Technological intervention in drug residues in food of animal origin

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Abstract
Consumers are increasingly concerned about the safety of their food and uncertain about food production practices. Veterinary drugs are important for conventional and also for ecological animal breeding to optimise animal health. The compounds are administered to prevent or treat diseases or to promote growth. Potential threats to human health related to meat and dairy products exist through means of drug residues. A residue is a trace of a substance, present in a matrix. The use of veterinary drugs is regulated by international and national legislation and either banned with zero tolerance or permitted up to specific concentration levels in the final food product. Residue analysis is a relatively young discipline and a very broad area, including banned substances as well as registered veterinary medicinal products. The objective of this study is to shed light in the trends in the analysis of residues of veterinary drugs in meat and milk of food producing animals out of historical perspectives. The analysis of drug residues in animal food products has known a tremendous evolution during the past 35–40 years. In the future, it can be foreseen that this technological intervention will proceed in the direction of the use of more and more sophisticated and expensive instruments for detection of drug residues in food of animal origin.

Keywords: Drug, Residue, Meat, Dairy

The analysis of drug residues in foods of animal origin is a relatively new discipline. Initially, residue analysis started in the late 1960s. In most European countries research on residues and the application in regulatory control on slaughter animals started later [1]. A drug residue may be defined as a trace of a drug, present in a matrix (e.g. meat, milk, etc.) after its administration to an animal. The determination of trace residues and contaminants in food has been of growing concern over the past few years. Residual drugs like antibacterial in food constitute a risk to human health, as they can contribute to the transmission of antibiotic-resistant pathogenic bacteria through the food chain. So, to ensure...
food safety EU and USA regulatory agencies have established lists of forbidden or banned substances and tolerance levels for authorized veterinary drugs including antibacterial. In addition, the EU Commission Decision 2002/657/EC have set requirements about the performance of analytical methods for the determination of veterinary drug residues in food and feedstuffs.

The substances involved may be divided into two major classes according to council directive 96/23/EC [2]: Group A and Group B substances. Group A involves the growth promoters used for animal fattening and may be subdivided into four major groups: anabolic steroids, thyreostats, beta-agonists and Annex IV substances. Corticosteroids (CoST) are also treated as A substances. Group B contains the veterinary drugs or veterinary medicinal products (VMPs): antibacterial substances, other VMPs as anthelmintics, coccidiostats, carbamates and pyrethroids, sedatives, non-steroidal anti-inflammatory drugs (NSAIDs) and other pharmacologically active substances. The analytical requirements for both groups are different. For banned (A) substances the emphasis lays on the identification of the substances in a large number of matrices (e.g. meat, urine, hair) in a concentration as low as possible (zero tolerance principle). In this case, at first qualitative multi-residue methods have to be developed and secondly quantitative methods. Recent developments in the use and abuse of growth promoters have been reported [3]. For B substances having a Maximum Residue Limit (MRL), methods for the quantitative determination of the substances in edible matrices only (e.g. milk, meat, liver, egg) have to be worked out. In most cases the MRLs of B substances are a magnitude 10–100 times higher than the recommended concentrations (minimum required performance levels; MRPLs) of the A substances [4]. A recent review on the analytical strategies for residue analysis of veterinary drugs and growth promoting agents in food producing animals was published [5].

**Historical perspective**

During the past years, the use of powerful mass spectrometric detectors in combination with innovative chromatographic technologies has solved many problems related to sensitivity and selectivity for analysis of drug residues [6]. In the early 70s thin layer chromatography (TLC) was the method of choice for the qualitative detection of banned substances (thyreostats and certain anabolics at that time). The reasons therefore were the specificity, the simplicity of development in two dimensions and the possibility of reaching low limits of detection for an acceptable budget (often using fluorescence detection). The
only alternative with acceptable limits of detection was Gas chromatography (GC) with electron capture detection (ECD). High Performance Liquid Chromatography (HPLC) with UV detection was introduced in the middle 70s, but the first instruments were expensive and not robust. UV detection does also not match the specificity and limits of detection needed for A substances. Fluorescence detectors were only introduced later. However, for the quantitative determination of B substances UV detection and post column derivatisation was often used. During the 90s more and more affordable gas chromatography–mass spectrometry (GC–MS) apparatus appeared on the market and the transition from TLC (and HPLC) to GC–MS methods was ongoing. At end of the 90s, LC–MS belonged more and more to the mandatory standard equipment of a residue laboratory. Recent years 20s the LC-MS/MS are more in use.

