International Journal of Agriculture, Environment & Biotechnology
Citation: IJAEB: 7(2): 331-342 June 2014
DOI: 10.5958/2230-732X.2014.00252.6

Entomology

Current Trends in Extraction Methodologies for Pesticide Residues in Food Matrices

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Paper No. 216 Received: November 20, 2013 Accepted: February 28, 2014 Published: May 28, 2014

Abstract

Inevitable presence of pesticides in foods demands development of efficient multiresidue methods for risk evaluation. Extraction of pesticide trace contaminants from foodstuffs is a laborious task. Development of reliable sample preparation procedures, characterized by the simplicity of both the operations and the devices involved in analytical process is need of the hour. Effective minimization of sample sizes; and the amount of solvents used in extraction is also a priority. Traditional solvent dedicated approach such as liquid- liquid extraction have been taken over by integrated techniques (SFE, SPE, MSPD) and automated microextraction based methods. Moreover, introduction of solventless techniques have become a benchmark to so-called "green chemistry", in analytical perspective. The review accounts upcoming trends and aspects of extraction methodologies, involved in pesticide analysis of food and future prospects in the view of same.

Highlights

- Recent advancements and introduction of microextraction techniques; SBSE, LPME in extraction method have given them edge over labor intensive, traditional, solid- liquid extractions.
- Automation of existing and novel extraction techniques aim at high throughput analysis.

Keywords: food sample, miniaturisation, automation

Deliberate change in food composition (Rakshit *et al.*, 2010) through pesticide application and its impact on public health emphasizes the need for efficient analysis. Regulatory limits set by legislative authorities' demand lowering of quantitation range of analytes, for trace and ultratrace level determination. Multiresidue analysis of pesticides demands multistage operations from sampling, sample preparation, extraction-partitioning, clean up; to final analysis (Self, 2005). Extraction process involved in sample preparation is the first major limiting step in the pesticide residue analysis with the aim to simplify a sample for further examination. Extraction in food sample requires removal of the pesticides from the matrix, following, subsequent removal of matrix interferences. Efficiency of this process depends on number of factors that largely include; extraction method, matrix type, comminution, pH, extraction solvent/s, water content, sample- solvent ratio, temperature, time of extraction, pressure, amount and type of salt added (Otles,2005). The recovery of pesticides, its stability and selectivity of procedure is principally a subset

of these factors; influencing the overall method performance. Importance of extraction cannot be overlooked keeping in view the impact of error generated in this analytical step that, even best suited separations cannot rectify (FAO/OMS, 1994). This paper examines relative advancements and improvisations, so far incorporated in existing as well as newly developed extraction approaches to synchronize them well with updated analysis thereby to achieve the analytical goals of trueness, precision, accuracy and proficiency.

On-Going Trends in Extraction

Prevailing trends in extraction methods intend towards effective miniaturization and hyphenation of ongoing procedures with an aim to achieve increased throughput. Automation would be beneficial in terms of decreased manual intervention and hence method performance time. It is preferable that sample preparation be achieved in minimal possible steps, as to decrease the possibility of contaminations or losses likely, during sample handlings.

Liquid-Liquid Extraction

Liquid-liquid extraction; LLE is officially most desirable method for extraction of pesticides from aqueous samples. Being a straight forward technique LLE requires minimum mechanistic skills complying with its inherent ease of use and alleviates baggage of special equipments as needed with other instrumental approaches. Sample preparation steps in LLE are subsequently reduced if extract is sufficiently free from matrix interferences (Puri, 2014 deatiled by author in an unpublished work) .

Extraction yield can hence be enhanced either by increasing the volume of solvent or by repetitive extraction with small portions of solvent (Mol *et al.,* 1995). A potential measure to improve the selectivity of extraction has been to utilize combination of solvents (Lacassie *et al.,* 1998). In general, less polar organic solvents such as hexane, DCM, acetonitrile favor extraction of more non polar pesticides through LLE. Mixture of these low polarity solvents into a water miscible solvent such as acetone can facilitate extraction of range of pesticides including highly polar ones. Lacassie *et al.,* (1998) used combination solvents for isolation of pesticides in apples and pears.5:2:3 ratio of acetone/DCM/ hexane provided advantage of extraction along with liquid –liquid partitioning effect LLP; maintaining method sensitivity and was equally efficient for low polar and non polar pesticide extraction.

