Sections in This Document

1. Introduction

The topics and exercises in this section are fundamental to the FDA Office of Regulatory Science (ORS) regulatory science-focused training process. They are intended for use during initial training of all analysts new to ORS to ensure a foundational level of understanding of the laboratory's role in consumer health protection.

Other sections of this manual cover training activities, which should be completed within approximately the first year of employment with FDA. These activities will complement the local laboratory training process in identified areas such as the Office of Regulatory Affairs (ORA) Quality Management System, major analytical technologies and methods, laboratory safety, etc.

The first year is an important one for career employees in the Federal service. During this period employees learn how to serve best in their role of protecting the public’s health; a role for which no educational institution or prior job/lab experience fully prepares them. During this first year new employees are carefully evaluated by their supervisors. Laboratory management is responsible for keeping the FDA scientific staff at the high level of competence and dedication it has historically demonstrated.

Exercises in this section depend on the extensive use of reference material by the trainee. The exercises reference many sources of information. Although most are relatively common, other sources may have to be substituted and supplemental material added. The trainee is encouraged to use the FDA Library resources, the Internet, the FDA Intranet (SharePoint online), and other information sources for reference.

2. FDA Laws and Regulations

The FDA is the U.S. federal regulatory agency for an extensive range of food and health-related products, including drugs, medical devices,

tobacco products, cosmetics, food for pets and livestock, dietary supplements, and electronic products that emit radiation. Its primary role is to ensure these products meet certain quality standards before they are introduced to the U.S. market.

The FDA was first created to enforce the Pure Food and Drug Act of 1906. In this capacity, the FDA was charged with protecting the health of the US public, to ensure the quality of its food, medicine, and cosmetics. Before this time, the United States government had no formal oversight of these products and left issues of quality and purity to the individual manufactures, or at times, individual states. You can go to the public FDA internet site for information on FDA history.

The FDA has been an operating division of the Department of Health and Human Services (HHS) since 1988. There are several other agencies within HHS doing work that intersects with that of the FDA. These include the Centers for Disease Control and Prevention (CDC), National Institutes of Health, and the Biomedical Advanced Research and Development Authority.

Although the FDA is no longer part of the Department of Agriculture (USDA), its role is still tethered to that of the USDA, which houses the Food Safety and Inspection Service and the National Institute of Food and Agriculture. The FDA also closely coordinates with the Environmental Protection Agency on issues including food waste and pesticides in foods.

Laws enforced by the FDA include the Federal Food, Drug, and Cosmetic Act (FD&C Act), the Food Safety Modernization Act (FSMA), and over 30 other acts, amendments, provisions, and laws.

An overview of regulatory information for the FDA can be found at Laws Enforced by FDA | FDA

2.1. FD&C Act and Amendments

The Federal Food, Drug, and Cosmetic Act (FD&C Act) is a federal law enacted by Congress as part of the United States Code, Title 21. This Act, some of its amendments, and other federal laws establish the legal framework within which FDA operates.

The Act, which was passed by Congress in 1938, replaced the Food and Drug Act passed in 1906. Since 1938, the Act has been amended several times.

The FD&C Act, as amended, with its general and enabling regulations, provides the basic authority for most of our regulatory operations. The most current edition as of the date of this revision of the US Code was published March 2022 [As Amended Through P.L. 117–103, Enacted March 15,

2022]. The Act and associated supplements will be discussed with the analyst by their training supervisor, or designee, and, later, in greater detail at FDA training sessions. It is important the analyst acquire an understanding of its provisions because those provisions will play a significant part in decisions the analyst will make during their career.

Further information for accessing current versions and amendments of the FD&C Act can be found on the FDA Library's SharePoint site devoted to this topic.

The analyst should become familiar with the Code of Federal Regulations , and the Federal Register to remain current on any amendments to the Act.

2.2. The United States Code

The United States Code (USC) should not be mistaken for the Code of Federal Regulations (CFR)

The United States Code is made up of the official federal statutes of the United States. It is arranged into 54 broad titles according to subject matter. The organization of the Code was originally established by Congress in 1926 with the enactment of the act of June 30, 1926, chapter 712. It includes laws passed by Congress, also called statutes, and is published by the Office of the Law Revision Counsel of the U.S. House of Representatives.

Normally, a new edition of the Code is issued every six years, with annual cumulative supplements identifying the changes made by Congress since the last "main edition" was published.

2.3. Code of Federal Regulations

The Code of Federal Regulations (CFR) contains all the rules and regulations directed by executive agencies and are arranged by subject or “title”. Title 21, Chapter I is the portion of the CFR that that provides rules for the FDA. It is a subject arrangement of regulations and is updated annually.

The purpose of the CFR is to present the official and complete text of agency regulations in one organized publication and to provide a comprehensive and convenient reference for all those who may need to know the text of general and permanent federal regulations. Criminal penalties may also fall under Title 18.

FDA personnel responsible for pesticide analyses also refer to Title 40 I Subchapter E Part 180 for tolerances and exemptions for Pesticide Chemical Residues in food established by the Environmental Protection Agency and enforced by FDA.

References to the CFR are cited as the title number, the abbreviation CFR, the word “part” or the symbol “§” for section, and the number of the part or section, e.g., "21 CFR § 121.3(d)."

2.4. Federal Register

The Federal Register (FR) is the official journal of the federal government of the United States that contains government agency rules, proposed rules, and public notices. A regulation will first be published in the Federal Register and later be included in the appropriate volume of the CFR.

It is published every weekday, except on federal holidays. Each daily issue is organized into four categories:

- Presidential Documents (executive orders and proclamations)
- Rules and Regulations (including policy statements and interpretations of rules by federal agencies)
- Proposed Rules (including petitions to agencies from the public)
- Notices (such as scheduled hearings and meetings open to the public and grant applications)

Regulations published in the FR are effective on the date stated in the FR unless "stayed," i.e., not to be put into effect for some reason. If a regulation is "stayed," the FR publishes that notice.

3. Analytical Methods

The analyst will use methods from several sources to determine regulatory compliance. These include methods described in official compendia, manuals relating to analytical categories, and FDA compliance manuals.

The FD&C Act and CFR Title 40 cite publications that contain methods to determine if a product conforms to legal requirements. The compendia cited have gained official status within the Federal judicial system as methods of choice.

