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Rapid Method for Measuring Rotenone in Water at Piscicidal Concentrations

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Abstract

A high-performance liquid chromatography (HPLC) procedure that is rapid, specific, and sensitive (limit of detection <0.005 mg/liter) was developed for monitoring application and degradation rates of rotenone. For analysis, a water sample is buffered to pH 5 and injected through a Sep Pak® C_{18} disposable cartridge. The cartridge adsorbs and retains the rotenone which then can be eluted quantitatively from the cartridge with a small volume of methanol. This step effectively concentrates the sample and provides sample cleanup. The methanol extract is analyzed directly by HPLC on an MCH 10 reverse-phase column; methanol : water (75:25, volume : volume) is the mobile phase and flow rate is 1.5 ml/minute. The rotenone is detected by ultraviolet spectrophotometry at a wavelength of 295 nm.

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Rotenone, the active constituent of derris root, has been used widely as an insecticide and piscicide. Its use for removing undesired fish populations in the United States began in the 1930s (Schnick 1974). Several analytical procedures have been reported for the analysis of rotenone, including colorimetry (Gross and Smith 1934; Goodhue 1936), infrared spectrometry (Delfel 1976), thin-layer chromatography (Delfel and Tallent 1969), gas chromatography (Delfel 1973), and high-performance liquid chromatography (HPLC) (Bushway et al. 1975; Freudenthal and Emmerling 1977; Bowman et al. 1978; Kobayashi et al. 1980). These procedures are either costly, time-consuming, insensitive, or lack specificity for monitoring concentrations of rotenone in water during fish-eradication projects.

We describe a simple, rapid HPLC procedure with a sample-concentrating step that can be used to determine residues of rotenone in water at piscicidal concentrations.

Methods

Apparatus

1. HPLC—Varian 5000 equipped with varichrom ultraviolet-light detector and optional Model CDS-111L data system.[1] Operating conditions:
   stationary phase—30 cm × 4 mm Varian micropak MCH-10 reverse phase;
   mobile phase—methanol : water (75:25, volume : volume);
   flow rate—1.5 ml/minute; chart speed—1 cm/minute;
   wavelength—295 nm;
   attenuation—0.04 absorbance full scale.
2. Sep Pak® C_{18} disposable cartridges from Waters Associates, Incorporated.
3. Vortex stirrer.
4. Disposable syringes (50 ml).
5. Test tubes (15 ml) with Teflon-lined screw caps.

Reagents

1. Methanol—HPLC grade.
2. Water—HPLC grade.
3. Rotenone—purified grade from Aldrich Chemical Company; 0.01 g/100 ml methanol (made fresh daily).
4. Acetic acid (glacial)—American Chemical Society (ACS) reagent grade, 0.2 M; 11.6 ml diluted to 1 liter with water.
5. Sodium acetate—ACS reagent grade, 0.2 M; 2.72 g of $C_2H_3O_2Na \cdot 3H_2O$ diluted to 100 ml with water.
6. Buffer reagent—0.1 M; 14.8 ml of 0.2 M acetic acid + 35.2 ml of 0.2 M sodium acetate diluted to 100 ml with water.

Procedure

1. Precondition Sep Paks with 2 ml methanol and 5 ml water according to instruction sheet supplied by manufacturer.
2. Add 1 ml buffer reagent for each 50 ml of water sample (if expected concentration is less than 0.02 mg/liter, more than 50 ml of sample may have to be extracted).

[1] Mention of commercial products does not imply endorsement by the United States Government.

3. Attach preconditioned Sep Pak to 50-ml syringe with plunger removed.
4. Transfer sample to syringe, insert plunger, and force sample through Sep Pak at a rate of not more than 40 ml/minute. Discard water.
5. Remove Sep Pak, remove plunger, and replace Sep Pak on syringe.
6. Add 2 ml methanol, insert plunger, and slowly force methanol through Sep Pak into test tube.
7. Cap tube and mix on vortex stirrer.
8. Analyze by HPLC against 50 ml of a standard containing a known concentration of rotenone in water solution processed as above.

Results and Discussion

The ultraviolet spectrum of rotenone has an absorption maximum at 295 nm (Fig. 1). A monochromatic detector (254 nm) can be used, but a considerable loss of sensitivity and potential loss of specificity will occur. Performance of monochromatic detectors can be enhanced by the use of 313-nm filters, but a grating monochrometer set at 295 nm provides optimal results.

As indicated in step 1 of the procedure, the Sep Paks must be prerinsed with methanol followed by water before they are used in the analyses. Previous tests (Dawson 1982) indicated that, for best results, this step should not be completed more than 2 hours before an analysis.

Rotenone recovery is influenced by the rate water samples flow through the Sep Paks during extraction and by the elution rate of methanol. The recovery was less than 70% at a flow of 100 ml/minute but exceeded 90% at flows of 40 ml/minute or less. Several volumes of methanol were evaluated for most efficient elution of adsorbed rotenone from Sep Paks. Small volumes of methanol provided more concentrated samples, but recoveries were consistently better when 2 ml or more of methanol were used for elution.

