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Effects of co-existed proteins on measurement of pesticide residues in blood by gas chromatography–mass spectrometry

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ABSTRACT

Accurate measurement of pesticides in biological fluids such as blood is important for quantifying environmental exposures. Beyond sample enrichment and separation, the method presented here is focused on studies of interactions between pesticides and co-existed proteins. It was experimentally demonstrated that entrapped or adsorbed pesticide residues within the folded native structures of proteins were poorly recovered using direct solvent extraction solely. We described here an effective approach termed Enzymatic Digestion-Organic Solvent Extraction (eDOSE) that utilizes the enzymatic approach to disrupt the folded structures of proteins and release entrapped or adsorbed pesticide residues. In this approach, samples were first reduced, alkylated, tryptically digested and then diluted 10 times before the subsequent extraction using an n-hexane solution. Resultant pesticide residues were determined by capillary gas chromatography coupled with a mass spectrometer. Mean recoveries of the 5 organophosphorus pesticides pre-spiked in fish blood including diazinon, parathion-methyl, malathion, parathion-ethyl and ethion were 85%, 95%, 84%, 103%, and 43% respectively using eDOSE strategy but only 24%, 45%, 40%, 27%, and 29% respectively using direct solvent extraction approach. The eDOSE approach was effective for demonstrating the critical role of folded native structure of serum albumin in adsorption of exogenous chemicals. It provides an alterative means for denaturation of proteins when the target analytes are not stable in acidic solution or entrapped within the protein aggregates caused by organic solvents such as acetone that have been applied for protein denaturation. The eDOSE approach should be able to combine with other advanced techniques of enrichment and separation for more efficient and accurate measurement of target compounds present in the context of complex biological systems. This approach can provide wide applications to the analysis of a variety of small molecules including environmental pesticide residues and metabolites as well as other toxins present in cells, tissues and biofluids.

1. Introduction

The wide application of pesticides has attracted more and more efforts on the risk assessment of long-term exposure to trace levels of these chemical pollutants [1–3]. Blood is one of the most accessible biological fluids for determining the physiological status in response to environmental exposures [4–6]. However, analysis of blood is challenging due to the presence of co-existed proteins, lipids, sugars and small metabolites with a huge dynamic range of more than 10 orders of magnitude in concentration.

Gas chromatography and liquid chromatography coupled with mass spectrometry are widely used instrumental approaches for qualitative and quantitative analysis of pesticide residues [7–9]. With the advances in hardware and software development, these instrumental methods are toward high sensitivity and versatility while sample preparation methods are still facing every kind of difficulties. The major problems are the concentration of low abundance pesticide residues and interferences from huge amounts of complex biological matrices [10–12]. The use of tandem MS/MS, Selected Ion Monitor (SIM) or Selected Reaction Monitor (SRM) can reduce matrix effects to some extent by minimizing effects of co-eluting compounds on the ionization processes [13–14]. In the past years, lots of efforts have been focused on the development of sample clean-up and separation techniques. Generally recoveries of pesticide residues can be increased by an improved separation of target analytes and background compounds using liquid–liquid extraction (LLE) [15–17], solid phase extraction (SPE) [18,19], matrix solid phase dispersion (MSPD) [20–21] or additional chromatographic clean-up steps using size exclusion [22–23], ion exchange [24–25] or coupled-column chromatography [26–27]. Isotopically labeled internal standards [28] and matrix-matched standards [10] have also been considered as useful approaches to

