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Quantitative determination of famotidine in human maternal plasma, umbilical cord plasma and urine using high-performance liquid chromatography - mass spectrometry

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Abstract

The liquid chromatography with electrospray ionization mass spectrometry for the quantitative determination of famotidine in human urine, maternal and umbilical cord plasma was developed and validated. The plasma samples were alkalized with ammonium hydroxide and extracted twice with ethyl acetate. The extraction recovery of famotidine in maternal and umbilical cord plasma ranged from 53% to 64% and 72% to 79%, respectively. Urine samples were directly diluted with the initial mobile phase then injected into the HPLC system. Chromatographic separation of famotidine was achieved by using a Phenomenex SynergiTM Hydro-RPTM column with a gradient elution of acetonitrile and 10 mM ammonium acetate aqueous solution (pH 8.3, adjusted with ammonium hydroxide). Mass Spectrometric detection of famotidine was set in the positive mode and used a selected ion monitoring method. Carbon-13-labeled famotidine was used as internal standard. The calibration curves were linear ($r^2 > 0.99$) in the concentration ranges of 0.631-252 ng/mL for umbilical and maternal plasma samples, and of 0.075-30.0 µg/mL for urine samples. The relative deviation of method was less than 14% for intra- and inter-day assays, and the accuracy ranged between 93% and 110%. The matrix effect of famotidine in human urine, maternal and umbilical cord plasma is less than 17%.

Keywords

Famotidine; LC-MS; pregnancy; umbilical cord plasma; urine

Introduction

Gastroesophageal reflux disease (GERD) and its major symptom, heartburn, occur anywhere within or throughout pregnancy and affects 40 to 85% of women (Gill *et al.*, 2009; Baron *et al.*, 1993). For the last 30 years, cimetidine and ranitidine, histamine-2 receptor antagonists (H₂RAs) have been extensively used during pregnancy to treat symptoms of GERD when lifestyle and dietary modifications alone or along with antacids have failed (Richer *et al.*, 2003; Ali *et al.*, 2007).

The newer H₂A famotidine gained wide clinical acceptance in non-pregnant patients because it is more potent than cimetidine and ranitidine and has less effects on hemodynamics (Howard *et al.*, 1985). However, famotidine has not been the medication of

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choice for the pregnant patient due to the limited information on its safety and efficacy in this patient population. Therefore, one of the aims of the recent investigation sponsored by the NICHD Obstetric-Pharmacology Research Network (OPRU) is to determine the pharmacokinetics of famotidine in pregnant patients.

Several methods have been reported on the quantitative determination of famotidine in human plasma and urine including capillary electrophoresis (Perez-Ruiz *et al.*, 2002) and liquid chromatography with UV (LC-UV) (Zarghi *et al.*, 2005; Dowling *et al.*, 1999; Zarzhi *et al.*, 1998; Zhong *et al.*, 2011) as well as mass spectrometry (LC-MS) (Zhong *et al.*, 2011; Campanero *et al.*, 2001; Sun *et al.*, 2009). In one method, using LC-MS, human protein was precipitated followed by quantitative determination of famotidine using selective ion monitoring (SIM) (Campanero *et al.*, 2001). Under these experimental conditions, the lower limit of quantification (LLOQ) was 1 ng/ml (Campanero *et al.*, 2001). However, due to the extremely high polarity of the compound in the acidic mobile phase used, the retention time of famotidine was 1.87min on the 250mm HPLC column. In order to increase the retention of famotidine thus enhancing its separation, Zhong *et al.* (Zhong *et al.*, 2011) described a normal-phase liquid chromatography tandem mass spectrometry method coupled with solid-phase extraction (SPE) to analyze famotidine in human plasma. In addition, trifluoroacetic acid (TFA) was used as an additive in the mobile phase (Zhong *et al.*, 2011). However, the authors did not report on the effect of TFA on the signal of the compound because it is known to be an ion-suppression reagent in LC-MS/MS (Shou *et al.*, 2005). In another HPLC-UV method used to determine concentration of famotidine in human plasma and urine, the retention time of famotidine was increased by the addition of ion-pair reagents such as heptanesulfonic acid (Dowling *et al.*, 1999) or sodium dodecyl sulphate (Zarzhi *et al.*, 1998). However, these ion-pair reagents might contaminate the MS detector. Furthermore, to date and to the best of our knowledge, there have been no reports on the quantitative analysis of famotidine in human umbilical cord plasma.

