THE DEVELOPMENT OF GREEN MICROEXTRACTION METHODS FOR THE DETERMINATION OF PHENOLIC ACIDS IN HONEY AND BEVERAGES

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by

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Specially dedicated to:

My Dad,

My Mum,

My Wife ,Son and daughter

Brother and Sisters,

My Relatives and Friends

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LIST OF ABBREVIATIONS

AP	Acceptance phase
BET	Brunauer-Emmett-Teller
BGE	Background electrolyte
ACN	Acetonitrile
CZE	Capillary zone elecrophoresis
DAD	Diode array detection
DLLME	Dispersive liquid liquid microextraction
DP	Donor phase
EF	Enrichment factor
FID	Flame ionization detection
Fl	Fluorescence detection
GC	Gas chromatography
HF	Hollow fiber
HPLC	High performance liquid chromatography
HS	Head space
i.d	internal diameter
IS	Internal standard
LC	Liquid chromatography
LLE	Liquid- liquid extraction
LOD	Limit of detection

Log p	Log Octanol-water partitioning coefficient
LOQ	Limit of quantitation
LPME	Liquid phase microextraction
M	Molar
MeOH	Methanol
MS	Mass spectrometry
MS/MS	Tandem mass spectrometry
MΩ	Mega ohms
o-	Ortho
p-	Para
PDMS	Polydimethylsiloxane
Pka	Acid dissociation constant
r^2	Regression coefficient
rpm	Rate per minute
RSD	Relative standard deviation
S/N	Signal -to-noise ratio
SDME	Single drop microextraction
SDME	Single drop microextraction
SLE	Supported liquid extraction
SPE	Solid phase extraction
SPME	Solid phase microextraction
UPLC	Ultra high pressure liquid chromatography
UV	Ultraviolet detection

VALLME	Vortex assisted liquid-liquid microextraction
π-π	Pi-Pi interaction
S/N	Signal -to-noise ratio
SDME	Single drop microextraction

PEMBANGUNAN KAEDAH PENGEKSTRAKAN MIKRO HIJAU BAGI PENENTUAN ASID FENOLIK DI DALAM MADU DAN MINUMAN

ABSTRAK

Teknik bantuan ultrabunyi-penyerakan matrik fasa pepejal (UAMSPD) telah dibangunkan bagi penentuan dua belas asid fenolik (sinnamik, m-komerik, klorogenik, siringik, ferulik, o-komerik, benzoik, p-komerik, vanilik, p-hidroksibenzoik, kafeik, 2,4-dihidroksibenzoik) di dalam madu menggunakan elektroforesis zon rerambut (CZE) dan pengesan pancaran foto diod. Dalam keadaan optimum, sampel (0.5 g) diserakkan dengan Amberlite XAD-2 (2 g), diikuti dengan cucian dengan air berasid (pH 2.0) dan seterusnya dibilas dengan metanol (8 mL). Purata perolehan semula kaedah UAMSPD–CZE bagi madu yang ditambahkan larutan piawai adalah di dalam julat 71.0-106 %. Had pengesanan (LODs) adalah daripada 0.036-0.132 μg g^{-1}. Kaedah ini telah berjaya digunakan bagi beberapa jenis madu Malaysia. Kaedah pengekstrakan yang selektif, ringkas, efisien dan mesra alam yang berasaskan kepada setiltrimetilammonium bromida-bentonit yang telah diubahsuai (CTAB-bentonit) sebagai penjerab di dalam pengekstrakan fasa pepejal-mikro (μ-SPE) telah dibangunkan bagi penentuan dua belas asid fenolik di dalam sampel jus. Pengubahsuaian permukaan bentonit telah disahkan menggunakan FTIR, TEM dan BET. Beberapa parameter yang mempengaruhi pengekstrakan telah dikaji dan dioptimumkan (amaun penjerab, pH sampel, masa pengekstrakan, kelajuan pengacauan, pelarut penyahserapan, isipadu dan masa). LODs dan had pengkuantitian masing-masing adalah 0.21-2.1 dan 0.63-0.62 μg L^{-1}. Kaedah yang dicadangkan telah berjaya digunakan bagi

penentuan asid fenolik di dalam sampel jus. Teknik penyediaan sampel lain menggunakan pasangan ion bantuan vortek pengekstrakan mikro cecair-cecair-cecair (IPVALLLME) bagi penentuan asid ellagik di dalam sampel madu telah berjaya dibangunkan. Asid ellagik di dalam larutan sampel telah ditukarkan kepada larutan kompleks pasangan ion (menggunakan tridesilamina sebagai reagen pasangan ion yang telah dilarutkan di dalam pentanol) dan diekstrak menggunakan bantuan vorteks diikuti dengan pengekstrakan semula ke dalam fasa akues 0.03 M KOH. Faktor pengkayaan dan LOD masing-masing adalah 184 dan 0.2 µg L^{-1}. Kaedah yang dicadangkan telah digunakan bagi penentuan asid ellagik di dalam sembilan belas madu Malaysia dengan berlainan jenis bunga. Kaedah pengekstrakan mikro cecair-cecair-cecair bantuan vorteks diikuti dengan kromatografi cecair berprestasi tinggi-pengesan pancaran diod (HPLC-DAD) telah dibangunkan bagi penentuan empat belas asid fenolik di dalam sampel madu, teh ais, dan minuman kopi. Pengekstrakan telah dijalankan menggunakan pelarut organik ternari (1-pentanol, propil asetat dan 1-heksanol) dengan isipadu yang kecil (400 µL) telah diserakkan ke dalam sampel akues (10 mL) dan dibantu dengan goncangan vorteks (2500 rpm selama 45 saat). Analit telah diekstrak semula daripada pelarut organik menggunakan 0.02 M KOH (40 µL) dengan kelajuan dan masa vorteks masing-masing adalah 2500 rpm dan 60 saat. Dalam keadaan ini, faktor pengkayaan sebanyak 30-193 telah tercapai. Secara keseluruhannya kaedah pengekstrakan mikro ini adalah bersesuaian dengan prinsip kimia analitsis yang hijau, terutamanya di dalam pengurangan pelarut organik, masa dan juga ringkas.