For clean extracts a liquid –liquid partitioning (Saha *et al.,* 2012), post- extraction clean up-concentration step or both are required, especially in case of lipid matrices (Argauer *et al.,* 1997). One possible measure to delimit intensive clean up is by use of SPE minicolumns (Obana *et al.,* 1999). While elimination of LLP is likely through use of solvent mixtures such as, acetone/DCM/hexane, 1:2:1 (Huang *et al.,* 2007); online extraction effect of ethyl acetate based procedures (Pihlstrom *et al.,* 1999) can eradicate both LLP as well as clean up requirements. In addition to this use of sensitive mass spectrometric detections GC-MS^n, LC-MS^n obviates the need of extensive clean up prior to analysis (Liapis *et al.,* 2003). In a proposed method for multiresidue extraction from vegetables sample, 10 µl of LLE extract was directly injected for analysis without additional clean up step. A guard column alongwith carbofrit inserted glass liner in GC in MS-MS detection mode was efficient enough for selective determination (Vidal *et al.,* 2002). For extraction of polar pesticides different solvents were evaluated in a liquid chromatography based method (Mol *et al.,* 2003). It was found that ethyl acetate produced favourable response with respect to matrix effect with resultant extraction efficiency in range of 81-101%. At fortification level 0.01and 0.5 mg/kg level R.S.Ds lower than 11% were obtained.

Simple pH adjustment can improve efficacy and selectivity of LLE procedures. pH adjustment particularly enable better extraction of certain pesticide groups such as chlorophenoxycarboxylic acids, aryloxyphenoxypropanoic acids that are not amenable to techniques such as MSPD (Jehlickova *et al.,* 1991; Hopper *et al.,* 1992). In two separate works by Aguera *et al.,* (2004); Jansson *et al.,* (2004) NaOH was added to ethyl acetate and sodium sulphate mixture for multiclass determination of pesticides from fruits and vegetables. At pH < 4.5, addition of NaOH enabled extraction of more basic pesticides especially carbamates, N-methylcarbamates from acidic fruits (Jansson *et al.,* 2004) improving overall recoveries to 88 - 98%. Possible breakdown of certain pesticides in selective matrix, indicate matrix influence on the procedure. Large amount of solvents utilized for subsequent extractions makes automation difficult in case of LLE. Application of miniaturized procedure can reduce solvent requirements as well as environment concern. This can be achieved simply by reducing earlier dimensions or by developing completely novel techniques such as LPME; discussed forth. Online methods provide commendable scope for LLE miniaturization. An online microextraction method for isolation of 17 fungicides is exploited in this regard

(Navarro *et al.,* 2000). Solvent extraction was performed using acetone-DCM (1:1), 30 ml for grapes sample, 20ml for must and wine samples. High selectivity with individual LOD's less than nanogram range was obtained. Acceptable recoveries within limits and reproducibility upto 14% were obtained.

Supercritical Fluid Extraction

Supercritical fluid extraction **(**SFE) is suitable for extraction of target compounds from relatively dry samples. Use of supercritical fluid with high isolation and purification effect is utmost advantage of this technique. Supercritical fluid can efficiently diffuse through matrix as a gas and effectively dissolve analytes like a fluid without any solvent load (Lehotay, 1996). This prevents need of an exuberant evaporation step required in many traditional methods.

Method development is more difficult in SFE since more parameters need to be optimized. It also has disadvantage of manual operation in comparison to fully automated methods such as PLE (refer PLE). SFE results avidly depend on selectivity of matrix and nature of analyte, optimisation of extraction condition is hence advocated with each new class of pesticide or new matrix (Lehotay, 1996). Better recovery of several analytes in selective food matrices than others has been reported by Rissato and colleagues (2005). At temperature condition 70 °C, extraction pressures 44.935 MPa, and flow rate of 1.5 ml min-1 for SFE; pesticides not amountable by classical extractions such as imazalil, tebuconazole, triadimefon, chlorpyrifos and cypermethrin could easily be determined. Control of simple variables such as temperature, pressure and solvent polarity allow extension of extraction range of SFE. At lower pressures, constant temperatures are more efficient for less polar, lighter compounds, whereas higher pressures can extract large, more polar analytes. Elevated temperatures usually improve recoveries of compounds mainly due to better desorption from matrix. As an exception, decomposition of captan, captafol, chlorthalonil and diclorofluanid have been reported at higher temperatures yielding recoveries <70% (Ono *et al.,* 2006). It is proposed that pH adjustments using phosphoric acid can be done for such problematic compounds. Din *et al.,* 1997 investigated the use of modifiers, ion-pairing agents in an attempt to improve sulphonamide recoveries at lower density. Recoveries of the ionic metabolites were increased by up to 72% when employing tetramethylammonium hydroxide for ion pairing in-situ with SFE. Figure 1, lists several strategies involved to improvise SFE performance.