Recoveries of rotenone were evaluated at various pH values to determine whether or not acidity of water samples affected the utility of the method. Water samples were fortified with 0.08 mg/liter of rotenone and buffered to pH 5, 7, and 9 before the Sep Pak extraction. Recoveries of rotenone from the buffered samples

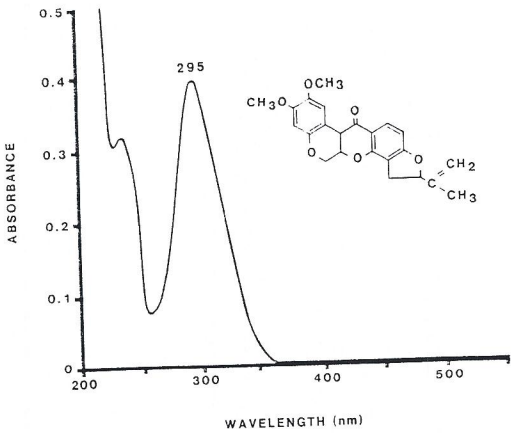

FIGURE 1.—*Chemical structure and ultraviolet spectrum of rotenone (10 mg/liter) in methanol:water (75:25, volume:volume).*

were 98, 94, and 73%, respectively, indicating that acidification is essential for optimal performance of the Sep Paks.

A water sample volume of 50 ml is sufficient for the analysis of rotenone concentrations of 0.02 mg/liter or greater. However, as much as 200 ml of sample may have to be extracted to achieve a sensitivity of 0.005 mg/liter. The limiting factors for greater sample concentration are the tedium of extracting large volumes of sample and possible interferences that may develop in samples as a result of the extraction and concentration of contaminants. Recently, J. T. Baker Chemical Company developed the Baker-10® extraction system, in which similar adsorption chromatography is used and the tedium of analysis is reduced by a vacuum manifold that extracts up to 10 samples simultaneously.

Samples extracted on Sep Paks are stable for only a few hours. However, if the sample is eluted from the Sep Pak and stored in the methanol eluate, the samples are stable for up to 2 days. For best results, samples should be kept cool and in the dark.

Retention time for rotenone from a sample of spiked pond water injected on the reverse-phase column was 5.7 minutes (Fig. 2). Unfortified pond water had no interfering peaks. The pen deflection at 2 minutes in Fig. 2 is the solvent injection peak.

Water samples from ponds treated with rotenone in summer and late fall were analyzed for residues of rotenone by this HPLC method (unpublished data). No interference problems

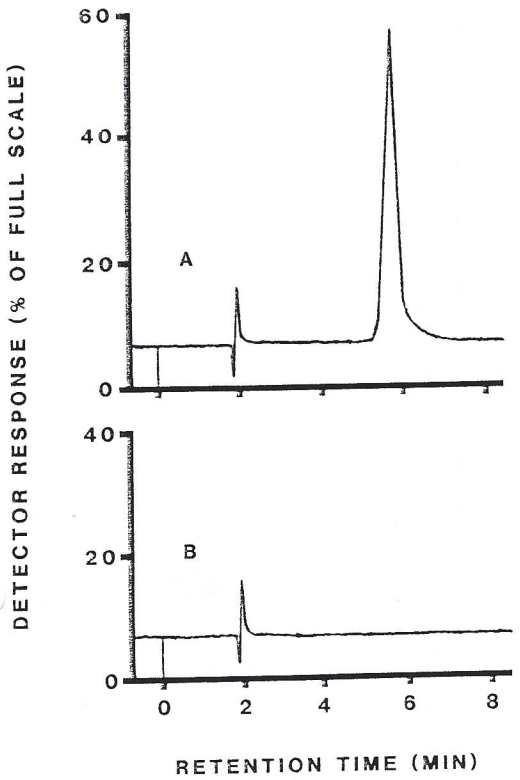

FIGURE 2.—*Liquid chromatograms of (A) pond water sample fortified with rotenone (0.1 mg/liter) and (B) unfortified pond water: mobile phase—methanol : water (75: 25, volume : volume); flow rate—1.5 ml/minute; wavelength—295 nm; attenuation—0.04 absorbance full scale. The water sample (50 ml) was concentrated 25 times on Sep Pak by elution with 2 ml methanol.*

were encountered and the measured concentration agreed closely with that calculated on the basis of the application rate.

Five replicate samples of pond water fortified with rotenone (0.1 mg/liter) were analyzed to evaluate the consistency of the method. The mean percentage of recovery and standard error were 97.6 ± 1.6.

The use of a micro-processor data system, such as a Varian CDS-111L, greatly facilitates the analysis by integrating peak areas and converting values directly into concentration units.

