

Chapter 4

Preparation of PE Samples

Manufacturing PE samples of authenticated compositions requires careful planning so that PE samples achieve their intended purposes. The preparation procedures for USACE PE samples described in this chapter include the following:

- Matrix selection.
- Selection of chemicals and reagents.
- Facility and equipment requirements.
- General and specific preparation procedures including fortified, real-world, and commercial PE samples.
- Handling and storage requirements.
- Packing and shipping guidelines.

4.1. Selection of PE Sample Matrices.

Three types of sample matrices commonly used to prepare PE samples are: reagent water, clean soils, and real-world environmental matrices. Guidelines for matrix selection include the following:

- Use the sample preparation methods required for project samples to determine the matrix type of PE samples.
- Use water PE samples for laboratories involved in analysis of aqueous samples.
- Use both water and soil PE samples for laboratories involved in analysis of soil samples. It is USACE policy that a laboratory must pass both water and soil PE samples to be considered for validation of soil sample analysis.

4.1.1. Reagent Water Matrix. Use reagent water that is free of contaminants of concern at MDL for preparation of aqueous PE samples by following these guidelines:

- Prepare by distillation or other equivalent processes, then polish with a mixed bed of ion exchange materials, a 0.2- μ m membrane filter, and/or an organic adsorption system.
- Use high-purity reagent water (ASTM Type I or II grade of reagent water) immediately after production; any kind of storage will cause some form of purity degradation.
- Monitor and document reagent water quality daily.

4.1.2. Clean Soil Matrix. Various types of soils that are free of contaminants of concern at MDL can serve as clean soil matrices. Select suitable clean soils according to target analytes, DQO, sample preparation, and analytical methods. In addition, some specific considerations include:

- Soil composition and texture impact preparation and PE sample performance. Coarse texture and large grain size affect homogeneity and representativeness of subsamples.
- High alkalinity affects the stability of acidic target analytes.
- Organic and metal contents may vary; however, avoid soils that are very reactive to acids or other reagents used for sample preparation.
- Contaminated soils may be used after purification with acid wash, solvent extraction, or thermal treatment. However, avoid excessive thermal treatment because it may destroy organic matter and soil structure. The resulting matrix would not be site-specific or similar to untreated soil. Refer to Appendix C for two procedures for soil cleaning.
- Background soils that contain minute amounts of indigenous contaminants and/or interferents may be more practical for PE sample preparation.
- Background soils collected from project sites mimic field samples.

4.1.3. Real-World Matrices. To mimic field samples, real-world environmental matrices, such as tap water, surface water, ground water, soils, and sediments can also be used to prepare PE samples. Use a well characterized, contaminated environmental matrix in one of three ways: as it is, fortify it with target analytes, or dilute it with a clean environmental matrix to desired analyte concentrations. The target analytes of real-world contaminated soils usually possess special speciation or partition in sample matrices or penetrate into the micropores of soil particles where they are tightly bound. These factors make the PE samples more challenging.

4.2. Selection of Chemicals and Reagents.

Considerations for chemical and reagent selection discussed in this section include purity, traceability, concentration, certified reference material, chemical composition, and matrix selection.

4.2.1. Purity. All chemicals shall be proven free of contaminants and interferences or be of sufficient purity to meet the intended purpose. Guidelines for purity include the following:

- Use reagents that conform to the specifications of the Committee on Analytical Reagents of the American Chemical Society (ACS). Other grades may be used if the reagent's purity permits its use without adverse impacts on the bias and precision of subsequent analyses.
- Use acids of reagent grade or better except for those used for ICP and GFAA work, which need to be of high-purity grade or equivalent.
- Use solvents of chromatographic grade or better.
- Document all critical information about chemicals and reagents.

4.2.2. Traceability. The quality and concentrations of all chemicals, reagents, and materials used for production of PE samples should be traceable to national or international standards. Document traceability of materials in these ways:

- Use unique identifiers for all purchased and in-house prepared reagents to establish and maintain traceability throughout PE sample manufacturing.
- Maintain a log book for all chemicals, reagents, and materials used for production.

