

Current Trends in Liquid-Liquid Microextraction for Analysis of Pesticide Residues in Food and Water

Sara C. Cunha, J.O. Fernandes and M. Beatriz P.P. Oliveira
*REQUIMTE, Department of Bromatology, Faculty of Pharmacy,
University of Porto, Rua Aníbal Cunha 164 4099-030 Porto
Portugal*

1. Introduction

Since the middle of last century, the use of organic synthetic pesticides became a widespread practice, in order to better prevent, control and destroy pests. Despite their usefulness in the increment of food production, the extensive use of pesticides during production, processing, storage, transport or marketing of agricultural commodities can lead to environmental contamination and to the presence of residues in food. Real and perceived concerns about pesticide toxicity have promoted their strict regulation in order to protect consumers, environment and also the users of pesticides. Thus, reliable and accurate analytical methods are essential to protect human health and to support the compliance and enforcement of laws and regulations pertaining to food safety.

The first analytical methods for pesticide analysis were developed in the years 1960s, employing an initial extraction with acetone, followed by a partitioning step upon addition of a non-polar solvent and salt; these methods involved complex and solvent-intensive cleanup steps. Moreover, the instruments available for analysis of the target compounds had a relative low selectivity and sensitivity. The development of technology and robotic in the 1990s allied to the aim to reduce manual interference and to allow sample preparation during non-working time, has boosted the development of automated sample preparation techniques such as supercritical fluid extraction and pressure liquid extraction. Though initially very promising, these techniques have not succeeded in the field of pesticides analysis for various reasons, namely high price and low reliability of the instruments, and inability to extract different pesticide classes in foods with the same efficiency, often requiring separate optimization for different analytes. Later, a successful simplification of "traditional" solvent sample preparation, QuEChERS (quick, easy, cheap, effective, rugged, and safe) was presented by Lehotay and collaborators (Anastassiades et al., 2003). This procedure, involving a simple extraction/partition using acetonitrile and salts followed by a simple dispersive cleanup, has been adopted for the analysis of many pesticide residues in food (Cunha et al, 2010). Two similar QuEChERS methods achieved the status of Official Method of the AOAC International (Lehotay, 2007) and European Committee for Standardization (CEN) standard method EN 15662 (Standard Method EN 15662). Unfortunately, the analysis of QuEChERS extracts in acetonitrile by GC-MS is not totally straightforward. Several facts can occur: degradation of the GC column by the polar solvent,

vapor overload of the insert liner due to the high thermal expansion coefficient, contamination of the system by co-extractives (Hetmanski et al., 2010), and reduced enrichment factors.

Recently, the development of new analytical equipment, namely tandem mass spectrometers coupled to LC and GC systems, allowed improvements in the sensitivity, selectivity, and speed of analysis. Although the prohibitive costs of such equipments make them unattainable to many groups working in this field. Such improvements in sensitivity and selectivity could also be accomplished by innovative sample preparation techniques recently introduced, most of them with the added benefit to be easy to execute, cost-effective, and environmental friendly. Cloud point extraction, single-drop microextraction, hollow fiber liquid phase microextraction, and dispersive liquid-liquid microextraction, are examples of liquid-liquid microextraction techniques that have emerged in recent years in the field of sample preparation and are being used increasingly. The major advantage of microextractive techniques is the use of only microliters of solvents instead of several hundred milliliters in the classical liquid-liquid extraction. In addition, due to the compatibility of the solvents used and the low volumes involved, samples are easily transferred to the next step of analysis, liquid or gas chromatography. The aim of this work is to review the application of liquid-liquid microextraction techniques in the analysis of pesticide residues in food and water and to compare its use with other well-established sample preparation techniques. Special emphasis will be given to articles published in the last four years. Principles, advantages and relative merits of each technique will be also summarized and discussed.

2. Analytical tools for determination of pesticide residues in food and water

Pesticide analysis is almost invariably accomplished by means of a chromatographic technique, either GC or LC coupled to universal (MS, MS/MS) or selective detectors (ECD, electron-capture detector; NPD, nitrogen phosphorus detector; FPD, flame photometric detector; UV, ultraviolet detector; and FLD, fluorimetric detector), following an adequate sample preparation step. Regardless the type of chromatographic technique employed, sample preparation remains as the limiting step to reach desired performance parameters, due to the low legally established levels and the complex nature of the matrices in which the target compounds are present typically in low amounts. As a rule, the physico-chemical methods used to obtain a pesticide extract able to be chromatographically analyzed consist in the extraction/isolation of the target analytes by an appropriate extraction technique followed by some purification and concentration steps. The classical procedures are often time consuming, laborious and environmental unfriendly, taking into account the large volume of organic solvents usually required. Recently, as referred in the Introduction section, new techniques have been introduced, offering consistently high enrichment factors and consequently higher sensitivity for the analytes of interest, together with a significant reduction of organic solvent consumption as well as extraction time. The most relevant techniques in this field are further detailed in the following sections.

2.1 Sample preparation

2.1.1 Cloud-point extraction (CPE)

Watanabe and collaborators, introduced in 1976 cloud-point extraction (CPE), a promising new separation and extraction technique, as an alternative to classical procedures with

organic solvents (Paleologos et al., 2005). CPE or micelle-mediated extraction, is based on the capacity exhibited by aqueous micellar solutions of some surfactants to form the cloud point, or turbidity, phenomenon that occur when the solution is heated or cooled above or below certain temperature. The temperature at which this phenomenon occurs is known as the cloud-point temperature or micelle-mediated extraction (Carabias-Martínez et al., 2000). Surfactants are amphiphilic molecules, which have a polar moiety (the head), hydrophilic in nature, linked to a hydrophobic portion (the tail). In aqueous solution, and at low concentrations, surfactant molecules are found in monomer form, although dimers and trimers have also been detected (Paleologos et al., 2005).

When the surfactant concentration is increased above a certain threshold, called "critical micellar concentration" (CMC), the surfactant molecules become dynamically associated to form molecular aggregates of colloidal size. These aggregates, containing between 60 and - 100 monomers, are called micelles and are at equilibrium with a surfactant concentration in the solution close to the CMC. Depending on the nature and concentration of the surfactant, as well as on the solvent used, another series of structures may be formed, organized as inverse micelles, microemulsions, vesicles, monolayers, or bilayers (Carabias-Martínez et al., 2000).

To date, liquid-liquid phase separation based on non-ionic or zwitterionic surfactant micelles (i.e., CPE) are employed, while the use of charged surfactant species is still scarce (Paleologos et al., 2005). Sanz et al. (2004) used non-ionic surfactants such as polyoxyethylene 10 lauryl ether and oligoethylene glycolmonoalkyl ether (GenapolX-080) at 95°C for 15 min to extract eight organophosphorus pesticide residues (chlorpyrifos, diazinon, dimethoate, ethoprophos, malathion, methidathion, parathion methyl and paration ethyl) from water, which were analyzed by HPLC-UV. The authors obtained an enrichment factor of 20, recoveries between 27 and 105%, and limits of detection (LOD) lower than 30 µg/L. In 2008, Santalad et al. presented a simple and rapid spectrophotometry method based on acid-induced anionic surfactant micelle-mediated extraction (acid-induced cloud-point extraction) coupled to derivatization with 2-naphthylamine-1-sulfonic acid to determine carbaryl residues in water and vegetables. In this work, sodium dodecyl sulphate (the extractant), was combined with 2-naphthylamine-1-sulfonic acid derivatization, allowing the extraction at low temperature (45°C). The proposed method showed good analytical features with low LOD (50 µg/L), good precision with a relative standard deviation (RSD) of 2.3%, and high recoveries when applied in samples (85%).

Notwithstanding the capacity to concentrate the analytes and the good recoveries achieved with CPE, its application in the extraction of pesticide residues in food matrices is restricted, in part due to the physico-chemical properties of the surfactant. As it is viscous, it cannot be injected directly to conventional analytical instruments, so it has to be diluted with an aqueous or organic solvent to reduce its viscosity, thus impairing the anticipated theoretical preconcentration factors. Moreover, surfactant-bearing chromophores interfere with UV detection by overlapping with the analyte signal. This problem can be solved by diluting the surfactant-rich phase with an organic solvent prior to injection into the chromatographic column, increasing the portion of organic solvent in LC mobile phases or using fluorescence detection (Paleologos et al., 2005).

2.1.2 Single drop microextraction (SDME)

Drop-drop microextraction was first introduced, in 1996, by Liu & Dasgupta, (1996). They extracted sodium dodecyl sulphate ion pairs by a microdrop (1.3 µL) of a water-immiscible

organic solvent, suspended in a larger aqueous drop. At the same year, Jeannot and Cantwell introduced a technique that they termed as solvent microextraction in which the extraction medium was a droplet (8 μL) of 1-octanol held at the end of a Teflon rod and suspended in a stirred aqueous sample solution. After extraction for a prescribed time, the Teflon rod was withdrawn from the aqueous solution; the organic phase sampled with a microsyringe and injected into a GC system. In this work, the authors also proposed equilibrium and kinetic theories to explain this microextraction procedure. Subsequently, the technique was changed to allow simultaneous extraction and injection of analytes, by introducing as support a microsyringe, where the organic phase was suspended at the needle tip (Jeannot & Cantwell, 1997) (Figure 1).

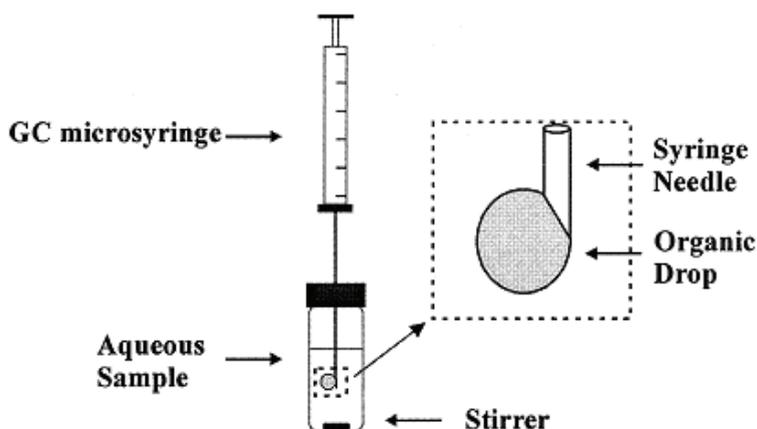


Fig. 1. Schematic illustration of direct immersion single-drop microextraction (from Xu et al., 2007).

