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# Enzyme-Linked Immunosorbent Assay (ELISA) and Sol−Gel-Based Immunoaffinity Purification (IAP) of the Pyrethroid Bioallethrin in Food and Environmental Samples†

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Enzyme-linked immunosorbent assay (ELISA) and sol−gel-based immunoaffinity purification (IAP) methods for the pyrethroid bioallethrin were developed and applied for monitoring bioallethrin in spiked food, soil, and dust samples. Attempts to determine bioallethrin content in fruit and vegetable extracts revealed high variability between sample preparations and marked interferences with the assay. Sol−gel IAP followed by solid-phase sample concentration was effective in removing the interfering components and resulted in high recovery of bioallethrin from spiked crude acetonic extracts of fruits and vegetables, even in the presence of high extract concentrations (28%). Solid-phase treatment alone failed to remove the interfering components from the spiked sample. Gas chromatography−mass spectrometry analysis of the IAP samples revealed bioallethrin as a doublet unsolved peak because of the *cis* and *trans* isomer present in the standard with confirmation of its mass. Unlike fruit and vegetable extracts, soil and dust samples did not interfere with the ELISA, and the bioallethrin content in those samples could be determined with high precision without the need of any further purification.

**KEYWORDS: Pyrethroid; bioallethrin; ELISA; sol−gel; immunoaffinity purification; residue monitoring**

## INTRODUCTION

Synthetic pyrethroid insecticides have been used in agricultural, domestic, and veterinary applications for more than 4 decades, and they account for approximately 25% of the worldwide insecticide market (*1*). The increasing use of synthetic pyrethroids, compared to other classes of insecticides, is attributed to their remarkably high insecticidal activity and their generally assumed low acute toxicity to mammals. Although these compounds are widely considered safe for mammals, studies have shown recently that short- and long-term neonatal and later adult exposure to synthetic pyrethroids may cause developmental neurotoxic and immunotoxic effects that may lead to spontaneous behavioral aberrations, changes in the muscarinic cholinergic system, impairment of memory and learning, lymph node and spleen damage, and carcinogenesis (*2*−*4*). The widespread use of pyrethroids in agriculture, horticulture, and forestry increases human exposure via the diet and occupational and domestic routes. This, together with the potential risks that pyrethroids pose to mammals, nontarget invertebrates, and aquatic organisms (*5*, *6*), which may be exposed to field runoff or drift from aerial and ground-based spraying, raises an urgent need for large-scale monitoring of these compounds in agricultural produce and the environment.

Many methods have been developed and employed for the detection of pyrethroids: they include capillary gas chromatography (GC) with electron-capture detection (*7*, *8*), GC coupled with mass spectrometry (MS) (*9*−*11*), high-performance liquid chromatography (HPLC) with a postcolumn photoderivatization and fluorimetric detection (*12*), and high-performance thin-layer chromatography (HPTLC) (*13*). Although chemical analytical methods are sensitive, precise, and reproducible and can accommodate multiresidue samples, they are expensive (in equipment, materials, and human resources), involve the use

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of large volumes of toxic solvents that require costly storage and disposal arrangements, necessitate the application of long and complicated concentration and cleanup procedures, and typically cannot be performed on site. In addition, the sensitivity of the relevant analytical methods for some pyrethroids is lower than that for other pesticides and, in some cases, lower than the limit of detection required for their monitoring (i.e., the legally set maximum residue limit). In attempts to develop highly sensitive methods and to meet the increasingly stringent regulations and demands for continuous monitoring of pyrethroid residues in food and in the environment and to expand monitoring programs, alternative sensitive, specific, cost-effective methods that enable simple, large-scale sample handling and analysis in the laboratory and on-site were sought. Immunoassays (IAs), especially solid-phase enzyme IAs (EIAs), such as the enzyme-linked immunosorbent assay (ELISA), fulfill most of the above requirements.

In the past 2 decades, the development of EIAs for agricultural and environmental applications has shown impressive acceleration in methods development and some of the assays have been recognized and even implemented for screening of large-scale pesticide residue monitoring and environmental research. Several hundred assays have been described in the literature, and many commercial kits for assaying pesticides are available (*14*−*16*). Pyrethroids definitely are a good example for this trend as indicated by the large number (over 25) of ELISAs that have been developed in just over a decade. These assays are in a variety of formats, using polyclonal, monoclonal, or recombinant antibodies (Abs) for monitoring type-I and type-II pyrethroids (individual compounds and class-specific), as well as their metabolites in various foods, beverages, soil, water, and human fluid samples (*17*−*40*).

Despite the massive use of EIAs for residue monitoring, a high-volume application of these methods is still not feasible, because several major technical barriers limit their wide use. One problem is the lack of simple, efficient, and reproducible methods for recovering the target compounds from the sample matrix. Another problem arises from matrix interference. Fruit, vegetable, and other food extracts, as well as soil samples, contain organic and inorganic substances that may interfere directly or indirectly with the assay, increasing variability and decreasing efficiency and monitoring capability (*19*, *29*, *39*). Such matrix interferences may affect the Ab−analyte binding, increase nonspecific binding of reagents, inactivate the Ab or reporting enzyme, or introduce a high background because of sample pigmentation or autofluorescence. The varied and complex sample matrices encountered in environmental monitoring, the low concentrations of the analytes within the matrix, and the presence of compounds that interfere with the analytical method raised the need for a highly specific, rapid, and cost-effective method for purification and concentration of the tested materials, which would be compatible with the simplicity and cost-effectiveness of the EIAs. Immunoaffinity purification (IAP), which offers single-step isolation and purification of individual compounds or classes of compounds from liquid matrices, emerged recently as a preferred method for trace analysis of biologically significant compounds. IAP introduces many advantages but still needs to be adapted to accommodate environmental and agricultural samples.

In the past few years, we have developed a novel IAP method based on the entrapment of Abs in a ceramic $SiO_2$ sol−gel matrix for cleanup and concentration of target analytes from environmental and agricultural samples (*41*−*46*). In those studies, we proved the ability of the entrapped Abs to bind pure standard analytes from aqueous solutions. In the present study, we extend the application of the sol−gel IAP method and describe the employment of sol−gel entrapped anti-pyrethroid monoclonal Abs (Mabs) for IAP of the pyrethroid bioallethrin from food samples (crude acetonic extracts of tomatoes, cucumbers, and strawberries).

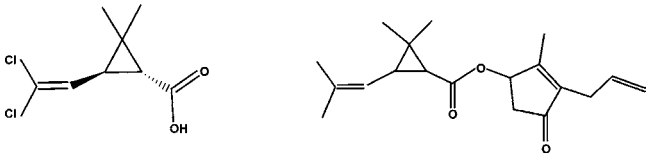


**Figure 1.** Structures of the hapten (1-*trans*-permethric acid) used for TPA–protein–conjugate preparation and the pyrethroid bioallethrin.

## MATERIALS AND METHODS

**1. Immunochemical Methods.** *Antibodies*. A monoclonal anti-allethrin Ab [Mab 1/E2 protein A purified, 4 mg/mL in double-distilled water (DDW) termed anti-bioallethrin Ab herein] generated in the laboratory of Prof. Hock as previously described (*37*) was used throughout the study.

