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Multi-residue method for the determination of 16 recently used pesticides from various chemical groups in aqueous samples by using DI-SPME coupled with GC–MS

Maciej Tankiewicz [a,*], Calum Morrison [b], Marek Biziuk [a]

[a] Department of Analytical Chemistry, Faculty of Chemistry, Gdańsk University of Technology, ul. G. Narutowicza 11/12, 80-233 Gdańsk, Poland
[b] School of Science, University of the West of Scotland, Paisley Campus, Paisley PA1 2BE, UK

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ABSTRACT

A simple and solvent-free multi-residue method has been optimized to determine 16 currently used pesticides from different chemical groups in aqueous samples. The extraction of analytes was carried out with direct immersion solid-phase microextraction (DI-SPME) and for the identification and quantitative determination gas chromatography coupled with mass spectrometry (GC–MS) was applied. Two commonly used adsorbent coatings have been applied and compared: 100 μm of polydimethylsiloxane (PDMS) and 85 μm of polyacrylate (PA). The method development parameters of DI-SPME, analyte desorption and GC–MS analysis have been outlined along with the final experimental conditions. When the optimum extraction conditions were applied (extraction time 60 min, 10% (w/v) NaCl solution, 45 °C) the limits of detection (LODs) were in the range of 0.015–0.13 μg L^{-1} and the relative standard deviations (RSDs) were between 1.9 and 9.6%. The developed analytical method was successfully applied to the analysis of natural water samples from the following sources: river, sea, canal and rain.

1. Introduction

The wide and large number of pesticides applications has resulted in an extension beyond agricultural applications into many other parts of the environment [1]. These compounds pose a particular threat in natural waters and the various processes they undergo in the aquatic environment may cause them to be converted into substances of greater toxicity [2]. Despite numerous merits, pesticides may also be toxic, mobile and capable of bioaccumulation. These compounds have been detected in all types of water circulating in the ecosystem, creating a potential source of exposure to life and health of all living organisms. Therefore, constant monitoring of these xenobiotics in the environment is needed [3,4].

Pesticides may be present in the environment at low concentrations, in complex matrices, meaning sample preparation and analysis is time-consuming. In addition to national guidelines, international legal regulations imposed by the EU concerning the permissible level of pesticide residues in water [5], have driven the development and improvement of novel analytical techniques towards multi-residue analysis, low limits of detection and the use of small sample volumes. The improvements of existing techniques are aimed at miniaturization, automation, and the use of solvent-free techniques at the sample preparation stage, that are consistent with Green Chemistry principles [6].

Multi-residue methods have been proposed for the simultaneous determination of pesticides in water [7,8]. However, compounds to be determined are often present at low concentrations and have diverse physicochemical properties (e.g. polarity, solubility, volatility, and acidic/base characteristics) and illustrated in Table 1 [9]. Therefore extraction and analysis may be difficult [10].

Traditionally the developed analytical methods for the analysis of pesticides in water samples are based on liquid–liquid extraction (LLE) [11,12] and solid-phase extraction (SPE) [13,14], which require several steps and large quantities of solvents for sample preparation and extraction of the analytes. Recently, single-drop microextraction (SDME) [15,16] and solid-phase microextraction (SPME) [17,18] have been applied to the determination of pesticides in water. SDME is practically solvent free, using a single drop of organic solvent, but difficulties may be encountered: maintaining a stable organic drop, air bubble formation, long timescale for extraction equilibrium. SPME is a quick, universal technique, does not require the use of organic solvents and is applied in the determination of various classes of pesticides in aqueous media or in other samples [19]. It is sensitive and

* Corresponding author. Tel.: +48 58 347 21 10; fax: +48 58 347 26 94.
E-mail addresses: mtelk84@gmail.com, maciej.tankiewicz@gmail.com (M. Tankiewicz).

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Table 1
Basic properties of the pesticides selected for the study [9].

Compound	Chemical formula	Substance group	Action	Molecular mass [g mol^{-1}]	Solubility in water at 20 °C [mg L^{-1}]	log P[a] at pH 7, 20 °C	Monitored ions[b]
Fenoxaprop-ethyl	$C_{18}H_{16}ClNO_5$	Aryloxyphenoxypropionate	Herbicide	361.78	0.90	4.3	183, 168, 140
Tau-fluvalinate	$C_{26}H_{22}ClF_3N_2O_3$	Pyrethroid (isomer mix)	Insecticide, acaricide	502.90	0.0010	7.0	250, 206, 252
Fenpropidin	$C_{19}H_{31}N$	Unclassified	Fungicide	273.46	530	2.6	98, 99, 273
Malathion	$C_{10}H_{19}O_6PS_2$	Organophosphate	Insecticide, acaricide	330.36	148	2.8	173, 127, 93
Tetraconazole	$C_{13}H_{11}Cl_2F_4N_3O$	Triazole	Fungicide	372.15	157	3.6	336, 338, 159
Haloxyfop-R-methyl	$C_{16}H_{13}ClF_3NO_4$	Aryloxyphenoxypropionate	Herbicide	375.70	7.9	4.0	316, 288, 375
Profenofos	$C_{11}H_{15}BrClO_3PS$	Organophosphate	Insecticide, acaricide	373.63	28	1.7	208, 139, 337
Fluazifop-butyl	$C_{19}H_{20}F_3NO_4$	Aryloxyphenoxypropionate	Herbicide	383.36	1.0	4.5	282, 254, 383
Prothioconazole	$C_{14}H_{15}Cl_2N_3OS$	Triazolinthione	Fungicide	344.26	300	3.8	186, 188, 125
Cyproconazole	$C_{15}H_{18}ClN_3O$	Triazole	Fungicide	291.78	93	3.1	222, 139, 224
Carfentrazone-ethyl	$C_{13}H_{14}Cl_2F_3N_3O_3$	Triaolinone	Herbicide	412.19	22	3.4	312, 340, 330
Diclofop-methyl	$C_{16}H_{14}Cl_2O_4$	Aryloxyphenoxypropionate	Herbicide	341.19	0.39	4.8	253, 340, 120
Bifenthrin	$C_{23}H_{22}ClF_3O_2$	Pyrethroid	Insecticide, acaricide	422.88	0.0010	6.6	181, 165, 166
Metconazole	$C_{17}H_{22}ClN_3O$	Triazole	Fungicide	319.83	30	3.8	125, 83, 70
Pyriproxyfen	$C_{20}H_{19}NO_3$	Unclassified	Insecticide	321.37	0.37	5.4	136, 226, 96
Alpha-cypermethrin	$C_{22}H_{19}Cl_2NO_3$	Pyrethroid	Insecticide	416.30	0.0040	5.5	181, 163, 209

[a] Octanol–water partition coefficient at pH 7, 20 °C.
[b] Quantitation based on first m/z listed.

convenient for use in the field or laboratory since equilibrium is quickly attained by adjusting factors including: temperature, fiber type and exposure time, volume of sample, salt concentration, pH and agitation [20].

