Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1061 (2004) 11–18
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Sensitive trace enrichment of environmental andiandrogen vinclozolin from natural waters and sediment samples using hollow-fiber liquid-phase microextraction

Dimitra A. Lambropoulou*, Triantafyllos A. Albanis

Laboratory of Environmental Technology, Department of Chemistry, University of Ioannina, Ioannina 45110, Greece

Received 15 September 2004; received in revised form 13 October 2004; accepted 25 October 2004

Abstract

The presence of vinclozolin in the environment as far as the endocrine disruption effects in biota are concerned has raised interest in the environmental fate of this compound. In this respect, the present study attempts to investigate the feasibility of applying a novel quantitative method, liquid-phase microextraction (LPME), so as to determine this environmental andiandrogen in environmental samples such as water and sediment samples. The technique involved the use of a small amount (3 μL) of organic solvent impregnated in a hollow fiber membrane, which was attached to the needle of a conventional GC syringe. The extracted samples were analyzed by gas chromatography coupled with electron-capture detection. Experimental LPME conditions such as extraction solvent, stirring rate, content of NaCl and pH were tested. Once LPME was optimized, the performance of the proposed technique was evaluated for the determination of vinclozolin in different types of natural water samples. The recovery of spiked water samples was from 80 to 99%. The procedure was adequate for quantification of vinclozolin in waters at levels of 0.010 to 50 μg/L ($r > 0.994$) with a detection limit of 0.001 μg/L ($S/N = 3$). Natural sediment samples from the Aliakmonas River area (Macedonia, Greece) spiked with the target andiandrogen compound were liquid–liquid extracted and analyzed by the methodology developed in this work. No significant interferences from the samples matrix were noticed, indicating that the reported methodology is an innovative tactic for sample preparation in sediment analysis, with a considerable improvement in the achieved detection limits. The results demonstrated that apart from analyte enrichment, the proposed LPME procedure also serves as clean-up method and could be successfully performed to determine trace amounts of vinclozolin in water and sediment samples.

Keywords: Water analysis; Sediment analysis; Liquid phase microextraction; Andiandrogen; Endrocrine disruptor compounds; Vinclozolin

1. Introduction

Recently, a wide variety of chemicals, that have been identified to disrupt endocrine system of higher life forms, such as fish, wildlife and even humans, have attracted considerable attention worldwide. The so-called endocrine disrupting chemicals (EDCs) can act as hormone mimics or antagonists to disrupt the reproductive system in humans and animals. The recently observed trends of these reproductive abnormalities as declining sperm counts, cryptorchidism, hypospadias and testicular cancer also have been linked to EDCs [1,2].

Vinclozolin [3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione] (Fig. 1) which is one of the known EDCs, is a dicarboximade fungicide, which is used in the United States and Europe to control fungus diseases in various crops such as lettuce, kiwi, canola, snap beans, onions, as well as grass and ornamentals. Several investigators have suggested that vinclozolin has antiandrogen activity, thus impairing the development of the reproductive systems in male rat pups [3–6]. Pre- and perinatal exposure to low doses of vinclozolin alters sex differentiation in male rats and frequently induces undescended testes located within an ectopic cremaster sac anterior to the abdominal wall [3–5]. Kelce et al. [4] has reported that vinclozolin acts as an antiandrogen by altering the androgen binding to

* Corresponding author. Tel.: +30 26510 98363; fax: +30 26510 98795.
E-mail address: dlambro@cc.uoi.gr (D.A. Lambropoulou).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.10.064

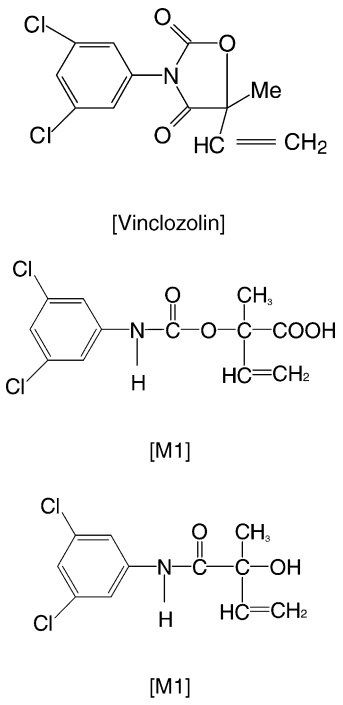

Fig. 1. Structural formula of vinclozolin and its primary metabolites M1 and M2.

androgen receptor (AR) and subsequently also altering the AR-dependent gene expression. Moreover, due to the fact that vinclozolin was found to develop tumours in animal experiments, its genotoxicity has also been discussed [7–10]. On the basis of these observations, it is unclear whether this compound is causally associated with human adverse effects since well-defined test guidelines detecting their adverse effects have not been validated.