3.1. Official Compendia

Official methods are those in compendia specified in the FD&C Act and prescribed in the Code of Federal Regulations. Official methods are used, whenever found, or unless otherwise specified, for either original or check analysis, when regulatory actions are based on analytical findings.

Official methods always have higher status and are preferred over non-official methods. However, the method is only official for the product matrix and performance range specified in the published method.

Several official methods can be accessed either directly through the CFR or by setting up accounts within the FDA Library Resources intranet website to more easily retrieve them.

Examples of some common CFR sources of methodology are included here for reference:

A. AOAC [21CFR 2.19]

B. United States Pharmacopeia (USP) [FD&C Act, 201 (g)(1), 201 (j), 501 (b)]

C. Pesticide Analytical Manual (PAM) [40 CFR 180.101 (c)]

D. Food Chemicals Codex [21 CFR 170.30]

Methods in manufacturer's Applications and Petitions that have been approved have "official" status. These include New Drug Applications (NDA), Abbreviated New Drug Applications (ANDA), New Animal Drug Applications (NADA), Food Additive Petitions (FAP), and Pesticide Petitions (PP).

3.2. Other Method Sources

In addition to official compendia, FDA also relies on other compendia, specialized manuals, and method sources that pertain to analytical categories. These additional sources contain both official and unofficial methods which are generated by FDA, other agencies, professional associations, and trade associations.

The FDA-produced method sources are a collection of analytical laboratory methods used by FDA to help ensure consumer safety. For example, Foods Program Compendium of Analytical Laboratory Methods | FDA lists some methods generated for specific FDA analytical laboratory program areas. Older methods that FDA no longer uses or whose method validation status are posted for a fixed and limited duration (subject to renewal) may also appear on this intranet page.

Certain methods may be directed for use by sources other than the CFR and the Act. The Compliance Manuals | FDA , Import Assignments, and special Assignments may dictate a particular method to be used. FDA laboratory analysts must consult with their supervisor, team lead, or other authority when unsure of method selection.

Consistency in method selection is important, as more than one laboratory may perform the analysis. Proper method selection is the responsibility of the analyst, with concurrence from their supervisor and laboratory management.

3.3. Laboratory Information Bulletins

Laboratory Information Bulletins (LIBs) are not official methods of analysis. They are the FDA mechanism for the rapid dissemination and exchange of scientific information with the agency. While they provide information that appear to be methods of analysis, they do not encompass all parameters required for an official and approved method of analysis. They can, however, form the foundation to develop a validation plan for a proposed study submitted for approval.

3.4. Method Validation

Validation of a method is a planned and documented process to establish its performance characteristics. The performance characteristics or the validation parameters of the method determine the suitability for its intended use. They define what the method can do under optimized conditions of matrix solution, analyte isolation, instrument settings, and other experimental features. Method validation often includes the determination of the following: system suitability, linearity region, accuracy, precision, specificity, detection limit, ruggedness, and recovery.

For more information, analysts should refer to *MAN-000050 ORA Lab Manual Vol. III Section 6 - ORS Method Development and Validation Program (III-06).*

3.5. Method Verification

When using official methods, which have already gone through the rigorous validation process, the laboratory must confirm they can perform the method as written by closely duplicating the basic criteria established. This is accomplished with a method verification. The essential parameters to confirm are accuracy, precision, detection limits (MDL, LOQ, LOD), Linearity, and Measurement of Uncertainty. Method verification is usually performed only once when planning to implement a method not performed previously within a laboratory.

Methods for a verification study need proof of validation from a recognized certification process such as AOAC Peer Verification or Performance Testing.

Certification of method validation is essential when using test kits as an additional method source.

Refer to *MAN-000037 ORA Lab Manual Vol. II - Methods, Method Verification and Validation (ORA-LAB.5.4.5)*

4. FDA Sample Requirements

The FDA sample is a representative portion of a product that is to be analyzed to determine something about the quality of the batch or lot sampled. To make

the sample representative, certain sampling procedures are used. Examples of these can be found in the Investigations Operations Manual (IOM), the pertinent compliance program, or assignment.

The sample serves both as analytical raw material and as physical evidence in a court of law. It is seldom known whether a sample will serve as legal evidence, so it is presumed, from the beginning that it will. This means the sample is treated with great care with respect to its identity, integrity, and chain-of-custody.

Sample integrity is an important concept to be thoroughly understood by all persons handling samples, from the sample collector, the sample custodian or evidence specialist, to the physical science aide, to the analyst. When a sample is introduced into evidence, FDA establishes it is the correct sample (identity) and it has not been changed in any way prior to receipt by the analyst, and that it represents the state of the lot as originally sampled. To say "not to our knowledge" is not sufficient. Everyone in the chain of custody must be able to prove the identity and integrity of the sample was maintained, conclusively.

4.1. Sample Accountability

Before the analyst analyzes an FDA sample, the training supervisor will instruct the analyst in the policies and procedures to be followed to preserve sample identity and integrity and to show continuity of sample handling. Sample accountability calls for entries on the analytical worksheet and in the web application, such as Field Accomplishments and Compliance Tracking System (FACTS) or ALIS, to document who handled the sample and when and that sample integrity was maintained.

FACTS was developed to centralize the data gathered by ORA into one nation-wide system. Therefore, FACTS is recognized as FDA's automated system for field assignments, firm information, compliance actions, and time reporting. The Automated Laboratory Information System (ALIS) is ORA's national laboratory database used to manage sample testing and updates FACTS as necessary. Using FACTS and ALIS will be an essential part of the analyst's responsibility to report analytical information on all work products.

Basic FACTS and ALIS training will be provided during the initial training period.

The FACTS User Guide is the original training manual developed when FACTS was first deployed in the late 1990s, therefore should be used as reference only. It has not been revised to include changes through subsequent releases; however, provides basic information when first introduced to this system.

4.2. Reporting Sample Analysis

The analyst is responsible for a complete and accurate analysis of the sample and a written or electronic report of the analysis. All analytical data is to be recorded on the currently approved version of the FDA worksheet designed for the purpose (the Analyst Worksheet: FDA-431 or Program specific harmonized worksheet), and on supporting documents, such as instrument charts, which are attached to the worksheet.

