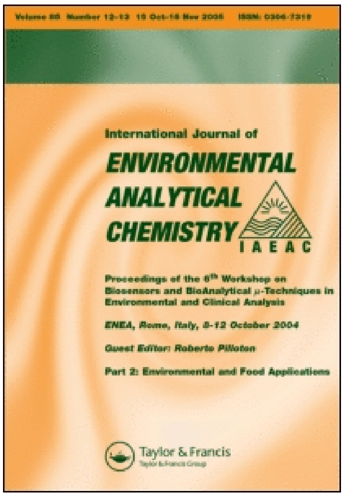

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Determination of Carbendazim in Water, Soil and Sediment Samples by RPLC with and Without Column Switching and Various Spectrometric Detection Modes

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DETERMINATION OF CARBENDAZIM IN WATER, SOIL AND SEDIMENT SAMPLES BY RPLC WITH AND WITHOUT COLUMN SWITCHING AND VARIOUS SPECTROMETRIC DETECTION MODES

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This study presents the developed and applied methods for the determination of carbendazim in environmental samples originating from several field studies.

For water samples sample pretreatment consisted of a solid phase extraction (SPE) on cartridges packed with 200 mg SDB-1. In case of solid samples the performance of microwave assisted solvent extraction (MASE) and classical ultrasonic extraction with acetone-ethyl acetate were studied. The latter technique was selected because of the reduced time of manual operations. Instrumental analysis of extracts of water samples was performed on-line with coupled column reversed phase liquid chromatography (LC/LC) and UV detection (280 nm) allowing to assay carbendazim to a level of at least 0.1 µg/l. Improved column life time was obtained by performing the favorable LC separation of carbendazim at high pH on newly developed 5 µm Extend-pH bidentate C18 material.

The combination of a short column packed with 5 µm Inertsil ODS-5 and a mobile phase at low pH material was most adequate as the regards the robust and fast processing of extracts of solid samples and allowed in most cases the screening of carbendazim in soils and sediments to a level of 10 µg/kg.

The developed procedures yield overall recoveries for carbendazim of 101, 80 and 71 % in water (levels, 0.1–1.2 µg/l; n=12), soil (levels, 10 and 100 µg/kg; n=22) and sediments (levels, 10 and 100 µg/kg; n=11), respectively, with a repeatability and reproducibility below 7 % for all method/matrix combinations. Soil samples with aged residues (level, 100 µg/kg; n=10) provided an overall recovery of 71% and no significant decrease of carbendazim was observed during nine weeks of storage in the refrigerator.

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Confirmation of carbendazim was successfully carried out with LC-APCI/PI/MS, a technique that also showed a performance in quantification comparable to that of UV detection, Data are presented of a field study on the mobility of carbendazim in soil, sediments and surface water in agricultural-use areas.

Keywords: Surface water; soils; sediment; pesticide; carbendazim; field study; SPE; LC columns at high pH; column switching; MASE; LC-MS

INTRODUCTION

For many decades carbendazim has been used in agriculture as a systemic fungicide. In The Netherlands it is mainly used in the culture of peas, beans, mushrooms, wheat and potatoes, but also for the post-harvest protection of seeds and bulbs and for the treatment of soils[1]. The amount of application in 1990 was estimated to be 61 tons with an emission to surface water of 0.84 tons[1].

As a result of emission, long term use and the property of being slowly transformed, carbendazim residues are frequently found in surface waters[1] and, hence, effective environmental monitoring of carbendazim with efficient and reliable analytical methodology will be necessary,

Being a low volatile and polar compound with basic and favorable spectrometric properties, instrumental analysis usually is performed with reversed phase liquid chromatography (RPLC) in combination with UV detection[2–11], UV and fluorescence detection (FD) in tandem[12,13], FD[14,15], or mass spectrometric detection[16–21].

Sample pretreatment involving water samples requires a concentration step which has been performed off-line by means of evaporation[2] or solid phase extraction[5,16] or on-line by means of large volume injection and column switching[3,4,17,18].

In case of solid samples such as soils[16,18] and foodstuffs[6–15,19–21], sample pretreatment usually consists of an extraction-concentration step employing a rather polar organic solvent or mixture followed by a cleanup step. Conventionally, this is done by a rather laborious two-stage liquid liquid partition procedure using a subsequent acidic and basic aqueous solution for cleanup and extraction, respectively[6,7,14]. More efficiently, cleanup can be performed by means of a solid phase extraction using cartridges packed with strong cation exchange-bonded silica[8,9], aminopropyl-bonded silica[16], diol-bonded silica[13] or protein G immunoaffinity material[18]. The latter two sample pretreatment steps [13,18] are fully integrated in the analytical procedure allowing the automated processing of crude extracts.

TABLE I Information on columns and mobile phases used

Code name column	*Packing material*	*Dimension ($l \times$ i.d. in mm)*	*Used as*[a]	*Mobile phase*[b]
Hyp-1	5 µm Hypersil ODS	50 × 4.6	C-1	M_{amm}-1
Hyp-2	5 µm Hypersil ODS	150 × 4.6	C-2	M_{amm}-2
Extend-1	5 µm bidentate C18	50 × 4.6	C-1	M_{amm}-1
Extend-2	5 µm bidentate C18	150 × 4.6	C-2	M_{amm}-2
Inertsil	5 µm Inertsil ODS-3	50 × 4.6	C-1, C-2	M-1 and M_{TFA}

a. C-1, first separation column; C-2, second separation column.

b. M-1, methanol-water (35.65, v/v); M_{amm}-1, acetonitrile-0.5% ammonia in water (10–90, v/v); M_{amm}-2, acetonitrile-0.5% ammonia in water (20–80, v/v); M_{TFA}, acetonitrile-0.01% TFA in water (10:90, v/v); all flow rates set at 1 ml/min.

