

NOVEL ADVANCEMENTS IN ASSISTED TECHNIQUES AND MICROEXTRACTION OF PESTICIDE RESIDUES FROM FOOD SAMPLES

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ABSTRACT

Pesticide agrochemicals have long been used for crop protection to improve agricultural productivity. Wide ranges of pesticide combinations have been developed so as to restrain fast evolving pest spectrum. Multiclass pesticide combinations used for crop treatment create problem of persistence in the agro-environment posing potential health hazard to the consumer and ecosystem. This accentuates need for development of efficient pesticide multiresidue methods for risk evaluation. Newer trends in analysis of pesticides in food intend at use of solvent minimized extractions with scope of automation and hyphenation to bring fast, accurate and sensitive analysis. Accelerated extraction techniques based on microwave, ultrasonication and microsolvent approaches such as liquid phase, solid phase microextraction LPME, SPME respectively; underlies the objectives of green analysis as well provide scope for integration. Additionally, these novel 'solvent-free' extraction methods vouch for their efficacy and selectivity in comparison to their 'classical' counterparts. Present paper extensively updates developments in modern, assisted instrumental extraction approaches along with novel aspects of microextraction techniques introduced in pesticide multiresidue analysis of foods. Noteworthy advantages and pitfalls of extraction modules based on these techniques are also evaluated.

KEYWORDS : Food, Microextraction, Ecotoxicity, Hyphenation, Automation

Pesticides are tenaciously present in food items. Unwarranted use of pesticides in and on food and increasing public concern emphasizes; need for safety assessments in compliance to set Maximum Residue Limits, MRL's. Multiresidue analysis of pesticides has two aspects; one there is need to determine wide range of multiclass pesticides in a single run, as fast as possible; since the products are perishable and number of samples is high. Moreover, there is a need to achieve reliability in the determination of such residues at the low concentration levels established by the legislation as the MRL's allowed. Both the aspects must involve low costs and low environmental contamination such as reduced solvent consumption, and must have accuracy and representativeness. In pesticide multiresidue methods selectivity based on multistep isolation (sample preparation, extraction, partitioning/clean up) (Self, 2005) and detection principles are frequently used. Sample preparation step is one of the foremost sources of variance in an analytical outcome. Effective isolation of analyte is dependent on choice of suitable extraction procedure keeping in view nature of analyte and its interaction with food matrix. Variable composition of foodstuff essentially those with high moisture or lipid content, makes it a complex matrix for sampling purposes (Curren and King, 2002). Variability in composition of food matrices can cause physiological changes in food that can affect

sampling. This heterogeneity in composition determines interaction of analyte with food matrix and ultimately the choice of method preferable for analyte detection (as depicted in table1).

In order to improve the quantitative analysis it is therefore necessary to remove the matrix interference by sample pre-treatment, based on effective extraction/clean-up steps. Traditional method based on liquid-liquid extraction, LLE have remained as backbone of many official standard procedures. However, such off-line procedures suffer from time and labor demand, high cost and poor reproducibility. In addition, there is need of greater volumes of expensive hazardous solvents posing problem of ecotoxicity and disposal. Keeping in view the importance of sample preparation; over the time, conventional techniques have been advanced upon by automated extraction systems and sophisticated auxiliary techniques such as MAE, USE; of importance to improve extractions of allied techniques. Additionally, in recent years, analysis of pesticide residues in food has incorporated novel microextraction methodologies SPME, LPME; DLLME, SDME, MASE miniaturizing or practically scaling down sampling needs aiming at to automation and integration of analytical systems (Ahmed,2001), and to sufficiently match sample preparations to modern, sophisticated analytical instrumentations. Lately, bioanalytical systems based on biosensors have made venture into multiresidue analytical

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Table 1 : Relative Scope of Analytical Extraction Techniques

| Technique | Sample | Analyte Nature/Range | Comments |
|-----------|------------------|------------------------------------|--|
| USE | Solid | Semi Polar, thermo stable | dried samples required to avoid emulsion formation |
| MAE | Solid | Polar, medium polar, thermo stable | clean up required prior to analysis |
| SPME | Aqueous, Gaseous | Polar to non polar | high sample throughput integrates sampling, extraction, preconcentration, sample introduction to single step |
| LPME | Aqueous | Less polar analytes, thermo stable | limited for highly polar analytes |

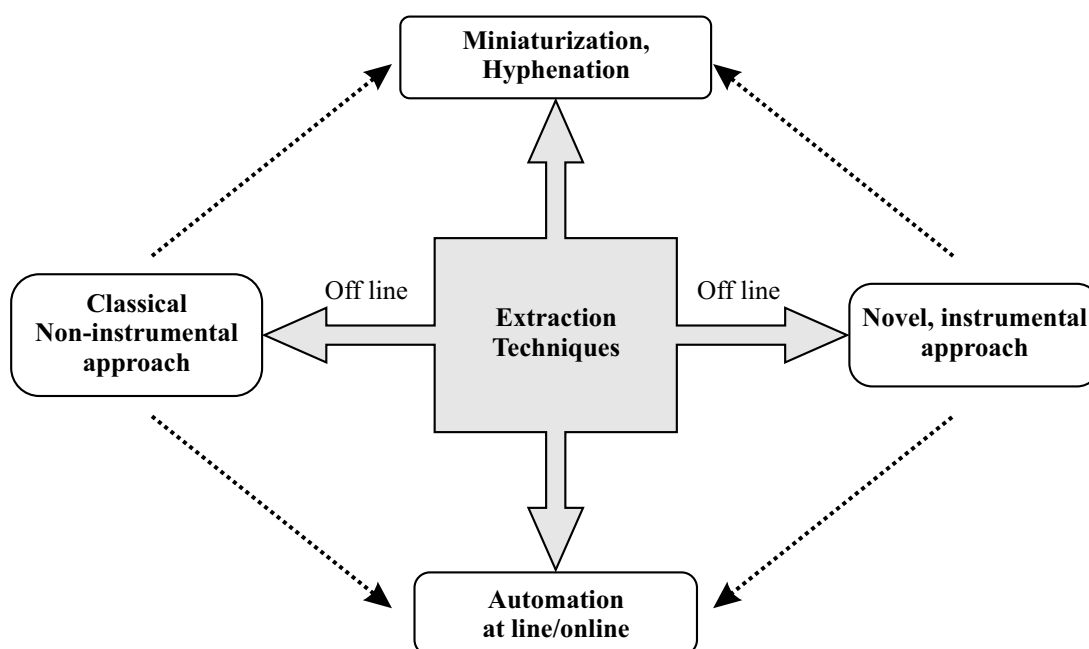


Figure 1 : Overall Trend in Extraction Methodologies in Pesticide Residue Analysis

determinations. With the advent of powerful determination techniques the reliability of analytical outcomes duly rests on efficient extractions much as before. The paper examines advancements in extraction techniques based on enhanced fluid extraction USE, MAE, FMAE as well development of recent microextraction procedures based on use of headspace modalities, polymeric membranes, organic drop and novel extraction phases. Growing trends in extraction techniques employed for analysis of pesticide residue in foods are outlined in Figure 1. Fervently, many efforts have also been directed towards devising extraction

methods with built-in clean-up capabilities.

Assisted Extraction Techniques

Assisted Extraction techniques such as ultrasonication based solvent extraction and microwave assisted extraction are considered auxiliary techniques to mainstream extraction procedures.

Ultrasound Assisted Solvent Extraction

Ultrasound assisted solvent extraction USE, is function of acoustic cavitation, mechanical agitation and thermal effect. Low frequency sound waves are used to physically disrupt the matrix, enhancing analyte transfer to

solvent. Ultrasound assisted solvent extraction is a preferred procedure for pesticide extraction from solid matrices. The technique has gained attention since it is rapid, inexpensive, with possibility of running several simultaneous extractions. Sometimes multiple extraction steps are required for increased transfer of analytes. Extraction efficiency is dependent on extraction time, solvent type, volume and number of steps involved. Decrease in extraction time compromises method efficacy whereas much longer extraction time is found to augment interferences from matrix (Ping et al., 2005). A lower recovery of polar pesticides; dichlorvos and dimethoate is reported (Pengyan and Yusong, 2005) due to increased water solubility of extract. Presence of water during extraction decreases method performance hence a sample drying step is recommended.

