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Matrix-effects of vegetable commodities in electron-capture detection applied to pesticide multiresidue analysis

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Abstract

The influence of the sample matrix in the analysis of pesticides in vegetable samples has been studied in order to determine if the matrix content introduces a systematic or proportional (or both) bias in the measurements. Experiments have been carried out during a 4-month period, in which calibration curves, prepared in solvent and in vegetable matrix, were prepared and analysed. A statistical treatment has been applied in order to: (i) check the stability of such calibrations during the period studied; (ii) compare both solvent and matrix-matched calibrations; and (iii) obtain a correction function. Applying the correction function to the results obtained with a solvent calibration it is possible to make a prediction of the values obtained applying a matrix-matched calibration. The performance of the correction function has been validated with recovery data. Finally the uncertainty derived from the use of each calibration plot and the correction function has been calculated.

Keywords: Vegetables; Food analysis; Pesticides

1. Introduction

Greenhouse production of crops requires pesticide applications. Chlorothalonil (tetrachloroisophthalonitrile), chlozolinate [ethyl (±)-3-(3,5-dichlorophenyl)-5-methyl-2,4-dioxo-oxazolidine-5-carboxylate], dichlofluanid (*N*-dichlorofluoromethylthio-*N*′,*N*′-dimethyl-*N*-phenylsulfamide), iprodione [3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxoimidazolidine-1-carboxamide], nuarimol [(±)-2-chloro-4′-fluoro-α-(pyrimidin-5-yl)benzhydryl alcohol], procymidone [*N*-(3,5-dichlorophenyl)-1,2-dimethylcyclopropane-1,2-dicarboximide], triadimefon [1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one] and vinclozolin [(*RS*)-3-(3,5-dichlorophenyl)-5-methyl-5-vinyl-1,3-oxazolidine-2,4-dione] are pesticides of different nature used as fungicides in agriculture. Chlozolinate, iprodione, procymidone and vinclozolin belong to the dicarboximide family, dichlofluanid is an *N*-trihalomethylthio, nuarimol is a pyrimidinyl carbinol, triadimefon is an azole fungicide compound and chlorothalonil derives from 1,3-benzenedicarbonitrile [1].

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A regulated use of pesticide is necessary. In the European Union (EU) the legislative basis for establishing the maximum residue levels (MRLs) of pesticides that may be found in food commodities is Directive 93/58/EEC of 1993 [2], which is adapted in each State member (Royal Order 280/1994) [3].

An approach among the reliability of analytical information [4] addresses the total variance of results as the summation of the variance resulting from two main sources, the analytical process as a whole, considering the results of several analyses of aliquots of a certified reference material (CRM), and the second, the variance derived from the sample heterogeneity and the diversity of matrixes. The quantification of pesticides can be affected by co-extractives existing in the matrix. Adsorption and/or decomposition of analytes in the chromatographic system are described as the likely sources of such effects [5,6]. These co-extractives may modify the analytical resolution, increasing in this way the level of random errors, and/or introducing a systematic effect on the analytical results both, constant affecting the blank, or proportional, affecting the analytical sensitivity [7].

Matrix effect is also described as one of the main sources of uncertainty in multiresidue analytical methods (MRMs) [8], including those derived from the injection port contamination and amount of matrix-components left in purified extract.

Matrix effect is being considered as a key point in method validation, the EU provides guidance [9] on residue analytical methods which represent the minimum validation requirements for residue analytical methods. In certain cases it may be essential to validate methods on a larger scale, an increased number of fortification levels or additional test matrices. This guidance states that recovery data must be submitted for representative sample matrices, and must distinguish between different crop groups, depending on the water, fat or acid content. The method must be validated to each commodity group in which the use of the plant protection product is allowed. Thus the potential for matrix effects to occur should be assessed at method validation. Matrix effects are notoriously variable in occurrence and intensity but some techniques are particularly prone to them. These effects derive from various physical and chemical processes and may be difficult or impossible to eliminate. They may be observed as increased or decreased detector responses, compared with those produced by simple solvent solutions of the analyte.

Recent related papers in different fields of pesticide analyses [9–13] include the matrix effect in the calibration step preparing the calibration solutions with extracts from blank samples (matrix matched calibration). This is considered as an effective way for avoiding errors derived from the matrix effects in the quantification of the analytes. Nevertheless, this procedure does not provide the magnitude of the effect of co-extractives and introduces an important increase in the cost and time of the analyses. Hill and Reynolds [9] reported that the effect of co-extractives (if any) on the analyte response obtained should be assessed by comparing matrix-matched standards with those prepared in solvent. For validation purposes, the presence or absence of matrix effects should be demonstrated over the concentration range of interest and the default should be to use matrix-matched calibration unless it is demonstrated to be unnecessary.

