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Short communication

Simultaneous determination of desloratadine and its active metabolite 3-hydroxydesloratadine in human plasma by LC/MS/MS and its application to pharmacokinetics and bioequivalence

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Abstract

A rapid and simple liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method was developed and validated for the simultaneous determination of desloratadine and its active metabolite 3-hydroxydesloratadine concentrations in human plasma. After liquid–liquid extraction with ethyl ether for sample preparation, the chromatographic separation was achieved on a CAPCELL PAK C18 column (50 mm × 2.0 mm, 5 μm, Shiseido). [$^2H_4$]desloratadine and [$^2H_4$]3-OH desloratadine were used as internal standards. A mobile phase consisted of 5 mM ammonium formate in water, methanol and acetonitrile (50:30:20). Detection was by positive ion electrospray tandem mass spectrometry on a Sciex API3000. A quadratic regression (weighted 1/concentration) gave the best fit for calibration curves over the concentration range 0.05–10 ng/mL for both desloratadine and 3-OH desloratadine. The method was shown to be accurate, rapid and sufficiently sensitive to be successfully applied to a pharmacokinetic and bioequivalent study.

Keywords: Desloratadine; 3-Hydroxydesloratadine; LC/MS/MS; Liquid–liquid extraction

1. Introduction

Desloratadine, a major active metabolite of loratadine, is a selective, potent, orally active, peripheral H_1 receptor antagonist, while 3-OH desloratadine is a major active metabolite of both desloratadine and loratadine. Clinical studies have demonstrated that desloratadine 5 mg (as compared with loratadine 10 mg) effectively relieves the signs and symptoms of seasonal allergic rhinitis in patients with this disease [1,2]. Following oral administration of desloratadine 5 mg once daily for 10 days to normal healthy volunteers, the mean time to maximum plasma concentration (T_{max}) occurred at approximately 3 h post-dose, and the mean steady-state peak plasma concentration (C_{max}) and area under the concentration–time curve (AUC) were observed to be 4 ng/mL and 56.9 ng h/mL, respectively. Neither food nor grapefruit juice had an effect on the bioavailability (C_{max} and AUC) of desloratadine [3].

Several analytical methods are available for the determination of desloratadine levels. El-Sherbiny et al. [4] and Qi et al. [5] reported that the limit of quantification of desloratadine in pharmaceutical preparations using HPLC with UV detection was 2 ng/mL [4] and 0.1 ng/mL [5], respectively. Liu et al. [6] reported that the limit of quantification of desloratadine in dog plasma using HPLC–UV was 5 ng/mL. Yin et al. [7] reported an HPLC method followed by fluorescence detection that had a minimum detection limit of 0.5 ng/mL. Following oral administration of desloratadine 5 mg once daily for 10 days, a mean peak plasma concentration of 4 ng/mL was observed at peak dose [3]. Given the low maximum plasma concentration, it was essential that the bioanalytical method be refined to a lower limit of quantification (LLOQ) of 0.05 ng/mL in order to measure both desloratadine and 3-OH desloratadine. Yang et al. [8] and Shen et al. [9] developed methods for the simultaneous determination of desloratadine and 3-OH desloratadine using a liquid chromatographic-tandem mass spectrometric (LC/MS/MS) method that had an LLOQ of 0.025 ng/mL. In their methods [8,9], they both used automated 96-well solid phase extraction for sample preparation, which was able to offer much

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cleaner sample extracts than protein precipitation or "dilute and shoot". In the present study, a one-step liquid–liquid extraction with ethyl ether was used, which was not only able to resolve the problems of ion suppression and matrix effects in protein precipitation, but was also rapid and robust. Yang et al. [8] used a gradient elution program, and the run time was 6 min. In the present study, an isocratic elution program was performed, and the run time was 3 min, which improved the throughput of this analytical method without compromising the robustness and selectivity of the existing assay. The purpose of the present study was to improve the LC/MS/MS method for the simultaneous determination of desloratadine and 3-OH desloratadine in human plasma, to support a bioequivalence study after oral administration of 5 mg desloratadine.

2. Experimental

2.1. Reagents and chemicals

Desloratadine (99.3% pure, Lot: 100643-71-8), 3-OH desloratadine (96.33% pure, Lot: 76214-141-4), $^{2}H_4$-desloratadine (95.6%, Lot: 75508-114-8) and $^{2}H_4$-3-OH desloratadine (96.3%, Lot: 77047-91-21) were obtained from Schering-Plough Co. (Shanghai, China). Methanol and acetonitrile, both HPLC grade, were obtained from Sigma–Aldrich Chemical Co. (St. Louis, MO, USA). Ethyl ether, ammonium formate and sodium hydroxide were reagent grade. Deionized/distilled (DI) water was prepared from tap water in our own department.

