Sudan University of Science and Technology
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Optimization and validation of an analytical method for the determination of macro and micro elements in multivitamin/multimineral formulations

التحسين والتحقق من مصداقية طريقة تحليلية لتقدير العناصر الماكرو والميكرو في مستحضرات متعددة الفيتامينات/متعددة المعادن

A thesis submitted in fulfillment for the degree of Doctor of Philosophy in Chemistry

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ (1) خَلَقَ الْإِنْسَانَ مِنْ عَلَقٍ (2) اقْرَأْ وَرَبُّكَ الْأَكْرَمُ

(3) الَّذِي عَلَّمَ بِالْقَلَمِ (4) عَلَّمَ الْإِنْسَانَ مَا لَمْ يَعْلَمْ (5)

صدق الله العظيم

Dedication

To my parents, for their support and kind gesture displayed in diverse ways to the success of this work, and to family for their patience, love and care.

List of publications

Albadri, A., Mohamed El Mukhtar, A. and Ahmed, H.E. (2019), "Optimized Microwave Digestion method for some micro and macro elements in pregnant women and diabetics multivitamin/multielement preparations", *Journal of Chemical and Pharmaceutical Research*, Vol. 11 No. 12, pp. 6–17.

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Abstract

The goal of the study was to optimize microwave decomposition conditions (Acid mixture, sample weight, radiation power, radiation period) to determine several minerals (Fe, Mg, Zn, Cu Mn, Cr and Se) in multivitamin/multimineral formulations. Optimum conditions were found to be 0.1 g of sample powder, acid mixture 5 ml of nitric acid, 0.5 ml of hydrochloric acid and 1 ml of hydrogen peroxide H_2O_2 and microwave conditions. A pretreatment, using a three-steps heating program: starting irradiation from 10 minutes at 500W, for 16 minutes at 600W and then cooling at room temperature. A reagent of the oxidizing acids mixture was suitable for determining the seven minerals studied without subsequent manipulation of the digestion product. The accuracy of the procedure was verified, and recoveries of iron, zinc, manganese, magnesium, copper, chromium and selenium were found to be in the percentage range 98.59 ± 0.36-100.91 ± 0.61, 99.05 ± 0.46-101.19 ± 0.56, 97.30 ± 0.1-100.57 ± 0.09, 97.50 ± 5.63-99.10 ± 3.62, 98.93 ± 0.05-100.12 ± 0.057, 99.136 ± 0.01-100.13 ± 0.02, and 98.54 ± 0.07-99.85 ± 0.03, respectively. The optimized method was validated and applied to the determination of iron, zinc, manganese, magnesium, copper, chromium and selenium in eight available multivitamin/multimineral capsules obtained from the local pharmacies.

The developed microwave digestion procedure was used to determine thirteen macro and micro elements Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn in the multivitamin/multimineral formulations, employing ICP-MS, and optimized by factorial design 3^3, The microwave digestion conditions, temperature, acid mixture volume and radiation period were selected as factors. Moreover, a multiple response was built to establish a compromise condition between the elements. The optimum conditions were found to be 160ºC, 6.5ml, 20min for temperature, acid mixture volume and radiation period, respectively. The procedure was validated using standard reference materials (SRM3280) and applied to determine of calcium, cobalt, chromium, copper, iron, potassium, magnesium, manganese, sodium, nickle, selenium, vanadium and zinc in six commercial multivitamin/multimineral samples obtained from the local pharmacies. Recoveries were found to be in the range 88 – 107%.

Moreover, ninety-three samples of widely used multivitamin/multimineral for pregnant and diabetic formulations available in the Sudan were analyzed by inductively

coupled plasma mass spectrophotometry (ICP-MS), to estimate values of thirteen macro and micro elements (Calcium, Cobalt, Chromium, Copper, Iron, Potassium, Magnesium, Manganese, Sodium, Nickle, Selenium, Vanadium and Zinc) and the concentration levels were compared with those labelled. Concentration levels were acceptable for Ca, Co, Cu, Fe, K, Mg, Na, Ni, and V, but six products contained mineral levels much higher than those labelled, for Mn, Zn, Cr and Se. Although many elements were not labelled in the leaflet of the multivitamin/multimineral products, some were experimentally determined. Those elements could be introduced in the composition of the product, during formulation processing from excipients and water, such as K (from 0.657 mg/caps. to 0.024 mg/caps.), and Ca (from 2.083 mg/caps. to 6.686 mg/caps.) in addition to their salts as iodide, selenite and molybdate.

المستخلص

الهدف من الدراسة تحسين ظروف ميكروويف الهضم (نوع خليط الأحماض، وزن العينة، زمن تعرض العينة لأشعة الميكروويف، طاقة اشعة الميكروويف)، لاستخلاص بعض المعادن (الحديد والماغنسيوم والخارصين والنحاس والمنجنيز) في مستحضرات متعددة الفيتامينات/ متعددة المعادن. تم التوصل الي الظروف المثلى لهضم هذه العناصر بواسطة جهاز الميكروويف وهي 0.1 غرام من العينة واستخلصها بواسطة 5مل من حمض النيتريك و0.5 مل من حمض الهيدروكلوريك، 1 مل من بيروكسيد الهيدروجين. وتخضع لبرنامج تسخين بالميكروويف من ثلاث خطوات: يبدأ بتعرض العينة لأشعة بطاقة 500 واط لمدة 10 دقائق؛ ثم تبدأ بالارتفاع الي 600واط وذلك لمدة 16 دقيقة؛ ثم التبريد الي درجة حرارة الغرفة. وكان كاشف خليط الأحماض المؤكسدة مناسب لتحديد المعادن السبعة التي تمت دراستها دون الحاجة لمعالجة لاحقة لمنتج الهضم. تم التحقق من صحة الطريقة، حيث تم استعادة الحديد والخارصين والمنجنيز والماغنسيوم والنحاس والكروم والسلينيوم من العينات بنسبة مئوية في المدي ما بين 98.59 ± 0.36 - 100.91 ±0.61 ، و99.05 ± 0.46 - 101.19± 0.56، و 97.30 ± 0.1 - 100.57 ±0.09، و 97.50 ± 5.63 - 99.10 ± 3.62، و 98,93 ± 0.05 - 100.12 ± 0.057، و 99.136 ± 0.01 - 100.13 ± 0.02، و98.54 ± 0.07 - 99.85 ± 0.03، على التوالي، تم أيضاً التحقق من مصداقية الطريقة وتطبيقها لتقدير كل من الحديد والماغنسيوم والخارصين والنحاس والمنجنيز والكروم والسلينيوم في ثماني عينات من متعددة الفيتامين/ متعددة المعادن المتوفرة في الصيدليات المحلية.

تم تطوير طريقة الهضم بواسطة الميكروويف لتقدير ثلاثة عشر ماكرو وميكرو عنصر (الكالسيوم والكوبلت والكروم والنحاس والحديد والبوتاسيوم والماغنسيوم والمنجنيز والصوديوم والنيكل والسلينيوم والفناديوم والخارصين) في متعددة الفيتامين/متعددة المعادن، وذلك باستخدام جهاز البلازما المقترن بمطياف الكتلة، وتم التحسين باستخدام منظومة التحليل الإحصائي الكاملة 3^3، العوامل المؤثرة في الهضم بالميكروويف التي تم اختيارها هي درجة حرارة وحجم خليط الحمض وفترة الإشعاع. وعلاوة على ذلك، تم بناء استجابة متعددة لوضع شرط توفيقي بين العناصر، وجد أن الظروف المثالية هي 160 درجة مئوية، 6.5 مل، 20 دقيقة لدرجة الحرارة، وحجم خليط الحمض

وفترة الإشعاع على التوالي. تم التحقق من مصداقية طريقة التحليل باستخدام المادة المرجعية القياسية (SRM3280) كما تم تطبيقها لتقدير الكالسيوم والكوبلت والكروم والنحاس والحديد والبوتاسيوم والماغنسيوم والمنجنيز والصوديوم والنيكل والسلينيوم والفناديوم والخارصين في سته عينات من متعددة الفيتامين/متعددة المعادن، التي تم الحصول عليها من الصيدليات المحلية. ووجد أن النسبة المئوية لاسترجاع جميع العناصر في المدي من 88 - 107٪.

بالإضافة الي ذلك، تم تحليل ثلاثة وتسعون عينة من المكملات الغذائية متعددة الفيتامين/متعددة المعادن والتي تستخدم على نطاق واسع للنساء الحوامل ومرضي السكري والمتوفرة في السودان. وذلك باستخدام جهاز البلازما المقترنة بمطياف الكتلة، من أجل تقييم ثلاثة عشر عنصرًا (الكالسيوم والكوبلت والكروم والنحاس والحديد والبوتاسيوم والماغنسيوم والمنجنيز والصوديوم والنيكل والسلينيوم والفناديوم والخارصين) ومقارنة التركيزات مع محتوي التركيزات على عبوة المكملات الغذائية متعددة الفيتامين/متعددة المعادن. كانت مستويات التركيز مقبولة لكل من الكالسيوم والكوبلت والنحاس والحديد والبوتاسيوم والماغنسيوم والصوديوم والنيكل والفناديوم، ولكن في عدد ستة من المنتجات التي تم تحليلها وجدت تحتوي على عناصر بمستويات أعلي بكثير من الملصق على العبوة وذلك للمنغنيز والخارصين والكروم والسلينيوم. بالرغم من أن كثير من العناصر لم تذكر في اللاصقة على العبوة تم تقديرها عملياً، كما يتوقع دخولها في التركيبة عن طريق تصنيع المنتج او عمليات المعالجة المختلفة أثناء التصنيع أو المواد الفعالة او الماء، مثل البوتاسيوم (من 0,675 ملجم/ كبسولة الي 0,024 ملجم/ كبسولة) والكالسيوم (من 2,083 ملجم/ كبسولة الي 6,686 ملجم/ كبسولة) بالإضافة الي املاحها مع اليود والسيلينايت والملبدات.

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List of abbreviations

Abbreviation	Term	Page no.
AAS	Atomic absorption spectrometer	25
AEs	Adverse effects	22
ANOVA	analysis of variance	41
API	Active pharmaceutical ingredient	55
BBD	Box behnken designs	41
CCD	Central composite design	41
CCD	Charge coupled device	47
CPS	Counted per second	54
CS	Control sample	80
CVD	Cardiovascular disease	2
DM	Diabetes mellitus	19
DM	Doehlert matrix	41
DOE	Design of experiments	41
DRC	Dynamic reaction cell	52
EAR	Estimated average requirement	23
FAAS	Flame atomic absorption spectrometer	28
FES	Flame emission spectrometer	25
FDA	Food and drug administration	22
FSANZ	Food standards Australia and New Zealand	22
GFAAS	Graphite furnace atomic absorption spectrometer	48
GLUT4	Glucose transporter 4	8
GMP	Good manufacturing practice	62
HCL	Hallow cathode lamp	46
HOMA-IR	Homeostatic model assessment for insulin resistance	17
HPMC	Hydroxypropylmethylcellulose	67
ICH	International conference on harmonization	97

List of abbreviations (cont.)

Abbreviation	Term	Page no.
ICP-AES	Inductively coupled plasma atomic emission spectrometry	55
ICP-MS	Inductively coupled plasma mass spectrometry	26
ICP-OES	Inductively coupled plasma optical emission spectrometry	25
IUGR	Intrauterine growth restriction	18
LDL	Low density lipoprotein	6
m/z	Mass to charge ratio	49
MR	Multiple response function	42
MVM	Multivitamin/multimineral	2
ng/l	Nano-gram / liter	15
NHMRC	National health and medical research council	17
NIH	National institutes of health	2
NIST	National institutes of standards and technology	31
PFA	Perfluoroalkoxy alkanes	30
ppb	Parts per billion	48
PTFE	Polytetrafluoroethylene	32
Ptt	Parts per trillion	48
PUFAs	Polyunsaturated fatty acids	6
RCC	Residual carbon content	31
RDA	Recommended dietary allowance	2
RDI	Recommended daily intake	22
RF	Radio frequency	51
RSM	Response surface methodologies	41
SOD	Superoxide dismutase	10
T2D	Type 2 diabetes	7
UL	Upper intake level	2
UV	Ultraviolet visible	46
µg/l	Microgram/liter	48

CHAPTER ONE

Introduction and literature review

CHAPTER ONE

1.1 INTRODUCTION AND LITERATURE REVIEW

1.1.1 Introduction

An increasing number of individuals take multivitamin/multimineral supplements (MVM) to maintain good health and to be protected from different diseases (e.g., cardiovascular disease [CVD], cancer and cognitive decline). The US Department of Agriculture/Department of Health and Human Services 2010 Dietary Guidelines for Americans acknowledge that "supplements containing combinations of certain nutrients may be beneficial in reducing the risks of some chronic diseases when used by special populations," yet also state that excessive use of certain supplements has the potential to be harmful (National Academies of Sciences and Medicine, 2017). This may in particular be the case for single supplements in concentrations exceeding the normal recommended dietary allowance (RDA), as recently documented (Sudfeld *et al*., 2014), or those with an imbalanced composition. This raises the question whether an adequately (within 100% of RDA) composed MVM is safe (Biesalski and Tinz, 2017).

1.1.2 Definition of MVM supplements

According to a National Institutes of Health (NIH) State of the Science Panel, an "MVM refers to any supplement containing 3 or more vitamins and minerals but no herbs, hormones, or drugs, with each component at a dose less than the tolerable upper level determined by the Food and Nutrition Board as the maximum daily intake likely to pose no risk for adverse health effects" (Moyer, 2014). In an NIH fact sheet regarding MVM, a more differentiated approach defines groups of MVM as follows (Biesalski and Tinz, 2017):

1- Many MVM are taken once daily and contain all or most recognized vitamins and minerals at levels close to daily values, RDAs, or adequate intakes. Basic formulations are for broad-spectrum use. Formulations for special populations such as children, pregnant women and seniors, provide the same vitamins and minerals in amounts tailored to those populations' specific needs.

2- Other MVMs contain vitamins and minerals at levels substantially higher than the recommended values and may also include other nutritional and herbal ingredients. These are sometimes packaged in multiple-pill packs to be taken each day.
3- Specialized MVMs, used, for example, to enhance performance or improve immune function or for weight control, are often composed of vitamins and minerals in combination with herbal or specialty ingredients such as coenzyme Q10, probiotics, and glucosamine. These may also include nutrients at levels substantially above recommended levels.

Using the NIH State of the Science Panel and the Agency for Healthcare Research and Quality definition of a multivitamin as containing three or more vitamins and/or minerals (Biesalski and Tinz, 2017), the supplements listed in the second and third groups above are indeed MVM. However, to exclude specific formulations or doses of vitamins or minerals near the tolerable upper intake level (UL), we decided to focus our research only on MVM as defined in the first groups. This is in accordance with the definitions of MVM per the National Health and Nutrition Examination Survey (NHANES) III (Biesalski and Tinz, 2017; Radimer *et al.*, 2004) and the National Health Interview Survey (Moss *et al.*, 1989).

1.1.3 Vitamins

Vitamins and essential minerals are involved in numerous metabolic processes (develop and function normally). Vitamins are organic compounds which the human organism cannot or only insufficiently produce, apart from a few exceptions. The known vitamins include A, C, D, E, K and the B vitamins: thiamin (B_1), riboflavin (B_2), niacin (B_3), pantothenic acid (B_5), pyridoxal (B_6), cobalamin (B_{12}), biotin and folate/folic acid. They play, a major part in the electrolyte and water metabolism, and are indispensable for the immune system as well as for the development and function of bones, muscles and teeth. They are needed for the visual process and the nervous system and are also involved in blood coagulation and processes of reproduction, cell division and differentiation ((NCCIH), 2018, Santos Júnior *et al*., 2017). Vitamins are classified according to their solubility, Water-Soluble Vitamins (Vitamin B_1, Vitamin B_2, Niacin, Vitamin B_6, Folate, Panthotenic acid, Biotin, Vitamin B_{12} and Vitamin C), Fat-Soluble Vitamins (Vitamin A, Beta carotene (provitamin A), Vitamin D, Vitamin E and Vitamin K) ((BfR), 2005).

1.1.3.1 Vitamin A

Widely known for its importance to good vision, vitamin A also supports the immune system and is necessary for a healthy pregnancy (Organization, 2004).Vitamin A (retinol) is an essential nutrient needed in small amounts by humans for the normal functioning of the growth development; maintenance of epithelial cellular integrity and reproduction. These dietary needs for vitamin A are normally provided for as preformed retinol (mainly as retinyl ester) and provitamin A carotenoid (Organization, 2004). Vitamin A deficiency is rare in the United States; it is largely a problem of developing countries((NCCIH), 2019).

1.1.3.2 Vitamin B

The B complex family of vitamins is made up of eight B vitamins, each of which performs a different important function throughout the body (Table 1.1) Vitamin B_9 (folic acid) can help prevent birth defects of the brain and spine, known as neural tube defects. It is recommended that all women who are capable of becoming pregnant consume 400 µg per day of folic acid from fortified foods or from dietary supplements. Vitamin B_{12} is important for nerve function and development. A deficiency can cause symptoms such as numbness, weakness, difficulty walking, yellowed skin and memory loss. The elderly, vegetarians and people who have undergone weight loss surgery are at risk of developing a vitamin B_{12} deficiency (Organization, 2004).

1.1.3.3 Vitamin C

Vitamin C is a powerful antioxidant because it can donate a hydrogen atom and form a relatively stable ascorbyl free radical (Weber *et al.*, 1996). Vitamin C also scavenges reactive nitrogen oxide species to prevent nitrosation of target molecules (Tannenbaum *et al.*, 1991). The importance of vitamin C in stabilizing various plasma components such as folate, homocysteine, proteins and other micronutrients has not been properly evaluated. When blood plasma is separated from erythrocytes, vitamin C is the first antioxidant to disappear (Organization, 2004). Vitamin C is necessary for growth and repair of tissues in all parts of the body. You may have also seen ads touting the benefits of vitamin C during cold and flu season, but these claims continue to be the source of great debate. Although research shows

that vitamin C supplements do not reduce the risk of getting the common cold for most people, regular vitamin C supplement intake may help to shorten cold duration and reduce symptom severity. Using vitamin C supplements after cold symptoms begin does not appear to be helpful (Douglas et al., 2000).

Table 1.1 Vitamins B family

Vitamin Name	Vitamin Abbreviation	Functions
Thiamin	**B_1**	Produces cellular energy from food; required for the synthesis of DNA and RNA.
Riboflavin	**B_2**	Helps the body produce energy; affects enzymes that influence the muscles, nerves, and heart.
Niacin	**B_3**	Energy production; helps keep the skin, nervous system, and digestive system healthy.
Pantothenic acid	**B_5**	Influences normal growth and development.
Pyridoxine	**B_6**	Helps break down protein; helps maintain the health of red blood cells, the nervous system, and parts of the immune system.
Biotin	**B_7**	Helps break down protein and carbohydrates; helps the body make hormones.
Folic acid, folate	**B_8**	Helps the cells in the body make and maintain DNA; important for the production of red blood cells.
Cobalamin	**B_{12}**	Plays a role in the body's growth and development, and nerve function.

1.1.3.4 Vitamin D

Vitamin D is required to maintain normal blood levels of calcium and phosphate, which are in turn needed for the normal mineralization of bone, muscle contraction, nerve conduction, and general cellular function in all cells of the body. It is also modulating the transcription of

cell cycle proteins, which decrease cell proliferation and increase cell differentiation of a number of specialized cells of the body. Vitamin D is both a nutrient in food and a hormone our bodies make through sun exposure (Organization, 2004).

1.1.3.5 Vitamin E

The major biological role of vitamin E is to protect Polyunsaturated fatty acids (PUFAs) and other components of cell membranes and low-density lipoprotein (LDL) from oxidation by free radicals. It is the major lipid-soluble antioxidant in the cell antioxidant defence system and is exclusively obtained from the diet**,** it is used for cell communication, to strengthen the immune system, and to form red blood cells. Much like vitamin D helps the body use calcium, vitamin E helps the body use vitamin K. In the past, it was believed that taking vitamin E supplements also might prevent a variety of diseases, including heart disease, cancers and Alzheimer's disease. However, other research provides little evidence that taking vitamin E supplements prevents these diseases, and the risks and benefits of taking vitamin E are still unclear (Organization, 2004).

1.1.3.6 Vitamin K

Vitamin K is an essential fat-soluble micronutrient, which is needed for a unique post-translational chemical modification in a small group of proteins with calcium-binding properties, collectively known as vitamin K-dependent proteins or Gla proteins. Vitamin K is a group name for a number of compounds that help the body make proteins necessary for blood clotting. Because of this role, vitamin K is used to reverse the anticoagulant effects of blood thinners when too much is given. For this reason, people taking blood thinners may need to be careful about how much vitamin K they take in. Vitamin K is also given to newborns who do not have enough of it naturally occurring to prevent clotting problems (Chan *et al.*, 2004).

1.1.4 Minerals

Minerals are divided into two groups Essential (macro) and Trace (micro) minerals, which is related to the quantity required and found in the body**.** Macro elements are the minerals of

which the body needs more amounts and are more important than any other elements. Trace elements constitute a minute part of the living tissues and have various metabolic characteristics and functions (Farrukh, 2012; Siddiqui *et al.*, 2014).

1.1.4.1 Macro elements

Macro elements have multiple roles within the body. They work together with vitamins and initiate hormone production as well as speeding up the metabolic processes. They are accepted as essential for human health and have diverse metabolic characteristics and functions (Matsumura *et al.*, 2000) The macro elements are sodium (Na), calcium (Ca), magnesium (Mg), potassium (K), chlorine(Cl) and phosphorous (P), which are required in larger quantities (Farrukh, 2012; Siddiqui *et al.*, 2014).

1.1.4.1.1 Sodium and Potassium

Sodium and potassium work together in muscle contraction and nerve transmission, and they play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, blood clotting and muscle contraction. Electrolyte imbalance resulting from kidney failure, dehydration, and fever and vomiting has been suggested as one of the contributing factors toward complications observed in diabetes and other endocrine disorders (Hussain *et al.*, 2009; Siddiqui *et al.*, 2014). Sodium, which is a predominant extracellular cation, normally in controlled physiological conditions lies in the range of 136–145mEq/l (136–145mmol/l) despite large variations in salt and water intake. The normal range of serum potassium value in the serum is very narrow and it ranges from 3.5 to 5mmol/l (Kratz *et al.*, 2004). Studies on electrolyte imbalances in association with diabetes have reported an inverse relationship between sodium and potassium levels in diabetic coma (Rohrscheib *et al.*, 2005). This association may be based on the movement of electrolytes between intra- and extracellular space dependent on impaired insulin action (Saito *et al.*, 1999). A significant reduction in serum sodium level was reported in T2D patients especially among insulin-treated patients. No significant association was found between T2D and serum potassium (Al-Rubeaan *et al.*, 2011).

1.1.4.1.2 Calcium

Calcium is responsible for strong bones and teeth, accounts for ninety percent of the calcium in the body whereas the other ten percent is circulating in fluids in order to ionise calcium. During pregnancy, maternal calcium homeostasis is integral to supporting fetal bone health and development (Wilson *et al.*, 2018; Farrukh, 2012). This is largely dependent on maternal vitamin D status and the vitamin D metabolic pathway, which, during pregnancy, maintains adequate transfer of calcium to the growing fetus. Currently, it is recommended that pregnant women consume >1000 mg calcium in order to meet increased fetal calcium requirements (Wilson *et al.*, 2018). Hypocalciuria (reduced calcium in the urine) has been associated with pre-eclampsia, and measuring urinary calcium to creatinine ratio in early pregnancy has been suggested as a possible predictor of pre-eclampsia. For this reason, calcium supplementation is recommended as a preventative measure for pre-eclampsia (Kazerooni and Hamze-Nejadi, 2003).

The elevated cytosolic calcium will lead to the pathogenesis of complications of T2D which in turn may interfere with normal insulin release, especially in response to a glucose load. The normal range of calcium in the serum is from 0.9 to 1.05mg/l (2.2–2.6mmol/l) (Kratz *et al.*, 2004). In addition calcium is essential for insulin-mediated intracellular processes in insulin-responsive tissues such as skeletal muscle and adipose tissue; any alteration in calcium may contribute to peripheral insulin resistance via impaired insulin signal transduction, leading to decreased glucose transporter 4 (GLUT4) activity (Siddiqui *et al.*, 2014; Sárközy *et al.*, 2014).

1.1.4.1.3 Magnesium

Magnesium has many roles including supporting the functioning of the immune system and assisting in preventing dental decay by retaining the calcium in tooth enamel; it has an important role in the synthesis of proteins, fat and nucleic acids, glucose metabolism as well as membrane transport system of cells. Magnesium also plays a role in muscle contraction and cell integrity. The normal level of serum magnesium ranges between 0.18 and 0.3mg/l (0.8–1.2mmol/l) (Kratz *et al.*, 2004)**.** Magnesium deficiency is associated with pre-eclampsia, and pre-term delivery (Chien *et al.*, 1996), and possibly with low birth weight. Of

the trials in developed country settings, some found fewer pre-term births and less intrauterine growth retardation with magnesium supplementation during pregnancy (Conradt *et al.*, 1985; Ludwig and Gabriele, 1988; Black, 2001). A randomized controlled trial indicated that oral magnesium supplementation may improve insulin sensitivity even in nondiabetic subjects with normal magnesium status. This emphasizes the need for an early optimization of magnesium intake to prevent insulin resistance and subsequently T2D (Siddiqui *et al*., 2014; Mooren *et al.*, 2011).

1.1.4.2 Micro elements

The trace element is a dietary mineral that is needed for the proper growth, development, and physiology of the organism. Trace elements participate in tissue, cellular and subcellular functions; these include immune regulation by humoral and cellular mechanisms, nerve conduction, muscle contractions, membrane potential regulations, and mitochondrial activity and enzyme reactions. In fact, although the trace elements are essential components of biological activities, the excessive levels of these elements can be toxic for the body health and may lead to many fatal diseases, such as cancers (Al-Fartusie and Mohssan, 2017). Trace elements include the transition metals iron ((Fe), copper (Cu), zinc (Zn), cobalt (Co), chromium (Cr), manganese (Mn), vanadium (V), Nickel (Ni) and molybdenum (Mo)) and the nonmetals (selenium (Se), fluorine (F), sulfur (S), and iodine (I)). All of these belong to the category of micronutrients, which are needed by the human body in very small quantities (generally less than 100mg/day) (Fraga, 2005; Farrukh, 2012).

1.1.4.2.1 Iron

Fe is a chemical element with symbol Fe, the common oxidation states are Fe^{+2} and Fe^{+3} and atomic number 26 and has been known since the beginning of time. It is by mass the most common element on Earth, forming much of Earth's outer and inner core, and the fourth most abundant elements after oxygen, silicon, and aluminum, respectively. It has an atomic weight of 55.8. Fe is the most abundant metal in the human body (Al-Fartusie and Mohssan, 2017). The sufficient supply of iron is essential for the functioning of many biochemical processes, including electron transfer reactions, gene regulation, binding and transport of oxygen, regulation of cell growth, and differentiation, antioxidant defences, DNA synthesis and is

also involved in the proper function of immune system. Anemia, as a result of iron deficiency, is highly prevalent in women worldwide (Stevens *et al.*, 2013; Farrukh, 2012), the normal range of iron in the adult is 6–17 μg/l. In addition, two prospective studies have identified an independent association between baseline elevations in iron stores and the incidence of diabetes. Elevated iron stores may induce diabetes through a variety of mechanisms, including oxidative damage to pancreatic β cells, impairment of hepatic insulin extraction by the liver, and interference with insulin's ability to suppress hepatic glucose production (Stevens *et al.*, 2013; Mastroiacovo *et al.*, 1999).

Iron deficiency in pregnancy has been associated with increasing perinatal morbidity and mortality, and it is recommended that pregnant women increase their daily iron intake to support increased requirements. Despite uncertain evidence surrounding routine iron supplementation, all women should have their hemoglobin and ferritin assessed at the first antenatal visit and again around 28 weeks' gestation. Women who are identified as anemic should be appropriately investigated and treated with iron supplements if iron deficiency is the identified cause (Fraga, 2005; Achebe and Gafter-Gvili, 2017).

1.1.4.2.2 Copper

Copper is a chemical element with symbol Cu and atomic number 29. It is in the top of group 11, of the periodic table, above silver and gold, the common oxidation states are Cu+ and Cu+2. It has an atomic weight of 63.5. Cu is a reddish metal with a face-centered cubic crystalline structure. It is malleable, ductile, and an extremely good conductor of both heat and electricity (second only to silver in electrical conductivity) (Al-Fartusie and Mohssan, 2017; Haynes, 2014).

The human body only contains about 150 mg of this vital mineral. The established RDA for Cu in normal healthy adults is 2 mg/day (Copper, 1980). Cu is absorbed in the gut and then transported to the liver bound to albumin. After processing in the liver, Cu is distributed to other tissues in a second phase. Cu transport in liver involves the protein ceruloplasmin, which carries the majority of Cu in blood (Al-Fartusie and Mohssan, 2017; Roger, 2011). Cu is an essential constituent of several enzymes such as cytochrome oxidase, monoamine oxidase, catalase, peroxidase, ascorbic acid oxidase, lactase, tyrosinase, and superoxide dismutase (SOD). Moreover, due to its presence in a wide variety of enzymes,

Cu is involved in many metabolic reactions. Cu is an essential micronutrient necessary for the hematologic and neurologic systems. It is necessary for the growth and formation of bone, formation of myelin sheaths in the nervous systems, helps in the incorporation of Fe in hemoglobin and assists in the absorption of Fe from the gastrointestinal tract and in the transfer of Fe from tissues to the plasma (Al-Fartusie and Mohssan, 2017).

The normal level of total copper in the body ranges between 7 and 14 μg/l (11–22 μmol/l) (Kratz *et al.*, 2004). Cu deficiency is rare among healthy people, but it may occur among infants. The most common symptoms of Cu deficiency include fatigue, anemia, and a decreased number of white blood cells. Sometimes, osteoporosis develops or nerves are damaged. Nerve damage can cause tingling and loss of sensation in the feet and hands. Muscles may feel weak. Some people become confused, irritable, and mildly depressed. It has been found that the most common cause of Cu deficiency is the remote gastrointestinal surgery, such as gastric bypass surgery, due to malabsorption of Cu. A deficiency of copper results in glucose intolerance, decreased insulin response and increased glucose response. It is associated with hypercholesterolemia and atherosclerosis. Copper possesses an insulin-like activity and promotes lipogenesis. Recent studies showed that no statistical difference is found in the level of copper in both diabetic and healthy patients (Kazi *et al.*, 2008; Ekmekcioglu *et al.*, 2001; Siddiqui *et al.*, 2014). On the other hand, Menkes disease is a genetic disorder of Cu deficiency, involving a wide variety of symptoms, that is often fatal (Kaler *et al.*, 2010). Acquired Cu deficiency is mainly attributable to nutritional deficiency and may be seen in malnourished low-birth weight infants, newborns and small infants. Cu deficiency has also been reported to develop after intractable diarrhea and prolonged parenteral or enteral nutrition. However, since Cu supplementation of intravenous and eternal nutritional formulas was made mandatory, the incidence of Cu deficiency has decreased dramatically (Al-Fartusie and Mohssan, 2017; Tsugutoshi, 2004).

1.1.4.2.3 Zinc

Zn is a chemical element with symbol Zn and atomic number 30. It is the first element of group 12 of the periodic table. This element was discovered by German chemist Andreas Sigismund Marggraf in 1746 at Germany. It has an atomic weight of 65.4, the common oxidation states are Zn^{+} and Zn^{+2}. Zn is the second metal present in the human body (about

2.5 g), after Fe (about 4 g) but before copper (Cu) (about 0.2 g). It is found throughout the entire body system, with half in the muscle tissue. The established recommended daily amount (RDA) for Zn is 8 mg/day for women and 11 mg/day for men (Bales and Ritchie, 2009). It is also found in most vitamin mineral supplements as sulfate, citrate, or oxide and these are inexpensive and bioavailable sources (Al-Fartusie and Mohssan, 2017; Bales and Ritchie, 2009).

Zn is an essential trace element that functions as a cofactor for certain enzymes involved in metabolism and cell growth; it is found in nearly 300 specific enzymes (Osredkar and Sustar, 2011). As a component of many enzymes, Zn is involved in the metabolism of proteins, carbohydrates, lipids, and energy. Zn is vital for the healthy working of many of the body's systems; it plays an essential role in numerous biochemical pathways. It is particularly important for healthy skin and is essential for a healthy immune system and resistance to infection. Zn plays a crucial role in growth and cell division where it is required for protein and DNA synthesis, in insulin activity, in the metabolism of the ovaries and testes, and in liver function (Al-Fartusie and Mohssan, 2017; Osredkar and Sustar, 2011).

The normal range of zinc in serum/plasma is reported as 8.4–15.9 μg/l. Zn deficiency may occur due to insufficient dietary intake. It was reported that nearly two billion people in the developing world are deficient in Zn (Prasad, 2003). Zn deficiency is a serious problem in many developing countries. Zn deficiency is ranked as the 5th leading risk factor in causing disease, especially diarrhea and pneumonia in children, which can lead to high mortality rates in these underdeveloped regions. Other severe deficiency symptoms include stunted growth and impaired development of infants, children, and adolescents. Early Zn deficiency also leads to impaired cognitive function, impaired immune function, behavioral problems, memory impairment, and problems with spatial learning and neuronal atrophy. Public health programs involving Zn supplementation and food fortification could help overcome these problems (Al-Fartusie and Mohssan, 2017; Lassi *et al.*, 2016). In more severe cases, Zn deficiency causes hair loss, delayed sexual maturation, impotence, hypogonadism in males, eye and skin lesions, weight loss, delayed healing of wounds, taste abnormalities, and mental lethargy can also occur (Al-Fartusie and Mohssan, 2017). Zinc may be involved in the regulation of insulin receptor-initiated signal transudation mechanism and insulin receptor synthesis (Tang and Shay, 2001). Its supplementation in patients with T2D improves insulin

secretion, while suppresses glucagon and glucose-6-phosphatase levels. In certain studies, the effect on serum insulin by zinc supplementation has been contradicted (Gunasekara *et al.*, 2011; Siddiqui *et al.*, 2014).

1.1.4.2.4 Cobalt

Cobalt is a chemical element with symbol Co and atomic number 27. It has an atomic weight of 58.9. Cobalt common oxidation state includes Co^{+2} and Co^{+3}, although compounds with oxidation states ranging from −3 to +5 are also known. Co is an essential trace element for the human body, where it is a key constituent of cobalamin (the scientific name of vitamin B_{12}). It also has a substantial role in the formation of amino acids and neurotransmitters (Al-Fartusie and Mohssan, 2017). It has been found that the cobalt deficiency is associated with disturbances in vitamin B_{12} synthesis. It might cause anemia and hypothyroidism, as well as increase the risk of developmental abnormalities and failure in infants (Battaglia *et al.*, 2009). The excess level of this metal in the human body might cause hypothyroidism and overproduction of erythrocytes, fibrosis in lungs and asthma (Lombaert *et al.*, 2008; Al-Fartusie and Mohssan, 2017).

Hyperglycemia is associated with excessive free radical generation and oxidant stress and reduction in the antioxidant status. Normal serum values of cobalt are less than 0.5 μg/l (Lauwerys and Hoet, 2001). In an animal study, glycemia-lowering effect of cobalt chloride ($CoCl_2$) on diabetic rats decreased the systemic glucose production, increased tissue glucose uptake, or made a combination of the two mechanisms. It is also reported that cobalt alone or with a combination of ascorbate decreases lipid peroxidation in diabetic rats in various organs such as the liver, kidney, heart, and aorta (Yildirim and Büyükbingöl, 2003). Compared with nondiabetic subject's serum concentration of cobalt is decreased in T2D (Flores *et al.*, 2011).

1.1.4.2.5 Chromium

Chromium is a chemical element with symbol Cr and atomic number 24. It is a steely-gray, lustrous, hard, and brittle metal. It has an atomic weight of 52.0; the common oxidation states are Cr^{+2}, Cr^{+3} and Cr^{+6}. Cr levels in biological matter have been studied extensively. It has been found that chromium produces significant increases in enzyme activity, serves an

important function in carbohydrate metabolism, stimulation of fatty acid and cholesterol synthesis from acetate in the liver, and improves sugar metabolism through the activation of insulin (Siddiqui *et al.*, 2014; Al-Fartusie and Mohssan, 2017). Dietary reference intakes for chromium were established. Adequate intake of chromium is 35 mg/day for adult males and 25 mg/day for adult females. Normal concentration of chromium in the serum of adult is 0.05–0.5 μg/l (1–10 μmole/l) (Tietz, 1995). Serum chromium shows an inverse relationship with serum glucose and its depletion during pregnancy may be responsible for insulin insensitivity leading to gestational diabetes in pregnancy (Idonije *et al.*, 2011).Chromium concentrations were significantly reduced in blood of T2D patients as compared to control subjects of both genders but urinary levels of these elements were found to be higher in the diabetic patients than in the age-matched healthy controls (Kazi *et al.*, 2008; Al-Fartusie and Mohssan, 2017).

1.1.4.2.6 Manganese

Manganese is a chemical element with symbol Mn and atomic number 25 and has an atomic weight of 54.9, The common oxidation states are +2, +4, and +7, but the less common +3, +5, and +6 states are easily prepared. The average human body contains about 12 mg of Mn. It is one of the most important nutrients for human health, about 43% of it is found in the skeletal system, with the rest occurring in soft tissues including liver, pancreas, kidneys, brain, and central nervous system (Emsley, 2011). Mn helps the body to form connective tissue, bones, blood-clotting factors, and sex hormones (Palacios, 2006; Fraga, 2005). It also plays a role in fat and carbohydrate metabolism, calcium absorption, and blood sugar regulation (Avila *et al.*, 2013; Henn *et al.*, 2010; Siddiqui *et al.*, 2014). Mn is also necessary for normal brain and nerve function. In addition, Mn is a key component of enzyme systems, including oxygen-handling enzymes. It is a component of the antioxidant SOD, which helps fight free radicals (Al-Fartusie and Mohssan, 2017).

The RDA for Mn is 2.3 mg/day for adult males and 1.8 mg/day for adult females (Trumbo *et al.*, 2001). The normal range of manganese in the adult blood is from 0.59 to 0.75 μg/l (Tietz, 1995).Although Mn is necessary for humans to survive, health problems will also occur when the uptake exceeds the normal level. It has been shown that the abnormal concentrations of Mn in the brain, especially in the basal ganglia, are associated with

neurological disorders similar to Parkinson's disease. The National Academy of Sciences established a tolerable upper intake level of 11 mg for total daily Mn intake for human adults(Trumbo *et al.*, 2001).

On the other hand, it has been found that the low levels of Mn in the body (deficiency of Mn) can causes hypercholesterolemia (Walter *et al.*, 1991), impaired glucose tolerance, dermatitis, changes in hair color, skeletal abnormalities, infertility, deafness, and impaired synthesis of vitamin K-dependent clotting factors (Kazi *et al.*, 2008; Siddiqui *et al.*, 2014). In fact, Mn is available in a wide variety of forms, including Mn salts (sulfate and gluconate) and Mn chelates (aspartate, picolinate, fumarate, malate, succinate, citrate, and amino acid chelate). Mn supplements can be taken as tablets or capsules, usually along with other vitamins and minerals in the form of a multivitamin (Roger, 2011).

