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Original Paper

A simple and inexpensive "solvent in silicone tube extraction" approach and its evaluation in the gas chromatographic analysis of pesticides in fruits and vegetables

A novel, simple, and inexpensive approach to sorptive extraction, which we call solvent in silicone tube extraction (SiSTEx), was applied to pesticide residue analysis and its effectiveness and efficiency were evaluated. In SiSTEx, which is a form of open tubular sorptive extraction, a piece of silicone tubing (4 cm long, 1.47 mm ID, 1.96 mm OD in this study) is attached to the cap of a 20 mL glass vial that contains the aqueous sample. The tubing is plugged at the end dangling in the sample solution, and MeCN (*e.g.*, 40 μL) added by syringe to the inner tube volume through a septum in the cap. A stir-bar is used to mix the sample for a certain time (*e.g.*, 60 min), which allows chemicals to partition into the tubing where they diffuse across the silicone and partition into the MeCN. The final MeCN extract is then analyzed for the concentrated analytes. In this study, the SiSTEx approach was evaluated for the analysis of organophosphorus (OP) and organochlorine (OC) pesticides in fruits and vegetables using GC/pulsed flame photometric (PFPD) and halogen specific (XSD) detectors for analysis. The produced samples were initially extracted by a rapid MeCN procedure, and 5 mL of the initial extract was diluted four-fold with water to undergo sorptive extraction for 60 min. The final extract was analyzed by GC/PFPD + XSD for 14 OP and 22 OC pesticides. This simple approach was able to detect 26 of the 36 pesticides at 10 ng/g or less original equivalent sample concentration with average reproducibility of 11 %RSD. For those 26 pesticides, a 44-fold lower detection limit on average was achieved in matrix extracts using SiSTEx despite the four-fold dilution with water.

Keywords: Fruits / Gas chromatography / Pesticide / Solvent in silicone tube extraction / Sorptive extraction / Vegetables

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1 Introduction

1.1 Sorptive extraction

Sorptive extraction is an analytical sample preparation approach that entails the partitioning of chemicals from gaseous or liquid samples into a polymeric phase, typically polydimethylsiloxane (PDMS) or another polyorganosiloxane (commonly called silicone). The chemicals with high affinity for the sorptive phase tend to be nonpolar and have a high octanol/water partitioning coefficient ($K_{o/w}$). For more details, a recent review article provides an overview of sorptive extraction techniques [1].

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Abbreviations: C_{18}, octadecylsilane; **DDD**, dichlorodiphenyldichloroethane; **DDE**, dichlorodiphenyldichloroethylene; **DDT**, dichlorodiphenyltrichloroethane; **ECD**, electron capture detector; **EtAc**, ethyl acetate; **GCB**, graphitized carbon black; **HAc**, acetic acid; **HCH**, hexachlorocyclohexane; $K_{o/w}$, octanol/water partitioning coefficient; **LCL**, lowest calibration level; **LOD**, limit of detection; **LOQ**, limit of quantitation; **LVI**, large-volume injection; **MASE**, membrane-assisted solvent extraction; **MeCN**, acetonitrile; **MeOH**, methanol; **MMLLE**, microporous membrane liquid–liquid extraction; **NaAc**, sodium acetate; **n/a**, not applicable; **nd**, not detected; **OC**, organochlorine pesticide; **OP**, organophosphorus pesticide; **PDMS**, polydimethylsiloxane; **PFPD**, pulsed flame photometric detector; **PSA**, primary secondary amine; **QuEChERS**, quick, easy, cheap, effective, rugged, and safe; **SBSE**, stir-bar sorptive extraction; **SiSTEx**, solvent in silicone tube extraction; **SPME**, solid-phase microextraction; **XSD**, halogen specific detector

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Solid-phase microextraction (SPME) is the most common form of sorptive extraction, which was introduced in 1989 by Pawliszyn and collaborators [2, 3], and more than 1900 papers have been published on SPME for a wide variety of applications. In SPME, the polymer is coated onto a fused-silica fiber rod, which can be retracted into a low-volume housing when not in use. The main advantageous feature of SPME is that its syringe-like holder can be used with standard GC injectors to thermally desorb the sample extract from the SPME fiber. Other advantages of SPME include: (1) compact and simple device; (2) multiresidue capability with selective extraction/cleanup; (3) rugged method of injection in GC; (4) easily automated; (5) low LODs for nonpolar analytes; (6) elimination of organic solvents; and (7) possible use of a small sample size.

However, a simple listing of the general advantages of SPME is deceiving. The most appropriate applications of SPME relate to the qualitative analysis of volatile components in gaseous samples. The cost of each device is relatively high; storage of the SPME extracts on the coatings is problematic; reanalyses require reextractions rather than just reinjections; and direct extraction of liquids can contaminate the coatings. In the analysis of pesticides in agricultural products, the most significant deficiency of SPME concerns the logistics of sample size, homogenization, and initial extraction to provide an aqueous liquid or headspace region that is conducive for the SPME procedure. Another major limitation with SPME (and sorptive extraction, in general) is that sensitivity is highly compound dependent.

The most important overall advantage of sorptive extraction is the potential for exceptionally low detection limits due to the concentration of analytes from a relatively large sample volume into a very small polymer volume. Alternate sorptive extraction techniques have been devised to improve upon certain practical aspects of SPME and to lower detection limits. In an approach known as SnifProbe [4], which is especially useful for the analysis of gaseous samples, a 15 mm piece of 0.53 mm ID GC capillary column is used for open tubular sorptive extraction. Another concept is that stir-bar sorptive extraction (SBSE) [5 – 10], which has been commercialized as Twister®, is designed for the analysis of aqueous samples. Another sorptive extraction concept used for aqueous samples entails the use of thick-film silicone rubber traps to absorb the analytes [11 – 13].

The commercial SBSE device is akin to SPME on a stir-bar, and is thus designed for the extraction of relatively nonpolar organic contaminants in water. The mixing action of a magnetic stirrer to speed the equilibration is more effective than the automated vibration approach in SPME and can handle larger sample volumes, which in combination with the larger PDMS volume, gives greater sample capacity and lower detection limits than SPME [5, 10].

Ideally, extraction of analytes from the sample to the extracting medium should be complete, instantaneous, and selective. In sorptive extraction, the process is not so much an extraction as an equilibration that occurs over the course of time. The time involved to achieve equilibrium between the polymer and sample phases is often on the order of hours or even days, not minutes [1, 2]. Therefore, a fixed time must be employed to provide a consistent, *albeit* incomplete, partitioning of the analytes into the sorptive polymer. Different temperatures, phases, volumes, time, sample treatments (*e.g.*, addition of salt) can increase recoveries or speed the equilibration in sorptive extraction, but in reality, the nature of the extraction process itself limits its usefulness.

A fundamental difficulty with all of the sorptive extraction approaches to date is that different analytes have different partitioning ratios and kinetics between the sample matrix and the sorptive material. The multiresidue capability of sorptive extraction is thus limited to nonpolar analytes. In the case of chemical residue and contaminant analysis in food and environmental matrices, a wide polarity range of chemicals often needs to be monitored, which extends beyond the range of the sorptive extraction approach.

Also, matrix effects can dramatically affect partitioning constants in sorptive extraction, thus making quantitation problematic and inconsistent. To achieve the most accuracy, an isotopically labeled internal standard for each analyte is needed, but this is not feasible in multiresidue applications or with detection methods other than MS. Instead, matrix-matched calibration or the method of standard additions is used to provide reasonable quantitation [5, 9]. The difficulty with these approaches in sorptive extraction, however, is that unlike traditional solvent-based methods in which a liquid extract solution is produced for use as a matrix for calibration standards or standard additions, the polymer cannot be used as a liquid extract. Thus for quantitation, the entire extraction procedure must be repeated for each calibration level whereby calibration solutions are spiked into matrix blanks or replicate samples (for matrix-matching calibration or method of standard additions, respectively). Extraction times are typically 60 min, and are commonly done sequentially rather than in batches, so quantitation is generally a time-consuming and difficult chore in sorptive extraction methods. This is why its main use has been for screening rather than quantitation of organic contaminants in samples [2, 5].

Another potential drawback of common sorptive extraction approaches relates to the need for thermal desorp-

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tion of the extracts in the polymer. Specialized thermal desorption units or injection ports are required for SBSE, which add to the complexity and cost of the analysis. Furthermore, this thermal desorption step, particularly with the thick film of SBSE, requires higher injection temperature, which can be detrimental in the analysis of thermally labile compounds. Additionally, "PDMS bleed" occurs in the chromatograms due to the thermal extraction of the sorbent. SPME can be used with standard split/splitless injection, providing one of the few advantages for it over the newer SBSE technique.

