Determination of Pesticide Residues in Water and Plant Material by Gas and Thin-Layer Chromatography

Report

by

Signe Tomsone

Department of Environmental Assessment
Swedish University of Agricultural Sciences
Box 7050, SE-750 07 Uppsala

2003

Rapport 2003:14

ABSTRACT

The isolation and determination of up to 21 different pesticide residues from fortified water and *Carum carvi* (L.) seeds is presented. Pesticides were extracted from water and seed samples by using liquid-liquid and solid-phase extraction methods. The suitability of HyperSep Retain solid-phase extraction columns for water analysis was evaluated. The water analysis recoveries of 70 to 120% were usually obtained, but the yields of atrazindesispropyl, 2,6-dichlorobensamide and dichlobenil were lower. It was observed that HyperSep Retain sorbent in some columns capture water. The first results indicated that HyperSep Retain columns are reusable. Pesticide residues from the fortified herbal samples were extracted by ethyl acetate using two different extracting procedures following cleanup on solid-phase extraction columns (ENV+) usually used in the analyses of pesticides in water. The recovery results indicated that ENV+ columns could be used for cleanup of plant extracts. To evaluate extraction efficiency different methods of thin layer chromatography were used for the determination of the analytes extracted from the fortified seeds.

INTRODUCTION

The objective of this study is to apply methods for pesticide residues analysis to medicinal plants.

The market of medicinal plants in Latvia contain local herbs that have been cultivated, or collected from wild nature, as well as imported products. Herbs are used in comparatively small amounts, however pesticide residues therein can potentially cause damage to health.

For control of the fate of pesticides the analysis of residues in different matrices are developed. In this study the main attention is paid to extraction, cleanup, determination and results evaluation. Water was choosen as model object because it is considered to be the simplest medium.

Liquid-liquid extraction (LLE) and solid-phase extraction (SPE) of water was used for sample preparation. LLE is considered to be effective since it extracts both water soluble and less soluble pesticides. The method is based on different solubility of pesticides in water and organic solvents (Åkerblom, 1995). The main advantage of SPE is small consumption of solvent. The principle of this method is the retention of analytes by sorbent of similar polarity following their elution with a solvent (Åkerblom, 1995).

The SPE columns examined are C2 EC/ENV+ and HyperSep Retain. The C2 EC is a non-polar sorbent containing trimethylsilyl groups. The ENV+ and HyperSep Retain sorbent is made up by styrene-divinylbenzene co-polymer. The C2 EC/ENV+ dual phase columns have been widely applied for ground water analysis in Dept. of Environmental Assessment (Swedish University of Agricultural Sciences). The main disadvantage of these columns is clogging. Therefore the examination of the suitability of HyperSep Retain columns was started. Determination of the recovery of analytes from fortified water samples was done by using different thin layer chromatography (TLC) and gas chromatography (GC) methods.

There are successful experiments of SPE column application for fruit and vegetable cleanup (Pihlström and Österdahl, 1999). Such methods have been applied to some medicinal plants too (Lino et al., 1997). The idea is to develop rapid and simple cleanup for plant extracts instead of gel permeation chromatography. This paper presents the results of attempts to use SPE columns for the cleanup of herbal extracts.

METHODS

1. Chemicals

All pesticide standards were provided by Dr. Ehrenstorfer. Stock solutions of standards were prepared in acetone. Solvents were provided by Merck.

2. Extraction

2.1. Liquid – liquid extraction of water

The sample bottles with water were weighted. Approximate sample volume was 1 l. Samples used for recovery studies were fortified with known amount of pesticide standard mixture (Mix A 324:1; Appendix 1). All samples were transferred to separating funnels. Ethion (standard solution M 86:cp concentration 4.144 μg/ml) with final concentration 0.166 μg/ml was used as internal standard. Sodium chloride 100 g was added to each sample, the funnels were shaken to dissolve NaCl. Dichlormetane (50 ml) was added and the samples were shaken for 2 min using the shaker. The phases were allowed to separate. The lower phase of dichlormetane was filtered through 10–15 g of sodium sulfate into a round-bottomed flask. Each empty sample bottle was rinsed with 50 ml of dichlormetane that was transferred to the separating funnel. The bottles were re-weighted and sample volumes were calculated. The shaking of samples and filtering was repeated as before. Another 50 ml of dichlormetane was added to the each sample into the separating funnels. The shaking of samples and filtering was done as previously. Finally the sodium sulfate was rinsed with 10 ml of dichlormetane. The extracts were evaporated to 1 ml at 37 °C using a BUCHI rotary vacuum evaporator. Cyclohexane (5–10 ml) was added to the extract for 2 times during evaporation to get rid of dichlormetane since the boiling point of cyclohexane is 83 °C and 39.75 °C for dichlormetane. The concentrates were transferred into the graded test tubes. The flasks were rinsed with acetone that was transferred into the test tubes. Acetone was evaporated under a gentle stream of nitrogen. The sample end volume was adjusted to 2.0 ml with cyclohexane/acetone (9:1).

2.2. Solid-phase extraction of water

A. *Preparation of sample*

MilliQ water and river water was used for recovery studies. Sampling of river water was carried out in Tensta 25 km north of Uppsala on the 2nd of April 2000. The water (approximate volume 1 l) was collected in glass bottles. Method 52:0 version 12 from Dept. of Environmental Assessment (Swedish University of Agricultural Sciences) was used to analyze the water samples. Two different pretreatment of the water samples were tested:

version A1. 5 ml methanol, 1 g sodium chloride was added per liter of water sample;

version A2. 1 g sodium chloride was added per liter of water sample.

Ethion and terbutylazin were used as internal standards by adding 40 μl of standard mixture Mix M 622:a (ethion 4.144 μg/ml, terbutylazin 0.635 μg/ml). The MilliQ water samples used for recovery studies were fortified with known amount of pesticide standard mixture Mix A 566:b (Appendix 2) or Mix S (Appendix 3), but the river water samples – with pesticide standard mixture NPSTD-I (Appendix 4).

B. *Preparation of columns*

Three types of solid-phase extraction columns 700 mg C2 EC/ENV+ from International Sorbent Technology, 200 mg HyperSep Retain from ThermoQuest, or 250 mg ENVI-Carb from SUPELICO were used. A VacMaster sample processing station was used to maintain the columns during extraction.

The columns were handled in two different manners:

version B1. The columns were not weighted before preparation;

version B2. The columns were weighted before preparation.

The columns were pre-treated in three different ways:

version B1`. The columns were pre-washed with 5 ml of ethylacetate/acetone (1:1), dried with vacuum for 30 seconds. Then the columns were washed with 5 ml of methanol and rinsed with 10 ml of MilliQ water;

version B2`. The columns were washed with 2.5 ml of ethylacetate/acetone (1:1), dried with vacuum for 30 seconds and rinsed with 5 ml of MilliQ water;

version B3`. The columns were washed with 5 ml of ethylacetate/acetone (1:1), dried with vacuum for 30 seconds and rinsed with 10 ml of MilliQ water.

C. *Extraction*

About 1 l of sample was passed through the columns at flow rate:

version C1. 22-33 ml/min;

version C2. 11-17 ml/min.

Vacuum (a reduced pressure of -10 to -15 in. Hg) was applied. The columns were washed with 10 ml of MilliQ water and dried with nitrogen. The analytes were eluted by gravity with 2 x 2.5 ml ethylacetate/acetone (1:1). The eluate was collected in graded test tubes. Vacuum (a reduced pressure of -10 in. Hg) was applied in the end for 30 seconds to be sure that all eluate was collected. Cyclohexane (1 ml) was added to the extract and the solution was evaporated under a stream of nitrogen to 0.5 ml. The volume was adjusted with cyclohexane/acetone (9:1) to 1.0 ml.

The design of solid-phase extraction is shown in experimental lay-out.

2.3. Extraction and cleanup of herbs

A. *Preparation of sample*

Carum carvi (L.) seeds from Latvia were used as matrix. Approximately 2.2 g of dry seeds per sample were mashed with pestle and mortar. A portion of the powder (2.0 g) was transferred into 50 ml flasks with glass stopper. The samples used for recovery studies were fortified with known amount of pesticide standard mixture Mix C (Appendix 5). Samples were shaken for 5 minutes using an INFORS AG shaker. Then flasks with fortified samples were opened and kept in a fume hood for 10 minutes while the acetone (solvent for pesticide standard mixture) was evaporated. MilliQ water 2.5 ml was added to each sample (for blank and recovery studies), then samples were shaken for 30 minutes.

