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10.1 Food Standards

"Food Standards" is our common designation for Standards of Identity, Standards of Quality, and Standards of Fill of Container promulgated under the FD&C Act. They are to be distinguished from grade standards often referred to as "U.S. Standards," which are promulgated by the U.S. Department of Agriculture.

10.1.1 Introduction

Food within our jurisdiction, represented as or purporting to be a food for which standards have been set under the Act, must comply with the requirements of the standard, or it is misbranded. For this reason, FDA Food Standards have been called mandatory.

In contrast, "U.S. Standards" are voluntary. It is not required that foods be labeled to show these grades. For instance, a canner who is packing canned corn that only comes up to "U.S. Grade C" requirement has the option to omit any reference to grade on the labels. However, if such canned corn is mislabeled as "U.S. Grade B", its labeling is "false or misleading".

This section is intended to acquaint the analyst with food standards, the law authorizing such standards, their basis, the procedures and techniques used in determining compliance with the standards, and the significance of results. The work in this section need not be taken in order. This introduction, however, should be read and discussed before examining samples in the succeeding exercises.

The work in this section will be almost entirely unfamiliar to a trainee. The methods are rigidly prescribed and are to be followed meticulously. Although these methods have a more or less empirical flavor, facts determined by such methods are called for in legal actions. The methods are published in the Code of Federal Regulations (CFR) or as final in the Federal Register prior to codification in the CFR. The specified method must be used to determine compliance with the standard, and the analytical report references the method as cited in the regulation.

The purpose of food standards is to maintain the integrity of food products so consumers get what they reasonably expect. So that they will be meaningful to the layperson, standards, as far as is practical, are established under the common or usual name of the food product.

Because food standards have legislative effect, they are phrased in such definite terms and with such precision as to stand up in court when challenged. Identity standards specify the required ingredients and the optional ingredients that are permitted in the food. Ingredients not recognized in the standard are not permitted. Some ingredients are specified quantitatively. For example, the identity standard requires oleomargarine to have not less than 80 percent fat as determined by a specified method.

Quality standards specify the quality factors covered by the standard and objective methods for measuring these factors. For example, green beans that are highly fibrous do not have good eating quality. The quality standard for canned green beans sets a limit for fiber and prescribes the method to be followed to determine the fiber content.

Fill of container standards prescribe how much of the defined product must be in the container. This standard has been promulgated only for certain fruits, vegetables, fish, shellfish, and nuts that are canned, packed in glass, or packed in semi rigid containers. Fill standards vary widely, from those without methods (e.g. canned peaches) to those where both method and apparatus are

specified in detail (e.g. press weight for tuna). Except for certain canned fruits, fill standards specify the method and apparatus to be used to determine compliance. Some standards (e.g. canned applesauce) prescribe "sampling and acceptance" procedures, which state the number of units to be examined for a specified size lot and the number of units that exceed the acceptance level before the lot is below standard.

10.1.2 Exercises

The trainee should carefully read sections of the Act applicable to food standards 401, 403(g) and (h), and 701(e)(1) and examine CFR Parts 130-169 to become familiar with the products that have been standardized.

Not any time has been planned in the Food Standards program area; therefore the laboratory will have the option of performing the following exercises.

10.1.2.1 Canned Vegetables and Fruits

A. Introduction

This exercise involves the determination of a canned food's compliance with a defined standard.

B. Assignment

The trainer will supply one or more of each of the following categories of canned foods: a canned corn or tomato product, canned peas or beans, and a canned fruit. Compliance with the standard should be determined according to applicable sections of the CFR.

C. References

1. *Code of Federal Regulations*. (2003). Title 21, Pts. 145-Canned Fruits, 155-Canned Vegetables, and 156-Vegetable Juices. Washington DC: Office of the Federal Register National Archives and Records Administration.
Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfrv2_01.html
2. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition. (1998). *Bacteriological analytical manual* (8th ed., chap. 21). Arlington, VA: Association of Official Analytical Chemists.

D. Questions

1. What are food standards? How do they differ from grade standards?
2. What consumer protection was presumed in the promulgation of food standards?

3. When is the packing liquid included in net weight calculations?
4. Define edible portion.
5. What is the basis for determining the solids content by use of the refractive index?
6. Why isn't the product allowed to "drain" completely in a drained weight determination? Why is two minutes specified in 21 CFR 145.3(n)?
7. What is the basis for the determination of alcohol in soluble solid in canned vegetables?
8. What are the requirements for the fill of a container?
9. Can the net contents be satisfactory and fill of container fail?
10. Define "degrees Brix."

10.1.2.2 Cheese

A. Introduction

This exercise involves preparing a composite, checking compliance with standards, and determining a food additive.

B. Assignment

The trainer will provide one or more types of cheese.

1. Determine moisture and fat in duplicate by the method specified in the CFR.
2. Determine the sorbic acid content according to the method designated by the trainer.

C. References

1. *Code of Federal Regulation*. (2003). Title 21, Pt. 133-Cheeses and Related Cheese Products. Washington DC: Office of the Federal Register National Archives and Records Administration.
Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr133_01.html
2. (1990). *AOAC official methods of analysis*. (15th ed., sections 926.08, 948.12, 969.19, 977.11 (17th ed., sections 33.7.03 - 33.7.06); 933.05, 974.09, 920.125 (17th ed., sections 33.7.17 - 33.7.19); 971.15, 974.10 (17th ed., sections 47.3.36 - 47.3.37). Arlington, VA: Association of Official Analytical Chemists.

3. Maxstadt, J. J., Karasz. A. B. (1972). Rapid spectrophotometric method for determination of sorbic acid in fresh dairy products. *Journal of Association of Official Analytical Chemists* 55, 7-8.
4. Joslyn. M. A. (1950). *Food analysis* (pp.124-125). New York: Academic Press.
5. Meyer. L. H. (1960). *Food chemistry* (pp. 306-315). New York: Reinhold Publishing Co.
6. U.S. Department of Agriculture. (1953). *Cheese varieties* (USDA Handbook No. 54,,: reprinted 1987). Alexandria, VA: National Cheese Institute.

D. Questions

1. Why is speed essential in sample preparation?
2. Differentiate between a cheese spread and cheese food.
3. Why are preservatives added? Must they be declared on labels?
4. List two kinds of cheese in which sorbic acid is permitted and include the conditions under which it might be used. List two kinds of cheese for which sorbic acid is not permitted.
5. What is a processed cheese?
6. In the CFR procedure, is the moisture content directly determined?
7. In the cheese fat extraction, what other substances may be found?
8. Why is petroleum ether used for the extraction?

10.1.2.3 Jams, Jelly, and Orange Juice

A. Introduction

These foods are highly susceptible to adulteration for economic gain. An economic cheat involves the substitution of a cheap, easily obtainable ingredient, such as water, for a more expensive one, such as fruit. Tests have been devised to check certain indices or ratios of indices related to product composition to determine their compliance with standards.

B. Assignment

The trainer will provide one or more of this type of product.

1. Make the following determinations:
 - a. Net contents
 - b. Soluble solids by refractometry
 - c. Ash
 - d. Potassium oxide (K₂O) by atomic absorption spectrometry
2. Compare results with established standards.

C. References

1. *Code of Federal Regulations*. (2003). Title 21, Pts. 146-Canned Fruit Juices and 150-Fruit Butters, Jellies, Preserves, and Related Products. Washington DC: Office of the Federal Register National Archives and Records Administration.
Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfrv2_01.html (CFR, Chap. 1, Parts 100-169, Table of Contents)
2. Joslyn, M. A. (1950). *Food analysis* (pp.133-141). New York: Academic Press.
3. *Journal Association of Official Agriculture Chemists* (1938). 21, 506; (1961). 44, 633; (1964). 47, 1068.
4. *Journal Association of Official Analytical Chemist*. (1968). 51, 1203; (1969). 52, 1704, 1152, (1973). 56, 743.
5. *Food Control Statement*. No. 2; No. 76, pp. 47-72.
6. Cruess, W.V. (1958). *Commercial fruit and vegetable products* (pp. 426-462, 465-488). New York: McGraw Hill.
7. Hart, F. L., Fisher. H. J. (1971). *Modern food analysis* (pp. 223-230, 238-244). New York: Springer-Verlag.
8. Kermit, M. F., Rogers, G. R. (1969). Chemical composition of Florida orange concentrates. *Journal of Agricultural Food Chemistry*, 17, 1119-1122.

