Developing new reference materials for effective veterinary drug-residue testing in food-producing animals

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Reliable analysis of veterinary drug residues in animal-derived foodstuffs represents an important measure to ensure consumer protection. European legislation addresses this issue (e.g., by defining maximum residue limits, specifying sampling and monitoring plans, and stipulating performance and validation criteria for analytical methods). Certified reference materials (CRMs) constitute an important tool for method validation and method-performance verification. This paper addresses the current status of legislation, describes characteristics of analytical methods in veterinary drug-residue testing and then focuses on the challenges and considerations in developing new RMs in this field.

Keywords: Animal food product; CCα/CCβ; Method validation; Reference material; Veterinary drug residue

Abbreviations: BCR, Community Bureau of Reference; CCα, Decision limit; CCβ, Detection capability; CRL, Community Reference Laboratory; CRM, Certified reference material; DAD, Diode array detector; DG SANCO, Health and Consumer Protection Directorate General (European Commission); DMZ, Dimetridazole; DSC, Differential scanning calorimetry; EC, European Commission; EEC, European Economic Community; ELISA, Enzyme-linked immuno-sorbent assay; ERM, European Reference Material; EU, European Union; FID, Flame ionization detector; GC, Gas chromatography; HMMNI, 2-Hydroxymethyl-1-methyl-5-nitroimidazole; IDMS, Isotope dilution mass spectrometry; IEC, International Electrotechnical Commission; IMEP, International Measurement Evaluation Program; IRMM, Institute for Reference Materials and Measurements; ISO, International Organization for Standardization; LC, Liquid chromatography; LOD, Limit of detection; LOQ, Limit of quantification; MNZ, Metronidazole; MNZ-OH, Hydroxymetronidazole; MRL, Maximum residue limit; MRPL, Minimum required performance limit; MS, Mass spectrometry; NMR, Nuclear magnetic resonance; NRCP, National Residue Control Plan; NRL, National Reference Laboratory; PLE, Pressurized liquid extraction; PT, Proficiency testing; ppb, Parts per billion; Q-TOF, Quadrupole–time-of-flight; RIA, Radio immuno-assay; RM, Reference material; RNZ, Ronidazole; SPE, Solid-phase extraction; TCA, Trichloric acid; TLC, Thin-layer chromatography; UV, Ultra-violet; WHO, World Health Organization; WTO, World Trade Organization.

1. Introduction

Veterinary drugs are used to treat animal diseases and as prophylactic agents. A large variety of substances is used (e.g., antibiotics, anthelmintics, anticoccidials, sedatives, and steroidal as well as non-steroidal hormones). Besides their desired activities, many of these substances also exhibit adverse properties, when used in a wrong or an abusive way (e.g., with a withdrawal period that is too short, or using growth-promoting hormones to accelerate animal-meat production). Veterinary drug-residue control therefore represents an important measure in ensuring consumer protection.

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European legislation addresses this issue (e.g., stipulates sampling and monitoring plans, definition of maximum residue limits (MRLs) for approved veterinary medicinal products and minimum required performance limits (MRPLs) for some of the testing procedures to detect banned substances, and stipulation of the performance characteristics of analytical methods).

The development, optimization, and validation of suitable analytical methods are important elements of assuring reliable veterinary drug-residue testing. A variety of methodologies is available for both screening and confirmatory analysis (e.g., enzyme-linked immunosorbent assays (ELISAs), gas chromatography (GC), mostly coupled to element-specific detectors (nitrogen/phosphorous detector, electron capture detector) or a mass spectrometer (GC-MS), as well as liquid chromatography coupled to UV or diode array detectors (LC-UV or LC-DAD), and LC coupled to a mass spectrometric system (LC-MS or LC-MS^2)). Several hundred thousand samples are analyzed each year in the European Union (EU), comprising mainly bovine, porcine and poultry samples, but also aquaculture products (e.g., farmed fish (trout, salmon) and shrimp), various matrices (e.g., muscle tissue, plasma and liver), and a huge variety of analytes (e.g., tetracyclines, nitroimidazoles, nitrofurans, β-agonists and chloramphenicol). Many of these drugs and/or their metabolites are water-soluble, non-volatile, and polar, which makes them good candidates for LC separations. Meanwhile, LC-MS^2 methods are available for a large variety of veterinary drugs and their metabolites and are increasingly used, especially for confirmatory analysis, as this methodology allows unequivocal identification of what are usually minute quantities (low-ppb levels) in complex matrices.

Proper method validation is crucial to assure accuracy and reliability in measurements. Reference materials (RMs) and, in particular, certified RMs (CRMs) represent valuable tools for method validation and performance verification. The development of CRMs is time-consuming and often challenging; several issues have to be taken into consideration when developing new CRMs for veterinary drug-residue testing:

- choice of the animal species and matrix;
- type of material (incurred matrix material, calibrant and matrix blank);
- analyte or metabolites and their target concentrations in the material;
- selection and order of processing steps;
- commutability aspects (closeness to real-world sample);
- design of homogeneity and stability studies;
- suitable analytical methodology for characterization measurements; and, finally,
- value-assignment process (certified value and associated uncertainty).

In this overview, we highlight the framework of current EU legislation and analytical methodology for veterinary drug-residue control, and then discuss in detail the impact and the role of RMs in this field, as well as the necessary considerations and challenges for the development of new CRMs.

2. EU legislation

The EU has established and implemented legislation aimed at streamlining the process of registration and authorization of veterinary medicinal products, establishing MRLs of veterinary medicinal products in foodstuffs of animal origin, harmonizing the validation and performance characteristics of analytical methods and the interpretation of results, and stipulating the monitoring measures. However, a recent ''reflection paper on residues of food-stuffs in animal origin'' from DG SANCO [1] critically reviewed the current situation and concluded that legislation needs to be adapted and streamlined to make it fit for purpose to face new challenges [2] (e.g., to comply with Regulation (EC) No. 178/2002 (''food law'') [3], and with international trade law as negotiated at WTO level). We briefly summarize the items of legislation linked to the need for RMs in the following paragraphs.

Council Regulation (EEC) 2377/90 [4] lays down the procedure for establishing MRLs of veterinary medicinal products in foodstuffs of animal origin. The MRL indicates the maximum residue concentration in foodstuffs that can be tolerated (consumer protection). The basic concept is that all veterinary medicinal products are placed in one of the following four lists represented by Annexes I – IV in the Regulation:

I substances with established MRL;
II substances for which no MRL was established (no special protection of public health required);
III substances with provisional MRLs; and,
IV banned substances.

A central part in the control measures is implementation of at least one National Reference Laboratory (NRL) per Member State [5] and the definition and responsibilities of four Community Reference Laboratories (CRLs) [6] in the EU. Each CRL is responsible for particular groups of substances and has certain tasks and functions assigned (e.g., support for NRLs in implementing quality-assurance schemes based on ISO/IEC 17025, organization of laboratory comparisons and comparative tests, and technical and scientific assistance to the European Commission (EC)).

Commission Decision 2002/657/EC [7] is probably the key document of legislation to be consulted by analytical laboratories in veterinary drug-residue control. It lists performance criteria and other requirements for separation and detection techniques for both screening and