These anti-androgenic and toxicity concerns over vinclozolin as well as the possible environmental persistence of its primary metabolic products 2-{[3,5-dichlorophenyl)-carbamoyl]oxyl}-2-methylbutenoic acid (M1) and 3′,5′-dichloro-2-hydroxy-2-methylbut-3-enanlide (M2) (Fig. 1) prompted the development of analytical methodologies so as to determine the presence and concentration of this environmental antiandrogen in different environmental matrices.

For many years, sample pre-treatment for isolation and/or enrichment of vinclozolin from aqueous solution have been invariably done by liquid–liquid extraction (LLE) [11] and especially solid-phase extraction (SPE) [12,13]. Although these techniques provide adequate analyte enrichment, they require large amounts of solvents are labour intensive and often require evaporation of solvent and reconstitution in order to provide sufficient preconcentration.

In response to the problems with traditional sample preparation techniques, several novel microextraction techniques have been developed. Among these methods solid-phase microextraction (SPME), has attracted increasing attention for water analysis of organic micropollutants [14,15]. Today, SPME is recognised as an attractive alternative to other traditional extraction methods such as LLE and several published reports deal with the development and validation of SPME methods in vinclozolin analysis [16–18].

An alternative to SPME is single drop microextraction (SDME), which was recently and successfully developed in several laboratories to extract aqueous samples. SDME is based on the traditional LLE technique but involves only a few microliters of organic solvent as extractant [19–21]. In this microextraction system, a single microdrop of solvent (as extraction phase) suspended on the tip of a conventional microsyringe, immersed in a contaminated water sample. Although the SDME have proved to be a simple, inexpensive, fast and virtually solvent-free sample pre-treatment technique, problems of drop stability and low sensitivity were often encountered [22,23].

The quest for novel micro-LLE methods have never ceased and a new microextraction method, termed liquid-phase microextraction (LPME), using porous polypropylene hollow-fibers was recently introduced [23–27]. In one of the possible configurations, analytes were extracted from a sample solution through pores of a porous hollow fiber (HF) of polypropylene into a small volume of an organic solvent under magnetic stirring. This fiber configuration is considered an evolution of SDME, because the organic microdrop is protected by the hollow fiber. LPME is attractive in terms of sensitivity, precision, analysis time, and relative simplicity and is proved to be a promising tool for the analysis of organic compounds in water samples.

In this light, the objective of this study is to exploit the potentiality of vinclozolin for hollow fiber LPME to water and sediment samples. Some LPME related parameters have been optimized and the proposed procedure was applied to determine the environmental antiandrogen in natural samples. To the best of our knowledge, this is the first work describing an application of HF-LPME method for the determination of vinclozolin in environmental samples. The proposed method combines the high selectivity, the low cost and the less time consuming for trace enrichment of target analyte in water samples. In addition the optimized methodology for solid samples is proposed as an efficient alternative analytical tool and offers more advantages over the well-known established methods.

2. Experimental

2.1. Chemicals and materials

The Accurel Q 3/2 polypropylene hollow fiber membrane was purchased from Membrana GmbH (Wuppertal, Germany). The inner diameter was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm.

All solvents (pesticide-grade) were supplied by Pestiscan (Labscan, Ltd, Dublin, Ireland) and sodium chloride from Merck (Darmstadt, Germany). Vinclozolin was purchased from Riedel-de Häen (Germany). Sea-nine 211 (internal stan-

dard) was a kind offer by Rohm & Haas (Philadelphia, PA, USA). Humic acids (HA) were purchased from Fluka (Steinheim, Germany). A toluene solution (1 mg/L) of Sea-nine 211 was prepared and used as the internal standard. Stock standard solutions were prepared in methanol with concentration levels of 1000 μg/L for each compound and were stored in a freezer at about −20 °C. Working solutions were prepared by dilution of stock standards with deionised water. Water from the GFL (2108) water purification system (GFL, Germany) was used.

A 10-μl Hamilton gas-tight syringe (Hamilton, Bonaduz, Bonaduz, Switzerland) model 1701 NRN, with a bevel needle tip (length: 5.1 cm, O.D.: 0.071 cm, I.D., 0.015 cm) was used to introduce the acceptor phase, support the hollow fibre and act as an injection syringe.

2.2. Sample collection

River water samples were obtained from Aliakmonas River (Macedonia, Greece), groundwaters from the main area of Ioannina (Greece) and sea water samples from Ionian Sea (N.W. Greece). The water samples were collected in glass bottles and used without previous treatment or filtration. Sediment samples, used in the investigations, collected from the Aliakmonas River estuary. Before extraction, sediment samples were sieved (pore size 2 mm i.d.), homogenized, and then suction-dried on a Buchner funnel in order to remove most of the water. A fraction of sample was dried in a desiccator until constant mass was achieved. Sediment samples were prepared by spiking appropriate amounts of the diluted working standards solutions to get final concentrations of 10–250 ng/g sediment. All samples were free of the target andiandrogen as found by previous analysis which was using the SPME technique [16] and acetone/SPME procedure [18] for water and sediment samples, respectively.