1.1.4.2.7 Vanadium

Vanadium affects various aspects of carbohydrate metabolism including glucose transport, glycolysis, glucose oxidation, and glycogen synthesis (Orvig *et al.*, 1995; Nakai *et al.*, 1995). Vanadium exists in several valence states, with vanadate (+4) and vanadyl (+5) forms being the most common in biological systems. In animal models, vanadium has been shown to facilitate glucose uptake and metabolism, facilitate lipid and amino acid metabolism, improve thyroid function, enhance insulin sensitivity, and negatively affect bone and tooth development in high doses. Vanadium acts primarily as an insulin mimetic agent, although enhanced insulin activity and increased insulin sensitivity have also been noted. Before about twenty year research suggests that insulin may be required for its effects (Poucheret *et al.*, 1998; Cam *et al.*, 2000). One of the obstacles in using vanadium for glucose management is that it is known to be harmful to humans. The normal range of vanadium in blood or serum is from 17 to 118 ng/l (Heinemann and Vogt, 1996). An elevated vanadium level is also reported in diabetic persons in a study with different blood fractions (Ekmekcioglu *et al.*, 2001). In subjects with T2D, vanadium increased insulin sensitivity, glucose oxidation and glycogen synthesis but hepatic glucose output was suppressed (Halberstam *et al.*, 1996; Cohen *et al.*, 1995).

1.1.4.2.8 Nickel

Nickel is a chemical element with symbol Ni and atomic number 28. Ni is a silvery-white metal, hard, malleable, and ductile metal. It is of the Fe group and it is a fairly good conductor of heat and electricity. It has an atomic weight of 58.7. It is well accepted that nickel is an essential ultra-trace nutrient in humans (Kasprzak, 1987; Welch, 1981). Although the biological function of nickel is still somewhat unclear in human body; however, nickel is found in the body in highest concentrations in the nucleic acids, particularly RNA, and is thought to be somehow involved in protein structure or function. It has been speculated that nickel may play a role, as a cofactor, in the activation of certain enzymes related to the breakdown or utilization of glucose. Ni may aid in prolactin production and thus be involved in human breast milk production (Anke *et al.*, 1995; Al-Fartusie and Mohssan, 2017). More research is needed to reveal the properties of this interesting mineral in the human body. Long-term nickel inhalation may cause serious health problems, including cancer (Barceloux and Barceloux, 1999).

There is no RDA has been established for nickel. Nevertheless, it has been reported that the estimated daily intake of nickel from food and water worldwide is 80-130 μg/day (Roger, 2011). It has been found that humans may be exposed to nickel during breathing air, eating food, or smoking cigarettes. Skin contact with nickel-contaminated soil or water may also result in nickel exposure. In fact, small quantities of nickel are essential for the body, but when the uptake is too high it can be a danger to human health. Studies have shown that acute exposure of human body to nickel may cause several health problems such as liver, kidney, spleen, brain and tissue damage and vesicular eczema; lung and long-term nickel inhalation may cause nasal cancer (Al-Fartusie and Mohssan, 2017; Barceloux and Barceloux, 1999).

Ni deficiency has not been shown to be a concern in humans; despite this, it may cause biochemical changes, such as reduced Fe resorption that leads to anemia. It can disturb the incorporation of calcium into skeleton and lead to parakeratosis-like damage, which finds expression in disturbed Zn metabolism. It was found that nickel deficiency particularly affects carbohydrate metabolism (Al-Fartusie and Mohssan, 2017). More researches are required to see the benefits of, and what effects nickel deficiency can cause on the human body.

1.1.4.2.9 Selenium

Selenium is a chemical element with symbol Se and atomic number 34 and has an atomic weight of 78.97. It is of the group 16 of the periodic table with properties that are intermediate between the sulfur and tellurium elements, and exist in oxidation states −2, +2, +4, and +6. Selenium is an antioxidant, important in supporting immune function and reducing cellular stress and is involved in the complex system of defense against oxidative stress through selenium-dependent glutathione peroxidases and other selenoproteins (Siddiqui *et al.*, 2014; Wilson *et al.*, 2018). The normal selenium concentration in the serum is less than 0.8 μg/l (Shils and Shike, 2006).

Selenium deficiency in pregnancy has been associated with miscarriage, pre-eclampsia and fetal growth restriction, and selenium supplementation has beneficial effects on hypertension. Thus, the NHMRC recommends an additional 10 – 15 micrograms/day selenium for pregnant women, and most pregnancy supplements contain selenium (Fraga, 2005; Wilson *et al.*, 2018). In addition, selenate, an inorganic form of selenium, mimics insulin activity in experimental models. Selenium is known to act as an antioxidant and peroxynitrite scavenger when incorporated into selenoproteins. This antioxidant property of selenium prevents the development of complications in diabetic patients. While in other studies higher serum selenium concentrations were associated with a higher prevalence of diabetes (Bleys *et al.*, 2007), in another study the mean selenium concentrations in T2D patients with and without complication were significantly lower than those in healthy controls (Siddiqui *et al.*, 2014; Durak *et al.*, 2010).

1.1.5 Use of multivitamin/multimineral supplements (MVM)

“MVM supplements for a healthy population?” MVM supplements are safe but according to the Physicians’ Health Study PHS II, without a real benefit. That raises the question: “Who should supplement and why?” Generally spoken, micronutrients can compensate inadequate supply or transient micronutrient gaps. The majority of the participants of PHS II celebrated a healthy life style (few smokers, most with a normal weight, a high vegetable intake, and regular exercise) and a supplement seems not really necessary. However, in cases of sudden disease or periods of inadequate dietary diversity a supplement reduces the risk for

micronutrient gaps. A diet with poor quality and an unhealthy lifestyle can never be compensated by any supplement (Biesalski and Tinz, 2017; Gaziano *et al.*, 2012).

Pregnant and lactating women experience a higher demand for most micronutrients, not only iron and folate as usually mentioned. Based on meta-analyses supplementation with a MVM is superior over folate and iron alone with respect to a lower incidence of malformations and small for gestational age newborns (Goh *et al.*, 2006). In particular, young females practicing a vegetarian or vegan diet should be advised to take an MVM in case of a planned pregnancy.

In different experimental diabetes models and clinical studies involving limited number of patients, several data are available on the effect of individual vitamins, minerals, trace elements, or the combination of limited number of them (Guerrero-Romero and Rodriguez-Moran, 2009). The individual components of the MVM preparation were investigated by some others in different diabetic animal models or patient populations. Daily doses of all components of the preparation were set below the human upper safe level (Sárközy *et al.*, 2014).

1.1.5.1 Multivitamin/multimineral (MVM) supplements in pregnancy

Pregnancy is a dynamic state characterised by major changes to maternal physiology and anatomy in order to accommodate the growth of the fetus and placenta. Adjustments in nutrient metabolism are key to supporting not only the fetus, but also the mother. It is important that pregnant women maintain adequate levels of essential vitamins and minerals. Collectively known as micronutrients, these dietary components support virtually all aspects of cellular and metabolic activity, including cell proliferation, apoptosis and differentiation, as well as tissue growth and homeostasis (Gernand *et al.*, 2016). Deficiencies in certain micronutrients can have consequences on pregnancy outcome (Black, 2001; Wilson *et al.*, 2018).

Pregnancy complications, including pre-eclampsia, gestational hypertension, intrauterine growth restriction (IUGR) and preterm birth, affect one in five first- pregnancies and predict lifelong morbidity and mortality for both mother and child, (Roberts, 2010). The cause of many of these complications is largely unknown. However, there is abundant literature looking at associations between pregnancy complications and deficiencies in

vitamin D, folate, vitamin B_{12}, iodine, iron, zinc and selenium (Gernand *et al.*, 2016). However, suboptimal micronutrient status may still affect the risk of adverse pregnancy outcomes because many physiological pathways can be disrupted by even the smallest perturbations in micronutrient homeostasis. Thus, governing bodies like the Institute of Medicine (Trumbo *et al.*, 2001) and the National Health and Medical Research Council (NHMRC) of Australia (Capra, 2006) recommend pregnant women increase their daily intake of most micronutrients, most of these micronutrients are included in the leading Australian and England pregnancy and diabetic multivitamin formulations available in Sudan as shown in Table (1.2).

At present, only folic acid and iodine are recommended for routine supplementation for all women. Intake of other vitamins and minerals, such as iron, calcium and vitamin D, are dependent on a woman's abilities to meet recommended dietary intakes based on nutritional intake alone, or on identified nutritional deficiencies. Although multivitamin use is common in pregnancy, there is a lack of data supporting widespread use. Pharmacists should be aware of special considerations regarding vitamin and mineral supplementation in pregnancy and be prepared to provide balanced and up-to-date information to women (Wilson *et al.*, 2018).

1.1.5.2 Multivitamin/multimineral (MVM) supplements in Diabetes

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (Siddiqui *et al.*, 2014; Organization, 2013). The prevalence of diabetes in the age groups between 20 to 70 years worldwide was estimated to be 8.3% in 2013 and 10.1% in 2035. The total number of adult with diabetes is projected to rise from 382 million in 2013 to 592 million in 2035. Type 2 diabetes can be prevented or delayed through healthy diet, regular physical activity, maintaining a normal body weight, and avoiding smoking (Organization, 2013). Moreover, MVM preparations appeared on the market as medical food for diabetics as shown in table 1.2; however, direct associations of macro and trace elements with diabetes mellitus (DM) have been observed in many research studies (Nourmohammadi *et al.*, 2000). Insulin action on reducing blood glucose was reported to be potentiated by some trace elements as

chromium, magnesium, vanadium, zinc, manganese, molybdenum, and selenium (Siddiqui *et al.*, 2014).

Table 1.2 Pregnancy and diabetic multivitamin/multimineral formulations available in Sudan

Product and source / Contents and dose	Wellwoman (Vitabiotics), England	Pregnacare (Vitabiotics), England	Diabetone (Vitabiotics), England	Pregnancy Platinum (Nature's Own), Australia
Dose (tablets/day)	1	1	1	1
Micronutrient content Folic acid (µg)	400	260	500	500
Vitamin B_{12} (µg)	20	2.2	9	0
Vitamin B_1 (µg)	10	1.5	15	0
Vitamin B_2 (µg)	5	1.6	5	0
Vitamin B_3 (µg)	36	17	45	0
Vitamin E (µg)	30	10	30	0
Vitamin K (µg)	90	60	0	0
Vitamin D (µg)	5	10	5	5
Calcium (mg)	0	-	50	20
Iron (mg)	12	15	8	5
Iodine (µg)	-	140	100	250
Zinc (mg)	12	15	15	12
Selenium (µg)	100	-	100	16.25
Magnesium (mg)	100	150	100	-
Chromium (µg)	50	-	200	-
Copper (µg)	1500	1000	1	-

Values represent the amount of each element found in one label.

The proposed mechanism of trace elements enhancing insulin action includes activation of insulin receptor sites, serving as cofactors or components for enzyme systems involved in glucose metabolism (Vincent, 2000), increasing insulin sensitivity, and acting as

antioxidants preventing tissue peroxidation (Kruse-Jarres and Rükgauer, 2000). It is also reported that the metabolism of several trace and macro elements alters T2D and these elements might have specific roles in the pathogenesis and progress of this disease.

1.1.6 Safety of multivitamin/multimineral supplements (MVM)

To compensate for deficiencies of macro and trace elements in the human diet, a number of multivitamin preparations have appeared on the market. These dietary supplements are recommended for application in the treatment and prophylaxis of the deficiency of macro and trace elements. The intake of these preparations has increased significantly in recent decades. Regulation No. 178/2002 of the European Parliament and the Council hold supplement manufacturers and distributors responsible for the content of the dietary preparations (Commission, 2002). These supplements should contain only what is on the label and should not contain any harmful or undesirable substances, such as toxic metals. The safety of multivitamin dietary supplements depends on various factors including the manufacturing process and the purity and origins of the raw ingredients. Several studies proved that multivitamin materials may contain high levels of certain elements (Krawczyk, 2014; Avula *et al.*, 2011).

In high-income countries in Europe and the United States, multivitamin are the most commonly used MVM. In particular, the increasing numbers of healthy elderly are the major consumers of multivitamin to improve or maintain their health. However, concerns are raised that multivitamin might create harmful effects, “and their long-term safety is in question”. The European Food Safety Authority (Verkaik-Kloosterman *et al.*, 2012; Biesalski and Tinz, 2017) assesses the safety of supplement use based on the risk of exceeding an established upper limit, defined as the maximum level of total chronic daily intake of a nutrient (from all sources) judged to be unlikely to pose a risk of adverse health effects to humans. Nutrients possess some characteristics that distinguish them from other food chemicals for the purpose of risk assessment. Nutrients are essential for human well-being within a certain range of intakes, and there is a long history of safe consumption of nutrients at the levels found in balanced human diets. Additionally, for some nutrients there may be experience of widespread chronic consumption (e.g., from dietary supplements) at levels significantly above those obtained from endogenous nutrients in foods without reported adverse effects.

Data on adverse effects of nutrients are also often available from studies in humans, which helps to reduce uncertainty factors. Furthermore, many nutrients are subject to homeostatic regulation of body content through adaptation of absorptive, excretory, or metabolic processes, and this provides a measure of protection against exposures above usual intakes from balanced diets (Verkaik-Kloosterman *et al.*, 2012; Biesalski and Tinz, 2017).

There is indeed a strong difference regarding safety issues if MVM with concentrations at or below the RDA are compared with MVM having doses near the UL or with one or more components that exceed the RDA. The major risk, if any, of MVM at or below RDA may be an insufficient supply of one or more minerals that might not cover the individual need in case of a higher demand or inadequate supply through using. MVM with concentrations above the RDA may exert adverse effects (AEs), particularly with long-term use (Biesalski and Tinz, 2017). Increase in the intake of certain nutrients as therapeutics or through food may lead to high concentrations of these elements resulting in toxicity of the body. Trace elements are essential components of biological structures, but at the same time they can be toxic beyond the concentration needed for their biological functions. The toxicity can be extended to other non-essential elements of very similar atomic characteristics that can mimic the reactivity of a trace element.

1.1.6.1 Recommended daily intake RDI of minerals according to age and gender

The Recommended daily intake (RDI) of metals is related directly to age, and gender. The requirements for babies, toddlers, children, adolescents, and elderly vary with gender and country due to soil type. These requirements are continually being reviewed in the light of more research that is undertaken by food regulating bodies such as Food Standards Australia and New Zealand (FSANZ), United Stated of America, Food and Drug Administration (FDA), and European Authorities. The work done by these bodies, as well as research on different age groups in particular locations in many countries, to assist in maintaining and improving the health of the various groups and the population in general (Farrukh, 2012), as shown in table 1.3. The Institute of Medicine has determined dietary reference intakes (DRIs): Recommended dietary allowances and Adequate intakes, for 10 minerals (Medeiros, 2007),

related directly to age, gender, and requirements. as shown in table 1.4. This table taken from the DRI reports, presents recommended dietary allowances (RDA) in **bold type** or adequate intakes (AI) in ordinary type followed by an asterisk (*). An RDA is the average daily dietary intake level sufficient to meet the nutrient requirements of nearly all (97-98 percent) healthy individuals in a group. It is calculated from an estimated average requirement (EAR). If sufficient scientific evidence is not available to establish an EAR, and thus calculate an RDA, an AI is usually developed. For healthy breastfed infants, the AI is the mean intake. The AI for other life stage and gender groups is believed to cover the needs of all healthy individuals in the group, but lack of data or uncertainty in the data prevent being able to specify with confidence the percentage of individuals covered by this intake (Farrukh, 2012).

Table 1.3 Dietary reference intakes (DRIs)

Major minerals (macro mineral)	**Recommended Daily Intake (RDI)**
Calcium (Ca)	1000 mg
Magnesium (Mg)	350 mg
Potassium (K)	3500 mg
Sodium (Na)	2400 mg
Trace minerals	
Chromium (Cr)	120 µg
Copper (Cu)	2 mg
Iron (Fe)	15 mg
Manganese (Mn)	5 mg
Molybdenum (Mo)	75 µg
Selenium (Se)	35 µg
Zinc (Zn)	15 mg
Nickel (Ni)	ND
Vanadium (V)	ND
Cobalt (Co)	ND

Table 1.4 Dietary reference intakes (DRIs), recommended dietary allowances and adequate intakes elements

Life Stage group		Ca(mg/d)	Cr(mg/d)	Cu (mg/d)	Fe(mg/d)	Mg(mg/d)	Mn(mg/d)	Se (mg/d)	Zn (mg/d)	K(g/d)	Na(g/d)
Infants	**0–6 month**	210*	0.2*	200*	0.27*	30*	0.003*	15*	2*	0.4*	0.12*
	7–12 month	270*	5.5*	220*	**11**	75*	0.6*	20*	**3**	0.7*	0.37*
Children	**1–3 year**	500*	11*	**340**	**7**	**80**	1.2*	**20**	**3**	3.0*	1.0*
	4–8 year	800*	15*	**440**	**10**	**130**	1.5*	**30**	**5**	3.8*	1.2*
Males	**9–13 year**	1,300*	25*	**700**	**8**	**240**	1.9*	**40**	**8**	4.5*	1.5*
	14–18 year	1,300*	35*	**890**	**11**	**410**	2.2*	**55**	**11**	4.7*	1.5*
	19–30 year	1,000*	35*	**900**	**8**	**400**	2.3*	**55**	**11**	4.7*	1.5*
	31–50 year	1,000*	35*	**900**	**8**	**420**	2.3*	**55**	**11**	4.7*	1.5*
	51–70 year	1,200*	30*	**900**	**8**	**420**	2.3*	**55**	**11**	4.7*	1.3*
	> 70 year	1,200*	30*	**900**	**8**	**420**	2.3*	**55**	**11**	4.7*	1.2*
Females	**9–13 year**	1,300*	21*	**700**	**8**	**240**	1.6*	**40**	**8**	4.5*	1.5*
	14–18 year	1,300*	24*	**890**	**15**	**360**	1.6*	**55**	**9**	4.7*	1.5*
	19–30 year	1,000*	25*	**900**	**18**	**310**	1.8*	**55**	**8**	4.7*	1.5*
	31–50 year	1,000*	25*	**900**	**18**	**320**	1.8*	**55**	**8**	4.7*	1.5*
	51–70 year	1,200*	20*	**900**	**8**	**320**	1.8*	**55**	**8**	4.7*	1.3*
	> 70 year	1,200*	20*	**900**	**8**	**320**	1.8*	**55**	**8**	4.7*	1.2*
Pregnancy	**14–18 year**	1,300*	29*	**1,000**	**27**	**400**	2.0*	**60**	**12**	4.7*	1.5*
	19–30 year	1,000*	30*	**1,000**	**27**	**350**	2.0*	**60**	**11**	4.7*	1.5*
	31–50 year	1,000*	30*	**1,000**	**27**	**360**	2.0*	**60**	**11**	4.7*	1.5*
Lactation	**14–18 year**	1,300*	44*	**1,300**	**10**	**360**	2.6*	**70**	**13**	5.1*	1.5*
	19–30 year	1,000*	45*	**1,300**	**9**	**310**	2.6*	**70**	**12**	5.1*	1.5*
	31–50 year	1,000*	45*	**1,300**	**9**	**320**	2.6*	**70**	**12**	5.1*	1.5*

1.1.7 Multivitamin/multimineral elemental analysis

Elemental profiling specially of elements can be divided into three subgroups (Farrukh, 2012):

1. Easy to determine routinely by several techniques (e.g. iron and zinc).
2. Not always easy to assay, particularly at low concentrations.
3. Expert handling (e.g. chromium, manganese, and nickel) which require a high level of analytical expertise because of the low concentrations present, detection limit problems, matrix interferences, incomplete recoveries and related methodological difficulties. At low concentrations, the analysis of minerals presents considerable difficulties, depending on whether the matrix is simple or complex. Multivitamin/multimineral contain some trace elements at very high concentrations and are generally easy to analyse.

The implications of these various conditions are important when single minerals are analysed, something that may present an array of problems (Soriano *et al.*, 2007).

1.1.7.1 Sample preparation techniques

The traditional techniques for elemental sample preparation are time consuming and require large amounts of reagents, which are expensive, generate hazardous waste, and might contaminate the sample with the reagents. Advances in sample preparation over the last few decades have been propelled by the advance of microwave-assisted acid digestion (Andrade Korn *et al.*, 2008; Sneddon *et al.*, 2006), ultrasound-assisted, extraction and slurry preparation, and direct solid sampling analysis (Andrade Korn *et al.*, 2008).

Sample preparation remains the major limiting step in analytical throughput. Dry ashing may take 2–3 days to prepare an analytical solution and some elements can be lost by volatilization. Conventional acid digestions are typically faster (3–4 h) than dry ashing but need permanent operator attention. Microwave digestion is usually performed with nitric acid in high-pressure vessel (at temperature above the boiling point of nitric acid) and is generally complete within 1 h (Dolan and Capar, 2002; Krejčová *et al.*, 2006). An alternative and relatively less reported technique is the slurry nebulization, involving the direct aspiration of suspended sample directly into and AAS, FES or ICP-OES (Krejčová *et al.*, 2006; Ebdon *et al.*, 1997)

Quality control and safety in the sample demands reliable methodology that is both rapid and easily transferable. In order to minimize the uncertainty in sample preparation a number of factors need to be considered. As statistically the degree of uncertainty in a method is directly related to the number of stages involved, a minimization of that number should reduce the uncertainty proportionally. Automation and mechanisation of processes also leads to a reduction in uncertainty. Automated procedures are generally more reproducible than manual methods and will also decrease the staff and time spent on sample preparation, which is often the bottleneck in analytical laboratories (Andrade Korn *et al.*, 2008; Capote and De Castro, 2007).

1.1.7.2 Sample digestion

Knowledge of the chemical composition of samples is a prerequisite for understanding their characteristics. The major task of sample digestion is to convert the form of sample into one suitable for chemical analysis. This digestion is produced by suppling energy, such as heat; by using chemical reagent such as acid; or by a combination of the two methods. Generally, after the sample is digested, the component of interest is in the solution as a soluble salt. The major advantage of the solution the excellent homogeneity. It represents the original solution composition even in microliter volume. Despite a considerable amount of new research on sample introduction techniques, solution sampling remains the preferred method for most modern instrumental techniques, such as inductively coupled plasma mass spectrometry (ICP-MS) and inductively coupled plasma atomic emission spectrometry (ICP-AES). Most solid multivitamin/multimineral samples cannot be dissolved in water, and sample digestion methods, such as acid digestion, fusion, or ashing, are required. Sample digestion is thus a fundamental and critical stage in the process of sample analysis, and it is often the limiting factor for sample throughput, especially with the rapid development of modern multielement measurement instrumentation (Navarro *et al.*, 2008; Hu and Qi, 2014)

The complexity of sample materials makes it necessary to choose a sample digestion method that is compatible with the specific objective of the analysis (Chao and Sanzolone, 1992; Hu and Qi, 2014). In reality, the choice of a digestion method should consider and be consistent with the chemical and physical composition and properties of the sample, the elements to be analysed, the sample size required, the precision and accuracy desired, the treatise on sample throughput needed, the suitability of the resulting

digestion matrix for the analysis method, the apparatus and laboratory facilities available, the economic aspects including the reagent and labor consumption and the safety considerations. There is an extensive literature on the various digestion methods for multivitamin/multimineral samples (Hight *et al.*, 1993, Soriano *et al.*, 2007, Krejčová *et al.*, 2006, Frentiu *et al.*, 2012). Despite numerous studies, the solution chemistry involved in many digestion methods is not well understood, and the knowledge in this area lags far behind developments in analytical instrumentation (Matusiewicz, 2003). Sample digestion thus remains a popular research theme for analytical chemistry.

1.1.7.2.1 Dry ashing techniques

Dry ashing is a sample preparation method generally convenient to be applied for subsequent macro and trace elements determination in general materials. Dry ashing or oxidation is usually performed by placing 0.1–1 g of the sample in an open vessel and removing the organic matter from the samples by thermal decomposition, normally in the presence of an ashing aid, using a muffle furnace. Typical ashing temperatures are 450 to 550 ºC at atmospheric pressure, and the ash residues are dissolved in an appropriate acid. The degree of volatilisation loss is a limiting factor and depends on (i) the applied temperature, (ii) the form in which the analyte is present in the sample, and (iii) the chemical environment in the ashing stage. Oxidizing reagents may be used as ashing aids in order to prevent the volatilization of analytes and also to speed up the ashing process. High-purity magnesium nitrate and magnesium oxide are commonly used for that purpose (Hoenig, 2001).

The application of dry ashing methods is simple and large quantities of food samples may be treated at the same time. This procedure permits the preconcentration of trace elements in the final solution, which is useful when very low concentrations are to be determined. The ash is also completely free of organic matter, which is a prerequisite for some analytical techniques. The addition of an ashing aid, on the other hand, increases the content of inorganic salts significantly, which might be a problem for the subsequent determination of trace elements, and it might also contribute to contamination, necessitating careful blank control (Andrade Korn *et al.*, 2008).

1.1.7.2.2 Wet ashing techniques

Wet digestion methods include sample decomposition by an acid or mixtures of acids, carried out in open vessels, in tubes, on a hot plate or in an aluminum heating block or in closed vessels at elevated pressure (digestion bombs) with thermal or microwave heating. Microwave-assisted digestion is an attractive method, especially for small samples. Extreme care should be exercised in using sealed pressure vessels since there is much anecdotal evidence of these vessels rupturing occasionally during conventional or microwave assisted digestion of organic materials (Andrade Korn *et al.*, 2008).

The applicability of this technique is strictly dependent on the type of sample: carbohydrates are easily mineralized with nitric acid at 180ºC, while fats, proteins, and amino acids cause incomplete digestion due to the relatively low oxidation potential of nitric acid at 200ºC; these materials require the addition of sulfuric and/or perchloric acid with all the problems related to their use at high temperature and pressure.

The type of acid used in the preparation procedure can have important consequences in the measurement step. It is commonly known that in all atomic spectrometric techniques nitric acid is the most desirable reagent. In spite of occasionally observed signal suppression in its presence (e.g., in ICP OES), no severe analytical problems are encountered in practice with nitric acid at concentrations up to 10%, sometimes higher, in all atomic spectrometric techniques as long as its concentration is similar in calibration and sample solutions. Hydrogen peroxide, added in most mineralization procedures, is also rarely responsible for analytical problems (Arruda, 2007). The presence of hydrochloric acid is not troublesome in ICP OES analysis; however, its exclusive use is kind of prohibited in GF AAS analysis because of the possible formation of volatile and difficult-to-dissociate analyte chlorides that could cause vapor phase and/or spectral interference. Because of its high viscosity, utilization of sulfuric acid is usually avoided in spite of its efficiency in digestion of organic matrices. Its presence is particularly undesirable in analytical techniques where the sample introduction is by nebulization (FAAS, ICP-OES, ICP-MS) (Andrade Korn *et al.*, 2008).

1.1.7.2.3 Ultrasonic techniques

Some conjectural approaches keep up the application of ultrasound irradiation to assist metallic species extraction from various solid samples, such as intense disturbance

imposed by acoustic wave propagation, disruptions produced by microjets at the collapse of cavitation bubble, as well as the products generated by volatile species sonodegradation. The application of ultrasound to assist sample preparation points to some singularities that align to the feature of expeditious preparation methods and low reagent consumption. Ultrasound speeds up sample preparation once it diminishes solvent gradient concentration in the solid-liquid interface, yields unstable species into the irradiated medium, and, sometimes, increases sample surface area due to solid erosion.

Ultrasound has been employed for sample preparation in order to improve analytical throughput; however, chemical information of samples submitted to ultrasonic irradiation can be severely compromised since the collapse of cavitation bubble results in a strong local temperature increase and free radical production, which could provoke analyte loss and gross analytical errors. Analyte losses were also observed for spectrometric determinations contrasting the results obtained for various metals in samples pretreated with ultrasound devices with other sample preparation techniques and certified materials.

1.1.7.2.4 Microwave assisted acid digestion techniques

In 1975, Abu-Samra *et al.* (1975) described one of the first uses of microwave heating for the rapid wet acid digestion of sample materials (Abu-Samra *et al.*, 1975). This discovery stimulated the long-term development of microwave technology for the preparation of all types of samples for analysis (Hu and Qi, 2014; Chen *et al.*, 2008). Currently, microwave technology is being applied not only in analytical chemistry but also in organic synthesis, inorganic reactions, preparation of catalysts, and other fields (Chen *et al.*, 2008).

This technology has now advanced to the point where it is revolutionizing chemical sample preparation and chemical synthesis (Chen *et al.*, 2008; Hu and Qi, 2014). Microwave digestion makes use of electromagnetic radiation with a typical frequency of 2450 MHz to generate heat. Compared with classical heating, microwave heating is many times more efficient. When irradiated by microwave energy, polar molecules and ions are energized via mechanisms of dipole rotation and ion conductance, respectively (Gilman and Engelhart, 1989; Hu and Qi, 2014).

The radiating energy is absorbed by both the digestion medium and the sample molecules, which enhances the chemical reaction that completes the decomposition of the sample. Furthermore, localized internal heating of individual sample particles can cause

these particles to burst, thus allowing new surfaces to come into contact with reagents increasing the dissolution rate (Hu and Qi, 2014; Nadkarni, 1984).

The high dielectric constant of the reagent facilitates the absorption of the radiating energy. Aqueous solutions of acids absorb microwave radiation to a lesser extent than water alone does. It has been shown that the efficiency of absorption of MW radiation decreases according to the following series: nitric, hydrofluoric, sulfuric, and hydrochloric acids (Hu and Qi, 2014). Microwaves only heat the liquid phase, while vapors do not absorb microwave energy. Thus, very high temperatures can be reached at relatively low pressures, which is a key advantage of microwave technology (Matusiewicz, 2003).

The vessel material also plays a significant role in microwave dissolution techniques. Glassy carbon and platinum vessels cannot be used with this method, but quartz glass and plastics, such as Teflon PFA and polycarbonate, are suitable because they cause no loss of energy to the vessel and allow for quick and efficient heating. Modern microwave digestion systems monitor both the pressure and temperature in the container. The electronic controls of these systems allow for very reproducible digestion conditions, which also reduces the need for operator attention.

Microwave (MW) assisted digestion with nitric acid, nitric and hydrochloric acids without or with the addition of hydrogen peroxide is a widely used technique for the dissolution of pharmaceutical and environmental samples. Microwave heating has several advantages over conventional heating on a hot plate, etc., as the energy is generated in the digestion mixture and not transferred by conduction. Among the key advantages of MW-assisted digestion are the much shorter digestion times and the reduced need for aggressive reagents to obtain complete digestion.

There are two different systems available for MW assisted digestion, pressurized closed-vessel systems and open focused MW systems that work under atmospheric pressure. Microwave-assisted digestion in closed vessels under pressure has gained popularity as a simple and fast dissolution technique that minimizes acid consumption, the risk of sample contamination, and loss of volatile elements. One of the limitations is the time required for cooling before the vessels can be opened, which may take hours, depending on the type of equipment used. The main advantages of focused MW radiation are safety, versatility, control of microwave energy released to the sample, and the possibility for programmed addition of solutions during the digestion. However, loss of volatile elements cannot be excluded in open-vessel digestion and results for low-level

elements might be affected by the high amount of reagents used and hence the increased risk of sample contamination. This risk can be minimized by using vapor phase acid digestion, which has proven to be very effective in minimizing the residual carbon content (RCC) (Arruda, 2007; Sneddon *et al.*, 2006; Andrade Korn *et al.*, 2008).

Dispite these advantages, only a few works were found reporting the use of extraction approaches for elemental determination in multivitamin tablets (Canfranc *et al.*, 2001; Soriano *et al.*, 2007). Closed-vessel microwave-assisted digestion of solid samples with concentrated acids or acid mixtures has also been employed in several studies for the determination of metallic elements in complex matrices (Cassella *et al.*, 1999; Sastre *et al.*, 2002). This technique has also been successfully applied in sample preparation for the determination of Cr and other elements in MVM supplements (Sołtyk *et al.*, 2003). Also, microwave radiation was used as energy source for the decomposition of 95 dietary supplement products and NIST reference materials with concentrated nitric acid aiming the determination of As, Cd, Hg and Pb (Dolan *et al.*, 2003). Application of extraction procedures has gained a considerable space in the field of sample preparation (Oliveira, 2003; Soriano *et al.*, 2007).

1.1.7.2.5 Direct solid sampling analysis

Direct solid sampling (SS) analysis is the oldest technique for the determination of metals by spectrometric techniques using arc or spark emission and, together with X-ray fluorescence spectrometry, it is still the most widely used technique in metallurgical laboratories nowadays. Among the techniques that can be used for direct SS in combination with AAS, ICP-OES, and ICP-MS are laser ablation and electrothermal atomization or vaporization.

From these alternatives, GF AAS has been shown to be the most attractive technique for the direct analysis of solid samples, mainly because of the absence of a nebulizer system, which simplifies the introduction of the solid material into the atomizer (Arruda, 2007). Direct SS analysis offers a number of advantages, such as the reduced sample preparation time and hence a faster analysis, higher accuracy, as errors due to analyte loss and/or contamination are dramatically reduced, higher sensitivity due to the absence of any dilution, and the absence of any corrosive or toxic waste. Another advantage is the long residence time of the sample in the GF AAS atomizer, which usually makes possible complete volatilization of the particles independent of their size and

complete atomization of the analyte. Moreover, it shows quite low limits of detection, which is highly desirable in trace analysis. Most of the disadvantages that have been mentioned for direct SS analysis using GF AAS are actually no longer valid. There are reliable tools available nowadays both for manual and automatic introduction of solid samples into the graphite furnace, and it has been shown that in most cases aqueous standards can be used for calibration also in direct SS analysis. The only limitations that have to be mentioned are the relatively short linear working range of AAS, which usually limits direct SS analysis to the determination of low trace concentrations, and the imprecision of the results, which is typically of the order of 10% due to the inhomogeneity of natural samples (Andrade Korn *et al.*, 2008).

1.1.7.2.6 Slurry sample preparation

Slurry sampling was considered to have certain advantages over direct solid sampling, since it is possible to change the slurry concentration by simple dilution, hence combining the advantages of solid and liquid sampling. Another advantage that has been claimed is that aqueous standards may be used for calibration. However, the stabilization of the slurry, its homogeneity, particle size, and sedimentation also have to be considered (Andrade Korn *et al.*, 2008; Sneddon *et al.*, 2006).

1.1.7.2.7 Digestion reagents

Strong oxidizing acids (HNO_3, $HClO_4$, H_2SO_4), non-oxidizing acids (HCl, HF, H_3PO_4), and hydrogen peroxide (H_2O_2) are generally used for sample dissolution, and appropriate combinations of acids have been used successfully to decompose various solid samples, such as pharmaceutical and environmental samples (Matusiewicz, 2003; Hu and Qi, 2014). All these acids are corrosive in nature, especially when heated and concentrated, and should be handled with extreme caution to prevent injury and accidents. Concentrated acids with the requisite high degree of purity are commercially available, and they can be further purified using subboiling distillation methods if needed (Yuan *et al.*, 2000). The physical properties of the common reagents used in sample preparation are summarized in table 1.5.

Hydrofluoric acid

Hydrofluoric acid (HF) is the most effective mineral acid for breaking up strong Si–O bonds to form SiF_6^{-2} ions in acidic solution. Silicates are converted to volatile SiF_4, which will be lost in open vessel digestion procedures. HF by itself is more effective in the digestion of silicate sample minerals than when mixed with another acid. However, HF is rarely used as the sole reagent because some salts are poorly soluble in this acid (Potts and Cresser, 1987). HF is almost always mixed with other oxidizing acids such as HNO_3 and/or $HClO_4$ to ensure complete dissolution and to produce uniformly high oxidation states in the final solutions. Even diluted HF solutions will etch glass, so plastic labware (preferably PTFE or Teflon) is essential. It should also be noted that HF is one of the most hazardous mineral acids used in the laboratory, and it is both highly corrosive and toxic. Any HF spills on the skin should be immediately washed with copious cold water, and the affected area should be treated with a gel containing monosodium glutamate (Potts and Cresser, 1987). Contact with HF does not cause an immediate burning sensation or pain but readily penetrates deep tissue and causes intense pain after an hour or more. HF will cause irreparable damage to the skin and eyes and should never be used without full safety precautions. Recently, NH_4F has been proposed to replace HF for the acid digestion of samples(Hu and Qi, 2014). A clear advantage of NH_4F-assisted acid digestion is that it does not require handling the very corrosive and toxic HF. Notably, the conventional subboiling purification procedure is not effective at removing As impurities in HF. This limit may be related to the presence of volatile As species such as AsF_3 (boiling point of 63 ºC). To remove this volatile As species, it is recommended to employ a boiling procedure for hydrofluoric acid prior to the conventional subboiling purification procedure (Hu *et al.*, 2005; Hu and Qi, 2014).

Nitric acid

Nitric acid (HNO_3) is one of the most widely used digestion reagents and the most widely used primary oxidant for the decomposition of organic matter. Hot and concentrated HNO_3 (16 M and 68%) is a strong oxidizing agent that will liberate trace elements from many materials as highly soluble nitrate salts. The oxidizing properties of nitric acid are lost when it is diluted below approximately 2 M. The most important application of HNO_3 in sample analysis is to decompose both carbonate and sulfide minerals (usually in association with HCl). Nitric acid matrices are the best acid medium for ICP-MS analysis.

Its constituents (H_2, N_2, and O_2) are already present in air entrained by the plasma, and the range of polyatomic ions are not increased significantly by the addition of an HNO_3 matrix(Hu and Qi, 2014), and it also does not interfere with most determinations. Additionally, nitric acid is available commercially in sufficient purity.

Table 1.5 General physical properties of the common reagents used in digestion sample preparation

Reagent	Formula	Concentration (%)	Molarity (M)	Density (Kgl^{-1})	Boiling point (ºC)	Comments
Hydrofluoric acid	HF	48	29	1.16	112	38.3% HF, azeotrope
Nitric acid	HNO_3	68	16	1.42	122	68% HNO_3, azeotrope
Hydrochloric acid	HCl	36	12	1.19	110	20.4% HCl, azeotrope
Perchloric acid	$HClO_4$	70	12	1.67	203	72.4% $HClO_4$, azeotrope
Sulfuric acid	H_2SO_4	98	18	1.84	338	98.3% H_2SO_4
Phosphoric acid	H_3PO_4	85	15	1.71	213	Decomposes to HPO_3
Hydrogen peroxide	H_2O_2	30	10	1.12	106	

Hydrochloric acid

Concentrated hydrochloric acid (HCl) is the most frequently used halogen acid for the dissolution of samples. Unlike HNO_3, HCl is a weak reducing acid and is not generally used to digest organic materials. It is an excellent solvent for carbonates, phosphates, many metal oxides, and metals. For example, due to its reducing properties and the complexing ability of Cl^-, HCl is a better solvent for dissolving iron and manganese oxides than HNO_3. For silicate analysis, HCl is generally used in combination with other acids, such as HF and HNO_3, although some basic silicate minerals can be completely or

partially decomposed by HCl alone. At elevated temperatures and pressures, many silicates and other refractory oxides, sulfates, and fluorides are attacked by HCl to produce soluble salts. HCl is the preferred acid medium to dissolve residues that remain after acid digestion or melts of alkali fusion for later analysis using atomic absorption spectrometry (AAS) (Potts and Cresser, 1987). Unlike for atomic absorption techniques, HCl is not a suitable sample matrix for ICP-MS analysis because chloride-bearing polyatomic ions cause major interferences (e.g., ArCl, ClO, and ClOH) with As and V (75As and 51V) and with many other trace elements (Cr, Fe, Ga, Ge, Se, Ti, and Zn) to a lesser extent (Jarvis, 1991). Hydrochloric acid can be effectively removed from sample solutions by repeated evaporation to incipient dryness with HNO_3 because the boiling point of the HCl azeotrope (110 ºC) is below that of the HNO_3 azeotrope (122 ºC). However, it should be noted that there may be potential losses of the volatile metal chlorides (As, Sb, Sn, Se, Ge, and Hg) if HCl is used in acid digestion procedures.

