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Analytical Methods

Simultaneous determination of ten steroid hormones in animal origin food by matrix solid-phase dispersion and liquid chromatography–electrospray tandem mass spectrometry

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ABSTRACT

An UPLC–MS/MS method for determination of ten steroid hormones in animal origin food has been developed with pretreatment of the samples by matrix solid-phase dispersion (MSPD). The MSPD conditions, including the dispersing sorbents, elution solvents, ratio of sorbent to sample and the volume of the elution solvent have been investigated and optimised, and the method has been evaluated and validated. The results showed that the developed method has satisfactory linearity between the MS/MS responses of the analytes and the concentration of the steroid hormones, and the limits of the detection can reach 0.01 μg/kg for most of the analytes. The spiking recoveries of the steroid hormones in chicken, pork, beef and sausage samples were between 76.8% and 98.7% with RSDs lower than 10%. The results demonstrated that the developed approach has high sensitivity and repeatability, and can rapidly determinate the trace residues of steroid hormones in complex food matrices.

1. Introduction

Steroid hormones are endocrine-disrupting chemicals with biological activity, and they have been illegally abused in animal husbandry for several decades due to their effects on the improvement in feed conversion efficiency and growth promotion. However, many evidences have indicated that the steroid hormones in animal origin food have potential toxic and carcinogenic on human health, and their residues, as well as their metabolites, may be relevant to a variety of diseases including cancer of the breasts, ovaries and prostate (Krieger, 2008), therefore, the illicit uses of these compounds have been restricted and banned in European and many other countries. So development of sensitive and reliable analytical methods for monitoring the residuals of steroid hormones in foodstuffs is particularly required.

Immunoassays have been traditionally used for the determination of steroid hormones (Hampl & Starka, 1989), whereas they lacked the function of structural validation for the target analytes (Sokoll, Wians, & Remaley, 2004) and cannot simultaneously determine multi-residues in complex matrices. Gas chromatography coupled to mass spectrometry (GC–MS) has been early adopted for the quantitative determination of steroid hormone residues in foodstuffs (Daeseleire, Vandeputte, & Van Peteghem, 1998). Unfortunately, this technique generally requires pre-derivatization processes for the analytes (Trinh, Harden, Coleman, & Khan, 2011), which would be tedious, time consuming, and moreover, not all components can be easily derivatized. High performance liquid chromatography (HPLC) combined with various mass spectrometric techniques, including reactive Desorption Electrospray ionization (Huang, Chen, Zhang, Cooks, & Ouyang, 2007), atmospheric pressure chemical ionisation and atmospheric pressure photoionization (Leinonen, Kuuranne, & Kostiainen, 2002), accurate mass time-of-flight, Fourier transform ion cyclotron resonance (Nielen, van Engelen, Zuiderent, & Ramaker, 2007; Song, Li, Zeng, Liu, & Xie, 2011), linear ion trap mass spectrometry (Strahm, Saudan, Sottas, Mangin, & Saugy, 2007), have been tried to determine the residues of steroid hormones in complex food samples, especially, the HPLC-electrospray ionisation tandem mass spectrometry (Blasco, Van Poucke, & Van Peteghem, 2007; Farke, Rattenberger, Roiger, & Meyer, 2011; Guedes-Alonso, Sosa-Ferrera, & Santana-Rodriguez, 2013; Penning, Lee, Jin, Gutierrez, & Blair, 2010; Regal, Vazquez, Franco, Cepeda, & Fente, 2009; Yang, Shao, Zhang, Wu, & Duan, 2009) has been extensively utilised over the past decade due to its excellent sensitivity, high selectivity and specificity. However, most of these approaches need multiple-steps sample pretreatment procedures, which would be more complex and time-consuming, and some techniques were more suitable for screening and identification of the target analytes, while their linear dynamic ranges were narrow for quantitatively determination of the steroid hormones.

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Sample pretreatment procedures play a vital role for determining trace residues of target analytes in complex matrices (Zhang, Liu, Xie, & Qiu, 2005). Liquid–solid extraction (Shao et al., 2005) and liquid–liquid extraction (Alnouti et al., 2005) have been generally employed as the preliminary step before purification and enrichment of multi-residue hormones by solid phase extraction (SPE). In order to improve the efficiency of extraction, accelerated solvent extraction (Hooijerink, van Bennekom, & Nielen, 2003), microwave-assisted extraction and supercritical fluid extraction (Tomsikova et al., 2012) were sometimes attempted. However, these procedures consumed large volumes of organic solvents, which were not environmental friendly. Some novel sample pretreatment techniques have been investigated recently for improving the selectivity and effects of purification and enrichment for steroid hormones, such as solid-phase microextraction (Aufartova et al., 2011), and molecular imprinted polymers (Baggiani, Baravalle, Giovannoli, Anfossi, & Giraudi, 2010; Yin et al., 2012; Zhang & Hu, 2010) as the sorbents of SPE procedures. On-line solid-phase extraction coupled to HPLC–MS/MS can also simplify the sample pretreatment procedures (Guo et al., 2013; Snow, Damon-Powell, Onanong, & Cassada, 2013), which were more suitable for liquid samples.

