

# MASS SPECTROMETRIC APPROACHES TO SURVEY RESIDUES OF ILLEGAL GROWTH PROMOTERS IN FOOD PRODUCING ANIMALS

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Since 1 January 1989, according to Directive 88/146/EEC replaced later by Directive 96/22/EC, the European Commission (EC) prohibits the administering to a farm animal by any means whatsoever of, *inter alia*, of substances having a thyrostatic, oestrogenic or gestagenic action for growth promotion purposes. As a result, the use of the hormones oestradiol, testosterone, progesterone, zeranol, trenbolone acetate and melengestrol acetate alone or in combinations for growth promotion purposes in meat production is prohibited. The prohibition covers both the use of these hormones for domestic production and imports from third countries of meat from animals treated with these hormones. Even if banned substances are sometimes reduced and summarized to "hormones", the reality is different. In Europe, five class of compounds are identified in the regulation, as listed in community directive 96/23/EC: stilbens (group A1), thyrostats (A2), steroids (A3) including estrogenic, androgenic and progestagenic substances, resorcylic acid lactones (A4), and  $\beta$ -agonistic drugs (A5). The control of growth promoters in meat producing animals is probably one of the most challenging areas in the field of chemical residue control in food, when considering the wide number of target substances, the variability of their chemical structures, their concentration level, as well as the variability of the biological matrices. It obliges the analyst to rely his strategy on analytical methods combining both specificity and sensitivity, respectively. Whereas, Enzyme Linked Immuno Sorbent Assay (ELISA) and Radio Immuno Assay (RIA) are still sometimes used for screening purpose, mass spectrometric methods constitute the obliged strategy during confirmatory processes, technically and strategically speaking. Until recently, the standard technique for growth promoters analysis has been gas chromatography (GC)–MS. This required the derivatization of the analytes using silylation, acylation, oxime/ silylation reactions, depending on the individual properties of the compounds. LC–MS provides a universal detector, since anabolic compounds may be analyzed without derivatization; this approach proved to be very efficient especially for trenbolone-like steroids, stanozolol,  $\beta$ -agonistic drugs, corticosteroids, thyrostats, as well as their glucurono- and sulfo-conjugates. The rules governing screening and confirmation of analytical methods for veterinary drug residues and their validation have been revised by the EU [2002/657/EC decision]. The rules have been extended to include a number of MS techniques, which have gained in popularity over the last decade, e.g. liquid chromatography (LC)–MS, and acquisition techniques such high resolution selected ion monitoring (HR-SIM) and selected reaction monitoring (SRM). Today, minimum required performance levels (MRPL) are lower and lower so that competent methods must be more sensitive (pg level), more robust whatever the biological matrix (hair, faeces, urine, tissue...), more specific (HR-MS and MS/MS) and relevant to challenging analytes such as ecdysteroids, endogenous steroids, growth hormone (rST). The first applications of metabolomic are since recently available in this domain; indirect biomarkers determination to characterize animal exposure to these illegal substances will be illustrated thanks to the use of high-resolution mass spectrometry (LC-HRMS, FT-orbitrap).