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Journal of Chromatography A, 909 (2001) 29–36

JOURNAL OF CHROMATOGRAPHY A

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Application of on-site solid-phase microextraction in aquatic dissipation studies of profoxydim in rice

Ralf Eisert*, Scott Jackson, Arno Krotzky

BASF Corporation, Agricultural Products Center, 26 Davis Drive, Research Triangle Park, NC 27709-3528, USA

Abstract

The application of a manual operated solid-phase microextraction (SPME)–HPLC interface is discussed for the analysis of thermally labile analytes in aqueous matrices. The technique has been applied on-site at a flooded rice field to demonstrate its potential for real time extraction of the herbicide profoxydim. Thus, compounds which would otherwise easily degrade in the aqueous matrices within hours or days could be determined more accurately. The fibers were shipped back to the laboratory with express delivery where the target analyte was desorbed from the fiber and determined by HPLC–UV analysis. The SPME method was characterized by significant ruggedness where conventional techniques such as liquid–liquid extraction and solid-phase extraction require additional shipping and handling costs and time-consuming multiple sample preparation steps. In general, any delay in shipping the aqueous samples to the laboratory has the potential for sample degradation and a loss in accuracy when using non on-site extraction techniques. Fifty μm Carbowax–templated resin coatings were most suitable for coupling SPME to HPLC in order to achieve a high sensitivity for polar analytes. The SPME technique was characterized by a good sensitivity and a precision less than 10% RSD. The SPME–LC–UV method was linear over at least three orders of magnitude while achieving a limit of detection in the lower μg/l range. The on-site SPME method has shown significantly increased accuracy. Profoxydim was determined at concentrations of ca. 180 μg/l 3 h after an application on a flooded bare soil field.

Keywords: Solid-phase microextraction; Field extraction methods; Pesticides; Profoxydim

1. Introduction

The coupling of solid-phase microextraction (SPME) to high-performance liquid chromatography (HPLC) was chosen to investigate a new type of on-site sample preparation for the analysis of organic compounds in aqueous matrices such as flooded rice fields. SPME has previously been applied very successfully to the analysis of pesticides under laboratory conditions [1–6]. The technique can be either coupled with gas chromatography (GC) for thermally stable compounds [2,4,5] or with HPLC for thermally labile compounds [7–9]. The latter coupling has been already proven to be a very efficient way of hyphenating miniaturized sample preparation to instrumental analysis. Besides pesticides, the technique has been successfully applied to the analysis of erythromycin [7], polycyclic aromatic hydrocarbons (PAHs) [8], surfactants [9], corticosteroids [10], acylcarnitines [11], and metal ions [12]. The SPME method, in general, is characterized by simple handling steps, high precision, potential for automation and integration of the sample preparation in the instrumental analysis [13–15]. Thus, a high sample throughput can be achieved. The manual sample preparation and en-

*Corresponding author. Present address: BASF AG, Agricultural Center, P.O. Box 120, D-67114 Limburgerhof, Germany. Tel.: +49-621-60-27656; fax: +49-621-60-27092.

0021-9673/01/$ – see front matter
PII: S0021-9673(00)01026-8

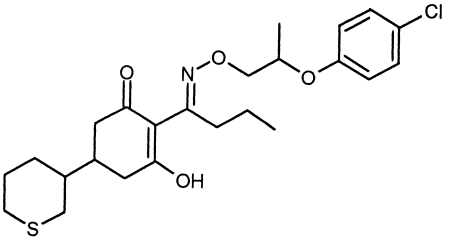
richment by SPME allows a unique strategy of performing the sample preparation part right where the sample is collected in the environment and not where the laboratory is located. The design of the fiber, its easy handling and almost insignificant mass makes SPME very suitable for on-site analysis without moving the entire laboratory equipment into the field. Complete transfer of the entire method, e.g., SPME–GC into the field, seems only justified if miniaturized equipment such as micro-GC which is based on fast GC can be used. Górecki and Pawliszyn reported the potential of on-site SPME when coupled to fast GC using a field portable system for trace analysis of volatile organic compounds (VOCs) [16]. To date, the SPME–GC coupling can be performed fully automated by using a modified SPME autosampler [5,17–19]. However, there is only one prototype for a fully automated SPME–HPLC system reported in the literature [20] which is not based on commercial fiber technology.