B. *Extraction*

To caraway samples 6 g of sodium sulfate was added. The samples were extracted with 15 ml of ethylacetate by shaking for 15 minutes following sonification in ultrasonic bath for 2-3 minutes. The extraction was repeated twice with 10 ml of ethylacetate. After each of the extraction the extract was decanted and filtered through 3 g of sodium sulfate into the same 250 ml E-flasks using vacuum. During the first extraction part of the samples were left in ethylacetate overnight at room temperature after sonification. The combined extracts were filtered through Munkell filter paper No. 3 into round bottomed flasks. The E-flasks were rinsed two times with 2 ml of ethylacetate. The extracts were evaporated to approximately 0.5 ml at 37 °C using a BUCHI rotary vacuum evaporator. The concentrate was transferred into graded test tubes. The round bottomed flasks were rinsed two times with small amounts of ethylacetate. Cyclohexane was added to obtain an ethylacetate/cyclohexane ratio of 1:4, which resulted in a final volume of sample of about 5 ml.

C. *Cleanup*

Solid-phase extraction columns containing 200 mg of sorbent ENV+ from International Sorbent Technology and 200 mg of sorbent HyperSep Retain from ThermoQuest were used for cleanup. A VacMaster sample processing station was used to maintain the columns during cleanup. The columns were conditioned with 10 ml of ethylacetate/cyclohexane (1:4). The samples was passed through the columns. The columns were washed with 1 ml of ethylacetate/cyclohexane (1:4). The solutions were collected in the test tubes. Pesticides were eluted by gravity with 3 ml of ethylacetate in graded test tubes, vacuum (a reduced pressure of -10 in. Hg) was applied in the end for 30 seconds to be sure that all eluate was collected. The solutions were evaporated to 1.0 ml under streams of nitrogen.

3. Detection

3.1. Thin layer chromatography (TLC)

3.1.1. Hill reaction for herbicides inhibiting photosynthesis

The samples were analyzed according to Ambrus (1996).

A. *Reagents*

About 15 g of two weeks old wheat leaves were cut into 2-4 mm long pieces and transferred into a mortar. About 3 ml of glycerine, 8 ml of MilliQ water and 3 g of quartz sand was added. The leaves were mashed with a pestle until a fairly homogenous pulp was obtained. The homogenate was pressed through a 4 layer cotton gauze to obtain a chloroplast suspension.

Borax buffer solution was made from a mixture of 350 ml 0.05 M borax solution (9.5 g borax dissolved in 500 ml water) and 150 ml 0.1 M HCl.

DCPIP reagent was made from 100 mg of 2,6-dichlorophenol-indophenol sodium salt dissolved in 250 ml of borax buffer solution.

Detecting reagent was made from 20-25 ml of chloroplast suspension mixed with approximately 20 ml of DCPIP reagent. The bluish-green color was adjusted with DCPIP reagent

dropwise until corresponding to the color pH 9-10 on a universal pH paper scale. This amount was enough for two 10 x 20 cm plates. The reagent should be prepared immediately before spraying.

B. *Detection*

Silicia gel 60 TLC plates 10 x 20 cm form Merck were used. The plates were activated at 105 °C for 30 minutes. Different amounts of sample extracts and standard solutions were spotted on the sorbent layer with a syringe. Pesticides were eluted with ethylacatate. The plates were dried in room temperature and sprayed uniformly with the reagent. The plates were placed about 20 cm below a 60 W ordinary bulb lamp for a few minutes. The inhibition should occur within 10 minutes. The spots showing inhibition were blue against a greenish background. Analytes were identified according to Rf values.

3.1.2. Enzyme inhibition with cow liver extract and β-naphthyl-acetate substrate (EβNA) for phosphoric and thio-phosphoric acid esters and carbamate pesticides

The samples were analyzed according to Ambrus (1996).

A. *Reagents*

Fresh cow liver was cut into small pieces. Liver (10 g) was homogenized together with 90 ml of MilliQ water. The homogenate was centrifuged at 4000 min^{-1} for 10 minutes. The supernatant was collected into 10 ml portions and placed into deep-freezer until use. The enzyme concentrate was diluted 3 times with MilliQ water before use.

β-Naphthyl-acetate (12.5 mg) was dissolved in 10 ml of ethanol using ultrasonification for some seconds.

Echtblau-salt (10 g) was dissolved in 16 ml of MilliQ water.

A mixture of 10 ml of β-naphthyl-acetate solution and 16 ml of Echtblau-salt solution was used as substrate solution

B. *Detection*

Silicia gel 60 TLC plates 10 x 20 cm form Merck were used. The plates were activated in 105 °C for 30 minutes. Different amounts of sample extracts and standard solutions were spotted on the sorbent layer with a syringe. Pesticides were eluted with ethylacatate. The plates were dried in room temperature.

The bromine vapor treatment was carried out in a developing tank where the flask with bromine and the TLC plate was carefully placed. The flask was opened and the tank covered for 5 minutes. The plate was removed and left in the hood for 30 minutes to take away the excess of bromine. Then the plate was sprayed with the cow liver extract and placed into the oven at 37 °C for 30 minutes. Finally the plate was sprayed with the substrate solution. Enzyme inhibition was detected as white spots occurring against a pink background. Analytes were identified according to Rf values.

3.1.3. Enzyme inhibition with horse blood serum and acetylthiocholine iodide substrate (EAcI) for organophosphorous and carbamate insecticides

The samples were analyzed according to Åkerblom (1995).

A. *Reagents*

Deep frozen horse serum was used as source of choline esterase.

Tris buffer (0.05 M, pH 7.1) was made from 3.0 g of tris(hydroxymethyl)-aminomethane dissolved in 100 ml of water, pH was adjusted with about 23.3 ml 1.0 M HCl. Solution vas diluted with water to 500 ml.

S-Acetylthiocholine iodide solution with concentration 0.01 g/ml in water was used as substrate solution.

2,6-Dichlorphenol-indophenol solution with concentration 0.5–1 mg/ml was prepared in water by dissolving for 30 minutes and filtering.

B. *Detection*

Silicia gel 60 TLC plates 10 x 20 cm form Merck were used. The plates were activated at 105 °C for 30 minutes. Different amounts of sample extracts and standard solutions were spotted on the sorbent layer with a syringe. Pesticides were eluted with ethylacatate. The plates were dried in room temperature.

The bromine vapor treatment was carried out in a developing tank where the flask with bromine and the TLC plate was carefully placed. The flask was opened and the tank covered for 45 seconds. The plate was removed and left in the hood for 30 minutes to take away the excess of bromine. The plate was sprayed with the serum mixed with tris buffer (1:1) and placed into the oven at 32-35 °C for 30 minutes. Then the plate was sprayed with s-acetylthiocholine iodide solution and replaced in oven for 15 minutes. Finally the plate was sprayed with 2,6-dichlorphenol-indophenol solution. Enzyme inhibition was detected as blue spots occurring against a white background. Analytes were identified according to Rf values.

3.2. Gas chromatography (GC)

3.2.1. Gas chromatography with electron capture and nitrogen-phosphorous detection

Samples were analyzed on a Hewlett Packard model 5890 gas chromatograph equipped with an electron capture detector (ECD) and a nitrogen-phosphorous detector (NPD) and two fused silica capillary columns (CP-Sil 19 CB and CP-Sil 5 CB, with dimensions of 20 m x 0.32 mm i.d. and 0.25 μm film thickness, provided by Chrompack) attached to a split-splitless injector. Two microliters of sample was injected splitless. The injector temperature was 250 °C, the ECD temperature was 290 °C, and the NPD temperature was 300 °C. The oven temperature was set to 90 °C for 1 minute, increasing 30 °C /min to 180 °C and then 4 °C /min to 260 °C, where it was held for 12 minutes for ECD, but 6 minutes for NPD. Nitrogen was used as carrier and make up gas for ECD and NPD. The detectors signals were collected and evaluated using Chromeleon software from Scandinaviska Genetec. Reference standards were injected in the start and end of each run consisting of 2 samples. Ethion was used as internal standard.

