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Short communication

Quantitative determination of epinastine in plasma by high-performance liquid chromatography

Hisakazu Ohtani[a,*], Hajime Kotaki[a], Yasufumi Sawada[b], Tatsuji Iga[a]

[a]*Department of Pharmacy, The University of Tokyo Hospital, Faculty of Medicine, The University of Tokyo, 3-1 Hongo 7-chome, Bunkyo-ku, Tokyo 113, Japan*

[b]*Faculty of Pharmaceutical Sciences, Kyushu University, 1-1 Maidashi 3-chome, Higashi-ku, Fukuoka City, Fukuoka 812, Japan*

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Abstract

A high-performance liquid chromatographic (HPLC) method for the quantitative determination of epinastine, a non-sedating histamine H_1 receptor antagonist, in rat plasma, was developed. A 100-μl volume of plasma sample was spiked with a solution of internal standard (diphenidol) and extracted with dichloromethane under alkaline conditions. The extract was applied onto the HPLC system and detected by ultraviolet absorption at a wavelength of 220 nm. The linearity of the calibration curve was preserved over the concentration range of 20–1000 ng/ml. Both intra-assay variation and relative error were less than 5% for the plasma sample containing 50 ng/ml or 1000 ng/ml of epinastine hydrochloride. The analytical method presented here should be useful for the investigation of the pharmacokinetic properties of epinastine, which is of clinical significance.

Keywords: Epinastine

1. Introduction

Epinastine (EPN), (±)-3-amino-9,13b-dihydro-1*H*-dibenz[*c*,*f*]imidazo[1,5-*a*]azepine (I, Fig. 1), is a newly developed, non-sedating, histamine H_1 antagonist that is used as an anti-allergic agent or for the treatment of asthma. EPN is a clinically valuable agent as it possesses weak potency on the central nervous system (CNS) because of its low rate of entry into the CNS [1–3]. This drug was reported to be mainly excreted into urine and faeces in an unchanged form [4]. Therefore, the kinetics of EPN may be relatively more resistant to drug-induced metabolic inhibition or hepatic dysfunction compared with other conventional non-sedating antihistamines, such as terfenadine or astemizole, whose metabolism is highly altered by metabolic inhibition or hepatic dysfunction [5–7].

As these pharmacokinetic properties of EPN make it a potential replacement for conventional non-sedating antihistamines, it is of great clinical relevance that its precise pharmacokinetic properties are investigated under several clinical states, such as hepatic or renal dysfunction. Thus, an analytical method for the determination of EPN is of urgent concern. Only Azuma et al. [8], in their report focused on a preclinical Phase I study, refer to a procedure for the determination of EPN. However,

*Corresponding author.

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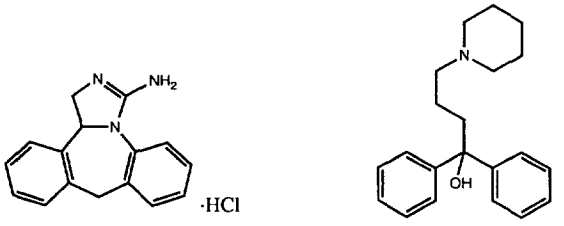

I. epinastine · HCl II. diphenidol

Fig. 1. Chemical structures of (I) epinastine hydrochloride and (II) diphenidol.

their procedure is lacking in some important aspects. They did not employ an internal standard as an analytical carrier and they did not carry out assay validation. Moreover, their method is complicated because it requires fluorescent labeling.

In this paper, we describe a reliable and simple high-performance liquid chromatographic (HPLC) method with ultraviolet (UV) detection for the quantitative determination of EPN in plasma.

2. Experimental

2.1. Standards and reagents

EPN hydrochloride was kindly provided by Nippon Boehringer Ingelheim (Hyôgo, Japan). The internal standard, diphenidol (II, Fig. 1), was purchased from Sigma (St. Louis, MO, USA). Dichloromethane and methanol were of HPLC reagent grade and were purchased from Wako Pure Chemical Industries (Osaka, Japan). All other compounds used were of reagent grade and supplied by Wako Pure Chemical Industries. Water used in this study was distilled deionized water.

EPN hydrochloride and diphenidol were dissolved in water at a concentration of 0.5 mg/ml and stored, as stock solutions, in the dark at 4°C.

The internal standard solution was prepared from the stock solution to give a concentration of 0.6 μg/ml in water. The blank plasma was a mixture obtained from three male Sprague–Dawley rats that weighed from 390 to 520 g.

