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REVIEW

Review of SBSE Technique for the Analysis of Pesticide Residues in Fruits and Vegetables

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Abstract Stir bar sorptive extraction (SBSE) is a microextraction technique, introduced to overcome the problem of limited extraction capacity and fragile fiber coatings inherent in the solid phase microextraction technique. The major limitations of the SBSE technique are that only polydimethylsiloxane has been commercially available, this reduces its use to non-polar analytes, and its tedious reconstitution step which can lead to loss of analytes and introduction of impurities. The current trend has been aimed at the use of other materials, some of which are commercially available, such as restricted access materials, carbon adsorbents, molecularly imprinted polymers, ionic liquids, microporous monoliths, sol–gel prepared coatings and dual phase material. This has greatly helped in widening the applications of SBSE for pesticide analysis in fruits and vegetables and other matrices. The introduction of a thermal desorption unit which eliminates the reconstitution step of the stir bar in organic solvents before instrumental analysis has helped to automate the extraction method online with gas chromatography. This paper reviews the use of SBSE in pesticide residues analysis in fruits and vegetables, with a view on sample preparation steps, method optimization and validation of analytical figures of merit.

Keywords Gas chromatography · Mass spectrometry · Stir bar sorptive extraction · Pesticide residues · Fruits · Vegetables

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Introduction

Stir Bar Sorptive Extraction (SBSE)

SBSE is a microextraction technique similar to solid phase microextraction (SPME) [1–4], but with a greater extraction capacity. It helps to overcome the small volume of the coated SPME fiber for a better enrichment factor and it delivers better sorptive-phase mass and higher surface area as a result of larger volume of the polydimethylsiloxane (PDMS) [5–7]. In the SBSE technique, a 10–40 mm long magnetic stir bar coated with thick layer (about 50–300 μL) of PDMS liquid phase is used as the extracting phase [8–10]. The mechanisms of SBSE are similar to those of SPME but differ in the design of extraction system, with SBSE having higher enrichment factor, which is determined by the amount of extractive phase. The extracted analytes are adsorbed on the PDMS coated rod, by stirring the sample solution with the rod for a given time. The rod is removed from the sample and the adsorbed analyte can be desorbed thermally into GC system, which provides high chromatographic resolution and better sensitivity or by means of liquid solvent into LC system for improved and better selectivity [6, 11–13].

The major limitation of SBSE technique is the polarity of PDMS (non-polar liquid), implying that it is best used for the analysis of low polar analytes, because the recovery will be low for highly polar analytes [11, 14]. The low recovery obtained in highly polar analytes is due to the weak hydrophobic interaction. Other limitations include longer desorption time (due to the large volume of the PDMS), and the need to reconstitute the extracted analytes on the stir bar, since it cannot be injected directly into the split/splitless injector port of the GC [12, 13, 15]. The tedious reconstitution step can lead to loss of analytes and

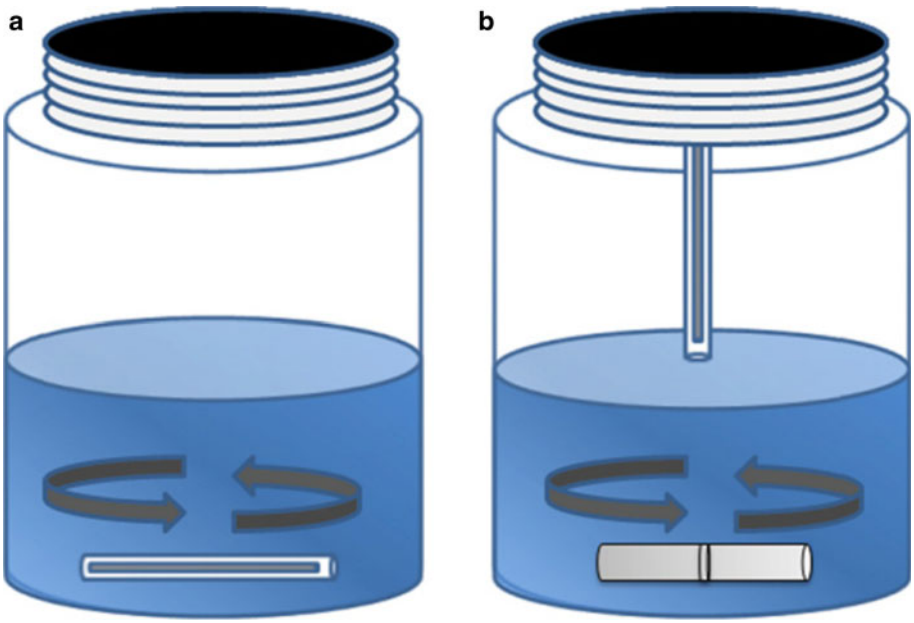


Fig. 1 Schematic diagram of the extraction modes in SBSE **a** DI and **b** HS [12]

