Talanta 117 (2013) 250–257

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Comparative evaluation of ELISA kit and HPLC DAD for the determination of chlorpyrifos ethyl residues in water and sediments

P.O. Otieno [a,b,*], P.O. Owuor [a], J.O. Lalah [d], G. Pfister [b], K.-W. Schramm [b,c]

[a] Department of Chemistry, Maseno University, P.O. Box 333, 40105 Maseno, Kenya
[b] Helmholtz Center Munich – German Research Center for Environmental Health (GmbH) Molecular EXposomics (MEX), Ingolstädter Landstreet 1, D-85764 Neuherberg, Germany
[c] TUM, Wissenschaftszentrum Weihenstephan fuer Ernaehrung und Landnutzung, Department fuer Biowissenschaften, Weihenstephaner Steig 23, 85350 Freising, Germany
[d] Department of Chemical Sciences and Technology, Technical University of Kenya, P.O. Box 42528, 00200 City Square, Nairobi, Kenya

ARTICLE INFO

Article history:
Received 19 June 2013
Received in revised form
9 September 2013
Accepted 10 September 2013
Available online 18 September 2013

Keywords:
Chlorpyrifos ethyl
ELISA
HPLC
Sediment
Validation
Water quality

ABSTRACT

Enzyme linked immunosorbent assay (ELISA) kit is a versatile, cheap and relatively available tool that can be used in remote areas. In this study, performance of ELISA kit was evaluated in terms of accuracy, recovery, precision, sensitivity, cross reactivity and matrix interference for pesticide residue determination in water and sediment samples. This method was compared with high performance liquid chromatography (HPLC) which is not a commonly available analytical technique for chlorpyrifos ethyl residue analysis in developing countries. The ELISA kit had limits of detection (LOD) of 0.37 $\mu g\ L^{-1}$ and 0.42 $\mu g\ Kg^{-1}$ dry weight (dw), for chlorpyrifos ethyl in water and sediment samples, respectively using deionized water and a control sediment sample. Mean percentage recoveries and coefficients of variation (CV) for ELISA kit varied from $96.0 \pm 5.8\%$ to $108.0 \pm 3.4\%$ for water and sediment samples. Comparison between ELISA and HPLC analysis results using water and sediment samples from Lake Naivasha showed no significant difference in results ($p \leq 0.05$). Strong correlations ($r_2 = 0.9878$ water samples and $r_1 = 0.9670$, $p < 0.0001$ for sediment samples, $n = 48$) were reported between the methods for the two samples analyzed. Bland–Altman bias plot analysis showed that the two methods were in agreement within 95% confidence interval of limits -2.9 to 3.8 and -2.2 to 3.6 for water and sediment, respectively. Given the high sensitivity reported and the obtained acceptable limits of coefficient of variation and percentage recovery, ELISA appears to be a suitable rapid analytical tool in analysis of chlorpyrifos ethyl in water and sediment samples. Results demonstrate comparability to HPLC and could complement conventional tools in regular monitoring program particularly in developing countries. This will hasten results delivery for ecological risk assessment and timely execution of mitigation measures.

1. Introduction

Pesticides are useful in agricultural production and their application increases the agricultural outputs since they protect crops from pests' infestations. Despite their importance in agriculture, repeated and indiscriminate use of some pesticides present considerable risk to non-target populations especially wildlife and aquatic organisms [1]. Pesticides residues in surface water above certain limits can impair the health of aquatic organisms and humans [2]. One of the factors that influence widespread environmental contamination by pesticide is global climate change. Regions experiencing increased precipitation have lower levels of air pollution but experience enhanced deposition of airborne persistent organic pollutants and increased pesticides run-off [1]. Rapid monitoring and evaluation of aquatic quality is, therefore, necessary in the wake of storms, floods and ecological changes for the purpose of gathering data for environmental risk assessment. Development and use of immunochemical methods for detection and quantification of pesticide residues through interaction of target analyte and specific antibodies has been a notable achievement in rapid monitoring chemical contaminants in the environment [3]. As an immunochemical technique, it offers a simple, rapid and cost effective determination of pesticide residues fruits and environmental samples [4]. The kits are also portable and can be used to analyze numerous samples simultaneously compared to conventional chromatographic techniques [5].

High acute toxicity and persistence of chlorpyrifos ethyl in sediments makes it a potential risk to human and aquatic organisms. Recent studies have reported that chlorpyrifos ethyl residues in food pose a significant risk to infants, children and expectant mothers and thus the need for continuous monitoring in aquatic ecosystem [4,6]. Chlorpyrifos ethyl (O,O-diethyl O-3,5,6-trichloro-2-pyridyl phosphate) is among the organophosphates that are commonly used in the small and large scale floricultural and

* Corresponding author at: Department of Chemistry, Maseno University, P.O. Box 333, 40105 Maseno, Kenya. Tel.: +254 0725 896858.
E-mail address: tudor20082000@yahoo.com (P.O. Otieno).

0039-9140/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.talanta.2013.09.014

horticultural farms in the catchment of Lake Naivasha [7]. It is possible that the pesticide residues from the agricultural fields may be draining into the lake and therefore, regular monitoring is advised.

Though contamination of surface waters has significantly decreased in developed countries, developing countries continue to experience elevated levels of contamination due to pesticide overuse [8] and probably lack of regular monitoring programs for timely intervention. Increased pollution of surface waters in many developing countries may also be due to difficulty in carrying out chemical analysis which is often caused by inadequate facilities, impure reagents, and financial constraints [6]. This probably explains why despite heavy application of acutely toxic and moderately persistent pesticides especially in the catchment of Lake Naivasha, there is no regular monitoring of pesticides. In California, for example, the Department of Pesticide Regulation has regular surface water monitoring program that monitors and protects surface waters from contamination and provide timely mitigation measures to pesticide pollution [9]. However, such an initiative is lacking in Kenya and possibly in other poor countries.