Specific method based on LC coupled to tandem MS is developed by Peruga et al., 2012 for effective determination of dithiocarbamate fungicide thiram in fruits and vegetables. Thiram degradation along sample treatment can be minimized by operating at low temperature condition (5°C) and adding anhydrous Na₂SO₄, NaHCO₃ as pH regulator and EDTA for enzymatic activity reduction. Appreciable recoveries, between 80% - 106%, and RSD's below 10% were obtained at low fortification levels providing high selectivity. For removal of lipidic and high-molecular-mass interferences gel permeation chromatography GPC is applied. More often a less needed clean up step, can be performed using SPE-florisil cartridge (Granby et al., 2004). Ultrasonic extraction has been used as auxiliary technique to matrix solid -phase dispersion extraction MSPD; this combines extraction-cleanup in single step. This method is extended for determination of organochlorines and herbicides in fruit and vegetable juices. Combination of USE to GC, HPLC-UV, TLC, and LC-MS-MS has been reported in various works.

Lately, USE has explored its avenues in real sample analysis. A single step analysis of six multiresidue pesticides was performed for application of the USE method to field samples of Chinese leafy vegetables (Pan et al., 2008). The extract did not require clean-up prior to a

LC-MS-MS determination. Proposed procedure enabled recovery over 83% and LOQ less than 1.4 µg/kg. Using 40 ml of ethyl acetate for 35 min sonication in one step analysis; extraction time and solvent consumption reduced by drastically as compared to a homogenized extraction method.

USE is more supportive to online coupling than microwave procedures. A micro on-line extraction method based on ultrasonication; for the extraction of insecticide residues in grapes, must and wine was used by (Oliva et al., 2000). 5 ml sample was extracted with 20 ml, 1:1 acetone DCM mixture. Extract was finally filtered and concentrated in isoctane-toluene (1:1) for GC analysis. Lower detection limits upto nanograms were obtained for organophosphate pesticides in must and wine. Using a biosensor based approach for monitoring of propoxur and carbaryl; (Xavier and colleagues, 2000) eliminated need for organic solvent and tedious preconcentration steps. The technique is noteworthy in achieving appreciable reproducibilities.

Newer initiatives in sonication techniques involve their coupling to novel micro-level extraction procedures. Application involving use of sonication assisted extraction to dispersive liquid liquid microextraction DLLME has been recently reported for OP's in summer fruits (Pirsaheb et al., 2013). Ultrasound-assisted solvent extraction followed by DLLME based on the solidification of floating organic drop USE-DLLME-SFO (Pirsaheb et al., 2013) can eliminate need for post extraction clean-up or evaporation; providing a rapid and small-scale extraction combined to sensitive HPLC-UV analysis.

Microwave Extractions

Microwave extraction applications involve two methodologies; with 'closed vessel' microwave assisted solvent extraction systems or using 'open system' focused microwaves under atmospheric pressure.

An open vessel uses focused monomode radiation to achieve precise, localized and homogenous heating; FMAE, compared to drastic heating in closed system; MAE. Temperature is maintained to solvent boiling point. Simultaneous use of clean solvent and focused microwaves favors mass transfer, shortening extraction time. Unlike MAE; FMAE does not require moisture adjustments. Water is removed during initial cycles via azeotropic distillation.

Table 2 : Novel Strategies in SPME and USE for Pesticides in Various Food Matrices

| Technique/ Food | Pesticide | Extraction Conditions | Determination | Reference |
|--|-------------------|------------------------------|----------------------------------|---------------------|
| SPME | | Sorbent | | |
| Radish | ¹ OCs | calix [4] arene fiber (60µm) | GC-ECD | Dong et al. 2005 |
| Food samples | ² OP's | crown ether fiber (40µm) | GC-FPD | Yu et al. 2004 |
| Cabbage | OPPs | PDMS (100µm) | SNO ₂ gas sensor | Huang et al. 2004 |
| Vegetables | herbicides | PA (85µm) | GC-MS | Berrada et al. 2004 |
| Wine | Polar pesticides | PPY cartridge | in tube SPME- HPLC-MS, online | Wu et al. 2002 |
| USE | | Solvent | | |
| Honey | atrazine,simazine | benzene/water (1:1) | TLC | Rezic et al. 2005 |
| Fruit, cereals | Carbamates | methanol/water (95:5) | LC-MS ² | Granby et al. 2004 |
| Grapes, must, wine | OPPs | ³ ac/dcm (1:1) | GC-ECD/NPDonline | Oliva et al. 2000 |
| Corn | Multiclass | dcm | GC-MS | Pengyan et al. 2005 |
| Onion, lettuce | Carbaryl | phosphate buffer(pH=7) | fiber optic biosensor | Xavier et al. 2000 |
| Herbs | OCs | petroleum ether | GC-ECD | Ping et al. 2005 |
| 1 organochlorines ; 2 organophosphorous ; 3 acetone; 4 dichloromethane | | | | |

Sample manipulation prior to or during extraction is thus outmoded in FMAE. As a modification to conventional Soxhlet; FMAE has overcome its shortfalls. Stronger interactions between analytes and matrix are acted upon by focused microwaves improving the recoveries over standard methods. Lipid interferences in matrix required thorough post extraction clean up of sample. Jointly, a precolumn and carbofrit plug in glass liner injector was introduced to increase sensitivity and selectivity of GC-MS-MS. A procedure for polar, non-GC amenable pesticides is described by (Falqui-Cao and colleague, 2001). For this integration of FMAE-SPME coupled to high pressure liquid chromatography is suggested. Focused monomode microwaves accounted for reduction in strong matrix diminishment effects whereas use of water as extraction solvent made the technique environmentally endurable. Similar coupling of FMAE to SPME has been done for determination of pyrethroid pesticides in strawberries (Sanusi et al., 2004). Addition of 50% aqueous ACN co-solvent under microwave conditions enhanced sensitivity of detection making direct thermal desorption of fibers possible, for GC-MS analysis.

Microwave assisted solvent extraction (MAE) operates under controlled temperature and pressure conditions. Simultaneous heating by microwaves in closed system allow solvent to heat above their boiling point. Microwave assisted solvent extraction requires use of

safety features such as temperature control vessels or sensors monitoring solvent leaks to prevent temperature and pressure build ups. Commercial and household systems are available for MAE. In its novel prospect MAE has incorporated use of new self-designed microwave absorption tube (Zhao et al., 2012) for extraction of seven pesticides from vegetable samples. Microwave absorption tube was made by sealing graphite powder in glass tube and used to heat samples directly. The extracts were directly analyzed by GC-MS without any clean-up process. The effects of some experimental parameters on extraction efficiency were investigated and optimized. 3.0 g of sample, 25 mL of hexane and three microwave absorption tubes were added in the microwave extraction vessel, the extraction was carried out under 425 W irradiation power at 70 °C for 8 min.

Microwave procedure shows dependence on matrix and analyte character (Sanghi and Kannamkumarath, 2004). Method optimization parameters need to be evaluated with each new matrix for MAE. As shown by (Sanghi and coworker, 2004); lowering acetone ratio in hexane- acetone solvent mixture considerably compromised recoveries of organochlorines from vegetable matrix. Addition of 5% water to solvent (Papadakis et al., 2006) improved recovery value of OC pesticides indicating effect of moisture on extraction efficiency. Although longer extraction time and higher power levels extract larger

interferences, elevated temperatures improve recoveries as long as analytes are stable at those temperatures. Contrastingly thiophanate methyl converts to carbendazim with microwave treatment (Singh et al., 2007). Cooling of extract at ambient temperature is required in MAE. A filtration (Moreno et al., 2007) or centrifugation step (Papadakis et al., 2006) or both (Bouaid et al., 2000) are performed post extraction for removal of coextracts. Additional interferences are removed through post extraction clean up. Less extensive clean up can be done using glass wool, florisil or activated silica column. Selective extractions using SPE and SPME allow extract clean up and preconcentration in one-go.

