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11.1 Introduction

This program provides training in the four distinct areas of nutrient analysis. These four areas are water-soluble vitamins, fat-soluble vitamins, proximate analysis, and metals. The training presented in this section is basic training for an analyst in the Atlanta Center for Nutrient Analysis (ACNA).

Each unit represents the four major areas of nutrient analysis. The units do not include the entire range of methodology performed in ACNA. The trainer will follow the preset unit for a particular assay. The trainer will demonstrate the method with the trainee. After the method has

been demonstrated the trainee will run the method with trainer assistance. The final test will be the trainee's ability to run the method independently.

The nutrition compliance programs have guidance and instructions for sample collection and analysis. For infant formulas (CP 7321.006) and medical foods (CP 7321.002), analytical methods are specified in the compliance program. Infant formulas and other standardized food have specifications in the Code of Federal Regulations (CFR) for nutrient content. Therefore, before beginning any sample analysis, the analyst should review the compliance program corresponding to the sample collected for analysis.

11.2 Historical Background

In 1973, the Food and Drug Administration (FDA) issued regulations requiring nutritional labeling on food for the following: food containing one or more added nutrients, food with labeling or advertising claims about the food's nutritional properties, or its usefulness in the daily diet. Nutritional labeling was voluntary for almost all other foods. In 1975, voluntary nutritional labeling went into effect. In 1984, sodium was added to the list of required nutrients, and potassium to the list of optional nutrients, on the nutrition panel. Effective in 1985, the new regulation also defined terms such as "low sodium." In 1990, FDA proposed extensive food labeling changes due to growing concern over diet, health, and labeling uniformity. These changes, now known as the NLEA, (The Nutritional Labeling and Education Act), included mandatory nutrition labeling for most foods, standardized serving sizes, and uniform use of health claims. Final regulations and health claims became effective in 1993; nutrient content claims became effective in 1994. In 1994, The Dietary Supplement Health and Education Act (DSHEA) was passed requiring mandatory nutritional labeling of dietary supplements.

The Atlanta Center for Nutrient Analysis (ACNA), established in 1976, performs nutrient profiles on all food products and dietary supplements. ACNA was established to analyze the products that had voluntary nutritional labeling. The mission of the branch has changed to accommodate the changes in regulations over the last 26 years. In addition to voluntary (then later mandatory) nutritional labeling for foods and supplements, the Infant Formula Act of 1980 impacted ACNA as well. In 1978 and 1979, two formulas were found to be deficient in chloride. The manufacturer wanted to reduce the amount of sodium in the formula, and reduced the levels of chloride below the levels found in human milk. After that incident, the Act was passed and placed infant formula in a category close to drugs in terms of strictness of quality. ACNA analyzes all infant formula to determine adherence to the Act.

ACNA performs laboratory analysis of samples for all FDA districts. The compliance program areas covered include 7321.002 Medical Foods, 7321.006 Infant Formulas, 7321.005 Domestic

NLEA, 7321.007 Import NLEA, 7321.008 Dietary Supplements and 7304.839 Total Diet Study (folic acid).

11.3 Sources of Methodology

Samples are analyzed using compendium methods as applicable. Compendium methods take precedence over use of other methods. Methods contained in the Association of Official Analytical Chemists (AOAC), United States Pharmacopeia (USP), or National Formulary (NF) are considered compendia methods. If a compendium method is not found, the Center for Food Safety and Applied Nutrition (CFSAN) is consulted. Whether a method is compendia or non-compendia, it is to be validated with recovery and reproducibility studies, use of positive and negative controls, use of Standard Reference Material, or in-house quality assurance/quality control materials. If a product is labeled as USP, the correct USP analytical method is used for analysis.

ACNA routinely encounters products that have no official method for the particular matrix. Use of AOAC procedures with modifications, memos of analysis (in-house methods), and standard operating procedures with proper controls and CFSAN approval have been used over the years.

