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Journal of Chromatography B, 657 (1994) 125–131

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Sensitive gas–liquid chromatographic method for the determination of loratadine and its major active metabolite, descarboethoxyloratadine, in human plasma using a nitrogen–phosphorus detector

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(First received November 8th, 1993; revised manuscript received March 1st, 1994)

Abstract

A sensitive gas–liquid chromatographic (GLC) method was developed for the determination of loratadine, a long-acting tricyclic antihistamine, and its active metabolite, descarboethoxyloratadine, in human plasma. The method involved extraction with organic solvent at neutral and alkaline pH. The organic layer from the neutral pH extraction was evaporated to dryness, reconstituted and injected into the GLC system. On the other hand, to the organic layer from the alkaline pH extraction trifluoroacetic anhydride was added. Following addition of H_2O, the mixture was centrifuged and the organic layer was evaporated to dryness, reconstituted and injected onto the GLC system that was equipped with a nitrogen specific detector and a fused-silica capillary column. The linearity for both loratadine and descarboxyloratadine were demonstrated with $r \geq 0.998$ at concentrations ranging from 0.1 to 30 ng/ml. The results showed that the GLC method was accurate (bias $\leq 12\%$) and precise (coefficient of variation, C.V., $\leq 12\%$) for loratadine and descarboethoxyloratadine. The limit of quantitation was 0.1 ng/ml for loratadine with a C.V. of 9.2% and for descarboethoxyloratadine with a C.V. of 5.3%. The GLC method described has been demonstrated to be useful for the determination of loratadine and descarboethoxyloratadine in plasma samples of pediatric volunteers following oral administration of a single dose of 10 mg of loratadine syrup.

1. Introduction

Loratadine, ethyl 4-(8-chloro-5,6-dihydro-11H-benzo[5,6]-cyclohepta[1,2-b]pyridin-11-ylidene)-1-piperidinecarboxylate (SCH 29851) (Fig. 1) is a long-acting tricyclic anti-histamine with selective peripheral H1-receptor antagonistic activity, devoid of significant effects on the central or autonomic nervous system [1–5]. It is effective in the treatment of seasonal/perennial rhinitis and urticaria [6,7]. Loratadine is rapidly and extensively metabolized in man, forming descarboethoxyloratadine, a pharmacologically active metabolite, which is further oxidized to several products [8]. The mean elimination half-lifes of loratadine and descarboethoxyloratadine are 8–11 h and 17–24 h, respectively [9].

Plasma concentrations of loratadine and descarboethoxyloratadine have been determined in clinical and preclinical studies using a radioim-

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0378-4347/94/$07.00
SSDI 0378-4347(94)00150-4

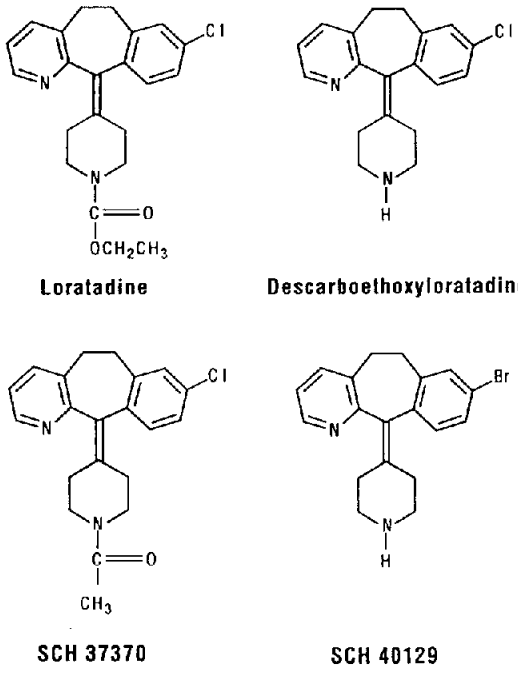

Fig. 1. Chemical structures of loratadine, its major metabolite descarboethoxyloratadine and the analogs, SCH 37370 and SCH 40129 (internal standards).

munoassay (with a limit of quantitation of 0.6 ng/ml) and a high-performance liquid chromatographic (HPLC) method (with a limit of quantitation of 0.3 ng/ml), respectively [10,11].