Current initiatives in MAE involve hyphenation of technique to more specific and sensitive procedures intended to increase extraction efficiencies. Miniaturized microwave-assisted extraction based on use of non-polar solvent has been employed by coupling it to a micro-SPE procedure (Wang et al., 2013). Microwave micro-SPE device is used for the determination of OP's in vegetable and fruit samples. Absorbing microwave μ -SPE device was prepared by packing activated carbon with microporous polypropylene membrane envelope used as sorbent in μ -SPE as well as microwave absorption medium. This allows direct sample heating with combined extraction-cleanup effect eliminating the need of solvent or sorbent switching. Simultaneous extraction of miniature sample (1.0 g) was carried out under 400 W irradiation power at 60°C for 10 min. The extracts obtained by MAE- μ -SPE were directly analyzed by GCMS. Recoveries in the range of 93.51-104.6%, and RSD's lower than 8.7% were obtained. MAE together with solid phase microextraction (SPME) has merged extraction speed with concentration. (Cai and coworkers, 2003) investigated different SPME fibres in a method to determine organochlorines in Chinese tea. A novel sol-gel polyphenylmethyl siloxane (PPMS) fiber provided good sensitivities and selectivity over other commercial ones. High responsiveness of PPMS was owing to its 3-D network and greater phenyl groups for reactivity. Until recently, microwaves have incorporated micellar extraction (Moreno et al., 2007) using surfactants and online one step 'in-situ' extractions (Chen et al.,

2002). These are a step forward in improvement of method performance.

Novel Micro Extraction Techniques

Solid Phase Microextraction

Solid phase microextraction is sorptive extraction based; solvent exclusive approach preferably used for isolation of volatile and semivolatile analytes. SPME is similar to reversed out gas chromatographic column. SPME involves, use of a fiber coated with an extracting phase, a polymeric sorbent which adsorbs extractants that are thereby desorbed into analytical instrument for determination. Seen as a direct derivative of solid phase extraction; SPME differs from it in view of equilibrium based non-exhaustive extraction achieved within rapid time-span. Moreover, this technique does not suffer from the clogging or channeling problems encountered with SPE. SPME achieves micro-dimensions in terms of sample size (upto few grams) and solvent usability that is practically negligible. The quantity of target compound extracted by the fiber is proportional to its concentration in the sample so long as equilibrium is reached or, in case of short time pre-equilibrium, with help of convection or agitation.

Array of commercial fibre coatings with variable thickness are available, including non-polar PDMS (100, 30, 7 μ m), polar polyacrylate PA (85 μ m), coated porous particle phase PDMS- Carboxen (75, 85 μ m), Carbowaxdivinylbenzene CW-DVB (70 μ m), semi-polar mixed phases like PDMS-DVB (60, 65 μ m).

Optimum coating type for broad range of analytes requires experimentation with different fibres. (Rial et al., 2002) found bipolar mixed phase DVB-CAR-PDMS suitable for determination of fungicides in white wine. Good precision values within range of 5% and low detection limits were obtained. Type of coating, its thickness and partitioning ratio of analytes have influence on equilibration time. Novel stationary phases such as, bisbenzo crown ether/hydroxyl-terminated silicone oil (OH-TSO) SPME (Yu et al., 2004), 5,11,17,23-tetra-tert-butyl-25,27-diethoxy-26,28 dihydroxycalix [4] arene /hydroxy-terminated silicone oil coated SPME fiber (C[4]/OH-TSO) coating (Dong et al., 2005) based on sol- gel technology have been developed to provide greater scope for SPME.

Table 3 : Operational Advancements in MAE Methods in Pesticide Analysis of Food

| A. MAE - Closed vessel system | | | | | | |
|--|-----------------------|---------------------------|------------------|------------------|--|-------------------------|
| Pesticide Group | Matrix | r% | RSDs | Detection | Comments | Author |
| Organochlorines | seaweeds | 80.5-104.3, 73.9-111.5 | <5.3%, <10.3% | HPLC-UV | microwave assisted micellar extraction (MAME) coupled to SPME, SPE | Moreno et al. 2007 |
| Organochlorines | horticultural samples | ~100 | - | GC-ECD, GC-MS | SPE cleanup on carbon cartridge | Lopez-Mahia et al.2005 |
| Organochlorines | Chinese tea | 39.05-94.35 | <16% | GC-ECD | Porous PPMs fiber for SPME | Cai et al. 2003 |
| Organochlorines | sesame seeds | >80 | <15% | GC-MS | florisil (1 g) clean up, water/ACN(5:95) for extraction | Papadakis et al.2006 |
| B. FMAE - Open vessel system | | | | | | |
| Pesticide Group | Matrix | LOD | RSDs | Detection | Conditions/ Comments | Author |
| carbendazim, azoxystrobin, diethofencarb, bupirimate, napropamide, Pyrethroids, deltamethrin, acrinathrin, bifenthrin, cyhalothrin | strawberries | 17-22 ng/gm | 4.1-7.3 % | HPLC-DAD | 25gmsample, water solvent , 30 w, 7 min/SPME | Falqui -Cao et al. 2001 |
| | strawberries | <14 µg/kg | 1.2-14.2% | GC-MS | 25 gm, water-ACN(1:1),30W, 5mm/SPME concentration | Sanusi et al.2004 |

Owing to many special characteristics such as higher surface area, strong hydrophobic and π - π interaction, and cavity-shaped cyclic molecular structure, these coatings shows far higher extraction efficiency to most of the tested analytes than the commercial ones; with LODs even lower than required range. Moreover, most of the sol gel based fibres are characterized by their high thermal (380°C) and solvent stability as well as a longer lifetime (200 times), thus preventing usual sample carry over effects readily associated with commercial coatings.

Routinely, SPME applications are hyphenated to GC by direct thermal-desorption of analytes, usually volatiles; from coated fiber to hot chromatographic inlet. Alternatively, coupling with HPLC and LC-MS broadens range of SPME essentially for thermally labile, non-volatile compounds; not compliant to GC. For this fiber is desorbed into organic solvent (static desorption) or a mobile phase (dynamic desorption) at a special SPME-HPLC interface prior to LC analysis. An automated SPME-LC system, known as in-tube SPME (Wu et al., 2002) uses an open-tubular capillary column as the SPME device. In-tube procedures are effective for cleaner samples which are particulate-free else pretreatment steps such as centrifugation; filtration and/or dilution are required prior to extraction. Online in-tube SPME reduces analysis times, offering better accuracy and precision than offline manual techniques. Capillary electrophoretic analysis has been explored for SPME in fruit juice samples. Online preconcentration with normal stacking (NSM), field-enhanced sample injection (FESI), and stacking with matrix removal (SWMR) for CE-UV analysis are investigated (Hernandez-Borges et al., 2005), it is established that in comparison to CE-UV, CE-MS (Hernandez-Borges et al., 2004) provided better LODs at ng/ml levels. Direct immersion SPME procedures are preferred for extractions from liquid or liquified solid samples. (Berrada et al., 2004) obtained an aqueous extract of vegetables carrot, onion and potatoes for easy estimation of phenylurea herbicides. SPME was performed on 85 µm polyacrylate fiber, effect of salt addition and pH was determined. Different pH levels at pH 4 and 11 enabled SPME hyphenated GC MS quantification of thermally liable phenylurea as their representative metabolites. Influence of pH on SPME

analysis of pesticides in juice and beverages is less defined, since they behave as buffered matrices (Millan et al., 2003) close to neutral pH. For headspace analysis of liquid samples, the addition of salt is suggested to decrease the solubility of pesticides in the liquid matrix, enabling its concentration in vapor phase. Salting out of pesticides in direct immersion analysis is usually not required, since it is associated with reduction in recoveries (Jimenez et al., 1998) as noticed in case of honey samples.