Determination of the occurrence and levels of pesticide in surface waters is critical in monitoring and regulatory programs. Although techniques such as gas chromatography (GC), liquid chromatography (LC) and HPLC hyphenated to mass spectrometry (MS) are recommended in the determination of a large number of residues and their metabolites in a single run [10], the methods are time consuming, expensive and usually require laborious clean-up procedures prior to analysis. Development and use of ELISA tests kit may be a suitable monitoring strategy tool in assessing the risk of exposure of human and aquatic ecosystem to pesticide residue contamination. However, there are still skeptism about the use of ELISA in pesticide residue analysis. New ELISA kit has been developed by Glory Biosciences of Shanghai, China and it is not known if its performance is satisfactory and comparable to the HPLC DAD, a conventional instrument routinely used in pesticide residue determination especially in sediment and water. The objective of this study was to evaluate the performance of ELISA kit and assess its comparability to micro-HPLC DAD in the determination of chlorpyrifos ethyl in water and sediment samples.

2. Materials and methods

2.1. Study design

The performance of ELISA kit was evaluated in terms of accuracy, sensitivity, precision and matrix effect. Cross reactivity of structurally similar analogues was also determined and percent cross reactivity evaluated. Water and sediment samples from Lake Naivasha were used to validate the kit by statistically comparing results obtained from the kit and HLPC DAD.

Samples for evaluation of ELISA kit and comparability studies were collected from Lake Naivasha. The lake is located on the floor of Eastern Rift Valley and lies between 0°42′–0°50′S and 36°16′–36°26′E at an altitude of 1890 m above the sea level. The lake catchment area of about 4200 km^2 is dominantly used for intensive farming requiring intensive use of pesticides. The topography of the catchment area predisposes the lake to incoming pesticide residues and other organic contaminants from agricultural activities in the region through the run-off that follows periods of heavy precipitation.

2.2. Sampling

Surface sediments (5 cm in depth) and water samples collected from 12 different sampling sites in Lake Naivasha using targeted-systematic sampling design. Three replicates of water and sediment samples were collected from sampling sites during the rainy (July 2010) and dry season (December 2010). Sediment samples (200 g) were collected from 5 cm below the lake bed. The sediments were sieved to remove stones and other material. They were homogenized and transferred into a pre-cleaned high density polyethylene plastic before wrapped with aluminum foil. Water samples were transferred into acetone-rinsed bottles. To avoid cross contamination of the samples the stainless steel spatula used was cleaned using ethanol followed by site water before re-use. Pesticide free water and sediment were collected, from the upper part of River Malewa as controls. This section of the river was chosen because there was no farming activity in the nearby area. All the samples were split for ELISA and HPLC analysis, kept in a cool box packed with ice and transported to the Department of Molecular Exposomics, at Helmholtz Zentrum Munchen, Germany for analysis.

2.3. Chemicals

Chlorpyrifos ethyl standard was purchased from Dr. Ehrenstorfer, Germany and acetonitrile super gradient HPLC grade was obtained from Alfa Aesar, Karlsruhe, Germany. Analytical grade methanol, n-hexane, ethyl acetate, dichloromethane and anhydrous sodium sulfate were supplied by Promochem (Wessel, Germany). Bond Elut™ solid phase extraction disposable cartridge columns were supplied by Agilent Technologies while chlorpyrifos ethyl ELISA kits was purchased from Glory Biosciences, Shanghai, China.

2.4. ELISA analysis

2.4.1. Preparation for performance evaluation

Evaluation of ELISA kit for accuracy, precision, sensitivity assays was done by spiking pesticide-free sediment samples and deionized water with chlorpyrifos ethyl standard made from a stock solution (100 $\mu g\ L^{-1}$). Resultant concentrations of 0.5, 0.8, 1.0 and 2.0 $\mu g\ L^{-1}$ were run in four replicates. In carrying out inter-assays, the analysis was repeated on four different days and percentage coefficient of variation determined while intra precision assays were carried out in a day. The lowest spiked concentration of 0.5 $\mu g\ L^{-1}$ was used to determine method detection limit (MDL) which was computed by multiplying the appropriate one tailed 99% *t*-statistic by the standard deviation of minimum of seven replicates of spiked control sample matrix at concentration 1–5 times the estimated MDL [11]. The accuracy was evaluated by calculating the percent recovery from the precision assays. Calibration standard curve on a semi-log scale was then drawn by preparing and analyzing standards of concentrations 0.2, 0.4, 0.5, 0.8, 1.0, 2.0, 4.0, 5.0, 10, and 20 $\mu g\ L^{-1}$ in duplicate.

Six replicates of negative control samples (blank) were run in order to determine the limit of detection (LOD). Potential matrix effect of the kit was determined by comparing the results obtained by running deionized water and fortified samples at 5 ng g^{-1}. The results were used to generate matrix interference index. The correction factor used to normalize the concentrations was then determined. Performance evaluation was done as described by Sullivan and Goh [9] and Sullivan et al. [12].