2.3. Liquid-phase microextraction process

2.3.1. Water extraction

Before use the hollow fiber was ultrasonically cleaned in acetone for several minutes in order to remove any contaminants. After being dried, the hollow fiber was cut manually and carefully into 1.3 cm lengths prior to use. A 3.0 μL aliquot of organic solvent (typically toluene) was withdrawn into the syringe followed by an equal volume of water. The needle tip was inserted into the hollow fiber, and the assembly was immersed in the organic solvent for ∼10 s in order to impregnate the solvent into the pores of the fiber wall. Because the hollow fiber used was hydrophobic, the fiber channel could have been filled with organic solvent as well. After solvent impregnation, the water in the syringe was injected carefully to flush the hollow fiber in order to remove the excess organic solvent from the interior (this procedure was performed while the fiber was remaining immersed in the organic solvent). The prepared fiber was removed from the solvent and subsequently immersed in the aqueous sample. Finally, the organic solvent in the syringe was injected carefully and completely into the hollow fiber. The experimental results indicated that the residue water inside the hollow fiber had no effect on extraction efficiency and precision. The volume of aqueous solution was 5 mL in an 7 mL vial.

The sample was continuously stirred at room temperature (25 °C) with a magnetic stirrer to facilitate the mass transfer process and to decrease the time required for the equilibrium to be established. The stirring speed was fixed at 800 rpm. Higher stir speed was not selected for the next experiments taking into consideration occasional difficulties in the quantification on the target analyte due to the formation of air bubbles in the hollow fiber and the loss of the organic solvent. After 20 min extraction, the analyte-enriched solvent was withdrawn into the syringe and then injected into the GC system with electron-capture detection (GC-ECD) for analysis. The used fiber was discarded and a fresh one was used for the next experiment.

2.3.2. Sediment extraction

Extraction of the vinclozolin from sediment samples was performed by mixing 5 g of sample with 10 ml of acetonitrile–methanol (9:1, v/v) (2:1 ratio solvent/sediment) followed by a subsequent sonication in an ultrasonic water bath at room temperature for 30 min. After the sonication the extracts were centrifuged at 4000 rpm for 5 min and the supernatant liquid was carefully evaporated to 0.05 mL by a gentle stream of nitrogen. Afterwards this extract was added to a volume of 5 mL of distilled water in order to get the sample in an aqueous phase and to be able to perform the preconcentration through the LPME technique. Thus the sediment samples could be treated as the water samples.

2.4. 2.4.GC-ECD analysis

Both water and sediment extracts were analyzed by a Shimadzu 14B capillary gas chromatograph equipped with an ECD system working at 300 °C. Analytes were separated with a DB-1 column (J & W Scientific, Folsom, CA, USA), 30 m × 0.25 mm i.d., contained dimethylpolysiloxane with a phase thickness of 0.25 μm (splitless mode). The temperature program used in the analysis was: from 80 (2 min) to 290 °C (10 min) at 21 °C/min. The injection temperature was 250 °C. Helium was used as the carrier at 1.5 mL/min and nitrogen was used as make-up gas at 35 mL/min.

3. Results and discussion

3.1. Optimization of LPME in aqueous samples

In the time being, most of the studies dealing with the hollow-fiber LPME of pesticides from water solutions are focused on the triazine [25], organophosphorous [27] and organochlorine compounds [28,29] and to the best of our knowledge, no previous study has ever been target in the

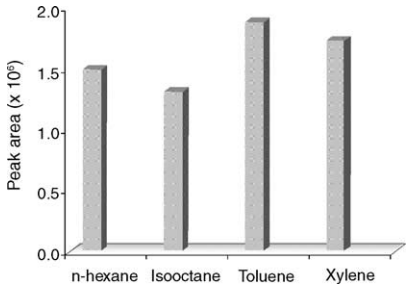

Fig. 2. Effect of extraction solvent on LPME efficiency.

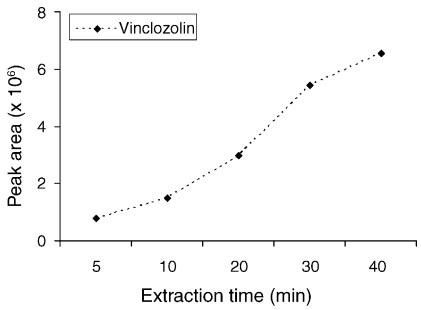

Fig. 3. Effect of extraction time on LPME efficiency.

investigation of environmental antiandrogens such as vinclozolin in natural waters. Thus, the effect of different experimental parameters in the yield of the microextraction was studied in detail. Measurements were carried out using the GC-ECD system. All optimization experiments were performed with water samples spiked with 1 μg/L of the target antiandrogen compound.

