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# Determination of Abamectin Residue in Paprika by High-Performance Liquid Chromatography

Wen-Ming Xie[1,2], Kwang-Yong Ko[1], Sung-Hun Kim[1],
Hee-Ra Chang[3], and Kyu-Seung Lee[1]*

[1]Department of Agricultural Chemistry, Chungnam National University, Daejeon 305-764, Korea
[2]College of Resources and Environmental Sciences, Jilin Agricultural University, Changchun 130-118, China
[3]Environmental Chemistry Team, Korea Institute of Toxicology, Daejeon 305-343, Korea
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**ABSTRACT: Reversed-phase high-performance liquid chromatography (HPLC) techniques were developed to quantify abamectin (ABM) in paprika (*Capsicum annum*). Separation was achieved on a $C_{18}$ ODS column with a mobile phase of acetonitrile/water (96/4, v/v) mixture in an isocratic elution at the flow rate of 1.2 mL/min for avermectins (AVMs). The retention times were 8.0 and 9.7mins for AVM $B_{1b}$ and AVM $B_{1a}$, respectively. Residual AVMs (sum of AVM $B_{1a}$, AVM $B_{1b}$ and 8,9-Z-AVM $B_{1a}$) in the vegetable were extracted with acetonitrile, and the silica solid-phase extraction cartridges were used to purify the extracts. AVMs were derivatized using trifluoroacetic acid and 1-methylimidazole, and the derivatives were determined with a fluorescence detector (excitation at 365 nm and emission at 470 nm). High and consistent recoveries, ranging from 93% to 115%, were obtained for AVM $B_{1a}$ and 8, 9-Z-AVM $B_{1a}$ at fortified levels of 20 μg/kg and 200 μg/kg for paprika. The limit of quantitation (LOQ) was 2 μg/kg. The residual levels of AVMs in paprika in a field experiment from one day to seven days after the last application decreased from 18.40 to 7.59 μg/kg. The half-life ($T_{1/2}$) of AVMs in paprika was 1.47 days.**

**Key Words:** Avermectin, paprika, residue, HPLC, fluorescence detection

## INTRODUCTION

Paprika (*Capsicum annum*) is one of the most important vegetables being cultivated in Korea. The rich coloring of paprika not only enhances the visual appeal of the vegetable, but leads to use as a fascinating flavoring source in pickling. Paprika is also used as a colorizing agent in foods and cosmetics. The paprika crop is usually attacked by a variety of pests, which multiply rapidly, and cultivators apply a combination of several types of pesticides to control these pests. In order to control the spider mite pests, one of the major pests in paprika cultivation, some pesticides including abamectin (ABM) have been applied. ABM is a highly pesticidal agent that contains a macrocyclic lactone derived from the soil bacterium *Streptomyces avermitilis*[1]. ABM is the product developed by Merck Co. Inc. as acaricide, insecticide and nematicides for crop protection[2]. Among ABMs, a mixture of different avermectins (AVMs), AVM $B_1$ is the major active ingredient and consists of a mixture of two homologs. AVM $B_1$ contains not less than 80% AVM $B_{1a}$ and not more than 20% AVM $B_{1b}$. These two components differ only by one methylene unit (-$CH_2$-) at the 25th carbon position; AVM $B_{1a}$ contains a sec-butyl group and AVM $B_{1b}$ contains an isopropyl group as shown in Fig. 1. AVM $B_{1a}$ has been shown to degrade to the 8,9-Z-AVM $B_{1a}$ on citrus fruits. The photolysis of AVM $B_{1a}$ in organic solution, aqueous solution or as a film also results in the