11.4 Nutrient Analysis

11.4.1 Water Soluble Vitamins (Chemical Analysis)

I. Thiamine (B₁)

A. Objective

The analyst will become familiar with Thiamine (B₁) through thiamine reading materials and exercise questions. The analyst will be able to perform thiamine procedure as written in the AOAC.

B. References

1. *Methods of vitamin assay* (3rd ed., chap. 6, pp. 123-142). Interscience Publishers
2. Sebrell and Harris. *The vitamins* (Vol. V, chap. 15 on Thiamine).
3. Combs, G. F., Jr. *The vitamins* (chaps. 1, 3, 10, 19).

4. Eitenmiller, R. R., Ph.D., and Landen, W. O. (1999). *Vitamin analysis for the health and food sciences*. Boca Raton, FL: CRC Press.
5. *AOAC official methods of analysis* (current ed.). Gaithersburg, MD: Association of Official Analytical Chemists International.
6. Other reading material that analyst can find through Internet and library searches.

Websites for Thiamine Information

www.britannica.com

www.sciencekomm.at/journals/food.html

C. Pre-Assay Questions

1. What is a vitamin?
2. Name examples of fat-soluble and water-soluble vitamins.
3. What are the recommended daily allowances of thiamine in adults, infants, and lactating and pregnant women?
4. What are other names for thiamine?
5. List some natural food products that are considered rich sources of thiamine.
6. What form of thiamine is present in animal and plant products?
7. What is the function of thiamine in the body?
8. What are the effects of thiamine deficiency in man?
9. Are there any toxicity effects when high dosages of thiamine are taken? If so what are the effects?
10. Discuss the solubility of thiamine in water, alcohol, and organic solvents.
11. Discuss the stability of thiamine with respect to light, pH, oxidizing and reducing agents.
12. Draw a structure of the thiamine molecule.

D. Exercise

Analyst will read pertinent information pertaining to thiamine and answer questions on thiamine. The analyst will perform thiamine procedure on Standard Reference Material (SRM1846) and a practice sample.

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?
2. What precautions should one take during the procedure to protect the analyte and analyst?
3. What is the final concentration of the sample extract in the procedure?
4. What are some stopping points where the procedure can be interrupted and stored overnight without affecting the results?

II. Riboflavin (B₂)

A. Objective

The analyst will become familiar with riboflavin (Vitamin B₂) through reading materials and exercise questions. The analyst will be able to perform the riboflavin method as written in the AOAC.

B. References

1. *Methods of vitamin assay* (3rd ed., chap 7, pp. 147-166). Interscience Publishers.
2. Sebrell and Harris. *The vitamins* (Vol. V, chap. 14 on Riboflavin).
3. Combs, G. F., Jr. *The vitamins* (chaps. 1, 3, 10, 19).
4. Eitenmiller, R. R., Ph.D., and Landen, W. O. (1999). *Vitamin analysis for the health and food sciences*. Boca Raton, FL: CRC Press.
5. *AOAC official methods of analysis* (current edition). Gaithersburg, MD: Association of Official Analytical Chemists International.

6. Other references that analyst finds through library and Internet searches.

Websites for Riboflavin Information:

www.bodywise.com/products/ingredients/riboflavin.htm

www.britannica.com

www.sciencekomm.at/journals/food.html

C. Pre-Assay Questions

1. Is Riboflavin a water-soluble or fat-soluble vitamin?
2. What are the recommended daily allowances of riboflavin in adults, infants, and lactating and pregnant women?
3. What are other names for riboflavin?
4. List some natural food products that are considered rich sources of riboflavin.
5. What form of riboflavin is present in animal and plant products?
6. What is the function of riboflavin in the body?
7. What are the effects of riboflavin deficiency in man?
8. Are there any toxicity effects when high dosages of riboflavin are taken? If so, what are the effects?
9. Discuss the solubility of riboflavin in water, alcohol, and organic solvents.
10. Discuss the stability of riboflavin with respect to light, pH, oxidizing and reducing agents.
11. Draw a structure of the riboflavin molecule.

D. Exercise

The analyst will read pertinent information pertaining to riboflavin and answer questions. Analyst will perform riboflavin procedure on Standard reference Material (SRM1846) and a practice sample.