It can be concluded that despite the fact that more reliable calibration may be obtained with matrix-matched calibration, this is only the way to compensate for matrix effects but does not eliminate the underlying cause. The intensity of an effect may differ from one matrix or sample to another, and also according to the concentration of matrix. Furthermore, where matrix effects could occur and blank sample material is not available for matrix-matching, isotope dilution or standard addition may be used. In most cases, if the techniques used are not inherently-free from such effects, calibration should be matrix-matched routinely, unless an alternative approach can be shown to provide equivalent or superior accuracy [14–16].

In this paper a methodology for demonstrating the matrix effects in the quantification of pesticide residues in vegetables, is presented. Solvent calibration (SC) and matrix-matching calibration (MC) have been used with the purpose of showing the matrix-effects. These calibrations types are compared statistically and when it is found that matrix exerts an effect in the quantification of pesticides, a ‘‘Correction Function’’ might be calculated simplifying the problem of the matrix-effect. The study includes

the reliability of the use of the correction function for estimating the concentration of analyte from data obtained using solvent calibration, the stability of such correction function during a 4-month period, and finally the uncertainty that this procedure implies.

2. Experimental

2.1. Chemicals

All pesticide standard reference materials were obtained from Dr. Ehrenstorfer (Augsburg, Germany). The following pesticides were tested: chlorthalonil, chlozolinate, dichlofluanid, iprodione, nuarimol, procymidone, triadimefon and vinclozolin.

The solvents used for dissolving and extracting were *n*-hexane, cyclohexane and dichloromethane (residue analysis grade, Panreac, Barcelona, Spain). Anhydrous sodium sulfate for residue analysis was purchased from Panreac.

2.2. Commodities

Tomato, pepper, green bean, aubergine, courgette, cucumber, melon and watermelon were the vegetable matrices for which the matrix effect has been established. All of them were obtained from greenhouses which had not been treated with any pesticide.

2.3. Analytical procedures

Two gas chromatographs were used: a Perkin-Elmer Model 8500 and a Hewlett-Packard Model 5890 both equipped with electron-capture detection (^{63}Ni ECD) systems. Chromatographic conditions were as follows: injector temperature, 250 °C; detector temperature, 350 °C; initial oven temperature, 180 °C for 5 min, raised at 3 °C min^{-1} to 250 °C, and then held at 250 °C for 2 min. The carrier gas was nitrogen at 10 ml min^{-1}. A fused-silica semicapillary (HP-1) column containing 100% methylpolysiloxane as stationary phase, 25 m×0.53 mm I.D., 1.0 μm film thickness, was used for the separation in the Perkin-Elmer Model and a fused-silica capillary (HP-1) column containing 100% dimethylpolysiloxane as stationary phase, 60 m×0.25 mm I.D., 0.22 μm film thickness was used for the separation in the Hewlett-Packard model.

2.4. Extraction procedure

The extracting method used for the routine analyses of samples, was similar to that used by Martínez Vidal and co-workers [17,18], consisting of mixing 50 g of a chopped sample with anhydrous sodium sulfate and dichloromethane, then homogenising and filtering the mixture. The solvent is removed under vacuum at 40 °C in a rotary evaporator until almost dry and then at the point of dryness with a slight N_2 stream, being dissolved with 20 ml of a cyclohexane–*n*-hexane (1:4, v/v) mixture containing 0.200 mg l^{-1} dieldrin as internal standard. The matrix content in the extract is 2.5 g ml^{-1}. Blank extracts used for the preparation of matrix-matched calibrations were prepared in the same way but dissolving in a final volume of 5 ml of the mixture without the internal standard.

2.5. Recovery study

The recovery study was carried out by spiking 50 g of vegetal sample, which had not been treated with the pesticides, with a mixture of working standard solutions that contained all pesticides at the second concentration level of the calibration curves (0.500 mg l^{-1} for iprodione, procymidone and vinclozolin, 0.025 mg l^{-1} for chlorothalonil, 1.000 mg l^{-1} for dichlofluanid and 0.100 mg l^{-1} for the rest of pesticides). After the evaporation of the *n*-hexane by using a nitrogen stream, the sample was extracted as it is explained above and injected into the GC–ECD system (1 μl).

2.6. Preparation of calibration curves

Firstly, a stock solution of each pesticide was prepared in *n*-hexane obtaining the primary calibration solutions. From those primary solutions, the secondary standard solution of lower concentration containing all pesticides was prepared by dilution with *n*-hexane. They were stored in a refrigerator at 4°C.

