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Solid-phase microextraction coupled with high performance liquid chromatography using on-line diode-array and electrochemical detection for the determination of fenitrothion and its main metabolites in environmental water samples

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Abstract

The aim of this study was to develop a methodology for the analysis of the insecticide fenitrothion and its two main environmental metabolites, fenitrooxon and 3-methyl-4-nitrophenol. For this purpose, a solid-phase microextraction (SPME) method coupled to high performance liquid chromatography (LC) was optimized. Two on-line detectors, diode array (DAD) and direct current amperometrical (DCAD) were used in order to determine sensitivity and selectivity. The effects of the extraction parameters, including exposure and desorption time, pH, temperature, salt concentration and desorption mode on the extraction efficiency were studied. A satisfactory reproducibility for extractions from samples at 20 ppb-level with RSD < 12.5% ($n = 10$) was obtained. The calibration graphs were linear in the range of 10–1000 $\mu g\,l^{-1}$ and detection limits for the target compounds were between 1.2 and 11.8 $\mu g\,l^{-1}$ depending on which detector was used. The method was applied for determining fenitrothion and both its metabolites in river waters which run through forest areas near to aerial application of the pesticide.

Keywords: SPME–HPLC; Fenitrothion; Pesticides; Waters

1. Introduction

Due to the indiscriminate use of pesticides, serious problems in the environment are emerging and these are an important risk to human health. It has been confirmed that the most serious cause of groundwater pollution in European countries is due to agricultural chemicals.

Hence, an increasing number of analytical methods are being developed to detect the presence of those compounds and their degradation products, some of which are more toxic that the original pesticide.

Fenitrothion is widely used as an organophosphorus insecticide. The level of global production is unknown. However, the global manufacturing capacity has been estimated to be between 15,000 and 20,000 tonnes [1]. Fenitrothion is mainly used in agriculture for controlling chewing and sucking insects on rice, cereals, fruits, vegetables, stored grains, and cotton. It also used for the control of flies, mosquitoes and cockroaches in public health programmes and/or indoor use. In rainy forest areas it is applied in the pest control of *Thaumetopea pityocampa* (pine processionary caterpillar) and *Eriosoma lanigerum* (affecting black poplar forestry populations).

Fenitrothion is in widespread use because of its relatively rapid decomposition and low accumulation in the biological food chain. Fenitrothion (FNT) is degraded in the environment mainly through hydrolysis and photodegradation, producing as major metabolites 3-methyl-4-nitrophenol (MNP) and fenitrooxon (FNO) [2]. FNT and its oxygenated analogue FNO, contain the usual toxicities found in organophosphate pesticides, while recently MNP has been identified in the OECD, (Organisation for Economic Co-operation and Development) HPV (High Production Volume) Chemical

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Programme as a potential risk to humankind due to its genotoxicity and thus presumed carcinogenicity [3].

These degradation compounds are partially soluble in water and they can also enter the environment. For this reason there is a need to determine low levels of FNT and FNO and trace levels of MNP in water.

Routine methods used in pesticide residue analysis are often time and solvent consuming due to the steps involved in sample preparation before chromatographic analysis, although modern trends in analytical chemistry have led to the simplification and increasing automation of preliminary analytical operations, particularly as regards extraction steps [4,5].

The most frequently used methods to determine this pesticide are gas chromatography (GC) with nitrogen-phosphorus [6], electron-capture [7] or mass spectrometric [8] detection and liquid chromatography (LC) with diode-array [9] (DAD) or electrochemical [10] detectors (ED). These methods require previous extraction and cleaning stages of the sample which are usually based mainly on supercritical fluid extraction (SFE), solid-phase extraction (SPE) and liquid–liquid extraction (LLE) [11,12].

Solid-phase microextraction (SPME), due to its simple, fast, and solvent-less features, has received growing interest from many areas [13,14], including its applications in pesticide analysis [15]. SPME allows the simultaneous extraction and preconcentration of analytes from a sample matrix [16]. The analytes are extracted by adsorption over the fiber, which is directly exposed to the samples or to the headspace. Finally for SPME–GC the fiber is placed in the hot injector of the gas chromatograph, where the analytes are thermally desorbed.

In SPME–GC analysis, thermal desorption at high temperature creates practical problems such as degradation of the polymer, and furthermore, many non-volatile compounds cannot be completely desorbed from the fiber. Solvent desorption is thus proposed as an alternative method of SPME–HPLC coupling. An organic solvent (static desorption mode) or the mobile phase (dynamic mode) is used to desorb the analytes from the SPME fiber.