3.1.1. Extraction solvent

The type of organic solvent in LPME technique is an essential consideration for efficient analyte preconcentration. As in LLE the principle "like dissolves like" is applied. The water immiscible solvent used should have been fulfilling several requirements including the high solubility for the target analyte, the good immobilization in the hollow fiber pores, the low solubility in water and finally the compatibleness with the capillary GC column. Based on the above considerations, four organic solvents, including *n*-hexane, isooctane, toluene and xylene were evaluated for extraction efficiencies. Toluene showed the best extraction performance among the four extraction solvents in terms of analyte peak areas while the hexane showed the lowest efficiency (Fig. 2). Thus, the toluene was used in further experiments taking into account not only the good selectivity of vinclozolin but also the low solvent losses compared to the other organic solvents tested, as well as the ability to be easily immobilized in the pores of the fibers within few seconds [25].

3.1.2. Exposure time

The effect of extraction time on vinclozolin congener extraction efficiency was investigated by monitoring the variation of analytical signal with exposure time over 5, 10, 20, 30, and 40 min. Fig. 3 shows that the analytical signal increases with sampling time in the range of 1-30 min and after 30 min the rate of increase slows down. It can be pointed out however, that equilibrium has not been reached even after 40 min. This extraction kinetic is similar to those generally observed by SPME, which normally takes considerable time before reaching equilibrium [16]. The longer sampling times were not studied taking into account possible solvent dissolution. It is remarkable though that as far as the quantitative analysis is concerned, it is not necessary for the analytes to reach the equilibrium. In this case, allowing sufficient mass transfer into the acceptor phase (organic solvent) in an exact reproducible extraction time is adequate. Thus, from a practical point of view, in this work an extraction time of 20 min was adopted for further studies to keep the total extraction time comparable with the chromatographic run time (maximum sample throughput).

3.1.3. Salt

In order to investigate the effect of ionic strength, a series of spiked samples with various concentrations of NaCl at a range of 0–30% (w/v) were extracted. Although the effect of salt addition in HF-LPME technique was not discussed widely, some contradictory results had been reported for some organic micropollutants such as pesticides [25,30], phthalates [22] and polycyclic aromatic hydrocarbons [30]. In our case, the obtained results showed that the addition of NaCl hampered the transport of the analytes to the extracting acceptor phase. It seemed like that the existence of the hollow fiber affected the kinetics of the partitioning of analyte between organic solvent and aqueous solution. Similar observations concerning the effect of salt on the LPME analysis was also made by other researchers [19,20]. Thus, all remaining extraction experiments were performed without salt addition on the water samples.

3.1.4. pH effect

The investigation of pH effect on vinclozolin extraction by LPME was undertaken in order to find a pH value at which the extraction of the andiandrogen compound would have been enhanced in general or was not significantly decrease (Fig. 4). There was no considerable impact on the extrac-

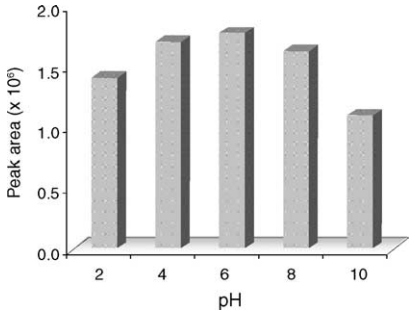

Fig. 4. Effect of pH on LPME efficiency.

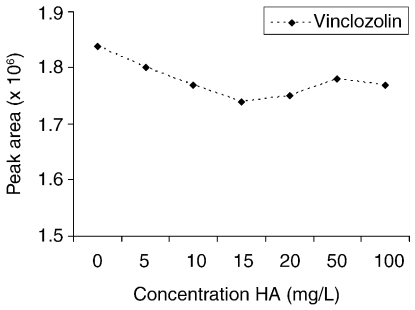

Fig. 5. Effect of addition of humic acid on LPME efficiency.

tion yield of vinclozolin while varying the sample pH value from 2 to 8. On the contrary, at higher pH values (pH 10) the extraction yield of the target analyte was decreased. This effect may be attributed to the higher hydrolysis rate of dicarboximide fungicides in alkaline media [31]. On the basis of these results, no adjustment of pH was made for subsequent experiments since neutral pH conditions were suitable for extraction. Moreover, under neutral conditions the application of LPME in sediment samples was made more favorable, since it would have been difficult to adjust the pH at small sediment extract volumes (5 mL).

3.2. Humic acids

In this study, an attempt was made to trace the effect of humic acids in the extraction efficiency of aqueous vinclozolin solution when using HF-LPME method. The concentration of HA was varied at a range of 0–100 mg/L levels. The results showed that the presence of HA in the sample cause insignificant influence on the extraction efficiency in the tested concentration HA levels (0–100 mg/L) when a 1 mg/L amount of vinclozolin was added (Fig. 5). This effect may be attributed to the selectivity of the hollow fiber. The small pore size of the membrane wall allows the low-molecular-mass target analytes to diffuse through while excluding high-molecular-mass interfering compounds. This means that humic acids which typically have molecular masses up to several million cannot be extracted into the organic solvent. A similar effect of HA has been also reported concerning the application of HF-LPME technique for the determination of triazines [25].