2.2. Solution preparation

Stock solutions of desloratadine and 3-OH desloratadine were prepared in methanol at a concentration of 100 μg/mL (corrected for purity). Intermediate and spiking solutions were prepared from the stock solutions in methanol. Separate stock solutions of desloratadine and 3-OH desloratadine were used to prepare spiking solutions for the quality control (QC) samples. Drug-free plasma was used to prepare standard and QC pools. A total of eight standard points and three QC concentrations were used to span the concentration range of 0.05–10 ng/mL. The pools were stored at −20 °C until used. $^{2}H_4$-desloratadine and $^{2}H_4$3-OH desloratadine internal standard working solutions were prepared at a nominal concentration of 50 ng/mL in methanol.

2.3. Sample preparation

Plasma samples (1 mL) or QC were added into 10 mL labeled centrifuge tubes. To each of these centrifuge tubes, with the exception of the blank plasma samples, 20 μL of the working internal standard solution (50 ng/mL [$^{2}H_4$]desloratadine and 50 ng/mL [$^{2}H_4$]3-OH desloratadine) was added. The plasma samples were then mixed with 0.15 mL sodium hydroxide (0.1 mol/L) and 6 mL ethyl ether, and extracted with a rotary mixer for 5 min at room temperature. The mixture was centrifuged for 5 min at 2500 rpm and the upper organic phase was transferred into a new glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted with 200 μL of mobile phase, and an aliquot of 10 μL was injected into a high-performance liquid chromatograph equipped with an MS–MS detector.

2.4. LC/MS/MS analysis

The liquid chromatography separation system consisted of the LC-10AD VP (pump), DGU-14AM and SIL-HTc (autosampler) (Shimadzu Corporation, Kyoto, Japan). The separation column was a CAPCELL PAK C18 column (50 mm × 2.0 mm, 5 μm) (Shiseido Corporation, Tokyo, Japan). The pre-column was an Octadecyl C_{18} (4.0 mm × 3.0 mm) (Phenomenex Corporation, Torrance, CA, USA). An XW-80 vortex was obtained from Shanghai Medical University Apparatus Co. (Shanghai, China). The Biofuge 28RS centrifuge was obtained from Heraeus Sepatech Co. (Osterode, Germany). The isocratic mobile phase consisted of 50% solvent A (5 mM ammonium formate in water) and 50% solvent B (methanol:acetonitrile = 3:2). The flow rate was set to 0.20 mL/min. The injection volume was 10 μL and the run time was 3 min. Representative retention times for desloratadine, [$^{2}H_4$]desloratadine (internal standard [IS]), 3-OH desloratadine and [$^{2}H_4$]3-OH desloratadin (IS) were 1.42, 1.40, 1.19 and 1.18 min, respectively.

A Sciex API 3000 LC/MS/MS system (Foster City, CA, USA), operating under Analyst 1.4 software, was used. The electrospray ion source was run in a positive ionization mode for all experiments. The typical ion source parameters were: declustering potential (DP): 45 V, collision energy (CE): 30 V, focusing potential (FP): 250 V, entrance potential (EP): 9 V, collision cell exit potential (CXP) 7 V, spray voltage: 4000 V and ion temperature: 400 °C. Nebulizer gas (NEB), curtain gas (CUR) and collision gas (CAD) were set to 90, 60 and 100 psi, respectively. Nitrogen gas was used for NEB, CUR and CAD. The sample was analyzed via selected reaction monitoring (SRM). The monitoring ions were set as m/z 311.1 → 259.1 for desloratadine, m/z 315.1 → 263.1 for [$^{2}H_4$]desloratadine, m/z 327.1 → 275.1 for 3-OH desloratadine and m/z 331.1 → 279.1 for [$^{2}H_4$]3-OH desloratadine. The scan dwell time was set at 0.15 s for every channel. Sciex Analyst version 1.4 software performed data collection and peak integration.

3. Results

3.1. Mass spectrometry

Both analytes and their own ISs responded best to the positive ionization mode, with the protonated molecular ions $[M+H]^+$ as the major species. Product ion spectra and fragmentation pathways of desloratadine, $^{2}H_4$-desloratadine, 3-OH desloratadine and 2H43-OH desloratadine are shown in Fig. 1. The MRM acquisitions were performed at unit resolution using the transition m/z 311.1 → 259.1 for desloratadine, m/z 315.1 → 263.1 for [$^{2}H_4$]desloratadine, m/z 327.1 → 275.1

