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DETERMINATION OF CARBENDAZIM RESIDUES IN FRUITS, VEGETABLES AND CEREALS BY HPLC WITH COLUMN SWITCHING

Monika Michel[1], *Bogusław Buszewski*[2]

[1]Institute of Plant Protection, Regional Experimental Station,
Pesticide Residue Laboratory
Żwirki i Wigury 73, 87-100 Toruń, Poland
e-mail: M.Michel@ior.poznan.pl
[2]Nicholas Copernicus University,
Department of Environmental Chemistry and Ecoanalytics,
Faculty of Chemistry
Gagarin 7, 87-100 Toruń, Poland
e-mail: bbusz@chem.uni.torun.pl

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Abstract: Method for determination of carbendazim residues in fruits, vegetables and cereals was described. The compound was extracted with methanol-hydrochloric acid mixture, and after liquid-liquid partition step with dichloromethane, was determined by high performance liquid chromatography (HPLC) with column switching and ultraviolet (UV) detection. The average recoveries of carbendazim from fortified sample were from 68.7% ± 4.3% to 92.6% ± 4.5%, the coefficients of variation were from 2.9% to 6.3%, and the limits of quantification at λ = 279 nm were from 0.02 mg/kg to 0.2 mg/kg.

Key words: carbendazim residue, food analysis, column switching, HPLC

INTRODUCTION

Carbendazim (**M**ethyl **B**enzimidazol-2-yl**C**arbamate, MBC) is effective against a wide range of pathogens of cereals, grapes, vegetables, fruits, rice, sugarbeet and ornamental plants. This fungicide is used as pre-harvest treatment on fruits and vegetables (absorbed by roots and green tissues of plants), as a seed dressing, and as post-harvest agent to control storage rots and moulds (Regis-Rolle and Bauville 1993). The structure of carbendazim is presented in figure 1. Other related fungicides, e.g. benomyl and thiophanate-methyl, are degraded to carbendazim, not only in contact with water and under moist conditions in soil, but also when applied to plants. Therefore, their residues are expressed as carbendazim (Tab. 1).

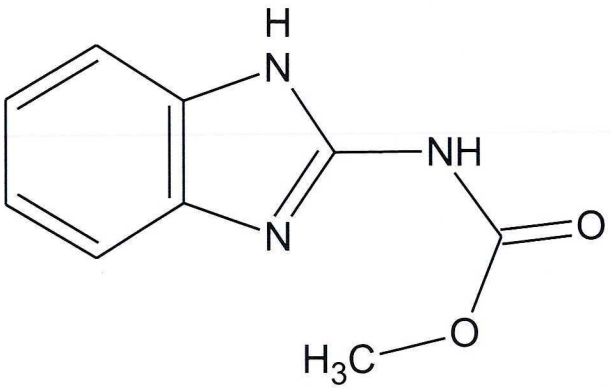

Fig. 1. Structure of carbendazim

Thermal instability of carbendazim does not permit its analysis by gas chromatography, unless it is transformed into thermally stable derivative (Tharsis et al. 1997). Therefore, the most common analytical method for analysis of carbendazim is liquid chromatography coupled with ultraviolet (UV) (Di Muccio et al. 1995; Di Muccio et al. 1999), fluorescence (Levine et al. 1998; Hiemstra et al. 1995), electron capture, and electrochemical detection methods, which present low selectivity. On the other hand, photodiode array UV (UV-PDA) (Arenas et al. 1996; Garrido et al. 1997; Sanchez-Rasero et al. 1991; Michel 2001) and mass spectrometry (MS) detectors (Lacassie et al. 1999; Fernandez-Alba et al. 2000) were frequently used and provided a selective detection.

Many of the methods published involved extraction and clean-up procedures, including solvent partitioning, solid phase extraction (SPE) (Buszewski et al. 1996), supercritical fluid extraction (SFE), which are laborious and time consuming (Bicchi et al. 1989; Ohlin and Jansson 1998; Navickiena and Ribeiro 1999). Nevertheless, there is still a lack of analytical methods that offer low quantification limits whatever the plant material. Some procedures are very sensitive but quite specific for a sample, while a few methods, suitable for a wider range of matrices, suffer from lack sensitivity.

Table 1. Maximum Residue Limits (MRL) for carbendazim in Polish legislation (Rozporządzenie MZiOS 1997)

Pesticide	MRL (mg/kg)	Commodity
CARBENDAZIM	5.0	citrus fruits
	1.0	other fruits and vegetables
(sum of carbendazim, benomyl and thiophanate-methyl expressed as carbendazim)	0.1	cereal grains
	1.0	mushrooms
	0.1	meats
	0.1	milks
methyl benzimidazol-2-ylcarbamate	0.1	eggs
	0.1	potatoes
MBC	0.1	tea

Column switching with HPLC system is still not very much in vogue in pesticide residue analysis, so it is an interesting object of study (Hogendoorn 1993; Michel et al. 2001; Michel and Buszewski 2001). The possibility to perform an automated and efficient clean-up of extract samples is a highly desirable option in analysis. The relevant aspects in applying column switching in our work were to increase chromatographic resolution, selectivity and sensitivity, to enrich trace amounts of the sample, to protect sensitive UV-PDA detector, and to speed up the column stabilisation.