Common materials used for coating fibers include: polydimethylsiloxane (PDMS), polyacrylate (PA) and also mixtures of: polydimethylsiloxane and polydivinylbenzene (PDMS–DVB), carbowax and polydivinylbenzene (CW–DVB), polydivinylbenzene and carbowax and polydimethylsiloxane (DVB–CAR–PDMS), carbowax and molecularly imprinted resin (CW–TPR) [21–23]. The most popular fibers used for the extraction of pesticides from water samples are PDMS and PA [3,4].

Most pesticides are volatile and thermally stable, and therefore are amenable to gas chromatography (GC). In contrast to GC, procedures based on application of liquid chromatography (LC) technique have the advantage of being suitable for thermally unstable and polar/ionic pesticides. The detection by mass spectrometry (MS) is most suitable for multi-residue analysis of pesticides belonging to different chemical groups and when coupled to GC provide high selectivity and low levels of detection [18]. It can be used both to identify unknown substances and to determine just a few ions. Moreover is easier to acquire and use with comparison to tandem MS, therefore is preferable for pesticide analysis in water samples [24–29].

A weakness of SPME is that the commercially available fibers cover a small range of polarity, manifesting in a lack of selectivity of the extraction process. Recently there has been an increase in interest in the preparation of tailor-made fibers with the aim of providing certain selectivity to the extraction process [30]. However, most of these studies do not include analytical validation data for recently used pesticides from different chemical groups, relating mainly to individual groups of compounds.

The objective of this study was to develop and apply a multi-residue methodology for simultaneous extraction of 16 recently used pesticides from various chemical groups in water samples using direct immersion SPME followed by GC–MS analysis. Polydimethylsiloxane (PDMS) and polyacrylate (PA) fibers were compared. The pesticides selected for the study belong to the following chemical groups: aryloxyphenoxypropionates (fenoxaprop-ethyl, haloxyfop-R-methyl, fluazifop-butyl, diclofop-methyl), pyrethroids (tau-fluvalinate, bifenthrin, alpha-cypermethrin), organophosphates (malathion, profenofos), triazoles (tetraconazole, cyproconazole, metconazole), triazolinthione (prothioconazole), triaolinone (carfentrazone-ethyl) and unclassified (fenpropidin, pyriproxyfen). To the best of our knowledge, in the literature to date contains no previous works that report this approach. Table 1 shows the physicochemical characteristics and types of action of selected pesticides in this study. The analyte properties vary significantly and the developed multi-residue method for all of the studied pesticides incorporated significant method development in terms of extraction (time, temperature, agitation rate, and sample volume), and desorption in the gas chromatograph injector. The analytical procedure was validated and applied to natural water samples from river, sea, canal and rain collected around Paisley region, Scotland, UK. The developed analytical method here is a simpler, lower cost and less labor intensive sample preparation technique, than conventional techniques such as LLE and SPE for the simultaneous determination of pesticides in environmental samples by GC. The results show that the selectivity of developed methodology is sufficient to analyze environmental samples and can be used in monitoring studies to control the content of selected pesticides in water samples.

2. Experimental

2.1. Chemicals and reagents

Certified standards of tau-fluvalinate (isomer mix), fenpropidin, malathion, tetraconazole, profenofos, fluazifop-buthyl, cyproconazole, carfentrazone-ethyl, bifenthrin, metconazole, pyriproxyfen and alpha-cypermethrin were purchased from Ultra Scientific, Kingstown, USA. All standards were methanol solutions at the concentration 100 μg mL^{-1} and purities greater than 97%. Fenoxaprop-ethyl, haloxyfop-R-methyl, prothioconazole and diclofop-methyl were dissolved in acetonitrile at the same concentration level (purity 98.5%) and

acquired from Dr. Ehrenstorfer GmbH, Germany. The chemical formula, substance group, molecular mass and physicochemical properties of studied pesticides are presented in Table 1.

Methanol and acetonitrile were supplied by Fisher Scientific (Loughborough, UK). External standard used in this study was triphenylphosphate (TPP) at the concentration 500 μg mL^{-1} in MTBE (Supelco, Bellefonte, USA). Sodium chloride salt (99%) and potassium dihydrogen phosphate (99+%) were purchased from Sigma-Aldrich (Poole, UK). The reagents were analytical or higher grade and solvents were HPLC grade. The water used in this study was obtained from an ultrapure water purification system Elgastat, Elga Ltd. (Bucks, UK).

A standard stock solution of pesticides was prepared in methanol at the concentration 10 and 1 mg L^{-1} and stored at 4 °C. The standard aqueous samples were produced by spiking purified water with the standard solution at different concentration levels. The working standard solution was prepared by spiking purified water with the standard solution at 10 mg L^{-1} concentration level for each pesticides and stored at 4 °C. This standard was used both for matrix spike, in order to optimize the extraction conditions and in the validation study in different concentration levels from 0.05 to 200 ng mL^{-1}. The calibration standards at the concentrations 0.05; 0.1; 0.2; 2.0; 20; 100 and 200 ng mL^{-1} were prepared by dilution of the working standard directly into the matrix. Each aqueous solution (standard or real) was spiked by external standard TPP at a concentration of 10 ng mL^{-1}.