*연락저자:
Tel: +82-42-821-6375 Fax: +82-42-822-5781
E-mail: kslee@cnu.ac.kr

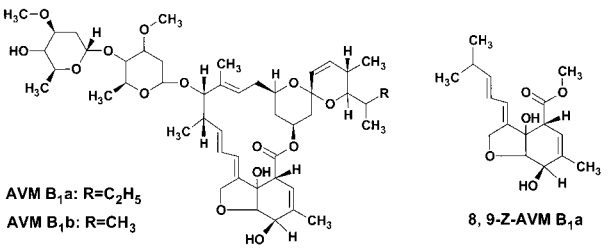


**Fig. 1. Chemical structures of avermectin $B_{1a}$, $B_{1b}$ and 8,9-Z-avermectin $B_{1a}$.**

formation of 8, 9-Z-AVM $B_{1a}$. Most AVMs are highly lipophilic substances and are dissolved in most organic solvents, but are poorly soluble in water[3]. In the environment, AVMs are quickly degraded (half-life, 4-21 h) by oxidative and photo-oxidative mechanisms when exposed to light in water on a thin biological surfaces (e.g. leave) or bounded to the soil particles[4].

The high molecular weight of the AVMs (>800 daltons) leads liquid chromatography as the most suitable chromatographic technique for determination. Liquid chromatographic methods using ultraviolet (UV) detection and fluorescence detection for the AVMs residue in different kind samples were reported. Johan described the determination of AVMs in lettuce and cucumber by high performance liquid chromatography (HPLC) with UV detector[5]. However, the method was not sensitive enough (limit of detection 40 μg/kg). So, HPLC with fluorescence detection following a fluorescent derivatization of the parent compounds using trifluoroacetic acid and a basic catalyst (such as methylimidazole) has been a common analytical method for monitoring ABM residues in tissue, milk, fruits, and vegetables[6-10]. Liquid chromatography/tandem mass spectrometry (LC/MS/MS) analysis was developed for the determination of AVMs in milk, liver, muscle, and food commodities[11-15]. The ELISA method was also used to detect ABM, IVM, and EPR residues in bovine liver tissues, with a limit of quantization of 1.06 ng/mL for all kinds of three AVMs[16].

Of all above methods, fluorescence detection offered the most specific method with the lowest detection limit. However, the methods published have been applied for measurements in complex matrices like plants and animal tissues. The very low concentrations in these tissues required a very laborious procedure including extraction, several clean-up steps, and derivatization before final HPLC-fluorescence detection. So the main purpose of this study was to develop a rapid, sensitive, and quantitative technique for the determination of AVMs in paprika. Since concentration of the sample extracts often led to severe interference by concurrent co-extracts, rigorous purification of sample extracts must be required. Therefore, this study was mainly focused on the development of an efficient but simple analysis method to detect the residual level and clarification the dissipation patterns of AVMs residue in paprika under greenhouse condition.

## EXPERIMENT

### Sample preparation

The field experiment on paprika was conducted under greenhouse condition at Bulgok-Myeon, Deajeon City of Korea in 2004. The paprika seedlings were transplanted in the middle of June. The plots were randomly distributed in the greenhouse, and the temperature was kept at 13-33℃ and the humidity at 66-100%. Abamectin (EC, 20% a.i.), was applied as 2000-time dilute spray with water when mite outbreak was found in September. There were a total of three applications with the interval of seven days before the fruits were sampled for residue analysis after the last application. Each treatment, including control, was replicated four times in a randomized block design. Paprika samples were plucked randomly from each treatment at different time intervals (1, 3, 5 and 7 days). Approximately 500 g of the sample homogenate was retained in the labeled plastic bags. The samples were stored at or below -24℃ until analyzed. Before subsampling for analysis, the samples were partially thawed and mixed thoroughly, whenever appropriate to ensure that a representative sample was aliquoted.

### Reagents and equipment

All reagents were analytical quality. Water, methanol, and acetonitrile (UV and pesticide residue grade) were obtained from Burdick & Jackson (Muskegon, USA). Trifluoroacetic anhydride and 1-methylimidazole were obtained from Sigma (St. Louis, MO, USA). The solid-phase extraction was done with Sep-Pak Vac silica cartridges (1,000 mg, 8 mL) obtained from Waters Associates (Milford, MA).The AVM standard containing 85.5% w/w AVM $B_{1a}$ and 6.9% w/w AVM $B_{1b}$ was obtained from Syngenta AG NOA. The degradation

compound 8, 9-Z-AVM $B_{1a}$ was also supplied by Syngenta.

