DETERMINATION OF MINERAL CONTENTS IN THAI HERBS USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY

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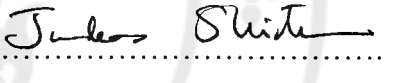

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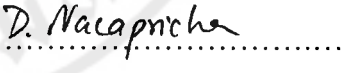

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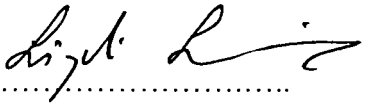

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SARAWUT LUNVONGSA: DETERMINATION OF MINERAL CONTENTS IN THAI HERBS USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY. THESIS ADVISOR : JUWADEE SHIOWATANA, Ph. D., DUANGJAI NACAPRICHA, Ph. D. 105 p. ISBN 974-664-238-3

The presence of minerals in food can have both good and bad consequences. Several minerals are known to be essential for human life while others are toxic. Determination of mineral contents of edible plants is therefore of considerable interest. This work attempted to determine 24 metals in 17 common Thai herbs and 5 herb capsules using a multielemental analytical technique, Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The performance of ICP-MS has been investigated as a function of a number of parameters associated with sample uptake rate and plasma operation. 1.1 mL/min sample uptake rate, 1300 watts RF power and 0.95 L/min nebulizer gas flow rate were the optimum conditions obtained. Indium was used as the internal standard in order to compensate for the difference in analyte signals resulting from sample preparation error and nebulization changing efficiency. The isobaric elemental interferences were corrected by selecting suitable isotopes and applying the appropriate correction equations.

The microwave digestion of the plant samples in a mixture of HNO_3 and H_2O_2 was explored. This digestion procedure is simple and quick and gives low reagent blank, also minimizing interference effect.

The accuracy of the method was assessed by analysis of a certified reference material, SRM 1515: Apple leaves. The analytical results showed that, twelve elements (Al, B, Ba, Co, Cu, Mg, Mn, Na, P, Pb, Sr and V) could be determined with good accuracy. The results of Mo, Ni, Sb and Zn were lower than the certified values probably due to incomplete digestion. As, Cr, Fe and Se gave higher values than certified values possibly owing to positive polyatomic interferences. The isotope ratios after mass bias correction of $^{53}Cr/^{52}Cr$, $^{54}Fe/^{57}Fe$ and $^{82}Se/^{77}Se$ were found to be -60.6, -63.4 and -63.9 % their natural ratios, respectively, indicating the interference problem of Cr, Fe and Se measurement. Separation by anion exchange resin was successfully used to eliminate (Cr and Fe) and reduce (As and Se) interferences from the existing anions. Upon treatment, acceptable recoveries were obtained for Cr and Fe but As and Se could not be determined with good accuracy although the results were improved. Ca and K could not be determined with good accuracy because of inconsistency in blank intensities, and were therefore determined using Flame Atomic Absorption Spectrometry (FAAS).

The method developed was applied to the analysis of 17 selected common Thai herbs, collected from three locations (Kabin buri, Pinklao and Kingpetch), 5 selected herb capsules.

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ศราวุธ ลันวงษา : การวิเคราะห์ปริมาณแร่ธาตุต่างๆในพืชสมุนไพรไทยด้วยเทคนิคอินดักทีพลีคัพเปิลพลาสมาแมสสเปกโตรเมตรี (DETERMINATION OF MINERAL CONTENTS IN THAI HERBS USING INDUCTIVELY COUPLED PLASMA MASS SPECTROMETRY) : คณะกรรมการควบคุมวิทยานิพนธ์ : ยุวดี เชี่ยววัฒนา, Ph. D., ดวงใจ นาคะปรีชา, Ph. D. 105 หน้า. ISBN 974-664-238-3

แร่ธาตุที่มีอยู่ในอาหารส่งผลทั้งในแง่ดีและไม่ดีต่อร่างกายมนุษย์ หลายชนิดมีความจำเป็นบางชนิดกลับก่อให้เกิดโทษถึงแม้ว่าจะได้รับในปริมาณน้อย การวิเคราะห์ปริมาณแร่ธาตุเหล่านี้ที่มีอยู่ในพืชผักจึงได้รับความสนใจเป็นอย่างมาก ในงานวิจัยนี้ได้ศึกษาวิธีวิเคราะห์ปริมาณแร่ธาตุต่างๆ 24 ชนิด ในตัวอย่างผักพื้นบ้าน 17 ชนิดและยาเม็ดสมุนไพร 5 ชนิดด้วยเทคนิค ICP-MS การศึกษาประสิทธิภาพของ ICP-MS ที่มีผลมาจากอัตราเร็วในการดูดสารละลายและปัจจัยที่มีผลต่อสภาวะของพลาสมาพบว่าที่อัตราเร็วของการดูดสารละลายเท่ากับ 1.1 มิลลิลิตรต่อนาที ค่า RF power เท่ากับ 1300 วัตต์ และค่าอัตราเร็วของ Nebulizer gas เท่ากับ 0.95 ลิตรต่อนาทีเป็นสภาวะที่ดีที่สุด อินเดียมถูกใช้เป็นสารเทียบมาตรฐานในการปรับค่าสัญญาณของธาตุที่สนใจที่อาจผิดผลาดไปในขั้นตอนการเตรียมสารละลายตัวอย่างหรืออาจคาดเคลื่อนไปในขั้นตอนของการผลิตละอองฝอย ปัญหาการรบกวนของธาตุที่มีน้ำหนักเท่ากันได้ทำการแก้ไขโดยเลือกไอไซโทปอื่นที่เหมาะสมและหักลบส่วนเกินด้วยสมการคณิตศาสตร์

การตรวจสอบผลการย่อยตัวอย่างพืชในกรดผสมระหว่างกรดไนตริกและไฮโดรเจนเปอออกไซด์ด้วยเครื่องไมโครเวฟพบว่าวิธีการย่อยมีความสะดวก รวดเร็ว ปริมาณ Reagent blank ต่ำและยังสามารถลดผลการรบกวนที่อาจเกิดขึ้นได้

การประเมินผลความถูกต้องของวิธีการวิเคราะห์ได้ศึกษาโดยการวิเคราะห์สารอ้างอิงมาตรฐานใบแอบเปิล SRM 1515 พบว่าธาตุ 12 ชนิด (Al B Ba Co Cu Mg Mn Na P Pb Sr และ V) ให้ผลการวิเคราะห์ที่ถูกต้อง ส่วน Mo Ni Sb และ Zn ให้ผลการวิเคราะห์ต่ำกว่าค่ารับรองทั้งนี้อาจเป็นเพราะว่าธาตุเหล่านี้ถูกย่อยได้ไม่สมบูรณ์นัก ส่วน As Cr Fe และ Se ให้ผลการวิเคราะห์สูงกว่าค่ารับรองซึ่งอาจเป็นผลจากการรบกวนของ Polyatomic ions ทั้งนี้การวัดสัดส่วนไอโซโทปของธาตุที่สงสัยหลังจากการแก้ค่าความผิดผลาดจากความแตกต่างของน้ำหนักแล้วพบว่าสัดส่วนของ $^{53}Cr/^{52}Cr$, $^{54}Fe/^{57}Fe$ และ $^{82}Se/^{77}Se$ มีค่าที่แตกต่างจากสัดส่วนธรรมชาติเท่ากับ –66.6 –63.4 และ –63.9 % ตามลำดับ แสดงว่าเกิดปัญหาการรบกวนขณะการวิเคราะห์ธาตุเหล่านี้ เรซินชนิดแลกเปลี่ยนไอออนลบแยกไอออนลบสามารถแก้ปัญหาการรบกวนที่เกิดขึ้นกับการวิเคราะห์ Cr และ Fe ได้และลดปัญหาการรบกวนลงได้บางส่วนสำหรับการวิเคราะห์ As และ Se ด้วยวิธีการนี้สามารถวิเคราะห์ Cr และ Fe ได้ด้วยความถูกต้องที่ดีแต่ยังได้ค่าที่สูงกว่าความเป็นจริงสำหรับ As และ Se ส่วน Ca และ K ไม่สามารถวิเคราะห์ปริมาณที่ถูกต้องได้ถูกต้องด้วย ICP-MS เนื่องจากความไม่คงตัวของสัญญาณ Reagent blank ดังนั้นจึงวิเคราะห์ด้วย FAAS

วิธีการที่ได้พัฒนาขึ้นนี้ได้นำมาประยุกต์ใช้เพื่อทำการวิเคราะห์ปริมาณของแร่ธาตุต่างๆในพืชผักพื้นบ้านจำนวน 17 ชนิดที่เก็บตัวอย่างมาจากสามแหล่งคือ กบินทร์บุรี ปิ่นเกล้าและกิ่งเพชรและยาเม็ดสมุนไพรอีก 5 ชนิด

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LIST OF ABBREVIATIONS

ICP-MS	Inductively Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ETAAS	Electrothermal Atomic Absorption Spectrometry
FAAS	Flame Atomic Absorption Spectrometry
HPLC	High Performance Liquid Chromatography
NAA	Neutron Activation Analysis
ETV	Electrothermal Vaporization
HG	Hydride Generation
mg/Kg	Milligram per kilogram
µg/Kg	Microgram per kilogram
mg/L	Milligram per liter
µg/L	Microgram per gram
ng/g	Nanogram per gram
m/z	Mass per charge
M	Molarity
v/m	Volume by mass
L/min	Litter per minute
mL/min	Milliliter per minute
°C	Temperature in degree of Celsius
SRM	Standard Reference Material
et al	Et.alli (Latin), and others

CHAPTER I

INTRODUCTION

Over the long time of earth formation, minerals have moved from rocks to soil, then to plants, to animals, and to human. Living organisms are dependent on a number of these basic elements. The human body continuously assimilates a variety of inorganic elements from food and the environment. Some of them are closely related to human health and illness, as their deficiency or excess results physiological and metabolic changes. Therefore, the analysis of inorganic elements in foods either naturally occurring or contaminated is necessary for the evaluation of their impacts on human health.

Many elements are usually present in agricultural products because of the industrialization and the pollution of the biosphere. The elements were absorbed from the soil, the residue from fertilizer pesticide treatment, and other industrial and domestic operations. It is, therefore, important to determine these elements in food plants.

1.1 Classification of Minerals in the Body

Most of the metals in the body come from food [1]. However, not all the metals that human ingests are retained. Some of them are lost by the excretion in faeces, urine, sweat, hair and skin. The amount of the metal absorbed by human body

from foods depends to some extents on eating behavior and the state of health and genetic composition, as well as other factors, such as vitamins in food.

❖ Occurrence of Minerals in the Body

Body minerals are found in several forms in places related to their functions. There are two basic forms in which minerals occur in the body [2]:

i. Free form: ions and particles may exist freely as ions, which carry an electrical charge in body fluids, such as sodium in tissue fluids, regulating the water balance.

ii. Combined form: minerals may exist as combined with other minerals, such as calcium with phosphorus in bone; or combined with other organic substances, such as iron with heme and globin in hemoglobin.

Minerals as nutrients occur in varying amounts in the human body. They can be divided into two classes according to the individual amounts which are required for normal body function as shown in table 1.1.

Table 1.1 Major minerals and trace elements in human nutrition [2]

Major minerals (required intake over 100 mg/day)	**Trace elements**	
	Essential (required intake under 100 mg/day)	**Essentiality unclear**
Calcium (Ca)	Iron (Fe)	Silicon (Si)
Phosphorus (P)	Iodine (I)	Vanadium (V)
Sodium (Na)	Zinc (Zn)	Nickel (Ni)
Potassium (K)	Copper (Cu)	Tin (Sn)
Magnesium (Mg)	Manganese (Mn)	Cadmium (Cd)
Chloride (Cl)	Chromium (Cr)	Arsenic (As)
Sulfur (S)	Cobalt (Co)	Aluminium (Al)
	Selenium (Se)	Boron (B)
	Molybdenum (Mo)	
	Fluorine (F)	

1.1.1 Major Minerals

Elements are referred as major minerals not because they are more important, but simply because they occur in large amounts in the body. Seven major minerals include calcium, phosphorus, sodium, potassium, magnesium, chloride, and sulfur.

1.1.2 Trace Elements

Trace elements comprise eighteen elements (Table 1.1). These minerals are not unimportant, but they occur in traces in the body. They are also essential for their specific task.

To some extent of major minerals (or macronutrients) and essential trace elements (or micronutrients), they have three main functions in the body as follows:

i. Constituents of bone and teeth.

ii. Soluble salts that regulate the composition of body fluids and cells.

iii. Essential adjuncts to many enzymes and other functional proteins.

Besides the essential elements, there are other metals, which are widely distributed in small amounts in living tissues. Some of them appear to assist in vital activities but their absences does not cause any visible ill effects. These have been called 'beneficial' or 'essential unclear' elements [1].

1.2 Biological Function of Inorganic Elements

Among many metals found in the body, some of them are known to be essential for normal biological functions. The absence of them results in the appearance of characteristic pathological deficiency symptoms. Most of other metals present in the body no functional significance [1]. The great efforts made by organisms to absorb, accumulate, transport and store inorganic elements have been adjusted by their important and otherwise not guaranteed function. Kaim [3] described the functions of metals (as ions and its compounds), which are listed below.

A. Structural Function

The assembly of hard structures in the form of endoskeletons or exoskeletons via biomineralization certainly falls into this category. Another aspect of this structural function is that cell membranes require the presence of metal ions to cross-link the organic 'filling material', and thus maintain the membrane integrity. Mainly the elements, Ca, Mg (as dications) and P, O, C, S, Si, F (as parts of anions), represent solid state/structural functions.

B. Information Transfer

Simple atomic ions are superbly suited as charge carriers for very fast information transfer. Starting with a transmembrane concentration gradient which has to be actively maintained by integral membrane ion pumps, information units in the form of electrical potential jumps can be created via diffusion. Electrical impulses in nerves as well as more complex trigger mechanism are thus initiated with the fastest possible effect by sudden fluxes, diffusion control of atomic, i.e. chemically and biologically nondegradable inorganic ions of different size and charge of Na^+, K^+, and Ca^{2+}.

C. Catalyst Formation

Metabolism and degradation of organic compounds in organism often require acid or base catalysis. Since the physiological pH is generally limited to about 7, except stomach, the rate enhancement of such reactions cannot be accomplished by simple proton or hydroxide catalysis but requires lewis acid/ lewis base catalysis involving metal ions. Many hydrolytically active enzymes thus contain the relatively small, positively charge metal ions, Zn^{2+} and Mg^{2+}.

D. Short-term Energy Converter

The transfer of electrons which is essential for the short-term energy conversion in organisms is mainly, but not exclusively, dependent on redox active metal centers. A number of corresponding redox pairs has thus been found, some of which involving oxidation states that seem quite unusual under physiological conditions (marked in **bold**). Specific modifications induced by bioligand are largely responsible for the stabilization of such unusual oxidation states. Biologically relevant oxidation states are in particular the following oxidation states of redox active metals: $Fe^{I}/Fe^{II}/\mathbf{Fe^{IV}}$, $\mathbf{Cu^{I}}/Cu^{II}$, $Mn^{II}/\mathbf{Mn^{III}}/Mn^{IV}$, $\mathbf{Mo^{IV}}/Mo^{V}/Mo^{VI}$, $\mathbf{Co^{I}}/Co^{II}/Co^{III}$, $\mathbf{Ni^{I}}/Ni^{II}/\mathbf{Ni^{III}}$.

E. Activator

The activation of small, highly symmetrical molecules with large bond energies places stringent demands on the required catalysis. The ability of transition metal centers to provide unpaired electrons and to simultaneously accept and donate electronic charge allow organisms to carry out energetically and mechanistically difficult reactions under physiological condition, such as;

-The reversible uptake, transport, storage and conversion (Fe and Cu) also generation (Mn) of the paramagnetic dioxygen molecule, $^3O_2$.

-The fixation of molecular nitrogen and its conversion to ammonia (Fe, Mo, V).

-The reduction of CO_2 with hydrogen to give methane (Ni, Fe).

F. Constitution of Enzyme

Typical organometallic reactivity such as reductive alkylation or the facile generation of radicals for rapid rearrangement of substrate molecules is found for cobalamin coenzymes which contain a σ bond between the transition metal cobalt and primary alkyl groups.

1.3 Mineral Contents of Foods

The presence of minerals in food can have both good and bad consequences. Several minerals are known to be essential for human life while others are toxic, even in small amounts. Elemental food composition data are important to both consumers and health professionals. The determination of metals in food can be of interest to food processors, nutritionist, toxicologist, and wide variety of other scientists [1]. For nutritional evaluation, the focus on mineral contents is beneficial to human health. Due to the growing trend toward increasing consumer awareness, it seem likely that in the near future food labels will contained food composition data for all of the elements of nutritional interest [4]. The main metal content of food may come from ingredients such as vegetables (plants), salts etc. Other situation, the contamination during food process, food metals container. The metal come from plant foodstuffs is being considered. While all plant will, if placed in a nutritionally balanced soil, take up nutrients to the extent they need for growth, other possess a special ability which enables them to take up and accumulates, sometimes to very high level, certain specific elements [1]. For Thai foods, common Thai herbs are commonly used as an ingredient. The knowledge of mineral content in these plants is useful in nutritional evaluation.

1.4 Determination of Mineral Contents in Plant Materials

The determination of minerals in food plants is challenging because of the wide range of concentrations present, ranging from ng/g (such as Co and Cr) to percent levels (such as Na and K). To obtain accurate elemental results, sampling and sample preparation, developments in analytical instrumentation and methodology should be considered.

Methods widely used for the identification and quantification of elemental concentrations of biological sample, include Flame Atomic Absorption Spectrometry (FAAS) [5-9], Electrothermal Atomic Absorption Spectrometry (ETAAS) [5,10], Neutron Activation Analysis (NAA) [5-14], Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) and Inductively Coupled Plasma Mass Spectrometry (ICP-MS) [4,15-24]. From the literature, multielement methods are becoming increasingly prevalent for the investigation elements in biological samples (foods, and plants).

1.4.1 Flame Atomic Absorption Spectrometry

The most common analytical technique for investigation of nutritional minerals in food is FAAS [20]. This is because FAAS is simple to perform and has a low running cost. The sensitivity of FAAS for Na, K, Ca, Mg, and Zn is adequate for their determination in most food items [25].

1.4.2 Electrothermal Atomic absorption spectrometry

ETAAS is the most sensitive among atomic spectrometric method for large number of elements. Another advantage of ETAAS is possibility of direct analysis of solid samples without prior decomposition. Risk of loss and contamination are therefore reduced. When only one or two elements with low concentration are determined, ETAAS should be used.

1.4.3 Inductively Coupled Plasma Mass Spectrometry

Among the techniques widely used, ICP-MS has many advantages. ICP-MS not only offers multielement detection possibility, but also offers large dynamic ranges, which are important when analyzing a wide variety of minerals in biological samples. Although the elements of widespread nutritional interest do not typically require the excellent detection capabilities of ICP-MS, the ability to reliably detect and quantify ultratrace levels of metal could lead to an increased understanding of their role and impact on human health [4].

Although ICP-MS has many advantages over other techniques, one of more serious problem is mass spectral interference in which the mass of an undesirable species coincides with a mass of the elemental interest. There are several sources of mass spectral interference, as described below.

1.4.3.1 Interferences in ICP-MS Measurements

Interference is anything that causes the signal from an analyte to be different from the same signal for the same concentration of that analyte in a calibration solution.

As with all other atomic spectrometric analytical techniques, interfernces of various types can occur during routine ICP-MS determinations. Interferences associated with sample introduction using a nebulizer/spray chamber are common to atomic absorption, optical emission ICP and ICP-MS. These are termed transport interferences and is one of the non spectral interferences. In addition, interferences can occur from the presence of other isotopes or elements with the same atomic weight or mass number as an analyte of interest. These are termed mass spectral interferences.

1.4.3.1.1 Non Spectral Interferences

Sample introduction effects when using a nebulizer/spray chamber system are common in ICP-MS determinations. These interferences are generally the result of either sample matrix effects that influence surface tension and viscosity resulting in aerosol formation and the ion formation in the plasma. Complex sample matrices may interfere with the focusing of ions into the sample and skimmer cone orifices, and with the focusing and transporting of ions through the ion lenses and quadrupole.

1.4.3.1.2 Spectral Interferences

Spectral interferences are the result of other chemical species (isotopes or ions) which are present at the same atomic mass as an analyte of interest. Mass spectral interferences generally occur from the sources listed in the following:

i. Elemental Isobaric Overlaps

Since most elements have more than one naturally occurring isotope, it is possible for the mass spectrum of an isotope of one element to directly overlap that of an isotope of another element. These interferences are termed isobaric overlap. The selecting of alternate isotope can overcome this problem.

ii. Plasma Induced Polyatomic Ions

The presence of atmospheric gasses or the argon carriers are sources of potential interfering ions as the result of reaction with other analyte or matrix components. These interfering ions occur as polyatomic, molecular ions. The following table lists the most severe, common overlaps which occur.

