ADVANCES IN ANALYTICAL MICROEXTRACTION METHODS TOWARDS GREEN CHEMISTRY

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Abstract

Membrane supported liquid-phase microextraction is one of the emerging techniques in liquid-phase microextraction (LPME) system. In this system, a polypropylene membrane impregnated with an organic solvent to accommodate or protect micro-volumes of acceptor solution. This novel methodology proved to be an extremely simple, low-cost and virtually solvent-free sample-preparation technique that parallel with green chemistry principles. LPME provides a high degree of selectivity and enrichment and additionally eliminating the possibility of carry-over between runs. In addition, LPME tolerates wide pH range that makes the technique advantageous and attractive to be used in applications that would not be suitable for solid phase extraction (SPE) or solid phase microextraction (SPME). Due to the small volume of the extracting solvent, the extracted samples do not require further concentration prior to analysis and thus total analysis time is considerably decreased in comparison to traditional liquid-liquid extraction procedure. This technique is apparently favorable especially in fulfilling the green chemistry obligations.

Keywords: Green chemistry, microextraction, liquid phase microextraction

1.0   Green Chemistry

Many approaches for minimizing the environmental impact of a chemical process involve reducing the quantities of chemicals consumed in that process. This may be by replacing stoichiometric reagents with catalytic ones, or running the reaction without any

solvent at all. In many organic reactions, the second option may not be possible, as the solvents is involved in the reaction either directly, or indirectly by stabilizing transition states or acting as a heat transfer medium. On too may occasions, sadly, little consideration is given to the choice of solvent: it is chosen because it worked in the last reaction, or because it is readily available.

Where a solvent must be used, water is, without doubt, the most acceptable in terms of cost and environmental impact. However, despite its large liquid range and extremely high specific heat capacity, it is frequently overlooked as a solvent for organic reactions, perhaps because of a misplaced belief that organic reactions must require an organic solvent. All of these issues were used to improve a method of analytical to green chemistry. Lately, basic of green chemistry has become an interesting topic for discussion and debate that used for researchers [1-3].

Green chemistry is the design, development, and implementation of chemical products and processes to reduce or eliminate the use and generation of substances hazardous to human health and environment [4]. This definition is further elucidated by the Twelve Principles of Green Chemistry that are used as a design framework. They are;

1. Prevent waste
2. Design safer chemicals & products
3. Design Less Hazardous Chemicals Syntheses
4. Use renewable feedstocks
5. Use catalysts, not stoichiometric reagents
6. Avoid chemical derivatives
7. Maximize atom economy
8. Use safer solvents & reaction conditions
9. Increase energy efficiency
10. Design chemicals & products to degrade after use
11. Analyze in real time to prevent pollution
12. Minimize the potential for accidents

A green chemical should be synthesized in a safe and energy efficient manner; its toxicity should be minimal, whereas its biodegradation should be optimal. Lately, its impact to the environment should be as low as possible.

This paper presents the application of liquid phase microextraction (LPME) as a very powerful and efficient sample preparation technique for identifying and applied to the analysis some analytes.

2.0   Sample Preparation

In recent years, the development of fast, precise, accurate and sensitive methodologies has become an important issue. Despite the great technological advances, most analytical instruments cannot handle sample matrices directly and as a result, a sample-preparation step is commonly introduced. For organic trace analysis, this step mainly comprises extractions, which serve to isolate compounds of interest from a sample matrix. The ultimately purpose, the concentration of target compounds is enhanced (enrichment) and the presence of matrix components is reduced (sample clean up). The analysis of trace level analytes involves the discreet steps of extraction, concentration and clean-up prior to detection by an instrument [5].

Traditional liquid-liquid extraction (LLE) is a versatile sample-preparation technique, prescribed in many standard analytical methods. Despite its widespread use, it is considered to be time-consuming, tedious and multistage operation, where problems of emulsion formation obstruct automation. More importantly though, the use of large amounts of toxic organic solvents influences trace analysis, poses a health hazard to laboratory personnel and results in the production of hazardous laboratory waste, thus adding extra operational costs for waste treatment [6-10].

