve of potassium (I) solution in the laboratory prepared standard mixtures; [Y= 0.0325X -0.0012, $r^2 = 0.9953$, n=6].

Table 10: Analytical parameters in the determination of laboratory prepared mixtures of calcium (II), magnesium (II) and potassium (II) standard solutions by FAAS.

Standard Solution of	Conc (μg/ml)	λ (nm)	a ± SD	b ± SD	r	r^2	LOD (μg/mL)	LOQ (μg/mL)
			Linear regression equation parameters					
Mg (II)	0.001- 0.0225	285.2	0.00002 ± 0.0050	0.566 ± 0.0299	0.9982	0.9964	0.00267	0.00889
Ca (II)	0.01- 3.00	422.7	0.00039 ± 0.00313	0.0468 ± 0.0016	0.9976	0.9952	0.0201	0.3694
K (I)	0.05- 1.50	766.5	-0.0012 ± 0.0033	0.0325 ± 0.0034	0.9976	0.9953	0.06471	1.0157

Table 11: Actual and predicted amounts of calcium (II), potassium (I) and magnesium (II) ternary mixtures of sample solution determined by FAAS.

Metal ions	Real conc. (μg/ml)	Found (μg/ml) Mean ± SD	RSE	Recovery (%)	RSD (%)
Ca (II)	2.00	1.9761 ± 0.0239	1.195	98.81	1.2099
	2.50	2.4698 ± 0.0313	1.220	98.79	1.2673
	3.000	2.9945 ± 0.0146	0.183	99.45	1.4675
Mg (II)	0.0175	0.0174 ± 0.0002	0.571	99.5	0.4874
	0.020	0.0197 ± 0.0003	1.500	98.72	0.7150
	0.0225	0.0224 ± 0.0001	0.444	99.40	0.6059
K (I)	1.000	0.9880 ± 0.0121	1.200	98.80	1.2278
	1.250	1.2296 ± 0.0209	1.632	98.36	1.6893
	1.500	1.4815 ± 0.0187	1.233	98.77	1.2622

Maximum concentrations of calcium (II), potassium (I) and magnesium (II) in dosage mixtures were determined by FAAS method of analysis and the accuracy was then calculated from the test results as the percentage of analyte recovered. The excellent recoveries obtained presented in Tables 12 indicates that good accuracy and precision of the FAAS method. The effect of co-existing ions on the determination of Ca (II), Mg (II) and K (I) were investigated and there is no interference due to specific and characteristic wave length for each metal ions. In the linear dynamic range of the concentration of each metal ion, one component calibration was performed for standard solutions of Ca (II), Mg (II) and , K (I) and the correlation coefficients, the determination coefficients, limits of detections and limit of quantitations were highly significant presented in Table 11. The analysis results of the concentration of each metal ions in the mixture solutions of dosage forms presented in Table 12, indicates that good recoveries of greater than 98.3%.

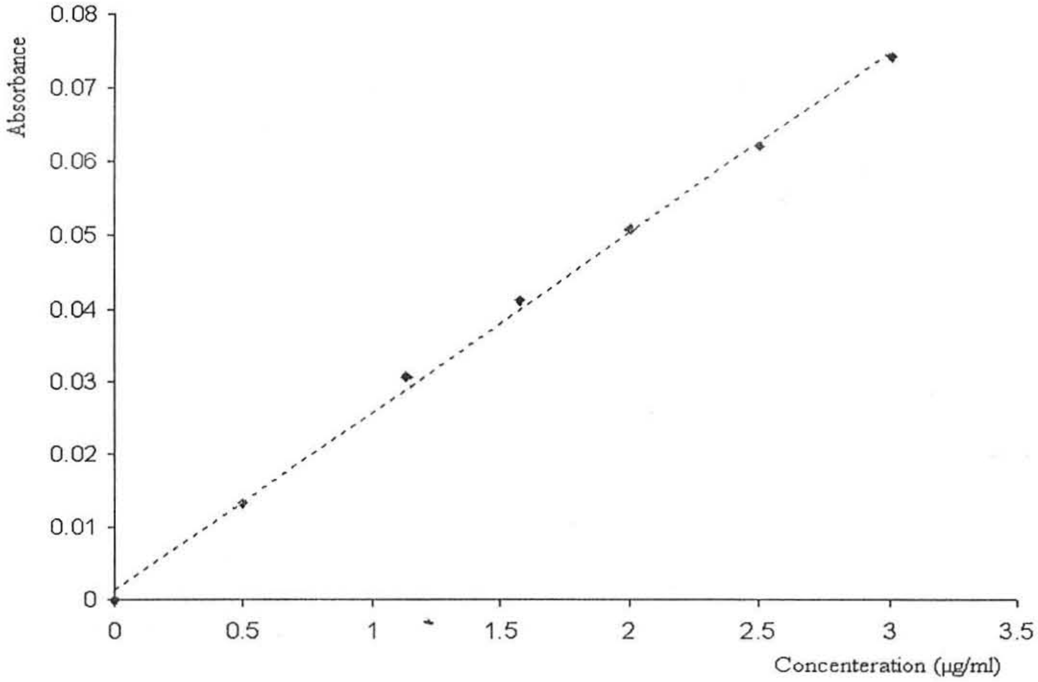

Figure 12: Calibration curve for total standard chromium (III and VI) solution in the determination of viminova tablets; [Y= 0.0246X +0.0015, $r^2 = 0.9972$, n=6].