*A. Preparation of 1R-trans-Permethric Acid (TPA)−Protein Conjugate (37).* The first step was conversion of the TPA chloride (**Figure 1**) to the non-chloride form (TPA-hapten). The second step involved coupling of the TPA-hapten to the protein. Conversion of TPA chloride (Dr. K. Naumann, Bayer AG, Leverkusen, Germany) to the non-chloride form was carried out by the addition of 1.1 M KOH to a stirring solution of 0.5 M 1*R* TPA chloride until a clear solution was obtained. The solution was extracted 3 times at room temperature with 200 mL of ether; the organic phase was adjusted to pH 1.0 by adding concentrated HCl, during which a precipitate was formed. The precipitate was washed with DDW to remove excess HCl and then dried at 50 °C.

Coupling of the TPA-hapten to ovalbumin (OV) was performed as follows. TPA-hapten (62 mg), *N*,*N*′-dicyclohexylcarbodiimide (DCC, 620 mg) and *N*-hydroxysuccinimide (NHS, 173 mg) were dissolved in 4 mL of dimethyl formamide (DMF) and stirred for 18 h at room temperature. At the end of the incubation, the TPA reaction mixture was centrifuged at 11000*g* for 20 min at room temperature. The supernatant was collected and added (dropwise) to 132 mg of OV dissolved in 7 mL of 0.13 M carbonate buffer at pH 9.6. The reaction mixture was incubated for 3 h at room temperature and then dialyzed against 5 L of DDW for 3 days. The solution was changed 3 times daily. The dialyzed solution was collected and centrifuged at 11000*g* for 20 min at room temperature. The pellet was dissolved in 3 mL of 0.05 M carbonate buffer at pH 9.6 and sonicated on ice for 10 min. The protein content was determined with the Bradford reagent (Bio-Rad Laboratories GmbH, Germany) according to the instructions of the manufacturer. The hapten protein conjugate (TPA−OV, 0.6 mg/mL) was stored aliquoted at −20 °C and served as the coating antigen in the ELISAs.

*B. Bioallethrin Competitive ELISA.* Microtiter plates (Nunc MaxiSorp ELISA plates) were coated with 200 $\mu$L of the conjugate (TPA−OV, diluted 16000, in 0.05 M carbonate buffer at pH 9.6) or with an equivalent amount of OV (diluted in the same buffer) and incubated overnight (ON) at 4 °C. After the ON incubation, wells were washed 3 times with phosphate-buffered saline (PBS; 10 mM $NaH_2PO_4$ and 150 mM NaCl) at pH 7.2 containing 0.1% Tween-20 (PBST), and 250 $\mu$L of 0.1% (w/v) OV in PBS were added to the wells to block nonspecific binding sites. The plates were kept for 1 h at room temperature and washed with PBST as above. Next, 100 $\mu$L of an unknown sample (5 serial dilutions, in duplicates), 12 serial dilutions of a bioallethrin standard ranging from 0.25 to 500 ng/well (**Figure 1**, CAS number 584-79-2, Dr. Ehrenstorfer GmbH, Augsburg, Germany, catalog number 106100), or quality control (QC) bioallethrin samples (5 serial dilutions, in duplicates, ranging from 31.25 to 500 ng/well) diluted in PBS (unless otherwise indicated) were added to the wells together with 100 $\mu$L anti-bioallethrin Mab (diluted in PBST 1:64000).

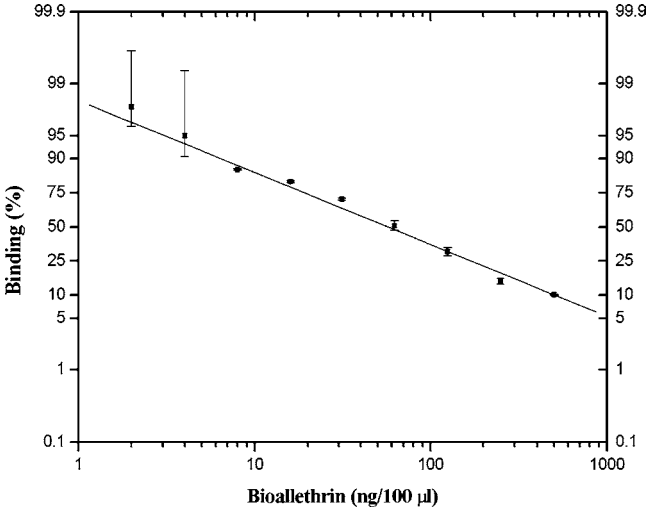


**Figure 2.** Representative standard curve of bioallethrin ELISA. Each data point represents the mean ± standard deviation (SD) ($n = 2$). $R = -0.99645$; $R^2 = 0.99291$.

In addition to the standard curve and unknown samples, each microplate contained a set of six wells that determined the maximal binding in the absence of any competing analyte (designated as 100% binding). Another set of six wells (coated with only the carrier protein OV instead of the TPA−OV conjugate) determined the nonspecific binding of the reaction components to the microplate and to each other (designated as the assay background samples). Plates were incubated for 1 h at room temperature and washed as above, and 200 µL of goat anti-mouse conjugated to horseradish peroxidase (HRP whole molecule, affinity isolated, Sigma), diluted 1:3000 in PBST, was added. Plates were incubated for 2 h at room temperature in a light-protected box on an orbital shaker set at 150 rpm. At the end of the incubation, microplates were rinsed with PBST as above, and 200 µL of 1-Step Ultra TMB-ELISA substrate (Pierce, Rockford, IL) were added to the wells. The reaction was stopped after 10−20 min by the addition of 100 µL of 4 M sulfuric acid. The absorbance was monitored with an ELISA reader (Multiscan Multisoft microplate reader Labsystems) at 450 nm. Under the above conditions, the $I_{50}$ (defined as the analyte concentration that decreases Mab binding by 50% to the immobilized TPA−OV conjugate on the plate) was 682 ± 180 ng/mL ($n = 14$) and the limit of detection (LOD; $I_{20}$, defined as the analyte concentration that decreases Mab binding by 20% to the TPA−OV conjugate) was 169 ± 66 ng/mL ($n = 14$) (**Figure 2**). The above values were higher than those obtained with the same Mabs by Pullen and Hock (*37*), most likely because of differences in the hapten−OV conjugate employed in the two laboratories. However, for the purpose of our study, these values were adequate. In cases where food samples were tested, the assay was performed in the presence of 5% ethanol (final concentration). Soil and dust samples were tested by ELISA in the presence of 5% acetone. The presence of 5% ethanol or acetone did not change the sensitivity or LOD of the assay, and the $I_{50}$ and $I_{20}$ values obtained were similar to those listed above. Higher ethanol or acetone concentrations (>5%) interfered with the assay. The overall precision of the method was determined according to the percentage of deviation of the QC samples from the theoretical value and was found to be 84%.

*C. Determination of the Cross-Reactivity (CR) Pattern of the Bioallethrin Mabs.* CR was determined by the ability of various pyrethroids (allethrin, cypermethrin, cyfluthrin, fenpropathrin, permethrin, and fenvalerate, purchased from Dr. Ehrenstorfer GmbH, Augsburg, Germany) to compete with the adsorbed TPA−OV on the plate. The data obtained indicated (**Table 1**) that bioallethrin exhibited the highest affinity in binding to the Mabs. The Mabs also reacted with allethrin (41%) and to some extent (16 and 9%) with cypermethrin and cyfluthrin, respectively. None of the other compounds were recognized by the Mabs.

