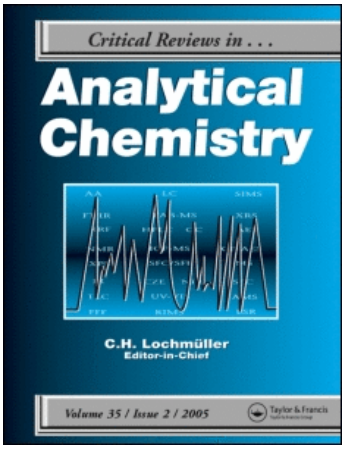

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New Trends in Solid-Phase Microextraction

Ralf Eisert[a]; Janusz Pawliszyn[a]
[a] Department of Chemistry, University of Waterloo, Waterloo, Ontario, Canada

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New Trends in Solid-Phase Microextraction

*Ralf Eisert and Janusz Pawliszyn**

Department of Chemistry, University of Waterloo, Waterloo, Ontario, N2L 3G1, Canada

* Author to whom correspondence should be addressed.

ABSTRACT: Recently introduced solid-phase microextraction (SPME) method has been applied to many different applications including analysis of air, water, and soil, both organic and inorganic target analytes. Several new devices based on SPME were developed to facilitate the application for air monitoring, integrated sampling, fast gas chromatography, solid-phase microextraction coupled to high-performance liquid chromatography, on-line screening systems, and automation of the entire sample preparation apparatus. This paper discusses recently published SPME methods in this field to outline the high potential of this unique microsampling technique. In addition, solid-phase microextraction is compared to classical sample preparation methods to demonstrate the advantages of this micro technique and new application fields of this technique.

KEY WORDS: solid-phase microextraction (SPME), environmental samples, automation, (fast) GC, microcolumn separations, LC/MS, pesticides, air analysis, on-line screening, field analysis, HPLC.

I. INTRODUCTION

To date solid-phase microextraction (SPME) has been applied for many different areas in analytical chemistry [1]. The basic concept of SPME was first described by Belardi and Pawliszyn [2]. This new extraction technique is based on the partitioning of the analyte between the extracting phase immobilized on a fused-silica fiber and the matrix (air, water, etc.). After equilibrium is reached or a well defined time the absorbed compounds are thermally desorbed by exposing the fiber into the injection port of a gas chromatograph or redissolved in an organic solvent, respectively, if coupled to HPLC. The technique was made practical by placing the fiber in a microsyringe [3] which was commercialized in 1993 by Supelco as SPME device illustrated in Figure 1. The initial work on solid-phase microextraction was exclusively done by SPME/GC coupling [3–9]. In 1995 the first interface for SPME/HPLC coupling was described [10]. Within the first years from the introduction of SPME it was mainly used for new analytical methods using this simple and easy extraction method. The early applications included air, water and soil analysis [6,11,12]. To date, SPME is mainly used with a manual SPME device coupled to GC. New devices are capable to perform the entire analysis including the sample preparation fully automated which significantly increases the throughput of the analytical method. Automation of the entire SMPE/GC system was achieved by modifying a commercial GC autosampler [4, 13]. Furthermore, mathematical models were developed to describe the mass transfer onto the fiber and diffusion phenomena which determine the kinetics of extraction's [1,5]. However, the most appli-

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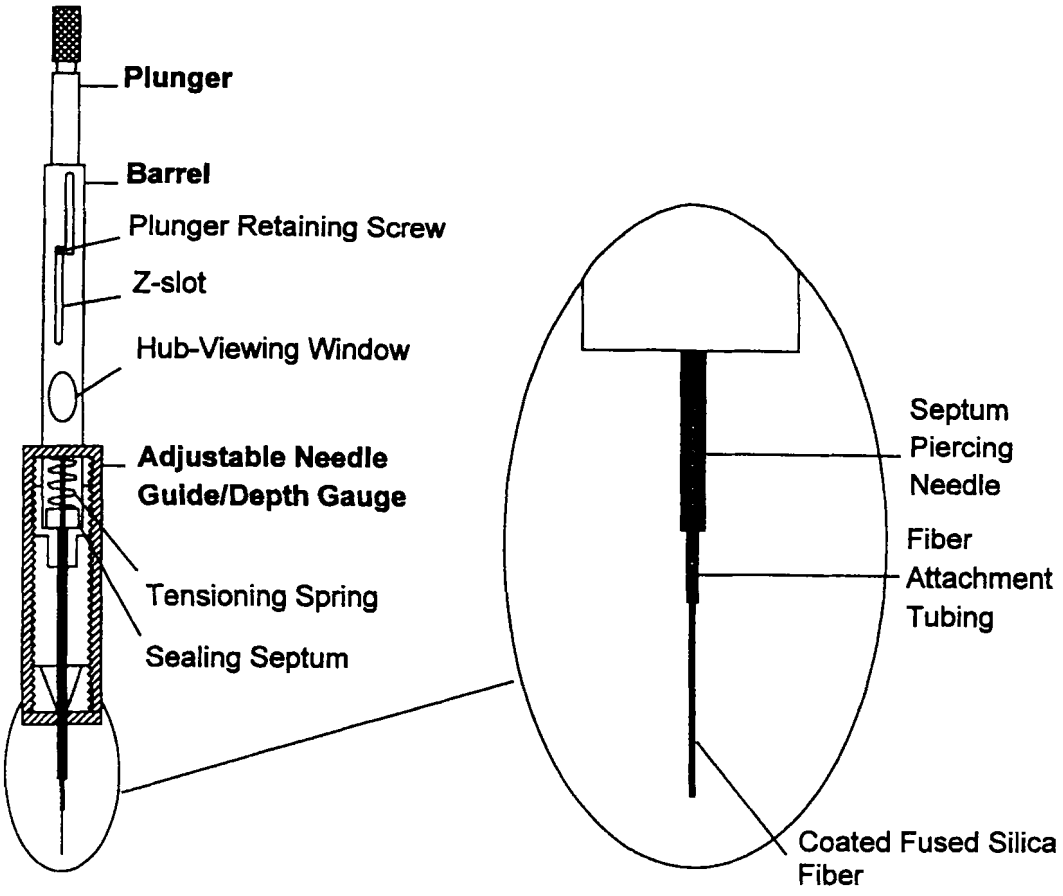

FIGURE 1. Schematics of the SPME fiber and exposure. From reference [1]. With permission.

cations of SPME are still based on the manual device. Insufficient temperature control and a lack for agitation techniques slowed down the initial rapid growth of automated SPME/GC applications. Within the past two years, many new devices and coupling techniques were developed to overcome initial problems, e.g., agitation for the automated SPME device [14], field portable devices [15], and analysis of thermally labile organic compounds by successful automated hyphenation to HPLC [16] and inorganic target analytes [17–19].

This review summarizes all recently published articles covering the above mentioned new topics where SPME methods are successfully established. It mainly focuses on new applications in environmental and biomedical analytical chemistry, automated SPME, interfacing to HPLC, automated in-tube SPME/HPLC, calibration techniques based on physico-chemical properties of the target compounds, analysis of soil samples, SPME-LC/MS of sludge samples, field sampling devices, on-site analysis and automated on-line systems. Solid-phase microextraction is not restricted to screening purposes. Different calibration methods are leading to a high precision in quantitative analysis. The fast transfer of the sample from the extraction to separation and detection underlines its high potential for applications where the storage time of classical extraction and sample preparation techniques failed to determine the sample composition. A new area, real-time analysis, which is an important aspect for flavor and perfume analysis opens a wide application field for SPME methods.

Furthermore, the simple handling of the entire solvent-free sample preparation technique should be considered a main advantage when on-site and field analysis is under investigation. SPME provides unique characteristics when sampling and determination of target compounds in remote areas and/or instant decisions (accidents) or proof (forensic) data are recommended.

II. THEORY

The theory of solid-phase microextraction covers the principal processes of SPME applying basic fundamentals of thermodynamics and mass transfer. The simplified mathematical relations which are reported in more detail in the literature [1,5] will be briefly discussed here to provide insight and practical help for method optimization. The theory was initially developed for ideal extraction conditions which are very accurate for trace concentrations and simple matrices like air and groundwater, however, they can be used for more complex and heterogeneous matrices to a first approximation.

A. Kinetics

The extraction time can be estimated by solving differential equations describing mass transfer conditions in the system. The kinetics of the extraction process determines the speed of extraction. The theory of mass transport is mostly based on Fick's second law of diffusion describing mass balance in a dynamic system [20].

1. Direct Extraction

A typical exposure of the fiber when sampling semi- and non-volatile compounds is direct extraction from a homogeneous aqueous phase. In theory there is no headspace present. Practically there is always a small headspace volume in the sampling vial, which shows no significant deterioration of the analytes in the aqueous phase. Under perfect agitation conditions, e.g., magnetic stirring, the aqueous phase moves very rapidly with respect to the fiber. Theoretically all analyte molecules present in the sample should have easy access to the fiber coating. The time to reach equilibrium is significantly lower compared to the same experiment done for static (no agitation) conditions. Figure 2 shows a typical result obtained when both exposure techniques are compared. In this case the equilibration time for compounds with higher coating/sample distribution constants, K_{fs}, values, such as parathion, is reduced from more than 2 h to 35 min (see Figure 2). From the theory [5] the time required to reach equilibrium is infinitely long. However, in practice a change in mass extracted cannot be determined if it is smaller than the experimental error, which is typically 5%. Thus, the equilibration time is assumed to be achieved when 95% of the equilibrium amount of the analyte is extracted from the sample.

Independently from the agitation level and method (magnetic stirring, fiber vibration, flow-through cell, etc.), fluid contacting a fiber's surface is always stationary, and as the distance from the fiber surface increases, the fluid movement gradually increases until it corresponds to the bulk flow in the sample. The model describing the mass flow is using this boundary layer concept [5] assuming that in a defined zone surrounding the fiber no convection occurs and perfect agitation is everywhere in the bulk solution. This static layer is called a *Prandtl* boundary layer (see Figure 3) [21]. The thickness of the *Prandtl* boundary layer depends on the agitation speed and viscosity of the fluid. Thus, agitation methods are very successful in reducing the thickness of the layer which determines the mass transfer into the fiber

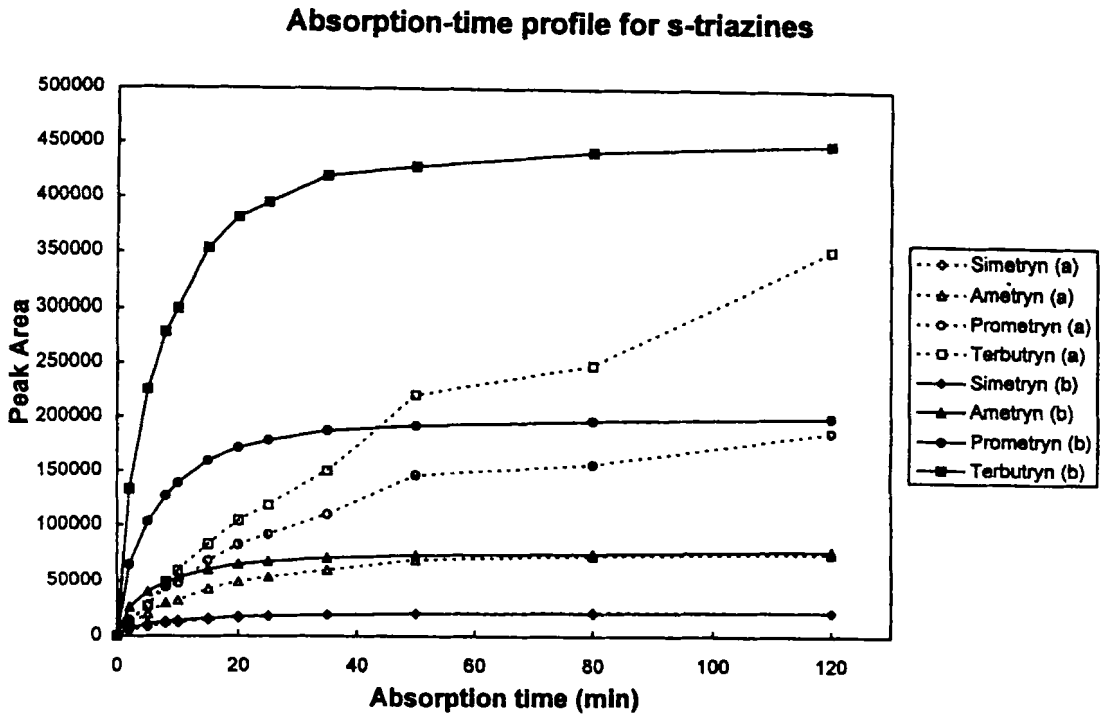

FIGURE 2. Absorption-time profiles for four s-triazines and parathion using (a) static absorption conditions and (b) fiber vibration method (for 2 ml vials). The equilibrium is reached substantially faster for agitation techniques. From reference [57]. With permission.