The analysis of residues of A and B substances in meat producing animals is a very broad area. Also in related areas, such as human doping analysis or analysis of pesticides and contaminants, an analogous evolution of methods is still going on. At the end of the 60s, beginning of the 70s only TLC and GC with electron capture detection (ECD) were used for the detection of small concentrations of organic substances in complex matrices, next to RIA methods. The analytical output of instruments consisted of a non-digital photograph of a TLC plate or a chromatogram of a single response of a detector on a strip chart recorder. Very sensitive methods were developed but the clean-up of the sample was mostly complicated and as a consequence sample throughput very small. For TLC, pre as well as post-development derivatisations were used. In GC pre-column derivatisation was necessary. For ECD detection mostly polyfluorated reagents were used. In the middle of the 70s also the first HPLC apparatus appeared in the laboratories. Since then more and more analysis shifted to HPLC and also a differentiation between an analytical strategy for A and B substances could be made. For A substances the desire for more specificity and lower limits of detection intensified in the 80s by the introduction of GC–MS using selected ion monitoring.

**Applications**

A systematic approach to the development of a method was reported that combines continuous solid-phase extraction and gas chromatography-mass spectrometry for the simultaneous determination of 20 pharmacologically active substances including
antibacterials (chloramphenicol, florfenicil, pyrimethamine, thiamphenicol), nonsteroideal anti-inflammatories (diclofenac, flunixin, ibuprofen, ketoprofen, naproxen, mfenamic acid, niflumic acid, phenylbutazone), antiseptic (triclosan), antiepileptic(carbamazepine), lipid regulator (clofibric acid), β-blockers (metoprolol, propranolol), and hormones (17R-ethinylestradiol, estrone, 17β-estradiol) in milk samples. Method for the simultaneous determination of about 100 veterinary drug residues (avermectines, benzimidazoles, quinolones, nitromidazoles, β-lactams, macrolides, triphenylmethan dyes, sulfonamides, tetracyclines) is also reported. The method has been developed and validated complying with the guidelines for the implementation of decision 2002/657/EC for the application in routine analysis of meat and fish samples. Simultaneous determination of 130 residual veterinary drugs and their metabolites in bovine, porcine and chicken muscle using liquid chromatography coupled with Mass Spectrometry was also reported. The author has also develop method for the detection of residues of veterinary drugs like Tetracylines groups, Enrofloxacin, Ciprofloxacin, Ceftiofur, Oxyclozanide, Albendazole, Fenbendazole using HPLC and pesticides like Endosulfan and Chlorpyrifos using GC in Pork.

Conclusion

Veterinary drugs are important for conventional and also for ecological animal breeding to optimise animal health. The compounds are administered to prevent or treat diseases or to promote growth. Depending on the specific compound and its application the use of veterinary drugs is regulated by international and national legislation and either banned with zero tolerance or permitted up to specific concentration levels in the final food product. Abuse of veterinary drugs and consequently its residues in food become an increasing problem due to economic pressure on farmers and changes in breeding practices. Public perception and the growing awareness of this issue have raised the interest among regulators to check for such residues. The analytical determination is a huge challenge, since there is a high variety in structure and chemical properties of the compounds; furthermore the protein rich commodities are extremely difficult matrices leading in time consuming and cost effective procedures in the laboratory. At the moment a broad range of analytical methods is in use to cover the whole spectrum of relevant analytes; differentiating screening and confirmatory analytical technologies such as biosensors, immunoassays and liquid chromatography coupled with mass spectrometry. Methods have to be extensively validated to be used in accordance to the requirements of the Commission Regulation (EU) No 37/2010.
with regard to the MRLs. In all cases, concentration levels of drug residues in the ppb concentration range or even lower have to be detected.

**Bibliography:**

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