Approval of any deviations from established reporting requirements, such as in the case of an emergency outbreak, is to be obtained through the Office of Regulatory Science. *MAN-000047 ORA Lab Manual Vol. III Section 3 - Recording of Results Analyst Worksheet (III-03)* provides detailed instructions for preparation of worksheets, both for routine analysis and approved deviations.

Before the analyst analyzes a sample, including samples collected solely for training purposes, the training supervisor will instruct the analyst on how to prepare a worksheet.

4.3. Notebooks

Notebooks may be kept to record data and observations not related to samples. During training, tabulations of assignments and reports on completed work or training sessions, notebooks may be useful.

The notebook is the analyst's record of observations made during work in the laboratory. It is subject to the limitations outlined in section 2.5 of *MAN-000047 ORA Lab Manual Vol. III Section 3 - Recording of Results Analyst Worksheet (III-03).* Data generated during a sample analysis and related to a sample is to be recorded only on the approved analytical worksheet or its attachments, never on scrap paper or in the notebook.

5. Training Progress and Analyst QMiS Completion Status

In addition to agency training, each employee must be trained in local practices, policies, and procedures. Laboratory analysts new to ORS must complete an ORS regulatory science-focused training process during which they demonstrate initial competence in their core lab functions in accordance with both enterprise and ORS local program office/lab processes.

5.1. Level I Analyst Certification

FDA/ORA laboratory analysts hired after January 1, 2002, are required to complete a standard training curriculum, and must achieve Level I Analyst Certification through the ORA Office of Training, Education, and Development (OTED), as well as demonstrate technical competence in keeping with ISO 17025 accreditation requirements, regardless of education level or prior laboratory experience. Laboratory analysts include, but are not limited to, all the following positions: biologists, chemists, engineers, entomologists, interdisciplinary scientists, microbiologist, etc.

Laboratory science technicians, support assistants, and sample evidence specialists do not fall within the category assigned as analysts, therefore they are not eligible to attain Level I Analyst Certification.

ORA/ORS management may use Level I Analyst Certification as part of the demonstration of competence for laboratory accreditation purposes. For those laboratory analysts hired prior to January 2002, there is a process for experienced analysts to achieve Level I Analyst Certification.

Refer to *MAN-000035 ORA Lab Manual Vol. II - Personnel Training and Competency Management (ORA-LAB.5.2)* for more information on Level 1 Analyst Certification and additional specifications for training of laboratory analysts.

5.2. Quality Assurance and documentation

FDA ORS has implemented a national quality assurance program (QAP) that is accredited to requirements outlined in the ISO 17025 General requirements for the competence of testing and calibration laboratories**.**
The ORA Laboratory Manual (LM) outlines how the ORS Laboratories must meet these requirements.

Each FDA laboratory has a quality assurance program (QAP) tailored to the accreditation and LM requirements pertaining to its analytical program and discipline. FDA analysts are expected to perform all activities in accordance with the QAP.

The ORA Quality Management Information System (QMiS) is a software application where the LM, procedures, and forms are maintained. Each ORS laboratory has a Quality System Manager (QSM) and Quality System Specialist (QSS) who will ensure all analysts within their laboratory have access to and basic training in the use of QMiS to complete the study of documents within the analyst's curriculum.

Upon a laboratory analyst's initial successful completion of all documents in QMiS as specified in their standard analyst curriculum, a standardized ORS attestation form will be completed and signed by the laboratory analyst and their immediate supervisor. The attestation form must be uploaded to OTED's learning system to capture completion of the analyst curriculum items located in QMiS so that OTED can reference the form when reviewing requests for Level I Analyst Certification. Local laboratory management must maintain the attestation form as part of the laboratory analyst's training records as part of their initial competency demonstration.

5.3. Laboratory Safety

Analysts must observe the safety and waste disposal processes in place for their laboratories. The analyst is to understand all instructions before beginning any training exercise or activity within the laboratory. Nothing is to be put down a sink drain unless specified as allowable under the waste disposal program.

Methods in the AOAC Official Methods of Analysis contain safety references which identify hazards and guidelines to avoid or protect against them. Review these in addition to equipment instructions and references found within the method before beginning the analysis.

Also consult the Safety Data Sheet (SDS) for safety notes. Safety and cross contamination precautions are used with all chemicals. Special care is to be taken to prevent injury from toxic chemicals, explosions, fires, and corrosive chemicals. Some chemicals or organisms may demand special precautions such as use of protective clothing or use of an enclosed area or balance.

Due to the COVID-19 pandemic of 2020, the laboratory may have special safety requirements that also include working within office and administrative areas of the laboratory.

Each laboratory has its own safety and waste disposal programs; the supervisor should give copies of these.

Read the general laboratory safety instructions found in *MAN-000045 ORA Lab Manual Vol. III Section 1 - Environmental Health and Safety (III-01)* and in the laboratory's program.

5.4. Instrumentation and Equipment

All instruments and equipment used in individual laboratories have manufacturer manuals that the analyst should become familiar with in addition to ORS and/or laboratory specific SOPs or Work Instructions outlining the calibration and maintenance requirements.

5.5. Administrative and regulatory procedures

Analysts must also be familiar with proper laboratory administrative and regulatory procedures.

Field Management Directives (FMD), Regulatory Procedures Manual (RPM), and Compliance Policy Guides (CPGs) are FDA publications that contain pertinent information such as the laboratory research directives, product recall information, and other statements of FDA regulatory procedures. These on-line manuals can be found on the FDA Intranet site.

6. General References

The following list is neither comprehensive nor exclusive; however, contains a few general reference titles that may be useful to the FDA Analyst.

A. The FDA Library Home page offers several resources for access to scientific publications and training resources.

B. MAN-000048 ORA Lab Manual Vol. III Section 4 - Basic Statistics and Data Presentation (III-04)

C. Handbook of chemistry and physics (current ed.). Boca Raton, FL: CRC Press.

D. Lange's Handbook of chemistry. New York: McGraw-Hill.

E. The Merck index. Rahway, NJ: Merck & Co.

F. Youden, W.J., Steiner. E.H. (1997). Statistical manual of the AOAC (5th printing). Arlington, VA: AOAC International.

G. Meier, P.C., Zund, R.E. (2000). Statistical methods in analytical chemistry (analytical chemistry Vol. 123, 2nd ed.). New York: John Wiley & Sons.