D. Questions

1. What is jam?
2. What is an imitation jelly?
3. What orange products are standardized?

4. Why are K₂O and ash used as criteria to determine fruit content?
5. Are the literature values of K₂O and ash definitive for fruit?

10.1.2.4 Mixed Nuts

A. Introduction

Packaged mixed nuts present several unique opportunities for economic cheating: labeling the product with a misleading vignette; representing the product as containing a high percentage of expensive nuts, although it actually contains a majority of relatively cheap peanuts; or presenting the product in a large container, labeling the net weight correctly, but substituting packaging material for a large excess volume. The analyst is to be aware of the law in examining any product for standards.

B. Assignment

The trainer will provide a sample of mixed nuts. Examine a minimum of six units for net weight and compliance with food standards. (Check the CFR for the number of units required to be analyzed to determine compliance with the standard.)

C. References

1. *Code of Federal Regulation*. (2003). Title 21, Pt. 164-Tree Nut and Peanut Products. Washington DC: Office of the Federal Register National Archives and Records Administration.
Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr164_01.html
2. *Quantity of Contents Compendium*.

D. Questions

1. Be prepared to discuss the sections of the Act that apply to the examples of cheating given in the Introduction.
2. Differentiate between misbranding and adulteration in regard to question 1.
3. Why do the standards specify both amount and type of nuts?
4. How does the shape of the container affect the net weight determination?
5. What is the maximum amount of peanuts allowed in mixed nuts?

6. What, if any, is the relationship between the vignette and the contents?

10.1.2.5 Egg Noodles

A. Introduction

Eggs are the most expensive ingredient in egg noodles. If the egg content does not meet the standard, the product cannot be shipped legally in interstate commerce.

B. Assignment

1. Prepare a well-mixed composite.
2. Determine total solids and moisture in duplicate.
3. Determine the cholesterol content by the AOAC digitonin and fluorometric methods. Compare the results.

C. References

1. *Code of Federal Regulations*. (2003). Title 21, Pt. 139-Milk and Cream. Washington DC: Office of Federal Register National Archives and Records Administration. Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr139_01.html
2. AOAC official methods of analysis. (1990). (15th ed., sections 926.06, 926.07 (17th ed. sections 32.5.01 - 32.5.02) 954.03, 969.14 (17th ed., sections 32.5.13 - 32.5.14); and related AOAC references. Arlington, VA: Association of Official Analytical Chemists.
3. (1926). *Journal Association of Official Agricultural Chemists*, 9, 397.
4. (1954). *Journal Association of Official Agricultural Chemists*, 37, 408.
5. (1967). *Journal Association of Official Analytical Chemists*, 50, 850-851.
6. (1968). *Journal Association of Official Analytical Chemists*, 51, 590.
7. (1969). *Journal Association of Official Analytical Chemists*, 52, 319.
8. Woodman, A.G. (1945). *Food analysis* (pp. 182-183). New York: McGraw Hill.
9. Triebold, H. O., Aurand, L. W. (1963). *Food composition and analysis* (pp. 104-105). Princeton, NJ: Van Nostrand.

10. Jacobs, M. B. (1951). *The chemistry and technology of food and food products* (Vol. 2, pp. 994-1021). New York: Interscience.
11. Feiser, L. F., Feiser, M. (1959). *Steroids* (p. 30). New York: Reinhold.
12. Hart and Fisher. (1971). *Modern food analysis* (pp. 1, 3, 77, 79, 87, 88).
13. Meyer. (1960). *Food chemistry* (pp. 3-10, 162-68).

D. Questions

1. Are eggs the only possible source of cholesterol in this product?
2. Does the label have an ingredient statement? Why?
3. Do all noodle products contain eggs?
4. Compare the two drying methods. Which one is to be used to determine compliance with the standard for total solids?
5. What is accomplished in each step of AOAC 954.03?
6. Why is there a precaution in AOAC 954.03 to retain solid material in the separator?
7. How would an analyst cope with an emulsion if it formed?
8. What is the difference between macaroni and noodles?

10.2 Food and Color Additives

Food and color additives are defined in the Act in Sections 201(s) and 201(t), respectively. However substances generally recognized as safe (GRAs) under proposed conditions of use, sanctioned prior to the 1958 Food Additives Amendment, or subject to the provisions of Section 408 (Pesticide Chemicals) of the Act, are exempt from the definitions.

10.2.1 Introduction

In 1958, the Food Additives Amendment became Section 409 of the Act. This section requires that safety be established on a proposed additive. The use of any food additive--direct, indirect, or GRAS--must be authorized by regulation in 21 CFR 170. Within Section 409 is the so called "Delaney clause," which restricts use of cancer inducing substances (carcinogens) in food: ". . . no additive shall be deemed to be safe if it is found to induce cancer when ingested by man or

animal, or if it is found, after tests which are appropriate for the evaluation of the safety of food additives to induce cancer in man or animal. . . ."

There are exceptions to this restriction on the use of carcinogens, which relate to their use in animal feeds for food producing animals.

In 1960, the Color Additive Amendment became Section 706 of the Act. It established the same safety criteria for proposed color additives as for food additives. Regulations for enforcement are in 21 CFR Parts 70-82.

In 1990, the Nutrition Labeling and Education Act (NLEA) was passed. It requires the declaration of all certifiable color additives on the product label with a few exceptions; for example butter, cheese, and ice cream are exempt from certified color labeling requirements except for FD&C Yellow No. 5 and No. 6. See CFR 101.22(k)(1).

Analytical exercises are included under this topic for food and color additives in consumer products.

Before beginning the exercises, the trainee should review the pertinent sections of the Act and the regulations to become familiar with the types of analyses that may be needed.

10.2.2 Food Additives Exercises

This section includes exercises for food additives, salt (sodium chloride), sodium nitrite in smoked fish, sulfites in dried fruit, monosodium glutamate, artificial sweeteners (saccharin & cyclamates) and borates in caviar.

10.2.2.1 Salt and Sodium Nitrite in Smoked Fish

A. Introduction

This exercise has been developed to provide an introduction to food additives, food additive regulation, and the principles of analysis for common chemical preservatives such as sodium chloride and sodium nitrite.

B. Assignment

The trainer will provide a sample of smoked fish. Determine water phase salt and nitrites as described:

1. Sample Preparation

Prepare each subsample separately for analysis according to AOAC 937.07(e), except

take the loin muscle as defined in 21 CFR 161.190(a)(3) for the sample portion. i.e.. "the longitudinal quarter of the great lateral muscle (loin muscle) freed from skin, scales, visible blood clots, bones, gills and viscera and from the nonstriated part of such muscle." Grind the loin muscle according to AOAC 937.07(a), paragraph 2. Store the sample as directed in AOAC 937.07, first paragraph, and use for analysis in parts 2 and 3, below.

2. Water Phase Salt

- a. Analyze each of the prepared subs by AOAC 952.08A for moisture content (total solids) and AOAC 937.09 for salt content. (See 21 CFR 172.177.)
- b. Calculate salt content in the water phase of the loin muscle according to the formula:
$$\% \text{ Salt in water phase of loin muscle} = [\% \text{ NaCl} (\text{NaCl} + \% \text{ moisture})] \times 100$$

3. Sodium Nitrite

Analyze each of the prepared subs for nitrite using AOAC 973.31. Report nitrite as ppm sodium nitrite in loin muscle.