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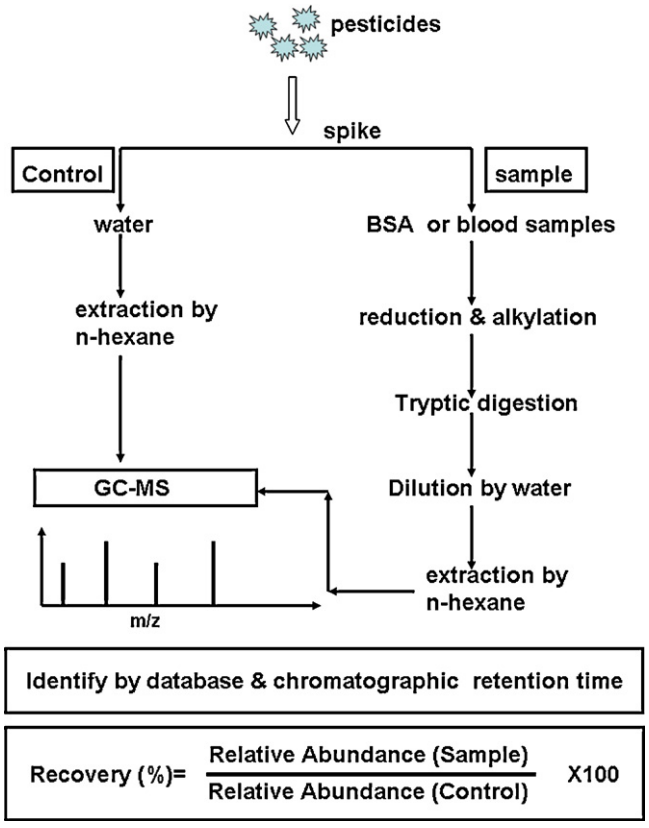

Fig. 1. Overall workflow of eDOSE approach.

compensate the consequences of matrix effects on the reliability of the experimental data. In such cases, major limitations come from the difficulties in compensating different matrices for the analysis of different unknown multi-residues as well as their metabolites. The complex biological background exerts dynamic and versatile effects on co-existed compounds.

In this work, efforts were focused on the studies of interactions between pesticide residues and high abundance proteins present in blood. The mixture of diazinon, malathion, ethion, parathion-methyl and parathion-ethyl was spiked in samples for the evaluation of different sample preparation methods. It has been found that recoveries of target pesticide residues were very low unless the folded native structures of co-existed proteins are chemically and enzymatically disrupted. As shown in Fig. 1, we proposed an approach termed eDOSE (Enzymatic Digestion-Organic Solvent Extraction) that combines protein degradation/denaturation with subsequent dilution and liquid–liquid solvent extraction to demonstrate the critical role of the folded native structure of serum albumin in adsorption and entrapment of exogenous pesticide residues. Because the eDOSE approach is aimed to investigate the effects of co-existed blood proteins on measurement of exogenous pesticide residues, we did not use other techniques for enrichment of targeted compounds in order to avoid additional unexpected adsorption on other sorbents. In this work, organic solvent n-hexane was used for subsequent extraction of targeted compounds after chemical/enzymatic digestion of blood proteins. To quantitatively evaluate the effects of blood proteins, pure water was used as a control. Relative recoveries were defined as the ratios of the relative abundance of target compounds isolated from the samples over that from the control water. This method has been applied to detect the organophosphorus pesticides pre-spiked in blood of *Carassius auratus* fish. It significantly reduces sample losses and improves the recoveries of pesticide residues in the complex biological context of fish blood. Taken together the advantage of other techniques for enrichment and separation, it should be able to efficiently isolate and identify exogenous compounds in blood in even lower level.

2. Experimental

2.1. Chemicals and materials

n-Hexane (HPLC grade) was purchased from Kermel (Tianjin, China). Methanol (HPLC grade), acetone (HPLC grade), NH_4HCO_3, sodium citrate, anhydrous Na_2SO_4 of analytical reagent quality were purchased from Guoyao, China. Non-defatted bovine serum albumin (BSA), dithiothreitol (DTT), iodoacetamide and trypsin were purchased from Sigma–Aldrich (MO, USA). Bradford protein assay kit was purchased from Thermo Scientific (IL, USA). Dimethyl phthalate is of analytical reagent quality and was purchased from ACROS (New Jersey, USA). Water was obtained from a Milli-Q purification system (Millipore, MA). Standard mixture of diazinon, malathion, ethion, parathion-methyl, parathion-ethyl was purchased from Dr. Ehrenstorfer GmbH (Augshurg, Germany).

