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Application of a sensitive liquid chromatographic/tandem mass spectrometric method to a pharmacokinetic study of allylestrenol in healthy Chinese female volunteers

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ABSTRACT

A sensitive, specific and rapid liquid chromatographic/tandem mass spectrometric (LC/MS/MS) assay for the determination of allylestrenol in human plasma was established. Plasma samples were extracted by *tert*-butyl ether and separated by LC/MS/MS using a Phenomenex Curosil-PFP column (250 mm × 4.6 mm ID, dp 5 μm) with a mobile phase of methanol–water (95:5, v/v). The analytes were monitored with atmospheric pressure chemical ionization (APCI) by selected reaction monitoring (SRM) mode. The linear calibration curves covered a concentration range of 0.04–20.0 ng/mL with lower limit of quantification (LLOQ) at 0.04 ng/mL. The mean extraction recovery of allylestrenol was greater than 81.8%. The intra- and inter-day precisions were less than 1.3% and 3.1% respectively, determined from quality control (QC) samples of three representative concentrations. The method has been successfully applied to determining the plasma concentration of allylestrenol and a clinical pharmacokinetics study in healthy Chinese female volunteers.

1. Introduction

Allylestrenol, 17α-allylestr-4-en-17β-ol (Fig. 1A), is a synthetic steroid which bears progestational activity. In clinic, it was initially used in case of progesterone deficiency, for treating threatened and recurrent miscarriage, and to prevent premature labour [1]. Later, it was used as antiandrogen therapy for treating prostate cancer or hyperplasia as well [2,3]. Recently, problems of the adverse effects in clinic usage, e.g. sexual disfunction [4] have been considered to prescribe allylestrenol. Therefore, it is important, especially for clinic usage, to know the pharmacokinetic parameters of allylestrenol in human being. However, the pharmacokinetic study of allylestrenol has rarely been reported although decades of its prescription in clinic.

So far as we know, only two paper up to now reported their traditional HPLC and gas chromatography assays, which is not suitable for pharmacokinetic study owing to the detection limitation, for the determination of allylestrenol from its marketed formulation [5,6].

In this study, we developed a sensitive, specific, and rapid LC/MS/MS assay for the determination of allylestrenol in human plasma based on optimizing the sample extraction and the mass spectrometric condition, with an LLOQ of 0.04 ng/mL and a lineal detection range of 0.04–20 ng/mL. Then, we successfully applied this method to a pharmacokinetic study involving 20 healthy Chinese female volunteers after a single oral dose of 20 mg allylestrenol.

2. Experimental

2.1. Chemical and reagents

Allylestrenol was obtained from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), and Mifepristone (internal standard, IS, Fig. 1B) provided by Zizhu Pharmaceutical Factory Co. Ltd. (Beijing, China). The test drug of allylestrenol tablets (5 mg/tablet) was kindly provided by the Fourth Pharmaceutical Co. Ltd. (Changzhou, China). Methanol and *tert*-butyl ether of HPLC grade were purchased from Merck (Darmstadt, Germany). Water used to prepare all aqueous solutions throughout the study was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA). Other chemicals and reagents are of analytical grade. Heparinized blank human plasma was obtained from Xijing hospital (Xi'an, China).

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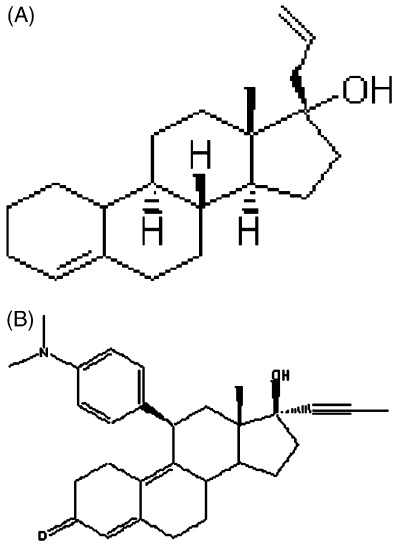

Fig. 1. Chemical structure of allylestrenol (A) and mifepristone (B).

2.2. Instrumentation and analytical conditions

The LC/MS/MS system consisted of a Surveyor LC pump, a Surveyor auto-sample, a TSQ Quantum Ultra AM triple–quadrupole tandem mass spectrometer with an ion max source, and the Xcalibur 1.1 software for data acquisition and analysis (Thermo Finnigan, San Jose, CA). Separation of the analytes from plasma was done at 25 °C on a Curosil-PFP column (250 mm × 4.6 mm ID, dp 5 μm; Phenomenex, Torrance, CA, USA). A mobile phase of methanol–water (95:5, v/v) at a flow rate of 1.0 mL/min was employed.