E. 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?
2. What precautions should one take during the procedure to protect the analyte and analyst?
3. What is the final concentration of the sample extract in the procedure?
4. What are some stopping points where the procedure can be interrupted and stored overnight without affecting the results?

III. Vitamin C

A. Objective

This training module is designed to train the employee to analyze vitamin C (ascorbic acid). This module covers two methods. These methods include the following:

1. 2,6-dichloroindophenol titrimetric method, and
2. total vitamin C microfluorometric method.

The employee will read background material on vitamin C, answer questions, and receive the methodology training.

B. References

1. Deutsch, M. J., and Weeks, C. E. (1965). Microfluorometric assay for vitamin C. *Journal Association of Official Analytical Chemists*, 48, 1248.
2. Deutsch, M. J. (1967). Assay for vitamin C: a collaborative study. *Journal of Association of Official Analytical Chemists*, 50, 798.
3. Horowitz, W. (Ed.). (2000). *AOAC official methods of analysis*. (17th ed., sects. 50.1.09 (985.33), and 45.1.15 (967.22)). Gaithersburg, MD: Association of Official Analytical Chemists International.
4. Eitenmiller, R. R., Ph.D., and Landen, W. O. (1999). *Vitamin analysis for the health and food sciences* (chap. 6, p. 223). Boca Raton: CRC Press.
5. Combs, G.F. (1992). *The vitamins* (chap. 9, p. 225). New York: Academic Press Inc.

C. Pre-Assay Questions

1. What is the %DV for vitamin C in adults, children, and infants?

2. What is disease related to vitamin C deficiency?
3. (a) 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 2 main biologically active forms of vitamin C?
4. What is the stability of L-ascorbic acid in solution?

D. Exercise

There are two commonly used methods for vitamin C in ACNA:

1. AOAC 17th ed. 50.1.09 (985.33) for infant formulas and medical foods, and
2. AOAC 17th ed. 45.1.15 (967.22) for all other matrixes.

The methods differ at the quantitation step and the forms of ascorbic acid that is measured. The trainee will read and make an outline of each procedure. The trainer will demonstrate each method. The trainer and trainee will perform each of the methods with various sample matrixes and controls. (In house controls such as infant formula, medical food, dietary supplement, and SRM 1846 are usable, as well as, samples received in the laboratory that has been previously analyzed. These samples or controls may be spiked at different levels unknown to the trainee.). The trainee will perform the method and meet the criteria of the method. When proficiency is demonstrated, the trainee will run official samples.

E. 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?
2. (a) What is the purpose of Norit?
(b) What is the purpose of 0-phenylenediamine?
3. What are the stopping points in each method?
4. What are the sources of interference in each method, and how does an analyst avoid them?

11.4.2 Water Soluble Vitamins (Microbiological Analysis)

I. Folic Acid

A. Objective

This module is designed to train the employee to analyze folic acid. This module covers three methods. These methods include the following:

1. a single enzyme digestion for the analysis of medical foods and infant formula,
2. a trienzyme digestion for all other food products, and
3. an alkaline digestion for dietary supplements.

The employee will read background material on folic acid, answer questions, and receive the methodology training.

