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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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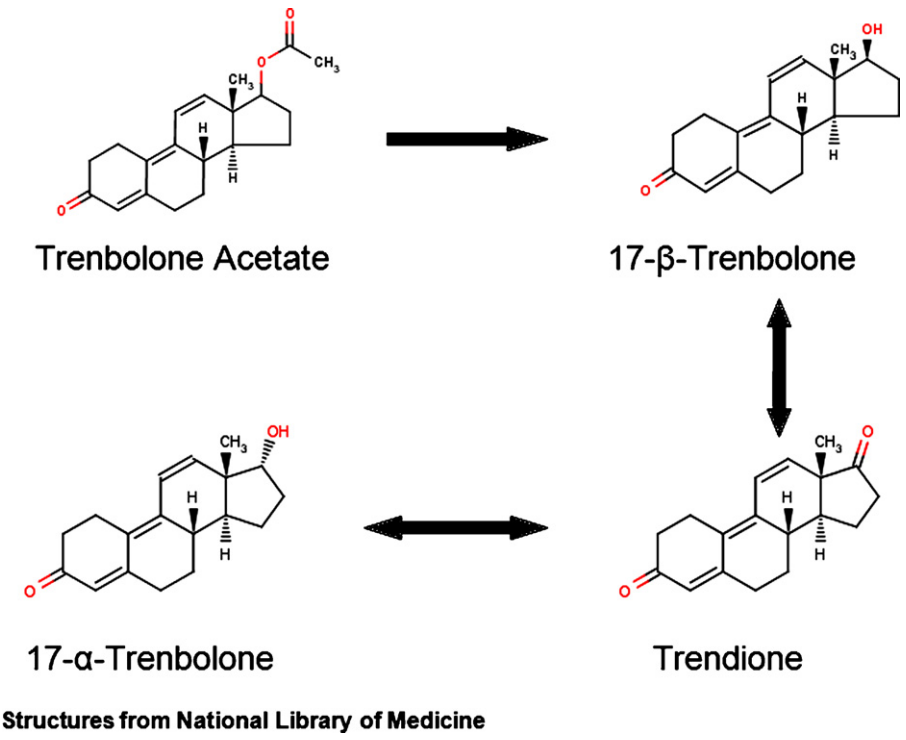

Fig. 1. Metabolism of trenbolone acetate (TbA) in the heifer.

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

proceeded for a period of approximately 30–90 min, depending upon the concentration of PM in the air. PM concentrations were highly variable, ranging from 420 to 22,000 μg/m^3, with a mean of 5200 ± 630 μg/m^3. Total masses collected ranged from 0.050 to 0.273 g with a mean of 0.122 ± 0.057 g. Samples were collected from fourteen individual feedyards, with a minimum of two samples from each feedyard. A total of 55 samples were collected. After collection, filters were transported to the lab in round metal tins obtained from SKS (Watervliet, NY, USA) and kept inside static resistant zip lock bags obtained from VWR (West Chester, PA, USA). Filters were reconditioned in a desiccator for a minimum of 24 h, weighed, and mass of PM determined. Samples were stored at −80 °C for a maximum of 2 weeks before proceeding to extraction.

2.4. Sample preparation

TSP samples underwent PLE followed by a series of SPE clean up prior to LC–MS analysis (Fig. 2). Each step is detailed below.

2.4.1. Isotope addition

Each PM-laden filter was spiked with 5 ng of 17βTb-d3 dissolved in 20 μL of ACN:water (50:50, v/v) to allow analyte quantification by isotope dilution procedures. Spiked filters were kept in a sealed vessel at room temperature for 10 min before proceeding to PLE.

2.4.2. PLE

Each PM-laden filter was rolled and placed lengthwise into a 33-mL cell. Void volume of the cell was filled with Ottawa sand. The extraction solvent (50:50 ACN:water, v/v) was used in the following program: pressure 1500 psi; temperature 60 °C, heat 5 min; static 5 min; flush 50%; purge 60 s; cycles 4. PLE was performed with an ASE-200 pressurized liquid extractor (Dionex, Sunnyvale, CA, USA). Extracts collected from the PLE underwent three SPE purification steps as described below.