introduction of contaminants. The introduction of a thermal desorption unit (TDU) online to the GC system [16] has helped to address this problem. The polarity of PDMS has been addressed by employing in situ derivatization [17] or the use of other phases such as restricted access materials (RAMs), carbon adsorbents, ionic liquid, dual phase materials, porous monoliths, molecularly imprinted polymers (MIPs), microporous membranes and sol–gel prepared coatings [14, 17–30], has helped to extend the technique for the analysis of a wide range of analytes from complex matrixes.

The SBSE techniques can also be carried out in both direct immersion (DI) and headspace (HS) mode [12, 31], depending on the complexity of the sample matrix (Fig. 1). Like SPME, sorptive extraction depends on the partition coefficients of the analyte between the coated stir bar and the sample matrix. The partition coefficients have been correlated with the octanol/water distribution coefficient of the target analyte, which is an indication of the efficiency of SBSE to extract a given analyte [11, 12, 32–34]. It has been found that high recovery can be achieved in hydrophobic solutes, while hydrophilic solutes results in low recovery [34], depending on the analyte's octanol/water partition coefficients ($K_{o/w}$). The SBSE technique can also the carried out in batch extraction and sequential analysis, this involves simultaneous extraction, separation and detection of up to 60 samples on stir bar plates and subsequent injection into the fast GC system. The fast GC system [called low thermal mass (LTM) GC] provides fast temperature programming rates and rapid cool-down, thus increasing the analysis throughput and short equilibration time [35].

Theory of SBSE

In SBSE, the extraction equilibrium depends on the phase ratio (β), and the volume of the coating on the stir bar (PDMS). This can be represented by the distribution coefficient of the analyte between PDMS and the sample matrix, using Eq. 1 which can be used in combination with the mass-balance equation (Eq. 2) [12], to calculate the extraction recovery (R %), under full equilibrium conditions [11, 33, 36], provided all factors have been fully optimized.

$$K_{\mathrm{PDMS,w}} = \frac{C_{\mathrm{PDMS}}}{C_{\mathrm{S}}} = \frac{m_{\mathrm{PDMS}}}{m_{\mathrm{w}}} \times \frac{V_{\mathrm{w}}}{V_{\mathrm{PDMS}}} = \frac{m_{\mathrm{PDMS}}}{m_{\mathrm{w}}} \times \beta \quad (1)$$

$$m_{\mathrm{w,o}} = m_{\mathrm{PDMS}} + m_{\mathrm{w}} \quad (2)$$

combining Eqs. 1 and 2, yields Eq. 3

$$R = \frac{m_{\mathrm{PDMS}}}{m_{\mathrm{w,0}}} = \frac{K_{\mathrm{PDMS,w}}}{K_{\mathrm{PDMS,w}} + \beta} \quad (3)$$

where C_{PDMS} is the concentration of analyte in the PDMS coated stir bar, C_{S} is the concentration of analyte in the sample matrix, $m_{\mathrm{w,o}}$ is the initial mass of the target analyte in the sample matrix, m_{PDMS} and m_{w} are the masses of the target analyte in the coated stir bar and in the sample matrix respectively, while β is the phase ratio, which is defined as the ratio of volume of the stir bar coating and the volume of sample solution. It can be observed in Eq. 3, that increase in the volume of stir bar coatings increases the phase ratio and improves the extraction efficiency.

The variation of the concentration $C_{\mathrm{PDMS}}(t)$, of the target analytes in the stir bar coatings as a function

extraction time t, gives the kinetic of extraction and can be expressed as [12]:

$$C_{\mathrm{PDMS}}(t) = C_{\mathrm{w},0} \times \frac{k_1}{k_2} \times (1 - e^{-k_2 \times t}) \quad (4)$$

where $C_{\mathrm{w},0}$ is the initial concentration of analyte is the sample matrix and k_1 and k_2 are the uptake and elimination rate constant respectively.