THE DEVELOPMENT OF GREEN MICROEXTRACTION METHODS FOR THE DETERMINATION OF PHENOLIC ACIDS IN HONEY AND BEVERAGES

ABSTRACT

Ultrasound-assisted matrix solid phase dispersion (UAMSPD) technique was developed for the determination of twelve phenolic acids (cinnamic, *m*-coumaric, chlorogenic, syringic, ferulic, *o*-coumaric, benzoic, *p*-coumaric, vanillic, *p*-hydroxybenzoic, caffeic, 2, 4-dihydroxybenzoic) in honey by using capillary zone electrophoresis (CZE) with photodiode array detector. Under the optimum conditions, sample (0.5 g) was dispersed with Amberlite XAD-2 (2 g), followed by washing with acidified water (pH 2.0) and subsequently rinsed with water to remove sugars and were finally eluted with methanol (8 mL). The average recoveries of the UAMSPD-CZE method from samples spiked to honey were within the range (71.0- 106 %). The limits of detection (LODs) ranged from 0.036 to 0.132 μg g^{-1}. The method was successfully applied to several Malaysia honey. A selective**,** simple, efficient and environmental friendly extraction method based on cetyltrimethylammonium bromide-modified bentonite (CTAB–bentonite) as sorbent in the micro-solid phase extraction (μ-SPE) has been developed for the determination of twelve phenolic acids in juice samples. The surface modification of bentonite was confirmed using FTIR, TEM and BET. Several parameters that affected the extraction were studied and optimized (i.e., sorbent amount, pH of sample, extraction time, stirring speed, desorption solvent, volume and time). The limit of detection and limit of quantitation were 0.21 – 2.1, 0.63 - 0.62 μg L^{-1}, respectively. The proposed method was successfully applied for the determine of phenolic acid in juice samples. Another sample preparation technique using

ion-pair vortex assisted liquid – liquid- liquid microextraction (IPVALLLME) for the determination of ellagic acid in honey samples was successfully developed. Ellagic acid in the sample solution was converted into an ion-pair complex (using tridecylamine as ion-pairing reagent that was dissolved in pentanol) and then extracted using the assistance of vortex, followed by back-extraction in the aqueous phase with 0.03 M KOH. Enrichcement factor and limit of detection were 184 and 0.2 µg L^{-1}, respectively. The proposed method was applied for the determination of ellagic acid in twenty one Malaysian honey with different floral origins. A vortex-assisted liquid-liquid–liquid microextraction method followed by high performance liquid chromatography-diode array detection (HPLC-DAD) has been developed for the determination of fourteen phenolic acids in honey, iced tea and canned coffee drink samples. The extraction was performed using a small volume (400 µL) of ternary organic solvents (1-pentanol, propyl acetate and 1-hexanol) dispersed into the aqueous sample (10 mL) and assisted by vortex agitation (2500 rpm for 45 s), the analytes were next back-extracted from the organic solvent using 0.02 M KOH (40 µL) with vortex speed and time of 2500 rpm and 60 s, respectively. Under these conditions, enrichment factors of 30–193-fold were achieved. On the whole, these microextraction methods are in agreement with green analytical chemistry principles, especially in terms of reduction of organic solvent, time and simplicity.

CHAPTER ONE

INTRODUCTION

1.1 Green Analytical Chemistry

Green chemistry uses different chemistry techniques to improve and develop the process, synthesis and use chemicals which reduce or eliminate products and by-products that reduce risks to humans and the environment (Anastas, 1999). Green Chemistry technologies were recently developed for the industrial sector for both economic benefits as well as environmental benefits by chemical companies in the world. Different sectors such as pharmaceuticals, organic, inorganic and analytical chemistry recently successfully applied green chemistry technologies (Anastas, 1999). Green analytical chemistry (GAC) term has been introduced for the first time by Namiesnik (Namieśnik, 2001). Over the last few years, tremendous research concentrated on all aspects of sample analysis to improve existing techniques so that they are effective, cheap, efficient, fast and environmentally friendly. Therefore, GAC is key to convert the chemurgical paradigm to ecological paradigm in analytical chemistry field as shown in Figure 1.1.

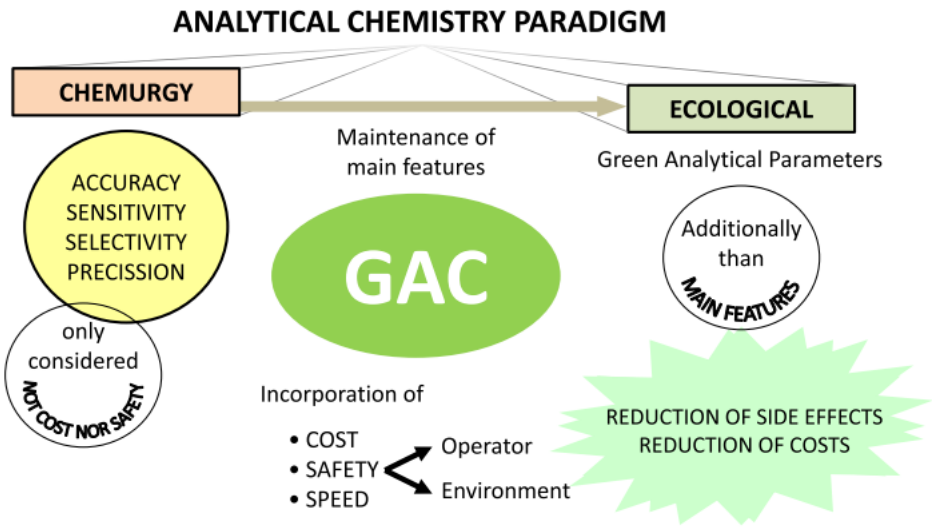

Fig. 1.1 The transfer of Green Analytical Chemistry from chemurgical paradigm to ecological paradigm (Armenta et al, 2015).

