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Use of Electrochemical Biosensor and Gas Chromatography for Determination of Dichlorvos in Wheat

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Two methods for the determination of dichlorvos in durum wheat by electrochemical assay and gas chromatography, respectively, have been developed. Dichlorvos, an organophosphorus anticholinesterase pesticide, was extracted from wheat with hexane, and the filtered extract was directly analyzed by gas chromatography with nitrogen−phosphorus flame detection (NPD). Recoveries of dichlorvos from milled wheat spiked at 0.25−1.5 μg/g ranged from 96.5 to 100.9%, and the limit of detection was 0.02 μg/g. The electrochemical assay was based on the detection of choline, the acetylcholinesterase product, via a choline oxidase biosensor. An aliquot of the filtered hexane extract was partitioned with phosphate buffer solution, and the organic layer was evaporated prior to electrochemical analysis. A limit of detection of 0.05 μg/g of dichlorvos was obtained with mean recoveries of 97−103% at spiking levels of 0.25−1.5 μg/g. A good correlation ($r = 0.9919$) was found between the results obtained with the electrochemical and those obtained with the gas chromatographic methods. The electrochemical method was peer-validated in two laboratories that analyzed 10 blind samples (5 duplicates), including a blank and 4 spiked samples with dichlorvos at levels of 0.25, 0.60, 1.00, and 1.50 μg/g. Within-laboratory repeatability (RSD_r) and between-laboratory reproducibility (RSD_R) ranged from 5.5 to 7.8% and from 9.9 to 17.6%, respectively.

KEYWORDS: Bioassay; screen-printed electrodes; acetylcholinesterase; dichlorvos; wheat

INTRODUCTION

Organophosphorus compounds (OP) are substances widely used in agricultural practices as pesticides having low environmental persistence and high efficacy. These compounds act by inhibition of acetylcholinesterase (AChE) activity, resulting in accumulation of acetylcholine (ACh) at cholinergic receptor sites, thereby excessively stimulating the cholinergic receptors in both insects and mammals, including humans (*1*). This can lead to various clinical implications and high acute toxicity (*2*).

Dichlorvos (2,2-dichlorovinyl dimethyl phosphate) is one of the most widely used pesticides worldwide in the storage of agricultural products such as corn, rice, and durum wheat, finding widespread use in most European countries (*3*). European Union regulation foresees a maximum residue limit (MRL) for dichlorvos in durum wheat at 2.0 μg/g (*4*). Dichlorvos, apart from affecting the central nervous system (CNS) by inhibiting AChE, is also classified as a probable human carcinogen on the basis of the effects observed in mice and rats. Therefore, the U.S. Environmental Protection Agency (EPA) proposed cancellation of most uses of the dichlorvos pesticide and proposed restrictions on retained uses. In particular, because of dietary cancer risk, the EPA proposed canceling uses of dichlorvos in processed agricultural commodities that are stored in bulk, packages, or bags including durum wheat and its derivates such as flour and pasta (*5*).

Sensitive, rapid, and reliable determination of dichlorvos in environmental samples is therefore important for protecting the environment and human health. Gas chromatographic (GC) procedures are currently used as reference methods for the determination of dichlorvos in air, vegetables, water, soil, and wastes and in a variety of foods (*6*−*9*). Most of them are multiresidue methods and have been validated with collaborative studies on several matrices excluding wheat. The determination of dichlorvos in durum wheat by GC−nitrogen−phosphorus flame detection (NPD) has been reported by Crisp and Tarrant, who used methanol as extraction solvent and cleanup on a charcoal column (*10*).

Despite their very good sensitivity and reliability, these methods are time-consuming and above all require expensive instrumentation and have to be performed by highly trained personnel.

Biochemical sensors could be a reliable and promising alternative to conventional methods because of their simplicity, ease of use, and acceptable sensitivity and selectivity. Recently,

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many studies have focused on the development of low-cost screen-printed electrode biosensors, which are well suited for mass production and portable devices (*11*). This kind of biosensor can be applied to the determination of pesticide inhibition on AChE coupled with the electrochemical determination of the enzyme product (*12*−*22*). In recent years an important achievement in AChE biosensor development has been the use of recombinant enzymes to design more sensitive and selective biosensors, but they are not commercially available (*23*−*29*).

