

Lukman Bola Abdulra'uf
Ala' Yahya Sirhan
Guan Huat Tan

Department of Chemistry,
University of Malaya, Lembah
Pantai, Kuala Lumpur, Malaysia

Received May 4, 2012
Revised August 27, 2012
Accepted August 27, 2012

Review Article

Recent developments and applications of liquid phase microextraction in fruits and vegetables analysis

The sample preparation step has been identified as the bottleneck of analytical methodology in chemical analysis. Therefore, there is need for the development of cost-effective, easy to operate, and environmentally friendly miniaturized sample preparation technique. The microextraction techniques combine extraction, isolation, concentration, and introduction of analytes into analytical instrument, to a single and uninterrupted step, and improve sample throughput. The use of liquid-phase microextraction techniques for the analysis of pesticide residues in fruits and vegetables are discussed with the focus on the methodologies employed by different researchers and their analytical performances. Analytes are extracted using water-immiscible solvents and are desorbed into gas chromatography, liquid chromatography, or capillary electrophoresis for identification and quantitation.

Keywords: Liquid-phase microextraction / Microextraction / Pesticide residues
DOI 10.1002/jssc.201200427

1 Introduction

The joint Food and Agricultural Organization and World Health Organization (FAO/WHO) Food Standard established the Codex Alimentarius Commission that set the maximum residue limit (MRL) for pesticides and other contaminants in fruits and vegetables. Other bodies including the European Union (EU) and United State Environmental Protection Agency (EPA) have also set directives for the MRL of pesticide residues on fruits and vegetables [1]. The pesticide residues are present at trace concentration levels (usually in mg/kg or less) and thus different MRL directives for pesticides were set at low concentration levels for various fruit and vegetable samples. The various legislations were proposed in order to check the misuse of pesticides in the environment and curb the adverse effects of the pesticides on human health, there-

fore the need for their accurate assessment [2]. The accurate evaluation of pesticide residues is aimed at quality control and quality assurance measures to regulate pesticide residues in foods.

The presence of the pesticide residues at trace levels embedded in complex food samples has encouraged the development of various environmental friendly sample preparation techniques that make use of microliter volume of solvents. Sample preparation step has been identified as the bottleneck of any analytical methodologies in the determination of contaminants from foods. Prior sample preparation before instrumental analysis helps to extract, isolate, and concentrate the analytes of interest from complex food matrices containing other interfering high molecular mass compounds. Therefore, the current trend of sample preparation techniques is focused on the simplifications, miniaturization, and combination of different steps, such as extraction, concentration, isolation of analytes, cleanup, and instrumental analysis in one single step. The microextraction techniques, including solid-phase microextraction (SPME) [3–5], microextraction in packed sorbent [6–9], and liquid-phase microextraction (LPME) [10–30], allow for greater sensitivity, low detection limit, and are fast and economical with high sample throughput.

The different microextraction techniques were developed in order to overcome the limitations inherent in the conventional sample preparation, which are tedious, time consuming, multistep, and use large volume of solvents and samples. They were developed to corroborate the recent advances in analytical instrumentations, such as gas chromatography (GC), liquid chromatography (LC), and capillary electrophoresis (CE), coupled to different detectors, and are highly sensitive, efficient, and compatible with various microextraction techniques. They are used for both qualitative and

Correspondence: Dr. Lukman Bola Abdulra'uf, Department of Chemistry, University of Malaya, Lembah Pantai, Kuala Lumpur, WP 50603, Malaysia
E-mail: lbarchem@yahoo.com; abdulraflukman@siswa.um.edu.my
Fax: +60-3-79674193

Abbreviations: DI, direct immersion; DLLME, dispersive liquid–liquid microextraction; EU, European Union; LLSME, liquid–liquid–solid microextraction; LPME, liquid-phase microextraction; MIP, molecularly imprinted polymer; MM-LLE, microporous membrane liquid–liquid extraction; MRL, maximum residue limit; MWCNT, multiwalled carbon nanotube; OCP, organochlorine pesticide; PHWE, pressurized hot water extraction; SDME, solvent drop microextraction; SLM, supported liquid membrane; sMEKC, sweeping electrokinetic chromatography; SPME, solid-phase microextraction; USAEME, ultrasound-assisted emulsification microextraction.

quantitative analysis of pesticide residues and other contaminants in food samples. The introduction of SPME by Pawliszyn and co-workers [3] opened the floodgates of interest in the development of microextraction techniques

In this review, various types of LPME techniques as they are used for the extraction and subsequent chromatographic analysis of pesticide residues in fruits and vegetables are discussed. The review includes the different modes of LPME such as solvent drop microextraction (SDME), hollow-fiber LPME (HF-LPME), and dispersive liquid–liquid microextraction (DLLME), factors affecting LPME, their advantages, limitations, and future trend.

2 LPME

LPME, also called solvent microextraction technique, was developed in order to overcome some problems inherent in SPME, such as low recommended operating temperature of coated fibers, swelling of fiber in organic solvent, fiber breakage due to its fragility, stripping of the coating, and possible bending of the needles [2, 31, 32]. LPME is a recently developed rapid and less-expensive sample preparation technique, performed between microliter volume of water-immiscible solvent called the acceptor phase and an aqueous sample called the donor phase, containing the analytes of interest [31]. The technique is classified majorly into SDME, HF-LPME, and DLLME depending on the methodology employed and only differs in the manner the extracting solvent is supported.

2.1 Factors affecting LPME

The development of the methods for the extraction and analysis of pesticides by the LPME technique, like in SPME, requires the optimization of the parameters related to the donor and acceptor phases. The following factors must be optimized for effective and efficient extraction: organic solvents type, volume of donor and acceptor phase solution, agitation, salt addition (ionic strength), pH, extraction time, and temperature [28].

The most important factor is the selection of an organic solvent (and dispersive solvent in DLLME). The organic solvent should be of high purity, less volatile, high boiling point, and high density, so as to prevent solvent evaporation and ensure analyte concentration. The solvents should also be water immiscible for extraction of analytes from an aqueous sample and should be compatible with chromatographic instruments [12, 33, 34]. The LPME extraction like SPME is a nonexhaustive extraction method, thus an appropriate extracting/dispersive solvents volume must be selected to prevent shrinking. The use of large volume of solvents yields better instrumental response, and improves extraction efficiency, but it is difficult to manipulate.

Agitation increases the extraction efficiency and decreases the extraction time, by increasing the interfacial contact area and diffusion distance. It also leads to an increase in

aqueous mass transfer, exposes the analytes to the extraction solvent, and increases the partitioning of analytes between the sample matrices and the extracting solvent. Agitation can be achieved through magnetic stirring, vortex and mechanical vibration, or syringe plunger motion [12].

Salt addition enhances extraction efficiency by reducing the solubility of the analytes in the sample, especially for the moderately polar and low molecular weight analytes, and has little effect on nonpolar analytes. The reduction of analytes solubility enhances their partitioning into the microdrop. It has been observed that salt addition can also have an adverse effect on the extraction efficiency, by changing the physicochemical properties (e.g. viscosity or surface tension) of the aqueous sample and the extraction film [12, 34]. Therefore, most of the published work on LPME has been conducted without salt addition. Salt addition, thus, depends on the polarity of target analytes.

It is also used to adjust the pH of sample matrices, which helps to suppress the ionization of target analytes, reduces the analyte solubility, and enhances their extractability [31, 34, 35]. pH adjustment helps to promote the formation of the molecular state of the target analytes [34]. Extraction of basic pesticide is done in alkaline medium while acidic pesticides are best extracted in acidic medium at low pH. Researchers have used a wide range of pH in the extraction of various classes of pesticide residues from fruit and vegetable samples.

The optimization of extraction temperature is important especially in the headspace mode, but a higher temperature may affect the stability of the microdrop in SDME and high solvent loss in DLLME and HF-LPME; for this reason, most of the reported analyses have been carried out by direct immersion (DI) mode at room temperature. The sample and headspace volume should be maintained constantly at minimal level, since larger sample volumes will require longer extraction time. In the headspace mode, the optimized volume should be at a level that allows the microdrop to be suspended over the stirred sample, while in the DI mode, the sample volume should only be enough to avoid the sample coming in contact with the septum [12, 34, 35]. The presence of air bubbles should also be avoided in the syringe to avoid errors in the analysis. LPME is an equilibrium extraction, in which analyte concentration increases with time till an equilibrium time where the analyte concentration remains constant with time, and therefore the extraction time must also be carefully optimized.

2.2 SDME

SDME is a microextraction technique that is based on the suspension of a single droplet of water-immiscible organic solvents (typically 0.5–3 μL) from the tip of a microsyringe needle in an aqueous solution [28, 31, 36], thus drastically reducing the volume of organic solvents used. The transport of target analytes from the sample matrix to the extraction solvent is limited by slow diffusion rate and the analytes are distributed between the microdrop of the solvent at the tip

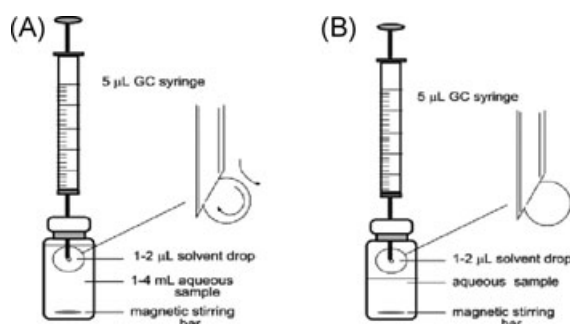


Figure 1. Solvent drop microextraction in (A) direct immersion (DI) and (B) headspace (HS) mode. Reprinted with permission [35].

of the syringe and the sample solution [12, 18]. The analyte, which is extracted by passive diffusion onto the droplet, is retracted back into the syringe and injected directly into the analytical instruments (GC, HPLC, or CE). The evaporation and reconstitution of analytes before injection is eliminated and the technique provides highly enriched extracts of the analytes [36].

