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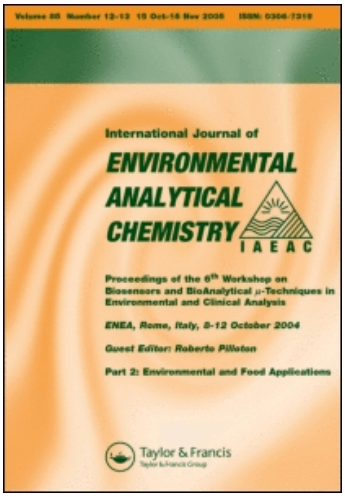

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Matrix effect in gas chromatographic determination of insecticides and fungicides in vegetables

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MATRIX EFFECT IN GAS CHROMATOGRAPHIC DETERMINATION OF INSECTICIDES AND FUNGICIDES IN VEGETABLES

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A study of the matrix-induced effect was performed for 16 common pesticides, most frequently found in monitoring studies in tomato, pepper and cucumber, using a simple multiresidue method with gas chromatography (GC) and electron-capture (ECD) or nitrogen-phosphorus (NPD) detection, without a previous cleanup step. Anomalously high gas chromatography responses and subsequently very high recoveries for several pesticides in the extracts were obtained by a conventional calibration with pesticide solution in ethyl acetate. Sample matrix enhancement varied from little to no effect for some pesticides (e.g. chlorpyrifos, pirimicarb) to >200% in the case of certain susceptible pesticides (captan, procymidone, iprodione). Pronounced matrix effects were observed at low concentration levels of analyte for all the ECD-detected pesticides. The use of matrix-standards solutions was found to reduce the recoveries of most pesticides to the levels of 70–110% acceptable for residue analysis.

Keywords: Matrix effect; Pesticide analysis; Vegetables; Gas chromatography

INTRODUCTION

A wide variety of insecticides and fungicides are used worldwide for pest control in agricultural commodities. The use of plant protection products provides a variety of foodstuff in sufficient quantity and good quality but it also has the disadvantage of the potential appearance of pesticide residues in fresh produce. A regulated use of pesticides is necessary and in the EU DIR 93/58 EEC of 1993 is the legislative basis for establishing Maximum Residue Levels (MRLs) of pesticides that may be found in food commodities [1]. For monitoring and control purposes under DIR 91/414 EEC, accurate and precise analytical methods are required. DIR 96/46/EC addresses the development of analytical methods and sets the minimum validation requirements for residue analytical methods [2]. Quality Control Procedures are also suggested to ensure the results of monitoring data [3]. Pesticide residue analysis in agricultural commodities compared to other organic trace analysis has some peculiarities: (i) in

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the same sample a wide range of analytes may be determined at very different concentration levels, as established by the MRLs; (ii) there is a wide range of commodities with different matrix effects in the determination of the analytes; (iii) Certified Reference Materials (CRMs) are not available. Pesticide residue analysis is routinely carried out by means of multiresidue methods (MRMs) for extraction and cleanup followed by a final chromatographic determination step. Although for monitoring purposes MRMs represent an effective means to screen a large number of samples for a variety of pesticides in a short time, it is practically impossible to obtain simultaneously optimum recoveries for all of them because of the broad range of their physicochemical properties and the different effect of each matrix on the final chromatographic step. The quantification of pesticides can be affected by co-extractives existing in the matrix. The presence of impurities in analyzed samples can cause problems at the detector and even more at the injector site. These co-extractives may modify the analytical resolution, thus increasing the level of random errors and/or introducing a systematic effect on the analytical results that affects the sensitivity of the analysis [4]. Matrix-induced response enhancement, described first by Erney *et al*. [5], is a phenomenon observed during analysis of real samples containing some matrix components and it has been reported in various residue studies for different matrices [6–10]. Active sites in the injection liner, which adsorb and/or induce thermal degradation of certain analytes, are the main source of the matrix enhancement effect. These phenomena can explain recoveries largely exceeding 100%, which are reported for some pesticides in studies utilizing calibration standards dissolved in solvent [5,11]. In pesticide residue analysis, recoveries of most analytes are usually different from 100% raising the question of whether a correction factor is necessary. This approach is not realistic taking into account the diversity of concentration ranges and the large number of analytes in a variety of different composition matrices and consequently a validation procedure would be necessary for each analyte/matrix pair. For this reason EC/7826/VI/97 considers acceptable recovery factors in the range 70–110%, supporting the establishment of MRLs on this basis [3]. The matrix effect is also described as one of the main sources of uncertainty in MRMs including those derived from injection port contamination and the amount of matrix components left in a purified extract [12]. The definition of uncertainty indicates that results should be given without systematic errors and it can be estimated from the detailed description of the operating procedure of the analytical method. Consequently, the matrix effect is being considered as a key point in method validation. The intensity of an effect may differ from one matrix or sample to another or according to the concentration of the analyte or the matrix. For validation purposes possible effects of the matrix on chromatographic transmission must be addressed and the presence or absence of matrix effects should be demonstrated over the concentration range of interest [3]. Therefore, matrix-induced effects are well recognized in pesticide residue analysis and depend on the chromatographic system, the type and chemical structure of the substrate and the physicochemical properties of each chemical compound, and may be difficult or impossible to eliminate [13]. Proper handling and maintenance of the gas-chromatographic system or the use in each case of an appropriate analytical procedure could reduce chromatographically enhanced responses. In the first approach, the use of on-column injection or pulsed splitless injection [14,15] and frequent changes of coated liners and maintenance of the GC system have been reported to minimize the effect, although these techniques add to the expense of the analysis. Several studies

