Determination of loratadine in human plasma by HPLC with solid phase extraction method

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ABSTRACT: OBJECTIVE To establish a method for the determination of loratadine in human plasma using a solid-phase extraction and reversed-phase HPLC. **METHODS** The sample was treated with ODS-C_{18} solid-phase extraction column, Zorbax SB-C_{18} column (4.6mm × 250mm, 5μm) was used, 50 mmol · L^{-1} ammoninium dihydrogen phosphate solution (adjust pH to 4.0 with H_3PO_4)-acetonitrile (62:38) were served as mobile phase with 1.2mL · min^{-1} flow rate. The detection wavelength was 275nm. **RESULTS** Loratadine concentration presented a good linear range of 1 ~ 100 ng · mL^{-1} (r = 0.999 6), the recovery was between 82.63% ~ 92.41%, the relative standard deviations of within-day and between-day were less than 10%. The minimal detectable concentration in plasma was 1ng · mL^{-1}. **CONCLUSION** This method is rapid, simple and accurate for the determination of loratadine levers in human plasma. It was suitable for the pharmacokinetics and bioavailability study of Loratadine.

KEY WORDS: HPLC; loratadine; drug concentration in plasma

固相萃取—高效液相色谱法测定氯雷他定的血药浓度

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摘要: 目的 建立固相萃取-高效液相色谱法测定氯雷他定血药浓度的方法。**方法** 采用 ODS-C_{18} 小柱处理样品, Zorbax SB-C_{18} 硅烷键合硅胶柱 (4.6mm × 250mm, 5um), 流动相: 50mmol · L^{-1} 磷酸二氢铵缓冲液 (磷酸调 PH4.0)-乙腈 (62:38), 流速: 1.2mL · min^{-1}, 检测波长: 275nm。**结果** 线性范围 1 ~ 100 ng · mL^{-1} (r = 0.999 6), 回收率在 82.63% ~ 92.41%, 日内、日间 RSD < 10%, 最低定量浓度为 1ng · mL^{-1}。**结论** 该法简便、快速、准确, 适用于氯雷他定药代动力血和生物等效性研究。

关键词: 高效液相色谱法; 氯雷他定; 血药浓度

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1 Introduction

Loratadine is a selective peripheral histamine H_1-receptor antogonist devoid of any substantial effects on the central and autonomic nervous system. Loratadine mainly used on allergic rhinitis, acute and chronic urticaria, allergic skin disease. In this paper, we determined the drug concentration in plasma of loratadine by HPLC with solid phase extraction (SPE) method and provided using basis for clinic.

2 Experimental

2.1 Apparatus

The HPLC employed in this work was a Agilent 1100 system which included vacuum degasser, quaternary pump, autosampler, thermostatted column compartment, diode-array detector and work station. The detection wavelength was set at 275 nm. A Zorbax SB-C_{18} reversed-phase column (4.6 × 250mm, 5μm particle size) was used for seperation. A RP-C_{18} guard column was fitted up-stream of the analytical column. The BF2000 SPE system and Zorbax ODS-C_{18} SPE column were used in sample preparation.

2.2 Chemicals and reagents

Loratadine tablets was provided by Shanghai Schering-Plough Pharmaceutical Co. Ltd. Loratadine standard was supplied by Tianjing Pharmacy Research Institute. Methanol of HPLC-grade were purchased from Tedia Company. The other reagents were analytical-grade. A stock solution of Loratadine (5μg · mL^{-1}) was prepared in methanol and was stable for 3 months when stored at 4℃.

2.3 Chromatographic condition

The mobile phase consisted of 50 mmol · L^{-1} ammoninium dihydrogen phosphate solution (adjust pH to 4.0 with H_3PO_4)-acetonitrile (62:38), flowing through the column at a constant flow rate of 1.2mL · min^{-1}.

2.4 Sample preparation

The 2mL methanol and 1mL water were added into the ODS-C_{18} SPE column successively to make the column active. Then 1mL plasma was added into column, flowing through the column

with 1mL water and 1mL 5% methanol successively to eliminate impurity. 1mL methanol was added into column and collected the drop solution, transferred it into autosampler vials and injected 50μL for HPLC analysis.

2.5 Calibration linearity

From above stock solutions various dilutions were made with blank plasma to get solutions of 100, 50, 25, 10, 5, 2, 1 ng · mL^{-1}. The standard curve was constructed by plotting the ratio of peak area of Loratadine to the known concentration of Loratadine added to drug free plasma. The curves were linear in the range of 1 ~ 100 ng · mL^{-1} with a correlation coefficient of r = 0.999 6.

2.6 Precisions and recoveries

To assess the recovery of the method, the drug was added to blank plasma (1, 10, 50μg · L^{-1}) and assayed according to the method mentioned above, the recoveries were calculated by using the peak area ratio of the detected to the added amounts of the drug.

2.7 Subjects

Twelve Chinese health male volunteers were involved in this study. The mean age was 22.2 years and mean body weight was 60.5kg. On the basis of medical history, clinical examination and laboratory investigation (haematology, blood chemistry and urine analysis), no subject had a history or evidence of cardiovascular, hepatic, renal, gastrointestinal or haematologic deviations or any acute or chronic disease or drug allergy. The subjects were instructed to abstain from taking any medication for two weeks and informed consents were obtained from the subjects after explaining the nature and purpose of the study.

2.8 Study design

After an overnight (12h) lasting, each volunteer received the tablet of loratadine (40mg) with 250mL water. No food was allowed until 4h after dose administration. Water intake was allowed after 2h and low fat standard meals were provided 4h and 10h post dose. Approximately 3mL blood samples were drawn into heparinize tubes through an indwelling cannula at 0.25, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0, 24.0h after dosing for determination of plasma concentration of loratadine. The blood samples were centrifuged at 2000r · min^{-1} for 10min and plasma was separated and kept frozen at −20℃.