3.2.2. Gas chromatography with mass spectrometric detection (GC/MS)

Samples were analyzed on a Hewlett Packard model 5890 gas chromatograph attached to a TRIO-I model mass spectrometer. The GC was equipped with a CP-Sil 5 column, with dimensions of 60 m x 0.25 mm i.d. and 0,25 μm film thickness, provided by Chrompack. The split-splitless injector temperature was 250 °C. Two microliters of sample were injected splitless. Oven temperature was 90 °C for 1 min, increased at 30 °C /min to 210 °C and then 4 °C /min to 300 °C where it was held for 8 min. Nitrogen was used as a carrier gas. The detector signals were collected and evaluated using MassLab version 1.3 software. The MS was operated in selected ion monitoring mode with two or three ions per compound monitored. Terbutylazine D5 was used as internal standard. Standards were injected in the beginning, after each three samples and at the end of the run consisting of 20 samples. Results were calculated from calibration curves.

EXPERIMENTAL LAY-OUT

1. Pesticide residues analysis in water

1.1. Liquid – liquid extraction

Matrix: MilliQ water (about 1 l)
Samples: 1 for recovery test (Mix A324:1 1.5 ml was added)
1 for matrix blank
Detection: TLC – Hill reaction (spotted amount: sample 20; 40 μl, Mix A 324:1 20 μl, atrazine (0.2 μg/ml) 10 μl)
GC/ECD, GC/NPD

1.2. Solid-phase extraction

1.2.1. Recovery studies of HyperSep Retain and 2C EC/ENV+ columns

C2 EC/ENV+	3 replicates for recovery test
HyperSep Retain	3 replicates for recovery test and 1 matrix blank
Matrix:	MilliQ water
Preparation of sample:	version A1, Mix A 566:b 100 μl was added to recovery samples
Preparation of columns:	version B1 and version B1`
Extraction:	version C2; HyperSep Retain columns were eluted the second time with another 2,5 ml of ethylacetate / acetone (1:1) to be sure that all analytes were recovered, eluate was collected in fresh graded test tubes and proceeded further according to prescription
Detection:	TLC – Hill reaction (spotted amount: sample 20; 50 μl, Mix A 566:b 5; 2 μl) GC/MS

1.2.2. Recovery studies of HyperSep Retain columns depending on different flow rate

Matrix:	MilliQ water
Preparation of sample:	version A1
Preparation of columns:	version B1 and version B1`
Extraction:	version C1 (Mix A 566:b 100 μl in 2 replicates, 50 μl in 3 replicates) version C2 (Mix A 566:b 100 μl in 2 replicates, 50 μl in 2 replicates, 1 matrix blank) Columns were eluted the second time with another 2,5 ml of thylacetate / acetone (1:1) to be sure that all analytes were recovered, eluate was collected in fresh graded test tubes and proceeded further according to prescription, except – sample end volume was 0.5 ml.
Detection:	TLC – Hill reaction (spotted amount: sample 20; 50 μl, Mix A 566:b 2 μl) GC/MS (the extract of the second eluate was not run)

1.2.3. HyperSep Retain and ENVI-Carb column system use for recovery studies

Matrix:	MilliQ water
Preparation of sample:	version A2, Mix S 100 μl in 4 replicates
Preparation of columns:	version B2 and version B3`
Extraction:	version C2
Detection:	TLC – Hill reaction (spotted amount: sample 20 μl, Mix SD* 10; 25 μl)

Mix SD* Mix S/acetone (1:4)

Figure 1 shows HyperSep Retain and ENVI-Carb column system.

1.2.4. Changes in HyperSep Retain column preparation

Matrix:	MilliQ water
Preparation of sample:	version A2, Mix A 566:b 50 μl in 3 replicates, 1 matrix blank
Preparation of columns:	version B1 and version B2`
Extraction:	version C1
Detection:	TLC – Hill reaction (spotted amount: sample 20 μl, Mix A 566:b 2 μl)

1.2.5. Reuse of HyperSep Retain columns

Once used dry HyperSep Retain columns

Matrix:	MilliQ water
Preparation of sample:	version A1, Mix A 566:b 50 μl in 3 replicates, 1 matrix blank
Preparation of columns:	version B1 and version B1`
Extraction:	version C1
Detection:	TLC – Hill reaction (spotted amount: sample 20 μl, Mix A 566:b 2 μl)

1.2.6. HyperSep Retain column use for pesticides isolation from surface water

Matrix:	river water
Preparation of sample:	version A1, NPSTD-I 10 μl in 1 replicate, 30 μl in 1 replicate, 1 matrix blank
Preparation of columns:	version B1 and version B1`
Extraction:	version C2

2. Analysis of pesticide residues in medicinal plants

Recovery studies of ENV+ columns used for cleanup

Extraction:	2 samples fortified with Mix C 100 μl; 2 matrix blank
Detection:	TLC – Hill reaction, EβNA and EAcl (spotted amount for sample and Mix C1* 20 μl)

Mix C1* pesticides from Mix C in concentrations equal to fortified sample

RESULTS AND DISCUSSION

1. Pesticide residue analysis in water

Liquid-liquid extraction for recovery check was performed for MilliQ water fortified with 17 pesticide standards. Photosynthesis inhibitors were identified by TLC Hill reaction. Expected pesticides were atrazine, desetylatrazin, terbutylazine, linuron and hexazion. Figure 2 shows the fingerprint of fortified sample in 2 concentrations. According to Rf values it is possible to identify atrazine and linuron. The concentration of atrazine in the third track (spotted amount of 20 μl) is below detection limit (1 ng). Terbutylazine, hexazion and desetylatrazin were not possible to identify due to low concentration in sample. Therefore it should be advantageous to concentrate sample to a final volume lover than 2 ml.

For better identification and quantification samples were analyzed on a GC/ECD and GC/NPD equipped with CP-Sil 5 CB and CP-Sil 19 CB columns. ECD is selective towards molecules containing electronegative atoms or groups and NPD to molecules containing nitrogen or phosphorus. The retention time of linuron and cyanazin was the same when sample was analyzed on NPD using the column CP-Sil 5CB therefore in GC chromatograms they appeared as one peak. The recovery data depending on used column and detector are summarized in Table 1. In the majority of cases, the recoveries were within acceptable levels (70-120%).

The first step of solid-phase extraction study was based on recovery comparison of C2 EC/ENV+ and HyperSep Retain columns. For this purpose MilliQ water was fortified with 21 pesticide standards. Photosynthesis inhibitors were identified by TLC Hill reaction. Expected pesticides were atrazine, desetylatrazin, atrazindesispropyl, diuron, terbutylazine, hexazion, isoproturon, metamitron, metribuzin, propyzamid and simazine. The chromatogram of fortified sample applied in 2 concentrations is shown in Figure 3. Diuron, desetylatrazin and simazine were identified by Rf values. Metribuzin, terbutylazine and atrazine were eluted as one zone, therefore it is necessary to try other TLC systems or multifold development in the same system to get better separation. To identify hexazion, isoproturon, metamitron and propyzamid their standard solutions should be run. The fingerprint shows (Fig. 3) that the second eluate from both columns does not contain pesticides in detectable amounts. In general detection by Hill reaction gives the similar recoveries from C2 EC/ENV+ and HyperSep Retain columns (Fig. 3). For more reliable results the other chromatographic systems has to be tried.

The detection by GC/MS was used to examine the difference between recoveries of all pesticides from C2 EC/ENV+ and HyperSep Retain columns. The recoveries for both columns were within acceptable levels (70-120%), except diuron for C2 EC/ENV+ and diuron, atrazindesispropyl and 2,6-dichlorobensamide for HyperSep Retain columns (Table 2). The chromatograms show that there were no pesticides in the second eluate.

Next step for HyperSep Retain column examination was to compare recoveries by using different flow rates. For the experiment MilliQ water fortified with the same pesticide standards as previously were run through the columns at flow rate 11-17 ml/min and 22-33

ml/min. Figure 4 illustrates the fingerprint of photosynthetic inhibitors from fortified samples in two concentrations. As before diuron, desetylatrazin and simazine is possible to identify by Rf values. Photosynthesis inhibitors were not found in second eluate (Fig. 4). According to the chromatograms the difference in recoveries between samples passed through HyperSep Retain columns with the rate 11-17 and 22-33 ml/min is not found (Fig. 4).