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suggest extensive cleanup procedures in order to reduce or eliminate co-extractives [6,13]. In a recent study it was concluded [13] that although extensive cleanup of various vegetable matrices with a combination of anion-exchange cartridges and graphitized carbon black, reduced the matrix enhancement effect they did not eliminate it, and the enhancement factor remained >20%. Moreover, extensive cleanup steps could minimize the matrix effect but may result in the partial loss of some compounds and an increase in the time and cost of analysis, while also causing some adverse effects such as the masking of residue peaks by co-eluted matrix components and the occurrence of false positives [6]. High pesticide recoveries (120–>300%) in various food commodities have been reported in the literature, which were corrected to the acceptable range of 70–120%, using matrix-matched standard calibration solutions. Recent related papers in different fields of pesticide analyses include the matrix effect in the calibration step, preparing the calibration solutions with extracts from blank samples (matrix-matched calibration) [5–7, 9, 16–18]. In most cases a cleanup step is also included in the extraction procedure. This is considered as an effective way of avoiding errors derived from matrix effects in the quantitation of the analysis. Recently, in order to avoid the use of matched calibration curves for routine analysis, the estimation and use of a correction function was suggested for cases in which the stability of the whole analytical process is ensured [12].

The aim of this study was to evaluate the influence of the co-extractives from tomato, pepper and cucumber matrices on the gas chromatographic responses of the insecticides and fungicides most often detected in monitoring studies and to investigate the effectiveness of calibrating with matrix-matched standards without previous cleanup steps for a multiresidue routine method. Also, the stability of this type of calibration and the influence of repeated use of matrix solutions on the response stability/repeatability of GC system was checked.

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EXPERIMENTAL

Chemicals and Materials

Pesticide standards (bifethrin, captan, chlorothalonil, chlorpyrifos, chlorpyrifos methyl, deltamethrin, iprodione, endosulfan-a, fluvalinate, methamidophos, parathion, parathion methyl, permethrin, pirimicarb and vinclozolin), of more than >95% purity, were obtained from Riedel-de Haën (Hanover, Germany). Stock solutions were prepared in ethyl acetate and working standard mixtures were obtained with appropriate dilution before use. Propanol-2, ethyl acetate and toluene were of pesticide grade (Riedel-de Haën, Hanover, Germany, and J. Baker Deventer, Holland). Anhydrous sodium sulfate (Merck, Darmstadt, Germany), Celite (Aldrich) and Nuchar C 190 N (Riedel-de Haën) were suitable for residues analysis. Tomatoes, cucumbers and peppers were obtained either from our experimental plots cultured without pesticide treatments, or from the retail market for organic farming produce.

Gas Chromatographic Analysis

A Hewlett-Packard 5890 gas chromatograph equipped with an electron-capture detector and a Hewlett-Packard 6890 gas chromatograph with a nitrogen–phosphorus

detector was used. Both instruments were equipped with electronic pressure control (EPC), split/splitless injector and a 30 m × 0.32 mm capillary column coated with a 0.25-μm thick film of 5% phenylmethylsiloxane (HP-5) attached to a 50-cm deactivated precolumn. HP 3365 Series II Chemstation software was used for instrument control and data acquisition. The extract (2 μL), corresponding to 1 mg of original matrix, was injected in the splitless mode with the purge valve on at 0.7 min and injector temperature at 250°C for both GC systems. The GC operating conditions for ECD compounds were: oven temperature program with initial temperature 80°C, 20°C/min ramp to 180°C, held for 3 min and finally 4°C/min ramp to 250°C, held for 10 min; carrier gas (He), constant flow rate 1 mL/min; detector temperature 310°C; nitrogen was used as an auxiliary gas. For NPD compounds: oven temperature program with initial temperature 80°C, 20°C/min ramp to 180°C, held for 3 min and finally 2°C/min ramp to 190°C, held for 10 min; detector temperature 310°C; carrier gas (He), with constant flow rate 3 mL/min; H_2 and air flows at 3 and 60 mL/min respectively; helium was used as auxiliary gas.

Quantitation was carried out using calibration curves obtained both with standards in ethyl acetate and standards in matrix extracts.

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Extractions

Parathion, parathion methyl, chlorpyrifos, chlorpyrifos methyl, pirimicarb and iprodione were extracted from samples with ethyl acetate in the presence of anhydrous sodium sulfate and determined in the filtered extract without cleanup by GC-NPD [19]. For the extraction of chlorothalonil, vinclozolin, captan, endosulfan-a, bifethrin, permethrin, fluvalinate and deltamethrin a 50-g sample was homogenized with a mixture of 100 mL toluene and 50 mL propanol-2 for 3 min with a Polytron. The propanol-2 was removed by washing twice with 250 mL of 2% sodium sulfate solution. 50 mL of the toluene phase was mixed with 5 g of an adsorbent mixture of Celite 545 and Nuchar C 190N (1 : 3 parts by weight). After filtration, the pesticides were determined by GC-ECD [19].

