Enzyme-Linked Immunosorbent Assay for the Quantitation of the Fungicide Tetraconazole in Fruits and Fruit Juices

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An antibody-immobilized ELISA test for the detection of the triazole tetraconazole is described. The minimum detection limit was 2 ng/mL (ppb), and the linearity of the assay extended from 1.9 to 1000 ng/mL (ppb), with a variation of absorbance (ΔOD) of ∼0.83 ± 0.04. Good reproducibility and reliability of the ELISA assay was found for fruit commodities spiked with different amounts of tetraconazole. The effect of fruit matrices on the sensibility of the ELISA was investigated, and a matrix interference index (I_m) is proposed for quantitation of tetraconazole recovery in the analyzed fruit homogenates. Analysis of fruit juices was optimized to minimize matrix interference. This study could represent an approach for validation of ELISA tests for analysis of tetraconazole in fruit commodities and it should be adaptable to other food matrices.

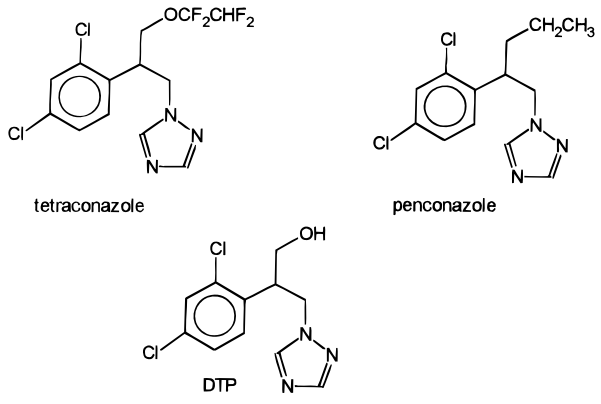
Keywords: *Immunoassay; ELISA; fungicide; tetraconazole; fruit analysis; matrix interference*

INTRODUCTION

The determination of the presence and level of pesticide residues in foods is fundamental in monitoring and regulatory programs. Residues are usually separated from foods by solvent extraction followed by a sequence of cleanup steps necessary for quantitation by instrumental analysis such as liquid or gas chromatography (Corely *et al.*, 1974; Ivie *et al.*, 1980; Monem and Mumma, 1981; Schaleppi *et al.*, 1991). To simplify the analytical procedures and to increase the number of samples processed, in the last years attention was focused on the development, testing and validation of enzyme immunoassays (EIA), which in principle can be rapid and easily performed tests. The basic theory and methodologies of EIA have been the subject of a number of reviews (Kaufman *et al.*, 1991; Hefle, 1995), and the general requirements, advantages, and disadvantages of the application of enzyme linked immunosorbent assays (ELISA) to food analysis have been discussed in detail (Hammock and Mumma, 1980; Newsome, 1986a; Schwalbe-Fehl, 1986; Harrison and Hammock, 1989; Ellis, 1989; Hall *et al.*, 1990). In the last decade, immunoassays began to be employed in the quantitative determination of drug and pesticide residues, and there is increasing interest in their applications to food analysis (Gazzaz *et al.*, 1992; Bushway and Fan, 1995).

Triazoles are a family of fungicides registered for use on several crops in the European countries and in the United States (Koller, 1992). The triazoles contain, as the fundamental structural moiety, a 1,2,4-triazole ring and very often a phenyl group bearing one or two halogens in position 2 and 4 that are connected by a short chain (one or three atoms). A third group (R) bound to this chain characterizes each single commercial compound. In contrast to their outstanding activity and extensive use, the analytical methods for triazole residue determination in foods and in the environment are mostly restricted to liquid and gas chromatography (Cabras *et al.*, 1992; Mendes, 1991; Reynaud *et al.*, 1991). Only a few ELISA's have been developed for the detection of triazoles in foodstuffs; that is, triadimefon in fruit samples (Newsome, 1986b) and myclobutanil in water, soil, and fruit samples (Székács and Hammock, 1995). In both cases the sensitivity of the assays, expressed as LOD (limit of detection), were in the range of high parts per billion values.