Factors Affecting SBSE

In the development of the SBSE method, the same factors as in SPME [3, 37–39] needed to be optimized for efficient extraction, high recovery and enrichment factors, and low detection limits. These factors include the type, volume and size of the stir bar coating, extraction time, pH, salt addition, stirring rate, extraction temperature, sample volume and addition of organic solvents [10, 12, 13].

The volume of stir bar coatings affects the efficiency of the SBSE technique. The higher the volume of stir bar coatings the higher the sensitivity for more polar compounds with low octanol/water partition coefficients, but the volume was found to have no significance difference for low polar or non-polar analytes with high octanol/water partition coefficients values [12, 14, 40, 41].

SBSE is an equilibrium extraction technique; therefore, the extraction time must be fully controlled and optimized. The extraction time, studied to obtain an equilibrium extraction, can be determined at different stages of method development, but its optimization is better conducted after other variables have been fixed and optimized [12], since equilibrium extraction yield better sensitivity and precision. In order to minimize extraction and analysis time, SBSE can be conducted under non-equilibrium conditions, but this could lead to low precision and sensitivity [33, 40–43].

The pH and ionic strength of the sample matrix is important in method development. The pH should be adjusted in analytes with acidic or basic properties, in order to obtain the analytes in a non-ionic form. The pH adjustment should be between 3 and 9, to avoid degradation of the coatings on the stir bar [12, 44, 45]. The addition of inert salts such as NaCl modify the ionic strength of the sample matrix, and improve extraction by decreasing the solubility of polar analytes [16, 35, 46], while it was observed to reduce extraction efficiency of non-polar analytes [42], as a result of an increase in viscosity of the sample matrix [47], occupation of the coated stir bar surface by the salt [44], and ion-pairing interactions between the non-polar analytes and the inert salts [12].

The agitation of the sample matrix increases the diffusion of analytes by decreasing the thickness of the boundary layer between the analytes in the sample matrix and the coated stir bar. The agitation rate must be carefully optimized, because higher stirring rates (>750 rpm) may lead to air bubble formation and can cause stripping of the coatings on the stir bar due to its contact and friction against the bottom of the sample vial [22]. Higher stirring rates (≥1,000 rpm) can be achieved when using coatings that are stronger than PDMS [44, 45], without stripping of the coating on the stir bar.

Extraction at an elevated temperature increases the partitioning of analytes between the coated stir bar and the sample matrix, which increases extraction efficiency, thereby decreasing the equilibrium time [22]. Although temperature has dual effects, an increase in temperature also decreases the sorption distribution coefficients and the lifetime of the coated stir bar [12, 22]. Therefore, compromise must be made between extraction efficiency and lifetime. Thus, an adequate and optimal extraction temperature must be selected based on the nature of the target analytes and the nature of the sample matrix.

Finally, the extraction efficiency depends on sample volume which is related to the phase ratio. Higher sample volumes increase chromatographic response for non-polar analytes, while it has no significance effect on the extraction of polar analytes [12]. The presence of organic solvents such as methanol and acetonitrile in the sample matrix helps to minimize loss of analytes due to the adsorption on the walls of sample vials [48]. Organic solvent addition can also decrease extraction efficiency by increasing the solubility of more polar analytes. The volume of organic solvent must be minimized (about 5 %), at higher percentage, and depending on the nature of the sample matrix, it reduces the affinity of the coated stir bar for the target analytes [49].

Applications of SBSE in Pesticide Residues Analysis in Fruits and Vegetables

The use of the SBSE technique has been employed by many researchers in the extraction and subsequent chromatographic analysis of different classes of pesticide residues in fruit and vegetable samples (Table 1), although the method was originally developed for the extraction of contaminants in water samples.

The SBSE was first used for the analysis of 10 pesticide residues in orange by Blasco and his coworkers. The method was compared with matrix solid-phase dispersion (MSPD) in terms of recoveries, relative standard deviation and limits of detection. The orange sample was prepared by chopping and homogenized 200 g representative samples. For SBSE procedure, a 5 g portion was weighed and placed into 25 mL flask and homogenized 5 mL each of methanol and water (containing 50 mg mL^{-1} of NaCl) for 15 min. The mixture was then filtered and the resulting filter cake

Table 1 Stir bar sorptive extraction for analysis of pesticide residues in fruits and vegetable