7. Orientation Exercises

The analyst becomes familiar with laboratory apparatus and glassware, preparation of standard solutions, and proper techniques common to many analytical procedures.

The completion of all or only parts of the orientation exercises listed in this section is left to the discretion of local management. Many of these exercises may not be applicable to the scientific discipline to which analysts are assigned. Therefore, these orientation exercises may be modified to meet the needs of the local laboratory.

7.1. Analytical Balances

A. Objectives

1. To determine how different laboratory balances function, their weight ranges, and their limitations.
2. To develop techniques for accurate weighing.
3. To determine which balances to use for a defined purpose (i.e., top loader vs. analytical vs. microbalance).

B. Assignment

1. Determine the sensitivity at maximum load and at half maximum load of each type of balance assigned. Determine the minimum mass detected by balance(s).
2. Weigh a nickel coin on the balance, remove the coin, and re-zero the balance. Repeat this process three times. Then repeat the process using another similar balance as well as a different type of balance. How do the weights compare? Repeat the process using a 100 mL beaker.
3. Weigh five different nickels. Determine the average mass, the standard deviation (SD), the % relative standard deviation (%RSD), and the % error if a standard nickel should weigh 5.000g.
4. Obtain a standard set of weights and calibrate a top-loader balance using 0.5, 1, 5, 10 25, 50, and 100g weights. Plot the percent error against each weight tested. Are these errors within the limits set by the balance manufacturer?

C. References

1. American Society for Testing and Materials International. (2003). E 617-18 Standard Specification for Laboratory Weights and Precision Mass Standards. Retrieve from Standards (sharepoint.com)

2. U.S. Pharmacopoeia and National Formulary (current ed.). (General Chapters, section 41, Weights and Balances, pp. 1689-1681). Gaithersburg, MD: Association of Official Analytical Chemists International.
3. Skoog, D. A., West, D. M., Holler, F. J., Crouch, S. R. (2000). Analytical chemistry an introduction (7th ed.). Philadelphia: Saunders College Publishing.
4. USP General Notices and Requirements. Can be obtained at Account Information (sharepoint.com)

D. Questions

1. Define precision. How is it related to accuracy? How does one measure precision?
2. What is the USP definition of “accurately weighed” and how does it relate to “measurement uncertainty”. How is the measurement uncertainty of a balance determined?
3. What do values of +/- 1 SD of +/- 2 SD, and of +/- 3 SD tell us?
4. What is the relationship between SD and % RSD? What is the benefit of using %RSD?
5. Describe two possible ways in which samples could be contaminated during weighing.
6. What are some of the common errors in weighing and describe how to minimize them?

7.2. pH Meters

A. Objectives

To identify the principles involved in conducting an accurate potentiometric test by:

1. Studying the operating principles of a pH meter and its electrodes.
2. Examining the various electrodes and their use as well as examining other ways of determining pH.
3. Carrying out simple determinations of pH.

B. Assignment

1. Review references, SOPs, and operating manuals for each type of pH meter in the laboratory.

2. Prepare and measure with a pH meter the pH of one or more of the buffers described in AOAC or USP/NF as directed by the trainer. Compare each measured pH to the theoretical value of the buffer(s). Plot buffer value versus measured pH.
3. List and describe several electrodes in the laboratory. Discuss the use and proper care of each.
4. List and describe each type of pH-indicating test paper used in the laboratory. What is the range of each? What is its accuracy? What are the storage parameters?
5. List and describe several common pH end-point indicators in the laboratory.
6. Draw and label a simplified schematic of a pH meter with reference and indicating electrodes.
7. Measure the pH of deionized water. Why is the pH reading unstable? Why is the pH reading different than 7.00?

C. References

1. AOAC official methods of analysis. (Current ed.). Sections on "Buffer Solutions for Calibration of pH Equipment" and "Standard Buffers and Indicators for Colorimetric pH Comparisons" in Appendix: "Standard Solutions and Certified Reference Materials." Gaithersburg, MD: Association for Official Analytical Chemists International.
2. U.S. Pharmacopeia and National Formulary (current ed.). (General Chapters, Section 791, "pH.", "Indicator and Test Papers." and "Buffer Solutions."). Gaithersburg, MD: Association for Official Analytical Chemists International.
3. Beyon, R. J., Easterby, J. S. (1996). Buffer solutions (ISBN 0199634424). Oxford, UK: BIOS Scientific Publishers.

D. Questions

1. Calculate the pH of a 0.075 M solution of acetic acid; assume the Ka = 1.8x10-5 for acetic acid. Prepare such a solution and measure the pH, calculate the % error, and give at least three reasons for the error.
2. Would a pH measurement in MeOH or in 50% MeOH - H2O be accurate? Why or why not?
3. Define buffer capacity.

4. List several factors involved in selecting a buffer for a given procedure.
5. What is the useful range in buffering capacity about the pKa of the weak acid?
6. Describe how to prepare a liter of 0.0100 M phosphate buffer at pH = 8.00 using only 10.0 M H3PO4 and a concentrated NaOH solution.
7. Discuss the use of an "equivalent weight" of an acid or base.
8. What kinds of determinations, other than pH, may be made using a pH meter? Give two examples.

7.3. Volumetric Glassware

A. Objectives

1. To develop good technique in the handling and use of volumetric glassware.
2. To calibrate one or more pieces of laboratory volumetric equipment, to review National Institute of Standards and Technology (NIST) and USP/NF requirements for volumetric glassware and compare these to the calibration values obtained.

B. Assignment

1. Review references.
2. Obtain a density vs. temperature table for water and accurately calibrate each piece of volumetric glassware supplied by the trainer (e.g., volumetric pipet, volumetric flask, buret). Calculate the % error for each measurement and compare to stated accuracy.
3. Measure the delivery times of two or three pipets of assorted volumes using a stopwatch. Compare with NIST, USP. etc., standards and manufacturers' specifications.
4. Convert the volume of one of the measurements at temperature of measurement to the volume at 20°C and 25°C.
5. Be prepared to explain the complete calibration operation, including all steps in the calculation, the use of significant figures, precision, and accuracy.

