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Hollow fiber membrane-protected solid-phase microextraction of triazine herbicides in bovine milk and sewage sludge samples

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Abstract

A porous polypropylene hollow fiber membrane (HFM)-protected solid-phase microextraction (HFM-SPME) procedure in conjunction with gas chromatography/mass spectrometric analysis for use in the determination of triazine herbicides in bovine milk samples is described. A 65-μm polydimethylsiloxane-divinylbenzne (PDMS–DVB) SPME fiber was protected by an HFM. HFM-SPME experimental parameters such as fiber type, extraction time, extraction temperature and salt concentration were investigated and optimized. The relative standard deviations for the reproducibility of the optimized HFM-SPME method varied from 4.30 to 12.37%. The correlation coefficients of the calibration curves were between 0.9799 and 0.9965 across a concentration range of 0–200 $\mu g\,l^{-1}$. The method detection limits for triazines in bovine milk were in the range of 0.003–0.013 $\mu g\,l^{-1}$ and limits of quantification were in the range of 0.006–0.021 $\mu g\,l^{-1}$. The suitability of HFM-SPME was extended to the analysis of the herbicides in sewage sludge samples. The results demonstrate that HFM-SPME was an efficient pretreatment and enrichment procedure for complex matrices.

Keywords: Solid-phase microextraction; Milk; Pesticides; Triazines

1. Introduction

Triazines are ubiquitous environmental pollutants in the environment at varying concentrations and therefore, therefore there is an increasing environmental concern with regard to these compounds [1]. United States Environmental Protection Agency (USEPA) and European Union (EU) legislation have established a maximum herbicides residue level in drinking water in the range of parts-per-billion. In the EU, the maximum allowed limit for each individual herbicide, has been set at 0.1 $\mu g\,l^{-1}$ [2] but in the USEPA, the maximum allowed level of atrazine is 3 $\mu g\,l^{-1}$ [3].

Sample preparation is critical in complex sample analysis and includes both analyte preconcentration and sample cleanup. Sample preparation is traditionally carried out by liquid–liquid extraction or by solid-phase extraction, followed by additional sample cleanup steps [4]. An attractive alternative technique that is solventless, solid-phase microextraction (SPME), has been developed by Arthur and Pawliszyn [5]. The technique combines simultaneous extraction and preconcentration of analytes from various matrices. In most cases, SPME is carried out with direct immersion for clean aqueous samples, and in the headspace for dirty matrices [6]. In direct immersion SPME (DI-SPME), the fiber is directly exposed to the sample. A disadvantage of this approach is the decrease in the life time of the fiber. This is caused by the influence of salt addition, pH adjustment or coexisting compounds of the complex matrix. On the other hand, in headspace SPME (HS-SPME) mode [6], the fiber is placed above the liquid or matrix to be sampled and exposed to the vapor phase. HS-SPME is often the technique of choice if the analytes are appreciably volatile or can be made volatile with moderate heating of the sample [7]. However, when HS-SPME procedure is applied, most of the

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non-volatile compounds are excluded from the determination. This limits the scope of the procedure in comparison to DI-SPME.

Normally, the use of SPME to determine pesticides in complex matrixes (food samples, soil samples, biological fluids) requires sample pretreatment or modification of the sampling protocol in order to simplify the matrix and to prevent damage to the fiber [8,9]. Extraction of organic pollutants from complex matrices such as bovine milk and sewage sludge is difficult task due to the high content of proteins, fat in bovine milk and suspended particles in sewage samples. The complexity of the sample can affect the recovery of the analytes, and also the analytical method precision, the accuracy, and the sample compatibility with a subsequent chromatographic technique [10]. When a sample contains non-volatile and high molecular interfering compounds, such as proteins, humic acids and fatty material, analysis using either DI-SPME or HS-SPME is difficult. To overcome these difficulties, porous cellulose fiber protected direct SPME was used by Zhang et al. [11] for the analysis of polycyclic aromatic hydrocarbons. This appears to be the only report of protected-SPME so far.

In this work, polypropylene hollow fiber membrane was studied as a protective material during SPME. We termed this approach hollow fiber membrane-protected solid-phase microextraction (HFM-SPME). It was applied to the extraction of triazine herbicides from complex matrices including bovine milk and sewage sludge. To the best of our knowledge, this is the first report of triazine analysis using SPME in these matrices.

