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Short-Time Derivatization Method for Analysis of Abamectin in Water Using High-Performance Liquid Chromatography Coupled to Fluorescence Detector

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Abstract

A method using a short-time derivatization step for the assessment of abamectin in water is presented. Abamectin derivative stable up to 7 days was obtained. Some regions where orange crops are present have received abamectin doses, aiming to increase the productivity and to combat pests and weeds, even when its residues reach the aquatic environment and interfere on water quality. Water samples from Jacaré-Pepira River (Brotas City, Brazil) nearby orange crops around urban zone, were evaluated for the presence of abamectin. The analytical method was validated resulting recovery around 108%, precision of 12%, accuracy of 104%, correlation coefficient of 0.9945, and detection and quantification limits of 0.1 μg·L^{-1} and 0.2 μg·L^{-1}, respectively. Stable abamectin derivative was reached after 60 min of derivatization at room temperature (25˚C). No abamectin residues were found into samples.

Keywords

Abamectin, Derivatization, SPE, HPLC-FLD, Water

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1. Introduction

Avermectin B_1 is a macrocyclic lactone naturally produced by actinomycete bacteria (*Streptomyces avemitilis*). It is a neurotoxin used as a pesticide in agriculture and as an acaricide and anthelminthic agent for livestock. Known as abamectin (ABA) in Brazil, it has high biological activity against parasites that attack different crops, being orange one of them [1]-[4]. The name abamectin refers to the mixture of two homologues (**Figure 1**), avermectin B_{1a} (≥80%) and avermectin B_{1b} (≤20%) [5]. Both homologues degrade by oxidative and photo-oxidative processes, having half-lives shorter than 24 h in aquatic environments [3] [6]. The transformation product 8,9-Z-avermectin B_{1a}, as well as avermectins B_{1a} and B_{1b} are considered to be of toxicological importance [7]. The European Union [8] indicates ABA as an expression of the sum of those three substances and establishes maximum residue limits (MRLs) only in food, animal and vegetables matrix, with values ranging from 0.01 to 1.0 $mg \cdot kg^{-1}$. Therefore, no environmental matrix is considered, such as in Brazil. The Brazilian Health Surveillance Agency (ANVISA) does not consider avermectin B_{1b} an important residue to be controlled.

Liquid chromatography coupled to fluorescence detector (LC-FLD), ultraviolet detector (LC-UV) and mass spectrometer (LC-MS), as well as immunochemistry assays have been reported as analytical methods for avermectins analyses [9]-[11]. Most of them use a derivatization step while analyzing complex matrices, such as milk [11] [12]. Reemtsma *et al.* [10] developed a LC-MS method for determining ABA in water; however, no derivatization step was used. Recently, Novelli *et al.* [13] reported a study evaluating the abamectin toxicity to some organisms. ABA was quantified in aqueous solution using a method based on solid phase extraction followed by determination in high performance liquid chromatography couple to fluorescence detector (SPE/HPLC-FLD).

Although the US-EPA adopted methods based on LC-FLD for analyzing those substances, many researchers [9] [12] reported difficulties mainly regarding the abamectin derivatization step. Avermectin B_{1a} and its isomer 8,9-Z-avermectin B_{1a} are converted to identical fluorescents derivatives in the presence of trifluoroacetic anhydride (TFAA), making the fluorescence detector unable to discriminate between them [14].

Among the analytical techniques used for the analyses of macrocyclic lactones, Berendsen *et al.* [12] and Danaher *et al.* [15] highlighted the selectivity and the sensitivity of HPLC-FLD and HPLC-MS. However, both authors reported problems with the analytes derivatization step, mostly regarding the derivatives stability and the length of this step in the sample preparation.

In comparison to older pesticides, abamectin is effective using lower doses; nevertheless, ecotoxicological assessments have found high ecotoxicity towards fishes [2] [3] [6]. Tisler and Erzen [6] assessed the abamectin ecotoxicity towards algae (*Scenedesmus subspicatus*), bacteria (*Vibrio fischeri* NRRL-B-11177), daphnids (*Daphnia magna*), and fishes (*Danio rerio*). According to the EU classification criteria for dangerous substances, the authors concluded that abamectin was highly toxic for aquatic organisms. In Brazil, Novelli *et al.* [13] reported that abamectin showed high ecotoxicity towards aquatic organisms, such as zooplankton (*Daphnia similis*), insects (*Chironomus xanthus*), and fishes (*Danio rerio*). Although the use of ABA has increased lately, mainly due to its attractive properties, there is little information about analytical methods for its identification in water [13] [16] [17].