As can be observed in Fig. 1, the SDME extraction can be carried out in DI mode (in which the droplet is suspended directly into the aqueous sample), most suitable for the analysis of medium's polar or nonpolar analytes, and headspace mode (where the droplet is suspended in the headspace of the sample solution), suitable for the analysis of volatile or semivolatile analytes [35]. The SDME technique was first reported by Liu and Dasgupta [10], using 1.3 µL chloroform suspended in a large aqueous solution containing a methylene blue active substance and later by Jeannot and Cantwell [11] for the extraction of 4-methylacetophenone in aqueous solution, using 8 µL of *n*-octane suspended at the end of a Teflon rod. He and Lee [37] also reported the extraction of 1,2,3-trichlorobenzene and pentachlorobenzene in aqueous solution using 5 µL of toluene drop suspended at the tip of a conventional microsyringe in dynamic and static SDME modes. The extraction technique was found to be simple, flexible, and can easily be coupled to chromatographic instruments. Its coupling with GC, LC, and CE has been reported and reviewed extensively, while it has also been successfully coupled with ICP-MS, ET-AAS, FI-AAS, MALDI-MS, and MS [35, 38].

2.2.1 Application of SDME in pesticide residues analysis in fruits and vegetables

SDME technique has been successfully applied for the analysis of pesticide residues in fruit and vegetable samples, although few studies have been published. Table 1 shows the applications of SDME in the extraction of different classes of pesticide residues in fruits and vegetables.

A method was developed for the analysis of multiclass pesticides in tomato sample by Amvrazi and Tsiropoulos [39]. The tomato sample was prepared by homogenizing 500 g of chopped raw tomato sample, then 2 g of the homogenized sample was transferred into a glass centrifuge tube and

Table 1. Solvent drop microextraction for analysis of pesticides in fruits and vegetable

Pesticides class	Matrix	Org. solv., vol. (µL)	Ext. time (min)	Ext. temp. (°C)	Ionic eff. NaCl (%)	pH	Agitation (rpm)	LOD	LOQ	Rel. Rec. (%)	RSD (%)	Linearity	EF	Det. method	Ref.
13 MCPs	Tomato	Toluene, 1.6	25	n.r.	n.r.	n.r.	350	0.6–30 µg/kg	n.r.	29.9–58.3	6.3–12	5–500 µg/kg	n.r.	GC-NPD	[39]
21 MCPs	Apple, grape	Toluene, 1.6	25	n.r.	n.r.	4	250	3–200 µg/kg	10–660 µg/kg	65–100	4–17	n.r.	11–328	GC-MS	[40]
6 TFs	Grape juices	HeOH/Hex, 30	21	n.r.	1	n.a.	n.r.	2–112 µg/L	8–375 µg/L	71–106	2.9–4.5	0.01–10 µg/mL	141–214	GC-FID	[41]
12 MCPs	Tomato	Toluene, 1.6	30	n.r.	n.r.	4	250	0.1–116 µg/kg	0.5–382 µg/kg	65–91	6.9–17.9	5–5000 µg/kg	0.7–812	GC-NPD	[42]
9 OCPs	Cabbages, cauliflower	<i>p</i> -Xylene/acetone, 1.0	30	n.r.	n.r.	n.r.	400	0.05–0.2 ng/mL	n.r.	65–100	8.7–18.1	0.05–50 ng/mL	n.r.	GC-MS	[43]
6 OPPs	Apple, pear, orange juices	Toluene, 1.5	20	Room temp	n.a.	5–6	600	0.21–0.56 ng/mL	n.r.	77–113	0.6–13.4	0.5–50 ng/mL	23–109	GC-FPD	[45]
5 OPPs, 3 OCPs	Cucumber, strawberry	Toluene, 1.5	15	Room temp	n.a.	n.r.	800	1–1000 µg/L	6–3000 µg/L	71–95	4.7–13.6	1–100 µg/mL	n.r.	GC-ECD	[46]
7 SFs, 6 OF	5 fruits, 2 juices	Octanone/undecanone, 10	30	50	10	5	n.r.	0.01–0.31 ng/mL	n.r.	79–117	2.5–9.9	0.06–300 ng/mL	81–1602	GC-MS	[47]

Org. Solv., organic solvent; Ext. time, extraction time; Ionic eff., ionic effect; Rel. Rec., relative recovery; EF, enrichment factor; Det. method, detection method; MCPs, multiclass pesticides; TFs, triazole fungicides; OCPs, organochlorine pesticides; OPP, organophosphorus pesticides; SFs, strobilurin fungicides; OF, oxazole fungicides; HeOH, hexanol; Hex., hexane; n.r., not reported; n.a., not adjusted.

25 mL of aqueous organic solvent solution was added followed by homogenization and centrifugation. Seven milliliters of the supernatant solution was placed into 10-mL glass vial and the analytes were extracted using the developed DI-SDME procedure. The optimized conditions were as follows: donor sample solution containing a mixture of water/acetone (90:10, v/v%), 1.6 μ L of toluene as the extracting solution, agitated at 350 rpm for 25 min. The extraction was conducted by immersing the microdrop in the stirred solution, and the extracted analytes were desorbed into GC-NPD (NPD, nitrogen phosphorous detector). The method performance obtained by internal standard calibration curve in blank spiked tomatoes gave limit of detection (LOD) between 0.6 and 30 μ g/kg, linearity from 5 to 500 μ g/kg, with correlation coefficients greater than 0.975. The matrix effect was also investigated by comparing the recoveries of the target analytes in spiked tomato and courgette samples, and the average recoveries range from 29.9 to 58.3% (RSD = 6.3–12%, where RSD is relative standard deviation) and 28 to 98.5% (RSD = 5.1–8.9%), respectively. It was found that there were higher relative peak areas in matrix-matched donor solution obtained from tomato, and a positive effect was observed for all the investigated pesticides.

A multivariate method for the analysis of 21 multiclass pesticide residues in apple and grape samples was also developed by Amvrazi and Tsiropoulos [40]. The developed method was based on the extraction of fruit samples, prepared by weighing 2 g of homogenized fruit in a glass tube and mixed with 2 mL of mixture of acetone/water (10:90, v/v%), buffered with sodium acetate and acetic acid. After the homogenization of the mixture followed by centrifugation, 7 mL of the supernatant was placed into 10-mL glass vial equipped with a PTFE-coated magnetic stir bar. The mixture was subjected to DI-SDME procedure for 25 min, stirred at 250 rpm using 1.6 μ L of toluene as extracting solvent, and injected into GC-MS at 250°C. The method developed was validated using grape and apple sample, and the matrix-matched calibration curve constructed showed linearity between the limit of quantitation (LOQ) and $50 \times \text{LOQ}$ μ g/mL with correlation coefficient greater than 0.97. The LODs ranged from 0.0004 to 0.2 and 0.0003 to 0.2 μ g/mL for apple and grape, respectively, while the LOQ was between 0.001 and 0.66 μ g/mL for both samples. The recoveries were between 65 and 91% in apple and 69 and 110% in grape with RSD of 4–17% and enrichment factor (EF) was between 11 and 328. The multivariate experimental factorial design allows for the determination of optimal conditions and the effect of their interactions on the extraction efficiency, and all the parameters can be optimized simultaneously.

A method for the determination of six triazoles in water and grape juice was proposed by Farajzadeh et al. [41]. The proposed method was based on DI-SDME in a narrow-bore glass tube and subsequent desorption into GC-FID (FID, flame ionization detector). The procedure involved the extraction of the target analytes from 23 mL of aqueous sample of grape juice containing 1% NaCl in an inverted narrow-bore glass using 30 μ L drop of mixture of hexanol and hexane

(50:50, v/v%). The microdrop was allowed to pass through the aqueous solution from one end of the tube to the other for 21 times by manually reversing the tube upside-down. The analytical performance of the method determined in grape juice sample showed wide linear range between 0.001 and 10 μ g/mL with RSD >0.994. The LOD and LOQ ranged from 0.006 to 0.112 μ g/mL and 0.010 to 0.375 μ g/mL, respectively. The recoveries were between 71 and 99% with RSD of 1–7% and EF between 141 and 214. The sample matrix was found to have no effect on the extraction efficiency and thus no dilution was performed. The developed method was compared to other extraction methods such as QuEChERS (quick easy cheap effective rugged safe), SPE-DLLME (SPE, solid phase extraction), and SPE, and the SDME was found to have comparable or better extraction efficiency.

Amvrazi et al. [42] also studied the analytical performance of SDME in the determination of 12 multiclass pesticides in tomato. The method was validated using a previously developed method [39], under ISO 17025 norms and SANCO Guide, and compared with modified acetone-partition extraction procedure. The analytical method validated using matrix-matched internal standard calibration curve gave LODs from 0.1 to 116 μ g/kg, LOQs between 0.5 and 382 μ g/kg, and enrichment factor (EF) ranging from 0.7 to 812. The recoveries were between 65 and 91% with RSD of 6.9–17.8% and linearity ranges from 5 to 5000 μ g/kg with correlation coefficients greater than 0.985. The method showed a negative matrix effect for most of the pesticide investigated and is a more selective method than acetone-partition extraction.