The HPLC analysis was carried out with a Shimadzu CLASS $LC_{10}$ system (Shimadzu, Kyoto, Japan) equipped with a SPD-$M_{10}$Avp photo-diode array detector in the range of 200-400 nm and an Rf-10Axl fluorescence detector. The HPLC separation was conducted using a Symmetry $C_{18}$ stainless column (3.9 mm i.d.×150 mm, Waters Associates). The mobile phase was eluted with acetonitrile/water (96/4, v/v) solution at the flow rate of 1.2 mL/min after degassing with Shimadzu DGU-$20A_3$. The column temperature was maintained at 30℃. An aliquot of 10μL was injected using SIL-$10_A$ auto-sampler.

## Extraction and purification

To 5.00 g of homogenized sample in a flask 50 mL acetonitrile was added, and then it was extracted for 30 minutes by shaking with a flash shaker (KMC-1205SL, Vision Scientific Co.). The extract was filtered through a Buchner funnel and the filter cake was washed with 50 mL fresh acetonitrile. The filtrate was concentrated in vacuum to 10-20 mL and transferred to a 1 L separatory funnel. To the concentrate, 10 mL of saturated sodium chloride solution and 500 mL of water were added. The container was washed with 100 mL of dichloromethane, and the solution was poured into the separator. Then mixture was shaken vigorously for 60 seconds and left to standard until the layers separated. The dichloromethane layer was transferred to a flask and the water layer remained was extracted with another 25 ml dichloromethane and combined the dichloromethane part. About 15 g of sodium sulfate was added, a stopper was placed on the flask, and the solution was shaken vigorously. Care was taken such that the extract remains with the sodium sulfate lese than one hour to minimum losses of pesticides by adsorption. Then the extract was transferred to a round-bottomed flask and was evaporated to dryness on a rotary evaporator (Buchi, Rotavapor R-124) at 40℃.

Solid phase extraction was followed to clean up the extract based on the method described by Johan[5], the residue was dissolved in 2 mL of ethyl acetate and 3 mL of hexane was added to prepare 40% ethyl acetate in hexane. The contents of the round-bottomed flask were mixed and sampled to a conditioned silica cartridge. The round-bottomed flask was washed two times with 1 mL of 40% ethyl acetate in hexane, and the washings were also applied to the cartridge. The cartridge was washed with 8 mL of 40% ethyl acetate in hexane and were eluted with 10 mL of 50% ethyl acetate in methanol. The last part of elute in a glass tube was evaporated to dryness with $N_2$ on a nitrogen evaporator (N-EVAP, Organomaion Associates, JNC, USA) at 50℃.

## Derivatization and analysis

Fluorescent derivatization was performed according to the method described by Diserens[6]. Deriving reagent I was prepared by adding one volume of trifluoroacetic anhydride to two volumes of acetonitrile in a brown-glass flask. Deriving reagent II was made by adding one volume of 1-methylimidazole to one volume of acetonitrile in a brown-glass flask. The two reagents were stored at 4℃ and prepared fresh at least once each week. For standards and samples, 300 μL of the reagent I and 200 μL of the reagent II were added to the reaction vial, which was sealed with a stopper, and mixed well on vortex mixer. The reacting mixture was cooled to room temperature and the derivatives of AVMs by HPLC were determined.

Stock standard solutions of AVM $B_{1a}$, $B_{1b}$ and 8,9-Z-AVM $B_{1a}$ were prepared in acetonitrile and stored at 4℃. Aliquots of the stock solution were diluted with acetonitrile to provide working standards ranging from 11.9 to 1196 ng/mL for AVM $B_{1a}$ and 0.966 to 96.6 ng/mL for AVM $B_{1b}$.

The specified standard solution containing AVMs was piped into a 2 mL sampling vial and evaporated to dryness under a gentle stream of nitrogen. The derivatization was performed as described above.

The AVM residues were identified and quantified by comparing with the retention time and peak height of standard AVMs. It was demonstrated that derivatization of AVM $B_{1a}$ and 8, 9-Z-AVM $B_{1a}$ isomer produced the identical derivatives. Thus, the peak at the retention time of $B_{1a}$ represents the total amount for $B_{1a}$ and its degradation products. Because $B_{1b}$ is about 20% of the active ingredient, its residue level is normally quite low and is always lower than the sum of AVM $B_{1a}$ and 8, 9-Z-AVM $B_{1a}$ residue level.

