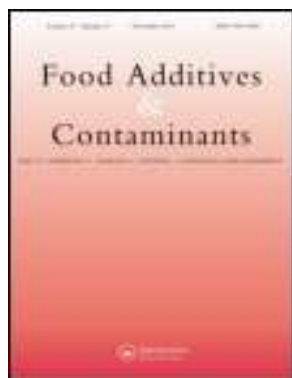


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Determination and confirmation of metronidazole, dimetridazole, ronidazole and their metabolites in bovine muscle by LC-MS/MS

Rodrigo H.M.M. Granja^{a*}, Alfredo M.M. Nino^a, Karine V.G. Reche^a, Fabio M. Giannotti^a, Andreia C. de Lima^a, Amaryllis C.B.A. Wanschel^b and Alessandro G. Salerno^{a,b}

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Nitroimidazoles are a class of veterinary drugs used for the treatment and prevention of certain bacterial and protozoal diseases in poultry, swine dysentery and genital trichomoniasis in cattle. Since the safety assessment of nitroimidazoles showed them to be genotoxic, carcinogenic and mutagenic, a number of nitroimidazoles have been banned for therapeutic purposes in different countries. Moreover, nitroimidazoles have also been extensively used as growth promoters in food-producing animals. Due to their efficacious improvement in meat production and feed conversion, deliberate use still exists. Therefore, the illegal use of nitroimidazoles in animal husbandry must be monitored. A sensitive method based on LC-MS/MS for the simultaneous determination and confirmation of five banned nitroimidazole drugs including metronidazole, ronidazole, dimetridazole, metronidazole-OH (metabolite of metronidazole), and 2-hydroxymethyl-1-methyl-5-nitroimidazole (metabolite of ronidazole and dimetridazole) in bovine muscle, using ronidazole- d_3 as an internal standard, was developed and validated. After extraction with ethyl acetate and evaporation, the nitroimidazoles were reconstituted in petroleum ether and purified, and LC-MS/MS analysis was performed. The method was validated according to Brazilian Regulation 24/2009 (equivalent to European Union Decision 2002/657/EC). Parameters such as decision limit ($CC\alpha$), detection capability ($CC\beta$), precision, accuracy, uncertainty and ruggedness were determined. Average accuracy of the five nitroimidazoles from bovine muscle fortified at 5 levels (0.5, 1.0, 1.5, 2.0 and 2.5 $\mu\text{g kg}^{-1}$) ranged from 96% to 103%. The calculated $CC\alpha$ ranged from 0.0 to 0.17 $\mu\text{g kg}^{-1}$; $CC\beta$ ranged from 0.08 to 0.41 $\mu\text{g kg}^{-1}$. A complete statistical analysis was performed and the results indicate that the method is robust when subjected to day-to-day analytical variations.

Keywords: nitroimidazoles; metabolites; drug residues; bovine muscle; LC-MS/MS

Introduction

Nitroimidazoles are a class of imidazole derivatives that have therapeutic uses as antibacterials against anaerobic bacteria and antiprotozoal agents. They are traditionally used for the prevention and treatment of histomoniasis in turkeys, trichomoniasis in pigeons, genital trichomoniasis in cattle or haemorrhagic enteritis in pigs. In addition to their antimicrobial effects, nitroimidazoles are also useful as growth promoters due to their efficacious improvement in meat production and feed conversion.

The commonly used nitroimidazole drugs include metronidazole (MNZ), ronidazole (RNZ), dimetridazole (DMZ), metronidazole-OH (MNZOH, the metabolite of MNZ), and 2-hydroxymethyl-1-methyl-5-nitroimidazole (HMMNI, the metabolite of RNZ and DMZ). The safety assessment of nitroimidazoles showed them to be genotoxic, carcinogenic and mutagenic (Voogd et al. 1979; Dobias et al. 1994). Nitroimidazoles have been banned in Europe: MNZ (613/98/EC), RNZ (3426/93/EC), and DMZ (1798/95/EC); and prohibited in the United States, Japan, China and Brazil. However, deliberate use still exists and the illegal use of nitroimidazoles in animal husbandry must be monitored.

Several different methods have been published for the determination of nitroimidazoles residues in feed and other biological matrices, such as LC-MS (Cannavan & Kennedy 1997; Daeseleire et al. 2000; Boison et al. 2012; Tolgyesi et al. 2012; Xia et al. 2012), LC-MS using atmospheric pressure chemical ionisation (APCI) (Sams et al. 1998), GC (Wang 2001), and GC-MS (Polzer & Gowik 2001). However, these available methods have drawbacks, especially for use in developing countries, such as Brazil. Some are expensive, labour intensive, requiring extensive sample pre-treatments, and cannot be used for routine analysis. Additionally, these methods have not been tested specifically in bovine muscle matrices. Brazil ranks as the world's largest beef exporter and it has the largest commercial cattle herd. Therefore, it is necessary to develop a specific and cheap method using bovine muscle samples produced in that country, and not just applying methods already developed.

MS is the preferred technique for confirmatory residue analyses because of the specificity provided by mass-related data. To detect nitroimidazole compounds with higher sensitivity and specificity, we developed a simple, rapid and reliable LC-MS/MS method. It was shown to be

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