However, despite the stated limitations of SBSE, it provides a favorable combination of high analyte enrichment for a certain polarity range of analytes, plus reduced matrix interference through selective cleanup. The combination of these factors translates to lower detection limits. Consequently, a new sorptive extraction approach is desirable that could combine its advantages but overcome its limitations.

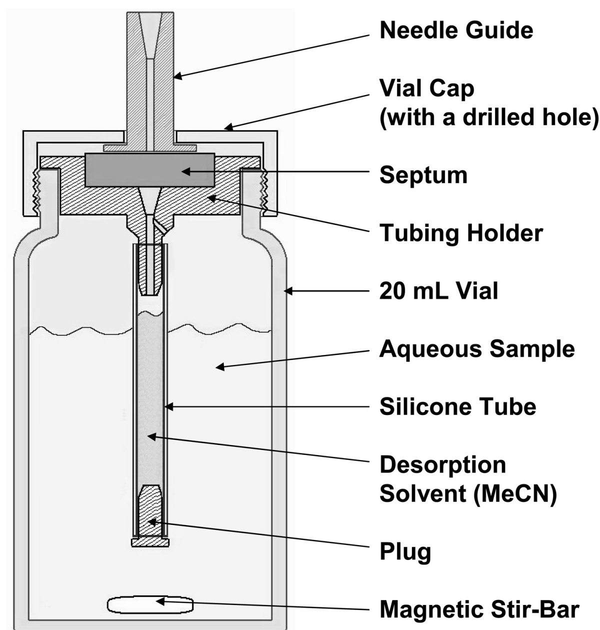

Figure 1. Drawing of the SiSTEx device used in this study.

1.2 Solvent in silicone tube extraction for pesticide analysis

The use of membranes for sample enrichment is another form of extraction that has been extensively explored [14–16]. The commonality in membrane extraction is that the liquid sample is separated from the extracting liquid by a microporous film (*e.g.*, filter disk or dialysis membrane as in semipermeable membrane device (SPMD)) or a nonporous film (*e.g.*, PDMS). The former technique is often known as microporous membrane liquid–liquid extraction (MMLLE), and the use of a nonporous film in this approach can be considered another form of sorptive extraction. In either case, the objective of membrane extraction is to isolate the analytes into the liquid at the other side of the membrane.

MMLLE can offer the same advantages of standard liquid–liquid extraction with added advantages of using smaller solvent volume to attain potentially higher enrichment. However, the sample liquid and extracting liquid make contact through the microporous membrane in MMLLE; thus, the extraction solvent cannot be miscible with the sample solvent. This means that water-miscible solvents such as MeCN cannot be used with water in MMLLE. This solvent immiscibility problem is alleviated with the use of nonporous membranes such as silicone [17, 18]. Furthermore, the membrane can be a tube, which provides inner and outer regions to separate the two liquids rather than a flat film. We have chosen to call this method "solvent in silicone tube extraction" (SiSTEx), and decided to evaluate it as an easier and less costly alternative to SBSE.

Figure 1 shows the SiSTEx device used in this study. A short piece of silicone tubing is attached to the cap of a 20 mL glass vial that contains the aqueous (or gaseous) sample. The tubing has been plugged at the end dangling in the sample, and MeCN is added by syringe to the inner tube volume through a septum in the cap. A magnetic stir-bar is used to mix the sample for a fixed time, which allows chemicals to partition into the tubing where they diffuse across the silicone and partition into the MeCN. The MeCN extract is then analyzed for the concentrated analytes. In this way, a standard split/splitless GC injector is used with SiSTEx. The SiSTEx device shown in Fig. 1 was designed to enable automation but the use of an autosampler was not attempted in this study.

Recently Popp *et al.* [19–23] investigated and developed a similar concept as SiSTEx, which they call "membrane-assisted solvent extraction" (MASE) [19–23]. MASE uses a dense polypropylene membrane bag filled with 800 μL organic solvent fitted into the cap of a 20 mL headspace vial, and large-volume injection (LVI) of 100–400 μL in GC is then used to analyze the water or aqueous samples. In contrast, our SiSTEx method and research is focused on the use of silicone tubing (PDMS) for the extraction of samples, and lower solvent volumes in the tube without the need for LVI in analysis. We also chose to apply the concept to the analysis of organic solvent extracts, such as pesticide residues extracted from fruits and vegetables, rather than water samples only.

Traditionally in pesticide residue monitoring of fruits and vegetables, large sample sizes (*e.g.*, 10 kg) are collected in the field and chopped into a homogeneous slurry in the laboratory. A smaller, representative por-

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tion (*e.g.*, 10–100 g) is extracted using an organic solvent, such as acetone, MeCN, methanol (MeOH), or ethyl acetate (EtAc). The chopped produce samples cannot be extracted directly in sorptive extraction approaches because the sample needs to be a liquid, and water is not a good extraction medium of pesticides for some practical and physicochemical reasons. Thus, an initial extraction has to be conducted with an organic solvent prior to any form of sorptive extraction.

Sandra *et al.* chose to use MeOH as the initial extraction solvent in an SBSE procedure for analysis of pesticides in produce and babyfood [5]. In our approach, we wanted to use the MeCN-based extraction known as the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method because it has several unique qualities and advantages over traditional extraction methods for pesticides [24–28]. A drawback with the QuEChERS method *versus* traditional methods that we hoped to resolve in this study involved the potentially higher detection limit for QuEChERS extracts when traditional split/splitless injection (as opposed to LVI) is used for GC analysis. LC/MS-MS is used for the analysis of polar and thermally labile pesticides in QuEChERS extracts to achieve <5 ng/g detection limits [26–27], and a similar capability for GC-amenable pesticides without LVI is desirable. Solvent evaporation and exchange can be performed to concentrate the extracts in an attempt to lower detection limits, but SiSTEx would be preferable if it can provide cleaner extracts in an easier procedure with higher enrichment factors.

In this study, we intended to evaluate several different interesting aspects in the analysis of pesticide residues in fruits and vegetables. Foremost, we wished to evaluate the SiSTEx concept and test it in a real-world application. Secondarily, we proposed to investigate the applicability of the QuEChERS method in combination with SiSTEx in an attempt to lower detection limits for organophosphorus pesticide (OP) and organochlorine pesticide (OC) pesticides in a clean and rugged process without resorting to solvent evaporation or exchange.

2 Experimental

2.1 Chromatographic instrumentation and conditions

An Agilent (Little Falls, DE, USA) 6890 GC, fitted with a 6890 enhanced parameters autosampler, standard split/splitless injector, and Models 5360 halogen specific detector (XSD) and 5380 PFPD from OI Analytical (College Station, TX, USA), was used for analysis. A 3 m, 0.25 mm ID uncoated guard column and a 30 m, 0.25 mm ID, 0.25 μm film thickness RTX-5 SIL MS analytical column from Restek (Bellefont, PA, USA) was used for GC. A 1 : 1 flow splitter connected the analytical column through a two-hole ferrule to two pieces of 0.25 mm ID uncoated capillary tubing leading to the detectors. For the PFPD, 20 cm of tubing was employed, and 14 cm was used for the XSD. Flow measurements determined that ≈67% of the flow and GC eluent went to the XSD, and ≈33% to the PFPD. Splitless injection (2 μL) at 250°C in a 4 mm ID double tapered deactivated glass liner was used with a pressure pulse of 70 psi for 1.5 min followed by 2 mL/min constant flow of He carrier gas. The oven temperature program was 85°C for 1.5 min, 8°C/min to 290°C, and held for 3 min. The computerized instrument control and collection used version A.06.01 ChemStation software (Agilent).

The PFPD was controlled by WinPulse software (OI Analytical) and the PFPD controller which required manual adjustment of gas flows. An Agilent Model 35900E A/D was used for the PFPD signal to be analyzed on the ChemStation. H_2 flow was ≈9 mL/min and air flow was ≈25 mL/min through the PFPD. Small flow adjustments were made to optimize phosphorus response with the PFPD, which also entailed use of a 3 mm combustor, 300°C temperature, GC-495 filter, and Model R1924 photomultiplier tube with 525 V setting. PFPD pulse frequency was 3.1 Hz according to the WinPulse readout, and phosphorus signal was collected with 4 ms gate delay and 11 ms gate width. The XSD with nonventing option was used for detection of OC pesticides. The temperature of the XSD base was set at 300°C and the detector controller at 1100°C. The air flow was set at 55 mL/min.