2.2. Water samples

The developed analytical procedure was tested using water from various sources (canal, sea and rain) to examine the applicability of the DI-SPME method for samples containing different values of the pH, content of salt and solid particles, etc. Water from a river was used in this study to calculate the recovery of analytes at two different concentration levels (10 μg L^{-1} and 100 μg L^{-1}) and check the impact of particulate matter on analysis results and SPME fiber. The higher value of concentration was dictated by the upper limit of linearity range of some studied pesticides. All sample collection points were situated on the central lowlands region of Scotland, UK. Water samples collected from different areas were analyzed in triplicate using the SPME optimized procedure and GC–MS. Based on the data from Science and Advise for Scottish Agriculture (SASA) organisation Coordinating the Agenda for Marine, Environment and Rural Affairs Science (CAMERAS) initiative [31] the investigated pesticides were chosen. This area is agricultural importance, being especially devoted to crops cultivation. Renfrewshire and North Ayrshire regions are extensively agriculture, used for arable crops including cereals, oilseeds, potatoes, peas, beans, barley, wheat, oats and triticale. Farming of cattle is also important in this region, especially sheep farming above Largs (North Ayrshire). In 2010, the fungicides accounted for 46% of the pesticide active ingredients applied, herbicides 38%, growth regulators 12%, seed treatments 2%, insecticides 1% and molluscicides < 0.5%. The most widely used pesticides to crop protection were prothioconazole, alpha-cypermethrin, bifenthrin, tau-fluvalinate and carfentrazone-ethyl, which was confirmed by the analysis of real water samples. All water samples were collected into glass bottles (250 mL) without a concave bottom and used without previous treatment or filtration. The samples were stored in darkness at 4 °C and were analyzed within 24 h of collection. The pH of studied water samples were in the range of 4.9 (rain)–7.4 (sea).

2.3. SPME procedure

The SPME was performed using holders for manual use and fiber, which were obtained from Supelco (Bellefonte, USA). Two of the most popular commercially available fiber coatings were used in this experiment: 100 μm polydimethylsiloxane (PDMS) and 85 μm polyacrylate (PA) respectively. Since the selected pesticides belong to various chemical groups, with significant variations in their polarity, these fiber phases were chosen to ensure optimal extraction efficiency. Conditioning was according to manufacturer's instructions before use and for each fiber blank desorption was performed. Three milliliter of aqueous sample (standard or real) was placed in 4 mL glass screw cap vial with PTFE/silicone septa (Supelco, Bellefonte, USA), containing 10% (w/v) sodium chloride salt and without pH adjustment. The fiber was immersed directly into the solution, at the selected temperature and time, retracted into a needle, and desorbed into the injection port of gas chromatograph for 5 min at 250 °C. The fiber remained for 4 min to eliminate the possible residues on the fiber. Extraction was supported by magnetic stirring at the highest possible level and a fiber blank experiment was performed.

2.4. Gas chromatography mass spectrometry (GC–MS)

GC analyses were performed using HP6890 gas chromatograph (Hewlett-Packard, PA) with a split/splitless injector coupled with a MSD 5973 mass spectrometer connected to MSD Chemstation (Agilent Technologies). Analytes were desorbed from the SPME fiber in a gas chromatograph splitless injection port. The injection port was fitted with a 0.75 mm i.d. injection liner (Supelco) with the split–splitless purge valve opened at 1 min after injection. The desorption temperature and time of analytes were optimized. The depth of the SPME needle in the injection port was also selected (3 cm) and controlled. The injector temperature was 250 °C. Chromatographic separation was carried out with a TR-5 MS 5% phenyl (equiv) polysilphenylene–siloxane capillary column (30 m × 0.25 mm × 0.25 μm thickness)) supplied by Thermo Scientific (UK). Helium (CP grade, 99.999%) was used as a carrier gas and was supplied by BOC Gases Europe (Guildford, UK). The flow of carrier gas was 1 mL min^{-1}, with constant flow conditions being observed throughout. The temperature program was as follows: 70 °C (hold 2 min), 25 °C min^{-1} to 220 °C, 2 °C min^{-1} to 240 °C, then 5 °C min^{-1} to 270 °C (hold 2 min). Duration of the temperature program was 26 min. The transfer line temperature was maintained at 280 °C. The mass spectrometer was operated in the electron impact ion (EI) mode with a source temperature of 230 °C. The electron energy was 70 eV.

The optimization of temperature program and chromatographic resolution was performed in the SCAN mode at the concentration level of 10 μg mL^{-1} for each pesticide. In order to quantify the pesticides in water samples (standard or real), selected ion monitoring (SIM) mode was then chosen and three specific ions were selected for each analyte. Table 1 presents selected ions, which were chosen to monitoring. The first ion was used for measurement and the other two for confirmation. For the pesticides tau-fluvalinate and alpha-cypermethrin, which show stereoisomerism, two peaks were detected for each one, corresponding to the *cis (Z)* and *trans (E)* isomers.

3. Results and discussion

3.1. Gas chromatography (GC) separation

A universal column dedicated to semi-volatile compounds was selected for separation. In order to achieve separation of all analytes the temperature program and carrier gas flow were optimized. Firstly, an experiment with a constant temperature increase to obtain the retention times of analytes, and then introduced three different temperature ramps in order to achieved full separation of analytes.

The flow rate conditions tested were 1, 1.5, 2 and 3 mL min^{-1}, with 1 mL min^{-1} giving the best results.

3.2. Optimization of SPME method

The different parameters that affect the extraction efficiency were monitored using SIM acquisition mode. The fiber coating, the time and temperature for the desorption of analytes, exposure time of the fiber in the aqueous sample, the temperature of the extraction process, the effect of pH and the ionic strength of the sample were taken into consideration. For optimization studies the solutions were prepared by spiking purified water with the standard solution at 10 mg L^{-1} for each pesticide. Each data point is the average of three independent measurements.

3.2.1. Fiber coating and extraction mode

Liquid polymer coating fibers, 100 μm PDMS and 85 μm PA, were used to extract selected pesticides. The chromatograms shown in Fig. 1 illustrate that the 85 μm PA fiber extracts all analytes in comparison with 100 μm PDMS, which allowed to extract only 11 analytes. Although PA fiber exhibited better extraction efficiency for the most studied analytes, especially for tau-fluvalinate, haloxyfop-R-methyl, diclofop-methyl and pyriproxyfen. Therefore, 85 μm PA fiber was selected for the subsequent experiments.

