

## 11.5 Appendix - Answer Key

### 11.4.1 Water Soluble Vitamins (Chemical Analysis)

#### I. Thiamine (B<sub>1</sub>)

##### C. Pre-Assay Questions

1. **What is a vitamin?** A vitamin is an organic compound that is a natural component of foods and in minute amounts is essential for normal physiological function. Absence or inadequate amounts in the diet can cause specific known deficiency syndromes.
2. **Name examples of fat-soluble and water-soluble vitamins.** Fat-soluble: vitamins A, E, D, and K. Water-soluble: thiamine, riboflavin, niacin, folic acid, B<sub>6</sub>, B<sub>12</sub>, pantothenic acid, and vitamin C.
3. **What are the recommended daily allowances of thiamine in adults, infants, and lactating and pregnant women?** Adults: 1.5mg, infants: 0.5 mg, pregnant and lactating women: 1.7 mg.
4. **What are other names for thiamine?** Vitamin B<sub>1</sub>, aneurin.
5. **List some natural food products that are considered rich sources of thiamine.** Brewers and bakers yeast, liver, cereal grains
6. **What form of thiamine is present in animal and plant products?** Plants: free thiamine. Animals: present in the phosphorylated forms with the predominant form being thiamine diphosphate.

7. **What is the function of thiamine in the body?** Thiamine acts as a coenzyme in the body, it serves as an essential co-factor in multienzyme alpha-ketoacid dehydrogenase complexes.
8. **What are the effects of thiamine deficiency in man?** The effects of deficiency are known as beriberi which can be followed by mental confusion, muscular weakness, enlarged heart, and death.
9. **Are there any toxicity effects when high dosages of thiamine are taken? Is so, what are the effects?** No toxic effects associated with high dosages, but can cause gastric upset.
10. **Discuss the solubility of thiamine in water, alcohol, and organic solvents.** Water, 100g/100mL; 95% Ethanol, 1g/100mL; 100% Ethanol, 0.3g/100mL; insoluble in acetone, benzene, hexane, and chloroform.
11. **Discuss the stability of thiamine with respect to light, pH, oxidizing and reducing agents.** In pH solutions of 3.5, the vitamin will withstand sterilization temperatures. In the dry form of the vitamin, it is stable and not sensitive to atmospheric oxidation. In solution, thiamine is sensitive to reduction and oxidation. The oxidation of the vitamin will produce its inactive form called thiochrome.
12. **Draw a structure of the thiamine molecule.** See the Merck Index.

#### *E. Post Assay Questions*

1. **In the procedure for thiamine what are possible sources of error? Could substances within the sample matrix interfere with the thiamine determination?** The sources of error could be calculation errors; errors in making reagents, instrument errors, and components in the sample matrix that could inhibit accurate determination.
2. **What precautions should one take during the procedure to protect the analyte and analyst?** Keep the sample away from direct sunlight, make sure pH is correct, work under a hood, and wear gloves, safety glasses, and lab coat
3. **What is the final concentration of the sample extract in the procedure?** AOAC 17<sup>th</sup> ed. 50.1.08 (986.27) states 15 ug; AOAC; AOAC 17<sup>th</sup> ed. 45.1.06 (953.17) states 0.2 ug.
4. **What are some stopping points where the procedure can be interrupted and stored overnight without affecting the results?** AOAC 17<sup>th</sup> ed. 50.1.08 (986.27) states: after the 3 hour enzyme hydrolysis, after filtration, and after collection from column, that the sample extracts can be placed in refrigerator overnight. AOAC 17<sup>th</sup> ed. 45.1.06 (953.17) states that after the filtration step samples can be placed in refrigerator overnight.