1.1.1 Principle of green analytical chemistry

Twelve principles of green chemistry were suggested by Anastas and Warner (Anastas et al., 1998) from these twelve principles of green chemistry only six of them can be applied to GAC, which are:

(i) Prevention.

It is better to prevent waste generation rather than cleaning-up or treating of waste after being formed.

(ii) Safer Solvents and Auxiliaries.

The auxiliary substances that are mainly used (e.g., separation agents, solvents, etc.) should be eliminated or reduced wherever possible.

(iii) Design for Energy Efficiency.

The energy used should be minimized and recognized for their economic and environmental impacts (e.g., synthetic procedure should be conducted at ambient pressure and temperature if that are possible).

(iv) Reduce Derivatives.

Derivatization process should be avoided or reduced if possible.

(v) Real-Time Analysis for Pollution Prevention.

Analytical methodologies have to be improved so that the analysis can be conducted in real time to minimize the generation of hazardous substances.

(vi) Inherently Safer Chemistry for Accident Prevention.

Inert chemical substances should be chosen and preferred to minimize the risk of chemical accidents such as explosion and fire.

Thus, the main goals of new analytical methods is to reduce and eliminate chemical substances (e.g., reagents, solvents, additives, preservatives) which can reduce the production of wastes, to improve the reliability of method, to minimize the energy consumption, to reduce analysis time, and reduce cost (Tiwari et al., 2015). Common strategies to achieve these goals are using micro scale sample preparation, new separation and detection methods.

The modern instrumental methods is also preferred to reduce energy and amount of chemical reagents consumed especially when the analytical method is automated, miniaturized, and safety in operation with minimum waste production (GAC principles # ii, iii, vi). Moreover, the combination between modern sample treatment with new separation methods and innovative methods of detection can improve the efficiency and reduce the energy (GAC principles # iii, v). Direct analytical techniques with universal detectors such as mass spectrum or evaporative light scattering or solventless sample treatment can also be used.

Reducing or elimination of toxic organic solvents in analytical methods is an important principle in GAC (principle # vi). The main target of using alternative solvents such as ionic liquids and supercritical fluids is not only the replacement the toxic solvent (e.g., benzene, chloroform) but also improving the sensitivity, reliability, selectivity and reduce the analysis time (Liu et al.,2005**).** Moreover, the alternative solvents are safe to the operator. Classifications of some common organic solvents (Table 1.1**)** were reported by Furusawa (Furusawa et al., 2004).

Table 1.1 Classification of common organic solvents used in analytical chemistry (Furusawa, 2004).

Solvent	Poison class[a]	Harmful class[b]
Acetone	5	Xn
Acetonitrile	2	T
Chloroform	1	T
Dichloromethane	4	Xn
Diethyl ether	4	Xn
Ethanol	-	-
Ethyl acetate	4	T
n-Heptane	5	-
n-Hexane	4	Xn
Methanol	3	T

Toxicity classification, 1 = very strong toxin (mutagenic, carcinogenic and teratogenic), 2 = very strong toxin, 3 = strong toxin, 4 = solvent considered harmful. 5 = solvent with low hazard potential (negligible hazard), - = not toxic classification, Harmful classification based on Japanese Reagent Chemical Association, Xn = harmful, T= toxic, - = not toxic

1.1.2 Green Approaches in Sample Preparation Techniques

In spite of progress in instrumentation, they still cannot handle complex samples. Sample preparation remain as important step in the analytical process. The main objectives of sample preparation is to convert or remove the matrix and interferents so that they are suitable for analysis. Removing the matrix and potential interferents not only increases the selectivity but also increases the sensitivity of the method.

Usually, the analytical procedure involves four major steps, i.e., sample preparation, separation, detection and identification, as shown in Fig. 1.2.

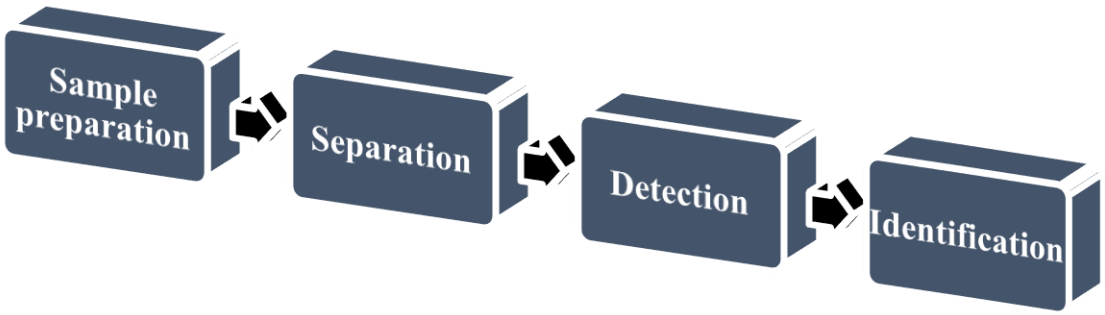

Fig. 1.2 Steps that are involved in analytical procedure (Koel et al., 2006)

Unfortunately, there is no universal sample preparation that is available due to variation of sample matrices. Sample preparation has long been recognized as bottleneck in analytical analysis. Classical sample preparation techniques such as liquid liquid extraction (LLE) and solid phase extraction (SPE) are time consuming, slow, consumes large amounts of organic solvents, generate wastes and are labor intensive.

Over the past several decades, more attention has been placed on the development of simple, fast, accurate, inexpensive, environmental friendly and automated analytical methods to replace the existing methods.

Miniaturized extraction techniques (referred to as microextraction) involve the use of small amounts of sample and extraction solvents.

Two categories of microextraction techniques were developed:

(i) Solid-based microextraction (e.g., matrix solid phase dispersion (MSPD), micro-solid phase extraction (μ-SPE), solid phase microextraction (SPME))

(ii) Liquid-based microextraction (e.g., single drop microextraction (SDME), hollow fiber liquid phase microextraction (HF-LPME), dispersive liquid-liquid microextraction (DLLME), vortex assisted liquid-liquid microextraction (VALLME).