Matrix limitation and troubles affecting the accuracy of results have been reported for the application of AChE inhibition assays to pesticide detection; therefore, a multistep preparation of samples is required to reduce these problems (*30*, *31*).

More recently, an acetylcholinesterase inhibition assay for the detection of dichlorvos in durum wheat has been developed. The calculated limit of detection (LOD) with commercial AChE was 0.45 μg/g, and the average recovery of dichlorvos from samples spiked at 2.0 μg/g was 75%. However, the method was applicable only to whole grain kernels, leading to possible nonhomogeneous testing samples (*32*). The homogeneity of test sample before analysis is important to obtain reliable analytical results; therefore, accurate grinding of samples is crucial.

In the present paper we report a method using a choline oxidase amperometric biosensor (screen-printed electrode) as measuring device for quantitative determination of dichlorvos in samples of ground wheat with contamination levels below the European regulatory limit. The method was peer validated by two laboratories to establish the accuracy, within-laboratory repeatability, and between-laboratory reproducibility characteristics. The biosensor results were compared with those obtained with a rapid GC-NPD method that was also developed within the present study.

MATERIALS AND METHODS

Reagents and Apparatus. Hexane and acetone were purchased from J. T. Baker. AChE (EC 3.1.1.7) was obtained from electric eel (type IV V-S, 970 units/mL) and choline oxidase from *Alcaligenes* sp. (EC 1.1.3.17). All other reagents were obtained from Sigma-Aldrich (Milan, Italy) and used without further purification. Dichlorvos was purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany). All aqueous solutions were prepared using Milli-Q-treated water (Milan, Italy). A mill, model MLI-204 Buehler (Milan, Italy), was used to grind (<1 mm particle size) durum wheat samples.

Electrochemical experiments were carried out using a PalmSens hand-held potentiostat equipped with PalmSens PC software for the elaboration of current data (PalmSens, Amsterdam, The Netherlands). The cell consisted of a circular graphite working electrode, a silver pseudo-reference electrode, and a graphite counter electrode. The cell was printed on a planar polyester substrate. These screen-printed electrodes (SPE) were produced by PalmSens.

A Varian model CP3800 gas chromatograph, equipped with a Varian model 8200 autosampler and a nitrogen−phosphorus flame detector (NPD) (Varian, Turin, Italy), was used for GC analyses. The GC column was a Crossbond 5% diphenyl 95% dimethyl polysiloxane capillary column (30 m × 0.32 i.d. mm × 0.25 μm) with an RTX-5w/Integra guard column (Restek Corp., Bellefonte, PA). Chromatographic data were elaborated with the Star Chromatography Workstation software, version 5.51 (Varian).

Spiking and Calibrant Solutions. For electrochemical detection, four dichlorvos spiking solutions (12.5, 30, 50, and 75 μg/mL) for recovery experiments and five working calibrant solutions (0.5, 1.0, 2.0, 4.0, and 8.0 μg/mL) for method validation were prepared by appropriately diluting with hexane aliquots of a dichlorvos stock solution. The latter was prepared by dissolving in hexane 11.1 mg of dichlorvos in a volumetric flask that was then made up to a final volume of 10.0 mL and was kept at 4 °C. A matrix-assisted calibration curve was obtained from working calibrant solutions. In particular, 25 μL of each working calibrant solution was added to 200 μL of blank wheat extract (hexane). After the addition of 100 μL of phosphate buffer solution (PBS), hexane evaporation, and subsequent addition of AChE solution (75 μL) and ACh solution (75 μL, see below), the concentrations of dichlorvos in PBS solutions were 0.05, 0.10, 0.20, 0.40, and 0.80 μg/mL. A matrix-assisted calibration curve for GC analysis was obtained from the same stock solution by adding the relevant dichlorvos aliquots (four points) to blank wheat extract (hexane) in order to cover the range 0.05−1.00 μg/mL.