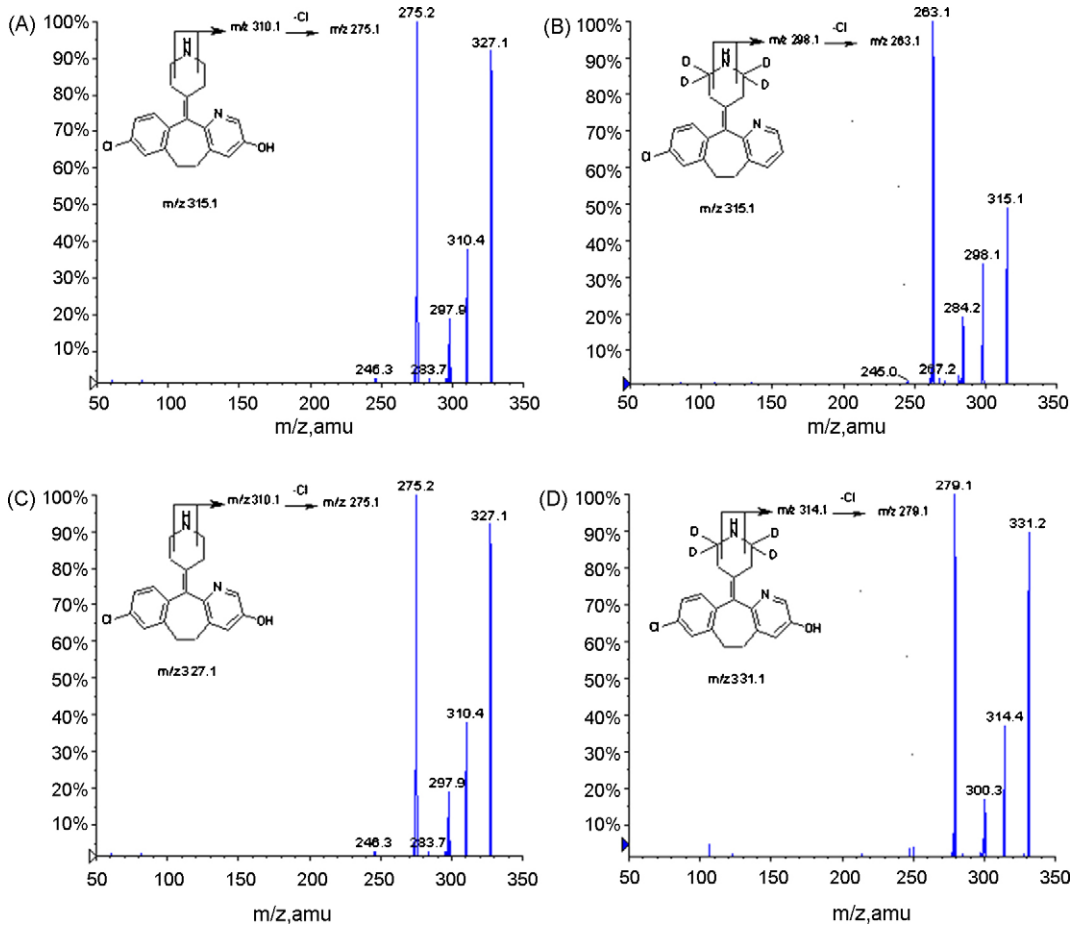

Fig. 1. Full-scan product ion spectra of $[M+H^+]$ and fragmentation pathways for (A) desloratadine, (B) $^2H_4$-desloratadine, (C) 3-OH desloratadine and (D) $^2H_4$-3-OH desloratadine.

for 3-OH desloratadine and *m/z* 331.1 → 279.1 for [$^2H_4$]3-OH desloratadine. The mass parameters were optimized by observing the maximum response obtained for the product ions.

3.2. Chromatograph

A short Octadecyl C_{18} pre-column was used to filter the sample, which was able to reduce the matrix effect. The inclusion of 0.15 mL sodium hydroxide (0.1 mol/L) was able to raise the recovery on the solvent extraction of desloratadine and 3-OH desloratadine, while hydrochloric acid reduced it. The inclusion of 5 mM ammonium formate buffer reduced matrix effects and peak tailing, without decreasing response. The isotope compound used as IS reduced potential matrix effects.

3.3. Method validation

3.3.1. Standard curve

After the liquid–liquid extraction and LC/MS/MS conditions had been defined a full validation was performed to assess the performance of the method. Eight-point calibration standard curves, ranging from 0.05 to 10 ng/mL desloratadine and 3-OH desloratadine, were used in duplicate in each analytical run. Peak area ratios of desloratadine or 3-OH desloratadine to their own ISs were used for regression analysis. A weighted ($1/x$) quadratic regression model, where x was the concentration of desloratadine or 3-OH desloratadine, was fitted to each standard curve. The signal to noise ratio of 0.05 ng/mL desloratadine was 80 and that of 3-OH desloratadine was 52. If the signal to noise ratio were to be five, the detection limits of desloratadine and 3-OH desloratadine would be lower than 0.005 ng/mL. The %CV at each level of desloratadine varied from 1.53 to 4.84, and that of 3-OH desloratadine varied from 1.84 to 5.16. The mean of the absolute values of percentage deviation from the theoretical value of desloratadine and of 3-OH desloratadine varied from 0 to 14.40 (LLOQ) and from 0 to 13.20 (LLOQ), respectively. The %CV of the five slopes of desloratadine was 2.93 and that of 3-OH desloratadine was 7.79. The lowest coefficient of determination (γ) among the five calibration curves of desloratadine was 0.9990 (mean = 0.9995) and that of 3-OH desloratadine was 0.9991 (mean = 0.9994) (Table 1). Thus, the calibration curves did not exhibit any non-linearity within the chosen range. Based on the standard data presented here, it was concluded that the calibration curves used in this method were precise and accurate for the simultaneous mea-

