Bull Environ Contam Toxicol (2009) 82:415–418
DOI 10.1007/s00128-008-9589-0

Determination of Trenbolone Residual in Bovine Liver by Liquid Chromatography–Mass Spectrometry

Hai Wang · Zijian Wang · Bing Shao · Ziru Liu · SuYing Liu

Received: 21 December 2007 / Accepted: 9 October 2008 / Published online: 25 October 2008

Abstract A sensitive method was developed and validated for the quantitation of trenbolone residues in bovine liver. Target analytes were extracted from liver homogenate using solid phase cartridges, separated on a Phenyl column and detected using an electrospray ionization mass spectrometer operating in positive ion mode. The mean recovery of the analytes was between 62% and 69%. The method provided detection capabilities of 1 μg/kg for trenbolone. The method is suitable for application in veterinary drug residue in surveillance programme.

Keywords Trenbolone · LC–ESI–MS · Bovine liver · Residue

H. Wang · Z. Wang
State key laboratory of environmental aquatic chemistry, Research Center for Eco-Environmental Sciences, Chinese Academy of Science, Shuangqing Street, Haidian District, Beijing 100085, China

H. Wang (✉) · S. Liu
Quality control and inspection center for animal products, Ministry of agriculture, China animal disease control center, Maizidian Street, Chaoyang District, Beijing 100026, People's Republic of China
e-mail: cadc2008@126.com

B. Shao
Institute of Nutrition and Food Hygiene, Beijing Center for Disease Prevention and Control, Hepingli Zhongjie, Dongcheng District, Beijing 100013, China

Z. Liu
Beijing Research Institute of Chemical Engineering and Metallurgy, 145 Jiukeshu, Tongzhou District, P.O. Box 234, Beijing 101149, China

Trenbolone is a synthetic anabolic steroid used in animal husbandry practices as potent growth-promoter. Abuse of trenbolone can result in endocrine disruption (Kirkwood et al. 1985). Therefore there is need to detect and quantify its residue in various food matrices.

Analytical method for trenbolone was particularly challenging due to its high sensitivity and selectivity required. Radioimmunoassay (Hoffmann 1983), thin-layer chromatography (Laitem et al. 1978) and liquid chromatography with fluorescence detection (Naoki et al. 2000) had been applied to trenbolone detection. However these methods could not provide satisfactory sensitivity or they could easily provide false positive results. GC–MS is a sensitive technique for ammoassay of hormones, but it is time-consuming because it requires derivatization. Due to its structure (Fig. 1), trenbolone gives difficulties with most common derivatizing agents (Daeseleire et al. 1991). In comparison, LC–MS should be a promising technique for trenbolone residual analysis for its high selectivity, sensitivity and no need for derivatization. Liver is the recommended target matrix for the residue control of trenbolone in cattle. In this study, an LC–ESI–MS method to detect trenbolone in bovine liver was described.

Materials and Methods

Methanol, acetonitrile, hexane, chloroform, 1-propanol, and ethyl acetate were all pesticide residue grade from Fisher (New Jersey, USA). Formic acid (99%), acetic acid (99%) were from Acros Organics (New Jersey, USA). Water was purified using a Mill-Q system (Millipore, USA) trenbolone were purchased from Sigma (St.Louis, MO, USA). Sep-Pak silica and amino-propyl solid phase extraction cartridges (3 cc, 500 mg) were purchased from

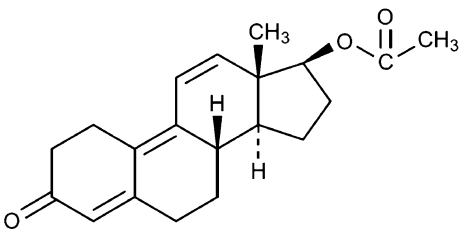

Fig. 1 Molecular structures of trenbolone

Waters Co. (Milford, MA, USA). Stock solutions were prepared for all standard substances at 1000 mg/L in methanol.

Ten grams of liver was weighed, and put into a 100 mL glass conical flask. Ten milliliters 0.2 mol/L acetate buffer (pH 5.2) was added and the samples were homogenized with an ultra turrax machine for about 1 min. About 35 mL methanol was added to homogenize the sample. Each mixture was centrifuged at 2000*g* for 10 min. The supernatant was decanted into a separatory funnel and extracted with 20 mL *n*-hexane twice to remove the parts of fat. The upper layer was discarded (*n*-hexane) and 5 mL 1-propanol was added to prevent foaming during evaporation. Methanol was evaporated at 50°C with a rotary evaporator. One hundred milliliters of water was added and the aqueous solution was subjected to solid phase extraction (SPE).