2.4.3. Solid phase extraction for clean-up

QMA cartridges were used to remove anionic interferences. Inclusion of the QMA prior to HLB also prevented HLB cartridges from clogging due to unfiltered particles and anionic contaminants. QMA cartridges were conditioned with 5 mL of ACN:water (50:50, v/v). Each extract was directly added onto a QMA cartridge and the filtrate was collected. The flow was approximately 5 mL/min. QMA cartridges were rinsed with 6 mL of ACN:water (50:50, v/v). Each HLB cartridge was sequentially primed with 6 mL of methyl tert-butyl ether, MeOH, and MeOH:water (5:95, v/v). The filtrate from the QMA cartridge was diluted to 500 mL with nanopure water, loaded into a reservoir and passed through the HLB at a flow rate of 5 mL/min. This step allowed the isolation of steroidal compounds and removal of highly polar compounds. The HLB was subsequently washed with 6 mL of 2% formic acid in MeOH:water (5:95, v/v), followed by 6 mL of MeOH:water (5:95, v/v), and 6 mL of MeOH:water (50:50, v/v). The HLB cartridge was air dried with 20 mm Hg vacuum for 30 min before being eluted with 5 mL (2 × 2.5 mL) of acetone. This cleaned extract was evaporated to dryness under a nitrogen stream at 35 ± 5 °C. Each extract was re-suspended in 3 mL of hexane:$MeCl_2$ (60:40, v/v). Florisil cartridges were conditioned with 4 mL of hexane:$MeCl_2$ (60:40, v/v). The reconstituted extract from HLB clean-up was loaded onto the Florisil cartridge under gravity. The Florisil was necessary to remove contaminants that led to insolubilities in the final solution of MeOH:water (60:40, v/v). Each Florisil cartridge was washed with 4 mL of hexane:$MeCl_2$ (60:40, v/v) followed by 1 mL of EtOAc:hexane (60:40, v/v) to remove interfering compounds. Analytes were eluted from Florisil cartridges in 7 mL (3 × 2.33 mL) of EtOAc:hexane (60:40, v/v). This eluate was evaporated to dryness under nitrogen stream at 35 ± 5 °C. Extracts were reconstituted in 1 mL of MeOH:water (60:40, v/v), filtered through 0.2 μm regenerated cellulose syringe filter, and analyzed by LC–MS/MS.