B. References

1. U.S. Food & Drug Administration, Atlanta Center for Nutrient Analysis, Southeast Regional Laboratory. Memo of analysis for total folate analysis (internal document).
2. Horowitz, W. (Ed.). (2000). *AOAC Official Methods of Analysis* (17th ed., sections 50.1.21 (992.05), 45.2.03 (944.12), and 45.2.01 (960.46). Gaithersburg, MD: Association of Official Analytical Chemists International.
3. U.S. Food & Drug Administration, Southeast Regional Laboratory. (2000, August 2). Total diet program: folate in foods. Standard Operating Procedure N/AM/4/1/94.
4. Martin, J., Landen, W. O., Soliman, A., and Eitenmiller R. R., Ph.D. (1990). Application of trienzyme extraction for total folate determination in foods. *Journal of Association of Official Analytical Chemists*, 73, 805.
5. Rader, J., Weaver, C., and Angyal, G. (1998). Use of microbiological assay with trienzyme extraction for measurement of pre-fortification levels of folates in enriched cereal-grain products. *Food Chemistry*, 62, 451.
6. Souza, S. and Eitenmiller, R. R., Ph.D. (1990). Effects of different enzyme treatments on extraction of total folate from various foods prior to microbiological assay and radioassay. *Journal of Micronutrient Analysis*, 7, 37.
7. Aiso, K. and Tamura, T. (1998). Trienzyme treatment for food folate analysis: optimal pH and incubation time for alpha-amylase and protease treatments. *Journal Nutr. Sci. Vitaminol.*, 44, 361.
8. Handout: Structures and Properties of Folic Acid.

9. Handout: Microbiological Determination of Vitamins.
10. Mills, J. (2000). Fortification of foods with folic acid – how much is enough? *New England Journal of Medicine*, 342, 1442.
11. Williams, R. (1994, May). FDA proposes folic acid fortification. *FDA Consumer*.
12. U.S. Food and Drug Administration. (1999, March-April). Folic acid awareness. *FDA Consumer*.
13. Eitenmiller, R. R., Ph.D. and Landen W. O., W. 1999. *Vitamin Analysis for the Health and Food Sciences*. Boca Raton: CRC Press.
14. Combs, G. (1992). *The vitamins*. San Diego, CA: Academic Press Inc.
15. Spallholz, J., Boylan, L., and Driskell, J. *Nutrition chemistry and biology* (2nd ed.). Boca Raton, FL: CRC Press
16. Glenn, C. (1997). Putting folates to work. *Food Formulating*, p. 47.
17. Rader, J., Weaver, C., and Angyal, G. (2000). Total folate in enriched cereal-grain products in the United States following fortification. *Food Chemistry*, 70, 275.
18. U.S. Food & Drug Administration. (2000). Letter regarding *dietary supplement health claim for folic acid with respect to neural tube defects*. Docket No.: 91N-100H.
19. U.S. Food and Drug Administration, Office of Public Affairs. (2000). *History of FDA's total diet study*.
20. U.S. Food and Drug Administration. (1996). Folic acid to fortify U.S. food products to prevent birth defects. *HHS News*, P96-3.
21. U.S. Food and Drug Administration. (1997). FDA announces name changes for lower-fat milks and folic acid fortification for bakery products. *HHS News*, P97-47.
22. U.S. Food and Drug Administration, Office of Public Affairs. (1996). *Folic acid fortification*.

C. Pre-Assay Questions

1. Why did the Food and Drug Administration decide to fortify cereal-grain products with folic acid?

2. What is the %DV (folic acid) for adults, infants, and children?
3. What are the four predominant forms of naturally occurring folates?
4. What is the stability of folic acid?
5. What are the symptoms/consequences of folic acid deficiency?
6. What are the consequences of too much folic acid in the diet?
7. What are good sources of folic acid in foods?

D. Exercise

There are three commonly used methods for folic acid in ACNA:

- 1) Memo of Analysis: total folate analysis (AOAC 17th ed. 50.1.21(992.05),
- 2) Folic acid in vitamin preparations: AOAC 17th ed. 45.2.03(944.12), and
- 3) Folate in foods: SOP N/AM/4/1/94.

The three methods differ in the digestion procedures, the microbiology portion of the assays are similar. The trainee will read and make an outline of the three different procedures. The trainer will then demonstrate each method. The trainee and trainer will perform each of the methods with various sample matrixes and controls. (In-house controls such as an infant formula, dietary supplements, and SRM 1846 are usable, as well as, samples received in the laboratory that have been previously analyzed.) The trainee will then demonstrate proficiency in the methods by performing them independently with proper samples and controls. After proficiency is established, the trainee may be requested to analyze official samples. The trainer will also demonstrate operation of the automated dilutors, pipettors, and the Autoturb II reader.

