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Determination of fexofenadine in human plasma and urine by liquid chromatography–mass spectrometry

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Abstract

A sensitive method was developed to determine fexofenadine in human plasma and urine by HPLC–electrospray mass spectrometry with MDL 026042 as internal standard. Extraction was carried out on C_{18} solid-phase extraction cartridges. The mobile phases used for HPLC were: (A) 12 m*M* ammonium acetate in water and (B) acetonitrile. Chromatographic separation was achieved on a LUNA CN column (10 cm×2.0 mm I.D., particle size 3 μm) using a linear gradient from 40% B to 60% B in 10 min. The mass spectrometer was operated in the selected ion monitoring mode using the respective MH^+ ions, m/z 502.3 for fexofenadine and m/z 530.3 for the internal standard. The limit of quantification achieved with this method was 0.5 ng/ml in plasma and 1.0 ng in 50 μl of urine. The method described was successfully applied to the determination of fexofenadine in human plasma and urine in pharmacokinetic studies.

Keywords: Fexofenadine

1. Introduction

Fexofenadine (Fig. 1), the active metabolite of terfenadine, is a non sedating H_1 antihistaminic drug. It is used for the treatment of seasonal allergic rhinitis and chronic urticaria. Fexofenadine is a substrate of the OATP-A uptake transporter [1] and of the efflux transporter P-glycoprotein, which determines absorption, tissue distribution and effects of a broad variety of drugs (for review see Ref. [2]). In spite of the fact that fexofenadine is metabolized only to a minimal extent in humans [3], several drug interactions have been reported. Coadministration of fexofenadine with ketoconazole or erythromycin resulted in increased plasma concentrations of the H_1-antagonist [3,4]. Since ketoconazole and erythromycin are not only inhibitors of CYP3A4, but also inhibitors of drug transporter function [1,5], the underlying mechanism of these drug interactions appears to be inhibition of drug transporter-mediated fexofenadine elimination. Moreover, fexofenadine oral clearance was significantly increased by rifampin [6] suggesting induction of P-glycoprotein and possibly other transport processes as underlying mechanism.

For pharmacokinetic studies on the potential impact of mutations in genes of transporter proteins on fexofenadine disposition, a sensitive assay for the determination of fexofenadine in human plasma was required. Two assays based on high-performance

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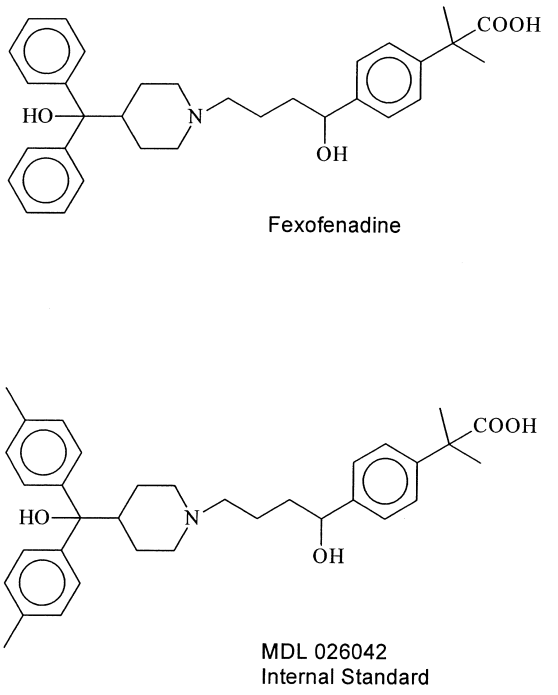

Fig. 1. Structures of fexofenadine and the internal standard MDL 026042.

liquid chromatography (HPLC) with fluorescence detection have been published [7,8], but the reported limits of quantification (LOQs) of 8.2 or 5 ng/ml, respectively, are not sensitive enough for the exact determination of the terminal half-life and the oral clearance CL_O in single dose pharmacokinetic studies. Typical concentrations of fexofenadine at 24 h after therapeutic doses of the drug are below 10 ng/ml. Due to the long half-life of fexofenadine (15 h) the exact calculation of the clearance requires the determination of fexofenadine in plasma samples up to 72 h after dosing. Methods utilising HPLC with electrospray mass spectrometry (MS) have been reported for the analogue terfenadine [9,10] with significant improvement in sensitivity compared to HPLC methods.