Most applications involving solid food matrices such as fruits and vegetables utilize headspace SPME (Fytianos et al., 2006). Presence of interferences in such complex samples is more profound and can compromise method sensitivity due to irreversible binding with fibre coatings. To prevent this, aqueous dilution and small amount of organic solvent addition to the sample makes quantification of analyte possible. Methanol (Lambropoulou et al., 2003) or mixture of methanol, acetone in ratio 1:1 (Kin et al., 2006) is generally preferred for solvent dilution. Matrix effects can be curtailed by using matrix matched calibration, standard addition procedure or less frequently used protective membranes that prevent adsorption of matrix components on the fibre. In their work (Millan et al., 2003) analyzed red wine samples for organochlorines utilizing SPME coupled to HPLC diode array detection. Spiked wine samples were passed through 0.45 μm membrane filter to separate analyte from particulate interferences thus obtaining relatively cleaner chromatograms.

SPME presents some unique advantages over existing conventional sample preparation techniques (USE, MAE) in terms of solvent-free, integrated, single step miniaturized extractions. However the technique suffers at many instances (Yu et al., 2004) in extracting multiple analytes with equivocal accuracy in single run, a requirement for real sample analysis. In this front, SPME requires development of more focused initiatives which are yet anticipated.

Liquid Phase Microextraction Procedures

Liquid phase microextraction is considered as a novel derivative of liquid liquid extraction. LPME involves automated, micro- LLE procedures using minimal amount of extraction solvents that enable direct instrumental

transfer. Based on LLE principles the technique involves various modalities that ensure high enrichment and better selectivity evading cost factor and laborious solvent reduction steps of conventional LLE. The technique combines several advantages of miniaturized solid phase microextraction procedure as well, but; much unlike SPME its applicability is restricted towards semisolid and solid matrices. Although lately, this schism has been lessened through various LPME applications extended for organosulfur and organophosphorous pesticides in solid samples involving fruits, vegetable and green tea beverage; illustrated in work by (Xiong and Hu, 2008).

LPME technique is a myriad of variants categorized as one. These largely include solvent microextractions (DLLME, discrete-drop based procedures SDME, HS-SDME, DI-SDME, and CFME) and membrane based methodologies, classified in figure 2.

Dispersive Liquid-Liquid Microextraction

In dispersive liquid-liquid microextraction, extraction solvent mixed with a dispersant is rapidly injected into a conical tube containing microlitres of aqueous sample. Gentle shaking allows partitioning out of analyte from aqueous sample into finely dispersed extraction phase. DLLME utilizes ternary solvent system that involves water from aqueous sample, a moderately polar disperser solvent and water immiscible extraction solvent to isolate analyte from sample. Depending on the density of extractant compared to aqueous sample phase; analytes can be sedimented out or collected from surface of partitioned solvent through solidification of floating drop. Widely; high density, chlorinated organic extraction solvent such as chlorobenzene/ chloroform/ carbon tetrachloride and tetrachloroethane have been used in DLLME whereas acetonitrile (ACN), acetone, ethanol and MeOH are usual choice as dispersant solvents. Newly reported applications of DLLME are based on use of less toxic, low density organic solvents such as 1-octanol (Wang et al., 2013), 1-undecanol (Rodríguez-Cabo et al., 2011). In one such application (Sanagi et al., 2012) developed DLLME method based on solidification of floating organic droplet (DLLME-SFO) for the analysis of triazines herbicides simazine, atrazine, sebumeton and cyanazine. The DLLME-SFO method applied to sugarcane samples

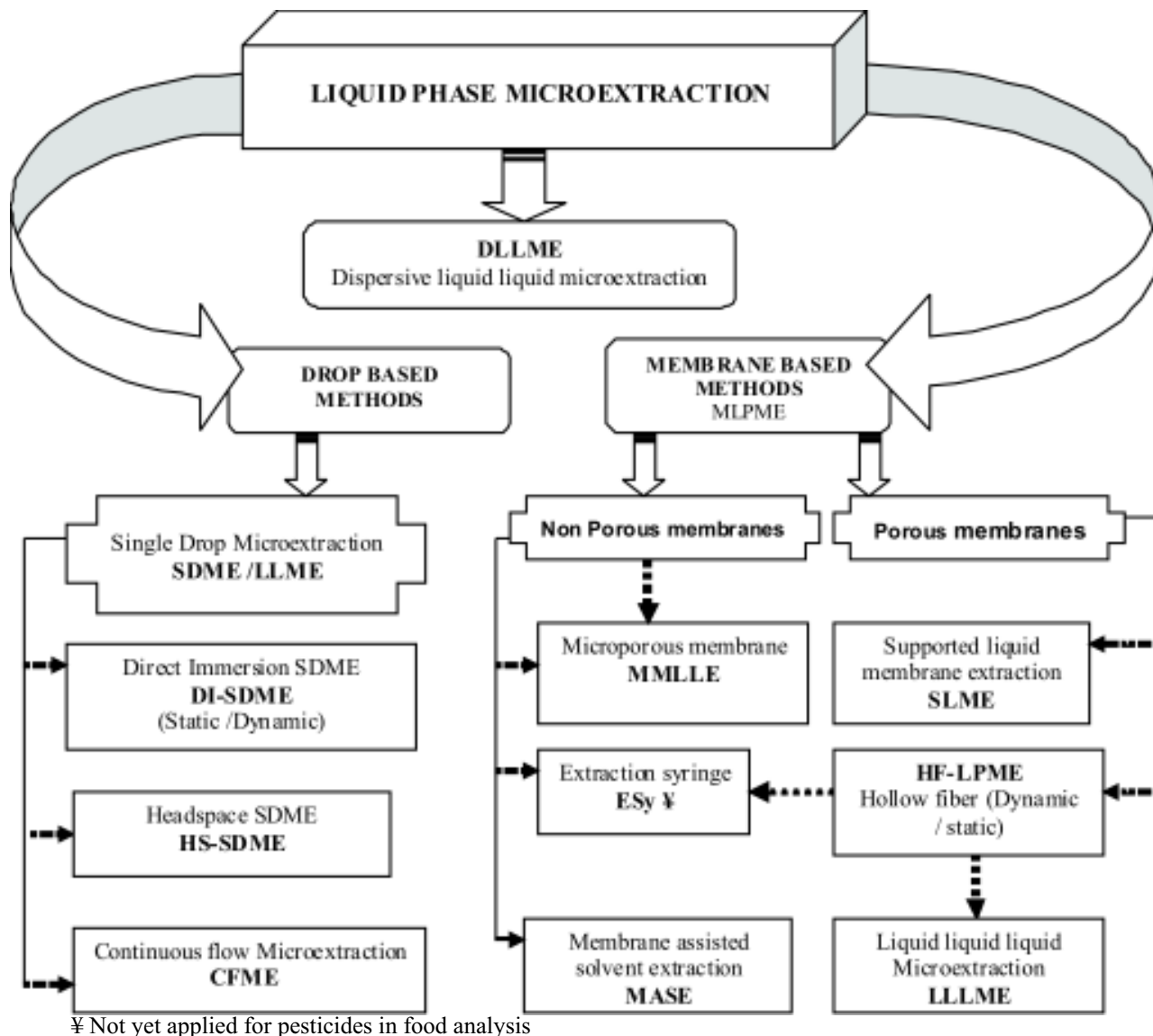


Figure 2 : Summarizing Liquid Phase Microextraction Techniques in Pesticide Analysis