Extraction. A test portion size of 10 g of ground durum wheat sample was extracted with 20 mL of hexane in a 100 mL flask by shaking for 30 min with a model 711 VDRL orbital shaker (Asal, Milan, Italy). Subsequently, the supernatant was filtered through Whatman no. 4 filter paper (Whatman International Ltd., Maidstone, U.K.), and 10 mL of filtrate was collected and used either directly for GC analysis or after cleanup for electrochemical bioassay.

Gas Chromatographic Analysis. One microliter of sample extract (equivalent to 0.5 mg of matrix) was injected in splitless mode at 250 °C. The oven temperature was as follows: 2 min at 70 °C, raised from 70 to 170 °C at 27 °C/min, and finally 5 min at 170 °C. The carrier gas was He at a pressure of 14.6 psi. The temperature of the NPD detector was held at 300 °C. Quantification was performed by measuring the dichlorvos peak area and comparing it with the matrix-assisted calibration curve (see above).

Sample Preparation for Electrochemical Bioassay. An aliquot (200 μL) of the filtered extract was transferred to a 2 mL micro-test tube (Eppendorf), and 25 μL of hexane and 100 μL of a 50 mM phosphate buffer solution (pH 7.4), KCl 100 mmol/L (PBS), were added and then mixed by vortex for 1 min. The mixture was allowed to separate into two layers. The test tube was placed into a Pierce Reach-Therm heating module (Pierce, Rockford, IL) set at 50 °C, and the upper organic layer (hexane) was removed by evaporation. Finally, the remaining extract in PBS was mixed by vortex for 1 min and then analyzed by electrochemical bioassay.

Biosensor Preparation. The choline oxidase biosensor was constructed using disposable screen-printed electrodes as reported by Del Carlo et al. (*23*) and Ricci et al. (*34*). In particular, the working electrode was modified with Prussian Blue catalyst and with the biorecognition element (choline oxidase). Prussian Blue modification of the screen-printed electrode was made by in loco addition of a mixture of potassium ferricyanide [$K_3Fe(CN)_6$] and ferric chloride ($FeCl_3$) in 0.01 mol/L HCl. Choline oxidase was immobilized on the surface of the Prussian Blue screen-printed electrode with the cross-linking method using a mixture of glutaraldehyde, Nafion, and bovine serum albumin (*34*).

Inhibition Assay. The inhibitory effect of dichlorvos on AChE was evaluated by comparing the decrease of the current intensity produced by the reduction of the Prussian Blue electrochemical mediator.

The biochemical−electrochemical pathway used to determine the inhibition consisted of two enzymatic reactions (eqs 1 and 2) generating a chemical oxidation (eq 3) that was determined by cathodic chronoamperometry (eq 4).

$$\mathrm{ACh} + \mathrm{H_2O} \rightarrow \text{acetic acid} + \text{choline} \quad \text{(enzyme I: AChE)} \qquad (1)$$

$$\text{choline} + 2\mathrm{O_2} + \mathrm{H_2O} \rightarrow 2\mathrm{H_2O_2} + \text{betaine} \quad \text{(enzyme II: choline oxidase)} \qquad (2)$$

$$\mathrm{Fe\ (II)} + \mathrm{H_2O_2} \rightarrow \mathrm{Fe\ (III)} + 2\mathrm{OH^-} \quad \text{(chemical oxidation)} \qquad (3)$$

$$\mathrm{Fe\ (III)} + \mathrm{e^-} \rightarrow \mathrm{Fe(II)} \quad \text{(electrochemical reduction)} \qquad (4)$$

Both standard and sample extract solutions were analyzed according to the following experimental scheme: first, the current intensity of a blank sample extract was measured, and then the current intensity of either the standard or the sample extract was measured. The current intensity of the blank sample (I_0) and of the contaminated sample or standard solution (I_I) was used to calculate the percent inhibition according to the following formula:

$$I\,(\%) = 100 \times (I_0 - I_I)/I_0$$

The assay consisted in the addition of 75 μL of a AChE solution (0.42 unit/mL in PBS) to 100 μL of the sample extract in PBS solution. The incubation was allowed to proceed for 10 min, and thereafter an aliquot of 75 μL of a 1.0 mM ACh solution in PBS was added; after 2 min, 100 μL of this solution was placed on the biosensor surface and the steady-state current recorded for 2 min. The total time of the electrochemical assay was 15 min. The final concentrations of AChE and ACh in solution were 0.125 unit/mL and 0.3 mM, respectively.