Based on our own survey of the literature, the insecticide FNT and some of its metabolites have been extracted by SPME–GC from water [17–19], from different fruits [17], and vegetables [20], and also from must and wine [21]. However, to date, there is no publication about SPME coupled with HPLC for the simultaneous determination of FNT, FNO and MNP.

Furthermore, the expected concentrations of these compounds in natural water, after the spray application of fenitrotion in nearby forest areas, are very low. Some studies [22] have estimated that concentrations of FNT in lotic (flowing) aquatic systems after operational spraying have ranged from 1.3 to 127 $\mu g\,l^{-1}$, and usually declined to less than 1.0 $\mu g\,l^{-1}$ within 24–48 h. Maximum concentrations of FNT in lentic (standing water) systems have usually occurred within 2 h of the start of operational spraying and have ranged from 0.38 to 2500 $\mu g\,l^{-1}$. The necessary sensitivity at these levels of concentration may be reached, as will be shown later, through SPME/HPLC–DAD for the compounds FNT and FNO, but not for MNP, the expected concentrations of which are even lower. Thus, it is necessary to use an on-line electrochemical detector which enables the detection threshold to be lowered [10,23].

The present study is undertaken to establish a suitable and sensitive method for the simultaneous determination of the insecticide and its major metabolites in a water matrix. This research is focused on the application of the SPME technique coupled with HPLC. The combination of the DCAD with DAD enables the combining of the advantages of both detection systems and it can result in a good analytical method for carrying out environmental studies which monitor the impact of FNT after aerial or spray applications.

2. Experimental

2.1. Instrumentation

The isocratic LC system used was a Hewlett-Packard (Palo Alto, CA, USA) series 1050 pump. The DAD was a Hewlett-Packard Model 1040. An EG&G model 400 (Princeton Applied Research, Oak Ridge, USA) direct current amperometrical detector (DCAD) in a thin-layer configuration, equipped with a glassy carbon electrode and a Ag/AgCl reference electrode was used. The chromatograms were recorded with a 714 IC-metrodata workstation (Metrohm, Herisau, SW).

The connection between the two detectors is accomplished by connecting the exit of the optical cell of the DAD with the entry of the electrochemical cell of the DCAD with a stainless steel tube of 0.25 mm I.D. and 10.0 cm of length. It must be guaranteed that the internal pressure of the system does not exceed 24.1 MPa in order to avoid the breaking of the quartz cell of the DAD.

SPME was performed with commercially available polymeric coated fibers and housed in the appropriated manual holder (Supelco, Bellefonte, PA, USA). For magnetic stirring, 4-ml US Environmental Protection Agency (EPA) screw-cap vials supplied with a PTFE-lined septum (Kimble Glass, Vineland, NJ, USA) and a 0.51 cm stir bar were used. The magnetic stirrer was a Metrohm 728 Model.

The SPME-interface for HPLC (Cat. No. 57350-U Supelco, Bellefonte, PA, USA) includes a desorption chamber and a six-port Valco valve (Valco Instruments, Houston, TX, USA).

2.2. Reagents

All the solvents used in this study were HPLC grade and tested for spectral purity: acetonitrile (Scharlau, Barcelona, Spain), methanol (Merck, Darmstadt, Germany) and 2-propanol (Riedel de Häen, Seelze, Germany). LC-grade water

was prepared by purifying demineralized water in a Milli-Q water filtration system (Millipore, Milford, MA, USA). All solvent and samples were filtered though a 0.22 μm Millipore membrane filters type GVWP and mobile phases were degassed by a Selecta Ultrasounds System (Selecta, Barcelona, Spain) and with helium before utilization.

The supporting electrolyte used in the mobile phase was prepared by mixing 5×10^{-4} mol l^{-1} citric acid and 1×10^{-3} mol l^{-1} disodium hydrogenophosphate to yield the desired pH, both chemicals grade (Merck, Darmstadt, Germany).

FNT and MNP standards were supplied by Dr. Ehrenstorfer (Augsburg, Germany) with a certified purity higher than 99.0%. FNO standard was supplied by ChemService (West Chester, PA, USA).

The river water samples used in the matrix effect study were obtained at three sampling points, situated in areas away from centers of urban or industrial contamination and close to forest areas which had been treated with FNT: Pedroso river ($x = 485488.23$, $y = 4666585.31$, Burgos, Spain), Zeberio river ($x = 512388.33$, $y = 4777402.87$, Vizcaya, Spain) and Cadagua river ($x = 502924.06$, $y = 4775092.33$, Vizcaya, Spain).

2.3. Preparation of the standards and the spiked water samples

The standard certified of FNO contained 100 mg l^{-1} in hexane, and the standards of FNT and MNP were used to prepare a 10 ml stock solution containing 1000 mg l^{-1} of each analyte in methanol. The stock solutions were preserved at −42 °C in a freezer. A stock mixed standard solution of 10 mg l^{-1} was prepared weekly. The stock mixed standard was diluted daily to the required concentration.

