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Confirmation of total florfenicol residues by LC-MS/MS: risk assessment based on false-negative results when non-hydrolysis was performed

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Florfenicol (FF) is a synthetic, broad-spectrum systemic antibiotic. The metabolic pathways are well documented, with the main route of elimination being in the urine. Is mainly metabolized to florfenicol amine (FFA) as final marker residue via three main intermediate metabolites: flofenicol alcohol, florfenicol oxamic acid and monochloroflorfenicol and can therefore be found in the muscle of animals intended for human consumption (1).

Listed in Table 1 of Regulation (EU) 37/2010, its veterinary medicine use is authorized in the European Union (EU) in all food-producing species, except for animals from which milk and eggs are produced for human consumption. Maximum residue limits (MRLs) in muscle are set to 100 µg.kg-1 for poultry; 200 µg.kg-1 for bovine, ovine and caprine; 300 µg.kg-1 for porcine; and 1000 µg.kg-1 for fin fish. MRL for all other food producing species is established to 100 µg.kg-1. Its determination is based on the marker residue defined as the sum of the FF and its metabolites measured as FFA. There are two possibilities: determine the concentration of each metabolite and express it in FFA equivalents; or perform an acid hydrolysis to convert all FF residues into FFA. This second option is recommended, because a significant proportion of FF residues are protein-bounded and therefore not extractable when hydrolysis is not used (2).

A reliable quantitative LC-MS/MS method was fully developed and validated, according to the new Regulation (EU) 2021/808, for determination of total FF residues as FFA residue marker using hydrolysis step in all muscle food-producing species. Chromatographic conditions enables the monitoring of FFA and its parent compound to ensure the total conversion during hydrolysis step. Furthermore, mass spectrometry conditions include an isotopically labelled internal standard to minimize matrix effects and obtain an accurate quantification with regard to the different species and the concentration levels studied, ranging from 0.1 to 1.5 MRLs.
This method was compared to a similar protocol but without this specific step, focusing exclusively on the FF and its main metabolite: FFA. Carried out on naturally contaminated samples, results demonstrated a proven risk of false-compliant results when a hydrolysis step is not applied.

Keywords: Total florfenicol residues, Determination by hydrolysis step, Risk assessment for food safety

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