The DI-SDME technique was used to investigate the presence of organochlorine pesticides (OCPs) in vegetable samples (cabbage, cauliflower, and Chinese cabbage) by Zhang et al. [43]. An aliquot of 5 g of chopped sample was processed, and the liquefied juice was extracted by 5 mL acetone in ultrasonic vibration for 1.5 h, followed by dilution with appropriate volume of the aqueous donor sample. The target analytes were extracted from 2 mL donor solution, using 1.0 μ L microdrop of mixture of *p*-xylene/acetone (80:20, v/v%) for 30 min at a stirring rate of 400 rpm and desorbed into a GC-MS (MS, mass spectrometry) system. The analytical characteristics determined under the optimized conditions show a linearity ranging from 0.05 to 50 ng/mL with correlation coefficients greater than 0.993 and LODs were between 0.05 and 0.2 ng/mL. The relative recoveries were found in the range of 74–95% (RSD = 10.4–18.5%), 72–87% (RSD = 8.7–18.1%), and 65–100% (RSD = 9.9–16.3%) for cabbage, cauliflower, and Chinese cabbage, respectively. It was observed that the proposed method gave good extraction efficiency and low LOD. The method precision obtained indicated that the proposed method could be used for the analysis of OCPs from vegetable samples.

Organophosphorus pesticides were determined in orange juice sample using DI-SDME-GC-FID procedure developed by Zhao et al. [44]. The orange juice sample was fortified with water and centrifuged. The dilution of the donor solution with water (1:25, v/v) was found to enhance the relative recoveries of all the target analytes. The effect of sample filtration

was also investigated and was found to have no significant effect on the recoveries. The target analytes was extracted with 10 μ L drop of toluene, for 15 min, stirred at 400 rpm. The method showed recoveries ranging from 73 to 108% with RSD of 4.6–14.1% and LODs between 0.98 and 2.2 μ g/L. Good linearity with correlation coefficients greater than 0.98 were obtained for all the investigated pesticides.

Xiao et al. [45] compared the EFs of two types of direct SDME: static-SDME and cycle-flow SDME procedures, for the analysis of organophosphorus pesticides in fruit (apple, pear, and orange) juice samples. The static-SDME showed a better EF (23–109) than the cycle-flow SDME (2–15), therefore static-SDME coupled to GC-FID system was used for method validation. The fruits samples were comminuted and homogenized in a blender and the resulting supernatant was filtered and spiked with a 50 and 200 ng/mL of standard pesticide mix. The fortified juice samples were analyzed after dilution with water at ratio of 1:100. The mixture was extracted using 1.5 μ L drop of toluene at room temperature, stirred at 600 rpm for 20 min at pH of 5–6, and desorbed. Salt addition was found to reduce extraction efficiency and was not added for validation studies. The analytical figure of merit: LODs, linear range, RSD, average recoveries, and correlation coefficients were 0.21–0.56 ng/mL, 0.5–50 ng/mL, ≥ 0.9995 , 77–113%, and 0.6–13.4%, respectively. This study showed that SDME analysis of pesticide residues in juice sample does not require any preliminary sample preparation when the dilution factor is carefully optimized.

Chai and Tan [46] compared HS-SDME with SPME and SPE in the analysis of organophosphorus and OCPs in strawberry and cucumber samples. The spiked samples for the extraction techniques were prepared using the same method prior to the analysis. A total of 100 g of pesticide-free samples was weighed and finely chopped, an aliquot of 30 g was weighed in a 150-mL beaker, and spiked with known amount of mixed standard at three concentration levels. The mixture was kept at room temperature for 1 h, and was homogenized with 30 g of distilled water and placed in 15-mL glass vial. The target analytes were extracted in HS-SDME mode with 1.5 μ L drop of toluene at room temperature, stirred at 800 rpm for 15 min, and desorbed into GC-ECD (ECD, electron capture detector). The SDME method validation gave linearity in the range of 1–100 μ g/mL ($R^2 \geq 0.987$), mean recoveries ranged from 75–95 (RSD = 4.7–13.6), while the LOQs and LODs ranged from 0.006 to 3 μ g/mL and 0.001 to 1 μ g/mL, respectively. The addition of salt to the donor solution was observed to reduce extraction efficiency, which is due to the reduction in diffusion of analytes to the extracting solvent. It was concluded that HS-SDME enables extraction of analytes from more complex matrices, simpler to perform, free from carry-over effect, and time effective, but HS-SPME is more efficient with better linearity, LOD, LOQ, and precision.

A method was also developed for the extraction of seven strobilurin and six oxazole fungicides in fruit (peach, peach and grape, pineapple and carrot) and juice (musts and canned fruit) samples by Viñas et al. [47]. The authors compared SDME with ultrasound-assisted emulsification microextrac-

tion (USAEME) technique by using EU Commission Decision (2002/657/EC) as a guideline for method validation. The sample of different fruit and juice samples was crushed to obtain a homogenous puree containing 10% m/v of NaCl at pH 5, and the mixture was then extracted with 10 μ L drop of 1:1 mixture of octanone and undecanone at 50°C, for 30 min, and desorbed into GC-MS for separation, identification, and quantification. Both methods gave similar average recoveries (SDME = 79–117%, RSD = 2.5–9.9%, and USAEME = 80–119%, RSD = 2.1–10%), which shows that matrix effect was corrected using method of standard addition for quantification. The SDME method gave LODs in the range of 0.01–0.31 ng/mL, while linearity ranged from 0.06 to 300 ng/mL. The EF ranged from 81 to 1602 for SDME and 142 to 1141 for USAEME. The USAEME was found to be advantageous for the extraction of target analytes when considering the equilibrium time (4 min) and extraction temperature (room temperature). The two techniques provide high and comparable selectivity, reproducibility, and EF.

It can be observed that most of the authors used toluene as the acceptor phase, which was attributed to its better characteristics: more stable, less toxic, moderate viscosity, higher extraction efficiency, higher EF, and its suitability for chromatographic system.

2.3 HF-LPME

HF-LPME (Fig. 2), also called liquid–liquid–liquid microextraction, is a multiphased microextraction system, which was developed to enhance the stability of the organic solvent [14, 15] and acts as a support for the acceptor (extracting) solvent. It is based on the principle of a supported liquid membrane (SLM) [14, 48] in which the organic solvent is used to fill both the wall pores and the HF lumen. It makes use of a polymeric membrane that forms a barrier between the solvent and the sample [49], and acts as a support for the small volumes of extracting solvents. It involves filling of few microliters of solvent inside the pores (about 0.2 μ m in size) and lumen of a semipermeable polypropylene HF attached to a syringe.

The HF-LPME can be carried out in either the static or dynamic mode. In the static mode, the acceptor phase is introduced in the lumen followed by the immersion of the fiber into the aqueous sample by a syringe, while in the dynamic mode the HF is attached to a syringe connected to programmable pump [18, 27, 31], which has also been developed for multiple extraction for up to eight sample vials simultaneously by the use of multiple channel syringe pump. This allows the continuous pumping of fresh solvent through the HF lumen [16, 17].

The extraction can be carried out in two or three phases. In the two-phase mode, the aqueous sample (donor) makes contact directly with the organic solvents (acceptor) through the membrane pores, and the analytes are extracted into the organic solvent present in the HF porous wall and inside the lumen of the HF [48], by a repeated pushing and pulling of the

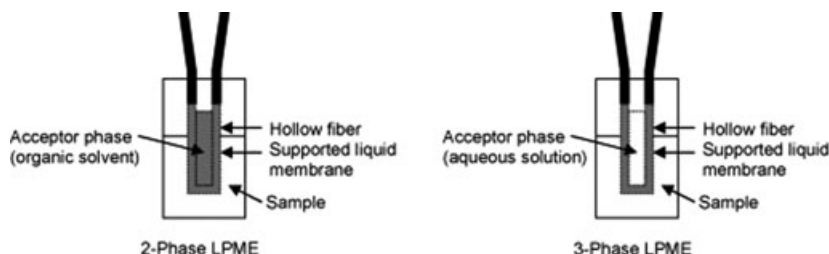


Figure 2. Hollow-fiber liquid-phase microextraction in two- and three-phase systems. Reprinted with permission [48].

microsyringe plunger [50]. The mass transfer of the analytes is driven by the diffusion of the analytes from the sample matrix into the organic solvent and is easily compatible to GC analysis. When the pores are prefilled with an organic solvent, which provides an SLM, and the acceptor solution is an aqueous solution, a three-phase system is formed, which is more compatible to HPLC or CE system [14, 48, 51, 52]. The solvent used must be compatible with the membrane, so as to ensure that the pores in the wall of the membrane are completely filled [31].

The technique can also be carried out in the headspace mode, but most researchers have used the DI mode for efficient preconcentration of the analytes. The limitation of this technique is the issue of carry-over [53]; therefore, a new membrane should be used for each extraction. The HF-LPME membranes are capable of accommodating larger volumes of organic solvents, which increases the efficiency of the extraction, while the pore acts as a filter that prevents interference of large molecular weight molecules present in the sample matrix.

2.3.1 Applications of HF-LPME

The use of HF-LPME for the analysis of pesticide residues from water samples has been extensively reported, where nonpolar organic solvents are used as the acceptor phase [34]. Only a limited number of studies have been performed on the analysis of pesticide residues from food samples, as shown in Table 2. HF-LPME was first applied to pesticides analysis in grapes by Luthje et al. [54], which was called pressurized hot water extraction-microporous membrane liquid–liquid extraction (PHWE-MMLLE) coupled to GC/MS. The MMLLE was used as a trapping step after PHWE and the water from the PHWE is then directed to the donor side of the membrane unit where the analytes are extracted onto the acceptor solution. The MMLLE was thus used to clean and concentrate the extract before on-line transfer to the GC. The optimized conditions include 1 ml/min of donor flow rate, at 120°C of PHWE for 40 min using toluene as the extraction solvent. Analytical performance yields an average recovery in the range of 9–26% (RSD = 1–6%), the low recovery was attributed to the presence of microporous membrane. The EFs ranged from 24 to 75, indicating an efficient partitioning between the target analytes and the acceptor phase. The LOQ ranged from 0.3 to 1.8 µg/kg, with linearity between 0.015 and 3 mg/kg and correlation coefficients greater than 0.973. It was observed that the chromatographic behavior of the on-line PHWE-MMLLE-

GC/MS was significantly better compared with liquid–solid and ultrasonic extractions with off-line analysis, indicating the selectivity of the developed method.

