INSTRUCTIONS FOR FIELD USE OF SPIKE SOLUTIONS FOR ORGANIC-ANALYTE SAMPLES 5.3.2

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A matrix spike is a type of quality-control sample used to evaluate the effects of sample matrices on the performance of an analytical method. Matrix spikes are used most often for quality control of organic-analyte samples because the analytical methods for organic-analyte samples involve extraction and analysis steps that can be affected by other chemicals in the sample (referred to as the sample matrix). For example, naturally occurring organic matter can be co-extracted with the organic analytes in the sample and interfere with the gas chromatographic analysis. For some methods, extreme levels of pH, ionic strength, or the concentration of naturally occurring organic matter in the sample enhance the detector response, resulting in biased results for the analyte concentration.

Matrix spikes can be prepared at the sampling site or in the laboratory. If spiked at the sampling site, matrix spikes are called **field matrix spikes**. If spiked at the USGS National Water-Quality Laboratory (NWQL), matrix spikes are called **laboratory matrix spikes**. Field matrix spikes are used to monitor the stability of the organic analytes in the sample bottles, from the time of collection at the site to the time of extraction and analysis at the laboratory, in addition to determining potential bias from the sample matrix. Organic analytes can be degraded by microorganisms in the sample, or undergo chemical reactions such as oxidation or hydrolysis. Many organic-analyte sampling methods do not use a preservative to prevent sample degradation and loss because the preservatives can negatively affect some (although not all) of the analytes. Instead, samples are shipped to the laboratory and extracted within a short time period. This time period is called the sample holding time, and is determined by the laboratory to represent a reasonable time for samples to be shipped and stored until analysis, with minimal change in analyte concentration. **Field matrix spikes can be used to verify that the sample holding times were sufficient for the sites sampled during the course of a project.**

This section of NFM5 provides detailed instructions for using organic-analyte spike solutions that meet the criteria for NWQL methods as part of the USGS quality-assurance and quality-control (QA/QC) protocol for incorporating field spikes into water-quality investigations. Information obtained from the analysis of matrix-spike samples can be used to determine the recovery, bias, and variability of analytes based on sampling conditions encountered at specific sites. Field spiking is not designed to replace routine laboratory QC programs or the Organic Blind Sample Project that currently is administered by the USGS Branch of Quality Systems (http://bqs.usgs.gov/obsp/). Although field spiking is a useful component of quality assurance for all laboratories used by the USGS, the instructions in this document were developed specifically for the analytical methods used at the NWQL and for the spike solutions provided by the NWQL through One-Stop Shopping (the NWQL-supported national field supply service (NFSS) for USGS employees). The number and type of matrix spike samples collected depend on the data-quality objectives and the requirements of individual projects. National programs, such as the National Water-Quality Assessment (NAWQA) Program, might have additional requirements.

- Laboratory matrix spikes provide information about bias from sample matrices.
- Field matrix spikes (samples that are spiked at the field site) provide information about bias from degradation during shipping and storage, in addition to bias from the sample matrices.

Field staff might collect a combination of a laboratory spike (for water samples only; not for solid phase extraction cartridges shipped to the laboratory), a field spike, and a field-spike replicate to provide the most information relating to the performance of the overall method. The relative percent difference between the duplicate spiked-sample results is calculated and used to assess variability at the spike concentration. Some projects may determine that only laboratory spikes, or only one field spike, will provide sufficient data for the analytes of interest. **In all cases, field staff must collect a regular (unspiked) sample along with the spiked sample(s) to determine the ambient levels of any organic analytes in the samples.**

5.3.2.A Supplies and Materials

Be sure to procure the equipment and supplies required for sample matrix spiking before the sampling trip.

Spike kits can be assembled from components listed in table 5.3.2-1 and can be obtained from One-Stop Shopping or from commercial laboratory-supply vendors. The micropipette and Teflon® squeeze bottle can be re-used. **Matrix spike solutions and glass capillaries are used only once.** About 30 glass capillaries are contained in a N1300 kit, enough to perform 30 spikes. The composition of each spike solution is described in separate Certificates of Analysis, available to USGS employees by laboratory schedule from the NWQL Intranet Web site (*click on "Technical Information" and scroll to the link "Organic Spiking Solutions"*).

Spike solutions are prepared and distributed by the NWQL (either through USGS One-Stop Shopping or by special request to NWQL LabHelp for those schedules not listed in One-Stop Shopping). Solutions in a 500-microliter (µL) methanol or ethyl acetate solution are usually supplied in flame-sealed, 2-milliliter (mL) amber glass ampoules, although in some cases screw-cap 2-mL vials are used. For most analytical schedules, concentrations of the spike solutions are designed to provide 0.1 microgram per liter (µg/L) of each analyte in a water sample by injecting 100 µL of the spike solution into a 1-L filtered water sample.

