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Simultaneous Determination of Hexoestrol, Diethylstilbestrol, Estrone and 17-Beta-estradiol in Feed by Gas Chromatography-mass Spectrometry

Huo Feng, Li Ning, and Lin Xiao-Li

Institute of Veterinary Drug and Food Control, Harbin 150010, China

Abstract: A method was developed for the simultaneous determination of four kinds of estrogens (hexoestrol, diethylstilbestrol, estrone, and 17-beta-estradiol) in feed by gas chromatography-mass spectrometry (GC-MS). After the sample was extracted by ethyl ether and cleaned-up on HLB phase extraction column, four kinds of estrogens were derived and quantified in gas chromatography-mass spectrometry. The results showed that the linear detectable ranged from 2.5 ng·mL^{-1} to 250 ng·mL^{-1} for hexoestrol and from 5 ng·mL^{-1} to 500 ng·mL^{-1} for three other estrogens with the correlation coefficients (R^2) were no less than 0.990. The recoveries were in the range of 76.34%-96.33% and the relative standard deviation was no more than 22.7%. The limits of quantitation (LOQ) for all analytics were between 10 ug·kg^{-1} and 20 ug·kg^{-1}. The method was accurate and sensitive and could meet the actual requirements for the analyses of feed samples.

Key words: gas chromatography-mass spectrometry, hexoestrol, diethylstilbestrol, estrone, 17-beta-estradiol, feed

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Introduction

In recent years, food safety problems have become increasingly prominent in China. In the food safety problems, the residues of estrogen in animal food are thought to be a new problem and now drawing the attention from the government. Feed is the main way that livestock ingestes of exogenous estrogens. Their continual but undetectable effects can accumulate slowly, and finally lead to irreversible change on livestock and human beings. If human have consumed of the animal food for a long time, they would have diseases such as precocious puberty, abnormal second feature, cancers and tumors (Wang *et al*., 2011; Hashemi and Davoodi, 2010; Hernandez *et al*., 2004). Therefore, the quality of feed is the important basis for protection of human food safety. The government documents clearly prohibite from using the estrogenic drugs into the feed in order to promote rapid growth of animals and other related purposes. However, very few studies about the determination of estrogens in the feed in China had been reported. Generally, previous studies only focused on one specific class (Florou-Paneri *et al*., 2006; Aksit *et al*., 2006; Basmacioglu *et al*., 2004), probably due to the challenges for simultaneous analyses of different estrogenic compounds with different physicochemical characteristics. There were many methods for the residues of the estrogens in animal food and feed including high performance liquid chromatography (HPLC) (Charles and Huang, 2009; Chen *et al*., 2004), gas chromatography (GC) (Gao *et al*., 2001; Giannenas *et al*., 2005), and enzyme-linked immunosorbent assay (ELISA) (Govaris *et al*., 2004). However, those methods officially issued in China are not suitable for simultaneously qualitative

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Huo Feng (1967-), male, Master, senior engineer, engaged in the research of feed and veterinary detection. E-mail: 13845101908@163.com

and quantitative determination of multiple estrogens in feed. The gas chromatography-mass spectrometry (GC-MS) is the most suitable method because of its specificity and selectivity (Guo and Lin, 2003) . To our best knowledge, it is the first study on the occurrence of four estrogens in the feed in China. It developed a sensitive and highly specific analytical method for simultaneous determination of four kinds of estrogens by gas chromatography-mass spectrometry.

Materials and Methods

Materials

All the standards were of chromatography grade (>95%), and purchased from Dr. Ehrenstorfer in Germany. BSTFA with 1% TMCS (CFCQ-270121) was provided by Supelco (Bellefonte, PA. USA) HPLC grade methanol, acetonitrile, acetone were provided by Dikma (USA) ammonia, ammonium acetate, hydrochloric acid, ethyl acetate, n-hexane, diethyl ether, chloroform, sodium hydroxide (analytical grade)

Main instruments

Gas chromatography-mass spectrometry was provided by Agilent Company in USA, AB204-N Electronic analytical balance and AB265-S Electronic analytical balance were purchased from Metter Toledo in Germany. VELOCITY 18R high-speed refrigerated centrifuges were obtained from Jouan Company in Australia. N-EVAP-24 blowing nitrogen concentration apparatus was produced by Organization Company in USA.

Methods

Sample extraction: 10 mL ammonium acetate buffer solution (0.2 moL·L^{-1}) was added into 50 mL polypropylene centrifuge tube containing 5 g feed sample, mixed with 1 min. In each tube, 10 mL ethyl ether was added. The samples were capped, vigorously shaken for 1 min, and then centrifuged at 10 000 r·min^{-1} for 5 min. The ether layer was drawn into another tube and evaporated to dryness on the rotary evaporator at 30℃. We added 5 mL chloroform into residue, mixed with 1min, added 5 mL sodium hydroxide solution (1 moL·L^{-1}), vortexed for 1 min, and then centrifuged at 10 000 r·min^{-1} for 5 min, the upper layer was transferred to another tube. Then added 1 mL methanol into the tube and adjusted pH of samples to 6-6.5 with 1 mol·L^{-1} hydrochloric acid.

First, SPE cartridge was conditioned with 5 mL ethyl acetate, 5 mL methanol and 5 mL hydrochloric acid (pH=3) into HLB SPE column. Then, the sample was introduced to the tube, at a flow rate of 2-5 mL·min^{-1}. After being washed with 5 mL water-methanol (9 : 1, v/v) and 5 mL hexane, the cartridge was dried under vacuum and eluted with 5 mL methanol, at a flow rate of 1-2 mL·min^{-1}. The eluted solution was collected and dried with nitrogen at 50℃.

Derivatization: an aliquot of 100 μL BSTFA with 1% TMCS was added to the sample, vortexed for 1 min, and then incubated at 70℃ for 60 min. After dried with nitrogen, 200 μL isooctane was added to dissolve the residue.

Analytical standards: the stock solution of hexoestrol (0.5 mg·mL^{-1}) was prepared by dissolving 12.5 mg hexoestrol into 25 mL methanol. Similarly, the stock solution of diethylstilbestrol, 17-beta-estradiol and estrone (1 mg·mL^{-1}), was prepared by dissolving 25 mg standards into 25 mL methanol. The multi-component standard solution was prepared by diluting the stock solution of hexoestrol (0.5 mg·mL^{-1}) into 2.5, 5, 12.5, 25, 50, and 250 ng·mL^{-1}, separately, diluting three compounds of the stock solution into 10, 25, 50, 100, and 500 g·mL^{-1}.

Derivatization of standard solution

Drawing 100 uL mixed standard solutions and drying on the rotary evaporator at 50℃, derivative sample was treated with the same measurement on the instruments.

Instrument conditions

GC conditions

High purity helium (>99.999%) was used as a carrier