With the objective of stating the matrix influence

and whether it is possible to obtain a correction function for each pesticide, two different types of calibration curves were prepared as follows:

(1) Calibration curves prepared in solvent (solvent calibration, SC): four standard solutions were prepared as a calibration set, at concentrations of 0.050, 0.100, 0.150 and 0.200 mg l^{-1} for chlozolinate, nuarimol and triadimefon, at 0.250, 0.500, 0.750 and 1.000 mg l^{-1} for iprodione, procymidone and vinclozolin, at 0.013, 0.025, 0.038 and 0.050 mg l^{-1} for chlorothalonil and at 0.500, 1.000, 1.500 and 2.000 mg l^{-1} for dichlofluanid. These concentration ranges were chosen on the basis of the maximum residue levels in vegetables allowed by the European regulations for such pesticides in the studied commodities. They were prepared taking 50, 100, 150 and 200 μl of the secondary standard solution, adding the internal standard (dieldrin) and diluting to 2 ml with *n*-hexane. A 1-μl volume of these solutions was injected into the instrument.

(2) Calibration set solutions prepared in vegetable matrix (matrix-matched calibration, MC): these solutions were prepared as described above but adding 0.5 ml of blank extract of each commodity and the internal standard, before filling up to the final volume of 2 ml. These extracts were obtained by applying the extracting method, explained above, to commodities, which had not been treated with any pesticide, and the matrix content in the standard solution is the same as in real and spiked samples.

2.7. Analytical procedure

Each experiment consisted of sequentially injecting a blank extract of each commodity, and the four standard solutions of each calibration set, starting by the solvent calibration, and then each vegetable matrix matched standard solution. Once injected all calibrations and samples, vials were replaced by others containing aliquots of the same solutions and the experiment repeated twice, obtaining in this way three curves in SC and three curves in MC. This experiment was repeated three times, in a 4-month period. The four experiments were carried out in the period corresponding to December 1999, March, April and May 2000. The experiments of December, March and May were performed in the Hewlett-Packard GC model, while the April experiment was carried out in other laboratory with the Perkin-Elmer GC system.

The purpose of these experiments was in the first place to check the repeatability of chromatographic signals under repeatability conditions, that is to say, same operating conditions, instrumental and short period of time; and under reproducibility conditions, i.e., during 6 months, batches of reagents, gases, operators and instruments changed. These data are necessary for the studies that are explained in the following sections.

3. Results and discussion

Tomato, pepper, green bean, aubergine, courgette, cucumber, melon and watermelon have been selected as vegetable commodities in order to study the matrix effect in the quantification of residues of chlorothalonil, chlozolinate, dichlofluanid, iprodione, nuarimol, procymidone, triadimefon and vinclozolin. The water, fat or sugar content of each commodity is different (Table 1) [19].

Analysis of covariance (ANCOVA) [20–22] was applied in order to compare slopes and intercepts obtained with SC and MC. Data from each calibration set (three replicates) are fitted to straight lines using least-squares method. An *F* statistic is calculated (F_{cal}) for comparing the slopes and then the intercepts of each calibration curve obtained for the analytes. Such *F* calculated is defined as the quotient of S_N^2 and S_D^2 (S_N^2 being the variance due to the difference between the reduced and full variability of residuals and S_D^2 the full variability of residuals):

Table 1
Fat, sugar and water content in the commodities studied

	Fat (g)	Sugar (g)	Water (g)
Tomato	0.30	3.00	94.20
Pepper	0.20	3.70	94.00
Green bean	0.20	5.00	89.60
Aubergine	0.18	2.66	93.00
Courgette	0.20	6.00	96.50
Cucumber	0.20	1.90	96.70
Melon	Tr	6.00	92.40
Watermelon	Tr	4.50	97.60

Data refer to 100 g. Tr: Trace amount.

$$F = \frac{S_N^2}{S_D^2} = \frac{\dfrac{SS_{res}^R - SS_{res}^F}{p-1}}{MS_{res}^F}$$

where p is the number of slopes to be compared, SS_{res}^F (full sum of squares) is calculated as the sum of the squares of the residuals with respect to the regression line and the mean square MS_{res}^F is the quotient between the full sum of squares of residuals and the full degrees of freedom (the sum of the degrees of freedom of each regression), finally the reduced sum of squares SS_{res}^R is given by the sum of the residuals resulting from a pooled regression performed with all the regression lines divided by the reduced degrees of freedom (the number of calibration levels in each calibration plot − 2).

F-calculated (F_{cal}) were less than the F-tabulated (F_{tab}) values, considering a 95% confidence level either for the slopes or for the intercepts. It can be concluded that calibrations can be maintained in the short period of time and therefore a unique regression either for the SC or for each MC can be performed with the 12 calibration data (four concentration levels, three replicates).

Data showed dichlofluanid in tomato as an exception because it did not show differences between both types of calibrations. In the rest of cases, there is a significant difference between SC and MC, with a probability of less than 5%, which means that the matrix content introduces a systematic bias in the case that only the intercepts are different, and/or proportional bias, when either slopes or intercepts are different (Table 2).