The demand for easy to handle, fast, and efficient sample preparation methods which can be directly coupled to either GC or HPLC is very high. The potential for the extraction of samples in the field makes SPME unique compared to many of the classical methods which are very time consuming and involve multiple steps and dedicated apparatus. The SPME method is based on a polymer (extracting sorbent) immobilized on a fused-silica fiber. The fiber is exposed to the (liquid) sample where the analyte partitions into the sorbent until a steady state (equilibrium) is reached.

This article shows the on-site application of SPME, trying to avoid time-consuming sample shipment and later sample preparation in the laboratory. Thus, an easy to handle and rugged method was developed to achieve sample preparation and enrichment using the SPME fibers on-site. The next step was shipping the light fibers back to the laboratory where they were analyzed. Different types of sample storage (temperature) conditions during the transport to the laboratory were investigated to achieve optimum results and trace back potential degradation of the compound or aging of the sample which was already extracted when shipped back to the laboratory. All fibers were send back to the laboratory from the field using overnight express delivery. By comparison of the mass of the fibers vs. the mass of the aqueous samples which is typically 1 g (fiber) vs. 100–1000 g (water sample) one can immediately conclude cost savings achieved by mass reduction. However, this was not the major driving force for this innovated on-site analysis approach.

2. Experimental

2.1. Reagents



Standards of profoxydim ($C_{24}H_{32}ClNO_4S$, formula M_r 466.04, CAS No. 139001-49-3) were prepared in the laboratories of BASF (Ludwigshafen, Germany) and used as received. The active ingredient was of 99.1% purity. Standard solutions were prepared in methanol at 1 mg/ml and further diluted for stock solutions if needed. In addition to profoxydim which shows a relatively fast degradation in methanolic and even aqueous solution depending on the pH, the lithium salt of the active ingredient was used as well. Thus, a conversion factor of 0.977 (molecular mass of profoxydim divided by the molecular mass of the corresponding lithium salt) was applied to convert the lithium analyte data to the corresponding profoxydim data. Fresh methanolic stock solutions were prepared at least every week and kept in the fridge. HPLC-grade water, methanol, and acetonitrile were purchased from Burdick & Jackson (Muskegon, MI, USA) and used as received. Sodium chloride and formic acid were obtained from EM Science (Gibbstown, NJ, USA).

2.2. Solid-phase microextraction

A commercial SPME interface from Supelco (Bellefonte, PA, USA) was used in this study (see Fig. 1). The interface was connected to the LC system. An automated six-port valve was employed

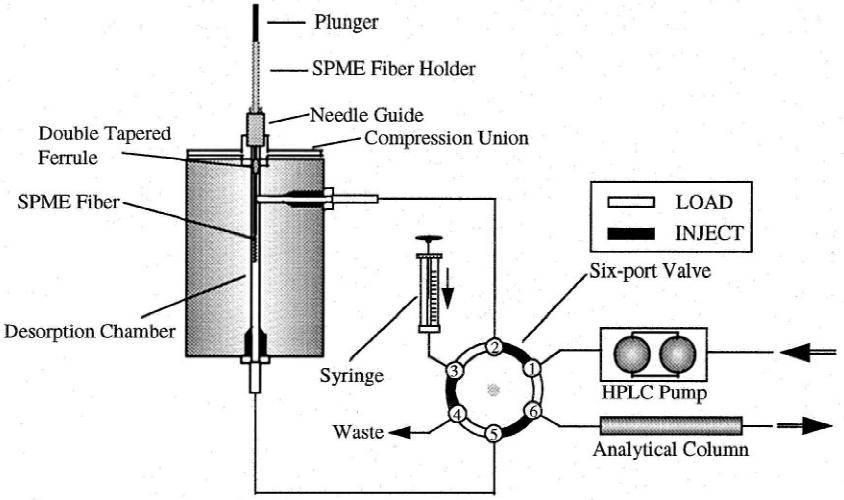


Fig. 1. SPME–LC set-up used in this study.