Pesticides class	Matrix	Org. solv., vol (mL)	SB phase	Ext. time (min)	Ext. temp. (°C)	NaCl (%)	pH	St. rate (rpm)	LOD (μg kg^{-1})	LOQ (μg/kg)	Rel. rec. (%)	RSD (%)	LR (μg/kg)	Detector	References
10 MCPs	Orange	MeOH, 5	PDMS	120	NR	5	NR	900	NR	1–50	8–84	4–16	1–6,000	LC–MS	[16]
17 MCPs	Lettuce, pear, grape	MeOH, 30	PDMS	60	NR	NR	NR	1,000	NR	NR	43–75	4.6–8.8	5–200 μg L^{-1}	TD-GC–MS	[50]
5 MCPs	Grape	H_2O, 25	PDMS	120	NR	30	NR	900	NR	10	15–100	10–19	10–1,000	LC–MS	[51]
85 MCPs	Tomato, cucumber, green soybean, grape	MeOH, 100	PDMS	60	R.T.	NR	NR	1000	0.63–26	NR	NR	NR	4–100	TD-GC–MS	[48]
12 OPPs	Cucumber, potato	AcO, 15	PDMS-OH	30	30	30	NR	600	0.007–0.15	NR	NR	NR	0.25–50	TD-GC-TSD	[22]
8 MCPs	Strawberry, lettuce, tomato	AcO/H_2O, 10	PDMS	240	NR	40	NA	900	1 mg kg^{-1}	NR	12–47	3–17	1–100 mg kg^{-1}	MEKC-DAD	[52]
11 MCPs	Strawberry, apple, lettuce, tomato	AcO/H_2O, 10	PDMS	60	NR	NR	NR	850	0.5–30	NR	NR	5–25	NR	TD-GC–MS	[53]
52 MCPs	28 Fruits and vegetables	MeOH/H_2O, 10	PDMS	60	NR	NR	NR	1000	NR	NR	NR	NR	NR	NR	[54]
8 OPPs	Grape, peach	H_2O, NR	PPESK	30	40	NA	NR	600	0.17–10.3 ng L^{-1}	NR		1.6–20	20–1,000 ng L^{-1}	TD-GC–MS	[42]
6 OFs	Grape, peach, strawberry, multifruit juices	AcO/AcOH, 5	PDMS	30	60	40	5	1700	0.05–2.5 ng mL^{-1}	0.15–8 ng mL^{-1}	95–113	5.3–7.9	0.5–250 ng mL^{-1}	UPLC	[55]
7 SFs	7 Fruits	EtOH, 2	PDMS	20	45	5	NR	2000	0.3–2.0	0.9–6.7	88–101	2–9	0.01–5 μg mL^{-1}	HPLC–DAD	[56]
11 MCPs	Fruit-base soft drinks	MeOH, 5	PDMS	360	30	NR	NR	1400	7–68 ng L^{-1}	NR	38–113	NR	NR	TD-GC–MS	[57]
20 OCPs	Potato, tomato, green pepper	ACN	PDMS	180	20	NR	NR	1000	0.01–8.68	NR	10–110	0.3–33	0.1–5,000	LD-LVI-GC–MS	[34]

MCPs Multiclass pesticides, *Org. solv* organic solvent, *vol* volume, *SB phase* stir bar phase, *Ext. time* extraction time, *Ext. temp.* extraction temperature, *St. rate* stirring rate, *LOD* limit of detection, *LOQ* limit of quantification, *Rel. rec.* relative recovery, *RSD* relative standard deviation, *LR* linearity range, *OPPs* organophosphorus pesticides, *OFs* oxazole fungicides; strobilurin fungicides, *AcO* acetone, *AcOH* acetic acid, *TD* thermal desroption, *EtOH* ethanol, *PDMS* polydimethyl siloxane, *PPESK* poly(phthalazine ether sulfone ketone), *PDMS-OH* hydroxyl terminated-PDMS, *LC–MS* liquid chromatography-mass spectrometry, *TD-GC–MS* thermal desorption-gas chromatography-mass spectrometry, *MEKC* micellar elctrokinetic chromatography, *DAD* diode array detection, *HPLC* high performance liquid chromatography, *UPLC* ultra-performance liquid chromatography, *NR* not reported, *NA* not adjusted

was washed with 5 mL water, while the filtrate of the extract was transferred into 25 mL glass beaker containing the PDMS coated stir bar and extracted for 2 h at stirring rate of 900 rpm. After extraction, the stir bar was removed and placed into water for 1 min, and then into 2 mL vial containing 500 μL of acetonitrile. The extracted analytes were desorbed into the solvent ultrasonically and was injected into LC–MS system.

