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A validated HPLC-ESI-MS method for the determination of loratadine in human plasma and its application to pharmacokinetic studies

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A liquid chromatographic-mass spectrometric (LC-MS) assay was developed and validated for the determination of loratadine in human plasma using reversed-phase HPLC combined with electrospray ionization (ESI) mass spectrometry. The analysis involved a simple liquid-liquid extraction. The organic extract was then evaporated and the residue was reconstituted in mobile phase. The reconstituted solution was injected into an HPLC system and was subjected to reverse-phase HPLC on a 5-μm ODS-3 column at a flow-rate of 0.2 ml/min. The mobile phase comprised of acetonitrile-ammonium acetate (pH 4.0; 0.02 M, using formic acid to adjust) using gradient elution. Loratadine was detected in the single ion monitoring (SIM) mode. Standard curves were linear over the concentration range of 0.2–100 ng/ml. The mean predicted concentrations of the quality control (QC) samples deviated by less than 10% from the corresponding nominal values; the intra-assay and inter-assay precision of the assay were within 12% relative standard deviation. The extraction recovery of loratadine was more than 80%. The validated assay was applied to a pharmacokinetic study of loratadine in human plasma following the administration of a single loratadine tablet (40 mg).

1. Introduction

Loratadine is rapidly absorbed and extensively metabolized in the liver and great individual variability in loratadine concentration occurs among patients. A sensitive and simple loratadine determination method for pharmacokinetic studies and for therapeutic drug monitoring is desired. Several techniques had been reported for loratadine quantification in plasma samples: radioimmunoassay (RIA) (Hilbert et al. 1987), gas chromatography (GC) (Johnson et al. 1994; Martens 1995) and HPLC (Zhong and Blume et al. 1994; Kunicki 2001). However, these methods were not satisfactory with respect to sensitivity, feasibility and reliability and were tedious in sample preparation. In order to provide more guidance to the reasonable use of this drug and to study the pharmacokinetic profile in Chinese healthy volunteers, a more sensitive and rugged HPLC-ESI-MS method has been developed and is now reported in this paper. Based on our method, the pharmacokinetic parameters of loratadine were studied using blood samples of 18 healthy male volunteers after a single oral dose of 40 mg loratadine.

2. Investigations and results

Fig. 1. shows typical mass spectra obtained for loratadine and internal standard SCH 37370. The spectra were obtained with a fragmentor voltage set at 50 V. Loratadine and internal standard gave protonated precursor molecular ions $[M + H]^+$ in the MS mode. The major ions observed were m/z 383 for loratadine and m/z 353 for internal standard.

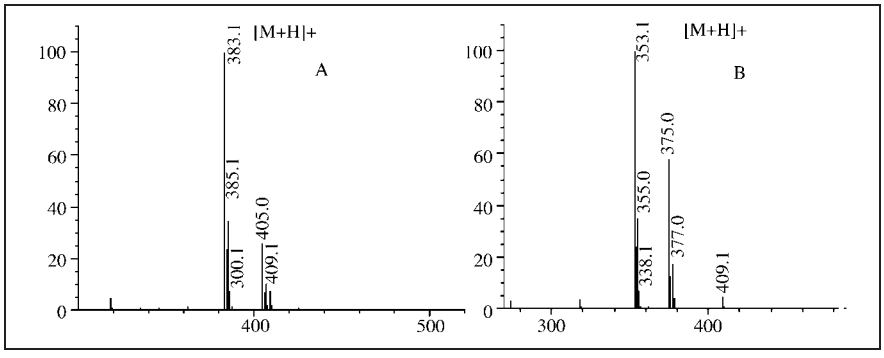


Fig. 1: Positive ion electrospray mass spectra obtained for injections of (A) loratadine, (B) SCH 37370. Fragmentor voltage 70 V, scanning range from m/z 70–500