SPE techniques are perhaps the most popular in sample preparation especially for organic analysis. However, most official methods still use LLE techniques such as those published by the U.S. Environmental Protection Agency (USEPA). The principle of SPE is based on sorption of analytes on a sorbent. The aqueous sample solution passes the SPE column, and the analytes are first trapped on the sorbent and then eluted with a suitable small volume of organic solvent. Extraction and enrichment of the analytes is thereby simultaneously achieved [7]. Advantages of SPE over LLE are the smaller amounts of organic solvents employed, the minimal manual work and the availability of automated equipment. The selectivity of SPE is usually worse than that of LLE, on the other hand,

problems may occur because of the poor retention of polar compounds into the solid-phase sorbent.

For the past few years, research activities have been focused toward the development of efficient, economical and miniaturized sample preparation technique. Among these, solid phase microextraction (SPME) is most widely developed. SPME has successfully redressed the limitations inherent in the traditional LLE method. With SPME, a small amount of extractant phase, dispersed on a solid support (fiber), is exposed to the sample [8]. Target analytes partition between the sample matrix and the extractant phase, and after a well-defined period of time, the fiber is transferred to a gas chromatograph (GC) or to a high performance liquid-chromatography (HPLC) interface for analysis. However, the main problems commonly encountered with SPME are the limited lifetime of the SPME fiber, their relatively fragile and easily broken while handling, expensive and the possibility of carry-over between analyses [9].

One of the emerging techniques in the area of sample preparation is liquid-phase microextraction (LPME), where a membrane impregnated with an organic solvent is used to accommodate or protect micro-volumes of acceptor solution [10]. This novel methodology proved to be an extremely simple, low-cost and virtually solvent-free sample-preparation technique, which provided a high degree of selectivity and enrichment by additionally eliminating the possibility of carry-over between runs.

One of the main methodologies that evolved from the solvent microextraction approach is the single drop microextraction (SDME). Although SDME is a simple, low-cost, and fast extraction technique and applicable even for headspace extraction, it requires careful and elaborate manual operations. The problems of drop stability have often been reported in the literature. More importantly however, the sensitivity and the precision of the SDME methods developed are rather poor. This is because prolonged extraction times and faster stirring rates are not recommended, since they typically result in drop dissolution and/or stability [11].

To analyze very low concentrations of analytes such as pesticides in aqueous solution, we need sample preparation method. The sample preparation method is a very important step before analysis. The position of sample preparation in the analysis process is shown in Figure 1. The sample preparation step usually constitutes an extraction process to isolate the analyte from the sample matrix and enrichment of analyte [12].
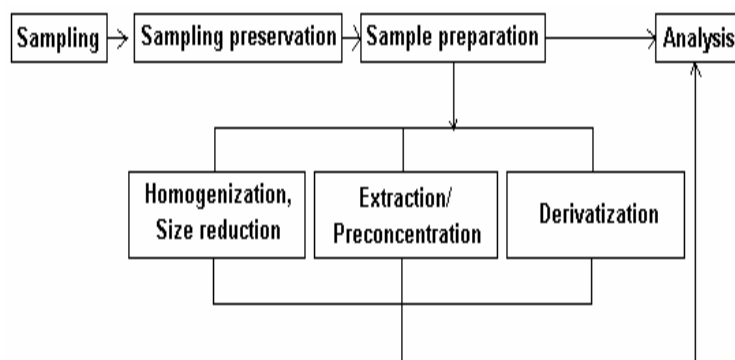
Figure 1: Diagram of analysis process [12]

3.0   Liquid Phase Microextraction (LPME)

In LPME, the organic (acceptor) phase is protected by the fiber and it appears that the presence of the hollow fiber decelerates the process of organic solvent dissolution into the bulk solution. Another factor contributing to the improved sensitivity of LPME is that the surface area for the rod-like configuration of the two-phase LPME system is larger than the spherical one adopted by the drop-based SDME method. By increasing the contact area between the sample donor solution and the organic acceptor phase, the rate of analyte extraction is increased.

A major advantage of LPME over SPME is that the range of compounds amenable to this technique can be extended by simply changing from the two-phase to the three-phase LPME mode and by adjusting the composition of the different phases. In addition, the low-cost per unit allows the use of each fiber for only one extraction. The disposable nature of the hollow fiber totally eliminates the possibility of sample carry-over and ensures high reproducibility, as the chemistry of each extraction unit is not affected by its previous usage. However, as the hollow-fiber segments are cut manually, variations in length are very likely. In addition, variations in wall thickness are possible and such fluctuations may alter analyte enrichment, especially when the two-phase approach is used [13].

