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Development of a high-performance liquid chromatographic method for the determination of mifepristone in human plasma using norethisterone as an internal standard: application to pharmacokinetic study

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Abstract

Objective: The objective of this study was to develop a simple, sensitive, stable and validated HPLC method for the determination of mifepristone levels in human plasma.

Methods: Solid-phase extraction cartridges were used to extract plasma samples. Separation was carried out on a C_{18} column maintained at 20°C with acetonitrile–water (80:20, v/v) as mobile phase at a flow rate of 0.6 mL/min. Norethisterone was employed as the internal standard. Dual wavelength mode was used, with mifepristone monitored at UV 302 nm and norethisterone at 240 nm.

Results: The calibration curve was linear in the concentration range of 5–10 000 ng/mL, with linear correlation coefficient r being 0.9999. The limit of detection for the assay was 3 ng/mL. The inter-day accuracy ranged from 92.4% to 98.4% and precision 3.6% to 11.4%. The intra-day accuracy ranged from 92.1% to 100.6% and precision 4.7% to 12.2%. The absolute recovery was 91.7–100.1%. Plasma samples were stable for at least 1 month if stored at −20°C. This validated HPLC method was successfully applied to pharmacokinetic study of mifepristone in human plasma samples collected from volunteers after oral administration of 10 mg mifepristone.

Conclusion: The simple, accurate and stable method allows the sensitive determination of mifepristone in human plasma at the nanogram level. It could be applied to assess the plasma level of mifepristone in women up to 5 days after oral administration of 10 mg mifepristone.

Keywords: Mifepristone; High-performance liquid chromatography; Norethisterone; Internal standard; Solid-phase extraction; Pharmacokinetic

1. Introduction

Since it was synthesized at Roussel Uclaf in the early 1980s [1], mifepristone has come to the attention of researchers due to its various medical and scientific uses [2]. As a derivative of the progestin northisterone, it has a high affinity for the progesterone and glucocorticoid II (GRII) receptors and a low affinity for the androgen receptor. Thus, it could be used as a contraceptive, as a treatment for endometriosis and progesterone-sensitive tumors [3,4], and to terminate pregnancy as a competitive inhibitor of progesterone. Recent studies also indicated that mifepristone might play an important role in the treatment of a number of neuropsychiatric disorders [5]. Its pharmacokinetics is characterized by rapid absorption, a long half-life of 25–30 h and high serum concentrations following ingestion of doses of ≥100 mg of the drug [6]. As the three most proximal metabolites of mifepristone all retain considerable affinity toward human progesterone and glucocorticoid receptors; thus, many recent clinical studies on pregnancy termination and emergency contraception have focused on the decrease of the dose of mifepristone from 200–600 mg to 2–100 mg [6–8]. Hence, it is necessary to develop a more simple, sensitive and reliable method to determine lower levels of mifepristone in plasma.

A variety of methods for the determination of serum mifepristone levels have been developed, mainly based on radioimmunoassay (RIA) [9], radioreceptorassay (RRA) [10,11] and high-performance liquid chromatography

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(HPLC) [12–15]. Compared with the HPLC method, the direct RIA and RRA methods have higher sensitivity although they have some disadvantages including: not specific for the parent mifepristone and its metabolites because of the cross-reacting metabolites, using radioactive materials, running costly and time-consuming experimental procedure and producing waste difficult to dispose, etc. Hence, the HPLC method is more suitable for detailed analysis of the pharmacokinetics and metabolism of mifepristone. However, the HPLC methods reported have some deficiencies. For example, Heikinheimo et al. [12] used chromosorb column chromatography followed by HPLC separation, with limit of detection (LOD) of 40 ng/mL and limit of quantification (LOQ) of 250 ng/mL. The method reported by He et al. [13] was a liquid–liquid extraction with UV detection, with LOD of 36 ng/mL and LOQ of 240 ng/mL. Sensitivities of the above two were poor and operations were cumbersome. The one described by Stith and Hussian [14] was a solid-phase extraction with UV detection, with the assay linear in the range of 10–1000 ng/mL. No LOD and LOQ were reported, and the internal standard RTI-3021-003 was difficult to acquire. In addition, the samples studied in the research project were not human plasma but serum samples taken from captive coyotes, the components of which are affirmatively different from those of the former. Recently, a method was developed by our group [15], which was linear in the concentration range of 10–20,000 ng/mL with LOD being 5 mg/mL. However, it was not an internal standard method but an external one. As it is well known, quantitative precision and accuracy of the internal standard method are higher than those of an external standard method because it could avoid the effect of instrument fluctuation and operation error.

