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Liquid chromatography–tandem mass spectrometry analysis of 17α-trenbolone, 17β-trenbolone and trendione in airborne particulate matter

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ABSTRACT

Trenbolone acetate (TbA) is a potent synthetic anabolic steroid that was approved by the FDA as a growth promoter in beef cattle in 1987. Given the endocrine-modulating activity of TbA and its metabolites in all vertebrates, a sensitive and reliable analytical method is needed to detect TbA and related residues in environmental matrices. We have developed a method that incorporates solid phase extraction and liquid chromatography–tandem mass spectrometry (LC–MS/MS) for the simultaneous determination of the three major TbA metabolites (trendione, 17β-trenbolone, 17α-trenbolone) in total suspended particulate matter (TSP) samples. Sample preparation involved pressurized liquid extraction followed by cleanup on solid-phase extraction cartridges. The procedure was optimized to obtain maximum recovery and minimum signal suppression/enhancement from matrix effects. Analytes were separated with a Phenomenex Gemini-NX C18 analytical column (150 mm × 2.0 mm, 3 μm particle size) using an aqueous methanol gradient at a flow rate of 0.2 mL/min. Column effluent underwent positive electrospray ionization (ESI). Two or more diagnostic product ions were acquired from analyte specific precursor ions for unambiguous confirmation and quantification. The method detection limit was 3.27–4.87 ng/g of particulate matter (PM). Method accuracy, determined with analyte recoveries, ranged between 68% and 117%, and method precision, expressed as relative standard deviation, was below 15% at spiked levels of 6.67, 33.3, and 167 ng/g PM. Analysis of TSP samples demonstrated the presence of the target species associated with PM in the vicinity of beef cattle feeding operations.

1. Introduction

A wide variety of industrial chemicals and pesticides have been identified as endocrine-disrupting chemicals (EDCs) in recent years [1]. The endocrine-related activity of most EDCs is inadvertent: they were not synthesized with the purpose of altering endocrine-related physiological processes. In contrast, synthetic compounds designed for the specific purpose of modulating endocrine activity are used extensively in agriculture, and thus may be introduced into the environment.

Accelerated growth rates may be achieved by treating livestock with steroids. More than 90% of beef feedyard cattle receive some type of steroid growth promoting implant during their lifetime [2]. The synthetic androgenic steroid trenbolone acetate (TbA), approved by the FDA in 1987 [3], is one of the most widely used. Administered as a subcutaneous implant, TbA is hydrolyzed to the active form 17β-trenbolone (17βTb) [4]. In heifers, the primary metabolic route is oxidation of 17βTb to trendione (TbO) followed by a reduction to 17α-trenbolone (17αTb), with the majority excreted as 17αTb (Fig. 1) [4,5]. In a metabolism study using tritiated TbA in a heifer, TbO, 17βTb, and 17αTb were found as 0.9%, 0.9%, and 34.7%, respectively, of excreted radioactivity in the bile [4].

Trenbolone (TbOH) is inherently lipophilic and shares structural similarities related with all steroids. Log Kow of TbO, 17βTb, and 17αTb is 2.63, 3.08, and 2.72, respectively [6]. Log Koc for TbO, 17βTb, and 17αTb is 3.38, 3.08, and 2.77, respectively [6]. Trenbolone metabolites also have relatively low vapor pressures (VP) of 8×10^{-11} Torr and 7×10^{-10} Torr for 17βTb and 17αTb, respectively [3]. Given these properties, it can be concluded that TbOH metabolites will associate with organic matter in upper soil layers of feedyard pens. These compounds are also not likely to be found in gas phase, given the low VP of 17βTb and 17αTb. Because of these physicochemical characteristics, run-off has been considered the primary mechanism for offsite transport of TbOH and thus the majority of research to evaluate trenbolone in the environment has been limited to aquatic ecosystems [7–11]. In much of the US, however, beef cattle operations are located in semi arid to arid climates, with limited surface water and less frequent runoff events, limiting entry into aquatic ecosystems. Under these conditions, we hypothesized that TbOH could be transported via particulate matter (PM) generated from feedyards. PM transport could provide a pathway

