VETERINARY DRUG RESIDUES

Development and Validation of a Liquid Chromatography-UV Detection Method for the Determination of Sulfonamides in Fish Muscle and Shrimp According to European Union Decision 2002/657/EC

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Sulfonamides are one class of antimicrobial agents used in aquaculture production. Sulfonamides are often overused because they are inexpensive and readily available. Their presence at a concentration above the legal limits is a potential hazard to human health. Brazilian authorities have included in the National Regulatory Monitoring Program the control of the three most widely used sulfonamides in aquaculture production, i.e., sulfathiazole, sulfamethazine, and sulfadimethoxine. An LC method with UV detection for the determination of residual sulfonamides in fish muscle, using sulfapyridine as an internal standard has been developed and validated. The validation was performed according to the Brazilian Regulation 24/2009 (equivalent to European Union Decision 2002/657/EC). The method meets the Brazilian regulatory requirement that establishes criteria and procedures for determination of parameters such as decision limit (CCα), detection capability (CCβ), precision, and recovery. For fish muscle, CCα was determined at 3.63, 2.91, and 7.46 µg/kg for sulfathiazole, sulfamethazine, and sulfadimethoxine, respectively. CCβ was 9.39, 14.54, and 9.39 µg/kg for sulfathiazole, sulfamethazine, and sulfadimethoxine, respectively. For shrimp, CCα was 11.5, 8.67, and 4.46 µg/kg for sulfathiazole, sulfamethazine, and sulfadimethoxine, respectively. CCβ was 18, 11.93, and 5.24 µg/kg for sulfathiazole, sulfamethazine, and sulfadimethoxine, respectively. A complete statistical analysis was performed on the results obtained. The results indicate that the method is robust when subjected to day-to-day analytical variations.

Aquaculture production has notably increased in the last decades, mainly due to intensive farming. Together with market globalization, this gives rise to the spreading of several fish diseases, increasing the demand for veterinary drugs for aquatic species. Many classes of antibiotics are commonly used in aquaculture worldwide to treat infections caused by a variety of bacterial pathogens of fish (1). Sulfonamides are a very important class of antibacterial compounds widely used in veterinary practice for therapeutic, prophylactic, and growth-promoting purposes. The potential hazards associated with the presence of antibiotics in edible tissues from aquaculture include allergies, toxic effects, and acquisition of drug resistance by pathogens in the human body (2), as well as potential carcinogenicity (3). Residues of these compounds in foods of animal origin, including those raised in aquaculture, intended for human consumption are of toxicological and regulatory concern. These concerns have led to the establishment of tolerance levels for residues remaining in the tissue and treatment schemes that take into account withdrawal periods for treated fish. The Committee for Veterinary Medicinal Products of Europe recommended that sulfonamides be entered into Annex I of Council Regulation No. 2377/90 (4) with a maximum residue level (MRL) of 100 µg/kg in fish muscle. The MRLs are regarded as the sum of all sulfonamides. The Brazilian Ministry of Agriculture demanded the monitoring of sulfathiazole (STZ), sulfamethazine (SMZ), and sulfadimethoxine (SDTX) residues (Normative Instruction 24 from 9/08/2011 published at Brazilian Official Gazzette 154, p.10, 11/09/2011) for muscle fish (5), specifically, tilapia (also known as St. Peter's fish, the main species cultivated in Brazil), and shrimp.

Reaching these limits requires the development of sufficiently sensitive and selective methods for determination of the antibiotic residues in food. Several quantitative methods for sulfonamides in fish have been reported using LC (6–9), immunoassay (10), and MS detection (11). But many of the methods cannot simply be extrapolated to Brazil due to different species, environmental conditions, and high cost. In contrast, LC-UV is a mature, robust, and reliable technology capable of meeting the requirements in terms of sensitivity, specificity, and method performance to achieve the objective of residue or multiresidue screening. This technology could allow laboratories, which do not have the capability to analyze using expensive mass spectrometric detectors, to continue to provide confirmatory analysis for sulfonamides using a second chromatographic column.

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