An HLB cartridge was conditioned sequentially with 6 mL methanol containing 50 mmol/L triethylamine, 6 mL methanol, and 6 mL water. The aqueous extract was applied to the cartridge at a flow rate of 3–4 mL/min. The glass reservoir and cartridge were rinsed with 2 × 4 mL water. The cartridge was dried with high purity nitrogen. The crude analyte was eluted with 10 mL methanol containing 50 mmol/L triethylamine. The eluate was dried under a gentle nitrogen stream. The residue was dissolved by ultrasonication for 30 s with 0.5 mL chloroform, and 5 mL *n*-hexane was added. The solution then was then passed through a Sep-Pak Silica solid phase extraction cartridge conditioning 6 mL *n*-hexane. Five milliliters of *n*-hexane was used to wash the interferences. The analyte was eluted sequentially with 6 mL water-saturated ethyl acetate. The eluate was dried under a gentle nitrogen stream, and the residue was redissolved with 2 mL methanol-ethyl acetate (40:60, v/v). The methanol-ethyl acetate solution was applied to amino-propyl solid phase extraction cartridges conditioned with 4 mL methanol-ethyl acetate (40:60, v/v) and 4 mL water-saturated ethyl acetate. The eluate was collected and another 2 mL methanol-ethyl acetate (40:60, v/v) was used to rinse the analytes. The eluate was dried under a gentle nitrogen stream. The residue was reconstituted with 0.5 mL mobile phase and mixed in a vortex stirrer.