In a previous paper (Forlani *et al.*, 1992) we reported the development of an ELISA test for the determination of the triazole fungicides tetraconazole and penconazole that involves using a polyclonal antibody generated against the hemisuccinate of 2-(2,4-dichlorophenyl)-3-(1*H*-1,2,4-triazol-1-yl)propanol (DTP) conjugated to bovine serum albumin (BSA). DTP is the major degradation product of tetraconazole (Isagro, unpublished results), and it was well recognized in the developed test (Forlani *et al.*, 1992). Although this ELISA (Forlani *et al.*, 1992) showed a low detection limit for tetraconazole in ethanol, its application for quantitating this commercial triazole in foodstuffs was unreliable (Forlani, 1992). To develop an ELISA suitable for reliable and easy quantification of tetraconazole traces in foodstuffs with the polyclonal antibody described in Forlani *et al.* (1992), a new ELISA format was selected. In the present work, we report an investigation of the performance of this new assay, including the definition of an index of matrix interference on selected fruits and fruit juices spiked with different amounts of tetraconazole.



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EXPERIMENTAL PROCEDURES

Reagents. Horseradish peroxidase (HRP), BSA, *o*-phenylendiamine, and most chemicals were purchased from Sigma Chemical Company (St. Louis, MO). Tween 20 was from Fluka (Buchs, Switzerland), and porcine gelatin was from Bio-Rad (Hercules, CA). Tetraconazole and DTP were a kind gift of Isagro (Milano, Italy). Penconazole, hexaconazole, propiconazole, and triadimefon were supplied by Labservice (Bologna, Italy). The antiserum, raised with the hemisuccinate of DTP (FF18) conjugate to BSA as the immunogen, was the same as in Forlani *et al*. (1992) and was used as such.

Hapten−Peroxidase Conjugate. The hemisuccinate of DTP (FF18) synthesized as previously described (Forlani *et al*., 1992) was used as hapten for coupling to HRP. To a small vial containing a magnetic stir bar were added *N*-hydroxysuccinimide (NHS, 3.15 mg), 1-ethyl-3-(dimethylaminopropyl)-carbodiimide·HCl (EDAC, 5.2 mg), and FF18 (9.3 mg). After drying with nitrogen gas (20−30 min), 1 mL of dimethylformamide (DMF) was added, and the mixture was stirred at 19 °C overnight to obtain the activated ester. The HRP solution (10 mg protein in 1 mL of 0.1 M carbonate buffer, pH 9.5) was rehydrated overnight at 4 °C with gentle stirring. The ester was then added to the HRP solution in small aliquots (5 μL each). After each addition, the mixture was stirred for 30 min at 4 °C. The cloudy mixture, obtained by addition of ~20 μL of ester, after a further 60 min of reaction, was chromatographed on a small Sephadex G-25 column (1 × 10 cm) equilibrated with 0.1 M phosphate buffer, pH 7. The hapten−HRP conjugate was eluted as a brownish fraction with the same buffer. The hapten−HRP conjugate activity, measured by the method of Maehly (1954), ranged from 300 to 400 units/mL. The HRP−conjugate was stored at −20 °C in the presence of glycerol (1:1, v/v).

ELISA. Polystyrene microtiter plates (Nunc Immunoplate) coated by overnight incubation at 4 °C with 0.1 mL/well of anti BSA-FF18 serum diluted 1:1000 in sodium carbonate buffer, pH 9.6, were used through all the experiments. The plates were washed four times with phosphate-buffered saline [PBS; 15 mM KH_2PO_4 (pH 7.4), 0.15 M NaCl] containing 0.2% Tween 20 (PBS-T) and then saturated with 3% porcine gelatin in PBS (0.15 mL/well). After incubation for 90 min at 37 °C, the unbound material was removed by four washes with PBS-T.