A short description of these microextraction methods are next discussed.

1.1.2.1 Solid-Based Microextraction Techniques

1.1.2.1 (a) Matrix Solid Phase Dispersion (MSPD)

The MSPD technique was introduced for the first time in 1989 by Barker and Long (Barker et al., 1989) for the extraction of organic compounds from solid, semi-solid and viscous samples. The technique is widely used for extraction and purification due to its selectivity, flexibility and the ability to perform the extraction and clean-up in one step (Capriotti et al., 2013) The method is based on the disruption and dispersion of the sample by mechanical blending with solid support material (e.g, octadecylsilyl silica C18, florisil, silica gel, carbon nanotubes). The mechanical forces generated by dispersing the sample over the surface of the support solid material (hydrophobic and hydrophilic interaction) to produce a new phase material which is suitable for the extraction process.

The homogenous blended mixture is then transferred and packed into a MSPD column. Suitable solvent is used for the elution of interferents and target analytes by using a vacuum manifold (Capriotti et al., 2010) (Fig. 1.3). The method offers several advantages over the traditional extraction methods such as being simple, flexible, requiring small amount of solvent and sorbent, and low costs. The MSPD method has been frequently applied in different fields particularly for the analysis of pesticides, drugs, herbicides and other pollutants from vegetables, fruits and animal tissues (Wu et al., 2008). Recently, different sorbent materials with large surface area such as multi-walled carbon nanotubes (MWCNTs) or high selective sorbents such as molecularly-imprinted polymers (MIP) (Capriotti et al., 2013) were used. Furthermore, solvent at high pressure and temperature to enhance the extraction efficiency can be used. Extraction of the target analyte with solvent under sonication bath before the elution process improves the extraction (Moreda-Piñeiro et all., 2009). Miniaturization of the MSPD method has been reported (Moreda-Piñeiro et al., 2009) by using small amount of sample and solvent.

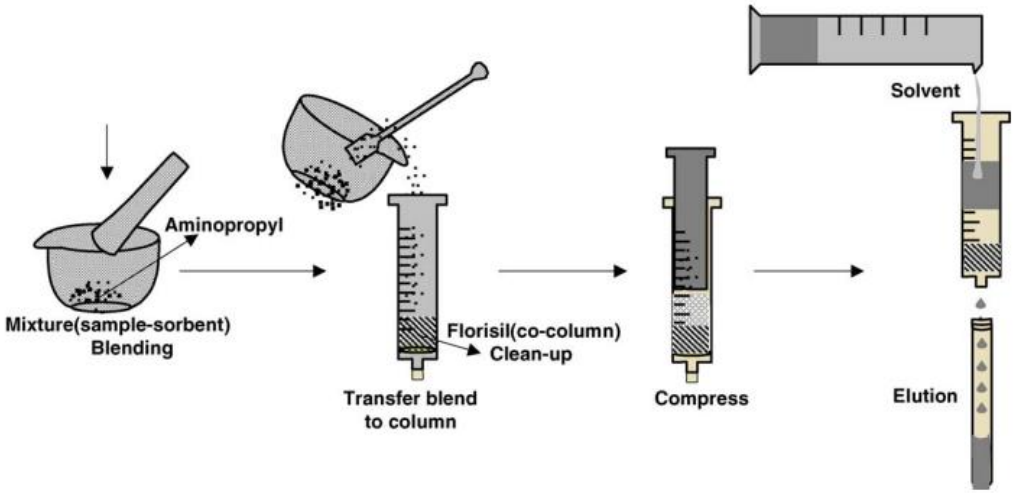

Fig. 1.3 Schematic diagram of the MSPD procedure (Ferrer et al., 2005)

1.1.2.1 (b) Micro Solid Phase Extraction (µ-SPE)

The µ-SPE technique involves the trapping of small amount of sorbent (about 5 mg) in a porous polypropylene membrane (Fig. 1.4). Due to the porosity and hydrophobicity character of the membrane, interferences from macro-molecules are minimised. During the extraction process, µ-SPE device is tumbled into the stirred sample and analytes are able to diffuse from the sample solution to the sorbent. After the extraction process is completed, desorption (often assisted by ultrasonication) is carried out by immersing the µ-SPE device in a suitable organic solvent. The µ-SPE technique is simple and effective for the extraction of compounds from complex samples.

Distinct advantages over the traditional SPE is the omitting of time consuming steps such as conditioning, cleaning, wetting and elution, reducing the solvents consumption (Asgharinezhad et al., 2014). Moreover, µ-SPE technique exhibits high reusability where single extraction device can be used for up to 30 extraction times. The µ-SPE technique was successfully applied for the determination of drug residues in wastewater (Basheer et al., 2007), organochlorine pesticides in drinking and agriculture waters (Ahmadi et al., 2008), ochratoxin A (OTA) in coffee (Lee et al., 2012 A), estrogens in cyst fluid samples (Kanimozhi et al., 2011), biogenic amines (BAs) in orange juice (Basheer et al., 2011) and persistent organic pollutants (POPs) in tissues (Basheer et al., 2008). Newer sorbents such as multi-walled carbon nanotubes (Basheer et al., 2006), molecularly imprinted polymer (Feng et al., 2009), graphite fibers (Xu et al., 2008) have been used.