to control the desorption times and injection to the analytical column which was part of the LC set-up used (see Section 2.3). Fifty μm Carbowax–templated resin (CW–TPR) fibers from Supelco were exclusively used in this study. The new fibers were conditioned for 10 min in pure methanol and desorbed once using a standard desorption procedure which includes the exposure to the solvent gradient prior to extraction. During sample extraction, the entire fiber was immersed into the aqueous solution. The fiber is placed perpendicular to achieve maximum agitation of the surrounding aqueous sample which was stirred at 650 rpm on a HP-15 Variomag from H+P Labortechnik (Oberschleissheim, Germany) multipoint magnetic stirplate or at 1000 rpm on a magnetic stirplate Series 400 HTS from VWR Scientific (Morrisville, NC, USA). Subsequently, the fiber was inserted into the desorption chamber of the SPME–LC interface while starting the LC program (see Section 2.3). The desorption chamber was previously filled with 100% methanol by flushing the desorption chamber with a total volume of 250 μl MeOH. Excessive methanol was removed from the double tapered ferrule. A 5-min static desorption was sufficient for the desorption of the profoxydim. After this period, the entire content of the desorption chamber was flushed onto the HPLC column by means of the mobile phase gradient flow. The protecting needle guide of the fiber was always kept in the headspace of the sample to prevent salt accumulation in the needle which could cause clogging of the fiber plunger mechanism.

The final SPME method which was applied in the field was designed to allow easy sample handling in the field combined with sufficient ruggedness. A 15-ml volume of the aqueous samples was placed in 40-ml amber US Environmental Protection Agency (EPA) vials (Supelco) which were then filled with 15 ml ultra pure water. A 3-g amount of NaCl was added and the fiber was exposed into the sample for 30 min. A maximum of 15 samples could be processed in parallel with the set-up shown (multipoint stirplate). The fibers were protected during the shipment until the analysis (desorption step) using an LB-2 septa from Supelco to seal the protecting needle which hosts the fiber.

2.3. Liquid chromatography

HPLC investigations were carried out using the Magic 2002 equipped with a UV detector (Michrom Bioresources, Auburn, CA, USA). A Supelcosil LC-18 15 cm×2.1 mm (5 μm) HPLC column was operated at 0.2 ml/min using the following water–acetonitrile [both containing 0.1% (v/v) formic acid] gradient program. The LC system is software controlled by Magic LC control ver. 1.1 software (Michrom Bioresources). The six-port valve is ini-

tially switched to the LOAD position. The desorption chamber for the SPME fibers is at ambient pressure. The initial 100% water solvent condition was held for 5 min during the static desorption of the analyte from the fiber. After 5 min the six-port valve was automatically switched to the INJECT position. A linear gradient from 5 to 15 min changes the solvent mixture to acetonitrile–water (90:10). These conditions are kept for additionally 5 min before the system returned to the initial conditions within 1 min. The program kept the initial conditions for additional 4 min. Thus, after a total turnaround time of 25 min including the exposure of the fiber and finishing the equilibration of the system under the initial conditions, the LC system was ready for the subsequent desorption. The compounds were detected at 290 nm. Data acquisition and processing were provided by a personal computer interfaced to the detector using the EZChrom chromatography data system ver. 6.6 from Michrom Bioresources. Aliquots of 5 μl of standard mixtures were injected via a 5-μl loop for calculating the amounts extracted by the fiber. In this (calibration) set-up, the SPME–LC interface was replaced by a 5-μl injection loop.