Table 1.2 Plasma induced polyatomic ions

Analyte	% Abundance of isotope	Interfering ions
$^{32}S^+$	95.0	$^{16}O_2^+$
$^{39}K^+$	93.3	$^{38}ArH^+$
$^{40}Ca^+$	96.9	$^{40}Ar^+$
$^{56}Fe^+$	91.7	$^{40}Ar^{16}O^+$
$^{80}Se^+$	49.6	$^{40}Ar_2^+$

iii. Matrix Solvent Induced Polyatomic Ions

The solvent or acid in which the sample dissolved can also be a source of interfering polyatomic ions. Chlorine from hydrochloric or perchloric acid and sulfur from sulfuric acid all form polyatomic ions with argon and other plasma gases. For organic solvents, carbon and oxygen can form polyatomic ions. The major solvent induced overlaps are listed in Table 1.3

Table 1.3 Matrix solvent induced molecular ion overlaps

Analyte	% Abundance of isotope	Interfering ions
$^{28}Si^+$	92.2	$^{12}C^{16}O^+$
$^{44}Ca^+$	2.0	$^{12}C^{16}O_2^+$
$^{48}Ti^+$	73.8	$^{32}S^{16}O$
$^{51}V^+$	99.7	$^{35}Cl^{16}O^+$
$^{64}Zn^+$	48.6	$^{32}S^{16}O_2^+$, $^{32}S_2^+$
$^{75}As^+$	100.0	$^{40}Ar^{35}Cl^+$

iv. Matrix Induced Polyatomic Ions

Sample constituents may also generate polyatomic ions, usually in combination with oxygen. Oxides of some heavier elements can be formed in the plasma/spectrometer interface. Typically, oxides are formed in relatively low concentrations, with even a worst case being only a few percent of the parent isotope. In sufficient concentrations, however, oxide-forming elements can interfere with determinations of analytes at higher masses.

Argon also can combine with major sample constituents to form argides. For example, common major elements in biological samples include Na, Cl, and S can produce polyatomic species $ArNa^+$, $ArCl^+$ and ArS^+, respectively. Wu *et al* [26] reported the basic spectral interferences from reagent background and biological sample, as listed in following Table 1.4

Table 1.4 Basic spectral interferences in ICP-MS for elements in biological samples (percentage natural relative abundance in parentheses) [26]

Element	Isobaric elemental ions	Isobaric polyatomic ions	
		H_2O, HNO_3	Biological matrix
^{24}Mg (78.99)			$^{12}C_2$ (97.817)
^{25}Mg (10.0)			$^{12}C_2H$ (97.797), $^{12}C^{13}C$ (2.176)
^{26}Mg (11.0)			^{12}CN (99.504), $^{13}C_2$ (0.012)
^{28}Si (92.23)		$^{14}N_2$ (99.202)	^{12}CO (98.702)
^{29}Si (4.67)		$^{14}N_2H$ (99.187)	
^{30}Si (3.10)		^{14}NO (99.401)	
^{35}Cl (75)			^{34}SH (4.199)
^{43}Ca (0.135)			
^{44}Ca (2.086)		$^{14}N_2{}^{16}O$ (99.503)	$^{12}CO_2$ (98.505)
^{45}Sc (100.0)		$^{14}N_2{}^{16}OH$ (99.988)	$^{12}CO_2H$ (98.49), $^{13}CO_2$
^{46}Ti (8.0)	^{46}Ca (0.0004)	$^{14}NO_2$ (99.202)	
^{47}Ti (7.3)			$^{35}Cl^{12}C$ (74.966), $^{31}P^{16}O$ (99.800)
^{48}Ti (73.8)	^{48}Ca (0.187)		$^{32}S^{16}O$ (94.81), $^{31}P^{16}OH$ (99.785)

Table 1.4 Basic spectral interferences in ICP-MS for elements in biological samples (percentage natural relative abundance in parentheses) [26] (continued)

Element	Isobaric elemental ions	Isobaric polyatomic ions	
		H_2O, HNO_3	Biological matrix
^{49}Ti (5.5)			$^{37}Cl^{12}C$ (23.934), $^{35}Cl^{14}N$ (75.497), $^{32}S^{16}OH$ (94.796)
^{50}Ti (5.4)	^{50}V (0.25)		$^{35}Cl^{14}NH$
^{51}V (99.75)			$^{35}Cl^{16}O$ (75.648), $^{37}Cl^{14}N$ (24.103)
^{52}Cr (83.789)			$^{35}Cl^{16}OH$ (75.637), $^{40}Ar^{12}C$ (98.504)
^{53}Cr (9.501)			$^{37}Cl^{16}O$ (24.303)
^{54}Fe (5.80)	^{54}Cr (2.365)	$^{40}Ar^{14}N$ (99.202)	$^{37}Cl^{16}OH$ (24.148)
^{55}Mn (100.0)		$^{40}Ar^{14}NH$ (99.187)	$^{39}K^{16}O$ (93.11)
^{56}Fe (5.80)		$^{40}Ar^{16}O$ (99.401)	$^{40}Ca^{16}O$ (96.776)
^{57}Fe (2.20)		$^{40}Ar^{16}OH$ (99.386)	$^{40}Ca^{16}OH$ (96.762)
^{58}Ni (68.077)	^{58}Fe (0.33)		$^{42}Ca^{16}O$ (0.639)
^{59}Co (100.0)			$^{42}Ca^{16}OH$ (0.639), $^{43}Ca^{16}O$ (0.14)
^{60}Ni (26.233)			$^{44}Ca^{16}O$ (2.096), $^{43}Ca^{16}OH$ (0.14)
^{61}Ni (1.14)			$^{44}Ca^{16}OH$ (0.095)
^{62}Ni (3.63)			$^{46}Ca^{16}O$ (0.003)
^{63}Cu (39.17)			$^{31}P^{16}O_2$ (99.60), $^{35}Cl^{14}N_2$ (75.195)
^{64}Zn (48.6)	^{64}Ni (0.926)		$^{32}S^{16}O_2$ (94.62), $^{31}P^{16}O_2H$, $^{32}S_2$ (90.25), $^{48}Ca^{16}O$ (0.18)
^{65}Cu (30.83)			$^{32}S^{16}O_2H$ (94.606), $^{33}S^{16}O_2$ (0.873), $^{32}S^{33}S$ (0.76), $^{48}Ca^{16}OH$ (0.18)
^{66}Zn (27.9)			$^{34}S^{16}O_2$ (7.986), $^{34}S^{32}S$ (4.565),
^{67}Zn (4.1)			$^{35}Cl^{16}O_2$ (75.497)
^{68}Zn (18.8)		$^{40}Ar^{14}N_2$ (99.805)	$^{35}Cl^{16}O_2H$ (75.486), $^{40}Ar^{12}C^{16}O$ (98.307)
^{75}As (100.0)		$^{36}Ar^{38}ArH$ (0.001)	$^{40}Ar^{35}Cl$ (75.52), $^{37}Cl_2H$ (5.855), $^{40}Ar^{34}SH$ (4.183)
^{77}Se (7.63)		$^{38}Ar_2H$ (0.598)	$^{40}Ar^{37}Cl$ (24.103), $^{40}Ca^{37}Cl$
^{82}Se (8.73)		$^{40}Ar_2H_2$ (99.172)	$^{12}C^{35}Cl_2$ (56.824), $^{34}S^{16}O_3$ (4.175), $^{33}S^{16}O_3H$ (0.795)
^{86}Sr (9.86)			$^{12}C^{37}Cl_2$ (5.792)
^{88}Sr (82.58)			
^{94}Mo (9.3)	^{94}Zr (17.38)		
^{95}Mo (15.9)			
^{96}Mo (16.7)	^{96}Ru (5.52)		
^{97}Mo (9.6)			
^{98}Mo (24.13)	^{98}Ru (1.99)		$^{63}Cu^{35}Cl$ (52.378)

Although theoretically predictable the actual extent of the interference at a given m/z value depends on the design and the operational conditions of the ICP-MS as well as the concentration of the sample matrix constituents [26]. For this reason, an interference investigation over whole mass range is necessary prior to ICP-MS measurement.

1.4.3.2 Methods to Overcome Interferences

Several methods can be used to compensate for matrix induced signal suppression or enhancement. For a well-defined matrix, it is simple to match the composition of the standard solutions with the sample solutions, and thus obtain the analytical accuracy. For a matrix of unknown composition, standard addition method was commonly favored. However, these methods might not be enough for compensating for the sensitivity difference between samples and standards. For elements with interference problem, suitable method to overcome interference is applied. These methods are detailed in the following section.

1.4.3.2.1 Internal Standardization

Internal standards are commonly employed for quantitative ICP-MS measurements in order to compensate for plasma related matrix effects, system drift, difference in sample introduction rates, etc. Ideally, the selected internal standard should be close in both mass and ionization potential to analytes of interest.

1.4.3.2.2 General Methods to Overcome Spectral Interferences

Several methods can be used to overcome the polyatomic interferences. Several publications proposed method for minimize these problem by mathematical correction, mixed gas plasma and hydrocarbon addition, matrix separation, alternative sample introduction or by use of high resolution mass spectrometer. Briefly details of these methods are listed as follow.

- **Mathematical Correction**

Mathematical correction is one of the simplest methods to correct for polyatomic interferences by elemental correction equations or multivariate correction methods. This method requires a thorough knowledge of the interference that is likely to be encountered with particular matrices.

- **Mixed Gas Plasma and Hydrocarbon Addition**

Mixed gas plasma and hydrocarbon addition can be used to reduce the levels of Ar- and Cl-containing polyatomic ions. This is because the added gas competitive formation of nitride or carbides helped to minimize these interfering species. This method may be limited by carbon deposit on the sampler and skimmer cones, thereby clogging the orifices and substantially affecting the sampling process.

❖ Separation Method

The first row transition metals and As suffer many spectral interferences from species containing chlorine, sulfur and sodium atoms. Often a good solution to the problem is to simply separate the analytes from these matrix components. Separation method serves to reduce the concentration of matrix elements in sample to levels that do not interfere with the trace determination of analytes.

❖ Alternative Sample Introduction

Introduction of sample into plasma plays a key role in the production of ions and interfering species. Many techniques are used for introducing gaseous (hydride generation, gas chromatography), liquid (HPLC) and solid (ETV, Laser ablation) samples and transporting analytes to the plasma. These techniques may suffer from limited applicability such as applicability for only one or a few elements.

❖ High Resolution Mass Spectrometer

One of most effective methods to overcome spectral interferences is to use a mass spectrometer with sufficient resolution to resolve between species that have similar m/z. High resolution mass spectrometer have led to a procedure that takes full advantage of the low-level multielement analysis capability of ICP-MS. The various methods used are summarized in table 1.5

Table 1.5 Methods for reduction of mass spectral interferences

Method	Sample	Analyte	Reference
Mathematical correction	Urine	Ni	31
	Wine	Pb, Cd, Cr, V, Bi, Li, Ba, Rb, Mn, Fe, Cu, Ni, Sr, B, Cs, As and Se	32
	Soil, sediment and coal fly ash	Al, Ag, As, Ba, Bi, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Sn, Sr, Th, Ti, Tl, U, V and Zn	33
	Plant and grain	Al, As, B, Ba, Ca, Cd, Co, Cr, Cu, Fe, Li, Mg, Mn, Mo, Ni, Pb, Sb, Se, Sn, Sr, Th, Ti, Tl, U, V and Zn	26
Mixed gas plasma and hydrocarbon addition	Standard	As and V	28
	Human serum and urine	As and Se	29
	Shrimp	As and Se	30
Separation method	Rice flour	Cr, Fe, Cd and Pb	18
	Water, urine, bovine muscle and bovine liver	Cu, As, Se, Cd, In, Hg, Tl and Bi	27
	Plants	B, Al, Cu, Zn, Mn, Fe, Cr, Cd, Pb and S	23

Table 1.5 Methods for reduction of mass spectral interferences (continued)

Method	Sample	Analyte	Reference
Alternative sample introduction system	Shrimp (HPLC-ICP-MS)	As and Se	30
	Liver (HG-ICP-MS)	Se	34
	Water and urine (HG-ICP-MS	As	21
	Sea water (ETV-ICP-MS)	As and Se	35
High resolution mass spectrometer	Estuarine and sea water	Al, Ba, Cd, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb, Sb, U, V and Zn	36

1.5 Sample Preparation and Dissolution

1.5.1 Preparation of Samples

Sample preparation serves to bring the elements of interest into a chemical and physical forms suitable for the method of determination; eliminate interfering substances; and in some cases enrich the elements of interest. Sample preparation may involve physical operations such as drying and grinding and /or chemical operations such as digestions. These procedures are frequently potential sources of contamination and losses [37]

1.5.2 Dissolution of Samples

In ICP-MS and FAAS system, an aqueous nebulization sample introduction system is equipped. The sample in solution form is needed. Therefore, all samples were usually acid digested prior to measurement. Sample digestion is an important step in elemental analysis. It can take a long preparation time and cause systematic error to the analysis [38]. General methods of sample digestion widely used for subsequent elemental determination are dry ashing, wet ashing, and wet digestion. These procedures are summarized as follow.

1.5.2.1 Dry Ashing

Dry ashing is simple and inexpensive. Large number of samples can be treated at the same time and the quantity of the sample is not limited. Usually the sample is heated in a crucible of platinum, silica or pyrex to a relatively high temperature, between 400°C and 700°C, in a muffle furnace. Dry ashing is not recommended for samples with trace metal contents unless special furnace, and very accurate temperature control methods are employed. The more volatile metals such as lead, mercury, and cadmium may be partially lost at even low temperature.

1.5.2.2 Wet Ashing

Wet digestions can be carried out in open vessels or in close systems. In open system, increased consumption of reagents requires the use of especially pure reagents. Losses of volatile elements during wet digestion can be minimized by reflux systems or by open system coupled with volatile element trapping. Closed systems minimized consumption of reagent and avoid losses of volatile elements.

1.5.2.3 Microwave Digestion

To replace wet ashing and dry ashing with a faster, cleaner, and more reproducible technique that is free of external contamination, alternative has recently focused on the microwave oven as a means of sample dissolution [39].

The wet digestion in a closed system employing microwave energy has many advantages such as high percent recoveries, reduced amounts of acid, minimal preparation time, and low potential for contamination. Therefore, it is highly recommended for the digestion of biological samples.

1.5.3 Type of Acid Use

Inorganic acid most commonly employed for oxidation of organic matter, singly or combination, are HCl, HNO_3, H_2SO_4, and $HClO_4$. The major organic constituents of matrix have been destroyed resulting solution consisting of the entire spectrum of chemical elements in various forms. In addition to matrices effect, the number of spectral overlaps is dramatically increased by acid use for the digestion propose. Among these acids, HNO_3 produces the least background spectral interference in the ICP-MS analysis [40]. Other acids such as H_2SO_4, and $HClO_4$ generally cause more spectral interferences than HNO_3. Many studies have used a mixture of HNO_3 and H_2O_2 as digestion reagent and reported good recoveries for certain elements in various biological samples analyzed by ICP-MS. This is because H_2O_2 can oxidize organic matrices and reduce total dissolve solid content in the solution with remaining products of only H and O atoms. Therefore, H_2O_2 should be used together with HNO_3 for sample digestion with subsequent ICP-MS measurement.

1.6 Aims of this Work

The aim of this work is to develop an analytical method for investigation of mineral in Thai herbs using inductively coupled plasma mass spectrometer and flame atomic absorption spectrometer. Details of the study include:

1. Optimization of ICP-MS conditions.
2. Development of sample digestion procedure using a microwave digestion system.
3. Validation of the method using a certified reference material.
4. Study and removal of interfernces in ICP-MS measurement.
5. Analysis of 13 common Thai herbs collected from different sites and five medicinal plants and their medicinal remedies in capsules.

Information on elemental composition for plant samples was expected to be useful for nutritional and/or toxicity evaluation. For medicinal plant, knowledge of mineral contents can provide the information for mineral supplementation effect on disease therapy.

CHAPTER II

EXPERIMENTAL

In this chapter, instrumentation, chemical, general procedure of preparation of solutions and sample pretreatment are presented. Optimization of ICP-MS conditions for simultaneous multielemental detection, evaluation of analytical method and methods to overcome interference problems in ICP-MS determination also presented.

2.1 Instrumentation

2.1.1 Inductively Coupled Plasma Mass Spectrometer (ICP-MS)

All ICP-MS measurements were performed using a Perkin Elmer SCIEX model ELAN 6000. The system uses inductively coupled plasma as an ion source for a quardrupole mass spectrometer with the interface system to link them together. All sample solutions are pumped up through the Gilson peristaltic pump and fed into the nebulizer to convert the liquid into fine aerosols. The vacuum system for mass spectrometer operation consists of an interface roughing pump, (Model 32513) and a turbo backing pump (mode DIOE). For cooling purpose; the CFT-75 Neslab was the recommended chiller for the ELAN 6000. The instrument control and data handling were done by a Dell Computer with a Hewlett Packard model Desk Jet 870

Cxi to print out all the analytical data. The ICP-MS operating parameters for all elements are shown in Table 2.1

Table 2.1 ICP-MS operating parameters

Plasma conditions	
*RF power	1300 watts
*Nebulizer flow rate	0.95 L/min
Auxiliary gas flow rate	0.9 L/min
Plasma gas flow rate	15 L/min
Mass spectrometer setting	
Analog stage voltage	-2550 volts
Pulse stage voltage	1400 volts
AC rod offset	-5 volts
Sampler cone	Nickel
Skimmer cone	Nickel
Measurement parameter	
Mode	Peak hopping
Resolution	Normal
Dwell time/measurement/isotope	20 ms
Number of sweeps per reading	65
Number of replicate	3
Sample introduction	
*Sample uptake rate	1.1 mL/min

*Values selected from the modified optimization.

2.1.2 Flame Atomic Absorption Spectrometer (FAAS)

The FAAS for Ca and K measurements were obtained using a Perkin Elmer atomic absorption spectrometer Model 3100 with Deuterium background correction system. The operating parameters are given in Table 2.2

Table 2.2 FAAS operating parameters for Ca and K measurement.

Element	Wavelength (nm)	Slit width (nm)	Flame type
Ca	422.7	0.7	Air-acetylene
K	766.5	0.7	Air-acetylene

2.1.3 Microwave Digestion System

All sample digestion were performed using a Milestone, microwave digestion system model MLS-1200 Mega. This system was equipped with MLS-1200 MEGA plus EM-45 Exhaust Module for operations with MDR Microwave Digestion Rotors, the MD Rotors consisting of 6 vessels. The operating parameters for microwave digestion are given in Table 2.3

Table 2.3 Operating parameters of microwave digestion system

Step	Power (watts)	Time (min)	Pressure (psi)
1	200	5	NA
2	250	5	NA
3	600	5	NA
4	0	5	NA

NA: Pressure during the digestion was not indicated

2.1.4 Other Equipment and Apparatus

2.1.4.1 Analytical Balance

A Precisa 40SM-200A (Zurich, Switzerland) was used to weigh samples and chemicals in the preparation of sample and standard solution. Analytical balances were calibrated before use.

2.1.4.2 Sub-boiling Distillations System

High purity nitric acid used in this work was obtained by purification of an AR grade nitric acid using a sub-boiling distillation. The sub-boiling distillation system was a Seaster Chemicals (Seattle, WA, USA) which was installed and operated in lamina flow hood.

2.1.4.3 Vacuum Manifold System

Vacuum manifold system (model DOA-4104-BN, GAST, Benton Harbor, USA) was used for the removal of anions by ion exchange micro column.

2.2 Chemical and Reagents

Chemicals and reagents used in this work were purchased from various sources, as shown in Table2.4

Table 2.4 List of chemicals and reagents

Chemicals	Companies
Nitric acid, HNO_3	J. T. Baker, USA
Hydrogen peroxide, H_2O_2	BDH, Pool, England
Mixed standard solution 100 mg/L	AccuTrace, AccuStandard, USA
Indium nitrate, $In(NO_3)_3$	Fluka, Switzerland
Sodium hydroxide, NaOH	Merk, Germany
Anion exchange resin, Amberlite	Fluka, Switzerland
Anion exchange resin, A-641	J. T. Baker, USA
Certified Reference Material, SRM 1515 (Apple leaves)	National Institute of Standards and Technology: NIST, USA

2.3 Preparation of Reagents and Standard Solution

2.3.1 Preparation of Standard Solution

All standard solutions were prepared in 2% high purity nitric acid using high purity deionized water. The high purity deionized water was used directly from a Milli-Qplus water purification (ion exchange) system fed with distilled water. All standard and chemical preparations were carried out in the cleanest environment available in order to minimize contamination.