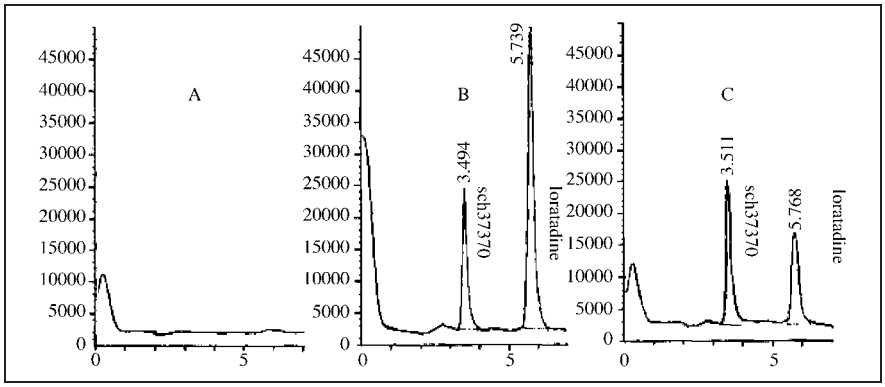


Fig. 2: Representative HPLC-ESI-MS chromatograms of blank plasma (A) and spiked plasma with I.S. (B), a subject after intake of loratadine (C)

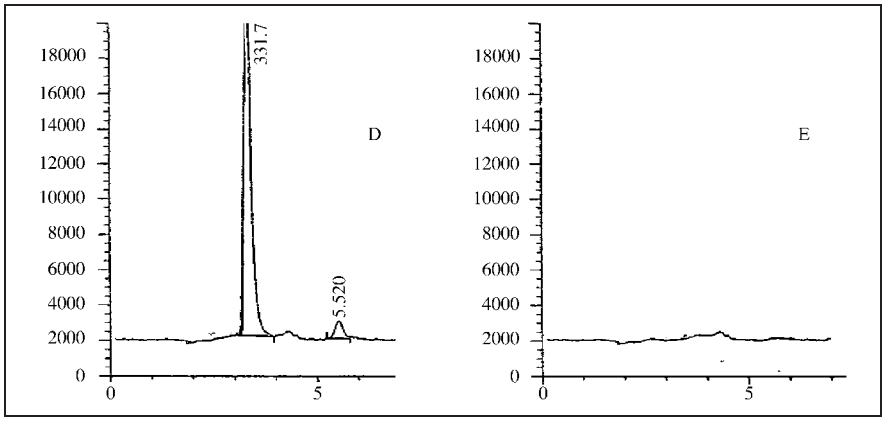


Fig. 3: HPLC-ESI-MS chromatograms of LOQ samples (0.2 ng/ml) (D) and blank plasma (E)

Table 1: Inter-day precision in the slope and intercept of standard curves (r = 0.9991–0.9995)

Days	Slope	Intercept	Correlation
1	0.0412	0.0361	0.9995
2	0.0455	0.0125	0.9991
3	0.0417	0.0225	0.9994
4	0.0427	0.0312	0.9995
5	0.0402	0.0327	0.9992
Mean ± SD	0.0423 ± 0.0020	0.0270 ± 0.0095	0.9993 ± 0.0002
RSD (%)	4.79	35.3	0.02

Table 2: Intra- and inter-day precision and accuracy of loratadine spiked in human plasma by LC-MS (n = 5)

	Actual concentration (ng/ml)	Detected concentration (Mean ± SD) (ng/ml)	Accuracy (error, %)
Intra-day	0.2	0.42 ± 0.04	5.0
	4.0	3.86 ± 0.45	−3.5
	60.0	64.32 ± 6.39	7.2
Inter-day	0.2	0.41 ± 0.03	2.5
	4.0	4.18 ± 0.29	4.5
	60.0	62.44 ± 6.03	4.1

High selectivity was found in the SIM mode for the determination of drugs in plasma samples. The representative chromatograms of blank plasma (A) and spiked plasma samples (20 ng/ml) (B) are shown in Fig. 2. The analytical peaks of loratadine and internal standard were resolved with good symmetry, the retention time of loratadine and internal standard were 5.7 min and 3.5 min respectively, no endogenous sources of interference were observed at the retention time of the analyte. A sample from a subject after intake of loratadine (t = 0.5 h) is also shown in Fig. 2. The representative chromatograms of LOQ samples (0.2 ng/ml) (D) and blank plasma (E) are shown in Fig. 3. The detection limit was 0.1 ng/ml (S/N = 3).