2.2. Overall workflow of the proposed eDOSE approach

The aim of this work was to study the interactions between high abundance blood proteins and low abundance co-existed small molecules. As shown in Fig. 1, 300 ng organophosphorus pesticides were spiked in 500 μl bovine serum albumin or fish blood as samples. Serum albumin or blood samples were first reduced by DTT (dithiothreitol) and alkylated by iodoacetamide before overnight tryptic digestion. The folded native structures of proteins were extensively destroyed by this chemical/enzymatic digestion approach. In order to further reduce the interactions between peptides and pesticide residues, samples were diluted 10 times with water. In this work, subsequent liquid–liquid extraction using n-hexane was applied to isolate organophosphorus pesticides from the aqueous phase. The resultant mixtures of organophosphorus pesticides were separated and identified by capillary gas chromatography directly coupled with a quadrupole mass spectrometer. Identification of target analytes was achieved by both database searching and comparison of the chromatographic retention time of standards with that of the experimental results. Accurate quantification of each analytes can be obtained using stable isotope labeled internal standards. Due to the availability of all these compounds, dimethyl phthalate was used as an alternative internal standard for relative quantification because it was not found in the original fish blood samples in this work and its chromatographic retention time does not interference with that of target analytes. Relative intensities of each organophosphorus pesticide were determined as the ratios of the peak area of each organophosphorus pesticide acquired in SIM mode over that of the internal standard. As a control, the same amount of organophosphorus pesticides was spiked in pure water and the same procedure as that of blood samples and bovine serum albumin samples was performed. Recoveries of pesticide residues were defined as the ratios of the relative intensities of pesticide residues extracted from blood samples or bovine serum albumin samples over that from the control water.

2.2.1. Sample preparation

The concentration of the stock solution of bovine serum albumin (BSA) was 30 μg/μl in water. Fresh blood was obtained from *C. auratus* fish and 10% sodium citrate was used to avoid blood coagulation. The protein concentration of blood samples was determined by Bradford protein assay protocol according to the guidelines of the provider. All blood samples were diluted with water to reach a concentration of 30 μg/μl. Both BSA solution and diluted fish blood

were pipetted into different clean glass tubes and stored in 500 μl aliquots at −20 °C for subsequent processes. The standard mixture of organophosphorus pesticides including diazinon, malathion, ethion, parathion-methyl, parathion-ethyl was prepared in acetone solution because acetone is miscible with water. The concentration of this standard mixture was 10 ng/μl. In order to demonstrate the effects of the folded native structure of blood proteins on the adsorption of exogenous compounds, 300 ng (30 μl) standard mixtures of these five organophosphorus pesticides were spiked in samples before or after the proteins were reduced, alkylated and tryptically digested.

2.2.2. Chemical and enzymatic disruption of the folded native structures of proteins

Disulfide bonds in serum albumin or other blood proteins were reduced by 15 mM DTT (dithiothreitol) in 20 mM NH_4HCO_3 solution at 37 °C for 1 h and then iodoacetamide was added to reach a concentration of 25 mM. The solution was incubated in dark at room temperature for another 2 h. After the treatment of reduction and carbamidomethylation, blood proteins was further enzymatically cleaved by trypsin at a concentration of 50:1 (protein vs trypsin) overnight in a 37 °C water bath.

2.2.3. Investigation of the interactions of blood proteins with exogenous chemicals by spiking organophosphorus pesticides in native or denatured samples

(1) 300 ng standard mixtures of organophosphorus pesticides were spiked in 500 μl native bovine serum albumin solution (30 μg/μl) or blood samples and extensively mixed by vortexing before they were stored in −20 °C freezer. Then samples were processed by the following procedures individually. (a) The samples without any pre-fractionation were reduced by DTT, alkylated by iodoacetamide and tryptically digested in 20 mM NH_4HCO_3 solution overnight and then further diluted 10 times with water. (b) Instead of enzymatic digestion, water soluble organic solvent acetone was applied to disrupt the folded native structures of proteins. Four times, the sample volumes of cold acetone (−20 °C) were added into the glass tubes and stored at −20 °C overnight. (c) Without disrupting the folded native structures of proteins, direct liquid–liquid extraction of pesticides by n-hexane solvent was performed. For all (a)–(c) samples, 400 μl of n-hexane solution was subsequently added on the top of the sample and then the extraction was performed by 5 min of vortexing and 1 h of shaking at 180 rpm.

