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Determination of abamectin residues in fruits and vegetables by high-performance liquid chromatography

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Abstract

A rapid and sensitive HPLC method has been developed and validated for the determination of abamectin residues (avermectin B_{1a} and B_{1b}, as well as the metabolite 8,9-*Z*-avermectin B_1) in apples, pears and tomatoes. Residues are extracted with acetonitrile. The diluted extract is cleaned up on a C_{18} solid-phase extraction cartridge. Abamectin residues are derivatised with trifluoroacetic acid and 1-methylimidazole and determined by reversed-phase liquid chromatography with fluorescence detection (excitation: 365 nm and emission: 470 nm). High and consistent recoveries, ranging from 88 to 106%, were obtained, at spiking levels of 10, 20 and 50 μg/kg, when analysing apples, pears and tomatoes.

Keywords: Fruits; Vegetables; Food analysis; Abamectin; Pesticides; Avermectins; Lactones

1. Introduction

Abamectin (Fig. 1) is a product which was developed as an insecticide and acaricide with contact and stomach action [1]. It belongs to the family of avermectins which are macrocyclic lactones produced by the actinomycete *Streptomyces avemitilis*. Abamectin consists of ≥80% of avermectin B_{1a} and ≤20% of avermectin B_{1b} [1].

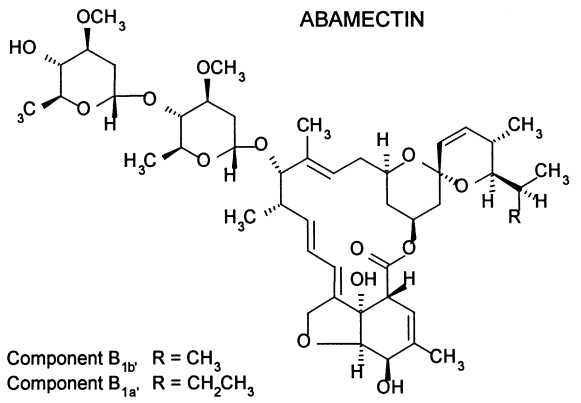

Fig. 1. Structure of abamectin.

Abamectin is notably used on apples, pears, vegetables (cucumbers, tomatoes, etc.) and cotton. Its use is increasing as a replacement for other acaricides, like amitraz [*N*-methylbis(2,4-xylyiminomethyl)amine], especially on apples and pears. As these fruits are often used as ingredients for baby foods, it is important to have a suitable method for the determination of low levels of abamectin.

Abamectin residues degrade by both oxidative and photochemical action to various products when applied to crops. However, the only residues of toxicological significance are avermectin B_1 and the metabolite 8,9-*Z*-avermectin B_1 [2]. Maximum residue limits (MRLs) are thus given as the sum of avermectin B_{1a}, avermectin B_{1b} and of 8,9-*Z*-avermectin B_1. The Codex Committee on Pesticide

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Residues under the Joint FAO/WHO Food Standards Programme have set MRLs of 0.01 to 0.02 mg/kg for fruits and tomatoes [3].

A high-performance liquid chromatography (HPLC) method using UV detection was reported for the analysis of abamectin residues in vegetables [4], but it was not sensitive enough to check compliance with legislation as the detection limit was only about 0.04 mg/kg. Other methods [5–8] prescribe the use of fluorescence detection after derivatisation with trifluoroacetic anhydride. In this way, a good sensitivity is obtained. For the extraction, it is prescribed either to use a mixture of acetonitrile, water and hexane and to take the hexane layer for further analysis [5,7] or to extract only with acetonitrile after adding salt to obtain phase separation between water and acetonitrile [6]. Subsequently, clean-up is performed on an aminopropyl or NH_2 solid-phase extraction (SPE) cartridge.