It should be mentioned that the zones appeared even of samples fortified with 50 µl of pesticide standard mixture and applied to the TLC plate in volume of 20 µl (Fig. 4). In this case amount of analytes on the plate was 0.2-0.9 ng (except 7.7 ng of metamitron). For applied pesticides 1 ng is considered as detection limit for Hill reaction (unpublished data from Dept. of Environmental Assessment, Swedish University of Agricultural Sciences). The obtained results indicate that the TLC method is very sensitive.

The extracts were run on GC/MS to see recoveries of all pesticides. The recoveries for both flow rate were within acceptable levels (70-120%). Only propachlor, atrazindesispropyl, 2,6-dichlorobensamide and especially dichlkobenil were recovered in too low amounts (Table 3). The samples fortified with two times smaller amount of pesticides (50 µl of pesticide standard mixture Mix A 566:b) shows disturbed peaks on chromatograms therefore these results are not included in the calculation of recoveries.

There may be several reasons of low recoveries for atrazindesispropyl, 2,6-dichlorobensamide and dichlobenil: HyperSep Retain sorbent does not retain the analytes, 200 mg of sorbent is too low amount for one liter of sample or ethylacetate/acetone (1:1) does not eluate retained pesticides. In order to solve the problem of low recoveries the extraction system from 3 SPE columns was made (Fig. 1). Two HyperSep Retain columns were used to increase the amount of sorbent. The ENVI-Carb column at the bottom was used to retain analytes in case if HyperSep Retain sorbent had passed them through. The recoveries were examined using MilliQ water fortified with 7 pesticide standards. There were 4 photosynthetic inhibitors expected to identify by TLC, Hill reaction. The fingerprint (Fig. 5) shows 3 zones in tracks A, A1, A2 and A3 representing recoveries from the first HyperSep Retain column. For the pesticides identification there are necessary to spot standard solutions. It is possible to conclude that three pesticides were retained by the first column. However it should be mentioned that one zone is noticed in tracks B1 and B3 representing the recoveries from the second HyperSep Retain column. The spot is unclear due to too low amount of analyte or it is a disturbance appeared during development. The tracks A, A1, A2 and A3 contain zones with approximate Rf 0.72, that are not in tracks of standards (Fig. 5). The reason could be matrix effect. To avoid obscurity like obtained in this experiment, the matrix blank should be spotted within every set of samples. The extracts should be run on GC, to obtain recovery results of all analytes. Only then it will be possible to draw out initial conclusions about the suitability to use HyperSep Retain columns for atrazindesispropyl, 2,6-dichlorobensamide and dichlobenil extraction from water.

The changes in HyperSep Retain column preparation were done in order to try to improve recovery efficiency. The column preparation in preliminary experiments was done

according to the prescription for C2 EC/ENV+ columns (Dept. of Environmental Assessment, Swedish University of Agricultural Sciences). Methanol there is used for conditioning of C2 EC sorbent containing trimethylsilyl groups. The HyperSep Retain sorbent consists of polystyrene-divinylbenzene therefore the column preparation in this experiment was done without methanol. However according to ChromTech instructions methanol is recommended for HyperSep Retain column conditioning. Other change in column preparation was reduced volume of ethylacetate/acetone (1:1) and MilliQ water because the amount of HyperSep Retain sorbent was 3.5 times less (200 mg) comparing to C2 EC/ENV+ sorbent (700 mg). The TLC Hill reaction results presented in Figure 6 shows seven zones per track obtained from fortified MilliQ water samples. The same number of zones per track is in the fingerprints where HyperSep Retain columns were prepared in the same manner as C2 EC/ENV+ columns (Fig. 3, 4). It seems that the changes in HyperSep Retain column preparation did not influence the recovery of analytes. However since the Hill reaction provides only photosynthetic inhibitors, the other detection methods using TLC and GC should be applied.

Possibility to reuse SPE tubes can provide a good cost economy. Therefore the recoveries from fortified MilliQ water samples passed through used HyperSep Retain columns were checked. The fingerprint of photosynthetic inhibitors shows seven zones in track (Fig. 7). Seven zones per track can be seen in chromatograms obtained in previous experiments either, where samples were run through new columns (Fig. 3, 4). After the first results it seems that HyperSep Retain columns could be used a second time. However to confirm reusability of the columns it could be necessary to carry out additional experiments and detection methods using GC.

The ability of HyperSep Retain columns to retain pesticides from surface water was tested by analytes extraction from fortified river water. The recoveries should be evaluated by different detection methods using TLC and GC.

It should be noticed that HyperSep Retain sorbent in some columns captured water. To solve this problem columns were weighted before experiment and after drying with nitrogen. Despite the less column weight after drying comparing to initial weight, some sorbents still contain water. In these cases the water from extracts were taken away by sodium sulfate. Nevertheless it caused the loses of analytes resulted in lover recoveries.

To be sure that HyperSep Retain columns are suitable for wide application in pesticide isolation from water samples additional tests are required. The unclear points are:

1. capture of water by sorbent,
2. atrazindesispropyl, 2,6-dichlorobensamide and dichlobenil retention or passing through sorbent (pesticides that are especially interesting in ground water analysis),
3. the use of columns for pesticides isolation from ground water,
4. reusability of the columns.

2. Pesticide residues analysis in medicinal plants

To evaluate the extraction method and suitability of SPE columns for cleanup of herbal extracts, the recoveries from fortified *Carum carvi* (L.) seed samples were of interest. In the first assay the ENV+ columns were checked. For the pesticide detection TLC methods were used.

Three photosynthesis inhibitors were expected to be recovered from the pesticide mixture used for fortification. Prometryn, simazine and diuron are identified by Rf values in the fortified sample where 3 extractions were done immediately one after another (Fig. 8). Only diuron was recovered from sample extracted overnight. The obtained results showed that pesticide extraction overnight decreased recoveries. Matrix blank does not contain photosynthesis inhibitors in detectable amounts (Fig.8).

Figure 9 shows fingerprint of TLC results using EβNA method. Expected pesticides are paration, mevinphos and carbaryl. The chromatogram presents four zones in tracks of fortified samples, matrix blanks and matrix blanks spotted together with pesticide standards mixture. It could be assumed that matrix blank contains the same pesticide residues as were used for fortification. Spot with approximate Rf 0.32 appears in the tracks where herbal sample is applied. It could be pesticide residue or any plant component.

Paration and dichlorvos is expected to be identified by TLC using EAcl method. Figure 10 illustrates the chromatogram with a zone corresponding to paration in tracks representing pesticide standards mixture and matrix blank spotted together with standards mixture. Dichlorvos is not identified even in spotted standards mixture, although the applied amount on TLC plate is 30 ng that is six times above detection limit for EAcl method. One possible reason could be the degradation of dichlorvos in standard solution during storage, other – the choline esterase solution used for TLC plate handling was too concentrated. Fingerprint shows (Fig. 10) that paration is not detected in fortified samples and matrix blank. It is contrary the results obtained using EβNA method. To clarify the possible presence of paration in caraway seeds the samples should be analyzed using other TLC systems or GC.

The obtained results showed that extraction of fortified herbal samples three times immediately repeated one after another improved recovery efficiency. The recovery results allowed to expect that ENV+ could be used for cleanup of herbal extracts. It should be useful to continue the studies of SPE column application for herbal extract cleanup. According to SPE column use for plant material cleanup promising results had achieved with fruits and vegetables (Pihlström and Österdahl, 1999). To complete the results the studies should be developed considering following points:

1. to try several herbs,
2. to fortify herbal samples with more pesticides in several concentrations,
3. to improve extraction,
4. to compare recoveries from different SPE columns and traditionally used gel permation chromatography cleanup,

5. to try different solvents for sample extracts pretreatment to evaluate the capacity of column to retain pesticides,
6. to try other eluents,
7. to evaluate recoveries using different detection methods by TLC and GC.

REFERENCES

Åkerblom M. (1995) Environmental monitoring of pesticides. Guidelines for the SADC Region

Ambrus A. (1996) Development and validation of cost effective screening methods for pesticide residues in vegetables. IAEA Report of technical contract 8908.

Lino M. C., Noronha de Silva M. I. (1997) Extraction and clean-up methods for the determination of organochlorine pesticide residues in medicinal plants. J. Chromatotogr. A, 769, 275-283.