C. References

1. *Code of Federal Regulations*. (2003). Title 21, Pts. 110-Current Good Manufacturing Practice in Manufacturing, Packing, or Holding Human Food, 170-Food Additives, and Section 172.177-Sodium nitrite used in processing smoked chub. Washington DC: Office of Federal Register National Archives and Records Administration. Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfrv2_01.html and from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfrv3_01.html
2. AOAC official methods of analysis. (1990). (15th ed., section 937.07 (17th ed., 35.1.01), 952.08A (17th ed., sect. 35.1.13) , 937.09 (17th ed., sect. 35.1.18) , 973.31 (17th ed., sect. 39.1.21). Arlington VA: Association of Official Analytical Chemists.
3. U.S. Food & Drug Administration, Office of Enforcement. (2000, August). Compliance policy guides manual. Compliance Police Guide 7308.15, Chubs, Hot Process Smoked with Added Nitrite, p. 276.
4. U.S. Food & Drug Administration, Office of Enforcement. (2000, August). Compliance policy guides manual. Compliance Policy Guide 7308.18, Tuna, Sable, Salmon, Shad--Smoked Cured. Adulteration Involving Food Additives, Sodium Nitrite, p. 283.
5. (1987). Food additive analytical manual. (Vol. 2, Sodium Nitrate, Sodium Nitrite).

Arlington, VA: Association of Official Analytical Chemists.

6. (1970). *FDA Papers*, 4, 25.
7. (1981). *Food chemicals codex* (3rd ed., pp. 1-5, 293-95). Washington. DC: National Academy of Sciences Press.
8. (1946). *Ind. Eng. Chem. Anal. Ed.*, 18, 96.
9. Jenkins, G. L., Knevel. A. M., DeGangi, E. (1976). *Quantitative pharmaceutical chemistry* (7th ed., chap. 7). New York: McGraw Hill.
10. Cox. H. E. Pearson, D. (1962). *Chemical analysis of foods* (chaps. 1 and 30). New York: Chemical Publishing Co.
11. U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition. (2003, May 16). Everything Added to Food in the United States (EAFUS): A Food Additive Database. *Food Ingredients and Packaging*. Retrieve from <http://www.cfsan.fda.gov/~dms/eafus.html>

D. Questions

1. What are the three conditions that are to be met for a preservative to be used according to good manufacturing practices?
2. List 10 standardized foods for which the addition of chemical preservatives has been permitted by regulation.
3. Which fish or fish products may legally contain nitrates or nitrites? List the maximum legal level for each product and describe how the retail container must be labeled.
4. Where is "water phase salt" defined? How does water phase salt vary with moisture content? Write the equation used for calculating the salt concentration in the water phase.
5. Why is water added to the portion taken for analysis by AOAC 952.08A?
6. How would one show that all the volatile components had been evaporated from the tissue?
7. Show the reactions involved in the titrimetric analysis for total chloride.
8. Why is the HNO₃ solution boiled before the indicator is added?

9. AOAC 937.09B(b) states "With 10g sample each mL 0.1N AgNO₃ = 0.058% NaCl."
How is this factor obtained?
10. What is the structure of the colored product formed during the nitrite analysis? Show the reactions involved.
11. How has the order of addition of reagents been shown to affect this type of analysis?
12. What is the correlation between pH and color stability?

10.2.2.2 Sulfites

A. Introduction

Sulfur dioxide and sodium and potassium bisulfites and metabisulfites are used in foods as antioxidants and bleaching agents. Sulfites are commonly used as preservatives in dried fruits and as bleaching agents in the processing of maraschino cherries.

This exercise instructs the trainee to determine the amount of sulfite, calculated as sulfur dioxide, present in a preserved food.

B. Assignment

The trainer will provide a sample of dried fruit preserved with sulfides. Determine the amount of sulfite present as sulfur dioxide using the Monier-Williams Procedure (with Modifications), also referred to as the Optimized Monier Williams Procedure published in the Federal Register Vol. 51, No.131. July 9, 1986, pp. 25017-20. Also perform the gravimetric determination ,AOAC. 962.16C.

C. References

1. *Federal Register*. (1986, July 9). (Vol. 51, No. 131, pp. 25012-26).
2. AOAC official methods of analysis. (1990). (15th ed., sects. 975.32, 962.16C, (17th ed., sects. 47.3.38 - 47.3.39). Arlington, VA: Association of Official Analytical Chemists.
3. (1990). AOAC official methods of analysis (15th ed., sections 987.04, 990.28 (17th ed., sects. 47.3.42 - 47.3.43). Arlington, VA: Association of Official Analytical Chemists.
4. (1989). Determination of sulfites in foods by simultaneous nitrogen purging and differential pulse polarography. *Journal Association of Official Analytical Chemists*, 72(3), 476 – 480.

5. Papazian, R. (1996, December). Sulfites: safe for most, dangerous for some. *FDA Consumer Magazine*. Retrieve from <http://www.cfsan.fda.gov/~dms/fdsulfit.html>
6. Warner, C. R., Ph.D., Diachenko, G. W., Ph.D., Bailey, C. J., M.Ed. (Ed.). *Sulfites: An Important Food Safety Issue* (reprinted from *Food Testing & Analysis*, August/September 2000).
Retrieve from <http://www.cfsan.fda.gov/~dms/fssulfit.html>

D. Questions

1. Why doesn't the HCl distill into the U tube or sulfur dioxide absorber? What is an azeotrope?
2. What reaction occurs in the distilling flask? In the U tube? In the sulfur dioxide absorber?
3. What is the purpose of the gravimetric determination?
4. What purpose does the pyrogallol/KOH gas wash serve?

10.2.2.3 Monosodium Glutamate

A. Introduction

Any substance used in food must be of a food grade quality. The "Food Chemicals Codex" as initiated in 1958 to compile standards for food grade chemicals. In 1971, FDA officially recognized the Codex in the regulations. (See 21 CFR 170.30(h)(1) and 570.30.)

This exercise has been developed to test a common food additive for conformity with certain Codex specifications.

B. Assignment

Perform these monograph tests: identification, assay specific rotation, pH (of 1 in 20 solution), and loss on drying. Do single determinations for identification and duplicate determinations for the others. If the laboratory does not have a mercury lamp, determine the specific rotation using the sodium D line only.

C. References

1. (1981). *Food Chemicals Codex* (3rd ed., p. 203).
2. *Code of Federal Regulations*. Title 21, Sects. 101.35-?, 182.1-Substances that are generally recognized as safe (as food additives), and 582.1-Substances that are generally

recognized as safe (for drugs).

Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfrv2_01.html

Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr182_01.html

Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfrv6_01.html

3. *Handbook of food additives* (pp. 495-98).
4. Laboratory Information Bulletin (LIB) 166 series (comments on Codex methods).
5. FDA and Monosodium Glutamate. Retrieve from <http://www.cfsan.fda.gov/~lrd/msg.html>

D. Questions

1. What other names are used for "monosodium glutamate"? What name must be used on a food label? Cite the regulation that supports the answer.
2. How much monosodium glutamate is permitted in foods?
3. Why is monosodium glutamate titrated in a non-aqueous solvent system and not in water? Show the chemical reactions involved.
4. Define optical activity, optical rotation, angular rotation, and specific rotation.

10.2.2.4 Artificial Sweeteners

A. Introduction

Based on a Canadian study, which showed that saccharin was the cause of bladder cancer in rats, FDA banned all uses of saccharin except as a tabletop sweetener in 1977. A two year moratorium was declared under the Saccharin Study and Labeling Act which lifted the ban and required any food containing saccharin to carry a warning statement on the label. This moratorium has been extended until 2002.

Cyclamate was banned for use in 1970 by FDA due to studies linking the sweetener to bladder cancer.

B. Assignment

This exercise has been developed to test a food for the presence of saccharin and cyclamates.

