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Does further clean-up reduce the matrix enhancement effect in gas chromatographic analysis of pesticide residues in food?

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Abstract

Sample extracts of apples, peas, green beans, oranges, raspberries, clementines, carrots, and wheat obtained using the Food and Drug Administration (acetone extraction) and Canadian Pest Management Regulatory Agency (acetonitrile extraction) multiresidue methods for pesticides were subjected to clean-up using different solid-phase extraction (SPE) cartridges in an attempt to reduce or eliminate the matrix enhancement effect. The matrix enhancement effect is related to the blocking of active sites on the injector liner by matrix components, thereby increasing signal in the presence of matrix versus standards in solvent in which the pesticides themselves interact with the active sites. Graphitized carbon black (GCB) was often used in combination with various anion-exchange SPE cartridges. The extracts were then spiked with organophosphorus insecticides. These process standards were then compared to standards in acetone of the same concentration using gas chromatography with flame photometric detection or ion trap mass spectrometric detection. Sample matrix enhancement varied from little to no effect for some pesticides (e.g. chlorpyrifos, malathion) to >200% in the case of certain susceptible pesticides. The GCB removed color components but showed little effect in reducing matrix enhancement by itself. The anion-exchange cartridges in combination with GCB or not, substantially reduced the matrix enhancement effect but did not eliminate it. Published by Elsevier Science B.V.

Keywords: Food analysis; Matrix enhancement effect; Clean-up methods; Solid-phase extraction; Pesticides

1. Introduction

Matrix induced enhancement is a phenomenon commonly encountered in the gas chromatographic (GC) analysis of pesticides in foods [1–10]. Active sites in the GC system, mainly in the injection liner, which adsorb and/or induce thermal degradation of certain analytes, are the main source of the matrix enhancement effect [2]. In a standard solution of pesticide analytes in solvent, more active sites are available to the pesticides than when the injected solution also contains matrix components which act to block the active sites. Thus, the injection efficiency for the affected trace analytes is greater in the presence of co-extracted matrix components than in solvent-only solutions. This leads to a greater response and erroneously high calculated pesticide

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concentration if standards in pure solvent solutions are used for calibration.

Some of the factors that may affect sample matrix enhancement include: the nature of the pesticide, the nature of the matrix, the pesticide-to-matrix ratio, and the GC system [1]. Several precautions (with varying degrees of success) may be taken to overcome or reduce the matrix enhancement effect, which include (1) use of standards in blank matrix (matrix-matched standards) (2) use of a method of standard additions (3) extensive clean-up to reduce matrix components (4) use of similarly affected internal standards (such as deuterated pesticides) (5) use of on-column or other means of injection in GC to avoid the effects of active sites (6) 'priming' the GC system by loading matrix components in an attempt to fill active sites and (7) compensation of the calculated results by a 'matrix enhancement factor'. Ideally, the use of inert surfaces in the GC system would eliminate the matrix enhancement effect, but silica-based surfaces, even fused-silica, contain a large number of active sites (e.g. hydroxyl groups, metal ions). The use of coated liners and/or alternative injection techniques, such as pulsed splitless injection [10] or on-column injection, can be very effective in reducing the effect, but the added expense, system incompatibilities, and/or lack of ruggedness often makes these options less popular. Priming of the GC system does not work well for repeated injections and can lead to poor chromatography and more GC maintenance.

The use of standards in blank extracts is the most common option followed by many laboratories due to the ease of use and effectiveness of the approach [11]. Its drawbacks can include the need for blank extracts, greater potential for analyte degradation [12], and the extra labor potentially involved. Furthermore, the current regulatory policy in the US Environmental Protection Agency (EPA) and Food and Drug Administration (FDA) related to pesticide residues in food does not permit the use of standards in matrix for calibration. Furthermore, current EPA and FDA policies do not permit the correction of results using surrogate standards or other types of recovery correction procedures. The use of internal standards is permissible for US regulatory purposes, but in the case of matrix enhancement, due to differences in the strength of the effect dependent on the pesticide, each pesticide would need its own deuterated internal standard. Multiple deuterated pesticides are expensive, unavailable, and/or impractical in multiresidue analysis.

Therefore, the remaining option for US federal regulatory agencies is to perform extensive clean-up of extracts in an attempt to remove matrix components and reduce or eliminate the matrix enhancement effect. Unfortunately, this leads to additional time, labor and costs. This study is designed to determine the effectiveness of extract clean-up using various solid-phase extraction (SPE) cartridges to reduce matrix enhancement in the analysis of food samples. The effects on a variety of organophosphorus (OP) pesticides were also compared using different extraction procedures, GC systems, and analyte concentrations.