E. Post-Assay Questions

1. What are the analytical procedures used in ACNA for determining folic acid? How do they differ?
2. Explain the use of the three enzymes in the SOP N/AM/4/1/94 procedure.
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?
4. What are the stopping points in each method?

5. What are the precautions used in each method to preserve the integrity of the samples/vitamin?
6. What are the hazardous materials and dangers in each method?

II. Niacin, Biotin, Vitamin B₆ (Pyridoxine), Vitamin B₁₂ (Cyanocobalamin) and Pantothenic Acid

A. Objective

The purpose of this training is to provide the analyst training to analyze water-soluble vitamins by AOAC microbiological methods and memo of analysis. Trainee will familiarize with Media (Broth and Agar) preparation, inoculum preparation, and instruments, reagents, and supplies used in the procedures.

B. Assignment

1. Review references and answer questions.
2. Review laboratory chemical hygiene plan, hazardous waste plan, and SRL Quality Assurance Plan.
3. Perform each assay using SRM 1846 and Infant Formula Control-198 (Milk based) along with training sample [see Exercises below].
4. Measure at least one recovery by spiking the sample with known amount of standard.
5. Read samples on Autoturb and Spectronic 20.
6. Calculate results manually (graph) and by using computer program.
7. Familiarize with weekly microorganism transfers to maintain cell line, Agar to Agar, and Agar to broth.

C. References

1. Combs, G. F. The vitamins: fundamental aspects. *Nutrition & Health*.
2. Eitenmiller, R. R., Ph.D., and Landen, W. O., Jr. Vitamin analysis for the health & food sciences.

3. Angyal, G. Method for the microbiological analysis of selected nutrients.
4. Horowitz, W. (Ed.). (2000). *AOAC official methods of analysis* (17th ed.). Gaithersburg, MD: Association of Official Analytical Chemists International.
5. *Journal of Association of Official Analytical Chemist* articles.
6. Any text which covers “Vitamins” & “Vitamin analysis” in the lab and library.
7. Handouts from trainer.
8. *Merck Index*.

D. Questions

1. Discuss the stability and solubility of each vitamin with respect to light, pH, oxidation, reduction, water, and organic solvents.
2. Name the diseases caused by the deficiency of each vitamin. Discuss symptoms of the diseases caused by each vitamin.
3. List some natural food products that are considered rich sources for each vitamin.
4. What is the recommended concentration of each vitamin in the final sample assay extract?
5. Indicate the stopping points, where an analyst can interrupt the procedure for overnight storage without affecting the results.
6. Identify the test organisms listed below and indicate the vitamins assayed by each.
(a) L.l. (b) S. f. (c) L.p. (d) L.c. (e) S.u.
7. How are the test organisms kept viable?
8. What are an inoculated and an uninoculated blank?
9. Define heat treatment and sterilization?
10. Why is lactic acid bacteria widely used in microbiological assays?

11. Why it is possible to use a test organism to assay more than one vitamin?
12. Why transfer bacteria weekly?
13. What is Lactobacillus agar, Lactobacillus broth, agar slants, agar stabs, 0.9% Saline, vitamin B₆ broth, and rinse and define the purpose of each.
14. How long do stab cultures stay active?
15. How often does one transfer stab cultures?
16. How long does one grow most bacteria for an inoculum?
17. What is the incubating temperature range for most assays? Is temperature critical to an assay?
18. What is the growth time for microbiological assays? Is the growth time critical to an assay?
19. What is the recommended temperature for storing agar, slants stabs?
20. Discuss the purpose of basal media in the assay.
21. What are the criteria of an official assay?
22. What is the advantage of an eight point standard curve versus a five point standard curve?
23. What precautions should be taken when performing microbiological assays to avoid contamination and invalidating the assays?
24. What are the differences between titrimetric and turbidimetric assays?
25. How does one determine if a sterilization cycle is complete and has rendered the items inside as sterile?
26. What are the three major factors that affect the growth of microorganisms?
27. How does one determine when the standard has reached its maximum growth?

