Stir-Bar Sorptive Extraction of Trace Organic Compounds from Aqueous Matrices

Frank David,1 Bart Tienpont2 and Pat Sandra,1,2
1Research Institute for Chromatography, Kortrijk, Belgium,
2University of Ghent, Ghent, Belgium.

Stir-bar sorptive extraction is a new solventless sample preparation method for the extraction and enrichment of organic compounds from aqueous matrices. The method is based on the same principles as solid-phase microextraction (SPME). Compared with SPME, a relatively large amount of extracting phase is coated on a stir bar. Solutes are extracted into the coating, based upon their octanol–water partitioning coefficient and upon the sample–extraction medium phase ratio. The technique has been applied successfully to trace analysis in environmental, biomedical and food applications. Users can obtain extremely low detection limits.

To determine organic trace compounds in aqueous matrices, an analytical method normally requires an extraction and enrichment step before analysts can perform chromatographic separation and detection. During the extraction and enrichment step, the trace solutes are isolated from the matrix, and the concentration of the solutes is increased to enable their identification or quantification. In environmental, food safety, biomedical and other analyses, analysts use a variety of extraction and enrichment techniques. These methods are based upon liquid–liquid extraction, solid-phase extraction (SPE), liquid–gas extraction methods such as purge-and-trap and liquid–gas equilibrium techniques such as static headspace.

Miniaturization has become a dominant trend in analytical chemistry. Typical examples of miniaturization in sample preparation techniques are micro-liquid–liquid extraction (or in-vial liquid–liquid extraction), disc–cartridge SPE, on-line SPE and solid-phase microextraction (SPME). In combination with state-of-the-art analytical instrumentation, the overall method can result in faster analysis, higher sample throughput, lower solvent consumption and less manpower per sample while maintaining or even improving sensitivity. In particular, the reduction of solvent consumption in analytical laboratories has an important effect on analytical costs. In most instances, miniaturized sample preparation techniques can also be automated and coupled on-line to the analysis. On-line coupling of extraction and analysis, whereby the whole extract is transferred to the analytical system, results in a higher sensitivity and lower potential analyte loss. Alternatively, analysts can use smaller sample volumes.

In the past, analytical chemists paid a lot of attention to solventless sample preparation techniques based upon sorptive extraction. These techniques include SPME and stir-bar sorptive extraction. Sorptive extraction has proven to be an interesting and environmentally friendly alternative to liquid extraction. In sorptive extraction, the analytes are extracted from the matrix (mostly aqueous) into a non-miscible liquid phase. In contrast to extraction with adsorbents in which the analytes are bound to active sites on a surface, the total amount of extraction phase is important in sorptive extraction, not the surface only. The most widely used sorptive extraction phase is polydimethylsiloxane. This phase is well known as a stationary phase in gas chromatography (GC), is thermostable, can be used in a broad temperature range (220 °C–320 °C), and has interesting diffusion properties. Extraction with polydimethylsiloxane can therefore be compared with a micro-liquid–liquid extraction. After extraction, the solutes can be introduced quantitatively into the analytical system by thermal desorption. This process provides high sensitivity because the complete extract can be analysed.

Baltussen and co-workers3 recently reviewed the principles and applications of sorptive extraction. The main difference between SPME and stir-bar sorptive extraction is the much higher mass of polydimethylsiloxane available in the latter, which results in high recoveries and higher sample capacity.

In this “Sample Prep Perspectives” column, we will describe the principles of stir-bar sorptive extraction and present some typical applications in different analytical areas.

Stir-Bar Sorptive Extraction Principle

Different research groups in the mid 1980s4–7 described the extraction of organic compounds from an aqueous or gas phase using open-tubular traps coated with thick polydimethylsiloxane films. However, practical limitations — such as low sample capacity and low breakthrough...
Sorptive extraction by nature is an equilibrium technique, and for water samples the extraction of solutes from the aqueous phase into the extraction medium is controlled by the partitioning coefficient of the solutes between the silicone phase and the aqueous phase. Recent studies have correlated this partitioning coefficient with the octanol–water distribution coefficients (K_{o/w}). Although not fully correct, the octanol–water distribution coefficient gives a good indication if and how well a given solute can be extracted with SPME or stir-bar sorptive extraction.

