Multiresidue Analytical Procedure for Insecticides Used by Organic Farmers

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A multiresidue procedure for the insecticides used by organic farmers has been developed. Rotenone, cevadine, and veratridine (the major components of sabadilla), pyrethrin I and pyrethrin II (the major components of pyrethrum), and ryanodine and dehydroryanodine (the major components of ryania) can be separated by high-performance liquid chromatography (HPLC) and detected and quantified by atmospheric pressure chemical ionization mass spectrometry (APCI/MS) in the selected ion monitoring mode. Piperonyl butoxide, a material sometimes used together with rotenone or pyrethrum to enhance toxicity, can also be detected and quantified by this procedure. The analytes are extracted with acetonitrile/water and are cleaned up with a C_{18} solid-phase extraction cartridge. Rotenone, piperonyl butoxide, and the two major sabadilla components could be detected (signal-to-noise ratio = 10) in lettuce, cucumber, and cabbage at 1−6 ppb. Pyrethrin I and the ryania components could be detected between 10 and 171 ppb in these vegetables, whereas pyrethrin II was generally less sensitive, with a limit of detection as high as 200 ppb in cabbage. Recoveries were in the 72−124% range. Percent coefficients of variation ranged from 2 to 17.

Keywords: *Organic farming; sabadilla; veratridine; cevadine; ryania; dehydroryanodine; ryanodine; rotenone; pyrethrum; pyrethrin I; pyrethrin II; piperonyl butoxide; high-performance liquid chromatography (HPLC); atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI/LCMS)*

INTRODUCTION

Despite high cost, sales of organically grown produce nearly doubled to $7.6 billion between 1989 and 1994 (Burros, 1996). The vast majority of consumers of this produce are unaware that organic farmers are permitted to use certain insecticides (rotenone, ryania, sabadilla, and pyrethrum) and that there are gaps in the databases concerning the chronic toxicity of these materials as well as their effects on the environment or on nontarget species. Rotenone is a selective, nonspecific pesticide highly toxic to fish, with 96-h LC_{50} values of 23 and 2.6 ppb to rainbow trout and channel catfish, respectively (Kidd and James, 1991). Rotenone is toxic to bees when used together with pyrethrin (Kidd and James, 1991). One of the organic farming product catalogues we obtained listed a general purpose spray for vegetables and herbs that consisted of just such a combination. In a 2-year feeding study of rotenone at the National Cancer Institute, there was equivocal evidence of parathyroid gland tumors in male rats (National Toxicology Program, 1986). Pregnant rats fed rotenone at 5 mg/kg rotenone gave birth to a significant number of offspring with skeletal deformities, although no effects were observed at twice this dose (National Research Council, 1983). Ryania is an insecticide made from the ground stems of *Ryania speciosa*, a plant native to South America (Ruest et al., 1985). There is essentially no information in the literature on its carcinogenic, teratogenic, mutagenic, or endocrine-disrupting effects on either young or adult animals. Nor is there such information for sabadilla insecticide (Zang et al., 1997). It is ironic that despite the paucity of chronic toxicity information for these insecticides, they are used on crops purchased mainly by consumers who are fearful of ingesting foods with traces of synthetic pesticide residues.

There is no published methodology for ryania in food. Newsome and Shields (1980) used high-performance liquicd chromatography (HPLC) to determine rotenone in tomato and lettuce with estimated limits of detection of approximately 25 and 100 ppb, respectively. Dawson and Allen (1988) reported on the use of HPLC (with UV detection at 295 nm) for the determination of rotenone with sensitivities of 25 ppb in sediments and 5 ppb in fish tissue. Ho and Budde (1994) reported a detection limit of 4 ppb for rotenone in river water using HPLC with particle beam mass spectrometry. Ryan et al. (1982) were able to detect pyrethrin I and pyrethrin II in fruit with detection limits of 10 and 30 ppb, respectively, using gas chromatography with an electron capture detector. Piperonyl butoxide was determined in wheat and barley by HPLC with fluorometric detection at a limit of detection of 100 ppb (Isshiki et al., 1978). A method for the two major components of sabadilla (veratridine and cevadine) in lettuce and cucumber was recently published (Zang et al., 1997). We now expand on this methodology to include the insecticides used by organic farmers and piperonyl butoxide. The latter has been included in the multiresidue procedure because it is employed as a synergist in some

