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Determination of Rotenone and Rotenonone in Fresh Water by HPLC

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Reversed-phase HPLC and low-wavelength UV absorbance detection are used to determine the botanical insecticide rotenone and a degradation product in fresh water. The method exhibits sufficient sensitivity to allow its use in regulatory analyses and sufficient speed to represent an improvement upon existing methods.

INTRODUCTION

Rotenone is a naturally occurring poison and insecticide that is extracted from derris root. In its commercial form, it is used for scientific fish-gathering (collecting large numbers of fish rapidly), fish population control, and in aerosol insecticides. In the presence of light and air, rotenone (1,2,12,12a-tetrahydro-2-isopropenyl-8,9-dimethoxy-[1]-benzopyrano-[3,4-b]-furo-[2,3-b][1]benzopyran-6[6a,H]-one) degrades to dehydrorotenone and rotenonone (1,2-dihydro-2-isopropenyl-8,9-dimethoxy-[1]-benzopyrano-[3,4-b]-furo-[2,3-b][1]-benzopyran-6,12-dione) among other compounds. Figure 1 illustrates the structures of rotenone and rotenonone. Some concern over unlawful use of the compound for fish-gathering arises from the fact that it is also poisonous to mammals: it inhibits mitochondrial electron transport (1).

Rotenone is oxidized and reacted with thymol (2) in the classical determination by UV/VIS spectrometry, with a reported sensitivity of 0.01 mg. *The United States Environmental Protection Agency Manual of Chemical Methods for Pesticides and Devices* and *The Pesticide Analytical Manual* do not promulgate a residue method (2,3). Bowman, Holder, and Bone reported a reversed-phase residue procedure that they applied to the determination of four rotenoids in animal feed and tissues (4). A method for determination of several rotenoids in formulated pesticides was described by Bushway and Hanks (5). Both of these procedures used wavelengths above 254 nm (295 nm and 280 nm, respectively) for detection, and the multiresidue nature of the approach was emphasized in each method. Fish and game authorities, residue analysts, and public health laboratories, among others, can make use of the method presented here to determine rotenone in water with minimum sample preparation. The method is characterized by speed, good precision along both retention and response axes, and good sensitivity.

EXPERIMENTAL

All solvents used in this study were either pesticide-residue grade or HPLC grade (J.T. Baker Chemical Corp., Phillipsburg, New Jersey). The instrument used for rotenone determinations was a high performance liquid chromatograph model 344 equipped with both model 160 and model 165 detectors. The output from the detector was integrated and displayed on a CR1A recording integrator that was controlled by a model 421 system controller. A 250 mm × 4.6 mm, 5-μm Ultrasphere Octyl column was used (all equipment and columns from Beckman Instruments, Altex Scientific Operations, Berkeley, California). Samples of brackish water were obtained from areas where fish were being collected after rotenone was applied. A 1-L water sample was extracted with three 150-ml portions of 15% methylene chloride in hexane. The mixed solvent was concentrated to about 0.1 ml and replaced with 8 ml of acetonitrile in a modified Kuderna-Danish concentrator (Southern Scientific, Micanopy, Florida). The resulting mixture was subjected to further concentration to remove any remaining hexane or methylene chloride by replacing the macroconcentrator flask and reflux column with a micro-Snyder column and reducing the mixture's volume to about 0.5 ml. A Swinney filter with glass-fiber prefilter and 0.45-μm final filter was prepared by washing it with acetonitrile. The filter was attached to a 5-ml gas-tight glass syringe (Hamilton Co., Reno, Nevada). The concentrate was washed into the syringe with acetonitrile, and the resulting mixture was filtered into a 10-ml volumetric flask. The

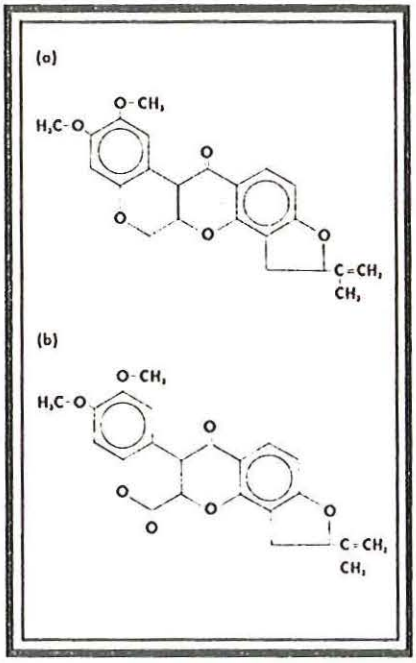

FIGURE 1: Structures of (a) rotenone and (b) rotenonone.

filtration apparatus was rinsed with acetonitrile, and the washings were filtered into the volumetric flask. The filtrate and washings were made up to 10.00 ml and were analyzed without further preparation.