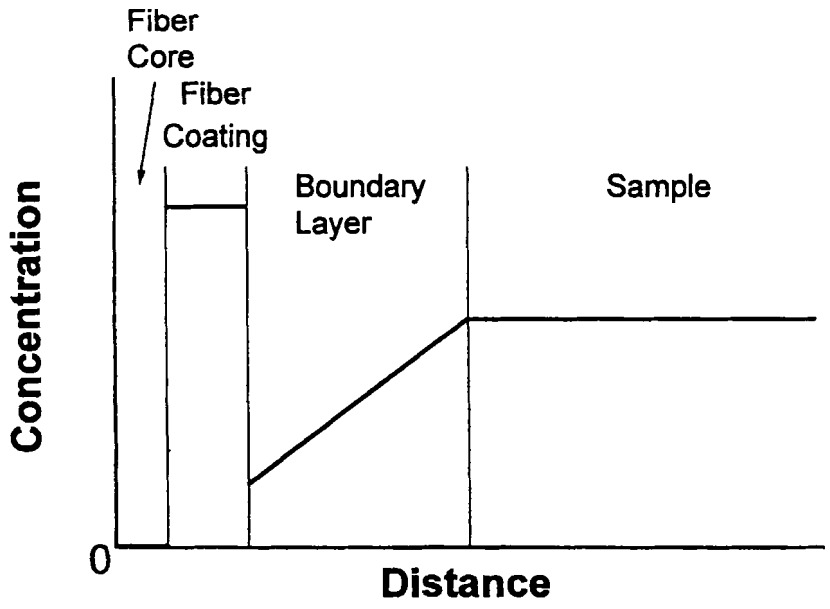

FIGURE 3. Boundary layer concept. From reference [1]. With permission.

coating. Thus, the equilibration time is significantly decreased.

2. Desorption

After the extraction of the analytes is complete, the fiber containing the analytes is transferred to the injection port of a GC or HPLC instrument. During the desorption process, the analyte diffuses from the coating into the stream of carrier gas (GC) or the solvent fluid (HPLC). The desorption process is inverse to the absorption from a well-agitated solution. The initial concentration in the fluid should be zero and a high linear flow rate surrounding the fiber is necessary to fulfill these requirements. The high linear flow is important for instant removal of the analytes from the vicinity of the coating. Practically these requirements can be achieved inside a very narrow insert placed in the heated GC injector or a narrow tube used for the injection chamber when SPME is coupled to HPLC. In practice however, the flow of the mobile phase has an finite value reducing the desorption process.

B. Thermodynamics

SPME is a multiphase equilibration process. In many cases the extraction system is very complex, such as an aqueous sample containing suspended solid particles including various adsorption interactions with analytes plus a gaseous headspace. The system can be simplified by initial assumptions which characterize the system significantly. The amount extracted by the fiber at equilibrium conditions can be derived from thermodynamic principles. The detailed model is discussed in the literature [1,7]. The amount absorbed on the fiber, n, can be expressed by:

$$n = \frac{K_{fs} V_f C_0 V_s}{K_{fs} V_f + K_{hs} V_h + V_s} \qquad (1)$$

where C_0 is the initial concentration of the analyte in the matrix; V_s, V_h and V_f are the volume of the sample, the headspace and the coating and K_{fs}, K_{hs} are defined as the coating/sample and headspace/sample distribution constants. The Equation (1) states, as expected from the equilibrium conditions, that the amount of analyte extracted is independent of the location of the fiber in the system. It may be placed in the headspace or directly in the sample as long as the volume of the fiber coating, headspace, and sample are kept constant. For aqueous samples with no headspace the equation can be simplified to:

$$n = \frac{K_{fs} V_f C_0 V_s}{K_{fs} V_f + V_s} \qquad (2)$$

Both Equations (1 and 2) describe the mass absorbed by the polymeric coating after equilibrium has been reached. For a first approximation (K_{fs} is small) Equation 2 can be further simplified:

$$n = K_{fs} V_f C_0 \qquad (3)$$

For large sample volumes (V_s (K_{fs} V_s) the amount extracted is directly proportional to the initial concentration C_0 in the sample. It should be emphasized that for compounds with high K_{fs} values the sample volume, V_s, significantly contributes to the amount extracted. Thus, this first approximation is not valid. The fact is more important if SPME will be used to determine physico-chemical data such as K_{fs} values of the compounds.

C. Quantitation

The fiber blank will verify that neither the instrument nor the SPME apparatus is contaminated by analytes or interfering compounds. The amount absorbed by the fiber coating can be expressed by the equations

shown above. Depending on the exposure and matrix which is used the equation can be simplified. In practice, different calibration methods are applicable to SPME. The external calibration can be easily applied for homogenous and very clean samples such as air or groundwater [22,23]. Using equilibrium conditions, and knowing the K_{fs} values of the compounds, the initial concentration can be determined. For complex matrices or variable matrix composition standard addition techniques which compensate the matrix effect or isotopically labeled standards should be used which typically show a higher precision and more reliable data [24]. For non-equilibrium conditions and unknown K_{fs} values of the compounds the system can be calibrated using spiked (aqueous) samples. However, matrix effects are not compensated in this method which is limited to very clean samples.

1. LTPRI for Air and Water

In addition to experiment the external calibration can be performed using physicochemical parameters or chromatographic results. The concept of retention indexes from linear temperature-programmed GC can be applied to estimate air/coating distribution coefficients and calibrate the SPME method based on a single injection [25,26]. In this approach the column phase is identical to the SPME coating [25]. It was successfully applied to the analysis of organic compounds in air [25] and water [27]. The calibration technique is based on a simple concept which is used to estimate distribution coefficients (K_{fa}) between the matrix (air) and the poly(dimethylsiloxane) SPME fiber coating. The technique uses the linear temperature-programmed retention index system (LTPRI). A linear relationship ($r^2 = 0.99989$) between the log K for a series of compounds (n-alkanes) and LTPRI was determined [25] see Equation 4:

$$\log K = a + b(LTPRI) \qquad (4)$$

where a is the y-intercept and b the slope of the calibration curve (detector response).

Thus, the K value of an unknown compound can be established in a single GC run. Determination of the LTPRI of this compound leads automatically to its K value. The concept was developed for 29 isoparaffinic compounds and a group of 33 aromatic compounds and applied for determining a complex mixture of gasoline [25]. The results obtained were compared to standard procedures and showed identical results.

III. OPERATING PRINCIPLES AND OPTIMIZATION PROCESS

A. Extraction Modes

The details of optimization process are discussed in Reference [1]. Based on the sample matrix, analyte volatility and its affinity to the matrix, the extraction mode should be selected. In general, there are three different exposure techniques: headspace or air, direct sampling from the aqueous phase and direct exposure using membrane protected extraction [28]. For very volatile compounds (VOCs) such as BTEX or Purgeables direct air sampling or headspace sampling can be considered. Headspace is preferred due to faster equilibration times. The selectivity is higher when dirty samples are analyzed. Clean aqueous samples such as groundwater can be extracted in the direct extraction mode especially if semi- and nonvolatile compounds will be extracted. For very dirty samples the fiber can be protected using a membrane protection. Table 1 summarizes the criteria for the right selection of the fiber coating. For aqueous samples, very polar compounds such as strong acids and bases are very difficult to extract. The adjustment of the pH value and addition of salt can be considered to extract

TABLE 1
Sampling Mode Selection Criteria

Sampling mode	Analyte property	Matrices
Direct	Medium to low volatility	Gaseous samples, Liquid (preferably simple)
Headspace	High to medium volatility	Liquid (including complex), soils
Membrane protection	Low volatility	Complex samples

these compounds which have a high affinity toward the matrix.

1. Coatings

To date (spring 1997), several different coating materials are available from Supelco. There are three different poly (dimethylsiloxane) (PDMS) films of different thickness (7, 30, and 100 μm), 85 μm poly(acrylate) (PA), 65 μm poly(dimethylsiloxane)/divinylbenzene (DVB), 75 μm Carboxen/PDMS, 65 μm Carbowax/DVB, and 50 μm Carbowax/template resin fibers. For the right selection of the polymer coating the general principle of "like dissolves like" applies. PDMS coated fibers are typically the first choice. They are very rugged and liquid coatings are used as GC stationary phases withstanding temperatures up to 300°C. This coating is apolar showing a high affinity for non-polar compounds. Very thin fiber coatings should be used whenever the sensitivity is sufficient. The extraction time is shorter for a thinner coating and smaller distribution constants of the analytes. The PA fiber shows a high affinity for polar compounds such as phenols [8] and polar pesticides [23]. PA is a solid polymer, thus the equilibration times are higher compared to the liquid PDMS fibers. The mixed phase coatings are more suitable for volatile compounds. The take-up of this fibers is significantly higher compared to PDMS. When changing from PDMS to Carbowax using the mixed phase coatings more polar analytes can be extracted such as ketones and alcohols.

2. Agitation

The diffusion process in air is very fast and only limited by the diffusion in the coating compared to diffusion in water. Many volatile analytes reach equilibrium within 5 min when sampling from air or headspace. The equilibrium for semi-volatile compounds using direct exposure of the fiber to the aqueous sample takes up to 2 hours under static absorption conditions. The equilibration time is significantly reduced when agitation is applied during the extraction process [14,29]. The effectiveness of the agitation process determines the equilibration time in aqueous samples. Magnetic stirring is the most commonly used agitation technique. However, one has to ensure that the rotation speed of the stir bar is constant and the base plate is thermally isolated from the vial containing the sample. Otherwise, heating of the sample might result in loss of precision.

Headspace sampling is very fast and magnetic stirring does not affect the diffusion from the headspace to the fiber, however, when the concentration in the headspace is significantly reduced by the SPME fiber exposure, the mass transport between the aqueous sample and the headspace slows down the extraction process. Agitation facilitates the equilibrium between the headspace and the aqueous

phase during the SPME sampling, thus reducing the depletion of the headspace concentration.

The alternatives which will be discussed in detail further below should be considered especially for automated or on-line systems such as fiber vibration and flow-through cell design facilitating a high linear flow at the fiber surface [14]. The fiber vibration technique is implemented by Varian in the new agitated SPME autosampler for GC.

3. Optimizing Extraction Conditions

To achieve a high precision of the SPME method the conditions including temperature, pH, salt concentration, sample volume, and the extraction time should be standardized. This is more important if real samples, like surface water, have to be analyzed where these parameters may vary. The following chapter briefly summarizes parameters which can significantly affect the extraction yield.

a. Temperature

The temperature affects both the sensitivity and the extraction kinetics. The increase in the extraction temperature causes an increase in the extraction yield, but simultaneously a decrease in the distribution constant. The temperature and especially the control of the temperature is very important for air and headspace analysis. An internally cooled fiber SPME device was developed by Zhang et al., which eliminates the sensitivity loss [30]. This device is not commercially available and reduces the selectivity of the fiber coating. The device is operated like a "cooled finger" leading to exhaustive extraction conditions.

b. Salt Addition and pH Adjustment

Addition of salt such as NaCl and $NaSO_4$ can either increase or decrease the amount extracted depending on the compound and the salt concentration. In general, the amount extracted increases with increasing salt concentration in the aqueous phase and increased polarity of the compound. The sensitivity can be significantly increased for polar analytes such as triazine pesticides by a factor of up to ten [14].

The dissociation equilibria in the aqueous phase are strongly affected by the pH adjustment. The adjustment of the pH in the aqueous sample can improve the sensitivity for acid and basic analytes. The decrease of the pH results in concentration increase of basic species present in the sample. In practice, it is very difficult to implement large pH change with the direct extraction approach since high and low pH damages the coating. A typical range for varying the pH is 2 to 10. Appropriate buffer should be used to ensure high reproducibility if basic and acid compounds are present in the sample.

4. Sample Volume

The volume of the sample should be selected based on the estimated distribution constant K_{fs}. As expressed in Equation (1) the sensitivity is affected by the volume of the sample if the distribution constant of the analyte is very high. A large sample volume (10 ml) should be used when extracting compounds with high K_{fs} values. Furthermore, thin coatings should be considered to reduce the equilibration time for these analytes. To increase the sensitivity of headspace extraction the volume of the gaseous phase should be minimized. Very volatile compounds will be accumulated in the headspace, resulting in a substantial loss in sensitivity when the headspace volume is large. Furthermore, a very precise evaluation of the volume is necessary if K values have to be determined [31].

