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Determination of olopatadine, a new antiallergic agent, and its metabolites in human plasma by high-performance liquid chromatography with electrospray ionization tandem mass spectrometry

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Abstract

A rapid, sensitive and specific assay method has been developed to determine plasma concentrations of olopatadine hydrochloride (A) and its metabolites, M1 (B), M2 (C) and M3 (D), using high-performance liquid chromatography with electrospray ionization tandem mass spectrometry (LC–ESI-MS–MS). Olopatadine, its metabolites, and internal standard, KF11796 (E), were separated from plasma using solid-phase extraction (Bond Elut C_{18} cartridge). The eluate was dried, reconstituted and injected into the LC–ESI-MS–MS system. The calibration curves showed good linearity over the ranges 1–200 ng/ml for olopatadine and M3, and 2–100 ng/ml for M1 and M2, and the method was thoroughly validated and applied to the determination of olopatadine and its metabolites in plasma collected during Phase I clinical trials. Furthermore, the assay values were compared with those determined by the radioimmunoassay method, which has been routinely used to determine olopatadine in plasma.

Keywords: Olopatadine

1. Introduction

(*Z*) - 11 - [(3 - Dimethylamino)propylidene] - 6, 11-dihydro-dibenz[*b*,*e*]oxepin-2-acetic acid hydrochloride, olopatadine hydrochloride (A) [1], is a new antiallergic drug, which has been found to be effective in some experimental animal models and humans [2–4].

Olopatadine is metabolized to M1 (B) and M2 (C) via oxidative N-demethylation and to M3 (D) via N-oxidation [5]; the pharmacological activity of M1 was almost equal to that of the parent drug while M3 had only one-tenth the activity. After oral administration of ^{14}C-olopatadine hydrochloride to rats and dogs, these metabolites were produced in very small quantities and the major component in plasma was the unchanged drug [5]. In addition, the urinary excretion of unchanged drug after oral administration of olopatadine hydrochloride to rats, dogs and humans was 27.3, 51.6 and 58.7%, respectively [5,6]. There is very little metabolism of olopatadine in rats and dogs, so these metabolites make very little contribution to its pharmacological effect.

A radioimmunoassay (RIA) method for the de-

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termination of olopatadine in plasma had been developed previously to support the pharmacokinetic studies in humans [6] and some animals [7]. This RIA method was very sensitive with a lower limit of quantification (LLOQ) of 0.1 ng/ml in plasma. However it had several problems, such as cross-reactivity of metabolites and low precision, and so it was considered that plasma concentrations of olopatadine measured by RIA needed to be confirmed by an alternative method. Furthermore, it was important to be able to monitor the pharmacologically active metabolites in plasma during clinical trials. Recently, high-performance liquid chromatography (HPLC) with tandem mass spectrometry (MS–MS) has been widely applied to the determination of a number of compounds in biological fluids and it has been found to be a highly sensitive, specific and rapid technique [8–10].

In this paper, we describe an assay for olopatadine and its metabolites in human plasma by HPLC with electrospray ionization tandem mass spectrometry (LC–ESI-MS–MS). We also report the plasma concentrations of olopatadine and its metabolites after oral administration of olopatadine hydrochloride to humans, and the comparison of the plasma concentrations of olopatadine measured by RIA and LC–ESI-MS–MS.

2. Experimental

2.1. Chemicals

Olopatadine hydrochloride, M1, M2, M3 and compound KF11796 (E) (internal standard, I.S.) were synthesized in our institute (Fig. 1). Bovine serum albumin (BSA) was purchased from Seikagaku Kogyo (Tokyo, Japan). Methanol and acetonitrile of HPLC grade and acetic acid were purchased from Kanto Chemicals (Tokyo, Japan). Distilled deionized water was obtained from a Milli-Q water purification system (Millipore, Bedford, MA, USA).