2. Experimental

2.1. Materials

Acetone, acetonitrile (MeCN), petroleum ether, dichloromethane (DCM), and toluene were pesticide residue grade. Pesticide standards were obtained from the EPA (Fort Meade, MD, USA). Figs. 1 and 2 provide the chemical structures of the OP pesticides used in the study. Individual stock standard solutions (0.25–1.0 mg/ml) of each standard were prepared in acetone. Mixed standard solutions were prepared from the stock solutions with acetone to achieve concentrations of 2.5 and 10.0 μg/ml. Ethion, which was unaffected by the matrix enhancement effect, was used as an internal standard in the solutions. Matrices included apple, green bean, clementine, orange, raspberries, pea, and wheat samples, which were previously analyzed and found to be free of the targeted pesticides at detectable levels, and which were obtained from the FDA pesticide surveillance program at the Baltimore District Laboratory.

SPE cartridges used in the study were all 500 mg and consisted of: Envi-Carb graphitized carbon black (GCB) (Supelco, Bellefonte, PA, USA); Bond Elut primary/secondary amine (PSA) (Varian, Harbor City, CA, USA), aminopropyl ($-NH_2$) (Varian), and strong anion-exchange (SAX) (Varian). All other

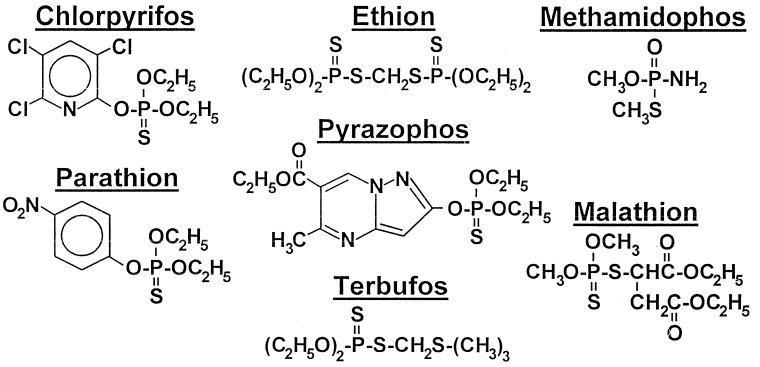

Fig. 1. Chemical structures of the OP pesticides in the study that did not exhibit notable matrix enhancement effects.

materials were as described in the Pesticide Analytical Manual [13].

2.2. Extraction

Samples were extracted using both the US FDA method [13,14] and Canadian Pest Management Regulatory Agency (PMRA) method [15]. For the FDA method, 100-g sample portions were each blended with 200 ml acetone and filtered through sharkskin filters. From each extract, 80-ml portions (equivalent to 27.5 g each) were subjected to two DCM–petroleum ether partitioning steps to separate the water, with NaCl added to the aqueous layer after the first partitioning. For the PMRA method, 60-g samples were weighed into 250-ml PTFE centrifuge bottles and blended with 120 ml of MeCN using a Tissumizer homogenizer (Tekmar; Cincinnati, OH, USA). Excess NaCl was added, and samples were blended again and centrifuged. The upper layers from the duplicate samples were dried with anhydrous Na_2SO_4. Extracts were evaporated prior to SPE (or not) and, in both cases, the final extract concentrations were 10 g commodity per 1 ml of solution.

2.3. SPE clean-up

The combination of cartridges chosen for the study consisted of: GCB, GCB+–NH_2, GCB+SAX, GCB+PSA, GCB+PSA+SAX, and PSA alone. The GCB cartridge was the top cartridge when it was utilized. Each clean-up procedure was conducted in triplicate, and controls (in triplicate) in which no

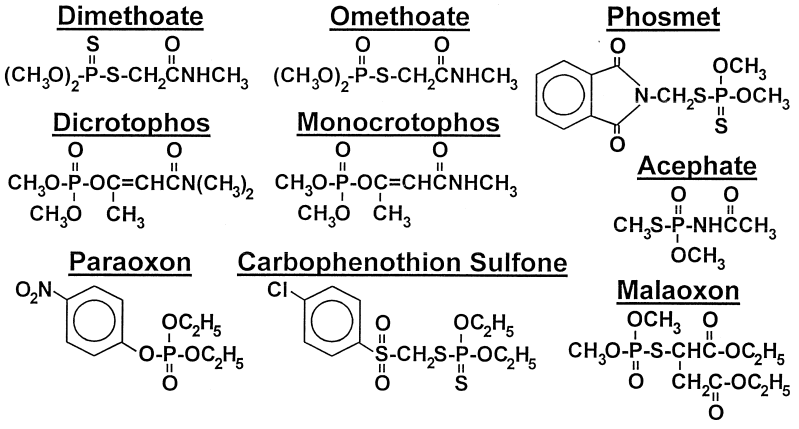

Fig. 2. Chemical structures of the OP pesticides in the study that exhibited notable matrix enhancement effects.

clean-up was performed were set aside for later analysis.

