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Rapid determination of abamectin in lettuce and cucumber by high-performance liquid chromatography

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ABSTRACT

A rapid, sensitive and reliable method is presented for the determination of trace amounts of abamectin in lettuce and cucumber. Abamectin consists of $\geqslant 80\%$ avermectin B1a and $\leqslant 20\%$ avermectin B1b. Vegetables were extracted with ethyl acetate and the extract was purified by solid-phase extraction using Sep-Pak silica cartridges. The purified extracts were analysed by high-performance liquid chromatography with a 5-μm Zorbax ODS column and UV detection under isocratic conditions. The method yields recoveries for avermectin B1a and B1b of 76–109% in the 0.054–0.54 mg/kg range. The limit of detection of the method is 40 μg/kg each of avermectin B1a and B1b in vegetables.

INTRODUCTION

The avermectins, a family of pesticidal agents, are macrocyclic lactones produced by the actinomycete *Streptomyces avermitilis* [1]. The avermectin structures were elucidated by Albers-Schönberg *et al.* [2], and some of the biological activities have been reviewed [3]. Abamectin is the commercial product that is being developed as an insecticide and acaricide with contact and stomach action. Abamectin consists of $\geqslant 80\%$ avermectin Bla and $\leqslant 20\%$ avermectin Blb (Fig. 1).

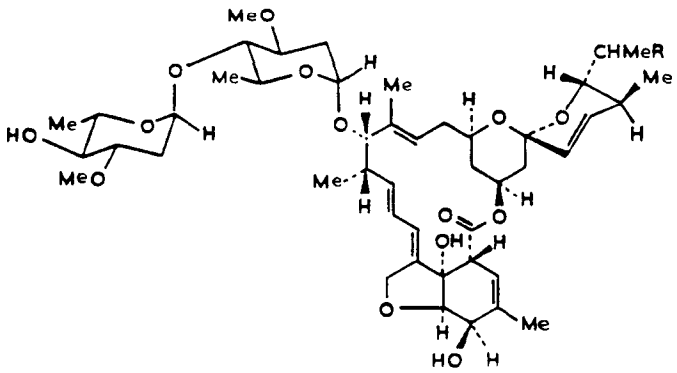

Fig. 1. Structures of the two major components of abamectin: Bla ($R = C_2H_5$), $\geqslant 80\%$, and Blb ($R = CH_3$), $\leqslant 20\%$. $Me = CH_3$.

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Several methods have been described for determining avermectins. Tolan *et al.* [4] and Tway *et al.* [5] described the determination of avermectins in plasma and ivermectin in tissue, respectively. Their methods are sensitive but also time consuming because of the elaborate clean-up steps and derivatization required. Fox and Fink [6] 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 *et al.* [7] and Maynard *et al.* [8] described the determination of avermectin Bla in citrus fruits. The methods are sensitive (detection limits 0.1 and 5 μg/kg, respectively), but radiolabelled avermectin (^{3}H 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 (avermectin Bla and Blb) in lettuce and cucumber. The vegetables were extracted with ethyl acetate and the extracts were purified by solid-phase extraction. Separation and detection were performed using reversed-phase HPLC with ultraviolet detection. Recovery experiments were carried out in lettuce and cucumber.

EXPERIMENTAL

Reagents

All reagents were of analytical-reagent grade. 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 Assoc. (Milford, MA, U.S.A.). An abamectin standard in glycerol containing 1.074% (w/w) avermectin Bla and 0.125% (w/w) avermectin Blb 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/l Bla and 6.28 mg/l Blb 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). A 50-g amount of chopped vegetables was weighed into a 250-ml centrifuge tube and 100 ml of ethyl acetate were added. The mixture was macerated with an Ultra Turrax for 1 min. The extract was centrifuged at 2000 *g* for 1 min and 50 ml of the supernatant were 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, then 3 ml of hexane were added. The contents of the flask were mixed and applied to a Sep-Pak silica cartridge. The flask was washed twice with 1 ml of 40% ethyl acetate in hexane and the washings were applied to the cartridge. The cartridge was first washed with 8 ml of ethyl acetate–hexane (40:60), then was eluted with 5 ml of ethyl acetate–methanol (50:50). This eluate was evaporated to dryness on a rotary evaporator at 40°C and the residue was dissolved in 1 ml methanol. Of this solution, 25 μl were injected into the liquid chromatograph.

