Journal of Chromatography A, 1347 (2014) 122–128

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

High-throughput screening of pesticide and veterinary drug residues in baby food by liquid chromatography coupled to quadrupole Orbitrap mass spectrometry

Wei Jia[a,b], Xiaogang Chu[a,b,*], Yun Ling[b], Junrong Huang[a], James Chang[c]

[a] College of Chemistry & Chemical Engineering, Shaanxi University of Science & Technology, Xi'an 710021, China
[b] Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing 100123, China
[c] Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA 95134, USA

ARTICLE INFO

Article history:
Received 10 March 2014
Received in revised form 19 April 2014
Accepted 22 April 2014
Available online 1 May 2014

Keywords:
Multi-residue methods
Q-Orbitrap
Targeted-screening
Baby food
Validation

ABSTRACT

A new analytical method was developed and validated for simultaneous analysis of 333 pesticide and veterinary drug residues in baby food. Response surface methodology was employed to optimize a generic extraction method. Ultrahigh-performance liquid chromatography and electrospray ionization quadrupole Orbitrap high-resolution mass spectrometry (UHPLC-ESI Q-Orbitrap) was used for the separation and detection of all the analytes. The method was validated by taking into consideration the guidelines specified in Commission Decision 2002/657/EC and SANCO/12571/2013. The extraction recoveries were in a range of 79.8–110.7%, with coefficient of variation <8.3%. The 333 compounds behave dynamic in the range 0.1–1000 $\mu g\,kg^{-1}$ concentration, with correlation coefficient >0.99. The limits of detection for the analytes are in the range 0.01–5.35 $\mu g\,kg^{-1}$. The limits of quantification for the analytes are in the range 0.01–9.27 $\mu g\,kg^{-1}$. This method has been successfully applied on screening of pesticide and veterinary drugs in ninety-three commercial baby food samples, and tilmicosin, fenbendazole, tylosin tartrate and thiabendazole were detected in some samples tested in this study. The present study is very useful for fast screening of different food contaminants.

1. Introduction

Pesticide and veterinary drugs are usually small molecular weight chemicals essential for treating infections, increasing production, and improving animal husbandry. However, the potential presence of contaminants and drug residues is an important issue in the field of food and animal feed safety [1,2]. Infants and children represent a vulnerable risk group of the population in terms of multi-residue toxicity and stringent regulations have been set to protect them from dietary exposure to these chemicals [3–5]. Baby foods combine a wide range of different matrices: cereal-based food (CBF), meat-based food (MBF), powdered milk-based infant formulae (PMBIF), non-fatty based on fruit (FBF) and vegetable (VBF) [6,7]. The European Commission (Directives 2006/141/EC and 2003/89/EC) specified the general Maximum Residue Levels (MRLs) of 10 $\mu g\,kg^{-1}$ for any individual pesticide residue in baby food. As a result of lack of regulation for veterinary drug residues, a "zero-tolerance" policy is applied for the veterinary drug residues in baby food, which means that the presence of these compounds is illegal at any level [8]. Consequently, these regulated limits require the analysis of extremely high number multi-residues in baby food [9–11].

Therefore, efficient, sensitive and accurate methods have been developed for the analysis of each group of residues separately. The determination of pesticide or veterinary drugs in baby food has been based on pressurized liquid extraction (PLE), liquid–liquid extraction (LLE), matrix solid-phase dispersion (MPSD) or modified QuEChERS methodology [12–17]. Some of these procedures have been followed by a clean-up step based on solid-phase extraction (SPE) or dispersive SPE, and subsequent chromatographic analysis by ultra-high-performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS) [12,14].

Normally, most of these methods are focused on specific groups of residues, not being suitable for wide-scope multi-residue analysis. To be able to analyze pesticide and veterinary drugs with a wide variety of physicochemical properties simultaneously, non-selective, generic sample-preparation procedures are applied. The

* Corresponding author at: Institute of Food Safety, Chinese Academy of Inspection and Quarantine, Beijing 100123, China. Tel.: +86 010 85778904; fax: +86 010 857707750.
E-mail addresses: foodjiawei@aliyun.com, foodjiawei@yahoo.com.cn (X. Chu).

http://dx.doi.org/10.1016/j.chroma.2014.04.081
0021-9673/

most frequently reported generic sample-preparation methods are "dilute and shoot" and QuEChERS methods [18–21]. A clear drawback of these strategies is the occurrence of abundant matrix effects, which compromise method selectivity, detection limits, maintenance frequency and quantitative aspects [22,23]. Nevertheless, the lack of selectivity in generic sample preparation can be compensated by selectivity in instrumental analysis. Although in terms of sensitivity, the use of LC or GC coupled to triple quadrupole tandem MS (QqQ) is usually preferred. However, QqQ instruments are not sufficiently selective and only provide unit mass resolution. In each retention time window, the number of transitions to be acquired can as such be kept relatively low. Using this approach, the number of analytes that can be acquired in one single run is limited [24–26]. An attractive alternative is the use of full scan high resolution mass spectrometry (theoretically, no limitations in number of monitored analytes). Most published high resolution mass spectrometry based multi-class, multi-residue methods are based on Orbitrap, time-of-flight (TOF), or quadrupole-time-of-flight (QqTOF) [27–29]. Typically, Orbitrap mass spectrometry is expected to provide resolving powers of not higher than 100,000–200,000 with detection times of 1–2 s [30]. From last year the role of UHPLC-Q-Orbitrap and related techniques is increasingly built up as enabling tool in food safety analysis for it can provide product-ion spectra with accurate mass measurement, that permit unequivocal confirmation of detailed structural information. In spite of the potential value of the application, to the best of our knowledge, so far no one has reported the application of Q-Orbitrap mass spectrometry combined with high performance liquid chromatography for simultaneous determination for a group of pesticide and veterinary drugs in foods [31,32].

