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Development of a new generic extraction method for the analysis of pesticides, mycotoxins, and polycyclic aromatic hydrocarbons in representative animal feed and food samples

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Abstract

Various generic extraction methods have been used to determine pesticide residues, mycotoxins, and polycyclic aromatic hydrocarbons (PAHs) in food and animal feed to ensure consumer safety. However, these methods cannot extract all relevant compounds at an acceptable rate of recovery. This study presents a new extraction method. This new method facilitated the identification of 231 compounds, including 196 pesticides, 11 mycotoxins, and 24 PAHs over a broad range of polarities. These compounds were identified in various sample matrices, including those that are lipid-rich. The processed sample is first extracted with water, acetonitrile, formic acid, and heptane. The addition of ammonium formate results in separation into three phases and enables analysis of the aqueous phase. Solid-phase extraction clean-up procedures were performed as necessary followed by analysis by liquid or gas chromatography and mass spectrometry. Analyte recoveries were typically in the range of 70 – 120% with relative standard deviations below 20%.
Keywords: Generic extraction method, pesticides, mycotoxins, polycyclic aromatic hydrocarbons (PAHs), liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS)

Chemical compounds studied in this article


1 Introduction

Pesticide residues and contaminants, including mycotoxins and polycyclic aromatic hydrocarbons (PAHs), are often detected in food and animal feed and are of concern for both human and animal health. Pesticides are routinely applied to plants to control pests and to improve crop yields, while mycotoxins are natural contaminants in food and animal feed resulting from fungal growth. Food processing may result in contamination with PAHs, which are produced by the incomplete combustion of organic material (Plaza-Bolaños et al., 2017).

Regulations are in place that establish maximum limits for a wide variety of pesticide residues and contaminants typically found in food and feed. As such, there are more than 1000 compounds associated with numerous different chemical groups that need to be analysed in sources of food and animal feed (Silano & Silano, 2017) as part of existing monitoring programs (EU 2017/660).

Several analytical methods can be used to identify and quantify this large variety of chemical compounds. However, as analytical methods typically target only a limited number of chemical compounds, samples routinely require multiple rounds of analysis (Anastassiades et al., 2003;
In an effort to reduce the number of methods needed to perform a complete chemical analysis, recent trends have focused on the development of multi-residue and multi-class methods based on chromatographic separation and mass spectrometric detection.

Popular generic extraction methods used for this purpose include QuEChERS (an acronym for Quick Easy Cheap Effective Rugged Safe) and its various modifications, including Dilute and Shoot and the Swedish Ethyl acetate method. Amongst the shortcomings of these methods, they cannot be used to detect highly polar pesticides and certain mycotoxins, including the fumonisins. These compounds are difficult to recover and require specialized methods that have been directly tailored for these purposes.

Non-polar analytes such as PAHs are particularly troublesome when performing analyses of lipid-rich samples and require additional, specifically-dedicated procedures. As such, none of the generic methods currently described in the literature can be used for simultaneous identification and analysis of pesticides, highly-polar herbicides, fumonisins, and PAHs in food and feed matrices.
The present study aimed to develop a generic sample preparation method that could be used to analyse pesticides, mycotoxins, and PAHs in various food and feed matrices, including fish feed, maize, mealworm, sunflower seeds, and wheat. For this purpose, a modification of a QuEChERS-based method (González-Curbelo et al., 2014) was proposed to facilitate concurrent identification and quantitative analysis of highly-polar, medium-polar, and non-polar analytes in several different matrices. A three-phase solvent partitioning system is presented based on initial extraction with water, acetonitrile, and heptane. The matrix components and targeted analytes are dissolved and partitioned into one or two of the three resulting solvent phases.

This approach facilitates the determination of a broad scope of analytes with different polarities. If necessary, multi-phase extraction and phase separation is followed by a versatile solid-phase extraction clean-up procedure. All three solvent phases were then evaluated with suitable chromatography/mass spectrometry protocols. This new method will be referred to as “WAHSPE”, which is an acronym that represents its core concepts, including the use of water, acetonitrile, and heptane (WAH) as solvents in combination with solid-phase extraction (SPE).

Similar to QuEChERS, the WAHSPE method demonstrates flexibility with respect to the sorbents that may be used for clean-up procedures as well as with other aspects of the extraction procedure. By contrast, the use of the WAHSPE method permits the detection and evaluation of analytes over a more extensive range of polarities. Therefore, the study hypothesizes that the newly developed WAHSPE method will facilitate simultaneous analysis of both polar and non-polar compounds in relevant complex matrices, including those with high-fat content. As such, the method may have the capacity for quantitative extraction of a larger number and greater variety of compounds than
what can be achieved with any other of the methods that are currently available, including QuEChERS and SweEt.

2 Materials and methods

2.1 Chemicals and Reagents

2.1.1 Chemicals

Analysis of PAHs and polar pesticides was performed at Wageningen University, The Netherlands. Acetonitrile (liquid chromatography-mass spectrometry [LC-MS] grade) and heptane (high-performance liquid chromatography [HPLC] grade) were purchased from Biosolve (Valkenswaard, The Netherlands). Formic acid (>99%), and ammonium formate (>99%) were purchased from Sigma-Aldrich (Zwijndrecht, The Netherlands). Analyses of pesticides and mycotoxins were performed at the Technical University of Denmark. Acetonitrile (HPLC grade S) was obtained from Rathburn (Walkerburn, UK), and heptane and formic acid were purchased from Merck (Darmstadt, Germany). Water was obtained from a Milli-Q water purification system from Millipore (Molsheim, France).