- **Use the spike solutions as soon as possible after opening to prevent changes in concentration caused by evaporation of the solvent.**
- **Do not save an unused portion of the spike solution for re-use** (*see section 5.3.2.F on disposal of used spiking materials).*
- **Keep matrix-spike ampoules chilled at less than (<) 6°C at all times—even in the field.** Many of the compounds included in the spike solutions are unstable and can degrade rapidly at room temperature. **Store the ampoules in a freezer (preferred) or refrigerator, and on ice in a cooler during the site visit.**

CAUTION: Perform spiking in a well-ventilated area to avoid inhaling vapors from the spike solution, and wear gloves and other protective gear to avoid contact with skin and eyes. Organic-analyte spike solutions contain toxic compounds that are either known to cause or are suspected of causing cancer and other diseases.

Table 5.3.2–1. Equipment and Supplies for Preparing Matrix Spikes for Organic-Analyte Samples.

[µL, microliter; mL, milliliter; oz, ounce; na, not available from One-Stop Shopping—purchase on the open market]

One-Stop Item Number	Description
N1370	Pipette, fixed volume, 100 µL (also called Microdispenser)
N1300	Kit, glass capillaries, baked/replacement, 100 µL (kit contains 30 capillaries)
N1124	Bottle, polyethylene, natural, 500 mL/16 oz, wide-mouth, with cap, sold individually (used for waste)
Disposable Gloves	
Q520FLD	Glove, Softwear®, white, nitrile, small
Q522FLD	Glove, Softwear, white, nitrile, medium
Q524FLD	Glove, Softwear, white, nitrile, large
Q526FLD	Glove, Softwear, white, nitrile, extra large
Organic-Analyte Spike Solutions for National Water Quality Laboratory (NWQL) Analytical Schedules	
N1420	Field spike solution, schedules 2003, 2032, 2033
N1430	Field spike solution, schedules 1433 and 4433
N1470	Field spike solution, schedules 2001 and 2010
N1490	Field spike solution, schedule 2060
N1510	Surrogate solution, schedule 2010
Miscellaneous Equipment/Supplies	
na	Teflon® squeeze bottle, 250-mL (for dispensing methanol rinse solvent)
na	American Chemical Society (ACS) pesticide-grade (high purity) methanol
na	Department of Transportation (DOT) Exemption packaging for hazardous waste shipping to contain waste material generated at a field site; for example, see http://hazplus.com/.
na	Breakers for removing ampoule tips

5.3.2.B Unpacking Equipment and Spike Preparations

Spike solutions should be placed into chilled storage promptly after arrival from the NWQL (for example, in a refrigerator or on ice in a cooler). Glass ampoules containing spike solutions can be chilled, stored, and transported to the field in their original containers; however, if the original containers are too bulky, glass ampoules can be placed inside of a zip-top plastic storage bag cushioned by foam or bubble wrap. When unpacking glass ampoules, work over a tray, shallow box, or similar surface to minimize the risk of ampoules breaking or rolling off of a countertop.

The spike solutions are assigned a 5-digit National Water Information System (NWIS) lot number for tracing the contents and concentrations of the spike solutions. The lot number, analytical schedule, and expiration date are listed on the ampoule label. Verify that the correct product was shipped, and check the expiration date before use. One current (2009) analytical schedule (Schedule 2060) has a spike solution that is shipped in two glass ampoules (an acid and a base fraction); both ampoules are used to prepare a spike for Schedule 2060.

The glass capillaries used to transfer the spike solution should be stored inside their sealed container (40-mL glass vial with screw cap), and the micropipette should be stored in its padded box inside doubled zip-top plastic storage bags or a dedicated storage container. The Teflon tip of the micropipette should be protected from contamination with aluminum foil or a glass vial. Spiking equipment should be stored away from extreme temperatures or acid vapors, which can lead to degradation of the rubber O-ring in the pipette.

A freezer is recommended for long-term storage of spike solutions.

5.3.2.C Sample Coding and Bottle Labeling

Field staff may wish to collect additional (replicate) spikes or laboratory spikes. If so, replicates must be clearly labeled to minimize confusion during login, and to prevent respiking of the samples in the laboratory. Identify on the bottle labels the field-spike samples and field-spike replicate samples as "FS" and "FSR," and the sample to be spiked in the laboratory as "LS."

