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Optimization of a matrix solid-phase dispersion method for the determination analysis of carbendazim residue in plant material

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Abstract

The objective of this paper was to prove that matrix solid-phase dispersion (MSPD) coupled with high performance liquid chromatography (HPLC) and column switching could be used for the determination and quantification of carbendazim residue in plant samples. By comparing results obtained after optimization of the extraction conditions on an acidic silica gel column, it was determined that sorption and retention of carbendazim were achieved via specific interactions. The method of standard additions was used for quantitative analysis. Its performance was evaluated and validated: the detection limit (UV-Vis detection at $\lambda = 279$ nm) was 0.02 μg/g, the relative standard deviations (R.S.D.) were between 2.7 and 4.1% and the recoveries were ranging from 84.3 to 90.7% at the 0.04, 0.08 and 0.1 μg/g fortification levels. The method was successfully tested on cereal samples, and the results obtained with the present off-line MSPD–HPLC procedure were found to compare well with those obtained with procedure involving LLE.

Keywords: Optimization; Matrix solid-phase dispersion; Carbendazim

1. Introduction

Present-day agricultural practice requires the use of many chemicals to increase crop yields [1]. Therefore, it has been a growing interest in the detection and determination of pesticide residues in agricultural produce intended for human consumption. In view of this, it was considered desirable to devise an analytical procedure in which the repetition of time-consuming operations and costs were minimized.

To eliminate some of the difficulties associated with solvent extraction of pesticide residues a rapid and simple method based on a modified version of the extraction procedures of solid-phase extraction (SPE) called matrix solid-phase dispersion (MSPD) [2–4] with the direct determination of carbendazim by high performance liquid chromatography (HPLC) with column switching are presented. Carbendazim (methyl benzimidazole-2-ylcarbamate, MBC) is a systemic fungicide with protective and curative action. It is registered for use in various crops, for example, in cereal, fruit, stored fruit, and as a seed dressing [5]. Other related fungicides, e.g. benomyl and thiophanate-methyl, are degraded to carbendazim. The commercial introduction of these pesticides still leads to the need for rapid, selective and sensitive analytical methods for the control of environmental pollution levels, especially in a staple foodstuff like cereals.

Nowadays, in our laboratory, the determination of carbendazim in cereal is performed according to a previously described method [6,7]. The method essentially consists in methanol–hydrochloric acid (HCl) mixture extraction, LLE partitioning with dichloromethane, and determination by HPLC with column switching and UV detection. Such procedure is time-consuming and tedious, and generally not kept pace with advances in analytical technology.

This paper assessed the solid-phase, eluting and volume solvent used in the MSPD extraction. The application and mechanism of sorption on modified silica gel for sampling carbendazim from the cereal is also discussed.

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2. Experimental

2.1. Reagents and samples

All chemicals and solvents were analytical-reagent grade. HPLC-grade methanol was obtained from Merck (Darmstadt, Germany). Deionized water was purified by Maxima water purification system (ELGA, High Wycombe, England). These solvents were filtered through 0.45 μm Nylon 66 Membranes (Supelco Inc., Bellefonte, PA, USA) and degassed using helium sparging. Methanol, dichloromethane and HCl were purchased from POCh (Gliwice, Poland).

Carbendazim was of analytical-pesticide grade purchased from IPO (Warsaw, Poland) and was dissolved in methanol at a concentration of 200 μg/ml to prepare stock standard solution. Working standard solutions were prepared by diluting the stock standard solution with methanol–water mixture. All solutions were stored at 4 °C.

The samples of cereal were collected from private producers.

2.2. Apparatus

The following apparatus were used: laboratory mill type WZ-1 (ZBPP, Bydgoszcz, Poland), rotary evaporator (Büchi, Flawill, Switzerland), mortar and pestle, funnel. Extraction columns were from polypropylene cartridge, 130 mm × 25 mm i.d. with a glass wool plug (Pharma-Plast A/S, Rodby, Denmark).