The extraction of polar analytes was poor and thus was not used in method validation. The quantification was performed using a standard addition calibration in a matrix matched sample solution. The linearity was between 0.001–6 mg kg^{-1} ($r^2 > 0.995$) and 0.008–10 mg kg^{-1} ($r^2 > 0.998$) for SBSE and MSDP respectively and the LOQs respectively ranged from 0.001 to 0.05 mg kg^{-1} and from 0.008 to 0.12 mg kg^{-1} for SBSE and MSPD. The recovery was very low in SBSE and ranged from 8 to 84 % with RSD between 4 and 16 % with MSPD having better recovery, and ranged from 47 to 96 % (RSD = 1–15 %). The MSPD was found to have better extraction efficiency for the target analyte, and although SBSE involves the use of a small solvent and has high sensitivity, it has a low enrichment factor of polar compounds [16].

An SBSE method coupled to thermal desorption retention time locked (RTL) capillary GC–MS has been employed for the screening of 17 multiclass pesticides (MCPs) in lettuce, pear, grape and baby food samples. The samples (15 g) were accurately weighed into a 100 mL flask, followed by the addition of 30 mL of methanol, homogenized for 15 min, then placed in an ultrasonic bath for 15 min. A 10 mL fraction of the homogenized mixture was transferred into a 20 mL vial and centrifuged for 5 min at 5,000 rpm. The supernatant (1 mL) formed was then placed in a 20 mL headspace vial and 10 mL of water was added, followed by insertion of a 10 mm long stir bar with 0.5 mm PDMS layer. The mixture was stirred for 60 min at 100 rpm, after which the stir bar was removed with tweezers, dipped in bi-distilled water, placed on lint-free tissue to remove residual droplets and finally placed in the liner of a TDU thermal dispersion system of GC–MS. The performance of the developed method was estimated at the optimal extraction conditions and gave linearity ranging from 5 to 200 μg L^{-1} with correlation coefficients greater than 0.992, while recoveries ranged from 43 to 75 % with RSD between 4.6 and 8.8 %. The low recoveries were attributed to the degradation of the target analytes during sample enrichment or in the TDU injection system [50].

The SBSE approach has been used for the extraction of five fungicides (bitertanol, flutiafol, tridimefon, tebuconazole, carboxin and pyrimethanil) residues at low μg kg^{-1} levels in grape samples. The developed SBSE method was also compared with a solid-phase extraction technique. The grape sample was prepared by chopping and homogenizing 200 g of a representative portion of the sample. A 5 g portion was then weighed into a 250 mL glass beaker and homogenized with 25 mL of water containing 30 % NaCl, by sonication for 15 min. The resulting suspension was then filtered and the filtrate was transferred into a 50 mL glass beaker containing the PDMS stir bar and was stirred for 2 h at 900 rpm. After extraction the coated stir bar was desorbed into 1 mL of methanol in a 2 mL vial by sonication for 15 min and injected into LC–MS system. The mean recovery ranged from 15 to 100 % with RSD from 10 to 19 % in SBSE while it ranged from 60 to 100 % and 7 to 17 % respectively for SPE. The LOQ was 10 μg kg^{-1} for the investigated analytes. The linearity which was estimated at the LOQ and 100 times the LOQ gave a correlation coefficient that is greater than 0.995 for SBSE and greater than 0.994 for SPE. The better analytical performance showed by SPE compared to SBSE was shown to result from the equilibrium nature of the adsorption of the coated stir bar, while the SPE is a non-equilibrium adsorption [51].

The optimization of a multi-residue screening of 85 pesticides of various classes (OPPs, CPs, OCPs and PPs) in vegetables (tomato, cucumber, green soybean and spinach), fruit (grape) and green tea was developed based on SBSE coupled to a TD-RTL-GC–MS operating in scanned mode. The developed method involved the extraction of the target analytes in a 25 g of sample with 100 mL of methanol in a 250 mL flask shaken ultrasonically for 20 min. A fraction of the resulting mixture was placed in a 40 mL vial, centrifuged at 3,000 rpm for 5 min and 10 mL of the methanol extract was then transferred into a 20 mL headspace vial followed by addition of 10 mL of Milli-Q purified water. A 10 mm stir bar coated with a 0.5 mm layer of PDMS was inserted into the diluted sample solution, and the extraction was performed for 60 min at a stirring rate of 1,000 rpm at room temperature. After extraction, the stir bar was removed, dipped in Milli-Q water, dried with a lint-free tissue and placed in a glass insert of TDU system. The developed method was validated using a standard addition and matrix match calibration methods in order to compensate for the effect of sample matrix. The method linearity ranged from 4 to 100 μg kg^{-1} with correlation coefficient greater than 0.99 for most of the investigated pesticides. The LODs were between 0.63 and 26 μg kg^{-1} [48].

