Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Pharmaceutical and Biomedical Analysis
34 (2004) 1013–1019
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

High-performance liquid chromatographic method for the bioequivalence evaluation of desloratadine fumarate tablets in dogs

Lihe Liu [a], Meiling Qi [b,*], Peng Wang [c], Haozhi Li [a]

[a] *School of Pharmacy, Shenyang Pharmaceutical University, Shenyang, PR China*
[b] *School of Chemical Engineering and Materials Science, Department of Chemistry, Beijing Institute of Technology, 5 South Street, Zhong Guan Cun, Beijing, PR China*
[c] *Shenyang Pharmtech Institute of Pharmaceuticals, Shenyang, PR China*

Received 23 August 2003; received in revised form 7 November 2003; accepted 13 November 2003

Abstract

A simple HPLC method was developed for the determination of desloratadine in dog plasma and was used for evaluating the bioequivalence of desloratadine fumarate tablets and desloratadine tablets in dogs. Chromatographic separation was performed on a Hypersil CN column (150 mm × 5.0 mm, 5 μm) using a mixture of methanol, acetonitrile and phosphate buffer (pH 5.5; 0.01 mol/l) (35:35:30, v/v/v) as mobile phase delivered at a flow rate of 0.8 ml/min. The detection was set at 241 nm. The limit of quantitation was 5.0 ng/ml. The calibration range was from 5.0 to 800.0 ng/ml. Inter- and intra-day precision ranged from 1.8 to 3.8% and from 2.2 to 9.0%, respectively. The recovery of desloratadine from dog plasma ranged from 78.8 to 82.0%. The developed method was applied to the bioequivalence studies of desloratadine fumarate tablets (test preparation) and desloratadine tablets (reference preparation) in five dogs. Pharmacokinetic parameters t_{max}, C_{max}, AUC_{0-t}, $AUC_{0-\infty}$, $t_{1/2}$ were determined from plasma concentration-time profiles of both preparations. The analysis of variance (ANOVA) did not show any significant difference between the two preparations and 90% confidence intervals fell within the acceptable range for bioequivalence. Based on these statistical inferences it was concluded that the two preparations exhibited comparable pharmacokinetic profiles and that desloratadine fumarate tablets was bioequivalent to desloratadine tablets.

Keywords: Desloratadine fumarate; HPLC; Bioequivalence

1. Introduction

Desloratadine (Fig. 1A), the major metabolite of loratadine, is a selective peripheral histamine H_1-receptor antagonist devoid of any substantial effects on the central and autonomic nervous system [1]. Compared with loratadine, desloratadine shows a higher affinity for histamine H_1 human receptors. In vivo, desloratadine exhibits two- to three-fold greater oral potency over loratadine in inhibiting the histamine-induced wheal and flare skin response in the guinea-pigs [2].

On account of the poor solubility and instability of desloratadine to light and heat, desloratadine fumarate

* Corresponding author. Tel.: +86-10-68912668; fax: +86-10-68913293.
E-mail addresses: mlqi@bit.edu.cn, qi_ml@sohu.com (M. Qi).

0731-7085/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2003.11.002

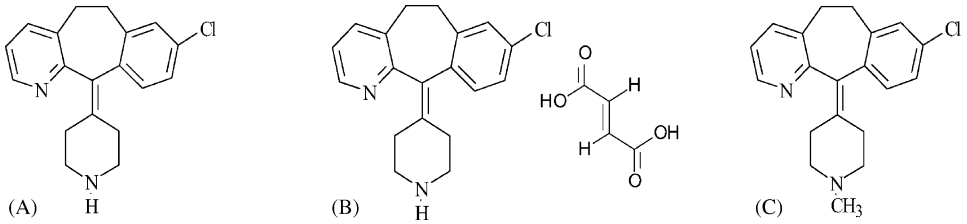

Fig. 1. Chemical structures of (A) desloratadine, (B) desloratadine fumarate and (C) *N*-methyl-desloratadine (internal standard).