2.3.2 Labware Cleaning

All calibrated flasks, pipette tips, transfer and graduated pipette, glasswares, and containers (Low density polyethylene (LDPE)) were pre-cleaned, soaked with 10% nitric acid solution, for a minimum of 24 hours, and then rinsed with deionized water. Microwave digestion vessels were detergent-washed and soaked in 10% high purity nitric acid solution. Prior to digestion, the vessels were cleaned by filling with 5 ml of concentrated nitric acid, microwave heated at conditions given in Table 2.3 and then rinsed thoroughly with deionized water.

2.4 Treatment of Samples

The plant samples, common Thai herbs, medicinal plants and herb capsules, were obtained from difference sources. The samples investigated are listed in Table 2.5

Table 2.5 Thai name and scientific name of plant samples

Sample symbol	Thai name	Scientific name	Part analysed
A	ใบมะกรูด	*Citrus hystrix DC.*	Leaves
B	ใบกะเพรา	*Ocimum sanctum L.*	Leaves
C	ใบแมงลัก	*Ocimum basilicum forma citratum Back*	Leaves
D	ใบตำลึง	*Coccinia grandis Voigt*	Leaves
E	ใบโหระพา	*Ocimum basilicum L.*	Leaves
F	ใบชะอม	*Acacia insuavis Lace*	Leaves
G	ใบสะระแหน่	*Mentha arvensis L.*	Leaves
H	กระชาย	*Gastrochilus panduratus (Ridl.) Schltr*	Roots
I	ขิง	*Zingiber officinale Roscoe*	Stems
J	ข่า	*Alpinia galanga (L.) Swilld.*	Stems
K	ตะไคร้	*Cymbopogon citratus (DC.)*	Leaves
L	หอมแดง	*Allium ascalonicum L.*	Leaves
M	กระเทียม	*Allium sativum L.*	Leaves
N	ใบสะเดา	*Azadirachta indica (A.) Juss Var siamensis Valeton*	Leaves
O	ใบขี้เหล็ก	*Cassia siamea Lam.*	Leaves
P	บอระเพ็ด	*Tinospora crispa Miers ex Hook. F. & Thoms*	Stems
Q	เหงือกปลาหมอ	*Acanthus ebracteatus Vahl*	Leaves

2.4.1 Preparation of Samples

All plant samples were carefully cleaned by washing with tap water to remove soil and dust, then washing with deionized water and soaking in 1 % nitric acid solution for 15 minute. The samples were then rinsed with deionized water twice. All cleaned plant samples were dried in clean oven at 65±5 °C for three days. The dry samples were grind in agate mortar, and then dried again in the oven at 65°C for 24 hours.

2.4.2 Dissolution of Samples

Microwave Digestion

Approximately 0.25xx g of samples were weighed accurately into PTFE vessels. 3 mL of concentrated nitric acid and 2 mL of 30% H_2O_2 were added to each vessel. The sample vessels together with reagent blank vessels were sealed and digested using the following heating program (see Table 2.3). After the vessels cooled down, all digestates were transferred into 50.0 mL calibrated flasks and 1 mL of 1000 µg/L In solution were added then diluted to 50.0 mL with deionized water.

2.5 Optimization of ICP-MS Conditions

The ICP-MS was daily optimized according to the manufacturer's recommendation. The modified optimization was performed by the use of mixed standard solution containing 10 ng/mL each of Ba, Ce, Mg, Pb and Rh. The three-optimization

parameters, including the effect of sample uptake rate on the signal of analytes (Mg, Rh and Pb), effect of RF power and nebulizer gas flow rate on oxide formation and sensitivity of the analyte. The optimization procedure was performed follow the sequence below;

1.) The Sample Uptake Rate

Sample uptake rate was adjusted by increasing speed of peristaltic pump, which pump the mixed standard solution into plasma. The signal of Mg, Rh and Pb were observed. The greatest sensitivity of Mg, Rh and Pb was selected.

2.) Nebulizer Gas Flow Rate and RF power

The effect of nebulizer gas flow rate and RF power on oxide formation (a ratio of CeO^+ to Ce^+, often as a percentage (%CeO)) and signal of Mg, Rh and Pb were studied by feed the mixed standard solution into plasma at the uptake rate obtained from step 1.). By varying nebulizer gas flow rate from 0.80 to 0.95 mL/min at the each value of RF power are 1000, 1100, 1200, 1300 and 1400 watts. The pair of nebulizer gas flow rate and RF power, which give a lowest %CeO was selected.

Also, the best sensitivity of Mg, Rh and Pb were observed simultaneously. The RF power that provides the greatest sensitivity of Mg, Rh and Pb was selected.

The optimum operating conditions obtained were used for investigation in this work. All operating parameters including sample uptake rate, RF power and nebulizer gas flow rate are shown in Table 2.1

2.6 Evaluation of Analytical Method

In trace elemental analysis, evaluation of accuracy and precision of an analytical method is usually based on the measurement of certified reference materials. For reliable evaluation the certified reference material used must be similar to that of the sample.

In this work, analytical precision and accuracy was established by triplicate analysis of a certified reference material supplied by National Institute of Standards and Technology (NIST) SRM 1515; Apple leaves. This certified reference material was analyzed, using digestion procedure (see Section 2.4.2) and ICP-MS measurement with optimum conditions obtained (see Section 2.5). Comparison of mean analytical results obtained with certified values were performed in order to evaluation of analytical method.

2.7 Removal of Interferences

The interferences encountered in ICP-MS can be subdivided into two categories, nonspectral interference and spectral interference [41]. The methods to overcome were studied and discussed in section 3.2.2. The method of investigation was described below.

2.7.1 Internal Standardization Procedure

Internal standard is commonly use to overcome nonspectral interference for quantitative ICP-MS analysis. In this work, Indium (In) was used as internal standard. A 1 mL aliquot of In solution, 1000 μg/L, was added to 50 mL of each blank and standards. For reagent blank and sample solution, In was added to them after digestion steps and then was diluted to 50.0 mL with deionized water. In the correction step, all analyte signals were compensated with the signals of In which were simultaneously measured.

To overcome spectral interference, mathematical correction procedure, separation method (anion removal by ion exchange resin) and use of alternative sample preparation method were studied. These procedures were described below.

2.7.2 Mathematical Correction Procedure

A single standard solution containing 100 μg/L of Ca, 100 μg/L of Cl were prepared in 2% HNO_3. This standard solution was used to derive the correction coefficients for Ca and Cl interferences as presented in Table 2.6, as well as to calibrate the system for the determination of Fe, As, Cr, and Se. The corrections were automatically by correction equation obtained which put on the software. Analytical results obtained with and without correction were presented in section 3.2.2.2.

The correction equation for correction of interference of $^{40}Ca^{16}OH$ on ^{57}Fe was determined. The correction factor was obtained by measuring a 100 µg/L solution of Ca and calculating the ratio of the signals at m/z 60 of $^{43}Ca^{16}OH$ and m/z 43 of Ca. The extent of the CaOH interference was measured at m/z 60 and m/z 43 as, opposed to m/z 57 and m/z 40, because of inability to measure the Ca signal at mass 40 due to the very large Ar background. The correction equations were calculated as described below.

$$^{57}\mathbf{Fe} = {}^{57}\mathbf{M} - {}^{40}\mathbf{Ca}^{16}\mathbf{OH}$$

$$= {}^{57}M - \left(\frac{Ab\,{}^{40}Ca^{16}OH}{Ab\,{}^{43}Ca^{16}OH}\right) \times {}^{43}Ca^{16}OH$$

$$= {}^{57}M - \left(\frac{Ab\,{}^{40}Ca}{Ab\,{}^{43}Ca}\right) \times {}^{43}CaOH$$

Because $$\frac{{}^{43}CaOH}{{}^{43}Ca} = \chi \quad \Rightarrow {}^{43}CaOH = \chi^{*}\,{}^{43}Ca$$

Therefore $$^{57}Fe = {}^{57}M - \left(\frac{Ab\,{}^{40}Ca}{Ab\,{}^{43}Ca}\right) \times \chi^{*}\,{}^{43}Ca$$

$$^{57}\mathbf{Fe} = {}^{57}\mathbf{M}\text{-}\mathbf{1642.970} * {}^{43}\mathbf{Ca}$$

Ab = Abundance

In the same way, the correction factor was obtained by measuring a 100 µg/L solution of Cl. The correction equation used for Fe, As, Cr and Se determination are summarized in Table 2.6

Table 2.6 Interference correction equations

Element	Correction equation	Interferences
Correction for Ca interferences:		
^{57}Fe	$^{57}Fe = {}^{57}M - \frac{Ab^{40}Ca}{Ab^{43}Ca} \times \frac{^{43}Ca^{16}OH}{^{43}Ca} \times {}^{43}Ca$ $^{57}Fe = {}^{57}M - 718.0815 \times 2.2880 * {}^{43}Ca$ $^{57}Fe = {}^{57}M - 1642.970 * {}^{43}Ca$	$^{40}Ca^{16}OH$
Correction for Cl interferences:		
^{75}As	$^{75}As = {}^{75}M - \frac{Ab^{35}Cl}{Ab^{37}Cl} \times \frac{^{40}Ar^{37}Cl}{^{37}Cl} \times {}^{37}Cl$ $^{75}As = {}^{75}M - 3.1271 \times 1.5499 \times 10^{-4} * {}^{37}Cl$ $^{75}As = {}^{75}M - 0.0004847 * {}^{37}Cl$	$^{40}Ar^{35}Cl$
^{53}Cr	$^{53}Cr = {}^{53}M - \frac{Ab^{37}Cl}{Ab^{35}Cl} \times \frac{^{16}O^{35}Cl}{^{35}Cl} \times {}^{35}Cl$ $^{53}Cr = {}^{53}M - 0.03198 \times 2.6673 \times 10^{-3} * {}^{35}Cl$ $^{53}Cr = {}^{53}M - 0.000853 * {}^{35}Cl$	$^{16}O^{37}Cl$
^{77}Se	$^{77}Se = {}^{77}M - \frac{Ab^{37}Cl}{Ab^{35}Cl} \times \frac{^{40}Ar^{35}Cl}{^{35}Cl} \times {}^{35}Cl$ $^{77}Se = {}^{77}M - 0.3198 \times 1.299 \times 10^{-4} * {}^{35}Cl$ $^{77}Se = {}^{77}M - 0.00036135 * {}^{35}Cl$	$^{40}Ar^{37}Cl$

2.7.3 Anion Removal by Ion Exchange Resin

A column with anion exchange resin was used to eliminate existing anions. Conditioning of anion exchange resin and preparation of the resin columns are described below.

2.7.3.1 Preparation of Anion Exchange Resin in OH^- Form

A 150 g of anion exchange resin was stirred with 500 mL 2 M NaOH in a covered of polyethylene beaker overnight. The solution was replaced with fresh 2 M NaOH and then back to stirring for 2 hours. Finally, the resin was washed with deionized water until neutral and then placed in a glass funnel connected to a vacuum pump. The resin was dried by drawing air through the funnel.

2.7.3.2 Preparation of Anion Exchange Resin in NO_3^- Form

A 150 g of anion exchange resin was washed with 500 mL 1 M HNO_3 in a covered polyethylene beaker overnight. Fresh solution of 1 M HNO_3 500 mL were added to the resin and then stirred for two hours. The solution was replaced with 500 mL of 1 M HNO_3. This resin slurry was packed in a 25 mL syringe.

2.7.3.3 Preparation of Columns

The resin slurry as prepared in 2.7.3.2 (approximately 2, 4, 6, 8, 10, 12, 14, and 20 g) were introduced into 25 mL syringe bottom-lined with glass filter frit. The columns with resin were placed on vacuum manifold connected to a vacuum pump. All columns were then washed with 1 M HNO_3 in order to remove residual chloride and dry by drawing air through the column.

2.7.3.4 Procedure for Removal of Matrix Anions

The column as prepared in 2.7.3.3 was placed on vacuum manifold connected to a vacuum pump. 10 mL of sample solution was loaded manually using a syringe. Vacuum pump was turned on and adjusted such that, the sample solution emerging from the end of the column was collected (in a 30 mL polyethylene container) with a flow rate of 1.5 mL/min. Thereafter, the columns were washed by passing 30 mL of 1 M HNO_3 in order to remove retained matrices and dried.

2.7.4 Alternative Sample Preparation

To reduce the interference from the acids and reagents used in acid digestion, dry ashing was tried. An approximately 0.25 g sample was accurately weighed in a platinum crucible. 3 mL of HNO_3 and 1 mL of 1000 μg/L of In were added in each crucible. All crucible together with reagent blank were placed in a

muffle furnace and heated for 16 hours at 500°C. After the crucible cooled down, 1 mL of HNO_3 were added and subsequently heated on hot plate. Finally, all digestates were transferred into 50 mL volumetric flask and diluted to 50 mL with deionized water.

CHAPTER III

RESULTS AND DISCUSSION

The chemical study of medicinal plants has been mainly concentrated on their active organic compounds. The investigation of mineral contents in food plants is useful for nutritional or toxicity evaluation. This work presents the investigation into the inorganic mineral composition of some common Thai herbs and medicinal plants. Details of study include, digestion method of plant sample in section 3.1 and analytical method development including optimization of ICP-MS conditions, method validation, study and removal of interferences in section 3.2. Finally, evaluation of analytical result of mineral contents in plant samples from difference sources will be discussed in section 3.3.

3.1 Dissolution of Sample

The use of nitric acid for the digestion of biological and food samples has been widely favored for ICP-MS analysis [40]. This is because nitric acid contains only the H, O and N atoms, which produce the least background spectrum. Other acids such as HCl or H_2SO_4 generally cause more spectral interferences than HNO_3 as reported by Tan and Horlick [40]. With H_2O and reactive oxygen being the composition products, H_2O_2 has become the preferred additional oxidizing reagent used in digestion for subsequent

ICP-MS analysis, in order to reduce carbon contents in the digestate as reported by Krushevska [20].

The minimum amount of concentrated HNO_3 required for digestion was studied, in order to use a small dilution factor and to have a sufficiently low concentration of HNO_3 (1-2 %) in final solutions for ICP-MS analysis. It was found that digestion was incomplete when 1 and 2 ml of HNO_3 was used for a 0.25xx g samples while 3 ml of HNO_3 was sufficient for complete digestion.

The major concern in the use of HNO_3 and H_2O_2 for digestion is the contamination from their impurities. Although high purity HNO_3 and H_2O_2 were used, the study on contribution of reagent blank is important, in order to evaluate the result of trace levels. The comparison of concentration of metal in reagent blank and digested solution of SRM 1515 (overall dilution factor of 200), are shown in Table3.1

From Table 3.1, the results of As, Cd, Co, Cr, Ni, Pb, Se, V and Zn for reagent blank analysis showed higher value than that of solution of SRM 1515 (shaded figures in Table 3.1). Accurate measurement of these elements will be limited by high contribution of reagent blank. To reduce the reagent blank levels, all sample preparation was carried out under the cleanest environment available and all labware were carefully cleaned before use. In addition, the reagent blank was daily prepared together with sample. By so doing, reagent blanks can be reduced as shown in the right column of Table 3.1.

Table 3.1 Results of reagent blank, 3 ml HNO_3 + 2 ml H_2O_2 digested and diluted in the same manner as SRM sample.

Element	Concentration of digested solution of SRM 1515 * (μg/L)	Reagent blank solution**(μg/L)	New reagent blank solution***(μg/L)
Al	1430	2.29±0.95	2.35±0.89
As	0.190	0.36±0.024	0.045±0.001
B	135	30.97±4.37	28.5±4.2
Ba	245	1.19±0.075	1.10±0.060
Be	Not certified	0.041±0.0078	0.035±0.002
Cd	0.0650	0.093±0.031	0.055±0.014
Co	0.065	0.11±0.067	0.033±0.005
Cr	1.5	2.19±0.35	0.990±0.025
Cu	28.2	3.13±0.67	2.51±0.34
Fe	415	29±2.8	22±1.60
Li	Not certified	0.096±0.008	0.088±0.007
Mg	13550	7.1±2.4	2.60±0.234
Mn	270	0.59±0.20	0.253±0.015
Mo	0.470	0.042±0.0097	0.0292±0.002
Na	120	37±3.2	38±2.5
Ni	4.55	3.0±1.9	1.41±0.03
P	7950	16±2.2	13±1.5
Pb	2.35	2.3±1.1	2.05±0.38
Sb	0.0650	0.023±0.0037	0.0144±0.001
Se	0.250	0.43±0.10	0.099±0.005
Sr	125	1.15±0.19	0.788±0.079
V	1.30	1.60±0.15	1.01±0.011
Zn	62.5	198±42	31.3±1.5

*Calculated from certified values of SRM with the overall dilution factor of 200 (v/m).
**Mean±Standard deviation, (n=6), derive from the between run of reagent blank solutions.
***Mean±Standard deviation, (n=6), derive from the within run of reagent blank solutions.

3.2 Analytical Method Development

Samples of plants contain many minerals in varying amounts, from ng/g to mg/g levels. ICP-MS provides simultaneous multielement capability with wide liner dynamic ranges and low detection limits. Therefore, it is considered for the investigation in this work. For elements with high concentration, FAAS was used.

3.2.1 ICP-MS Determinations

In order to achieve accurate ICP-MS determination of elemental concentration in complex biological samples, optimization and method validation were performed. Analytical performances of each element including calibration and detection limit study will also be presented in this section.

3.2.1.1 Optimization of ICP-MS Conditions

The sensitivity achievable by ICP-MS for any element depends upon its isotope abundance, its degree of ionization in the ICP, the ion lens voltages, and parameters associated with sample introduction and plasma condition [42]. The effect of plasma operating parameters on analyte signals has been reported by Horlick *et al.* [43], using the SCIEX ICP-MS Elan Model, and Long and Brown [44], using the PlasmaQuad Model. These parameters included nebulizer gas flow rate, auxiliary flow rate, RF power and distance of the orifice from the top of load coil. Although ion signals are dependent on all these parameters, the two most critical are the nebulizer

gas flow rate and plasma power. In this work, three parameters have been investigated. The optimizations were performed under the procedure as described in the section 2.5. The signal of Mg, Rh and Pb (representing low, medium and high masses respectively) were observed to obtain the best sensitivity. The %CeO (representing oxide formation) as a function of nebulizer gas flow rate and RF power, was observed to obtain as low %CeO as possible.

Relationship of sample uptake rate and analyte signals were plot in Figure 3.1

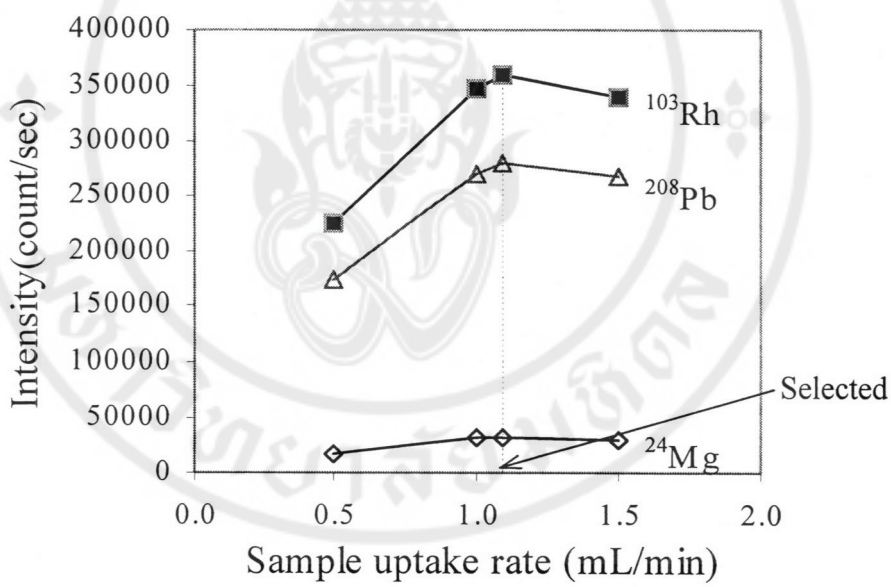


Figure 3.1 Optimization of ICP-MS instrumental parameters, showing the effect of sample uptake rate on Mg, Rh and Pb signals. (RF power = 1050 watts, nebulizer gas flow rate = 0.85 L/min)

According to Figure 3.1, as the sample uptake rate is 1.1 mL/min, (RF power = 1050 watts, nebulizer gas flow rate = 0.85 L/min), the signal of analyte for all three different mass number showed highest values. Thus, 1.1 mL/min sample uptake rate was selected.

