High-Performance Liquid Chromatography–Electrospray Ionization-Mass Spectrometric Determination of Emedastine Difumarate in Human Plasma and Its Pharmacokinetics

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Abstract

A selective and sensitive method employing high-performance liquid chromatography (HPLC)–electrospray ionization (ESI)-mass spectrometry is developed and validated for the determination of emedastine difumarate in human plasma. With naphazoline hydrochloride as the internal standard, emedastine difumarate is extracted from plasma with ethyl acetate. The organic layer is evaporated, and the residue is redissolved in the mobile phase. An aliquot of 10 µL is chromatographically analyzed on a prepacked Phenomenex Luna 5u CN 100A (150 × 2.0-mm i.d.) column, using a mobile phase comprised of methanol–water (20mM CH_3COONH_4, pH 4.0) (80:20, v/v). Standard curves are linear (r^2 = 0.9990) over the concentration range of 0.05–30 ng/mL and had good accuracy and precision. The within- and between-batch precisions did not exceed 15% for the relative standard deviation. The lower limit of detection is 0.01 ng/mL. The validated HPLC–ESI-MS method is successfully used to study emedastine difumarate pharmacokinetics in 12 healthy volunteers.

Introduction

Emedastine difumarate, [1-(2-ethoxyethyl)-2-(hexahydro-4-methyl-1-H-1,4-diazepin-1-yl) benzimidazole difumarate], a second-generation H_1-receptor antagonist, has been shown to suppress anaphylactic shock, passive cutaneous anaphylaxis, and allergic rhinitis in various experimental models in rats and guinea pigs (1,2). In man, emedastine qualified as a suitable alternative to reference H_1-receptor antagonists because of its high therapeutic index and less potent effects on the circulation central nervous system compared with established antihistamines (3). Since 1993, emedastine has been licensed in Japan to treat allergic rhinitis and urticaria. Currently, two dosages (2 mg b.i.d. or 4 mg o.d.) are administered (4).

The therapeutic dose of emedastine difumarate was only 4 mg every day, given orally. The test tablet in this experiment was an extended-release formulation, so the concentration of emedastine in plasma was extremely low (4,5). A sensitive analytical method was needed for its determination. A radioimmunoassay (RIA) method for the determination of emedastine difumarate in serum and urine has previously been reported (4–6). Although the RIA method was sensitive, it was not selective enough for a pharmacokinetic study. Besides, a capillary gas chromatography (with a nitrogen-sensitive detector) method has been reported for the determination of emedastine difumarate in dog plasma (7). The lower limit of detection (LLOD) (1 ng/mL) reported in this study was not adequate to monitor the therapeutic levels needed. In this paper, a simple, selective, and highly sensitive method by using high-performance liquid chromatography (HPLC) coupled with electrospray ionization (ESI)-single quadrupole mass spectrometry (MS) is described for the determination of emedastine difumarate in human plasma.

Experimental

Reagents

Emedastine difumarate extended-release tablets (batch no. 20040329) and emedastine difumarate reference standard (99.0% purity) were supplied by Shandong Z.D.F.R.D. Pharmaceutical (Shandong, P.R. China), and Naphazoline

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hydrochloride reference standard [internal standard (IS), 98.5% purity] was supplied by Jiangsu Institute for Drug Control (Nanjing, P.R. China). HPLC-grade methanol was purchased from VWR (Darmstadt, Germany). Other chemicals were all of analytical grade and were used as received. Water was purified by redistillation before use.

Liquid chromatography conditions

Separation was performed using a Shimadzu LC-10AD HPLC system (Kyoto, Japan) equipped with an autosampler (SIL-HTc). The column was a Phenomenex Luna 5u CN 100A (150 × 2.0 mm i.d.) and was operated at 35°C. The mobile phase consisted of methanol–water (20mM CH_3COONH_4, pH 4.0) (80:20, v/v) and was set at a flow rate of 0.2 mL/min.

