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Short communication

Pharmacokinetic study of orphenadrine using high-performance liquid chromatography–tandem mass spectrometry (HPLC–MS/MS)☆

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Abstract

We developed and validated a simple, rapid, and accurate HPLC–MS/MS method with simple protein precipitation for the determination of orphenadrine. Injection-to-injection running time was 3 min with a retention time of orphenadrine of 1.1 min. The linear assay range was 1–200 ng/mL ($r^2 > 0.99$). The intra- and inter-assay imprecisions were CV 0.6–4.2% and CV 1.6–6.1%, respectively. The accuracy, extraction recovery, specificity and stability were satisfactory. Using the measured plasma concentrations of orphenadrine in 24 healthy subjects, pharmacokinetic profiles of orphenadrine were evaluated (AUC_{0-72}, 1565 ± 731 ng h/mL, C_{max} 82.8 ± 26.2 ng/mL, T_{max} 3.0 ± 0.9 h, elimination half-life 25.8 ± 10.3 h).

Keywords: Orphenadrine; HPLC–MS/MS; Pharmacokinetic

1. Introduction

Orphenadrine (*N*,*N*-dimethyl-2(*o*-methyl-alpha-phenylbenzyloxy)ethylamine; Fig. 1) is a close structural analogue of diphenhydramine. It is widely accepted as a skeletal muscle relaxant and is used as a therapeutic agent in the treatment of Parkinson disease and the neuroleptic syndrome.

The high prevalence of anticholinergic misuse has been previously reported [1]. There is also reported cases of orphenadrine poisoning [2–4]. Blood concentrations of orphenadrine greater than 500 ng/mL may cause toxic reactions. In addition, blood levels have been reported to be positively related to the improvement of neuroleptic-induced extrapyramidal side effects [5]. However, until recently, there has been little information available on the pharmacokinetics of orphenadrine. The reported pharmacokinetic profile of orphenadrine was derived from less than 10 subjects [6,7]. Determination of blood orphenadrine levels and understanding of its pharmacokinetics may be helpful in assessing poisoning in patients as well as monitoring of patients on chronic therapy. To date, the orphenadrine measurements on plasma samples have been performed either by HPLC–UV [2,6], GC [7] or GC/MS [3], all labor-intensive and time-consuming methods.

The purpose of this study was to develop simple and reliable HPLC–MS/MS method for determination of plasma orphenadrine, to apply this technique to bioavailability studies, and to examine pharmacokinetic parameters of orphenadrine in 24 healthy subjects.

2. Experimental

2.1. Reagents, instruments, and analytical conditions

Orphenadrine hydrochloride was supplied by Fluka (Steinheim, Switzerland). Diphenhydramine hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). All solvents were of HPLC grade and were obtained from JT Baker (Phillips-

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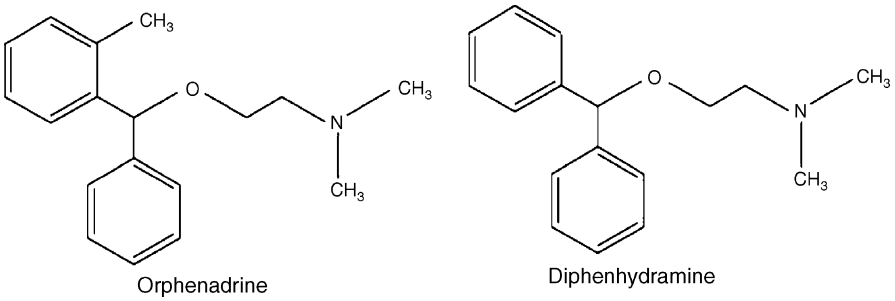

Fig. 1. Structures of orphenadrine and I.S. (diphenhydramine).

burg, NJ, USA). HPLC grade water was prepared using a Mili-Q system (Milipore, Molsheim, France).

Analyses were performed on a Quattro Micro API tandem mass spectrometer (Waters, Manchester, UK) equipped with Waters 2795 Alliance HPLC system (Waters, Manchester, UK).

The column used was a XTerra MS C18 (2.1 mm × 50 mm × 5 μm) (Waters, Manchester, UK) and was maintained at 55 °C. Mobile phases consisted of water containing 2 mM ammonium acetate and 0.1% formic acid (mobile A) and methanol containing 2 mM ammonium acetate and 0.1% formic acid (mobile B). The flow rate was 0.2 mL/min and the final injection volume of each sample was 10 μL.