Attracted by its simplicity, we have tested the method described by Guggisberg et al. [8] for the analysis of ivermectin in meat and adapted for the determination of abamectin in fruits and vegetables. Only acetonitrile is used for the extraction and clean-up is performed on a C_{18} cartridge after dilution of the extract with water.

The metabolite 8,9-*Z*-avermectin B_1 gives the same derivative as that obtained from avermectin B_{1a} when derivatised with trifluoroacetic anhydride [5]. Therefore, it is possible to determine avermectin B_{1a}, the metabolite 8,9-*Z*-avermectin B_1 and avermectin B_{1b} with the procedure described.

2. Experimental

2.1. Principle

The avermectins were extracted with acetonitrile and clean-up was performed on a C_{18} Bond-Elut cartridge. Derivatization of samples and standards was done with trifluoroacetic anhydride and 1-methylimidazole and the derivatives were determined by HPLC with fluorescence detection.

2.2. Chemicals

Acetonitrile and water were of HPLC grade (LiChrosolv) and were obtained from Merck (Darmstadt, Germany). Triethylamine was of analytical-reagent grade and was purchased from Merck. Trifluoroacetic anhydride and 1-methylimidazole were obtained from Sigma (Buchs, Switzerland). An abamectin standard in acetonitrile (10 ng/μl) was bought from Dr. Ehrenstorfer (Augsburg, Germany).

2.3. Preparation of reagents

Derivatization reagent 1: add one volume of trifluoroacetic anhydride to two volumes of acetonitrile in a brown-glass flask. Keep this solution at +4°C. Prepare fresh every week.

Derivatization reagent 2: add one volume of 1-methylimidazole to one volume of acetonitrile in a brown-glass flask. Keep this solution at +4°C. Prepare fresh every week.

Conditioning solution for C_{18} Bond Elut cartridge: add 30 ml of acetonitrile to 70 ml of water and add 0.1 ml triethylamine. Mix well.

HPLC mobile phase: acetonitrile–water (94:6, v/v).

2.4. Abamectin standard solutions

Stock solution for HPLC: pipette 1 ml of abamectin solution (10 ng/μl) and dilute to 50 ml with acetonitrile. Shake well to mix. This solution contains 200 ng/ml. Keep this solution at +4°C.

Remarks: All standard solutions should be made in acetonitrile or methanol. The solubility in water is very low (<10 ng/ml) [2].

2.5. Material and apparatus

Polytron: Polytron aggregate, Typ PT 10, Kinematica, Switzerland. HPLC system: Waters 600E multisolvent delivery system (Waters). Detector: fluorescence detector, Waters 474. HPLC Column: RP18, Merck Endcapped LiChrocart (12.5 cm×4 mm, 5 μm). C_{18} Bond-Elut cartridge (3 ml/500 mg), Varian, cat. No. 1210-2028. Condition just before use as follows: connect the C_{18} Bond-Elut cartridge to the vacuum manifold. Rinse the cartridge with 5 ml of acetonitrile and then with 5 ml of the

conditioning solution for SPE (acetonitrile–water–triethylamine). Do not let sorbent run dry during conditioning and before applying sample. Glassware: abamectin being sparingly soluble in water, it is important to rinse all the glassware with ethanol or acetone before use.

2.6. Analytical procedure

Weigh 5.00 g of homogenised sample in a 50-ml centrifuge tube, add 10 ml acetonitrile and extract for 1 min by blending with a Polytron (10 000 rpm). Allow to decant and transfer the supernatant liquid to a 50-ml graduated cylinder. Extract the residue a second time with 10 ml acetonitrile and combine the extracts in the 50-ml cylinder. Add 50 μl triethylamine and bring the volume to 50 ml with distilled water.

Transfer the entire final test portion extract (50 ml) to the C_{18} cartridge fitted with adapter and 50 ml reservoir. Apply vacuum and discard the eluent. Allow the cartridge to dry by passing air during 10 min. Elute the avermectins with 5 ml acetonitrile and collect in a 5-ml amber micro-reaction vial. Evaporate the extract just to dryness under a stream of dry nitrogen using a heating block at 50°C.

