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# Green approaches in sample preparation of bioanalytical samples prior to chromatographic analysis

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ABSTRACT

Sample preparation is considered to be the most challenging step of the analytical procedure, since it has an effect on the whole analytical methodology, therefore it contributes significantly to the greenness or lack of it of the entire process.

The elimination of the sample treatment steps, pursuing at the same time the reduction of the amount of the sample, strong reductions in consumption of hazardous reagents and energy also maximizing safety for operators and environment, the avoidance of the use of big amount of organic solvents, form the basis for greening sample preparation and analytical methods.

In the last decade, the development and utilization of greener and sustainable microextraction techniques is an alternative to classical sample preparation procedures. In this review, the main green microextraction techniques (solid phase microextraction, stir bar sorptive extraction, hollow-fiber liquid phase microextraction, dispersive liquid – liquid microextraction, etc.) will be presented, with special attention to bioanalytical applications of these environment-friendly sample preparation techniques which comply with the green analytical chemistry principles.



## 1. Introduction

The World Commission on Environment and Development (1987) has defined sustainability to be "the satisfaction of the needs of present generation, while the welfare of future generations is also taken into consideration to the same extent making them able to meet their own needs" [1]. The idea of green chemistry has its roots in sustainable development. Green chemistry is about atom economy, energy efficiency and it is focused on the design of chemical products and procedures that minimize the use and generation of hazardous substances [2]. In 1998, P. Anastas and J. Warner determined a set of principles to which a chemical procedure should correspond in order to be considered as environmentally friendly [3].

Green analytical chemistry (GAC) emerged from green chemistry in 2000 and it refers to the role of analytical chemists in making clever combination of environment-friendly and cheap methodologies. Sample preparation is a critical part of the analytical process and it has been the spotlight of thorough research from the GAC perspective in the past two decades since it affects the whole analytical methodology. Sample preparation must ensure a quantitative recovery of target analytes avoiding contamination and providing matrix isolation leading to reduction of interferences and matrix effects during the measurement step [4].

Liquid liquid extraction (LLE) and solid phase extraction (SPE) are the most commonly used sample preparation procedures. Main drawbacks of the liquid extraction procedure include the consumption of high amounts of hazardous reagents causing environmental problems, difficulty in coupling with the analytical instruments, limited selectivity leading to the extraction of many interfering components from the sample. In some extent, SPE reduces these disadvantages but great amounts of sample are still required for both procedures, a real problem when it comes to biological samples.

Therefore, microextraction techniques have been proposed as an alternative to classical sample preparation procedures since solid-phase microextraction (SPME) was developed by Pawliszyn and his coworkers in the 1990s [5]. At the same time period, attention was focused to the utilization of small volumes of liquids for analytical extractions, namely liquid phase microextraction (LPME). In particular this field was introduced in 1996 using organic droplets suspended from the tip of a microsyringe [6].The replacement of classical extraction procedures with microextraction techniques is a new concept in which the amount of sample

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and reagents used significantly decreases and the sustainability and speed of methods are improved.

Green microextraction techniques have advantages over classical techniques (LLE or SPE) including utilization of less solvents and minimal sample number and size, reduction of the sample treatment steps, reduction in consumption of hazardous reagents and energy maximizing at the same time the safety for operators and the environment, and generation of less waste [4].

Microextraction techniques find increasing applications in the sample preparation step before chromatographic determination of analytes in complex biological samples, such as saliva, blood, plasma, serum, urine, hair, cerebrospinal fluid, etc. Regarding biological samples, sample preparation prior to chemical analysis is required because the target analytes are present in low concentrations levels,often lower than the limits of detection of the analytical instrument. Moreover most biological samples are not compatible with the analytical instruments and they are too complex for direct analysis.

In this review, the main microextraction techniques, which comply with the green analytical chemistry principles, based on the solid phase and the liquid phase will be presented and selected recent applications of these environmentally friendly sample preparation techniques in biological samples will be described.

## 2. Microextraction techniques

### 2.1. Solid-phase microextraction (SPME)

Solid phase microextraction (SPME) is one of the most widely used green and solventless or with minimum solvent use sample preparation techniques applied in analytical practice for isolation/preconcentration of a wide range of analytes. Due to the fact that SPME fulfills the requirements of green chemistry such as simplicity in operation and reduction of analysis time saving energy at the same time, low solvent consumption, use of small sample size, direct use for sampling of river, lake etc, reuse of the fiber many times making SPME green also in terms of coating preparation and capability of full automation and because of its undoubted advantages over conventional procedures such as use of SPME under pre-equilibrium conditions with short extraction time, avoiding the use of complicated equipment, effectiveness, cost minimization, high sensitivity (possibility of determination at the ppt level), it has gained huge attention [5]. It is also impressively simple to couple with different instrumental techniques, usually with liquid chromatography (LC), gas chromatography (GC), high-performance liquid chromatography (HPLC) in the on-line or off-line modes [7].

