Boldenone and Related Steroids are Formed in The Faeces of Veal Calves

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Abstract

The use of NBoldenone as a growth promoter in meat-producing animals is prohibited in the European Union (Directive 96/22/EC). Boldione (ADD), boldenone, and boldenone esters (primarily the undecylenate form) are all commercially available as anabolic preparations for humans, horses, and cattle. Boldenone metabolites have been discovered naturally in cattle since the late 1990s. According to EU regulations, an unambiguous demonstration of boldenone administration in bovine urine must be provided based on the identification of boldenone in the corresponding conjugate fraction. A method for measuring intact 17-boldenone-sulphate has been developed and validated in accordance with current standards.

The analytical procedure included direct extraction–purification of the target analyte on octadecylsilyl cartridges, as well as direct detection of the phase II metabolite via liquid chromatography (negative electrospray), tandem mass spectrometry (QqQ), or high resolution mass spectrometry (OrbitrapTM). On the triple quadrupole, the decision limit (CC) and detection capability (CC) were 0.2 g L1 and 0.4 g L1 respectively, and 0.1 g L1 and 0.2 g L1 on the hybrid system. The method was successfully used to analyse incurred samples collected in various experiments. 17-Boldenone-sulphate levels were detectable up to 36 hours after boldione administration or 30 days after intramuscular injection of 17-boldenone undecylenate.

Keywords: NBoldenone • Undecylenate

Introduction

Given the similarity of the ring A structure of prednisolone and prednisone on the one hand and androstadienedione on the other, the transformation of cortisol and cortisone into prednisolone and prednisone in cattle faeces was investigated. A simple method was used, involving only a 1:100 dilution of cattle faeces, spiking with 400 ng/mL cortisol, cortisone, or cortisol glucuronide, and incubating the suspension. The HPLC-MS3 analysis was used to detect the alleged 1 dehydrogenation of the glucocorticoids.

Boldenone has been detected in a growing number of biological samples from various European Union Member States for several years. The question arose as to whether the increased number of boldenone discoveries was due to illegal animal treatment or whether, in some cases, boldenone could be of endogenous origin. Boldenone, for example, is formed from phytosterols found in vegetable fat. Due to the crises caused by bovine spongiform encephalopathy and polychlorobiphenyl, the substitution of animal fat (as beef tallow) in animal feed by vegetable fat may be significant in this regard. Phytosterol-enriched products are sometimes recommended as animal feed [1].

Boldenone (Bol) has frequently been the subject of discussion in residue analysis as an anabolic steroid with low androgenic activity. Boldenone (bBol), also known as 1-dehydrotestosterone, differs from the main circulating androgen-testosterone (bT) only by one double bond at the 1-position. Important steroids must be monitored carefully.

The bBol epimer -boldenone (aBol), androstadienedione (boldione, ADD), androstenedione (AED) are all related to bBol and bT . Bol is known to be administered to cattle to promote growth, owing to the structural similarity between Bol and testosterone (T).

The phenomenon of increased boldenone detection could also be linked to the increased analytical capabilities, such as better limits of detection (LODs), of European laboratories over time. This issue was investigated by several authors. The majority of the experience was gained about the presence or 'absence' of boldenone and metabolites in urine from cattle and veal calves. In comparison to other countries in the European Union, Belgium has extensive experience with the analysis of boldenone in bovine faeces samples [2].

Faeces samples positive for -Bol in routine screening also contained ADD and androst4-ene-3,17-dione (AED), but only some of these samples also contained -Bol. AED, which is both a precursor and a conversion product of the natural hormone testosterone, was also found in some faeces samples that tested negative for -Bol and ADD. Preliminary data show that oral administration of ADD to veal calves results in ADD, -Bol, and -Bol residues in urine, but oral administration of some phytosterols does not. Several studies are still being conducted to determine a link between the consumption of specific feed components such as phytosterols and the presence of boldenone and related substance residues in bovine sample matrices.

As a result, several laboratories have conducted and continue to conduct studies to identify specific metabolite markers (either phase I or phase II) that meet strict criteria such as always being detectable regardless of the boldenone form or route of administration and never being detectable in "so-called" endogenous cases. 6-Hydroxy-17-boldenone (androsta-1,4-diene-6,17-diol-3-one) has been identified as a potentially good candidate metabolite for signing boldenone administration. Nonetheless, this strategy based on a low abundance metabolite necessitates a highly sensitive approach to avoid erroneous false-positive conclusions. Van Poucke et al. recently described a new analytical strategy that allows the separation, detection, and quantification of several boldenone metabolites in calf urine to differentiate between endogenously formed and exogenously administered boldenone [3].

In terms of phase II metabolites, a group of European experts agreed that the presence of 17-boldenone conjugates in the urine of veal calves was unmistakably the mark of illegal treatment. Two additional papers. demonstrated the utility of 17-boldenone conjugates as definitive markers for distinguishing treated from untreated animals. The sulpho-conjugate fraction was thus found to be interesting because very few steroids were excreted in this manner, making the detection of 17-boldenone-sulphate in the corresponding extract highly specific. This fraction is also described as being very stable upon storage.

Following these preliminary findings, the current study focused on the development of a high-throughput protocol for the direct measurement of boldenone sulpho-conjugate metabolite. This novel methodology, based on LC–HRMS (linear and orbital trap) and/or LC-MS/MS (triple quadrupole), has demonstrated detection and identification of boldenone-sulphate at ultra-trace levels as low as 1 g L1.

This method has been validated in accordance with current European standards (2002/657/EC) and applied to the analysis of various urine samples collected from treated and non-treated animals. Following that, a kinetic of elimination was performed to assess the delay in detectability after treatment [4].

References

1. Schänzer, W., "Donike M. Metabolism of boldenone in man: gas chromatographic/mass spectrometric identification of urinary excreted

metabolites and determination of excretion rates." *Biological Mass Spectrometry*. 1992;21(1):3-16.

2. Buiarelli, F., et al. Determination of trenbolone and its metabolite in bovine fluids by liquid chromatography-tandem mass spectrometry. *J Chromatogr B*. 2003;784(1):1-5.
3. Popot, M.A., et al. First mass spectrometric detection of boldenone in horse mane samples. *Chromatographia*. 2003;57(3):255-260.
4. Arioli, F., et al. Evaluation of bolden formation and related steroids transformations in veal faeces by liquid chromatography/tandem mass spectrometry. Rapid Communications in Mass Spectrometry: Rapid Mass Spectrom. 2008;22(2):217-23.

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