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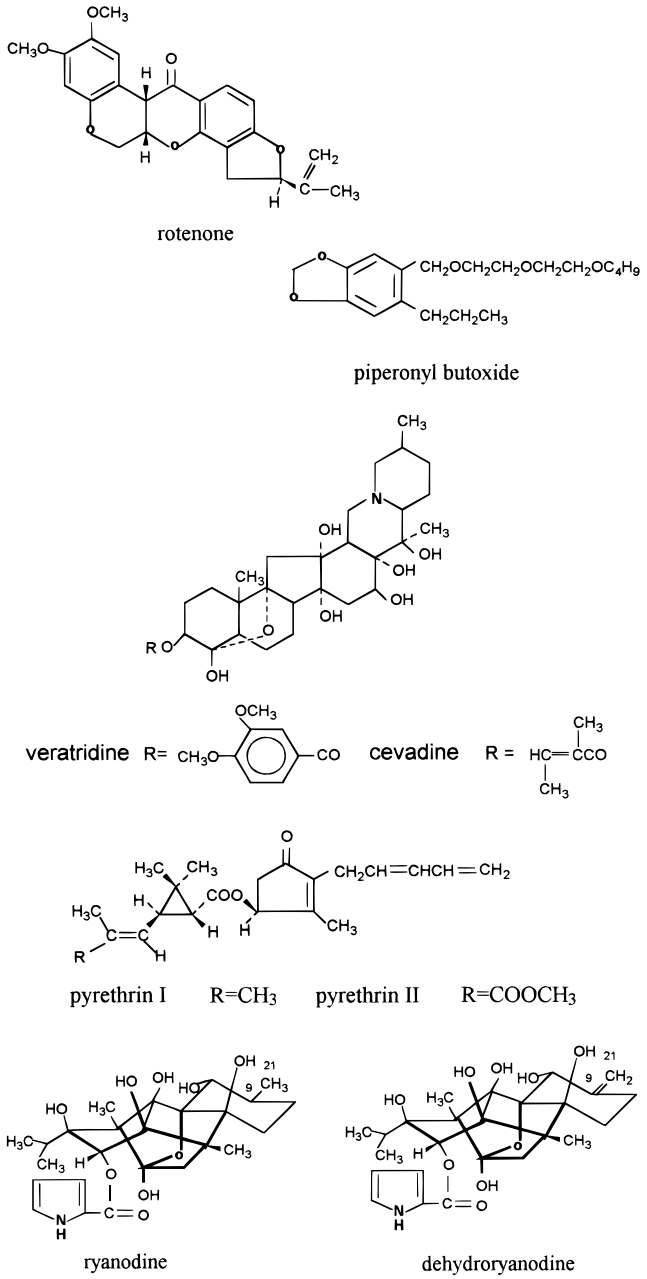

commercial pyrethrum and rotenone formulations. Piperonyl butoxide is a synthetic chemical and should not be used by farmers who sell organic produce.

MATERIALS AND METHODS

Chemicals. Veratrine (a mixture of alkaloids consisting of 38% veratridine, 59% cevadine, and 3% other alkaloids) and ryania (a mixture of 53% dehydroryanodine and 47% ryanodine) were purchased from Sigma Chemical Co., St. Louis, MO. Pyrethrum (a mixture of 49% pyrethrin I and 42% pyrethrin II) was purchased from Chem Service, West Chester, PA. Piperonyl butoxide and rotenone were purchased from Aldrich Chemical Co., Milwaukee, WI. Water, acetonitrile, and methanol (all of optima grade), caffeine, and ammonium acetate were purchased from Fisher Scientific, Springfield, NJ.