2.4.2. Cross reactivity studies

Specificity assays were performed to determine the potential of structurally related compounds and metabolites that could compete for the antibody binding sites [12]. Standard solutions of chlorpyrifos-ethyl (1.00 mg L^{-1}), chlorpyrifos-methyl (1.01 mg L^{-1}) diazinon (1.04 mg L^{-1}), pirimiphos-methyl (0.95 mg L^{-1}), bromiphos-methyl (0.99 mg L^{-1}), chlorpyrifos-oxon (1.02 mg L^{-1}), 3,5,6-trichloro-2-pyridinol (1.04 mg L^{-1}), diethylthiophosphate (1.05 mg L^{-1}), diethylphosphate (1.02 mg L^{-1}), and diclofethion (0.89 mg L^{-1}) were used to prepare spikes for selectivity assays. Fortified

samples were made from 100 mg L^{-1} working stock solutions prepared in deionized water. Working solutions of concentration running to eight orders of magnitude were serially prepared and run in triplicate. Concentrations of 0.03, 0.15, 0.75, 3.75, 18.75, 100, 500, and 2000 μg L^{-1} were used to obtain standard curve. Assays were done as earlier described and percent cross reactivities (%CR) were determined by using the formula below [9].

$$\%\ CR = (IC_{50}\text{Chlorpyrifos ethyl}/IC_{50}\text{structural analogue}) \times 100$$

where IC_{50} is the amount of substance that displaces 50% of the labeled antigen (kit enzyme conjugate) compared to the IC_{50} of the structural analogue (unlabeled antigen). IC_{50} values for each cross reactant were generated from four-parameters logistic fit of experimentally determined absorbance vs spike concentration data (GraphPad Prism 6 Demo) according to Sullivan et al. [12].

2.4.3. Preparation of water samples for ELISA analysis

Water samples were removed from the refrigerator and allowed to attain room temperature. Afterwards, 10 mL of water sample was first filtered using 0.2 μm Whatman glass microfiber filters to remove particulates that could give false positive results. The filtered water sample (2 mL) was transferred into a 5 mL test tube and 2 mL of methanol added to make 50% aqueous methanol solution. Using Eppendorf pipettes, 100 μL of negative controls, calibrators and 100 μL of samples to be analyzed were placed in the wells in the microtitre plate. Chlorpyrifos–horseradish peroxidase (HRP-labeled enzyme conjugate) (100 μL) and chlorpyrifos ethyl antibody (200 μL) were deposited into the wells and vortexed for 3 min then allowed to incubate for 15 min at room temperature. The contents of the wells were decanted to dispose off unbound antibodies and antigens. Washing solution was added to totally remove the unbound molecules then 250 μL chromogen (3,3′,5,5′-tetramethylbenzidine) was added to the wells in order to form color. Finally 250 μL of sulfuric acid (0.5%) was added to stop the reaction before the results were read on Tecan Microtiter plate reader spectroflour at 415 nm. Since the HRP-labeled chlorpyrifos ethyl analogue is in competition with unlabeled chlorpyrifos ethyl in the samples for antibody sites, the color development is inversely proportional to the concentration of chlorpyrifos ethyl in the samples. The extraction was done according to the instruction of the manufacturer and method used by Sullivan et al. [12] and Rubio et al. [13].

2.4.4. Extraction of sediment samples for ELISA analysis

Sediment samples were air-dried in the laboratory to a constant weight. The samples were ground to a free flowing powder using a pestle and a mortar to achieve homogeneity. Ten grams of the sample were placed in a vial and extracted by adding 20 mL of methanol. The vial was vortexed for 30 s and placed in a rotary shaker (250 oscillations min^{-1}) for 30 min. Afterwards, the vial was centrifuged for 10 min at 2600 rpm. Supernatant (1 mL) was placed in another vial followed by the addition of 1 mL of deionized water to make 50% methanol solution. The sample was diluted 10 times (0.1 mL added to 0.9 mL of 50% methanol) before the assay to reduce the expected concentration to the kit's limit and reduce the sample matrix interference. In performing recovery assays, blank sediment samples were fortified with chlorpyrifos ethyl standard to yield a final concentration of between 0.5 and 2.0 μg Kg^{-1}. The samples were then air dried and ground in a mortar and with a pestle before undergoing the same extraction procedure and analysis. The results were multiplied by the dilution factor to determine the actual concentrations.

2.4.5. Extraction of water samples for HPLC analysis

For HPLC analysis, the pH of 100 mL water samples was adjusted to 5 with the addition of 0.6 mL acetic acid. In addition, 50 μL of 2 mg L^{-1} terbuthylazine, was added for extraction of chlorpyrifos ethyl. Samples were degassed using ultrasonicator for 5 min before solid-phase extraction (SPE). SPE cartridge (Bond-Elut™ PPL 200 mg Varian, Agilent Technologies, USA) was fixed with 25 mm Acrodisc® glass fiber prefilter (1 μm glass fiber membrane) and mounted on Supelco Visiprep 12-place vacuum manifold. Afterwards, the cartridge was conditioned by 2 ml ethyl acetate, 2 ml of methanol and finally by 0.6 mL acidified deionized water in that sequence. The cartridge was equilibrated by 1.5 mL acidified water before loading samples to the column at a flow rate of 20 mL min^{-1} using a vacuum pump. Thereafter, the column was post-washed by 1 mL solvent mixture of deionized water and methanol (1:1) and then dried under vacuum for 15 min. The analyte was eluted with 1.5 mL ethyl acetate, twice at a flow rate of 2 mL min^{-1} followed by evaporation in a stream of nitrogen gas at 40 °C to near dryness before re-constituted under vortex in 1 mL acetonitrile–water mixture (8:2). The extract was filtered using 0.2 μm acrodisc syringe filter before analysis by micro-HPLC DAD. For recoveries assays, 100 mL control water samples were fortified with standards at concentration range of 0.5–2 μg L^{-1} with the addition of internal standards. Extraction was performed according to method used by Schramm et al. [14].

