# Determination of loratadine in human plasma by HPLC with fluorescence detector and study on its bioavailability

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**Abstract**: **Aim** To establish an HPLC-fluorescence method for determination of loratadine in human plasma and evaluate its relative bioavailability. **Methods** An Alltech-$C_{18}$ column and a mobile phase of acetonitrile-water-glacial acetic acid-triethylamine (90∶100∶6∶0.15) were used. The fluorescence detector was set at $E_x$ 274 nm, $E_m$ 450 nm. The flow rate was 1 mL·min$^{-1}$. **Results** The calibration curve was linear over a concentration range of 0.2 - 30 μg·L$^{-1}$. The limit of quantification was 0.2 μg·L$^{-1}$. The average method recoveries varied from 96 % to 98 %. The results showed AUC, $T_{max}$, $C_{max}$ and $T_{1/2}\beta$ between the testing tablets, testing capsules and reference tablets had no significant difference ($P > 0.05$). Relative bioavailabilities were 107 % ±17 % and 100 % ±14 % respectively. **Conclusion** The three formulations were bioequivalent.

**Key words**: loratadine ; bioavailability ; fluorescence detection ; HPLC
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# 氯雷他定血药浓度的 HPLC 荧光检测法及生物等效性研究

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摘要 : 目的 建立高效液相色谱法荧光检测血浆中氯雷他定(loratadine)含量的方法 ,以评价氯雷他定的相对生物利用度。方法 色谱柱为 Alltech $C_{18}$ ,4.6 mm × 150 mm ;流动相为乙腈-水-冰醋酸-三乙胺(90∶100∶6∶0.15) ;流速为 1 mL·min$^{-1}$ ;荧光检测器测定波长 , $E_x$ = 274 nm , $E_m$ = 450 nm。结果 HPLC 测定线性范围为 0.2 ~ 30 μg·L$^{-1}$ ,最低定量限 0.2 μg·L$^{-1}$ ,方法回收率为 96 % ~ 98 %。人体生物利用度结果表明 ,实验片 、胶囊与对照片间的 AUC , $T_{max}$ , $C_{max}$ 和 $T_{1/2}\beta$ 均无显著性差异($P > 0.05$) ,两者的相对生物利用度分别为 107 % ±17 %和 100 % ±14 %。AUC 和 $C_{max}$ 经可信区间法检验生物等效。结论 3 种制剂生物等效。

关键词 : 氯雷他定 ; 生物利用度 ; 荧光检测 ; HPLC

Loratadine is an antihistamine drug used as first-line agent for the treatment of urticaria and rhinitis[1] (Figure 1). The drug is rapidly absorbed and extensively metabolized in liver, and descarboethoxyloratadine is the major metabolite. Thus, great individual variability in level of loratadine occurs among patients. A sensitive and simple loratadine determination method for bioavailability studies and therapeutic drug monitoring is thus in need. Several techniques have been reported for loratadine quantification in plasma samples, such as radioimmunoassay, gas chromatography (GC)[2], high performance liquid chromatography (HPLC) with ultraviolet (UV) detection[3], mass spectrometry (MS)[4], fluorescence detection[5] and chemiluminescence detection[6]. The method using fluorescence detection has been reported by Zhong[5] with an LOQ of only 0.5 μg·L$^{-1}$.

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This paper presents a simple and sensitive procedure for loratadine determination in human plasma , which has been successfully applied to bioavailability study .

## Materials and methods

**Chemical and reagents** Loratadine tablets and capsules ( each contains 10 mg loratadine , Nanjing A Pharmaceutical Co , Ltd .) ; reference tablets ( clatitin , 10 mg/tablet , Shanghai Schering-plough Pharmaceutical Co , Ltd .) ; the internal standard chlorimipramine ( Figure 1) was obtained from Shanghai Novartis Pharmaceutical Cor . , Ltd ; acetonitrile , water , glacial acetic acid were of analytical grade or HPLC grade .

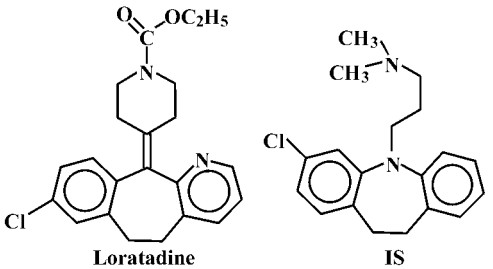



Figure 1 Chemical structures of loratadine and chlorimipramine ( IS)

**Chromatographic condition** The HP1090 HPLC system was used with RF10A ( Shimadazu) fluorescence detector. The samples were separated on a Alltech-$C_{18}$ (150 mm × 4.6 mm ID, 5 μm) column. The mobile phase was a mixture of acetonitrile-water-glacial acetic acid-triethylamine (90∶100∶6∶0.15) at a flow-rate of 1 mL• $min^{-1}$. The parameters of the detector were as follows: $E_x$ 274 nm; $E_m$ 450 nm; Gain 2; Ratio 1; Range 1; Sens 1; Response 4.