2. Experimental

2.1. Materials and chemicals

Atrazine (purity 98%), simazine (purity 99%), and propazine (purity 98%) were purchased from ChemService (West Chester, PA, USA); secbumeton (purity 96%), sebuthylazine (purity 98.6%), and desmetryn (purity 99.5%) were from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Internal standard, fluoranthene d-10 (purity 98.6%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Stock standard solutions were prepared in acetone at concentration levels of 500 μg ml^{-1} and stored at −20 °C. Working solutions were prepared by dilution of stock standards with methanol. The Accurel Q 3/2 polypropylene hollow fiber membrane was purchased from Membrana GmbH (Wuppertal, Germany). The inner diameter was 600 μm, the thickness of the wall was 200 μm, and the pore size was 0.2 μm. HFM-SPME was performed with Carbowax–divinylbenzene (CW–DVB), polydimethylsiloxane (PDMS)–DVB, PDMS and polyacrylate (PA) fibers housed in a manual holder (Supelco, Bellefonte, PA, USA). All SPME fibers were conditioned in a GC injection port under recommended conditions.

2.2. Bovine milk and sewage sample preparation

Fresh full-cream bovine milk samples were purchased off the shelf and stored at 4 °C. A small portion of the bovine milk samples (50 ml) was spiked with mixture of herbicide standards and used for method evaluation. After spiking, pH and ionic concentrations were varied. The sample was then stirred with a glass rod and allowed to equilibrate at room temperature for 5 min. The samples were extracted by HFM-SPME as described below. Sewage sludge samples were collected near a sewage treatment plant. Sewage sludges were sampled in glass bottles precleaned with acetone. The bottles were covered with aluminium foil and transported under cooled conditions to the laboratory and stored in the dark at −20 °C until analysis. The particle size distribution and other physical/chemical characteristics of the sludge samples were not determined.

2.3. HFM-SPME

Performing SPME directly in a bovine milk sample is inherently more difficult owing to the presence of interfering compounds. The HFM-protected SPME setup is illustrated in Fig. 1. The commercially available polypropylene HFM was used without any pretreatment. The internal diameter of the HFM (600 μm), is large enough to accommodate the SPME stainless steel protective tubing for the fiber. The SPME fiber assembly was inserted into 7-cm long (one end sealed by flame) HFM so that the latter completely covered the stainless steel tubing and the polymeric fiber. A long-neck 10-ml vial was filled with 5 ml of the bovine milk sample (30% (w/v) sodium chloride and adjusted to pH 10). The HFM-protected SPME fiber was exposed to the sample solution for 40 min to attain extraction equilibrium. During extraction,

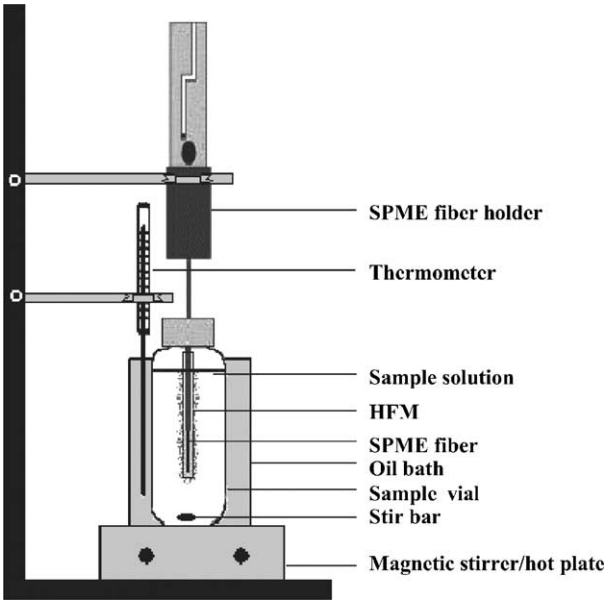

Fig. 1. Schematic of HFM-SPME.