## ***II. Riboflavin (B<sub>2</sub>)***

### **C. Pre-Assay Questions**

- 1. Is Riboflavin a water-soluble or fat-soluble vitamin?** Riboflavin is a water-soluble vitamin.
- 2. What are the recommended daily allowances of riboflavin in adults, infants, and lactating and pregnant women?** Riboflavin for adults: 1.7mg; infants 0.6mg; lactating and pregnant women 2.0mg.
- 3. What are other names for riboflavin?** Vitamin B<sub>2</sub>, ovoflavin, lactoflavin, riboflavine.
- 4. List some natural food products that are considered rich sources of riboflavin.** Bakers' yeast, broccoli, spinach, breads, and cereals.
- 5. What form of riboflavin is present in animal and plant products?** In animals, riboflavin is present in fungi cells. It is in the form of ovoflavin in eggs, in human milk it is FAD, and in other living cells and tissues it is present as FMN.
- 6. What is the function of riboflavin in the body?** Riboflavin participates in oxidation–reduction functions, and it aids in electron transport, catabolism of amino acids and the production of uric acid.
- 7. What are the effects of riboflavin deficiency in man?** Riboflavin deficiency results in lacrimation, seborrheic dermatitis, purple swollen tongue, burning and itching of eyes.
- 8. Are there any toxicity effects when high dosages of riboflavin are taken? Is so, what are the effects?** No toxicity reported in humans.
- 9. Discuss the solubility of riboflavin in water, alcohol, and organic solvents.** Riboflavin is soluble in water 0.12g per 100ml, in ethanol at .0045g per 100ml, and it is insoluble in acetone, benzene, hexane, and chloroform.
- 10. Discuss the stability of riboflavin with respect to light, pH, oxidizing and reducing agents.** It is stable in strong mineral acids, is oxidized by chromic acid, and is destroyed by KMnO<sub>4</sub>. It is unstable in alkaline solution. It is sensitive to both visible and ultraviolet light. It becomes fluorescent in neutral solution of pH 6.7 to 6.8.
- 11. Draw a structure of the riboflavin molecule.** Refer to the Merck Index or the Method of Vitamin Assay for picture of structure on pg. 147 of Chapter 7.

### *A. Post Assay Questions*

1. **In the procedure for riboflavin what are possible sources of error? Could substances within the sample matrix interfere with the riboflavin determination?** Sources of error could be calculations of sample weights, error in making reagent, dilution errors. There could also be other substances present in the matrix of the sample that could hinder accurate results.
2. **What precautions should one take during the procedure to protect the analyte and analyst?** To protect the analyte, protect the analyte from unnecessary light, make sure solutions are made properly and that pH is at proper level. Use proper equipment such as flask, filter paper. To protect the analyst, work under a hood, wear protective equipment such as gloves, goggles, and lab coat.
3. **What is the final concentration of the sample extract in the procedure?** 0.1ug/ml.
4. **What are some stopping points where the procedure can be interrupted and stored overnight without affecting the results?** After 30-minute digestion, after the first pH step and first dilution; after filtration of extract; and after the final dilution step the solutions can be place in a refrigerator overnight.

### *III. Vitamin C*

#### **C. Pre-Assay Questions**

1. **What is the %DV for vitamin C in adults, children, and infants?** Adults 60mg; children 40 mg; infants 35mg.
2. **What is disease related to vitamin C deficiency?** Scurvy.
3. **What is the trivial name for vitamin C? (b) What is the definition of vitamin C? (c) What are the primary dietary sources of vitamin C? (d) What are the two main biologically active forms of vitamin C?** a) L-ascorbic acid. b) Refers to compounds exhibiting full or partial biological activity of L-ascorbic acid. (c) Citrus fruits, potatoes, tomatoes, fortified foods. (d) D-hydroascorbic acid, L-ascorbic acid.
4. **What is the stability of L-ascorbic acid in solution?** Very unstable.