Matrix solid-phase dispersion (MSPD) can combine the steps of homogenisation, disruption, extraction and purification into one procedure (Barker, 2000; Zou, Liu, Xie, Han, & Zhang, 2005), and has been proven to be an effective technique for sample pretreatment of various biological systems ranged from solid, semi-solid to highly viscous samples. As a beneficial alternative to other approaches, MSPD has been successfully applied to the analysis of pesticides (Garcia-Rodriguez, Cela-Torrijos, Lorenzo-Ferreira, & Carro-Diaz, 2012; Rallis, Sakkas, Boumba, Vougiouklakis, & Albanis, 2012), veterinary drug (Lu et al., 2012), and other pollutants (Garcia-Mayor, Gallego-Pico, Garcinuno, Fernandez-Hernando, & Durand-Alegria, 2012; Pavlovic, Perisa, & Babic, 2012; Zou et al., 2008) from various complex matrices.

In the present work, MSPD with ultra performance liquid chromatography–tandem mass spectrometry (UPLC–MS/MS) was applied to the extraction and determination of 10 steroid hormones residues in food matrices. Several sample pretreatment parameters, including the sorbent of MSPD, the rinsing and eluting solvents, and the conditions for separation and determination of the steroid hormones have been optimised. After verification, the optimised approach has been evaluated to the simultaneous detection of the target steroids in real samples. The developed method can rapidly determine the residues of steroid hormones in complex matrices with high sensitivity and reproducibility.

2. Experimental

2.1. Chemicals and apparatus

The ten steroid hormones: Testosterone was purchased from Acros Organics (New Jersey, USA). 4-Androstene-3,17-dione, Stanozolol, 17α-Hydroxyprogesterone, Prednisone, Prednisolone were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Megestrol acetate and Medroxyprogesterone Acetate were from TCI Chemicals (Shanghai, China). Progesterone and Hydrocortisone were provided by Aladdin Chemistry. Co., Ltd. (Shanghai, China). The chemical structures of the above compounds are presented in Fig. S1.

Ethyl acetate, n-hexane, methylene dichloride were of HPLC-reagent grade, and provided by Sinopharm Chemical Reagent Beijing Co., Ltd. (Beijing, China). Silica gel (40–60 μm) and C18 (40–60 μm) were obtained from Merck (Darmstadt, Germany), and Florisil (40–60 μm) was obtained from J&K Chemical. Ltd. (Beijing, China). Doubly deionized water (DDW, 18 MΩ/cm) obtained from Millipore Milli-Q purification system (Barnstead Corp., Boston, USA) was used throughout the experiments. HPLC grade methanol and acetonitrile were obtained from Merck (Darmstadt, Germany). Formic acid (purity >99.0%) was obtained from Waters (USA) and all the other reagents used were analytical grade.

The SPE cartridge (6 mL) and sieve plates were purchased from Jiruisen (Beijing, China). Separation was carried out using a solid-phase extraction equipment, 12 port vacuum manifolds (Supelco, Bellefonte, PA, USA). EVA 30A Nitrogen blowing instrument (Polytech. Co. Ltd. Beijing, China) was used for concentration.

2.2. Preparation of standard stock solutions and samples of animal origin food

Standard stock solutions of the ten compound at 1.00 mg/mL were prepared by dissolving individual target in 10.0 mL acetonitrile–water (1:1, v/v) and stored at 4 °C in refrigerator. Working solutions (0.100 ng/mL–1.00 μg/mL) were obtained by subsequent dilution of the stock solution with acetonitrile–water (1:1, v/v). Tuning solutions (100 ng/mL) were freshly prepared in acetonitrile–water (1:1, v/v) containing 0.1% formic acid.

The blank or negative animal origin food samples (chicken, pork, beef and sausage) used for method optimisation and development were provided by Chinese Academy of Inspection and Quarantine. A representative portion of the samples (about 200 g) was homogenised and stored at 4 °C for analysis.

2.3. LC/MS/MS conditions

Identification and quantification of analytes were performed on an Acquity UPLC system equipped with a Xevo TQ MS tandem mass spectrometer (Waters Co., USA).The Acquity UPLC BEH C18 column (1.7 μm, 50 × 2.1 mm) was used for the LC separation. The column oven was set to 40 °C, and the flow rate of mobile phase was 0.3 mL/min, and the injection volume was 10 μL, using full loop mode for sample injection. Acetonitrile (A) /water containing 0.1% formic acid (B) were used as mobile phase. For separation of the ten steroid hormones, a linear gradient was applied in the following manner: the initial composition of the mobile phase was 90% B (v/v), and decreased to 10% B in 5 min, hold in 10% B for 1 min, and then increase from 10% to 90% B in 0.2 min) and stabilized in 90% B for another 1 min.

The mass spectrometer was operated in positive electrospray ionization using multiple reaction monitoring (MRM) mode. Nitrogen was used as the nebulizing, desolvation and cone gas. The capillary voltage was held at 3.7 KV, the flow of the desolvation gas was 650 L/h. The source temperature and desolvation gas temperature were set at 150 and 350 °C, respectively. Instrument operation and data acquisition were processed using Waters MassLynx software. The mass transitions and collision energy used for determination of the ten steroid hormones, and their quantitative ion pair (parent and daughter ions) were listed in Table 1. The precisions of the instrument for the ten analytes were below 1.0% (RSDs, $n = 6$).

2.4. Optimisation of the matrix solid-phase dispersion procedures

The blank or negative animal tissue samples (chicken) were used for optimisation of the MSPD procedures, including the factors of dispersing agents, the ratio of sorbent to sample, elution solvent and the volume of the elution solvent.

The spiking level of each steroid hormone was 100 μg/kg, and the recovery of each analyte was obtained by ratio for the peak area of the analyte to that for the corresponding standard concentration of the analyte.