(Fig. 1B) was developed to achieve improvement of the above properties. Desloratadine fumarate exhibits good solubility and high stability in vitro. But it is unknown if desloratadine fumarate can show comparable pharmacokinetic behavior with desloratadine in vivo. Therefore, an analytical method is needed for the determination of the concentrations of desloratadine in plasma to evaluate the bioequivalence between the two drugs.

A recent literature survey revealed that some methods have been used for the determination of desloratadine in plasma, such as high performance liquid chromatography (HPLC) [3,4], gas chromatography (GC) [5] and HPLC-MS [6–8]. HPLC methods are preferred in most of laboratories for its high efficiency of separation and accuracy. But the HPLC methods available for the determination of desloratadine in plasma adopted either fluorescence detection or mass spectrometry detection. With fluorescence detection, a complex and time consuming process of derivatization was often involved. With mass spectrometry detection, it was often costly and not suitable for ordinary laboratories. This paper describes a simple, specific and accurate HPLC method with ultraviolet detection for the determination of desloratadine in dog plasma and its application to bioequivalence evaluation of desloratadine fumarate tablets and desloratadine tablets in dogs.

2. Experimental

2.1. Reagents and chemicals

Desloratadine (ca. 99.8% purity), *N*-methyl-desloratadine (ca. 99.6% purity) and desloratadine fumarate were from Shenyang Pharmtech Institute of Pharmaceuticals (Shenyang, China). Desloratadine fumarate tablets (each tablet containing 6.9 mg of desloratadine fumarate, equivalent to 5 mg desloratadine) as test preparation and desloratadine tablets (each tablet containing 5 mg desloratadine) as reference preparation were also from Shenyang Pharmtech Institute of Pharmaceuticals. HPLC-grade methanol and acetonitrile were purchased from Shandong Yuwang Reagent Company (Jinan, China). Toluene, isopropyl alcohol, sodium hydroxide and potassium dihydrogen phosphate were all analytical-grade chemicals from Shenyang Reagent Company (Shenyang, China).

2.2. Instrumentation

Chromatographic separation was performed with an HP series 1100 chromatographic system equipped with a G1310A iso pump, a G1314A variable UV-Vis detector, a 3395 integrator and a G1328A manual injector with 20 μl loop (Agilent, USA). A Shimadzu UV-2201 UV-Vis double-beam spectrophotometer (Shimadzu, Japan) was used for scanning and selecting the detection wavelength.

2.3. Drug administration and sample collection

This study was based on a single-dose, randomized, two-treatment and two-period crossover design. In the morning of phase I, after an overnight fast (10 h), five dogs (weighing from 14 to 21 kg) were given single dose of either two desloratadine fumarate tablets (a dose equivalent to 10 mg desloratadine) or two desloratadine tablets (a dose equivalent to 10 mg desloratadine). No food was allowed until 4 h after dose administration, while water intake was free. About 4 ml of blood samples were collected from the foreleg vein before (0 h) and at 0.5, 1, 1.5, 2, 3, 4, 6, 9, 12, 24, 36, 48 and 58 h after dosing. Plasma was separated by centrifugation at 3000 × *g* for 10 min and kept frozen at −20 °C until analysis. After a washout period of 7

days, the study was repeated in the same manner to complete the cross-over design.

2.4. Sample preparation

To 1.0 ml of dog plasma, 100 μl of methanol, 100 μl of *N*-methyl-desloratadine solution (internal standard, 3 μg/ml) and 100 μl of 1 mol/l sodium hydroxide were added. To this mixture, 2 ml of a mixture of toluene-isopropyl alcohol (95:5, v/v) was added, vortex mixed for 5 min and centrifuged at 3000 × *g* for 5 min. The separated organic phase was evaporated to dryness under N_2 at room temperature. The residue was reconstituted with 100 μl of mobile phase and 20 μl was injected onto the HPLC column. Standard samples were prepared by spiking blank plasma with known amount of desloratadine and used for construction of calibration curves.

