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Note

High-performance liquid chromatographic analysis of rotenone and rotenonone in water by direct injection

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Rotenone is a naturally occurring insecticide found in many leguminous species of the genera *Derris, Lonchocarpus, Amorpha* and *Tephrosia*[1]. Its use is primarily for house plants, home gardens and to control fish populations. In fish control, it becomes important to be able to monitor residue levels in water both for safety and law enforcement purposes.

Because of rotenone's nonvolatility, the best methods for analysis have been high-performance liquid chromatographic (HPLC). The majority of these HPLC procedures developed thus far have been for rotenone in formulations[2–6]. However, recently two methods have been developed for residue levels of rotenone and its degradation products in animal feed and tissue[1] and water[7]. The method for water[7] appears to be extremely sensitive, but requires a tedious solvent extraction procedure with methylene chloride–hexane.

A technique is described here whereby rotenone and rotenonone (a degradation product of rotenone in water) can be detected at the low ppb* level by direct injection into an HPLC system.

EXPERIMENTAL

Solvents and pesticides

Acetonitrile and water were HPLC grade (Fisher Scientific, Medford, MA, U.S.A.). Tetrahydrofuran (THF) was Fisher Certified grade. Rotenone, 97.3% pure, was obtained from the Environmental Protection Agency, Research Triangle Park, NC, U.S.A. Rotenonone, 99% pure, was a gift from Norman Delfel, United States Department of Agriculture, Peoria, IL, U.S.A.

Water samples

Water samples were of three types: drinking, stream and pond. The drinking water was obtained at the laboratory. Pond water was from two sources in Eastern Maine[1], Mud Pond which is affected by acid rain with a pH 4.0 (ref. 2), and Salmon Pond which is a normal pond with a pH of 5.8. Stream water was from Clifton Stream off Route 9.

* The American billion (10^9) is meant.

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Standard preparation

Stock solutions of rotenone and rotenonone were prepared in THF at concentrations of 1.92 mg/ml and 1.70 mg/ml, respectively. All solutions used for direct analysis were prepared by appropriate dilutions of these stock solutions into 100 ml of the appropriate water sample.

Apparatus

The HPLC system consisted of a 6000 A pump (Waters Assoc., Milford, MA, U.S.A.), a U6K injector (Waters Assoc.), a variable-wavelength UV detector (Schoeffel, Westwood, NJ, U.S.A.) and a Houston Instruments (Austin, TX, U.S.A.) dual pen recorder. The column, 25 cm × 5 mm I.D. was a Zorbax ODS (HPLC Technology, Palos Verdes Estates, CA, U.S.A.).

Operating conditions

LC parameters were: mobile phase, acetonitrile–water (70:30); flow-rate, 1.3 ml/min; column temperature, ambient; wavelength, 210 nm; attenuation, 0.04 a.u.f.s.; chart speed, 0.4 in./min.

Analytical procedure

Water samples as received and spiked were filtered (2 ml) through a 0.45 μm millipore aqueous filter (Waters Assoc.) and injected (200 μl) directly into the HPLC system.

RESULTS AND DISCUSSION

Results from the direct injection of two types of spiked water samples, pond and drinking, are given in Table I. Spiked samples varied in concentration from 15 to 60 ppb for rotenone and 17 to 68 ppb for rotenonone. All water samples were chromatographed without clean-up, concentration or derivatizing steps which makes this procedure very simple and rapid. The precision is fine for rotenone with percent coefficients of variation ranging from 3.17 to 7.58% (Table I). However, the percent coefficients of variation for the rotenonone were much higher, 4.72 to 16.50% (Table

TABLE I

DIRECT ANALYSIS OF ROTENONE AND ROTENONONE

Rotenone			*Rotenonone*		
Water source	*Level spiked (ppb)*	*C.V. (%)**	*Level spiked (ppb)*	*C.V. (%)**	*Blank for both***
Drinking	15	7.58	17	16.50	N.D.
Drinking	30	5.51	34	8.20	N.D.
Drinking	60	3.17	68	12.74	N.D.
Mud Pond	15	5.16	17	12.09	N.D.
Mud Pond	30	5.69	34	4.72	N.D.
Mud Pond	60	6.58	68	7.53	N.D.

* Coefficient of variation (C.V.) based on the mean of five samples analyzed.

** None detected (N.D.) at a limit of 7.5 ppb rotenone and 8.5 ppb rotenonone.

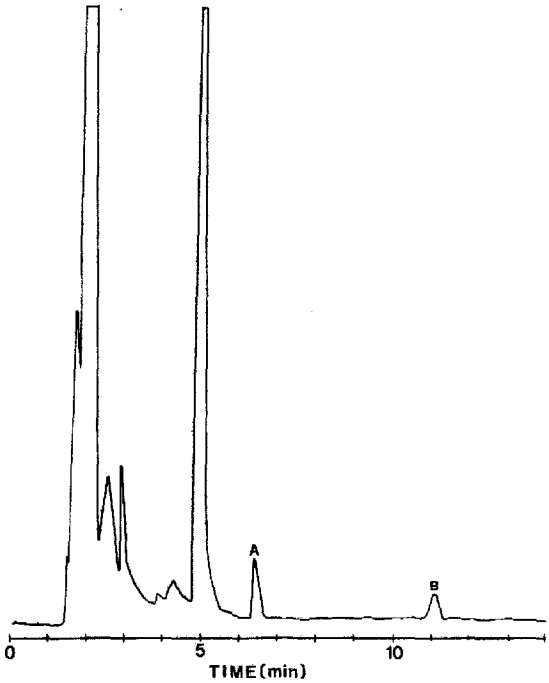

Fig. 1. Liquid chromatogram of 200 μl of Mud Pond water spiked with 30 ppb rotenone (A) and 34 ppb rotenonone (B). For operating conditions see Experimental.

I) indicating a greater variation for rotenonone although not unreasonable for a residue method. One possibility for the rotenonone variation may be its degradation in water.

A typical chromatogram of a pond water sample spiked at the second lowest ppb level is shown in Fig. 1. Analysis time was approximately 11 min with rotenone eluting first. Besides the extensively studied samples of drinking water and Mud Pond, two other samples (Salmon Pond and Clifton Stream) were also spiked at the lowest concentration of each compound. The blanks showed no detectable rotenone or rotenonone while the spiked samples were similar to those in Table I indicating that this method is good for several water sources.

The lowest concentration of each substance detected in this study was 15 ppb for rotenone and 17 ppb for rotenonone, but it should be possible to quantify levels of 7.5 ppb and 8.5 ppb, respectively. With a new type of UV detector, it may be possible to quantify rotenone and rotenonone in the low ppt* by direct injection. McGown[7] using a Beckman UV detector and a solvent concentration step was able to quantify pg amounts of rotenone and rotenonone at 229 nm which is not as sensitive as 210 nm or even 294 nm. Thus, if McGown's results are correct, it should be possible to detect low ppt levels of each substance by direct-injection HPLC.

Both compounds were shown to be linear (peak height *vs.* concentration) over a wide concentration range. Rotenone was observed to be linear from 15 to 300 ppb while rotenonone was linear from 17 to 415 ppb.

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* The American trillion (10^{12}) is meant.

In order to detect and quantify rotenone and rotenonone in water at concentrations lower than those possible by direct injection, trace enrichment using a C_{18} Sep-Pak cartridge was studied. Water spiked with both compounds was passed through a Sep-Pak, but neither compound was retained at an adequate level.

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