Instrumentation. Conditions for atmospheric pressure chemical ionization liquid chromatography mass spectrometry (APCI/LCMS) were as follows: Chromatography was carried out using a Varian 9012 solvent delivery system (Varian Analytical Instruments, Sugar Land, TX) equipped with a Varian 9050 UV−vis detector and interfaced to a Micromass Platform II mass spectrometer (Altrincham, U.K.) equipped

Table 1. Time-Scheduled SIM

analyte	channel, mass (*m*/*z*)	retention time window (min)
caffeine (internal standard)	195	0−17
dehydroryanodine	345	19−27
ryanodine	347	19−27
rotenone	395	29−41
cevadine	592	29−41
veratridine	674	29−41
piperonyl butoxide	177	34−50
pyrethrin I	166	34−50
pyrethrin II	166	34−50

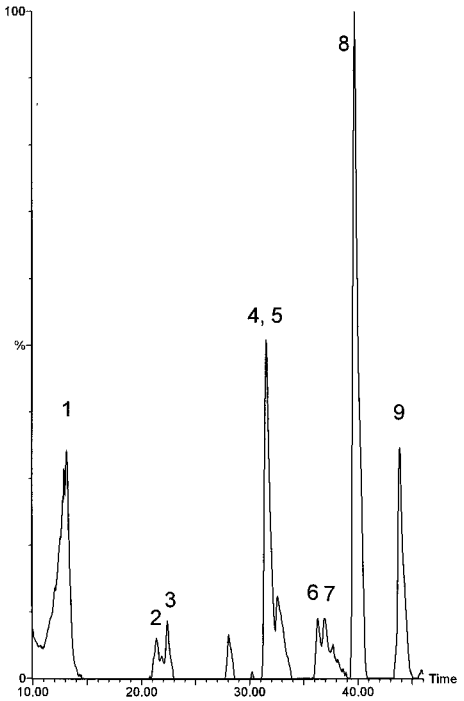

Figure 1. Total ion chromatogram obtained by APCI/LCMS from a mixture containing 100 ng of caffeine (1), 53 ng of dehydroryanodine (2), 47 ng of ryanodine (3), 100 ng of rotenone (4), 38 ng of veratridine (5), 840 ng of pyrethrin II (6), 59 ng of cevadine (7), 100 ng of piperonyl butoxide (8), or 980 ng of pyrethrin I (9).

with an APCI source. The HPLC column was a Supelco (Supelco, Inc., Bellefonte, PA) Rx-C_{18} (4.6 mm × 250 mm, 5 μm particle size). The initial mobile phase composition of methanol/0.01 M ammonium acetate (10:90) was programmed linearly to 40% methanol and 60% 0.01 M ammonium acetate in 10 min, to 75:25 in 25 min, and to 95:5 in 50 min. The flow rate was 0.9 mL/min.

The APCI source and nebulizer temperatures were 150° and 400 °C, respectively. The flow rates of the nitrogen drying gas and the nitrogen APCI sheath gas were 240 and 100 L/h, respectively. The mass spectrometer was operated in the APCI positive ion mode, with APCI corona discharge pin maintained at 3.0 kV; ion sampling cone voltage was set at 25 V. The scan range was from 150 to 700 amu with a scan rate of 1 s for full-scan determinations. For quantification in vegetables, time-scheduled selected ion monitoring (SIM) with dwell time of 2.0 s and span of 0.3 amu was performed (Table 1).

