PESTICIDE FORMULATIONS

Modification of Liquid Chromatographic Method for Analysis of Rotenone Formulations

RODNEY J. BUSHWAY
University of Maine, Department of Food Science, Orono, ME 04469

Modifications have been made on the official first action liquid chromatographic method for analysis of rotenone formulations. Methanol–water was replaced with acetonitrile–water as the eluant and methanol was substituted for dioxane as the extracting solvent. The extraction procedure has been changed from 1½ h of rotary shaking to 5 min of sonication. The rest of the procedure is identical to the published method. For 9 different products analyzed 6 times each, the percent coefficients of variation were all below 2.40. Five of these 9 samples were previously used in the rotenone collaborative study. A comparison of results from both studies showed that the modifications did not affect the percent rotenone found. It is recommended that these changes be tested in a mini-collaborative study.

In 1982, a reverse phase liquid chromatographic analysis of rotenone formulations was successfully collaborated and adopted official first action (1). Once completed, several collaborators suggested possible ways to improve the method. Also, interferences from naphthalene and sulfur occurred when a pesticide manufacturer tried to use the collaborated method on a formulation mixed with these 2 chemicals. Naphthalene was not resolved from rotenone, and sulfur eluted late, affecting other injections.

Because of the suggested improvements and the formulations problem, an investigation was undertaken to determine if the official LC method for rotenone would be changed to make it better and more versatile. This paper describes these improvements.

METHOD

Apparatus and Reagents

(a) *Liquid chromatograph.*—Waters Associates (Milford, MA) 6000A pump, U6K septumless injector, Schoeffel variable wavelength UV detector (Westwood, NJ), and Omniscribe recorder (Houston Instrument, Austin, TX). Operating conditions: injection volume, 5 μL; flow rate, 1.1 mL/min; wavelength, 280 nm; absorbance range, 0.4 AUFS; recorder setting 10 mV; chart speed, 1.0 cm/min.

(b) *Chromatographic column.*—Partisil 5 ODS-3, 5 μm particle size, stainless steel, 25 cm × 4.6 mm id (Whatman Inc., Clifton, NJ).

(c) *Mobile phase.*—Acetonitrile–water (70 + 30). All solvents LC grade (Fisher Scientific, Fair Lawn, NJ).

(d) *Sample extraction solvent.*—Purified grade methanol (Fisher Scientific).

(e) *Rotenone standard solution.*—Weigh 20 mg 99% rotenone (Penick Co., Lyndhurst, NJ) into actinic 50 mL volumetric flask and dilute to volume with purified grade methanol. Actinic glassware is necessary because rotenone degrades readily in most types of light.

Preparation of Sample

(a) *Dust.*—Weigh sample equivalent to 20 mg rotenone into glass-stopper Erlenmeyer flask. Pipet in 50 mL methanol and sonicate in sonic bath (Bransonic 32, Fisher Scientific) 5 min while swirling gently. Let settle, and filter 5–10 mL aliquot through 0.45 μm filter (Millipore Corp., Bedford, MA). Inject 5 μL into liquid chromatograph.

Received July 7, 1983. Accepted September 13, 1983.

(b) *Liquid.*—Weigh sample equivalent to 20 mg rotenone into glass-stopper Erlenmeyer flask. Pipet in 50 mL methanol and shake to dissolve. Filter through 0.45 μm filter (Millipore Corp.). Inject 5 μL into LC system.

Determination

Inject standard, followed by 2 injections of sample. Finally inject another standard. Measure peak heights, then average and substitute into formula below:

$$\% \text{ Rotenone} = (H/H') \times (W'/W) \times \% \text{ purity of std}$$

where H and H' = average peak heights of sample and standard, respectively; W' = g rotenone standard/50 mL and W = g sample extracted.

Results and Discussion

After the rotenone collaborative study, participating scientists made numerous suggestions as to possible improvements for the procedure. Many of these suggested changes could be incorporated into the collaborative report without further study. However, 2 possible modifications required extensive investigation before they could be adopted into the procedure.

The first suggested change was replacing the solvent system (methanol–water) with acetonitrile–water; such a modification would reduce the back-pressure on the column. High pressure can shorten the normal life span of a column, injector, and pump. Back-pressure decreased from 2300 psi (methanol–water) to 800 psi (acetonitrile–water).

Modifying the extraction procedure was the other major change, resulting, in part, from a mistake by one of the collaborators in which methanol was used for extracting instead of dioxane and, in part, from a radial compression method (2) which was developed using sonication for the extraction technique. These led to an extraction method involving a 5 min sonication step with methanol in place of the 1½ h dioxane procedure.

The effects of these changes on rotenone analysis were evaluated. Preliminary work was performed using a sample previously analyzed by collaborators and found to be the most difficult because of interfering compounds. When the formulation was analyzed using acetonitrile–water, rotenone eluted in 6.7 min while it eluted in 10.2 min with methanol–water. Other compounds were also affected by the solvent changes. Rotenolone, deguelin, and tephrosin are resolved from each other in methanol–water, but tephrosin co-chromatographed with a large peak believed to be an aromatic hydrocarbon which elutes before rotenone. Analysis of this rotenone sample with acetonitrile–water only partially separated rotenolone and tephrosin. The aromatic hydrocarbon and deguelin co-chromatographed, but the hydrocarbon eluted after rotenone. A comparison of percent rotenone values in this formulation agreed well between the solvent systems.