The competition step for the standard curves was performed as follows: 0.05 mL of PBS containing 0.05% Tween 20 and 0.1% gelatin (PBS-T-G), 2 μL of a standard of the selected triazole (see next section for standard solution preparation) at concentrations ranging from 3 ng/mL to 12.5 mg/mL, and 0.05 mL of hapten−HRP conjugate (1:5000 in PBS-T-G) were sequentially added to each well. In the case of food analysis, 0.05 mL of fruit extracts (see section on sample preparation) and 0.05 mL of hapten−HRP conjugate (1:5000 in PBS-T-G) were added to each well. In both cases, after overnight incubation at 4 °C and four washes with PBS-T, the peroxidase activity was measured after the addition of 0.2 mL/well of a 0.32-mg/mL solution of *o*-phenylendiamine in 0.1 M citrate buffer, pH 5.0, containing 0.03% H_2O_2. The reaction was stopped after 10 min with 4 N H_2SO_4 (0.05 mL/well), and the absorbance at 490 nm was measured in a Bio-Rad 3550 microplate reader interfaced with a Microplate Manager Data Analysis Software. Background binding was assessed by a blank in which the coating with antibody was omitted. Standard curves were calculated from the raw data with a four-parameter (sigmoidal) equation (Rodbard, 1981).

Standard Solutions Preparation. For standard curves, stock solutions of tetraconazole, DTP, penconazole, hexaconazole, propiconazole, and triadimefon in ethanol (25 mg/mL) were diluted to 3 ng/mL in 1:2 dilution steps. For fruit sample analysis, tetraconazole standards were prepared in solutions obtained from fruit homogenized and then treated with either methanol or methanol followed by an ether/hexane (1/9) washing step. Tetraconazole concentrations ranged from 10 to 0.025 ppm.

Sample Preparation. Fruit samples were prepared as described in a paper by Newsome *et al*. (1993) with some modifications. Commercial apples, pears, peaches, and apricots (four varieties for each fruit) from a local organic market were homogenized in a Braun blender. Aliquots of 10 g of each homogenate were fortified with tetraconazole at concentrations of 10, 5, 2.5, 1.25, 0.4, 0.2, 0.1, 0.05, and 0.025 ppm, and incubated at room temperature overnight. Each sample was then stirred with 60 mL of methanol and refluxed for 30 min. Solids were removed by filtration through Whatman No. 1 paper, and each filtrate was divided in two aliquots. The first one (6 mL) was directly dried in a Speed Vac (Savant). The second one (10 mL), washed once with 10 mL of ether/hexane (1/9), was then centrifuged at 1250*g* for 5 min to discard the ether/hexane fraction. An aliquot of 6 mL of the obtained solution was dried in the Speed Vac (Savant). The dried material obtained from each procedure was dissolved in 1 mL of PBS-T-G and stored at −20 °C until analysis by ELISA.

Commercial apple, pear, peach, and apricot juices were simply diluted in PBS-T-G. To minimize matrix interference and increase the colorimetric signal, different dilutions were tested.