Anhydrous Na_2SO_4 (≈2 cm) was added above the sorbent bed to the top SPE cartridge. Anhydrous $MgSO_4$ was found to be more effective at drying the MeCN extracts than Na_2SO_4, [9] but in keeping with the Canadian method Na_2SO_4 was used in this study. When tandem cartridges were used for clean-up, the cartridges were connected using adapters. Prior to the addition of the extract, the cartridge(s) were placed on a vacuum manifold and washed with 5.0 ml of MeCN–toluene (3:1, v/v) (or MeCN in the case of PSA alone). Labeled 15-ml graduated centrifuge tubes were placed in the manifold to collect the extracts from the cartridge(s). For both acetone and MeCN extracts, 0.5 ml (equivalent to 5 g commodity) was added to the top cartridge, and flow was adjusted to 1–2 drops/s under vacuum. When all of the solvent passed through the SPE cartridge(s), 15 ml of MeCN–toluene (3:1, v/v) was added to elute the pesticides (in the case of PSA alone, 10 ml of MeCN was used). The eluate was evaporated to <1 ml with the aid of a stream of nitrogen at 40°C. Then, ≈10 ml acetone was added, and the eluate was again evaporated to <1.0 ml to remove any residual MeCN and toluene. The final volumes of the extracts were 2.0 ml in acetone. Prior to analysis, 100 μl of 2.5 or 10.0 μg/ml of pesticide mix solution was added to give the desired pesticide concentrations. Triplicate control extracts (5 g sample per 2 ml acetone+100 μl pesticide mixtures) that did not undergo clean-up were also prepared. Similarly, pesticide standard solutions of the same concentrations in the same volumes of acetone were prepared to measure the enhancement effect.

2.4. Analysis

Gas chromatography (GC) coupled with a flame photometric detection (FPD) system or an ion trap mass spectrometric (ITMS) detection system was used for analysis of the extracts. For GC–FPD, an HP-5890 Series II (Hewlett-Packard, Palo Alto, CA, USA) was utilized. Its analytical column was 30 m×0.53 mm I.D., 1.5 μm film thickness DB-5 widebore capillary column (J&W Scientific, Folsom, CA, USA). The He carrier gas head pressure was 50 p.s.i. (1 p.s.i.=6894.76 Pa) at 12 ml/min flow-rate through the column at 200°C, and He make-up gas through the FPD was 10 ml/min. Detector temperature was 225°C, and injection temperature was 220°C using direct splitless injection of 2 μl into a 4-mm I.D. liner. The oven temperature program was 130°C for 1 min, and 6°C/min until the final temperature of 225°C was reached and held for 17 min.

For GC–ITMS, analysis was performed using a Finnigan ITS40 (San Jose, CA, USA). A narrowbore Rtx-5 ms (Restek, Bellefonte, PA, USA), 30 m×0.25 mm I.D., 0.25 μm film thickness, capillary column coupled to a 5-m phenylmethyl deactivated (Restek) guard column (0.25 mm I.D.) was employed in the separation. A Model 1093 (Varian, Walnut Creek, CA, USA) septum programmable injector (SPI) was used for the 1-μl injection volume into a silanized high-performance type insert. The temperature programming of the SPI was 50°C for 6 s followed by ramping to 260°C at 200°C/min. The GC was set to 10 p.s.i. He column head pressure (34 cm/s at 50°C), and the oven program was 50°C initial temperature for 6 s, ramped to 270°C at 10°C/min, and held until 30 min total time elapsed. The transfer line temperature was 270°C, and the ion-trap manifold temperature was 220°C. Positive ion chemical ionization mode with methane was used for the analysis of the pesticides.

In all cases, peak areas divided by peak areas of the internal standard were used for quantitation. A set of extracts usually consisted of the fifteen extracts that had undergone clean-up (five different SPE procedures in triplicate), three that had not undergone clean-up, and four to ten injections of duplicate standards in acetone interspersed throughout the sequence. The analysis sequences always ended with the analysis of the extracts that had not undergone clean-up, and the injection port liner (and guard column in the GC–ITMS) was replaced prior to the next sequence. The chromatographic response enhancement recovery (in terms of percentage) was the signal of the standards in the extracts divided by the signal of the standards in acetone at the same concentrations.