B. Desorption Interfaces

Two desorption techniques are used for SPME to transfer the absorbed amount from

the fiber coating to a chromatographic column for separation and detection. For coupling to gas chromatography the fiber is exposed to the heated injection port of a GC and thermal desorption releases the analytes from the fiber. When coupled to HPLC, the fiber is placed in a small desorption chamber, a piece of HPLC tubing, where desorption into an organic solvent releases the compounds from the coating [10,32].

1. Thermal Desorption in GC

A narrow bore insert is required for fast desorption using a splitless injector or a septum-programmable injector (SPI). Hot on-column injection can be used in addition. The narrow bore insert sustains a high linear flow around the fiber during the desorption, thus reducing the desorption time. The highest possible desorption temperature which is amenable for the target analytes and the fiber coating should be used for a fast transfer of the analytes. The injection band can be sharpened by using a thick film column, cryofocusing, or retention gap. Cryotrapping is necessary for a limited number of applications. In general, a 1 μm thick column is sufficient for sharpening of the injection band width of volatile organic compounds. The fiber should be exposed immediately after the introduction to the insert and placed at the heated part of the injector (depth). Possible carryover of unknown compounds with a high affinity to the fiber present in real samples can be easily removed when using a split/splitless injector. The sample introduction is performed in the splitless mode. After the desorption of the target analytes the fiber is still kept in the injector for an additional time operating the injector in the split mode (purge on).

2. Solvent Desorption in HPLC

For the manual SPME/HPLC a commercial interface is recently available from Supelco. It is based on the initial design [10] used for coupling SPME with HPLC (loop type injection). The appropriate solvent selection and the flow determine the desorption process. The manual SPME/HPLC interface uses the initial mobile phase composition for the desorption. The linear flow rate should be maximized by choosing a small ID tubing. This is very important since the volumetric flow rate in HPLC is very low. The main disadvantage of this injection concept is carryover which is mostly related to desorption conditions. The elution power of the initial eluent composition is not sufficient for a quantitative desorption of the absorbed analytes, e.g., high molecular weight compounds (PAHs). Heating of the interface might help to increase the driving force of desorption, but this is limited by thermal stability of the target compounds. In addition the desorption chamber can be filled with a pure organic solvent which increases the desorption power. However, peak broadening might be increased, too. A complete separation of the sample desorption step from the transfer to the HPLC column can help to overcome these effects. This approach is used in the later discussed automated in-tube SPME system. Figure 4 shows the instrumental setup for the manual SPME/HPLC interface. The commercial version is only slightly different from this. The number of different fibers available for SPME/HPLC is still limited. The coating used must be extremely inert to protect it during the desorption step. Crosslinked films are very stable during the desorption when exposed to organic solvents such as methanol. So far, a limited number of coating materials are available for SPME/HPLC which fulfill those criteria. The 7-μm PDMS fiber shows a high affinity to high molecular weight compounds such as PAHs. Increasing the desorption temperature (heating tape surrounding the desorption chamber) reduces carryover which is often determined for PAHs at ambient temperatures. The 50-μm Carbowax template resin fiber shows a high

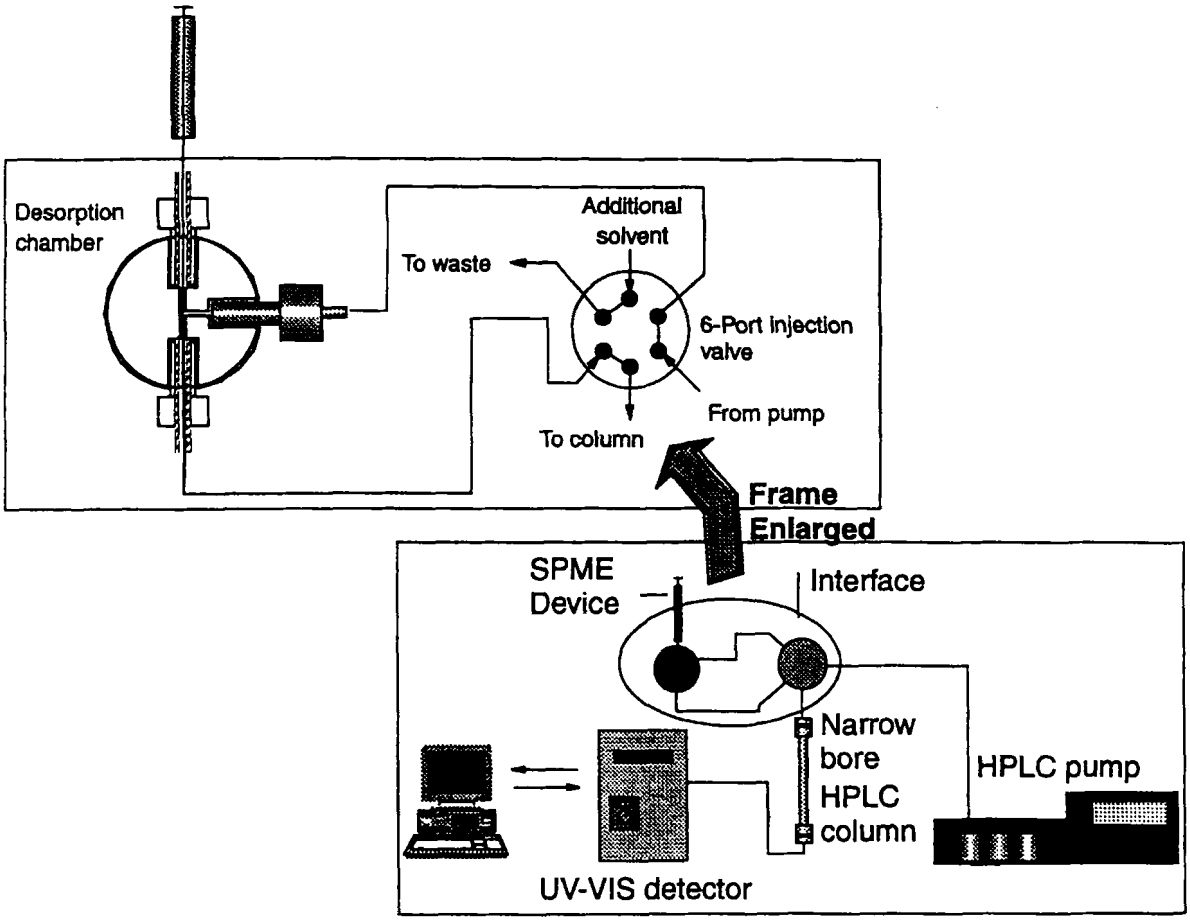

FIGURE 4. Instrumental setup of the manual SPME/HPLC interface. From reference [10]. With permission.

efficiency for polar analytes such as polar carbamates. The PDMS fibers are showing the best long-term stability and sufficient ruggedness can be achieved.

IV. AUTOMATION

In the past, optimization of SPME methods has mostly concentrated on the effects of matrix conditions such as the influence of pH, salt content of an aqueous sample, the presence of polar, low molecular weight solvents (e.g. methanol) [23], or humic material [33]; competition between main and trace compounds; and the use of agitation techniques [14]. For many applications automation is very important. Sample preparation techniques which cannot be automated are less often used for routine analysis, even if they offer other attractive features, like high selectivity or sensitivity. However, no suitable automation of the entire SPME method, especially for SPME-HPLC, is the most often cited disadvantage of SPME. To date, the applications of SPME-HPLC are all based on the manual device and interface. So far, SPME has been automated only for the GC sample preparation with static absorption, fiber vibration, and direct aqueous or headspace sampling using a commercial GC autosampler from Varian.

Solid-phase microextraction is based on the equilibration of the analyte between the sample and the stationary phase of the SPME fiber, determined by the distribution con-

stant of the analyte. Therefore, the diffusion of the analyte into the polymer represents the most important step for the mass transfer in the fiber. The accumulation of analytes in the fiber is controlled by diffusion in the (aqueous) matrix and the diffusion into the fiber. Depending on the extraction conditions, i.e., dynamic mode, the diffusion in the aqueous sample becomes nearly negligible. Without intensive mixing of the aqueous solution, the equilibration time increases considerably. In the static case, transport of the analyte is limited by diffusion in both the aqueous phase and the aqueous layer surrounding the fiber surface; during the absorption process, the concentration outside this layer steadily decreases, thus reducing the mass transfer into the fiber. In the dynamic case using agitation, a thin layer of water still remains on the surface of the fiber coating. Thus, the final equilibration time is determined by diffusion through this layer but no longer by the diffusion of the analytes in the aqueous sample. A typical example for the influence of intensive agitation of the aqueous solution on the extraction efficiency is shown in Figure 2. When using static absorption conditions for the analysis only compounds with low coating/sample distribution constants, K_{fs} values, such as simetryn, reach equilibrium within 2 h (see Figure 2 (a) curves). The equilibrium is reached for all four s-triazines after 35 min when using agitation during the absorption (see Figure 2 (b) curves). Consequently, a high precision with RSD values below 5% was achieved.

A. SPME-GC

The fiber vibration method works very efficiently when using the small 2 ml vials. The results show a faster equilibrium for the 2 ml vials than for the 16 ml vials. The vibration of the needle is transferred to the SPME fiber in the sample. Equilibrium was achieved for all five compounds within 35 min when using the 2 ml vials. Even the 16 ml vials show an efficient extraction, but it took more than 80 min for all compounds until the equilibrium was achieved. The fiber vibration method works very efficiently. Furthermore, we have to keep in mind that for these experiments there was no operator necessary. The entire SPME analysis is automated completely for the fiber vibration method. This shows the great potential of this technique for routine analysis. The analysis of semi-volatiles such as pesticides seems to be a promising field for the automated SPME-GC technique.

In general, the results obtained for the 2 ml and 16 ml vials show a similar trend when using different extraction conditions, except for the total amount extracted [14]. Sonication is mentioned in addition to the extraction processes used in this study. As expected and shown in the past [29] it is a very efficient sample agitation technique for SPME. For example, very volatile organic compounds (VOCs) such as toluene, equilibrate in less than 1 min, which is very close to the theoretical prediction. The major disadvantage of the sonication technique is sample heating during the extraction and the loss of analytes caused by sonication induced decomposition. To date, sonication is not available for automated SPME and the number of applications using sonication is negligible.

The results of checking the precision for five repetitive injections (n = 5) when using the 2 ml vials are summarized in Table 2. During the 30 min absorption time, most of the investigated compounds reach their equilibrium under agitation conditions. The precision obtained for the 2 ml vials when using the fiber vibration method can be considered optimal. The RSD was <3% for all compounds when agitation was used. The results of checking the precision for five repetitive injections (n = 5) when using the 16 ml vials are summarized in Table 3. The precision obtained for static absorption conditions is as poor as observed for the 2 ml vials. For semi-volatile compounds such as s-triazines

TABLE 2
Equilibration Times for All Investigated Compounds Obtained under Different Absorption Modes

No.	Compound	Molecular formula	Molecular mass	K_{ow}	Solubility in water [mg/l]	Equilibration time [min]						
						2 ml sta[a]	2 ml stir[a]	2 ml vib[a]	16 ml sta[b]	16 ml stir[b]	16 ml vib[b]	40 ml flow[c]
1	Simetryn	$C_8H_{15}N_5S$	213.32	347	450	60	30	30	80	25	40	30
2	Ametryn	$C_9H_{17}N_5S$	227.35	955	185	>120	35	30	>120	30	50	30
3	Prometryn	$C_{10}H_{19}N_5S$	241.37	3236	33	>120	30	35	>120	30	80	35
4	Terbutryn	$C_{10}H_{19}N_5S$	241.37	5495	25	>120	30	35	>120	50	80	35
5	Parathion	$C_{10}H_{14}NO_5PS$	291.27	6761	11	>120	30	20	>120	35	35	25

[a] The results were obtained for 2 ml vials with 1.4 ml sample volume.
[b] The results were obtained for 16 ml vials with 12 ml sample volume.
[c] The results were obtained for 40 ml vials with 10 ml sample volume using the flow-through extraction cell.

Abbreviations: flow = SPME fiber is exposed to the flow-through extraction cell, **sta** = static absorption with no agitation of the aqueous sample, **stir** = mixing of the aqueous sample using magnetic stirring, and **vib** = fiber vibration method using autosampler agitation.

From reference [14]. With permission.