2.2 Materials

The pesticide standards in this study were obtained from Chemservice (West Chester, PA, USA), EPA National Pesticide Repository (Fort Meade, MD, USA), or Dr. Ehrenstorfer GmbH (Augsburg, Germany). Fruit and vegetable samples were purchased from a local organic food store. MeCN, toluene, EtAc, and MeOH were all analytical grade from Burdick and Jackson (Muskegon, MI, USA), and water was obtained from a Barnstead (Dubuque, IA, USA) water purification system in the laboratory. Certified anhydrous $MgSO_4$, American Chemical Society (ACS)-grade anhydrous sodium acetate (NaAc), and ACS-grade NaCl were obtained from Fisher (Fair Lawn, NJ, USA), ICN Biochemicals (Cleveland, OH, USA), and Mallinckrodt (Paris, KY, USA), respectively. The $MgSO_4$ was baked for 5 h at 500°C in a muffle furnace to remove phthalates and residual water prior to usage. Primary secondary amine (PSA) (Varian, Harbor City, CA, USA), octadecylsilane (C_{18}) (J.T. Baker, Phillipsburg, NJ, USA), and graphitized carbon black (GCB) (Supelco, Bellefonte, PA, USA) were used in dispersive-SPE cleanup experiments. Glacial acetic acid (HAc) was obtained from Mallinckrodt. Ultrahigh purity He, H_2, and air for GC/PFPD + XSD were supplied by Air Products (Allentown, PA, USA).

Pesticide stock solutions in toluene or MeCN were prepared at ≈2000 ng/μL and stored in sealed dark glass vials

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in the freezer. Working mixtures for the 36 pesticide analytes of 20 and 0.5 ng/μL in MeCN were prepared for preliminary experiments to evaluate the sorptive extraction approach. Once LODs had been assessed, a pesticide spiking mixture in MeCN was prepared in which analyte concentrations depended on LOD. Level A analytes were 0.25 ng/μL, which consisted of bromophos, chlorothalonil, chlorpyrifos, coumaphos, diazinon, endosulfan sulfate, ethion, hexachlorobenzene, heptachlor, lindane, *p,p′*-dichlorodiphenyldichloroethane (DDD), *p,p′*-dichlorodiphenyldichloroethylene (DDE), phosalone, and pirimiphos-methyl; Level B pesticides were 2.5 ng/μL, which consisted of atrazine, azinphos-methyl, bifenthrin, captan, λ-cyhalothrin, dichlofluanid, fenvalerate, folpet, *p,p′*-dichlorodiphenyltrichloroethane (DDT), permethrin, procymidone, tolylfluanid, and vinclozolin; and Level C analytes were 25 ng/μL, which consisted of acephate, deltamethrin, dichlorvos, dimethoate, methamidophos, mevinphos, omethoate, tebuconazole, and trifluralin. This solution was diluted as needed in spiking experiments to achieve the desired relative pesticide concentrations in validation experiments. The same pesticide solution was used for spiking as for calibration.

For SiSTEx, Helix Medical (Carpenteria, CA, USA) silicone tubing (1.47 mm ID, 1.96 mm OD) was used. Depending on the experiment, a 3.0–4.0 cm length of tubing was placed in a specially designed cap for use with standard 20 mL glass liquid scintillation vials, as shown in Fig. 1. The concept and device was designed and manufactured at Tel Aviv University, Israel, and tested at the United States Department of Agriculture (USDA) Eastern Regional Research Center. We had four caps to work with in experiments in this study, thus typically four replicates were done at the same conditions, or a single parameter was changed with four levels of variation. Teflon coated stir-bars were added to the solutions during sorptive extraction, and standard magnetic stirrers were used to induce the mixing. To add and remove the final extract from the inside of the tubing, 25 μL Hamilton (Bonaduz, Switzerland) syringes were used, and low-volume inserts were placed in the autosampler vials to contain the extracts prior to GC analysis.

2.3 Method

Mixed comminuted portions of fruits and vegetables (equal parts of apple, strawberry, plum, peach, carrot, potato, green pepper, and cauliflower) were first extracted by the buffered QuEChERS method [27], and those extracts were then used in SiSTEx. The QuEChERS method entails the vigorous shaking of a 15 g sample plus 15 mL MeCN containing 1% v/v HAc along with 7.5 g anhydrous $MgSO_4$/NaAc (4:1 w/w) for 1 min in a 50 mL fluoroethylenepropylene (FEP) centrifuge tube. The tube is then centrifuged at 3450 relative centrifugal force (rcf), and a portion of the initial extract (upper buffered MeCN layer) is transferred to a 20 mL glass liquid scintillation vial, which is taken to 20 mL volume with water (volumes depended on the experiment being performed; 5 mL was used in the final method). For most experiments, the extract was spiked with pesticides in MeCN at this point to make the desired concentration in the extract (or solvent solution). A stir-bar was placed in the vial, and the vial was capped with the silicone tubing in place. Then, the sample solution was stirred at room temperature (22°C) for the designated period of time (60 min in the final method). At the beginning of the SiSTEx process, a fixed volume (40 μL) of solvent (MeCN) was added to the inner part of the tubing by syringe through the septum in the vial cap using the needle guide (see Fig. 1). The chemicals in the sample extract, based on their partitioning coefficients, would partition into the outside of the silicone tubing from the MeCN/water solution, then diffuse through the tubing, and partition into the small volume of organic solvent in the inner part of the tube. At the completion of the extraction time, the final extract is transferred by syringe to the autosampler vial (fitted with a small volume insert) for GC/PFPD + XSD analysis.

To account for volume losses and fluctuations, four internal standards were employed: d_{10}-parathion, d_6-α-hexachlorocyclohexane (HCH), chlorpyrifos-methyl, and triphenylphosphate, which were dissolved at 50 ng/mL in the MeCN used for the final extracts.

For presentation of the data and quantitation, the desired effect of the approach was to increase the concentration of the pesticide analytes from the original MeCN extract into the final MeCN extract. With this in mind, we often calculated results in terms of an "enrichment factor," which is the difference in response normalized to the internal standards in the final extract *versus* the original QuEChERS extract. Thus, an enrichment factor of 2.0 indicates that the analyte in the final extract is twice as concentrated as it was in the original extract (with respect to internal standards in both instances). Note that the real enrichment factors were higher by whatever aqueous dilution factor was used in the experiment since the initial QuEChERS extracts had to be diluted in water for sorptive extraction. When other approaches were employed for quantitation, they are described in Section 3.

3 Results and discussion

3.1 Pesticides selection and GC/PFPD + XSD conditions

In our evaluation of SiSTEx, we chose to analyze OP and OC pesticides by GC using PFPD and XSD simultaneously in a split column flow configuration. The PFPD has well-documented advantages over the traditional flame

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Table 1. Pesticides included in the study and other pertinent information. Solubility in water and log $K_{o/w}$ values taken from [37], except where italicized, in which case the values were obtained from multiple sources on the Internet