3. Results and discussion

Prior to the field experiments, the SPME–HPLC method was optimized under laboratory conditions. An identical set-up which was later used on-site was employed to achieve identical conditions (field vs. laboratory). Factors such as the amount of salt in the aqueous sample, exposure and desorption time were investigated. Fig. 2 shows the extraction time profile of the compound studied. Within 45 min equilibrium conditions were established using magnetic stirring at 1000 rpm. The extraction under field conditions was performed at 650 rpm using a multiple stirplate set-up. Thus, the equilibration took slightly longer (ca. 1 h). However, to achieve optimum overlapping of the time it takes to extract a sample and to run the HPLC analysis a 30-min extraction time was chosen for all future experiments. Faster sample turnaround times were achieved (30 min vs. 60 min) by a minor loss of sensitivity of ca. 20% as a result of lower extraction efficacy. The precision achieved under these conditions, see Table 1, was still <10% RSD and sufficient for the analytical purpose. The precision was determined for two different configurations. First, the intra-fiber precision which typically yields higher precision. For six repetitive extractions the intra-fiber precision was determined to be 3.1% RSD. Second, the inter-fiber precision which is more significant in an on-site scenario where more than one fiber was used. The inter-fiber precision for three fibers yields 6.9% RSD (see Table 1). In all extractions performed within this study the precision (inter-fiber) never exceeded the 10% RSD value which demonstrates the high reproducibility of the method and almost identical fiber capacities (sorption volumes). The limit of detection (LOD) of the method using UV absorption at 290 nm for the detection of profoxydim and a salt concentration of 0.1 g NaCl/ml was in the lower μg/l range (see Table 1). The linearity was characterized by the square of the coefficient of correlation better than 0.998 over at least three orders of magnitude in concentration. The addition of salt to the aqueous sample shows a significant effect on the extraction yield which is demonstrated in Fig. 3. The extraction yield (peak area) is increased by factor of 10 when changing the NaCl concentration from 0 to 0.33 g/ml. Thus, for further improvements of the sensitivity the sodium chloride concentration which was typically kept at 0.1 g/ml can be increased. However, in some cases the protecting needle might be clogged by salt particles at high NaCl concentrations. One has to consider a larger gap between the aqueous sample an the resting position of the protect-

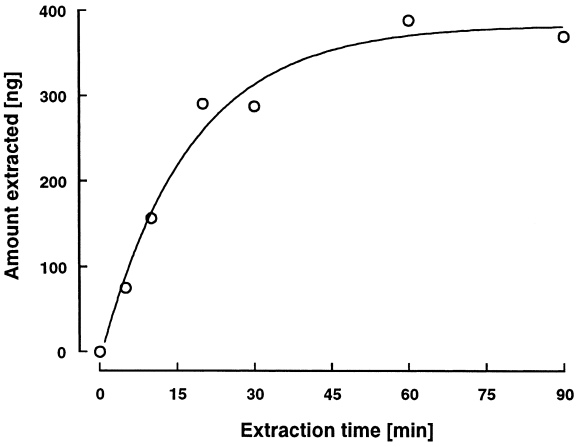


Fig. 2. Exposure time profile using a 50 μm CW–TPR fiber (500 μg/l spiking level, 1000 rpm).

Table 1
Precision, limit of detection and inter-fiber repeatability of the SPME–HPLC method for profoxydim

Compound	Precision (RSD, %)		LOD (μg/l)[c]	
	Intra-fiber[a]	Inter-fiber[b]	At 290 nm	At 240 nm
Profoxydim	3.1	6.9	5	3.3

[a] A single fiber was used in this study ($n=6$).
[b] A set of three fibers was used to determine the precision ($n=6$).
[c] The LOD was determined applying a signal-to-noise ratio of 3.

