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Analytical Methods

Multi-class method for the determination of nitroimidazoles, nitrofurans, and chloramphenicol in chicken muscle and egg by dispersive-solid phase extraction and ultra-high performance liquid chromatography-tandem mass spectrometry

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ABSTRACT

This study describes the development of a multiresidue method for the efficient identification and quantification of nitroimidazoles, nitrofurans, and chloramphenicol in chicken and egg. After derivatization of nitrofuran metabolites, dispersive-solid phase extraction was used for the extraction of target analytes. An optimization strategy involved the selection of sorbents and extraction solutions for dispersive-solid phase extraction in order to achieve acceptably high recoveries and reduce co-extractives in the final extracts. Analytes were determined by ultra-high performance liquid chromatography-tandem mass spectrometry, in one single injection with a chromatographic run time of 7.5 min. Mean recoveries ranged from 86.4% to 116.7% and interday precision was lower than 18%. The limits of quantification were between 0.1 and 0.5 μg/kg, which were satisfactory to support surveillance monitoring. Finally, the method was applied to real samples, and metabolite of furazolidone, metronidazole and its metabolite, dimetridazole and its metabolite were detected in both chicken and egg samples.

1. Introduction

Nowadays, there is increasing concern about the health and safety impact of the extensive use of veterinary drugs in livestock husbandry, which can result in residues in edible animal products. In order to ensure human food safety, many countries set strict tolerance levels for these compounds and perform surveillance programs. Among the major groups of compounds to be monitored, some zero tolerance substances, like nitroimidazoles (NIIMs), nitrofurans (NFs), and chloramphenicol (CAP), are prohibited globally (European Commission, 2010) due to their carcinogenic and mutagenic potency (World Health Organisation, 1989, 1993) or the risk of causing aplastic anemia (Festing, Diamanti, & Turton, 2001). The European Commission has established the minimum required performance level (MRPL) at 0.3 μg/kg and 1 μg/kg for CAP and NFs (European Commission, 2003), respectively, and recommended value was 3 μg/kg for NIIMs (Zeleny, Schimmel, Ulberth, & Emons, 2009b). Consequently, methods to detect and quantify the residues of these compounds in animal tissues must be very sensitive as well as selective.

The most important NFs are furazolidone, furaltadone, nitrofurazone and nitrofurantoin, which are metabolized rapidly after their administration (Chu & Lopez, 2005). Their toxic metabolites, 3-amino-2-oxazolidinone (AOZ), 3-amino-5-morpholinomethyl-2-oxazolidinone (AMOZ), semicarbazide (SEM), and 1-aminohydantoin (AHD), are strongly bound to proteins and highly stable for long periods (McCracken, Blanchflower, Rowan, McCoy, & Kennedy, 1995). For this reason, the analysis of NFs is based on the determination of their main metabolites (Conneely et al., 2003; Leitner, Zöllner, & Lindner, 2001). The most frequently used NIIMs included metronidazole (MNZ), dimetridazole (DMZ), ronidazole (RNZ), and ipronidazole (IPZ). Their major metabolites, 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI), 1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole (MNZOH), 1-meth

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yl-2-(2′-hydroxyisopropyl)-5-nitroimidazole (IPZOH), should be covered in the analytical method because they are also carcinogenic and mutagenic. In the vast majority of methods, liquid chromatography coupled to tandem quadrupole mass spectrometry (LC-MS/MS) was applied for the determination of these three groups of compounds in different matrices (Finzi, Donato, Sucupira, & De Nucci, 2005; Fraselle, Derop, Degroodt, & Van Loco, 2007; Mottier, Huré, Gremaud, & Guy, 2006; Rodziewicz & Zawadzka, 2008; Verdon, Couedor, & Sanders, 2007). Although a number of analytical methods have been described for the determination of NIIMs (Cronly, Behan, Foley, & Malone, 2009; Gadaj et al., 2014; Mitrowska, Posyniak, & Zmudzki, 2010; Mohamed et al., 2008; Polzer, Stachel, & Gowik, 2004; Zeleny, Harbeck, & Schimmel, 2009a), NFs (Barbosa, Freitas, Mourão, da Silveira, & Ramos, 2012; Bock, Stachel, & Gowik, 2007; Khong et al., 2004; Lopez, Feldlaufer, Williams, & Chu, 2007; Mottier et al., 2005; Rodziewicz, 2008; Zhang, Qiao, Chen, Wang, & Xia, 2016), and CAP (Berendsen, Essers, Stolker (A.)A.M., & Nielen, & M.W.F., 2011; Forti, Campana, Simonella, Multari, & Scortichini, 2005; Guy et al., 2004; Lu et al., 2012; Mohamed et al., 2007; Mottier, Parisod, Gremaud, Guy, & Stadler, 2003; Rønning, Einarsen, & Asp, 2006; Santos et al., 2005) separately, few multi-class methods were available for the analysis of these three groups of analytes in animal-food products. Zhai et al. (2015) developed a micellar electrokinetic capillary chromatography (CE) method to analyze CAP, NFs and their metabolites in fishery tissues. Since only CE was applied, the method couldn't provide confirmatory information of the analytes and the sensitivity of the method couldn't fulfill the requirement of MRPL of CAP and NFs. Kaufmann, Butcher, Maden, Walker, and Widmer (2015) reported the use of ultra-high performance liquid chromatography coupled to high resolution mass spectrometry (UHPLC-HRMS) for the detection of NFs and CAP in animal food products. Orbitrap instrument adopted in the method provided highly selective and sensitive confirmatory signals. Shendy, Al-Ghobashy, Alla, and Lotfy (2016) described a modified QuEChERS method for simultaneous determination of NFs and NIIMs in honey, but for NIIMs, only RNZ and DMZ were analyzed in the method. In the literature, no method is available for simultaneous determination of NIIMs, NFs, and CAP in animal origin matrices.