2.5. Chromatographic conditions

Separation was achieved on a Hypersil CN column (150 mm × 5.0 mm, 5 μm) from Elite (Dalian, China). The mobile phase consisted of a mixture of methanol, acetonitrile and potassium dihydrogen phosphate buffer (pH 5.5; 0.01 mol/l) (35:35:30, v/v/v). The flow rate was maintained at 0.8 ml/min. Chromatography was performed at room temperature. The detection was made at 241 nm. The run time was about 14 min.

2.6. Data analysis

Pharmacokinetic analysis was performed by means of a model independent method. The maximum concentration (C_{max}) and the corresponding peak time (t_{max}) were determined by the inspection of the individual drug plasma concentration-time profiles. The terminal elimination rate constant (k_e) was determined by least-square regression analysis of terminal log-linear portions of the plasma concentration-time profile ($k_e = -2.303 \times$ slope). The elimination half-life ($t_{1/2}$) was calculated as $0.693/k_e$. The area under the curve to the last measurable concentration (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve extrapolated to infinity ($AUC_{0-\infty}$) was calculated as$AUC_{0-t} + C_t/k_e$, where as C_t is the last measurable concentration. The relative bioavailability (*F*) of desloratadine fumarate tablets was calculated as follows: $F = AUC_{test}/AUC_{reference}$, where the subscripts indicate the preparation.

2.7. Statistical analysis

For the purpose of bioequivalence analysis, AUC was considered as a primary parameter. Bioequivalence was evaluated by means of an analysis of variance (ANOVA) for cross-over design and calculating standard 90% confidence intervals (CI) of the ratio of test/reference using log-transformed data. The products were considered bioequivalent if the difference between compared parameters fell within 80–125%. The acceptance range for C_{max} may be wider than that for AUC, particularly for drugs having highly variable peak concentrations; the recommended range for C_{max} is 70–143% [9–11].

3. Results and discussion

3.1. Development of the HPLC method

Different liquid-liquid extraction (LLE) conditions were evaluated with various aqueous pH values and different organic solvents for the extraction of the drug. As a result, a 0.1 ml of 1 mol/l sodium hydroxide solution was found to be optimal for the extraction. Five kinds of organic solvents were evaluated: ethyl acetate, diethyl ether, chloroform, toluene and toluene-isopropyl alcohol (95:5, v/v). Among them, toluene-isopropyl alcohol yielded the highest recovery.

The influence of the percentage of methanol in mobile phase on the chromatographic behaviors of desloratadine and *N*-methyl-desloratadine was studied. It was found that with the increase of methanol, the retention time of both desloratadine and *N*-methyl-desloratadine was decreased. Since acetonitrile possesses lower viscosity and good solubility with many components, the incorporation of acetonitrile favors the separation. After several trials, the mobile phase consisting of the mixture of methanol–acetonitrile–phosphate buffer (0.01 mol/l), (35:35:30, v/v) achieved the purpose of this study. It was also found that the pH value of the buffer had no significant influence on the

