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Liquid chromatographic method development for determination of fungicide epoxiconazole enantiomers by achiral and chiral column switching technique in water and soil

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Abstract

High-performance liquid chromatography (HPLC) in both chiral isocratic and achiral–chiral column switching mode was employed for optimization of separation conditions, separation and determination of fungicide epoxiconazole in real samples. Two enantiomers of commercially available triazole fungicide epoxiconazole (BAS 480 F), first registered in 1993, were resolved for the first time on a microcrystalline cellulose triacetate (MCTA). A low-cost home-packed chiral column (150×3 mm, 15–25 μm, MCTA, Merck) enabled baseline enantiomeric resolution of two enantiomers of the fungicide epoxiconazole produced commercially. The effects of concentration of organic modifiers (methanol, ethanol) in mobile phase, flow-rate and temperature were studied. The isocratic chiral HPLC method allows determination of the enantiomers in tap and surface water within the range 1–1000 mg/l by direct injection (20 μl) of the sample. Using the achiral (C_{18})–chiral (MCTA) column-switching technique and 1-ml sample volume, injection of 0.050 mg/l of epoxiconazole enantiomers can be conveniently determined by UV detection at 230 nm. The same method applied to methanolic soil extracts allows determination of 0.2 mg/kg of epoxiconazole enantiomers in addition to the other 10 commonly used pesticides in fortified soils.

Keywords: Enantiomer separation; Column switching; Water analysis; Soil; Method development; Environmental analysis; Epoxiconazole; Pesticides

1. Introduction

Specific biological activity of a chiral pesticide molecule results in increased efficiency of the applied product. Activity is usually fixed to one and/or more enantiomer(s), which consequently leads to decrease in applied dose. These facts result in reduction of environmental load, reduction of material and energy demand during manufacturing and economisation of product consumption, management and distribution [1]. Method development and analysis of the enantiomers of pesticides by chiral separation techniques, especially chromatography, follows similar trends as chiral analysis of pharmaceuticals and clinical analysis developed more than decade ago [2]. Liquid chromatography is used during investigation of new chiral compounds in several fields as are separation of racemic mixtures

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for primary tests, preparation of both isotopically labeled stereoisomers (monitoring of metabolism), preparation of auxiliary reagents in pure enantiomer form, analysis and isolation of chiral metabolites and ''chiral'' quality control of formulated agrochemicals [3]. However, there are relatively few published applications on chiral HPLC in environmental trace analysis of chiral pesticides [3,4].

During our work on Environmental Geoinformation System feasibility study [23], we were faced with the problem of monitoring of a selected group of pesticides which were used during 1999–2000 at the monitored test field for crop protection. Among them was also the licenced agrochemical preparation Tango (BASF, AG, Ludwigshafen/Rhein) containing epoxiconazole (125 g/l) and tridemorph (375 g/l) applied as fungicidal agent. The test field is situated in an area close to the Danube river which has suffered in the past from flood disasters. Now the flow is regulated and the area is protected, but the underground water level is in some periods only 0.5 m depth. Environmental changes (biodegradation and conversion) of the pesticides including epoxiconazole in both soil and water were and still are of academic interest, because the Danube river gravel and sand sediments is the largest underground basin of high quality water in the region. This reason in addition to the lack of literature information on epoxiconazole chiral analysis led us to the method development.

Enantiomers of various triazole fungicides, except epoxiconazole (Fig. 1), were directly separated by HPLC in normal-phase mode. Diniconazole and uniconazole enantiomers were initially separated using HPLC on a Sumichiral OA-2200 [5], later the authors used Sumichiral OA-4100 [6] and also Chirex 3020 [7] was successfully used. Diniconazole and uniconazole enantiomers were also separated on β- and γ-cyclodextrin bonded columns [8,9], or other chiral stationary phases (CPSs) [10]. Becliconazole, econazole and miconazole were baseline resolved by Chiralpak AD and Chiralcel OD columns in hexane-rich mobile phases [11]. Enantiomers of triadimefon and the four stereoisomers of triadimenol were resolved on Chiralpak OT [12]. Alternatively, enantiomers of triadimefon can be resolved by Chiralcel OJ [13]. Resolution of all four stereoisomers of propiconazole was achieved on a Chiralcel OD-H [14]. Hexaconazole and some triazolylalcohols were completely separated into enantiomers on a polysaccharide stationary phase [15].