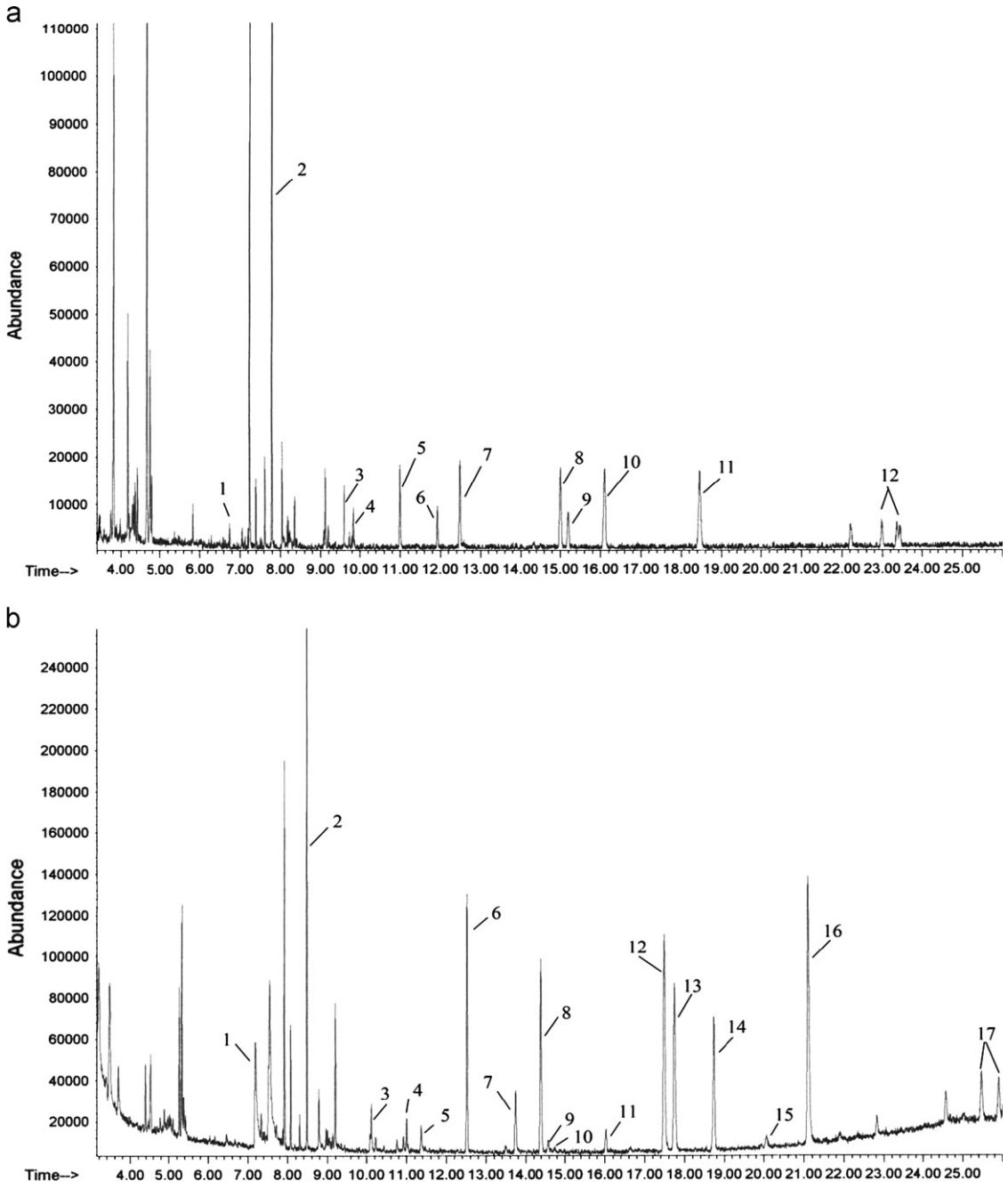

Fig. 1. Chromatograms obtained for spiked water samples at the concentration level 10 mg L^{-1} for each pesticide by using DI-SPME–GC–MS with different fibers; (a) for 100 μm PDMS: (1) fenoxaprop-ethyl, (2) tau-fluvalinate, (3) fenpropidin, (4) malathion, (5) haloxyfop-R-methyl, (6) profenofos, (7) fluazifop-butyl, (8) diclofop-methyl, (9) TPP (external standard), (10) bifenthrin, (11) pyriproxyfen, and (12) alpha-cypermethrin; (b) for 85 μm PA: (1) fenoxaprop-ethyl, (2) tau-fluvalinate, (3) fenpropidin, (4) malathion, (5) tetraconazole, (6) haloxyfop-R-methyl, (7) profenofos, (8) fluazifop-butyl, (9) prothioconazole, (10) cyproconazole, (11) carfentrazone-ethyl, (12) diclofop-methyl, (13) TPP (external standard), (14) bifenthrin, (15) metconazole, (16) pyriproxyfen, and (17) alpha-cypermethrin.

In addition, the mode of extraction method was also investigated. Conventionally, DI-SPME is more sensitive than HS-SPME and it is thus the method of choice for the analysis of clean aqueous samples without suspended matter, which can damage the fiber. However, in the case of the more volatile compounds DI mode is less sensitive than HS mode. These two extraction modes were evaluated and DI-SPME successfully extracted all the 16 pesticides (Fig. 1) while HS-SPME was able to extract only five compounds: fenoxaprop-ethyl, tau-fluvalinate, fenpropidin, bifenthrin and pyriproxyfen. The DI mode was then selected for the development of the method.

3.2.2. Desorption of analytes

Pesticide compounds were extracted into the fiber at the 10 mg L^{-1} concentration level and desorbed for 3, 5 and 7 min at injector temperatures ranging from 210 to 260 °C (10 °C increments) with optimal conditions being 5 min desorption at 250 °C. The desorption process was then performed twice for each extraction using three different concentrations of each pesticide, i.e., 10, 1, and 0.1 mg L^{-1}. Peak areas between replicate chromatograms were within 0.5% suggesting that carryover can be neglected since it is smaller than the experimental errors.

3.2.3. Extraction time

An evaluation experiment was performed using different extraction times from 25 to 80 min at the ambient temperature with continuous stirring. In this study the selected time periods were 25, 40, 60 and 80 min. Under the above studied optimum conditions, adsorption-time profiles were generated for each pesticide and are presented in Fig. 2. Each data point is the average of three independent measurements.