MS conditions

MS detection was performed using a Shimadzu LCMS-2010A quadrupole MS with an ESI interface. The ESI source was set at positive ionization mode. The $(M+H)^+$ ion (*m/z* 303.15) was used for emedastine and the $(M+H)^+$ ion (*m/z* 211.10) was used for naphazoline as the detection ions, respectively. The MS operating conditions were optimized as follows: nebulizer gas rate, 1.5 L/min; curved desolvation line temperature, 250°C; block temperature, 200°C; and probe voltage, +4.5kV. The quantitation was performed via peak-area ratio (peak area of emedastine/peak area of the IS). Data acquisition and processing were accomplished using Shimadzu LCMS solution Software for the LCMS-2010A system.

Stock solutions

Stock solutions of emedastine difumarate and naphazoline hydrochloride were prepared in HPLC-grade methanol at a concentration of 1.0 mg/mL and were stored at 4°C. Working solutions of emedastine difumarate were prepared daily in HPLC-grade methanol by appropriate dilution at 2.5, 5.0, 15.0, 50.0, 150.0, 250.0, and 500.0 ng/mL and 1.0 and 1.5 µg/mL. The stock solution of naphazoline hydrochloride was further diluted with HPLC-grade methanol to prepare the working IS solution containing 100 ng/mL of naphazoline hydrochloride.

Sample preparation

For extraction of emedastine from a 1 mL plasma sample, 10 µL IS solution (100 ng/mL), 100 µL 1M NaOH, and 5 mL ethyl acetate were added to the plasma sample, and the mixture was vortexed thoroughly for 2 min. After centrifugation (1600 × *g*, 10 min), the organic layer was transferred to another 10-mL centrifuge tube and evaporated to dryness under a gentle stream of nitrogen gas in a water bath at 40°C. The residue was redissolved in 100 µL mobile phase, and vortexed for 30 s. After centrifugation (7470 × *g*, 8 min), an aliquot of 10 µL was injected into the LC–MS system.

Assay specificity

The specificity of the method was tested by analyzing blank plasma samples from healthy humans obtained from five sources. Each blank sample was tested for interference by using the proposed extraction procedure and chromatographic–MS conditions and compared with those obtained from an aqueous solution of analyte at a concentration near to the lower limit of quantitation (LLOQ).

Calibration curve

A calibration curve was generated to confirm the linear relationship between the peak area ratio of emedastine–naphazoline versus the concentration of emedastine. One milliliter of blank control human plasma, 10 µL IS solution (100 ng/mL), and 20 µL of one of the previously-mentioned emedastine working solution was added in the 10-mL centrifuge tube, respectively, to yield final concentrations of 0.05, 0.1, 0.3, 1, 3, 5, 10, 20, and 30 ng/mL. These samples, with known amounts of emedastine, were extracted as described in the Sample preparation section. In each run, a blank plasma sample was also analyzed.

Quality control samples

Quality control (QC) samples were prepared in blank control plasma at concentrations of 0.1, 5, and 10 ng/mL. The following procedures were the same as previously described. QC samples at three concentrations (0.1, 5, and 10 ng/mL) were prepared and analyzed at the same time as the test samples. The total amount of the QC samples was 5% of the test samples in the same batch.

Precision and accuracy

Precision and accuracy were assessed by determining QC samples at three concentrations (0.1, 5, and 10 ng/mL). Within-batch precision and accuracy were determined by repeated analysis of a group of standards on one day (n = 5). Between-batch precision and accuracy were determined by repeated analysis on three days (n = 5 series per day, n = 15 in total). The concentration of each sample was determined using the standard curve prepared and analyzed on the same day.

LLOD and LLOQ

The LLOD and the LLOQ were determined as the concentrations with a signal-to-noise ratio of 3 and 10, respectively.

Stability

The stability of emedastine difumarate in frozen plasma (–20°C) of three concentrations (0.1, 5, and 10 ng/mL) were studied over 2 months. The stability of the processed samples left at room temperature (25 ± 3°C) for 24 h after evaporating to dryness under nitrogen gas was studied. The stability of the processed samples left in the autosampler (4°C) for 24 h after redissolved in 100 µL mobile phase was also studied.

