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Short communication

Liquid chromatographic determination of fexofenadine in human plasma with fluorescence detection

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Abstract

A simple and sensitive method was developed for determination of fexofenadine by liquid chromatography with fluorescence detection. Fexofenadine in human plasma was extracted on a C18 bonded-phase extraction cartridge. The mobile phases were: (A) 0.05 M KH_2PO_4 buffer/acetonitrile/methanol (60:35:10, v/v/v) and (B) 0.05 M KH_2PO_4 buffer/acetonitrile (40:60, v/v). Chromatographic separation was achieved on an ODS-80A column (150 mm × 4.6 mm i.d., particle size 5 μm) using a linear gradient from A to B in 10 min. The peak was detected using a fluorescence detector set at Ex 220 nm and Em 290 nm, and the total time for a chromatographic separation was ∼17 min. The validated quantitation ranges of this method were 1.0–500 ng/ml with coefficients of variation of 0.6–9.1%. Mean recoveries were 72.8–76.7% with coefficients of variation of 2.7–5.8%. This method is successfully applicable for therapeutic drug monitoring in patients treated with clinical doses of fexofenadine and for analyses within pharmacokinetic studies.

Keywords: Fexofenadine; Fluorescence detection; Solid-phase extraction; Pharmacokinetics

1. Introduction

Fexofenadine, an active metabolite of terfenadine, is a selective histamine H1 receptor antagonist, and is clinically effective in the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria as a first-line therapeutic agent, such as loratadine and cetirizine [1]. In clinical trials fexofenadine did not prolong the QT interval or decrease the heart rate, unlike terfenadine, astemizole and ebastatine [1].

Recently, it is increasingly evident that drug transporters including P-glycoprotein and the organic anion transporting polypeptide (OATP) has a pivotal role in pharmacokinetics of numerous drugs with therapeutic implications [2,3]. Fexofenadine is regarded as a substrate of P-glycoprotein and OATP based on several in vitro studies [4,5], and drug-drug and drug-food

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interaction reports [6–8]. Therefore, it is important that a highly sensitive analytical method for fexofenadine is developed to investigate the significant implication and mechanisms of transporter-mediated drug interaction.

Because fexofenadine disposition does not fit one compartment model, lower concentration is important to precisely define the pharmacokinetic parameters of fexofenadine. Although several HPLC methods with fluorescence detection for the determination of fexofenadine concentrations have been previously reported [9,10], their respective quantification limits (8.2 or 5 ng/ml) are not sufficiently sensitive to apply for non-compartment model. Hofmann et al. [11] have reported the determination of fexofenadine in human plasma by liquid chromatography–mass spectrometry (LC–MS) with a low quantification limit (0.5 ng/ml). However, LC–MS methods are very expensive for routine analysis of therapeutic concentration of fexofenadine. Therefore, new methods with both simple and sensitive determination of fexofenadine are required. In the present study, we describe a simple and sensitive HPLC method with fluorescence detection for determination of fexofenadine in plasma using a solid-phase extraction (SPE). The limit of quantification obtained allows measurement of fexofenadine plasma concentrations up to 48 h after an administration of fexofenadine 120 mg as a representative clinical dose.

2. Experimental

2.1. Reagents and materials

Fexofenadine and diphenhydramine (Fig. 1) as an internal standard (I.S.) were kindly donated by Aventis Pharma (Tokyo, Japan) and Tanabe Pharmaceutical Co. Ltd. (Osaka, Japan), respectively. Solid-phase extraction cartridge, Bond Elut C18® (500 mg, 3 ml) was purchased from Varian (Harbor City, CA, USA). Solvents used were of HPLC grade (Wako Pure Chemical Industries, Osaka, Japan). All reagents and chemicals of analytical grade were purchased from Wako Pure Chemical Industries (Osaka, Japan). Water was deionized and purified using a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Drug solutions

Stock solutions of fexofenadine and I.S. for generating standard curves were prepared by dissolving an appropriate amount of each compound in methanol to yield concentrations of 1.0 mg/ml. A very high working standard solution of fexofenadine and I.S. (10 μg/ml) were obtained by diluting each stock solution with methanol 100 times. Middle (1.0 μg/ml) and low (0.1 μg/ml) working standard solutions of fexofenadine were obtained by further diluting the working standard solution with methanol 10 and 100