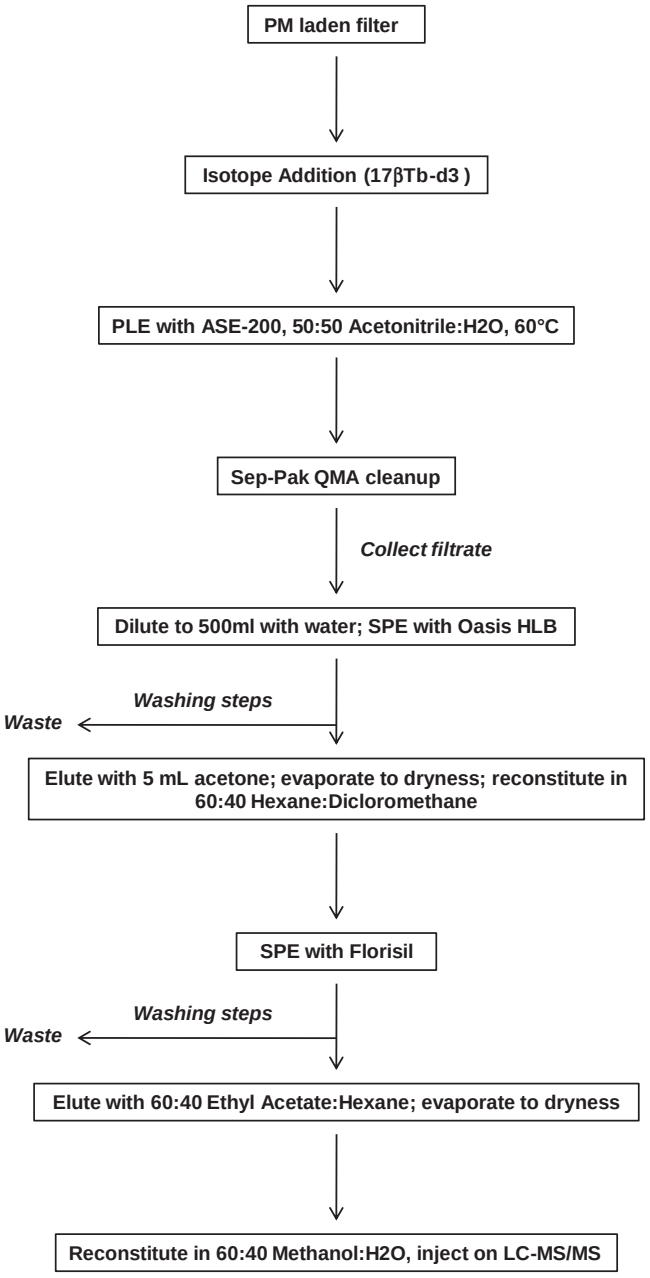

Fig. 2. Analytical procedure for the determination of trenbolone in particulate matter samples.

2.5. LC–MS/MS analysis

A Thermo Fisher LC–MS/MS system (San Jose, CA, USA) consisting of a Surveyor LC pump, Surveyor autosampler, a LCQ Advantage ion trap mass spectrometer, and a Xcalibur data system was used to identify and quantify steroids of interest. Reversed-phase LC was accomplished using a 150 mm × 2.0 mm Gemini-NX C18 column with a 3 μm particle size (Phenomenex, Torrance, CA, USA). Gradient separations were achieved at 40 °C with aqueous MeOH containing 2.0 mM of ammonium formate as the additive. The gradient began at 40% MeOH, increased to 100% MeOH over the time interval of 5–30 min. The gradient was held at 100% MeOH for 10 min, then the column was regenerated at 40% MeOH for 5 min

Table 1
Ion fragments used to identify and quantify trenbolone related steroids.

Analyte	Retention time (min)	Precursor ion (*m*/*z*)	Collision energy (%)	Product ions (*m*/*z*)	Ion ratios
Trendione	12.5	269.3	38	225.1, 251.2	100:27
17β-Trenbolone	14.1	271.3	36	199.1, 227.1, 253.2	15:18:100
17α-Trenbolone	16.9	271.3	36	199.1, 227.1, 253.2	9:7:100
17β-Trenbolone-d3 (ISTD)	14.0	274.3	36	199.1, 227.1, 256.2	15:18:100

Table 2
Essential optimized parameters for ESI-MS/MS analysis of trenbolone metabolites.

ESI source	Ion optics
Mode: positive ionization	Tube lens offset (V): 30.00
Source voltage (kV): 4.50	Multiple 1 offset (V): −2.00
Sheath gas flow rate (L/h): 80.00	Multiple 2 offset (V): −5.50
Auxiliary gas flow rate (L/h): 30.00	Multiple RF amplifier (Vp-p): 560.00
Capillary temperature (°C): 300	Inter multipole lens voltage (V): −16.00
Capillary voltage (V): 32.00	Trap dc offset voltage (V): −10.00

prior to the next injection. The flow rate was 0.2 mL/min and the injection volume was 25 μL. MS analyses were conducted with an electrospray (ESI) interface in positive-ionization mode. Nitrogen served as the sheath and auxiliary gas for the ion source. Product ions were generated from precursor ions by using helium as the damping gas (Table 1). The ESI-MS/MS was tuned by infusing standard steroids in 50% aqueous MeOH containing ammonium formate at 5 μL/min. Other important ESI-MS/MS operating conditions, including spray voltage, sheath gas flow, and capillary temperature, were optimized for the greatest product ion response using the program LCQ TunePlus (Table 2).