Recovery

The extraction recovery of emedastine was determined by comparing the emedastine–IS peak area ratios obtained from extracted plasma samples with those from standard solutions at the same concentration. This procedure was repeated for the three different concentrations of 0.1, 5, and 10 ng/mL (n = 5)

Clinical study method

Volunteer selection

The study involved six healthy male volunteers (mean age

35 ± 4, mean weight 66 ± 5 kg, and mean height 171 ± 4 cm) and six female volunteers (mean age 30 ± 5, mean weight 55 ± 6 kg, and mean height 161 ± 4 cm).

Study design

The study included three parts: a single-dose, multiple-dose, and food effect study. The single-dose study was an open-label, randomized, complete three-way crossover study. Each subject received the following doses: 2, 4, and 8 mg. The subjects were randomly distributed into three groups (A, B, and C) and were administrated according to the latin squares (Table I). Venous blood was drawn at pre-specified time points during the administration days.

In the multiple-dose study, 12 volunteers were administered emedastine difumarate for 5 consecutive days, according to 2 mg b.i.d. (dose at 7:00 and 19:00), and the last dose was given on the morning of day 6. The volunteers fasted for 2 h before and after administration. Venous blood was drawn on the morning of days 4 and 5 before administration to make sure the steady state appeared. During day 6, blood was drawn again following the pre-specified time points after the last dose.

In the food effect study, the volunteers were randomly distributed into two groups (E and F). One group of subjects was given a light meal 4 h after administration. Another group of subjects were permitted to have meal immediately after the administration. The volunteers were administered as seen in Table II. Venous blood was drawn at pre-specified time points in the administration days. There was a 7-day washout period between every two treatment periods.

Sampling

Blood samples (4 mL) were collected immediately before dose and at 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 15, 24, and 36 h. The blood samples were collected into the heparinized collection tubes and were gently mixed. Plasma was separated by centrifugation (at 1600 × *g* for 5 min). The aliquots of plasma were transferred to other tubes and stored at –20°C until analysis.

Table I. Design of Drug Administration in Single-Dose Study

	Treatment period		
Group	1	2	3
A	a*	b	c
B	b†	c	a
C	c‡	a	b

* a = 2 mg.
† b = 4 mg.
‡ c = 8 mg.

Table II. Design of Drug Administration in Food Effect Study

	Treatment period	
Group	1	2
E	e*	f
F	f†	e

* e = food.
† f = fasting.

Result and Discussion

IS selection

It was necessary to use an IS to get a high accuracy when HPLC was equipped with MS as the detector. Naphazoline hydrochloride was adopted in the end because of its similarity of retention action, ionization, and extraction efficiency to emedastine. The structures of the emedastine difumarate and naphazoline hydrochloride are shown in Figure 1.

Sample preparation

Liquid–liquid extraction cannot only purify, but also concentrate the sample. Ethyl acetate, dichlormethane–ethyl acetate (20:80,v/v), and *n*-hexane–isopropanol (95:5, v/v) were all considered. Ethyl acetate was found to be the most suitable because of the highest extraction efficiency. Alkalifying and acidifying the sample was also considered. Alkalization by 100 µL NaOH (1M) was adopted at last because of the high recovery and no interference.

Chromatographic condition optimization

The shape of the peak was more symmetrical when ammonium acetate was added to the water phase of the mobile phase. Several different concentrations of ammonium acetate were investigated, and the result of 20mM ammonium acetate was

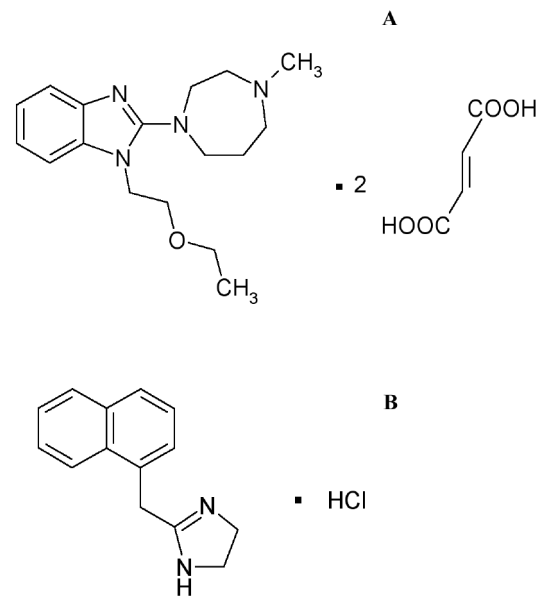

Figure 1. The structures of emedastine difumarate (A) and naphazoline hydrochloride (B).

ideal. The acidification of the mobile phase permitted better detection of the analytes in positive molecular ions.