One advantage of SDME over other liquid extraction techniques is the small volume of organic solvent required. Additionally, in this technique, analytes with high partition coefficient can reach high concentrations, since they are transferred by diffusion from a significant volume of sample (1-5 mL) to a small micro-extract (5-50 μL).

Since its introduction, different modes of SMDE have been developed, in order to improve extraction efficiency, such as direct SDME, headspace SDME (HS-SDME) and continuous-flow microextraction (CFME).

Direct SDME consists of suspending a microdrop of organic solvent at the tip of a syringe, which is immersed in the aqueous sample. An alternative approach was described as dynamic technique by He & Lee (1997), in which organic solvent repetitively forms a film inside the syringe barrel by continuously pulling and pushing of the syringe plunger. Extraction takes place between the sample solution and the organic film (He & Lee, 2006).

Direct SDME has extensively been used for the direct extraction of pesticide residues from aqueous samples (Table 1). Xiao et al. (2006) evaluated two types of SDME, static and dynamic, in extraction of six organophosphorus pesticides (OPPs) (dichlorvos, phorate, fenitrothion, malathion, parathion, quinalphos) from water and fruit juice. Significant parameters affecting SDME performance such as extractant solvent, solvent volume, stirring rate, sample pH and ionic strength were evaluated. The authors verified that static SDME

procedure allowed an enrichment factor of the six OPPs nearly 100 fold, which were much better than the results obtained with the dynamic mode. The optimized static SDME procedure in conjugation with GC-FPD allowed good detection limits ranging from 0.21 to 0.56 $\mu\text{g/L}$. In the same year, Zhao et al. (2006) also optimized a SDME procedure for extraction of seven OPPs (ethoprophos, diazinon, parathion methyl, fenitrothion, malathion, isocarbophos and quinalphos) in orange juices with analysis under GC-FPD. An effective extraction was achieved by suspending during 15 min a 1.6 μL drop of toluene to the tip of a microsyringe immersed in a 5 mL donor aqueous solution with 5 % (w/v) NaCl and stirred at 400 rpm. The seven OPPs were extracted from orange juice samples with good limits of detection (below 5 $\mu\text{g/L}$). However, better detection limits for 13 OPPs pesticides (ranging from 0.001 to 0.005 $\mu\text{g/L}$) in water were obtained by Ahmadi et al. (2006) using SMDE with a modified 1.0 μL microsyringe and GC-FPD, compared to 10 μL microsyringe used in the works above referred. By using a 1.0 μL microsyringe the repeatability of the drop volume and the injection were improved, due to the maximum volume of microsyringe without dead volume. On the other hand, the modification of the needle tip caused increasing cross section of it and increasing adhesion force between needle tip and drop, thereby increasing drop stability and allowing a higher stirrer speed (up to 1700 rpm). The method used 0.9 μL of carbon tetrachloride as extractant solvent, 40 min of extraction time, stirring at 1300 rpm and no salt addition. The potential of SMDE was also investigated by Liu et al. (2006) in the extraction of four fungicides from water and wine samples. Additionally, SDME has been applied in the extraction of organochlorine pesticides (OCPs) in various matrices (Table 1). Qia & He (2006) introduced a funnel from SDME to extract 11 OCPs and 2 pyrethroid pesticides from tea samples and analyze by GC-ECD. More recently, Cortada et al. (2009a) proposed a SDME procedure comprising a 2 μL toluene microdrop exposed for 37 min to 10 mL aqueous sample without salt addition and stirred at 380 rpm to extract eight OCPs from wastewater followed by GC-MS analysis.

Contrary to the aqueous samples, vegetable and fruits, being mostly in solid or heterogeneous form do not allow direct extraction with SDME. However, it is possible to use SDME after a previous pretreatment. Nine OCPs (β - λ - α -, σ - BHC, dicofol, dieldrin, DDD, DDE, and DDT) were extracted with SDME from fresh vegetable (cabbage, cauliflower, Chinese cabbage) after an adequate mixture of sample aliquots with acetone using an ultra-sonic vibrator. An effective extraction was achieved by suspending a 1.0 μL mixed drop of *p*-xylene and acetone (8:2 w/v) to the tip of a microsyringe immersed in a 2 mL donor sample solution and stirred at 400 rpm (Zhang et al., 2008). SDME technique coupled with GC-NPD and GC-ECD has also been successfully applied for the determination of multiclass pesticides in vegetable samples (tomato and courgette) by Amvrazi & Tsiropoulos (2009). Donor sample solution preparation from solid vegetable tissues was achieved in one step with the minimum amount of organic solvent (10% acetone in water) and optimum SDME was accomplished using a toluene drop (1.6 μL) under mild stirring for 25 min.

HS-SDME is very similar to direct SDME except that a microdrop of a high boiling extracting solvent is exposed to the headspace of a sample. This technique allows rapid stirring of the sample solution with no adverse impact on the stability of the droplet. Additionally, as in headspace-solid phase microextraction (HS-SPME), non-volatile matrix interferences are strongly reduced, if not totally eliminated. In this mode, the analytes are distributed among three phases, the water sample, the headspace and the organic drop (Xu et al., 2007). Aqueous phase mass transfer is the rate determining step in the extraction

process as explained by Theis et al. (2001). Hence, a high stirring speed of the sample solution facilitates mass transfer among the three phases. A HS-SDME was optimized for the extraction of organochlorine and organophosphorous pesticide residues in food matrices (cucumbers and strawberries) (Kin & Huat, 2009). The extraction was achieved by exposing 1.5 μL toluene drop to the headspace of a 5 mL aqueous solution in a 15 mL vial and stirred at 800 rpm. The analytical parameters, such as linearity, precision, LOD, limits of quantification (LOQ), and recovery, were compared with those obtained by HS-SPME and solid-phase extraction. The mean recoveries for all three methods were all above 70% and below 104%. HS-SPME was the best method with the lowest LOD and LOQ values. Overall, the proposed HS-SDME- GC-ECD method was acceptable for the analysis of pesticide residues in food matrices.

CFME was introduced by Liu & Lee, 2000, in order to improve the mass transfer between aqueous and organic phases. The technique is based in the continually refreshing of the surface of the immobilized organic drop used as extractant solvent by a constant flow of sample solution delivered by an HPLC pumping system (Xu et al., 2007). Both diffusion and molecular momentum resulting from mechanical forces contribute to its effectiveness. With the use of an HPLC injection valve, precise control of the solvent drop size could be achieved, avoiding the introduction of undesirable air bubbles. Another advantage was the high enrichment factor that can be achieved, requiring smaller volumes of aqueous samples for extraction (Xu et al., 2007). He & Lee (2006) reported the combination of CFME with HPLC to extract and determine the widely-used organonitrogens and OPPs (simazine, fensulfothion, etridiazole, mepronil and bensulide) (Table 1). CFME employs a single organic solvent drop of carbon tetrachloride (3 μL) positioned at the tip of a polyether ether ketone (PEEK) tubing, which is immersed in a continuous flowing aqueous sample solution in a 0.5-mL glass chamber. The PEEK tubing acts as the organic drop holder and fluid delivery duct. Analytes are partitioned between the organic drop and the bulk sample solution. Important extraction parameters including type of solvent, volume, sample solution flow rate, extraction time, pH and the addition of salts were investigated. Detection limits lower than 4 $\mu\text{g/L}$ were obtained for all analytes.

As mentioned above several parameters affect the rates and efficiencies of SDME techniques such as: i) analyte properties, ii) solvent acceptor, iii) drop volume, iv) agitation, v) ionic strength, vi) extraction time. A detailed discussion of these important parameters can be found in the literature (Jeannota et al., 2010). i) Analyte properties: low molecular weight, volatile and semi volatile analytes are extractable by headspace (HS-SDME). Direct immersion (DI-SDME) extraction is appropriate for non polar or moderately polar high molecular weight, semi volatile chemicals. Highly polar chemicals may need to be derivatized to ensure recovery, especially when the matrix is aqueous. ii) Extractant solvent: the extractant solvent in SDME is usually a pure or mixed hydrophobic solvent (*n*-hexane, benzene, toluene, dichloromethane, *n*-butanol, etc.), although some authors have reported the use of a hydrophilic solvent mixture as extractant solvent (*p*-xylene:acetone). iii) Drop volume: the use of a large drop results in an increase of analyte extracted. However, larger drops (>3 μL) are difficult to manipulate and less reliable. Difficulties with drop size variations are minimized if the drop size used is about 1 μL . iv) Ionic strength: addition of salts (such as NaCl or Na_2SO_4) to the sample may improve the extraction of analytes since high ionic strength reduces their water solubility. However, apart from the salting-out effect, the presence of salt can change the physical properties of the extraction film, thus reducing the diffusion rates of the analytes into the drop. v) Agitation of the sample: the

time required to thermodynamic equilibrium can be reduced by agitation. Three sample agitation methods are available: stirring, vibration and vortexing. Stirring, using a magnetic stir bar, is effective with stirring rates of 300–600 rpm for DI-SDME and 500–1000 rpm for HS-SDME. The limitations of higher stirring rates are the dislodgement of the drop by the sample solution or splashing when using headspace. Vibration and vortex stirring, used with some autosamplers, are also effective, with the limitation that the agitation cannot occur while the drop is exposed at the needle tip. vi) Extraction time: extraction efficiency increases with longer extraction times in most of SDME techniques. The extraction time should be enough to extract an adequate amount of analyte by the microdrops. Times between 5 and 45 min are commonly used, longer times may cause drop dissolution.