In comparison the SDME and the technique of SPME into a polymer film coated on a fused silica fiber reveals that the proposed method was less affected by the sample matrix. In SDME [32] and SPME [33] the extraction efficiency of analytes decreases dramatically when the humic acids in the solution were over 100 mg/L, since the single drop or the fiber is in direct contact with the humic acids, which compromises the extraction.

Based on the above observations, it is apparent that porous hollow fiber functions as a filter in “dirty” samples and HF-LPME technique could be successfully performed in more complex samples, which contain solid or high-molecular-mass materials such as soil and sludge for determining the target antiandrogen compound.

3.3. Matrix in water samples and evaluation of the LPME method

A matrix intereference study was carried out to compare the calibration graph obtained for distilled water and for each type of natural water. The equations of the least-squares linear regression obtained with distilled water and with environmental samples were similar, and the covariance analysis showed that the calculated F values were lower than the tabulated ones, indicating that both straight lines were parallel ($p < 0.05$). These results were also supported by the experiments with humic acids proving that interferences were negligible in the tested environmental samples.

Recovery efficiency and precision of this method was carefully evaluated for each matrix using six replicates at very low concentration (ppt) in order to match the lowest allowed concentration of this pollutant within the European Union (EU) (0.1 μg/L—this level is equal to the limit issued by the EU for the presence of each pesticide in a drinking water). The recovery and precision data are reported in Table 2. The relative recoveries varied from 90 to 99% with a R.S.D. values between 4.3 and 6.2%.

Recoveries not showing dependence on the concentration for the different spiked levels assayed. As a model, recoveries and R.S.D. obtained for river water, spiked at different concentrations, are summarized in Table 3. Results were better or similar for natural water samples with other microextraction techniques such as SPME [16,17].

The good precision exhibited for vinclozolin by LPME in different types of water samples indicating that the manually cut hollow fiber had no significant effect on the precision. In addition the protection offered by the hollow fiber made the solvent drop stable, and the effect of the matrix on the extraction solvent was eliminated.

3.4. Analytical performance of LPME in aqueous samples

Once established the experimental LPME conditions, a number of performance parameters such as linearity, detection limits and precision were evaluated for the target andiandrogen compound in water samples.

The linearity of the method was tested over a range between 0.010 and 50 μg/L using five concentration levels and analyzing each level in triplicate. After plotting the mean peak areas versus sample concentration to generate calibration curves, a statistical regression model was applied to obtain the corresponding values for slope and intercept for the target analyte. Vinclozolin exhibited good linearity with square regression coefficient (R^2) higher than 0.9954. The calibration curves exhibited a slow rise at intercept value in the tested concentration levels. This effect could be ascribed to the high enrichment factor of the target analyte by the pro-

Table 1
Analytical characteristics of LPME method in water samples

	Vinclozolin
t_r (min)	14.26
Linearity concentration (μg/L)	0.010–05
Slope ($\times 10^6$)	3.24
Intercept ($\times 10^6$)	0.74
Correlation coefficient (r^2)	0.9954
LOD (μg/L)	0.001
LOQ (μg/L)	0.004
Preconcentration factor[a]	150.4
Extraction efficiency (%)[b]	3

[a] Calculated as the ratio of the final analyte concentration in the extraction solvent to the analyte concentration in the original sample ($n = 3$).
[b] Calculated as the percent of the total analyte present in the original sample that was extracted into the fiber ($n = 3$).

Table 2
Relative extraction recoveries[a] (%) and R.S.D. obtained for each water sample matrix spiked at 0.1 μg/L concentration level

Antiandrogen compound	Underground water	River water	Sea water	Lake water
Vinclozolin	98.7 (3.6)	94.0 (4.1)	92.3 (4.8)	90.0 (6.2)

[a] Since the LPME is an equilibrium rather than an exhaustive extraction method, "% recovery" refers to the andiandrogen compound concentrations determined rather than the actual percent of antiandrogen compound extracted by the LPME analysis.

posed method (Table 1), as well as the enhanced selectivity and sensitivity which were observed by using electron capture detection system. At this point, it should be mentioned that more reliable and quantitative results were obtainable, even for environmental water samples, after the selection of a relatively narrower dynamic linear range of concentration levels for the calibration study.

Table 3 also shows the detection limits calculated as the lowest concentration of an analyte giving a signal of three times the base line of the chromatogram. The obtained results show that the proposed method allows detection of vinclozolin at concentrations lower than 0.001 μg/L.

The overall precision of the method was evaluated by performing repeatability and reproducibility experiments by carrying out six replicates of a sample during 1 day ($n = 6$, intra day precision), spiked at a level of 0.1 μg/L of the target andiandrogen and two replicates at three different days (inter-day precision), over a period of 1 week. Results were similar in both cases with coefficient of variations below 12.0%.