2.3. Chromatography

The HPLC system were consisted of a CM 3500 and 3200 pumps, diode array detector (DAD) type SM 5000 (TSP, Riviera Beach, FL, USA) set at $\lambda = 279$ nm; programmable, 6 port column switching valve type WEC6WK (VICI, Valco Instruments, Houston, TX, USA); 100 μl injection loop (Supelco Inc., Bellefonte, PA, USA); Rheodyne injector model 7125 (Rheodyne, Cotati, CA, USA). The data were collected and analyzed with LCtalk computing system (TSP LCtalk™ HPLC software, version 2.03.02).

Separating columns, CC-1 clean-up was Supelcosil LC-8-DB, 150 mm × 4.6 mm i.d. and 5 μm particle size (Supelco Inc., Bellefonte, PA, USA), and AC-2 analytical column was Alltima C18, 250 mm × 4.6 mm i.d. and 5 μm particle size (Alltech, Carnforth, UK). Chromatographic conditions were presented in Table 1. Column switching procedure was described in details in reference of Michel et al. [8] and Michel and Buszewski [9].

Table 1
HPLC conditions

Parameter	Description
Detector	DAD
Wavelength (nm)	$\lambda = 279$
Columns parameters	CC-1: Supelcosil LC-8-DB, 150 mm × 4.6 mm i.d., 5 μm AC-2: Alltima C18, 250 mm × 4.6 mm i.d., 5 μm
Injection volume (μl)	100
Mobile phase (v/v)	A: methanol–water (45:55) B: methanol–water (60:40)
Flow rate (ml/min)	1
Column switching time (min)	ca. 10–11
Retention time (min)	ca. 18
Running (min)	25

2.4. Procedure

2.4.1. Preparation of acidic silica gel adsorbent

Kieselgel 60 extrapure, particle size 0.063–0.200 mm (70–230 mesh ASTM) (Merck, Darmstadt, Germany) was reactivated prior to use at 500 °C for 2 h, cooled in a dessicator, and kept tightly closed. One hundred grams of the silica were placed in three 300 ml Erlenmeyer flasks. To each of the flasks 5, 10 and 15 ml of 1 M HCl, respectively, were added. The flasks were stoppered and shaked for 30 min.

2.4.2. Extraction

Approximately 100 g of the sample was ground using laboratory mill. Five grams of the ground sample were accurately weighted and were added to the mortar with an eight grams of an acidic silica gel. All were ground to obtain the consistency of the free-flowing powder. The extraction column was plugged with glass wool, the powdery sample was transferred through a widemouth polypropylene funnel. The residue of carbendazim was extracted with eluent, methanol–dichloromethane mixture (1:5, v/v, 120 ml), and was collected in round-bottomed flasks. The solvent was evaporated to dryness using a rotary evaporator, and the dry residue was dissolved in HPLC mobile phase methanol–water (45:55, v/v).

3. Results and discussions

As was earlier mentioned for trace analysis of xenobiotics residue in cells different extractions techniques are used. For plant material the LLE and MSPD are generally employed because of simplicity, low cost and acceptable analytical results.

The extraction efficiency of classical LLE technique was compared with those obtained by using MSPD extraction method of separating carbendazim from cereal material.

The idea of separation mechanisms which rule in the case of solid-phase extraction was the base to elaboration practical technique of sample preparation—MSPD [10–14]. In this method the analytes to be extracted are partitioned between a solid and a liquid (rather than between two immiscible liquids as in LLE) and these analytes must have a greater affinity for the solid phase than for the sample matrix

(retention or adsorption step). Compounds retained on the solid phase can be removed at a later stage by eluting with a solvent with a greater affinity for the analytes (elution or desorption step) [15].

