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Determination of amantadine and rimantadine in chicken muscle by QuEChERS pretreatment method and UHPLC coupled with LTQ Orbitrap mass spectrometry

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ABSTRACT

A novel sample pretreatment method was developed for the quantitative determination of amantadine and rimantadine in chicken muscle tissues by ultra high performance liquid chromatography coupled with high resolution LTQ Orbitrap mass spectrometry (UHPLC-LTQ Orbitrap MS). The samples were pretreated by modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method, using acetonitrile (1% acetic acid, v/v) as extraction solution and C_{18} sorbent for clean-up. The separation was carried out on a Waters ACQUITY UPLC HSS T3 column (150 mm × 2.1 mm, 1.8 μm particle), using a mobile phase of acetonitrile and 0.1% aqueous formic acid solution. LTQ Orbitrap MS with resolving power of 60 000 was applied for analysis of the samples. Amantadine and rimantadine were identified from their accurate mass (within 5 ppm) and retention times from the acquired full-scan chromatogram and quantified by their peak areas. The linear range for the determination of the analytes was 1–100 μg/kg. Limits of detection (LODs) for amantadine and rimantadine were 1.02 μg/kg and 0.67 μg/kg, respectively. The intra- and inter-day accuracy ranged from 87.5% to 102.4%, and 82.5% to 105.8% for amantadine, and 95.3% to 97.4%, and 89.4% to 93.2% for rimantadine, respectively. The precision of intra- and inter-day was between 3.9–6.3% and 5.95–13.9% for amantadine, 6.0–7.45% and 7.8–12.4% for rimantadine, respectively. Finally, the method was applied for the determination of these antiviral agents in routine samples, and amantadine residue was detected in some cases.

1. Introduction

Amantadine and rimantadine are antiviral agents used for the treatments of influenza A virus infections [1,2]. Due to the potential resistance to these compounds for human beings [3], amantadine and rimantadine have been banned as antiviral agents during poultry farming in many countries including USA [4] and China since 2005. Nevertheless, these antiviral drugs are still illegally used for the treatment of avian influenza in poultry farming (especially in chicken farming) in China occasionally [5]. Therefore, it is necessary to establish a sensitive method for the analysis of these antiviral agents in chicken muscle tissues.

Up to now, different methods have been published for the determination of amantadine and rimantadine in biological fluids samples such as plasma and urine [6–15], including gas chromatography with electron-capture detection (GC-ECD) [6], gas chromatography with mass spectrometry (GC–MS) [7], high-performance liquid chromatography with fluorescence detection (HPLC-Flu) [8–12], HPLC with UV detection (HPLC-UV) [13], capillary zone electrophoresis [14] and HPLC coupled with mass spectrometry (HPLC-MS) [15]. As these two compounds have no prominent UV absorption and fluorescence properties, it is usually required derivatization to increase its sensitivity by HPLC-UV and HPLC-Flu. Compared with HPLC-UV and HPLC-Flu, HPLC-MS has the advantage of simple sample treatment without derivatization and high sensitivity and selectivity. HPLC coupled to triple quadrupole mass spectrometry (QQQ) is currently widely used in residue analysis. A number of compounds can be analyzed by QQQ under selected reaction monitoring (SRM) mode [16–19]. When applying SRM mode, at least two transitions are required because monitoring only one transition might result in false positive identifications [20]. The application of SRM is limited for some cases when analyte ions with low molecular weight and with only one transition. High resolution mass spectrometry (HRMS) such as TOF MS

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and LTQ Orbitrap MS provides higher resolution, higher sensitivity, and higher scan speed than QQQ. For LTQ Orbitrap, excellent selectivity could be achieved with resolution up to 100 000, by which the target analytes could be easily distinguished from interferences of matrix backgrounds [21]. More recently, TOF MS and LTQ Orbitrap MS have been increasingly utilized in food analysis [20]. However, there were few reports about determination amantadine and rimantadine in chicken muscle using HRMS.

The samples were biological fluids in normal such as plasma and urine owing to the most studies was focused on the pharmacokinetic of amantadine and rimantadine. Liquid–liquid extraction and solid-phase extraction were commonly used for sample pretreatment in plasma and urine. However, these pretreatment methods are not suitable for chicken muscle tissues. The QuEChERS method (acronym for Quick, Easy, Cheap, Effective, Rugged and Safe), is a promising sample preparation method for the determination of residues in different food matrices [22,23]. QuEChERS method is based on a single extraction with acetonitrile followed by partitioning with salt and purifying the extract using dispersive solid-phase extraction (d-SPE). d-SPE does not require SPE apparatus, cartridges, vacuum, preconditioning of column, and many steps in experiment. It is quicker, cheaper, and provides better interaction with the sorbent for cleanup than traditional SPE. The QuEChERS method was originally applied for the analysis of pesticide residues in plant tissues [22,24], and now it has been widely used for the extraction of compounds with a wide polarity range, including both pesticide residues in plant origin food and veterinary drugs residues in animal origin matrices [25], such as bovine milk, liver [26], shrimps [27], fish [17] and chicken muscle tissue [18]. It was reported that dozens of animal drug residues including quinolones, fluoroquinolones, sulfonamides, macrolides, anthelmintics, avermectins, nitroimidazoles, ionophores and dinitrocarbanilide can be extracted using QuEChERS method. However, as for as we know, this approach has not been applied for the extraction of amantadine and rimantadine in chicken muscle tissues.

