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An HPLC method for the determination of ng mifepristone in human plasma

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Abstract

An HPLC method was developed and validated for the determination of mifepristone in human plasma. C_{18} solid-phase extraction cartridges were used to extract plasma samples. Separation was by C_{18} column; mobile phase, methanol–acetonitrile–water (50:25:25, v/v/v); flow rate, 0.8 ml/min; UV detection at 302 nm. The calibration curve was linear in the concentration range of 10 ng/ml to 20 μg/ml ($r = 0.9991$). Within- and between-day variability were acceptable. The limit of detection for the assay was 6 ng/ml. Plasma samples were stable for at least 7 days in the state of plasma or residue treated at −20 °C. The method was simple, sensitive and accurate, and allowed to determine ng mifepristone in human plasma. It could be applied to assess the plasma level of mifepristone in women receiving low oral doses of mifepristone.
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Keywords: Mifepristone; High performance liquid chromatography

1. Introduction

Since synthesized by researchers at the pharmaceutical company Rochelle Uclaf (Paris, France) [1], mifepristone has been used for a variety of medical and scientific purposes [2], especially in terminating pregnancy as a competitive inhibitor of progesterone. Its pharmacokinetics is characterized by rapid absorption, a long half-life of 25–30 h, and high micromolar serum concentrations following ingestion of doses of >100 mg of the drug [3]. Because its metabolites which still retain considerable affinity toward human progesterone and glucocorticoid receptors has the same biological actions as mifepristone itself, 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 [3–5]. Thus, developing a more sensitive and validated method to determine lower levels of mifepristone in plasma is becoming more and more important.

Various determining methods based on radioimmunoassay (RIA) [6,7], radioreceptorassay (RRA) [8,9] and high performance liquid chromatography (HPLC) [10–12] have been used to measure serum mifepristone levels. Due to the cross-reacting metabolites, the direct RIA and RRA methods were not specific for the parent mifepristone and its metabolites. In addition, their disadvantages were obvious, such as: using radioactive materials, running costly and time-consuming experimental procedure and bringing waste difficult to dispose, and so on. Therefore, the HPLC method is more suitable for detailed analysis of the pharmacokinetics and metabolism of mifepristone. However, the sensitivity of previous references on HPLC methods was not satisfied. The first one developed by Heikinheimo et al. [10] used Chromosorb column chromatography followed by HPLC separation, with limit of detection (LOD) 40 ng/ml and limit of quantification (LOQ) 250 ng/ml. Another one reported by Chang-hai et al. [11] was a liquid–liquid extraction with UV detection, with LOD 36 ng/ml and LOQ 240 ng/ml. Recent one described by Stith and Hussian [12] 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 this work, we wanted to develop a simpler, more sensitive HPLC method to detect lower human plasma concentration following ingestion of lower dose of mifepristone.

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2. Experimental

2.1. Materials

Mifepristone was purchased from Zizhu Pharmaceutical Co. Ltd. (Beijing, China). Its purity was reported as ≥98%, and only one peak could be found under the selected experimental conditions. HPLC-grade methanol and acetonitrile were purchased from Carledon (Georgetown, Ont., Canada). Water was distilled twice and then deionized by ion-exchange resin and then reverse osmosis membrane in a HB-RO/10 deionzation ultrapure system (Huibang Co., Hangzhou, Zhejiang, China). All other chemicals and solvents were analytical reagent and obtained from common commercial sources. Solid-phase extraction cartridges (Oasis HLB 1 ml, 30 mg), the HPLC separation column (Waters Spherisorb, 250 mm × 4.6 mm i.d., 5 μm particle size, C_{18}) and the guard column (Waters Nova-Pak, C_{18}) were all purchased from Waters (Milford, MA, USA).