**2. Sol−Gel Immunoaffinity Purification.** *A. Column Preparation and Sample Loading.* The entrapment was carried out by a two-step procedure in which hydrolysis was followed by polymerization of tetramethylsilane (TMOS; Aldrich, 99%, Karlsruhe, Germany) as previously described (*47*). Briefly, an acidic silica sol solution was obtained by mixing TMOS with 2.5 mM HCl in DDW at a molar ratio of 1:8 in the presence of 10% polyethylene glycol (PEG-400, with average molecular weight of 400 g/mol, corresponding to approximately seven methylene units in the chain, Merck, Germany). The mixture was stirred for 1 min until a clear solution was obtained and was then sonicated for 30 min in an ELMA ultrasonicator bath (model T-460/H, 285 W, 2.75 L, Singen-Hohentwiel, Germany). The reaction was performed in a well-ventilated fume hood.

**Table 1.** CR of the Bioallethrin Mab with Different Pyrethroid Compounds[a]

| compound | CR (%) | compound | CR (%) |
|---|---|---|---|
| bioallethrin | 100 | fenpropathrin | 0 |
| allethrin | 41 | permethrin | 0 |
| cypermethrin | 16 | fenvalerate | 0 |
| cyfluthrin | 9 | | |

[a] CR represents the ratio (expressed as a percentage) between the concentration of the tested compound that causes a 50% decrease in the binding of the Mab to the coating antigen adsorbed onto the microplate and the concentration of bioallethrin causing the same inhibition.

Anti-bioallethrin Mabs (10 or 100 µL corresponding to 40 and 400 µg, respectively) were premixed with 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid buffer (HEPES, 99.99%, Sigma) at pH 7.5 to a final volume of 0.5 mL. An equivolume amount was added to a prehydrolyzed TMOS mixture. The solution was mixed quickly for 5 s, and gelation occurred within 1−2 min. After 30 min, the gels (total volume of 1 mL) were washed with 2 mL of HEPES buffer at pH 7.5 and were kept wet (with 2 mL of HEPES buffer at pH 7.5 on top) at 4 °C until use. Best results were obtained with gels that were stored ON at 4 °C and used on the second day after preparation. The gels exhibited high stability and could be used for over 2 months after preparation.

*B. Binding and Elution of Bioallethrin from Sol−Gel IAP Columns.* Wet gels were thoroughly crushed, transferred into inverted 5 mL plastic syringes, and packed in 1 mL columns (1.3 cm in diameter and 0.8 cm in height). The sol−gel columns were washed with 50 mL of PBS prior to sample application. For optimal binding, columns were kept under buffer at all times during the experiment. A total of 100 ng or 5 µg of bioallethrin standard (unless otherwise indicated) was applied, in a volume of 1 mL of PBS, to the sol−gel columns, which were doped with either 10 or 100 µL, respectively, of anti-bioallethrin Mab (unless otherwise indicated). Unbound bioallethrin was removed by washing with 10 mL of PBS. In some experiments, the bioallethrin content in the washout fraction was monitored by ELISA. Elution was performed with 10 mL of absolute ethanol (PESTI-S, Bio-Lab, Jerusalem, Israel). The eluted fraction was further concentrated by means of a solid-phase column as described below.

*C. Solid-Phase Extraction (SPE): Sample Application and Elution.* Sol−gel-eluted samples were diluted to a final concentration of 10% ethanol in PBS. Oasis SPE columns (Waters, Milford, MA) were preconditioned by two washes with 5 mL of absolute ethanol or acetone (PESTI-S, Bio-Lab, Jerusalem, Israel), followed by two washes with 5 mL of PBS. Samples were loaded on the columns, which were then washed with 5 mL of 10% ethanol in PBS. Elution was carried out with 1 mL of ethanol or acetone (for ELISA or GC−MS analysis, respectively). Bioallethrin content was determined either by ELISA or GC−MS as described below. The ELISA determination was preceded by diluting the sample to a concentration of 10% to reduce the final concentration of the organic solvent in the assay to 5% (a level that does not interfere with the assay).

**3. Analytical Methods:** *GC−MS.* The sample extracts and standard solutions were analyzed by 70 eV electron impact (EI) GC−MS. A Hewlett-Packard GC−MS was operated in the selected ion monitoring (SIM) mode. Data acquisition and processing were performed with a ChemStation data system. The GC column was a DB-5 fused-silica capillary (60 m × 0.32 mm, 0.25 µm film thickness). Helium was used

as the GC carrier gas. After injection, the GC column was set at 70 °C for 1 min, with the temperature programmed to 190 °C at 25 °C/min, 230 °C at 10 °C/min, and 290 °C at 4 °C/min. Peaks monitored (expressed as *m*/*z*) were the molecular ion peaks and their associated characteristic fragment ion peaks (123, 124, and 136 for *cis*- and *trans*-bioallethrin and 94 and 188 for the internal standard (IS), phenanthrene-$d_{10}$). Identification of the target analytes (*cis*- and *trans*-bioallethrin) was based on their GC retention times relative to the IS and the relative abundances of the monitored ions. Quantification was performed by comparing the integrated ion current response of the target ions to those of the IS. The average response factors of the target ions were generated from the standard calibrations (*48*). In brief, the $R_f$ value was obtained using $R_f = (A_S/A_{IS})(C_{IS}/C_S)$ from the analyses of standard solutions used for generating the calibration curve. Note that $A_S$ and $A_{IS}$ refer to the area counts of the quantification ions of target analytes and the IS, respectively, and $C_S$ and $C_{IS}$ are the concentration values of target analytes and the IS, respectively. The concentration of target analytes ($C_S$) in the samples was obtained from $(A_S/A_{IS})(C_{IS}/R_{f\ avg})$, where the $R_{f\ avg}$ value was the average $R_f$ value generated from the analyses of standard solutions.

**4. Sample Analysis.** *A. Preparation of Fruit and Vegetable Samples.* All of the fruits and vegetables used in the study were organically grown. Fruits and vegetables were washed thoroughly and frozen in 50 g batches at −80 °C until use. Before use, samples were thawed, mixed with 100 mL of acetone, and homogenized in a food blender for 1 min. The mixture was filtered through Whatman filter paper (Grade 41: 20−25 $\mu$m) in a Büchner funnel. The filtrate was centrifuged twice at 500 *g* for 20 min at room temperature. The supernatant was collected, transferred to Teflon-capped glass vials, and stored for up to 1 month at −20 °C prior to analysis.

*B. Spiking of Fruit and Vegetable Samples.* Shortly before use, samples (10 mL) were evaporated to 0.35× the original volume, i.e., 3.5 mL, to obtain a 2.8-fold concentrated sample. The evaporation occurred under a stream of $N_2$ in a Reactiv-Vap evaporator equipped with Teflon-coated needles (Pierce, Rockford, IL). A freshly prepared bioallethrin standard (100 ng or 5 $\mu$g) was added to 1 mL of the evaporated extract (equivalent to 1.4 g of the original fruit or vegetable). The samples were incubated for 1 h at room temperature.