The relationship of RF power, %CeO and analyte signal (Mg, Rh and Pb) were presented as a family of plots of response versus nebulizer gas flow rate at difference power setting (1000-1400 watts) as shown in Figure 3.2

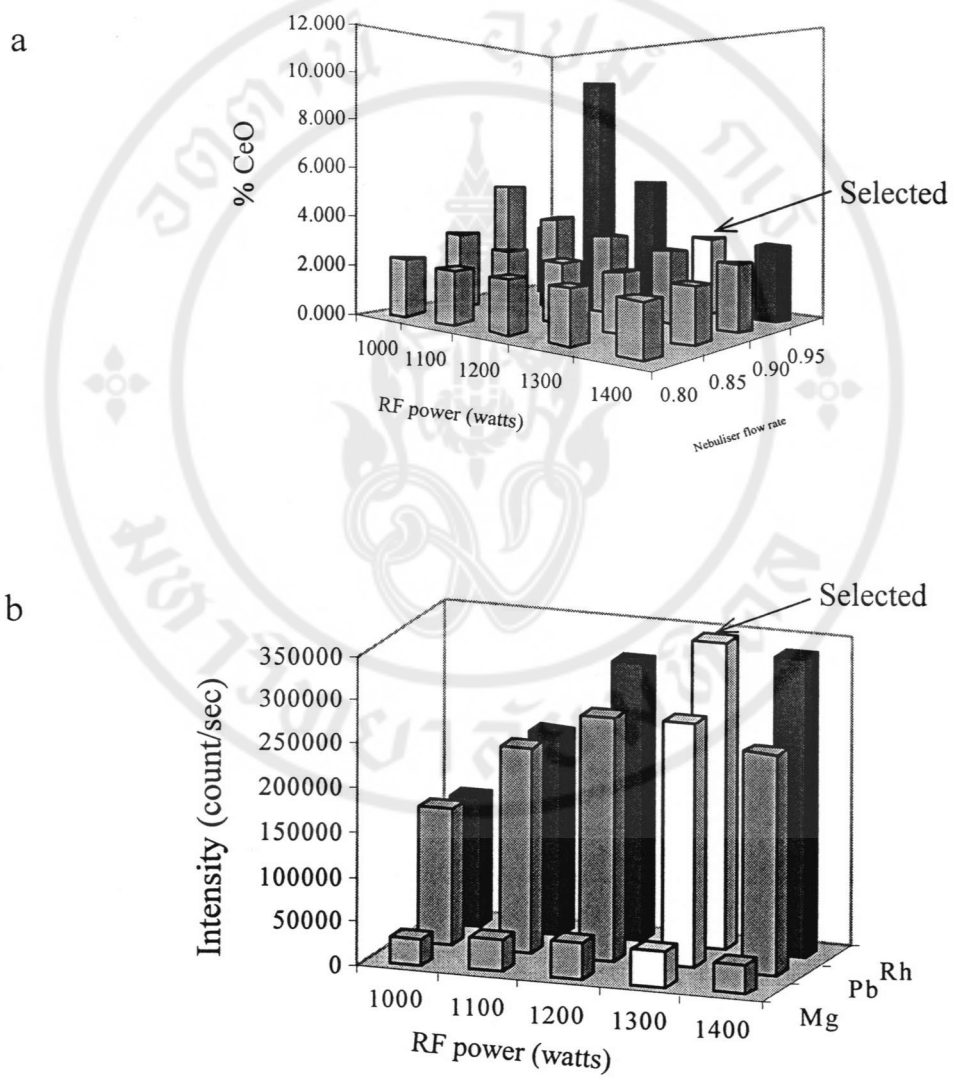


Figure 3.2 The effect of RF power and nebulizer gas flow rate on %CeO and signal of Mg, Rh and Pb:

a.)Percentage of CeO at varying RF power and nebulizer gas flow rate, at sample uptake rate1.1 mL/min.

b.)Mg, Rh and Pb signal as a function of varying RF power, at sample uptake rate of 1.1 mL/min. and nebulizer gas flow rate of 0.95 L/min.

The highest nebulizer gas flow rate was required in order to achieve the best sensitivity of analytes. When the nebulizer gas flow rate increased, unwanted oxide (CeO) is oxide formation (as seen in %CeO) increased accordingly (Figure 3.2 a.). However, %CeO decreased when RF power increased as seen on Figure 3.2 a. It can be concluded that the percentage of CeO falls as the power increases and the nebulizer gas flow rate decreases. Thus 1300 watts of RF power and 0.95 L/min for nebulizer gas flow rate, which produces the lowest CeO, were selected. The effect of RF power on sensitivity of analytes is shown in Figure 3.2 b., when the RF power increases the signals of Mg, Rh and Pb increase until the RF power of 1300 watts. The greatest sensitivity was achieved by the plasma power is 1300 watts, at the nebulizer gas flow rate, 0.95 L/min.

In conclusion, the best sensitivity for analytes was obtained when the sample uptake rate and nebulizer gas flow rate were1.1 mL/min and 0.95 L/min respectively and when the RF power was 1300 watts. In addition, these conditions produce low oxide formation. Therefore, they were used for investigation through out this work.

3.2.1.2 Selection of Analytical Mass

As already mentioned (in Section 1.4.2.1), the interferences from polyatomic ion species containing C, Cl and Ca are severe in ICP-MS analysis for biological samples. According to the publication of Wu *et al* [26]. Interferences

from carbon include the isotopes of ^{52}Cr ($^{40}Ar^{12}C$), ^{82}Se ($^{12}C^{35}Cl_2$) and for ^{11}B (tail interference by the strong ^{12}C signal). Other major isotopes suffering interference from carbon species include ^{44}Ca ($^{12}C^{16}O_2$), ^{53}Cr ($^{40}Ar^{13}C$), and ^{60}Ni ($^{12}C^{16}O_3$). Thus, selecting ^{10}B, ^{53}Cr and ^{77}Se as the measured isotope should reduce the carbon interference.

Plant samples contain high levels of Ca and Cl. The interference from CaO and CaOH species were found in the determination of ^{57}Fe ($^{40}Ca^{16}OH$), ^{59}Co ($^{43}Ca^{16}O$) and ^{60}Ni ($^{44}Ca^{16}O$) as reported by Wu *et al* [26]. Major interferences from Cl in ICP-MS analysis include ^{51}V ($^{35}Cl^{16}O$), ^{53}Cr ($^{37}Cl^{16}O$), ^{75}As ($^{40}Ar^{35}Cl$) and ^{77}Se ($^{40}Ar^{37}Cl$). In this work, in order to achieve accurate ICP-MS element concentration, separation method was applied to remove matrix interference for the determination of ^{75}As, ^{53}Cr, ^{57}Fe, and ^{77}Se (see Section 3.2.2.2.2).

The plant samples contain relatively high amount of Mg. Thus, ^{25}Mg (an isotope with low relative abundance of 10.00%) was selected. For the Cu determination, ^{65}Cu was selected to avoid $^{23}Na^{40}Ar$ interference on ^{63}Cu. For Zn determination, ^{66}Zn was selected because the interferences at m/z 66 are much less severe than those at m/z 64, 67 and 68 (see Table 1.4). For the isotopes at higher mass, the isotope with higher abundance was selected because spectral interference is generally less.

3.2.1.3 Validation of Method Using Certified Reference Material

The accuracy of plants analyzes by ICP-MS technique was evaluated by analyzing a certified reference material. The National Institute of Standard and Technology (NIST) certified reference material SRM 1515, which is apple leave sample certified for trace metals was used.

The certified reference material were acid digested using microwave system following by ICP-MS determination. Results were shown in Table 3.2 and the correlation plot in Figure 3.3

Table 3.2 Results for the analysis of certified reference material, SRM 1515 (Apple leaves)

Element	Mass	Certified (mg/Kg)	Found (mg/Kg)[@]	%Difference
Al	27	286 ± 9	263 ± 39	-8
As	75	0.038± 0.007	0.095± 0.007	150
B	10	27 ± 2	28 ± 2	4
Ba	138	49 ± 2	44 ± 0.4	-10
Be	9	Not certified	0.017± 0.002	-
Ca[&]	-	15260 ± 150	16300 ± 760	7
Cd	114	0.013± 0.002	< 0.000093	-
Co	59	0.09*	0.098± 0.006	9
Cr	53	0.3*	1.55 ± 0.08	417
Cu	65	5.64 ± 0.24	5.53 ± 0.23	-2
Fe	57	83 ± 5	133 ± 2	60
K[&]	-	16100 ± 200	14968 ± 824	-7
Mg	25	2710 ± 80	2877 ± 52	6
Mn	55	54 ± 3	54.5 ± 1	1
Mo	98	0.094 ± 0.13	0.07± 0.002	-25
Na	23	24.4 ± 1.2	25.4 ± 2.5	4
Ni	62	0.91 ± 0.12	0.61 ± 0.04	-33
P	31	1590 ± 110	1460 ± 94	-8
Pb	208+207+206	0.470± 0.024	0.481± 0.07	2
Sb	121	0.013*	0.008±0.002	-28
Se	77	0.050± 0.009	0.197±0.012	294
Sr	88	25 ± 2	23.7 ± 0.17	-5
V	51	0.26 ± 0.03	0.263±0.019	1
Zn	66	12.5 ± 0.3	9.67 ± 0.2	-23

[@]Mean ± standard deviation (n=3)
[&]Values obtained using FAAS (n=6)

*Reference values

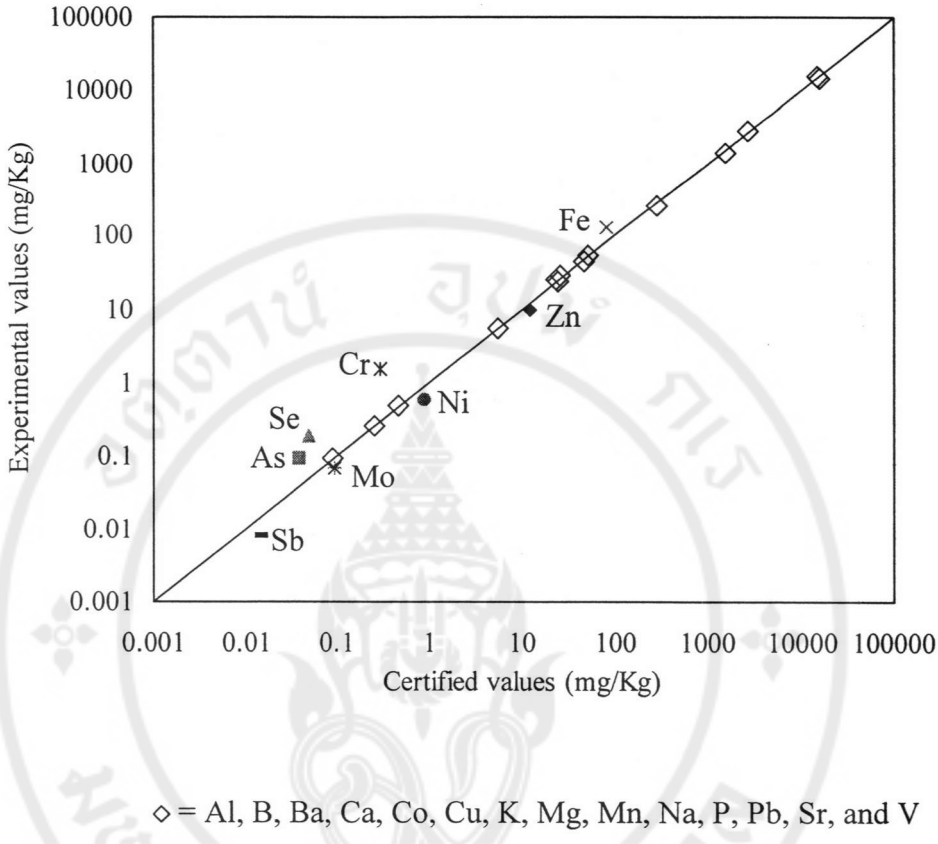


Figure 3.3 Analytical results obtained for SRM 1515 plotted against the certified values.

Figure 3.3 shows a correlation plot of concentrations measured and the certified values for several selected elements in the previously mentioned reference materials, using the calibration curve quantitation technique. As demonstrated by this plot, fourteen elements (Al, B, Ba, Ca, Co, Cu, K, Mg, Mn, Na, P, Pb, Sr, and V) show good agreement with the certified values. Four element, Mo, Ni, Sb and Zn gave lower amounts than certified values probably according to incomplete digestion, whereas As, Cr, Fe and Se gave higher values possibly owing to positive polyatomic interferences. The elements showing excessively higher value than certified values were

identified as having interference problem. Correction of polyatomic interference for As, Cr, Fe and Se was performed and presented in section 3.2.2.2.

3.2.1.4 Calibration Curve

Quantification of mineral in plant samples was carried out using the internal standard method to correct for small variations between each run of sample nebulization plasma and detection conditions. In this work, indium (^{115}In) solution was used as the internal standard because it has no polyatomic interference problem at its mass number. It is also not present in normal plant and reagents used.

Measurement of calibration standard mixture of 23 metals, 27 isotope, under the condition as described in Table 2.1, gave linear response as shown in Table 3.3. Intensities of elemental signals are plotted against its concentration and correlation coefficients between 0.9985 and 1.0000 were obtained.

Table 3.3 Equation of calibration curve in ICP-MS measurement

Element	Mass	Regression equation	Regression, R^2
Al	27	y=17726x-29169	0.9997
As	75	y=2131.9x-760.42	0.9996
B	10	y=285.96x-79.583	0.9995
Ba	138	y=31547x-55465	0.9996
Be	9	y=928.76x+214.08	0.9999
Cd	114	y=5141.7x+1342.3	0.9999

Table 3.3 Equation of calibration curve in ICP-MS measurement (Continued)

Element	Mass	Regression equation	Regression, R^2
Co	59	y=26755x-25892	0.9996
Cr	52	y=12978x+48388	0.9985
Cr	53	y=1665x+819.47	0.9995
Cu	65	y=7327.7x+245.26	0.9998
Fe	57	y=941.37x-5069	0.9990
Li	7	y=4720.5x+1078.8	0.9998
Mg	25	y=10068x-38235	0.9997
Mn	55	y=34159x-31869	0.9997
Mo	98	y=6038.3x+1628	0.9998
Na	23	y=17662x-334846	1.0000
Ni	58	y=3414.7x-1913.8	0.9999
P	31	y=6025.6x-4449.2	0.9992
Pb	208	y=9850.2x+6396.6	0.9997
Pb	207	y=4078x+2305.4	0.9999
Pb	206	y=5066.8x+2794.2	0.9998
Sb	121	y=5710.4x+983.05	1.0000
Se	77	y=211.48x+1.3174	0.9999
Se	82	y=278.45x-22.91	1.0000
Sr	88	y=26123x+11957	0.9994
V	51	y=15768x+5344.9	0.9997
Zn	64	y=3470.7x-3902	0.9999
Zn	66	y=2092.3x-2218	0.9999

y= signal (count/sec.)

x= concentration (μg/L)

3.2.1.5 Limits of Detection

The detection limit of an analytical method is dependent on the degree of unwanted noises and the sensitivity of detection. The most generally accepted qualitative definition of detection limit is that it is the minimum concentration of analyte that can be detected at a known confidence level. For quantitative definition, detection limit is the lowest quantities of analyte giving an instrument signal significantly different from the blank signal. The limit of detection for the analytes were determined using operating conditions given in Table 2.1. Six replicate measurements of reagent blank were performed. The three times of the standard deviation of the reagent blank signals and the instrument detection limits and method detection limits were derived from the equation below.

$$\textbf{Instrument detection limit (IDL)} = \frac{3 \times SD_{\text{reagent blank}}}{\text{Slope of calibration}}$$

The method detection limit was calculated in the following equation.

Method detection limit (MDL) = *Dilution factor × IDL

$$*\text{Dilution factor} = \frac{\text{Final volume (50mL)}}{\text{Weight of test sample}}$$

Table 3.4 shows the instrument detection limit and method detection limit for the interested metals.

Table 3.4 Instrument detection limits (IDLs) and method detection limits (MDLs)

Element	*IDL (μg/L)	**MDL, Solid (mg/Kg)
Al	0.025	0.005
As	0.015	0.003
B	0.025	0.005
Ba	0.021	0.004
Be	0.042	0.008
Cd	0.061	0.012
Co	0.022	0.0044
Cr	0.070	0.014
Cu	0.149	0.030
Fe	0.521	0.104
Li	0.023	0.0046
Mg	0.108	0.022
Mn	0.016	0.0032
Mo	0.054	0.011
Na	0.225	0.045
Ni	0.289	0.058
P	0.218	0.044
Pb	0.342	0.068
Sb	0.045	0.009
Se	0.25	0.05
Sr	0.08	0.016
V	0.051	0.010
Zn	0.82	0.164

*Derived from three times the standard deviation of reagent blank solutions, (n=6)

**Calculate from the IDL (in solution) in μg/L multiplied by the overall dilution factor of 200 (v/m)

3.2.2 Polyatomic Interferences in ICP-MS Measurements

The spectral interferences in ICP-MS measurement have been investigated thoroughly in several studies [18,21,23,26-36]. Elimination of these spectral interferences is necessary to obtain accurate results. Recognition and reduction of mass spectral interference will be presented in this section. Isotope ratio measurement with mass bias correction is also used to recognize the polyatomic interference problem.

3.2.2.1 Identification of Elements with Possible Interferences by Isotope Ratio Measurements

According to the results of the analysis of the certified reference material, as given in Table 3.2., As, Cr, Fe and Se showed 60-417% higher amounts than certified values. These differences can be considered as owing to polyatomic interferences. An approach to recognize spectral interference problem in ICP-MS is to measure, more than one isotope and the isotopic ratios of the analyte in the sample matrix of interest are calculated to compare with the natural ratio [45]. Discrepancies from natural isotope ratio are usually an indication of spectral interference problem. The isotope ratios of the elements in question (As, Cr, Fe and Se) were calculated as presented below.

In order that isotope ratio measurement can be used to indicate the presence of polyatomic interference, prior corrections for known elemental

isobaric interferences were necessary and were performed automatically by the ELAN software. The results of isotope ratio measurement, after correction of elemental isobaric interference, were corrected for mass bias correction. The correction equations of elemental isobaric interference for interested element are given in Table 3.5

Table 3.5 Correction equations for isobaric elemental interference

Element	Mass	Correction equation
Ba	138	^{138}M- 0.000903 * La 139 – 0.002825 * Ce 140
Ba	136	^{136}M- 0.002147 * Ce 140 – 0.337121*Xe129
Cd	114	^{114}M- 0.026826 * Sn 118
Fe	54	^{54}M- 0.028226 * Cr 52
Mo	98	^{98}M- 0.110588 * Ru 101
Ni	58	^{58}M- 0.003053 * Fe 56
Se	82	^{82}M- 1.013447 * Kr 83
Sn	120	^{120}M- 0.013447 * Te 125
Zn	64	^{64}M- 0.035313 * Ni 60

The results of isotope ratio measurement on unspiked digested SRM 1515 compared with the expected values are shown in Table 3.6. The ratio of lead was not measured because lead having isotope abundance vary in nature, so this method can not be used to indicate spectral interference problem in lead.

Table 3.6 Results of isotope ratio measurement for SRM 1515 solution.

Ratio	Natural ratio	Measured* ratio	%difference from** natural ratio
$^{11}B/^{10}B$	4.0251	4.5775	13.7
$^{138}Ba/^{136}Ba$	9.1291	8.7001	-4.7
$^{111}Cd/^{114}Cd$	0.4455	0.5752	29.1
$^{53}Cr/^{52}Cr$	0.1134	0.0558	-50.8
$^{65}Cu/^{63}Cu$	0.4457	0.5045	13.2
$^{54}Fe/^{57}Fe$	2.6363	0.9548	-63.8
$^{26}Mg/^{24}Mg$	0.1393	0.1755	26.0
$^{25}Mg/^{24}Mg$	0.1266	0.1507	19.0
$^{95}Mo/^{98}Mo$	0.6598	0.6291	-4.7
$^{62}Ni/^{60}Ni$	0.1385	0.0758	-45.3
$^{123}Sb/^{121}Sb$	0.7434	0.7962	7.1
$^{82}Se/^{77}Se$	1.1442	0.4518	-60.5
$^{87}Sr/^{88}Sr$	0.0848	0.0900	6.1
$^{86}Sr/^{88}Sr$	0.1194	0.1266	6.0
$^{66}Zn/^{64}Zn$	0.5741	0.6398	11.4

*Results of SRM 1515 solution measurement

**Calculated from [(Measured ratio / Natural ratio)*100]-100

Both interferences and/or mass bias are contributive in the error of the isotope ratio measurement. To use isotope ratios to indicate interference problem, the effect of mass bias should be corrected as presented in the following section.

