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Fast agitated directly suspended droplet microextraction technique for the rapid analysis of eighteen organophosphorus pesticides in human blood

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ABSTRACT

A new sample preparation technique named as fast agitated directly suspended droplet microextraction (FA-DSDME) was proposed as an improved version of directly suspended droplet microextraction (DSDME) for the extraction and pre-concentration of wide-range organophosphorus pesticides (OPPs) from human blood prior to liquid chromatography tandem mass spectrometric (LC–MS/MS) analysis. In this method, instead of protecting the unwanted rupturing of extraction droplet (organic solvent), it was deliberately splintered into fine droplets by providing automated high-speed agitation to the biphasic extraction system (extraction solvent and sample solution). Fine organic droplets were then recollected into one, not by using a centrifuge machine but just by giving a very slow stirring to the bottom of the extraction system. The present method has surmounted the problem of prolonged extraction time associated with old DSDME. Under optimum extraction conditions, the method showed good sensitivity with low detection limits ranging from 0.0009 to 0.122 $\mu g\,L^{-1}$. Mean recoveries were achieved in the range of 86–109% at three levels of spiking concentration (low, middle and high) from linearity range of individual analyte. Intra-day and inter-day precisions were ≤4.68 and ≤9.57 (%RSD) respectively. Enrichment factor (EF) for each analyte varied from 30 to 132 which prove the ability of this technique to pre-concentrate the extracted analytes up to a good extent. The sample matrices have shown an insignificant influence on method's sensitivity. The proposed method may find immense use in epidemiological, toxicological, regulatory and forensic laboratories.

1. Introduction

Among all classes of pesticides, organophosphorus pesticides (OPPs) are majorly affecting our population, leading to lethality and persistent health problems [1–3]. Especially in Asian countries, pesticide poisonings (intended and accidental), are quite common due to easy accessibility to pesticides [2]. As a result of ceaselessly increasing poisoning problems due to OPPs, there is an urgent need to develop a sensitive, reliable and cost-effective sample preparation method for their trace analysis in biological fluids. A number of sample preparation methods have been reported for the quantitative evaluation of pesticides in biological samples [4–12]. However, most of them suffer from disadvantages as they involve multiple steps viz. de-proteinization, plasma separation, multistep extraction to achieve good recovery, concentrating large volumes of extraction solvent, making them expensive and time consuming [13]. A few years ago, a novel microextraction technique named as dispersive liquid liquid microextraction (DLLME) was developed involving a rapid injection of a mixture of extraction solvent and dispersive solvent into the aqueous sample solution. The rapid injection caused the cloud formation consisting of infinitely fine droplets of extractant diffused entirely into the donor phase, leads to instantaneous extraction of targeted compounds [14]. Certainly, DLLME is a sensitive and fast pre-concentration technique but the major limitations are; firstly, the DLLME process requires halogenated extraction solvents, hazardous to the user and

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environment. Secondly, the extra use of polar co-solvents (dispersive solvent) caused the extraction solvent and/or targeted analytes to solubilize into the sample solution itself, which would generate unwanted variations among successive experiments [15]. Thirdly, it requires centrifugation to separate the extracted layer from sample solution which unnecessarily enhanced the processing time. Furthermore, DLLME is entirely a manual process and all efforts made to automate the process have not yet been achieved beneficial results [16–18].

Recently, air assisted liquid liquid microextraction (AALLME) – a new pre-concentration method came into the existence, which overcomes the unnecessary utilization of dispersive solvent in DLLME process. This technique involves the repeated syringe plunging of extractant and the sample solution for achieving immediate extraction [15]. Although, the use of dispersive solvent has been diminished but all other demerits remained the same. And the processing becomes much tiring due to repeated injection, might not be a suitable alternative. To circumvent these problems, there is a need to develop an automated pre-concentration method such as directly suspended droplet microextraction (DSDME); one of the valuable modifications which was started progressively since after the development of liquid phase microextraction, LPME in 1996 [19–21]. Unfortunately, DSDME suffered with the drawback of unwanted dislodgement of extraction solvent's droplet at high agitation speed makes it very tricky to recollect completely. And the concluding results vary according to the pattern in which the solvent's droplets are dispersed and collected.