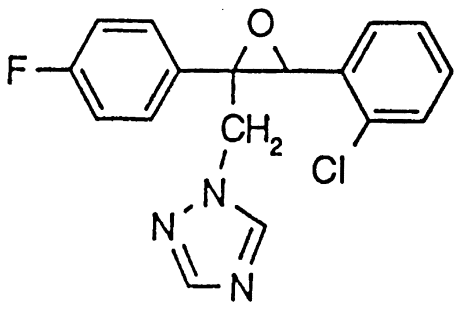

Fig. 1. Structural formula of epoxiconazole.

Because in our previous work [16] we did not succeed in chiral separation of epoxiconazole [17] using Whelk O1 column, in our present work we focused on the solution of several problems involving the study of the chromatographic behavior and optimization of the chiral separation of the fungicide epoxiconazole on microcrystalline cellulose triacetate (MCTA) [18,21,22]. Emphasis was also given to the development of the analytical procedure for the determination of the fungicide epoxiconazole in tap water, underground water and soil employing the column-switching technique. The HPLC method was applied for analysis of water and soil samples from the monitored test field.

The development of an HPLC method of analysis of the residues of 11 pesticides [asulam, atrazine, 2,4-D (2,4-dichlorophenoxyacetic acid), PCA (5-amino-4-chloro-2-phenyl-3-pyridazone), propazine, simazine, MCPA (4-chloro-2-methyl phenoxyacetic acid), mecoprop, chlortoluron, metoxuron and epoxiconazole] of different chemical structures in soil and water was solved. HPLC method development was interconnected with the investigation of several methods for isolation of the whole group of pesticides from soil samples containing 1.0–0.1 mg/kg levels of pesticide residua [23]. The soil samples were obtained from the test field in south-western Slovakia.

Epoxiconazole (BAS 480F), first registered by BASF in 1993, is used as the active substance in several commercial formulations (Tango, Duett, Opus, Opus Team from BASF). It acts as an

inhibitor of C-14 demethylase in sterol biosynthesis. It is used as a broad-spectrum fungicide with preventive and curative action for control of diseases caused by Ascomycetes, Basidiomycetes and Deuteromycetes in cereals, sugar beet, peanuts, oilseed rape and ornamentals [17].

As far as we know, no article has been published in available literature on achiral and/or chiral analysis of epoxiconazole (Fig. 1) in technological, environmental or other samples.

2. Experimental

2.1. Instrumentation

Study of the retention behavior and evaluation of spectral characteristics of the selected group of pesticides including epoxiconazole was carried out by the HPLC system LaChrom (Merck–Hitachi, Darmstadt, Germany) consisting of L-7100 pump provided by quarternary low-pressure gradient, L-7200 autosampler, L-7300 column oven, L-7450A diode-array detector, L-7480 fluorescence detector, D-7000 interface, PC data station with software HSM ver.3.1 and L-7612 solvent degasser.

For study of the retention behavior and optimization of epoxiconazole enantiomer separation, we used the HPLC system LiChrograph (Merck–Hitachi, Darmstadt, Germany) consisting of L-6200A Intelligent Pump provided by ternary low-pressure gradient and high-pressure dynamic mixer, manual injection valve Rheodyne model 7125 with 20-μl sample loop (Rheodyne, Cotati, CA, USA), L-4250 UV–Vis Detector and PC-based data acquisition system CSW ver.1.0, 20-bit A/D converter (DataApex, Prague, Czech Republic).

Air-cooled precision GC oven type F-11 (Perkin-Elmer) was used for thermostating (±0.1 °C) the chiral column.

Trace analysis of epoxiconazole in tap, underground or surface water or soil extracts required an additional Knauer pump (Berlin, Germany). Injection valve Valco N 60 with 1-ml sample loop (VICI, Palo Alto, CA, USA) and switching valve Rheodyne model 7010 (Rheodyne, Cotati, CA, USA) were also used for large volume injection and/or transfer of the analyte from achiral (LaChrom) to chiral column (LiChrograph).

Achiral and chiral column switching method uses alternatively direct transfer technique according to Ramsteiner's systematic approach to column switching (Fig. 1 in Ref. [19]), or reversed transfer technique (backflush) (Fig. 3 in Ref. [19]). For analysis of epoxiconazole in addition to the other pesticides present in methanolic extracts after the extraction of fortified soil samples, we used the direct transfer technique, whereas the reversed transfer technique (backflush) was successfully used for the analyte enrichment and/or concentration from water samples.