Despite its simplicity, easy implementation, and low cost, SDME techniques have some limitations, for example: i) direct immersion requires careful and intricate manual operation because of problems of drop dislodgment and instability; ii) complex matrices requires a pretreatment or extra filtration step; iii) sensitivity and precision of SDME methods even acceptable need further improvement. The main issue lies with the adverse consequences of prolonged extraction time and fast stirring rate, since they may result in drop dissolution and/or dislodgement; and iv) SDME is not yet suitable as routinely applicable online preconcentration procedure (Xu et al., 2007).

2.1.3 Hollow-fiber liquid-phase microextraction (HP-LPME)

Pedersen-Bjergaard & Rasmussen introduced hollow-fiber based liquid-phase microextraction (HP-LPME) in 1999, to improve the stability and reliability of SDME techniques (Pedersen-Bjergaard & Rasmussen, 1999). In HP-LPME the extracting phase was placed inside the lumen of a porous polypropylene hollow fiber. The fiber had a porosity of 70% with a pore size of 0.2 μm , a wall thickness of 200 μm and an internal diameter of 600 μm . A supported liquid membrane was formed by dipping the hollow fiber into a suitable organic solvent. The solvent penetrates the pores of the hollow fiber and bound by capillary forces to the polypropylene network comprising the fiber wall. The high porosity enabled immobilization of a considerable volume of solvent as a thin film, e.g. a 1 cm length of the fiber was able to immobilize ca. 8 μL of solvent as a 200 μm film within the polypropylene network. The extracting phase (acceptor solution) which was placed into the lumen of the fiber was mechanically protected inside the hollow fiber and it was separated from the sample by the supported liquid membrane (organic solvent), thus preventing its dissolution into the aqueous sample. In LPME (HP-LPME), analytes are extracted from an aqueous sample, through the organic solvent immobilized as supported liquid membrane (SLM), into the acceptor solution placed inside the lumen of the hollow fiber. Subsequently, the acceptor solution is removed by a micro-syringe and further analyzed (Pedersen-Bjergaard & Rasmussen, 2008). Chemical principles of HP-LPME are similar to those employed in supported liquid membrane (SLM), but the techniques differ in terms of instrumentation and operation.

According to the analyte to be extracted, HP-LPME can be performed either in two-phase or three-phase modes. In the two-phase LPME sampling mode, analyte is extracted from an aqueous sample (donor phase) through a water-immiscible solvent immobilized in the pores of the hollow fiber into the organic solvent (acceptor phase) present inside the hollow fiber (Figure 2). In the three-phase LPME sampling mode, analyte is extracted from an aqueous solution (donor phase) through the organic solvent immobilized in the pores of the hollow

Table 1. Applications of SDME in the extraction of pesticide residues.

Analytes	Sample	Extractant solvent	Volume of organic solvent (μL)	Stiring speed (rpm)	Extraction time (min)	Detector	LOD/LOQ
Organochlorine (1) Triazole (1) Azole (2)	Water (river) and wine (red wine)	Toluene	1.6	800	30	GC-ECD	LOD: 0.0009-0.0015 $\mu\text{g/L}$ LOQ: n.r.
Organophosphosphate (7)	Juice (orange)	Toluene	1.6	400	15	GC-FPD	LOD: 0.98-2.20 $\mu\text{g/L}$ LOQ: n.r.
Organophosphosphate (6)	Water (lake) and juices (apple, orange and pear)	Toluene	1.5	600	20	GC-FPD	LOD: 0.21-0.56 $\mu\text{g/L}$ LOQ: n.r.
Organophosphosphate (13)	Water (farm, river and well)	Carbon tetrachloride	0.9	1300	40	GC-FPD	LOD: 0.002-0.020 $\mu\text{g/L}$ LOQ: n.r.
Triazine (1) Organophosphosphate (2) Thiadiazole (1) Benzanilide (1)	Water	Carbon tetrachloride	3.0	n.a.	10	HPLC-UV	LOD: 0.6-4.0 $\mu\text{g/L}$ LOQ: n.r.
Organochlorine (9)	Vegetable (cabbage, cauliflower, Chinese cabbage)	Acetone:p xylene (2:8v/v)	1.0	400	30	GC-MS	LOD: 0.05-0.20 $\mu\text{g/L}$ LOQ: n.r.
Organophosphosphate (9) Anilinopyrimidine (1) Dicarboximide (1) Triadiazine (1) Strobilurin (1) Juvenile hormone mimic (1)	Vegetables (courgette, tomato)	Toluene	1.6	350	25	GC-NPD/EC D	LOD: 0.03-30 $\mu\text{g/L}$ LOQ: n.r.
Organochlorine (18)	Water and wastewater	Toluene	2.0	380	37	GC-MS	LOD: 0.022-0.10 $\mu\text{g/L}$ LOQ: 0.074-0.33 $\mu\text{g/L}$

EF, enrichment factor; n.r., not reported; n.a., no adjustment

fiber (organic phase) into another aqueous phase (acceptor phase) present inside the lumen of the hollow fiber (Figure 2). The organic phase serves in this case as a barrier between the acceptor and the donor aqueous solutions, preventing mixing of these two phases. Whereas two-phase mode has been mainly used for hydrophobic compounds, further analyzed by GC, three-phase mode has been preferably used for ionisable compounds, using LC or capillary electrophoresis (CE) as analytical techniques (Psillakis & Kalogerakis, 2003).

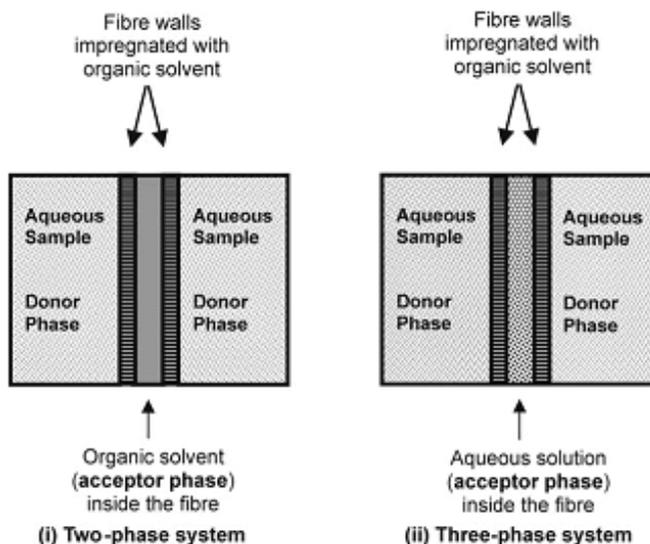


Fig. 2. Schematic illustration of 2- and 3-phase LPME (from Pedersen-Bjergaard & Rasmussen, 2008).

HP-LPME even providing high enrichment, an easy cleanup, low solvent consumption and making possible the direct analysis by chromatography of the acceptor phase requires long extraction times, which is perhaps the major disadvantage of the technique. Normally, extraction time range between 15 and 45 min for sample volumes below 2 mL, whereas for 1 L samples even 2 h may be required to reach equilibrium (Pedersen-Bjergaard & Rasmussen, 2008).

Recently, some proposals have been made in order to speed up the throughput of the procedure, either by treating many samples in parallel, carrying out the extraction under non-equilibrium condition (Ho et al. 2002), or using the so called dynamic hollow fiber protected liquid phase microextraction (DHFP-LPME). The latter technique was successful applied by Huang & Huang (2006) in the extraction of OCPs from green tea leaves and ready-to-drink tea prior to GC-ECD analysis. In this work, six OCPs (heptachlor, aldrin, endosulfan, *p,p'*-DDE, dieldrin and *o,p'*-DDT) were extracted and concentrated to a volume of 3 μ L of organic extracting solvent (1-octanol) confined within a 1.5 cm length of hollow fiber. The effects of extractant solvent, extraction time and temperature, sample agitation, plunger speed, and salt concentration on the extraction performance were investigated. Good enrichments were achieved (34–297 fold) with this method, and good repeatabilities of extraction were obtained, with RSDs below 12.57%. Detection limits were below 1 μ g/L for ready-to-drink tea and below 1 μ g/g for green tea leaves. The application of HP-LPME to a

large number of pesticides representatives of several chemical classes was reported by Bolaños et al. 2008. In this study 50 pesticides were extracted from alcoholic beverages (wine and beer) to a volume of 5 μL of organic extracting solvent (1-octanol) confined within a 2 cm length of hollow fiber followed by ultra-high pressure liquid chromatography coupled to tandem mass spectrometry (UHPLC-MS/MS), without any further clean-up step. Using optimized conditions, low detection limits (0.01–5.6 $\mu\text{g/L}$) and acceptable linearity ($R^2 > 0.95$) were obtained. Recently, a liquid-phase microextraction (LPME) based on polypropylene hollow fiber was evaluated for the extraction of the fungicides (thiabendazole, carbendazim and imazalil) from orange juices (Barahona et al., 2010). Each sample aliquot (3 mL) was previously alkalized with NaOH until reach a pH of 10–11, and the analytes were further extracted through a supported liquid membrane (SLM) of 2-octanone into 20 μL of a stagnant aqueous solution of 10 mM HCl inside the lumen of the hollow fibre. Subsequently, the acceptor solution was directly subjected to analysis by LC-MS and capillary electrophoresis (CE). The LC-MS provided better sensitivity than CE allowing a LODs below 0.1 $\mu\text{g/L}$.

As described in the works above mentioned several parameters should be optimized in order to obtain the maximum efficiency such as i) fiber, ii) organic solvent, iii) extraction time, iv) temperature, v) agitation, vi) ionic strength and vii) pH (Psillakis & Kalogerakis, 2003).