3.5. Enrichment factor

In an analysis, it is desired to evaluate the initial concentration of analytes in an aqueous sample solution based upon the measured value of the analyte concentration in the organic acceptor phase at sampling time. Similar to that in SPME [14,15] an exhaustive extraction does not occur in LPME. Instead the analyte is partitioned between the bulk aqueous solution and the organic acceptor phase [31].

In the present study the amount of vinclozolin in the hollow fiber following extraction was calculated using the peak area ratio measurements and the calibration curves of the standards only. From that data the enrichment factor and extraction efficiency for the target analyte were determined and are presented in Table 1. The enrichment factor is defined as the ratio of C_0/C_v, where C_0 is the concentration of analytes in the organic phase after extraction and C_v is the original concentration of vinclozolin in the aqueous phase and is calculated using the average of the three trials obtained for 0.1 mg/L concentration level. Extraction efficiency was calculated from the average peak area ratio of the three replicate trials at the same concentration level.

3.6. Application of LPME to sediment samples

Once the procedure for the determination of andiandrogen vinclozolin in water was developed, we indented to extend the applications of the LPME to other sample matrices. The determination of vinclozolin in solid samples posses a significant challenge to the analyst, since sample matrixes are complex and their extraction requires an extensive sampling handling, including the submission of liquid extracts to one or several clean-up steps, using normal sorbents prior to the determination of the analyte in the analytical instrument. Moreover, the environmental occurrence of vinclozolin in sediment samples due to its widespread use in agriculture as well as to its endocrine disruptor effect on biota make its determination an emerging environmental issue in these matrices.

In the present study the use of traditional LLE coupling with LPME as analytical procedure for further enrichment and quantitative determination of vinclozolin in sediments was evaluated. For this purpose optimal conditions for the extraction of vinclozolin from sediments have been searched varying factors likely to affect the selectivity and sensitivity of the target compound toward the proposed methodology.

In order to avoid loss of the target analyte and increase the extraction efficiency, a suitable selection of solvents to optimize procedure of extraction and preconcentration of analyte is indispensable. Acetone, acetonitrile and methanol are some of the most common organic solvents usually used for the extraction of pesticides from solid samples such as soils [34–36]. In this study the mixed solution acetonitrile–methanol (9:1) was selected as the extraction

Table 3
Relative extraction recoveries (%) and R.S.D. obtained for river water sample matrix spiked at different concentration levels

Antiandrogen compound	Spiking level (μg/L)				
	0.05	0.1	0.250	0.5	1.0
Vinclozolin	93.4 (5.7)	91.0 (4.7)	94.3 (4.5)	96.3 (4.6)	92.6 (5.3)

Table 4
Mean recoveries (%, $n = 3$) and limits of detection (LODs) of vinclozolin in spiked sediments obtained from LPME method

Antiandrogen compound	Linear range[a] (r)	Recovery (%)[a]		LOD (ng g^{-1})	(R.S.D.) (%)
		100 (ng g^{-1})	200 (ng g^{-1})		
Vinclozolin	0.995	94 (5.7)[c]	96 (5.4)[c]	0.5[b]	6.1[d]

[a] Linear curves were constructed using five samples between 10 and 250 ng/g (10, 50, 100, 150 and 250 ng/g).
[b] Since the LPME is an equilibrium rather than an exhaustive extraction method, "% recovery" refers to the andiandrogen compound concentrations determined rather than the actual percent of antiandrogen compound extracted by the LPME analysis.
[c] Relative standard deviation (R.S.D) ($n = 3$).
[d] Calculated from the chromatograph of the sample spiked at 10 ng/g concentration level.

solvent from the extraction of vinclozolin from sediments taking into account the results which had been presented by our group in previous work [18]. Apart from the good selectivity of vinclozolin with acetonitrile-methanol, the selection of this mixed elution solution is related to the evaporation step. Volatility of acetonitrile and methanol is lower than that of acetone make the evaporation step of eluates to near dryness by the gentle stream of nitrogen gas easier and reproducible, resulting in hardly any loss of the analyte. Besides both solvents they can be mixed well with the dissolution phase (water), and the sediment extracts can be treated as the water samples.

To test the suitability of the method for quantitative analysis, linearity, precision, recoveries, and limits of detection were investigated. The results are listed in Table 4. Calibration of vinclozolin was obtained with GC-ECD following the proposed extraction procedure using the optimal conditions with each set of extracts. Matrix matched calibration curve was performed in blank sediment extracts to check any difference in sensitivity. The latter calibration curve was used for the validation experiments and quantification in sediment samples. Calibration curves were recorded with five replicates and the LPME procedure showed a good linear behavior in the tested range, with correlation coefficient of 0.993.

