Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

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

Full Length Article

Optimization for QuEChERS extraction of mycotoxins and veterinary drugs by response surface methodology for application to egg and milk

Jian Zhou[a,b], Jiao-Jiao Xu[a,*], Jin-Mi Cong[b], Zeng-Xuan Cai[a], Jing-Shun Zhang[a], Jun-Lin Wang[a], Yi-Ping Ren[c,*]

[a] Lab of Physicochemical Research, Department of Physicochemical & Toxicology, Zhejiang Provincial Center for Disease Control and Prevention, Zhejiang 310051, China
[b] Zhejiang Provincial Key Lab of Health Risk Appraisal for Trace Toxic Chemicals, Ningbo Municipal Center for Disease Control and Prevention, Ningbo 315010, China
[c] National Center for Food Safety Risk Assessment Application Technology Cooperation Center, Yangtze Delta Region Institute of Tsinghua University, Zhejiang 314006, China

ARTICLE INFO

Article history:
Received 12 June 2017
Received in revised form
19 November 2017
Accepted 21 November 2017
Available online xxx

Keywords:
QuEChERS
Egg
Milk
Mycotoxins
Veterinary drugs
Response surface methodology

ABSTRACT

A multiclass method was proposed for the simultaneous determination of various classes of veterinary drugs ($n = 65$), mycotoxins and metabolites ($n = 39$) in egg and milk by ultra-high performance liquid chromatography-tandem mass spectrometry. The contaminants were extracted by QuEChERS-based strategy including salt-out partitioning and dispersive solid-phase extraction for cleanup further. With the aim of maximizing throughput and extraction efficiency, Plackett-Burman design was employed initially for screening significant variables. And response surface methodology based on central composite design was conducted to achieve optimal conditions in details: 3.35% (*v*/*v*) of formic acid in acetonitrile, 1.2 g of NaCl, 0.5 g of anhydrous NaAc, 300 mg of C18 and 140 mg of primary secondary amine. Satisfactory analytical characteristics in validation, in aspects of accuracy (70%–105% for mycotoxins and quinolones, 55%–80% for sulphonamides and 40%–105% for other veterinary drugs), precision (inter-day RSDs < 14%) and sensitivity (LOQs ranged from 0.01 μg/kg to 31 μg/kg), were achieved under the optimized conditions. The matrix effects were evaluated and compensated by the use of matrix-matched calibration curves ($R^2 > 0.987$). In practice, 45 eggs and 30 milk samples were investigated by the established method, of which positive finding aflatoxin in milk and sterigmatocystin in eggs.

1. Introduction

Veterinary drugs have been widely prescribed for both therapeutic and prophylactic reasons. But for improper usage, non-respect of withdrawal periods and cross-contamination, the drugs could be transferred and accumulated in animal-derived products [1]. Because of high consumption of animal-derived products, like egg and milk, the presence of drug residues poses a risk to public health, which could cause problems of allergy, antibiotic resistance and even carcinogenic character.

Mycotoxins contamination is another serious worldwide problem on food and feed, which contaminated 25% of grain and oil crops approximately every year reported by FAO. The mainly concerned toxins includes aflatoxins, ochratoxin A and trichothescenes, which were classified in group 1, group 2 and group 3 by the International Agency of Research on Cancer, respectively. These substrates could be transferred to animal-derived food by contaminated feed and bio-transformed into various metabolites, e.g. aflatoxin M_1 and hydrolyzed fumonisin B_1/B_2, which also represents related parental compound toxicity and risk to human health [2–4].

The maximum residue limits (MRLs) for veterinary drugs and maximum limit (MLs) for mycotoxins in food and feed have been established in EU [5,6] and China [7,8]. Hereinto, MRLs for QNLs and SAs in animal-derived food are in the range of 10 μg/kg to 1900 μg/kg, and 100 μg/kg for total amount, respectively. However,

Abbreviations: ACN, acetonitrile; CCD, central composite design; ESI, electrospray ionization; FA, formic acid; LOQ, limit of quantification; ME, Matrix effect; MeOH, methanol; MRL, maximum residue limit; MRM, multiple-reaction-monitoring; PSA, primary secondary amine; QNL, quinolone; RSM, response surface methodology; S/N, signal-to-noise; SA, sulphonamide; SPE, solid-phase extraction.