*C. Sample Analysis.* Spiked samples (five serial dilutions ranging from 1:10 to 1:160 in PBS containing 10% ethanol) were tested for bioallethrin content by the ELISA before and after sol−gel IAP and solid-phase concentration. Spiked samples subjected only to solid-phase concentration were tested in parallel. A bioallethrin standard was subjected to the same treatments to determine the efficiency of the sol−gel IAP process. Unspiked samples that were prepared in a similar manner were also subjected to the same treatments and tested before and after the IAP and concentration of the extract for the presence of exogenous (external contaminants) or endogenous bioallethrin-like immunoreactive (IR) compounds. ELISAs, before and after sol−gel IAP and/or solid-phase concentration, were performed as described above, except that the standard and QC samples were in PBS containing 10% ethanol. Sample interference with the assay (e.g., the presence of components that adsorb at 450 nm or nonspecific binding of the sample to the microplate in a manner that increased nonspecific binding of the other reaction components) was monitored by the ELISA as described above, except that wells were coated with OV instead of TPA−OV in an amount equivalent to that of the conjugate. The values obtained in these wells (i.e., the background levels of the assays) were averaged and subtracted from each value of the standard, the QC sample, and the respective unknown sample.

*D. Preparation of Soil and Dust Samples.* Soil samples were mixed thoroughly before removing aliquots for sample preparation and analysis. Soil samples (~4 g) were extracted twice by sonication for 15 min with 10 mL of 10% diethyl ether in hexane. The extracts were concentrated to a final volume of 2 mL in hexane. The concentrated hexane extract was split into two portions: portion I for GC−MS and portion II for ELISA. For GC−MS analysis, the sample extract was spiked with 10 $\mu$L of IS and transferred to a GC vial.

Indoor floor dust samples were separated into fine and coarse particles by sieving. Only the fine dust particles, less than 150 $\mu$m, were analyzed. Dust samples (~0.5 g) were extracted twice by sonication for 15 min with 10 mL of 10% diethyl ether in hexane. The extracts were concentrated and split into two portions for GC−MS and ELISA analysis as described above for soil samples. For GC−MS analysis, the sample extract was processed through a Florisil column with 12 mL of 15% diethyl ether in hexane and 6 mL of dichloromethane (DCM). The extracts were concentrated with the final volume adjusted to 1 mL of hexane, spiked with 10 $\mu$L of IS, and transferred to a GC vial for GC−MS analysis.

*E. Spiking of Soil and Dust Samples.* Samples (1 mL) were evaporated to dryness shortly before use and dissolved to the original volume with PBS containing 10% acetone. A freshly prepared bioallethrin standard (5 $\mu$g) was added to 1 mL of the extract, and the samples were incubated for 1 h at room temperature. The spiked samples (ranging from undiluted to 1:16 diluted in PBS containing 10% acetone) were tested for bioallethrin content by ELISA as described above, except that the standard curve and QC samples were in PBS containing 10% acetone. Similarly prepared unspiked samples used as controls to determine any matrix interference with the ELISA underwent a similar analysis.

**5. Data Transformation.** Bioallethrin contents of the ELISA samples were calculated from a bioallethrin standard curve after linearization of the data by transformation to a logit-log plot by means of Microcal Origin software, version 6.0 (Microcal Software, Inc., Northampton, MA). All samples were tested in duplicate in four or five dilutions that were within the range of the standard curve. Slopes of all samples were tested for parallelism with the standard curve, using a test for homogeneity of slopes regression according to Sokal and Rohlf (*49*). Only samples whose regression lines were parallel to the standard curve were considered.

**Table 2.** Bioallethrin Binding and Elution Recoveries from Sol–Gel Columns[a]

| column | total nanograms on column | free (ng) | bound (ng) | eluted | recovery (%) |
|---|---|---|---|---|---|
| A. Sol–Gel IAP | | | | | |
| Mabs (60 $\mu$L) | 3000 | 820 | 2180 | nt | 73 |
| Mabs (100 $\mu$L) | 5000 | 630 | 4370 | nt | 87 |
| B. Sol–Gel Followed by Oasis SPE | | | | | |
| Mabs (100 $\mu$L) | 5000 | 688 | 4312 | 3733 | 87 |

[a] The data in part A represent an experiment in which bioallethrin was applied on the column and the unbound analyte present in the washout fraction was monitored. No elution was carried out, and the amount of bound analyte was determined from the difference between the total amount of bioallethrin applied on the column and that detected in the wash rinse. Recovery represents the ratio (expressed as a percentage) between the amount of bound analyte and the total amount applied on the column. The data in part B represent an experiment in which bioallethrin was applied on the sol–gel column and the eluted fraction was further concentrated by means of a solid-phase Oasis column. The amount of bound bioallethrin was calculated as above. Recovery represents the ratio (expressed as a percentage) between the amounts of eluted and bound bioallethrin. The results represent one of four independent experiments, where the recovery ranged from 85 to 105%. nt, not tested; IAP, immunoaffinity purification; SPE, solid-phase extraction.

## RESULTS

**1. Bioallethrin Recovery from Sol−Gel Columns.** The first part of the study examined the extent of binding of standard bioallethrin (in PBS) to sol−gel-entrapped anti-bioallethrin Mabs. The data in **Table 2A** indicate a binding of 73 and 87% in the presence of 60 or 100 $\mu$L, respectively, of anti-bioallethrin Mabs. The nonspecific binding of bioallethrin to an empty (nondoped) column was in the range of 8−13%. In previous studies, we found that efficient elution from sol−gel IAP columns was obtained with 10−20 mL of organic solvent (*50*, *51*). Unfortunately, this resulted in a 10-fold dilution of the eluted analyte with an organic solvent that was not tolerated by the ELISA.

**Table 3.** Interference of Unspiked Vegetable and Fruit Extracts with ELISA

| extract dilution | extract (%)[b] | interference (%)[a] tomato exp. 1 | tomato exp. 2 | cucumber exp. 1 | cucumber exp. 2 | strawberry exp. 1 | strawberry exp. 2 |
|---|---|---|---|---|---|---|---|
| 1:2 | 140 | 18 | 72 | 22 | 27 | 100 | 54 |
| 1:4 | 70 | 4 | 52 | 16 | 25 | 100 | 44 |
| 1:8 | 35 | 0 | 6 | 17 | 25 | 100 | 27 |
| 1:16 | 17.5 | 0 | 2 | 3 | 23 | 100 | 26 |
| 1:32 | 8.8 | 0 | 0 | 4 | 21 | 100 | 3 |
| 1:64 | 4.4 | 0 | 0 | 0 | 24 | 100 | 6 |
| 1:128 | 2.2 | 0 | 0 | 2 | 29 | 100 | 0 |
| 1:256 | 1.1 | 0 | 0 | 0 | 24 | 100 | 0 |
| 1:512 | 0.55 | 0 | 0 | 0 | 27 | 100 | 0 |
| 1:1024 | 0.27 | 3 | 0 | 0 | 25 | 100 | 6 |
| 1:2048 | 0.14 | 8 | 0 | 0 | 25 | 100 | 0 |
| 1:4096 | 0.07 | 3 | 0 | 0 | 32 | 87 | 0 |

[a] The data represent the degree of interference, defined as 100 minus the absorbance (optical density, OD) signal ratio (in percentage) obtained in the presence and absence of the fruit and vegetable extracts at each dilution. [b] Extract percentage represents the content of the fruit or vegetable in the tested sample. The values were calculated on the basis of the preparation procedure described in the Materials and Methods. The extract was concentrated 2.8-fold in the course of its preparation, and a 1:2 dilution of the concentrate is equivalent to a 1.4-fold concentrated extract or 140%.

