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Determination of carbaryl, carbofuran and methiocarb in cucumbers and strawberries by monoclonal enzyme immunoassays and high-performance liquid chromatography with fluorescence detection An analytical comparison

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Abstract

Carbaryl, carbofuran and methiocarb are three of the most important *N*-methylcarbamate pesticides. In the present work, the application of laboratory-developed monoclonal antibody-based enzyme-linked immunosorbent assays (ELISAs) to the determination of these compounds in fruits and vegetables is described. Cucumbers and strawberries were spiked with the three carbamates at 10, 50 and 200 ppb. After extraction and clean-up, samples were analyzed by immunoassay and by HPLC with post-column derivatization and fluorescence detection (US Environmental Protection Agency Method 531.1). Results obtained by ELISA correlated well with those obtained by HPLC, both in terms of accuracy and precision. Recoveries were in the 60–90% range by ELISA and in the 50–90% range by HPLC, depending on the particular combination of commodity, pesticide, and fortification level under consideration. ELISAs were also applied to the analysis of non-purified sample extracts with excellent results: recoveries close to 100% were obtained, while maintaining similar precision values. This approach avoids the use of solid-phase extraction columns, saves time, and considerably increases the sample throughput. Results clearly indicate that the developed immunoassays may be suitable for the quantitative and reliable determination of carbaryl, carbofuran and methiocarb in fruits and vegetables even without including clean-up steps. These considerations make these ELISAs very useful analytical tools for monitoring and regulatory programs, without the need of complex and expensive instrumentation.

Keywords: Food analysis; Fruits; Vegetables; Immunoassays; Pesticides; Methylcarbamates; Carbamates

1. Introduction

Since their commercial introduction in the early 1960s, *N*-methylcarbamate pesticides began to be used worldwide as substituents of the organochlorine compounds because of their high effectivity as insecticides and nematicides, their relatively low mammalian toxicities in many cases, and their low bioaccumulation potentials. Consequently, *N*-methylcarbamates currently constitute a class of chemicals widely used in agriculture to combat a high number of pests in a great variety of crops, and their residues may be encountered in fruits and vegetables. Even though pesticides are indispensable chemicals in modern civilization, people are aware

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of their potential toxicity for animals and humans, so the presence of their residues in foodstuffs poses a potential hazard for consumers. Therefore, to watch over the safety of our food supply, government agencies of most countries established maximum residue levels (MRLs) of pesticides and set up monitoring programs of pesticide residues on crops. The actual effectivity and reliability of these surveillance programs greatly depend on the number of samples analyzed and on the variety of pesticides and crops covered. In this respect, most control laboratories mainly focused their monitoring programs on the analysis of organochlorine, organophosphorus and organonitrogen pesticides. It is well-known that the polarity and thermal instability of most *N*-methylcarbamates make their sensitive and accurate determination by traditional gas chromatographic methods difficult. Based on the pioneering work by Moye et al. [1], further modified by Krause [2] for use on food samples, high-performance liquid chromatography (HPLC) with post-column derivatization and fluorescence detection [US Environmental Protection Agency (EPA) Method 531.1] is the prevalent technique for the analysis of *N*-methylcarbamate pesticides. Although sensitive and well-established, the method requires complex and expensive instrumentation that has to be managed by highly qualified personnel. Thus, in most control laboratories where this equipment is available, *N*-methylcarbamates are not fully implemented in routine pesticide screening programs, so only a small proportion of the analytical work devoted to residue monitoring in food samples concerns these agrochemicals.

In the last decade, different laboratories worldwide have developed immunoassays for a large number of pesticides belonging to different chemical classes [3–5]. The contribution of our group to this field includes immunoassays to organophosphorus [6–8], organochlorine [9], and *N*-methylcarbamate pesticides [10–12]. Immunoassays are analytical methods based on the interaction of an analyte with an antibody that recognizes it with high affinity and specificity. They are simple, cost-effective and field-portable, do not require sophisticated instrumentation, and are able to accurately and precisely analyze a large number of samples simultaneously. All these features make immunoassays very promising analytical tools in pesticide monitoring programs, particularly for those chemicals difficult and/or costly to determine by conventional chromatographic techniques, or for specific pesticides that deserve especial attention because of their toxicity, extensive use or high frequency of appearance in foodstuffs. Nevertheless, immunoassays have not currently reached a wide acceptance among analytical chemists as alternative and/or complementary methods for the analysis of agrochemicals in fruits and vegetables. This situation may be ascribed to the lack of rigorous and comprehensive validation studies in foodstuffs, so the misconception that enzyme-linked immunosorbent assays (ELISAs) are adequate for the determination of pesticide residues in water but not in food samples is still a widespread belief.

