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Determination of ribavirin in chicken muscle by quick, easy, cheap, effective, rugged and safe method and liquid chromatography-tandem mass spectrometry

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ABSTRACT

A new analytical method for the determination of ribavirin in chicken muscle using a QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) method and liquid chromatography-tandem mass spectrometry (LC–MS–MS) was developed and validated. Samples were extracted with acidified methanol (methanol:acetic acid, 99:1, v/v). The extract was further purified by QuEChERS method using primary-secondary amine (PSA) and C_{18}. Finally, the extract was dried by nitrogen under 45 °C and reconstituted in water. The separation was performed on a Hypercarb analytical column under a gradient elution. The mobile phase was composed of water buffered with ammonium acetate (2.0 mM) and acetonitrile. The proposed method was validated according to the European Commission Decision 2002/657/EC. The values of the decision limit (CCα) and the detection capability (CCβ) were 1.1 and 1.5 μg/kg, respectively. The mean recoveries of ribavirin ranged from 94.2% to 99.2%. The repeatability (expressed as coefficient of variation, CV_r) of the method ranged from 4.5% to 4.9% and the reproducibility (CV_R) of the method ranged from 4.8% to 5.4%. The method is demonstrated to be suitable for the determination of ribavirin in chicken muscle in conformity with the current EU performance requirements through validation. The total time required for the analysis of one sample, including sample preparation, was about 45 min.

1. Introduction

Ribavirin is a synthetic purine nucleoside analogue with antiviral activity [1]. It is indicated that ribavirin was mainly used for the treatment of human respiratory syncytical viral infections and hepatitis C. However, ribavirin is still illegally used for the prevention and treatment of avian influenza in poultry farming in China occasionally [2]. The illegal use of ribavirin may result in unsafe residues in animal-derived food. Moreover, the illegal use may lead to developing resistance to human beings through the food chain and affect the effectiveness of human optional drugs against the influenza virus. Therefore, it is necessary to develop a selective and sensitive method to quantify ribavirin in fluids, tissues and food derived products from poultry.

Some studies had showed that ribavirin will be phosphorylated to ribavirin mono-phosphat, ribavirin diphosphate and ribavirin tri-phosphate in vitro studies in various cells using [^{3}H]- or [^{14}C] ribavirin [3–5]. So, some methods for determining ribavirin and its metabolites have been developed in human biological samples and experimental animal tissues using liquid chromatography (LC) with ultraviolet (UV) [6–8] and mass spectrometric detection [9–20]; and capillary electrophoresis (CE) [21] to carry out pharmacokinetic studies. However, the pre-treatment methods of determination of ribavirin usually were complicated and time-consuming because the combination of enzymatic hydrolysis and solid phase extraction (SPE) was often used for complex biological tissue samples. So far, to our knowledge, methods for determination of ribavirin in chicken tissues is still scarce because the high polarity of ribavirin and it is difficult to purify in tissue samples [13]. Although Berendsen et al. have recently developed a LC–MS/MS method for determination of antiviral drugs including ribavirin in poultry muscle with efficient separation between ribavirin and the endogenous interferences, but the sample preparation involve multiple SPE procedures and is very time-consuming [19]. Moreover, no relevant evidence had been provided to explain whether enzymatic hydrolysis should be used to extract ribavirin from chicken tissues until now. So, it is a challenge to establish a simple, selective, accurate and sensitive method for determination of ribavirin in chicken tissues.

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The present study reports the development of a simple, reliable and sensitive method for the determination of ribavirin in chicken samples. This method is based on modified QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) procedures followed by LC–MS/MS analysis after determing ribavirin in chicken tissue in incurred samples. The established QuEChERS method uses 1% acetic acid methanol (MeOH) extraction followed by a dispersive solid-phase extraction (d-SPE) cleanup procedure with primary-secondary amine (PSA) and C_{18} sorbent. The method was fully validated according to 2002/657/EC. The validation parameters tested include linearity, specificity, CCα, CCβ, recovery, precision and stability. Based on the results of the validation, the method proved to be fit for the purpose of quantitative analysis of ribavirin in chicken muscle samples.

2. Materials and methods

2.1. Materials and reagents

MeOH (LC grade) and acetonitrile (ACN, LC grade) were purchased from Fisher Chemicals (Fairlawn, USA). Formic acid (88.0%) was obtained from the Tedia Company Inc (Fairfield, USA). Acetic acid (99.9%) and ammonium acetate (99.5%) were analytical grade and purchased from Shanghai Chemical Reagent Co., Ltd. (Shanghai, China). Ribavirin and ribavirin-$^{13}C_5$ were purchased from Toronto Research Chemicals Inc. (North York, ON, Canada). Acid phosphatase (200 U/mL) was purchased from Megazyme International Ireland Ltd. (Bray, Wicklow, Ireland). The PSA (40–63 μm) and C_{18} (40–63 μm) were purchased from Shanghai ANPEL Scientific Instrument Co., Ltd. (Shanghai, China).