To reduce the amount of the organic solvent to a level that would be tolerated by the ELISA (i.e., a final concentration of 5% or less), it was necessary to eliminate the organic solvent and to concentrate the sample after its elution from the sol−gel IAP column. Evaporation either under vacuum or nitrogen was not very effective, because both methods resulted in a loss of bioallethrin that sometimes exceeded 50% (data not shown). We therefore, used solid-phase concentration in combination with the sol−gel columns, to obtain the analyte in 1 mL of solvent. To determine whether the sol−gel eluates could indeed be concentrated by the solid-phase columns and eluted at high efficiencies, bioallethrin in 10% ethanol was applied to Oasis solid-phase columns and the elution with absolute ethanol or acetone was monitored by ELISA. The data indicated that the average recovery of bioallethrin (3000 ng) from the solid-phase Oasis columns was >90% ($n = 20$) in either solvent (data not shown). The combination of both IAP and Oasis columns resulted in a recovery of 87% (**Table 2B**).

**2. Interference of Tomato, Cucumber, and Strawberry Acetonic Extracts with the ELISA.** Crude acetonic extracts of fruits and vegetables are heavily pigmented and may interfere with colorimetric ELISAs. The extracts may also contain components that could affect analyte−Ab interactions and cause false-negative or false-positive outcomes. To determine the extent of the interference of the tested extracts, unspiked acetonic extracts of strawberries, tomatoes, and cucumbers were prepared and their interference with the assay was determined by comparing the ratio between the signal obtained in their presence and absence. To determine the reproducibility of the results, experiments were performed with extracts obtained from fruits and vegetables obtained and prepared for analysis on different days. As can be seen in **Table 3**, different extracts interfered with the ELISA to differing extents and extracts prepared from different batches differed in their interference. Tomato and cucumber extracts interfered with the assay only at high concentrations, and in some cases (e.g., cucumber extract in experiment 2 in **Table 3**), the interference, although moderate, could not be eliminated up to a dilution of 1:4096. Strawberry extracts were much more problematic than tomato and cucumber extracts. The differences between strawberry extracts were much greater and interfered strongly with the ELISA up to a dilution of 1:4096 (experiment 1 in **Table 3**).

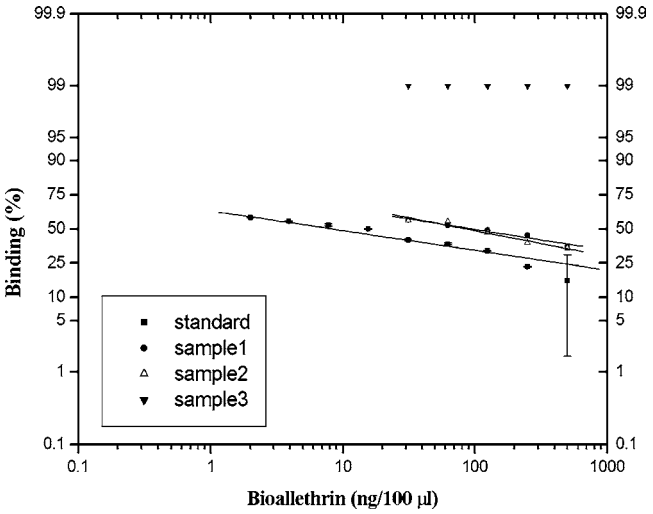


**Figure 3.** Determination of bioallethrin content in spiked acetonic extracts of strawberry, before and after sol–gel/solid-phase IAP and concentration. Bioallethrin recovery was monitored by ELISA (performed in PBST plus 5% ethanol). Strawberry samples were tested at five serial dilutions ranging from 1:10 to 1:160 (equivalent to 28–1.75% extract). Bioallethrin standard curve (■; $R = -0.97076$; $R^2 = 0.94237$); quality control (●; $R = -0.99781$; $R^2 = 0.99562$); spiked strawberry samples after IAP on sol–gel and concentration on Oasis solid-phase (△; $R = -0.95408$; $R^2 = 0.91026$); and untreated spiked strawberry sample (▼). Note that the IAP sample curve parallels the standard curve, whereas the untreated sample curve does not. Each data point represents the mean ± SD ($n = 2$). Results represent one of three independent experiments.

**3. Sol−Gel IAP of Bioallethrin from Spiked Fruit and Vegetable Samples.** The interference of the above extracts with the bioallethrin ELISA as well as the high variability among extracts indicated the need to purify the samples prior to the immunochemical analysis. An extremely high dilution may minimize the matrix interference in the assay but may result in an inability to detect low-level bioallethrin residues in real samples. Therefore, extracts spiked with bioallethrin were applied on sol−gel IAP columns, and the bioallethrin content of each eluate was monitored by ELISA and validated by GC−MS.

Two sample matrices were chosen for sol−gel IAP: strawberry and tomato. The following samples were tested for bioallethrin content: (i) untreated spiked extracts, (ii) spiked extracts that had undergone sol−gel IAP followed by solid-phase concentration, (iii) spiked extracts that had undergone only solid-phase concentration, and (iv) standard bioallethrin that had undergone both (sol−gel/solid-phase and solid-phase alone) treatments. Unspiked extracts that had undergone all of the above treatments were also tested.

The data in **Figures 3** and **4** show that the regression lines of the spiked strawberry and tomato samples were parallel to those of the bioallethrin standard curve following sol−gel IAP purification. In contrast, samples that did not undergo IAP did not parallel the standard curve. Thus, the data clearly demonstrate the high efficiency of the combined sol−gel/solid-phase method in removing interfering components from the strawberry and tomato matrices in a manner that enabled quantitative determination of bioallethrin in the spiked samples even in the presence of high concentrations (28%) of the extract. A quantitative analysis of the bioallethrin recovery from spiked

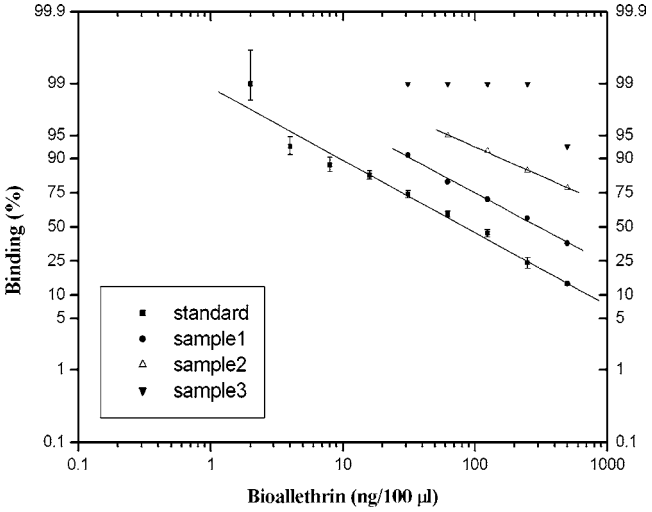


**Figure 4.** Determination of bioallethrin content in spiked acetonic extracts of tomato, before and after sol–gel/solid-phase IAP and concentration. Bioallethrin recovery was monitored by ELISA (performed in PBST plus 5% ethanol). Tomato samples were tested at five serial dilutions ranging from 1:10 to 1:160 (equivalent to 28–1.75% extract). Bioallethrin standard curve (■; $R = -0.98134$; $R^2 = 0.96302$); quality control (●; $R = -0.99714$; $R^2 = 0.99428$); spiked tomato samples after IAP on sol–gel and concentration on Oasis solid-phase (△; R=-0.99754; $R^2 = 0.99508$); and untreated spiked tomato sample (▼). Note that the IAP sample curve parallels the standard curve, whereas the untreated sample curve does not. Each data point represents the mean ± SD ($n = 2$). Results represent one of three independent experiments.