3. Results and discussion

Matrix enhancement was difficult to study because

the GC system changed as matrix compounds were progressively deposited in the injection port and front of the analytical column. The effect of changing the injection liner and/or column was also unpredictable. In one case, when a new column was placed in the GC–FPD system, there was little or no enhancement. The amount of enhancement in this system increased after a few weeks of repeated injections, and then decreased again even though the injection liner was very dirty and the chromatography of the late eluting compounds was seriously deteriorating. In this instance, replacing the dirty injection liner with a new one did not result in an increase in the enhancement effect on this GC–FPD instrument, but in similar situations previously, matrix enhancement was observed when the injection liner was changed. In the case of the GC–ITMS instrument, the matrix enhancement effect was always observed, but to a varying extent depending on the liner and condition of GC–ITMS system. We believe that variability in the surface activity of different GC liners (due to manufacturing differences and usage patterns) is the source of this problem. In the comparison of results, more weight was placed within the same set of samples than among different GC injection sequences.

3.1. SPE clean-up

In this study, extracts of fresh fruit, vegetable, and grain samples were obtained using the FDA and PMRA methodologies. The SPE cartridges included in this study have been demonstrated previously to provide high recoveries of many pesticides [15–19]. However, to eliminate pesticide elution as a variable, the extracts were spiked with OP insecticides after clean-up (or not) and these process standards were then compared to neat standards at the same spiking concentration in acetone. The extracts from both extraction methods were heavily pigmented, containing large amounts of matrix coextractants. Other studies have demonstrated the effectiveness of GCB for removing pigments from food extracts [16–18]. In this study, a carbon SPE clean-up of the extract was also effective in removing the majority of the coextracted pigment in the extract. The combination of an anion-exchange SPE column (SAX, PSA, or $-NH_2$) in tandem with GCB resulted in a clean-up that removed the remaining visible pigment.

Several different combinations of SPE cartridges were evaluated to determine if clean-up could reduce or eliminate the matrix enhancement effect. Tables 1–5 present the results from experiments conducted using different SPE clean-up cartridges and their combinations for different commodities and analyzed with the two instruments. The order of the pesticides listed in the tables is based on the recovery factors without clean-up in the upper row of the column farthest to the right. The columns are grouped by SPE cartridge (e.g. all clean-up procedures that contain PSA are adjacent to each other) to enable easier comparison of the effects of clean-up with different cartridges.

GCB clean-up resulted in the most dramatic visible change to the extracts, but it had little effect on reducing the matrix enhancement effect (Table 1). This was observed most notably in the cases of omethoate, monocrotophos, malaoxon, dimethoate, phosmet, and acephate which all exhibited a 20–50% higher signal due to the matrix enhancement effect. Paraoxon gave a small enhancement effect of $<10\%$ in this experiment. No pesticide gave noticeably reduced matrix enhancement after GCB clean-up alone. Therefore, although GCB is very useful for removing pigment, the pigment did not play a role in matrix enhancement.

When GCB was used in combination with one of the weak anion-exchange SPE columns, PSA or $-NH_2$, a definite reduction in matrix enhancement occurred, as shown in Tables 1–3. The use of PSA alone was also evaluated and in nearly all cases was found to reduce the matrix enhancement effect versus no clean-up (Tables 1–3 and 5). No difference in the matrix enhancement effect was observed between the use of PSA alone or its combination with GCB (Tables 1–3), but the extracts after PSA clean-up alone still contained visible pigmentation.

The SAX column, used in combination with GCB, appeared to be slightly less effective than the weak anion-exchange columns in reducing matrix enhancement (most notably in Table 3). In the GC–FPD results, the combination of GCB, SAX, and PSA appeared to be slightly better than using GCB and PSA (or $-NH_2$) or PSA alone. Therefore, SAX either removed somewhat different components than the

Table 1
Chromatographic response enhancement recoveries [(matrix standard/matrix free solvent standard)×100%] using GC–FPD analysis of apples extracted with the FDA method after SPE clean-up (or not) and spiked with the FPD pesticide mix (0.070–0.600 mg/kg) — numbers in bold are >110% and italicized numbers had RSD values >10% (otherwise, RSD was <10%)

Pesticide	Average recovery, (%) $n=3$						
	GCB+ $-NH_2$	GCB+ SAX	GCB+SAX +PSA	GCB+ PSA	PSA	GCB	None
Omethoate	**120**	**123**	108	**116**	**115**	***141***	**145**
Monocrotophos	**122**	**124**	108	**116**	***114***	***145***	**143**
Malaoxon	105	**111**	100	103	105	**126**	**132**
Dimethoate	**121**	**116**	107	**113**	**114**	**126**	**130**
Phosmet	***120***	*108*	92	**110**	***111***	**120**	**129**
Acephate	**112**	**117**	104	108	*105*	**125**	**122**
Paraoxon	98	102	97	98	100	106	109
Malathion	99	100	99	100	100	104	106
Parathion	97	99	100	99	98	99	100
Methamidophos	103	106	105	103	*98*	106	99
Pyrazophos	**111**	97	100	103	*103*	105	99
Terbufos	99	101	105	102	99	100	99
Average	109	109	102	106	105	**117**	**118**

weak anion-exchange columns, or the greater capacity of the SAX+PSA combination helped to reduce the matrix enhancement effect. In any case, none of the SPE cartridges or combinations tested were able to eliminate the matrix enhancement effect.