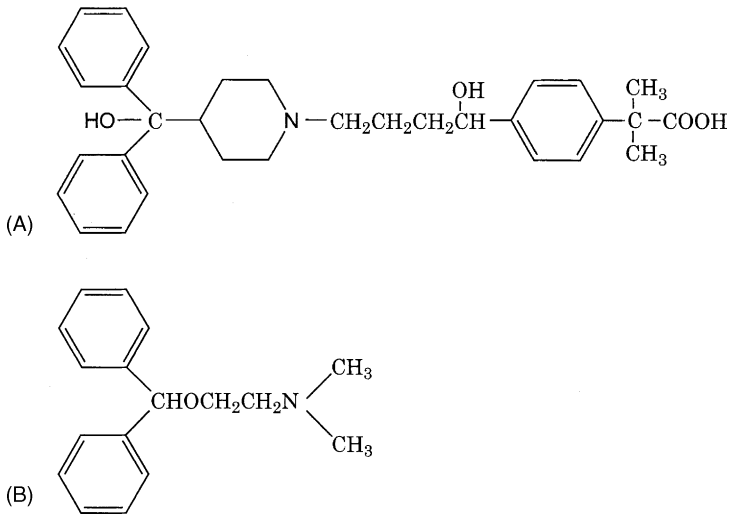

Fig. 1. Chemical structures of fexofenadine (A) and diphenhydramine (B) as an internal standard.

times, respectively. According to FDA Guideline [12], stability of stock solutions was confirmed at 4 °C for at least 3 months. Drug-free plasma from healthy donors was used for the validation studies. Calibration curves were prepared by spiking 10–50 μl of working solutions in 1 ml of blank plasma (final volume) to yield the final concentrations of 1, 5, 10, 50, 100, 200, and 500 ng/ml for each analysis. Standard curves were prepared daily and constructed by linear regression analysis of the compounds/internal standard peak-height ratio versus the respective concentration of fexofenadine. Stock solution of fexofenadine was separately prepared for quality controls in the same manner for standard curves. Quality control samples were obtained by spiking 10–50 μl of working standard solutions in 1 ml of blank plasma (final volume) to yield the final concentrations range of 1, 50 and 500 ng/ml for fexofenadine, which were stored at −20 °C until analysis. All standard curves were checked using these quality control samples.

2.3. Sample collections

Two tablets containing 120 mg of fexofenadine (fexofenadine tablets, 60 mg) were orally administered to each of 10 healthy volunteers. Their mean (range) age was 23 (21–24 years old) and body weight was 64 (54–85) kg. Blood samples were obtained before and at 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 48 h after the dosing. Blood samples were collected in heparinized tubes and centrifuged immediately at 2500 × *g* for 10 min. The plasma was stored at −20 °C until analysis. The study protocol was approved by Ethical Committee of Hirosaki University School of Medicine and a written informed consent was given by each subject.

2.4. HPLC analysis

The HPLC system consisted of two Shimadzu LC-10AD high-pressure pumps, a Shimadzu CTO-10A column oven, Shimadzu a SCL-10AVP system controller, Shimadzu Work station CLASS-VP chromatography integrator, Shimadzu RF-10AXL fluorescence detector and Shimadzu SIL-10A autoinjector (500 μl injection volume) (Kyoto, Japan). The mobile phases were: (A) 0.05 M KH_2PO_4 buffer/acetonitrile/methanol (60:35:10, v/v/v) and (B) 0.05 M KH_2PO_4 buffer/acetonitrile (40:60, v/v). Chromatographic separation was achieved on an Inertsil® ODS-80A column (150 mm × 4.6 mm i.d., particle size 5 μm; GL Sciences Inc., Tokyo, Japan) at a flow rate of a 1 ml/min using a linear gradient from A to B in 10 min for detection and with mobile phase (B) from 10 to 17 min for clean-up. The temperature of the column was 40 °C. The fluorescence detector was set at an excitation wavelength of 220 nm and an emission wavelength of 290 nm. In a typical chromatogram, the retention times of fexofenadine and I.S. were 4.5 and 7.1 min, respectively, and the total time for a chromatographic separation was ∼17 min. The peak area was used for the quantification of fexofenadine.

2.5. Extraction procedure

I.S. (diphenhydramine) 10 μl of a 10 μg/ml solution diluted with 1 ml of 0.2 M acetate buffer (pH 4.0) were added to plasma sample (1 ml). Sample purification was performed by solid-phase extraction on C18 mini-columns (Bond Elut® C18, 3 ml, 500 mg packing). The cartridges were preconditioned with methanol (2 ml), water (2 ml) and 1.5 ml of 0.2 M acetate buffer (pH 4.0). After loading the samples, the cartridges were washed with water (2 ml), methanol-water (50:50, v/v, 2 ml) and methanol (1 ml). After drying the cartridges with air flow, fexofenadine and I.S. were eluted with 50 mM triethylamine in methanol (1 ml). The eluates were dried with air flow and the residue was dissolved in 300 μl of mobile phase A used as an extract.

