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SHORT COMMUNICATION

Fast Separation Ultra-Performance Liquid Chromatography for Determination of Pre-Column Derivative Abamectin and Ivermectin Residues in Vegetable

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Abstract A new residue method for quantification of abamectin and ivermectin in vegetable is described in the article. The derivative process is devised that acylating chemical is firstly performed by *N*-methylimidazole (MI) and trifluoroacetic anhydride (TFAA), then which reacted with hydroxyl function of abamerctin to make fluorescence. The influence of triethylamin (TEA) is examined. Separation is resolute by a short column of 1.7 μm size and operated at high pressure values (10.000 psi). The optimal chromatographic condition and the highest sensitivity are achieved by acetonitrile: water (95: 5), 0.4 mL/min, 0.2 μL injector. The detection limits of abamectin and ivermectin are 1 μg/kg respectively.

Keywords Abamectin · Ivermectin · UPLC · Fluorescence

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Introduction

Macrocyclic lactones are probably one of the anti-parasitic agents most applied in vegetable and fruit protection; among them, avermectins constitute an important group of compounds. Abamectin and ivermectin (22, 23-dihydro-Abamectin), which belongs to the avermectins family, is a neurotoxin used both as insecticide and acaricide.

Concerning a particular use of pesticides, abamectin and ivermectin have demonstrated to be effective in the control of the perseae mite, *Oligonychus perseae* in vegetable. In China, Maximum residue limits of abamectin in vegetable are 0.01 mg/kg (GB/T 2763-2005). HPLC with UV detection method was reported for the analysis of abamecetin residue in vegetable, due to the compound lack of ultraviolet chromospher, it was not sensitive enough to check compliance with the legislation at the limit of detection was only about 0.04 mg/kg [1–3].

Although recently liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods had been described for the analysis of avermectins [1, 6–8], fluorescence detection is still the most commonly applied detection technique with respect to the limit of detection and quantification. A number of analytical methods for the determination of avermectins in vegetable and fruit have been described mostly using high-performance liquid chromatography (HPLC) and fluorescence detection [4, 5]. The methodology in biological sample with HPLC/FD had recently been reviewed by Danaher et al. [9]. From the mechanical reaction studied, Berendsen et al. [10] had described in detail the derivative process obtaining either a trifluoro-acetylester

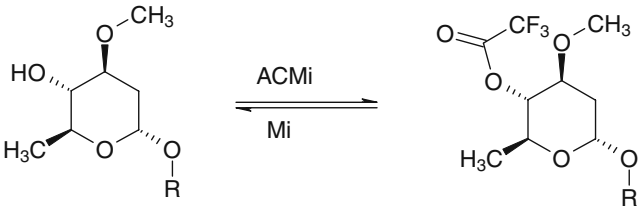

Fig. 1 Formation of a trifluoroacetylester (flu-TFA) derivatization or a hydroxyl (flu-OH) derivatization in the equilibrium

(flu-TFA) or a hydroxyl (flu-OH) derivatization (Fig. 1), and an unstable formation of flu-TFA was observed in common derivative procedure, it was very likely that flu-TFA was slowly hydrolysis for the flu-OH.

Due to the high number of compounds to be separated the run times will become relatively long. Novel low-dead-volume, high-pressure (1,000 bar) LC equipment provides strategies to improve resolution while maintaining or even shortening run times. This technique is called ultra performance LC (UPLC). Ultra-performance liquid chromatography (UPLC) based separations, using columns packed with small particles (1.7 μm), mobile phases at high linear velocities and operating at high pressure values (15.000 psi), have been shown to give superior chromatographic resolution, high speed of analysis and increased sensitivity [15, 16].

Therefore, the aim of this study is to develop a pre-column derivatization of abamectin and ivermectin, and fast separation by using ultra-performance liquid chromatography technique.

Experimental

Chemicals and Samples

All chemicals were of analytical reagent grade and used as received. Acetonitrile (HPLC-grade) were from Merck (Darmstadt, Germany). Derivatization reagents: trifluoroacetic anhydride (TFAA) *N*-methylimidazole (MI), triethylamin (TEA) and abamectin (B1a) (purity 97.5%) and ivermectin (purity 97.5%) were bought from Sigma–Aldrich (Madrid). Standard solutions of abamectin and ivermectin were prepared in acetonitrile (1,000 $mg.L^{-1}$) and kept in the dark under refrigeration at 4 °C. Working mixtures of pertinent concentrations were prepared daily by appropriate combination and dilution. Distilled water was deionized using a Milli-Q gradient system A10 (Millipore, Bedford, MA, USA).