E. Exercise

The trainee will read each method and make an outline of procedure. The trainer will demonstrate and discuss different aspects of each method with trainee. The trainee will perform each method with or without trainer help, but under his/her supervision. The trainee will run SRM 1846 and a training Sample. Upon satisfactory completion of training, trainee will run official sample.

The methods routinely used in ACNA are as follows:

Pantothenic Acid: Memo of Analysis.

Biotin: Memo of Analysis.

Niacin: Memo of Analysis for Biotin (Same extract can be used to assay niacin).

- AOAC 17th Ed 50.1.19 (985.34) RTF milk based IF.
- AOAC 17th Ed 45.2.04 (944.13) Vitamin Preparation.

Vitamin B₆

- AOAC 17th Ed 50.1.18 (985.32) RTF milk based IF.
- AOAC 17th Ed 45.2.08 (961.15) Food products.

Vitamin B₁₂

- AOAC 17th Ed 50.1.20 (986.23) RTF milk based IF.
- AOAC 17th Ed 45.2.02 (952.20) Vitamin Preparation.

Outline of training program is as follows:

Week # 1: Training assignment

- Review references and answer questions
- Review laboratory chemical hygiene plan, hazardous waste plan, and SRL Quality Assurance Plan.

Week # 2: Niacin (SRM & Duplicate Training Sample)

- Extraction
- Set up tubes
- Preparation of inoculum, media
- Reading sample & standard on Spectronic 20 & Autoturb II
- Calculation by regression analysis manually (graph)
- Calculation by computer program
- Complete worksheets

Week # 3: Vitamin B₆ (SRM & Duplicate Training Sample)

- Extraction
- Set up tubes
- Preparation of inoculum, media
- Reading sample & standard on Autoturb II
- Calculation by computer program.
- Complete worksheets
- Discuss extraction procedure for Biotin & Pantothenic Acid.

Week # 4: Vitamin B₁₂ (SRM & Duplicate Training Sample)

- Extraction-, Set up tubes,Preparation of inoculum, media
- Reading sample , standard on Autoturb II
- Calculation by computer program.
- Complete worksheets.

Week # 5: To determine dry cell weight for one vitamin

- Use niacin or folic acid or any other vitamin
- Calibrate Autoturb or Spectronic 20
- Check contamination during assay
- Draw a curve plotting % transmittance reading for each level of diluted cell suspension against cell content (mg dry wt) of respective tubes).

11.4.3 Fat Soluble Vitamins (HPLC)

I. Vitamin K

A. Objective

This training module is designed to train the employee to analyze Vitamin K. This method covers the AOAC 999.15 procedure, which is the official procedure used in ACNA. The employee will read background material on Vitamin K, answer questions, and receive the methodology training.

B. References

1. Horowitz, W. (Ed.). *AOAC official methods of analysis*. (2000). (17th ed., sect. 50.1.25 (999.15). Gaithersburg, MD: Association of Official Analytical Chemist International.
2. Indyk, H. E., and Woollard, D.C. (2000). Determination of vitamin K in milk and infant formula by liquid chromatography: collaborative study. *Journal of Association of Official Analytical Chemists International*, 83(1), 121-130.

3. Indyk, H. E., Woodllard, D. C. (1997, May). Vitamin K in milk and infant formulas: determination and distribution of phyloquinone and menaquinone-4. *Analyst*, 122, 465-469.
4. Simplified Vitamin K Assay Procedure.
5. HP 1100 Operating Instructions for Vitamin K.
6. Misc. attachments and e-mail messages.
7. Horowitz, W. (Ed.). (2000). *AOAC official methods of analysis*. (17th ed., sect. 50.1.06 (previous method used in Atlanta Center for Nutrient Analysis). Gaithersburg, MD: Association of Official Analytical Chemists International
8. Hewlett Packard books for the HP1100 ChemStation (particularly Understanding Your ChemStation) (Note: Hewlett Packer is now Agilent).
9. Eitenmiller, R. R., Ph.D., and Landen, W. O. (1999). *Vitamin analysis for the health and food sciences*. Boca Raton: CRC Press.
10. Combs, G. (1992). *The vitamins*. San Diego, CA: Academic Press Inc.