Homogenized biological samples with silica bonded supports provoke disruption of the architecture of the sample, serve sample homogenization, exhaustive extraction, fractionation and purification in a simple process and provide a unique column support material for subsequent isolation of the dispersed compounds. The phase induces a lot of chemical interactions basis of hydrophobic–hydrophilic interactions within the sample components [16]. The interactions involve the analyte with the solid support, the analyte with the bonded phase, the analyte with the dispersed ma-

Table 2
Standard deviations, limits of determination and limits of detection obtained by LLE and MSPD extractions of fortified cereal

Extraction technique	Fortification level (μg/g)	S.D. (%)	Limit of determination (μg/ml)	Limit of detection (μg/g)
LLE	0.04	3.7	0.04	0.02
	0.08	3.8	0.04	0.02
	0.1	4	0.04	0.02
MSPD	0.04	2.6	0.04	0.02
	0.08	2.3	0.04	0.02
	0.1	3.7	0.04	0.02

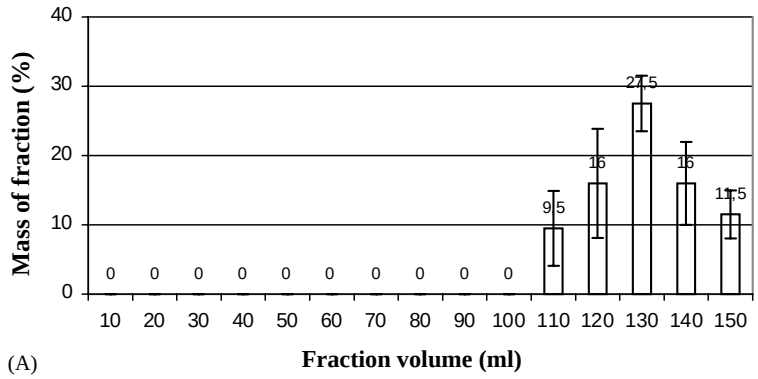

Fig. 1. The elution profiles from MSPD column: (A) 5 ml, 1 M HCl + 100 g silica gel; (B) 10 ml, 1 M HCl + 100 g silica gel; (C) 15 ml, 1 M HCl + 100 g silica gel.

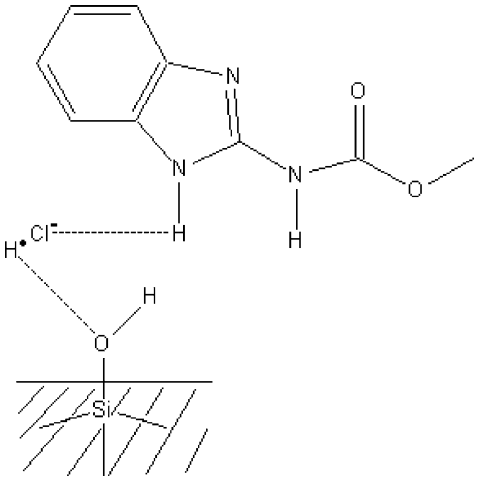

Fig. 2. The sorption of carbendazim on an acidic silica gel support.

trix, the matrix with the solid support, the matrix with the bonded phase, all of the above components interacting with the elution solvents, and the dynamic interactions of all of the above occur simultaneously. Factors influencing in SPE and chromatography generally work in MSPD.

Qualification of conditions to conduct the MSPD was executed by the choice of HCl amount in silica gel and volumes of solvent to elute carbendazim (see Section 2.4.1).

Three MSPD columns with different amount of HCl in 100 g of silica gel and spiked cereal samples were tried. Elution solvent mixture was added to each column and 10 ml fractions were collected. Each fraction was analyzed to determine the amount of carbendazim present. Because when acidified silica gel was used good recovery was achieved, the cartridges were compared in order to determine the maximum extraction conditions. The fraction containing carbendazim are shown in Fig. 1. The shape of elution profile in the two first cases are almost identical and take the typical Gaussian profile. In the third case the elution profile is strongly flattened. To much eluting solvent in the cases 1 and 3, more than 150 ml, were needed. Therefore, the MSPD column containing 10 ml of HCl in 100 g of silica gel was found to be the most suitable for routine analysis. The volume of eluting solvent required to obtain the highest recoveries was found to be 120 ml.

The differences in eluting profiles are consequences in sorption of carbendazim molecules on residual silanols. This is probably due to the chemical characters of carbendazim, it is a weak organic base, $pK = 11.7$ [17], which means that different strength of isolation mechanism are observed [18]. Possibly, the higher recovery may be obtained on the packing with great (but not too many) numbers of residual silanols.

