Chloramphenicol and its analogues thiamphenicol and florfenicol are antibiotics with broad-spectrum. However, the clinical use of chloramphenicol has been discontinued in many countries because of its adverse reactions and side effects on humans, such as aplastic anemia. Thiamphenicol and florfenicol appear to be viable substitutes in veterinary medicine, however high levels should be avoided. To ensure consumers' safety, sensitive and reliable methods are needed for the routine monitoring of these compounds. Liquid chromatography with mass spectrometry detection (LC-MS/MS) is very useful as it allows separation, quantification and confirmation of different analytes with high sensibility. The objective of this study was to optimize a method for simultaneous analysis of three amphenicols using LC-MS/MS. Chromatography was performed on an Agilent 1200 HPLC and Aqua C18 column (50x2.0 mm, 5 µm). The injection volume was 10 µL. Studies indicated that the best mobile phase was (A) water + 0.1% formic acid and (B) methanol + 0.1% formic acid at a flow rate of 0.4 mL/min and a gradient elution of: 0 min (90%A:10%B); 3 min (90%A:10%B); 5 min (80%A:20%B); 10 min (80%A:20%B) and 12 min (90%A:10%B). A mass spectrometer AB SCIEX QTRAP 5500 was connected to the LC via electrospray ionization interface. The analysis was performed in the negative ionization mode and the samples were analyzed by multiple reaction monitoring. The optimized LC-MS/MS method provided good resolution among peaks (Rs > 2.0) and distinct ions for monitoring. It was fast and reliable for the analysis of amphenicols.

Financial support: Fapemig, CNPq, FINEP.