Therefore, the objective of this investigation was to develop and validate a sensitive, selective and rapid LC-MS method for the quantitative determination of famotidine in maternal plasma, umbilical cord plasma and urine of pregnant patients under treatment with famotidine.

Experimental

Chemicals

All chemicals were purchased from the following companies: famotidine, Sigma Chemical Co. (St. Louis, MO); the internal standard $^{13}\text{C}_3$ -famotidine, Toronto Research Chemicals, Inc. (North York, Canada); HPLC-grade acetonitrile, ammonium hydroxide, ethyl acetate and ammonium acetate, Fisher Scientific (Fair Lawn, NJ).

Liquid chromatographic conditions

Analysis of famotidine was carried out with an LC-MS instrument consisting of a Waters® 600E multi-solvent delivery system and a 717 auto-sampler controlled by Empower™ 2 Data Software (Waters, Milford, MA). The separation of famotidine was performed with a Phenomenex Synergi™ Hydro-RP™ column (150 × 4.6 mm, 4 μm) connected to a Phenomenex C₁₈ guard column (4 × 3.0 mm). The mobile phase consisted of acetonitrile and an aqueous solution of 10 mM ammonium acetate (pH=8.3, adjusted with NH₄OH). Elution was achieved by a linear gradient elution starting with 14% of acetonitrile at time zero to 32% of acetonitrile at 6 min and a flow rate of 1.0 mL/min. The column was equilibrated for 9 min at each interval. The total eluent from the column was split at a 4:1 ratio and hence, the flow directed to the mass spectrometer was equivalent to 200 μL/min.

Mass spectrometric conditions

The mass spectrometer (Waters EMD 1000 single-quadrupole, Milford, MA) equipped with an electrospray ion source (ESI) was operated in positive mode. MS parameters were as follows: capillary voltage, 3.5 kV; cone voltage, 45 V; source temperature, 90°C; desolvation temperature of 350°C; desolvation gas flow rate, 500 L/h; and cone gas flow rate of 100 L/h. Famotidine and its internal standard were monitored by selected ion monitoring (SIM) at m/z 189 for famotidine, and m/z 192 for the internal standard.

Preparation of stock and working standard solutions

Stock solutions of famotidine and its internal standard were prepared in 30% methanol. The working standard solutions of famotidine for the analysis of maternal and umbilical cord plasma samples were prepared in the range between 6.31 ng/mL to 2.52×10^3 ng/mL, and between 0.188 µg/mL and 75.0 µg/mL for urine samples. The working solutions of internal standard (IS) were prepared at a final concentration of 83.3 ng/mL for analysis of maternal and umbilical cord plasma samples and 6.25 µg/mL for urine samples. All the stock solutions were stored at 4°C.

Preparation of calibration and quality control (QC) samples

The calibration samples for maternal and umbilical cord plasma samples were prepared by adding 15 µL of famotidine and 15 µL of IS working solutions into 150 µL blank maternal or umbilical cord plasma samples to achieve a final famotidine concentration at 0.613, 1.26, 2.25, 6.31, 63.1, 126, 189 and 252 ng/mL.

The calibration samples for the urine samples were prepared by adding 10 µL of famotidine and 10 µL of IS working solutions into 25 µL blank urine samples to achieve a final famotidine concentration range of 7.50×10^{-2} , 0.150, 0.300, 0.750, 7.50, 15.0, 22.5 and 30.0 µg/mL.