Bearing in mind the lack of works related to the determination of several classes of pesticide and veterinary drugs in baby food, in this paper, we describe the development of a cost-effective, time-efficient and easy-to-use sample preparation method based on QuEChERS for the simultaneous extraction of more than 330 pesticide and veterinary drugs in different types of baby foods. Coupled with an optimized UHPLC-Q-Orbitrap method, this method was successfully applied on screening of multi-residues in baby food from local market.

2. Experimental

2.1. Chemicals and reagents

Pesticide and veterinary drugs analytical standards were purchased from LGC Standards (Teddington, UK), Fluka (Buchs, Switzerland), Sigma–Aldrich (Steinheim, Germany), Dr. Ehrenstorfer GmbH (Augsburg, Germany) and Witega (Berlin, Germany). Individual stock standard solutions (500–1000 mg L^{-1}) were prepared, depending on the specific solubility properties, by dissolving the appropriate quantity of the compound mainly in acetone (ACE), methanol (MeOH) or acetonitrile (MeCN), and were stored at −20 °C. HPLC-grade ACE, MeCN and MeOH were sourced from J.T. Baker (Deventer, Holland). A solution (100–300 mg L^{-1}) for each family of pesticide and veterinary drug was prepared from corresponding individual stock standard solution in MeCN or MeOH. Then, a multi-compound working solution at a concentration of 10 mg L^{-1} of each compound was prepared by combining suitable aliquots of each individual standard stock solution and diluting them with MeOH. Acetic acid, formic acid (FAc), ammonium formate, sodium acetate, sodium chloride and anhydrous sodium sulfate (Na_2SO_4) were of analytical grade and purchased from Sigma–Aldrich (Steinheim, Germany). Ultrafree-MC centrifugal filter devices (0.22 μm) of Millipore (Millipore, Brussels, Belgium) were used. Trifluoro acetic acid was obtained from Fluka (Buch, Switzerland). Ultrapure Water (resistivity, 18.2 MΩ) was purified on a Milli-Q Plus apparatus (Millipore, Brussels, Belgium).

2.2. Instrumentation

The UHPLC-Q-Orbitrap system consisted of an Accela 1250 LC pump and a CTC Analytics PAL open autosampler coupled with a Q Exactive mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). The system was controlled by Exactive Tune 1.1 and Xcalibur 2.2 software (Thermo Fisher Scientific, San Jose, USA).

2.3. Analytical procedure

2.3.1. Sample preparation

The sample preparation protocol was developed using modified literature protocols and personal experience [31,32]. After homogenization on a Polytron PT-2000 (Kinematica, Switzerland) for 30 s, 5.0 g of each sample was weighed in polypropylene centrifuge tubes (50 mL), fortified with the three hundred and thirty-three different analytes and let to stand for 15 min. 10 mL volume of a MeCN/water solution (84/16, v/v) with 1% acetic acid was added as an extraction solvent and the tube was tightly capped and vigorously mixed for 1 min using a vortex (Scientific Industries, New York, USA) mixer at maximum speed. Na_2SO_4 (6 g), sodium acetate anhydrous (1.45 g) and ceramic homogenizers were added to the tube, to induce phase separation. After that, the tube was immediately shaken for 1 min, and then centrifuged for 5 min at 2264 × *g* at 4 °C (Beckman Couler, Brea, USA). An aliquot of the final upper layer (200 μL) was transferred into a Mini-UniPrep vial, 300 μL MeOH and 500 μL 8 mM ammonium formate buffer were added. After the vial was capped, vortexed for 30 s. 1 mL of the sample extract was taken and filtered through a Millex-GN nylon filter (0.22 μm, Pall Corporation, Harbor, USA). The cleaned extract was collected in a vial for injection into the UHPLC-Q-Orbitrap system.

A total of ninety-three different baby foods (including VBF, MBF, CBF, FBF and PMBIF) were analyzed. These samples were obtained from different markets and all of them were analyzed following the procedure described above. Those samples found to contain no response at the retention times of reference compounds or metabolite were selected for use as negative controls and stored at 4 °C prior to analysis.

2.3.2. Experimental design for response surface methodology (RSM)

Response surface methodology (RSM) was employed to investigate the variations in recovery rates with respect to the preparation of conditions including extraction solvent volume, the amounts of sodium acetate, and MeCN. The optimal composition of the 3 variables was determined by using a central composite design (CCD) approach. In this work, the full CCD consisted of (1) a complete two-factorial design; (2) n_0, center point ($n_0 > 1$), and (3) two axial points on the axis of each design variable at a distance of $\alpha = 2.000$ from the design center. Hence, a total number of design points of $N = 2^k + 2k + n_0$ was used. The actual variable was coded to facilitate multiple regression analysis. The complete design consisted of 15 combinations including seven replicates of the center point with five degrees of freedom for calculation of errors in the experiments. The optimal values of response *Y* (individual recovery of interest compounds) were obtained by solving the regression equation and by analyzing the response surface contour plots. Table 1 indicates the coded and CCD-processed variables for the optimization of the QuEChERS method for samples. The resulting 15 experiments were carried out randomly. The goodness of fit of the regression model and the significance of parameter estimates were