HPLC analysis

A Spectra Physics 8100 liquid chromatograph with an SP 8400 UV detector and

an SP 4100 computing integrator was used. The column was a DuPont Zorbax ODS (25 cm × 4.6 mm I.D.) operated at ambient temperature. A Newquard RP-2 precolumn (15 × 3.2 mm I.D.) (Brownlee Labs.) was used to prevent contamination of the analytical column. The mobile phase was methanol–water (90:10) at a flow-rate of 1.0 ml/min. The UV detector was operated at 245 nm. Quantification 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 pipette 500-μl aliquots of abamectin standard solutions (54.0 mg/l of Bla and 6.28 mg/l of Blb or 5.40 mg/l of Bla and 0.628 mg/l of Blb) to a 50-g portion of chopped vegetables, which corresponds to 0.54 mg/kg of Bla and 0.0628 mg/kg of Blb or 0.054 mg/kg of Bla and 0.00628 mg/kg of Blb (which is below the limit of detection). To another 50-g portion nothing was added. After a few minutes the samples were analysed as described above. Recoveries were calculated as the difference between the amounts of avermectin Bla or Blb found in the spiked and in the non-spiked samples expressed as a percentage of the amount of avermectin Bla and Blb added.

RESULTS AND DISCUSSION

Fig. 2 shows a typical chromatogram of an abamectin standard solution. We used three different ODS columns: Zorbax ODS, LiChrosorb ODS and Nucleosil ODS. Of these columns, only the Zorbax ODS column yielded a good separation between avermectin Bla and Blb. Fig. 3 and 4 show chromatograms of lettuce extracts with and without abamectin and cucumber extracts with and without abamectin, respectively. The limit of detection, defined as three times the baseline noise was calculated to be 25 ng per injection, which corresponds to 40 μg/kg of avermectin Bla and Blb.

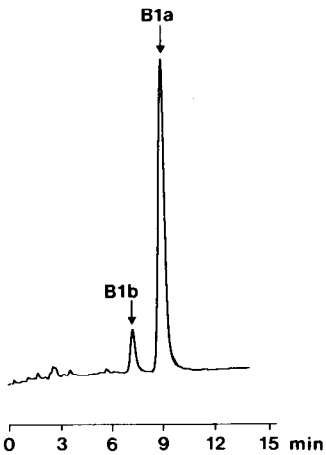

Fig. 2. Chromatogram of abamectine standard. Amounts injected: 810 ng of avermectin Bla and 94.2 ng of avermectin Blb.

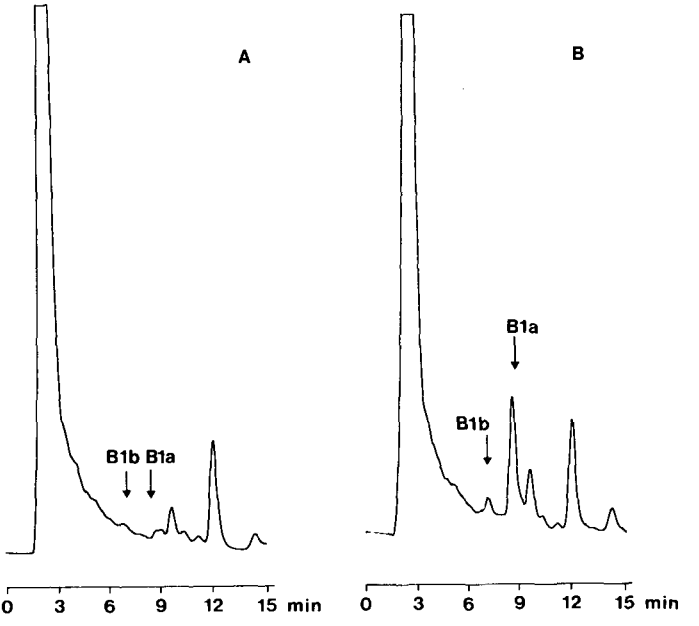

Fig. 3. Chromatograms of (A) unfortified lettuce extract and (B) lettuce extract fortified with 540 μg/kg of avermectin Bla and 62.8 μg/kg of avermectin Blb. Avermectin Bla and Blb peaks represent injected amounts of 337.5 and 39.25 ng of avermectin Bla and Blb, respectively.