3.1. Correction function

Regressions obtained with SC and MC can be correlated using a correction function (CF): $C_{CF} = \overline{A} + \overline{B}\, C_{SC} \approx C_{MC}$. The CF is a straight line, being the correction coefficients, A and B, the mean value of the slopes and intercepts of the regression lines (SC), obtained in each experiment performed in December, March, April and May. Table 3 shows the correction coefficients of CF (A and B) obtained for the pesticides in the different matrices.

The test of Dixon was applied to the obtained correction coefficients in order to test if any of them can be considered as outliers, in such case a new correction function is calculated without considering the anomalous experiment. This is the case of nuarimol in every matrixes, chlorothalonil, and chlozolinate in aubergine; chlozolinate in watermelon, triadimefon in tomato and green bean and dichlofluanid in pepper. In these cases the experiment performed in April was denoted an outlier, and kept out of the calculations. This experiment was performed with another instrument, which indicates that the matrix effect is instrument specific. Therefore, we can calculate $C_{CF,}$ which provides an estimation of the concentration that would be obtained using a matrix matched calibration (C_{MC}) from results obtained with a solvent calibration. In such a case, the stability of the correction function is limited to the instrument and it is not applicable to other instruments taking into account that the experiment carried out in April used different instrumentation and has been denoted an outlier.

3.2. Uncertainty estimation

Table 3 also shows the uncertainty associated to the estimated concentration $u(C_{CF})$, which may be calculated by applying the variance propagation rules to the CF and calculating each of the terms that are in the expression [23]:

$$u^2(C_{CF}) = u^2(A) + C_{SC}^2 u^2(B) + B^2 u^2(C_{SC}) + 2r_{A,B} C_{SC} u(A) u(B)$$

with $u(A)$ the uncertainty due to the correction coefficient A. It is calculated as the contribution derived from the regression line [$u(A)$function] and the uncertainty derived from the precision [$u(A)$precision]:

$$u(A) = \sqrt{u^2(A)_{func} + u^2(A)_{prec}}$$

$$u^2(A)_{func} = \frac{\sum u_k^2(A)_{func}}{r}$$

$$u_k^2(A)_{func} = u^2\left(\frac{a_S - a_M}{b_M}\right) = \left(\frac{a_S - a_M}{b_M}\right)^2 \cdot \left[\frac{u^2(a_S) + u^2(a_M)}{(a_S - a_M)^2} + \frac{u^2(b_M)}{b_M^2}\right]$$

Table 2
Comparison of calibrations SC with VMC for the experiment realised in December

	F_{cal}			F_{cal}	
	Slope	Intercept		Slope	Intercept
Chlorothalonil			Nuarimol		
Tomato	95.82	185.30	Tomato	120.64	1262.74
Pepper	66.68	145.98	Pepper	221.46	1244.75
Green bean	72.40	168.53	Green bean	421.78	2781.28
Aubergine	93.91	100.98	Aubergine	327.84	3443.88
Courgette	150.82	161.98	Courgette	366.11	2556.96
Cucumber	508.60	1665.24	Cucumber	241.38	2071.78
Melon	129.88	296.65	Melon	216.28	2570.42
Watermelon	13.06	68.87	Watermelon	958.47	6814.04
Chlozolinate			Procymidone		
Tomato	6.11	46.66	Tomato	6.17	14.56
Pepper	91.89	462.03	Pepper	94.79	363.80
Green bean	228.30	1090.75	Green bean	142.03	638.56
Aubergine	43.32	372.29	Aubergine	94.53	1178.26
Courgette	206.39	1021.28	Courgette	104.31	680.25
Cucumber	21.84	115.22	Cucumber	8.03	179.14
Melon	67.45	488.12	Melon	96.40	1447.10
Watermelon	114.73	656.07	Watermelon	564.67	4725.76
Dichlofluanid			Triadimefon		
Tomato	0.24	0.44	Tomato	40.21	441.67
Pepper	88.00	460.15	Pepper	336.99	1821.33
Green bean	248.01	1496.82	Green bean	411.99	3091.61
Aubergine	69.73	341.38	Aubergine	227.43	4171.30
Courgette	310.40	1939.57	Courgette	162.19	807.98
Cucumber	39.54	102.75	Cucumber	56.99	624.38
Melon	611.93	3235.95	Melon	230.66	2478.29
Watermelon	603.14	3158.24	Watermelon	239.12	2103.59
Iprodione			Vinclozolin		
Tomato	35.20	61.68	Tomato	6.65	65.03
Pepper	6.20	81.73	Pepper	203.68	770.65
Green bean	64.51	336.42	Green bean	213.80	917.59
Aubergine	15.21	2421.93	Aubergine	50.90	330.92
Courgette	31.57	314.78	Courgette	69.53	264.13
Cucumber	18.37	310.97	Cucumber	8.87	29.15
Melon	17.94	197.27	Melon	62.13	613.90
Watermelon	54.95	202.01	Watermelon	190.20	1203.99

$F_{tab}=4.35$ for the slopes. $F_{tab}=4.33$ for the intercepts.

$$u^2(A)_{prec}=\frac{s^2(A)}{r}=\frac{\sum(A_k-\bar{A})^2\cdot(r-1)}{r}$$

where $k=1, 2, 3, 4$ (the number of each experiment); $r=4$ (the number of experiments); a and b are the intercept and the slope of each calibration curve, respectively; A is the intercept of the correction function.

