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VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD FOR CARBENDAZIM RESIDUES QUANTIFICATION IN TOMATOES

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Abstract: *A high performance liquid chromatography method for carbendazim residues determination was adapted to the condition of our laboratory and validated for tomatoes samples. Carbendazim eluted at 3.13 minutes. The signal was linear over the concentration range 1 to 15 µg/ml with correlation coefficient 0.999092. The detection limit and the quantitation limit values were 0.002 mg/kg and 0.02 mg/kg respectively. Relative standard deviation of repeatability was 3.47% and 4.78% and recovery was 99.67% and 113,11% for two levels of concentration. The adapted method allowed a simple and rapid separation and quantification of carbendazim in tomatoes by high performance liquid chromatography.*

Keywords: *carbendazim, tomatoes, validation parameters, HPLC.*

1. Introduction

It is well known the human health impact of pesticide exposure: asthma, diabetes, Parkinson's disease, leukaemia, cancer [1].
Carbendazim (Metil N-benzimidazol-2-carbamat) belongs to carbamate pesticide class and is a fungicide used in fruit and vegetable growing and viticulture. Carbendazim is not approved for use in the European Union [2], but is used in Brazil, China and some other non-EU/EEA countries to preserve agricultural crops. For this reason carbendazim residues in food are monitored in the European Union. Carbendazim residues are determined by high performance liquid chromatography (HPLC) coupled with UV, diode array, fluorescence or MS detectors, or by gas chromatography [3-10].
The aim of this study was to adapt a HPLC method for carbendazim residues determination described by Phansawan et al. [10] for tomatoes samples using our equipments, and to develop an internal validation study focused on the following performance characteristics: linearity, accuracy, precision (repeatability), sensitivity (detection limit, quantitation limit).

2. Materials and method

2.1. Samples
Tomatoes (hybrid F1 Primadona) free of carbendazim were obtained from the Department of Horticultural Cultures in Protected Areas, HORTING Institute, Romania, and were kept at -20^0 C before analysis.

2.2. Reagents and standards
Carbendazim (97 %) was purchased from Aldrich. A stock solution (250 µg/ml) was prepared in methanol and used for the preparation of working standard solutions necessary for calibration curve (1, 2.5, 5, 7.5, 10, 12.5 and 15 µg/ml). All other reagents used were p.a. grade and solvents were HPLC grade.

2.3. The analytical procedure has been adapted in the Chemistry and Biochemistry Laboratory, HORTING Institute, following a previously published protocol [10]. Tomatoes samples were well blended, and 5 g sample were extracted in 25 ml methanol. The extracts were filtered through Whatman No. 1 paper; the filtrates were cleanup on OASIS MCX cartridges (Waters, Ireland) following the manufacturer instructions, then were concentrated using a TurboVap equipment (Caliper LifeSciences), so that the injected volume contained an amount of carbendazim within the linear range of the diode array detector. Finally the samples were filtered through 45 µm filters prior to injection. In our case, the chromatographic separation was performed using a LichroCART Purospher RP-18 column (250 * 4 mm), with 5µ particle size (Merck KGaA, Germany) and, the mobile phase consisted of water and methanol (25:75,v/v) under isocratic chromatographic conditions, with a flow rate of 1 ml/min. The column temperature was set at 20^{0}C.
Carbendazim was detected at 286 nm by the diode array detector. The data acquisition and processing used software ChromQuest 4.2. (ThermoFinnigan). The results were statistically processed using GraphPad Prism (version 5.00, GraphPad Software Inc., San Diego, 2007).

3. Results and Discussion

Carbendazim peak eluted at 3.13 minute in standard solutions (Fig. 1), and that is important if analysis time is of a higher priority. Increasing of organic solvent in mobile phase, we obtained a retention time (RT) shorter than Phansawan et al. [10], with a value around 12.5 minutes.
A good separation of carbendazim peak for tomatoes spiked samples was also obtained (Fig. 2).
The signal was linear over the concentration range 1.0 - 15.0 µg/ml, with correlation coefficient of 0.999092 (Fig.3); this range covers the EU maximum residue level tolerance for carbendazim in tomatoes (0.3 mg/kg) [2].
The accuracy of the method was examined through the results of the recovery by means of spiking procedure. For the recovery assays known amounts of carbendazim were added to the tomatoes samples to achieve 1 mg/kg (level 1) and 0.5 mg/kg (level 2) respectively.
Good recovery was obtained: 99.67% and 113.11% respectively (table 1).
Han et al. [9] reported recovery values between 72.0%-86.5% for tomatoes samples spiked with carbendazim at level of 5-50 ng/g. Phansawan et al. [10] reported also good recoveries of carbendazim from spiked pooled vegetable samples (including tomatoes) ranged from 92.5% to 96.0 % at spiked levels of 0.05-0.30 mg kg^{-1}.

The precision was evaluated by relative standard deviation (RSD) at two levels of concentration previously presented. RSD was 3.47% and 4.78% respectively (table 1). RSDs that ranged from 2.1% to 7.5 % were obtained by Phansawan et al. [10] for different carbendazim amounts.