Pihlström T., Österdahl B.-G. (1999) Analysis of pesticide residues in fruit and vegetables after cleanup with solid-phase extraction using ENV+ (polystyrene-divinylbenzene) cartridges. J. Agric. Food Chem. 47, 2549-2552.

Table 1. Recoveries using liquid-liquid extraction of fortified MilliQ water with GC/ECD or GC/NPD determonation (n=1)

pesticide	fortified (µg/l)	recovery (%)	detector/column
dichlobenil	0.060	83	ECD/CP-Sil 5 CB
		88	ECD/CP-Sil 19 CB
2,6-dichlorbensamide	0.150	80	ECD/CP-Sil 5 CB
		65	ECD/CP-Sil 19 CB
desetylatrazin	0.075	84	NPD/CP-Sil 19 CB
dimetoat	0.076	100	NPD/CP-Sil 5 CB
		83	NPD/CP-Sil 19 CB
gamma-HCH	0.015	80	ECD/CP-Sil 19 CB
atrazine	0.149	67	NPD/CP-Sil 5 CB
		11	NPD/CP-Sil 19 CB
terbutylazine	0.074	99	NPD/CP-Sil 19 CB
pirimicarb	0.075	73	NPD/CP-Sil 5 CB
metalaxyl	0.301	73	NPD/CP-Sil 5 CB
		85	NPD/CP-Sil 19 CB
cyanazine	0.149	54	ECD/CP-Sil 5 CB
		99	ECD/CP-Sil 19 CB
metazachlor	0.150	91	ECD/CP-Sil 5 CB
		98	ECD/CP-Sil 19 CB
propichonazol	0.150	100	ECD/CP-Sil 5 CB
		105	ECD/CP-Sil 19 CB
hexazion	0.150	86	NPD/CP-Sil 5 CB
		92	NPD/CP-Sil 19 CB
bitertanol	0.300	92	NPD/CP-Sil 5 CB
		83	NPD/CP-Sil 19 CB
prochloraz	0.300	93	ECD/CP-Sil 5 CB
		54	ECD/CP-Sil 19 CB
esfenvalerate	0.149	100	ECD/CP-Sil 5 CB
		101	ECD/CP-Sil 19 CB

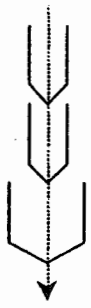
Table 2. Recoveries of fortified MilliQ water using C2 EC/ENV+ and HyperSep Retain columns with GC/MS detection

pesticide	C2 EC/ENV+ recovery (%)	s. d. (n=3)	HyperSep Retain recovery (%)	s. d. (n=3)
diuron	215	19	159	24
dichlobenil	91	16	103	39
propachlor	91	0	107	14
atrazindesisopropyl	119	2	48	2
desetylatrazin	120	2	96	8
2,6-dichlorbensamide	127	9	45	3
metabenztiazuron	126	21	141	32
simazine	107	3	107	2
atrazine	105	5	110	8
terbutylazine	88	2	102	3
propyzamid	102	2	106	5
pirimicarb	114	21	121	19
metribuzin	107	8	108	10
etofumesate	96	5	100	6
cyanazine	103	4	100	4
fenpropimorf	95	7	72	18
metazachlor	92	5	104	6
metamitron	80	1	65	6
propikonazol	88	10	100	9
hexazion	72	19	94	14

Table 3. Recoveries of fortified MilliQ water using different flow rate through HyperSep Retain columns with GC/MS detection (Mix A 566:b 100 μl in 1 litre of water)

pesticide	flow rate11-17 ml/min recovery (%)	s. d. (n=2)	flow rate 22-33 ml/min recovery (%)	s. d. (n=2)
diuron	107	38	103	15
dichlobenil	x	x	8	7
propachlor	52	21	57	11
atrazindesisopropyl	42	16	42	8
dsetylatrazin	102	35	91	3
2,6-dichlorbensamide	44	13	44	7
metabenztiazuron	138	45	119	20
simazine	119	40	107	32
atrazine	105	45	104	20
terbutylazine	100	42	106	14
propyzamid	109	23	102	18
pirimicarb	107	38	99	20
metribuzin	116	29	97	12
etofumesate	118	34	101	5
cyanazine	174	60	109	6
fenpropimorf	202	90	104	22
metazachlor	114	30	111	14
metamitron	82	309	67	7
propikonazol	114	34	121	3
hexazion	104	25	109	10

x - recovered in very low amount

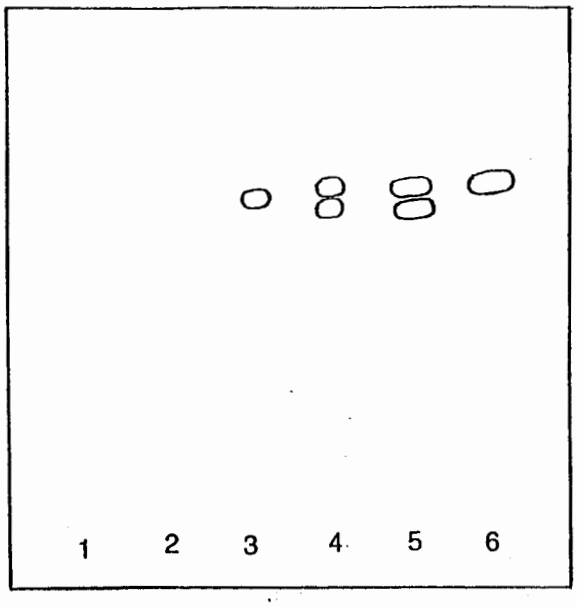


HyperSep Retain (A)

HyperSep Retain (B)

ENVI-Carb (C)

Figure 1. Two HyperSep Retain and ENVI-Carb column system



Rf average	pesticide
0.79	atrazine
0.71	linuron

Figure 2. Chromatogram of MilliQ water sample fortified with pesticide standard mixture Mix A 324:1 (see Appendix 1). Hill reaction.

Sample application:

track 1 - matrix blank 20 µl;

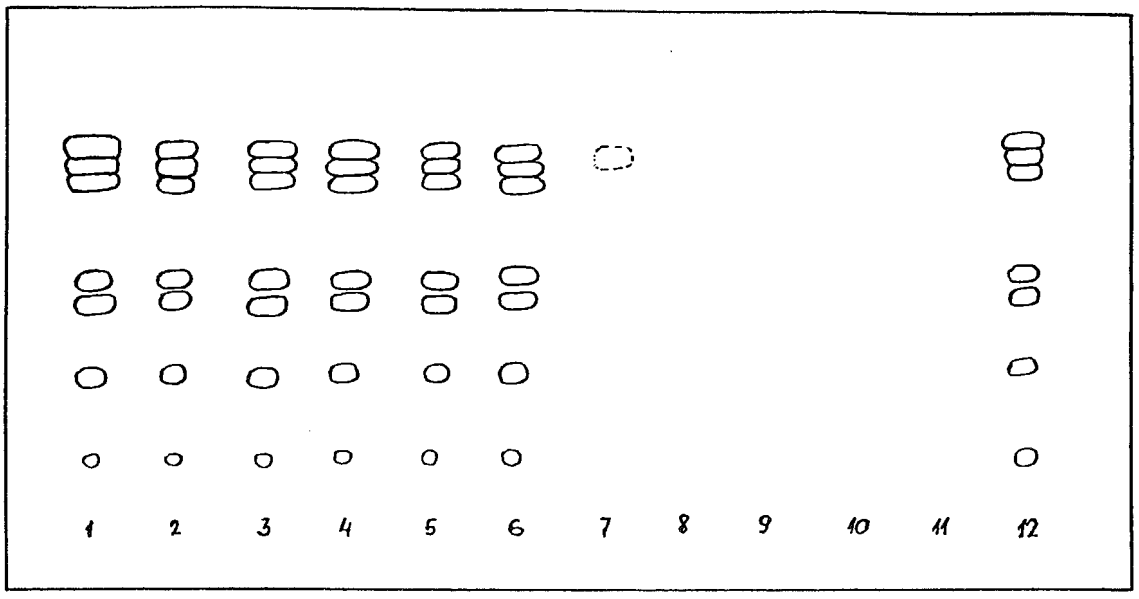
track 2 - matrix blank 40 µl;

track 3 - fortified sample 20 µl;