Extractions of pesticides from soil samples were done with the aid of a KS 125 shaker (IKA Labortechnik, Junke and Kunkel GmbH, Germany).

2.2. Methods

Microcrystalline cellulose triacetate (MCTA) was slurry packed according to the procedure in Refs. [20,21] at pressures not exceeding 10 MPa. Eleven compact glass columns 150×3 mm I.D. were packed and evaluated for efficiency, selectivity and permeability.

Separation conditions for mixture of 11 pesticides were optimized with the aid of HPLC method development software ChromSword 1.5 Auto for Windows (Merck, Darmstadt, Germany). The chosen optimal conditions for baseline separation of asulam, PCA, metoxuron, simazine, chlortoluron, atrazine, 2,4-D, MCPA, propazine, MCPP and epoxiconazole were as follows: Analytical column Purospher RP 18e (125-4)mm was guarded by precolumn (Purospher RP 18e (4-4)mm). Composed gradient, mobile phase A: aqueous phosphate buffer (pH 2.5, 20 m*M*), B: methanol. Gradient program was from 0 to 5.0 min isocratic 95%A and 5% B, from 5.1 to 35.0 min, a linear increase from 95% A and 5% B to 46%A and 54%B, from 35.1 min, a linear decrease to initial conditions in 5 min. Injection volumes by the autosampler were 40 and 100 μl, respectively, for phases A and B. Wavelength range of DAD: 200–400 nm; monitoring wavelength, 254 nm; column oven temperature 32.0±0.1 °C. Mobile phase flow-rate was 1.00 ml/min.

Enantioseparation was studied and optimized

using LiChrograph instrument and MCTA columns. Basic chromatographic parameters were studied within the ranges: flow-rate from 0.050 to 0.400 ml/min, mobile phase was composed of binary mixtures of methanol and water, alternatively ethanol and water in volume fractions from 100 to 50% of organic modifier. Mobile phases were mixed by the instrument. Column temperature ranged from 30 to 61 °C. Chosen optimal conditions were at flow-rate 0.095 ml/min, mobile phase containing 87% of methanol and 13% of water, column temperature 30.0±0.1 °C, wavelength 230 nm.

Calibration solutions were prepared daily fresh by dilution from stock solutions of pesticides prepared at 1000 mg/l concentration level in methanol and stored at −18 °C. There was no hydrolysis of epoxiconazole observed in water (pH 7–5) during 12 days at room temperature. Calibration graphs were obtained in concentration ranges 0.5–1000 and 0.5–50 mg/l, respectively for trace analysis of epoxiconazole in distilled, tap or surface water samples for 20 μl injected sample volume. For column switching experiments with 20-μl injection 0.5–4 mg/l range of epoxiconazole in tap water was chosen, whereas 0.025–0.25 mg/l range was evaluated for 1-ml injection volume.

The soil extraction procedure involved weighing 2 g of air-dried homogenized soil sample fraction with particle diameters less than 1 mm into the extraction vessel. After the addition of 3 ml of methanol/water mixture (95:5, v/v) the sample was shaken for 1 h at 500 movements per min. The extract (after 30 min standing) was ultrafiltered (0.2-μm ultrafilter Anotop 10, Merck) and transferred to the 5-ml volumetric flask. The extraction step was successively repeated with another 2-ml portion and the final volume was adjusted exactly to 5-ml volume by the extraction mixture. The procedure was applied to the spiked (11 pesticides added at levels of 2.5 mg/kg each) and non-spiked soil samples, respectively.

2.3. Materials

The target group of pesticides and purity of standards was:

1. Asulam 98% [IC] methyl-(4-aminobenzenesulfonyl)-carbamate
2. PCA 98% [IC] 5-amino-4-chloro-2-phenyl-3-pyridazone
3. Metoxuron 99.4% [DE] 3-(3-chloro-4-metoxyphenyl)-1,1-dimethylurea
4. Simazine 98% [IC] 2-chloro-4,6-bis(ethylamino)-1,3,5-triazine
5. Chlortoluron 99.0% [IC] 3-(3-chloro-4-methyl)-1,1-dimethylurea
6. Atrazine 98% [IC] 2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine
7. 2,4-D 98% [IC] 2,4-dichlorophenoxyacetic acid
8. MCPA 99.1% [DE] 4-chloro-2-methylphenoxyacetic acid
9. Propazine 98% [IC] 2-chloro-4,6-bis(isopropylamino)-1,3,5-triazine
10. MCPP 99.1% [DE] racemic, 2-(4-chloro-2-tolyloxy) propionic acid
11. Epoxiconazole 99.5% [RH] (2*RS*, 3*SR*)-1-[3-(chlorophenyl)-2,3-epoxy-2-(4-fluorophenyl) propyl]-1H-1,2,4-triazole