The repeatability study was performed by extracting sediment extracts obtained from sediments spiked at 100 ng/g (six replicates). The R.S.D. was calculated to be 6.1%

The proposed microextraction method was used for the determination of vinclozolin in sediment extracts obtained from sediments spiked at 100 and 250 ng/g concentration levels. The total relative recovery of the method, including sediment extraction, LPME extraction, transfer to GC and GC analysis was better than 72% for the target andiandrogen compound and the R.S.D. values were ranged between 5.5 and 6.7%.

In general, precision in the case of sediment samples was similar with that of water samples. This means that deviations come mainly from the application of LPME itself and from the extraction of the sediment samples, since as mentioned

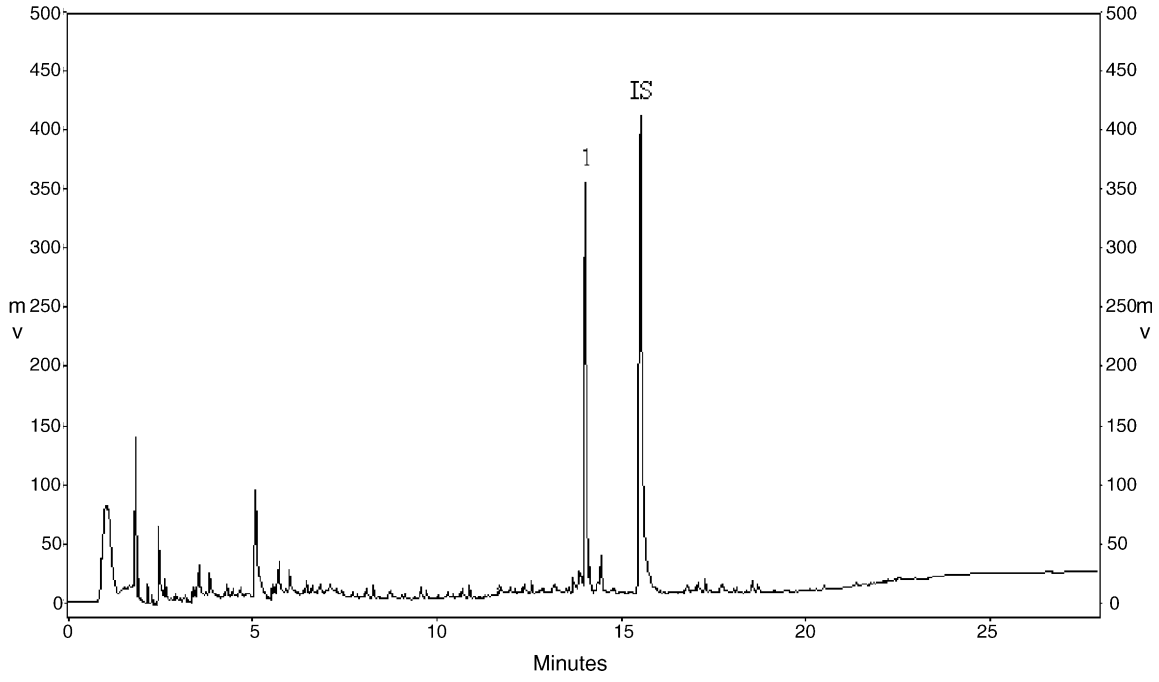

Fig. 6. GC/ECD chromatogram of vinclozolin obtained from LPME analysis in spiked river water sample at concentration level of 1 μg/L. [Vinclozolin, Sea-nine 211 (IS)].

before the matrix complexity and interference of sediment extracts had negligible effect on the extraction efficiency of vinclozolin. The obtained precision values are better or similar to other values present in the literature for the determination of vinclozolin in solid samples using other extraction techniques [18].

The combination of these methodologies appears as a good alternative approach in sediment analysis since limit the volume of organic solvent which is required for all extraction steps at 10 ml approximately and increase greatly the enrichment factor of the andiandrogen compound in the solvent drop which is finally introduced in the GC system. In addition to enrichment, hollow-fiber LPME also served as a technique for sample clean-up because of the selectivity of the membrane, which prevented large molecules and extraneous materials, such as humic acids, hydrocarbons and inorganic material in solution, from being extracted into organic solvent.

Fig. 6 shows a representative chromatogram corresponding to the HF-LPME-GC-ECD analysis from a sediment organic extract spiked at 100 ng/g level in the sediment sample.

4. Conclusions

The results of this study demonstrate that a selective trace enrichment of environmental andiandrogen vinclozolin from natural water samples can be achieved by hollow fiber LPME method. The newly developed microextraction technique has distinct advantages over conventional methods with respect to extraction time and volume of solvents required, where a high level of precision and detection limits are readily achieved, exceeding the requirement for vinclozolin analysis in aqueous samples.