Because of the correlated results obtained from the preliminary investigation with acetonitrile and methanol, a full scale study was performed using 5 samples from the 1982 collaborative study along with 4 others. These 9 samples were

Table 1. Analysis of rotenone formulations

Sample	Formulated, %	Modified method		Collaborative method	
		Found, %	CV, %	Found, %	CV, %
1. Liquid	5.0	4.55*	1.65	4.76*	2.47
2. Dust	1.0	1.00	1.58	0.99	3.19
3. Dust	5.0	4.86	1.96	4.87	2.54
4. Dust	20.0	17.57*	1.16	18.14*	2.62
5. Dust	0.75	0.88	1.68	0.89	3.19
6. Liquid	0.75	0.83	1.98		
7. Dust	0.20	0.18	2.28		
8. Dust	1.0	1.08	1.52		
9. Dust	34.0	38.84	2.39		

*Means are significantly different at the 0.01 level by the *t*-test.

analyzed 6 times each on 3 different days (2 samples per day). The results are given in Table 1. As can be seen, the reproducibility was excellent with this modified procedure. The percent coefficients of variation ranged from 1.16 to 2.39. The samples analyzed using both the official and modified official methods were quite close in agreement. However, 2 samples, 1 and 4, had significantly different means. A mini-collaborative would point out any possible discrepancies.

Sample 7, a louse powder formulated with sulfur and naphthalene, could not be analyzed by the conditions set forth in the collaborative study (Figure 1). Naphthalene was not resolved from rotenone, while sulfur was extracted into the dioxane and eluted 1 h later to cause interferences. However, when the acetonitrile–water system was used in conjunction with methanol for extraction, the naphthalene peak was separated from rotenone, and the sulfur was barely extractable in methanol, causing no problems. There is a small sulfur peak (0.4 cm) that elutes at 31 min.

Rotenone is sometimes formulated with other pesticides such as carbaryl (1-naphthol may be present as a degradation product), folpet, captan, difolatan, methoxychlor, piperonyl butoxide, pyrethrins, naphthalene, sulfur, and other rotenoids (deguelin, tephrosin, rotenolone, and isorotenone). None of these compounds interfered with this modified rotenone analysis.

In conclusion, these modifications of the official first action LC method for rotenone formulations result in a better method because of less pressure on the system, faster analysis time, elimination of peroxides, and ability to analyze formulations containing sulfur and naphthalene. It is suggested that this modified method be tested in a mini-collaborative study.

References

(1) Bushway, R. J. (1983) *J. Assoc. Off. Anal. Chem.* **66**, 796–800
(2) Bushway, R. J. (1983) *J. Assoc. Off. Anal. Chem.* **66**, 793–795

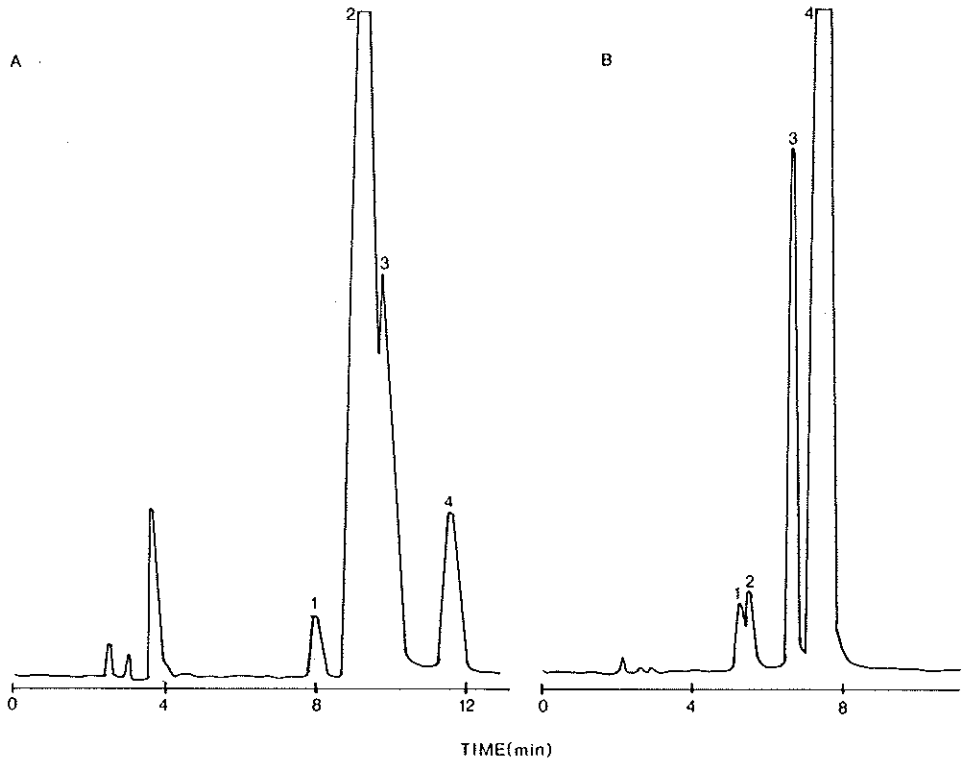

Figure 1. Liquid chromatograms of (A) rotenone formulation 7 chromatographed using methanol–water (70 + 30). Peaks: 1, rotenolone; 2, tephrosin plus naphthalene; 3, rotenone; 4, deguelin. (B) rotenone formulation 7 chromatographed using acetonitrile–water (70 + 30). Peaks: 1, rotenolone; 2, tephrosin; 3, rotenone; 4, deguelin plus naphthalene. Chromatographic conditions described in text.