In the same way the uncertainty associated to the slope, the correction coefficient B [$u(B)$], is also calculated as the contributions of $u(B)$function and $u(B)$precision. $r_{A,B}$ is the uncertainty due to the covariance between slope and intercept. Finally, the uncertainty associated to the correction function [$u(C_{CF}$] is obtained as the sum of all the above contributions, resulting a second degree equation,

Table 3
Parameters used for the estimation of $u(C_{CF})$

	B	$u(B)$	A (mg/l)	$u(A)$ (mg/l)	C_{SC} (mg/l)	$r_{A,B}$	$u(C_{CF})_{rel}$ (%)
Chlorothalonil							
Tomato	1.3449	0.0552	−0.0058	0.0018	0.023	0.817	15.3
Pepper	1.2808	0.0567	−0.0042	0.0018	0.023	0.598	14.5
Green bean	1.2826	0.0518	−0.0047	0.0017	0.023	0.507	14.0
Aubergine*	1.3525	0.0593	−0.0109	0.0018	0.027	−0.960	11.6
Courgette	1.3009	0.0504	−0.0086	0.0019	0.026	0.892	16.6
Cucumber	1.7674	0.0885	−0.0062	0.0024	0.018	0.918	18.2
Melon	1.4595	0.0609	−0.0069	0.0021	0.022	0.903	16.7
Watermelon	1.1421	0.0464	−0.0085	0.0015	0.029	−0.484	12.3
Chlozolinate							
Tomato	0.8996	0.0371	−0.0032	0.0055	0.116	−0.353	10.0
Pepper	0.6502	0.0271	0.0113	0.0049	0.136	−0.104	9.2
Green bean	0.5943	0.0227	0.0082	0.0041	0.153	−0.553	8.2
Aubergine*	0.7326	0.0291	−0.0073	0.0048	0.147	−0.161	10.4
Courgette	0.6497	0.0247	0.0093	0.0041	0.141	0.489	9.8
Cucumber	0.8143	0.0327	0.0078	0.0053	0.115	−0.138	9.6
Melon	0.7310	0.0298	0.0017	0.0067	0.133	0.893	13.1
Watermelon*	0.5541	0.0229	0.0027	0.0046	0.179	−0.420	9.1
Dichlofluanid							
Tomato							
Pepper*	1.4675	0.0579	−0.0859	0.0626	0.749	−0.695	9.8
Green bean	1.9584	0.0863	−0.0623	0.0824	0.545	−0.519	11.0
Aubergine	1.4846	0.0580	−0.0942	0.0645	0.746	−0.890	9.3
Courgette	1.8766	0.0726	−0.0422	0.0757	0.566	0.078	12.1
Cucumber	1.3261	0.0501	−0.1481	0.0596	0.845	0.541	13.1
Melon	2.7210	0.1071	−0.2326	0.1018	0.454	−0.203	14.3
Watermelon	2.6248	0.0971	−0.2046	0.0972	0.455	0.291	15.3
Iprodione							
Tomato	0.6174	0.0431	0.0778	0.0411	0.694	0.347	13.4
Pepper	0.7617	0.0483	−0.0798	0.0338	0.787	0.991	16.6
Green bean	0.7940	0.0527	−0.1091	0.0493	0.715	−0.821	11.7
Aubergine	0.6695	0.0486	−0.3451	0.0644	1.267	−0.761	16.0
Courgette	0.6653	0.0425	−0.0677	0.0307	0.857	0.682	15.3
Cucumber	0.7339	0.0452	−0.0720	0.0339	0.792	−0.477	11.5
Melon	0.7012	0.0543	−0.0865	0.0419	0.853	0.215	16.4
Watermelon	0.5987	0.0459	0.0047	0.0317	0.844	−0.250	11.6
Nuarimol							
Tomato*	0.6699	0.0236	−0.0116	0.0042	0.169	0.451	11.2
Pepper*	0.4848	0.0187	0.0008	0.0045	0.205	0.027	9.9
Green bean*	0.4118	0.0151	0.0006	0.0041	0.245	−0.619	8.8
Aubergine*	0.5374	0.0191	−0.0175	0.0038	0.219	−0.423	10.3
Courgette*	0.4828	0.0167	−0.0024	0.0036	0.208	−0.823	8.5
Cucumber*	0.5791	0.0210	−0.0091	0.0040	0.187	−0.334	9.9
Melon*	0.4840	0.0176	−0.0220	0.0043	0.257	−0.627	10.4
Watermelon*	0.3789	0.0120	−0.0025	0.0030	0.274	−0.220	9.1