Extraction with pressurized hot (subcritical) water[12] and supercritical fluid extraction (SFE)[12,15] seems to be attractive as regards selectivity because they allow the direct processing of crude food extracts with LC-UV/FD.

MS detection[17–21] offers high selectivity and analysis of extracts of several types of non-fatty foodstuff samples has been performed without an additional cleanup step[19–21], however, in case of soil samples a cleanup appeared to be necessary[17,18].

Unfortunately, the methods mentioned above do not comprise an easy accessible and or a cost-effective methodology for the assay of carbendazim in both aqueous (ditch surface water) and solid (soil and sludge) samples involved in our studies. In addition, the RPLC separation of carbendazim is frequently encountered by a severe peak tailing caused by unwanted interaction of the basic analyte with residual silanol groups[22,23], which reversibly effects both selectivity and sensitivity[3–5,7,14–21]. We successfully used Hypersil ODS columns with a mobile phase containing 0.5% ammonia (pH of about 11) for the efficient separation of basic compounds such as benzimidazole fungicides[2,6] and ethylenethiourea[24].

An important development in this field are silica-based bidentate-C18 stationary phases which combine excellent column efficiency and superior stability at high pH[25,26]. For more than one decade we have successfully applied coupled column RPLC (LC/LC) in the trace analysis of many polar compounds in environmental samples[27]. In comparison to a one-column separation or precolumn switching, LC/LC can enhance considerably both selectivity and sensitivity. Applications, the theoretical and practical aspects as regards the technique and method development has been recently reviewed and discussed in detail[27].

This work presents our developed cost-effective methods for the monitoring of carbendazim in various types of environmental samples. Analytical methodology clearly demonstrates the viability of LC/LC, the use of bidentate C-18 columns as well as various spectrometric detection modes.

The results of a field study focussed at the measurement of drift and spray deposition of carbendazim at normal agricultural use in The Netherlands will be presented.

EXPERIMENTAL

Reagents

Carbendazim (content > 99 %) was from Dr. S. Ehrenstorfer (Promochem, Wesel, Germany). HPLC-grade acetone, acetonitrile and methanol, and nano-grade ethyl acetate and dichloromethane were from J.T. Baker (Deventer, The Netherlands). Analytical grade sodium sulfate (anhydrous), trifluoroacetic acid (TFA) and hydrochloric acid (HCl) were from Merck (Darmstadt, Germany). Ammonia (sp. gr. 0.91; 25% NH_3) was purchased from BDH (Poole, UK). HPLC-grade water was obtained by purifying demineralized water in a Mili-Q system (Millipore, Bedford, MA, USA).

A 10% hydrochloric acid and 0.5% ammonia solution were prepared by making a dilution (v/v) of the concentrated solution in HPLC water.

A stock standard solution (about 100 µg/ml) was prepared by weighing 25 mg in a 250 ml volumetric flask and dissolving it in acetone using an ultrasonic bath. For spiking, the stock standard solution was diluted in acetone. For LC-analysis, the stock standard solution was diluted in a solution corresponding to the mobile phase of the first column. The diluted solutions were kept in the refrigerator at 4°C.

Columns packed with 5 µm Hypersil (Shandon, Runcorn, Cheshire, UK), 5 µm Inertsil ODS-3 (GL Science Inc., Tokyo, Japan) or 5 µm Extend-pH bidentate C18 (Zorbax, Hewlett Packard, Newport, DE, USA) were used in the one-column LC mode (LC) and/or the coupled-column LC mode (LC/LC) as a first, C-1, and/or a second, C-2, analytical separation column. The dimensions of the columns and the various types of application modes are given in Table I.

Mixtures of acetonitrile-0.5% ammonia in water (10:90 and 20:80; v/v), a mixture of acetonitrile-0.01% TFA in water, pH 3.0 (10:90; v/v) and a mixture of methanol-water (35:65; v/v) were used as a first, M-1, and/or the second, M-2, mobile phase in the various methods listed in Table II.

TABLE II Information on LC conditions[a] used in LC methods for various types of samples

Sample type	*LC- Method*	*C-1/M-1*	*C-2/M-2*	*Volumes (ml) for cleanup/transfer*	*Detection mode*[b]	*Fig.*
surface water	Ia	Hyp-1/ M_{amm}-1	Hyp-2/ M_{amm}-2	3.8/0.8	UV (FLD)	1A
surface water	Ib	Extend-1/ M_{amm}-1	Extend-2/ M_{amm}-2	3.8/0.8	UV (FLD)	1B
surface water	Ic	Inertsil/M-1			APCI/PI/MS	4
soil/sediment	IIa	Intersil/ M_{TFA}			UV (FLD)	3
soil/sediment	IIb	Intersil/ M_{TFA}	Intersil/ M_{TFA}	3.6/08	UV (FLD)	

a. See information given in Table I.
b. UV, ultra violet detection, FLD, fluorescence detection, MS, mass spectrometric detection.