In the last few years we have developed monoclonal antibody-based ELISAs for three of the most important *N*-methylcarbamate pesticides, i.e., carbaryl, carbofuran and methiocarb (Fig. 1). Some of these immunoassays have been applied to pesticide analysis in water [13] and fruit juices [11,14]. In the present work, the first results obtained on the application of these ELISAs to the determination of residues of those three pesticides in fruits and

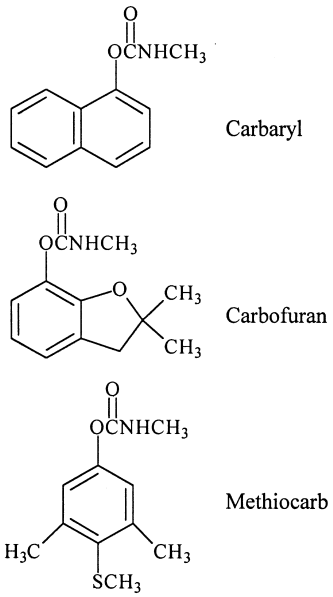

Fig. 1. Chemical structures of the *N*-methylcarbamate pesticides analyzed.

vegetables are described. Cucumbers and strawberries were spiked with the carbamates at several levels and fortified samples were analyzed by ELISA. Results were compared in terms of precision and accuracy with those generated by high-performance liquid chromatography (HPLC) as the reference method. Moreover, the possibility of eliminating the sample clean-up step for ELISA determination was also evaluated.

2. Experimental

2.1. Chemicals and immunoreagents

Enzyme immunoassay-grade horseradish peroxidase (HRP) for enzyme tracer preparation was purchased from Boehringer Mannheim (Barcelona, Spain) and *o*-phenylenediamine (OPD) for ELISA colour development was obtained from Sigma (Madrid, Spain). The production of monoclonal antibodies (MAbs) specific to carbaryl, carbofuran and methiocarb, as well as the synthesis and preparation of the corresponding HRP–hapten conjugates, was carried out in our laboratory as previously reported [10–12]. LIB-CNH45 MAb and HRP-CPNU tracer were used for carbaryl analysis, LIB-BFNB67 MAb and HRP-BFNH tracer were used for carbofuran analysis, and LIB-MXNB31 MAb and HRP-MCNH tracer were used for methiocarb analysis. The particular combination of immunoreagents used for the analysis of each chemical, as well as their concentrations, are shown in Table 1.

Dichloromethane, acetone and light petroleum for pesticide residue analysis and HPLC-grade methanol and acetonitrile were obtained from Scharlau (Barcelona, Spain). Water for HPLC analysis was produced using a Barnstead Nanopure II system (Dubuque, USA). Reagent-grade *o*-phthaldialdehyde (OPA), 2-mercaptoethanol, sodium hydroxide and sodium tetraborate decahydrate were from Merck (Darmstadt, Germany).

OPA reagent was prepared fresh daily by dissolving 50 mg of *o*-phthaldialdehyde in 5 ml of methanol. This solution was transferred to a 500-ml volumetric flask and diluted to mark with 0.05 *M* sodium borate. After filtering and degassing, 25 μl of 2-mercaptoethanol was added.

Standards of carbaryl, carbofuran and methiocarb (≥99.9%) were from Riedel-de Haën (Seelze, Germany). Standard stock solutions of each analyte (20 ppm in methanol) were made by dilution of 100 m*M* solutions prepared by dissolving 30–40 mg of pesticide standard in dimethylformamide (DMF). Stock solutions were stored in amber-coloured flasks at −20°C and used every day to prepare fresh working standard solutions for ELISA and HPLC determinations, as well as pesticide mixtures for fortification studies.