The HF-SLM method was developed by Romero-González et al. [55] for simultaneous determination of 23 multiclass pesticide residues in vegetable (cucumber, tomato, and pepper) samples. A representative portion of the samples was chopped and homogenized, and an aliquot of 10 g was placed in a 20-mL vial followed by addition of 1.5 g (15%) of NaCl and 5 mL of buffer at pH 4. Prior to LC/MS analysis, the target analytes were extracted from the sample for 60 min at a stirring rate of 40 oscillations/min, using diethyl ether containing 1% of trioctylphosphine oxide as the organic solvent phase and an acceptor phase made up of 20% methanol in 100 mM of HCl. The quantification performed by matrix-matched calibration yields LOD and LOQ ranging from 0.06 to 2.7 µg/kg and 0.2 to 9.0 µg/kg, respectively. The method linearity was between 10 and 200 µg/kg with correlation coefficients greater than 0.9910. The sample matrix was found to have no influence on the extraction efficiency and the donor samples were not diluted with water. The developed procedure was found to yield good analytical performance, which allows determination of pesticides below MRLs.

Hu et al. [56] developed HF-LPME method called liquid–liquid–solid microextraction (LLSME) coupled to HPLC, based on porous membrane protected molecularly imprinted polymer (MIP) coated SPME silica fiber (solid phase) for the extraction of triazine pesticides from sludge water, milk, urine, and water melon. The MIP-coated fiber was used as the acceptor phase protected by HF filled with toluene. The target analytes were first extracted from the donor phase through an organic solvent phase and were then extracted onto the MIP-coated SPME fiber, thereby integrating the advantages of high selectivity of SPME and the enrichment and sample cleanup of the HF-LPME into a single device. The method performance for watermelon gave good recovery in the range of 74–103% with RSD of 1.2–9.5%. The developed method was compared with MIP-SPME and HF-LPME (using *n*-octanol as the acceptor phase) in terms of linearity, correlation coefficients, LODs, and the result shows that the developed method (LLSME) has the best performance. The linearity ranged from 0.02 to 10 µg/L ($R^2 \geq 0.9956$), 0.5 to 10 µg/L ($R^2 \geq 0.9949$), and 0.5 to 100 µg/L ($R^2 \geq 0.9994$) for LLSME, MIP-SPME, and HF-LPME, respectively. The LODs for LLSME (0.006–0.02 µg/L) were lower than those of MIP-SPME (0.18–0.30 µg/L) and HF-LPME (0.08–0.20 µg/L). The RSD for the target analytes was in the range of 2.3–8.5, 1.5–8.7, and

Table 2. Hollow-fiber liquid-phase microextraction for analysis of pesticides in fruits and vegetable.

Pesticides class	Matrix	Org. Solv., vol.	Acceptor sol./vol (μL)	Ext. time (min)	Ext. time temp (°C)	Ionic eff. NaCl	pH	Agitation	LOD	LOQ	Rel. rec.	RSD	Linearity	EF	Det. method	Ref.
6 MCPs	Grape	Toluene, 3 mL	n.r.	40	120	n.r.	n.r.	n.r.	n.r.	0.3–1.8 μg/kg	9–28%	1–6%	15–300 g/kg	24–75	GC-MS	[54]
23 MCPs	Cucumber, tomato, pepper	DHE/TOPO, n.r.	MeOH/HCl, n.r.	60	n.r.	15%	4	40 osc/min	0.06–2.7 μg/kg	0.2–9.0 μg/kg	n.r.	n.r.	10–200 μg/kg	n.r.	LC-MS	[55]
9 THs	Water melon	Toluene	MIP fiber	30	n.r.	n.r.	n.r.	n.r.	0.08–0.02 μg/L	n.r.	74–103%	1.2–9.5%	0.02–10 μg/L	n.r.	HPLC	[56]
13 OCP	Tomato, strawberry	Octanol, 20 μL	Toluene/hexane, 30	59	60	2.91 g	2–4	n.a.	0.5–12.79 μg/kg	1.69–42 μg/kg	59–123%	5–15%	2–230 μg/kg	n.r.	GC-ECD	[21]
3 DFs	Orange juice	2-Octanone, 20 μL	HCl	30	n.r.	n.r.	8–12	1000 rpm	0.05–0.1 μg/L	0.17–0.33 μg/L	17–33.7 μg/L	8.6–14.8%	0.1–10 μg/L	n.r.	CE, LC/MS	[19]

Acceptor sol., acceptor solution; DFs, diazole fungicides; OCP, organochlorine pesticide; THs, triazine herbicides; DHE, dihexylether; TOPO, trioctylphosphine oxide.

2.5–7.8% for LLSME, MIP-SPME, and HF-LPME, respectively. The method was found to be highly selective, effective, and suitable for the extraction of pesticide residues in complex sample matrices.

A simple and low-cost method based on the simultaneous application of HF-LPME-GC-ECD was developed by Bendendo and Carasek [21] for the analysis of OCPs in river water, tomato, and strawberry samples. A fresh sample of tomato and strawberry was cut into pieces and 100 g was homogenized. An aliquot of 1 g was weighed in 100-mL flask and spiked with appropriate amount of pesticide standard solution and left for 3 days. The spiked sample was then suspended with 15 mL deionized water and was pH adjusted. The sample was then subjected to ultrasonic vibration for 10 min and then centrifuged for 8 min. The resulting supernatant was placed in 20-mL glass vial containing 2.91 g of NaCl and was extracted using 1.5-cm-long microporous membrane containing 20 μL of 1-octanol at 59°C for 60 min at pH 2–4. The acceptor phase containing the extracted analytes was desorbed into GC injector using 30 μL of mixture of toluene and hexane (60:40, v/v%). The LOD was in the range of 0.5–1.15 and 1.53–12.79 μg/kg for strawberry and tomato, respectively, while the LOQ was between 1.69 and 3.85 μg/kg for strawberry and 5.49 and 42.53 μg/kg for tomato. The method linearity and relative recovery (RSD) were in the range of 2–230 μg/kg ($R^2 > 0.992$) and 59–123% (RSD = 5.0–15%), respectively. The developed method was compared with MMLLE and was found to more efficient.

Barahona et al. [19] report the analysis of postharvest diazole fungicides (thiabendazole, carbendazim, and imazalil) residues from orange juice sample using HF-LPME. The method was coupled to CE-DAD for method development but LC-MS was used for estimation of analytical method performance, due to poor LOD of the CE-DAD (DAD, diode array detector). The analytes were extracted through an SLM containing 20 μL of 2-octanone and an acceptor phase containing 10 mM of HCl for 30 min, stirred at 1000 rpm. The pH of the donor phase was adjusted to alkaline condition by adding 0.5 mL of 120 mM of NaOH. The developed method yields linearity between 0.1 and 10 μg/L, with correlation coefficients greater than 0.998. The recoveries ranged from 17 to 33% (RSD = 8.6–14.8). The LODs were between 0.05 and 0.1 μg/L, while the quantitation were ranged from 0.17 to 0.33 μg/L, which were found to be consistent with the MRL permitted for pesticides in drinking water. Although the recoveries obtained were not quantitative enough, the method selectivity was found to be suitable for the estimation of LOD in drinking water.

2.4 DLLME

DLLME is a recently developed microextraction technique introduced by Assadi and co-workers [13], which also makes use of microliter volume of a mixture of extraction solvent and dispersive solvent with high miscibility in the donor and acceptor solutions [33]. This helps to minimize dislodgement

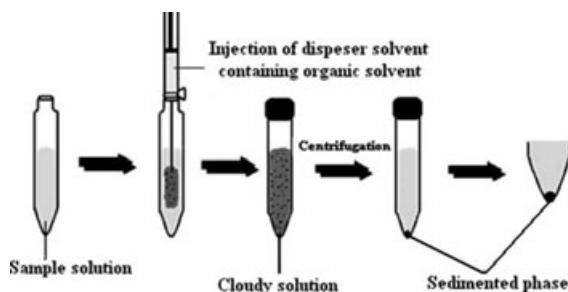


Figure 3. Steps in dispersive liquid-liquid microextraction. Reprinted with permission [33].

of the organic solvent drop inherent in SDME. It involves the formation of a cloudy solution when an appropriate mixture of high-density water-immiscible extraction solvent and dispersive solvents is injected rapidly into an aqueous solution [13, 33], containing the analytes of interest. The hydrophobic solutes are then enriched in the extraction solvents, which are dispersed into the bulk aqueous solution and the mixture is centrifuged, thus, making DLLME a two-step extraction technique. After centrifugation, a sedimented phase is settled at the bottom of a conical tube and used for chromatographic analysis with or without further treatment (Fig. 3). The choice of the type and volume of dispersive solvent is as important as that of the extraction solvent, because it helps the extraction solvent to form fine droplets in the sample matrices. The nature of the fine droplet has been found to enhance extraction efficiency, because of the abundant surface contact between the droplet and the analytes, thus the mass transfer of analytes into the extraction solvent is speeded up [31, 33] and the extraction is time independent.