In the present report, our aim was to develop a simpler, more sensitive and accurate HPLC method to detect lower human plasma concentration following ingestion of lower dose of mifepristone.

2. Materials and methods

2.1. Chemicals

Mifepristone and norethisterone used as the internal standard (IS) were donated by XianJu Pharmaceutical Co. Ltd. (Zhejiang, China). Their purities were reported as ≥99%, and only one peak could be found under the selected experimental conditions. HPLC-grade acetonitrile was purchased from Tedia Company, Inc. (Fairfield, OH, USA). Water was distilled twice and then deionized by an HB-RO/10 deionzation ultrapure system (Huibang Co., Hangzhou, Zhejiang, China). All other chemicals and solvents were analytical reagents and obtained from local commercial sources.

2.2. Apparatus

The HPLC system consisted of an LC-10A system (Shimadzu Co., Kyoto, Japan) equipped with two Shimadzu LC-10AT$_{VP}$ pumps, a Shimadzu CTO-10A column oven, a Shimadzu SPD-10A$_{VP}$ UV detector, a Shimadzu DGU-20A3 on-line degasser and a Rheodyne manual injector (Rheodyne Inc., Cotati, CA, USA) fitted with a 20-μL loop. Data acquisition and integration were processed with the N2000 computer software package (Zhida Co., Hangzhou, Zhejiang, China).

2.3. Chromatographic conditions

The HPLC separation was achieved using a C_{18}-bonded phase (ODS, 150×4.6 mm ID, 4.6 μm particle size, Shimadzu) at 20°C. The mobile phase was a mixture of acetonitrile–water (80:20, v/v). The effluent was monitored at a flow rate of 0.6 mL/min. The wavelength of UV detector was operated at 302 and 240 nm using dual wavelength mode, the former for mifepristone and the latter for norethisterone. Extraction cartridges (Oasis HLB 1 mL, 30 mg) were purchased from Waters (Milford, MA, USA).

2.4. Standard and quality control (QC) solutions

Stock solutions of both mifepristone and IS (1.0 mg/mL) were prepared by dissolving 100 mg of mifepristone and IS in 100 mL acetonitrile, respectively, and then stored in clean brown glass bottles at −20°C lightproof. Working solutions of different concentrations were prepared from fresh from stock solutions prior to each analysis, by adding the appropriate volume of water to prevent precipitation of proteins when added to the plasma. Calibration standards in human plasma in the range of 0.005–10 μg/mL of mifepristone and 1 μg/mL of IS were prepared by spiking aliquots of stock solution or working solutions to blank human plasma. QC samples were also prepared as above at the concentration of 10, 100 and 1000 ng/mL. These calibration standards and QC solutions were then treated as follows.

2.5. Sample collection

Sample collection was carried out in a governmental hospital, and the study was approved by the local government hospital ethical committee. Five healthy women, 21-30 years of age, volunteered for the study. They all had regular menstrual cycles, with cycle length varying from 26 to 31 days. All were of normal weight, the body mass index varying from 19.4 to 25.2 kg/m^2. Prior to their participation, each woman signed an informed consent document. Institutional review board approval was obtained for administration of the mifepristone and data analysis. Following an overnight fast, a single dose of 10 mg mifepristone was ingested by each volunteer at 0 h, and then human plasma samples were collected at 0, 1, 6, 24, 48, 72, 96 and 120 h. All samples were stored at −20°C until analysis.

2.6. Sample preparation and extraction

All samples, including blanks, standards, QCs and unknowns, were extracted using the above-mentioned solid-phase extraction cartridges. Each cartridge was