2.4.6. Extraction of sediment samples for HPLC analysis

Extraction and analysis of sediment samples was done as described by Konda and Pásztor, [15,11]. The sediments were air-dried in the laboratory to constant weight then homogenized. Sediment sample (10 g) was placed in Erlenmeyer flask and 50 μL of 2000 ng mL^{-1} terbuthylazine internal standard was added. Afterwards, 50 mL of a mixture of dichloromethane/n-hexane, (7:3 v/v) was added before sealing the flask and placing it horizontally on an Orbital shaker (280 oscillations min^{-1}) for 1 h. The sediment was extracted twice by shaking for 15 min with 50 mL of a mixture of dichloromethane and n-hexane; (4:1 v/v). The mixture was filtered through PTFE filter and anhydrous sodium sulfate in a Buchner funnel mounted on a round bottomed flask using a vacuum filter grip. The three extracts were pooled in a round bottom flask and reduced to near dryness using rotary evaporator. The residue was re-dissolved in 5 mL acetonitrile in readiness for clean up. In carrying out recovery experiment, 10 g of air dried soil sample was weighed and fortified with standards to give resulting concentrations of 0.5–2 μg Kg^{-1}. Fortified sediment samples were homogenized and air dried to constant mass before extracted through the same procedure.

Clean up was done using Strata™ SPE cartridge obtained from phenomenex, USA. The cartridge was conditioned by first adding 5 mL of methanol followed by 5 mL of deionised water before loading the extract onto it. After extract had run out, the cartridge was washed using methanol/water mixture in the ratio of 3:7 before drying under vacuum for 10 min. The analyte was eluted by 3 mL of ethyl acetate and the extract eluted by evaporation to near dryness in a gentle stream of nitrogen gas. The residue was re-dissolved in 1 mL of acetonitrile/water (8:2) then filtered using acrodisc 0.2 μm filters before micro-HPLC-DAD analysis.

2.5. Conditions of micro-HPLC

Analysis was done using Water® CapLC™ system (Waters Milipore, MA) equipped with an autosampler, and UV photodiode-array detector, linked to PC running Mass Lynx 4.0 software. The Atlantis D C_{18} (3 μm particle size) analytical column was used with dimensions (150 mm × 0.3 mm I.D) and fitted with a guard column

(2.1 mm × 1.8 μm I.D) at the temperature of 30 °C. The injection volume was 2 μL and eluted at a constant flow rate of 4 μL min^{-1} with as helium carrier gas. The analytes were eluted with a gradient solvent system of acetonitrile:water (2:8 v/v) (A) and acetonitrile (B) with initial composition of 0% B, increasing to 20% at 1 min, 100% at 8 min then to 0% at 16 min. Qualitative determination was done at 230 nm and the analyte was identified by comparing its retention time with the spectra of the standard solutions. The quantification was done by use of the response of internal standard. The limit of detection was determined to be 0.10 μg L^{-1} and 0.11 μg Kg^{-1} for water and sediment samples, respectively.

2.6. Statistical analysis

Statistical analysis was performed using Medcalc and SAS statistical software (Duncun's Multiple Range Test) which was used to determine ANOVA and summary statistics (mean standard deviation and coefficient of variation). Linear regression analysis was used to determine the correlation between the two analytical techniques. Bland Altman Bias plot was used to determine the 95% confidence limits of agreement and the bias between ELISA kit and micro-HPLC results.

3. Results and discussion

3.1. Performance evaluation of ELISA kit

3.1.1. Standard curves and sensitivity

The analytical method validation of ELISA kit was carried out to determine its performance before its application as an analytical method in field samples. A standard linear curve for the chlorpyrifos-ethyl was drawn on a semi-log scale and results showed a significant linear correlation with a regression coefficient of $r=0.993$ ($r^2=0.986$) at $p<0.05$) (Online Resource 1). LOD is computed based on a formula of 85% B/Bo where B/Bo is the mean absorbance of a given sample (B) divided by mean absorbance of the negative control (Bo) [12]. However, in this study the LOD was experimentally determined as three times the standard deviation of six replicates negative control. The results were converted to concentration units by substituting them in the calibration curve equation.

The LOD was 0.37 μg L^{-1} and 0.42 μg Kg^{-1} dw for water and sediment samples, respectively compared to the manufacturer's LOD of 0.5 μg L^{-1}. The LOD, which is the amount of the analyte that can achieve 85% B/Bo inhibition, was validated by a line drawn from the 85% B/Bo mark on the standard curve on a semilog scale. The study demonstrated that the kit had a higher sensitivity than was reported by the manufacturer (0.5 μg L^{-1}) as confirmed by the obtained strong correlation coefficient of $r=0.993$. In water analysis, the ELISA kit's LOD was, however, higher (0.37 μg L^{-1}) than the one observed (0.11 μg L^{-1}) in HPLC DAD analysis. Although ELISA kit is widely reported as sensitive with fairly lower LOD compared to conventional instruments [12,16], the contrary results were obtained.

In the evaluation of ELISA and GC–MS in pesticides residue analysis in North California, GC–MS a conventional instrument was more sensitive than ELISA [17]. The result of this earlier study is comparing to the present finding, where HPLC was more sensitive than ELISA kit. Since ELISA kit is generally considered as more sensitive than conventional instruments [9], this finding could be a due to a technical error or simply inherent low sensitivity of the kit compared to HPLC. Despite the relatively lower sensitivity compared to HPLC DAD, it is possible to use the kit in screening and quantitative determination of pesticide residue especially in developing countries where use of conventional instrument is expensive and in some instances lacking due to limited resources.