Good linearity was observed over the concentration range of 0.2 to 100.0 ng/ml plasma (r = 0.9991–0.9995). The RSD (n = 5) of the slope calculated with calibration curve data was 4.79%, showing a good repeatability (Table 1).

The intra- and inter-day precision and accuracy are shown in Table 2. The RSD of loratadine ranged from 9.5% to 11.6% for intra-day and 6.9% to 9.7% for inter-day, respectively. The R.E. of loratadine ranged from −3.5% to 7.2% for intra-day and −2.6% to 4.5% for inter-day, respectively.

The change in the efficiency of ionization that could be attributable to components of the sample matrix was estimated by comparing analyte peak areas obtained from unextracted preparations of the analytes with those obtained from plasma samples spiked with an equivalent amount of each analyte. The average signal suppression was 4.6% at 0.2 ng/ml, 2.4% at 4.0 ng/ml, and 1.9% at 60 ng/ml for loratadine and 5.1% for the internal standard.

Freshly prepared solutions showed no evidence of degradation for either loratadine or the internal standard. No significant degradation was observed for any analytes during the sample processing and extraction, including the dry down procedure. The stability of the sample solution in the autosampler at 4 °C was also assessed. Loratadine in sample solution was found to be stable for approximately 24 h since the found concentrations were within 95–106% of the initial concentrations. The results obtained after three freeze-thaw cycles demonstrated that 96.6 ± 4.3% of the initial content of loratadine were recovered and that the analytes were stable under these conditions. Plasma samples collected from clinical studies of loratadine were evaluated before and after storage at −18 °C for stability and found to be stable for at least 3 months.

The mean absolute recoveries for loratadine were 80%, 83%, and 85% at the 0.2, 4.0, and 60.0 ng/ml concentra-

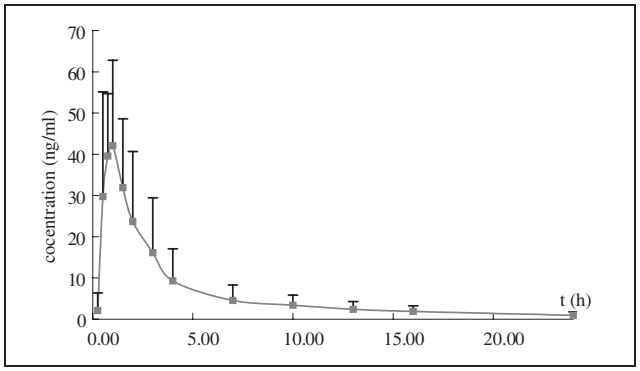
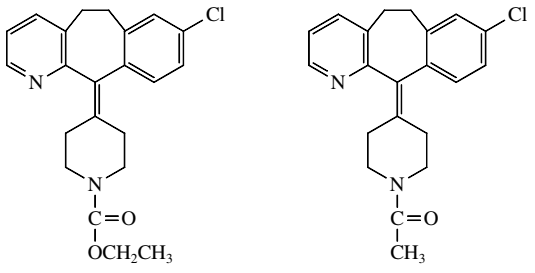


Fig. 4: The mean loratadine plasma concentration curves after oral administration of 40 mg loratadine tablet. (n = 18)



LORATADINE SCH 37370

Chemical structure of Loratadine and internal standard SCH 37370

tion, respectively (n = 3). The mean absolute recovery for internal standard was 78% at the 20.0 ng/ml concentration (n = 3).

The present HPLC-ESI-MS method of loratadine was for the first time employed to determine the pharmacokinetic parameters of loratadine in volunteers' plasma samples. After a single oral dose of 40 mg loratadine in 18 healthy volunteers, concentration versus time profiles were constructed for up to 24 h. Fig. 4. shows the mean ± SD plasma concentration-time profile of loratadine. Pharmacokinetic parameters were estimated using standard noncompartmental methods. The mean maximum loratadine plasma concentration was 52 ± 22 ng/ml, t_{max} was 0.8 ± 0.27 h, $AUC_{0-24\,h}$ was 147 ± 92 ng · h/ml, $AUC_{0-\infty}$ was 161 ± 106 ng · h/ml, and $t_{1/2}$ in the terminal elimination phase was 8.7 ± 2.6 h.

