



Analytical Methods

QuEChERS-based extraction with dispersive solid phase extraction clean-up using PSA and ZrO₂-based sorbents for determination of pesticides in bovine milk samples by HPLC-DAD



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ABSTRACT

In this study, a quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction technique was adapted to develop a simple sample treatment for multi-residue pesticide analysis in milk samples. The proposed method is based on liquid–liquid partitioning with acetonitrile followed by dispersive solid phase extraction clean-up using primary secondary amine along with zirconia-coated silica particles for extract purification. Identification and quantification of 30 pesticides was conducted via high performance liquid chromatography with diode-array detection (HPLC-DAD). Recoveries were from 70 to 100% for the vast majority of the analytes, with relative standard deviations less than 20% being observed. HPLC-DAD provided suitable linearity, precision and accuracy. For 28 of 30 analytes in the study method limit of quantification values (mLOQs) comply with the most recent European Union guidelines for the maximum residue levels (MRLs) in milk. Negligible matrix effect was observed due to efficient extract clean-up with ZrO₂-based sorbents.

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1. Introduction

Pesticides (more than 1000 active substances available) are extensively used for the control of weeds, diseases, and pests of cultivated plants. Most of these compounds are persistent and stable under environmental conditions (Rejczak & Tuzimski, 2015a). Fat solubility of most of these substances promotes bioaccumulation through the food chain. Consequently, pesticide residues may be found in bovine milk as a result of consumption of contaminated feedstuffs or water by cattle. Bovine milk is an essential component in the diet of children and the elderly. It is a good source of proteins, fat, vitamins and minerals. Therefore, contamination of milk with pesticide residues is a matter of serious concern (Bedi, Gill, Aulakh, & Kaur, 2015).

In order to ensure food safety, legislation such as the European Union (EU) directives describe the maximum residue levels (MRLs) for pesticides in or on food and feed of plant and animal origin (Regulation (EC) NO 396/2005). Accordingly, accurate analytical methods are needed to monitor pesticide residues in foodstuffs at a lower concentration level than the established MRLs. Milk and other dairy products, considered as products with significant

fat amounts, are challenging for pesticide residue analysis. Some of the lipids are co-extracted with organic solvent and may strongly interfere with the analytes. On the other hand, some of fat-soluble nonpolar pesticides may persist in fat-rich sample and give poor recoveries (Rejczak & Tuzimski, 2015b). Therefore, the choice of an appropriate sample preparation method influences considerably the reliability and accuracy of the analysis (Martins, Amaya, Waliszewski, Colín, & García Fabila, 2013).

Several methods have been proposed for the extraction of pesticides from milk samples and extract clean-up, such as liquid–liquid extraction (LLE) (Azeredo et al., 2008; Kampire, Kiremire, Nyanzi, & Kishimba, 2011; Mueller, Harden, Toms, Symons, & Fürst, 2008; Qu, Suri, Bi, Sheng, & Fu, 2010), solid phase extraction (SPE) (Liu et al., 2010; Chen et al., 2014), gel permeation chromatography-solid phase extraction (GPC/SPE) (Zheng et al., 2014), solid-phase microextraction (SPME) (Cardeal & Dias Paes, 2006; González-Rodríguez, Arrebola Liébanas, Garrido, Martínez Vidal, & Sánchez López, 2005), solid matrix dispersion (SMD) (Di Muccio et al., 1997), dispersive solid-phase extraction (d-SPE) (Dagnac, Garcia-Chao, Pulleiro, Garcia-Jares, & Llompard, 2009), quick, easy, cheap, effective, rugged and safe (QuEChERS) method (Jeong, Kwak, Ahn, & Jeong, 2012; Lehotay, Mastovská, & Yun, 2005; Selvi, Paramasivam, Rajathi, & Chandrasekaran, 2012; Yang et al., 2014), cloud point extraction (CPE) (Liu et al., 2014), microwave assisted ionic liquid microextraction (MAILME) (Gao

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et al., 2010), hollow fiber membrane-protected solid-phase microextraction (HFM-SPME) (Bashheer & Lee, 2004), coupled microwave-assisted extraction-solid phase extraction (MAE/SPE) (Fang, Lau, Law, & Li, 2012), pressurized liquid extraction (PLE) (Mezcua et al., 2007), and diphasia dialysis extraction (García, Santaefemia, & Melgar, 2012).

In general, traditional procedures are time-consuming, labor-intensive, complicated, expensive and produce considerable quantities of wastes (Martins et al., 2013). On the other hand, some of modern extraction procedures can provide difficulties during application and validation for a wide variety of analytes with different physicochemical properties. High-throughput multiresidue methods operating in a routine contaminant control of food products are often based on the QuEChERS approach (Rejczak & Tuzimski, 2015b). Due to great flexibility of QuEChERS, different modification may be introduced to the procedure to ensure its robustness, even for residue analysis in complex matrixes (Rejczak & Tuzimski, 2015b). Tailoring the use of d-SPE sorbents is the most important for adapting the method for samples with significant fat content (e.g. raw bovine milk) (Rejczak & Tuzimski, 2015b). Jeong et al. elaborated a QuEChERS-based method for pesticides determination in milk (Jeong et al., 2012). Authors made an optimisation of d-SPE clean-up step by response surface methodology (RSM) resulting in application of primary secondary amine (PSA) and octadecyl gel (C18) for this purpose. Nevertheless, the recovery rates for the most lipophilic analytes were decreased, because they were removed by C18 along with other fatty compounds (Jeong et al., 2012).