A more sensitive and rugged chromatographic method was required for the determination of loratadine and descarboethoxyloratadine in biological fluids after therapeutic doses (10 mg/day) in order to facilitate pharmacokinetic studies of the drug in humans. This report describes a new and more sensitive gas–liquid chromatographic method for the analysis of loratadine and descarboethoxyloratadine in human plasma which has been used in clinical pharmacokinetic studies.

2. Experimental

2.1. Chemicals

Loratadine, descarboethoxyloratadine, SCH 37370 (internal standard) and SCH 40129 (internal standard) were supplied by Schering-Plough (Kenilworth, NJ, USA). Cyclohexane, toluene, methanol, butanol-2, hexane and methyl-*tert.*-butyl ether were obtained from Burdick and Jackson (Muskegon, MI, USA). Potassium phosphate dibasic, octanol-1, acetone, and sodium hydroxide were obtained from Fisher Scientific (Fairlawn, NJ, USA), and trifluoracetic anhydride was obtained from Pierce Chemicals.

2.2. Drug administration and plasma sample collection

Seven male and six female pediatric volunteers (aged between 8 and 12 years, mean = 10.5 yr; weighing between 77 and 134 lb, mean = 103.8 lb) participated in the study. Each volunteer underwent a physical examination, electrocardiography and laboratory tests (hematology, blood chemistry and urinalysis) after which they were judged to be suitable for the study. Written informed consent was obtained from the parent/guardian and child (if possible) upon entering the study. Each volunteer received a 10-mg dose of loratadine syrup. Blood samples were collected at various time periods after dosing up to 72 hr.

2.3. Sample preparation

To a 1-ml aliquot of plasma were added 50 μl of internal standard solution (containing 0.3 μg/ml of SCH 37370 and 0.2 μg/ml of SCH 40129 as internal standard), 5.5 ml of hexane–methyl-*tert.*-butyl ether (50:50, v/v), and 0.5 ml of 1.0 *M* phosphate buffer pH 7. The mixture was vortex-mixed for 20 min and then centrifuged (2000 rpm, 10 min). The aqueous layer was retained for analysis of the metabolite descarboethoxyloratadine, whereas the organic layer was transferred to another tube and evaporated to dryness in a vortex evaporator. To the residue one drop of octanol-1 and 200 μl of cyclohexane–butanol-2 (98.5:1.5, v/v) were then added. After mixing, the organic layer was transferred to another tube and evaporated to 3–4 μl in a Speed Vac Concentrator. A 1-μl aliquot of the mixture was injected onto the gas–liquid chromatograph.

For analysis of the metabolite descarboethoxyloratadine, 5 ml of methyl-*tert.*-butyl ether–toluene (15:85, v/v) and 0.5 ml of 0.5 *M* NaOH were added to the aqueous layer. The mixture was mixed thoroughly and then centrifuged (2000 rpm, 10 min). The organic layer was transferred to another tube to which was added 50 μl of trifluoroacetic anhydride and then gently shaken for 15 min. Following addition of 1 ml of water, the mixture was vortex-mixed and centrifuged (2000 rpm, 10 min). The organic layer was transferred to another tube and evaporated to dryness. To the residue was added one drop of octanol-1 and 200 μl of cyclohexane–butanol-2 (98.5:1.5, v/v). The sample was thoroughly mixed and evaporated to 3–4 μl in a Speed Vac Concentrator. A 1-μl volume of the mixture was injected onto a gas–liquid chromatograph.

2.4. Chromatography

The gas–liquid chromatographic system consisted of a Varian Model 3700 GLC equipped with TSD (nitrogen specific detector) and a fused-silica capillary RTX 20 column (30 m × 0.53 mm I.D.) containing 20% phenyl methyl bonded silicone. The temperatures for the column, injector and detector were 290°C, 320°C and 320°C, respectively. Helium was used both as carrier gas (flow-rate 14 ml/min) and make-up gas (flow-rate 3 ml/min). The hydrogen pressure was maintained at 5 psi and the air flow-rate was 175 ml/min. The bead current for the detector was maintained between 4.5 and 6.5 amp and bias voltage was set at 4 V. A Hitachi D-2000 integrator was used at a chart speed of 5 mm/min and 1 V full scale.

