10.6.30A

AOAC Official Method 998.01
Synthetic Pyrethroids in Agricultural Products

Multiresidue Gas Chromatographic Method

First Action 1998

(Applicable to determination of 8 synthetic pyrethroid pesticides in wheat, oranges, and tomatoes. *See* Table **998.01** for the results of interlaboratory study.)

A. Principle

Fruits and vegetables are extracted with acetone, and grains are extracted with acetonitrile-water. Analytes are partitioned into hexane, evaporated to dryness, and dissolved in hexane. The extract is partitioned with acetonitrile and cleaned up on a deactivated Florisil® column with 6% ethyl ether in hexane. Analyte concentrations are determined by gas chromatography with electron capture detection (GC-ECD) and comparison with calibration standards. The appropriate standard concentration is determined by screening test extracts before analysis.

B. Apparatus and Reagents

(**a**) *Gas chromatograph*.—With autosampler and ECD, operating conditions: injection port temperature, 280°C; ECD temperature, 300°C; He carrier gas, 29 cm/s; N make-up gas, 30 mL/min; column temperature program: 50°C for 1 min, to 205°C at 30°C/min, to 240°C at 1°C/min, hold 2 min at 240°C; splitless injection, opening splitter 0.8 min after injection; split vent flow, 22 mL/min; purge flow, 9 mL/min (Hewlett-Packard, now Angilent Technologies, Palo Alto, CA USA; Model 5890A replaced by 6890, autosampler Model 7673, and integrator Model 3393A, or equivalent). Silanized glass wool must not be used in the GC inlet adapter. Difference in detector response between 2 successive injections of the same standard solution should not exceed 6%. Peak resolution should be as indicated in Figure **998.01A**.

(**b**) *GC column*.—30 m × 0.25 mm id, 0.10 μm film thickness (DB-5, 5% phenylmethylpolysiloxane, J&W Scientific, 91 Blue Raven Rd, Folsom, CA, 95630-4714 USA, or equivalent).

(**c**) *Homogenizer*.—Model T25 230V, 600W, 800-24000 rpm (Janke & Kunkel, Janke-8-Kunkel-Str. #10 D-79219, Germany), or equivalent.

Table 998.01 Interlaboratory study results for determination of 8 synthetic pyrethroid pesticides in wheat, oranges, and tomatoes by multiresidue capillary gas chromatographic method

Pesticide	Spike, mg/kg	Recovery, %	s_r	s_R	RSD_r, %	RSD_R, %	r	R
Wheat								
Bifenthrin	0.105	91.8	0.0074	0.0104	7.60	10.71	0.0209	0.0294
Fenpropathrin	0.210	95.2	0.0126	0.0129	6.34	6.46	0.0356	0.0366
Cyhalothrin	0.105	96.9	0.0068	0.0084	6.66	8.19	0.0194	0.0238
Permethrin	1.909	95.0	0.1364	0.2536	7.46	13.89	0.3856	0.7174
Cypermethrin	0.382	97.6	0.0340	0.0537	9.02	14.21	0.0962	0.1518
Fenvalerate	1.909	100.2	0.1692	0.2134	8.82	11.08	0.4758	0.6034
Fluvalinate	0.954	92.9	0.0814	0.1556	9.18	17.52	0.2300	0.4402
Deltamethrin	0.954	99.4	0.1029	0.1692	10.84	17.74	0.2911	0.4784
Oranges								
Bifenthrin	0.105	93.3	0.0051	0.0077	5.19	7.88	0.0144	0.0218
Fenpropathrin	0.210	99.4	0.0122	0.0142	5.82	6.78	0.0346	0.0402
Cyhalothrin	0.105	96.1	0.0070	0.0103	6.84	9.66	0.0196	0.0278
Permethrin	0.954	97.7	0.0537	0.0558	5.72	5.94	0.1520	0.1578
Cypermethrin	1.909	97.5	0.1138	0.2212	6.11	11.86	0.3221	0.6256
Fenvalerate	1.909	100.6	0.1201	0.2052	6.23	10.70	0.3396	0.5806
Fluvalinate	1.909	88.1	0.1451	0.2988	8.67	17.78	0.4104	0.8451
Deltamethrin	0.095	99.9	0.0111	0.0172	11.72	18.13	0.0314	0.0486
Tomatoes								
Bifenthrin	0.105	96.0	0.0032	0.0056	3.20	5.66	0.0090	0.0160
Fenpropathrin	0.954	96.0	0.0328	0.0554	3.58	6.04	0.0930	0.1568
Cyhalothrin	0.105	95.0	0.0038	0.0071	3.81	7.18	0.0108	0.0202
Permethrin	0.954	97.4	0.0302	0.0519	3.27	5.59	0.0856	0.1466
Cypermethrin	0.477	97.7	0.0290	0.0385	6.20	8.26	0.0822	0.1088
Fenvalerate	0.954	100.5	0.0462	0.0772	4.84	8.04	0.1308	0.2184
Fluvalinte	0.954	88.2	0.0534	0.0884	6.28	10.48	0.1509	0.2502
Deltamethrin	0.191	101.5	0.0158	0.0188	8.09	9.70	0.0446	0.0532