2.4.1 Applications of DLLME

Table 3 shows the applications of SDME in the extraction of different classes of pesticide residues in fruits and vegetables using the DLLME technique. Zang et al. [57] employed DLLME for the analysis of captan, folpet, and captafol in apple sample. The sample of apple (20 g) was accurately weighed into a centrifuge tube, followed by addition of zinc acetate dehydrate solution (which prevents degradation of captan and folpet) and 20 μL of internal standard. The mixture was homogenized, centrifuged, and the supernatant was filtered with Buchner funnel under reduced pressure. The filtrate was diluted with double-distilled water and an aliquot of 5 mL was transferred into a glass tube and centrifuged again and the supernatant was then transferred into a tube containing 1 mL of the disperser solvent (acetone). The mixture was gently shaken, centrifuged, and then transferred to a glass tube followed by addition of 9 μL of chlorobenzene (extraction solvent), and the mixture was shaken again for 3 min and centrifuged at 5000 rpm for 5 min. The chlorobenzene sediment was formed at the bottom of the tube, and 1- μL aliquot of the sedimented phase was taken and injected into GC for analysis. Under the optimized condition, the method

linearity ranged from 10 to 100 $\mu\text{g}/\text{kg}$ ($R^2 \geq 0.9982$), LODs ranged from 3 to 8 $\mu\text{g}/\text{kg}$, with recoveries and EF between 93 and 107% (RSD = 4.6–6.4) and 824 and 912, respectively. Salt addition was found to have no significant effect on the recoveries but slightly decrease the EF and was not added for method validation.

Cunha et al. [58] optimized a multidimensional GC/MS using DLLME for trace analysis of 24 multiclass residual pesticides in apple sample. A 50 g of homogenized sample was weighed into a plastic tube and centrifuged for 5 min, and an aliquot (5 g $\approx$ 5 mL) of the upper layer was transferred into a 25-mL screw-cap plastic tube and spiked with 25 μL of triphenylphosphate (IS, internal standard). Then a mixture of 400 μL of acetone (dispersive solvent) and 100 μL of carbon tetrachloride (extracting solvent) was rapidly injected into the sample tube, closed, and manually shaken for 1 min, followed by centrifugation at 5000 rpm for 2 min. After centrifugation, the sedimented phase (85 μL) was transferred into a vial with 100 μL vial insert, and 2 μL of the extract was injected into the MD-GC/MS system. Under the optimized conditions, the linearity ranged from 0.04 to 0.188 mg/kg ($R^2 \geq 0.9950$), and the LOD and LOQ ranged from 0.06 to 2.20 and 0.2–7.3 $\mu\text{g}/\text{L}$, respectively, while the EF and recoveries (RSD) were between 35 and 101 and 60 and 105% (1–20%), respectively.

Fu et al. [59] developed a DLLME coupled to HPLC-fluorescence detection for the analysis of pesticide (carbaryl and triazophos) residues in water and fruit (apple, grape, and peach) juice samples. A total of 30 mL of fruit juice samples was centrifuged, and the supernatant was filtered, followed by 1:1 dilution of 25 mL of the filtrate with deionized water to reduce matrix effect. An aliquot of 5 mL of the mixture was subjected to the DLLME procedures under the optimized conditions (extraction solvent/tetrachloromethane (15 μL), dispersive solvent/ACN (1 mL), no salt addition, extraction time <5 s). The analytical performance of the method evaluated under this conditions yield linearity ranging from 0.1 to 1000 ng/mL ($R^2 \geq 0.991$), the LOD ranged from 12.3 to 16.0 pg/mL. The recovery varied from 86.3 to 105.3% (RSD = 1.11–9.67%) and the EF was in the range of 87.5–275.6. The developed method was compared to other LPME techniques [60, 61], and it showed lower RSD, LODs, and much wider linear range, while the extraction was very short and does not require any special approach and instrument in the pretreatment step.

Ravelo-Perez et al. [62] employed a room temperature ionic liquid based DLLME method for the extraction of trace amount of eight pesticide residues from banana. A sample of banana (1 kg) was homogenized and 1g was extracted with ACN, followed by filtration, evaporation, and reconstitution of the extract in 10 mL of distilled water containing 25% NaCl (w/v) at pH 2.7. DLLME procedure was performed using 88 mg of 1-hexyl-3-methylimidazolium hexafluorophosphate ([C₆MIm][PF₆]) as extracting solvent and 714 μL of methanol as the disperser solvent. The mixture was centrifuged at 4000 rpm for 20 min, immediately the dispersive and extraction solvents were added to the sample at room temperature. The linearity of the method ranged from 0.043 to 6.83 mg/L with

Table 3. Dispersive liquid–liquid microextraction for analysis of pesticides in fruits and vegetable

Pesticides class	Matrix	Org. solv., vol	Disperser sol., vol (μL)	Ext. time	Ext. temp (°C)	Ionic eff. NaCl (%)	pH	Centrifuge (rpm)/min	LOD	LOQ	Rel. rec. (%)	RSD (%)	Linearity	EF	Det. method	Ref.
3 DCFs	Apple	CB, 9 μL	ACO, 1000	3 min	n.r.	n.a.	n.r.	5000/5	3–8 μg/kg	n.r.	93–107	4.6–6.4	10–100 μg/kg	824–912	GC-ECD	[57]
		CTC, 100 μL	ACO, 400	1 min	n.r.	n.a.	n.r.	5000/2	0.06–2.20 μg/L	0.2–7.3 μg/L	60–105	1–20	0.04–0.18 μg/kg	35–101	MD-GC-MS	[58]
OP CP	Grape, apple, peach juices	TCE, 15 μL	ACN, 1000	5 s	Room temp	n.a.	n.r.	5000/2	12.3–16 pg/mL	n.r.	86–105	1.11–9.67	0.1–1000 pg/mL	87.5–275.6	HPLC-FLD	[59]
8 MCPs	Banana	RTIL, 88 mg	MeOH, 714	1 s	Room temp	25	2.7	4000/20	0.32–4.66 μg/kg	n.r.	53–97	2.3–8.7	0.043–6.83mg/L	n.r.	HPLC-DAD	[62]
		Grape, plum	RTIL, 88 mg	MeOH, 714	1 s	Room temp	25	2.7	4000/20	0.65–6.33 μg/kg	n.r.	66–105	1.4–9.1	0.01–6.83 mg/L	n.r.	HPLC-DAD
NI	Tomato	TCE, 30 μL	USD	6 min	n.r.	n.a.	n.r.	4000/5	0.45 mg/kg	n.r.	87–100	< 4.5	375	LC-UV	[64]	
		Pear juice	TCE, 30 μL	MeOH, 3500	2.5 min	n.r.	n.a.	4.5	4000/5	2.2–3.1 μg/kg	n.r.	92–107	< 4	0.009–15.2 μg/kg	344	GC-FID
6 CPs	Apple	CF, 60 μL	ACO, 6000	1 min	n.r.	n.a.	n.r.	5000/5	2–3 μg/kg	n.r.	85–113	4.3–7.4	6–500 μg/kg	491–1834	MEKC-DAD	[66]
		Pear juice	IL, 50 μL	MeOH, 600	n.r.	Room temp	n.a.	6.3	4000/5	0.01–0.05 μg/L	n.r.	78–86	1.1–1.7	5–1000 μg/L	300	HPLC-UV-Vis
CP PF	Apple	1-UnOH, 10 μL	ACO, 400	3 min	n.r.	28	n.r.	4000/2	1.4–1.6 μg/kg	n.r.	83–101	4.8–8.3	8–800 μg/kg	n.r.	LC-DAD	[80]
4 NI	Cucumber, tomato	CTC, 200 μL	ACN, 2500	30 s	n.r.	8	n.r.	n.r.	0.5–1.0 μg/kg	n.r.	84–97	3.7–6.2	5–300 μg/kg	110–243	HPLC-DAD	[70]
12 CPs	Banana, pineapple, tomato	CF, 800 μL	MeOH, 1500	5 min	n.r.	n.a.	7.5	5000/2	1–7 μg/L	6–24 μg/L	78–105	3.6–8.9	4–1000 μg/kg	n.r.	MEKC-DAD	[71]
14 MCPs	Apple	1-DoOH, 8 μL	ACO, 400	2 min	6	n.a.	n.a.	4000/2	2.8–18.5 ng/L	9.3–53.8 ng/L	79–102	3.2–7.6	10–2000 ng/L	409–1089	GC-ECD	[73]
13 OPPs	Tomato	CB, 60 μL	ACO, 5000	n.r.	n.r.	n.a.	n.r.	5000/4	0.1–0.5 μg/kg	n.r.	n.r.	7–10	0.5–1000 μg/kg	n.r.	GC-FPD	[74]
6 DCFs	Pear, grape, apple, strawberry	TCE, 14 μL	ACN, 800	30 s	n.r.	1	n.r.	3200/5	0.02–0.12 μg/kg	n.r.	81–98	3.1–7.8	0.5–40 μg/kg	685–820	GC-ECD	[76]

Disperser sol., disperser solution; DCFs, dithiocarbamide fungicides; MCPs, multiclass pesticides; OPP, organophosphorus pesticide; CPs, carbamate pesticides; NI, neonicotinoid insecticide; PPs, pyrethroid pesticides; PF, pyrimidine fungicide; CB, chlorobenzene; CTC, carbontetrachloride; TCE, tetrachloroethane; RTIL, room temperature ionic liquid; IL, ionic liquid; CF, chloroform; 1-UnOH, 1-undecanol; ACO, acetone; MeOH, methanol; USD, ultrasound dispersion.

correlation coefficients greater than 0.994. The recovery determined at three spiked levels was between 53 and 97% (RSD = 2.6–8.7%), except for thiophanate and carbofuran (53–63%). The LOD ranged from 0.320 to 4.66 $\mu\text{g/kg}$, which is below the harmonized EU MRLs established for bananas.

The same authors also used room temperature ionic liquids (1-hexyl-3-methylimidazolium hexafluorophosphate, $[\text{C}_6\text{MIm}][\text{PF}_6]$) for the extraction of eight multiclass pesticides in table grape and plum samples [63]. Using the previously optimized conditions for banana samples [62], the mean recovery was in the range of 72–100% (1.4–9.1%) and 66–105% (1.9–8.5%) for table grape and plum, respectively, except for thiophanate and carbofuran (64–75%). The linearity ranged from 0.01 to 6.83 mg/L and the LODs in table grape and plum ranged between 0.651 and 5.44 and 0.902 and 6.33 $\mu\text{g/kg}$, respectively. The target analytes found in the samples were at the levels that do not present any threat for the consumer, since they were below the MRLs established by the EU.