2.1.2 Standards

The mycotoxins aflatoxin B1 (AFB1), aflatoxin B2 (AFB2), aflatoxin G1 (AFG1), aflatoxin G2 (AFG2), ochratoxin A (OTA), fumonisin B1 (FB1), fumonisin B2 (FB2), zearalenone (ZEN), deoxynivalenol (DON), HT-2 toxin (HT-2), and diacetoxyscirpenol (DAS) were obtained from Sigma-Aldrich (Buchs, Switzerland). PAHs were obtained from Cambridge Isotope Laboratories (Tewksbury, MA, USA) and DSP-Systems (Ede, The Netherlands). Pesticide standards were purchased from Dr.
Ehrenstorfer (Augsburg, Germany), Sigma-Aldrich (Taufkirchen, Germany), Medical Isotopes (Pelham, NH, USA), Chem Service (West Chester, PA, USA), HPC Standards GmbH (Cunnersdorf, Germany), and CDN Isotopes (Thaxted, UK). A more detailed list of reagents is included in Supplementary Material A.

2.2 Sample material

Cereals were chosen as the sample matrix, as they are currently amongst the most crucial of the food commodities available worldwide. Fish feed, mealworm, and seeds were chosen to represent complex high fat-containing matrices. A full list of all sample materials, including fish feed, mealworm, maize, sunflower seeds, and wheat as well as methods used for sample processing before extraction is included in Supplementary Material A, Table A.7. Maize was obtained as “popcorn maize” from Netto (Lyngby, Denmark). Mealworm, sunflower seeds, and wheat were from the Dutch feed monitoring program and provided by The Netherlands Food and Consumer Product Safety Authority. Fish feed was obtained from BioMar A/S (Brande, Denmark).

2.3 Sample preparation

Mealworm, sunflower seeds, and wheat were milled at room temperature using a Retsch Grindomix GM 200. The processed mealworm and wheat samples were stored at -20 °C and the processed sunflower seed samples were stored at room temperature. The fish feed sample, CPK2, was homogenized by cryo-milling (with liquid nitrogen) using a Retsch ZM 200 with a sieve (1 mm). The processed fish feed sample was stored at -20 °C. The maize sample was homogenized at room temperature using a Retsch ZM 200 with a sieve (0.25 mm) and stored at -20 °C.

2.4 WAHSPE (Water Acetonitrile Heptane Solid Phase Extraction) method
The WAHSPE method begins with a three-solvent extraction procedure and includes a primary extraction with acidified acetonitrile/water and heptane followed by salt-induced partitioning into three phases (water, acetonitrile, and heptane). This is followed by one or more clean-up steps as needed (none, dispersive solid-phase extraction (dSPE), or solvent switch followed by SPE). This procedure is depicted schematically in Fig. 1.

2.4.1 Extraction

A 5.0 g aliquot of milled sample was transferred into a 50 mL polypropylene tube (Sarstedt AG & Co. KG, Nümbrecht, Germany). For analysis of polar pesticides, isotopically-labelled standards were added at this stage. Samples were extracted with 10.0 mL of 5% formic acid in acetonitrile, 10.0 mL of Milli-Q water, and 10.0 mL of heptane. The mixture was shaken end-over-end for 1 hour with a mechanical shaker (Reax 2 overhead shaker, Heidolph, Schwabach, Germany).

2.4.2 Phase separation

Five g of ammonium formate was added to induce phase separation (from two to three phases). The mixture was shaken for 1 min, followed by centrifugation for 10 min at 5,000 $\times g$ and 20 ℃. After centrifugation, the three phases were as follows: heptane at the top, acetonitrile in the middle, and water at the bottom. Sample matrix accumulated between the phases and at the bottom of the tube. Each of the three phases was isolated and transferred into separate 15 mL polypropylene tubes (Sarstedt AG & Co. KG, Nümbrecht, Germany).

It should be noted that, although 10 mL of each solvent (water, acetonitrile, and heptane) was initially added to the sample to promote extraction, phase separation induced by the addition of solid ammonium formate resulted in altered solvent volumes. This was due to the partial miscibility
of the phases and the dissolution of ammonium formate in the water phase. To address this issue, the resulting phase volumes were determined in an extraction procedure performed in the absence of sample (i.e., reagent blank); the volume of each phase was determined with adjustable-volume micropipettes with a precision of 0.1 mL (10 replicates). After phase separation, the aqueous, acetonitrile, and heptane phases contained volumes (± standard deviation) of 16.4 (± 0.1) mL, 7.2 (± 0.1) mL, and 9.3 (± 0.1) mL, respectively. The volumes of the three different phases were taken into account in the calculation of analyte recovery in experiments performed without internal standards (see section 2.6.6).

2.4.3 Clean-up

For LC-MS analysis, the water and acetonitrile phases were cleaned-up as follows: 400 µL of the respective phase (acetonitrile or water phase) were filtered using a filter vial (0.2 µm, Separa, ThermoFisher Scientific, Roskilde, Denmark). No additional clean-up procedures were performed. The filtered extracts were placed in separate autosampler vials (Carl Roth, Malente, Germany) with inserts and analysed by LC-MS.