UPLC–FL

A Waters Acquity UPLC system (Waters, Massachusetts, USA) equipped with an auto-sample, a quaternary pump

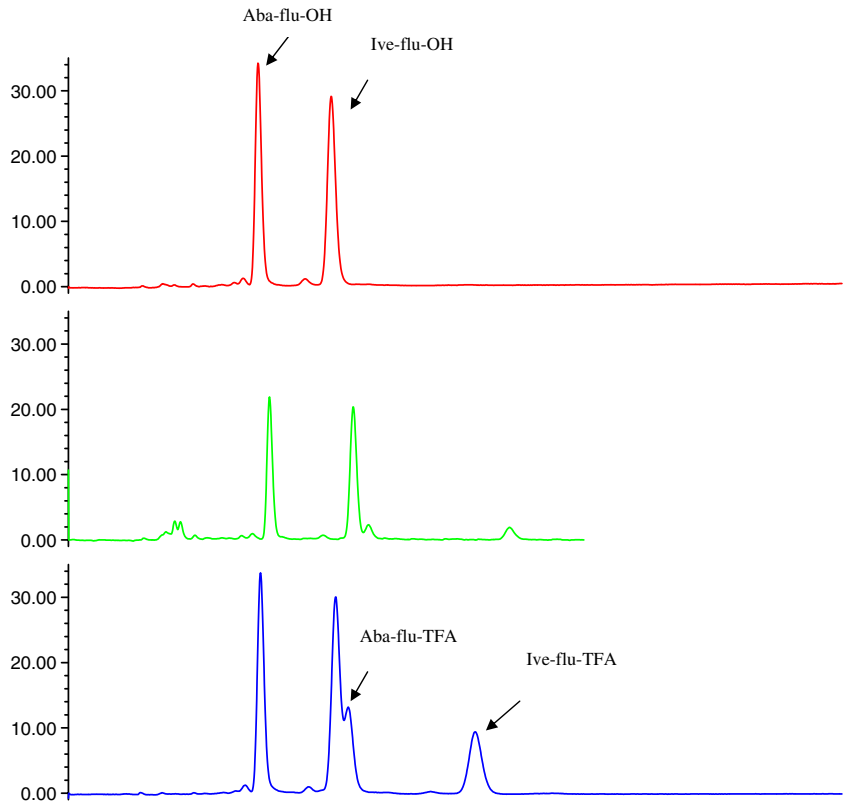

Fig. 2 showed that the peak of TFA-ester derivatization (flu-TFA) disappeared in different TEA concentration

Table 1 Figures of merit of the UPLC–FL method

	Abamectin	Ivermectin
*t*R intraday precision (n=5)	0.72%	1.12%
*t*R interday precision (n=20)	2.10%	2.73%
Areas intraday precision (n=5)	3.53%	4.47
Areas interday precision (n=20)	6.62%	7.31%
LOD (μg/kg)	1	1
LOQ (μg/kg)	3	3
Calibration equation (n=5)	Y=1.71 e^4X+2.62 e^3	Y=2.43e^4X+6.67e^3
Determination coefficient, *R*2	0.9952.	0.9936
Linear range tested (μg/L)	1–50	

system and a FLR fluoroescence detector, thermostated column compartment, degasser and empower software II. The analytical column was BEH Phenyl (50 mm×2.1 mm, 1.7 μm) from Waters Co. The mobile phase consisted of water: acetonitrile (5:95 *v/v*) and was pumped at isocratic rate 0.4 mL.min^{-1}. The fluorescence detector was set at excitation wavelength 365 nm and emission wavelength 470 nm.

Extraction Procedure

Fresh vegetables (500 g) were prepared, using a food processor (HR1821, Philips), and mixed thoroughly. Twenty gram sample was weighed into a 250-mL conic flask, mixed with 100 mL acetone: water (1:1, *v/v*) by using high speed homogenizer at 10,000 rpm (IKA T-25, Germany) for 1.5 min. The solution was completely transferred into funnel and extracted by hexane (50 mL). Collecting the organic solvent, the water phase was extracted twice by hexane (25 mL) again. The organic layer was evaporated to dryness on a vacuum at 40 °C.

Derivatization of Samples and Standards Solution

The residues obtained from the extract procedure will be derivative reaction. For the derivatization, 100 μL MI–acetonitrile (1:1 *v/v*), 150 μL TFAA–acetonitrile (1:2 *v/v*) and 100 μL TEA were added orderly; the standard solution was carried out in the same way. The solution was swirled at 2 min in vortex machine (TGL-16 L, Hunan, China); the mixture was diluted with 1 mL methanol, 1 mL water and 1 mL ethyl acetate and was centrifuged in 3,000 g at 5 min. The organic layers were injected for UPLC–FL analysis.

MI–acetonitrile and TFAA—acetonitrile solutions under nitrogen gas were prepared daily.

Results and Discussion

As it had been previously indicated, Berendsen et al. [10] had studied in detail the derivative process of abamectin using TFAA and MI, proposing two methods for obtaining two stable derivative products: a trifluoroacetylester (flu-TFA) or a hydroxyl derivatization (flu-OH). An unstable formation of ABA-flu-TFA was observed in common derivative procedure [9–13]. Based on these results it was very likely that most laboratories [12, 13] analyzed for flu-TFA while the slow hydrolysis of the flu-OH. It was anticipated that the flu-TFA derivatization and the corresponding flu-OH were in equilibrium (Fig. 1).