4.2.3. Concentration. Determine the concentration or purity of all starting materials by various procedures that are listed below in an order of increasing uncertainty.

- True or known concentrations as determined by specific formulations (e.g., manufacture or dilution.)
- Certified reference values as determined by multiple definitive methods of different quantitation principles.
- Reference values as determined by analysis along with standards traceable to national reference standards.
- Consensus values as determined by a group of expert laboratories that have demonstrable competence in the analytical methods to be evaluated.

Use multiple definitive methods as defined below to minimize systematic errors in determining analyte concentrations. Definitive measurement methods are methods that have been rigorously proven and demonstrated to be accurate within specified limits. Definitive methods provide certified reference values that are directly traceable to fundamental units of measurements (e.g., mass, length, time, volt, ohm, etc.) or indirectly related to the fundamental units through physical or chemical theory expressed in exact mathematical equations. Theoretically, any methods of high precision and accuracy might be qualified as definitive methods for any combinations of analyte and matrix. In practice, however, a definitive method must have, at least, sufficient documentation to prove its accuracy and use indirect means to establish measurement errors in real sample matrices.

4.2.4. Certified Reference Materials. When commercially available reference materials are utilized as PE samples or used to prepare PE samples, follow these guidelines:

- Be sure that the acceptance limits for reference materials and methods being evaluated were established using the same or equivalent sample preparation and analysis methods. This is especially critical for commercially available solid reference materials where the certified concentrations are often the total concentrations instead of the recoverable concentrations given by most USEPA methods.
- Establish acceptance limits for target analytes in soil PE samples based on the same preparation and analysis methods to be evaluated. Otherwise, results may not be equivalent to those of the methods being evaluated.

4.2.5. Chemical Compositions. Determine chemical compositions using these guidelines.

- Consider the types of cation/anion pairs, the oxidation states of cations, the ligands, the hydrations, etc., for inorganic standards.
- Consult literature for solubility, stability, and chemical and physical properties of a standard, especially if the standard contains compounds that are not previously used in a chosen solvent.
- Collect current results of Karl Fisher analysis to determine the concentration of PE samples if the standard is hygroscopic.

4.2.6. Selection of Matrix. After matrix selection:

- Verify that a current purity assay of the matrix is on file. If one is not, determine and document the purity of the matrix with a minimum of two definitive methods of different measurement methodologies by a minimum of two reliable laboratories.
- Use reagent water meeting ASTM Type I for inorganic analyses and water meeting Type II for organic analyses.

4.3. Facility and Equipment Requirements.

Laboratories providing PE samples must meet these standards:

- USACE validation according to the requirements in EM 200-1-1.
- Compliance with ISO Guides 25 (17025) and 34, ISO 9000 series of standards, and ASTM Standard D5522 for facilities, equipment, and quality systems used in PE sample preparation and testing.

Standard for facilities, balances, ovens, thermometers, pH meters, and volumetric glassware, are outlined below.

4.3.1. Facility. Facility requirements include the following:

- Provide laboratory security by limiting access and using a secured storage area.
- Monitor the laboratory for proper air flow, ventilation, humidity, temperature, and lighting.
- Provide adequate work space such that PE samples can be prepared and tested safely and efficiently in order to minimize the possibility of cross contamination.
- Provide a stable power supply, sufficient exhaust hoods, and proper storage facilities.
- Use adequate procedures and facilities for the collection, storage, and disposal of chemical wastes.

4.3.2. Balances. Standards for balances include:

- Use appropriate balance ranges for applications.
- Check each balance daily (or with use) with two ASTM Class 1 or equivalent weights that bracket the expected weight range. Check analytical balances monthly with a series of Class 1 weights and document the results. Analytical balances should have readabilities of 0.01 to 0.1 mg to meet measurement requirements.
- Correct any variance of greater than 0.1% between expected weights and actual weights.
- Check top-loading balances with, at a minimum, ASTM Class 2 or equivalent weights within $\pm 1\%$. Top-loading balances should have readability of 0.001 to 0.01 g.
- Use Class 1 and 2 weights calibrated within the last five years and traceable to NIST reference standards.