Disposable 3 ml SPE cartridges containing 200 mg of styrene-diphenyl-benzene (SDB) polymer packing material (40 µm) were obtained from J.T. Baker. The cartridges were preconditioned with 3 ml of methanol, 3 ml of acetone, 3 ml of methanol and 6 ml of 0.1 % HCl in water (pH = 2 ± 0.1), respectively.

Equipment

A Baker-10 system was used to perform SPE. The HPLC system consisted of a Model 231 XL autosampler from Gilson (Villiers-le Bel, France) equipped with an additional six-way programmable high pressure valve (type 7010, Rheodyne, Cotati, CA, USA) for column switching, a Model 305 and a Model 306 isocratic LC pump from Gilson, a Model 118 UV detector from Gilson and a Model FP-920 fluorescence detector from Jasco Corporation (Tokyo, Japan).

Microwave Assisted Solvent Extraction (MASE) experiments were performed with a MES-1000, 950-W laboratory Microwave Extraction System (CEM, Mathews, NC, USA) configured with a 12-position carousel.

LC/MS was performed on a Series 1100 benchtop LC/MS of Hewlett Packard applying Atmospheric Pressure Chemical Ionisation (APCI) in the positive ionization (PI) mode. The LC columns were kept at 30°C with a laboratory made column oven connected to a Model 1441 circulating water system from Braun (Melsungen, Germany). Quantitative measurements of peak heights were made with the PC-1000 integrator system of Thermoquest employing a Model 800 DP integrator from Fisons,

An ultrasonic bath Model 104H and a centrifuge Model 2–15 were from Ney Dental International (Bloomfield, CT, USA) and Sigma (Osteroode, Germany), respectively.

Pretreatment sediment/soil samples

Recovery experiments

A selected reference clay soil to perform recovery experiments was characterized by a water content 17.9%, a clay content of 28,0%, a sand content of 46%, an organic carbon content of 3.8 % an organic matter content of 5.8% and a pH 7.5. Sediment samples were prepared by adding 40% water to the reference clay sample following by mechanical shaking in a closed vessel for one hour.

Freshly spiked soil and sediment samples were prepared by weighing 10.0 g of material into a glass bottle followed by the addition of an appropriate volume (= 2.0 ml) of spiking solution; the samples were allowed to stand overnight before extraction. Fortifications were made at levels of 10 and 100 µg/kg, respectively.

Soil samples with aged residues were prepared by spiking 10 g of clay soil at a level 100 µg/kg. After overnight standing, they were stored in the refrigerator at about 4°C; sample analysis was performed after 1, 2, 3, 5 and 9 weeks.

Extraction

Before extraction, sediment samples were transferred into a glass tube and centrifuged for 15 min (480 g). After decanting the water, the sample was manually homogenized by stirring. 10.0 g of soil or sediment sample were weighted into an erlenmeyer flåsk of 100 ml. Next, 25 ml of ethyl acetate-acetone (25:75; v/v) solution were added, the vessel was closed, shaken and placed in an ultrasonic bath during 30 min. The organic extract was filtered over a funnel containing about 10 g of sodium sulfate and 5 ml of extract were transferred to a rotavapor flask. After solvent evaporation using a water bath temperature of 40°C and a gentle stream of nitrogen, the residue was redissolved in 1 ml of acetonitrile-0.01% TFA in water (10–90; v/v). The solution was pipetted into an autosampler vial of 2 ml.

Pretreatment of water samples

Concentration method A

100 ml of water sample were brought into a 250 ml rotavapor flask and evaporated to a volume of about 1 ml at a water bath temperature of 80°C. 200 µl of acetonitrile were added and after swirling, the content was transferred with a Pasteur's pipette into a calibrated tube and made up to a volume of 2.0 ml with 0.5% ammonia in water solution and pipetted into an autosampler vial of 2 ml.

Concentration method B

After adding 250 µl of a solution of 10% HCl in water to 100 ml of sample (pH = 3 ± 0.1), the volume was percolated through a preconditioned 200 mg SDB-1 cartridge at a flow of approximately 4 ml/min. After sample loading the cartridge was dried by passing air for about 15 min. The cartridge was transferred to the top of a calibrated tube, and by means of slight over-pressure, 4 ml of methanol were passed through the cartridge and collected in the tube.

Prior to the LC-UV (FLD) the methanol extract was evaporated to dryness using a warm water bath and a gentle stream of nitrogen. The residue was dissolved by first adding 200 µl of acetonitrile, followed by 1800 µl of 0.5% ammonium in water; the solution was pipetted into an autosampler vial of 2 ml.

For the LC-MS-confirmation analysis, 500 µl of extract were transfered to an autosampler vial and 175 µl of 0.6% formic acid in water were added in order to lower the pH to about 5 ± 0.5.

LC analysis

Information on applied LC and LC/LC methods and conditions are given in Table II. Independent of type of sample or method, flow rates were set at 1 ml/min and 100 µl of extract obtained after the applied sample pretreatment were injected on the column (LC mode) or on the first column (C-1) in the LC/LC mode. UV detection was performed at a wavelength of 280 nm and in case of tandem fluorescence detection (optional) wavelengths of 285 and 317 nm were used for excitation and emission, respectively,

Quantification of carbendazim was done by external calibration with standard solutions. Confirmation and quantification (optional) of carbendazim was carried out with LC-APCI/PI/MS (LC conditions of LC-method Ic) using the settings of 4 µA and 80 V for the corona current and fragmentor voltage, respectively. Under these conditions two ions were obtained for carbendazim: $[M\text{-}H]^+$ at m/z 192 and a fragment ion at m/z 160.

