MEASUREMENT OF SOME ESSENTIAL ELEMENTS IN INFANT FORMULAS, INFANT FOLLOW-ON FORMULAS AND FORTIFIED BABY FORMULAS USING ICP-OES

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ABSTRACT

MEASUREMENT OF SOME ESSENTIAL ELEMENTS IN INFANT FORMULAS INFANT FOLLOW-ON FORMULAS AND FORTIFIED BABY FORMULAS USING ICP-OES.

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Infancy is a period in which a rapid physical, biological, immunological, and mental growth and development occur. Providing appropriate nutritional resources for the infants are crucial for their healthy growth and development. Insufficient food intake or poor bio-availability may lead to certain deficiencies and dysfunctions. Major and trace elements have important effects and functions on human biology and genetics. As these elements are not produced in the body, they are taken by the foods

In the present study, it was aimed to decide, develop and validate the analysis methods for sodium, magnesium, phosphorus, potassium, calcium, iron and zinc in infant formulas, infant follow-on formulas and fortified baby formulas, and determine them using ICP-OES.

In the present study, microwave digestion, which is a quite effective method for digestion of food products, was used for sample preparation. The samples were analyzed using 2 different ICP-OES instruments. The accuracy of the method was

determined using BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011Wheat CRM (China) CRM. Analysis results of the CRMs were found in confidence interval of the certified value of the CRMs. Student t-test was applied at 95% confidence levels to check accuracy. It is concluded that there is no significant difference between the experimental values and certified values at 95% confidence level. Detection limits were found to be 15 μg/L, 39 μg/L, 45 μg/L, 24 μg/L, 3 μg/L, 3 μg/L and 3 μg/L for sodium, magnesium, phosphorus, potassium, calcium, iron and zinc respectively for ICP-OES 2100 DV. Detection limits were found to be 45 μg/L, 72 μg/L, 150 μg/L, 75 μg/L, 90 μg/L, 15 μg/L and 18 μg/L for sodium, magnesium, phosphorus, potassium, calcium, iron and zinc respectively for ICP-OES 5300 V.

The analyses results of the formulas found in the present study were compared with the boundaries specified by international authorities, national authorities and regulations; consistent results were obtained.

The analyses results of the formulas were also evaluated statistically using the Student's t-test. The results were compared with the reference values specified by the manufacturers and it was revealed that results were not significantly different at 95 % confidence level. In addition, the results of the same sample obtained in two different ICP-OES instruments were compared. There is a significant difference between the experimental and certified values at 95% confidence level. However, null hypothesis at 99% confidence level can be accepted through no significant difference between the certified and experimental values.

Keywords: ICP-OES, infant formula, Student's t test, Turkish Food Codex.

ÖZ

BEBEK MAMALARI, BEBEK DEVAM MAMALARI VE EK BEBEK MAMALARINDA BULUNAN BAZI TEMEL ELEMENTLERİN ICP-OES İLE ÖLÇÜMÜ

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Bebeklik fiziksel, biyolojik, bağışıklık, zihinsel, gelişmenin ve büyümenin çok hızlı bir şekilde gerçekleştiği dönemdir. Bu dönemdeki bebekler için uygun beslenme olanakları sağlamak, sağlıklı gelişmeleri ve büyümeleri için kritik derecede öneme sahiptir. Yetersiz beslenme ya da besinlerden biyoyararlanım oranının az olması vücut fonksiyonlarında bozukluklara neden olabilir. Temel elementler ve eser elementler insan genetiği ve biyolojisi üzerinde önemli etkilere ve fonksiyonlara sahiptir. Bu elementler vücutta üretilemediği için gıdalar aracılığı ile vücuda alınmaktadır.

Bu çalışmada, ilk 6 ay boyunca tüm beslenme ihtiyaçları anne sütü ile sağlanan bebeklerin beslenmesinde kullanılan bebek mamaları, bebek devam mamaları ile ek bebek mamalarında sodyum, potasyum, magnezyum, kalsiyum, fosfor, çinko, demir elementlerinin ICP-OES ile tayin yöntemlerinin belirlenmesi ve geliştirilmesi amaçlanmaktadır.

Çalışmada numunelerin hazırlanması için gıda ürünlerinde çok etkili bir yöntem olan Mikrodalga ile çözme yöntemi kullanılmıştır. Numuneler 2 farklı ICP- OES cihazı kullanılarak analiz edilmiştir. Yöntemin doğruluğu BCR-063R Yağsız Süt Tozu CRM (IRMM, EU) ve GBW 10011 Buğday Unu CRM (China) kullanılarak gösterilmiştir. Sertifikalı referans malzemelerin analiz sonuçları sertifika değerlerinde belirtilen güven aralığında bulunmuştur. Doğruluğu kontrol etmek için % 95 güvenirlilik seviyesinde Student t-test yapılmıştır ve elde edilen deney sonuçlarıyla sertifika değerlerinin birbirinden farklı olmadığı tespit edilmiştir. ICP-OES 2100 DV cihazıyla yapılan analizlerde sodyum, magnezyum, fosfor, potasyum, kalsiyum, demir ve çinko için tayin sınırı sırasıyla 15 µg/L, 39 µg/L, 45 µg/L, 24 µg/L, 3 µg/L, 3 µg/L and 3 µg/L bulunmuştur. ICP-OES 5300 V cihazıyla yapılan analizlerde sodyum, magnezyum, fosfor, potasyum, kalsiyum, demir, çinko, için tespit limiti sırasıyla 45 µg/L, 72 µg/L, 150 µg/L, 75 µg/L, 90 µg/L, 15 µg/L and 18 µg/L olarak bulunmuştur.

Bebek mamalarının analizi ile elde edilen sonuçlar uluslararası ve ulusal otoriteler ve mevzuatlar tarafından belirlenen limitlerle karşılaştırılmış ve yasal limitlere uygun değerler elde edilmiştir.

Bebek mamalarının analizi ile elde edilen sonuçlar üreticilerin beyan ettikleri referans değerlerle Student's t test kullanılarak istatistiksel olarak karşılaştırılmış ve % 95 güvenirlilik seviyesinde farklı olmayan sonuçlar elde edilmiştir. Ayrıca çalışmada 2 farklı ICP-OES cihazından elde edilen sonuçlar birbirleriyle karşılaştırılmış ve % 99 güvenirlilik seviyesinde farklı olmayan sonuçlar elde edilmiştir.

Anahtar Kelimeler: ICP-OES, bebek maması, Student's t test, Türk Gıda Kodeksi.

Dedicated to my family (mine & yiğit ozan)

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ABBREVIATIONS

$[Ca10(PO4)_6(OH)_2]$	Hydroxyapatite
ADP	Adenosine Diphosphate
AES	Atomic Emission Spectrometry
AI	Adequate Intakes
ATP	Adenosine Triphosphate
CTD	Charge-Transfer Device
DCP	Direct Current Plasma
DNA	Deoxyribonucleic Acid
DRIs	Dietary Reference Intakes
EIE	Easily Ionizable Elements
FAAS	Flame Atomic Absorption Spectrometry
FES	Flame Emission Spectrometry
GD	Glow Discharge
GFAAS	Graphite Furnace Atomic Absorption Spectrometry
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectrometry
LDR	Linear Dynamic Range
LOD	Limit of Detection
LOL	Limit of Linearity
LOQ	Limit of Quantification
MIP	Microwave Induced Plasma

MW	Microwave
NFT	Nutritional Facts Table
PFA	Perfluoroalkoxy Vinyl Ether Teflon
PTFE	Polytetrafluoroethylene
RDA	Recommended Dietary Allowances
RNA	Ribonucleic Acid
RSD	Relative Standard Deviation
TSE	Türk Standardları Enstitüsü
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Nutrition constitutes one of the most important components of human life cycle in the first year of life as it is a sensitive period in the human development of the nervous, reproductive, digestive, respiratory and immune systems **(Pandelova et al., 2012).** Nutrition begins in the mother's womb and continues throughout the life and malnutrition can lead to poor quality of life, diseases, and even mortality in advanced stages.

Elements are of particular importance in the nutrition of infants. Since elements are not produced in the human body, humans should obtain the essential elements through their diets. Elements play several roles in various body functions including mineralization of the bones, enzymatic reactions, hormone secretion, and protection of cells and lipids in biological membranes **(Schlenker and William, 2003; Taylor et al., 2004)**. Deficiency of dietary elements causes physiological and structural abnormalities that are preventable and likely to be reversible with administration of elements **(Gibson and Hotz, 2000; Melo, et al., 2008).**

The main mineral source in the first six months of infants' life is the breast milk. As a primary nutrient source, milk and infant formulas have an important part in infant and child nutrition **(Lesniewicz et al., 2010, Ikem et al., 2002)**. As milk is composed of water, macronutrients (fats, carbohydrates and proteins) and micronutrients (vitamins and minerals), it is almost a complete food product **(Mc Cance, 1993)**.

Breastfeeding has been recommended as the best choice of feeding of babies by the World Health Organization (WHO) **(WHO, 2008),** and human milk, which is the natural and optimal nutrient source for infants, cannot be completely replaced by commercially manufactured infant formula **(Bocca et al., 2000; Rossipal and Krachler, 1998; Wappelhorst et al., 2002)**. However, with its importance in infants' diet, infant formulas are an alternative to breast-milk particularly **(Pandelova et al., 2012)** in the absence or insufficiency of breastfeeding.

Most available formulas are composed of the essential elements of milk to satisfy nutritional requirements **(Goldhaber, 2003).** Thus, analyzing and confirming the chemical as well as the elemental composition of the commercial milk formulas are of importance to verify the nutritional properties and to control the levels of potentially toxic elements **(Lesniewicz et al., 2010).**

1.1. Infant Formula

Infant formula is a liquid thermal processed long-lasting product or a powdered or a granulated product prepared by adding water before consumption which satisfies nutritional requirements of healthy and normal weight infants until the first 4 to 6 months and is manufactured close to the breast milk in terms of composition by mixing proteins, fat, carbohydrates and, preservatives using appropriate technology in order to provide normal growth and development **(TSE, 2006).**

1.2. Infant Follow-on Formula

Infant follow-on formula that constitute mainly the liquid intake of gradually varying diets of babies beginning from 6 months, unless another month is recommended depending on the growth and development requirements of baby by an independent

healthcare worker specialized only in maternal and children nutrition for special nutrition **(Turkish Food Codex, 2009)**.

1.3. Fortified Baby Formula

Fortified Baby Formulas basically made up of one or more crushed cereals and/or legumes and/or plants consisting starch in their root or trunk, which are used as complementary foods to breast milk or infant formulas or follow-on formulas or to accustom young children to their daily diet in their advanced ages **(Turkish Food Codex, 2007).**

1.4. Chemical Properties of the Formulas

Baby formulas are mainly composed of proteins, fats, carbohydrates, lactose, vitamins, elements, food additives (e.g., stabilizers, emulsifiers, acidity regulators, antioxidants), nucleotides and amino acids. General chemical composition of the baby formulas including proteins, fats, carbohydrates, and lactose are presented in the **Table 1.1**.

Allowed quantity of the elements in the infant formulas are presented the **Table 1.2** and the allowed compounds of these elements are presents **Table 1.3**.

Table 1.1 Chemical composition of the infant formulas (adapted from TS 11983, TSE)

Properties	The mass of formula (in g) that provides			
	100 kcal		100 kJ	
	Lowest	Highest	Lowest	Highest
Proteins				
The formulas that are made from cow's milk proteins[1]	1.80	3.00	0.43	0.72
The formulas that are made from partially hydrolysed proteins[2]	1.80	3.00	0.43	0.72
The formulas that are made from soy protein isolates or mixture of soy protein isolates and cow's milk[3]	2.25	3.00	0.54	0.72
Fats[4]	4.40	6.00	1.05	1.43
Carbohydrates[5]	9.00	14.0	2.15	3.35
Lactose[6]	4.50	-	1.08	-

1. The mass of the proteins of cow's milk = the mass of N in the cow's milk x 6.38 and serum protein / casein ratio have to be 1.
2. The mass of the proteins for soy protein isolates and partially hydrolyzed proteins = the mass of N x 6.38.
3. Only soy protein isolates can be used in this formula.
4. Fat content of the formula cannot contain sesame oil and cottonseed oil.
5. The mass of the saccharose cannot be more than 20 % of the total carbohydrates.
   The starch in the formula cannot be more than 2 g/100 mL and cannot be more than 30 % of the total carbohydrates.
6. If the soy proteins are more than 50 % in the total proteins, there is no restriction for lactose content.

1 cal = 4.184 j

The average mass of the infant formulas used in the present study that provides 100 kcal is 20 g.

Table 1.2 Allowed mass of the elements in the infant formulas (adapted from TS11983, TSE)

Elements	The mass of formula that provides			
	100 kcal		100 kJ	
	Lowest	Highest	Lowest	Highest
Sodium, mg	20	60	4.8	14.3
Potassium, mg	60	160	14.3	38.2
Chloride, mg	50	160	12.0	38.2
Calcium[1]**,** mg	50	140	12.0	33.5
Phosphorus, mg	25	90	6.0	21.5
Magnesium, mg	5.0	15	1.2	3.6
Iron[2]**,** mg	0.5	1.5	0.12	0.36
Iron[3]**,** mg	1.0	2.0	0.24	0.48
Iodine, μg	10	50	1.2	12.0
Copper, μg	35	100	8.4	24.0
Zinc[4]**,** mg	0.5	1.5	0.12	0.36
Zinc[5], mg	0.75	2.4	0.18	0.60
Manganese, μg	1.0	100	1.2	24.0
Selenium[6]**,** μg	1.0	9.0	1.2	2.2

1. Calcium/Phosphorus ratio shall be between 1.2 and 2.0.
2. The iron mass in which the formulas that made of cow's milk protein and fortified with iron. The fortified iron has to be iron (II).
3. The iron mass in which the formulas that made of soy protein or soy protein and cow's milk protein mixture.
4. The zinc mass in which the formulas that made of cow's milk protein and fortified with iron.
5. The zinc mass in which the formulas that made of soy protein or soy protein and cow's milk protein mixture.
6. Only applied for the selenium fortified formulas.

1 cal = 4.184 j

The average mass of the infant formulas used in the present study that provides 100 kcal is 20 g.

Table 1.3 Compound sources of the elements in the infant formulas (adapted from TS11983, TSE)

Elements	Allowed Structure	
Calcium	Calcium Carbonate	Calcium Glycerophosphate
	Calcium Chloride	Calcium Lactate
	Calcium Citrate	Calcium Salts of the O-phosphoric Acid
	Calcium Gluconate	Calcium Hydroxide
Magnesium	Magnesium Carbonate	Magnesium Gluconate
	Magnesium Chloride	Magnesium Salts of the O-phosphoric Acid
	Magnesium Oxide	Magnesium Hydroxide
	Magnesium Citrate	Magnesium Sulphate
Iron	Iron (II) Citrate	Iron (III) Ammonium Citrate
	Iron (II) Gluconate	Iron (II) Fumarate
	Iron (II) Lactate	Iron (III) Diphosphate
	Iron (II) Sulphate	Iron (III) Pyrophosphate
Copper	Copper Citrate	Copper Lysine Complexes
	Copper Gluconate	Copper Carbonate
	Copper Sulphate	
Iodine	Potassium Iodide	Sodium Iodide
	Potassium Iodate	
Zinc	Zinc Acetate	Zinc Citrate
	Zinc Chloride	Zinc Gluconate
	Zinc Lactate	Zinc Oxide
	Zinc Sulphate	
Manganese	Manganese Carbonate	Manganese Sulphate
	Manganese Chloride	Manganese Gluconate
	Manganese Citrate	
Sodium	Sodium Bicarbonate	Sodium Carbonate
	Sodium Chloride	Sodium Lactate
	Sodium Citrate	Sodium Salts of the O-phosphoric Acid
	Sodium Gluconate	Sodium Hydroxide
Potassium	Potassium Bicarbonate	Potassium Carbonate
	Potassium Chloride	Potassium Lactate
	Potassium Citrate	Potassium Salts of the O-phosphoric Acid
	Potassium Gluconate	Potassium Hydroxide
Selenium	Selenium Selenate	Selenium Selenite

1.5. Elements

All elements required for health are essential nutrients as they cannot be produced in the body from other compounds. Essential elements are classified based on the mass required by the body as major elements (required in mass greater than 100 mg/day) or trace elements (required in mass less than 100 mg/day). Calcium, phosphorus, magnesium, sodium, chloride and potassium are six major elements that are required by the body. In addition, the body requires at least eight trace elements, which are vital for health and are therefore essential nutrients, including iron, copper, iodine, selenium, chromium, manganese, molybdenum, and zinc **(Mc Guire and Beerman, 2013)**.

Table 1.4 General characteristics of major elements (McGuire and Beerman, 2013)

Food Sources	Seafood, meat and dairy products tend to be the best source Vegetables and legumes are sometimes good sources Whole-grain products are better sources than milled products
Digestion	Very little needed
Absorption	Occurs mostly in small intestine but sometimes also in large intestine Bioavailability sometimes influenced by nutritional status and interactions with other dietary components
Circulation	Circulated from the small intestine to the liver in the blood, and then to the rest of the body
Functions	Many are cofactors for enzymes, some of which are involved in energy metabolism Some have major structural roles such as maintaining bone and tooth health Involved in nerve and muscle function Electrolytes involved in fluid balance
Toxicity	Rare and usually associated with excess supplemental or medicinal intake

Table 1.5 General characteristics of trace elements (McGuire and Beerman, 2013)

Food Sources	Amount often depends on mineral content of soil Found in all food groups Whole-grain products tend to contain more than refined cereal products Amount not influenced by cooking
Digestion	Very little needed
Absorption	Occurs mostly in small intestine but also in stomach Bioavailability sometimes influenced by form nutritional status and interactions with other dietary components
Circulation	Via blood from the gastrointestinal tract to the liver and throughout the body
Functions	Cofactor for enzymes, some of which are involved in redox reactions Components of non-enzymatic proteins Structural roles (such as mineralization)
Toxicity	Rare; generally associated with genetic disorders, excessive supplement intake, or environmental exposure

1.5.1. Sodium and Functions of Na in the Body

Sodium (Na) is a soft, silvery-white, highly reactive metal belonging to alkali metals family, and has an atomic number of 11. Sodium acts a major role in fluid balance. In the body, water naturally moves to regions that are highly concentrated in Na^+ and/or Cl^-; thus, the body provides the maintenance of fluid balance by selectively moving these electrolytes to regions requiring more water **(Mc Guire and Beerman, 2013)**. In addition to its role in body fluid balance, Na is of importance in nerve function and muscle contraction, in both of which potassium (K^+) also play a role. Moreover, Cl is also required in several body functions such as production of hydrochloric acid (HCl) in the stomach, elimination of carbon dioxide (CO_2) by lungs, and optimal immune function **(Mc Guire and Beerman, 2013)**.

On the other hand, high intakes of NaCl may lead to elevated blood pressure, a major risk factor for heart disease and stroke **(Conlin, 2005, Meneton et al., 2005, Jaitovich and Bertollo, 2010, Sanada et al., 2011)**.

1.5.2. Magnesium and Functions of Mg in the Body

Magnesium (Mg) is an alkaline earth metal with an atomic number of 12 and oxidation number of +2. The majority of Mg in the body plays a role in the bone metabolism, particularly in formation of bone structure **(Mc Guire and Beerman, 2013)**. Mg, which is found in the body in the form of Mg^{2+}, is of vital for energy metabolism. With its positive charge, Mg can be used for stabilizing the enzymes and neutralizing the negatively charged ions. Its substrates include ATP and ADP that are typically found as negatively charged ATP^{2-} and ADP^{3-}. The role of Mg in the stabilization of these high energy compounds shows its importance in the energy metabolism **(Mc Guire and Beerman, 2013)**. Overall, Mg takes part in more than 300 chemical reactions, particularly those involved in DNA and RNA metabolism, and affects nerve and muscle function, particularly in cardiac tissue **(Bo and Pisu, 2008, Gums, 2004).**

In cases of Mg deficiency, abnormal nerve and muscle function may be observed, particularly in cardiac tissue. Accordingly, it is considered that risk for cardiovascular diseases, type 2 diabetes mellitus and even migraine headaches may be increased due to mild Mg deficiency **(Alghamdi et al., 2005, Belin and He 2007, Conlin, 2005, Meneton et al., 2005, Jaitovich and Bertorello 2010, Sanada et al., 2011).**

1.5.3. Phosphorous and Functions of P in the Body

Phosphorus (P) is a multivalent nonmetal of the nitrogen group with atomic number of 15. Being an important component of phospholipids, P has numerous structural and functional roles. P is required for the structure of biological membranes and acts in the transportation of lipids in the body through lipoproteins. Additionally, P is also essential for the protein synthesis and energy metabolism as it is a component of genetic material (DNA) and ATP **(Mc Guire and Beerman, 2013)**. Phosphorus-containing compounds also have a role in the maintenance of blood pH (acid-base balance) by acting as buffers accepting and donating hydrogen ions**.** In the body, P is involved in numerous metabolic reactions, in which "phosphorylated" molecules are produced through transformation of phosphate groups from one molecule to another. Phosphorylation leads some molecules to be activated while some others to be inactivated. Besides, phosphorus together with Ca is also needed to form hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], which is the mineral matrix forming the bones and teeth. Deficiency in P results in appetite loss, anemia, muscle weakness, and poor bone development.

On the other hand, its toxicity causes soft tissue mineralization, particularly renal tissues **(Mc Guire and Beerman, 2013)**.

1.5.4. Potassium and Functions of K in the Body

Potassium (K) is a soft silvery-white alkali metal with an atomic number of 19. K cations have important role in neuron function, brain and nerve functions, and influences osmotic balance between cells and the interstitial fluid. K cations act on osmotic balance through the Na^+/K^+-ATPase pump, which mediates the distribution of potassium cations in all animals (but not in all plants) **(Campbell, 1987)** The Na^+/K^+-ATPase pump creates an electrochemical gradient over the cell membrane

through a process called as Na-K Pump in which three sodium ions are pumped out of the cell and two potassium ions are pumped into the cell with the use of ATP.

K deficiency leads to muscle weakness, constipation, irritability and confusion, and there are also some studies suggesting that it is associated with insulin resistance **(Colussi et al., 2007, Mc Carty et al., 2005)**. Irregular heart rhythms, muscular weakness, decreased blood pressure, and difficulty in breathing are also observed due to potassium deficiency in severe cases. **(Mc Guire and Beerman, 2013)** Although K deficiency is not frequently observed; heavy use of diuretics may lead to excessive urinary K loss. In addition, excretion of excessive amounts of water in the body also leads to electrolyte loss. This can cause hypokalemia, i.e. low blood K **(Mc Guire and Beerman, 2013)**.

On the other hand, high levels of K in the blood can be life-threatening by causing some conditions such as cardiac arrest and potassium toxicity **(Kallen et al., 1976).** Less severe symptoms associated with high levels of K in the blood include tingling of the feet and hands and muscular weakness **(Mc Guire and Beerman, 2013)**.

1.5.5. Calcium and Functions of Ca in the Body

Calcium (Ca) is a soft gray alkaline earth metal with an atomic number of 20. Being one of the components of hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], a large crystal-like molecule, Ca acts in the structural formation of bones and teeth. Combining with other minerals including fluoride and magnesium, hydroxyapatite forms the structural matrix of bones and teeth and provides a storage depot for Ca **(Mc Guire and Beerman, 2013)**.

In addition to its role in the maintenance of skeleton structure, Ca regulates several other activities in the body such as stimulating blood clot formation together with vitamins, facilitating muscle contractions, transmission of nerve impulses, providing healthy vision, regulating blood glucose, and cell differentiation **(Chin, 2005, Mihayli, 2004, Thorneloe and Nelson, 2005, Barnes and Kelly, 2004, French and Zamponi, 2005, Senin et al., 2002).**

Ca is not directly involved in energy metabolism; however, it plays as a cofactor for several enzymes required for energy metabolism. Moreover, researchers are also recently investigating the additional roles of Ca in the body; for instance, there is increasing evidence that risk of cardiovascular disease and some forms of cancer may be reduced by adequate Ca consumption **(Alonso et al., 2009; Manios et al., 2009; Wilkinson et al., 2007; Zemel et al., 2009)**.

Adequate Ca intake is of crucial throughout the life cycle. Ca deficiency in children may lead to a disease called rickets, which is more frequently resulted from vitamin D deficiency. Poor bone mineralization and characteristic "bowed" bones, particularly, in the legs are observed in children with rickets. In adults, a calcium-poor diet can result in osteopenia, a moderate loss of bone mass, and even osteoporosis, a severe bone loss **(Mc Guire and Beerman, 2013)**. A low Ca level in blood can result in muscle pain and spasms, as well as a tingling sensation in the hands and feet. More serious Ca deficiency leads muscles to tighten and become unable to relax, i.e. it causes a condition called tetany **(Mc Guire and Beerman, 2013)**.

On the other hand, overconsumption of Ca may cause its deposition in soft tissues, such as muscle and kidney. High intake of Ca is also related to impairments in kidney function, formation of kidney stones, and it can hinder the bioavailability of some other nutrients including iron and zinc **(Hallberg et al., 1992).**

1.5.6. Iron and Functions of Fe in the Body

Iron (Fe) is a metal in the first transition series with an atomic number of 26. Transportation of O_2 and CO_2 through hemoglobin is the most important function of the Fe in the body. Hemoglobin, the most abundant protein in erythrocytes, is composed of four protein subunits, namely globins, and four iron-containing heme groups. During their circulation through the blood vessels, erythrocytes come in contact with O_2 in the lungs, and then O_2 attaches to Fe atoms in hemoglobin. Thereafter, oxygenated blood circulates from the lungs to the heart, where it is pumped to other tissues in the body, Thus, hemoglobin transports the O_2 to the cells for aerobic energy metabolism. After hemoglobin releases the O_2, it binds to CO_2, and thus deoxygenated, carbon dioxide-rich blood is transported to the lungs and it is eliminated upon exhalation **(Mc Guire and Beerman, 2013)**. O_2 reservation via myoglobin, another iron-containing heme protein, is another function of the Fe. Unlike hemoglobin, myoglobin is found in muscle cells. Acting as an O_2 reservoir, myoglobin releases O_2 to muscle cells when they require to produce ATP for physical activity **(Mc Guire and Beerman, 2013)**.

In addition to its role in O_2 transport to cells, Fe has also important roles in the energy metabolism. Cytochromes, heme-containing protein complexes, function in the electron transport chain and acts as electron carriers. Accordingly, being basic component of the cytochromes, Fe acts indirectly in the conversion of adenosine diphosphate (ADP) to adenosine triphosphate (ATP) and in water production **(Mc Guire and Beerman, 2013)**. Fe also serves as a cofactor for several nonheme-containing enzymes participating in electron transport chain, citric acid cycle and gluconeogenesis **(Mc Guire and Beerman, 2013)** and for various enzymes that play roles in the metabolism of drugs and removal of toxins, i.e. cytochrome P450 enzymes. Activity of cytochrome P450 enzymes is considered to affect risk for many chronic diseases such as cancer and cardiovascular diseases **(Coon, 2005; Masson et al., 2005)**. Fe as a cofactor has also several additional important roles. Being a cofactor of antioxidants enzymes, hey act in stabilizing the free radicals, in

protecting DNA, cell membranes, and proteins from oxidative damage. Fe is also vital for optimal growth and development as it is a cofactor for an enzyme required for DNA synthesis **(Mc Guire and Beerman, 2013)**.