2.2. ELISA instrumentation

Ninety-six-well ELISA polystyrene plates (High Binding Plates, cat. No. 3590) were from Costar (Cambridge, USA). ELISA plates were washed with a 96PW microplate washer from SLT (Salzburg, Austria), and absorbances were read in dual-wavelength mode (490–650 nm) with a Emax microplate

Table 1
Concentrations of immunoreagents and sample dilution requirements for the immunoassays to carbaryl, carbofuran and methiocarb

	ELISA		
	Carbaryl	Carbofuran	Methiocarb
Antibody	LIB-CNH45	LIB-BFNB67	LIB-MXNB31
Concentration (μg ml^{-1})	1.5	1.0	0.75
Tracer	HRP-CPNU	HRP-BFNH	HRP-MCNH
Concentration (ng ml^{-1})	70	100	400
Sample dilution			
10 ppb	1/20	1/5	1/40
50 ppb	1/80	1/15	1/180
200 ppb	1/300	1/60	1/750

reader from Molecular Devices (Sunnyvale, USA). Data processing was performed using Sigmaplot software from Jandel Scientific (San Rafael, USA).

2.3. HPLC instrumentation

All the equipment for HPLC analysis was supplied by Waters (Milford, USA). The instrument consisted of a so-called carbamate analysis system (which includes a quaternary analytical pump and the reaction coils and oven required for post-column *N*-methylcarbamate hydrolysis and derivatization) equipped with a 600E system controller, a 715 variable-volume injector and autosampler, two pumps for delivering the OPA and NaOH solutions to the post-column reaction coils, an in-line degassing system, and a 474 fluorescence detector for signal monitoring. Data acquisition and processing was performed on a Digital Venturis 486 computer using Waters Millenium software, version 2.15.2.

2.4. Sample fortification and extraction

Cucumbers and strawberries were bought from a local market and analyzed for incurred residues of *N*-methylcarbamate pesticides by HPLC. No incurred residues were detected. A mixture of carbaryl, carbofuran and methiocarb, each at 200 ppb, was prepared in dichloromethane from the individual stock solutions at 20 ppm. This mixture was further diluted in dichloromethane to provide fortification solutions at 2, 10 and 40 ppb. Seventy-five ml of these pesticide mixtures was added to 15 g of well-mixed, chopped crop sample, so cucumbers and strawberries were spiked at 10, 50 and 200 ppb. After 10 min, organic solvent was evaporated at reduced pressure in a water bath kept at 30°C.

Sample extraction and purification were performed according to de Kok and Hiemstra [15]. Fortified samples were homogenized with 30 ml of acetone in a centrifuge bottle for 30 s with a Ultra-turrax T-25 apparatus from Hanke and Junkle. Thirty ml of dichloromethane and 30 ml of light petroleum were subsequently added, and the mixture was homogenized for another 60 s. After centrifuging for 5 min at 4000 rpm, the organic layer was transferred to a graduated cylinder to determine the recovered volume. Two portions of 20 ml were transferred to conical-bottom flasks, and the solvent was evaporated to dryness at reduced pressure in a water bath at 30–35°C. One of the aliquots was redissolved in 10 ml of water, pH 3, while the other one was redissolved in 10 ml dichloromethane and purified on a 1-g Bond-Elut aminopropyl-bonded silica extraction column (Varian/Analytichem, Harbor City, USA). Extracts in water were determined only by ELISA, whereas cleaned up (column purified) extracts were analyzed by both HPLC and ELISA for direct comparison of method performance. Solid-phase extraction (SPE) cartridges were conditioned with 10 ml dichloromethane. After applying the extract, pesticides were eluted from the column with 5 ml dichloromethane and 10 ml of dichloromethane–methanol (99:1). Although this type of column is very efficient to clean up the sample from matrix components, *N*-methylcarbamates are not fully retained under these conditions. Therefore, to avoid pesticide losses in this step, the collection of the eluent was started at the same time as the sample was applied to the column. Finally, the eluent was evaporated to dryness, redissolved in 10% methanol in water, pH 3, and filtered through Gelman Acrodisc GHP filters (13 mm, 0.45 μm). Both purified and non-purified sample extracts were stored at −20°C in the dark until analysis (typically one week). In order to evaluate method reproducibility, six replicates of each matrix were spiked at each of the fortification levels mentioned above.