A set of calibration of seven standards was prepared at concentrations from 0.01 to 1.0 mg l^{-1} by diluting the stock mixed standard in water. Stock and working mixed standard were preserved at 4 °C in a refrigerator.

Spiked water samples for the extraction procedure study were prepared by adding an appropriate amount of a working standard solution to 3 ml of river water. The spiked samples were stirred and left to stand for 15 min to allow the solution to stabilize. All the analytical measurements were carried out in triplicate.

2.4. Chromatographic conditions

The column was a Spherisorb ODS2 (150 mm × 4.1 mm, 5 μm) with a guard column (10 mm × 4 mm) from Tracer (Barcelona, Spain). The mobile phase was acetonitrile/citric acid–disodium hydrogenophosphate buffer (50:50) pH 6.5, at a flow rate of 1.0 ml min^{-1}. It was chosen to provide good chromatographic separation and the ionic strength required by electrochemical detection. All analytes were eluted in less than 10 min and detected with good peak resolution when the appropriate wavelength was chosen.

2.5. Conditioning of fibers for HPLC use, and SPME procedure

A previous stage of fiber conditioning is necessary to ensure good selectivity and sensitivity results. The best results are obtained through conditioning the fiber with the mobile phase and then with the solvent to which it will be exposed. Then, the SPME fibers were conditioned and placed in the desorption chamber allowing the mobile phase to pass through the interface (dynamic mode) for 40 min and secondly were immersed in water with magnetic stirring for 10 min.

The SPME step was carried out by introducing 3 ml of aqueous samples into 4-ml screw-cap vials. The water samples contained 150 g l^{-1} of Na_2SO_4. The samples were stirred with a magnetic stirrer before and during extraction. The fiber was immersed in the sample for an appropriate time period at selected temperature. Then, the fiber was withdrawn into the needle, the needle was removed from the septum and was introduced into the chromatographic system by the SPME–HPLC interface. The analytes were desorbed from fiber by dynamic mode.

As a precautionary measure, as the fiber was desorbed in organic solvents and buffers, it was cleaned with water for 5 min and dried for two minutes prior to starting the next extraction.

3. Results and discussion

3.1. Detection conditions

Under the above-described chromatographic conditions, all the analytes were simultaneously determined by DAD at 200 nm, except FNO, which was detected at 275 nm (its secondary maxima of absorbance) in order to avoid interferences which coelute at this retention time.

The electrochemical detection of MNP was in DC mode. Oxidation of the phenolic groups on carbon electrodes in the presence of nucleophiles such as water, have been studied sufficiently [24]. In the majority of cases a cetonic group is generated in a reaction involving 1 H^+ and $2e^-$.

Hydrodynamic voltammograms were used to establish the optimum pH and potential conditions for the MNP electrochemical determination. The graphs were derived by injecting 20 ng of MNP in mobile phase and varying the potential between 0.8 and 1.5 V and the pH between 5 and 7. As can be seen in Fig. 1, the most suitable pH for its determination was 6.5, using an oxidation potential of 1.2 V. At pH 5.0 similar levels are reached, but in this case the oxidation potential is higher, causing an increase in background noise and a loss of peak resolution.

The effect of the buffer molarity on the electrochemical response of the DCAD was examined, using citrate/phosphate buffer ranging from 10^{-4} to 5×10^{-2} M. It was observed that peak height increases when the ionic

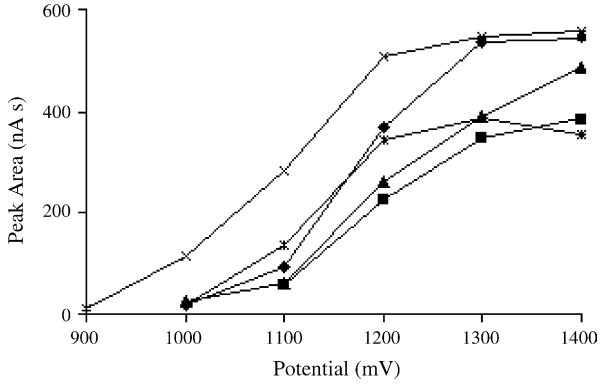

Fig. 1. Study of influence of pH and potential by hydrodynamic voltammogram of MNP. Amount injected: 20 ng; injection volume: 20 μl; flow rate: 1.0 ml min^{-1} acetonitrile/citrate–disodium hydrogen phosphate buffer (50:50): (♦) pH 5, (■) pH 5.5, (▲) pH 6, (⨯) pH 6.5, (*) pH 7.

strength increases, but an increase of background was also observed. On the other hand ionic strengths in the mobile phase of more than 5×10^{-2} M, generate considerable increases of pressure in the chromatographic system, which is particularly noted in the HPLC-SPME interface, its weakest point. A 10^{-3} mol l^{-1} concentration was selected as an optimum value of the buffer molarity thereby guaranteeing a stable measurement and an appropriate pressure in interface.