showed excellent recoveries 95.7-116.9% with high enrichment upto 322. Using 10 µL of 1-undecanol as extraction solvent, ACN as disperser solvent (100 µL) and salt addition (NaCl, 5%) for 3 min repeatabilities 0.03-5.1% and linearity, LOD's in low parts per billion ranges were obtained. In yet another DLLME based procedure for determination of fungicides, (Rodríguez-Cabo and group, 2011) used binary mixture of acetone and 1-undecanol (0.5, 0.05 mL respectively) to extract analytes from (1:1) diluted red and white wine samples. Floating phase of 1-undecanol was solidified and separated from the hydro-alcoholic matrix after centrifugation and then melted at room temperature for injection into GCMS system. The method

showed limits of quantification between 0.2 and 3.2 mg/ ml and linear responses for concentrations up to 300 mg/ ml. Efficiency of DLLME procedure was found to be nearly independent of matrix characteristics. Apart from 1-undecanol as usual solvent for DLLME-SFO, 1-dodecanol has also been investigated for simultaneous analysis of organochlorines and pyrethroid pesticides in peach juice, pulps and peels (Matsadiq et al., 2011). Optimization of extraction conditions was achieved taking 8 µL of extractant volume, acetone as disperser solvent with <2 min extraction time; without the need of salt addition.

Non-organic solvents have substituted volatile organics in DLLME based techniques. Recent initiatives in

DLLME focus on use of novel extraction solvents such as Ionic Liquids. Ionic liquids ILs are considered as environmentally durable benign solvents having characteristics of high thermal stability, nonflammability, ultralow detectable vapor pressure, wide temperature range and good solubility for organic as well as inorganic compounds. Moreover, their physicochemical properties (melting point, density, viscosity and polarity) can be easily modified by combination of suitable ions. Therefore IL is regarded as 'green' alternative to volatile organic solvents largely used in sample preparations. Use of ILs as extractants has been found to be especially important in DLLME as well as in other LPME procedures. Key operational modes distinguished for IL-DLLME are conventional; in-situ; temperature-controlled; ultrasound-assisted/microwave or vortex-assisted IL-DLLME. The dispersive solvent in these modes can be an organic solvent, a hydrophilic ionic liquid, a surfactant or even use of disperser is solvent totally avoidable (Trujillo-Rodríguez et al., 2013). In this regard a novel IL-DLLME procedure involving organic solvent-free emulsification microextraction coupled to HPLC for acaricides - chlorfenapyr, fenpyroximate and spirodiclofen was investigated by Zhang and group, 2013. Dispersion and extraction efficiency of IL, 1-hexyl-3-methylimidazolium bis (trifluoromethylsulfonyl) imide ([C6MIM][NTf2]) was enhanced using ultrasound assisted- temperature controlled microextraction thereby obliterating need of dispersive organic solvent. Using the proposed method real fruit juice samples were successfully analyzed at 10 µg/ L and 30 µg/ L fortification levels. While almost all IL-DLLME applications are based on liquid chromatographic determination no work until yet had been available using GC- analysis due to non compatibility of IL's with gas chromatographic column. Lately, in a suggestive application by (Zhang and Lee, 2010); handling of the ionic liquid in a normal GC was made possible by exposing the IL extract as a headspace microdrop (attached to the syringe needle tip) to permit volatilization of the analytes. In this manner, the spent ionic liquid could be recovered from GC port, eliminating the contamination caused by direct IL application into GC. Proposed procedure can pave way for ionic liquid based DLLME and other LPME applications

HS-LPME, SDME to be coupled well with GC analysis. A variant to DLLME; air-agitated liquidliquid microextraction (AALLME) makes use of fine extraction solvent drops repeatedly aspirated and dispensed with a syringe into the aqueous samples several times. Compared to DLLME; AALLME method excludes use of disperser solvent and much less volume of organic solvent is used, resulting in higher enrichments upto 713-808 times (Farajzadeh and Khoshmaram, 2013).

Dispersive liquid-liquid microextraction is limited towards polar, less lipophilic analytes. So far, DLLME is seen as a clean-liquid-sample extraction technique biased towards simple matrices like aqueous sample and juice samples. The technique suffers in extracting analytes from complicated; solid matrices since it is prone to matrix interferences. This problem is proposedly overcome by conjoining DLLME to other concentration techniques such as SPE; that result in markedly enhanced enrichments. On these lines; a dual microextraction procedure hyphenating IL-DLLME to dispersive microsolid-phase extraction (D-µ-SPE) for determining pyrethroids in honey samples is developed using magnetized nanoparticles as SPE phase (Li et al., 2013). The method eliminates need of a disperser DLLME solvent and even chemical modification on SPE nanoparticles is not required. Overall advantage of procedure is its resulting high recoveries due to improved analyte enrichments. Many DLLME procedures are supportably based on dispersive-SPE methodology; QuEChERS. Reportedly, acetonitrile extracts obtained from QuEChERS served as disperser DLLME solvent for extraction of mutiresidues in ginseng samples (Chen et al., 2013). Alternately, efficiency of dispersive liquid-liquid microextraction is enhanced by coupling it to sensitive analytical techniques such as LCMS/MS, ultra high pressure liquid chromatography tandem MS, UHPLC-MS/MS (Chen et al., 2013). DLLME coupled sweeping MEKC has capability of providing enrichments high as 4,000-10,000 fold (Zhang et al., 2012) with enhanced reproducibility and recoveries; within short analysis time.

Comprehensive analytical techniques enable multiclass pesticide quantification down to sub-ppt levels (Ho et al., 2013) with limited usage of organic solvents; remarkable enrichment factors obtained in short analytical

Table 4 : Recent LPME Applications in Pesticide Analysis of Food

| Pesticides | Sample | Technique | Extraction/Disperser Solvent | Analysis | Recovery (%) | LOD | Reference |
|---------------------|-------------------------------|------------------------|---|-----------------------|--------------|-------------------------------|------------------------|
| DLLME | | | | | | | |
| Neonicotinoids | cucumber | DLLME | chloroform/ACN | MEKC | 79.7-98% | 0.8 - 1.2 ng g ⁻¹ | Zhang et al. 2012 |
| Triazine herbicides | sugarcane | DLLME-SFO | 1-undecanol/ACN | GC-MS | 95.7-116.9% | 0.037-0.008ppb | Sanagi et al. 2012 |
| OCP's | peach juice, pulp, peel | DLLME-SFO | 1-dodecanol/acetone | GC-ECD | 73-106% | 2.8-18.5 ng L ⁻¹ | Matsadiq et al. 2011 |
| Multiclass | ginseng | QuEChERS - DLLME | chloroform/ACN | UHPLC-MS ² | 70-120% | 0.01-1.0 µg kg ⁻¹ | Chen et al. 2013 |
| Pyrethroids | honey | IL-DMME - D-µ-SPE | [C ₆ MIM][NTf ₂] | HPLC-VWD* | 86.7-98.2% | 0.03-0.05µg L ⁻¹ | Li et al. 2013 |
| AA-LLME | | | | | | | |
| Triazoles | cucumber, grape juice, tomato | AA-LLME | toluene | GC-FID | 100-113% | 0.53-1.13 ng mL ⁻¹ | Farajzadeh et al.2013 |
| OPP | fruit juice | AA-LLME | chloroform | GC-FPD | 79-113% | 0.02-0.6 µg L ⁻¹ | You et al. 2013 |
| Triazoles | grape juice | AA-LLME | 1,2 dibromoethane | GC-FID | 92-105% | 0.20 -1.1 ng mL ⁻¹ | Farajzadeh et al. 2013 |
| MASE | | | | | | | |
| OPP | wine, fruit juice | MASE | cyclohexane | LVI-GC/MS | 47-100% | 1-23 ng L ⁻¹ | Schellin et al. 2004 |
| Multiclass | sugarcane juice | MASE | cyclohexane | LVI-GC/MS | 13.6-103.1% | 0.004-0.56µg L ⁻¹ | Zuin et al. 2006 |
| SDME | | | | | | | |
| OCP, OPP | strawberry, cucumber | HS-SDME | toluene (1.5µl) | GC-ECD | 71.8-95.8% | 10-200 µg L ⁻¹ | Kin and Huat 2009 |
| OPP | fruit juice, water | DI, static/ cycle flow | toluene (1.5µl) | GC-FPD | 77.7-113.6% | 0.21-0.56 ng mL ⁻¹ | Xiao et al. 2006 |
| OPP | orange juice | DI, static | toluene (1.6 µl) | GC-FPD | 76.2-108% | <5 µg L ⁻¹ | Zhao et al. 2006 |

*VWD Variable wavelength detector

run thereby opening avenues for automation of DLLME procedures.