2.5. ELISA determinations

N-Methylcarbamates were analyzed in separated ELISA plates using a specific pair of immunoreagents (antibody and tracer) for each one of the analytes. Nevertheless, most of the ELISA conditions were exactly the same for the three immunoassays, and in fact they were performed simultaneously. Specific details about immunoreagent concentrations and sample dilution requirements are summarized in Table 1. Working standard solutions in the appropriate range for each immunoassay were prepared from the individual stock solutions by serial dilution in assay buffer (100 m*M* sodium phosphate, 137 m*M* NaCl, pH 7.2). ELISAs were performed as follows: 96-well microtiter plates were coated by adding 100 μl/well of the antibody solution in 50 m*M* carbonate

buffer, pH 9.6. After overnight incubation at room temperature, plates were washed four times with 0.15 *M* NaCl containing Tween 20 (0.05%, v/v). At this stage ELISA plates were ready for carbamate analysis. Fifty μl of standards or sample extracts adequately diluted in assay buffer were added to triplicate wells, followed by 50 μl/well of a solution of enzyme tracer in assay buffer containing 0.1% bovine serum albumin (BSA) and 0.02% thimerosal. Plates were incubated for 1 h, washed as before, and finally the colour was developed by adding 100 μl/well of a 2 mg ml^{-1} OPD solution in reaction buffer (25 m*M* sodium citrate, 62 m*M* sodium phosphate, 0.012% H_2O_2, pH 5.4). After 10 min at room temperature, the enzymatic reaction was stopped by adding 100 μl of 2.5 *M* sulphuric acid, and the absorbance was read. Absorbance values from standards were mathematically fitted to a four-parameter logistic equation, and the analyte concentration of samples was determined by interpolation of the mean absorbance on the resulting standard curve.

2.6. HPLC determinations

Analytical separation of pesticides was performed on a Waters C_{18} (4 μm particle size) 150×3.9 mm I.D. reversed-phase column used in conjunction with Waters Nova-Pak C_{18} Guard-Pak inserts. The column oven temperature was kept at 30°C, and the sample injection volume was 400 μl. Mobile phase flow-rate was kept at 1.5 ml min^{-1}. Chromatographic runs were performed using the ternary gradient profile shown in Table 2. Post-colum oven temperature was kept at 80°C. Both NaOH and OPA solutions were delivered to the hydrolysis and derivatization system at 0.5 ml min^{-1}. Detection of pesticides as fluorescent isoindole derivatives was carried out using 339 and 445 nm as wavelengths for excitation and emission, respectively. Sample concentrations were automatically calculated by the software using the external calibration method. Calibration standards of pesticide mixtures at 100, 25 and 5 ppb were prepared in water, pH 3 from the individual stock solutions and run at the beginning of the analysis and every ten samples.

3. Results and discussion

Immunochemical determinations of carbamates in fortified samples were carried out by using three laboratory-developed monoclonal immunoassays. On the basis of previous characterization studies, these immunoassays are highly specific for their respective target compound, since not even pesticide metabolites are in fact recognized. Each immunoassay was performed in a different ELISA plate, so samples were quantitatively analyzed for a single residue in each plate, irrespective of the presence of the two other analytes.

An eight-point standard curve was included in each ELISA plate to better estimate analyte concentrations. Representative ELISA standard curves for carbaryl, carbofuran and methiocarb are shown in Fig. 2. As it corresponds to competitive assays, signal was inversely proportional to analyte concentration and standard points fitted to a sigmoidal equation. The central section of the curve with a nearly linear response was assumed as the operative working range of the assay. A common way to express immunoassay sensitivity is by calculating the I_{50} value, that is, the analyte concentration that reduces the assay signal to 50% of the maximum

Table 2
Gradient conditions for *N*-methylcarbamate analysis by HPLC

Time (min)	Water (%)	Methanol (%)	Acetonitrile (%)	Curve[a]
Initial	88	12	0	–
4.00	88	12	0	1
4.10	68	16	16	3
16.10	30	35	35	10
19.00	88	12	0	9

[a] Curve numbers refer to the gradient curve shapes as described in the technical booklet of the Waters 600E controller. 1=Instant transition; 3=convex curve; 9, 10=concave curves.