3.1.2. Intra and inter assay reproducibility

The mean coefficient of variation for intra assays and inter assay for the control water ranged between 2.4–15.9% and 8.4–15.0%, respectively (Online Resource 2). Similarly, the coefficient of variations for intra and inter-assay for sediment samples were between 6.3–11.8% and 5.3–14.0%, respectively (Online Resource 2). Although the coefficient of variation for inter assay was above 10%, it was still within acceptable limit. The higher value reported could be attributed to variation in volumes of the reagents added. In general, the observed coefficients of variation within the day (intra-assay) and between the days (inter-assays) were below 20%, a limit recommended by EPA [18]. This result confirms the appropriateness of ELISA kit in pesticide residue analysis. The inter and intra assay variations were within the recommended limits and the observed variability could be due to lack of uniformity in antibody coating material and the wells within the microtiter plates [12]. Presented CV (%) ranges were comparable to a range of 5.1–15.2% obtained in an evaluation of commercial ELISA kit for diazinon [9].

The lowest spiked concentration of 0.5 μg L^{-1} and 0.5 μg Kg^{-1} dw for water and sediment sample, respectively were used to calculate the minimum detection limit (MDL). Using this method the MDL was 0.24 μg L^{-1} and 0.24 μg Kg^{-1} dw for water and sediment samples, respectively. The values are satisfactory and comparable to experimentally determined LODs of 0.37 μg L^{-1} for water and 0.42 μg Kg^{-1} dw sediment, respectively. In terms of reproducibility, the result demonstrates that ELISA kit can be used as an analytical tool in pesticide residue analysis.

3.1.3. Determination of accuracy

The accuracy of the ELISA kit was determined by computing the mean percent recoveries for spiked deionised water and control water sample obtained from the upper section of River Malewa (Online Resource 2). Mean percent recovery for spiked control sediment samples conducted at four different concentration levels is similarly presented in the same tables in the Online Resource 2. The mean recoveries for the spiked samples in field control and deionized water were 96.0 ± 4.2% and 101.1 ± 7.3% (Online Resource 2), respectively. Although the calculated mean percent recoveries for deionized and river water samples were within recommended limits, it was evident that river water had higher mean percent recovery compared to deionized water, which demonstrated possible presence of matrix interference in the latter sample. For sediment samples, the mean percent recovery was 108.0 ± 3.4% and the average coefficient of variation for the recovery assays ranged between 3.5% and 13.7% (Online Resource 2). The values obtained in this study are comparable (90.6–108.0%) to those obtained by Deng et al. [19] and Sullivan et al. [12]. The mean percentage recoveries and coefficient of variation obtained in this study are within the EPA criteria for evaluation of analytical methods of less than 20% (CV) and between 80% and 120% for mean percent recovery [3]. The results thus demonstrated utmost accuracy of ELISA kit in quantitative determination of chlorpyrifos ethyl residues in water and sediment samples. It further demonstrated that ELISA kit can be used for diagnostic procedures.

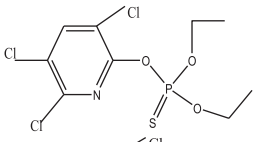
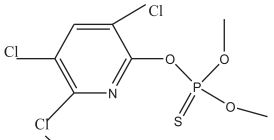
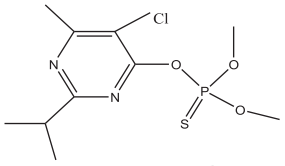
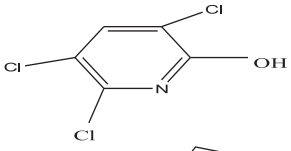
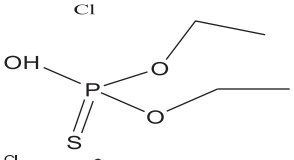
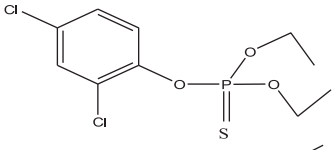
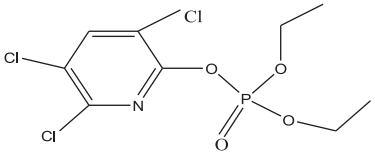
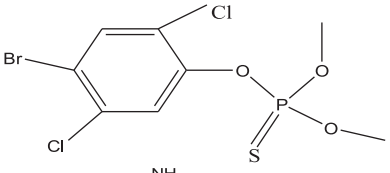
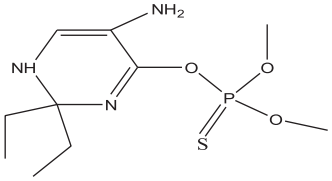
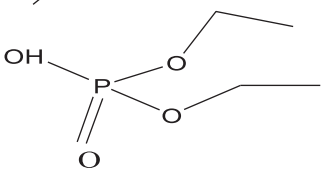
In a related study of accuracy of ELISA kit for determination of chlorpyrifos ethyl, a mean percent recovery of 98.7 ± 7%, [16] was obtained, a value which is close to the result presented here. Given that the obtained CV (%) and recovery of ELISA kit results were within acceptable limits the kit can adequately be used in qualitative determination of pesticide residues in water and sediment samples.

