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Analysis of pesticide and veterinary drug residues in baby food by liquid chromatography coupled to Orbitrap high resolution mass spectrometry

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ABSTRACT

Pesticide and veterinary drug residues have been simultaneously determined in several baby foods as meat, fish and vegetable-based baby food. A generic extraction method without clean-up step was applied. Moreover, the use of a representative matrix for proper quantification of all target compounds was studied and the best results were obtained when vegetable-based baby food was used as representative matrix, allowing the reliable quantification of more than 300 compounds. The method was validated and good recoveries were obtained for most of compounds at concentrations higher than 50 μg kg^{-1}. Limits of detection (LOD) ranged from 0.5 to 50 μg kg^{-1}, whereas limits of quantification (LOQ) were established between 10 and 100 μg kg^{-1}. Limits of identification (LOIs) ranged from 0.5 to 50 μg kg^{-1}. This method was applied to the analysis of 46 different baby food samples and no positive samples were found.

1. Introduction

There are significant economic benefits associated with the use of pesticides and veterinary drugs (VDs), but the inadequate use of these substances can provoke a transfer to the food chain, producing negative consequences in the consumer health [1]. Special attention must be paid to children and infants because they are a vulnerable risk group [2] due to their poor immunologic system and low weight [3–5].

The European Commission only has set up maximum residue levels (MRLs) for some chemicals and contaminants, such as pesticides and mycotoxins in processed cereal-based baby food and infant formulae, at 10 μg kg^{-1} or even at lower concentrations (4–8 μg kg^{-1}) [6]. This Directive (Commission Directive 2006/141/EC) put emphasis on the control of pesticides or transformation products (including metabolites) of pesticides with a maximum acceptable daily intake of 0.5 μg kg^{-1} body weight. Moreover, some pesticides shall not be used in agricultural production intended for the production of baby foods [7]. Regarding MRLs of pharmacologically active substances (including antibiotics) general MRLs are established for foodstuffs of animal origin [8] and the "zero tolerance" principle should be applied to those pharmaceuticals and foodstuffs for which MRLs have not been established [9].

The wide variety of infant food matrices and their complexity, as well as the high number of compounds that should be monitored, requires the application of reliable, high-throughput and efficient analytical methods [10]. There are studies related to the determination of several classes of VDs [2,5,9,10] and pesticides [11–15] in baby food, and one work focused on the development of a multiresidue method, where VDs and pesticides are analysed simultaneously by quadrupole (Q)-Orbitrap-MS [16] has been recently published, For that purpose, the development and application of generic extraction procedures are necessary. Moreover, the quantification process should be as fast and reliable as possible. In this sense, the use of high performance liquid chromatography coupled to Orbitrap mass spectrometry (UHPLC-Orbitrap-MS) [17] is sufficient to enable the detection, quantification and accurate mass measurement of a wide range of residues in complex matrices as baby food. However it is well-known that when electrospray ionization (ESI) is employed, matrix effect must be taking into account [18,19] and precision, linearity and sensitivity of the results are significantly affected, because matrix compounds can be responsible for the modification of the analytes behaviour into the electrospray source. This matrix effect can decrease (suppression) or increase (enhancement) the instrumental response of the analyte, and therefore, the analysis of a real

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sample or a pure analyte standard solution does not provide the same instrumental response [20]. Moreover, post-interface signal suppression has sometimes been observed when Orbitrap analyser is used, and this is mainly due to a high abundance of matrix multiple charged proteins in the C-trap located before the analyser [21].

The most commonly strategies used to compensate this matrix effect are based on several calibration procedures as matrix-matched calibration, standard addition method or isotopically labelled standards [22], being matrix-matched calibration the most widely used in routine laboratories. Other approaches to minimize matrix effect are the development of efficient extraction procedures, which can decrease the extraction of interferents [23], or the use of the dilution of the sample. In this sense, and despite the simultaneous extraction of other compounds present in the matrix, "dilute and shoot" method could be an interesting approach because of the dilution of the extracted compounds [24], minimizing matrix effect [25].

On the other hand, there is a wide variety of baby food with different compositions (i.e. meat or fish-based baby food, etc), and therefore, different matrix-matched calibrations should be performed for each type of baby food in order to achieve a reliable quantification. This is time-consuming, especially for routine laboratories, and thus, the use of a representative matrix is desirable in order to minimize the quantification process and associated quality controls [26].

The aim of this study was the analysis of more than 350 pesticides and VDs in several types of baby food based on a variety of raw materials (meat, fish and vegetables) evaluating matrix effect in all matrices, as well as the possibility of using a representative matrix for quantification, decreasing time-consuming.