Both gas chromatography (GC) and liquid chromatography (LC) have been operated for the analysis of pesticides in food samples (Rejczak & Tuzimski, 2015a). Most of current LC based methods rely on QqQ-MS/MS operating in the selected reaction monitoring (SRM) mode, which enables accurate identification and quantification of targeted analytes (Rejczak & Tuzimski, 2015a). However, it does not change the fact that a critical aspect of pesticide residue analysis is the purification process, which is required to isolate the residues from matrix components and reduce matrix effect (Jeong et al., 2012). Application of high performance liquid chromatography with diode-array detection (HPLC-DAD) is less expensive than LC-MS/MS, but the latter offers the advantage of identifying the compounds of interest with a higher level of confidence (Tuzimski & Rejczak, 2016). HPLC-DAD may be useful technique for residue analysis in the case when matrix effect is negligible (Watanabe, Kobara, Baba, & Eun, 2014). For complex samples it may be achieved with application of alternative sorbents in QuEChERS d-SPE step (Rejczak & Tuzimski, 2015b). Zirconium dioxide based sorbents were already applied for extract purification of fat-rich samples by different authors (Lozano et al., 2014; Rejczak & Tuzimski, 2015a; Sapozhnikova & Lehotay, 2013; Tuzimski & Rejczak, 2014, 2016). These innovative dispersive phases demonstrate ability to extract more fat than traditional PSA and C18 sorbents and show greater analyte recovery and better reproducibility (Rejczak & Tuzimski, 2015b).

In this work, the authors proposed a rapid, efficient, and reliable method for extraction of 30 pesticides from raw bovine milk samples, based on modified QuEChERS procedure. The novelty of the experiments concern possibility of utilizing relatively new and innovative zirconium based sorbents (Z-Sep and Z-Sep Plus) for clean-up of milk extracts. Evaluation of the efficiency of dispersive-SPE clean-up step applying different d-SPE sorbent and their combinations was performed. Average recovery; repeatability and matrix effect were accounted for finally selected extraction conditions. Validation of the chromatographic method (linearity, limits of detection (LODs) and quantification (LOQs)) was also carried out.

2. Experimental

2.1. Chemicals and reagents

2.1.1. Pesticides standards

Standards for the 30 pesticides under investigation (Table 1), such as metamitron, hexazinone, lenacil, methabenzthiazuron, isoprotruron, buturon, linuron, aziprotryne, terbutryn, bitertanol, procymidone, fenitrothion, neburon, chlorfenvinphos, hexaflumuron, propaquizafop, lufenuron, flufenoxuron, trifluralin and alphacypermethrin were obtained from Dr. Ehrenstorfer-Schäfers (Augsburg, Germany); those of monuron, fluometuron, prometryn and clofentezine were obtained from Sigma-Aldrich (Supelco, Bellefonte, PA, USA); those of fenuron, metribuzin, propachlor, terbuthylazine, vinclozolin and bromopropylate were obtained from the Institute of Organic Industry (IPO, Warsaw, Poland).

The standard purity indicated by the manufacturers for all of the reference standards was $\geq 97\%$. Individual stock standard solutions (400 mg L^{-1}) were prepared in methanol or acetone and were stored at $6 \pm 2^\circ\text{C}$. A pesticides standard mixture containing all the analytes (10 mg L^{-1}) was prepared by combining suitable aliquots of each individual standard stock solution and diluting them with gradient grade acetonitrile (MeCN, E. Merck, Darmstadt, Germany).

2.1.2. Solvents and mobile-phase solutions

Acetonitrile (MeCN), methanol (MeOH), and acetone (Ac) were pro chromatography grade and were obtained from E. Merck (Darmstadt, Germany). Deionized water ($0.07\text{--}0.09 \mu\text{S cm}^{-1}$) was obtained by means of Hydrolab System (Gdansk, Poland) in our laboratory.

2.1.3. QuEChERS salts and sorbents

Anhydrous magnesium sulphate (MgSO_4) and sodium chloride (NaCl) were obtained from POCH (Gliwice, Poland). Primary secondary amine (PSA) as well as zirconium dioxide based sorbents (Z-Sep and Z-Sep Plus) were purchased from Sigma-Aldrich (Supelco, Bellefonte, PA, USA).

2.2. QuEChERS-based extraction procedure

Raw bovine milk samples were purchased from local farms. For the extraction, 20 mL of milk samples were transferred into polypropylene (PP) centrifugation tubes. Then, 16 mL MeCN was added and closed tubes were vigorously shaken manually for approximately 1 min. Subsequently, 2 g NaCl and 8 g anhydrous MgSO_4 were added and the tubes were immediately shaken to prevent coagulation of MgSO_4 . The tubes were centrifuged (Centrifuge MPW-223e, Warsaw, Poland) at 6000 rpm (3480 rcf) for 5 min. The acetonitrile layer (12 mL) of each tube was obtained with a pipette, and evaporated to dryness under a fume hood. The evaporated extracts were reconstituted in 1.2 mL MeCN and transferred into 12-mL PP tubes containing 125 mg PSA, 25 mg Z-Sep, and 5 mg Z-Sep Plus. The tubes were shaken vigorously for 1 min and centrifuged (6000 rpm, 3480 rcf) for 5 min. The supernatants (800 μL) were collected and evaporated to dryness under a fume hood. Afterwards, remaining residues were reconstituted in 200 μL MeCN and transferred into a vial. Final extracts were stored at $6 \pm 2^\circ\text{C}$ before the analysis via HPLC-DAD. The flowchart of the procedure is presented (Fig. 1).

2.3. RP-HPLC procedure

For the LC analysis, an Agilent Technologies 1200 HPLC system with a quaternary pump was used. The HPLC apparatus was equipped with a ZORBAX Eclipse XDB-C18 150 mm \times 4.6 mm

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