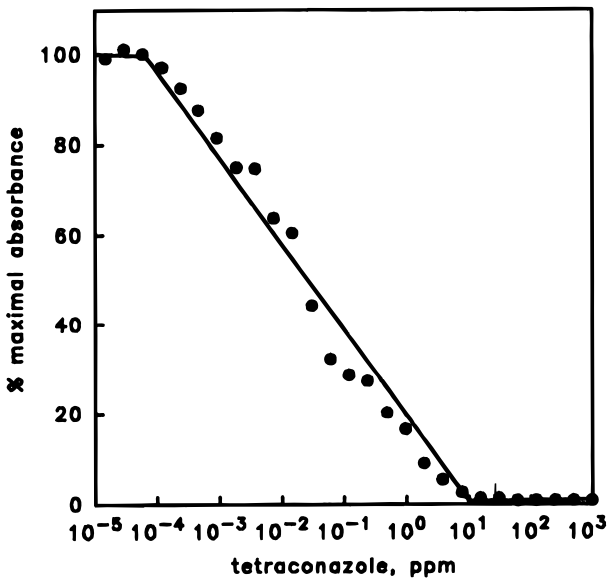

Figure 1. Typical standard curve for tetraconazole determined by the optimized ELISA. Points represent the mean values measured in eight ELISA plates over a 3-month period, in which two replicates were assessed for each tetraconazole concentration.

RESULTS

A typical standard curve obtained with tetraconazole as analyte is shown in Figure 1. Taking into account the standard error of the experimental data, the linear range of reliable detection extended from 2 to 1000 ng/mL (ppb), the ΔOD being 0.83 ± 0.04. The background was near zero, and the sensitivity (i.e., the concentration of analyte that give 50% inhibition, I_{50}) of this assay was 22 ± 2 ppb. Comparison of the results obtained on tetraconazole with those obtained by the format described in Forlani *et al*. (1992) showed that the new format enhanced the assay sensitivity (5-fold) and it can be applied in a more extended range of analyte concentration (2−1000 versus 12−850 ng/mL). In addition, the new format resulted in a significant background decrease, and the obtained curve (Figure 1) showed a useful slope for accurate quantitation of tetraconazole.

The cross-reactivity of some triazole compounds with respect of tetraconazole was also investigated, and the results are summarized in Table 1. The developed ELISA appears to be even more specific than the previous one (Forlani *et al*., 1992) for tetraconazole with

Table 1. Cross-Reactivity with Various Triazole Fungicides[a]

fungicide	I_{50}[b] (ppm)	SD	CV%	cross-reactivity[c]
tetraconazole	0.022	0.002	9	100
DTP	0.100	0.031	31	22
penconazole	0.156	0.021	14	14
hexaconazole	13.1	1.0	8	0.17
propiconazole	68.3	13.3	20	0.03
triadimefon	n.d.[d]			

[a] Values represent the mean ± SD of 10 experiments. [b] Expressed as the analyte concentration (ppm) that inhibits the assay by 50%. [c] Defined as the percentage ratio of the I_{50} values of tetraconazole and of the given triazole. [d] Not detected.

Table 2. Index of Matrix Interference (I_m) for Various Fruit Extracts[a]

extraction procedure	I_m			
	apple	pear	peach	apricot
methanol[b]	9.7 ± 0.9	12.9 ± 1.0	21.0 ± 1.3	18.3 ± 1.2
methanol plus wash[c]	7.3 ± 0.8	10.8 ± 0.7	7.3 ± 0.9	8.8 ± 0.9

[a] Values represent the mean of 24 experiments (six for each fruit varieties). [b] Analyte-free fruits extracted with methanol only. [c] Analyte-free fruits extracted with methanol and than washed with ether/hexane (1/9).

respect to other triazoles, including the parent compound DTP.

The maximum permitted residue levels in Italy for tetraconazole are 0.5 ppm in apple and pear and 0.2 ppm in peach. The high sensitivity of the new ELISA test (detection limit, 2 ppb) prompted us to explore the possibility of its application for quantitating tetraconazole in foodstuffs. Detection limits have been determined as the minimal concentrations of analyte at which the signal produced exceeds three times the background.

