

Method validation for the determination of Chloramphenicol, a banned veterinary antibiotic in shrimps, by using liquid chromatography-tandem mass spectroscopy

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Chloramphenicol is one of the most widely used antibiotics for both food and domestic animals. It is a broad spectrum antibiotic which interferes with protein synthesis of many Gram-negative and Gram-positive bacteria and has toxic effects on human. Maximum Residue Level (MRL) of Chloramphenicol in shrimp is $0.5 \mu\text{g kg}^{-1}$. In Europe, Chloramphenicol is currently banned from use on animals used as food sources and animal feed products. Although unauthorized, Chloramphenicol was used in Shrimp culture in Asia, where shrimp are cultured primarily for the European market. The main objective of the present study was to develop a simple and rapid method to analyse Chloramphenicol residues in shrimps using Liquid Chromatography-tandem mass spectroscopy (LC-MS/MS). Shrimp samples were homogenised and directly extracted with acetonitrile. HPLC grade standard Chloramphenicol (98%) was used for all experiments. The chromatographic separation was carried out on a C-18 column under isocratic elution conditions. The mass analyser was operated in

the negative polarity of multiple reaction monitoring (MRM) mode. The mass spectrum of the precursor ion was found at $m/z = 320.9$ and product ions were obtained at $m/z = 256.9$ and 152.0 . The linearity of the plot was obtained between $0.05 \mu\text{g L}^{-1}$ – $100 \mu\text{g L}^{-1}$ and the r^2 value was greater than 0.99. The obtained average recoveries against seven replicates at 2, 45 and $90 \mu\text{g kg}^{-1}$ levels were 85, 110 and 115%, respectively. Accuracy of the proposed method was within the range of $(-2.74) - (+2.74)$. The LOQ and LOD values were $0.076 \mu\text{g kg}^{-1}$ and $0.055 \mu\text{g kg}^{-1}$ respectively. The validation parameters demonstrated good linearity, accuracy, precision, and recovery. The LOD and LOQ of Chloramphenicol in shrimps showed greater sensitivity with respect to required MRL. Moreover, the proposed method provides a simple sample preparation in a shorter time. Hence, this study has proven to be efficient and suitable for monitoring Chloramphenicol in shrimps.

Key words: Chloramphenicol, Shrimp, LC-MS/MS, MRM, Accuracy