#### *A. Post-Assay Questions*

1. **What are the two main analytical procedures for vitamin C in ACNA? What form of vitamin C does each method measure?** (1.) 2, 6-Dichloroindophenol titrimetric method AOAC (985.33). This method measures L-ascorbic acid. (2.) Vitamin C (total)

microfluorometric method AOAC (967.22). This method measures L-ascorbic acid and dehydroascorbic acid.

2. **(a) What is the purpose of Norit? (b) What is the purpose of o-phenylenediamine?** (a) Norit oxidizes L-ascorbic acid to dehydroascorbic acid. (b) O-phenylenediamine is used to produce a highly fluorescent and easily measurable compound.
3. **What are the stopping points in each method?** The major stopping point of each method is after the addition of meta-phosphoric acid. The solution is stable up to 7 days.
4. **What are the sources of interference in each method, and how does an analyst avoid them?** Microfluorometric method: high starch products (the way to avoid the starch is to extract it with cold ethanol). Titration: minerals interfere (the way to avoid this is the addition of EDTA).

## ***11.4.2 Water Soluble Vitamins (Microbiological Assays)***

### ***I. Folic Acid***

#### **C. Pre-Assay Questions**

1. **Why did the Food and Drug Administration decide to fortify cereal-grain products with folic acid?** To ensure that women of childbearing age would get the desired 400 ug/day of folic acid in their diet.
2. **What is the %DV (folic acid) for adults, infants, and children?** 0.1 mg infants, 0.1mg children, 0.4 mg adults
3. **What are the four predominant forms of naturally occurring folates?** 5-methyl-H<sub>4</sub>PteGlu<sub>5</sub>, tetrahydrofolate pentaglutamate, pteroylglutamic acid, 5, 10-methenyl-H<sub>4</sub>PteGlu.
4. **What is the stability of folic acid?** Slightly soluble in acid form, very soluble in salt form in solutions of dilute alkali, hydroxides, sulfuric and hydrochloric acid. Folic acid is stable in alkali solutions.
5. **What are the symptoms/consequences of folic acid deficiency?** Neural tube defects.
6. **What are the consequences of too much folic acid in the diet?** Masking of pernicious anemia in the elderly.
7. **What are good sources of folic acid in foods?** Liver, fortified cereals.

### ***E. Post-Assay Questions***

- 1. What are the analytical procedures used in ACNA for determining folic acid? How do they differ?** AOAC methods for infant formula and dietary supplements as well as the tri-enzyme method for foods. The three methods differ in sample preparation and digestion time. These differences help to liberate the different forms of folic acid.
- 2. Explain the use of the three enzymes in the SOP N/AM/4/1/94 procedure.** The protease and amylase help liberate bound folate. The conjugase helps in the hydrolysis of polyglutamyl folates.
- 3. What two microorganisms are used to assay folic acid and what are the concentrations of their respective standard curves? Which one is used for dietary supplements?** The two organisms are *Streptococcus faecalis* (dietary supplements) and *Lactobacillus casei*. The concentration of the standard curves is 0.16ng/mL to 0.08ng/mL respectively.
- 4. What are the stopping points in each method?** The stopping points are after the various dilutions.
- 5. What are the precautions used in each method to preserve the integrity of the samples/vitamin?** Minimizing exposure to light, air, and oxygen.
- 6. What are the hazardous materials and dangers in each method?** The use of autoclaves, acids, and alkaline solutions.

### ***II. Niacin, Biotin, Vitamin B<sub>6</sub> (Pyridoxine), Vitamin B<sub>12</sub> (Cyanocobalamin) and Pantothenic Acid***

#### ***A. Questions***

- 1. Discuss the stability and solubility of each vitamin with respect to light, pH, oxidation, reduction, water, and organic solvents.**

**Niacin:** Soluble in water, insoluble in ethyl ether. Stable in its dry form and aqueous solutions, unaffected by light or pH.

**Vitamin B<sub>6</sub>:** Soluble in water, acetone, and alcohol, slightly soluble in ether and chloroform. Stable in heat, concentrated alkali and hydrochloric acid. Destroyed by light.