Table 1
Mean inter-batch back-calculated standard and standard curve results of desloratadine and 3-OH desloratadine in human plasma

	Mean inter-batch back-calculated standard results								Standard curve result		
	STD1 (0.05 ng/mL)	STD2 (0.1 ng/mL)	STD3 (0.2 ng/mL)	STD4 (0.5 ng/mL)	STD5 (1.0 ng/mL)	STD6 (2.0 ng/mL)	STD7 (5.0 ng/mL)	STD8 (10 ng/mL)	Slope	*Y*-intercept	γ
Desloratadine											
Run ID											
I	0.0537	0.0963	0.187	0.484	1.03	2.01	5.28	9.71	0.875	0.0103	0.9992
II	0.0554	0.095	0.179	0.504	1.000	2.16	4.71	10.1	0.891	0.0082	0.9990
III	0.0501	0.103	0.19	0.508	0.973	2.1	4.89	10.0	0.946	0.0080	0.9998
IV	0.0543	0.104	0.183	0.490	0.966	1.98	5.21	9.86	0.900	0.0394	0.9996
V	0.0572	0.101	0.181	0.471	0.99	1.98	5.08	10.0	0.901	0.0427	0.9998
Mean	0.0541	0.0999	0.184	0.491	0.992	2.05	5.03	9.93	0.903	0.0217	0.9995
S.D.	0.0026	0.0040	0.004	0.015	0.025	0.08	0.23	0.15	0.026	0.0177	0.0004
%CV	4.84	4.02	2.43	3.06	2.55	3.93	4.65	1.53	2.93	NA	0.036
%Dev	8.28	−0.14	−8.00	−1.72	−0.82	2.30	0.68	−0.66			
n	5	5	5	5	5	5	5	5	5	5	5
3-OH desloratadine											
Run ID											
I	0.052	0.094	0.202	0.494	1.01	2.12	4.74	10.1	0.996	0.00378	0.9993
II	0.0499	0.103	0.201	0.471	1.03	2.06	4.77	10.2	1.010	0.00617	0.9995
III	0.0524	0.102	0.191	0.498	0.96	2.07	4.92	10.1	1.040	−0.0133	0.9998
IV	0.0566	0.099	0.186	0.477	0.99	2.08	4.71	10.2	1.190	0.0429	0.9992
V	0.0551	0.097	0.192	0.470	1.00	1.96	5.32	9.75	1.130	0.0497	0.9991
Mean	0.0532	0.0989	0.194	0.482	0.998	2.06	4.89	10.1	1.0732	0.0179	0.9994
S.D.	0.0027	0.0038	0.0069	0.013	0.026	0.06	0.25	0.19	0.0836	0.0271	0.00028
%CV	4.99	3.86	3.54	2.72	2.61	2.88	5.16	1.84	7.79	NA	0.0278
%Dev	6.40	−1.10	−2.80	−3.60	−0.16	2.90	−2.16	0.70			
n	5	5	5	5	5	5	5	5	5	5	5

Note: Calibration curves were weighed 1/concentration. NA, not applicable; conc, concentration; STD, standard.

surement of desloratadine and 3-OH desloratadine in human plasma.

3.3.2. Accuracy and precision

Inter- and intra-batch accuracy and precision for assays were characterized by the three levels of QCs run on three sequential batches in six replicates. For desloratadine and 3-OH desloratadine, the low-level QC was 0.05 ng/mL (equivalent to the LLOQ), the medium QC was 1.0 ng/mL and the high QC was 5.0 ng/mL. Accuracy was assessed by calculating the percentage deviation from the theoretical concentration. Precision was determined by calculating the coefficient of variation for inter- and intra-run replicates.

Table 2 shows a summary of the individual QC data obtained in the three runs for the validation. As can be seen, the assay for desloratadine and 3-OH desloratadine were both accurate and precise between runs and within individual runs for each level. The greatest mean inter-run coefficients of variation were 9.54% and 7.48% for the low-level QCs of desloratadine and 3-OH desloratadine, respectively. All QC levels for desloratadine and 3-OH desloratadine had inter- and intra-run coefficients of variation of less than 10%. The deviation from the expected concentration, as a measurement of accuracy, ranged from –4.83% to ∼+5.30% and −5.03% to ∼+8.70% for desloratadine and 3-OH desloratadine, respectively. The coefficients of variation were all within 10%. These results indicated that the method was precise and accurate.