- i) Fiber: the fiber should be hydrophobic and compatible with the organic solvents used. Such requirements are met by fibers based on polypropylene; most of them have 600 μm of inner diameter, compatible with the volumes (μL) of the acceptor solution required for microextraction.
- ii) Organic solvent: a fundamental step in the optimization of the LPME methods is the selection of the organic solvent. Some properties need to be considered in their choice including: water-immiscibility, to prevent the organic phase dissolution in the aqueous (donor) phase; low volatility, to avoid organic phase loss during extraction; compatibility with the fiber used; easy immobilization within the pores of the hollow fiber; and high solubility for target analytes.
- iii) Extraction time: mass-transfer is a time-dependent process, increasing with the time of extraction. In practice to ensure high sample throughputs sampling times are shorter than the total chromatographic run time.
- iv) Agitation: agitation of the sample is routinely applied to accelerate the extraction kinetics. Increasing the agitation rate of the donor solution enhances extraction, the diffusion of analytes through the interfacial layer of the hollow fiber is facilitated, and the repeatability of the extraction method is improved.
- v) Temperature: with increasing temperature, the diffusion coefficients also increase in response to decreased viscosity. Thus the time required to reach equilibrium decrease. On the other hand, partition coefficients for the acceptor phase decrease, reducing the amount of analyte extracted. Therefore, the speed of extraction could be improved at costs of a loss of sensitivity. Typically, LPME is performed at room temperature in order to avoid possible bubbles problems and evaporation of the solvent during extraction, since the amount of solvent used is very small (20 μL).
- vi) Ionic strength: depending on the nature of the target analytes, addition of salt to the sample solution can decrease their solubility and therefore enhance extraction because of the salting-out effect, in particular for polar analytes. Among the salts mainly used sodium chloride is the most common.
- vii) pH: sample pH is crucial for efficient extraction of acidic and basic analytes. pH adjusting results in a greater ratio of distribution, ensures high enrichment factors and high recovery of the analytes of interest. Adjustments in pH can increase the extraction efficiency, since both the balance dissociation and the solubility of acids and bases are directly affected by sample pH.

HP-LPME provides in general an acceptable sensitivity in the analysis of pesticide residues. However, extraction procedure requires the presence of the analytes in liquid solutions, being its application usually restricted to liquid samples. Moreover the technique is difficult or even impossible to automate, the time of extraction could be considered too long and the operator skills should be high in order to get reproducible results.

2.1.4 Dispersive liquid-liquid microextraction (DLLME)

Dispersive liquid-liquid microextraction (DLLME) was developed by Assai and co-workers in 2006 (Rezaee et al., 2006). Consists in the rapid addition to an aqueous sample (in a conical test tube) of a mixture of two selected solvents (few microliters of a water-immiscible high density extractant solvent jointly with a dispersive solvent with high miscibility in both extractant and water phases). The aim is to form a cloudy solution of small droplets of extractant solvent which are dispersed throughout the aqueous phase. In consequence of the very large surface area formed between the two phases, hydrophobic solutes are rapidly and efficiently enriched in the extractant solvent and, after centrifugation, they can be determined in the phase settled at the bottom of the tube. The resultant sedimented phase is read for direct analysis by GC or LC.

Since its introduction, DLLME has gained popularity as a simple, fast and reliable tool for sampling preparation of a variety of analytes, as can be seen in recent reviews (Xiao-Huan et al., 2009; Ojeda & Rojas, 2009; Rezaee et al. 2010; Herrera-Herrera et al., 2010). DLLME has extensively been used for direct extraction of pesticides from aqueous samples such water, fruit juice and wine (Table 2). The first study using DLLME in pesticide residues was applied in the extraction of 13 OPPs (phorate, diazinon, disolfotane, methyl parathion, sumithion, chloropyrifos, malathion, fenthion, profenphose, ethion, phosalone, azinphose-methyl, co-ral) from river water (Berijani et al., 2006). In this study a mixture of 12.0 μL of chlorobenzene (extractant solvent) and 1.00 mL of acetone (dispersive solvent) was rapidly injected in 5 mL of aqueous sample. The sedimented phase (about 5 μL) collected after centrifugation (2 min at 5000 rpm) was analyzed by GC-FPD. Some important parameters, such as kind of extractant and dispersive solvents and their volumes, extraction time, temperature and salt effect were investigated. Under the optimized conditions, enrichment factors and extraction recoveries were high, ranging between 789–1070 and 78.9–107%, respectively. LODs ranged between 3 and 20 pg/mL for most of the analytes. Other classes of pesticides were extracted by DLLME from water such as triazine herbicides, amide herbicides, phenylurea herbicides, organochlorines, pyrethroids and carbamates (Table 2). In most of the reported studies only one chemical class of pesticides was evaluated, being the number of pesticide residues scarce (less than eighteen analytes). However, in a recent publication different classes of pesticides namely triazole fungicides, isoxazolidinone herbicides and carbamates were simultaneously evaluated, although the number of analytes pertaining at each class has been reduced (three) (Caldas et al., 2010). After optimization of the parameters that influence the extraction efficiency, such as the type and volume of the dispersive and extractant solvents, extraction time, speed of centrifugation, pH and addition of salt, the extraction of pesticide residues from 5 mL of water was achieved with a mixture of 2.0 mL acetonitrile (dispersive solvent) containing 60 μL of carbon tetrachloride (extractant solvent), followed by centrifugation at 2000 rpm for 5 min; the analysis was performed by LC-MS/MS. The recoveries of pesticides in water at spiking levels between 0.02 and 2.0 $\mu\text{g/L}$ ranged from 62.7% to 120.0%. RSDs varied between 1.9% and 9.1%. LOQs of the method considering a 50-fold preconcentration step were 0.02 $\mu\text{g/L}$. The LODs of the method were not reported in this study.

The application of the DLLME procedure in the extraction of pesticide residues in food samples is reported in only few papers, probably due to the complexity of food matrices (Table 2). Montes et al. (2009) used DLLME for preconcentration of seven fungicides (metalaxyl-M, penconazole, folpet, diniconazole, propiconazole, difenoconazole and azoxystrobin) in wine samples after extraction with SPE. A direct use of DLLME as extraction procedure followed by GC-MS analysis was performed by Cunha et al. (2009) to determine 24 pesticide residues, belonging at eight different chemical classes, in juice fruits. In order to avoid the precipitation of some components of the matrix, which make unsuitable the application of DLLME as referred by Montes et al (2009), samples were centrifuged prior extraction. As can be seen in Figure 3, the optimized DLLME procedure

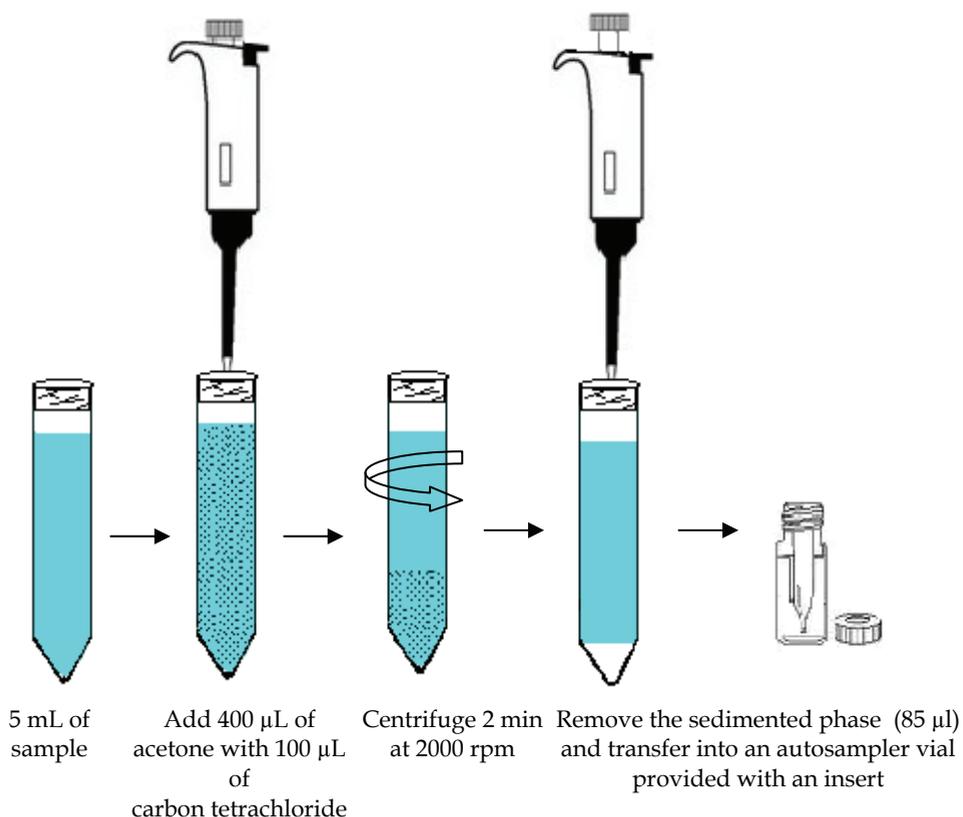


Fig. 3. Diagram of the dispersive liquid-liquid microextraction procedure used by Cunha et al. (2009).

consisted in the formation of a cloudy solution promoted by the fast addition to the sample (5 mL) of a mixture of carbon tetrachloride (extractant solvent, 100 μL) and acetone (dispersive solvent, 400 μL). The tiny droplets formed and dispersed were sedimented (85 μL) in the bottom of the conical test tube after centrifugation at 2000 rpm for 2 min. More than the parameters that influence the extraction efficiency of DLLME such as type and

volume of extractant solvent, type and volume of dispersive solvent and salt addition, other factors that could restrict the analytical performance, such as matrix effects or robustness of the method were evaluated according the Sanco guidelines (2007). Under the optimized conditions mean recoveries for apple juice spiked at three concentration levels ranged from 60% to 105% and the intra-repeatability ranged from 1% to 21%. The LODs of the 24 pesticides ranged from 0.06 to 2.20 $\mu\text{g/L}$. In 2 of a total of 28 analysed fruit juice samples residues of captan were found, although at levels below the maximum legal limit established by European Union (Figure 4).