**Vitamin B<sub>12</sub>:** Soluble in water, alcohol, and phenol, insoluble in most organic solvents. Stable at low pH.

**Biotin:** Soluble in water, ethyl alcohol, insoluble in petroleum ether, chloroform and ether. Stable in strong acids, but unstable in alkali.

**Pantothenic acid:** soluble in water, ethyl acetate, glacial acetic acid, slightly soluble in ether, amyl alcohol, and insoluble in benzene and chloroform. Stable in calcium and sodium salt forms.

2. **Name the diseases caused by the deficiency of each vitamin. Discuss symptoms of the diseases caused by each vitamin.**

**Niacin:** Pellegra symptoms--insomnia, anorexia, anemia.

**Vitamin B<sub>6</sub>:** Symptoms--dermatitis, microcytic anemia, irritability, depression, convulsions

**Vitamin B<sub>12</sub>:** Symptoms--weakness, nervous system degeneration, confusion, depression.

**Biotin:** Symptoms--anorexia, nausea, depression.

**Pantothenic acid:** "Tingling Foot Syndrome," other symptoms include gastric distress, nausea, insomnia, emotional instability, muscular weakness.

3. **List some natural food products that are considered rich sources for each vitamin.**

**Niacin:** organ meats, poultry, fish, nuts, cereals, grains.

**Vitamin B<sub>6</sub>:** bananas, seeds, rice, beef, pork, poultry.

**Vitamin B<sub>12</sub>:** beef, pork, lamb, seafood, eggs.

**Biotin:** organ meats, egg yolks, soybeans.

**Pantothenic acid:** all meats, egg yolks, whole grains, molasses.

4. **What is the recommended concentration of each vitamin in final sample assay extract?**

**Niacin:** 0.01-0.02 ug/mL.

**Vitamin B<sub>6</sub>:** 1.0 ng/mL.

**Vitamin B<sub>12</sub>:** 0.014 ng/mL.

**Biotin:** 0.1-0.04 ug/mL.

**Pantothenic acid:** 0.01ug/mL.

5. **Indicate the stopping points, where an analyst can interrupt the procedure for overnight storage without affecting the results.** With all assays, one can stop after the digestion step and subsequent dilutions.

6. **Identify the test organisms listed below and indicate the vitamins assayed by each.**

(a) L.l.: *Lactobacillus (leichmannii) delbrueckii*: vitamin B<sub>12</sub>.

(b) S. f.: *Streptococcus faecalis*: folic acid, riboflavin.

(c) L.p.: *Lactobacillus plantarum* : niacin, biotin, pantothenic acid.

(d) L.c.: *Lactobacillus casei*: folic acid.

(e) S.u.: *Saccharomyces pastorianus (uvarum)*: vitamin B<sub>6</sub>.

7. **How are the test organisms kept viable?** Growth on slants and stabs, -70 °C storage.
8. **What is an inoculated and an uninoculated blank?** Inoculated blank: broth or media, no vitamin and inoculated with test organism. Uninoculated blank: broth or media, no vitamin, no test organism.
9. **Define heat treatment and sterilization?** Sterilization is the treatment of samples at high temperature and pressure to kill most microorganisms. Heat treatment is not sterilization, will only kill a few organisms.
10. **Why is lactic acid bacteria widely used in microbiological assays?** The acids produced by the organisms are a good measure of growth.
11. **Why it is possible to use a test organism to assay more than one vitamin?** Some organisms have more than one selectable marker.
12. **Why transfer bacteria weekly?** To keep the culture viable.
13. **What is Lactobacillus agar, Lactobacillus broth, agar slants, agar stabs, 0.9% Saline, vitamin B<sub>6</sub> broth and rinse, and define the purpose of each.**

**Lactobacillus agar:** sterile culture media (solid) containing specific nutrients at concentrations ideal for *Lactobacillus* type organisms to grow.