Recovery Study

The recovery study was carried out by spiking 50 g of the homogenized sample, which had not been treated with pesticides, with working standard solutions of pesticides at three fortification levels, corresponding to the LOQ, the MRL value established for each pesticide or ten times the LOQ and an intermediate concentration level, with three replications for each level. After evaporation of the solvent, the samples were extracted according to the previously described procedure.

Preparation of Calibration Curves

A stock solution of each pesticide in ethyl acetate at a concentration of 1 mg/mL was prepared. These solutions were used for the preparation of a working standard solution containing all pesticides at 100 μg/mL. Two different types of calibration curves were prepared, one in the solvent and another in the vegetable matrix, as follows: (i) Six working standard solutions at different concentrations were prepared by serial dilution with ethyl acetate for the preparation of the solvent calibration curves (SC); (ii) Another

six working standard solutions at the same concentrations were prepared by serial dilution, using extract of tomato, cucumber and pepper matrices for the preparation of the matrix calibration curves (MC). These extracts were obtained from the extraction of vegetables following the described analytical procedure. The matrix content in the standard solution is the same as in the spiked samples.

RESULTS AND DISCUSSION

Analysis

In order to determine the matrix effect on the sensitivity of the detector (calibration curve), standard solutions containing 0.50 mg/mL of each pesticide were prepared in mixtures with different solvent : matrix extract proportions. The ethyl acetate : matrix ratios were 1 : 100; 20 : 80; 50 : 50; and 80 : 20. Relative responses [(response in matrix extract/response in neat solvent) × 100] in the obtained mixtures are presented in Table I. In almost all cases a tendency to higher responses was observed for the mixtures with higher extract content. For instance, neat tomato extracts (1 : 100) provided relative responses about 163, 180 and 210% for iprodione, captan and deltamethrin, respectively. For this reason, all the matrix-matched calibration curves were prepared in 100% matrix extract.

Calibration curves prepared either in solvent or in matrix extract (three replications for each set of standards) were linear with correlation coefficients $R^2 > 0.9648$–0.9999. The investigated pesticides, along with the linear dynamic concentration range, the retention times (Rt), the limit of detection (LOD) and the limit of determination (LOQ) are shown in Table II. LOD and LOQ values and the sensitivity of the method were calculated from the results of the recovery experiments, according to the analysis proposed by Thier and Zeumer [20]. These values were calculated for each pesticide on the basis of standard deviations of the blanks at the lowest for each pesticide and matrix fortification level, with $f = 4$ degrees of freedom at 95% confidence level. Consequently, the LOQ values correspond to the lowest examined fortification level. Analysis of covariance [21] was applied in order to compare the slopes and intercepts of curves and a Fischer coefficient (F) statistic was calculated for each case. In most of the cases a significant difference for slopes considering a 95% confidence level was found between solvent calibration and matrix calibration curves (t-test for slopes and intercepts) suggesting that matrix constituents introduce a proportional bias. The statistical parameters are presented in Table III. An exception was observed for chlorpyriphos methyl in all the matrices, permethrin-*cis*, iprodione, parathion and methamidophos in tomato, pirimicarb and parathion methyl in cucumber and iprodione in pepper.

Recovery Study

To assess the performance of an analytical method, several criteria have to be considered. The pesticide recoveries should be in the range 70–110% with relative standard deviations (RSDs) $< 20\%$ [3]. In this study recovery experiments were conducted at three spiking levels. Each pesticide was fortified at its LOQ level, at the MRLs level or at 10 times the LOQ level and at a third intermediate level. Table IV presents

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TABLE I Relative response (%) in various solvent : matrix extract ratios ($c = 0.5$ mg/kg)

Pesticide	*Matrix*	*Extract percentage*			
		20	*50*	*80*	*100*
Pirimicarb	*Tomato*	89	104	100	104
	Pepper	103	105	101	100
	Cucumber	99	100	94	105
Chlorpyrifos	*Tomato*	97	114	111	113
	Pepper	106	115	114	114
	Cucumber	98	101	101	109
Chlorpyrofos methyl	*Tomato*	107	108	105	107
	Pepper	102	109	106	109
	Cucumber	98	104	103	103
Parathion methyl	*Tomato*	113	125	129	123
	Pepper	135	141	140	140
	Cucumber	107	126	127	144
Parathion	*Tomato*	108	112	111	111
	Pepper	121	122	120	126
	Cucumber	130	135	140	148
Iprodione	*Tomato*	135	140	169	163
	Pepper	137	147	151	151
	Cucumber	92	95	104	124
Chlorothalonil	*Tomato*	124	145	168	189
	Pepper	96	99	106	104
	Cucumber	104	97	101	104
Vinclozoline	*Tomato*	117	121	133	147
	Pepper	88	93	96	98
	Cucumber	86	89	92	104
Captan	*Tomato*	148	163	187	180
	Pepper	113	104	148	156
	Cucumber	96	162	173	209
Procymidone	*Tomato*	92	95	104	124
	Pepper	137	150	163	169
	Cucumber	143	143	143	148
Endosulfan-a	*Tomato*	122	146	149	142
	Pepper	101	103	114	113
	Cucumber	92	95	96	106
Bifethrin	*Tomato*	123	142	159	152
	Pepper	99	115	122	122
	Cucumber	92	102	98	106
Permethrin-*cis*	*Tomato*	130	146	146	150
	Pepper	129	132	145	150
	Cucumber	91	100	116	115
Permethrin-*trans*	*Tomato*	177	177	176	179
	Pepper	127	139	153	160
	Cucumber	104	99	104	108
	Tomato	133	148	157	162
Fluvalinate-I	*Pepper*	141	156	163	176
	Cucumber	115	124	125	137
Fluvalinate-II	*Tomato*	130	139	153	167
	Pepper	161	173	180	192
	Cucumber	113	132	132	134
Deltamethrin	*Tomato*	182	199	203	210
	Pepper	182	184	185	206
	Cucumber	114	117	123	135