Field samples

The study involved the sampling of soil, surface (ditch) water, and sediment of seven Fields (I-VII). At field II collection of water samples was failed because of the absence of water in the adjacent ditches.

Ditch water and sediment sampling

At each field one ditch was selected for sampling. If more ditches were present a field ditch was selected that was preferably down-wind of the spray cloud and where there was no spray/crop free zone. The absence of a spray free zone prevailed above the down-wind criterion.

From each ditch four water samples (1, 2, 3, 4) were taken at a depth of 5 cm below the water surface with a mutual distance of 25 meters. The first sample spot was at least 25 meters from the edge of the field crop. Samples were taken just before spraying (t_A), just after spraying, (t_B), and 5 days later (t_C). The sample spots were determined arbitrarily each time of sampling.

At the last day of the ditch water sampling sediment samples were taken on the same spot with a multisampler device.

Soil sampling

Soil samples over a depth of 10 cm were taken with a gonge-anger device shortly after application with an amount of active ingredient of 0.1 and 0.25 kg/ha for Field I and Fields II-VII, respectively. Of a selected field the area consisted of at least 5000 m^2. The area is situated in such a way that the borders are at least ten meters away from the border of the field and from often-used wheel tracks. The selected area is divided into 4 equal sections. On each transect, a slanting line (Transect Sight Line) was drawn. The corner points were determined randomly with the help of the computer program UNCSAM, according to the Standard Operation Procedure No 647 of the Laboratory of Soil and Ground water Research. On each line 10 samples were taken on randomly predetermined sampling spots and divided over 4 sample buckets according to a random scheme.

RESULTS AND DISCUSSION

Method development for water samples

Until recently water samples were analysed with an unpublished procedure involving a concentration step of the sample with a rotating evaporator and coupled-column RPLC (LC/LC) with UV detection at 280 nm for the instrumental analysis of crude extracts. The LC/LC-method (Ia, Table II) employs the stationary/mobile phase combination of Hypersil ODS/0.5% ammonia for the efficient elution of carbendazim and the separation power of two analytical columns (LC/LC) in the column switching mode. The powerful cleanup of this approach allowed the assay of carbendazim in crude extracts of water samples at the required level of at least 0.1 µg/l in less than 15 min.

The good performance of the existing method is nicely illustrated in Figure 1A showing the LC/LC-UV analysis of a concentrated ditch water sample containing 0.55 µg/l of carbendazim.

Recently the operational method was further optimised on two critical points: sample pretreatment and column lifetime.

Enhancement of sample pretreatment was focussed at the replacement of the rather laborious concentration method (A, see Experimental) by a solid phase extraction (SPE) procedure. The performance of SPE cartridges packed with 500 mg Bakerbond C18, 200 mg Bakerbond SDB-1, 500 mg Superclean Envi-Carb (Supelco) and 500 mg Bondelute C_{18}-OH (Varian) was tested by pre-

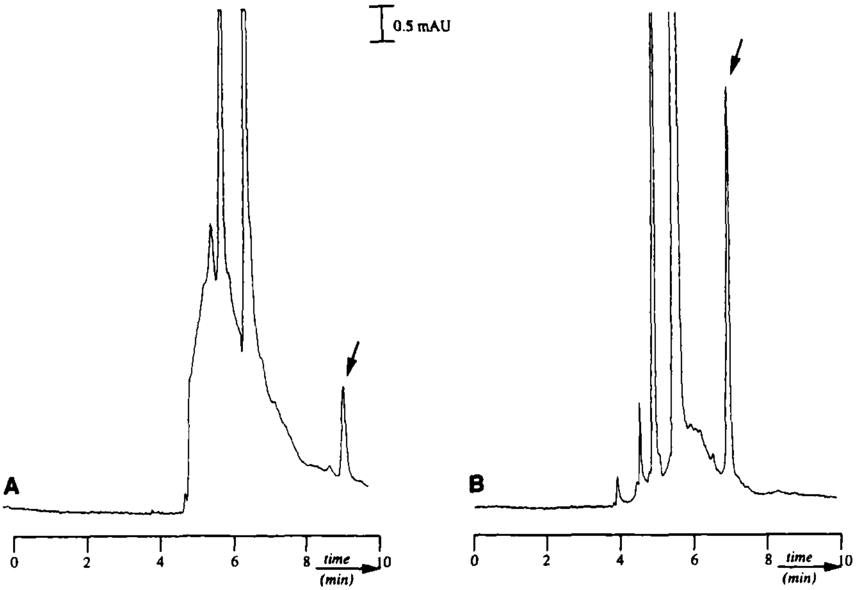

FIGURE 1 LC/LC-UV (280 nm) of 100 μl of an extract of ditch water samples containing carbendazim, employing different extraction methods and LC columns. A, Extraction method A (rotavapor) and LC-method Ia (5 μm Hypersil columns) of a sample containing 0.50 μg/l of carbendazim. B, Extraction method B (SPE) and LC-method Ib (5 μm Extend-pH bidentate C18 columns) of a sample containing 0.60 μg/l of carbendazim (see Experimental and Tables I and II)

colating 100 ml of blank and spiked (level of 1 μg/l) tap water samples adjusted to pH 3.