In the previous work, we reported an HPLC-MS/MS method for simultaneous determination of NIIMs and NFs in swine muscle (Xia et al., 2008). In this paper, we present the development and validation of a rapid multiclass UHPLC-MS/MS method using dispersive-solid phase extraction (d-SPE) procedure, capable of qualitative and quantitative determination of three classes of prohibited veterinary drugs including NIIMs, NFs, and CAP, in chicken muscle and egg samples. The newly proposed method was optimized to cover more analytes and obtain higher throughput. Applicability of the method was demonstrated in the analysis of 46 commercial samples from local markets.

2. Experimental

2.1. Materials and reagents

HPLC grade methanol (MeOH), acetonitrile (ACN), formic acid, hexane, and ethyl acetate (EtOAc) were obtained from Fisher Scientific Inc. (Pittsburgh, PA, USA). Hydrochloric acid was obtained from Alfa-Aesar (Ward Hill, MA, USA). 2-nitrobenzaldehyde (2-NBA) and diatomaceous earth (Celite® 545) were purchased from Sigma-Aldrich (St. Louis, MO, USA). C_{18} (50 μm) was purchased from Bonna-Agela Technologies (Beijing, China). Water was purified using a Milli-Q Synthesis system from Millipore (Bedford, MA, USA). Syringe filter was purchased from Pall Corporation (Ann Arbor, MI, USA).

The analytical standards AOZ, AMOZ, AHD, SEM, AOZ-d_4, AMOZ-d_5, AHD-$13C_3$, SEM (13C, $15N_2$), RNZ, MNZOH, HMMNI, IPZ, IPZOH, HMMNI-d_3, and MNZOH-d_2 were obtained from WITEGA (Berlin, Germany). RNZ-d_3 and DMZ-d_3 were acquired from RIVM (Bilthoven, Netherlands). IPZ-d_3, IPZOH-d_3, and MNZ-d_3 were provided by the EU Reference Laboratory for Residues of Veterinary Drugs (Berlin, Germany). CAP, MNZ, and DMZ were purchased from Sigma-Aldrich. CAP-d_5 was purchased from Dr. Ehrenstorfer (Augsburg, Germany).

Standard stock solutions (1 mg/mL) were prepared by dissolving 10 mg of individual compounds in 10 mL of methanol with the exception of SEM (13C, $15N_2$), which was dissolved in water. Intermediate standard solutions of each compound (10 μg/mL) were prepared by dilutions with methanol from stock solutions. These solutions were stored at −20 °C and were stable for at least 6 months. Mixed working standard solutions for spiking blanks (0.5, 0.1, 0.01 μg/mL) were obtained by appropriate dilutions with methanol. Working standard solutions of CAP (0.5, 0.1, 0.01 μg/mL) were prepared separately. Mixed working internal standard (IS) solution was prepared in methanol at 0.5 μg/mL. These solutions were stored at −20 °C and were stable for at least 8 weeks.

2.2. Sample preparation

For routine testing samples, a previously homogenized sample (2.00 ± 0.02 g) was weighed into a 50 mL centrifuge tube. For spiked samples, 50 μL of working solutions at each concentration were added to blank samples. Eight μL of working IS solution (0.5 μg/mL) was spiked into each sample to obtain the concentration of 2.0 μg/kg. After vortexing, samples were allowed to stand for 30 min in the darkness. Ten mL of 0.2 M hydrochloric acid solution and 200 μL of 0.1 M 2-NBA freshly prepared in methanol were added and vortex-mixed for 1 min. The samples were placed in a shaker at 200 rpm and incubated overnight at 37 °C. After cooling to room temperature, 4 g of C_{18} and 4 g of diatomaceous earth were added into the samples, and then 10 mL of hexane was added and shaked at 200 rpm for 5 min. The mixtures were centrifuged at 10,000 rpm for 5 min, and the aqueous layer and hexane layer were discarded. The samples were extracted with 15 mL of ethyl acetate by vortexing for 1 min and centrifuged at 10,000 rpm for 5 min. The supernatant was collected and the extraction was repeated with 15 mL of ethyl acetate. The combined extraction solutions were evaporated to dryness in a water bath at 40 °C under nitrogen. The dried extract was reconstituted in 1.0 mL of 5% methanol in 0.1% formic acid, vortex-mixed for 1 min, and filtered through a 0.2 μm syringe filter into a glass LC vial.

2.3. Instrumental conditions

LC analyses were performed on a Waters Acquity ultra-performance liquid chromatography system with column oven temperature maintained at 40 °C, using an Acquity BEH C_{18} column (50 mm × 2.1 mm i.d., 1.7 μm particle size) (Waters, Milford, MA, USA). The mobile phase was constituted by solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The injection volume was 5 μL. The flow rate was 0.4 mL/min with a linear gradient at the following conditions: 0–0.5 min, 98% A; 0.5–2 min, 98–85% A; 2–4.5 min, 85–60% A; 4.5–5.5 min, 60–1% A; 5.5–6 min, 1% A; 6–6.1 min, 1–98% A; 6.1–7.5 min, 98% A.