2. Materials and methods

2.1. Chemicals and reagents

Pesticides and VDs analytical standards were purchased from Sigma-Aldrich (Madrid, Spain), Riedel-de-Haën (Seelze, Germany), Fluka (Steinheim, Germany), Dr. Ehrenstorfer GmbH (Ausburg, Germany), Witega (Berlin, Germany), Santa Cruz (Santa Cruz, CA, USA) and European Pharmacopoeia (Strasbourg, France). Individual stock standard solutions (100–450 mg L^{-1}) were prepared in methanol, acetone or acetonitrile, and they were stored at 5 °C or −18 °C (VDs). LC–MS grade methanol, acetonitrile and acetone were obtained from Fluka. VDs were grouped in families and a solution for each of them was prepared from corresponding individual stock standard solutions in methanol or acetonitrile, whereas two multi-pesticide solutions (corresponding to typical LC and GC-amenable compounds) were prepared in acetone or methanol. Then, a multi-compound working solution containing all the analytes (0.41 mg L^{-1}) was prepared by combining suitable aliquots of each individual standard stock solution and diluting them with LC–MS-grade methanol. This solution was kept at −18 °C. Formic acid (purity >98%) and ammonium formate (purity >99%) were obtained from Panreac (Barcelona, Spain). LC–MS water was provided by Scharlau (Barcelona, Spain). Extra-Bond Florisil cartridges (500 mg, 3 mL) were purchased from Scharlau (Barcelona, Spain). For accurate mass calibration, a mixture of caffeine, Met-Arg-Phe-Ala acetate salt (MRFA) and Ultramark 1600 (Proteo Mass LTQ/FT-Hybrid ESI positive mode calibration mix) and a mixture of acetic acid, sodium dodecyl sulphate, taurocholic acid sodium salt hydrate and Ultramark 1621 (fluorinated phosphazines) (ProteoMass LTQ/FT-Hybrid ESI negative mode calibration mix) from Thermo Fisher Scientific (Rockford, IL, USA) were used in the Orbitrap analyser.

2.2. Apparatus

For the extraction procedure, a rotary agitator from Heidolph (Schwabach, Germany) and an analytical AB204-S balance (Mettler Toledo, Greinfesee, Switzerland) were used. Centrifugations were performed in a Consul 21 high-volume centrifuge from Olto Alresa (Madrid, Spain).

2.3. UHPLC-Orbitrap-MS analysis

The separation of the analytes was carried out using a Transcend 600LC (Thermo Scientific Transcend™, Thermo Fisher Scientific, San Jose, CA, USA) equipped with an analytical column Hypersil GOLD aQ C18 column (100 mm × 2.1 mm, 1.7 μm particle size) from Thermo. The mobile phase consisted of 0.1% (v/v) formic acid and ammonium formate 4 mM in water (eluent A) and 0.1% (v/v) formic acid and ammonium formate 4 mM in methanol (eluent B). The analysis started with 95% of eluent A. After 1 min, this percentage was linearly decreased to 0% in 7.0 min. This composition was held during 4.0 min and increased again up to 95% in 0.5 min, followed by a re-equilibration time of 1.5 min (total running time=14.0 min). The flow rate was 0.3 mL min^{-1} and the column temperature was set at 30 °C. Aliquots of 10 μL of the sample extract were injected into the chromatographic system.

The UHPLC system was coupled to a single stage Orbitrap mass spectrometer (Exactive™, Thermo Fisher Scientific, Bremen, Germany) operating with a heated electrospray interface (HESI-II, Thermo Fisher Scientific, San Jose, CA, USA), in positive (ESI+) and negative ionization mode (ESI−) using the previously developed parameters [27]. Mass spectra were acquired using four alternating acquisition functions: (1) full MS, ESI+, without fragmentation (the higher collisional dissociation (HCD) collision cell was switched off), mass resolving power=25000 FWHM; scan time=0.25 s; (2) all ions fragmentation (AIF), ESI+, with fragmentation (HCD on, collision energy=30 eV), mass resolving power=10000 FWHM; scan time=0.10 s; (3) full MS, ESI−using the settings explained for (1); and (4) AIF, ESI−using the settings explained for (2). Mass range in the full scan experiments was set at *m/z* 70–1000. All the analyses were performed without lock mass. Mass accuracy was carefully monitored as follows: checked daily with the calibration mix solution (see Section 2.1); evaluated (once a week) and calibrated when necessary (every two weeks at least). Data were acquired using matrix-matched external calibration mode and they were processed using Xcalibur™ version 2.2.1 (Thermo Fisher Scientific, Les Ulis, France) with Qual and Quan-browser. Genesis peak detection was applied. ToxID™ 2.1.1 (automated compound screening software, Thermo Scientific) was used for screening and LCQuan™ 2.6 software (Thermo Scientific) was used for quantification during method validation and sample analysis.

2.4. UHPLC-QqQ-MS analysis

These analyses were carried out using an Agilent series 1290 RRLC instrument (Agilent, Santa Clara, CA, USA) equipped with a binary pump (G4220A), a high-performance autosampler (G4226A), an autosampler thermostat (G1330B) and a column compartment thermostat (G1316C). The system was coupled to an Agilent QqQ mass spectrometer (6460A) with a Jet Stream ESI ion source (G1958-65138). For the chromatographic separation of the extracts, a Zorbax Eclipse Plus C18 column (100 mm × 2.1 mm, 1.8 μm particle size) from Agilent was used. Column temperature was set at 30 °C and injection volume was 5 μL. Chromatographic