3. Discussion

Different methods (RIA, HPLC, GC, GC-MS) exist for the determination of loratadine in human plasma. RIA (Hilbert et al. 1987) is not specific since anti-loratadine plasma also cross-reacts with the major metabolite of loratadine. Furthermore, the RIA requires both the synthesis of tritium-labeled compound and the production of specific anti-serum. Therefore, RIA is not the method of choice for the evaluation of loratadine pharmacokinetics. The sample preparation of GC method is very tedious (Johnson et al. 1994). In this method, 1 ml of plasma was extracted by organic solvent and evaporated to 3–4 µl, and 1 µl of the mixture was injected into the gas chromatograph. The relative high injection volume brings a lot of contaminations to the GC column and the robustness of this method is not good. The GC-MS method (Martens 1995) did not have the sufficient sensitivity to properly evaluate the pharmacokinetics of loratadine in human since the limit of quantitation was 0.5 ng/ml. The HPLC-Fluorescence method (Zhong and Blume 1994) requires a derivatisation procedure prior to the analysis and the analytical procedure is complicate. Also there were limited data on precision and accuracy. Recently an HPLC-UV method was established (Kunicki 2001). In order to improve its sensitivity, its wave length was set at 200 nm. The column used in this method had to be regenerated after each run by eluting the column with various solvent mixtures to remove biological residues from the column. Furthermore, it was difficult to reproduce the method.

All the methods previously described require a total volume of 1–2 ml of plasma for the determination of loratadine. However, only 0.5 ml of plasma was needed in the method proposed here. The requirement of a smaller volume of plasma is an important factor, especially when the pharmacokinetics of loratadine are evaluated in the pediatric population.

In conclusion, the established HPLC-MS method is very sensitive, precise, selective and useful to monitor low plasma levels of loratadine. So the method is suitable for quantitative analysis are required in human pharmacokinetic studies.

4. Experimental

4.1. Chemicals and reagents

Loratadine standard was provided by the National Institute for the Control Pharmaceutical and Biological Products (Beijing, China). SCH 37370, used as internal standard, was supplied by Shanghai Schering-Plough Pharmaceutical Co. Ltd.(Shanghai, China). Methanol and acetonitrile of HPLC-grade were purchased from Shanghai No. 1 Chemical Reagents Factory. Ammonium acetate, formic acid, hexane and methyl-*tert*-butyl ether were all analytical-grade and water was Milli-Q grade.

4.2. Chromatographic conditions

The LC-MS experiments were conducted with an HP 1100 LC-DAD-MS system (Hewlett-Packard corporation, Palo-Alto CA, USA). The system components included a binary pump, a UV-visible Diode Array Detection (DAD) system and a HP 1100 mass spectrometric detector. The source was a nebulizer assisted electrospray unit incorporation a proprietary orthogonal spraying configuration. Analyses were performed on an Inertsil ODS-3 reverse column (5 µm particle size, 2.1 × 150mm i.d.) purchased from GL Sciences Inc. (Japan).

Each chromatographic run was carried out at a flow rate of 0.2 ml/min with a binary mobile phase consisting of acetonitrile (A) and ammonium acetate (pH 4.0; 0.02 M, using formic acid to adjust). The run started with a step gradient profile of 80% A for 7 min, then increased up to 100% A in 1 min, isocratic at 100% A for 4 min, and finally down to 80% A in 1 min. After re-equilibration at 80% A for 5 min, the next sample was injected.

The mass spectrometer was run in positive ion mode and tuned for unit mass resolution in the mass range used in the experiments. Full scan spectra were obtained by scanning masses between m/z 200 and 800. The exact source conditions were: drying gas temperature 350 °C, drying gas flow 10 L/min, nebulizer pressure 30 p.s.i.g., quadrupole temperature 100 °C and capillary voltage 4000 V. Nitrogen was used exclusively as drying gas. The ion for loratadine was m/z 383 and that for the internal standard SCH 37370 was m/z 353. Both ions had a dwell time of 280 ms per ion. After the acquisition of the resultant SIM, chromatograms were integrated by the HP Chemstation software.

