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Multiclass pesticide residue analysis in fish muscle and liver on one-step extraction-cleanup strategy coupled with liquid chromatography tandem mass spectrometry

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ABSTRACT

The analysis of pesticide residues in fish samples is challenging due to the low concentrations and large number of analytes that need to be monitored and quantified in a complex matrix. This is the first report providing a novel one-step extraction-cleanup strategy for simultaneous analysis of over 340 pesticides in a fatty fish and liver matrix, coupled with liquid-chromatography tandem mass spectrometry. The samples of fish muscle and liver were prepared according to the modified QuEChERS (quick, easy, cheap, effective, rugged and safe) procedure, wherein the extraction and cleanup protocol were integrated into one step. Among the tested cleanup dispersive solid phase extraction sorbents (C18, primary-secondary amine, Z-Sep), chitin yielded the best results. Spike-in experiments were carried out at three different spiking levels in fish and liver to determine the recovery, precision and limits of detection of the method as well as the matrix effect. The method's detection limits ranged from 0.05 to 1.2 μg kg^{-1}, while recoveries of most pesticides were in the range of 70–120% with associated precision - relative standard deviations below 20%. A linear relationship was observed within the range of 0.005–1 mg kg^{-1}, and the correlation coefficient was $R^2 > 0.997$. Expanded measurement uncertainty was estimated to be between 7% and 52%, on average. Matrix effects were evaluated and were not significant for the vast majority of pesticides. The validated method was employed in the analysis of 54 real fish and liver samples in which 10 different pesticides with concentrations ranging from 0.005 to 0.047 mg kg^{-1} were detected.

1. Introduction

Fish may be used as valuable indicators of ecosystem conditions because they accumulate contaminants directly from the water and diet. On the other hand, fish are a valuable source of nutrients in the diet of humans and animals. Fish are commonly consumed by humans and the presence of contaminants can cause health problems (Granados-Galván et al., 2015; Robinson et al., 2016; Wang et al., 2013; Wine et al., 2012; Yohannes et al., 2014).

Indirect exposure of fish to pesticides used in agriculture is a major problem among food producers. For example, organochlorine pesticides are still detected at low levels (0.1–139 ng g^{-1}) in some fish, fish products and seafood (LeDoux, 2011). Especially lipophilic pesticides, such as highly chlorinated (e.g. DDT, lindane) and certain pyrethroids (e.g. cypermethrin) can bioaccumulate in fatty tissues and enter the food chain (Panseri et al., 2013; Stenerson and Claus, 2013). Fish and other aquatic organisms may be harmed by pesticide-contaminated water. This undesirable effect of pesticides was discussed by many countries, many pesticides were withdrawn, but the global tendency of spreading pesticide use has increased overall (Lamberth et al., 2013). In recent years, pesticides have generally become less toxic and persistent and more species-specific, reducing their negative effect on the environment and human health. In addition, individual countries and organizations establish restrictive law on the use of pesticides. According to European Union law, plant protection products that can be used in the EU fall within the scope of Regulation (EC) No. 1107/2009 (EC, 2009). This Regulation lays down rules for the authorisation of plant protection products in commercial form and for their placement on the market, use and control within the EU. EU Member States monitored pesticide residues in surface and ground water systems within the EU-Water Framework Directive 2000/60/EC (EC, 2000). Moreover, EU has established rules specifying maximum residue levels of pesticides in or on food and feed of plant and animal origin (EC, 2005).

Analysis of a wide range of pesticides in the matrix of fish is a difficult task and a challenge. Despite advances in chromatographic

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separation and dynamic development of instrumental methods (e.g. mass spectrometry techniques), sample preparation is still one of the most important steps in the analytical protocol, and effective sample treatment is essential for achieving good analytical results (Nunez et al., 2012). Optimal sample preparation methods should be fast, accurate, and cheap, performed without loss, and yield extract as free of interferences as possible.

The extraction methods of pesticides from fish or fish products commonly used organic solvents of different polarity, which in turn causes of contamination of the extract of fatty matrix components. A sample cleanup step is therefore necessary by carrying out techniques such as freezing, liquid-liquid partitioning, solid phase extraction (SPE), gel permeation chromatography (GPC) or matrix solid phase dispersion (MSPD) prior to chromatographic analysis (LeDoux, 2011; Panseri et al., 2013; Lehotay et al., 2005; Łozowicka et al., 2012a, 2012b).

The QuEChERS (quick, easy, cheap, effective, rugged and safe) approach is flexible and can be modified depending on the composition of the matrix and the properties of the analyte. Therefore, in the last decade, QuEChERS has become a very popular method for extraction and cleanup of pesticides in plant matrices: fruits and vegetables (Koesukwiwat et al., 2010; Kwon et al., 2012; Lehotay, 2011; Łozowicka et al., 2016; Rajski et al., 2013).