(2) In contrast, organophosphorus pesticides were spiked in the solution of tryptic peptides resulting from the chemical/enzymatic disruption of serum albumin or blood proteins followed by the same processes as described previously. As a control, the same amount of pesticides was spiked in 500 μl pure water. Subsequently, the same liquid–liquid extraction procedure was performed to isolate pesticides from the aqueous phase.

For all cases, the glass tubes were allowed to stand for 5 min after liquid–liquid extraction. Then 300 μl of the top n-hexane layers were pipetted into new glass vials and dried. Finally, 70 μl of n-hexane solutions were added to each vial and 5 μl of dimethyl phthalate solution (24 ng/μl in n-hexane) was spiked as an internal standard. Anhydrous Na_2SO_4 was added to absorb trace water in the samples. All prepared samples were stored in −20 °C freezer for further GC–MS analysis.

2.2.4. Comparison of ultrasound-assisted solvent extraction with liquid–liquid solvent extraction by vortexing and shaking

In this work, liquid–liquid extraction of pesticides by n-hexane solvent was performed by 5 min of vortexing and 1 h of shaking at 180 rpm. However, ultrasound-assisted liquid–liquid solvent extraction has also been compared. Glass tubes containing samples and n-hexane solvent were put in a water bath ultrasonicator (100 W, 40 kHz, Kunshan, China) and subjected to ultrasound irradiation. The major concerns of ultrasonic approach are the elevated high temperature and foaming.

2.2.5. Separation and identification of organophosphorus pesticides by GC–MS

1.5 μl of a hexane solution, equivalent to 4.5 ng of organophosphorus pesticides prepared by the described procedures, was separated by capillary gas chromatography (Rtx-5MS, 0.25 μm in thickness, 30 m × 0.25 mm i.d., USA). The column oven temperature was programmed from 60 °C to 150 °C at the rate of 50 °C/min (held at 150 °C for 5 min), and then elevated to 250 °C at the rate of 10 °C/min. Finally, the column was then maintained at 250 °C for 10 min. During the whole process of analysis, the injector temperature was kept at 220 °C. Splitless injection was performed with 1 min sampling time. The capillary gas chromatographic column is directly coupled with a quadruple mass spectrometer (GC-MS QP2010PLUS, Shimadzu, Japan). The interface temperature was set at 270 °C and the ion source was maintained at 200 °C. 70 eV ionization energy of impact ionization (EI) was used to fragment the eluent from capillary GC. Mass spectra were recorded in full scan mode with mass-to-charge ratio (m/z) ranging from 50 to 600 unit.

Organophosphorus pesticides were identified by searching against NIST database and comparing the chromatographic retention time of standard organophosphorus pesticides with that of the experimental results. Supplementary Figure 1 presents the mass spectra of the 5 pesticides and the internal standards in which the ions used for SIM experiments (Selected Ion Monitoring) are indicated.

3. Results and discussion

3.1. Principles of eDOSE strategy

Recoveries of pesticide residues present in complex background of blood are usually low due to a variety of reasons including not only the technical difficulties in enrichment of low abundance pesticide residues but also the elimination of matrix effects. Approaches such as different kinds of liquid–liquid extraction (LLE), solid phase extraction (SPE) or even coupled-column chromatographic methods can generally reduce or avoid matrix effects. But the investigation of interactions between pesticide residues and co-existed biological context and subsequent effects on the accurate measurement of pesticides are still under-represented. The eDOSE approach (as shown in Fig. 1) was aimed to pursue such goals. It is mainly based on the extensive disruption of the interactions by chemical/enzymatic approaches followed by liquid–liquid extraction of target analytes.