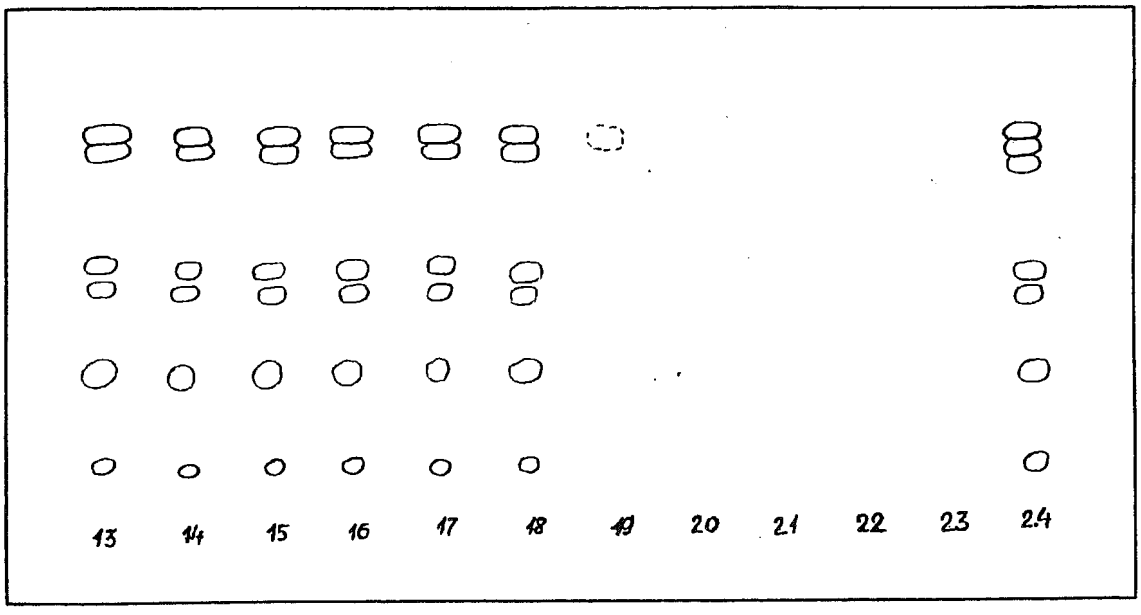
track 4 - fortified sample 20 µl;

track 5 - Mix A 324:1 20 µl;

track 6 - atrazine (0,2 µg/ml) 10 µl



Rf average	pesticide
0.82	
0.80	
0.75	metribuzin terbutylazin atrazine
0.52	simazine
0.50	desetylatrazin
0.29	diuron
0.09	



Rf average	pesticide
0.77	
0.73	
0.71	metribuzin terbutylazin atrazine
0.49	simazine
0.44	desetylatrazin
0.26	diuron
0.08	

Figure 3. Chromatograms of MilliQ water samples fortified with pesticide standard mixture Mix A 566:b (see Appendix 2). Hill reaction.

Sample application:

track 1, 2, 3 - C2 EC/ENV+ 50 µl;

track 4, 5, 6 - HyperSep Retain 50 µl;

track 7 - matrix blank 50 µl;

track 8, 9, 10 - second eluate from HyperSep Retain 50 µl;

track 11 - second eluate of matrix blank from HyperSep Retain 50 µl;

track 12 - Mix A 566:b 5 µl;

track 13, 14, 15 - C2 EC/ENV+ 20 µl;

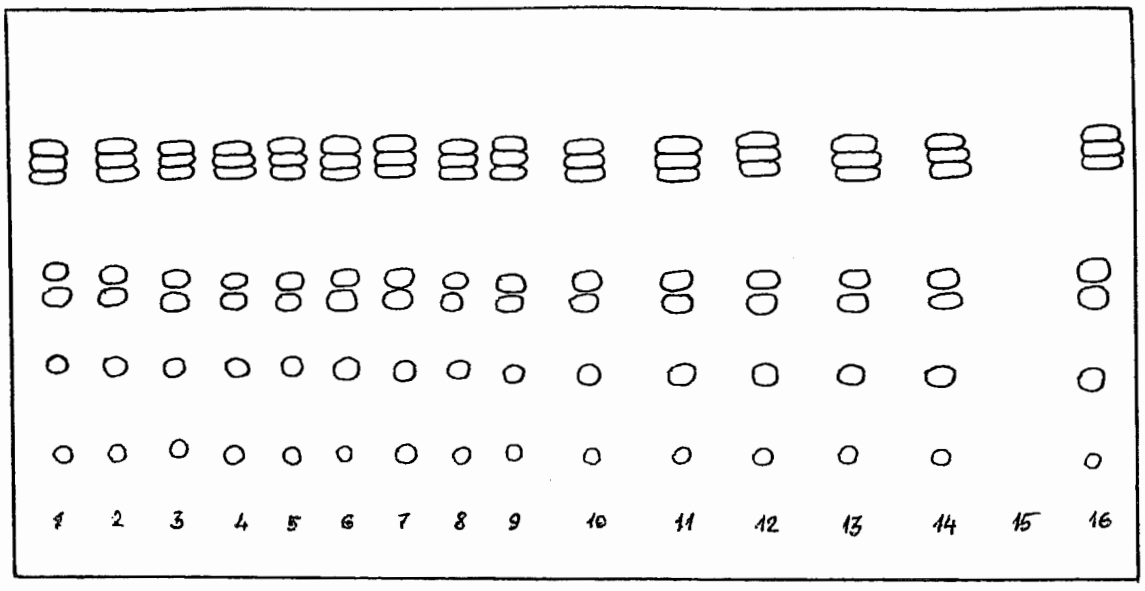
track 16, 17, 18 - HyperSep Retain 20 µl;

track 19 - matrix blank 20 µl;

track 20, 21, 22 - second eluate from HyperSep Retain 20 µl;

track 23 - second eluate of matrix blank from HyperSep Retain 20 µl;

track 24 - Mix A 566:b 2 µl



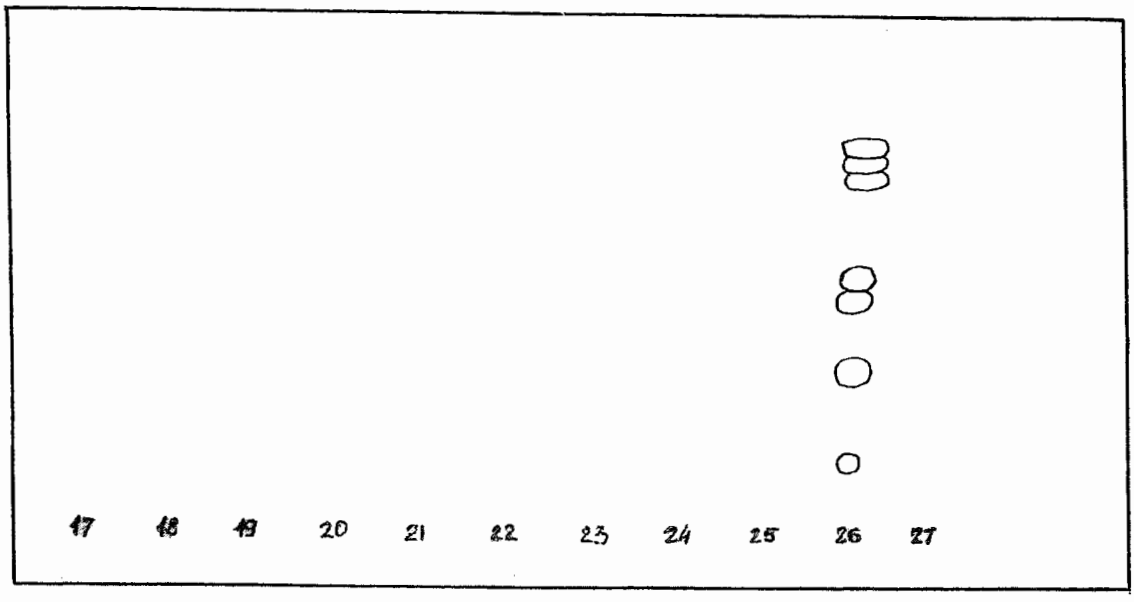


Figure 4. Chromatograms of MilliQ water samples fortified with pesticide standard mixture Mix A 566:b (see Appendix 2). Hill reaction.