4.3. Standard solutions

The stock standard solution of loratadine was prepared as follows: loratadine standard 50 mg was firstly dissolved in 50 ml methanol, then diluted with methanol to a volume of 10 ml leading to a concentration of loratadine of 0.1 mg/ml. This stock solution was further diluted with methanol to obtain working standard solutions for validation and calibration. An internal standard SCH 37370 stock solution was also prepared in methanol. This solution was further diluted with methanol to prepare the working standard solution containing 0.2 µg/ml of SCH 37370.

4.4. Sample preparation

To a 0.5 ml of aliquot plasma 50 µl of internal standard solution (containing 0.2 µg/ml of SCH 37370) and 50 µl NaOH were added (1 mol/L) for a

few seconds, then 3.0 ml of hexane-methyl-tert-butyl ether (50 : 50, V/V) were added. The mixture was vortex-mixed for 3 min and then centrifuged at 3500 rpm at ambient temperature for 10 min. The organic layer was transferred to another tube and evaporated to dryness under a stream of nitrogen at 60 °C. Finally, the residue was reconstituted in 100 μl mobile phase, transferred into autosampler vials and 10 μl were injected for HPLC-MS analysis.

4.5. Validation test

4.5.1. Linearity and calibration curve

To make calibration standards, each 25 μl of loratadine standard solution in methanol was added to 0.5 ml of drug-free plasma. The spiked concentrations of the calibration standards were 0.2, 0.4, 1.0, 4.0, 10.0, 20.0, 60.0, 100.0 ng/ml. The samples were then processed as described in section 4.4. Three replicate analyses were performed for each calibrator to evaluate linearity. The calibration curves were constructed by plotting the peak-area ratio of loratadine to internal standard versus spiked concentration. The calibration curves were calculated by least-squares regression.

4.5.2. Specificity and interference

Chromatograms of the sample prepared with human blank plasma were visually inspected for peaks from endogenous sources which might correspond to the loratadine and I.S. peaks. The standard plasma sample extracts were dissolved in 100 μl mobile phase and 10 μl aliquot was injected into the chromatograph to determine the detection limit (S/N = 3).

4.5.3. Accuracy and precision

Samples at each of the three concentrations (0.2, 4.0, 60 ng/ml plasma, n =5) were prepared and assayed to determine the intra-day accuracy expressed as relative error (R.E.), and the precision was used as relative standard deviation (RSD). The same method was used in five days for inter-day assay.

4.5.4. Evaluation of matrix suppression effects

The matrix effect (co-eluting, undetected endogenous matrix compounds that may influence the analyte ionization) was investigated by extracting 'blank' biological fluids from five different sources, and reconstituting the final extract in mobile phase containing a known amount of the analyte. The matrix effects were determined at concentrations of 0.2, 4.0, 60.0 ng/ml (n = 5). The reconstituted extracts were analyzed and the peak areas of the analytes were compared. The matrix effect of internal standard at a concentration of 20 ng/ml was also evaluated.

4.5.5. Recovery determination

The absolute recovery of loratadine was determined by direct comparison of peak areas from extracts versus spiked post-extraction samples at 0.4, 4, and 60 ng/ml.

4.6. Pharmacokinetic study

The HPLC-MS method developed was used to investigate the plasma profile of loratadine after single 40 mg oral dose of loratadine. A clinical study on 18 Chinese healthy male volunteers (age from 18 to 24 years old) was conducted under fasting conditions. Informed consent was obtained from the subjects after explaining the nature and purpose of the study. The protocol was approved by the Base for Drug Clinical Trial of SDA of Shanghai No. 2 Medical University, Shanghai, China. Following written informed consent, each volunteer received the loratadine tablet with 250 ml of water. No food was allowed until 4 h after dose administration. Water intake was allowed after 2 h and low-fat standard meals were provided at 4 h and 10 h post dose. Blood samples were drawn into heparinized tubes through an indwelling cannula before (0 h) and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 7, 10, 13, 16, 24 h after dosing for determination of plasma concentration of loratadine. The blood samples were centrifuged at 3500 rpm for 15 min, plasma was separated and kept frozen at −18 °C in coded polypropylene tubs until analysis.

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