#	Pesticide	t_R, (min)	Type/ detector(s)	Molecular formula	Log $K_{o/w}$	Solubility in H_2O, (mg/L)
1	Dichlorvos	6.340[a]	OCP/both	$C_4H_7Cl_2O_4P$	1.9	18 000
2	Methamidophos	6.421	OP/PFPD	$C_2H_8NO_2PS$	−0.8	200 000
3	Mevinphos	9.428	OP/PFPD	$C_7H_{13}O_6P$	0.13	*600 000*
4	Acephate	9.640	OP/PFPD	$C_4H_{10}NO_3PS$	−0.89	790 000
5	Omethoate	11.941	OP/PFPD	$C_5H_{12}NO_4PS$	−0.74	*25 000*
6	Trifluralin	12.950	OF/XSD	$C_{13}H_{16}F_3N_3O_4$	4.8	0.221
7	d_6-α-HCH	13.170	OC/XSD	$C_6D_6Cl_6$	3.5[c]	8.52[c]
8	Hexachlorobenzene	13.326	OC/XSD	C_6Cl_6	5.3	0.035
9	Dimethoate	13.790	OP/PFPD	$C_8H_{12}NO_3PS_2$	0.70	23 800
10	Atrazine	14.025	OC/XSD	$C_8H_{14}ClN_5$	2.5	33
11	Lindane	14.150	OC/XSD	$C_6H_6Cl_6$	3.5	8.52
12	Diazinon	14.585	OP/PFPD	$C_{12}H_{21}N_2O_3PS$	3.3	60
13	Chlorothalonil	14.725	OC/XSD	$C_8Cl_4N_2$	2.9	0.81
14	Chlorpyrifos-methyl	15.700[a]	OCP/both	$C_7H_7Cl_3NO_3PS$	4.2	2.6
15	Vinclozolin	15.756	OC/XSD	$C_{12}H_9Cl_2NO_3$	3.0	2.6
16	Heptachlor	15.881	OC/XSD	$C_{10}H_5Cl_7$	*5.0*	0.056
17	Pirimiphos-methyl	16.445	OP/PFPD	$C_{11}H_{20}N_3O_3PS$	4.2	10
18	Dichlofluanid	16.577	OC/XSD	$C_9H_{11}Cl_2FN_2O_2S_2$	3.7	1.3
19	Chlorpyrifos	16.893[a]	OCP/both	$C_9H_{11}Cl_3NO_3PS$	4.7	1.4
20	d_{10}-Parathion	19.963	OP/PFPD	$C_{10}H_4D_{10}NO_5PS$	3.8[c]	11[c]
21	Bromophos	17.343[a]	OCP/both	$C_8H_8BrCl_2O_3PS$	*5.1*	*40*
22	Tolylfluanid	17.809	OC/XSD	$C_{10}H_{13}Cl_2FN_2O_2S_2$	3.9	0.9
23	Procymidone	18.040	OC/XSD	$C_{13}H_{11}Cl_2NO_2$	3.1	4.5
24	Captan	18.135	OC/XSD	$C_9H_8Cl_3NO_2S$	2.8	3.3
25	Folpet	18.143	OC/XSD	$C_9H_4Cl_3NO_2S$	3.1	0.8
26	*p,p′*-DDE	19.192	OC/XSD	$C_{14}H_{10}Cl_4$	*5.7*	*0.04*
27	*p,p′*-DDD	20.200	OC/XSD	$C_{14}H_{10}Cl_4$	*5.5*	0.02
28	Ethion	20.260	OP/PFPD	$C_{10}H_{15}O_3PS_2$	*5.1*	2
29	*p,p′*-DDT	20.930	OC/XSD	$C_{14}H_9Cl_5$	*6.2*	*0.003*
30	Endosulfansulfate	21.020	OC/XSD	$C_9H_6Cl_6O_4S$	–	–
31	Tebuconazole	21.359	OC/XSD	$C_{16}H_{22}ClN_3O$	3.7	36
32	Triphenylphosphate	21.533	OP/PFPD	$C_{18}H_{15}PO_4$	4.7	1.9
33	Bifenthrin	22.107	OC/XSD	$C_{23}H_{22}ClF_3O_2$	6.0	0.1
34	Phosalone	22.950[a]	OCP/both	$C_{12}H_{15}ClNO_4PS_2$	4.0	3.05
35	Azinphos-methyl	22.996	OP/PFPD	$C_{10}H_{12}N_3O_3PS_2$	3.0	28
36	λ-Cyhalothrin	23.334	OC/XSD	$C_{23}H_{19}ClF_3NO_3$	6.9	0.005
37	Permethrins	24.26[b]	OC/XSD	$C_{21}H_{20}Cl_2O_3$	6.1	0.006
38	Coumaphos	24.477[a]	OCP/both	$C_{14}H_{16}ClO_5PS$	4.1	1.5
39	Fenvalerates	26.53[b]	OC/XSD	$C_{25}H_{22}ClNO_3$	5.0	*0.02*
40	Deltamethrin	27.313	OC/XSD	$C_{22}H_{19}Br_2NO_3$	4.6	*0.0002*

a) PFPD t_R (XSD t_R is 0.04 – 0.07 min earlier).
b) Mid-point between two isomer peaks.
c) Value from nondeuterated compound.

photometric detector (FPD), particularly for OP pesticides, in terms of multielement detection, lower detection limits, greater selectivity, and capability to either analyze sulfur and phosphorus simultaneously or suppress sulfur interferences in the phosphorus-only mode [29 – 33]. The XSD also has a key advantage over electron capture detector (ECD) with respect to selectivity, which means that although it possesses worse sensitivity for OC pesticides than ECD, it often achieves lower detection limits in real samples due to reduced chemical noise from the matrix [34, 35]. GC/FPD + ECD [8], GC/PFPD + MS [32], and GC/PFPD + μECD (μECD, micro electron capture detector) [36] (among other combinations) have been employed previously for the analysis of OP and OC pesticides, but this study provides the first publication in a scientific journal of GC/PFPD + XSD for the application.

Table 1 lists the pesticides included in this study, their average retention time in the final method, type of pesticide, quantitative detector used, molecular formula, log $K_{o/w}$, and solubility in water. The molecular formula is given to indicate the number of phosphorus, sulfur, chlorine, bromine, and fluorine atoms in the molecules that are detectable to different extents by the PFPD and/

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Figure 2. Test injection of 1 ng of each pesticide in a MeCN mixture on the GC/PFPD + XSD system (≈ 1/2 ratio to PFPD/XSD).

or XSD. The PFPD was set to optimize for the detection of P, and although S could also be detected in a second PFPD signal channel, its response was suppressed with the GG 495 filter and it was not used in this study. In the analysis of real-world samples, the combination of phosphorus and halogen information provided by the PFPD + XSD could be very helpful in making analyte identifications using more than just t_R, especially if MS is not available to the analyst.

According to manufacturer, the PFPD is ten-fold more sensitive to P than the XSD is to Cl (detectabilities of 0.1 pg P/s *vs*. 1 pg Cl/s for the PFPD and XSD, respectively). Fortunately, twice as much of the GC column effluent went to the XSD than to the PFPD, which still gave a five-fold lower P detectability in this configuration *versus* Cl. The selectivity of the PFPD is also ten-fold better in avoiding carbon interferences (P/C $>10^5$ for the PFPD and Cl/HC $>10^4$ for the XSD). Thus, the XSD was unable to perform as well for the detection of Cl as the PFPD for the detection of P.

Figure 2 gives a typical GC/PFPD + XSD chromatogram in the separation of the pesticide test mix in MeCN. In practice, the drift in the XSD response was excessive and uncontrollable, especially in comparison with the PFPD, which was very stable. As Fig. 2 shows, the XSD responded to column bleed, unlike the PFPD, and detection of late-eluting pyrethroids (fenvalerate and deltamethrin) was problematic with the XSD. Furthermore, the XSD responded more than expected to some analytes that did not have a halogen, as may be observed in the example chromatogram, but it had a weaker than desired response for halogens other than Cl. For example, trifluralin has three F atoms in the molecule, yet it gave such a weak response in the XSD that we could not integrate it at the 1 ng injection level.

The pesticides chosen in the study included a wide cross section of GC-amenable OP and OC residues that are sometimes found in fruit and vegetable samples [37, 38]. The $K_{o/w}$ and water solubility values in Table 1 give an approximation of the polarity of the pesticides, which plays a critical role in sorptive extraction. Their physicochemical properties in terms of $K_{o/w}$ values, water solubilities, structures, volatilities, *etc.* vary to the widest extent possible for GC/PFPD + XSD analysis. The pesticide analytes were not chosen to demonstrate the optimal utility of SiSTEx and the detection method, but they were selected based on actual residue monitoring needs for fruits and vegetables. However, LC/MS-MS is also used in the QuEChERS method to detect many of the same pesticides as in GC, such as acephate, atrazine, azinphos-methyl, dichlofluanid, dichlorvos, dimethoate, methamidophos, mevinphos, omethoate, tebuconazole, and tolylfluanid [26, 27]. Coelutions were avoided as much as possible in the GC method to give a reasonably short chromatographic runtime of < 35 min.

3.2 Method optimization experiments

Once the chromatographic and detection conditions were optimized for the analysis of the chosen pesticides, experiments were performed to determine the effects of different parameters in the SiSTEx approach. For the sake of simplicity, we chose to limit our experiments to

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one type of silicone tubing (1.47 mm ID, 1.96 mm OD) and 20 mL sample volume in glass liquid scintillation vials. Also, room temperature (22°C) was used in all experiments. Furthermore, the use of the QuEChERS method for initial sample preparation was a central component of the study, and the method was not modified from the published protocol [27, 39].

MeCN has advantages for extraction of pesticides from fruits and vegetables over other solvents [24], including MeOH as employed in a previous study using sorptive extraction [5]. For one, the MeCN extracts from the QuEChERS method have undergone a salting out step with $MgSO_4$ and NaAc to separate the water from the sample, which results in higher pesticide concentrations and less matrix coextractives in the MeCN extract. For another, buffering is used thereby providing greater stability of key analytes (*e.g.*, captan, folpet, dichlofluanid, tolyfluanid), improving recoveries and reproducibility in results, and overcoming pH differences [27]. This procedure has been shown to yield high recoveries of a wide range of pesticides [24–28], and this aspect of the overall method did not need to be repeated. Thus, the pesticides were spiked into blank QuEChERS extracts to isolate the sorptive extraction step in the overall sample preparation method.