For GC-MS analysis, 1 mL of the acetonitrile phase was transferred to an Eppendorf vial containing 250 mg primary secondary amine (PSA), 150 mg MgSO₄, and 25 mg C18 for dSPE clean-up. The Eppendorf tube was vortexed for 30 s and centrifuged at 10,000 × g for 10 min. Four hundred µL of the resulting supernatant was then transferred into a filter vial (0.2 µm, Separa, ThermoFisher Scientific, Roskilde, Denmark). Two hundred µL of the filtered supernatant was then transferred to a 1.5 mL autosampler vial (Carl Roth, Malente, Germany) with a glass insert and acidified with 10 µL of 1% formic acid in acetonitrile. One mL of the heptane phase was then transferred into a 10 mL
glass test tube (Biotage, Uppsala, Sweden) followed by the addition of 1 mL of acetonitrile. Isotopically-labelled internal standards were added at this stage to facilitate the evaluation of PAHs. The $^{13}$C-PAH internal standard mix was added to the heptane phase of the spiked samples (see section 2.6.1) at a concentration of 10 ng/mL before performing evaporation for the solvent switch (Fig. 1); this allows for adjustments due to variations in evaporation. The mixture remained under a nitrogen stream at 40 °C until the heptane phase had evaporated, leaving acetonitrile and residual lipids. The acetonitrile extract and the residual lipids were subjected to clean-up using the SPE column, EZ-Pop NP (12 mL, Sigma-Aldrich, St. Louis, MO, USA). Before clean-up, the column was conditioned with 10 mL acetone and dried for 10 min under vacuum. The acetonitrile extract and the residual lipids were then loaded onto the column. Acetonitrile (15 mL) was added, and the retained analytes were eluted into a 20 mL glass test tube. The eluate was placed in a nitrogen evaporator and the volume was reduced to 1 mL under a nitrogen stream at 40 °C for approximately 2 hours. The sample was then transferred to an autosampler vial (Carl Roth, Malente, Germany) with an insert and analysed by GC-MS.

2.4.4 Instrumental analysis

The GC-MS and LC-MS analyses were performed using five different instruments in two different laboratories. The key analytical parameters are summarized in Supplementary Material A, Table A.8. The quantification was performed by interpolation from the mean of two bracketing calibration curves. Accurate masses of mycotoxins and PAHs were used in analyses performed on high-resolution instruments. Tandem mass spectrometry was used for the analysis of polar and non-polar pesticides; two transitions were used for quantitation and identification. Three examples of pesticide analysis in samples of fish feed are included in Supplementary Material B.
2.4.5 Matrix-specific details

The WAHSPE procedure was slightly modified when used for the analysis of several specific analyte groups, and in some cases, not all phases were analysed. For procedures designed to detect pesticides in fish feed, the cleaned-up heptane and acetonitrile extracts were mixed with a solution of internal standards in acetonitrile together with additional acetonitrile to reach a final internal standard concentration of 0.010 µg mL⁻¹. The internal standard solution was used for quality control only; the water phase was not analysed. The extracts were subjected to clean-up as described above for GC-MS and were analysed by both GC-MS and LC-MS. For the analysis of polar pesticides in sunflower seeds and wheat, the water phase and the acetonitrile phase were analysed by LC-MS after phase separation. Internal standards were added before the extraction procedure and used for quantification, and the heptane phase was not analysed. For the evaluation of PAHs in mealworm and sunflower seeds, only the acetonitrile and heptane phases were analysed. For procedures used to detect mycotoxins in maize, only the acetonitrile and water phases were analysed. The acetonitrile phase was concentrated to increase sensitivity; 5 mL was evaporated under a nitrogen stream at 40 °C to a final volume of 500 µL.

2.5 GC-flame-ionization detection (FID) for the determination of residual fat content in extracts subjected to clean-up procedures.

Triplicate samples of sunflower seeds were prepared according to the extraction method previously described (Section 2.4) without the clean-up steps. In each triplicate experiment, 1 mL or 3 mL of the heptane phase was transferred into a glass tube and 1 mL of acetonitrile was added. The mixture was
evaporated under a nitrogen stream at 40 °C until the heptane phase had evaporated, leaving acetonitrile and residual lipids. The residual lipids and acetonitrile were transferred to an EZ-Pop NP SPE column to remove the lipids as previously described (see Section 2.4.3). The cleaned-up extract was transferred to a vial with an insert. GC-FID was performed to analyse the samples for residual lipids (Capuano et al., 2014).

2.6 Validation

2.6.1 Spiking of test samples

Five different materials, including fish feed, maize, mealworm, sunflower seeds, and wheat were chosen to evaluate the effectiveness of the new extraction protocol. Fish feed and sunflower seed samples had a lipid content >20%; the lipid content of mealworms was measured at >10%, and that of wheat and maize, < 10%. The spiking solution was added to the samples 20 min before extraction.

2.6.2 Spiking fish feed with pesticides

Fish feed samples were spiked with a mix of 189 pesticides at 5, 10, and 50 µg kg⁻¹; 6 replicate samples at each concentration were evaluated (see Supplementary Material A, Standards).

2.6.3 Spiking sunflower seeds and wheat with polar pesticides

Sunflower seeds and wheat were spiked with polar pesticides, with five replicates each of two different concentrations for each analyte. Spike levels are summarized in Supplementary Material A, Table A.6.

2.6.4 Spiking maize with mycotoxins
The mycotoxins AFB1, AFB2, AFG1, AFG2, OTA, FB1, FB2, ZEN, DON, HT-2, and DAS were spiked into the milled maize sample, with 6 replicates each at two different concentrations. Spike level 1 was 2.0 µg kg\(^{-1}\) for AFB1 and AFG1, 0.5 µg kg\(^{-1}\) for AFB2 and AFG2, 1 µg kg\(^{-1}\) for OTA, 200 µg kg\(^{-1}\) for FB1 and FB2, 100 µg kg\(^{-1}\) for ZEN, 200 µg kg\(^{-1}\) for DON, and 10 µg kg\(^{-1}\) each for HT-2 and DAS. Spike level 2 was 8.0 µg kg\(^{-1}\) for AFB1 and AFG1, 2.0 µg kg\(^{-1}\) for AFB2 and AFG2, 10 µg kg\(^{-1}\) for OTA, and 100 µg kg\(^{-1}\) each for HT-2 and DAS.