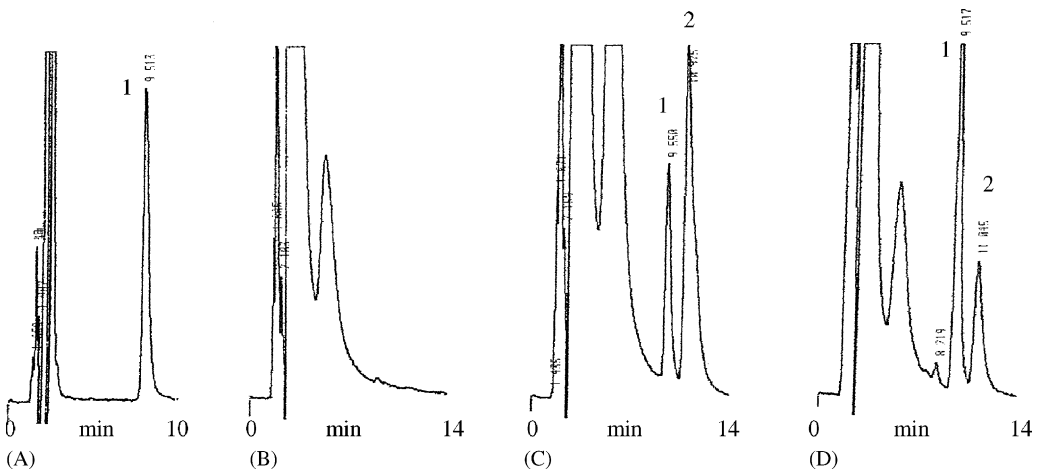

Fig. 2. Typical chromatograms for method selectivity and sample determination: (A) standard solution (2 μg/ml); (B) blank dog plasma; (C) blank plasma spiked with 154 ng/ml desloratadine and 230 ng/ml IS; (D) a plasma sample collected from foreleg vein at 2 h after oral administration desloratadine fumarate tablets. Peaks: 1, desloratadine; 2, *N*-methyl-desloratadine (IS).

chromatographic separation in the range of 5.0–7.0, the final pH selected was 5.5.

3.2. Selectivity

The method was evaluated for selectivity by analysis of dog plasma samples. Typical chromatograms are shown in Fig. 2. Desloratadine and IS exhibited retention times of ca. 9.5 and 11.0 min, respectively. Baseline resolution was achieved without interference of endogenous substances from the blank dog plasma.

3.3. Linearity

The calibration curve was constructed by plotting the peak-height ratios (y) of desloratadine to the IS against desloratadine concentrations (x) in dog plasma. The linear range was from 5.0 to 800.0 ng/ml. The typical regression equation was $y = 1.09 \times 10^{-2} + 3.19 \times 10^{-3}x$, $r > 0.997$.

3.4. Limit of quantitation

Limit of quantitation (LOQ) is the concentration above which quantitation can be carried out with adequate accuracy and precision (CV, coefficient of variation, <20%). The LOQ of desloratadine was found to be 5.0 ng/ml.

3.5. Accuracy and precision

The accuracy and precision of this method were examined using dog plasma samples freshly prepared at desloratadine concentrations of 10.0, 200.0 and 800.0 ng/ml. Six replicate determinations were made for each concentration and CV served as measure of the precision. The accuracy was determined by comparing the measured concentrations with the expected concentrations of desloratadine in spiked blank dog plasma and given as relative error (RE). As shown in Table 1, the inter-day precision and accuracy of the method are less than 3.8 and 8.7% ($n = 18$), respectively. The intra-day precision and accuracy are less than 9.0 and 9.5% ($n = 18$), respectively. The results demonstrate the acceptable accuracy and precision of the method.

3.6. Recovery

The extraction recovery of desloratadine from dog plasma was determined by comparing peak heights from spiked plasma with those from standard solutions. The spiked plasma was prepared by spiking plasma with known amounts of desloratadine. The standard solutions were prepared in methanol and can be directly analyzed without any purification. The mean recoveries of desloratadine from dog plasma were 82.0 ± 4.9, 80.9 ± 4.4 and $78.8 \pm 1.5\%$ ($n = 6$)

Table 1
Accuracy and precision of the developed HPLC method for the determination of desloratadine in dog plasma

	Concentration (ng/ml)		
	10.0	200.0	800.0
Inter-day ($n = 18$)			
Mean ± S.D. (ng/ml)	10.5 ± 0.4	203.2 ± 5.5	730.3 ± 12.8
CV[a] (%)	3.8	2.7	1.8
RE[b] (%)	4.9	1.6	−8.7
Intra-day ($n = 18$)			
Mean ± S.D. (ng/ml)	10.1 ± 0.9	201.9 ± 4.4	723.8 ± 18.3
CV (%)	9.0	2.2	2.5
RE (%)	0.9	1.0	−9.5

[a] CV: coefficient of variation.
[b] RE: relative error.

for the final spiked desloratadine concentration of 10.0, 200.0 and 800.0 ng/ml, respectively. For the IS (3 μg/ml), the recovery was 85.1 ± 0.6% ($n = 6$).