**Lactobacillus broth:** sterile liquid culture media with same criteria as above.

**Agar slants:** same as Lactobacillus agar except when prepared while media is still hot and in a liquid state, the culture tubes containing the media are placed at an angle to create a larger surface area for organisms to grow.

**Agar stabs:** same as Lactobacillus agar prepared in culture tubes, the tubes are allowed to remain upright when they solidify at room temperature post sterilization.

**0.9% saline:** this solution is equivalent to natural saline, used to wash any nutrients from growth media out of cells.

**Vitamin B<sub>6</sub> Broth:** this broth is the sterile culture media containing specific nutrients to support *Saccharomyces pastorianus*.

**Rinse:** used in B<sub>6</sub> assay to wash cells.

14. **How long do stab cultures stay active?** 1-2 months.
15. **How often does one transfer stab cultures?** 1 month (approx. 30 days).
16. **How long does one grow most bacteria for an inoculum?** 12-24 hours.



17. **What is the incubating temperature range for most assays? Is temperature critical to an assay?**  $37 \pm 0.5^\circ \text{C}$  for bacteria and  $30 \pm 0.5^\circ \text{C}$  for yeasts.
18. **What is the growth time for microbiological assays? Is the growth time critical to an assay?** 12-24 hours. Yes, growth time is very critical.
19. **What is the recommended temperature for storing agar, slants stabs**  $4^\circ \text{C}$
20. **Discuss the purpose of basal media in the assay.** Provides the necessary, non-selective nutrients for growth.
21. **What are the criteria of an official assay?** Eight out of 12 tubes.
22. **What is the advantage of an eight point standard curve versus a five point standard curve?** More points allow for less estimation between definitive points on the standard curve.
23. **What precautions should be taken when performing microbiological assays to avoid contamination and invalidating the assays?** Use aseptic techniques when making transfers or inoculating. Clean dilutor between samples. Make sure the water bath is clean. Autoclave tubes at the correct temperature and pressure.
24. **What are the differences between titrimetric and turbidimetric assays?** A titrimetric assay is an experiment using acid producing bacteria to determine the response of bacteria in the designated growth media to a selective growth required substance such as a vitamin. Turbidimetric assays serve the same purpose except the measurement is of cell density.
25. **How does one determine if a sterilization cycle has been is complete and has rendered the items inside as sterile?** Add a sterilization indicator in with the samples or media during an autoclave cycle. The uninoculated blank is also an indicator.
26. **What are the three major factors that affect the growth of microorganisms?** Oxygen source, temperature, nutrient source.
27. **How does one determine when the standard has reached its maximum growth?** After overnight growth (usually 16 hrs.) take a percent (%) transmittance reading at 550nm using the standard with the highest concentration (the uninoculated blank is used to zero the instrument). Once every hour afterwards take the same reading, a plateau of growth is reached when the successive percent (%) transmittance readings change by less than 2%.

## ***II. Vitamin K***

### ***C. Pre-Assay Questions***

1. **What is the %DV (Vitamin K) for adults, infants, and children?** Infants 5ug/day, adults 80 ug/day.
2. **What are the forms of vitamin K and where do they come from (source)?** Phylloquinone K1 is the most common natural form produced by plants. Menadione K3 is a synthetic form.
3. **What is the stability of Vitamin K?** Stable with most food processing procedures, slightly unstable if heated at frying temperatures, exposure to light and alkaline conditions can destroy it.
4. **What are the symptoms/consequences of too much or too little vitamin K in the diet for adults and children or infants?** Liver disease.
5. **What are good sources of vitamin K in foods?** Green leafy vegetables, legumes, and vegetable oil.