Fig. 3 presents a good demonstration of the differences between GCB, SAX, and PSA in the clean-up of wheat extracted using the PMRA method. The figure is a GC–ITMS total ion chromatogram of the extract after no clean-up, clean-up using GCB+SAX, and clean-up using PSA alone. The chromatograms are scaled appropriately to allow direct comparison of the intensity of the responses. The use of GCB+SAX did very little to remove the major interferences in the chromatogram; only a few small peaks were removed toward the end of the chromatogram. However, PSA did an excellent job of removing the large matrix components, and the pigment was much reduced as well. The matrix components were not detected in GC–FPD and did not interfere in its analysis (see Table 2), but the large interferences in the wheat made GC–ITMS analysis very difficult for co-eluting pesticides except when PSA was used for clean-up.

Table 2
Chromatographic response enhancement recoveries [(matrix standard/matrix free solvent standard)×100%] using GC–FPD analysis of wheat extracted with the PMRA procedure and pesticides spiked at 0.200 mg/kg — numbers in bold are >110% and italicized numbers had RSD values >10% (otherwise, RSD was <10%)

Pesticide	Average recovery (%), $n=3$			
	GCB+SAX+ PSA	GCB+ PSA	PSA	None
Carbophenothion sulfone	**270**	***260***	**280**	***320***
Phosmet	***118***	**127**	**131**	***137***
Dicrotophos	**110**	**112**	**114**	**118**
Paraoxon	107	108	108	**114**
Malaoxon	107	108	104	106
Chlorpyrifos	100	100	99	100
Malathion	98	99	99	99
Average	**122**	**131**	**134**	**142**

Table 3
Chromatographic response enhancement recoveries [(matrix standard/matrix free solvent standard)×100%] using GC–ITMS analysis of apples extracted with the FDA and PMRA methods after SPE clean-up (or not) and spiked with pesticides at 0.2 mg/kg — numbers in bold are >110% and italicized numbers had RSD values >10% (otherwise, RSD was <10%)

Pesticide	Method	Average recovery (%) $n=3$					
		GCB+ $-NH_2$	GCB+ SAX	GCB+SAX +PSA	GCB+ PSA	PSA	None
Dicrotophos	FDA	***160***	**200**	**160**	**160**	**170**	**230**
	PMRA	***240***	**260**	**230**	**230**	***220***	***340***
Carbophenothion sulfone	FDA	**112**	**120**	**119**	**123**	**117**	**130**
	PMRA	109	**134**	**135**	**123**	**129**	108
Paraoxon	FDA	**111**	**126**	**119**	**117**	104	**116**
	PMRA	**118**	**137**	***124***	**123**	***137***	**210**
Phosmet	FDA	**127**	**114**	*109*	**131**	**128**	**140**
	PMRA	**118**	**150**	***141***	**139**	**160**	**160**
Malaoxon	FDA	**160**	**210**	***180***	**180**	**150**	**190**
	PMRA	**260**	**340**	***270***	***280***	***300***	**600**
Malathion	FDA	108	108	104	108	108	104
	PMRA	102	106	*106*	108	107	97
Chlorpyrifos	FDA	103	100	102	102	*93*	*107*
	PMRA	94	96	92	89	97	99
Terbufos	FDA	96	100	106	97	87	66
	PMRA	94	96	*97*	98	97	69
Averages	FDA	**121**	**135**	**125**	**128**	**121**	**136**
	PMRA	**141**	**160**	**149**	**148**	**160**	**210**

3.2. Pesticide concentration

Hajšlová et al. stated that the concentration of analyte relative to the matrix material played a large role in the amount of matrix enhancement and that larger amounts of enhancement would be encountered with lower concentrations of analyte [1]. In this study, the effect of analyte concentration on enhancement was evaluated in experiments with the two different GC systems, and with extracts that were subjected to SPE clean-up or not. In the case of GC–ITMS, a 4-fold difference in pesticide concentrations (0.05–0.2 mg/kg) was used, and in the case of GC–FPD, the experiment involved three spiking levels with 20-fold differences in concentrations among analytes. The results from the GC–ITMS experiment did not show a notable increase in the matrix enhancement effect except in the cases of malathion and carbophenothion sulfone. Whereas malathion exhibited the expected trend that the 0.05 mg/kg concentrations gave a 6–19% higher recovery factor than the 0.2 mg/kg level, carbophenothion sulfone actually gave a 10–24% lower matrix enhancement effect at the 0.05- than at the 0.2-mg/kg level for the extracts that underwent SPE clean-up. Otherwise, the general lack of recovery differences between the 0.05 and 0.2 mg/kg concentrations was an unexpected result and the experiment was repeated with the GC–FPD.