* Corresponding authors.
E-mail addresses: jjxucdc@163.com (J.-J. Xu), renyiping@263.net (Y.-P. Ren).

https://doi.org/10.1016/j.chroma.2017.11.050
0021-9673/

no specific criteria have been legally established for mycotoxins in animal-derived matrices, due to the lack of representative data and studies. In this case, it is of great importance to develop generic, accurate and sensitive methods for these contaminants analysis in food of animal origin.

With the increasing number and class of organic residues and contaminants to be found in raw materials and/or processed food, consumers have strong willing to get more information about toxicological implications and other potential risk relating to the presence of these residues and contaminants. In this sense, it is essential to approach reliable and suitable analytical methods that allow to analyze a huge number of compounds simultaneously, including several classes or more than one family.

To date, few studies have been proposed for simultaneous determination of multi-classes in food and feed matrices, especially for veterinary drugs and mycotoxins together. Mol et al [9] proposed one of the first methods for the simultaneous analysis of pesticides, mycotoxins and plant toxins in feed and several types of food matrices, as well as Dominicis et al [10] established targeted screening method applied for bakery ingredients and food commodities. A similar approach was used by Capriotti et al [11] for the simultaneous determination of antimicrobials and mycotoxins in egg. In order to achieve a suitable elution of the target compounds and to reach good sensitivity, more than one injection was performed. Zhan et al determined 255 veterinary drugs, pesticides and mycotoxins in milk [12], 220 chemical residues and mycotoxins in infant formula [13] and 226 veterinary drugs and other contaminants in muscle [14]. However, two or three injections were necessary, analysis time was longer. And in the previous reports, there is less residues' or contaminants' metabolites considered.

Bearing in mind that complex components in matrices and a great variability in physicochemical properties of residues and contaminants, generic extraction and chromatographic conditions would be used and optimized. Besides of solid phase extraction and dilute-and-shoot for purification and enrichment, QuEChERS (acronym of Quick, Easy, Cheap, Effective, Rugged, and Safe) methodology was a significant method for high-throughput determinations and widely utilized in multi-classes targets pretreatment [11,15]. It was initially proposed for pesticide residues [16], and had subsequently been expanded to antibiotics, food additives and mycotoxins due to the merits of rapidity, universality and effectiveness. For individual class of veterinary drugs or mycotoxins analysis, Stubbings et al. established a multiclass UPLC–MS/MS procedure in animal tissue using QuEChERS approach [17], as well as 10 mycotoxins in eggs at trace level reported by Frenich et al. [18]. And Moretti et al. developed a multiclass method for screening and confirmatory analysis of 62 antibiotics in milk [19]; Capriotti et al. proposed a multiclass screening method based on QuEChERS technique for the determination of antimicrobials and mycotoxins in eggs [11]. Although there is a growing tendency to develop multiclass methods in the field of food safety, the greater diversity in the chemical properties between veterinary drugs and mycotoxins, has impeded progress to combine them into analytical experiments.

Otherwise, in the most of previous studies, every single factor was usually optimized using one-factor-at-a-time approach whilst all the other factors were fixed at a constant level [20,21]. The single-dimensional optimization is incapable of distinguishing the importance of each factor and reaching the true optimum, due to the ignorance of the interaction effects among factors [22]. In this context, statistical experimental approaches, e.g. fractional factorial (Plackett-Burman) design and response surface methodology (RSM) could optimize the parameters collectively and eliminate the limitations of conventional optimization process. Plackett-Burman design provides an effective way to identify the main factors among a large number of variables and reduce the number of experiments. RSM, which includes factorial design and regression analysis, helps to design the fitted models, estimate the interaction effects and determine the optimum conditions, respectively [23].