strawberry extracts revealed that bioallethrin could be fully recovered after sol−gel/solid-phase treatment (sample 5 in **Table 4**), in a similar manner to that of standard bioallethrin (sample 2 in **Table 4**) and that the components that interfered with the ELISA could be removed. The solid-phase treatment alone failed to remove the interfering components that impaired the determination of bioallethrin (samples 6 and 9 in **Table 4**), despite the ability of the method to fully recover the bioallethrin standard (sample 3 in **Table 4**). A closer examination of the data showed that the solid-phase-treated samples caused severe background interference in the assay (sample 6 in **Table 4**). This interference was in the same range as that of the untreated spiked or unspiked samples (samples 4 and 7 in **Table 4**) and could not be removed by the solid-phase treatment alone. Sol−gel IAP reduced the background interference significantly (samples 5 and 8 in **Table 4**). No endogenous bioallethrin could be found in the concentrated extract of the unspiked samples that had undergone sol−gel/solid-phase IAP (sample 8 in **Table 4**). The above data clearly indicate that binding of bioallethrin with the sol−gel entrapped Mab was not hampered by the presence of the crude extract and that the method is highly efficient for analyte recovery from the crude acetonic extracts. Similar results were obtained with the tomato acetonic extract.

For further validation of the above data and to prove the chemical identity of the sol−gel/solid-phase-eluted compound, spiked and unspiked strawberry samples were subjected to GC−MS analysis before and after IAP. The above sol−gel/solid-phase experiment was repeated with the strawberry extracts. However, because the GC−MS had a much lower detection level than the ELISA, much smaller amounts of bioallethrin and sol−gel-entrapped bioallethrin Mabs could be used. The experiment was performed in the following manner. Strawberry extract was spiked with 100 ng/mL bioallethrin and was applied to a sol−gel IAP column that contained 10 µL of anti-bioallethrin Mab. The resulting eluate was subjected to solid-phase concentration. Purification of the spiked extract by the solid-phase column alone (i.e., without prior purification on sol−gel) was used to compare the purification efficiency of the solid-phase method with that of the combined sol−gel/solid-phase procedure. A bioallethrin QC sample and unspiked samples that underwent the same treatment served as positive and negative controls as above. All of the samples, together with a bioallethrin standard QC sample, were subjected to GC−MS analysis.

The GC−MS analysis identified *cis*- and *trans*-biollethrin in spiked strawberry samples and in the standard solutions (parts **A** and **C** of **Figure 5**, respectively) as a doublet peak (unresolved peak for *cis*- and *trans*-bioallethrin). The identification of the target analyte was based on the relative retention time to the IS and the major confirmation ions at *m/z* 123 and 136 (**Figure 5D**). Traces of bioallethrin were found in the unspiked sample (**Figure 5B**). Quantitative analysis of the recoveries, however, revealed somewhat lower values than those obtained by ELISA (**Table 5**). Examination of the recovery of bioallethrin from spiked strawberry samples with combined sol−gel/solid-phase columns revealed a recovery of 15% (sample 2 in **Table 5**). A similar efficiency, of 13%, was obtained with standard bio-

**Table 4.** Recovery of Bioallethrin from Spiked Strawberry Extracts Following Sol–Gel IAP[a]

| sample/treatment | recovery (ng) | recovery (%) | Bg $OD_{450\ nm}$ |
|---|---|---|---|
| bioallethrin (standard) | | | |
| (1) untreated bioallethrin standard (QC) | 5000 | 100 | 0.2–0.3 |
| (2) bioallethrin standard after sol–gel and solid-phase concentration | 5400 | 108 | 0.2–0.3 |
| (3) bioallethrin after solid-phase concentration | 4600 | 92 | 0.2–0.3 |
| spiked strawberry extract | | | |
| (4) spiked extract (untreated) | nd | 0 | 0.7–0.8 |
| (5) spiked extract after sol–gel and solid-phase concentration | 7200 | 144 | 0.2–0.3 |
| (6) spiked extract after solid-phase concentration | nd | 0 | 1.2–1.8 |
| unspiked strawberry extract | | | |
| (7) unspiked extract (untreated) | nd | | 1.2–1.8 |
| (8) unspiked extract after sol–gel and solid-phase concentration | <17 | | 0.3–0.4 |
| (9) unspiked extract after solid-phase concentration | nd | | 1.3–2.0 |

[a] Extracts were spiked with 5 µg of bioallethrin and applied on sol–gel columns that contained 100 µL of Mab. The bioallethrin content was determined by ELISA. All samples were tested at five serial dilutions ranging from 1:10 to 1:160 (equivalent to 28–1.75% extract). Recovery was calculated as the ratio (expressed as a percentage) between the eluted amount and the amount applied on the column. The samples in which bioallethrin could not be detected were those in which the pigmentation of the sample generated a high background that masked the ability to detect the enzymatic reaction. Results represent one of three independent experiments. Bg, background; nd, not detected; OD, optical density.