Another advantage of LPME over SPME is that the small pore size ensures microfiltration, thus yielding very clean extracts. When the SPME is used in complex matrices, a sample-pre-treatment step (such as filtration), high salt concentration and pH

adjustment are applied to the sample solution can damage the SPME fiber. This is not the case for LPME, where the ionic strength of the sample solution and high or low pH values do not influence the repeatability of the method or the condition of the hollow fibre [13-15].

There are two sampling modes that can be used with LPME, namely two phase and three phase sampling modes (Figure 2) [15, 16]. In two-phase LPME sampling mode, analyte "i" is extracted from an aqueous sample (donor phase) through a water-immiscible solvent immobilized in the pores of the hollow fiber into the same organic solvent (acceptor phase) present inside the hollow fiber. The extraction process of the two-phase LPME for analyte i may be illustrated as follows:

$$i_d \leftrightarrow i_{org}$$

It is characterized by the distribution ratio $K_{org/d}$, which is defined as the ratio of the concentration of analyte i in the organic and donor phase at equilibrium conditions.

In the three-phase sampling mode, analyte i is extracted from an aqueous solution (donor phase) through the organic solvent immobilized in the pores of the hollow fiber (organic phase) into another aqueous phase (acceptor phase) present inside the lumen of the hollow fiber. The organic phase in this case serves as a barrier between an acceptor and the donor aqueous solution, preventing mixing of these two-phase. The three-phase sampling mode is usually combined with a high performance liquid chromatography (HPLC) or a capillary electrophoresis (CE) system, as the acceptor phase is aqueous.

Overall, the three-phase LPME extraction process for analyte i may be illustrated as follows;

$$i_d \leftrightarrow i_{org} \leftrightarrow i_a$$
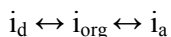
The three-phase LPME process is characterized by $K_{org/d}$ and $K_{a/org}$, which are the distribution ratio equilibrium between the organic phase and the donor phase, and the acceptor solution and the organic phase, respectively [16].
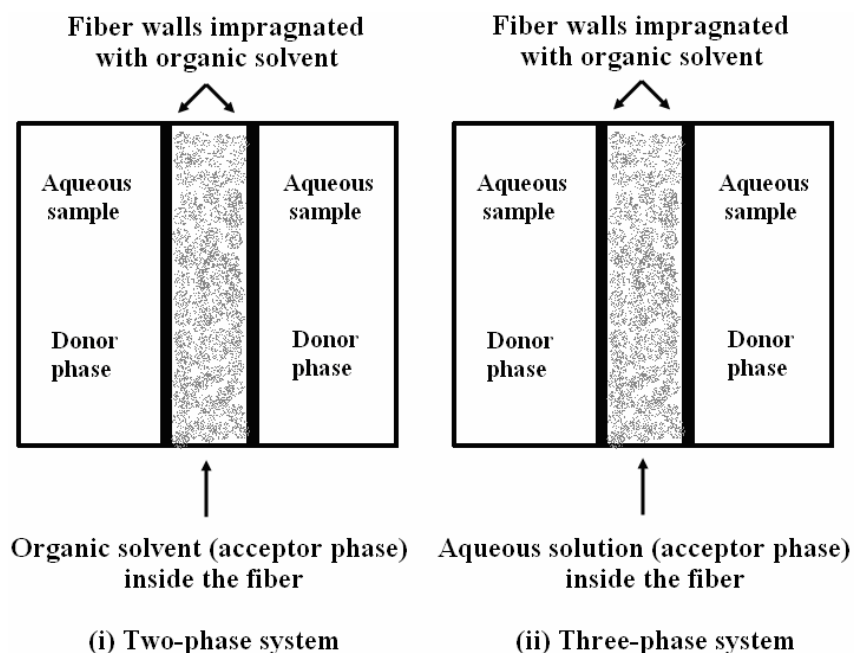
Figure 2: Two-phase and three-phase sampling modes LPME [11]

The overall distribution ratio $K_{a/d}$ between the acceptor and the donor phase can be written as:

$$K_{a/d} = K_{org/d} \cdot K_{a/org}$$

Adjustment of the composition of the donor and acceptor phase is critical for successful three-phase LPME. Large Ka/d (>> 1) can be achieved when the analytes in the acceptor phase are converted by reactions, such as protonation or complexation, to species that will have very small affinity for the organic phase. In this way, back extraction of analytes for the acceptor to the donor solution is prevented. Hence, the three-phase system will also extract acidic or basic compound having a low $K_{org/d}$, as long as the $K_{a/org}$ value is high, thus preserving the requirement for large overall distribution ratio. This is very important, because the applicability range of LPME may be extended to ionisable analytes having a low $K_{org/d}$ by simply changing from two-phase to three-phase LPME sampling mode [16].