the recoveries along with RSDs obtained by a conventional standard calibration and a matrix-standard calibration, respectively, in tomato, pepper and cucumber spiked at three concentration levels. Anomalously high recoveries were obtained by a conventional calibration with pesticide solutions in the organic solvent. A variable influence

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TABLE II Investigated pesticides, detectors, linear dynamic concentration range, retention times (Rt), limits of determination (LOQ) and limits of detection (LOD)

Pesticides	*Linear dynamic range* (μg/mL)	*Rt* (min)	*LOQ* (mg/kg)	*LOD* (mg/kg)
NPD				
Methamidophos	0.5–5	3.742 ± 0.082	0.1	0.01
Pirimicarb	0.005–2	8.659 ± 0.009	0.01	0.003
Parathion methyl	0.005–2	9.345 ± 0.011	0.01	0.003
Chlorpyrifos	0.005–2	9.307 ± 0.009	0.01	0.003
Chlorpyrifos methyl	0.005–2	11.191 ± 0.012	0.01	0.003
Parathion	0.005–2	11.260 ± 0.031	0.01	0.003
Iprodione	0.1–10	20.353 ± 0.013	0.2	0.02
ECD				
Chlorothalonil	0.05–2.5	10.071 ± 0.033	0.1	0.02
Vinclozoline	0.001–0.25	11.035 ± 0.015	0.002	0.0007
Captan	0.05–7.5	11.26 ± 0.013	0.1	0.06
Procymidone	0.005–2.5	14.281 ± 0.014	0.01	0.003
Endosulfan-*a*	0.001–2.5	14.813 ± 0.006	0.002	0.0005
Bifethrin	0.005–2.5	21.256 ± 0.003	0.01	0.003
Permethrin-*cis*	0.01–2.5	25.023 ± 0.006	0.1	0.02
Permethrin-*trans*	0.01–2.5	25.341 ± 0.013	0.1	0.02
Fluvalinate-I	0.05–5	31.437 ± 0.013	0.1	0.05
Fluvalinate-II	0.05–5	31.795 ± 0.014	0.1	0.05
Deltamethrin	0.005–2.5	33.679 ± 0.022	0.01	0.002

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of matrix on the calculated recoveries was observed, depending on the type of matrix, the physicochemical properties of each pesticide, and its concentration in the sample. Distinct matrix-induced effects could be seen, especially in the cases of iprodione, procymidone and captan, where the recoveries were very high for the three matrices and at all the spiking levels. Similar effects have been reported for pesticides determined in orange, wheat and cabbage in which more polar compounds (such as iprodione, procymidone and captan) tended to exhibit a higher matrix enhancement [6].

In normal tomato-matrix concentrations all the ECD-detected pesticides spiked at the lowest level gave more than 110% enhanced response, with captan, pyrethroids and chlorothalonil being among the worst. The phenomenon was less evident at higher fortification level and for the NPD-detected compounds, where only methamidophos and iprodione gave an enhanced response. Similarly, in green pepper and cucumber extracts the responses of ECD-detected compounds were higher than 126% with the exception of vinclozolin and chlorothalonil respectively. For "NPD" pesticides the phenomenon was less evident. The highest recoveries were observed for parathion methyl (122–325%), mostly in pepper extracts. Similarly, matrix enhancement (197%) was observed for parathion only at the lowest spiking level of cucumber, and for chlorpyrifos at the lowest spiking level of pepper. No matrix enhancement was observed for chlorpyrifos methyl and pirimicarb. It was speculated that compounds which contain P=O bonds (acephate, methamidophos) rather than P=S bonds (chlorpyrifos, parathion) tended to exhibit more matrix effects [13].

For pyrethroids, matrix enhancement was observed in all the matrices. For bifethrin the recoveries were very high in pepper (132–158%) and cucumber (135–158%), in contrast to tomato where the recoveries were high (135%) only at the spiking level of 10 μg/kg. As regards permethrin-*cis*, high recoveries were observed in pepper (126–143%), while in cucumber and tomato the effect was evident only at the level

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TABLE III Statistical parameters for comparison of calibration curves prepared in solvent (SC) and matrix-matched extract (MC)