C. References

1. *Code of Federal Regulations*. (2003). Title 21, Sections 180.37-Saccharin, ammonium saccharin, calcium saccharin, and sodium saccharin, 189.135-Cyclamate and its derivatives. Washington DC: Office of Federal Register National Archives and Records Administration.
Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr180_01.html
Retrievelfromhttp://www.access.gpo.gov/nara/cfr/waisidx_01/21cfr189_01.html
2. (1990). AOAC official methods of analysis (15th ed., sect. 969.27 (17th ed., 47.6.04), Non nutritive Sweeteners in nonalcoholic Beverages - Qualitative Thin Layer Chromatography Method). Arlington, VA: Association of Official Analytical Chemists.
3. (1990). AOAC official methods of analysis (15th ed., sect. 990.18 (17th ed., 27.1.50). Saccharin in Food - Differential Pulse Polarographic Method). Arlington, VA: Association of Official Analytical Chemists.
4. (1990). AOAC official methods of analysis (15th ed., section 979.08 (17th ed., 29.1.14). Benzoate, Caffeine, and Saccharin in Soda Beverages - LC Method). Arlington, VA: Association of Official Analytical Chemists.
5. HPLC determination of selected food additives. Laboratory Information Bulletin, LIB No. 3053.
6. HPLC determination of saccharin, benzoate, sorbate, and dulcin. Laboratory Information Bulletin, LIB No. 3140.
7. Sugar Substitutes.
Retrieve from <http://www.cfsan.fda.gov/~dms/fdsugar.html>
8. ORA Import System, FIARS, *Import Alert 21-04 Detention Without Physical Examination and Increased Surveillance of Dried Fruits*.
Retrieve from http://www.fda.gov/ora/fiars/ora_import_ia2104.html

D. Questions

1. The referenced TLC method is for beverages, what modification would be made for a non-liquid sample? What potential problems may be incurred using a modified method?
2. In terms of the TLC extraction procedure, what is the purpose of adding 5 ml of 50% NaOH solution?
3. In terms of HPLC, what may occur if there is a change in the composition of the mobile phase?

4. In terms of HPLC how critical is the detection wavelength?
5. What is the salt of benzoic acid? What pH does sodium benzoate optimally function? Which is more soluble, sodium benzoate or benzoic acid?
6. What is meant by a regulated food additive? Provide examples.

10.2.2.5 Borates

A. Introduction

Boric Acid has been prohibited for use in foods as a preservative.

B. Assignment

This exercise has been developed to test food products, such as caviar, for the presence of boric acid.

C. References

1. (1990). AOAC official methods of analysis. (15th ed., sect. 970.33 (17th ed. , chap. 47, 47.3.07) Boric Acid and Borates in Food - Turmeric Qualitative Test). Arlington VA: Association of Official Analytical Chemists.
2. Determination of boron in caviar by inductively -coupled argon plasma- atomic emission spectrophotometry. Laboratory Information Bulletin, LIB No. 3843.
3. U.S. Food & Drug Administration, Office of Enforcement. (2000, August). Compliance policy guides manual. Compliance Policy Guide 540.150: Caviar, Use of Term-Labeling, p. 275. Retrieve version from http://www.fda.gov/ora/compliance_ref/cpg/cpgfod/cpg540-150.html

D. Questions

1. "Caviar" is made from what type of fish?
2. When the roe of other fish types are used, what labeling requirement must be followed?
3. What is the purpose of adding boric acid?

10.2.3 Color Additives Exercises

A. Introduction

The purpose of this exercise is to introduce the trainee to the regulations for color additives.

B. Assignment

Perform the following laboratory exercises: analyses of a bakery product (cookie or cake) using the Graichen Method (reference #3 below), hard candy or beverage using the Sep Pak Method (reference #4 below) and a lipstick using Newburger's method (reference #5 below) for the presence of color additives. Review the references and answer the questions that follow.

C. References

1. *Code of Federal Regulations*. (2003). Pts. 70-Color Additives, 71-Color Additive Petitions, 73-Listing of Color Additives Exempt from Certification, 74-Listing of Color Additives Subject to Certification, and 81-General Specifications and General Restrictions for Provisional Color Additives for Use in Food, Drugs, and Cosmetics. Washington DC: Office of Federal Register National Archives and Records Administration.
Retrieve from http://www.access.gpo.gov/nara/cfr/waisidx_01/21cfrv1_01.html
2. Compliance Program Guidance Manual, current programs that direct the analyses of food, cosmetics, and drugs for colors.

Imported Foods-Food and Color Additives
<http://www.cfsan.fda.gov/~comm/cp09006.html>

Domestic Food Safety Program
<http://www.cfsan.fda.gov/~comm/cp03803.html>

Imported Cosmetics Program
<http://www.cfsan.fda.gov/~comm/cp29002.html>

Domestic Fish and Fishery Program
<http://www.cfsan.fda.gov/~comm/cp03842.html>

3. Hendry, F. A. F. and Houghton, J. D. (Eds.). (1996). *Natural food colorants* (2nd ed.) Blackie Academic & Professional.

4. Marmion, D. (1991). *Handbook of U.S. colorants, foods, drugs, cosmetics and medical devices* (3rd ed.). New York: John Wiley and Sons, Inc.
5. *Journal Association of Official Agricultural Chemists*. (1962). 45, 767; 1963, 46, 1022 (revised 1975, 58, 278 82).
6. (1990). AOAC official methods of analyses. (15th ed., sect. 988.13 (17th ed., chap. 46, 46.1.05). Arlington, VA: Association of Official Analytical Chemists.
7. (1977). *Newburger's manual of cosmetic analysis* (2nd ed., chap. 19). Arlington, VA: Association of Official Analytical Chemists.
8. Polyamide column clean up of water soluble food dyes for TLC and IR determination. *Laboratory Information Bulletin*, LIB No. 934.
9. Separation and identification of color additives in foods by reverse phase HPLC. *Laboratory Information Bulletin*, LIB No. 3036.
10. High performance TLC of 15 synthetic Colors. *Laboratory Information Bulletin*, LIB No. 3073.
11. LC determination and TLC identification of synthetic colors in unsweetened powdered soft drink mixes. *Laboratory Information Bulletin*, LIB No. 3093.
12. Determination of color additives in food products. *Laboratory Information Bulletin*, LIB No. 3420.
13. Center for Food Safety and Nutrition, Office of Cosmetics and Colors: Color Additives. Retrieve from <http://www.cfsan.fda.gov/~dms/col-toc.html> (table of contents)
Retrieve from <http://www.cfsan.fda.gov/~lrd/colorfac.html> (Color Additives Fact Sheet)
14. European Codes: Food and Color Additives.
Retrieve from <http://members.tripod.com/rh.net/page2/additives.htm>
15. EEC designation numbers for color and food additives. *Laboratory Information Bulletin*, LIB No. 3252.
16. Import Alerts Involving Color Additive Violations: U.S. Food & Drug Administration, Center for Food Safety and Applied Nutrition, Office of Cosmetics and Color Additives, Facts Sheet September 24, 2001, revised December 26, 2002, *Import Refusals and Import Alerts Involving Color Additive Violations*. Retrieve version at <http://www.cfsan.fda.gov/~dms/col-imp.html>

17. Office of Regulatory Affairs Import System, *FIARS*, Import Alert: Foods containing Illegal/Undeclared Colors.
Retrieve at http://www.fda.gov/ora/fiars/ora_import_ia4502.html (IA 45-03 Detention Without Physical Examination and Guidance of Foods Containing Illegal and/or Undeclared Colors.)
Retrieve from http://www.fda.gov/ora/fiars/ora_import_ia2302.html (IA 23-02 Detention Without Physical Examination of Melon Seeds)
18. *Color Index* (3rd ed., Vol. 1- 9). Research Triangle Park, NC: American Association of Textile Chemists and Colorists (AATCC).
19. Silverstein, R. M., Bassler, G. C., Morrill, T. C. (1981). *Spectrophotometric identification of organic compounds* (4th ed., pp. 308-09). New York: John Wiley and Sons.
20. Marmion, D. M. (1979). *Handbook of U. S. colorants for foods, drugs, and cosmetics*. New York: John Wiley and Sons.
21. (1965). *Remington's Pharmaceutical Sciences* (13th ed., pp. 1381- 1391). (Note: Most of this information has been deleted from later editions.). Easton, PA: Mack Publishing Co.