TABLE 3
Precision Achieved with Autosampler SPME-GC/FID Method of Five Selected Pesticides using 2 ml and 16 ml Vials and Three Different Absorption Modes

		Precision (RSD [%])					
No.	Compound	Static[a]	Magnetic stirring[a]	Fiber vibration[a]	Static[b]	Magnetic stirring[b]	Fiber vibration[b]
1	Simetryn	0.9	2.6	1.4	1.2	4.2	3.2
2	Ametryn	3.0	1.3	1.1	2.6	0.7	3.4
3	Prometryn	7.0	1.1	1.1	4.1	0.7	3.9
4	Terbutryn	7.8	2.9	1.0	4.9	3.1	4.9
5	Parathion	11.3	2.3	0.8	5.4	0.8	4.1

[a] Precision achieved for 2 ml vials from five repetitive extractions of 1.4 ml samples (n = 5). The concentration was 300 µg/l for each compound. A 30 min absorption time was used.
[b] Precision achieved for 16 ml vials from five repetitive extractions of 12 ml samples (n = 5). The concentration was 300 µg/l for each compound. A 30 min absorption time was used.

From reference [14]. With permission.

the precision is increased by using magnetic stirring in comparison to static absorption. This absorption process for four s-triazines and parathion when using a flow-through cell shows similar efficiency as other agitation techniques. The samples were pumped at a flow rate of 10 ml/min, achieving a high linear flow of the aqueous sample inside the Teflon tubing where the SPME fiber was positioned during the absorption. The precision (n = 5) achieved for this technique is satisfactory (RSD < 8%).

B. SPME-HPLC

Recently, solid-phase microextraction (SPME) was successfully coupled to high-performance liquid chromatography [10]. However, the efficiency of this analytical method, in terms of sample throughput, still suffers from its manual operation. Furthermore, the selectivity obtained for the analysis of very polar compounds is still limited due to small number of commercially available fiber coatings (selectivity) that can withstand the aggressive HPLC conditions (solvents). An automated SPME-HPLC system was recently developed [15]. Polar thermally labile analytes such as phenylurea pesticides, were selected for microextraction directly from an aqueous sample. A piece of an ordinary capillary GC column with its coating (Omegawax 250) was used for the absorption of analytes from the aqueous sample (in-tube solid-phase microextraction) [15]. A needle hosts the capillary when it is pierced through the septum of the vial containing the spiked aqueous sample. The aqueous samples were stored in 2 ml vials on the tray of a commercial autosampler. A sample of 25 µl was aspirated and dispensed several times from the sample into the capillary using a syringe. After the extraction the absorbed analytes were released from the coating by aspiring methanol into the column and then dispensing the methanol into the HPLC injector loop. The absorption-time profiles, the amounts absorbed by different coatings, linearity, and precision were studied under different sampling conditions using spiked aqueous samples. SPME selectivity for polar compounds, which represent an important compound class for water analysis, can be improved by using more polar column coatings such as Carbowax instead of poly (dimethylsiloxane) coated columns. Compared to the manual version this automated

micro SPME/HPLC system could increase performance, efficiency (throughput), and reproducibility. Furthermore, the desorption step is quantitative, i.e., no carryover was detected. This entire method for automated SPME sample preparation is easy to apply and controlled by a commercial autosampler from LC Packings which was modified to operate in-tube SPME. The automated SPME-HPLC device obtains RSD for all investigated compounds below 6%.

Direct extraction from the aqueous matrix, with the SPME fiber directly exposed to the aqueous matrix, is usually used for semivolatile compounds, including polar pesticides such as triazines. The SPME fiber methods developed so far have had limited effectiveness for thermally labile compounds, the likes of phenylurea and carbamate pesticides. These compounds could be extracted from an aqueous matrix only with a very polar coating like Carbowax. Besides a high polarity of the SPME fiber, it has to be stable against solvents used in HPLC, for example methanol or acetonitrile. The capacity (total volume times partition coefficient of the stationary phase) of the commercially available fibers is low. Thus, the SPME-HPLC method is characterized by a low sensitivity, low ppb range when using UV detection. Sensitivity can be increased instrumentally by using hyphenated techniques, in particular LC-MS.

The newly developed automated SPME-HPLC method uses a flow-through process which is expected to reduce the total extraction time per sample and increase the precision of the entire method. The open tubular column which is used for the direct absorption of the target analytes from the aqueous sample is enclosed in a needle device and can automatically be exposed to a vial containing the sample. The device combines features of earlier developed SPME devices where the inner surface of a syringe was coated with a polymer and microcolumn LC. The absorption equilibrium will be achieved faster because the extraction process involves agitation by sample flow in and out of a column.

1. Dynamic In-Tube SPME

The in-tube SPME consists of a piece of fused silica capillary, internally coated with a thin film of extraction phase (a piece of open tubular capillary GC column), or a capillary packed with extracting phase dispersed on an inert supporting material (a piece of micro-LC column) [15]. In these geometric arrangements, the concentration profile along the axis, x, of the tubing containing the extracting phase as a function of time, t, can be described by adopting the expression for dispersion of a concentration front:

$$C(x,t) = \frac{1}{2} C_0 \left[1 - erf \frac{x - \frac{ut}{1+k}}{\sigma\sqrt{2}} \right] \quad (5)$$

where u is linear velocity of the fluid through the tube, k is the partition ratio defined as:

$$k = K_{fs} \frac{V_f}{V_V} = 4K_{fs} \frac{d_s}{d_c} \quad (6)$$

where K_{fs} is a coating/sample distribution constant, V_f is the volume of extracting phase, V_V is a void volume of the tubing containing the extracting phase, d_s is the stationary phase film thickness, and d_c is the diameter of the column bore. σ is the mean square root dispersion of the front. The calculated concentration time profiles inside the capillary can be used to estimate the absorption time of the target analytes.

2. Autosampler Operation and Interface Design

The autosampler software was manually programmed using user defined programs to

control the SPME absorption and desorption technique. For desorption, pure methanol from a second vial was flushed through the SPME unit and directly transferred into the injection loop. The HPLC injection loop was built of a 56 cm long PEEK tubing (300 µm ID) which had a total volume of 40 µL. The instrumental set-up is illustrated in Figure 5.

The 2 ml vials were filled with 1.4 ml sample for the absorption of the compounds from the aqueous sample. The first step in the method was to rinse the GC capillary with methanol and it still contained methanol before the first absorption step. A sample volume of 25 µL (total volume of the syringe used in this study) was aspirated from the sample vial at a flow rate 63 µL/min. Then the same sample volume was dispensed back into the vial. Usually, these two steps were repeated ten times. After the absorption step the six-port valve was switched to the LOAD position. 38 µL methanol was aspirated from a solvent vial and transferred to the injection loop for the desorption of the extracted analytes from the capillary coating. The six-port valve was switched to the INJECT position and a trigger was sent to the PC for starting the data acquisition. The

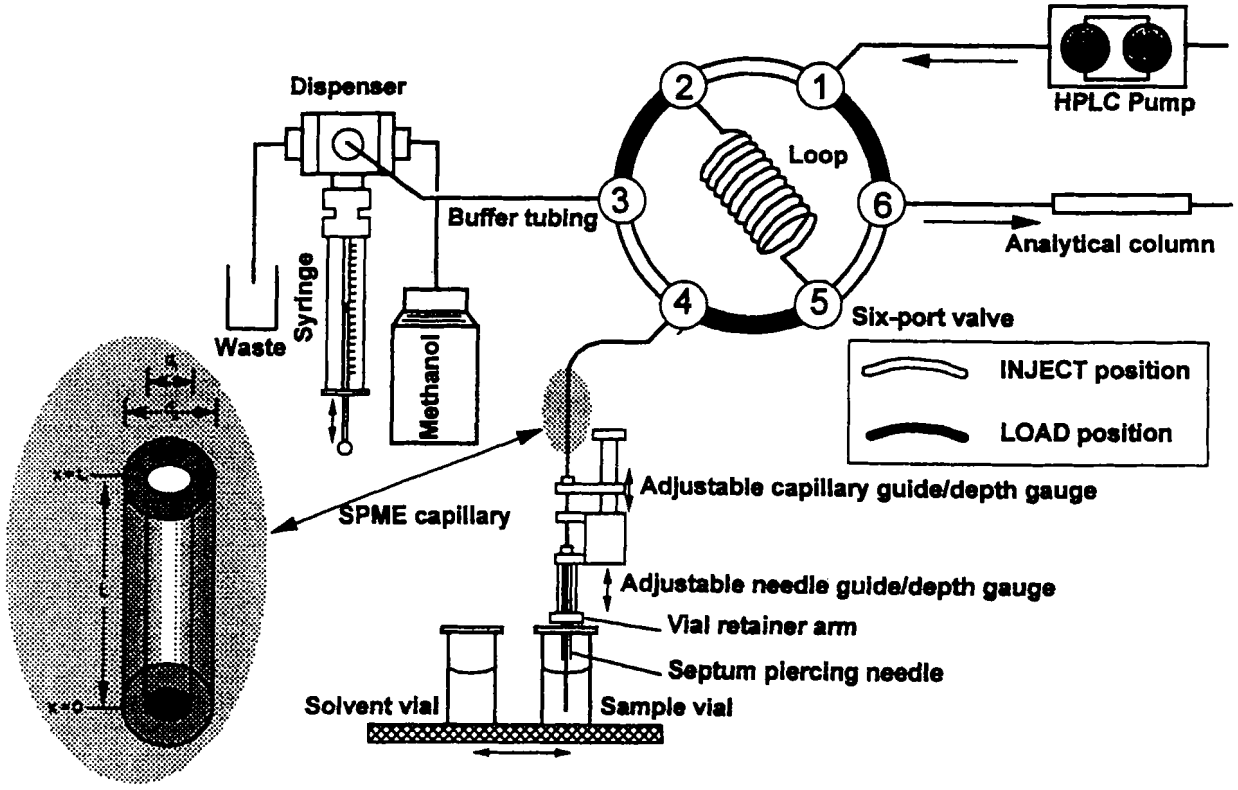

FIGURE 5. Instrumental set-up of the new on-line SPME/HPLC interface based on an in-tube SPME capillary technique. A piece of GC column (in-tube SPME) hosts in the position of the former needle capillary. The aqueous sample is frequently aspirated from the sample vial through the GC column and dispensed back to the vial (INJECT position) by movement of the syringe. After the extraction step the six-port valve is switched to the LOAD position for the desorption of the analytes from the in-tube SPME by flushing methanol from another vial through the SPME capillary. The volume is transferred to the loop. After switching the Valco valve to the INJECT position an isocratic separation using a mixture of 60/40 acetonitrile/water was performed. A detailed view of the in-tube SPME capillary is included at the left side of the figure. From reference [15]. With permission.

transferred from the loop to the analytical column by the isocratic eluent mixture. During the analysis of the first sample a subsequent sample could be extracted.

In our final experimental design the sample is aspirated and dispensed instead of a one-way flow. The contamination of the buffer tubing makes the flow-through approach less efficient compared to the repeated aspirate/dispense mode. The increase of the flow rate of the sample is a significant factor which determines the equilibration time. However, the increase of this parameter is limited by practical factors. Furthermore, the total volume which can be aspirated in one step is limited by the syringe volume. A continuous one-way aspiration system shows no significant advantages and further improvement of the extraction efficiency. The aspiration concept, however, could improve the automated in-tube SPME handling substantially by obtaining a higher precision.

The absorption-time profiles of six phenylurea pesticides are shown in Figure 6 using the automated in-tube SPME-HPLC system. The absorbed compounds are first desorbed and then transferred into an HPLC loop. The injection into the analytical column is similar to the injection of methanolic standards. The separation of the desorption step from the injection to HPLC step is a big advantage compared to the manual SPME-HPLC interface. Using this procedure a different solvent mixture can be used for the desorption which shows a higher eluting power compared to the initial solvent composition of the HPLC eluent.