Determination of Percent Recovery. Recoveries were determined three separate times for each vegetable. To 50 g of chopped vegetable (lettuce, cabbage, or cucumber) was added 50 μL each of 0.1 mg/mL methanol solutions of veratrine, rotenone, or piperonyl butoxide, 125 μL of a 0.1 mg/mL solution of ryania, and 375 μL of a 0.1 mg/mL solution of pyrethrum. The solutions were distributed on the vegetables as equally as possible. The syringes were rinsed with methanol and the rinsings added to the vegetable. The spiked vegetables were allowed to stand for 30 min and then homogenized with 10

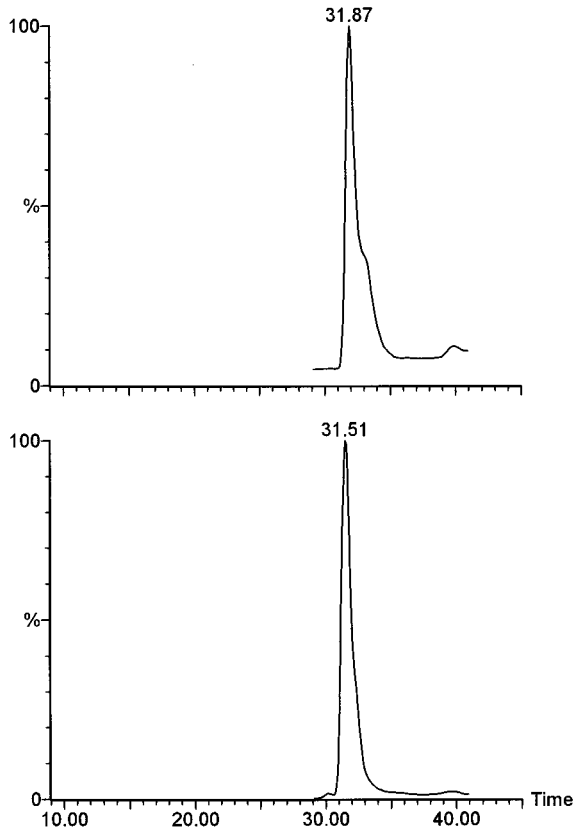

Figure 2. Mass chromatography of veratridine (upper trace) and rotenone (lower trace).

mL of water and 90 mL of acetonitrile in an explosion-proof blender for 1 min at the low-speed setting. The mixture was filtered through an 11 cm diameter, 1.5 μm pore size glass filter, and the volume of the filtrate was measured. One-fifth of the filtrate (by volume) was evaporated under reduced pressure on a flash evaporator (Buchler Instruments, Fort Lee, NJ) to remove all of the acetonitrile. A Supelco Envi-18 SPE 6 mL cartridge was placed on a Visiprep solid-phase extraction vacuum manifold (Supelco) and conditioned first with 6 mL of methanol and then with 6 mL of water. The vegetable extract was loaded onto the cartridge and passed through at a flow rate of 1−2 mL/min. The cartridge was washed with 6 mL of water and dried under vacuum for 5 min. After that, the cartridge was eluted with 3 mL of methanol, and the eluate was dried under a gentle stream of nitrogen. Four microliters of internal standard stock solution (250 ng/μL caffeine) was added to the final concentrate, and the volume was adjusted to 1 mL with methanol. One hundred microliters of the methanol solution was injected into the HPLC. There were no significant differences in peak shape between 100, 50, and 10 μL injections. To calculate the recovery, the area ratio of each component to internal standard (caffeine) either in spiked vegetable or in reference standard solution was determined. Each area ratio (average of three samples) in the spiked vegetables was compared to the corresponding ratios (average of three determinations) obtained from injecting 100 μL of a solution containing 1 μg of veratrine, 1 μg of rotenone, 1 μg of piperonyl butoxide, 1 μg of caffeine, 2.5 μg of ryania, and 7.5 μg of pyrethrum per milliliter of methanol.

Determination of Limit of Detection (LOD). To 50 g of chopped vegetable (lettuce, cabbage, or cucumber) was added 5 μL each of 0.1 mg/mL methanol solutions of veratrine, or rotenone, piperonyl butoxide, 25 μL of a 0.1 mg/mL solution of ryania, and 100 μL of a 0.1 mg/mL solution of pyrethrum. The vegetables were then analyzed as above. The ratio, R, of the height of the peak to the height of the maximum noise peak in the retention time window of the pesticide(s) was determined from the SIM chromatograms. To determine the LOD, at a signal-to-noise ratio of 10, R was divided into the spiked pesticide concentration, C, multiplied by 10:

$$\mathrm{LOD} = 10C/R$$

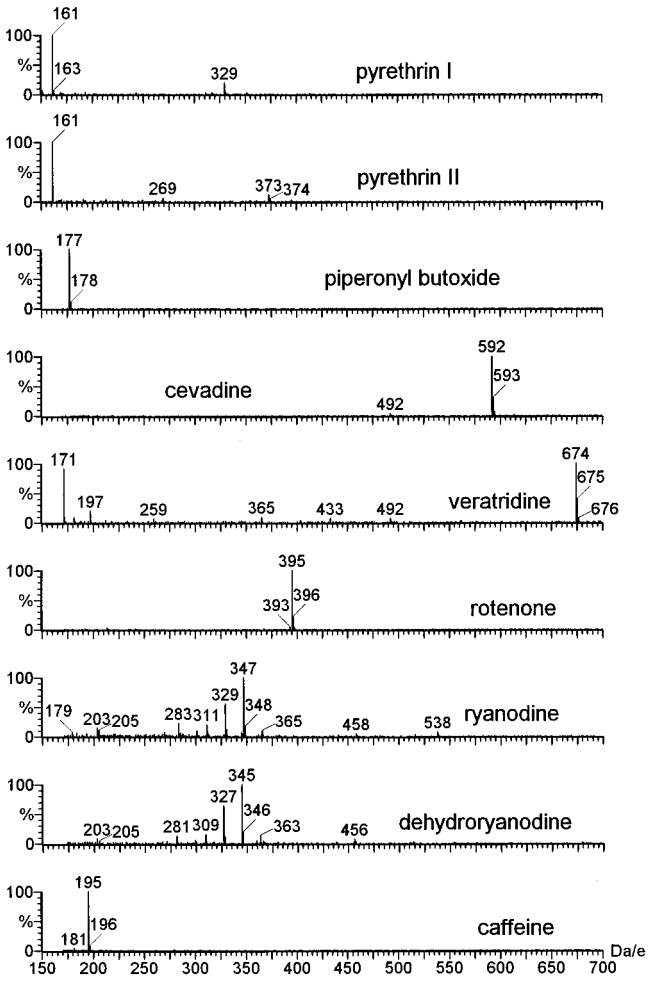

Figure 3. APCI mass spectra of the analytes and caffeine (internal standard).

RESULTS AND DISCUSSION

Figure 1 is the total ion chromatogram of a mixture containing 100 ng of caffeine, 53 ng of dehydroryanodine, 47 ng of ryanodine, 100 ng of rotenone, 38 ng of veratridine, 840 ng of pyrethrin II, 59 ng of cevadine, 100 ng of piperonyl butoxide, and 980 ng of pyrethrin I at 13.15, 21.37, 22.40, 31.51, 31.87, 36.32, 36.93, 39.70, and 43.86 min, respectively. Veratridine and rotenone are not well separated under our HPLC conditions. Since the analytes are detected on the basis of mass, they can still be detected even if both are present, as can be seen in Figure 2.

The APCI mass spectra of the analytes and the internal standard are shown in Figure 3. The mass spectrum of pyrethrin I has an M + H ion at m/z 329 and a base peak at m/z 161 (from the loss of chrysanthemic acid). Pyrethrin II forms the same base peak by an analogous fragmentation as well as an M + H ion at m/z 373. The mass spectrum of piperonyl butoxide does not exhibit an M + H ion at m/z 339 but has a base peak at m/z 177 due to loss of HOC_2H_4O $C_2H_4OC_4H_9$. Cevadine exhibits an M + H ion at m/z 592, whereas veratridine exhibits an M + H ion at m/z 674. The peak at m/z 492 in both spectra arises from loss of the ester group. The other peaks in the spectrum of veratridine are not from the veratridine as deter-