Periodically, after every 10 determinations, a sweeping of between −1 and 1 V with a scan rate of 200 mV s^{-1} was applied, in order to clean the electrode surface of possible adsorbed species. This has enabled the electrodic response to be maintained at high levels of reproducibility, without the loss of sensitivity over time.

3.2. Selection of adequate fiber coating

A preliminary and qualitative assay was performed in order to evaluate the available fibers carbowax-templated resin (CW-TPR), polydimethylsiloxane-divinylbencene (PDMS-DVB) and polyacrylate (PA) coated fibers. To select the best fiber coating, the extraction was performed in 60 min and desorption was carried out in dynamic mode during 5 min. We found that with the most polar CW-TPR fiber there was no signal for FNO. On the other hand, with the less polar PA fiber only there was a signal for FNT. So, PDMS-DVB fibers were chosen as an extraction coating due to the good results obtained in the aforementioned assay.

3.3. Optimization of desorption process

Two modes of desorption were evaluated for PDMS-DVB fiber: dynamic and static desorption with samples containing 0.5 mg l^{-1} of each analyte. To compare both modes of desorption an extraction time of 45 min and ambient temperature were initially selected for the extraction step.

In static mode, several trials were carried out with different solvents for each desired period of time (2–10 min). Static desorption using acetonitrile, water and mobile phase were evaluated by the addition of 500 μl of each into the desorption chamber with a luer-tipped glass syringe. All trials carried out presented carry-over, thereby revealing the static desorption system to be somewhat inefficient for this study.

In the dynamic mode, after the extraction process, the SPME fiber was placed into the desorption chamber and the valve was immediately switched from the load to the inject position and the mobile phase at 1 ml min^{-1} was passed though the desorption chamber for a time ranging from 2 to 7 min in order for complete dynamic desorption to be carried out. A desorption time of 5 min was selected, as after this period of time peak areas did not increase significantly and in a subsequent analysis no peaks appeared at the retention time of the analytes, neither was there any evidence of carry-over. After 5 min, the fiber could be removed from the desorption chamber and prepared for a further extraction.

3.4. Optimization of the extraction process

The efficiency of analyte extraction by SPME can vary widely depending upon the matrix influence [25], the appropriate time period for the extraction [26], the temperature of absorption, the addition of salt to the sample and/or the pH of the sample [27,28]. To optimize the extraction process, the water samples were spiked containing 0.5 mg l^{-1} in each compound and the desorption parameters were fixed at the previously optimized values.

To ensure extraction efficiency of analytes from a sample, one of the important steps in the development of a SPME method is to determine the time needed before an extraction process reaches equilibrium between the sample matrix and the coating of a fiber. Triplicate water samples containing all the analytes were extracted with PDMS-DVB fiber for periods of time ranging from 10 to 150 min. Fig. 2 shows the extraction time profiles obtained for the analytes. For routine analysis it is not necessary to reach a complete equilibrium as long as the exposure time of the fiber is kept exactly constant [27]. With 60 min, the obtained response for all of them exhibited good reproducibility and it was considered suitable for

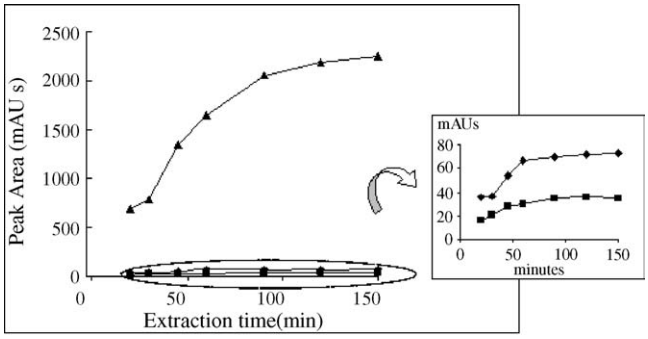

Fig. 2. The effect of the equilibrium time on the chromatographic response. The fiber was exposed to samples containing 0.5 mg l^{-1} of each compound. Each point is the average of the three data points: (♦) MNP, (■) FNO, (▲) FNT.