Generally, SFE of polar compounds is enhanced by addition of modifiers to the supercritical fluid (dynamic modifier) or the matrix (static modifier).A short static extraction time may improve recoveries especially for pesticides that are difficult to extract (Stefani *et al.,* 1997). Methanol as a static modifier improved recoveries of polar pesticides acephate and methamidofos (Aguilera *et al.,* 2003), on the other hand evaporation losses from acetone modification, has been reported for volatile pesticides dichlorvos, butylate, dichlobenil and 2, 6-dichlorobenzamide (Ono *et al.,* 2006). Combination of both static and dynamic modifiers methanol and acetone respectively, gave best result (Nerin *et al.,* 1998). Percentage of modifier should be kept as low as possible since presence of modifier reduces extraction selectivity and require a cleanup step prior to analysis. Water available in SFE itself acts as modifier causing polar pesticides to partition into (Kaihara *et al.,* 2000). Excessive water is unsafe for SFE in two ways. Water in sample can phase out polar compounds, in CO_2 it may cause restrictor plugging by ice formation and carried to chromatographic system. Removal of water can be done through lypholisation or adsorbent addition.SFE gives low recoveries for most polar as well as most non polar pesticides. Hydromatrix, cellulose, CF-1 and celite (Hopper and King, 1991) partially retain polar pesticides methamidophos, omethoate and acephate. Ratio of drying agent to sample effects recovery. Apart from its ratio, manner of mixing of adsorbent is also found to affect extractability of SFE. Kaihara *et al.,* (2000) followed stepwise addition of a dispersant then a drying agent in the sample to achieve reproducible results.

Quantitative extractions for fortified samples require mild SFE conditions, while at such instances low extraction efficiency result in case of real samples (Eller *et al.,* 1997). Analyte degradation and intense analyte-matrix interaction might be the cause of lower yields in field-incurred samples and can be eliminated using strong SFE conditions. For most food matrices SFE enable to obtain extracts that can be directly analysed without additional clean up. Integration of SFE to various separation techniques can provide greater scope for automation reducing operator intervention. SFE has been coupled to online chromatographic systems GC, LC and SFC through an interface. Automated online coupling of SFE/SFC/MS has been done for determination of carbamates (Voorhees *et al.,* 1998). System provides an on-line integration of extraction-cleanup and determination step.

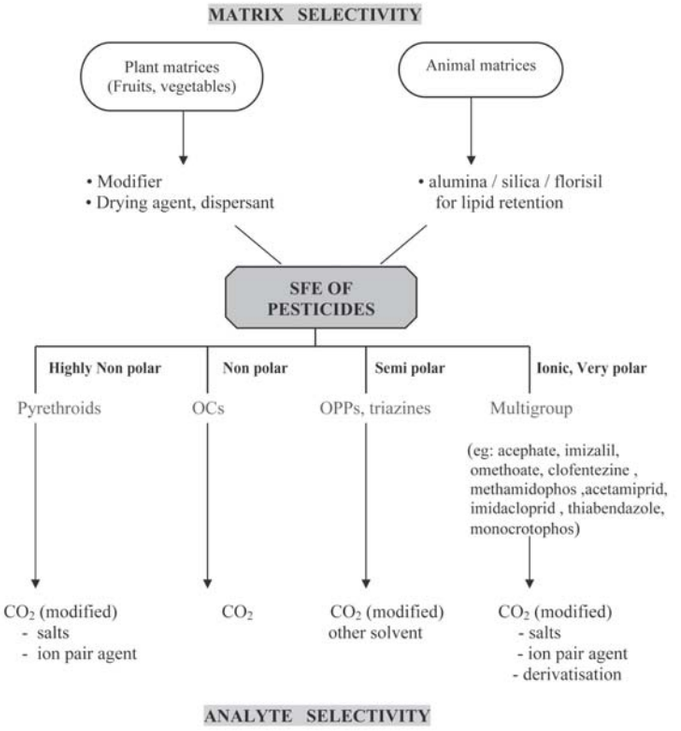

Fig. 1: Improvisation of extracton selectivity of SFE (Reproduced from Camel, 1998)

Pressurized Liquid Extraction

Pressurized liquid extraction, alternately called as acceleraled solvent extraction or pressurized fluid extraction is advancement in extraction of trace analytes from solid matrices. Pressure and temperature largely affect PLE efficacy similar to SFE, albeit selectivity constraints are less since PLE is relatively independent of matrix and analyte character and there are less parameters to be optimized.

Possibility to use preferably all traditional solvents and solvent mixtures extend its analytical range from polar to non-polar analytes. Thorough dessication of high moisture content samples is needed prior to extraction while a cleanup step is essentially required for fatty extracts. Automated offline GPC clean up was found to be more effective than SPE clean up for fatty vegetable matrix (Moreno *et al.,* 2006). PLE methods use high temperature and pressures (upto 200^0C, 20 MPa respectively); most thermally labile compounds tend to degrade at such intense conditions. At 110^0C thermal degradation of malathion, permethrin and diclofop-methyl has been reported in vegetable samples by Adou *et al.,* (2001).