RESULTS AND DISCUSSION

Figure 2 shows a chromatogram of the analytical standards of rotenone (75 pg) and rotenonone (90 pg). The reference mixture must be kept cool, in the dark, and under an inert gas to reduce the rate of oxidation of rotenone to rotenonone. New reference solutions were prepared weekly. Any change in the relative composition of the mixture was regarded as evidence of decomposition of the standard, which was then replaced immediately. Figure 3 illustrates a chromatogram of a water extract that demonstrates considerable oxidation of rotenone. The 5-µl injection was equivalent to 0.5 ml of the original sample. Rotenone was detected at a concentration of 794 pg/ml, and the concentration of rotenonone was 586 pg/ml. It was not possible to calculate the original concentration of rotenone because the rate constant for oxidation is not known, nor was the exact time of application of rotenone to the water.

Authentic blanks (water from the same region, but without detectable quantities of the analytes) fortified with rotenone were carried through the preparation described above. Recovery of rotenone varied from 86% to 102%. The low recoveries were partially attributed to oxidation, but oxidation was significant only if the samples were allowed to sit for more than 8 hr. Approximately 5% of the rotenone was oxidized to the ketone in this period. Purging the fortified water or its extracts with nitrogen retarded oxidation, but refrigeration under nitrogen was found to be the best method of preservation.

Figure 4 shows a typical analytical working curve, optimized for sensitivity and logarithmically compressed, that demonstrates linearity over almost four orders of magnitude. The relative standard deviation of each point is better than ±1%, and the instrumental limit of detection for rotenone is 50 pg.

Substitution of a 2-mm i.d. Ultrasphere Octyl column into an otherwise identical system produced a fivefold decrease in the instrumental limit of detection with a moderate loss of peak symmetry because of detector-cell-volume/flow-rate mismatch. A twofold decrease in instrumental limits of detection was realized by substituting both a 2-mm column and a low-volume flow cell into the system.

A method that allows the determination of residues of two rotenoids has been presented. The method is quick in comparison with other methods reported in the literature and is sensitive enough to allow its use as a residue method.

ACKNOWLEDGMENT

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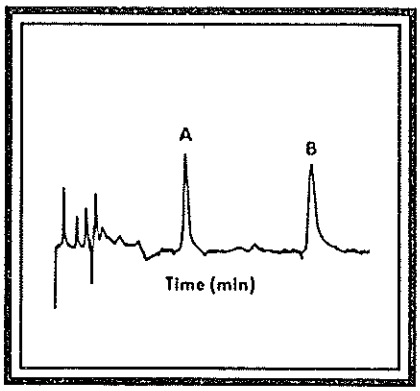

FIGURE 2: Chromatogram of rotenone and rotenonone reference mixture. Column: 250 mm × 4.6 mm, 5-µm Ultrasphere Octyl; mobile phase: acetonitrile/water (65:35); flow rate: 1 ml/min; detection: UV 229 nm (0.005 AUFS); sample size: 5 µl. Peaks: A = rotenone (t_R = 6.26 min), B = rotenonone (t_R = 12.29 min).

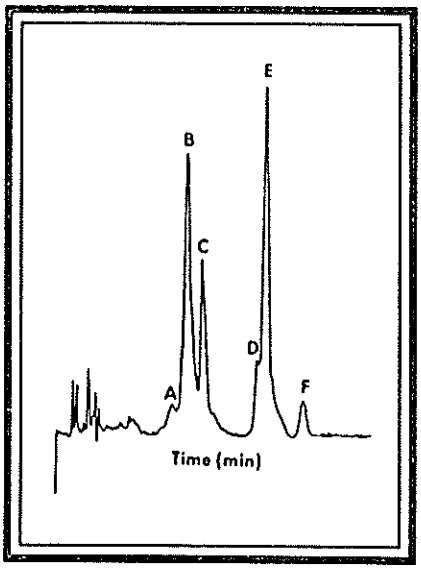

FIGURE 3: Chromatogram of brackish water extract. Chromatographic conditions same as in Figure 2. Peaks: A = unknown coextractable (t_R = 5.5 min), B = rotenone (t_R = 6.18 min), C = unknown coextractable (t_R = 7.03 min), D = unknown coextractable (t_R = 9.65 min), E = unknown coextractable (t_R = 9.98 min), F = rotenonone (t_R = 12.23 min).

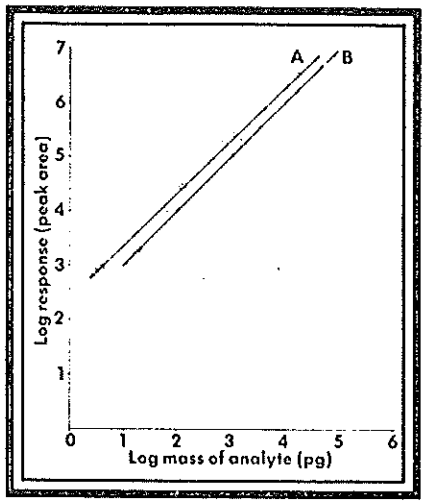

FIGURE 4: Analytical working curves for rotenone and rotenonone determination. A = rotenonone, B = rotenone.

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