Providentially, it was confirmed with the above-mentioned techniques that rapid agitation works as a crucial parameter in the extraction process. Thus, a new advancement was made to get a better extraction in a very short time via deliberately spattering and rejoining of organic droplets at varying but automated agitation of the sample solution and then solidification of the extracted droplet for easy collection. Most of the shortcomings of DLLME, AALLME and DSDME technique were approached to surmount in the present study. The proposed method 'Fast Agitated Directly Suspended Droplet Micro Extraction (FA-DSDME)' chiefly involves the combination of two microextraction techniques, i.e. DSDME/LPME and solidified floating organic drop microextraction (SFODME) [22–29]. The key benefits of this innovative technique are (i) the automated agitation of binary liquid extraction system which tends to minimize the inaccuracy of the results, occurred due to manual processing, (ii) unlike DLLME and AALLME, temperature support can also be employed to further enhance the extraction efficiency, (iii) the controlled stirrings for splitting and rejoining the organic droplets have avoided the use of dispersive solvents and also unnecessitate the use of centrifuge machine, (iv) it's application does not require any prior treatment i.e. de-proteinization/plasma separation, on blood sample, and (v) the entire process involves only one step to extract the targeted analytes as well as to separate and pre-concentrate the extracted phase.

2. Experimental

2.1. Chemicals and materials

Analytical grade standards of all organophosphorus pesticides (chlorpyrifos, chlorpyrifos-methyl, dichlorvos, dimethoate, fonofos, ethion, malathion, methidathion, monocrotophos, paraoxon-methyl, phorate, phorate sulfone, phorate sulfoxide, phosalone, pirimiphos-ethyl, pirimiphos-methyl, quinalphos and triazophos) of highest purity (>99.9%) were procured from Sigma Aldrich (Bellefonte, PA, USA). All solvents (1-dodecanol, 2-dodecanol, 1-undecanol, n-hexadecane and methanol) were from Merck (Darmstadt, Germany). Glass vials of 7.0 mL capacity, magnetic stir fleas and micro-syringe (50 μL) were obtained from Sigma Aldrich (Bellefonte, PA, USA). Electronically controlled temperature and stirring module was purchased from Sigma Aldrich. Ultra pure water was obtained from in-house water purification system having conductivity of 18 mΩ (Milli-Q, Millipore Corp., MA, USA). Human blood samples were collected from the villagers at the time of pesticides spraying on agricultural fields in or around Lucknow city after obtaining approval from Institutional Human Ethics Committee and informed consent from the subjects. All the samples were stored at −20 °C until processed for the analysis. No minors/children participants were involved in this study.

2.2. Preparation of standard solution

An individual standard stock solution of 1000 μg mL^{-1} was prepared by dissolving accurately weighed (10 mg) individual pesticide in 10 mL of methanol. The working standard solutions of lower concentration (0.1 μg mL^{-1}) were prepared by diluting successively with methanol. All the standards were stored in a refrigerator at 4 °C, when not in use.

2.3. LC–MS/MS conditions

Liquid chromatographic analysis was performed on UPLC system (Acquity-Waters, Miliford, USA) coupled to an API-4000 mass spectrometric system (AB ScieX) with an electrospray ionization (ESI) source. Analysis was done within 2.0 min on a Acquity UPLC® BEH C-18 column (50 mm × 2.1 mm, 1.7 μm particle size) under an isocratic elution of mobile phases, 5% – A (0.1% formic acid in water) and 95% – B (0.1% formic acid in methanol) at a constant flow of 0.3 mL/min. Definite sample volume (10 μL) was injected using an auto sampler of the UPLC.

Instrumental processing was controlled by Analyst™ software (Version 1.4.1, AB ScieX, Foster City, CA, USA). The ESI was operated in positive mode with the source temperature at 300 °C, and source voltage at 5500 V. The nebulizer gas (GS1), turbo gas (GS2), collisionally activated dissociation gas (CAD) and curtain gas (CUR) were programmed at 40 psi, 60 psi, 8 psi and 10 psi respectively. Acquisition was performed over three time periods for each sample. The mass spectrometric analysis was executed in selected reaction monitoring (SRM) mode by keenly optimizing the dwell time varied from ≥15 to ≤50 ms as shown in Table S1 (Supplementary data). The suitable dwell times were adjusted in order to maintain appropriate number of data points (≥13) per chromatographic peak. The pause time and target scan time were set to 3.0 ms and 1.0 s correspondingly. Optimal conditions for the dependent characteristics of the mass spectrometer for qualitative and quantitative analysis of individual analyte are summarized in Table S1. Each analyte was quantified on the basis of quantifier ion (high intensity peak), which was presented in bold letters in Table S1.

2.4. Data handling and processing

The results obtained from chromatographic analysis based on the peak area for individual analyte, were evaluated and tested using, Microsoft Excel, 2007. Quantization was done by inferring the integrated peak areas of individual analyte of unknown concentration into the calibration graph formula of their respective analyte of known concentration. Using paired *t*-test, *p*-values were calculated which were if found <0.05, then only can consider to be insignificants the differences among repetitive tests ($n-3$ or more). Otherwise, the tests were regarded as not valid to rely on their executed interpretations.