4.3.3. Ovens and Furnaces. Guidelines for oven and furnace use include:

- Check the temperature of each drying oven before and after each use to verify the correct operating temperature for the given operation.
- Check and document the extent of temperature swings at different operating temperature ranges.
- Verify the temperature at least annually in the operating ranges for muffle furnaces.

4.3.4. Thermometers. Standards for thermometers include:

- Check the calibration of each mercury or alcohol thermometer at least annually against an NIST traceable thermometer. Check at two separate temperatures bracketing the expected temperature range.
- Calibrate electronic and dial-type thermometers at least quarterly against an NIST traceable thermometer.
- Use thermometers with $\pm 1^\circ\text{C}$ accuracy.

4.3.5. pH Meters. Guidelines for pH meter use include:

- Calibrate pH meters with buffer solutions prepared with NIST primary buffer salts or commercial secondary buffer solutions traceable to NIST's standards.
- Standardize the pH meter at least daily with two buffers that bracket the expected pH range. The bracket must be no more than three to four pH units wide.
- Use pH meters with ± 0.1 pH unit accuracy.

4.3.6. Volumetric Glassware. Standards for volumetric glassware use include:

- Make all volumetric measurements with ASTM Class A glassware. ASTM Class A pipets and calibrated microsyringes must be used for delivering and spiking during preparation of PE samples. Variable pipettors can be used if they are gravimetrically verified to be in calibration; however, glass pipets are preferred.
- Use volumetric glassware that yields $\pm 1\%$ accuracy.

4.4. Preparation of PE Samples.

The goal of preparation is that PE samples resemble routine field samples in appearance, characteristics, analyte content, and concentration level. PE samples can be prepared in two ways:

- Spiking known amounts of analytes into a clearly defined homogeneous matrix (known as fortified PE samples).
- Defining homogenized real-world samples (known as real-world PE samples).

Both of these methods as well as commercially prepared PE samples are discussed in this section.

4.4.1. General Preparation Procedure. Regardless of the type of PE samples, the general procedure for preparing PE samples is outlined below. Some of these steps, such as (6), (7), (11), etc., may be exempted when additional batches of the same or similar PE samples are prepared.

- (1) Determine matrix type, analytical method, and analytical instrumentation.
- (2) Calculate the total amount of PE samples needed by volume or weight.
- (3) Select analytes, interferences, solvents, and preservatives.
- (4) Decide the concentration of each component.
- (5) Select stock materials and calculate appropriate amounts to add.
- (6) Write step-by-step instructions (i.e., SOPs).
- (7) Perform an error analysis and define performance requirements.
- (8) Obtain stock materials.
- (9) Prepare PE samples.
- (10) Verify the concentration of each component in PE samples.
- (11) Verify the composition of PE samples by multilaboratory referee analyses.
- (12) Establish performance acceptance limits of each PE sample.
- (13) Characterize any indigenous level of target analytes and/or interferences when using real-world materials. Additional interlaboratory analyses are needed to establish the acceptance limits of real-world PE samples.

4.4.2. Fortified PE Samples. General guidelines regardless of matrix are given below followed by fortification techniques for aqueous and soil PE samples.

4.4.2.1. General guidelines. Prepare fortified PE samples of any matrix using these guidelines:

- Minimize variations between PE samples by preparing a large amount of PE sample and subsampling it to create individual PE samples.
- Preparing individual samples if analyte loss to containers is a major concern. For example, hydrophobic petroleum hydrocarbons tend to separate from water matrix and stick to the container wall. Therefore, the container must be rinsed with extraction solvent to enhance the recovery of petroleum hydrocarbons. So use individually spiked PE samples.
- Use experienced senior chemists to prepare spiked samples and improve reproducibility.
- Prepare aqueous PE samples of one liter for semivolatile organic analyses and 40 mL volatile organic analyses.
- Prepare aqueous PE samples for inorganic analyses from 200 mL to one liter, depending upon the target analytes and analytical methods.
- Prepare a trip blank to accompany volatile organic samples for each analytical method.

4.4.2.2. Aqueous PE Samples. Aqueous PE samples can be prepared by either gravimetric or volumetric methods depending on the physical properties of bulk materials.