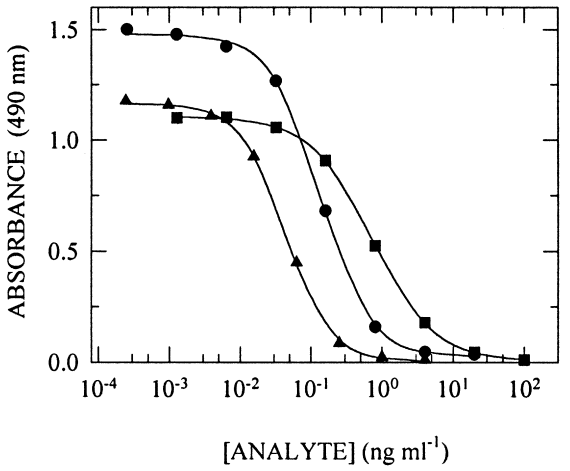

Fig. 2. Representative ELISA standard curves for carbaryl (●), carbofuran (■) and methiocarb (▲). Immunoassay conditions are described in Section 2.5.

value. With regard to this criterion, the most sensitive of the three assays for standards was the ELISA for methiocarb, with a I_{50} value of 0.044 ng ml^{-1}, followed by the ELISAs for carbaryl (I_{50}=0.13 ng ml^{-1}) and carbofuran (I_{50}=0.79 ng ml^{-1}).

Cucumbers and strawberries were chosen as model matrices to evaluate the suitability of these immunoassays for the analysis of vegetables and fruits. Apart from their relevance for the compounds under study, these matrices were chosen because they do not present interferent compounds in the chromatographic area of interest, thus allowing an optimal quantification of the analytes (Figs. 3 and 4). Six replicates of each matrix were spiked at 10, 50 and 200 ppb. These fortification levels are well below the MRLs for carbaryl, carbofuran, and methiocarb in these crops, which are in the 0.2–1.0 ppm range depending on the particular pesticide/commodity combination.

After extraction and clean-up, samples were analyzed by ELISA and HPLC. Due to the rather narrow working range and high sensitivity of these immunoassays, particularly those of carbaryl and methiocarb, the analyte concentration in the final extract was too high as to be properly quantified, even for the 10 ppb fortification level. Therefore, whereas for HPLC