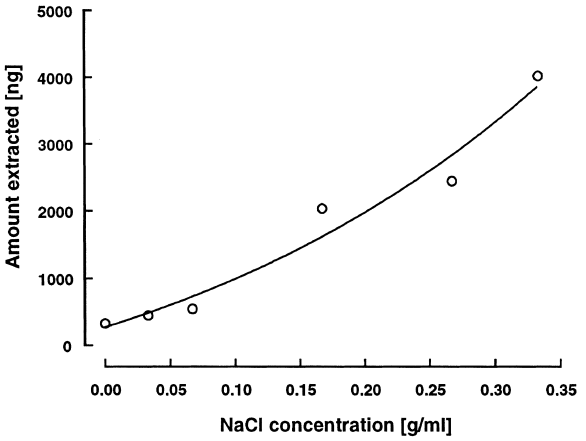


Fig. 3. Effect of salt on the extraction yield of the compound under investigation (500 μg/l spiking level, 1000 rpm, 30 min extraction time).

ing needle during the extraction step or an additional wash step with ultra pure water prior to desorption to avoid mechanical damage to the fiber. Furthermore, the pH shows a significant effect on the extraction yield. Fig. 4 shows an increased extraction yield when lowering the pH of the solution. In general, a high extraction yield was achieved at pH values equal or below 6. The pH of the environmental samples studied was ranging from pH 5 to 6. Thus, optimum extraction yields could be obtained without further pH adjustment. However, if different samples of varying pH are analyzed pH adjustment using adequate buffer solutions are necessary for achieving accurate results.

Moving the sample preparation step to the field required additional testing of the SPME method. The storage of the extracted profoxydim compared to the stability of the analyte in the aqueous sample was investigated over a 4-day period. Table 2 summarizes the results of the on-fiber storage vs. the aqueous sample storage stability of the target compound. Three replicates were employed for each set. Besides very comparable precision results which were obtained under all conditions the compound shows no degradation when stored on the fiber for a few days. All fibers were kept at ambient temperature (ca. 21°C) during the storage experiment. Once extracted, the compound shows relative stability for several days at ambient temperatures. The on-fiber storage can be further increased by keeping the fiber in the fridge. The day 1 samples, of the aqueous sample storage experiment shows no loss of analyte (see Table 2). However, after 4 days a 12% decrease in extraction yield was determined. The sample starts degradation in water within a few days. One metabolite peak which could be determined with the same SPME method could be identified when the profoxydim peak area was starting to decrease. Without extra protection (storage conditions) the initial concentration of the aqueous sample decreases as a result of degradation processes. However, if the

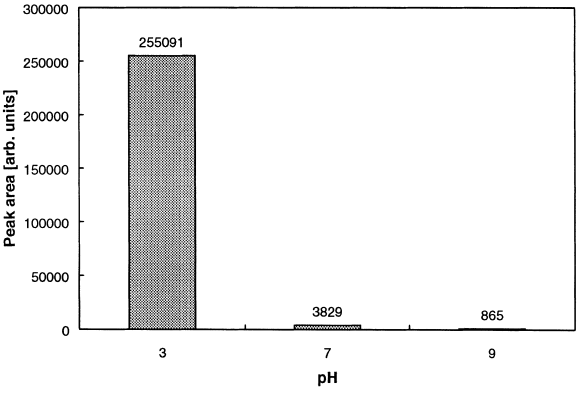


Fig. 4. Effect of varying the pH on the extraction yield of profoxydim. A 1000-μg/l sample was stirred at 1000 rpm during the 30 min extraction time at ambient temperature.