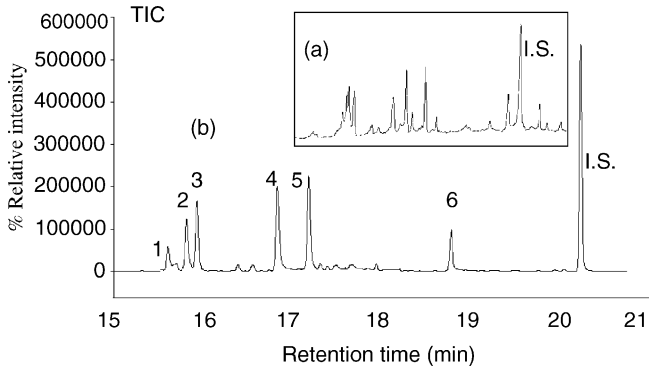

Fig. 2. GC–MS chromatogram of a spiked bovine milk sample (20 μg l^{-1} of each analyte) obtained by (a) direct-SPME; and (b) HFM-SPME. Peaks: (1) simazine; (2) atrazine; (3) propazine; (4) secbumeton; (5) sebuthylazine; (6) desmetryn; and internal standard (IS) (fluoranthene d-10, 50 μg l^{-1}).

the polymeric fiber was immersed in the sample solution, as in conventional SPME (about half of the HFM-protected stainless steel tubing was also immersed). Each sample was stirred vigorously (130 rad s^{-1}) during the sorption step using a stir bar. After extraction, the HFM was discarded. The metallic tubing of the SPME fiber holder and the fiber were gently wiped with soft tissue to remove water droplets. No problems with interference by water were observed and thermal desorption of the analytes was achieved as normal, by inserting the SPME fiber into the GC injection port (held at 280 °C) for 5 min [12]. All desorptions were performed in the splitless mode. The fibers were reused for up to 50 analyses.

Attempts were made to extract bovine milk samples without protecting the SPME fiber. These led to fatty material deposition on the GC column and some peak tailing (Fig. 2a). On the other hand, HFM-SPME gave good separation and well resolved peaks (Fig. 2b). It can also be anticipated that the lifetime of the column would be considerably shortened under these conditions.

2.4. GC–MS analysis

Analysis of triazines was performed on a Shimadzu (Tokyo, Japan) QP2010 GC–MS system. The GC was fitted with DB-5 column (30 m, 0.32 mm i.d., 0.25 μm) from J&W Scientific (Folsom, CA, USA). Helium was used as the carrier gas at a flow rate of 1.8 ml min^{-1}. The following temperature programme was employed: Initial temperature 90 °C for 4 min; increased at a rate 25 °C min^{-1} to 160 °C, held for 2 min; then another increase at 2 °C min^{-1} to 180 °C and a third increase at 20 °C min^{-1} to 280 °C, held for 5 min. The injector temperature was 280 °C, and all injections were made in splitless mode. The detector was scanned over the *m*/*z* range 50–500 to confirm the retention times of the analytes. For determination of triazines, selected ion monitoring (SIM) was performed. For confirmation of triazine ions tentatively identified by SIM, two characteristic fragment ions were monitored in addition to the molecular ion. The interface temperature was set at 200 °C.

3. Results and discussion

3.1. Optimization of HFM-SPME

Prior to the application of HFM-SPME to the determination of triazine herbicides from bovine milk samples, it was necessary to optimize the HFM-SPME parameters that were deemed to be important for efficient extraction, such as extraction time, extraction temperature, sample pH and the ionic concentration of the sample. These are parameters that are usually considered in extraction work of the nature. The precision and linearity relating to the calibration plots were investigated.

The type of SPME fibers used plays an important role in the extraction and analysis of analytes. Four different commercially available SPME fibers were evaluated for HFM-SPME with bovine milk samples. The moderately polar PDMS–DVB fiber gave better efficiency [13] than the non-polar PDMS, as expected. They gave comparable results with more polar PA and Carbowax–DVB fibers (data not shown). Therefore, the 65-μm PDMS–DVB fiber was used for further HFM-SPME investigations of triazine extraction from bovine milk samples.

The adsorption profile of the triazine herbicides in bovine milk sample on the PDMS–DVB was determined by extracting the analytes for 10–60 min at room temperature. The highest extraction was achieved at 40 min for all analytes although the standard deviation was higher than with longer extraction time (Fig. 3). However, during long extraction times, fatty and protein materials were more likely to clog the pores of the HFM and reduce the diffusion of the analytes, resulting in lower extraction. Therefore, 40 min was most suitable for extraction.

Increasing the sampling temperature can normally speed up mass transfer and allow HFM-SPME to extract more analytes although in one study, it was reported that increase in temperature caused low analyte enrichment [14]. Five temperatures ranging from 40 to 90 °C were studied in this work.