Qiao et al. [64] reported an ultrasonic-based DLLME method for the extraction of trace level of imidacloprid in tomato samples. Samples of tomato were homogenized and the fruit was centrifuge for 10 min. The supernatant was filtered and diluted with equal volume of ultra-pure water and was subjected to ultrasonic dispersion for 6 min. The filtered solution (5 mL) was transferred into 10-mL centrifuge tube followed by rapid injection 30 μL of tetrachloroethane (extraction solvent). The mixture was shaken vigorously for 5 s and subsequently ultrasonic vibrated for 10 min. The mixture was then centrifuged at 4000 rpm for 5 min and the sedimented phase was transferred to another tube, dried with nitrogen, and reconstituted with 100 μL of methanol for LC analysis. Under optimal condition, the linearity ranged from 6 to 100 $\mu\text{g/L}$ ($R^2 = 0.9980$), the average recovery was between 87.6 and 110% (RSD < 4.5), and the EF was 375-fold. The LOD was 0.45 mg/kg for the target analyte, which indicated that the proposed method could be used for the analysis of imidacloprid in tomato, with good sensitivity and accuracy.

Du et al. [65] also developed an ultrasonic-assisted DLLME for simultaneous determination of cypermethrin and permethrin residues in pear juice using GC-FID. Pear samples were juiced by food squeezer, filtered under vacuum, and centrifuged for 10 min. An aliquot (5 mL) of the supernatant was transferred into a centrifuge tube and mixed with 3.5 mL of methanol (disperser solvent). The sample solution was shaken, then filtered, and 30 μL of tetrachloroethane was added to form a ternary system, which was vortex for 30 s and emulsified by ultrasound for 2 min to get a cloudy solution. The solution was further centrifuged at 4000 rpm for 5 min and the sedimented phase was used for chromatographic analysis. The EF for cypermethrin was 344-fold while that of permethrin was 351-fold. Linearity was observed in the range of 0.009–15.2 $\mu\text{g/kg}$ ($R^2 \geq 0.9993$). The LODs were 3.1 and 2.2 $\mu\text{g/kg}$ for cypermethrin and permethrin, respectively. The recoveries determined at three spiked levels ranged from 92.1 to 107.1% (RSD < 4%). The salt addition was found to have effect on the recoveries and EF, while the pH of the donor sample was kept at 4.5. The ultrasound system was found

to accelerate the formation of a fine cloudy solution, which increased the efficiency and reduced extraction time.

A rapid and sensitivity method was developed based on DLLME coupled with sweeping electro-kinetic chromatography (sMEKC), for the analysis of six carbamate pesticides in apple by Zhang et al. [66]. The sample was prepared by homogenizing 20 g of chopped apple sample and subsequently centrifuged for 10 min. The supernatant was transferred to a Buchner funnel and filtered twice under reduced pressure. The filtrate was diluted to 25 mL with double-distilled water and a 5-mL aliquot was transferred to 10-mL screw-cap glass tube and was centrifuged for 10 min. The supernatant was further transferred into another tube followed by rapid addition of 1 mL of acetone (disperser solvent) containing 60 μL of chloroform (extraction solvent) and shaken manually for 1 min. After centrifugation at 4000 rpm for 5 min, the sedimented phase was completely transferred into another test tube, evaporated to dryness, and reconstituted with 20 μL of double-distilled water for CE analysis. The method validation estimated using a six-point calibration curve gave linear range of 6–500 ng/g ($R^2 \geq 0.9952$), with EF and recovery ranging from 491 to 1834 and 85 to 113% (RSD = 4.3–7.4%), respectively, and the LODs between 2 and 3 ng/g. The EF of the DLLME-sMEKC when compared with that of DLLME (74–151) and sMEKC (7.1–10.9) showed that the DLLME-sMEKC provided about 500- to 800-fold sensitivity enhancement without obvious loss of resolution.

He et al. [67] reported the use of a new 1,3-dibutylimidazolium hexafluorophosphate ($[\text{BBIIm}][\text{PF}_6]$) ionic liquid, as an extraction solvent for preconcentration of organophosphorus pesticides in water and pear juice samples, optimizing the effect of different parameter on the extraction efficiency. The pear sample was squeezed by a juice extractor, the juice was filtered and the filtrate was diluted (1:3) with double-distilled water, and subjected to DLLME procedure. Under optimal conditions (50 μL of the extraction solvent ($[\text{BBIIm}][\text{PF}_6]$), 600 μL of disperser solvent (methanol), no salt addition, 5 min of centrifugation at 4000 rpm, room temperature and no pH adjustment), the linearity of the method ranged from 5 to 1000 $\mu\text{g/L}$ ($R^2 \geq 0.9988$). The LODs were 0.01–0.05 $\mu\text{g/L}$, the recoveries were between 78.6 and 86.8%, and the RSD at three spiked levels ranged from 1.1 to 2.7%, while the EF was over 300-fold. The recovery value (92.7–109.1%) obtained in real pear sample shows that the sample matrix had little effect on the proposed method.

A DLLME method was developed based on solidification of a floating organic droplet combined with LC-DAD, for simultaneous analysis of diethofencarb and pyrimethanil in apple pulp and peel by Zhou et al. [68]. The sample of 0.5 g of spiked apple was weighed into microwave tube containing 400 μL of acetone (disperser solvent), and irradiated at 80°C for 30 min. The ACN extract was removed from the tube and injected rapidly into a 10 mL screw-cap test tube containing 5 mL of double-distilled water followed by addition of 1.4 g of NaCl and 10 μL of 1-undecanol (extraction solvent). The tube was shaken manually for some seconds and centrifuged at 4000 rpm for 2 min, and the less-dense 1-undecanol droplet

rose to the surface of the aqueous solution. The test tube was cooled in an ice bath to freeze the organic drop and the drop was removed using small medical biceps. The drop was then placed in polychloroprene rubber tube at room temperature and 5 mL of the melted drop was collected and used for HPLC analysis. The method linearity ranged from 8 to 800 $\mu\text{g/kg}$ ($R^2 \geq 0.9916$), LODs were 1.4 and 1.6 $\mu\text{g/kg}$ for pyrimetani and diethofencarb, respectively. The recovery ranged between 83. and 101.3% ($\text{RSD} = 4.8\text{--}8.3\%$), which demonstrated that the developed method was not significantly affected by matrix effect. The proposed method was observed to give comparable results when compared with HS-SPME-GC-MS method [69].

Wu et al. [70] introduced the use of dispersive SPE (DSPE) cleanup followed by DLLME for the extraction of neonicotinoid insecticides in vegetable (cucumber and tomato) samples prior to HPLC-DAD analysis. The method involved the use of multiwalled carbon nanotubes (MWCNTs) as DSPE sorbent for the removal of color-interfering substances from the samples. The spiked vegetable samples were prepared by homogenizing in a laboratory homogenizer. An aliquot of 10 g portion of the homogenized was transferred into a 50-mL centrifuge tube, followed by addition of 10 mL ACN, 4 g of MgSO_4 , and 1 g of NaCl. The mixture was vigorously shaken manually for 1 min and the extract was centrifuged for 5 min. The resulting supernatant was transferred to a 10-mL centrifuge tube containing 250 mg of MWCNTs and 250 mg of primary secondary amine, and the extract was vortexed for 30 s followed by centrifugation for 5 min.

For the DLLME, a 2.5-mL aliquot of the supernatant from the DSPE step above containing ACN, which also serves as the disperser solvent, was transferred into 50-mL centrifuge tube containing 10 mL of water, 0.8 g of NaCl, and 200 μL of CHCl_3 (extraction solvent). The mixture was vortexed for 30 s followed by centrifugation for 5 min and the sedimented phase was then transferred into a test tube, evaporated to dryness, reconstituted with 20 μL of methanol, and 15 μL was injected into the HPLC system. The linearity of the method ranged from 5 to 300 ng/g, with the correlation coefficients ranging from 0.9989 to 0.998 and the EF between 110 and 243. The LODs were from 0.5 to 1.0 ng/g while the recoveries ranged between 84.6 and 97.5%, with RSD from 3.7 to 6.2%. It was observed that the combination of the DSPE procedure helped to achieve better sample cleanup, which was possible by the use of MWCNT-primary secondary amine dual sorbent.

A DLLME method coupled to MEKC was proposed by Moreno-Gonzales et al. [71] for the extraction and preconcentration of 12 carbamate pesticides in fruit juice samples. A total of 50 mL of fruit juice at pH 7.5 was placed in a falcon tube and centrifuged for 10 min. An aliquot of the upper layer was placed in another tube followed by rapid addition of 1.5 mL of methanol (disperser solvent) and 800 μL of chloroform (extraction solvent), and shaken mechanically for 5 min, then centrifuged at 4000 rpm for 2 min. The sedimented phase was removed and collected in a glass vial, while the chloroform phase was evaporated to dryness. The final residue was redissolved with 500 μL of mixture of 100 mM boric acid and 5% ACN and then injected into electrophoretic sys-

tem. The proposed method gave linearity in the range between 4 and 1000 $\mu\text{g/L}$ with correlation coefficients greater than 0.991, while the recoveries, LODs, and LOQs ranged from 78 to 105% ($\text{RSD} = 3.6\text{--}8.9\%$), 1 to 7 $\mu\text{g/L}$, and 6 to 24 $\mu\text{g/L}$, respectively. Three agitation modes (vortex, manual, and mechanical) were compared and no significant differences were observed on the recoveries, but mechanical shaking gave the best reproducible results and was selected. The method was found to provide similar sensitivity compared to SPME-HPLC-UV method [72].