2.6. Data analyses of pharmacokinetics

The peak concentration (C_{max}) and concentration peak time (T_{max}) were obtained directly from the original data. The area under the plasma concentration–time curve (AUC0-∞), the elimination half-life values ($T_{1/2}$) and the steady-state volume of distribution (Vd_{ss}) were determined by non-compartment model with WinNonlin® software (Pharsight Co., Cary, NC, USA).

3. Results and discussion

3.1. Chromatography

A representative chromatogram of an unextracted working aqueous solution containing fexofenadine and

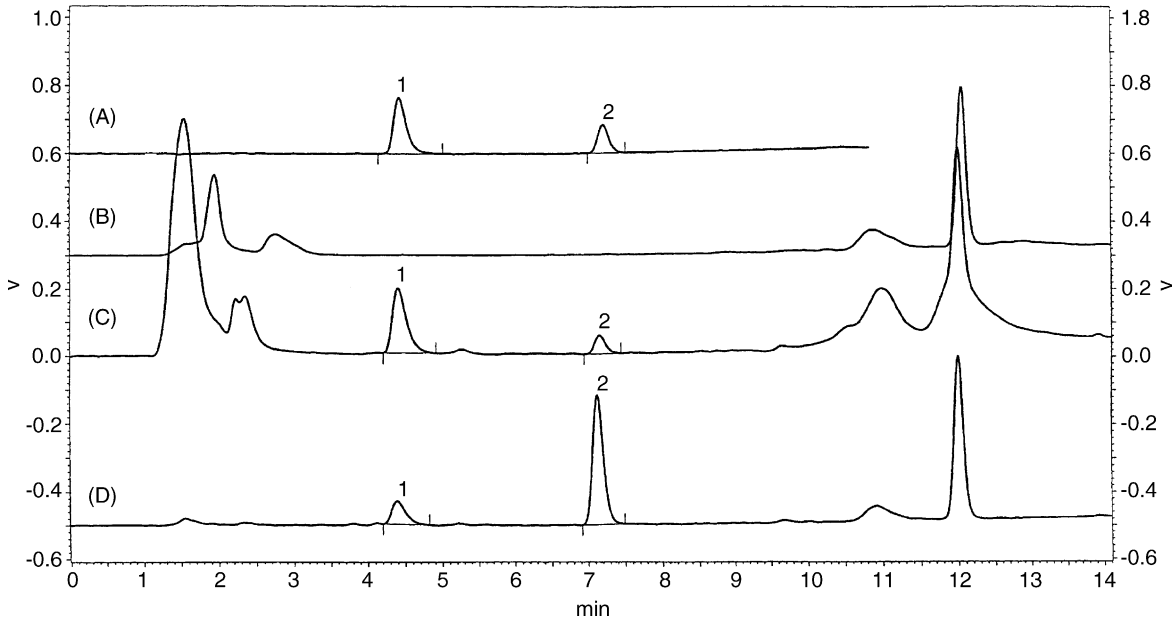

Fig. 2. The chromatogram of references without extraction (50 ng) (A), an extracted blank plasma sample (B), an extracted sample spiked with 50 ng/ml of fexofenadine and I.S. (C), extracted plasma samples obtained from one volunteer receiving 120 mg fexofenadine (D). Peaks: (1) diphenhydramine, (2) fexofenadine.

diphenhydramine (I.S.) is shown in Fig. 2A. The chromatogram of an extracted blank plasma sample is shown in Fig. 2B, while the chromatogram of an extracted sample spiked with 50 ng/ml of fexofenadine and I.S. is shown in Fig. 2C. Several compounds were well separated from each other and from the front of the solvent peaks. The chromatograms of extracted plasma samples obtained from one volunteer receiving 120 mg fexofenadine did not show interference peaks (Fig. 2D).

3.2. Recovery and linearity

Recovery from plasma was calculated by comparing the peak areas of pure standards prepared in purified water, and injected directly into the analytical column with those of extracted plasma samples containing the same amount of the test compound ($n = 6$ each). Mean recoveries were 72.8–76.7% with coefficients of variation of 2.7–5.8% at three different concentrations ranging from 1 to 500 ng/ml for fexofenadine (Table 1). Calibration curves were linear over the concentrations range from 1 to 500 ng/ml for fexofenadine ($r = 0.999$) (Table 2).

3.3. Sensitivity

The limit of detection was defined, as analyte responses are at least three times the response compared to blank response. The lowest standard on the calibration curve was defined as the limit of quantification as an analyte peak was identifiable, discrete, and reproducible with a precision of 20% and accuracy of 80–125%. The limits of detection and quantification were 1.0 ng/ml.

