Hammond Bay Biological Station
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SOP No. LAB 303.8
Date: 26 February 2019
Replaces: 16 November 2017
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STANDARD OPERATING PROCEDURES
LABORATORY

PROCEDURE TITLE: Niclosamide determination using the HPLC (high performance liquid chromatography) method and Waters µBondapak Column

SCOPE: Describes HPLC methods to determine concentrations of Niclosamide in water

PROCEDURE:

A. Equipment:
1. Waters HPLC system
2. Appropriately sized screw cap vials
3. Waters µBondapak C-18 reverse phase column (150 x 3.9 mm, 150 µm)
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 Niclosamide Standards:
1. Prepare   ~100 mg/L Niclosamide stock solution using N, N-dimethylformamide (SOP No. LAB 423.0)
2. Using this stock solution prepare at least 3 working standards using either filtered lake water or buffered HPLC grade water as the diluent. The concentrations of the Niclosamide standards should bracket the concentration of samples to be analyzed.

Calculation of working Niclosamide standards:

$$\frac{mg}{L} Niclosamide = \frac{(mL\ stock\ soln.)(\frac{mg}{L}\ stock\ soln.)}{100 mL}$$

D. Method:
1. Inject blank, standards and samples using following parameters:
   a) Isocratic flow 2.0 mL/min (1.5 mL/min mobile phase A and 0.5 mL/min mobile phase B)
   b) 10 uL injection
   c) 4 minute run time, retention time ~2.5 minutes
   d) 330 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