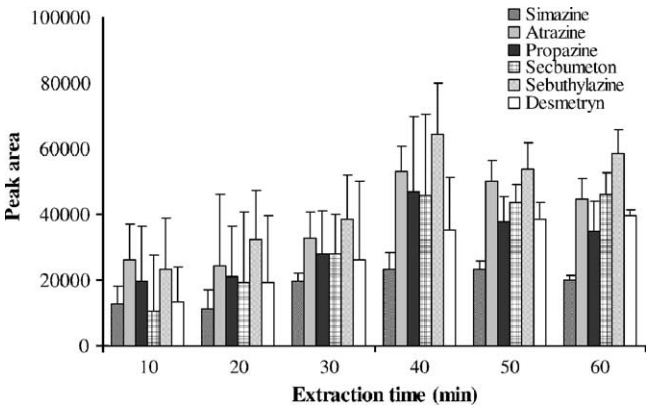

Fig. 3. Effect of exposure time on the extraction of triazines from bovine milk samples by HFM-SPME at room temperature with PDMS–DVB fibers (concentration, 20 μg l^{-1} of each analyte) and 130 rad s^{-1} stirring speed (n = 3) (no adjustment of sample pH and ionic strength).

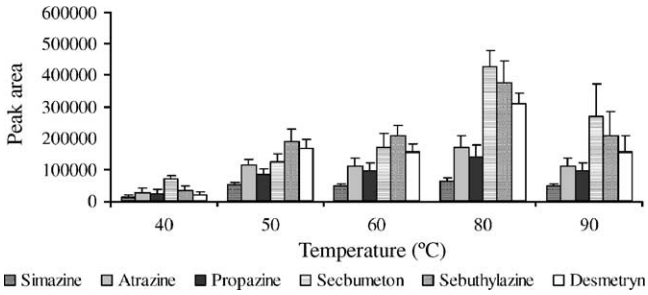

Fig. 4. Effect of bovine milk sample temperature on extraction efficiency of HFM-SPME. Concentration, 20 $\mu g\,l^{-1}$ of each analyte. Extraction time was 40 min and extraction was performed with PDMS–DVB fiber at 130 $rad\,s^{-1}$ stirring speed (n = 3).

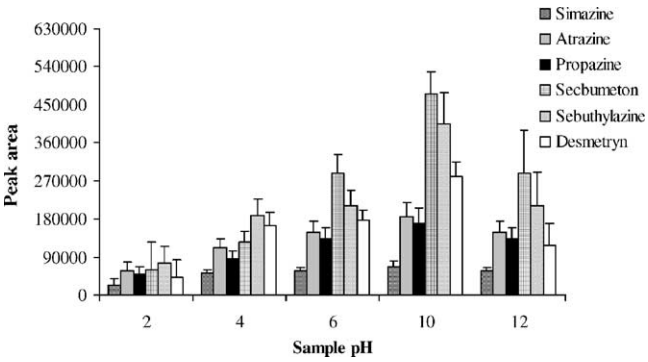

Fig. 5. Effect of pH of bovine milk sample solution on HFM-SPME. Concentration, 20 $\mu g\,l^{-1}$ of each analyte. Extraction time, 40 min, at 130 $rad\,s^{-1}$ stirring speed and sample temperature 80 °C (n = 3).

As shown in Fig. 4, simazine, atrazine and propazine show the highest extraction efficiencies at 80 °C. The figure also shows that higher temperatures (>80 °C) were not suitable to increase extraction efficiencies. Since sorption is an exothermic process, poor analyte enrichment (based on peak areas), in this case, predominated over increased mass transfer at elevated temperatures. Thus, 80 °C was optimum and was used for subsequent experiments.

The influence of extraction pH on HFM-SPME over the range of 2–13 was investigated. Sample pH may play an important role to reduce protein binding with organic contaminants [15]. At pH 10 we obtained higher responses than at strongly acidic or basic conditions (Fig. 5). This could be due to hydrolysis of triazines under strongly acidic or basic aqueous environments [16,17].