We have developed a sensitive LC–MS assay which enables the quantification of fexofenadine in human plasma up to 72 h after a single oral dose of 180 mg of fexofenadine hydrochloride. The achieved LOQ of 0.5 ng/ml for fexofenadine is a significant improvement over current published HPLC assays with fluorescence detection.

2. Experimental

2.1. Materials

Solvents used were of HPLC quality; chemicals were of analytical grade. Methanol and acetonitrile were purchased from Baker (Gross-Gerau, Germany), ammonium acetate, acetic acid and potassium hydroxide from Merck (Darmstadt, Germany), and triethyl amine from Sigma (Taufkirchen, Germany). Fexofenadine hydrochloride and the internal standard MDL 026042 (Fig. 1) were obtained from Aventis Pharma (Frankfurt a.M., Germany). Solid-phase extraction (SPE) cartridges, Bond Elut C_{18} (500 mg) were supplied by Varian (Darmstadt, Germany).

2.2. Sample preparation

Samples (1 ml of plasma or 50 μl of urine diluted with 950 μl of water) were spiked with 50 ng of internal standard (25 μl of a 2 ng/μl solution) and diluted with 1 ml of acetate buffer (0.2 *M*, pH 4.0).

Sample purification was performed on a Baker spe 24 G system using C_{18} SPE cartridges. The cartridges were preconditioned with 2 ml each of methanol and water and 1.5 ml of acetate buffer (0.2 *M*, pH 4.0). After sample load the cartridges were washed with water (2 ml), methanol–water (50:50, v/v, 2 ml) and methanol (1 ml). After drying the cartridges by suction, fexofenadine and the internal standard were eluted with 50 m*M* triethylamine in methanol (1 ml). The eluates were dried with nitrogen and the residue dissolved in 150 μl of the mobile phase, containing 40% B. An aliquot of 10 μl was used for LC–MS analysis.

2.3. HPLC–MS analysis

A HP Series 1100 LC–mass-selective detection system (Hewlett-Packard, Waldbronn, Germany) with a binary pump, a degasser, an autosampler and a mass-selective detector equipped with an electrospray ion source was used. The mobile phases for HPLC were: (A) 12 m*M* ammonium acetate in water and (B) acetonitrile. Chromatographic separation was achieved on a LUNA CN column (10 cm×2.0 mm I.D., particle size 3 μm; Phenomenex, Aschaffenburg, Germany) at a flow-rate of 0.25 ml/min

using a linear gradient from 40% B to 60% B in 10 min.

The mass spectrometer was tuned with the autotune procedure provided by the HP Chemstation software. Electrospray parameters were as follows: capillary voltage 3500 V, drying gas flow 11 l/min nitrogen, drying gas temperature 350°C, nebulizer pressure 50 p.s.i. g, fragmentor 140 V (1 p.s.i.= 6894.76 Pa). The mass spectrometer was operated in the selected ion monitoring mode (SIM resolution high) using the respective MH^+ ions, m/z 502.3 for fexofenadine and m/z 530.3 for the internal standard. Peak width was set at 0.30 min.

2.4. Standardisation and validation

Standard curves were prepared by adding increasing amounts (0.5, 1, 5, 10, 50, 100 and 200 ng) of fexofenadine to drug-free plasma or urine and extracting the samples as described above. Standard curves were evaluated by weighted ($1/x$) linear regression based on internal standard calibration and were obtained by plotting peak-area ratios against the amount of the substance. The concentration of fexofenadine in unknown samples was obtained from the regression line. All standardisation was performed with HP Chemstation software (Hewlett-Packard).