DLLME is more suitable for the extraction of analytes from aqueous samples; nonetheless, some authors have applied this process in solid samples after an adequate pretreatment. Zhao et al. (2007) applied DLLME as a concentration procedure after a previous extraction with QuEChERS of OPPs (ethoprophos, parathion methyl, fenitrothion, malathion, chlorpyrifos and profenofos) from watermelon and cucumber. Hence, 1 mL of the extract obtained after homogenization of 10 g of sample with 10 mL of acetonitrile, 4 g MgSO_4 , and 1 g NaCl, was added with 27 μL of chlorobenzene and rapidly injected in 5 mL of water. Then 1 μL of 18 μL of sedimented phase obtained by centrifugation of the mixture at 4000 rpm for 3 min was analyzed by GC-FPD. The optimized method allowed recoveries between 67 and 111%, repeatability between 2 and 9% and LODs ranging from 0.010 to 0.190 $\mu\text{g/kg}$, for all the target pesticides. In other study, Zang et al. (2008) applied the DLLME procedure directly in the extraction of captan, folpet and captafol from apples. The developed procedure consisted in the injection of a mixture containing chlorobenzene (extractive), and acetone (dispersive) directly into an aqueous extract of apple samples, obtained after homogenization with a solution of zinc acetate dehydrate and dilution with water. Under the optimum conditions, high enrichment factors for the targets were achieved ranging from 824 to 912. The recoveries of fungicides in apples ranged from 93.0 to 109.5% and the RSD ranged from 3.8 to 4.9%. The LODs were between 3.0 and 8.0 $\mu\text{g/kg}$.

To date, the majority of the applications related to DLLME involve the use of solvents of high, density commonly chlorinated solvents (e.g. chlorobenzene, carbon tetrachloride and tetrachloroethylene) as extractant solvents. However, the use of ionic liquids (IL) as extractants has been found to be especially important in DLLME as well as in other microextraction procedures (in order to replace the volatile ones used during sample preparation procedures) because of their negligible vapor pressure, good solubility for organic and inorganic compounds, no flammability, high thermal stability, wide temperature range as a liquid phase, etc. (Han & Armstrong, 2007; Ravelo-Pérez et al., 2009). One of the main drawback of the use of IL in DLLME is the impossibility to make use of GC in the analysis, due to the adverse effects of these solvents in the chromatographic system. IL-DLLME has been applied in the extraction of a high variety of pesticides in water and food matrices such as fruits and honey, as can be seen in Table 2. DLLME based on IL was initially applied by Zhou et al. (2008a), to extract five pyrethroid pesticides (cyhalothrin, deltamethrin, fenvalerate, taufluvinate and biphenthrin) in different types of water samples (tap, river and reservoir water, and groundwater). In this study, the sample (10 mL) was heated at 80 $^{\circ}\text{C}$ after addition of 45 μL of 1-hexyl-3-methylimidazolium hexafluorophosphate [C6MIM][PF6]. The IL mixed with the solution entirely at this temperature and thereafter the solution was cooled with ice-water for a certain time. The IL and the aqueous phase were separated after centrifugation and the IL phase injected into the

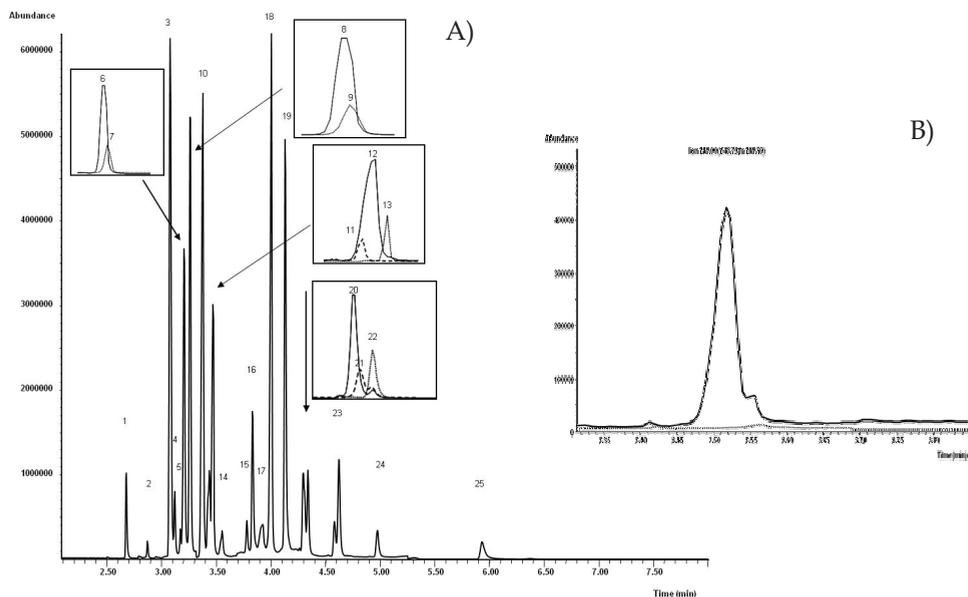


Fig. 4.A) Chromatogram of spiked blank apple juice with 24 OPPs B) Overlay of extracted ion chromatograms in SIM mode for captan (ion 149) obtained in not contaminated (---) and contaminated (—) (0.541 $\mu\text{g/L}$) apple juice samples using DLLEM extraction and MDGC-MS analysis (from Cunha et al., 2009).

HPLC-UV. In this study good recoveries were obtained (76.7– 135.6%) and LODs were in the range 0.28–0.6 $\mu\text{g/L}$. In a further work, the same group used a similar procedure, using [C6MIM][PF6] as extractant solvent in DLLME at 80°C for determine traces of methylparathion and phoxim in water (Zhou et al., 2008b). A new IL-DLLME procedure was introduced by Liu et al. (2009) for the extraction of four insecticides (fipronil, chlorfenapyr, buprofezin, and hexythiazox) from water. The proposed procedure combined extraction and concentration of the analytes into one step, avoiding heating and cooling steps, so reducing extraction time. Thus, a mixture of 0.052 g [C6MIM][PF6] and 0.50 mL methanol (dispersive solvent) was quickly injected into the sample (5.0 mL). Then, the mixture was centrifugated at 4000 rpm for 10.0 min, and 19 μL of sedimented phase were diluted with 50 μL methanol and 10 μL of the mixture analysed by HPLC-UV. Under the optimized conditions, good enrichment factors (209–276) and accepted recoveries (79–110%) were obtained for the extraction of the target analytes in water samples. The LODs for the four insecticides ranged from 0.53 to 1.28 $\mu\text{g/L}$.

The application of IL-DLLME to solid samples is scarce as referred above for the classical DLLME. Usually, it is necessary a previous pretreatment of the sample in order to obtain an aqueous extract before extraction. In a recent work Wang et al. (2010) developed an IL-DLLME/HPLC-UV method for the extraction and determination of triazines in honey. A mixture of 175 μL of [C6MIM][PF6] (extractant solvent) and 50 μL of 10% Triton X 114 (dispersive solvent) was rapidly injected into 20 mL aqueous honey sample, obtained by dissolution of 2 g of honey with 20 mL of water. The detection limits for chlortoluron,

prometon, propazine, linuron and prebane were 6.92, 5.84, 8.55, 8.59 and 5.31 $\mu\text{g}/\text{kg}$, respectively.

Another type of extractant solvents used in DLLME are low density solvents such as undecanol, 1-dodecanol, 2-dodecanol and n-hexadecane, which are usually less toxic than the chlorinated solvents. An interesting work was developed by Leong & Huang (2009) for the determination of OCPs in water samples. The method is based on the solidification of a floating organic drop (DLLME-SFO) and it is combined with GC-ECD. The dispersive solvent (200 μL of acetonitrile) containing 10 μL of hexadecane (HEX) was rapidly injected into 5.0 mL water sample. After centrifugation, the fine HEX droplets ($6\pm 0.5 \mu\text{L}$) floating at the top of the screw-capped tube were solidified through ice and then transferred into a vial to be injected into GC. Under optimum conditions, enrichment factors and extraction recoveries are high ranging between 37–872 and 82.9–102.5%, respectively. LODs ranged between 0.011 and 0.110 $\mu\text{g}/\text{L}$ for most of the analytes. Recently Chen et al. (2010) reported a low-density extractant solvent-based, termed solvent terminated (ST) DLLME to determine carbamate pesticides (carbofuran, tsumacide, isoprocarb, and pirimicarb) in water by GC-MS/MS. Hence, 0.50 mL of acetonitrile containing 15 μL of toluene were rapidly injected in 5 mL of water. After dispersing, the obtained emulsion was quickly cleared into two phases when an aliquot of acetonitrile (0.5 mL) was introduced as a chemical demulsifier into the aqueous bulk. Therefore, the developed procedure does not need centrifugation to achieve phase separation. Under the optimized conditions, the LODs for all the target carbamate pesticides were in the range of 0.001–0.50 $\mu\text{g}/\text{L}$ and the precisions were in the range of 2.3–6.8%.

In order to achieve such a wide range of applications, several parameters have to be taken into account to optimize DLLME to extract pesticide residues, such as i) type and volume of extractant solvent, ii) type and volume of dispersive solvent, iii) extraction time, and iv) effect of salt addition. i) Extractant solvent: the extractant solvents should be immiscible with water, and they must possess both good solubility for analytes and good chromatographic behavior. They can either have higher or lower density than water and the volume used ranged between 10 to 100 μL . Lower volumes of extractant solvent enhance enrichment factor, although reducing the volume of sedimented phase, could give problems of reproducibility. ii) Dispersive solvent: the dispersive solvent should be miscible with both aqueous sample and extractant solvent and possess the capacity to decrease the interfacial tension of extractant solvent in order to make the droplet size smaller, increasing the extraction efficiency. Acetone, methanol and acetonitrile can be used as dispersive solvents at volumes ranging from 0.5 mL to 2 mL. iii) Extraction time: in DLLME after mixture of the three components (sample, extractant and dispersive solvent) the equilibrium is achieved in few seconds due to the large contact surface between tiny drops of extractant solvent and the sample. Nevertheless, in most of the studies the extraction time ranged from 1 to 5 min. iv) Salt addition: salt addition can improve extraction yield in DLLME, particularly for those analytes with lower solubility, as a result of a “salting out” effect. This effect is prevailing in DLLME when NaCl is employed.

DLLME has generally showed a very good performance to extract pesticide residues from water and aqueous extracts of food samples, but it is desirable to extend this application to more complex matrices and to a large number of pesticide residues using standard guidelines for the validation of the methods.

