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An improved dispersive solid-phase extraction clean-up method for the gas chromatography–negative chemical ionisation tandem mass spectrometric determination of multiclass pesticide residues in edible oils

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ABSTRACT

An improved sample preparation using dispersive solid-phase extraction clean-up was proposed for the trace level determination of 35 multiclass pesticide residues (organochlorine, organophosphorus and synthetic pyrethroids) in edible oils. Quantification of the analytes was carried out by gas chromatography–mass spectrometry in negative chemical ionisation mode (GC–NCI-MS/MS). The limit of detection and limit of quantification of residues were in the range of 0.01–1 ng/g and 0.05–2 ng/g, respectively. The analytes showed recoveries between 62% and 110%, and the matrix effect was observed to be less than 25% for most of the pesticides. Crude edible oil samples showed endosulfan isomers, *p,p′*-DDD, α-cypermethrin, chlorpyrifos, and diazinon residues in the range of 0.56–2.14 ng/g. However, no pesticide residues in the detection range of the method were observed in refined oils.

1. Introduction

Oil is one of the important ingredients used in the preparation of our food. The edible oils are rich in saturated and unsaturated fatty acids, triglycerides, antioxidants such as tocopherols, and other fat soluble vitamins (Gunstone, 2011). Pesticides such as organophosphates, carbamates and synthetic pyrethroids are used worldwide to improve the productivity of crops. The used pesticides often contaminate the seeds and hence will be extracted into the oils during the extraction process. Although chlorinated pesticides are banned in most countries, they are persistent pollutants in the environment, due to previous applications. Pesticide contamination of oils possesses several health hazards and reduces the export quality. Hence, it is very important to monitor the levels of pesticide residues in crude and refined edible oils.

Some countries have established maximum residue limits (MRLs) for each pesticide residue in different edible oils (crude and refined). The European Union standards (http://ec.europa.eu/sanco_pesticides/public/index.cfm?event=substance.resultat&s=1), the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) (http://www.codexalimentarius.net/pestres/data/pesticides/index.htmL) have defined MRLs in edible oils for various pesticide residues in the range of 0.002–2 mg/kg. However, MRL values for pesticides in edible oils have not yet been formulated in several countries including India.

Determination of pesticide residues in fat/lipid matrices has been reported in some recent reviews (Chung & Chen, 2011; García-Reyes et al., 2007; Gilbert-López, García-Reyes, & Molina-Díaz, 2009). Sample preparation is the crucial step in the methodology as the extraction of hydrophobic molecules from hydrophobic matrices is tedious work. Several sample preparation methods, such as liquid–liquid extraction (LLE), solid-phase extraction (SPE), dispersive solid-phase extraction (DSPE), matrix solid-phase dispersion (MSPD) and QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) have been reported for extraction of pesticide residues from oil samples. Comparative studies on the efficiency of different sample preparation techniques for extraction of pesticide residues from fat/lipid matrices have also been reported (Gilbert-López, García-Reyes, Fernández-Alba, & Molina-Díaz, 2010a; Lacina et al., 2012; Muhamad, Zainudin, & Abu Bakar, 2012; Łozowicka, Jankowska, Rutkowska, & Kaczynski, 2009).

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Some laborious extraction methods based on LLE followed by SPE clean-up procedures for the determination of organochlorine pesticides (OCP) in various oils have been reported (Roszko, Szterk, Szymczyk, & Waszkiewicz-Robak, 2012). Among the described extraction methods, solvent extraction (SE) followed by low-temperature fat precipitation (LTFP) is still the most preferred method (Jiang, Li, Jiang, Li, & Pan, 2012; Muhamad et al., 2012; Nguyen, Lee, & Lee, 2010; Sobhanzadeh & Nemati, 2013). The application of QuEChERS procedures (Cunha et al., 2007; Sack et al., 2011) after SE, followed by LTFP showed highly promising results when compared to other methods. Although methods like MSPD, DSPE, SPE, microwave-assisted extraction (MWE) and headspace solid-phase microextraction (SPME) have been reported, they have some important limitations. MSPD methods are not applicable for a large volume of matrix as it proportionately needs large amount of extraction phase. Headspace SPME methods are only useful for volatile pesticides and the recovery is very low. The fibres are easily saturated by oil vapours and their stability decreases over time. Although DSPE is a simple and efficient method for extraction of analytes from aqueous media, its application to hydrophobic and viscous media like oils does not provide encouraging results, due to the poor movement of extraction media in the matrix and the reduction in its active surface area due to irreversible coating of matrix on the surface. However, DSPE can be used as an efficient clean-up method for removal of interfering substances from the matrix after solvent extraction, by applying suitable sorbents, such as primary secondary amine (PSA) and graphitized carbon black (GCB) as described in QuEChERS method. A large amount of oil is often retained on SPE phases, which consists of some polar fatty acids and glycerides and their clean-up is often very difficult. Hence, the recovery of the analytes in SPE is often low and hence is not frequently used.