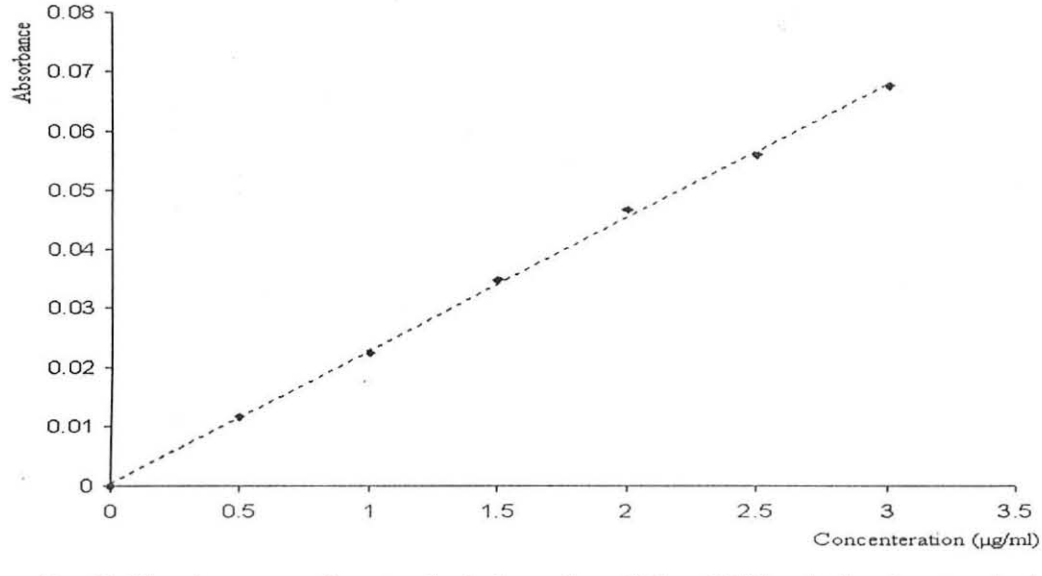

Figure 13: Calibration curve for standard chromium (III and VI) solution by standard addition method in the determination of viminova tablets; [Y= 0.0225X +0.0004, $r^2 = 0.9992$, n=6].

Table 12: Analytical parameters in the determination of chromium (III and VI) standard solution by FAAS technique.

Standard Solution of	Conc. (μg/ml)	λ (nm)	Linear regression equation parameters					
			a ± SD	b ± SD	r	r^2	LOD (μg/mL)	LOQ (μg/mL)
Cr (III and VI)	0.01-3.00	357.9	0.0015 ± 0.002095	0.0246 ± 0.001078	0.9986	0.9972	0.02559	0.85285
Cr (III and VI)	0.01- 3.00	357.9	0.0004 ± 0.007316	0.0225 ± 0.00376	0.9996 a *	0.9992	0.03754	0.32515

Table 13: Actual and predicted values of total chromium (III and VI) sample solution in the commercial dosage forms of viminova tablets determined by FAAS.

Metal ions	Real conc. (μg/ml)	Found (μg/ml) Mean ± SD	RSE	Recovery (%)	RSD (%)
Cr (III and VI)	1.50	1.4865 ± 0.0135	0.900	98.65	1.3709
	2.00	1.9765 ± 0.0237	1.175	98.83	1.1991
	2.50	2.4688 ± 0.0315	1.248	98.75	1.2759
Cr (III and VI) a *	1.50	1.4819± 0.0204	1.207	99.40	0.6855
	2.00	1.9913 ± 0.0088	0.435	99.57	0.4414
	2.50	2.4872 ± 0.0129	0.512	99.49	0.5737

Where a * refers results obtained by standard addition method.

The total chromium was determined in pharmaceutical dosage forms of Viminova tablets by the methods of FAAS. In the Statistical evaluation of standard total chromium solution, the correlation coefficients, the sensitivity represented by the slope indicated in Table 13, the recoveries and %RSD presented in Table14 of total chromium obtained from the dosage forms are highly significant. The amount of analyte originally in the sample was determined from a calibration curve, thus the correlation coefficients are strongly correlated to the true value and to one another, the higher values of the percentage recovery, the lower values of the relative standard error and %RSD presented in Table 14 ascertains the precision of the analytical method. Herewith, in the ultra trace determination of total chromium, the standard addition method is suitable and shows preferable analytical results shown in Table 13. The values of LOD, LOQ and RSD of total Cr (III and VI) determined by FAAS in Viminova tablets solutions are highly reproducible and no significant variation. In all the cases the RSD % values are not more than 2% that indicates the repeatability and the accuracy of the analytical method.

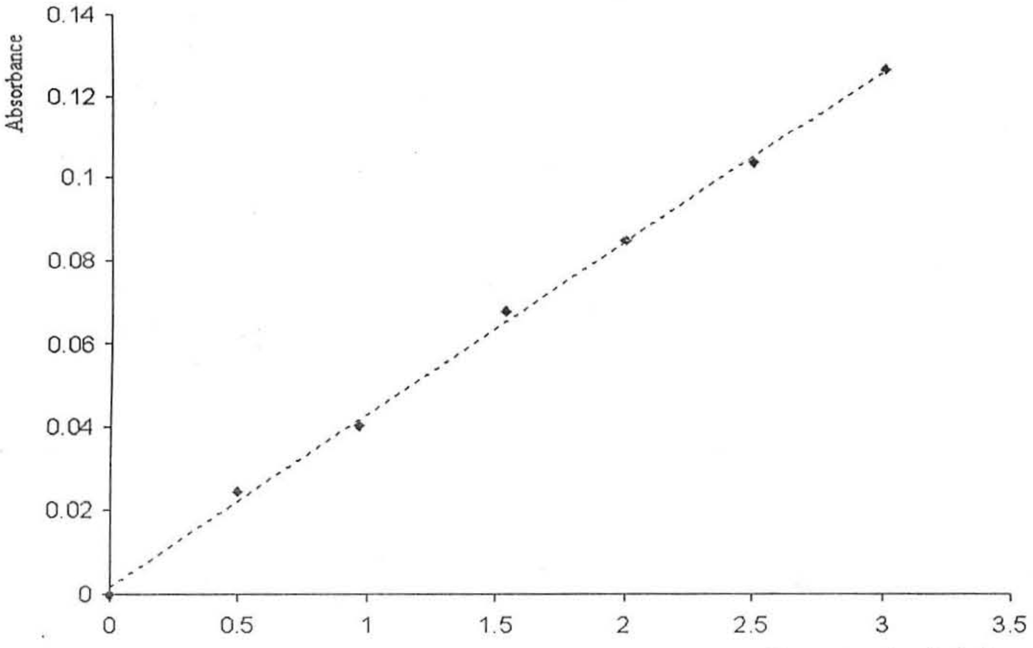

Figure 14: Calibration curve for intera-day calcium (II) standard solution in the determination of maxamine forte tablets; [Y= 0.0415X +0.0016, $r^2 = 0.9972$, n=6].