The biosensor was previously polarized in PBS at −50 mV versus Ag/AgCl pseudo-reference electrode. After each measurement, the biosensor surface was rinsed with PBS. A calibration curve was obtained in the range of 0.05−0.80 μg/mL. The highest point of the calibration curve corresponded to 2.0 μg/g of dichlorvos in wheat, that is, the MRL established in the European Union. Using this experimental protocol, the amount of matrix loaded on the working electrode was 40 mg. For quantitative analysis of samples contaminated at levels close to or higher than 2.0 μg/g, a 3-fold-diluted extract was used, and quantification was performed on the basis of a new calibration curve using a 3-fold-diluted matrix extract.

Validation of the Electrochemical Biosensor Method. The electrochemical method was validated in two laboratories according to the AOAC guidelines for peer-verified method program (*33*). The two laboratories were the Institute of Sciences of Food Production ISPA-CNR (Bari, Italy) and the Department of Food Science, University of Teramo (Teramo, Italy). Aliquots of 10 g of ground blank durum wheat were weighed in conical flasks for the preparation of blank and spiked samples at four dichlorvos spiking levels. Five sets of duplicate samples were obtained by spiking with 200 μL each of hexane (blank) or dichlorvos spiking solutions to obtain contamination levels of 0.25, 0.60, 1.00, and 1.50 μg/g. The spiking solvent was removed by evaporation at room temperature for 1 h, and then each spiked sample was extracted with hexane. The preparation and analysis of blank and spiked samples (total of 20 samples in two laboratories) were performed in the two testing laboratories, which received blind spiking solutions. For further validation GC analyses were performed on a series of 10 blind samples by one of the laboratories and compared with electrochemical results.

Statistical Analysis. Origin ver. 6.0 software (OriginLab Corp., Northampton, MA) was used for statistical analysis.

RESULTS AND DISCUSSION

Recently, dichlorvos has been determined in durum wheat kernels by electrochemical assay using a choline oxidase biosensor and PBS as extraction solvent and electrochemical measuring buffer (*32*). However, such an extraction procedure (using whole wheat kernels) might lead to inadequate homogeneity of testing samples. Moreover, aqueous solvents cannot be used for extraction of ground wheat samples due to the formation of a slurry, preventing filtration of adequate amounts of extract. To apply the electrochemical method to ground wheat samples, as generally required in food analysis, a nonaqueous extraction solvent was required. The extraction of pesticides from different matrices is usually carried out with organic solvents, such as methanol, acetone, acetonitrile, hexane, ethyl acetate, or a combination of them (*6*−*10*). To select a good extraction solvent for dichlorvos, the GC-NPD technique was used to check the recovery and repeatability characteristics of the extraction procedure. When GC conventional methods for the determination of pesticides in nonfatty foods (*35*−*37*) were applied, the mean recoveries of dichlorvos were not higher than 40−45% in our laboratory. A possible explanation for these low recoveries could be that dichlorvos is lost during cleanup of the sample extract. To avoid possible loss of analyte during cleanup steps, we decided to inject the filtered extract into the GC apparatus without further purification. The use of methanol as extraction solvent (*10*) resulted in a dirty extract, whereas the mixture of acetone/hexane (Ac/Hex) 1:1 v/v gave a clean extract with a good recovery and a limit of detection (signal-to-noise 3:1) of 0.02 μg/g. In particular, using this extraction solvent, mixture recoveries of dichlorvos from durum wheat spiked at level of 0.3, 0.5, and 0.7 μg/g were 100 ± 12, 100 ± 2, and 114 ± 7%, respectively. With the aim of analyzing the Ac/Hex extracts with the electrochemical assay and considering that AChE activity is strongly influenced by the presence of the solvent used, it was necessary to remove completely the organic solvent (by evaporation) and dissolve the dichlorvos residue in PBS. However, the evaporation step resulted in low dichlorvos recovery (≤30%), probably due to partial solubilization of the dichlorvos in PBS after evaporation of the organic solvent. In fact, when the dried residue was redissolved in Ac/Hex and injected in GC, the dichlorvos recoveries were acceptable, ranging from 89 to 107%.