Matsadiq et al. [73] compared the residual level of multi-class pesticides (poly chlorinated biphenyl (PCB), organochlorine pesticide (OCP), and pyrethroid pesticides (PP)) in peach juice, pulp, and peels using DLLME based on solidification of floating organic droplet method coupled to GC/ECD. The peach pulps were cut into pieces, and then put into stainless blender to homogenize. The homogenized fruit was filtered and the juice and pulpy residues were separately collected and the pulp was dried. The peels were also cut, homogenized, and were vacuum freeze dried. After drying, the peach pulps and peels were powdered, sieved, and stored at 4°C before use. The juice sample was diluted with ultra-pure water before analysis. A total of 5 mL of acetone was added to 0.5 g of spiked peach and pulp samples in a separate microwave tube and was irradiated for 30 min at 6°C. After extraction, 0.4 mL of acetone was rapidly injected into the test tube containing the irradiated sample, 5 mL of water, followed by addition of 8 μL of dodecan-1-ol (extraction solvent), and the tube was placed in a water bath under ultrasonic sound for 2 min.

The mixture was centrifuged and the extraction solvent floats to the surface of the aqueous sample. The test tube was cooled and the floating organic solvent was removed and then placed in a 200- μL polychloroprene rubber tube at room temperature. After melting of the organic drop, it was diluted with methanol (1:10) and the mixture was injected into GC system. A total of 5 mL of the spiked juice sample was also mixed with 8 μL of dodecan-1-ol and 0.4 mL of acetone, and the subsequent procedure was carried out in the peel and pulp samples. Using the optimal conditions, the linearity ranged from 10 to 2000 ng/L in juice sample and 1–20 $\mu\text{g/kg}$ in pulp and peel samples with correlation coefficient greater than 0.99. The relative recoveries in juice, pulp, and peels were in the range of 79–102% ($\text{RSD} = 3.2\text{--}7.6\%$), 73–105% ($\text{RSD} = 2.6\text{--}11.8\%$), and 81–106% ($\text{RSD} = 3.2\text{--}7.8\%$), respectively. The LODs and LOQs were between 2.8 and 18.5 and 9.3 and 53.8 ng/L in juice, and 0.23 and 1.75 and 0.76 and 5.77 $\mu\text{g/L}$ in pulp and peel samples, respectively, while the EF ranged from 409 to 1089. The result of the blank juice, peel, and pulps analyzed indicated that the pyrethroid residues did not penetrate into the pulp and juice but are deposited on the peels of the fruits.

Bidari et al. [74] developed an ultrasound-assisted solvent extraction-DLLME method for the extraction of 13 organophosphorus pesticides from tomato sample. The method involved spiking 1 g of previously homogenized tomato sample with the pesticide standards followed by addition of 5 mL acetone (disperser solution) and 10 μL of

5 $\mu\text{g/L}$ triphenylphosphate (IS) in methanol and extracted in an ultrasonic bath for 35 min. After the extraction, 60 μL of chlorobenzene (extraction solvent) was added and the mixture was gently shaken and then centrifuged for 2 min. One milliliter of the acetone extract was then added to a test tube containing 5 mL of ultra-pure water, and the cloudy solution formed was centrifuged at 5000 rpm for 4 min. One microliter of the sedimented phase was removed and injected directly into the GC system. The method validated under the optimized conditions showed good linearity that ranged from 0.5 to 1000 $\mu\text{g/kg}$ with correlation coefficients greater than 0.9917. The LODs ranged from 0.1 to 0.5 $\mu\text{g/kg}$ and the repeatability estimated in terms of RSD was between 7 and 10%. The method was compared to HS-SPME [75] and other extraction methods in term of LOD linearity, volume of extraction solvent, RSD, and sample amount, and they were found to be comparable or lower than some of the methods.

Huo et al. [76] investigated the use of DLLME for the determination of six fungicides in fruit samples (pear, grape, apple, and strawberry) using GC-ECD. The samples of fruits were separately homogenized and 20 g of the homogenized samples was placed in a centrifuge tube and centrifuged for 5 min. After centrifugation, the supernatant was filtered into 10-mL volumetric flask and was made up to the mark with double-distilled water. A total of 5 mL of the solution was transferred into 10-mL test tube followed by addition of 1% w/v of NaCl and rapid injection of 0.8 mL of ACN (disperser solvent) and 14 μL of tetrachloroethane (extraction solvent), shaken for 30 s followed by centrifugation at 3200 rpm for 5 min. The sedimented phase was then transferred into a small vial and 1 μL was injected into a GC-FID system. The method validation estimated under the optimized conditions gave linearity in the range of 0.5–40 $\mu\text{g/kg}$ with correlation coefficients greater than 0.9902. The EF ranged from 685 to 820 while the recoveries were between 81.3 and 98.4%, with RSD ranging from 3.1 to 7.8%, and the LODs of the developed method were in the range of 0.02–0.12 $\mu\text{g/kg}$. The method performance was found to fit the requirements for the determination of selected fungicides in real fruit samples.

3 Limitations, future trends, and conclusion

The use of LPME technique is emerging as a very reliable sample preparation method, while employing little or no solvent. The advantages over the traditional method include their simplicity of operation, rapid sampling, low cost, high recovery and EF, and being environmentally friendly. The major limitations of the technique include low volume and instability of the microdrop solvent, possible loss of organic solvent in SDME, the presence of porous membrane, use of a large amount of solvent for analyte elution in HF-LPME. The difficulty of the automation, use of large volume of dispersive solvent, which usually decreases the partitioning between the analyte and the organic solvent, and solvent dissolution are

the major drawbacks in DLLME. The use of more selective, efficient, and versatile extraction procedure and increasing interest in overcoming the aforementioned limitations and trend toward automation will provide better integration of sampling and instrumental analysis, which can be used for a wide range of analytes. The limitations still remain when the technique is used in the analysis of pesticides in food matrices, thus dilution of sample with water or other solvents, which helps to reduce matrix effects by increasing diffusion of analytes into the extractant solvent, should be carefully optimized. Although most of the LPME applications employed GC, since water-immiscible solvents are used, recent development has been geared toward the use of HPLC for thermally labile and nonvolatile pesticides that are not amenable to GC analysis. Future trend is aimed at the use of less-toxic solvents such as ionic liquids, and supramolecular solvents as the extraction solvents instead of chlorinated solvent that are highly toxic. The application of LPME technique can be applied to a wide range of pesticide residues ranging from polar, nonpolar, volatile, to semivolatile, provided all factors are carefully optimized.

Other microextraction techniques for the extraction and analysis of pesticide residues from fruits and vegetables have also been developed. The techniques make use of little or no solvents, such as SPME using sol gel and MIP-coated fibers, microextraction in parked solvent, stir-bar sorptive extraction [77]. These techniques have been compared with LPME and were found to have similar analytical performance for the analysis of Sudan dye in food samples. [78]. In another study, HS-SPME was found to be more efficient in terms of linearity, precision, LOD, and LOQ, but LPME is simpler without any carry-over effect [46, 75, 79] for the extraction of pesticide residues from fruit and vegetable samples. All microextraction techniques have shown to be very effective and efficient with good analytical figure of merit, in the analysis of pesticide residues and other contaminants from environmental samples [56, 77, 80], but their direct comparison is a difficult task [32, 81].

The financial support of University of Malaya through the IPPP Research Grant No: PV009/2011A and RG025–09AFR is gratefully acknowledged. The authors also thank Elsevier® for reprint of figures used in this review.

The authors have declared no conflict of interest.

4 References

- [1] European Union (EU), Directorate General for Health & Consumers, European Commission, Brussels 2011.
- [2] Abdulra'uf, L. B., Hammed, W. A., Tan, G. H., *Crit. Rev. Anal. Chem.* 2012, 42, 152–161.
- [3] Arthur, C. L., Pawliszyn, J., *Anal. Chem.* 1990, 62, 2145–2148.
- [4] Pawliszyn, J., *Solid Phase Microextraction: Theory and Practice*, VCH, New York 1997.