❖ Mass Bias Correction

The mass bias, the deviation of the measured isotope ratio from the actual value (natural ratio), occurs because the sensitivity of the instrument varies with mass owing to differences in ion transmission [46]. Using a quadrupole ICP-MS the mass bias can be greatest more than 10% for element with mass number < 10, about 1-5% for mass number in the range of 20-120 and only <1% for heavier element [47].

The mass bias correction is determined by measuring a standard of known isotopic ratio, normally a natural abundance standard. In this work, corrections for mass bias were made with 100 μg/L natural abundance solution of each element of interest. Table 3.7 shows isotope ratio obtained from measurement of 100 μg/L standard solution element of interest with and without correction.

Table 3.7 Isotope ratios and mass bias correction for same elements with interference problem.

Ratio	Natural ratio	Measured ratio		%difference between SRM and natural ratio	%difference** between SRM solution and standard solution
		SRM 1515 solution	Standard* solution		
$^{11}B/^{10}B$	4.0251	4.5775	4.2206	13.7	8.5
$^{138}Ba/^{136}Ba$	9.1291	8.7001	8.9141	-4.7	-2.4
$^{111}Cd/^{114}Cd$	0.4455	0.5752	0.4701	29.1	22.4
$^{53}Cr/^{52}Cr$	0.1134	0.0558	0.1417	-50.8	-60.6
$^{65}Cu/^{63}Cu$	0.4457	0.5045	0.4759	13.2	6.0
$^{54}Fe/^{57}Fe$	2.6363	0.9548	2.6052	-63.8	-63.4
$^{26}Mg/^{24}Mg$	0.1393	0.1755	0.1848	26.0	-5.0
$^{25}Mg/^{24}Mg$	0.1266	0.1507	0.1551	19.0	-2.8
$^{95}Mo/^{98}Mo$	0.6598	0.6291	0.6394	-4.7	-1.6
$^{62}Ni/^{60}Ni$	0.1385	0.0758	0.1587	-45.3	-52.2
$^{123}Sb/^{121}Sb$	0.7434	0.7962	0.7648	7.1	4.1
$^{82}Se/^{77}Se$	1.1442	0.4518	1.2509	-60.5	-63.9
$^{87}Sr/^{88}Sr$	0.0848	0.0900	0.0942	6.1	-4.5
$^{86}Sr/^{88}Sr$	0.1194	0.1266	0.1321	6.0	-4.2
$^{66}Zn/^{64}Zn$	0.5741	0.6398	0.6195	11.4	3.3

*100 μg/L standard solution in 2%HNO_3

**Mass bias correction.; calculated from

[(Measured ratio of SRM 1515 solution/Standard solution)*100]-100

In Table 3.7, it was found that the measured isotope ratios after mass bias correction did not conform to the natural ratios with percent differences of 63.9-29.7% for some elements. These differences indicate spectral interference problem on one isotope or both for each of the isotope pairs of B, Cd, Ni, Cr, Fe and Se.

The ^{11}B / ^{10}B ratio of SRM 1515 solution was higher than the natural ratio, indicating probable positive interference on ^{11}B isotope. The interfering species of ^{11}B is likely to come from the tail of strong ^{12}C signal [26]. Plant sample with wet digestion in closed system was reported to contain high carbon concentration. Use of ^{10}B can avoid interference in B determination.

The ^{111}Cd / ^{114}Cd ratio showing higher values than the natural ratio may have suffered from incorrect measurement. This is because very low concentration of Cd was found in the SRM. The ^{111}Cd / ^{114}Cd ratio of SRM 1515 solution was higher than that of natural ratio, indicating interference on one or both isotope of Cd. $^{95}Mo^{16}O$ can overlap on ^{111}Cd measurement. This problem was found and corrected by Wu *et al* by mathematical correction [33]. The selecting of ^{114}Cd can avoid $^{95}Mo^{16}O$ interference.

$^{44}Ca^{16}O$ may have interfered on ^{60}Ni isotope because the ratio of ^{62}Ni / ^{60}Ni in SRM 1515 solution was found to be lower than the natural ratio. Vaughan *et al.* [31] reported interference on Ni determination for samples with high calcium levels through formation of these Ca polyatomics with spectral overlapping on every isotope of Ni, but the effect on ^{62}Ni is relatively low. Thus, ^{62}Ni isotope was selected.

Overlap of ^{54}Fe, ^{56}Fe, ^{57}Fe and ^{58}Fe can come form $^{40}Ar^{14}N$, $^{40}Ar^{16}O$, $^{40}Ca^{16}OH$ and/or $^{40}Ar^{16}OH$, and ^{58}Ni, respectively. These polyatomic ion species were more severe using normal plasma for Fe determination. The $^{40}Ar^{14}N$ interference on ^{54}Fe originates from HNO_3 and from sample matrix itself. Oxygen from atmosphere, water and HNO_3 are contributing to polyatomic species $^{40}Ar^{16}O$ which overlap the ^{56}Fe isotope. ^{58}Ni is major elemental interference on ^{58}Fe, with 68.077 % abundance whereas ^{58}Fe occurs in nature with 0.33 % abundance. Therefore, ^{58}Fe was not a good choice for measurement. $^{40}Ar^{16}OH$ is an interfering species on ^{57}Fe but it can be corrected by subtracting the reagent blank signal. Ca contents in the sample can cause formation of the $^{40}Ca^{16}OH$ species, which can also interfere on ^{57}Fe. The ^{54}Fe / ^{57}Fe ratio of SRM 1515 solution showed much discrepancy with the natural ratio, this is because of the spectral overlapping on every isotope of Fe.

The relatively high contributions of C and Cl in the digestates are also contributing polyatomic species on Cr measurement. For isotope ratio measurement of ^{53}Cr / ^{52}Cr in SRM solution, it showed much lowers value than natural ratio. These indicating interference on one or both isotope, ^{53}Cr and ^{52}Cr. $^{40}Ar^{12}C$ and $^{35}Cl^{16}OH$ are two possible interfering species on ^{52}Cr and $^{37}Cl^{16}O$ can interfere ^{53}Cr isotope.

Selenium occurs in nature with six isotopes, ^{74}Se (0.96), ^{76}Se (9.12), ^{77}Se (7.5), ^{78}Se (23.61), ^{80}Se (49.96), and ^{82}Se (8.84). Four isotopes of Se are interfered by argon dimer, ^{76}Se ($^{40}Ar^{36}Ar$), ^{78}Se ($^{40}Ar^{36}ArH_2$), ^{80}Se ($^{40}Ar_2$), and ^{82}Se ($^{40}Ar_2H_2$). Thus these were not selected for Se analysis. ^{74}Se, ^{77}Se, and ^{82}Se isotopes are

interfered by Cl polyatomic ion species, $^{37}Cl_2$, $^{40}Ar^{37}Cl$, and $^{12}C^{35}Cl_2$ respectively. The overlap of ^{77}Se by $^{40}Ar^{37}Cl$ probably accounts for much lower than expected ^{82}Se / ^{77}Se ratio observed for the SRM solution.

In order to confirm interference problem on ^{75}As, which is the only isotope of arsenic, comparison between observed value and certified value was made. The results of SRM 1515 analysis showed much higher value than the certified value, indicating the possible positive interference on ^{75}As. Wu *et al.* [26] reported interfering species of ^{75}As as $^{40}Ar^{35}Cl$ with relative abundance of 75.52 %, $^{37}Cl_2H$ with 5.855 % abundance, and $^{40}Ar^{34}SH$ with 4.183 % abundance.

In conclusion, B, Cd, Ni could be measured by selecting suitable isotope (^{10}B, ^{114}Cd, and ^{62}Ni). In addition, the analytical results for B was in good agreement with the certified values. Although Cd can not be detected, whereas Ni gave lower amounts than certified values probably according to incomplete digestion (see Table 3.2). For element with interference problem (As, Cr, Fe, and Se), in order to achieve accurate ICP-MS measurement, separation technique was applied to remove the matrix causing interference species prior to ICP-MS analysis. This separation method will be presented in the following section.

3.2.2.2 Methods to Overcome Polyatomic Interferences

Chloride, Sulfur, Calcium are macronutrient of plants occurring in high level. Their contents in sample are a potential interference in the determination by ICP-MS [22].

Various approaches can be followed to reduce the effects of polyatomic ion interference. These approaches are summarized in Table 1.5. In this work three approaches, mathematical correction procedure, separation method and alternative sample preparation (dry ashing) were studied and will be discussed in this section.

3.2.2.2.1 Use of Mathematical Correction Equations

The correction equations for isobaric elemental interferences on ^{138}Ba, ^{136}Ba, ^{114}Cd, ^{54}Fe, ^{98}Mo, ^{58}Ni, ^{82}Se, ^{120}Sn and ^{64}Zn were automatically incorporated in the software provided by the manufacturer as shown in Table 3.5. The correction equation for the polyatomic interference of $^{40}Ca^{16}OH$ on ^{57}Fe, $^{40}Ar^{35}Cl$ on ^{75}As, $^{40}Ar^{37}Cl$ on ^{77}Se and $^{37}Cl^{16}O$ on ^{53}Cr were determined and described in section 2.7.2.1. The analytical results for the analysed SRM 1515 solution obtained with and without the correction equation were shown together in Table 3.8.

Table 3.8 Results obtained for As, Cr, Fe and Se in SRM 1515 (Apple leaves) with and without correction equation

Element	Certified (mg/Kg)	Found (mg/Kg)[@]	
		Without correction	With correction
^{75}As	0.038±0.007	0.128±0.006	0.125±0.005
^{53}Cr	0.3*	0.477±0.03	0.463±0.05
^{57}Fe	83±5	132±1	6809±86
^{77}Se	0.05±0.009	0.983±0.014	0.955±0.015

* Reference value

[@] Mean ± Standard deviation, (n=3)

According to Table 3.8, using the corresponding correction equation given in Table 2.6, the results for ^{75}As, ^{53}Cr and ^{77}Se were still far from agreement with the certified values. This is because in all equations, the measurement of the signals of Cl at m/z 35 and 37 to derive the coefficients use in the interference correction equation (see section 2.7.2), can severely suffer from S interference. ^{34}SH and ^{36}SH can interfere ^{35}Cl and ^{37}Cl, respectively and therefore can be contributive to the error of coefficient obtained in all correction equation for element with Cl interference.

In this work, ^{57}Fe was selected for the Fe determination rather than ^{54}Fe and ^{56}Fe which suffered severe interference from $^{40}Ar^{14}N$ or $^{40}Ar^{16}O$. To evaluate the method, the SRM 1515 was analysed. The Ca levels in the SRM 1515 may be as high as 1.5% that was high potentially produce $^{40}Ca^{16}OH$ interference species on the determination of Fe at m/z 57. The $^{40}Ca^{16}OH$ interference on ^{57}Fe was corrected using the correction equation as shown in Table 2.6 and the result have shown in Table 3.8. The corresponding interference correction coefficients listed in Table 2.6 were the measured percentage ratios of the signals of $^{40}Ca^{16}OH$ to ^{43}Ca in standard solution at m/z 60 values to that at m/z 43. When the correction equation was applied to Fe determination, high %recovery was obtained. This was because the measurement at m/z 60 of $^{43}Ca^{16}OH$ was interfered by ^{60}Ni. Therefore, Fe cannot be determined using just mathematical correction equation.

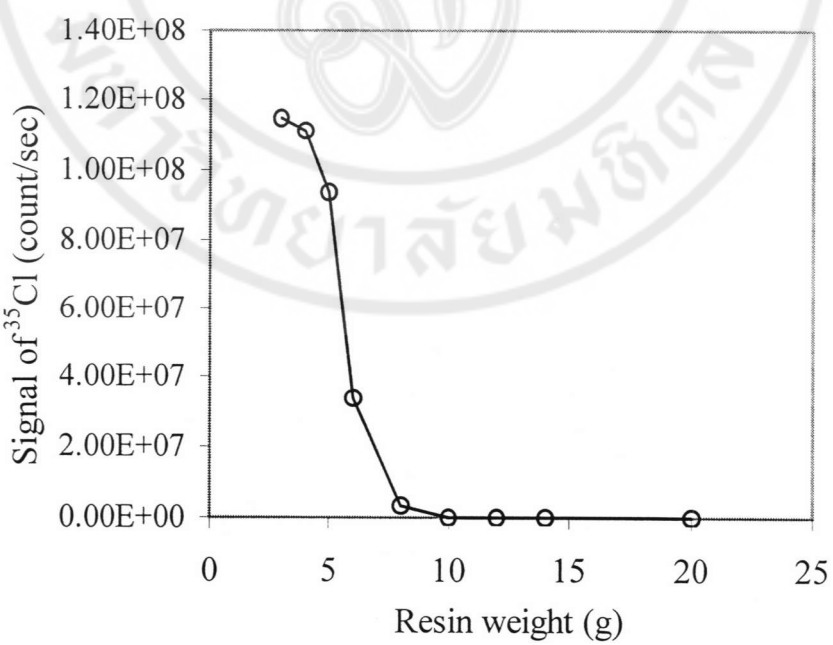
3.2.2.2.2 Removal of Matrix Ions by Separation Method

In this section, anion exchange resin was used for removal of concomitant anions in the sample solution prior to ICP-MS determination in order to reduce polyatomic interference. The SRM solution was used as the sample for their study.

i.) Effect of Amount of Resin on Chloride Removal

In anion removal step, the sample volume should not exceed the maximum capacity of the anion exchange resin column. For ICP-MS determination, the approximately sample volumes of 6 mL is needed to obtain three replicate readings. For this reason, optimal amount of resin was investigated for 10 mL sample volume. The effect of amount of resin on Cl removal is shown in Figure 3.4.

Figure 3.4 Effect of varying amount of resin on chloride removal of 10 mL for SRM 1515 solution



From Figure 3.4, the result showed that 10 g of anion exchange resin was enough for complete removal of chloride in 10 mL of the SRM 1515 digestate. This amount was selected for treatment of samples before ICP-MS measurement.

ii.) Analysis of Certified Reference Material

Having demonstrated that Cl can be removed by anion exchange resin, it was necessary to validate the method using a certified reference material. Firstly, the capability of the resin to remove chloride ions from SRM 1515 digestates was tested by measurement of untreated 2%HNO_3 compare with 10 mL treated and untreated of the SRM 1515 digestates. The results shown in Figure 3.5 indicated that the amount of Cl in SRM solution after treatment is lower than the untreated 2% HNO_3. The determination of elements with polyatomic interference problem (As, Cr, Fe and Se) for the SRM before and after treatment with resin were performed. The analytical results are shown in Table 3.9.

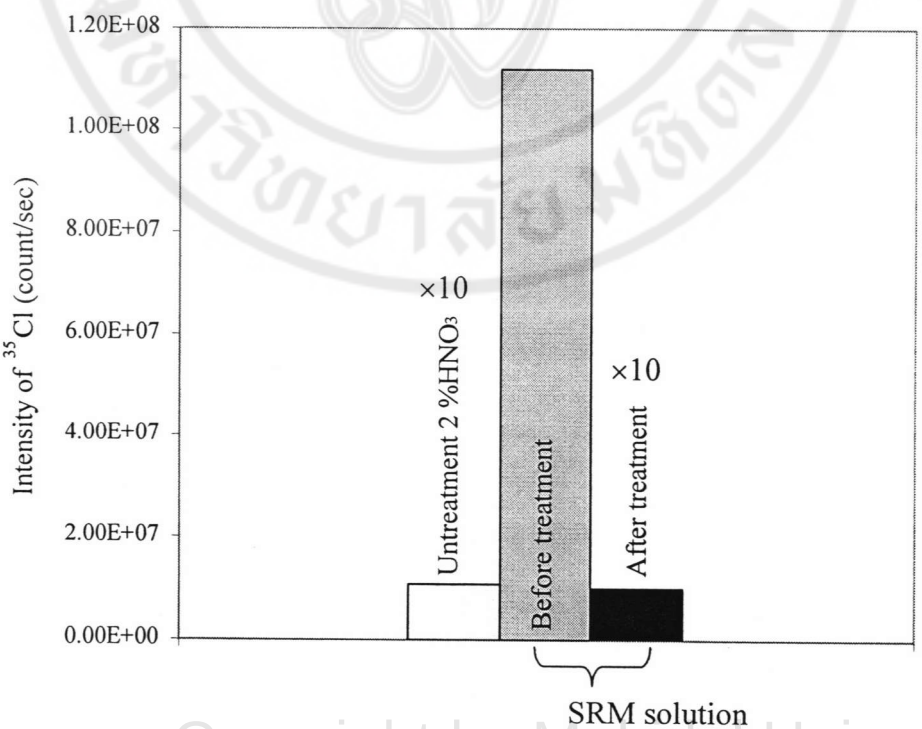


Figure 3.5 Signal of chloride, monitored at m/z 35 of untreated 2 % HNO_3 and SRM 1515 solution, with and without treatment with resin.

Table 3.9 Elemental concentration of ^{75}As, ^{53}Cr, ^{57}Fe, ^{77}Se and ^{82}Se, in SRM 1515 after treatment with anion exchange resin.

Elements	Certified (mg/Kg)	Found (mg/Kg)[@]		%Recovery
		Untreated with resin	Treated with resin	
^{75}As	0.038±0.007	0.223±0.0049	0.128±0.021	337
^{53}Cr	0.3*	0.454±0.0396	0.39±0.028	130
^{57}Fe	83±5	132±2	84±10	101
^{77}Se	0.05±0.009	0.627±0.0286	0.223±0.014	446
^{82}Se	0.05±0.009	0.344±0.0131	0.126±0.036	252

*Reference value

[@]Mean ± standard deviation, (n=3)

The use of anion exchange resin could reduce 99% free chloride ions in the SRM solution. This treatment could reduce the interference effect in ^{75}As, ^{53}Cr, ^{57}Fe, ^{77}Se and ^{82}Se measurement to obtain 337, 130, 101, 446 and 252 %recovery, respectively. The results for ^{53}Cr and ^{57}Fe are satisfactory but those of ^{75}As, ^{77}Se and ^{82}Se are still higher than the certified values.

This proposed ion-exchange method was very efficient for removing chloride ions and may remove other anions commonly found in plant digestates. Interference from chloride ions and matrices of sample on ^{53}Cr and ^{57}Fe were completely removed but not those on ^{75}As, ^{77}Se and ^{82}Se.

3.2.2.2.3 Alternative Sample Preparation Method

Dry ashing was used as an alternative sample preparation, to avoid introduction of reagent matrices causing polyatomic interferences in ICP-MS measurement. Certified reference material was used to validate this method. The analytical results obtained for the SRM 1515 were given in Table 3.10.

Table 3.10 Results obtained for the analysis of SRM 1515 using dry ashing digestion method

Element	Certified values (mg/Kg)	Found (mg/Kg)[@]	%Recovery
^{75}As	0.038±0.007	0.107±0.00052	282
^{53}Cr	0.3*	0.387±0.127	129
^{57}Fe	83±5	118±2.5	142
^{77}Se	0.05±0.009	0.429±0.013	858
^{82}Se	0.05±0.009	0.109±0.003	218

*Reference value

[@] Mean ± Standard deviation, (n=2)

Note: analytical results for twenty elements are given in Appendix II.

Table 3.10 shows the results of duplicate analyses of the SRM 1515. The result of ^{53}Cr shows good agreement with reference value whereas As, Fe and Se are still higher than certified values. Thompson and Ward [8] have developed dry ashing method for plant samples and obtained reasonable agreement with the certified values for most of the analytes, but low recoveries were observed for As and Cr. This was thought to be due to loss of this element during the ashing stage

of the digestion process. Disagreement of As and Cr recoveries between this work and Thompson's work possibly due to different digestion procedure. In this work, with and without nitric acid addition to the sample before ashing was explored. The result showed that, ashing without nitric acid addition 69 % recovery was found for Cr. This was because dues to loss of Cr in ashing step. Thompson ashed the sample without nitric acid and low recovery of As and Cr were found. It was probably owing to this reason.

3.2.3 FAAS Determinations

Ca and K concentration in plant samples are too high to be measured using ICP-MS. FAAS technique was used for these elements. The measurement was performed under the condition given in Table 2.2. Equations of calibration for Ca and K determination are presented in Table 3.11

Table 3.11 Equations of calibration of Ca and K

Element	Equation of calibration	Regression, R^2
Ca	y=0.0202x-0.018	0.9996
K	y=0.0784x-0.0374	0.9943

To evaluation FAAS method for Ca and K, it was necessary to validate the technique using the certified reference material. The results obtained are already given in Table 3.2. The results are in good agreement with the certified values.

To summarize the analytical procedure for determination of minerals contents in plant samples; Twenty elements Al, B, Ba, Be, Cd, Co, Cu, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Sb, Sn, Sr, V and Zn can be directly measured using ICP-MS after digestion. Anion removal by ion exchange resin was needed for Cr and Fe determination. For element with high concentration (Ca and K), FAAS should be used. These procedures were used to obtain mineral contents of 13 common Thai herbs and 5 medicinal plants as presentd in the following section.