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METHOD ROTE.H2O

ANALYSIS OF WATER SAMPLES FOR ROTENONE BY HPLC (HIGH PERFORMANCE LIQUID CHROMATOGRAPH)

1.0 SCOPE AND APPLICATION

1.1 This procedure is applicable to the analysis of water for rotenone and rotenolone.

2.0 SUMMARY OF METHOD

2.1 This method involves the concentration of the water sample on Sep-Pak C-18 column followed by extraction into methanol and analyzed by HPLC.

2.2 Limits of detection for rotenone and rotenolone is 2 ppb.

3.0 INTERFERENCES

3.1 No interferences are observed with the method.

4.0 APPARATUS AND MATERIALS

4.1 HPLC - Varian 5500 equipped with UV detector, autosampler and integrator.

HPLC conditions:

Column: Varian MCH-5, 4 mm x 15 cm.
Guard column: Varian MCH-5, 4mm x 4cm.
Detector: UV - 295 nm.
Temp.: 25°C.
Mobile phase: Methanol:water (75:25), H_2O contain 1% MeOH.
Flow: 1.5 mL/min.
Pressure: 280 atm. with guard column.
Rotenone RT = 3.8 min.
Run time: 12 min.

4.2 Mixing cylinders: 500 mL, 100 mL, 10 mL.

4.3 Sep Pak C-18 columns, J.T. Baker, #JT-7020-6.

4.4 Vacuum box for Sep-Paks.

4.5 Test tubes, 10 mL with teflon-lined screw caps.

4.6 Gelman filters, 0.45u.

4.7 Pipets: volumetric, 2 mL, 5 mL.

4.8 Pipets: graduated, 1 mL, 10 mL.

4.9 Volumetric flasks, 1 liter, 100 mL.

5.0 REAGENTS

5.1 Methanol - pesticide grade

5.2 Water - HPLC grade

5.3 Acetic acid, reagent grade

5.4 Sodium acetate, JT Baker, #3460-01

5.5 Rotenone standard, Chem Services

5.6 Rotenone standard, Ultra Scientific

6.0 SAMPLE COLLECTION, PRESERVATION, AND HANDLING

6.1 All samples are kept cold at 4°C until analysis.

7.0 PROCEDURE FOR SAMPLE PREPARATION

7.1 Preparation of buffer reagent 0.1M:
14.8 mL of 0.2M acetic acid plus 35.2 mL of 0.2M sodium acetate, dilute to 100 mL with DI H_2O.

Acetic acid, 0.2 M: 11.6 mL/1-liter H_2O

Sodium acetate, 0.2 M: 2.72 g $C_2H_3O_2Na.3H_2O$/100 mL H_2O.

7.2 Add 8 mL of buffer to 400 mL of water sample.

7.3 Precondition Sep-Pak C-18 column with 2 mL of methanol, then 5 mL of H_2O.

7.4 Pass the buffered water sample thru the preconditioned Sep-Pak @ <40 mL per minute.

7.5 Place 10 mL vial in the vacuum box, previously calibrated to 4 mL.

7.6 Elute with 2 x 2 mL MeOH, final volume should be exactly 4 mL.

7.7 Filter the extract thru a 0.45u Gelman filter.

7.8 Analyze with HPLC for rotenone and rotenolone.

8.0 ANALYTICAL PROCEDURE

8.1 Rotenone is detected with HPLC under the conditions described in Apparatus Section 4.1.

8.2 The peak area of the standard rotenone is compared with the peak area of the rotenone in the water sample and the concentration of the rotenone in the water sample is calculated by peak area ratio.

8.3 Calculations:

$$C = \frac{DxGxI}{AxExH}$$

C = Concentration of rotenone in the water sample in ug/L (ppb).
D = Final volume of the sample extract before injection (4 mL).
G = Sample peak area.
I = Weight of standard in picograms.
A = Volume of water sample (400 mL).
E = Sample volume injected in microliters (10 uL).
H = Standard peak area.

9.0 QUALITY CONTROL

9.1 Calibration standards are run with similar concentration range as the samples, should have a 3-point curve.

9.2 Dilute samples if they are out of range of the standard curve.

9.3 Run a mid-point standard between every ten samples.

9.4 Employ one blank per vacuum box batch to determine if contamination or interfering peaks are present.

9.5 Run one reagent spike for every 10 samples when possible or per vacuum box batch.

9.6 Obtain and prepare a second standard designated as "reference standard" using a second chemical supplier. This reference standard should be made up independently of the standard used for calibration and should be analyzed as a blind sample to verify the accuracy of the calibration standards.

10.0 METHOD PERFORMANCE

10.1 Spiked samples at the level of 10 to 100 times the detection limit should give recoveries greater than 80%.

11.0 REFERENCES

11.1 Dawson, V., P. Harmon, D. Schultz, and J. Allen, 1983. Rapid method for measuring rotenone in water at piscicidal concentrations. Trans. Am. Fish. Soc. 112; 725-728.

SOP Section Approval:_____

SOP Final Approval:_____