

## VETERINARY DRUG RESIDUES

# Validation of a High-Performance Liquid Chromatographic Method with UV Detection for the Determination of Ethopabate Residues in Poultry Liver

RODRIGO H.M.M. GRANJA, ALFREDO M. MONTES NIÑO, ROBERTO A.M. ZUCCHETTI, ROSARIO E. MONTES NIÑO, and ALESSANDRO G. SALERNO

Microbóticos Laboratories, Avenida Santa Isabel, 2120 Campinas, São Paulo, Brazil

**Ethopabate is frequently used in the prophylaxis and treatment of coccidiosis in poultry. Residues of this drug in food present a potential risk to consumers. A simple, rapid, and sensitive column high-performance liquid chromatographic (HPLC) method with UV detection for determination of ethopabate in poultry liver is presented. The drug is extracted with acetonitrile. After evaporation, the residue is dissolved with an acetone–hexane mixture and cleaned up by solid-phase extraction using Florisil columns. The analyte is then eluted with methanol. LC analysis is carried out on a C18 5  $\mu$ m Gemini column, 15 cm  $\times$  4.6 mm. Ethopabate is quantified by means of UV detection at 270 nm. Parameters such as decision limit, detection capability, precision, recovery, ruggedness, and measurement uncertainty were calculated according to method validation guidelines provided in 2002/657/EC and ISO/IEC 17025:2005. Decision limit and detection capability were determined to be 2 and 3  $\mu$ g/kg, respectively. Average recoveries from poultry samples fortified with 10, 15, and 20  $\mu$ g/kg levels of ethopabate were 100–105%. A complete statistical analysis was performed on the results obtained, including an estimation of the method uncertainty. The method is to be implemented into Brazil's residue monitoring and control program for ethopabate.**

Coccidiosis is a parasitic disease caused by protozoa resident in the intestinal epithelium. It occurs wherever animals are housed in overcrowded areas that are contaminated with coccidial oocysts. Historically, poultry have shown the greatest susceptibility to coccidiosis, because of the intensive nature of most of the poultry industry (1). The symptoms of coccidiosis in poultry may be one or more of the following: poor weight gain, bloody diarrhea, high mortality,

reduction in feed and water intake, emaciation, and reduced egg production (2).

A wide range of drugs is available for the prevention and treatment of coccidiosis. Ethopabate (Figure 1), methyl 4-acetamido-2-ethoxybenzoate, is a coccidiostat commonly used prophylactically via animal feeds to prevent an otherwise costly outbreak of coccidiosis (3). It has a synergetic effect with some anticoccidial drugs (4). The residue presents a potential health risk to consumers.

The use of ethopabate as a feed additive is banned in many countries, and in Brazil a maximum residue limit (MRL) established at 15  $\mu$ g/kg. There is a considerable lack of analysis data on ethopabate residues in poultry liver, which has proved to be an excellent matrix for the detection of ethopabate residues. To control the illegal use of ethopabate, a variety of analytical methods have been developed for different biological materials, based on gas chromatography (5, 6), column high-performance liquid chromatography with UV detection (HPLC-UV; 7), and HPLC with fluorescence detection (8). The methods are, however, not sufficiently sensitive when applied to Brazilian poultry liver samples.

We have developed an analytical method for the monitoring of ethopabate residues in poultry liver with cleanup of the sample by solid-phase extraction (SPE) on a Florisil column. Analyses were carried out by HPLC-UV. The method is simple, sensitive, and rapid, and can be used in a regulatory laboratory with a high throughput of samples.

Validation was conducted according to the 2002/657/European Commission (EC) guidelines (9) and in agreement with the International Organization for Standardization/International Electrotechnical Commission (ISO/IEC) 17025:2005 (10). The identity of eventual ethopabate found in routine samples can be confirmed by HPLC/tandem mass spectrometric (HPLC/MS/MS) systems. Parameters such as decision limit ( $CC\alpha$ ), detection capability ( $CC\beta$ ), precision, recovery, and ruggedness were determined for ethopabate. Typical measurement uncertainty values were estimated in order to comply with ISO 17025 requirements.