As requirement for Fe increases during growth and development, infants, growing children, teenagers and pregnant women are more typically suffer from to Fe deficiency. Pica, the eating of nonnutritive substances such as dirt, and clay, is considered to be associated with Fe deficiency **(Young, 2010).** Being most common in pregnant women, the rate of pica disease has been reported to be seen between 8% and 65% of pregnant **(Young, 2011)**.

Impaired Fe status leads to fatigue and impaired physical work performance and has effects on behavior and intellectual abilities in children **(Best et al., 2011)**. Impairment of body temperature regulation may also be caused by mild Fe deficiency **(Rosenzweigh and Volpe, 1999**). Mild Fe deficiency may negatively affect the immune system in cold conditions **(Cunningham-Rundles et al., 2005; Wang et al., 2010).** In pregnancy, mild Fe deficiency has been suggested to be associated with an increased risk of premature delivery, low birth weight, and maternal mortality **(Gambling, 2003)**. In case of severe Fe deficiency, microcytic hypochromic anemia, which is characterized by small, pale red blood cells, occurs when the body is unable to produce enough heme, and thereby hemoglobin. Fatigue, difficulties in mental concentration and compromised immune function are among the signs and symptoms of this condition, as in other types of anemia **(Mc Guire and Beerman, 2013)**.

Excess Fe is deposited in soft tissues such as the liver, heart and muscles and impairs their function. Excessive Fe supplementation has been suggested to cause cardiovascular disease and some forms of cancers by researchers **(Ganz and Nemeth, 2011)**.

1.5.7. Zinc and Functions of Zn in the Body

Zinc (Zn), the first element of group 12 of the periodic table, is a metallic chemical element with an atomic number of 30. In addition to being a cofactor for more than 300 enzymes in the body, Zn is also known to be required for growth, reproduction, immunity, gene expression and protein synthesis. However, mechanisms by which it influences human health are still being investigated. Hundreds of biological activities require Zn as a cofactor. For instance, enzymes participating in RNA synthesis and alcohol metabolism require Zn. Zn is also used to stabilize the structure of many proteins regulating gene expression. These proteins contain zinc fingers, three-dimensional structure, which turn on and off specific genes and regulates transcription. These proteins cannot function without Zn. Zinc fingers are of importance for the initiation of protein synthesis critical to cell maturation, growth and immune function **(Prasad, 2009)**. Zn also serves as a potent antioxidant **(Eibl et al., 2010, Powell, 2000)** and appears to be important in stabilization of cell membranes.

Zn supplements are often recommended to cure the common cold. Although this claim is not supported by some investigators, Zn was concluded to be effective in common cold, particularly in children, in a recent systematic review **(Caruso et al., 2007, Kurugöl et al., 2007, Kurugöl et al., 2006, Singh and Das 2011).**

A defect in the protein transporting Zn into enterocytes results in a genetic abnormality called acrodermatitis enteropathica. As a result of this condition, a lower amount of Zn is transported into the enterocyte, Zn is lost in the feces, all of which in turn leads to secondary Zn deficiency **(Mc Guire and Beerman, 2013)**. Proper growth is not observed in babies with acrodermatitis enteropathica; in these babies, the disease is characterized by severely red and scaly skin, particularly around the scalp, eyes, and feet **(Kury et al., 2002, Wang et al., 2010)**.

In humans, Zn deficiency is first documented in the 1968s in Egypt and Iran, where plant-based diets high in phytates inhibiting zinc absorption are common **(Hambidge, 2000).** Mild Zn deficiency is associated with decrease in appetite; accordingly, it increases illness and reduces growth, particularly in children, whereas severe Zn deficiency results in skin irritation, diarrhea and delayed sexual maturation **(Mc Guire and Beerman, 2013)**.

On the other hand, excess Zn is associated with poor immune function, depressed levels of high density lipoprotein cholesterol, as well as impaired copper status **(Mc Guire and Beerman, 2013)**.

1.6. Methods for Determination of Elements

All materials including foods contain elements ranging in concentration from percentage levels to trace and ultra-trace levels **(Dean, Ando, 1997).**

There have been many techniques that are used in the elemental analysis of many matrices. These techniques include UV-VIS spectrometry, polarimetry, stripping voltammetry, X-ray fluorescence, neutron activation analysis, capillary zone electrophoresis, and complexometry **(Tolg, 1987, Skurikhin, 1989, Lavi and Alfassi, 1990, Bersier et al., 1994, Pretswell et al., 1995)**, several of which have been used mainly for trace element speciation **(Florence, 1986),** and for the collaborative certification of international reference milk powders **(Griepink et al., 1984).**

Atomic spectrometry has been established as the keystone for trace element analysis in foods, with flame atomic absorption spectrometry (FAAS), graphite furnace atomic absorption spectrometry (GFAAS), and flame emission spectrometry (FES)

are being the predominant techniques in use**.** However, the development of inductively coupled plasma-optical emission spectrometry (ICP-OES) including charge-transfer device (CTD) detectors has provided an opportunity for the development of a flexible, user-programmable, simultaneous multi-elemental capability and facilitated elevated spectral information and sample throughput **(Mc Kinstry, 1999)**. ICP-OES is a feasible alternative to the above-mentioned techniques since CTD has lower detection limits, higher quantum efficiency, wider linear dynamic range (LDR) and inherent simultaneity as compared with photomultiplier detectors **(Hanley et al., 1996)**. Moreover, inductively coupled plasma - mass spectrometry (ICP-MS) is very popular technique for trace element analysis in foods since has very low detection limits and speed.

There exist many analytical figures of merit in element determination including calibration, detection limit and quality control. Accuracy, precision, LOD and LOQ are important analytical figures of merit in the determination of trace elements in food samples **(Şenol, 2010**; **Dean and Ando, 1997)**.

1.7. Sample Treatment

Sample preparation is not only remained to be the major limiting step **(Mc Kinstry, 1999)** but is also probably the most neglected area in analytical chemistry **(Dean, Ando, 1997)**. Nevertheless, sample treatment is an important step for the separation of the analyte from the matrix and for avoiding matter that is likely to be react with metal ions or chemical reagents and interfere with analyte during measurements **(Şenol, 2010).**

Although trace element pre-concentration (e.g., chromatography, solvent extraction and co-precipitation) can be used in sample treatment, dry ashing, wet ashing and Microwave digestion are the most commonly used methods for the sample treatment of food matrix.

1.7.1. Dry Ashing

The organic matter is digested in the matrix by ashing, in which the sample is heated in a muffle furnace in the presence of air at 400-800^{o}C **(Dean, Ando, 1997)**. In dry ashing, after weighing fresh or dried sample in suitable crucibles, the sample is placed in the furnace **(Şenol, 2010)** and temperature is increased up to 400-800 ^{0}C according to type of the sample matrix, and a heating programme is followed. After decomposition of the ash, i.e. inorganic residue, it is dissolved in suitable acid or acid mixture and transferred to a flask before analyses **(Dean, Ando, 1997)**.

Complete destruction and elimination of the organic matter is the major advantage of dry ashing **(Şenol, 2010).** On the other hand, the disadvantages of the method include the loss of volatile elements such as mercury, lead, cadmium, calcium, arsenic, antimony, chromium and copper **(Dean, Ando, 1997),** reactions of the analyte with the crucible material, and sample contamination from combustion residues **(Şenol, 2010)**.

1.7.2. Wet Ashing

Wet ashing, which is a decomposition method using concentrated acids [HCl, H_2SO_4, HNO_3, $HClO_4$, HF and mixture of HNO_3/HCl (1:3)] with often assistance of H_2O_2 is carried out using open or closed vessels depending on the nature of the heat source. Heat sources may include conventional ways (electrical and gases) or Microwave

(MW) **(Dean, Ando, 1997)**. The major disadvantages of wet ashing are the loss of the element species by volatilization with the use of open vessels for digestion **(Dean, Ando, 1997)** and requirement of constant operator attention due to possible occurrence of contamination **(Şenol, 2010)**.

1.7.3. Microwave Digestion

The use of microwave (MW) systems is an alternative and a well-known approach for digestion of food samples **(Dean, Ando, 1997)**. There exist two types of commercially available MW systems which are open vessel MW and closed vessel MW systems.

Open vessel MW systems run under atmospheric pressure and are not affected by pressure build-up. These systems allow flexibility in treatment control, such as delivery of digestion reagents at any stage of the procedure without vessel cooling or opening **(Şenol, 2010).** The usage of open vessels for digestion can result in problems such as loss of the element species due to volatilization **(Dean, Ando, 1997)** and the needs for acids with high boiling points for complete decomposition of the sample matrix **(Şenol, 2010).**

As compared with open vessel MW systems, closed vessel MW systems (**Figure 1.1**) are more advanced. In these systems, samples are placed inside closed vessels. These closed vessels are usually made up fluorinated polymers such as polytetrafluoroethylene (PTFE) (**Figure 1.2**) or perfluoroalkoxy vinyl ether (PFA) **(Dean, Ando, 1997)**.

MW heating decomposes the sample matrix, leading to the evaporation of the gases produced during decomposition and of the digestion acids. As a result, the pressure

inside the vessel is increased, which in turn causes an elevation in the boiling point of the reagents, and thereby decomposition efficiency is increased **(Şenol, 2010).** The main advantage of the closed vessel MW systems is the high heating efficiency (**Figure 1.3**); however, excessive occurrence of pressure can result in the rapture of sealed vessels **(Şenol, 2010),** which can be considered as the main disadvantage of these systems.

Additional features of commercial systems include PTFE-lined cavity, a safety vent; these systems also have the ability to measure both temperature and pressure inside a single vessel **(Dean, Ando, 1997)**. All these features make these systems safe and efficient.

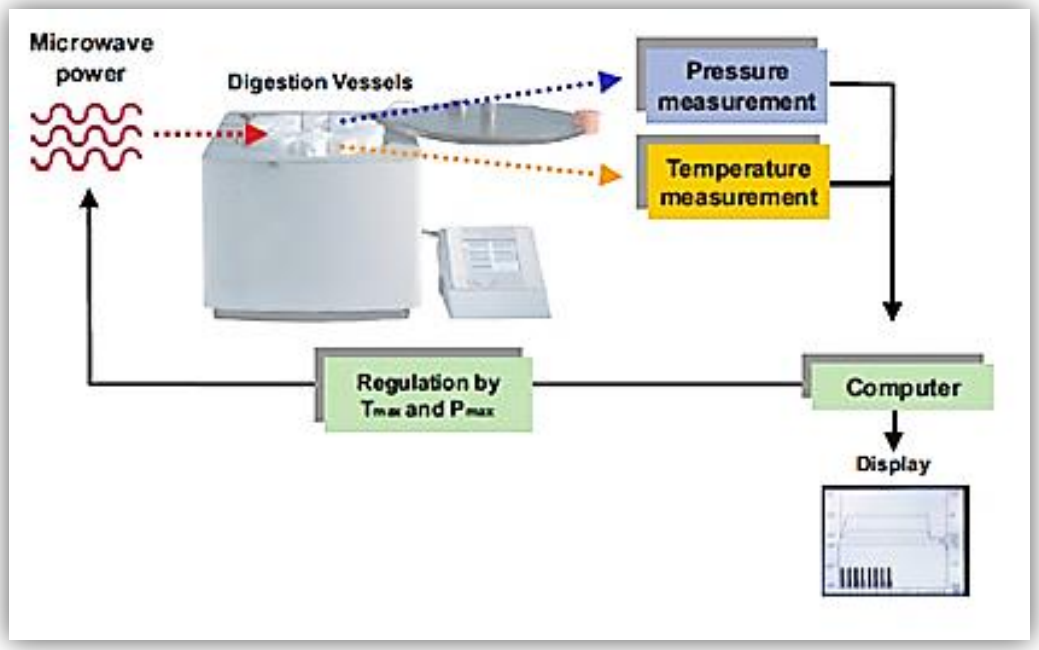

Figure 1.1 Closed vessel microwave digestion system. **(Berghof, 2011)**

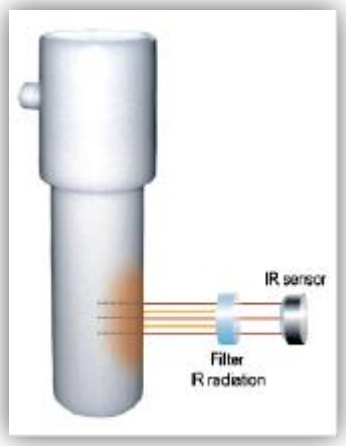

Figure 1.2 Polytetrafluoroethylene (PTFE) and heat control of closed system. **(Berghof, 2011)**

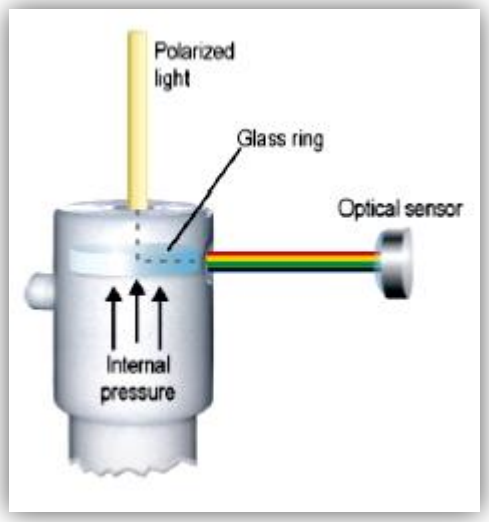

Figure 1.3 Pressure and pressure control in Polytetrafluoroethylene (PTFE) **(Berghof, 2011)**

1.8. Theory of Atomic Emission Spectroscopy

The emission signals in visible region are readily detectable by the human eye. Accordingly, the qualitative and quantitative use of emission signals is rather early as compared with the other analytical techniques using signals not readily detectable by human senses like absorption **(Aras and Ataman, 2006).** Atomic Emission

Spectrometry (AES) is one of the oldest analytical techniques. AES has 3 different techniques according to type of the source as flame emission, arc and spark, and plasma.

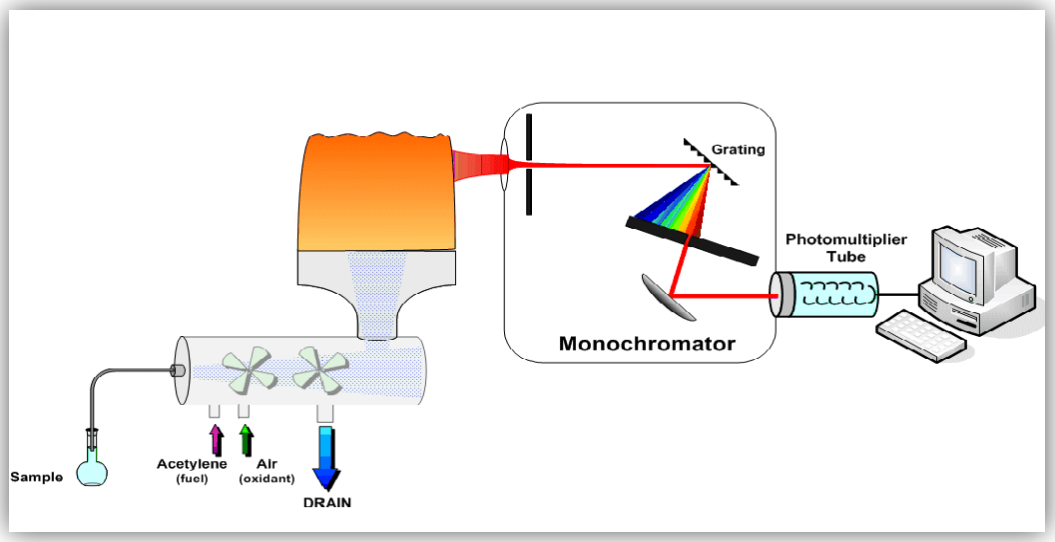

Figure 1.4 Schematic representation of AES block diagram **(http://www.cofc.edu).**

Flame is the oldest spectroscopic radiation source and has good spatial and temporal stability. In the determination of the alkali elements, flame atomic emission spectrometry still remains the most sensitive technique **(Broekaert, 2003)**. Alkali and alkaline earth elements have quite long analytical wavelenghts, corresponding to low-excitation energies; thus, excited states are sufficiently populated even in an air-propane flame with an average temperature of 2200 K **(Aras and Ataman, 2006).** Nevertheless, the usage of flame emission spectrometry is limited due to the insufficiency of this temperature for many other elements in the periodic table. Additionally, interferences originating from the formation of stable compounds **(Broekaert, 2003)** and small variations in flame temperature leads to significant alterations in excited state populations, and thus random errors occur in atomic emission signals due to flame T fluctuations **(Aras and Ataman, 2006).**

Arc and spark, which is another emission source used in AES, provides reaching a temperature of as high as 6000 K. Electrical arcs have wider applicability on the periodic table and provide high sensitivity than the flame sources **(Aras and Ataman, 2006).** In addition to trace analyses, arcs are also used for analysis of pure substances in the requirement of the highest power of detection. However, they may be hampered by poor precision **(Broekaert, 2003)**.

Researches are being conducted to design hotter sources due to the requirement of higher temperatures for better population of the upper electronic states. The high temperature required up to 10000 K has been achieved by plasma sources, particularly those using Argon (Ar) and Helium (He), resulting in higher sensitivity in AES **(Aras and Ataman, 2006).** Many plasma sources have been reported including direct current plasma (DCP), microwave induced plasma (MIP), inductively coupled plasma (ICP) and glow discharge (GD); among them, the most common source is the inductively coupled plasma (ICP) with Ar gases today **(Aras and Ataman, 2006).** Ar ICP source is advantageous over flame, arc, spark and other plasma sources of emission spectroscopy techniques in several aspects. Almost all molecular bonds can be broken by the hot source (almost 6000 K) and atomization of the refractory carbides and oxides occurs. Energy is sufficient even appreciable ionization of the analyte atoms. Signal-to-Noise (S/N) is better at a certain height above the load coil, in which the background emission is low. Plasma structure allows almost no self-absorption; thus, linear dynamic range is wide as much as 10^4. The high temperature results in the presence of only few molecular species. No combustion products occur as in the flames, leading the environment to be chemically inert. Reduction of free atom population due to molecular reactions is minimized; chemical interferences rarely occur. Population of many upper electronic states occurs due to the high temperature medium, leading to many possibilities for the wavelength choice for an analyte. While this situation provides more analytical choices in the spectral domain, since the same conditions are also valid for all the other species, the resulting spectrum is rich in potentially interfering lines. Similar to the other AES techniques, multi-element determination is also performed by ICP-OES; this determination is performed in the sequential mode by the use of

monochromators or in a simultaneous mode by the use of polychromators. Besides, atomization, ionization and excitation of atoms and/or ions result from the high temperature medium and the rather long residence times (e.g.,2 ms for analyte species) is observed in the plasma **(Aras and Ataman, 2006**).

As compared with the flame AAS and ICP-OES have similar or better detection limits. One of the most important advantages of ICP-OES is the determination of refractory elements such as boron, aluminium, silicon, tungsten, zirconium, thallium and rare earth elements; these analytes pose difficulties in combustion flames.

1.9. Sample Introduction Systems

Sample introduction into plasma is often named as the weakest ring of the chain or the *Achille's heel* for the system **(Aras and Ataman, 2006).** The use of nebulizer is the most common method utilized for liquid sample introduction in AES (**Figure 1.5)** Converting an aqueous sample into an aerosol by the action of the carrier gas is the main function of the nebulizer. Viscosity and surface tension of the aqueous samples, both of which have an impact on the carrier gas and uptake rate, affects their nebulisation **(Dean and Ando, 1997)**.

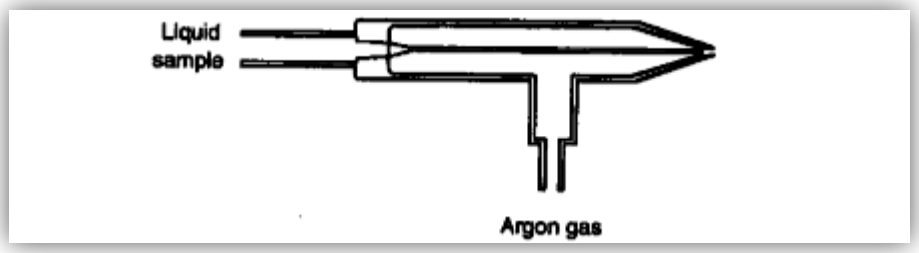

Figure 1.5 Schematic diagram of the pneumatic concentric nebulizer **(Dean, Ando, 1997).**

If introduced into the plasma source directly, the coarse aerosols generated by the nebulisers would either extinguish or induce cooling of the plasma. This in turn would lead to severe matrix interferences unless a spray chamber (**Figure 1.6**) is added to the system. Spray chamber reduce the aerosol size towards an ideal particle size **(Dean and Ando, 1997)**.

An ideal spray chamber should pose all of the following features: a large surface area for the induction of collisions and fragmentation of the coarse aerosol, minimal dead volume for the prevention of sample dilution, and easy removal of condensed sample to waste without inducing pressure pulsing **(Dean and Ando, 1997)**.

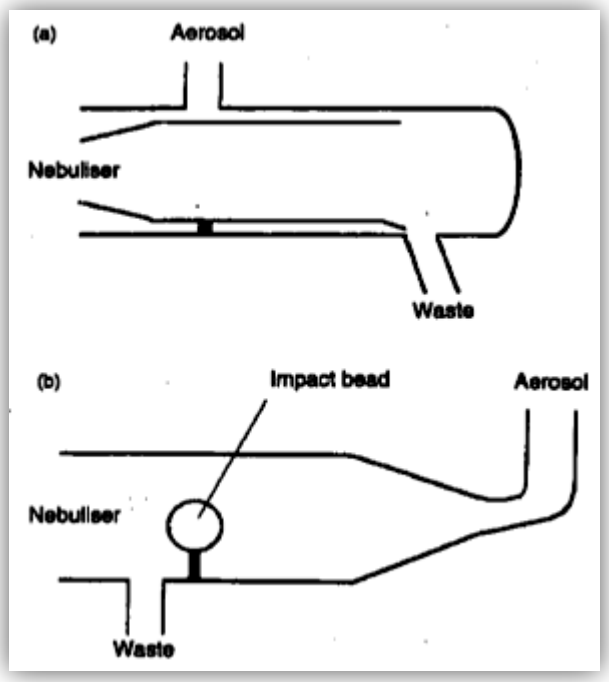

Figure 1.6 Types of spray chamber; (a) Scott double-pass type; (b) impact-bead spray chamber **(Dean, Ando, 1997).**

1.10. Detection Systems and Measurement Modes in ICP-OES

In practice, there are two main alternatives; sequential and simultaneous instruments are available in ICP-OES determination based on the needs of the analyst and capital cost of the instrument.

When a monochromator with a single exit slit and a single detector is employed, dispersing device rotates to select the desired wavelenght on this exit slit. While the sample is continuously introduced into plasma, measurement on each selected line is made for a time period that is sufficient to give a good Signal to Noise (S/N) value; multi-element detection is possible in a sequential mode (**Figure 1.7**) **(Aras and Ataman, 2006).**

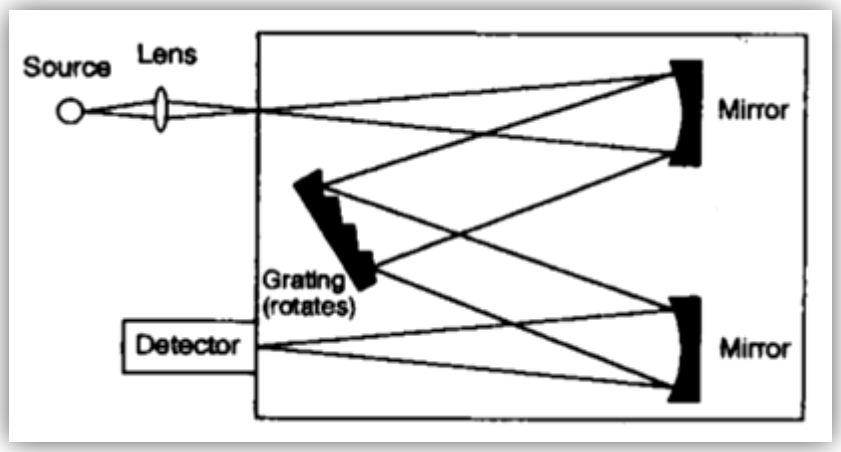

Figure 1.7 Optical layout of the Czerny-Turner Spectrometer (Sequential mode) **(Dean, Ando, 1997).**

In order to provide multi-element detection in a simultaneous mode, some of the ICP-OES instruments are equipped with a polychromator; several exit slits, and detectors at selected wavelengths. None of the components are required to be moved in these systems as shown in **Figure 1.8 (Aras and Ataman, 2006).**

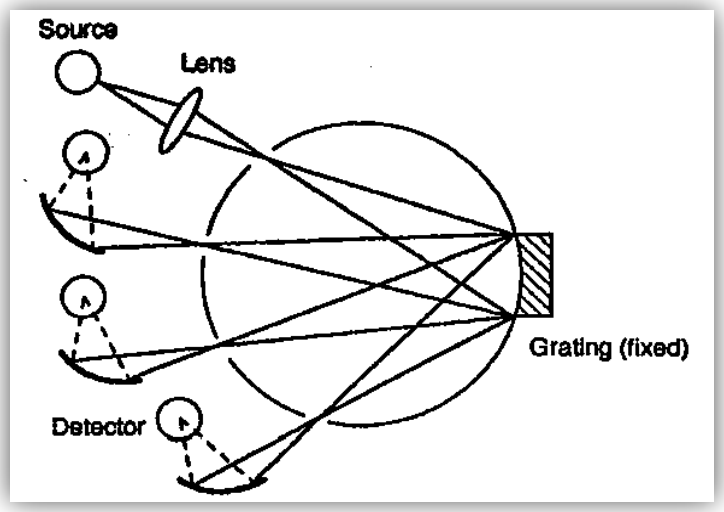

Figure 1.8 Optical layout of the Paschen-Runge Spectrometer (simultaneous mode) **(Dean, Ando, 1997).**

Sequential ICP-OES instruments are associated with lower cost and flexibility in choice of analyte; however, these instruments are not used for fast multi-element analysis. Simultaneous ICP-OES instruments on the other hand, are costly; however, they are able to produce large number of data in unit time; the selections for wavelength and therefore the chemical matrix to work on are limited and should be determined before the instrument is designed. This limitation was experienced for ICP-OES designs with multi-slits as shown in **Figure 1.8**; however with the present instruments having array detectors, this disadvantage is not valid anymore.