- Use gravimetric methods for very volatile materials or those with unreliable density values.
- Use volumetric methods when bulk materials have low vapor pressures and reliable density values.
- Prepare aqueous PE samples on the day of shipment, usually early in the week to allow adequate preparation time for participating laboratories to perform digestions, extractions, cleanups, etc., before the weekend.

4.4.2.2.1. Gravimetric Method. A gravimetric method can be used for both liquid and solid fortifications. Follow these procedures for a gravimetric method:

- Determine the density of the solution so that volume-to-weight conversions can be calculated if weights will be used for calculation.
- Calculate the mass of starting materials to be weighed for the specified volume of solution. The prepared concentration should be within $\pm 1\%$ of the target concentration of the solution.
- Use this formula:

$$M (g) = \frac{V_s (mL) \times C (\mu g/mL)}{10^6 (\mu g/g) \times P}$$

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where:

 M = mass of the starting material required V_s = volume of the final solution desired C = concentration of the final solution desired P = purity as a fraction of the starting material

Purity used in the calculation could be a certified value from the manufacturer or vendor.

- Perform weighing in a glove box flushed with high purity nitrogen gas to reduce the risk of contamination.
- Check the balance with NIST traceable calibration masses on the day the standard is prepared to ensure accuracy of weighing.
- Record the results in the balance logs.
- Guard against static charges on the balance or the beaker influencing the balance readings with an antistatic device.
- Remove the beaker from the balance and pipet some solvent (~3 mL) into the beaker to aid the dissolution and prevent evaporation of the weighed samples as soon as balance door is closed and weight is measured.
- Cover the top of the beaker to avoid contamination.
- Record the lot numbers of all reagents and starting materials.
- Designate a unique identification number for the solution.
- Date and initial the entry.

4.4.2.2.2. Volumetric Method. A volumetric method can only be used for liquid fortification.

Follow these procedures for a volumetric method:

- Calculate the volume of each bulk required.
- Allow all solutions to warm to ambient temperature and mix them thoroughly to ensure homogeneity and accurate volumetric measurement. The prepared concentration should be within $\pm 1\%$ of the target concentration of the solution.
- Use this formula:

$$V \text{ (mL)} = \frac{V_s \text{ (mL)} \times C \text{ (\mu g/mL)}}{d \text{ (g/mL)} \times 10^6 \text{ (\mu g/g)} \times P}$$

where:

 V = volume of the bulk required V_s = volume of the final solution desired C = concentration of the final solution desired d = density of the bulk P = purity as a fraction of the bulk

- Obtain starting materials and stock solutions at appropriate concentration levels to minimize the amounts required and to remain in the realm of accurate measurements of laboratory ware. Avoid dilutions requiring odd sizes of volumetric ware.
- Keep volume measurements between 50 : L and 1 L and weights between 10 mg and 1 kg.
- Choose the more concentrated solutions of stock solutions to minimize potential contamination.
- Use minimal headspace and exposure to the atmosphere while preparing volatile liquids.
- Use diluents that already contain any required preservatives so that final volumes are not altered by preservation.

4.4.2.3. Soil PE Samples. Soil PE samples are prepared with clean soils in bulk or in small quantity. Use these guidelines for determining sample size.

- Use bulk quantity PE samples for stable target analytes and subsample for use over an extended time period.
- Use small quantity PE samples for less stable target analytes and prepare as individual PE samples on an as needed basis. Currently, small quantity PE samples are only used for explosives PE samples where preweighted, clean soils in extraction vials are spiked with target analytes.

4.4.2.3.1. Preparation of Clean Soils. To prepare clean soils, follow these steps:

- Collect a large quantity of soil from a clean site.
- Check soil cleanliness to ensure that no contaminants or interferents exist at concentrations above the detection limits of the analytical methods being evaluated.
- Remove rocks, sticks, and other foreign material and dry soil at ambient temperature to approximately 2 - 4% moisture.
- Grind, mix, and run dried soils through a fine screen to remove large particles.
- Determine grinding and mixing times by checking particle size and soil consistency after short interval runs.
- Sieved soil should be homogeneous with the consistency of fine powder and specific particle sizes.
- Use the sample mass required for PE samples and the maximum sampling errors allowed (15%) to determine maximum particle size (according to Pierre Gy's Sampling Theory). See Appendix D for the calculation of maximum allowable particle size that can be accommodated by a given sample mass at various sampling errors. (A No. 50 mesh sieve generally yields materials of adequate particle size.)
- Keep the overall sampling error as low as practically feasible but it should not exceed 15%.

