Preparation of Reference Materials for Veterinary Drug-Residue Testing

MIRYAN BALDERAS\textsuperscript{1}, NORMA GONZALEZ\textsuperscript{1*}, ESTHER CASTRO\textsuperscript{1}, MARÍA ELENA GONZALEZ\textsuperscript{2}, FRANCISCO OLVERA\textsuperscript{2}

Directorate of Materials Metrology\textsuperscript{1}, Sub-directorate of Analytical Technology Transfer\textsuperscript{2}
Centro Nacional de Metrologia\textsuperscript{1}, Centro Nacional de Servicios de Constatacion en Salud Animal\textsuperscript{2}
km 4.5 Carretera a los Cues, Mpio. El Marques, Queretaro, C.P. 76246\textsuperscript{1}, Carretera Federal Cuernavaca - Cuautla No. 8534, Col. Progreso Morelos, Jiutepec, C.P. 62550\textsuperscript{2}
MEXICO
ngonzale@cenam.mx http://www.cenam.mx

Abstract: Clenbuterol is a synthetic drug which is widely used in human and veterinary medicine; however this β-agonist may be used illegally as a growth promoter in meat-producing livestock. The use of clenbuterol as growth promoter is prohibited in Mexico, as well as in other countries, since its residues may lead to a health risk for consumers. Reliable analysis of clenbuterol residue in animal-derived foodstuffs represents an important measure to ensure consumer protection. Mexican regulatory agencies address this issue improving the laboratory measurements reliability. Certified reference materials (CRMs) constitute an important tool for method validation and method performance verification which are crucial to assure accuracy and reliability in measurements. This paper addresses the preparation of CRMs candidate of clenbuterol in liver starting from an incurred material to support Mexican laboratories measurements.

Key-Words: Clenbuterol residues, veterinary drug residues, reliable measurements, certified reference materials.

1 Introduction

The use of β-agonists such as clenbuterol for growth promoting purposes in farm animals has been banned in many countries including the European Union [1], Mexico [2], between others, because of their adverse effects on human health, such as food poisoning associated with the residues in liver. In 2002, food poisoning cases with clenbuterol increase and they were associated to the consumption of bovine liver [3]. Consequently, the Mexican government issued a regulation that included the technical specifications for a control program in the use of β-agonists in meat-producing livestock [4]. Since then, the National Service of Agrifood Health, Safety and Quality (SENASICA) has been executing programs to avoid the use of clenbuterol and the approval of laboratory techniques activities.

Laboratories involved in the control of veterinary drug residues in edible products have to demonstrate the reliability of their data and analytical performance. Where available, the use of matrix-based certified reference materials may help to achieve this goal. Therefore, it is highly desirable to improve the accuracy of β-agonist residue measurements, thereby making the comparability of test results possible between different laboratories in all Mexico. One of the most important ways of establishing a common basis for accurate measurement is to have readily available reference materials.

Herein, we produced two clenbuterol candidate reference materials from bovine liver, a negative control and a positive standard. Bovine liver was selected as matrix for monitoring slaughtered animal.

2 Problem Formulation

The use of clenbuterol as a repartitioning agent in meat producing animals is prohibited in Mexico. In order to implement measures to monitor the prohibition the Ministry of Agriculture, Livestock, Food, Fisheries (SAGARPA) elaborate a residue-control plan for the collection of samples and their laboratory analysis in all Mexico. The National Centre of Verification Services in Animal Health (CENAPA), which is the reference laboratory in animal health of SAGARPA considered necessary to improve the accuracy of clenbuterol
quantification and thereby make the test results comparable between different Mexican laboratories. The certified reference materials (CRMs) give measurement laboratories a means to validate analytical methods, to assess the quality of the measurement results and to demonstrate their traceability to stated references such as the SI units. Therefore, the availability of clenbuterol certified reference materials in Mexico was priority issue. The development of matrix CRMs is time-consuming and often challenging, several issues have to be taken into consideration when developing CRMs for veterinary drug-residue testing. The preparation of these CRMs for clenbuterol considered the choice of animal species and matrix, the type of material, analyte and its target concentration in the material, selection and order of processing steps. Incurred material is the most closely resembles real-world samples and allows proper assessment of the extractability. The most suitable approach to achieving incurred material is controlled feeding of the clenbuterol to bovine. Clenbuterol is characterized by high bioavailability when administered by oral route [5] and liver is generally considered as the target organ for the control of residues of xenobiotics.

3 Problem Solution
The aim of this work was to prepare CRMs of clenbuterol in bovine liver to support Mexican laboratories measurements. The most suitable approach to achieving incurred materials is controlled feeding of the substance in question to animals. A clenbuterol-contained material was obtained from an orally administrated bovine. In liver, clenbuterol itself is the main form of clenbuterol residues. In Mexico, this β-agonist is forbidden and there is not a maximum residue limits and clenbuterol target concentration was estimated considering the sensitivities of the methods. The homogeneity which is one of the basic requirements for a candidate reference material can be achieved by a thorough mixing of the powdered material. Reference materials of any type must be sufficiently uniform regarding the certified properties when sub-sampled. In other words, the between-sample variation in properties of the samples should be as low as possible. The minimization of the between-sample variation was carried out by subsequent riffling using a rotating sample divider and a cross-riffling scheme was applied (see Fig 1) [6].

Fig. 1 Recombination table of the cross-riffling scheme.

Before homogeneity study is carried out, a small batch of containers with clenbuterol-free bovine liver material and clenbuterol containing bovine liver were assayed for clenbuterol content. Both materials were reconstituted according to the assembled protocols and were quantified by LC-MS/MS analysis following acid hydrolysis and mixed-mode polymeric sorbent clean up [7]. Clenbuterol-free bovine liver material was found to contain below of detection limit. Mass fraction measured in clenbuterol containing bovine liver was 10.79 ng/g (SD 0.91). Bottled materials were kept at -40 °C.

2.1 Treatment of animals
Two Aberdeen Angus adult bovine (300 – 400 kg) were provided by CENAPA herd. Bovines were provided ad libitum access to alfalfa hay and water and provided 1 kg of grain concentrate per day throughout the adaptation and study periods. One bovine was orally administered a feed pellet containing clenbuterol (20 µg/kg live weight per day). The second bovine was used as control. The treatment was applied during 25 days and the animals were slaughtered on day 26. Livers were taken and frozen at -40°C until processing.

2.2 Material processing
Each liver was thawed, ground using a knife mill and frozen at -50°C. Approximately 4.5 kg of each ground liver was lyophilized in one batch for 95 h at 0.1 Pa. About 1.2 kg of dried liver was obtained. Freeze dried materials were ground using a cryogenic vibratory disc mill and sieved through 300 µm to 212 µm stainless steel sieves. Material was homogenized using a riffler and bottled under nitrogen atmosphere.
4 Conclusion
The availability of clenbuterol-containing certified reference materials would facilitate comparisons between different analytical methods (performed in different laboratories) and would provide useful information in the event of trade disputes concerning clenbuterol residues in bovine liver.

Two clenbuterol RMs from bovine livers were produced. DMR-498a clenbuterol-free bovine liver and DMR-499a clenbuterol-containing bovine liver. Homogeneity and stability studies will be performed for these materials.

5 Acknowledgements
This project was financially supported by the sector funds SAGARPA-CONACYT. We thank Judith Sainz for performing materials processing and Karina Cordova Ramirez, Marco Antonio Hernandez, Luis Angel Laguna, Miguel Angel Trujillo and Eduardo Hernández for technical support.

References:
[6] A.M.H. van der Veen, DM.G. Nater, Sample preparation from bulk samples: an overview,