Limit of detection (LOD) is given as the concentration of the analyte that gives an absorbance signal three times higher than background noise, while limit of quantitation (LOQ) is given as the lowest concentration of analyte that can be determined with an acceptable accuracy in terms of methods of analysis. Using the signal to noise ratio registered for standard carbendazim solutions by high performance liquid chromatograph, we calculated LOD and LOQ values **(**table 1). LOD and LOQ (0.002 mg/kg and 0.02 mg/kg respectively) were comparable with those obtained by Phansawan et al. [10] (0.003 mg kg^{-1} and 0.03 mg/kg respectively). LOD value was greater than that reported by Han et al. [9] (0.55 ng/g).

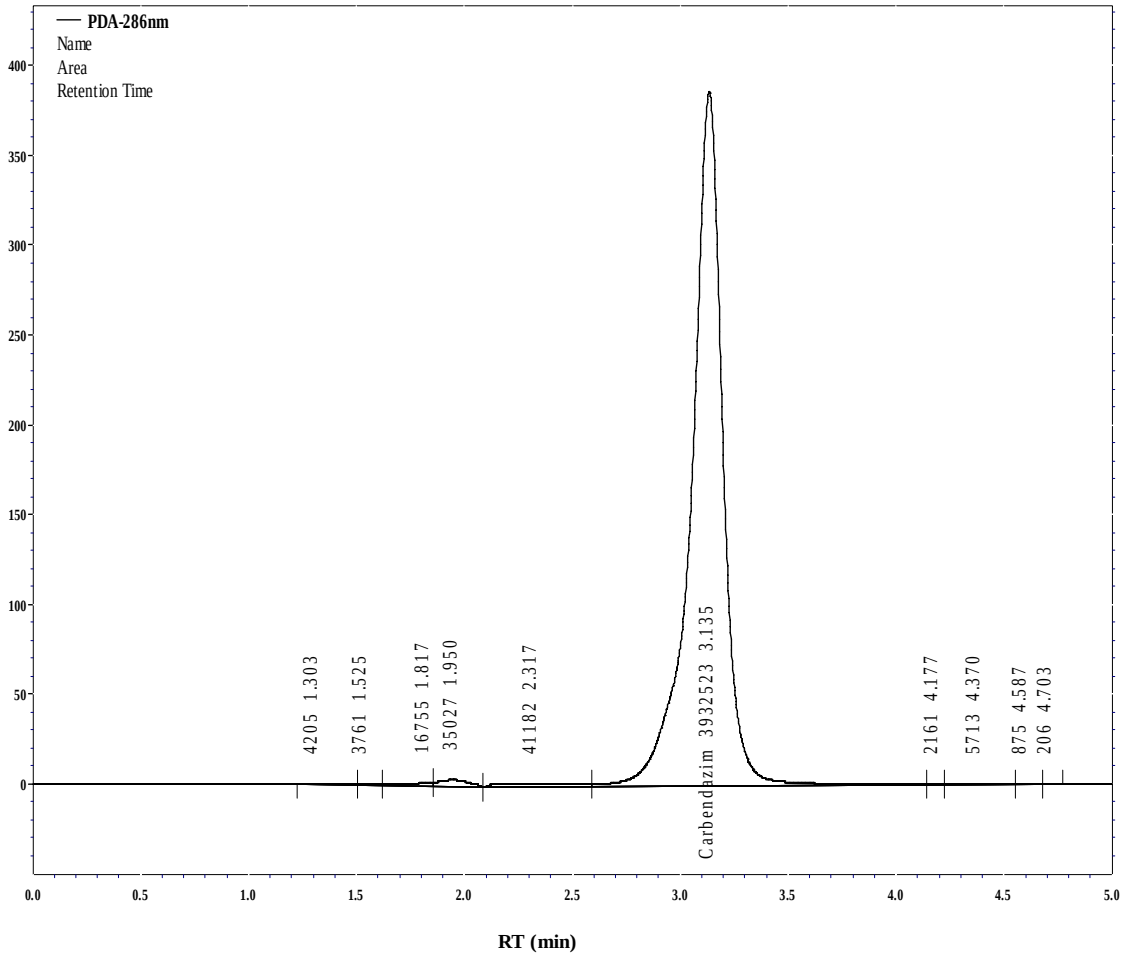

Fig. 1. Chromatogram of carbendazim standard solution (10 µg/ml)