D. Exercise

Discuss with the trainer:

Briefly describe the analysis of a temporary tattoo for colors using Neuberger's method of analysis.

E. Questions

1. What is a color additive?
2. Define these terms: straight color, lake, diluent.
3. What are "certified" colors?
4. Under what conditions may color additives be used in the area of the eye, in injections, or in surgical sutures?
5. List the restrictions, if any, for the following colors:
 - a. Citrus Red No.2
 - b. Orange B

- c. Titanium dioxide
 - d. FD&C Blue No. 1
 - e. FD&C Red No. 3
 - f. FD&C Red No. 40
 - g. FD&C Yellow No. 5
6. What are chromophores and auxochromes? Identify the chromophores and auxochromes in two of the colors listed in the previous question.
 7. What is hypochromic shift? Give the effect of hypochromic shift for two of the colors in question 5.
 8. Give one example of a triphenylmethane color.
 9. List the Colour Index Number (C.I. No.) and common name for FD&C Red No. 1, FD&C Red No. 3, D&C Orange No. 3, FD&C Green No. 3, FD&C Yellow No. 7, D&C Yellow No. 9, FD&C Blue No. 2, and FD&C Violet No. 1.
 10. A label of a food product declares "...E123..." as an ingredient. Is this product permitted in the U.S.?
 11. A label of a food product declares "...Sunset Yellow FCF, Erythrosine Lake, Allura Red AC." Is this product correctly labeled? If not, why? If the importer relabels the product, would it be permitted in the U.S.?
 12. How many synthetic colors are approved for use in cosmetics? Which of these colors are permitted in eye area cosmetics?
 13. A lipstick sample was analyzed using Neuburgers, 19.1. One bright pink band of color, exhibiting orange fluorescence, was detected. Using Table 12 in Neuburgers (page 119), what color(s) was(were) detected? Are the possible color(s) detected, permitted for use?
 14. Discuss solubility in terms where one would expect to find the following colors:
 - Natural color such as riboflavin or saffron
 - Tartrazine
 - Sudan 1
 - D&C Red 19 or DC Red 28
 - Red #3

10.3 Appendix – Answer Key

10.1.2.1 Canned Vegetables and Fruits

1. **What are Food Standards? How do they differ from grade standards?** “Food Standards” is FDA’s common designation for Standards of Identity, Standards of Quality, and Standards of Fill of Container promulgated under the FD&C Act. They are written to promote honesty and fair dealing in the interest of consumers. Food Standards are written by FDA and are found in 21 CFR 130-169; industry must comply with these or their products are deemed misbranded. The US Department of Agriculture establishes Grade Standards for fruits and vegetables to designate quality: "U.S. Grade A" and "U.S. Grade B". Industry compliance is voluntary. The U.S. Department of the Interior also uses these grade designations for fishery products. Such grade designations are not required by the Federal Food, Drug, and Cosmetic Act to be stated on the labels, but if they are stated, the product must comply with the specifications for the declared grade.
2. **What consumer protection was presumed in the promulgation of food standards?** Consumers are protected from adulterated and misbranded foods by use of standards.
3. **When is the packing liquid included in net weight calculations?** The packing liquid is included in the net weight calculations when it is also used by the consumer.
4. **Define edible portion.** Edible portion is any portion of a food product that is fit to be eaten.
5. **What is the basis for determining the solids content by use of the refractive index?** The refractive index is used in determining the solids for liquid samples containing only soluble solids. A refractometer is used and the observed value is corrected to 20 °C. This reading is compared to the same refractive index value of a standard sucrose concentration; solids in the sample solution are reported as percent by weight of sucrose.
6. **Why isn't the product allowed to "drain" completely in a drained weight determination? Why are two minutes specified in 21 CFR 145.3(n)?** For the analyst “drained” completely is an empirical term that would be impossible for an analyst to time accurately and results would vary for one lab to another. Products are not drained completely in order to avoid leaving products on a sieve for a non-uniform time and to prevent evaporation. Two minutes are specified in the CFR in order to insure a constant basis for measurement and comparison.
7. **What is the basis for the determination of alcohol-insoluble solid in canned vegetables?** The basis for determination of alcohol-insoluble solid in canned vegetables is consistency and to determine if product is excessively liquid which would make the product substandard. In this method the definition of Solids in Canned Peas is alcohol insoluble material.
8. **What are the requirements for the fill of container?** Food containers must be so made, formed, or filled so they are not misleading (FD&C Act, Sec. 403(d)). Fill of container is the

minimum quantity of the solid food in the container after processing: not less than 90 percent of the total capacity of the container.

9. **Can the net contents be satisfactory and fill of container fail?** When the volume of product settles during shipment the correct net contents may no longer properly fill the container. When this is expected, the manufacturer may include a comment on the label to that effect.
10. **Define "degrees Brix."** A unit of measure on the Brix scale for measuring the density or concentration of sugar (sucrose) in solution, determined as percent by weight. It is used to measure the density of the packing medium of fruits and vegetables, or strength of juices.

10.1.2.2 Cheese

1. **Why is speed essential in sample preparation?** Speed is essential in sample preparation to prevent moisture loss, which would affect the results in the fat calculations of cheeses.
2. **Differentiate between a cheese spread and cheese food.** A cheese food is the food prepared by comminuting and mixing, *without* the aid of heat, one or more optional ingredients into a homogeneous mass. A cheese spread is the food prepared by comminuting and mixing, *with* the aid of heat, one or more optional ingredients into a homogeneous mass.
3. **Why are preservatives added? Must they be declared on labels?** Preservatives are added to slow or prevent growth of microorganisms which spoil food or cause disease. They must be declared on label according to 21 CFR 101 and 130.
4. **List two kinds of cheese in which sorbic acid is permitted and include the conditions under which it might be used. List two kinds of cheese for which sorbic acid is not permitted.** Sorbic Acid is permitted for use in cold pack cheeses in an amount not to exceed 0.3 percent by weight and in pasteurized process cheeses in an amount not to exceed 0.2 percent by weight. Sorbic Acid is not permitted for use in Nuworld and Roquefort cheeses.
5. **What is a processed cheese?** A processed cheese is the food prepared by comminuting and mixing, with the aid of heat, one or more of the optional cheese ingredients, into a homogeneous mass that is spreadable.
6. **In the CFR procedure, is the moisture content directly determined?** The CFR procedure uses an indirect method and moisture loss is determined, not content.
7. **In the cheese fat extraction, what other substances may be found?** Other product ingredients, such as oil-soluble natural colors, may be extracted if present.
8. **Why is petroleum ether used for the extraction?** Petroleum ether is used because fats and

oils are readily soluble in this solvent and it is easily evaporated.