3.2.1 Choice of desorption solvent

The first aspect of the approach to be studied was the choice of solvent to desorb the pesticides from the tubing prior to GC analysis. In a previous study of solvent suitability for pesticide analysis by GC, toluene was found to be the best overall solvent in terms of pesticide stability, solubility, and injection aspects [40]. EtAc and MeCN were also acceptable for a wide range of analytes, particularly if MeCN was slightly acidified with 0.1% HAc and dark autosampler vials were used [40]. Acetone and MeOH were shown to be poor choices as solvents in pesticide analysis by GC methods.

Although toluene was the first choice as the desorption solvent and final extract solution, it was found to flow into and through the tubing within a matter of minutes due to its relatively low polarity. In a simple experiment, when 25 μL of toluene, EtAc, or MeCN was placed in the tubing, 0 μL toluene, 5 μL EtAc, and 15 μL MeCN remained after 30 min. Rather than leaving the solvent in the tube during the entire sorptive extraction time, we considered adding toluene at the end of the process for merely 1 min to desorb the polymer, but the toluene disappeared too quickly even in that case. Based on these results, we chose to use MeCN as the desorption solvent for the final extracts.

The autosampler needed at least 25 μL of solvent in the vials containing the inserts to make a 2 μL injection including preliminary washing steps. Thus, we needed to add 40 μL MeCN at the beginning of a 60 min SiSTEx process to consistently get back >25 μL. To account for MeCN volume losses, the added MeCN contained 50 ng/mL of each internal standard (d_{10}-parathion and triphenylphosphate for the PFPD, d_6-α-HCH for the XSD, and chlorpyrifos-methyl for both detectors). These were used for volume correction only, and chlorpyrifos-methyl was the primary internal standard used, and the others served for quality control and could be used if a problem was noted with chlorpyrifos-methyl.

To determine if MeCN was a strong enough solvent to desorb the pesticides (including the internal standards) from the tubing, 30 μL of a 500 ng/mL MeCN pesticide mixture solution was placed in the tubing for 60 min. The chromatographic peak areas of the pesticides in the MeCN (23 μL remained after 60 min) were compared with peak areas from the pesticides in the original solution. Taking volume loss into account, 96% of triphenylphosphate, 94% of the d_6-α-HCH, 93% of chlorpyrifos-methyl, and 91% of the d_{10}-parathion remained in the MeCN; thus the validity of using them as internal standards was reasonable.

All pesticide analytes were recovered >90% from the MeCN desorption solvent in the experiment except: chlorpyrifos (89%), bromophos (88%), λ-cyhalothrin (88%), vinclozolin (88%), *p,p′*-DDD (86%), endosulfan sulfate (80%), bifenthrin (78%), *p,p′*-DDE (70%), heptachlor (68%), deltamethrin (64%), and hexachlorobenzene (49%). Plots of pesticide recoveries *versus* their $K_{o/w}$ and water solubility values gave poor correlation (R^2 <0.2). Certain pesticides independent of their $K_{o/w}$ have high affinity for MeCN, certain pyrethroids in particular [41], and despite that some pesticides did not fully partition into the MeCN, the partitioning process is reproducible at fixed conditions.

To verify if the pesticides had partitioned into the tubing, 35 μL of MeCN was added to the same tube for 30 min and analyzed for the pesticides (the tube had been removed from the vial and the previous MeCN had been taken from the tube). Recoveries of the pesticides listed above were: chlorpyrifos (9%), bromophos (10%), λ-cyhalothrin (not detected, nd), vinclozolin (3%), *p,p′*-DDD (6%), endosulfan sulfate (10%), bifenthrin (7%), *p,p′*-DDE (20%), heptachlor (20%), deltamethrin (nd), and hexachlorobenzene (26%). Thus, MeCN was able to extract nearly 100% of most pesticides in the polymer, and in the worst case (hexachlorobenzene), 50% of the pesticide was recovered. A second addition of MeCN to the tubing would increase extraction efficiency, but this was not done in the final method because that would dilute the final extracts moreso than gain recoveries and act to increase detection limits.

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The kinetics of the partitioning between the MeCN and tubing phase was found to be very quick. The same pesticide recoveries occurred if 40 μL MeCN was added for 1, 30, or 60 min to the tubing. However, it was more convenient to add the MeCN at the beginning of the sorptive extraction process than at the end, and the MeCN volume loss over time actually lowered detection limits because of a higher final concentration. For 1 min desorption times with EtAc and toluene, no differences in the pesticide recoveries were observed *versus* those with MeCN. In all subsequent experiments, 40 μL MeCN added at the beginning of the SiSTEx process was employed.

3.2.2 Use of stirring and NaCl in SiSTEx

Sorptive extraction depends on the extent of the partitioning of the analytes between the polymer and sample and the kinetics of that process. The kinetics of SPME is typically diffusion limited, thus agitation or stirring of the solution is often performed to reduce the concentration gradient that forms around the polymer surface in the sample as partitioning occurs [2]. A major advantage of SBSE over SPME for water samples is that stirring is conveniently inherent in the approach. To increase the amount of analyte that partitions into the polymer, addition of NaCl to the aqueous solution often reduces the affinity of chemicals to the water phase and increases the partitioning into the polymer (*i.e.*, a salting out effect occurs). Stirring and salting out are common techniques to aid the sorptive extraction process, and we wanted to determine the efficacy of these techniques in the SiSTEx approach.

In the stirring experiment, 1 mL MeCN containing 0.5 ng of each pesticide was diluted to 20 mL with water. The extraction time was 40 min and the tubing length was 3.5 cm. When the stir-bar was used, stirring was conducted to induce a visible vortex in the solution. The results indicated that stirring increased the pesticide signals by an average factor of 5.8 over the course of 40 min. The highest factors were for those pesticides that were least soluble in water, such as permethrin (10.6-fold improvement), bifenthrin (9.6), *p,p′*-DDT (8.3), heptachlor (8.3), and *p,p′*-DDD (8.1). The stirring was critical in helping to keep those pesticides in solution in better contact with the tubing surface. In the case of more polar pesticides, such as dichlorvos (1.6-fold improvement), diffusion was not the limiting factor because they simply do not partition readily into the silicone. Unquestionably, stirring was needed in the initial sorptive extraction step of the SiSTEx procedure, and all other experiments in this study entailed stirring of the aqueous solutions.

In the salting out experiment, the same conditions were used as in the stirring experiment, except 0, 1, 2, and 5 g NaCl was added to the sample solution. Unfortunately, the addition of the NaCl caused the MeCN to separate from the water phase in the sample, and the interpretation of these results were not straightforward. Moderately polar pesticides, such as dichlorvos, azinphos-methyl, procymidone, and tebuconazole, yielded an increased enrichment factor as more salt was added to the sample. The most nonpolar pesticides (chlorpyrifos, hexachlorobenzene, heptachlor, and DDD, DDE, and DDT) other than pyrethroids, gave the opposite trend. The pyrethroids (bifenthrin, λ-cyhalothrin, deltamethrin, fenvalerates, and permethrins) gave a curious effect whereby the enrichment increased as NaCl was added, but not to the extent as had been expected compared to the results when NaCl was not added. The semipolar pesticides (*e.g.*, lindane, chlorothalonil, bromophos) exhibited no clear trend in their enhancement factors *versus* NaCl content.

Ultimately, we chose not to add NaCl to the MeCN extracts in the final SiSTEx method. The NaCl complicated the procedure and the improvement gained for the few moderately polar pesticides did not compensate for the losses of the most nonpolar pesticides. Most of the pesticides were not appreciably affected by the NaCl, thus it was easier to avoid the extra step. Usually when NaCl is used in sorptive extraction, the sample consists of water only, and the NaCl does not form two phases as it did in our case when MeCN was present.

3.2.3 %MeCN in the aqueous sample

As in the case of SBSE in which MeOH extracts are *diluted* with water to achieve a *higher* enrichment factor [5], the QuEChERS MeCN extracts must be diluted with water for the same reason in SiSTEx. This adds a complication in the SiSTEx approach for fruit and vegetable (and other solid sample types) as opposed to simpler extraction methods in water alone. The presence of MeCN in the aqueous sample affects the partitioning coefficients between the polymer and sample for each pesticide. Since a greater volume of MeCN is present in the glass vial, the pesticides have a higher affinity for the solution and less partitioning could occur into the polymer. However, the undiluted MeCN on the other side of the tubing pulls the pesticides across the polymer, thereby reducing the concentration of the pesticides in the polymer and inducing more analyte to partition into the polymer from the solution. If enough time is given, an equilibrium will form in which pesticides reach a steady concentration in all three phases, but predominantly in the MeCN for which the pesticides have the highest affinity (except for hexachlorobenzene which would be dispersed ≈1:1 between the MeCN and polymer).