Perchloric acid

Perchloric acid ($HClO_4$) is one of the strongest mineral acids. Hot and concentrated perchloric acid has powerful oxidizing and dehydrating properties, and it will react explosively with organic compounds. For this reason, it is best to pretreat samples containing organic material or organic samples with HNO_3 or an HNO_3 –$HClO_4$ mixture. Perchlorate salts are generally highly soluble and stable in aqueous solutions, but some alkali (K, Rb, and Cs) perchlorates are exceptions to this general rule. The high boiling point of the acid ensures a more efficient attack of refractory minerals by improving the efficiency of HF and the more complete removal of HF during evaporation stages. Unlike HCl, chlorine ions introduced during the digestion procedure in the form of $HClO_4$ are difficult to be removed by evaporation and will be harmful to the determination of low levels of As and V in ICP-MS (Jarvis, 1991). Some salts of $HClO_4$ are spontaneously flammable in the anhydrous form. Therefore, $HClO_4$ must be used in a specially designed hood that is equipped with wash down facilities for cleaning after use (Potts and Cresser, 1987).

Sulfuric acid

Concentrated sulfuric acid (H_2SO_4) has dehydrating and mildly oxidizing abilities and the highest boiling point (338 ºC for the 98.3% acid) of the mineral acids. Cold concentrated

sulfuric acid has an extremely high affinity for water, and it will produce a significant amount of heat when diluted. Therefore, this acid must always be added slowly to excess water and not vice versa. Sulfuric acid is a highly effective reagent in combination with HF for the decomposition of most resistant minerals, such as zircon, chromite, monazite, cryolite, and many naturally occurring fluorides (Potts and Robinson, 2003). Unfortunately, some inorganic sulfates have low solubilities (e.g., Ba, Ca, Pb, and Sr), and volatilization of trace elements (Ag, As, Ge, Hg, Re, and Se) may occur during the digestion of some samples (Sulcek and Povondra, 1989; Hu and Qi, 2014). Furthermore, H_2SO_4 is very difficult to remove by evaporation (several days) due to its high boiling point (338 ºC). The viscosity of this acid results in transport effects during sample introduction in ICP-MS. This acid also causes severe sulfur polyatomic ion interferences and attacks the nickel sampler cones in the ICP-MS instrument (Hu and Qi, 2014)t. For these reasons, H_2SO_4 has not been widely used for the decomposition of samples.

Phosphoric acid

Phosphoric acid (H_3PO_4) is somewhat limited in its use in analytical chemistry because phosphate ions can cause interferences by complexing or precipitating some of the elements to be analysed (Chao and Sanzolone, 1992). Possible difficulties encountered in ICP-MS analysis are the presence of polyatomic species of P, transport effects induced by the high viscosity of the acid, and rapid erosion of the nickel sampler cone. H_3PO_4 undergoes a series of condensation reactions to form condensed phosphoric acid upon heating. Condensed phosphoric acid alone or with $HClO_4$ can decompose 70 natural minerals among sulfides, oxides, silicates, and carbonates (Hu and Qi, 2014). Further investigations are needed to explore the full potential of H_3PO_4 as an acid decomposition agent.

Hydrogen peroxide (H_2O_2)

Typically, concentrations of about 30% hydrogen peroxide are used in digestions, but more recently 50% concentrations are available. Hydrogen peroxide alone can react explosively with many organics, especially in the more concentrated form. Hydrogen peroxide is usually combined with an acid because its oxidizing power increases as the acidity increases. The combination of hydrogen peroxide and sulfuric acid forms monoperoxosulphuric acid (H_2SO_5), a very strong oxidizing reagent (Walter and Chalk,

1997). Because of its oxidizing power, hydrogen peroxide is frequently added after the primary acid has completed a pre-digestion of the matrix. The hydrogen peroxide can complete the digestion and the potential safety hazards previously described are minimized. In this regard, hydrogen peroxide is used similarly to perchloric acid, but perchloric acid has been shown to decompose at 245°C in a microwave closed vessel developing dangerous amounts of gaseous byproducts and tremendous excess pressure. Using these acids after the primary digestion of organic matter is completed is one way to avoid potentially violent reactions (Walter and Chalk, 1997).

Procedures using a low volume of acids or dilute acid solutions generally require H_2O_2 as an auxiliary oxidant agent. Hydrogen peroxide addition has been investigated, and a decrease in both solid residue and residual carbon content (RCC) was observed for sample digestates obtained using a higher volume of this reagent (Araújo *et al.*, 2002). In addition, the use of higher amounts of H_2O_2 caused a buildup of pressure, leading to efficient organic-matter oxidation at lower temperatures. However, for volumes higher than 5.0 ml, the solid residue and RCC remained constant, showing that high volumes of H_2O_2 are not imperative for efficient sample digestion (Araújo *et al.*, 2002; Domínguez-Perles *et al.*, 2014). Some procedures recommended a mixture containing 4:1 v/v HNO_3 / H_2O_2 (both as concentrated reagents); others showed that a mixture composed of 2.0 ml of concentrated HNO_3 combined with 1.0 ml of H_2O_2 is efficient in digesting up to 300 mg of biological samples (Carrilho *et al.*, 2001; Levine *et al.*, 1999). The possibility of decreasing volumes of reagents is attractive from the point of view of minimizing residues, reducing cost, decrementing blank values, and generating digestate solutions and is better suited to introduction by nebulization. The feasibility of using dilute acid solutions and a reduced volume of hydrogen peroxide originates from both the high pressure and high temperature reached in closed vessels. Higher temperatures cause more complete destruction of the sample matrix and improved accuracy (Gouveia *et al.*, 2001).

1.1.7.2.7 Digestion vessel materials

Many different vessel materials are used to handle samples during sample digestion in laboratories. The vessel material must be chosen carefully according to its nature (e.g., resistance to acids and alkalies, heat resistance and conductance, surface properties, reactivity, mechanical strength, and contamination), the sample components to be analysed, and the analytical requirements. Table 1.6 lists the preferred vessel materials

for sample digestion. These materials have gained considerable popularity for use in the handling of samples.

1.1.7.2.8 Contamination from the digestion process

The influence of contamination on the analytical results becomes increasingly important with decreasing concentrations of the analyte. Modern analytical methods and instrumentation make possible the measurement of extremely low concentrations of elements in complex matrices. In many cases, the blank level determines the lower limit of detection, which has increased the importance of digesting samples in a manner that keeps them as contamination-free as possible.

Contamination can occur from the reagents, the vessel materials, and the environment during the digestion process and is often an unforeseen barrier in sample analyses that can lead to false results. Liquid reagents, such as water and acids, are most important for sample preparation, and they are generally available in high-purity grades. If required, these reagents also can be further purified using subboiling distillation. In contrast, solid reagents are difficult to purify and result in comparatively high blank levels.

Many different vessel materials have been used to handle samples during sample preparation. Contaminants can be desorbed from impurities on the surfaces of the vessels or leached out from the vessel materials. It has been reported that the degree of contamination from the commonly used vessel materials is in the following order: polyethylene (low density) < fluorocarbons (e.g., Teflon, PTFE, and Tefzel) < quartz (synthetic) < polyethylene (high density) < quartz (natural) < platinum < borosilicate (Hu and Qi, 2014). Although containers made of linear polyethylene or Teflon introduce the least amount of contamination for most trace element analysis, these containers should be carefully cleaned with HCl and HNO_3 before use (Moody and Lindstrom, 1977). Simple acid washing of the bottles prior to use does not solve the contamination problem for all elements. For example, Ta contamination from perfluoroalkoxy Teflon vessels (Makishima *et al.*, 1999), Ba, Zn, and Cr contamination from high-density polyethylene bottles (Reimann *et al.*, 2007) and Sb contamination from polyethylene terephthalate bottles (Shotyk *et al.*, 2006) occur via leaching of the contaminants from the containers. Reimann *et al.* (2010) reported that dark-colored containers leach more materials than clear containers do for most elements, and this observation is independent of the container

material (Reimann *et al.*, 2010). Therefore, great care is required when choosing containers, reagents, and the apparatus used for sample digestion.

Environmental contamination is caused by airborne particles and gaseous matter. To keep contamination risks low during chemical treatment in open systems, work should be performed in clean rooms equipped with laminar-flow clean benches (Hu and Qi, 2014).

Table 1.6 Preferred vessel materials for sample digestion

Materials	Maximum temperature (ºC)	Comments
Borosilicate glass	800	Resistant to most acids, but should not be used with HF or boiling H_3PO_4 or alkaline solutions
Porcelain	1100	Popular material used for ashing purpose
Quartz	1200	The most suitable material for the wet digestion of organic materials
Platinum	1500	Resistant to attack by most acids and fusion reagents. Heats up and cools down rapidly, making it excellent for ash determinations
Glassy carbon	500	An inexpensive material for alkaline fusions with low melting point agents
Polytetrafluorethylene (PTFE)	250	Generally used for closed digestion vessels
Perfluoroalkoxy (PFA)	250	Commonly used in microwave digestion vessels
High-density polyethylene (HDPE)	120	Typically used for containment of the diluted sample digestion solution
Low-density polyethylene (LDPE)	80	
Polypropylene (PP)	135	

1.1.8 Chemometrics

Chemometrics and factorial designs have been used to select the best digestion technique for a particular purpose, i.e., to choose the best combination of reagents; reagent volumes, sample weight, digestion times and power settings (Mohd *et al.*, 1992; Kokot *et al.*, 1992; Kokot *et al.*, 1992; Zhou *et al.*, 1996; Zhou *et al.*, 1997). This is of particular value in a multielement situation when no single digestion procedure gives good results for all the elements required and a method is needed to obtain the best overall performance. In addition, related digestion programs with the nature of the sample matrix using an empirical modelling approach (Feinberg *et al.*, 1994). A preliminary study using Kjedahl nitrogen determinations in food samples to define reference digestion procedures was found to be very effective for precisely defined samples. However, for complex foods the model needed further development (Lamble and Hill, 1998; Feinberg *et al.*, 1994).

1.1.8.1 Multivariate

Currently, multivariate optimization techniques have often been used in analytical chemistry, and they have been subjects for the publication of several review papers (Bezerra *et al.*, 2008; Bezerra *et al.*, 2016; Candioti *et al.*, 2014; Ferreira *et al.*, 2019). In analysis, these tools can be employed for evaluation preliminary of the factors (factor screening) and also for determination of the critical conditions of these. So, the optimization techniques can be divided into two kinds of designs (Ferreira *et al.*, 2019).

1.1.8.1.1 Tools for evaluation preliminary of the factors

The main technique employed for factor screening is the two-level full factorial design, whose number of experiments established by the matrix is determined by the expression (2^k), being (k) the number of factors investigated. It allows the determination of the effects and the significances of the factors and their interactions of the processes. However, when the number of factors is large, the use of two-level full factorial design becomes unacceptable. So, in this case, the factorial designs (2^{k-x}) may be the most recommended, where (x) is the reduction of the number of experiments. These designs have the disadvantage that the effects of the main factors are confounded with the effects of interactions of other factors. A strategy to improve interpretation and decrease the risk of error is to establish designs whose the main effects are confounded with effects of high-

order factor interactions. This approach is defined by the design resolution that is represented by Roman numerals. Thus, for a fractional factorial of resolution III, the main effects are not confounded with other main effects, but they are confounded with two-factor interactions. In a fractional factorial of resolution IV the main effects are confounded with three-factor interactions, and also two-factor interactions are confounded with other two-factor interactions (Ferreira *et al.*, 2019; Friedrich *et al.*, 2016; Massart *et al.*, 1998).

1.1.8.1.2 Response surface methodologies (RSM)

The response surface methodologies (RSM) is called the Design of Experiments (DOE) establish quadratic models, and they allow the obtaining of the critical conditions of the factors (maximum or minimum). The more employed RSM by analytical chemistry are central composite design (CCD), Doehlert matrix (DM), three-level factorial (3^K) and Box Behnken designs (BBD). All these tools have their advantages and drawbacks. During the optimization of experimental factors using RSM, the validation study of the quadratic model is obligatory because in this case, the critical conditions of the method are being determined in this step. So, the analysis of variance (ANOVA) has been one of the best options for this evaluation. Also, the model obtained should not have lack of fit to ensure the efficiency of the optimization. By another hand, for the linear models, this requirement is lower because the two-level factorial designs full or fractional are employed for preliminary evaluation of the factors (Ferreira *et al.*, 2019, Massart *et al.*, 1998).

The full three-level factorial design (3^k) is a response surface methodology, which has as a disadvantage the number required for obtaining of the quadratic model. Besides that, this design also contemplates experiments with all factors at the negative or positive level (Ferreira *et al.*, 2019; Massart *et al.*, 1998).

1.1.8.1.3 Applications of multivariate optimization techniques in analytical chemistry

The applications of the chemometric or multivariate tools in analytical chemistry can be divided into two approaches: optimization of the experimental conditions during the sample preparation step and also optimization of the instrumental variables of analytical

techniques. Eventually, these tools have also been used in the validation step of the analytical methods in the robustness tests.

1.1.8.1.4 Applications of experimental designs in the sample preparation steps

The employ of chemometric tools for the optimization of sample preparation procedures in food analysis is quite diverse. Between these, report analytical strategies using microwave assisted radiation (Andrade and Lanças, 2017; Bagheri *et al.*, 2016; Khajeh and Sanchooli, 2010; Maria das Graças *et al.*, 2005; Marval-Leon *et al.*, 2012), ultrasound assisted radiation (Machado *et al.*, 2017), sample solubilization using tetramethylammonium hydroxide (de Figueiredo *et al.*, 2018; Torres *et al.*, 2016), enzymatic extraction (Bayar *et al.*, 2018), pressurized water extraction (Moras *et al.*, 2017), besides preconcentration procedures involving liquid-liquid extraction (Biata *et al.*, 2017; Khazaeli *et al.*, 2017), cloud point extraction (dos Santos Costa *et al.*, 2015; Heidarizadi and Tabaraki, 2016; Rezende *et al.*, 2011), dispersive liquid-liquid microextraction (Yamini *et al.*, 2010), solid phase extraction in their several forms (Andrade and Lanças, 2017; Arabi *et al.*, 2016; Dahaghin *et al.*, 2017; Kakavandi *et al.*, 2017; Seidi and Fotouhi, 2017) and others (Wei *et al.*, 2016; Gao *et al.*, 2017).

1.1.8.2 Multiple response modeling

Many analytical methods have been proposed for determination of a single species; however, very often the objective of the processes is the extraction and or quantification of more than one species. So, multiple responses are required to establish conditions of compromise between the quantified analytes. This way, the desirability function (D) has been often employed for optimization of methods for analysis using multiple responses (Derringer and Suich, 1980). Between these, we can cite some references: (Heidarizadi and Tabaraki, 2016; Ferreira *et al.*, 2017; Martí *et al.*, 2017; Setyaningsih *et al.*, 2017). Additionally, another multiple response function (MR) with a mathematic approach very simple was also proposed (Ferreira *et al.*, 2007). This function also has been used for optimization of analytical strategies developed for food analysis (Santos *et al.*, 2009; Santos *et al.*, 2014; Sousa *et al.*, 2014). Recently a comparison between the function MR and the desirability function D was reported. The results obtained in the generated models by the application of the two multiple response functions in the experimental data were quite similar (G Novaes *et al.*, 2016).

1.1.9 Analytical techniques

Analytical techniques such as atomic absorption spectrophotometry (AAS) (flame and flameless), atomic emission spectroscopy (direct-current and inductively coupled plasma), chemical and electro-analytical methods, chromatography, mass spectrometry (in different modes) nuclear-activation techniques and X-ray fluorescence offer sufficiently low detection limits to make them suitable for investigating a variety of biomatrices. Low detection limits alone are not sufficient to answer all the questions as analytical data on trace elements are mostly regarded with skepticism. Ignorance of various interferences, e.g. matrix-related problems, flaws in sample and standard preparation and inadequate calibration procedures all contribute to this regrettable situation. The analyst is therefore by far the most important component of any analytical system (Ma, 2004; Hiefje, 2000).

1.1.10 Choice of type of assay

The analyst is faced with the choice between multielement and single-element assays, which is affected by a number of factors. Thus, even though sometimes only partly quantitative, multielement assays are useful in obtaining simultaneous elemental composition profiles of a given specimen. For example, the non-destructive procedures offer the possibility of generating data simultaneously (including repeated determinations on the same test portion) for several elements for purposes of comparison. They also offer the possibility of internal quality control so that unusual situations involving any specific element can be evaluated. Moreover, in a carefully designed study, multielement assays can provide very useful information at relatively low cost. However, some elements must be determined alone because of serious analytical problems. Clinical, environmental and nutritional laboratories dealing with specific elements frequently need single-element assays. In a laboratory performing a wide range of analyses, therefore, a combination of both single- and multi-element capability may be essential for effective functioning (Farrukh, 2012).

1.1.11 Choice of analytical techniques

The choice of an analytical technique depends on a number of factors, including (Farrukh, 2012):

1. Susceptibility to matrix effects.
2. Range of elements covered.
3. Detection limits.
4. Suitability for the matrix of interest.

The susceptibility of an analytical technique to matrix effects depends on the sample composition. With some matrices, these effects are of major importance, but others can be avoided by a modification of the technique. The usefulness of an analytical method for trace-element analysis, also depends on the range of elements covered and the order of magnitude of its detection limits for the elements at the top and bottom of its sensitivity range. Detection limits will not be the same for all elements, so that simultaneous elements determination will require compromises in experimental conditions that will affect the accuracy and precision of at least a few elements. Even when there is a method of choice for the analysis of a particular element, its performance will depend on the concentration of the element in question and that of others in the matrix (Toelg, 1988). Concentration ranges also vary widely between different types of sample (Kumpulainen, 1980). These changes in relationships between elements may necessitate modifications to the technique for specific applications in order to maintain optimum performance and prevent any decline in detection limits. The most important criterion of the suitability of a method, however, is whether it is appropriate for the matrix of interest (Jones, 1992; Watson, 1998; Bernazzani, 2001). Many techniques have been utilised for the elemental analysis of a range of matrices, including less common stripping voltammetry, X-ray fluorescence, neutron activation analysis, capillary zone electrophoresis or wide extended flame atomic absorption spectrometry (F-AAS), graphite furnace spectrometry (GF-AAS), flame emission spectrometry and multielement inductively coupled plasma-emission spectrometry (ICP-OES) and inductively coupled plasma-mass spectrometry (ICP-MS) (Krejčová *et al.*, 2006).

1.1.11.1 Atomic absorption spectrophotometry (AAS) basic principle and instrumentation

Every atomic absorption spectrometer presents the same basic components; however, each manufacturer differentiates the configuration due to the analytical demand and according to technological advance. Figure 1.1 shows the main components of an atomic absorption spectrometer (Filho *et al.*, 2011; Farrukh, 2012).

The radiation source may be continuous, emitting from visible to infrared wavelengths or from lines that emit discreet lines, specifically from each chemical element. The modulator helps to differentiate radiation emitted by radiation lamp coming from the environment and, mainly, from the atomization system. The atomization system removes analyte atoms in solution and generates atomic vapor composed of atoms in ground state, putting them between the source and the detector to absorb the radiation emitted. The monochromator is responsible for the selection of photons due to the wavelength that will reach the detector. The detector transforms the energy of photons into a proportional electronic signal and amplifies it. The signal intensity obtained is treated by systems for data acquisition and processing (Filho *et al.*, 2011; Farrukh, 2012).

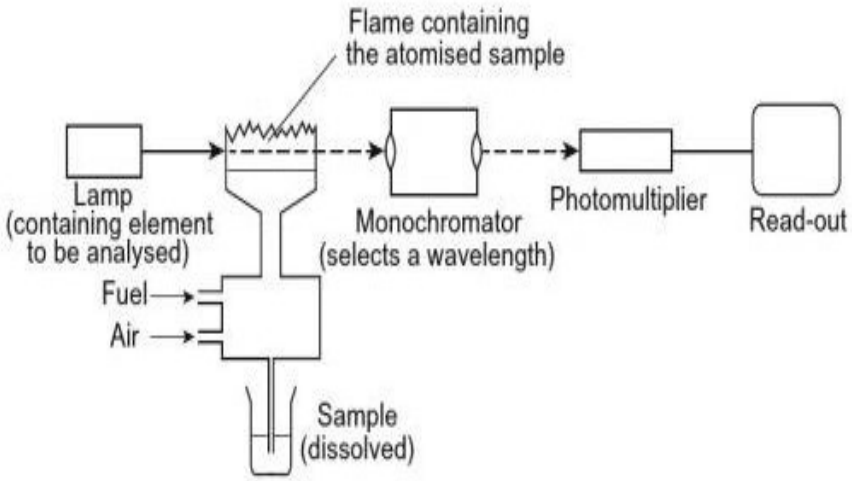

Figure 1.1 The main components of an atomic absorption spectrometer AAS

At this point, it is important to stand out that the element cannot be directly determined by the atomic absorption spectrometer; even though the system provides that response. A spectrometer measures the amount of electromagnetic energy coming from the source before and after passing through a sample; this is, an indirect measurement of

the absorption of energy by atoms present in the sample. To obtain a final result, a series of physical and chemical phenomenon should occur under controlled conditions and the measurement performed should be treated by an appropriate mathematical model. As each component of the equipment plays a part on this work (Filho *et al.*, 2011; Farrukh, 2012).

1.1.11.1.1 Hallow cathode lamp radiation source

Hollow cathode lamp (HCL) as shown in figure 1.2 consists of a sealed glass tube filled with inert gas, argon or neon, where an anode (positive pole) and a cathode (negative pole) are placed. The cathode contains an element whose spectrum is required basically in a pure source or an appropriate alloy. For elements that emit radiation in the ultraviolet region, the front window of emission is made of quartz because the glass of the tube absorbs UV radiation. When an appropriate difference of power is applied, some atoms of Argon (filling gas) are ionized (Ar^{+}) and accelerated in the cathode direction where they are struck enough with force to eject atoms of the element of interest (M^{o}). These atoms are struck by other filling gas ions and pass from the ground state into the excited state (M*), which will return rapidly to the ground state by emitting photon with a characteristic wavelength (Farrukh, 2012).

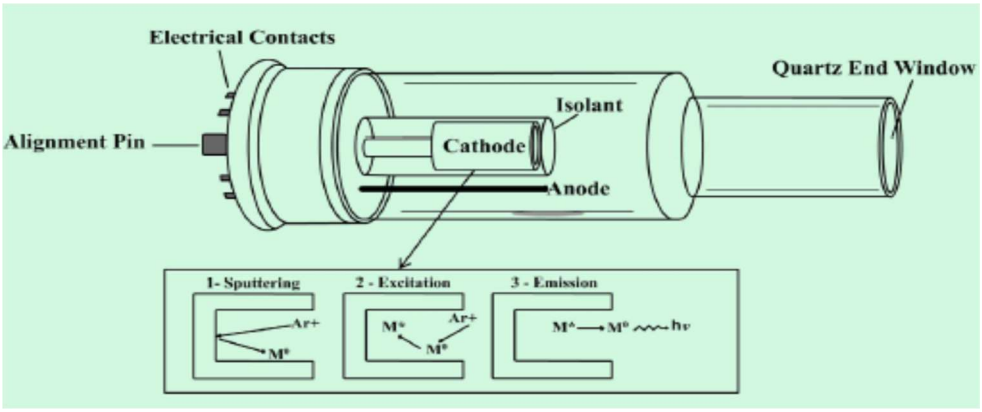

Figure 1.2 The hollow cathode lamp

1.1.11.1.2 Atomization system

Although there are some different techniques used to work with solid and gaseous samples in most of the atomic absorption equipments, these samples are introduced in solution. pre-mixture chamber, actually, most commonly used in FAAS. The solution

containing the analyte is aspired by a nebulizer, mixed in a nebulization chamber with the gases. The obtained mixture is directed to the burner, where they are burned, occurring sample atomization and absorption. The nebulizer has an orifice for gases and a central capillary tube where the solution to be analysed is introduced. Three more basic kinds atomizers can be used to produce ground state atoms, which are used for specific techniques. These atomizers were Hydride Generation, Cold Vapor Mercury and Graphite Furnace Atomic Absorption (Filho *et al.*, 2011).

1.1.11.1.3 Monochromator

The monochromator is responsible for selecting the appropriate wavelength for analysis. It is a hermetical closed box with entrance and exit slits of 0.2 to 2nm, lenses and mirrors to focus the radiation and a dispersed element that can be a prism or a diffraction grating or a combination of them. It is filled up with inert gas to avoid ultraviolet radiation absorption by the air and normally has the walls painted in matte black to avoid reflection and scattered radiation. The polychromatic radiation enters through the entrance slit, it is separated and only the chosen wavelength of radiation reaches the exit slit and goes on to the detector (Farrukh, 2012).

1.1.11.1.4 Detector

The detector is placed in front of the exit slit and receives the determined photons by the monochromator. It transforms lightning energy in an electrical signal, that is amplified and measured. Detectors mostly used in old equipment's are photo-multiplier valves. With modern equipment's, they are being replaced by a system based on solid-state detectors type CCD (charge coupled device). They are similar to digital cameras sensors with a set of sensitive pixels to electromagnetic radiation in the UV regions and visible that transforms the energy of photons coming from the monochromator into a digital signal (Filho *et al.*, 2011).

1.1.11.1.5 Acquisition of data processing

In the beginning, the signal obtained in the detector was read in an analogical amperemeter. The value was written down and then, all the calculations were done manually. Nowadays, everything is made by a microcomputer equipped with a specific program for operational control and data processing. Each manufacturer develops a

program according to the characteristics and implemented accessories in the spectrometry, so that everything can be done by software (Farrukh, 2012).

1.1.11.2 Graphite furnace atomic absorption spectrometer (GFAAS)

The graphite furnace atomic absorption spectrometer (GFAAS) and flame atomic absorption spectrometer (FAAS) measurement principle is the same. The difference between these two techniques is the way the sample is introduced into the instrument. In GFAA analysis, an electrothermal graphite furnace is used instead. The sample is heated stepwise (up to 3000ºC) to dry. The advantage of the graphite furnace is that the detection limit is about two orders of magnitude better than that of AAS. The analysis of different species of a given element is important because different oxidation states of the same element may present different toxicities and, consequently, different risks. Therefore, sequential extraction procedures for the separation and further analysis of a species have been developed for several metals (Filho *et al.*, 2011).

The graphite furnace atomizer has several advantages over a flame furnace. First it accepts solutions, slurries, or solid samples. Second, it is a much more efficient atomizer than a flame furnace and it can directly accept very small absolute quantities of sample. It also provides a reducing environment for easily oxidized elements. Samples are placed directly into the graphite furnace and the furnace is electrically heated in several steps to dry the sample, ash organic matter, and vaporize the analyte atoms. It accommodates smaller samples but it is a difficult operation, since the high energy that is provided to atomize the sample particles into ground state atoms might also excite the atomized particles into a higher energy level, thus lowering the precision.

The three-step sample preparation for graphite furnaces is as follows (Filho *et al.*, 2011):

A. Dry - evaporation of solvents (10–100s)

B. Ash - removal of volatile hydroxides, sulfates, carbonates (10–100s)

C. Fire/Atomize - atomization of remaining analyte (1s)

1.1.11.3 Inductively coupled plasma-mass-spectrometry (ICP-MS) principle and instrumentation

Inductively coupled plasma-mass-spectrometry (ICP-MS) is accepted as the most powerful multielement analytical technique available today to determine low-concentrations (range: ppb = parts per billion = µg/l) and ultra-low-concentrations of

elements (range: ptt = parts per trillion = ng/l), capable of true multielement determinations within minutes. The advantages of the inductively coupled plasma-mass-spectrometry (ICP-MS) technique above atomic absorption spectroscopy (AAS) or inductively coupled plasma optical emission spectrometry (ICP-OES) are extremely low detection limits, a large linear range, possibilities to detect isotope composition of elements (Linge and Jarvis, 2009)**.**

The components of an ICP-MS instrument are shown in figure 1.3 A sample travels through six main steps during an analysis (Linge and Jarvis, 2009): -

• The sample is converted into a suitable form for introduction into the plasma.

• The sample is ionised in the plasma.

• Ions are extracted from the plasma.

• Ions are focussed and transported to the mass spectrometer.

• The mass spectrometer is used to separate the ions based on mass-to-charge ratio (m/z).

• Ions are counted to quantify the amount of each in the original sample.

Like for the AAs, the sample solution is introduced into the device by means of a peristaltic pump. There it becomes nebulized in a spray chamber. The resulting aerosol is injected into an argon-plasma that has a temperature of 6000-8000 K. Inside the plasma torch, solution is removed from the sample and also atomization and ionization occur. Only a small amount part of the ions produced in the plasma further penetrate to the mass spectrometer part.

Mass-spectrometer part consists of an interface (in particular a “sampler cone” and a skimmer cone), in which a small amount of the free ions generated by the plasma are transmitted. During this process the ions migrate from an environment with extremely high temperature and atmospheric pressure to a compartment at room temperature and high vacuum (< 0,001Pa). Electrostatic lenses that focus (positive) ions onto the entry to the true mass-spectrometer. The true mass-spectrometer in the general instrumentation device has a quadrupole, composed of 4 metal rods which separate the ions on account of their mass by a kind of resonance principle. An electro-multiplier (aspecic type of detector) containing active surfaces, which enhances the signal from one colliding ion so that a measurable pulse is generated. Electronics that counts and sorts the pulses and relates them to the corresponding mass. This selection can be accomplished in milliseconds, so that a complete spectrum can be acquired within one second (Coplen *et al.*, 2002; Böhlke *et al.*, 2005).

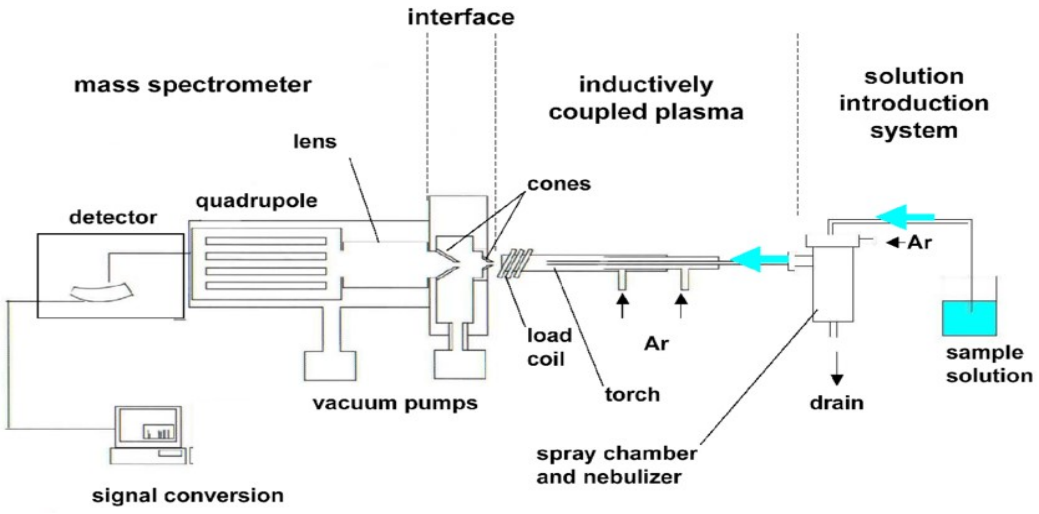

Figure 1.3 The components of ICP-MS instrument

1.1.11.3.1 Sample introduction

A wide variety of techniques have been developed for the introduction of solids, liquids and gases into ICP-MS instrumentation. While each method may be fundamentally different, they have the same goal: to sweep the sample of interest into the ICP torch in a gaseous or aerosol form for analysis (Nakahara, 2005; Günther and Hattendorf, 2005). However, discussion in this research will be limited to liquids, the most common sample types analysed by ICP-MS. However, the samples must be introduced to the torch in either a gaseous form or an aerosol form. Therefore, liquid samples require sample nebulization. The liquid sample is pumped from a vial, via a peristaltic pump, into the nebulizer. Liquid droplets are formed on the tip of a needle, where they become nebulized due to argon gas flowing through a second needle perpendicular to the sample needle. A small amount of aerosol created is swept into the torch, but the majority of sample condenses on the walls of the nebulizer and is wasted to the drain. The most commonly used nebulisers are pneumatic, where the aerosol is formed by the action of a high-speed gas jet over a tip of a small orifice (Linge and Jarvis, 2009).

Although some nebuliser types self-aspirate without external pumping, most are operated in conjunction with a peristaltic pump, ensuring a constant flow of liquid irrespective of solution viscosity or the vertical distance that the liquid must be lifted. Pumping also means that liquid uptake is independent of nebuliser gas flow and the nebuliser can be operated at the flow rate for its optimum performance (Bazilio and Weinrich, 2012).

1.1.11.3.2 Torch

The ICP torch is essentially a series of three concentric quartz tubes that are placed into a water cooled induction coil (typically made of copper metal) connected to a radio frequency generator. The basic design of the ICP torch is illustrated in figure 1.4. Argon gas is continuously flowing throughout the quartz torch, and a radio-frequency (RF) generator provides power to the RF coil at oscillating frequencies. Plasma (an electrical conducting gaseous mixture) generation occurs when the argon gas is seeded with a spark from a Tesla unit. The spark ionizes some of the argon, and the cations and electrons produced from that accelerate towards the RF coil. The cations and electrons collide with other argon molecules during this acceleration, creating high temperatures. With ample argon supplied, the plasma will reach equilibrium and remain at a constant temperature of about 6,000°C for the duration of analysis(Bazilio and Weinrich, 2012).

The aerosol produced via nebulization enters this high temperature plasma, where it is first dried to a solid, and then heated to a gas, referred to as atomization. These atoms will continue to travel through the plasma, absorbing energy until they release an electron, becoming ionized, referred to as ionization. These newly formed ions then travel out of the torch and come to the interface (Linge and Jarvis, 2009).

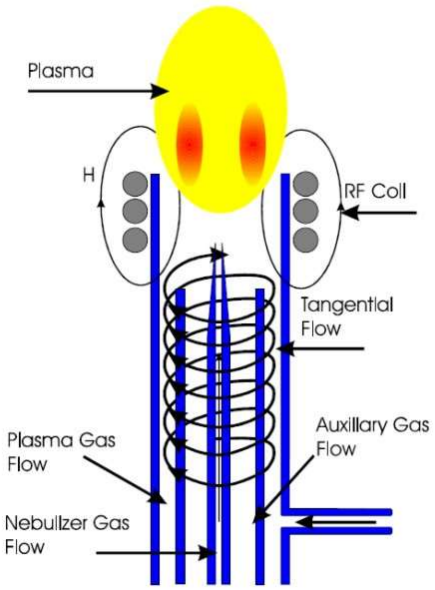

Figure 1.4 The basic design of the ICP torch

1.1.11.3.3 Interface

Generally speaking, the interface can be described as the point at which sample from the ICP portion of the instrument is introduced to the mass spectrometry (MS) portion of the instrument. The interface portion of the instrument serves to allow the ICP and MS portions to be coupled. The first component the sample matrix confronts after ionization in the ICP torch is the sampler cone. This is a water cooled cone with a small orifice, allowing for the hot plasma gas to enter a depressurizing chamber. In this chamber, rapid cooling, and thus rapid expansion, of the gas occurs. A fraction of this gas then passes through a skimmer cone, and into a chamber that is maintained at a vacuum of that of the MS. This two-step pressure reduction allows the ionic gas to enter the MS at proper temperature and pressure (Bazilio and Weinrich, 2012).

1.1.11.3.4 Ion focussing and transmission

The lenses that focus and transport ions to the mass analyser are typically a series of metal pates or rings, each with a specific voltage. The lens stack may be located in the high vacuum region or directly behind the skimmer in order to "pull" the ions from the interface region. The exact dependence of the signal on the voltage of each lens will depend on the design and arrangement of the lens stack. The lens stack also removes neutral species and photons from the ion beam that would otherwise be registered as additional ion counts by the detector (LaFreniere *et al.*, 1987). A circular disk, or photon stop, may be incorporated into the lens stack in the direct line of the ion beam. Applying a voltage to the photon stop will deflect ions around the disk, while stopping photons or neutral species (Linge and Jarvis, 2009).

1.1.11.3.5 Reaction cell

The Agilent ICP-MS is equipped with a dynamic reaction cell (DRC). The DRC is located in the vacuum chamber between the lens and the quadruple. Chemical modification of the ion beam to eliminate interferences occurs in the DRC when operating in DRC mode. The type of reaction gas and pressure is set by the user in the computer software. Interference is prevented by interrupting the sequence of reactions that would otherwise create interference. When DRC mode is off, the DRC is a multipole device which transfers the ions to the MS analyser chamber (Bazilio and Weinrich, 2012).

1.1.11.3.6 Ion separation

To quantify each element, the ions must be separated from the ion beam and counted. A mass spectrometer is able to distinguish between different ions based on mass-to-charge ratio (m/z). The most common mass spectrometer used in ICP-MS is the quadrupole mass filter (Figure 1.5), consisting of four metal rods that are suspended in parallel to, and equidistance from, the ion beam. Each rod is electrically connected to the rod directly opposite and voltages are applied to both rod pairs. Ions entering the quadrupole travel down the central axis and the voltages applied to the rods cause the ions to oscillate. The magnitude of the oscillations are influenced by both the mass and charge of the ion. Extreme oscillations cause the ion to be ejected from the stable transmission region, striking the rods or the inside of the quadrupole housing. The rod voltages are optimised to ensure that only ions of a single m/z have a stable path and exit the quadrupole and the mass filter must be switched to sequentially filter for each m/z of interest (Bazilio and Weinrich, 2012; Linge and Jarvis, 2009).