2.2. Instruments and chromatographic conditions

The HPLC system consisted of a liquid chromatograph LC 10AD (Shimadzu, Kyoto, Japan) equipped with the UV spectrophotometric detector, SPD 10A (Shimadzu). The chromatogram was drawn using a pen recorder, Model U-125S158 (Shimadzu). The separation was performed on a reversed-phase column (Cosmosil 5C18-MS; 15 cm×4.6 mm) (Nacalai Tesque, Kyoto, Japan). The column was maintained at ambient temperature. The mobile phase consisted of 0.3% (v/v) triethylamine (pH adjusted to 4.5 with phosphoric acid) and methanol (64:36, v/v) and pumped at a constant rate of 1.3 ml/min. The absorbance of the eluent was monitored at a wavelength of 220 nm.

2.3. Extraction procedure

Plasma and internal standard solution (100 μl each) were pipetted into a 10-ml glass centrifuge tube and made alkaline by the addition of 600 μl of 0.1 *M* Na_2CO_3. The compounds were extracted using 5 ml of dichloromethane, by shaking with a reciprocal shaker for 10 min. After the centrifugation at 1000 *g* for 8 min, 4 ml of the lower organic phase were transferred into another centrifuge tube and dried using a rotary evaporator at 45°C. The residue was dissolved into 50 μl of mobile phase and 20 μl were applied onto the HPLC system.

2.4. Assay validation and calculations

The plasma was spiked with EPN hydrochloride to obtain final plasma concentrations of 0, 20, 50, 100, 300 and 1000 ng/ml and these were used for the calibration. A constant volume (100 μl) of internal standard solution was added and assayed as described in Section 2.3.

The intra-assay validation was performed with five aliquots of plasma at concentrations of 50 or 1000 ng/ml of EPN hydrochloride, and the accuracy and the coefficient of variation were determined. The concentration was determined from the peak-height ratio of EPN hydrochloride to the internal standard. Moreover, the inter-day coefficient of variation for the slope of the calibration lines was also calculated, to validate the inter-assay reproducibility.

The absolute extraction recovery of EPN and internal standard was determined by comparing the heights of the respective peaks from extracted standard plasma containing 500 ng/ml of EPN hydrochloride and internal standard to those of unextracted standard solutions.

2.5. Animal experiment

A male Sprague–Dawley rat weighing 350 g was used for the experiment. The left femoral artery and vein were cannulated with polyethylene tubing. EPN hydrochloride (2 mg/kg dose) was intravenously injected into the left femoral vein for 20 s. The blood samples (250 μl each) were obtained from the left femoral artery at 2, 5, 15, 30, 60, 120, 180, 240 and 360 min after injection. The sample was immediately centrifuged to obtain 100 μl of plasma and the plasma was stored at −20°C until assayed.

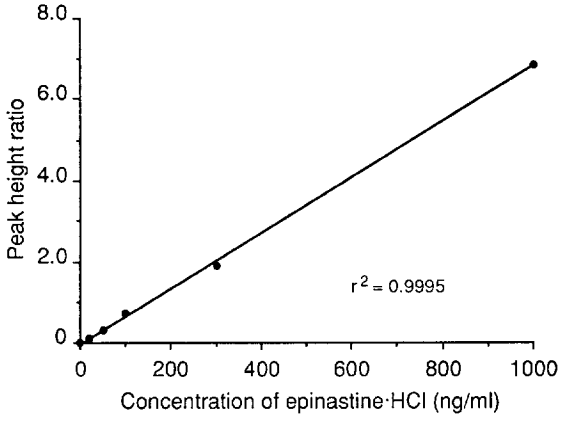

Fig. 3. Typical calibration curve for epinastine.

