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RESEARCH PAPER

Identification of Major Metabolites of Salbutamol in Swine Urine and Plasma Using Ultra-High Performance Liquid Chromatography-Electrospray Time of Flight Mass Spectrometry

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Abstract: The excretion of urinary and plasmic metabolites of salbutamol was studied using ultra performance liquid chromatography electrospray ionization quadrupole time-of-flight tandem mass spectrometry, after a single intragastric gavaged dose administration with salbutamol. The software of Agilent Mass Hunter Metabolite ID was employed to find and identify the chemical structure of metabolites of salbutamol. Five metabolites from salbutamol were identified. The metabolites identified in swine urine included glucuronide conjugate salbutamol, *N*-oxide-salbutamol, hydroxyl-salbutamol, methoxyl-salbutamol and dehydrated hydroxyl-salbutamol. Whereas, only the parent drugs, glucuronide conjugate salbutamol and *N*-oxide salbutamol were observed in plasma.

Key Words: Salbutamol; Ultra-high performance liquid chromatography-electrospray time of flight mass spectrometry; Metabolites

1 Introduction

Liquid chromatography/electrospray ionization mass spectrometry (LC/ESI-MS), due to its selectivity and high sensitivity, has been widely applied in drugs and metabolites analysis in various complex biological matrixes concluding a large number of interfering components such as whole blood, plasma, urine, bile and biological tissue. The use of LC-MS not only avoids complex, tedious, time-consuming sample preparation procedure, but also separates and identifies the trace of drug metabolites. Triple-quadrupole time-of-flight (Q-TOF) is moderate-resolution mass spectrometry which provides accurate molecular mass of parent drug and metabolites. Combined with the Agilent Mass Hunter Data Acquisition software, the identification of metabolite molecular formulae (elementary composition) and their accurate mass fragments for assisting in structural characterization can be rapid and reliably achieved in biological samples[1,2].

β_2-Adrenoceptor agonist could relax bronchial, blood vessels, intestinal and uterine smooth muscle, increase the cilia movement frequency and adjust the mucosa cilia clearance[3]. In addition to promoting protein synthesis, β_2-agonist had the functions to increase fat lipolysis and inhibit adipogenesis. Salbutamol, named [2-(tert-butylamino)-1-(4-hydroxy-3-hydroxy methylphenyl) ethanol], also known as albuterol, was a synthetic potent selective β_2-adrenoceptor agonist commonly used for relieving the acute symptoms of asthma[4]. Higher doses of salbutamol could promote protein deposition and increase fat biolysis in livestock raising[5]. However, residues of salbutamol and its metabolites, which were likely more poisonous than the parent compound, were

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toxic to humans through consumption animal products[6]. Thus, these drugs were used illegally in the livestock industry as growth promoting agent.

In recent years, there were several published papers reporting in vivo metabolism of salbutamol in human beings. The metabolism of salbutamol in human body was reported and the major route was depending on sulphation which generated the carcinogenic sulphate conjugate metabolite[7–9]. Following intraperitoneal administration, four metabolites from salbutamol were identified in rat urinary, including salbutamol glucuronide, methoxy-salbutamol glucuronide conjugate, hydroxyl-salbutamol and *N*-oxide salbutamol[10]. Drug metabolism path between different species is different, even the different dosage of drug metabolism for the same animal are also different[9]. Now study on the biotransformation and metabolic mechanism of salbutamol in livestock is rare, in addition to Montrade's research that reported the salbutamol oxidation metabolites in bovine urine performing on high resolution mass spectrometry[11]. The ultra-performance liquid chromatography electrospray ionization quadrupole time-of-flight tandem mass spectrometry combined with Agilent MassHunter Metabolite ID software were applied for the identification of metabolites which is based on the search of typical biotransformation with their corresponding accurate mass shift and the use of common diagnostic fragment ions from the parent drugs.

2 Experimental

2.1 Chemicals and reagents

Salbutamol analytical standard (purity, ≥ 99.0%) was purchased from Dr. Ehrenstorfer GmbH (Germany). Acetonitrile and methanol (HPLC grade) were obtained from Sigma Aldrich (USA). The HPLC water was prepared through a Milli-Q system (Millipore, MA, USA). All other chemicals and reagents were of analytical grade supplied by Guoyao Chemical Co. (Shanghai, China). 0.22-μm Filter Unit was from Bonna-agela Technologies, China.

2.2 Collection of animal samples

Animal study was conducted following the guidelines of the Care and Use of Laboratory Animals, which was approved by the Institutional Animal Care and Use Committee.

The study was performed on male Changbai swine weighing 25–30 kg from COFCO Tianjin Meat Product Limited, China. The animal were housed under standardized conditions of light and temperature and allowed *ad libitum* accessed to standard food and water. After acclimating to the environment for 1 week, the animals were fasted for 12 h with free access to water before experiment. Then the swines were intragastric gavaged with salbutamol at a dose of 10 mg kg^{-1} BW. The vehicle solution for Salbutamol was saline solution. After drug administration, blood samples were collected at 1, 2, 3, 4, 6 and 12 h, and urine samples were collected from 0–6 h, 6–12 h, 12–24 h, 24–48 h after oral administration. The plasma samples were separated by centrifugation at 3000 *g* for 10 min. All samples were stored at –80 ºC until analysis.

2.3 Sample preparation

Approximately 200 μL of urine or blood samples and 600 μL of acetonitrile were added into each centrifuge tube, and the obtained mixture was then vortexed for 30 s and centrifuged at 10000 rpm (at 4 ºC) for 10 min. The organic layer was carefully removed into a new centrifuge tube, and evaporated to dryness under a steam of nitrogen at 37 ºC. The residues were then reconstituted in 500 μL 30% acetonitrile followed by centrifugation at 10000 rpm for 10 min before analysis. Subsequently 5 μL of supernate was injected into the LC-QTOF system to be analyzed.

2.4 Q-TOF-MS analysis

Chromatographic separations were carried out on a Agilent Plus C18 column (100 mm × 2.1 mm, 1.8 μm, Agilent, USA) using a Agilent LC system. The mobile phase consisted of 0.2% acetic acid in water as solvent A and acetonitrile as solvent B. The gradient elution program was as follows: initial, A:B(95:5, *V/V*); 0–12 min, a linear gradient from 5%–98%, B and kept for 2 min; then back to initial composition within 2 min and held another 5 min for column re-equilibration at a rate of 0.3 mL min^{-1}. The column temperature was maintained at 40 ºC. The injected volume of the test sample was set at 5 μL. Q-TOF detection (Agilent, USA) equipped with an electrospray ionization source (ESI) was performed in positive ion mode under the optimized conditions such as 4.0 kV of capillary voltage, 40 psi of nebulizer pressure, 6.0 L min^{-1} of drying gas flow rate at 325 ºC, 130 V of fragmentor voltage with mass spectra scan range of *m*/*z* 100–1000. The continuous accurate-mass calibration for instrument was performed by using a second sprayer with a reference solution containing purine (*m*/*z* 121.05) and HP-921 (*m*/*z* 922.01).

2.5 Q-TOF data analysis

The full-scan data were recorded with Agilent Mass Hunter Data Acquisition software, and the metabolites were searched using Agilent Mass Hunter Metabolite ID software which was set to automatically identify the possible metabolites by comparing the samples with the control and according to the molecular mass of salbutamol and metabolism pathways (the mass spectrum detection error < 10 mDa).

3 Results and discussion