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Multiresidue analysis of pesticides in animal liver by gas chromatography using triple quadrupole tandem mass spectrometry

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Abstract

Two methods for extracting organochlorine (OCs) and organophosphorus (OPs) pesticides from animal liver have been developed. The determination was carried out by gas chromatography with electron impact ionization tandem mass spectrometry (GC–(EI-)MS/MS) using a triple quadrupole (QqQ) analyzer. First, a liquid–solid extraction performed with a high-speed homogenizer (Polytron) using ethyl acetate as solvent, and a subsequent clean-up by gel permeation chromatography (GPC) was applied, determining 34 pesticides. Secondly, a matrix solid phase dispersion (MSPD) extraction with octadecylsilyl (C_{18}) sorbent combined with a Florisil clean-up and ethyl acetate elution was performed, analyzing 25 compounds. These methodologies have been tested and compared in the sample pre-treatment due to the fatty nature of the matrix. The GPC method was finally selected and validated, yielding recoveries in the range 70–115%, with precision values expressed as relative standard deviation (RSD) lower or equal to 20%, at the spiking levels of 25 and 50 $\mu g\,kg^{-1}$, and limits of quantification (LOQs) lower than the maximum residue levels (MRLs) set by the European Union in animal products, except for isofenphos. Linearity was also studied ranging between 5 and 300 $\mu g\,kg^{-1}$ for most of pesticides. This method was applied to the analysis of real liver samples of chicken, pork and lamb.

Keywords: Liver; Pesticide; Gel permeation chromatography; Matrix solid phase dispersion; Gas chromatography; Tandem mass spectrometry; Triple quadrupole

1. Introduction

Pesticides haven been widely used all over the world because they have allowed the development of agricultural and farming production by controlling a wide range of pests and diseases. However, it is well known that the application of these substances can bring along injuries for human health as well as for the environment [1–4]. Organochlorine (OCPs) and organophosphorus (OPPs) pesticides are two groups of compounds extensively used due to their high effectiveness and low price in the control of a variety of pests. OCPs are easy to bioaccumulate into fatty tissues as fat meat, egg yolk or liver [5–7] due to their lipophilic nature and great stability, because of that, they are considered as persistent organic compounds (POPs) [8]. They can easily reach the food chain with the consequent risk for health [1,2,9,10]. Despite of OPPs are less persistent than OCPs, they can also reach the food chain and the European Union has set maximum residue limits (MRLs) in fatty matrices of animal origin [11–14].

Liver is one of the lipophilic tissues of the animal anatomy in which pesticides can be found, especially OPPs which are metabolized in this organ [2,15]. Therefore, it is a matter of interest to have advanced analytical methods in order to identify and determine pesticide residues in liver, and in fatty matrices in general, at trace levels.

Nowadays, one of the trends in analytical chemistry is the development of rapid multiresidue methods which allow determining a variety of active substances in a single injection [9,16–20]. Nevertheless, methods applied to determine pesticide residues in fatty food can require many steps and analysis time. The procedure normally includes three steps: extraction, clean-up and gas chromatography (GC) determination among which clean-up is the most laborious [8,21,22]. Pesticides extraction is also a critical point, and different methods are used to isolate pesticides from fatty matrices, for example, Soxhlet extraction [22], accelerated solvent extraction (ASE) [7,18,23] that is the name of the pressurized liquid extraction (PLE) system commercialized by Dionex, solid phase extraction (SPE) [24,25], supercritical fluid extraction (SFE) [25,27], microwave-assisted extraction (MAE) [24,26,28], matrix solid phase extraction (MSPD) [26,29–33] or the combined use of MSPD and ASE [34].

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The aim of this work is the development of a robust and reliable analytical method to extract OCPs and OPPs from liver by comparing two approaches: firstly, a separated extraction and clean-up procedure with a liquid–solid extraction followed by a gel permeation chromatography (GPC) clean-up; in second place, a strategy which combines both extraction and clean-up steps, an extraction by MSPD followed by a Florisil cleaning. The first applied approach tries to perform a single extraction with ethyl acetate followed by a GPC clean-up, obtaining an extract clean enough for the later chromatographic analysis. GPC may be is one of the best techniques in pesticide residue analysis, as reported in bibliography [7,9,35–39]. Since the point of view of the work topic, this technique has been previously applied in the determination of only OPPs residues in human tissues but using a non-automated GPC system, and with a chromatographic analysis time of almost 30 min [38]. In the present work, analysis time has been reduced until around 15 min and the GPC clean-up is automated; moreover, this method analyzed both OPPs and OCPs.

MSPD was the second methodology tested since is very simple, cheap, fast and adequate in pre-treatment of solid, semisolid and highly viscous samples as liver; however, its main disadvantage is the impossibility of automating the extraction step. This methodology has been successfully applied in the analysis of antibiotics, pesticides and pollutants in foodstuffs [26,29,30,34]. However, applications of MSPD in fatty matrices to extract pesticides are limited: analysis of 4 OCPs in animal fats [31]; analysis of 12 pesticides in olives and olive oil [32] and analysis of 20 OCPs in egg [33]. This work is a new approach in the use of the MSPD technique in pesticide residue analysis. In our case, an octadecylsilyl (C_{18}) sorbent was chosen due to the non-polar character of the majority of the target pesticides, and a variety of solvents and clean-up SPE cartridges were studied.