2.2. Chromatographic system

The HPLC system consisted of a Waters-600 pump, a Rheodyne manual injector with final volume loop of 20 μl and a Waters 2487 UV absorbance detector. System management and hardware interface for data acquisition were carried out by the Empower computer software package from Waters. The mobile phase was a mixture of methanol–acetonitrile–water (50:25:25, v/v/v). The effluent was monitored with UV detector at 302 nm at 0.8 ml/min. The column temperature was regulated at 30 °C. Data were collected and integrated using Waters Empower software, and analyzed using SPSS 10.0 software.

2.3. Preparation of stock and working solutions

Stock solutions of mifepristone were prepared in mobile-phase solution and stored in cleaned brown glass bottles. They were stared at −20 °C until they were used for preparing working solution by adding the appropriate volume of mobile-phase solution. Tests showed that it was stable for at least 10 days (Table 4). Working solutions of different concentrations were prepared from above-mentioned stock solutions fresh just prior to use.

2.4. Preparation of calibration standard and QC samples in plasma

Calibration standards in plasma at the concentration range of 10 ng/ml to 20 μg/ml were prepared by spiking aliquots of stock solutions and working solutions to 0.5 ml of blank human plasma. QC samples were also prepared as above at concentration of 10, 50 and 600 ng/ml. These calibration standards and QC samples were then treated using the same procedure as described in Section 2.5.

2.5. Sample preparation and extraction procedure

Ten volunteered healthy women of childbearing age received single oral dose of 25 mg mifepristone at 0 h, and human blood samples were collected at 72 h. Another five ones received single oral dose of 50 mg mifepristone for 3 successive days, and thereafter samples were collected just prior to the first dose (0 h) and after the last dose (0.5, 24 and 48 h). Samples were collected by venipuncture into a glass tube containing heparinate anticoagulant. The plasma was separated by centrifugation for 5 min at 3000 × g at room temperature, and then it was harvested and stored at −20 °C until analysis.

All samples, including blanks, unknowns and standards, were extracted using the above-mentioned solid-phase extraction cartridges. Each cartridge was activated by 1 ml methanol and then balanced by 1 ml water just prior to use. Samples were then passed through it without lab vacuum and washed with 1 ml 5% methanol. Though it was claimed that this kind of cartridge could extract 1 ml sample, it was found that recovery would decrease to some extent in some case. Thus, volume of samples was selected as 0.5 ml instead of 1 ml to avoid it. The analytes were eluted with 1 ml 100% methanol and collected in clean glass tubes. The eluent was evaporated to dryness under −0.08 MPa lab vacuum at 37 °C, and the extraction residue was reconstituted in 100 μl of 100% methanol prior to injection into the HPLC system. About 50 μl aliquot was manually injected to insure that the 20 μl loop of the injector was totally full.

3. Results and discussion

3.1. Specificity

A series of typical chromatograms were shown in Fig. 1. Fig. 1A was the chromatogram of mifepristone in mobile-phase solution. Elution time was 12.64 ± 0.11 min ($n > 20$). Fig. 1B was the chromatogram of blank plasma. Although there were characteristic peaks for the biological matrix before 12 min, there were not any interfering peaks between 12 and 15 min, depicting no interfering peaks at the corresponding elution time of mifepristone. Fig. 1C was the chromatogram of standard plasma spiked with 10 ng/ml of mifepristone, in which peak for 10 ng/ml of mifepristone could be identified easily and accurately. Fig. 1D was the chromatogram of human plasma collected at 72 h from a volunteered healthy women received single oral dose of 25 mg mifepristone.

3.2. Standard curve and sensitivity of the method

Calibration standards in plasma at the concentration of 0.01, 0.05, 0.1, 0.2, 1, 2, 10 and 20 μg/ml were prepared by mixing aliquots of stock solution and working solution with 0.5 ml blank human plasma. The calibration standard curve for mifepristone in plasma was constructed by plotting peak height y (mV) versus mifepristone concentration in plasma x (μg/ml). The calibration standard curve of assay for mifepristone showed an acceptable linearity in the concentration range from 0.010 to 20 μg/ml, with the coefficient of correlation (r) being 0.9991. The regression equation was $y = 10.515x - 0.031$.