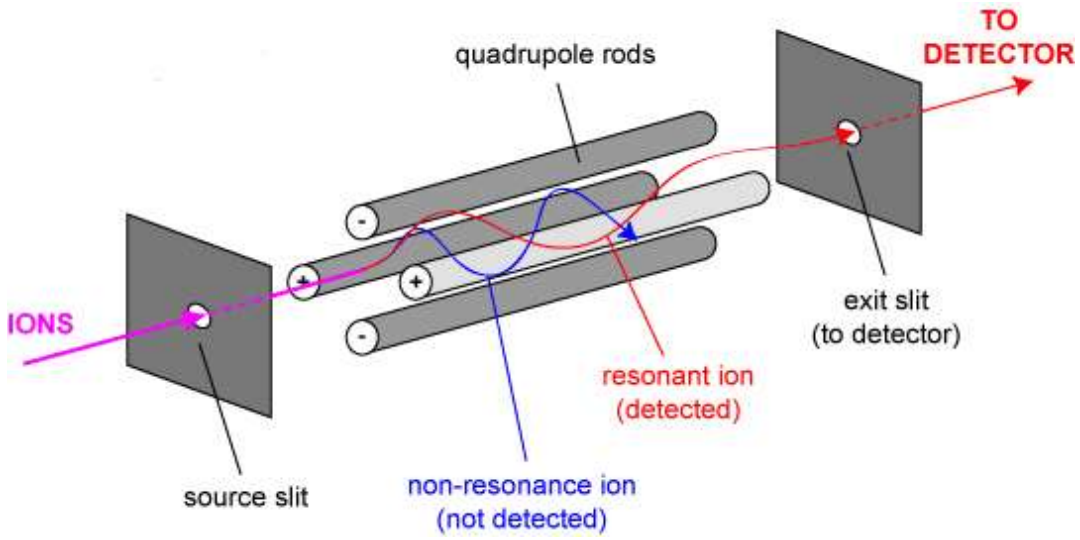

Figure 1.5 Quadrupole mass filter

1.1.11.3.7 Ion counting

Individual ions are counted by pulse counting, where each ion is converted into a discrete electrical pulse. The number of pulses is related to the number of analyte ions present in the sample and can be converted into an absolute concentration by comparing the signal from a sample with that from a calibration reference sample. The two main pulse counting

detectors used in ICPMS instruments channel electron multiplier and discrete dynode electron multiplier both employ electron multiplication (Linge and Jarvis, 2009).

1.1.11.3.8 Data and figures

The signal that is processed by the detection electronics and sent to the computer for data processing. The data resulting from the analysis of a sample consists of the number of ions counted at fixed m/z (or channels), as selected by the operator. Normally the total ions counted are converted to ions counted per second (CPS) to give counts which are independent of counting time at each channel. The operator may choose to count ions at a single channel for each isotope (i.e., peak hopping), or to count at multiple channels across each m/z (i.e., peak scanning). Measuring multiple channels (normally 6 or more) of all m/z produces a full mass spectrum.

1.1.12 Analytic method development and validation

Method validation often evolves from method development. Method development can take a number of forms. At one extreme, it may involve adapting an existing method, making minor changes so that it is suitable for a new application. It requires a lot of effort, and there is a degree of doubt initially to whether the method will be successful. It involves working on various ideas simultaneously and then finally picking one of those. Various steps involved in method development and validation are: (Method development plan definition, background information gathering, laboratory method development, generation of test procedure, methods validation protocol definition, laboratory methods validation, validated test method generation and validation report**)** (Kumar *et al.*, 2012).

1.1.12.1 Method validation

Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice. It is the process of defining an analytical requirement, and confirms that the method under consideration has performance capabilities consistent with what the application requires. Use of equipment that is within specification, working correctly and adequately calibrated is fundamental to the method validation process. Likewise, the operator carrying out the studies must be competent in the analysis under

study and have sufficient knowledge of the method/analysis to draw conclusions from the observations as the validation work proceeds. Quite often method validation evolves from method development and so the two activities are often closely tied, with the validation study employing the techniques and steps in the analysis as defined by the method development (Kalra, 2011; Kumar *et al.*, 2012). The parameters for method validation have been defined in different working groups of national and international committees and are described in the literature. Unfortunately, some of the definitions vary between the different organizations. An attempt at harmonization was made for pharmaceutical applications through the ICH where representatives from the industry and regulatory agencies from the United States, Europe and Japan defined parameters, requirements and, to some extent, methodology for analytical methods validation (Kalra, 2011; González and Herrador, 2007). The method validation parameters that are applicable to most methods are (Kumar *et al.*, 2012; Chan *et al.*, 2004; González and Herrador, 2007):

1.1.12.1.1 Accuracy

The accuracy of an analytical method is the extent to which test results generated by the method and the true value agree. Accuracy can also be described as the closeness of agreement between the value that is adopted, either as a conventional, true or accepted reference value, and the value found. It is established by quantitation of the sample against a reference standard for API, or spiking placebo with API for drug product. It can also be determined by comparison of results from alternate measurement techniques. Calibration is the most important step in bioactive compound analysis. A good precision and accuracy can only be obtained when a good calibration procedure is adopted. Results of the accuracy study should be reported as percent recovery of the known amount added or the difference between the mean assay result and the accepted value (Kumar *et al.*, 2012).

1.1.12.1.2 Linearity

The linearity of an analytical method is its ability to elicit test results that are (directly or by means of well-defined mathematical transformations) proportional to the concentration of analytes in samples within a given range or proportional by means of well-defined mathematical transformations. Linearity may be demonstrated directly on the test substance by preparing a series of dilution of a standard stock solution or by using separate weighing of synthetic mixtures of the test product components, using the

proposed procedure. Test results should be evaluated by appropriate statistical methods, for example, by calculation of a regression line by the method of least squares. Acceptability of linearity data is often judged by examining the correlation coefficient and y-intercept of the linear regression line for the response versus concentration plot (A correlation coefficient of >0.999 is generally considered as evidence of acceptable fit of the data to the regression line. The y-intercept should be less than a few percent of the response obtained for the analyte at the target level) (Kumar *et al.*, 2012).

1.1.12.1.3 Precision

Is the degree of agreement between replicate analyses of a homogenous sample, usually measured as the relative standard deviation (RSD) of a set of replicates, Samples may be analysed on different days, by different analysts, on different instruments, or in different laboratories. There are three levels of precision validation evaluations repeatability, intermediate precision, and reproducibility. Repeatability (intraday precision) is a measure of precision under the same conditions over a short period of time. Intermediate precision is a measure of precision within the same laboratory by different operators, using different instruments, and making measurements on different days. Reproducibility (interday precision) assesses precision between two or more laboratories (Kalra, 2011).

1.1.12.1.4 Limit of detection (LOD)

The limit of detection (LOD) is the lowest concentration of the analyte in a sample that can be detected but not necessarily quantified. Several approaches for determining the detection limit (DL) are possible, depending on whether the procedure is a noninstrumental or instrumental. Noninstrumental methods are based on the visual evaluation. The DL is determined by the analysis of samples with known concentrations of the analyte and by establishing the minimum level at which the analyte can be reliably detected. Instrumental method based on signal-to-noise, this approach can only be applied to analytical procedures which exhibit baseline noise or may be expressed as: $DL = 3\sigma/S$, where σ is the standard deviation of the response, S is the slope of the calibration curve, the slope S may be estimated from the calibration curve of the analyte (Kumar *et al.*, 2012).

1.1.12.1.5 Limit of quantitation (LOQ)

It is the concentration level above which the concentration can be determined with acceptable precision [usually relative standard deviation (RSD) < 10–25%] and accuracy. Several approaches for determining the quantitation limit (QL) are possible, depending on whether the procedure is a noninstrumental or instrumental. Limit of quantification is evaluated based upon the visual evaluation which is a type of noninstrumental method, using this method, QL is determined by the analysis of the samples with known concentrations of analyte and by establishing the minimum level at which the analyte can be quantified with acceptable accuracy and precision. instrumental method based on the signal-to-noise approach, this approach can only be applied to analytical procedures that exhibit baseline noise or the QL may be expressed as: $QL = 10\sigma/S$, where σ is the standard deviation of the response, S is the slope of the calibration curve, the slope S may be estimated from the calibration curve of the analyte (Kumar *et al.*, 2012).

1.1.12.1.6 Specificity

Can be established by a number of approaches, depending on the intended purpose of the method. The ability of the method to assess the analyte of interest in a drug product is determined by a check for interference by placebo. Specificity can be assessed by measurement of the API in samples that are spiked with impurities or degradants, if available. If API-related compounds are not available, drug can be stressed or force-degraded in order to produce degradation products. In chromatographic separations, apparent separation of degradants may be confirmed by peak purity determinations by photodiode array, mass purity determinations by mass spectroscopy (MS), or by confirming separation efficiency using alternate column chemistry. During forced degradation experiments, degradation is targeted at 5 to 20% degradation of the API, in order to avoid concerns about secondary degradation.

1.1.12.1.7 Robustness

Is typically assessed by the effect of small deliberate changes to chromatographic methods on system suitability parameters such as peak retention, resolution, and efficiency. Experimental factors that are typically varied during method robustness evaluations include: (i) age of standards and sample preparations, (ii) sample extraction time, (iii) variations to pH of mobile phase, (iv) variation in mobile phase composition,

(v) analysis temperature, (vi) flow rate, (vii) column lot and/or manufacturer, and (viii) type and use of filter against centrifugation. Robustness experiments are an ideal opportunity to utilize statistical design of experiments, providing data-driven method control.

1.1.13 Methods cited in literature for the determination of elements in MVM

1.1.13.1 Development methods for determination elements in MVM

The development of reliable methods for the determination of elements in these supplements is an important issue because of their widespread consumption. Several analytical techniques and different strategies for sample preparation have been used for such purpose (Krejčová *et al.*, 2006). Sample preparation still represents the most sensitive and time consuming step in most analytical procedures. Only total digestion enables the determination of total metallic content in different types of samples. In spite of its versatility total digestion exhibits some drawbacks depending on the experimental procedure utilized. Dry ashing procedures are very simple to perform but some elements can be lost by volatilization. In turn, wet acid digestion is faster than dry procedures but requires large amounts of reagents and constant supervision (Oliveira, 2003).

Analyses of MVM samples have usually been performed after total digestion of samples. Canfranc *et al.* (2001) determined Fe and Mo in pharmaceutical preparations by flame atomic absorption spectrometry (FAAS), after dry ashing of samples at 600 °C. No volatilization of the analytes under study was observed at this temperature. Hight *et al.* (1993) utilized boiling acids and acid mixtures containing HNO_3, $HClO_4$, H_2SO_4 and H_2O_2 for total dissolution of dietary supplements before measurement of 36 elements in 42 samples.

A multicommutation flow system was developed for the spectrophotometric determination of Fe, Zn, Ca and Mg in multivitamin samples. Total acid digestion of samples was carried out with a mixture of concentrated HNO_3 and $HClO_4$ (Rocha *et al.*, 2001).

Soriano *et al.* (2007) developed a method for the determination of Cu, Fe, Mn and Zn in multivitamin/multimineral tablets, by flame atomic absorption spectrometry (FAAS), after extraction of the analytes with diluted hydrochloric acid solution. Several

parameters that could influence the extraction process such as acid extraction solution concentration and nature, mixing mode (ultrasonic or magnetic stirring), extraction time and sample composition were evaluated. The obtained results showed that Fe, Mn and Zn were easily extracted with 1 mol/l HCl solution after 5 min of mixing with either ultrasonic or magnetic stirring for all studied samples. On the other hand, Cu extraction appeared to be more complex since it could only be extracted at the same conditions for silicate-free samples. For samples containing silicates the time of contact between solid sample and extraction solution presented remarkable influence, being necessary up to 12 h to achieve quantitative recovery with 1 mol/l HCl solution. The developed methodology was applied in the determination of Cu, Fe, Mn and Zn in seven commercially available multivitamin/multimineral tablets. The results obtained with the developed method were compared with those obtained after total digestion of 0.12g samples using a closed-vessel microwave oven device under conditions of Step 1: 300 W, 2 min. Step 2: 720 W, 5 min. Step 3: 200 W, 5 min with 5 ml conc HNO_3.

A method for multimineral (Ca, Cr, Cu, Fe, K, Mg, Mn, Na, P and Zn) determination in multivitamin/multimineral, by atomic emission spectrometry in a medium power radiofrequency capacitively coupled plasma (275 W) and low Ar consumption (0.4 l/min), was proposed by Frentiu *et al.* (2012). Determinations were performed on commercially available 1.5g tablets powder and a standard reference material after acidic (15 ml of HNO_3 69%, 3 ml of H_2O_2 30% and 25 ml demineralized water) high-pressure microwave assisted digestion and using the standard additions procedure. The detection limits were 0.003mg g^{-1} (Na) - 1.5mg g^{-1} (P) and were not depreciated by the non-spectral interference of mineral matrices of K, Ca, Mg and Na except Zn and P. The concentrations obtained corresponded generally to the labelled contents with recovery in the range of 90% – 107% and 1.0% – 13.0%, respectively. The proposed technique could be an advantageous alternative to the more expensive inductively coupled plasma atomic emission spectrometry in the quality control of multivitamin/multimineral formulations.

Sołtyk *et al.* (2003) were determined the macro- and microelements Ca, Cr, Cu, Fe, Mg, Mn, Mo, P, Se and Zn in multimineral and multivitamin preparations and in pharmaceutical raw material. Inductively coupled plasma mass spectrometry (ICP-MS) and electrothermal atomic absorption spectrometry (ET AAS) were used throughout the study. The examined (0.2-0.6 g of tablet mass and 0.16 g of capsule) samples were

dissolved in a high-pressure microwave system (The maximum pressure and temperature during mineralization were 45 at and 155 ºC) using 3ml concentrated nitric acid. The effect of the carbon residue in the digest solution on the determination result was eliminated by introducing an equation correcting the ArC^+ interference with 52Cr.

Kahoun *et al.* (2006) utilized a slurry sampling technique for elemental analysis of multivitamins preparations using inductively coupled plasma-emission spectrometry (ICP-OES). For the sake of comparison, 0.3-0.5 g of samples were mineralized by microwave oven (power setting of 80% for 10 min and at 100% for 10 min. The maximum total output of the microwave generator was 700 W). Slurry concentration range of 0.1% – 0.2% m/v in 6% v/v HNO_3, was used. The calibration was performed by water standard solutions; slurry standards and standard additions were tested for determination of the above-mentioned elements in slurries. The method gave good precision for macro elements (RSD ranging from 5% to 10%). For in-home control sample, the measured concentrations were in satisfactory agreement with independent laboratories. For the analysed multivitamin preparations, the elemental concentration obtained was compared with that declared by producer. The concentrations of Ca, Mg, P, K, Fe, Mn, Zn, Cu and of Cr, Ni and V were determined in the range 1000–100,000 and 5–50 μg/g, respectively. The slurry ICP-OES analysis was found to be suitable for quality control monitoring of multivitamin preparations and could be useful as a routine procedure.

Additionally, Krawczyk *et al.* (2014) analysed three different commercially available multivitamin dietary supplements by high-resolution continuum source graphite furnace atomic absorption spectrometry (HR-CS GFAAS) with slurry sampling. The concentrations of Cr, Cu, Fe, Mn, and Se were determined and compared with that stated by producers. and concentrations of several toxic elements (As, Cd, and Pb) were determined. Microwave-assisted high pressure Teflon bomb digestion (heated for 20 min at 300 W) was used to determine total amounts of elements in samples containing 1 ml of 30%H_2O_2, then 4 ml of concentrated HNO_3 and 1.5 ml concentrated HF were added. Samples were prepared as slurries at a concentration of 0.1% (m/v) for macro elements (Cr, Cu, Fe, Mn, and Se) and at a concentration of % (m/v) for trace elements (As, Cd, and Pb) in acidic media (3M HNO_3). The influence of acid concentration, Triton X-100 addition, sonication time, and sonication power on absorbance was investigated. The accuracy of this method was validated by analyses of NRCC LUTS-1 (Lobster hepatopancreas), NRCC DORM-1 (Dogfish Muscle), NRCC DOLT-2 (Dogfish Liver),

NBS SRM 1570 (Spinach Leaves) and NBS SRM 1573 (Tomato Leaves) certified reference materials. The measured elements contents in these reference materials (except NRCC DOLT-2) were in satisfactory agreement with the certified values according to the t-test for a 95% confidence level.

Also, Santos *et al.* (2017) were used inductively coupled plasma optical emission spectrometry (ICP OES) for multielement analysis in five multimineral preparations from a dissolution test, in accordance with the United States Pharmacopeia (USP 34 method): apparatus 1, 75 rpm and 900 ml of 0.1 mol l^{-1} HCl. Element releases in all samples (minimum-maximum in %) were: Ca (14.5-28.2), Cr (54.5-68.0), Cu (2.9-10.0), Fe (4.0-34.3), Mg (6.0-25.2), Mn (2.6-51.2), V (0.0-51.4) and Zn (1.5-107.3). The concentrations of Al, Ba, Cd, Co, Mo, Pb and Se were below the limit of detection of ICP OES. Accuracy was assessed by microwave digestion and recovery values of 94%-102%. USP 34 method indicates that not less than 75% of the elements described on the product label must be dissolved in 1 h. Only the release of Zn met the recommendations. The results indicated the need for greater quality control in multivitamin preparations. The dissolution test was validated in order to contribute to Brazilian and other pharmacopoeias.

1.1.13.2 Monitoring the elements in MVM

The dietary supplement manufacturer is responsible for ensuring that a dietary supplement is safe before it is marketed and that product label information is truthful and not misleading. A multi-element analysis method that is applicable to a large variety of food supplements and easy to use is needed to verify accuracy of element content on multivitamin labels (Krawczyk, 2014).

The concentration of heavy metals like nickel, manganese, zinc, chromium, copper and iron in multivitamin drugs sampled from an indigenous market of a well reputed manufacturer employing atomic absorption spectrophotometer (AAS) was determined. Intake of nickel in excess results in different diseases like liver, stomach and kidney disorder. Saleem *et al.* (2016) was focused to know the concentration of toxic metals in multivitamins tablets and syrups. Study of different tablets and syrups from different companies showed varying levels of toxic metals. Variable concentration of nickel was found in tested products ranging from 0.8 – 92.28 µg/g. The concentration of zinc in tested sample was 0.37 to 980.93µg/g which was within the permissible limit.

Most of tested multivitamins samples contain cobalt concentration less than the recommended daily intake (Saleem *et al.*, 2016).

Thirty-five different commercially available multivitamin/multimineral (MVM) dietary supplements in tablet, capsule, liquid or powder form for children, women, men, young and adult consumption were analysed by collision/reaction cell ICP-MS for their inorganic elemental compositions including Na, Mg, K, Ca, V, Cr, Mn, Fe, Ni, Cu, Zn, As, Se, Cd, Hg, and Pb. Samples were digested with concentrated nitric and hydrochloric acid (8:2) using a closed vessel microwave system. The validity of the applied method was assessed by the analysis of standard reference materials (SRM 3280, SRM 1566b) and of spiked samples. Special emphasis was given to the percentage deviation of calculated daily intake of each analysed element from their corresponding label claim. Additionally, for toxic elements calculated daily intake values were compared with those of the regulatory guideline values (e.g., recommended dietary allowance). The results revealed that all analysed products had calculated daily intake of As, Cd, Pb and Hg concentrations lower than those of the regulatory limits. The percentage differences between the calculated and claimed daily intake values varied from 20% (moderate) to >30% (significant) for the potentially toxic elements, especially Cr, Se, Mn, and Zn. Furthermore, it was not uncommon for the same product to have high, as well as low, elemental compositions compared to their corresponding claimed values (Avula *et al.*, 2011).

1.1.14 The objectives of the research

The main objective of this study is to validate and optimize a suitable acid mixture and closed microwave digestion (with pressure control) condition for the simultaneous determination of elements in the multivitamin/multimineral (MVM) pharmaceutical formulations are being used for pregnant women and diabetic patients collected from local pharmaceutical markets in Sudan without the need for additional handling. The procedure will be chosen by selecting the most appropriate conditions from several experiments to provide suitable recoveries of element concentrations.

The specific objectives are:

- To optimize the oxidation mixture, radiation power, radiation period, and sample weight for extraction of some macro and micro elements (Fe, Mg, Zn, Cu, Mn, Cr, and Se), from multivitamin/multimineral (MVM) pharmaceutical formulations of

pregnant women and diabetic patients using HNO_3, HCl and H_2O_2, followed by their AAS (flame and electro thermal) determination.

- To develop an easy and fast procedure for determination of thirteen elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn) in multivitamin/multimineral (MVM) pharmaceutical formulations used for pregnant women and diabetic patients, by ICP-MS after optimization of microwave digestion conditions with DOE (Design of Experiments) and to evaluate the method accuracy using standard reference material (SRM 3280).
- To evaluate simultaneously the thirteen elements in the multivitamin/multimineral (MVM) pharmaceutical formulations available in Sudan pharmacies and to ensure that the concentration conform with those labelled by the manufactures.

CHAPTER TWO

Materials and methods

CHAPTER TWO

2.1 MATERIALS AND METHODS

2.1.1 Chemicals and reagents

2.1.1.1 AAS standard solutions

Scharlau (Gota Perez, Spain) individual standard solutions (1000µg/ml), containing the minerals of interest (Fe, Mg, Zn, Cu, Mn, Cr and Se), were used for construction of flame and thermal graphite atomic absorption calibration curves, using 3% HNO_3 for dilution.

2.1.1.2 ICP-MS tuning and standard solutions

ICP-MS stock tuning solution containing Ce, Co, Li, Tl and Y (10 µg/ml), and multi-element calibration standard solution (10µg/ml) containing the minerals of interest (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn) were obtained from Agilent Technologies (Palo Alto, CA, USA), and they were used for construction of ICP-MS calibration curves.

2.1.1.3 Acids and reagents

Concentrated analytical grade nitric acid (HNO_3, 65%), concentrated analytical grade hydrochloric acid (HCl, 36%), and hydrogen peroxide (H_2O_2, 6% w/v) were obtained from Scharlau (Gota Perez, Spain), and they were used for MVM digestion.

2.1.1.4 Multivitamin/multimineral standard reference material tablets (SRM3280)

Standard reference material (SRM 3280) is intended primarily for use in validating analytical methods for the determination of vitamins, carotenoids, and elements in dietary supplement tablets and similar matrices. This SRM can also be used for quality assurance when assigning values to in-house control materials. A unit of SRM 3280 consists of five bottles, each containing 30 tablets. The SRM is provided as whole tablets because some of the vitamins are coated or encapsulated to provide stability and grinding would compromise this coating. Each tablet weighs approximately 1.5 g containing Ar (0.132

µg/g), B (0.141 mg/g), Ca (110.7mg/g), Cd (80.15 µg/g), Co (0.8 µg/g), Cr (93.7µg/g), Cu (1.4 mg/g), Fe (12.35 mg/g), K (53.1mg/g), La (0.7 µg/g), Mg (67.8mg/g), Mn (1.44mg/g), Mo (70.7µg/g), Na (330µg/g), Ni (8.4µg/g), Pb (0.2727µg/g), Sb (0.159µg/g), Se (17.42µg/g), Si (2010µg/g), Sn (11µg/g), Sr (29.8µg/g) Ti (5400µg/g), V (8µg/g) and Zn (10.15mg/g). Obtained from the National Institute of Standards and Technology (NIST; Gaithersburg, MD, USA), namely multivitamin/multimineral tablets (SRM 3280).

2.1.1.5 Sampling of multivitamin/multimineral tablets/capsules (MVM)

The samples of the multivitamin/multimineral formulations are used for pregnant women and diabetic patients available in Sudan, contains all or most recognized minerals (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn) and vitamins, at levels close to daily intake values. and they are imported from aboard. They were collected randomly from different pharmaceutical markets (Khartoum, Omdurman and Khartoum North) in Sudan at the time of study, table 2.1 shows a full detail of all multivitamin/multimineral formulation samples used for pregnant women and diabetic patients (**93** different MVM). All samples were kept in the original package until the execution of the experiments.

A. Multivitamin/multimineral formulations (MVM) were used for diabetics: 45 samples collected randomly from local Sudanese pharmaceutical markets, mainly from Khartoum State.
B. Multivitamin/multimineral formulations (MVM) were used for pregnant women: 48 samples collected randomly from local Sudanese pharmaceutical markets, mainly from Khartoum State.

Table 2.1 Details of collected samples of multivitamin/multimineral formulations for pregnant women and diabetic patients from different pharmaceutical markets in Sudan

Sample No.	Type of formulation	Country of origin	Used for	Elements constituents
1	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
2	Coated Capsules	Italy	Male	Ca, Cr, Cu, Mg, Mn, Se and Zn
3	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
4	Coated Capsules	UK	Female/Male	Cr, Cu, Fe, Mg, Mn, Se and Zn
5	Hard gelatin Capsules	France	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Se and Zn
6	HPMC Capsules	UK	Male	Cr, Cu, Fe, Mg, Mn, Se and Zn
7	Hard gelatin Capsules	Switzerland	Female	Ca, Cr, Cu, Fe, Mg, Mn, Se and Zn
8	Hard gelatin Capsules	UK	Female	Cr, Cu, Fe, Mg, Mn, Se and Zn
9	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Se and Zn
10	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Se, V and Zn
11	Coated Capsules	USA	Female	Cr, Cu, Mg, Mn, Se and Zn
12	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
13	Coated Capsules	Canada	Female/Male	Cu and Zn

Table 2.1 (Cont.)

Sample No.	Type of formulation	Country of origin	Used for	Elements constituents
14	Coated Capsules	UK	Female/Male	Mg and Zn
15	Hard gelatin Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
16	HPMC Capsules	UK	Female	Cu, Fe and Zn
17	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
18	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Se and Zn
19	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Se, V and Zn
20	Coated Capsules	USA	Female	Cr, Cu, Mg, Mn, Se and Zn
21	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
22	Coated Capsules	Canada	Female/Male	Cu and Zn
23	Coated Capsules	UK	Female/Male	Mg and Zn
24	Hard gelatin Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
25	HPMC Capsules	UK	Female	Cu, Fe and Zn
26	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn

Table 2.1 (Cont.)

Sample No.	Type of formulation	Country of origin	Used for	Elements constituents
27	Coated Capsules	USA	Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se, Zn and V
28	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
29	Coated Capsules	Italy	Male	Ca, Cr, Cu, Mg, Mn, Se and Zn
30	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
31	Coated Capsules	UK	Female/Male	Cr, Cu, Fe, Mg, Mn, Se and Zn
32	Hard gelatin Capsules	France	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Se and Zn
33	HPMC Capsules	UK	Male	Cr, Cu, Fe, Mg, Mn, Se and Zn
34	Coated Capsules	USA	Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se, Zn and V
35	Coated Capsules	USA	Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se, Zn and V
36	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
37	Coated Capsules	Italy	Male	Ca, Cr, Cu, Mg, Mn, Se and Zn
38	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
39	Coated Capsules	UK	Female/Male	Cr, Cu, Fe, Mg, Mn, Se and Zn

Table 2.1 (Cont.)

Sample No.	Type of formulation	Country of origin	Used for	Elements constituents
40	Hard gelatin Capsules	France	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Se and Zn
41	HPMC Capsules	UK	Male	Cr, Cu, Fe, Mg, Mn, Se and Zn
42	Hard gelatin Capsules	Switzerland	Female	Ca, Cr, Cu, Fe, Mg, Mn, Se and Zn
43	Hard gelatin Capsules	UK	Female	Cr, Cu, Fe, Mg, Mn, Se and Zn
44	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Se and Zn
45	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Se, V and Zn
46	Coated Capsules	USA	Female	Cr, Cu, Mg, Mn, Se and Zn
47	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
48	Coated Capsules	Canada	Female/Male	Cu and Zn
49	Coated Capsules	UK	Female/Male	Mg and Zn
50	Hard gelatin Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
51	HPMC Capsules	UK	Female	Cu, Fe and Zn
52	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn

Table 2.1 (Cont.)

Sample No.	Type of formulation	Country of origin	Used for	Elements constituents
53	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Se and Zn
54	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Se, V and Zn
55	Coated Capsules	USA	Female	Cr, Cu, Mg, Mn, Se and Zn
56	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
57	Coated Capsules	Canada	Female/Male	Cu and Zn
58	Coated Capsules	UK	Female/Male	Mg and Zn
59	Hard gelatin Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
60	HPMC Capsules	UK	Female	Cu, Fe and Zn
61	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
62	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
63	Coated Capsules	Italy	Male	Ca, Cr, Cu, Mg, Mn, Se and Zn
64	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
65	Coated Capsules	UK	Female/Male	Cr, Cu, Fe, Mg, Mn, Se and Zn

Table 2.1 (Cont.)

Sample No.	Type of formulation	Country of origin	Used for	Elements constituents
66	Hard gelatin Capsules	France	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Se and Zn
67	HPMC Capsules	UK	Male	Cr, Cu, Fe, Mg, Mn, Se and Zn
68	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
69	Coated Capsules	Italy	Male	Ca, Cr, Cu, Mg, Mn, Se and Zn
70	Coated Capsules	Canada	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
71	Coated Capsules	UK	Female/Male	Cr, Cu, Fe, Mg, Mn, Se and Zn
72	Hard gelatin Capsules	France	Female/Male	Ca, Cr, Cu, Fe, K, Mg, Mn, Se and Zn
73	HPMC Capsules	UK	Male	Cr, Cu, Fe, Mg, Mn, Se and Zn
74	Hard gelatin Capsules	Switzerland	Female	Ca, Cr, Cu, Fe, Mg, Mn, Se and Zn
75	Hard gelatin Capsules	UK	Female	Cr, Cu, Fe, Mg, Mn, Se and Zn
76	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Se and Zn
77	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Se, V and Zn
78	Coated Capsules	USA	Female	Cr, Cu, Mg, Mn, Se and Zn

Table 2.1 (Cont.)

Sample No.	Type of formulation	Country of origin	Used for	Elements constituents
79	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
80	Coated Capsules	Canada	Female/Male	Cu and Zn
81	Coated Capsules	UK	Female/Male	Mg and Zn
82	Hard gelatin Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn
83	HPMC Capsules	UK	Female	Cu, Fe and Zn
84	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
85	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Se and Zn
86	Coated Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Se, V and Zn
87	Coated Capsules	USA	Female	Cr, Cu, Mg, Mn, Se and Zn
88	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn
89	Coated Capsules	Canada	Female/Male	Cu and Zn
90	Coated Capsules	UK	Female/Male	Mg and Zn
91	Hard gelatin Capsules	USA	Female	Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se and Zn

Table 2.1 (Cont.)

Sample No.	Type of formulation	Country of origin	Used for	Elements constituents
92	HPMC Capsules	UK	Female	Cu, Fe and Zn
93	Hard gelatin Capsules	UK	Female	Cu, Fe, Mg and Zn

2.1.2 Equipment

2.1.2.1 Water purification system

For all analytical procedures (Dilution of MVM digestion samples and preparation calibration curves standard solutions) deionized purified water (0.055 μS/cm), was obtained by Barnstead water purification system ASTM Type II (Thermo Electron LED GmbH, Germany).

2.1.2.2 Analytical balance

Nimbus (NBL 254i) highly sensitive electronic analytical balance, from Adam Equipment Co.ltd., U.K, with weighing chamber to prevent the samples from being affected by air currents (readability 0.01mg, capacity 250 g) was used for samples weight.

2.1.2.3 Microwave assisted acid digestion system

A START D microwave digestion system from Milestone (Germany), consisting of reaction sensors for pressure, temperature control, monochrome touch-screen industrial grade controller screen, and high-pressure digestion PTFE vessels 100 ml was used for total digestion of MVM samples as shown in figure 2.1. The maximum power and temperature for this instruments are 1200W and 300°C, respectively. It is noteworthy that A START D is equipped with non-contact temperature monitoring and infrared control in all containers, as well as direct monitoring of pressure in the reference vessel, and direct monitoring of pressure control up to 100 bar in the reference vessel. Microwave cavity is entirely made of 18/8 stainless steel housing with multi-layer PTFE plasma coating applied at over 350°C; it's equipped with a heavy duty air flow system, placed above the microwave cavity. The air flow rapidly cools the external surfaces of the vessels. An acid resistant flexible hose connects the exhaust fan to a fume hood ensuring a safe working environment. Total of safety interlocks 4 micro-switches to prevent microwave emission with door open. Figure 2.2 shows the start D closed microwave assisted digestion system.

Figure 2.1 High-pressure digestion vessels 100 ml PTFE

Figure 2.2 Start D closed microwave assisted digestion system

2.1.2.4 Atomic absorption

Atomic absorption spectrometer (Varian, Mulgrave, Australia) consist of AA240FS flame (fast sequential), GTA 120 (graphite tube atomizer) and single hollow cathode lamps were used for determination of Fe, Mg, Zn, Cu, Mn, Cr and Se. The equipment had been used after optimization by blank solution (3% HNO_3) as recommended by the manufacturer. Figure 2.3 shows Varian atomic absorption spectrometer.

Figure 2.3 Varian atomic absorption spectrometer (AAS) instrument

2.1.2.5 Inductively coupled plasma-mass-spectrometry (ICP-MS)

Agilent ICP-MS 7800 (Model G8421A, USA) consists of per pump (ISIS) sample introduction, Aqueous solution plasma ignition mode, x-lens ion lenses model (Micromist Nebulizer), helium (He) collision, quadrupole mass-spectrometer, and orthogonal detector system (ODS). Figure 2.4 shows the main components of ICP-MS 7800 which was used for determination of selected element. The equipment was operated under the optimum conditions, which are shown in table 2.2.

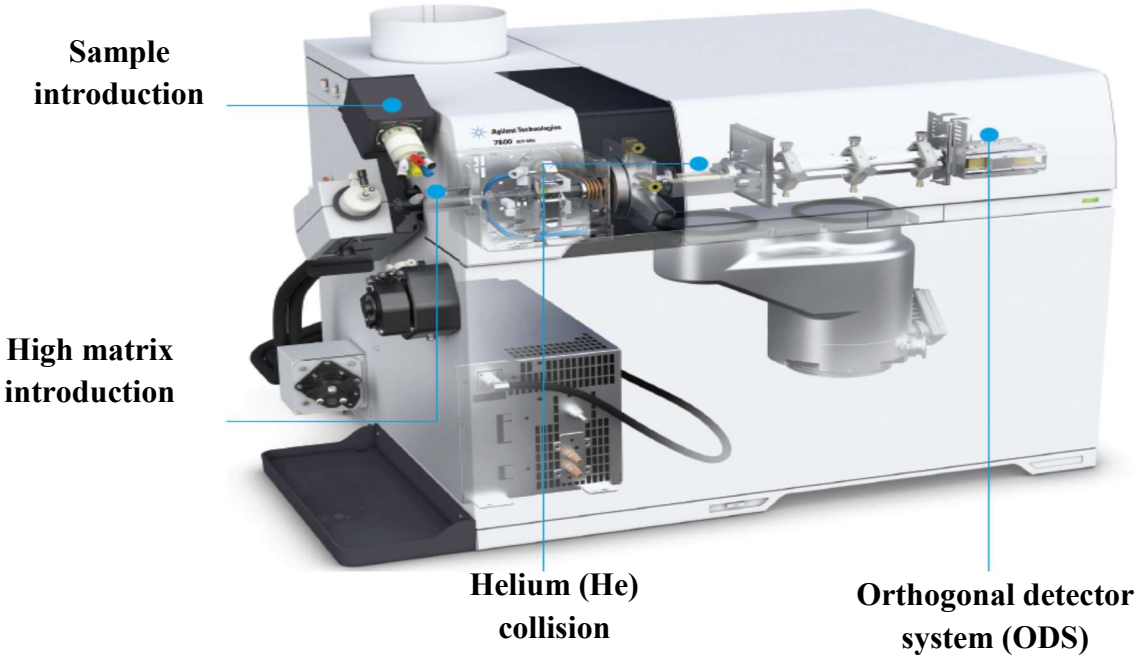

Figure 2.4 The main components of ICP-MS 7800

2.1.2.6 Other equipment

All glassware were cleaned with 5% nitric acid, rinsed with deionized water, and dried at 60°C.

Volumetric flasks – polypropylene, covering three volume 50ml, 100ml and 1000ml. as needed for preparation and storing samples, standards and reagents.

Micropipettes – variable, covering three ranges 1 - 10 µl, 10-100 µl, and 100-1000 µl.

Containers – polypropylene 100 ml, all working standard solutions and digested samples, were stored in polypropylene containers.

Agate mortar and pestle – Size: internal diameter 70 mm (2.8"); external diameter 90 mm (3.5") and inner depth 21 mm (0.8").

Table 2.2 ICPMS operation condition for determination of element constituents in MVM

ICP-MS condition	Value	
RF power (W)	1550	
Sample Depth (mm)	8.0	
Sample up-take (sec)	30	
Nebulizer type	MicroMist	
Carrier gas (l/min)	1.05	
Make up gas (l/min)	0.15	
Plasma gas (l/min)	15	
Nebulizer Pump (rps)	0.1	
S/C temp(°C)	2	
He or H_2 gas (ml/min)	No gas Mode	He Mode Mode
	0	5
Extract 1 (V)	0	0
Extract 2 (V)	160	160
Omega Bias (V)	-90	-90
Omega Lens (V)	10	10
Cell Enrance (V)	30	30
Cell Exit (V)	-50	-60
Oct P RF (V)	160	160
Oct P Basic (V)	-8	-18
Energy Discrimination (V)	5	3
Points/peak	3	
Repetitions	3	
Integration time /mass (sec)	0.3	

2.2 METHODS

2.2.1 Optimization of acid mixtures and microwave digestion by AAS

2.2.1.1 Sample preparation procedures

Owing to the lack of reference materials at the time of start study, MVM solid preparations were purchased from Vitabiotics (London, England) which were used for the home control (CS) samples. Prior to analysis, a set of 20 capsules were manually crushed with an agate mortar and pestle, homogenized and sieved through a plastic sieve with a pore diameter of 1 mm.

2.2.1.2 Acid mixtures

Total digestion of the samples was performed by mixing about 0.4 g of the sample powder with various mixtures of HNO_3, HCl and H_2O_2 (Table 2.3), in order to find the most effective mixture for elements extraction from MVM. The containers were closed, placed into the oven cavity and subjected to the following three-step heating program: (1) irradiation for 10 minutes at a power of 500 W; and (2) irradiating for 10 minutes at a power of 700 W; (3) After cooling the containers were opened and their contents were filtered and quantitatively transferred into 100 ml polypropylene volumetric flasks. The volumes were made up to the mark with purified water. The concentrations of the elements of interest (Fe, Mg, Zn, Cu, Mn, Cr, and Se) were determined by AAS in the solution after appropriate dilutions to the corresponding linear calibration curves ranges.

2.2.1.3 Optimization of microwave digestion

The objective of the optimization of the experimental conditions of the digestion procedure is to examine the effect of the microwave power, the duration of radiation and variation of the mass ratio of the sample to the oxidant mixture volume. In six separate runs, the digestion powers of the second stage of the microwave oven were changed to 400, 500, 600, 700, 800 and 900 W, and all other parameters were kept constant (sample mass 4g, radiation period 10min). The radiation period of the second stage of the microwave were changed to 10 minutes, 13 minutes, 16 minutes, 19 minutes, 22 minutes

and 25 minutes. all other parameters were kept constant (sample mass 0.4g, radiation power 600W). Finally, in different vessels, 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 g of sample were measured and dissolved in 5 ml of HNO_3, 0.5 ml of HCl and 1 ml of H_2O_2 using digestion powers of the second stage 600W for 16min. Each sample was replicated three times and a blank digestion was performed in the same manner (digestion conditions) and contained the same acid concentration in the final solution. The concentrations of the elements of interest (Fe, Mg, Zn, Cu, Mn, Cr, and Se) were determined by AAS in the solution after appropriate dilutions to the corresponding linear calibration curves ranges.

