Multi-residue analysis of ceftiofur, sulfonamides and fluoroquinolones in milk by LC-FL/DAD with electrospray mass spectrometer confirmation

Isabela Maia Toaldo\textsuperscript{a}, Gabriel Zandonadi Gamba\textsuperscript{a}, Lidia Almeida Picinin\textsuperscript{a}, Gabriel Rubensam\textsuperscript{b}, Rodrigo Hoff\textsuperscript{b}, Marilde Bordignon-Luiz\textsuperscript{a*}

\textsuperscript{a} Programa de Pós-Graduação em Ciência dos Alimentos, Laboratório de Bioquímica de Alimentos, Departamento de Ciência e Tecnologia de Alimentos, Universidade Federal de Santa Catarina, Florianópolis, Brasil

\textsuperscript{b} Ministério da Agricultura, Pecuária e Abastecimento, Laboratório Nacional Agropecuário – LANAGRO/RS, Porto Alegre, Brasil

The large-scale application of antibacterial drugs is a worldwide concern. The fluoroquinolones, sulfonamides and cephalosporins, mainly ceftiofur, are especially applied in dairy cattle for mastitis treatment. The misuse of these drugs raises the risk of their residues in milk, representing a human health hazard and technological problem for dairy industry. A novel and simplified procedure for simultaneous determination of ceftiofur (CEF), four fluoroquinolones (FQ) and six sulfonamides (SA) antibacterials in milk using LC-FL/DAD was developed and validated (Commission Decision 2002/657/EC). Confirmation was performed by LC-ESI-MS/MS analysis. The analytes were extracted from milk by liquid-liquid extraction with acidified ultrapure water and directly analyzed in the chromatograph. The SA compounds were pre-column derivatized with fluorescamine for fluorescence detection. Analyte identities were confirmed by LC-ESI-MS/MS, through liquid-liquid extraction in the fortified matrix. Ninety milk samples from two state capitals in the South region of Brazil were analyzed. The method provided good results regarding the analytical parameters, with correlation coefficients ($r^2$) greater than 0.98 for all the analytes and recovery rates up to 98% for all the studied drugs. For the LOD and LOQ limits, low values were obtained.
for all analytes, demonstrating good specificity. The intra-day and inter-day precisions were below or equal to 7.40 and 10.13, respectively. Milk samples showed no detection for the studied antimicrobials. The multi-residue method comprises an efficient alternative for residue analysis in milk, with its applicability confirmed by analysis of real milk samples from different origins.