Pesticides	*Tomato*			*Cucumber*			*Pepper*		
	F_{cal}	*Slope*, *b*	s_b	F_{cal}	*Slope*, *b*	s_b	F_{cal}	*Slope*, *b*	s_b
Chlorothalonil	9.2737	2357561	139567.5	8.0225	2960579	38378.27	7.3562	3105905	16865.14
Vinclozoline	16.785	1120943	10409.03	6.465	1518421	34684.14	4.1349	1903493	18723.43
Captan	9.7112	185618.2	4313.108	25.671	219507.3	2652.143	7.2475	152139.3	2097.189
Procymidone	7.2627	321053	5939.165	3.9908	364071	9050.668	4.494	383892	12465.89
Endosulfan-a	9.9224	3440278	55204.3	2.8305	2871111	48802.42	22.455	3571462	11942.1
Bifethrin	16.958	553938.6	8672.187	9.133	516264.2	3368.327	26.872	586157.3	3215.205
Permethrin-*cis*	0.8451	45630.97	1002.526	15.249	40760.53	349.8996	54.73	47871.27	199.3458
Permethrin-*trans*	12.068	180581.1	617.4359	9.9318	139929.4	1301.013	29.624	171250.6	1398.566
Fluvalinate-I	6.5646	254360.5	5450.01	15.133	160449.3	1911.93	41.261	230190.7	2383.207
Fluvalinate-II	10.089	258977.7	5951.412	20.805	155043	686.7215	23.927	201575	2923.906
Deltamethrin	30.188	569072.6	8119.192	4.1269	338937	18535.78	7.9189	395169	21813.09
Iprodione	0.8043	378.06	6.1709	3.7827	343.5031	2.665	0.3141	374.9998	11.79729
Parathion	1.8682	1084.22	4.928	5.9402	1209.637	9.67	4.8435	1180.112	11.82775
Parathion-methyl	5.3573	835.0154	7.002	1.6131	747.9981	11.00739	10.448	962.9	8.857
Chlorpyrifos	3.1787	1089.934	9.8502	2.898	897.6227	18.4831	4.8547	1149.96	14.99835
Chlorpyrifos-methyl	1.5898	1177.507	23.57271	1.9295	1054.158	25.077	2.5372	1192.972	11.58547
Pirimicarb	13.825	50.544	1.456	1.3408	49.969	1.058	9.5995	67.659	1.849
Methamidophos	0.5074	419.586	26.0958	4.437	578.046	15.66037	5.8648	629.9419	12.41822

*$F_{tab} = 2.776$ for degree of freedom $f = 4$ (six concentration levels, three replicates) at 95% confidence level.

TABLE IV Percentage recovery (RSD) of pesticides obtained by a conventional solvent calibration and a matrix-standard calibration on tomato, pepper and cucumber spiked at different concentrations