There are essentially two different configurations (A and B) of arranging the fiber during extraction with the two-phase or three-phase LPME sampling modes (Figure 3) [11]. In configuration A, two conventional medical syringe needles are inserted through a septum

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and the two ends are connected with a piece of hollow fibre. After impregnation, the hollow fiber is immersed in the donor solution. The acceptor solution is then injected in the lumen of the hollow fibre with the help of a microsyringe and once extraction is completed, the acceptor solution is collected in microvials by applying a small head pressure on the inlet or withdrawn with the help of a microsyringe and submitted to analysis. This configuration is called U-shaped.

In configuration B, only one end of the hollow fibre is used for injection/collection of the acceptor solution and the other one is left suspended in the donor-sample solution. In general, when configuration B is used, the free end of the hollow fibre can be flame-sealed. Prior to immersion in the donor solution, the fiber should be dipped in the organic solvent to impregnate it.
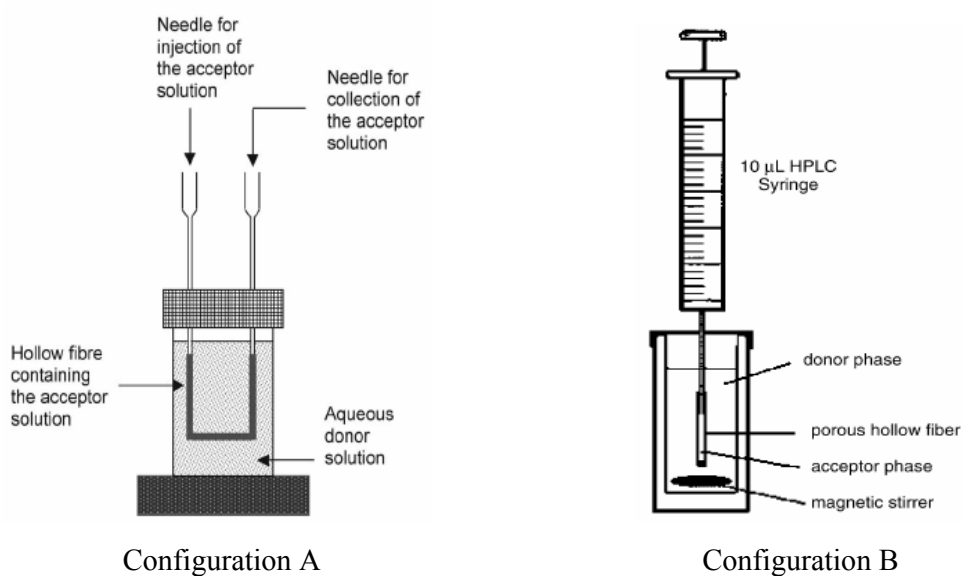

Configuration A   Configuration B

Figure 3: Configuration A and B in LPME [11]

3.1   Cone Shaped LPME

LPME has seen a recent development where instead of using a hollow polypropylene fibers, a cone-shaped membrane was used to immobilized the acceptor phase (Figure 4). This method, however, requires relatively larger amounts of organic solvent as acceptor phase, and

thus the enrichment factor as the main purpose of extraction step is rather modest (55 - 59 folds) [17].

