Nitroimidazoles (NMZs) are effective antiprotozoal agents and for this reason they are used in the prophylactic and therapeutic treatment of histomoniasis and coccidiosis in avian species. Studies suggested possibly mutagenicity and carcinogenicity of these compounds in humans. Due to their toxicological characteristics, the use of nitroimidazoles in food-producing animals was prohibited, therefore this drugs have been included in Table 2 of the Commission Regulation (EU) No 37/2010. In this Table there are compounds for which no Maximum Residue Levels can be fixed. However in 2007 the European Union Reference Laboratories published a Guidance Document setting recommended concentration/levels for prohibited substances without maximum residue limits. For NMZs and their metabolites CCα for confirmatory methods should be lower than 3 µg/kg in plasma, retina and eggs was fixed. A very important tool to minimize and to control possible illicit use of nitroimidazoles in avian species is the availability of suitable and validated analytical methods. The validation of a method for the simultaneous determination of residues of NMZs in whole eggs by UPLC-MS/MS is presented. This technique is able to combine the speed of a screening technique with the reliability of a confirmatory method. The compounds considered in this validation study were carnidazole, dimetridazole, ipronidazole, metronidazole, ronidazole, ternidazole and tinidazole. In addition the metabolites 2-hydroxymethyl-1-methyl-5-nitroimidazole, hydroxy-irpentidazole (HMMNI) and hydroxy-metronidazole were considered. Labeled internal standards were used for quantification of analytes. The validation study was performed according to the Commission Decision 2002/657/EC using the alternative matrix-comprehensive in-house validation approach based on designed Interval Plus software (QuoData GmbH, Dresden, Germany). Experiments were carried out at the concentration range of 0-20 µg/kg for all substances. The outcomes of experimental design were CCα, CCβ, recovery at CCα, reproducibility at CCα and measurement uncertainty. The results of validation study showed that the method is able to identify and to quantify the presence of NMZs and their metabolites in eggs at the recommended level required in the official control of residues.