MS condition optimization

MS analysis was carried out using ESI. It was found that the *m/z* value of 303.15 $[M+H]^+$ and 211.10 $[M+H]^+$ were associated with the parent compound of emedastine and naphazoline. They also had a high abundance and stability. Therefore, the *m/z* of 303.15 and 211.10 in SIM mode was set for the detection. The retention times of emedastine and naphazoline (IS) were 7.2 and 6.2 min, respectively, which is shown in Figure 2D. They were well separated, which avoided the interference of ionization between them.

Assay performance

Assay performance of the present method was assessed by the following criteria: specificity, linearity, precision, accuracy, LOQ, LOD, stability, and recovery. The chromatograms were visually inspected for interfering chromatographic peaks from endogenous substances. No visual interference was observed. (Figure 2A).

Standard curves of nine concentrations of emedastine difumarate, ranging from 0.05–30 ng/mL, were extracted and assayed. Blank samples were not used to construct the calibration function. The qualification on emedastine difumarate in plasma samples was carried out by determining the slope (*b*), intercept (*a*), and regression (*r*) of the standard curves of the peak area ratio of emedastine–naphazoline (R) versus the concentration of emedastine (C). Using linear regression analysis, the data confirmed linear relationships over the selected concentration rage. Five standard curves in five days were constructed. The mean standard curve was typically described by the least-square equation:

$$C = 1.4456 \times R - 0.0091 \ (r^2 = 0.9990) \qquad \text{Eq.1}$$

Table III. Regression Parameters for the Calibration Curves of Emedastine Difumarate in Human Plasma

Batch	Slope (*b*)	Intercept (*a*)	r^2
1	1.5016	–0.0185	0.9986
2	1.4208	–0.0073	0.9958
3	1.4088	–0.0047	0.9956
4	1.3996	–0.0051	0.9991
5	1.4556	–0.0081	0.9980