Wet samples require high pressures that can enhance efficiency of PLE. In a study on organophosphates, orange juice samples provided poor precision and low recovery values, between 37-50% for polar pesticides methamidophos and acephate. Higher extraction pressure slightly added to recovery of these pesticides, whilst increasing extraction time or temperature did not show any effect (Obana *et al.,* 1997). Increase in temperature improves extraction kinetics at the cost of an increase in lipid coextracts for fatty matrices (Pihlstrom *et al.,* 2002). Direct hyphenation of PLE to chromatographic techniques is still under evaluation. Offline coupling of extraction to a chromatographic and an immunochemical method is done by Chuang *et al.,* (2001), in baby food samples. Extraction conditions were evaluated, ACN solvent at 80^0C under 2000 psi pressure was best suited followed by a clean up using SPE column. ASE-GC-MS and ASE-ELISA provided better results compared to an offline SFE. PLE can be combined online with LC/GC but so far, application based on organic solvents has not appeared. Direct transfer and online trapping is a problem when dealing with large solvent volumes in PLE extracts.

Selectivity of PLE is improved using sensitive separation and determination methods (Suchan *et al.,* 2004). In this respect, high resolution -GC was used for determination of organochlorines in fish samples. Different selectivity of parallel ECD columns used in gas chromatograph provided efficient monitoring and confirmation of less chlorinated analytes. Similarly, effectiveness of low pressure GC column has been investigated in high fat containing matrix by Moreno *et al.,* (2006). LP-GC improves analysis speed, curbing problem of analyte peak tailing. It's coupling to tandem MS enhanced sensitivity of PLE with LOD and LOQ values were much lower than set MRL's. LC-MS^n has come up as a powerful tool for unequivocal identification of PLE extracts. LC-IT-MS^3 has been applied to the identification and confirmation of carbosulfan and its metabolised products in citrus (Soler *et al.,* 2006). The product ion full scan mode provided unambiguous identification, even for cases in which standards were not available.

Matrix Solid- Phase Dispersion

Matrix solid phase dispersion extraction is a method for isolation of pesticides in foodstuffs with in-situ clean up effects for direct extract purity. MSPD has been applied to a broad range of target pesticides in diverse range of foods. Being a solid phase extraction based strategy MSPD samples are blended by adding suitable solid phase dispersant, into a glass mortar or pestle. Unlike SPE, samples are dispersed throughout the column, thus the extraction efficiency is enhanced as entire sample is exposed to extractant. Due to its clean up properties MSPD is used to improve upon LLE procedures. Being an equally effective method for solid and liquid samples MSPD has been sought as possible substitute to SPE and LLE methods.

Variety of solid sorbents including florisil, alumina (Filho *et al.,* 2006), aminopropyl (Garcia-Reyes *et al.,* 2007), diatomaceous earth (Chu *et al.,* 2005) and silica based sorbents (Garcia de Llasera *et al.,* 2005) have been used to homogenize samples. In general, 1:1 sorbent to sample ratio is used in MSPD, although 2:1 (Garcia-Reyes *et al.,* 2007), 4:1 ratios have also been investigated. It is believed that higher sorbent amount enable well dispersion of sample, attaining smaller particle sizes that improves extraction (Dorea *et al.,* 2004).MSPD offers faster performance with better reproducibility for polar pesticides such as imidacloprid, trichlorfon and carbendazim not amenable to SBSE microextration (Blasco *et al.,* 2002).

Down the time, MSPD has explored various aspects in improving extraction capacity. Miniaturisation is one such step that provides better analytical prospects and environmental concern. MSPD microextraction has been achieved by reducing sample size (upto 0.5 -10 mg), along with reduction in solvent and sorbent amounts. Despite well adopted microextractions, large volume MSPD has been examined for multiresidue analysis for 266 pesticides. 'Macro' procedure is applied to 10 gm apple juice sample, homogenized into inert sorbent and leached with 160 ml solvent mixture of hexane to DCM; 1:1.The procedure is labor taxing since longer pretreatments are demanded but added advantage to inhibit emulsion formation gives it edge above traditional MSPD procedures (Chu *et al.,* 2005). Choice of sorbent and solvent depends on analyte nature and matrix properties. Preferentially, sample matrix did not influence recoveries if lipid and protein content of matrix were less than 0.3% and 1.5% respectively (Torres *et al.,* 1997).