Table 2.3 Combination of oxidizing acidic mixtures for extraction of elements from MVM

Mixture Number	65% HNO_3 ml	37% HCl ml	6% H_2O_2 ml
1	3	0	0
2	4	0	0
3	5	0	0
4	5	0.3	0
5	5	0.5	0
6	5	0.7	0
7	5	0.5	1
8	5	0.5	2
9	5	0.5	3
10	5	0	2
11	5	0	3

2.2.1.4 Method validation

The objective of method validation was to demonstrate that the method is suitable for its intended purpose as it is stated in ICH guidelines. The method was validated for linearity, precision (repeatability, inter-day and intra-day precision) and accuracy.

2.2.1.5 Application of the method

For applicability of method validation, eight capsule samples of MVM for pregnant woman and diabetic patients were digested under optimum conditions and determined by AAS.

2.2.2 ICP-MS method

In this section microwave digestion procedure for the determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn in multivitamin/multimineral (MVM) used for pregnant women and diabetic patients employing ICP-MS was optimized by factorial design 3^3 (27 runs). The microwave digestion condition temperature (160, 180 and 200ºC), acid mixture volume (3.9, 5.2 and 6.5ml) and radiation period (10, 15 and 20 min) were selected as factors.

2.2.2.1 Samples and reference material preparation procedures

Prior to analysis, a set of 20 capsules were taken from MVM reference material standard (SRM 3280) bottles (four capsules from each of five bottles) and were manually crushed with an agate mortar and pestle to obtain the homogenous powder. The homogenous powder was sieved through a plastic sieve with a pore diameter of 1mm. Later, 0.1g capsule powder were weigh in PTFE vessels for 27 factorial runs and the conditions including acid mixture (5ml HNO_3: 0.5ml HCl :1ml H_2O_2) volume, microwave temperature and radiation periods were set according to factorial design run. The radiation power was 600W. The digested sample was filtrated and was diluted to 100 ml with deionized water.

A set of 20 commercial solid MVM capsule samples each for pregnant women and diabetic patients were homogenised out in the same way as for reference material standard, pretreated and measured by ICPMS after optimization and method validation.

2.2.2.2 Optimization strategy

A recent investigation reported that acid mixture (5ml HNO_3, 0.5ml HCl and 1ml H_2O_2), sample weight 0.1g, and radiation power 600W are effective enough to extract MVM samples (Albadri *et al.*, 2019). Therefore, full factorial design (3^3) with 27 runs was performed in the current study. Three factors including acid mixture volume (Acid

Vol.), radiation period (Radiation Per.) and microwave temperature (Temp.), were chosen as the variables to obtain the influence of the factors, interaction of factors, and optimum conditions to extract elements simultaneously. The factorial levels are given in table 2.4, and the experimental work was carried on a reference standard MVM SRM 3280.

2.2.2.3 Validation method

The procedure was validated through the analysis of linearity, precision and accuracy parameters, for quantitative analysis of the interesting elements in MVM samples. Linear eight-point calibration curves (0.1 - 150 ng/ml) for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn were constructed from multielement standard solution; the data of calibration curve including correlation coefficient (R^2) detection limits (DL), and background equivalent concentration (BEC) of detector response were calculated.

To determine accuracy of the method, reference materials SRM 3280 was digested under the optimum microwave conditions (acid volume (6.5ml), and radiation (20min) with temperature (160ºC)) for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. Accuracy was expressed as the recovery percentage (R%) of each element. The element concentration values were found and compared with their concentration values in certificate of SRM 3280.

Precision of the method expressed as relative standard deviation (RSD%) was evaluated as repeatability intra-day and inter-day. Repeatability intra-day was calculated after analysing reference materials SRM 3280 ten times in one day. In order to study the repeatability inter-day, furthermore, the reference material SRM 3280 was analysed during three consecutive days.

Table 2.4 Variables and levels for the factorial design 3^3

Factor	Low	Central point	High
Acid mixture volume (Acid Vol.) ml	3.9	5.2	6.5
Microwave temperature (Temp.)°C	160	180	200
Radiation period (Radiation Per.) min	10	15	20

2.2.2.4 Application of the method

To investigate applicability of validation of ICPMS method, six MVM capsule samples for pregnant women and diabetic patients were digested under optimum conditions acid mixture volume (Acid Vol.), Radiation period (Radiation Per.), and microwave temperature (Temp.) and concentration of elements Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn were determined.

2.2.2.5 Statistical analysis

Statistical process including relative standard deviation**,** recoveries**,** standard deviation, and multiple response (MR) were calculated using the Excel Software, Windows version 2010. Factorial design analysis, and analysis of variance (ANOVA) were processed using MINTAB 19.

2.2.3 Monitoring thirteen elements (macro and micro) in MVM available in Sudan

The concentration of thirteen macro and micro elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn) in MVM preparations for pregnant women and diabetic patients available in Sudan pharmaceutical markets were simultaneously determined, each type was collected.

A set with 10 tablets for each sample was manually grinded with an agate mortar and pestle, homogenised and sieved through a 1mm pore diameter plastic sieve.

Approximately 0.1g capsules/tablets powder were weigh accurately in PTFE vessels and digested with 6.5ml acid mixture under microwave digestion conditions radiation power 600W, radiation period 20min, and microwave temperature 160ºC.

After cooling, the vessels were opened and their contents were filtered into 100mL volumetric flasks. The volumes were made up to the mark with purified water. The concentrations of the metals of interest (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn) were determined by ICPMS in the solution after suitable dilutions to fit working linear ranges.

CHAPTER THREE

Results and discussion

CHAPTER THREE

3.1 RESULTS AND DISCUSSION

3.1.1 Oxidant mixture and microwave digestion optimization by AAS

3.1.1.1 Oxidant mixture

When examining the efficiency of the oxidant mixture using HNO_3 alone for digestion, a yellow color and white solid residue was observed. However, on increasing the volume of HNO_3 the color partially disappeared and the residue diminished. But on using a mixture of HNO_3, HCl, and H_2O_2, the yellow color completely disappeared. Some earlier investigators reported the same observation including Araújo *et al.* (2002). Table 3.1 shows recoveries under different oxidant mixtures. Less recoveries were (67% - 91%) observed when HNO_3 (3ml) was used; increasing HNO_3 volume (5 ml to 7 ml) did not affect recoveries of elements except those for Se, Zn, and Cu (R%>94%). An obvious increase in recoveries was observed for some elements Se, Mn, Mg, and Cr after using a mixture of HNO_3 (5 ml), HCl (0.3 ml) and adding more HCl (0.5 to 0.7 ml) showed gradual increase in recoveries (R% between 86% - 96%) because chlorides are in general terms soluble (Dolan *et al.*, 2003). An improved extraction efficiency of elements was observed when an oxidant mixture HNO_3, HCl, and H_2O_2 was used. However, excellent recoveries (94% - 101%) were observed by using an oxidant mixture combination of 5 ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2. To ensure that a complete release of remaining elements bound to white solid residue, any increase in the H_2O_2 volume gives negative effect on recovery of most elements. HNO_3 can also be used together with HCl and H_2O_2 to improve the performance of digestion, and avoid interference with the residual in agreement with results reported by Dolan *et al.* (2003) and Saavedra *et al.* (2004). Poor recoveries were observed when a mixture of HNO_3, and H_2O_2 was used (R% between 45% and 93%). This experiment demonstrated that the oxidant mixture No.7 (5 ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2) used in microwave digestion of MVM was significant for almost all the elements. Therefore, it was chosen as the best overall combination for the microwave digestion process as shown in figure 3.1.

Table 3.1 Effect of 11 oxidant mixtures on recoveries% of some elements using microwave digestion, weight 0.4 g MVM, radiation power 700 W and radiation period 10 min

Sample	Acid mixture	Recovery Fe %	Recovery Zn %	Recovery Cr%	Recovery Se %	Recovery Mn %	Recovery Mg %	Recovery Cu %
Sample ID M1 of 3	Oxidant mixture 1	89.52	93.03	93.61	67.94	91.22	90.52	90.05
Sample ID M2 of 3	Oxidant mixture 2	91.30	93.92	92.64	70.10	90.38	89.95	89.76
Sample ID M3 of 3	Oxidant mixture 3	90.56	94.04	91.05	71.73	89.64	89.77	90.99
Sample ID M4 of 3	Oxidant mixture 4	89.41	93.47	95.01	97.27	91.72	92.57	86.53
Sample ID M5 of 3	Oxidant mixture 5	90.75	96.83	95.40	96.15	92.25	94.62	89.66
Sample ID M6 of 3	Oxidant mixture 6	89.68	95.19	95.15	94.81	92.23	89.83	96.57
Sample ID M7 of 3	Oxidant mixture 7	93.98	96.99	97.98	97.84	101.64	99.74	94.99
Sample ID M8 of 3	Oxidant mixture 8	89.47	85.34	84.77	80.40	75.65	85.74	88.42
Sample ID M9 of 3	Oxidant mixture 9	90.39	84.09	89.91	89.06	80.37	90.96	91.65
Sample ID M10 of 3	Oxidant mixture 10	88.47	92.19	45.18	81.64	79.07	89.76	91.24
Sample ID M11 of 3	Oxidant mixture 11	92.33	93.64	80.66	78.45	86.39	92.07	91.87

Recoveries were calculated by the flowing equation:

Recovery (R%) = (C_{obs} /C_{nat})×100

Where $\boldsymbol{C_{nat}}$ *is the element concentration in the control sample.*

$\boldsymbol{C_{obs}}$ *is the element concentration observation by procedure in the control sample.*

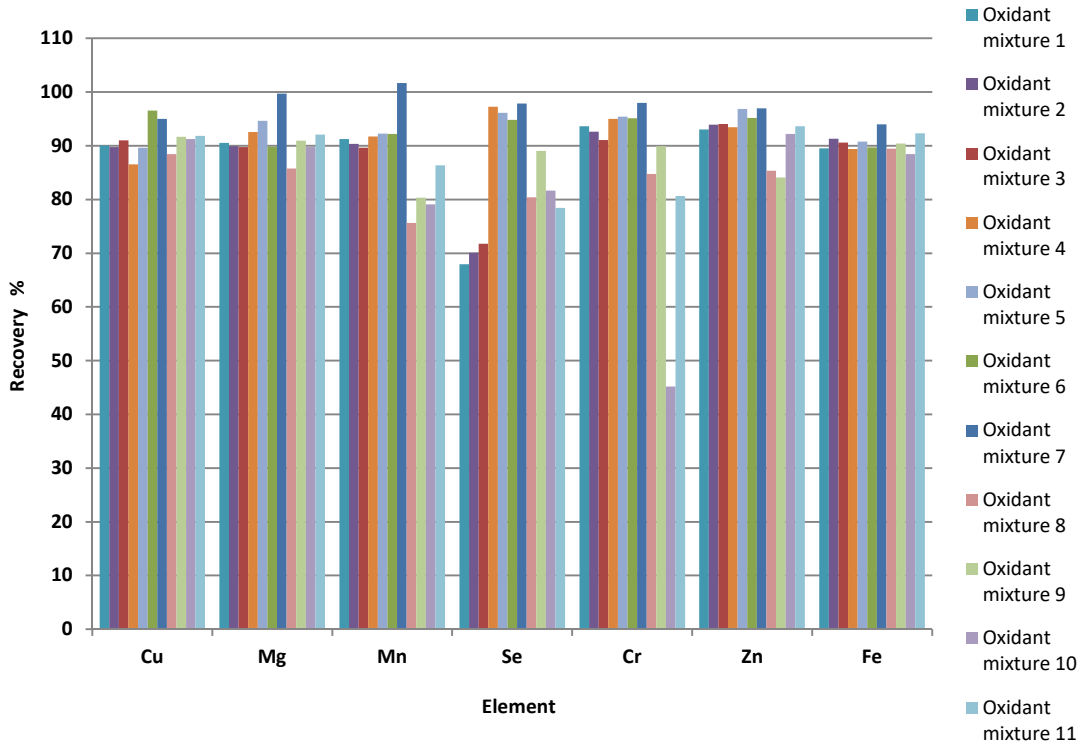

Figure 3.1 Effect of 11 oxidant mixtures on recoveries% of some elements using microwave digestion, weight 0.4 g MVM, radiation power 700 W and radiation period 10 min

3.1.1.2 Optimization of microwave digestion conditions

3.1.1.2.1 Radiation power

Radiation power is known to be an attractive parameter of effect on microwave based sample digestion procedures. For the optimization of the extraction radiation power in the current study, the impact of six radiation powers (400, 500, 600, 700, 800 and 900 W) were studied, at the second stage of the microwave program, and elements recoveries (Fe, Mg, Zn, Cu, Mn, Cr, and Se) were calculated under constant other factors (0.4 g MVM sample, 5 ml HNO_3, 0.5 ml HCl, and 1 ml H_2O_2 and 10 min radiation period) as shown in table 3.2.

From figure 3.2 It can be seen that modified radiation power from 400 to 600W shows gradual improved extraction efficiency for all tested elements (R% 94 - 101%). However, elements Fe, Zn, and Cu did not show much effect between the six investigated radiation power levels. Therefore, the radiation power 600 W selected as the optimum

radiation power in the second step. From the previous study (Krejčová *et al.*, 2006) radiation power 700 W was selected, and authors did not examine other radiation power.

Table 3.2 Effect of radiation power on recoveries % of some elements using microwave digestion, weight 0.4g MVM, radiation period10min, oxidant mixture (5 ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2)

Sample	Radiation Power (W)	Recovery Fe %	Recovery Zn %	Recovery Cr%	Recovery Se %	Recovery Mn %	Recovery Mg %	Recovery Cu %
Sample ID P1 of 3	400 W	90.29	95.64	96.37	81.18	85.85	96.46	94.85
Sample ID P2 of 3	500 W	89.53	95.83	96.18	83.82	90.45	95.25	96.43
Sample ID P3 of 3	600 W	93.44	99.55	99.38	94.53	93.10	101.60	97.28
Sample ID P4 of 3	700 W	92.23	98.26	98.93	87.33	85.56	101.19	93.71
Sample ID P5 of 3	800 W	91.25	98.73	96.02	83.15	85.40	100.66	93.75
Sample ID P6 of 3	900 W	90.71	93.16	83.46	83.53	80.26	94.26	87.23

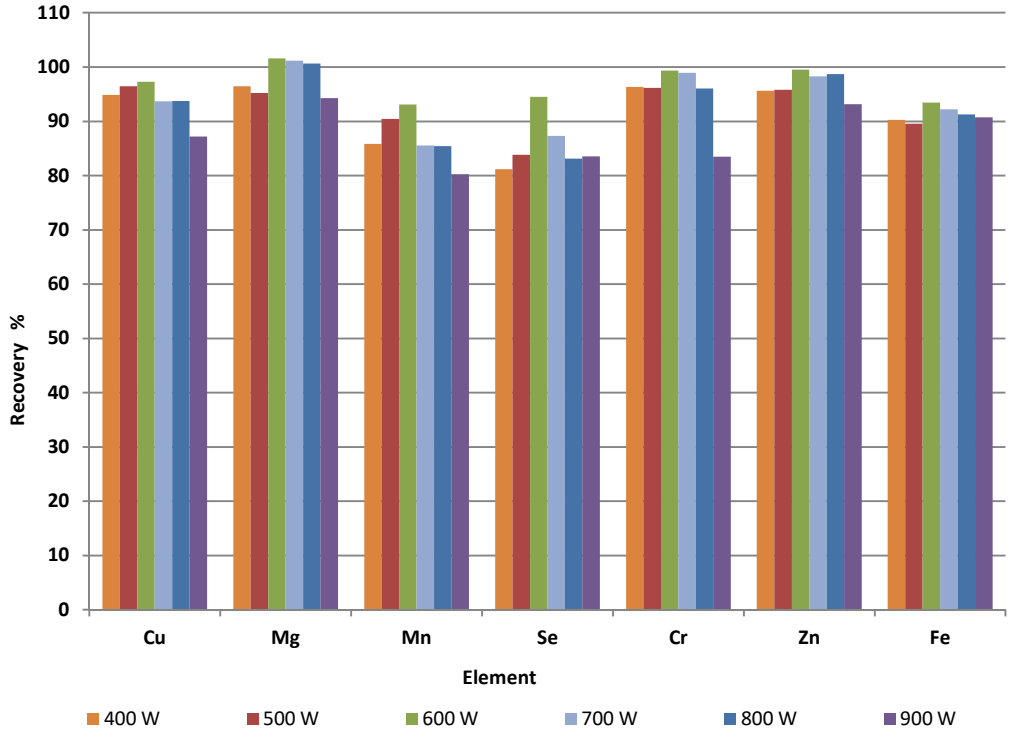

Figure 3.2 Effect of radiation power on recoveries% of some elements using microwave digestion, weight 0.4g MVM, radiation period 10min, oxidant mixture (5 ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2)

3.1.1.2.2 Radiation period

Radiation period is one of the important features of microwave sample digestion methods; their rapidness are compared to that of the conventional methods. Therefore, radiation period is one of the factors that needed to be evaluated for the microwave digestion of MVM. The factor of radiation period was examined from 10 to 25 min and the recoveries against radiation periods are illustrated in figure 3.3. From this figure it can be seen that recoveries of elements were not affected by change of radiation period from 10 to 25 min (R% 97-102%), less effect was observed for Se, Zn, and Cu, and good recoveries were obtained when samples were radiated for 16 min at second step microwave program. Therefore, this radiation period was selected as an optimum radiation period (Table 3.3).

Table 3.3 Effect of radiation period on recoveries% of some elements using microwave digestion, weight 0.4 g MVM, oxidant mixture (5ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2), and radiation power 600 W

Sample	Time (min)	Recovery Fe %	Recovery Zn %	Recovery Cr%	Recovery Se %	Recovery Mn %	Recovery Mg %	Recovery Cu %
Sample ID T1 of 3	10	100.418	94.595	94.334	93.457	96.175	98.530	85.505
Sample ID T2 of 3	13	99.425	98.886	93.540	92.199	95.264	100.044	86.072
Sample ID T3 of 3	16	102.062	99.724	94.050	98.388	97.555	100.787	101.400
Sample ID T4 of 3	19	100.367	98.284	90.294	95.945	94.910	99.321	98.742
Sample ID T5 of 3	22	100.716	100.448	90.573	97.458	94.672	97.705	99.668
Sample ID T6 of 3	25	96.523	100.647	87.219	93.161	89.777	94.708	99.077

3.1.1.2.3 Sample weight

Under optimum radiation period, and radiation power, the effect of MVM weight was studied with a mixture of 5 ml of HNO_3, 0.5 ml of HCl and 1 ml of H_2O_2, and it was evaluated in a range of 0.05 to 0.5 g. Figure 3.4 shows that the change in the sample amount from 0.05 to 0.1 g had an effect on efficiency of extracting recoveries of Se and Cr, but had less effect on that of Fe, Zn, Cu and Mn, and had no effect on that of Mg. However, an increase in the sample amount from 0.1 to 0.5 g showed a drastic decrease in extraction efficiency of all elements studied. These observations are also similar to those reported in the literature (Mketo *et al.*, 2016; Ghanemi *et al.*, 2014; Nomngongo

and Ngila, 2014). The weight of 0.1 g sample was chosen as the best overall weight for the microwave digestion process (Table 3.4).

The optimum conditions of oxidant mixture were 5 ml of HNO_3, 0.5 ml of HCl and 1 ml of H_2O_2, 0.1 g MVM sample, microwave program (1) 10 min irradiation at 500 W power; and (2) 16 min irradiation at 600W power; (3) cooling. As discussed earlier optimum conditions were utilized in all subsequent experiments in order to complete validation (precision, sensitivity, accuracy, LOD and LOQ) of the proposed method.

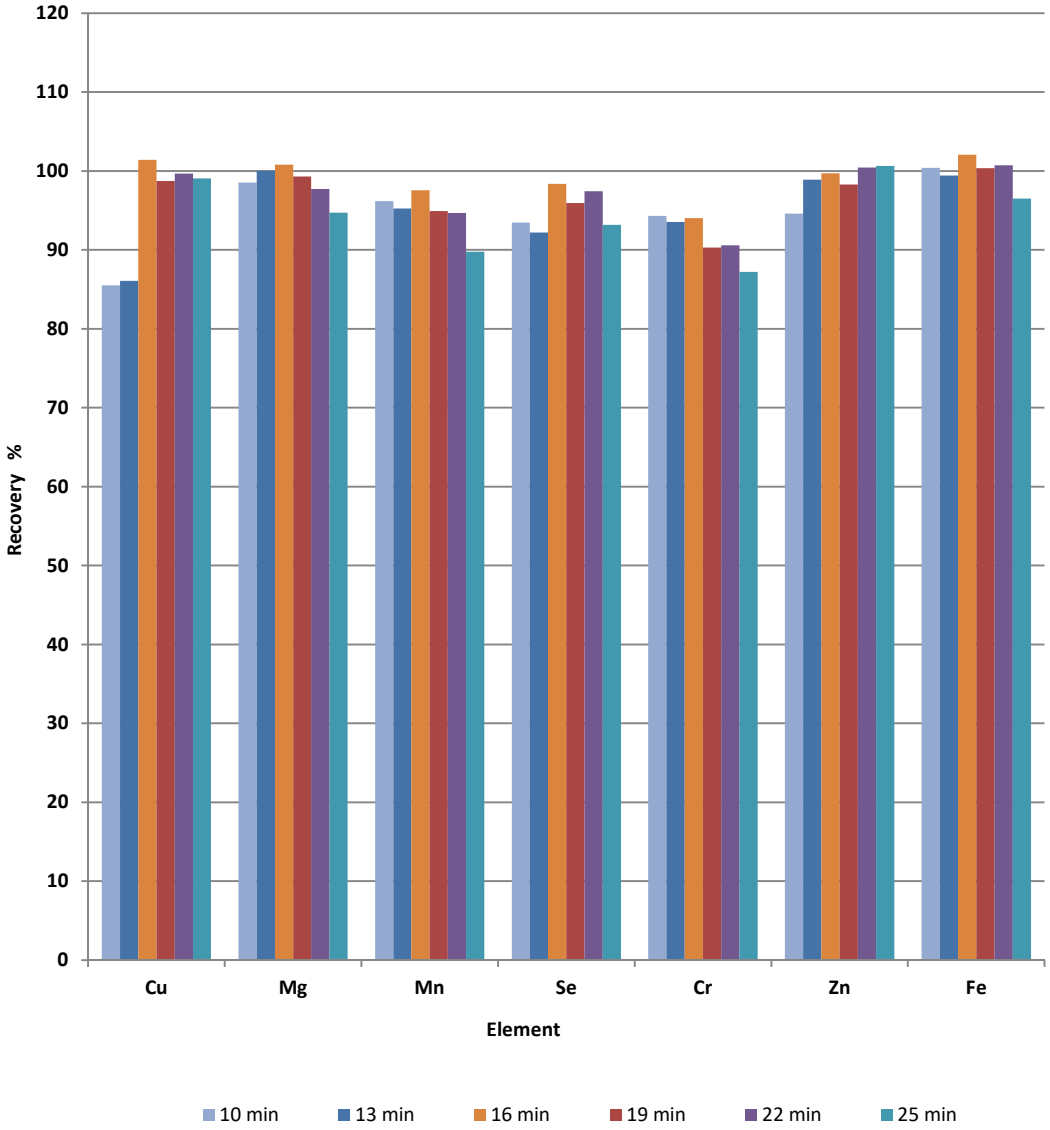

Figure 3.3 Effect of radiation period on recoveries% of some elements using microwave digestion weight 0.4 g MVM, oxidant mixture (5ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2), and radiation power 600 W

Table 3.4 Effect of MVM weight on recoveries% of some elements using microwave digestion at optimum radiation period, radiation power and oxidant mixture (5 ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2)

Sample	Weight (g)	Recovery Fe %	Recovery Zn %	Recovery Cr%	Recovery Se %	Recovery Mn %	Recovery Mg %	Recovery Cu %
Sample ID W1of 3	0.05	96.12	102.33	98.25	103.72	92.60	90.73	99.59
Sample ID W2 of 3	0.1	99.34	98.54	100.91	99.46	100.56	99.69	98.66
Sample ID W3 of 3	0.2	98.26	97.31	98.19	87.51	99.50	96.70	96.77
Sample ID W4 of 3	0.3	97.67	98.25	90.92	82.15	99.53	96.32	94.47
Sample ID W5 of 3	0.4	94.63	94.69	86.54	82.98	94.68	96.12	92.74
Sample ID W6 of 3	0.5	92.67	92.78	85.75	80.42	94.23	95.52	92.43

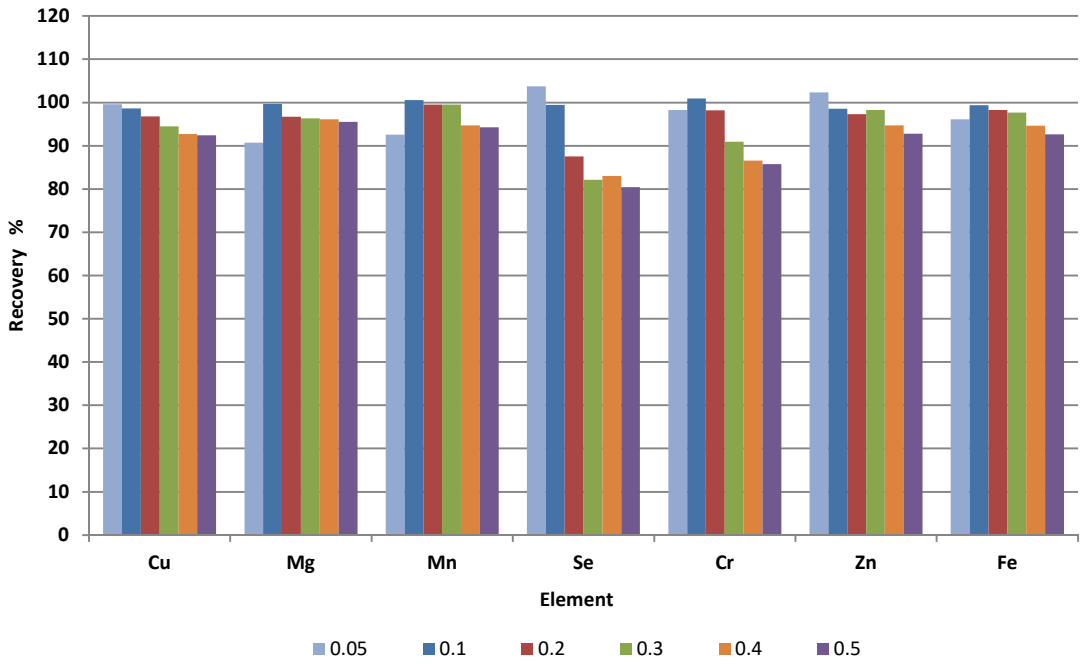

Figure 3.4 Effect of MVM weight on recoveries% of some elements using microwave at optimum radiation period, radiation power and oxidant mixture (5 ml HNO_3, 0.5 ml HCl and 1 ml H_2O_2)

3.1.1.3 Validation of the method

3.1.1.3.1 Calibration curve

For quantitative analysis of the elements in MVM samples, calibration curves were constructed for each element at different concentration levels as shown in figures 3.5, 3.6, 3.7, 3.8, 3.9, 3.10 and 3.11. The data of calibration curve including linearity range, slope, and correlation coefficient, are presented in table 3.5, the linearity is satisfactory in all cases with correlation coefficients (R^2) ranging from 0.9999 to 0.9951.

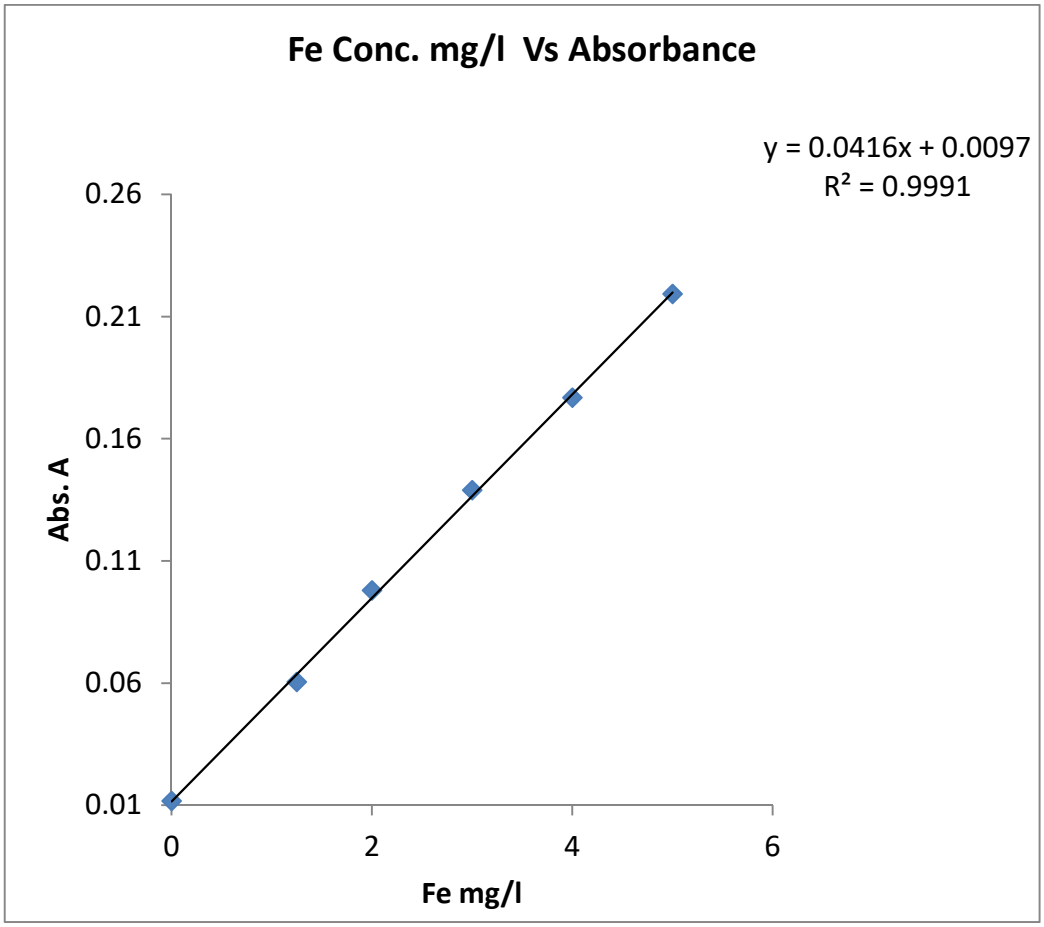

Figure 3.5 Calibration curve of iron (Fe) measured

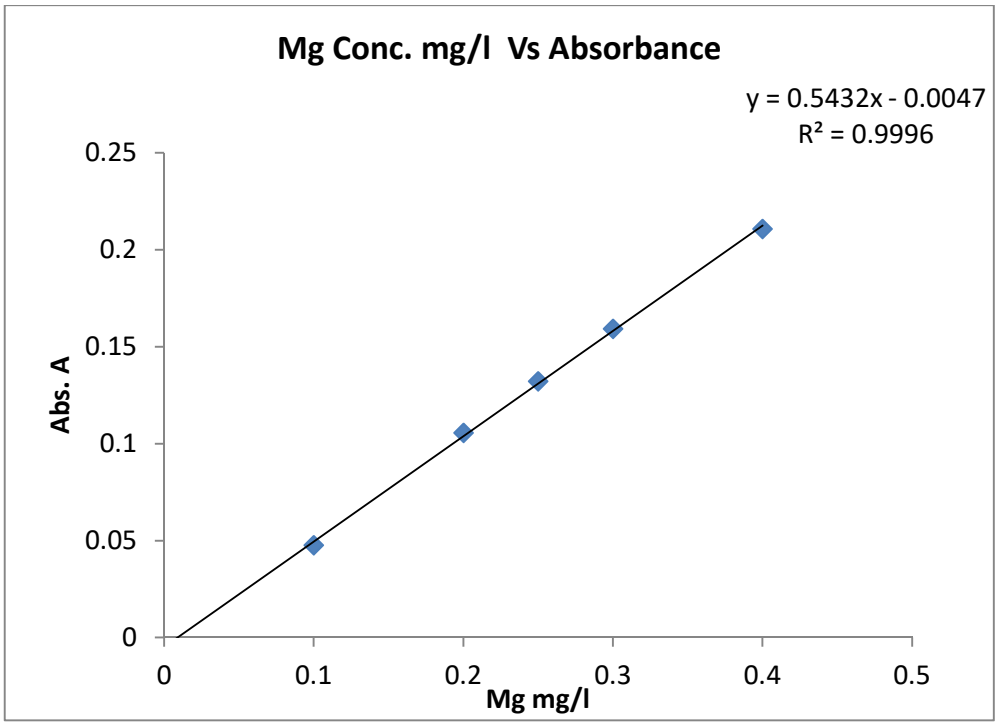

Figure 3.6 Calibration curve of magnesium (Mg) measured

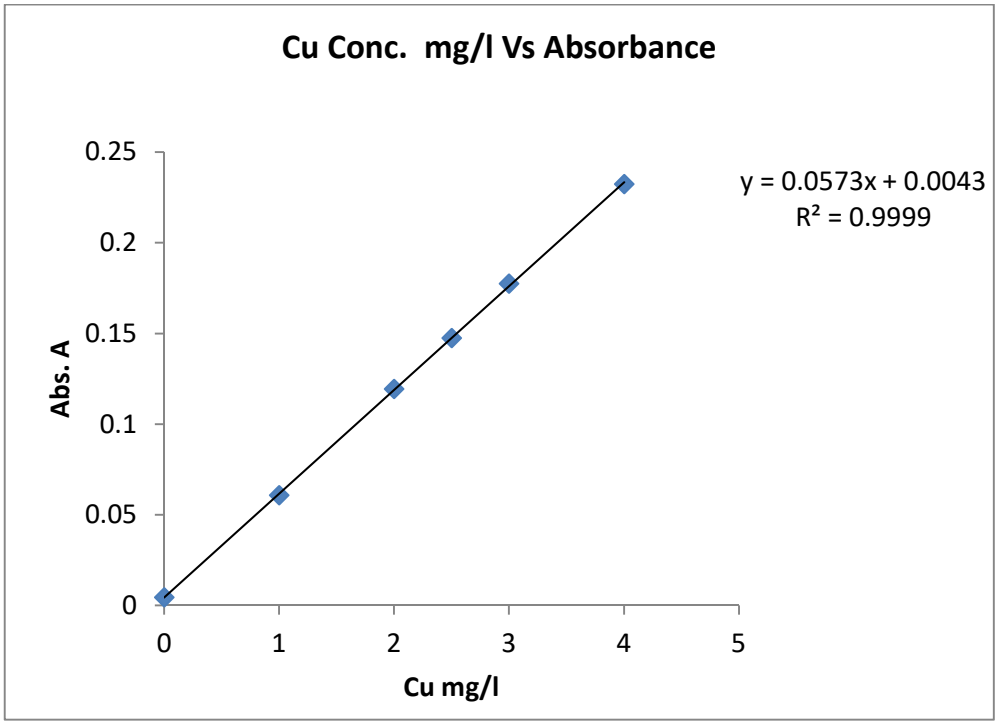

Figure 3.7 Calibration curve of copper (Cu) measured

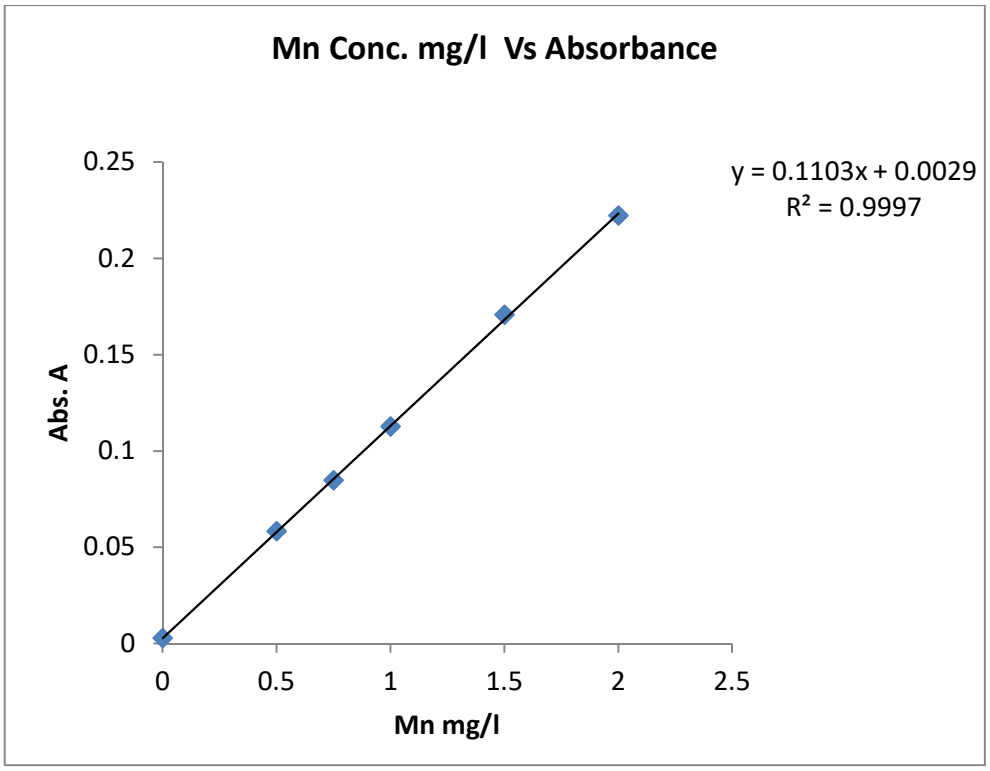

Figure 3.8 Calibration curve of manganese (Mn) measured

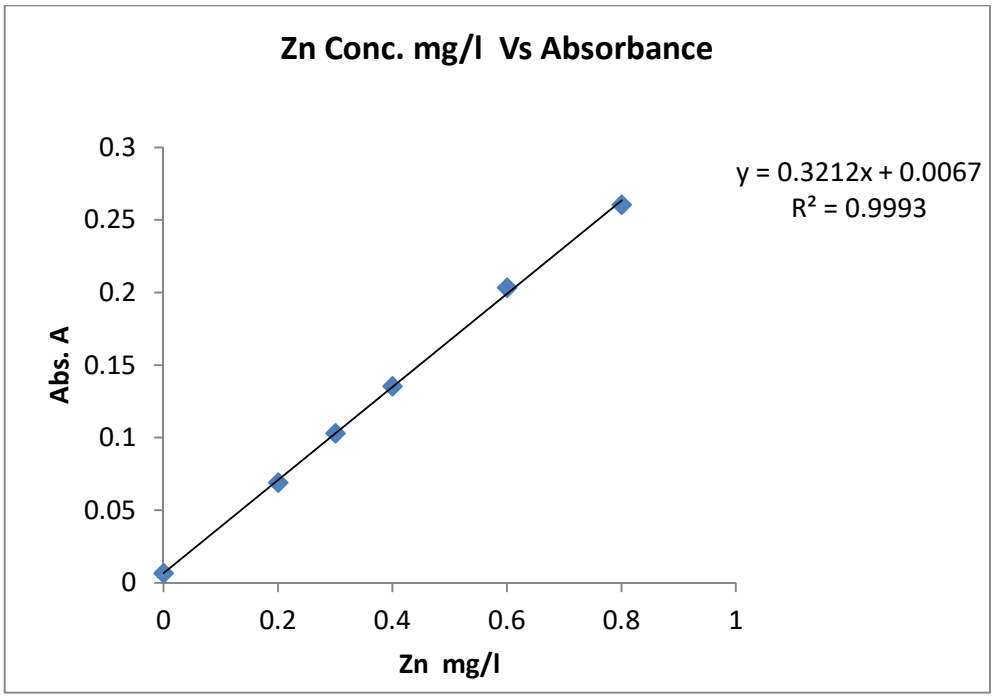

Figure 3.9 Calibration curve of zinc (Zn) measured

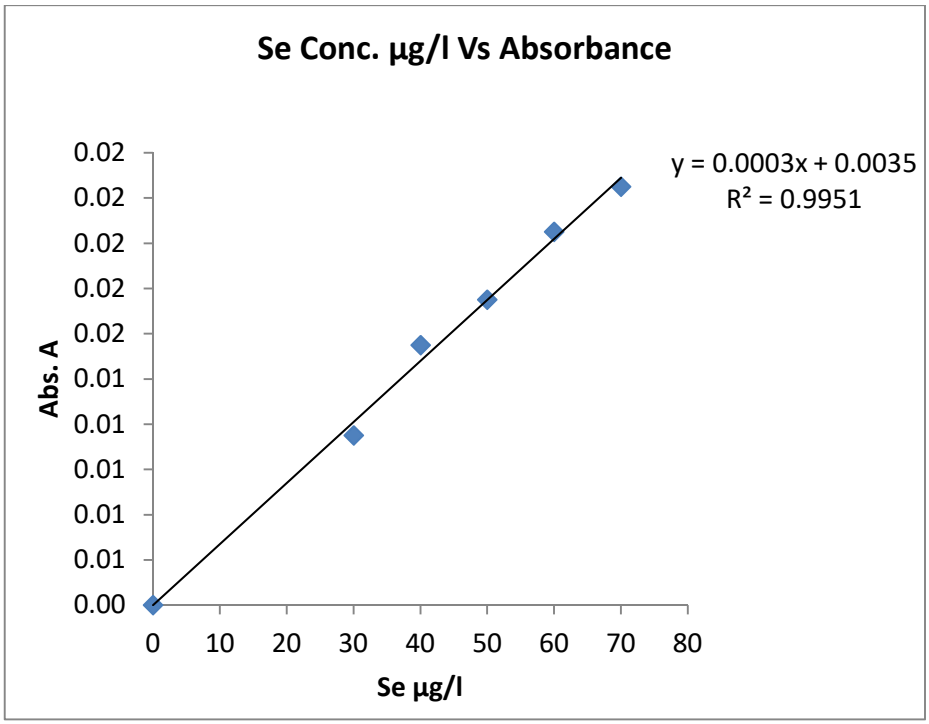

Figure 3.10 Calibration curve of selenium (Se) measured

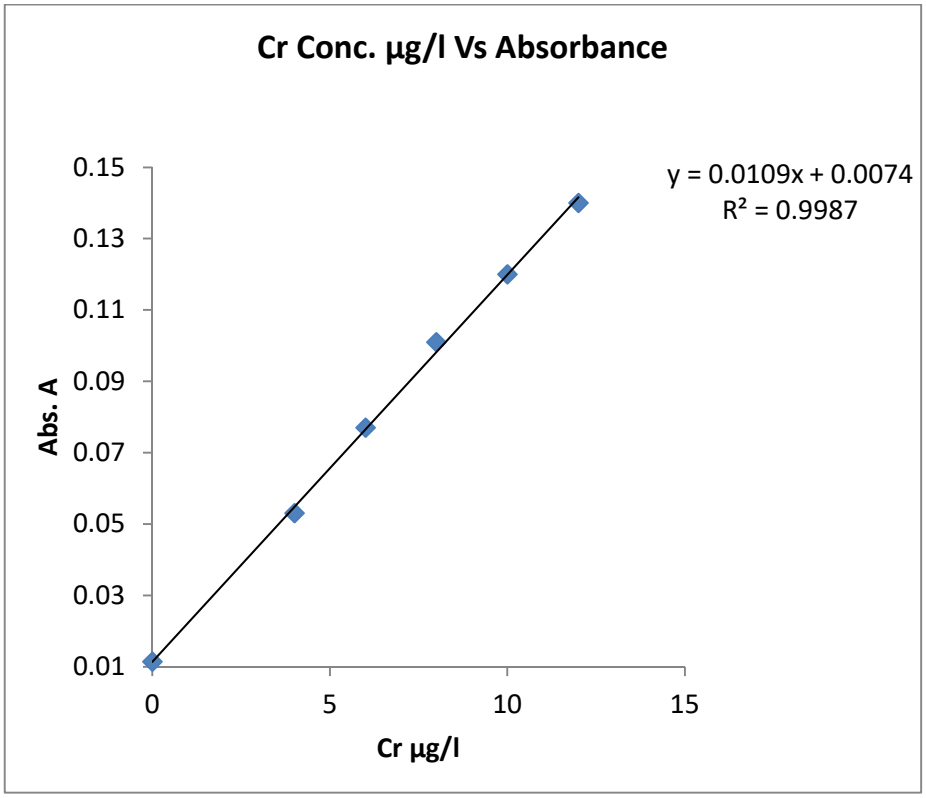

Figure 3.11 Calibration curve of chromium (Cr) measured

Table 3.5 Calibration curve data, including linearity range, slope, intercept, and correlation coefficient of some elements in MVM