The application of ELISAs for routine pesticide analysis in foods requires an investigation on the effect of food matrices and of the extraction procedures (i.e., use of organic solvents). Apples, pears, peaches, and apricots of different varieties from a local organic market were homogenized, and the corresponding extracts were prepared by methanol treatment. The effect of the fruit matrix was investigated by comparing the ELISA absorbance of the unspiked fruit extracts (blank A) with that of the simple buffer (blank B). In both cases, no analyte was present, so no competition was expected, thus making the absorbances of blank A and blank B the highest for each experiment. Higher absorbance values were obtained for blank B than for blank A because the matrix produces a sort of inhibition of the reaction of coated antiserum and hapten−HRP conjugate. To take into account this phenomenon, an index of matrix interference (I_m) was calculated with the following formula:

$$[(OD_{BlankB} - OD_{BlankA})/OD_{BlankB}] \times 100 = I_m \quad (1)$$

The values of I_m calculated from eq 1 for various fruit extracts are shown in Table 2. The coefficient of

Table 3. Percentage Differences between the Added and the Calculated Amounts of Tetraconazole in Spiked Fruit Extracts[a]

tetraconazole spiked concn, ppm	tetraconazole recoveries in extracts from							
	apple		pear		peach		apricot	
	ppm[b]	% error[c]	ppm[b]	% error[c]	ppm[b]	% error[c]	ppm[b]	% error[c]
10	11.1	11.2	11.3	12.8	12.0	19.6	11.6	16.3
5	5.43	8.5	5.60	11.2	5.95	19.0	5.92	18.3
2.5	2.76	10.2	2.86	14.5	2.98	19.3	2.94	17.5
1.25	1.41	12.4	1.43	14.3	1.49	19.4	1.49	19.3
0.4	0.45	12.9	0.43	12.1	0.48	19.6	0.47	18.1
0.2	0.23	14.4	0.22	11.1	0.24	18.8	0.24	17.7
0.1	0.112	12.9	0.110	11.0	0.119	19.4	0.116	15.9
0.05	0.056	12.3	0.055	11.5	0.060	19.7	0.059	18.4
0.025	0.028	10.7	0.028	10.9	0.030	19.1	0.029	16.9
av % error		11.7 ± 1.7		12.2 ± 1.3		19.3 ± 0.3		17.6 ± 1.1

[a] Samples have been extracted with methanol only. [b] Values obtained from standards prepared in extract from fruit homogenates, data are the mean of 24 experiments (six for each four fruit varieties) with CV values, not shown, always <8−12%. [c] Values represent percentage errors between the tetraconazole concentration determined by ELISA versus tetraconazole spiked concentration.

Table 4. Percentage Differences between the Added and the Calculated Amounts of Tetraconazole in Spiked Fruit Extracts[a]

tetraconazole spiked concn, ppm	tetraconazole recoveries in extracts from							
	apple		pear		peach		apricot	
	ppm[b]	% error[c]	ppm[b]	% error[c]	ppm[b]	% error[c]	ppm[b]	% error[c]
10	11.2	11.7	10.9	9.4	10.7	7.6	11.2	10.2
5	5.38	7.5	5.45	8.9	5.40	7.9	5.47	9.5
2.5	2.70	7.9	2.78	11.2	2.70	7.4	2.78	11.0
1.25	1.39	11.3	1.38	10.0	1.35	7.9	1.38	10.4
0.4	0.45	12.7	0.43	8.3	0.43	8.1	0.44	8.8
0.2	0.22	12.2	0.22	11.3	0.21	7.3	0.22	9.3
0.1	0.111	11.2	0.111	10.8	0.106	6.6	0.111	10.6
0.05	0.055	9.7	0.055	10.2	0.054	7.6	0.056	11.4
0.025	0.027	9.3	0.027	9.8	0.027	8.6	0.027	8.3
av % error		10.4 ± 1.8		10.0 ± 1.0		7.7 ± 0.5		9.9 ± 1.0

[a] Samples were extracted with methanol and washed with ether/hexane (1/9). [b] Values obtained from standards prepared in extract from fruit homogenates; data are the mean of 24 experiments (six for each four fruit varieties) with CV values, not shown, always <9−12%. [c] Values represent percentage errors between the tetraconazole concentration determined by ELISA versus tetraconazole spiked concentration.