An increasing efficiency was observed for all the pesticides when the longer extraction time was used up to a maximum of 60 min for the optimal time. After this time the extraction efficiency decreased, except tau-fluvalinate, haloxyfop-R-methyl, fluazifop-butyl, diclofop-methyl and pyriproxyfen. For these pesticides efficiency was better at 80 min of extraction, but low efficiencies were noted for other analytes. Therefore, the extraction process time of 60 min was chosen for further analysis.

3.2.4. Extraction temperature

Higher temperature can increase adsorption of analytes and in consequence reduce extraction time. The influence of temperature on the area of peaks for each pesticide was investigated varying the temperature of extraction between 24 °C and 90 °C with a constant optimum extraction time of 60 min and the highest possible stirring. In this experiment room temperatures, 45, 70 and 90 °C were chosen for investigation. The detector responses (areas) for each pesticide with different extraction temperatures are presented in Fig. 3.

Fig. 3 shows that temperatures above room temperature increases the extraction efficiency. Increasing the temperature improved the mobility of selected pesticides through liquid phase and higher detector responses were obtained for all analytes until 45 °C. At higher temperatures the ability of fiber to adsorb begins to decrease, except fenpropidin, bifenthrin and alpha-cypermethrin. For these analytes the highest extraction efficiency at 70 °C was observed and optimal extraction of bifenthrin occurred at 90 °C. Since adsorption is an exothermic process and disfavored at high temperature the optimum extraction efficiency was achieved at the temperature of 45 °C and was therefore selected for the subsequent experiments.

3.2.5. Salt addition

The influence of sodium chloride (NaCl) salt additives to SPME procedure was investigated by comparing the extraction efficiency of samples with different concentrations of NaCl. In this study 5, 10 and 20% (w/v) of salt was used. Higher salt content has not been taken into consideration because of problems with solubility of NaCl. Fig. 4 illustrates effect of NaCl additives on detector responses (areas).

The addition of salt into the aqueous sample prior to the extraction process increased the ionic strength of the solution. As a consequence, the diffusion of the analytes is favored and extraction time is reduced. As can be seen in Fig. 4 increase in peak areas was observed with the increasing of NaCl concentration up to 10% (w/v).

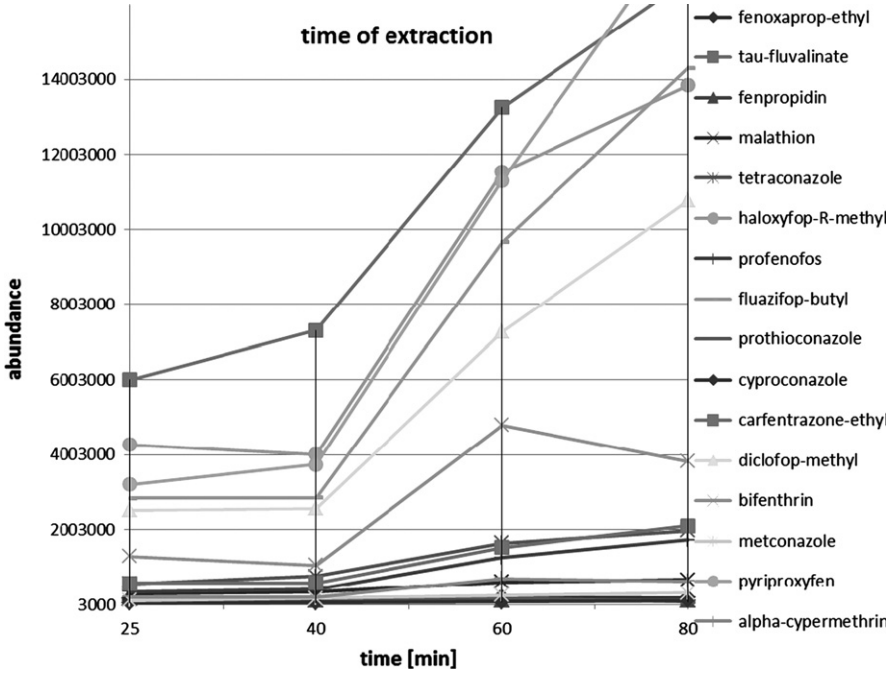

Fig. 2. Effect of extraction time from 25 to 80 min at ambient temperature on extraction efficiency: desorption time 5 min at 250 °C, concentration of each pesticide 10 mg L^{-1}, $n=3$.

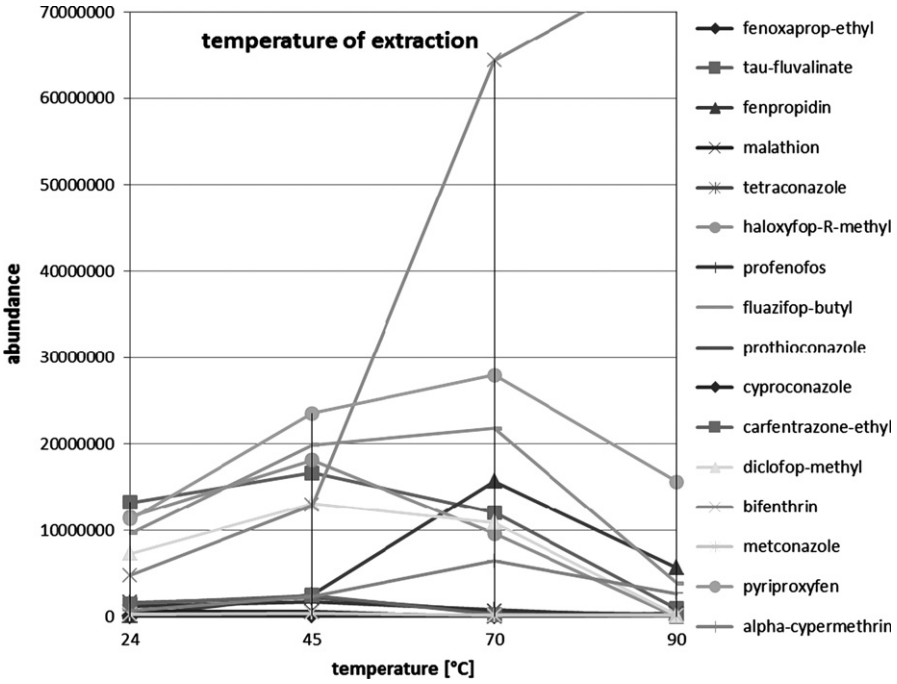

Fig. 3. Effect of extraction temperature from 24 °C to 90 °C on extraction efficiency: extraction time 60 min, concentration of each pesticide 10 mg L^{-1}, $n=3$.