3 Results and discussion

3.1 Performance of the HPLC system

The analytical peaks of loratadine was resolved with good symmetry (Fig. 1). Under the condition, retention time was 7.7 min for loratadine and no endogenous sources of interference observed at the analytic retention time.

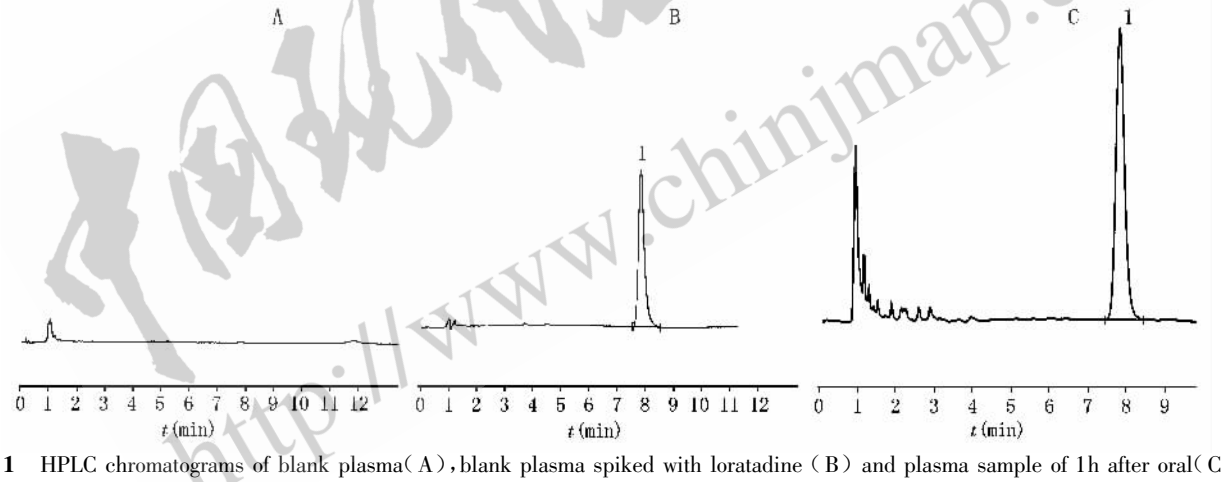

Fig 1 HPLC chromatograms of blank plasma(A), blank plasma spiked with loratadine (B) and plasma sample of 1h after oral(C)

图1 空白血浆(A),空白血浆加氯雷他定(B)以及口服人体氯雷他定1h后(C)血浆HPLC色谱图

1. 氯雷他定(Loratadine, 7.7min)

3.2 Linearity, precision and recoveries

Loratadine concentration presented a good linear range of 1 ~ 100 ng · mL^{-1}, r = 0.9996 (n = 7). The linear regression equation was $Y = 0.0696C + 0.0121$. The detection limit of assay had been evaluated as 1ng · mL^{-1} at a sign to noise of 3:1.

Precision data were obtained by the repeated analysis of plasma samples prepared according to the method mentioned. The methodological recoveries and the absolute recovery of the HPLC method were high and stable (Table 1, Table 2).

3.3 Drug concentration in plasma study of loratadine

Tab 1 The method precision and recovery of loratadine in plasma (n = 5)

表1 测定血浆中氯雷他定的方法精密度和回收率(n = 5)

added	Recovery ± RSD%		Precision RSD%	
μg · L^{-1}	within day	between day	within day	between day
1.0	85.17 ± 8.86	82.63 ± 10.10	4.8	6.8
10.0	87.53 ± 6.63	86.55 ± 7.32	2.6	3.4
50.0	89.42 ± 4.85	92.41 ± 4.42	2.2	3.6

The concentration-time curves of the loratadine after a single dose of 4 0 mg was shown in Fig 2 . The parameters were deter -

Tab 2 The absolute recovery of loratadine in plasma($n=5$)

表 2 测定血浆中氯雷他定的绝对回收率($n=5$)

Conc($\mu g \cdot L^{-1}$)	Recovery(%)	RSD(%)
1.0	76.52 ± 7.51	9.45
10.0	81.76 ± 7.72	8.90
50.0	83.62 ± 4.99	5.49

mined by the methods mentioned and the pharmacokinetic parameters were calculated with 3P87 program. The C_{max}, t_{max}, $t_{1/2}$, AUC_{0-t}, and $AUC_{0-\infty}$ were (51.48 ± 20.35) ng · mL^{-1}, (0.80 ± 0.37) h, (8.46 ± 2.20) h, (146.74 ± 90.43) ng · h · mL^{-1}, (150.24 ± 99.38) ng · h · mL^{-1}.

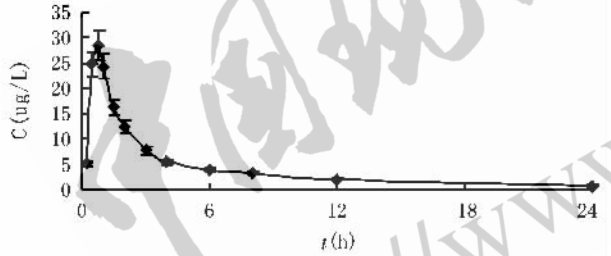

Fig 2 Plasma concentration-time curve of loratadine after a single oral dose of 40mg

图 2 单次口服 40mg 氯雷他定的血药浓度-时间曲线

In conclusion, the assay provided excellent recovery and was linear over a wide range of analyte concentrations. The established SPE-HPLC method was selective, sensitive, precise and enough to monitor the low plasma levels of the drug.

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