For the sake of this discussion, let us assume that 100% of the pesticides are transferred from the liquid sample through the tubing to the MeCN final extract. This mimics a simple volume concentration step, which is the

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most obvious competing procedure for analyte enrichment in this situation. Then, the maximum enrichment factor is based on the amounts of MeCN added to the inside and the outside of the tube. If 1 mL (1000 μL) MeCN extract is diluted with water to serve as the sample, and 40 μL ACN is placed inside the tube, then the maximum enrichment factor is 25 (1000/40). If 10 mL QuEChERS extract is diluted with water, then the maximum enrichment factor is 250. This theoretical limit continues to increase as more MeCN extract is added and/or less MeCN is added into the tube, but in practice with this approach, the sample volume was limited to 20 mL and ≈25 μL was needed to make the injection. Also, limits will be reached in which the partitioning equilibrium will shift toward the larger volume of MeCN solution in the sample. Increasing sample volume may reduce this effect, but in any event, kinetics of the extraction process will not permit the theoretical limit to be achieved for most analytes in a reasonable time frame.

In an experiment, we added 1, 3, 4, 5, and 8 mL MeCN containing 500 ng/mL pesticide concentrations to the vials brought up to 20 mL with water. The samples were stirred for 40 min with 3.5 cm long pieces of tubing containing 40 μL of the 50 ng/mL internal standards solution in MeCN. Table 2 gives the theoretical maximum enrichment factors as discussed above and lists the results of the experiment. The pesticides are ordered in terms of highest to lowest enrichment factors for the 5:15 MeCN/water ratio data. The maximum theoretical enrichment for the 5:15 dilution factor is 125, and the enrichment factor of 47 obtained for fenvalerates is quite good considering the 40 min extraction time and the greater amount of MeCN outside than inside the tube. As mentioned previously, the XSD had trouble detecting deltamethrin and fenvalerates in direct MeCN extracts, but the large enhancement factor provided much help in lowering its detection limit.

As Table 2 indicates, the largest enrichment factors (30–47) were achieved for the pyrethroids (fenvalerates to bifenthrin), mostly in the 25% MeCN sample. The most nonpolar OCs are the next group on the list (*p,p′*-DDE to hexachlorobenzene), and they achieved their highest concentration enhancements (20–30) in the 25–40% MeCN sample range. The semipolar group (bromophos to procymidone) yielded enrichment factors of 3–20, with the maximum value most often occurring in the 15% MeCN sample. The most polar analytes (atrazine to omethoate) gave enrichment <2 in all of the samples. As in any sorptive extraction approach with silicone, SiSTEx is not suitable for this last group of pesticides, which tend to have water solubilities >100 mg/L and log $K_{o/w}$ values <3 (but this is not a rule).

Table 2. Enrichment factors of the pesticides in the final extract with respect to the original 500 ng/mL solution (normalized to chlorpyrifos-methyl) in 1, 3, 4, 5, and 8 mL MeCN that was diluted to 20 mL with water and extracted under stirring for 40 min with 3.5 cm silicone tubing. Highest enrichment factor is given in bold text for each pesticide

Pesticide	MeCN/water sample ratio 1/19	3/17	4/16	5/15	8/12
Theoretical Max.	25	75	100	125	200
Fenvalerates	1.4	21	21	**47**	16
Deltamethrin	0.82	19	25	**46**	14
λ-Cyhalothrin	1.4	19	20	**46**	20
Permethrins	1.7	17	19	**45**	40
Bifenthrin	1.3	8.6	11	28	**30**
p,p′-DDE	2.1	13	13	25	**31**
p,p-DDD	4.2	17	15	**23**	14
Endosulfan sulfate	2.4	14	13	**23**	**23**
Heptachlor	3.6	14	13	21	**24**
Hexachlorobenzene	2.7	12	10	18	**20**
Bromophos	3.6	12	**13**	**13**	11
Chlorpyrifos	3.2	11	**12**	**12**	11
Tolylfluanid	6.6	**17**	11	12	3.4
Dichlofluanid	5.1	**20**	7.4	12	3.2
Lindane	5.7	**14**	11	11	4.8
Ethion	2.6	9.7	**10**	9.8	7.0
p,p-DDT	5.4	**15**	10	9.2	1.9
Chlorothalonil	5.6	**14**	8.3	9.2	3.4
Pirimiphos-methyl	3.2	9.7	**9.8**	8.6	5.3
Vinclozolin	5.1	**11**	7.5	7.8	2.7
Diazinon	2.8	9.0	**9.1**	7.6	4.0
Phosalone	3.8	**11**	10	7.4	1.7
Folpet	4.8	**11**	5.1	6.4	2.2
Coumaphos	3.0	**8.6**	7.3	4.3	0.83
Captan	2.4	**5.6**	3.0	3.4	1.2
Procymidone	1.2	**3.0**	1.1	2.0	0.78
Atrazine	nd	**1.8**	nd	1.3	0.22
Azinphos-methyl	1.1	**1.9**	1.3	0.86	0.24
Dichlorvos	0.21	**0.56**	0.44	0.50	0.28
Tebuconazole	nd	**0.23**	0.15	0.16	0.07
Mevinphos	0.01	nd	0.03	**0.04**	nd
Acephate	nd	nd	nd	nd	nd
Dimethoate	nd	nd	nd	nd	nd
Methamidophos	nd	nd	nd	nd	nd
Omethoate	nd	nd	nd	nd	nd
Average	2.4	9.5	8.3	**13**	8.3

The same experiment was repeated using a QuEChERS extract of mixed fruits and vegetables. The same trend in the data occurred leading to the same conclusion. In selecting the final conditions, a compromise had to be made between highest enrichment factors of the most nonpolar pesticides, which occurred at 25–40% MeCN, and the semipolars, which occurred at 15–20% MeCN. Fortunately, most pesticides reached a plateau of sorts from 20 to 25% MeCN, which eased the decision. Ultimately, the 25% MeCN sample solution was chosen for the final method because it gave the highest average enrichment factor overall (as shown in Table 2).

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Table 3. Sorptive extraction parameters for different tubing lengths used in experiments

Parameter	SiSTEx approach Tubing length (cm)			SBSE approach Dimensions (mm length × mm thick)			
	3.0	3.5	4.0	10 × 0.5	10 × 1	20 × 0.5	20 × 1
Surface area (mm^2)	185	216	246	63	94	126	188
Polymer vol. (μL)	40	46	53	24	63	47	126
Inner vol. (μL)	51	59	68	n/a	n/a	n/a	n/a

n/a, not applicable.

3.2.4 Sorptive extraction time and tubing length

In theory, longer time and greater polymer volume can increase the enrichment factor in SiSTEx, at least until an equilibrium is reached. Greater surface area also increases the rate of extraction [2], but this aspect was not fully evaluated in this study. However, a pair of experiments was conducted to test these factors.

In the first experiment, 10, 20, 30, 40, 60, and 90 min extraction time under constant stirring was tested for 3.5 cm long tubes. The kinetics of the sorptive extraction process is typically on the order of hours or days until equilibrium is reached [2], and our application was not likely to be an exception. As expected, the results showed that the enrichment factors continued to increase as up to 90 min was given for the extraction, and the enrichment factors were typically less than a third of the theoretical maximum even for the nonpolars (as shown in Table 2). Just as Sandra *et al.* [5] chose to perform sorptive extractions for 60 min, we also felt that was a reasonable length of time to conduct the extractions in parallel. Perhaps higher enrichment factors could have been achieved with longer extraction times, but this was not practical in our application.

To increase the polymer phase volume and surface area in the sorptive extraction approach, we used different lengths of tubing. Due to the constraints of tubing length that could easily fit in the 20 mL vial, and still hold 40 μL of desorption solvent, we could only evaluate 3–4 cm tubes. Table 3 gives parameters of the polymer for 3.0, 3.5, and 4.0 cm lengths of the tubing chosen for the study. For comparison purposes, the given parameters are listed for the SBSE dimensions that are commercially available.

The experimental results of tubing lengths indicated that 4.0 cm length gave 56 and 49% greater responses for all the pesticides on average than the 3.0 and 3.5 cm long tubes, respectively, in the given extraction time (60 min). Some of the pesticides were extracted equally well or some better than others in each of the 3.0 and 3.5 cm tube lengths, but all of the pesticides gave higher responses in the case of the 4.0 cm tubes. This indicated that the longer tubing length was better than the shorter lengths.