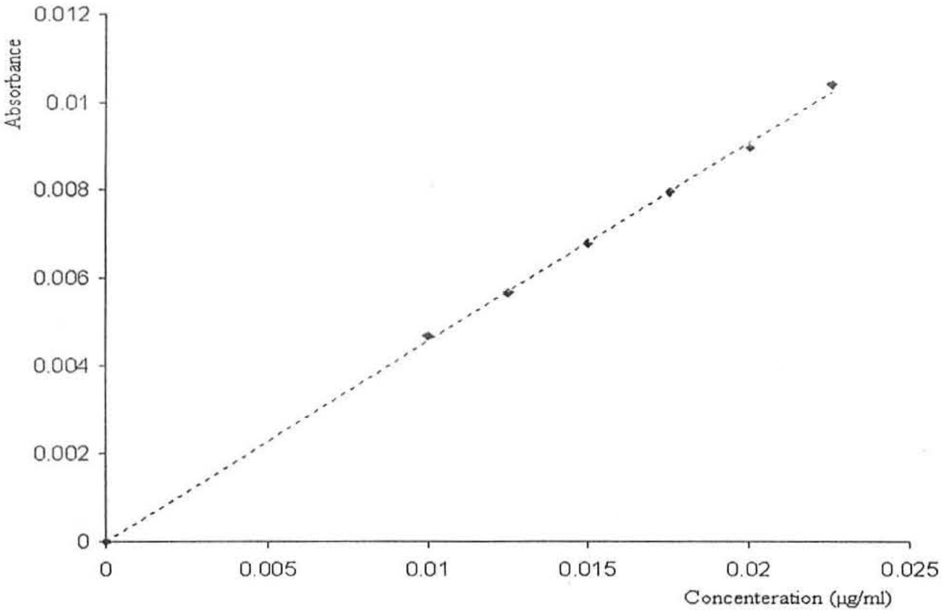

Figure 15: Calibration curve for intera-day magnesium (II) standard solution in the determination of maxamine forte tablets; [Y= 0.04593X -0.0001 ,r^2 = 0.9955, n=6].

Tablet 14: Analytical parameters for the intra day (n =3) determination of calcium (II) and magnesium (II) standard solutions by FAAS technique

Standard Solution of	Conc. (µg/ml)	λ (nm)	Linear regression equation parameters a ± SD	 b ± SD	 r	 r^2	LOD (µg/mL)	LOQ (µg/mL)
Mg (II)	0.001- 0.0225	285.2	-0.0001 ± 0.0002	0.04593 ± 0.0135	0.9977	0.9955	0.00124	0.00414
Ca (II)	0.01- 3.00	422.7	0.0016 ± 0.0025	0.0415 ± 0.00129	0.9986	0.9972	0.08947	1.1156

Table 15: Actual and predicted amount in an intera-day (n =3) determination of calcium (II) and magnesium (II) in the maxamine forte tablets sample solution by FAAS.

Metal ions	Real conc. (μg/ml)	Found (μg/ml) Mean ± SD	RSE	Recovery (%)	RSD (%)
	2.000	1.9713 ± 0.0288	1.450	98.57	1.4610
Ca (II)	2.500	2.4772 ± 0.0314	0.912	99.09	1.2688
	3.000	2.9694 ± 0.0306	1.020	98.98	1.0315
	0.1750	0.01739± 0.00012	0.629	99.38	0.6837
Mg (II)	0.0200	0.01992± .000101	0.400	99.61	0.5105
	0.0225	0.02243± 0.00007	0.311	99.70	0.3134

In an intra-day precision, the determinations of Ca (II) and Mg (II) in maxamine forte tablets, the analyses were performed using concentrations at three levels, 2, 2.5, and 3 μg/mL of calcium (II) and 0.0175, 0.020 and 0.0225 μg/mL of magnesium (II). Each concentration was analysed in triplicate (*n* = 3) and intra-day precision was found to be less than 2%RSD and good recoveries of greater than 98.57 % for all samples of Ca (II) and Mg (II) presented in Table 16, in all days indicates the accuracy of analytical method. The analyses results of the actual concentrations at different times with in the same day were very close to the real concentrations of Mg (II) and Ca (II) in maxamine forte tablets indicating the reliability of the analytical method. Thus, the Ca (II) and Mg (II) values were found to be typical and comparable to the different times of analyses determined by a FAAS. This comparison verifies the accuracy of the FAAS method.