The extraction and quantitative determination of 12 OPPs in cucumber and potato samples using a sol–gel prepared hydroxyl terminated PDMS coating, coupled to a TD-GC-TSD (thermionic specific detector) was reported. The sample matrix spiked with various concentrations of target analytes were prepared by homogenizing 10 g of each sample in a mortal followed by addition of 15 mL of acetone and extracted for 30 min. The resulting mixture

was transferred into a vial and centrifuged for 5 min at 4,000 rpm. After extraction, which was repeated twice, the combined extracts were evaporated in a rotavapour to 5 mL and a 2 mL aliquot of the extract was placed in a vial, diluted to 20 mL with purified water containing 30 % of NaCl and was subjected to SBSE procedure. The stir bar coating prepared using sol–gel process with thickness of 20 μm was used for extraction of target analytes at 30 °C for 30 min at a stirring rate of 600 rpm. The performance of the developed method was estimated by analyzing standard solution to give a linear range of 0.25–50 ng g^{-1} with correlation coefficients greater than 0.99. The LODs ranged from 0.007 to 0.15 ng g^{-1} and 0.0056 to 0.098 ng g^{-1} in cucumber and potato, respectively [22].

A study which compared SBSE and SPE for the determination of eight pesticide residues in strawberry, lettuce and tomato samples using MEKC coupled to a diode array detector (DAD) was also reported. A representative portion of the samples was chopped and 100 g was stored in closed containers in a freezer before analysis. A 5–15 g portion of the sample was spiked with working pesticide standards, placed in 250 mL beaker and homogenized in an ultrasonic bath for 15 min, followed by addition of 10 mL of an acetone/water (50:50, v/v) mixture containing 40 % of NaCl and homogenized again for 15 min. The resulting suspension was filtered, the cake filter was washed with 20 mL of acetone; the filtrate was subjected to the SBSE procedure in a 50 mL glass beaker containing the coated stir bar and was extracted for 4 h at a stirring rate of 900 rpm. After the extraction, the stir bar was removed and transferred into 2 mL vial containing 1 mL of methanol to desorb the extracted analytes, which was then concentrated to dryness under a stream of nitrogen and redissolved with 0.5 mL of buffer solution.

The method linearity was between 1 and 100 mg kg^{-1} with correlation coefficients higher than 0.996. The recoveries were between 12 and 47 % with RSDs between 3 and 17 %, while the LOQ was 1 mg kg^{-1} for all the studied pesticides. The SBSE was found to be better than SPE in terms of linearity, repeatability and cleaner chromatogram, but gave lower recoveries and high LOQ which is found to be too high to meet the MRLs of the investigated pesticides in the sample analyzed. In this study, SPE was found to provide higher extraction efficiency, robustness, better sensitivity and rapid extraction than the SBSE [52].

An organochlorine pesticide residue and chlorobenzene was found in five fruit and vegetable samples using accelerated solvent extraction (ASE) combined with SBSE and SPME coupled to GC/MS. The fresh fruit and vegetable samples were chopped and homogenized for 10 s at a speed of 7,000 rpm. A 10 g portion of the homogenized samples were spiked with the working standard in a 100 mL glass beaker and allowed to equilibrate for 1 h, followed by mixing of 5 g of cellulose flakes and was subjected to ASE using a mixture of water/acetone (90:10, v/v) as the extraction solvent. Then 10 mL of the resulting solution was transferred into 15 mL glass vial and then subjected to SBSE for 60 min at stirring rate of 850 rpm, using 10 mm long stir bar coated with 1 mm PDMS layer. After the extraction the stir bar was removed, dipped into organic free water and dabbed with a lint-free tissue and placed into TDU system online with GC/MS. The evaluation of method performance of the developed ASE-SBSE method gave LOD ranging from 0.5 to 30 μg kg and RSD between 5 and 25 % [53].