2.6. Method validation

In order to determine the accuracy and precision of the developed LC–MS/MS method, 150 mg of lab generated (LG) dust was collected onto a four-inch Hi-Q filter and spiked with 1.0 ng, 5.0 ng, or 25.0 ng of each analyte along with 5 ng of 17βTb-d3. To create LG dust, soil from a small scale experimental feedyard without trenbolone use was collected, sieved, and agitated to produce airborne PM, which was collected with the same samplers used in field studies. All filters were spiked with a 50 μL volume of different concentration solutions to achieve target spike concentrations. Spiked filters stood at room temperature for 10 min until all solvent was evaporated and were processed according to the sample preparation procedure and LC–MS/MS analysis described above. This was done on three different days with four to seven replicates (Table 3). Solvent blanks and filter matrix blanks were included in all validation sample sequences and subsequent environmental sample sequences.

3. Results and discussion

3.1. Extraction

Shaking, sonication, and PLE were compared to determine the most efficient extraction procedure. Due to the size of the glass fiber filters on which the PM was collected and their ability to retain solvent, a large volume of solvent (>50 mL) was required to fully wet the filter and allow for extraction in shaking and sonication techniques. PLE allowed for the use of less solvent and provided good efficiency for each compound, therefore PLE was chosen for extraction.

Based on methods for steroid extraction from soil and manure [14,22], MeOH was initially utilized as the extraction solvent, however because of the large 33 mL PLE extraction cell, the final extract was near 50 mL. The HLB SPE directly following extraction requires the sample to be diluted to ∼5% organic, thus samples had to be diluted to 1 L. To reduce the organic content of the extract, 1:1 ACN:water was compared to MeOH as the extraction solvent with satisfactory results, and 1:1 ACN:water was chosen as the extraction solvent.

3.2. Sample clean-up

During method development, use of the three separate SPE steps was deemed necessary to accomplish our goals. If HLB was used without prior QMA clean-up, extraction times for HLB cartridges ranged from approximately 1.5 h to over 6 h, as build up of organic contaminants and physical clogging of the cartridge slowed flow. This variability led to unequal flow rates through HLB cartridges and long delays in sample processing. Use of the QMA cartridge allowed sample uniformity and the ability to maintain similar flow rates and processing times for HLB extraction. After HLB extraction, if samples were reconstituted in mobile phase, excessive precipitates formed. With this amount of precipitate, syringe filtering was exceedingly difficult, and it was unknown what portion of target analytes could be associating with the precipitate opposed to the sample solvent. The use of the Florisil SPE reduced the formation of precipitates considerably and led to more uniformity from sample to sample. As such, the Florisil was also necessary for consistent sample clean up.

3.3. LC–MS/MS analysis

Linearity of the LC–MS/MS response was evaluated by making three separate calibration curves containing all three analytes in the concentration range 0.5–25 ng/mL and each containing 5 ng/mL of the ISTD 17βTb-d3. At least six calibration points were used to establish calibration curves, and each curve had a coefficient of determination greater than 0.99. Compound identification was based upon product/precursor ion ratios as well as congruence of retention times for each analyte in standard solutions and sample

Table 3
Performance of analytical method for steroid quantification in 150 mg particulate matter. Trials were performed on separate days (interday) or by separate technicians (interoperator). Recoveries were determined by comparing calculated concentrations. Recoveries are corrected for 17βTb-d3 response.