C. References

1. http://www.nist.gov/
2. https://www.nist.gov/pml/weights-and-measures/resources

3. https://www.nist.gov/pml/weights-and-measures/publications
4. Harris, D.C. (May 2015). Quantitative chemical analysis (9th. ed.). W. H. Freeman and Company, New York.
5. U.S. pharmacopoeia 25 and national formulary 20. (General Chapter, Section 31, “Volumetric Apparatus.”). Taunton, MA: Rand McNally.
6. Leveson, D. J. (2002). Rounding of Numbers, How, When and Why. Retrieve version from http://academic.brooklyn.cuny.edu/geology/leveson/core/linksa/roundoff.html

D. Questions

1. Can all volumetric glassware be NIST certified?
2. What do the letters "TC" and "TD" signify?
3. Why should freshly boiled water be used in calibrating glassware?
4. How would someone know when a piece of volumetric glassware is not clean? What method would be used to clean it?
5. List the manufacturer’s % accuracy of the following types of volumetric equipment: Class A 10 mL pipette, Class A 100 mL volumetric flask, 10 mL Mohr pipette, 1 mL Auto Pipette, 10 µL Auto Pipette, 100 mL graduated cylinder, 250 mL graduated beaker.
6. List several of the variables involved in correctly using a 10 mL volumetric pipette.

7.4. Standard Solutions

A. Objectives

To identify the principles and techniques involved in conducting a successful, accurate standardization by:

1. Using one or more official methods for standardization of volumetric solutions.
2. Preparing one or more standard laboratory solutions for later use or for other analysts.
3. Preparing an accurate dilution of a standard solution.

B. Assignment

1. Review references.

2. Prepare a solution of 0.1 N NaOH, 0.1 N HCl, or other solutions as assigned by the trainer. Using an official method (AOAC or USP/NF), standardize the solution. Perform the analysis in triplicate and calculate the %RSD.
3. Prepare100 mL of 0.01N NaOH from the standardized 0.1N NaOH to within 1% accuracy.

C. References

1. AOAC official methods of analysis. (Current edition.). (Appendix: “Standard Solutions and Certified Reference Materials.”). Gaithersburg, MD: Association of Official Analytical Chemist International.
2. U.S. Pharmacopeia 25 and National Formulary 20 (current ed.). (General Chapters, Section on “Volumetric Solutions.”). Taunton, MA: Rand McNally.
3. Volumetric Solutions: Preparation and Standardization
   a) General Information: http://pubs.acs.org/reagents/reagent2000/sec_e000.html
   b) Preparation and Standardization: http://pubs.acs.org/reagents/reagent2000/sec_e001.html

D. Questions

1. What is a primary standard? What are its essential properties? Why was the standard dried?
2. What conditions are to be met to maximize the accuracy of a standardization?
3. Define the normality factor and discuss its use.
4. Why couldn’t one accurately weigh out solid NaOH to make the 0.1 N standard NaOH solution?
5. Define a "1 in 2" solution; a "(1 + 2)" solution; a 10% W/W KI solution; a 10% V/V methanol - water solution; a 5% W/V acetic acid solution; a saturated solution. Calculate the normality and molarity of a 10% H2SO4 solution.

7.5. Thermometers

This exercise is to be modified for different analytical disciplines and may be integrated with other exercises.

A. Objectives

1. To familiarize the analyst with different types of liquid-in-glass thermometers.
2. To establish the proper use of each type of thermometer.
3. To familiarize the analyst with the methods used to calibrate thermometers.

B. Assignment

1. Review references for care, handling, and calibration of liquid-in-glass thermometers.
2. Calibrate one each of the following (if the laboratory uses them): partial immersion, total immersion, complete immersion, and maximum recording thermometers using two standards for each thermometer.

C. References

1. American Society for Testing and Materials International. (1984, March). E 77-84 Standard (pp. 59 – 72).
2. Wise, J. A. NIST Special Publication 819, A procedure for the Effective Recalibration of Liquid-in-Glass Thermometers.

D. Questions

1. Define partial immersion, total immersion, and complete immersion as related to thermometers.
2. How can an analyst recognize the different types (partial, total, or complete) of immersion thermometers?
3. How does a maximum recording thermometer differ from a complete immersion thermometer?
4. What is the best method for reuniting a separated mercury column?
5. Why should an open flame not be applied to a thermometer to raise the temperature?
6. Why are two or more standards generally used to calibrate a thermometer?
7. Why is the boiling point of pure H2O generally not used as one of the two calibrating standards?
8. How many significant figures can be obtained from the temperature reading of a normal thermometer at room temperature?

9. List another typical device for measuring the temperature besides a glass thermometer.

7.6. Microscopes

This exercise is to be modified for different analytical disciplines and may be integrated with other exercises.

A. Objective

1. To acquaint the analyst with the proper care and use of the light microscope, i.e.:
   a) Study the operation of the microscope parts.
   b) Learn the correct procedures for cleaning the microscope.
   c) Learn the procedure for light alignment of the microscope.
   d) Learn the method for determining the microscope factor of the microscope.
   e) Learn the method of calibrating an ocular micrometer.

B. Assignment

1. Review reference material found in the lab on the microscope.
2. Clean the objectives, condenser, and oculars.
3. Properly align the microscope.
4. Determine the microscope factor of the microscope. Prepare a slide and determine the microscopic count of a food sample.
5. Calibrate an ocular micrometer. Measure the length and width of some stained bacteria.

C. References

1. (1998). Bailey and Scott's diagnostic microbiology (10th ed., chap. 11, pp.134-150). St. Louis, MO: Mosby Co.
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D. Questions

1. Define the following types of illumination: bright field, dark ground, phase contrast, and fluorescence.
2. Where is each form of illumination used?
3. What are the two ways someone can tell an oil immersion lens from a high dry lens?
4. List and describe each objective in the nosepiece.
5. List and describe the ways the light source may be used to gain the best resolution.
6. List and describe the cleaning procedure for each microscope used in the laboratory.