- [5] Pawliszyn, J., in: Pawliszyn, J. (Ed.), *Handbook of Solid Phase Microextraction*, Elsevier, Waltham, MA 2012, pp. 13–59.
- [6] Abdel-Rehim, M., *J. Chromatogr. B* 2004, 801, 317–321.
- [7] Abdel-Rehim, M., *Anal. Chim. Acta* 2011, 701, 119–128.
- [8] Altun, Z., Abdel-Rehim, M., Blomberg, L. G., *J. Chromatogr. B* 2004, 813, 129–135.
- [9] El-Beqqali, A., Kussak, A., Abdel-Rehim, M., *J. Chromatogr. A* 2006, 1114, 234–238.
- [10] Liu, H., Dasgupta, P. K., *Anal. Chem.* 1996, 68, 1817–1821.
- [11] Jeannot, M. A., Cantwell, F. F., *Anal. Chem.* 1996, 68, 2236–2240.
- [12] Jeannot, M. A., Przyjazny, A., Kokosa, J. M., *J. Chromatogr. A* 2010, 1217, 2326–2336.
- [13] Rezaee, M., Assadi, Y., Milani Hosseini, M.-R., Aghaee, E., Ahmadi, F., Berijani, S., *J. Chromatogr. A* 2006, 1116, 1–9.
- [14] Pedersen-Bjergaard, S., Rasmussen, K. E., *Anal. Chem.* 1999, 71, 2650–2656.
- [15] Pedersen-Bjergaard, S., Rasmussen, K. E., Grønhaug Halvorsen, T., *J. Chromatogr. A* 2000, 902, 91–105.
- [16] Pezo, D., Salafranca, J., Nerín, C., *J. Chromatogr. A* 2007, 1174, 85–94.
- [17] Salafranca, J., Pezo, D., Nerín, C., *J. Chromatogr. A* 2009, 1216, 3731–3739.
- [18] Asensio-Ramos, M., Ravelo-Pérez, L. M., González-Curbelo, M. Á., Hernández-Borges, J., *J. Chromatogr. A* 2011, 1218, 7415–7437.
- [19] Barahona, F., Gjellstad, A., Pedersen-Bjergaard, S., Rasmussen, K. E., *J. Chromatogr. A* 2010, 1217, 1989–1994.
- [20] Basheer, C., Alnedhary, A. A., Rao, B. S.M., Lee, H. K., *Anal. Chim. Acta* 2007, 605, 147–152.
- [21] Bedendo, G. C., Carasek, E., *J. Chromatogr. A* 2010, 1217, 7–13.
- [22] Chen, P.-S., Huang, S.-D., *Talanta* 2006, 69, 669–675.
- [23] Cheng, J., Xiao, J., Zhou, Y., Xia, Y., Guo, F., Li, J., *Microchim. Acta* 2011, 172, 51–55.
- [24] Cheng, J., Zhou, Y., Zuo, M., Dai, L., Guo, X., *Int. J. Environ. Anal. Chem.* 2010, 90, 845–855.
- [25] Farajzadeh, M. A., Bahram, M., Vardast, M. R., Bamorowat, M., *Microchim. Acta* 2011, 172, 465–470.
- [26] Hou, L., Lee, H. K., *J. Chromatogr. A* 2004, 1038, 37–42.
- [27] Hou, L., Shen, G., Lee, H. K., *J. Chromatogr. A* 2003, 985, 107–116.
- [28] Kokosa, J. M., Przyjazny, A., Jeannot, M. A., *Solvent Microextraction: Theory and Practice*, John Wiley, Hoboken, NJ 2009.
- [29] Dean, J. R., *Extraction Techniques in Analytical Science*, John Wiley, West Sussex 2010.
- [30] Rios, A., Escarpa, A., Simonet, B. M., *Miniaturization of Analytical Systems: Principles, Designs and Applications*, John Wiley, West Sussex 2009.
- [31] Sarafraz-Yazdi, A., Amiri, A., *TrAC, Trends Anal. Chem.* 2010, 29, 1–14.
- [32] Nerin, C., Salafranca, J., Aznar, M., Batlle, R., *Anal. Bioanal. Chem.* 2009, 393, 809–833.
- [33] Rezaee, M., Yamini, Y., Faraji, M., *J. Chromatogr. A* 2010, 1217, 2342–2357.
- [34] Lambropoulou, D. A., Albanis, T. A., *J. Biochem. Biophys. Methods* 2007, 70, 195–228.
- [35] Jain, A., Verma, K. K., *Anal. Chim. Acta* 2011, 706, 37–65.
- [36] Kataoka, H., *Anal. Bioanal. Chem.* 2010, 396, 339–364.
- [37] He, Y., Lee, H. K., *Anal. Chem.* 1997, 69, 4634–4640.
- [38] Xu, L., Basheer, C., Lee, H. K., *J. Chromatogr. A* 2007, 1152, 184–192.
- [39] Amvrazi, E. G., Tsiropoulos, N. G., *J. Chromatogr. A* 2009, 1216, 2789–2797.
- [40] Amvrazi, E. G., Tsiropoulos, N. G., *J. Chromatogr. A* 2009, 1216, 7630–7638.
- [41] Farajzadeh, M. A., Djozan, D., Khorram, P., *Talanta* 2011, 85, 1135–1142.
- [42] Amvrazi, E. G., Papadi-Psillou, A. T., Tsiropoulos, N. G., *Int. J. Environ. Anal. Chem.* 2010, 90, 245–259.
- [43] Zhang, M., Huang, J., Wei, C., Yu, B., Yang, X., Chen, X., *Talanta* 2008, 74, 599–604.
- [44] Zhao, E., Han, L., Jiang, S., Wang, Q., Zhou, Z., *J. Chromatogr. A* 2006, 1114, 269–273.
- [45] Xiao, Q., Hu, B., Yu, C., Xia, L., Jiang, Z., *Talanta* 2006, 69, 848–855.
- [46] Chai, M. K., Tan, G. H., *J. Chromatogr. Sci.* 2009, 47, 694–699.
- [47] Viñas, P., Martínez-Castillo, N., Campillo, N., Hernández-Córdoba, M., *J. Chromatogr. A* 2010, 1217, 6569–6577.
- [48] Pedersen-Bjergaard, S., Rasmussen, K. E., *J. Chromatogr. A* 2008, 1184, 132–142.
- [49] Hyötyläinen, T., Riekkola, M.-L., *Anal. Chim. Acta* 2008, 614, 27–37.
- [50] Lambropoulou, D. A., Konstantinou, I. K., Albanis, T. A., *J. Chromatogr. A* 2007, 1152, 70–96.
- [51] Pedersen-Bjergaard, S., Rasmussen, K. E., *J. Chromatogr. B* 2005, 817, 3–12.
- [52] Rodríguez, A., Pedersen-Bjergaard, S., Rasmussen, K. E., Nerín, C., *J. Chromatogr. A* 2008, 1198–1199, 38–44.
- [53] Krylov, V. A., Krylov, A. V., Mosyagin, P. V., Matkivskaya, Y. O., *J. Anal. Chem.* 2011, 66, 331–350.
- [54] Luthje, K., Hyötyläinen, T., Rautiainen-Rama, M., Riekkola, M. L., *Analyst* 2005, 130, 52–58.
- [55] Romero-González, R., Pastor-Montoro, E., Martínez-Vidal, J. L., Garrido-Frenich, A., *Rapid Commun. Mass Spectrom.* 2006, 20, 2701–2708.
- [56] Hu, Y., Wang, Y., Hu, Y., Li, G., *J. Chromatogr. A* 2009, 1216, 8304–8311.
- [57] Zang, X., Wang, J., Wang, O., Wang, M., Ma, J., Xi, G., Wang, Z., *Anal. Bioanal. Chem.* 2008, 392, 749–754.
- [58] Cunha, S. C., Fernandes, J. O., Oliveira, M. B. P. P., *J. Chromatogr. A* 2009, 1216, 8835–8844.
- [59] Fu, L., Liu, X., Hu, J., Zhao, X., Wang, H., Wang, X., *Anal. Chim. Acta* 2009, 632, 289–295.
- [60] Xiong, J., Hu, B., *J. Chromatogr. A* 2008, 1193, 7–18.
- [61] Zhang, J., Lee, H. K., *J. Chromatogr. A* 2006, 1117, 31–37.

- [62] Ravelo-Pérez, L. M., Hernández-Borges, J., Asensio-Ramos, M., Rodríguez-Delgado, M. Á., *J. Chromatogr. A* 2009, **1216**, 7336–7345.
- [63] Ravelo-Perez, L. M., Hernandez-Borges, J., Herrera-Herrera, A. V., Angel Rodriguez-Delgado, M., *Anal. Bioanal. Chem.* 2009, **395**, 2387–2395.
- [64] Qiao, F., Zhang, X., Wang, M., Kang, Y., *Chromatographia* 2010, **72**, 331–335.
- [65] Du, J., Yan, H., She, D., Liu, B., Yang, G., *Talanta* 2010, **82**, 698–703.
- [66] Zhang, S., Li, C., Song, S., Feng, T., Wang, C., Wang, Z., *Anal. Methods* 2010, **2**, 54–62.
- [67] He, L., Luo, X., Jiang, X., Qu, L., *J. Chromatogr. A* 2010, **1217**, 5013–5020.
- [68] Zhou, X., Xie, P.-f., Wang, J., Zhang, B.-b., Liu, M.-m., Liu, H.-l., Feng, X.-h., *J. Chromatogr. A* 2011, **1218**, 3571–3580.
- [69] Navalon, A., Prieto, A., Araujo, L., Víchez, J. L., *J. Chromatogr. A* 2002, **975**, 355–360.
- [70] Wu, Q., Li, Z., Wang, C., Wu, C., Wang, W., Wang, Z., *Food Anal. Methods* 2011, **4**, 559–566.
- [71] Moreno-González, D., Gámiz-Gracia, L., García-Campaña, A. M., Bosque-Sendra, J. M., *Anal. Bioanal. Chem.* 2011, **400**, 1329–1338.
- [72] Yang, X. M., Wang, O., Wang, M. Z., Hu, Y. X., Li, W. N., Wang, Z., *J. Chromatogr. Sci.* 2008, **46**, 751–756.
- [73] Matsadiq, G., Hu, H.-L., Ren, H.-B., Zhou, Y.-W., Liu, L., Cheng, J., *J. Chromatogr. B* 2011, **879**, 2113–2118.
- [74] Bidari, A., Ganjali, M. R., Norouzi, P., Hosseini, M. R.M., Assadi, Y., *Food Chem.* 2011, **126**, 1840–1844.
- [75] Lambropoulou, D. A., Albanis, T. A., *J. Chromatogr. A* 2003, **993**, 197–203.
- [76] Huo, X., Li, Q., Lin, X., Chen, X., Bi, K., *Chromatographia* 2011, **73**, 313–319.
- [77] Tan, G. H., Abdulra'uf, L. B., in: Soundararajan, R. P. (Ed.), *Pesticides – Recent Trends in Pesticide Residue Assay*, InTech, Rijeka, Croatia 2012, pp. 171–190.
- [78] Yu, C., Liu, Q., Lan, L., Hu, B., *J. Chromatogr. A* 2008, **1188**, 124–131.
- [79] Garrido Frenich, A., Romero-González, R., Martínez Vidal, J. L., Martínez Ocaña, R., Baquero Fera, P., *Anal. Bioanal. Chem.* 2011, **399**, 2043–2059.
- [80] Zhou, Y., Han, L., Cheng, J., Guo, F., Zhi, X., Hu, H., Chen, G., *Anal. Bioanal. Chem.* 2011, **399**, 1901–1906.
- [81] Tankiewicz, M., Fenik, J., Biziuk, M., *Talanta* 2011, **86**, 8–22.