Quantitative analyses were performed with the multiple reaction monitoring (MRM) mode (*m/z* 270.3 → 181.1 for orphenadrine, *m/z* 256.3 → 167.0 for I.S. diphenhydramine). The operating conditions of MS are shown in Table 1.

2.2. Preparation of calibrators and quality control samples

A standard stock solution of orphenadrine (10 μg/mL) was prepared in methanol. Blank plasma (pooled plasma from 10 blood donors) was used for the preparation of calibrator and control samples.

Calibrator and control samples with the highest concentration of orphenadrine were prepared by spiking the standard stock solution; then samples were further diluted with blank plasma to obtain final orphenadrine concentration levels of: 2.5, 5.0, 10, 50, 100 and 200 ng/mL.

2.3. Sample preparation

Ten microliters of plasma samples or calibrators were added to 40 μL 0.1 M $ZnSO_4$ solution. The mixture was placed in a vortex mixer for 30 s and 100 μL of 50% acetonitrile containing I.S. (diphenhydramine, 2 ng/mL) was added. After mixing in a vortex mixer for 30 s, it was centrifuged for 2 min at 14,000 rpm. The supernatant was transferred to another tube and 10 μL was injected onto the HPLC column.

2.4. Assay validation

The specificity of the method was evaluated by analyzing pooled plasma obtained from 10 healthy blood donors and blank plasma from the 24 study subjects before drug administration. The ion suppression effect of the method was investigated using pooled blank plasma sample, injected onto the LC–MS/MS system after protein precipitation, in parallel with continuous post-column infusion of orphenadrine and I.S. in the electrospray source.

The intra- and inter-day precision and accuracy were determined with five replicates at six concentrations (2.5, 5, 10, 50, 100 and 200 ng/mL of orphenadrine).

Table 1
MS operating conditions

Ionization mode	ES+		
Scan type	Multiple reaction monitoring		
Channel reaction	Dwell time (s)	Cone voltage (V)	Collision energy (eV)
270 → 181 for orphenadrine	0.2	15.0	13.0
256 → 167 for IS	0.2	15.0	13.0
Gas cell pressure (E^{-3} mbar)	3.5		
Capillary voltage (kV)	0.6		
Source temperature (°C)	130		
Desolvation temperature (°C)	350		
Desolvation gas flow (L/h)	700		
Resolution	Q1:14.5, Q3: 14.5		
Multiplier (PM) voltage (V)	650		