Silica is the most commonly used adsorbent. The polar sites of silica adsorb moderately polar compounds dissolved in organic solvents with ϵ° (eluotropic strength) values less than approximately 0.38, like carbendazim [16]. This analyte can be eluted from the silica column with solvents having ϵ° greater than approximately 0.6 (methanol–dichloromethane, 1:5, v/v; $\epsilon^{\circ} = 0.63$). In general, basic compounds are retained more strongly on the mildly acidic silica. Silica gel contains HCl molecules adsorbed on the surface form hydrogen bonds with the active silanols. As a result, the adsorbent activity depends on the content of those molecules. A polar interaction via hydrogen bonding takes place between a hydrogen of the amino group in carbendazim and the oxygen of a silanol group (Fig. 2) [19]. In the study referred as [20], it was shown, that only biological active centre of benzimidazole fungicides is the moving proton from amino group of imidazole ring. The interaction with the acidic silica gel caused the retaining of the carbendazim by the adsorbent. Elution is accomplished with a polar solvent, such as methanol–dichloromethane mixture, which forms strong hydrogen bonds with the silanol groups, thus displacing the carbendazim.

The whole procedure involving MSPD extraction was validated for cereal samples fortified at levels 0.04, 0.08 and 0.1 μg/g. Therefore, grinded cereal samples were spiked with adequate working standard solution volume prior to extraction. Up to five replicate analyses were run at all fortification levels. Table 2 shows standard deviation (S.D.) values, limit of detection and limit of determination for LLE, obtained earlier [6], and MSPD extraction techniques. Fig. 3 presented the average recoveries and relative standard deviations (R.S.D.) values. The average recoveries for LLE were >71.2% and for MSPD >84.3%. R.S.D. for LLE and MSPD were 5.2 and 2.7–4.1%, respectively. Standard deviation values ranged from 3.7 to 4% for LLE and 2.3–3.7% for MSPD. A detection limit was found in both cases about

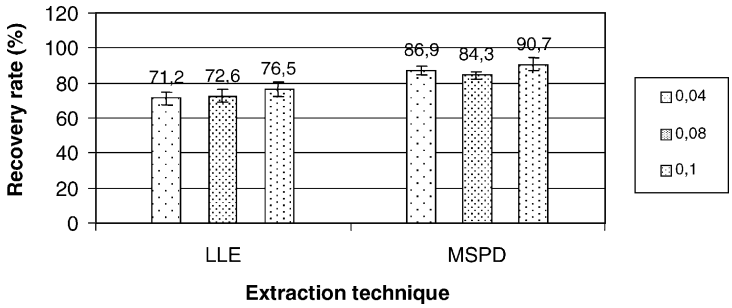

Fig. 3. The comparison of recovery rates and R.S.D. of carbendazim residue obtained from optimization parameters of LLE and MSPD extraction techniques.