3.2.5 Cleanup

In theory, higher enrichment factors can be achieved faster than in SiSTEx or other forms of sorptive extraction by evaporating the solvent to reduce the QuEChERS extract volumes. There are multiple reasons that this is not so practical: (1) Coextracted matrix materials also become more concentrated by an equal ratio as the analytes, which can adversely affect the GC analysis; (2) losses of volatile analytes, such as dichlorvos, will occur to some extent; (3) it is problematic to work with final volumes <500 μL in typical glassware designed for solvent evaporation; and (4) the initial QuEChERS extract contains ≈14% water, and if the extract is evaporated to be less than about 200 μL, then this would lead to a final extract of 100% water, evaporation difficulties, and GC injection problems.

The SiSTEx approach is not necessarily rapid, but it provides cleanup of relatively polar matrix components. Unfortunately, as shown in the bottom trace in Fig. 3, siloxanes from the tubing are also introduced to the final extracts, which gave interfering peaks on the XSD chromatograms (the peaks were identified as siloxanes by using GC/MS and mass spectral libraries). Soaking and/or sonicating the silicone tubing in MeOH for 60 min prior to their use tended to reduce the background in the XSD chromatograms from the SiSTEx approach. In any event, these types of coextractives were often the limiting source of noise for many pesticides detected by the XSD. The PFPD did not show significant interferences in either case.

When GC/MS and LC/MS-MS are used for analysis in the QuEChERS method, cleanup of the extracts is conducted using dispersive-SPE [24–28]. This entails the addition of 1 mL extract to a minicentrifuge tube that contains 150 mg of anh. $MgSO_4$ (to reduce water content from ≈14 to ≈2%) and 50 mg PSA (to remove fatty acids and other carboxylic acids). The tube is then agitated briefly to mix the sorbent with the extract and centrifuged prior to analysis. Depending on the sample composition and

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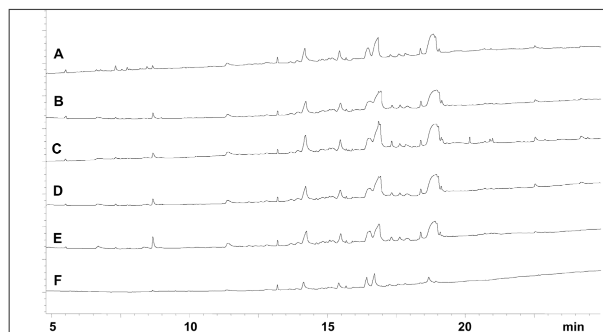

Figure 3. GC/XSD chromatograms of (F) MeCN desorption solvent from SiSTEx of an empty vial, and (A) SiSTEx of mixed blank fruit and vegetable QuEChERS extracts without cleanup, and (B) after dispersive-SPE cleanup with $MgSO_4$, (C) $MgSO_4$ + PSA, (D) $MgSO_4$ + PSA + C_{18}, and (E) $MgSO_4$ + PSA + C_{18} + GCB.

analytes, C_{18} and GCB may also be used in dispersive-SPE to remove other matrix components [24, 28]. This is an easy, fast, inexpensive, and effective cleanup approach that helps to improve the ruggedness of GC/MS and LC/MS-MS analyses.

We evaluated whether cleanup of fruit and vegetable extracts was needed in SiSTEx. Figure 3 shows the effect of dispersive-SPE using the least (none) to the most stringent cleanup ($MgSO_4$ + PSA + C_{18} + GCB) in GC/XSD chromatograms from the SiSTEx of QuEChERS extracts of mixed blank fruits and vegetables (PFPD chromatograms showed no significant interferences in any case). As the figure shows, few differences can be observed in the chromatograms from using the SiSTEx method with different degrees of cleanup or without it. The SiSTEx method effectively avoided the same chromatographic interferences as removed by the dispersive-SPE approach, but siloxane interferences were added by the approach. In the final method, we chose to use the initial QuEChERS extracts without dispersive-SPE cleanup prior to performing SiSTEx.

3.3 Validation

The final SiSTEx method was optimized based on the results from the method development studies described in Sections 3.1 and 3.2. This method involved the use of 4.0 cm silicone tubes containing 40 μL MeCN (including internal standards) for the extraction of 5 mL QuEChERS fruit/vegetable extracts mixed with 15 mL water for 60 min under continuous stirring conditions at room temperature. This method was evaluated to estimate detection limits, quantitative aspects, and precision.

3.3.1 Detection limits

The determination of LOD (S/N = 3) and LOQ (S/N = 10) was difficult for the XSD in this study due to the drifting baseline and presence of interferences. A convenient alternative approach that we adopted was to use the lowest calibration level (LCL) as an assessment of analyte detectability, which is the lowest calibration standard concentration that could be integrated with confidence.

Figure 4 gives an indication of how this was accomplished. The figure also shows the advantage of using the SiSTEx procedure to lower the detection limits for many pesticides that could not be detected normally in the QuEChERS extracts without conducting a solvent evaporation step and/or LVI. The figure also further demonstrates the difficulties experienced with the XSD in the study compared to the PFPD. Perhaps the ECD or electrolytic conductivity detector (ELCD) would have performed better in the application after all, but this possibility remains to be evaluated. We further note that the LCL values discussed below are method and detector dependent parameters, and for example, without the splitting of sample into the two detectors the values with the PFPD could be lowered by a factor of 3, while the XSD is not among the most sensitive detectors.

Based on the enrichment factors (see Table 2) and estimated LCLs from experiments using MeCN rather than QuEChERS extracts in SiSTEx, a new spiking solution was prepared with pesticides at 0.25, 2.5, or 25 ng/μL in MeCN for use in the fortification experiments of mixed fruit/vegetable extracts. The LCLs were determined by sequentially fortifying the mixed fruit and vegetable QuEChERS extracts at lower and lower concentrations until the pesticide peaks could no longer be reliably integrated.

Table 4 presents the results from these experiments. The LCLs in the mixed fruit/vegetable QuEChERS extracts (without cleanup) appear in the second column, and extracts analyzed using the final SiSTEx method appear in the third column. The LCL decreased for 25 of the 36 pesticides with an average 50-fold lower value for the 20 of those pesticides for which the enrichment factor could be calculated (values for chlorothalonil, dichlofluanid, fenvalerates, permethrins, and trifluralin could not be determined due to matrix interferences in the straight QuEChERS extracts). The pesticides that can be reproducibly detected at <10 ng/g meets the European baby food directive [42], and based on this experiment, 12 of the 36 pesticides could be quantified at ≈5 ng/g in the QuEChERS extracts without using SiSTEx, whereas the use of SiSTEx doubled that number.

As expected, the LCLs increased in SiSTEx for the relatively polar pesticides, acephate, atrazine, azinphos-methyl, dimethoate, methamidophos, mevinphos, and

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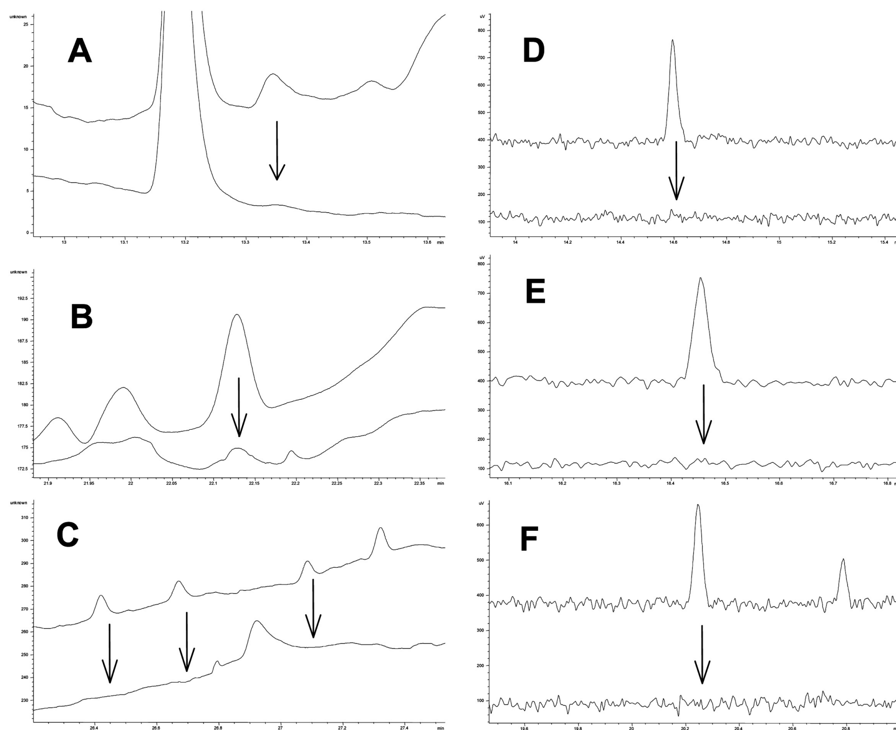