### ***B. Post-Assay Questions***

1. **What are the differences in the new and old procedures?** Old procedures used UV, newer procedures utilize fluorescence. This presents a problem because most matrices have UV interfering compounds. The new procedures use post-column reduction columns packed with zinc metal reducing quinones to hydroquinones, which are detected through fluorescence.
2. **What is the purpose of the buffer, the Lipase, the alcohol solution, the K<sub>2</sub>CO<sub>3</sub>, and the hexane?**
  - Buffer: make conditions favorable for lipase activity
  - Lipase: hydrolyzes fat and fatty acids
  - Reagent alcohol: stops enzyme activity
  - Potassium carbonate: denatures protein, destroys enzyme
  - Hexane: used to extract the vitamin
3. **Explain the post column reductor. What precautions are necessary in the use of this piece of equipment?** A post-column reductor is a solid phase reductive column with metallic zinc particles. It reduces non-specific fluorescence compounds.
4. **What is the purity factor and how is it determined?** It is the measured absorbance of working standard at 248nm, determined by calculating purity divided by the theoretical absorbance at 248nm, of standard at the same concentration for purity.

5. **Why would one use a slightly elevated column temperature instead of ambient temperature?** To keep temperature stable.
6. **Explain the system suitability Test.** Relative Standard Deviation <2.0. See United States Pharmacopoeia for further definition.
7. **What does using a “forced zero” in the linear regression curve mean?** Linear regression line should touch zero in order to calculate linear regression.
8. **If a sample calculates to have a fluorescence value above the highest standard point value, how can one get it on the curve?** 1:1 dilution.
9. **What are the stopping points in the assay?** After the hexane extraction, take an aliquot, evaporate to dryness, and blanket with nitrogen. It can be kept for three days in refrigerator.
10. **What are the precautions used in the method to preserve the integrity of the sample/vitamin?** UV/HPLC grade solvents should be used, pH meter calibration should be accurate, zinc column should be packed properly, and water should not touch zinc column.
11. **What are the hazardous materials and dangers in the method?** Hexane, dichloromethane, and methanol are carcinogenic solvents. Gloves should be worn and work done in a hood.

#### ***11.4.4 Proximate Analysis (Various Methods)***

##### ***C. Questions***

##### **II. Cholesterol**

1. **What is Cholesterol and where it is found (source)?** There are several definitions of cholesterol, which are as follows: Cholesterol is a soft, waxy, fat-like substance that occurs naturally in all parts of the body and is needed to function normally. A pearly, fat like steroid alcohol,  $C_{27}H_{45}OH$ , crystallizing in the form of leaflets or plates form, found in animal fat & oils, bile, blood, brain tissue, milk, and yolk of eggs. Cholesterol is a fatty substance, (a lipid) that is an important part of the outer lining (membrane) of cells in the body of animals.
2. **What are the health claims approved by FDA linking cholesterol to disease?** Dietary saturated fat, cholesterol, and risk of coronary heart disease. (21 CFR 101.75). *Sample claim:* “While many factors affect heart disease, diets low in saturated fat and cholesterol may reduce the risk of this disease.”

**3. Describe toxicity of pyridine and benzene. What are the waste codes and what they mean?** Benzene is a colorless, flammable, toxic liquid with a characteristic aromatic odor. Benzene is a confirmed human carcinogen and a possible human mutagen. Long-term exposure to high levels of benzene in air can cause leukemia, cancer of blood. Ingestion of benzene causes a burning sensation; inhalation causes irritation of respiratory system. Benzene is a serious poison by all routes of exposure.

Pyridine is harmful. It is a highly flammable liquid. It has with a nauseating odor. Short-term exposure may result in permanent injury. Inhalation causes irritation of the respiratory system and may affect the central nervous system. Short-term exposure causes irritation, headache, drowsiness, dizziness, and loss of coordination. Long-term exposure additionally causes nausea, vomiting, and diarrhea. Their waste code is “ D” which means they are regulated at the ppm level.

