

## STANDARD OPERATING PROCEDURES LABORATORY

**PROCEDURE TITLE:** TFM field standard concentration determination using the HPLC (high performance liquid chromatography) method and Waters  $\mu$ Bondapak Column

**SCOPE:** Describes HPLC methods to determine concentrations of TFM in water

### PROCEDURE:

- A. Equipment:
1. Waters HPLC system
  2. Appropriately sized screw cap vials
  3. Waters  $\mu$ Bondapak C-18 reverse phase column (150 x 3.9mm, 10  $\mu$ m)
  4. Inline filter and/or guard column filter
  5. Filtration system for filtering lake water, if used
- B. Reagents:
1. Mobile phase A: HPLC water (747 mL), HPLC methanol (250 mL), ammonium acetate (770 mg), acetic acid (3.0 mL)
  2. Mobile phase B: HPLC methanol (997 mL), ammonium acetate (770 mg), acetic acid (3.0 mL)
- C. Prepare TFM Standards:
1. Prepare ~1000 mg/L TFM stock solution using SOP No. LAB 423.0
  2. Using the TFM stock solution prepare at least 3 working TFM standards using either filtered lake water or buffered HPLC grade water as the diluent. The concentrations of the TFM standards should bracket the TFM samples to be analyzed.

Calculation of working TFM standards:

$$\text{mg/L TFM} = \frac{(\text{mL stock soln.})(\text{mg/L stock soln.})}{100 \text{ mL}}$$

- D. Method
- :
1. Inject blank, standards and samples using following parameters:
    - a) Isocratic flow 1.5 mL/min (1.0 mL/min mobile phase A

and 0.5 mL/min mobile phase B)

- b) 10 uL injection
- c) 3-minute run time, retention time ~2 minutes
- d) 300 nm
- e) Adjust flow rate and mobile phase ratio for best peak shape and separation
- f) Choose one sample for triplicate injection to demonstrate repeatability.
- g) Use Waters software to analyze results
- h) Standards must have a correlation coefficient  $> 0.995$
- i) Report mean, standard deviation, %RSD and 95% confidence interval for the triplicate injection
- j) Record calibration results and operating procedures in log book
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