3.4. Precision and accuracy

Intra- and inter-day precision and accuracy were evaluated by assaying quality controls with different concentrations of fexofenadine. Intra- and inter-day

Table 1
Extraction recovery of analytes from plasma ($n = 6$)

Analyte	Concentration added (ng/ml)	Recovery (%)	CV (%)
Fexofenadine	1	72.8	2.7
	50	76.7	5.8
	500	76.3	3.9

Table 2
Individual and mean values for slope, intercepts, bias and correlation coefficients of five calibration curves for analyte

Analyte	Curve	Slope	Intercepts	Bias (%)	r
Fexofenadine	1	0.00760	0.00591	−9.1 to 1.5	0.9997
	2	0.00937	0.01028	−3.3 to 7.3	0.9998
	3	0.00981	0.00242	−7.8 to −1.1	0.9996
	4	0.00836	0.00027	−8.8 to 4.5	0.9998
	5	0.00925	0.01552	−7.1 to 3.1	0.9999
	Mean	0.00888	0.00688		0.9998
	S.D.	0.00079	0.00549		
	S.E.	0.00035	0.00245		

The ranges of bias (%) between the measured value and the theoretical value are shown in each point of calibration curve.

Table 3
Presicion (CV) and accuracy (relative error) for determination of analytes in spiked plasma ($n = 6$)

Analyte	Concentration added (ng/ml)	Inter-day		Intra-day	
		CV (%)	Relative error (%)	CV (%)	Relative error (%)
Fexofenadine	1	4.9	−1.2	9.1	4.2
	50	4.5	−2.3	3.7	0.3
	500	0.6	−0.4	1.3	0.2

precisions were assessed by analyzing six quality controls samples at each concentration on the same day and mean values of two quality controls for 6 days, respectively. Intra- and inter-day relative standard deviations were less than 9.1 and 4.9%, respectively (Table 3). Accuracy was expected as percent error (relative error) [(measured concentration − spiked concentration)/spiked concentration] × 100 (%), while precisions was quantitated by calculating intra- and inter-CV values.

3.5. Drug concentrations in human plasma

Fig. 3 shows mean plasma concentration versus time curves of fexofenadine after an oral administration of fexofenadine (120 mg) in 10 subjects. The pharmacokinetic parameters of fexofenadine are summarized in Table 4.

The sensitivity in the present simple HPLC method was comparable with a previous method using LC–MS analysis [11], enabling the monitoring of fexofenadine plasma concentrations up to 48 h after an administration of fexofenadine 120 mg in all volunteers. Consequently, precise pharmacokinetic parameters could be obtained from plasma concentrations during the elimination phase of the drug, from 12 to

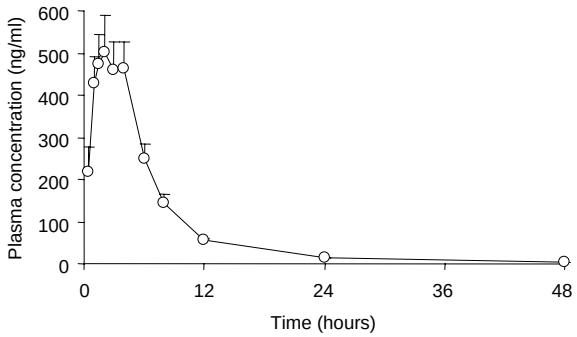

Fig. 3. Plasma concentration–time curves (mean ± S.D.) of fexofenadine from 0 to 48 h in 10 healthy volunteers after a single-oral dose of fexofenadine 120 mg.

Table 4
Pharmacokinetic parameters of fexofenadine after a oral 120 mg dose in 10 healthy volunteers

Parameters	Mean ± S.D.
C_{max} (ng/ml)	610 ± 222
T_{max} (h)	2.1 ± 0.9
$T_{1/2}$ (h)	9.6 ± 2.9
AUC $(0 - \infty)$ (ng h/ml)	3808 ± 1266
Vd_{ss} (l/kg)	5.0 ± 2.6

AUC, area under plasma concentration–time curve; C_{max}, peak concentration; T_{max}, time to C_{max}; $T_{1/2}$, elimination half-life, Vd_{ss}; steady-state volume of distribution.

48 h after administration. These results can apply to clinical pharmacokinetic studies in patients receiving fexofenadine treatment.

4. Conclusion

The HPLC procedure described for determination of fexofenadine is suitable for routine analysis of patients receiving fexofenadine. Satisfactory validation data were achieved for linearity, precision and recovery. The limit of quantification obtained allows measurement of therapeutic concentrations of fexofenadine.

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