The reproducibility and accuracy of the method was established by analysing quality control samples, prepared like the calibration samples. The intra-assay precision and accuracy was assessed by measuring the concentration of fexofenadine in six identical samples (0.5, 1.0, 100 and 200 ng/ml plasma, or 1.0 and 200 ng/50 μl urine) extracted and analysed on a single day. Inter-assay precision and accuracy was determined from the results of three different quality control samples (1.0, 10 and 200 ng/ml plasma) which were extracted and analysed on different days. The LOQ was determined as the lowest concentration with a relative standard deviation (RSD) and a bias of $<20\%$ ($n=6$).

2.5. Dilution of plasma samples

Plasma samples with a concentration above the highest LOQ had to be diluted. To validate the dilution several plasma samples from a kinetic study were used. From these samples 150, 300 and 500 μl were taken and blank human plasma was added to a volume of 1 ml. These samples were analysed as described above.

2.6. Pharmacokinetic study

The LC–MS assay developed was used to investigate the pharmacokinetic parameters after a single oral dose of 180 mg of fexofenadine hydrochloride (Telfast, Aventis Pharma). Blood samples were drawn before drug intake and at different time points until 72 h after administration of the drug. Urine was collected before drug administration and at 0–72 h after administration. The study had been approved by the local ethics committee (Landesärztekammer Baden-Württemberg). All volunteers gave their written informed consent prior to participating in the study.

3. Results and discussion

3.1. Sample preparation

Fexofenadine can be extracted from plasma samples by liquid–liquid extraction [8] or SPE [7] with similar extraction recoveries of 81–93, or 78–88%, respectively. We decided to use SPE, as it gives the possibility of automatization. SPE was performed similar to a described method [7]. Extraction recoveries were determined by comparing the peak areas from extracted standards in human plasma to the peak areas of unextracted standards. Recoveries (mean±standard deviation) were found to be 89.1±3.3 or 88.9±2.9% for 10 or 200 ng/ml, respectively ($n=6$).

3.2. LC–MS analysis

Since no stable isotope-labelled internal standard was available the analogue with a 4′-methyl group in each phenyl ring (MDL 026042) was used as internal standard (Fig. 1). Both substances show simple mass spectra in positive electrospray ionization (Fig. 2) with the protonated molecular ions (MH^+) as the base peak and two minor fragment ions which represent the loss of one or two mole-

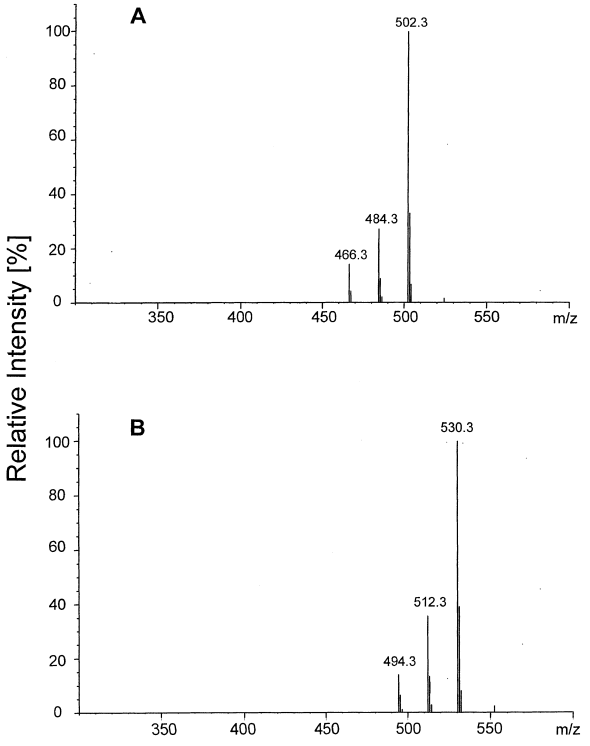

Fig. 2. Mass spectra of fexofenadine (A) and the internal standard (B) obtained by positive electrospray ionization.

cules of water. Electrospray parameters were optimized for maximum intensity of the MH^+ ion.

