High-performance Thin-layer Chromatography for the Determination of Cetirizine in Human Plasma and Its Use in Pharmacokinetic Studies

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Abstract

A rapid and sensitive high-performance thin-layer chromatography (HPTLC) assay has been developed for the measurement of cetirizine in human plasma and its utility for pharmacokinetic study has been evaluated.

In the proposed HPTLC method, protein-bound cetirizine was freed by proteolysis of plasma proteins by incubating the plasma with 0·35% pepsin and then extracting with 2 mL pH 5·0 phosphate buffer, followed by 4 mL chilled chloroform. The chloroform layer was separated and concentrated. An aliquot of the extract was then spotted on precoated silica-gel 60 F_{254} plates using a Camag Linomat IV autosampler. Quantification was with the help of a dual-wavelength TLC scanner. The proposed method had a recovery of 98% and the lowest amount of cetirizine that could be detected was 50 ng.

The method was applied for the determination of the plasma levels and pharmacokinetic parameters of cetirizine after oral administration of two marketed preparations in healthy volunteers and the pharmacokinetic parameters determined by the proposed method were in agreement with previously reported values.

Cetirizine, a second-generation long-acting peripheral histamine H_1-receptor antagonist, is a piperazine derivative and carboxylated metabolite of hydroxyzine (Fig. 1) (Estelle et al 1991). Cetirizine is currently used in the management of various allergic reactions and it has no sedative effects (Desager et al 1993). Cetirizine also has an anti-inflammatory effect and inhibits recruitment of inflammatory cells to the site of allergic reaction (Estelle et al 1991). Cetirizine is excreted unchanged in the urine and only small amounts of metabolites have been recovered from blood, faeces and urine (Wood et al 1987).

Several analytical methods have been reported for pharmacokinetic studies of cetirizine using GLC and HPLC (Gengo et al 1987; Matzke et al 1987; Baltes et al 1988; Lefebvre et al 1988; Watson et al 1989; Desager et al 1993). The aim of the present study was to detect cetirizine in human plasma samples by high-performance thin-layer chromatography (HPTLC).

Materials and Methods

Apparatus
Samples were spotted using a Camag Linomat IV autosampler onto TLC plates (Silica gel 60 F_{254}, E. Merck, Darmstadt, Germany) which were developed in a 25 × 25 × 12 cm glass chamber. Scanning was with a Shimadzu dual-wavelength scanner (Model CS 930, Shimadzu, Kyoto, Japan).

Chemicals
Cetirizine dihydrochloride was obtained from Dr Reddy's Laboratory, India, and pepsin (0·35%, pH 1·2, 108 units mg^{-1} from Sigma, St Louis, MO, USA) was used for proteolysis. Phosphate buffer (pH 5·0) and chloroform (analytical grade) were used for extractions. Chloroform and methanol (-

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analytical grade) were used for TLC development.

Standard solutions
A stock solution of cetirizine (1 mg mL^{-1}) in methanol was prepared. Working standard solutions were obtained by diluting the stock solution to concentrations ranging from 10 to 100 μg mL^{-1}.

Procedure
In a 10-mL graduated glass centrifuge tube, cetirizine working standard (10 μg mL^{-1}) was added in volumes of 0, 20, 40, 80 and 100 μL to 1 mL drug-free plasma to provide calibration standards of 0 (no cetirizine added), 200, 400, 800 and 1000 ng mL^{-1}. A 20-μL volume of cetirizine working standard (100 μg mL^{-1}) was added to 1 mL drug-free plasma to provide a calibration standard of 2000 ng mL^{-1}. All test samples were incubated for 2 h at 36°C with 0·5 mL pepsin (proteolytic enzyme) solution (Fox & Foster 1957; Plummer 1985). After incubation, precipitation of total plasma protein was accomplished by the addition of 2 mL phosphate buffer (pH 5·0) and extracted with 4 mL chloroform. The mixture was shaken on a
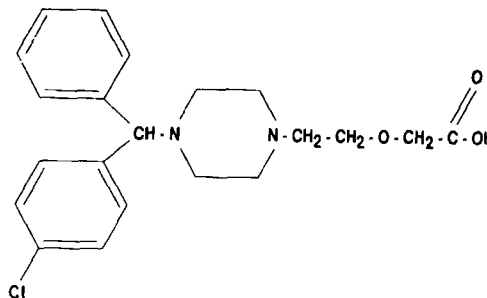
FIG. 1. Chemical structure of cetirizine dihydrochloride.