Another classification regarding the measurement mode has been related to the manner by which the ICP sources viewed. The early designs, the ICP torch was mostly positioned in a vertical manner and a portion of plasma was radially by the entrance slit of spectrometer; this is presently called side-on or radial view as shown in **Figure 1.9 (Aras and Ataman, 2006).**

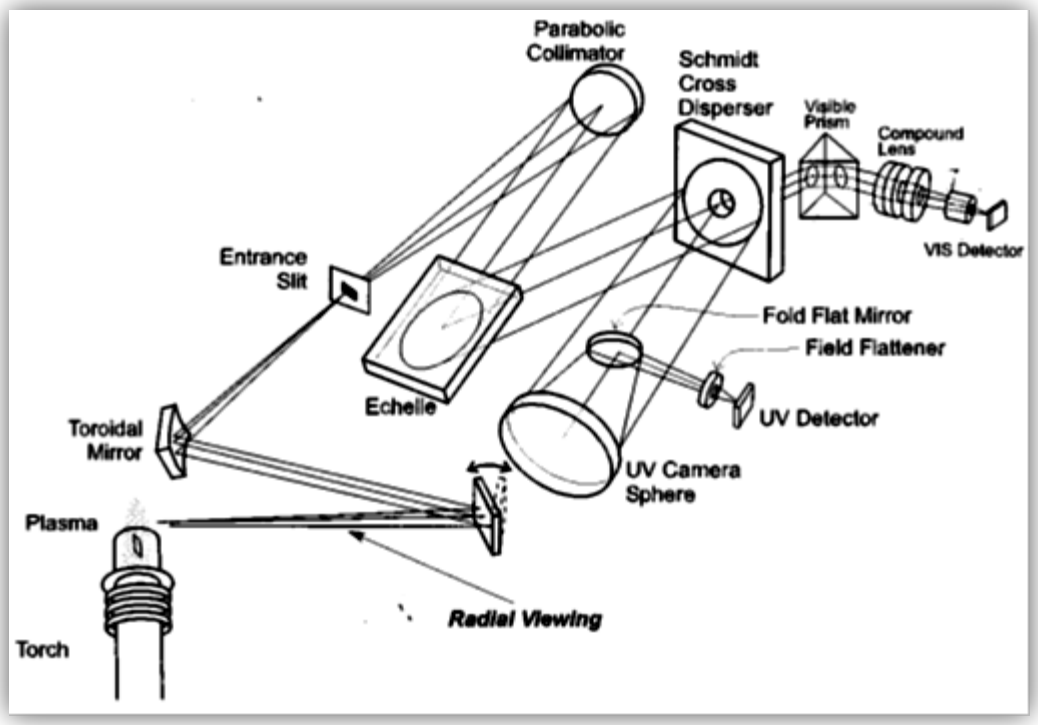

Figure 1.9 Schematic diagram of the Radial View Optical System **(PerkinElmer, 2007)**

Later on, another mode of plasma viewing has become popular; in this sort of design, the plasma positioned in a horizontal manner and is viewed along its long axis; the term used for this sort of measurement are end on or axial viewing as shown in **Figure 1.10 (Aras and Ataman, 2006).**

Some instruments provide both of these options; either can be chosen in a dual as shown in **Figure 1.11** and **Figure 1.12** view design.

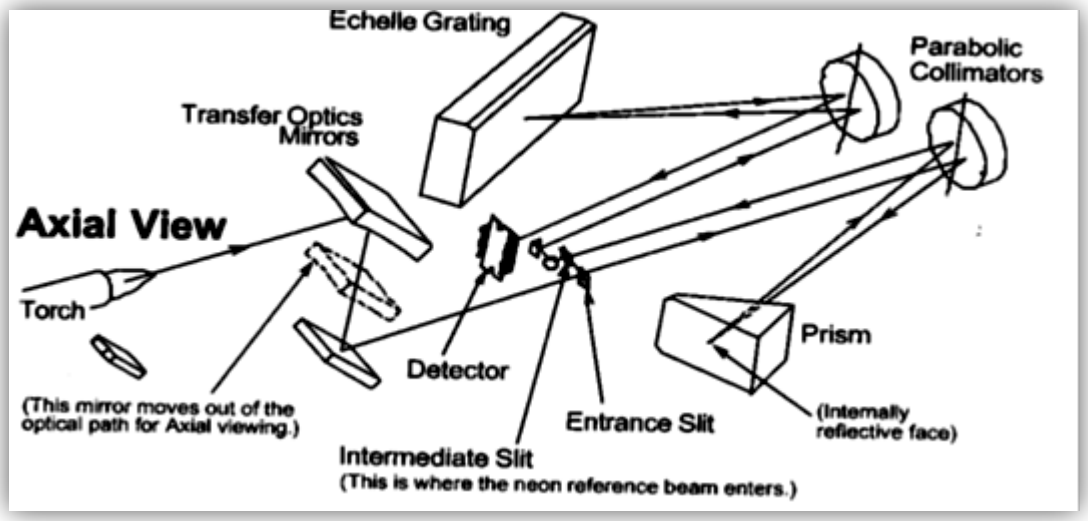

Figure 1.10 Schematic diagram of the Axial View Optical System **(PerkinElmer, 2004)**

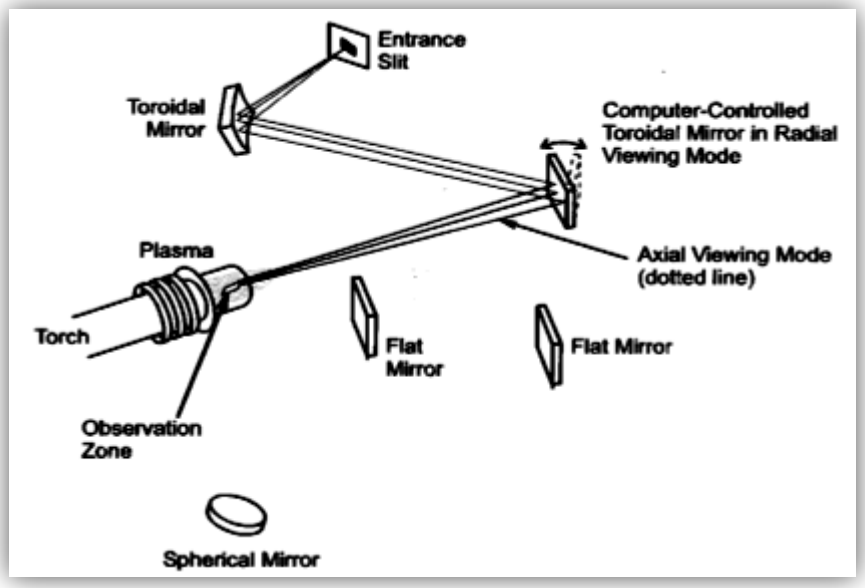

Figure 1.11 Schematic Diagram of the Dual View Optical System in Axial view mode **(PerkinElmer, 2004)**

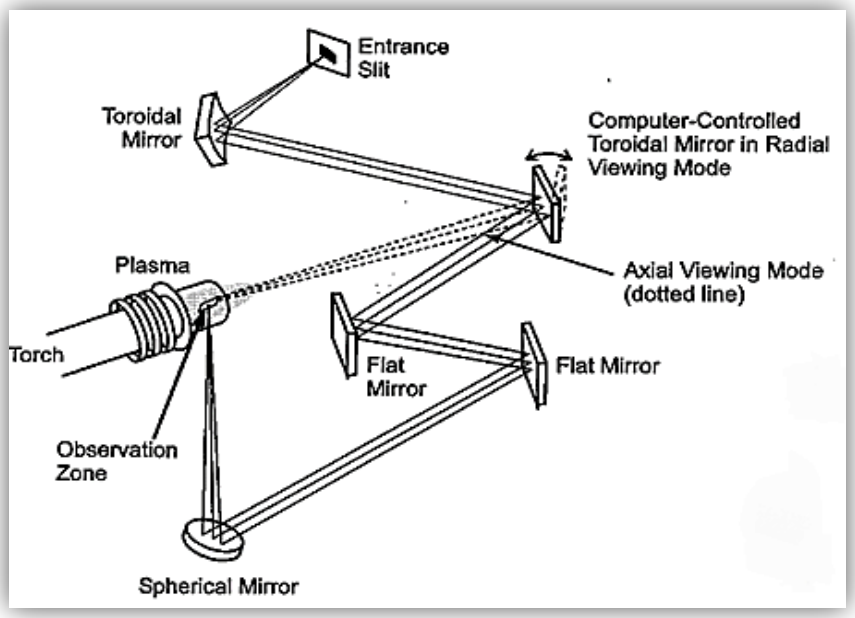

Figure 1.12 Schematic diagram of the Dual View Optical System in radial view mode **(PerkinElmer, 2004).**

Axial viewing provides a sensitivity enhancement of typically 5-20 times since a longer source path-length is viewed; but this system is more prone to non-spectral interferences. On the other hand, radial systems are relatively much free of interferences although its sensitivity is lower **(Aras and Ataman, 2006).**

1.11. Interferences in ICP-OES

Interferences in ICP-OES can be classified as spectral and non-spectral origin **(Aras and Ataman, 2006).** Three types of spectral overlap can be identified; (1) direct wavelength coincidence with an interfering emission line (**Figure 1.13**), (2) partial overlapping of the emission line of interest with an interfering line in close proximity (**Figure 1.14**), (3) the presence of an elevated or depressed background continuum (**Figure 1.15**) **(Dean and Ando, 1997)**.

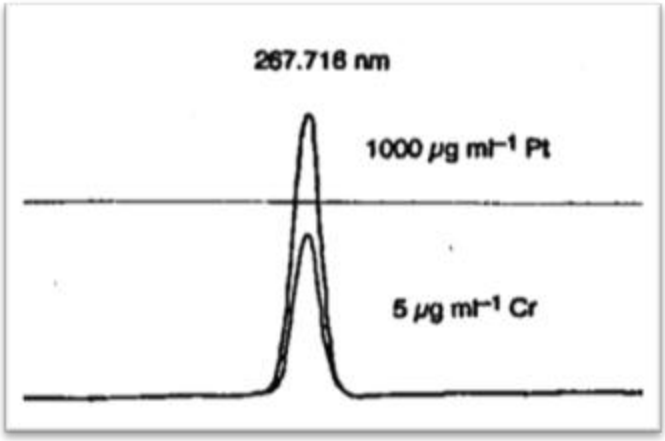

Figure 1.13 Direct wavelength coincidence with interfering emission line **(Dean, Ando, 1997).**

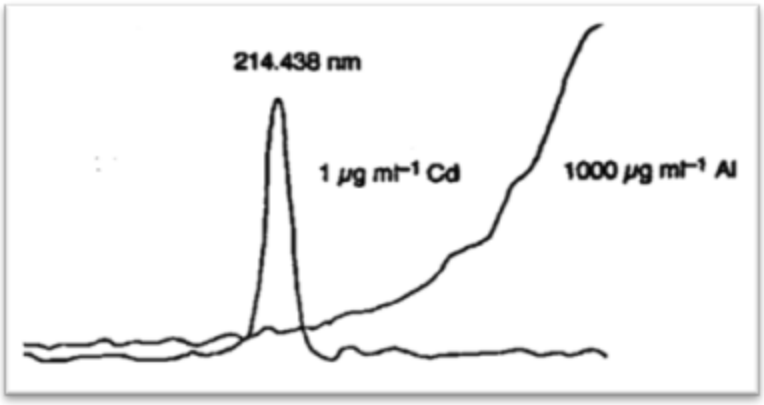

Figure 1.14 Partial overlapping of the emission line of interest with an interfering line in close proximity (Dean, Ando, 1997).

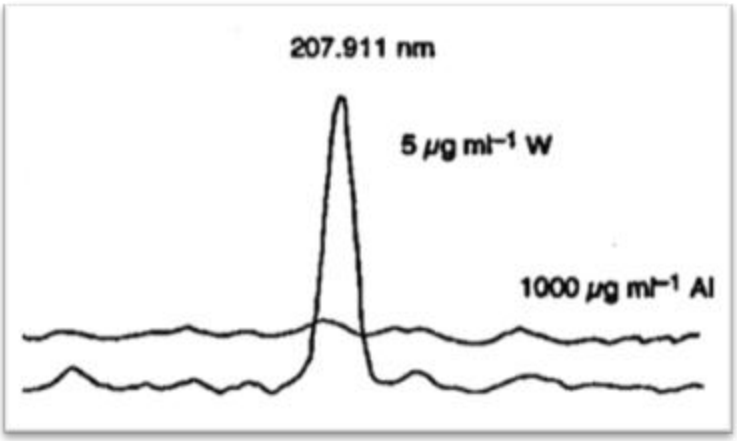

Figure 1.15 The presence of an elevated or depressed background continuum (Dean, Ando, 1997).

The usual remedy to alleviate a spectral interference is to either increase the resolution of the spectrometer, or to select an alternative spectral emission line.

One of the non-spectral interferences is the alterations in Ar plasma equilibria. Plasma conditions may be affected by the presence of easily ionizable elements (EIE's) such as calcium and sodium. High amounts of these elements may have to be introduced into plasma as a result of the natural sample matrix or fusion methods used to dissolve solid samples. Presence of EIE's in relatively large quantities in plasma upset equilibrium conditions and thus both suppressing and enhancing effects in complex ways may take place for analyte signals in ICP-OES **(Dean and Ando, 1997)**. Non-spectral interferences are often associated with the sample introduction process. For aqueous solutions, peristaltic pumps are used to assure a constant sample flow to nebulizer; however due to differences in solution viscosity nebulization efficiencies may not be the same for sample and standard. Standard addition or matrix matching techniques should be used to eliminate such interferences.

1.12. Aim of the Study

In this study, it was aimed to decide, develop and validate the analysis methods for Na, Mg, P, K, Ca, Fe and Zn, elements, play important roles in various body function and not synthesized in the human body, in infant formulas, infant follow-on formulas and fortified baby formulas, using ICP-OES.

Moreover, it was aimed to compare the obtained results with the limits regulated by international and national authorities. Furthermore the obtained results compared with the reference values specified by the manufacturers. In addition, the results of the same sample obtained in two different ICP-OES device systems were compared with each other, as well.

CHAPTER 2

EXPERIMENTAL

2.1. Chemicals and Reagents

In the present study, chemicals and reagents were of analytical grade or high purity. Calibration standards were prepared using 1000 mg/L commercial stock solutions; the following medium were used: Zn and Fe in 2 % (v/v) HNO_3 (Merck, Germany) Ca, Mg, K and Na in 0.07 % (v/v) HNO_3 (Inorganic Ventures, USA), and P in H_2O (Inorganic Ventures USA).

Working standard solutions were prepared in 1 % (v/v) HNO_3 by successive dilutions. Dilutions were performed using 18.2 MΩ-cm deionized water obtained by a Puris Expe - CB Water System (Mirae st, Rep. of Korea). Pro-analysis grade HNO_3 (Merck, Germany) was utilized to acidify working standard solutions.

All solutions were prepared using two Eppendorf micropipettes covering the range from 10 µL to 100 µL and 100 µL to 1000 µL. Polyethylene and polypropylene containers were used to prevent working solutions from any contamination due to glassware. Before each use, all glass containers and labware were immersed in acid tanks containing 10% (v/v) HNO_3 for at least 24 hours and then washed with deionized water.

2.2. Samples

Infant formulas, infant follow-on formulas and fortified baby formula samples were collected from a supermarket in Ankara, Turkey. The samples used in the present study were from four different brands, which are commercially available and purchasable by everybody. One of the samples was composed of organic products. Infant formulas and infant follow-on formulas were milk and milk-protein based, whereas fortified baby formulas were cereal- and fruit-based. The samples were presented in the **Table 2.1.**

2.3. Instruments

2.3.1. Inductively Coupled Plasma Optical Emission Spectrometer

In the present study, two different ICP-OES system was used. First, ICP-OES 2100 DV (Perkin Elmer, USA) which is sequential instrument with charge coupled detector (CCD) and operated in dual view configurations. Secondly, ICP-OES 5300 V (Perkin Elmer, USA) which is simultaneous instrument with segmented array charge couple detector (SCD) and radial view configurations. Background correction was done automatically for both devices; any residual deviations are compensated for by measuring a neon reference spectrum simultaneously with each measurement of an analytical emission line. The neon spectrum passes through the echelle system with the analytical radiation and illuminates the top array of the detector. The neon spectrum acts as a wavelength scale to enable active wavelength correction.

Different types of food samples, including infant formulas, were being analyzed in TSE laboratories for 5 years with ICP-OES 2100 DV and ICP-OES 5300 V. Therefore, profound information about the emission lines has already known.

Determination of Na, K, Mg, Ca, P, Zn and Fe was performed by selecting the emission line at which the highest S/N ratio was achieved, and the emission lines for each element are listed in **Table 2.2.**

Table 2.1 Types of infant formula (IF), infant follow-on formula (IFF) and fortified baby formula (FBF) analyzed in the present study

Sample[1]	Sample Information
IF-A-1	0-6 months, milk based
IF-A-2	0-6 months, milk based
IF-B-1	from birth, milk based
IF-C-1	from birth, milk based
IF-D-1	from birth, organic, milk based
IFF-A-1	from 6. month, milk based
IFF-B-1	from 6. month, milk based
IFF-C-1	from 6. month, milk based
IFF-D-1	from 6. month, organic, milk based
IFF-A-2	from 9. month, milk based
IFF-B-2	from 9. month, milk based
IFF-C-2	from 9. month, milk based
IFF-D-2	from 10. month, organic, milk based
IFF-B-3	from 12. month, milk based
FBF-A-1	Cereal-based with milk, semolina and fruits,4-36 months
FBF-A-2	Cereal-based with 8 different cereal and honey, from 6.month
FBF-B-1	Cereal-based with milk fruits and rice,4-36 months
FBF-B-2	Cereal-based with rye and corn,5-36 months
FBF-B-3	Cereal-based with milk, biscuits and oat,6-36 months
FBF-C-1	Cereal-based with milk, and rice, started from 4.month
FBF-C-2	Cereal-based with milk, fruits and 8 different cereal, 6-36 months

[1]In the present study, coding system was used to identify the formula samples. In the coding system IF represents Infant Formulas, IFF represents Infant Follow-on Formulas and FBF represents Fortified Baby Formulas. Capital letters A, B, C and D belong to different brand names and number next to capital letters was used to distinguish more than one sample belonging to that brand name and sample class.

Table 2.2 Emission lines of the Na, Mg, P, K, Ca, Fe and Zn selected in the present study, for both ICP-OES 2100 DV and ICP-OES 5300 V instruments.

Elements	Wavelength (nm)
	ICP-OES 2100 DV (Axial) & ICP-OES 5300V (Radial)
Na	589.592
Mg	285.213
P	213.617
K	766.490
Ca	317.933
Fe	238.204
Zn	206.200

2.3.2. Ultrasonic Water Bath

It has been suggested that while dissolving organic substances in HNO_3 + H_2O_2, the substances are firstly treated with HNO_3 alone to be warmed or evaporated to dryness before addition of the peroxide **(Bock, 1979)**. In the present study prior to microwave digestion of infant formulas, ultrasonic pre-treatment was performed to homogenize for 20 minutes twice by using WiseClean (DAIHAN Wisd, Rep. of Korea) ultrasonic water bath at room temperature.

2.3.3. Microwave Digestion System

Digestion of BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011 Wheat CRM (China) was performed using a Berghof Speedwave MWS 3^+ microwave dissolution system with a 12 DAP-60+ digestion bombs set. Optimization studies of the sample preparation and microwave programming will be explained in detail in Section 2.4.1.3.

2.4. Procedures

Procedures contain two parts. The first part consists of analytical procedures; the optimization of ICP-OES analyses system, calibration, and the sample preparation studies. The second part contains the studies for determination of the analytical figures of merit; accuracy, precision, LOD and LOQ. In accordance with this purpose, Certified Reference Material (CRM) analyses were carried out

2.4.1. Analytical Procedure

2.4.1.1. Optimization Studies for ICP-OES Analysis Systems

The present study was not designed as a method development study, it was rather a study conducted for the analysis of some elements found in the infant formulas, infant follow-on formulas and fortified baby foods marketed in Ankara; thus, the methods, which were previously proven to be effective, were utilized in the present study by optimizing and adapting them according to the properties of the devices.

In a previous study conducted by Mc Kinstry *et al.* (1999), elements in milk and infants formula including Ca, Cu Fe, K, Mg, Mn, Na, P, and Zn were analyzed using radial ICP-OES system. The operating conditions of the device used by Mc Kinstry *et al.* (1999) in that particular study are presented in **Table 2.3.**

In Ikem *et al.* (2002) study, infant formulas were analyzed by the use of axial ICP-OES system. In that particular study, Ikem *et al.* (2002) analyzed 26 different elements, including Ca, Fe Mg Na and Zn, which were also analyzed in the present study. The operating conditions for ICP–OES in determination of metals for infant formulas used by Ikem *et al.* (2002) are presented in **Table 2.4.**

Table 2.3 Instrument operating parameters for ICP-OES during (a) instrument assessment and (b) routine use (Mc Kinstry et al., 1999)

Parameters	(a)	(b)
View Mode	Radial	Radial
View Distance	15 mm	15 mm
Plasma Gas Flow	15 L/min	15 L/min
Auxilary Gas Flow	0.5 L/min	0.5 L/min
Source Equilibrium Time	15 s	15 s
RF Power	1100 W	1350 W
Nebulizer	0.95 L/min	0.80 L/min
Sample Aspiration Rate	1.5 mL/min	1.8 mL/min
Read	Peak Area	Peak Area
Number of Replicates	10	3
Background Correction	Manual point selection	Manual point selection
Read Delay	40 s	40 s
Rinse Delay	30 s	30 s

Table 2.4 Instrument operating conditions applied for elements determination by ICP-OES (Ikem et al.,2002)

Parameters	Value
View Mode	Axial
View Distance	15 mm
Plasma Gas Flow	15 L/min
Auxilary Gas Flow	0.5 L/min
Source Equilibrium Time	15 s
Pump Flow Rate	1 mL/min
Detector	Charge array
RF Power	1300 W
Nebulizer	0.8 L/min
Sample Aspiration Rate	1 mL/min
Read	Peak Height
Number of Replicates	3
Background Correction	Manual point correction
Read Delay	120 s
Rinse Delay	20 s

In a recent study, Zand *et al.* (2011) also used axial ICP-OES system for the analysis of 8 different elements in baby foods in the United Kingdom. These 8 elements also included Ca, Fe, Mg, Na, and Zn, which were analyzed in the present study. The operating conditions used by Zand *et al.* (2011) are presented in **Table 2.5.**

The devices used in the present study had similar features to the devices used in the study by Mc Kinstry et al. (1999), Ikem et al. (2002) and Zand et al. (2011) only minor changes were performed on the operating conditions used by Mc Kinstry et al. (1999), Ikem et al. (2002) and Zand et al. (2011). Validity of the study conditions was tested using Quality Control (QC) solutions after the calibration studies.

The optimum values for the present study for ICP-OES 5300 V (Perkin Elmer, USA) and ICP-OES 2100 DV (Perkin Elmer, USA) devices, which perform measurements on the radial and axial axis, respectively are summarized in **Table 2.6**

Table 2.5 Instrument operating parameters applied for determination of elements by ICP-OES (Zand et al.,2011)

Parameters	**Value**
View Mode	Axial
View Distance	15 mm
Plasma Gas Flow	15 L/min
Auxilary Gas Flow	0.2 L/min
Source Equilibrium Time	15 s
Pump Flow Rate	1.5 mL/min
Detector	Segmented array change coupled device
RF Power	1300 W
Nebulizer	0.8 L/min
Sample Aspiration Rate	1.5 mL/min
Read	Peak Area
Number of Replicates	3
Background Correction	2-Point
Read Delay	60 s
Rinse Delay	30 s

Table 2.6 Operating conditions for the ICP-OES 2100DV and ICP-OES 5300 V in the present study.

Parameters	ICP 2100 DV	ICP 5300 V
View Mode	Axial	Radial
View Distance	15 mm	15 mm
Plasma Gas Flow	15 L/min	15 L/min
Auxilary Gas Flow	0.2 L/min	0.2 L/min
Source Equilibrium Time	15 s	15 s
Pump Flow Rate	1.5 mL/min	1.5 mL/min
Detector	CCD	SCD
RF Power	1300 W	1300 W
Nebulizer	0.65 L/min	0.80 L/min
Sample Aspiration Rate	1.5 mL/min	1.5 mL/min
Read	Peak Area	Peak Area
Number of Replicates	3	3
Background Correction	2-Point	2-Point
Read Delay	60 s	60 s
Rinse Delay	30 s	30 s

2.4.1.2. Calibration Studies for ICP-OES Systems

2.4.1.2.1. Calibration Studies for Na

In the calibration studies for Na, concentration of the calibration standards ranged from 0.05 mg/L to 15 mg/L for the axial system and from 0.15 mg/L to 45 mg/L for the radial system, and linear calibration graphs were obtained.

In order to determine the limit of the linearity (LOL), the concentration at which the calibration curve departs from linearity by a specified amount, in the axial system, calibration standards with concentrations ranging from 0.05 mg/L to 50 mg/L were used. A deviation from linearity in calibration curve was observed in concentrations above 15 mg/L. In addition, “saturation” signal was observed on the device in

concentrations above 25 mg/L. LOL was determined as 15 mg/L for axial system. In dynamic range study performed for the radial system, calibration standards with concentrations ranging from 0.15 mg/L to 50 mg/L were used and LOL was determined as 45 mg/L for radial system.

The lowest limit of the dynamic range was determined as limit of quantification (LOQ), the lowest concentration at which quantitative measurements for both radial and axial systems. Detailed results of the LOQ for Na were given in Section 2.4.2.1.

2.4.1.2.2. Calibration Studies for Mg

In the calibration studies for Mg, calibration standards with concentrations ranging from 0.1 mg/L to 50 mg/L for axial system and 0.25 mg/L to 100 mg/L for radial system were used, and linear calibration graphs were obtained.

In order to determine the LOL in the axial system, calibration standards with concentrations ranging from 0.1 mg/L to 100 mg/L were used and LOL was determined as 50 mg/L for axial system. In dynamic range study performed for the radial system, calibration standards with concentrations ranging from .25 mg/L to 150 mg/L were used. LOL was determined as 100 mg/L for radial system.

The lowest limit of the dynamic range was determined as LOQ for both radial and axial systems and detailed results of the LOQ for Mg were given in Section 2.4.2.1.

2.4.1.2.3. Calibration Studies for P

In the calibration studies for P, calibration standards with concentrations ranging from 0.2 mg/L to 50 mg/L for axial system and 0.5 mg/L to 100 mg/L for radial system were used, and linear calibration graphs were obtained.

In order to determine the LOL in the axial system, calibration standards with concentrations ranging from 0.2 mg/L to 100 mg/L were used. LOL was determined as 50 mg/L for axial system. In dynamic range study performed for the radial system, calibration standards with concentrations ranging from 0.5 mg/L to 150 mg/L were used and LOL was determined as 100 mg/L for radial system.

The lowest limit of the dynamic range was determined as LOQ for both radial and axial systems and detailed results of the LOQ for P were given in Section 2.4.2.1.

2.4.1.2.4. Calibration Studies for K

In the calibration studies for K, calibration standards with concentrations ranging from 0.1 mg/L to 50 mg/L for axial system and 0.25 mg/L to 100 mg/L radial systems were used, and linear calibration graphs were obtained.

In order to determine the LOL in the axial system, calibration standards with concentrations ranging from 0.1 mg/L to 100 mg/L were used and LOL was determined as 50 mg/L for axial system. In dynamic range study performed for the radial system, calibration standards with concentrations ranging from 0.25 mg/L to 150 mg/L were used. LOL was determined as 100 mg/L for radial system.