However, it is very important in this respect to realize that the sorptive equilibrium is also dependent upon the phase ratio and thus on the amount of polydimethylsiloxane applied. This relationship is shown in Equation 1.

\[ K_{o/w} = K_{PDMS/w} = \frac{C_{PDMS}}{C_{w}} = \left( \frac{m_{PDMS}}{m_{w}} \right) \left( \frac{V_{w}}{V_{PDMS}} \right) \quad [1] \]

The distribution coefficient between polydimethylsiloxane and water \( K_{PDMS/w} \) is defined as the ratio between the concentration of a solute in the polydimethylsiloxane phase \( C_{PDMS} \) over the concentration in the water \( C_{w} \) at equilibrium. This ratio is equal to the ratio of the mass of the solute in the polydimethylsiloxane phase \( m_{PDMS} \) over the mass of the solute in the aqueous phase \( m_{w} \) times the phase ratio \( \beta \), with \( \beta = V_{w}/V_{PDMS} \).

The recovery, expressed as the ratio of the extracted amount of solute \( m_{PDMS} \) over the original amount of solute in the water \( m_{0} = m_{w} + m_{PDMS} \), is thus dependent upon the distribution coefficient \( K_{PDMS/w} \) and on \( \beta \), as described in Equation 2.

\[ \frac{m_{PDMS}}{m_{0}} = \frac{K_{PDMS/w}}{1 + \left( K_{PDMS/w} / \beta \right)} \quad [2] \]

Using this equation, analysts can calculate the theoretical recovery for a solute with a known partition coefficient and a given phase ratio. From Equation 2, it is also clear that the extraction efficiency increases with increasing \( K_{PDMS/w} \). Because \( K_{PDMS/w} \) is similar to the octanol–water distribution coefficient \( K_{o/w} \), chemists can predict extraction efficiencies. Besides the \( K_{PDMS/w} \) factor, the phase ratio \( \beta = V_{w}/V_{PDMS} \), the sensitivity is increased by a factor of 50 to 250. The theoretical extraction efficiency reaches 100% for solutes with low \( K_{o/w} \) values, for example, values less than 10 000. In stir-bar sorptive extraction, 25–125 µL polydimethylsiloxane coatings are used. Consequently, the sensitivity is increased by a factor of 50 to 250. The theoretical extraction efficiency reaches 100% for solutes with low \( K_{o/w} \) values, for example, values less than 10 000. In stir-bar sorptive extraction, 25–125 µL polydimethylsiloxane coatings are used. Consequently, the sensitivity is increased by a factor of 50 to 250. The theoretical extraction efficiency reaches 100% for solutes with low \( K_{o/w} \) values, for example, values less than 10 000.

La figure 1 montre l’influence de \( K_{o/w} \) et de la phase ratio sur l’efficacité d’extraction. Pour SPME, la quantité de polydimethylsiloxane est d’environ 0.5 µL. Cela rend difficile la récupération de soluts à l’octanol–water pour \( K_{o/w} \) valeurs inférieures à 10 000. En extraction par barde extractante, 25–125 µL de polydimethylsiloxane sont appliqués. Par conséquent, la sensibilité est augmentée d’un facteur de 50 à 250. L’efficacité d’extraction théorique atteint 100% pour des soluts à \( K_{o/w} \) valeurs inférieures à 10 000.
second part of the stir bar is a thin glass jacket that covers the magnetic stirring rod. The third and outermost part is the layer of polydimethylsiloxane sorbent into which the analytes are extracted. The glass layer is essential in the construction of a high-quality stir bar. It effectively prevents decomposition of the polydimethylsiloxane layer, catalysed by the metal of the magnetic rod.

**Extraction procedure:** Stir-bar sorptive extraction of a liquid sample is performed by placing a suitable amount of sample in a headspace vial or other container. A polydimethylsiloxane-coated stir bar is added and the sample is stirred for 30–240 min. The extraction time is controlled kinetically; determined by sample volume, stirring speed and stir bar dimensions; and must be optimized for a given application. Optimization is normally accomplished by measuring the analyte recovery as a function of the extraction time. Optimum conditions are obtained when no additional recovery is observed when the extraction time is increased further.