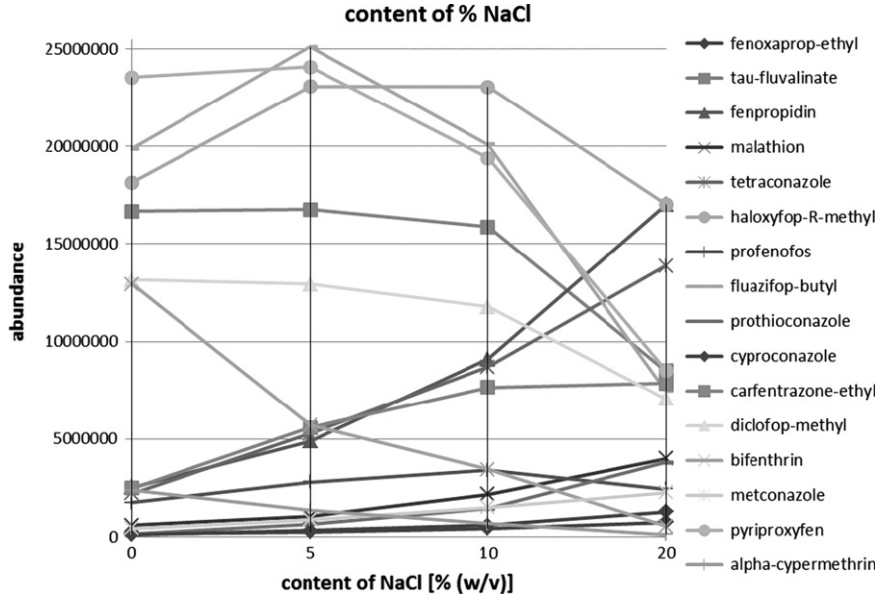

Fig. 4. Effect of content of sodium chloride (NaCl) salt (%) (w/v) on extraction efficiency: extraction time 60 min, temperature 45 °C, concentration of each pesticide 10 mg L^{-1}, $n=3$.

For higher content of NaCl the extraction efficiency decreased. Particularly for alpha-cypermethirn, bifenthrin, fluazifop-butyl and pyriproxyfen addition of salt had a negative influenced on detector responses (areas). Thus, the optimum concentration of salt for the extraction of selected pesticides was 10% (w/v) and was chosen for further analysis.

3.2.6. *The pH of the solution*

In this study, the pH of solution was varied from 4 to 9.5 and adjusted by adding hydrochloric acid or phosphate buffer, respectively. According to the chemical characteristic of the selected pesticides, the pH has different effects on detector responses (areas) as shown in Fig. 5.

The results indicated that the best extraction efficiency was obtained without addition of buffer in pH around 6.5 for most of the studied pesticides. Only for fenpropidin and prothioconazole extraction efficiency increased slightly when the pH value increased up to 9.5. The detector responses decreased significantly when the pH value decreased with the peak of fenpropidin almost disappearing at pH 4. Therefore, analysis was carried out using a constant pH of 6.5, since most pesticides have an acceptable response at this value.

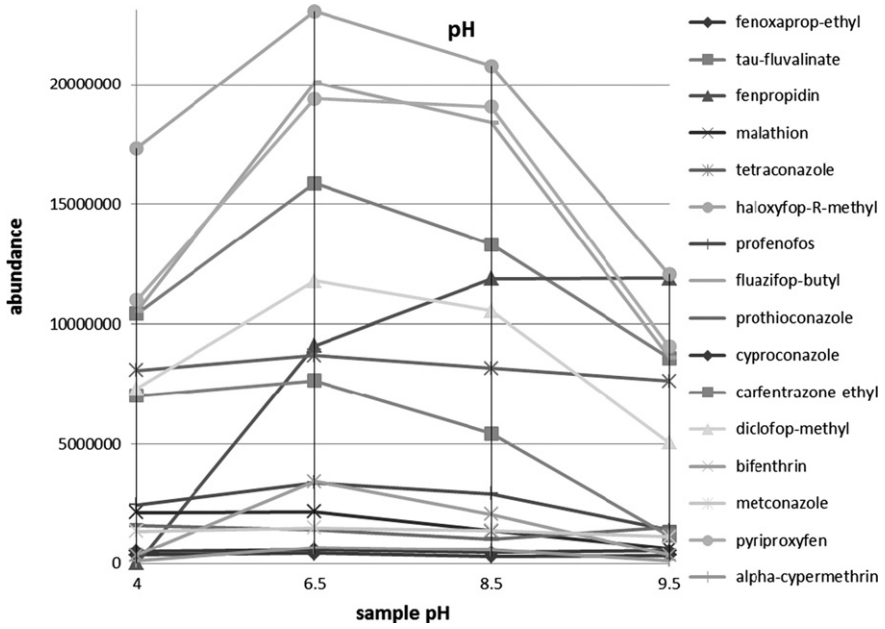

Fig. 5. Effect of varying the pH of the solution on extraction efficiency: extraction time 60 min, temperature 45 °C, content of NaCl salt 10% (w/v), the highest possible stirring, concentration of each pesticide 10 mg L^{-1}, $n=3$.

3.3. Method validation

The optimized conditions were used for validating the DI-SPME method for quantitative analysis of selected pesticides (linear range, detection limit, and precision). The working standard solutions for the calibration study were prepared by spiking purified water with the standard solution in the concentration range of 0.05–200 μg L^{-1} for each analyte. Linear range for pesticides was studied by replicate analysis of the standard stock solutions. Linear calibration curves for all analytes over seven calibration levels were constructed using 10 μg L^{-1} TPP as external standard. The linear regression values were calculated with the average peak areas of three replicate injections for each analyte. Table 2 shows the linear regression for each pesticide with coefficient of determination in the range from 0.982 (tetraconazole) to 0.997 (profenofos).