2.5. Calculation

Chromatographic peaks were identified on the basis of their respective retention times. A standard curve was constructed for loratadine using the peak-height ratio of loratadine to its internal standard (SCH 37370). A separate standard curve was constructed for descarboethoxyloratadine using the peak-height ratio of descarboethoxyloratadine to its internal standard (SCH 40129). The ratio for each standard was plotted against the known standard concentration (ng/ml) and a standard curve generated using the equation, $y = mx + b$, where y is peak-height ratio, x is concentration (ng/ml), m is the slope and b is the intercept.

3. Results

3.1. Loratadine

A typical chromatogram for loratadine and its internal standard (SCH 37370) extracted from plasma is shown in Fig. 2. The retention time was 3.2 min for loratadine and 3.7 min for SCH 37370. The standard curve for loratadine was obtained by plotting the ratio of the peak height of loratadine to that of the internal standard (SCH 37370) against the concentrations (0.1 to 30 ng/ml) of loratadine added ($y = 0.1158x + 0.002801$). There was a good linear relationship between the peak-height ratio and the plasma concentration, with a correlation coefficient of 0.9990. The limit of quantitation of the GLC method was 0.1 ng/ml, the lowest concentration validated with a small coefficient of variation (9.2%) (Table 1). There were no interfering peaks in control plasma that occurred at the retention time of either loratadine, its internal standard or its metabolite, indicating that the GLC analysis for loratadine was specific.

Intra-day precision of the method was validated at eight concentrations. Five plasma samples at each concentration were analyzed by the GLC procedure described. The results demon-

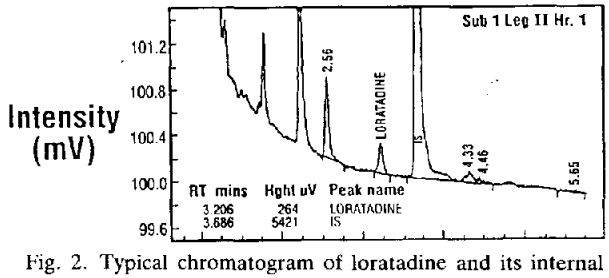

Fig. 2. Typical chromatogram of loratadine and its internal standard is (SCH 37370) extracted from human plasma. y-Axis represents the intensity of peak (mV), x-axis represents the retention time (min).

Table 1
Within-day reproducibility for loratadine

Concentration added (ng/ml)	Concentration found (mean ± S.D., $n = 5$) (ng/ml)	C.V. (%)
0.1	0.10 ± 0.009	9.2
0.2	0.21 ± 0.015	7.4
0.4	0.41 ± 0.046	11.2
1	0.97 ± 0.033	3.4
5	4.69 ± 0.242	5.2
10	9.8 ± 0.44	4.5
20	20.6 ± 0.86	4.2
30	32.1 ± 0.86	2.7

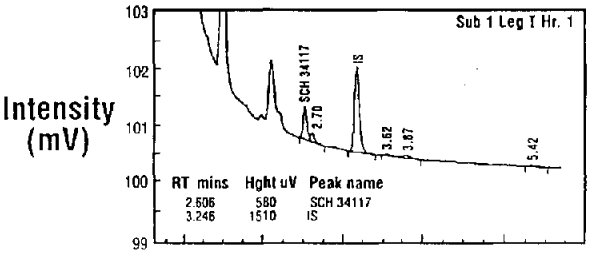

Fig. 3. Typical chromatograms of descarboethoxyloratadine and its internal standard (I.S., SCH 40129) extracted from human plasma. y-Axis represents the intensity of peak (mV), x-axis represents the retention time (min).

strated that the GLC method was accurate (bias less than 12%) and reproducible (C.V. less than 12%) (Table 1). Inter-day precision of the method was validated with plasma samples that were spiked with 0.4, 3 or 25 ng/ml of loratadine and analyzed in duplicate on each of five consecutive days. Inter-day precision was very good with a coefficient of variation of less than 9% (Table 2).