The purpose of this work is the development of a simple and sensitive method for the determination of amantadine and rimantadine in chicken muscle tissues. The developed method involves an optimize QuEChERS method in the sample pre-treatment step followed by determination by UHPLC LTQ-Orbitrap MS.

2. Experimental

2.1. Reagents and materials

Acetonitrile and methanol (HPLC grade) were from Merck (Darmstadt, Germany). Formic acid, acetic acid and ammonium acetate (purity > 99%) were from TEDIA (Fairfield, OH, USA). Sodium chloride and anhydrous magnesium sulfate (analytical grade) were supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Sorbents including Cleanert C_{18} (40–60 μm), Cleanert graphitized carbon black (GCB, 120–140 mesh), Cleanert NH_2 (40–60 μm), Cleanert Alumina-N (150 mesh), Florisil (60–100 mesh), and primary-secondary-amine (PSA, 40–60 μm) were obtained from Bonna-Agela Technologies (Tianjin, China). Certified analytical standards of amantadine and rimantadine were obtained from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Individual stock standard solutions (1000 μg/mL) of amantadine and rimantadine were prepared by dissolving the appropriate amount of each analyte in methanol and were stored in the dark at −20 °C. A 1 μg/mL working standard solution containing both compounds was prepared by appropriate dilution of the stock standard solutions with methanol. Standard solutions for UHPLC LTQ Orbitrap-MS analysis (1–100 μg/L) were prepared daily from dilution of the 1 μg/mL working standard solution in acetonitrile/0.1% formic acid aqueous solution (3:7, v/v). Ultrapure water was obtained from a Milli-Q Elix system (Millipore, Beaford, MA, USA).

2.2. Instrument conditions

The analysis was performed on an Accela liquid chromatographic system (Thermo Scientific, San José, USA) coupled to LTQ-Orbitrap XL MS (Thermo Scientific Bremen, Germany). The data was collected and analyzed by the Thermo Fisher Xcalibur software package (version 2.1.0).

The UHPLC separations were operated on a Waters ACQUITY UPLC HSS T3 column (150 mm × 2.1 mm, 1.8 μm particle size, Waters, Wexford, Ireland) under gradient conditions with mobile phase of 0.1% formic acid aqueous solution (eluent A) and acetonitrile (eluent B) at a flow rate of 0.25 mL min^{-1} at 30 °C. The initial mobile phase composition was 80% of eluent A and 20% of eluent B. The content of eluent B was increased by a linear gradient from 20% to 50% in 6 min and increased from 50% to 90% in 0.1 min and held for 2.5 min. The analytical column was then equilibrated at the initial conditions for 4 min. The total run time for analysis was 12 min. The injection volume was 10 μL.

LTQ Orbitrap MS equipped with an ESI source was operated in positive ionization mode using the following operating parameters: electrospray voltage 4.5 kV, sheath gas flow rate 30 abu, auxiliary gas flow rate 8 abu, capillary temperature 350 °C, capillary voltage 47 V, tube lens offset 130 V. Instrument calibration was performed externally prior to each sequence with a calibration solution. Accurate mass spectra of $[M+H]^+$ ions were recorded from 100 to 500 m/z, the mass resolution power of the mass analyzer was set to 60 000 ($m/\Delta m$). Nitrogen (99.95%) was used as sheath gas, aux gas and served as the collision gas in the HCD cell and the bath gas in the C-trap.

2.3. Samples and sample extraction procedures

Chicken breast samples were obtained from local supermarket (Beijing, China) and the samples were homogenized (1000 rpm, 2 × 15 s) by a homogenizer (Grindomix GM 200, Retsch GmbH) and then stored at −20 °C until analysis. Blank samples were confirmed to be free of targeted analyte residues by UHPLC-LTQ Orbitrap MS after sample preparation by the procedures investigated.

Extraction and cleanup were based on the QuEChERS method and modified as follows: 3.0 g homogenized chicken muscle samples were weighted into 50 mL polypropylene centrifuge tubes, followed by addition of 6 mL of H_2O, and then vortexed by IKA MS 3 basic (IKA, Staufen, Germany) at 3000 rpm for 30 s. Subsequently, 10.0 mL of 1% HAc in acetonitrile was added to each tube and the tube was vortexed for 1 min. After that, 3 g $MgSO_4$ and 1 g NaCl was added to each tube and the tube was shaken vigorously for 1 min. The sample was centrifuged at 7000 rpm for 5 min at 15 °C. A 6.0 mL of the upper acetonitrile layer was transferred into 15 mL tube which containing 150 mg sorbent for cleanup. The tube was vortexed for 30 s and centrifuged at 1500 rpm for 10 min at 15 °C. An aliquot of 4.0 mL of the upper layer was transferred into 10 mL centrifuge tube and evaporated to dryness by nitrogen-evaporator (N-EVAP^{TM} 116, Organomation Association Incorporated, South Berlin, USA) at 45 °C water bath. Samples were then redissolved with 0.8 mL of ACN/H_2O (0.1%, FA) (7: 3, v/v), filtered through 0.22 μm cellulose acetate filters and transferred into autosampler vials for analysis.

2.4. Method validations

Calibration curves were conducted using mixed working standard solutions by plotting the peak area at concentrations of 1,