Add 300 μl of the derivatisation reagent 1 and 200 μl of the derivatisation reagent 2 to the reaction vial. Mix well on vortex mixer. Allow to cool at room temperature (about 5 min) and determine avermectins by liquid chromatography.

Remarks: depending on the product, it may be necessary to centrifuge before transferring the liquid

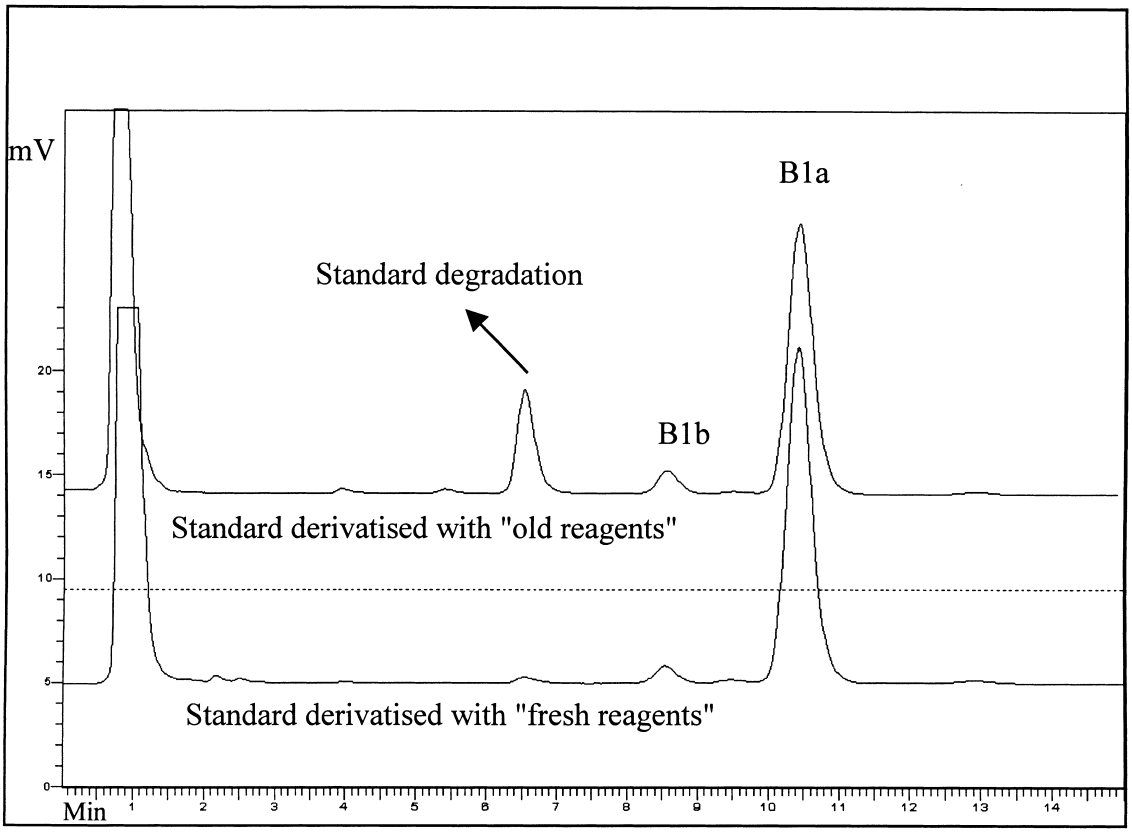

Fig. 2. Standard derivatised with old reagents gives an extra-peak. HPLC conditions: RP18 Merck Endcapped LiChrocart (12.5 cm×4 mm, 5 μm), acetonitrile–water (94:6, v/v), 1.5 ml/min, fluorimeter, excitation: 365 nm/emisssion: 470 nm.

extract to the measuring cylinder. Make sure that the acetonitrile–water ratio is 4:6. A higher concentration of acetonitrile will lead to losses of abamectin during transfer of extract to the SPE cartridge (C_{18} Bond-Elut).