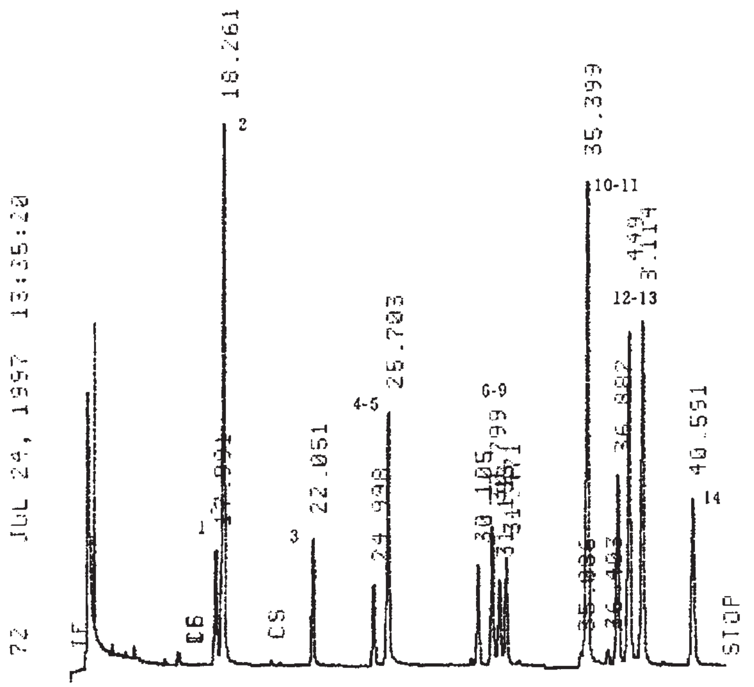

Figure 998.01A—Typical chromatogram of a mixed standard of 8 synthetic pyrethroids (peak number from left to right): (1) bifenthrin (0.11 ng); (2) fenpropathrin (1.00 ng); (3) λ-cyhalothrin (0.11 ng); (4–5) permethrin (1.00 ng); (6–9) cypermethrin (0.50 ng); (10–11) fenvalerate (1.00 ng); (12–13) fluvalinate (1.00 ng); and (14) deltamethrin (0.20 ng).

(**d**) *Mechanical shaker*.—Speed, 50–300; osallations/min. 50–280; SA31 (Yamato Scientific Co., Tokyo, Japan), or equivalent.

(**e**) *Rotary evaporator*.—Buchi EL 131 with Buchi 461 water bath (Flawil, Switzerland) and NEOCOOL Circulator BC55, BP-51 aspirator (–5–20°C) (Yamato, Japan), or equivalent.

(**f**) *Solvents*.—Acetone, hexane, acetonitrile, and ethyl ether; pesticide grade, or redistill in all-glass apparatus and check for interferences by GC–ECD, (**a**).

(**g**) *Sodium sulfate, anhydrous*.—Heat at 650°C for 4 h. Cool in desiccator.

(**h**) *Eluting solvent*.—6% ethyl ether in hexane. Mix 60 mL ethyl ether, (**f**), with 940 mL hexane, (**f**).