3.1.4. Matrix effects

Immunoassays are rapid and easy to use for both environmental water and sediment sample analysis since they do not

require elaborate sample preparation and clean up. However, ELISA methods have high potential for non-specific binding between non-analytes and antibodies from other related OP and CB that may be present in the samples [13]. This may result into false positive signals that sometimes affect the expected absorbance reading. To take care of the matrix effects, [20] two standard curves one prepared in control matrix such as distilled water and the other in the matrix of interest and results compared. The slope of the curve prepared in the matrix of interest is expected to be less than that in control system. In the present study, an alternative was used where experimentally determined absorbance values for matrix blank were normalized against those of the blank

Table 1
Cross reactivity of chlorpyrifos ethyl antibody to similar structural analogues

Analogue	Structure	IC_{50} ($\mu g\ L^{-1}$)	%Cross reactivity
Chlorpyrifos-ethyl		5.2	100
Chlorpyrifos-methyl		26.4	19.7
Diazinon		199.6	2.6
3,5,6-trichloro-2-pyridinol		346.7	1.5
Diethylthiophosphate		655.1	< 1.0
Diclofethion		533.8	< 1.0
Chlorpyrifos-oxon		1080.8	< 1.0
Bromiphos methyl		768.4	< 1.0
Pirimiphos-methyl		661.9	< 1.0
Diethylphosphate		521.1	< 1.0

control matrix to give an index of matrix interference I_m [21].

$$I_m = \frac{ABS_{blank\ A} - ABS_{blank\ B}}{ABS_{blank\ A}}$$

Where, ABS blank A is the mean absorbance determined by running control matrix (DI water) and ABS blank B is the mean absorbance determined by running blank sample matrix (river water).

Quantitative assessment of matrix interference was conducted by normalizing matrix blank with respect to absorbance of the blank control matrix (deionized water). The calculated I_m was used to get the correction factor (N) [9].

$$N = 100 - lm/100$$

Using this method, the I_m was 0.072 for water and 0.105 for sediment samples, respectively. These values were used to calculate correction factors, which were determined to be 0.9993 and 0.9990 for water and sediment samples, respectively. In order to account for matrix effect, which was also observed earlier in recovery studies, all the concentrations were corrected by multiplying all the obtained raw concentrations by the correction factor. However, variability in microtiter wells may have contributed to variation in final absorbance. Overestimation as a result of interfering substances is a common occurrence in ELISA analysis and can easily be observed in the slope of the regression curves [3,11,16] as witnessed in the comparative evaluation between ELISA and HPLC results. Due to matrix effect and presence of other components in environmental samples filtration or clean-up are recommended before ELISA analysis to minimize interferences.

3.1.5. Cross reactivity

Cross reactivity often affect the analytical results by indicating the presence of the target analyte while it is actually absent or elevating the concentration above the true levels [16]. In order to address this concern, inhibitory concentration 50 (IC_{50}) for analytes and possible compounds that might cause cross reactivity were evaluated. The IC_{50} values generated from the semi-log calibration curves refers to the concentration of the structural analogue which displaces 50% of labeled antigen compared to the IC_{50} of the analyte. Below is the formula used to determine percent cross reactivities (%CR) [9].

$$\%\ CR = (IC_{50}\text{Chlorpyrifos ethyl}/IC_{50}\text{structural analogue}) \times 100$$

Ten similar analogues were used for this assay and chlorpyrifos methyl showed significant cross reactivity (19.7%; Table 1).

Chlorpyrifos methyl may result into overestimation of the concentration, though its use was not common in the study area and should not be a serious concern. From the results chlorpyrifos ethyl antibodies were selective and could be suitable in pesticide residue analysis. However, higher reactivities like that of chlorpyrifos methyl may result into overestimation as the antibodies may not accurately differentiate between the actual analyte from its analogue [12]. In general, specificity of each kit toward a specific target pesticide and other likely compounds that might cross react need to be established before the kit is recommended for use in the field, otherwise false results may be generated.

3.1.6. Comparative evaluation of ELISA and HPLC results

Water and sediment samples were split for ELISA and HPLC DAD analysis and the results (Online Resources 3) were statistically compared to assess the comparability of ELISA kits and HPLC DAD by determining significant difference and limits of agreement between the two methods. Regression analysis performed to compare ELISA and HPLC results (Fig. 1) showed a strong correlation between the two methods for water and sediment samples. A strong significant correlation ($r=0.9670$; regression line $y=1.0574x+0.0962$; p-value <0.0001; $n=48$) was obtained for water samples (Fig. 1A). A similar significant correlation ($r=0.9878$; p-value <0.0001) between the two methods was observed for sediment samples analysis (Fig. 1B). The correlation observed in the results is a clear demonstration of the positive relationship between the two analytical methods. However, a positive bias toward ELISA was evident in the slope of the curve, which was greater than one (Fig. 1A and B). The bias is a serious analytical concern and is a clear indication of overestimation [10] which may have been due to potential effects of the metabolites of the organophosphate and carbamates or other components present in the matrices. Such biases can be minimized by incorporating clean up step during sample preparation.

Apart from regression analysis, additional comparative evaluation tests were carried out. Paired-two tailed t-test was performed and the calculated t value (2.02) with $p \leq 0.056$ for water samples