**Clinical study** Three formulations of loratadine were administered to 24 healthy male volunteers in a balanced 3 × 3 Latin square design experiment ( Table 1) , separated by one week washout period. The volunteers fasted overnight for at least 10 h before the administration of drug (10 mg × 2) and 4 wore hours after dose. The participants did not take any medication, cigarette , wine or tea for at least two weeks prior to and during the days of the study. Venous blood samples ( 3 mL) were drawn into heparinized tubes before and 0.25 , 0.5 , 0.75 , 1 , 1.5 , 2 , 2.5 , 3 , 4 , 5 , 6 , 8 , 12 h after the drug was administrated. The blood samples were centrifuged at 1 100 r• $min^{-1}$ for 15 min and the plasma samples were frozen at - 20 ℃ before analysis .

**Table 1 Balanced 3 × 3 Latin square design**

| Volunteer | First period | Second period | Third period |
|---|---|---|---|
| A | R | Tl | T2 |
| B | Tl | T2 | R |
| C | T2 | R | Tl |
| D | T2 | Tl | R |
| E | R | T2 | Tl |
| F | Tl | R | T2 |
| G | R | Tl | T2 |
| H | Tl | T2 | R |
| I | T2 | R | Tl |
| J | T2 | Tl | R |
| K | R | T2 | Tl |
| L | Tl | R | T2 |
| M | R | Tl | T2 |
| N | Tl | T2 | R |
| O | T2 | R | Tl |
| P | T2 | Tl | R |
| Q | R | T2 | Tl |
| R | Tl | R | T2 |
| S | R | Tl | T2 |
| T | Tl | T2 | R |
| U | T2 | R | Tl |
| V | T2 | Tl | R |
| W | R | T2 | Tl |
| X | Tl | R | T2 |

R: Reference tablets; Tl : Experimental tablets; T2: Experimental capsules

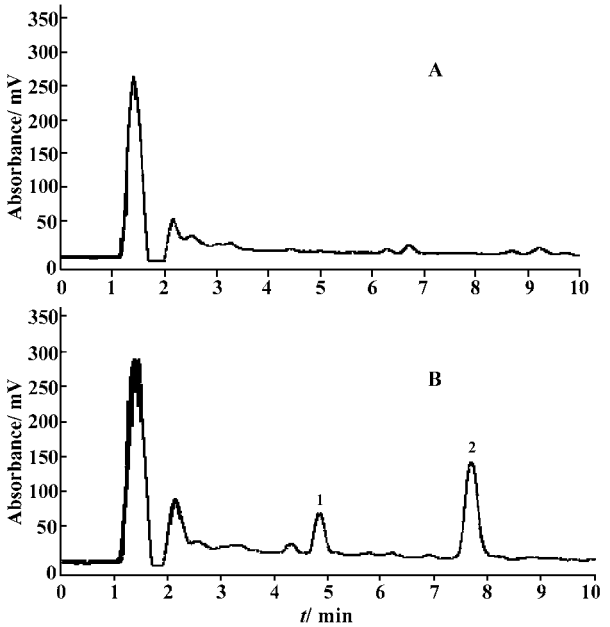
**Sample preparation** One mL volume of plasma was transferred to a 10 mL glass centrifuge tube , mixed with 50 μL of internal standard solution ( 5 mg• $L^{-1}$) and 0.1 mL of sodium acetate solution ( 50 g• $L^{-1}$) , followed by 0.5 mL of methanol and 5 mL of *n*-hexane . The sample was vortexed for 3 min and centrifuged at a speed of 4 000 r• $min^{-1}$ for 10 min . The organic layer was quantitatively ( 4 mL) transferred to another 10 mL centrifuge tube and evaporated to dryness in a water bath at 60 ℃ under a stream of nitrogen . The residue was reconstituted in 50 μL methanol , after a high speed centrifugalization of 16 000 r• $min^{-1}$ , a 20 μL aliquot was injected into HPLC for analysis .

## Results

### 1 Separation

Loratadine and internal standard were well separated from the biological background under the described chromatographic conditions , and the retention time were 7.5 min and 4.9 min , respectively ( Figure 2) . The peaks were of good shape and completely resovled . No

interference from plasma matrix was observed. The limit of quantification was 0.2 μg·L$^{-1}$.