The lowest limit of the dynamic range was determined as LOQ for both radial and axial systems and detailed results of the LOQ for K were given in Section 2.4.2.1.

2.4.1.2.5. Calibration Studies for Ca

In the calibration studies for Ca, calibration standards with concentrations ranging from 0.1 mg/L to 50 mg/L for axial system and 0.3 mg/L to 100 mg/L for radial system were used, and linear graphs were obtained.

In order to determine the LOL in the axial system, calibration standards with concentrations ranging from 0.1 mg/L to 100 mg/L were used. LOL was determined as 50 mg/L for axial system. In dynamic range study performed for the radial system, calibration standards with concentrations ranging from 0.3 mg/L to 150 mg/L were used and LOL was determined as 100 mg/L for radial system.

The lowest limit of the dynamic range was determined as LOQ for both radial and axial systems and detailed results of the LOQ for K were given in Section 2.4.2.1.

2.4.1.2.6. Calibration Studies for Fe

In the calibration studies for Fe, calibration standards with concentrations ranged from 0.01 mg/L to 10 mg/L for axial system and from 0.05 mg/L to 12 mg/L for radial system were used, and linear calibration graphs were obtained.

In order to determine the LOL in the axial system, calibration standards with concentrations ranging from 0.01 mg/L to 15 mg/L were used and LOL was

determined as 10 mg/L for axial system. In dynamic range study performed for the radial system, calibration standards with concentrations ranging from 0.05 mg/L to 15 mg/L were used. LOL was determined as 12 mg/L for radial system.

The lowest limit of the dynamic range was determined as LOQ for both radial and axial systems and detailed results of the LOQ for Fe were given in Section 2.4.2.1.

2.4.1.2.7. Calibration Studies for Zn

In the calibration studies for Zn, calibration standards with concentrations ranging from 0.01 mg/L to 5 mg/L for axial system and 0.05 mg/L to 10 mg/L for radial system were used, and calibration graphs were obtained.

In order to determine the LOL in the axial system, calibration standards with concentrations ranging from 0.01 mg/L to 10 mg/L were used. LOL was determined as 5 mg/L for axial system. In dynamic range study performed for the radial system, calibration standards with concentrations ranging from 0.05 mg/L to 15 mg/L were used and LOL was determined as 10 mg/L for radial system.

The lowest limit of the dynamic range was determined as LOQ for both radial and axial systems and detailed results of the LOQ for Zn were given in Section 2.4.2.1.

Concentrations of the calibration standards and LOL for Na, Mg P, K, Ca, Fe and Zn in ICP-OES 2100 DV and ICP-OES 5300 V analyses system are given **Table 2.7** and **Table 2.8** respectively.

Table 2.7 Calibration interval and LOL values for ICP-OES 2100 DV analyses system in present study.

Elements	Calibration Interval (mg/L)	# of Calibration Point	LOL(mg/L)
Na	0.05 – 15	7	15
Mg	0.13 – 50	7	50
P	0.15 – 50	7	50
K	0.08 – 50	7	50
Ca	0.01 – 50	7	50
Fe	0.01 – 10	7	10
Zn	0.01 – 5	7	5

Table 2.8 Calibration interval and LOL values for ICP-OES 5300 V analyses system in present study.

Elements	Calibration Interval (mg/L)	# of Calibration Point	LOL(mg/L)
Na	0.15 – 45	7	45
Mg	0.24 – 100	7	100
P	0.50 – 100	7	100
K	0.25 – 100	7	100
Ca	0.30 – 100	7	100
Fe	0.05 – 12	7	12
Zn	0.06 – 10	7	10

2.4.1.3. Determination of the Sample Preparation Method

In the present study, sample preparation method was chosen among three different sample digestion techniques. Firstly, dry ashing with open vessel system had some disadvantageous such as sample loss and more time requirement. Secondly, wet ashing technique with open vessel had some problems about providing homogenization for food matrix. Moreover, sample loss, time and using too much chemicals were other disadvantageous of this technique. Wet and dry ashing techniques with open vessel are time consuming and are not advantageous with respect to digestion efficiency except for freedom of using larger sample sizes.

Accordingly, microwave digestion technique was used for certified reference materials and optimization studies were conducted with different heat programs.

2.4.1.3.1. Microwave (MW) Digestion System Programming

In order to completely digest the samples using the MW system, the methods suggested by the manufacturer of the microwave digestion system (Berghof) were studied and the ideal method that would be used for all CRM and samples of the present study was determined by performing required optimizations.

2.4.1.3.1.1. MW Heat Programming 1

According to this method, which was suggested by the manufacturer for digestion of milk powder, approximately 0.2 g of BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011 Wheat CRM (China) were weighed and placed into the digestion vessels (DAP 60S) separately. 5 mL of 65 % (w/w) HNO_3 were added to the vessels, the vessels were shaken carefully for 30 s, and the cap of the vessels were closed after a waiting period of 20 minutes. The prepared vessels were placed into the device, and the digestion process was then started according to the following procedure (**Table 2.9**).

Table 2.9 MW heating program 1

	Steps			
	1	**2**	**3**	**4**
T (^{o}C)	150	200	100	-
T_a (min)	5	5	1	-
Time (min)	10	20	10	-

T_a: Ramp Time to reach the related temperature.

Vessels were cooled to room temperature in order to avoid foaming and splashing. Obtained clear and colourless solutions were diluted to different volumes using distilled water and were measured in both ICP systems. The results are presented in **Table 2.10** and **Table 2.11.**

Table 2.10 Concentrations of elements in BCR-063R Skim Milk Powder CRM samples that prepared according to MW heating program 1

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**BCR-063R Skim Milk Powder (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	4370±31	4016±45	92	4102±50	94
Mg	1263±24	1118±40	86	1188±35	94
P	11100±130	10457±150	94	10278±120	93
K	17680±190	15980±150	90	16017±160	91
Ca	13490±100	12134±120	90	12213±100	91
Fe	2.32±0.23	2.03±0.32	88	2.10±0.25	91
Zn	49.0±0.6	45.0±1.1	92	44.0±1.5	90

Table 2.11 Concentrations of elements in GBW 10011 Wheat CRM samples that prepared according to MW heating program 1

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**GBW 10011 Wheat (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	17±5	15±2	88	15±3	88
Mg	450±70	400±40	89	418±50	93
P	1540±70	1379±50	90	1439±60	93
K	1400±6	1303±9	93	1338±10	96
Ca	340±20	298±20	88	318±30	94
Fe	18.5±3.1	15.9±1.1	86	16.2±2.0	88
Zn	11.6±0.7	9.9±1.0	85	10.5±1.2	90

2.4.1.3.1.2. MW Heat Programming 2

According to this method, which was suggested by the manufacturer for digestion of milk powder, approximately 0.3 g of BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011Wheat CRM (China) were weighed and placed into the digestion vessels (DAP 60S) separately. 3 mL of 65 % (w/w) HNO_3 and 3 mL of 30 % (w/w) H_2O_2 were added to the vessels, the vessels were shaken carefully for 30 s, and the cap of the vessels were closed after a waiting period of 20 minutes. The prepared vessels were placed into the device, and the digestion process was then started according to the following procedure (**Table 2.12**).

Table 2.12 MW heating program 2

	Steps			
	1	**2**	**3**	**4**
T (^{o}C)	145	180	100	-
T_a (min)	10	5	1	-
Time (min)	5	10	10	-

T_a: Ramp Time to reach the related temperature.

Vessels were cooled to room temperature in order to avoid foaming and splashing. The obtained clear and colourless solutions were diluted to different volumes using deionized water and were measured in both ICP systems. The results are listed in **Table 2.13** and **Table 2.14**.

Table 2.13 Concentrations of elements in BCR-063R Skim Milk Powder CRM samples that prepared according to MW heating program 2

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**BCR-063R Skim Milk Powder (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	4370±31	3972±40	90	4005±45	92
Mg	1263±24	1098±40	87	1087±30	86
P	11100±130	10151±130	92	10087±130	91
K	17680±190	14982±160	85	14890±150	84
Ca	13490±100	11857±130	88	11963±120	87
Fe	2.32±0.23	1.99±0.32	86	1.98±0.40	85
Zn	49.0±0.6	43.0±1.5	88	43.0±1.5	88

Table 2.14 Concentrations of elements in GBW 10011 Wheat CRM samples that prepared according to MW heating program 2

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**GBW 10011 Wheat (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	17±5	15±2	88	14±2	82
Mg	450±70	389±50	86	401±40	89
P	1540±70	1296±60	84	1325±70	86
K	1400±6	1280±7	91	1308±7	93
Ca	340±20	278±16	82	302±22	89
Fe	18.5±3.1	14.6±1.5	80	15.2±2.5	82
Zn	11.6±0.7	9.5±1.0	82	9.8±1.0	85

2.4.1.3.1.3. MW Heat Programming 3

According to this method, which was suggested by the manufacturer for digestion of milk powder, approximately 0.2 g of BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011 Wheat CRM (China) were weighed and placed into the digestion vessels (DAP 60S) separately. 4 mL of 65 % (w/w) HNO_3 and 1 mL of 37

% (w/w) HCl were added to the vessels, the vessels were shaken carefully for 30 s, and the cap of the vessels were closed after a waiting period of 20 minutes. The prepared vessels were placed into the device, and the digestion process was then started according to the following procedure (**Table 2.15**).

Table 2.15 MW heating program 3

	Steps			
	1	**2**	**3**	**4**
T (^{0}C)	150	190	100	-
T_a (min)	5	5	1	-
Time (min)	10	20	10	-

T_a: Ramp Time to reach the related temperature.

Vessels were cooled to room temperature in order to avoid foaming and splashing. It was observed that the obtained solutions, particularly the wheat solutions, were not clear. In addition, the obtained solutions were not completely digested and were green in color due to undissolved organic structures. The obtained solutions were diluted to different volumes using deionized water and were measured in both ICP systems. The results are presented in **Table 2.16** and **Table 2.17**.

Table 2.16 Concentrations of elements in BCR-063R Skim Milk Powder CRM samples that prepared according to MW heating program 3

		ICP-OES 2100 DV		**ICP-OES 5300 V**	
Elements	**BCR-063R Skim Milk Powder (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	4370±31	3908±45	89	3951±40	90
Mg	1263±24	1023±35	81	996±25	79
P	11100±130	10025±120	90	9987±100	90
K	17680±190	14152±150	80	14023±130	79
Ca	13490±100	11024±120	82	11127±120	83
Fe	2.32±0.23	1.76±0.50	76	1.81±0.50	78
Zn	49.0±0.6	42.2±1.2	86	41.8±2.0	85

Table 2.17 Concentrations of elements in GBW 10011 Wheat CRM samples that prepared according to MW heating program 3

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**GBW 10011 Wheat (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	17±5	13±2	77	13±2	77
Mg	450±70	373±40	83	396±30	88
P	1540±70	1201±50	78	1216±50	79
K	1400±6	1195±5	85	1201±5	86
Ca	340±20	261±15	77	278±20	82
Fe	18.5±3.1	13.5±2.0	73	14.1±2.0	76
Zn	11.6±0.7	9.1±1.0	79	9.3±1.0	80

2.4.1.3.1.4. MW Heat Programming 4

According to this method, which was suggested by the manufacturer for digestion of milk powder, approximately 0.5 g of BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011Wheat CRM (China) were weighed and placed into the digestion vessels (DAP 60S) separately. 2 mL of 65 % (w/w) HNO_3 and 3 mL of 30 % (w/w) H_2O_2 were added to the vessels, the vessels were shaken carefully for 30 s, and the cap of the vessels were closed after a waiting period of 20 minutes. The prepared vessels were placed into the device, and the digestion process was then started according to the following procedure (**Table 2.18**).

Table 2.18 MW heating program 4

	Steps			
	1	**2**	**3**	**4**
T (^oC)	145	170	190	100
T_a (min)	2	5	2	1
Time (min)	5	10	15	10

T_a: Ramp Time to reach the related temperature.

Vessels were cooled to room temperature in order to avoid foaming and splashing. It was observed that the obtained solutions, particularly the skim milk powder solutions, were not sufficiently clear. In addition, the obtained solutions were not completely digested and were green in color due to undissolved organic structures. The obtained solutions were diluted to different volumes using deionized water and were measured in both ICP systems. The results are presented in **Table 2.19** and **Table 2.20**.

Table 2.19 Concentrations of elements in BCR-063R Skim Milk Powder CRM samples that prepared according to MW heating program 4

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**BCR-063R Skim Milk Powder (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	4370±31	3286±60	75	3118±50	71
Mg	1263±24	958±50	76	926±30	73
P	11100±130	8765±140	79	8621±120	78
K	17680±190	12314±170	70	12256±150	69
Ca	13490±100	9453±140	70	9542±120	71
Fe	2.32±0.23	1.58±0.10	68	1.62±0.20	70
Zn	49.0±0.6	35.1±3.0	72	35.7±3.0	73

Table 2.20 Concentrations of elements in GBW 10011 Wheat CRM samples that prepared according to MW heating program 4

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**GBW 10011 Wheat (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	17±5	12±2	71	12±2	71
Mg	450±70	325±30	72	312±30	69
P	1540±70	1108±70	72	1025±60	67
K	1400±6	1002±10	72	998±15	71
Ca	340±20	241±20	71	229±20	67
Fe	18.5±3.1	12.9±1.5	70	12.1±2.0	67
Zn	11.6±0.7	8.2±1.0	71	7.9±1.0	68

2.4.1.3.1.5. MW Heat Programming 5

According to this method, which was suggested by the manufacturer for digestion of wheat, approximately 1 g of BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011Wheat CRM (China) were weighed and placed into the digestion vessels (DAP 60S) separately. 10 mL of 65 % (w/w) HNO_3 and 2 mL of 30 % (w/w) H_2O_2 were added to the vessels, the vessels were shaken carefully for 30 s, and the cap of the vessels were closed after a waiting period of 20 minutes. The prepared vessels were placed into the device, and the digestion process was then started according to the following procedure (**Table 2.21**).

Table 2.21 MW heating program 5

	Steps			
	1	**2**	**3**	**4**
T (^oC)	160	170	100	100
T_a (min)	10	5	1	1
Time (min)	15	10	10	0

T_a: Ramp Time to reach the related temperature.

Vessels were cooled to room temperature in order to avoid foaming and splashing. It was observed that the obtained solutions, particularly the skim milk powder solutions, were not completely digested. In addition, undissolved sample residues were observed in the vessels. Thus, analyses of the samples were not performed

2.4.1.3.1.6. MW Heat Programming 6

According to this method, which was suggested by the manufacturer for digestion of wheat, approximately 0.5 g of BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011Wheat CRM (China) were weighed and placed into the digestion vessels (DAP 60S) separately. 6 mL of 65 % (w/w) HNO_3 and 2 mL of 30 % (w/w)

H_2O_2 were added to the vessels, the vessels were shaken carefully for 30 s, and the cap of the vessels were closed after a waiting period of 20 minutes. The prepared vessels were placed into the device, and the digestion process was then started according to the following procedure (**Table 2.22**).

Table 2.22 MW heating program 6

	Steps			
	1	**2**	**3**	**4**
T (^{0}C)	170	190	210	100
T_a (min)	2	1	1	1
Time (min)	15	10	10	10

T_a: Ramp Time to reach the related temperature.

Vessels were cooled to room temperature in order to avoid foaming and splashing. It was observed that the obtained solutions, particularly the skim milk powder solutions, were not completely clear. In addition, samples were not completely digested and were green in color due to undissolved organic structures. The obtained solutions were diluted to different volumes using deionized water and were measured in both ICP systems. The results are listed in **Table 2.23** and **Table 2.24**.

Table 2.23 Concentrations of elements in BCR-063R Skim Milk Powder CRM samples that prepared according to MW heating program 6

		ICP-OES 2100 DV		**ICP-OES 5300 V**	
Elements	**BCR-063R Skim Milk Powder (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	4370±31	3518±45	81	3523±50	81
Mg	1263±24	993±40	79	986±40	78
P	11100±130	8996±130	81	8891±120	80
K	17680±190	12867±150	73	12785±130	72
Ca	13490±100	9816±130	73	9777±120	73
Fe	2.32±0.23	1.69±0.10	73	1.71±0.10	74
Zn	49.0±0.6	38.3±3.0	78	37.3±2.0	76

Table 2.24 Concentrations of elements in GBW 10011 Wheat CRM samples that prepared according to MW heating program 6

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**GBW 10011 Wheat (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	17±5	13±2	77	13±2	77
Mg	450±70	358±25	79	342±19	76
P	1540±70	1136±60	74	1152±48	75
K	1400±6	1096±12	78	1100±14	79
Ca	340±20	252±13	74	251±21	74
Fe	18.5±3.1	13.1±1.0	71	13.6±1.0	74
Zn	11.6±0.7	8.9±1.0	77	8.6±1.0	74

2.4.1.3.1.7. Optimization Studies for Microwave Digestion System

Based on the above-mentioned results, the use of approximately 0.2 g sample and the use of HNO_3 and H_2O_2 were considered useful in order to achieve complete digestion. Moreover, prior to microwave digestion of infant formulas, ultrasonic pre-treatment was performed to increase the efficiency of digestion.

First, 4.0 mL of 65 % (w/w) HNO_3 was added onto 0.2 g of GBW 10011 Wheat CRM or BCR-063R Skim Milk Powder CRM and the mixture was then kept in ultrasound water bath for 20 min at room temperature. After addition of 1.0 mL of 30 % (w/w) H_2O_2, the mixture was kept in ultrasound water bath for an additional 20 min at room temperature. No heating was performed; however, an increase of around 20°C in temperature was observed at the end of the process. Thereafter, microwave digestion was performed in microwave oven using the program summarized in **Table 2.25** which was same with the MW heating program 6. Different form previous heating programs MW heating program 6 took the longest time (45 min) and was reached the highest temperature (210 ^{0}C)

The obtained solutions were clear and colourless. The entire sample was observed to be digested with this method. Thereafter, the digest was diluted to 30 mL for ICP-OES 2100 DV and 60 mL for ICP-OES 5300 V using deionized water.

Table 2.25 MW heating program used in present study

	Step			
	1	**2**	**3**	**4**
T (^{o}C)	170	190	210	100
T_a (min)	2	1	1	1
Time (min)	15	10	10	10

T_a: Ramp Time to reach the related temperature.

Measurements of three replicates and one blank were performed. The results of the analysis, which are presented in **Table 2.26** and **Table 2.27,** were close to the certified value.

Table 2.26 Concentrations of elements in BCR-063R Skim Milk Powder, (mg/kg) CRM samples that prepared according to MW heating program used in present study.

		ICP-OES 2100 DV		**ICP-OES 5300 V**	
Elements	**BCR-063R Skim Milk Powder (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	4370±31	4321±71	99	4328±68	99
Mg	1263±24	1226±25	97	1200±30	95
P	11100±130	11283±226	102	10957±118	99
K	17680±190	17531±170	99	17107±270	97
Ca	13490±100	13396±122	99	13416±111	99
Fe	2.32±0.23	2.34±0.10	101	2.45±0.28	106
Zn	49.0±0.6	46.0±1.6	94	48.9±1.1	100

Table 2.27 Concentrations of elements in GBW 10011 Wheat, (mg/kg) CRM samples that prepared according to MW heating program used in present study.

		ICP-OES 2100 DV		ICP-OES 5300 V	
Elements	**GBW 10011 Wheat (mg/kg)**	**Present Study (mg/kg)**	**Accuracy (%)**	**Present Study (mg/kg)**	**Accuracy (%)**
Na	17±5	17±1	100	17±2	100
Mg	450±70	420±11	93	423±45	94
P	1540±70	1479±72	96	1527±30	99
K	1400±6	1340±41	96	1343±46	96
Ca	340±20	340±14	100	350±33	103
Fe	18.5±3.1	18.0±0.9	97	18.1±0.6	98
Zn	11.6±0.7	11.6±0.5	100	11.8±1.4	102

2.4.2. Determination of the Analytical Figures of Merit; LOD, LOQ, Accuracy and Precision

2.4.2.1. Determination of LOD and LOQ

LOD (limit of detection), is the lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit (generally 99 %). Experimentally, an analyte concentration at which S/N ratio is 3.3 is accepted as LOD. **(Say et al., 2009)** The following equation was used for calculation:

$$\mathrm{LOD} = \frac{3.3\mathrm{SD}}{\mathrm{m}}$$

where SD presents the standard deviation of the blank solution (solution not containing the analyte) or the analyte solution with a known limited concentration in the calibration graph; m presents the value of the slope of the used calibration curve; the coefficient of 3.3 presents the S/N ratio. In different applications, LOD is also accepted as a concentration value at which the S/N ratio is 3 **(Say et al., 2009).**

In the present study, 10 blank solutions were measured in order to calculate the LOD values of the method used. The standard deviations of the calculated values were estimated and the value of S/N ratio was accepted 3 for LOD.

LOQ (limit of quantification) is a concentration not within the linearity limits or the lowest concentration of the linearity at which amount of an analyte can be precisely and accurately detected. Experimentally, an analyte concentration at which S/N ratio is 10 is accepted as the quantification limit. The following equation is used for calculation; **(Say et al., 2009)**

$$\mathrm{LOQ} = \frac{10\mathrm{SD}}{\mathrm{m}}$$

In order to calculate the LOQ values of the method used in the present study, 10 different blank solutions were tested. The standard deviations of the calculated values were estimated and the value of S/N ratio was accepted 10 for LOQ. Calculated LOQ values for Na, Mg, P, K, Ca Fe and Zn were also used as the lowest limit of the dynamic range in the calibration studies.

LOD and LOQ values of the samples were obtained by multiplying the LOD and LOQ values in mg/L units by dilution factors which were 150 in the present study. Since 0.2 g of sample were digested in 4.0 mL of 65 % (w/w) HNO_3 and 1.0 mL of 30 % (w/w) H_2O_2 and after digestion, they were diluted with deionized water to 30.0 mL in volumetric flasks for the analyses. By this way, the units were converted into mg/kg in order to make more accurate evaluations.

LOD and LOQ values of the Na, Mg, P, K, Ca, Fe and Zn that obtained by using ICP-OES 2100 DV is smaller than the values obtained from the ICP-OES 5300 V whereby ICP-OES 2100 DV has axial view configuration and ICP-OES 5300 V has

radial view configuration. The detailed LOD and LOQ values of the samples for Na, Mg, P, K, Ca, Fe and Zn are listed in **Table 2.28** and **Table 2.29.**

Table 2.28 LOD and LOQ values for ICP-OES 2100 DV analyses system in present study, n=10.

Elements	LOD (mg/L)	LOQ (mg/L)	LOD (mg/kg)	LOQ (mg/kg)
Na	0.015	0.05	2.25	7.50
Mg	0.039	0.13	5.85	19.5
P	0.045	0.15	6.75	22.5
K	0.024	0.08	3.60	12.0
Ca	0.003	0.01	0.45	1.50
Fe	0.003	0.01	0.45	1.50
Zn	0.003	0.01	0.45	1.50

Table 2.29 LOD and LOQ values for ICP-OES 5300 V analyses system in present study, n=10.

Elements	LOD (mg/L)	LOQ (mg/L)	LOD (mg/kg)	LOQ (mg/kg)
Na	0.045	0.15	6.75	22.5
Mg	0.072	0.24	10.8	36.0
P	0.150	0.50	22.5	75.0
K	0.075	0.25	11.3	37.5
Ca	0.090	0.30	13.5	45.0
Fe	0.015	0.05	2.25	7.50
Zn	0.018	0.06	2.70	9.00

2.4.2.2. Accuracy and Precision

2.4.2.2.1. Accuracy

Accuracy is defined as the closeness of the observed results to the true value. In the present study, the accuracy of the method was checked by using experimental results of the BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011Wheat

CRM (China). CRM samples of 0.2 g were digested in 4.0 mL of 65 % (w/w) HNO_3 and 1.0 mL of 30 % (w/w) H_2O_2 by a MW digestion system. Digested solutions were diluted to 30.0 mL and 60.0 mL with deionized water and analyzed by ICP-OES 2100 DV and ICP-OES 5300 V devices, respectively.

During the analyses direct calibration method was used with three replicates and one blank measurement. Analysis results were in confidence interval of the certified value of the CRMs. % RSD values of the analyses for Na, Mg, P, K, Ca, Fe and Zn were determined as smaller than 10 for both CRM and for both ICP-OES systems. Student t-test was applied at 95% confidence levels. It is concluded that there is no significant difference between the experimental values and certified values at 95% confidence level. The experimental results and certified values are listed in **Table 2.30** and **Table 2.31.**

Table 2.30 The experimental results, and certified values for ICP-OES 2100 DV analyses system.

Elements	**GBW 10011 Wheat (mg/kg)**	**Present Study (mg/kg)**	**BCR-063R Skim Milk Powder (mg/kg)**	**Present Study (mg/kg)**
Na	17±5	17±1	4370±31	4321±71
Mg	450±70	420±13	1263±24	1226±25
P	1540±70	1479±72	11100±130	11283±226
K	1400±60	1340±41	17680±190	17531±170
Ca	340±23	341±13.9	13490±100	13396±122
Fe	18.5±3.1	18.0±0.9	2.32±0.23	2.34±0.10
Zn	11.6±0.7	11.6±0.5	49.0±0.6	46.1±1.6

Table 2.31 The experimental results and certified values for ICP-OES 5300 V analyses system

Elements	GBW 10011 Wheat (mg/kg)	Present Study (mg/kg)	BCR-063R Skim Milk Powder (mg/kg)	Present Study (mg/kg)
Na	17±5	17±2	4370±31	4328±68
Mg	450±70	423±45	1263±24	1200±30
P	1540±70	1527±30	11100±130	10957±118
K	1400±60	1343±46	17680±190	17507±270
Ca	340±23	350±33	13490±100	13419±111
Fe	18.5±3.1	18.1±0.6	2.32±0.23	2.55±0.25
Zn	11.6±0.7	11.8±1.1	49.0±0.6	48.9±1.1

2.4.2.2.2. Precision

Precision is defined as a measure of repeatability for a method developed. Precision is generally expressed as percent relative standard deviation (% RSD). In the present study, precision is determined in three steps using BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011Wheat CRM (China).

2.4.2.2.2.1. Injection Repeatability

During the injection repeatability studies, one BCR-063R Skim Milk Powder CRM (IRMM, EU) and one GBW 10011Wheat CRM (China) sample was prepared by MW digestion system. Samples were tested ten times separately and straight in the same operating conditions with the baby formulas' analyses conditions. Standard deviation, relative standard deviation and percent relative standard deviation were calculated.

2.4.2.2.2.2. Intraday Repeatability

During the intraday repeatability studies, CRM samples were re-prepared within a day in the same operating conditions with the baby formulas' analyses conditions and tested five times separately. Standard deviation, relative standard deviation and percent relative standard deviation were calculated.

2.4.2.2.2.3. Interday Repeatability

During the interday repeatability studies, CRM samples was freshly prepared in the same operating conditions with the baby formulas' analyses conditions for three consecutive days and tested three times. Standard deviation, relative standard deviation and percent relative standard deviation was calculated.