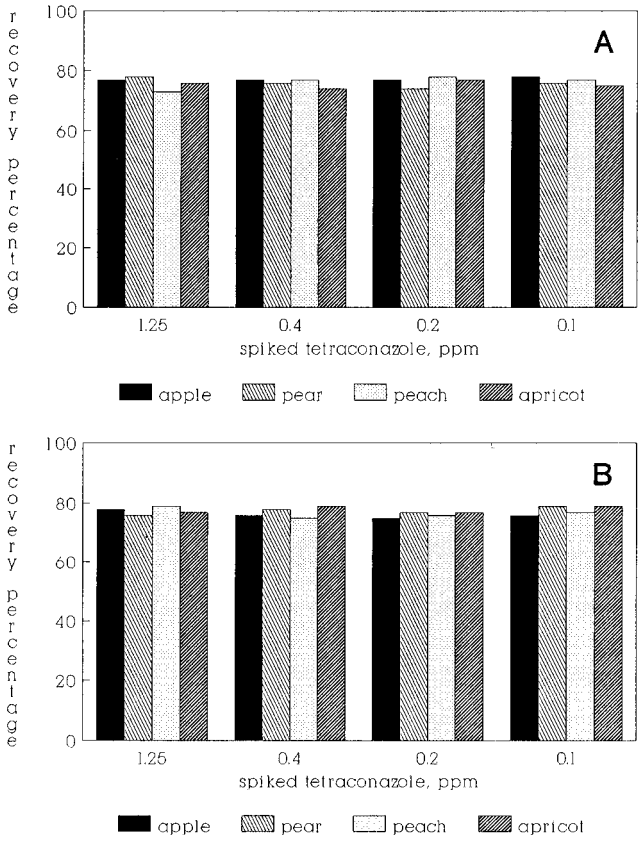

Figure 2. Recovery percentage of tetraconazole from fruit samples treated with (A) methanol only or (B) with the washing passage in ether/hexane (1/9). Recovery percentages were calculated by eq 3 and represent the mean of 24 experiments (six for each fruit variety) carried out over 4 months.

variation (CV) values were always <10%, and no significant differences in I_m values for analyte-free fruit extracts, either washed or not washed with ether/hexane, were found for apple and pear varieties. The washing procedure with ether/hexane, on the other hand, significantly lowered the I_m for peaches and apricots. To assess the reliability of the calculated I_m, the same fruit extracts were spiked with known amounts of tetraconazole. Methanol extracts obtained from apple, pear, peach, and apricot homogenates were spiked with tetraconazole concentrations ranging from 0.025 to 10 ppm. The extracts were analyzed by ELISA, and the tetraconazole amounts were calculated by the standard curve included in every run. The percentage differences between the added and calculated amounts (Table 3) appeared to be independent of the amount of tetraconazole added and had an average value comparable with the I_m values calculated from eq 1 (Table 2). The highest percentage errors as well as calculated I_m values were found for peaches and apricots.

To evaluate the possible effect of different sample preparation, samples from the same fruits were prepared by methanol extraction followed by the washing step with ether/hexane. The results obtained (Table 4) paralleled those shown in Table 3 because the percentage differences between the added and determined amounts of tetraconazole were essentially the same all over the concentration range of spiked tetraconazole. Also in these samples, as in those extracted with methanol only, a correlation between the percentage error and the calculated I_m values was found.