8. Document History

Revision #	Status* (D, I, R)	Date	Author Name and Title	Approving Official Name and Title
1.2	R	06/06/2008	LMEB	LMEB
1.3	R	02/14/2013	LMEB	LMEB
1.4	R	05/02/2014	LMEB	LMEB
02	R	06/30/2020	LMEB	LMEB
03	R	05/04/2022	LMEB	LMEB
04	R	Refer to QMiS	LMEB	LMEB

* - D: Draft, I: Initial, R: Revision

9. Change History

Revision #	Change
1.2	1.6.2 second paragraph, changed Volume III, Section Two to Section One. 1.8.1 C. Reference 1., updated website 1.8.3 C. References 2. and 3., updated websites 1.8.3 C. References 4 deleted
1.3	Header – Division of Field Science changed to Office of Regulatory Affairs 1.3.3, 2nd paragraph – Division of Field Science changed to Office of Regulatory Affairs 1.4.3, 1st paragraph – Division of Field Science changed to Office of Regulatory Affairs
1.4	Contents – added LIMS to 1.4.4 1.4.2-1.4.4 – revised to include reference to LIMS 1.5.2.B.2. – added or LIMS
02	• Section 2.1: Added FOOD SAFETY MODERNIZATION AMENDMENT (2011). Moves food manufacturers, distributors, and processors toward a proactive relationship with food safety. Grants mandatory recall authority for foods. • Section 3.4: Added to first paragraph: There are many sources of internal (FDA) and external (ISO, AOAC) guidelines for conducting method validation activities. • Section 4.1: Added “assignment” to “the pertinent compliance program or assignment” • Updated the following references in Sections 7.3, 7.4, 8.1, and 8.3: o Skoog, D. A., West, D. M., Holler, F. J., Crouch, S. R. (2013). Fundamentals of analytical chemistry (9th ed.). Cengage Learning. o Skoog, D. A., Holler, F. J., Crouch, S. R. (2018). Principles of instrumental analysis, (7th ed.). Cengage Learning. o Poole. C. F. (2003). The Essence of Chromatography. New York: Elsevier. o Skoog, D. A., West, D. M., Holler, F. J., Crouch, S. R. (2000). Analytical chemistry an introduction (7th ed.). Philadelphia: Saunders College Publishing. o Harris, D.C. (May 2015). Quantitative chemical analysis (9th. ed.). W. H. Freeman and Company, New York. • Document reformatted and a few other clarifications and minor revisions made.
03	• 2.1: Replaced his or her with "their respective" • 4.1: Replaced "Inspections" with "Investigations" • 4.3: Changed reference from Vol. II to Vol. III sub-section Recording Analytical Information, Observations and Findings • 5.1: Changed reference from Vol. II to Vol. III • 7: Removed reference, as it is no longer available • 8.3 C: References 2 and 3 replaced with correct links • 8.4 C: Reference 2 typo of Pharmacopeia
04	Complete reformat of information. Edits made concerning L1 analyst certification. Resource/reference links updated.

10. Attachments

List of Attachments

Attachment A - Answer Key

Analytical Balances

1. Define precision. How is it related to accuracy? How does one measure precision?

Precision is a measure of how closely the measured values are grouped. Measurements with good precision have a tight or close grouping. Accuracy, on the other hand, is a measure of how closely the average value of the measurements is to the true value. Ideally, we strive for good accuracy and good precision. It is possible to have poor accuracy and good precision, good accuracy, and poor precision (luck), and poor accuracy and poor precision. We usually use standard deviation (SD) or a derivative of standard deviation to measure accuracy. Precision is expressed as relative standard deviation (RSD) or relative percent difference (RPD) of duplicate samples.

$$SD = \sqrt{\frac{\sum (X_i - X)^2}{N-1}}$$

Where:

X = Average Value

Xi = Individual Value

n = Number of Runs

RSD is calculated from standard deviation and mean recovery, when the standard deviation is derived from multiple recovery results.

$$RSD = CV = 100 \times \frac{\sigma}{X}$$

Where:

RSD = Relative Standard Deviation
CV = Coefficient of Variation
σ = Standard Deviation
X = arithmetic mean of the measurements

2. What is the USP definition of "accurately weighed" and how does it relate to "measurement uncertainty". How is the measurement uncertainty of a balance determined?

Accurate weighing is to be performed with a weighing device whose measurement uncertainty does not exceed 0.1% of the reading. We can

determine the accuracy of a balance by comparing the balance reading with the stated value of the standard mass used. Measurement uncertainty is the combination of random and systematic error. Measurement uncertainty is satisfactory if three times the standard deviation (of not less than ten replicate weighings) divided by the amount weighed, does not exceed 0.001.

3. **What do values of +/- 1 SD of +/- 2 SD, and of +/- 3 SD tell us?**

   Measurements with an accuracy range of +/- 1 SD tells us that if the analyst continues to perform the measurements using the same technique, then the results will fall within this range 68% of the time. +/- 2 SD = 95 % and +/- 3 SD = 98 %.

4. **What is the relationship between SD and %RSD? What is the benefit of using %RSD?**

   One benefit of %RSD is that the precision is normalized to a 100-point scale which allows us to readily compare precisions of two methods or analysts regardless of the analysis concentration range.

5. **Describe two possible ways in which samples could be contaminated during weighing.**

   There are several possible contamination routes during weighing. An analyst could contaminate a sample during weighing by placing a contaminated spatula into the sample, by placing the sample on or into a contaminated holder during weighing, by dropping some lint/hair/skin or sneeze into the sample while weighing, or by opening a bottle of chemicals near the sample being weighed. When performing trace analysis, it is possible for just a microgram of contaminant to be important, and a microgram is about 100 times less massive than a fingerprint!

6. **What are some of the common errors in weighing and describe how to minimize them?**

   There are several possible ways that an error in weighing can occur. A few potential errors are:

   - Misreading of the balance,
   - Balance not level,
   - Not cleaning the surface of the balance first,
   - Touching the weighed object with moist hands,
   - Leaving the balance doors open during weighing,
   - Using a balance that has not been calibrated or was not calibrated successfully,

- Not cooling the sample down to near room temperature,
- Not removing a static charge from the sample,
- Excess vibration or air currents from people or nearby equipment, and
- Prolonged time sample left on pan adds/loses moisture.

pH Meters

1. **Calculate the pH of a 0.075 M solution of acetic acid; assume the Ka = 1.8x10-5 for acetic acid. Prepare such a solution and measure the pH; calculate the % error and give at least three reasons for the error.**

   Possible reasons for error are: a) Errors involved in making the 0.075 M acetic acid solution from concentrated acetic acid. b) The concentrated acetic acid may not be 100 % acetic acid. c) The pH meter was not calibrated properly. d) Equilibrium calculations have at least a 10% error due to the use of M instead of activity; activity depends upon ionic strength, concentration, and temperature.