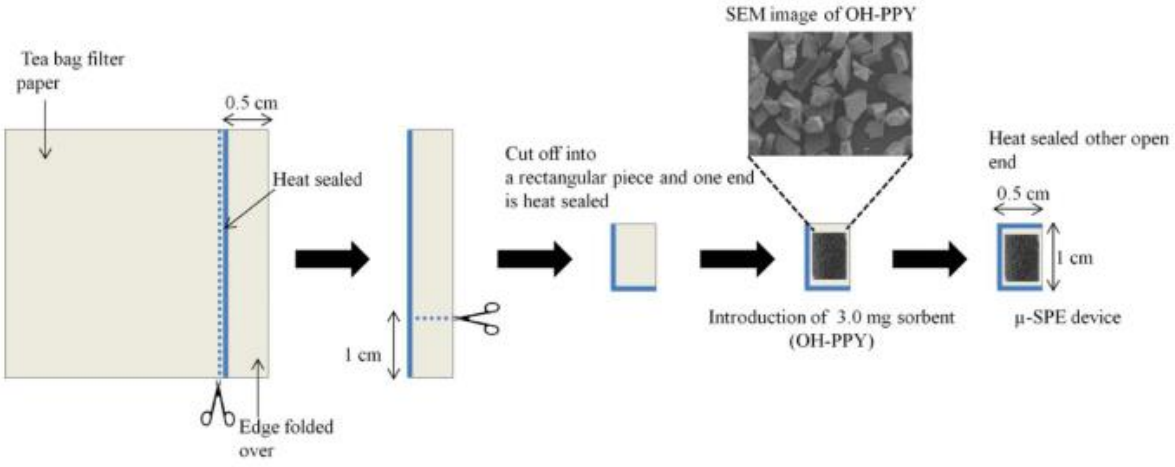

Fig. 1.4 Schematic of µ-SPE device preparation (Pelden, et al., 2014)

1.1.2.1 (c) Solid Phase Microextraction (SPME)

The SPME technique was first introduced in 1990 by Arthur and Pawliszyn (Liu and Dasgupta, 1996). The technique uses a polymer coated fiber that can be used to extract the compounds from liquid solution (direct immersion mode) or gas samples (headspace mode) headspace region or solution (Fig. 1.5) (Flanagan et al., 2008). The SPME method uses two main steps: (i) partitioning of the analytes between sample solution and stationary phase which is coated on the SPME fiber, (ii) desorption of the analyte from the SPME fiber to a gas chromatography injector or stripped with special interface at the high performance liquid chromatography inlet (Risticevic et al., 2009).

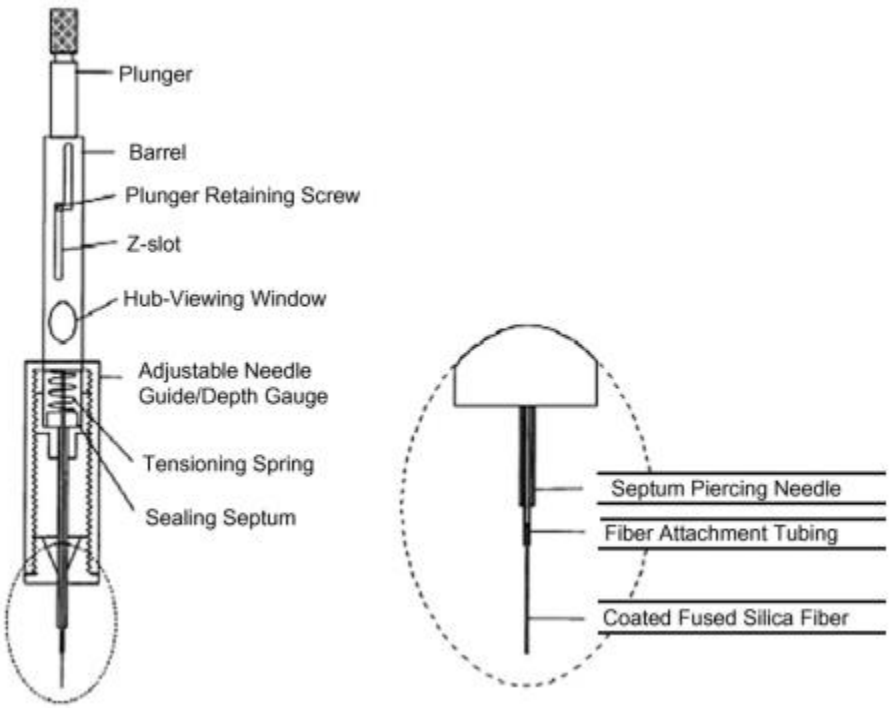

Fig. 1.5 Design of commercial SPME device **(**Chen et al., 2008).

The SPME technique was classified into two types:

(i) Direct immersed solid phase microextraction (DI-SPME)

In DI-SPME, the SPME fiber is directly immersed into the extraction vial during the extraction. It can be applied with or without derivatization process.

(ii) Head space solid phase microextraction (HS-SPME)

In this technique, the SPME fiber is exposed in head space region of sample vial and thereby, preventing the contamination on the surface of fiber from sample matrices. (Nováková et al., 2009**)**. SPME technique widely used with GC but less was reported with HPLC. This is due to the stripping step that is needed to extract the analytes from the fiber and inject into the HPLC system. The in-tube SPME technique was developed by Eisert and Pawliszyn 1997 to overcome these problems (Eisert et al., 1997). It is based on the use of a short piece of fused silica capillary GC column which was internally coated with specific stationary phase. The analytes are adsorbed in the open tube short column by the repeated aspirating and dispensing sample solution. The sorbed analyte is desorbed and transferred from the tube to the HPLC column (Fig 1.6) (Eisert et al., 1997). The SPME technique was applied for the determination of analytes in biological, pharmaceutical, clinical, environmental and food samples (Nerín et al., 2009). The technique offers several advantages over the classical method such as simple, rapid, easily automated, solvent free, small volume of sample used, ability to extract polar and non-polar analytes in different sample matrices (Nováková et al., 2009). However, the range of coated fiber stationary phases and their capacity are

limited. The fiber itself is fragile and have limited lifetime. In addition, sample carryover effects from sample to sample is often encountered (Risticevic et al., 2009).