The aim of this study was to set up a sensitive and convenient HPLC method, to reduce labour and organic solvents, no buffer control conditions, and the use of column switching liquid chromatography with UV-PDA detection with special attention to the elimination of co-extracted interferences for the routine analysis of thermally labile and polar pesticides with satisfactorily low quantification limits.

MATERIALS AND METHODS

Reagents, Materials and Apparatus

Methanol was *for HPLC* grade from J.T. Baker (Deventer, the Netherlands). Deionized water was purified by Maxima water purification system (ELGA, High Wycombe, England). Both solvents were filtered through 0.45 μm Nylon 66 Membranes (Supelco, Bellefonte, PA, USA). Methanol and dichloromethane were residue analysis grade, and distilled-in-glass if necessary. The extraction mixture was methanol-hydrochloric acid (83:17, v/v). Inorganic compounds were all reagent grade.

Carbendazim standard (purity 99.0%, Dr. Ehrenstorfer, Augsburg, Germany) was used for fortification and quantitation. Stock solution of carbendazim (200 μg/mL) was prepared in *for HPLC* grade methanol. The calibration and working standard solutions of carbendazim were prepared by diluting from stock solution with methanol-deionized water (40:60, v/v). These solutions were stored in the refrigerator at 277 K over a three-month period.

The HPLC system consisted of a CM 3500 and 3200 pumps, autosampler Milton Roy type 713 (AS), UV-PDA detector type SM 5000 set at $\lambda = 279$ nm (TSP, Riviera Beach, FL, USA); programmable, 10 port (or equivalent) column switching valve type WEC10WK (VICI, Valco Instruments, Houston, TX, USA); 100 mL injection loop (Supelco, Bellefonte, PA, USA); Rheodyne Pneumatic Sample Injector Model 7126 (RH) (Rheodyne, Cotati, CA, USA). The data were collected and analysed with LCtalk computing system (TSP LCtalk™ HPLC software, version 2.03.02).

Extraction

After homogenisation of 1 kg of fruits, vegetables or cereals, 10 g portions were sampled, 100 mL of methanol-hydrochloric acid were added and shaken. After vacuum filtering, methanol was evaporated and remaining phase was partitioned with dichloromethane. Dichloromethane was evaporated to dryness, and residues were redissolved in the HPLC mobile phase before injection.

LC Analysis

Separating columns were Supelcosil LC–8 DB, 150 x 4.6 mm id, 5 μm (Supelco, Bellefonte, PA, USA) and Zorbax Rx-C8, 250 x 4.6 mm id, 5 μm (Rockland Technologies, Nuenen, The Netherlands). Mobile phases were A, HPLC methanol-deionized water (45:55, v/v) and B, HPLC methanol-deionized water (60:40, v/v). Flow-rates for both pumps were 1 mL/min; injection volume was 100 μL. Typical chromatograms are presented in figure 2.