(**i**) *Insecticide standards*.—Purity, ≥90%. Prepared from neat material (Guangzhou Nanfang Scientific Instrument Co., Guangzhou, China; Chem Service, Inc., P.O. Box 559, West Chester, PA 19381-0599, USA, www.chemservice.com; or equivalent). Prepare individual standard stock solutions in hexane at 10.0 mg/100 mL (deltamethrin, cypermethrin, 250 µg/mL), and 50 mg/mL (fenpropathrin, permethrin, fenvalerate, and fluvalinate, 500 µg/mL. Prepare a mixed standard solution for determining the Florisil elution pattern by pipetting 5 mL of each of bifenthrin and λ-cyhalothrin, 10 mL of each deltamethrin, cypermethrin, fenpropathrin, permethrin, fenvalerate, and fluvalinate individual standard stock solutions into 100 mL volumetric flask and diluting to volume with hexane.

(**j**) *Deactivated Florisil*.—60–100 mesh, pesticide grade (Alltech Associates, Inc., 2051 Waukeegan Rd, Deerfield, IL 60015-1899, USA), or equivalent. Activate at 650°C 4 h in muffle furnace. Transfer to oven at 130°C and let stand 5 h. Store in glass-stoppered bottles or in air-tight desiccator and let cool overnight. Deactivate Florisil by carefully adding 5% (w/w) distilled water. Shake 1 h on mechanical shaker and let stand overnight. Store in sealed container at room temperature. Deactivated florisil is stable for up to 7 days.

(**k**) *Acetonitrile saturated with hexane*.—Add 300 mL acetonitrile and 100 mL hexane to 500 mL separatory funnel. Shake vigorously, with frequent venting, 2 min. Let layers separate. Drain acetonitrile layer into a storage bottle.

C. Determination

(*1*) *Florisil eultion pattern*.—Place small plug of glass wool at bottom of 400 × 22 mm id glass column. Add 1 cm layer of anhydrous Na_2SO_4, **B**(**g**). Add ca 50 mL hexane to column, then add 10 g deactivated Florisil, **B**(**j**), and tap sides of column for even packing. Top with 1 cm layer of anhydrous (Na_2SO_4, **B**(**g**). Prewash column with ca 50 mL hexane. Do not let column dry until elution is complete. Place 1.0 mL of the mixed standard solution, **B(l)**, on the Florisil column and elute the synthetic pyrethroids with 6% eluting solvent, **B**(**h**), as in **C**(*5*). Inject 1 µL alquots onto GC, **B**(**a**), and determine the recovery of each insecticide. Figure **998.01B** lists elution order of analytes. A recovery of ca 95% of fluvalinate is expected which depends upon the activity of the Florisil and volume of eluting solvent, **B**(**h**), which may range from 130 to 200 mL. Adjust volume to achieve ca 95% fluvalinate recovery.

(*2*) For extraction from high-moisture (≥75%) products, weigh 50.0 g (to nearest 0.1 g) chopped product into homogenizer jar, **B**(**c**); add 120 mL acetone; and homogenize 3 min at 18,000 rpm. Suction filter through 12 cm Büchner funnel with filter paper into 500 mL suction flask. Rinse homogenizer with two 25 mL portions acetone

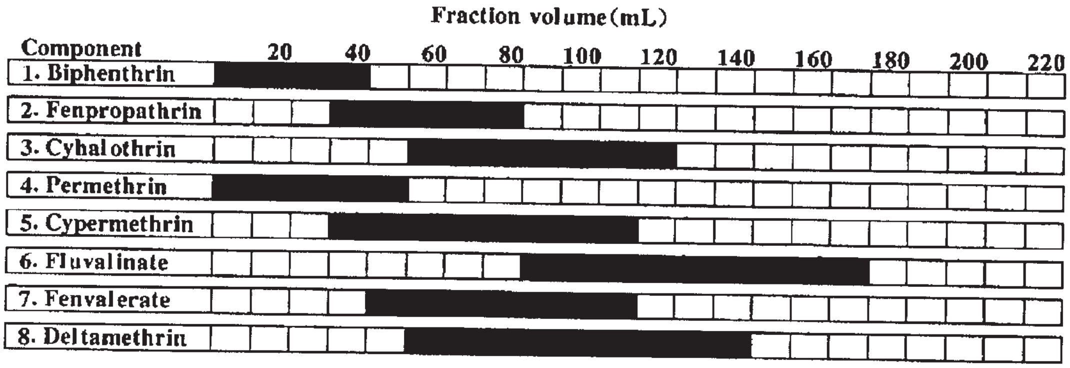

Figure 998.01B—Pattern of elution of insecticides from 5% water deactivated Florisil column by 6% eluting solvent.

and use rinses to wash residues in Büchner funnel. Transfer filtrate to 500 mL separatory funnel. Wash suction flask with two 10 mL portions of acetone, adding washes to separatory funnel.