Element	Unit	External Calibration linear Range	Slope	Intercept	R^2
Fe	mg/l	1.2-5.0	0.0416	0.0097	0.9991
Zn	mg/l	0.2-0.8	0.3212	0.0067	0.9993
Mg	mg/l	0.1-0.4	0.5432	0.0047	0.9996
Mn	mg/l	0.5-2.0	0.1103	0.0029	0.9997
Cu	mg/l	1.0-4.0	0.0573	0.0043	0.9999
Se	µg/l	30.0-80.0	0.0003	0.0035	0.9951
Cr	µg/l	4.0-12.0	0.0109	0.0074	0.9987

3.1.1.3.2 Precision

Precision (intra-day repeatability and inter-day reproducibility) is expressed as the relative standard deviation (RSD %) of ten independent analyses of the spiked samples of MVM product. Repeatability values ranged from 0.0018 to 0.0329% for Fe, Mg, Zn, Cu, Mn, Cr, and Se. In order to study the intra-day repeatability, furthermore, the MVM samples were analysed ten times during five consecutive days. In order to study the inter-day reproducibility values ranged from 0.0028 to 0.0626% for Fe, Mg, Zn, Cu, Mn, Cr, and Se as shown in table 3.6, the lowest precision values reflect imprecision of total procedure.

3.1.1.3.3 Limit of detection (LOD) and Limit of quantification (LOQ)

The limit of detection (LOD) and limit of quantification (LOQ) for all elements were calculated as 3 and 10 times, respectively, the standard deviation of the response estimated by the standard deviation of *y*-intercepts of regression lines divided by the slope of the calibration curve as suggested by the International Conference on Harmonization

(ICH) (Maroto *et al.*, 2001; Moreno *et al.*, 2008). LOQs and LODs were expressed as µg of metal per mL of solution as shown in table 3.6 LOQs and LODs value reflect the lowest amount of elements which can be determined accurately by the procedure.

Table 3.6 Wavelength (nm), precision, limit of detection (LOD), and limits of quantification (LOQ) for some analysed elements in MVM

Element	Wavelength (nm)	LOD µg/ml	LOQ µg/ml	Precision	
				(RSD%) Intra-day	(RSD%) Interday
Fe	248.3	0.241147	0.803832	0.030982	0.062624
Zn	213.9	0.030813	0.102709	0.003289	0.006483
Mg	285.2	0.013344	0.044482	0.001801	0.008789
Mn	279.5	0.0467	0.155668	0.011635	0.017223
Cu	324.8	0.068446	0.228152	0.02807	0.035696
Se	196	0.00995	0.033166	0.004819	0.006028
Cr	357.9	0.000795	0.002649	0.002277	0.0028

3.1.1.3.4 Accuracy

In this study, because of the lack of sufficient MVM certified reference material (Krejčová *et al.*, 2006), the sample was spiked with the analyte in order to determine a possible proportional bias derived from the sample pretreatment and matrix interference; accuracy was expressed as the recovery percentage of the analyte (Maroto *et al.*, 2001). A solution with known analyte concentration, depending on the actual concentration of elements in a sample, was added to the samples of MVM prepared in triplicate according to three different concentration levels. In order to determine the recovery (R%) for each mineral, the following formula was used:

$$R\% = \frac{C_{obs} - C_{native}}{C_{spiked}} \times 100$$

Where:

C_{native} is the analyte concentration in the unspiked control sample.

C_{obs} is the analyte concentration of an element in the spiked sample.

C_{spiked} is the analyte concentration in the solution added to sample (Maroto *et al.*, 2001; Moreno *et al.*, 2008; Hopfer *et al.*, 2013).

According to the results present in table 3.7, the recoveries for spiked samples were in range of 97-101%. The agreement of the results shows that both the proposed mineralization process of samples and the quantitative determination of elements are correct.

Table 3.7 Recovery% ± RSD (%) of some elements in spiked MVM samples of three different concentration levels

Element	Spike Concentration level 1		Spike Concentration level 2		Spike Concentration level 3	
	Added µg/mg	Recovery (%) ± RSD (%)	Added µg/mg	Recovery (%) ± RSD (%)	Added µg/mg	Recovery (%) ± RSD (%)
Fe	0.02	98.59 ± 0.36	0.04	100.91 ± 0.61	0.08	99.72 ± 0.14
Zn	0.07	101.19 ± 0.56	0.125	99.05 ± 0.46	0.25	100.27 ± 0.47
Mn	0.05	97.30 ± 0.11	0.1	100.57 ± 0.09	0.2	100.55 ± 0.10
Mg	0.7	99.10 ± 3.62	1.25	97.50 ± 5.63	2.5	98.36 ± 2.92
Cu	0.03	98.93 ± 0.05	0.06	99.10 ± 0.04	0.120	100.12 ± 0.057
Cr	0.02	99.79 ± 0.01	0.04	99.136 ± 0.01	0.08	100.13 ± 0.02
Se	0.002	98.54 ± 0.07	0.004	99.85 ± 0.03	0.008	99.64 0.05

3.1.1.3.5 Application of the developed method

In order to demonstrate the applicability of the developed method for the quality control of MVM capsules for pregnant women and diabetic patients, applied to the determination of Cu, Fe, Mn, Mg, Zn, Se and Cr element in different samples purchased from the local market were determined. The results of these experiments are shown in table 3.8. The average concentration of the determined elements corresponded generally to the labelled content and gave an average recovery range of 96% - 104% with relative standard deviation of the repeatability of 1 to 0.0003%. The best results were obtained for Fe, Mg, Zn, Cu, and Mn. Recoveries were also acceptable for Se and Cr, but in two formulations (Se in MVM3 and Cr in MVM7) were found much higher than those labelled, which are marked as **bold** in table 3.8, two formulations samples (MVM 3 and MVM 8) contain, however, unlabelled Fe and Cr, respectively, (Table 3.8).

3.1.2 Factorial design optimization of microwave digestion for macro and micro elements by ICP-MS

3.1.2.1 Optimization of microwave digestion

The microwave digestion conditions temperature (temp.), acid volume (Acid Vol.), and radiation period (Radiation Per.) were optimized to maximize multiple determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. In addition, a factorial experiment was carried out by design of experiments (DOE) using Minitab19. A number of 27 measurements were performed (3^3), as shown in table 3.9. It was found that concentration values for Ca, Fe, Mg, and Na were always at maximum concentration in experiment run 7. However, other elements including Co, Mn, Ni, and Zn showed maximum concentration values in experiment run 9. On the other hand, elements Cr, Cu, K, Se, and V had maximum concentration values in runs 26, 6, 12, 14, and 10, respectively. Considering these points, a factorial design was framed using the multiple response (MR) which was based on the normalization of the concentrations by the highest values to find a single condition that lets the multi-element determination. This assumption reported by other authors (Dos Anjos *et al.*, 2018; de Santana *et al.*, 2016). Table 3.9 shows that the greatest value of MR was observed in experiment run 9 at a digestion temperature of 160ºC, acid volume of 6.5 ml, and at a radiation period of 20 min.

Table 3.8 Comparison of labelled and measured, by AAS, of levels of some elements in some commercial products of MVM for pregnant women and diabetic patients

MVN formulation / Minerals		Fe	Zn	Mg	Mn	Cu	Cr	Se
MVM1	Labelled mg/caps	8	15	100	2	1	0.02	0.01
	Found mg ± RSD%	8.03 ± 0.12	15.70 ± 0.41	100.18 ± 0.66	2.02 ± 0.095	1.00 ± 0.04	0.023 ± 0.0009	0.095 ±0.003
MVM 2	Labelled mg/caps	6	15	50	2	1.5	0.05	0.015
	Found mg ± RSD%	5.92 ± 0.08	15.07 ± 0.22	50.62 ± 0.47	2.02 ± 0.12	1.49 ± 0.03	0.044 ± 0.002	0.016 ± 0.009
MVM3	Labelled mg/caps	___	14	100	2	1.1	0.025	0.025
	Found mg ± RSD%	1.91 ± 0.2	14.47 ± 0.25	103.19 ± 0.85	1.99 ± 0.13	1.08 ± 0.48	0.026 ± 0.0004	**0.030 ± 0.004**
MVM 4	Labelled mg/caps	10	5	50	1	0.5	0.04	0.03
	Found mg ± RSD%	9.90 ± 0.16	4.95 ± 0.09	51.57 ± 0.90	0.98 ± 0.03	0.48 ± 0.02	0.037 ± 0.0006	0.03 ± 0.007
MVM5	Labelled mg/caps	10	5	50	1	0.5	0.04	0.03
	Found mg ± RSD%	10.01 ± 0.12	4.89 ± 0.07	50.10 ± 1.15	1.00 ± 0.03	0.49 ± 0.01	0.039 ± 0.0008	0.03 ± 0.005
MVM 6	Labelled mg/caps	8	15	50	3.5	1.5	0.025	0.05
	Found mg ± RSD%	7.96 ± 0.11	14.97 ± 0.75	50.50 ± 1.08	3.46 ± 0.25	1.51 ± 0.06	0.025 ± 0.0003	0.044 ± 0.002
MVM7	Labelled mg/caps	12	12	100	2.5	1.5	0.05	0.1
	Found mg ± RSD%	12.26 ± 0.09	11.78 ± 0.19	99.94 ± 0.78	2.52 ± 0.03	1.47 ± 0.06	**0.06 ± 0.002**	0.11 ± 0.011
MVM 8	Labelled mg/caps	10	1	10	2.5	2	_	0.05
	Found mg ± RSD%	10.08 ± 0.21	0.98 ± 0.03	9.73 ± 0.44	2.54 ± 0.04	1.98 ± 0.05	0.01 ± 0.001	0.06 ± 0.005

Mean of 3 successive measurements

Table 3.9 Design of microwave digestion conditions by DOE full factorial 3^3, and multiple response (MR)

Std Order	Run Order	Temp.	Acid Vo.	Radiation Per.	Ca conc. mg/caps	Co conc. µg/caps	Cr conc. µg/caps	Cu conc. mg/caps	Fe conc. mg/caps	K conc. mg/caps	Mg conc. mg/caps	Mn conc. mg/caps	Na conc. µg/caps	Ni conc. µg/caps	Se conc. µg/caps	V conc. µg/caps	Zn conc. mg/caps	MR
1	1	160	3.9	10	104.28	0.81	92.66	1.44	12.44	56.97	66.5	1.48	323.7	8.28	17.12	7.83	10	11.60
2	2	160	3.9	15	96.01	0.71	88.43	1.36	10.89	49.05	63.58	1.3	309.44	7.15	7.87	7.25	8.64	10.25
3	3	160	3.9	20	106.04	0.83	71.77	1.52	12.76	58.8	66.21	1.4	322.24	8.34	16.6	7.3	10.07	11.40
4	4	160	5.2	10	103.17	0.73	89.78	1.55	11.33	52.34	64.31	1.28	312.99	8.62	8.98	8.12	10.41	11.02
5	5	160	5.2	15	98.13	0.74	73.28	1.4	11.41	56.24	65.76	1.49	320.08	8.03	17.9	7.02	9.7	11.03
6	6	160	5.2	20	115.98	0.81	70.31	1.67	12.57	62.03	65.67	1.45	319.64	7.77	16.72	7.3	9.39	11.46
7	7	160	6.5	10	121.66	0.97	80.15	1.48	13.19	58.63	70.33	1.46	342.33	8.61	18.06	7.3	10.4	11.87
8	8	160	6.5	15	102.19	0.74	83.8	1.6	11.4	53	65.01	1.37	316.44	8.36	16.56	8.31	10.11	11.31
9	9	160	6.5	20	118.36	1.00	82.11	1.41	12.58	56.16	70.24	1.49	341.89	8.89	15.79	8.34	10.74	11.90
10	10	180	3.9	10	119.03	0.8	97.27	1.56	12.27	52.14	70.05	1.42	340.97	7.62	17.08	9.1	9.2	11.80
11	11	180	3.9	15	100.94	0.75	87.9	1.52	11.57	59.83	66.84	1.48	325.34	7.07	17.92	6.52	8.55	11.11
12	12	180	3.9	20	97.76	0.72	59.78	1.29	11.06	66.16	64.9	1.37	315.89	6.59	8.47	7.21	7.96	10.20
13	13	180	5.2	10	112.52	0.79	80.94	1.41	12.22	53.25	68.71	1.37	334.42	7.78	4.4	8.79	9.4	11.00
14	14	180	5.2	15	99.65	0.69	80.43	1.46	10.69	64.52	64.85	1.41	315.62	6.68	29.72	6.99	8.08	11.17
15	15	180	5.2	20	108.13	0.77	78.48	1.58	11.95	58.4	66.54	1.37	323.88	7.08	17.34	8.05	8.56	11.21
16	16	180	6.5	10	101.77	0.84	94.8	1.5	12.99	56.17	64.26	1.36	312.76	7.71	17.06	7.26	9.31	11.37
17	17	180	6.5	15	97.15	0.75	92.72	1.45	11.55	55.3	65.57	1.33	319.16	7.48	17.19	6.5	9.04	10.94
18	18	180	6.5	20	113.11	0.77	83.09	1.49	11.85	54.66	67.8	1.43	330	7.4	4.24	8	8.94	10.87
19	19	200	3.9	10	120.12	0.83	76.8	1.52	12.85	52.24	67.42	1.48	328.14	7.34	17.15	7.49	8.86	11.38
20	20	200	3.9	15	105.26	0.76	89.34	1.36	11.73	55.69	69.57	1.47	338.59	7.19	17.78	7.3	8.69	11.20
21	21	200	3.9	20	105.98	0.8	75.79	1.46	12.28	50.04	69.33	1.46	337.47	6.71	17.58	6.75	8.11	10.94
22	22	200	5.2	10	94.12	0.7	76.67	1.36	10.78	55.02	63.68	1.32	309.95	6.77	17.24	8.05	8.18	10.53
23	23	200	5.2	15	104.11	0.8	81.5	1.52	12.29	61.59	66.01	1.4	321.3	7.51	17.56	8.95	9.07	11.47
24	24	200	5.2	20	96.75	0.72	97.49	1.41	11.17	52.56	64.29	1.33	312.92	6.32	17.69	6.36	7.63	10.57
25	25	200	6.5	10	96.41	0.74	76.88	1.51	11.5	55.79	67.58	1.22	328.91	7.29	17.83	7.86	8.81	10.95
26	26	200	6.5	15	111.29	0.78	82.16	1.37	11.99	55.57	67.81	1.46	330.07	6.99	17.85	8.37	8.45	11.28
27	27	200	6.5	20	104.17	0.75	93.12	1.41	11.62	59.4	67.76	1.37	329.82	7.4	16.52	6.82	8.94	11.18

Two-way analysis of variance (ANOVA) was used to study the significance of the variance. According to ANOVA, the results (Table 3.10) indicted that the percentage of contributions (PC) values reflected multiple element digestion, and were significantly affected (87.83%) by design mode conditions. The highest effect was observed in the case of interaction of temperature with radiation period (33.47%), acid volume with radiation period (23.96%) and the lowest value is the interaction of temperature with acid volume (11.49%). Besides, ANOVA results show the *p*-value (probability value) which indicates the significant differences in multiple responses with each factor. When the *p*-value is lower than 0.05, it demonstrates that the model is statistically significant (Khajeh, 2012). Additionally, the interactions of acid volume with radiation period model, and interaction of temperature with radiation period model are statistically significant with p-value 0.047 and 0.02, respectively.

Table 3.10 Results of ANOVA using MR values, considering temperature (temp.), acid volume (Acid Vo.) and radiation period (Radiation Per.)

Source	DF	Seq SS	PC	Adj SS	Adj MS	F-Value	*p*-value
Model	18	4.2431	87.83%	4.2431	0.23573	3.21	0.048
Linear	6	0.9137	18.91%	0.9137	0.15229	2.07	0.168
Temp.	2	0.4128	8.55%	0.4128	0.20642	2.81	0.119
Acid Vo.	2	0.2701	5.59%	0.2701	0.13507	1.84	0.22
Radiation Per.	2	0.2308	4.78%	0.2308	0.11538	1.57	0.266
2-Way Interactions	12	3.3294	68.92%	3.3294	0.27745	3.78	0.034
Temp.*Acid Vo.	4	0.5549	11.49%	0.5549	0.13872	1.89	0.206
Temp.*Radiation Per.	4	1.617	33.47%	1.617	0.40425	5.5	0.02
Acid Vo. * Radiation Per.	4	1.1575	23.96%	1.1575	0.28938	3.94	0.047
Error	8	0.5877	12.17%	0.5877	0.07346		
Total	26	4.8308	100.00%				

DF: Degree of freedom, Seq SS: sequential sum of squares, PC: percentage of contributions, Adj SS: Adjusted sums of squares, Adj MS: Adjusted mean squares, F-Value: F distribution and p-alue: probability value

Pareto charts (Figure 3.12) was used to study the major factors, and effect values in digestion obtained from 3^3 factorial designs, including temperature (A), acid volume (B) and radiation period (C), and their interactions (AC, BC, AC). The results show that two interaction factors, temperature with radiation period (AC) and acid volume with radiation period (BC) bars, exceed a vertical reference line (95% confidence interval) indicating statistically significance of AC, and BC at the level. However, the major effects (A, B, C) and interaction of temperature and acid volume (AB) are not statistically significant. The results reflect that these variables have a synergistic effect on MR response. This means that, the use of BC at higher levels, and temperature at the lower levels may result in a better analytical response. Mketo *et al.* (2016) in their observations obtained the same result. The interaction plot for MR confirm that higher acid volume (6.5ml), and higher radiation period (20 min) with lower temperature (160ºC) bring about better analytical response (Figure 3.12).

According all of these observations by MR value and statistical analysis, acid volume (6.5 ml), and radiation period (20 min) with temperature (160ºC) were chosen as optimum conditions for the determination of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn.

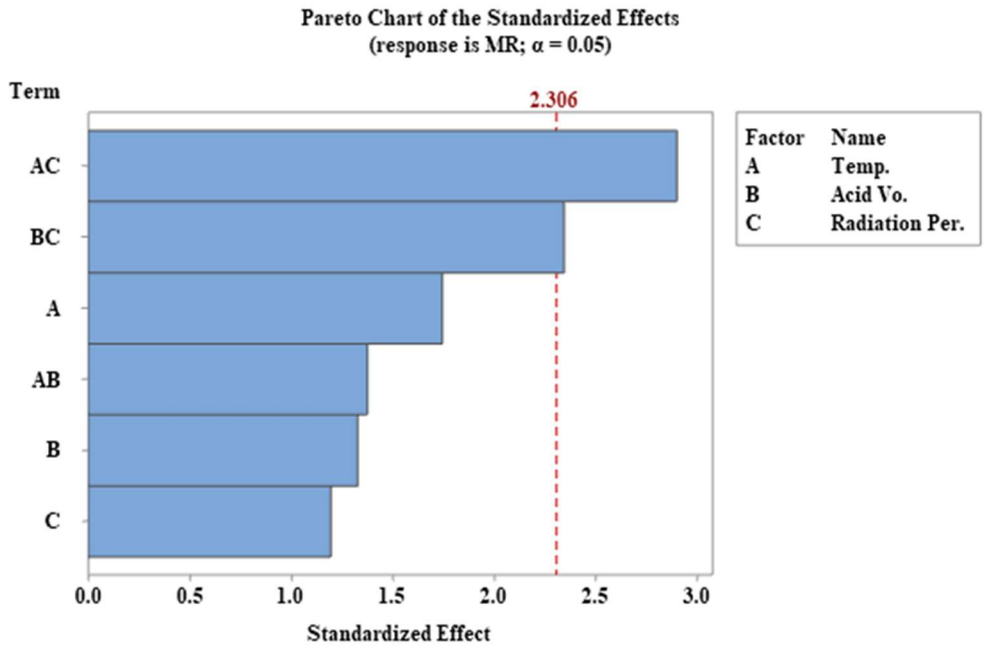

Figure 3.12 Pareto chart of major effects and interaction obtained from 3^3 factorial designs. The vertical line defines the 95% confidence interval (A: Temperature, B: Acid volume, C: Radiation period)

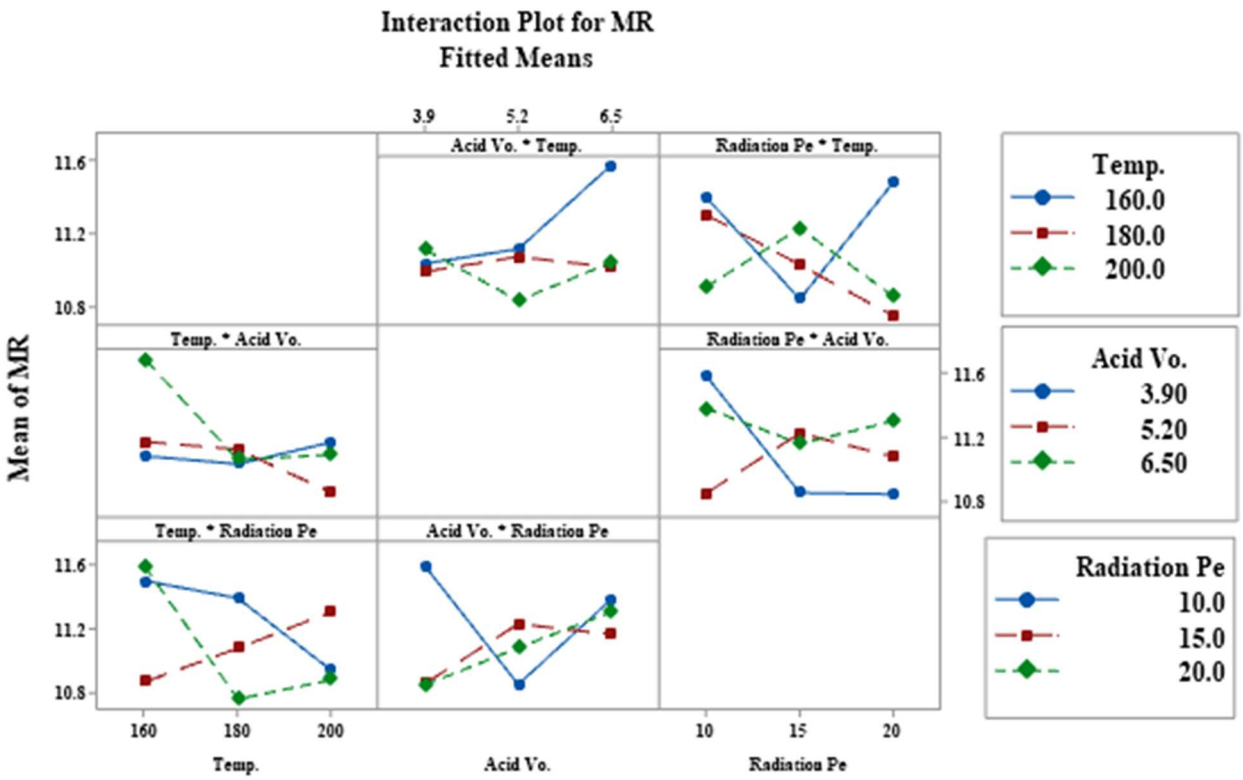

Figure 3.13 Interaction plot for MR responses and factor levels effect of the factorial design 3^3

3.1.2.2 Validation of the method

3.1.2.2.1 Calibration curve

For quantitative analysis of the interesting elements in MVM samples, linear eight-point calibration curves (0.1 - 150 ng/ml) for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn were constructed from multi-element standard solution figures 3.14 - 13.26. And figure 13.27 shows a typical mass spectrum of a reference sample. The data of calibration curve including ICP modes, elements masses, correlation coefficient (R^2) detection limits (DL), and background equivalent concentration (BEC) of detector response are presented in table 3.11. As it is shown in the table, the correlation coefficient is satisfactory in all cases with correlation coefficients (R^2) in range from 0.985 to 0.999.