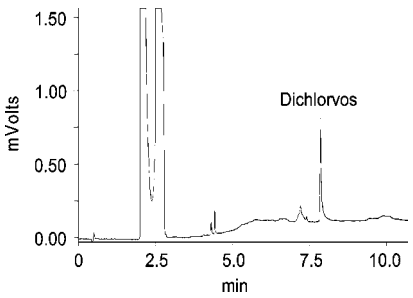

Figure 1. GC-NPD chromatogram relevant to wheat durum sample spiked with 0.6 μg/g of dichlorvos (DDPV). The retention time of the dichlorvos was 7.9 min.

Table 1. Recovery and Repeatability Data Obtained with Durum Wheat Spiked at Different Levels of Dichlorvos and Analyzed by GC-NPD Method

spiking level (μg/g), $n = 6$	GC mean recovery (%)	RSD_r (%)
blank		
0.25	97.1 ± 0.3	0.3
0.60	97.2 ± 5.8	5.9
1.00	96.5 ± 2.9	3.0
1.50	100.9 ± 0.2	0.2

To overcome this problem, a liquid−liquid partitioning of organic solvent with PBS was carried out. This procedure increased the efficiency of dichlorvos transfer from organic extraction solvent to PBS avoiding drying of the extract. Nevertheless, this procedure could not be applied with the Ac/Hex mixture because acetone passed into the PBS phase, thus influencing the AChE activity. To overcome this problem, hexane was tested as extraction solvent. Recovery efficiency and repeatability of results were checked by GC-NPD analysis of filtered sample extract from spiked wheat. **Figure 1** shows the chromatogram of durum wheat spiked with 0.6 μg/g of dichlorvos eluting with a retention time of 7.9 min. The limit of detection was 0.02 μg/g, and good recovery and repeatability results were obtained as shown in **Table 1**. In particular, recoveries ranged from 96.5 to 100.9%, showing that the extraction method with hexane was suitable for a quantitative extraction of dichlorvos from ground wheat.

The main advantage of the GC method reported herein is its rapidity coupled with an acceptable limit of detection. The method also showed good repeatability, which is comparable with that obtained with other published GC methods (*6*−*10*). These methods have been used for the analysis of air, vegetables, water, soil, wastes, and a variety of food commodities with limits of detection ranging from below picograms per gram to a few

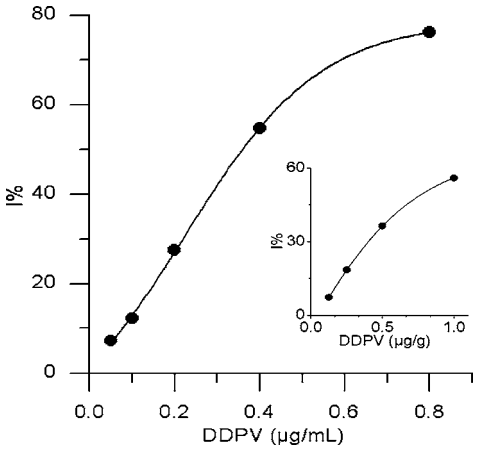

Figure 2. Dichlorvos (DDPV) calibration curve in matrix extract obtained by loading 40 mg of matrix equivalent on the working electrode. (Inset) DDPV calibration curve obtained using the blank extract after dilution (1:3). Data are reported as micrograms per gram of DDPV in wheat.

micrograms per gram depending on the matrices and cleanup procedure used. The LOD of the method developed herein is 4 times greater than the one reported by Crisp and Tarrant, who used a cleanup step with a charcoal column before GC determination of dichlorvos in wheat (*10*). However, the limit of 0.02 μg/g could be reasonably acceptable, taking into account the limit accepted by official organization, and the lack of a cleanup step allows the total time of analysis to be drastically reduced. In fact, as several extractions can be realized simultaneously, once the filtered extracts are obtained, a large number of analyses can be performed by using an autosampler.