For dry or low-moisture products such as grains, weigh 20.0 g (to nearest 0.1 g) into homogenizer jar, **B**(**c**), add 150 mL acetonitrile–water (2 + 1), **B**(**k**), and homogenize at 18,000 rpm 5 min. Proceed as for *High-Moisture Products*, **C**(*2*), with acetonitrile–water, **B**(**k**), in place of acetone.

(*3*) Add 60 mL hexane to separatory funnel containing extract. Shake vigorously, with frequent venting, 5 min. Add 200 mL 4.0% aqueous NaCl (w/v) and mix vigorously ca 30 s. Let layers separate and discard aqueous layer. Pass hexane layer through glass funnel containing glass wool plug and ca 15 g Na_2SO_4 **B**(**g**), collecting extract in 250 mL round-bottom flask. Rinse separatory funnel with two 20 mL portions of hexane and pass rinses through glass wool into round-bottom flask.

(*4*) Evaporate contents of round-bottom flask to dryness on rotary evaporator, **B**(**e**), at 40°C. Redissolve residue in 10 mL hexane and transfer to 125 mL separatory funnel. Rinse round-bottom flask with two 5 mL portions hexane and add rinse to separatory funnel. Add 30 mL acetonitrile-saturated hexane, **B**(**k**), and shake vigorously, with frequent venting, 5 min. Let layers separate, and drain acetonitrile phase into 250 mL round-bottom flask. Add 30 mL acetonitrile-saturated hexane to hexane phase in separatory funnel and shake vigorously, with frequent venting, 5 min. Let phases separate and drain acetonitrile layer into same 250 mL round-bottom flask. Repeat 30 mL acetonitrile extraction and collect acetonitrile. (*Note*: Accurately measure volumes of acetonitrile and hexane for optimal results.) Evaporate acetonitrile extract to dryness on rotary evaporator at 60°C. Dissolve residue in 5 mL hexane. (*Note*: Ensure acetonitrile is evaporated completely to dryness.)

(*5*) Transfer extracts to column and let level fall until just above Florisil packing. Rinse the 250 mL round-bottom flask with two 10 mL portions of hexane, add each rinse to column, and let run through column. Elute pyrethroid residues with same volume of 6% eluting solvent, **B**(**h**), used in Florisil elution standardization, **C**(*1*), collecting eluate at 3 mL/min in 250 mL round-bottom flask. Evaporate eluate to less than <50 mL on rotary evaporator at 40°C, and transfer to 50 mL volumetric flask. Dilute to volume with hexane so that the final concentration is 1.0 g/mL for fruits and vegetables or 0.4 g/mL for grains.

(*6*) Tentatively identify residue peaks by comparing retention times from extract to those of standards solution. According ca concentration of pyrethroids in extracts, select the standard solution with peak heights similar to those of the extracts. Inject a standard solution with peak height ± 25% of analyte peak height. Dilute and reanalyze if analyte concentration exceeds 500 µg/mL. Calculate amounts of each pyrethrin in test extract with the following equation:

$$\text{Residue, mg/kg} = C_{\text{Std}} \times (H_{\text{Ex}}/H_{\text{Std}}) \times (V_{\text{Std}}/V_{\text{Ex}}) \times (D/W)$$

where C_{Std} = standard concentration (µg/mL), H_{Ex} = peak height in extract, H_{Std} = peak height in standard, V_{Std} = standard volume injected (µL), V_{Ex} = extract volume injected (µL), D = dilution volume (mL), and W = test portion weight (g).

For pyrethroids containing multiple isomers, quantitate by addition of concentrations from multiple peaks in both standards and tests.

Reference: *J. AOAC Int.* **82**, 186(1999).

CAS-82657-04-3 (bifenthrin)
CAS-39515-41-8 (fenpropathrin)
CAS-91465-08-6 (λ-cyhalothrin)
CAS-52645-53-1 (permethrin)
CAS-52315-07-8 (cypermethrin)
CAS-51630-58-1 (fenvalerate)
CAS-69409-94-5 (fluvalinate)
CAS-52918-63-5 (deltamethrin)