3.2. Descarboethoxyloratadine

A typical chromatogram of descarboethoxyloratadine and its internal standard (SCH 40129) extracted from plasma is shown in Fig. 3. The retention time was 2.9 min for descarboethoxyloratadine and 3.2 min for SCH 40129. The standard curve for descarboethoxyloratadine was obtained by plotting the ratio of the peak height of descarboethoxyloratadine to that of the internal standard (SCH 40129) against the concentrations (0.1 to 30 ng/ml) of descarboethoxyloratadine added ($y = 0.1327x - 0.002233$). There was a good linear relationship between peak-height ratio and plasma concentration, with a correlation coefficient of 0.9982. The limit of quantitation was 0.1 ng/ml, the lowest concentration tested with a small coefficient of variation, (5.3%) (Table 3). There were no endogenous interfering peaks in control plasma that occurred at the retention time of descarboethoxyloratadine, loratadine and internal standard,

Table 2
Between-day reproducibility for loratadine

Assay day	Concentration (ng/ml)					
	0.4 ng/ml		3 ng/ml		25 ng/ml	
	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
1	0.37	0.48	2.85	3.21	26.8	25.7
2	0.40	0.44	2.86	2.93	24.0	21.9
3	0.41	0.36	2.70	2.84	26.9	25.4
4	0.34	0.36	3.02	3.08	25.7	24.5
5	0.39	0.36	2.82	2.89	23.6	24.8
Mean	0.38		2.92		24.9	
S.D.	0.031		0.147		1.52	
C.V.(%)	8.2		5.0		6.1	

Table 3
Within-day reproducibility for descarboethoxyloratadine

Concentration added (ng/ml)	Concentration found (mean ± S.D., $n = 5$) (ng/ml)	C.V. (%)
0.1	0.10 ± 0.005	5.3
0.2	0.20 ± 0.011	5.5
0.4	0.39 ± 0.016	4.1
1	0.92 ± 0.053	5.8
5	5.00 ± 0.191	3.8
10	10.1 ± 0.54	5.3
20	20.8 ± 0.77	3.7
30	30.5 ± 0.46	1.5

indicating that the GLC method was specific for the analysis of descarboethoxyloratadine.

Intra-day precision of the method was validated at eight concentrations. Five plasma samples at each concentration were analyzed by the GLC procedure described. The results demonstrated that the GLC method was accurate (bias less than 10%) and reproducible (C.V. less than 6%) (Table 3). Inter-day precision of the method was validated using ten plasma samples spiked with 0.4, 3 and 25 ng/ml of descarboethoxyloratadine and analyzed in duplicate on each of five consecutive days. Inter-day precision was very good with coefficient variation less than 7% (Table 4).

3.3. Assay feasibility

The methods described above were used to analyze plasma samples from pediatric volunteers following oral administration of a single dose of 10 mg of loratadine syrup. Mean plasma concentration–time curves for loratadine and descarboethoxyloratadine are shown in Fig. 4. Loratadine was rapidly absorbed. Plasma concentrations of loratadine and descarboethoxyloratadine reached a maximum one hour after dosing and thereafter decreased rapidly with time. At 24 h, plasma concentrations of loratadine in all subjects were below the limit of quantitation (0.1 ng/ml). On the other hand, plasma concentrations of descarboethoxyloratadine at 48 h were still above the limit of quantitation (0.1 ng/ml) in twelve of the fourteen volunteers.

4. Discussion

Radioimmunoassay (RIA) has been used to determine plasma concentrations of loratadine in

Table 4
Between-day reproducibility for descarboethoxyloratadine

Assay day	Concentration (ng/ml)					
	0.4 ng/ml		3 ng/ml		25 ng/ml	
	Assay 1	Assay 2	Assay 1	Assay 2	Assay 1	Assay 2
1	0.45	0.44	3.28	3.19	26.2	25.2
2	0.43	0.39	3.03	3.30	25.5	27.1
3	0.47	0.46	3.56	3.57	28.1	26.4
4	0.42	0.44	3.51	3.37	26.5	26.4
5	0.44	0.42	3.23	3.36	28.0	27.1
6	0.37	0.74	3.12	3.45	28.2	27.6
7	0.42	0.43	3.24	3.21	27.0	26.0
Mean	0.43		3.32		26.8	
S.D.	0.027		0.163		0.94	
C.V.(%)	6.3		4.9		3.5	

