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Pesticide residues in chicken eggs – A sample preparation methodology for analysis by gas and liquid chromatography/tandem mass spectrometry

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ABSTRACT

A sample preparation method was developed for the analysis of chicken eggs to determine 97 GC and 81 LC amenable residues, including organophosphates, organochlorines, pyrethroids, triazoles, carboxyl-containing compounds, and the indicator PCBs. Hereby, considerations were given to the recoveries of the analytes, the method's suitability for routine analysis, and the assessment of the clean-up effect, for which a simple thin layer chromatography was implemented to visualize the most important lipid classes.

The procedure consisted of (I) the extraction by matrix solid phase dispersion, and the clean-up by means of (II) small-scale gel permeation chromatography (GPC) and (III) two different solid phase extractions (SPE) for GC and LC amenable analytes, as well as (IV) the quantification using GC–MS/MS and LC–MS/MS. Cyclohexane/ethyl acetate was chosen as extraction solvent due to its suitability for extracting strong non-polar but also more polar analytes. The classical GPC was scaled down to ensure a 50% lower solvent consumption. The comprehensive investigation of conventional and modern zirconium-oxide-coated SPE materials resulted in the selection of octadecyl-modified silica (C18) combined with primary secondary amine using acetonitrile as elution solvent for GC amenable analytes and pure C18 in combination with acidified methanol for LC amenable pesticides.

Compared to the currently established EN 1528 method the sample preparation proposed offered a higher sample throughput and a lower solvent consumption. Furthermore, for the first time the clean-up effectiveness of the sample preparation steps was documented as shown by means of thin-layer chromatography.

The validation of chicken eggs proved the fulfillment of the quality control criteria for 164 of the 178 analytes tested, mostly at the lowest validated level of 5 μg/kg for pesticides and 0.5 μg/kg for the single indicator PCBs. However, the analysis of strongly polar analytes was still problematic, which could be attributed to the extraction and the GPC step. Nevertheless, the successful investigation of EU proficiency test materials (EUPT AO 07–09) confirmed the comparability of the results with the currently established sample preparation procedures and demonstrated the potential of the applicability of the presented method to other matrices as exemplified for lean poultry meat and fatty liquid cream.

1. Introduction

Pesticide residue analysis is focused primarily on fruits and vegetables as pesticides are employed predominantly in crop production. Nevertheless, pesticide residues can also be detected in food of animal origin due to different input sources. (I) For many years, the accumulation of persistent organic pollutants such as

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DDT or PCBs in animal organisms has been under special consideration, resulting in numerous findings, e.g. in chicken eggs and boar meat [1]. (II) Today, research is also focused on the uptake of pesticides from residue-containing feed by animal organisms [2,3]. (III) Furthermore, pesticides can also be applied directly to the animal sector according to the definition by the Food and Agriculture Organization of the United Nations, in which pesticides are also substances used "for preventing (. . .) any pest, including vectors of human or animal disease" [4]. Accordingly, the direct treatment of animals with insecticides such as organochlorines, organophosphates, and carbamates as well as with acaricides such as pyrethroids is employed in order to avert diseases. (IV) In addition, insecticides can also be applied during the processing of food of animal origin, e.g. in dairy factories [5,6]. Consequently, due to the different input sources, pesticides can be transferred to animal organisms and form residues, which can be determined in different products of animal origin. In line with the principles of Good Agricultural Practice and in order to protect consumer health, maximum residue levels (MRL) were set in regulation (EC) No. 396/2005 [7], whose compliance is checked by the official food control and the food business operator. Thus, effective analysis methods for the determination of pesticides in food of animal origin are required.

The primary challenge for the modern multi-residue analysis of food of animal origin is to cover a large polarity spectrum of pesticides. Mainly non-polar compounds have been monitored over years in residue analysis due to their tendency to accumulate in fatty tissue. Today, more and more polar pesticides have to be investigated additionally due to the possible transfer from feed. This is further complicated by the increased inclusion of polar metabolites in the residue definition according to regulation (EC) No. 396/2005. Secondly, due to the high proportion of lipids and proteins, the matrix in food of animal origin is a more challenging analytical task in comparison to the matrix in fruits and vegetables. Liquid whole chicken eggs mainly consist of ~74% water, ~12% proteins and ~10% lipids. The lipid fraction comprises 66% triglycerides, 28% phospholipids and 6% cholesterol, cholesterol esters, and minor lipid compounds such as fat-soluble (pro-)vitamins (especially tocopherols and carotenoids) [8]. The different lipid classes are known to interfere strongly with the analysis system, resulting in non-reproducible signals. High amounts of triglycerides can form visible lipid drops in the GC inlet or even in the pre-column or analytical column; free fatty acids coelute with the pesticides; high cholesterol concentrations can result in a significant carry-over effect during GC analysis; also, ion suppression could be observed during LC analysis due to phospholipids [2,9]. Thus, particularly lipids have to be removed by means of an effective clean-up before residue analysis.