Table 2. Applications of DLLME in the extraction of pesticide residues

Analytes	Sample	Extractant solvent	Dispersive solvent	Detector	LOD/LOQ	Re
Organophosphate (13)	Water (river and farm)	Chlorobenzene (12 μ L)	Acetone (1.0 mL)	GC-FPD	LOD: 0.003-0.020 μ g/L LOQ: n.r.	9
Organophosphate (6)	Watermelon and cucumber	Chlorobenzene (27 μ L)	Acetonitrile (1.0 mL)	GC-FPD	LOD: 0.5-20 μ g/kg LOQ: n.r.	6
N-methylcarbamate (1)	Water (river and lake)	Tetrachloroethane (20 μ L)	Methanol (0.5 mL)	HPLC-UV	LOD: 1.0 μ g/L LOQ: n.r.	9
Organophosphate (1) Phthalimide (1) Carboximide (1)	Apples	Chlorobenzene (9 μ L)	Acetone (1.0 mL)	GC-ECD	LOD: 3.0-8.0 μ g/kg LOQ: n.r.	9
Pyrethroid (3)	Water (river, well and tap)	Chlorobenzene (15 μ L)	Acetone (1 mL)	GC-ECD	LOD: 0.04-0.10 μ g/L LOQ: n.r.	7
Pyrethroid (5)	Water (tap, river, reservoir and ground)	[C ₆ MIM][PF ₆] (45 μ L)	n.a.	HLPC-UV	LOD: 0.28-0.6 μ g/L LOQ: n.r.	7
Organophosphate (2)	Water (rain, river, reservoir and ground)	[C ₆ MIM][PF ₆] (50 μ L)	n.a.	HLPC-UV	LOD: 0.17-0.29 μ g/L LOQ: n.r.	8
Organophosphate (5) Carbamate (1)	Water (tap, lake, river, well and farm) green tea and tea leaves	Carbon tetrachloride (10 μ L)	Methanol (0.8 mL)	GC-FPD	LOD: 0.21-3.05 μ g/L LOQ: n.r.	7

Table 2. Applications of DLLME in extraction of pesticide residue (cont.)

Analytes	Sample	Extractant solvent	Dispersive solvent	Detector	LOD/LOQ	Re
Organophosphate (13) Pyrethroid (3) Phthalimide (1) Dicarboximide (1) Phenylamide (1) Cyclodiene (1) Anilino-pyrimidine (1) Strobilurin (1)	Juice (apple)	Carbon tetrachloride (100 μ L)	Acetone (0.4 mL)	GC-MS	LOD: 0.06-2.20 μ g/L LOQ: 0.2-7.3 μ g/L	6
Carbamate (5)	Water (surface)	Trichloromethane (40 μ L)	Acetonitrile (1 mL)	HPLC-DAD	LOD: 0.1-0.4 μ g/L LOQ: n.r.	8
Phenyurea (8)	Water (river, tap and well)	Carbon disulfide (103 μ L) and toluene (45 μ L)	Acetone (2 mL)	HPLC- UV/DAD	LOD: 0.01-0.5 μ g/L LOQ: n.r.	8
Carbamate (1) Organophosphate (1)	Water (river and tap) and juice (apple, peach and grape)	Tetrachloroethane (15 μ L)	Acetonitrile (1.0 mL)	HPLC-FLD	LOD: 0.0123-0.016 μ g/L LOQ: n.r.	8
Organochlorine (5)	Water (river, sea and reservoir)	Tetrachloroethylene (5.2 μ L)	tert-butyl methyl ether (7.8 μ L)	GS-MS	LOD: 0.0004-0.0025 μ g/L LOQ: n.r.	5
Pyrazole (1) Thiazolidine (2) Pyrrole (1)	Water (tap, lake and fountain)	[C ₆ MIM][PF ₆] (0.052 g)	Methanol (0.5 mL)	HPLC-DAD	LOD: 0.53-1.28 μ g/L LOQ: n.r.	7
Organophosphate (4)	Water (river, tap, rain and well)	[C ₆ MIM][PF ₆] (35 μ L)	Methanol (1.0 mL)	HPLC-UV	LOD: 0.1- 5.0 μ g/L LOQ: n.r.	8

Table 2. Applications of DLLME in extraction of pesticide residue (cont.)

Analytes	Sample	Extractant solvent	Dispersive solvent	Detector	LOD/LOQ	Re
Benzimidazole (1) Carbamate (2) Dicarboximide (1) Quinazoline (1) Triazole (1) Diphenyl ether (1) Thiazolidine (1)	Bananas	[C ₆ MIM][PF ₆] (88 mg)	Methanol (0.714 mL)	HPLC-DAD	LOD: 0.320-4.66 µg/kg LOQ: n.r.	5
Organochlorine (18)	Water (river, surface and tap) and wastewater	Tetrachloroethylene (10 µL)	Acetone (1.0 mL)	GC-MS	LOD: 0.001-0.025 µg/L LOQ: n.r.	5
Organophosphate (1) Strobilurin (1) Phenylamide (1) Triazole (1) Conazole (1) Azole (1)	Wine (red and white)	1,1,1-trichloroethane (100 µL)	Acetone (1 mL)	GS-MS	LOD: n.r. LOQ: 0.020-0.020 µg/L	7
Organochlorine (6)	Water (lake and tap)	Hexadecane (10 µL)	Acetonitrile (0.2 mL)	GC-ECD	LOD: 0.011-0.109 µg/L LOQ: n.r.	8
Organophosphate (10)	Tea	N-hexane (24 µL)	Acetonitrile (0.5 mL)	GC-FPD	LODs: 0.030-1.00 µg/kg LOQ: n.r.	8
Triazole (2) Carbamate (1)	Water (tap)	Carbon tetrachloride (60 µL)	Acetonitrile (2.0 mL)	LC-MS/MS	LOD: n.r. LOQ: 0.02 µg/L	6
Carbamate (4)	Water (lake)	Toluene (50 µL)	Acetonitrile (1.0 mL)	GS-MS	LOD: 0.001-0.050 µg/L LOQ: n.r.	9
Triazine (5)	Honey	[C ₆ MIM][PF ₆] (175 µL) and 10% Triton X 114 (50 µL)	n.a	HPLC-DAD	LOD: 5.31-8.59 µg/kg LOQ: n.r.	6

EF, enrichment factor; n.r., not reported; n.a. no adjustment

2.2. Analysis

The determination of pesticide residues in water and food matrices has traditionally been performed by GC, due the high number of theoretical plates of the columns employed and the variety and selectivity capabilities of the detectors than can be coupled such as ECD, NPD, and FPD. Among the detectors used, MS is the preferred tool for determination of multi class pesticide residues because it permits: i) the simultaneous quantification and identification of detected analytes; ii) the detection of a wide range of analytes independently of its elemental composition; iii) mass-spectrometric resolution of co-eluting peaks; and iv) potentially faster analysis time (Cunha et al., 2010).

To increase sample throughput during GC analysis, which would consequently reduce the laboratory operating costs, several approaches were evaluated such as the reduction of: column length, column inner diameter or column stationary film thickness; and the utilization of fast temperature programming, low-pressure and multicapillary columns (Maštovská & Lehotay 2003). In practice a combination of two or more approaches is very often applied to enhance the speeding-up effect with the less sacrifice in sample capacity and/or separation efficiency. Sample capacity influences the limit of detection and the sensitivity, for example. Separation efficiency influences performance characteristics such as selectivity, detection limit (through the level of chemical noise) and, of course, accuracy of the analytical results. Multidimensional GC system with Deans switch heart-cutting represents a very interesting technical solution, which not only responds adequately to the demand of increased speed of analysis, capacity and separation efficiency, but also provided an enhancement in robustness. This technique is based essentially on the transfer of selected effluent fractions from a first to a second column for MS analysis and transfer of fractions without analytical interest to a restrictor column for waste (see Figure 5) (Cunha et al., 2009; Cunha & Fernandes, 2010). A devoted transfer device (Deans switch), situated between the two columns, enables the entire procedure.

Recently a dual GC column system involving a short wide-bore capillary column connected by a Deans switch device to a narrower and longer second chromatographic column was successful applied in determination of 24 pesticide residues in fruit juice (Cunha et al., 2009). This system allowed a gain in the speed of chromatographic analysis, providing an efficient sample injection and column introduction of the analytes with limited interferences, high sample capacity, and sharp and symmetric peak shapes without loss of resolution.

Notwithstanding the recent advances in GC-MS systems, the analysis of polar, non-volatile or/and thermally labile pesticides by this technique is limited, usually requiring chemical derivatization. LC-MS/MS has become a standard approach in developed countries to expand the range of pesticides quantified and identified in complex matrices.

3. Conclusions

Microextraction methods usually require both smaller sample size and organic solvent volumes when compared with the conventional methods. The main advantages of these procedures are the high degree of enrichment for the analytes in complex matrices, which enable detection limits down to the levels required by the regulatory bodies to the analysis of pesticide residues in water and food. Additionally, given the compatibility of the solvents used, and the low volumes involved, the procedures are easily associated with gas or liquid chromatography. Most of microextraction applications are employed in aqueous samples for the extraction of nonpolar or moderately polar high molecular weight analytes. Although

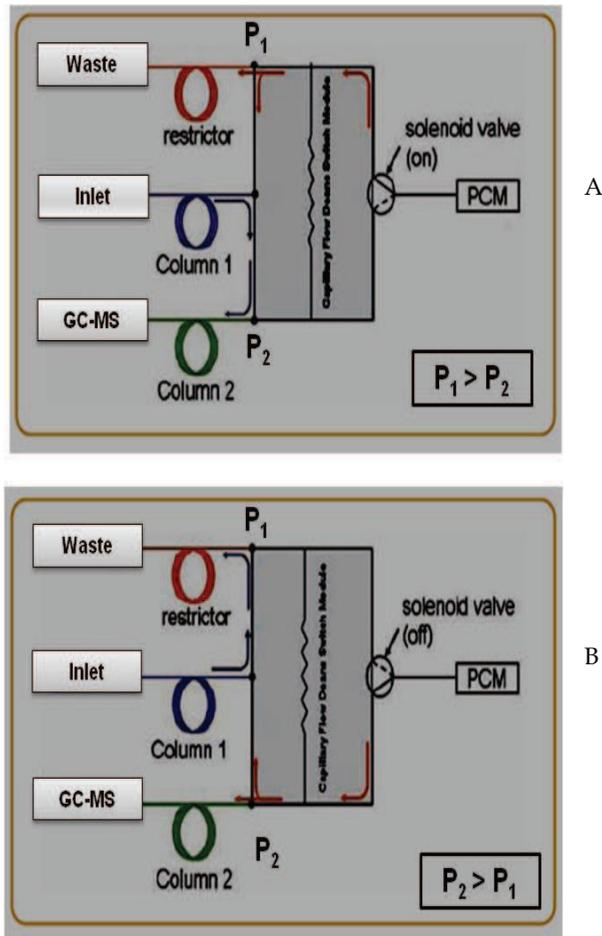


Fig. 5. Deans switch GC-MS system. (A) The solenoid valve is in the on position, allowing effluent to flow to the 2D GC separation column prior to MS detection. (B) The solenoid valve is in the off position and effluent from the primary column is flowing to the exit gas line. (Adapted from Agilent).

some attempts were made for the extraction of analytes in solid matrices and also for the extraction of polar analytes, is still expected an increment along this line in the future. On other hand, despite their high-throughput, the automation of most of microextraction procedures presented seems to be very difficult and has not yet been achieved, thus new developments in this area are required.