The calculated calibration curves showed good linearity range up to 100 μg L^{-1} for prothioconazole, cyproconazole, bifenthrin and alpha-cypermethrin, and up to 200 μg L^{-1} for fenoxaprop-ethyl, tau-fluvalinate, fenpropidin, malathion, tetraconazole, haloxyfop-R-methyl, profenofos, fluazifop-butyl, carfentrazone-ethyl, diclofop-methyl, metconazole and pyriproxyfen. Coefficient of variability (percentage of relative standard deviation, CV %) was the average value of different concentrations of studied pesticides in the linear range and was in the range from 1.5% (fenoxaprop-ethyl) to 10% (metconazole), which is considered as good method precision.

The sensitivity of the DI-SPME method was considered in terms of limit of detection (LOD). LODs were calculated from calibration functions [32] using the following equation:

$$LOD = \frac{3.3S}{b} \quad (1)$$

where S is the residual standard deviation of the calibration function and b is the slope of the first linear function. As it can be seen in Table 2 the method allows detection of the selected pesticides in water samples at concentrations lower than 0.13 μg L^{-1}. LODs were in the range from 0.015 μg L^{-1} (fenoxaprop-ethyl) to 0.13 μg L^{-1} (cyproconazole). The limits of quantitation (LOQs) defined as 3 times the LOD were analyte-dependent and range from 0.045 μg L^{-1} (fenoxaprop-ethyl) to 0.38 μg L^{-1} (cyproconazole).

Reproducibility of the method was examined by extracting five water samples from river spiked at two different concentration levels 10 μg L^{-1} and 100 μg L^{-1} under optimized experimental conditions. As shown in Table 3, RSDs vary between 0.020 (profenofos) and 0.092 (pyriproxyfen) for the pesticides at the concentration 10 μg L^{-1} and 0.019 (profenofos)—0.096 (haloxyfop-R-methyl) for the pesticides at the concentration 100 μg L^{-1}, indicating that the reproducibility of this extraction method is satisfactory.

The mean recoveries obtained for the studied pesticides spiked at two concentration levels 10 and 100 μg L^{-1} in water samples are shown in Table 3. The relative recovery was applied because SPME is a non-exhaustive extraction technique since absolute recovery is more appropriately used for exhaustive extraction techniques. The recovery of analytes at the concentration level 10 μg L^{-1} ranged between 84% (pyriproxyfen) and 119% (bifenthrin). For analytes added to the water samples at the concentration level 100 μg L^{-1} the recovery was in the range of 82% (cyproconazole)–114% (carfentrazone-ethyl). Fig. 6 displays the chromatogram obtained for spiked water sample from river at the concentration level 10 μg L^{-1} for each pesticide by using DI-SPME–GC–MS.

The recovery of all analytes ranged between 82% and 119%. It should be noted that results obtained using a PA fiber indicate that the procedure shows good agreement for the extraction of selected pesticides in natural waters, in terms of the concentration of analytes spiked in the samples, giving comparable recoveries. This demonstrates that DI-SPME is not influenced by the non dissolved particles or low volatile compounds contained in natural waters which may act competitively for the adsorption sites of the fiber.

3.4. Applications

The developed procedure was applied to determine the selected pesticides in aqueous samples from river, canal, sea and rain. Water samples were collected in the central lowland region of Scotland, UK (area of study), in accordance with item 2.2.

Table 2
Basic validation parameters obtained for each pesticide by using DI-SPME–GC–MS.

Analyte	Retention time (min)	Equation	Coefficient of determination R^2	Limit of detection LOD ($\mu g\ L^{-1}$)	Limit of quantification LOQ ($\mu g\ L^{-1}$)	Linearity range ($\mu g\ L^{-1}$)	Coefficient of variability CV (%)
Fenoxaprop-ethyl	6.668	$y=0.057x+0.013$	0.995	0.015	0.045	0.045–200	1.5
Tau-fluvalinate	7.814	$y=3.172x-0.484$	0.994	0.042	0.13	0.13–200	1.6
Fenpropidin	9.633	$y=0.238x+0.1$	0.989	0.074	0.22	0.22–200	4.8
Malathion	9.845	$y=0.123x+0.034$	0.991	0.058	0.17	0.17–200	7.4
Tetraconazole	10.131	$y=0.303x+0.146$	0.982	0.12	0.35	0.35–200	7.1
Haloxyfop-R-methyl	11.054	$y=2.661x-0.601$	0.996	0.062	0.19	0.19–200	1.7
Profenofos	11.991	$y=0.241x-0.037$	0.997	0.056	0.17	0.17–200	3.3
Fluazifop-butyl	12.551	$y=1.648x-0.413$	0.990	0.10	0.30	0.30–200	8.4
Prothioconazole	12.649	$y=0.174x-0.014$	0.994	0.046	0.14	0.14–100	5.4
Cyproconazole	12.794	$y=0.058x-0.005$	0.991	0.13	0.38	0.38–100	3.1
Carfentrazone-ethyl	13.890	$y=0.346x+0.017$	0.996	0.10	0.30	0.30–200	2.7
Diclofop-methyl	15.095	$y=1.293x-0.379$	0.991	0.055	0.16	0.16–200	6.3
Bifenthrin	16.198	$y=0.122x-0.008$	0.991	0.064	0.19	0.19–100	8.9
Metconazole	17.403	$y=0.059x+0.009$	0.994	0.043	0.13	0.13–200	10
Pyriproxyfen	18.431	$y=1.518x-0.485$	0.991	0.029	0.087	0.087–200	3.4
Alpha-cypermethrin	23.086; 23.452	$y=0.025x-0.002$	0.992	0.12	0.37	0.37–100	1.7

Table 3
Mean recovery and RSD value of selected pesticides in real water samples from river by using DI-SPME–GC–MS ($n=5$).