Spike Level	Trial	*N*	Analyte percent recovery (mean ± SD)		
			17αTb	17βTb	TbO
1 ng; 6.67 ng/g PM	1	5	97.4 ± 14.9	107.5 ± 9.4	93.2 ± 4.9
5 ng; 33.3 ng/g PM	1 (interday)	4	97.4 ± 12.1	84.1 ± 13.5	104.3 ± 13.9
	2 (interday)	7	100.2 ± 8.5	87.9 ± 12.4	98.5 ± 10.2
	3 (interoperator)	7	104.3 ± 3.0	94.1 ± 10.8	103.5 ± 8.27
25 ng; 167 ng/g PM	1	5	94.9 ± 10.4	107.8 ± 10.0	93.8 ± 8.2

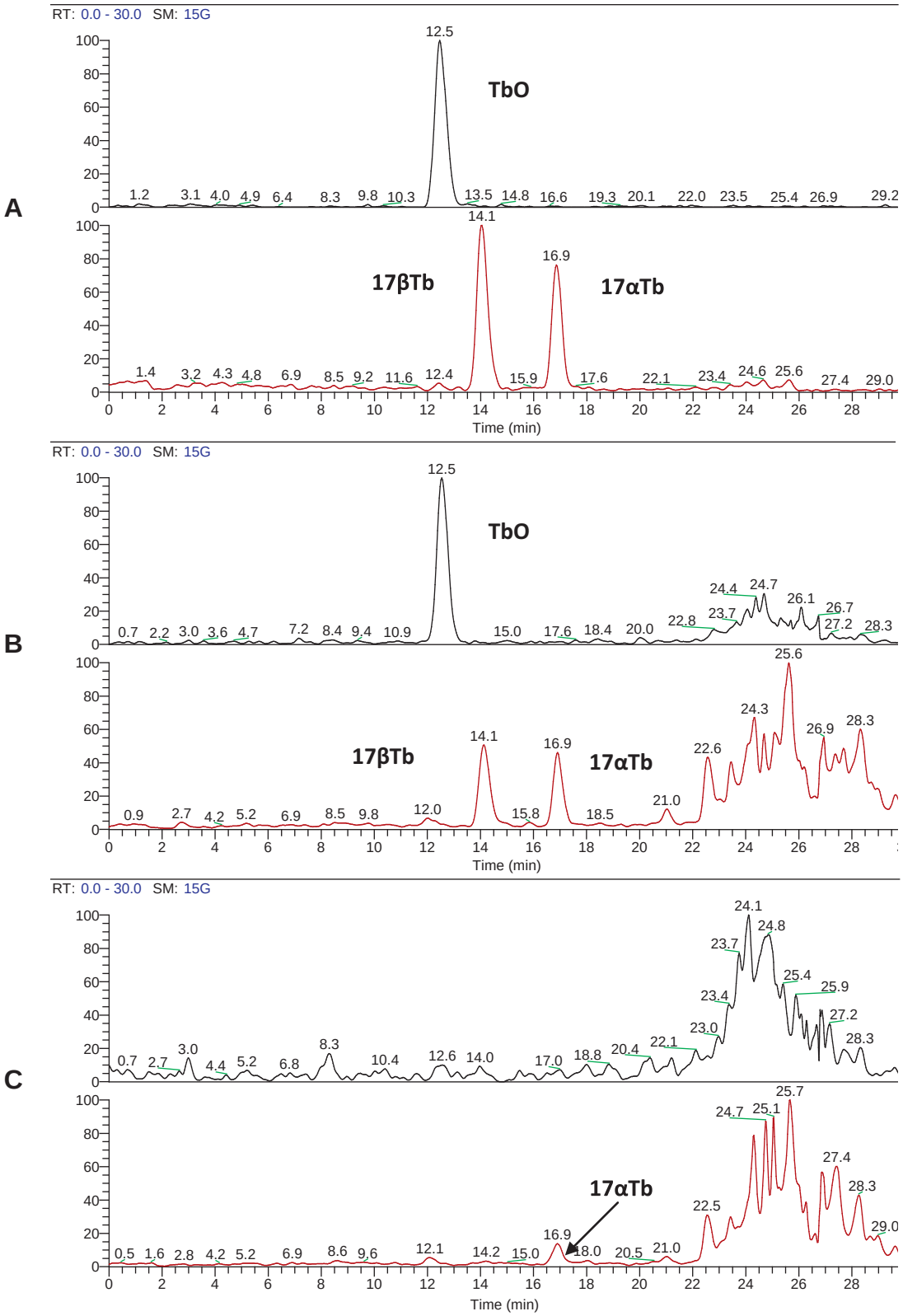

Fig. 3. LC–MS/MS analysis of: 200 pg trendione (TbO), 17β-trenbolone (17βTb), 17α-trenbolone (17αTb) in calibration standards (A); 25 μL injection of feedyard PM sample spiked with 33.3 ng/g TbO, 17βTb, 17αTb (B); 25 μL injection of feedyard PM sample containing 17αTb at a quantified concentration of 3.87 ng/g PM (C).

extracts. Good separations and sensitivity were achieved for the analytes of interest (Fig. 3).

Instrument detection limits (IDL) were determined by injecting progressively lower concentrations until a minimum signal:noise ratio of 3 was obtained. The IDL for TbO, 17βTb, and 17αTb was 0.5 ng/mL, 0.3 ng/mL, and 0.3 ng/mL, respectively. Limits of quantitation (2 × IDL) for TbO, 17βTb, and 17αTb were 1.0 ng/mL, 0.6 ng/mL, and 0.6 ng/mL, respectively. Method detection limits (MDL) for each analyte were determined from low concentration spikes onto 150 mg LG dust. MDLs, based upon a constant 150 mg

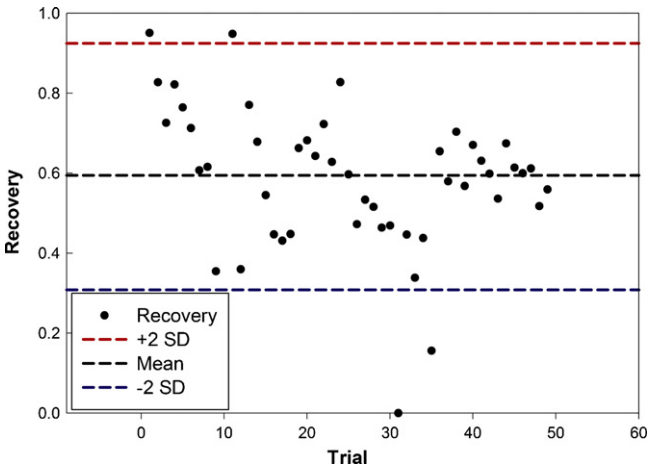