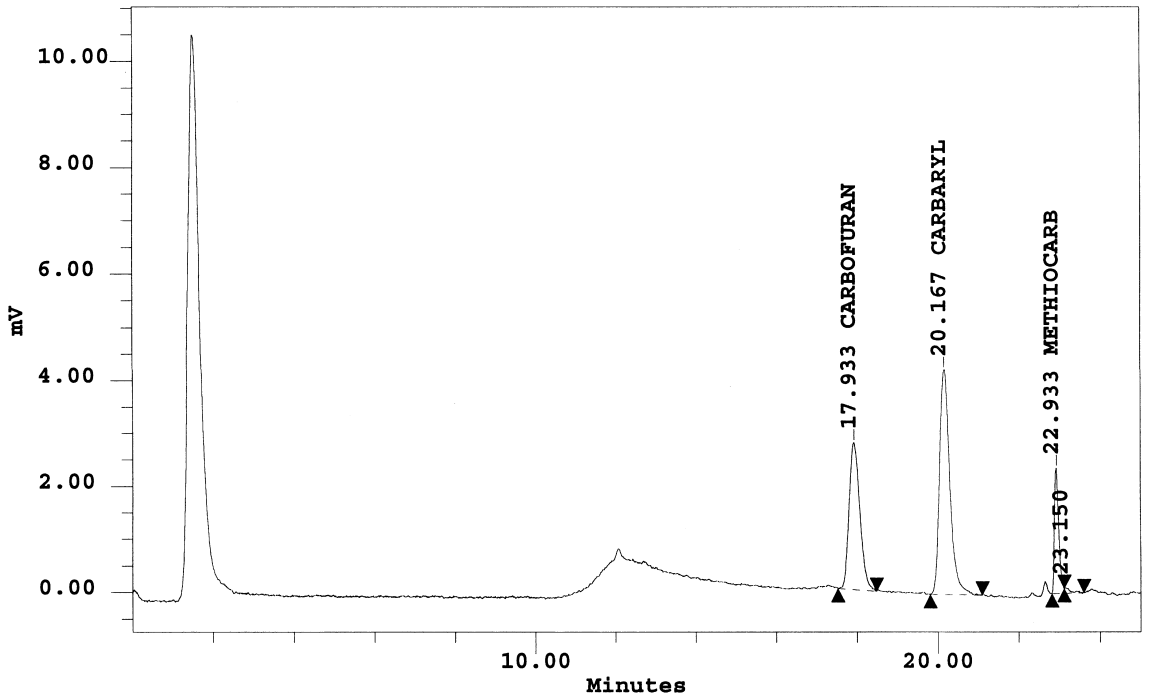

Fig. 3. HPLC chromatogram of an extract of a cucumber sample fortified with a mixture of carbaryl, carbofuran and methiocarb at 0.05 ppm each. HPLC conditions are described in Section 2.6.

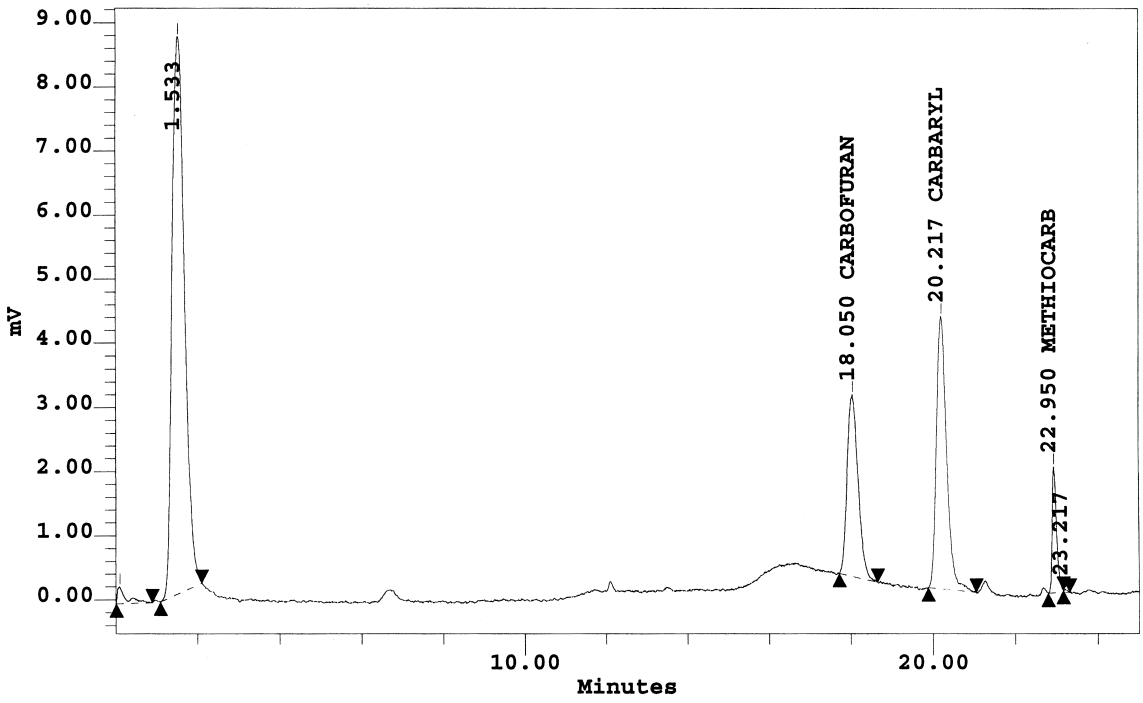

Fig. 4. HPLC chromatogram of an extract of a strawberry sample fortified with a mixture of carbaryl, carbofuran and methiocarb at 0.05 ppm each. HPLC conditions are described in Section 2.6.

analyses 400 μl of sample extract were directly injected, samples had to be previously diluted in assay buffer for ELISA, so that they entered the working range of the standard curves. An additional reason to dilute samples for ELISA was the solvent composition used to finally redissolve the sample extract (methanol–water, 10:90), which is optimal for HPLC analysis but is not for antibody–analyte interactions. The dilution approach was preferred over the possibility of using different solvents for ELISA and HPLC analysis because in this way the comparison between methods could be more properly established.