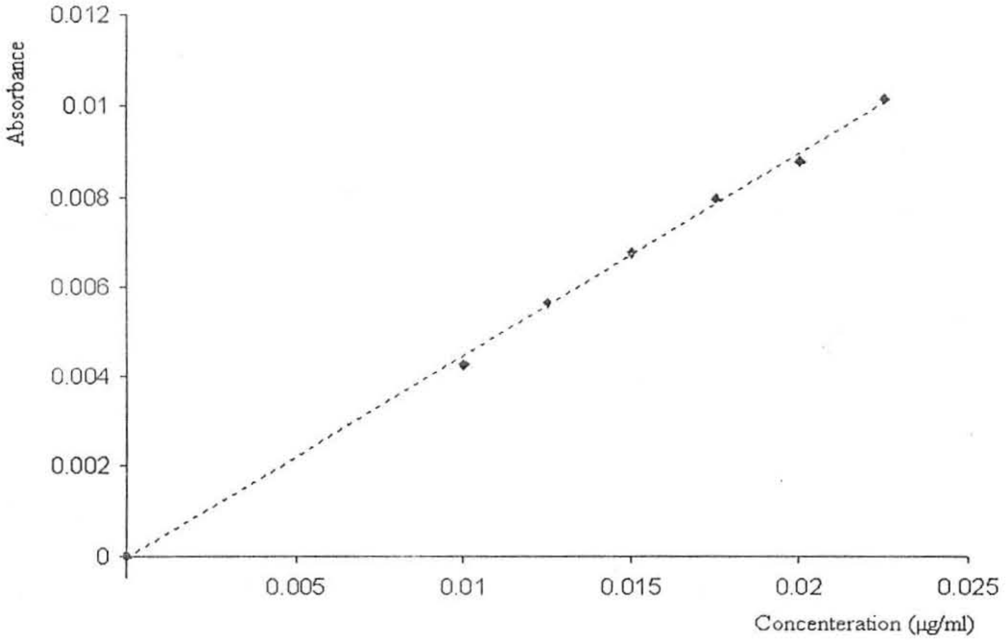

Figure 16: Day-1, Calibration curve for inter-day magnesium (II) standard solution in the determination of maxamine forte tablets; [Y= 0.04593X -0.0001, $r^2 = 0.9955$, n=6].

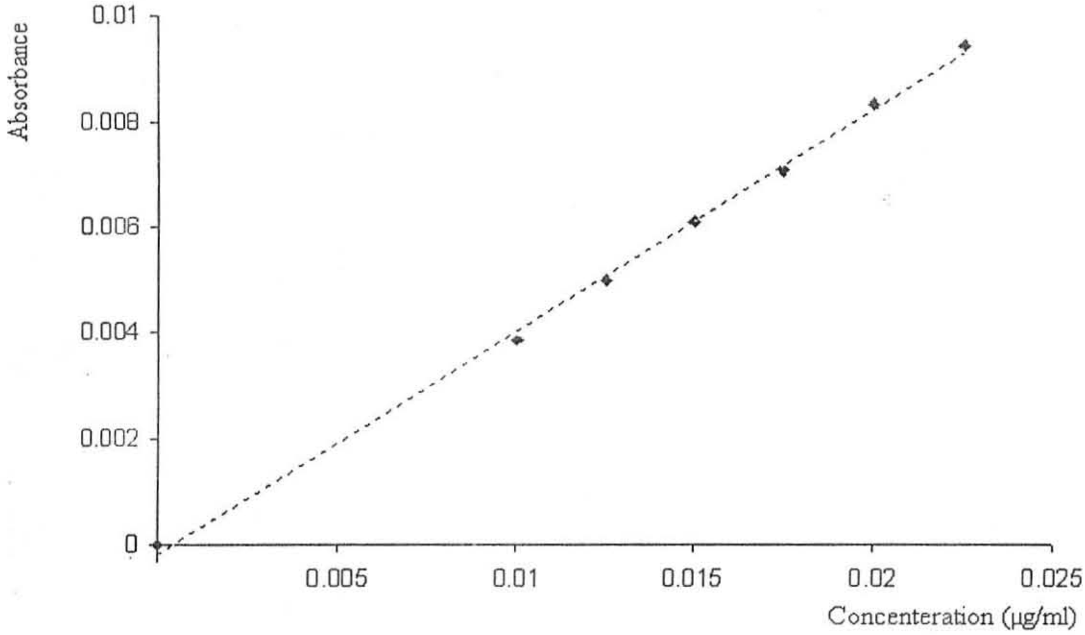

Figure 17: Day-2, Calibration curve for inter-day magnesium (II) standard solution in the determination of maxamine forte tablets; [Y= 0.04313X -0.0003, $r^2 = 0.9938$, n=6].

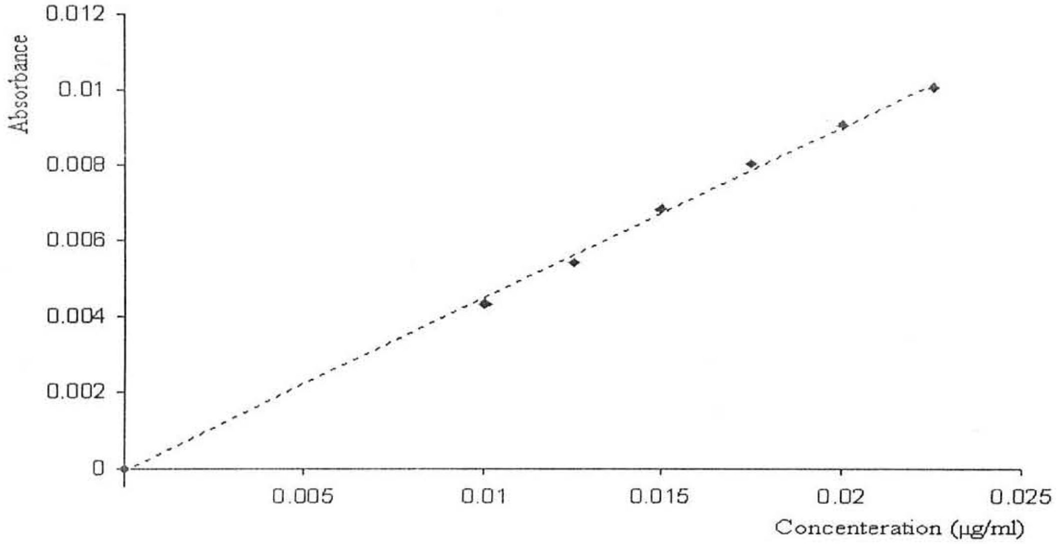

Figure 18: Day-3, Calibration curve for inter-day magnesium (II) standard solution in the determination of maxamine forte tablet; [Y= 0.04604X -0.002, r^2 = 0.9981, n=6].

Table 16: Analytical parameters for the inter day (n = 3) determination of magnesium (II) of the standard solution by FAAS technique.