In general, the precision obtained using an Omegawax 250 capillary was <6% RSD (n = 10). Table 4 shows the precision for all compounds investigated under different con-

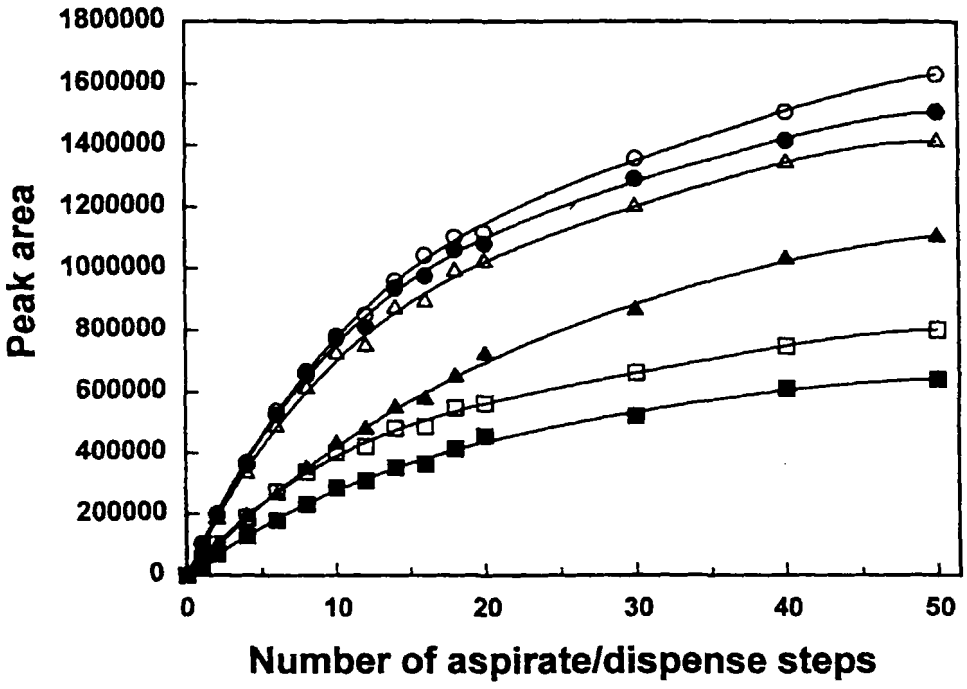

FIGURE 6. Absorption-time profiles for six phenylurea pesticides using a 60 cm long piece of an Omegawax 250 GC column tubing (0.25 mm ID and 0.25 µm film thickness) and up to fifty aspirate/dispense steps at a sample flow rate of 63 µl/min from a 1.4 ml aqueous sample with a concentration of 1000 µg/l. Peak assignment: ○ = neburon, ● = linuron, △ = diuron, ▲ = monuron, □ = siduron, ■ = fluometuron. From reference [15]. With permission.

TABLE 4
Precision and Limit of Detection (LOD) of the In-Tube SPME/HPLC-UV System for Six Phenylureas

							Precision			
No.	Compound	Molecular formula	M_n[a]	K_{OW}	RT [min]	$w_{1/2}$ [s]	Omegawax RSD [%][b,c]	Omegawax RSD [%][b,d]	SPB–5 RSD [%][b,e]	LOD [µg/l][f]
1	Monuron	$C_9H_{11}ClN_2O$	198	87	3.69	10.4	5.4	3.1	8.3	3.3
2	Fluometuron	$C_{10}H_{11}F_3N_2O$	232	263	4.28	9.6	5.6	3.7	4.5	3.3
3	Diuron	$C_9H_{10}Cl_2N_2O$	232	479	4.70	8.7	2.6	3.1	3.2	2.7
4	Siduron	$C_{14}H_{20}N_2O$	232	1230	5.74	10.6	2.7	2.6	4.2	3.8
5	Linuron	$C_9H_{10}Cl_2N_2O_2$	248	1585	6.72	12.7	2.1	2.8	1.9	2.8
6	Neburon	$C_{12}H_{16}Cl_2N_2O$	274	6310	10.08	17.9	1.6	1.9	2.6	4.1

[a] Mn = nominal mass.
[b] from ten repetitive injections (n = 10).
[c] The concentration in the aqueous sample was 10,000 µg/l for each compound (for an Omegawax 250 capillary).
[d] The concentration in the aqueous sample was 1000 µg/l for each compound (for an Omegawax 250 capillary).
[e] The concentration in the aqueous sample was 1000 µg/l for each compound (for a SPB–5 capillary).
[f] using a signal-to-noise ratio of S/N = 3. The concentration in the aqueous sample was 10 µg/l for calculating the LOD values for an Omegawax 250 capillary.

From *reference [15]. With permission.*

ditions for n = 10. The method was linear over at least three orders of magnitude. The coefficient of correlation achieved was better than 0.99.

Using ten aspirate/dispense steps the limit of detection (LOD) was determined for all compounds which was determined below 5 µg/l (see Table 4). This limit can be optimized by increasing sampling time to achieve equilibrium conditions. Furthermore, the LOD can be lowered by using narrow bore HPLC columns achieving sharper peaks or more sensitive detection methods such as mass spectrometry (MS) in the selected-ion monitoring (SIM) mode. When using hyphenated detection techniques, e.g. LC-MS, the LOD is expected to be below the 0.1 µg/l level.

The SPME absorption and desorption steps are fully automated with the HPLC autosampler. Once programmed, an operator can fill the autosampler tray with the samples and all following steps are controlled by the software. The newly developed instrument described [15] is expected to show good potential for routine analysis. Furthermore, the selectivity for target analytes, such as drugs or chiral target compounds, could be increased by using very polar or chiral coating materials.

For more specific analysis of metabolites and unknown compounds more selective hyphenated detection systems, e.g., LC-ESI/MS or LC-API/MS can be evaluated in the future. A modified continuous flow setup could be helpful for on-line monitoring of target compounds for process control using a modified in-tube SPME device.

The automated micro SPME-HPLC system can be applied for routine analysis of polar thermally labile compounds and easy coupling to µ-LC and capillary electrophoresis (CE).

C. On-line Screening System

A prototype of an analytical system allowing the quasi-continuous monitoring of organic contaminants in surface water which should be also applicable to the analysis of sewage water was developed [34]. It consists of a flow-through cell and an automated solid-phase microextraction (SPME) unit, coupled in-line to a gas chromatograph (GC). This system combines the advantages of SPME as a simple, fast, sensitive, and solvent-free sample introduction technique, with the advantages of on-line processing of aqueous samples as a less time-consuming, efficient, and continuous technique. Organochlorine pesticides and triazine herbicides were selected as test analytes for the system evaluation. The flow-through cell was shown to provide a successful way for automated on-line SPME coupled in-line to GC with a repeatability of ca. 10% RSD for the investigated triazines.

The sample is pumped continuously through the flow-through cell mounted on a commercial GC-autosampler (see Figure 7). The fiber is dipped in regular intervals into the flowing sample. In this first laboratory prototype the sample placed in a brown-glass bottle was pumped through the cell using a peristaltic pump. In the future a bypass of a river or a sewage effluent will be pumped through the cell thus allowing the direct and quasi-continuous monitoring of organic compounds at trace levels in surface waters.

A flow-through cell made of glass was constructed by us for the on-line solid-phase microextraction. This cell was mounted onto the commercial autosampler modified to take up the cell as shown schematically in Figure 8. For this arrangement nine positions for autosampler vials had to be removed. The glass flow-through cell forms a half-circle with the absorption position in the middle. At this position which is arranged exactly at the site of one of the original autosampler vials the cell is sealed by a septum. The fiber fixed to the SPME-autosampler dips directly through the seal into the cell. This allows the use of the normal software procedure to control the entire absorption and desorption steps also for this flowing sample arrangement. The sample was pumped through the cell

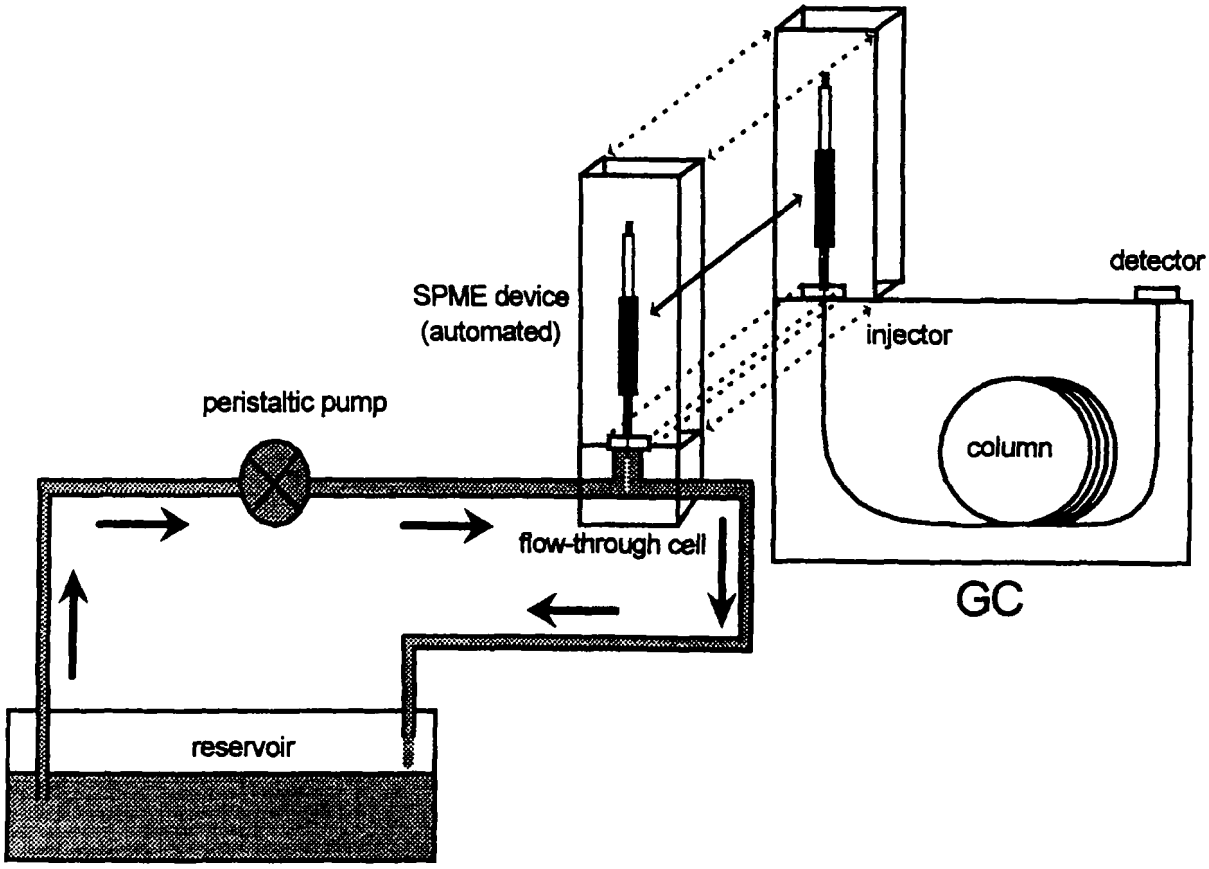

FIGURE 7. Schematic illustration of an automated analytical system for on-line SPME enrichment GC. From reference [34]. With permission.

with a peristaltic pump. The aqueous sample was pumped in a loop (see Figure 7) at a flow rate of c. 300 ml/min.

First, the fiber is exposed to the sample pumped at a constant flow through the cell for a given period. Second, the fiber is withdrawn from the sample and introduced automatically into the GC-injector, where thermal desorption occurs. After thermal desorption the fiber is withdrawn from the injector and again automatically dipped into the flowing sample. While the next sample is absorbed by the fiber the preceding sample is chromatographed by the GC. This overlapping of absorption and chromatography reduces the average time for an overall analysis of each sample.

In this first prototype of an automatic system for quasi-continuous analysis of contaminated water the flowing surface or sewage water was simulated by a spiked aqueous sample stored in a reservoir and pumped through the cell continuously. The in-line SPME-GC system works fully automatically using the normal software control of the autosampler and gas chromatograph. Extensive testing of the system developed showed a good performance in particular with respect to reproducibility and ruggedness.