The SBSE coupled to RLT-GC/MS and automated mass spectral deconvolution was employed for the identification of pesticide residues in 28 fruit and vegetable samples. Next, 30 mL of methanol was added to 15 g of sample, homogenized for 3 min and extracted in ultrasound for 10 min followed by centrifugation for 10 min at 4,500 rpm. An aliquot of 1 mL of the resulting supernatant was mixed with 9 mL of distilled water and was extracted using a 0.5 mm thick and 10 mm long PDMS coated stir bar for 1 h at 1,000 rpm. The stir bar was rinsed with distilled water and dried on a lint-free tissue then placed in a glass desorption tube. The chromatograms obtained were evaluated in MSD Chemstation by the calculated command using a homebuilt quantitation database, which contained 150 pesticides. The method identified 52 pesticides in 16 samples and the contaminated samples were further examined by comparing the quality of evaluation done by the quantitation database (QD), RTL-RS (result screener) and mass spectral deconvolution software (AMDIS). The AMDIS software was found to support most of the decision making process and perform best in the identification and discovering of positive hits and proved to be more reliable and can efficiently reduce time taken for data analysis [54].

A novel poly(phthalazine ether sulfone ketone) (PPESK) stir bar coating prepared by immersion precipitation was reported for the extraction of four OCPs in seawater and eight OPPs in grape and peach samples. The new coatings showed better extraction efficiency, higher thermostability (290 °C), long lifetime and ability to extract analytes of varying degree of polarities. The fruit sample were diluted with water (1:20, v/v) and spiked with the working standard solution of the OPPs and was extracted with the prepared PPESK coated stir bar at 40 °C for 30 min stirred at 600 rpm. After the extraction, the extracted analytes were desorbed into the TDU of GC-ECD system. The method validated estimated using a six-point calibration curve gave linearity between 20 and 1000 ng L^{-1} with r^2 greater than 0.99. The LODs were in the range of 0.17–2.25 and 2.47–10.3 ng L^{-1} in grape and

peach juices, respectively, with RSD of 1.6–20 %. The PPESK coated stir bar was found to have a porous homogenous surface structure and a sponge-like sublayer which gave it a better adsorption mechanism and exhibited better selectivity and higher extraction capability [42].

A method was developed for the extraction of oxazole fungicide residues in wines and juices (grape, peach, strawberry and multifruit) based on the comparison of SBSE and membrane-assisted solvent extraction (MASE), coupled to ultra-performance liquid chromatography (UPLC). A 5 mL sample of juice was measured into a 15 mL vial, followed by addition of 5 mL of 0.2 M acetone/acetic acid buffer (pH 5) and 2 g of NaCl. The stir bar was introduced into the sample and was stirred for 30 min at 1,700 rpm at a temperature of 60 °C. After the extraction, the stir bar was rinsed with distilled water and dried with tissue paper. The stir bar was transferred into an HPLC vial with a glass micro-insert and the extracted analytes were desorbed into 100 μL of mixture of acetonitrile/water (80:20, v/v) for 10 min. The stir bar was removed and the 20 μL of the mixture was injected into UPLC.

The method performance was estimated using least-square linear regression analysis of the chromatogram peak area versus analyte concentration at six concentration levels using external standard calibration method. The validation parameters include linearity (0.5–250 ng mL^{-1}), correlation coefficients ($r^2 = 0.999$), LODs (0.05–2.5 ng mL^{-1}), LOQs (0.15–8 ng mL^{-1}), recoveries (95–113 %) and RSDs (5.3–7.9 %). The SBSE method showed better extraction efficiency in terms of sensitivity, repeatability and analyte recovery than the MASE [55].

A stir bar microextraction procedure was developed for the extraction of seven strobilurin fungicides in fruit (apple, pear, grape, orange, lemon, peach and plum) samples using LC-DAD. The fruit samples (5 g) were accurately weighed into a capped 50 mL glass polycarbonate centrifuge tube, followed by addition of 2 mL of ethanol and the mixture was sonicated at room temperature for 1 min and then centrifuged for 5 min at 6,000 rpm. The resulting supernatant was diluted to 14 mL with 0.04 M acetate buffer solution and 5 % NaCl and was subjected to the optimized SBSE procedure. The stir bar was immersed into the solution and was stirred 2,000 rpm for 20 min at temperature of 45 °C. After extraction, the bar was rinsed in distilled water and dried with tissue paper, and the dried stir bar was placed in a LC vial with micro-insert containing 100 μL of acetonitrile/water (50:50, v/v) mixture for 15 min. The stir bar was removed and 20 μL was injected into LC system.

