

# Liquid Chromatography/Tandem Mass Spectrometry Method To Determine Boldenone in Bovine Liver Tissues

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**Boldenone, an androgenic steroid, is forbidden for use in meat production in most countries worldwide. Residues of this drug in food present a potential risk to consumers. A sensitive LC/MS/MS method for analysis of 17 $\beta$ -boldenone using boldenone-d3 as an internal standard was developed. An enzymatic hydrolysis and extraction using ethyl acetate, methanol, and hexane were performed in the sample preparation. Parameters such as decision limit (CC $\alpha$ ), detection capability (CC $\beta$ ), precision, recovery, and ruggedness were evaluated according to the Brazilian Regulation 24/2009 (equivalent to European Union Decision 2002/657/EC) and International Organization for Standardization/International Electrotechnical Commission 17025:2005. CC $\alpha$  and CC $\beta$  were determined to be 0.17 and 0.29  $\mu$ g/kg, respectively. Average recoveries from bovine liver samples fortified with 1, 1.5, and 2  $\mu$ g/kg were around 100%. A complete statistical analysis was performed on the results obtained, including an estimation of the method uncertainty. The method is considered robust after being subjected to day-to-day analytical variations and has been used as a standard method in Brazil to report boldenone levels in bovine liver.**

**B**oldenone (androsta-1,4-diene-17 $\beta$ -ol-3-one) is an androgenic steroid that has one more double bond in position 1 than testosterone (androst-4-ene-17 $\beta$ -ol-3-one) and improves the growth and food conversion in food producing animals (1). For years, boldenone has been increasingly detected in a number of biological samples in different European Union (EU) member states (2). The question thus arose about whether this increased number of boldenone findings was due to the illegal treatment of animals or if, in some circumstances, boldenone could be of endogenous origin (3). The control of its illegal use has been based either on

17 $\beta$ -boldenone or 17 $\alpha$ -boldenone (its main metabolite in cattle) identification in hair, feces, or urine (4–6). Boldenone is banned in the EU by Council Directive 96/22/EC (7) and in Brazil, which has set a minimum required performance limit (MRPL) at 1  $\mu$ g/kg of boldenone residues in bovine liver (8).

A variety of analytical methods for the determination of 17 $\beta$ -boldenone and metabolites in cattle have been developed based on GC/MS (9) and LC/MS/MS for analysis of urine, feces, muscle, serum (10–16), and hair (5). However, no methods are described for detection of boldenone in bovine liver. Liver tissue is important for the detection of residues considering longer withdrawal periods, and a method for its analysis can be used in a regulatory laboratory.

Here we report confirmatory LC/MS/MS methodology for the determination of boldenone in bovine liver. The method proposed does not use SPE, an advantage considering costs, particularly in the case of developing countries such as Brazil. SPE cartridges are expensive in comparison to liquid–liquid extraction, and SPE is more complicated. The method is validated in accordance with Brazilian Regulation 24/2009 (17), which is equivalent to EU Decision 2002/657/EC (18), and with International Organization for Standardization (ISO)/International Electrotechnical Commission (IEC) 17025:2005 guidelines (19), and it is being considered for use in official monitoring programs in Brazil. Parameters evaluated in the validation were decision limit (CC $\alpha$ ), detection capability (CC $\beta$ ), precision, recovery, and ruggedness.

## Experimental

### Samples

Bovine liver samples were collected in different states of Brazil. The samples were frozen or kept at 4°C and analyzed within 24 h.

### Chemicals and Materials

- (a) *Methanol*.—LC grade (Carlo Erba, Milano, Italy).
- (b) *Acetonitrile*.—LC grade (Carlo Erba).
- (c) *Sodium acetate*.—Analytical grade (Nuclear, Sao Paulo, Brazil).
- (d) *Formic acid*.—LC grade (Synth, Sao Paulo, Brazil).

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