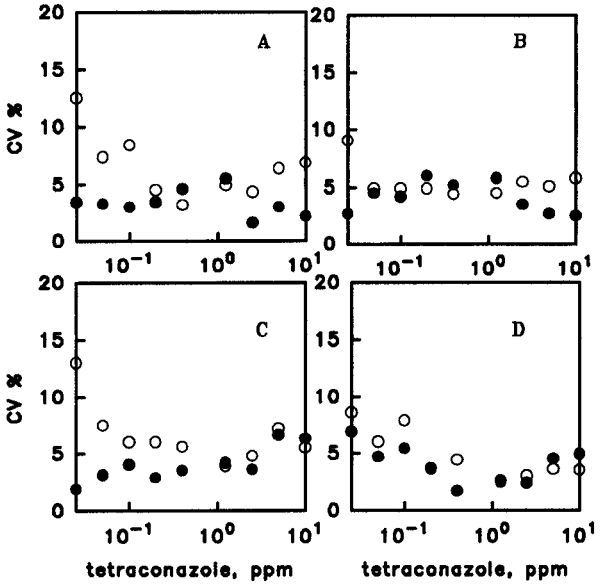

Figure 3. Coefficient of variation (CV) values of percentage recovery of tetraconazole from the analyzed fruit commoditie (A) apple; (B) pear; (C) peach; and (D) apricot homogenates, (○) with or (●) without the ether/hexane-washing step. CVs express the standard deviation as a percentage of the mean values of the percentage of recovery calculated from 24 ELISA plates in which two replicates were assessed for each tetraconazole concentration. The experiments were carried out over 4 months.

These results prompted us to exploit the calculated I_m values as a correction factor for direct quantitation of tetraconazole. Taking into account the matrix effect, the actual concentration of the analyte in the samples can be calculated with

$$C_x = C_{\text{determined}}\,[(100 - I_m)/100] \qquad (2)$$

where C_x is the actual analyte concentration and $C_{\text{determined}}$ is the analyte concentration determined by the ELISA standard curve.

In the case of slightly water-soluble pesticide residues (i.e., triazoles), extraction procedures are necessary, thus making important the evaluation of the recovery yields. The homogenates of the fruits used in this study, after spiking with tetraconazole, were treated either with methanol only or with methanol and then ether/hexane (9/1). The tetraconazole recovery, quantitated by ELISA in each fruit sample (24 replicates) was calculated with the formula

$$C_x/C_t \times 100 \qquad (3)$$

where C_t is the amount of tetraconazole added to fruit homogenates.

In Figure 2, the recovery yields found for the various fruit samples are shown without (hystogram A) or with (hystogram B) the ether/hexane treatment. The tetraconazole recovery was 75−80% of the spiked amount for both extraction procedures, and no significant difference was found among the various fruits. For sake of clarity, only four concentrations of tetraconazole are reported in Figure 2, but comparable results were obtained over the range of 0.025 to 10 ppm. No false positive was found in the control ELISA performed with samples not spiked with tetraconazole.

The reliability of ELISAs for routine analysis of tetraconazole residues in food samples depends on both percentage recovery and CV values for various assays.

Table 5. Percentage Recovery of Tetraconazole from Fruit Juice Samples[a]

tetraconazole spiked concn, ppm	tetraconazole recoveries from											
	apple			pear			peach			peach		
	%	SD	CV	%	SD	CV	%	SD	CV	%	SD	CV
10	98	3	2.5	99	1	1.1	97	2	2.2	95	2	2.0
5	95	3	2.6	97	2	2.2	94	1	1.1	97	1	0.5
2.5	96	2	2.2	95	2	1.6	98	2	2.0	97	3	0.5
1.25	98	2	1.5	97	2	2.1	100	2	2.0	95	3	2.6
0.4	96	2	2.0	96	1	1.0	97	3	2.7	99	1	1.1
0.2	95	2	2.1	96	2	1.8	98	3	2.5	100	3	3.0
0.1	98	1	0.6	98	2	2.0	95	2	1.9	96	1	0.9
0.05	100	2	2.2	100	3	3.0	97	3	3.1	98	3	2.5
0.025	98	2	2.0	98	3	2.5	96	2	2.2	99	2	2.0

[a] Data are the mean of 12 experiments.