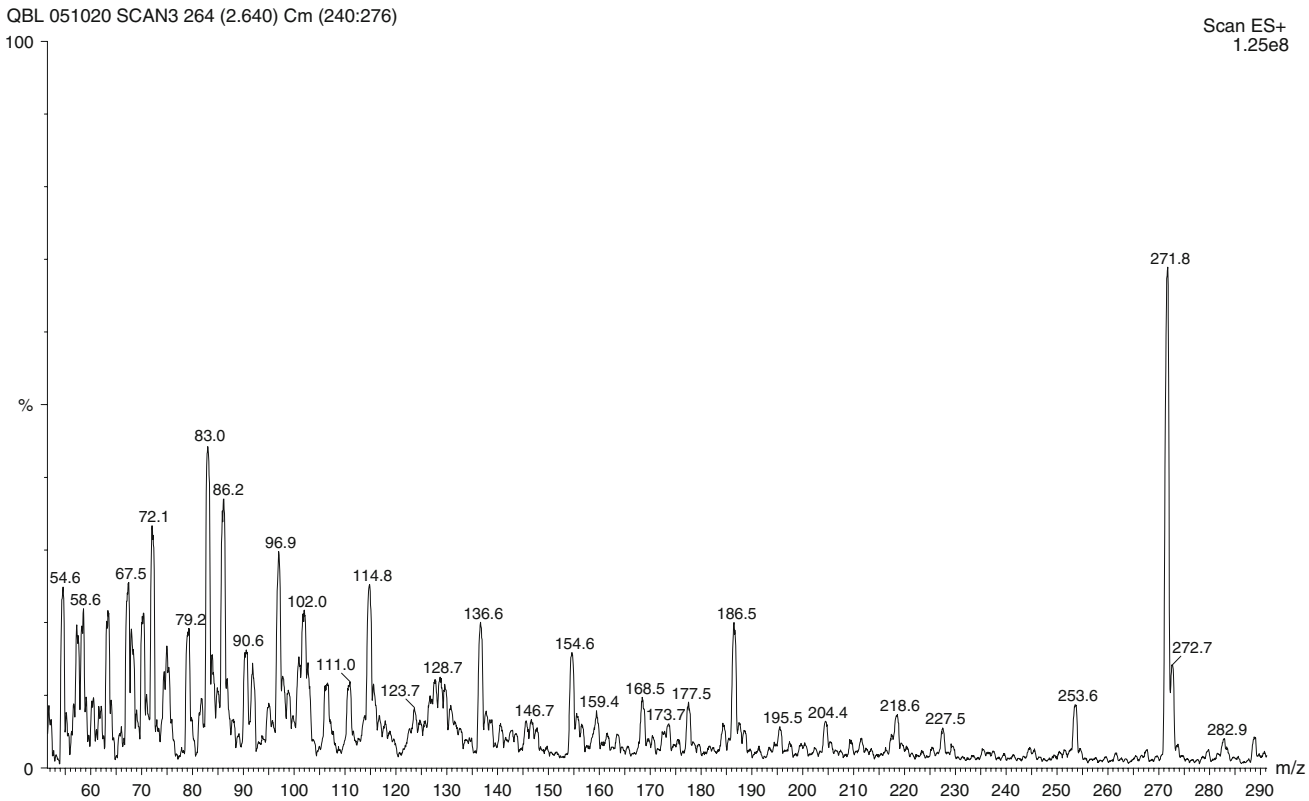

Fig. 2 Full scan spectra of trenbolone

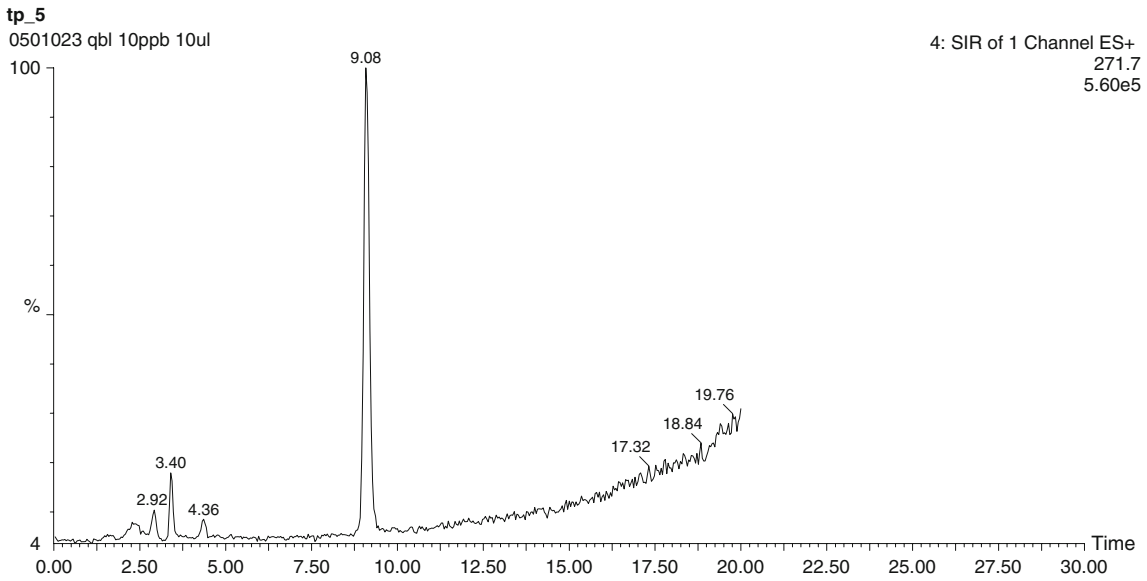

Fig. 3 LC–MS chromatogram of trenbolone standard solution (10 μg/L)

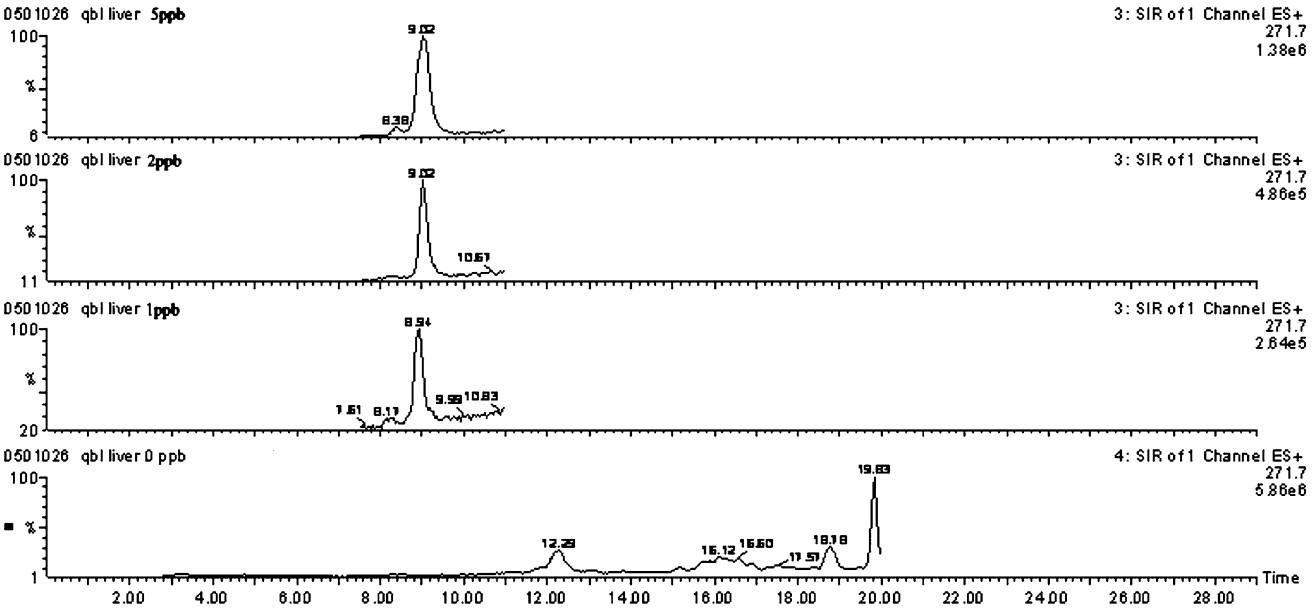

Fig. 4 LC–MS chromatograms of spiking liver sample containing 5, 2, 1, 0 μg/kg of trenbolone

