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Multi-class, multi-residue analysis of trace veterinary drugs in milk by rapid screening and quantification using ultra-performance liquid chromatography–quadrupole time-of-flight mass spectrometry

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ABSTRACT

A simple and rapid multi-class multi-residue analytical method was developed for the screening and quantification of veterinary drugs in milk 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 almost 20 classes including lincomycins, macrolides, sulfonamides, quinolones, tetracyclines, β-agonists, β-lactams, sedatives, β-receptor antagonists, sex hormones, glucocorticoids, nitroimidazoles, benzimidazoles, nitrofurans, and some others. A modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) procedure was developed for the sample preparation without the 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 compound in a wide concentration range with a correlation coefficient (R^2) of 0.9973 to 0.9999 (among them $R^2 > 0.999$ for 73 of 90 analytes). The range of the limit of quantification for these compounds in the milk ranged from 0.10 to 17.30 μg/kg. The repeatability and reproducibility were in the range of 2.11 to 9.62% and 2.76 to 13.9%, respectively. The average recoveries ranged from 72.62 to 122.2% with the RSD (n = 6) of 1.30 to 9.61% at 3 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. An accurate mass database for the confirmation and identification of the target compounds was established. The applicability of the screening method was verified by applying to real milk samples. The proposed analytical method allows the identification and confirmation of the target veterinary drugs at trace levels employing quick analysis time. Certain veterinary drugs were detected in some cases.

Key words: veterinary drug, milk, QuEChERS, ultra-performance liquid chromatography, quadrupole time-of-flight mass spectrometry

INTRODUCTION

Veterinary drugs have been widely used in medical and veterinary practice to treat and prevent diseases and to enhance growth rate and feed efficiency. If they are not used correctly, the practice could lead to the presence of veterinary drug residues in foods of animal origin. Their control is highly important for the agricultural environment and food industry. Bovine milk is one of the most important components of the human diet. The risk of veterinary drug residues in milk is significant. To ensure human food safety, the European Union has set a maximum residue limit (**MRL**) of 4 to 30 μg/kg for penicillins, 20 to 100 μg/kg for cephalosporins, 30 to 100 μg/kg for quinolones, and 100 μg/kg for sulfonamides (SA) in milk (European Union, 2010). These low values require the development of analytical methods that are sensitive enough to monitor and determine these drugs in bovine milk.

Recently, Garrido Frenich et al. (2014) provided an overview of current methods based on ultra-high-performance liquid chromatography coupled to mass-spectrometry analyzers (**UPLC-MS**), for the determination of organic contaminants and residues in food. Currently, UPLC-MS has become a very common tool to analyze drug residues in various food matrices. Several papers are available on the analysis of veterinary drugs in milk, but most focus on only few compounds or one class of antibiotics, such as quinolones (Hermo et al., 2008), penicillins (Martínez-Huélamo et al., 2009), β-lactams (Kantiani et al., 2009), and ionophores (Thompsona et al., 2011). These single-class methods are relatively easy for the optimization of both extrac-

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tion and instrumental parameters due to the similar physical and chemical properties of targeted drugs from the same family. For 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 UPLC-MS/MS methods concerning milk have been reported for the multi-residue determination of β-lactams and veterinary drugs (Aguilera-Luiz et al., 2008; Turnipseed et al., 2008), pesticides and mycotoxins (Aguilera-Luiz et al., 2011), some anthelmintic drugs and phenylbutazone (Kaufmann et al., 2011), and 18 residues of antibiotics, coccidiostats, and corticosteroids (Nebot et al., 2012). Recently, the developed generic and rapid method was able to analyze 8 classes of veterinary drug, but with average relative recoveries from 63 to 141%, the method accuracy needs to be improved further (Zhan et al., 2012). The UHPLC-Orbitrap-MS can offer efficient performance for screening purposes, and it can also effectively provide adequate quantification/confirmation values of veterinary drugs in the positive milk samples (Romero-González et al., 2011).

Ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry (**UPLC-TOF-MS**) also showed very good performances for quantification and allowed the determination of majority of compounds below MRL in milk (Ortelli et al., 2009). Liquid chromatography–quadrupole–time-of-flight–mass spectrometry (**LC-Q-TOF-MS**) was used for the analysis of sulfamethazine, flunixin, cephapirin, or enrofloxacin and metabolites in milk (Turnipseed et al., 2011). The UPLC-QTOF-MS method was able to quantify 34 or screen up to 59 drugs in milk (Wang and Leung, 2012), and to investigate the veterinary drugs belonging to 9 families (Kang et al., 2014). The screening levels of detection for more than 150 veterinary drug residues in milk were determined by the LC-Q-TOF-MS method, and more than half of those tested can be detected at concentrations of 10 ng/mL or less; 72% can be found in milk when present at 100 ng/mL (Turnipseed et al., 2014). Recently, UPLC-Q-TOF-MS was applied for qualitative multi-residue screening of 143 veterinary drugs and pharmaceuticals in milk and fish tissue (Dasenaki et al., 2015), and satisfactory results were obtained at concentration levels of 15 ng/mL in milk and 20 μg/kg in fish (>60% of the compounds detected and identified). However, the sensitivity of these methods needs to be improved further for trace residue analysis.

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. Because milk is a complex matrix due to its high protein and fat content, which often interfere in analytical procedures, special focus has been placed on sample preparation (extraction and cleanup). The previous sample pretreatments focused on a few compound or a single class of veterinary drugs. For multi-class, multi-residue analysis, it is difficult to develop a common extraction procedure and chromatographic conditions. In general, after protein precipitation and centrifugation, solid-phase extraction can be applied to determine fluoroquinolones in milk (Zheng et al., 2014), veterinary drugs in milk (Stolker et al., 2008), and mycotoxins and pesticides in milk (Aguilera-Luiz et al., 2011); however, these methods are time consuming and require large volumes of organic solvent. Recently solid-phase microextraction has been reported for monitoring estrogens in milk powder, low solvent consumption, and ease of coupling with chromatographic instruments (Lan et al., 2014). A dispersive liquid–liquid microextraction method was used for the determination of quinolones and β-lactams in raw cow milk (Junza et al., 2014). To simplify the sample pretreatment procedure, the quick, easy, cheap, effective, rugged, and safe (**QuEChERS**) method is a streamlined approach that makes it easier and less expensive for analytical chemists to examine pesticide residues in food (Schenck and Hobbs, 2004). However, it is necessary to modify QuEChERS procedure for different target analytes and matrices. A new sample preparation procedure loosely based on QuEChERS methodology was used for the extraction of nitroimidazoles, sulfonamides, fluoroquinolones, quinolones, ionophores, and dinitrocarbanilide in animal tissues (Stubbings and Bigwood, 2009). A modified QuEChERS procedure for the preparation of milk sample was reported to determine 18 veterinary drugs, but with limits of detection (**LOD**) ranging from 1 to 4 μg/kg (Aguilera-Luiz et al., 2008). A total of 21 veterinary drugs were extracted from milk samples using a modified QuEChERS method, and no further cleanup steps were necessary (Martínez Vidal et al., 2010). It should be pointed out 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, for the analysis of multi-residue, multi-class veterinary drugs with physical and chemical performances in complex matrices.

A total of 90 veterinary drugs selected for the study belong to almost 20 classes of veterinary drugs including lincomycins, macrolides, sulfonamides, quinolones, tetracyclines, β-agonists, β-lactams, sedatives, β-receptor