Table 3. Continued

	B	$u(B)$	A (mg/l)	$u(A)$ (mg/l)	C_{SC} (mg/l)	$r_{A,B}$	$u(C_{CF})_{rel}$ (%)
Procymidone							
Tomato	0.8918	0.0403	0.0004	0.0335	0.566	0.099	11.6
Pepper	0.6100	0.0281	0.0870	0.0254	0.666	0.417	10.0
Green bean	0.5029	0.0234	0.0670	0.0235	0.882	0.294	9.8
Aubergine	0.7270	0.0376	−0.0642	0.0287	0.761	0.526	13.7
Courgette	0.6429	0.0334	0.0084	0.0293	0.748	0.207	11.7
Cucumber	0.9026	0.0390	0.0033	0.0319	0.542	0.849	13.1
Melon	0.6170	0.0284	−0.0618	0.0301	0.927	−0.120	11.6
Watermelon	0.4312	0.0197	−0.0199	0.0222	1.201	0.738	12.0
Triadimefon							
Tomato*	0.7915	0.0303	−0.0097	0.0047	0.137	−0.917	9.0
Pepper	0.5416	0.0183	0.0070	0.0037	0.175	0.932	10.0
Green bean*	0.4365	0.0185	−0.0083	0.0048	0.246	−0.562	9.8
Aubergine	0.5439	0.0196	−0.0479	0.0043	0.270	−0.299	13.2
Courgette	0.5169	0.0192	0.0050	0.0042	0.188	−0.182	9.0
Cucumber	0.6983	0.0283	−0.0167	0.0051	0.166	0.007	11.7
Melon	0.5785	0.0232	−0.0154	0.0046	0.198	0.670	12.5
Watermelon	0.4588	0.0171	−0.0176	0.0041	0.258	−0.185	10.8
Vinclozolin							
Tomato	0.9239	0.0409	−0.0040	0.0297	0.545	−0.558	9.5
Pepper	0.6824	0.0259	0.0717	0.0218	0.624	0.294	9.2
Green bean	0.6421	0.0221	0.0644	0.0178	0.675	0.748	9.3
Aubergine	0.7615	0.0299	0.0037	0.0236	0.653	0.477	10.9
Courgette	0.7059	0.0316	0.0559	0.0274	0.623	−0.652	8.3
Cucumber	0.8585	0.0391	0.0469	0.0297	0.542	0.605	11.5
Melon	0.7423	0.0283	−0.0412	0.0226	0.735	0.926	12.1
Watermelon	0.5850	0.0249	0.0007	0.0242	0.844	0.596	11.4

Correction coefficients (B, A) and their associated uncertainties [$u(A)$, $u(B)$]. Correlation coefficients between A and B ($r_{A,B}$). Concentration calculated with the solvent calibration and uncertainty associated to the estimated concentration C_{CF}.
* The number of experiments was three.

which is only function of C_{SC} for a given value of uncertainty $u(C_{SC})$, in such way that when C_{SC} increases the uncertainty also increases. Table 3 shows the uncertainty obtained in each case considering $u(C_{SC})=8\%$ for all pesticides (this uncertainty value is a mean of those obtained in Ref. [23]). When the analyte concentration is obtained using a calibration prepared in solvent and applying to the result the correction function (instead of quantifying with the matrix-matched calibration), the uncertainty obtained ranged between 8.2 and 18.2%, being in most of cases less than 13%, which means that the use of the CF increases the uncertainty by about 5% in most cases.

3.3. Validation

The validation of each correction function was performed comparing the recovery rates obtained with the 100%. This was carried out in different ways: (a) as the quotient between the amount of analyte found (with SC) and the spiked amount; (b) as the quotient between the amount of analyte found (with MC) and the spiked amount; (c) quantifying the chromatographic signals with the solvent calibration (as in the first case) and then applying the correction function to the obtained results.

A statistical test (t-test) was applied to the mean of recovery rates ($\overline{\Re}$) (12 replicates) in order to check if