10.1.2.3 Jams, Jelly, and Orange Juice

1. **What is jam?** A viscous or semi solid food, from a mixture of one or more fruits, mixed with or without water, and concentrated with or without heat. The soluble solids content of the finished product should not be less than 65 per cent. The standards of identity for jams and jellies (21 CFR 150) require that these products be prepared by mixing not less than 45 parts by weight of certain specified fruits (or fruit juice in the case of jelly), and 47 parts by weight of other designated fruits, to each 55 parts by weight of sugar or other optional nutritive carbohydrate sweetening ingredient.
2. **What is an imitation jelly?** A food is an imitation if it is a substitute for, and resembles another food, but is nutritionally inferior to that food (21 CFR 101.3(e)). Nutritional inferiority includes any reduction in the content of an essential nutrient that is present in a measurable amount.
3. **What orange products are standardized?** Orange juice that is fresh, pasteurized, or with preservatives; or made from concentrate. Also juice that is frozen, concentrated, canned, frozen concentrated, canned concentrated, or un-concentrated for manufacturing. Refer to 21 CFR 146.
4. **Why are K_2O and ash used as criteria to determine fruit content?** K_2O is a pyrolysis product of the predominant mineral found in fruits. The ash content reflects the pyrolysis product of all minerals found in the fruit.
5. **Are the literature values of K_2O and Ash definitive for fruit?** The authentic literature values for K_2O and Ash data are fairly uniform for a given fruit. They may be used as a guide for sample data evaluation.

10.1.2.4 Mixed Nuts

1. **Be prepared to discuss the sections of the Act that apply to the examples of cheating given in the Introduction.** (To discuss with the trainer.)
2. **Differentiate between misbranding and adulteration in regard to question 1.**

A food shall be deemed to be "adulterated" if:

It bears or contains any poisonous or deleterious substance which may render it injurious to health.

It bears or contains any added poisonous or added deleterious substance (other than a substance that is a pesticide chemical in or on a raw agricultural commodity; or processed food, a food additive, a color additive, or a new animal drug) that is unsafe within the meaning of section 406 of The Act.

It consists in whole or in part of any filthy, putrid, or decomposed substance, or if it is otherwise unfit for food.

It has been prepared, packed, or held under unsanitary conditions whereby it may have become contaminated with filth, or whereby it may have been rendered injurious to health.

A food shall be deemed to be “misbranded” if:

Its labeling is false or misleading in any particular, or in the case of a food to which section 411 of the Act applies, its advertising is false or misleading in a material respect or its labeling is in violation of section 411(b)(2).

It is offered for sale under the name of another food.

Its container is so made, formed, or filled as to be misleading.

In the case of nuts with misleading vignettes, misrepresentation of product, and incorrect net weight, misbranding charges apply.

3. **Why do the standards specify both amount and type of nuts?** Standards specify amount and type of nuts to prevent misbranding and economic fraud.
4. **How does the shape of the container affect the net weight determination?** The shape of the container dictates the method used to determine the net volume in cubic centimeters to calculate the net weight for the container.
5. **What is the maximum amount of peanuts allowed in mixed nuts?** In mixed nuts, each nut ingredient shall be present in a quantity not less than 2 percent and not more than 80 percent by weight of the finished food.
6. **What, if any, is the relationship between the vignette and the contents?** If the label bears any pictorial representation (vignette) of the mixture of nuts, it must depict the relative proportions of the nut ingredients contained in the finished food.

10.1.2.5 Egg Noodles

1. **Are eggs the only possible source of cholesterol in this product?** In egg noodles, concentrated glyceryl monostearate may be another possible source of cholesterol. Any fat ingredient obtained from an animal may be a source of cholesterol.

2. **Does the label have an ingredient statement? Why?** The label of an egg noodle must have an ingredient statement *when optional ingredients* such as onions, garlic salt, gum gluten, or glyceryl monostearate are added.
3. **Do all noodle products contain eggs?** As defined in the standard, all noodle products should contain egg; in noodles, the total solids of egg or egg yolk are not less than 5.5 percent.
4. **Compare the two drying methods. Which must be used to determine compliance with the standard for total solids?** One method uses a vacuum oven with drying to a constant weight at temp 98-100 ° C (~ 5 hrs.) and the second uses an air oven at 130 ° C for 1 hour. CFR 21.139.150(a)4 specifies the vacuum oven method.
5. **What is accomplished in AOAC 954.03?** Quantification of sterols (mostly cholesterol) by extraction and precipitation of the unsaponifiable fat portion of the macaroni product.
6. **Why is there a precaution in AOAC 954.03 to retain solid material in the separator?** To avoid loss of cholesterol before the extraction is completed, and to help keep insoluble material from causing an emulsion.
7. **How would one cope with an emulsion if it formed?** If an emulsion forms, leave it in the separatory funnel and wash, by swirling gently, the ether solution using two portions of water. Discard the aqueous phase and continue the analysis on ether solutions.
8. **What is the difference between macaroni and noodles?** The difference between macaroni and noodles is the egg content. In macaroni the total solids from egg white, frozen egg white, or dried egg white is not less than 0.5 and not more than 2.0 percent. In noodles, the total solids of egg or egg yolk are not less than 5.5 percent.

10.2.2.1 Salt and Sodium Nitrite in Smoked Fish

1. **What are the three requirements that must be met for a preservative to be used according to good manufacturing practices?** The three requirements for preservative use are the following: generally recognized as safe for such use (a food additive is covered by food additive regulations prescribing conditions of safe use); not used in such a way as to conceal damage or inferiority or to make the food appear better or of greater value than it is; and proper declaration on the food label in which used.
2. **List 10 standardized foods for which the addition of chemical preservatives has been permitted by regulation.** Milk and milk products, cereal, fish, macaroni, cheeses, canned vegetables, nuts, fruits bakery goods, and cacao.

3. **Which fish or fish products may legally contain nitrates or nitrites? List the maximum legal level for each product and describe how the retail container must be labeled.** The fish or fish products that may contain nitrates or nitrites are smoked cured sablefish, salmon, shad, and chubs at levels not to exceed (NTE) 200 ppm sodium nitrite and NTE 500 ppm sodium nitrate. In smoked tuna fish products the level of sodium nitrite is NTE 10 ppm. The container label must bear the name and concentration of the additive.

4. **Where is "water phase salt" defined? Water phase salt (WPS) is defined as the salt content of the water phase portion of the edible portion of the finished smoked product, as measured in the loin muscle (21 CFR 172.177). Generally, WPS should not be less than 3.5%. However, consult Compliance Programs 7303.844 and 7303.842 Domestic/Imported Fish and Fisheries Products for specific requirements, or the HACCP program (Fish and Fisheries Products Hazards & Controls Guide). How does water phase salt vary with moisture content? The water phase salt content is inversely proportional to the moisture content. Write the equation used for calculating the salt concentration in the water phase.**

$$\% \text{ salt in water phase} = \% \text{ salt} / (\% \text{ salt} + \% \text{ water}) \times 100.$$

5. **Why is water added to the portion taken for analysis by AOAC method 952.08A?** Water is added for the purpose of mixing and to help uniformly distribute the sample portion across the surface of the drying pan. A smooth layer of sample promotes even drying.

6. **How would one show that all the volatile components had been evaporated from the tissue?** If no significant change in weight is observed after an additional period of drying then all volatiles have been evaporated.

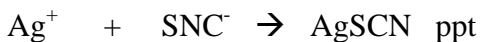
7. **Show the reactions involved in the titrimetric analysis for total chloride.**

The procedure uses a modification of the old Volhard Titration.

For fish and fish products, the organic matter is oxidized by heating with HNO₃; the Cl⁻ is precipitated by the addition of Ag⁺. This results in an excess of Ag⁺.



The excess Ag⁺ is then titrated with SCN⁻ (thiocyanate).



