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Quantitative determination of 26 steroids in eggs from various species using liquid chromatography–triple quadrupole-mass spectrometry

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Abstract

A method for analyzing 26 types of steroids in egg matrix was developed. The method used liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) in electrospray ionization mode (ESI). The procedure involved extraction with acetonitrile and removal of phospholipids by zinc chloride (ZnCl_2) followed by SPE cleanup with a Plexa cartridge. The effect of ZnCl_2 on phospholipid removal was directly observed using the post column infusion procedure. The SPE washing and elution conditions were optimized using a shallow gradient procedure. The free and conjugated steroids forms were determined using enzyme hydrolysis. The developed method resulted in satisfactory precision ($\text{RSD} \leq 15\%$), and the limits of quantification were between 0.05 and 25 ng/g depending on the steroid types. The recoveries ranged from 63.2% to 121.5%. Finally, the developed method was successfully applied to compare the steroids in eggs from different species (i.e., hen, duck, quail and pigeon eggs) or different raising system (i.e., normal vs. organic eggs). The steroids can be clearly clustered according to species and raising system. The hierarchical clustering analysis indicated similarity of the steroids among the species. The developed method is sensitive and useful for detection and quantification of steroids in eggs and can be used for residue control programs. In addition, the observed steroid content will provide a fundamental reference for food risk assessment analysis.

Keywords: Steroids; Eggs; Liquid chromatography-tandem mass spectrometry; Principal component analysis

1. Introduction

Steroidal hormones control the physiological functions of mammals. These hormones act as body chemical messengers that are transported in the blood and activate signal transduction via binding to androgen, estrogen, or corticosteroid receptors. In addition, these hormones are vital to normal development, maturation and cell senescence of the body. However, the toxic effect of steroidal hormones has been well documented for human exposure to certain circumstances or excessive amounts. Animal products are a major source of human exposure to steroids. Because steroidal hormones can increase the performance of food-producing animals [1, 2], many steroidal hormones as well as synthesized steroids have been illegally used in the animal husbandry industry. The occurrence of steroidal hormone residue in animal products negatively influences human health. Therefore, monitoring of the illicit use of hormones has been performed by many countries' national

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