Place the laboratory-spike ampoule into the sturdy plastic vial that is supplied from the laboratory and secure it with a rubber band (do not use tape) either to the container of the corresponding sample or in the protective foam sleeve with the sample bottle.

Laboratory Login and Analytical Services Request Form 5.3.2.D

Review of data entered into the Laboratory Information Management System (LIMS) enables the NWQL to monitor results of the spiking program.

Use the following procedures to fill out the laboratory analytical services request form:

1. For both field and laboratory spikes, enter "1" for "Sample Type" and either "WSQ" (surface water) or "WGQ" (ground water) for "Sample Medium."
2. In the fields for "Parameter Code (P Code)" and "Value" enter parameter code "99106" and a value of either "10.00" for field spikes or "20.00" for laboratory spikes.
3. In the fields for "Parameter Code (P Code)" and "Value" enter parameter "99104" (Reference Material or Spike Source, Code Number) and the 5-digit NWIS-I lot number—located on the vial label—in order to track lot numbers in the NWIS-I database. The value for NWQL for parameter "99103" is "10."
4. Under "Comments" indicate whether the sample was spiked in the field or is to be spiked at the laboratory.

The additional NWIS/QWDATA coding required to completely identify spiked samples for the NAWQA Program is provided in the NAWQA water-quality sample-coding outline, found on the NAWQA Intranet Web page under "Field Technical Support" (*click on "SWQA Site and Sample Coding"*).

Laboratory spikes and field spikes require additional, separate Analytical Services Request (login) forms. Because the database currently in use at the NWQL does not allow for more than one sample with the same combination of schedule, station ID, date, and time, it is necessary to vary at least one of these fields (for example, the time) within a set of samples. This usually is done by adding one minute to the time of collection designated for each of the spikes.

Whenever samples are collected to be field and (or) laboratory spiked it is important to collect an unspiked environmental sample for analysis to determine the ambient concentrations of any organic analytes that might be present. This sample should be coded as a regular sample, and the parameter code "99111" can be used to indicate "QC sample associated with this environmental sample."

5.3.2.E Field Spiking Instructions

Use the following procedure when preparing a field-matrix spike sample:

1. Put on disposable gloves. Wearing gloves is a reminder to not handle the glass capillary tip, and will protect hands from accidental spills of methanol or spiking solutions.
2. Wear protective eyewear to protect eyes from accidental splashes of methanol, spike solutions, or shards of broken glass.
3. Loosen (but do not remove) the black knurled nut (the collet) on the micropipette (fig. 5.3.2–1). The nut compresses a rubber O-ring that grips the glass capillary.
4. Position the orange Teflon tip of the micropipette plunger over the wide-mouth polyethylene jar that is being used as a waste container and rinse the tip with a few drops of methanol from the squeeze bottle. This will lubricate the tip so it will slide through the glass capillary more easily. Gently shake off any residual methanol left on the tip. Be careful not to drip methanol on the O-ring, as the methanol could cause corroding.
   - Remove the cap from the vial containing the precleaned glass capillaries. The glass capillaries have colored bands inscribed on the outside of the glass—thin alignment bands and a wider insertion band.
   - The alignment bands are used to check the position of the Teflon tip inside the glass capillary, while the wider insertion band is used to indicate that the end of the capillary has been fire polished to prevent damage to the Teflon tips when inserted into the glass capillary. The capillaries are shipped as a kit in a 40-mL glass vial with their wide band near the top (open) end of vial.

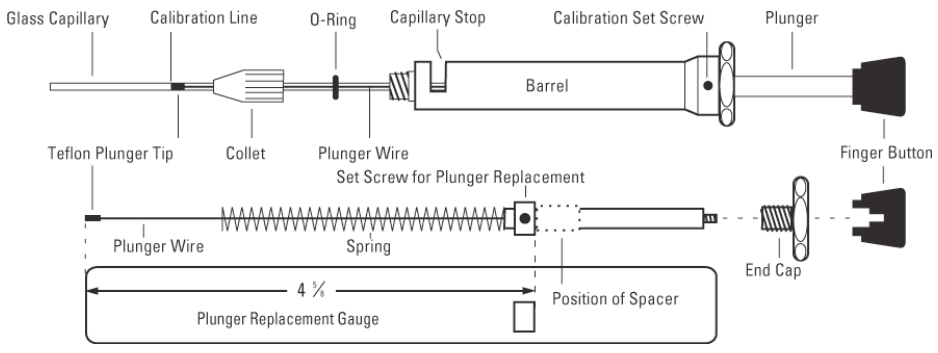

Figure 5.3.2–1. Diagram of a Drummond fixed-volume micropipette and available replacement parts. (Diagram provided by Drummond Scientific Company and published with permission.)