### 2.6.5 Spiking mealworm and sunflower seeds with PAHs

Mealworm and sunflower seed samples were spiked with a mixture of 24 PAH standards at a concentration of 1 µg mL\(^{-1}\) in toluene. Experiments included 5 replicates at two spike levels, including 20 and 200 µg kg\(^{-1}\) for the sunflower seed samples and 25 and 250 µg kg\(^{-1}\) for the mealworm samples. A mix of 22 \(^{13}\)C-isotopically-labelled PAHs (50 µL at 0.5 µg mL\(^{-1}\) in isoctane) was added to 1 mL of the heptane extract (equivalent to 50 µg kg\(^{-1}\)). See Fig. 1 for an overview of this method.

### 2.6.6 Quantification

Quantification was based on a matrix-matched calibration for pesticides in fish feed and mycotoxins in maize. For fish feed, pesticide calibration standards were prepared in acetonitrile extracts of a blank sample at concentrations including 0.10, 0.33, 1.0, 3.3, 10, and 33.3 µg kg\(^{-1}\), to quantify the pesticides in the acetonitrile phase. For analysis of pesticides in the heptane phase, pesticide calibration standards were prepared in the heptane extracts of a blank sample, after solvent change to acetonitrile and clean-up by SPE, at concentrations including 0.10, 0.33, 1.0, 3.3, 10, and 33.3 µg kg\(^{-1}\).
For maize, calibration standards were prepared in water extracts of blank samples for FB1, FB2 (at 10 concentrations over a range from 5 to 200 µg kg⁻¹), and DON (range from 10 to 400 µg kg⁻¹). Calibration standards in maize were prepared in blank acetonitrile samples for AFB1, AFB2, AFG1, AFG2, OTA, ZEN, DON, HT-2, and DAS that included 10 different concentrations in the following ranges: AFB1 and AFG1, from 2 to 80 µg kg⁻¹; AFB2 and AFG2, from 0.5 to 20 µg kg⁻¹; OTA, from 0.25 to 10 µg kg⁻¹; ZEN, from 5 and 200 µg kg⁻¹; DON, from 10 to 400 µg kg⁻¹; and HT-2 and DAS, from 5 and 200 µg kg⁻¹. For the polar pesticides (in sunflower seeds and wheat) and PAHs (in mealworms and sunflower seeds), isotopically-labelled internal standards were used for quantification. Calibration was performed using multi-level solvent standards (for concentrations, see Supplementary Material A, Table A.2) containing the internal standards. For quantification, the response ratios of the native analytes to their corresponding internal standards were calculated.

3 Results and discussion

This work aimed to develop a generic extraction method that would facilitate the analysis of pesticides, mycotoxins, and PAHs over a wide range of polarities in both low- and high-fat matrices. Hydrophobicity (log K<sub>ow</sub>) values for the analytes tested in this study ranged from -4.6 for diquat (PubChem, Diquat) to 7.3 for dibenzo(α,h)pyrene (PubChem, Dibenzo[α,h]pyrene). The analytes were classified as follows: high polarity (log K<sub>ow</sub> < 2.5), intermediate polarity (2.5 ≤ log K<sub>ow</sub> < 4), and low polarity (log K<sub>ow</sub> ≥ 4), as described by Hepperle et al. (2015). The extraction/partitioning method featured here generates three solvent phases (water, acetonitrile, and heptane) that were analysed at two different laboratories using existing LC-MS and GC-MS methodologies.
Analytes were selected to demonstrate a comparison between the WAHSPE and QuEChERS procedures. This study included compounds that present challenges to standard chemical analysis, including several already shown to be incompatible with QuEChERS methodology. Compounds in this group include some highly polar pesticides (with log Kow values below -2) and non-polar analytes in matrices with a high lipid content (Rejczak & Tuzimski, 2015). Diquat and paraquat were selected as highly polar pesticides and organochlorine pesticides and PAHs as non-polar analytes.

3.1 Method development

The WAHSPE procedure was inspired by a method originally published by González-Curbelo et al. (González-Curbelo et al., 2014). The original procedure was modified by (1) the addition of a heptane phase, (2) the use of formic acid to adjust the pH, and (3) induction of phase separation with ammonium formate salts. The pH adjustment with formic acid was included to improve the stability of pesticides that are chemically labile under neutral or basic conditions.

The acetonitrile phase was analysed by LC-MS or GC-MS, depending on the specific analytes of interest. For LC-MS analysis, low background signals were obtained upon injection of the acetonitrile phase obtained from a blank maize-extraction sample. Evaluation of total ion chromatograms (TICs), obtained by LC-full scan high-resolution mass spectrometry (HRMS), revealed the sporadic appearance of background peaks with medium intensity values. However, the numbers and intensities of the matrix peaks identified in these extracts were sufficiently low so that the use of clean-up methods other than filtration was unnecessary. By contrast, the acetonitrile phase needed to undergo clean-up to remove lipids and water before analysis by GC-MS. Primary secondary amine (PSA), MgSO₄, and C18 were used for sample clean-up according to an established method (Tienstra
& Mol, 2018). The use of this clean-up procedure resulted in a low overall background on GC-full scan HRMS TICs, which revealed a limited number of peaks of medium intensity.

The water phase was analysed by LC-MS. This was facilitated by the selection of ammonium formate rather than a non-volatile salt to induce phase separation. Injection of the filtered water phase alone resulted in TICs with a small number of peaks, typically of medium intensity. As such, no clean-up or salt removal was required. Samples were subjected to filtration only before injections were performed.