The pesticide standards were obtained alternatively from Dr Ehrenstorfer GmbH (Augsburg, Germany) denoted as DE, Riedel-deHaen Laborchemikalien GmbH and Co (Seelze, Germany) denoted as RH and Institute for Chemical Technology (Bratislava, Slovakia) denoted as IC.

Note that in spite of two chiral centers (four theoretical enantiomers, two diastereoisomers) the fungicide epoxiconazole is produced (BASF, Germany) as one enantiomeric pair.

The mixture of 11 pesticides was separated on achiral column: Purospher RP 18e 125×4 mm, 5 μm, guarded by precolumn Purospher RP 18e 4×4 mm, 5 μm (Merck, Darmstadt, Germany). Column switching experiments were done alternatively with the above mentioned column, or short column Separon SGX C_{18}, 30×3 mm, 5 μm (Tessek, Prague, Czech Republic) and chiral column 150×3 mm (Tessek) compact glass column laboratory packed with microcrystalline cellulose triacetate (MCTA, 15–25 μm particle diameter, Merck, Darmstadt, Germany).

The solvents methanol, ethanol, 2-propanol (Merck, Darmstadt, Germany) were of gradient grade purity. Water for gradient HPLC was prepared by Labconco Pro-PS unit (Labconco, Kansas City, USA). Phosphoric acid (Lachema, Brno, Czech Republic) and sodium hydroxide (Merck, Darmstadt,

Germany) of pro analysi grade were used for preparation of buffered mobile phases.

3. Results and discussion

3.1. Optimization of chiral separation conditions

Optimization of the chiral separation of commercially available fungicide epoxiconazole on cellulose triacetate column was done with respect to the chosen optimization criteria: resolution R_s should be higher than 1.10 and analysis time should not exceed 60 min, preferably from a practical point of view it should be around 30 min.

Optimized HPLC separation for resolution (R_s) equal to 1.15 is shown in Fig. 2A. Under the optimal separation conditions (described under Methods), analysis is done in 25 min. The first enantiomer E1 elutes at a retention time of 16.0 min with a retention factor of 1.16 (calculated with a dead time of 7.40 min from the first disturbance of the baseline), the second E2 elutes at 19.4 min with the retention factor 1.62. Under these conditions, the column porosity is 66.3%. The value of the resolution can be increased up to 1.7 by a decrease in flow-rate to 0.05 ml/min as can be seen in Fig. 2B, however the duration of analysis is 56 min. A flow-rate decrease to approximately half the initial value increases the

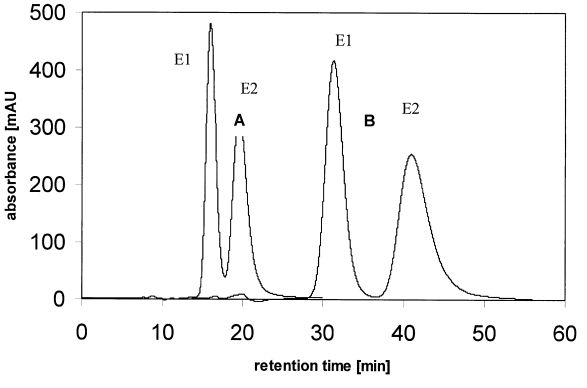

Fig. 2. Chromatograms of the epoxiconazole enantiomer separation showing the influence of mobile phase flow-rate to the separation. A = 0.095 ml/min, R_s = 1.15; B = 0.050 ml/min, R_s = 1.7. Concentration of the analyte was 500 μg/ml, injection volume 20 μl, binary mobile phase 87% methanol and 13% water, detection at 230 nm, temperature 30 °C.