Table 2
On-fiber and aqueous sample storage results at ambient temperature in the laboratory

	Day 0	On-fiber storage time		Aqueous sample storage time	
		Day 1	Day 4	Day 1	Day 4
Mean (%)[a]	100	101	98	101	88
Mean (area counts)[b]	462 176	466 928	454 052	466 928	405 847
SD	37 957	33 495	30 366	33 495	30 242
RSD (%)	8.2	7.2	6.7	7.2	7.5

[a] Normalized results: area counts of day 0≡100%.
[b] Using three replicates at the 1000 μg/l spiking level.

profoxydim is stored under dry conditions on the SPME fiber the degradation is significantly slower. Thus, an on-fiber storage of the extracted analyte is considered stable (still achieving accurate analytical results) to accomplish sample shipment from the field to the laboratory within 1 week.

Fig. 5 shows two SPME–HPLC–UV chromatograms. The lower trace indicates a typical blank run. No significant carryover could be detected. The upper trace shows a spiked water sample. Table 3

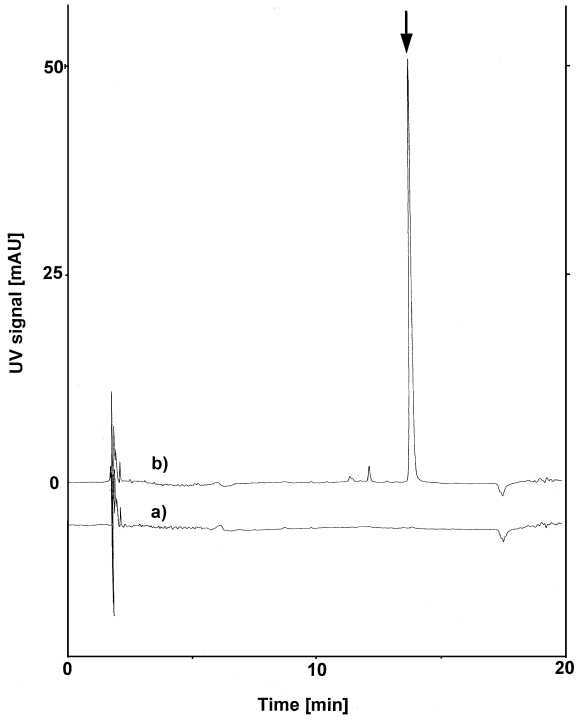


Fig. 5. SPME–HPLC–UV chromatogram at 290 nm of (a) a fiber blank and (b) a spiked water sample (100 μg/l spiking level, 0.3 g NaCl/ml, 190 min extraction time).

summarizes the results obtained from the on-site study. Three different conditions were compared for the fiber storage during shipment back to the laboratory and further storage in the laboratory. The first set (No. 1, see Table 3) was always kept at ambient temperature. It shows a very poor precision and low concentrations of profoxydim determined. While we know all circumstances, especially temperature conditions, before and after we send the samples, we can only assume the conditions during the express mail delivery overnight. There is always a good chance that the box containing the fibers was exposed to sunlight and significantly heated up which could explain the low concentrations determined and poor precision. However, a similar set was kept in the fridge immediately upon arrival in the laboratory before it was further analyzed 10 days later. This set (No. 2) shows very similar results to the last set (No. 3) where the fibers were always kept in a cooling chain. Set No. 3 was shipped back on ''blue ice'' (cooling package) and immediately kept in the fridge upon arrival in the laboratory. The concentration determined for the aqueous sample taken and extracted in the field were highest for this set. Thus, keeping the fibers close to 0°C protects the target analyte from further degradation or even slows down diffusion into surrounding air. This is important if the fibers are not immediately analyzed upon arrival in the laboratory. As shown previously, the compound shows no degradation when kept at ambient temperatures for a few days (see Table 2), however, keeping the fibers in the fridge extends the time limit which still allows an accurate analysis. The on-site SPME data (see Table 3) were compared to aqueous samples from the same spot which were shipped back to the laboratory with a cooling package and then later analyzed by SPME–HPLC in the labora-

Table 3
On-site field results using a SPME–HPLC method[a]

	Sample 1	Sample 2	Sample 3	Non-field data
No. of samples	5	5	5	5
Mean concentration (μg/l)	46.7	166.6	178.0	
RSD (%)	80.0	8.4	3.1	
Concentration (μg/l) of water sample from the same site (RSD, %)[b]				134.7 (5.1)

[a] Different storage conditions; No. 1: samples were shipped and kept at ambient temperature after shipment to the laboratory, No. 2: samples were shipped at ambient temperature and kept in fridge after shipment, No. 3: samples were shipped on ''blue ice'' and kept in fridge after shipment.

