Rapid Determination of Abamectin in Lettuce and Cucumber Using High-Performance Liquid Chromatography

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A rapid, sensitive, and reliable method is presented for the determination of trace amounts of abamectin in lettuce and cucumber. Abamectin consists of ≥80% avermectin B_{1a} and ≤20% avermectin B_{1b}. Vegetables were extracted with ethyl acetate, and the extract was purified by solid-phase extraction using Sep-Pak silica cartridges. The purified extracts were analyzed by HPLC with a 5-μm Zorbax ODS column and UV detection under isocratic conditions. The method yields recoveries for avermectins B_{1a} and B_{1b} of 76–109% in the 0.054–0.54 mg/kg range. The limit of detection of the method is 40 μg of avermectins B_{1a} and B_{1b}/kg of vegetable.

The avermectins, a newly discovered family of pesticidal agents, are macrocyclic lactones produced by the actinomycete *Streptomyces avermitilis* (Burg et al., 1979). The avermectin structures have been elucidated by Albers-Schönberg et al. (1981), and some of the biological activities have been reviewed (Campbell et al., (1983). Abamectin is the commercial product that is being developed as an insecticide and acaricide with contact and stomach action. Abamectin consists of ≥80% avermectin B_{1a} and ≤20% avermectin B_{1b} (Figure 1).

Several methods have been described in the literature to determine avermectin quantitatively. Tolan (1980) and Tway (1981) described the determination of, respectively, avermectins in plasma and ivermectin in tissue. The methods are sensitive but also time-consuming because of elaborate cleanup steps and derivatization. Fox and Fink (1985) described the determination of ivermectin in feeds by high-performance liquid chromatography (HPLC). However, the method is not sensitive (detection limit 6 mg/kg). Iwata (1985) and Maynard (1989) described the determination of avermectin B_{1a} in citrus fruits. The methods are sensitive (detection limit 0.1 and 5 μg/kg, respectively), but radiolabeled avermectins (^3H and ^{14}C respectively) were used.

The purpose of this study was to develop a rapid, sensitive, and quantitative method for the determination of abamectin (avermectins B_{1a} and B_{1b}) in lettuce and cucumber.

The vegetables were extracted with ethyl acetate. The extracts were purified by solid-phase extraction. Separation and detection were performed by using reversed-phase HPLC with ultraviolet detection. Recovery experiments were carried out in lettuce and cucumber.

EXPERIMENTAL PROCEDURES

Reagents. All reagents were of analytical quality. Ethyl acetate, hexane, and methanol were obtained from Merck (Darmstadt, Germany). The solid-phase extraction was done with Sep-Pak silica cartridges (0.8 g) obtained from Waters Associates (Milford, MA). An abamectin standard in glycerol formal containing 1.074% w/w avermectin B_{1a} and 0.125% w/w avermectin B_{1b} was obtained from Merck Sharp & Dohme (Haarlem, The Netherlands). Abamectin standard solutions were prepared by diluting a stock standard solution of abamectin containing 54.0 mg of B_{1a}/L and 6.28 mg of B_{1b}/L in methanol. The standard solutions were stored in the dark at 4 °C. Under these conditions the standard solutions were stable for at least 4 months.

Extraction Procedure. The vegetables were chopped with a cutting machine (Stephan). Exactly 50 g of chopped vegetables was weighed into a 250-mL centrifuge tube. Exactly 100 mL of ethyl acetate was added. The mixture was macerated with an Ultra Turrax for 1 min. The extract was centrifuged at 2000*g* for 1 min. Exactly 50 mL of the supernatant was transferred to a round-bottomed flask and evaporated to dryness on a rotary evaporator (Buchi 011) at 40 °C. The residue was dissolved in 2 mL of ethyl acetate. Next, 3 mL of hexane was added. The contents of the round-bottomed flask were mixed and applied to a Sep-Pak silica cartridge. The round-bottomed flask was washed two times with 1 mL of 40% ethyl acetate in hexane, and the contents of the flasks were applied to the cartridge. The cartridge was washed first with 8 mL of 40% ethyl acetate in hexane and then eluted with 5 mL of 50% ethyl acetate in methanol. This eluate was evaporated to dryness on a rotary evaporator at 40 °C and the residue dissolved in 1 mL of methanol. Of this solution 25 μL was injected into the liquid chromatograph.

