

**THE DEVELOPMENT OF THE METHOD FOR ENRAMYCIN RESIDUES
DETERMINATION IN POULTRY SAMPLES
USING ULTRA-EFFICIENT LIQUID CHROMATOGRAPHY
WITH TANDEM-MASS SPECTROMETRIC DETECTION**

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The manuscript presents the results of the development and introduction of UPLC-MS/MS method for the determination of residues of polypeptide antibiotic enramycin in chicken muscle tissues. Polypeptide antibiotics are often used in poultry for the treatment and prevention of various infectious diseases. Sometimes these drugs are added to feed at levels of sub-therapeutic concentrations as antimicrobial growth promoters, throughout the entire period of growth, which may cause the appearance of antibiotic-resistant strains of pathogenic bacteria and reduce the effectiveness of known antibiotics for human treatment. For this reason, growth promoters such as polypeptide antibiotics: bacitracin, colistin, virginamycin, enramycin, are prohibited for use as feed additives, in particular in the European Union. Since enramycin is banned in European countries, its maximum residue level is not legally established. However, in Japan and Korea, MRL for enramycin in chicken meat and offal is at the level of 30 µg/kg. Enramycin (enduracidine) is a polypeptide antibiotic consisting of 17 amino acids. It is produced by bacteria *Streptomyces fungicidicus*. Enramycin is effective against gram-positive bacteria; it has a growth promoting effect on pigs and chickens, including broilers. The main components of enramycin are enramycin A and enramycin B, which differ only on one –CH₂– group. Their ratio may vary, but predominantly enramycin A content is ~ 45-70 % and the enramycin B ~ 30 %. Very few methods had previously been published for the analysis of polypeptide residues, in particular, of enramycin, in food of animal origin. Therefore, due to the problem of antibiotic resistance, as well as the increased requirements to the safety control of food products of animal origin, in particular poultry, we have developed and introduced the method of simultaneous quantitative determination of enramycin A and enramycin B in chicken muscle tissues by rapid, highly sensitive and highly selective UPLC-MS/MS technique.

Sample preparation procedure consists of the homogenization of chicken muscles tissues, liquid-liquid extraction by means of aqueous and methanolic solutions of trifluoroacetic acid, purification by octadecyl cartridges for solid-phase extraction, samples concentration by means of evaporation, reconstitution in mobile phase and filtration. Since enramycin A and enramycin B have large molecular weights: 2355 and 2369 a.m.u., respectively, these polypeptides can form two-, three-, and four-charged molecular parent ions (precursor ions) in ionization mode ES⁺. Typically, three-charge ions $[M + 3H]^{3+}$ are the most intensive in the mass spectrum of polypeptides, therefore, for quantification of enramycin residues we used four MS/MS transitions of each of three-charge parent ions: 786 m/z for enramycin A and 791 m/z for enramycin B (product ions were common for both analytes, viz. 95, 122, 688 and 1090 m/z). Reversed phase chromatographic separation was performed in gradient mode during 6 min using 0.1 % formic acid in water and 0.1 % formic acid in methanol as mobile phases. Determination of enramycin content (total enramycin A and enramycin B) in examined poultry meat samples was carried out using the calibration curves, built on enramycin standard solutions in the range of concentrations from 0 to 100 µg/kg, that is, from 0 to 70.0 µg/kg for enramycin A and from 0 to 30, 0 µg/kg for enramycin B according to the certificate of quality of enramycin standard.

The main advantages of the developed method are the rapid chromatographic separation, high selectivity and high sensitivity. The limit of detection of enramycin A is 5 µg/kg and enramycin B is 4 µg / kg, which competes with previously published UPLC-MS/MS methods for the determination of polypeptide antibiotics in animal tissues. The method has been approved in the analysis of real and fortified samples of poultry meat (CV ~ 20 %).

Keywords: POLYPEPTIDE ANTIBIOTICS, ENRAMYCIN, RESIDUES, UPLC-MS/MS, POULTRY MEAT.