Standard Solution of Mg (II)			Linear regression equation parameters					
	Conc. (µg/ml)	λ (nm)	a ± SD	b± SD	r	r^2	LOD (µg/mL)	LOQ (µg/mL)
Day-1	0.001- 0.0225	285.2	-0.0001 ± 0.00019	0.04593 ± 0.013519	0.9977	0.9955	0.001241	0.004141
Day-2	0.001-0.0225	285.2	-0.0003 ± 0.00029	0.04313 ± 0.01715	0.9969	0.9938	0.00203	0.00668
Day-3	0.001- 0.0225	285.2	-0.002 ± 0.00045	0.04604 ± 0.02691	0.9990	0.9981	0.002945	0.00982

Table 17: Actual and predicted amount in an inter-day (n =3) determination of magnesium (II) in the maxamine forte tablets sample solution by FAAS technique.

Metal ions	Real conc. (μg/ml)	Found (μg/mL) Mean ± SD	RSE	(%) Recovery	RSD (%)
Mg (II) Day-1	0.01750	0.0173983± .000201	0.581	99.47	0.95949
	0.0200	0.019637± .0003800	1.815	98.19	1.9351
	0.0225	0.022455± .0000490	0.200	99.80	0.2187
Mg (II) Day-2	0.0175	0.0173894± .000111	0.632	99.37	0.6395
	0.0200	0.0197433± .000378	1.284	98.72	1.91557
	0.0225	0.0223987± .000113	0.450	99.55	0.5049
Mg (II) Day-3	0.0175	0.0172978± 0.000213	1.155	98.84	1.2285
	0.020	0.019688± 0.000313	1.560	98.44	1.5878
	0.0225	0.022429± 0.0001022	0.314	99.73	0.4555

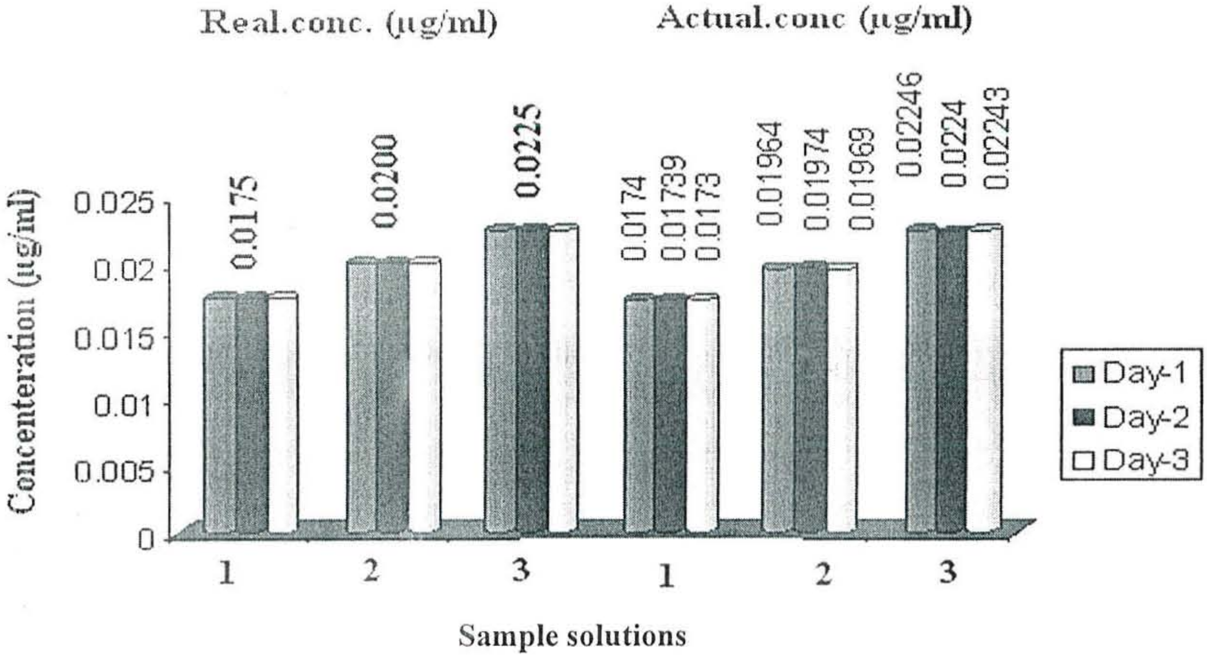

Figure 19: Comparisons between the real and the actual concentrations of standard Mg (II) solution of an inter-day determination in maxamine forte tablets.

An inter-day precision determination of Mg (II) in maxamine forte tablets unlike that of intra-day determination of Mg (II) conducted on three separate days and the %RSD found to be less than 2% presented in Table 18 in the low, middle and high concentrations. Good recovery in an inter-day determination of greater than 98% and lower values of RSD% indicates the higher precision of the analytical method. In the comparison between inter-day determinations of the actual with the claimed, the actual with the actual concentrations in the separate days of Mg (II) Figure 22 in maxamine forte tablets shows the reproducibility that ascertain the precision of the analytical method. An inter-day determination was undertaken to assess precision of the analytical method in and, to confirm the analyses results with the concentration stated by the manufacturer.