CAUTION: Organic-analyte spike solutions contain toxic compounds. Perform spiking in a well-ventilated area; avoid inhaling the fumes, and wear gloves at all times. Take adequate precautions to prevent contact with eyes and skin.

5. Insert the orange Teflon tip of the plunger into a glass capillary, wide alignment band first. The Teflon tip of the plunger wire can be partially inserted into a capillary while the tip is in the vial. This ensures that the Teflon tip always is inserted into the polished end of the glass capillary and minimizes handling of the glass capillary, which might contaminate the capillary (and the sample).

6. Remove the attached glass capillary from the vial and continue to slip the capillary through the O-ring and black nut until it rests against the stop on the barrel of the micropipette. When the capillary is properly seated, the lower alignment band will be adjacent to the end of the Teflon tip. The volume inside the capillary is 100 µL.

7. Tighten the nut firmly. Press the button of the micropipette and verify that the orange Teflon tip of the plunger slides through the glass capillary and exits out the end. If the glass capillary is not seated properly, the plunger tip will not exit the end of the glass capillary.

8. Place the micropipette on a clean aluminum foil-covered surface while the spike solution ampoule/vial is opened. Be careful to prevent contamination of the glass capillary.

9. Loosen the cap on the sample bottle.

10. Break open the ampoule containing the spike solution (or remove the screw-cap from the vial). As the ampoule is snapped open, aim the break away from the face to avoid breathing any vapors expelled. **RECOMMENDED: As a safety measure, use ampoule-tip "breakers"; these inexpensive tools are readily available and can be purchased from laboratory and medical supply vendors.**

11. Depress fully the finger button on the end of the micropipette and then immerse the capillary tip into the spike solution.

12. Release the finger button slowly as the micropipette is filled.

13. Withdraw the capillary from the spike solution. Make sure the capillary is full and that no droplets are retained on the outside of the capillary. **If the capillary is not full, return the capillary tip to the spike solution and repeat the capillary filling (steps 11 and 12 above).**

14. Remove the sample bottle cap and immerse the capillary tip into the sample, with the tip about 1 to 2 centimeters (cm) below the surface of the sample.

15. Expel the spike solution by rapidly depressing the finger button.

16. After dispensing the spike solution, with the button still depressed, withdraw the tip from the sample and touch the tip to the side of the bottle to allow any water drops to drain into the sample.

17. Replace and securely tighten the cap on the sample bottle; swirl the sample bottle to thoroughly mix.

18. Loosen the black nut one-half turn and remove the capillary from the micropipette.

19. Place the used capillary in a wide-mouth polyethylene bottle labeled for waste disposal.

20. Rinse the Teflon tip of the plunger with methanol to prepare for the next spike. Position the orange Teflon tip of the micropipette plunger over the wide-mouth polyethylene jar (the waste container) and rinse with a stream of methanol from the Teflon squeeze bottle. Be careful to prevent methanol from contacting the rubber O-ring on the micropipette plunger.

21. Use a new glass capillary for each spike. If spiking more than one matrix sample, start again with Step 3 and repeat the same process using 100 µL of the spike solution for the second water sample bottle. If a spike solution is contained in two glass ampoules (for example, schedule 2060), start again with Step 3, using the second ampoule for the same water sample bottle.
22. Place used ampoules/vials and any unused spike solution into a wide-mouth polyethylene waste bottle that is labeled for disposal. Ampoules contain toxic solutions that must be disposed of properly. Recommended: Place the polyethylene waste bottle inside a Hazardous Waste shipping container for safe transport of the waste material from the field site to the Science Center.
23. The spike solutions must be used at one site only. The solutions are not meant to be re-used after the ampoule has been opened.

5.3.2.F Disposal of Used Spiking Materials

When the project or field work has been completed, the field staff must carefully dispose of all waste materials in accordance with current local area or USGS Science Center guidelines for disposal of hazardous materials. Used spiking materials cannot be returned to the NWQL for disposal. For USGS employees, the NWQL Rapi-Note 03-043 provides additional information.

5.3.2.G Shipping Reminders

Field personnel are reminded to:

- Ship spiked samples to the laboratory so they arrive within the recommended holding time. USGS personnel can access holding-time information through the Intranet page of the NWQL by clicking on “Technical Information” and the “Holding time Table.”
- Ship samples on ice to maintain the sample temperature at < 6°C.