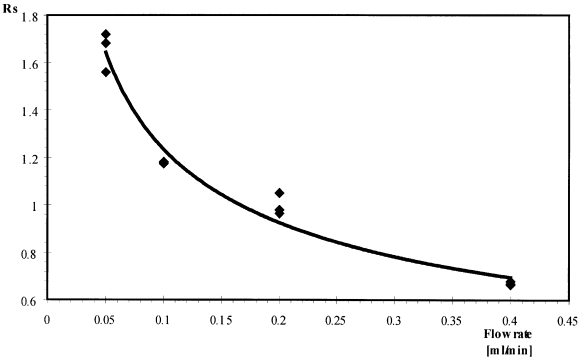

Fig. 3. Flow-rate dependence of resolution R_s of the two studied epoxiconazole enantiomers. Other conditions are identical with those in Fig. 2. Triple measurements were done at flow-rates of 0.05, 0.1, 0.2, 0.4 ml/min.

retention factor of E1 to 1.23 and E2 retention factor to 1.93. The resolution of both enantiomers on MCTA is dependent on flow-rate as is shown also in Fig. 3 and supports the observations of Rizzi [22] that relatively high efficiencies can be achieved even with large particles of MCTA, but at linear velocities much lower than are usual for silica-based chiral stationary phases.

Temperature was another parameter significantly influencing the retention and also the resolution as is evident from van't Hoff dependences measured for the epoxiconazole enantiomers (Fig. 4a) and corresponding chromatograms (Fig. 4b). At temperatures exceeding 72 °C, retention factor of the E2 is less than 0.67 and no resolution is observed.

A change in mobile phase composition also in-

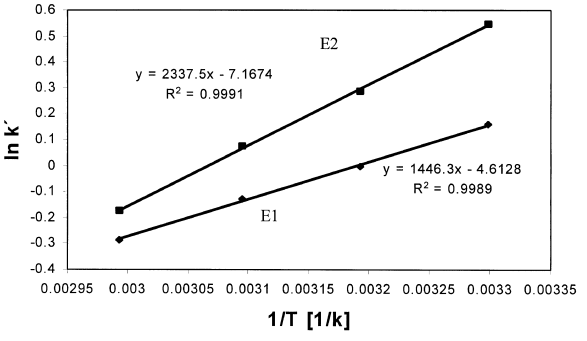

Fig. 4. van't Hoff plot of studied epoxiconazole enantiomers; ln k' is the natural logarithm of retention factors k', $1/T$ [1/K] denotes reciprocal absolute temperature in reciprocal Kelvins.

fluences basic figures of merit within the studied composition range; for instance, change in the proportion of methanol in water from 100 to 60% at a flow-rate of 0.05 ml/min decreases the resolution from 1.65 to 1.50. The selectivity decreases from 1.6 to 1.3, the average retention factor increases from 1.0 to 2.0 and the efficiency falls from around 1500 theoretical plates per column (t.p.p.c.) to 800. Packing procedure enables production of columns with efficiency around 1500 t.p.p.c. The overall cost of the column is low (approx. 20 USD) and can be disposed, or repacked after failure during real environmental sample analysis.

3.2. Quantitative analysis

Quantitative parameters for the direct chromatographic analysis were measured in a wide linear range of 1–1000 mg/l for 20-μl injection volume and sample dissolved in the mobile phase. Large sorption capacity of MCTA results in good linearity of the calibration curve evaluated by least-squares method of linear regression. For a linear model $Y = A + BX$ where Y is peak area (mV s) and X is the concentration of epoxiconazole (mg/l) evaluation resulted in $A = -930 \pm 534$, $B = 117.7 \pm 1.5$ and correlation coefficient $R = 0.9995$ for the first eluted enantiomer E1. For the second eluting enantiomer E2, the parameters are $A = -1196 \pm 700$, $B = 125 \pm 1.9$ and $R = 0.9993$. Number of data points was nine, representing the concentrations 1, 5, 10, 25, 50, 100, 250, 500, 1000 mg/l of racemic epoxiconazole. For practical analysis, a narrower range of the concentrations is usually sufficient, depending on the actual problem to be solved.

The linearity of calibration curves was verified for epoxiconazole spiked tap and/or surface water within the range 0.5–4 mg/l. This range was selected with respect to low solubility of epoxiconazole in water at 20 °C referred by Ref. [17] to be 6.63 mg/l. Resulting calibration curves with all parameters are shown in Fig. 5a,b.

Under the given conditions, low volume (20 μl) injection did not enable quantitation of both epoxiconazole enantiomers present in water samples below 0.5 mg/l concentration level without additional sample treatment. For this reason, we have studied other possibilities including off-line solid-phase extraction, on-line C_{18} short column preconcentration via reversed transfer technique from water samples and/or heart-cut direct transfer technique for analysis of effluent from high efficiency C_{18} column used for separation of selected pesticides extracted from fortified soils. In the last case, high compatibility of both mobile phases used in achiral and chiral separation systems and also extractant compatible with the mobile phases is essential for simple column switching method development.