3.7. Stability

The stability of desloratadine was evaluated by analyzing quality control samples (10.0, 200.0 and 800.0 ng/ml) which were stored for 10 h at 20 °C and 3 months at −20 °C, respectively. As shown in Table 2, the mean concentration of desloratadine did not change substantially when plasma samples were stored at 20 °C for up to 10 h. The difference from quality control samples was less than 5% after 3 months of storage at −20 °C.

Table 3
Pharmacokinetic parameters of desloratadine in five dogs after oral administration of desloratadine fumarate tablets (a dose equivalent to 10 mg desloratadine) and desloratadine tablets (10 mg desloratadine) (Mean ± S.D., $n = 5$)

Pharmacokinetic parameters	Desloratadine fumarate tablets (test preparation)	Desloratadine tablets (reference preparation)
AUC_{0-t} (ng h/ml)	5801.9 ± 4680.2	6293.7 ± 5305.0
$AUC_{0-\infty}$ (ng h/ml)	5981.1 ± 4751.4	6511.4 ± 5500.2
C_{max} (ng/ml)	388.1 ± 258.7	393.2 ± 315.4
t_{max} (h)	3.80 ± 1.48	3.20 ± 1.64
$t_{1/2}$ (h)	11.05 ± 3.06	11.19 ± 1.51
K_e (h^{-1})	0.067 ± 0.018	0.063 ± 0.009

3.8. Bioequivalence studies

The developed HPLC method yields satisfactory results for the determination of desloratadine in dog plasma samples and has been used successfully in a pilot bioequivalence study of desloratadine fumarate tablets and desloratadine tablets in dogs following oral administration. The mean plasma concentration-time profiles for desloratadine fumarate tablets and desloratadine tablets are shown in Fig. 3. The pharmacokinetic parameters of both preparations in dogs are given in Table 3. Both the mean values and standard

Table 2
Stability of desloratadine in dog plasma after 10 h at 20 °C and 3 months after stored at −20 °C

	Concentration(ng/ml)					
	10.0		200.0		80.0	
	1	2	1	2	1	2
10 h at 20 °C						
Found (ng/ml)	9.84	9.90	199.0	197.1	769.76	782.9
Mean (ng/ml)	9.87		198.0		776.3	
Recovery (%)	98.7		99.0		97.0	
Mean ± SD(%)			98.2 ± 1.1			
3 months at −20 °C						
Found (ng/ml)	9.41	9.69	197.8	193.6	752.9	774.8
Mean (ng/ml)	9.55		195.7		763.8	
Recovery (%)	95.5		97.8		95.5	
Mean ± S.D. (%)			96.3 ± 1.4			

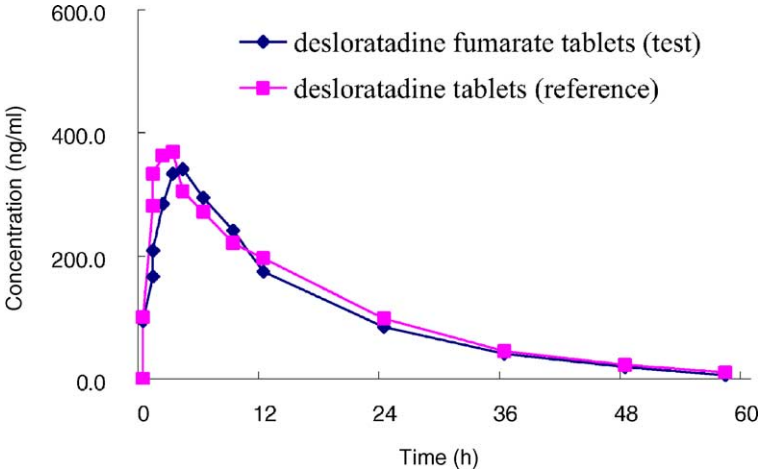

Fig. 3. Mean plasma concentration-time profiles of desloratadine in five dogs after oral administration of desloratadine fumarate tablets (a dose equivalent to 10 mg desloratadine) and desloratadine tablets (10 mg desloratadine).

deviation (S.D.) of AUC_{0-t}, $AUC_{0-\infty}$ and C_{max} were found to be very close between the two preparations, indicating that the plasma profiles generated by desloratadine fumarate tablets are comparable to those produced by desloratadine tablets.