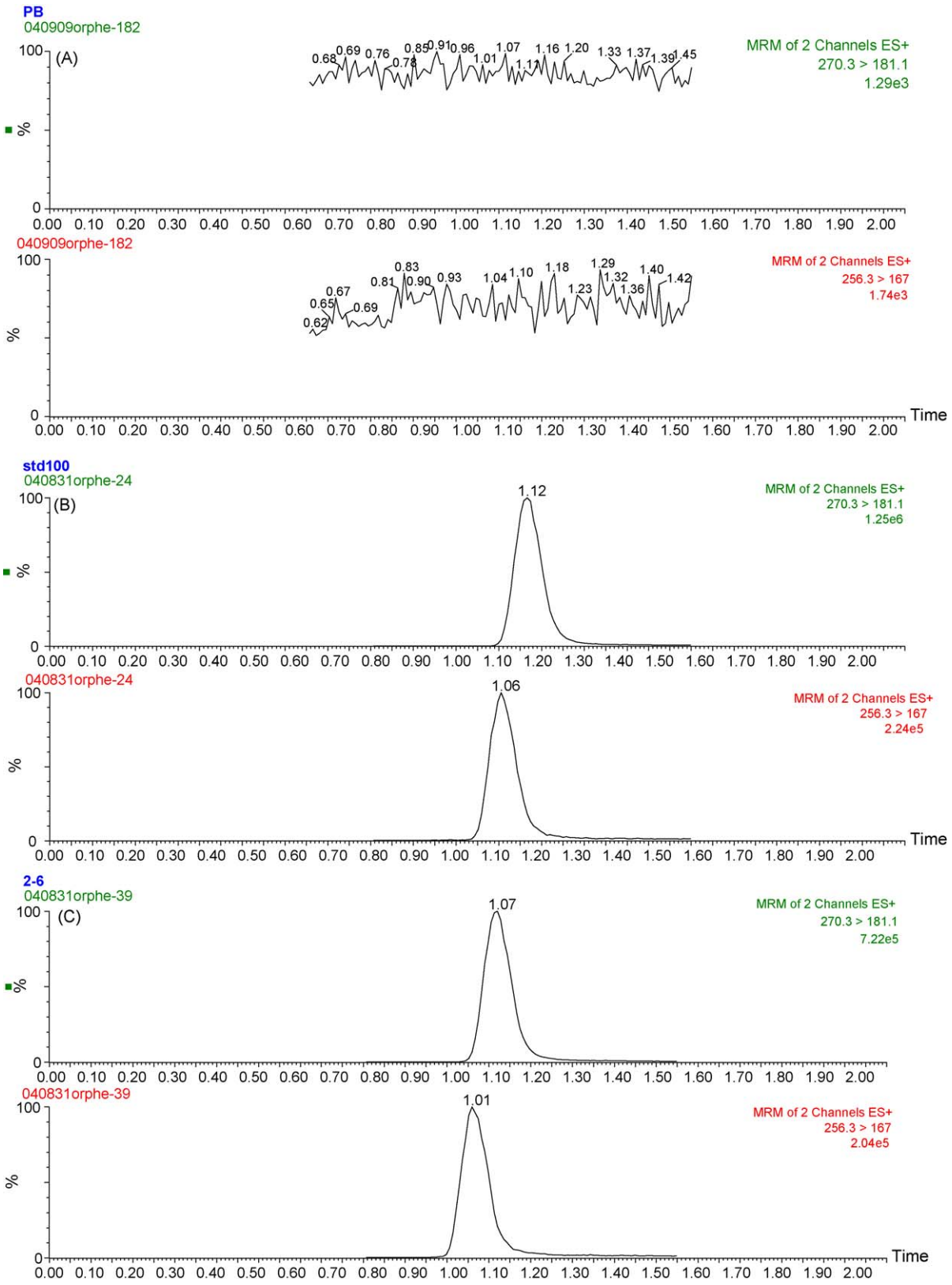

Fig. 2. Chromatograms of blank plasma (A), plasma spiked with orphenadrine (100 ng/mL) and I.S. (2 ng/mL) (B) and an extracted plasma sample from a healthy subject (6 h after oral administration of orphenadrine 50 mg) containing orphenadrine (55.5 ng/mL) along with I.S (2 ng/mL) (C).

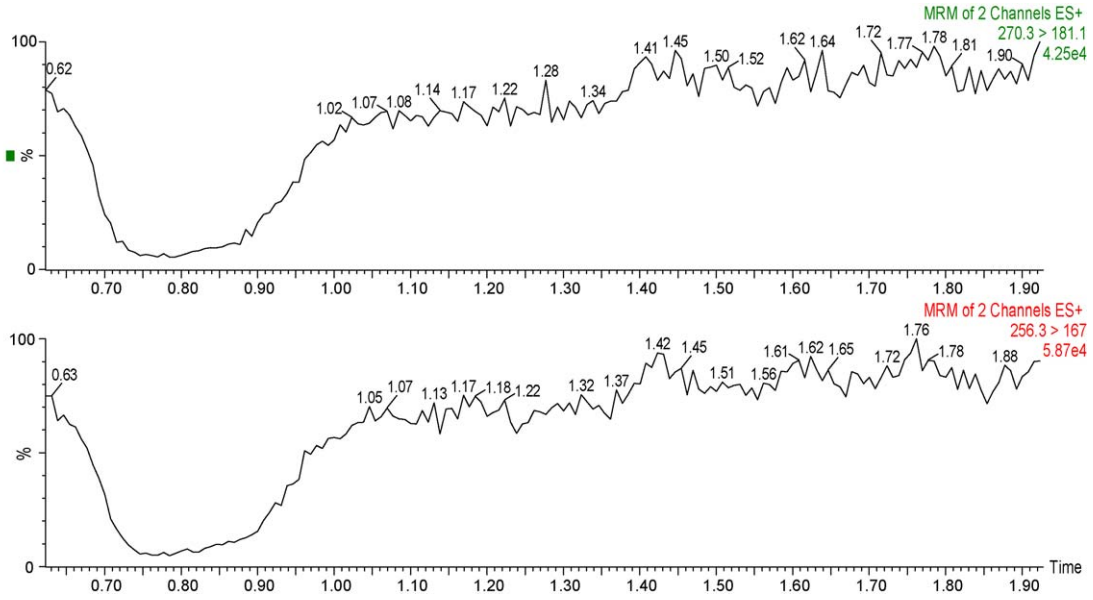

Fig. 3. Evaluation of ion suppression effect using pooled blank plasma sample.

A six-point standard curve of orphenadrine was prepared by spiking the blank plasma with appropriate amounts of orphenadrine. The calibration curve was generated for orphenadrine concentrations up to 200 ng/mL.