The limit of detection (LOD) for the assay of mifepristone, based on 3/1 of the signal-to-noise ratio, was 6 ng/ml. And the limit of quantitation (LOQ), based on 10/1 of the signal-

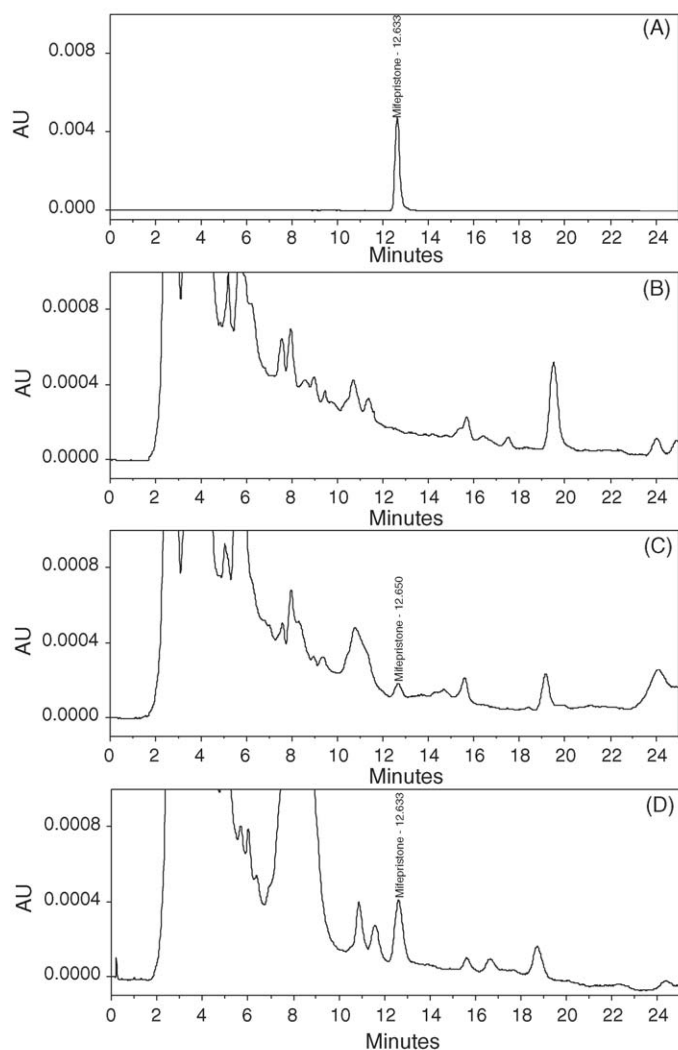

Fig. 1. HPLC chromatograms of mifepristone. (A) Mobile-phase solution spiked with 1000 ng/ml of mifepristone, (B) blank human plasma, (C) human plasma spiked with 10 ng/ml of mifepristone, and (D) human plasma collected at 72 h after single oral dose of 25 mg mifepristone (39 ng/ml).

to-noise ratio, was 20 ng/ml. In fact, satisfactory recovery (see Tables 2 and 3) could be achieved even the concentration was low as 10 ng/ml. Thus, we could conclude prudently that LOQ of this method could be extended down to 10 ng/ml. Clearly, if 50 μl loop of the injector was chosen, LOD could be extended down to about 2–3 ng/ml.

3.3. Extraction efficiency

Extraction efficiencies were calculated by comparing the mean peak heights of three extracted plasma samples ($n = 3$) to those of three non-extracted samples ($n = 3$). Extracted plasma samples were subjected to extraction, drying and reconstitution as described above. Non-extracted samples were prepared in methanol, and subjected to drying and reconstitution without the extraction procedure. As shown in Table 1, extraction efficiency varied from 94.7 to 101.2%. Very high extraction efficiency of mifepristone suggested that very little was lost during the extraction process.