Using methanol as solvent for elution, carbendazim was fully recovered on both C_{18}-OH and SDB-1 cartridges, however, under these conditions no recovery for carbendazim was obtained using the C18 and Envi-Carb cartridges.

Arbitrarily, SDB-1 cartridges were selected for further work. In comparison to the existing procedure the SPE method (B, see Experimental) is distinctly more efficient and, moreover, the obtained organic extracts are more appropriate as regards storage over a long period of time.

The second improvement was obtained by using the bidentate C_{18} columns. Beside an impressive long lifetime of more than 5 months of intensive use these columns provide an excellent separation of carbendazim.

An example of the improved method involving SPE and LC/LC with bidentate C18 columns is given in Figure 1B showing the analysis of an extract of a surface water sample containing 0.61 μg/l of carbendazim.

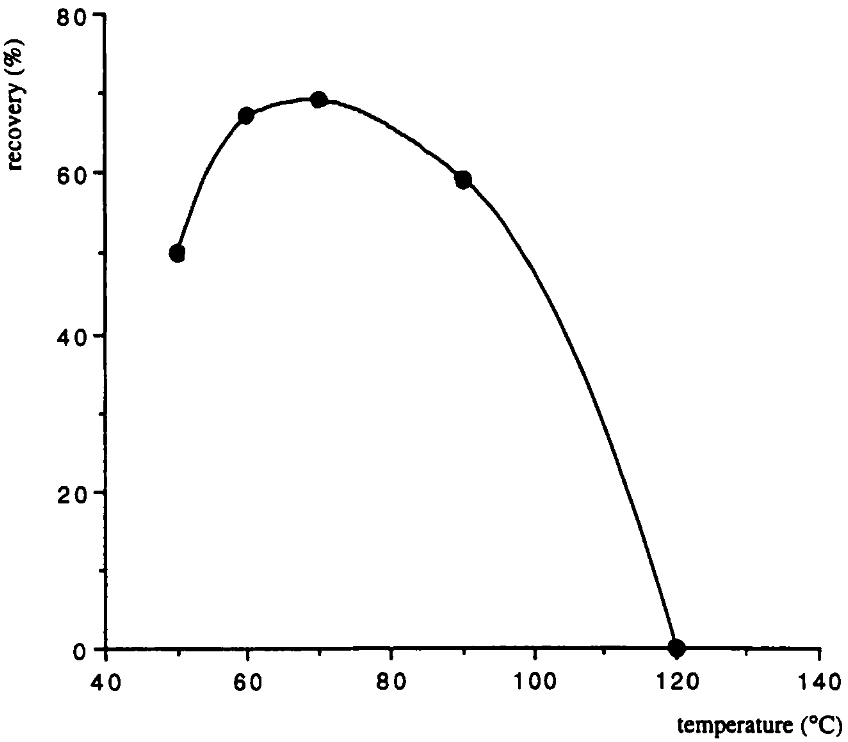

FIGURE 2 Recoveries of soil samples spiked at a level 100 μg/kg obtained with MASE at different temperatures; 100% power, extraction time of 20 min

Method development for soil and sediment samples

Because of our favorable experience on the efficient extraction of polar pesticides from soil with microwave-assistant solvent extraction (MASE)[28–30], the potential of this technique was firstly investigated. In our previous applications a mixture of dichloromethane-methanol (90:10; v/v) appeared to be an efficient and convenient extraction solvent. Unfortunately, losses of the amount of analyte of about 25% were observed during careful evaporation of 10 ml solvent. Therefore, a switch was made to other solvents, e.g. acetone and ethyl acetate, usually applied in the analysis of carbendazim in foodstuffs[6–10,13,14,19–21].

First recovery experiments were carried out with classical ultrasonication testing the solvents (i) acetone, (ii) ethyl acetate, (iii) acetone-ethyl acetate (50:50; v/v) and (iv) acetone-ethyl acetate (75:25; v/v). These experiments involved the extraction of 10 g of spiked clay soil (see experimental) with 20 ml