Using this approach 150 extractions were performed with a single fiber showing neither a significant deterioration of its performance nor any mechanical damage illustrating that the analytical system discussed here is characterized by satisfactory ruggedness. This ruggedness is of particular importance for an on-site operation of the system, e.g., at an effluent of a waste-water plant. In this case the automatic system may be run without intervention of an operator. Remote con-

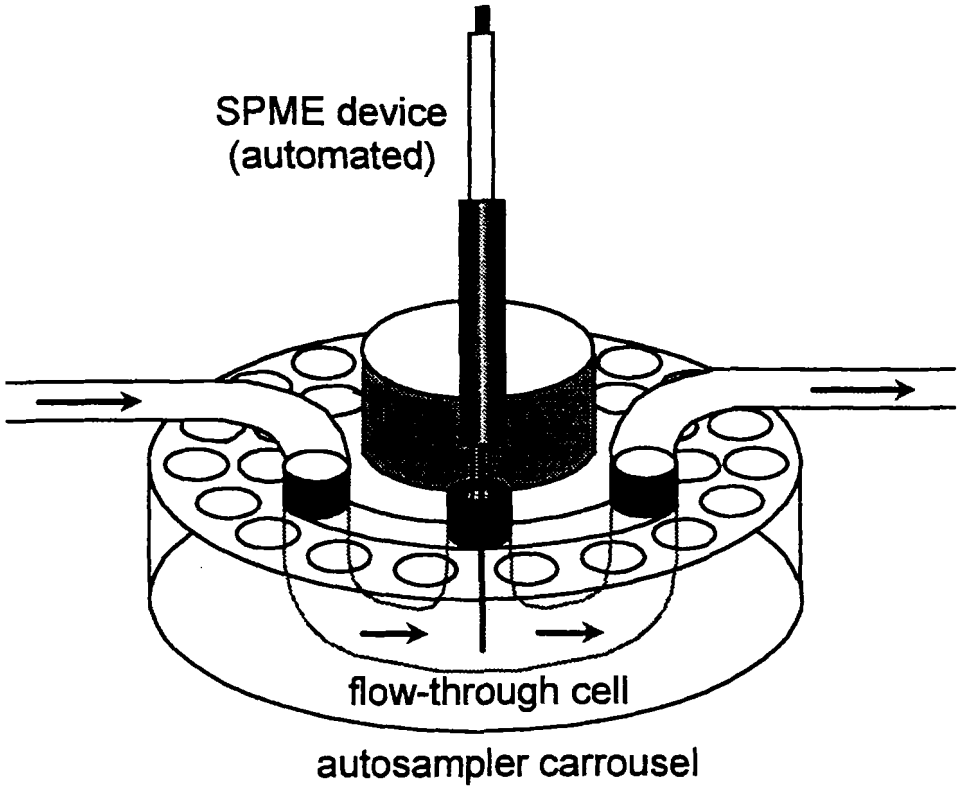

FIGURE 8. Detailed view of the flow-through cell mounted on an autosampler carrousel. From reference [34]. With permission.

trol is possible, e.g. by transmitting the data via modem to a laboratory. Furthermore, the daily performance of the system can be controlled by adding, continuously, a marker compound as internal standard which gives information on the overall performance of the entire system. Such a marker also allows quality control of the system.

The repeatability of this automatic enrichment and analysis system was tested using ten successive extractions achieving a reproducibility of 4–13% RSD for triazine herbicides.

V. APPLICATIONS BASED ON NEW SPME METHODS

A. Air Analysis by Integrated Sampling

In addition to the analyte concentration measurement at a well defined place in space and time, an integrating sampling is possible with a simple SPME device. This sampling is particularly important in field studies, when changes of the analyte concentration in time and space have to be taken into account [26]. For integrated sampling the SPME fiber is not directly exposed to the matrix. It is kept inside a protecting tubing (needle) without any flow of the sample through it. The extraction occurs through a static layer of gas present in the needle. The system can be designed similar to the in-tube SPME discussed for the SPME/HPLC system where an extracting phase is coating the interior of a tubing or a fiber coated with the stationary phase which is placed inside the protecting needle. The later device is the classical manual SPME fiber device which is used without exposing the fiber during the sampling. However, the position of the fiber inside the protecting needle is very important. It has to be placed very accu-

rately at the same position which represents the same volume of protecting gas. The mechanism of analyte transport into the extracting phase in such a set-up is determined only by the diffusion through the gaseous phase. Thus, the response is proportional to the integral of the analyte concentration over the time and space (when the needle is moved through space) [22]. The process is characterized by a linear concentration profile established in the tubing between the small needle opening with the area, A, and the position of the extracting phase which is located at the position Z from the opening. The amount of analyte extracted, dn, during the time interval, dt, can be derived from Fick's law of diffusion [35] and can be expressed by:

$$dn = AD_g \frac{dc}{dz} dt = AD_g \frac{\Delta C(t)}{Z} dt \quad (7)$$

where $\Delta C(t)/Z$ is a value of the gradient established in the needle between the needle opening and the position of the extracting phase, Z; $\Delta C(t) = C(t) - C_Z$, where C(t) is a time dependent concentration of the analyte in the sample in the vicinity of the needle opening, and C_Z is close to zero for a high coating/gas distribution constant capacity, then: $\Delta C(t) = C(t)$. D_g is the diffusion coefficient of the analyte in the gaseous phase. The concentration of analyte at the coating position in the needle, C_Z, will increase with integration time, but it will be kept low compared to the sample concentration. Thus, the accumulated amount over time can be calculated as:

$$n = D_g \frac{A}{Z} C(t) dt \quad (8)$$

This equation shows that the extracted amount of analyte is proportional to the integral of the sample concentration over time. This equation is valid for the assumption that the amount absorbed onto the extraction sorbent is a small fraction (below the RSD of the measurement, typically 5%) of equilibrium amount with respect to the lowest concentration in the sample. By varying the position of the sorbent in the tubing, the integration time can be extended. The sensitivity of the system can be increased by increasing the volume of the stationary phase or by using more selective coating materials with larger coating/gas distribution constants.

The integrated in-tube SPME sampling device was applied to field sampling in the past [26]. The concentration of styrene in indoor air at an industry due to the application of large quantities of vinyl ester resin was determined. The 30 min integrated sampling using a 100 μm PDMS fiber shows a good agreement with additional determinations based on direct SPME grab sampling and 30 min charcoal tube sampling results [26]. The concentrations determined were in the 100 μg/l range [26]. SPME for fast grab sampling and integrated sampling can be an alternative method to time consuming active charcoal sampling, which, furthermore, needs (toxic) solvents for the desorption process.

B. Analysis of Volatile Organic Compounds from Soil Samples

The analysis of volatile organic compounds from soil samples has been successfully applied using headspace SPME-GC methods [36–39]. Aromatic hydrocarbons and chlorinated hydrocarbons were determined by headspace SPME in soil samples from landfill sites [36]. Maximum xylene concentrations of 2.8 μg/g were determined [36]. BTEX compounds were quantified in soil samples from a railroad operation location at 1.0 μg/g [38]. The method was compared to a purge and trap technique. Table 5 shows the results of both techniques for the analysis of BTEX compounds determined in three soil samples. The correlation between the analytical methods was generally apparent [38]. To increase the release of organic target compounds methanol was added to

TABLE 5
Results of Two Analytical Methods for BTEX Applied to Contaminated Soils

		Compound Concentration [μg/g]				
Soil	**Method**	**Benzene**	**Toluene**	**Ethylbenzene**	**m/p-Xylene**	**o-Xylene**
S1	a)	2.92	3.07	42.5	169	48.3
	b)	2.63	3.40	43.7	180	43.0
S3	a)	t	0.33	24.9	60.5	2.23
	b)	0.32	0.47	19.8	47.0	1.69
S4	a)	0.82	1.46	27.8	104	2.62
	b)	0.78	1.25	20.4	96.9	2.28

Note: Method: a) purge and trap with MSD, b) SPME/ion trap MS. t = detected below the statistical instrument detection limit.

From reference [38]. With permission.

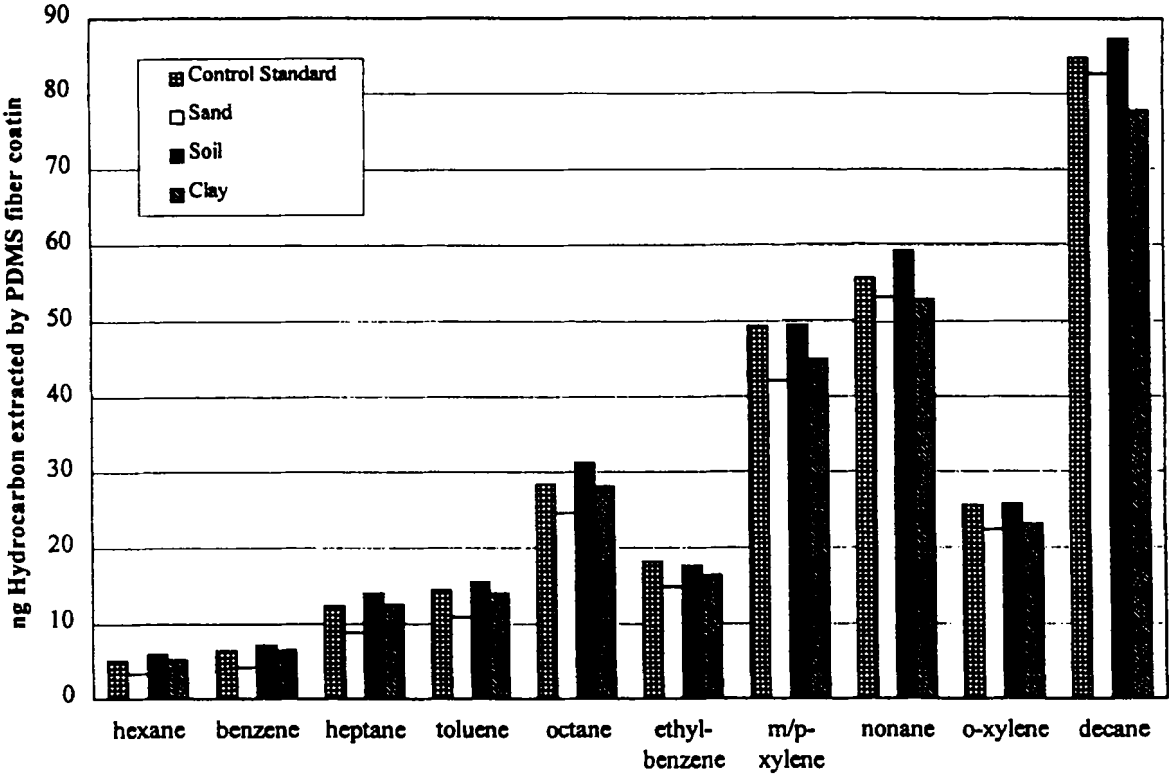

FIGURE 9. Methanol sonication of 130 μg/g individual hydrocarbon concentration in sand, soil, and clay, followed by SPME in the presence of molecular sieve at 23°C. From reference [39]. With permission.

the sample before the headspace SPME fiber exposure [37,39]. The analysis of hydrocarbons (n-alkanes) from sand and soil showed similar results when 10 μl of methanol was added [39]. Figure 9 illustrates the methanol sonication of 130 μg/g individual hydrocarbon concentration in sand, soil, and clay, followed by SPME in the presence of mo-

lecular sieve at 23°C. In the presence of 10 µl of methanol the extraction of n-alkanes from sand yields similar results as the n-alkanes from water [39]. Thus, signifying that they possess similar partitioning coefficients between the sample (water or sand) and the headspace. Therefore, Henry's law coefficients for the n-alkanes in water can be used as an estimate to describe the partitioning between sand and the headspace providing a polar modifier is added to the sand to release the compounds into the headspace. In the absence of a polar modifier, methanol, the n-alkanes yield similar to results from soil samples [39]. Soils highly adsorptive in nature are composed of large amounts of clay, organic carbon and other species which strongly adsorb organic target analytes. In this case the amount extracted is dependent on the matrix. The development of a matrix independent method becomes more important when analyzing different soil types. Zhang and Pawliszyn used an internally cooled SPME fiber apparatus to exhaustively extract the compounds from soil [30]. The soil was heated while the fiber was cooled by liquid CO_2. Furthermore, methanol extraction of a sample followed by the addition of the methanol extract into water and analysis by headspace SPME has been applied to the analysis of BTEX from wastewater samples [38]. Finally, hydrocarbons can be released from soil samples and the methanol extract will not be added to water but will be treated as an air sample. The advantages of analyzing the methanol extract as an air sample as opposed to a water sample is that addition of the methanol extract to water is limited to compounds which have high solubilities in water. Up to 10 µl of methanol the extraction yield was not affected. However, when increasing the amount of methanol the amount of extracted analyte decreased. Addition of a desiccant providing an optimum surface area to trap the methanol but no target compounds leads to an optimum amount of hydrocarbons extracted which was demonstrated for gasoline contaminated soil samples [39].

C. Analysis of Semivolatiles from Soil Samples by Hot Water Extraction

Efficient extraction of semi volatile organic compounds from soil samples can be obtained using supercritical (high temperature) water to extract the target analytes which are later extracted from water by SPME [40, 41]. Two techniques are reported in the literature for the determination of PAHs in soils and urban air particulate. Achieving recoveries of 60 to 140% the method uses supercritical water extraction at 250°C for 15–60 min [40]. The SPME fiber is exposed to the cooled water sample to trap the target analytes. The second method combines both steps, thus the fiber is placed inside the cell during the dynamic hot water extraction [41]. The method was validated using NIST standard material for PAHs in urban air particulate [41].

