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## **TECHNICAL OPERATING PROCEDURE**

### **PROCEDURE TITLE:**

Procedures for Conducting Liquid Chromatographic Analysis for TFM Active Ingredient

### **APPLICABILITY:**

Procedure applies to high performance liquid chromatographic (HPLC) analyses conducted in conjunction with lampricide treatments or toxicity tests.

### **PRINCIPLE:**

The waters of streams which receive applications of TFM are analyzed to provide information for adjustment of lampricide concentrations and to measure the extent of lampricide degradation following an application. Applications are controlled to assure the efficacy and safety of treatments. Concentrations of TFM are occasionally measured with a Waters Corporation HPLC system (IOP:015.x). Analyses for TFM conducted during toxicity tests utilize similar procedures.

### **SAMPLE COLLECTION AND PRESERVATION:**

Samples of water containing lampricides are collected by hand or by automatic water sampler (IOP:002.x and IOP:003.x). Water samples are usually analyzed immediately, but if storage is necessary the tubes and bottles are properly labeled and refrigerated. Subsequent analysis is completed as soon as possible.

### **EQUIPMENT REQUIRED:**

Sample collection

Sampling device for collection of mid-stream water samples

Sigma or ISCO brand automatic water sampler

Analysis for TFM

HPLC; Waters Corporation system  
Disposable glass screw-top culture tubes 16X100 mm  
Syringe (250 uL) and appropriate glassware for formulation of standard (TOP:019.x).

**POTENTIAL INTERFERENCES:**

Sampling must be done in culture tubes with Teflon-lined caps. Rubber-lined caps may introduce a co-eluting peak in the chromatogram.  
See IOP:015.x for additional information.

**SAFETY:**

Standard laboratory safety procedures are followed when handling reagents, otherwise no special precautions are required.

**DISPOSAL:**

Wastes from the HPLC are collected in a 4-L glass bottle. When filled, the bottle is capped and stored in the chemical storage locker until transported for disposal. This is considered hazardous waste.

**REAGENTS:**

Methanol, HPLC grade  
Distilled water, HPLC grade  
Acetic acid, glacial  
Sodium acetate  
TFM standard

**PROCEDURES:**

- I. Preparation of TFM standards: For preparation of standards used for all applications of analytical procedure see TOP:019.x or IOP:015.x.
- II. Preparation of instruments and essential equipment:
  - A. HPLC; Waters system
    1. Prepare carrier solution according to procedure in IOP:015.x. For TFM analysis begin with a ratio of 330 mLs 0.01 Molar buffer solution: 670 mLs methanol.
    2. Turn on system according to IOP:015.x. The initial flow of carrier solution should be set at 1.0 mL/min.
    3. Inject a water blank to determine background absorbance.
    4. Inject samples of standard to determine retention time. The retention time of TFM is less than that of niclosamide. The operating conditions of the HPLC must be adjusted to separate TFM from other compounds while maintaining peak shape and integrity. This may be difficult in certain natural waters, particularly those that contain high concentrations of humic acids. Adjust flow and carrier ratio to optimize separation.

- III. Sampling (see TOP:018x)
- IV. Conducting analyses for TFM (see IOP:015x)
- V. Adjustment of application rates
  - A. Primary applications
    - 1. Primary applications have no lampricide upstream of the application point, so a single analysis point is required to determine the concentration of lampricide that is being added to the stream.
    - 2. Calculations of new application rates are based on equations in Appendix J.
    - 3. The new rate is given to the applicator and the change is noted on the LAMPRICIDE ANALYSIS data form (Appendix M).
    - 4. Additional adjustments to the application rate are not made until sufficient time has elapsed for the change in application rate to appear as a stabilized change in concentration of TFM.
  - B. Maintenance applications
    - 1. Maintenance applications have lampricide downstream and usually upstream of the application, so samples are analyzed from two sites to determine the concentration of lampricide that the application is adding to the stream. Downstream analysis should be a spread of 3 or more samples to ensure uniform application of TFM.
    - 2. Calculations of new application rates are based on equations in Appendix J.
    - 3. The new rate is given to the applicator and the change is noted on the LAMPRICIDE ANALYSIS data form (Appendix M).
    - 4. Additional adjustments to the application rate are not made until sufficient time has elapsed for the change in application rate to appear as a stabilized change in concentration of TFM.
    - 5. The concentration reaching the application point is usually changing, so samples are collected at a minimal time interval to provide rapid updates of application rates. The minimum interval may be limited by the time necessary to conduct an application rate change.
- VI. Documentation and reporting
  - A. The method detection limit and limit of quantitation limit for the measurement of TFM concentrations are 2.4 ng/mL and 7.5 ng/mL, respectively (Hubert and Johnson, 2000).
  - B. Quality assurance steps
    - 1. Standards: see TOP:019.x

2. Instrument: complete entry in log book
- C. Data records
1. Stream treatments and analyses of municipal water supplies; concentrations of TFM are documented on the LAMPRICIDE ANALYSIS data form (Appendix M).
  2. Toxicity tests
    - a. Concentrations of TFM are recorded on the ANALYSIS data sheet (TOP:010.x).
    - b. Mean concentrations are calculated and recorded on the TOXICITY TEST REPORT data sheet (TOP:010.x).
  3. Other analyses (research assistance, degradation analysis)
    - a. Data are recorded in a convenient, usable form.
    - b. Data are retained with reports which result from or are based on the data.
    - c. Either of the forms used for analyses during stream treatments or toxicity tests may be used.

**REFERENCE:**

Determination of TFM residues in sediment and water collected from the mouth and littoral zone of the Milakokia River (Schoolcraft and Mackinac counties, Michigan) during and following a lampricide application. 2000. Project Completion Report submitted to the Great Lakes Fisher Commission. (Unpublished) 19 p.

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This procedure has been reviewed and approved by the undersigned representatives of the U.S. Fish and Wildlife Service and Fisheries and Oceans Canada.

REVIEWED/APPROVED \_\_\_\_\_ DATE \_\_\_\_\_  
Field Supervisor (U.S.)

REVIEWED/APPROVED \_\_\_\_\_ DATE \_\_\_\_\_  
Program Manager (Canada)

Revision No.	Date	Person(s) Responsible	Description
31	2/1/2021	Benson Solomon, Stephen Smith, Barry Scotland	Added 3 sample spread to ensure equal distribution in stream