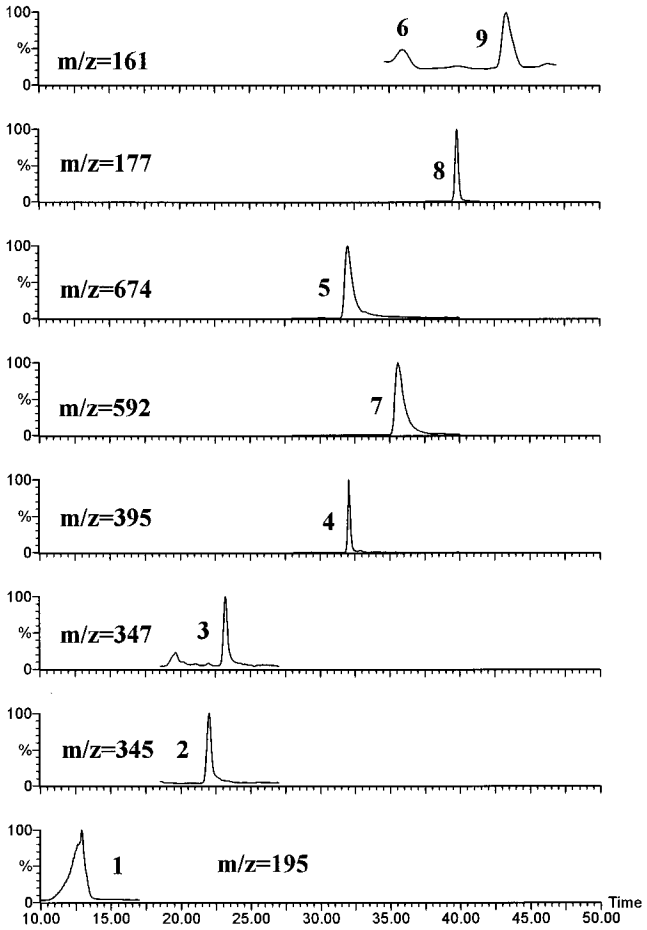

Figure 4. SIM chromatograms of spiked cucumber extract from recovery study showing the ions that were monitored: 1, caffeine; 2, dehydroryanodine; 3, ryanodine; 4, rotenone; 5, veratridine; 6, pyrethrin II; 7, cevadine; 8, piperonyl butoxide; 9, pyrethrin I.

Table 2. Recovery of Organic Insecticides in Lettuce, Cabbage, and Cucumber

	recovery (%) ± % CV[a]		
insecticide	lettuce	cabbage	cucumber
dehydroryanodine	109 ± 5	91 ± 7	99 ± 10
ryanodine	124 ± 2	102 ± 10	108 ± 9
rotenone	107 ± 17	99 ± 9	107 ± 6
cevadine	99 ± 4	105 ± 5	97 ± 9
veratridine	87 ± 11	98 ± 7	72 ± 8
piperonyl butoxide	87 ± 17	81 ± 2	92 ± 10
pyrethrin I	96 ± 9	110 ± 8	104 ± 5
pyrethrin II	101 ± 7	100 ± 9	119 ± 6

[a] % CV = percent coefficient of variation (three replicates).

mined by mass chromatography. Rotenone has an M + H ion at *m/z* 395. The mass spectrum of ryanodine exhibits no M + H ion. The peak at *m/z* 458 is due to loss of two water molecules, and the peak at *m/z* 347 is due to subsequent loss of pyrrole-2-carboxylic acid. Peaks at *m/z* 329 and 311 are due to additional losses of water. The fragmentation of dehydroryanodine is analagous to that of ryanodine.