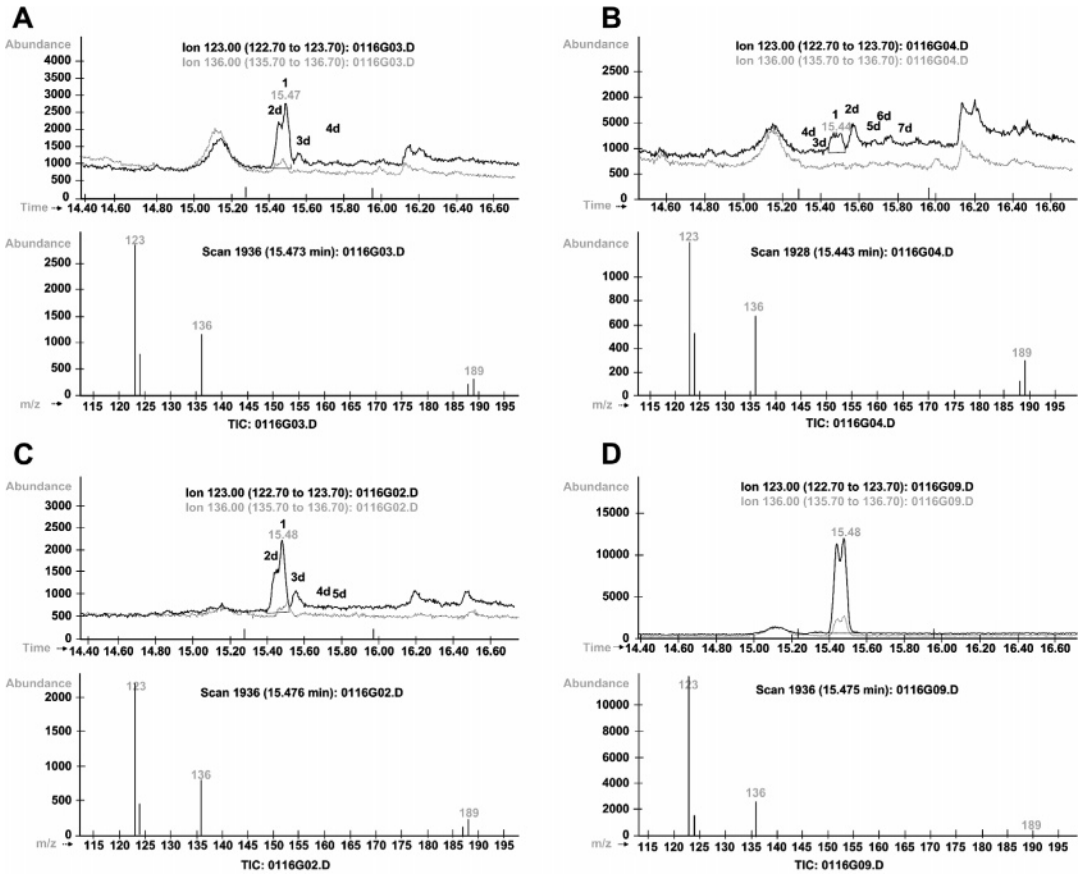


**Figure 5.** GC–MS analysis of spiked (A) and unspiked (B) bioallethrin strawberry samples after sol–gel/solid-phase treatment. Extracts (1 mL, concentrated 2.8 times) were spiked with 100 ng of bioallethrin and applied on a sol–gel column that contained 10 $\mu$L of Mab. Samples were eluted with ethanol, applied on a solid-phase column, and eluted as described in the Materials and Methods. Standard bioallethrin that underwent the same treatment (C) and an untreated bioallethrin standard (QC) sample (D) served as controls. Bioallethrin content was determined by GC–MS. Upper panel, GC chromatogram; lower panel, MS.

**Table 5.** GC–MS Recovery Analysis of Bioallethrin from Spiked Strawberry Extracts Following Sol–Gel IAP and Solid-Phase Concentration[a]

| | | | bioallethrin | |
|---|---|---|---|---|
| sample number | content | treatment | (ng/mL) | recovery (%) |
| 1 | bioallethrin standard | sol–gel plus solid phase | 13 | 13 |
| 2 | spiked strawberry extract | sol–gel plus solid phase | 16 | 15 |
| 3 | unspiked strawberry extract | sol–gel plus solid phase | 3 | 3 |
| 4 | bioallethrin standard | solid phase | 19 | 18 |
| 5 | spiked strawberry extract | solid phase | 49 | 47 |
| 6 | unspiked strawberry extract | solid phase | nd | |
| 7 | standard bioallethrin (QC) | | 104 | 100 |

[a] Recovery is expressed as the ratio (expressed as a percentage) between the amount detected in each sample and that of the QC standard (sample 7). All experimental details are as described in the caption for **Figure 5**. nd = not detectable at the detection limit of the GC–MS (2 ng).

allethrin that had undergone the same treatment (sample 1 in **Table 5**). The recovery of standard bioallethrin that had undergone solid-phase concentration alone was also low (18%, sample 4 in **Table 5**); the recovery of bioallethrin from spiked strawberry extracts with the solid-phase column alone was considerably higher (47%, sample 5 in **Table 5**) than that with the combination of the sol−gel plus solid-phase column. The reason for this is unclear. Note that the sample extracts generated a high background signal in the bioallethrin ELISA preventing quantitation (sample 6 in **Table 4**). As indicated above, our experiments revealed a very high recovery of bioallethrin from both sol−gel and solid-phase columns. One possible explanation for the low recovery in this set of experiments may be that the concentrations of bioallethrin in all of the previous experiments were 10 times higher than those used in this experiment. Thus, it may be that the recoveries obtained with the larger amounts of bioallethrin (3000−5000 ng) are different than those obtained when smaller amounts of the analyte are present. This may occur for either or both of the sol−gel or solid-phase columns.

**4. Determination of Bioallethrin Recovery from Spiked Soil and Dust Samples Using ELISA.** Spiked and unspiked soil and dust samples were analyzed in the ELISA to determine bioallethrin levels and to test for background interferences. All ELISA experimental designs were as described for the fruit and vegetable extracts. The data revealed that unspiked samples did not affect either the background signal or the assay itself. As can be seen in **Figure 6**, spiked soil and dust samples did not interfere with the ELISA performance (as indicated by the parallelism of the sample curves with those of the standard curve). As seen in **Table 6**, the sample contents could be determined with high precision without any further purification.

## DISCUSSION

In the present study, we developed an ELISA and a sol−gel-based IAP for the pyrethroid bioallethrin. The performance of both methods was tested with analytical standards and spiked food (strawberry, tomato, and cucumber), soil, and dust samples.

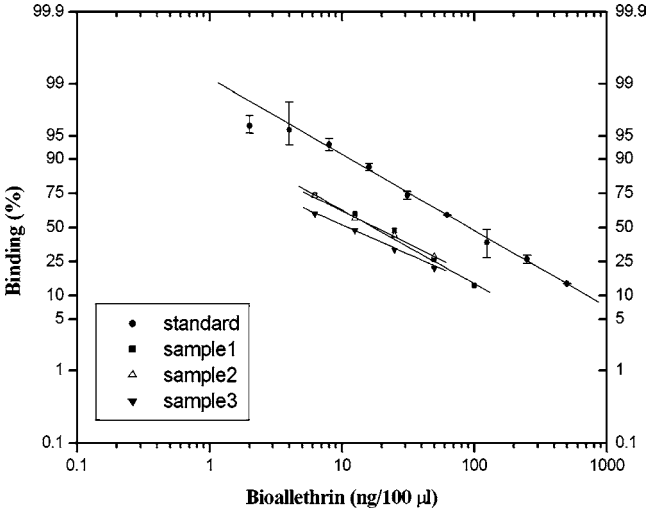


**Figure 6.** Determination of bioallethrin content in spiked soil and dust samples. Dust and soil samples were tested at five concentrations ranging from undiluted to 1:16 (100–6.25% extract). Bioallethrin standard curve (●; $R = -0.99541$; $R^2 = 0.99084$); quality control (■; $R = -0.98851$; $R^2 = 0.97715$); spiked dust 10632MS sample (△; $R = -0.99057$; $R^2 = 0.98122$); and spiked 10798 soil sample (▼; $R = -0.98653$; $R^2 = 0.97324$). Note that all three curves paralleled the standard curve. Each data point represents the mean ± SD ($n = 2$). Results represent one of two independent experiments.

**Table 6.** Recovery of Bioallethrin From Spiked Soil and Dust Samples[a]

| sample code | bioallethrin (ng/mL) | recovery (%) |
|---|---|---|
| QC | 5000 | 100 |
| SOL 10798 | 6000 | 120 |
| IFD 10630 | 5000 | 100 |
| IFD 10632MS | 5000 | 100 |

[a] Amount of spiked bioallethrin: 5000 ng/mL. Recovery is expressed as the ratio (expressed as a percentage) between the amount detected in each sample and that of the QC standard (sample 1). Results represent one of two independent experiments. SOL 10798, soil sample; IFD 10630 and IFD 10632MS, dust samples. All experimental details are as described in the caption for **Figure 6**.