Single Drop Microextraction

Another LPME approach single-drop microextraction SDME; requires miniscule amount of solvent as compared to DLLME. In single-drop microextraction a miniature drop of solvent (upto 1.6 μl) is suspended from the tip of a microsyringe either above the sample head space (headspace-SDME) or immersed discretely in the sample solution (Direct immersion-SDME) in which it is immiscible. The same microsyringe is then used to introduce the solvent extracted analytes into the chromatographic system for quantitation. SDME applications in pesticide analysis of food are listed in table 4.

HS-SDME is similar to traditional headspace procedure wherein volatiles are sampled from the vapors above the sample, thus outwitting the possibility of non-volatile sample matrix interferences. HS-SDME parallels the precision and analytical efficiency of HS-SPME added with advantage of wide solvent choice and cost benefits in comparison to restricted SPME fibres. As yet there has been dearth of literature focusing on use of HS-SDME to pesticide analysis. Recently, one work by Kin and Huat (2009) has explored possibility of HS-SDME towards extraction of organochlorine and organophosphorus pesticides from fruits and vegetables. Compared to SPE and HS-SPME; headspace-SDME was simple to perform, cost effective and free from memory effects largely encountered with solid phase fibres. Shorter extraction time achieved in HS-SDME; however, compromises sensitivity in terms of poor LOD and LOQ values in comparison to HS-SPME procedure. Strategic prolongation of extraction times can expectedly lower LOD but may result in drop dissolution and dislodgment for HS-SDME. Different modalities of SDME have been updated to improve selectivity of analytes. Xiao and group (2006) evaluated static and cycle-flow mode of direct immersion-SDME (DI-SDME) as a variant to continuous-flow microextraction, for determination of OPP's in water and fruit juice samples. Compared to cyclic mode, static system provided better sensitivity and precision values particularly for real samples. In the procedure salt addition

was avoided to prevent its overall negative effect on recoveries of most analytes. SDME of pesticides from complex matrices such as fruits, juices is susceptible to ionic strength, sugar interferences and pH of the sample solution. To overcome this, addition of salt and pre-extraction sample centrifugation - dilution (Zhao et al., 2006) upto 100- folds (Xiao et al., 2006) with distilled water is required. Most SDME applications utilize toluene as solvent of choice for extraction of pesticides from foods. This is due to compatibility of the solvent to GC based analysis. Lately, use of mixed solvent has been explored by (Zhang et al., 2008), thereof to expand extraction range of SDME. A novel organic drop (1 μl) comprising of p-xylene and acetone in 8:2 ratio was rendered to preconcentration of organochlorine pesticides coupling DI-SDME to GC-MS. This resulted in good recovery values, between 63.3 - 100%, with acceptable R.S.D values.

Membrane Liquid Phase Microextractions

Polymeric membranes packing extraction solvent at intersection of donor and acceptor phases are seen as better substitute to SDME. Membrane assemblies use porous or non-porous configurations to improve sensitivity and selectivity of extractions. Non-porous microporous membranes- MMLLE is a two-phase system with an aqueous and organic phase separated by a microporous hydrophobic membrane; aptly suited for hyphenation to on-line determination techniques. MMLLE provide advantage of multiple extractions along with possibilities of automation, though chances of cross contaminations are wide. Porous, hollow fiber- LPME applications are based on two phase or three phase modalities depending on presence of extraction phase in the lumen of fiber. In contrast to MMLLE; HF-LPME use inexpensive membranes capable of single extraction that prevent sample carry over effects, yet it is difficult to automate. Bolanos et al., 2008 suggested an HF-LPME approach based on UHPLCMS/MS determination using a final desorption solvent as an alternative to conventional two and three phase system. Analytes extracted from the sample in pores of hollow fiber is ultimately desorbed in methanol as suitable solvent and subjected to direct analysis. Selection of stripping solvent can be adjusted depending on varying polarity of the pesticides. Widely, most HF-LPME applications are based

on hollow polypropylene fibres of small pore size (0.2 μm) with high compatibility for range of organic solvents. Lately, polyvinylidene difluoride (PVDF) fibre with large membrane porosity has been applied to pesticide extraction in solid matrices. (Wang et al., 2012) developed a new method based on the use of PVDF hollow fibres for extracting pesticides from cucumbers. Pesticides extracted from the sample into the organic solvent are desorbed in a solvent mixture of methanol: water (1:1) prior to chromatographic analysis. Method was investigated for the usefulness of fibre sealing at ends through mechanical crimping. PVDF fibres resulted in higher extraction efficiency probably due to its higher porosity and solvent compatibility providing enrichment factor of the range 100-147.

Hollow fiber liquid phase microextraction (HF-LPME) is a much superior LPME technique for extraction of pesticides from solid samples. Xiong and Hu (2008) in their procedure compared performance of HF-LPME to dispersive liquid-liquid microextraction for analysis of organosulfur pesticides (OSPs) in environmental and beverage samples. HF-LPME was demonstrated to be more robust and sensitive choice than the DLLME for analysis of complicated samples. For real sample analysis, HF-LPME is more suitable for the analysis of a wide range of samples matrices without any prior filtration-dilution needed with DLLME.

Membrane-assisted solvent extraction MASE is a fully automated in vial membrane LPME technique. Extraction is brought by transfer of analytes from donor aqueous phase to acceptor solvent enclosed in a non porous membranous bag carried suspended in a headspace vial. An advantage of this technique is that it achieves clear phase separation (Schellin et al., 2004) by reducing the co-extraction of the matrix compounds from the samples from the samples. However the volume of the acceptor phase is typically much higher (100-800 μL) than the other LPME modalities and therefore large-volume injection (LVI), is required to reduce detection limits. The applicability of MASE combined with LVIGC-MS detection method to real samples has been examined by Schellin et al. (2004) for OPP's malathion, fenthion, bromophos, parathion-methyl, fenitrothion, bromophos-ethyl, fenamiphos, ethion to wine

and apple juice samples. Detection limits in the ng/l level were achieved using large-volume injection with injection volume of 100 μL .

CONCLUSION

Over the years, preference of analytical chemists has shifted toward short span, minimalistic extractions. Keenly there is an increasing need to integrate extractions with powerful chromatographic and electrophoretic determination for real time analysis of analytes and their sensitive estimation in single run. Moreover cleaner techniques such as microwave, sonication-assisted extraction using enhanced extraction kinetics have paved way to implement green chemistry principles solving out problems of ecotoxicity and solvent waste disposal. Time-tardy, non instrumental approaches have been replaced by instrumental alternatives and techniques with in-built isolation clean up capabilities (MASE, SDME). Promising novel extraction-cleanup techniques, such as solid-phase microextraction, liquid-phase microextraction provide the possibility of miniaturization due to smaller sample sizes used, automation, modularization, on-line, and onsite measurement, and high throughput; to match selective detections like mass spectrometry. Moreover, newer microextraction techniques have overcome constraints of selectivity and sensitivity, and provide high enrichment capabilities. Recent developments in food analysis have allowed full automation of extractions through direct online transfer of extracts; minimizing manual intervention, likely chances of contamination and analyte loss. Hyphenation of recently developed microextraction procedures has been reported to improve efficiency of extractions. In this context auxiliary extractions USE and MAE serve as good alternatives. Time since there is not any "universal" multiresidue method capable of analyzing all the possible combinations of pesticide/food. Developing trends in pesticide analysis of food; focus largely on removal of interferences from contributing matrix and subsequent recovery of analytes for their unequivocal estimation. In this respect, modern extraction methodologies offer tremendous promise. Accordingly considerable efforts need to be directed towards mining their full potential.