Simple alteration in water temperature ensures selective extraction of polar and medium-polar analytes such as atrazine and carbamates. Inspite of its in-built clean up properties some MSPD operations requires additional purification step. This can be achieved by extract evaporation followed by redissolution (Hercegova *et al.,* 2006), centrifugation (Libin *et al.,* 2007), or LLE from an aqueous extract into an organic solvent (Chu *et al.,* 2005) to a well availed SPE where in- line packing of sorbent (usually florisil or silica) in series of MSPD column can be utilised (Ferrer *et al.,* 2005).

Majority of MSPD applications using C_{18}, C_8 reverse phases use LC-MS determinations. Lately, use of these conjugated phase material has been extended for GC-MS methods (Navarro *et al.,* 2002). Tandem MS edges over simple LC-MS in its selectivity for analytes. Ultratrace estimations of analytes is made feasible with MSPD by use of such sensitive determinations .One another approach used efficient GC conditions for faster analysis. Fast GC was used for quantification of pesticide residues at ultratrace concentrations reducing analysis time to 7 min at constant resolution by reduction of column inner diameter (Domotorova *et al.,* 2005). Recoveries > 90% at 0.06 ng / kg concentration level and LOQs below 0.047 ng/ kg were reached utilizing ECD, except for diazinon. Except dimethoate all LOQs were lower than MRLs set for apple produce.

In an in-situ extraction of carbaryl in tomato samples, 'in-natura' determination (Caetano *et al.,* 2007) using an amperometric biosensor was compared to MSPD method.

Acceptable recoveries upto 83% could be obtained with LOD value of 3.2×10^{-6} g/l (much lower than that for biosensor) using an MSPD approach. Fervently, MSPD is taken for its resolution and robustness to produce higher recovery values with lower variabilities and practically eliminates problem of emulsion formation. Miniaturisation with automation of MSPD has been brought under one roof (Kristenson *et al.,* 2001) for determination of pyrethroid and organophosphorous pesticides in fruits. 25 mg homogenised sample was desorbed with 100µl ethyl acetate and subjugated to GC-MS. Recoveries exceeding 80% were obtained in apple samples amiss any clean up step. Effective miniaturizations provide room for automation of MSPD albeit little work has been done in this direction. As yet there is no considerable work that has implemented direct transfer of MSPD extracts onto a LC or GC apparatus.

Liquid Microextraction Methods

Miniaturisation and automation of liquid –liquid extraction can be well realized through liquid phase microextraction, LPME procedures .These include novel micro-LLE methods using immensely small amounts of extraction solvent that enable direct introduction for instrumental analysis. LPME has a defined range of application for compounds with partitioning coefficient between solvent and sample ; $K_{org/s}$ >500.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.

Recent approach termed as single drop microextraction uses solvent microdrop suspended from the tip of a conventional micro syringe and is immersed in a sample solution (Direct immersion- SDME) in which it is immiscible or suspended in the head space above the sample (HS-SDME). 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. So far, attention dedicated to HS-SDME in literature focus specifically on more volatile compounds but none dealing with pesticide analysis. Hitherto, HS-SDME methods are in nascent stages of development. Application of HS -SDME to pesticide analysis can possibly be realised through effective matrix and sample manipulations. In an application for analysis of OPP's in liquid sample Zhao *et al.,* (2006) optimized various parameters of DI –SDME; highest extraction efficiency was achieved using1.6 µl toluene microdrop at a stirring rate of 400 rpm for 15 min .Subsequent dilution of orange juice sample prior to extraction enabled ultratrace quantification of pesticides. Similar results were confirmed in another application considering water and fruit juice samples (Xiao *et al.,* 2006). Xiao and group evaluated DI-SDME in recycling mode as a variant to continuous-flow micro-extraction (CFME) cycling waste from sample back to sample vial. Compared to cyclic system, static SDME provided better sensitivity and precision values particularly for real sample analysis. In the procedure, a pH range 5-6 was found suitable for OPP extraction. Addition of salt gave variable results depending on type of analyte; salt addition was avoided to forgo its overall negative effect on recoveries of major analytes.