The heptane phase was analysed by GC-MS; this required the elimination of all co-extracted lipids. GC-FID analysis of the heptane phase from a sunflower seed extract revealed the presence of both di- and triglycerides (Supplementary Material A, Fig. A.1). An attempt at clean-up of the heptane phase using a published SPE method (Stenerson et al., 2015), did not yield satisfactory results, as di- and triglyceride peaks were still observed in the chromatogram. This problem was solved by performing a solvent switch from heptane to acetonitrile. One mL of the heptane phase was placed in a glass tube and acetonitrile was added; the heptane was evaporated until only acetonitrile and residual lipids remained. The remaining acetonitrile and residual lipids were subjected to clean-up by SPE (EZ-Pop NP) as described previously (Stenerson et al., 2015). The acetonitrile eluent resulting from this clean-up method was analysed by GC-FID; the resulting chromatogram was devoid of all di- and triglyceride peaks. An attempt to use a larger volume of the heptane phase (3 mL) to perform a solvent switch followed by SPE was hampered by the limited capacity of the SPE column; di- and triglycerides were again detected in the resulting chromatogram. Based on the samples used (sunflower seed, 40 % fat) and the amount of sample-equivalent in the heptane extract, the
apparent capacity of the SPE column was determined to be at least 0.2 g, but less than 0.6 g of sunflower oil.

Various LC-MS and GC-MS methods were used to evaluate sample extracts; this was due to the fact that the experimental work in this study was performed in different laboratories that focus on different sub-classes of residues and contaminants. However, results can be obtained with fewer LC- and GC-based methods than those used here. Specifically, four injections on two different systems will be sufficient to cover the entire spectrum of physicochemical properties presented by the analytes included in this study. Specifically, the water and the acetonitrile phases can be analysed by LC-MS, whilst the heptane and acetonitrile phases can be analysed by GC-MS.

3.2 Method validation

The validation parameters assessed in these trials include linearity, selectivity, limit of quantitation (LOQ), repeatability, trueness, and precision. Matrix effects were taken into account by using matrix-matched calibration standards or isotopically-labelled internal standards.

Linearity was tested by injecting at least six different concentrations of each compound, followed by evaluation of calibration curves and generation of correlation coefficients ($R^2$). Evaluations of all analytes resulted in good linearity with $R^2 > 0.98$. Selectivity was determined for each compound group by identification with HRMS or tandem mass spectrometry methods. LOQ was determined by analysis of at least five replicates at various concentrations; the lowermost concentration that yielded recoveries between 70 – 120 % with RSDs < 20 % was assigned as the LOQ. Trueness was determined by quantitative evaluation of each compound in blank samples spiked with known concentrations, followed by a determination of the percentage recovery. Method precision was
evaluated by measuring the RSDs of recovery of the replicates evaluated for each compound. For an overview of all results, see Supplementary Material C.

3.2.1 Pesticides in fish feed

A mixture of 189 pesticides, including those amenable to analysis using the QuEChERS procedure, were spiked into fish feed (22% fat content) at final concentrations of 5, 10, and 50 µg kg\(^{-1}\). Recoveries between 70 – 120% were determined at the highest pesticide concentrations (spike levels of 50 µg kg\(^{-1}\)) for 181 of the 189 pesticides evaluated. By contrast, recovery of carbendazim and omethoate was between 40 – 70%; recovery of an additional six pesticides (bixafen, coumaphos, dimoxystrobin, indoxacarb, methiocarb, procymidone) exceeded 120%. Reduced recovery of carbendazim and omethoate may relate to their partial water solubility, a factor that was not evaluated in this experiment. Recoveries > 120% might be the result of interference from other compounds that were integrated into the signals obtained from each of these pesticides.

Recoveries in the range of 70 – 120% were determined for 146 of the 189 pesticides at the mid-range dose (spike level of 10 µg kg\(^{-1}\)). However, at the lowest dose (5 µg kg\(^{-1}\)), only 139 of these pesticides were recovered at 70 – 120%. The observed discrepancies between recoveries at the highest and lowest spike doses may relate to limitations associated with instrument sensitivity.

Pesticides from the organochlorine group were detected in both the heptane and the acetonitrile phases, with combined recoveries between 96 – 118% for the spike levels of 50 µg kg\(^{-1}\) (Table 1). Most of the other pesticides evaluated in this study were amenable to QuEChERS extraction protocols; results from the WAHSPE method showed similar recoveries and LOQ values. As such, the validation acceptance criteria were met for most of the pesticides evaluated even at the lowest
spike levels as per the SANTE guidelines (European Commission, 2019). A LOQ of 5 µg kg\(^{-1}\) was achieved for all analytes evaluated. Of importance, a LOQ of 5 µg kg\(^{-1}\) is lower than the default maximum residue level for pesticides (at 10 µg kg\(^{-1}\)) set by the European Union (European Commission, 2005). As such, this method is thus appropriate for use in monitoring and control of pesticide residues in lipid-rich and complex matrices such as fish feed.

Most of the pesticides evaluated in this study were amenable to QuEChERS and were analysed in previously published studies (Anastassiades et al., 2003; González-Curbelo et al., 2014; Lehotay et al., 2005). For these compounds, QuEChERS and WAHSPE are equally suitable. However, the QuEChERS extraction method resulted in low recoveries of organochlorine pesticides in lipid-rich matrices (Cunha et al., 2007). By contrast, WAHSPE resulted in sufficient recoveries of these pesticides, thus demonstrating the advantage of the WAHSPE protocol for analyses of compounds in high-fat matrices.