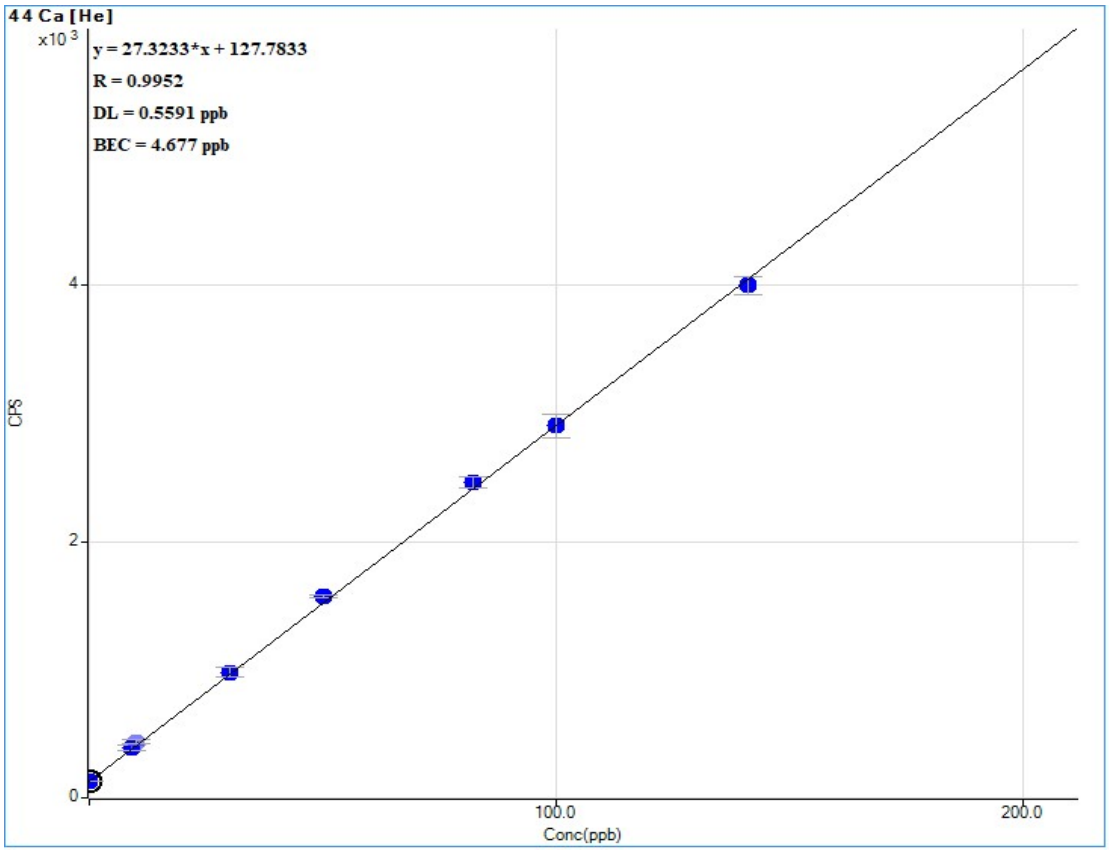

Figure 3.14 Calibration curve of calcium (Ca) by ICP-MS

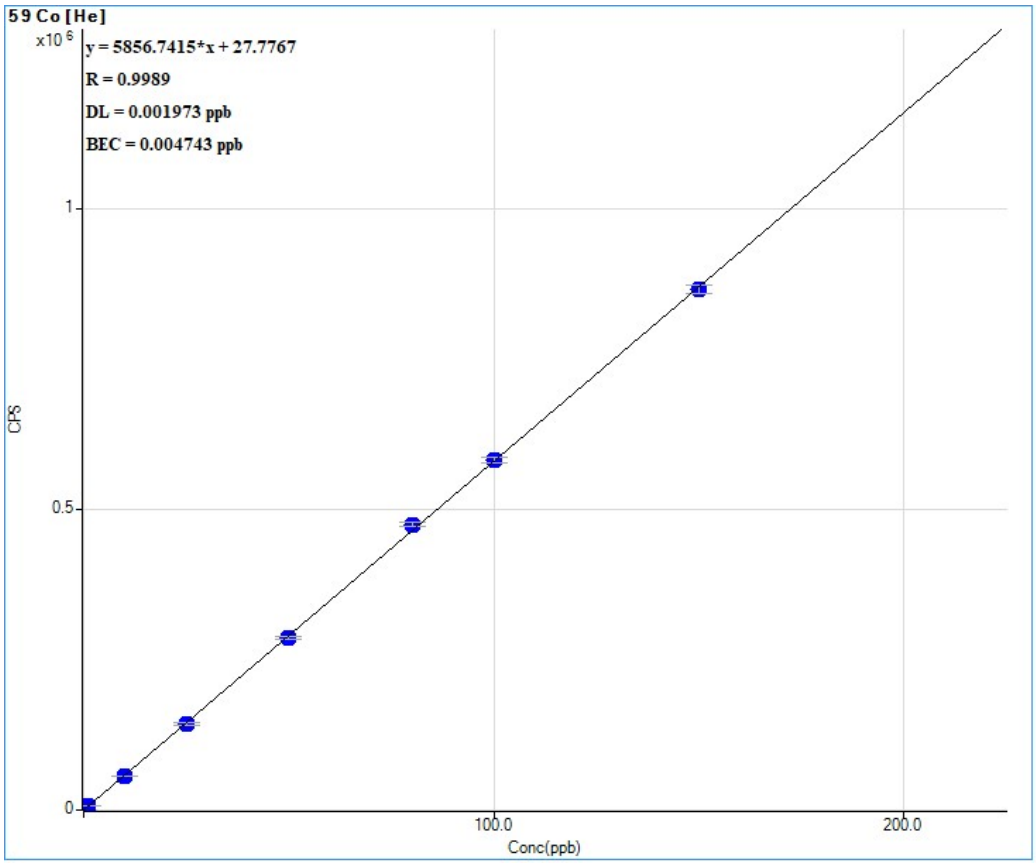

Figure 3.15 Calibration curve of cobalt (Co) by ICP-MS

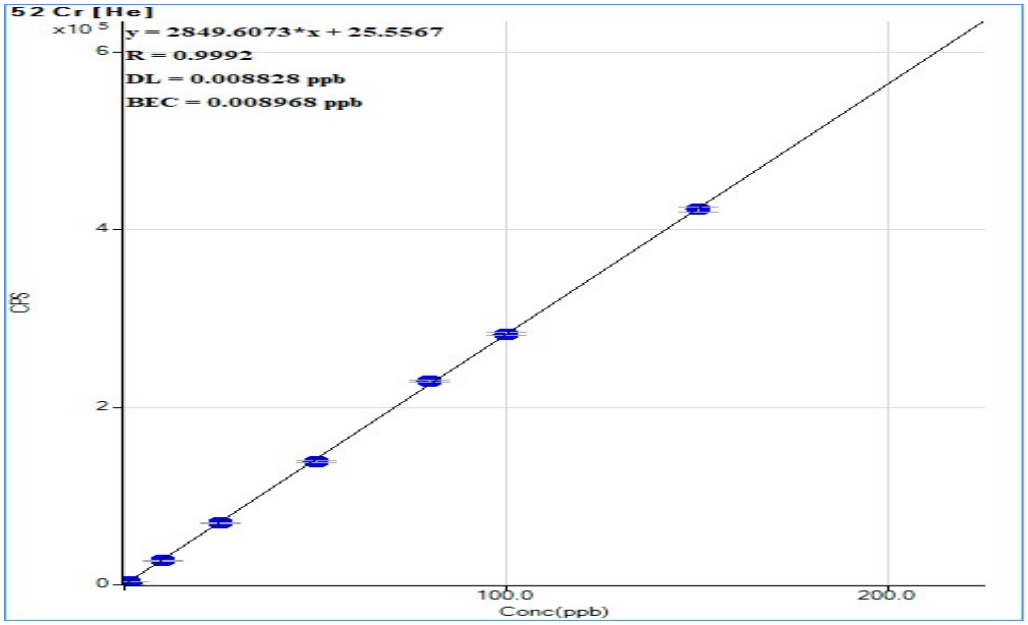

Figure 3.16 Calibration curve of chromium (Cr) by ICP-MS

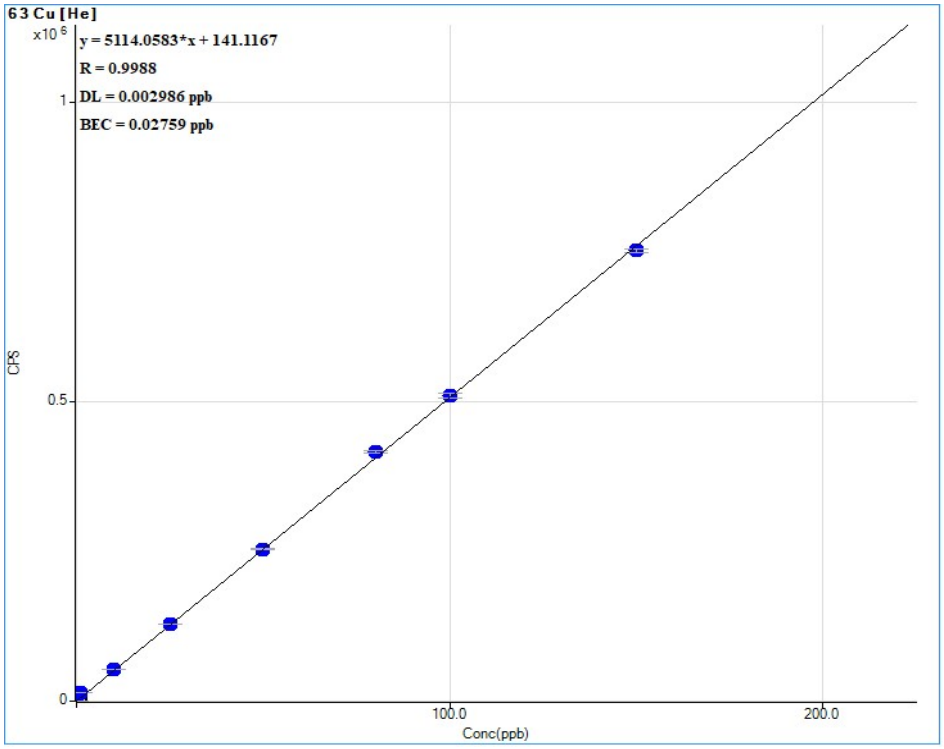

Figure 3.17 Calibration curve of copper (Cu) by ICP-MS

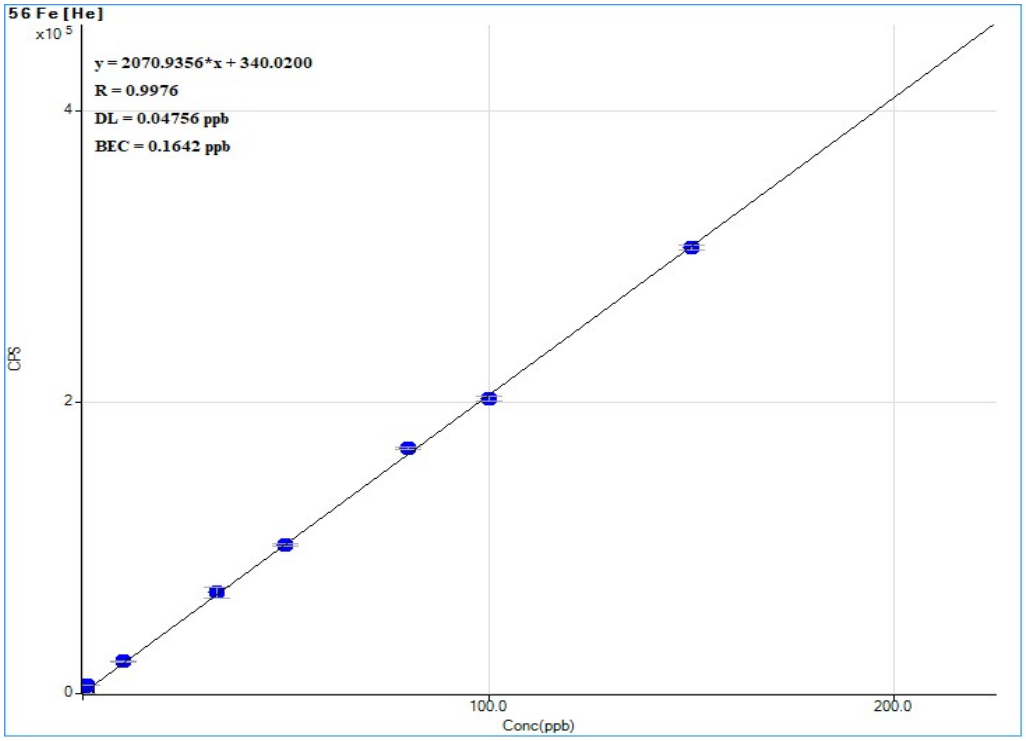

Figure 3.18 Calibration curve of iron (Fe) by ICP-MS

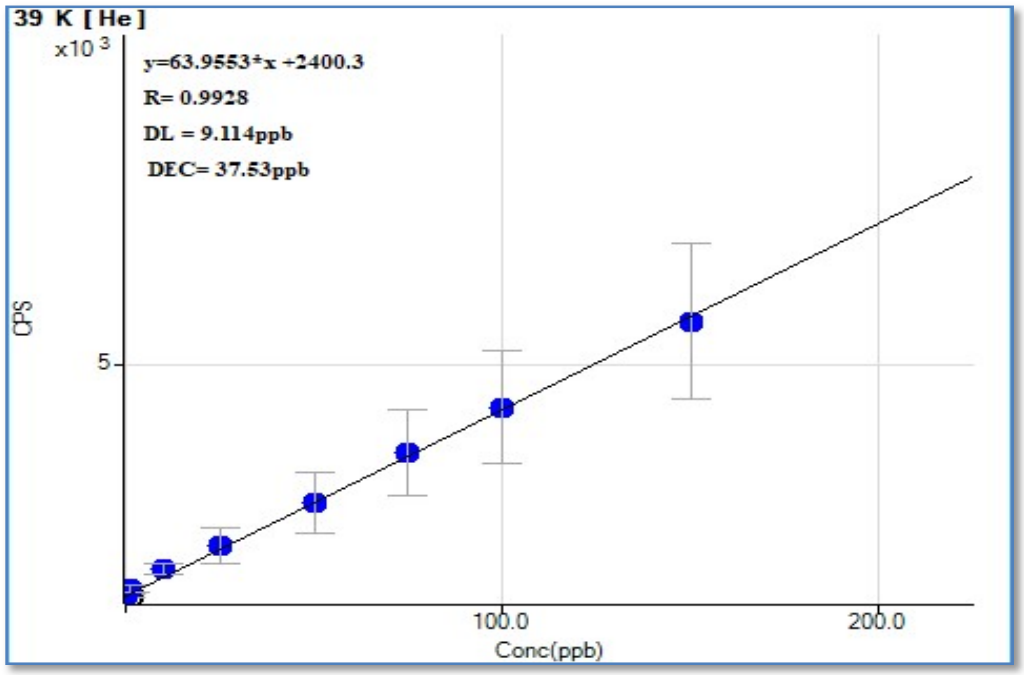

Figure 3.19 Calibration curve of potassium (K) by ICP-MS

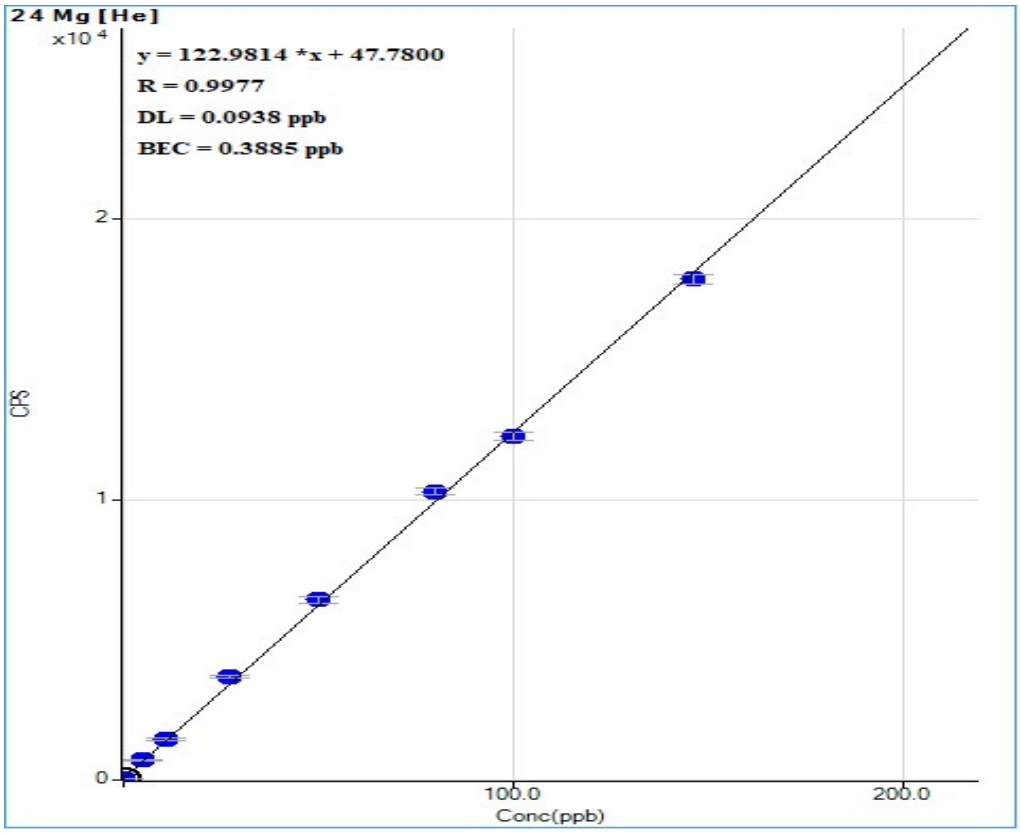

Figure 3.20 Calibration curve of magnesium (Mg) by ICP-MS

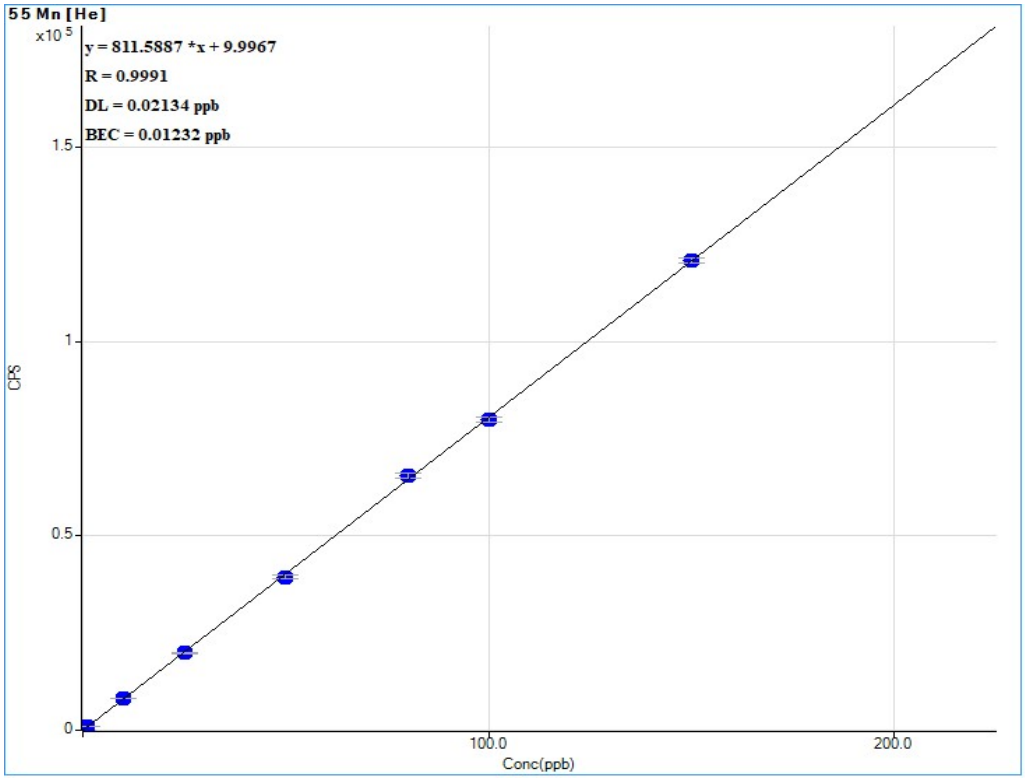

Figure 3.21 Calibration curve of manganese (Mn) by ICP-MS

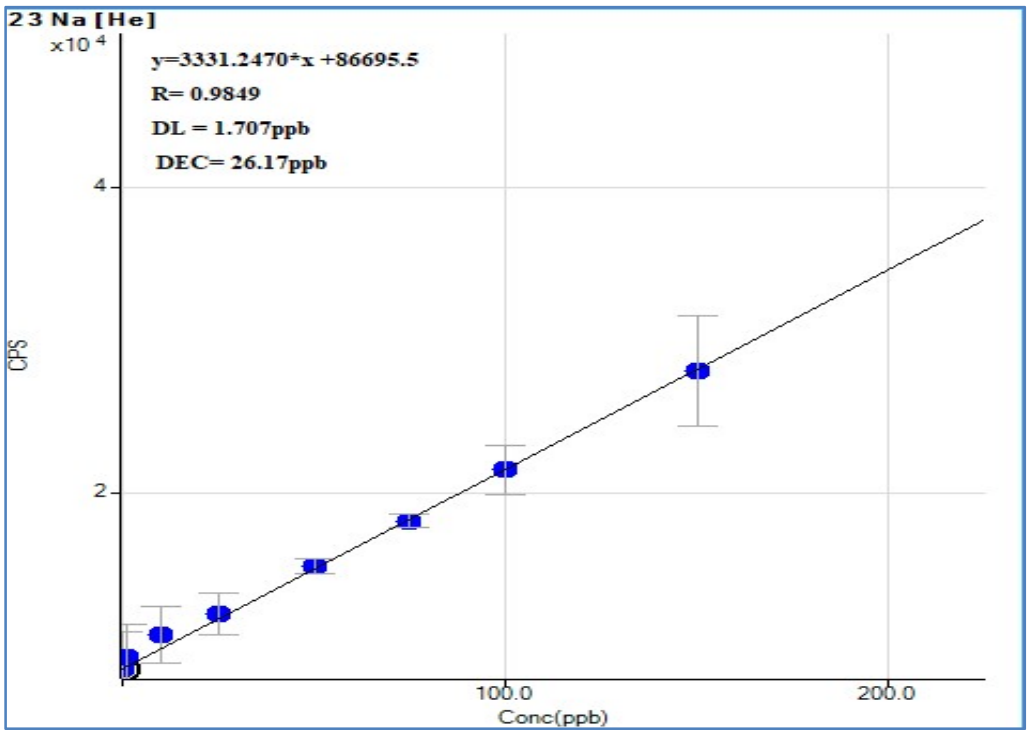

Figure 3.22 Calibration curve of sodium (Na) by ICP-MS

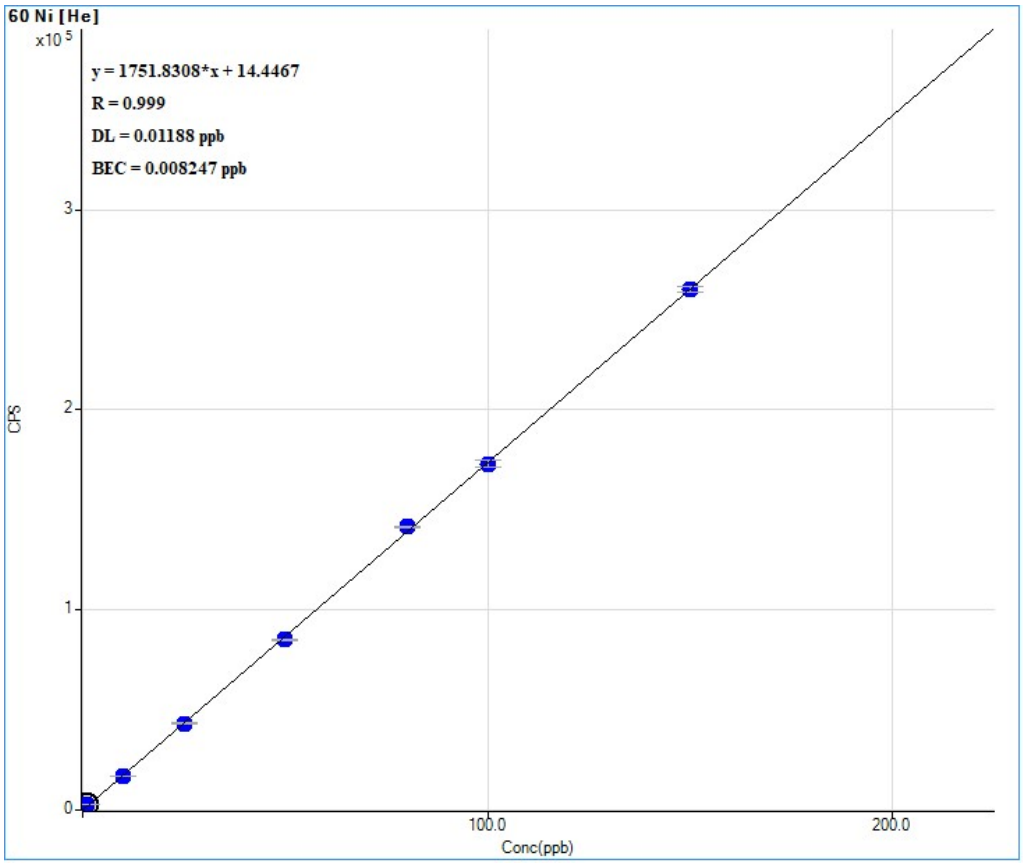

Figure 3.23 Calibration curve of nickel (Ni) by ICP-MS

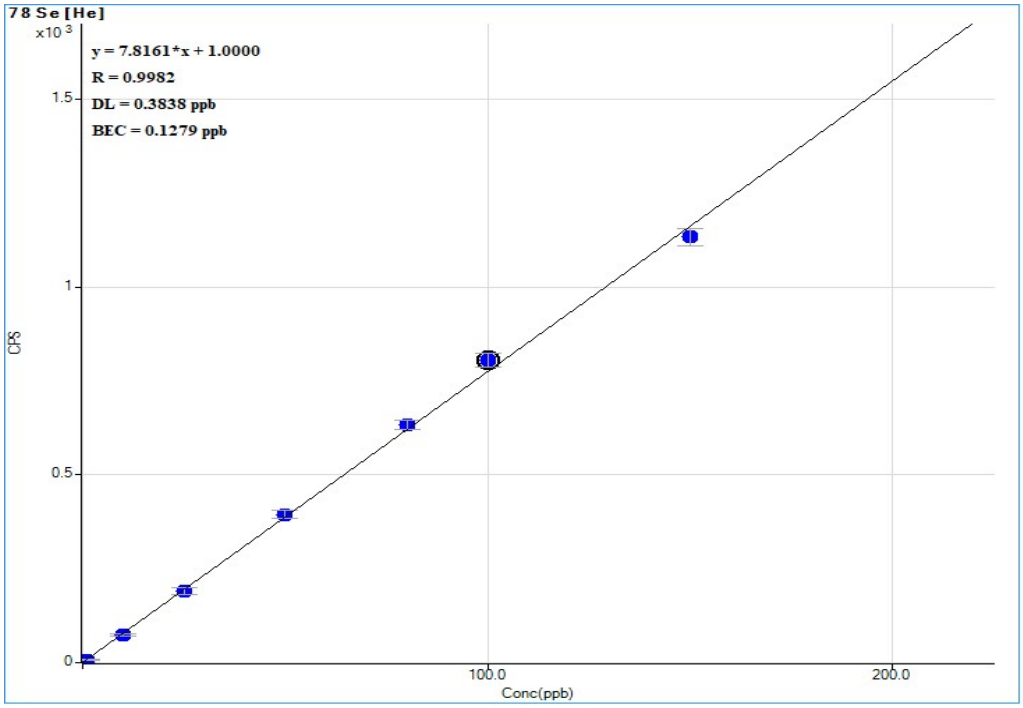

Figure 3.24 Calibration curve of selenium (Se) by ICP-MS

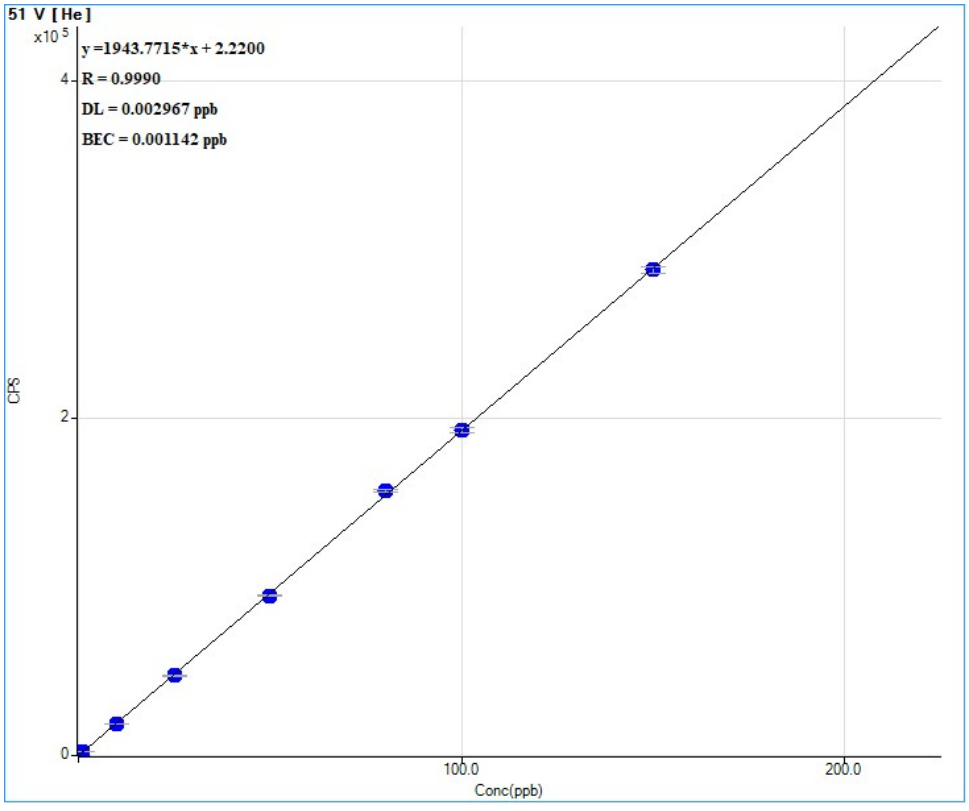

Figure 3.25 Calibration curve of vanadium (V) by ICP-MS

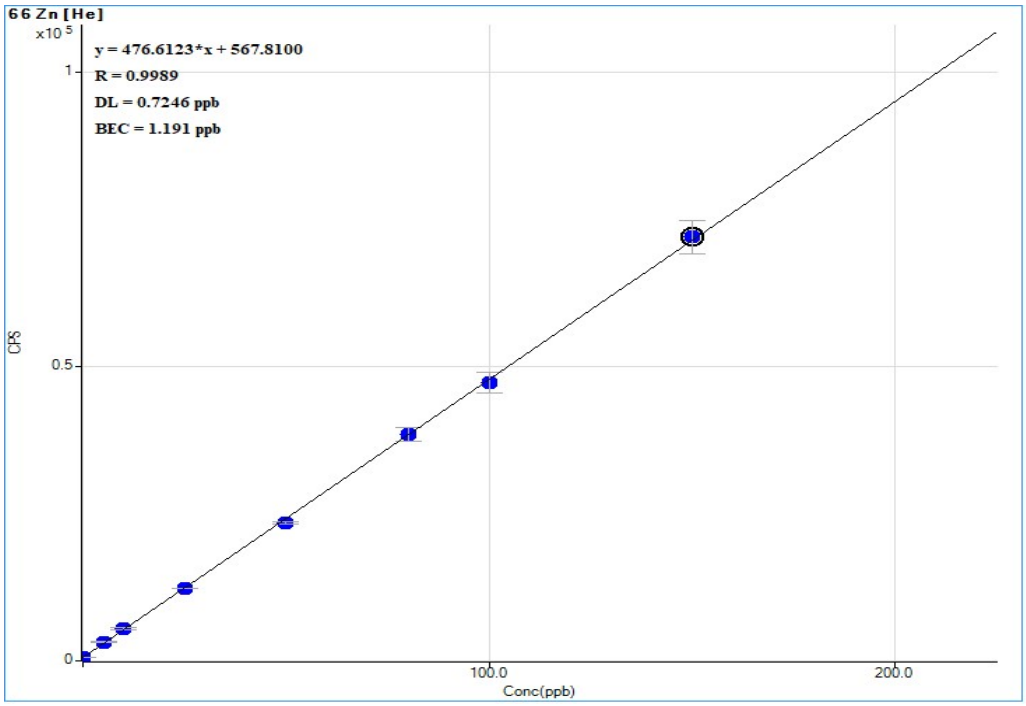

Figure 3.26 Calibration curve of zinc (Zn) by ICP-MS

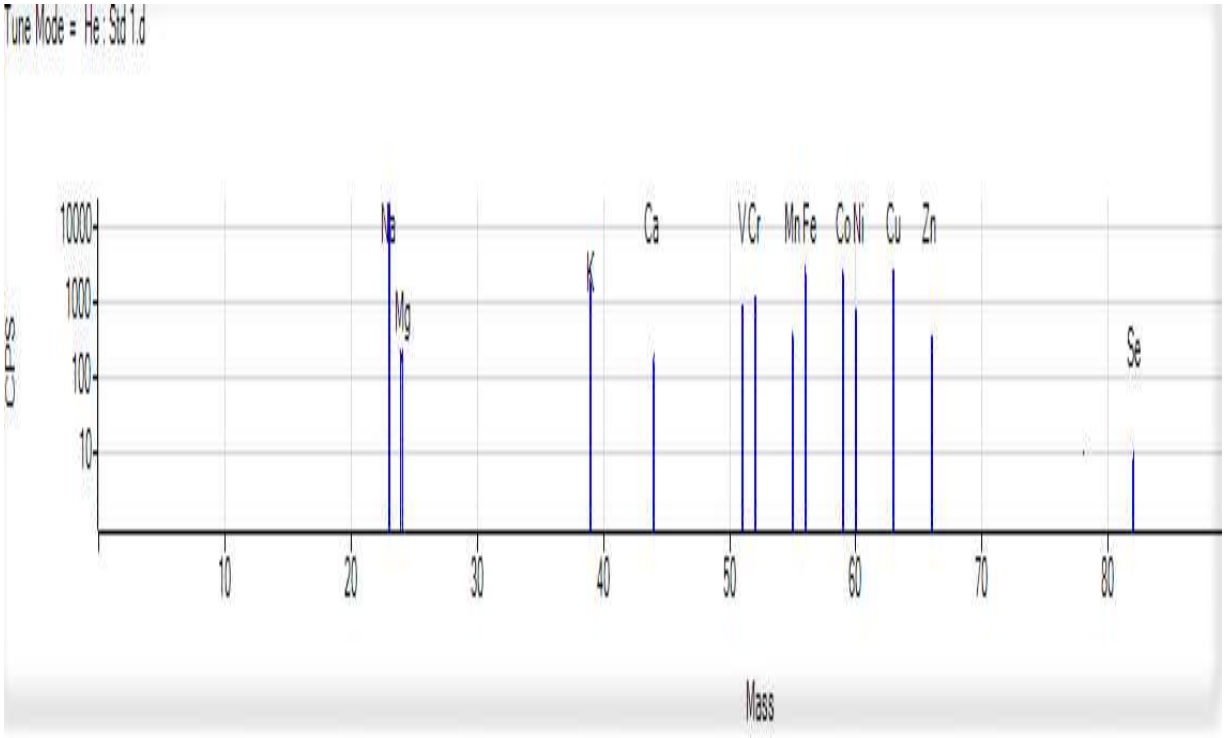

Figure 13.27 A typical mass spectrum of a reference sample

3.1.2.2.2 Accuracy

The accuracy of the procedure was confirmed by analysis of MVM standard reference materials SRM 3280 after digesting them under the optimum microwave conditions (acid volume (6.5ml), and radiation (20min) with temperature (160ºC)) for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. Accuracy was expressed as the recovery percentage (R%) of each element. The element concentration values were found and were compared with their concentration values in certificate of SRM 3280. According to the results present in table 3.12, the recoveries of all elements were founded in range of 88 – 107.5%. The agreement of the results shows that both the proposed mineralization process of samples and the quantitative determination of elements are correct.

Table 3.11 Calibration curve data (ICP modes, elements masses, correlation coefficient (R^2), detection limits (DL) and background equivalent concentration (BEC)) for the interesting elements

Calibration data / Element	Mode	Mass	R^2	DL (ng/ml)	BEC (ng/ml)
Ca	He	44	0.9952	0.5591	4.6767
Co	He	59	0.9989	0.0019	0.0047
Cr	He	52	0.9992	0.0088	0.0089
Cu	He	63	0.9988	0.0029	0.0276
Fe	He	56	0.9976	0.0476	0.1642
K	He	39	0.9928	0.9114	37.5306
Mg	He	24	0.9977	0.0938	0.3885
Mn	He	55	0.9991	0.0213	0.0123
Na	He	23	0.9849	1.7069	26.1724
Ni	He	60	0.9990	0.0119	0.0082
Se	He	78	0.9982	0.3838	0.1279
V	He	51	0.9990	0.0029	0.0011
Zn	He	66	0.9989	0.7245	1.1913

3.1.2.2.3 Precision

Precision of the method expressed as relative standard deviation (RSD %) was evaluated as repeatability intra-day and repeatability inter-day. The repeatability was calculated after analysing reference materials SRM 3280 ten times in one day. The repeatability values are ranged from 0.68 to 5.87% for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. In order to study the repeatability inter-day, furthermore, the reference material SRM 3280 are analysed during three consecutive days. However, the reproducibility values were ranged from 0.91 to 4.15% for Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn. The lowest precision values reflect imprecision of total procedure as shown in table 3.12.

Table 3.12 The method recoveries values of selected elements in SRM 3280, and precision (R. intra-day and R. inter-day) under the optimum conditions

Element	Element value in SRM 3820	Element value found ±RSD	Recovery % (R%)	R. intra-day RSD% (n=10)	R. inter-day RSD% (n=12)
Mg conc.mg/g	67.8 ± 4.0	70.24 ± 0.68	103.60	2.47	3.56
Cr conc. µg/g	93.7 ± 2.7	82.11 ± 1.13	87.63	2.45	3.10
Mn conc. mg/g	1.44 ± 0.11	1.49 ± 0.14	103.47	1.64	3.96
Cu conc.mg/g	1.4 ± 0.17	1.41 ± 0.27	100.71	0.68	0.91
Se conc. µg/g	17.42 ± 0.45	15.79 ± 0.91	90.64	1.24	1.29
Ni conc. µg/g	8.4 ± 0.30	8.89 ± 0.76	105.83	1.43	1.43
Co conc. µg/g	0.8 ± 0.01	0.86 ± 0.1	107.5	0.97	1.17
Na conc. µg/g	330± 20.0	341.9 ± 1.90	103.60	1.54	2.61
K conc.mg/g	53.1 ± 7.0	56.16 ± 1.47	105.76	1.72	1.89
Zn conc.mg/g	10.15 ± 0.81	10.74 ± 0.59	105.81	2.95	3.35
Fe conc.mg/g	12.35 ± 0.91	12.58 ± 1.03	101.86	4.72	4.15
V conc. µg/g	8 ± 2.0	8.34 ± 1.87	104.25	5.87	3.42
Ca conc.mg/g	110.7 ± 5.3	118.4 ± 2.99	106.92	1.67	1.96

3.1.2.3 Application of the method

The optimum conditions including temperature 160ºC, acid mixture volume 6.5ml, and radiation period 20min were applied to microwave digestion for determination of selected elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn.) in six commercial products of MVM for pregnant women and diabetic patients after validating the procedure, the results are shown in table 3.13. the concentration results were listed with relative standard deviation (RSD) of successive triplicate analysis by IC-MS. The values were compared with labelled content of each commercial product as shown in table 3.13. In general, the concentration of the determined elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn.) were in the range corresponded to manufacturer labels. However, exception was found in the situation of Cu in sample (MVM2), Mn, and Zn in sample (MVM3) the contents were found which were much higher than labelled. Some samples contain Ca, Fe, K, Na, Ni, and V. They did not mention their presence in label, and these situations are marked as **bold** *italic*, since they could come from excipients and their salts with some microelements (Table 3.13).

3.1.3 Monitoring of macro and micro elements in multivitamin/multimineral (MVM) for pregnant women and diabetic patients available in Sudan

For mutual comparison of the actual elements levels in multivitamin for pregnant women and diabetic patients, the element levels in ninety-three most commonly used multivitamin/multimineral (MVM) capsules in the Sudan were analysed. The digestion was carried through the previous validated developed high-pressure microwave digestion method. The amount of macro and micro elements (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn) were determined by ICPMS and compared with the amount declared by producer (Table 3.14).

The sample No. 1 represented as the control sample and categorized as reference material (NIST SRM 3280, multivitamin/ multielement tablets), was used to assess the declared content of elements compared with actual content. The element values were found to be similar to those declared in the certificate of SRM 3280.

Table 3.13 Comparison of determined element concentrations by ICP-MS, in commercial MVM capsules for pregnant women and diabetic patients with labelled contents

Preparation		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 1	Labelled mg/caps	168	-	0.04	0.5	10	40	50	1	0.05	-	0.03	5	-
	Found mg ± RSD %	167.2858 ±0.846	N/D	0.0409± 0.0020	0.5534 ±0.0399	10.3130 ±0.0350	40.0122 ±0.4819	50.0239 ±0.4874	1.0142 ±0.0661	0.0520 ±0.0129	N/D	0.0307 ±0.0032	5.1497 ±0.0199	N/D
MVM 2	Labelled mg/caps	160	-	0.025	1.1	-	-	100	2	-	-	0.025	14	-
	Found mg ± RSD %	158.9463 ±0.9546	N/D	0.0250 ±0.0014	1.2885 ±0.0425	***3.7398 ±0.5364***	***3.4092 ±1.3177***	100.4042 ±0.2038	2.0717 ±0.0839	***9.70181 ±1.23165***	N/D	0.0250 ±0.0256	14.4981 ±0.0543	N/D
MVM 3	Labelled mg/caps	168	-	0.04	0.5	10	40	50	1	0.05	-	0.03	5	-
	Found mg ± RSD %	168.1438 ±1.0194	N/D	0.0396 ±0.0018	0.5073 ±0.0017	10.1052 ±0.2932	40.3511 ±0.2476	53.0880 ±1.1875	1.11917 ±0.0472	0.0493 ±0.0066	N/D	0.0304 ±0.0015	5.2596 ±0.0990	***0.0117 ±0.0025***
MVM 4	Labelled mg/caps	-	-	0.2	1	8	-	100	2	-	-	0.1	15	-
	Found mg ± RSD %	***4.7268 ±0.7009***	N/D	0.2075± 0.0057	1.0588 ±0.0238	8.0520 ±0.1790	***2.3900 ±0.7945***	96.8274 ±1.6728	2.0568 ±0.0305	***3.7674 ±0.6786***	N/D	0.1019 ±0.0154	15.2289 ±0.0223	N/D
MVM 5	Labelled mg/caps	120		0.025	1.5	8	40.5	50	3.5	-	-	0.05	15	-
	Found mg ± RSD %	120.0708 ±0.3810	N/D	0.0261 ±0.0009	1.5669 ±0.0333	8.01378 ±1.7244	40.5056 ±0.5028	50.5589 ±0.2228	3.5132 ±0.0605	***0.0377 ±0.0068***	N/D	0.0505 ±0.0130	15.4886 ±0.0125	N/D
MVM 6	Labelled mg/caps	162	-	0.065	2	18	80	100	3.5	-	-	0.02	15	0.01
	Found mg ± RSD %	160.8123 ±1.2134	N/D	0.0660 ±0.0021	2.1915 ±0.0798	18.7374 ±0.1841	78.2441 ±1.1408	100.4622 ±0.3115	3.6743 ±0.1312	***7.5560 ±1.0701***	***0.0427 ±0.0131***	0.02192 ±0.0052	14.6164 ±0.6745	0.01062 ±0.0013

N/D : Not detected

The recoveries of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V and Zn were 99.4%, 108.3%,104.8%, 102.3%, 100.2%, 100.1%, 99.4%, 99.5%, 104%, 92.3%, ,105.7%, respectively, showing that they ranged from 92.3% to 108.3% for all elements.
The agreement of the results shows that both developed mineralization process of samples and quantitative determination method of elements were correct.

Macro and microelements are present in MVM at different concentrations and the determined average levels of the elements in the analysed samples agreed with manufacturer labels. However, there are no standard protocol and content uniformity requirements specified for multivitamin/multimineral formulations. The USP 32-NF 27 General Chapter < 905 > Uniformity of Dosage Units lists standard test methods for the quality of oral dosage forms. They are divided into different categories: performance test for oral drug products (Pharmaceuticals) and performance test for dietary supplements. The characterization and quality control of pharmaceutical dosage forms is much more closely controlled compared with dietary supplements. Also European Pharmacopeia regulations (European Pharmacopoeia, 5th edition, Supplement 5.2, Council of Europe, Strasbourg, 2005, Chapter 2.9.40) to determine whether the measured data is in agreement with the label claim (Avula *et al.*, 2011) were adapted recently. Based on this regulation, if the percentage deviation falls within 85% and 115%.

Apart from only six sample products, all the other MVM products showed content ranges of 96.5% to 100.9%, 91.8% to 110%, 90.4% to 111.9%, 89.5% to 112.2%, 97.6% to 103.6%, 98% to 103.3%, 91.4% to 105%, 91.4% to 112%, 85.4% to 115%, 90% to 110% and 93.4% to 107.9% for Ca, Cr, Cu, Fe, K, Mg, Mn, Na, Se, V and Zn elements, respectively, relative to the table claim (Table 3.14). However, MVM products of both 21 and 35, both 18 and 75, 16 and 44 showed element contents of 116.15% and 116.5%, 117.7% and 118.2%, 117.1% and 119.2%. for elements Mn, Zn, Cr, Se, respectively. However, Co was not labelled in all MVM product samples except MVM sample number 1 and 2. But irrespective of all other samples, it was practically detected in MVM sample numbers 1, 2, 15, 20, 47, 50 and 59. Also Ni was not labelled in all samples except in only one MVM sample number 1. But also irrespective of all samples it was practically detected in MVM samples number 1, 3, 10, 11, 12, 13, 14, 15, 16, 19 and 44. Several earlier investigators (Whittakar *et al.*,2001;

Frentiu *et al.*, 2012; SALEEM *et al.*, 2016) reported that the analytical values of element contents found were often higher or lower than those labelled.

3.1.3.1 Macro elements (Na, Mg, K, Ca)

Of the 93 products analysed 74, 3, 57 and 6 products contained Na, Mg, K and Ca ranging from 0.045 mg/caps to 1.5mg/caps, 3.14 mg/caps to 23.844 mg/caps, 0.024 mg/caps to 0.657 mg/caps and 2.083 mg/caps to 6.686 mg/caps, respectively (Table 3.14). Although Na and K are not included in the labelled constituents of the product their presence is detected since they could be introduced from the excipients together with small amounts of anions such as iodine, selenate and molybdate from their salts, and from water used in the formulation procession adding more elements including Na, K, Ca and Mg (Frentiu *et al.*, 2012).

3.1.3.2 Micro elements (V, Cr, Co, Mn, Fe, Ni, Cu, Zn, Se)

It has been well established that Se and Cr are often added at low concentrations (<0.2 mg/caps and <0.12 mg/caps, respectively) for nutritional purposes. This was also valid for the samples analysed during this study. Of the 93 samples analysed 5, 6, 8, 9, 12, 10 and 4 all samples showed content ranges from 0.022 mg/caps to 0.076 mg/caps, 0.106 mg/caps to 0.174 mg/caps, 0.019 mg/caps to 0.040 mg/caps, 0.073 mg/caps to 1.262 mg/caps, 0.202 mg/caps to 0.338 mg/caps, 1.76 mg/caps to 4.175 mg/caps, 0.007 mg/caps to 0.014 mg/caps and 0.368 mg/caps to 0.714 mg/caps for elements V, both Cr and Co, Mn, Cu, Fe, Ni and Se, respectively (Table 3.14). Tumir (2010) and Craner (1995) were reported that stainless steel used in food processing equipment may leach certain quantities of Ni, Cr and other metals, where the extent of the metal migration depends on various factors, such us pH, contact time, etc.