**4. How does one dispose the above two reagents?** All the material, including the container and any rinse waste that comes in contact with pyridine and benzene is collected as hazardous waste. Consult the chemical hygiene officer for any questions regarding hazardous waste..

**5. What are the stopping points in the assay?** The assay could be stopped after the saponification and methylation steps.

**6. What is the purpose of Benzene, p-nitrobenzoyl chloride, pyridine, and 20%aq KOH during the derivatization process?**

### ***11.4.5 Metals (Graphite Furnace, IMP, Atomic Absorption/Hydride Generation, IMP)***

#### ***C. Questions***

##### ***Background***

**1. How are sensitivity, and sensitivity check, and detection limits defined?** Sensitivity: Concentration of an element that is needed to produce a signal of 1% absorption (0.0044 absorbance units). Sensitivity check: concentration of element that will produce signal approximately 0.2 absorbance units under optimum conditions of the wavelength listed.

**2. How are limits of detection (LOD) and limits of quantitation (LOQ) defined?**

LOD:  $S/N = 3$

LOQ:  $S/N=10$

S= signal output that is measured as difference between sample and blank (avg.)

N= noise standard deviation of the fluctuations of the instrument output with a blank

3. **When dry ashing a sample, why is it necessary to char samples before the ashing step? How critical is temperature in the ashing procedures? What are good stopping places in the ashing procedure?** To prevent volatilization of materials and destruction of carbon. The temperature is critical to destroy the organic matter. Can stop after charring or leave the dishes in the oven overnight.
4. **What is the biochemical function of iron and sodium in the body?** *Iron* is used to make hemoglobin, acts as an oxygen carrier in the blood and muscle tissues and in enzyme catalysis. *Sodium* is absorbed by using ATP from the intestinal lumen. It counteracts calcium in muscular contraction and plays a role in osmotic balance.

### ***21 CFR and Compliance Program***

1. **What are the RDI values for iron, calcium, sodium, and selenium in adults? What are the Infant Formula Act requirements for these same elements?**

	<i>Adults</i>	<i>Infants</i>
Calcium	1000mg	50mg
Sodium	200mg	20mg
Selenium	70ug	
Iron	18mg	0.15mg

2. **List three foods that have standard requirements for minerals. Cite the requirements with the respective references.** Infant formula: ratio of calcium to phosphorous can be >1.1 and <2.0 21CFR 107.100(e). Iron-enriched macaroni products: 21CFR 139.117(b) (1) (2) (3). Iron-enriched rolls: 21CFR 136.115(a) (1) (2).
3. **Distinguish/define sodium-free, low sodium, and reduced sodium.** *Sodium free*: when a food contains less than 5mg of sodium per reference value serving. *Low sodium*: a food that has less than 140mg sodium/reference amount. *Reduced sodium*: food that contains less than 25% sodium per reference amount.
4. **A sample of crackers is labeled as low sodium with the sodium content declared at 35 milligrams per serving. The serving size is 30 grams. Is this product labeled correctly for sodium? The sample is found to contain 70 milligrams sodium per serving. Is the sample a violation for sodium according to the CP 7321.005/007 for NLEA? The sample is labeled correctly.**

### ***Elemental Analysis***

1. **What precaution is to be taken for perchloric acid digestions?** The digestion is not to proceed too rapidly or the sample may char. Perchloric acid when taken to dryness becomes explosive.
2. **Why is it necessary to expose the Se digestion in HCl after the wet digestion?** To prevent interference.
3. **What is the principle of the hydride generation method for Se analysis?** The addition of sodium borohydride, gaseous hydrides of the metal is formed. This is swept by argon purge and into a heated quartz cell, atomized vapor in cell produces a signal and the height is proportional to the amount of metal in sample.
4. **What is the primary difference between ICAP and AAS technology?** AAS has higher detection limits.
5. **What are some advantages of using the ICAP technology verses the GFAA technology for elemental analysis?** GFAA is useful when high temperatures are needed and interference is less than ICP.