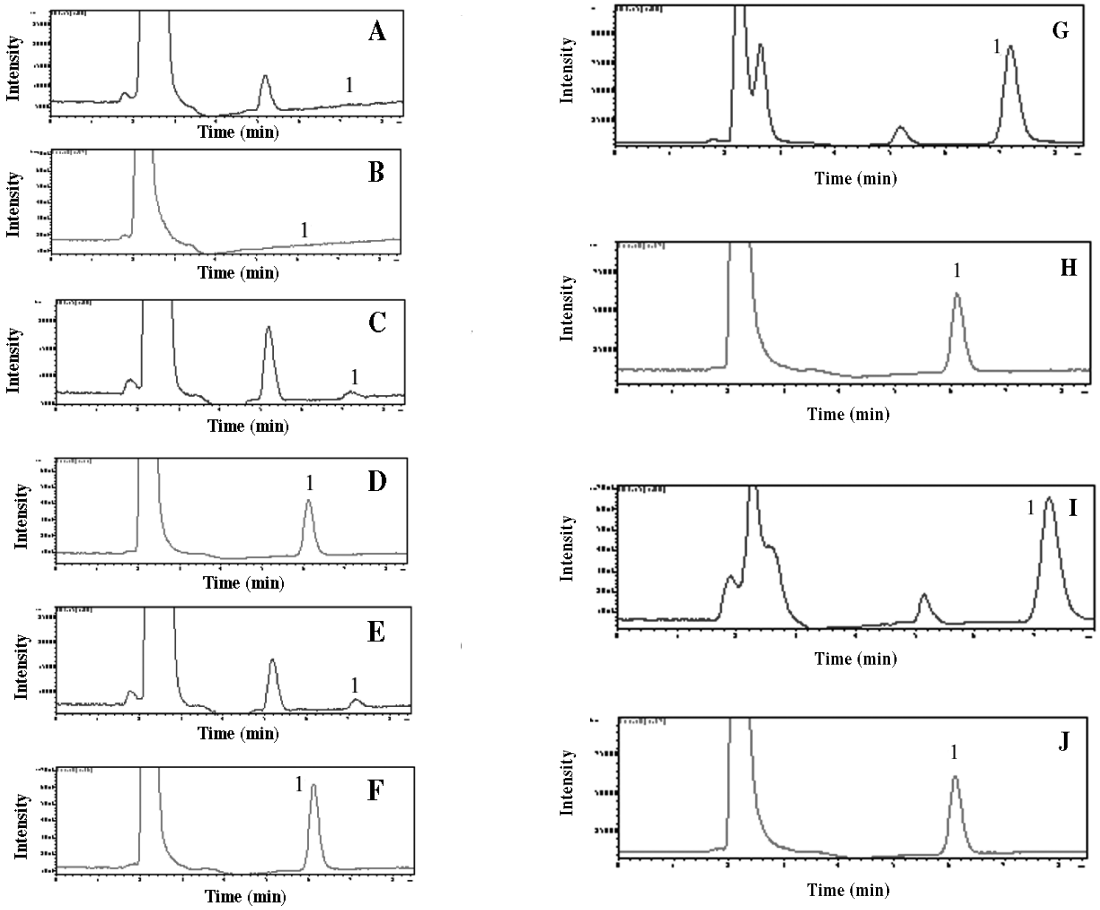

Figure 2. The retention times of emedastine difumarate and IS were 7.2 and 6.2 min, respectively. Blank plasma (A and B), LOD (C and D), LOQ (E and F), supplemented plasma (G and H), and volunteer plasma (I and J). (The peaks of emedastine difumarate and IS were labeled by 1).

Table IV. Precision and Accuracy of Within- and Between-Batch of Method for the Determination of Emedastine Difumarate by LC–MS

	Within-batch (n = 5)			Between-batch (n = 15)		
Add concentration (ng/mL)	Detected concentration (mean ± SD* ng/mL)	RSD (%)	Mean accuracy (%)	Detected concentration (mean ± SD ng/mL)	RSD (%)	Mean accuracy (%)
0.10	0.101 ± 0.009	9.37	100.62	0.099 ± 0.011	10.99	99.32
5.00	4.85 ± 0.32	6.60	97.04	4.87 ± 0.41	8.38	97.33
10.00	10.42 ± 0.59	5.64	104.16	10.46 ± 0.76	7.28	104.60

* SD = standard deviation.

Table V. Recovery of the Method for the Determination of Emedastine Difumarate by LC–MS (n = 5)

Added concentration (ng/mL)	Recovery (mean ± SD %)	RSD (%)
0.10	87.04 ± 7.14	8.45
5.00	89.30 ± 5.96	6.48
10.00	93.96 ± 4.14	4.15

Table VI. Data Showing Stability of Emedastine Difumarate

Added concentration (ng/mL)	0.10 Accuracy (mean ± SD %)	0.50 Accuracy (mean ± SD %)	10.00 Accuracy (mean ± SD %)
Long-term stability (2 months, –20°C)	111.16 ± 11.05	102.91 ± 7.23	99.82 ± 5.42
Short-term stability			
24 h, room temperature (25 ± 3°C)	107.77 ± 9.01	101.08 ± 11.22	100.32 ± 6.51
24 h, autosampler (4°C)	101.13 ± 10.03	103.78 ± 7.61	103.98 ± 7.10

The results of five standard curves are given in Table III. The LOQ of the method was 0.05 ng/mL, and the LOD was 0.01 ng/mL. The chromatograms of the LOQ and LOD are shown in Figures 2B and 2C. Data for within-batch and between-batch precision and accuracy of the method for emedastine difumarate is given in Table IV. The accuracy was within acceptable limits. The precision did not exceed 15% for the relative standard deviation (RSD). The data showed the good precision and accuracy of the method.