The lower limit of quantification (LLOQ) was defined as the lowest concentration with a signal-to-noise ratio of >10 and a precision of <CV 10%; this was verified by the analysis of 10 replicates.

Extraction recovery of orphenadrine and I.S. was determined at two different concentrations (2.5 and 200 ng/mL) representing the entire range of the calibration curve. The concentrations of the spiked plasma samples were compared with those of standards prepared in the mobile phase.

The short-term and long-term stability was investigated by analyzing the samples stored at an ambient temperature for 4 h and −70 °C for 30 days, respectively. The samples underwent three freeze–thaw cycles (−20 °C for at least 24 h and ambient temperature for 4 h) to evaluate the freeze–thaw stability. To test the post-preparative stability of orphenadrine and I.S., the prepared samples were placed into the autosampler at 10 °C for 6 h. Stock solution stability was also examined after storage at −20 °C for 30 days.

2.5. Pharmacokinetic study of orphenadrine in healthy subjects

The ethics committee approved this study. A total of 24 healthy volunteers had a physical examination and normal findings on routine laboratory tests. All volunteers were given 50 mg of orphenadrine orally after an overnight fast. Blood samples were obtained for up to 72 h (0, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 9, 12, 24, 48 and 72 h) after drug administration. We measured plasma concentrations of orphenadrine by HPLC–MS/MS as described above. For pharmacokinetic analysis, BA Calc 2002 (version 1.1.1, Seoul, Korea) was used.

Table 2
Precision and accuracy obtained for orphenadrine in plasma

Added concentration (ng/mL)	Number of observations	Measured concentration (ng/mL)	CV (%)	Accuracy (%)
Intra-day				
2.5	5	2.3	2.6	92.7
5.0	5	5.2	2.9	103.2
10.0	5	10.5	4.2	105.5
50.0	5	48.2	0.6	96.5
100.0	5	97.1	0.9	97.1
200.0	5	200.0	1.1	100.0
Inter-day				
2.5	5	2.4	3.8	95.4
5.0	5	5.7	6.1	114.8
10.0	5	11.0	3.4	110.3
50.0	5	47.1	2.0	95.6
100.0	5	94.8	1.6	94.8
200.0	5	207.8	2.4	103.9

Table 3
Extraction recovery of orphenadrine from plasma

Added concentration (ng/mL)	Number of observations	Measured concentration (ng/mL)	Recovery (%)
2.5	3	2.3	92.0
200	3	188.1	94.1

3. Results

Representative ion-pair LC–MS/MS chromatograms are shown in Fig. 2. Injection-to-injection running time was 3 min with retention time of 1.1 min for orphenadrine and 1.0 min for I.S. Analysis of plasma, from the 24 study subjects before orphenadrine administration and pooled plasma from 10 blood donors showed no interfering peaks at the retention times measured for orphenadrine and I.S. No ion suppression effects were observed at the retention times of the two analytes (Fig. 3).

The linear assay range was observed to be 1.0–200 ng/mL. The peak-area of orphenadrine compared to the I.S. (y) versus orphenadrine concentration (x) was plotted; the regression equation of the calibration curves was as follows: $y = 0.05987x - 0.01444$ ($r^2 > 0.99956$) (weighting factor $1/x$ was used). The LLOQ for orphenadrine was 1.0 ng/mL (CV 6.8%, S/N = 18). The accuracy was satisfactory (91.5–103.2%). The intra- and inter-assay CVs were 0.6–4.2% and 1.6–6.1%, respectively, for six levels of orphenadrine (Table 2).

The extraction recovery was estimated to be >92.0% as shown in Table 3. In all stability tests, the analytes were considered stable; as the measured concentration after the treatment was 0–12% of the nominal value (Table 4).

Plasma concentration–time profile after administration of orphenadrine (50 mg) in the 24 healthy subjects is shown in Fig. 4. Pharmacokinetic properties of orphenadrine were as follows; area under the curve from time zero to 72 h ($AUC_{0–72}$) 1565 ± 731 ng h/mL (range: 1128–3901 ng h/mL), maximum plasma concentration (C_{max}) 82.8 ± 26.2 ng/mL (range: 53.4–138.0 ng/mL), time to reach maximum plasma concentration (T_{max}) 3.0 ± 0.9 h (range: 2–5 h), elimination half-life 25.8 ± 10.3 h (range: 15.3–59.5 h). Significant interindividual differences were found in the half-life measurements (CV 39.9%), $AUC_{0–72}$ (CV 46.7%), C_{max} (CV 31.6%), and T_{max} (CV 28.2%).