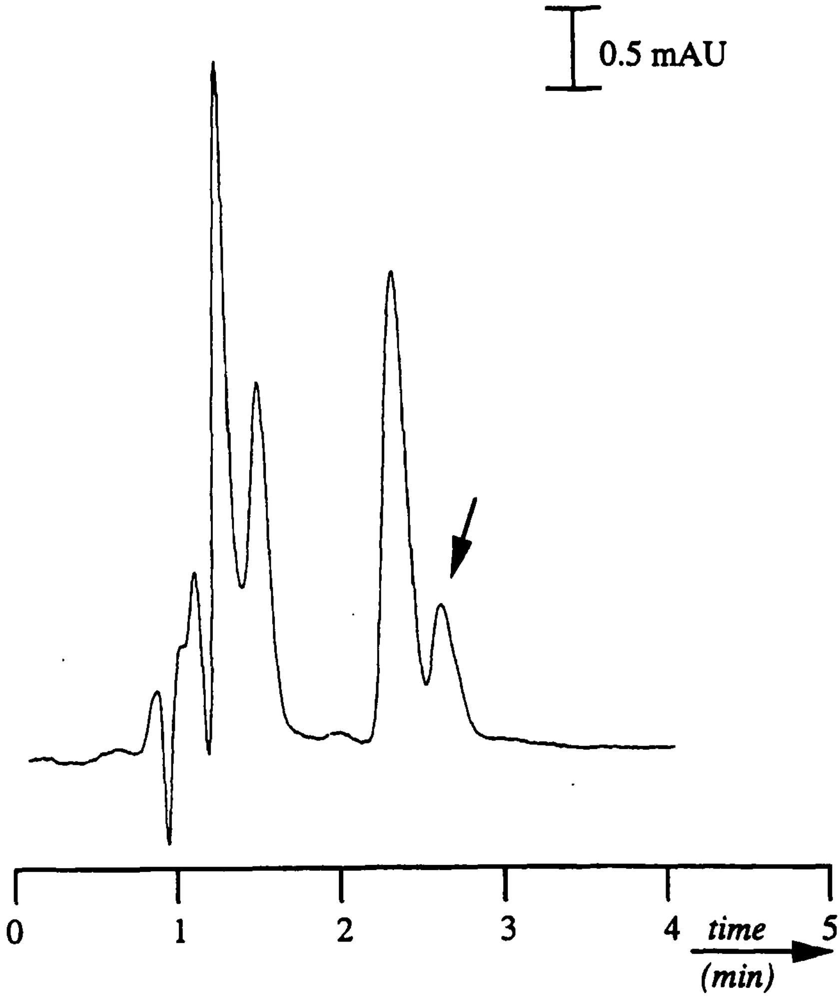

FIGURE 3 LC-UV (280 nm) of 100 µl of an extract of a soil sample containing 30 µg/kg of carbendazim. (LC-method IIa, see Tables II and III)

of solvent during 10, 20 and 30 min. It appeared that an extraction time of at least 20 min and solvent mixtures provided highest recoveries (range 60–80%).

Selecting acetone-ethyl acetate (75:25; v/v) as the extraction solvent, the performance of MASE at 100% power, 20 min extraction time and the temperature (T) as the varying parameter (range 50–120°C) was further investigated. As clearly displayed in Figure 2, the recovery of carbendazim depends on the temperature, a phenomena also encountered in the extraction of sulfonylurea[29] and phenylurea[30] herbicides from soils with MASE. In comparison to ultrasonic extraction, MASE did not substantially improve the recovery, the time of sample pretreatment and solvent consumption. Requiring less manual operation as regards the cleaning of the extraction vessels, the ultrasonic-based extraction procedure (see Experimental) was selected as the extraction technique for both soil and sediment samples.

Scouting LC/LC-UV experiments of soil and sediment extracts revealed that in comparison to the analysis of extracts of water samples, matrix interferences were distinctly less. Column switching appeared not to be really necessary and preferring simplicity the feasibility of a one-column separation without high pH was investigated. Based on valuable information[31], satisfactory results were obtained on 5 μm Inertsil ODS columns in combination with a mobile of low pH (Table II, LC-method IIa). Despite protonation (ionization) of the analyte this stationary/mobile phase combination provides sufficient retention of carbendazim and peak tailing is nicely prevented by ion-suppression of the residual silanol groups on the silica surface[22].

An illustration of the screening ability of this approach is given in Figure 3, showing the LC-UV analysis of an extract of a soil sample containing 30 μg/kg of carbendazim in less than 5 min.

The final method consisting of ultrasonic extraction and LC-method IIa could be efficiently applied for most of the samples involved in this study. In a few cases, however, LC/LC (LC-method IIb or LC-method Ib) had to be applied for the elimination of the excess of matrix interferences.

Performance methods

Validation of the methods applied for the various types of samples was performed by analyzing during each measurement sequence of real samples a few spiked samples consisting of tap water, reference soil or reference sediment (see also Experimental). The results of these validation experiments are summarized in Table III. As regards the analysis of water samples one clearly can see improved results on both recovery and variation of the improved procedure using SPE in comparison to the existing procedure employing the laborious rotating evaporation method.

For the freshly spiked solid samples, the somewhat lower average recovery of 69% obtained for sediments in comparison to soil (80%) is acceptable and repeatabilities and reproducibilities below 7% for both matrices clearly illustrate the good performance of the method.

An average recovery of 71% and values below 10% for both repeatability and reproducibility for the aged residues experiments demonstrate the suitability of the extraction technique and the possibility to store samples at least for a period of 9 weeks. Based on the data of Table III and inspection of the chromatograms (cf. Fig. 1 and 3) limits of quantification of carbendazim were estimated to be 0.1 µg/l and 10 µg/kg for water and soil, respectively.

TABLE III Overall results of carbendazim recoveries from environmental samples

Sample type	*Method*[a]	*Spiking levels*[b] *(µg/kg or µg/l)*	*n*	*Overall average recovery (%)*	*RSD*[c] *r (%)*	*RSD*[c] *r (%)*
Water	A	0.1 and 0.5	16	66	12.4	13.5
Water	B	0.1, 0.4 and 1.2	12	101	6.6	6.7
Soil (freshly spiked)		10 and 100	22	80	6.9	6.5
Soil (aged residues)		100	10	71	4.9	8.1
Sediment		10 and 100	11	69	5.3	5.1

a. A, rotavapor method; B, SPE method.
b. Soil and sediment, µg/kg; water, µg/l.
c. r, repeatability; R, reproducibility (intra-laboratory)

Despite selective on-line sample cleanup by means of LC/LC, additional confirmation will be necessary in case of positive samples when using UV detection at 280 nm. Unfortunately, for the matrices studied fluorescence detection (FLD) did not substantially enhanced selectivity, a phenomena which also has been encountered in the analysis of carbendazim in foodstuffs[12,13].