1: IS; 2: Loratadine

Figure 2 Chromatograms of blank plasma (A) and 10 μg·L$^{-1}$ loratadine and internal standard in plasma (B) under the running condition of Alltech-$C_{18}$ column and a mobile phase of acetonitrile-water-glacial acetic acid-triethylamine (90∶100∶6∶0.15)

## 2 Calibration

The calibration curve was obtained by analyzing five samples for each of seven tested concentrations (0.2, 0.5, 2, 5, 10, 20 and 30 μg·L$^{-1}$). The curve was linear in whole range tested (0.2 - 30 μg·L$^{-1}$) and described by $Y = 0.295\ 9X + 0.004\ 8$ ($r = 0.999\ 8$, $n = 5$), where $Y$ corresponds to the peak area ratio of loratadine and the internal standard and $X$ to the concentration of loratadine added over the whole range.

## 3 Recovery and precision

The absolute recovery of loratadine was determined at three different concentration levels (low, medium, high) by comparing extracted versus unextracted samples (the one which was dissolved by the mobile phase with the corresponding concentration). The method recovery was determined by comparing concentration obtained through the calibration curve versus that had been added (Table 2).

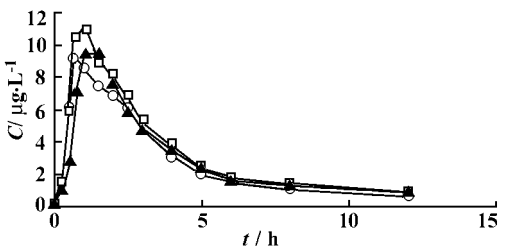
To evaluate the precision of the method, the samples at three different concentrations were analyzed both on the same day and different days.

## 4 Clinical application

Figure 3 depicts the mean (24 patients) plasma concentration-time curve of loratadine after per oral administration of loratadine tablets, loratadine capsules and claritin, respectively. As can be seen, the three curves are almost overlapped.

**Table 2 Recovery and precision**

| Content / μg·L$^{-1}$ | Absolute recovery / % | Method recovery / % | RSD / % | |
|---|---|---|---|---|
| | | | Intra-day | Inter-day |
| 0.5 | 96 ±6 | 98 ±6 | 6.29 | 6.02 |
| 5 | 93 ±4 | 96 ±4 | 4.35 | 4.55 |
| 20 | 95 ±6 | 98 ±3 | 2.64 | 3.68 |




○—○ Reference tablets; □—□ Loratadine tablets; ▲—▲ Loratadine capsules

Figure 3 Mean plasma concentration-time curve

Table 3 shows the main pharmacokinetic parameters ($AUC_{0-12\ h}$, $C_{max}$, $T_{max}$, $T_{1/2}\ \beta$) of loratadine. The Results demonstrate that the three formulations of loratadine were bioequivalent by variance analysis, test of confidence interval and 90 % confidential limit. And the relative bioavailability of the testing tablets and capsules were 107 % ±17 % and 100 % ±14 %, respectively.

**Table 3 Pharmacokinetic parameters of loratadine following oral administration (20 mg) of three formulations to 24 subjects ($\bar{x} \pm s$)**

| Parameter | R | T1 | T2 |
|---|---|---|---|
| $AUC_{0-12\ h}$/ mg·h·L$^{-1}$ | 34 ±25 | 37 ±28 | 33 ±23 |
| $AUC_{0-\infty}$/ mg·h·L$^{-1}$ | 38 ±28 | 42 ±32 | 39 ±28 |
| $C_{max}$/ μg·L$^{-1}$ | 14 ±7 | 13 ±8 | 13 ±7 |
| $T_{max}$/ h | 1.2 ±0.7 | 1.0 ±0.5 | 1.3 ±0.7 |
| $T_{1/2}$/ h | 3.6 ±2.0 | 4.3 ±3.0 | 4.1 ±2.0 |

## Discussion

The described method was established as a rapid, sensitive and selective RP-HPLC analysis of loratadine with fluorescence detection. The method improved upon previously reported methods for accurate measurement of loratadine in biological samples. And it needs only 1 mL of human plasma and LOQ is 0.2 μg·L$^{-1}$ better than that has been reported. Uncomplicated two-steps analytical

procedure based on simple liquid-liquid extraction followed by redissolving and evaporation gives clean probe for HPLC with satisfactory recovery.

Instead of using special synthetic loratadine analog as internal standard, we used commonly available chlorimipramine. Fluorescence detection is more sensitive than UV. Addition of 0.5 mL methanol is to prevent emulsification. Balanced 3 × 3 Latin square design is to eliminate the difference between periods.

The result indicates that there is great difference of bioavailability among individuals, because loratadine is metabolized to descarboethoxyloratadine predominantly by cytochrome P450 3A4 (CYP3A4) and cytochrome P450 2D6 (CYP2D6), which both have different activities between individuals.

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