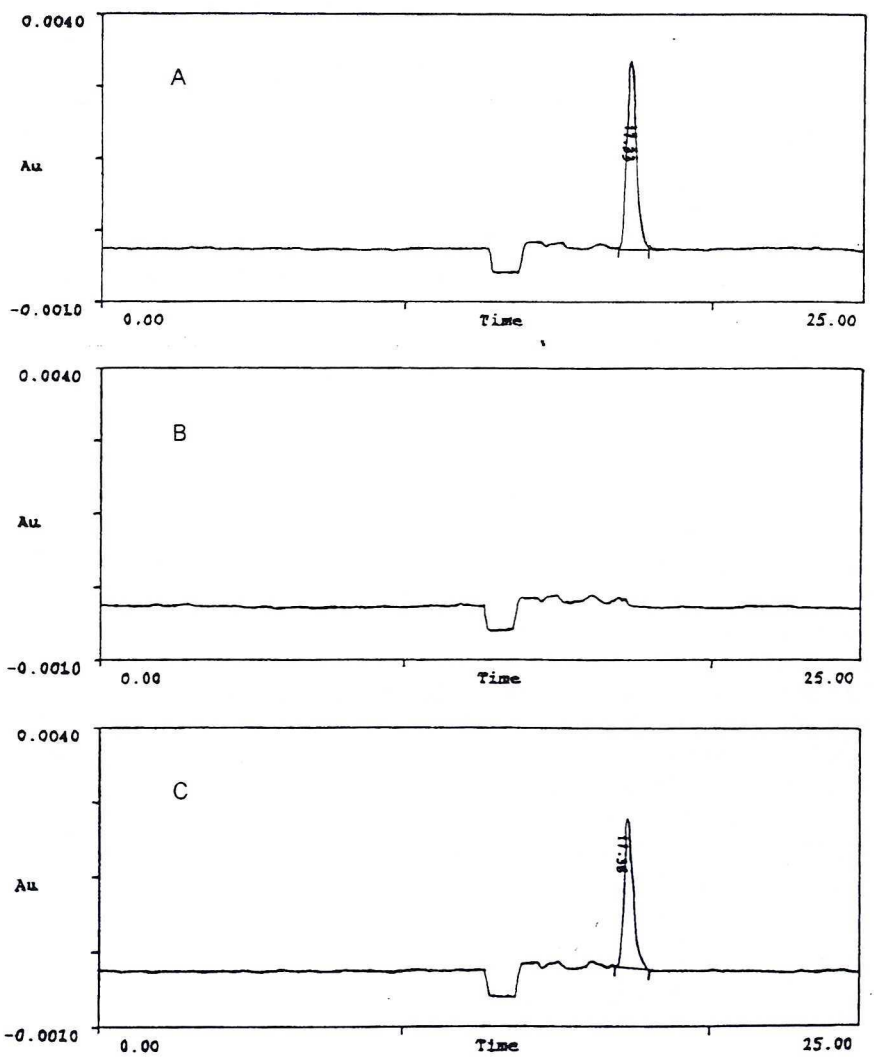

Fig. 2. Typical chromatograms: (A) – carbendazim standard 0.18 μg/mL; (B) – 10 g untreated control apples; (C) – 10 g control apples fortified at 0.3 μg/g

RESULTS AND DISCUSSION

Optimisation of Chromatographic Conditions

Stationary phase

Usual RP-HPLC conditions to analyse carbendazim need mobile phase modifiers to improve the peak shape and the column efficiency. Compound adsorption on stationary phase is due to interaction between nitrogen atoms of the benzimidazole molecules (weak base) and the acid residual silanol groups of the silica surface. Therefore, adding a competing amine to the mobile phase can improve the peak shape. However, chromatographic conditions become more complicated since it is necessary to use pH buffer to maintain pH to preserve silica chemical stability.

Another way checked in laboratory to improve poor chromatographic performance, revealed in broad and badly tailing peak, is to use the column switching procedure, without adding modifiers to the mobile phase.

Mobile phase

Different mobile phase compositions of methanol-deionized water for both columns were tested. Increase of water content involved increase of retention time, peak broadness and resolution. But increasing water content could be useful for separating fungicide peak from potential interfering matrix peaks, usually situated at the beginning of chromatograms. 55% water contents for the first column and 40% for the second were selected, ensuring good resolution and better sensibility.

Besides, injecting solutions of the same composition as the mobile phase for the first column brought about the best resolution, sensitivity and peak shape.

Validation of the Method

All validation procedures were performed using pesticide-free fruits, vegetables and cereals. Known amounts of carbendazim were introduced before extraction to samples in order to obtain concentrations ranging from 0.09 to 0.47 mg/kg. Recovery data were determined for each sample at two fortification levels by comparing the analyte peak height obtained after extraction of spiked samples and to ensure that the method would perform satisfactorily for a wide range of residue amounts, from detection to maximum residue limits. All the samples were analysed consecutively in the same day, for the same analyst to study repeatability, too. Standard deviations (SD) were not higher than 4.5% in this study. Recoveries were no less than 68.7%, and significant differences were not found at either of the two different fortification levels. The results of repeatability and recoveries were considered adequate to the validation of the method.

The detection limits (LOD) were determined as the lowest concentration giving response of three times the average of the baseline noise defined from three unfortified samples. The minimum detectable amount of carbendazim was 0.04 μg/mL. The limits of quantification (LOQ) were determined as the lowest concentration of a given pesticide giving a response that could be quantified with coefficient of variation (CV) of less than 20%.

The limit of quantification depended on the sample type because co-extractive interferences were quantitatively and qualitatively different according to plant material. Practical quantification limits were from 0.02 μg/kg for rye grain samples to

0.2 mg/kg for tomato samples. These values are well below the MRLs established in Poland.

All the parameters in the recovery studies are shown in table 2.