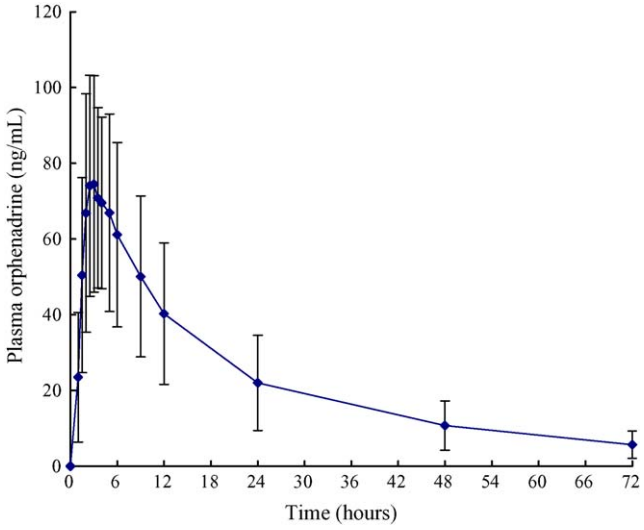

Fig. 4. Plasma concentration–time profile of orphenadrine in 24 healthy subjects following oral administration of orphenadrine (50 mg).

The lowest observed plasma concentrations in 24 subjects at the 72 h after a single oral dose of orphenadrine were 2.1–18.6 ng/mL.

4. Discussion

Orphenadrine is a widely used anticholinergic drug in the treatment of Parkinsonism. However, to date, blood level monitoring for orphenadrine levels is not routinely performed. In addition, there is no sufficient data on the pharmacokinetics of orphenadrine. In this study, the pharmacokinet-

Table 4
Stability of orphenadrine and I.S. in plasma and stock solution

Stability test	Added concentration (ng/mL)	Number of observations	Measured concentration (ng/mL)	Bias (%)
Short-term				
Orphenadrine	2.5	3	2.3	8.0
Orphenadrine	200	3	194.7	2.7
Long-term				
Orphenadrine	2.5	3	2.2	12.0
Orphenadrine	200	3	180.0	10.0
Freeze–thaw				
Orphenadrine	2.5	3	2.5	0.0
Orphenadrine	200	3	213.4	6.7
Post-preparative				
Orphenadrine	50	3	47.6	4.8
I.S.	2	3	1.9	5.0
Stock solution				
Orphenadrine	1000	3	938.3	6.2
I.S.	10000	3	9877.7	1.2

ics of orphenadrine was examined with a newly developed HPLC–MS/MS method.

Previously reported pharmacokinetic parameters of orphenadrine have been derived from very small numbers of subjects. The reported mean half-lives were 15.5 h after 100 mg of orphenadrine administration in five subjects [7] and 13.7 h in eight subjects in another study [6]. In our study, the mean elimination half-life was 25.8 ± 10.3 h, which is longer than that reported in previous papers. In addition, we found considerable interindividual difference among the 24 subjects studied. Our AUC_{0-72} (1565 ± 731 ng h/mL), C_{max} (82.8 ± 26.2 ng/mL) and T_{max} (3.0 ± 0.9 h) were comparable to those found in other studies [6,7].

Orphenadrine has a peripheral and central anticholinergic effect as well as a known cardiotoxic effect when taken in larger doses. The reported therapeutic concentration is in the plasma range of 100–200 ng/mL [3]. Blood concentrations >500 ng/mL may cause toxic reactions and >5000 ng/mL may be lethal [3]. Accidental intoxication in children and a few cases of death have resulted from overdose of orphenadrine in schizophrenic patients [2–4]. Therefore, determination of blood orphenadrine levels and improved understanding of its pharmacokinetics may be help assess poisoned patients as well as improve monitoring in patients on the chronic therapy where indicated.

Compared to the several analytical methods available for the determination of orphenadrine, our HPLC–MS/MS method is much more rapid, simple, sensitive, and specific. Injection-to-injection sample running time is only 3 min (retention times of orphenadrine was 1.1 min) and requires a minimal sample volume (10 μL of plasma). Our simple sample preparation method takes less than 5 min and provides clean chromatograms. There were no interfering peaks present in the clinical samples. We could also produce excellent recovery, precision, accuracy and stability, over a wide analytical range sufficient for the pharmacokinetic study of orphenadrine.

In summary, we established a simple, rapid, and reliable HPLC–MS/MS method for the measurement of orphenadrine. In addition, pharmacokinetic parameters for orphenadrine in healthy subjects were studied. These results can be used to examine the metabolism of orphenadrine and to ensure effective and safe therapy in patients.

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