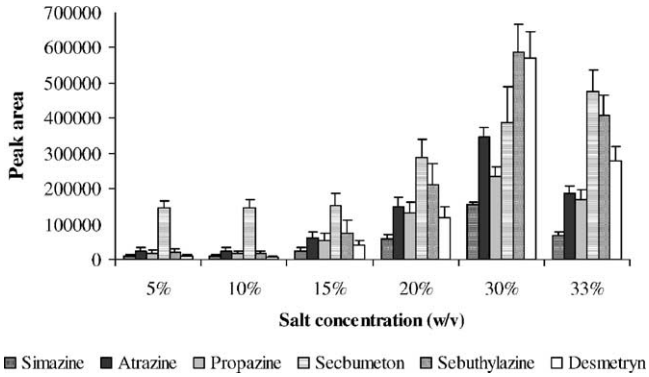

Fig. 6. Effect of sodium chloride concentration on HFM-SPME of triazines from bovine milk sample (concentration, 15 $\mu g\,l^{-1}$ of each analyte) at extraction temperature 80 °C for 40 min extraction with pH 10 and stirring speed was 130 $rad\,s^{-1}$ (n = 3).

The impact of sodium chloride addition to the bovine milk sample was investigated. As triazines are relatively polar analytes, the addition of salt proved to be very effective in increasing the extraction yield of triazines in HFM-SPME. Salt flocculates the bovine milk sample and decreases the solubility of the analytes in this matrix, enhancing the extraction by the fiber. Fig. 6 summarizes the results. Sodium chloride at >30% led to a decrease in the extraction of the triazines, possibly due to its effect on the HFM itself. 30% of sodium chloride was therefore selected as the optimum concentration.

3.2. Linearity, limits of detection, and repeatability

To evaluate the applicability of the proposed HFM-SPME procedure to bovine milk, the repeatability, linearity and limits of detection were investigated using the optimum extraction conditions. The performance of this method is shown in Table 1. To evaluate the linearity of the calibration plots, bovine milk samples were spiked with triazines to give final sample concentrations of 5, 10, 15, 30, 50, 75, 100 and 200 $\mu g\,l^{-1}$, and then extracted. The GC peak area counts were plotted against the respective triazine concentrations to generate calibration curves. The calibration plots were linear over the range of 0 $\mu g\,l^{-1}$ (blank) to 200 $\mu g\,l^{-1}$ with correlation coefficients (r) between 0.9799 and 0.9965 (Table 1).

Table 1
Relative recoveries, precision (R.S.D., n = 3), linearity, and LODs (S/N = 3) of HFM-SPME

Analyte	Spiked bovine milk samples (%)		Correlation coefficient (r)[a]	LODs ($\mu g\,l^{-1}$)	LOQs ($\mu g\,l^{-1}$)
	1 $\mu g\,l^{-1}$	20 $\mu g\,l^{-1}$			
Simazine	56.9 ± 4.4	94.1 ± 9.8	0.9923	0.007	0.020
Atrazine	61.5 ± 8.2	101.5 ± 5.4	0.9874	0.005	0.011
Propazine	98.2 ± 12.4	107.2 ± 4.3	0.9799	0.003	0.009
Secbumeton	77.3 ± 11.4	88.4 ± 4.6	0.9928	0.006	0.009
Sebuthylazine	84.2 ± 11.9	98.8 ± 2.8	0.9928	0.003	0.006
Desmetryn	86.1 ± 4.5	94.51 ± 0.5	0.9965	0.013	0.021

[a] Linearity range 0–200 $\mu g\,l^{-1}$.

Table 2
Extraction of triazines from sewage sludge samples by HFM-SPME (n = 3)

Analyte	Spiked sludge samples (20 μg l^{-1})		Spiked Sludge samples (100 μg l^{-1})		LODs (ng/g)
	Percent of relative recovery	R.S.D.%	Percent of relative recovery	R.S.D.%	
Simazine	106.2	1.1	95.6	6.2	0.005
Atrazine	98.6	7.1	95.5	5.7	0.003
Propazine	93.3	8.3	97.7	9.4	0.001
Secbumeton	113.2	2.2	107.8	7.3	0.004
Sebuthylazine	101.3	8.1	103.6	8.6	0.002
Desmetryn	105.9	12.3	107.5	12.9	0.009