Quality control (QC) samples were similarly prepared at high, medium, and low concentration levels for famotidine in maternal plasma, umbilical cord plasma and urine samples, as well as for the determination of the lower limit of quantification (LLOQ).

Preparation of human maternal plasma, umbilical cord plasma and urine samples

Maternal and umbilical cord plasma samples were prepared as follows: An IS working solution (15 µL) was added to 150 µL of plasma samples and vortexed for 30 sec. Then 50 µL of ammonium hydroxide (22% w/v) and 900 µL ethyl acetate were added to the samples, vortexed for 5 min and centrifuged at $12,000 \times g$ for 5 min at 4°C. The organic layer was then transferred to a clean tube. The aqueous layer (plasma) was re-extracted with 900 µL ethyl acetate as described above. The organic layers were then combined and dried under a stream of air at 40°C. The dried residues were reconstituted in 100 µL of the initial mobile phase and filtered by a 0.45 µm syringe filter. An aliquot of 50 µL of each sample was analyzed by the HPLC system.

Urine samples were prepared as follows: 10 µL of the IS working solution were added to 25 µL of the urine samples and diluted with 500 µL of the initial mobile phase. The solution was filtered by a 0.45 µm syringe filter. An aliquot of 50 µL of each sample was analyzed by the HPLC system.

Method validation

This analytical method of famotidine was validated for specificity, matrix effect, precision, accuracy, sensitivity, linearity and stability accordingly to the guidelines established by the FDA for bio-analytical method validation (FDA, 2001).

The selectivity of the method towards endogenous human biological samples was evaluated by analyzing blank maternal plasma, umbilical cord plasma and urine samples obtained from six patients. The SIM chromatograms of the blank samples were compared to LLOQ samples. The peak area of endogenous substances in blank samples co-eluting with the analytes was < 5% of the peak area of analytes at LLOQ concentration levels.

The extraction recovery of famotidine from the maternal and umbilical cord plasma samples was evaluated with the peak area of famotidine in QC samples versus the peak area of analytes in post-extracted samples at low, medium and high concentration levels. The recovery of famotidine extraction from urine samples was not evaluated because no extraction was performed in the preparation of these samples.

The matrix effect of famotidine and IS was evaluated quantitatively by calculating the Matrix Factor, which is defined as the ratio of the analyte peak area of post-extraction samples versus the analyte peak area of pure standards (Viswanathan *et al.*, 2007). The Matrix Factors of famotidine and its IS were investigated at low, medium and high concentrations in urine, maternal and umbilical cord plasma samples obtained from six patients. The variability of the Matrix Factor, as measured by the relative standard deviation (RSD) was <15% (Viswanathan *et al.*, 2007).

To evaluate the linearity of the method, calibration standards of famotidine at eight concentration levels were prepared in blank urine, blank maternal and umbilical cord plasma samples. The calibration curves were fitted by weighted least-squares linear regression of the internal ratio (peak area of the analyte/peak area of the IS) versus concentration. The weighting factors for the linear regression of plasma and urine samples were optimized according to a percentage relative error of each calibration samples (Almdida *et al.*, 2002). The correlation coefficient (r) >0.99 was desirable for all calibration curves. The limit of detection (LOD) was determined using a signal-to-noise (S/N) ratio of 3. The lower limit of quantification (LLOQ) was determined as the lowest concentrations that produced an S/N >10, and can be quantified with a RSD lower than 20% and accuracy in the range of 80-120% (FDA, 2001).

Intra-day and inter-day accuracy and precision of famotidine in urine, maternal and umbilical cord plasma samples were evaluated by the analysis of six replicates of each QC sample at high, medium, low and LLOQ concentration levels. For the intra-day assay accuracy and precision, QC samples were analyzed using a calibration curve prepared on the same day; for the inter-day assay accuracy and precision, the QC samples were analyzed on three consecutive days. The relative standard deviation for each concentration level should be less than 15%, except for LLOQ, which was < 20%. Accuracy of the method was evaluated as [mean obtained concentration/nominal concentration] $\times$ 100%. The accuracy is expected to be in the range of 85-115% of the nominal concentration, except for the LLOQ, which should be in the range of 80-120% (FDA, 2001).