2. **Would a pH measurement in MeOH or in 50% MeOH - H2O be accurate? Why or why not?**

   No, the pH measurements would not be accurate. The surface of the indicator electrode needs to be coated with H2O to function properly. Also, the % ionization of the acid is dependent upon the water content.

3. **Define buffer capacity.**

   A buffer is a solution of a weak acid or a weak base and its salt which resists a change in pH. The capacity of the buffer to resist a change in pH when either a strong acid or a strong base is added is a measure of the buffer capacity. One definition of buffer capacity is: the amount of a given acid or base that can be added to the buffer solution in order to change the pH by one unit.

4. **List several factors involved in selecting a buffer for a given procedure.**

   The main factor is the pH -- a buffer only works if the pH is within one unit of the pK of the weak acid or weak base -- so select a buffer that has a pK as close to the pH as possible. Another factor is buffer solubility: for example, phosphate buffers may not be soluble in aqueous acetonitrile solutions. Other factors relate to buffer cost, buffer toxicity, buffer influence on microorganism growth, etc.

5. **What is the useful range in buffering capacity about the pKa of the weak acid?**

   pH = pKa +/- 1.0. A buffer only works if the pH is within one unit of the pKa of the weak acid.

6. **Describe how one would prepare a liter of 0.0100 M phosphate buffer at pH = 8.00 using only 10.0 M H3PO4 and a concentrated NaOH solution.**

   Add 1.00 mL of the 10.0 M H3PO4 to a little less than 1.00 L of water. Add enough concentrated NaOH to bring the pH to 8.00 (use calibrated pH meter) and then bring the volume to 1.00 L with water.

7. **Discuss the use of an "equivalent weight" of an acid or base.**

   The equivalent weight is the mass of the acid or base that will neutralize one mole of either H+ or OH-. The use of equivalent weight, equivalents, and the corresponding Normality (N) is useful since one equivalent of a substance will react with one equivalent of another substance; this simplifies calculations since the balancing coefficients of the reaction do not enter into the calculation. The use of equivalent weight, equivalents, and the corresponding Normality (N), is an older concept and is frequently not taught in college chemistry classes.

8. **What kinds of determinations, other than pH, may be made using a pH meter? Give two examples.**

   One can use a pH meter as a potentiometer and measure the voltage developed at the electrodes in an oxidation-reduction reaction. Also, the pH meter can be used to measure the concentration of a given ion using an ion dedicated electrode.

Volumetric Glassware

1. **Can all volumetric glassware be NIST certified?**

   All volumetric glassware can be calibrated in the laboratory using accurately weighed deliveries of water since mass measurements can be more accurate than volume measurements (a chart of the densities of water at various temperatures is needed).

2. **What do the letters "TC" and "TD" signify?**

   The letters “TD” means “to deliver;” many volumetric pipets are calibrated to deliver a given volume after a given drain time. “TC” means “to contain;” many volumetric flasks are calibrated to contain a given volume.

3. **Why should freshly boiled water be used in calibrating glassware?**

   The density of the water does slightly depend upon the amount of dissolved gases, and boiling is a simple way to remove dissolved gases.

4. **How would one know when a piece of volumetric glassware is not clean? What method would one use to clean it?**

The most common way to determine the cleanliness of volumetric glassware is to drain water from the apparatus; if the water drains smoothly and does not bead up, then it is considered clean. Volumetric glassware can become “dirty” just by picking up surface organics from the air. One way to clean the glassware is to rinse the glassware with chromic acid. Chromic acid is a solution of potassium dichromate in aqueous sulfuric acid. This solution is toxic, corrosive, and a strong oxidizing agent (caution); it will oxidize and dissolve most organics present. Other methods include rinsing with strong acid or base (depending on nature of deposit), rinsing with an organic solvent, or good old-fashioned scrubbing followed by a thorough rinse.

5. **List the manufactures % accuracy of the following types of volumetric equipment: Class A 10 mL pipette, Class A 100 mL volumetric flask, 10 mL Mohr pipette, 1 mL Auto Pipette, 10 µL Auto Pipette, 100 mL graduated cylinder, 250 mL graduated beaker.**

   Note: The manufacturers’ tolerances for Class A glassware may be better than the required standard. Class A volumetric glassware are those whose capacity tolerances are accepted by the National Bureau of Standards, and these tolerances are listed in the USP under Volumetric Apparatus [31]. The actual answers depend upon the manufacturer of the equipment; some possible answers are: Class A 10 mL pipet = 0.02 mL; Class A 100 mL Volumetric Flask = 0.08 mL; 10 mL Mohr pipet = 0.04 mL; 1 mL Auto Pipet = 0.02 mL; 10 µL Auto Pipet = 0.5 µL; 100 mL Graduated Cylinder = 0.5 mL; 250 Graduated Beaker = 10 mL.

6. **List several of the variables involved in correctly using a 10 mL volumetric pipette.**

   Some variables involved in using a 10 mL volumetric pipet are: drain time; possible beads on the inner surface due to uncleanliness; temperature; bringing meniscus to the proper level; angle of drain; touching off last drop; rinsing of the pipet with the solution used; pipet calibration; etc.

Standard Solutions

1. **What is a primary standard? What are its essential properties? Why does one dry the standard?**

   A primary standard is one which is weighed out and the concentration is known to be better than 1% accuracy. Some essential properties of the primary standard are having a high purity; having a known purity; being stable under normal temperature and normal drying conditions; being stable under long storage conditions; and not being hygroscopic. Drying of the primary standard may be needed to bring it to a constant mass by removing any surface moisture.

2. **What conditions are to be met to maximize the accuracy of a standardization?**

   The standards are to be of desired and known concentrations; the standard reacts quickly and completely with the reagents of interest; the instruments and glassware used allow the desired accuracy; the techniques used by the analyst are to be reproducible and allow the desired accuracy; and preferably, the weight of standard substance and volume of standard solution should not be too small. In general, titrations are made directly to the end point. A back-titration with another standardized solution increases the possibility of error. The standardization of a solution against another standardized liquid should be avoided. Every standardization should be based on at least three parallel determinations.