Fig. 4. Quality control chart of steroid 17βTb-d3 analyses performed over a six month time frame. Note that it is difficult to differentiate signal alteration due to recovery versus matrix effects.

PM, were 4.87 ng/g, 3.27 ng/g, and 3.47 ng/g for TbO, 17βTb, and 17αTb, respectively. It should be noted that environmental samples varied in PM mass, which had a direct impact on detection limits. Masses ranged from 0.050 to 0.273 g with a mean of 0.122 ± 0.057 g. Based on these collected masses, the detection limits ranged from 33 to 182% of the reported MDL, with a mean of 82 ± 38% the reported MDL.

3.4. Method validation

Extraction of PM-laden filters provided reproducible recoveries (Table 3) when analyte responses were corrected for 17βTb-d3 responses. Matrix effect depressed 17βTb-d3 responses to approximately 80% of expected responses in spiked LG samples. Since 17βTb-d3 was the only readily available dueterated compound of our three target compounds, it was used as the internal standard for all compounds. Based upon the data from LG dust spikes and spiked environmental samples, adjustment factors were determined for TbO and 17αTb to account for signal enhancement differences of the single internal standard. Adjustment factors were determined by comparing the slope of standard curves in blank solvent and matrix from 0.5 to 25 ng/mL performed in triplicate. Relative to 17βTb, adjustment factors for 17αTb and TbO were 115 ± 2.7% and 109 ± 7.9%, respectively, and reported concentrations of environmental samples are all corrected using these factors.