Figure 4. Examples of chromatograms obtained from the SiSTEx analysis of QuEChERS mixed fruit and vegetable extracts (upper traces) *vs.* those using direct injection of the extracts without SiSTEx (lower traces). Plots A, B, and C are XSD chromatograms of 0.1 ng/mL HCB, 10 ng/mL bifenthrin, and 10 ng/mL fenvalerates and deltamethrin, respectively. Plots D,E, and F are PFPD chromatograms for 0.5 ng/mL diazinon, 0.5 ng/mL pirimiphos-methyl, and 0.1 ng/mL ethion.

omethoate, all of which can be analyzed at ≈5 ng/g by LC/MS-MS [26, 27]. The LCLs remained the same for *p,p′*-DDE, dichlorvos, lindane, and tebuconazole. The only curious result was in the case of *p,p′*-DDE, and this was because the DDE peak from the SiSTEx extracts from matrix occurred on the shoulder of a large interferant, presumably an enriched matrix component. Considering that *p,p′*-DDD and *p,p′*-DDT gave enrichment factors of 50 and 200, respectively, *p,p′*-DDE could be detected much more sensitively in the absence of the interfering peak.

The effects of matrix and cleanup provided by SiSTEx can be observed by comparing Figs. 2 – 4. Those pesticides most affected by interferences included the late-eluting pyrethroids (permethrins, fenvalerates, and deltamethrin). In the cases of captan, chlorothalonil, dichlofluanid, folpet, and tolylfluanid, SiSTEx provided lower LCLs and reduced degradation of the pesticides in the QuEChERS extracts over the course of the 60 min extraction time [26, 27, 40]. This was an unexpected and welcome outcome that the method worked reasonably well for those problematic pesticides.

3.3.2 Repeatability and reproducibility

The attempt was made to preliminarily assess quantitative aspects of the SiSTEx method. A series of five spikes were made in mixed fruit/vegetable matrix over the concentration range of 0.1 – 10, 1 – 100, and 10 – 1000 ng/g depending on the LCLs listed in Table 4. As we had determined that matrix makes a significant impact in the partitioning process for many of the pesticides, matrix-matched calibration was needed to perform quantitation. In a method using SBSE, Sandra *et al.* [5] employed the method of standard additions for quantitation, but this is too unwieldy in practice for so many pesticides and potential concentrations. In general, the PFPD gave satisfactory calibration plots, but due to the drifting baseline and interferences, the XSD gave highly variable quality of results depending on the analyte. The linear dynamic range of the XSD was also less than the PFPD, which posed another difficulty.

The precision of the SiSTEx method was determined by using the final procedure to analyze four batches of three replicate extractions *per* batch of mixed fruit/vegetable QuEChERS extracts spiked at 10, 100, or 1000 ng/g. %Repeatability represents the precision of the method within a single batch of analyses, and %reproducibility is the precision of the method among the four different batches. The %RSD values are given for each pesticide in Table 4 with respect to %repeatability (average RSD among the batches, $n = 4$) and %reproducibility (overall

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Table 4. Evaluation of the final SiSTEx method (using chlorpyrifos-methyl as the internal standard) for each pesticide. %Repeatability is average, %RSD of results among four batches of three mixed fruit/vegetable extracts spiked at the highest level in the calibration range, and %reproducibility is overall %RSD of the results

Pesticide	LCL without SiSTEx (ng/g)	LCL with SiSTEx (ng/g)	%Repeatability (n = 4)	%Reproducibility (n = 12)
Acephate[a]	100	>1000	int.	int.
Atrazine[b]	50	>100	int.	int.
Azinphos-methyl[a]	5	10	10	18
Bifenthrin[b]	50	5	10	13
Bromophos[a]	10	0.1	5	6
Captan[b]	10	1	7	10
Chlorothalonil[b]	int.	5	7	13
Chlorpyrifos[a]	5	0.1	6	5
Coumaphos[a]	10	1	10	9
λ-Cyhalothrin[b]	100	1	10	8
p,p′-DDD[b]	5	0.1	11	16
p,p′-DDE[b]	5	5[c]	14	31
p,p′-DDT[b]	100	0.5	5	8
Deltamethrin[b]	1000	25	7	8
Diazinon[a]	10	0.1	5	6
Dichlofluanid[b]	int.	5	8	12
Dichlorvos[a]	10	10	9	11
Dimethoate[a]	10	500	17	20
Endosulfan sulfate[b]	5	0.05	10	12
Ethion[a]	5	0.1	4	5
Fenvalerates[b]	int.	5	7	10
Folpet[b]	10	1	8	9
Heptachlor[b]	0.5	0.1	6	8
Hexachlorobenzene[b]	5	0.05	6	8
Lindane[b]	5	5	2	3
Methamidophos[a]	50	>1000	int.	int.
Mevinphos[a]	100	>1000	12	15
Omethoate[a]	100	>1000	int.	int.
Permethrins[b]	int.	50	9	16
Phosalone[a]	5	1	7	8
Pirimiphos-methyl[a]	5	0.1	5	7
Procymidone[b]	10	1	8	10
Tebuconazole[b]	100	100	9	13
Tolylfluanid[b]	5	2	5	8
Trifluralin[b]	int.	25	6	8
Vinclozolin[b]	10	5	4	7

a) PFPD used for analysis.
b) XSD used for analysis.
c) LCL limited by siloxane interferant; int. = interferences.

RSD, n = 12). As the results indicate, both forms of precision were quite good for nearly all pesticides. The average repeatability of the 32 detected pesticides was 8 ± 3 %RSD and reproducibility was 11 ± 5% RSD. Only four detected pesticides (*p,p′*-DDD, *p,p′*-DDE, dimethoate, and mevinphos) gave repeatability >10 %RSD, and five pesticides (azinphos-methyl, *p,p′*-DDD, *p,p′*-DDE, dimethoate, and permethrins) gave reproducibility >15 %RSD.

The satisfactory linearity of the calibration curves for the OP pesticides in PFPD and precision of the method when proper precautions were taken (matrix-matching) indicates that the sorptive extraction approach could be quantitative. Several different matrices and many replicates would have to be analyzed to better test the aspects of quantitation (or semiquantitative screening applications). This aspect was not investigated fully, however, because only four devices were available for experiments, and this report was only intended to be a preliminary investigation of the concept.

4 Concluding remarks

A new SiSTEx device was developed and was applied and evaluated for the analysis of a broad range of pesticides in fruit and vegetables. The SiSTEx concept possesses several advantages in the combination of simplicity, provision of sample enrichment, limited use of solvents, low cost, and applicability to a range of nonpolar and semipolar pesticides. Furthermore, high enrichment factors (low LOQs) could be achieved for several pesticides without the need for cleanup or concentration steps in the procedure.

The SiSTEx approach provides some important advantages over SBSE, which include: (1) the analysis involves the standard splitless GC injection of clean and highly concentrated liquid solvent extracts without the need for expensive added thermal desorption equipment; (2) the GC injection is done at relatively lower injector temperature than for SBSE thermal desorption, hence, it is better for the analysis of thermally labile pesticides; and (3) the silicone tubing used is cheap and could be replaced after every analysis, thus providing less carry over and lower cost of analysis. SiSTEx can also be used the same as SBSE for the analysis of air, head space, and compounds in pure or contaminated water.

Due to varying partitioning factors inherent to the sorptive extraction concept, SiSTEx does not provide the wide analytical scope to cover all pesticides that need to be routinely monitored in fruits and vegetables, but it works well to lower the LOQs for some important nonpolar pesticides. This approach could be used in conjunction with LC/MS-MS of the QuEChERS extracts to improve the overall scope of analysis. For example, good results were achieved for captan and folpet in the method, which are problematic in the QuEChERS method using GC/MS analysis and cannot be adequately detected by LC/MS-MS. Several semipolar and nonpolar OP pesticides were also reproducibly detected with LOQ < 10 ng/g using the PFPD. For OC pesticides, the XSD was not as selective or sensitive as desirable, but when instrument drift, matrix components, column bleed, or siloxanes did not interfere with detection, several semipolar and nonpolar OC pesticides could also be reproducibly detected with LOQ < 10 ng/g. Unfortunately, results for the late-eluting pyrethroids were disappointing in fruit/vegetable extracts using the XSD detection.

Overall, we believe that the SiSTEx method can be effectively used in the analysis of suitable selected target pesticides in fruit and vegetables with various detectors, including MS.

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