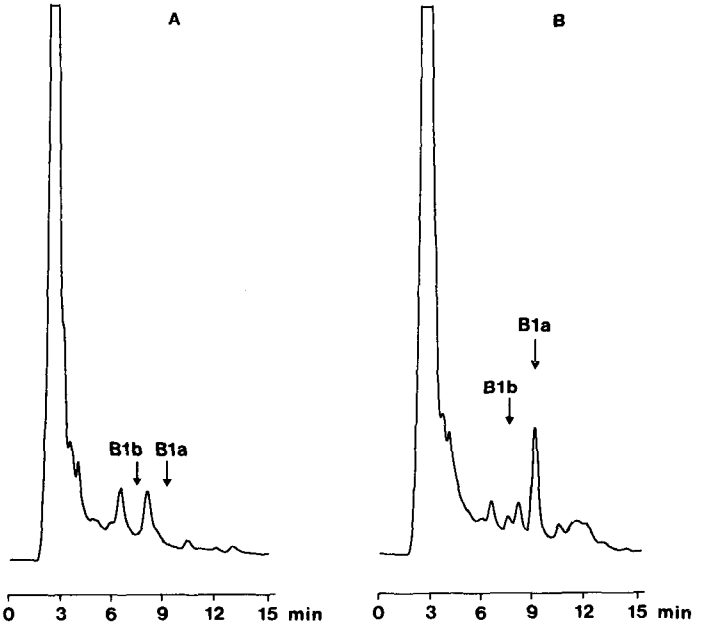

Fig. 4. Chromatograms of (A) unfortified cucumber extract and (B) cucumber extract fortified with 540 μg/kg of avermectin Bla and 62.8 μg/kg of avermectin Blb. Avermectin Bla and Blb peaks represent injected amounts of 337.5 and 39.25 ng of avermectin Bla and Blb, respectively.

TABLE I

RECOVERY STUDY OF ABAMECTIN ADDED TO CHOPPED LETTUCE

Avermectin Bla added (μg/kg)	Amount found (μg/kg)[a]	Recovery (%)	Avermectin Blb added (μg/kg)	Amount found (μg/kg)	Recovery (%)
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. ± S.D.		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. ± S.D.		103 ± 6			

[a] Non-fortified samples were all below the limit of detection (40 μg/kg).

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

The detector response is linear over a range of at least 25–1350 ng injected

TABLE II

RECOVERY STUDY OF ABAMECTIN ADDED TO CHOPPED CUCUMBER

Avermectin Bla added (μg/kg)	Amount found (μg/kg)[a]	Recovery (%)	Avermectin Blb added (μg/kg)	Amount found (μg/kg)	Recovery (%)
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. ± S.D.		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. ± S.D.		85 ± 3			

[a] Non-fortified samples were all below the limit of detection (40 μg/kg).

avermectin Bla (correlation coefficient $r = 0.9999$) and 15.7–157 ng injected of avermectin Blb ($r = 0.9999$).

Peaks were identified by comparing the retention times of the peaks on the sample chromatograms with those on the standard chromatograms. The results of a recovery study are given in Tables I and II. The lower fortification level of avermectin Bla in lettuce (Table I) resulted in more than a 100% recovery probably because of a small interfering peak. All other recoveries averaged *ca.* 85%, probably because of some loss in the sample clean-up. Mean recoveries were within the commonly accepted range of 80–110%. The relative standard deviations of the recovery determinations, were not higher than 7%, which is an acceptable precision for residue analysis.

CONCLUSION

Both avermectin Bla and Blb can be detected and determined in lettuce and cucumbers in one chromatogrphic run. The method is rapid, the extraction and clean-up are easy to carry out and derivatization is not necessary. The limit of detection for each component is 40 μg/kg. Clean-up by solid-phase extraction saves time and chemicals and the method should be easily automated with aid of the now commercially available sample pretreatment instruments.

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