vortex mixture for 2 min, and centrifuged for 10 min at 2500 rev min^{-1}. The supernatant was decanted into another 10 mL centrifuge tube and extracted again with 2 mL chloroform and centrifuged. The combined chloroform extract was evaporated to dryness at 45°C in a waterbath under dry nitrogen. The residue was reconstituted in 70 μL chloroform, vortexed for 30 s and 50-μL samples were spotted on TLC plates. Cetirizine (50, 200, 400, 800, 1000, 2000 and 5000 ng) reference standard was separately spotted on each TLC plate as external standard.

Plasma samples were prepared in an identical manner except for the addition of cetirizine.

Chromatography

Different compositions of solvents were tried for achieving optimum separation of cetirizine.

For the separation of interfering peaks all the samples were spotted at the middle of the plate. The plate was first developed in one direction in chloroform. The plates were then cut off 2 cm above the origin in the direction of solvent front so as to remove the interfering substances from plasma. The plates were turned through 180° and development was carried out using the solvent system chloroform:methanol (85:15) in which the drug had an R$_f$ value of 0.31, and was well-separated from other components in plasma. The volume of both the solvents in solvent system drastically affected the R$_f$ value as well as the separation from other components. For example, changing the methanol volume resulted in tailing of the spot and on changing the chloroform volume the spot moves closer to the solvent front. The TLC chamber was saturated with the solvent system to ensure a concentrated zone of the compound and hence better resolution. After development, the TLC plates were dried completely using a hot-air drier. Quantitation of cetirizine was by scanning with a dual-wavelength scanner.

Analysis of marketed formulations

The cetirizine content in the marketed samples of cetirizine tablets was analysed by using the same solvent system. A cetirizine tablet (10 mg) was dissolved in methanol and diluted to 100 and 250 μg mL^{-1}. Twenty microlitres of the respective dilutions were spotted onto the plate followed by development and scanning as described above.

Bioavailability study

The bioavailability study was carried out in eight healthy volunteers in the age group 25–51 years (mean age 34.13 ± 3.41) and having normal biochemical parameters. The study protocol was approved by the institutional review board and ethics committee of Cadila Laboratories, Research and Development unit. Two commercial brands of cetirizine dihydrochloride tablets (product A and product B) were selected for the study. One tablet of either brand was administered to each volunteer with 200 mL water, such that half the number of volunteers received product A and the rest received product B in the first part of a single blind cross-over study. The second part of the study was carried out after seven days. Identical conditions were maintained on both occasions. Venous blood samples were drawn into heparinized tubes before the drug administration and at 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0 and 24.0 h following the drug administration. All the blood samples were taken via an indwelling catheter. The samples were immediately centrifuged at 3000 rev

min^{-1} for 10 min, the plasma separated and frozen at −20°C until analysis.

The following pharmacokinetic parameters were determined to evaluate the two formulations: C$_{max}$, maximum plasma concentration (ng mL^{-1}) from the individual data; T$_{max}$, time (h) at which peak plasma levels were observed; and mean residence time (MRT), calculated according to the equation of Yamaoka et al (1978):

$$MRT = AUMC_{0-\infty}/AUC_{0-\infty} \qquad (1)$$

where AUMC$_{0-\infty}$ is the area under the first moment curve from time zero to infinity. Benet & Galeazzi (1979) defined AUMC$_{0-\infty}$ as:

$$AUMC_{0-\infty} = AUMC_{0-t} + (t/C_t)/k + C_t/k \qquad (2)$$

where C$_t$ is the plasma concentration of the drug at time t, k is the apparent elimination rate constant in the terminal phase. AUMC$_{0-t}$ is calculated by the trapezoidal rule.