After extraction, the stir bar is removed, dipped on a clean paper tissue to remove water droplets and introduced to an empty glass thermal-desorption tube. In some cases, we recommend rinsing the stir bar slightly with distilled water to remove adsorbed sugars, proteins or other sample components. This step will avoid the formation of non-volatile material during the thermal-desorption step. Rinsing does not cause solute loss, because the sorbed solutes are present in the polydimethylsiloxane phase. Finally, the solutes are thermally desorbed. Desorption temperatures are application dependent, primarily determined by the volatility of the solutes, and typically between 150 °C and 300 °C. Desorption can be accomplished in 5–15 min under a 10–50 mL/min helium flow. As an alternative to thermal desorption, analysts also can use liquid desorption. Sampling also can be performed in the headspace of a liquid or a solid sample.11

**Instrumentation:** In contrast to SPME, in which desorption is performed in the inlet of a gas chromatograph, stir-bar sorptive extraction is used in combination with a thermal-desorption system. Because more extraction phase is used, the desorption process is slower than that for a SPME fibre, and thus desorption combined with cold trapping and reconcentration is required. The whole process has been automated. Two systems are available commercially: the TDS-A classic thermal-desorption system and a specially designed Twister desorption unit (both from Gerstel). The systems can be mounted on gas chromatographs equipped with a CIS-4 programmed-temperature vaporizing inlet (Gerstel). The programmed-temperature vaporizing injector is operated as a cryotrap for cryogenic refocusing of the thermally desorbed analytes. Temperatures as low as −150 °C are used along with liquid nitrogen cooling. Both systems allow fully automated control of all desorption, trapping and injection conditions, including temperatures, flows and split or splitless modes.

**Applications of Stir-Bar Sorptive Extraction**

**Environmental analysis:** Stir-bar sorptive extraction has been applied successfully in environmental analysis. The main advantage of the technique is that it can be applied to volatile organic compounds (VOCs) and semivolatile compounds. In combination with liquid desorption and HPLC, the technique even can be used for non-volatile compounds. Depending upon their octanol–water partitioning, the compounds are extracted and enriched. Successful applications include volatile aromatic,2,11 halogenated solvent,2,11 polyaromatic hydrocarbon,12,13 chlorinated and brominated anisoles have 2-methylisoborneol, geosmin and their octanol–water partitioning, the compounds are extracted and enriched. Successful applications include volatile aromatic,2,11 halogenated solvent,2,11 polyaromatic hydrocarbon,12,13 chlorinated and brominated anisoles have

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![Figure 2](https://example.com/image.png)

**Figure 2:** Extracted ion chromatogram at m/z 91 from the analysis of VOCs in drinking water spiked at 5 ng/L. Stir-bar sorptive extraction was performed on a 50 mL sample using a 20 mm × 0.5 mm Twister stir bar. The analysis was performed by GC–MS in selected-ion monitoring mode.

**Table 1** provides an overview of stir-bar sorptive extraction applications.2,11–36 We will describe some typical applications below.

Recent research has paid attention to the presence of odorous compounds in drinking water. Compounds such as 2-methylisoborneol, geosmin and chlorinated and brominated anisoles have odour thresholds of less than 10 ng/L. Using
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stir-bar sorptive extraction these compounds can be extracted with high recovery from drinking water samples. In comparison to labour-intensive techniques such as closed-loop stripping, the stir-bar sorptive extraction method provides a much higher sample throughput with better sensitivity, reproducibility and accuracy.\textsuperscript{14,15}

Figure 3 shows the analysis of a water sample spiked with 2 ppt of 2-methylisoborneol, geosmin and 2,4,6-trichloroanisol. We analysed a 20 mL sample. The analysis was performed on a 30 m ×

0.25 mm, 0.25 µm df HP-5MS column (Agilent Technologies, Inc., Wilmington, Delaware, USA). Detection was performed by MS in selected-ion monitoring mode. The compounds were detected at the 2-ppt level. For some solutes, extremely low traces (picograms per litre or the parts-per-quadrillion level) could be detected using GC–MS in negative-ion chemical ionization mode.\textsuperscript{15}

Stir-bar sorptive extraction also can be combined with \textit{in situ} derivatization. For phenols, Tienpont and co-workers\textsuperscript{17} obtained excellent sensitivities by \textit{in situ} derivatization with acetic anhydride (acylation). Derivatization and extraction were performed simultaneously. The phenyl acetates were extracted more efficiently because the log $K_{ow}$ is higher than the value for the corresponding phenols.