Although ultrasound has been widely used to assist solvent extraction, it was observed in this work that the high temperature generated by ultrasonication results in reduced recoveries of organophosphorus pesticide due to hydrolysis (Supplementary Figure 2) and it has also been demonstrated in the literature [29]. Even worse recoveries were obtained when the ultrasonication time was increased to 3 min or longer due to the elevated temperature resulting from ultrasonication. Additionally, it has been observed that ultrasonication causes foam formation (Supplementary Figure 3) in polypeptide or protein solutions because of transportation, penetration and organization of molecules on the air/water interfaces [30], especially for concentrated protein solutions. As shown in Table 1, significantly better recoveries were observed for parathion-methyl, malathion,

Table 1
Analysis of the mixture of 5 standard organophosphorus pesticides spiked in water.

Pesticides	Relative abundance[a] (mean ± SD)	
	Ultrasound assisted liquid–liquid extraction	Liquid–liquid extraction by vortexing and shaking
Diazinon	0.31 ± 0.06	0.34 ± 0.01
Parathion-methyl	0.09 ± 0.01	0.20 ± 0.01
Malathion	0.16 ± 0.04	0.25 ± 0.01
Parathion-ethyl	0.19 ± 0.03	0.30 ± 0.01
Ethion	0.31 ± 0.10	0.35 ± 0.02

[a] Relative abundance was defined as the ratio of the target compound over that of the internal standard acquired in Selected Ion Monitoring (SIM) mode. Numbers of replicates: $n = 3$.

parathion-ethyl of which the relative intensities are 0.20, 0.25, 0.30 respectively by gentle vortexing and shaking in comparison with 0.09, 0.16, 0.19 respectively using ultrasound assisted extraction method. Better precision was also observed for vortexing and shaking method that was demonstrated by the standard deviations ranging from 0.01 to 0.02 for 5 spiked pesticides. Standard deviations ranging from 0.01 to 0.10 were obtained for ultrasound assisted method. Therefore the liquid–liquid extraction was performed by gentle vortexing and shaking in the following work.

3.2. Analysis of the 5 organophosphorus pesticides spiked in native or denatured bovine serum albumin solution

As we know, small molecules, especially insoluble exogenous chemicals such as pesticide residues, interact with different blood proteins and are transported around the body mainly by serum albumin, one of the most abundance proteins present in blood. Serum albumin was thus used as a model in this work for studies of the effects of proteins on the measurement of pesticide residues in blood. In order to investigate if the folded native structures of proteins exert detrimental effects on the measurement of co-existed small molecules, organophosphorus pesticides including diazinon, malathion, ethion, parathion-methyl, parathion-ethyl were spiked to native or denatured bovine serum albumin solutions.

Fig. 2(A)–(C) is the representative TIC (Total Ion Chromatogram) of the isolated organophosphorus pesticides that have been spiked in the original native solution, tryptic polypeptide solution, and 10 times diluted tryptic polypeptide solution of bovine serum albumin respectively. Table 2 summarizes the recoveries of these 5 organophosphorus pesticides. Experimental results indicated that poor recoveries ranging from 30% to 56% were obtained by direct extraction from the native folded albumin solution. Recoveries were significantly improved ranging from 88% to 112% when pesticides were spiked after the native folded structures of proteins were destroyed chemically/enzymatically. Further dilution of the resultant polypeptide solution did not lead to significantly greater recoveries ranging from 94% to 112%. The striking differences of