3.3.3. Specificity and matrix effects

Six different batches of control human plasma were analyzed, with and without standards, to determine whether any endogenous plasma constituents interfered with the analytes or the ISs. The degree of interference was assessed by inspection of SRM chromatograms. No significant interfering peaks from the plasma were found at the retention time or in the ion channel of analytes or the ISs (Fig. 2).

Blank plasma (1 mL) was added to a 10 mL labeled centrifuge tube, and then mixed with 0.15 mL sodium hydroxide (0.1 mol/L) and 6 mL ethyl ether. The tube was capped and vortexed for 5 min at room temperature. The sample was centrifuged for 5 min at 2500 rpm and the organic phase was transferred into a new glass tube and evaporated to dryness at 40 °C under a gentle stream of nitrogen. The residue was reconstituted in 20 μL of the working internal standard solution, 20 μL of a suitable concentration of the working standard solution for desloratadine and 3-OH desloratadine, and 160 μL of mobile phase. The concentrations were equal to the LLOQs for desloratadine, 3-OH desloratadine and ISs, respectively. Six replicates for desloratadine, 3-OH desloratadine and ISs were prepared. We replaced blank plasma with distilled water and prepared standard solution in the same way. The matrix effect was assessed by comparing the peak area of the former with the peak area of the corresponding standard solution. The means of the ratios for desloratadine, 3-OH desloratadine, IS [$^2H_4$]desloratadine and IS [$^2H_4$]3-OH desloratadine were 0.85, 0.81, 0.84 and 0.79, respectively. The

Table 2
Intra- and inter-assay accuracy and precision of desloratadine and 3-OH desloratadine in human plasma

	Desloratadine			3-OH desloratadine		
	Low (0.05 ng/mL)	Middle (1.0 ng/mL)	High (5.0 ng/mL)	Low (0.05 ng/mL)	Middle (1.0 ng/mL)	High (5.0 ng/mL)
Intra-assay accuracy and precision						
Mean (%)	0.0507	0.979	5.12	0.0531	0.977	5.11
S.D.	0.0041	0.046	0.26	0.0034	0.046	0.23
%CV	7.99	4.73	5.17	6.35	4.68	4.58
%Dev	1.42	−2.15	2.32	6.27	−2.31	2.10
n	18	18	18	18	18	18
Inter-assay accuracy and precision						
Run ID I						
Mean (%)	0.0522	1.00	5.19	0.0528	0.991	5.17
S.D.	0.0050	0.039	0.28	0.0034	0.031	0.307
%CV	9.54	3.91	5.48	6.45	3.08	5.94
%Dev	4.43	0.40	3.77	5.50	−0.88	3.40
n	6	6	6	6	6	6
Run ID II						
Mean (%)	0.0499	0.980	4.90	0.0523	0.990	4.92
S.D.	0.0032	0.045	0.19	0.0028	0.032	0.143
%CV	6.34	4.58	3.89	5.32	3.22	2.90
%Dev	−0.30	−2.02	−2.10	4.60	−1.00	−1.57
n	6	6	6	6	6	6
Run ID III						
Mean (%)	0.0501	0.952	5.27	0.0544	0.950	5.22
S.D.	0.0041	0.046	0.17	0.0041	0.062	0.09
%CV	8.21	4.81	3.25	7.48	6.51	1.81
%Dev	0.13	−4.83	5.30	8.70	−5.03	4.47
n	6	6	6	6	6	6

absolute recoveries were assessed by comparing the peak area of the QCs with the peak area of the corresponding standard solution. The absolute recoveries of three levels in plasma were 48.59 ± 3.36%, 47.21 ± 4.66% and 49.74 ± 3.51% for desloratadine, and 38.63 ± 2.96%, 44.16 ± 3.52% and 47.51 ± 4.01% for 3-OH desloratadine, respectively.

3.3.4. Stability

The stability of desloratadine and 3-OH desloratadine in human plasma was investigated using the low (0.05 ng/mL), medium (1.0 ng/mL) and high (5.0 ng/mL) QCs. Test conditions included three freeze–thaw cycles, room temperature (4 h), 40 °C (1 h) and −20 °C (40 days). Stability was also checked by extracting the appropriate QC, which had been maintained at a specific temperature (4 °C) for the specified time, and analyzing the extracts for desloratadine and 3-OH desloratadine. The stability of desloratadine and 3-OH desloratadine in the extract was tested after 24 h storage at 4 °C to allow for sample waiting or re-injection in the autosampler. The 4 h stability test was performed at ambient temperature, since the plasma samples could remain stable on the bench for up to 4 h after thawing or before freezing. The 1 h stability test at 40 °C was performed, since the plasma sample could evaporate stably under a gentle stream of nitrogen for up to 1 h. The 40-day stability test at −20 °C was performed, since the plasma samples could be stored stably in −20 °C refrigerator after collection. Deterioration of desloratadine or 3-OH desloratadine was defined as greater than a 15% difference in the tested sample versus control at the sample nominal concentration.