Therefore, additional confirmation for a selection of both positive and negative samples was carried out by re-analyzing the extracts with LC-APCI/PI/MS. LC/MS performed on the benchtop system appeared to be an easy accessible, robust and reliable technique offering high sensitivity and selectivity. Suitable LC/MS conditions (See Experimental and Table II) were established within two days. It appeared that the LC separation on a short Inertsil ODS column without the use of a buffer (Method Ic) is most optimal as regards the MS detection of carbendazim. An example of the LC-MS method is given in Figure, 4, showing the LC/MS re-analysis of the extract displayed in Figure 1B.

Because of the one column analysis of crude extracts without cleanup, one can expect that co-extracted matrix interferences effect the ionization of the analyte and, hence, reliable quantification when using external standards for calibration. This effect was investigated by the LC/MS analysis of extracts a large group of positive surface water samples originating from another study which were analyzed before with LC-UV (Table II, Method Ib). The data were evaluated with linear regression analysis using weighted least squares[32]. A linear relation was

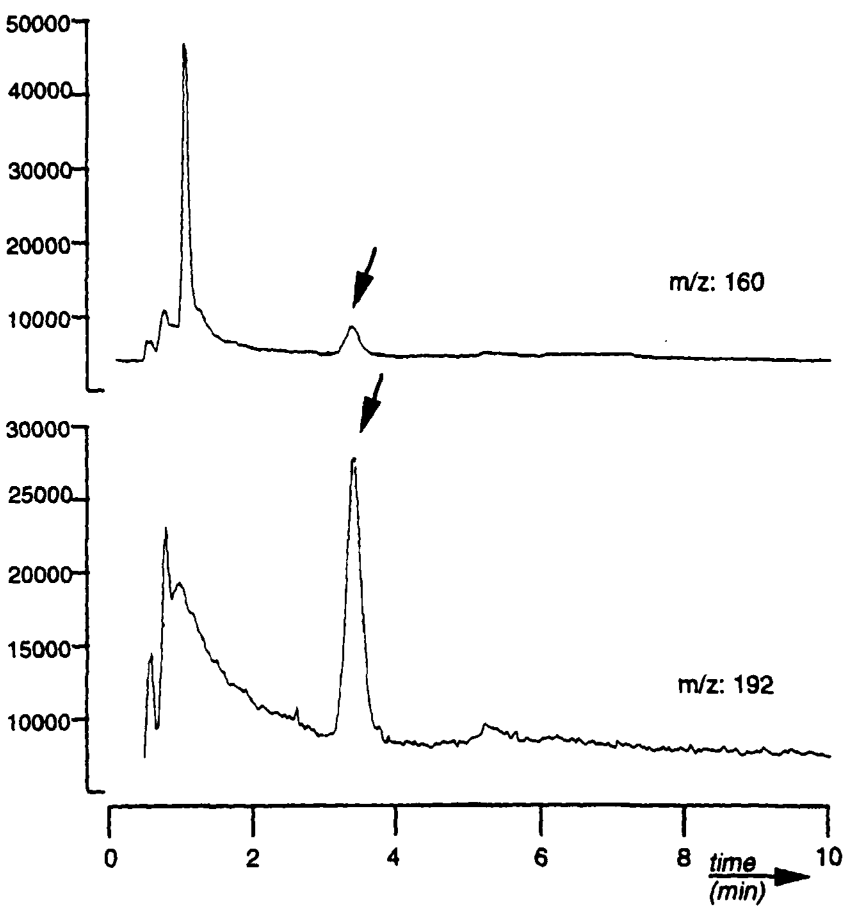

FIGURE 4 LC-APCI/PI/MS of 100 μl of an extract of surface water sample displayed in Fig. 2A and containing 0.60 μg/l of carbendazim (LC-method Ic, see Tables II and III)

found of $y = -0.012 + 0.998x$ in which y and x correspond to the value of LC-MS and LC-UV (reference method), respectively. The good correlation between the two methods is nicely illustrated in Figure 5, including the 95% confidence intervals.

An important aspect to emphasize is that the LC/LC approach was successful as regards the lifetime of the bidentate C18 columns. Under the wearing conditions for bonded silica's, viz. a mobile phase at pH 11 and large pressure variability, the decrease in the efficiency (N) of both the first and the second analytical column was below 10% during the five month period of intensive use.

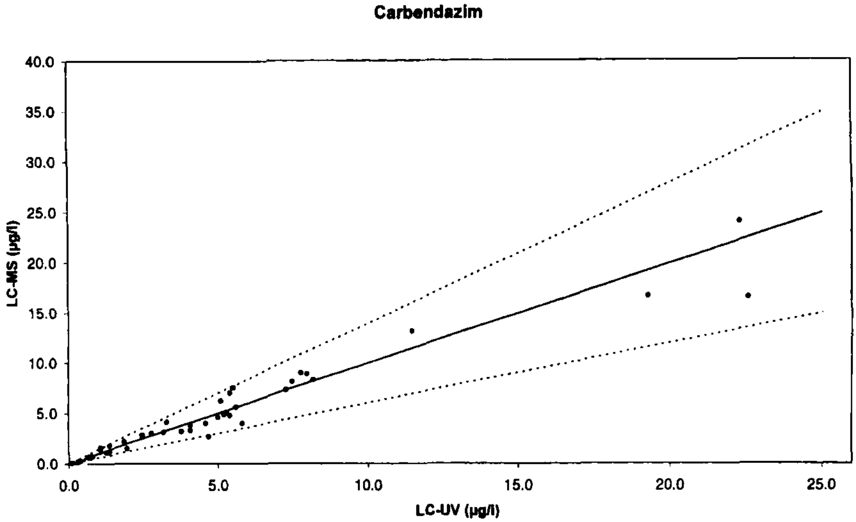

FIGURE 5 Comparison of the results of the LC-UV vs LC-MS method for the analysis of carbendazim in extracts of water samples with incurred residues. Bracket lines corresponds to 95% confidence interval

Data of field study

The aim of the field study discussed in this study was to obtain information on drift and spray deposition of carbendazim at normal agricultural use on seven locations in The Netherlands. Information on types of samples, sampling and sample location is given in the experimental section.