The stability of famotidine in samples of urine, maternal and umbilical cord plasma were investigated by analyzing replicate (n=3) QC samples at high and low concentrations. For the freeze-thaw stability studies, unprocessed QC samples were stored at -80°C for 24 h, thawed at room temperature ($22-25^{\circ}\text{C}$), and then the refrozen for 24 h at -80°C . After three freeze-thaw cycles, samples were analyzed. For the short-term temperature stability study,

unprocessed QC samples were analyzed after being kept at room temperature for 4 h, which exceeds the routine sample preparation time. Famotidine was considered stable if the RSD for each concentration was less than 15%, and the accuracy did not deviate by $\pm 15\%$ of the nominal concentration.

Application for a pilot clinical trial

This validated method was applied to support a clinical pharmacokinetic investigation of pregnant patients under treatment with famotidine (oral administration of 20 mg famotidine every 12 h). After reaching steady-state, maternal vein blood and urine were collected at the predetermined time points. Maternal blood and umbilical cord venous and arterial blood were obtained immediately after delivery from the Labor and Delivery ward of The John Sealy Hospital, the teaching hospital of the University of Texas Medical Branch at Galveston according to an approved protocol by the Institutional Review Board. The blood was collected into BD Vacutainer® tubes containing lithium heparin (87 USP units). Plasma was separated by centrifugation and all biological samples were kept frozen at -80°C until analysis. QC samples were prepared at low, medium and high concentration levels and assayed daily along with the clinical samples.

Results and discussion

Method development

Famotidine precursor and product ions were optimized by using infusion of the neat solution under both positive and negative modes of ESI. Campanero *et al.* (Campanero *et al.*, 2001) reported that the concentration of ammonium acetate and the pH of the mobile phase affected the intensity of the famotidine precursor ion (m/z 338). Therefore, the concentrations of ammonium acetate and pH were optimized according to the responses of the precursor and product ions of famotidine. Famotidine showed good responses and stability in the positive ionization mode for product ions at m/z 189 and 259, whereas the precursor ion (m/z 338) of famotidine showed lower sensitivity and higher baseline under the optimized MS conditions (Figure 1). On the other hand, famotidine product ion response was not significantly affected by changes in the concentration of ammonium acetate (range: 2–20 mM) or in the pH range of 3–9. The famotidine products ion at m/z 189 was chosen as the quantitative SIM fragment ion in order to avoid an interference peak from human plasma which was observed in at m/z 256. The product ion spectra of the internal standard (IS, $^{13}\text{C}_3$ -famotidine) is shown in supplementary data Figure 4. The product ion at m/z 192 was chosen as the SIM fragment ion for $^{13}\text{C}_3$ -famotidine.

The major disadvantage of prior famotidine assays by reverse-phase HPLC is its poor retention by the column because of its extreme polarity in acidic conditions. Attempts to improve the retention of famotidine on the column to achieve the desired separation from the early-eluting endogenous peaks have included the HPLC column switch technique (Zhong *et al.*, 1998), an ion-pair reagent (Dowling *et al.*, 1999; Zarzhi *et al.*, 1998), normal phase HPLC (Zhong *et al.*, 2011) and alkaline mobile phase (Anzenbacherova *et al.*, 2003). After considering the compatibility of the HPLC method and the MS detector, the alkaline mobile phase was selected. The optimized composition of the mobile phase and its pH value was acetonitrile and 10 mM ammonium acetate (pH 8.3, adjusted with NH_4OH). A gradient elution of 14% acetonitrile at 0 min to 32% acetonitrile at 6 min was applied to achieve a good separation of famotidine from the endogenous peaks in the human biological matrices used.