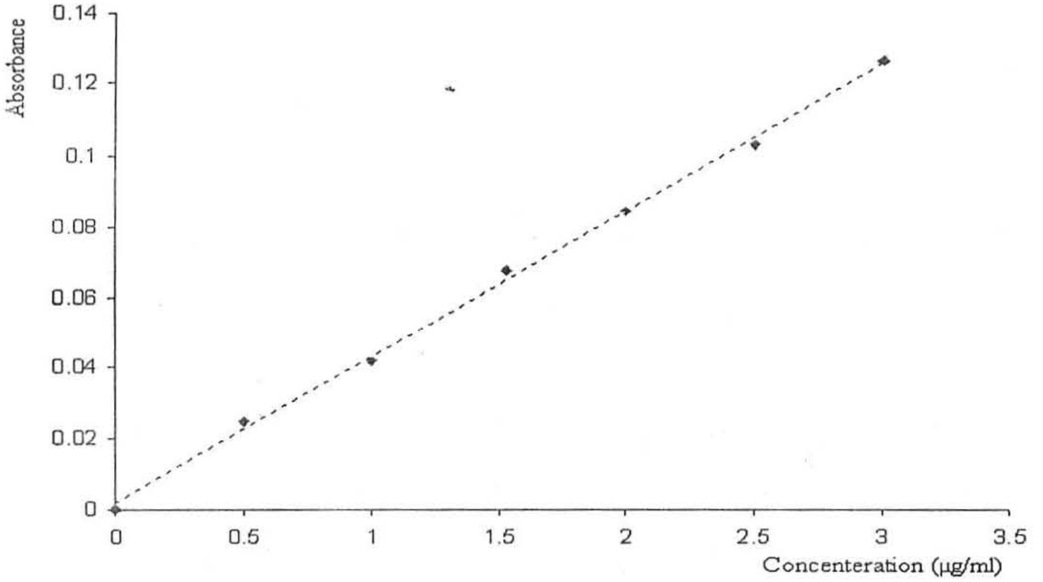

Figure 20: Day-1, Calibration curve for inter-day calcium (II) standard solution in the determination of maxamine forte table; [$Y= 0.0415X + 0.0016$, $r^2 = 0.9972$, $n=6$].

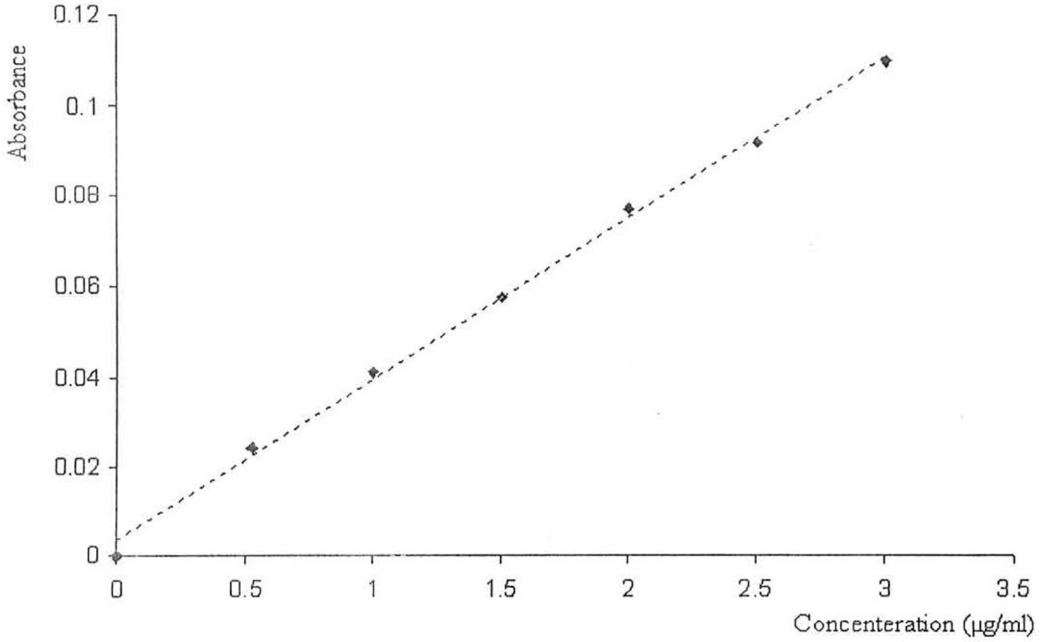

Figure 21: Day-2, Calibration curve for inter-day calcium (II) standard solution in the determination of maxamine forte table; [Y= 0.0356X + 0.004, $r^2 = 0.9961$, n=6].

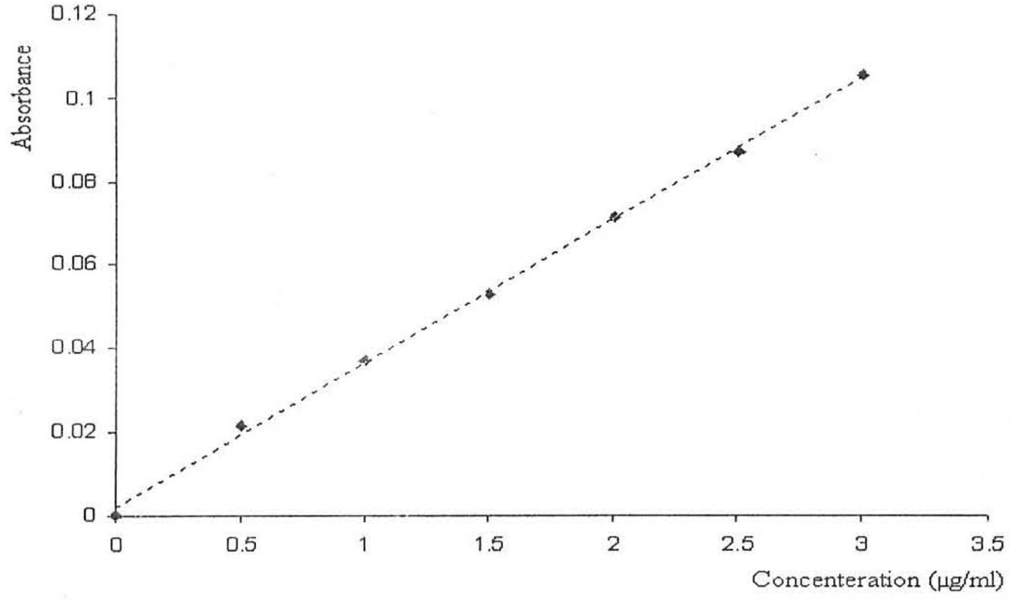

Figure 22: Day-3, Calibration curve for inter-day calcium (II) standard solution in the determination of maxamine forte table; [Y= 0.0346X + 0.0018, $r^2 = 0.9988$, n=6].

Table 18: Analytical parameters of an inter day (n=3) determination of calcium (II) standard solution by FAAS.