## Validation of analytical method

Recovery experiments were conducted by using the

control samples to assess the analytical method proposed for AVMs residues. Prior to extraction, the series of the control samples were fortified with AVMs standard solution in acetonitrile at specified concentrations according to the MRL in pepper and linear range. After standing for 2 hours, the analytical procedures mentioned above were carried out to produce recovery data.

## RESULTS AND DISCUSSION

### Method evaluation

AVMs and the degradation compound 8, 9-Z-AVM $B_{1a}$ were determined in paprika using the HPLC method with fluorescence detector. The Fig. 2 shows the UV absorption spectra of AVM $B_{1a}$ and fluorescence spectra of AVM $B_{1a}$ derivative. The absorbance spectra were shown to exhibit absorbance maxima at 244 nm for AVMs and 242 nm for 8, 9-Z-AVM $B_{1a}$. According to the excitation and emission spectra of AVM, 365 nm and 470 nm were chosen as excitation and emission wavelength to get the highest detection sensitivity.

The RP-HPLC system used for this method provided an excellent separation for all three compounds showing baseline separation. The representative chromatograms of a mixture of these three compounds and their derivatives are shown in Fig.3. The derivative formation was instantaneous[17]. When the aged reagents were used, the yield was decreased and an extra peak appeared[6]. Using acetonitrile-water (96:4, v/v) as mobile phase, derivatives eluted after 8.0 and 9.7 minutes and no interference was found in paprika extracts.

The standard curves for these AVM $B_{1a}$+8, 9-AVM $B_{1a}$ and AVM $B_{1b}$ exhibited excellent linearity over the following concentration ranges: AVM $B_{1a}$+8, 9-AVM $B_{1a}$, 11.97-1197 ng/mL, AVM $B_{1b}$, 1-96.6 ng/mL. The linear ranges were selected on the basis of expected residue concentration and the MRL of ABM in paprika. The linear correlation coefficients of calibration curves for AVM $B_{1a}$+8, 9-Z-AVM $B_{1a}$ and AVM $B_{1b}$ were 0.999 and 0.998, respectively.

Paprika samples spiked at two levels (20 and 200 μg/kg) of ABM were analyzed three times using the prescribed procedure for extraction, clean-up and derivatization. The results, summarized in Table 1, indicate that the recoveries, ranged from 93% to 115%, and the repeatability, less than 10% ,was satisfactory for trace residue analysis.

The lowest level of quantitation for AVMs in paprika was calculated as 2 μg/kg for AVM $B_{1a}$, including 8,9-Z-AVM $B_{1a}$ ,with a 5 g spiking sample portion. Considering the MRLs of AVMs in most vegetables, range from 10 to 50 μg/kg, the sensitivity was high enough for routine residue analysis of fruit and vegetable samples.

### Terminal residue of AVMs in the field experiment

ABM has been linked to a wide spectrum of human health hazards, ranging from short-term impacts to chronic impacts. Chronic health effects may occur years

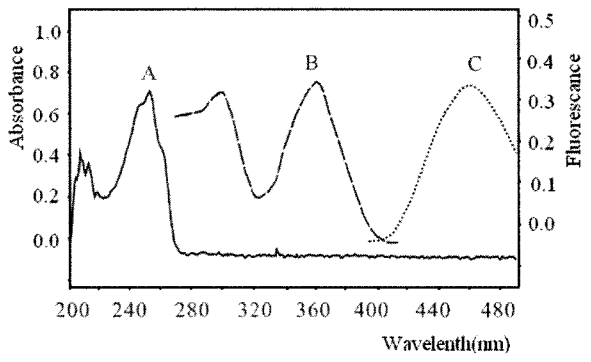


**Fig. 2. UV absorption spectra of avermectin (AVM) and fluorescence spectra of AVM derivative.**

A: UV absorption spectrum of AVM B1. B and C: excitation and emission spectrum of AVM B1 derivative