SPME has been increasingly used to research since it allows analysis of many kind of samples in the three physical states (liquid, gas and solid), is used for the extraction of volatile and nonvolatile compounds from different matrices and determines trace or ultra trace levels of organic and inorganic analytes from samples of complex matrices [7,8].

It is a simple two-stage technique which includes sorption of analytes into the sorbent phase and desorption (solvent or thermal) of analytes from the sorbent phase and their introduction into separation or detection devices for analysis [7,9].

SPME is based on the equilibrium of analytes between the sample and a fused-silica or metal fiber coated with an appropriate sorption material mounted on a syringe needle as shown in Fig. 1, therefore the type and the property of the coating it has an effect to the efficiency of extraction and the final sensitivity of the analysis [7,10]. Depending on position of the fiber in relation to the sample, microextraction can be applied in two modes [7]:

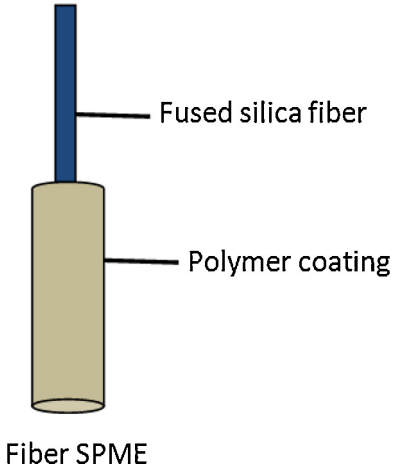


**Fig. 1.** The setting of the sorbent layer in SPME.

1) Direct (DI-SPME), where the fiber coating is exposed to gases or liquid samples and the analytes are transferred directly from the matrix to the stationary phase.
2) Indirect (adsorption from the headspace (HS-SPME)) where the fiber coating is exposed to the gaseous headspace above the sample and the analytes are transferred from the sample to the gas phase, which is in direct contact with the sample improving the selectivity of SPME, while avoiding contamination of the fiber and in this way prolonging its lifetime.

Choosing a suitable stationary phase, showing the highest affinity to target analytes, is considered quite difficult but at the same time is a main job for analytical chemists. One of the drawbacks of SPME is the restricted choice of commercially available stationary phases and fiber coatings used in this preparation technique despite the current requirements. Nowadays, the choice is limited to divinylbenzene (DVB), polydimethylsiloxane (PDMS), carboxen (CAR), polyethylene glycol (PEG) and Carbowax (CW) in different thicknesses and combinations [11,12].

The most commonly used sorbent is PDMS, which is an immobilized liquid at the extraction temperature, thermostable to around 300 °C, chemically neutral, with no active centers and a non polar material efficient for the extraction of non polar compounds. Analytes can be desorbed at medium temperatures, excluding analyte decomposition [12]. PDMS (100 μm) was selected as SPME fiber coating, as the result of the assessment of several polymer phases, for the analysis of persistent organic pollutants (POPs) in human serum by GC-EI-MS. The fiber was exposed for 50 min into a preheated and magnetically stirred vial which contained 1 mL serum, 1 mL deionized water, 200 μL $H_2SO_4$ and a magnetic bar and then it was inserted into the GC's injection port for 5 min at 230 °C. The proposed method involved a minimum organic solvent and the limits of detection for POPs pesticides were in the 0.22–5.41 μg/L range and the limits of quantification were in the 1.57–10.09 μg/L range [13].

Sometimes, stationary phases combine polar and non polar materials in order to achieve isolation and enrichment of mixtures of compounds of different groups which have wide polarity range (e.g ethers or alcohols). The most commonly used mixed-phase sorbents are carboxen/polydimethylsiloxane (CAR/PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) and Carbopack Z/PDMS [7,11]. Fluoride was determined in urine and plasma by HS-SPME and GC–MS/MS using CAR/PDMS as SPME fiber, 2-(bromomethyl) naphthalene for the derivatization and 2-fluoronaphthalene as an internal standard. Derivatization and