Nowadays, in the European Union, two particular sample preparation methods are applied for pesticide residue analysis in food of animal origin. The EN 1528 method has been established in Europe since 1996 [10], correlating with the German DFG S 19 method [11]. The QuEChERS method [12] is increasingly applied for the sample preparation of food of animal origin. The EN 1528 method was developed for fatty foods, including intensive clean-up by means of gel permeation chromatography (GPC) and solid phase extraction (SPE) via silica gel, resulting in a good separation of triglycerides; however e.g. cholesterol still remains in the final extract. Due to the high expenditure of time and human resources as well as the high consumption of solvents, this procedure hardly meets today's economical requirements. Furthermore, the EN 1528 method is predominantly aimed at the determination of non-polar analytes due to the non-polar extraction solvents. In contrast, the QuEChERS method was introduced in 2003 for the pesticide residue analysis of fruits and vegetables [13]. The procedure is based on a shaking extraction with acetonitrile and a clean-up with dispersive SPE by means of primary secondary amine (PSA). Hence, the modern sample preparation method is time-efficient and economical but was not designed for fatty matrices. With the QuEChERS method, especially polar pesticides are determined, which is attributed mainly to the polar extraction solvent.

With regard to fatty matrices, in the field of pesticide residue analysis mainly sample preparation methods for non-polar analytes or studies for more polar analytes based on the QuEChERS approach have been published within the past 5 years.

Concerning non-polar insecticides, the suitability of modern zirconium-oxide-coated SPE materials (ZrO_2-coated materials so-called Z-sep phases) for the clean-up of eggs was proved by Piatkowksa et al. [14]. Moreover, the clean-up with magnetic nanoparticles modified with functional moieties (e.g. silica, C18) was investigated in dispersive SPE for non-polar analytes [1,15]. Furthermore, Chung and Chen [16] gave a detailed overview of different extraction and clean-up methods for the determination of organochlorines in fatty foods and reported their advantages and disadvantages. At the European Pesticide Residue Workshop in 2014, Lippold et al. [17] presented a method for the analysis of non-polar pesticides in liver, which included the shaking extraction, the GPC and SPE using silica and a ZrO_2-coated phase. Hence, this method was aimed at a more time-efficient extraction and an improved clean-up effect compared to the established EN 1528 method.

In contrast, the QuEChERS approach, which is focused on more polar analytes, was frequently described for pesticide residue analysis of fatty foods. Chio et al. [3] proposed the additional use of graphitized carbon black (GCB) for egg samples due to the improved clean-up effect. Castillo et al. [18] investigated the fat removal by means of octadecyl-modified silica (C18) and freezing-out overnight. Rajski et al. [19] compared different SPE materials by means of the QuEChERS approach concerning their suitability for high oil vegetal commodities and demonstrated the suitability of modern ZrO_2-coated phases. However, the working group reported that the recoveries of non-polar analytes declined with an increasing fat content in the foods. This could be attributed to the polar solvent acetonitrile, which cannot extract non-polar analytes from the lipid phase sufficiently [20,21]. This problem was compensated by Unterluggauer et al. [22] by means of the procedural standard calibration within their modularized method for food of animal origin, which was based inter alia on the QuEChERS extraction.

In conclusion, in the literature several studies analyzing pesticides in food of animal origin were published, in which mainly single aspects of the sample preparation method were investigated for, mostly, a limited spectrum of pesticides in order to overcome the problems of the currently applied sample preparation methods. Hence, the main objective of the presented study was the development of an integrated analysis method which fulfills the essential requirements for the modern pesticide residue analysis in food of animal origin. The primary goal was to cover a large spectrum of analytes from strong non-polar to more polar pesticides. This should go in line with an effective matrix clean-up to remove interfering matrix compounds without analyte losses. Furthermore, modern analysis methods no longer require high sample weights or high sample equivalents due to the sensitive tandem mass spectrometry. Hence, contemporary sample preparation methods should not only be effective but also economical. Eventually, the analysis method should be in compliance with the legal demands according to the quality control criteria of the SANCO document [23] and with the requirements pursuant to regulation (EC) No. 396/2005 [7] with regard to the MRLs. Thus, this study presents the development of a multi-residue analysis method for chicken eggs, continuously considering the recoveries of the analytes, the matrix burden of the extracts, and the handling in routine analysis.