In recent years, the concept of using QuEChERS for sample cleanup has been successfully applied to foods other than plants, in fatty matrices such as: breads, milk, and oils, prior to pesticide residue analysis (Nollet and Toldra, 2015; Lehotay, 2011; Koesukwiwat et al., 2010). In addition, the range of analyzed compounds has been broadened from one group of pesticides to a wide range of pesticides. Still, this list is far from exhaustive. The extraction step uses acetonitrile or acidified acetonitrile, ethyl acetate and isooctane (instead of *acetonitrile)* and a salting-out effect with magnesium sulphate. Cleanup is realized using dispersive SPE (d-SPE), based on sorbents such as: primary-secondary amine (PSA) for the removal of polar pigments, acids and sugars, graphitized carbon black (GCB) for removal of color pigments, C18 as the only sorbent that was available for the removal of fats and non-polar components from samples, and the new Z-Sep or Z-Sep+ for removal of lipids and pigments. Typically, a combination of PSA/C18 is used for reduction of a fatty matrix (Lucci et al., 2012). A new cleanup sorbent, which has various bioactivities and functionalities, was recently developed. Chitin obtained from shrimp shell waste was applied in the d-SPE clean-up step according to the methodology elaborated for analysis of organic contaminants in drinking water treatment sludge by Cerqueira et al. (2014). This approach allowed the authors to obtain the most satisfactory recovery rates.

The important task was then to obtain good recoveries for a wide range of analyte polarities, because the application of some sorbents could result in substantial adsorption and loss of hydrophobic, hydrophilic and base- or acid-sensitive compounds. In addition, the QuEChERS extraction method for fish should allow for a sufficient level of sensitivity. This study also focuses on matrix effects (ME) present in fish muscle and liver and their interference in the analytical system depending on the sorbent used in the cleanup step. The matrix effect in a chromatographic system is an important analytical factor in pesticide analysis linked to overestimation or underestimation of analyte concentrations and is one of the major sources of errors in measurements. This effect has an influence on the chromatographic response (increase or decrease) to pesticide analytes prepared in the sample matrix versus solvent (Mol et al., 2008; Ferrer et al., 2011). Although most studies involving this analytical parameter thoroughly discuss the presence/absence of a matrix effect, the full mechanism of this phenomenon is still unidentified.

In the literature, there are many research papers employing the QuEChERS analytical procedure and LC-MS methods as the main stream for analysis of multiclass, multiresidue pesticide contaminants in a huge variety of foodstuffs (Kwon et al., 2012; Łozowicka et al., 2016; Marin et al., 2009; Rajski et al., 2013), but the one-step approach based on chitin is not widely described. The presented strategy was successfully applied to clean up other matrices in our previous study (Kaczyński and Łozowicka, 2016).

The challenge undertaken in this study was to introduce a novel concept of clean-up, called the one-d-SPE-step clean-up procedure, for determination of a wide range of pesticides with various polarities in complex fatty matrices based on the salting effect and chitin.

Analysis of pesticide residues in fish muscle and liver is challenging due to the low concentrations and large number of pesticide analytes that need to be monitored and quantified in a complex matrix.

The aim of this study was to test and validate a simple, fast and sensitive method for trace analysis of over 340 pesticides in fish muscle and liver. The analytical method was based on a modified QuEChERS method, where the application of chitin as a sorbent material in one-step solid phase extraction using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was evaluated. The validation study was performed based on recoveries, precision, sensitivity, linearity and matrix effect. The method was tested on muscle and liver of pike (*Esox lucius*) with good results.

2. Materials and methods

2.1. Materials and reagents

Acetonitrile, acetone and methanol were purchased from J.T. Baker (Deventer, The Netherlands). LC-MS grade formic acid (98% purity) was obtained from Merck (Dramstadt, Germany), ammonium formate (> 99%) from Fluka (Seelze-Hannover, Germany) and LC-grade water (18 MΩ cm) from a MilliQ water purification system (Millipore Ltd., Bedford, MA, USA). QuEChERS Extract Pouches containing 4 g magnesium sulphate, 1 g sodium chloride, 1 g sodium citrate dihydrate, 0.5 g disodium hydrogen citrate sesquihydrate were purchased from Agilent Technologies (Santa Clara, USA). Pre-weighed sorbent mixtures for different cleanup methods: 150 mg PSA +150 mg C18 + 900 mg anhydrous magnesium sulphate kit were purchased from Perlan Technologies (Santa Clara, USA); Z-Sep (120 mg) was purchased from Supelco (Bellefonte, PA, USA). Chitin from shrimp shells (0.28–0.46 mm) and GCB were purchased from Sigma-Aldrich (Steinheim, Germany).

2.2. Preparation of pesticide standard solutions

Pesticide standards were purchased from Dr. Ehrenstorfer Laboratory (Augsburg, Germany) and Sigma-Aldrich (Steinheim, Germany). The purities of the standard pesticides ranged from 96–99.8%. Internal standards (IS), atrazine-d_5, carbendazim-d_3 and isoproutron-d_6, were obtained from Sigma-Aldrich (Steinheim, Germany).

Stock solutions of pesticides (around 1000 μg mL^{-1}) were prepared separately by dissolving an accurately weighed amount of each reference standard in acetone. Intermediate stock standard mixtures in methanol, containing 10 μg mL^{-1} of each compound, were prepared by mixing appropriate quantities of the individual stock solutions. Working standard mixtures of 0.001–1.0 μg mL^{-1} were prepared in methanol from the above stock standard by serial dilution, and were then used for spiking as well as to prepare the solvent and matrix-matched calibration curves from the pesticides in the solvent and matrix. Matrix-matched calibration solutions were prepared by adding the appropriate amount of standard mixtures in solvent into matrix extracts. A solution of IS was prepared in methanol (0.5 μg mL^{-1}) and used to prepare working calibration solutions as well as during the procedure of spiking the samples. All stock and working standard solutions and IS were stored in a freezer at about −18 °C until analysis.