Limits of detection (LODs) of triazines in the bovine milk samples, calculated at a signal to noise ratio of 3 under MS-SIM conditions, were in the range of 0.003–0.013 μg l^{-1}. Limits of quantification (i.e. the standard deviation of the replicate samples multiplied by 10) [18] were in the range of 0.006–0.021 μg l^{-1} (Table 1). The LODs of the proposed method are comparable with DI-SPME of water samples [19]. Therefore, the proposed extraction procedure could be useful for trace level environmental analysis without further pretreatment. The repeatability study was performed by extracting bovine milk samples spiked at 1 and 20 μg l^{-1} of each compound (three replicates). HFM-SPME is not an exhaustive, but rather an equilibrium extraction procedure. Relative recoveries were calculated based on the extraction of spiked ultrapure water in comparison with spiked bovine milk sample at the same level of spiking. The recoveries varied between 57 and 107% and the relative standard deviations (R.S.D.) were calculated to be from 4.3 to 12.3%. Low extraction recoveries were obtained for low-concentration spiked (1 μg l^{-1}) samples (Table 1) for simazine and atrazine, probably due to their being partially bound to the matrix. For the higher-concentration spiked samples (20 μg l^{-1}), there were no significant differences in recovery between water and bovine milk samples.

3.3. Comparison of HFM-SPME with DI-SPME and HS-SPME

Optimized conditions from previous reports were used in this work for DI-SPME (without HFM protection) and HS-SPME [20,21]. Fig. 7 shows the comparison of HFM-SPME with DI-SPME (the later was used for extraction from a spiked ultrapure water sample, since it cannot be used for bovine milk samples), and HS-SPME. In view of the HFM protection of the SPME fiber, HFM-SPME gave noticeable benefits as compared to HS-SPME. In HFM-SPME, the extraction is slower than for DI-SPME due to analytes needing to diffuse though the HFM [11,22]. However, it seems probable that the HFM prevents substances such as fats, proteins and other substances from interfering with the extraction. HFM-SPME gave better enrichment than HS-SPME (see Fig. 7) which allowed us to determine sub-ppb level concentrations of triazines in bovine milk samples without further sample pretreatment.

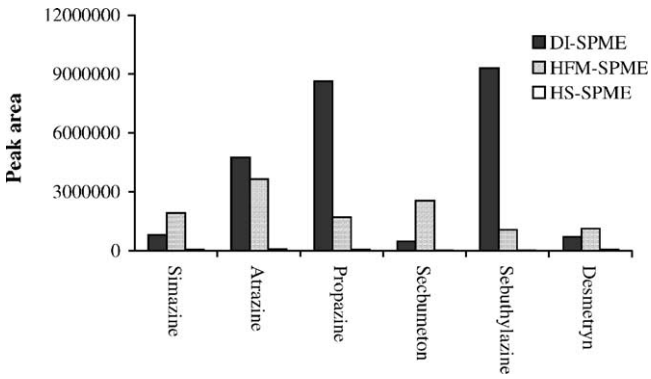

Fig. 7. Comparison of direct SPME (with out HFM protection) of ultrapure water with HFM-SPME and headspace-SPME of bovine milk samples. Concentration of each analyte, 50 μg l^{-1} (n = 3). Both DI-SPME and HS-SPME was performed at 30 min, room temperature and at 95 °C, respectively [20,21].

3.4. Extraction of sewage sludge samples

As an additional demonstration of the applicability of HFM-SPME developed in this study, sewage sludge samples (30 mg of sample/ml of water) were analysed. The data in Table 2 show the suitability of the procedure to the analysis of triazines from these samples. The percentage recovery for samples with two different spiked concentrations (20 and 100 μg l^{-1}) varied between 93.33 and 107.78% and the R.S.D.% were <13%. The present method was able to overcome the problems encountered in normal SPME because of the protection afforded by the HFM. Typically, in normal SPME, particles must be removed from samples by filtration before extraction. The HFM addresses this issue and also eliminates the possible damage to the fiber; high molecular-mass compounds and particulates were protected by the membrane from reaching the fiber. This prolongs fiber life and lowers the cost of analysis.

4. Conclusion

This study has demonstrated the feasibility of using a polypropylene hollow fiber membrane (HFM) protective sleeve for the SPME fiber in the microextraction of triazines from “dirty” and complex matrices. For milk sample, there is no need for defatting and deproteinization. The

HFM, apart from being protective of the SPME fiber, also acts as a barrier to interfering substances in the milk. Additionally, the applicability of HFM-SPME was extended to sewage sludge samples. As with milk samples, HFM-SPME of the latter achieved good enrichment, low limits of detection, i.e. lower than USEPA and EU requirements, and satisfactory relative standard deviations. This procedure has a disadvantage: unlike direct immersion or headspace SPME, automation cannot yet be implemented easily and conveniently. However, it is potentially ideal for the direct extraction of analytes from biological and other complex and "dirty" samples.

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