The aim of this present study was to develop a reliable and universal QuEChERS method coupling with ultra-high performance liquid chromatography tandem mass spectrometry (UPLC–MS/MS), for the simultaneous determination of 104 veterinary drugs (mainly QNLs and SAs), mycotoxins and its metabolites in egg and milk. To obtain the optimal extraction efficiencies, the statistical methodologies were implemented in details. The established method was expected to contribute to the risk monitoring and investigation of contamination level in eggs and milk.

2. Experimental

2.1. Standards and chemicals

HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). Formic acid (FA, purity > 99%) was purchased from Anaqua Chemicals (Houston, USA). Analytical grade of $MgSO_4$, NaCl, Na_2SO_4, anhydrous NaAc and QuEChERS sorbents (C_{18} and primary secondary amine (PSA)) were provided by local suppliers. Ultrapure water was obtained from Millipore (Bedford, MA, USA). Immunoaffinity columns for sterigmatocystin were provided by R-Biopharm (Glasgow, Scotland). Analytical standards of mycotoxins were purchased from Sigma-Aldrich (St. Louis, USA), Biopure (Tulln, Austria), Toronto Research Chemicals Inc. (Toronto, Canada), ALEXIS (Lausen, Switzerland) and Pribolab (Qingdao, China), respectively. The standards of hydrolyzed fumonisin B_1/B_2 were prepared by the basic hydrolysis of fumonisin B_1/B_2 as described in a previous report by G. Pagliuca et al [24]. The achieved product solution was further confirmed by UPLC–MS/MS and as a result, no residues of the parental fumonisin toxins were detectable. The standards of veterinary drugs were all purchased from Dr. Ehrenstorfer GmbH (Augsburg, Germany).

The standard stock solution of each analyte was prepared individually in the suitable solvents: ACN/0.02% FA aqueous solution (50/50, *v/v*) for QNLs; ACN for all other analytes. The solutions were stored at −20 °C in the dark and were stable for at least 6 months. Then, two different working solutions were prepared by combining suitable aliquots of each stock solution and diluting them with appropriate volume of ACN for mycotoxins and 50% ACN for drugs. The specific concentrations were set as follows: aflatoxin $B_1/B_2/G_1/G_2$ and ochratoxin A/B (500 ng/mL); sterigmatocystin (2 μg/mL); T-2 toxin, diacetoxyscirpenol, neosolaniol, deoxynivalenol-3-glucuronide and tentoxin (5 μg/mL); HT-2 toxin, de-epoxydeoxynivalenol, alternariol methyl ether, chaetoglobosin and ochratoxin α (10 μg/mL); deoxynivalenol, 3/15-acetyl-deoxynivalenol, fusarenone X and fumonisin B_1 (100 μg/mL); fumonisin B_2 (50 μg/mL) and fumonisin B_3 (25 μg/mL); zearalenone and its derivatives (6 μg/mL); aflatoxin M_1/M_2 (50 ng/mL); verruculogen and gliotoxin (40 μg/mL); cyclopiazonic acid (0.1 μg/mL); penicillic acid (12.7 μg/mL) and hydrolyzed fumonisin B_1/B_2 (11.2 μg/mL); veterinary drugs (1 μg/mL, except 200 ng/mL of amantadine and chloramphenicol, 100 ng/mL of metronidazole). In this paper, the concentration of aflatoxin B_1 was expressed as a representative for convenience.

2.2. Samples

Totally, 75 fresh samples including eggs ($n = 45$) and milk ($n = 30$) were purchased from local markets in Zhejiang province. Egg samples (both albumen and yolk) were homogenized thoroughly for 3 min using a blender (IKA, German). In spite of milk, as homogeneous liquid, no extra operation step was done. All the samples

Please cite this article in press as: J. Zhou, et al., Optimization for QuEChERS extraction of mycotoxins and veterinary drugs by response surface methodology for application to egg and milk, J. Chromatogr. A (2017), https://doi.org/10.1016/j.chroma.2017.11.050