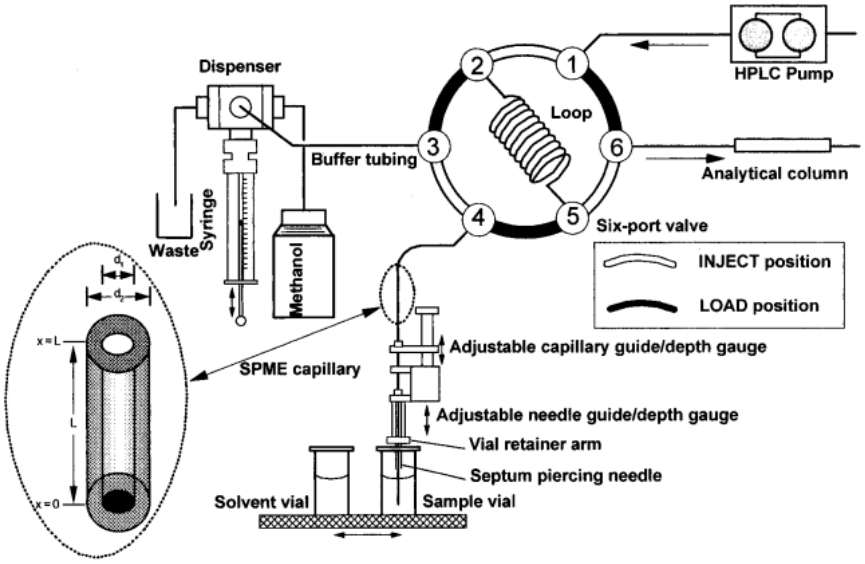

Fig. 1.6 Set-up of the on-line SPME-HPLC interface based on an in-tube SPME capillary technique (Eisert, et al., 1997).

1.1.2.2 Liquid Phase Microextraction Techniques

1.1.2.2 (a) Single Drop Microextraction (SDME)

The SDME technique was first introduced by Liu and Dasgupta in 1996 (Xu et al., 2007). It uses an immiscible single drop of organic solvent (1-3 µL) that was suspended at the tip of micro-syringe needle which is immersed in the aqueous solution with continues stirring during the extraction process (Fig 1.7). After the extraction is completed, the organic micro-drop is retracted back into the micro-syringe and injected directly into either capillary electrophoresis (CE), GC or HPLC column (Xu et al., 2007). Similar to the LLE, the extraction of compounds from the sample depends on the partition and diffusion of analytes between the aqueous phase (sample solution) and

organic phase (single organic micro-drop). Therefore, the extraction process depends mainly on equilibrium principle rather than on exhaustive extraction. Different parameters such as extraction temperature, stirring rate, type of solvent, salt-out effects, pH of solution and volume of single organic drop can also effect the extraction efficiency.

The SDME technique is simple, inexpensive, efficient, uses minimum organic solvent, fast without any sample carryover effects, low cost, preconcentration and extraction in one single step (Xu et al., 2007, Psillakis et al., 2002). Therefore, it has been used for the determination of analytes in biological application (Li et al., 2005), food (Zhao et al., 2006) and water samples (Ahmadi et al., 2006). Details of SDME applications and developments have been reported in several reviews (Psillakis and Kalogerakis , 2002, Xu et al., 2007).

On the other hand, the main problems of the SDME is the stability of the hanging single organic drop with high stirring rates that may dislodged the drop from the tip of mico-syringe needle (Psillakis and Kalogerakis , 2002). Moreover, air bubble formation due to the stirring process may hinder it automation to preconcentrate the analyte (Xu et al., 2007).

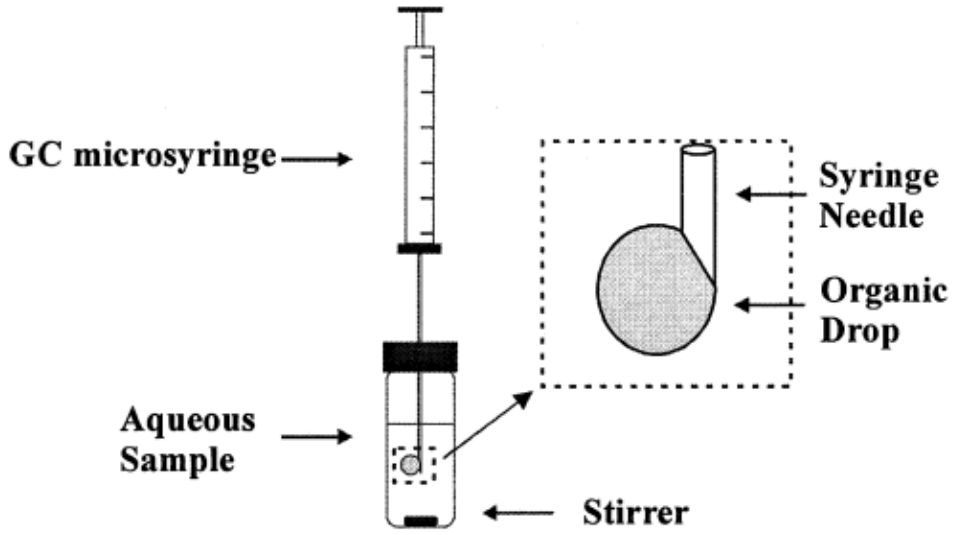

Fig 1.7. Schematic of the single-drop microextraction system (Psillakis and Kalogerakis , 2002).

1.1.2.2 (b) Hollow Fiber -Liquid Phase Microextraction (HF-LPME)

In 1999, Pedersen and Rasmussen introduced the HF-LPME technique. It uses a porous hollow fiber membrane (made from polypropylene) (Pedersen-Bjergaa et al., 1999). The major advantages of HF-LPME are its low cost, requiring minute amounts of organic solvent (2- 30 µL), good clean-up ability, high analyte enrichments (pre-concentration) within a relatively short time (Carasek and Merib , 2015). Moreover, the fibers are inexpensive and disposed after used, thereby overcome the sample carry-over problems between extractions. Additionally, the HF-LPME technique can be conducted over wide pH range (cannot be done using SPE technique that uses silica support) (Richoll and Colon, 2006).