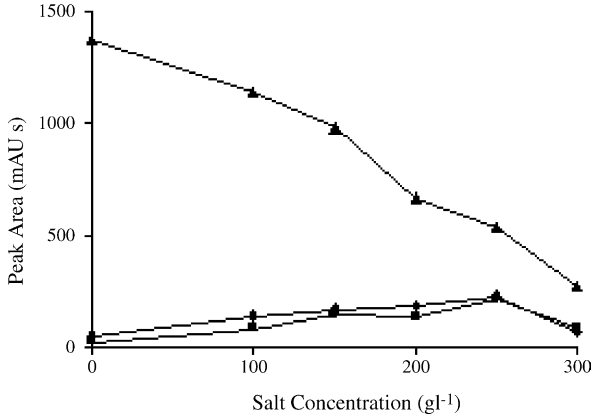

Fig. 3. The effect of salt addition on extraction efficiency. The fiber was exposed for 60 min to samples containing 0.5 mg l^{-1} of each compound. Each point is the average of the three data points: (♦) MNP, (■) FNO, (▲) FNT.

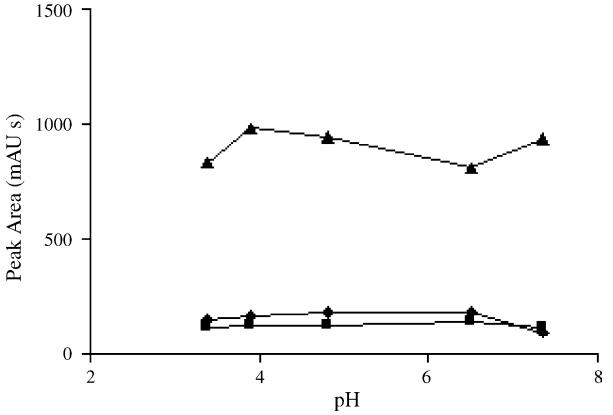

Fig. 4. The effect of pH on extraction efficiency. The fiber was exposed for 60 min to samples containing 0.5 mg l^{-1} of each compound. Each point is the average of the three data points: (♦) MNP, (■) FNO, (▲) FNT.

the determination of trace levels of FNT and metabolites in water. So therefore an extraction time of 60 min was selected although at this time equilibrium concentrations were not yet achieved for FNT.

Three different extraction temperatures, room temperature (21 ± 1 °C), 45 and 80 °C, were explored. The increase in extraction efficiency as temperature varies is negligible for the three compounds (1.9–4.3% at 80 °C) and moreover the analysis time increases as it is necessary to reach a thermal equilibrium. It was therefore decided to carry out all the measurements at room temperature.

Another extraction parameter which has a well-established effect in conventional extraction methods is the salting out effect obtained by adding ionic salts to the water sample. This effect has also been studied in SPME applications mainly by the addition of NaCl [29] and alternatively divalent salts such as Na_2SO_4 [30] or $MgSO_4$ [11]. A preliminary comparative study between NaCl and Na_2SO_4 was carried out. Although the addition of NaCl caused fewer problems on fiber cleaner process, the results obtained with NaCl were not satisfactory as only negligible increases in extraction efficiency were obtained. Otherwise the extraction efficiency was highly affected by Na_2SO_4 addition. Na_2SO_4 concentrations ranging from 0 to 30% (w/v) were studied. Area peaks increase when Na_2SO_4 is added even at 25%, except for FNT which is negatively affected because its area decreases dramatically. Fig. 3 shows the effect of salt addition on extraction efficiency. A compromise percentage of Na_2SO_4 was selected for further studies, 15% (150 g l^{-1}) was added to water samples.

The effect of pH was evaluated by mixing 0.1 M citric acid and 0.2 M disodium hydrogenophosphate to yield the desired pH. pH ranged between 3 and 8 was studied by addition of 50 μl of buffer mixes to water samples with 15% of Na_2SO_4. As can be seen in Fig. 4 no significative changes in the amount extracted were observed. The extraction process in water was carried out at sample pH.

The effect of organic modifier solvent content of the sample on absorption was studied by preparing a set of samples that contained methanol at concentration from 0 to 10%. In concordance to results obtained by others authors [30], the results obtained (Fig. 5) reveal that an increase in methanol concentration involves a fall in the extracting efficiency. The effect of another organic modifier solvent such as acetonitrile was also tested: in most cases, the extraction efficiencies decreased when acetonitrile was present in solutions. An