Cone shaped LPME describes a simple and inexpensive extraction technique which involves two-phase microextraction combined with micro-liquid chromatography (micro-LC) for the analysis of selected pesticides in water. In this novel procedure, a cone shaped Nylon membrane is used to protect the extracting solvent, thus permitting extraction only on the surface of the solvent immobilized in the membrane pores [17]. A round-shaped Nylon 66 membrane filters (200 μm thickness and 0.2 pore size) were cut into halves and each half was folded and sealed with flame into a cone shaped membrane with an open diameter of 13 mm and height of approximately 20 mm to fit the vial I.D (Figure 4).
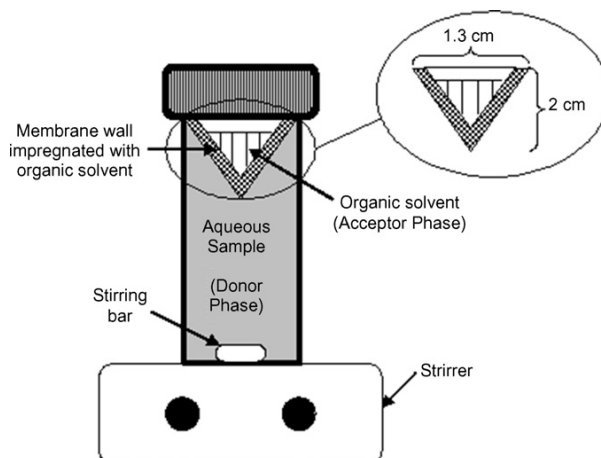

Figure 4: Schematic of Cone-Shaped LPME [17]

A 15 mL aliquot of sample solution was placed into a 15 mL sample vial. The membrane was immersed into organic solvent for approximately 10 s to allow the solvent to impregnate the pores of the membrane wall. After solvent impregnation, the CSM was quickly positioned in the sample vial that already contained the aqueous sample, and a 200 μL aliquot of organic solvent was pipetted into the membrane. The sample was continuously stirred with a magnetic stirrer at room temp to facilitate the mass transfer of the analytes between donor phase and acceptor phase. The agitation also significantly decreased the time required for the equilibrium to establish. After 20 min of extraction, the analyte-enriched solvent was withdrawn and transferred into 1.5 mL cone-shaped vial. The solvent was dried with gentle flow of nitrogen and redissolved with 100 μL of acetonitrile solution containing 1

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ppm prefonofos (I.S). A 0.5 µL of solvent was withdrawn into the syringe and injected into micro-LC system for further analysis [17].

In the research, four pesticides (hexaconazole, procymidone, quinalphos and vinclozolin) were considered as targeted analytes. Several important extraction parameters such as type of extraction solvent, agitation rate, pH value, total exposure time and effect of salt and humic acids were optimized. Enrichment factors of >50 folds were easily achieved within 20 min of extraction. The analytical data demonstrated relative standard deviation for the reproducibility of the optimized CSM-LPME method ranging from 6.3 to 7.5 %. The correlation coefficients of the calibration curves were at least 0.9995 across a concentration range of 2-100 µg/L. The detection limits for all analytes were found to be in the range of 1.1-1.9 µg/L [17].

3.2   Coated Fiber LPME

The polypropylene fiber used in the extraction process was prepared before coating. The 1.5 cm of polypropylene was cut manually and sealed in one edge by sealed machine, then washed by acetone for several minutes to remove any contaminants. The fiber was removed and allowed to dry completely. The fiber was put into a small vial and Fenton's reaction was carried out to activate the fiber surface. The Fenton's reaction was done by mixing $FeSO_4$ and H_2O_2 step by step in the vial that contained the fibers. Once completed, the fiber was illuminated by UV-ray for 1 h. After illumination process, fiber was dried in the room temperature and ready to coating.

Polydimethylsiloxane (PDMS) sol phase was prepared by mixing 300 µL MTMOS, 400 µL OH-TPDMS, 20 µL PMHS and 200 µL 95% TFA in a bullet shaped tube. Then, the tube was vortexed for 5 minutes before centrifuged at 12,000 rpm for 10 minutes. The centrifugation process will result in two layer solution which the top sol solution was used for further fiber coating. The fiber was dipped vertically into the sol solution for 15 minutes and was formed on the bar outer surface of the fiber end about 1.5 cm. The coating technique is illustrated in Figure 5. Then, the coated fibers were allows to dry in room temperature for 1 day and were end-capped in a 20% (v/v) methanolic solution of trimethylmethoxysilane (TMMS) for 1 minute.
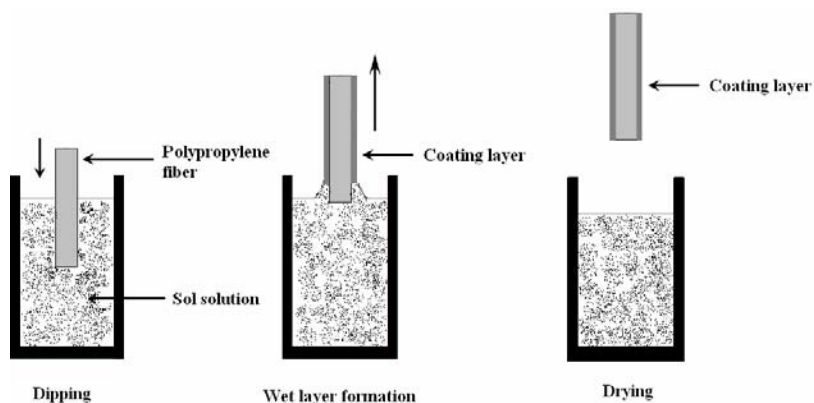
Figure 5: Sol-gel coating technique of PDMS fiber