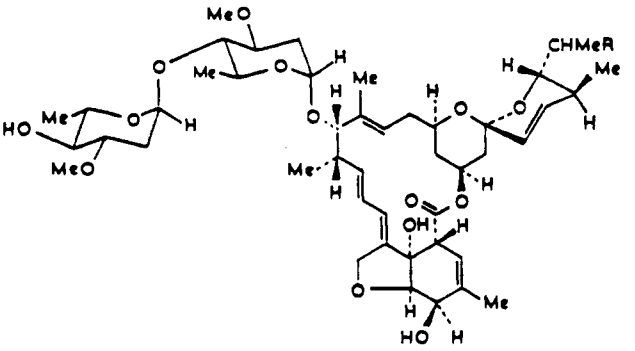

Figure 1. Structures of the two major components of abamectin: B_{1a} R = C_2H_5 (≥ 80%) and B_{1b} R = CH_3 (≤ 20%).

HPLC Analysis. A Spectra Physics 8100 liquid chromatograph with a SP 8400 UV detector and a SP 4100 computing integrator comprised the basic chromatographic instrumentation. The column was a Du Pont Zorbax ODS (4.6 mm × 25 cm) operated at ambient temperature. A precolumn Newquard RP-2 (3.2 × 15 mm) of Brownlee Labs was used to prevent contamination of the analytical column. The mobile phase was methanol–water (90:10). The flow rate was 1.0 mL/min. The UV detector was operated at 245 nm. Quantitation was performed by comparing sample peak heights with those obtained for standard solutions.

Recovery Experiments. The recovery of abamectin from vegetables was determined by applying with a pipet 500-μL aliquots of abamectin standard solutions (54.0 mg of B_{1a}/L and 6.28 mg of B_{1b}/L or 5.40 mg of B_{1a}/L and 0.628 mg of B_{1b}/L) to a 50-g portion of chopped vegetables, which corresponds to 0.54 mg of B_{1a}/kg and 0.0628 mg of B_{1b}/kg or 0.054 mg of B_{1a}/kg and 0.00628 mg of B_{1b}/kg (which is below the limit of detection). To another 50-g portion was added nothing. After some minutes, the samples were analyzed as described above. Recoveries

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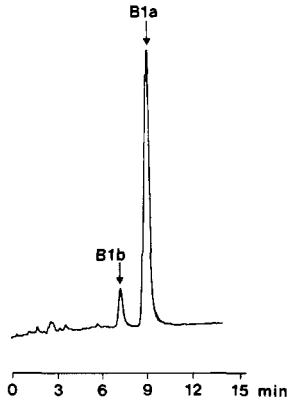

Figure 2. Chromatogram of abamectin standard. Amount injected: 810 ng of avermectin B_{1a} and 94.2 ng of avermectin B_{1b}.

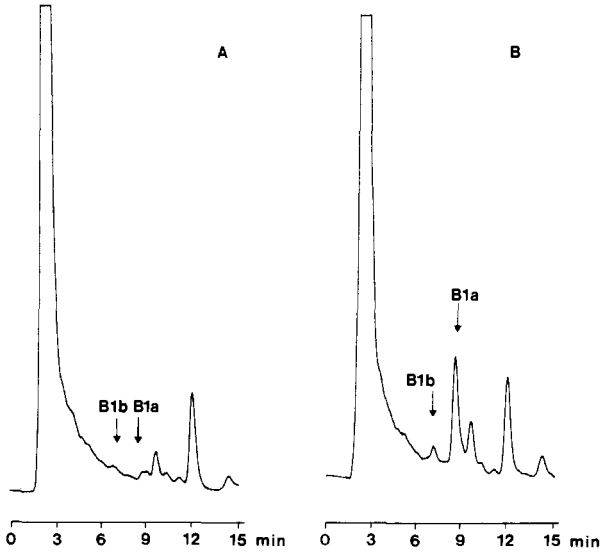

Figure 3. Chromatograms: (A) unfortified lettuce extract; (B) lettuce extract fortified with 540 μg of avermectin B_{1a}/kg and 62.8 μg of avermectin B_{1b}/kg. Avermectin B_{1a} and B_{1b} peaks represent an injected amount of, respectively, 337.5 and 39.25 ng of avermectins B_{1a} and B_{1b}.

(percent) were calculated as the difference between the amounts of avermectin B_{1a} or B_{1b} found in the spiked and in the nonspiked samples expressed as a percentage of the amount of avermectins B_{1a} and B_{1b} added.

RESULTS AND DISCUSSION

Figure 2 shows a typical chromatogram of an abamectin standard solution. In our research we used three different ODS columns: Zorbax ODS, Lichrosorp ODS, and Nucleosil ODS. Of these columns only the Zorbax ODS column yields a good separation between avermectins B_{1a} and B_{1b}.

Figures 3 and 4 show, respectively, chromatograms of lettuce extracts with and without abamectin and cucumber extracts with and without abamectin. The limit of detection, defined as 3 times the base-line noise, was estimated to be 25 ng/injection, which corresponds to 40 μg of avermectins B_{1a} and B_{1b}/kg.