To perform electrochemical analysis with the choline oxidase biosensor, dichlorvos needed to be transferred to PBS solution, thus avoiding any electrochemical interference by organic solvents. Therefore, the filtered hexane extract was submitted to liquid−liquid partitioning with PBS and the upper organic layer was removed by evaporation. Finally, the buffer solution containing dichlorvos was analyzed by the biosensor. Dichlorvos was easily measured in ground wheat by electrochemical bioassay at levels as low as 0.05 μg/g. Moreover, the use of hexane as extraction solvent should reduce the effect of water-soluble matrix components, potentially interfering with the assay. However, a residual matrix effect that reduced the assay sensitivity was still observed; therefore, calibration curves were prepared in matrix extracts for the accurate determination of dichlorvos.

As shown in **Figure 2** the calibration curve was almost linear in the range of 0.05−0.40 μg/mL, but at higher concentrations (between 0.40 and 0.80 μg/mL) quantification was shown to be less accurate. To overcome this problem, sample extracts producing inhibition either near or higher than the 0.40 μg/mL point of the calibration curve were appropriately diluted with hexane and analyzed again in order to produce inhibition that fell within the linear part of the calibration curve. For the quantification of dichlorvos in these samples it was necessary to prepare a new calibration curve with diluted blank extract in order to load the same amount of matrix on the working electrode.

The matrix-assisted calibration curve of dichlorvos was described by the following equation reported, which fits the inhibition data:

$$y = 78.63 - \frac{95.71}{\{1 + e[1 + (x + 0.22)/0.16]\}} \quad (R^2 = 0.9997)$$

The detection limit of 0.02 μg/mL (equivalent to 0.05 μg/g in wheat) was calculated using the I value ($I = 5.7\%$) obtained from the formula $I\ (\%) = I_0 - (I_0 - 3 \times \mathrm{SDI}_0)/I_0$ in the calibration curve equation, where SDI_0 is the standard deviation of three measurements of a blank sample. The calculated I_{50} was 0.360 μg/mL (equivalent to 0.90 μg/g in wheat). The inset in **Figure 2** shows the calibration curve of dichlorvos in the working range 0.05−0.40 μg/mL (equivalent to 0.125−1.000 μg/g in matrix) obtained using the blank extract after 1:3 dilution. The equation describing this curve is

$$y = 65.85 - \frac{170.65}{\{1 + e[1 + (x + 0.06)/0.16]\}} \quad (R^2 = 0.9999)$$

These equations were used for the calculation of dichlorvos in wheat sample extracts, where y is I (%) and x is the dichlorvos concentration (μg/mL). The amounts of matrix equivalent loaded on the working electrode to record the first and second calibration curves were 40 and 13.3 mg, respectively. The increase of matrix amount led to a different inhibition, showing a residual matrix effect. In particular, the inhibitions produced by 0.20 μg/mL (equivalent to 0.5 μg/g in matrix) dichlorvos were 34.9 and 27.5% with 13.3 and 40 mg of matrix, respectively.

The method was peer-validated by two laboratories, and the results of the validation test are reported in **Table 2**.

Table 2. Results of the Validation Test Performed by Two Laboratories for Determination of Dichlorvos in Ground Durum Wheat by Electrochemical Bioassay

	spiked level				
	blank	0.25 μg/g	0.60 μg/g	1.00 μg/g	1.50 μg/g
lab 1, rep 1	nd[a]	0.27	0.54	1.03[b]	1.71[b]
lab 1, rep 2	nd	0.24	0.48	1.18[b]	1.64[b]
lab 2, rep 1	nd	0.29	0.67	0.99[b]	1.38[b]
lab 2, rep 2	nd	0.29	0.63	0.93[b]	1.48[b]
mean recovery (%)	–[c]	108	97	103	103
S_r	–	0.015	0.036	0.081	0.061
RSD_r (%)	–	5.5	6.2	7.8	3.9
S_R	–	0.027	0.102	0.117	0.179
RSD_R (%)	–	9.9	17.6	11.4	11.5

[a] Not detected, LOD = 0.05 μg/g. [b] Data calculated with calibration curve obtained in diluted (1:3) blank extract. [c] Not applicable.