Calculated % RSD values for injection repeatability, intraday repeatability and interday repeatability were found between 1.0 and 5.2. Detailed % RSD values are presented in **Table 2.32** and **Table 2.33.**

Table 2.32 RSD % values of repeatability for ICP-OES 2100 DV

	BCR-063R Skim Milk Powder CRM			**GBW 10011Wheat CRM**		
Elements	**Injection %RSD**	**Intraday %RSD**	**Interday %RSD**	**Injection %RSD**	**Intraday %RSD**	**Interday %RSD**
Na	1.2	2.2	2.9	1.0	2.1	3.9
Mg	2.1	2.6	3.6	1.9	2.2	3.5
P	1.3	2.1	3.2	1.2	1.6	3.1
K	1.2	2.0	2.8	1.1	2.0	3.3
Ca	1.2	2.0	2.7	1.3	1.7	3.0
Fe	2.7	3.9	5.2	3.0	3.8	4.8
Zn	1.7	3.5	4.8	1.7	3.2	4.5

Table 2.33 RSD% values of repeatability for ICP-OES 5300 V

Elements	BCR-063R Skim Milk Powder CRM			GBW 10011Wheat CRM		
	Injection %RSD	Intraday %RSD	Interday %RSD	Injection %RSD	Intraday %RSD	Interday %RSD
Na	1.3	2.1	2.4	1.3	2.5	3.8
Mg	2.1	2.6	3.0	1.2	2.4	3.1
P	1.0	1.8	2.0	1.2	2.2	2.8
K	1.4	2.1	2.6	1.2	2.5	3.2
Ca	1.1	1.4	1.6	1.2	2.4	3.1
Fe	3.4	4.2	5.2	1.9	2.5	3.0
Zn	2.1	3.0	3.8	1.8	3.4	4.0

CHAPTER 3

RESULTS AND DISCUSSION

This chapter presents concentrations of the infant formulas infant follow-on formulas and fortified baby formulas, collected from a supermarket in Ankara which were analyzed by the method that was validated in the present study.

The samples, which were prepared as mentioned Chapter 2, were injected to the ICP-OES 5300 V (Perkin Elmer, USA), which performs measurements on the radial axis, via an autosampler and injected to the ICP-OES 2100 DV device (Perkin Elmer, USA), which performs measurements on the axial axis, via an autosampler. Data obtained from the detectors of the device were analyzed using both the Software system (WinLab 32^{TM} Version 5.2) of the device and the Microsoft Excel (Microsoft Corporation, USA) program.

3.1. Results of the Samples

In the present study, 21 different samples from 4 different brands of formula were used. Among these, 5 samples were infant formulas, 9 samples were infant follow-on formulas, and 7 samples were fortified baby formulas. The samples were purchased from supermarkets in Ankara. Infant formulas and infant follow-on formulas were milk and milk-protein based, whereas fortified baby formulas were cereal- and fruit-based.

As previously mentioned, approximately 0.2 g samples were weighed and prepared in microwave oven using 4 mL of 65 % (w/w) HNO_3 and 1 mL of 30 % (w/w) H_2O_2 and diluted to 30 mL for the analyses.

Three parallel analyses were carried out for each formula sample. The average of the results of the three analyses was calculated as the final result.

The experimental results were evaluated within each other for infant formulas, fortified baby formulas and in two groups for infant follow-on formulas. In the evaluation of the experimental results, it was used from the values on the nutritional facts tables (NFT) that gives you information on the amount of core nutrients and calories in an amount of food. NFT were provided by the manufacturer of the samples, and the accuracy values were calculated based on these values.

Consistency of the experimental results of the formulas with the values on the NFT and comparison of the results obtained in ICP-OES 2100 DV system with those obtained in ICP-OES 5300 V are presented with statistical methods (Student's t-test) in Section 3.2.

Evaluation of the results of the analyses with respect to daily requirements index for babies and tolerable upper intake levels are presented in the Section 3.3.

The experimental results of the formulas obtained in the present study were also compared with the values specified in the National Regulations and Standards and are discussed in the in the Section 3.4.

3.1.1. Results of the Infant Formulas

The obtained analyses results of the infant formulas were evaluated by taking the values on the NFT of the samples into consideration. The minimum and maximum mass values of the elements according to the NFT provided by the manufacturer are given in **Table 3.1.**

Table 3.1 Maximum and minimum mass of the elements (mg/kg) in IF according to the NFT provided by the manufacturer.

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1250	IF-A-1	1550	IF-C-1
Mg	360	IF-D-1	460	IF-C-1
P	1900	IF-C-1	2200	IF-D-1
K	4779	IF-A-1	5450	IF-D-1
Ca	3429	IF-A-2	3850	IF-C-1 , IF-D-1
Fe	38.6	IF-A-2	60.0	IF-C-1
Zn	36.4	IF-A-2	46.0	IF-C-1

According to the analyses performed using ICP-OES 2100 DV device on the axial view, the minimum and maximum mass of the elements are given **Table 3.2** and according to the analyses performed using ICP-OES 5300 V device on the radial view revealed the minimum and maximum mass of the elements are given **Table 3.3.**

Table 3.2 Maximum and minimum mass of the elements (mg/kg) in IF according to the analyses performed using ICP-OES 2100 DV

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1148	IF-B-1	1637	IF-C-1
Mg	366	IF-A-1	518	IF-C-1
P	1727	IF-A-1	2035	IF-D-1
K	4416	IF-B-1	5027	IF-C-1
Ca	3138	IF-A-1	3985	IF-C-1
Fe	34.2	IF-B-1	53.9	IF-C-1
Zn	33.2	IF-A-2	42.4	IF-C-1

Table 3.3 Maximum and minimum mass of the elements (mg/kg) in IF according to the analyses performed using ICP-OES 5300 V

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1120	IF-B-1	1547	IF-C-1
Mg	370	IF-B-1	519	IF-C-1
P	1947	IF-A-1	2214	IF-D-1
K	5040	IF-A-2	5306	IF-D-1
Ca	3553	IF-B-1	3816	IF-A-1
Fe	38.6	IF-B-1	59.1	IF-C-1
Zn	35.3	IF-B-1	47.1	IF-C-1

The detailed results of ICP-OES 2100 DV analyses of infant formulas for Na, Mg, P, K, Ca, Fe and Zn are given in **Table 3.4**, detailed results of ICP-OES 5300 V analyses for Na, Mg, P, K, Ca, Fe and Zn are given in **Table 3.5** and comparison of the results of that ICP-OES devices' are given in **Table 3.6**.

The accuracy of the infant formulas that calculated based on the values on the NFT ranged from 90% to 110%. The accuracy values outside this range are shown in **bold** in **Table 3.4** and **Table 3.5.**

Table 3.4 The results of the infant formulas analyses for Na, Mg, P, K, Ca, Fe, and Zn by using ICP-OES 2100 DV.

Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**
IF-A-1 (mg/kg)	4548±94	1157±45	34.0±1.5	3138±130	1727±75	366±7	35.0±1.7
Reference Value (mg/kg)	4779	1250	36.8	3456	1912	375	39.0
Accuracy (%)	95.2	92.6	92.4	90.8	90.3	97.5	90.0
IF-A-2 (mg/kg)	4451±120	1180±45	33.2±1.4	3418±74	1816±56	406±9	35.7±1.6
Reference Value (mg/kg)	4786	1286	36.4	3429	1929	379	38.6
Accuracy (%)	93.0	91.8	91.1	99.7	94.2	107.3	92.6
IF-C-1 (mg/kg)	5027±80	1637±40	42.4±2.0	3985±70	1907±48	518±14	53.9±2.6
Reference Value (mg/kg)	5000	1550	46.0	3850	1900	460	60.0
Accuracy (%)	100.5	105.6	92.1	103.5	100.4	**112.5**	90.0
IF-B-1 (mg/kg)	4416±150	1148±42	33.4±1.4	3235±95	1754±69	368±7	34.2±2.2
Reference Value (mg/kg)	4779	1250	36.8	3456	1912	375	39.0
Accuracy (%)	92.4	91.9	90.8	93.6	91.8	98.1	**87.7**
IF-D-1 (mg/kg)	4977±200	1412±40	37.4±1.6	3516±145	2035±67	396±15	50.9±2.0
Reference Value (mg/kg)	5450	1500	38.0	3850	2200	360	53.0
Accuracy (%)	91.3	94.1	98.5	91.3	92.5	110.0	96.1

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the nutrition facts table.
Detailed information about samples and coding system of the samples were given in Table 2.1.

Table 3.5 The results of the infant formulas analyses for Na, Mg, P, K, Ca, Fe, and Zn by using ICP-OES 5300 V.

Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**
IF-A-1 (mg/kg)	5180±165	1252±44	39.2±1.5	3816±89	1947±55	401±11	40.9±1.5
Reference Value (mg/kg)	4779	1250	36.8	3456	1912	375	39.0
Accuracy (%)	108.4	100.2	106.5	110.4	101.8	106.9	104.9
IF-A-2 (mg/kg)	5040±121	1203±44	38.0±1.2	3638±87	2005±56	401±11	40.9±1.6
Reference Value (mg/kg)	4786	1286	36.4	3429	1929	379	38.6
Accuracy (%)	105.3	93.6	104.3	106.1	104.0	105.9	106.1
IF-C-1 (mg/kg)	5228±102	1547±50	47.1±2.0	3725±80	2014±50	519±15	59.1±2.1
Reference Value (mg/kg)	5000	1550	46.0	3850	1900	460	60.0
Accuracy (%)	104.6	99.8	102.3	96.8	106.0	**112.8**	98.5
IF-B-1 (mg/kg)	5063±120	1120±55	35.2±1.4	3553±75	1986±56	370±8	38.6±1.5
Reference Value (mg/kg)	4779	1250	36.8	3456	1912	375	39.0
Accuracy (%)	105.9	90.0	96.1	102.8	103.9	98.7	99.0
IF-D-1 (mg/kg)	5306±103	1535±45	37.7±1.6	3721±75	2214±60	388±2	51.0±2.0
Reference Value (mg/kg)	5450	1500	38.0	3850	2200	360	53.0
Accuracy (%)	97.4	102.3	99.1	96.7	100.6	107.8	96.2

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the nutrition facts table.
Detailed information about samples and coding system of the samples were given in Table 2.1.

Table 3.6 Comparison of the ICP-OES 2100 DV with ICP-OES 5300 V analyses results for the infant formulas.

Sample	K	Na	Zn	Ca	P	Mg	Fe
IF-A-1[a] (mg/kg)	4548±94	1157±45	34.0±1.5	3138±130	1727±75	366±7.	35.0±1.7
IF-A-1[b] (mg/kg)	5180±165	1252±44	39.2±1.5	3816±89	1947±55	401±11	40.9±1.5
Reference Value (mg/kg)	4779	1250	36.8	3456	1912	375	39.0
IF-A-2[a] (mg/kg)	4451±120	1180±45	33.2±1.4	3418±74	1816±56	406±9	35.7±1.6
IF-A-2[b] (mg/kg)	5040±121	1203±44	38.0±1.2	3638±87	2005±56	401±11	40.9±1.6
Reference Value (mg/kg)	4786	1286	36.4	3429	1929	379	38.6
IF-C-1[a] (mg/kg)	5027±80	1637±40	42.4±2.0	3985±70	1907±48	518±14	53.9±2.6
IF-C-1[b] (mg/kg)	5228±102	1547±50	47.1±2.0	3725±80	2014±50	519±15	59.1±2.1
Reference Value (mg/kg)	5000	1550	46.0	3850	1900	460	60.0
IF-B-1[a] (mg/kg)	4416±150	1148±42	33.4±1.4	3235±95	1754±69	368±7	34.2±2.2
IF-B-1[b] (mg/kg)	5063±120	1120±55	35.3±1.4	3553±75	1986±56	370±8	38.6±1.5
Reference Value (mg/kg)	4779	1250	36.8	3456	1912	375	39.0
IF-D-1[a] (mg/kg)	4977±200	1412±40	37.4±1.6	3516±145	2035±67	396±15	50.9±2.0
IF-D-1[b] (mg/kg)	5306±103	1535±45	37.7±1.6	3721±75	2214±60	388±2	51.0±2.0
Reference Value (mg/kg)	5450	1500	38.0	3850	2200	360	53.0

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the nutrition facts table.
Detailed information about samples and coding system of the samples were given in Table 2.1.
[a]: ICP-OES 2100 DV [b] : ICP-OES 5300 V

3.1.2. Results of the Infant Follow-on Formulas

Infant follow-on formulas were analyzed in 2 groups according to the age of onset. The first group included those for infants over 6 months of age and the second group included those recommended for babies over 9 months of age.

The minimum and maximum mass of the elements of the samples included in the first group according to according to the NFT provided by the manufacturer are given in **Table 3.7**

Table 3.7 Maximum and minimum mass of the elements (mg/kg) IFF (1st group) according to the NFT provided by the manufacturer.

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1233	IFF-B-1	1600	IFF-C-1
Mg	329	IFF-B-1	520	IFF-C-1
P	2329	IFF-A-1	3550	IFF-C-1
K	4384	IFF-A-1	5900	IFF-D-1
Ca	4178	IFF-A-1	5300	IFF-D-1
Fe	68.5	IFF-A-1	73.0	IFF-C-1
Zn	34.9	IFF-A-1	47.0	IFF-C-1

According to the analyses of infant follow-on formulas performed using ICP-OES 2100 DV device on the axial viewing, the minimum and maximum mass of the elements of the samples included in the first group are given in **Table 3.8**

Table 3.8 Maximum and minimum mass of the elements (mg/kg) in IFF (1st group) according to the analyses performed using ICP-OES 2100 DV

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1123	IFF-B-1	1542	IFF-D-1
Mg	320	IFF-A-1	504	IFF-C-1
P	2093	IFF-A-1	3123	IFF-C-1
K	3988	IFF-A-1	5409	IFF-D-1
Ca	3988	IFF-A-1	4935	IFF-D-1
Fe	61.8	IFF-A-1	69.6	IFF-C-1
Zn	30.9	IFF-A-1	45.1	IFF-C-1

According to the analyses of infant follow on formulas performed using ICP-OES 5300 V device on the radial viewing, the minimum and maximum values of the elements of the samples included in the first group are given in **Table 3.9**

Table 3.9 Maximum and minimum mass of the elements (mg/kg) in IFF (1st group) according to the analyses performed using ICP-OES 5300 V.

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1123	IFF-B-1	1615	IFF-C-1
Mg	293	IFF-A-1	503	IFF-C-1
P	2167	IFF-A-1	3317	IFF-C-1
K	4267	IFF-A-1	6097	IFF-D-1
Ca	4013	IFF-A-1	5457	IFF-D-1
Fe	63.8	IFF-A-1	77.6	IFF-C-1
Zn	30.9	IFF-A-1	47.6	IFF-C-1

In the first group, the accuracy of the infant follow-on formulas that calculated based on the values on the NFT ranged from 90% to 110%. The accuracy values outside this range are shown in **bold** in **Table 3.13** and **Table 3.14.**

The minimum and maximum mass of the elements of the samples included in the second group according to the NFT are given **Table 3.10.**

Table 3.10 Maximum and minimum mass of the elements (mg/kg) IFF (2^{nd} group) according to the NFT provided by the manufacturer.

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1164	IFF-B-3	1800	IFF-C-2
Mg	308	IFF-B-3	500	IFF-C-2
P	2333	IFF-A-2	2867	IFF-B-2
K	4333	IFF-A-2	6250	IFF-C-2
Ca	4200	IFF-A-2	5200	IFF-B-2
Fe	66.7	IFF-A-2, IFF-B-2	73.0	IFF-C-2, IFF-D-2
Zn	34.7	IFF-A-2	52.0	IFF-C-2

According to the analyses of infant follow-on formulas performed using ICP-OES 2100 DV device on the axial viewing, the minimum and maximum values of the elements of the samples included in the second group are given in **Table 3.11.**

Table 3.11 Maximum and minimum mass of the elements (mg/kg) in IFF (2^{nd} group) according to the analyses performed using ICP-OES 2100 DV

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1068	IFF-B-3	1683	IFF-C-2
Mg	293	IFF-B-3	4820	IFF-C-2
P	2131	IFF-A-2	2613	IFF-B-2
K	4389	IFF-A-2	5912	IFF-C-2
Ca	3997	IFF-A-2	4886	IFF-B-2
Fe	61.3	IFF-A-3	75.5	IFF-D-2
Zn	28.2	IFF-A-2	45.4	IFF-C-2

According to the analyses of infant follow-on formulas performed using ICP-OES 5300 V device on the radial viewing, the minimum and maximum values of the elements of the samples included in the second group are given in **Table 3.12**

Table 3.12 Maximum and minimum mass of the elements (mg/kg) in IFF (2nd group) according to the analyses performed using ICP-OES 5300 V

Elements	Minimum Mass (mg/kg)	Sample	Maximum Mass (mg/kg)	Sample
Na	1110	IFF-B-3	1805	IFF-C-2
Mg	283	IFF-B-3	471	IFF-C-2
P	2272	IFF-A-2	2849	IFF-B-2
K	4218	IFF-A-2	5966	IFF-C-2
Ca	4156	IFF-A-2	5303	IFF-B-2
Fe	66.6	IFF-B-3	72.1	IFF-C-2
Zn	29.9	IFF-A-2	48.3	IFF-C-2

In the second group, the accuracy results calculated based on the values on the NFT ranged from 90% to 110%. Accuracy values outside this range were obtained in the analyses of Na (87.8%) and Zn (81.4%) in the IFF-A-2 sample and in the analysis of Zn (87.3%) in the IFF-C-2 sample and in the analysis of Fe (89.4%) in the IFF-B-3 sample using ICP-OES 2100 DV device on the axial view. In addition, a accuracy value outside this range was also obtained in the analysis of Zn in the IFF-A-2 sample using ICP-OES 5300 V device on the radial view (86.2%). The accuracy values outside this range are shown in **bold** in **Table 3.15** and **Table 3.16.**

The detailed results of ICP-OES 2100 DV analyses of infant follow-on formulas for Na, Mg, P, K, Ca, Fe and Zn are given in **Table 3.13** and **Table 3.15**, detailed results of ICP-OES 5300 V analyses for Na, Mg, P, K, Ca, Fe and Zn are given in **Table 3.14** and **Table 3.16**. Comparison of the results for ICP-OES devices are given in **Table 3.17** and **Table 3.18**.

3.1.3. Results of the Fortified Baby Formulas

The detailed results of ICP-OES 2100 DV analyses of fortified baby formulas for Na, Mg, P, K, Ca, Fe and Zn are given in **Table 3.19**, detailed results of ICP-OES 5300 V analyses for Na, Mg, P, K, Ca, Fe and Zn are given in **Table 3.20** and comparison of the results of that ICP-OES devices' are given in Table **3.21**

Table 3.13 The results of the infant follow-on formulas (1st group) analyses for Na, Mg, P, K, Ca, Fe, and Zn by using ICP-OES 2100 DV.

Sample	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**
IFF-A-1 (mg/kg)	3988±160	1341±50	30.9±1.7	3988±90	2093±99	320±10	61.8±2.8
Reference Value (mg/kg)	4384	1438	34.9	4178	2329	343	68.5
Accuracy (%)	91.0	93.2	**88.6**	95.5	90.0	93.5	90.2
IFF-C-1 (mg/kg)	5217±150	1492±53	45.1±2.0	4487±135	3123±120	504±10	69.6±2.2
Reference Value (mg/kg)	5550	1600	47.0	4800	3550	520	73.0
Accuracy (%)	94.0	93.3	95.9	93.5	**88.0**	97.0	95.3
IFF-B-1 (mg/kg)	5383±185	1123±52	35.6±1.7	4860±160	2662±95	329±8	68.6±2.1
Reference Value (mg/kg)	5822	1233	39.0	5205	2877	329	68.5
Accuracy (%)	92.5	91.1	91.1	93.4	92.5	100.0	100.2
IFF-D-1 (mg/kg)	5409±200	1542±45	35.1±1.6	4935±150	2879±78	446±9	66.6±2.2
Reference Value (mg/kg)	5900	1500	37.0	5300	3000	430	70.0
Accuracy (%)	91.7	102.8	95.0	93.1	96.0	103.7	95.1

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the nutrition facts table.
Detailed information about samples and coding system of the samples were given in Table 2.1.

Table 3.14 The results of the infant follow-on formulas (1st group) analyses for Na, Mg, P, K, Ca, Fe, and Zn by using ICP-OES 5300 V

Sample	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**
IFF-A-1 (mg/kg)	4267±99	1381±40	30.9±1.8	4013±100	2167±82	293±12	63.8±2.2
Reference Value (mg/kg)	4384	1438	34.9	4178	2329	343	68.5
Accuracy (%)	97.3	96.0	**88.5**	96.1	93.1	**85.6**	93.2
IFF-C-1 (mg/kg)	5226±133	1615±43	47.6±2.0	5191±158	3317±95	503±10	77.6±2.1
Reference Value (mg/kg)	5550	1600	47.0	4800	3550	520	73.0
Accuracy (%)	94.2	100.9	101.3	108.2	93.4	96.7	106.3
IFF-B-1 (mg/kg)	5491±139	1123±46	39.5±1.7	5055±110	2802±75	333±8	67.7±1.5
Reference Value (mg/kg)	5822	1233	39.0	5205	2877	329	68.5
Accuracy (%)	94.3	91.1	101.3	97.1	97.4	101.3	98.8
IFF-D-1 (mg/kg)	6097±120	1450±45	39.4±1.6	5457±124	3010±78	429±9	65.5±1.4
Reference Value (mg/kg)	5900	1500	37.0	5300	3000	430	70.0
Accuracy (%)	103.3	96.7	106.5	103.0	100.3	99.8	93.6

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the nutrition facts table.
Detailed information about samples and coding system of the samples were given in Table 2.1.

Table 3.15 The results of the infant follow-on formulas (2nd group) analyses for Na, Mg, P, K, Ca, Fe, and Zn by using ICP-OES 2100 DV

Sample	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**
IFF-A-2 (mg/kg)	4389±114	1287±69	28.2±2.6	3997±92	2131±90	323±8	61.9±2.4
Reference Value (mg/kg)	4333	1467	34.7	4200	2333	340	66.7
Accuracy (%)	101.3	**87.8**	**81.4**	95.2	91.3	95.1	92.8
IFF-C-2 (mg/kg)	5912±150	1683±52	45.4±4.0	4633±99	2529±96	483±8	68.4±2.5
Reference Value (mg/kg)	6250	1800	52.0	4400	2750	500	73.0
Accuracy (%)	94.6	93.5	**87.3**	105.3	92.0	96.6	93.7
IFF-B-2 (mg/kg)	5325±200	1161±32	36.3±3.1	4886±128	2613±106	336±7	64.7±2.1
Reference Value (mg/kg)	5800	1200	39.3	5200	2867	327	66.7
Accuracy (%)	91.8	96.8	92.4	94.0	91.2	102.8	97.1
IFF-B-3 (mg/kg)	4877±175	1068±41	35.5±3.0	4769±88	2379±88	292±7	61.3±3.0
Reference Value (mg/kg)	5274	1164	39.0	4658	2534	308	68.5
Accuracy (%)	92.5	91.7	90.9	102.4	93.9	94.8	**89.4**
IFF-D-2 (mg/kg)	4998±225	1397±38	35.3±3.2	4677±117	2482±115	443±10	75.5±3.5
Reference Value (mg/kg)	5550	1400	35.0	4950	2750	420	73.0
Accuracy (%)	90.1	99.8	100.9	94.5	90.3	105.5	103.4

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the nutrition facts table.
Detailed information about samples and coding system of the samples were given in Table 2.1.

Table 3.16 The results of the infant follow-on formulas (2nd group) analyses for Na, Mg, P, K, Ca, Fe, and Zn by using ICP-OES 5300 V

Sample	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**
IFF-A-2 (mg/kg)	4218±104	1400±34	29.9±2.2	4156±80	2272±50	313±11	67.2±2.4
Reference Value (mg/kg)	4333	1467	34.7	4200	2333	340	66.7
Accuracy (%)	97.3	95.5	**86.2**	99.0	97.4	92.1	100.8
IFF-C-2 (mg/kg)	5966±140	1805±32	48.3±4.1	4568±86	2624±56	471±12	72.1±2.5
Reference Value (mg/kg)	6250	1800	52.0	4400	2750	500	73.0
Accuracy (%)	95.5	100.3	92.9	103.8	95.4	94.2	98.7
IFF-B-2 (mg/kg)	5408±170	1154±22	37.2±3.0	5303±99	2849±49	328±7	70.6±2.3
Reference Value (mg/kg)	5800	1200	39.3	5200	2867	327	66.7
Accuracy (%)	93.2	96.2	94.6	102.0	99.4	100.4	105.8
IFF-B-3 (mg/kg)	4766±210	1110±31	36.2±3.0	4797±88	2484±48	283±12	66.6±2.7
Reference Value (mg/kg)	5274	1164	39.0	4658	2534	308	68.5
Accuracy (%)	90.4	95.3	92.8	103.0	98.0	91.8	97.2
IFF-D-2 (mg/kg)	5006±220	1446±28	32.6±2.5	4743±97	2627±57	383±15	72.0±3.0
Reference Value (mg/kg)	5550	1400	35.0	4950	2750	420	73.0
Accuracy (%)	90.2	103.3	93.2	95.8	95.5	91.2	98.7

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the nutrition facts table.
Detailed information about samples and coding system of the samples were given in Table 2.1.

Table 3.17 Comparison of the ICP-OES 2100 DV with ICP-OES 5300 V analyses results for the infant follow-on formulas (1st group).

Sample	K	Na	Zn	Ca	P	Mg	Fe
IFF-A-1[a] (mg/kg)	3988±160	1341±50	30.9±1.7	3988±90	2093±99	320±10	61.8±2.8
IFF-A-1[b] (mg/kg)	4267±99	1381±40	30.9±1.8	4013±100	2167±82	293±12	63.8±2.2
Reference Value (mg/kg)	4384	1438	34.9	4178	2329	343	68.5
IFF-C-1[a] (mg/kg)	5217±150	1492±53	45.1±2.0	4487±135	3123±120	504±10	69.6±2.2
IFF-C-1[b] (mg/kg)	5226±133	1615±43	47.6±2.0	5191±158	3317±95	503±10	77.6±2.1
Reference Value (mg/kg)	5550	1600	47.0	4800	3550	520	73.0
IFF-B-1[a] (mg/kg)	5383±185	1123±52	35.6±1.7	4860±160	2662±95	329±8	68.6±2.1
IFF-B-1[b] (mg/kg)	5491±139	1123±46	39.5±1.7	5055±110	2802±75	333±8	67.7±1.5
Reference Value (mg/kg)	5822	1233	39.0	5205	2877	329	68.5
IFF-D-1[a] (mg/kg)	5409±200	1542±45	35.1±1.6	4935±150	2879±78	446±9	66.6±2.2
IFF-D-1[b] (mg/kg)	6097±120	1450±45	39.4±1.6	5457±124	3010±78	429±9	65.5±1.4
Reference Value (mg/kg)	5900	1500	37.0	5300	3000	430	70.0

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the nutrition facts table.
Detailed information about samples and coding system of the samples were given in Table 2.1.
[a]: ICP-OES 2100 DV [b]: ICP-OES 5300 V

Table 3.18 Comparison of the ICP-OES 2100 DV with ICP-OES 5300 V analyses results for the infant follow-on formulas (2nd group).