Pesticide	*Spiking level* (mg/kg)					
	< 0.02 (LOQ) (mg/kg)		0.02–0.2		0.21–>2	
	Solvent	*Matrix*	*Solvent*	*Matrix*	*Solvent*	*Matrix*
Tomato						
Chlorothalonil	253 (7.9)	120 (1.1)	210 (7.2)	120 (8.3)	230 (4.4)	115 (4.3)
Vinclozoline	150 (9.9)	114 (4.4)	140 (13)	120 (4.2)	112 (9.4)	90 (12.3)
Captan	245 (1.7)	210 (0.6)	165 (3.3)	78 (2.8)	146 (12)	106 (12)
Procymidone	196 (8.2)	102 (4.8)	175 (1.7)	104 (1.9)	150 (1.1)	90 (1.4)
Endosulfan-a	150 (3)	80 (5.5)	95 (11)	85 (12)	115 (0.9)	114 (3)
Bifethrin	135 (4)	113 (17)	129 (10)	90 (9)	82 (1.6)	95 (4)
Permethrin-*cis*	256 (2.8)	106 (7)	120 (1.9)	102 (2.6)	103 (0.6)	95 (0.3)
Permethrin-*trans*	206 (4)	99 (5.7)	138 (1.5)	93 (1.5)	130 (8.3)	93 (8.5)
Fluvalinate-I	135 (2.2)	117 (2.9)	99 (3.3)	88 (3.5)	113 (11.5)	86 (11.5)
Fluvalinate-II	238 (0.8)	110 (0.9)	122 (6)	88 (6.6)	121 (5.4)	80 (5.5)
Deltamethrin	320 (2.5)	140 (2.2)	170 (17)	90 (14)	171 (5.7)	84 (5)
Iprodione	176 (4)	90 (5.5)	200 (10)	89 (13.6)	185 (6.8)	105 (7.8)
Parathion	97 (4.2)	101 (4.4)	90 (3)	94 (1.7)	85 (0.5)	85 (0.3)
Parathion-Methyl	97 (1)	100 (0.8)	110 (9.3)	100 (8)	105 (9)	90 (8.2)
Chlorpyrifos	111 (0.9)	80 (1.1)	106 (10)	93 (11)	102 (0.3)	91 (0.4)
Chlorpyrifos-methyl	111 (1.7)	95 (1.7)	99 (6)	92 (6)	105 (1.7)	99 (1.8)
Pirimicarb	114 (6.4)	85 (8.6)	108 (4.9)	100 (5.3)	106 (2.8)	100 (2)
Methamidophos	152 (1.8)	86 (2.3)	98 (2.3)	73 (3.9)	98 (8.5)	84 (14)
Pepper						
Chlorothalonil	154 (2)	80 (3.6)	100 (4.3)	93 (4.5)	88 (2.3)	86 (2.3)
Vinclozoline	70 (11)	75 (9.1)	77 (2.7)	77 (3.4)	80 (17)	80 (17)
Captan	257 (7.9)	120 (10.0)	185 (8.9)	110 (13.0)	104 (3.8)	90 (4.0)
Procymidone	160 (7.0)	96 (9.4)	170 (11.5)	110 (11.0)	105 (0.9)	70 (1.1)
Endosulfan-a	225 (3.3)	111 (5.0)	105 (11.5)	85 (15)	90 (13.0)	87 (12.7)
Bifethrin	210 (6.6)	120 (8.5)	130 (7.5)	103 (7.2)	114 (10.5)	92 (11.0)
Permethrin-*cis*	143 (12.5)	111 (19.0)	150 (13.6)	110 (13.0)	130 (5.0)	91 (5.5)
Permethrin-*trans*	151 (10.0)	91 (8.8)	150 (7.1)	100 (8.0)	150 (7.1)	102 (7.8)
Fluvalinate-I	198 (9.6)	105 (12.0)	170 (6.0)	95 (5.3)	134 (16.0)	80 (15.6)
Fluvalinate-II	200 (9.6)	86 (14.0)	180 (5.7)	95 (8.5)	142 (15.4)	86 (16.0)
Deltamethrin	233 (4.9)	120 (4.6)	150 (14.0)	97 (20)	158 (12.4)	86 (12.8)
Iprodione	227 (2.8)	90 (4.0)	150 (4.5)	88 (5.5)	150 (14.4)	89 (15.0)
Parathion	93 (5.4)	80 (6.0)	90 (0.8)	85 (0.8)	105 (1.9)	87 (1.5)
Parathion-methyl	320 (2.0)	90 (9.0)	120 (0.9)	85 (1.0)	125 (1.8)	83 (1.4)
Chlorpyrifos	130 (1.2)	85 (1.9)	119 (1.2)	97 (1.4)	111 (12.8)	93 (13.0)
Chlorpyrifos-methyl	114 (3.0)	82 (4.4)	112 (0.1)	99 (0.1)	108 (6.0)	99 (6.3)
Pirimicarb	117 (5.4)	80 (7.9)	105 (10.4)	97 (11.3)	118 (8.4)	102 (8.4)
Methamidophos	175 (0.8)	80 (2.8)	142 (2.4)	83 (0.6)	150 (2.6)	83 (8.7)
Cucumber						
Chlorothalonil	105 (5.8)	80 (7.9)	88 (0.45)	82 (1.7)	99 (3.4)	95 (4.0)
Vinclozoline	126 (6.0)	70 (7.9)	76 (8.0)	70 (7.6)	93 (9.0)	73 (8.9)
Captan	184 (2.2)	70 (4.6)	145 (3.6)	75 (10.8)	150 (3.7)	82 (3.2)
Procymidone	212 (8.2)	120 (1.1)	203 (10.0)	109 (12.0)	135 (4.5)	95 (4.6)
Endosulfan-a	200 (4.0)	85 (8.3)	135 (3.7)	90 (6.0)	100 (6.6)	72 (8.5)
Bifethrin	160 (9.9)	88 (3.3)	135 (12.0)	118 (12.7)	106 (5.5)	99 (5.8)
Permethrin-*cis*	140 (6.6)	90 (8.4)	120 (14.0)	96 (18.0)	125 (5.1)	102 (5.2)
Permethrin-*trans*	146 (9.6)	91 (14.0)	108 (8.8)	83 (9.3)	102 (3.8)	87 (4.0)
Fluvalinate-I	161 (16.0)	83 (3.3)	125 (14.0)	80 (19.0)	130 (11.0)	98 (10.0)
Fluvalinate-II	166 (5.6)	105 (7.9)	130 (5.4)	90 (6.8)	134 (7.9)	106 (8.1)
Deltamethrin	153 (3.2)	119 (3.1)	110 (9.9)	86 (9.8)	124 (6.5)	93 (6.5)
Iprodione	157 (0.2)	95 (0.9)	123 (4.7)	102 (5.2)	124 (6.1)	109 (6.3)
Parathion	197 (1.5)	95 (2.2)	91 (10.0)	98 (5.4)	96 (1.5)	98 (0.7)
Parathion-methyl	380 (3.1)	102 (10.8)	110 (5.7)	102 (10.8)	105 (1.4)	90 (2.0)
Chlorpyrifos	104 (4.3)	100 (6.0)	116 (3.5)	93 (3.4)	120 (4.0)	94 (3.7)
Chlorpyrifos-methyl	101 (2.6)	90 (2.6)	96 (2.0)	99 (2.0)	101 (6.4)	101 (6.8)
Pirimicarb	87 (1.7)	87 (1.7)	88 (2.6)	89 (2.6)	93 (2.1)	93 (1.9)
Methamidophos	151 (4.6)	80 (12.5)	132 (2.9)	83 (8.2)	135 (4.2)	90 (9.2)

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of 0.1 mg/kg. On the contrary, for permethrin-*trans* the recoveries were unacceptably high for all the spiking levels in tomato and pepper extracts. In the case of fluvalinate, matrix enhancement was observed for all the matrices except for tomato, where the recovery was 127% at the lowest concentration. Finally, for deltamethrin recoveries were more pronounced in tomato (171–320%) and pepper (148–233%), while the effect of cucumber was evident only at the lowest spiking level.