The results presented in Table 4 were conducted on the same sample set on GC–FPD, and a 20-fold concentration range was used. As in the case of the GC–ITMS, the GC–FPD results did not clearly demonstrate more matrix enhancement when lower concentrations of analyte were injected. A matrix enhancement effect related to concentration was anticipated for all affected pesticides, but the lack of differences for some pesticides (omethoate, acephate, methamidophos) and a possible effect for others (dimethoate, terbufos, pyrazophos) was a surprising result.

Interestingly, terbufos and pyrazophos gave higher relative responses in matrix at the lower concentrations. No matrix enhancement was observed for these pesticides at higher concentrations or in other experiments. It is possible that interferants were the source of the effects at the low concentrations as noted by the higher variability of these results.

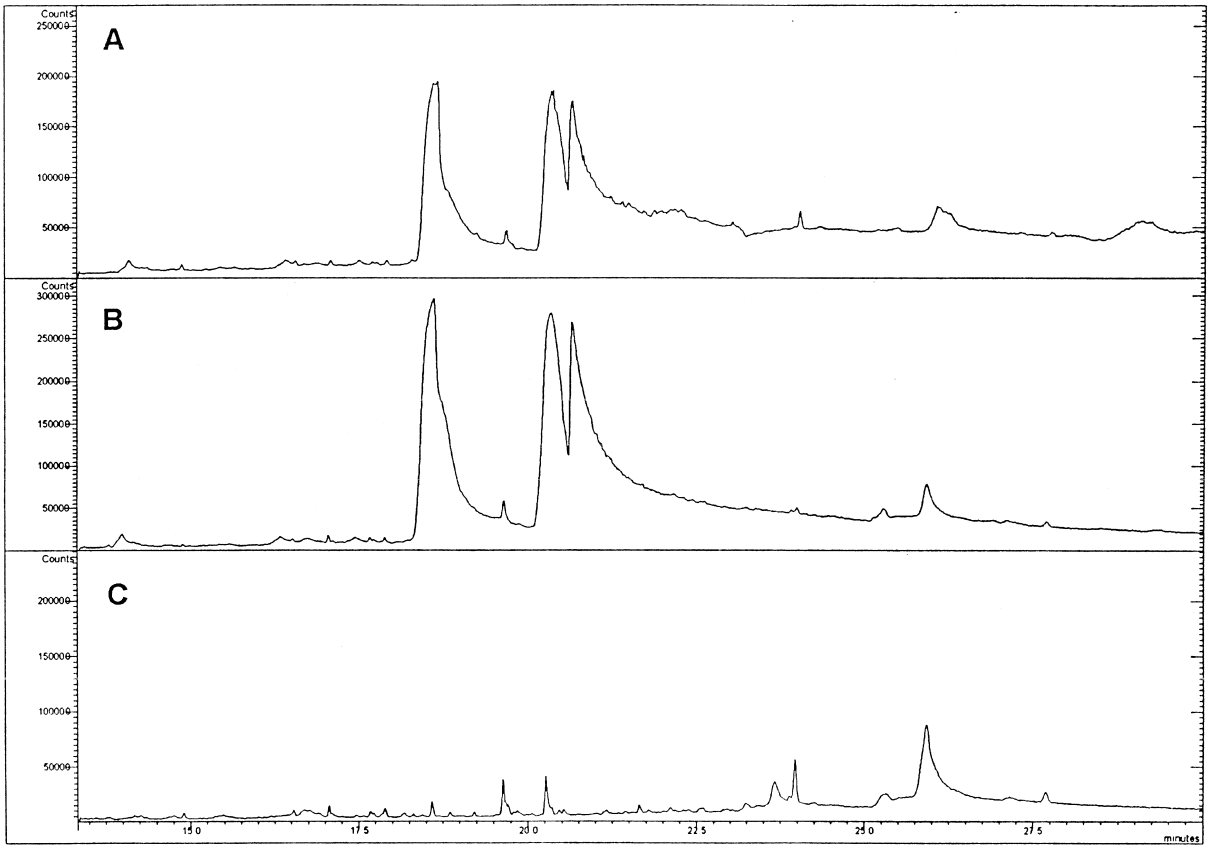

Fig. 3. GC–ITMS total ion chromatograms before and after SPE clean-up of blank wheat extracted using the PMRA method: (A) no clean-up (B) GCB+SAX clean-up (C) PSA clean-up.