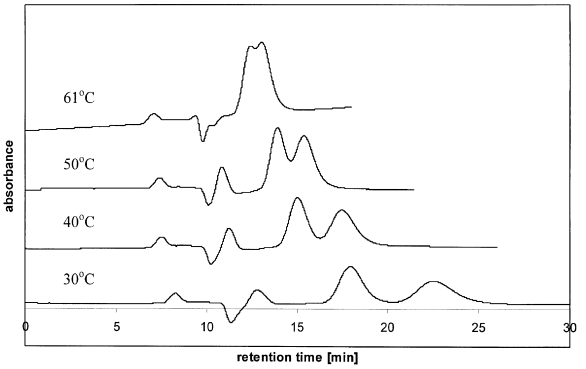

Fig. 5. Chromatograms of the epoxiconazole enantiomer separation at different temperatures. Concentration of the analyte was 10 μg/ml, injection volume 20 μl, binary mobile phase 87% methanol and 13% water, flow-rate 0.095 ml/min, detection at 230 nm.

3.3. On-line preconcentration

On-line preconcentration of epoxiconazole for trace analysis in tap water (produced by simple disinfection by chlorination of the underground water) aimed to decrease the detection limit and remove potential interfering impurities. These two goals are essential because MCTA offers good chiral selectivity, but rather restricted achiral selectivity and restricted retention of achiral substances under real conditions. The probability of interference caused by different groups of substances (co-extracted from water samples, as are e.g. humic substances) was tested by various substances and is relatively high.

Retention factors and preconcentration factors of epoxiconazole on short Separon SGX C_{18} column were measured by injection of 1-ml sample volume at two concentration levels (0.2 and 4 mg/l) into the

isocratic binary methanol/water mobile phases within the range 100% (v/v) methanol to 70% (v/v) methanol. The most suitable composition was 80% (v/v) methanol and 20% (v/v) water. At this mobile phase composition, epoxiconazole was focused on the top of the C_{18} column and quantitatively eluted as a narrow peak by the transient gradient created during water sample injection. The transient gradient of decreased elution power is formed at the front edge of the purely aqueous sample plug (1-ml volume) injected to the RP column with smaller dead volume (0.120 ml) than injected sample volume. The lipophilic constituents (the main one being the analyte) of the sample were focused at the top of the short column during the injection of the water sample and successively eluted by increased elution power of the isocratic mobile phase at the back edge of the water sample plug.

Optimal timing of the transfer step to MCTA column was calculated and successively verified as depicted in Fig. 6.

Calibration graphs were measured and evaluated by linear regression using least-squares method for both enantiomers in the range from 0.025 to 0.200 mg/ml. Calibration curves are linear with correlation coefficients of 0.998 and 0.992, respectively (Fig. 7). The limit of detection is estimated to be 0.010 mg/l which means that the method is suitable for trace analysis at low ppb level. This value was further decreased to 0.001 mg/l level by injection of 50 ml

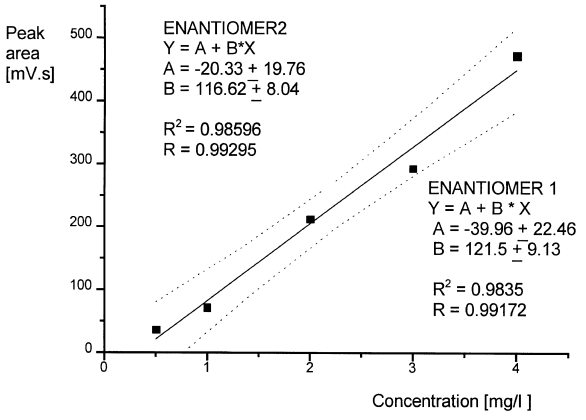

Fig. 6. Calibration curve for the first eluting enantiomer E1 and for the second eluting enantiomer E2 of epoxiconazole dissolved in tap water. For operational parameters, refer to Fig. 2.