Sample application:

track 1, 2 - flow rate 22-33 ml/min 20 µl (Mix A 566:b 100 µl in litre of water);

track 3, 4, 5 - flow rate 22-33 ml/min 20 µl (Mix A 566:b 50 µl in litre of water);

track 6, 7 - flow rate 11-17 ml/min 20 µl (Mix A 566:b 100 µl in litre of water);

track 8, 9 - flow rate 11-17 ml/min 20 µl (Mix A 566:b 50 µl in litre of water);

track 10, 11, 12 - flow rate 22-33 ml/min 50 µl (Mix A 566:b 50 µl in litre of water);

track 13, 14 - flow rate 11-17 ml/min 50 µl (Mix A 566:b 50 µl in litre of water);

track 15 - matrix blank, flow rate 22-33 ml/min 50 µl;

track 16 - Mix A 566:b 2 µl;

track 17, 18 - second eluate, flow rate 22-33 ml/min 50 µl (Mix A 566:b 100 µl in litre of water);

track 19, 20, 21 - second eluate, flow rate 22-33 ml/min 50 µl (Mix A 566:b 50 µl in litre of water);

track 22, 23 - second eluate, flow rate 11-17 ml/min 50 µl (Mix A 566:b 100 µl in litre of water);

track 24, 25 - second eluate, flow rate 11-17 ml/min 20 µl (Mix A 566:b 50 µl in litre of water);

track 26 - Mix A 566:b 2 µl;

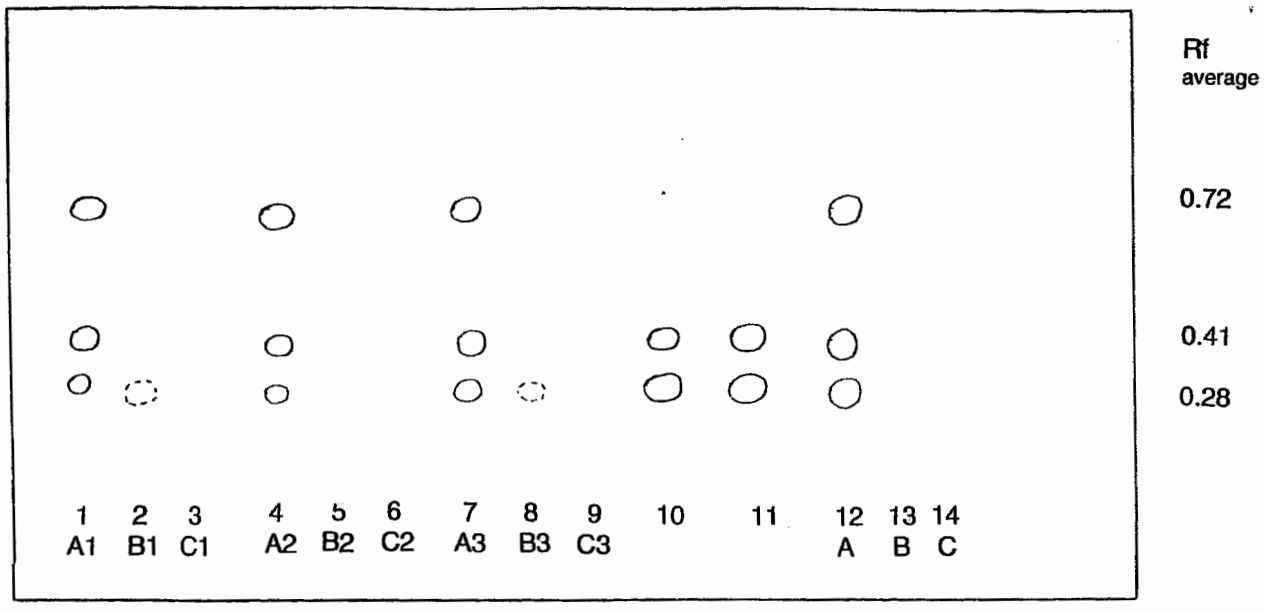


Figure 5. Chromatogram of MilliQ water samples fortified with pesticide standard mixture Mix S (see Appendix 3). Hill reaction.

Sample application:

track 1, 4, 7, 12 - first HyperSep Retain column (A) 20 µl;

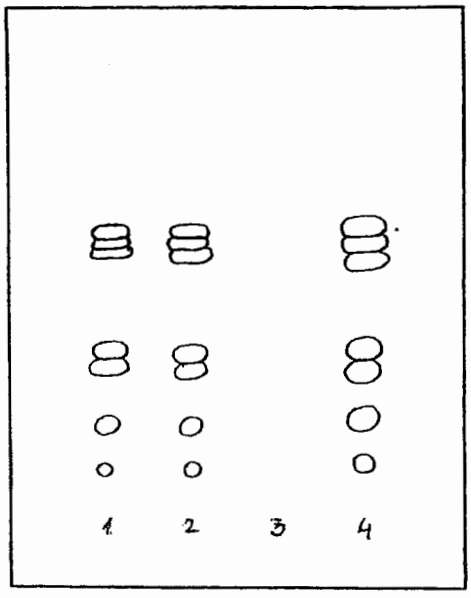
track 2, 5, 8, 13 - second HyperSep Retain column (B) 20 µl;

track 3, 6, 9, 14 - ENVI-Carb column (C) 20 µl;

track 10 - Mix SD 10 µl;

track 11 - Mix SD 25 µl

(Mix SD - Mix S/acetone 1:4)



Rf average	pesticide
0.68	
0.65	
0.61	metribuzin terbutylazin atrazine
0.36	simazine
0.30	desetylatrazin
0.17	diuron
0.05	

Figure 6. Chromatogram of MilliQ water samples fortified with pesticide standard mixture Mix A 566:b (see Appendix 2). Hill reaction.

Sample application:

track 1, 2 - fortified sample 20 µl;

track 3 - matrix blank 20 µl;

track 4 - Mix A 566:b 2 µl

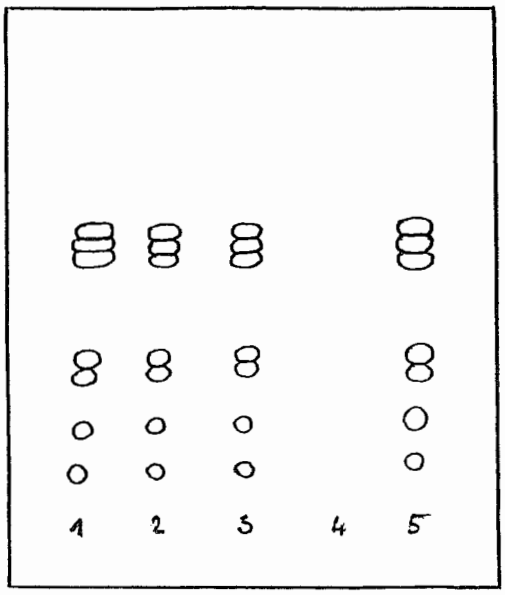


Figure 7. Chromatogram of MilliQ water samples fortified with pesticide standard mixture Mix A 566:b (see Appendix 2). Hill reaction.