Table 2. Method of validation studies

Parameter\Assortment	Abbr.	Mushroom	Field cucumber	Field tomato	Apple	Cherry	Black currant	Rye-grain	Wheat -grain
Fortification level (mg/kg)	FL	0.19	0.23	0.15	0.09	0.47	0.09	0.02	0.02
Number of recovery tests	n	4	4	4	4	4	4	4	4
Average recovery (%)	AR	75.2	90.4	92.6	83.1	68.7	82	84.7	82.1
Standard deviation (%)	SD	2.5	3.3	4.5	2.4	4.3	2.7	3.4	3.7
Coefficient of variation (%)	CV	3.3	3.7	4.9	2.9	6.3	3.3	4	4.5
Limit of quantification (mg/kg)	LOQ	0.1	0.1	0.2	0.1	0.07	0.09	0.02	0.03
Detection limit (μg/mL)	LOD	0.2	0.2	0.1	0.19	0.14	0.19	0.04	0.07

The calibration curve, obtained by plotting peak height (in LCtalk units) versus concentration of carbendazim (μg/mL) over the range from 0.15 to 3.74 μg/mL with UV detection set at 279 nm for 100 μL injections and passed through origin. The straight line obtained corresponds to the equation

$$y = 205220x + 7849 \quad (1)$$

and is presented in figure 3. The coefficient of correlation was r = 0.9993.

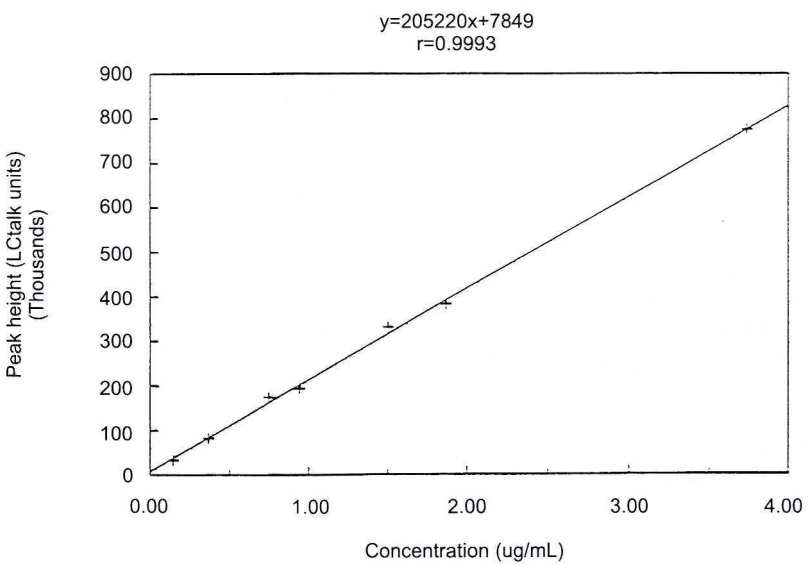

Fig. 3. Calibration curve for carbendazim

CONCLUSIONS

A modern analytical method should combine sensitivity, selectivity, and precision. Time-consuming and expensive clean-up or derivatization as well as toxic or ecologically critical substances should be avoided as far as possible. In this paper, we present an alternative method for the fast and sensitive determination of carbendazim in fruits, vegetables, and cereals. The simple extraction, elimination of a clean-up step, and use the column switching procedure can significantly reduce the analysis time and solvent consumption, resulting in cheaper analysis. Regarding our validation results and routine experience, we think that this analytical procedure is a mature technique for the carbendazim residue analysis in food and can readily be adapted to new matrices.

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Rozporządzenie Ministra Zdrowia i Opieki Społecznej z dnia 15 kwietnia 1997 r. w sprawie najwyższych dopuszczalnych pozostałości środków chemicznych stosowanych przy ochronie, przechowywaniu i transporcie roślin, które mogą znajdować się w środkach spożywczych lub na ich powierzchni, (Dz. U. Nr 43, poz. 273).

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POLISH SUMMARY

OZNACZANIE POZOSTAŁOŚCI KARBENDAZYMU W OWOCACH, WARZYWACH I ZBOŻACH TECHNIKĄ WYSOKOSPRAWNEJ CHROMATOGRAFII CIECZOWEJ HPLC Z SYSTEMEM PRZEŁĄCZANIEM KOLUMN

Przedstawiono procedurę analityczną oznaczania pozostałości karbendazymu w materiale roślinnym techniką wysokosprawnej chromatografii cieczowej HPLC z wykorzystaniem przełączania kolumn. Procedura ta opracowana została w celu wykorzystania w badaniach monitoringowych płodów rolnych i ogrodniczych traktowanych preparatami zawierającymi substancje czynne z grupy fungicydów benzimidazolowych: benomyl, karbendazym, tiofanat metylu. Średni odzysk karbendazymu z próbek fortyfikowanych mieścił się w granicach od 68,7% ± 4,3% do 92,6% ± 4,5%, współczynnik zmienności wynosił od 2,9% do 6,3%, a granica oznaczalności przy długości fali λ = 279 nm była od 0,02 mg/kg do 0,2 mg/kg.