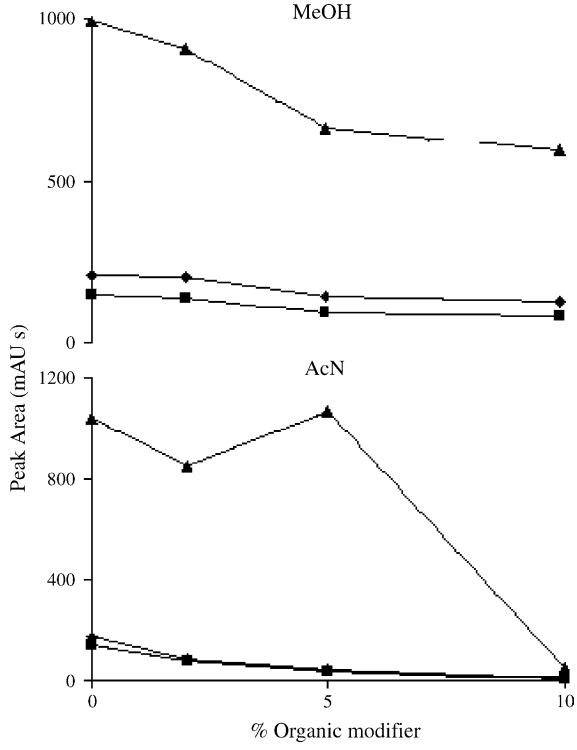

Fig. 5. Influence of the acetonitrile and metanol organic modifiers in SPME efficiency. The fiber was exposed for 60 min to samples containing 0.5 mg l^{-1} of each compound. Each point is the average of the three data points: (♦) MNP, (■) FNO, (▲) FNT.

Table 1
Linear regression analysis, detection limits and intra/inter-day relative standard deviations at two different levels of concentration of SPME/HPLC–DAD–DCAD determinations

DAD	Slope (mAU s/mg l^{-1})	Intercept (mAU s)	Correlation coefficients (r)	LOD ($\mu g\,l^{-1}$)	Intra-day RSD (%, $n = 10$)		Inter-day RSD (%, $n = 10$)	
					0.02 mg l^{-1}	0.1 mg l^{-1}	0.02 mg l^{-1}	0.1 mg l^{-1}
MNP	319.9 ± 19.2	6.2 ± 9.8	0.9950	11.8	6.05	4.09	7.34	4.88
FNO	249.3 ± 5.2	9.1 ± 2.4	0.9991	1.6	6.90	5.39	12.50	11.90
FNT	2304.9 ± 107.1	25.8 ± 49.9	0.9957	1.4	9.82	8.21	12.20	9.51
DCAD	Slope (nA s/mg l^{-1})	Intercept (nA s)	Correlation coefficient (r)	LOD ($\mu g\,l^{-1}$)	Intra-day RSD (%, $n = 10$)		Inter-day RSD (%, $n = 10$)	
					0.02 mg l^{-1}	0.1 mg l^{-1}	0.02 mg l^{-1}	0.1 mg l^{-1}
MNP	1301.3 ± 26.1	−6.3 ± 11.2	0.9992	1.2	7.50	3.21	8.12	9.49

increasing proportion of organic solvent in aqueous solution decreases the polarity of the aqueous sample, so the distribution constant decreases [31].

3.5. Detection limits, precision and linearity

The linearity correlation coefficients, precision data (RSDs), and method detections limits (LODs) are shown in Table 1. The linearity of this method for analyzing standard solution has been investigated over the range 0.01–1 mg l^{-1} at DAD and 0.005–1 mg l^{-1} at DCAD. All the correlation coefficients were better than 0.9950. The calculation of the detection limits was based on a 2 N/m ratio, where N is the noise and m is the slope of the respective calibration equation. As can be observed in Table 1, the limits of detection (LODs) vary from 1.4 to 11.8 $\mu g\,l^{-1}$ at the DAD detector. The MNP limit of detection at DCAD is 1.2 $\mu g\,l^{-1}$ (approximately ten times less than that obtained in the DAD).

With the aim of testing the precision of this SPME method, the intra-day relative standard deviations (RSDs) were determined by performing ten consecutive extractions at two levels of concentration under the selected conditions. The same standards were also analyzed at intervals over a 2-week period ($n = 10$) in order to determine the inter-day RSDs. The RSD values obtained shown in Table 1 can be deemed excellent compared to the values usually obtained with SPME–HPLC methods.

3.6. Matrix effect study

SPME–HPLC with on-line DAD and DCAD detection was applied to the determination of FNT and metabolites in river water to confirm its practicability and feasibility for the analysis of these analytes in river water samples. The river samples used in this study present no analytes peaks or interference in the chromatograms. Different concentrations of FNT and metabolites were then spiked into the river samples to investigate the matrix effect on this method. The recoveries of these analytes were hardly affected by the matrix of river water. The slope of the calibrated straight line in river water does not vary more than 5% compared to those obtained with deionized water. Therefore, a calibration curve based on simple aqueous standards can be used for other river samples.