Quantification of the pesticide residues is often performed by sensitive and selective techniques such as gas chromatography (GC) with electron capture detector (ECD), flame photometric detector (FPD), nitrogen phosphorus detector (NPD), and mass (MS)/tandem mass spectrometric detection (MS/MS) operated in electron impact ionisation (EI) and chemical ionisation (CI) modes. Liquid chromatography (LC)–mass spectrometry (MS) and gel permeation chromatography (GPC) have also been employed for the determination of polar pesticide residues (Botitsi, Garbis, Economou, & Tsipi, 2011; Chung & Chen, 2011). Often the LC–MS-based analysis methods have suffered interference from matrix compounds and furthermore the sensitivities of the methods were low. Although LC–MS-based analytical methods are well suited for polar pesticides, the preferred methods for analysis of non-polar pesticides such as organochlorines are still GC-ECD and GC–MS. The selectivity of the ECD is often low in the presence of interfering chemicals and hence unambiguous quantification of the pesticide residues is often not possible. GC–MS/MS methods offer more specificity and sensitivity and hence are more suitable for unambiguous quantification. Among the ionisation methods for GC–MS, CI is reported to be more suitable for the analysis of synthetic pyrethroids (SP) and organochlorine (OC) pesticides (Chaler, Vilanova, Santiago-Silva, Fernandez, & Grimalt, 1998; Feo, Eljarrat, & Barceló, 2011; Sichilongo, 2004). Negative chemical ionisation (NCI) is highly sensitive for those chemicals that possess elements/groups with high electron affinities. Hence the interferences from fatty acids in NCI mode are less when compared to positive chemical ionisation (PCI) and EI modes.

In the present work, an efficient sample preparation method based on solvent extraction followed by rapid low-temperature fat precipitation (RLTFP) and an improved DSPE clean-up procedure for the trace level determination of multiclass pesticide residues in three edible oils (sunflower oil, ground nut oil and rice bran oil) was proposed. The quantification of pesticide residues was carried out by GC–MS/MS in NCI mode using ammonia as reagent gas.

2. Materials and methods

2.1. Chemicals and Apparatus

Pesticides (organochlorine pesticides (ocs), such as α, β, γ and δ HCH isomers, α and β endosulfan isomers, α and γ chlordane isomers, heptachlor, heptachlor exoepoxide (HEE), *p*,*p*′-DDT, *p*,*p*′-DDD, *p*,*p*′-DDE, aldrin, endrin, dieldrin, endrin aldehyde, endrin ketone; organophosphorus (OP) pesticides, such as dichlorvos, phorate, diazinon, methyl parathion, fenitrothion, malathion, chlorpyrifos, parathion-ethyl, quinalphos, phosmet, profenofos, phosalone; synthetic pyrethroids (SP), such as allethrin, α-cypermethrin, β-cyfluthrin, flumethrin isomers and deltamethrin isomers), internal standard (IS) pentachlorobenzene (PCB), anhydrous reagent-grade magnesium sulfate ($MgSo_4$), and sodium chloride (NaCl) were purchased from Sigma–Aldrich (Bengaluru, KA, India). Optima LC–MS grade acetonitrile (ACN), methanol and ethyl acetate (EA) were purchased from Thermo Fisher Scientific (Fair Lawn, NJ). Analytical reagent-grade acetone was obtained from Merck (Mumbai, MH, India). Primary secondary amine (PSA) was purchased from Agilent technologies (Santa Clara, CA). Activated charcoal (AC, particle size <200 nm) was purchased from SD Fine Chemicals Ltd. (Mumbai, MH, India). The CI reagent gases methane, isobutane and ammonia were purchased from Bharuka Gases (Bengaluru, KA, India). N-Evap nitrogen evaporator was purchased from Organomation Associates Inc. (South Berlin, MA).

2.2. Preparation of stock solutions and working standards

Individual standard stock solutions were prepared at 1 mg/mL by dissolving 10 mg of each pesticide and internal standard PCB in 10 mL of ACN and were stored at −20 °C until use. Working standard solutions of all pesticides mixture were prepared at 4000, 2000, 1000, 400, 200, 100, 40, 20, 10, 4, 2, 1, 0.4 and 0.2 ng/mL concentration levels by adding appropriate aliquots of individual pesticide stock solutions. These concentrations were used for spiking the samples as and when needed.

2.3. Fortification and extraction procedure

Five grams of homogeneous blank edible oil (sunflower oil, rice bran oil and groundnut oil) samples were transferred into 50-mL centrifuge tubes. The samples were fortified with appropriate amount of pesticides solutions to produce final concentrations equivalent to 200, 100, 50, 20, 10, 5, 2, 1, 0.5, 0.2, 0.1, 0.05, 0.02 and 0.01 ng/g. Internal standard (PCB) was added to the samples at a fixed concentration of 1 ng/g. The fortified samples were mixed thoroughly by vortex mixer for 3 min and equilibrated at room temperature for 1 h. The analytes from the fortified samples were extracted with 10 mL of acetonitrile (ACN) by shaking them manually for two minutes and mixed vigorously by vortex mixer for 5 min. The samples were centrifuged at 10,000 rpm for 5 min and were placed in a dry ice bath containing acetone for 10 min for RLTFP. The supernatant ACN layer was collected immediately into 20-mL glass test tubes. The solvent was evaporated to dryness under gentle flow of nitrogen gas, using N-EVAP nitrogen evaporator equipment, and the residues were reconstituted in 3 mL of ACN.

2.4. DSPE clean-up method

The ACN fraction was subjected to the DSPE clean-up procedure. DSPE tubes were prepared by weighing 150 mg of PSA