Samples were chosen for this study based on the extensive use of pyrethroids for domestic and agricultural applications, their persistence in soil, and their lipophilicity that enable them to adsorb house dust. Attempts to determine the bioallethrin contents in crude acetonic extracts of strawberry, tomato, and cucumber revealed that the extracts impaired the quantitative validity of the assay (within a working range of 31.25−500 ng/50 $\mu$L of bioallethrin) and necessitated the purification of the analyte prior to the ELISA (**Table 3**). *A* sol−gel IAP was found to be effective for both analyte recovery and removal of interfering components. However, the sol−gel IAP had to be followed by a solid-phase concentration step. This step was necessary because the relatively high volume of eluate from the sol−gel IAP columns had to be reduced for the ELISA analysis; the low tolerance of the ELISA to the eluting organic solvents interfered with the ELISA at concentrations above 5%, necessitating sample dilution; and the high volatility of the analyte did not allow for concentration by evaporation under vacuum or nitrogen. The combination of sol−gel IAP with solid-phase concentration resulted in a high recovery of bioallethrin (87%, **Table 2B**) from spiked strawberry extracts (**Table 4**) and a high efficiency in removal of components that interfered with the ELISA (**Table 4** and **Figures 3** and **4**). Solid-phase treatment alone was not adequate to purify samples. As is clearly indicated in **Table 4**, spiked strawberry samples that were applied on the solid-phase columns without prior purification on the sol−gel column interfered strongly with the assay in a manner that prevented analyte quantification by the ELISA. GC−MS analysis clearly confirmed the chemical identity of bioallethrin, further validating the sol−gel method. Unlike crude fruit and vegetable extracts, soil and dust sample matrices did not interfere with the assay (**Figure 6**) and the bioallethrin content in those samples could be determined with high precision by the ELISA without the need for any further purification (**Table 6**). GC−MS results for the nonspiked soil and dust samples were <1 and 7.3 ng/mL, respectively.

Matrix interference is a major challenge when developing rapid streamlined analytical methods employing minimal sample preparations. Problems are frequently encountered with both instrumental and ELISA methods when analyzing food and environmental samples in which the matrix may quench the signal or adversely influence the analysis in other ways.

A large variety of food, environmental, and biological samples has been tested for pyrethroids or pyrethroid metabolites. Among those are various water samples (river and lake water, industrial tap water, and agricultural runoff) (*19*, *22*, *24*, *25*, *28*, *32*, *52*), urine (*18*, *27*, *29*), milk (*23*), tea (*25*), wine, fruits and vegetables (*20*, *25*), whole or ground wheat or barley grains (*32*, *38*, *39*), and soil (*25*, *32*). Several approaches were used in these studies to cope with the problem of matrix interference with ELISA detection. The most common approaches being sample dilution with an appropriate buffer (*18*, *20*, *27*, *29*, *32*) or SPE using $C_{18}$ (*19*, *24*, *28*, *52*), $C_8$ (*52*), or alumina columns (*39*) or multistep extraction procedures with organic solvents had to be employed (*23*).

Most water samples did not exhibit any matrix interference after a $C_{18}$ SPE and could be tested at concentration factors of 40−50 (*19*, *24*, *25*, *28*, *52*). Urine was more problematic and had to be diluted, usually by a factor of 1:25 to 1:100 (*18*, *29*), to a final concentration of 1−4%. However, in some assays, the urine sample had to be diluted by as much as 1:1000 to a final concentration of 0.1% (*27*) to overcome the matrix interference. In another study, urine samples had to undergo SPE on $C_{18}$ columns (*29*). Although SPE yielded good recovery and reduced the interferences, the method failed to eliminate all matrix effects. Dilution of the concentrated sample prior to the ELISA was required for accurate quantification. Sample dilution was also applied to red and white wine, to methanolic extracts of several fruits and vegetables (i.e., apple, banana, peach, cucumber, lettuce, and onion), and to whole and ground barley and wheat grains (*20*, *32*, *38*, *39*). In most cases, samples had to be diluted by a factor of 1:200 to a concentration of 0.5%; for white wine (*20*) and wheat grains (*32*), a concentration of 10% could be tolerated by the respective ELISAs. The same dilution factor of 1:10 was used for analysis of spiked soil samples (*25*, *32*). None of the above methods could be applied to milk, and samples at a concentration of 2 or 3% had to undergo a multistep extraction with an organic solvent before ELISA detection (*23*).

Our approach to the problem of matrix interference was based on a combination of sol−gel IAP with solid-phase concentration. The method was successful in eliminating matrix interferences and enabling precise analyte quantification and high recovery rates at much higher matrix concentrations than those attainable by the use of dilution or SPE alone. While most of the approaches resulted in extracts that could be tolerated in the

ELISA at single-digit percentage concentrations (e.g., 0.5% for methanolic extracts of food samples, 1−4% for urine, and 10% for soil and methanolic extracts of wheat grain), our approach yielded fruit and vegetable sample extracts that could be tolerated by the ELISA at a concentration of 28% without noticeable interference (**Figures 3** and **4** and **Table 4**). It should be noted that perhaps higher matrix percentages could have been tolerated by the ELISA, but the intolerance of the assay to ethanol concentrations higher than 5% and the inability to evaporate the solvent without the loss of the analyte necessitated dilution of all samples by 1:10 after their elution from the solid-phase columns. The inability to evaporate the samples by evaporation of the eluting solvent also required combining the sol−gel IAP with the solid-phase concentration of the samples. It is important to note that such a combination is not a general practice of the sol−gel IAP methods. All of our previous studies used analytes that could be concentrated by evaporation, and solid-phase concentration was not required. The ability to subject concentrated samples to ELISA detection resulting in highly precise data introduces a major advantage in residue analysis. It also demonstrates that the assay can be applied for accurate quantification of crude acetonic fruit and vegetable extracts without multistep extraction, even when the LOD is relatively high for a particular analyte.

Since our first paper on the employment of sol−gel-based IAP (*46*), the method has been widely applied by many laboratories, including our own, to a variety of environmental analytes and compounds of clinical and forensic interest. For a review, see ref *53*. All of the studies proved that sol−gel-entrapped Abs could serve as highly efficient, reproducible, stable, and reusable IAP devices for purification of environmental, forensic, occupational, and medical samples.

In summary, the significant advantages of the sol−gel method in comparison to other immobilization matrices and methods and the ability to use a wide range of compositions to entrap many different biomolecules introduce a unique, generic, and flexible IAP approach. The sol−gel method can be employed for almost any analyte. This is in contrast to other methods that can be applied to one compound but may be ineffective for others as in the case of basic alumina columns that were able to remove interferences in grain matrices for the pyrethroid permethrin (*39*) but were ineffective with the same matrix in a bioresmethrin ELISA (*38*). This advantage is in addition to those offered by the physical and chemical properties of the sol−gel, such as its amenability to modifications of the properties of the composite, the high biomolecule content that can be loaded onto it, and the improved properties of the entrapped biomolecules (e.g., high stability). Together, these characteristics and advantages result in a unique combination, with an immense application potential for IAP purposes. The sol−gel method can still be further improved in terms of the physical and chemical properties of the matrix. However, current IAP sol−gel technology provides rapid throughput and a high sample load at a low cost. The compatibility with both ELISA and instrumental analytical methods opens a promising novel approach for residue analysis in food and environmental samples. The sol−gel IAP in combination with ELISA may provide an alternative analytical approach for quantitative analysis. It also has the potential for use as a first screen to reduce the number of samples that require subsequent instrumental analyses, facilitating the analysis of a large number of samples that are required for environmental and food monitoring.

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