The effectiveness of the proposed method for the determination of FNT and metabolites in river samples was tested by performing replicate analyses of three different samples of

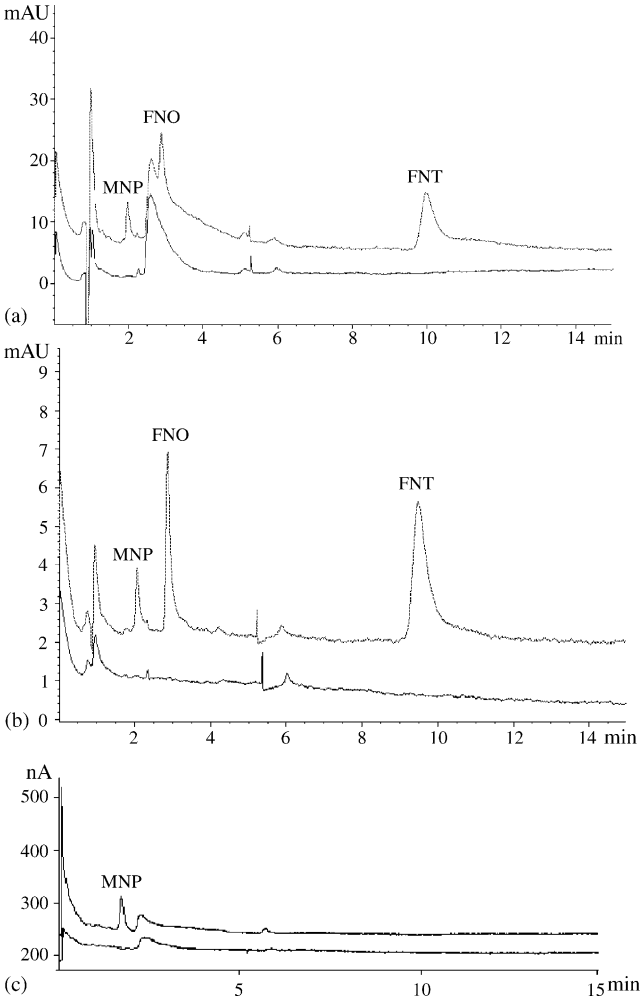

Fig. 6. SPME–HPLC chromatograms of a river water sample (Zeberio river). Normal lines: un-spiked sample, dotted lines: spiked samples containing 0.1 mg l^{-1}: (a) HPLC–DAD (λ = 200 nm); (b) HPLC–DAD (λ = 275 nm); (c) HPLC–DCAD (E = 1200 mV).

river samples (Zeberio river, Pedroso river and Cadagua river) all of them located in the North of Spain. The samples were taken at periods of 24, 48 and 168 h after the aerial application of FNT on nearby forest areas. The method of aerial pesticide application has been outlined previously [32]. As target analytes were not found in these river samples, triplicate aliquots of each sample were artificially spiked with two levels of concentration (25 and 100 $\mu g\,l^{-1}$) and subsequently analyzed using the proposed SPME–HPLC/DAD–DCAD method with the PDMS-DVB fiber. The average concentrations obtained in the analysis of these spiked samples with a 25 $\mu g\,l^{-1}$ correspond to mean recoveries ranging from 93.7 ± 9.9 to 108.2 ± 4.2% and the average concentrations obtained in the analysis of spiked samples with a 100 $\mu g\,l^{-1}$ correspond to mean recoveries ranging from 92.5 ± 2.8 to 104.1 ± 9.5% for a significance level of 0.05 in DAD detector. Using DCAD the average concentrations obtained in spiked samples with 25 $\mu g\,l^{-1}$ of MNP was 102.9 ± 8.1 and in spiked samples with 100 $\mu g\,l^{-1}$ was 97.1 ± 7.1%.

Fig. 6 shows the chromatograms of a river sample in which FNT, FNO and MNP were added at ppb levels.

4. Conclusions

A method for the determination of trace FNT and its main metabolites in aqueous solution by HPLC with on-line DAD and DCAD detection was developed. When SPME is combined with HPLC/DAD–DCAD, the overall sensitivity is greatly enhanced allowing for FNT and metabolites at very low levels to be easily analyzed. The technique developed in this study has been applied toward the quantification of these compounds in water from rivers which flow near to forest areas which have been treated with the pesticide. The presented experimental results clearly demonstrate that the combination of SPME with HPLC/DAD–DCAD can achieve low LODs, and be applied to determine target analytes in real samples.