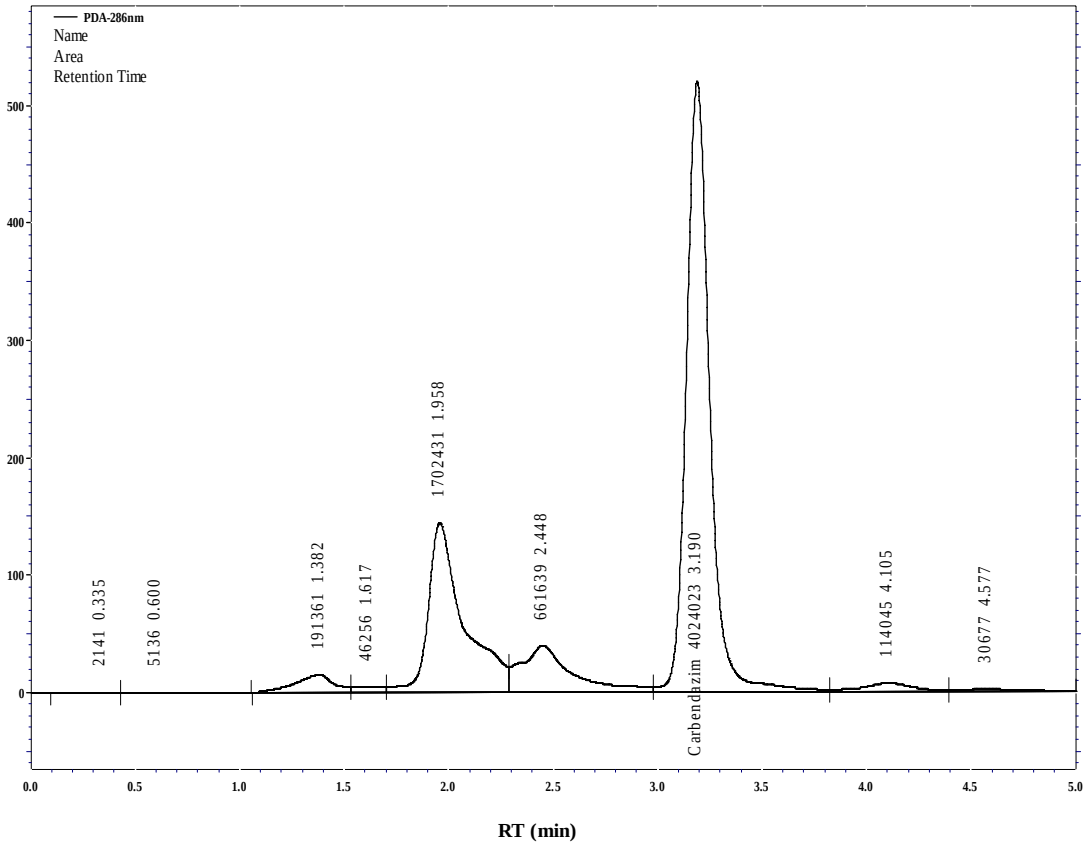

Fig. 2. Chromatogram of a tomato sample spiked with carbendazim (1 mg/kg)

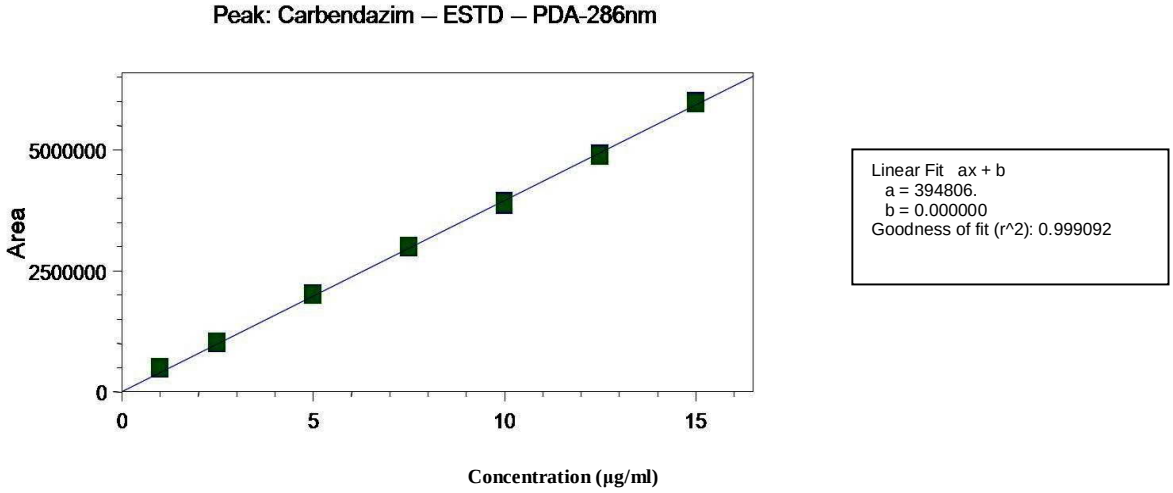

Fig. 3. Carbendazim calibration curve

Table 1.

Validation parameters for carbendazim determination by HPLC method

Precision (RSD(%))		Recovery(%)±RSD		LOD	LOQ
1mg/kg (level 1) (n=5)	0.5 mg/kg (level 2) (n=5)	1mg/kg (level 1) (n=5)	0.5 mg/kg (level 2) (n=5)	(mg/kg)	(mg/kg)
3.47	4.78	99.67±3.35	113.11±4.86	0.002	0.02

4. Conclusion

The adapted method is simple, fast, accurate, precise and sensitive in order to detect carbendazim residues in tomatoes samples below the maximum residue level of 0.3 mg/kg, according to the EU tolerance.

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All the authors declare no conflict of interest.

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