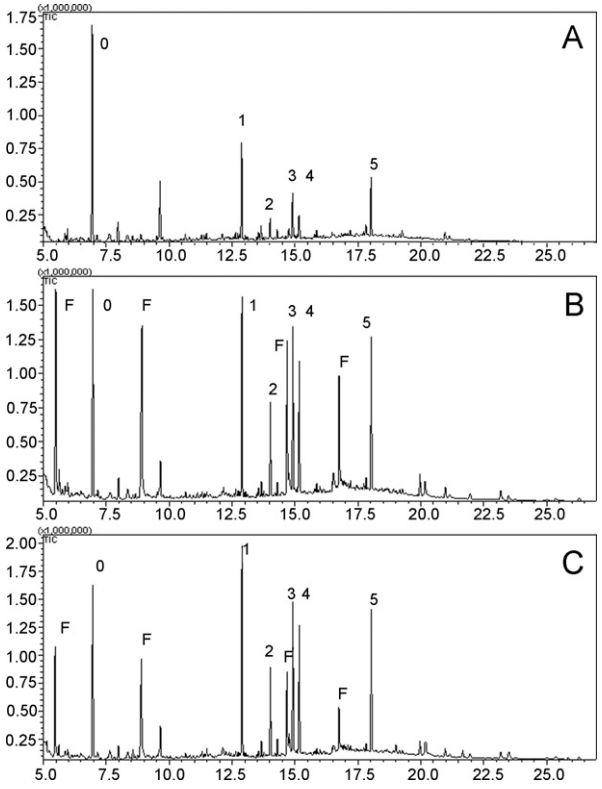

Fig. 2. Representative Total Ion Chromatogram (TIC) of isolated organophosphorus pesticides spiked in different solutions of bovine serum albumin (30 μg/μl). (A) Original native bovine serum albumin; (B and C) tryptic polypeptide solutions of bovine serum albumin that have been subjected to reduction, alkylation, and tryptic digestion. (B) Without dilution with water. (C) 10 times diluted with water. All extractions were performed by gentle vortexing and shaking. 0: internal standard dimethyl phthalate; 1: diazinon; 2: parathion-methyl; 3: malathion; 4: parathion-ethyl; 5: ethion; F: free fatty acids.

recoveries of pesticides spiked in the solution of folded native albumin and the solution of polypeptides have experimentally demonstrated the detrimental effect of albumin–pesticide interactions on the measurement of pesticides. When the folded native structure of albumin was destroyed chemically and enzymatically, this effect was minimized. It should also be noted that free fatty acids were observed in tryptically digested solution (as indicated in Fig. 3). These free fatty acids maybe released from complexes of serum albumin when the complexes were destroyed enzymatically [31]. It provides another evidence for the presence of an interacted network in the biological system.

Inversely, when pesticides were pre-spiked in folded native albumin solution, we have also demonstrated that afterward reduction, alkylation, enzymatic digestion and dilution of albumin solution were also effective to recover target compounds

Table 2
Relative recoveries of organophosphorus pesticides spiked in different solutions.

Pesticides	RA[a] (mean ± SD)				Relative recoveries (%)[b]		
	Water	BSA	Digested BSA	Digested and diluted BSA	BSA	Digested BSA	Digested and diluted BSA
Diazinon	0.34 ± 0.01	0.19 ± 0.02	0.30 ± 0.04	0.34 ± 0.03	56	88	100
Parathion-methyl	0.20 ± 0.01	0.06 ± 0.01	0.18 ± 0.03	0.19 ± 0.02	30	90	95
Malathion	0.25 ± 0.01	0.12 ± 0.02	0.28 ± 0.00	0.28 ± 0.01	48	112	112
Parathion-ethyl	0.30 ± 0.01	0.09 ± 0.02	0.31 ± 0.02	0.32 ± 0.02	30	103	107
Ethion	0.35 ± 0.02	0.17 ± 0.04	0.34 ± 0.02	0.33 ± 0.05	49	97	94

[a] RA: relative abundance was defined as the ratio of the target compound over that of the internal standard acquired in Selected Ion Monitoring (SIM) mode. Numbers of replicates: $n = 3$.
[b] Relative recoveries were determined as the ratios of the relative abundance of target compounds isolated from the samples over that isolated from the control water.