Table 3 shows that there was no deterioration for desloratadine or 3-OH desloratadine in plasma at any QC level for the three freeze–thaw cycles, suggesting that drug concentrations can be confidently determined in samples that have been thawed up to three times prior to analysis. Table 3 also shows that desloratadine and 3-OH desloratadine in plasma were stable following storage at 4 °C for 24 h, 40 °C for 1 h and −20 °C for 40 days.

3.4. Clinical application

The present method was used to determine the plasma concentrations of desloratadine and 3-OH desloratadine in a randomized, crossover bioequivalence study. Twenty-four healthy male volunteers received a single oral dose of 5 mg desloratadine syrup or tablet. The test and reference formulations were desloratadine syrup (Schering-Plough Co., Shanghai, China) and desloratadine tablet (AERIUS®, Schering-Plough Co., Shanghai, China), respectively. Each formulation was administrated with 240 mL water to subjects after 10 h overnight fasting on 2 treatment days, separated by a 1-week washout period. The blood sampling was carried out before drug administration and at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 36, 48, 72, 96 and 120 h after drug administration. Pharmacokinetic parameters were estimated using non-compartmental calculations. The parame-

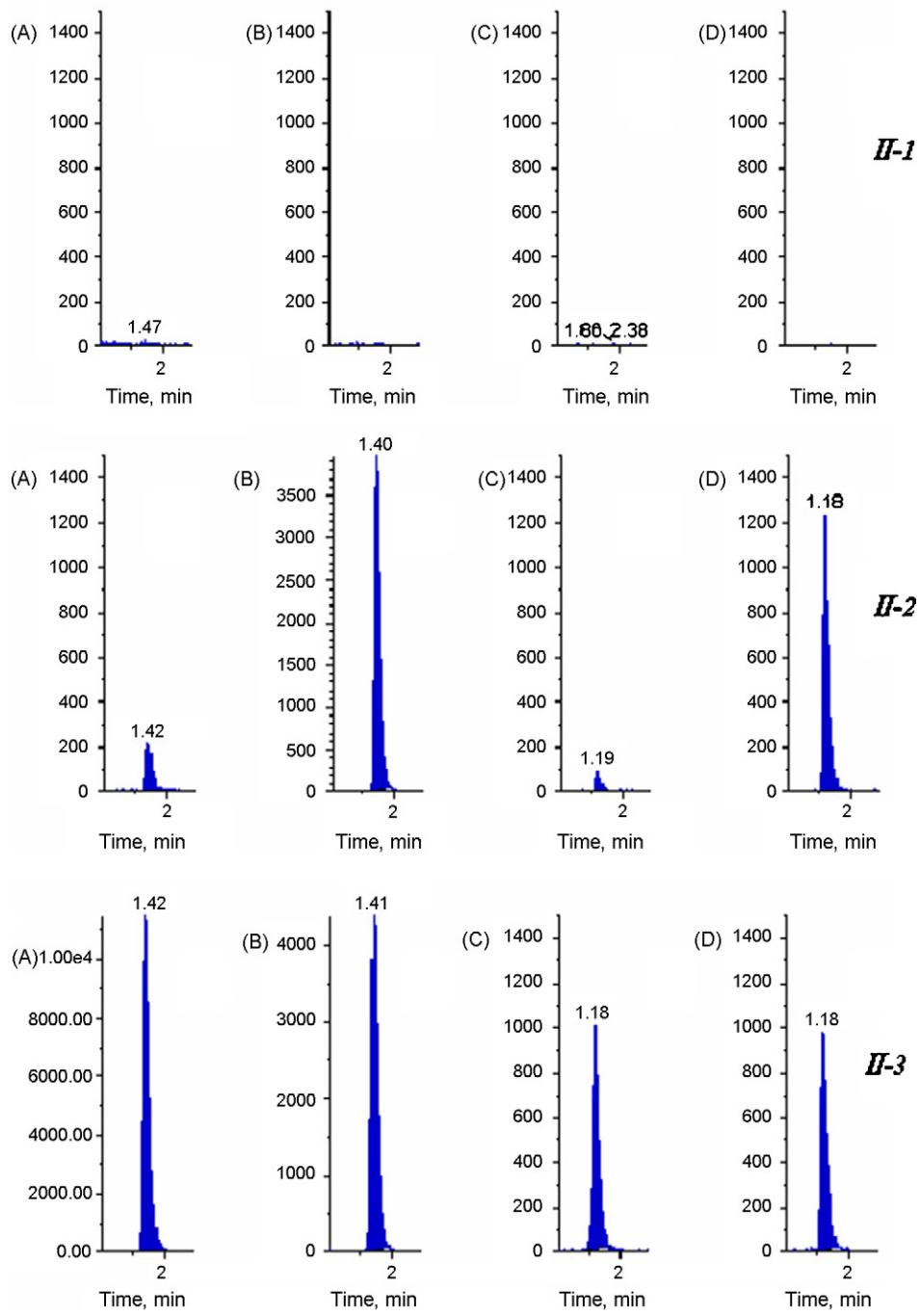

Fig. 2. Chromatography of desloratadine: 3-OH-desloratadine and internal standards (A) desloratadine; (B) [$^2H_4$]desloratadine; (C) 3-OH-desloratadine; (D) [$^2H_4$]3-OH-desloratadine. II-1: blank plasma; II-2: plasma spiked with desloratadine, 3-OH-desloratadine and internal standards of LLOQ; II-3: a human plasma of random number 07 collected 1.5 h after administration 5 mg desloratadine tablet.