Polymeric membrane supports at an interface between the donor and acceptor phases are seen as improved alternative to SDME. These membrane based LPME procedures vouch for their increased sensitivity and reproducibility, eliminating sample carry-over effects. Microporous membrane based extractions use pump system to percolate fresh sample onto the membrane. These have advantage of multiple extractions using single membrane at a time and are aptly suited for automation. Hollow fiber LPME, HF-LPME is a robust approach to membrane liquid phase micro extraction that does not require expensive membranes and tardy instrumentation. In HF-LPME single membrane cannot be extended for multiple extractions. This in turn prevents chances of cross contamination to occur. A novel alternative to these modalities (Bolanos *et al.,* 2008) has been use of a final stripping solvent such as methanol into which analytes extracted in the pores of fiber, from the sample; is ultimately desorbed and subjected to direct analysis. High selectivities for membrane based modalities are achieved at the expense of very slow extraction kinetics (Xiong and Hu, 2008). In this matter ternary solvent (water/disperser solvent/extraction solvent) based dispersive liquid- liquid microextraction assure efficient equilibrium based extractions. For complex matrix composition; DLLME procedures however demand prior filtration - dilution that often compromise with method sensitivity. This problem is proposedly overcome by use by adjuncting DLLME with other sample clean up techniques; such as SPE that result in enrichment upto 200 times (Montes *et al.,* 2009). Desirable initiatives are now exploring potentials of DLLME for complex solid matrices but much work need to be done to expand the technique for real sample analysis making it adept for automation.

Solid-Phase Extraction

Solid phase extraction is extensively used isolation, clean up procedure for purification and concentration of analytes from environmental and biological samples. SPE has gained importance due to wide choice of sorbents available for extraction of analytes with diverse polarity range. Analyte isolation is achieved using a particulate sorbent, packed into minicolumns commonly referred as cartridges or immobilized on a membrane as discs, the former being elaborately used for food analysis while disc configurations are mostly limited towards water samples. SPE process involves four distinct steps; conditioning of sorbent with an organic solvent , adsorption / sample application, washing of interferences and finally elution of analytes with a solvent compatible for analysis.

Preconditioning of SPE cartridge has been eliminated using commercially available cartridges such as Varian Nexus SPE used for high sugar foods and diatomaceous earth material Extrelut-NT20 (Muccio *et al.,* 2006) for analysis of neonicotinoids in agricultural produce. Choice of adsorbent used in SPE is determined by its selectivity for analytes, type of food matrix and nature of interferences. Reversed-phase (C8, C18), ion-exchange (anion / cation exchange), or normal-phase (silica, florisil, cyano, diol, amino) packings are widely used in various applications for analysis in food. Compared to most sorbents (alumina, diol, cyano), silica based sorbent are a widely preferred choice because of their high extract purity and obtainable recovery values (Otero *et al.*, 2003). SPE has innate inability to deal directly with solid samples; vegetable, fruits, grains. It thus requires prior homogenization, filtration/centrifugation and liquid liquid partitioning with water-miscible solvents to be performed. Matrix interferences can be removed using dual -SPE column in tandem (He and Liu, 2007). Very recently, suitable improvements to classical SPE have expanded the supremacy of this technique to solid food extractions. These include use of 'dispersive- SPE' procedures such as QuEChERS and disposable pipette extractions, DPX. A worthy advantage of QuEChERS is its useful approach for analysis of pesticides of diverse polarities (Anastassiades *et al.,* 2003) due to the ability of sorbent to bind matrix interferences strongly without interacting with target analytes. In DPX, the sorbent is contained inside a disposable pipette tip and is thoroughly mixed with sample solutions. Rigorous mixing for disposable pipette extractions uses less sorbent, eliminating solvent evaporation step, thus result in faster extraction of analytes compared to QuEChERS procedure (Guan *et al.,* 2010).

New materials used in SPE involve mixed mode sorbents as hybrid materials having both reverse phase and ion-exchange properties. Selective extraction has been achieved using Oasis- MAX mixed mode SPE material (Carpinteiro *et al.,* 2010) for LC–MS detection of fungicides in wine samples. Melo and group (2004) achieved better performance parameters using in-house amino functional PDMS material compared to C18 based and commercially available phases for multiclass fungicides in fruits. This procedure is equally effective for polar pesticides tebuthiuron, benomyl, and simazine. Very recently, carbon nanotubes have gained popularity over commonly used SPE formats. Speedy extraction capability of multiwalled carbon nanotube MWCNT compared to single walled nanostructures makes it promising sorbent for various solid-phase extractions (Lopez- Feria *et al.,* 2009). Aromatic organophosphorous compounds can strongly bind to the MWCNT surface due to its heterogeneous structure and strong affinity for phosphoric group enabling quick single step extractions (Du *et al.,* 2008).

Highly selective SPE based procedures utilize group or analyte specific stationary phases such as immunosorbents (IASPE) and molecularly imprinted polymers (MISPE) that eliminate need for much selective determinations, refer table 1.

