



Validation of a liquid chromatography with ultraviolet detection methodology for the determination of sulfonamides in bovine milk according to 2002/657/EC

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ABSTRACT

Sulfonamides are one class of antimicrobial agents used in milk production. Sulfonamides are often overused because they are inexpensive and readily available. Their presence at a concentration above the limit value is a potential hazard to human health. Brazilian authorities have included in the National Regulatory Monitoring Program, the control of the sulfonamides in bovine milk production. A simple, rapid, sensitive and reliable high-performance liquid chromatographic method with ultraviolet detection for the simultaneous determination of three sulfonamides (sulfathiazole, sulfamethazine, sulfadimethoxine) in bovine milk, using sulfapyridine as an internal standard has been developed and validated. The whole procedure was validated according to the Brazilian Regulation 24/2009 (equivalent to European Union Decision 2002/657/EC). This Regulation establishes criteria and procedures for determination of parameters such as decision limit ($CC\alpha$), detection capability ($CC\beta$), precision, recovery and ruggedness. The $CC\alpha$ was determined at 104.4, 102.2 and 105.0 $\mu\text{g}/\text{kg}$ for sulfathiazole, sulfamethazine, and sulfadimethoxine, respectively. $CC\beta$ was 108.8, 104.9 and 109.9 $\mu\text{g}/\text{kg}$ for sulfathiazole, sulfamethazine, and sulfadimethoxine, respectively. Average recoveries fortified with 50, 100 and 200 $\mu\text{g}/\text{kg}$ leads around 100%. A complete statistical analysis was performed on the results obtained and the results indicate that the method is robust when subjected to day-to-day analytical variations.

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1. Introduction

During recent decades the use of antibiotics and chemotherapeutics in animal husbandry has increased considerably and it inevitably results in the presence of residues in animal tissue and in milk. There is an increasing interest in development of proceeding to ensure safe and qualitative food products. Quality control of milk and its derivatives is a very demanding field, with a great need for development of more economical, time saving, and accurate analytical methods for ensuring product quality (Husain, 2010; Nada, Ilija, Igor, Jelena, & Ruzica, 2012).

Sulfonamides (SAs) are broad spectrum antibiotics frequently used in veterinary medicines. They are commonly used for the prevention and treatment of dairy cattle for several infectious diseases, prophylactic, or as feed additives to promote growth in farm animals (Corcia & Nazzari, 2002).

Milk is known as a nutritious wholesome food consumed globally. It is an inexpensive source of protein and calcium essential for promoting growth in children and general good health of the

population. However, due to poor hygienic conditions of many local dairy farms, cows often get ulcers or sores on their udders. This bovine condition is known as mastitis and requires antibiotics to treat and control the condition. Prevalent use of antibiotics in dairy farming such as unnecessary administration for disease control or not observing the withdrawal time after treatment can lead to the veterinary drugs residues in milk. The presence of sulfonamide residues in milk intended for human consumption is of toxicological and regulatory concern. Some of the compounds such as sulfamethazine are carcinogen (Littlefield, Sheldon, Allen, & Gaylor, 1990), and all of them can promote occurrence of the antibiotic-resistant bacteria, which leads to inefficiency of this medicine for the therapeutic use (Haagsma, Pluijmakers, Aerts, & Beek, 1987), as well as severe allergic reactions.

To ensure milk safety for the consumers, in Brazil, government authorities have established a monitoring program to determine SAs levels in meat, honey and milk. The maximum residue limit (MRL) in milk has been set at 100 $\mu\text{g}/\text{kg}$ of total sulfonamides (Ministry of Agriculture, Livestock and Supply, 2008).

A number of analytical high-performance liquid chromatography methods (HPLC) have been published which describe the analysis of sulfonamides (Bogialli, Curini, Corcia, Nazzari, & Polci,

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