2.2. Instruments and apparatus

The LC-MS/MS system consisted of a Waters Acquity UPLC system along with a Xevo^{TM} TQ triple-quadrupole Mass spectrometer (Milford, MA, USA). The vortex mixer was purchased from Fisher Scientific (Norcross, GA, USA). The centrifuger (3K 15) was purchased from Sigma Laborzentrifugen GmbH (Osterode am Harz, Germany).

2.3. Incurred chicken samples

Six broiler chickens (three males and three females), aged 5 weeks and weighing 1000–1200 g, were used in this study. The birds were administered ribavirin in drinking water at concentrations of 100 mg/L for 3 days. Then, the birds were slaughtered on day 3 after dosing with two hours withdrawal time. Incurred chicken samples were collected from each bird and stored at −20 °C until they were processed to analysis time.

2.4. Standard solutions

Individual stock solutions of ribavirin and ribavirin-$^{13}C_5$ (100 mg/L) were prepared in ACN and stored at −20 °C. Then, the two stock solutions were appropriately diluted to produce two individual intermediate standard solutions (1000 μg/L) with ACN. The intermediate standard solutions were used to prepare six calibration standard solutions (1.0, 2.0, 5.0, 10, 20 and 50 μg/L for ribavirin and each calibration standard solutin containing 10 μg/L for ribavirin-$^{13}C_5$) and four spiking standard solutions (100, 150 and 200 μg/L for ribavirin and 200 μg/L for ribavirin-$^{13}C_5$) with water.

2.5. Chromatographic conditions

Separation was carried out on a Hypercarb analytical column (100 mm × 4.6 mm, 7.0 μm) maintained at 35 °C. A gradient elution program was initiated using solvent A (2.0 mM ammonium acetate solution) and solvent B (ACN) at 5% A and held for 6.0 min before incorporating a linear gradient increasing to 95% B at 8.0 min and holding for 1.0 min. At 15.1 min, the gradient was programmed to initial conditions to re-equilibrate the column for 2.9 min (the total run time was 18 min). The flow rate was 0.30 mL/min. The injection volume was 10 μL in full loop injection mode.

2.6. Mass spectrometry conditions

Detection was carried out with a Waters Xevo^{TM} TQ triple-quadrupole MS fitted with an electrospray ionization (ESI) probe and operated in the positive ion mode. The capillary voltage was set at 3.8 KV, the ion source temperature was 150 °C, the desolvation gas temperature was 500 °C, the desolvation gas (N_2) flow rate was 1,000 L/h and the collision gas (argon) flow was 0.20 mL/min. Diagnostic MRM transitions were first selected using IntelliStart software. Data acquisition and processing were performed using MassLynx version 4.1 software. The selected MRM transitions were (m/z) 245.0 > 95.7 and 245.0 > 113.0 for ribavirin and (m/z) 250.0 > 113.0 for ribavirin-$^{13}C_5$. The dwell time for three MRM transitions was 0.50 s. The transition chosen for quantification was (m/z) 245.0 > 113.0. The cone voltage was 10 V for the three MRM transitions. As for the collision energy, it was 26 eV for (m/z) 245.0 > 95.7 and 9 eV for the other two MRM transitions.

2.7. Sample preparation

2.00 g chicken muscle samples were weighed into a 50 mL polypropylene centrifuge tube, and 100 μL of 200 μg/mL spiking standard solution followed by 10 mL of MeOH (1.0% acetic acid, v/v) were added. Each sample was homogenized for 1 min using a high-speed blender (Ultra-Tyrrax T25; IKA, Germany) and then centrifuged for 3 min at 5000 rpm. An aliquot (6 mL) of supernatant, collected in a 10 mL polypropylene centrifuge tube with 200 mg PSA and 200 mg C_{18} sorbent, was shaking vigorously on a vortex mixer for 30 s. After shaking, the sample was centrifuged at 5000 rpm for 2 min. Then, an aliquot (5.00 mL) of supernatant was transferred to a 5 mL glass tube and evaporated to dryness under a stream of nitrogen at 45 °C. The residue was reconstituted in 1.00 mL of water. The resulting solution was filtered through a 0.22 μm filter, and 10 μL of filtrate was injected into the LC.

2.8. Matrix effects

To evaluate the matrix effect, six concentrations (1.0, 2.0, 5.0, 10, 20, and 50 μg/L) of ribavirin were analyzed in the solvent and in the blank chicken muscle sample after the sample preparation procedure. The slope ratio was calculated by comparing the external matrix matched calibration slope of ribavirin with the solvent external calibration slopes.

2.9. Method validation

Validation process of the method was carried out according to the European Commission Decision 2002/657/EC [22] to test the fitness for the purpose of quantitative analysis.The selectivity of the method was investigated by analyzing 20 blank chicken muscle samples. Calibration curves were individually constructed (n = 6) using working standard solutions and matrix-matched standard solutions and by plotting the peak area ratio of the quantitative ion pair to internal standard at concentrations of 1.0, 2.0, 5.0, 10, 20,