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References

[1] International Program on Chemical Safety (ICPS-INCHEM), Report EHC 113: Fenitrothion. United Nations Environment Programme, Geneve, 1992.
[2] N. Mikami, K. Imanishi, H. Yamada, J. Miyamoto, Nippon Noyaku Gakkaishi 10 (1985) 263.
[3] Organisation for Economic Co-operation and Development, OECD, HPV Chemicals Programme, Draft initial hazard assessment of 3-Methyl-4-nitrophenol, Paris, 1994.
[4] J. Cook, M. Engel, P. Wylie, B. Quimby, J. AOAC Int. 82 (1999) 313.
[5] J. Sherma, J. AOAC Int. 82 (1999) 561.
[6] A. Prieto, G. Ettiene, D. Medina, I. Buscema, G. Gonzalez, L. Araujo, Food Addit. Contam. 16 (1999) 57.
[7] P.T. Holland, D.E. McNaughton, C.P. Malcolm, J. AOAC Int. 77 (1994) 79.
[8] M. Natangelo, S. Tavazzi, E. Benfenati, Anal. Lett. 35 (2002) 327.
[9] G.E. Miliadis, N.G. Tsiropoulos, P.G. Aplada-Sarlis, J. Chromatogr. A 835 (1999) 113.
[10] T. Galeano-Diaz, A. Guiberteau-Cabanillas, N. Mora-Diez, P. Parrilla-Vazquez, F. Salinas-Lopez, J. Agric. Food Chem. 48 (2000) 4508.
[11] M. Vitali, M. Guidotti, R. Giovinazao, O. Cedrone, Food Addit. Contam. 15 (1998) 280.
[12] G. Niessner, W. Buchberger, R. Eckerstorfer, J. Chromatogr. A 846 (1999) 341.
[13] H. Lord, J. Pawliszyn, J. Chromatogr. A 885 (2000) 153.
[14] J. Pawliszyn (Ed.), Applications of Solid Phase Microextraction, Royal Society of Chemistry, London, 1999.
[15] J. Beltran, F.J. Lopez, F. Hernandez, J. Chromatogr. A 885 (2000) 389.
[16] J. Dugay, C. Miege, M.C. Hennion, J. Chromatogr. A 795 (1998) 27.
[17] D.A. Lambropoulou, V.A. Sakkas, T.A. Albanis, Proceedings of the International Conference on Environmental Science and Technology, University of the Aegean, Department of Environmental Studies, Myrina, Lemnos Island, Greece, 2003, p. A523.
[18] H.M. Nguyen, H.V. Pham, W. Giger, M. Berg, Anal. Sci. 17 (2001) a375.
[19] D.A. Lambropoulou, T.A. Albanis, J. Chromatogr. A 922 (2001) 243.
[20] C.S. Tsoutsi, T.A. Albanis, Proceedings of the International Conference on Environmental Science and Technology, University of the Aegean, Department of Environmental Studies, Myrina, Lemnos Island, Greece, 2003, p. A911.
[21] C.G. Zambonin, M. Quinto, N. De Vietro, F. Palmisano, Food Chem. 86 (2004) 269.
[22] Pest Management Regulatory Agency Health Canada, Re-evaluation of Fenitrothion, Report PACR2003-08, Ontario, 2003.
[23] C. Garcia Pinto, J.L. Perez Pavon, B. Moreno Cordero, Anal. Chem. 67 (1995) 2606.
[24] M.D. Hawley, in: P.T. Kissinger, W.R. Heineman (Eds.), Laboratory Techniques in Electroanalytical Chemistry, Marcel Dekker, New York, 1996.
[25] T. Gorecki, A. Khaled, J. Pawliszyn, Analyst 123 (1998) 2819.
[26] M.C. Sampedro, O. Martin, C. Lopez de Armentia, M.A. Goicolea, E. Rodriguez, Z. Gomez de Balugera, J. Costa-Moreira, R.J. Barrio, J. Chromatogr. A 893 (2000) 347.
[27] D. Lambropoulou, T. Sakellarides, T. Albanis, Fresenius' J. Anal. Chem. 368 (2000) 616.
[28] A. Penalver, E. Pocurull, F. Borrull, R.M. Marce, J. Chromatogr. A 953 (2002) 79.
[29] S. Millan, M.C. Sampedro, N. Unceta, M.A. Goicolea, E. Rodriguez, R.J. Barrio, J. Chromatogr. A 995 (2003) 135.
[30] L. Cardenes, J.H. Ayala, A.M. Afonso, V. Gonzalez, J. Chromatogr. A 1030 (2004) 87.
[31] C.L. Arthur, L.M. Killam, K.D. Buchholz, J. Pawliszyn, J.R. Berg, Anal. Chem. 64 (1992) 1960.
[32] O. Baroja, N. Unceta, M.C. Sampedro, M.A. Goicolea, R.J. Barrio, J. Chromatogr. A 1059 (2004) 165.