For HPLC analysis of fexofenadine, mostly cyano columns were employed [7,8]. With the column investigated (3 μm CN, 2 mm I.D.) fexofenadine could be well separated from a small endogenous peak with an ammonium acetate buffer–acetonitrile gradient (Fig. 3A). Under the LC–MS conditions described, the peak shapes of both fexofenadine and internal standard were good and the retention times were 6.6 and 8.2 min, respectively (Fig. 3B). As the retention times of analyte and internal standard are different, ion suppression due to matrix effects could occur. To exclude ion suppression, another HPLC gradient was employed, where the peaks of fexofenadine and the internal standard are nearly completely overlapping with retention times of 3.9 and 4.0 min, respectively. The concentrations of fexofenadine were determined in about 50 plasma samples with both methods. In general, the differences were below 15%.

3.3. Validation

The linearity of the standard curves showed to be good over the entire concentration range measured: 0.5–200 ng/ml for plasma and 1.0–200 ng/50 μl for urine. In general, coefficients of correlation (r) greater than 0.998 were observed. Because of the highly selective detection method, there were no interfering peaks present in more than 20 blank samples investigated. An example of a blank plasma sample is shown in Fig. 3A.

Accuracy and precision of the method was determined with quality control samples as described in Section 2.4. The RSD and mean values of the deviation from the amount added (% bias) were calculated. The results for plasma are given in Table 1, for urine in Table 2. The data show good precision and accuracy of the method with an intra-day RSD and bias of below 10%, even at the LOQ. The inter-day RSDs are higher but still acceptable.

The results from the dilution experiments are given in Table 3. In four plasma samples with concentrations between 100 and 500 ng/ml the results are almost identical using 150, 300 or 500 μl with the RSD being far below 10%. In our pharmacokinetic study with a single dose of 180 mg of fexofenadine hydrochloride we used 150 μl of plasma from samples up to 12 h post-dosing, and 1 ml from 24 to 72 h. With this procedure the plasma concentrations measured were in the specified linear concentration range in 20 subjects. A chromatogram from a volunteer 72 h after treatment with 180 mg of fexofenadine hydrochloride is shown in Fig. 3B.

3.4. Assay application

The assay presented allowed for the determination of fexofenadine in human plasma and urine in pharmacokinetic studies. Representative plasma concentration–time curves of four volunteers receiving a single oral dose of 180 mg of fexofenadine hydrochloride are shown in Fig. 4. The corresponding pharmacokinetic parameters (calculated with TOPFIT 2.0, Gustav-Fischer-Verlag, 1993) are summarized in Table 4.

The sensitivity achieved with the assay described is at least 10 times better than with previously