C. Pre-Assay Questions

1. What is the %DV (Vitamin K) for adults, infants, and children?
2. What are the forms of vitamin K and where do they come from (source)?
3. What is the stability of Vitamin K?
4. What are the symptoms/consequences of too much or too little vitamin K in the diet for adults and children or infants?
5. What are good sources of vitamin K in foods?

D. Exercise

The method to be used is the AOAC 999.15. The trainer will demonstrate the assay procedure, then the trainee and the trainer will perform the assay including the High Performance Liquid Chromatography (HPLC) set-up and run. (In house controls such as the SRM1846 Infant Formula and a blank are included, as well as samples received in the laboratory that have been

previously analyzed.) The trainee will then demonstrate proficiency by performing the assay independently with proper samples and controls.

E. Post-Assay Questions

1. What are the differences in the new and old procedures?
2. What is the purpose of the buffer, the Lipase, the alcohol solution, the K_2CO_3 , and the hexane?
3. Explain the post column reductor. What precautions are needed in the use of this piece of equipment?
4. What is the purity factor and how is it determined?
5. Why would an analyst use a slightly elevated column temperature instead of ambient temperature?
6. Explain the system suitability test.
7. What does using a “forced zero” in the linear regression curve mean?
8. If a sample calculates to have a fluorescence value above the highest standard point value, how can one get it on the curve?
9. What are the stopping points in the assay?
10. What are the precautions used in the method to preserve the integrity of the sample/vitamin?
11. What are the hazardous materials and dangers in the method?

11.4.4 Proximate Analysis (Various Methods)

I. Cholesterol (HPLC)

A. Objective

This training module is designed to train analysts in the cholesterol assay published in *Journal of AOAC International* Vol. 77, No 5, 1994. The method is usable for a wide variety of food

matrixes. Under the Nutrition Labeling and Education Act (NLEA) of 1990, the amount of cholesterol is mandatory on the label if present in more than 2 mg/serving in products regulated by FDA. At least two health claims linking cholesterol to disease are approved. For regulatory compliance with the nutrition label, ACNA frequently receives samples for cholesterol analysis. The trainee will read references, answer questions, and receive methodology training. Upon satisfactory completion of training, trainee will demonstrate proficiency by running official samples.

B. References

1. Hamill, T. and Soliman, A. (1994). Determination of cholesterol by p-nitrobenzoate derivatization and liquid chromatography. *Journal Association of Official Analytical Chemists*, 77(5).
2. Simplified Cholesterol assay procedure and notes by analyst.
3. High Performance Liquid Chromatography Troubleshooting guides from Supelco.
4. High Performance Liquid Chromatography columns use and care by ANSYS Technologies Inc.
5. Attachments and Email messages regarding cholesterol waste.

C. Questions

1. What is cholesterol and where it is found (source)?
2. What are the health claims approved by FDA linking cholesterol to disease?
3. Describe toxicity of pyridine and benzene. What are the waste codes and what they mean?
4. How does one dispose the above two reagents?
5. What are the stopping points in the assay?
6. What is the purpose of benzene, p-nitrobenzoyl chloride, pyridine, and 20% aq KOH during the derivatization process?

D. Exercise

The method used for cholesterol assay in ACNA is Thomas Hamill's paper, which is published in *Journal of AOAC International* Vol. 77, No 5, 1994. Read the method thoroughly during training and make notes. The trainer will demonstrate the assay procedure. Trainee will watch the demonstration and then perform the assay independently. Trainer and trainee will run SRM 1845 (NIST), Training sample and Standard.

11.4.5 Metals Analysis (Graphite Furnace, Inductively Coupled Atomic Plasma (ICAP), Atomic Absorption/Hydride Generation)

I. Iron, Calcium, Phosphorus, Manganese, Magnesium, Zinc, Copper, Sodium, Potassium, Selenium, Chromium, Molybdenum

A. Objective

This module provides training in the four metal analysis methods. These methods include the analysis of different metal elements using the IACP technique, the Hydride Generator, and the Graphite Furnace Atomic Absorption (GFAA). The employee will read background material on metals, answer questions, and receive the methodology training.