Table 3.14 Monitoring of macro and micro elements in multivitamin/multimineral (MVM)for pregnant women and diabetic patients available in Sudan

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM1	Labelled mg/caps	169.72	**0.012**	0.144	2.15	18.39	81.4	104	2.21	0.521	0.013	0.0267	15.56	0.0123
	Found mg ± RSD %	168.808 ±0.068	0.013 ±0.001	0.1509 ±0.001	2.20 ±0.100	18.431 ±0.067	81.503 ±0.063	103.366 ±0.998	2.199 ±0.066	0.542 ±0.005	0.012 ±0.001	0.0273 ±0.003	15.832 ±0.643	0.013 ±0.002
MVM 2	Labelled mg/caps	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	167.986 ±0.069	0.019 ±0.003	0.0431 ±0.001	0.500 ±0.105	10.023 ±0.069	40.336 ±0.062	49.412 ±1.023	1.000 ±0.107	0.056 ±0.007	N/D	0.0327 ±0.002	5.358 ±0.683	N/D
MVM 3	Labelled mg/caps	160	0	0.025	1.1	0	0	100	2	0	0	0.025	14	0
	Found mg ± RSD %	159.997 ±0.068	N/D	0.0259 ±0.001	1.100 ±0.094	4.175 ±0.074	0.439 ±0.023	100.536 ±0.906	2.084 ±0.096	0.945 ±0.008	0.008 ±0.001	0.0255 ±0.002	14.846 ±0.653	N/D
MVM 4	Labelled mg/caps	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	168.027 ±0.071	N/D	0.0367 ±0.001	0.501 ±0.103	10.605 ±0.070	40.578 ±0.065	49.900 ±0.994	1.010 ±0.105	0.047 ±0.017	N/D	0.0313 ±0.002	5.396 ±0.682	N/D
MVM 5	Labelled mg/caps	0	0	0.2	1	8	0	100	2	0	0	0.1	15	0
	Found mg ± RSD %	N/D	N/D	0.2168 ±0.001	0.999 ±0.097	8.159 ±0.073	0.304 ±0.034	100.319 ±0.959	2.088 ±0.097	0.615 ±0.013	N/D	0.0992 ±0.002	15.062 ±0.695	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 6	**Labelled mg/caps**	120	0	0.025	1.5	8	40.5	50	3.5	0	0	0.05	15	0
	Found mg ± RSD %	120.003 ±0.076	N/D	0.0254 ±0.001	1.499 ±0.118	8.033 ±0.068	40.305 ±0.026	50.639 ±1.159	3.413 ±0.117	0.440 ±0.003	N/D	0.0507 ±0.002	15.570 ±0.649	N/D
MVM 7	**Labelled mg/caps**	0	0	0.05	1.5	6	0	50	3	0	0	0.15	15	0
	Found mg ± RSD %	6.686 ±0.080	N/D	0.0502 ±0.001	1.486 ±0.110	6.118 ±0.076	0.257 ±0.046	49.871 ±1.098	3.022 ±0.111	0.413 ±0.007	N/D	0.1526 ±0.002	15.644 ±0.762	N/D
MVM 8	**Labelled mg/caps**	100	0	0	2	10	0	10	2.5	0	0	0.05	1	0
	Found mg ± RSD %	99.991 ±0.073	N/D	N/D	2.049 ±0.125	9.896 ±0.072	0.311 ±0.065	10.216 ±1.229	2.317 ±0.124	0.067 ±0.007	N/D	0.0502 ±0.002	1.177 ±0.670	N/D
MVM 9	**Labelled mg/caps**	0	0	0.05	1.5	12	0	100	2.5	0	0	0.1	12	0
	Found mg ± RSD %	N/D	N/D	0.0500 ±0.001	1.478 ±0.103	11.600±0. 070	0.038 ±0.032	100.100 ±1.015	2.561 ±0.104	0.082 ±0.010	N/D	0.0989 ±0.001	12.026 ±0.681	N/D
MVM10	**Labelled mg/caps**	220	0	0.05	0.5	0	80	50	2.3	0	0	0.019	11	0
	Found mg ± RSD %	219.008 ±0.072	N/D	0.0504 ±0.002	0.491 ±0.114	N/D	80.273 ±0.066	50.129 ±1.095	2.335 ±0.112	0.978 ±0.004	0.009 ±0.001	0.0192 ±0.002	11.518 ±0.697	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 11	**Labelled mg/caps**	162	0	0.065	2	18	80	100	3.5	0	0	0.02	15	0.01
	Found mg ± RSD %	161.957 ±0.074	N/D	0.0624 ±0.002	2.013 ±0.107	18.021 ±0.067	80.196 ±0.061	100.711 ±1.071	3.514 ±0.109	1.302 ±0.005	0.008 ±0.001	0.0211 ±0.002	15.184 ±0.641	0.009 ±0.001
MVM 12	**Labelled mg/caps**	500	0	0.12	2	12	0	50	2	0	0	0.02	15	0
	Found mg ± RSD %	501.778 ±0.067	N/D	0.1253 ±0.002	2.019 ±0.122	12.313 ±0.068	0.591 ±0.018	50.942 ±1.191	2.091 ±0.124	1.523 ±0.005	0.014 ±0.001	0.0225 ±0.002	15.766 ±0.647	N/D
MVM 13	**Labelled mg/caps**	200	0	0	0	28	0	0	0	0	0	0	25	0
	Found mg ± RSD %	199.682 ±0.067	N/D	N/D	0.264 ±0.135	28.328±0.066	0.425 ±0.029	N/D	N/D	1.109 ±0.009	0.007 ±0.001	N/A	25.332 ±0.633	N/D
MVM 14	**Labelled mg/caps**	25	0	0	3	0	0	0	0	0	0	0	23.9	0
	Found mg ± RSD %	24.932 ±0.090	N/D	N/D	3.008 ±0.131	3.005 ±0.077	0.415 ±0.030	N/D	N/D	0.834 ±0.015	0.007 ±0.001	N/A	24.072 ±0.666	N/D
MVM 15	**Labelled mg/caps**	800	0	0	0	0	0	300	0	0	0	0	10	0
	Found mg ± RSD %	799.522 ±0.070	0.034 ±0.001	0.1568 ±0.002	0.304 ±0.132	3.729 ±0.075	0.541 ±0.029	300.123 ±1.153	0.163 ±0.159	1.350 ±0.008	0.009 ±0.001	N/A	10.048 ±0.670	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 16	**Labelled mg/caps**	200	0	0.2	1	18	20	100	6	5	0	0.1	15	0
	Found mg ± RSD %	199.565 ±0.080	N/D	0.2342 ±0.002	0.997 ±0.179	17.849 ±0.074	19.887 ±0.061	100.282 ±1.662	5.964 ±0.168	5.060 ±0.014	0.008 ±0.002	0.0854 ±0.002	15.027 ±0.705	N/D
MVM 17	**Labelled mg/caps**	0	0	0	1	15	0	0	0	0	0	0	12	0
	Found mg ± RSD %	N/D	N/D	N/D	1.004 ±0.138	14.987 ±0.072	0.153 ±0.020	N/D	N/D	0.256 ±0.022	N/D	0.3680 ±0.001	11.843 ±0.699	N/D
MVM 18	**Labelled mg/caps**	200	0	0	1	15	0	150	0	0	0	0	15	0
	Found mg ± RSD %	199.964 ±0.091	N/D	N/D	1.010 ±0.147	15.485 ±0.069	0.257 ±0.032	149.150 ±1.450	N/D	0.373 ±0.022	N/D	0.6544 ±0.001	14.771 ±0.679	N/D
MVM 19	**Labelled mg/caps**	220	0	0.05	0.5	0	80	50	2.3	0	0	0.019	11	0
	Found mg ± RSD %	219.084 ±0.078	N/D	0.0517 ±0.003	0.452 ±0.172	N/D	79.616 ±0.067	50.425 ±1.684	2.342 ±0.167	0.891 ±0.014	0.009 ±0.002	0.0183 ±0.001	11.326 ±0.709	N/D
MVM 20	**Labelled mg/caps**	162	0	0.065	2	18	80	100	3.5	0	0	0.02	15	0.01
	Found mg ± RSD %	161.988 ±0.077	0.019 ±0.004	0.0656 ±0.002	1.995 ±0.168	18.136 ±0.084	79.437 ±0.070	100.234 ±1.556	3.507 ±0.156	1.110 ±0.008	N/D	0.0220 ±0.001	14.788 ±0.713	0.010 ±0.004

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 21	**Labelled mg/caps**	500	0	0.12	2	18	0	50	2	0	0	0.02	15	0
	Found mg ± RSD %	501.778 ±0.067	N/D	0.1237 ±0.003	1.998 ±0.170	18.122 ±0.068	0.657 ±0.020	50.284 ±1.646	2.323 ±0.164	1.186 ±0.022	N/D	0.0230 ±0.001	14.972 ±0.651	N/D
MVM 22	**Labelled mg/caps**	200	0	0	0	28	0	0	0	0	0	0	25	0
	Found mg ± RSD %	199.742 ±0.065	N/D	N/D	0.205 ±0.224	28.005±0.068	0.372 ±0.023	N/D	N/D	0.818 ±0.010	N/D	N/A	24.582 ±0.649	N/D
MVM 23	**Labelled mg/caps**	25	0	0	3	0	0	0	0	0	0	0	23.9	0
	Found mg ± RSD %	25.037 ±0.107	N/D	N/D	2.999 ±0.172	1.760±0.088	0.405 ±0.020	N/D	N/D	0.613 ±0.046	N/D	N/A	23.880 ±0.719	N/D
MVM 24	**Labelled mg/caps**	800	0	0	0	0	0	300	0	0	0	0	10	0
	Found mg ± RSD %	799.563 ±0.102	N/D	0.1624 ±0.005	N/D	N/D	0.558 ±0.033	300.570 ±1.727	N/D	0.835 ±0.028	N/D	N/A	9.966 ±0.668	N/D
MVM 25	**Labelled mg/caps**	200	0	0.2	1	18	20	100	6	5	0	0.1	15	0
	Found mg ± RSD %	200.256 ±0.092	N/D	0.1985 ±0.004	1.119 ±0.176	17.958 ±0.070	19.594 ±0.008	100.015 ±1.660	6.144 ±0.185	4.919 ±0.039	N/D	0.0996 ±0.001	15.081 ±0.670	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 26	**Labelled mg/caps**	0	0	0	1	15	0	0	0	0	0	0	12	0
	Found mg ± RSD %	N/D	N/D	0.1058 ±0.002	1.015 ±0.176	14.872 ±0.068	0.572 ±0.048	5.376 ±1.658	N/D	0.289 ±0.017	N/D	0.5081 ±0.001	11.430 ±0.653	N/D
MVM 27	**Labelled mg/caps**	200	0	0	1	15	0	150	0	0	0	0	15	0
	Found mg ± RSD %	200.255 ±0.071	N/D	N/D	1.020 ±0.231	14.965 ±0.079	0.227 ±0.023	149.914 ±1.728	N/D	0.367 ±0.027	N/D	0.7144 ±0.001	14.633 ±0.705	N/D
MVM 28	**Labelled mg/caps**	75	0	0.15	2.15	19	80	104	2.2	0.5	0	0.025	6	0.0125
	Found mg ± RSD %	73.548 ±0.076	N/D	0.1444 ±0.002	2.11 ±0.174	19.223 ±0.070	81.279 ±0.062	103.977 ±1.685	2.247 ±0.170	0.504 ±0.032	N/D	0.0281 ±0.001	6.277 ±0.675	0.012 ±0.003
MVM 29	**Labelled mg/caps**	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	168.262 ±0.081	N/D	0.0402 ±0.004	0.496 ±0.188	10.549 ±0.077	39.113 ±0.067	50.612 ±1.711	0.973 ±0.173	0.053 ±0.046	N/D	0.0315 ±0.001	4.964 ±0.751	N/D
MVM 30	**Labelled mg/caps**	160	0	0.025	1.1	0	0	100	2	0	0	0.025	14	0
	Found mg ± RSD %	160.172 ±0.076	N/D	0.0232 ±0.003	1.097 ±0.180	4.486 ±0.076	0.502 ±0.011	100.483 ±1.719	2.006 ±0.172	0.830 ±0.027	N/D	0.0244 ±0.001	14.099 ±0.671	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 31	**Labelled mg/caps**	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	167.972 ±0.073	N/D	0.0415 ±0.003	0.500 ±0.188	10.124 ±0.069	40.562 ±0.059	50.612 ±1.727	1.094 ±0.174	0.053 ±0.043	N/D	0.0300 ±0.001	5.256 ±0.659	0.076 ±0.003
MVM 32	**Labelled mg/caps**	0	0	0.2	1	8	0	100	2	0	0	0.1	15	0
	Found mg ± RSD %	N/D	N/D	0.2088 ±0.002	1.000 ±0.177	7.909 ±0.074	0.224 ±0.022	100.099 ±1.728	2.049 ±0.172	0.379 ±0.034	N/D	0.0998 ±0.001	15.089 ±0.702	N/D
MVM 33	**Labelled mg/caps**	120	0	0.025	1.5	8	40.5	50	3.5	0	0	0.05	15	0
	Found mg ± RSD %	119.948 ±0.072	N/D	0.0260 ±0.004	1.502 ±0.181	8.023 ±0.067	40.929 ±0.059	50.249 ±1.720	3.496 ±0.172	0.341 ±0.026	N/D	0.0510 ±0.001	15.498 ±0.634	N/D
MVM 34	**Labelled mg/caps**	0	0	0.05	1.5	6	0	50	3	0	0	0.15	15	0
	Found mg ± RSD %	N/D	N/D	0.0504 ±0.002	1.489 ±0.177	5.979 ±0.071	0.199 ±0.025	49.469 ±1.726	3.072 ±0.172	0.294 ±0.028	N/D	0.1500 ±0.001	14.709 ±0.673	0.046 ±0.005
MVM 35	**Labelled mg/caps**	75	0	0.15	2.15	19	80	104	2.2	0.5	0	0.025	6	0.0125
	Found mg ± RSD %	73.607 ±0.081	N/D	0.1472 ±0.002	2.19 ±0.175	19.114 ±0.073	80.012 ±0.068	103.843 ±1.705	2.564 ±0.170	0.457 ±0.030	N/D	0.0257 ±0.001	6.026 ±0.711	0.011 ±0.008

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 36	**Labelled mg/caps**	75	0	0.15	2.15	19	80	104	2.2	0.5	0	0.025	6	0.0125
	Found mg ± RSD %	73.542 ±0.085	N/D	0.1439 ±0.003	2.16 ±0.181	19.062 ±0.077	79.431 ±0.070	103.295 ±1.720	2.206 ±0.172	0.507 ±0.045	N/D	0.0266 ±0.001	6.043 ±0.737	0.013 ±0.009
MVM 37	**Labelled mg/caps**	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	167.986 ±0.071	N/D	0.0403 ±0.004	0.498 ±0.183	9.029 ±0.070	40.132 ±0.061	49.479 ±1.705	1.039 ±0.171	0.049 ±0.029	N/D	0.0294 ±0.001	5.386 ±0.681	N/D
MVM 38	**Labelled mg/caps**	160	0	0.025	1.1	0	0	100	2	0	0	0.025	14	0
	Found mg ± RSD %	160.143 ±0.079	N/D	0.0252 ±0.006	1.101 ±0.174	N/D	0.489 ±0.013	100.612 ±1.675	2.069 ±0.167	0.835 ±0.026	N/D	0.0251 ±0.001	14.016 ±0.691	N/D
MVM 39	**Labelled mg/caps**	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	167.907 ±0.074	N/D	0.0410 ±0.004	0.501 ±0.175	10.082 ±0.071	40.057 ±0.064	50.435 ±1.665	1.082 ±0.167	0.051 ±0.039	N/D	0.0292 ±0.001	5.048 ±0.686	N/D
MVM 40	**Labelled mg/caps**	0	0	0.2	1	8	0	100	2	0	0	0.1	15	0
	Found mg ± RSD %	N/D	N/D	0.2002 ±0.003	0.999 ±0.176	7.963 ±0.075	0.330 ±0.023	99.442 ±1.720	2.078 ±0.171	0.470 ±0.013	N/D	0.1095 ±0.001	15.051 ±0.719	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 41	**Labelled mg/caps**	120	0	0.025	1.5	8	40.5	50	3.5	0	0	0.05	15	0
	Found mg ± RSD %	119.956 ±0.075	N/D	0.0266 ±0.001	1.499 ±0.088	8.015 ±0.071	40.873 ±0.081	50.923 ±0.874	3.538 ±0.091	0.242 ±0.008	N/D	0.0501 ±0.003	15.319 ±0.675	N/D
MVM 42	**Labelled mg/caps**	0	0	0.05	1.5	6	0	50	3	0	0	0.15	15	0
	Found mg ± RSD %	4.145 ±0.072	N/D	0.0489 ±0.001	1.496 ±0.093	5.933 ±0.071	0.192 ±0.072	49.020 ±0.951	3.044 ±0.096	0.354 ±0.022	N/D	0.1499 ±0.002	14.978 ±0.742	N/D
MVM 43	**Labelled mg/caps**	100	0	0	2	10	0	10	2.5	0	0	0.05	1	0
	Found mg ± RSD %	99.961 ±0.076	N/D	N/A	2.036 ±0.091	9.998 ±0.073	0.327 ±0.071	10.222 ±0.908	2.504 ±0.093	0.045 ±0.003	N/D	0.0499 ±0.002	0.972 ±0.702	N/D
MVM 44	**Labelled mg/caps**	0	0	0.05	1.5	12	0	100	2.5	0	0	0.1	12	0
	Found mg ± RSD %	N/D	N/D	0.0542 ±0.001	1.478 ±0.088	11.962 ±0.070	0.024 ±0.083	100.187 ±0.871	2.460 ±0.091	0.047 ±0.002	0.007 ±0.001	0.1192 ±0.002	12.038 ±0.673	N/D
MVM 45	**Labelled mg/caps**	220	0	0.05	0.5	0	80	50	2.3	0	0	0.019	11	0
	Found mg ± RSD %	220.388 ±0.072	N/D	0.0544 ±0.001	0.516 ±0.091	N/D	80.084 ±0.068	50.345 ±0.883	2.352 ±0.092	0.691 ±0.003	N/D	0.0195 ±0.002	11.040 ±0.675	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM46	**Labelled mg/caps**	162	0	0.065	2	18	80	100	3.5	0	0	0.02	15	0
	Found mg ± RSD %	162.000 ±0.067	N/D	0.0673 ±0.001	1.956 ±0.088	18.007 ±0.067	79.923± 0.064	100.023 ±0.873	3.555 ±0.090	0.690 ±0.003	N/D	0.0203 ±0.002	15.148 ±0.649	0.046 ±0.005
MVM 47	**Labelled mg/caps**	500	0	0.12	2	18	0	50	2	0	0	0.02	15	0.012
	Found mg ± RSD %	496.203 ±0.068	0.040 ±0.001	0.1239 ±0.001	2.045 ±0.088	18.339±0. 069	0.341 ±0.087	50.364 ±0.882	2.044 ±0.094	0.919 ±0.000	N/D	0.0200 ±0.002	15.751 ±0.677	0.011 ±0.008
MVM 48	**Labelled mg/caps**	200	0	0	0	28	0	0	0	0	0	0	25	0
	Found mg ± RSD %	200.550 ±0.064	N/D	0.1743 ±0.001	0.276 ±0.099	27.989±0. 064	0.193 ±0.057	N/D	N/D	0.620 ±0.007	N/D	N/D	25.264 ±0.621	N/D
MVM 49	**Labelled mg/caps**	25	0	0	3	0	0	0	0	0	0	0	23.9	0
	Found mg ± RSD %	24.833 ±0.076	N/D	N/D	2.996 ±0.090	3.494 ±0.080	0.177 ±0.105	N/D	0.135 ±0.205	0.551 ±0.004	N/D	N/D	23.833 ±0.720	N/D
MVM 50	**Labelled mg/caps**	800	0	0	0	0	0	300	0	0	0	0	10	0
	Found mg ± RSD %	800.294 ±0.070	0.021 ±0.001	0.1678 ±0.001	0.338 ±0.102	4.281 ±0.075	0.292 ±0.086	300.413 ±0.936	N/D	0.672 ±0.001	N/D	N/D	9.956 ±0.694	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 51	**Labelled mg/caps**	200	0	0.2	1	18	20	100	6	5	0	0.1	15	0
	Found mg ± RSD %	199.952 ±0.074	N/D	0.1926 ±0.001	1.045 ±0.095	17.396 ±0.070	19.708 ±0.071	99.380 ±0.918	6.025 ±0.093	5.018 ±0.003	N/D	0.0994 ±0.002	14.999 ±0.686	N/D
MVM 52	**Labelled mg/caps**	0	0	0	1	15	0	0	0	0	0	0	12	0
	Found mg ± RSD %	2.388 ±0.088	N/D	N/D	1.018 ±0.088	15.044 ±0.070	0.066 ±0.087	3.137 ±0.941	N/D	0.186 ±0.004	N/D	N/D	12.143 ±0.686	N/D
MVM 53	**Labelled mg/caps**	200	0	0	1	15	0	150	0	0	0	0	15	0
	Found mg ± RSD %	200.046 ±0.080	N/D	N/D	0.992 ±0.090	16.827 ±0.069	0.175 ±0.074	150.340 ±0.895	N/D	0.289 ±0.002	N/D	N/D	14.809 ±0.663	N/D
MVM 54	**Labelled mg/caps**	220	0	0.05	0.5	0	80	50	2.3	0	0	0.019	11	0
	Found mg ± RSD %	219.807 ±0.081	N/D	0.0524 ±0.001	0.499 ±0.092	N/D	80.324 ±0.077	50.327 ±0.913	2.333 ±0.092	0.642 ±0.002	N/D	0.0191 ±0.002	10.904 ±0.764	0.025 ±0.002
MVM 55	**Labelled mg/caps**	162	0	0.065	2	18	80	100	3.5	0	0	0.02	15	0.01
	Found mg ± RSD %	162.004 ±0.067	N/D	0.0686 ±0.001	2.034 ±0.096	18.001 ±0.070	80.051 ±0.063	100.831 ±0.927	3.573 ±0.098	0.729 ±0.004	N/D	0.0195 ±0.002	14.936 ±0.654	0.011 ±0.005

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 56	**Labelled mg/caps**	500	0	0.12	2	18	0	50	2	0	0	0.02	15	0
	Found mg ± RSD %	500.587 ±0.065	N/D	0.1177 ±0.001	1.986 ±0.109	18.107 ±0.068	0.377 ±0.070	50.914 ±1.101	2.000 ±0.116	0.889 ±0.001	N/D	0.0201 ±0.002	14.870 ±0.659	N/D
MVM 57	**Labelled mg/caps**	200	0	0	0	28	0	0	0	0	0	0	25	0
	Found mg ± RSD %	200.497 ±0.072	N/D	0.1553 ±0.001	0.230 ±0.113	27.981 ±0.072	0.175 ±0.116	N/D	0.269 ±0.144	0.598 ±0.005	N/D	N/D	24.355 ±0.703	N/D
MVM 58	**Labelled mg/caps**	25	0	0	3	0	0	0	0	0	0	0	23.9	0
	Found mg ± RSD %	24.128 ±0.081	N/D	N/D	3.000± 0.140	3.037 ±0.080	0.181 ±0.080	N/D	N/D	0.521 ±0.009	N/D	N/D	23.998 ±0.704	N/D
MVM 59	**Labelled mg/caps**	800	0	0	0	0	0	300	0	0	0	0	10	0
	Found mg ± RSD %	799.051 ±0.095	0.028 ±0.001	N/D	N/D	N/D	0.322 ±0.098	300.088 ±1.388	N/D	0.639 ±0.006	N/D	N/D	10.345 ±0.775	N/D
MVM 60	**Labelled mg/caps**	200	0	0.2	1	18	20	100	6	5	0	0.1	15	0
	Found mg ± RSD %	200.133 ±0.078	N/D	0.1968 ±0.002	1.046 ±0.144	18.073 ±0.072	20.723 ±0.093	99.662 ±1.374	6.158 ±0.207	5.040 ±0.008	N/D	0.0997	15.165 ±0.695	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 61	**Labelled mg/caps**	0	0	0	1	15	0	0	0	0	0	0	12	0
	Found mg ± RSD %	4.011 ±0.072	N/D	N/D	0.990 ±0.135	14.805 ±0.070	0.495 ±0.072	23.844 ±1.318	N/D	0.213 ±0.002	N/D	N/D	12.300 ±0.679	N/D
MVM 62	**Labelled mg/caps**	200	0	0	1	15	0	150	0	0	0	0	15	0
	Found mg ± RSD %	200.446 ±0.067	N/D	N/D	1.024 ±0.157	14.829 ±0.072	0.099 ±0.087	150.158 ±1.390	0.073 ±0.251	0.276 ±0.006	N/D	N/D	14.713 ±0.680	N/D
MVM 63	**Labelled mg/caps**	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	167.968 ±0.073	N/D	0.0430 ±0.002	0.503 ±0.137	10.073 ±0.070	40.573 ±0.071	50.370 ±1.342	1.034 ±0.142	0.046 ±0.009	N/D	0.0302 ±0.002	5.148 ±0.697	N/D
MVM 64	**Labelled mg/caps**	160	0	0.025	1.1	0	0	100	2	0	0	0.025	14	0
	Found mg ± RSD %	160.030 ±0.070	N/D	0.0261 ±0.002	1.096 ±0.149	3.537 ±0.077	0.213 ±0.068	100.358 ±1.484	2.077 ±0.154	0.658 ±0.004	N/D	0.0251 ±0.001	13.765 ±0.681	N/D
MVM 65	**Labelled mg/caps**	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	167.919 ±0.072	N/D	0.0401 ±0.002	0.500 ±0.143	10.024 ±0.071	40.517 ±0.069	50.261 ±1.407	1.044 ±0.148	0.050 ±0.013	N/D	0.0301 ±0.001	5.270 ±0.710	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 66	**Labelled mg/caps**	0	0	0.2	1	8	0	100	2	0	0	0.1	15	0
	Found mg ± RSD %	4.574 ±0.085	N/D	0.2088 ±0.002	0.995 ±0.130	8.315 ±0.074	0.132 ±0.083	100.529 ±1.293	2.002 ±0.125	0.311 ±0.006	N/D	0.0999 ±0.001	15.506 ±0.722	N/D
MVM 67	**Labelled mg/caps**	120	0	0.025	1.5	8	40.5	50	3.5	0	0	0.05	15	0
	Found mg ± RSD %	119.514 ±0.071	N/D	0.0275 ±0.002	1.501 ±0.151	8.039 ±0.070	40.703 ±0.067	50.536 ±1.506	3.521 ±0.148	0.251 ±0.009	N/D	0.0500 ±0.001	15.637 ±0.675	N/D
MVM 68	**Labelled mg/caps**	0	0	0.05	1.5	6	0	50	3	0	0	0.15	15	0
	Found mg ± RSD %	N/D	N/D	0.0538 ±0.002	1.504 ±0.130	6.232 ±0.075	0.106 ±0.075	49.711 ±1.297	3.096 ±0.130	0.222 ±0.004	N/D	0.1501 ±0.001	15.281 ±0.720	N/D
MVM 69	**Labelled mg/caps**	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	167.028 ±0.071	N/D	0.0378 ±0.002	0.504 ±0.148	10.891 ±0.069	40.107 ±0.068	50.247 ±1.481	0.914 ±0.157	0.047 ±0.006	N/D	0.0296 ±0.001	4.893 ±0.681	N/D
MVM 70	**Labelled mg/caps**	160	0	0.025	1.1	0	0	100	2	0	0	0.025	14	0
	Found mg ± RSD %	158.127 ±0.072	N/D	0.0236 ±0.003	1.099 ±0.167	N/D	0.209 ±0.087	100.032 ±1.659	2.020 ±0.170	0.612 ±0.007	N/D	0.0250 ±0.001	14.282 ±0.690	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 71	**Labelled mg/caps**	168	0	0.04	0.5	10	40	50	1	0.05	0	0.03	5	0
	Found mg ± RSD %	167.058 ±0.069	N/D	0.0385 ±0.002	0.500 ±0.146	9.691 ±0.069	40.040 ±0.067	50.232 ±1.453	1.047 ±0.148	0.053 ±0.012	N/D	0.0301 ±0.001	5.116 ±0.672	N/D
MVM 72	**Labelled mg/caps**	0	0	0.2	1	8	0	100	2	0	0	0.1	15	0
	Found mg ± RSD %	2.083 ±0.077	N/D	0.2030 ±0.002	1.003 ±0.132	7.914 ±0.069	0.205 ±0.075	100.240 ±1.311	2.068 ±0.132	0.347 ±0.003	N/D	0.1008 ±0.001	15.437 ±0.667	N/D
MVM 73	**Labelled mg/caps**	120	0	0.025	1.5	8	40.5	50	3.5	0	0	0.05	15	0
	Found mg ± RSD %	120.36 ±0.080	N/D	0.0251 ±0.002	1.496 ±0.152	8.159 ±0.068	40.519 ±0.092	50.447 ±1.510	3.599 ±0.149	0.239 ±0.008	N/D	0.0508 ±0.001	15.419 ±0.657	N/D
MVM 74	**Labelled mg/caps**	0	0	0.05	1.5	6	0	50	3	0	0	0.15	15	0
	Found mg ± RSD %	4.713 ±0.074	N/D	0.0482 ±0.003	1.502 ±0.166	5.370 ±0.072	0.158 ±0.090	50.289 ±1.646	3.002 ±0.168	0.213 ±0.007	N/D	0.1503 ±0.001	14.812 ±0.770	N/D
MVM 75	**Labelled mg/caps**	100	0	0	2	10	0	10	2.5	0	0	0.05	1	0
	Found mg ± RSD %	100.922 ±0.071	N/D	N/D	2.036 ±0.154	9.854 ±0.070	0.322 ±0.068	10.325 ±1.527	2.510 ±0.152	0.046 ±0.001	N/D	0.0510 ±0.001	1.182 ±0.674	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 76	**Labelled mg/caps**	0	0	0.05	1.5	12	0	100	2.5	0	0	0.1	12	0
	Found mg ± RSD %	N/D	N/D	0.0540 ±0.002	1.471 ±0.169	11.843 ±0.075	0.024 ±0.075	100.363 ±1.696	2.551 ±0.173	0.042 ±0.008	N/D	0.0956 ±0.001	11.204 ±0.727	N/D
MVM 77	**Labelled mg/caps**	220	0	0.05	0.5	0	80	50	2.3	0	0	0.019	11	0
	Found mg ± RSD %	219.170 ±0.069	N/D	0.0544 ±0.002	0.496 ±0.156	3.500 ±0.074	80.012 ±0.067	50.986 ±1.505	2.324 ±0.153	0.697 ±0.004	N/D	0.0193 ±0.001	11.354 ±0.674	N/D
MVM 78	**Labelled mg/caps**	162	0	0.065	2	18	80	100	3.5	0	0	0.02	15	0.01
	Found mg ± RSD %	161.652 ±0.074	N/D	0.0662 ±0.002	2.005 ±0.160	18.868 ±0.071	79.431 ±0.069	100.652 ±1.593	3.554 ±0.161	0.697 ±0.001	N/D	0.0200 ±0.001	15.346 ±0.691	0.011 ±0.001
MVM 79	**Labelled mg/caps**	500	0	0.12	2	18	0	50	2	0	0	0.02	15	0
	Found mg ± RSD %	500.755 ±0.077	N/D	0.1197 ±0.002	1.951 ±0.163	18.159 ±0.077	0.331 ±0.087	50.122 ±1.638	2.002 ±0.170	0.877 ±0.002	N/D	0.0200 ±0.001	15.262 ±0.746	N/D
MVM 80	**Labelled mg/caps**	200	0	0	0	28	0	0	0	0	0	0	25	0
	Found mg ± RSD %	199.848 ±0.069	N/D	N/D	0.216 ±0.185	28.980 ±0.070	0.147 ±0.106	N/D	0.305 ±0.242	0.589 ±0.005	N/D	N/D	24.795 ±0.684	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 81	**Labelled mg/caps**	25	0	0	3	0	0	0	0	0	0	0	23.9	0
	Found mg ± RSD %	24.195 ±0.077	N/D	N/D	2.996 ±0.166	N/D	0.234 ±0.049	N/D	0.104 ±0.325	0.549 ±0.006	N/D	N/D	24.031 ±0.693	N/D
MVM 82	**Labelled mg/caps**	800	0	0	0	0	0	300	0	0	0	0	10	0
	Found mg ± RSD %	799.908 ±0.077	N/D	N/D	0.258 ±0.189	N/D	0.164 ±0.144	299.983 ±1.734	N/D	0.627 ±0.007	N/D	N/D	10.016 ±0.785	N/D
MVM 83	**Labelled mg/caps**	200	0	0.2	1	18	20	100	6	5	0	0.1	15	0
	Found mg ± RSD %	199.924 ±0.069	N/D	0.2017 ±0.002	1.012 ±0.172	17.846 ±0.067	20.135 ±0.064	100.046 ±1.685	6.296 ±0.170	5.007 ±0.001	N/D	0.0992 ±0.002	14.628 ±0.663	N/D
MVM 84	**Labelled mg/caps**	0	0	0	1	15	0	0	0	0	0	0	12	0
	Found mg ± RSD %	N/D	N/D	N/D	0.996 ±0.174	15.258±0.068	0.085 ±0.088	N/D	0.075 ±0.244	0.198 ±0.001	N/D	N/D	11.779 ±0.670	N/D
MVM 85	**Labelled mg/caps**	200	0	0	1	15	0	150	0	0	0	0	15	0
	Found mg ± RSD %	200.203 ±0.091	N/D	N/D	0.996 ±0.174	15.023 ±0.075	0.113 ±0.111	150.284 ±1.731	N/D	0.249 ±0.012	N/D	N/D	14.730 ±0.738	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 86	**Labelled mg/caps**	220	0	0.05	0.5	0	80	50	2.3	0	0	0.019	11	0
	Found mg ± RSD %	219.727 ±0.072	N/D	0.0480 ±0.003	0.501 ±0.174	2.449 ±0.079	80.186 ±0.071	50.239 ±1.724	2.237 ±0.178	0.625 ±0.001	N/D	0.0195 ±0.002	10.621 ±0.696	N/D
MVM 87	**Labelled mg/caps**	162	0	0.065	2	18	80	100	3.5	0	0	0.02	15	0.01
	Found mg ± RSD %	159.662 ±0.092	N/D	0.0660	1.963 ±0.176	17.916 ±0.075	79.728 ±0.091	100.195 ±1.673	3.526 ±0.319	0.494 ±0.028	N/D	0.0205 ±0.002	14.558 ±1.062	0.009 ±0.025
MVM 88	**Labelled mg/caps**	500	0	0.12	2	18	0	50	2	0	0	0.02	15	0
	Found mg ± RSD %	499.076 ±0.070	N/D	0.1225 ±0.002	2.000 ±0.168	17.982 ±0.069	0.189 ±0.139	50.992 ±1.672	2.000 ±0.171	0.792 ±0.005	N/D	0.0201 ±0.002	14.660 ±0.674	0.022 ±0.001
MVM 89	**Labelled mg/caps**	200	0	0	0	28	0	0	0	0	0	0	25	0
	Found mg ± RSD %	199.864 ±0.068	N/D	N/D	0.202 ±0.180	28.217 ±0.068	0.121 ±0.119	N/D	N/D	0.555 ±0.001	N/D	N/D	23.171 ±0.655	N/D
MVM 90	**Labelled mg/caps**	25	0	0	3	0	0	0	0	0	0	0	23.9	0
	Found mg ± RSD %	24.433 ±0.083	N/D	N/D	2.996 ±0.173	2.724 ±0.078	0.190 ±0.076	N/D	N/D	0.487 ±0.014	N/D	N/D	23.651 ±0.699	N/D

Table 3.14 (cont.)

Formulation product		Ca	Co	Cr	Cu	Fe	K	Mg	Mn	Na	Ni	Se	Zn	V
MVM 91	**Labelled mg/caps**	800	0	0	0	0	0	300	0	0	0	0	10	0
	Found mg ± RSD %	799.481 ±0.091	N/D	N/D	N/A	N/D	0.262 ±0.099	300.144 ±1.702	N/D	0.619 ±0.007	N/D	N/D	9.876 ±0.709	N/D
MVM 92	**Labelled mg/caps**	200	0	0.2	1	18	20	100	6	5	0	0.02	15	0
	Found mg ± RSD %	199.917 ±0.081	N/D	0.2044 ±0.003	0.966 ±0.174	18.164 ±0.070	19.510 ±0.080	100.120 ±1.678	6.149 ±0.258	4.974 ±0.012	N/D	0.0200 ±0.001	15.105 ±0.675	N/D
MVM 93	**Labelled mg/caps**	0	0	0	1	15	0	0	0	0	0	0	12	0
	Found mg ± RSD %	4.891 ±0.068	N/D	N/D	0.994 ±0.173	14.974 ±0.067	0.483 ±0.068	4.455 ±1.712	1.262 ±0.175	0.203 ±0.001	N/D	N/D	12.286 ±0.644	N/D
MVM 94	**Labelled mg/caps**	200	0	0	1	15	0	150	0	0	0	0	15	0
	Found mg ± RSD %	199.965 ±0.069	N/D	N/D	1.004 ±0.105	15.003 ±0.077	0.081 ±0.127	149.791 ±0.912	N/D	0.268 ±0.007	N/D	N/D	14.977 ±0.709	N/D

3.2 Conclusions

Fast and accurate sample preparing method for multivitamin/multimineral (MVM) method was optimized and succeeding AAS analysis was validated. The analytical method presented satisfactory linearity, LOD, LOQ, accuracy, repeatability, and reproducibility for seven elements (Cu, Fe, Mn, Mg, Zn, Se and Cr).

A Factorial design of experiments (DOE) to determine microwave digestion conditions both for simultaneous multielement extraction of Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, Se, V, and Zn in MVM, and for determination by ICP-MS was applied. The digestion method was optimized and the determination method was validated giving satisfactory result.

Not some of the formulations had higher levels than those labelled, and consequently were of poor manufacturing quality but also, in several instances, some samples contained Ca, K, Na, Ni, and V whose presences was not mentioned in the label and therefore could cause health disorders and diseases. Moreover, the high metal levels found in several analysed products indicated the importance of strengthening the regulation and monitoring of the manufacture of dietary supplements as proposed by the USP (Pharmacopeial Forum, 2008). This problem is not only of local but also of general importance as analysed MVM samples were produced by both Sudanese and foreign manufacturers.

3.3 Recommendations

* Researcher should develop screening methods for simultaneous multielement of analysis toxic elements (Ar, Pb, Hg...etc.) and essential elements (macro and micro elements) in multivitamin/multimineral and raw materials.
* Researcher should develop screening methods for other multivitamin/multimineral formulation such as those for enhanced performance or energy, weight control, cardiovascular disease, cancer improved immune function, or management of menopause symptoms.
* Researcher should develop analytical methods for multivitamin/multimineral by using other analytical technique, such as chromatography, electrophoresis.
* Users should be aware about the accumulative behavior of macro and micro elements and other constituents so that they can take care in consuming multivitamin/multimineral.
* Health authorities should strictly adopt FDA, and WHO regulations and /or any other relevant regulations of distribution of multivitamin/multimineral products (MVM) to prevent the sale of products with harmful ingredients that jeopardizes consumer health.
* The government should ensure that these (MVM) contain only what is on the label and does not contain any harmful or undesirable substances, such as toxic metals, and essential elements concentration at the recommendation intake allowance (RIA).
* Manufacturers should be aware that the safety of multivitamin dietary supplements depends on various factors including the manufacturing process and the purity and origins of the raw ingredients.
* Manufacturers should be aware that multivitamin/multimineral raw materials may contain high levels of certain elements, especially Pb and As.

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