Food Control 60 (2016) 667–676

Contents lists available at ScienceDirect

Food Control

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

Rapid screening and quantification of multi-class multi-residue veterinary drugs in royal jelly by ultra performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry

Yaqian Zhang [a], Xiaomao Liu [b], Xiang Li [b], Jinjie Zhang [b], Yanzhong Cao [b], Ming Su [a], Zhihong Shi [a], Hanwen Sun [a, *]

[a] College of Chemistry and Environmental Science, Hebei University, Key Laboratory of Analytical Science and Technology of Hebei Province, Baoding 071002, China
[b] Qinhuangdao Entry-Exit Inspection and Quarantine Bureau, Qinhuangdao 066001, China

ARTICLE INFO

Article history:
Received 1 May 2015
Received in revised form
9 September 2015
Accepted 11 September 2015
Available online 14 September 2015

Keywords:
Ultra performance liquid chromatography
Quadrupole time-of-flight mass spectrometry
QuEChERS
Veterinary drug residues
Royal jelly

ABSTRACT

A simple and rapid multi-class multi-residue analytical method was developed for the screening and quantification of veterinary drugs in royal jelly by ultra performance liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (UPLC-QTOF-MS). A total of 90 veterinary drugs investigated belonged to more than 14 families such as lincomycins, macrolides, sulfonamides, quinolones, tetracyclines, β-agonists, β-lactams, sedatives, β-receptor antagonists, sex hormones, glucocorticoids, nitroimidazoles, benzimidazoles, nitrofurans, and the others. A modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) procedure was used for the sample preparation without solid-phase extraction step. The linearity, sensitivity, accuracy, repeatability, and reproducibility of the method were fully validated. The response of the detector was linear for each target compounds in wide concentration range (at least, two orders of magnitude) with correlation coefficient (R^2) of 0.9921–0.9999. The range of the limit of quantification for these compounds in the royal jelly ranged from 0.21 to 20 μg/kg. The repeatability and reproducibility were in the range of 3.01–11.6% and 5.97–14.9%, respectively. The average recoveries ranged from 70.21 to 120.1% with relative standard deviation of 1.77–9.90% at three concentration levels. For the screening method, the data of the precursor and product ions of the target analytes were simultaneously acquired under the All Ions MS/MS mode in a single run. A homemade database including the elemental composition, accurate masses, retention time, isotopic pattern data of the target ions the characteristic in-source fragment ions was utilized for the confirmation and identification of the target compounds. The applicability of the screening method was verified by applying to real royal jelly samples, and certain veterinary drugs were detected in some cases.

1. Introduction

Veterinary drugs have been widely utilized in medical and veterinary practice to treat and prevent diseases and to enhance growth rate and feed efficiency. If veterinary drugs are not used correctly, the practice could lead to the presence of veterinary drug residues in foods of animal origin. Royal jelly, one of the most important bee products, is a popular nutritional supplement. Royal jelly could be contaminated with pesticide and/or antibiotic residues resulting from treatments applied either inside beehives or in the agricultural environment. To protect honey consumers' health, maximum residue levels (MRLs) of many contaminants have been set to levels as small as parts per billion. Different national regulations have established maximum concentrations of pesticide residues permitted in honey, but the lack of homogeneity causes problems in international marketing and trade (Al-Waili, Salom, Al-Ghamdi, & Ansari, 2012). Germany, Italy, and Switzerland have set different MRLs for amitraz, bromopropylate, coumaphos, cyamizole, flumetrine, and fluvalinate (Bogdanov, 2006). The maximum

* Corresponding author.
E-mail address: hanwen@hbu.edu.cn (H. Sun).

http://dx.doi.org/10.1016/j.foodcont.2015.09.010
0956-7135/

limits of pesticide residues in honey are not included in the Codex Alimentarius (Codex Alimentarius, 1998). The European Union legislation has regulated the MRLs for pesticides and veterinary (EC Regulation, 2005), which are 0.01–0.5 mg/kg for some drugs (Gómez-Pérez, Plaza-Bolaños, Romero-González, Martínez-Vidal, & Garrido-Frenich, 2012). The US Environmental Protection Agency has established MRLs for amitraz (1 mg/kg), coumaphos (0.1 mg/kg), and fluvalinate (0.05 mg/kg) (Food and Drug Administration of the United States, 2003). However, MRLs for veterinary drugs in royal jelly have not been set up. Their control is highly important for the agricultural environment and food industry.

A high performance liquid chromatography–fluorescence detection method for the quantitative determination of single class antibacterial (fluoroquinolone) in royal jelly samples was developed (Zhou et al., 2009). Currently, ultra performance liquid chromatography coupled to mass spectrometry (UPLC-MS) has become a very common tool to analyze for drug residues in various food matrices (Garrido Frenich, Romero-González, & del Mar Aguilera-Luiz, 2014). Several UPLC-MS/MS methods have been reported for the determination of single class drug residues in royal jelly, such as chloramphenicol (Ishii, Horie, Murayama, & Maitani, 2006; Jiang et al., 2006; Xie et al., 2005; Zhou et al., 2008), chloramphenicol, thiamphenicol and florfenicol (Wang et al., 2007), eight quinolones (Lombardo-Agüí, García-Campaña, Gámiz-Gracia, & Cruces-Blanco, 2012), five macrolides (Xie, Ding. Xi, Qian, & Huang, 2007), and adenosine (Xue, Zhou, Wu, Fu, & Zhao, 2009). However these single-class methods are relatively easy to optimize for both extraction and instrumental parameters due to the similar physical and chemical properties of veterinary drugs from the same group. Due to the high number of veterinary drugs that need to be controlled, it is highly important to develop multi-class multi-residue methods that permit analysis for a variety of drugs with a single procedure.