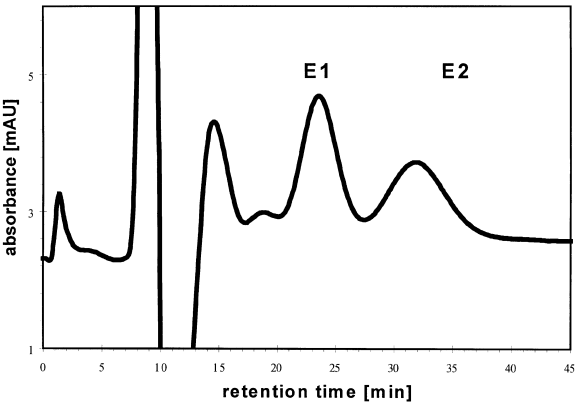

Fig. 7. Chromatogram of the chiral separation (MCTA) of the epoxiconazole (0.2 mg/l) in tap water after 30-fold preconcentration on Separon SGX C_{18} column from 1-ml volume of tap water by achiral–chiral column switching. For other conditions, refer to Fig. 2; E1, the first eluted enantiomer; E2, the second enantiomer.

of spiked water sample, but a 50-fold increase in injected volume did not result in the achieved decrease in limit of detection because both inorganic and organic constituents of tap water (humic substances at concentration levels 2 mg/l) interfered and displaced epoxiconazole from the C_{18} column. Under these conditions, the standard addition method is recommended to be used.

These aspects are under further investigation.

Chiral analysis of the epoxiconazole enantiomers in addition to 10 pesticides in soil by achiral–chiral column switching method required optimization of several procedures which will be described in detail in another article prepared for publication:

(a) Extraction of the pesticides. The highest recoveries of the whole group of pesticides were achieved by methanol and water (95:5) soil extraction; recovery of the extraction procedure of soil spiked at low ppm levels ranged from 80 to 98% depending on the pesticide. The recovery for the epoxiconazole was 92±5%, this value represents the average of five experiments.

(b) Optimized separation of the mixture of 11 pesticides in reversed-phase mode on Purospher RP C18e column under gradient elution conditions described in chapter Methods is shown in Fig. 8. For column switching, direct transfer technique of 250-μl fraction of epoxiconazole

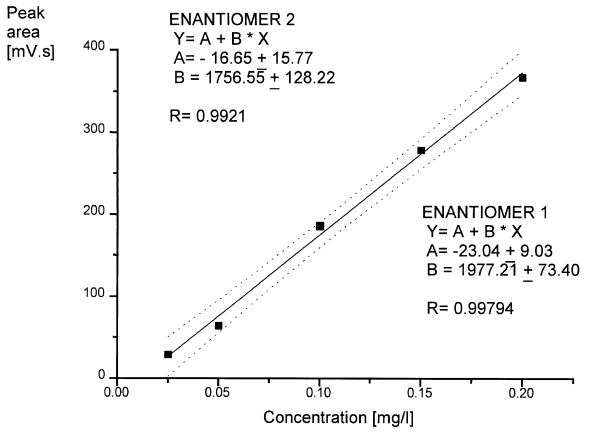

Fig. 8. Calibration curve parameters for both enantiomers, graphical representation is given for the first eluting enantiomer E1 for 1-ml sample injection volume and by application of the column switching technique. For other parameters, refer to Methods.

peak (heart-cut) from 30.8 to 31.05 min was accomplished as is evident from Fig. 8. The other pesticides were conveniently quantified in the achiral system except the asulam peak eluting at the solvent front and therefore it is not conveniently quantified. Detection limits ranged from 0.010 to 0.025 mg/l for a 200-μl injection [23].

(c) Optimized chiral separation of the transferred fraction is depicted in Fig. 9. Detection limits of 0.2 mg/kg were reached. Measured values were from two-point calibration. Non-spiked and spiked (fortified by the standard addition) soil samples were extracted according to the described procedure and both results were used for the detection limit estimate from peak heights ($2\times S/N$).

Calibration curves are described by parameters similar to those described in Fig. 5a,b. Resolution is lower than during analysis of aqueous samples, which is caused by transfer technique which must be further studied and refined.

However, no interferents transferred from the Purospher RP C18e column were observed due to the fact that all polar co-extracted soil constituents are eluted either near the void volume of the C_{18} column (soil fulvic acids, etc.) or after the epoxiconazole peak (humic acids) (Fig. 10).