Table 1
Extraction efficiency for mifepristone (mean ± S.D., $n = 6$)

Concentration (ng/ml)	10	200	2000
Extracted peak height (mV)	0.121 ± 0.003	2.078 ± 0.183	18.967 ± 0.354
Non-extracted peak height (mV)	0.124 ± 0.003	2.054 ± 0.075	20.031 ± 0.241
Extraction efficiency (%)	97.6	101.2	94.7

3.4. Validation

Validation of this method was performed using within- and between-day variability of QC samples at three concentration levels (10, 50 and 600 ng/ml). Within-day variability was determined in six replicates by 1 working day, while between-day variability in six replicates by 3 working days. The results were listed in Tables 2 and 3. This method showed good reproducibility and accuracy.

3.5. Stability of stock solution and plasma samples

The stability of stock solution was tested with one at the concentration of 10 μg/ml at −20 °C. It was evaluated by the difference from the initial concentration after being stored for different days, and expressed as the relative variation (%). As shown in Table 4, the relative variation was about 5%, which suggested that stock solution of mifepristone was stable at 4 °C for at

Table 2
Within-day analysis of mifepristone (mean ± S.D., $n = 6$)

Concentration added (ng/ml)	10	50	600
Concentration found (ng/ml)	10.7 ± 1.8	48.6 ± 4.5	591.6 ± 15.2
Accuracy (%)	107.0	97.2	98.6
Precision (R.S.D.%)	16.8	9.3	2.5

Table 3
Between-day analysis of mifepri stone (mean ± S.D., $n = 6$)

Concentration added (ng/ml)	10	50	600
Concentration found (ng/ml)	11.4 ± 2.3	46.8 ± 5.3	586.1 ± 49.8
Accuracy (%)	114.0	93.6	97.7
Precision (R.S.D.%)	20.2	11.3	8.5

Table 4
Stability of stock solution of mifepristone

Store days	1	3	5	7	10
Initial concentration (μg/ml)	10	10	10	10	10
Concentration found (μg/ml)	9.89	10.41	9.43	10.58	10.35
Relative variation (%)	1.1	4.1	5.7	5.8	3.5

Table 5
Stability of plasma samples of mifepristone by analyzing 600 ng/ml QC samples

Store state	Concentration found (μg/ml)		Relative variation (%)	
	3 days	7 days	3 days	7 days
Plasma at −20 °C	582.1	573.2	3.0	4.5
Residue at −20 °C	594.6	584.4	0.9	2.6
Residue at 4 °C	218.4	245.4	59.1	63.6

least 10 days. The stability of plasma samples was also observed by analyzing QC sample at the concentration of 600 ng/ml in different store states on different store days. The results were shown in Table 5. It was found that samples of mifepristone were stable in the state of plasma or residue treated at −20 °C for at least 7 days.

3.6. Measurement of mifepr is tone in human plasma

Plasma samples were collected at 72 h from 10 volunteered healthy women of childbearing age received single oral dose of 25 mg mifepristone at 0 h, and then analyzed using this method. The concentration was 41.2 ± 20.5 ng/ml (mean ± S.D., $n = 10$). This value was approximately consistent with about 0.1 μmol/l, the results of Heikinheimo et al. [3].

Plasma levels of mifepristone of the five volunteered healthy women as described in Section 2.5 were determined. The results were shown in Table 6.

Table 6
Plasma levels of mifepristone of the five volunteered women at different determining time

Code of the volunteered woman	Concentration (ng/ml)			
	0 h	0.5 h	24 h	48 h
1	<6	795	307	170
2	<6	525	131	34
3	<6	868	540	259
4	<6	541	120	111
5	<6	905	200	134

4. Conclusion

An HPLC method for the determination of ng mifepristone with UV detection in human plasma was developed and validated. This method showed good reproducibility and accuracy with the calibration curve ranges of 10–20,000 ng/ml. It was successfully applied to assess the plasma level of mifepristone in women of childbearing age received low oral doses of mifepristone.

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