REFERENCES

- Ahmed F.E., 2001. Analyses of pesticides and their metabolites in foods and drinks. *Trends Anal. Chem.* **20**:649-661.
- Berrada H., Font G. and Molto J.C., 2004. Application of solid-phase microextraction for determining phenylurea herbicides and their homologous anilines from vegetables. *J. Chromatogr. A* **1042**: 914.
- Bolanos P. P., Romero-Gonzalez R., Frenich A.G. and Vidal J.L.M., 2008. Application of hollow fibre liquid phase microextraction for the multiresidue determination of pesticides in alcoholic beverages by ultra-high pressure liquid chromatography coupled to tandem mass spectrometry. *J. Chromatogr. A* **1208**:16-24.
- Bouaid A., Martin-Esteban A., Fernandez P. and Camara C., 2000. Microwave-assisted extraction method for the determination of atrazine and four organophosphorus pesticides in oranges by gas chromatography (GC). *Fresenius J. Anal. Chem.* **367**:291-294.
- Cai L., Xing J., Dong L. and Wu C., 2003. Application of polyphenylmethylsiloxane coated fiber for solid-phase microextraction combined with microwave-assisted extraction for the determination of organochlorine pesticides in Chinese teas. *J. Chromatogr. A* **1015**:1121.
- Chen Y.I., Su Y.S. and Jen J.F., 2002. Determination of dichlorvos by on-line microwave-assisted extraction coupled to headspace solid-phase microextraction and gas chromatography-electron capture detection. *J. Chromatogr. A* **976**: 349355.
- Chen L., Yin L., Song F., Liu Z., Zheng Z., Xing J. and Liu S., 2013. Determination of pesticide residues in ginseng by dispersive liquid-liquid microextraction and ultra high performance liquid chromatography-tandem mass spectrometry. *J. Chromatogr. B* **917**: 71-77.
- Curren M.S.S. and King J.W., 2002. Sampling and sample preparation for food analysis. *Comprehensive Analytical Chemistry Volume 37*, Edited by J. Pawliszyn. (Elsevier Science), USA: 869-894.
- Dong C., Zenga Z. and Lia X., 2005. Determination of organochlorine pesticides and their metabolites in radish after headspace solid-phase microextraction using calix [4] arene fiber. *Talanta* **66**: 721-727.
- Falqui-Cao C., Wang Z., Urruty L., Pommier J.-J. and Montury M., 2001. Focused microwave assistance for extracting some pesticide residues from strawberries into water before their determination by SPME/HPLC/DAD. *J. Agric. Food Chem.* **49**: 5092-5097.
- Farajzadeh M.A., Mogaddam M.R.A. and Aghdam A.A., 2013. Comparison of air-agitated liquid-liquid microextraction technique and conventional dispersive liquid-liquid microextraction for determination of triazole pesticides in aqueous samples by gas chromatography with flame ionization detection. *J. Chromatogr. A* **1300**: 70-78.
- Farajzadeh M.A., Khoshmaram L., 2013. Air-assisted liquid-liquid microextraction-gas chromatography-flame ionization detection: A fast and simple method for the assessment of triazole pesticides residues in surface water, cucumber, tomato and grape juices samples. *Food Chem.* **141**: 1881-1887.
- Fytianos K., Raikos N., Theodoridis G., Velinova Z. and Tsoukali H., 2006. Solid phase microextraction applied to the analysis of organophosphorus insecticides in fruits. *Chemosphere* **65**: 2090-2095.
- Granby K., Andersen J.H. and Christensen H.B., 2004. Analysis of pesticides in fruit, vegetables and cereals using methanolic extraction and detection by liquid chromatography tandem mass spectrometry. *Anal. Chim. Acta* **520**: 165-176.
- Hernandez-Borges J., Rodriguez-Delgado M.A., Garcia-Montelongo F.J. and Cifuentes A., 2004. Highly sensitive analysis of multiple pesticides in foods combining solid-phase microextraction, capillary electrophoresis-mass spectrometry, and chemometrics. *Electrophoresis* **25**: 2065-2076.

- Hernandez-Borges J., Cifuentes A., Garcia-Montelongo F. J. and Rodriguez-Delgado M.A., 2005. Combining solid-phase microextraction and on-line preconcentration-capillary electrophoresis for sensitive analysis of pesticides in foods. *Electrophoresis* **26**:980-989.
- Ho Y.-M., Tsoi Y.-K. and Leung K.S.-Y., 2013. Highly sensitive and selective organophosphate screening in twelve commodities of fruits, vegetables and herbal medicines by dispersive liquidliquid microextraction. *Anal. Chim. Acta* **775**: 58-66.
- Huang X., Sun Y., Meng F. and Liu J., 2004. New approach for the detection of organophosphorus pesticide in cabbage using SPME/SnO₂ gas sensor: principle and preliminary experiment. *Sensor Actuat. B* **102**: 235-240.
- Jimenez J.J., Bernal J.L., del Nozal M.J., Martin M.T. and Mayorga A.L., 1998. Solid-phase microextraction applied to the analysis of pesticide residues in honey using gas chromatography with electron-capture detection. *J. Chromatogr. A* **829**: 269-277.
- Kin C.M. and 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. *J. Chromatogr. Sci.* **47**:694-699.
- Kin C.M., Huat T.G. and Kumari A., 2006. Method development for the determination of pesticide residues in vegetables and fruits by using solid-phase microextraction. *Malays. J. Chem.* **8**: 67-71.
- Lambropoulou D.A. and Albanis T.A., 2003. Headspace solid-phase microextraction in combination with gas chromatography-mass spectrometry for the rapid screening of organophosphorus insecticide residues in strawberries and cherries. *J. Chromatogr. A* **993**: 197-203.
- Li M., Zhang J., Li Y., Peng B., Zhou W. and Gao H. 2013. Ionic liquid-linked dual magnetic microextraction: A novel and facile procedure for the determination of pyrethroids in honey samples. *Talanta* **107**: 81-87.
- Lopez-Mahia P., Muniategui-Lorenzo S., Fernandez-Fernandez E., Prada-Rodriguez D., Barriada-Pereira M. and Gonzalez-Castro M.J., 2005. Determination of organochlorine pesticides in horticultural samples by microwave assisted extraction followed by GC-ECD. *Int. J. Environ. Anal. Chem.* **85**:325-333.
- Matsadiq G., Hu H.-L., Ren H.-B., Zhou Y.-W., Liu L. and Cheng J., 2011. Quantification of multi-residue levels in peach juices, pulps and peels using dispersive liquidliquid microextraction based on floating organic droplet coupled with gas chromatography-electron capture detection. *J. Chromatogr. B* **879**: 2113-2118.
- Millan S., Sampedro M.C., Unceta N., Goicolea M.A., Rodriguez E., Barrio R.J., 2003. Coupling solid-phase microextraction and high-performance liquid chromatography for direct and sensitive determination of halogenated fungicides in wine. *J. Chromatogr. A* **995**:135-142.
- Moreno D.V., Ferrera Z.S. and Rodriguez J.J.S., 2007. SPME and SPE comparative study for coupling with microwave-assisted micellar extraction in the analysis of organochlorine pesticides residues in seaweed samples. *Microchem. J.* **87**: 139-146.
- Oliva J., Barba A., Vela N., Melendreras F. and Navarro S., 2000. Multiresidue method for the rapid determination of organophosphorus insecticides in grapes, must and wine. *J. Chromatogr. A* **882**: 213-220.
- Pan J., Xia X.-X. and Liang J., 2008. Analysis of pesticide multi-residues in leafy vegetables by ultrasonic solvent extraction and liquid chromatography-tandem mass spectrometry. *Ultrason. Sonochem.* **15**: 25-32.
- Papadakis E.N., Vryzas Z. and Papadopoulou-Mourkidou E., 2006. Rapid method for the determination of 16 organochlorine pesticides in sesame seeds by microwave-assisted extraction and analysis of extracts by gas chromatography-mass spectrometry. *J. Chromatogr. A* **1127**: 6-11.