ANOVA for pharmacokinetic parameters (after log-transformation of the data) showed no statistically significant difference between the two preparations and two periods except that between individuals (Table 4). 90% CI also demonstrated that the ratio of these parameters of the two drugs lie within the FDA accepted range of 80–125% (70–143% for C_{max}) (Table 5). Based on the AUC_{0-t} values, the mean relative bioavailability of the desloratadine fumarate tablets to desloratadine tablets was found to be $97.3 \pm 14.2\%$ ($n = 5$). The above results demonstrate that the bioavailability of desloratadine fumarate remains unchanged compared with desloratadine in addition to better solubility and stability than desloratadine in vitro, which are favorable for its determination and storage.

Table 4
ANOVA results of the pharmacokinetic parameters in 5 dogs after a single oral dose of desloratadine fumarate tablets and desloratadine tablets

Parameters	F value		
	Between-preparation	Between-period	Between-individual
In $AUC_{0-\infty}$	0.05	0.88	22.55*
In AUC_{0-t}	1.02	8.55	549.84*
In C_{max}	0.02	4.53	33.27*
t_{max}	1.59	1.59	7.50

$F_{0.05(4,3)} = 9.12$; $F_{0.05(1,3)} = 10.13$.
* $P < 0.05$.

Table 5
Two-one sided tests and (1–2α) confidence interval analysis results of the parameters after a single oral dose of desloratadine fumarate tablets and desloratadine tablets

Parameters	t_1	t_2	90% CI	Point estimates (%)
AUC_{0-t}	4.00*	5.10*	87.7–108.1	97.4
AUC_{0-8}	4.77*	6.52*	88.8–105.1	96.6
C_{max}	2.88*	2.69*	77.2–134.8	102.0

$t_{(1-0.05)(3)} = 2.35$.
* $P < 0.05$.

4. Conclusions

The proposed HPLC method is simple, specific and accurate for the determination of desloratadine in dog plasma. This method also offers a rapid and simple sample preparation procedure, which facilitates the pharmacokinetic and bioequivalence studies of desloratadine fumarate tablets. Based on the pharmacokinetic and statistical results, it can be concluded that desloratadine fumarate tablets is bioequivalent to desloratadine tablets in dogs.

References

[1] A. Graul, P.A. Leeson, J. Castaòer, Drugs Future 25 (2000) 339–346.
[2] D.A. Handley, J.R. McCullough, Y. Fang, Ann. Allergy Asthma Immunol. 78 (1997) 164–169.
[3] J. Hilbert, E. Radwanski, R. Weglein, J. Clin. Pharmacol. 27 (1987) 694–698.
[4] D. Zhong, H. Blume, Pharmazie 49 (1994) 736–739.
[5] R. Johnson, C. Jeffrey, C.C. Lin, J. Chromatogr. B 657 (1994) 125–131.
[6] F.C.W. Sutherland, A.D. de Japer, D. Badenhorst, J. Chromatogr. A 914 (2001) 37–43.
[7] Z.Y. Xie, X.Y. Chen, Y.F. Zhang, Chin. J. Clin. Pharmacol. 18 (2002) 211–214.
[8] L. Yang, R.P. Clement, B. Kantesaria, L. Reyderman, F. Beaudry, C. Grandmaison, L. Donato, R. Masse, P.J. Rudewicz, J. Chromatogr. B 792 (2003) 229–240.
[9] Executive board of health ministries for gulf states, Approved guidelines on drug bioavailability and bioequivalence requirements in the executive board of health ministries for gulf states, 1998.
[10] WHO Expert Committee, Guidelines for good clinical practice (GCP) for trials on pharmaceutical products, the use of essential drugs. Sixth report of the WHO Export Committee, WHO Technical Report Series, No. 850, 1995.
[11] European Community, Investigation of Bioavailability and Bioequivalence: The Rules Governing Medicinal Products in European Community, vol. 3, 1991, pp. 149–169 (addendum 2).