2.7. Standard preparation

Pipette 250 μl, 500 μl and 1.25 ml of the standard solution containing 200 ng/ml into three 5-ml amber micro-reaction vials. Evaporate to dryness under a stream of nitrogen using a heating block at 50°C. Perform the derivatisation as described for the samples. These three standards contain, respectively, 50 ng, 200 ng and 500 ng.

Remarks: run freshly prepared standards with each series of samples because derivatised standards are not stable for more than one day (two in fridge).

2.8. Determination by HPLC

For HPLC determination, a RP18 Merck End-capped LiChrocart (12.5 cm×4 mm, 5 μm) column was used. The mobile phase consisted of acetonitrile–water (94:6, v/v) and the flow-rate was 1.5 ml/min. The entire system was allowed to stabilise for 15 to 20 min. The fluorescence detector was operated at 365 nm for excitation and 470 nm for emission. Aliquots of 50 μl of standard or test portion extract were injected.

Avermectin B_{1b} and avermectin B_{1a} elute, respectively, after about 8 and 10 min. The metabolite

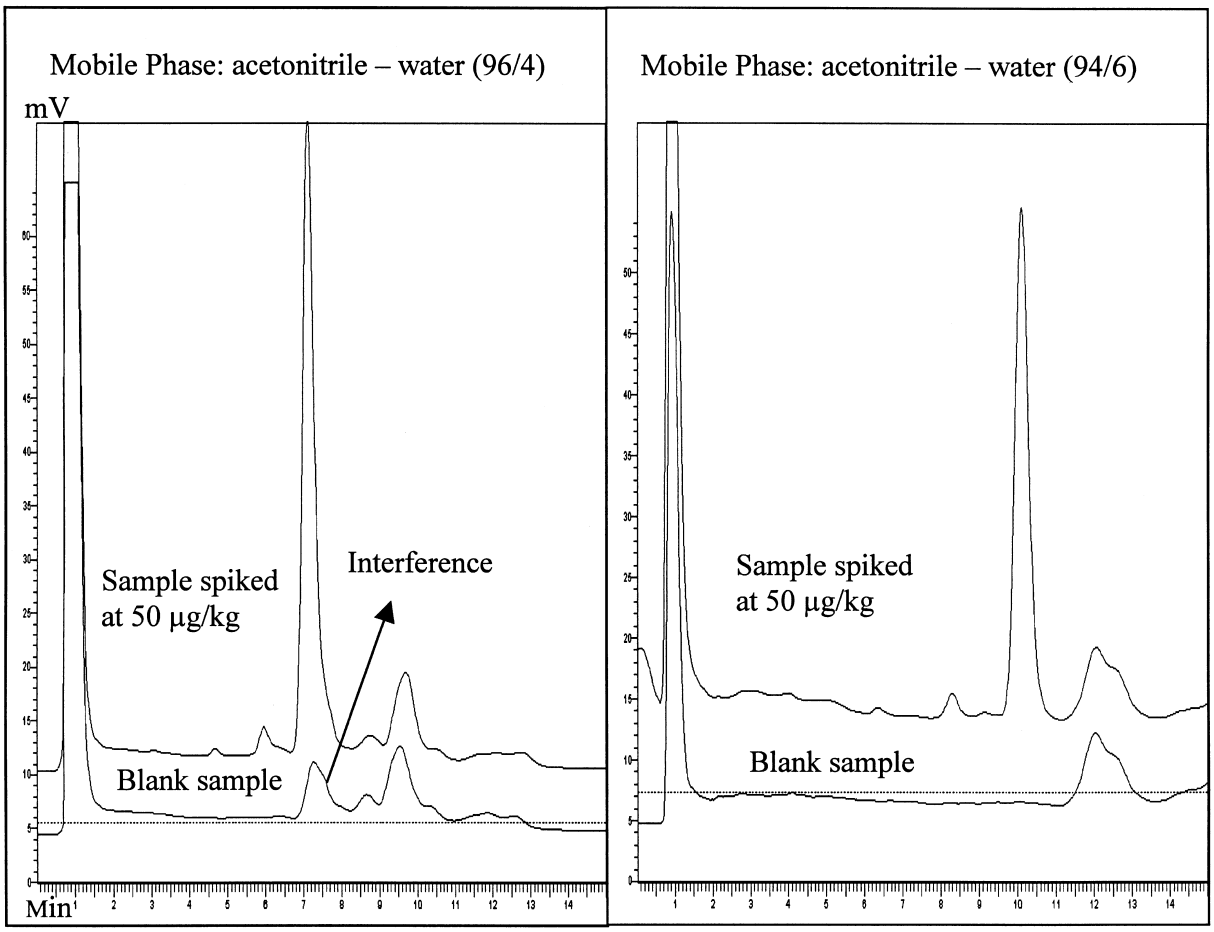

Fig. 3. Chromatograms obtained when performing HPLC analysis of tomato extracts using different mobile phases.

8,9-*Z*-avermectin B_1 yields the same derivative as that obtained with avermectin B_{1a}.

3. Results and discussion

3.1. Method refinement

Most of the published methods for the determination of abamectin have used either liquid–liquid partition to obtain residues in hexane prior to SPE on aminopropyl or NH_2 cartridges [4–7]. By using a C_{18} cartridge [8], it was possible to directly apply the diluted acetonitrile extract to the column. To avoid losses of abamectin residues, it is important to have no more than 40% acetonitrile in the clean-up step. A small amount of triethylamine was added to the extract to reduce absorption of the analyte on residual silanol groups of the SPE cartridge material. By comparing C_{18} cartridges from various suppliers, Bond Elut C_{18} cartridges from Varian were found to give better and more consistent recoveries.

The derivatisation reaction to produce the fluorescent derivatives is instantaneous [5]. When using reagents older than one week, it was found that the yield was lower and a peak eluting earlier appeared on the chromatogram (see Fig. 2). Thus, it is important to prepare each reagent fresh every week and to store them at +4°C. Derivatives being light sensitive, amber vials are used in the derivatisation step and for HPLC. At normal temperature, derivatives are stable for about 10 h. After this time, peak response tends to decrease significantly. However, derivatised extracts kept for 24 h at 4°C have not shown any deterioration.