In the HF-LPME technique, an organic solvent is immobilized in the pores of a polypropylene hollow fiber, hence, forming a supported liquid membrane. In the two

phase HF-LPME mode, the extract organic solvent is immobilized in pores of the hollow fiber. An aqueous acceptor phase is placed into its lumen (three phase-HF-LPME) (Fig 1.8).

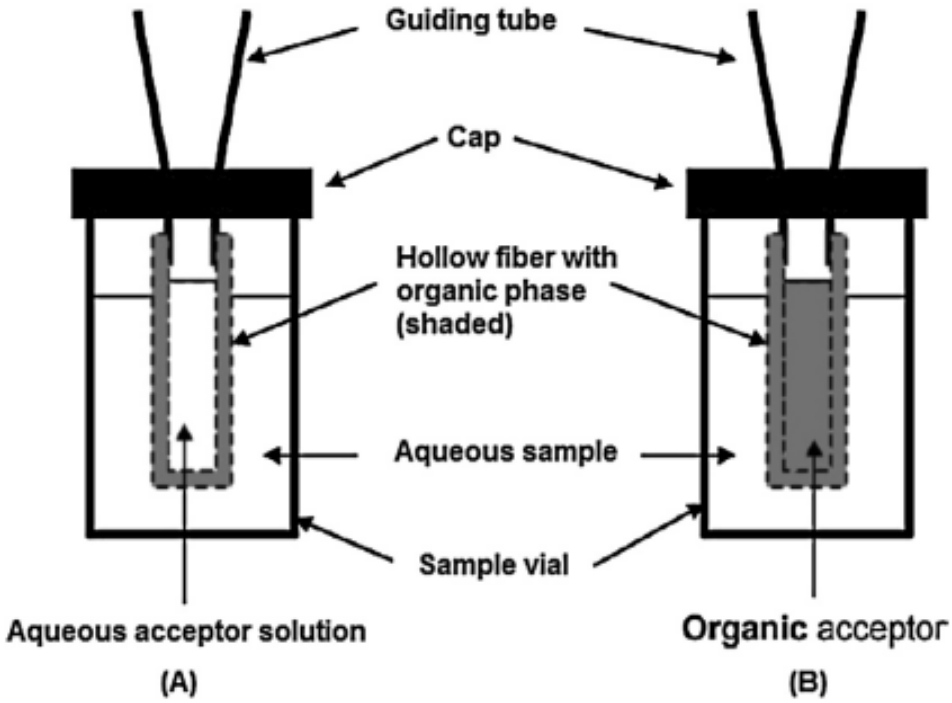

Fig. 1.8 Schematic of the HF-LPME (A) three phase and (B) two phase. **(**Han and Row, 2012).

The analytes are extracted from the aqueous sample solution through thin layer of organic solvent in the pores of the HF (SLM) into the acceptor solution (organic or aqueous) in the lumen of the HF (Fig. 1.9). After the extraction, the extract can be directly injected into the GC unit (for two phase-HF LPME) or the acceptor phase is aqueous solution (three phase-HF-LPME) which is suitable for HPLC or CE analysis

(Pedersen-Bjergaard and Rasmussen, 2008). The HF-LPME technique has been applied for the extraction and clean-up for a wide range of analytes in biomedical, food and environmental samples (Pedersen-Bjergaard and Rasmussen, 2008). This technique, however, is time consuming (≈ 20- 60 min). Furthermore, the leakage of organic solvent in the HF pores into the sample solution reduced the reproducibility of HF-LPME (Sarafraz-Yazdi and Amiri, 2010).

Additionally, using complex sample matrices can block the HF pores, thus reducing the repeatability of the extraction process (Sarafraz-Yazdi and Amiri, 2010).

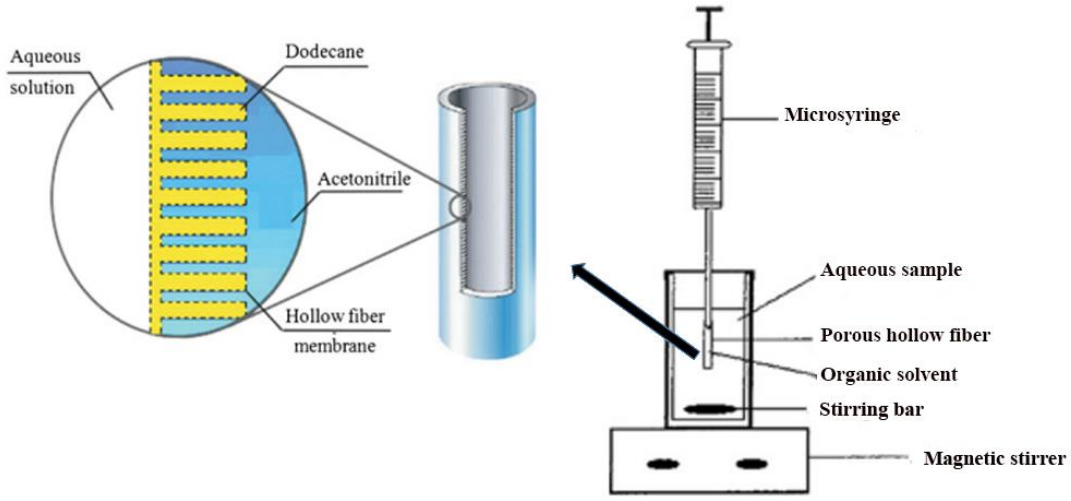

Fig. 1.9 Schematic HF-LPME diagram with the enlargement of the HF (modified from Ghambarian et al., 2012)

1.1.2.2 (c) Dispersive Liquid-Liquid Microextraction (DLLME)

The DLLME technique was proposed by Rezaee and co-workers in 2006 (Rezaee et al., 2006). In this technique, a mixture of extracting organic solvent (high density organic solvent) and small amount of water miscible organic solvent (dispersive solvent) was rapidly added into an aqueous sample. A cloudy solution (emulsion) due to the formation of micro droplets was formed. The analytes are rapidly extracted from the aqueous sample (donor phase) into organic extracting solvent (acceptor phase) due to the large contact surface between the donor and acceptor phases. After the extraction, the two phases were separated by centrifugation (the organic phase containing the analytes settled at the bottom of the conical centrifuge tube, collected) and subsequently analysed using appropriate technique (Rezaee et al., 2006). A schematic diagram of the DLLME technique is shown in Fig. 1.10.