The carbendazim residues found in the ditch water samples taken at times before (t_A), shortly after (t_B) and 5 days after (t_C) application are given Table IV which includes also the data of the sediments samples taken at t_C at the same spot of the water samples. For most of the Fields (III to VII), the data clearly show an increase in concentration at t_B and a significantly decrease at t_C. However, in Field I there is no significant increase in concentration at t_B, while a decrease can be observed at t_C. In Fields I, IV, VI and VII carbendazim was already found at t_A, viz. before application. The low concentrations in the ditch near Field III can be caused by relatively high flow stream of the water in comparison to the other locations.

In case of the sediments, carbendazim was found only at three locations (Field I, VI and VII). In comparison to Field I and VI, the ditch of Field VII had a distinctly less water content. This might explain the better consistency between residues found at this field as a result of the enhancement of the exchange of the analyte between water and soil providing a more homogeneously process over the total length of the ditch. The average concentrations and standard deviations of carbendazim in the series of soil samples (n=4) were 51±23, 209±16, 117±11, 74±14, 98±29, 98±14 and 65±10 μg/kg for the Fields I, II, III, IV, V, VI and VII, respectively.

TABLE IV Carbendazim residues in ditch water (μg/l) and sediment (μg/kg) samples of the field study collected before (t_A), shortly after (t_B) and 3 days after (t_C) application

Field-Sample code	*Water (t_A)*	*Water (t_B)*	*Water (t_C)*	*Sediment (t_C)*
I-1	5.1	6.1	2.9	21.3
I-2	5.2	4.9	2.9	< 10
I-3	5.1	4.6	2.6	< 10
I-4	5.4	5.2	2	< 10
III-1	< 0.1	n.s.[a]	0.1	< 10
III-2	< 0.1	n.s.[a]	< 0.1	< 10
III-3	< 0.1	n.s.[a]	0.2	< 10
III-4	0.1	n.s.[a]	< 0.1	< 10
IV-1	0.6	1.3	0.6	< 10
IV-2	0.2	1.3	0.6	< 10
IV-3	0.6	1.3	0.6	< 10
IV-4	0.2	0.8	0.3	< 10
V-1	<0.1	0.2	0.1	< 10
V-2	<0.1	0.8	< 0.1	< 10
V-3	<0.1	0.1	0.1	< 10
V-4	<0.1	0.2	0.1	< 10
VI-1	1.3[b]	128.1[b]	< 0.1	57.7
VI-2	n.s.[a]	n.s.[a]	1	54
VI-3	n.s.[a]	n.s.[a]	0.2	< 10
VI-4	n.s.[a]	n.s.[a]	< 0.1	< 10
VII-1	16.3[b]	17.8[b]	1.3	12.6
VII-2	45.0[b]	58.8[b]	0.5	12.5
VII-3	n.s.[a]	n.s.[a]	0.6	< 10
VII-4	n.s.[a]	n.s.[a]	0.5	11.2

a. n.s, not sampled.

b. sample taken by farmer.

It must be mentioned that on the field with highest concentration (Field II), carbendazim has been applied several times before the field study, while the on the field with lowest concentration (Field I) a lower amount of the fungicides was applied (see Experimental).

CONCLUSIONS

The combination of off-line solid phase extraction (SPE) and coupled-column liquid chromatography (LC/LC) with UV detection at 280 nm is an efficient approach for the screening of carbendazim in environmental water samples to a level of at least 0.1 µg/l. The rapid SPE of only 100 ml of sample and the on-line instrumental processing of the crude extracts in less than 10 min provides a high sample throughput of at least 40 samples per day. Columns packed with newly developed bidentate C18 material showed an excellent high-pH stability allowing the efficient LC separation of carbendazim at pH 11 for a long period of time.

In case of solid samples, a mixture of acetone-ethyl acetate (75:25; v/v) was selected as a suitable solvent for the extraction of carbendazim. Both classical ultrasonification and microwave assistant solvent extraction (MASE) demonstrates to be an adequate and rapid extraction technique. The selected classical ultrasonification technique tested with soil samples with aged residues shows a performance comparable to that obtained with freshly spiked samples. In most cases the instrumental analysis of soil extracts could be performed with a one-column separation.

Benchtop LC-APCI/PI/MS used for confirmation appeared to be a fast, robust and reliable technique offering the possibility to simultaneously perform in a cost-effective way confirmation and quantification of carbendazim in environmental samples. In the field study carbendazim was found in 46 out of 58 ditch water samples (range 0.1–128 µg/l), in 6 out of 24 sediment samples (range 11–60 µg/kg) and in all of 28 soil samples (range 30–230 µg/kg).

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