3.3 Mineral Contents of Plant Samples

In this section, analytical results obtained for 17 common Thai herbs from different sampling sites, 5 herb capsules were presented.

3.3.1 Thai Herbs

17 Thai herbs were purchased from 3 markets, Kabin buri, Pinklao and Kingpetch. All the plant samples are commonly used as vegetables in Thai foods.

3.3.1.1 Elemental Concentrations

The concentrations of 24 elements of 17 Thai herbs obtained from different sources are presented in Table 3.12 (for elements with higher levels) and Table 3.13 (for elements with lower levels).

Table 3.12 Analytical results (mg/Kg dry weight) of mineral contents for 13 Thai herbs.

Sample	K	Ca	P	Mg	Na	Mn	Ba	Zn	Fe	Al	Sr	Ni	B	Cu
*A1	18488± 241	20551± 197	242±6.33	949±20.83	22.72± 3.56	9.85± 0.144	297.0± 0.20	8.53± 0.002	48.2±0.92	56.08± 9.16	58.60± 0.711	0.924± 0.013	32.62± 2.41	1.94± 0.022
*A2	12211± 105	20856± 104	267.7± 1.74	1532±14	414±2.26	17.31± 0.065	137.1± 0.018	11.45± 0.28	47.15±± 1.35	86.29± 0.38	105±0.312	1.20± 0.080	26.50± 0.01	22.59± 0.693
*A3	14413± 171	11144±17	263±18.6	1186±43	35.94± 7.13	23.13± 1.00	2068±2.0	13.97± 1.03	75.41± 1.48	76.79±7.6	356±3.4	2.02± 0.124	39.57± 3.25	2.28± 0.169
^B1	4679±593	17255± 279	553±9.36	2832±62	9327±252	18.65± 0.47	59.12± 1.85	25.32± 0.82	31.83± 0.57	38.13± 0.91	43.16± 1.18	0.722± 0.066	6.25±0.99	8.51± 0.244
^B2	8379±302	9866±249	567±33.7	2504±80	10391± 539	128±2.83	234±9.30	33.77± 0.58	43.56± 1.58	67.18±2.4	76.34± 1.95	3.29± 0.138	34.13± 0.95	12.34± 0.265
^B3	21455± 638	14492± 107	491.24± 11.5	2136±4.8	5018±94.5	77.4±1.35	201±4.98	32.39± 0.55	46.8±0.44	35.79±2.8	18.30± 0.907	1.83± 0.031	16.72± 0.58	13.02± 0.25
*C1	30032± 398	9466±208	836.6± 9.96	2520±3.50	368±10.1	219±1.82	789±4.9	60.63± 1.74	48.14+0.7 3	151.86± 8.42	61.39± 0.77	1.68± 0.056	16.83± 0.47	17.19± 0.34
*C2	20636± 193	13580± 187	1183±54	5242±177	501±20.5	241±7.80	129±6.64	44.63± 2.14	54.23± 3.52	127±6.89	78.39± 1.66	2.78±0.12	21.43± 0.84	12.65± 0.68
*C3	22793± 399	15867± 158	618±1.85	3145±3.25	59.58± 0.61	46.30± 0.89	327±9.95	39.37± 0.25	41.8±0.81	60.0± 17.20	34.7± 0.033	1.21± 0.012	14.57± 0.114	16.3±0.18
^D1	36421± 362	2657±113	1328±8.24	2268±8	314±3.8	29.42± 0.42	83.36± 3.51	50.52± 0.38	26.2±4.12	56.07± 6.86	16.95± 0.74	1.63± 0.019	17.65± 1.20	10.67± 0.194
^D2	30280± 206	8506±84	1000±21	3005±64	461.8±9.6	56.65± 1.40	549±96	46.02± 2.03	43.32±1.5	80.93± 11.98	140.87± 3.24	6.02±0.30	18.50± 0.32	13.27± 0.74
^D3	34044±80	5954±88	1133±70	2574±209	224±16.8	37.64± 2.43	30.0±0.2	43.97± 2.56	27.93± 0.12	58.90± 7.83	29.72± 1.46	1.36±0.05	27.74± 1.50	8.45±0.41
*E1	20647± 345	15416±83	743±16	4089±92	173±2.39	282±11	69.58±3.3	56.50± 3.07	25.70± 1.34	41.85± 0.75	84.02± 2.10	4.30±0.13	16.83± 0.68	17.78± 0.74
*E2	16933± 389	22736± 332	910±132	6576±35	573±12.3	157±4.4	63.83± 2.30	59.60±2.0	46.22± 3.38	66.30± 1.27	136.6± 4.11	2.32± 0.066	19.89± 0.58	8.32±0.30
*E3	23349± 412	15393+43	946±15	4711±174	78.4±2.13	112±1.74	63.05± 1.76	39.64± 1.43	33.41± 4.42	33.18± 0.10	135±2.17	2.78± 0.153	17.69± 0.023	9.50±0.43

Table 3.12 Analytical results (mg/Kg dry weight) of mineral contents for 13 Thai herbs (continued)

Sample	K	Ca	P	Mg	Na	Mn	Ba	Zn	Fe	Al	Sr	Ni	B	Cu
*F1	19534± 267	1275±46	929±16	1200±27	91.56±4.3	25.78± 0.26	33.73± 1.90	43.20±1.0	28.66± 0.69	16.72± 0.26	2.50±0.07	3.01±0.11	20.53± 0.89	4.59± 0.104
*F2	23386± 377	1269±86	1187±9.7	1296±36	107±3.4	36.34± 0.62	30.16± 0.47	42.5±1.50	19.91± 0.44	29.28± 1.86	2.60±0.06	7.50±0.20	14.78± 0.06	4.46± 0.021
*F3	18805± 207	1074±29	1027±25	1263±41.3	43.15±1.5	21.40± 0.31	12.30± 1.13	33.42± 0.74	16.6±1.1	14.75± 0.78	3.40±0.35	1.08± 0.047	18.10± 0.17	4.41±0.18
^G1	23755± 156	11742± 117	552±14	2097±52	548±12.3	249±7.0	186±4.2	21.80± 0.60	138±10.0	660±56	74.8±0.92	1.85± 0.027	12.4±0.81	7.59±0.81
^G2	19127± 146	6931±105	422±8.9	2938±81	356±5.0	125±3.4	39±2.0	31.1±1.61	36.7±0.78	101±0.91	33.68±2.1	2.34±0.10	15.14± 1.19	9.25±0.52
^G3	23849± 266	5343±108	413±2.65	2701±26	390±5.4	38.3±0.90	40.4±0.80	21.7±0.65	31.97±0.5	73.0±0.85	16.0±0.32	0.50±0.03	11.92± 0.70	6.97±0.20
^H1	39688± 348	588±99	513±0.57	1654±60	80.5±1.50	64.14±8.5	137±5.20	31.32± 1.36	47.89± 1.43	486±3.6	7.39±0.22	0.36±0.02	5.14±0.75	2.90±0.09
^H2	23434± 342	671±90	285±10.1	1236±61	36±2.3	30±1.55	28.4±1.50	8.90±0.87	27±3.87	64.4±6.3	2.31±0.20	0.44± 0.030	2.82±0.42	1.37±0.09
^H3	30215± 314	591±85	426±5.82	1221±20	138±2.7	27.10± 0.40	24.90± 1.50	9.02±0.18	23.19±1.2	111±2.88	3.36±0.13	0.103± 0.027	5.58±1.84	1.69±0.05
*I1	25809± 483	944±16	123±7.7	1067±234	59.6±27	215±7.1	20.8±3.0	12.7±0.97	27±1.9	68.6±1.84	14±0.81	0.88±0.23	ND	3.31±0.79
*I2	24439± 617	685±34	130±5.6	1001±47	30.4±1.3	175±0.64	7.30±0.11	7.83±0.85	4.92±0.07	6.54±0.83	5.55±0.23	0.82± 0.017	11.5± 0.035	3.68±0.16
*I3	30233± 354	1136±37	135±1.30	1245±84	84.3±1.34	221±16.3	8.97±0.32	11.2±2.3	5.2±0.21	39.0±2.2	15.0±0.91	0.71±0.07	ND	2.81±0.14
*J1	34329± 255	1277±53	96.4±16	1470±83	600±75	36±6.3	8.50±1.4	45.4±11	45.34±3.2	62.7±9.9	8.95±1.6	0.69±0.19	4.24±2.9	4.24±0.62
*J2	28834± 130	754±37	77.0±3.1	1376±26	578±16	1010±20	0.74±0.03	110±3.4	7.30±0.05	42.0±3.8	9.97±0.15	2.10±0.16	7.68±0.13	4.10±0.07
*J3	32678± 626	1139±85	74±2.1	2745±116	243±2.3	1606±35	2.50±0.08	68.7±3.3	11.36± 1.83	46.4±0.37	11.6±0.65	1.65±0.06	ND	4.30±0.34

Table 3.12 Analytical results (mg/Kg dry weight) of mineral contents for 13 Thai herbs (continued)

Sample	K	Ca	P	Mg	Na	Mn	Ba	Zn	Fe	Al	Sr	Ni	B	Cu
^K1	27538± 194	3656±83	138±3.98	958±25	45.7±0.77	36.26± 2.12	18.5±0.66	22.5±2.1	25.89± 0.35	12.1±0.59	21.7±0.78	0.87±0.12	4.53±1.45	2.10± 0.058
^K2	17669± 175	2449±119	199±7.0	1185±77	9.95±0.54	41.48± 0.80	2.82±0.18	23.0±1.88	8.25± 0.427	3.72±0.18	30.0±1.73	0.86±0.08	1.30±0.30	2.50±0.15
^K3	9534±325	908±72	91.2±4.7	101±11	12.02± 2.81	220±28	20.2±1.5	19.1±2.91	5.10±0.18	2.58±0.08	9.261± 0.68	0.24±0.04	ND	1.06±0.17
*L1	17125± 176	1041±32	233±5.83	396±13	80.8±1.44	6.78±0.23	1.35±0.12	13.87± 0.086	8.16±0.33	7.69±2.63	3.48± 0.165	1.14±0.12	5.83±0.60	2.28± 0.213
*L2	11230± 155	1381±25	188±20	514±17	253±12.6	5.85±0.14	2.26±0.12	14.71± 0.47	6.35±0.79	2.93±0.35	24.1±0.14	1.82±0.36	9.13±0.25	3.20±0.10
*L3	21883± 200	1051±36	204±7.3	374±2.3	113±1.93	6.93±0.23	3.11± 0.001	20.17± 0.03	9.89±0.45	9.37±1.8	4.42±0.10	1.10± 0.037	7.58±0.12	3.80±0.03
^M1	12964±97	327±79	311±4.0	255±4.44	25.5±0.06	5.20±0.13	4.00±0.09	26.4±0.47	6.11±0.22	1.86±0.14	1.93± 0.007	1.69±0.05	0.027± 1.31	2.08±0.05
^M2	12431± 237	330±78	218±1.84	234±1.83	87.1±0.79	5.63±0.14	0.94±0.01	23.13± 0.35	6.99±0.61	3.81±0.19	1.23± 0.001	0.57± 0.003	2.07±0.63	2.62± 0.024
^M3	11747± 221	314±82	199±0.89	197±0.30	37.8±0.71	4.64±0.11	1.04± 0.033	18.5±0.20	5.76±0.26	1.90±0.12	0.73± 0.041	0.47±0.01	2.77±0.34	1.86± 0.043

A = *Citrus hystrix* G = *Mentha arvensis* M = *Allium sativum* ND=Not detectable

B = *Ocimum sanctum* H = *Gastrochilus pandurata* 1 = Kabin buri

C = *Ocimum basilicum* I = *Zingiber officnale* 2 = Pinklao

D = *Coccinia grandis* J = *Alpinia galanga* 3= Kingpetch

E = *Ocimum basilicum* K = *Cymbopogon citratus* *(n = 2)

F = *Acacia insuavis* L = *Allium ascalonicum* ^(n = 3)

Table 3.13 Analytical results (μg/Kg dry weight) of mineral contents for 13 Thai herbs

Sample	Co	Cr	V	Mo	Pb	Li	Be	Sb	Cd	Sn
*A1	48.1±3.25	142±22	303±10	ND	123±3.2	131±21	ND	ND	ND	ND
*A2	36.3±3.6	204±8	34.50± 3.30	ND	1160±25	2190±17	ND	11.35± 0.93	ND	ND
*A3	132±17	295±32	200±64	ND	417±248	11.60±14	ND	5.44±2.2	ND	ND
^B1	16.72± 3.50	214±13	ND	ND	219±28	788±27	ND	ND	ND	ND
^B2	138±8.50	202±24	125±17	ND	3876±236	721±178	ND	12.63± 1.10	13.2±0.29	ND
^B3	232±5.0	362±25	ND	ND	175±29	3114±127	ND	ND	ND	ND
*C1	387±9.13	1130±21	401±56	ND	590±44	29.7±7.29	ND	9.3±4.6	12.59±2.8	ND
*C2	147±7.56	252±5.8	254±4.99	ND	801±34	1645±40	ND	3.62±0.89	168±11	ND
*C3	239±1.01	230±8	ND	ND	122±2.6	ND	ND	ND	ND	ND
^D1	343±2.5	1360±38	ND	ND	1232±345	25.3±1.8	ND	3.43±0.49	13.8±32	ND
^D2	129±8.2	193±22	125±19	ND	455±28	122±12.4	ND	2.50±0.69	ND	ND
^D3	31.2±2.1	144±15	ND	337±23	128±48	718±20	ND	ND	ND	ND
*E1	559±26	1512±55	ND	ND	1040±31	360±21	29±3.8	1.48±0.74	5.4±2.8	ND
*E2	143±16	146±6.5	44.2±20	ND	514±17	930±19	ND	4.4±0.19	23.2±1.6	ND
*E3	537±24	111±21	48.2±17	ND	ND	236±9.6	ND	ND	ND	ND

Table 3.13 Analytical results (μg/Kg dry weight) of mineral contents for 13 Thai herbs (continued)

Sample	Co	Cr	V	Mo	Pb	Li	Be	Sb	Cd	Sn
*F1	126±2.5	158±9.9	ND	103±13	16.1±25	ND	ND	6.19±3.3	ND	ND
*F2	30.3±2.3	65±2.2	ND	ND	108±18.3	ND	ND	ND	37.56±29	ND
*F3	15.8±2.70	115±4.7	ND	313±4.15	6.60±5.0	ND	ND	ND	ND	ND
^G1	270±15	1079±63	1147±107	ND	147±17	569±21	ND	5.93±0.22	ND	ND
^G2	58.0±7.3	271±26	39.17±7.1	ND	831±534	822±16	ND	4.58±1.32	ND	ND
^G3	25.0±2.3	252±49	ND	ND	289±90	551±2.75	ND	1.65±0.75	ND	ND
^H1	139±3.24	758±140	988±27	ND	60.99±9.9	47.5±6.8	ND	38.15±1.4	23.86±3.9	ND
^H2	148±19	177±18	70.67±21	ND	50.1±34	ND	ND	8.40±1.98	ND	ND
^H3	42.4±2.56	278±16	378±25	ND	378±732	8.13±14	ND	25.12±1.8	ND	ND
*I1	267±49	618±34	63.22±143	112±49	204±179	ND	ND	ND	ND	ND
*I2	208±10.34	66±1.7	ND	72.3±0.64	122±148	ND	ND	ND	ND	ND
*I3	231±22	128±5	ND	82.3±0.99	260±126	ND	ND	ND	ND	ND
*J1	256±9.3	198±11	188±28	68.2±35	ND	35.0±10	ND	ND	ND	ND
*J2	991±38	308±5	98±14	78.9±10.4	76.5±81	311±38	ND	ND	24.2±15	ND
*J3	772±39	367±31	37.15±5.9	ND	556±81	400±4.4	ND	ND	10.9±5.1	ND

Table 3.13 Analytical results (μg/Kg dry weight) of mineral contents for 13 Thai herbs (continued)

Sample	Co	Cr	V	Mo	Pb	Li	Be	Sb	Cd	Sn
^K1	236±10	163±0.9	227±14.4	141±5.84	ND	ND	ND	ND	58.8±34	ND
^K2	205±6.2	322±7	104±6.6	419±33	91.4±254	8.2±2.3	ND	ND	338±4.8	ND
^K3	251±16	136±27	ND	0.59±2.3	416±288	ND	ND	ND	91.8±15.3	ND
*L1	214±27	267±12	ND	ND	26±114	ND	ND	ND	84.1±7.4	ND
*L2	210±13.0	42±2.6	ND	84±8	ND	ND	ND	ND	ND	ND
*L3	206±16	62.7±5.5	ND	43.5±16.4	ND	ND	ND	ND	11.4±6.1	ND
^M1	213±11	47.3±5.8	ND	52.3±2.07	17.9±182	ND	ND	ND	84.7±0.6	ND
^M2	204±7.3	88±8.2	ND	ND	9.92±42	ND	ND	ND	32±4.3	ND
^M3	194±21	45±5.7	ND	ND	ND	ND	ND	ND	262±16.3	ND

ND=Not detectable

A = *Citrus hystrix*
B = *Ocimum sanctum*
C = *Ocimum basilicum*
D = *Coccinia grandis*
E = *Ocimum basilicum*
F = *Acacia insuavis*
G = *Mentha arvensis*
H = *Gastrochilus pandurata*
I = *Zingiber officnale*
J = *Alpinia galanga*
K = *Cymbopogon citratus*
L = *Allium ascalonicum*
M = *Allium sativum*
1 = Kabin buri
2 = Pinklao
3= Kingpetch
*(n = 2)
^(n = 3)

Table 3.14 The ranges of mineral concentration (mg/Kg dry weight) for 13 Thai herbs

Sample	K	Ca	P	Mg	Na	Mn	Ba	Zn	Fe	Al	Sr	Ni	B	Cu
A	12200-18500	11000-20800	240-270	950-1100	20-400	10-25	130-2100	8-14	1.5-48	60-80	60-360	1-2	27-40	2-23
B	4600-21500	9800-17200	490-550	2100-2800	5000-9300	18-130	60-230	25-33	30-47	36-68	18-76	1-3	6-34	9-13
C	20600-30000	9500-16000	840-1200	3100-2500	60-500	50-220	130-790	40-60	41-50	60-150	35-80	1-3	14-17	13-17
D	30200-36400	2600-8500	1000-1300	2200-3000	220-460	30-56	85-550	43-50	26-43	56-80	17-140	1-6	18-27	8-11
E	16900-23300	15300-22700	740-950	4100-6500	78-570	110-280	63-69	40-60	25-46	33-66	85-136	2-4	16-19	8-10
F	18800-19500	1100-1280	930-1200	1200-1300	92-107	21-36	12-34	33-43	16-29	15-30	3-4	1-8	15-21	4-5
G	19000-23800	6300-11700	400-550	2100-3000	350-550	40-250	40-190	22-30	32-140	73-660	16-75	1-2	11-15	7-9
H	23000-39600	580-670	280-510	1200-1600	36-140	27-64	25-140	9-31	23-48	65-480	2-8	0.5-1	3-6	1-3
I	24400-30200	680-1130	120-140	1000-1200	30-85	180-220	7-21	8-13	5-30	7-70	6-15	0.7-0.9	12	3-4
J	28800-32600	750-1200	74-100	1300-1500	240-600	36-1600	1-3	45-110	7-45	42-62	9-12	1-2	4-8	4
K	9500-27500	900-3600	90-200	100-1200	10-46	36-220	3-20	20-23	5-26	3-12	10-30	0.2-0.8	1-5	1-3
L	17000-21800	1050-1400	180-230	370-510	80-260	6-7	1-3	13-20	6-10	3-10	4-24	1-2	8-10	2-4
M	11700-12900	300-330	200-310	200-250	25-87	5-6	1-4	19-26	6-7	2-4	1-2	1-2	0.5-3	1-3

Table 3.15 The range of mineral concentration (μg/Kg dry weight) for 13 Thai herbs

Sample	Co	Cr	V	Mo	Pb	Li	Be	Sb	Cd	Sn
A	36-130	140-300	34-200	ND	120-410	12-2190	ND	5-11	ND	ND
B	16-230	200-360	125	ND	180-3800	720-3100	ND	13	13	ND
C	150-380	230-1130	250-400	ND	120-800	30-1600	ND	4-9	4-9	ND
D	31-350	140-1360	125	330	120-1200	25-700	ND	3-4	3-4	ND
E	150-550	110-1500	44-48	ND	500-1000	240-930	30	1-5	5-23	ND
F	16-130	65-160	ND	103-310	7-110	ND	ND	6	38	ND
G	25-270	250-1100	40-1100	ND	150-830	550-820	ND	2-6	ND	ND
H	42-150	180-760	70-980	ND	50-380	8-48	ND	8-40	24	ND
I	230-270	66-620	63	72-110	120-260	ND	ND	ND	ND	ND
J	260-780	200-370	37-180	70-80	77-560	35-400	ND	ND	10-25	ND
K	200-250	140-300	104-230	1-420	91-420	8	ND	ND	91-340	ND
L	200-220	42-260	ND	44-85	26	ND	ND	ND	11-85	ND
M	200-210	45-88	ND	52	10-18	ND	ND	ND	32-260	ND

The ranges of mineral concentration for 13 Thai herbs are listed in Table 3.14 and Table 3.15.