D. Fast Gas Chromatography

Solid-phase microextrcation has been successfully used as the sample introduction technique for fast-gas chromatography. A modified injector was used which allows fast heating by applying capacity discharge to a heating wire [42]. The separation of BTEX was achieved in less than 9 s. Volatile organic compounds from EPA method 624 could be separated within 150 s. Flash heating was achieved by passing an electric current directly through a wire used in plane of the fiber or by using a hollow fiber equipped with an internal micro heater. The SPME fast GC set-up was installed on a portable field GC instrument which was in a field study as discussed in the following chapter [16]. Using a temperature-programmed separation Purgeables A and B could be separated within 1.5 min. Fast GC shows a high efficiency when coupled with an extraction technique which can be performed within the same time frame. It is not necessary to have

a fast GC when the long extraction time of the sample preparation method determines the total analysis time. SPME combines the unique feature of fast sample preparation and easy injection to GC system. Thus, the coupling of both techniques shows a significant increase in the sample throughput of the analysis. Furthermore, sample handling is very easy and rugged which makes the technique amenable for field sampling. Typical sample turnaround times are 5 min achieving a good precision of the method.

E. Field Analysis

A commercially available field portable gas chromatograph has been adapted to enable the use of SPME as the sample preparation and introduction technique for fast GC in the field [16]. Fast GC separations of BTEX compounds in less than 15s were reported. The instrument was tested in the field for the analysis of trichloroethene in soil samples. The SPME method was used for 500 samples analyzed within 10 days showing a high ruggedness of the method and instrumentation during on-site monitoring. Taking into account versatility of SPME, this technique enables preliminary screening of a wide range of compounds and/or samples (aqueous, gaseous, solid) directly in the field. These results are required to determine whether a suspect sample has to be drawn and further analyzed. Figure 10 shows a fast GC separation of four BTEX compounds which were extracted by SPME on a PDMS/DVB fiber using a modified photoionization detector (PID).

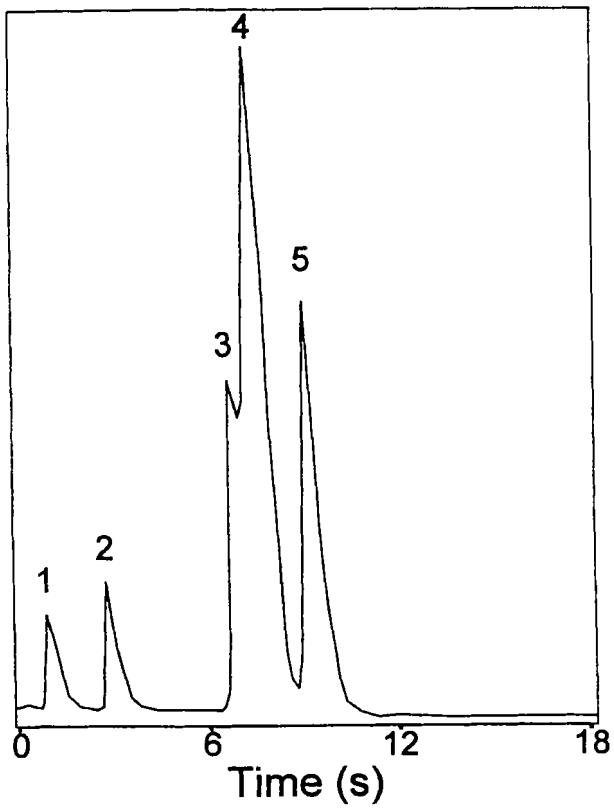

FIGURE 10. Fast gas chromatogram of BTEX compounds by SPME with a PDMS/DVB fiber. Peak assignment: 1 = benzene, 2 = toluene, 3 = ethylbenzene, 4 = m/p-xylene, 5 = o-xylene. From reference [1]. With permission.

F. Flavor and Pharmaceuticals

SPME shows a unique feature for fast and sensitive analysis of flavor compounds. Since only an insignificant amount of flavor is extracted, the composition of a product is not changed. Furthermore, SPME is capable of the determination of intermediates which are very unstable and must be immediately transferred after isolation to the analytical instrument for successful determination. The SPME fiber is instantly transferred to the GC or HPLC for separation and determination. Thus, it shows a high potential for the analysis of intermediate composure, drug discovery, and drug biodegradation.

Headspace SPME was used for the determination of volatile organic compounds which are frequently present in food or pharmaceutical products [43,44]. The determination of orange juice flavor compounds was achieved by headspace SPME/GC/FID [43]. A major advantage of SPME is the small sample amount necessary for the investigation. Flavor analysis of beverages to quantify caffeine in soft drinks [45], food products, spices, and oils had been investigated [46]. Quantitative Headspace SPME of apple volatiles was reported by Matich [47] for cooled stored apples. The method was compared to SPE and LLE results.

Drugs and body fluids are more difficult to analyze. However, a number of publications in the area of pharmaceutical applications such as amphetamines [48,49], valproic acid [50], and tricyclic antidepressants [51] have been reported. Recently, SPME was used for the determination of barbiturates coupled to capillary electrophoresis (CE) [52].

G. Multi-Residue Methods for Pesticides

Multi-residue methods have been developed for a large number of pesticides covering different compound classes [33,53]. One method based on the manual SPME device was developed for the determination of nitrogen- and phosphorous-containing pesticides (amines, anilides, phosphorothioates, and triazines) by solid-phase microextraction (SPME) coupled to gas chromatography-mass spectrometry (GC/MS) [33]. The 85 μm poly(acrylate) fiber was applied for direct sampling from the aqueous sample. The fiber is exposed to the sample for a given time and then directly introduced into the heated injector of the GC/MS where the analytes are thermally desorbed. The method was evaluated with respect to the limit of detection, linearity and precision. The limit of detection for the selected ion monitoring (SIM) mode depends on the compound and varies from 5 to 90 ng/l. The method is linear over at least three orders of magnitude with coefficients of correlation usually ≥0.996. In general, the coefficient of variation (precision) is < 10%. The partitioning of the analyte between the aqueous phase and the polymeric phase depends on the hydrophobicity of the compound as expressed by the octanol-water partitioning coefficient K_{ow}. The addition of sodium chloride has a strong effect on the extraction efficiency. This effect increases with decreasing hydrophobicity (increasing polarity) of the compound [23].

Real water samples were investigated and triazine herbicides could be identified using the SPME-GC/MS method. The triazines atrazine, simazine, and terbuthylazine were first identified and quantified in water samples from the effluent of sewage water plants by SPME-GC/NPD. For such a complex matrix GC/NPD is not sufficiently selective for an unambiguous identification at low levels (< 1 ppb) of pesticides. Selectivity was enhanced by using SMPE-GC/MS in the SIM mode with three characteristic ions for each pesticide. This method allows an unequivocal identification and quantification at low levels of pesticides in envi-

ronmental samples. The results were verified by an alternative method. The analysis of the sewage water samples by on-line solid-phase extraction (SPE) coupled to LC-MS indicated a good correlation to SPME results.

In addition, the concentration of humic acids which are normally present in surface water samples was varied between 0.1 and 100 mg/l which are typical DOC (dissolved organic carbon) values determined in surface water samples [33]. At high concentrations of humic acid such as 100 mg/l the response determined for simetryn and terbutryn shows a significant decrease > 20%. Thus, the method of external standards can be no longer applied. The reliability of the quantification can be improved by using internal standards or standard addition methods. In addition, isotopically labeled standards could be used. However, the concentrations typically observed in these matrices show no significant effect on the extraction yield. Thus, external standard calibration was used in the past for sewage water samples obtaining a sufficient precision. It is conceivable that a high content of organics (e.g., solvents) precludes an efficient extraction. To study this effect, different amounts of terbuthylazine, a compound with a high affinity to the polymeric fiber, were added in concentrations that ranged from 18 to 12,000 ng/ml. Although the concentration was varied over 3 orders of magnitude, no significant decrease in peak response for all the other investigated pesticides (which had been kept at a constant concentration level of 18 ng/ml) was observed. Thus, the effect of excess concentration of organics with high affinities to the SPME fiber on the extraction efficiency of other analytes in the low ppt — low ppb range is less pronounced than expected [33].

The use of liquid or solid polymers such as PDMS or poly(acrylate) coatings are based on absorption. The partitioning of the analytes is not significantly affected by high contents of matrix compounds with a high affinity to the fiber coating which was shown in the past [33]. However, the situation is different for the mixed porous polymer coatings such as DVB or Carbowax where adsorption phenomena determine the extraction process. The adsorption is significantly affected by competition phenomena especially when all active sites are occupied. The higher sensitivity obtained for these new fibers might be accomplished by significant competition at high matrix concentrations which results in a lower precision. One has to decide which point is more important for the analysis: sensitivity or precision.

Furthermore, SPME was successfully used for the determination of metolachlor in runoff and tile-drainage water [54]. In addition, this method was compared to solid-phase extraction and immunoassay analyses of metolachlor [55]. A good correlation of the three methods was found in this interlaboratory study. SPME and ELISA have a high potential for the determination of metolachlor in natural waters [55].

The applicability of SPME for the analysis of semi-volatile compounds in water was verified by an interlaboratory study on pesticide analysis [56]. The test was done at low ppb levels with participants from eleven laboratories in Europe and North America. Table 6 summarizes the statistical characteristics of the round robin test. The results of the test proved that SPME is an accurate and fast method of sample preparation and analysis [57].

H. Liquid Chromatography — Mass Spectrometry (LC/MS)

SPME has been successfully applied for the analysis of surfactants [32] which was mainly focusing on the capability of different fiber coatings to extract alkylphenol

TABLE 6
Statistical Characteristics of the Results Obtained by the Participating Laboratories for the Blind Sample

Compound	s_r	s_L	s_R	r	R	A.v.	C.I.	T.v.
Dichlorvos	2.06	5.04	5.44	5.83	15.40	27.30	27.3 ± 5.83	25 ± 1.35
EPTC	0.56	1.56	1.66	1.57	4.70	9.90	9.9 ± 1.57	10 ± 0.54
Ethoprofos	0.82	4.79	4.86	2.32	13.74	15.50	15.5 ± 2.32	17 ± 0.92
Trifluralin	0.27	0.57	0.63	0.76	1.79	1.60	1.6 ± 0.76	2 ± 0.11
Simazine	2.34	3.45	4.17	6.61	11.79	23.60	23.6 ± 6.61	25 ± 1.35
Propazine	1.21	2.04	2.37	3.42	6.71	9.50	9.5 ± 3.42	10 ± 0.54
Diazinon	0.63	2.13	2.22	1.79	6.29	8.20	8.2 ± 1.79	10 ± 0.54
M.chlorpyriphos	0.12	0.32	0.34	0.35	0.97	1.60	1.6 ± 0.35	2 ± 0.11
Heptachlor	2.03	2.89	3.53	5.75	10.00	8.90	8.9 ± 5.75	10 ± 0.54
Aldrin	0.54	0.73	0.91	1.53	2.58	2.00	2.0 ± 1.53	2 ± 0.11
Metolachlor	0.73	2.83	2.92	2.07	8.28	15.70	15.7 ± 2.07	17 ± 0.92
Endrin	0.87	3.00	3.13	2.47	8.85	8.80	8.8 ± 2.47	10 ± 0.54

Note: s_r — repeatability standard deviation, s_L — interlaboratory standard deviation, s_R — reproducibility standard deviation, r — repeatability, R — reproducibility, Av. — gross average, C.i. — confidence interval of the gross average, T.v. — confidence interval of the "true" value. All values expressed in µg/l.

From reference [55]. With permission.

ethoxylate surfactants like Triton X. The aromatic system of the molecule allow a sensitive UV detection of these compounds. Analytes with less UV-absorption response require much more effort for a comparative trace detection. Mass spectrometry offers several options when used to detect polar, thermally labile, and high molecular weight compounds at ppb level. Thermospray mass spectrometry as well as atmospheric pressure ionization mass spectrometry in combination with SPE and HPLC are established techniques in environmental analysis [58–62].

The first combination of solid-phase microextraction (SPME) and HPLC-electrospray ionization mass spectrometry (ESI-MS) was applied for the determination of polar water soluble components from sludge and sediments [63]. The extraction, desorption and detection conditions were examined for selected carboxylic acids, phthalates, and surfactants using Carbowax coated SPME fibers. Maximum extraction yields of the target compounds were obtained at extraction times between 1 and 15 hours using magnetic stirring (phthalates and surfactants) and a desorption period of two minutes when using a methanol/ethanol mixture of 80:20 (v/v) [63]. The main components of sludge and sediment samples analyzed could be identified as phthalates, fatty acids, non-ionic surfactants, chlorinated phenols and carbohydrate derivatives. Additional information for compound identification was obtained at different spray potentials for the mass spectrometric ionization. Switching from positive to negative ionization mode changes the selectivity and sensitivity of detection and substances of different nature could be detected.