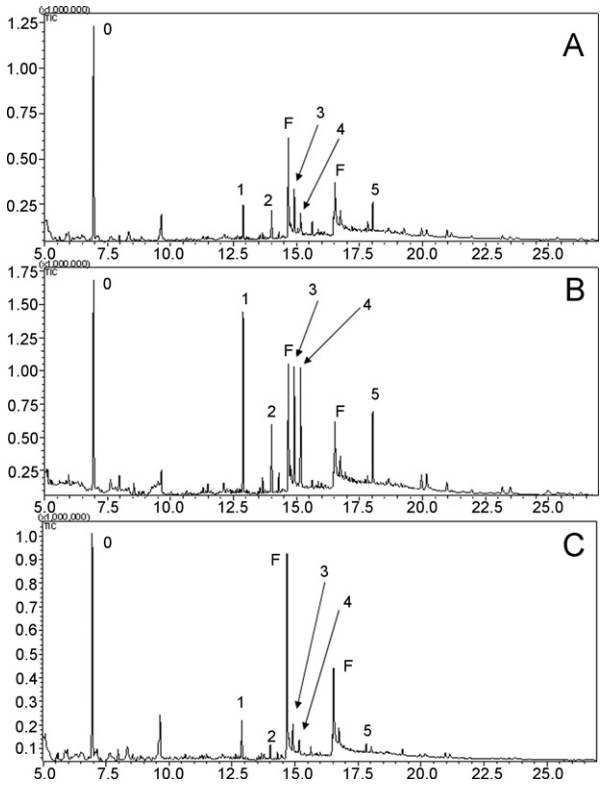

Fig. 3. Representative Total Ion Chromatogram (TIC) of isolated organophosphorus pesticides pre-spiked in blood of *Carassius auratus* fish using different approaches. (A) Direct extraction by n-hexane solvent; (B) extraction by n-hexane solvent after folded native structures of blood proteins were disrupted chemically and enzymatically; (C) extraction by n-hexane solvent after folded native structures of blood proteins were disrupted by water soluble organic solvent acetone. All extractions were performed by gentle vortexing and shaking. 0: internal standard dimethyl phthalate; 1: diazinon; 2: parathion-methyl; 3: malathion; 4: parathion-ethyl; 5: ethion; F: free fatty acids.

(Supplementary Figure 4 and Supplementary Table 1). It was shown that afterward treatment results in similar recoveries ranging from 85% to 109% as that of pre-treatment. Further dilution of the tryptic peptides slightly affects the recoveries of target compounds.

3.3. Analysis of the 5 organophosphorus pesticides spiked in blood of C. auratus fish

The composition of blood proteins and other co-existed molecules is much more comprehensive than that of pure albumin solution. Blood proteins are capsules for a variety of small molecules to transport around the body. Similar procedures as described previously were performed on whole fish blood without any pre-fractionation. As shown in Supplementary Figure 3, clear separation between the n-hexane phase and the aqueous phase was obtained when the extraction of pesticide residues from the blood sample was performed by vortexing and shaking after chemical and enzymatic treatment. Foam formation was observed by ultrasonication even after chemical and enzymatic treatments. Without degradation of the folded native structures of blood proteins, there was even more serious foaming by either ultrasonic extraction or direct solvent extraction. Table 3 shows that recoveries of target pesticide residues were significantly improved 1.5–3.8 times using eDOSE approach in comparison with direct extraction approach. 4 of the 5 organophosphorus pesticides achieved more than 80% recoveries except for ethion that has only 43% recovery due to unknown reasons [32].

Acidic solution and organic solvent have been routinely used for protein denaturation [33]. The eDOSE approach provides an alternative choice for dealing with acid-labile pesticides or entrapment of target analyses within protein aggregates caused by organic solvents. In this work, 4 times the blood sample volume of cold acetone was added to the sample tube before direct extraction by n-hexane solution. Experimental results as shown in Figure 3 and Table 3 indicated that there were decreased recoveries using acetone to unfold native blood proteins compared with that of chemical and enzymatic treatment. Although organic solvents are able to disrupt the native structures of proteins, co-precipitation and adsorption of pesticide residues within protein aggregates result in losses of target compounds. Moreover, addition of acetone increases the solubility of target compounds in the aqueous phase and causes decreased recoveries in the n-hexane phase.

The experimental results demonstrated that eDOSE strategy is an effective method to disrupt the folded native structures of blood proteins and is helpful for isolating exogenous compounds from complex biological context of blood. The complex matrices of biological samples exert versatile effects on the target compounds not only by an ion suppression in instrumental detection but also by a complex interaction with target compounds. Using chemical and enzymatic approaches, eDOSE strategy has demonstrated that target compounds can be entrapped or adsorbed within the folded native structures of blood proteins. Without disruption of the folded native structures of proteins, it is limited to efficiently recover target compounds by direct organic solvent extraction. In eDOSE strategy, enzymatic digestion in combination with generic solvent extraction was demonstrated to be capable of destroying the 3D structures of proteins and improving the recoveries of target compounds from complex matrices. The major advantages of eDOSE strategy are summarized in the following: (1) It is simple and effective, and no special apparatus is needed. The whole blood can be readily digested chemically and enzymatically without any

Table 3
Relative recoveries of 5 organophosphorus pesticides spiked in fish blood using different methods.