Liquid-liquid extraction (LLE) (Sun *et al.*, 2009; Dowling *et al.*, 1999) solid phase extraction (SPE) (Zhong *et al.*, 1998; Zhong *et al.*, 2011) and protein precipitation (PP)

(Zarghi *et al.*, 2005; Campanero *et al.*, 2001) have been previously reported as methods to extract famotidine from human plasma. In this investigation, both PP and LLE methods were investigated for famotidine extraction recovery. The protein precipitation of plasma by adding methanol or acetonitrile resulted in severe matrix effects. Therefore, LLE was pursued without protein precipitation. For the optimization of the LLE method, the pH and the following organic solvents additive were screened: n-heptane, ethyl acetate, dichloromethane, HCl, NaOH and NH₄OH. The high polarity of famotidine prevented its efficient extraction by an organic solvent under acidic conditions. However, in alkaline conditions, ethyl acetate exhibited good extraction recovery of famotidine. Although higher extraction recovery of famotidine could be obtained by adding NaOH to human plasma, this resulted in the observation of peaks which interfered with the retention time of famotidine peaks. Therefore, NH₄OH was chosen as a pH additive reagent for plasma extraction. In the present study, the optimized extraction recovery of famotidine was achieved by alkalization of plasma with NH₄OH, followed by twice extraction with ethyl acetate.

Assay performance and validation

Selectivity—The selectivity of the method was achieved by comparing SIM chromatograms of six different blank samples of maternal plasma, umbilical cord plasma and urine samples. Figure 2 shows typical SIM chromatograms of blank samples, blank samples spiked with famotidine and the IS, and samples obtained from pregnant patients treated twice a day with a 20 mg dose of famotidine. Endogenous metabolites in maternal plasma, umbilical cord plasma and urine samples did not interfere with the retention times of famotidine and its IS.

Extraction recovery and matrix effects—As shown in Table 1, the extraction recovery of famotidine from maternal and umbilical cord plasma ranged between 53.7% and 79.0% with a variation of < 10% at low, medium and high concentration levels. The Matrix Factor of famotidine in six samples from maternal plasma, umbilical cord plasma and urine at low, medium and high concentration levels ranged between 83% and 96%, with relative standard deviation < 12% (Table 2). These results indicate that there was negligible ion suppression or enhancement from the plasma or urine matrix under the selected conditions.

Linearity and sensitivity—Calibration curves for maternal plasma, umbilical cord plasma and urine samples were constructed by the internal standard method and fitted by weighted least-squares linear regression analysis of internal ratio versus concentration (Table 3). The weighting factor for the calibration curves was selected as 1/y and 1/y² for plasma (maternal or umbilical) and urine quantification, respectively. The calibration curve exhibited good linear regression ($r^2 > 0.99$) within the tested ranges. The lack of fit test of the regression between the concentration and the internal ratio was not significant as determined by the *F*-test at $\alpha = 0.05$ levels. Under the optimized conditions, the LODs were observed to be 0.252 ng/mL in human plasma and 0.015 μ g/mL in urine. The LLOQ was set as the lowest concentration of the calibration curves, and inter- and intra-day accuracy values were in the range of 93.1% to 110.3% with precision < 14% (Table 4).

Accuracy and precision—The results of accuracy and precision for the QC samples at high, medium and low concentration levels are shown in Table 4. The intra-day accuracy for the famotidine ranged between 94.6% and 106.1% with precision < 8.8%, and the inter-day accuracy ranged between 95.5% and 107.0% with precision < 9.3%. These results indicate acceptable reproducibility of the method used.

Stability—The short-term stability tests were performed for 4h at room temperature (22-25°C). The accuracy of the short-term stability tests ranged between 86.5% and 110.8%

with precision of < 9.4% at the low and high QC levels (Table 5). The accuracy after three freeze-thaw cycles ranged between 95.2% and 112.3%, with precision < 3%.