During analysis of field samples over a six month period, 17βTb-d3 responses were well controlled with a mean response 62.0 ± 18.1% in extracts from PM (Fig. 4). Within this distribution, 96% of responses fell between 30% and 101%. Any analyses falling outside 2SD of the mean recovery were evaluated for quality before inclusion. For example, samples 31 and 35 (Fig. 4) were omitted from field data analysis due to poor recovery of 17βTb-d3. These data demonstrate a method that can account for effects that a sample matrix may have on analytes of interest. After correcting each analyte recovery for 17βTb-d3 responses, we were able to reliably compute analyte concentrations in each PM sample.

Comparatively, Khan et al. [22] utilized soil microcosms to assess degradation of TbOH in agricultural soils. In their design, methanol extractions of spiked soil microcosms were followed by LC–MS/MS analysis. For spiked soils ranging from 0.04 to 10.0 mg/kg of applied TbOH, recoveries of 95–105% were reported. After correcting for 17βTb-d3, we experienced a similar level of recovery for each of the three analytes in the LG dust experiment (Table 3). While agricultural soils may offer a similar matrix to feedyard PM, our samples required a more extensive process of clean-up and concentration to detect target compounds via LC–MS/MS. Environmental PM samples exhibited a lower response of 17βTb-d3 than LG samples; however, as discussed, this response was well controlled and monitored (Fig. 4).

3.5. Analysis of feedyard PM samples

With this method, we determined that PM samples from 12 of 14 feedyards had TbOH concentrations above our MDL. 17αTb was the most commonly detected compound, detected in 45 of 55 samples from 12 of 14 feedyards at concentrations ranging from non-detect (ND)-26.5 ng/g PM with a mean of 4.88 ± 0.69 ng/g PM. TbO was found less frequently, being detected in 31 of 55 samples from 10 of 14 feedyards at concentrations ranging from ND-38.8 ng/g PM with a mean of 3.49 ± 0.74 ng/g PM. 17βTb was the least commonly quantified form of TbOH, being found in 25 of 55 samples from 6 of 14 feedyards at concentrations ranging ND-63.0 ng/g PM with a mean concentration of 3.45 ± 1.14 ng/g PM. For mean calculations, non-detects were given a value equal to one-half the MDL. Given the large number of non-detects for TbO and 17βTb, means are near or below the reported MDL. We realize more accurate methods such as maximum likelihood estimation could be utilized [30], but with the high percentage of non-detects, any method of mean calculation will have a substantial margin of error. In comparison to available literature, a previous study of trenbolone concentrations in solid dung found 17αTb ranging from 5 to 75 ng/g dung [5]. Our results compare well with this study, as TbOH concentrations in PM were expected be lower than concentrations in solid dung.

4. Conclusion

Determining the extent and magnitude of anabolic steroids in PM originating from beef cattle feedyards is a critical aspect of evaluating potential risks that these compounds may pose to human or ecological receptors. Successful implementation of this method will allow quantification of anabolic steroid content in PM, thereby providing data regarding exposure potential. Analytical determination of biologically active chemicals in the environment must include stable isotope internal standards, given the known modulation of ion production in LC–MS of extracts from environmental matrices [31]. It is difficult to predict the extent to which matrix effects suppressed ion production in each sample given the varied nature of samples that we collected. Further study of this phenomenon is warranted to better clarify the ionization behavior of TbOH isomers and TbO in LC–MS systems. The addition of 13-C labeled compounds, spiked prior to extraction, along with dueterated compounds spiked immediately prior to injection, would distinguish extraction efficiency from matrix effects; however, the acquisition of 13-C standards was beyond the financial scope of this project. It should be noted that spiking with isotopically labeled standards will confound assessment of androgenic or estrogenic potentials if PM extracts are used for biological assays. This could be alleviated by splitting filters before extraction. One portion could be used for residue analysis and the other for biological assays.

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