The transition elements Mn, Zn, Co, and Fe are known to exist in different organic complexed forms in plants. Some of them are biologically essential micronutrients to plants [12]. From the results of this work, the concentration of these elements varies from μg/Kg to mg/Kg. This is because the transition metals are differently distributed in soils.

Some elements are considered biologically essential to plants. It is also known that not all elements absorbed by plants are of importance to them. It is, therefore, not surprising that the plants studied in this work mostly contain appreciable amounts of biologically non-essential elements, (Ba, Sr, B, Pb, Li, Be, Sb and Cd), in the concentration range from μg/Kg to mg/Kg. This is because plants accumulate varying concentrations of elements from the soil with different degrees of contamination. Sn can not be detected in all plant samples.

3.3.1.2 Sampling Site Variation

The comparison of elemental concentrations of 24 elements in the same plant species from different sources is presented in Figure 3.6. From Figure 3.6 there is not much difference in the concentrations found for K, Ca, Mg, P, Na, Al, Cu, Cr, Co, Mn, Ni, Fe, Zn, Ba and Sr between localities, i.e. Kabin buri, Pinklao and Kingpetch. This is because these elements are needed by any plants. B, Pb, Be, Li, Sb,

Cd, V and Mo which plants do not need (Pb and Cd) or need very trace amount (B, Li, Sb, V and Mo) showed the slight difference between locations.

K, Ca, P, Mg and Na are found to be present at the major level. Mn, Ba, Zn, Fe, Al, Sr, Ni, B and Cu are at the minor level and Co, Cr, V, Mo, Pb, Li, Be, Sb, Cd and Sn are at the trace levels. The difference in the concentration of the various elements within the different plants is attributed to the preferential absorbability of a particular plant for the corresponding element and the mineral composition of the soil in which the plant grows as well as surrounding climatological conditions [7].

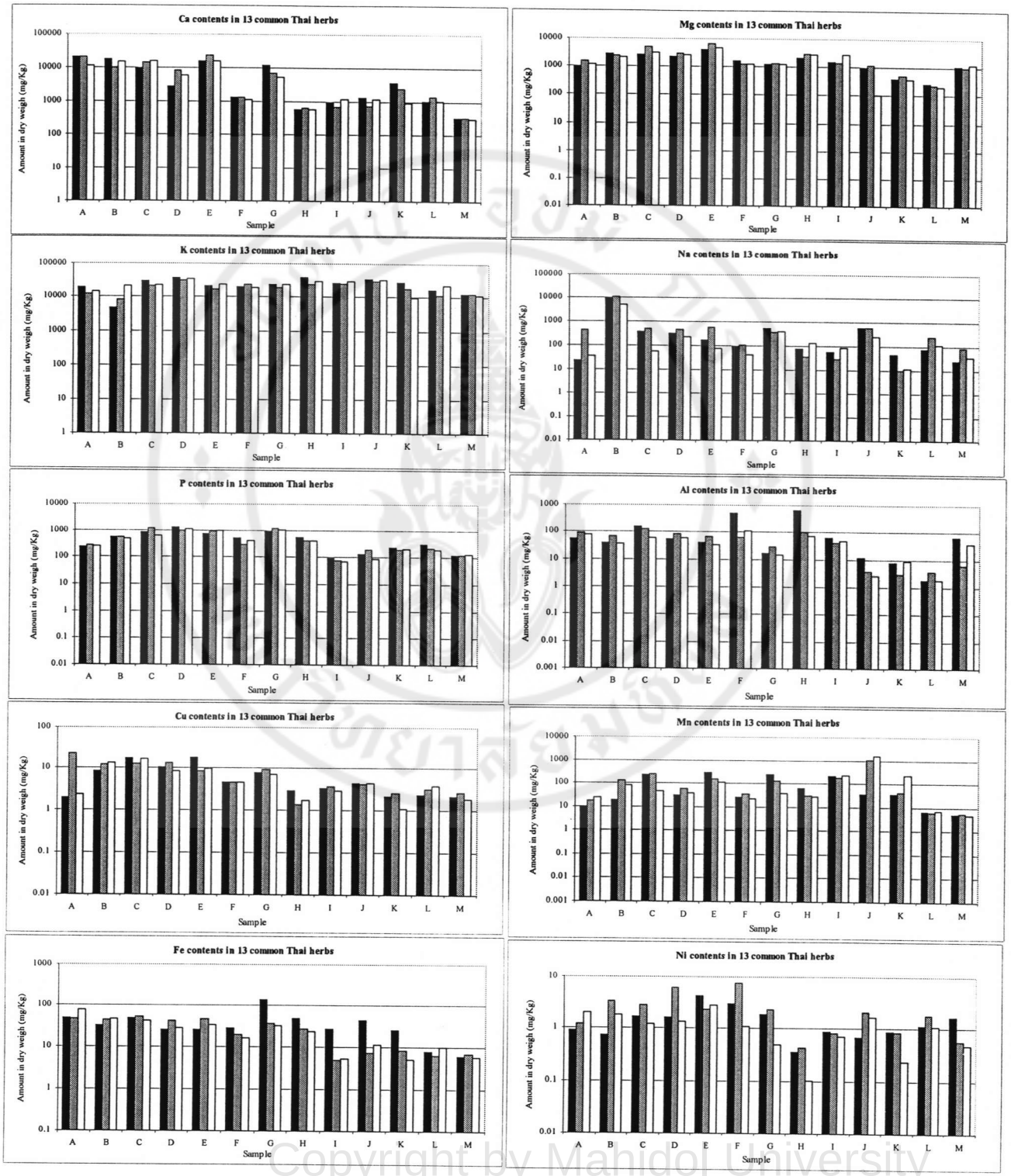


Figure 3.6 Comparison of mean concentrations of mineral contents in 13 Thai herbs

(Sampling sites; ■ = Kabin buri, ▨ = Pinklao, □ = Kingpetch)

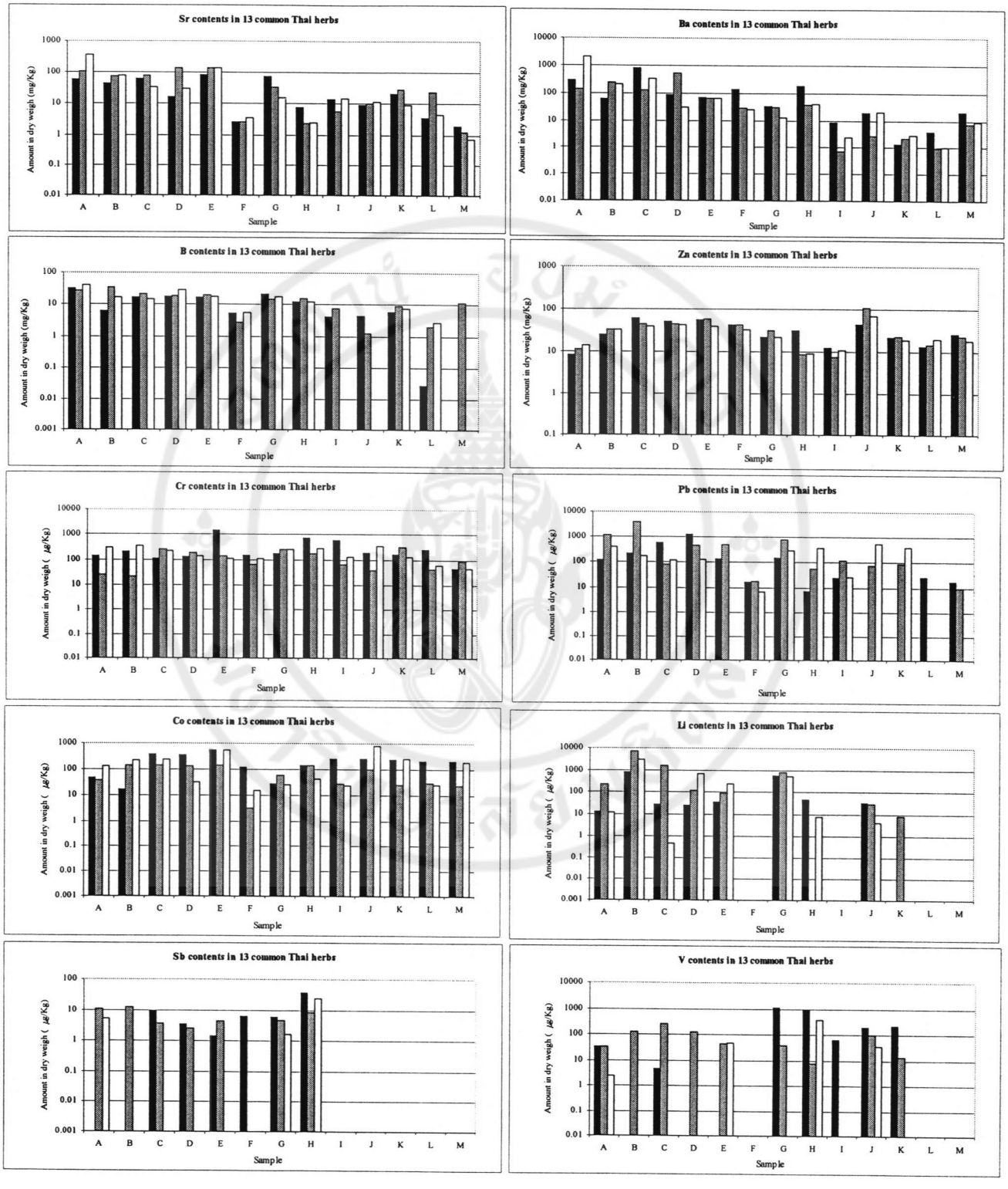


Figure 3.6 Comparison of mean concentrations of mineral contents in 13 Thai herbs (Sampling sites; ■ = Kabin buri, ▨ = Pinklao, □ = Kingpetch). Some elements in samples were not detectable and the bar graph was not seen. (Continued).

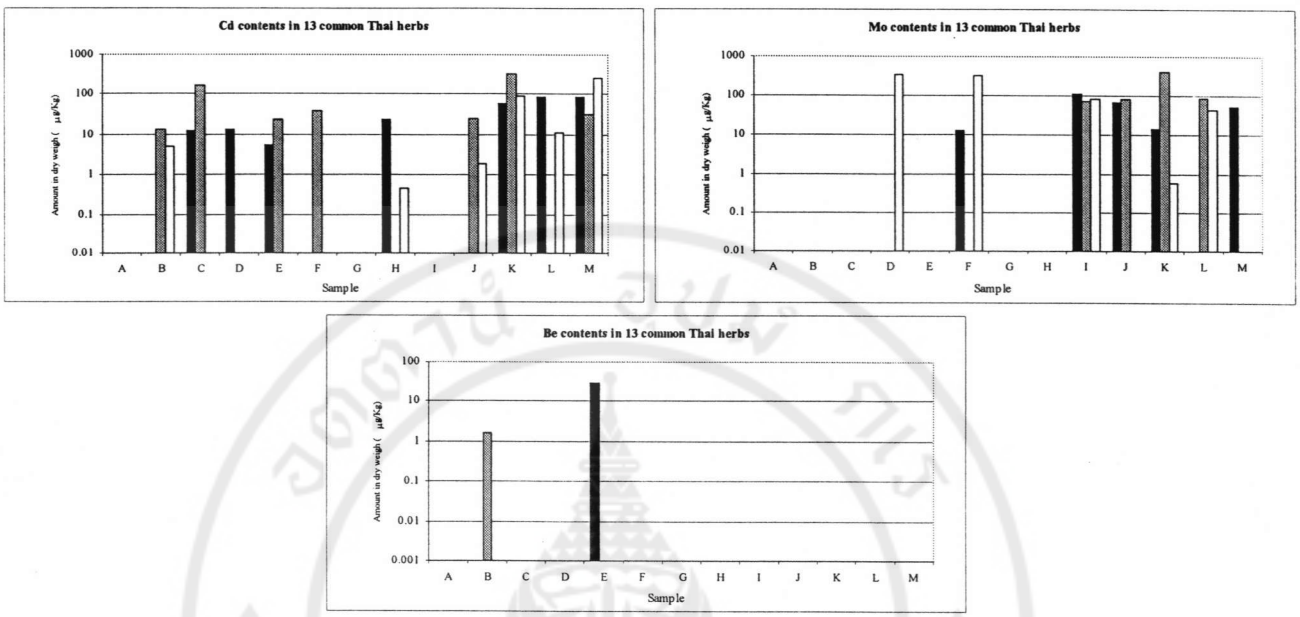


Figure 3.6 Comparison of mean concentrations of mineral contents in 13 Thai herbs (Sampling sites; ■ = Kabin buri, ▨ = Pinklao, □ = Kingpetch). Some elements in samples were not detectable and the bar graph was not seen. (Continued).

A = ใบมะกรูด ; *Citrus hystrix DC.*
B = ใบกะเพรา ; *Ocimum sanctum L.*
C = ใบแมงลัก ; *Ocimum basilicum forma citratum Back*
D = ใบตำลึง ; *Coccinia grandis Voigt*
E = ใบโหระพา ; *Ocimum basilicum L.*
F = ใบชะอม ; *Acacia insuavis Lace*
G = ใบสะระแหน่ ; *Mentha arvensis L.*
H = กระชาย ; *Gastrochilus panduratus (Ridl.) Schltr*
I = ขิง ; *Zingiber officinale Roscoe*
J = ข่า ; *Alpinia galanga (L.) Swilld.*
K = ตะไคร้ ; *Cymbopogon citratus (DC.)*
L = หอมแดง ; *Allium ascalonicum L.*
M = กระเทียม ; *Allium sativum L.*

3.3.2 Thai Herbs and Herb Capsules

The elemental analysis was carried out for 5 selected herbs commonly used in Thailand. The herb capsules are drugs prepared from the same type of the selected herbs. The capsules contain finely ground powder. The elemental composition of those samples and the comparison of elemental content between raw medicinal plants and the capsules are presented in the following section.

3.3.2.1 Elemental Concentrations

The determined concentrations of 24 elements in 5 herbs and herb capsules are presented in Table 3.16 (for elements with higher levels) and Table 3.17 (for elements with lower levels).

Medicinal plants contain both organic and inorganic constituents. It is known that certain inorganic mineral elements (K, Zn, Ca, trace of Cr, etc.) play an important role in the maintenance of normal glucose tolerance and in the release of insulin from β-cell of islets of Langerhans [48]. Potassium salts are partially responsible for the diuretical action of some drugs; they also increase the cardiotonic action of digitalis [12]. From the results of this work, it is evident that all plant species studied, (medicinal plants and local vegetables) have high natural potassium concentrations. It can be seen from the Table 3.14 that all herbs showed potassium amount in the same range of common Thai herbs.

Table 3.16 Analytical results (mg/Kg dry weight) of mineral contents for 5 herbs and herb capsules

Sample	K	Ca	P	Mg	Na	Mn	Ba	Zn	Fe	Al	Sr	Ni	B	Cu
*Np	19449± 161	16111± 109	128±4.10	456±27	27.4±0.35	37.61± 0.43	3.98±0.11	16.65± 0.71	88.6±3.74	17.21± 0.54	43.60± 0.25	0.95± 0.037	35.15±3.0	1.99±0.10
^Nc	19089± 209	3075±211	208±8.1	1026±60	112±5.5	8.19±0.67	1.39±0.07	9.92±0.93	20.64± 0.142	15.94± 1.74	5.03±0.32	0.54± 0.030	10.10± 0.76	4.04±0.32
*Op	22280± 304	1921±18	268±1.03	744.6± 11.8	25±1.56	9.02± 0.005	1.42± 0.044	16.9±0.39	10.32± 0.42	4.61±0.53	2.63±0.04	0.56± 0.005	11.26± 0.18	3.80±0.16
^Oc	6746±116	18879± 378	59.5±0.67	722±22	39.4±1.91	51.8±0.85	139±4.03	9.32±0.20	109±3.8	83.7±4.6	79.5±0.75	1.30±0.02	23.2±0.77	4.91± 0.137
^Pp	12389± 336	10955± 161	139±8.97	581±27	189±41	4.00±0.27	7.71±0.50	14.5±0.85	15.23± 0.94	9.45±1.10	70.34± 0.79	0.82±0.02	7.55±0.56	3.04±0.13
^Pc	14780± 129	5400±195	120±9.50	670±25	6.97±5.5	9.40±0.37	44.5±1.31	25.4±3.4	35.16±5.4	32.6±0.98	38.5±0.82	0.69±0.02	ND	5.70±0.15
^Qp	29774± 278	8482±368	139±4.5	1567±50	1677±54	55.4±0.64	6.66±0.38	36.2±1.69	58.66± 1.74	85.1±3.7	30.4±0.50	1.30±0.03	14.7±2.02	5.03±0.26
^Qc	4107±153	2926±114	31.0±1.08	834±6.4	2271±33	29.1±0.29	3.06±0.08	21.4±0.78	178±2.93	373±6.2	56.4±0.26	1.36±0.06	12.1±4.6	9.97±0.70
*Jp	34329± 255	1277±53	96.4±16	1470±83	600±75	36±6.3	8.50±1.4	45.4±11	45.34±3.2	62.7±9.9	8.95±1.6	0.69±0.19	4.24±2.9	4.24±0.62
^Jc	8609±229	5184±86	69.83± 6.55	1250±14	407±6.8	115±1.26	7.50±0.13	14.02± 0.89	152±5.83	201±2.43	57±0.46	1.21± 0.004	10.4± 0.160	4.11±0.41

N = *Azadirachta indica*

O = *Cassia siamea*

P = *Tinospora crispa*

Q = *Acanthus ebracteatus*

J = *Alpinia galanga*

*(n = 2)

^(n = 3)

Subscripts p and c indicate plant and capsule, respectively.

Table 3.17 Analytical results (μg/Kg dry weight) of mineral contents for 5 herbs and herb capsules

Sample	Co	Cr	V	Mo	Pb	Li	Be	Sb	Cd	Sn
*Np	228±3.6	682±36	98.3±10.3	137±7.2	ND	ND	ND	ND	ND	ND
^Nc	249±5.2	66.1±8.5	ND	ND	ND	ND	ND	ND	ND	ND
*Op	221±14	61.5±4.7	ND	398±24	ND	ND	ND	ND	ND	ND
^Oc	419±6.89	387±46	563±34	23.5±8.0	523±27	90.4±1.9	ND	ND	ND	ND
^Pp	261±6.6	127±17	8.5±28	37.7±11.5	512±99	47±2.8	ND	ND	ND	ND
^Pc	192±8.9	386±39	129±37	254±8.2	464±164	ND	ND	ND	ND	ND
^Qp	361±9.7	322±2.5	621±23	492±30	108±614	24.3±10.3	ND	ND	ND	ND
^Qc	584±5.1	2640±176	1248±86	666±86	2386±422	451±14	ND	ND	ND	1351±62
*Jp	256±9.3	198±11	188±28	68.2±35	ND	35.0±10	ND	ND	ND	ND
^Jc	302±13	1920±186	467±19	191±6.5	689±34	275±8.4	ND	ND	ND	59±21

N = *Azadirachta indica*

O = *Cassia siamea*

P = *Tinospora crispa*

Q = *Acanthus ebracteatus*

J = *Alpinia galanga*

*(n = 2)

^(n = 3)

ND= Not detectable

Subscripts p and c indicate plant and capsule, respectively.

3.3.2.2 Comparison of Thai herbs and Herbs Capsules

As the results in Figure 3.7, there was not much difference in the concentrations found for P, Mg, Ca, K, Na, Mn, Cu, Al, Zn, Fe, Ni, Cr, Co, Ba and Sr between Thai herbs and herb capsules. Cd and Be were not found for all medicinal plants and capsules. Mo, B, Sn, Li, Pb, and V showed differences between the plant samples and the capsules. Sn could not be detected for all selected Thai herbs, but it was found in *Tinospora crispa*, *Acanthus ebracteatus* and *Alpinia galanga* capsules (see Figure 3.7). This could be the contamination during the manufacturing process of the capsules.