The extraction recovery of the method was above 85% and shown to be consistent, precise, and reproducible. Data is shown in Table V. Table VI summarizes the data of long-term stability, short-term stability at room temperature, and autosampler of emedastine. All the results showed the stability behavior during these tests, and there were no stability-related problems during the samples routine analysis for the pharmacokinetic, bioavailability, or bioequivalence studies.

Applicability in pharmacokinetic study

In the single- and mutiple-dose studies,

Table VII. Pharmacokinetic Parameters of 12 Health Volunteers After Oral Administration of Emedastine Difumarate in Single-Dose, Multiple-Dose, and Food Effect Study

	Single dose			Multiple dose	Food-intake	Fasting
Parameters	2 mg (o.d.)	4 mg (o.d.)	8 mg (o.d.)	2 mg (b.i.d.)	2 mg (o.d.)	2 mg (o.d.)
$T_{1/2}$ (h)	6.66 ± 0.55	6.60 ± 0.98	6.71 ± 1.40	8.81 ± 4.28	6.16 ± 2.58	6.72 ± 3.85
MRT (h)	11.34 ± 1.04	10.84 ± 1.46	11.41 ± 1.78	12.37 ± 4.85	11.57 ± 3.08	11.72 ± 4.25
T_{max} (h)	3.83 ± 1.70	3.75 ± 1.54	4.17 ± 0.94	3.42 ± 1.08	5.00 ± 1.28	4.00 ± 0.74
C_{max} (ng/mL)	5.13 ± 1.73	9.64 ± 3.66	20.64 ± 6.32	9.24 ± 4.75	2.67 ± 1.06	5.81 ± 1.33
$AUC_{0\sim36}$ (ng h/mL)	52.87 ± 14.72	92.73 ± 45.45	238.59 ± 112.64		22.16 ± 13.72	40.35 ± 15.94
$AUC_{0\sim\infty}$ (ng h/mL)	54.61 ± 15.62	96.04 ± 47.41	248.16 ± 118.41		23.19 ± 13.95	42.86 ± 16.63
C_{min} (ng/mL)				4.29 ± 2.82		
C_{av} (ng/mL)				9.27 ± 4.75		
AUC_{ss} (ng h/mL)				5.52 ± 3.72		
DF				0.95 ± 0.35		

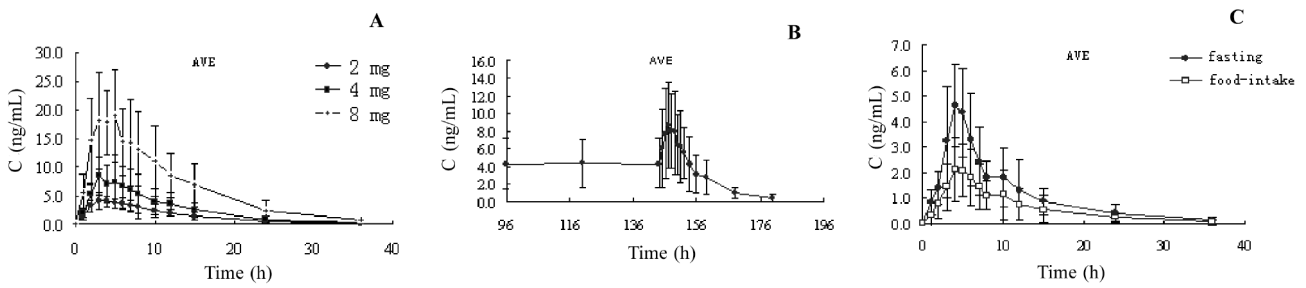

Figure 3. Mean drug plasma concentration-time curve of emedastine difumarate in 12 volunteers after oral administration of emedastine difumarate in a single-dose study (A), multiple-dose study (B), and food effect study (C).