The liquid chromatography mass spectrometer system consisted of a Waters Alliance 2695 LC system equipped with a Micromass (Manchester, UK) ZQ 4000 mass spectrometer. The mass spectrometer was operated in positive mode electrospray ionization. The capillary voltage was held at 2.8 kV. The cone voltage was 50 V. The multiplier voltage was 450 V. The nitrogen gas flow of the desolvation gas and cone gas were set to 450 and 0 l/h, respectively. The source temperature and desolvation gas temperature were held at 100 and 300°C.

Four most abundant product ions were monitored in SIM mode, they were 271.7, 199.0, 165.0 and 157.6, respectively. The CAPCELL PAK Phenyl column (250 mm × 2.0 mm i.d. 5 μm) was used for LC separation. The column temperature was 40°C, the flow rate was 0.2 mL/min, and the injection volume was 10 μL. Methanol and water were used as mobile phases. The initial composition was 35% of organic phase and 65% of water. After 3 min, the organic phase was programmed to linearly increase to 100% in 27 min and to hold for 5 min and then

Table 1 Intra-assay variation for the recovery of the analytes from bovine liver

Analyte	Fortification level μg/kg	Recovery (%)	
		Mean ± s ($n = 5$)	RSD
Trenbolone	1	65 ± 7.9	12.1
	2	67 ± 7.2	10.7
	5	72 ± 7.0	9.7

Table 2 Inter-assay variation for the recovery of the analytes from bovine liver

Analyte	Fortification level μg/kg	Recovery (%)	
		Mean ± s (n = 5)	RSD
Trenbolone	1	62 ± 8.5	13.7
	2	65 ± 7.5	11.5
	5	69 ± 7.3	10.6

decrease to the initial composition in 1 min. The column was then equilibrated for 20 min.

Results and Discussion

The method was found to be suitable for the determination of trenbolone (Figs. 2, 3, 4).

Linearity of the response was checked from a set of eight working standards ranging in concentration from 2.0 to 250 μg/L. Calibration curves were prepared by plotting the peak area versus the analyte concentration. Least-squares linear regression analysis was used to determine the slope. The curves were found to be linear over this range ($r^2 = 0.999$). The limits of detection was determined from representative blank samples. It was equal to three times signal-to-noise. The limit of quantitation for the method was 1 μg/kg. The accuracy and precision of the method were determined using liver samples fortified at levels of 1,2 and 5 μg/kg. Intra-assay variation was determined by analysing five samples within a single run, mean recovery of the analytes was between 65% and 72%. RSD were typically at less than 12.1% (Table 1). Inter-assay variation was determined by analysing samples on five different occasions to evaluate the run to run variation in the method. Mean recovery of analytes was between 62% and 69% with RSD at less than 13.7% (Table 2).

This method has been used to analyze 10 bovine liver samples available from local market. No trenbolone was detected among the samples.

A sensitive and reliable LC–ESI–MS method for the determination of trenbolone residue in bovine liver was described. This method avoided the most interferences in previous GC–MS caused by the derivatization. The limit of quantitation for the method was 1 μg/kg and suitable for inspection of its residue in animal products.

Acknowledgements Funding support from Natural Science Foundation of China (20621703) and the Foundation of Ministry of Science & Technology of China (2002BA906A75) are gratefully acknowledged.

References

Daeseleire E, De Guesquière A, Van Peteghem C (1991) Derivatization and gas chromatographic – mass spectrometric detection of anabolic steroid residues isolated from edible muscle tissues. J Chromatogr Biomed Appl 562:673–679. doi:10.1016/0378-4347(91)80617-L

Hoffmann B (1983) Use of radioimmunoassay procedures for the determination of sex hormones in animal tissues. J Steroid Biochem 19:947–951. doi:10.1016/0022-4731(83)90038-9

Kirkwood RN, Moller K, Smith WC, Lapwood KR, Garrick DJ (1985) The influence of allyl trenbolone (Regumate) on the timing, duration and endocrinology of parturition in sows. Anim Reprod Sci 9:163–171. doi:10.1016/0378-4320(85)90020-X

Laitem L, Gaspar P, Bello I (1978) Detection of trenbolone residues in meat and organs of slaughtered animals by thin-layer chromatography. J Chromatogr A 147:538–539. doi:10.1016/S0021-9673(00)85191-2

Naoki Y, Yumi A, Nobuyuki T (2000) Determination of α- and β-trenbolone in bovine muscle and liver by liquid chromatography with fluorescence detection. J Chromatogr B Biomed Sci Appl 739:363–367. doi:10.1016/S0378-4347(99)00557-5