Results and Discussion

Quantitation

The quantitation of the chromatograms was performed using the ratio of the peak area of the unknown to that of a standard. A representative standard curve of cetirizine was obtained by plotting the area under the peak of cetirizine against the concentration over the range 50–5000 ng. Standard curves of cetirizine were constructed on 10 different days to determine the variability of slopes and intercepts. The result showed little day-to-day variability of slopes or intercepts as well as a good linear relationship (r = 0.989) over the concentration range studied. The R$_f$ value of cetirizine was found to be 0.31 ± 0.05. Quantitation was carried out at $\lambda_{max} = 232$ nm.

Table 1. Precision data of the HPTLC assay for cetirizine.

Amount added (ng)	Peak area[a] (mean ± s.d.)	Coefficient of variation (%)
Intra-day		
200	4032.35 ± 158.89	3.94
800	16 278.64 ± 646.74	3.97
2000	40 901.00 ± 2155.4	5.27
Inter-day[b]		
200	4001.55 ± 155.09	3.88
400	8343.37 ± 335.64	4.02
1000	21 032.76 ± 1090.9	5.19

[a]Calculated for total concentration (integrated value). [b]One millilitre plasma contained added drug at the indicated concentrations.

Table 2. Accuracy and precision of HPTLC method for determination of cetirizine in plasma.

Added (ng mL^{-1})	Detected mean ± s.d., n = 5 (ng mL^{-1})	CV[a] (%)	Accuracy[b] (%)
200	192.01 ± 7.56	3.94	98.01
400	397.30 ± 15.98	4.02	101.40
800	775.17 ± 30.79	3.97	98.92
1000	1001.50 ± 51.95	5.18	102.24
2000	1947.60 ± 102.63	5.26	99.42

[a]Coefficient of variation. [b]After correction for recovery.

Sensitivity

The minimum quantifiable concentration of cetirizine in human plasma samples was 50 ng mL^{-1}.

Selectivity

Interference was evaluated by studying the effects of other drugs in the HPTLC system used; R_f values were found as follows: astemizole, 0·75; cetirizine , 0·31; loratadine, 0·64; and terfenadine, 1·0.

The identity of cetirizine in the plasma samples was confirmed by spraying the plate with bismuth subnitrate and potassium iodide solution, giving an orange spot for cetirizine at the appropriate R_f value.

Precision

The intra-day precision (random analytical variation) was evaluated by analysing drug-free plasma samples in triplicate, to which had been added cetirizine at concentrations of 200, 800 and 2000 ng mL^{-1}. All specimens used to study precision and bias were interspersed with clinical samples during analysis. The inter-day precision was determined by analysing 200-, 400- and 1000-ng standards simultaneously with subjects' plasma daily for five days (Table 1). The linearity of the detector response was tested by spotting standards in triplicate for each concentration over the range 50–5000 ng.

Recovery

The recovery of cetirizine from plasma was determined by comparing peak areas obtained from plasma to which had been added cetirizine (200, 400, 800, 1000 and 2000 ng mL^{-1}) with the peak areas obtained from standards (Table 2). The recovery of cetirizine in the extraction procedure from 1 mL plasma was
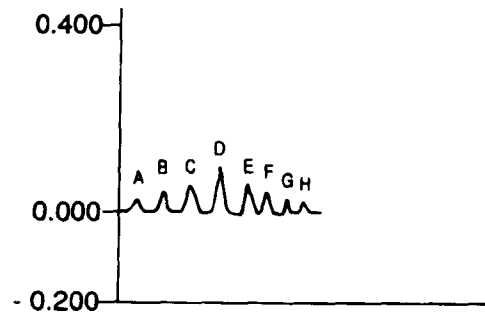