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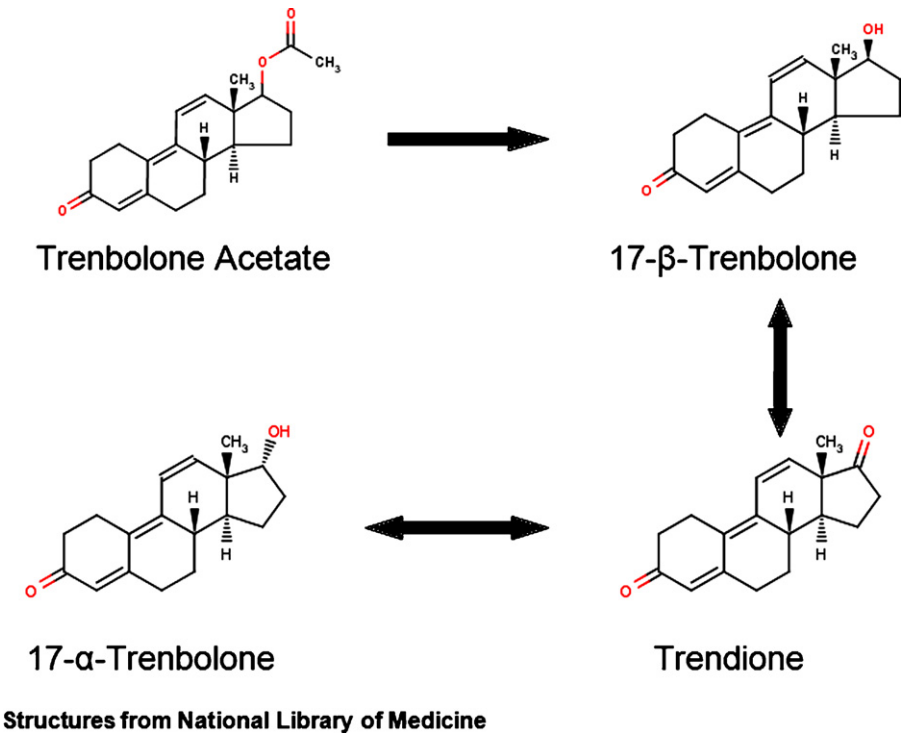

Fig. 1. Metabolism of trenbolone acetate (TbA) in the heifer.

of transport and subsequent TbOH exposure among human and/or ecological receptors, thus a sensitive analytical method was needed to detect and quantify TbO, 17βTb, and 17αTb in total suspended particulate matter (TSP) samples collected near beef cattle feedyards.

Analysis of TbOH from environmental matrices is difficult given the low, part per billion concentrations expected, and the complex, variable matrix feedyard PM provides. Available trenbolone immunoassays are designed for biological matrices and do not readily distinguish the individual trenbolone metabolites in complex environmental matrices [12–14]. Gas chromatography–mass spectrometry is a sensitive technique for analyzing hormones; however, it requires time consuming derivatizations, and not all hormones, including trenbolone, can be derivatized well [15–19]. Thus, liquid chromatography–tandem mass spectrometry (LC–MS/MS) was utilized as a more robust method of detection and quantitation. LC–MS/MS methods have been used to quantify TbOH in environmental matrices [6,20–22], but LC–MS/MS methods for trenbolone primarily address biological matrices [23–29]. While sharing characteristics of soil and cattle feces, PM from feedyards differs from each and provided its own challenges. Compared to other environmental or biological matrices, one unique characteristic of TSP samples is the presence of a filter on which the PM is collected. PM cannot be transferred from the filter without losing much of the sample, so the filter must be extracted as part of the whole. To address the challenges of this sample matrix, we have developed a specific method to simultaneously quantify TbO, 17βTb, and 17αTb in TSP samples using pressurized liquid extraction (PLE), solid-phase extraction (SPE), and LC–MS/MS.

2. Experimental

2.1. Chemicals and reagents

Steroid standards 17β-trenbolone (17β-hydroxyestra-4,9,11-triene-3-one, >99%) was purchased from Steraloids (Newport, RI, USA), and 17α-trenbolone (17α-hydroxyestra-4,9,11-triene-3-one, >98%) was from Cerilliant (Round Rock, TX, USA), while trendione (estra-4,9,11-trien-3,17-dione,) was synthesized and purified in-house (purity above 98.5% by LC–MS) according to a published procedure [22]. Deuterated internal standard (ISTD) 17β-trenbolone-d3 (17βTb-d3) was obtained from RIVM (Bilthoven, The Netherlands). Acetonitrile (ACN), acetone, methanol (MeOH), methyl tert-butyl ether, water, hexane, ethyl acetate (EtOAc), dichloromethane ($MeCl_2$) and ammonium formate (all HPLC grade) were obtained from VWR (West Chester, PA, USA). Formic acid (≥98%) and Florisil (6 mL, 1 g) cartridges were obtained from Sigma (St. Louis, MO, USA). Standard Ottawa sand (20–30 mesh) was from Fisher (Pittsburgh, PA, USA). Oasis hydrophilic–lipophilic balance (HLB) cartridges (6 mL, 500 mg) and Sep-Pak Vac Accell Plus quaternary ammonium (QMA) cartridges (6 mL, 1 g) were purchased from Waters (Milford, MA, USA). Regenerated cellulose syringe filters were obtained from Phenomenex (Torrance, CA, USA). Nanopure water was prepared with a Barnstead NANOpure Infinity UV system (Dubuque, IA, USA).

2.2. Standard solutions

Stock standard and ISTD solutions (1 mg/mL) were prepared in ACN and stored at −20 °C. Working standard solutions were prepared by dilution of the stock solution with methanol. Working standard solutions were stored at −20 °C.

2.3. Sample collection

Air sampling was conducted in the vicinity of feedlots in western Texas between April and August, 2009. TSP samples were collected using a Hi-Q CF-902-Digital Series Portable Air Sampler incorporated with four-inch FPAE-102 glass fiber filters (Hi-Q, San Diego, CA, USA). Filters were conditioned in a desiccator for a minimum of 24 h, then weighed. The volumetric flow rate of the air sampling unit was 0.62–0.99 m^3/min, and each unit recorded the total volume of air sampled. To obtain filter loading similar to masses used in method development (150 mg PM), collection