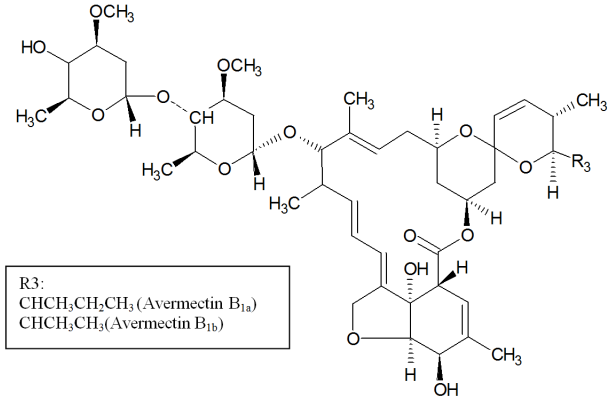

Figure 1. Molecular structure of avermectins.

In 2008, Brotas (São Paulo, Brazil), a city located in the studied region, had around 6 million orange trees, being the 5th largest cultivated area in São Paulo State, Brazil [4].

This paper describes a method for analyzing abamectin in surface natural waters and proposes an alternative derivatization step, which is faster and less energy-consuming, in comparison to others studies [15]. The method is applied in a region known by its ecotourism and aquatic activities in the Jacaré-Pepira River, whose watershed has a large number of orange crops.

2. Methodology

Triethylamine (TEA), tetrahydrofuran (THF) 1-methylimidazole (1MI, ≥99%), and abamectin (ABA, 99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Trifluoroacetic anhydride (TFAA) and dichloromethane (DCM) were purchased from Merck (Darmstadt, Germany) and Mallinckrodt Baker (Phillipsburg, NJ, USA), respectively. Previous reagents were of analytical grade. Ultrapure water (18.2 MΩ·cm at 25˚C) was obtained from a Milli-Q water purification system (Millipore Corporation, Billerica, MA, USA). SPE cartridges containing 500 mg of octadecylsilane (C-18, SampliQ series) adsorbents were purchased from Agilent Technologies® (West Lothan, UK). Stock and working solutions were prepared in acetonitrile (ACN) HPLC grade purchased from Merck (Darmstadt, Germany) and stored at −20˚C.

2.1. Chromatographic Settings

A high performance liquid chromatography device coupled to a fluorescence detector (HPLC-FLD, Agilent Technologies® 1200 series, Waldbronn, Germany) was used to analyze abamectin, with a Partisil 5 ODS-3 column (250 × 4.6 mm, 5 µm, Whatman®), at a controlled temperature of 25˚C. The excitation and emission wavelengths were 364 and 475 nm, respectively. An isocratic mobile phase comprised of ACN:H_2O:THF (80:14:6, in volume) was used, with a flow rate of 1.0 mL·min^{-1} and a run time of 14 min. The injection volume was 100 µL.

2.2. Sampling and Sample Preparation

Solid phase extraction (SPE) was used to prepare the sample prior to ABA determination with HPLC-FLD. Ultrapure water was used to prepare the spiked samples employed in the SPE development and method validation.

Surface water samples from the Jacaré-Pepira River were collected at four different points downstream, near to orange crops and the urban zone, sites where ABA could be used (**Table 1** and **Figure 2**). Dark glass bottles (1 L) were used to collect water samples, at a depth around 50 cm. Those bottles were previously washed with neutral detergent, tap water, and ultrapure water, sequentially.

Sample preparation started with the filtration of 200 mL of the samples through cellulose acetate membranes (0.45 µm × 47 mm, Millipore®, Brazil). Afterwards, it was percolated through an SPE cartridge containing C-18 adsorbent (500 mg), previously conditioned with 10 mL ACN and 5 mL ACN:H_2O:TEA (30:70:0.2, in volume). Then, the adsorbent was dried for 3 min and the analytes were eluted, into an amber flask, using 20 mL DCM. Except for filtration, all the other steps were held under 15 kPa vacuum. The eluate was dried under a gentle N_2 stream and reconstituted by adding 1 mL ACN to the flask. This extract was transferred to an amber 2 mL-vial wrapped in aluminum foil.

Table 1. Information of the sampled points.