Sample	K	Na	Zn	Ca	P	Mg	Fe
IFF-A-2[a] (mg/kg)	4389±114	1287±69	28.2±2.6	3997±92	2131±90	323±8	61.9±2.4
IFF-A-2[b] (mg/kg)	4218±104	1400±34	29.9±2.2	4156±80	2272±50	313±11	67.2±2.4
Reference Value (mg/kg)	4333	1467	34.7	4200	2333	340	66.7
IFF-C-2[a] (mg/kg)	5912±150	1683±52	45.4±4.0	4633±99	2529±96	483±8	68.4±2.5
IFF-C-2[b] (mg/kg)	5966±140	1805±32	48.3±4.1	4568±86	2624±56	471±12	72.1±2.5
Reference Value (mg/kg)	6250	1800	52.0	4400	2750	500	73.0
IFF-B-2[a] (mg/kg)	5325±200	1161±32	36.3±3.1	4886±128	2613±106	336±7	64.7±2.1
IFF-B-2[b] (mg/kg)	5408±170	1154±22	37.2±3.0	5303±99	2849±49	328±7	70.6±2.3
Reference Value (mg/kg)	5800	1200	39.3	5200	2867	327	66.7
IFF-B-3[a] (mg/kg)	4877±175	1068±41	35.5±3.0	4769±88	2379±88	292±7	61.3±3.0
IFF-B-3[b] (mg/kg)	4766±210	1110±31	36.2±3.0	4797±88	2484±48	283±12	66.6±2.7
Reference Value (mg/kg)	5274	1164	39.0	4658	2534	308	68.5
IFF-D-2[a] (mg/kg)	4998±225	1397±38	35.3±3.2	4677±117	2482±115	443±10	75.5±3.5
IFF-D-2[b] (mg/kg)	5006±220	1446±28	32.6±2.5	4743±97	2627±57	383±15	72.0±3.0
Reference Value (mg/kg)	5550	1400	35.0	4950	2750	420	73.0

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the NFT.
Detailed information about samples and coding system of the samples were given in Table 2.1.
[a]: ICP-OES 2100 DV [b]: ICP-OES 5300 V

Table 3.19 The results of the fortified baby formulas analyses for Na, Mg, P, K, Ca, Fe, and Zn by using ICP-OES 2100DV

Sample	K	Na	Zn	Ca	P	Mg	Fe
FBF-A-2 (mg/kg)	2343±48	199±4	10.0±1.0	3372±64	1377±64	384±7	89.1±4.0
Reference Value (mg/kg)	1500	200.0	21.0	3500	-	200	96.0
Accuracy (%)	156.2	99.5	47.6	96.3	-	192.0	92.8
FBF-A-1 (mg/kg)	5755±120	941±29	18.5±1.9	3329±64	2125±74	504±10	47.6±2.0
Reference Value (mg/kg)	6000	1000	21.0	4200	2300	500	50.0
Accuracy (%)	95.9	94.1	87.9	79.3	92.4	100.8	95.2
FBF-C-2 (mg/kg)	4350±95	1015±40	65.5±7.0	4648±99	3138±77	513±11	76.1±3.0
Reference Value (mg/kg)	-	1300	66.0	4890	-	501	100.0
Accuracy (%)	-	78.1	99.2	95.1	-	102.4	76.1
FBF-C-1 (mg/kg)	5209±112	1334±66	64.2±6.0	5143±102	3567±82	486±21	30.1±1.5
Reference Value (mg/kg)	-	1800	50.0	6610	-	440	100.0
Accuracy (%)	-	74.1	128.4	77.8	-	110.3	30.1
FBF-B-2 (mg/kg)	2066±41	100±2	7.6±0.8	3372±75	1103±45	359±17	92.8±4.2
Reference Value (mg/kg)	2100	100	-	3500	1200	300	100.0
Accuracy (%)	98.4	100.0	-	96.3	91.9	119.7	92.76
FBF-B-1 (mg/kg)	4164±85	1030±31	17.0±1.7	3193±52	2206±52	353±17	35.1±1.6
Reference Value (mg/kg)	4000	1100	23.0	4120	3000	500	38.0
Accuracy (%)	104.1	93.6	73.7	77.5	73.5	70.6	92.5
FBF-B-3 (mg/kg)	4259±73	947±28	34.8±3.5	2917±60	1970±50	364±17	37.5±1.7
Reference Value (mg/kg)	4300	1000	18.0	3600	2600	450	40.0
Accuracy (%)	99.1	94.7	193.4	81.0	75.8	80.8	93.6

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the NFT.
Detailed information about samples and coding system of the samples were given in Table 2.1.

Table 3.20 The results of the fortified baby formulas analyses for Na, Mg, P, K, Ca, Fe, and Zn by using ICP-OES 5300 V.

Sample	K	Na	Zn	Ca	P	Mg	Fe
FBF-A-2 (mg/kg)	2489±54	206±4	10.1±1.00	3524±84	1437±34	366±7	93.1±4.0
Reference Value (mg/kg)	1500	200	21.00	3500	-	200	96.0
Accuracy (%)	165.9	103.0	48.2	100.7	-	183.0	97.0
FBF-A-1 (mg/kg)	6129±85	972±19	18.3±1.9	3340±74	2167±34	487±10	46.1±2.0
Reference Value (mg/kg)	6000	1000	21.0	4200	2300	500	50.0
Accuracy (%)	102.2	97.2	87.1	79.5	94.2	97.4	92.2
FBF-C-2 (mg/kg)	4567±85	1027±20	68.5±7.0	4844±87	3417±47	492±11	76.5±3.00
Reference Value (mg/kg)	-	1300	66.0	4890	-	501	100.0
Accuracy (%)	-	79.0	103.7	99.1	-	98.2	76.5
FBF-C-1 (mg/kg)	5414±105	1394±26	65.8±6.4	5283±72	3641±52	458±9	30.9±1.5
Reference Value (mg/kg)	-	1800	50.0	6610	-	440	100.0
Accuracy (%)	-	77.4	131.6	79.9	-	104.1	30.9
FBF-B-2 (mg/kg)	2165±41	108±4	7.7±0.8	3532±65	1151±35	346±7	95.1±4.2
Reference Value (mg/kg)	2100	100	-	3500	1200	300	100.0
Accuracy (%)	103.1	108.0	-	100.9	95.9	115.3	95.1
FBF-B-1 (mg/kg)	4118±61	1063±21	17.8±2.1	3276±52	2260±32	334±7	36.4±1.6
Reference Value (mg/kg)	4000	1100	23.0	4120	3000	500	38.0
Accuracy (%)	103.0	96.6	77.4	79.5	75.3	66.8	95.8
FBF-B-3 (mg/kg)	4568±113	915±25	34.2±3.5	2912±60	2064±30	341±7	35.7±1.8
Reference Value (mg/kg)	4300	1000	18.0	3600	2600	450	40.0
Accuracy (%)	106.2	91.5	190.1	80.9	79.4	75.8	89.2

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in the NFT.
Detailed information about samples and coding system of the samples were given in Table 2.1.

Table 3.21 Comparison of the ICP-OES 2100 DV with ICP-OES 5300 V analyses results for the fortified baby formulas.

Sample	K	Na	Zn	Ca	P	Mg	Fe
FBF-A-2[a] (mg/kg)	2343±48	199±4	10.0±1.0	3372±64	1377±64	384±7	89.1±4.0
FBF-A-2[b] (mg/kg)	2489±54	206±4	10.1±1.0	3524±84	1437±34	366±7	93.1±4.0
Reference Value (mg/kg)	1500	200	21.0	3500	-	200	96.0
FBF-A-1[a] (mg/kg)	5755±120	941±29	18.5±1.9	3329±64	2125±74	504±10	47.6±2.0
FBF-A-1[b] (mg/kg)	6129±85	972±19	18.3±1.9	3340±74	2167±34	487±10	46.1±2.0
Reference Value (mg/kg)	6000	1000	21.0	4200	2300	500	50.0
FBF-C-2[a] (mg/kg)	4350±95	1015±40	65.5±7.0	4648±99	3138±77	513±11	76.1±3.0
FBF-C-2[b] (mg/kg)	4567±85	1027±20	68.5±7.0	4844±87	3417±47	492±11	76.5±3.0
Reference Value (mg/kg)	-	1300	66.0	4890	-	501	100.0
FBF-C-1[a] (mg/kg)	5209±112	1334±66	64.2±6.0	5143±102	3567±82	486±21	30.1±1.5
FBF-C-1[b] (mg/kg)	5414±105	1394±26	65.8±6.4	5283±72	3641±52	458±9	30.9±1.5
Reference Value (mg/kg)	-	1800	50.0	6610	-	440	100.0
FBF-B-2[a] (mg/kg)	2066±41	100±2	7.6±0.8	3372±75	1103±45	359±17	92.8±4.2
FBF-B-2[b] (mg/kg)	2165±41	108±4	7.7±0.8	3532±65	1151±35	346±7	95.1±4.2
Reference Value (mg/kg)	2100	100	-	3500	1200	300	100.0
FBF-B-1[a] (mg/kg)	4164±85	1030±31	17.0±1.7	3193±52	2206±52	353±17	35.1±1.6
FBF-B-1[b] (mg/kg)	4118±61	1063±21	17.8±2.1	3276±52	2260±32	334±7	36.4±1.6
Reference Value (mg/kg)	4000	1100	23.0	4120	3000	500	38.0
FBF-B-3[a] (mg/kg)	4259±73	947±28	34.8±3.5	2917±60	1970±50	364±17	37.5±1.7
FBF-B-3[b] (mg/kg)	4568±113	915±25	34.2±3.5	2912±60	2064±30	341±7	35.7±1.8
Reference Value (mg/kg)	4300	1000	18.0	3600	2600	450	40.0

Reference Value: Mass of the related element in mg/kg, provided by the manufacturer in NFT.
Detailed information about samples and coding system of the samples were given in Table 2.1.
[a]: ICP-OES 2100 DV [b]: ICP-OES 5300 V

3.2. Statistical Evaluation of Results

The validity of the experimental results of the formulas analysis was statistically evaluated using Student's t-test. The t critical value was chosen taking the 95% confidence level into consideration.

3.2.1. Statistical Evaluation of Analyses Result versus Nutritional Facts Tables of the Samples

Firstly, the values found in the analysis were compared with the values on the NFT of the samples. t values were calculated according to the following formula,

$$t = \frac{\bar{x} - \mu_0}{s/\sqrt{N}}$$

where $\bar{x}$ is the mean of the values found in the analysis, μ_0 is the reference value on the NFT of the samples, s is the standard deviation of the results of the analysis, n is the number of measurements.

The calculated t values and critical t value are presented in **Table 3.22.** to **Table 3.29** The results with t values greater than the t critical value are shown in **bold**.

The majority of the t values found in the infant formula and infant follow-on formula analysis were lower than the t critical value, which means that analyses results of the present study for infant formula and infant follow-on formula can be considered not significantly different from the reference value on the NFT provided by the manufacturers of the formulas at 95 % confidence level. On the other hand, experimental t values could not be calculated for some of the fortified baby formulas because of lack of information in the NFT that provided by the manufacturer.

Moreover, t values found in some of the fortified baby formula were higher than the t critical value which means analyses results of the present study for some baby fortified formula can be considered significantly different at 95 % confidence level.

Table 3.22 The experimental t values and critical t value of the infant formulas at 95% confidence level for ICP-OES 2100 DV, n=3.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IF-A-1	4.264	3.580	3.210	4.235	4.267	2.301	4.055	
IF-A-2	**4.831**	4.061	4.008	0.247	3.482	**5.298**	3.107	
IF-C-1	0.585	3.767	3.135	3.340	0.253	**7.114**	4.070	±4.303
IF-B-1	4.196	4.190	4.182	4.027	3.960	1.732	3.771	
IF-D-1	4.096	3.811	0.628	3.990	4.265	4.192	1.801	

Table 3.23 The experimental t values and critical t value of the infant follow-on formulas (1st group) at 95% confidence level for ICP-OES 2100 DV, n=3.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IFF-A-1	4.282	3.373	4.065	3.658	4.125	3.875	4.163	
IFF-C-1	3.845	3.529	1.671	4.016	**6.163**	2.702	2.685	±4.303
IFF-B-1	4.109	3.660	3.525	3.740	3.915	0.006	0.115	
IFF-D-1	4.252	1.617	2.024	4.215	2.687	3.098	2.685	

Table 3.24 The experimental t values and critical t value of the infant follow-on formulas (2nd group) at 95% confidence level for ICP-OES 2100 DV, n=3.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IFF-A-2	0.846	4.510	4.297	3.822	3.889	3.880	3.479	
IFF-C-2	3.903	3.897	2.862	4.076	3.987	3.702	3.187	
IFF-B-2	4.114	2.111	1.676	4.249	4.145	2.220	1.625	±4.303
IFF-B-3	4.045	4.067	2.061	2.194	3.056	3.939	4.174	
IFF-D-2	4.249	0.137	0.168	4.041	4.036	4.001	1.227	

Table 3.25 The experimental t values and critical t value of the fortified baby formulas at 95% confidence level for ICP-OES 2100 DV, n=3.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
FBF-A-2	**30.42**	0.476	**19.10**	3.464	ND	**45.50**	3.005	
FBF-A-1	3.536	3.542	2.315	**23.57**	4.096	0.675	2.096	
FBF-C-2	ND	**12.36**	0.124	4.234	ND	1.921	**13.79**	
FBF-C-1	ND	**12.23**	4.093	**24.91**	ND	3.753	**80.73**	±4.303
FBF-B-2	1.436	0.260	ND	2.956	3.734	**6.021**	2.986	
FBF-B-1	3.342	3.933	**6.164**	**30.88**	**26.45**	**15.00**	3.096	
FBF-B-3	0.973	3.279	**8.319**	**19.72**	**21.82**	*8.793*	2.598	

ND: Not detected

Table 3.26 The experimental t values and critical t value of the infant formulas at 95% confidence level for ICP-OES 5300 V, n=3.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IF-A-1	4.205	0.079	2.760	**7.008**	1.109	4.094	2.240	
IF-A-2	3.640	3.256	2.281	4.169	2.364	3.532	2.566	
IF-C-1	3.872	0.104	0.918	2.706	3.949	**6.813**	0.742	±4.303
IF-B-1	4.093	4.094	1.782	2.243	2.296	1.083	0.450	
IF-D-1	2.422	1.347	0.368	2.979	0.404	4.041	1.749	

Table 3.27 The experimental t values and critical t value of the infant follow-on formulas (1st group) at 95% confidence level for ICP-OES 5300 V, n=3.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IFF-A-1	2.039	2.484	3.878	2.859	3.417	**7.140**	3.685	
IFF-C-1	4.219	0.604	0.546	4.286	4.248	2.944	3.819	±4.303
IFF-B-1	4.124	4.137	0.499	2.369	1.725	0.916	0.924	
IFF-D-1	2.843	1.925	2.587	2.193	0.222	0.192	3.226	

Table 3.28 The experimental t values and critical t value of the infant follow-on formulas (2^{nd} group) at 95% confidence level for ICP-OES 5300 V, n=3.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IFF-A-2	1.921	3.396	3.763	0.953	2.125	4.251	0.361	
IFF-C-2	3.514	0.271	1.559	3.384	3.897	4.186	0.651	
IFF-B-2	3.994	3.622	1.218	1.802	0.625	0.329	2.922	±4.303
IFF-B-3	4.190	3.038	1.617	2.745	1.813	3.640	1.219	
IFF-D-2	4.283	2.846	1.649	3.696	3.738	4.272	0.566	

Table 3.29 The experimental t values and critical t value of the fortified baby formulas at 95% confidence level for ICP-OES 5300 V, n=3.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
FBF-A-2	**31.72**	2.598	**18.827**	0.495	ND	**41.07**	1.243	
FBF-A-1	2.629	2.552	2.461	**20.13**	**6.775**	2.252	3.412	
FBF-C-2	ND	**23.64**	0.609	0.916	ND	1.417	**13.55**	
FBF-C-1	ND	**27.05**	4.273	**31.92**	ND	3.464	**79.85**	±4.303
FBF-B-2	2.746	3.959	ND	0.853	2.425	**11.38**	2.037	
FBF-B-1	3.351	3.052	4.289	**28.11**	**40.06**	**41.07**	1.732	
FBF-B-3	4.108	**5.889**	**8.022**	**19.86**	**30.95**	**26.97**	4.218	

ND: Not detected

3.2.2. Statistical Evaluation of Analyses Results of ICP-OES 2100 DV versus ICP-OES 5300 V

The results of the formulas analyses performed by the ICP-OES 2100 DV device system were compared with those of the analyses performed by the ICP-OES 5300 V using the Student's t-test at 95 % confidence level and 99% confidence level.

These statistical analyses were performed by the Microsoft Excel (Microsoft Corporation, USA) using the following formulas,

$$s = \sqrt{\frac{\sum_{i=1}^{N_1}(x_{1i} - \bar{x}_1)^2 + \sum_{i=1}^{N_2}(x_{2i} - \bar{x}_2)^2}{N_1 + N_2 - 2}} \qquad t = \frac{|\bar{x}_1 - \bar{x}_2|}{s\sqrt{\left(\frac{1}{N_1} + \frac{1}{N_2}\right)}}$$

where s indicates the combined standard deviation of the results of the same sample measured by the two different device systems, N_1 and N_2 indicate the number of the measurements, $\bar{x}_1$ and $\bar{x}_2$ indicate mean values of the results of the analysis, x_{1i} and x_{2i} indicate the results of the analysis, t_c indicates the calculated t value.

As previously mentioned, when the results of the formula samples analyzed by two different device systems were compared with the reference values on the nutritional facts tables of the samples, it was observed that the majority of the results were not significantly different at 95 % confidence level. On the other hand, comparison of the results of the same formula sample obtained by two different analyses systems (ICP-OES 2100 DV and ICP-OES 5300 V) revealed that there is a significant difference between the experimental and certified values at 95% confidence level. However, null hypothesis at 99% confidence level can be accepted owing to no significant difference between the certified and experimental values.

The tabulated t values are presented in **Table 3.30** to **Table 3.38**. The results with calculated t values greater than the t critical value are shown in **bolds**.

Table 3.30 The calculated t values and critical t value of the infant formulas at 95% confidence level for ICP-OES 5300 V versus ICP-OES 2100 DV, df=4.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IF-A-1	**5.764**	2.614	**4.221**	**7.454**	**4.097**	**4.689**	**4.523**	
IF-A-2	**5.986**	0.627	**4.528**	**3.336**	**4.134**	0.622	**4.011**	
IF-C-1	2.686	2.435	2.866	**4.236**	2.674	0.127	2.700	±2.776
IF-B-1	**5.834**	0.711	1.697	**4.551**	**4.522**	0.326	**2.862**	
IF-D-1	2.533	**3.538**	0.184	2.175	**3.447**	0.748	0.037	

Table 3.31 The calculated t values and critical t value of the infant formulas at 99% confidence level for ICP-OES 5300 V versus ICP-OES 2100 DV, df=4.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IF-A-1	**5.764**	2.614	4.221	**7.454**	4.097	**4.689**	4.523	
IF-A-2	**5.986**	0.627	4.528	3.336	4.134	0.622	4.011	
IF-C-1	2.686	2.435	2.866	4.236	2.674	0.127	2.700	±4.604
IF-B-1	**5.834**	0.711	1.697	4.551	4.522	0.326	2.862	
IF-D-1	2.533	3.538	0.184	2.175	3.447	0.748	0.037	

Table 3.32 The calculated t values and critical t value of the infant follow-on formulas (1st group) at 95% confidence level for ICP-OES 5300 V versus ICP-OES 2100 DV, df=4.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IFF-A-2	2.568	1.082	0.028	0.322	0.997	**3.005**	0.997	
IFF-C-2	0.078	**3.122**	1.568	**5.867**	2.195	0.171	**4.579**	±2.776
IFF-B-2	0.808	0.000	**2.846**	1.740	2.003	0.643	0.631	
IFF-B-3	**5.109**	2.504	**3.261**	**4.646**	2.057	2.327	0.564	

Table 3.33 The calculated t values and critical t value of the infant follow-on formulas (1st group) at 99% confidence level for ICP-OES 5300 V versus ICP-OES 2100 DV, df=4.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IFF-A-2	2.568	1.082	0.028	0.322	0.997	3.005	0.997	±4.604
IFF-C-2	0.078	3.122	1.568	**5.867**	2.195	0.171	4.579	
IFF-B-2	0.808	0.000	2.846	1.740	2.003	0.643	0.631	
IFF-B-3	**5.109**	2.504	3.261	**4.646**	2.057	2.327	0.564	

Table 3.34 The calculated t values and critical t value of the infant follow-on formulas (2nd group) at 95% confidence level for ICP-OES 5300 V versus ICP-OES 2100 DV, df=4.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IFF-A-2	1.919	2.544	0.849	2.259	2.368	1.327	2.715	±2.776
IFF-C-2	0.456	**3.461**	0.883	0.859	1.481	1.429	1.793	
IFF-B-2	0.548	0.312	0.357	**4.463**	**3.500**	1.363	**3.253**	
IFF-B-3	0.712	1.412	0.314	0.390	1.814	1.320	2.287	
IFF-D-2	0.044	1.798	1.147	0.752	1.957	**6.347**	1.300	

Table 3.35 The calculated t values and critical t value of the infant follow-on formulas (2nd group) at 99% confidence level for ICP-OES 5300 V versus ICP-OES 2100 DV, df=4.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
IFF-A-2	1.919	2.544	0.849	2.259	2.368	1.327	2.715	±4.604
IFF-C-2	0.456	3.461	0.883	0.859	1.481	1.429	1.793	
IFF-B-2	0.548	0.312	0.357	4.463	3.500	1.363	3.253	
IFF-B-3	0.712	1.412	0.314	0.390	1.814	1.320	2.287	
IFF-D-2	0.044	1.798	1.147	0.752	1.957	**6.347**	1.300	

Table 3.36 The calculated t values and critical t value of the fortified baby formulas at 95% confidence level for ICP-OES 5300 V versus ICP-OES 2100 DV,df=4.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
FBF-A-2	**3.500**	2.174	0.190	2.493	1.434	**3.132**	1.246	
FBF-A-1	**4.405**	1.564	0.103	0.195	0.893	2.070	0.931	
FBF-C-2	**2.948**	0.480	0.518	2.576	**5.357**	2.360	0.171	
FBF-C-1	2.313	1.465	0.318	1.942	1.320	2.085	0.621	±2.776
FBF-B-2	**2.957**	**3.308**	0.265	**2.792**	1.458	1.234	0.671	
FBF-B-1	0.762	1.545	0.545	1.955	1.532	1.771	0.964	
FBF-B-3	**3.978**	1.477	0.210	0.102	**2.792**	2.139	1.268	

Table 3.37 The calculated t values and critical t value of the fortified baby formulas at 99% confidence level for ICP-OES 5300 V versus ICP-OES 2100 DV,df=4.

	$t_{experimental}$							
Samples	**K**	**Na**	**Zn**	**Ca**	**P**	**Mg**	**Fe**	$t_{critical}$
FBF-A-2	3.500	2.174	0.190	2.493	1.434	3.132	1.246	
FBF-A-1	4.405	1.564	0.103	0.195	0.893	2.070	0.931	
FBF-C-2	2.948	0.480	0.518	2.576	**5.357**	2.360	0.171	
FBF-C-1	2.313	1.465	0.318	1.942	1.320	2.085	0.621	±4.604
FBF-B-2	2.957	3.308	0.265	2.792	1.458	1.234	0.671	
FBF-B-1	0.762	1.545	0.545	1.955	1.532	1.771	0.964	
FBF-B-3	3.978	1.477	0.210	0.102	2.792	2.139	1.268	

3.3. Conformity Assessment of Analyses Results According to Dietary Reference Intakes (DRIs)

Formulas, used in this study, were also evaluated in respect to meeting the daily needs of infants during feeding and it is concluded that all formulas analyzed in the present study can easily compensate babies' daily element requirements. Recommended Dietary Allowances (RDA), Adequate Intakes (AI) and Tolerable Upper Intake Levels for babies, ages between 0-1 year, with respect to mass of Na, K, Ca, Mg, P, Fe, and Zn are given **Table 3.38** and **Table 3.39**

Table 3.38 Dietary Reference Intakes (DRIs): Recommended Dietary Allowances (RDA) and Adequate Intakes (AI) (in mg/day) for elements.

Age (years)	Na[a,b]	K[a,b]	Ca[a,b]	P[a,c]	Mg[a,c]	Fe[a,c]	Zn[a,c]
0-0.5	120	400	210	100	30	0.27	2
0.5-1	370	700	270	275	75	11	3

a- mg/day
b- Adequate Intakes (AI)
c- Recommended Dietary Allowances (RDA)

Table 3.39 Dietary Reference Intakes (DRIs): Tolerable Upper Intake Levels (mg/day) for elements

Age (years)	Na[a,b]	K[a,b]	Ca	P[a,b]	Mg[a,b]	Fe	Zn
0-0.5	ND	ND	1000	ND	ND	40	4
0.5-1	ND	ND	1500	ND	ND	40	5

a- mg/day
b- ND: not determinable due to lack of data of adverse effects in this age group and concern with regard to lack of ability to handle excess amounts. Source of intake should be from food only to prevent high levels of intake

3.4. Conformity Assessment of Analyses Results According to National Regulations and Standards

The experimental results of the formulas obtained in the present study were also compared with the values specified in the National Regulations and Standards. According to the Turkish Food Codex, the National Regulation, the mass of Na, K, Ca, Mg, P, Zn, and Fe elements are allowed in infant formulas and these mass were found in the present study were presented in **Table 3.40** and **Table 3.41,** the amounts of Na, K, Ca, Mg, P, Zn, and Fe elements are allowed in infant follow-on formulas and these amounts were found in the present study were presented in **Table 3.42, Table 3.43, Table 3.44** and **Table 3.45,** the mass of Na, K, Ca, Mg, P, Zn, and Fe elements are allowed in infant formulas and these mass were found in the present study were presented in **Table 3.46** and **Table 3.47.**

Table 3.40 The mass of elements, in mg, that determined by ICP-OES 2100 DV for infant formulas which provides 100 kcal and legal boundaries according to Turkish Food Codex.

Elements	The mass of formula that provides 100 kcal						
	Legal Boundaries		Experimental Results				
	Lowest	Highest	IF-A-1	IF-A-2	IF-C-1	IF-B-1	IF-D-1
Na (mg)	20	60	24.2	24.7	32.1	24.0	28.0
K (mg)	60	160	95.2	93.0	98.6	92.4	98.8
Ca (mg)	50	140	65.7	71.4	78.1	67.7	69.8
P (mg)	25	90	36.1	38.0	37.4	36.7	40.4
Mg (mg)	5	15	7.65	8.49	10.2	7.70	7.86
Fe (mg)	0.3	1.3	0.73	0.75	1.06	0.72	1.01
Zn (mg)	0.5	1.5	0.71	0.69	0.83	0.70	0.74

Table 3.41 The mass of elements, in mg, that determined by ICP-OES 5300 V, for infant formulas that provides 100 kcal and legal boundaries according to Turkish Food Codex.