As shown in **Table 2** mean recoveries of dichlorvos ranged from 97 to 108%, RSD_r values ranged from 5.5 to 7.8%, and RSD_R values ranged from 9.9 to 17.6%. The dichlorvos mean recovery was calculated for each spiking level as the mean of four measurements, $n = 4$ (see **Table 2**). No false negative or false positive results were obtained by the electrochemical assay. A good correlation between dichlorvos concentrations obtained by electrochemical biosensor and GC analysis was also found as shown in **Figure 3**. The correlation coefficient (r) was 0.9919, and data were fitted to the linear regression by the equation

$$c^{\mathrm{GC}} = -0.0431 + 1.1318c^{\mathrm{biosensor}}$$

Electrochemical biosensors have been previously used for the determination of organophosphorus pesticides in standard solutions and real samples such as fruits, vegetables, foods of animal origin, and cereals such as wheat and rice (*12*−*29*). However, application to real food samples for the detection of dichlorvos is lacking. In particular, detection of dichlorvos by amperometric sensors was reported with limits of detection of 1.6×10^{-6} mol/L (*15*) and 4.5×10^{-9} mol/L (*16*). Screen-printed electrodes modified with cobalt phthalocyanine were also reported to have detection limits of 10^{-8} mol/L (*17*) and 6×10^{-9} mol/L (*18*). A higher limit of detection for dichlorvos (6×10^{-6} mol/L) was reported using a dual amperometric−potentiometric bio-

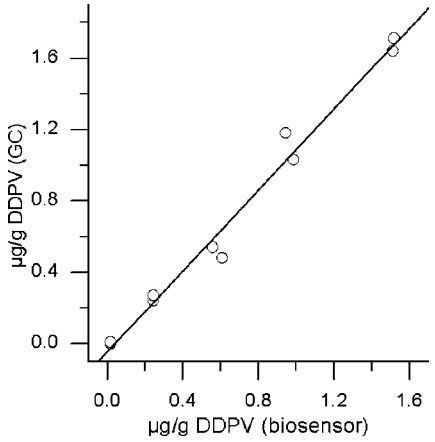

Figure 3. Comparison of dichlorvos (DDPV) contents in spiked durum wheat samples analyzed by GC and electrochemical biosensor.

sensor (*19*). Determinations of dichlorvos were also performed in buffer/ethanol solution, showing a detection limit of 0.5×10^{-6} mol/L (*20*). Biosensors prepared by immobilization of genetically modified AChE with a photosensitive polymer or by using metal chelate affinity gave limits of detection of 2.5×10^{-9} and 1.5×10^{-9} mol/L, respectively (*27*). Recently, the use of biosensors based on recombinant AChE led to exceptionally low dichlorvos limits of detection (10^{-17} mol/L) (*28*, *29*). Nevertheless, all of these results were obtained with solutions of dichlorvos, and no application to real samples has been reported. In particular, no data were reported on the recovery, repeatability, and reproducibility tests performed on food commodities, such as wheat, or on the matrix effect on these biosensors. The use of recombinant AChEs is a promising alternative due to their very low limit of detection but is limited because they are not commercially available.

The only electrochemical acetylcholinesterase inhibition assay for the detection of dichlorvos in food has been only recently developed, and the method, as already mentioned, was applicable only to whole grain kernels with an LOD of 0.45 μg/g (*32*). The method reported herein is based on the same electrochemical end determination; however, the different extraction procedure and sample preparation allowed a much lower LOD to be obtained, making the procedure applicable to ground wheat. This method allows determination of dichlorvos in ground wheat at levels as low as 0.05 μg/g (and up to 1.0 μg/g), which is 40 times lower than the European legal limit. The sensitivity of method might satisfactorily respond to the EPA requirement to eliminate dichlorvos from agricultural food commodities. Accurate determination of dichlorvos at levels >1.0 μg/g can also be performed by analyzing appropriately diluted sample extracts. The limit of detection obtained with the proposed procedure is 0.05 μg/g in durum wheat, corresponding to 9×10^{-8} mol/L in the final matrix extract solution. This limit is comparable with those reported above for commercial AChE. Recovery, RSD_r, and RSD_R values demonstrated that the electrochemical method proposed herein is robust and can be easily applicable to real samples of wheat. Finally, the electrochemical biosensor allows rapid measurements, is easy to use, and above all is inexpensive.

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