Information	Point 1	Point 2	Point 3	Point 4
Date	09/03/2010	09/03/2010	09/03/2010	09/03/2010
Time	9:55	10:30	11:13	11:51
Coordinates	22.26309˚S 48.22975˚W	22.29144˚S 48.12938˚W	22.28438˚S 48.10722˚W	22.30025˚S 48.11026˚W
pH	6.98	7.25	6.60	7.11
Condutivity (mS·cm^{-1})	0.033	0.031	0.019	0.032
Turbidity (uT)	10	10	10	10
Dissolved oxygen (mg·L^{-1})	9.75	8.25	8.25	6.02
Temperature (˚C)	21.7	21.9	21.9	22.2
Salinity (%)	0	0	0	0

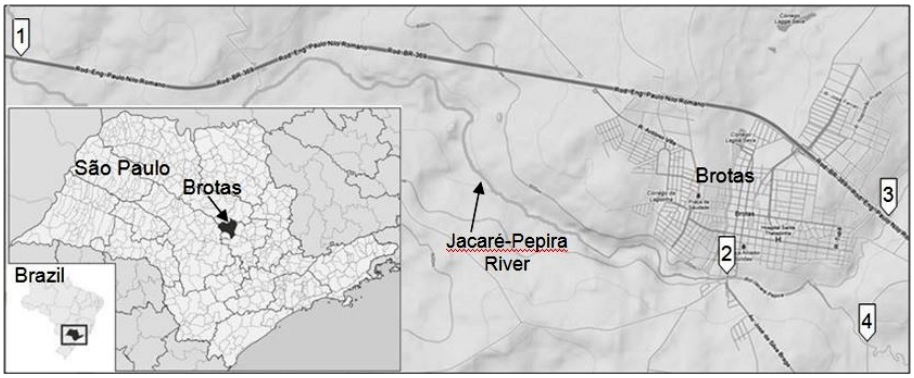

Figure 2. Sample points (1, 2, 3, and 4) in Jacaré-Pepira River, surrounding Brotas City.

2.3. Abamectin Derivatization Step

The derivatization step of ABA took place by adding 200 µL 1MI and 200 µL ACN:TFAA (2:1, in volume) to the vial. After 60 min at room temperature (25˚C), 100 µL of the sample was introduced into the chromatographic system.

During method development and prior to validation, all samples preparation steps were performed with ultrapure water spiked with ABA concentrations ranging from 0.2 to 10.0 $\mu g \cdot L^{-1}$.

2.4. Method Validation

Method validation assessed the parameters: selectivity, limit of quantification (LOQ), limit of detection (LOD), linearity, accuracy, precision, and recovery. The latter was performed using ultrapure water spiked with ABA concentrations of 0.2, 1.0, and 5.0 $\mu g \cdot L^{-1}$, in three replicates each one and according to the sample preparation described in Section 2.2.

Precision was calculated dividing the standard deviation (SD) of the instrumental responses (peak areas) by their average. Recovery was set as the peak area obtained for a spiked sample, divided by the peak area of the analyte standard solution. Accuracy was calculated in the same way, but the peak areas were previously turned into concentrations through the linearity equation of the method. The calculations regarded only the peak for avermectin B_{1a} due to its greater intensity in the HPLC-FLD analysis.

LOD was considered the concentration at which the abamectin signal was three times larger than the noise from the chromatogram baseline. LOQ was the concentration at which the analyte signal was five times higher than that noise.

3. Results and Discussion

In this study, a simple derivatization method, without heating, was used to provide derivatives with stability twice greater than the largest duration reported by Danaher *et al.* [15]. The repeatability of the method is obtained using less energy (no heat) and shorter times (60 min).

The derivatization duration was a critical step of the method. 15 min of reaction yielded chromatographic peaks with variable areas. Therefore, 30, 45, and 60 min of the derivatization reaction were tested. Among them, only a 60 min reaction yielded peaks stable enough during at least 7 days, after which a decrease of the analyte concentration was observed. Considering the total reaction time and the decrease of the derivative concentration after 7 days, it is believed that trifluoroacetyl ester (flu-TFA) derivative was the chromophore compound formed. Berendsen *et al.* [12] reported that flu-TFA was the most stable abamectin derivative in such reaction.

The validation results, LOD, and LOQ are shown in **Table 2**. The method selectivity and the retention times of avermectin B_{1a} (10.4 min) and avermectin B_{1b} (9.3 min) can be observed in **Figure 3**. It shows that no interfering signals from the sample matrix occurred in the same retention times of the analytes.

A procedure for the derivatization of abamectin was established at room temperature (25˚C) and during 60 min. Although the sampling points were located downstream and near some orange crops, ABA residues were not found in any of the samples analyzed (**Figure 4**). Similar works, performed in the same studied region, could not be found. Probably, abamectin residues are in concentrations below the method LOD.

Table 2. Validation data.