FIG. 2. HPTLC scans of cetirizine in patient samples at different time intervals after oral administration of 10-mg tablets. A 30 min, B 1 h, C 1·5 h, D 2 h, E 4 h, F 6 h, G 8 h, H 24 h.
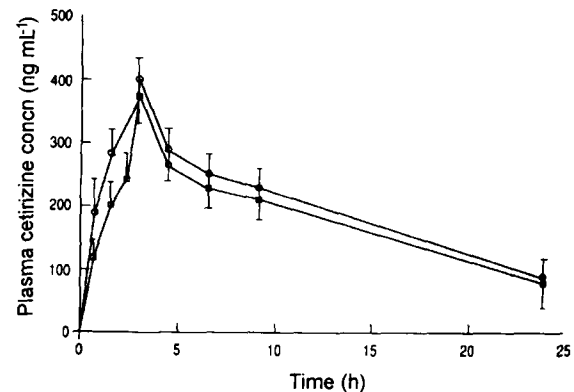

FIG. 3. Plasma concentration after oral administration of 10 mg cetirizine as two marketed products (product A, □; product B, ○). Each point represents the mean ± s.e. (n = 8, cross-over design).

Table 3. Pharmacokinetic parameters calculated from the plasma-time data obtained following administration of product A and product B to healthy volunteers.

Subject	C_{max}(ng mL^{-1})		T_{max} (h)		AUC_{0-24} (ng mL^{-1} h)		MRT (h)		Bioavailability (%)
	A	B	A	B	A	B	A	B	
1	239·73	78·00	2·00	2·00	2780·24	3290·00	13·16	10·57	84·5
2	189·08	290·00	2·00	2·00	6863·72	8000·00	15·17	11·81	85·79
3	455·00	334·35	2·00	2·00	5005·00	4953·51	14·66	13·29	101·04
4	338·70	454·00	2·00	4·00	5578·37	4004·50	19·81	4·95	139·30
5	457·70	185·00	2·00	2·00*	4050·08	3601·50	8·95	6·64	112·45
6	289·00	932·61	3·00	2·00	3296·75	2994·46	7·98	17·55	110·09
7	1454·43	205·05	2·00	2·00	2787·43	2050·89	8·74	15·81	135·17
8	513·00	360·07	2·00	2·00	5069·25	5072·58	9·81	16·62	99·93
Mean	399·33	380·14	2·12	2·25	4428·85	4495·93	12·29	12·16	108·53
± s.e.	32·13	62·18	0·12	0·23	481·36	620·23	1·37	1·52	6·73
CV (%)	22·76	46·27	15·5	29·4	30·47	39·02	31·49	35·46	17·16

Table 4. Analysis of variance for different pharmacokinetic parameters following administration of products A and B ($P = 0·05$).

Subject	Parameter	SSQ	D.F.	Variance	F
1	AUC_{0-24}	133 844·39	1	133 844·39	0·41 (NS)
	Residual	229 9061·17	7	328 437·31	
2	C_{max}	1473·79	1	1473·79	0·20 (NS)
	Residual	261 017·91	7	37 288·27	
3	MRT	0·07	1	0·07	0·022 (NS)
	Residual	216·85	7	30·98	

NS not significant.

found to be 97.95 ± 1.54 ($n = 5$). Percent recoveries of cetirizine from plasma incubated with pepsin for 0, 1, 2 and 3 h were found to be 56, 75, 96.2 and 73.6%, respectively.

Analysis of marketed formulations

The marketed products were analysed for cetirizine content using the proposed technique. The R_f values were found to be the same for tablets and standard cetirizine and there was no interference from the excipients. Cetirizine content was found to be 97.8 ± 2.5 and $98.3 \pm 1.8\%$ respectively for product A and B.

Bioavailability

Fig. 2 shows the concentration of cetirizine in plasma of individual volunteers at different time intervals. Fig. 3 shows the mean plasma concentration time profile following two brands of cetirizine tablets in eight healthy male volunteers. The pharmacokinetic parameters are shown in Table 3. The pharmacokinetic parameters were subject to two-way analysis of variance (Table 4). The results indicate that the pharmacokinetic profiles of the two formulations are identical and the bioavailability is not significantly different at $P = 0.05$, indicating the bioequivalence of the two products.

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