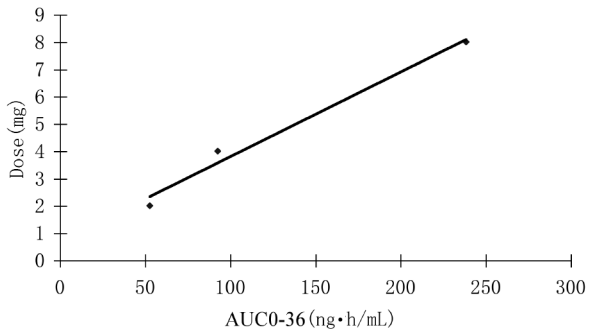

Figure 4. The linear relationship between AUC and dose i.

the mean plasma concentration-time curve of emedastine difumarate in 12 volunteers after oral administration of emedastine difumarate is shown in Figures 3A and 3B. The pharmacokinetic parameters are listed in Table VII. The data in single-dose study showed the linear relationship (r^2 = 0.9956, Figure 4.) between the area under the plasma concentration (AUC) time cuve and dose. There were no significant differences of mean residence time, $t_{1/2}$, and T_{max} between the three dosages, indicating a pharmacokinetic linearity of the three dosages within the studied dose range. Therefore, a preliminary conclusion that the absorption, distribution, metabolism, and excretion of emedastine in the human body coincide to the process of first order kinetics could be made. Although the mean value of C_{max} and AUC_{ss} of steady-state levels in a mul-

Table VIII. Pharmacokinetic Parameters of Different Genders of 12 Health Volunteers After Oral Administration of Emedastine Difumarate in Single-Dose, Multiple-Dose, and Food effect Study

		Single dose			Multiple dose	Food-intake	Fasting
Parameters	Gender	2 mg (o.d.)	4 mg (o.d.)	8 mg (o.d.)	2 mg (b.i.d.)	2 mg (o.d.)	2 mg (o.d.)
$T_{1/2}$ (h)	male	6.77 ± 0.55	7.00 ± 0.69	7.25 ± 1.11	10.47 ± 5.03	7.59 ± 2.86	7.36 ± 1.65
	female	6.55 ± 0.53	6.20 ± 0.89	6.17 ± 1.54	7.14 ± 2.90	4.74 ± 1.28	6.08 ± 5.38
MRT (h)	male	11.36 ± 1.04	11.35 ± 1.12	12.35 ± 1.17	14.27 ± 5.73	13.55 ± 3.02	13.35 ± 4.88
	female	11.32 ± 1.14	10.34 ± 1.68	10.46 ± 1.87	10.48 ± 3.22	9.58 ± 1.50	10.10 ± 3.09
T_{max} (h)	male	3.83 ± 1.70	3.67 ± 0.82	4.33 ± 1.03	3.17 ± 0.98	5.17 ± 1.60	4.17 ± 0.75
	female	3.83 ± 2.23	3.83 ± 2.14	4.00 ± 0.89	3.67 ± 1.21	4.83 ± 0.98	3.83 ± 0.75
C_{max} (ng/mL)	male	5.12 ± 1.73	10.87 ± 4.03	21.03 ± 8.29	9.49 ± 3.52	2.54 ± 1.39	6.11 ± 0.90
	female	5.13 ± 2.24	8.41 ± 3.11	20.26 ± 4.35	8.98 ± 6.10	2.80 ± 0.73	5.51 ± 1.70
$AUC_{0\sim36}$ (ng h/mL)	male	51.66 ± 14.72	99.16 ± 42.07	245.40 ± 129.60		20.90 ± 13.92	48.33 ± 16.88
	female	54.07 ± 18.69	86.30 ± 51.73	231.77 ± 104.90		23.43 ± 17.71	32.37 ± 11.03
$AUC_{0\sim\infty}$ (ng h/mL)	male	53.58 ± 15.62	100.82 ± 43.14	257.64 ± 135.37		22.54 ± 14.51	51.47 ± 17.00
	female	55.64 ± 19.95	89.25 ± 54.52	238.67 ± 110.91		23.84 ± 14.72	34.25 ± 11.91
C_{min} (ng/mL)	male				3.99 ± 1.78		
	female				4.60 ± 3.76		
C_{av} (ng/mL)	male				5.46 ± 2.03		
	female				5.58 ± 5.12		
AUC_{ss} (ng h/mL)	male				65.49 ± 24.40		
	female				66.94 ± 61.50		
DF	male				1.05 ± 0.35		
	female				0.85 ± 0.34		

tiple-dose study were higher than in single-dose study, the mean value of MRT, and T_{max} were similar to that in a single dose. Degree of fluctuate (freedom) less than 1 manifested the good delayed release character of the praeparatum.