Reproducibility and recovery values obtained for each crop, analyte and spiked level by the two analytical systems are shown in Table 3. Irrespective of the fortification level, mean recoveries by ELISA were 88.8%, 90.7% and 62.2% for carbaryl, carbofuran and methiocarb, respectively, in cucumbers, and 80.8%, 80.4% and 64.7% in strawberries. Nearly identical mean recoveries were obtained by HPLC. Moreover, method reproducibility was also very similar for both analytical systems, with relative standard deviations (R.S.D.s) ranging from 2.1 to 25.4% for ELISA and from 3.2 to 19.6% for HPLC. The fact that recovery values were below 100%, specially in the case of methiocarb, may be probably ascribed to pesticide losses during clean-up and evaporation/redissolution steps. Therefore, in terms of precision and accuracy, all three ELISAs can be considered as suitable as the reference method for the quantitative analysis of carbaryl, carbofuran and methiocarb in cucumbers and strawberries.

It is well known among analytical chemists that sample clean-up is the rate-determining step in the analysis of *N*-methylcarbamates by HPLC. The introduction of commercial apparatus for automated SPE has had an evident beneficial effect on sample throughput. However, the ideal situation would be to omit the sample clean-up step, as is usually the case for capillary gas chromatography (GC) multi-residue methods in routine analysis. One of the main advantages generally attributed to immunoassays is the possibility of reducing or even eliminating sample

Table 3
Analysis by ELISA and HPLC of purified sample extracts of cucumbers and strawberries spiked with carbaryl, carbofuran and methiocarb at 10, 50 and 200 ppb (n=6 replicates)

Matrix	Analyte	Fortification level (ppb)	ELISA			HPLC		
			Mean±S.D. (ppb)	Recovery (%)	R.S.D. (%)	Mean±S.D. (ppb)	Recovery (%)	R.S.D. (%)
Cucumber	Carbaryl	10	9.4±0.2	94.0	2.1	8.6±0.6	86.0	7.0
		50	43.0±2.8	86.0	6.5	42.9±2.0	85.8	4.7
		200	172.5±13.7	86.3	7.9	168.1±19.4	84.1	11.5
		Mean		88.8	5.5		85.3	7.7
	Carbofuran	10	8.7±0.9	87.0	10.3	9.0±0.4	90.0	4.4
		50	46.6±1.8	93.2	3.9	46.2±1.5	92.4	3.2
		200	183.5±14.7	91.8	8.0	181.4±12.1	90.7	6.7
		Mean		90.7	7.4		91.0	4.8
	Methiocarb	10	7.1±0.4	71.0	5.6	7.7±1.0	77.0	13.0
		50	31.3±4.1	62.6	13.1	32.0±2.6	64.0	8.1
		200	105.9±15.0	53.0	14.2	112.9±18.8	56.5	16.7
		Mean		62.2	11.0		65.8	12.6
Strawberry	Carbaryl	10	8.0±0.4	80.0	5.0	7.0±0.4	70.0	5.7
		50	39.4±5.1	78.8	12.9	33.6±3.8	67.2	11.3
		200	167.3±13.5	83.7	8.1	150.9±10.1	75.5	6.7
		Mean		80.8	8.7		70.9	7.9
	Carbofuran	10	6.8±1.3	68.0	19.1	8.1±0.7	81.0	8.6
		50	40.6±3.8	81.2	9.4	40.7±3.0	81.4	7.4
		200	183.9±14.7	92.0	8.0	174.0±7.6	87.0	4.4
		Mean		80.4	12.2		83.1	6.8
	Methiocarb	10	6.0±1.0	60.0	16.7	4.4±0.4	44.0	9.1
		50	32.7±8.3	65.4	25.4	24.0±4.7	48.0	19.6
		200	137.3±15.5	68.7	11.3	114.2±17.0	57.1	14.9
		Mean		64.7	17.8		49.7	14.5

purification steps in the analysis of pesticide residues in foodstuffs. Nevertheless, this issue should be adequately demonstrated rather than assumed, since it probably depends on the particular immunoassay under consideration and on the matrix complexity. Therefore, experimental work was undertaken to asses whether these immunoassays were able to quantitatively analyze non-purified samples. To this purpose, 20-ml portions of the original organic extracts were simply evaporated, redissolved in water, pH 3, and analyzed by ELISA after being properly diluted in assay buffer to bring samples into the standard working range of each immunoassay. Samples were analyzed unfiltered, with solid particles in suspension. Analytical results are shown in Table 4. A direct comparison with the reference method was not possible in this case because extracts had to be necessarily cleaned up for HPLC analysis. However, data could indeed be compared with those shown in Table 3, since purified and non-purified extracts came from the same spiked samples and both were processed in parallel. Mean recovery values were closer to 100% than those found with cleaned up samples, and method reproducibility also compared favourably. The high recovery value for carbofuran in cucumbers spiked at 10 ppb (219.0%)