In Fig. 1 the recoveries of ECD-detected compounds are illustrated for the three fortification levels and the three matrices. Though results vary for each pesticide, generally higher enhanced responses were measured at the lower fortification level. For instance, permethrin-*cis* and fluvalinate-II recoveries increased from 103 to 256% and 121 to 238% respectively, on decreasing the spiked concentration of tomato samples. Some compounds showed enhanced responses only at low concentrations. In case of chlorothalonil in pepper, a matrix effect was evident (154%) only at the lowest concentration, while no matrix enhancement was observed for this pesticide in cucumber. The recoveries of endosulfan-a were unacceptably high only at the spiking level of 2 μg/kg for all the matrices. The concentration of the analyte and the ratio of analyte concentration to matrix amount were found to be the most significant factors influencing the enhanced response.

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The matrix effect has been reported to depend on the type of matrix. In Fig. 2 the influence of the three matrices on the recovery for the lowest fortification level is illustrated. In the case of tomato, pepper and cucumber the observed differences do not seem to fit into a general rule similar for all the compounds. More pronounced increase in responses was observed in tomato and green pepper extracts in which a distinct matrix-induced effect could be seen for all the ECD-detected pesticides and for methamidophos and iprodione of NPD compounds. The phenomenon was evident for vinclozolin only for tomato extracts, where the recoveries were 135 and 140%, at the lower concentrations, while the pepper and cucumber extracts did not influence the recoveries. Similarly, the recoveries of chlorothalonil were very high (210–253%) in tomato. In cucumber although a matrix effect was evident, lower enhanced responses were obtained. In certain cases a matrix effect was observed for a pesticide in only one of the tested matrices, as for example with vinclozolin in tomato or parathion in cucumber. The recoveries of most pesticides decreased to the acceptable for residues analysis levels 70–110%, when matrix-standard calibration was used for the quantification (Table III). In various studies an improved accuracy of results has been found by the use of matrix-matched standards [6,7]. The improvement of responses measured in this study is correlated to the physicochemical properties of each pesticide. For example the recovery of captan, a polar thermally degraded molecule, did not decrease enough at the lowest spiking levels in tomato. In the case of chlorothalonil, the recovery in tomato samples showed a significant reduction (253–120%), without achieving the acceptable level of 110%. At the lowest spiking levels of tomato the recovery of fluvalinate-I did not change. Deltamethrin was the only compound for which recoveries did not reach the acceptable range, although recoveries at the lowest fortification level reduced significantly (from 320 to 140% in tomato samples, from 233 to 120% in pepper and from 153 to 119% in cucumber). It is possible in the case of tomato, that components of tomato matrices block the active sites in the inlet especially after repeated injections of a large number of samples.

Finally the influence of repeated matrix extract injections on the response stability and repeatability of the GC system over a prolonged period of time was explored.