Sample application:

track 1, 2, 3 - fortified sample 20 µl;

track 4 - matrix blank 20 µl;

track 5 - Mix A 566:b 2 µl

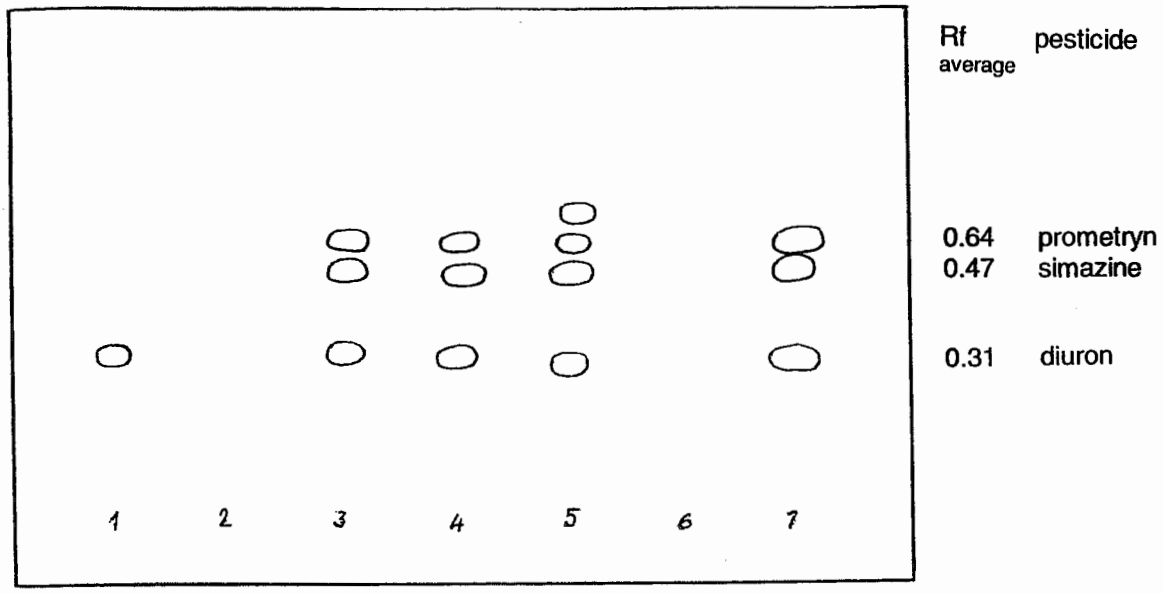


Figure 8. Chromatogram of *Carum carvi* (L.) samples fortified with pesticide standard mixture Mix C (see Appendix 5). Hill reaction.

Sample application:

track 1 – fortified sample extracted overnight 20 µl;

track 2, 6 – matrix blank 20 µl;

track 3, 7 – matrix blank 20 µl spotted together with Mix C1 20 µl;

track 4 – Mix C1 20 µl;

track 5 – fortified sample (3 extractions repeated immediately) 20 µl

(Mix C1 - pesticides from Mix C in concentrations equal to fortified sample)

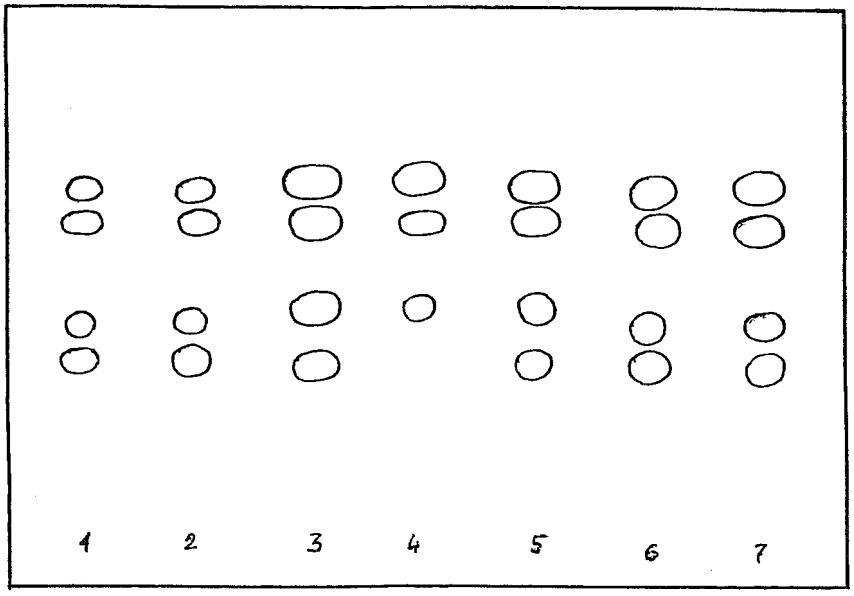


Figure 9. Chromatogram of *Carum carvi* (L.) samples fortified with pesticide standard mixture Mix C (see Appendix 5). EβNA method.

Sample application:

track 1 – fortified sample extracted overnight 20 µl;

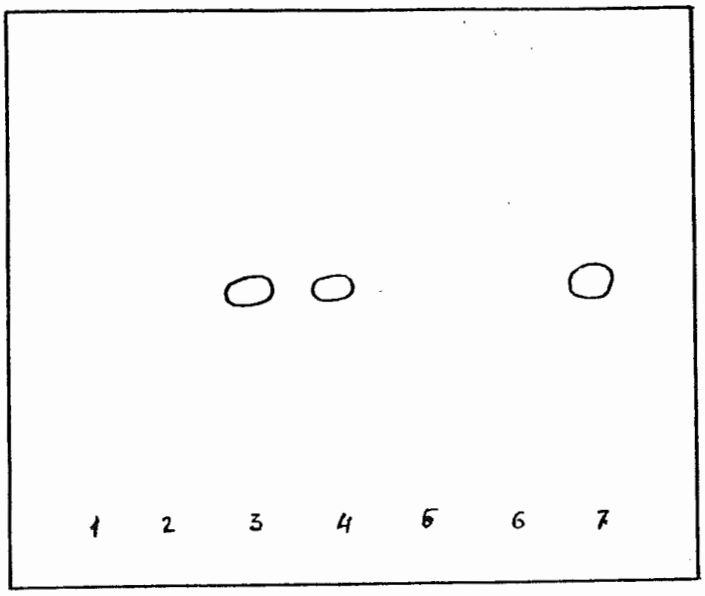
track 2, 6 – matrix blank 20 µl;

track 3, 7 – matrix blank 20 µl spotted together with Mix C1 20 µl;

track 4 – Mix C1 20 µl;

track 5 – fortified sample (3 extractions repeated immediately) 20 µl

(Mix C1 - pesticides from Mix C in concentrations equal to fortified sample)



Rf average | pesticide

0.62 | parathion

Figure 10. Chromatogram of *Carum carvi* samples fortified with pesticide standard mixture Mix C (see Appendix 5). EAcI method.

Sample application:

track 1 – fortified sample extracted overnight 20 µl;

track 2, 6 – matrix blank 20 µl;

track 3, 7 – matrix blank 20 µl spotted together with Mix C1 20 µl;

track 4 – Mix C1 20 µl;

track 5 – fortified sample (3 extractions repeated immediately) 20 µl

(Mix C1 - pesticides from Mix C in concentrations equal to fortified sample)

Appendix 1. Concentration of pesticides in standard mixture Mix A 324:1

pesticide	concentration µg/ml
dichlobenil	0.040
2,6-dichlorbensamide	0.101
desetylatrazin	0.050
dimetoat	0.050
gamma-HCH	0.010
atrazine	0.100
terbutylazine	0.050
pirimicarb	0.050
metalaxyl	0.200
linuron	0.200
cyanazine	0.100
metazachlor	0.100
propikonazol	0.100
hexazion	0.100
bitertanol	0.200
prochloraz	0.200
esfenvalerate	0.100

Appendix 2. Concentrations of pesticides in standard mixture Mix A 566:b

pesticide	concentration µg/ml
atrazine	0.204
desetylatrazin	0.406
atrazindesispropyl	0.756
2,6-dichlorbensamide	0.590
cyanazine	0.990
dichlobenil	0.216
diuron	0.378
etofumesate	0.612
fenpropimorf	0.779
hexazion	0.742
isoproturon	0.214
metabenztiazuron	1.174
metamitron	7.585
metachaklor	0.985
metribuzin	0.778
pirimicarb	0.391
propachlor	0.235
propikonazol	1.168
propyzamid	0.966
simazine	0.460
terbutylazine	0.215

Appendix 3. Concentrations of pesticides in standard mixture Mix S

pesticide	concentration µg/ml
dichlobenil	0.216
2,6-dichlorbensamide	0.589
diuron	0.378
propyzamid	0.966
metamitron	7.584
atrazindesispropyl	0.756
fenpropimorf	0.778

Appendix 4. Concentrations of pesticides in standard mixture NPSTD-I

pesticide	concentration µg/ml
chlorpyrfos-ethyl	29.79
dimethoate	20.00
bitertanol	210.45
cyanazine	29.13
carbofuran	25.73
atrazine	57.00
desetylatrazin	28.65
diuron	18.91
pirimicarb	39.00

Appendix 5. Concentrations of pesticides in standard mixture Mix C

pesticide	concentration µg/ml
diuron	3.0
prometryn	3.0
simazine	3.0
paration	3.0
mevinphos	15.0
carbaryl	6.0
dichlorvos	15.0