It is important to notice that the final determination was performed in both cases by GC coupled to electron impact ionization tandem mass spectrometry ((EI-)MS/MS) using a triple quadrupole (QqQ) analyzer, which makes possible a short pre-treatment sample due to its high selectivity and sensitivity [9,16,17]. QqQ detector is one of the latest advances in MS technology, as the reduced number of articles about it shows. The instrumental conditions of the GC–(EI-)MS/MS method were based on the ones previously developed in our laboratory [9]. In summary, the goal of this study is the development of a sample extraction methodology for the analysis of pesticides in animal liver by GC–(EI-)MS/MS.

2. Experimental

2.1. Chemical and reagents

Dichloran, hexachlorobenzene, vinclozoline, malathion, endosulfan ether, parathion-methyl, heptachlor, chlorpyriphos-methyl, parathion-ethyl, chlorpyriphos-ethyl, quinalphos, endosulfan lactone, chlorfenvinphos, heptachlor epoxide, dieldrin, fenamiphos, *p*,*p*′-DDT, *o*,*p*′-DDT, endosulfan beta, endosulfan sulphate, *p*,*p*′-DDE standards and the internal standard (I.S.), caffeine, were supplied from Dr. Ehrenstorfer GmbH (Augsburg, Germany); methamidophos, sulfotep, etrimfos, fenthion, pirimiphos-methyl, isofenphos, endosulfan alpha, *p*,*p*′-DDD and endrin standards were purchased from Riedel-de Haën (Seelze-Hannover, Germany); thionazin, lindane and endrin aldehyde were provided by Supelco (Bellefonte, PA, USA) and aldrin was obtained from Chemservice (West Chester, PA, USA); purity was always ≥99%. Pesticide-quality solvents (cyclohexane, ethyl acetate, dichloromethane, *n*-hexane, methanol and acetone) and anhydrous sodium sulphate (instrumental analysis quality) were supplied by Panreac (Barcelona, Spain), and GC-quality solvents (cyclohexane, ethyl acetate, acetonitrile) were purchased from Scharlau (Barcelona, Spain). Stock standard solutions (between 75 and 550 μg mL^{-1}) were prepared by exact weighting and dissolution in acetone, and were stored in a freezer (−30 °C). Working standard solutions were prepared by appropriate dilution with acetone and stored under refrigeration (4 °C). Preparative-grade (bulk) C_{18} SPE material with 40-μm particle size, 18% carbon load and end capped and SPE cartridges Bond Elute with 2 g Florisil and 12 mL of volume were provided by Varian (Harbour City, CA, USA). Alumina-N cartridges were supplied by Waters (Milford, MA, USA). Pesticide residue grade Florisil sorbent with 150–250-μm particle size and 60–100 mesh was purchased from Merck (Darmstadt, F.R. Germany). Washed and chemically pure glass wool was obtained from Panreac. Ten millilitres disposable plastic syringe barrels and plungers were obtained from Becton Dickinson (Franklin Lakes, NJ, USA).

2.2. Apparatus

The ProStar GPC system used (Varian) consisted of a 410 autosampler with a 24 vials (10 mL) tray, a 230 solvent delivery module, a 325 UV–vis detector with dual wavelength operation (set at 254 nm), a 704 fraction collector, and two on-line connected Envirogel GPC clean-up columns from Waters packed with polystyrene–divinylbenzene (150 mm × 19 mm I.D. and 300 mm × 19 mm I.D., respectively).

GC–MS analysis was performed with a Varian 3800 gas chromatograph with electronic flow control (EFC) and coupled to a Varian 1200 L triple quadrupole mass spectrometer. Samples were injected with a Combi Pal (CTC Analytics AG, Zwingen, Switzerland) using a 100 μL syringe, into an SPI/1079 split/splitless programmed temperature injector operated in the large volume injection technique. The glass liner was equipped with a plug of carbofrit (Resteck, Bellefonte, PA, USA). A fused silica untreated capillary column 2 m × 0.25 mm I.D. from Supelco (Bellefonte, PA, USA) was used as guard column connected to a Factor Four Capillary Column VF-5 ms analytical column (30 × 0.25 mm I.D. × 0.25 μm film thickness) from Varian Instruments (Sunnyvale, CA, USA). The mass spectrometer was operated in EI. The computer that controlled the system also held an (EI-)MS/MS library specially created for the target analytes under our experimental conditions. The mass spectrometer was calibrated weekly with perfluorotributylamine. After the ionization in the ionization source, ions were passed through a hexapole ion guide previous to the quadrupoles (*m*/*z* range 10–1500). The collision cell presents a curved shape forming a