Analyte	Spiked water at concentration 10 $\mu g\ L^{-1}$		Spiked water at concentration 100 $\mu g\ L-1$	
	Recovery (%)	RSD ($n=5$)	Recovery (%)	RSD ($n=5$)
Fenoxaprop-ethyl	102	0.047	94	0.029
Tau-fluvalinate	87	0.034	86	0.057
Fenpropidin	98	0.048	101	0.042
Malathion	101	0.048	90	0.025
Tetraconazole	104	0.086	103	0.067
Haloxyfop-R-methyl	93	0.024	92	0.096
Profenofos	90	0.020	101	0.019
Fluazifop-butyl	87	0.067	96	0.020
Prothioconazole	97	0.040	89	0.069
Cyproconazole	102	0.039	82	0.057
Carfentrazone-ethyl	101	0.032	114	0.015
Diclofop-methyl	96	0.045	94	0.034
Bifenthrin	119	0.035	99	0.057
Metconazole	109	0.035	105	0.021
Pyriproxyfen	84	0.092	89	0.072
Alpha-cypermethrin	102	0.032	112	0.043

First, a blank of each type of water samples was analyzed using the optimized conditions, in order to verify the presence of different peaks in the corresponding chromatogram at the same retention times as the pesticides being studied. Then the samples identified as containing pesticide residues were analyzed again with three replicates, in order to confirm and quantify levels of concentration detected analytes. The concentrations of individual pesticides were determined by the external standard method. None of the analytes were detected in the water samples from river, rain and sea. The results for analyses indicate that only water from canal was contaminated by pesticides. Fig. 7 shows the chromatogram obtained for the water sample from canal by using the DI-SPME–GC–MS.

The calculated contamination levels of tau-fluvalinate was 0.19 $\mu g\ L^{-1}$, prothioconazole was 0.16 $\mu g\ L^{-1}$, bifenthrin was 0.27 $\mu g\ L^{-1}$ and pyriproxyfen was 0.32 $\mu g\ L^{-1}$. The concentration level of alpha-cypermethrin could not be quantified because detected concentration was between the detection limit and the quantification limit of the method. The obtained results for water samples confirmed the data from Science and Advice for Scottish Agriculture (SASA) organisation about usage of pesticides in the study area. Detected pesticides are the most widely used in agriculture and their concentration levels were higher than the maximum limits established by the European Union 98/83/EC (0.1 $\mu g\ L^{-1}$) [5]. It can be quite significant, since the local population use these water sources for personal consumption.

4. Conclusion

The developed methodology has proved to be selective, sensitive and precise for the simultaneous determination of residues pesticides from various chemical groups (four aryloxyphenoxy-propionates, three pyrethroids, two organophosphates, three triazoles, one triazolinthione, one triaolinone and two unclassified) in water samples. The results of analysis of collected water samples in the study area indicate a potential risk of environmental contamination, because detected concentration levels of pesticides were higher than those established by the European Union legislation. The described procedure is a simpler, lower cost and less labor intensive sample preparation technique, than conventional techniques such as LLE and SPE for the simultaneous determination of pesticides in environmental samples by GC. The

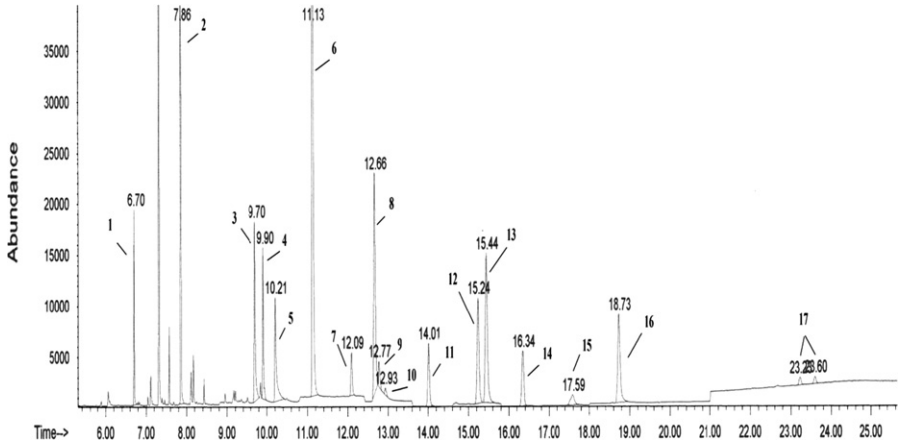

Fig. 6. Chromatogram obtained for spiked river water at the concentration level of 10 μg L^{-1} for each pesticide by using DI-SPME–GC–MS; (1) fenoxaprop-ethyl, (2) tau-fluvalinate, (3) fenpropidin, (4) malathion, (5) tetraconazole, (6) haloxyfop-R-methyl, (7) profenofos, (8) fluazifop-butyl, (9) prothioconazole, (10) cyproconazole, (11) carfentrazone-ethyl, (12) diclofop-methyl, (13) TPP (external standard), (14) bifenthrin, (15) metconazole, (16) pyriproxyfen, and (17) alpha-cypermethrin.

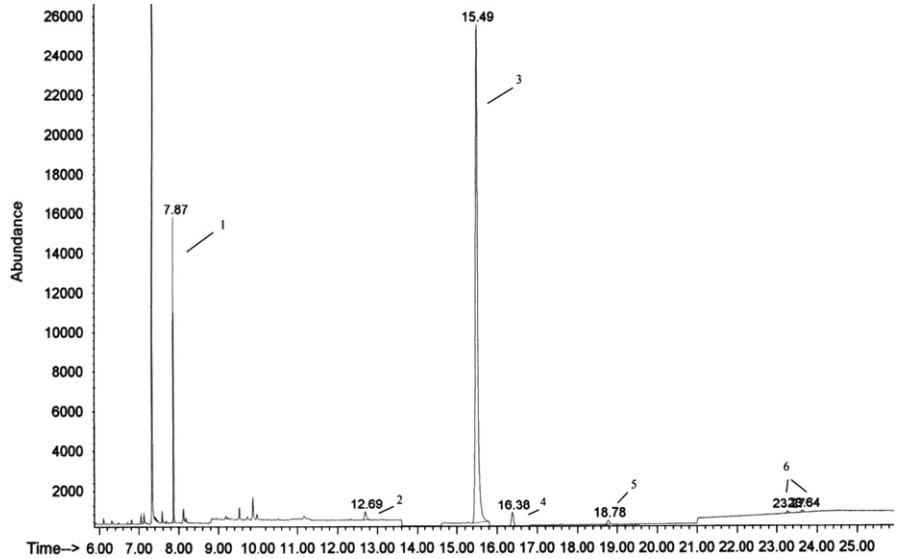

Fig. 7. Chromatogram obtained for real water sample from canal by using DI-SPME–GC–MS; (1) tau-fluvalinate, (2) prothioconazole, (3) TPP (external standard), (4) bifenthrin, (5) pyriproxyfen, and (6) alpha-cypermethrin.

lack of solvents and the ability to use the gas chromatograph injection port for thermal desorption makes SPME the ideal sampling device.

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