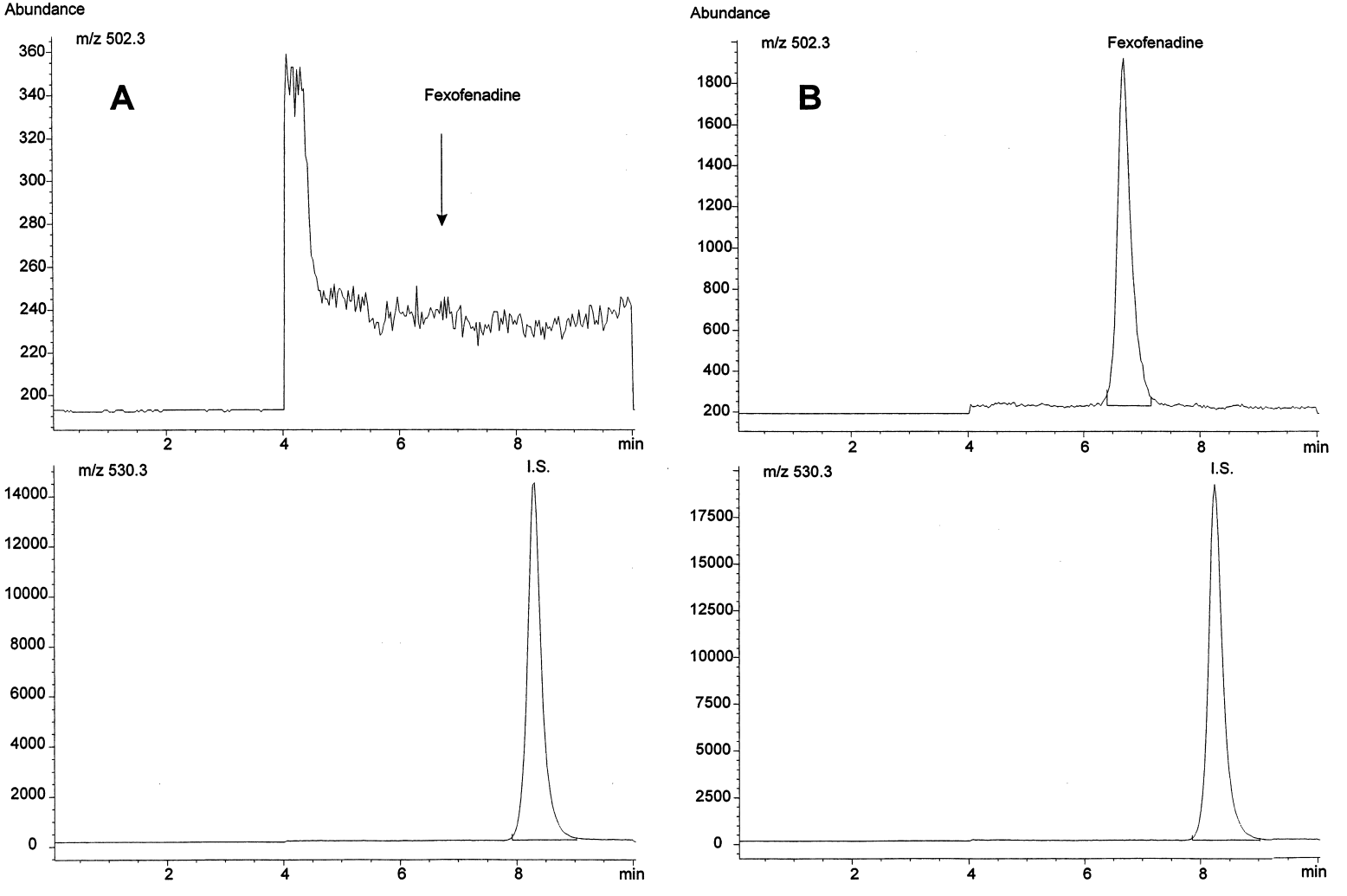

Fig. 3. Mass chromatograms of extracts from (A) blank human plasma, (B) plasma from a volunteer 72 h after a single oral dose of 180 mg fexofenadine hydrochloride containing 2.48 ng/ml fexofenadine.

Table 1
Intra-assay and inter-assay precision and accuracy for the determination of fexofenadine in plasma

Concentration added (ng/ml)	*n*	Concentration found (ng/ml)	Bias (%)	RSD (%)
Intra-assay				
0.5	6	0.532±0.034	6.5	6.4
1.0	5	1.09±0.10	8.8	9.6
100	6	108.8±5.0	8.8	4.6
200	6	197.3±9.7	−1.3	4.9
Inter-assay				
1.0	16	1.00±0.15	0.2	14.6
10.0	17	10.52±1.23	5.2	11.7
200	14	207.0±28.1	3.5	13.2

Table 2
Intra-assay precision and accuracy for the determination of fexofenadine in urine