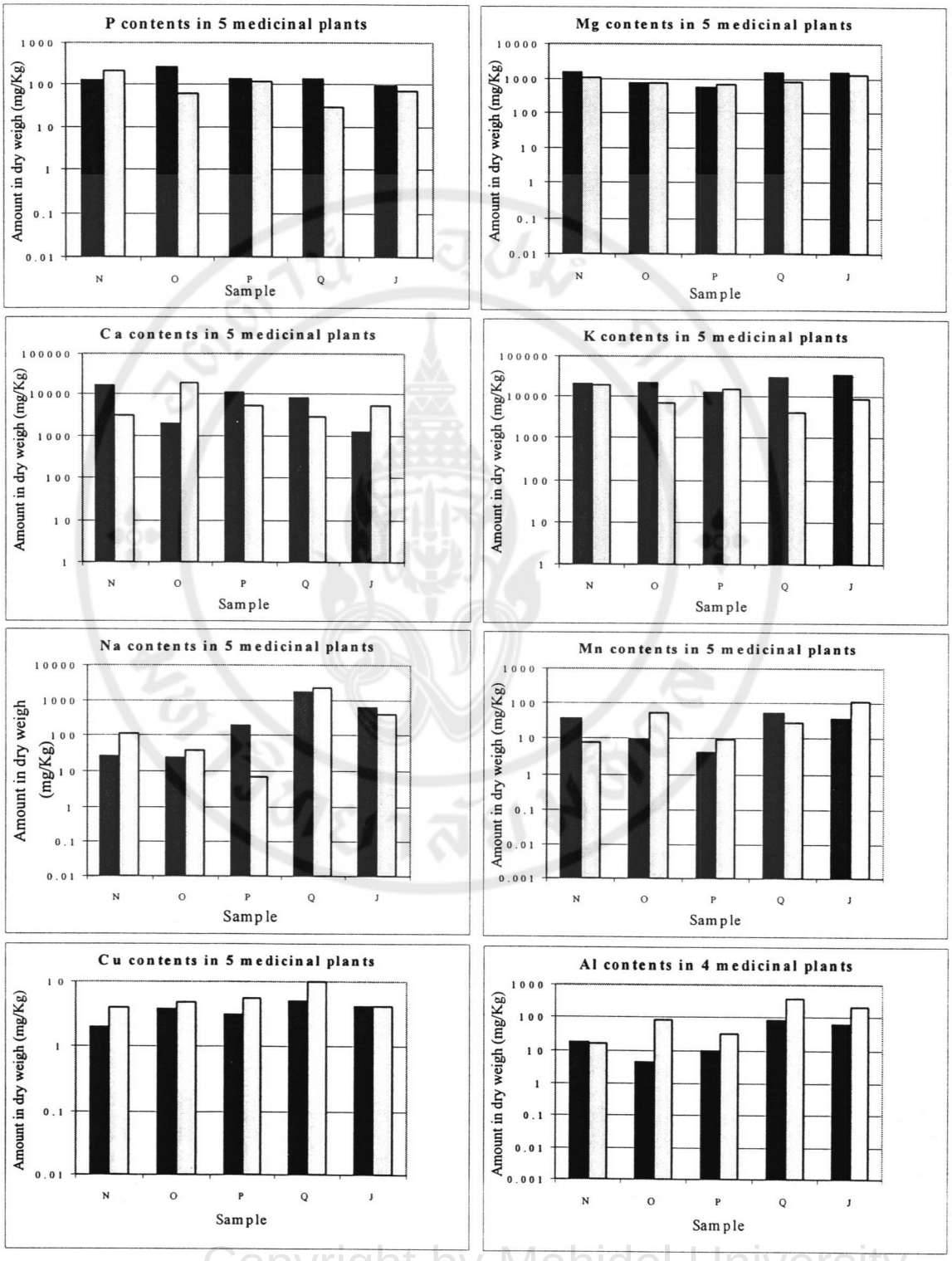


Figure 3.7 Comparison of mean concentrations of mineral contents in herbs and herb capsules (Sample; ■ = herbs, ⊡ = herb capsules).

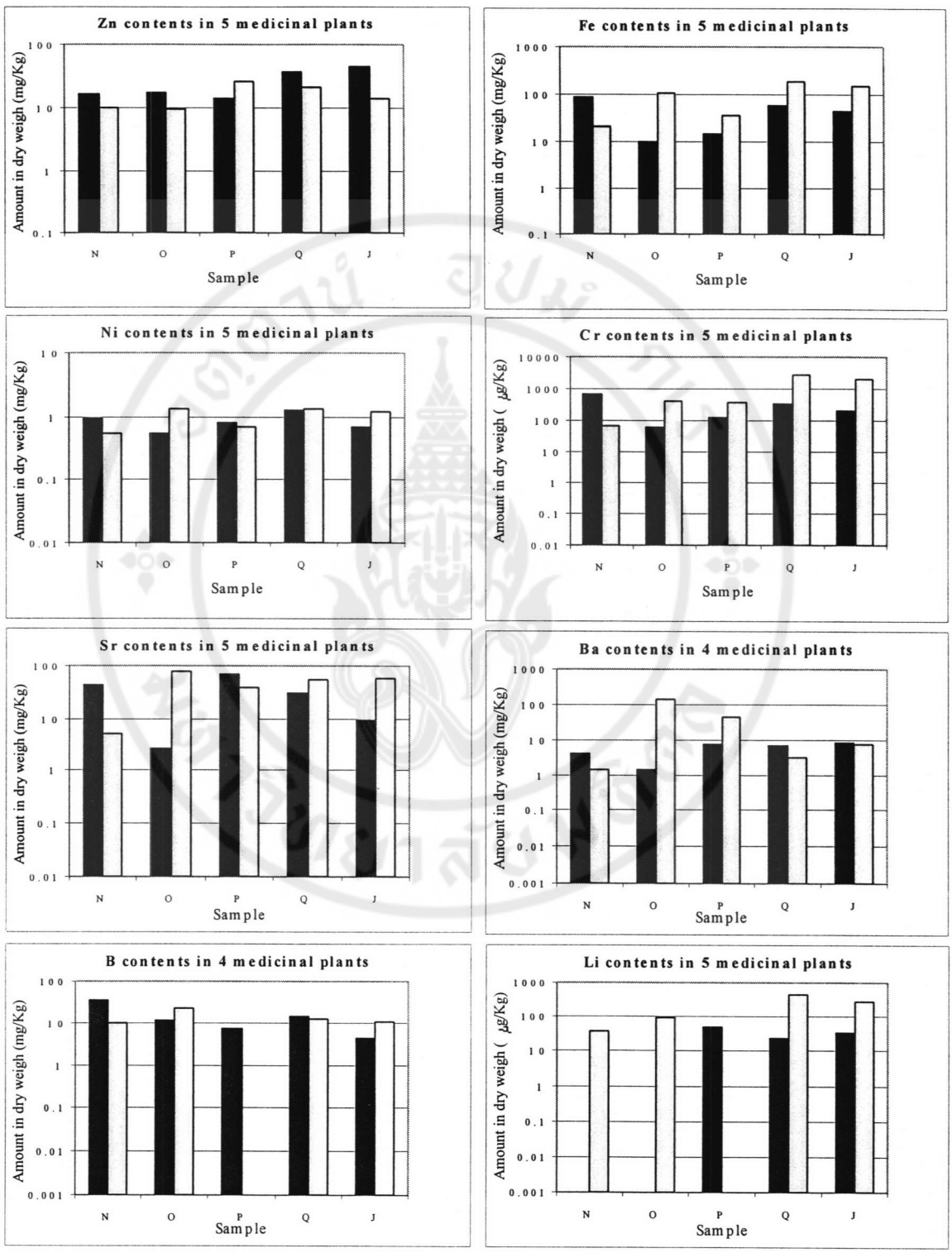


Figure 3.7 Comparison of mean concentrations of mineral contents in herbs and herb capsules (Sample;■ = herbs,⊡ = herb capsules). Some elements in same samples were not detectable and the bar graph was not seen. (continued).

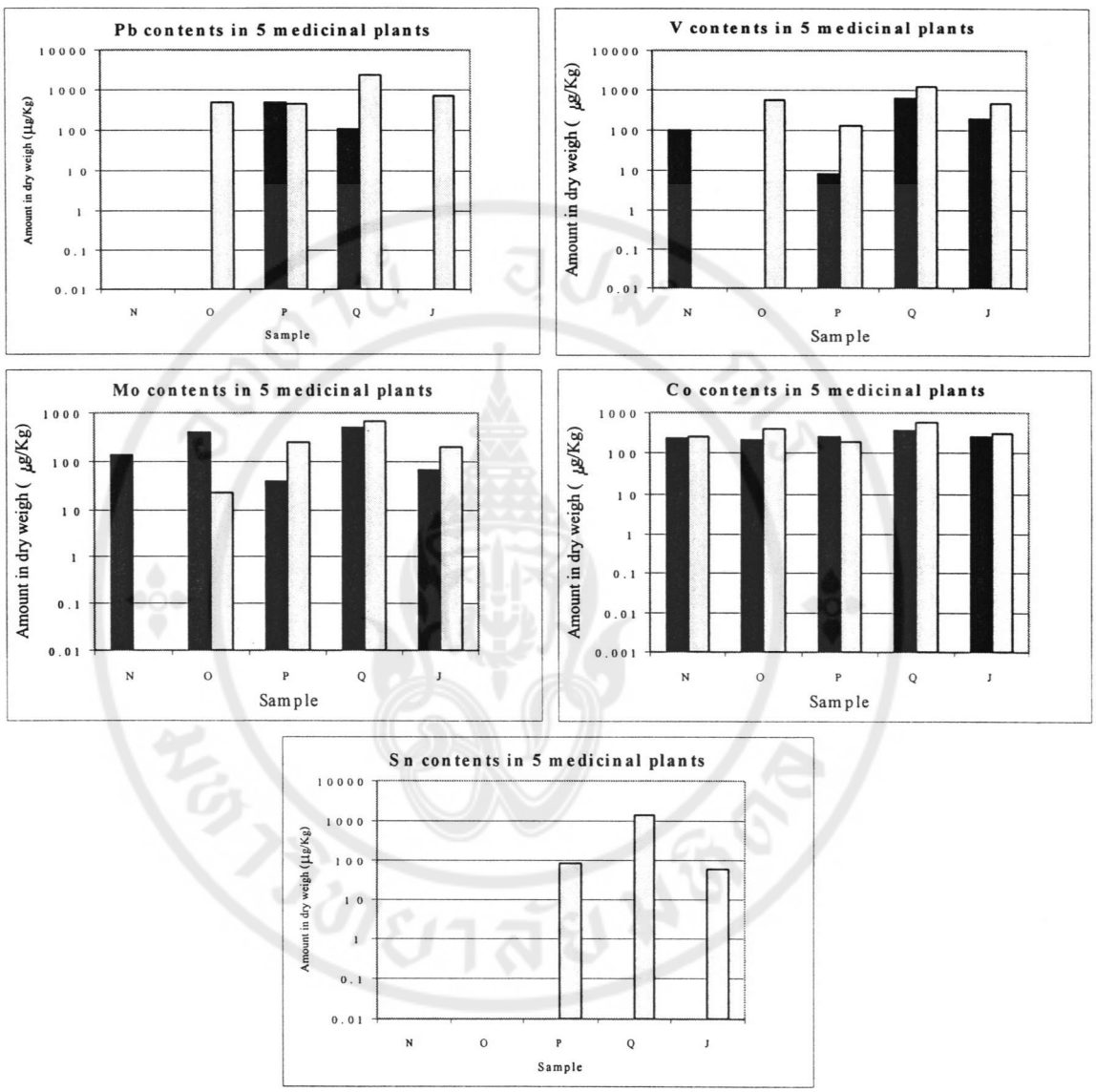


Figure 3.7 Comparison of mean concentrations of mineral contents in herbs and herb capsules (Sample; ■ = herbs,⊡ = herb capsules). Some elements in same samples were not detectable and the bar graph was not seen. (continued).

N = ใบสะเดา ; *Azadirachta indica (A.) Juss Var siamensis Valeton*

O = ใบขี้เหล็ก ; *Cassia siamea Lam.*

P = บอระเพ็ด ; *Tinospora crispa Miers ex Hook. F. & Thoms*

Q = เหงือกปลาหมอ ; *Acanthus ebracteatus Vahl*

J = ข่า ; *Alpinia galanga (L.) Swilld.*

CHAPTER IV

CONCLUSIONS

The results of the determination of 24 minerals in 17 Thai herbs and 5 herb capsules using the ICP-MS method can be summarized as follow:

The digestion procedures have been studied for plant samples. The results showed that, the microwave digestion of plant samples in concentrated HNO_3 and H_2O_2 gave lowest reagent blank values and minimal interference effect and therefore was selected. The digestion procedure is simple and rapid. The performance of ICP-MS was investigated as a function of a number of parameters associated with sample uptake rate and plasma operation. The RF power and the nebulizer gas flow rate are two parameters having the greatest sensitivity effect. 1.1 mL/min sample uptake rate, 1300 watts RF power and 0.95 L/min nebulizer gas flow rate were the optimum conditions obtained.

The sample solutions were analyzed by ICP-MS using optimal conditions. Indium was used as the internal standard in order to compensate for the difference in analyte signals resulting from sample preparation error and nebulization changing efficiency. The isobaric elemental interfernces were corrected by selecting suitable isotopes and applying the appropriate correction equations.

The accuracy of method was assessed by analysis of a certified reference material, SRM 1515 (Apple leaves). The analytical results have shown that twelve elements (Al, B, Ba, Co, Cu, Mg, Mn, Na, P, Pb, Sr and V) could be determined with good accuracy. Four elements (Mo, Ni, Sb and Zn) were slightly lower than the certified values probably due to incomplete digestion. As, Cr, Fe and Se gave extremely higher values than certified values, which may have suffered from positive polyatomic interferences from C, Ca and Cl containing species. Accurate measurement of ^{43}Ca was not accurate for inconsistency in blank intensities and ^{39}K was not possible because of interference come from the tail of strong ^{40}Ar signal. To avoid these problems, a FAAS method was used to measure Ca and K.

Isotope ratio measurement was also performed to confirm the polyatomic interferent problem for Cr, Fe and Se. The isotope ratios after mass bias correction of $^{53}Cr/^{52}Cr$, $^{54}Fe/^{57}Fe$ and $^{82}Se/^{77}Se$ were found to be -60.6, -63.4 and -63.9 % their natural ratios, respectively. This clearly indicated the interferent problem of Cr, Fe and Se measurement. Polyatomic interferences for As cannot be confirmed by isotope ratio measurement because As exists as one isotope.

A column with anion exchange resin was used to overcome the polyatomic interferent problem of As, Cr, Fe and Se. The interference was eliminated (for Cr and Fe) or reduced (for As and Se) by removing the existing anions from the digested sample solution before introducing the sample into the ICP-MS system. Upon treatment, acceptable recoveries were obtained for Cr and Fe but As and Se could not be determined with good accuracy although the results were improved.

The method developed was applied to analyze the mineral contents of 13 selected Thai herbs, collected from three locations (Kabin buri, Pinklao and Kingpetch), and 5 selected herbs and herb capsules. The elemental concentrations for 24 elements were given in Table 3.12-3.15. These data are considered to be useful for the study of dietary sources of essential elements and should enhance studies of the physiological significance of the essential and other trace elements in life process. For medicinal plants, these data will be useful in characterizing the plants. The known content of toxic elements warns the use of the herbs.

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APPENDIX I

Diagram of the ICP-MS, ELAN 6000 major sub-systems.

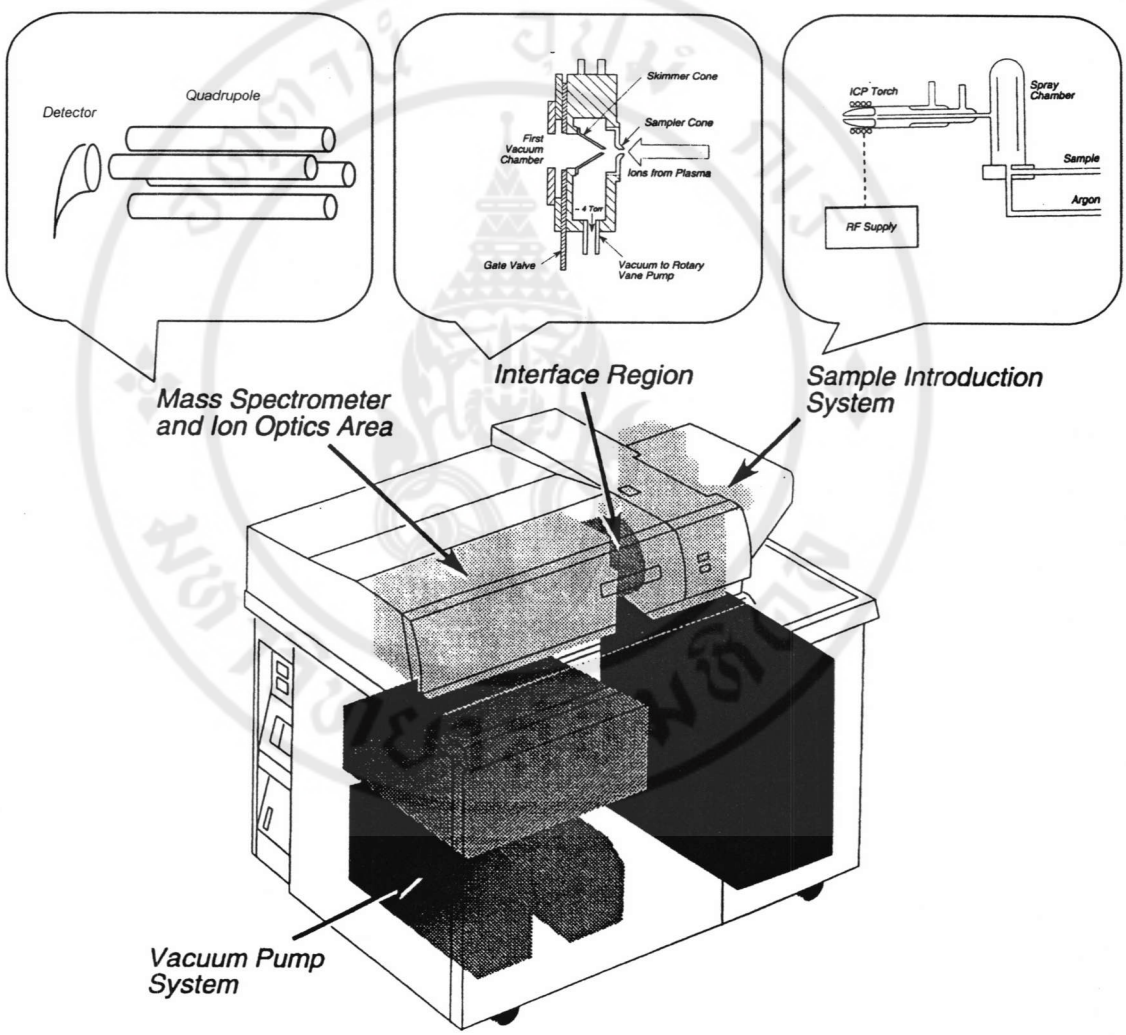

Figure 1A The ELAN 6000 major sub-system, sample introduction system, interface region, mass spectrometer and ion optics area.

APPENDIX II

Analysis of certified reference material using dry ashing digestion method

Table 1A Analytical results for the analysis of certified reference material, SRM 1515 (Apple leaves) using dry ashing digestion method

Element	Certified values (mg/Kg)	Found (mg/Kg)**
Al	286 ± 9	174.7 ± 3.2
B	27 ± 2	21.63 ± 0.70
Ba	49 ± 2	42.0 ± 0.52
Be	Not certified	0.012 ± 0.001
Cd	0.013± 0.002	Not Detectable
Co	0.09*	Not Detectable
Cu	5.64 ± 0.24	5.01 ± 1.0
Li	Not certified	0.135 ± 0.064
Mg	2710 ± 80	1937 ± 44
Mn	54 ± 3	46.5 ± 0.203
Mo	0.094 ± 0.13	0.162 ± 0.0061
Na	24.4 ± 1.2	24.2 ± 2.82
Ni	0.91 ± 0.12	1.54 ± 0.076
P	1590 ± 110	1100 ± 19.0
Pb	0.470± 0.024	0.561 ± 0.23
Sb	0.013*	0.109 ± 0.005
Sn	Not certified	0.050 ± 0.002
Sr	25 ± 2	19.93 ± 0.041
V	0.26 ± 0.03	0.191 ± 0.009
Zn	12.5 ± 0.3	11.5 ± 0.29

* Reference values

** Mean ± Standard deviation, (n=2)

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