The first experiments were carried out by using

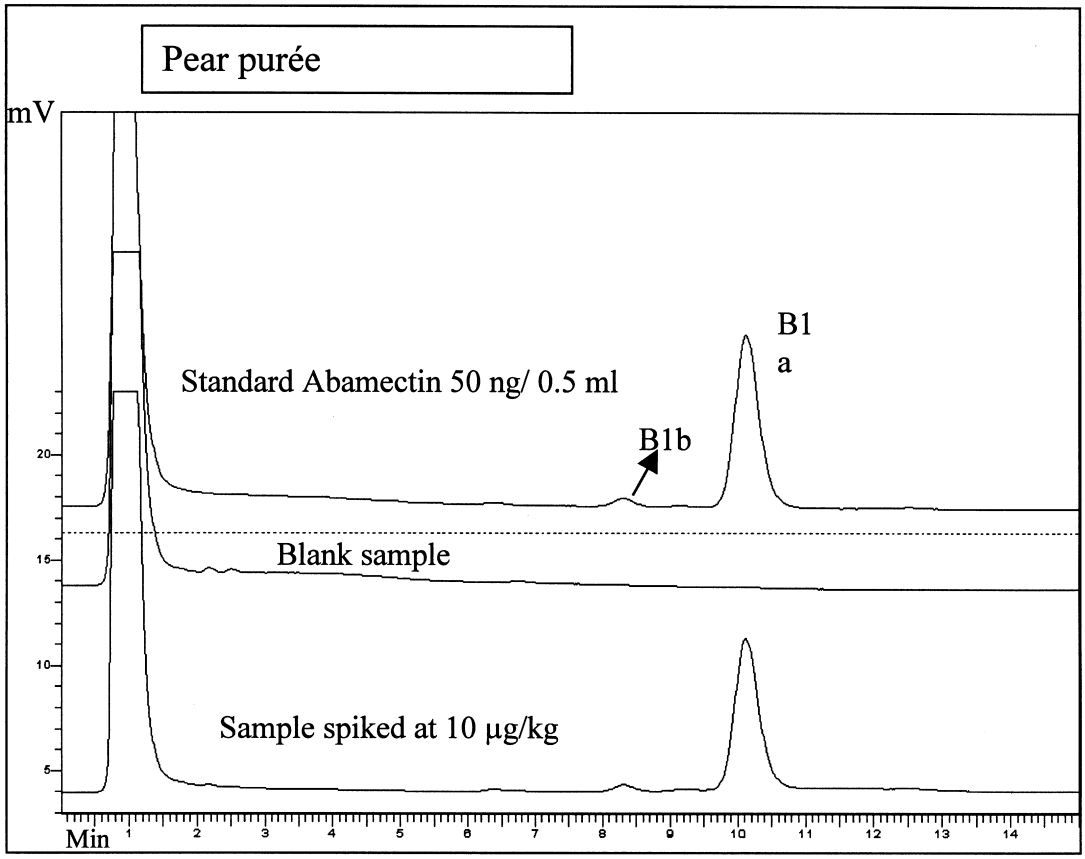

Fig. 4. Chromatograms obtained when analysing pear purée. HPLC conditions as in Fig. 1.

Table 1
Recoveries after adding 10, 20 and 50 μg/kg to apple, pear and tomato (five replicates)

Product	Spiking level (μg/kg)	Recoveries (%)	Average recoveries (S.D.)
Apple	10	98, 97, 98, 93, 96	96.4 (2.1)
	20	99, 97, 98, 92, 100	97.2 (3.1)
	50	98, 100, 100, 99, 97	98.8 (1.3)
Pear	10	104, 102, 88, 100, 103	99.4 (6.5)
	20	99, 98, 92, 97, 95	96.2 (2.8)
	50	92, 94, 99, 96, 92	94.6 (3.0)
Tomato	10	101, 98, 101, 100, 102	100.4 (1.5)
	20	106, 105, 103, 97, 97	101.6 (4.3)
	50	94, 94, 97, 94, 93	94.4 (1.5)

acetonitrile–water (96:4, v/v) as mobile phase. Derivatives eluted after 6 and 7.2 min and no interferences have been found when analysing apples and pears. However, when analysing tomato products, we observed interferences. By increasing the proportion of water to 6%, separation of peaks corresponding to interference and abamectin derivatives was readily obtained (see Fig. 3). The same mobile phase also yielded good results for apples and pears (see Fig. 4).

3.2. Method evaluation

As abamectin is sparingly soluble in water [2], all recoveries were run by adding abamectin in acetonitrile to the substrates of interest.

Test portions of apples, pears and tomatoes spiked at three levels (10, 20 and 50 μg/kg) of abamectin were analysed five times by using the prescribed procedure. The results, summarised in Table 1, indicate that the recoveries ranged from 88 to 106% and that the repeatability was satisfactory.

The limit of detection measured on working standard solutions corresponds to less than 1 μg/kg with a 5 g test portion. Under these conditions, the limit of quantitation, depending of the sensitivity of the fluorescence detector, is between 0.002 and 0.005 mg/kg.

4. Conclusion

The method presented is simple and rapid. Moreover, correct and repeatable results were obtained when analysing tomatoes, pears and apples at different spiking levels.

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