For the extraction process, a 10 μL SGE syringe (Sydney, Australia) with a cone tip was used to introduce the organic solvent as acceptor phase into the coated hollow fiber membrane polypropylene. The coated hollow fiber used 1.5 cm of length, a new coated hollow fiber was used each extraction to prevent the carry over effect. The experiment steps are as follows: The coated fiber was dipped in organic solvent (e.g. toluene) for 10 seconds to impregnate all pores of the fiber with organic solvents. Microsyringe that contains organic solvent as acceptor phase (3 μL toluene) was inserted through the septum of the sample vial. The microsyringe needle tip was then inserted into the coated hollow fiber segment and the assembly was immersed in the 11 mL sample solution that contain magnetic stirrer. Acceptor phase was injected into the coated hollow fiber to start the extraction. After extraction for prescribed time, the plunger of microsyringe was withdrawn and the acceptor phase obtained was transferred into a small vial (2 μL) to allow solvent to evaporate at room temperature. Then, 50 μL of acetonitrile was added to reconstitute the extract and 1 μL of reconstituted solution was injected for the HPLC analysis.

From the works that has been done, it showed that the coated fiber can extract analytes 1.5 times than uncoated fiber.

3.3 Three Phase LPME

Recent works in our laboratory are focusing on the development of three phase LPME for the analysis of some tricyclic antidepressant drugs and phenolic compounds. Three-phase LPME involves extraction from an aqueous sample matrix, through an organic phase in the

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pores of hollow fiber, immiscible with water and back into the new aqueous phase inside the lumen of the hollow fiber [18].

For extraction of basic compounds, pH of the samples has to be adjusted into the alkaline region to suppress analyte solubility, whereas pH in the acceptor solution should be low to promote analyte solubility. In this manner, the basic compounds may be easily extracted into the organic phase and further into the acceptor phase without back extraction to the organic phase again. By contrast, for acidic analytes, pH of the sample should be low and an acceptor should be utilized within the lumen of the fiber [16].

For the extraction, the fiber pores were impregnated with organic solvent by dipping the fiber into the solvent for 5 s and the excess of this solvent was removed by ultrasonicating the fiber in ultra pure water for 15 s. 24 µL of the acceptor phase (pH 2.1) was withdrawn into the microsyringe and its needle was inserted into the channel of the hollow fiber. The fiber was supported in the U shape configuration by two medical syringe needles (0.6 cm O. D.). Two syringe needles were inserted through the silicon septum; one served to introduce the acceptor solution into the hollow fiber prior to extraction while the second needle was utilized for collection of the acceptor solution after extraction. The whole fiber is totally immersed in the donor phase but above the magnetic stirrer bar, so that it would not be damaged during the stirring. The vials were placed on a multiple-station magnetic stirrer for 45 minutes at approximately 500 rpm and covered with a small piece of parafilm, in order to avoid contamination, and to reduce evaporation. After completion of extraction, the analyte-enriched acceptor phase was withdrawn and was directly injected into the high temperature HPLC system for further analysis.

From the present work, it is expected that the results obtained will be better in term of enrichment factors, partition coefficient and selectivity for the selected compounds.

Conclusions

Based on the works done in our laboratory, liquid phase microextraction has been proven as a simple and fast sample preparation technique. As the usages of organic solvents are minimal, LPME could be considered as an emerging green chemistry technique for analytical microextraction.

Acknowledgements

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