Berendsen [10] had demonstrated that the optimized experimental conditions, 100 μL MI –acetonitrile (1:1 *v/v*),

Table 2 Recoveries after adding 0.005, 0.01 and 0.05 mg/kg to samples (five replicates)

samples	Spiking level (mg/kg)	Blank value	Recovery%		R.S.D%	
			Abamectin	Ivermectin	Abamectin	Ivermectin
tomato	0.005	0	88.2	82.4	3.19	4.17
	0.01	0	90.7	92.4	5.29	6.11
	0.05	0	104.3	93.2	8.23	4.65
cucumber	0.005	0	78.5	79.1	6.17	4.21
	0.01	0	80.3	83.6	5.19	9.11
	0.05	0	92.1	101.7	3.14	2.48
cabbage	0.005	0	110.2	103.4	10.0	9.82
	0.01	0	94.6	90.8	8.92	7.35
	0.05	0	97.3	92.1	7.92	6.43

50 μLTEA, 150 μL TFAA–acetonitrile (1:2 *v/v*) and 50 μL TFA were added orderly, a 30-min incubation at 70 °C to form the flu-TFA.

Because an acylating chemical was formed by MI and TFAA, then it reacted with hydroxyl function of abamerctin to make fluorescence. So the derivative procedures were devised that MI and TFAA firstly made acylating chemical, and then the TEA was added to interrupt the reaction.

To simplify the derivative procedures, we tried to derivative reaction was performed at room temperature compared with at 70 °C, and tested the peak area by UPLC. Different reaction temperatures resulted in the same peak areas. It hypothesized that MI and TFAA directly mixed will gave out large amount of heat and can complete the derivative reaction instantaneously.

The effect of TEA was examined. The peak area of flu-TFA decreased slowly after TEA (25, 50 and 100 μL) added. Form Fig. 2, it found that flu-TFA was completely disappeared while 100 μL TEA added, so it was significant that the reaction reached respective maximum value. In the contrast to previous published results, the unsteady flu-TFA was not observed. So it implied that the process of flu-OH was not absolutely derivative from the flu-TFA hydrolysis.

Chromatographic Condition

The optimal chromatographic separation and sensitivity were tested with different organic solvent (methanol, acetonitrile, water). The optimal chromatographic separation and the highest sensitivity were achieved by using acetonitril: water (95: 5), 0.4 mL/min, 0.2 μL injector, the chromatographic separation was achieved in 3 min and chromatographic resolution was R=4.11.

In order to check the repeatability of the UPLC–FL method, a repeatability study was carried out at three levels of concentration (0.02 $mg.L^{-1}$) with five consecutive injections of each level in four different days (see Table 1). RSD values for the retention times were lower than 1.12% for the intraday precision and lower than 2.73% for the day-to-day precision; while RSD values for peak areas were lower than 4.47 and 7.31% for the intraday and interday precision, respectively. Table 1 also showed the calibration equation obtained as a result of the triplicate injection of five derivative standard concentrations (1, 5, 10, 25, 50 $\mu g.L^{-1}$). The calibration graph was a good linearity with a coefficient ($R2$) of 0.9952, 0.9936 (abamectin and ivermectin). The limit of detection (LOD) of the method, defined as the lowest concentration of abamectin and ivermectin that the analytical method can reliably detect at a signal-to-noise ratio (S/N) of 3, are 1 $\mu g.kg^{-1}$. The stability of the derivative standard solution was also checked keeping the samples in the refrigerator, which was a usual laboratory practice. It was found that the derivative solutions were stable for at least 3 days kept in the dark at 4°C, which was a good margin for daily laboratory work. Other derivative procedures proposed in the literature generally lack of enough stability of the derivatizations [12] or a stability of 24 h [14].

The accuracy and reproducibility of the whole method was evaluated by the development of a recovery study carried out at three concentration levels (0.005, 0.01 and 0.05 $mg.kg^{-1}$). For this purpose, tomato, cucumber and cabbage samples were prepared, 5 of which were spike 0.005 $mg.kg^{-1}$, 5 of which were spike 0.01 $mg.kg^{-1}$, other 5 of which were spike 0.05 $mg.kg^{-1}$, and others were unspiked sample. All experiments were carried out in quintuplicate at each level (results were shown in Table 2). As it can be seen in Table 2, recovery values were satisfactory, ranging from 78% to 110% with RSD lower than 10%. As it can be seen from the RSD values, the method was reproducible and applicable to the analysis of abamectin and ivermectin in vegetable. The LOQ of the whole method are 3 $\mu g.kg^{-1}$ (abamectin and ivermectin), respectively. These values are very similar to those reported in the literature for the HPLC–FL analysis of abamectin in other fruits samples [11].

Conclusion

In this study, there were significantly improved conditions that meet the requirements for the residue analysis of abamectin and ivermectin concerning speed and economy. The optimal chromatographic separation and sensitivity was successfully applied to the analysis of large amounts of samples.

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