Several LC–MS/MS methods were reported for the multi-class multi-residue analysis of honey, such as sulfonamides and chloramphenicol (Sheridan, Policastro, Thomas, & Rice, 2008), 27 antibiotic drugs belonging to sulfonamide, nitroimidazole and quinolone families (Galarini, Saluti, Giusepponi, Rossi, & Moretti, 2015), pesticides and veterinary drugs (Gómez-Pérez et al., 2012), as well as 42 antibiotic residues (Hammel, Mohamed, Gremaud, Le Breton, & Guy, 2008). The developed method was able to analyze up to 59 veterinary drugs of 7 families, however, aminoglycosides, β-lactams, penicillins, and tetracyclines were not analyzed by the method because of chromatographic retention issue and sample extraction challenges (Wang & Leung, 2012). As far as we know, no LC-MS/MS and LC-QTOF-MS methods specifically for multi-class multi-residue analysis of royal jelly have been proposed.

Despite the advances in separation and detection techniques, sample extraction is still a cornerstone of the analytical process, and effective sample preparation is essential to achieve reliable results and maintain instrument performance. The previous sample pre-treatments focused on one compound or a single class of veterinary drugs such as the chloramphenicol (Ishii et al., 2006; Jiang et al., 2006; Xie et al., 2005; Zhou et al., 2008), chloramphenicol, thiamphenicol and florfenicol (Wang et al., 2007), and quinolones in royal jelly (Lombardo-Agüí et al., 2012; Zhou et al., 2009). For multi-class multi-residues it difficult to develop a common extraction procedure and chromatographic conditions. In recent years, the studies on multi-class methods have increased, and related studies in honey have been carried out using solid-phase extraction step (Galarini et al., 2015; Sheridan et al., 2008), dispersive solid-phase extraction (Gómez-Pérez et al., 2012), and four subsequent liquid/liquid extraction steps (Hammel et al., 2008). To simplify the sample pretreatment procedure, the QuEChERS method is a streamlined approach that makes it easier and less expensive for analytical chemists to examine pesticide residues in food (Schenck & Hobbs, 2004). However, to the best of our knowledge, there was only a work using a QuEChERS method for the determination of quinolones alone in bee products (Lombardo-Agüí et al., 2012). Since.royal jelly has very complex matrices, it is difficult to extract multi-class multi-residue drugs from royal jelly. So that the original and reported relative QuEChERS methods need to be modified further including the type and amount of extraction solvent, the appropriate acid, salting-out procedure, and the type and amount of sorbent. Therefore, these parameters were optimized in this study.

A total of 90 veterinary drugs selected for the study belong to more than 14 families drugs including lincomycins(2), macrolides(7), sulfonamides(19), quinolones(18), tetracyclines(14), β-agonists(6), β-lactams (6), sedatives(2), β-receptor antagonists, sex hormones(5), glucocorticoids (6), nitroimidazoles (3), benzimidazoles (2), nitrofurans (4), and the others (6) (Table 1). The purpose of this study is to develop a modified QuEChERS method coupled with UPLC-QTOF-MS for the identification and quantification of the 90 residues in royal jelly. The effectiveness of the proposed method was confirmed by detecting trace residues in real royal jelly samples.

2. Materials and methods

2.1. Chemicals and solutions

Ninety veterinary standards (purity: ≥95%) listed in Table 1 were purchased from Dr.Ehrenstorfer Com. (Germany). Individual stock solutions (1000 mg/L) were prepared by dissolving 10 mg of each compound in 10 mL of methanol, and mixed standard solution at concentrations of 1 mg/L of each standard was prepared by additive mixing 10 μL of each stock solution and diluting to 10 mL with methanol. All these solutions were stored in an amber bottles at 4 °C. The penicillins are not very stable drugs and these compounds were therefore added to the mixed standard solution just before analysis.

HPLC-grade methanol, acetonitrile, ethyl acetate, dichloromethane, and formic acid were purchased from Dikma Technologies Inc. (Tianjin, China). Guaranteed reagent–grade acetic acid (36%), magnesium sulfate, and sodium acetate; Analytical reagent–grade sodium sulfate, disodium ethylenediamine tetraacetate, disodium hydrogen phosphate, hydrochloric acid, and citric acid were purchased from Guangfu reserch institute (Tiajin, China). ProElut QuEChERS kit including four sobents (150 mg PSA/900 mg $MgSO_4$,400 mg PSA/1200 mg $MgSO_4$, 400 mg PSA/400 mg C18/1200 mg $MgSO_4$, 150 mg PSA/150 mg C18/900 mg $MgSO_4$) were purchased from Dikma Technologies Inc. (Tianjin, China), and five sobents (C18, PAX, PSA, NH_2, and Alumina-N) were purchased from Tianjin Bonna-Agela Technologies Ltd. (China).

2.2. Instrumentation

LC-30A high performance liquid chromatography (Shimadzu Co., Japan) and QTOF5600 mass spectrometry (AB Com., USA) with electrospray ionization source (EIS) were used. A 3K-30 High speed refrigerated centrifuge (Segma Co., USA), TurboVap LV Cyclone concentrator (Caliper com., USA), G560E Vortex mixer (SI VORTEX-GENIE2 Com., USA), and SA300 horizontal oscillator (Yamato Com., Japan) were used for sample preparation.

2.3. UPLC-QTOF-MS analysis conditions

Separation of the analytes was achieved on an ACQUITY® UPLC BEH C_{18} column (100 mm × 2.1 mm i.d., 1.7 μm) at 40 °C with the