DLLME offer several advantages such as rapid, simple, low cost, relatively high enrichment factor and good recovery. The DLLME principle, applications and developments have been reviewed (Rezaee et al., 2010, Zgoła-Grześkowiak and Grzes´kowiak, 2011). DLLME technique has been applied to the extraction of analytes from different fields (e.g., food, environmental, biological) (Rezaee et al., 2010). However, there are some drawbacks such as using dispersion solvent that may reduce the partition coefficient and mass transfer of the analytes into the organic solvent. Furthermore, the high density organic solvent (e.g., carbon tetrachloride, chloroform and chlorobenzene), are considered as highly toxic and environmentally unfriendly.

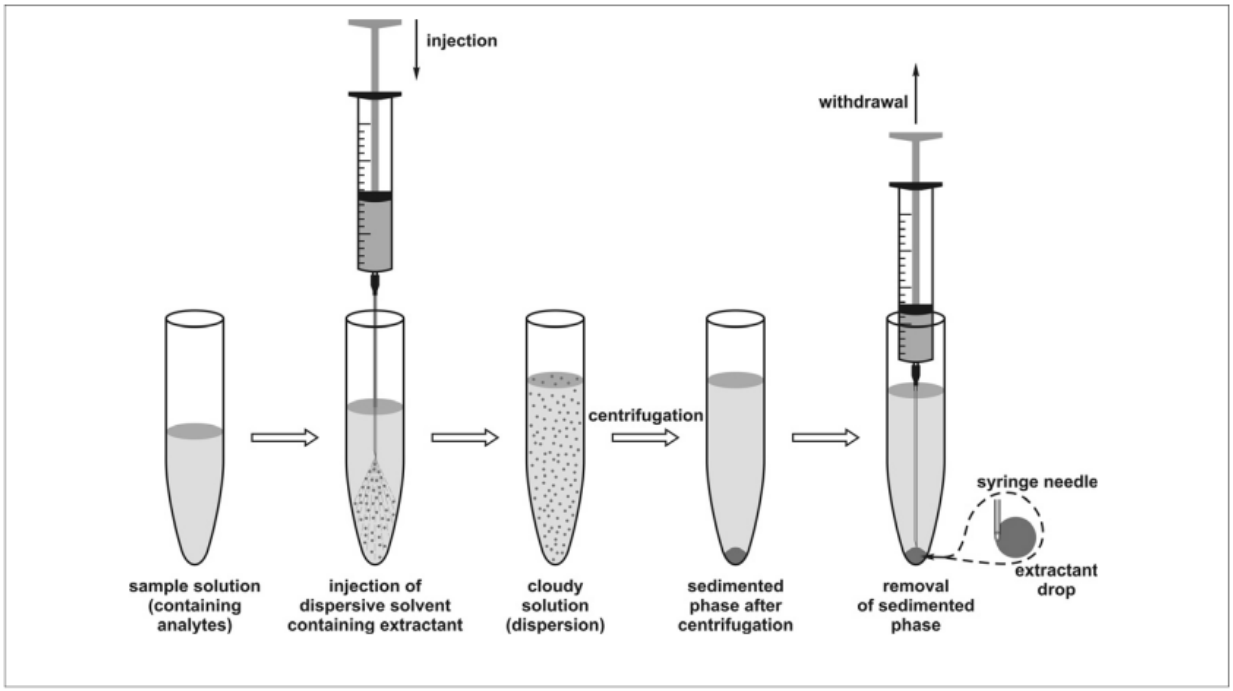

Fig. 1.10 Schematic diagram of the DLLME procedure (Grześkowiak, et al., 2011)

1.1.2.2 (d) Vortex-Assisted Liquid–Liquid Microextraction (VALLME)

The VALLME technique was introduced by Psillakis and Yiantzi et al. in 2010 (Yiantzi et al., 2010). In this method (Fig. 1.11), the dispersion of the organic solvent (lower density than water) into the aqueous sample solution is achieved by using vortex agitation, which forms a mild emulsion. This process enables the extraction of the analyte from the sample solution in a short time due to the large contact surface area and short diffusion distance between the two phases (organic and aqueous) (Bosch Ojeda and Rojas, 2014). This technique has been successfully applied for the extraction of analytes such as aliphatic amines (Chang et al., 2012), furfurals (Abu-Bakar et al., 2014)], polychlorinated biphenyls (Ozcan, 2011) pesticides (Yan et al., 2013;

Vichapong et al., 2013), herbicides (Li et al., 2012) alkyl phenols (Yiantzi et al., 2010) and phthalate esters (Zhang and Lee, 2013). As the extracts are organic-based, they are mostly analysed using GC (Zhang and Lee, 2012; Guo et al., 2013; and Yang et al., 2013) although HPLC (Yiantzi et al., 2010, Román et al., 2014) is also used to a small extent.

Problems that arise when analysed by HPLC are the extra peaks or broad analyte peaks were found due to the extracted organic solvent (Yiantzi et al., 2010, Chang et al., 2012). Alternatively, extra step such as evaporation, centrifugation and filtration before analysis or extending the conditioning of column by using gradient system after each run were attempted (Yiantzi et al., 2010, Lian et al., 2013, Chang et al., 2012). Based on these reasons, a three phase vortex-assisted liquid–liquid microextraction was introduced (Makahleh et al., 2015). In this technique, the analytes are extracted from the sample (aqueous) to the organic solvent (organic) by using vortex agitation and subsequently back-extracted into the acceptor phase (aqueous). Consequently, the acceptor phase is directly injected into the HPLC unit.