Sample preparation methodology for sediment samples with the combination of LLE followed by the optimized hollow-fiber LPME technique, is highly efficient and the recommended methodology is a selective tool for the separation and enrichment of vinclozolin from sediments. The small pore size allows the hollow fiber to function as a filter that prevents larger molecules and interfering compounds in matrixes to be extracted into the organic solvent. Based on this consideration, the LPME method is not only a good sample enrichment technique but also a sample clean-up procedure, which makes it applicable in complex matrices such as sediments extracts. The recommended procedure (LLE/hollow-fiber LPME) proved to be a promising analytical tool and could be effectively applied to other solid environmental matrices, regarding the good accuracy and selectivity.

References

[1] T. Colbom, F.S. vom Saal, A.M. Soto, Environ. Health Perspect. 101 (1993) 378.
[2] P. Preziosi, Pure Appl. Chem. 70 (1998) 1617.
[3] L.E. Gray Jr., J.M. Ostby, W.R. Kelce, Toxicol. Appl. Pharmacol. 129 (1994) 46.
[4] W.R. Kelce, C.R. Lambright, L.E. Gray Jr., K.P. Roberts, Toxicol. Appl. Pharm. 142 (1997) 192.
[5] C.J. Wolf, G.A. LeBlanc, J.S. Ostby, L.E. Gray Jr., Toxicol. Sci. 55 (2000) 152.
[6] C. Wong, W.R. Kelce, M. Sar, E.M. Wilson, J. Biol. Chem. 270 (1995) 1998.
[7] P. Hrelia, C. Fimognari, F. Maffei, F. Vigagni, R. Mesirca, L. Pozzetti, G. Cantelli Forti, Mutagenesis 11 (1996) 445.
[8] V. Stadie, Umweltmedizin 17 (1997) 84.
[9] C.G. Smith, Am. J. Ind. Med. 4 (1983) 107.
[10] J. Ostby, W.R. Kelce, C. Lambright, C.J. Wolf, P. Mann, L.E. Gray, Toxicol. Ind. Health 15 (1999) 80.
[11] J.W. Readman, T.A. Albanis, D. Barcelo, S. Galassi, J. Tronczynski, G.P. Gabrielides, Marine Poll. Bull. 34 (1997) 259.
[12] L. Brossa, R.M. Marcé, F. Borrull, E. Pocurull, J. Chromatogr. A 998 (2003) 41.
[13] A. Balinova, Anal. Chim. Acta 311 (1995) 423.
[14] D.S. Lough, S. Moltagh, J. Pawliszyn, Anal. Chem. 64 (1992) 1187.
[15] J. Pawliszyn, Applications of Solid Phase Microextraction, Royal Society of Chemistry, Hertfordshire, 1999.
[16] D.A. Lambropoulou, I.K. Konstantinou, T.A. Albanis, J. Chromatogr. A 893 (2000) 143.
[17] D.A. Lambropoulou, V.A. Sakkas, D.G. Hela, T.A. Albanis, J. Chromatogr. A 963 (2002) 107.
[18] D.A. Lambropoulou, T.A. Albanis, Anal. Chim. Acta 514 (2004) 125.
[19] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 68 (1997) 2935.
[20] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 235.
[21] M.H. Ma, F.F. Cantwell, Anal. Chem. 71 (1999) 388.
[22] E. Psillakis, N. Kalogerakis, J. Chromatogr. A 999 (2003) 145.
[23] E. Psillakis, N. Kalogerakis, Trends Anal. Chem. 21 (2002) 53.
[24] L. Zhao, H.K. Lee, Anal. Chem. 74 (2002) 2486.
[25] G. Shen, H.K. Lee, Anal. Chem. 74 (2002) 648.
[26] Ch. Basheer, V. Suresh, R. Renu, H.K. Lee, J. Chromatogr. A 1033 (2004) 213.
[27] S. Muller, M. Moder, S. Schrader, P. Popp, J. Chromatorgr. A 985 (2003) 99.
[28] C. Basheer, H.K. Lee, J.P. Obbard, J. Chromatogr. A 968 (2002) 191.
[29] Ch. Basheer, R. Balasubramanian, H.K. Lee, J. Chromatogr. A 1016 (2003) 11.
[30] Y. He, H.K. Lee, Anal. Chem. 69 (1997) 4634.
[31] J.S. Salau, R. Alonso, G. Batllo, D. Barceló, Anal. Chim. Acta 293 (1994) 109.
[32] D. Lambropoulou, E. Psillakis, T. Albanis, N. Kalogerakis, Anal. Chim. Acta 516 (2004) 205.
[33] D.A. Lambropoulou, V.A. Sakkas, T.A. Albanis, Anal. Bioanal. Chem. 963 (2002) 107.
[34] T.A. Albanis, D.A. Lambropoulou, V.A. Sakkas, I.K. Konstantinou, Chemosphere 48 (2002) 475.
[35] D.A. Lambropoulou, V.A. Sakkas, T.A. Albanis, J. Chromatogr. A 1010 (2003) 1.
[36] F. Hernandez, J. Beltran, F.J. Lopez, J.V. Gaspar, Anal. Chem. 72 (2000) 2313.