Figure 4 shows the SIM determinations in cucumber spiked to contain 368 ppb of pyrethrin I, 315 ppb of pyrethrin II, 133 ppb of dehydroryanodine, 118 ppb of ryanodine, 100 ppb of rotenone, 59 ppb of cevadine, 38 ppb of veratridine, and 100 ppb of piperonyl butoxide. Similar SIM chromatograms (not shown) were obtained from extracts of lettuce and cabbage spiked at the same

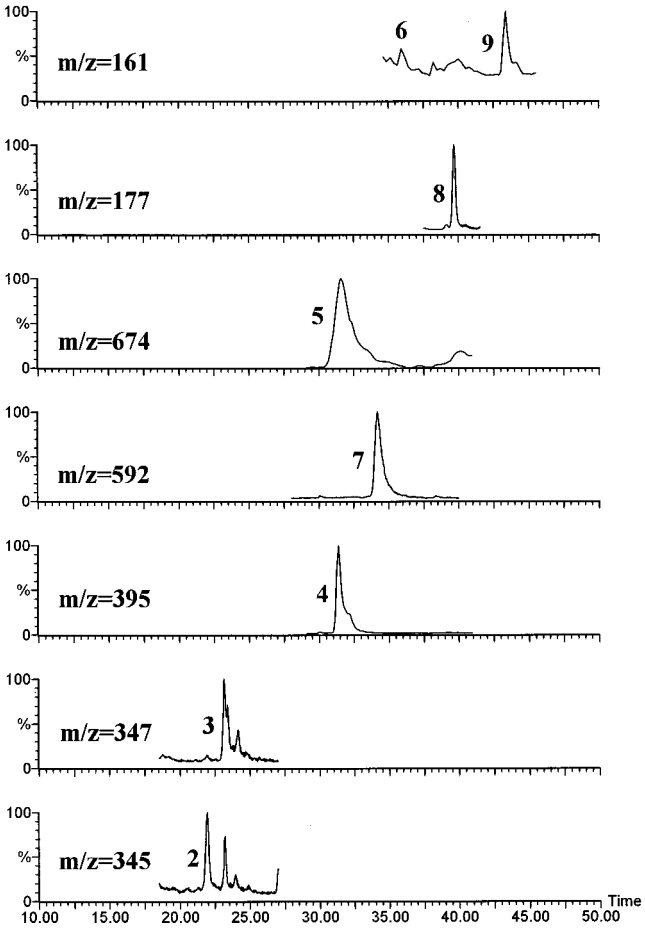

Figure 5. SIM chromatograms of spiked lettuce extract from LOD determination showing the ions that were monitored: 2, dehydroryanodine; 3, ryanodine; 4, rotenone; 5, veratridine; 6, pyrethrin II; 7, cevadine; 8, piperonyl butoxide; 9, pyrethrin I.

Table 3. LOD in Lettuce, Cabbage, and Cucumber at Signal to Noise Ratio of 10

	ppb		
insecticide	lettuce	cabbage	cucumber
pyrethrin I	46	71	25
pyrethrin II	180	200	140
dehydroryanodine	17	45	20
ryanodine	171	27	10
rotenone	1	5	2
cevadine	1	5	1
veratridine	1	6	3
piperonyl butoxide	1	3	6

levels. Table 1 shows the results of the recovery studies, which ranged from 72 to 124%. Precision, as given in Table 2, was quite good, with the vast majority of determinations at <10% coefficient of variation (CV).

Figure 5 shows the SIM determinations in lettuce at lower levels: pyrethrin I at 98 ppb, pyrethrin II at 84 ppb, dehydroryanodine at 27 ppb, ryanodine at 24 ppb, rotenone at 10 ppb, cevadine at 6 ppb, veratridine at 4 ppb, and piperonyl butoxide at 10 ppb. Results with cucumber and cabbage were essentially the same, with differences in sensitivity due to the mass spectral characteristics of the different interfering compounds found in different vegetables. Calculated LODs from these data at a signal-to-noise ratio of 10 are given in Table 3. This is a rather conservative criterion for sensitivity. For example, if we reported sensitivity at

a signal-to-noise ratio of 2 (as is done in many cases), then all of the LODs in Table 3 would be reduced by a factor of 5.

In conclusion, we have developed, for the first time, a multiresidue procedure for the determination of insecticides used by organic farmers in vegetables. The inclusion of piperonyl butoxide in the methodology may be of use to those who are interested in applying strict standards to organic produce.

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