ters were not further corrected for weight or administrated dose. After logarithmic transformation, AUC_{0-t} and C_{max} values were subjected to analysis of variance (ANOVA), including the terms for subjects, treatment (sequence) and the time period. For the evaluation of bioequivalence, 90% confidence intervals (CIs) for the relative differences were used between the test and reference formulations.

The mean desloratadine and 3-OH desloratadine plasma concentration–time curves are shown in the Figs. 3 and 4. The relevant pharmacokinetic (PK) parameters of desloratadine and 3-OH desloratadine for the two formulations are listed in Table 4. For the bioequivalence (BE) assessment, a standard BE range for basic PK parameters (including AUC_t and C_{max}) of 0.8–1.25 has been generally accepted. Table 5 shows the 90% CI obtained by ANOVA for these parameters after log-transformation of the data. No differences between the test and reference formulations were detected ($p > 0.05$ for the effects of the formulations in all tested parameters). Statistical analysis

Table 3
Stability results of desloratadine and 3-OH desloratadine in human plasma

	Desloratadine			3-OH desloratadine		
	Low (0.05 ng/mL)	Middle (1.0 ng/mL)	High (5.0 ng/mL)	Low (0.05 ng/mL)	Middle (1.0 ng/mL)	High (5.0 ng/mL)
Stability at 40 °C for 1 h						
Mean (%)	0.054	0.964	4.945	0.056	0.972	4.950
S.D.	0.001	0.079	0.304	0.004	0.025	0.198
%CV	2.11	8.22	6.15	6.48	2.62	4.00
%Dev	7.40	−3.60	−1.10	11.30	−2.80	−1.00
n	2	2	2	2	2	2
Stability at room temperate for 4 h						
Mean (%)	0.055	0.939	4.970	0.052	0.922	5.070
S.D.	0.003	0.086	0.170	0.000	0.059	0.071
%CV	4.80	9.19	3.41	0.00	6.37	1.39
%Dev	9.10	−6.10	−0.60	4.20	−7.85	1.40
n	2	2	2	2	2	2
Stability at 4 °C for 24 h						
Mean (%)	0.046	1.055	4.965	0.051	1.014	4.900
S.D.	0.004	0.064	0.290	0.002	0.107	0.071
%CV	8.79	6.03	5.84	3.76	10.60	1.44
%Dev	−8.30	5.50	−0.70	1.50	1.40	−2.00
n	2	2	2	2	2	2
Stability at −20 °C for 40 days						
Mean (%)	0.055	0.9665	4.600	0.049	0.891	4.575
S.D.	0.005	0.009	0.071	0.002	0.002	0.233
%CV	8.26	0.95	1.54	4.22	0.24	5.10
%Dev	9.60	−3.35	−8.00	−2.70	−10.95	−8.50
n	2	2	2	2	2	2
Stability after three freeze–thaw cycles						
Mean (%)	0.055	1.025	5.080	0.055	0.996	5.050
S.D.	0.000	0.021	0.141	0.000	0.034	0.198
%CV	0.26	2.07	2.78	0.26	3.41	3.92
%Dev	10.60	2.50	1.60	10.60	−0.40	1.00
n	2	2	2	2	2	2

of the two formulations with respect to AUC_t, $AUC_{infinity}$ and C_{max} demonstrated that the 90% CIs of AUC_t, $AUC_{infinity}$ and C_{max} were 91.61–103.97%, 91.98–102.94% and 86.04–99.92% for desloratadine, and 94.22–101.71%, 94.21–101.71% and 88.01–101.35% for 3-OH desloratadine, respectively, residing within the BE limit, which was 80–125% for the ratio of product averages (Table 5).

4. Discussion

An LC/MS/MS method with liquid–liquid extraction was developed and validated for the simultaneous determination of desloratadine and 3-OH desloratadine in human plasma over the concentration range of 0.05–10 ng/mL. Yang et al. [8] and Shen et al. [9] used automated 96-well solid-phase extraction

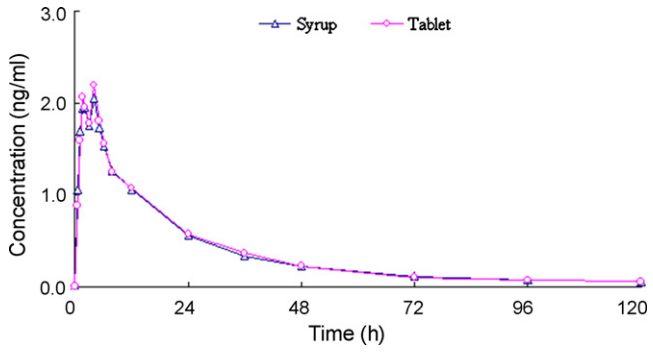

Fig. 3. Mean plasma concentration–time curve of desloratadine after receiving 5 mg single oral dose of desloratadine syrup and tablet in 24 healthy male volunteers.