- Pengyan L. and Yusong M., 2005. Determination of 36 pesticide residues in corn using gas chromatography mass spectrometry. *Chem. J. Internet [Online]* **7**: 13.
- Peruga A., Grimalt S., Lopez F.J., Sancho J.V. and Hernandez F., 2012. Optimization and validation of a specific analytical method for the determination of thiram residues in fruits and vegetables by LCMS/MS. *Food Chem.* **135**: 186-192.
- Ping L., Baoan S., Wubin S., Deyu H., Wei X., Linhong J., Gang L., Rongmao H. and Song Y., 2005. Ultrasonic extraction method for the gas chromatographic determination of organochlorine pesticides residue in some herbs. *Chem. J. Internet [Online]* **7**: 50.
- Pirsaheb M., Fattahi N. and Shamsipur M., 2013. Determination of organophosphorous pesticides in summer crops using ultrasound-assisted solvent extraction followed by dispersive liquid-liquid microextraction based on the solidification of floating organic drop. *Food Control* **34**: 378-385.
- Rezic I., Horvat A.J.M., Babic S. and Kastelan-Macan M., 2005. Determination of pesticides in honey by ultrasonic solvent extraction and thin-layer chromatography. *Ultrason. Sonochem.* **12**: 477-481.
- Rial O.R., Yague R.C., Cancho G.B. and Simal G.J., 2002. Solid-phase microextraction-gas chromatographic-mass spectrometric method for the determination of the fungicides cyprodinil and fludioxonil in white wines. *J. Chromatogr. A* **942**: 41-52.
- Rodriguez-Cabo T., Rodriguez I., Ramil M. and Cela R., 2011. Dispersive liquid-liquid microextraction using non-chlorinated, lighter than water solvents for gas chromatography-mass spectrometry determination of fungicides in wine. *J. Chromatogr. A* **1218**: 6603-6611.
- Sanagi M.M., Abbas H.H., Ibrahim W.A.W. and Aboul-Enien H.Y., 2012. Dispersive liquid-liquid microextraction method based on solidification of floating organic droplet for the determination of triazine herbicides in water and sugarcane samples. *Food Chem.* **133**: 557-562.
- Sanghi R. and Kannamkumarath S.S., 2004. Comparison of extraction methods by Soxhlet, sonicator, and microwave in the screening of pesticide residues from solid matrices. *J. Anal. Chem.* **59**: 1032-1036.
- Sanusi A., Guillet V. and Montury M., 2004. Advanced method using microwaves and solid-phase microextraction coupled with gas chromatography-mass spectrometry for the determination of pyrethroid residues in strawberries. *J. Chromatogr. A* **1046**: 35-40.
- Schellin M., Hauser B. and Popp P., 2004. Determination of organophosphorus pesticides using membrane-assisted solvent extraction combined with large volume injection-gas chromatography-mass spectrometric detection. *J. Chromatogr. A* **1040**: 251-258.
- Self R., 2005. Extraction of Organic Analytes from Foods - A Manual of Methods. RSC Food Analysis Monographs, Royal Society of Chemistry, Cambridge.
- Singh S.B., Foster G. D. and Khan SU, 2007. Determination of thiophanate methyl and carbendazim residues in vegetable samples using microwave-assisted extraction. *J. Chromatogr. A* **1148**: 152-157.
- Trujillo-Rodriguez M. J., Rocio-Bautista P., Pino V. and Afonso A.M., 2013. Ionic liquids in dispersive liquid-liquid microextraction. *Trends Anal. Chem.* **51**: 87-106.
- Urruty L. and Montury M., 1996. Influence of ethanol on pesticide extraction in aqueous solutions by solid-phase microextraction. *J. Agric Food Chem* **44**: 3871-3877.
- Wang J., Du Z., Yu W. and Qu S., 2012. Detection of seven pesticides in cucumbers using hollow fibre-based liquid-phase microextraction and ultra-high pressure liquid chromatography coupled to tandem mass spectrometry. *J. Chromatogr. A* **1247**: 10-17.

- Wang X., Cheng J., Zhou H., Wang X. and Cheng M., 2013. Development of a simple combining apparatus to perform a magnetic stirring-assisted dispersive liquid-liquid microextraction and its application for the analysis of carbamate and organophosphorus pesticides in tea drinks. *Anal. Chim. Acta* **787**: 71-77.
- Wang Z., Zhao X., Xu X., Wu L., Su R., Zhao Y., Jiang C., Zhang H., Ma Q., Lu C. and Dong D., 2013. An absorbing microwave micro-solid-phase extraction device used in non-polar solvent microwave-assisted extraction for the determination of organophosphorus pesticides. *Anal. Chim. Acta* **760**: 60-68.
- Wu J., Traga C., Lord H. and Pawliszyn J., 2002. Analysis of polar pesticides in water and wine samples by automated in-tube solid-phase microextraction coupled with high-performance liquid chromatography-mass spectrometry. *J. Chromatogr. A* **976**: 357-367.
- Xavier M.P., Vallejo B., Marazuela M.D., Moreno-Bondi M.C., Baldini F. and Falai A., 2000. Fiber optic monitoring of carbamate pesticides using porous glass with covalently bound chlorophenol red. *Biosens. Bioelectron.* **14**: 895-905.
- Xiao Q., Hu B., Yu C., Xia L. and 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.
- Xiong J. and 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. *J. Chromatogr. A* **1193**: 7-18.
- You X., Xing Z., Liu F. and Jiang N., 2013. Air-assisted liquid-liquid microextraction used for the rapid determination of organophosphorus pesticides in juice samples. *J. Chromatogr. A* **1311**: 41-47.
- Yu J., Wu C. and Xing J., 2004. Development of new solid-phase microextraction fibers by sol-gel technology for the determination of organophosphorus pesticide multiresidues in food. *J. Chromatogr. A* **1036**: 101-111.
- Zhang J., Liang Z., Guo H., Gao P., Lu R., Zhou W., Zhang S. and Gao H., 2013. Ionic liquid-based totally organic solvent-free emulsification microextraction coupled with high performance liquid chromatography for the determination of three acaricides in fruit juice. *Talanta* **115**: 556-562.
- Zhang S., Yang X., Yin X., Wang C. and Wang Z., 2012. Dispersive liquid-liquid microextraction combined with sweeping micellar electrokinetic chromatography for the determination of some neonicotinoid insecticides in cucumber samples. *Food Chem.* **133**: 544-550.
- Zhang J. and Lee H. K., 2010. Headspace ionic liquid-based microdrop liquid-phase microextraction followed by microdrop thermal desorption-gas chromatographic analysis. *Talanta* **81**: 537-542.
- 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. *J. Chromatogr. A* **1114**: 269-273.
- Zhao X., Xu X., Su R., Zhang H. and Wang Z., 2012. An application of new microwave absorption tube in non-polar solvent microwave-assisted extraction of organophosphorus pesticides from fresh vegetable samples. *J. Chromatogr. A* **1229**: 6-12.
- Zuin V. G., Schellin M., Montero L., Yariwake J.H., Augusto F. and Popp P., 2006. Comparison of stir bar sorptive extraction and membrane-assisted solvent extraction as enrichment techniques for the determination of pesticide and benzo[a]pyrene residues in Brazilian sugarcane juice. *J. Chromatogr. A* **1114**: 180-187.