Standard Solution of Ca (II)	Conc. (µg/ml)	λ (nm)	Linear regression equation parameters a ± SD	b ± SD	r	r^2	LOD (µg/ml)	LOQ (µg/ml)
Day-1	0.01- 3.00	422.7	0.0016 ± 0.00251	0.04115 ± 0.00129	0.9986	0.9972	0.08145	0.30482
Day-2	0.01- 3.00	422.7	0.004 ± 0.00099	0.0356 ± 0.00129	0.9980	0.9961	0.0837	0.27893
Day-3	0.01- 3.00	422.7	0.0018 ± 0.00095	0.0346 ± 0.00049	0.9994	0.9988	0.08272	0.27572

Table 19: Actual and predicted amount in an inter-day (n= 3) determination of calcium (II) in the maxamine forte tablets sample solution by FAAS technique.

Metal ions	Real.conc. (µg/ml)	Found (µg/ml) Mean ± SD	RSE	(%) Recovery	RSD (%)
Ca (II) Day-1	2.00	1.9809 ± 0.0201	0.955	99.05	1.0161
	2.50	2.4772 ± 0.0231	0.912	99.09	0.9325
	3.00	2.9678 ± 0.0322	1.073	98.98	1.0846
Ca (II) Day-2	2.00	1.9655 ± 0.0345	1.725	98.28	1.7542
	2.50	2.4940 ± 0.0162	0.24	99.73	0.6504
	3.00	2.9701 ± 0.0307	0.997	99.00	1.0336
Ca (II) Day-3	2.00	1.9751 ± 0.0250	1.245	98.77	1.3523
	2.50	2.4736 ± 0.0267	1.056	98.95	1.0798
	3.00	2.9498 ± 0.0511	1.673	99.00	1.0316

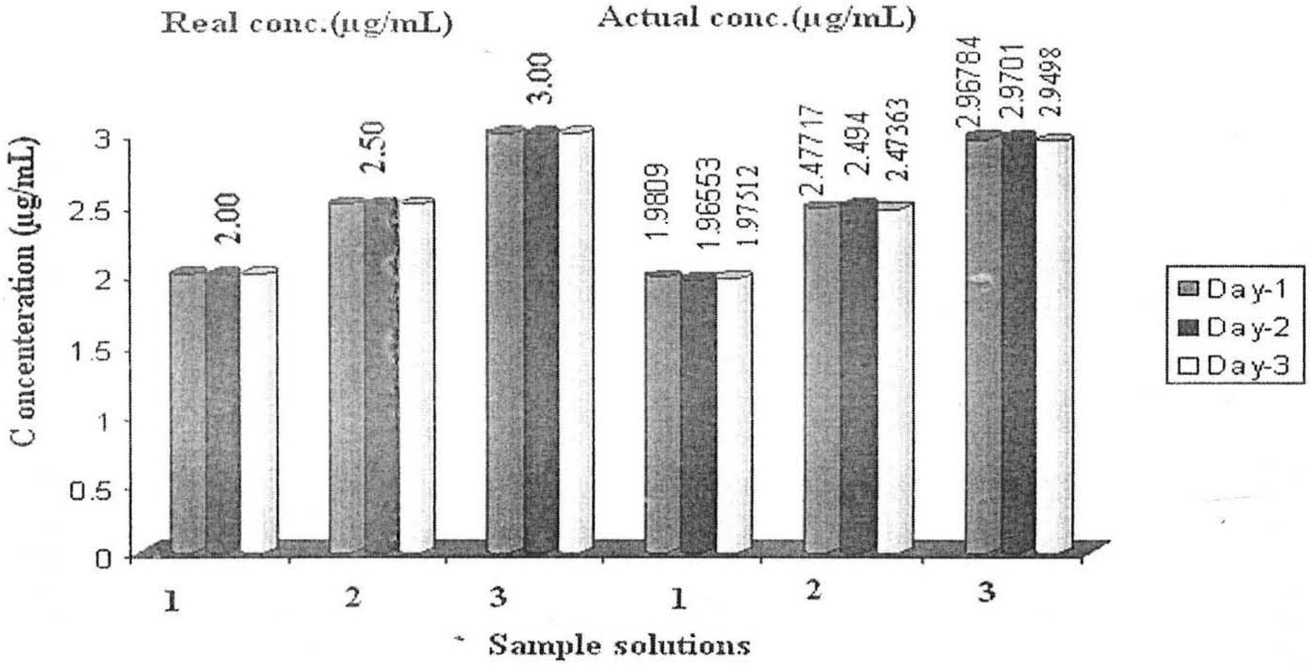

Figure 23: An inter-day comparisons between the real and actual concentrations of Ca (II) determination in maxamine forte tablets.

In an inter-day precision determination of Ca (II) in maxamine forte tablets were carried out using concentrations at three levels, 2, 2.5, and 3 μg/mL of calcium (II). The lower variability of the analyses results presented in Table 20 obtained in all three separate day measurements under the same conditions shows greater repeatability. The lower values of LOD obtained in the calibration curve of standard solution of Ca (II) presented in Table 19 shows the capabilities of detecting of the analytical technique at the lower concentration. Good recovery in an inter-day determination and lower values of RSD% (< 2%) indicates the high accuracy of the analytical method. In the comparison between inter-day determinations of the actual with the claimed, the actual with the actual concentrations in the separate days Ca (II) Table 20 in maxamine forte tablets shows reproducible results that ascertain the precision of the analytical method. Thus, this method shows comparable accuracy and repeatability, but different detection limits. The results obtained by FAAS technique are presented as arithmetic means ± standard deviations of trireplicate analysis. Reproducibility for an inter-day determination was evaluated and the relative standard deviations (RSD) based on the proposed method found to be between 0.6504% and 1.7542%.