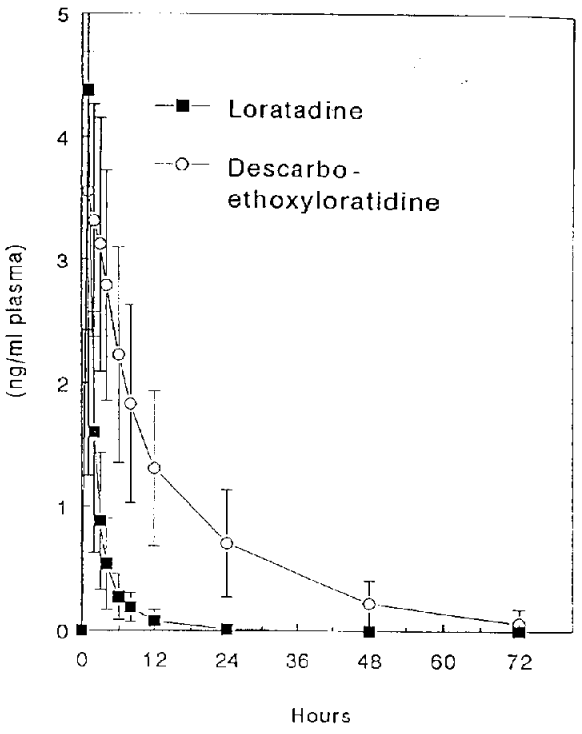

Fig. 4. Mean (n = 13) plasma concentration–time curves for loratadine and descarboethoxyloratadine in pediatric volunteers after an oral dose of 10 mg loratadine syrup. Bar represents the standard error.

healthy adult volunteers, nursing female volunteers and geriatric volunteers following a daily dose of 40 mg. The plasma samples were first extracted with organic solvent which was evaporated to dryness and then exposed to antisera for radioimmunoassay. The limit of quantitation (LOQ) was estimated to be 0.3 ng/ml, which was not sufficient for pharmacokinetic evaluation in volunteers receiving the recommended therapeutic dose of 10 mg once daily. An HPLC method was used to determine plasma concentrations of descarboethoxyloratadine in several clinical pharmacokinetic studies in volunteers who received a single 40-mg oral dose. However, the column used in the HPLC method had to be regenerated after each run by eluting the column sequently with various solvent mixtures to remove biological residues from the column. Furthermore, the manufacturer of the column had difficulty in producing columns with identical specifications, and it was very difficult to reproduce the method. Therefore, the development of a more robust and sensitive analytical method for loratadine and descarboethoxyloratadine was considered essential for further pharmacokinetic evaluation of loratadine.

In this report, we have described a gas–liquid chromatographic (GLC) method for the separate determination of loratadine and descarboethoxyloradine in human plasma. The limit of quantitation (LOQ) of the method was 0.1 ng per ml of plasma for both loratadine (with a C.V. of 9.2%) and descarboethoxyloratadine (with a C.V. of 5.3%). This LOQ was an improvement over the previously used RIA method for loratadine (0.3 ng/ml) and HPLC method for descarboethoxyloratadine (0.6 ng/ml). The new GLC method for loratadine is specific since the loratadine and descarboethoxyloratadine peaks are chromatographically resolved and are different from their respective internal standards. In contrast, a radioimmunoassay previously used for the determination of loratadine in human serum has not been fully validated with respect to the crossactivity of potential metabolites.

For radioimmunoassay of loratadine, an I^{125} ligand had to be synthesized every two months because of the relative short half-life of I^{125}. In addition, the yield of the synthesis was very low and unpredictable. Furthermore, the disposal of radioactive wastes has increasingly become an environmental problem, especially in New Jersey. These problems are circumvented by using the GLC method for loratadine described here.

Both the radioimmunoassay and the HPLC method required a total volume of two ml of plasma for the determination of loratadine and descarboethoxyloratadine. However, only one ml of plasma is needed for the GLC method. The requirement of a smaller volume of plasma is an important factor especially when the pharmacokinetics of loratadine are evaluated in the pediatric population.

The GLC methods for loratadine and descarboethoxyloratadine described in this paper have been demonstrated to be reproducible and robust with C.V.s for inter-day or intra-day analysis

less than 12% for loratadine and 6% for descarboethoxyloratadine. Furthermore, the LOQ of the GLC method for loratadine (0.1 ng/ml) is six-fold better than that of the radioimmunoassay (0.6 ng/ml). Clinical utility of this new reproducible and sensitive GLC method was demonstrated in the evaluation of the pharmacokinetics of loratadine in pediatric volunteers following a therapeutic oral dose of 10 mg.

5. References

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