\textbf{Biological fluids:} Stir-bar sorptive extraction also can be applied to the determination of organic compounds in biological fluids. Different classes of solutes have been extracted from serum, plasma or urine. These classes of solutes include phenols, steroids, fatty acids, drugs of abuse,\textsuperscript{18} barbiturates and benzodiazepines,\textsuperscript{19} phthalates and metabolites,\textsuperscript{21} and VOCs such as aldehydes and sulphur compounds.\textsuperscript{22} Special applications include the determination of polynuclear aromatic hydrocarbon (PAH) metabolites, such as hydroxy PAHs, in urine\textsuperscript{23} and the determination of PCBs in sperm.\textsuperscript{24}

Urine samples can be extracted directly or after enzymatic hydrolysis. \textit{In situ} derivatization also can be used. Blood samples, including serum and plasma, bile fluids and sperm, must be diluted with water or a buffer solution before extraction.

Figure 4 is a typical example that shows the determination of drugs of abuse in the urine of a drug-addicted patient. We extracted a 5 mL urine sample directly (without dilution) using a 10 mm × 0.5 mm Twister stir bar. Methadone (peak 1) and its metabolite I (peak 2) can be detected in the extracted-ion chromatogram [Figure 4(a)]. We also detected traces of metabolites of d-9-tetrahydrocannabinol (probably from cannabis use) (Figure 4b). Cocaine also was detected in Figure 4b. Although the log $K_{ow}$ of cocaine was rather low (log $K_{ow}$ = 2.17), the compound could still be detected by stir-bar sorptive extraction–thermal desorption–GC–MS at trace levels.

In combination with derivatization, polar

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure3}
\caption{Extracted ion chromatograms for 2-methylisoborneol (ion m/z 108), 2,4,6-trichloroanisole (ion m/z 197), and geosmin (ion m/z 112) from an analysis of odorous compounds spiked in drinking water at 2 ng/L. Stir-bar sorptive extraction was performed on a 20 mL sample using a 10 mm × 0.5 mm Twister stir bar. The analysis was performed by GC–MS in selected-ion monitoring mode.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure4}
\caption{Extracted ion chromatograms at (a) ions m/z 72 and 276, (b) ion m/z 182, and (c) ions m/z 231 and 330 showing the presence of drugs in urine of a drug-addicted patient. Stir-bar sorptive extraction was performed on a 20 mL sample using a 10 mm × 0.5 mm Twister. The analysis was performed by GC–MS in scanning mode.}
\end{figure}

\textbf{Peaks:} 1 = methadone, 2 = 2-ethylidene-1,5-dimethyl-3,3-diphenylpyrrolidine (methadone metabolite), 3 = cocaine, 4 = cannabidiol, 5 = cannabichromene, 6 = cannabielsoin.
solute also can be analysed, as demonstrated for the analysis of \(\alpha\)-aminohippuric acid, a uremic toxin related to renal dysfunction. The native compound is very polar (free amino and acid function, \(\log K_{ow} = 0.3\)), but after derivatization with ethyl chloroformate into the N-ethoxycarbonyl ethyl ester (\(\log K_{ow} = 3\)), Tienpont and colleagues \(^{18}\) obtained an efficient extraction.

**Food analysis:** Another successful application area of stir-bar sorptive extraction is the determination of minor food ingredients and food contaminants. Stir-bar sorptive extraction has been used for the determination of flavour and off-flavour compounds in different food matrices, including non-alcoholic and alcoholic beverages and dairy products. \(^{25-30}\) Food preservatives have also been analysed. \(^{31}\) In general, analysts encounter no problems with different food matrices if the fat content is less than 2–3%. In other instances, dilution is necessary. For samples that contain high levels of alcohol, dilution to a maximum ethanol concentration of 10% is necessary.