4. Acknowledgments

S.C.C. is grateful to "POPH-QREN- Tipologia 4.2, Fondo Social Europeu e Fondo Nacional MCTES". This research was supported by grant from the FCT project "PTDC/AGR-ALI/101583/2008" and Compete/ FEDER.

5. References

- Ahmadi, F.; Assadi, Y.; Hosseini, S.M.R. M. & Rezaee, M. (2006). Determination of organophosphorus pesticides in water samples by single drop microextraction and gas chromatography-flame photometric detector. *Journal of Chromatography A*, 1101, 1-2, 307-312.
- Amvrazi, E.G. & Tsiropoulos, N.G. (2009). Application of single-drop microextraction coupled with gas chromatography for the determination of multiclass pesticides in vegetables with nitrogen phosphorus and electron capture detection. *Journal of Chromatography A*, 1216, 1-2, 2789-2797.
- Anastassiades, M.; Lehotay, S.J.; Štajnbaher, D. & Schenck, F.J. (2003). Fast and easy multiresidue method employing acetonitrile extraction/partitioning and dispersive solid-phase extraction for the determination of pesticide residues in produce. *Journal AOAC Internacional*, 86, 2, 412-431.
- Barahona, F.; Gjelstad, A.; Pedersen-Bjergaard, S. & Rasmussen, K.E. (2010). Hollow fiber-liquid-phase microextraction of fungicides from orange juices. *Journal of Chromatography A*, 1217, 1-2, 1989-1994.
- Berijani, S.; Assadi, Y.; Anbia, M.; Hosseini M.R.M. & Aghaee, E. (2006). Dispersive liquid-liquid microextraction combined with gas chromatography-flame photometric detection. Very simple, rapid and sensitive method for the determination of organophosphorus pesticides in water. *Journal of Chromatography A*, 1123, 1-2, 1-9.
- Bolaños, P.P.; Romero-González, R.; Frenich, A.G. & Vidal, J.L.M. (2008). Application of hollow fiber liquid phase microextraction for the multiresidue determination of pesticides in alcoholic beverages by ultra-high pressure liquid chromatography coupled to tandem mass spectrometry. *Journal of Chromatography A*, 1208, 1-2, 16-24.
- Caldas, S.S.; Costa, F.P. & Primel, E.G. (2010). Validation of a method for determination of different classes of pesticides in aqueous samples by dispersive liquid-liquid microextraction with liquid chromatography-tandem mass spectrometric detection. *Analytica Chimica Acta*, 665, 55-62.
- Chen, H.; Chen, R. & Li, S. (2010). Low-density extraction solvent-based solvent terminated dispersive liquid-liquid microextraction combined with gas chromatography-

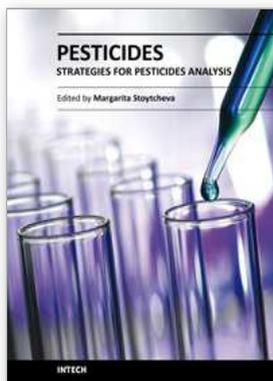
- tandem mass spectrometry for the determination of carbamate pesticides in water samples. *Journal of Chromatography A*, 1217, 1-2, 1244-1248.
- Cortada, C.; Vidal, L.; Tejada, S.; Romo A. & Canals, A. (2009a). Determination of organochlorine pesticides in complex matrices by single-drop microextraction coupled to gas chromatography-mass spectrometry. *Analytica Chimica Acta*, 638, 1, 29-35.
- Cortada, C.; Vidal, L.; Pastor, R.; Santiago, N. & Canals, A. (2009b). Determination of organochlorine pesticides in water samples by dispersive liquid-liquid microextraction coupled to gas chromatography-mass spectrometry. *Analytica Chimica Acta*, 649, 1, 218-221.
- Cunha, S.C. & Fernandes, J.O. (2010). Development and validation of a method based on a QuEChERS procedure and heart-cutting GC-MS for determination of five mycotoxins in cereal products. *Journal of Separation Science*, 33, 600-609.
- Cunha, S.C.; Fernandes, J.O. & Oliveira, M.B.P.P. (2009). Fast analysis of multiple pesticide residues in apple juice using dispersive liquid-liquid microextraction and multidimensional gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1216, 1-2, 8835-8844.
- Cunha, S.C.; Lehotay, S.J.; Mastovska, K.; Fernandes, J.O. & Oliveira, M.B.P.P. (2010). Sample preparation approaches for the analysis of pesticide residues in olives and olive oils. *Olives and Olive Oil in Health and Disease Prevention*, Editor Preedy V. R. & Watson R.R., Oxford: Academic Press, chap. 70 pp. 653-666.
- EN Standard Method EN 15662: Food of plant origin - determination of pesticide residues using GC-MS and/or LC-MS/MS following acetonitrile extraction/partitioning and clean-up by dispersive SPE - QuEChERS method (www.cen.eu accessed August 2010).
- Fu, L.; Liu, X.; Hu, J.; Zhao, X.; Wang, H. & Wang, X. (2009). Application of dispersive liquid-liquid microextraction for the analysis of triazophos and carbaryl pesticides in water and fruit juice samples. *Analytica Chimica Acta*, 632, 289-295.
- Han, X. & Armstrong, D.W. (2007). Ionic liquids in separation. *Accounts of Chemical Research*, 40, 1, 1079-1086.
- He, L.; Luo, X.; Xie, H.; Wang, C.; Jiang, X. & Lu, K. (2009). Ionic liquid-based dispersive liquid-liquid microextraction followed high-performance liquid chromatography for the determination of organophosphorus pesticides in water sample. *Analytica Chimica Acta*, 655, 52-59.
- He, Y. & Lee, H.K. (1997). Liquid-phase microextraction in a single drop of organic solvent by using a conventional microsyringe. *Analytical Chemistry*, 69, 15, 4634-4640.
- He, Y. & Lee, H.K. (2006). Continuous flow microextraction combined with high-performance liquid chromatography for the analysis of pesticides in natural waters. *Journal of Chromatography A*, 1122, 1-2, 7-12.
- Herrera-Herrera, A.; Asensio-Ramos, M.; Hernández-Borges, J. & Rodríguez-Delgado, M.A. (2010). Dispersive liquid-liquid microextraction for determination of organic analytes. *Trends in Analytical Chemistry*, 29, 7, 728-751.

- Hetmanski, M.T.; Fussell, R.; Godula, M. & Hübschmann, H.J. (2010). Rapid analysis of pesticides in difficult matrices using GC-MS-MS. *LCGC Europe*, July/August, 14-15.
- Ho, T.S.; Pedersen-Bjergaard, S. & Rasmussen, K.E. (2002). Liquid-phase microextraction of protein-bound drugs under non-equilibrium conditions. *Analytist*, 127, 608-613.
- Huang, S.P. & Huang, S.D. (2006). Dynamic hollow fiber protected liquid phase microextraction and quantification using gas chromatography combined with electron capture detection of organochlorine pesticides in green tea leaves and ready-to-drink tea. *Journal of Chromatography A*, 1135, 1-2, 6-11.
- Jeannot, M.A. & Cantwell, F.F. (1996). Solvent Microextraction into a Single Drop. *Analytical Chemistry*, 68, 13, 2236-2240.
- Jeannot, M.A. & Cantwell, F.F. (1997). Mass transfer characteristics of solvent extraction into a single drop at the tip of a syringe needle. *Analytical Chemistry*, 69, 2, 235-239.
- Jeannota, M.A.; Przyjazny, A. & Kokosa, J.M. (2010). Single drop microextraction—Development, applications and future trends. *Journal of Chromatography A*, 1217, 1-2, 2326-2336.
- Kin, C.M. & Huat, T.G. (2009). Comparison of HS-SDME with SPME and SPE for the determination of eight organochlorine and organophosphorus pesticide residues in food matrices. *Journal of Chromatography Science*, 47, 8, 694-699.
- Lehotay, S. J. (2007). Determination of pesticide residues in foods by acetonitrile extraction and partitioning with magnesium sulfate: collaborative study. *Journal AOAC Int.*, 90, 485-520.
- Leong, M.-I. & Huang, S.-D. (2009). Dispersive liquid-liquid microextraction method based on solidification of floating organic drop for extraction of organochlorine pesticides in water samples. *Journal of Chromatography A*, 1216, 1-2, 7645-7650.
- Liu, H. & Dasgupta, P.K. (1996). Analytical Chemistry in a drop. solvent extraction in a microdrop. *Analytical Chemistry*, 68, 11, 1817-2821.
- Liu, Y.; Hashi, Y. & Lin, J.M. (2007). Continuous-flow microextraction and gas chromatographic-mass spectrometric determination of polycyclic aromatic hydrocarbon compounds in water. *Analytica Chimica Acta*, 585, 2, 294-299.
- Liu, Y.; Zhao, E. & Zhou, Z. (2006). Single-Drop microextraction and gas chromatographic determination of fungicide in water and wine samples. *Analytical Letters* 39, 11, 2333-2344.
- Liu, Y.; Zhao, E., Zhu, W.; Gao, H. & Zhou, Z. (2009a). Determination of four heterocyclic insecticides by ionic liquid dispersive liquid-liquid microextraction in water samples. *Journal of Chromatography A*, 1216, 1-2, 885-891.
- Liu, Z.M.; Zang, X.H.; Liu, W.H.; Wang, C. & Wang, Z. (2009b). Novel method for the determination of five carbamate pesticides in water samples by dispersive liquid-liquid microextraction combined with high performance liquid chromatography. *Chinese Chemical Letters*, 20, 213-216.
- Martínez, R.C.; Gonzalo, E.R.; Cordero, B.M.; Pavón, J.L.P.; Pinto, C. G. & Laespada, E.F. (2000). Surfactant cloud point extraction and preconcentration of organic compounds prior to chromatography and capillary electrophoresis. *Journal of Chromatography A*, 902, 1-2, 251-265.