Table 4
Comparison of recovery rates obtained quantifying with SC with 100%

Pesticide	Commodity																							
	Tomato			Pepper			Green bean			Aubergine			Courgette			Cucumber			Melon			Watermelon		
	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}
Chlorothalonil	90.2	6.7	5.1	92.2	4.5	5.9	92.2	5.0	5.4	106.9	6.9	3.4	104.5	5.0	3.1	71.6	3.5	27.9	86.8	6.2	7.4	117.2	1.1	53.1
Chlozolinate	116.0	6.0	9.3	135.7	6.2	20.0	152.6	6.5	28.0	147.0	6.7	24.3	141.4	8.7	16.5	114.8	6.0	8.6	133.1	6.2	14.5	179.2	8.7	31.5
Dichlofluanid	100.4	4.3	0.3	74.9	4.5	19.4	54.5	2.8	56.8	74.7	3.7	24.0	56.6	2.3	65.4	84.5	3.9	13.7	45.4	1.6	116.9	45.5	1.7	114.1
Iprodione	138.9	7.3	18.6	157.5	9.2	21.6	143.1	6.8	22.1	253.5	12.5	42.4	171.5	6.8	36.6	158.3	6.2	32.4	170.6	8.2	30.0	168.7	6.4	37.4
Nuarimol	168.8	11.2	21.3	205.0	9.6	38.0	245.1	13.2	38.0	218.6	6.1	67.0	208.5	8.7	43.1	187.2	7.6	39.8	257.1	14.3	38.2	273.8	18.6	32.4
Procymidone	113.2	5.5	8.3	133.2	5.0	23.1	176.3	9.2	28.6	176.3	9.2	28.6	152.3	8.8	20.5	149.7	6.8	25.3	108.4	4.8	6.2	185.5	10.6	28.0
Triadimefon	136.6	8.7	14.7	175.3	8.7	30.0	246.5	12.1	42.1	270.0	15.2	38.7	188.1	8.0	38.4	166.1	8.4	27.4	198.1	11.6	29.2	258.3	11.6	29.2
Vinclozolin	109.1	6.7	4.7	124.8	6.3	13.7	135.0	8.6	14.1	130.6	6.3	16.9	124.6	4.2	20.2	108.4	5.7	5.1	147.1	9.7	16.9	168.9	9.5	25.1

$t_{tab}=2.2$. $\overline{\Re}$, Mean of recovery rates of the spiked samples quantified with SC. S_U, Standard deviation of recovery rates.

there is any significant difference between such recovery rates and 100% with a probability higher than 5%. As shown in Table 4, when SC is used, the value of the t calculated (t_{cal}) obtained is greater than the tabulated (t_{tab}) (except for dichlofluanid in tomato), which means that C_{SC} is different (actually an over-estimation in most cases) of the actual concentration due to the presence of the matrix effect. Therefore the recoveries obtained quantifying with SC were statistically different from the 100% recovery. When recovery rates ($\overline{\Re}$) were estimated with the corresponding MC (Table 5), they were not statistically different from 100% since t_{cal} is less than t_{tab} in all cases.

Finally, a paired-samples test was applied to the recovery rates obtained quantifying the results of spiked samples using MC and using the CF. For this purpose the mean of the differences between such results ($\overline{D}$), and their standard deviations S_D were obtained (Table 6). Any statistical difference was observed between both methods in all cases, with a probability greater than 5%. As shown in Table 6, t_{cal} was less than t_{tab} in all cases and therefore there is no significant difference between recovery rates obtained quantifying with MC and CF.

In conclusion the correction function allows an estimation of the concentration, quantifying with a calibration solution prepared in solvent, that does not differ from the spiked amount, recovery rates obtained in this way do not differ from the 100%.

Table 5
Comparison of recovery rates MC with 100%

Pesticide	Commodity																							
	Tomato			Pepper			Green bean			Aubergine			Courgette			Cucumber			Melon			Watermelon		
	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}	$\overline{\Re}$	S_U	t_{cal}
Chlorothalonil	100.0	5.2	0.0	101.1	5.3	0.7	97.7	3.7	2.1	98.3	4.0	1.6	99.9	4.7	0.1	98.5	4.4	1.2	101.5	5.5	0.9	101.0	5.5	0.6
Chlozolinate	100.3	4.5	0.3	100.1	3.6	0.1	98.2	4.2	1.5	101.0	3.2	1.1	99.8	4.0	0.2	99.8	2.8	0.3	100.5	5.9	0.3	100.4	4.4	0.3
Dichlofluanid	102.5	4.5	1.9	102.2	4.5	1.7	99.1	7.6	0.4	98.9	4.3	0.9	103.1	5.6	1.9	100.4	4.9	0.3	102.0	5.8	1.2	98.6	6.1	0.8
Iprodione	99.4	3.5	0.6	100.0	4.2	0.0	98.2	5.6	1.1	100.6	6.7	0.3	100.9	3.3	1.0	101.6	5.5	1.0	98.9	5.0	0.8	100.2	5.5	0.1
Nuarimol	97.3	6.8	1.4	101.4	3.5	1.4	100.9	5.5	0.6	101.8	3.7	1.7	100.0	6.4	0.0	101.0	5.7	0.6	99.4	5.8	0.4	100.1	5.5	0.1
Procymidone	99.1	7.5	0.4	98.8	6.0	0.7	99.2	6.3	0.4	100.8	6.0	0.5	101.1	3.5	1.0	99.0	4.7	0.7	99.1	4.2	0.7	97.7	4.6	1.8
Triadimefon	100.5	4.6	0.4	101.9	4.5	1.5	98.2	7.1	0.9	101.5	5.1	1.0	100.5	7.2	0.2	101.8	3.4	1.9	100.5	4.9	0.3	102.1	5.5	1.4
Vinclozolin	99.8	4.5	0.1	100.7	4.3	0.6	98.1	5.3	1.2	101.3	5.0	0.9	100.2	3.3	0.2	98.4	4.7	1.2	98.9	3.9	1.0	99.7	4.1	0.2

$t_{tab}=2.2$. $\overline{\Re}$, Mean of recovery rates of the spiked samples quantified with MC. S_U, Standard deviation of recovery rates.