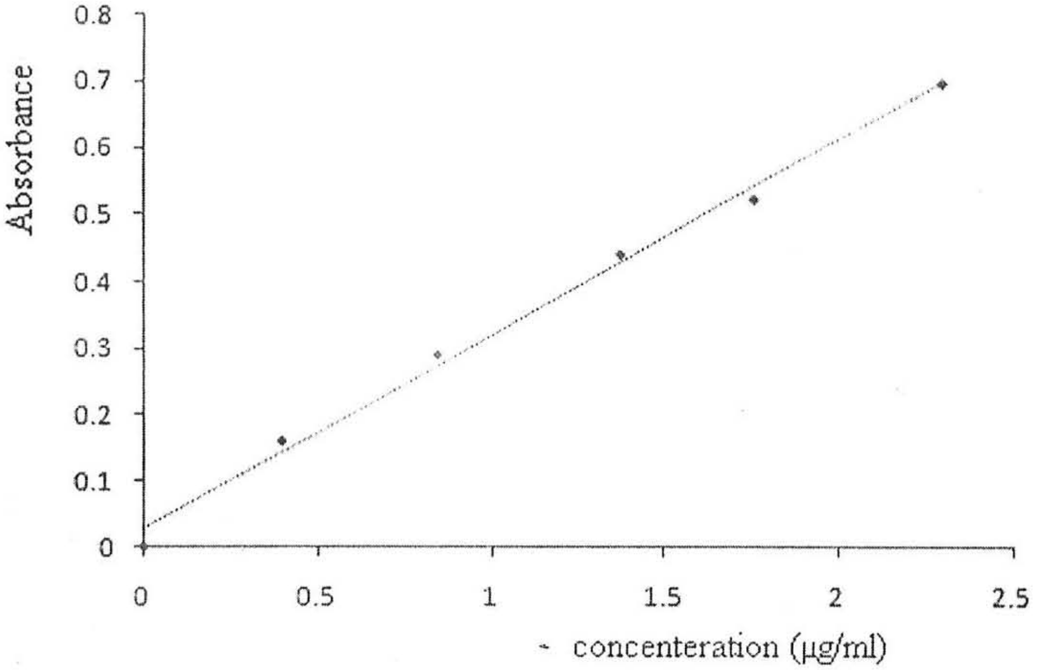

Figure 24. Calibration curve determination of standard chromium (III and VI) solution by the reported (UV-Visible spectrophotometric) method; [$y = 0.292x + 0.027$; $R^2 = 0.996$].

Table 20: Analytical parameters in the determination of total Chromium (III and VI) standard solution by UV-Visible spectrophotometer technique.

Standard Solution of	Conc. (µg/ml)	λ (nm)	Linear regression equation parameters				LOD (µg/mL)	LOQ (µg/mL)
			a ± SD	b ± SD	r	r^2		
Cr (III and VI)	0.01-3.00	357.9	0.027 ± 0.0117	0.292 ± 0.0066	0.998	0.996	0.120	0.400

Table 21: Actual and predicted amount determination of chromium (III and VI) in the viminova tablets sample solution by UV-Visible spectrophotometer technique.

Real conc. (μg/ml)	Mean ± SD (μg/ml)	% RSD	% Recovery	% RSE
1.5	1.37 ± 0.0259	1.89	91.3	8.50
2.0	1.806 ± 0.0550	3.05	90	9.70
2.5	2.33 ± 0.0785	3.50	89.2	10.30

The rapidity of brick red color development and sensitivity in reaction between Cr (VI) and the complexing agent 1, 5-diphenylcarbazaide is an advantage in analyzing of chromium (III and VI) but involves interference in many metal ions as Fe (III) and V (V). The spectrophotometric determinations were carried out with different concentrations of total chromium (III and VI) under optimum conditions and the coefficient of determination, LOD, and LOQ are highly significant.

The method offers less precision for the quantitative determination of metal ion of RSD of less than 4 %. Recovery studies were carried and found to be between (89-91.3) % that ascertains the less accuracy of the method. Herewith, the average amounts of metal ions claimed by the manufacturer of sample solution of viminova + tablet was not close to the values of (Mean ± SD) obtained by the spectrophotometric technique. The results obtained by the spectrophotometric method are less precise, accurate and imperfectly agreed with those obtained by direct flame atomic-absorption spectrometry (FAAS).

5. CONCLUSION

In this study, metal ions of Ca (II), Mg (II), K (I) and total Cr (III and VI) in six commercial brands of multivitamins with multimineral pharmaceutical dosage forms marketed in Ethiopia were determined by a FAAS technique for the quantitative determination. Sample digestion technique has been utilized for multi-element analysis of Ca, Mg, K and total Cr in multivitamins with multimineral preparations for FAAS.

Results obtained in the routine Kjeldahl digestion for FAAS analysis of multivitamin preparation show possible work simplification in preparation step. Method based on digestion using acids can result complete dissolution of the sample, evaporation of the more volatile compounds and avoids contamination problem.

The analysis results of correlation coefficients, LOD, LOQ and determination coefficients are highly significant. The results obtained revealed that FAAS method offers good precision for the quantitative determination of metal ions in an inter-day and intra- day RSD of less than 2%.

Recovery studies were carried and found to be between (98-101.07) % that ascertains the accuracy and precision of the method. Herewith, the average amounts of metal ions claimed by the manufacturers in each brands of multi-vitamins with multi-minerals were very close to the values of (Mean ± SD) obtained by the FAAS technique.

This method is simple as there is no need for solvent extraction, rapid as it only requires measurements of absorbance values at a single wavelength and reagent saving . FAAS estimates metal ion directly and independently of the other and selective due to the narrow width of absorption lines.

Thus, FAAS is suitable and used independently for the determination of Ca (II), Mg (II), K (I) and total Cr (III and VI) in the presence of each other and without prior separation of the excipients compared to the other reported spectrophotometric method.

This method has been successfully applied to the determination of metal ions in the dosage forms and opens up the possibility of applying the same technique to other metal ions in a variety of other aqueous solutions.

The clossness of the analytical results (precision) and the accuracy in all sample solutions were found to be less than 2% RSD and greater than 98% of recoveries which ascertain the repeatability and the reproducibility of FAAS technique. The lower cost per sample analysis and the faster time required to complete an analysis indicates the ease and suitability of analytical FAAS technique in the quantitative determination of metal ions in multi-vitamins with multi-mineral pharmaceutical formulations.

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DECLARATION

I, the undersigned, declare that this thesis is my original work and has not been presented for a degree in any other university.

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