- Maštovská, K. & Lehotay, S.J. (2003). Practical approaches to fast gas chromatography-mass spectrometry. *Journal of Chromatography A*, 1000, 1-2, 153-180.
- Moinfar, S. & Hosseini, M.-R.M. (2009). Development of dispersive liquid-liquid microextraction method for the analysis of organophosphorus pesticides in tea. *Journal of Hazardous Materials*, 169, 907-911.
- Montes, R.; Rodríguez, I.; Ramil, M.; Rubí, E. & Cela, R. (2009). Solid-phase extraction followed by dispersive liquid-liquid microextraction for the sensitive determination of selected fungicides in wine. *Journal of Chromatography A*, 1216, 1-2, 5459-5466.
- Ojeda, C.B. & Rojas, F.S. (2009). Separation and preconcentration by dispersive liquid-liquid microextraction procedure: A review, *Chromatographia*, 69, 11-12, Online First™, 14 April 2009.
- Paleologos, E.K.; Giokas, D.L. & Karayannis, M.I. (2005). Micelle-mediated separation and cloud-point extraction. *Trends in Analytical Chemistry*, 24, 5, 426-436.
- Pedersen-Bjergaard, S. & Rasmussen, K.E. (1999). Liquid-Liquid-liquid microextraction for sample preparation of biological fluids Prior to capillary electrophoresis. *Analytical Chemistry*, 71,14, 2650-2656.
- Pedersen-Bjergaard, S. & Rasmussen, K.E. (2008). Liquid-phase microextraction with porous hollow fibers, a miniaturized and highly flexible format for liquid-liquid extraction. *Journal of Chromatography A*, 1184, 1-2, 132-142.
- Psillakis, E. & Kalogerakis, N. (2003). Developments in liquid-phase microextraction. *Trends in Analytical Chemistry*, 22, 9, 565-574.
- Qia, L.L. & He, Y.Z. (2006). Funnel form single-drop microextraction for gas chromatography-electron-capture detection. *Journal of Chromatography A*, 1134, 1-2, 32-37
- Ravelo-Pérez, L.M.; Hernández-Borges, J.; Asensio-Ramos, M. & Rodríguez-Delgado, M.Á. (2009). Ionic liquid based dispersive liquid-liquid microextraction for the extraction of pesticides from bananas. *Journal of Chromatography A*, 1216,1-2, 7336-7345.
- Rezaee, M.; Assadi, Y.; Hosseini, M.-R. M.; Aghaee, E.; Ahmadi, F. & Berijani, S. (2006). Determination of organic compounds in water using dispersive liquid-liquid microextraction. *Journal of Chromatography A*, 1116, 1-2, 1-9.
- Rezaee, M.; Yamini, Y. & Faraji, M. (2010). Evolution of dispersive liquid-liquid microextraction method. *Journal of Chromatography A*, 1217, 1-2, 2342-2357.
- Sanco (2007). Method validation and quality control procedures for pesticide residues analysis in food and feed, European Commission Document SANCO/2007/3131, Brussels, 2007.
- Santalad, A.; Srijaranai, S.; Burakhama, R.; Sakai, T. & Deming, R.L. (2008). Acid-induced cloud-point extraction coupled to spectrophotometry for the determination of carbaryl residues in waters and vegetables. *Microchemical Journal*, 90, 50-55.
- Sanz, C.P.; Halko, R.; Ferrera, Z.S. & Rodríguez, J.J.S. (2004). Micellar extraction of organophosphorus pesticides and their determination by liquid chromatography. *Analytica Chimica Acta*, 524, 265-270.

- Saraji, M. & Tansazan, N. (2009). Application of dispersive liquid-liquid microextraction for the determination of phenylurea herbicides in water samples by HPLC-diode array detection. *Journal of Separation Science*, 32, 4186-4192.
- Theis, A.L.; Waldack, A.J.; Hansen, S.M. & Jeannot, M.A. (2001). Headspace solvent microextraction. *Analytical Chemistry*, 73,23, 5651-5654
- Tsai, W.C. & Huang, S.D. (2009). Dispersive liquid-liquid microextraction with little solvent consumption combined with gas chromatography-mass spectrometry for the pretreatment of organochlorine pesticides in aqueous samples. *Journal of Chromatography A*, 1216, 5171-5175.
- Wang, Y.; You, J., Ren, R., Xiao, Y.; Gao, S.; Zhang, H. & Yu, A. (2010). Determination of triazines in honey by dispersive liquid-liquid microextraction high-performance liquid chromatography. *Journal of Chromatography A*, 1217, 1-2, 4241-4246.
- Wei, G.; Li, Y. & Wang, X. (2007) Application of dispersive liquid-liquid microextraction combined with high-performance liquid chromatography for the determination of methomyl in natural waters. *Journal of Separation Science*, 30, 3262-3267.
- Xiao, Q.; Hu, B.; Yu, C.; Xia, L. & Jiang, Z. (2006). Optimization of a single-drop microextraction procedure for the determination of organophosphorus pesticides in water and fruit juice with gas chromatography-flame photometric detection. *Talanta* 69, 848-855.
- Xiao-Huan, Z.; Qiu-Hua, W.; Mei-Yue, Z.; Gu-Hong, X. & Zhi, W. (2009). Developments of dispersive microextraction technique. *Chinese Journal of Analytical chemistry*, 37, 2, 161-168.
- Xiao-Huan, Z.; Wang,C.; Gao, s.T.; Zhou, X. & Wang, Z. (2008). Analysis of pyrethroid pesticides in water samples by dispersive liquid-liquid microextraction coupled with gas chromatography. *Chinese Journal of Analytical Chemistry*, 36, 6, 765-769.
- Xiong, J. & Hu, B. (2008). Comparison of hollow fiber liquid phase microextraction and dispersive liquid-liquid microextraction for the determination of organosulfur pesticides in environmental and beverage samples by gas chromatography with flame photometric detection. *Journal of Chromatography A*, 1193, 1-2, 7-18.
- Xu, L.; Basheer, C. & Lee, H. K. (2007). Developments in single-drop microextraction. *Journal of Chromatography A*, 1152 1-2, 184-192.
- Zang, X.; Wang, J.; Wang, O.; Wang, M., Ma, J.; Xi, G. & Wang, Z. (2008). Analysis of captan, folpet, and captafol in apples by dispersive liquid-liquid microextraction combined with gas chromatography. *Analytical Bioanalytical Chemistry*, 392, 749-754.
- Zhang, M.; Huang, J.; Wei, C.; Yu, B.; Yang, X. & Chen, X. (2008). Mixed liquids for single-drop microextraction of organochlorine pesticides in vegetables. *Talanta* 74, 599-604.
- Zhao, E.; Han, L.; Jiang, S.; Wang, Q. & Zhou, Z. (2006). Application of a single-drop microextraction for the analysis of organophosphorus pesticides in juice. *Journal of Chromatography A*, 1114, 1-2, 269-273.
- Zhao, E.; Zhao, W.; Han, L.; Jiang, S. & Zhou, Z. (2007). Application of dispersive liquid-liquid microextraction for the analysis of organophosphorus pesticides in watermelon and cucumber. *Journal of Chromatography A*, 1175, 1-2, 137-140.

- Zhou, Q.; Bai, H.; Xie, G. & Xiao, J. (2008a). Temperature-controlled ionic liquid dispersive liquid phase micro-extraction. *Journal of Chromatography A*, 1177, 1-2, 43-49.
- Zhou, Q.; Bai, H.; Xie, G. & Xiao, J. (2008b). Trace determination of organophosphorus pesticides in environmental samples by temperature-controlled ionic liquid dispersive liquid-phase microextraction. *Journal of Chromatography A*, 1188, 1-2, 148-153.



Pesticides - Strategies for Pesticides Analysis

Edited by Prof. Margarita Stoytcheva

ISBN 978-953-307-460-3

Hard cover, 404 pages

Publisher InTech

Published online 21, January, 2011

Published in print edition January, 2011

This book provides recent information on various analytical procedures and techniques, representing strategies for reliability, specificity, selectivity and sensitivity improvements in pesticides analysis. The volume covers three main topics: current trends in sample preparation, selective and sensitive chromatographic detection and determination of pesticide residues in food and environmental samples, and the application of biological (immunoassays-and biosensors-based) methods in pesticides analysis as an alternative to the chromatographic methods for "in situ" and "on line" pesticides quantification. Intended as electronic edition, providing immediate "open access" to its content, the book is easy to follow and will be of interest to professionals involved in pesticides analysis.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Sara C. Cunha, J.O. Fernandes and M. Beatriz P.P. Oliveira (2011). Current Trends in Liquid-Liquid Microextraction for Analysis of Pesticide Residues in Food and Water, Pesticides - Strategies for Pesticides Analysis, Prof. Margarita Stoytcheva (Ed.), ISBN: 978-953-307-460-3, InTech, Available from: <http://www.intechopen.com/books/pesticides-strategies-for-pesticides-analysis/current-trends-in-liquid-liquid-microextraction-for-analysis-of-pesticide-residues-in-food-and-water>

INTECH

open science | open minds

InTech Europe

University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China

Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the [Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License](#), which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.