Immunoaffinity based adsorbents rely upon molecular recognition using natural antibodies with a strong affinity for the analyte or structurally related analytes including their metabolites. Immunosorbents in pesticide analysis have been developed for determination of triazines (Dalluge *et al.,* 1999), phenylureas (Pichon *et al.,* 2004) and imazalil fungicide (Watanabe *et al.,* 2001) in beverages & fruits with monoclonal or polyclonal antibodies covalently immobilized on cellulose, silica and CNBr- activated agarose supports respectively. Compared to classical reversed phase-SPE better selectivities were obtained by IAE. Sensitivity of immunosorbents is susceptible to organic solvents, pH and even large amount of high molecular weight interferences. Unlikely, cross reactivity of antibody in immunoassays can fervently result in false positives. Molecularly imprinted polymers MIP's, have been developed as synthetic antibody-mimics to overcome paucities of IAE sorbents. Online coupling of MIP based SPE to liquid and gas chromatography is possible; enabling full automation of procedure. Direct subjection of dilute sample is possible for online analysis using a multiport

Table 1: Emerging strategies in extraction methods for pesticides in various food matrices

Technique/Food	Pesticide	Extraction Conditions	Determination	Reference
SPE		**Sorbent**		
Orange juice	s-triazines	Immunosorbent	on-line SPE-GC-FID/NPD	Dalluge *et al.,* 1999
Lemons	ionisable pesticides	RP-cartridges	HPLC-UV	Prousalis *et al*., 2006
Bovine liver	Atrazine	MIP	HPLC, ELISA	Muldoon *et al*., 1997
Garlic	OP's	Multiwall Carbon nanotube	square-wave voltammetry	Du *et al*., 2008
SBSE		**Sorbent**		
Fruits, Vegetables	Multiclass	PDMS (0.5mm)	[5](RTL) GC-MS	Kende *et al*., 2006
Cucumber, potato	OPPs	OH –PDMS (20µm)	GC-[6]TSD	Liu *et al*., 2005
Fruit juice	OC's,OPP's	PPESK (250 µm)	GC-TSD	Guan *et al.,* 2008
MSPD		**Solvent, Sorbent**		
Rice	OPPs (2), OCs	et ac, neutral alumina	GC-ECD	Dorea andSobrinho,2004
Apples	Multiclass	et ac/ dcm, florisil	GC-ECD/MS	Domotorova *et al*., 2005
Fruits, Vegetables	Fungicides (8)	et ac, C_{18}-silica	GC-ECD/NPD, GC-MS	Navarro *et al.,* 2002
Olives	OPPs (9), OCs Pyrethroids, ureas Triazines	ACN, NH2 -propyl	LC-MS	Ferrer *et al*., 2005
SFE		**Solvent, Sorbent**		
Produce [9]HMX/$MgSO_4$/H_2O	Multiresidue	CO_2 (320 atm, 60^0C),	GC-ITD	Eller and Lehotay, 1997
Beef, chicken meat	Carbamates	CO_2 (219 atm, 90^0C), HMX	On-line SFE-SFC-MS	Voorhees *et al*., 2008
Fresh fruits, rice, Vegetables	Pesticides (18)	CO_2 (acetone modified) (300kg/cm^2, 40^0C), Arasorb®S-310	LC-MS	Kaihara *et al*., 2002
PLE		**Solvent, Sorbent**		
Fish	OCs	hx/ac (1:1), hx/dcm (4:1), anhy.Na_2SO_4	GC-ECD	Suchan *et al*., 2004
Avocado	Multiresidue (65)	et ac/cyclo hx (1:1), HMX	LP-GC-MS[2]	Moreno *et al.,* 2006
Oranges	Carbosulfan and metabolites	dcm ,anhy.Na_2SO_4	LC-MS[3]	Soler *et al*., 2006

[1]organophosphorous ; [2]organochlorines ; [3] acetone; [4] ethyl acetate ; [5] Real time locking; [6] thermionic specific detection ; [7] acetonitrile; [8] ion-trap mass spectrometric detector; [9] hydromatrix; [10]hexane
References Puri (2014) , unpublished work

switching valve system (Hantash *et al.,* 2006). SPE is more robust, rapid, and sensitive technique when compared to its improved upon counter parts such as SBSE. As a popular clean up – preconcentration approach for food analysis SPE has found tremendous potential for automation. Current initiatives in SPE aim towards development of novel selective phases for trace enrichments, which will require time demanding characterization and validation.

Stir-Bar Sorptive Extraction

A sorption based technique stir bar sorptive extraction (SBSE), marketed commercially by Gerstel under the trade name "Twister" utilizes magnetic bars (1 or 2 cm long) coated with a 0.5 or 1mm layer of polydimethylsiloxane (PDMS) phase to pre-concentrate analytes from liquid samples. A back extraction into suitable organic solvent can be carried out for SBSE for separation with; liquid chromatography (LC), gas chromatography or capillary electrophoresis (CE). Alternatively, SBSE extracts can be analysed thermally by online desorption on a GC or GC-MS. For this an extensive thermal desorption unit coupled to chromatograph is required. Despite scope of automation the desorption step requires stir bar to be transferred manually to the desorption unit. This might compromise proficiencies attributed to extended sorbent dimension.