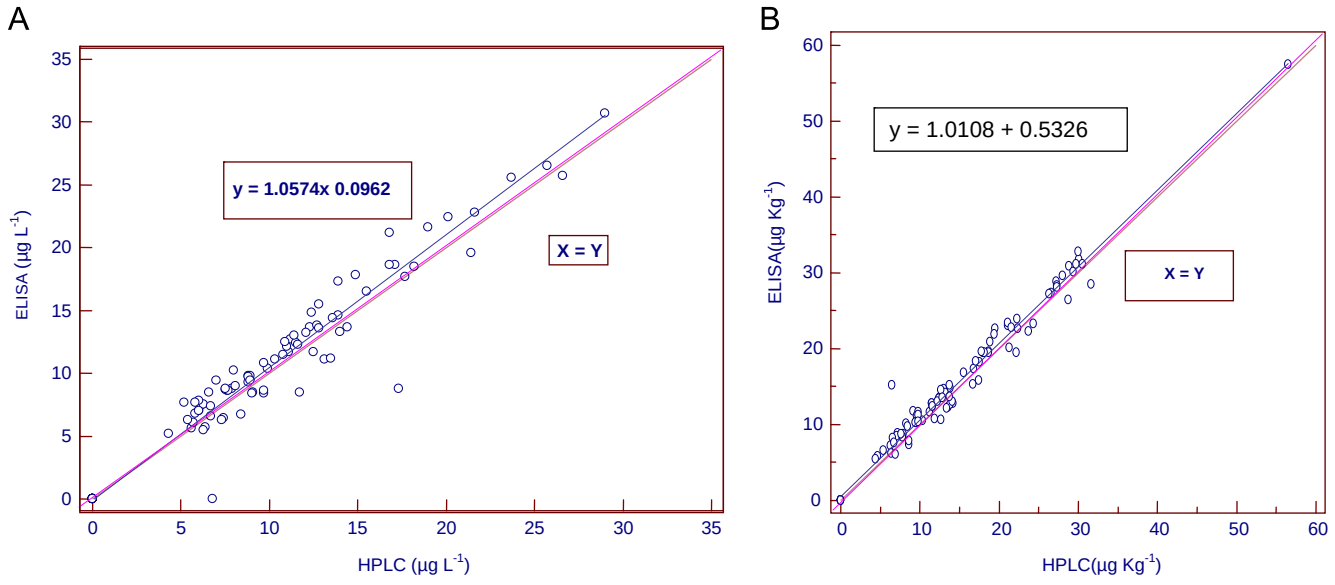


Fig. 1. Regression curve for ELISA kit and HPLC results for water sample analysis A ($r=0.9670$; regression line $y=1.0574x+0.0962$; p-value <0.0001; $n=48$) and sediment B ($r=0.9878$; $y=1.0108x+0.5326$; p-value <0.0001).

results ($n=48$) determined was less than the critical t value from the t standard distribution table at 95% confidence limit. Similarly, for sediment samples results, the computed t-value (2.0) with $p \leq 0.057$ for the sediment ($n=48$) was lower than the critical t-value. Based on these findings, it was concluded that there was no significant difference ($p \leq 0.05$) between the two methods hence the two methods could complement each other.

Use of regression analysis to compare ELISA and HPLC analytical methods in this study was found to be acceptable and in agreement with results obtained in previous studies [13]. However, in related comparative studies between ELISA and GC it was observed that it is not sufficient to use correlation coefficient as a measure of agreement and comparison between two methods [9,12,13,22]. This is because two methods may correlate strongly but show poor agreement since the correlation coefficient is only used to determine the strength of a relationship between two methods [11,22]. In this respect, Bland–Altman bias plot was obtained by use of Medcalc statistical software in order to determine the agreement of the two methods. The relationship between the measurement error and true value (mean of the two methods) and the bias for the two methods were assessed by the use of limit of agreement (Fig. 2). Limit of agreement is defined as the limit where 95% of the differences would lie if the two methods are in agreement [22]. A plot of the difference between the methods against their mean was drawn and limits of agreement at 95% confidence level were −2.2 to 3.6 (Fig. 2A) and −2.9 to 3.8 (Fig. 2B) for sediment and water samples, respectively.

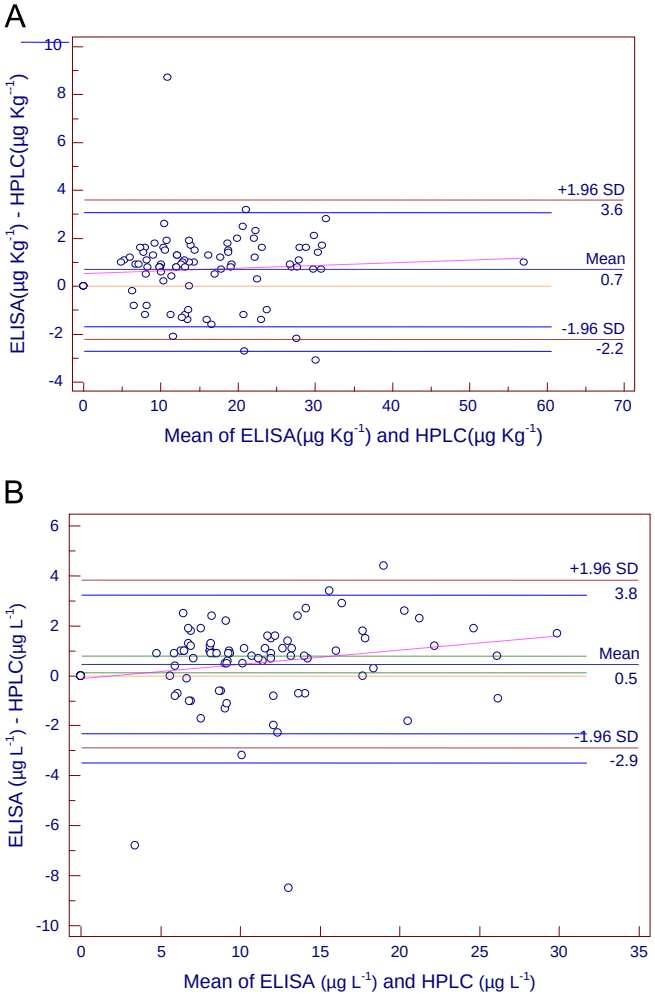


Fig. 2. Bland Altman bias plot for sediment (A) and water (B) samples.