3.2.2 Analysis of polar pesticides

Diquat, paraquat, mepiquat, cyromazine, chlormequat, difenzoquat, and trimesium were chosen as representative highly-polar pesticides and were used to spike extracts of wheat and sunflower seeds. Analyses of both the acetonitrile phase and the water phase resulted in combined recoveries between 80 – 141\% (Table 2). Diquat and paraquat were detected primarily in the water phase, whereas mepiquat, cyromazine, chlormequat, difenzoquat, trimesium were detected primarily in the acetonitrile phase. The lowest detectable concentration of these compounds was 5 µg kg\(^{-1}\) for diquat, chlormequat, and difenzoquat, 10 µg kg\(^{-1}\) for paraquat, cyromazine, and mepiquat, and 12.5 µg kg\(^{-1}\) for trimesium. Of note, none of these polar pesticides can be evaluated by the QuEChERS
method; they are typically analysed using specialized methods (Anastassiades et al., 2015; Rejczak & Tuzimski, 2015). As such, it is clear that the WAHSPE protocol is broad in scope and can be used for the evaluation of several classes of analytes that cannot be extracted using the QuEChERS method.

3.2.3 Analysis of mycotoxins

Recoveries from spiked samples of maize were determined by LC-MS analysis of the water and acetonitrile phases. FB1 and FB2 were recovered from the aqueous phase only, whilst DON was detected in both phases. The aflatoxins, OTA, ZEN, HT-2, and DAS were all recovered from the acetonitrile phase. Mycotoxins spiked at level 1 as described above were recovered at rates between 51 – 116%. Except for ZEN and FB2, all mycotoxins were recovered at satisfactory rates ranging from 70 – 120% (Table 3). The RSDs associated with these recoveries were between 5 – 25%.

The LOQs determined for these mycotoxins are adequate, indicating that this method can be used to test compliance with the limits on mycotoxin contamination of cereals by current EC regulations (EC 2006/1881), which include 2.0 μg kg\(^{-1}\) for aflatoxin B1, 4.0 μg kg\(^{-1}\) for the sum of aflatoxins, 5 μg kg\(^{-1}\) OTA, 1000 μg kg\(^{-1}\) for the sum of FB1 and FB2, 75 μg kg\(^{-1}\) for ZEN, and 750 μg kg\(^{-1}\) for DON (EC 1881/2006).

All mycotoxins evaluated in this study are amenable to extraction using the QuEChERS method, although some of the recoveries are challenging; relatively low recovery rates were obtained using the QuEChERS method for extraction of FB1, FB2, and DON (Dzuman et al., 2014). For most of our mycotoxin analytes, recoveries were in the range of 70 – 120%, demonstrating that the WAHSPE protocol is suitable for use in analyses of mycotoxin contamination.
3.2.4 Analysis of PAHs

To study the performance of the WAHSPE method for extraction of PAHs, samples of mealworm and sunflower seeds were spiked with 24 PAH compounds together with 22 isotopically-labelled PAH internal standards. The lowest spike levels (spike level 1) included 200 µg kg\(^{-1}\) for naphthalene and 20 µg kg\(^{-1}\) for all the other PAHs. Recoveries of PAHs at spike level 1 from the heptane phase of extracts of mealworm and sunflower seeds at spike level 1 were between 65 – 127% (RSD 1 – 13%) and 32 –104% (RSD 2 – 17%), respectively. For mealworms, 23 out of 24 analytes were recovered at rates of 50 – 120%; for sunflower seeds, 19 out of 24 were in this range (Table 4), which is satisfactory as per EC regulation 333/2007 (EC 333/2007) and the scientific opinion of the European Food Safety Authority (EFSA; Alexander et al., 2008).

The lowest spike levels tested in this study were higher than the LOQs required for food control, as the EU maximum levels for the PAH-4 in oil and benz[α]pyrene are 10 µg kg\(^{-1}\) and 2 µg kg\(^{-1}\), respectively ([EU] No. 835/2011). Nonetheless, the recoveries achieved using the WAHSPE method are acceptable. To reach the lower LOQ, it may be possible to concentrate the SPE eluent 5-fold by evaporation of the heptane phase clean-up to 200 µL instead of 1 mL; however, this may also result in more pronounced matrix effects. While non-polar compounds in lipid-rich matrices are challenging to analyse (Forsberg et al., 2011; González-Curbelo et al., 2015; Rejczak & Tuzimski, 2015; Wilkowska & Biziuk, 2011), these analytes can be recovered at sufficiently high rates when using WAHSPE extraction, thereby providing an important advantage over the QuEChERS method.

3.3 WAHSPE versus QuEChERS

The advantages of WAHSPE vs. QuEChERS extraction procedures include higher rates of recovery of both highly-polar and non-polar analytes in lipid-rich matrices. However, QuEChERS might be a more
efficient choice for applications limited to compounds of moderate polarity. WAHSPE generally requires several separate analyses to exploit its full potential, including two LC analyses and one or two GC analyses. The decision tree shown in Fig. 2 can be used as a guide when choosing between the more laborious, but comprehensive WAHSPE and the more straightforward QuEChERS procedures. The WAHSPE method is quite versatile and can be expanded to perform analyses in other challenging matrices by changing one or more of its elements as necessary; amongst various options, one can consider adjustments to the pH or SPE sorbents. The WAHSPE method presented here is a strong contribution to the field of food science as it facilitates screening for multiple compounds in a single sample and collection of more data with less effort required for sample preparation.

4 Conclusion

The results of this study demonstrate that the newly-developed WAHSPE method can be used to perform simultaneous extractions of both polar and non-polar compounds from both high and low fat-containing matrices. The efficacy of this method was demonstrated for 231 compounds, including pesticides, mycotoxins, and PAHs. Of particular note, the use of WAHSPE to extract polar pesticides was validated using both sunflower seeds and wheat. Likewise, the results validated the use of the method to extract pesticides from lipid-rich fish feed, mycotoxins from maize, and PAHs from lipid-rich mealworm and sunflower seeds. The recovery from all matrices and associated RSD values were in most cases satisfactory. Taken together, the results confirm the initial hypothesis and document that WAHSPE is highly effective at extracting more and a greater variety of compounds than either QuEChERS or SweEt.
Acknowledgments

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References


EU 2017/660. Commission implementing regulation (EU) 2017/660 of 6 April 2017 concerning a coordinated multiannual control programme of the Union for 2018, 2019 and 2020 to ensure


https://doi.org/10.1016/j.talanta.2013.02.059


https://doi.org/10.1016/j.foodchem.2015.11.070


Rejczak, T., & Tuzimski, T. (2017). 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. Food Chemistry, 217, 225–233. https://doi.org/10.1016/j.foodchem.2016.08.095


Figure captions

Fig. 1. Scheme of the WAHSPE procedure. For a detailed description of these individual steps, see Materials and methods.