CV values, however, are important to assess the application of the developed test for defined concentration of analyte in food samples. In Figure 3, the between-assay CV values, derived from ELISA curves obtained over a 4-month period, are plotted as a function of tetraconazole concentration for all the investigated fruit samples with or without ether/hexane treatment. The CV values were 5-7% for tetraconazole concentration ranging from 0.1 to 1.25 ppm, and they were not significantly different for the two treatments. Higher CV values were found at the lowest and highest concentrations investigated.

For the analysis of fruit juices by ELISA, no cleanup procedures are required (Itak *et al.*, 1993; Bushway *et al.*, 1989) because the matrix interference can be minimized by sample dilution. A proper dilution, however, is needed to avoid matrix interference and to handle samples useful for ELISA determination in light of the detection limit of the assay. At first, the analyses were performed by diluting the samples 100-fold with PBS, but the ΔOD of the curves was too low for reliable determinations. However, by reducing the dilution factor to 5, absorbance values and range of linearity useful for tetraconazole quantitation were obtained. In this case, the absorbance of Blank B and Blank A (eq 1) was identical, because the chosen dilution reduced matrix interference. All samples gave quantitative recovery over all the analyzed tetraconazole concentrations, and the intra- and interassay CV values were <4% (Table 5).

It is important to note that the concentration range of tetraconazole used in this study was chosen taking into account both the maximum permitted residue levels in apples (0.5 ppm), pears (0.5 ppm), and in peaches (0.2 ppm) and the range of linearity of the assay (Figure 1).

DISCUSSION

The quantitation of compounds not easily soluble in water (i.e., triazoles) requires procedures minimizing matrix interference, which can represent a possible drawback in analysis of foodstuffs by ELISA. The ELISA described here showed high sensitivity for tetraconazole, and, to simplify its application to food, we have studied a procedure to calculate the impact of matrix interference in fruit samples. To take into account the matrix interference in the analyzed foodstuff, the usual methods require either the construction of a calibration curve in the same food matrix (Rauch *et al.*, 1989) or the correction of the data with blanks made with the same matrix subjected to the entire extraction and ELISA procedure (Ferguson *et al.*, 1993; Newsome *et al.*, 1993). In the analysis of pesticide residues, these approaches are valid only if the standard matrix is completely free of any pesticide, a situation that can be difficult to obtain. Another approach was reported by Bushway *et al.* (1994) in which a proper buffer able to reproduce matrix interference was optimized. The standard solutions in this buffer made possible an internal correction without control matrices.

In the present study the "matrix interference" of fruit samples was investigated with the aim of defining a coefficient (I_m) useful to correct data after ELISA analysis. The I_m figures (Table 2) calculated for apples, pears, peaches and apricots from 24 experiments, six for each fruit variety, are comparable to the percentage error (Tables 3 and 4) in ELISA analysis in which the original fruit samples were absolutely not treated with any triazole pesticide. With this approach, the calculated I_m values can be considered a "true" matrix interference, thus allowing the determination of tetraconazole in apples, pears, peaches and apricots directly from the calibration curve in buffer using the eq 2.

Investigation of the workup procedure indicated that the ether/hexane washing step did not improve the recovery yields that are in the 75−80% range for all the analyzed samples. Also, the CV values indicated that further washing with ether/hexane is not necessary. The range of recovery is in good agreement with other reports (Usleber *et al.*, 1994; Yoshida *et al.*, 1991) in which ELISA tests were very sensitive but not actually quantitative. For fruit juices analysis the choice of a proper dilution factor minimized matrix interference and improved recoveries to quantitative yields. ELISA methods have the advantages of higher throughput and lower expense, making it possible to analyze large number of samples simultaneously, and the method can be applied in routine analysis for screening of residue levels in large numbers of fruit samples. Samples that appear to violate the maximum permitted residue levels would then be subjected to a confirmatory analysis by gas or high-performance liquid chromatography methods.

Our results represent an improvement for rapid and consistent ELISA analysis of tetraconazole residues. Although this study was restricted to fruit commodities, the method should be adaptable to other matrices for routine analysis of tetraconazole.

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