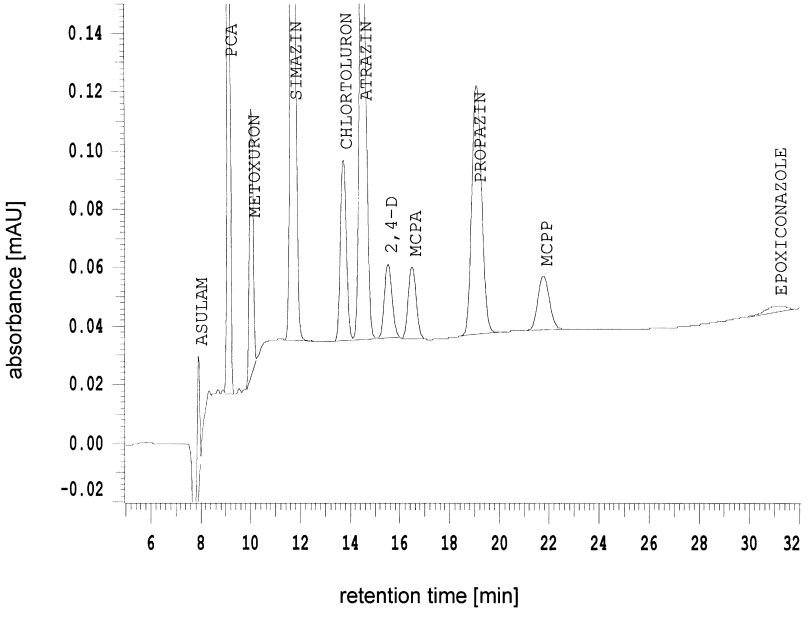

Fig. 9. Separation of 11 pesticides using a Purospher RP 18e column by gradient elution. Concentration of pesticides 2.5 mg/kg (each), standard addition to arable soil, injected volume 100 μl of methanol and water mixture (95:5 v/v) soil extract, recovery of extraction procedure ranged from 80 to 98% depending on pesticide (epoxiconazole 92±5%), UV detection 220 nm, flow-rate 1 ml/min, oven temperature 40 °C.

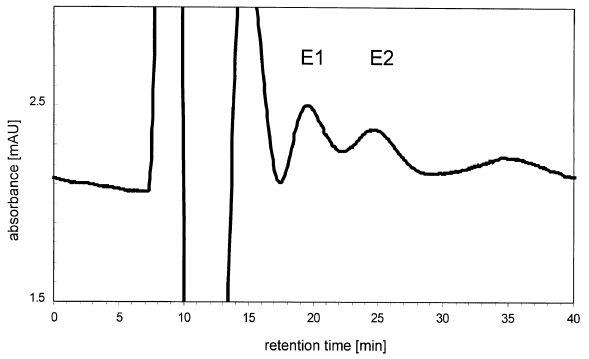

Fig. 10. Chromatogram of the chiral separation of the 0.25 ml epoxiconazole fraction volume (heart-cutting technique by column switching Purospher RP18e achiral–MCTA chiral column). Same conditions as in Fig. 2.

4. Conclusion

Selection of the stationary (MCTA) and mobile phases was appropriate; analyte can be separated by isocratic binary aqueous mobile phases having a composition from 100% methanol to 60% methanol.

For the separation conditions of the epoxiconazole enantiomers (in mode compatible with RP achiral separation) on a column packed with 15–25-μm particles of the MCTA, a mobile phase of 87% methanol and 13% water is optimal.

Linearity was measured in a wide concentration range (1–1000 mg/l) of the analyte in the mobile phase. Then the calibration lines of the epoxiconazole were measured in tap water in the range 4–0.5 mg/l for injection volumes of 20 μl and 1 ml, respectively (column-switching experiments). Calibration lines are linear with a correlation coefficient higher than 0.99.

By the combination of achiral and chiral column, the analyte was preconcentrated and separated from the interferents. Calibration lines were measured for epoxiconazole in tap water and in soil extracts in the range from 0.2 to 0.025 mg/l and no interference was observed from the compounds present.

The whole analysis time with column switching of achiral and chiral column (preconcentration of the epoxiconazole from water samples) takes less than 40 min. The retention time of the first eluted enantiomer is 23.6 min, and for the second is 31.7 min; resolution of the enantiomers is 1.17. Analysis of the epoxiconazole in fortified soils (2.5 mg/kg) in addition to 10 other commonly used pesticides provides comparable results and enables determination of epoxiconazole in soil at concentrations higher than 0.2 mg/kg. Overall retention time reproducibility of epoxiconazole (including the column switching) is ±3%RSD.

The application range of the developed method will be broadened to analysis of epoxiconazole in air and/or aerosol samples.

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