Fig. 2. Decision tree to be used to determine appropriate use of the QuEChERS or WAHSPE extraction procedures.

Supplementary Material captions

Supplementary Material A: Summary of additional materials, methods, and results

Supplementary Material B: Identification of pesticides in fish feed by GC-MS/MS

Supplementary Material C: Expanded results tables that include all recoveries and RSDs from the validation experiments
Table 1. Recoveries and repeatability using the WAHSPE procedure. Analysis of organochlorine pesticides in the heptane and acetonitrile phases of an extract of fish feed.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Recovery from the heptane phase (%) at 50 µg kg(^{-1})</th>
<th>Recovery from the acetonitrile phase (%) at 50 µg kg(^{-1})</th>
<th>Total recovery (%) at 50 µg kg(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aldrin</td>
<td>78 (19)</td>
<td>11 (14)</td>
<td>89 (17)</td>
</tr>
<tr>
<td>Dieldrin</td>
<td>59 (13)</td>
<td>55 (13)</td>
<td>114 (15)</td>
</tr>
<tr>
<td>Chlordane alpha-cis</td>
<td>67 (20)</td>
<td>37 (11)</td>
<td>104 (16)</td>
</tr>
<tr>
<td>Chlordane gamma-trans</td>
<td>71 (19)</td>
<td>42 (5)</td>
<td>113 (12)</td>
</tr>
<tr>
<td>DDD pp</td>
<td>43 (8)</td>
<td>64 (4)</td>
<td>107 (6)</td>
</tr>
<tr>
<td>DDE pp</td>
<td>78 (15)</td>
<td>21 (4)</td>
<td>99 (10)</td>
</tr>
<tr>
<td>DDT op</td>
<td>72 (12)</td>
<td>23 (5)</td>
<td>95 (9)</td>
</tr>
<tr>
<td>DDT pp</td>
<td>60 (17)</td>
<td>35 (6)</td>
<td>95 (12)</td>
</tr>
<tr>
<td>Endosulfan sulfate</td>
<td>not detected</td>
<td>106 (13)</td>
<td>109 (59)</td>
</tr>
<tr>
<td>Endosulfan alpha</td>
<td>59 (23)</td>
<td>46 (8)</td>
<td>105 (16)</td>
</tr>
<tr>
<td>Endosulfan beta</td>
<td>20 (48)</td>
<td>82 (7)</td>
<td>102 (28)</td>
</tr>
<tr>
<td>Endrin</td>
<td>52 (14)</td>
<td>49 (18)</td>
<td>101 (16)</td>
</tr>
<tr>
<td>HCH, alpha</td>
<td>31 (10)</td>
<td>72 (5)</td>
<td>103 (8)</td>
</tr>
<tr>
<td>HCH, beta</td>
<td>10 (21)</td>
<td>100 (4)</td>
<td>110 (13)</td>
</tr>
<tr>
<td>Heptachlor</td>
<td>74 (11)</td>
<td>23 (10)</td>
<td>97 (11)</td>
</tr>
<tr>
<td>Heptachlor epoxide</td>
<td>47 (9)</td>
<td>60 (13)</td>
<td>107 (11)</td>
</tr>
</tbody>
</table>

RSDs (%) are shown in parentheses.
Table 2. Recoveries and repeatability using the WAHSPE procedure to analyse seven selected polar pesticides found in sunflower seeds.

<table>
<thead>
<tr>
<th>Compound (spike level 1 and spike level 2 in µg kg⁻¹)</th>
<th>Analysed phase</th>
<th>Recovery determined at spike level 1 (%)</th>
<th>Recovery determined at spike level 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diquat (5 and 50)</td>
<td>water</td>
<td>91 (6)</td>
<td>82 (7)</td>
</tr>
<tr>
<td>Paraquat (10 and 100)</td>
<td>water</td>
<td>80 (4)</td>
<td>141 (6)</td>
</tr>
<tr>
<td>Mepiquat (10 and 100)</td>
<td>acetonitrile</td>
<td>119 (3)</td>
<td>116 (4)</td>
</tr>
<tr>
<td>Cyromazine (10 and 100)</td>
<td>acetonitrile</td>
<td>101 (19)</td>
<td>100 (3)</td>
</tr>
<tr>
<td>Chlormequat (5 and 50)</td>
<td>acetonitrile</td>
<td>114 (3)</td>
<td>99 (4)</td>
</tr>
<tr>
<td>Difenzoquat (5 and 50)</td>
<td>acetonitrile</td>
<td>101 (9)</td>
<td>97 (15)</td>
</tr>
<tr>
<td>Trimesium (12.5 and 125)</td>
<td>acetonitrile</td>
<td>99 (4)</td>
<td>100 (2)</td>
</tr>
</tbody>
</table>

RSDs (%) are shown in parentheses.
Table 3. Recoveries and repeatability of WAHSPE for the analysis of mycotoxins in maize.