A water slurry (4 ml) was prepared from 10 mg of each of the dried and homogenized solid samples. The slurries were saturated with sodium chloride and adjusted at pH 2 with 25% hydrogen chloride solution to force the dissociation equilibrium of polar compounds to the non-dissociated species.

The desorption was performed by injecting 100 µl of a methanol/ethanol (80:20)

mixture into the injection port before the fiber was placed and fixed in the desorption chamber. The use of atmospheric pressure ionization mass spectrometry avoids a prederivatization and guarantees a high sensitivity, especially, for ionic, thermally labile and polar compounds. The electrospray source was operated in positive as well as negative ionization mode to utilize the different selectivity of both options. In the positive mode (ESI^+) mainly cations are recorded or ions that dispose of sufficient proton affinities to produce the $[M+H]^+$ ions. The positive ESI mode proved to be an appropriate ionization mode for the phthalates (DBP and DEHP) studied. Negative spray conditions abstract protons from the analytes which leads to $[M-H]^-$ ions. This process is dominant for acidic compounds like fatty acids, diacids and alkylpolyglycoside.

The $[M+H]^+$ and $[M-H]^-$ ions were used for target analysis. These ions are of diagnostic value because of their dominance in the mass spectra. Furthermore, the efficiency of pure ethanol and octanol as desorbing solvents were examined to improve the desorption yields of fatty acids. However, the spray conditions for the MS ionization significantly changed and the sensitivity dropped down.

Two separate measurements using different spray potentials were carried out. One major component indicated by the ions m/z 133, 147 was detected in the chromatogram of the sediment analysis (see Figure 10).

Alkyldiacids like butanediocacid and succinates, adipic acid and the corresponding ester known as degradation products of biological processes (trace m/z 133, 147) were recorded. Alkylbenzenesulphonates (m/z 339), alkylcarboxylic ions with m/z 227, 255 (Figure 11, left) and 425 were identified. These compounds are released, e.g., from soap products and fat metabolism. The chromatogram traces of the corresponding SPME-ESI^- analysis show additional sediment constituents identified as surfactant and carboxylic compounds. In addition, trichlorophenol was identified in the sediment, a compound known for toxic relevance (Figure 12).

The method combination of SPME/LC-MS proved to be a very powerful instrumentation for the determination of water soluble compounds if current methods like GC/MS or HPLC-UV failed or special efforts are necessary for their detection (derivatisation).

Using a 10 mg sample leads to the identification of components like trichlorophenol, phthalate derivatives, alkylsulphonate and polyethylether surfactants, fatty acids, diacid esters and carbohydrate derivatives. Different ionization options of electrospray mass spectrometry (ESI^+ and ESI^-) were used for substance identification. Carboxylic acid ester as well as non-ionic surfactants are more effectively ionized by a positive spray potential. The negative spray mode was successful for the detection of chlorophenols, anionic surfactants and some carbohydrates. Carboxylic acids could be ionized with both methods.

VI. PERSPECTIVE FOR FUTURE APPLICATIONS

The new applications discussed in this review demonstrate the high potential of solid-phase microextraction for fast screening and precise quantification methods for mainly organic target analytes at trace levels. The following major directions for future investigations can be summarized. The direct air sampling and determination of indoor air using integrated sampling techniques can be used as an alternative method in industrial hygiene. The coupling of SPME with fast GC increases the throughput of sample handling and shows a high applicability for field sampling methods. The dimensions of the micro device used for SPME increases the potential for small sample volumes. The small fibers can be exposed directly inside a

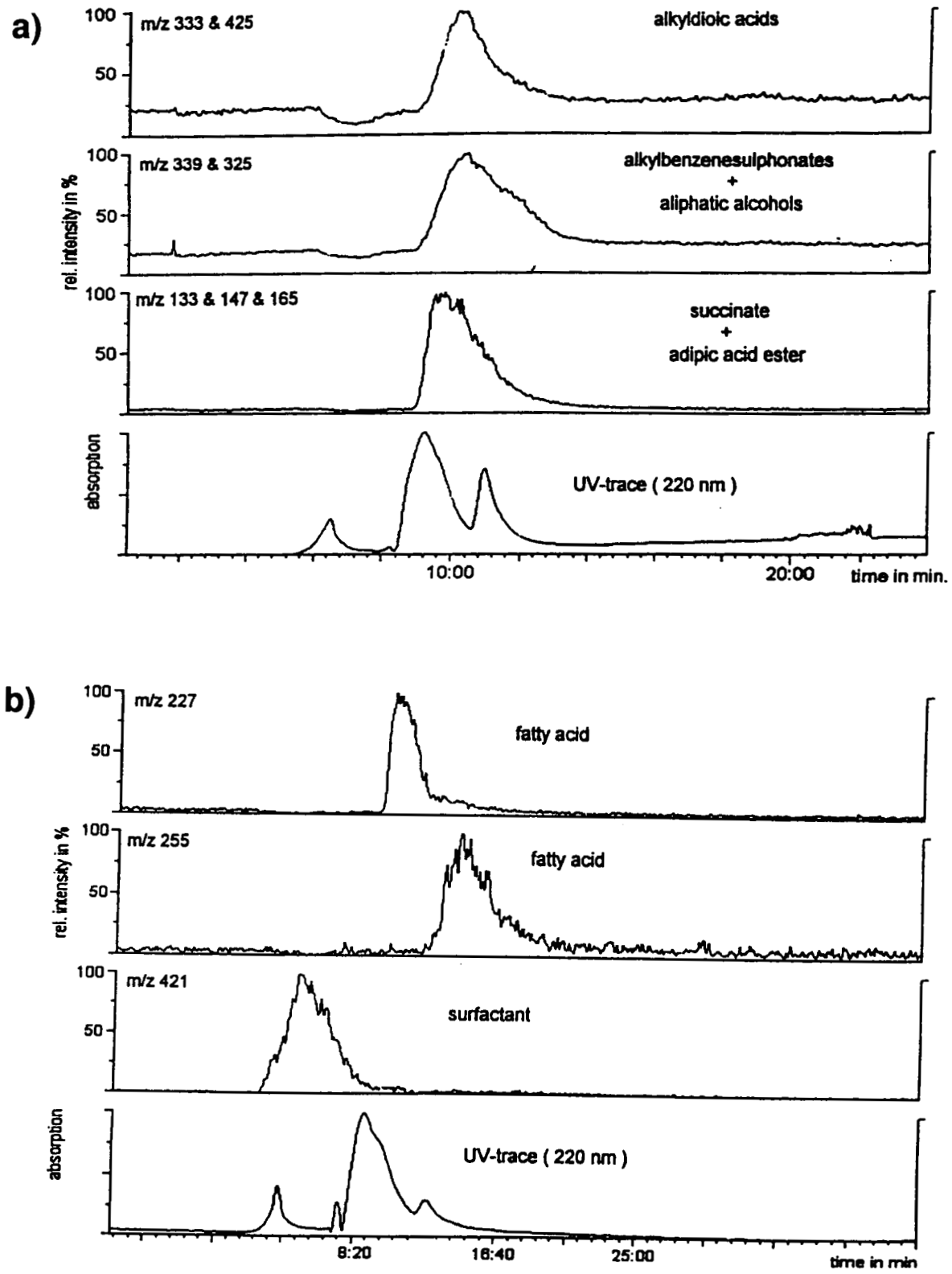

FIGURE 11. UV- and ion traces of a) the SPME-HPLC-ESI$^+$-MS analysis and b) the SPME-HPLC-ESI$^-$-MS analysis of the river sediment. From reference [62]. With permission.

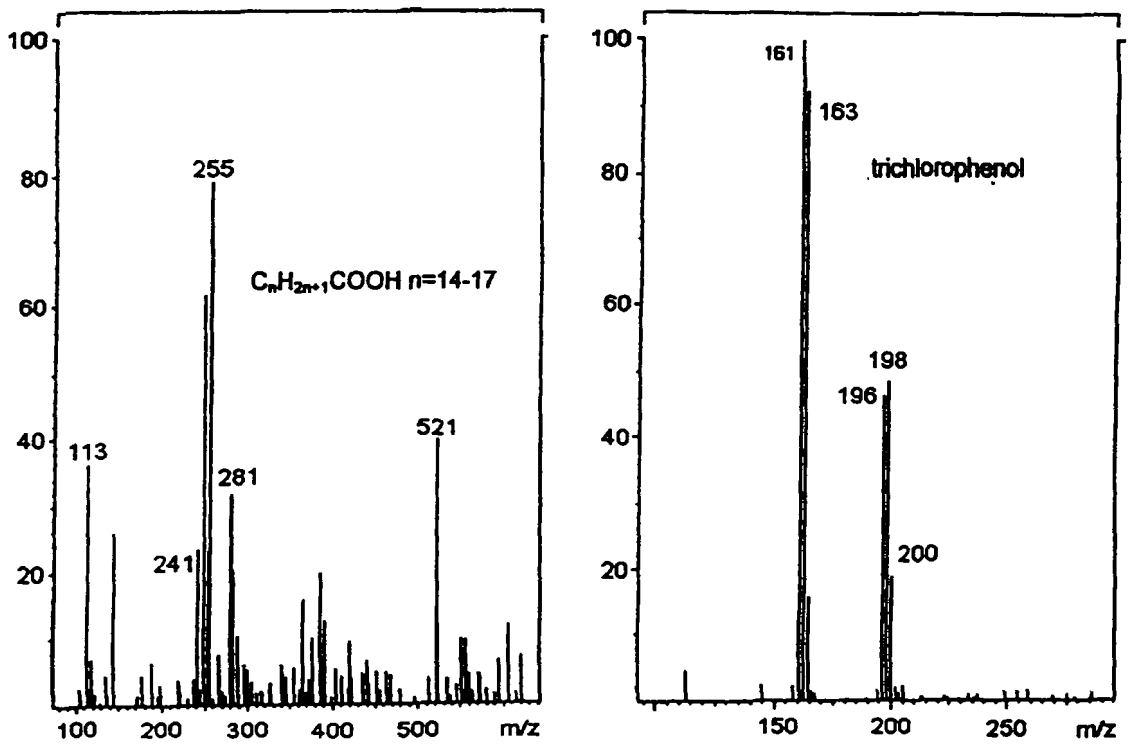

FIGURE 12. Mass spectra of a fatty acid mixture (left) and of trichlorophenol determined in the river sediment. From reference [62]. With permission.

flower for the determination of flavor compounds in air. The equilibrium technique of SPME and typically very small amounts extracted are important when in-situ monitoring without or less interference of the process is required. The non disturbing nature of SPME opens new application fields where exhaustive extraction techniques such as SPE are not amenable, for example, to study the fragrance spectra of flowers or to determine partitioning constants in complex systems without affecting the system [64]. In addition to more elegant derivatisation techniques which are already used for SPME, the automated in-tube SPME/HPLC method increases the potential for polar thermally labile compounds which can be determined in very small sample volumes. This opens the applications for drug analysis where the sample volume is limited and continuous monitoring becomes an important issue, such as intoxication, degradation, and metabolism studies. SPME has been successfully coupled to capillary electrophoresis (CE) analyzing PAHs [65] and barbiturates [52]. In addition, fully automated systems might be helpful for the design of continuous and quasi-continuous analysis apparatus which can be operated in remote field situations or on-site for process control or monitoring of river and surface water systems. Consequently, the data basis of these studies and its reliability will be increased. Fast GC can be easily performed in portable micro GCs when using SPME for the sample preparation. New coating materials, such as metal coatings and porous polymers will increase the number of specific applications. Furthermore, the technique is capable of the determination of physical constants such as distribution constants with a minimum or no interference with the target system. This approach was shown for the determination of distribution constants of chemicals in water in the pres-

ence of high dissolved organic matter (HOM) [64]. The SPME fiber technique can be used for direct coupling to MS and ICP/MS [19] gaining maximum selectivity for the determination of organic and inorganic target compounds. The direct coupling without any column separation technique increases the sample throughput significantly and increases sensitivity.

The unique, solvent-free, and easy sample preparation method of SPME has been successfully applied to many organic target analytes in environmental, bioanalytical and industrial hygiene studies. Further automation and miniaturization of the entire tool will keep the advantages of this extraction device.

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