Table 4
Analysis by ELISA of non-purified sample extracts of cucumbers and strawberries spiked with carbaryl, carbofuran and methiocarb at 10, 50 and 200 ppb (n=6 replicates)

Matrix	Analyte	Fortification level (ppb)	ELISA		
			Mean±S.D. (ppb)	Recovery (%)	R.S.D. (%)
Cucumber	Carbaryl	10	10.2±0.4	102.0	3.9
		50	49.2±1.6	98.4	3.3
		200	215.7±13.1	107.9	6.1
		Mean		102.8	4.4
	Carbofuran	10	21.9±3.5	219.0	16.0
		50	61.6±4.8	123.2	7.8
		200	180.4±8.7	90.2	4.8
		Mean		144.1	9.5
	Methiocarb	10	9.6±1.2	96.0	12.5
		50	55.6±2.6	111.2	4.7
		200	206.2±35.3	103.1	17.1
		Mean		103.4	11.4
Strawberry	Carbaryl	10	7.2±0.4	72.0	5.6
		50	46.5±1.9	93.0	4.1
		200	215.3±7.9	107.7	3.7
		Mean		90.9	4.5
	Carbofuran	10	12.1±0.9	121.0	7.4
		50	46.9±2.6	93.8	5.5
		200	198.5±11.0	99.3	5.5
		Mean		104.7	6.1
	Methiocarb	10	9.7±0.9	97.0	9.3
		50	43.9±3.4	87.8	7.7
		200	251.9±30.9	126.0	12.3
		Mean		103.6	9.8

could be caused by insufficient dilution of this matrix, since recovery values at 50 and 200 ppb were in fact acceptable, as well as those obtained for strawberries, even at 10 ppb. This case illustrates that although matrix effects are always present to a higher or lesser extent, they may be minimized simply by properly diluting the sample in assay buffer. Overall these results do not only prove that the clean-up step can be omitted for ELISA determinations without deleterious effects, but also that the accuracy of this approach is even better than that obtained by the usual analytical procedure, while maintaining comparable precision. Moreover, this finding supports the idea that pesticide losses occur during sample clean-up and evaporation and redissolution steps.

4. Conclusions

Results in this work clearly prove that the immunoassays herein presented are able to analyze carbaryl, carbofuran and methiocarb in cucumbers and strawberries at levels of regulatory relevance with accuracy and precision comparable to those obtained with the reference method. Although the analysis of lower levels of carbaryl and methiocarb with these immunoassays seems also feasible, this

possibility was not tested because the determination of the detection limits of the methods was out of the scope of this work. Immunoassays require minimum equipment and are easy to perform even by unskilled people. From pre-coated plates, immunoassays take 2 h to be performed, including proper dilution of the samples, with the possibility of analyzing dozens of samples simultaneously. An additional issue of major importance is the ability of these ELISAs to determine pesticides in crude sample extracts, which has several practical consequences. First, the time of analysis significantly decreases, which entails a higher sample throughput. Second, the cost of the analysis is reduced, as well as organic solvent consumption; and third, the same extract used for multi-residue methods is amenable to immunoassay analysis, so these ELISAs may be easily included as complementary methods in pesticide regulatory programs.

ELISAs have very often been considered as valuable methods for screening purposes. Although this is also an important application of the immunoassays herein described, it is worthy to note that the analytical quality of the data generated by these methods, together with the high specificity displayed by these ELISAs, also allows their use for confirmation purposes.

Overall, these results should reasonably contribute to increase the acceptance of immunological methods among analytical chemists involved in pesticide residue analysis in foods. Work is in progress to extend the application of these ELISAs to further fruits and vegetables. For analysts interested in immunoassay technology, limited amounts of experimental kits for the determination of carbaryl, carbofuran and methiocarb are available on request.

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