Concentration added (ng/50 μl)	*n*	Concentration found (ng/50 μl)	Bias (%)	RSD (%)
1.0	6	0.911±0.047	−8.9	5.2
200	6	209.7±3.8	4.9	1.8

Table 3
Concentration of fexofenadine in four plasma samples post-dosing using different plasma volumes for extraction

Plasma volume (μl)	Fexofenadine (ng/ml)			
	Sample 1	Sample 2	Sample 3	Sample 4
150	468.6	374.9	109.2	229.8
300	519.9	351.0	110.9	198.8
500	501.0	327.6	109.0	206.3
Mean	496.5	351.2	109.7	211.6
RSD (%)	5.2	6.7	1.0	7.6

The samples were diluted with blank human plasma to a final volume of 1.0 ml.

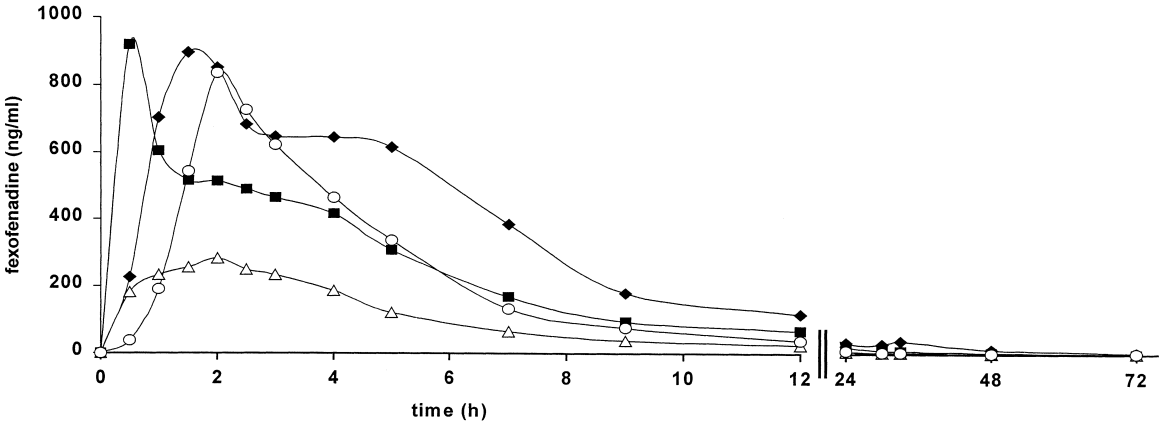

Fig. 4. Plasma concentration–time curves of fexofenadine in four volunteers after a single oral dose of 180 mg fexofenadine hydrochloride.

Table 4
Pharmacokinetic parameters (means±SD) after oral administration of 180 mg fexofenadine HCl to four healthy individuals

Parameter[a]		
$AUC_{0-\infty}$	(ng h/ml)	4107.5±1837.4
t_{max}	(h)	1.5±0.6
C_{max}	(ng/ml)	734.5±261.3
$t_{1/2}$	(h)	19.1±7.0
$Ae_{0-72\ h}$	(% of dose)	10.3±3.7
CL_O	(ml/min)	857.8±433.6
CL_R	(ml/min)	76.0±15.0
CL_{NR}	(ml/min)	781.7±419.3

[a] Parameters: AUC, area under the plasma concentration–time curve; t_{max}, time of peak concentration; C_{max}, peak plasma concentration; $t_{1/2}$, terminal elimination half live; $Ae_{0-72\ h}$, cumulative amount excreted in urine (0–72 h); CL_O, apparent oral clearance; CL_R, renal clearance; CL_{NR}, non-renal clearance.

published methods [7,8], enabling the monitoring of fexofenadine plasma concentrations up to 72 h after administration of a 180 mg oral dose of fexofenadine hydrochloride. The exact calculation of the terminal half-life of the drug and the oral clearance might be useful for a comparison of the influence of different mutations in the *MDR1* gene on fexofenadine pharmacokinetics.

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