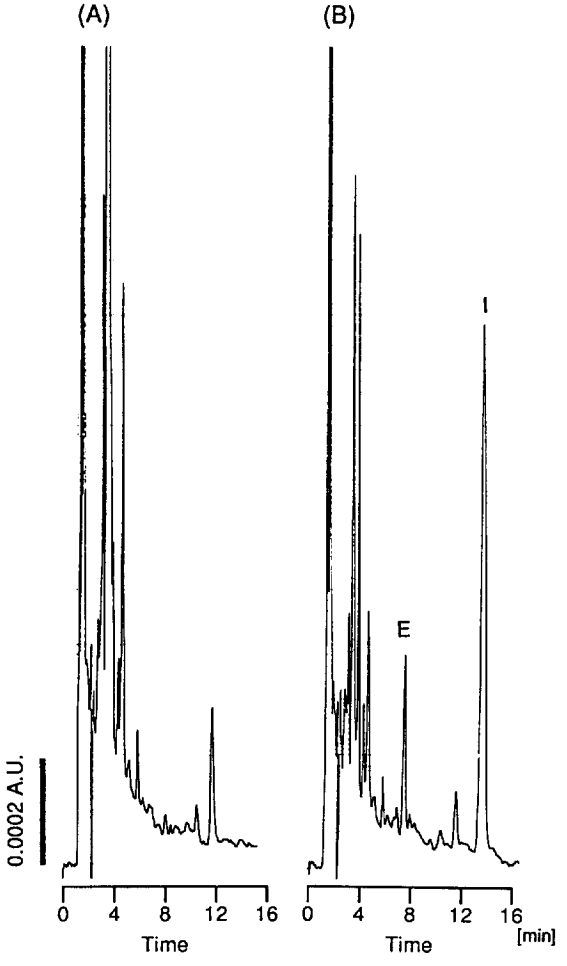

Fig. 2. Chromatograms of extracts from (A) blank rat plasma and (B) plasma containing 50 ng/ml of epinastine·HCl and internal standard. Peaks: E=epinastine; I=internal standard.

3. Result and discussion

Fig. 2 shows a typical chromatogram of blank rat plasma (A) and plasma spiked with internal standard and 50 ng/ml of EPN hydrochloride (B). For EPN and the internal standard, sharp peaks were obtained with retention times of 8.1 and 14.8 min, respectively. The chromatogram from blank plasma was free from interfering peaks at the retention time of EPN or internal standard.

Fig. 3 shows a typical calibration curve for EPN. The peak-height ratio was quite linear vs. the con-

Table 1
Accuracy and coefficients of variation for the determination of epinastine ($n=5$)

Concentration added (ng/ml)	Concentration determined (ng/ml)	C.V. (%)	Relative error (%)
50.0	52.3	2.0	4.6
1000	996	4.9	−0.4

Table 2
Interday variation of the assay

Day	Slope	r^2
1	6.447	0.994
2	6.565	0.998
3	6.691	1.000
4	6.237	0.999
Mean	6.485	
S.D.	0.167	
C.V. (%)	2.58	

centration of EPN hydrochloride, which ranged from 20 to 1000 ng/ml (r^2=0.9995).

The assay accuracy and the coefficient of variation are given in Table 1 and Table 2. The relative error and the intra-assay coefficient of variation were less than 5% for both high concentration (1000 ng/ml) and low concentration (50 ng/ml) samples (Table 1). The inter-assay coefficient of variation calculated from the slope of the calibration line was 2.58% (Table 2). The absolute extraction ratios for EPN and internal standard were 96.1±5.4% and 75.9±7.1%, respectively (mean±S.D.).

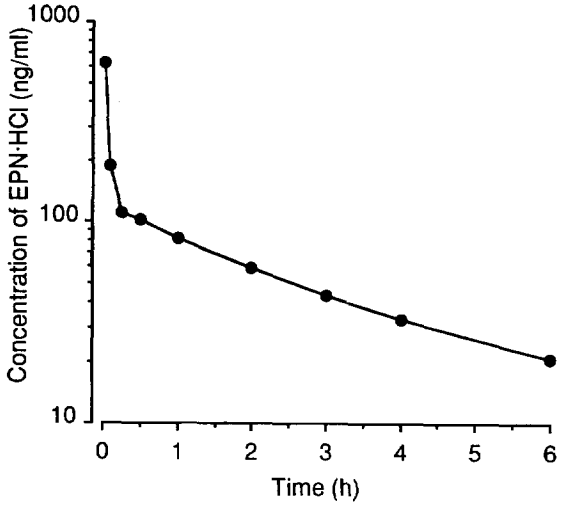

Fig. 4. Time course of EPN concentration in plasma after the intravenous injection of 2 mg/kg of EPN·HCl into a rat.

The detection limit for EPN hydrochloride from 100 μl of plasma was 4 ng/ml for S/N=2. The determination limit is considered to be 10 ng/ml from 100 μl of plasma. While the clinical concentration of EPN hydrochloride is considered to range from 10 to 30 ng/ml after a single oral dose of a 20-mg tablet of EPN hydrochloride [4], the procedure in this report should be useful for investigating the pharmacokinetic properties of EPN within the clinical concentration range.

The present method was applied to the determination of the concentrations of EPN in plasma after intravenous injection to a rat. The plasma concentration decreased with the passage of time in a biexponential manner, and the concentration was 21 ng/ml at 6 h (Fig. 4). These results demonstrated the usefulness of the present method for pharmacokinetic studies of EPN.

In conclusion, an analytical method using HPLC for the quantitative determination of EPN in small quantities of plasma was developed. Our procedure was suitable for examining the pharmacokinetic properties of EPN in a small animal.

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