Pesticides	RA[a] (mean ± SD)			Recoveries (%)[b]		
	Direct extraction	Extraction after digestion	Extraction after acetone denaturation	Direct extraction	Extraction after digestion (ratio to direct extraction)	Extraction after acetone (ratio to direct extraction)
Diazinon	0.08 ± 0.03	0.29 ± 0.02	0.13 ± 0.03	24	85 (3.5)	38 (1.6)
Parathion-methyl	0.09 ± 0.05	0.19 ± 0.01	0.14 ± 0.03	45	95 (2.1)	70 (1.6)
Malathion	0.10 ± 0.04	0.21 ± 0.02	0.18 ± 0.03	40	84 (2.1)	72 (1.8)
Parathion-ethyl	0.08 ± 0.03	0.31 ± 0.02	0.16 ± 0.03	27	103 (3.8)	50 (1.9)
Ethion	0.10 ± 0.03	0.15 ± 0.03	0.07 ± 0.03	29	43 (1.5)	20 (0.7)

[a] RA: relative abundance was defined as the ratio of the target compound over that of the internal standard acquired in Selected Ion Monitoring (SIM) mode. Numbers of replicates: $n = 3$.
[b] Relative recoveries were determined as the ratios of the relative abundance of target compounds isolated from the samples over that isolated from the control water.

fractionation and special treatment. (2) The recoveries of target compounds were greatly improved using eDOSE strategy compared with that of direct solvent extraction. It provides an alternative approach for denaturing proteins and releasing entrapped target compounds. (3) GC-based analysis provides the second dimension to further reduce the interferences from the complex biological context. The resultant polypeptides and co-existed sugars as well as other hydrophilic metabolites are not able to be extracted into non-polar organic solvents such as n-hexane that were eventually injected into GC–MS system for further separation and identification. (4) The eDOSE approach should be able to combine with other separation and enrichment techniques and bring out further improvement of recoveries. (5) The Selected Ion Monitoring (SIM) mode provided by mass spectrometers is a useful mass-selective detection method that further reduces the interferences from matrices. Finally, this eDOSE strategy has experimentally demonstrated that blood proteins do not just play important roles in many biological processes. The folded native structures of blood proteins actually entrap and adsorb exogenous and endogenous molecules and transport them around the body. The combination of eDOSE approach with advanced separation and enrichment techniques should provide a new avenue for more efficient and accurate measurement of target compounds in the complex context of blood.

4. Conclusion

The experimental results illustrate the perspective applications of the eDOSE strategy to the analysis of a variety of chemicals present not only in blood but also in cells, tissues and other biofluids. Enrichment and separation methods alone are limited to release analytes from the folded native structures of proteins. The eDOSE method has demonstrated the critical role of the folded native structures of blood proteins in adsorption and entrapment of exogenous compounds. Interferences from blood matrices can be minimized by chemical and enzymatic disruption of the folded native structures of blood proteins in combination with GC–MS based detection and SIM experiments of the mass spectrometer. Another reason that the eDOSE approach can greatly avoid the interferences from the context of blood faced by LC–MS based detection is that huge amounts of hydrophilic molecules cannot be extracted into the organic phases. Therefore, there are fewer possibilities for them to suppress the target analytes. Moreover, SIM experiment further solves the matrix effect by a mass-selective manner. In combination with other separation and enrichment approaches, eDOSE strategy should be useful to monitor the biological exposures to environmental pesticide residues and other toxins. Additionally, eDOSE approach also provides a new tool to investigate the interactions between proteins and small molecules that have been involved in many aspects of biological processes.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jchromb.2010.09.013.

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