3. **Define the normality factor and discuss its use.**

   Acid yields one, two, or three protons per formula. If the same number of formula weights of each, per liter, were dissolved the neutralizing capacities would not be the same. To express concentration in the terms of neutralizing capacity one does so according to the number of H3O+ or OH- in the solution. A solution with one mole H3O+ or OH- present for neutralization reactions is called “one normal solution.” That weight of a compound or substance which will supply one mole H3O+ or OH- for a neutralization reaction is called equivalent weight of the material. The equivalent weight of an acid is the formula weight divided by the number of protons per formula present for neutralization reaction.

4. **Why couldn’t one accurately weigh out solid NaOH to make the 0.1 N standard NaOH solution?**

   One can purchase a high and known purity NaOH product; so, why can’t we just weigh out 4.00 g of 100% NaOH and dissolve to 1.00 L in order to make 1.00 L of a 0.100 N solution? The reason why this is not possible is that NaOH is highly hygroscopic; NaOH will rapidly absorb up to 100% of its mass in water from the atmosphere. Even if one has a pure sample of NaOH it will pick up significant amounts of moisture during a 30 second weighing.

5. **Define a "1 in 2" solution; a "(1 + 2)" solution; a 10% W/W KI solution; a 10% V/V methanol - water solution; a 5% W/V acetic acid solution; a saturated solution. Calculate the normality and molarity of a 10% H2SO4 solution.**

   1 in 2 solutions (Usually written as 1:2 dilution) =1 volume liquid A + 1 volume liquid B (or sufficient volume of liquid B to make the volume of the finished solution two parts by volume). A 1 + 2 solution (Usually written as 1:3 dilution) = 1 volume of chemical A and 2 volumes of chemical B. A 10% W/W KI

solution = 10 g of KI added to 90 g of water. A 10% V/V methanol - water solution = 10 mL methanol + enough water to make 100 mL total. A 5% W/V acetic acid solution = 5 g of 100% acetic acid added to enough water to make 100 mL. A saturated solution = a solution containing the maximum amount of solute in solution, the undissolved solute in equilibrium with the solution.

Thermometers

1. **Define partial immersion, total immersion, and complete immersion as related to thermometers.**

   A partial immersion thermometer has just its lower portion - the mercury bulb up to the marked immersion line - immersed in the sample. A total immersion thermometer involves immersion of the thermometer to the top of the mercury column, with the remainder of the stem and the upper expansion chamber exposed to ambient temperature. A complete immersion thermometer is entirely immersed in the sample.

2. **How can an analyst recognize the different types (partial, total, or complete) of immersion thermometers?**

   The partial immersion thermometer is by far, the most common, and it will usually have an immersion line etched into it just above the mercury bulb; assume any unmarked thermometer will be a partial immersion type. The other two types will be plainly marked.

3. **How does a maximum recording thermometer differ from a complete immersion thermometer?**

   A maximum recording thermometer only indicates the highest temperature achieved.

4. **What is the best method for reuniting a separated mercury column?**

   The best way to reunite a separated mercury column is to place the thermometer into a very cold environment ¬-- liquid¬ N2 or dry ice in acetone; allow the mercury to unite in the bulb; and then slowly warm back up to room temperature.

5. **Why should an open flame not be applied to a thermometer to raise the temperature?**

   The intense heat from the flame will expand the thermometer rapidly and possibly crack the glass.

6. **Why are two or more standards generally used to calibrate a thermometer?**

   If one calibrates a thermometer at a single temperature, then it will be accurate at that temperature; however, it could easily be off at other temperatures -

especially at temperatures far from the calibration point. To help ensure that the thermometer is accurate over a selected range of temperatures, then one calibrates the thermometer at the lower portion of the range as well as at the upper portion of the range.

7. **Why is the boiling point of pure H2O generally not used as one of the two calibrating standards?**

   The boiling point of water depends upon the external pressure. For example, the BP of water at 760 Torr is 100 ºC, and the BP of water at 630 Torr is 94 ºC.

8. **How many significant figures can be obtained from the temperature reading of a normal thermometer at room temperature?**

   At a temperature of about 20 ºC, a typical thermometer can be interpolated to 0.2 ºC; so, three significant figures are possible.

9. **List another typical device for measuring the temperature besides a glass thermometer.**

   A thermocouple is a common temperature probe. It measures the temperature sensitive electric current flow through two dissimilar metals in contact with each other and converts the current to temperature.

Microscopes

1. **Define the following types of illumination: bright field, dark ground, phase contrast, and fluorescence.**

   Using bright field, object is illuminated with light. Using dark field, object reflects the light out of its surface and appears bright against a black background. In phase contrast microscopy, the unstained material can be viewed when light passes and is partially deflected by different thicknesses of the object. Fluorescence microscopy uses certain dyes. When the dye is excited by light of high energy (short wavelength UV), it emits the absorbed energy later as visible long wave light, and the stained object is thus illuminated against a dark background.

2. **Where is each form of illumination used?**

   Bright field is used with stained objects and fresh material. Dark field is used with thin motile objects. Phase contrast is used with unstained material. Fluorescence is used for special diagnostic tasks like antibody-antigen diagnostic reactions.

3. **What are the two ways one can tell an oil immersion lens from a high dry lens?**

   It is marked 100X oil immersion lens. Its tip moves up and down.

4. **List and describe each objective in the nosepiece.**

   Framework: basic framework includes arm and base.

   Lens system:

   a) Oculars: Two eyepieces, for the examiner to use to view object,

   b) the objectives usually three 10X, 45X and 100X, and

   c) the condenser located under the stage, it collects and directs the light.

   Nosepiece: to carry the lens system.

   Light source: usually in the base of most microscopes.

   Stage: horizontal platform that supports the microscope slide.

5. **List and describe the ways the light source may be used to gain the best resolution.**

   a) Change the wavelength of the light source by using filters. A blue filter should be used. This gives short wavelength and high resolution.

   b) Keep the condenser at its highest position, this allows maximum amount of light to enter the objective.

   c) Keep the diaphragm up, this increases the numerical aperture. (d) Use immersion oil with the 100 X lens.

6. **List and describe the cleaning procedure for each microscope used in the laboratory.**

   To be answered by individual instructor as requested.