4.4.2.3.2. Verification of Clean Soils. Use the following procedures to confirm the cleanliness of the prepared clean soil after processing.

- Randomly select multiple sets of clean soil samples for analysis by several reliable laboratories using the analytical methods being evaluated. An alternative is to have one reliable laboratory use several different analytical methods with similar or lower detection limits than those of reference methods.
- Instruct laboratories to prepare samples with different preparation methods known for good analyte recovery (within 70% to 130%).
- Accept the clean soil, if the concentrations of any native target analytes or interferences are below the MDL of the analytical methods to be evaluated or are negligible (i.e., <5%) in comparison with the concentrations of spiked target analytes. Otherwise, remove the trace contaminants if necessary with acid washing or solvent extraction, or find another, cleaner soil.
- Bring the pH value of any cleaned soil to neutral. Otherwise, certain spiked target analytes might be decomposed, transformed, and lost in recovery.

4.4.2.3.3. Fortification of Clean Soils. Clean soils can be fortified with target analytes by solid, liquid, or vapor fortification methods. The choice of a specific method depends on the chemical properties of target analytes and the stability of the PE samples.

4.4.2.3.3.1. Solid Fortification. Use solid fortification for analytes of low vapor pressure and solubility following these guidelines:

- Reduce the particle size of solid reagents to small particles of approximately the same size (ca. # 300 microns) before mixing. This makes blending easier, and components will be less prone to segregate during storage and transit.
- Use similar amounts of each component because it is very difficult to evenly distribute otherwise. If extremes in relative amounts cannot be avoided, do the blending in stages by spiking and blending a small quantity of the main component, then mixing and blending with the rest.
- Note that solid fortification works best if the specific gravities of the analyte compounds are similar to that of the host matrix (ca. 2.5 g/cm³ for soil) within reasonable limits.

4.4.2.3.3.2. Liquid Fortification. Use liquid fortification for soluble and less volatile analytes following these guidelines:

- Spray analyte solutions over homogenized soil in small increments.
- Mix the soil thoroughly and spray again after vaporization of the solvent.
- Repeat until all analyte solutions are used up.
- Rinse the spray bottles with more solvent and spray over the soil again to ensure that all target analytes are quantitatively transferred to the homogenized soil.
- Mix thoroughly after drying to avoid analyte deposits.
- Improve mixing using enough solvent to form a runny paste or mud. Stir the paste occasionally while drying and, when completely dry, re-grind and blend.

- An alternative to the above method is to soak soils with a large volume of analyte solution, remove the solvent, and dry, grind, and blend the soil.

4.4.2.3.3. Vapor Fortification. Use vapor fortification for volatile organic analytes following the vapor fortification technique developed by Alan Hewitt of USACE Cold Regions Research and Engineering Laboratory (CRREL) in Hanover, New Hampshire. Steps in this technique include:

- Place ampules of preweighed, homogenized soils in a sealed desiccator with desiccant for a minimum of three days.
- Transfer soils to another desiccator containing fortification solution of target analytes.
- Allow the soils equilibrate with the vapor of analytes for a minimum of 14 days.
- Remove the ampules and flame seal them quickly.
- Use vapor-fortified PE samples by breaking open the ampule in a purge cell containing distilled water, appropriate surrogates, and internal standards.

4.4.3. Real-World PE Samples. Guidelines for the collection, preparation, and fortification of real-world PE samples are outlined in this section.

4.4.3.1. Collection. Real-world environmental materials that have various contaminants of concern at significant concentrations can be prepared, certified, and used as PE samples. Due to stability concern, only soils or sediments are usually used for preparation of real-world PE samples. Collect materials using these procedures:

- Locate candidate contaminated sites through solicitation or project documents.
- Collect samples and analyze to verify the location and extent of contamination prior to collection of a large quantity of materials.
- Avoid environmental materials that contain numerous analytes at low concentrations because they may cause problems with the assessment of laboratory performance.