[b] Aqueous samples from the same site were shipped back to the laboratory on ''blue ice'' and analyzed by SPME–LC under laboratory conditions 3 weeks after the application day.

tory (on-site vs. laboratory extraction). The concentration (mean of five replicates) was 134.7 μg/l which is significantly lower compared to the on-site result 178.0 μg/l. After 3 weeks the aqueous sample already shows a loss of 25% which underlines the advantage of the on-site SPME method. Fig. 6 displays the SPME–HPLC–UV chromatogram of a water sample taken from a flooded field 3 h after the treatment with profoxydim in comparison to the pre-application blank and a fiber blank. The pre-application sample (Fig. 6b) shows no measurable amount of the target analyte. The sample was taken just 2 weeks after the last application which was performed on the bare soil plot. The plot stayed dry without any precipitation or irrigation for 2 weeks. No residues could be determined which underlines the fast degradation tendency of this herbicide. In addition, no interference from another unknown compound could be detected. The chromatogram of the post-application sample indicates a good baseline separation of profoxydim from unknown peaks. Thus, the selectivity of the SPME–HPLC–UV was sufficient for an unequivocal determination of the target analyte.

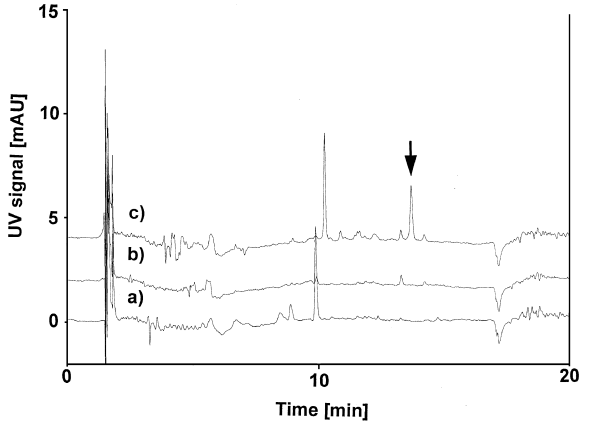


Fig. 6. SPME–HPLC–UV chromatogram at 290 nm of a water sample taken from a flooded rice field; (a) fiber blank, (b) pre-application water blank and (c) 3 h after the application (0.3 g NaCl/ml, 650 rpm, 30 min extraction time performed on-site).

4. Conclusions

Further improvements of the set-up such as using an autosampler for the desorption step of the SPME fibers could increase the sample throughput and handling of the instrumental analysis. The fiber can be used several times which lowers the costs per sample significantly. Otherwise the on-site SPME procedure would be more cost intensive. Each sample requires its own fiber. During our study the fibers were used for more than 20 extraction–desorption cycles without any significant loss of performance. In a typical field sample collection scenario up to 30 samples are collected per field plot and day. The extraction method described in this study takes roughly 45 min for a set of 15 samples. Thus, the technique is very suitable for field analytical measurements which require time efficient methods.

The reported SPME–HPLC–UV set-up is characterized by sufficient selectivity for an unequivocal determination of the target analyte in the matrix investigated. However, the current method can be easily applied for an SPME–HPLC–MS set-up. All parameters are suitable for LC–MS detection which

could be necessary if more interference peaks from soil or water are present in the samples under investigation. Thus, the selectivity of the method can be further enhanced using LC–MS coupling.

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