Elements	The mass of formula that provides 100 kcal						
	Legal Boundaries		Experimental Results				
	Lowest	Highest	IF-A-1	IF-A-2	IF-C-1	IF-B-1	IF-D-1
Na (mg)	20	60	26.2	25.1	30.3	23.4	30.5
K (mg)	60	160	108	105	103	106	105
Ca (mg)	50	140	79.8	76.0	73.0	74.3	73.8
P (mg)	25	90	40.7	41.9	39.5	41.6	43.9
Mg (mg)	5	15	8.39	8.38	10.2	7.74	7.70
Fe (mg)	0.3	1.3	0.86	0.86	1.16	0.81	1.01
Zn (mg)	0.5	1.5	0.82	0.79	0.92	0.74	0.75

Table 3.42 The mass of elements, in mg, that determined by ICP-OES 2100 DV for the 1st group of infant follow-on formulas that provides 100 kcal and legal boundaries according to Turkish Food Codex.

Elements	The mass of formula that provides 100 kcal					
	Legal Boundaries		Experimental Results			
	Lowest	Highest	IFF-A-1	IFF-C-1	IFF-B-1	IFF-D-1
Na (mg)	20	60	28.0	32.1	24.5	31.0
K (mg)	60	160	83.3	112	117	109
Ca (mg)	50	140	83.3	96.5	106	99.1
P (mg)	25	90	43.7	67.2	58.0	57.8
Mg (mg)	5	15	6.69	10.9	7.16	8.96
Fe (mg)	0.6	2.0	1.29	1.50	1.50	1.34
Zn (mg)	0.5	1.5	0.65	0.97	0.78	0.71

Table 3.43 The mass of elements, in mg, that determined by ICP-OES 5300 V for the 1st group of infant follow-on formulas that provides 100 kcal and legal boundaries according to Turkish Food Codex.

Elements	The mass of formula that provides 100 kcal					
	Legal Boundaries		Experimental Results			
	Lowest	Highest	IFF-A-1	IFF-C-1	IFF-B-1	IFF-D-1
Na (mg)	20	60	28.9	34.7	24.5	29.1
K (mg)	60	160	89.2	112	120	122
Ca (mg)	50	140	83.9	112	110	110
P (mg)	25	90	45.3	71.3	61.1	60.4
Mg (mg)	5	15	6.12	10.8	7.26	8.61
Fe (mg)	0.6	2.0	1.33	1.67	1.48	1.32
Zn (mg)	0.5	1.5	0.65	1.02	0.86	0.79

Table 3.44 The mass of elements, in mg, that determined by ICP-OES 2100 DV for the 2nd group of infant follow-on formulas that provides 100 kcal and legal boundaries according to Turkish Food Codex.

Elements	The mass of formula that provides 100 kcal						
	Legal Boundaries		Experimental Results				
	Lowest	Highest	IFF-A-2	IFF-C-2	IFF-B-2	IFF-B-3	IFF-D-2
Na (mg)	20	60	28.0	35.8	25.2	23.3	28.2
K (mg)	60	160	95.4	126	116	106	101
Ca (mg)	50	140	86.9	98.6	106	104	94.5
P (mg)	25	90	46.3	53.8	56.8	51.8	50.1
Mg (mg)	5	15	7.03	10.3	7.30	6.37	8.95
Fe (mg)	0.6	2.0	1.34	1.46	1.41	1.33	1.52
Zn (mg)	0.5	1.5	0.61	0.97	0.79	0.77	0.71

Table 3.45 The mass of elements, in mg, that determined by ICP-OES 5300 V for the 2nd group of infant follow-on formulas that provides 100 kcal and legal boundaries according to Turkish Food Codex.

Elements	The mass of formula that provides 100 kcal						
	Legal Boundaries		Experimental Results				
	Lowest	Highest	IFF-A-2	IFF-C-2	IFF-B-2	IFF-B-3	IFF-D-2
Na (mg)	20	60	30.4	38.4	25.1	24.2	29.2
K (mg)	60	160	91.7	126	118	104	101
Ca (mg)	50	140	90.4	97.2	115	105	95.8
P (mg)	25	90	49.4	55.8	61.9	54.1	53.1
Mg (mg)	5	15	6.80	10.0	7.13	6.17	7.74
Fe (mg)	0.6	2.0	1.46	1.53	1.53	1.45	1.45
Zn (mg)	0.5	1.5	0.65	1.03	0.81	0.79	0.66

Table 3.46 The mass of elements, in mg, that determined by ICP-OES 2100 DV for fortified baby formulas that provides 100 kcal and legal boundaries according to Turkish Food Codex.

Elements	The mass of formula that provides 100 kcal								
	Legal Boundaries		Experimental Results						
	Lowest	Highest	FBF -A-2	FBF -A-1	FBF -C-2	FBF -C-1	FBF -B-2	FBF -B-1	FBF-B-3
Na (mg)	-	-	5.17	22.2	24.2	32.3	2.58	24.1	21.9
K (mg)	-	160	60.9	136	104	126	53.1	97.3	98.6
Ca (mg)	-	180	87.6	78.5	111	125	86.7	74.6	67.5
P (mg)	-	-	35.8	50.1	74.9	86.4	28.4	51.5	45.6
Mg (mg)	-	40	9.97	11.9	12.3	11.8	9.23	8.24	8.42
Fe (mg)	-	3	2.31	1.12	1.82	0.73	2.38	0.82	0.87
Zn (mg)	-	2	0.26	0.44	1.56	1.55	0.19	0.40	0.81

Table 3.47 The mass of elements, in mg, that determined by ICP-OES 5300 V for fortified baby formulas that provides 100 kcal and legal boundaries according to Turkish Food Codex.

Elements	The mass of formula that provides 100 kcal								
	Legal Boundaries		Experimental Results						
	Lowest	Highest	FBF -A-2	FBF -A-1	FBF -C-2	FBF -C-1	FBF -B-2	FBF -B-1	FBF -B-3
Na (mg)	-	-	5.35	22.9	24.5	33.8	2.78	24.8	21.2
K (mg)	-	160	64.7	145	109	131	55.7	96.2	105
Ca (mg)	-	180	91.5	78.8	116	128	90.8	76.5	67.4
P (mg)	-	-	37.3	51.1	81.6	88.2	29.6	52.8	47.8
Mg (mg)	-	40	9.51	11.5	11.7	11.1	8.89	7.80	7.89
Fe (mg)	-	3	2.42	1.09	1.83	0.75	2.44	0.85	0.83
Zn (mg)	-	2	0.26	0.43	1.63	1.59	0.20	0.42	0.79

CHAPTER 4

CONCLUSION

In the present study, infant formulas, infant follow-on formulas and fortified baby formulas, which were purchased from supermarkets in Ankara, were analyzed and concentration of Na, K, Ca, Mg, P, Zn, and Fe elements were determined.

Microwave digestion was used for the preparation of the samples, and experimental analysis were performed using the ICP-OES device as it is associated with several advantages such as short duration of analysis, lower limit of detection (LOD), requirement of a small mass of analyte. During the application of the selected method, previous studies conducted i.e. the samples and devices similar to those used in the present study, were reviewed.

Accordingly, after performing necessary modifications, verification of the methods were carried out. During the verification of the method, BCR-063R Skim Milk Powder CRM (IRMM, EU) and GBW 10011 Wheat CRM (China) were used. Analyses results of the CRMs were found in the confidence interval of the certified values of the CRMs. Moreover Student t test was applied at 95 confidence level to check accuracy and it was concluded that there was no significant difference between experimental results and certified values at 95% confidence level.

Additionally, LOD, LOQ, and precision studies were also performed during the verification of the method. Detection limits were found to be 15 μg/L, 39 μg/L, 45 μg/L, 24 μg/L, 3 μg/L, 3 μg/L and 3 μg/L for sodium, magnesium, phosphorus, potassium, calcium, iron and zinc respectively for ICP-OES 2100 DV.

Detection limits were found to be 45 μg/L, 72 μg/L, 150 μg/L, 75 μg/L, 90 μg/L, 15 μg/L and 18 μg/L for sodium, magnesium, phosphorus, potassium, calcium, iron and zinc respectively for ICP-OES 5300 V. Precision is also determined using CRMs and % RSD values were found between 1.0 and 5.2.

After obtaining a valid method, samples were analyzed. During the analysis, two different ICP-OES analysis systems, one of which had axial view and the other which have radial view, were used for all samples. The analyses results of the infant formulas and infant follow-on formulas were compared with the reference values on the NFT specified by the manufacturers and were obtained between 90 % and 110 % accuracy. However, analyses results of the fortified baby formulas were compared with the reference values on the NFT specified by the manufacturers and were found between 30 % and 193%.

Statistical analysis was also performed in the present study. Analyses results of the formulas were compared with the reference values on the NFT specified by the manufacturers using the Student's t-test and it was concluded that there is no significant difference between the experimental and certified values at 95% confidence level.

In the present study, results of the same formula analyzed by two different devices were also compared with each other using the Student's t-test at 95% and 99% confidence levels. There is a significant difference between the experimental and certified values at 95% confidence level. However, null hypothesis at 99% confidence level can be postulated due to no significant difference between the certified and experimental values.

In addition, while the minimum mass of Na, K, Ca, Mg, P, Zn, and Fe elements in infant formulas that provides 100 kcal are allowed to be at least 20 mg, 60 mg, 50

mg, 5mg, 25 mg, 0.5 mg and 0.5 mg respectively, according to the National Standard TS 11583 (TSE, 2006). Experimental results of the infant formulas that used in the present study were found to be suitable for the values indicated in the National Standard TS 11583 (TSE, 2006)

The experimental results of the formulas obtained in the present study were also evaluated in terms of nutritional needs of babies and these results were compared with the recommended dietary intake (RDI), adequate intake (AI) and daily tolerable intake values specified by the National Academies of Sciences; Food and Nutrition Board. It was concluded that the formulas used in the present study can easily meet elements requirement of the babies.

In conclusion, the contents of the formulas marketing in Ankara are in accordance with the values specified in the National Regulations and Standards as well as specified by international legislations and reference values on the NFT specified by the manufacturers with respect to the mass of Na, Mg, P, K, Ca, Fe, and Zn elements.

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Zand, N., Chowdhry, B.Z., Zotor, F.B., Wray, D.S., Paul, A., Pullen, F.S. (2011). Essential and trace element content of commercial infant foods in the UK. *Food Chemistry*, 28, 123–128.