Numerous parameters differently affect analyte recoveries in SBSE. Often for hydrophobic analytes, salt addition results in decrease in recovery values while for polar analytes, salt additions (usually 30% NaCl) increases SBSE recoveries considerably. Multiresidue methods, considering analytes with varying polarities under single SBSE regime could thus be problematic. A probable solution is performing SBSE in multi-shot or dual mode. Using this approach,

Ochiai *et al.,* (2006) carried out two individual SBSE extractions simultaneously, for a 20 ml brewed green tea sample with 30% NaCl and another 20 ml aliquot without modification (100% sample solution). One extraction can be optimized for hydrophilic analytes of medium and high polarity with salt addition and the other extraction with addition of organic modifier (methanol, 20%) to the sample targeting hydrophobic analytes. Two extraction bars are then simultaneously desorbed with a thermal desorption system. The desorbed compounds are analyzed using low thermal mass LTM- fast GC-MS. Appreciable linearities with high sensitivity LOD's less than10 ng/ l are obtained for most of the target pesticides. Another adaptation of dual SBSE is described by Sasamoto *et al.,* (2007) in their work on 82 multiclass pesticides. Dual-column separation on fast GC was performed in a single injection; this enabled improved identification capability of analytes within short analysis time. Solid, non-fatty foods <3% fat; require a pre-exrtaction step with addition of solvent and subsequent aqueous dilution, prior to SBSE. Extraction of compounds present in higher concentrations can be problematic with SBSE and often results in matrix overload. SBSE coupled to retention time locked, GC–MS applications enable accurate identification of trace analytes from complex matrix profiles (Kende *et al.,* 2006). Retention Time Locking, a special feature of the Agilent MSD ChemStation software contains expected retention time of each compound thus can screen pesticides in samples without the need of standard calibrations.

So far, since PDMS an apolar sorbent has remained a universal SBSE phase, only those solvents which do not solublise PDMS can be adopted. Limited enrichment capability of PDMS for polar pesticides from complex matrices such as imidacloprid, carbendazim and trichlorfon for orange (Blasco *et al.,* 2002) and iprodione for wine samples (Sandra *et al.,* 2001) have been reported. Quoted constraints of PDMS phase restricts the widespread of this state-of- art technique for food analysis.Over the recent years focus has been shifted towards development of new generation phases, offering longer lifetime and thermal stabilities, particularly suitable for polar analytes. In this view combinational 'Twister' using dual- phase have been described (Barletta *et al.,* 2011). Combinational twisters can open avenues for class selective extractions based on analyte specific packings used as inner phase materials. Poly-phthalazine ether sulfone ketone (PPESK) coated stir bars have proved to show higher affinity towards polar compounds than PDMS coatings for isolation of OPP'S in fruit juices (Guan *et al.,* 2008).SBSE is highly reliable approach scarcely affected by matrix influence (Blasco *et al.,* 2004). However, the technique suffers at the level of extracting thermally susceptible, polar analytes with acceptable enrichment. Moreover, precision, reproducibilties and resolution offered by the technique are less at par with other reported extraction techniques (refer SPE). At any instance SBSE is a highly promising approach, with ease of rapid and sensitive extractions ideally suited towards ultratrace isolations from foods for routine applications.

Conclusion

Overriding concern of precision and accuracy requires development of analytical procedures to equip well with efficient detections. In this respect, the importance of sample preparation for pesticide multiresidue analysis has long been recognized. Over the years, a variety of applications for widely different analyte/ matrix combinations have been published to demonstrate the practicality of the various approaches. To date, there is no single committed method to cover most; if not all analytes and matrix combinations in food analysis. Matrix interference is hitherto a major roadblock when developing rapid streamlined analytical methods employing minimal sample preparations.

In recent years, the conventional methodologies are gradually being taken over by modern instrumental extraction techniques. They are typically more carefully designed and more complex. In general modern extraction techniques are frequently easier to operate compared to conventional methods but provide optimization challenge. Lately, focus has also been onto development of automated, computerized analytical instruments that aim at minimizing analysis time and data handling steps. Moreover, attention has been devoted to development of integrated analytical systems which can couple sample pretreatment; separation and detection in one go. Good selectivity is required to minimize the risk of detection problems, and rapid extraction should therefore aim at obtaining high-throughput analysis. Thus, there is need to further accelerate and automate sample pretreatments. With automation and computerization of analytical instruments, the onus for precision and accuracy rests on sample preparation; more than ever before. The main aims have been, and still remain, to achieve fast, accurate and sensitive analysis.

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