The matrix effect was estimated by comparing the slope of calibration curve of aqueous solution spiked with 70 ng g^{-1} of internal standard and that of the standard addition method and showed not significance difference. Thus, the use of an internal standard helps to compensate for the matrix effect. The calibration parameters obtained include linearity which ranges from 0.01 to 5 μg mL^{-1} with correlation coefficient greater than 0.996. The LODs and LOQs ranged from 0.3 to 2.0 ng g^{-1} and 0.9 to 6.7 ng g^{-1}, respectively, while the mean recoveries ranged from 88 to 101 % with RSD between 2 and 9 %. The analytical performance obtained for the developed method make it a useful, reliable and sensitive method for routine analysis of strobilurin in fruit samples and the use of internal standard for quantification helps to compensate for matrix effect and avoids the use of tedious standard addition method [56].

The performance of coupling the SBSE technique with TDU-GC/MS analysis with the statistical variance component model (VCM) was evaluated for the determination of 13 multiclass pesticide residues in fruit-based soft drink. The VCM statistical procedure was used to account for the contribution of matrix- and time-induced deviation to the uncertainty. The fruit-based soft drink matrix were mixed with methanol in different proportions (5 mL of sample matrix + 5 mL of methanol or 5 mL of water with 1, 2, 3 mL of methanol respectively) to form four solution, each of which were spiked with the standard solution. A 1 mm PDMS coated stir bar was inserted into the solution and extraction was performed at temperature of 30 °C for 6 h and stirred at 1,400 rpm.

The limit yielding of recovery of each analyte was evaluated, and was found to range from 38 to 113 and 17.5 to 103 % in real sample matrix and sample diluted with methanol (1:10) respectively with detection limit ranging from 7 to 68 ng L^{-1}. The Cochran test was used to check the heteroscedasticity of the experimental measurement at fixed matrix and various calibration concentrations and compared with the data set measured at fixed concentrations and varied amounts of the sample matrix. The Cochran test showed non-constancy of the measurement variances with the concentration level and the heteroscedasticity resulting in the use of different matrices was evident. It was concluded that the dispersion of the experimental data could be a result of instrumental uncertainties, handling of solutions and change of the sample matrix, and that matrix effects could be corrected using labeled or unlabeled internal standards. Therefore, it was suggested that for routine pesticide analysis in different matrices, the adopted analytical procedure most offer the best compromise between costs and benefits. Thus the VCM allowed the calculation of an inter-matrix detection limit and of an inter-matrix confidence interval of a discriminated concentration value [57].

The SBSE method was used for the analysis of trace levels of 20 OCP in nine vegetable samples using a liquid desorption and large volume injection-gas GC (LD/LVI-GC–MS). The LD procedure involves the use of 100 mg

aliquots of freeze-dried samples spiked with pesticides standards. The target analytes were pre-extrcated twice in 2 mL of methanol for 15 min by sonication. The mixture was then centrifuged for 5 min at 4,000 rpm and the supernatant obtained was transferred into a sample vial diluted with ultrapure water. The stir bar was then placed into the vial, closed, and the assay was then carried out in a 15 agitation point plate for 180 min at a stirring rate of 1,000 rpm. After extraction the stir bar was dried with a lint-free tissue and the extracted analytes were reconstituted with 1.5 mL of acetonitrile concentrated under gentle stream of nitrogen and redissolved with 120 mL of hexane followed injection of 20 μL of the hexane extract into the LVI-GC system. The developed method was validated using standard addition due to the presence of the matrix effect. The method's linearity was found to be between 0.1 and 5,000 μg kg^{-1}. The LODs ranged from 0.01 to 8.68 μg kg^{-1}. The liquid desorption was shown to be an efficient and effective back-extraction technique, cost-effective and it eliminates the use of expensive thermal desorption devices [34].

Conclusion

SBSE has provided a better extraction method in terms of higher extraction capacity, sensitivity and wide range of application. The main disadvantage of the method is the use of organic solvents which may be toxic. Another drawback is the inability to desorb the stir bar into a split/splitless injection port of GC, which added an extra step of desorbing the extracted analytes in a suitable organic solvent. The current trend aiming at improving on the limitations of SBSE include the use of a thermal desorption unit online with GC and the use of other sorbent materials other than PDMS which has helped to increase the application of the method to a wide range of environmental matrices. The use of restricted access materials (RAM), molecular imprinted polymer and immunosorbent materials as LC column for the analysis of pesticide residues in environmental samples has been reported [58, 59], and was found to be efficient in the analysis of acidic compounds in water containing high amount of organic compounds. This can also be extended to SBSE of pesticide residues in fruits and vegetables which contain suspended matters and dissolved organic compounds like pectin [60], which can interfere with the extraction process.

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