B. References

1. Instrumental Manual for the Perkin-Elmer 2100 and TJA ICAP 61E.
2. National Research Council. (1989). *Recommended dietary allowance* (10th ed.). Washington, DC: National Academy Press.
3. Perkin-Elmer. *Analytical methods for furnace Atomic Absorption Spectroscopy (AAS)*.
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11. *United States Pharmacopoeia XXI* (p. 189).

C. Questions

Background

1. How is sensitivity, sensitivity check and detection limits defined?
2. How are limits of detection (LOD) and limits of quantitation (LOQ) defined?
3. When dry ashing a sample, why is it needed to char samples before the ashing step? How critical is temperature in the ashing procedures? What are good stopping places in the ashing procedure?
4. What is the biochemical function of iron and sodium in the body?

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?
2. List three foods that have standard requirements for minerals. Cite the requirements with the respective references.
3. Distinguish/define: sodium-free, low sodium, and reduced sodium.
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?

Elemental Analysis

1. What precaution is to be taken for perchloric acid digestions?
2. Why expose the Se digestion in HCl after the wet digestion?
3. What is the principle of the Hydride Generation method for Se analysis?
4. What is the primary difference between ICAP and AAS technology?
5. What are some advantages of using the ICAP technology versus the GFAA technology for elemental analysis?

D. Exercise

This training exercise will consist of performing all determinations in duplicate. The employee is to assay the SRM 1846, any other assigned samples, and 2 method blanks.

1. Assay the samples for Calcium, Phosphorous, Magnesium, Iron, Copper, Manganese, Zinc, Sodium, and Potassium using the ICP technique. (Method: AOAC 17th ed. 50.1.15 (984.27)).
2. Assay the samples for Selenium. See Selenium SOP procedure.
3. Assay a medical food sample for Chromium and Molybdenum using GFAA. See memo of analysis for procedure.

11.5 Appendix - Answer Key

11.4.1 Water Soluble Vitamins (Chemical Analysis)

I. Thiamine (B₁)

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, B₆, B₁₂, 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 B₁, 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 17th ed. 50.1.08 (986.27) states 15 ug; AOAC; AOAC 17th 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 17th 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 17th ed. 45.1.06 (953.17) states that after the filtration step samples can be placed in refrigerator overnight.

II. Riboflavin (B₂)

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 B2, 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 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_4 . 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.

E. 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.

E. 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-H₄PteGlu₅, tetrahydrofolate pentaglutamate, pteroylglutamic acid, 5, 10-methenyl-H₄PteGlu.
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 B₆ (Pyridoxine), Vitamin B₁₂ (Cyanocobalamin) and Pantothenic Acid

D. 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 B₆: Soluble in water, acetone, and alcohol, slightly soluble in ether and chloroform. Stable in heat, concentrated alkali and hydrochloric acid. Destroyed by light.

Vitamin B₁₂: 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 B₆: Symptoms--dermatitis, microcytic anemia, irritability, depression, convulsions

Vitamin B₁₂: 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 B₆: bananas, seeds, rice, beef, pork, poultry.

Vitamin B₁₂: 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 B₆: 1.0 ng/mL.

Vitamin B₁₂: 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 B₁₂.

(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 B₆.

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 B₆ 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 B₆ Broth: this broth is the sterile culture media containing specific nutrients to support *Saccharomyces pastorianus*.

Rinse: used in B₆ 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°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.

E. 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 K₂CO₃, 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.

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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.

11.6 Document History

Version 1.3 Revision Approved: 02-14-13 Author: LMEB Approver: LMEB

Version 1.2 changes:

Sections 11.4.3, 11.4.4 expanded and answers added.

Table of Contents – 11.5 title changed; 11.6 added.

Version 1.3 changes:

Header – Division of Field Science changed to Office of Regulatory Science