<table>
<thead>
<tr>
<th>Compound (spike level 1 and 2 in µg kg⁻¹)</th>
<th>Analysed Phase</th>
<th>Recovery determined at spike level 1 (%)</th>
<th>Recovery determined at spike level 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aflatoxin B1 (2.0 and 8.0)</td>
<td>acetonitrile</td>
<td>100 (5)</td>
<td>102 (5)</td>
</tr>
<tr>
<td>Aflatoxin B2 (0.5 and 2.0)</td>
<td>acetonitrile</td>
<td>92 (5)</td>
<td>98 (6)</td>
</tr>
<tr>
<td>Aflatoxin G1 (2.0 and 8.0)</td>
<td>acetonitrile</td>
<td>107 (4)</td>
<td>103 (5)</td>
</tr>
<tr>
<td>Aflatoxin G2 (0.5 and 8.0)</td>
<td>acetonitrile</td>
<td>111 (8)</td>
<td>94 (3)</td>
</tr>
<tr>
<td>OTA (1 and 10)</td>
<td>acetonitrile</td>
<td>73 (25)</td>
<td>77 (7)</td>
</tr>
<tr>
<td>Fumonisin B1 (200)</td>
<td>water</td>
<td>112 (6)</td>
<td>Not determined</td>
</tr>
<tr>
<td>Fumonisin B2 (200)</td>
<td>water</td>
<td>51 (4)</td>
<td>Not determined</td>
</tr>
<tr>
<td>Zearalenone (100)</td>
<td>acetonitrile</td>
<td>63 (8)</td>
<td>Not determined</td>
</tr>
<tr>
<td>DON (200)</td>
<td>water and acetonitrile</td>
<td>72 (10)</td>
<td>Not determined</td>
</tr>
<tr>
<td>HT-2 (10 and 100)</td>
<td>acetonitrile</td>
<td>103 (7)</td>
<td>72 (10)</td>
</tr>
<tr>
<td>DAS (10 and 100)</td>
<td>acetonitrile</td>
<td>93 (6)</td>
<td>116 (8)</td>
</tr>
</tbody>
</table>

RSDs (%) are shown in parentheses. The recoveries of FB1, FB2, and DON were measured at spike level 1 only.
Table 4. Recoveries and repeatability of WAHSPE for the analysis of PAHs in samples of mealworm and sunflower seeds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Mealworm</th>
<th>Sunflower seeds</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>spike level 1 20 µg kg⁻¹ in %</td>
<td>spike level 2 200 µg kg⁻¹ in %</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>Recovery</td>
</tr>
<tr>
<td></td>
<td>spike level 1 20 µg kg⁻¹ in %</td>
<td>spike level 2 200 µg kg⁻¹ in %</td>
</tr>
<tr>
<td>5-Methylchrysene</td>
<td>84 (3)</td>
<td>62 (3)</td>
</tr>
<tr>
<td>Acenaphthylene</td>
<td>89 (4)</td>
<td>36 (1)</td>
</tr>
<tr>
<td>Acenaphthene</td>
<td>99 (3)</td>
<td>60 (1)</td>
</tr>
<tr>
<td>Anthracene+ Phenanthrene</td>
<td>106 (3)</td>
<td>62 (2)</td>
</tr>
<tr>
<td>Benz[a]anthracene+ Chrysene</td>
<td>79 (5)</td>
<td>54 (1)</td>
</tr>
<tr>
<td>Benzo[a]pyrene</td>
<td>108 (4)</td>
<td>75 (1)</td>
</tr>
<tr>
<td>Benzo[b+k+j]fluoranthene</td>
<td>127 (2)</td>
<td>92 (13)</td>
</tr>
<tr>
<td>Benzo[c]fluorene</td>
<td>75 (2)</td>
<td>76 (3)</td>
</tr>
<tr>
<td>Benzo[g,h,i]perylene</td>
<td>75 (4)</td>
<td>47 (1)</td>
</tr>
<tr>
<td>Cyclo pent[a,d]pyrene</td>
<td>105 (17)</td>
<td>60 (2)</td>
</tr>
<tr>
<td>Dibenzo[a,h]anthracene</td>
<td>65 (6)</td>
<td>45 (1)</td>
</tr>
<tr>
<td>Dibenzo[a,e]pyrene</td>
<td>117 (16)</td>
<td>74 (7)</td>
</tr>
<tr>
<td>Dibenzo[a,h]pyrene</td>
<td>92 (5)</td>
<td>55 (3)</td>
</tr>
<tr>
<td>Dibenzo[a,i]pyrene</td>
<td>69 (8)</td>
<td>55 (3)</td>
</tr>
<tr>
<td>Dibenzo[a,L]pyrene</td>
<td>96 (9)</td>
<td>86 (7)</td>
</tr>
<tr>
<td>Fluoranthene</td>
<td>120 (2)</td>
<td>66 (1)</td>
</tr>
<tr>
<td>Fluorene</td>
<td>119 (2)</td>
<td>104 (1)</td>
</tr>
<tr>
<td>Indeno[1,2,3-cd]pyrene</td>
<td>70 (5)</td>
<td>45 (3)</td>
</tr>
<tr>
<td>Naphthalene</td>
<td>Not detected</td>
<td>Not detected</td>
</tr>
<tr>
<td>Pyrene</td>
<td>99 (5)</td>
<td>47 (2)</td>
</tr>
</tbody>
</table>

RSDs (%) are shown in parentheses. All recoveries (from the heptane phase) were calculated with reference to ¹³C-labelled internal standards.
Highlights

- New three-phase extraction method to cover widest variety of analytes/food matrices
- Three phase extraction method using water, acidified acetonitrile, and heptane
- Suited for analyte/matrix combinations that are not amenable to established methods
- Clean-up with solid phase extraction to ensure gas chromatography compatibility
- Works from diquat in cereals to organochlorine pesticides in lipid-rich matrices
Development of a new generic extraction method for the analysis of pesticides, mycotoxins, and polycyclic aromatic hydrocarbons in representative animal feed and food samples

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