Application for clinical study

This validated analytical method was applied for the quantitative determination of famotidine in human urine, maternal and umbilical cord plasma samples obtained from pregnant patients (n=3). As shown in Figures 3A and 3C, the plasma concentrations of famotidine under steady-state conditions in three pregnant patients' prior drug administration ranged between 6.43 ng/mL and 45.8 ng/mL and the peak concentration ranged between 33.1 ng/mL and 97.3 ng/mL. The concentrations of famotidine in urine ranged between 1.37 µg/mL and 13.4 µg/mL. The concentrations of famotidine in the umbilical venous and arterial plasma were similar (Figure 3B), which suggests that the primary route of famotidine elimination by the fetus is its transfer to the maternal circulation.

Conclusion

This is the first report, to the best of our knowledge, on an LC-MS method for the quantitative determination of famotidine in human umbilical cord plasma. A good retention time (4.5 min) for famotidine was achieved by utilizing a reverse-phase HPLC column and optimizing the composition and pH of the mobile phase. The optimization of the conditions for sample preparation resulted in a sensitive method with an LLOQ < 0.7 ng/mL for human maternal and umbilical cord plasma, and an LLOQ < 0.08 µg/mL for urine samples. This validated method has been used to determine famotidine pharmacokinetics, and its distribution in pregnant patients under treatment with the medication in a clinical trial conducted by the Obstetric-Fetal Pharmacology Research Units Network (OPRU).

Supplementary figure 4. Product ion spectra of $[M+H]^+$ of ^{13}C -famotidine (internal standard).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Figure 1.
Product ion spectra of famotidine $[M+H]^+$.

Figure 2.

The selected ion monitoring (SIM) chromatograms for the determination of famotidine in samples of maternal plasma (**A1-A3**), umbilical cord plasma (**B1-B3**) and urine (**C1-C3**): (**A1**) chromatogram of blank maternal plasma; (**A2**) chromatogram of blank maternal plasma spiked with famotidine (6.31 ng/mL); (**A3**) maternal plasma of a pregnant patient under treatment with 20 mg famotidine (b.i.d.); (**B1**) chromatogram of blank umbilical cord plasma; (**B2**) chromatogram of blank umbilical cord plasma spiked with famotidine (6.31 ng/mL); (**B3**) umbilical cord plasma following delivery from a pregnant patient under treatment with 20 mg famotidine (b.i.d.); (**C1**) chromatogram of blank urine sample; (**C2**)

chromatogram of blank urine sample spiked with famotidine (750 ng/mL); (C3) urine sample of a pregnant patient under treatment with 20 mg famotidine (b.i.d.).

Figure 3.

Representative (A) famotidine plasma concentration-time profiles, (B) concentration of famotidine in maternal, umbilical venous and arterial plasma at delivery, and (C) famotidine urine concentration-time profile in pregnant patients (n=3) prescribed famotidine (20 mg orally, b.i.d.). The blood (A) and urine (C) were collected from late gestation pregnant patients under steady-state conditions. It should be noted that no plasma samples were collected from Subject No. 1 at the time of delivery.

Table 1

Extraction recovery of famotidine in maternal and umbilical cord plasma samples (n=3).

Specimen	Nominal concentration (ng/mL)	Mean Recovery ^a (%)	Precision (RSD, %)
Maternal plasma	1.26	53.7	9.0
	126	54.1	5.4
	252	63.9	8.2
Umbilical cord plasma	1.26	72.5	4.8
	126	76.5	9.8
	252	79.0	5.8

^a Recovery (%) = (peak area of QC sample/peak area of post-extraction sample)×100%.

Table 2

Matrix effect factor of famotidine in maternal plasma, umbilical cord plasma and urine samples (n=6).

Specimen	Nominal concentration	Matrix factor ^a (%)	precision (RSD, %)
Maternal plasma	1.26 ng/mL	91.8	1.5
	126 ng/mL	96.3	0.8
	252 ng/mL	95.2	1.8
Umbilical cord plasma	1.26 ng/mL	86.3	7.1
	126 ng/mL	83.4	1.6
	252 ng/mL	84.4	1.0
urine	0.150 µg/mL	90.2	9.1
	15.0 µg/mL	88.6	10.2
	30.0 µg/mL	89.9	11.3

^a Matrix Factor (%) = (peak area of post-extraction sample/peak area of pure standard)×100%.