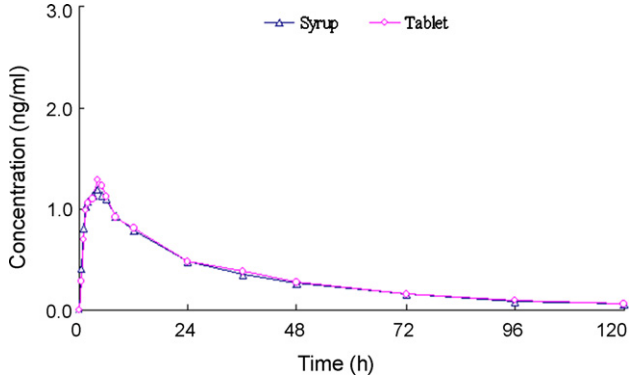

Fig. 4. Mean plasma concentration–time curve of 3-OH desloratadine after receiving 5 mg single oral dose of desloratadine syrup and tablet in 24 healthy male volunteers.

Table 4
Pharmacokinetic parameters of desloratadine and 3-OH desloratadine (mean ± S.D. of $n = 24$) of two types desloratadine formulations

Component	Parameters	Test (desloratadine syrup)	Reference (desloratadine tablet)
Desloratadine	AUC_t (ng h/mL)	41.35 ± 14.91	41.67 ± 12.04
	$AUC_{infinity}$ (ng h/mL)	43.60 ± 14.89	44.12 ± 11.99
	C_{max} (ng/mL)	2.28 ± 0.90	2.42 ± 0.80
	T_{max} (h)	3.21 ± 1.28	2.85 ± 1.32
	$t_{1/2}$ (h)	23.20 ± 8.16	23.25 ± 7.60
	K_e (h^{-1})	0.032 ± 0.008	0.033 ± 0.009
	F (%)	99.40 ± 19.01	
3-OH desloratadine	AUC_t (ng h/mL)	36.64 ± 9.60	37.35 ± 6.45
	$AUC_{infinity}$ (ng h/mL)	39.57 ± 9.91	40.29 ± 9.38
	C_{max} (ng/mL)	1.27 ± 0.40	1.35 ± 0.40
	T_{max} (h)	3.69 ± 1.26	4.13 ± 1.14
	$t_{1/2}$ (h)	29.5 ± 5.02	30.45 ± 7.15
	K_e (h^{-1})	0.024 ± 0.005	0.024 ± 0.006
	F (%)	98.79 ± 14.07	

Table 5
90% confidence interval for AUC_t, $AUC_{infinity}$ and C_{max} of desloratadine and 3-OH desloratadine after single 5 mg dose of two types desloratadine formulations (syrup and tablet) in 24 healthy male volunteers

Component	Pharmacokinetic parameters		
	AUC_t	$AUC_{infinity}$	C_{max}
Desloratadine	91.61–103.97%	91.98–102.94%	86.04–99.92%
3-OH desloratadine	94.22–101.71%	94.21–101.71%	88.01–101.35%

as sample preparation, which was able to offer clean sample extracts, although the devices may not be available in many pharmaceutical laboratories. Sutherland et al. [10] have published a manual liquid–liquid extraction LC/MS/MS method for the determination of loratadine and desloratadine. In their study [10], the samples were extracted from plasma with toluene, followed by back-extraction into formic acid (2%) for desloratadine. Due to the toxicity of toluene, we replaced this solvent with ethyl ether, which was also able to produce clean sample extracts. Yeh et al. [11] reported the determination of desloratadine with liquid–liquid extraction by ethyl ether, but they did not determine the concentration of 3-OH desloratadine. On one occasion, we tried to replace sodium hydroxide with formic acid, to acidify the plasma sample, but the extraction recovery was low. We used an isocratic elution program consisting of 5 mM ammonium formate in water, methanol and acetonitrile (50:20:30) at a flow rate of 0.20 mL/min, which reduced the total run time to 3 min. The method could be used for high-throughput bioanalysis.

The results in healthy volunteers are consistent with those of a pharmacokinetic study in healthy adults after 7.5 mg of oral desloratadine [12]. The mean values for the C_{max} and AUC_{0-t} of desloratadine after dosing with 5 mg of oral desloratadine were 2.28 ng/mL and 41.35 ng h/mL, respectively, in our study, compared with 3.30 ng/mL and 61.9 ng h/mL, repectively, after 7.5 mg of oral desloratadine [12].

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