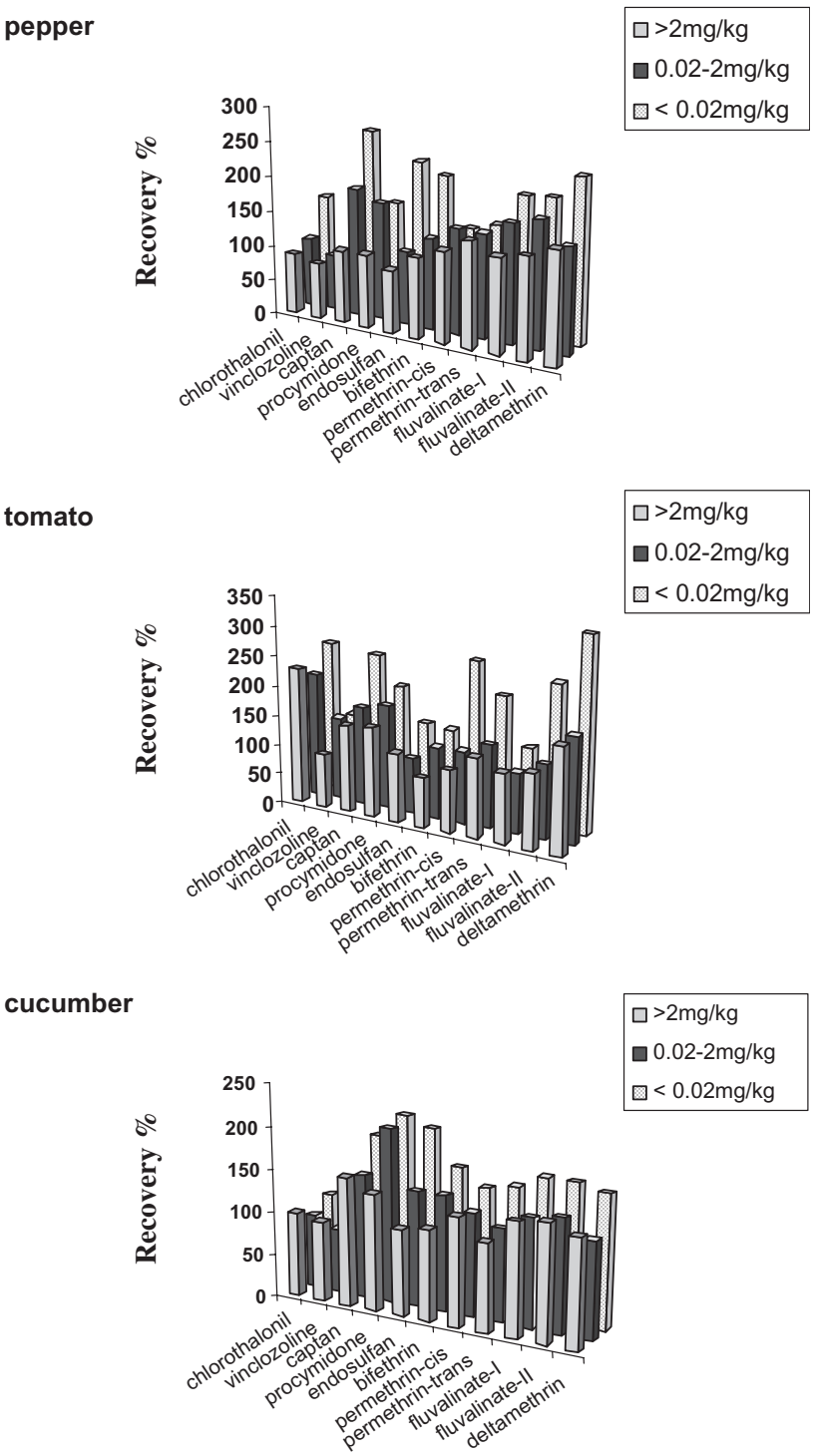

FIGURE 1 Percentage recoveries of ECD-detected compounds at three different fortification levels in tomato, green pepper and cucumber matrices.

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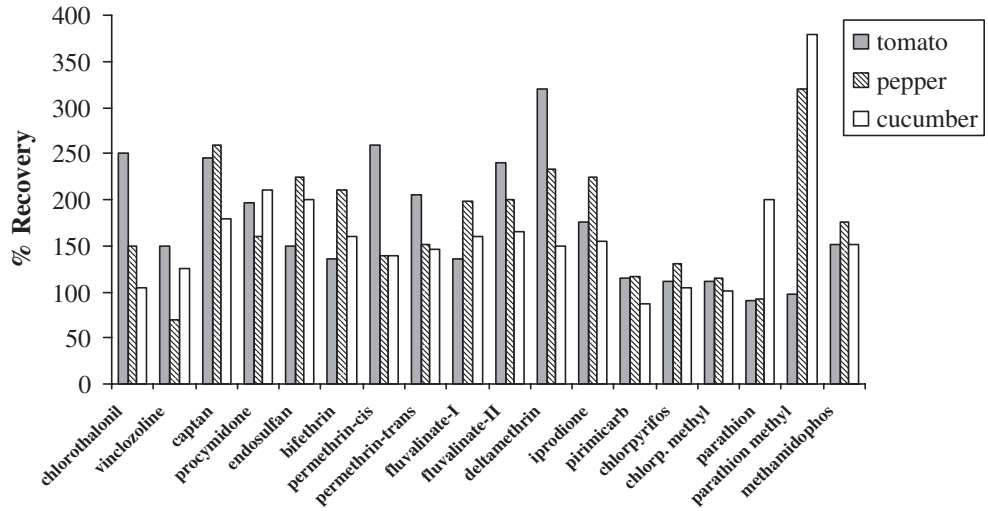

FIGURE 2 Influence of the three matrices on the recovery, at spiking concentrations 0.002–0.02 mg/kg (LOQ). Percentage recoveries obtained with solvent calibration.

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For this, at the end of each experiment, analysis of a 100 ppb standard mixture solution in solvent was performed. A significant increase of the ECD-detected compounds and iprodione was observed only after repeated injections of the tomato samples, indicating the need for GC system maintenance. The increase in responses ranged from 112% for bifethrin to 152% and 210% for fluvalinate and vinclozolin respectively. For the other two matrices and for the NPD compounds the differences in responses were within the values of standard errors.

CONCLUSIONS

The chromatographic response of the pesticides tested in this study increased significantly when the analytes were dissolved in the matrix extracts ($>80\%$) compared to neat solvent. Recovery values ranging from 120 to 340% were obtained for most of the analyzed compounds. The matrix effect was more pronounced for polar and thermally unstable compounds. Though results vary for each pesticide and substrate, generally recoveries were higher for decreasing concentrations in the sample. Calibration curves prepared with standards dissolved in matrix extracts were statistically significantly different to the corresponding curves from standards prepared in the solvent. Recovery values ranged from 70 to 110% in all cases when matrix-matched standards were used for calibration. An influence on the response stability/repeatability of the GC system was observed only after the analysis of tomato samples. The use of matrix-matched standards for calibration in the case of tomato, green pepper and cucumber gave reliable results with the simple analytical method used for routine analysis. In this way the suggested laborious and extensive cleanup steps [6,13] could be avoided in a routine analysis without losing accuracy. Since the presence and the intensity of the matrix effect in an analytical method may vary, it is necessary for validation purposes to explore the possible effects of each matrix on

chromatographic transmission and to evaluate the presence or absence of matrix effects over the concentration range of interest for each analytical method.

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