The differences were distributed within the limits of agreement, which demonstrated that the two methods were in agreement with acceptable positive bias of 0.7 (Fig. 2A) and 0.5 (Fig. 2B) for sediment and water samples, respectively. The bias observed in this study could be attributed to the cross-reactivity of the metabolites and the possible presence of related analogues in the matrix, ionic strength, organic particulates as well as the experimental errors [12].

4. Conclusions

Use of ELISA kit in quantitative determination of the levels of chlorpyrifos ethyl residues in water and sediment samples is accurate, convenient and has high precision. The performance of the ELISA kit method was satisfactory and could be suitable for screening and monitoring of chlorpyrifos ethyl residues in water and sediment since mean percent coefficient of variation and recovery were within the approved limits for analytical methods. ELISA kit may provide a better environmentally friendly means of carrying out environmental risk assessment of chlorpyrifos ethyl and other pesticide residues. Since the performance of ELISA kit was satisfactory, it is recommended as a complimentary analytical method in chlorpyrifos ethyl residue analysis especially in developing countries where cost of using conventional instrumentation in analysis is prohibitive and in remote locations where conventional laboratory facilities are not available. Use of ELISA kit in regular monitoring of pesticide residues in aquatic ecosystem will hasten results delivery for ecological risk assessment and timely execution of mitigation measures. The presence of bias might necessitate clean up procedure before analysis. Although the ELISA kit and micro-HPLC results were in agreement based on linear regression analysis, t-test and Bland Altman Bias plot statistical analysis, a positive bias was observed toward ELISA, and might be of concern. Additional study on ways of reducing consistent interference like clean up before analysis is therefore recommended.

Acknowledgment

Alexander von Humboldt Foundation and National Commission for Science and Technology are acknowledged for financial support. We also thank the Institute of Molecular EXposomics (MEX), Helmholtz Center Munich – German Research Center for Environmental Health (GmbH) for analysis of the samples.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.09.014.

References

[1] S. Bollmohr, J.A. Day, R. Schulz, Chemosphere 68 (2007) 479–488.
[2] K.E. Banks, S. Hernandez, Talanta 61 (2003) 257–265.
[3] B.D. Hammock, S.J. Gee, R.O. Harrison, F. Jung, M.H. Goodrow, Q.X. Li, A. D. Lucas, A. Szekas, K.M.S. Sundaram, Immunochemical Methods for Environmental Analysis, in: J.M. Van Emon, R.O. Mumma (Eds.), American Chemical Society Symposium Series, 1990, pp. 112–139.
[4] R.L. Ellis, Rapid Test Methods for Regulatory Programs, in: R.C. Beier, L. H. Stanker (Eds.), Immunoassays for Residue Analysis, Food Safety, vol. 621, ACS Symposium Series, American Chemical Society, Washishgton, DC, 1996, pp. 44–58.
[5] K. Wanatabe, K. Franz, R.M. Gersberg, Bull. South Calif. Acad. Sci. 107 (2008) 33–40.
[6] D. Pimentel, in: P. Rajinder, A. Dhawan (Eds.), Innovation-Development Process Pesticides and Pest Control, Integrated Pest Management. Springer+ Business Media, Houten, Netherlands, 2009, pp. 83–87.
[7] J. Mitoko-Ohayo, Occupational pesticide exposure among Kenyan Agricultural workers: an epidemiological and public health perspective (Ph.D. dissertation),

Kenya Medical Research Institute (KEMRI) and Department of Epidemiology and Public Health, Wageningen Agricultural University, Wageningen, The Netherlands, 1997.
[8] B.M. Kaufman, M.C. Clower, Assoc. Anal. Chem. 74 (1991) 239–247.
[9] J.J. SullivaN, S.K. Goh, J. Agric. Food Chem. 48 (2000) 4071–4078.
[10] S.G. Nunes, I.A. Toscano, D. Barceló, Trends Anal. Chem. 17 (1998) 79–86.
[11] USEPA Immunoassay Guidelines for Planning Environmental Projects, United State Environmental Agency, Office of Environmental Measurement and Evaluation, Region I, EPA-New England, 1996.
[12] J.J. Sullivan, Y.G. Chen, S.K. Goh, J. Agric. Food Chem. 55 (2007) 6407–6416.
[13] F. Rubio, J. Linda, J. Veldhuis, B.S. Clegg, J.R. Fleeker, J.C. Hall, J. Agri. Food Chem. 51 (2003) 691–696.
[14] K.-W. Schramm, W. Jaser, G. Welzl, G. Pfister, G.F. Wohler-Moorhoff, B. Hense, Ecotoxicol. Environ. Saf. 69 (2008) 437–452.
[15] L. Konda, Z. Pásztor, J. Agric. Food Chem. 49 (2001) 3859–3863.
[16] T.S. Lawruck, A.M. Gueco, C.A. Mihaliak, S.C. Dolder, G.E. Dial, D.P. Herzog, F.M. Rubio, J. Agric. Food Chem. 44 (1996) 2913–2918.
[17] A.E. Walker, R.E. Holman, R.B. Leidy, ELISA and GC–MS analysis of pesticide residues in North Carolina, J. Am. Water Resour. Assoc. 36 (2000) 67–74.
[18] F. Harnandez, R. Serrano, M.C. Miralles, N. Font, Chromatographia 429 (1996) 151–158.
[19] A. Deng, V. Kolář, R. Ulrich, M. Fránek, Anal. Bioanal. Chem. 3739 (2002) 685–690.
[20] A.J. Krotzy, B. Zeeh, Pure Appl. Chem. 67 (1995) 2065–2088.
[21] S. Cairoli, A. Arnoldi, S. Pagani, J. Agric. Food Chem. 44 (1996) 3849–3854.
[22] J.M. Bland, D.G. Altman, The LancetI (1986) 307–310.