The mobile phase composition is a critical parameter. It should be carefully chosen to avoid interfering matrix peaks. In our study we used two Zorbax ODS columns, a new one and one already used for other determinations. On the new column the optimal mobile phase composition was methanol–water (90:10) as described under Experimental Procedures. On the other column a mobile phase composition of methanol–acetonitrile–water (60:30:10) yields the best separation.

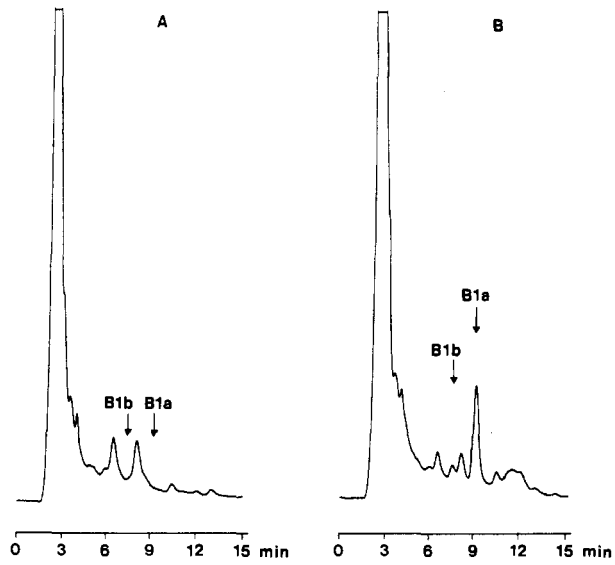

Figure 4. Chromatograms: (A) unfortified cucumber extract; (B) cucumber extract fortified with 540 μg of avermectin B_{1a}/kg and 62.8 μg of avermectin B_{1b}/kg. Avermectin B_{1a} and B_{1b} peaks represent an injected amount of, respectively, 337.5 and 39.25 ng of avermectins B_{1a} and B_{1b}.

Table I. Recovery Study of Abamectin Added to Chopped Lettuce (Micrograms per Kilogram)

avermectin B_{1a} added	amt found[a]	% rec	avermectin B_{1b} added	amt found	% rec
540	466	86	62.8	54.0	86
540	471	87	62.8	50.0	80
540	458	85	62.8	54.0	86
540	448	83	62.8	54.0	86
540	416	77	62.8	48.0	76
av ± SD		84 ± 4			83 ± 5
54.0	51.0	94			
54.0	55.0	102			
54.0	59.0	109			
54.0	54.0	100			
54.0	59.0	109			
av ± SD		103 ± 6			

[a] Nonspiked samples were all below limit of detection (40 μg/kg).

The detector response is at least linear from 25 up to 1350 ng of injected amount of avermectin B_{1a} (correlation coefficient 0.9999) and from 15.7 up to 157 ng of injected amount of avermectin B_{1b} (correlation coefficient 0.9999).

Peak identification was established by comparing the retention times of peaks in the sample chromatograms with those in the standard chromatograms. The experimental results of a recovery study are given in Tables I and II. The lower fortification level of avermectin B_{1a} in lettuce (Table I) resulted in more than 100% recovery, probably because of a little interfering peak. All other recoveries averaged approximately 85%, probably because of some loss in the sample cleanup. Mean recoveries were within the commonly accepted range of 80–110%. Relative standard deviations of recovery determinations, being a measure for the precision of the method, were not higher than 7%. This is a quite acceptable precision for residue analysis.

CONCLUSION

Both avermectins B_{1a} and B_{1b} can be detected and quantitated in lettuce and cucumbers in one chromatographic run. The method is rapid, the extraction and cleanup are easy to carry out, and derivatization is not necessary. The limit of detection for both components is

Table II. Recovery Study of Abamectin Added to Chopped Cucumber (Micrograms per Kilogram)

avermectin B_{1a} added	amt found[a]	% rec	avermectin B_{1b} added	amt found	% rec
540	473	88	62.8	50.0	80
540	515	95	62.8	50.0	80
540	473	88	62.8	59.0	94
540	437	81	62.8	52.0	83
540	437	81	62.8	52.0	83
av ± SD		87 ± 6			84 ± 6
54.0	46.0	85			
54.0	46.0	85			
54.0	47.0	87			
54.0	43.0	80			
54.0	47.0	87			
av ± SD		85 ± 3			

[a] Nonspiked samples were all below limit of detection (40 μg/kg).

40 μg/kg of vegetable. Cleanup by solid-phase extraction saves time and chemicals, and the method should be easily automated with the aid of the now commercially available sample pretreatment instruments.

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