Parameters	Results (n = 5)
Recovery*	75%, 87%, 109%
Limit of detection (LOD)	0.1 μg·L^{-1}
Limit of quantification (LOQ)	0.2 μg·L^{-1}
Linearity	Y = 8.38 + 615.56X
Determination coefficient (R^2)	0.9945
Precision (RSD—relative standard deviation)	12%
Accuracy	104%

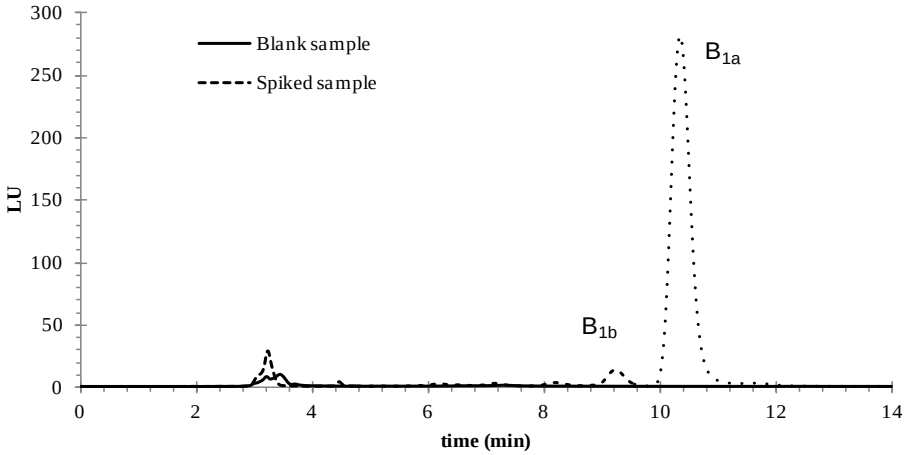

Figure 3. Method selectivity chromatogram comparing the blank matrix sample (—) to a 10 μg·L^{-1} spiked matrix sample (---). Chromatographic conditions: H_2O:ACN:THF (14:80:6, in volume), isocratic mode, column temperature of 25˚C ± 0.5˚C, flow rate of 1 mL·min^{-1}, injection volume of 100 μL, excitation λ = 364 nm, emission λ = 475 nm.

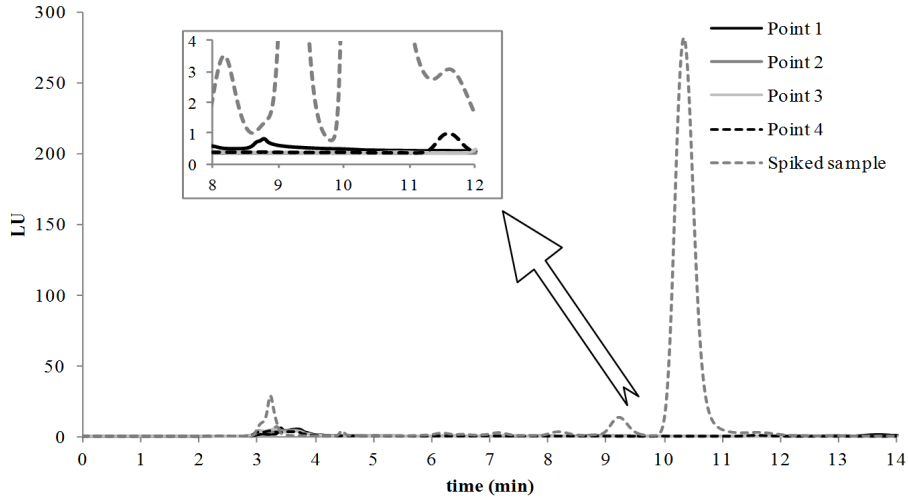

Figure 4. Chromatograms comparing the signals of the sampled points to a 10 μg·L^{-1} spiked sample (---). Chromatographic conditions: H_2O:ACN:THF (14:80:6, in volume), isocratic mode, flow rate of 1 mL·min^{-1}, injection volume of 100 μL, excitation λ = 364 nm, emission λ = 475 nm, column temperature of 25˚C ± 0.5˚C.

4. Conclusion

A procedure, at a room temperature and during 60 min, able to produce a stable abamectin derivative for seven

days was established, making it easy and fast for the analysis of abamectin in water. These method characteristics are the innovation presented in this paper. Abamectin residues were not found at concentrations comprised by the method, even though the assessed region was surrounded by orange crops. That occurs, in part, because abamectin residues have high degradation rates, almost disappearing within 48 h. The method presented here improves the analysis of abamectin in water, decreasing its running time and ensuring the repeatability of its results. This is a pioneer work in the studied region.

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