3.3. GC instrument

Table 5 directly compares the results of the same extracts analyzed using the different GC instruments. The GC–ITMS instrument tended to give a higher matrix enhancement effect than the GC–FPD instrument. For example, phosmet had as much as an 8-fold enhancement on the GC–ITMS, while its enhancement was less than 2-fold on the GC–FPD. This was believed to be due to the different type of liner and smaller diameter column in the GC–ITMS system. Furthermore, the ion trap MS itself was believed to contribute to the enhancement effect due to the metal surfaces in the detector and non-combustion detector approach. The efficiency of the FPD response was not believed to be affected by matrix components. Quenching of the response due to high levels of matrix components would have led to a reduction in the signal, which was not observed.

The GC–ITMS used septum-programmable injection (SPI) at 50°C while the GC–FPD used splitless injection at 250°C. The lower injection temperature which permitted injection of acetone as a liquid was believed to decrease analyte interactions with the liner in a previous study [6], but the interactions were still quite large in this study. Furthermore, the lower initial temperature was anticipated to reduce thermal degradation mechanisms [2], but the effects were still prevalent. On-column injection is one of the possible manners to avoid interactions with the

Table 4
Chromatographic response enhancement recoveries [(matrix standard/matrix free solvent standard)×100%] using GC–FPD analysis of apples, oranges, and raspberries extracted with the FDA method before and after SPE clean-up with GCB+SAX+PSA at three levels — numbers in bold are >110% and italicized numbers had RSD values >5% (otherwise, RSD was <10%)

Pesticide	Clean-up	Average recovery (%), $n=3$			Conc. (mg/kg)
		1×Conc.	5×Conc.	20×Conc.	
Monocrotophos	None	***200***	***180***	**170**	0.02
	GCB+SAX+PSA	**136**	***120***	***122***	
Omethoate	None	***160***	***170***	**160**	0.02
	GCB+SAX+PSA	***125***	***116***	***120***	
Malaoxon	None	***140***	***140***	***145***	0.03
	GCB+SAX+PSA	103	100	108	
Phosmet	None	***127***	***129***	***139***	0.03
	GCB+SAX+PSA	***118***	*101*	*102*	
Acephate	None	***139***	***147***	**134**	0.04
	GCB+SAX+PSA	**111**	**111**	***115***	
Dimethoate	None	***180***	***133***	**131**	0.005
	GCB+SAX+PSA	***180***	***116***	**113**	
Paraoxon	None	***126***	***114***	**117**	0.03
	GCB+SAX+PSA	106	102	108	
Pyrazophos	None	***125***	*106*	***112***	0.03
	GCB+SAX+PSA	***124***	*107*	*101*	
Methamidophos	None	*106*	***117***	108	0.003
	GCB+SAX+PSA	***111***	*109*	*108*	
Malathion	None	108	105	103	0.01
	GCB+SAX+PSA	98	98	100	
Parathion	None	***114***	103	101	0.004
	GCB+SAX+PSA	*107*	103	103	
Terbufos	None	***160***	**111**	99	0.003
	GCB+SAX+PSA	***160***	***113***	104	
Averages	None	**140**	**129**	**126**	
	GCB+SAX+PSA	**123**	108	109	

glass surfaces of a injector liner, but SPI is not a true on-column injection and matrix interactions were still observed.

3.4. Extraction method

As shown in Tables 3 and 5, the extracts from the FDA method, exhibited less matrix enhancement than the extracts from the PMRA method. This is not surprising since the FDA method uses an additional liquid–liquid partitioning clean-up step. MeCN may also more exhaustively extract the matrix than acetone [20], but the actual extraction solvent is a combination of water from the sample with the added miscible organic solvent.

Originally, the FDA method did not use an SPE clean-up step [13,14], but SPE clean-up steps were added to the method (which became known as the Luke II) in 1993, in part due to matrix interferences and enhancement effects associated with the use of GC–ITMS for analysis [19]. The Netherlands General Inspectorate for Health Protection uses the original Luke procedure followed by GC–ITMS for the majority of GC-amenable pesticides and employs matrix-matched standards to routinely determine a wide range of pesticides in food commodities [21].