Finally, stir-bar sorptive extraction has also been used for the determination of contaminants in food. Hoffmann and co-workers \(^{32}\) were able to determine trichloroanisole, the compound responsible for the musty odour and taste in wine, at very low nanograms-per-litre levels in wine samples.

Several applications have shown the determination of pesticides in food matrices. \(^{33-35}\) Recently, we presented a multiresidue screening method for non-fatty foods; that is, fruits and vegetables. \(^{36}\) The method is based on stir-bar sorptive extraction of a diluted methanol extract followed by thermal desorption and GC–MS analysis. Typically, we extracted a 15 g sample with 30 mL of methanol. From the extract, we diluted 1 mL with 10 mL of water and then extracted that solution with a stir bar for 60 min. After extraction, we washed the stir bar with a few millilitres of water to remove adsorbed sugars or non-volatile material, dried it on a clean tissue, and inserted it into a thermal-desorption tube. Thermal desorption is performed at 280 °C for 5 min. The compounds are trapped in the programmed-temperature vaporizing inlet at 250 °C and then injected by programming to 280 °C at 600 °C/min. The analysis is performed on an Agilent 6890 gas chromatograph and an Agilent 5973 mass-selective detector operated in scanning mode. The analytical conditions are identical to the conditions used for the retention-time-locked analysis of pesticides. \(^{37}\) Samples analysed under these conditions can thus be screened for the pesticides present in the Agilent RTL pesticide screener library. Based on the \(\log K_{ow}\) values of the 567 compounds present in the library, we have calculated that 374 pesticides can be extracted efficiently and screened using this method. The sensitivity of the method allows the detection of pesticides in the low parts-per-billion range. For most compounds and matrices, the limits of detection are less than 10 ppb.

Figure 5 is an example of this pesticide screening. We detected tolylfuand, endosulphan sulphate and bromopropyline in the extract of a pear sample. The measured concentrations were 59 ppb, 3 ppb and 190 ppb, respectively. This application also demonstrates that stir-bar sorptive extraction can be used for the analysis of solid samples after a preliminary extraction with a water-miscible solvent.

**Quantification in Stir-Bar Sorptive Extraction**

Quantification in stir-bar sorptive extraction can be performed in different ways, and the selection of the method is mainly dictated by the complexity of the sample. For example, both external standardization and internal standard addition can be used for tap water because matrix effects that contribute to the equilibrium are absent. For samples in which matrix effects contribute to the equilibrium — biological fluids, wastewater, beverages, fruits and vegetables — different methods can be used, including single-level calibration with a standard at a concentration close to that of the estimated concentration and prepared in a blank matrix to compensate for matrix effects, internal standard addition of deuterated or \(^{13}\)C-labeled target solutes and standard addition at three to six concentration levels. The first method requires a blank sample to compensate for matrix effects, but, as strange as this may seem, these samples are often difficult to obtain. For the second approach, labeled standards are commercially available for only a few solutes such as deuterated PAHs and \(^{13}\)C PCBs. The last method is by far the easiest to use in a routine environment, and it has been applied in the determination of dicarboximide fungicides in wines \(^{35}\) and in the quantification of pesticides in...
vegetables, fruits and baby food. Conclusion

Stir-bar sorptive extraction is a solventless extraction and concentration technique that can be used successfully to determine low traces of organic compounds in aqueous matrices, including water samples, biological fluids and food samples. We have obtained sensitivities of less than 1 ng/L, depending upon the solutes (log K_dW), sample volume, stir bar dimensions and GC–MS sensitivity.

References


Frank David is R&D manager at the Research Institute for Chromatography, President Kennedypark 20, B-8500, Kortrijk, Belgium, e-mail frank.david@richrom.com.

Bart Tienpont is a Ph.D. student at the Laboratory of Organic Chemistry, University of Ghent, Krijgslaan 281 S4, B-9000 Ghent, Belgium.

Pat Sandra is director of the Research Institute for Chromatography and a professor at the University of Ghent.

“Column Watch” editor Ronald E. Majors is the business development manager, consumables and accessories business unit, Agilent Technologies, Wilmington, Delaware, USA and is a member of LC•GC Europe Editorial Advisory Board.

Direct correspondence about this column to LC•GC Europe, Advanstar House, Sealand Road, Chester CH1 4RN, UK, e-mail dhills@advanstar.com.