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APPENDIX A

CALIBRATION CURVES USED IN THE PRESENT STUDY

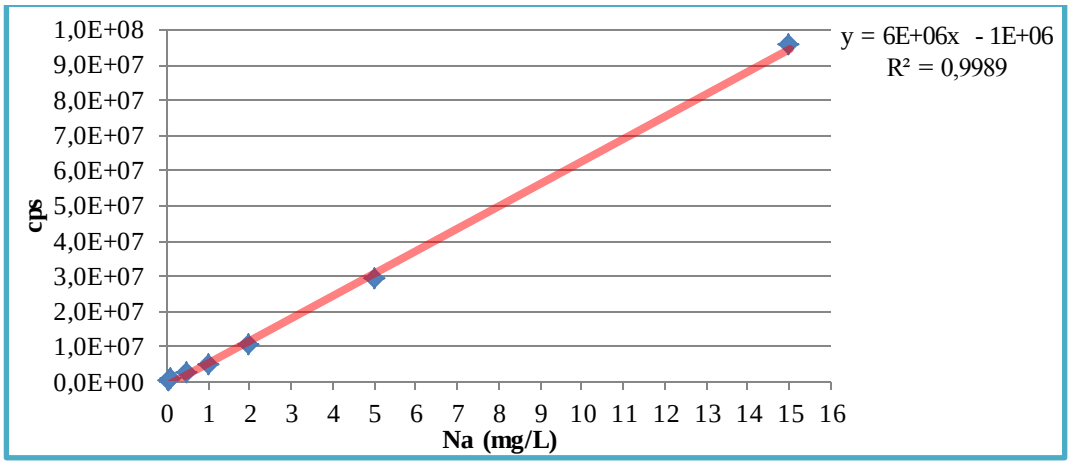


Figure A.1 Calibration plot for Na using ICP-OES 2100 DV

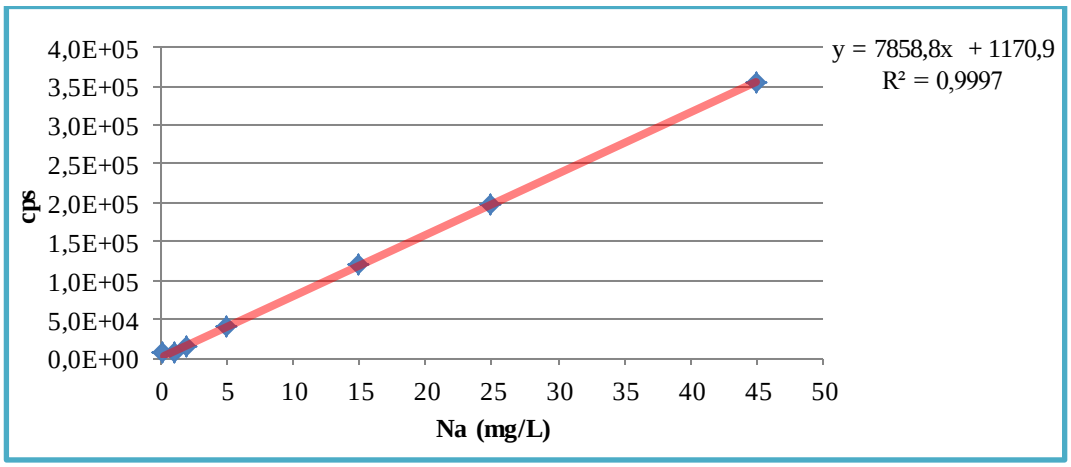


Figure A.2 Calibration plot for Na using ICP-OES 5300 V

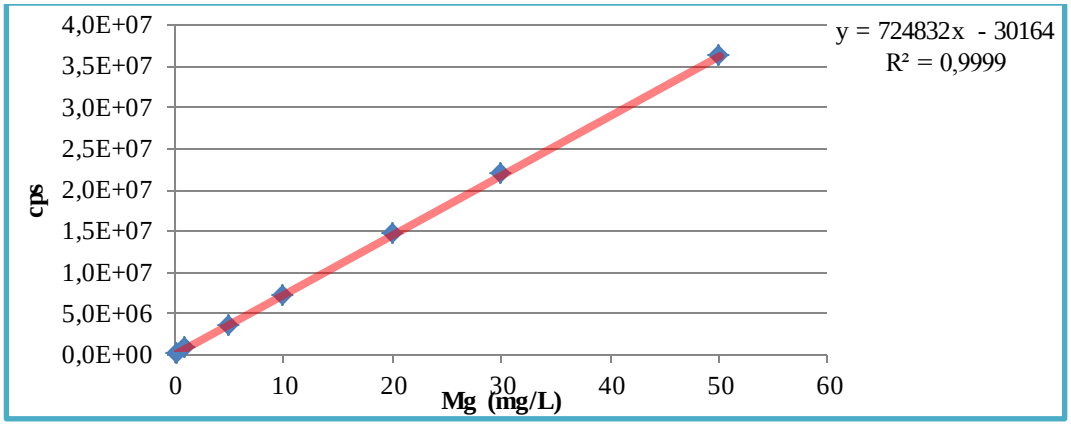


Figure A.3 Calibration plot for Mg using ICP-OES 2100 DV

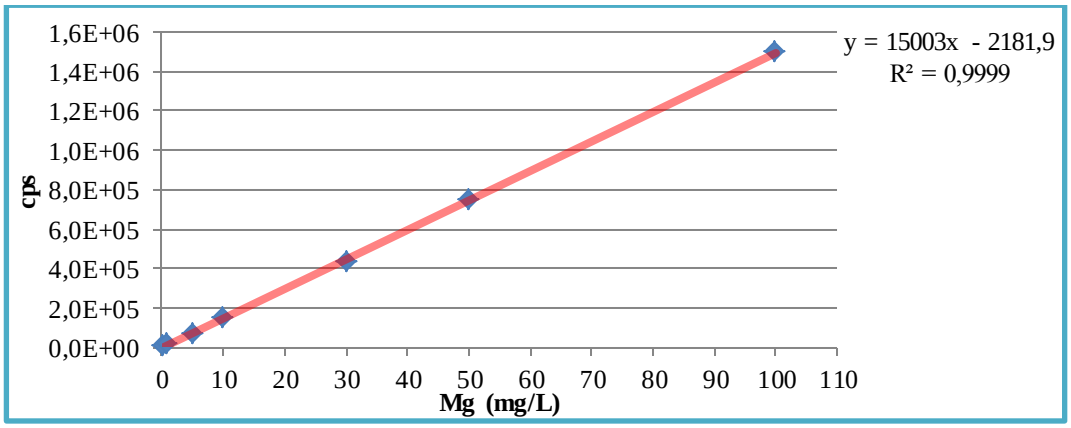


Figure A.4 Calibration plot for Mg using ICP-OES 5300 V

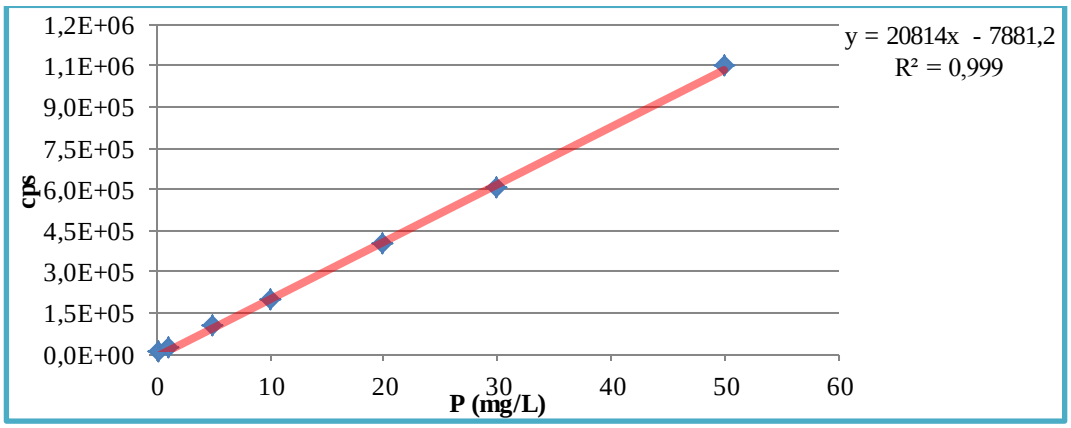


Figure A.5 Calibration plot for P using ICP-OES 2100 DV

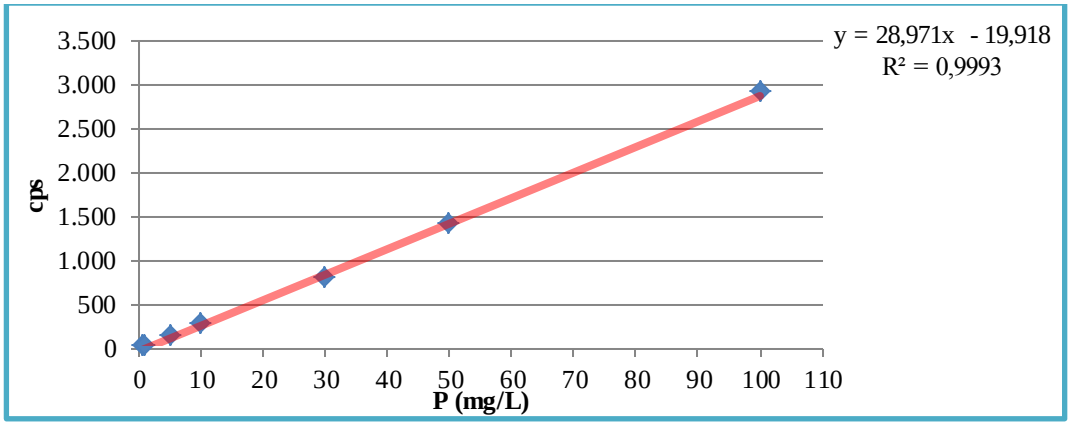


Figure A.6 Calibration plot for P using ICP-OES 5300 V

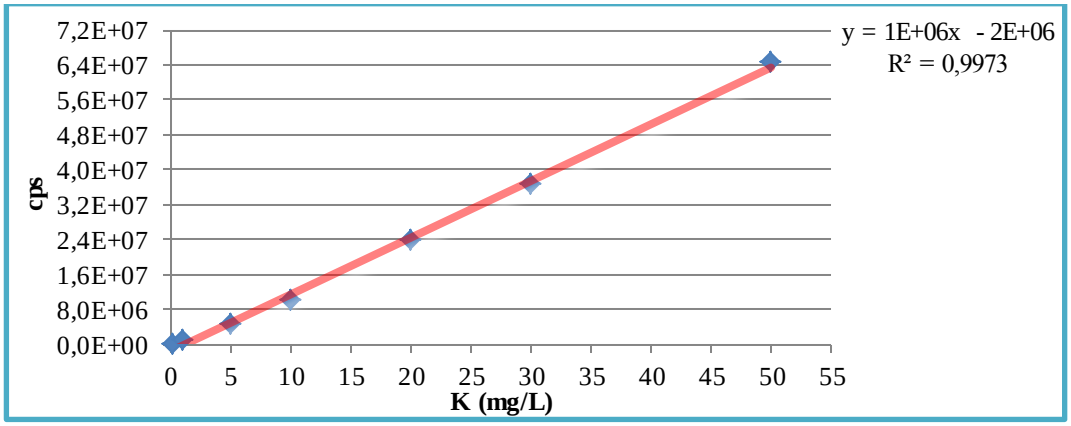


Figure A.7 Calibration plot for K using ICP-OES 2100 DV

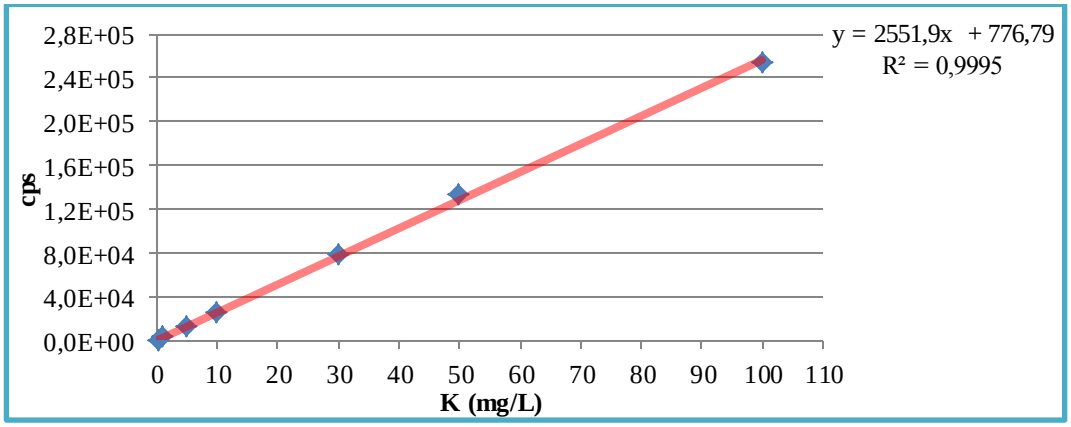


Figure A.8 Calibration plot for K using ICP-OES 5300 V

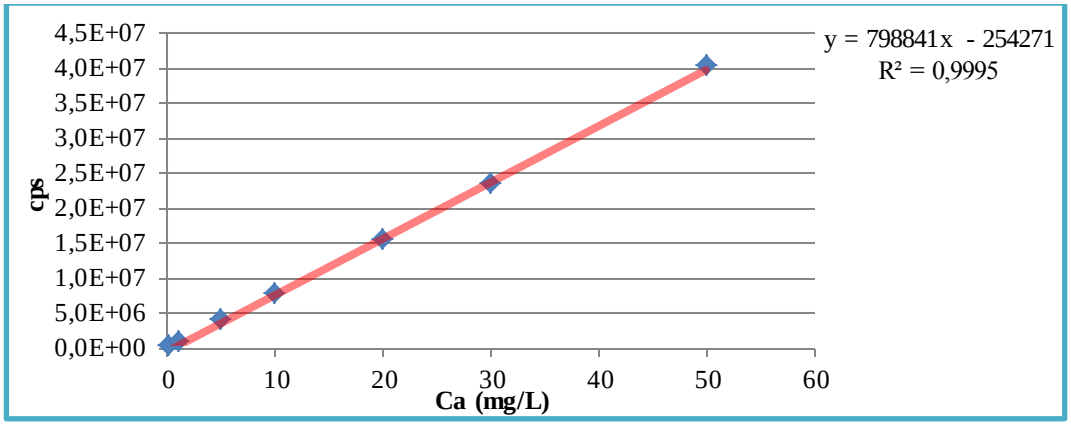


Figure A.9 Calibration plot for Ca using ICP-OES 2100 DV

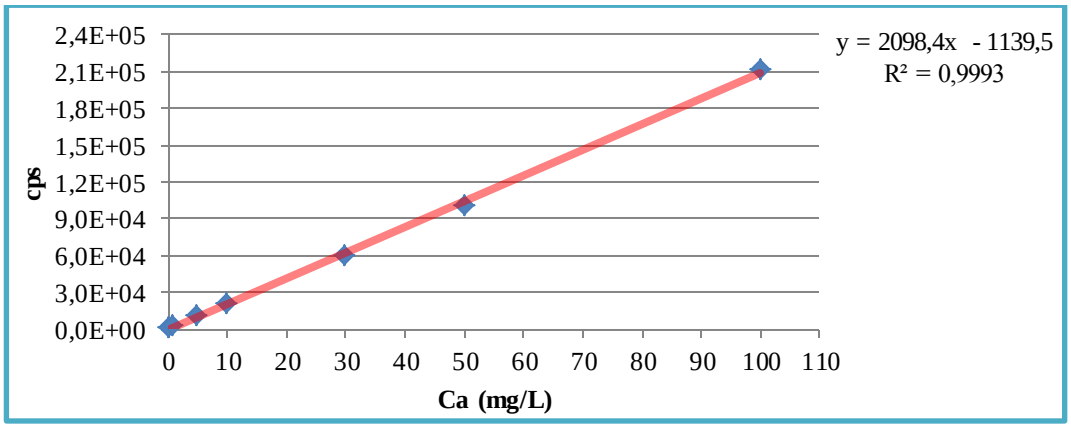


Figure A.10 Calibration plot for Ca using ICP-OES 5300 V

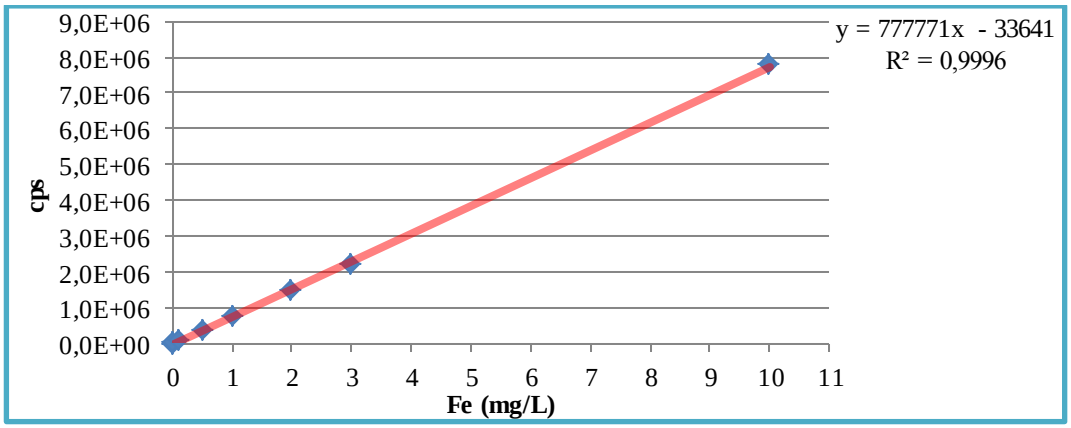


Figure A.11 Calibration plot for Fe using ICP-OES 2100 DV

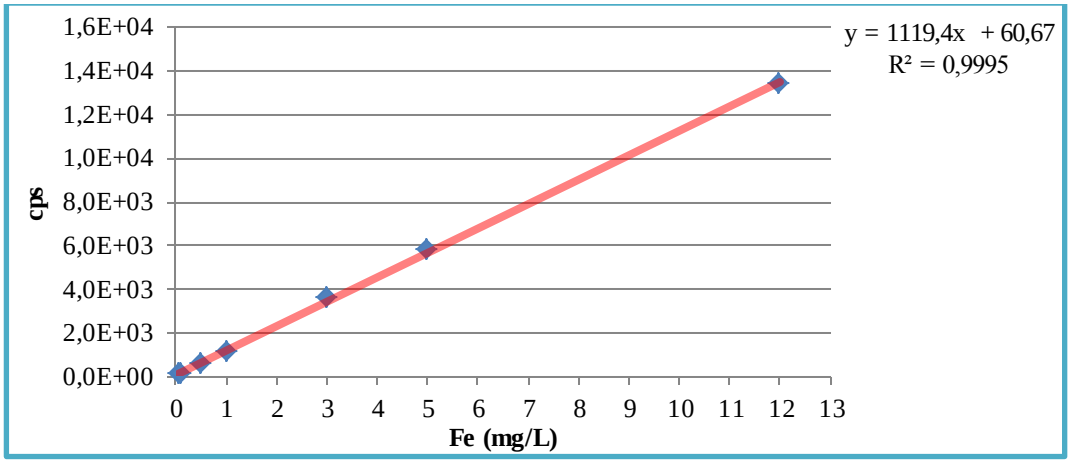


Figure A.12 Calibration plot for Fe using ICP-OES 5300 V

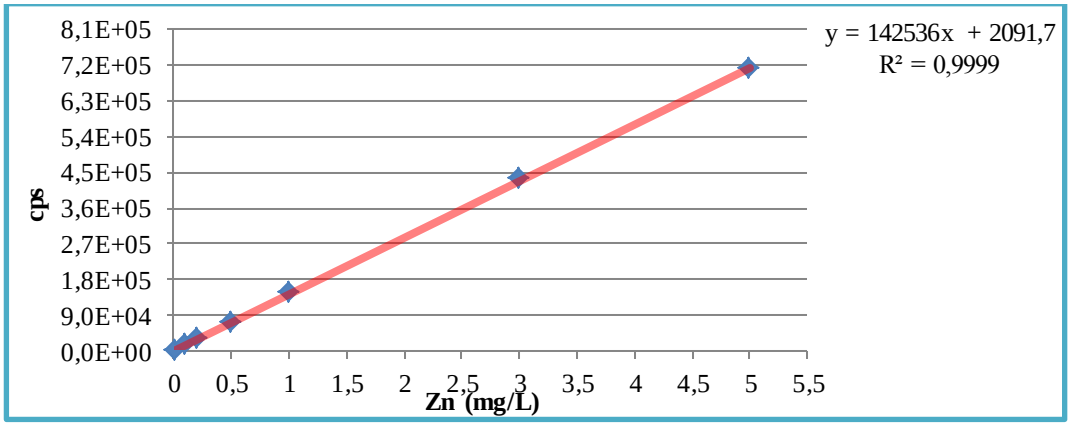


Figure A.13 Calibration plot for Zn using ICP-OES 2100 DV

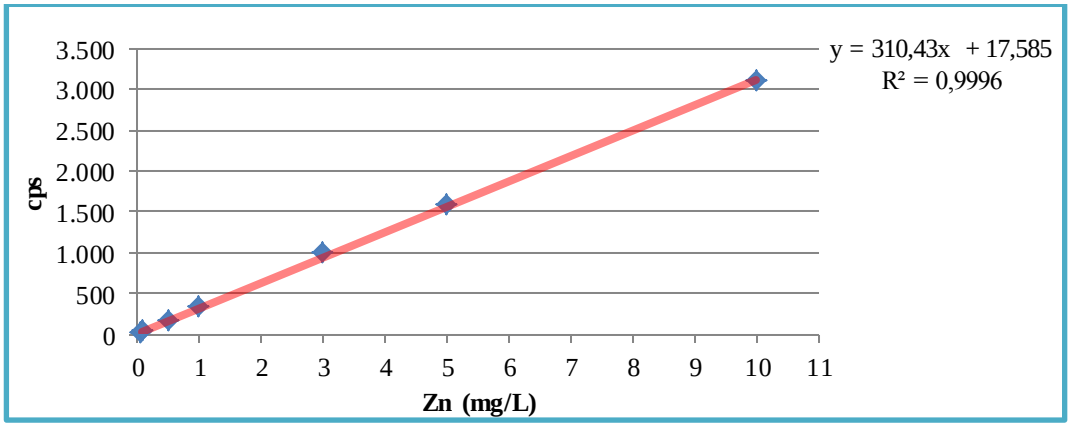
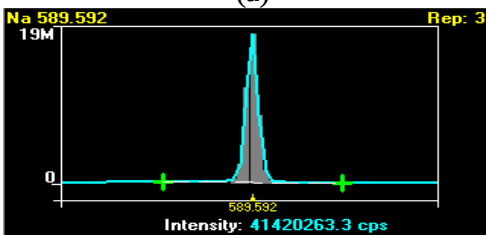


Figure A.14 Calibration plot for Zn using ICP-OES 5300 V

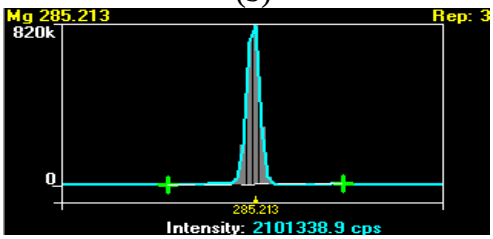
APPENDIX B:

EXAMPLES OF THE SPECTRAL WINDOWS FOR IF, IFF AND FBF SAMPLES OBTAINED BY ICP-OES 2100 DV AND ICP-OES 5300 V

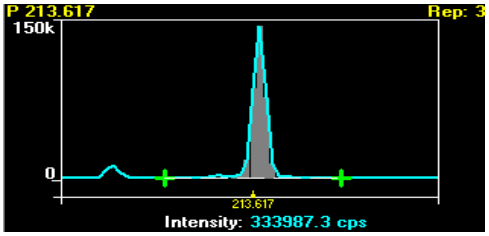
(a)



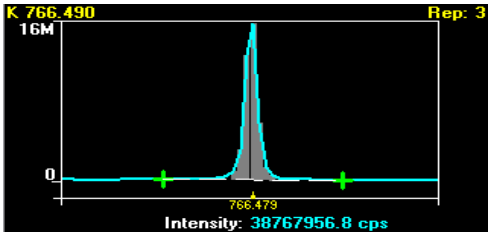
(b)



(c)



(d)



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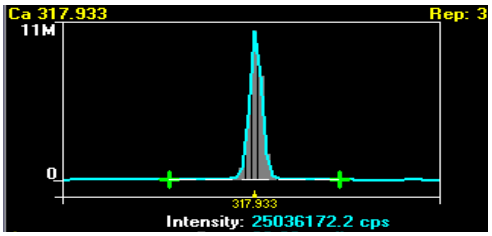

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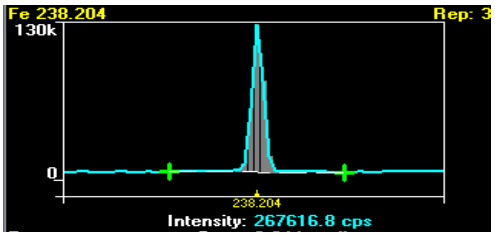


(g)

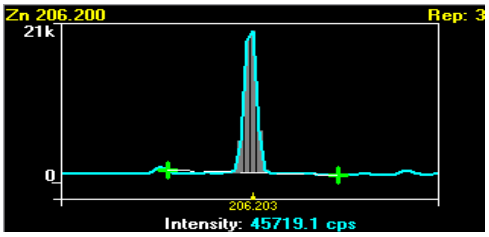
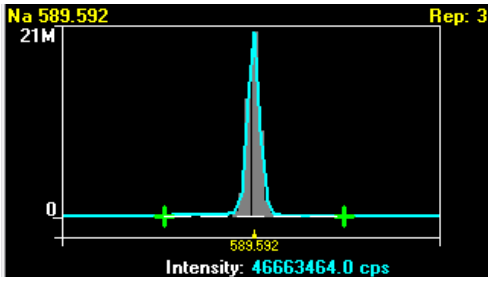
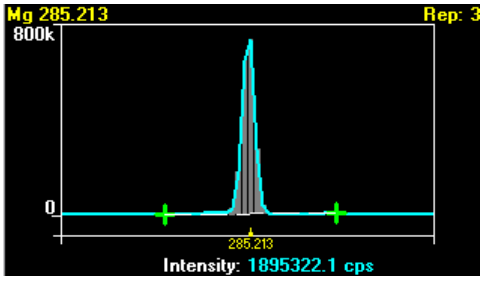


Figure B.1 Examples of spectral windows (a) Na, (b) Mg, (c) P, (d) K, (e) Ca, (f) Fe and (g) Zn for IF-B-1 samples obtained in ICP-OES 2100 DV

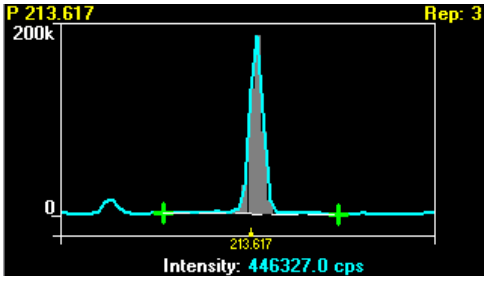
(a)



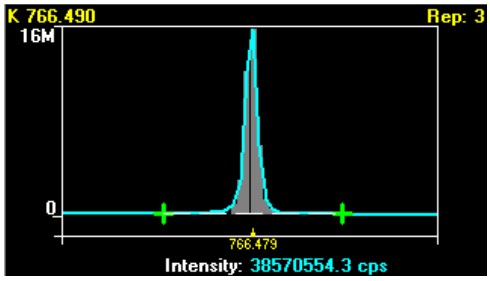
(b)



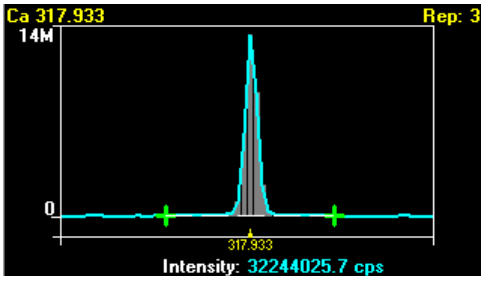
(c)



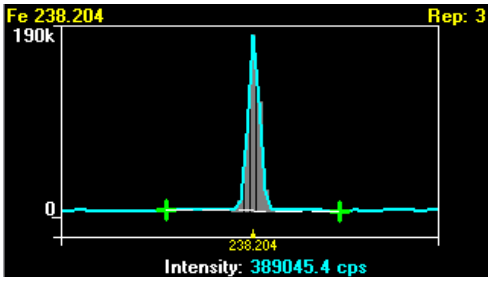
(d)



(e)



(f)



(g)

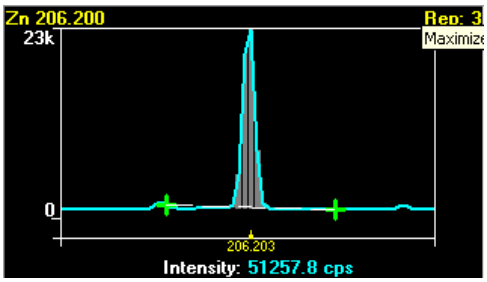
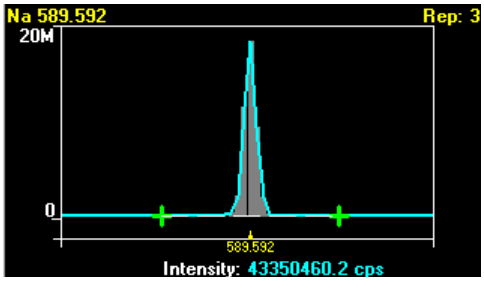
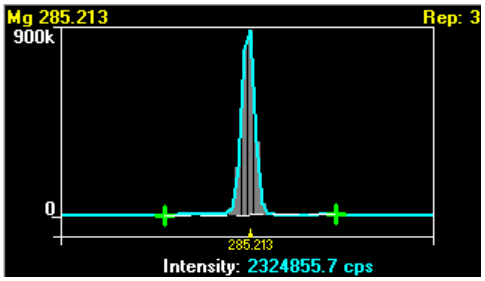


Figure B.2 Examples of spectral windows (a) Na, (b) Mg, (c) P, (d) K, (e) Ca, (f) Fe and (g) Zn for IFF-B-1 samples obtained in ICP-OES 2100 DV.

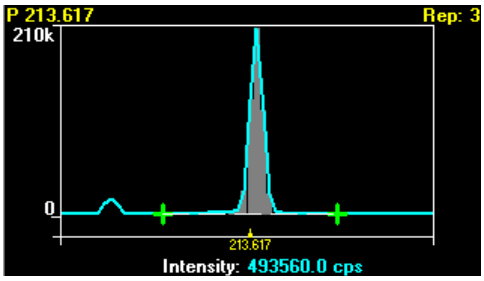
(a)



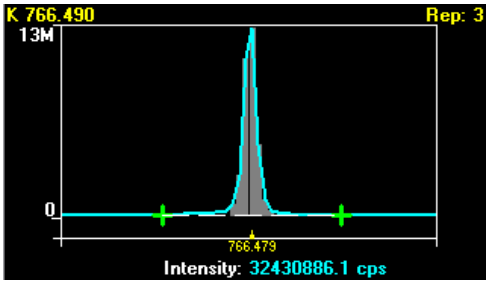
(b)



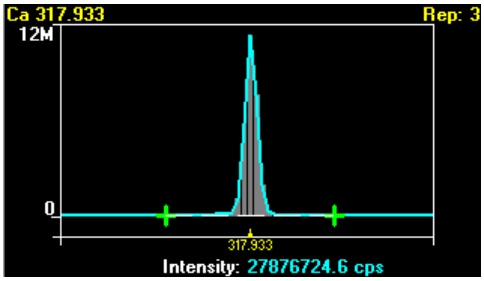
(c)



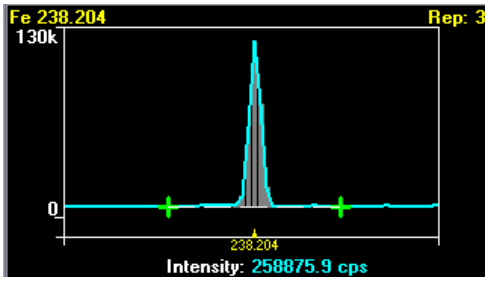
(d)



(e)



(f)



(g)

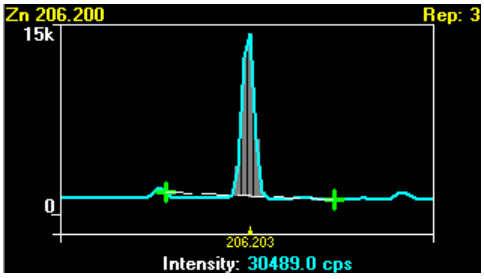
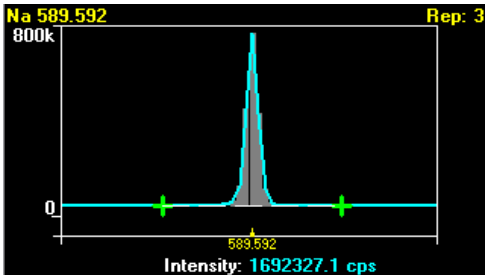
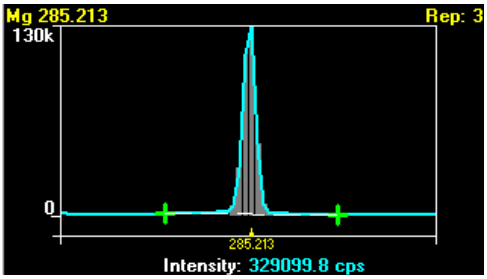


Figure B.3 Examples of spectral windows (a) Na, (b) Mg, (c) P, (d) K, (e) Ca, (f) Fe and (g) Zn for FBF-B-1 samples obtained in ICP-OES 2100 DV.

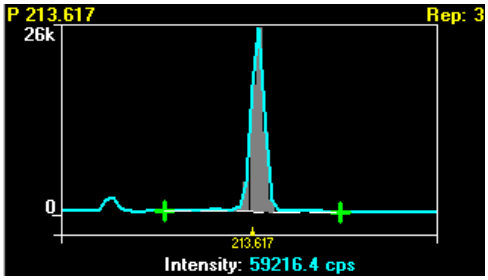
(a)



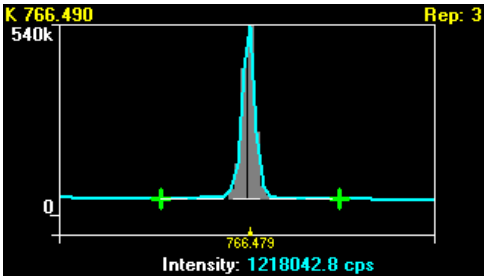
(b)



(c)



(d)



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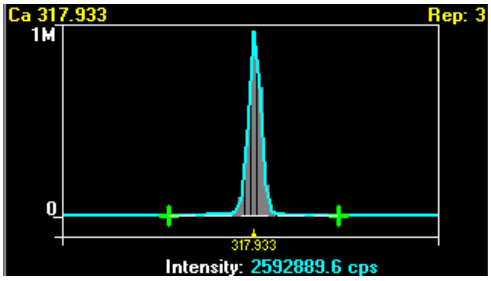
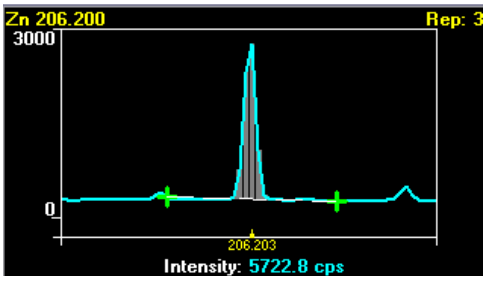


(f)



(g)

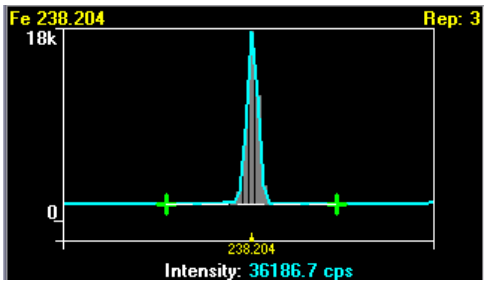
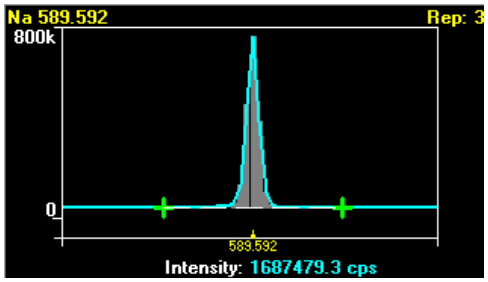
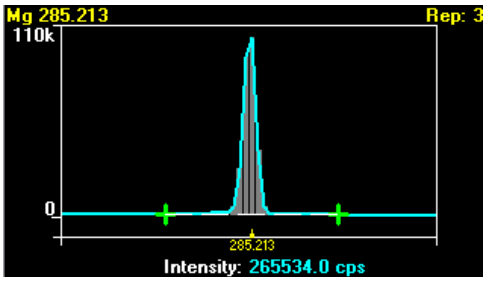


Figure B.4 Examples of spectral windows (a) Na, (b) Mg, (c) P, (d) K, (e) Ca, (f) Fe and (g) Zn for IF-B-1 samples obtained in ICP-OES 5300 V.

(a)



(b)



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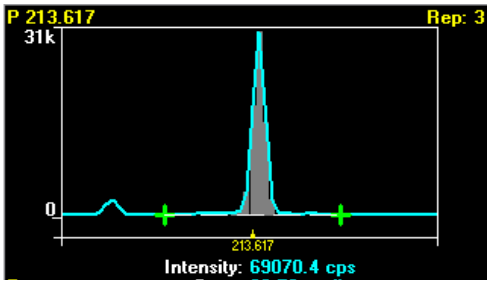
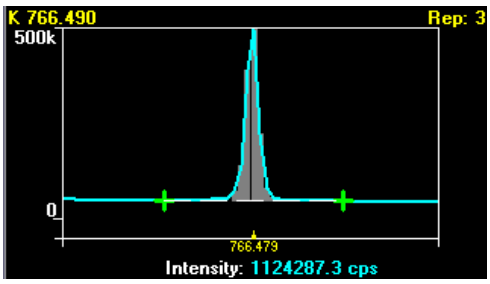
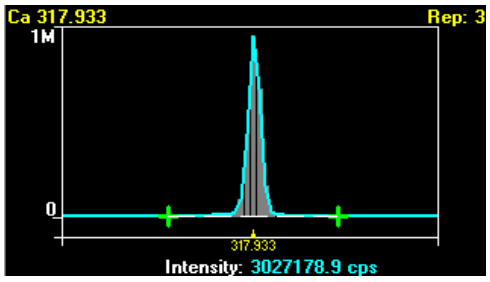


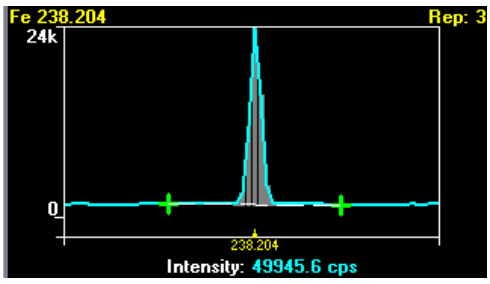
(d)



(e)



(f)



(g)

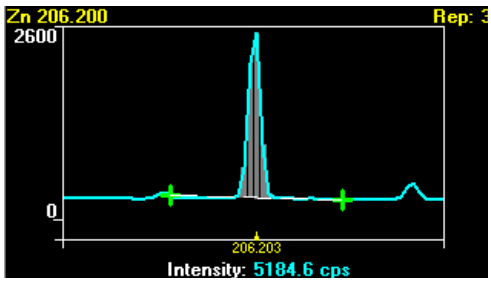
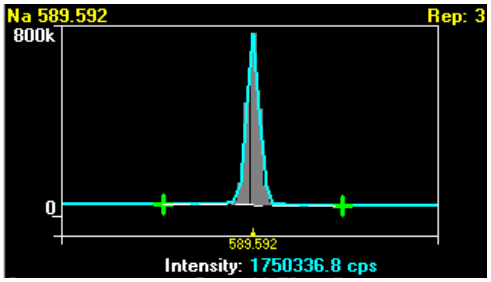
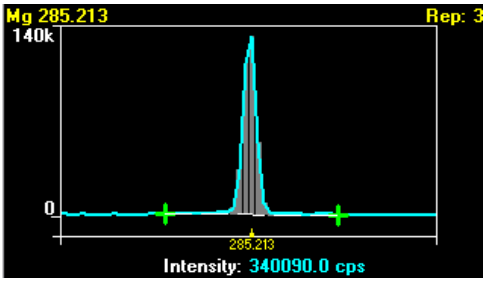


Figure B.5 Examples of spectral windows (a) Na, (b) Mg, (c) P, (d) K, (e) Ca, (f) Fe and (g) Zn for IFF-B-1 samples obtained in ICP-OES 5300 V.

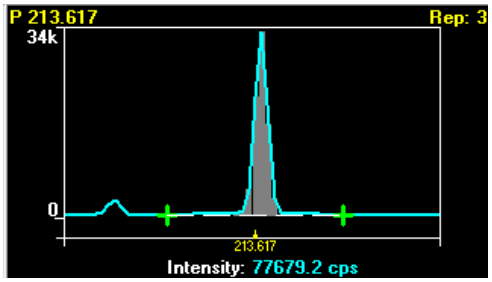
(a)



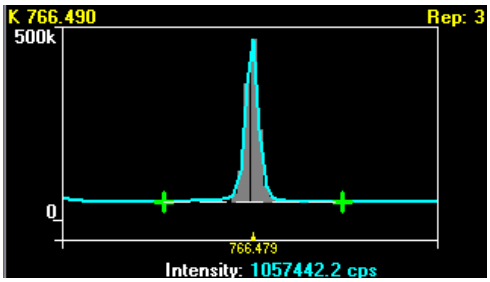
(b)



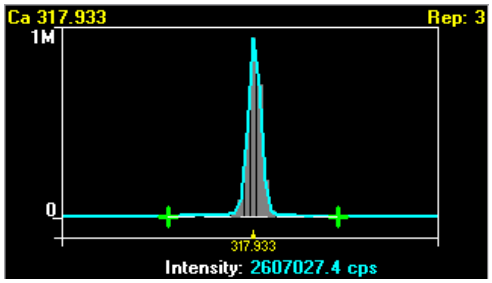
(c)



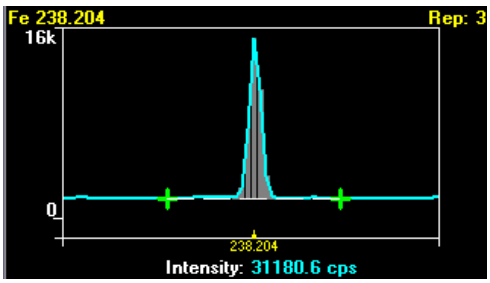
(d)



(e)



(f)



(g)

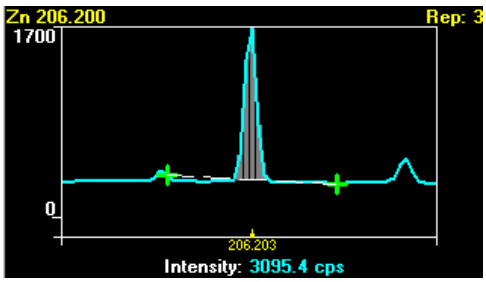


Figure B.6 Examples of spectral windows (a) Na, (b) Mg, (c) P, (d) K, (e) Ca, (f) Fe and (g) Zn for FBF-B-1 samples obtained in ICP-OES 5300 V.