Table 3

Calibration curves of famotidine in maternal plasma, umbilical cord plasma and urine samples (n=3).

Specimen	Regression equation ^{a,b}	Weighting	r^2	F -test ^c		Linear range	LOD
				F	p -value		
Maternal plasma	$y=0.026+0.080x$	$1/y$	0.992	0.68	0.667	0.631-252 ng/mL	0.252 ng/mL
Umbilical cord plasma	$y=0.020+0.120x$	$1/y$	0.997	1.16	0.375	0.631-252 ng/mL	0.252 ng/mL
urine	$y=0.015+0.359x$	$1/y^2$	0.987	1.77	0.170	0.075-30.0 μ g/mL	0.015 μ g/mL

^a In the regression equation $y = b + ax$, x refers to the concentration of famotidine in biological samples, and y refers to the peak area ratio of famotidine versus the IS.

^b The unit of concentration of famotidine in maternal and umbilical cord plasma is ng/mL; the unit of concentration of famotidine in urine samples is μ g/mL.

^c F -test for lack-of-fit.

Table 4

Intra-day and inter-day precision and accuracy of the method for maternal plasma, umbilical plasma and urine samples.

Specimen	Nominal concentration ^a	Intra-day (n=6)			Inter-day (n=3)		
		Mean obtained concentration ^a	Mean accuracy ^b (%)	Precision (RSD, %)	Mean obtained concentration ^a	Mean accuracy ^b (%)	Precision (RSD, %)
Maternal plasma	0.631	0.655	103.8	9.6	0.599	95.0	14.0
	1.26	1.20	95.0	8.8	1.24	98.2	9.3
	126	146	104.2	6.9	133	105.6	6.2
	252	247	98.1	7.7	244	99.6	7.7
Umbilical cord plasma	0.631	0.696	110.3	12.8	0.682	93.1	8.4
	1.26	1.26	100.4	7.9	1.26	100.4	6.3
	126	129	107.6	6.6	128	98.4	5.4
	252	240	95.4	4.2	243	103.9	4.5
urine	0.075	0.077	96.7	9.7	0.077	96.5	10.2
	0.150	0.159	106.1	6.0	0.160	107.0	7.9
	15.0	15.2	101.4	2.3	15.2	101.2	4.5
	30.0	28.4	94.6	2.1	28.6	95.5	3.0

^a The unit of concentration of famotidine in maternal and umbilical cord plasma is ng/mL; the unit of concentration of famotidine in urine samples is µg/mL.

^b accuracy (%) = (mean obtained concentration/nominal concentration)×100%.

Table 5

Stability of famotidine in maternal plasma, umbilical cord plasma and urine samples (n=3).

Specimen	Nominal concentration ^a	Freeze and Thaw stability (3 cycles, –80°C)			Short-term Stability (4h, 22-25°C)		
		Mean obtained concentration ^a	Mean accuracy ^b (%)	Precision (RSD, %)	Mean obtained concentration ^a	Mean accuracy ^b (%)	Precision (RSD, %)
Maternal plasma	1.26	1.20	95.2	1.4	1.15	91.4	7.5
	252	250	99.4	2.9	218	86.5	6.0
Umbilical cord plasma	1.26	1.40	111.0	1.3	1.40	110.8	3.4
	252	238	94.7	1.4	232	92.0	4.1
urine	0.150	0.168	112.3	1.6	0.153	102.0	9.4
	30.0	29.6	98.5	0.5	29.9	99.6	8.0

^aThe unit of concentration of famotidine in maternal and umbilical cord plasma is ng/mL; the unit of concentration of famotidine in urine samples is µg/mL.

^baccuracy (%) = (mean obtained concentration/nominal concentration)×100%.