One drop past the end point there is an excess of SCN⁻. This excess SCN⁻ then reacts with the Fe⁺³ to produce a colored ferric thiocyanate complex:



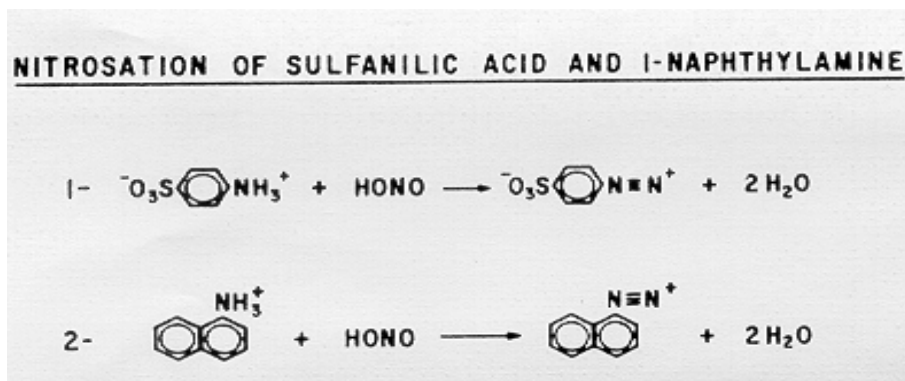
So, the total amount of Ag^+ is known and the excess Ag^+ after AgCl formation is known from the titration. Subtracting the moles of each yields the amount of Ag^+ that reacts with the Cl^- , which in turn tells us the amount of Cl^- . The Fe^{+3} does not do anything until there is an excess of SCN^- ; then it reacts and produces the colored FeSCN^{+2} complex which is used for the end point. The reddish-brown FeSCN^{+2} actually has a more complex formula. Bromide, iodide and cyanide are interferences. Also, if one does not do the titration quickly, then there will be an interference from the AgCl ($K_{sp} = 1 \times 10^{-10}$) reacting with the SNC^- since AgSCN has a $K_{sp} = 1 \times 10^{-12}$). The analyst should get an RSD of $< 5\%$ and a relative error of $< 2\%$.

8. **Why is the HNO_3 solution to be boiled before the indicator is added?** The HNO_3 solution is used to digest the protein and liberate the chloride in solution before the indicator is added.
9. **AOAC method 937.09B(b) states "With 10g sample each mL 0.1N $\text{AgNO}_3 = 0.058\%$ NaCl." How is this factor obtained?**

Assuming a 10.000 gram sample is used:

$$\frac{100\% \times 58.44 \text{ mg NaCl/meq N SCN}}{10,000 \text{ mg sample}} = 0.058\%$$

10. **What is the structure of the colored product formed during the nitrite analysis? Show the reactions involved.** The nitrosation of sulfanilic acid to form the diazonium salt of sulfanilic acid which the couples with 1-naphthylamine to form a pink azo compound.



11. **How has the order of addition of reagents been shown to affect this type of analysis?** Nitrite was added to sulfanilic acid and the mixture was allowed to stand 20 minutes before 1-naphthylamine was added. The resulting amount of azo dye was the same as that formed when nitrite was added to a mixture of sulfanilic acid and 1-naphthylamine.
12. **What is the correlation between pH and color stability?** Use of more acid and

substitution of acids were investigated and the amount of dye compound formed was not affected.

10.2.2.2 Sulfites

1. **Why doesn't the HCl distill into the U tube or sulfur dioxide absorber? What is an azeotrope?** The risk of interfering substances reaching the hydrogen peroxide trap (sulfur dioxide absorber) by aerosolization, co-distillation, or steam distillation has been reduced by condenser coolant temperatures, reflux rates, and nitrogen flow. The HCl is trapped in the reflux column. An azeotrope is a mixture of liquids that has a constant boiling point and cannot be separated by distillation.
2. **What reaction occurs in the distilling flask? In the U tube? In the sulfur dioxide absorber?** An oxidation reaction, $\text{SO}_2 \rightarrow \text{SO}_3$.
3. **What is the purpose of the gravimetric determination?** The gravimetric determination serves as a confirmatory test specific for sulfites.
4. **What purpose does the pyrogallol/KOH gas wash serve?** The pyrogallol/KOH wash serves as an oxygen scrubbing solution.

10.2.2.3 Monosodium Glutamate

1. **What other names are used for "monosodium glutamate?" What name must be used on a food label? Cite the regulation that supports the answer.** MSG is the sodium salt of the amino acid glutamic acid and a form of glutamate. "L-glutamic acid, sodium salt," and "sodium glutamate" are other names for MSG. When MSG is added to a food it must be included on the ingredient list as "monosodium glutamate," as required by 21 CFR 101.22(h)(5).
2. **How much monosodium glutamate is permitted in foods?** MSG is generally recognized as safe when used as a salt substitute in accordance with good manufacturing practice.
3. **Why is monosodium glutamate titrated in a non-aqueous solvent system and not in water? Show the chemical reactions involved.** MSG is titrated in a non-aqueous solvent because this condition is needed to protonate the amine; if water is present it will compete with this process:





4. **Define: optical activity, optical rotation, angular rotation and specific rotation.** *Optical activity* is a measure of the ability of substances to rotate plane-polarized light. Optical activity results from polarized light emerging in a continuum of planes at different intensities such that the plane of maximum intensity forms a measurable angle with the plane of incident light. Where this effect is large enough for precise measurement it serves as the basis for an assay or an identity test (and is expressed in degrees). *Optical rotation* is the change of direction of the plane of polarized light to either the right or left as it passes through a molecule containing one or more asymmetric carbon atoms. Substances that rotate the light plane clockwise (dextrorotatory) are designated by “+” or “*d*.” Substances that rotate the light plane counterclockwise (levorotatory) are designated by “-” or “*l*.” *Angular rotation* is the observed optical rotation, measured in degrees. *Specific rotation* is the angular rotation divided by length of the path in decimeters x concentration of solute in grams in 100 cc solution. The wavelength and temperature are usually specified. For a pure liquid, concentration is replaced by its density.

10.2.2.4 Artificial Sweeteners

1. **The referenced TLC method is for beverages, what modification would be made for a non-liquid sample? What potential problems may be incurred using a modified method?** For a non-liquid sample, add a suitable portion of hot water, let stand and then filter through filter paper. The problem of emulsions, change of salt content, etc. - preventing suitable extractability of the analyte of interest - can be encountered when modifying the method.
2. **In terms of the TLC extraction procedure, what is the purpose of adding 5 ml of 50% NaOH solution?** Modification of the nature of the aqueous phase to basic changes the solubility of the 4 analytes. This will promote their extracting into the ethyl acetate organic phase.
3. **In terms of HPLC, what may occur if there is a change in the composition of the mobile phase?** The chromatographic elution pattern will change and possibly the peak shape; the resolution between peaks may not meet the performance criteria, components may elute in the void volume or greatly increase elution time resulting in poor chromatography and efficiency.
4. **In terms of HPLC how critical is the detection wavelength?** The wavelength at which the analyte of interest absorbs is critical information. When using HPLC with a UV/VIS fixed detector and attempting to chromatograph several analytes at once, a wavelength that is common for the group of analytes should be selected. However, modern instrumentation allows for different wavelengths to be simultaneously or sequentially monitored throughout

the course of a chromatographic run.

5. **What is the salt of benzoic acid?** Sodium or potassium benzoate. **What pH does sodium benzoate optimally function?** pH 2.5 – 4.0 **Which is more soluble, sodium benzoate or benzoic acid?** Sodium benzoate is 180 times more soluble than benzoic acid.
6. **What is meant by a regulated food additive? Provide examples.** Benzoic acid, sodium benzoate, calcium benzoate, sorbic acid potassium sorbate, sodium sorbate and calcium sorbate. Their maximum allowable in a food product is regulated at 0.1%.

10.2.2.5 Borates

1. **"Caviar" is made from what type of fish?** Originally the name "caviar" was applied only to the eggs of the sturgeon prepared by a special process. [The eggs first prepared and most extensively used were those of the sturgeon, and to many people the term caviar was synonymous with sturgeon caviar.] The term caviar can properly be applied to any kind of fish eggs prepared by this special method.
2. **When the roe of other fish types are used, what labeling requirement must be followed?** The term "caviar" can be used on a product prepared according to the usual caviar method and made from the roe of whitefish - provided the name of the fish is given in conjunction with the word caviar on the product label.
3. **What is the purpose of adding boric acid?** Boric Acid is added to retard spoilage and to promote color retention (a color fixative). It is not allowed in caviar sold in the U.S.