In the food effect study, the mean plasma concentration-time curve of emedastine difumarate in 12 volunteers after oral administration of emedastine difumarate is shown in Figure 3C. MRT and $t_{1/2}$ obtained under food intake condition were similar to that obtained under fasting conditions, and T_{max}, C_{max}, and AUC obtained under food intake conditions were significantly lower than that obtained under fasting conditions. The result indicated that the elimination rate and degree of emedastine were cut down under the food intake condition. The pharmacokinetic parameters are listed in Table VII. Table VIII compares the parameters between male and female volunteers. A preliminary conclusion that emedastine is equally absorbed, distributed, metabolized, and excreted in both genders could be made. But future studies will be needed to support this conclusion.

Conclusion

The paper describes a sensitive, specific, accurate, and precise LC–MS method for the determination of emedastine difumarate in human plasma. The short analysis time makes the method suitable for analysis of large sample batches resulting from the pharmacokinetic, bioavailability, or bioequivalent study. The pharmacokinetic parameters on single- and multiple-dose as well as food effect studies in different genders can give some useful information for the development and proper clinical application of the medicine.

References

1. T. Fukuda, T. Saito, S. Tajima, K. Shimohara, and K. Ito. Anti - allergic effect 1-(2-ethoxyethyl)-(4-methyl-1-homopiperazinyl)-benzimidazole difumarate (KB-2413). *Arzneim-Forsch.* **34:** 805–10 (1984).
2. T. Saito, N. Nishimura, S. Tajima, T. Fukuda, and K. Ito. Anti-allergic effects of 1-(2-ethoxyethyl)-(4-methyl-1-homopiperazinyl)-benzimidazole difumarate (KB-2413). *Nippon Yakurigaku Zasshi* **89:** 55–62 (1987).
3. K. Tasaka, C. Kamei, S. Katayama, K. Kitazumi, H. Akahori, and T. Hokonohara. Comparative study of various H_1-blockers on neuropharmacological and behavioral effects including 1-(2-ethoxyethyl)-(4-methyl-1-homopiperazinyl)-benzimidazole difumarate (KB-2413). *Arch. Iternat. Pharmacodynam.* **280:** 275–91 (1986).
4. B. Jansen, U. Graselli, S. Dallinger, B. Kiss, V. Wacheck, H Schlagbauer-Wadl, A. Assandri, and M. Müller. Pharamacodynamics of the novel H_1-receptor antagonist emedastine in healthy volunteers. *Eur. J. Clin. Pharmacol.* **55:** 837–41 (2000).
5. M. Brunner, K. Kletter, A. Assandri, M. Ermanno Corrado, H.G. Eichler, and M. Müller. Pharmacokinetic and mass balance study of unblabelled and ^{14}C-labelled emedastine difumarate in healthy volunteers. *Taylor & Francis Health Sci.* **32:** 761–70 (2002).
6. C. Joukhadar, U. Herranz, T. Pernersoyrfer, A. Asssandri, N. Klein, C. Schrolnberger, E. Lackner, Hans G. Eichler, and M. Müller. Pharmacokinetics of emedastine difumarate, a New Anti-histaminic agent in patients with renal impairment. *Eur. J. Clin. Pharmacol.* **56:** 905–10 (2001).
7. T. Hamada, M. Kadowaki, Y. Nakamura, and N. Awata. Determination of the Antiallergic Agent KB-2413 in plasma by means of capillary gas chromatography with a nitrogen-sensitive detector. *Chem. Pharm. Bull.* **34:** 1168–71 (1986).

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