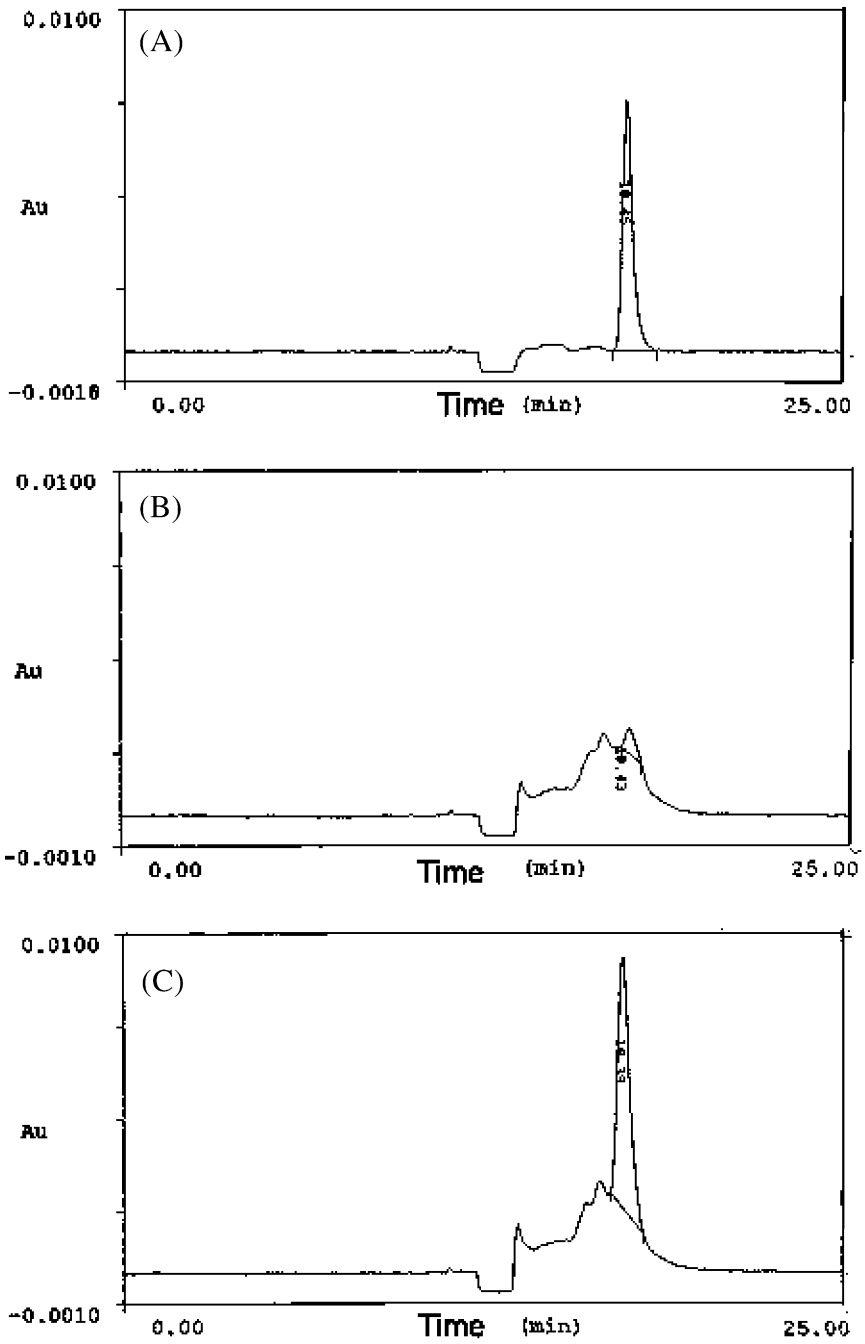

Fig. 4. Typical chromatograms: (A) carbendazim standard 0.4 μg/ml; (B) 5 g control wheat; (C) 5 g fortified wheat over the level 0.1 μg/g.

0.02 μg/g. This value is lower than Polish tolerance limit for carbendazim in cereals (0.1 μg/g).

Fig. 4 illustrates typical chromatograms of the carbendazim standard, unfortified and fortified wheat sample extracts using MSPD technique. Although no interfering peaks were observed on the chromatogram of the unspiked extracts obtained under the selected conditions.

The results shows that both present procedures work well at all levels. The average recoveries for LLE were lower, but values of R.S.D. were identical than in MSPD. Although the classical LLE method demonstrated their efficiency for carbendazim residue analysis in cereal, their results are always comparable with those obtained with the proposed MSPD procedure. Thus, laborious conventional LLE can be substituted by a rapid technique, since only a few minutes are enough to obtain the final extract for HPLC analysis [21,22].

4. Conclusions

The increasing number of samples to be tested, related to the growing concern over food safety, require methods

that must be simple, reliable and cheap. MSPD extraction technique and HPLC determination are appropriate for carbendazim analysis in cereals. The applicability of the procedure is demonstrated by the recovery studies. These data can provide qualitative as well as quantitative information about the influence of xenobiotics on the human health. Such investigations are the object of our work.

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