Table 6
Paired samples test between the recovery of the correction function and MC recovery

Pesticide	Commodity																							
	Tomato			Pepper			Green bean			Aubergine			Courgette			Cucumber			Melon			Watermelon		
	$\overline{D}$	S_D	t_{cal}	$\overline{D}$	S_D	t_{cal}	$\overline{D}$	S_D	t_{cal}	$\overline{D}$	S_D	t_{cal}	$\overline{D}$	S_D	t_{cal}	$\overline{D}$	S_D	t_{cal}	$\overline{D}$	S_D	t_{cal}	$\overline{D}$	S_D	t_{cal}
Chlorothalonil	1.806	9.7	0.6	−0.261	9.0	0.1	−1.722	7.6	0.8	−2.847	11.0	0.9	−1.036	6.3	0.6	−2.977	8.7	1.2	2.386	11.5	0.7	1.019	5.8	0.6
Chlozolinate	−0.867	7.7	0.8	0.589	5.9	0.3	−0.645	5.4	0.4	0.593	6.0	0.3	−1.367	7.6	0.6	−0.428	5.8	0.3	1.396	8.1	0.6	−1.498	4.8	1.1
Dichlofluanid				0.972	8.0	0.4	−1.345	10.8	0.4	−2.470	5.4	1.6	1.288	9.0	0.5	3.113	7.7	1.4	1.764	5.7	1.1	−0.235	6.7	0.1
Iprodione	0.065	6.6	0.0	−2.212	8.4	0.9	−0.035	10.0	0.0	2.361	7.5	1.1	2.222	6.1	1.3	1.486	7.9	0.7	0.055	5.5	0.0	−0.546	6.2	0.3
Nuarimol	−4.224	10.0	1.5	1.166	5.4	0.8	−0.591	5.1	0.4	1.931	5.2	1.3	1.724	7.7	0.8	1.719	5.1	1.2	−3.089	8.0	1.3	0.450	10.2	0.2
Procymidone	−2.955	8.1	1.3	0.114	7.3	0.1	−2.865	7.0	1.4	2.949	7.9	1.3	3.145	6.4	1.7	0.029	7.5	0.0	−2.959	8.4	1.2	−1.872	5.0	1.3
Triadimefon	2.104	8.5	0.9	−0.071	6.0	0.0	−0.954	8.0	0.4	2.522	11.3	0.8	−1.790	7.4	0.8	2.520	7.2	1.2	1.4	6.1	0.8	1.2	9.7	0.4
Vinclozolin	−0.152	7.3	0.1	1.315	5.1	0.9	−1.430	5.627	0.9	1.183	7.3	0.6	1.104	4.2	0.9	−3.989	7.8	1.8	−1.995	8.6	0.8	0.809	7.8	0.4

$t_{tab} = 2.2$. $\overline{D}$, Mean of the differences between recovery rates of the spiked samples quantified with MC and those calculated using CF. S_D, Standard deviation of the difference.

4. Conclusions

A methodology for characterising the matrix effect of different vegetable commodities for the quantification of pesticides using GC–ECD has been proposed.

The matrix effect leads in most of cases to systematic and/or proportional bias in the quantification of pesticides when a solvent calibration is used.

A correction function for each pesticide in each commodity has been obtained.

The stability of the correction functions has been checked during a 4-month period. The statistics shows that they maintain stables in a given conditions, during the time considered, in which minor changes in the working conditions, such as reagent batches and chromatographic maintenance occurred.

An estimation of the uncertainty associated with the use of the correction function showed in almost all cases that the additional uncertainty of the results is less than 8%. An exception is chlorothalonil in cucumber matrix which is 10%, however it is quite minor than the associated to other steps of the analytical method, such as the calibration step.

The use of the correction function can save in cost and time in pesticide residues laboratories, avoiding the extraction of blank samples, saving solvents, enlarging the life of chromatographic consumables and making the quantification using the simple solvent calibration easier. Nevertheless, the instrument dependency of the matrix-effect is evidenced, the experiment carried out in April with a different GC system resulted in an outlier, so that the transferability of the correction function should be considered cautiously. It only works in cases in which the stability of the whole analytical process is ensured.

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