10.2.3 Color Additives

1. **What is a color additive?** A color additive is a specific category of Food Additive defined as a dye, pigment or other substance - whether synthetic or derived from a vegetable, animal, mineral, or other source - which is added to impart color to a food, drug, cosmetic, or applied to the human body.
2. **Define these terms: straight color, lake and diluent.** A *straight color* is a color additive that has not been mixed or chemically reacted with any other substance. Straight colors

subject to certification are listed in 21 CFR Part 74. Straight colors exempt from certification are listed in 21 CFR Part 73. In addition, 21 CFR Section 81.10 and 81.30 identify straight colors whose listings or certain uses have been terminated or whose certificates have been cancelled. *Lakes* are pigments prepared by precipitating a soluble dye onto an insoluble reactive or adsorptive substratum or diluent. They are water-insoluble and more stable than straight dyes, and are ideal for product in which leaching of the color is undesirable (coated tablets and hard candies, for example). A *diluent* is any component of a color additive mixture that is not of itself a color additive and has been added to facilitate the use of the mixture as a coloring agent. The term *mixture* means a color additive made by mixing two or more straight colors, or one or more straight colors and one or more diluents.

3. **What are "certified" colors?** Certified colors are Color Additives from certified batches analyzed by FDA's Color Certification Branch, CCB, to assure the Code of Federal Regulations (21 CFR Part 74) specifications are met. These Certifiable Color Additives are synthetic organic dyes, pigments or lakes of a dye. In order to be used in foods, drugs, cosmetics, and medical devices manufactured or sold in the United States, the color additives must meet the following criteria: come from a certified batch, be allowed in the respective product, and the color additive name must be listed in the ingredients label.
4. **Under what conditions may color additives be used in the area of the eye, in injections, or in surgical sutures?** Color additives may be used in the area of eye, injections, or in surgical sutures only if the color additives are specifically certified for such use.
5. **List the restrictions, if any, for the following colors:**
 - a. **Citrus Red No.2** - Permanently listed for use only in coloring of skins of mature oranges. Limit of 2 ppm calculated on basis of whole fruit weight.
 - b. **Orange B** - Permanently listed for use in coloring surfaces and casings of frankfurters or sausages. Limit of 150 ppm by wt. of finished product.
 - c. **Titanium dioxide** - Limit: of 1.0% by wt of food.
 - d. **FD&C Blue No. 1** - For food, drug and cosmetic use, including drugs and cosmetics for eye area - GMP - 74.101, 74.1101, 74.2101, 82.101.
 - e. **FD&C Red No. 3** - For food and ingested drugs - GMP - 74.303, 4.1303. Lake use terminated 2-1-90; All cosmetic uses terminated 2-1-90.
 - f. **FD&C Red No. 40** - For food, drug and cosmetic use, including drugs and cosmetics for eye area - GMP - 74.340, 74.1340, 74.2340.
 - g. **FD&C Yellow No. 5** - For food, drug and cosmetics, including drugs and

cosmetics for eye area - GMP - 74.705, 74.1705, 74.2705, 82.705.

6. **What are chromophores and auxochromes? Identify the chromophores and auxochromes in two of the colors listed in the previous question.** *Chromophores* are groups of one or more unsaturated bonds that absorb light (in the ultraviolet or visible region) and thus produce colored substances. They are, in most cases, covalent unsaturated groups such as C=C, C=O, C=NH, CH=N, and N=N. Examples are FD&C Yellow No. 5 (N=N) and FD&C Blue No. 1 (N=CH). *Auxochromes* are functional groups that do not confer color to a substance themselves, but increase the coloring power of a chromophore. They contain functional groups, such as -OH, -NH₂, -SO₃H, -COOH, that don't exhibit absorption in visible wavelengths (however, auxochromes may absorb strongly in the far-ultraviolet region). When an auxochrome is combined with a chromophore in the same molecule, the chromophore absorption will typically shift to a longer wavelength (a bathochromic shift) and show an increase in intensity (a hyperchromic shift). Auxochromes provide an unshared pair of electrons in conjunction to the chromophore. Examples are FD&C Blue No. 1 (-SO₃) and Citrus Red 2 (-OH).
7. **What is hypsochromic shift? Give the effect of hypsochromic shift for two of the colors in question 5.** Hypsochromic shift is a change in wavelength maxima to a shorter wavelength, and thus change in color. For FD&C Red No. 40 and Citrus Red 2, the addition of base produces a change in color from red to orange and a wavelength maxima shift of approximately 50 nanometers.
8. **Give one example of a triphenylmethane color.** Brilliant Blue FCF (CI 42090, certifiable as FD&C Blue No.1) is an example of a triphenylmethane color. [Other triphenylmethane dyes, such as malachite green or gentian violet, are sometimes used as fungicides in the food industry.] Triphenylmethane colors consist of a common moiety of three phenyl groups attached to a central carbon (hence "methane"). Other functional groups are then attached to the phenyls and these determine the different colors.
9. **List the Color Index Number (C.I. No.) and common name for FD&C Red No. 1, FD&C Red No. 3, D&C Orange No. 3, FD&C Green No. 3, D&C Yellow No. 7, D&C Yellow No. 9, FD&C Blue No. 2, and FD&C Violet No. 1.**

FD&C Red No.1	CI 16155	Ponceau 3R
FD&C Red No.3	CI 45430	Erythrosine
D&C Orange No.3	CI 16230	Orange G
FD&C Green No.3	CI 42053	Fast Green FCF
D&C Yellow No. 7	CI 45350	Fluorescein
D&C Yellow No.9	CI 45350	Uranine K
FD&C Blue No.2	CI 73015	Indigotine
FD&C Violet No.1	CI 42640	Wool Violet 5BN

10. **A label of a food product declares "...E123..." as an ingredient. Is this product permitted in the U.S.?** E123 is the European code designation for Amaranth (a.k.a. CI 16185, former FD&C Red No.2) which is not permitted for use in the U.S.
11. **A label of a food product declares "...Sunset Yellow FCF, Erythrosine Lake, Allura Red AC." Is this product correctly labeled? If not, why? If the importer re-labels the product would it be permitted in the U.S.?** Artificial colors must be declared on the labels by listing the color additive name (*i.e. Red 40*) among the list of ingredients (21 CFR 101.22(k)(1)). When colors are declared using their common names (*i.e. Allura Red AC*), it is assumed that the colors used were not from a certified lot and the product can be detained based on adulteration. Use of Erythrosine Lake is not permitted in the U.S. and the product would not be permitted to be sold even if relabeled.
12. **How many synthetic colors are approved for use in cosmetics? Which of these colors are permitted in eye area cosmetics?** Presently there are thirty four synthetic colors approved for use in cosmetics, of which only four are permitted in eye area cosmetics – FD&C Yellow No.5, FD&C Red No. 40, FD&C Blue No.1, and D&C Green No.5.
13. **A lipstick sample was analyzed using Newburgers, 19.1. One bright pink band of color, exhibiting orange fluorescence, was detected. Using Table 12 in Newburgers (page 119), what color(s) was(were) detected? Are the possible color(s) detected, permitted for use?** A bright pink band of color exhibiting orange fluorescence under UV light can be due to either D&C Red No. 27, which is permitted for use in cosmetics, or it can be due to D&C Red No. 19, which is not permitted for use in the U.S.
14. **Discuss solubility in terms where one would expect to find the following colors:**
Natural color such as riboflavin or saffron- in the chloroform
Tartrazine- as it is water soluble, in the Resin in hexane solution
Sudan 1 – in the chloroform
D&C Red 19 or DC Red 28 – as it is oil soluble, in the chloroform
Red #3 – in the chloroform layer, once the solution is made basic in order to see the red color

10.4 Document/Change History

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Version 1.3 changes:

Answer Key 10.2.3, 2., straight color definition changed and mixture definition added