3.5. Commodity

An experiment was conducted to compare matrix

Table 5
Chromatographic response enhancement recovery [matrix standard/matrix free solvent)×100%] in GC–FPD and GC–ITMS of green beans extracted with the FDA and PMRA methods after SPE clean-up (or not) and spiked with pesticides at 0.200 mg/kg — numbers in bold are >110% and italicized numbers had RSD values >10% (otherwise, RSD was <10%)

Pesticide	Detector	Extraction method	Average recovery (%), $n=3$		
			GCB+SAX +PSA	PSA	None
Phosmet	ITMS	FDA	100	–	**640**
		PMRA	–	***180***	***810***
	FPD	FDA	100	**124**	**149**
		PMRA	*86*	**120**	**170**
Malaoxon	ITMS	FDA	105	**135**	**200**
		PMRA	86	**145**	**180**
	FPD	FDA	104	108	**118**
		PMRA	109	**113**	**114**
Dicrotophos	ITMS	FDA	**120**	**129**	**170**
		PMRA	**132**	**135**	**240**
	FPD	FDA	108	**112**	**129**
		PMRA	**118**	**120**	**126**
Carbophenothion sulfone	ITMS	FDA	*55*	90	**150**
		PMRA	53	90	**180**
	FPD	FDA	104	**113**	**121**
		PMRA	**121**	**126**	**170**
Paraoxon	ITMS	FDA	107	*109*	**144**
		PMRA	**115**	**113**	**129**
	FPD	FDA	106	107	**114**
		PMRA	**111**	**111**	**114**
Averages	ITMS	FDA	97	**116**	**260**
		PMRA	96	**133**	**310**
	FPD	FDA	104	**113**	**126**
		PMRA	109	**118**	**139**

enhancement results using clementine, apple, pea, and carrot extracts. Each commodity was analyzed in a different set on the GC–FPD instrument. No maintenance of the GC was conducted between sets, thus, the effect of time and condition of the GC system may have contributed to the results. The effects of the different commodities did not appear to be more variable than the day-to-day variability of the analyses. However, this does not imply that a standard in one matrix could give accurate results when used for calibration of a pesticide in a sample in a different matrix.

3.6. Pesticide structure

When spiked extracts were injected into the GC systems, certain analytes exhibited greater degrees of matrix enhancement than others. Tables 1–5 list the pesticides by the degree of the matrix enhancement effect with respect to the no clean-up results. Fig. 1 presents those pesticides that did not have a notable matrix enhancement effect in this study, and Fig. 2 gives those that were significantly affected. The pesticides in Fig. 2 all contain amides, sulfones, and/or P=O bonds. Pesticides that did not contain these groups were unaffected (except methamidophos). Pesticides that have multiple P=O and/or amides, such as dicrotophos, monocrotophos, and omethoate, were more greatly affected than other pesticides with only a single P=O or amide. Phosmet (a phthalimide) and carbophenothion sulfone were also more highly affected by matrix enhancement presumably due to the two =O bonds. The amount of the relative effect per functional group may be observed by comparing the results of compounds that have small structural differences, such as di-

methoate–omethoate, parathion–paraoxon, and malathion–malaoxon. These findings agree with those of Hajšlová et al. who noted that more polar compounds, and those containing P=O bonds rather than P=S bonds tended to exhibit more matrix enhancement [1]. Hajšlová also speculated that the presence of amine groups may also induce matrix enhancement, but in this study, using the GC–FPD system, the only pesticide to contain a primary amine, methamidophos, did not show high levels of matrix enhancement. This was very curious because methamidophos often exhibits very high matrix enhancement effects [8].

4. Conclusions

The purpose of this study was to evaluate and compare different SPE clean-up procedures and GC systems for OP pesticides in nonfatty foods. The matrix enhancement effect served to measure the amount of resulting clean-up by the different cartridges. However, as the case of GCB demonstrated, the matrix enhancement effect alone did not account for removal of some components, such as pigments, which did not lead to the enhanced injection efficiency in extracts. The GC–ITMS was found to be more sensitive to matrix enhancement than the GC–FPD system used in this study.

Another goal was to overcome the matrix enhancement effect through the use of more extensive clean-up. The use of GCB+SAX+PSA was found to reduce the matrix enhancement effect more than the other SPE approaches investigated, but even the use of three cartridges did not eliminate the effect. For most affected pesticides, the enhancement factor remained >20% despite the more extensive clean-up. SPE cartridges cost $1–2 each in the US which is often the highest material cost in an analytical method [9]. The use of GCB+PSA (or $-NH_2$) was found to be an effective combination that essentially matched the clean-up achieved in the three cartridge stack and required a third lower expense and somewhat less effort. The PSA alone was very useful in reducing the matrix enhancement effect, but did not remove pigment as well as GCB.

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