4.4.3.2. Preparation. The preparation process for real-world PE samples is similar to that of clean soil PE samples. Procedures are listed below:

- Remove extraneous materials such as rocks, sticks, etc.
- Dry, grind, and mix materials with mills or grinders to a powdery consistency with fine particle sizes. The maximum allowable particle size depends on the maximum allowable subsampling error and subsample size (See Appendix D). In general, soil materials sieved through No. 50 mesh (# 300 microns) have adequate particle sizes for most environmental analyses including mercury analysis, which requires a sample size of 0.2 grams.

4.4.3.3. Fortification. The content of real-world PE samples can be modified by fortification with target analytes or by dilution with clean matrices. The fortification techniques previously described may be used. (See Section 4.4.2.3.3.) After fortification or dilution, additional grinding and blending steps are necessary to ensure the homogeneity of the final products.

4.4.4. Commercially Available PE Samples. Commercially available PE samples may also be used for proficiency testing. Guidelines for use include:

- Use commercial PE samples after evaluation and approval by the Program Manager. This evaluation is based on national or international standards and requirements issued by ISO, ASTM, and USEPA. The evaluation covers manufacturers' preparation and certification of PE samples.
- Inform laboratories that they must explicitly follow manufacturers' instructions.
- Use only certified reference materials (CRM). NIST is the most widely used supplier of CRM. However, using NIST values for solid materials may sometimes lead to comparison errors on data obtained with USEPA organic and inorganic sample preparation methods. NIST expresses CRM values as total concentrations, but USEPA methods use values based upon extractable or leachable concentrations. Because of this, certified NIST values for solid CRM may not be used directly to determine acceptance limits of PE samples. However, NIST values do not pose a problem with analysis of water PE samples, where extractable or leachable concentrations approximate NIST certified total concentrations.
- Base acceptance criteria on extractable or leachable concentrations.

4.5. Handling and Storage of PE Samples.

Guidelines for storage of fortified and real-world PE samples are given in this section as well as packing and shipping considerations.

4.5.1. Fortified PE Samples. All PE samples should be handled and stored with extreme care to ensure stability, integrity, purity, and authenticity. Follow these storage guidelines:

- Preserve and store PE samples with short shelf-lives (most fortified PE samples) like normal field samples. (Most of these samples are prepared on as needed basis.)
- Use the same sample containers required by the analytical methods for storage. Containers are selected for their inertness to the contents and their ability to prevent sample loss.
- Store PE samples for organic analyses in amber glass containers to avoid leaching of plasticizers from plastic containers or loss of target analytes due to absorption by plastic containers. Amber glass is recommended since some analytes are light sensitive.
- Use plastic bottles for metals to avoid leaching of trace metallic impurities from glass containers. Phenolic caps should not be used to avoid potential sample contaminations.
- Do not group analytes requiring different preservatives together in the same sample container.

- Fill the bottles for volatile organic samples completely to prevent loss of volatiles.

4.5.2. Real-World PE Samples. Although real-world PE samples used by USACE are generally very stable with long shelf-lives, the homogenized soils should be stored in a cool, dark, and dry place to retard degradation. Any inert and hermetically sealable containers are acceptable for storage. If needed, remove influences of laboratory humidity by conditioning the homogenized soils with calcium sulfate desiccant.

4.5.3. Packing and Shipping. Follow these guidelines for packing and shipping to ensure the integrity and quality of the PE samples:

- Use appropriate packaging materials, documentation, and shipping labels for all PE samples according to USACE, USEPA, and DOT regulations and guidelines.
- Use special precautions on labeling and packing double blind PE samples make them indistinguishable from regular field samples. Use packaging and containers identical to those used by field personnel sending the same types of field samples.
- Ship PE samples with short holding times immediately after preparation to allow adequate time for the contract laboratory to prepare and analyze them.
- Use overnight delivery services for shipping of all PE samples.