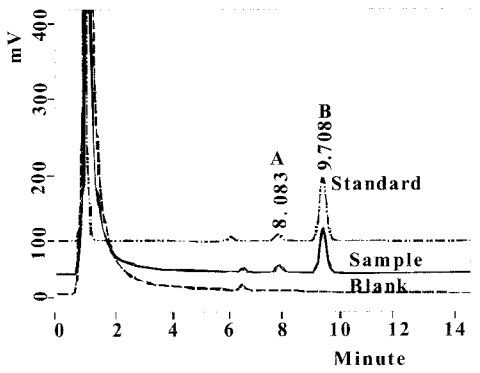


**Fig. 3. Chromatograms of AVM derivatives in standard and sample extract. A: AVM $B_{1b}$, B: AVM $B_{1a}$+8, 9-Z-AVM $B_{1a}$.**

HPLC conditions: RP Symmetry $C_{18}$ stainless column (3.9 mm i.d.×150mm, Waters Associates). The mobile phase, consisting of water in acetonitrile (4+96, v/v), was pumped at the total flow of 1.2 mL/min

**Table 1. Recoveries of avermectins (AVMs) from paprika**

| Pesticides | Fortified concentration (g/kg) | Recoveries (%) | Average recoveries (%) | RSD (%) |
|---|---|---|---|---|
| AVM $B_{1a}$+8, 9-Z-AVM $B_{1a}$ | 20 | 115.1, 105.1, 115.2 | 111.8 | 5.8 |
| | 200 | 93.3, 93.9, 93.7 | 93.6 | 0.3 |
| AVM $B_{1b}$ | 2 | 60.7, 60.1, 67.8 | 62.9 | 4.3 |
| | 20 | 115.3, 121.0, 129.0 | 121.8 | 6.9 |

**Table 2. Residual levels of avermectins (AVMs) in paprika after elapsed days of last application**

| Days after application | Residue (μg/kg, fresh weight) | | | Regression equation (half-life, d) |
|---|---|---|---|---|
| | AVM $B_{1a}$+8, 9-Z-AVM $B_{1a}$ | AVM $B_{1b}$ | Total | |
| 1d | 16.89 ± 1.22 | 1.42 ± 0.24 | 18.40 ± 1.10 | $C=17.91e^{-0.47t}$ (1.47) |
| 3d | 9.40 ± 0.91 | 0.79 ± 0.16 | 10.18 ± 0.99 | |
| 5d | 7.50 ± 0.70 | 0.57 ± 0.09 | 8.07 ± 0.66 | |
| 7d | 7.11 ± 0.61 | 0.48 ± 0.11 | 7.59 ± 0.71 | |

Mean values of four replicates with standard deviation

even after minimal exposure to them in the environment, or result from their residues ingested through vegetables, food, and water[19]. So, an acceptable daily intake (ADI) of 0.2 μg/kg body weight (b.w.) for ABM was allocated in 1994 on the basis of a no-observed-adverse-effect level (NOAEL) of 120 μg/kg b.w. per day in a multigenerational study of reproductive toxicity in rats, using a safety factor of 500. The increased safety factor was used because of concern about the teratogenicity of the 8, 9-Z-AVM $B_{1a}$, which is a photolytic degradation product that forms a variable proportion of the residue on crops. Estimate of ADI for humans (sum of AVMs and 8.S9-Z-AVM $B_{1a}$ isomer) is 2 μg/kg b.w..

Since the presence of these residues in fruits and vegetables can affect consumer health, the regulatory authorities have established MRL that are range from 0.01 to 10 mg/kg for most common vegetables and fruits. The MRL of ABM has not been established for paprika in Korea, but in Europe the MRL recommended for sweet pepper is 50 μg/kg. For this field experiment, the residue levels of AVMs in paprika were found to be rather lower than the MRL of sweat pepper even on the first day after application. For the untreated control, no AVMs were detected. The residual level was decreased gradually from 18.40 to 7.59 μg/kg for total AVMs in the first day and seventh day after application, respectively. The $T_{1/2}$ of AVMs in paprika is 1.47 days. As only trace residue was detected in the 7-day samples (<10 μg/kg), it might be stated that the AVM residue may not pose any residual toxicity problem in paprika, which also fits with the ABM application method and application rate in the region of Korea.

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