The MS/MS was detected by atmospheric pressure chemical ionization (APCI) source in positive mode. The injection volume was 50 μL. Separation was conducted under isocratic conditions and the total running time was within 6 min. The TSQ quantum parameters were optimized and set as following: charging current of 16.0 μA, capillary temperature of 200 °C, sheath gas pressure of 20 psi, auxiliary gas pressure of 10 psi, heater gas temperature at 420 °C and argon collision gas pressure of 1.3 mTorr. Typical product ion-scan spectra for the allylestrenol and the internal standard are shown in Fig. 2. Quantification was carried out using selected reaction monitoring (SRM) with ion transitions of *m*/*z* 241–91 with collision energy (CE) of 38 eV for allylestrenol, and *m*/*z* 430–372 with CE of 35 eV for mifepristone (IS).

2.3. Preparation of standard and quality control (QC) samples

Two stock solutions of allylestrenol were prepared by dissolving the accurately weighed reference substance in methanol. One solution was then serially diluted with methanol to give working solutions at the following concentrations: 0.40, 1.0, 2.0, 4.0, 10.0, 20.0, 40.0, 100.0, 160.0 and 200.0 ng/mL. The other stock solution was independently diluted in a similar way to achieve QC solutions at concentrations of 2.0, 40.0 and 160.0 ng/mL. Internal standard working solution (15 ng/mL) was prepared by diluting the 200 μg/mL stock solution of mifepristone with methanol. All the solutions were kept at 4 °C and were brought to room temperature before use.

Both the calibration standard samples and the quality control samples, which were used in the pre-study validation and during the pharmacokinetic study were prepared by spiking 0.5 mL blank plasma with 50 μL working solutions correspondingly.

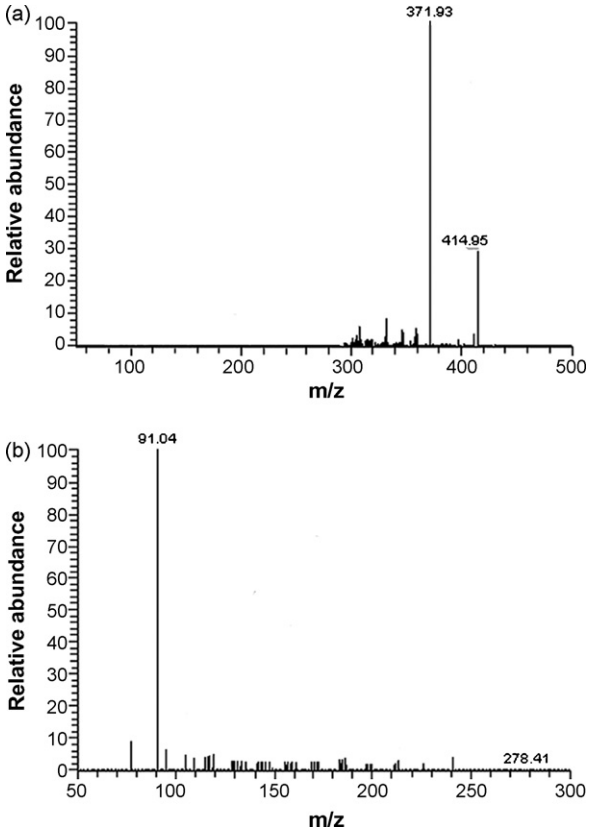

Fig. 2. Product ion mass spectra of $[M+H]^+$ of (A) miferpristone and (B) allylestrenol (internal standard).

2.4. Sample preparation

The frozen human plasma samples were thawed at ambient temperature, then a 0.5 mL aliquot plasma sample was mixed with 50 μL IS (1.5 ng/mL) solution and 100 μL of 0.1 M hydrochloric acid solution. The mixture was extracted with 5 mL of *tert*-butyl ether, vortex-mixed for 4 min, and then centrifuged at 1700 × *g* for 5 min. The 4 mL of organic layer was removed and evaporated under a stream of nitrogen gas in the thermostatically controlled water-bath maintained at 40 °C until completely dry. The residue was dissolved in 150 μL of mobile phase, vortex-mixed for 1 min, centrifuged at 6000 × *g* for 5 min, and a 50 μL of the supernatant was then injected into the LC/MS/MS for analysis.

2.5. Method validation

2.5.1. Selectivity

To investigate the selectivity of the method, human blank plasma samples from six different donors were pretreated and analyzed at LLOQ. LLOQ was defined as the lowest concentration of analyte within the acceptable precision and accuracy (five replicates with relative standard deviation (R.S.D.) below 20% and relative error (R.E.) within ±20%). Moreover, the analyte's response at this concentration level should be >5 times of the baseline noise.

2.5.2. Linearity of calibration curves and lower limits of quantification

Linearity was evaluated by assaying calibration curves in human plasma in four separate runs. Each validation run consisted of a set of the spiked calibration standards at 10 concentration levels of 0.040, 0.10, 0.20, 0.40, 1.0, 2.0, 4.0, 10.0, 16.0 and 20.0 ng/mL