2.2. Instrumentation and chromatographic conditions

HPLC was performed with a Jasco PU-980 solvent delivery system (Jasco, Tokyo, Japan) equipped with a Rheodyne Model 7125 injector (Cotati, CA, USA). A mobile phase of 10 mmol/l acetic acid–methanol (55:45, v/v) was pumped through a Develosil ODS HG-5 column (50×2 mm I.D., 5 μm; Nomura Chemical, Aichi, Japan) at a rate of 0.1 ml/min at ambient temperature. The exit of the column was connected to a Quattro tandem mass spectrometer (Micromass, Manchester, UK). The mass spectrometer was operated in the electrospray mode with an ion source temperature of 120°C, the capillary voltage was 4.0 kV, the counter current electrode voltage was 1.2 kV and the cone voltage was 20 V. Olopatadine, its metabolites and I.S. were detected in the positive mode by multiple reaction monitoring (MRM). Collision-activated dissociation (CAD) of the protonated molecules of olopatadine (*m/z* 338), M1 (*m/z* 324), M2 (*m/z* 310) and M3 (*m/z* 354) all yielded an ion of *m/z* 165 as the most abundant product ion; in the case of CAD of the protonated molecule of I.S. (*m/z* 352), the product ions of *m/z* 179 and 247 were the most abundant. For CAD, argon was used with a collision-cell gas pressure of 0.2 Pa. The collision energy was set at 20 eV and the instrument was programmed for a dwell time of 220 ms.

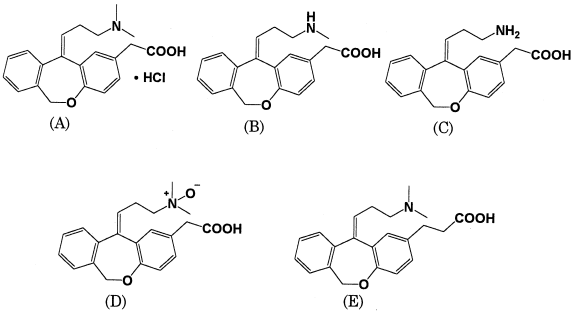

Fig. 1. Chemical structures of olopatadine hydrochloride (A), M1 (B), M2 (C), M3 (D) and internal standard (E).

2.3. Standard and quality control

A stock solution of olopatadine was prepared in water at a concentration of 500 μg/ml. Stock solutions of its metabolites and I.S. were prepared in 1% (v/v) acetic acid aqueous solution–acetonitrile (1:1, v/v) at concentrations of 500 μg/ml. The stock solutions of olopatadine and its metabolites were mixed and diluted with water to yield mixed stan-

dards solutions containing each 0.1, 1 and 10 μg/ml. The stock solution of I.S. was diluted with water to yield a solution of 0.2 μg/ml. The stock solution was stored at 4°C.

Quality control plasma containing olopatadine and its metabolites at concentrations of 1, 2, 4, 20, 100 and 200 ng/ml was prepared by transferring aliquots of the mixed standard solution to a tube containing control human plasma.

2.4. Administration and sample collection

Olopatadine hydrochloride (80 mg) was orally administered to six healthy male volunteers from 22 to 26 years of age after written informed consent was obtained. Blood samples were collected pre-dose and 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 24, 36 and 48 h after dosing and transferred to heparinized tubes. Blood samples were centrifuged to yield plasma, which were stored at −20°C until analyzed.

2.5. Sample preparation

0.25 ml plasma, 50 μl I.S. (0.2 μg/ml solution) and 250 μl water were vortex-mixed and transferred to a Bond Elut C_{18} cartridge (Varian, Harbor City, CA, USA). The cartridge had been pre-treated sequentially with 1 ml methanol, 2 ml water, 2 ml 1% (w/v) BSA aqueous solution and 5 ml water. After adding the plasma, the cartridge was washed with 2 ml water. Olopatadine, its metabolites and I.S. were eluted with 1 ml of methanol and the eluate was evaporated to dryness under a nitrogen stream. The residue was reconstituted with 50 μl mobile phase, and after filtering the sample through a 0.2-μm PTFE membrane filter (Millipore), 10 μl filtrate was injected into the LC–ESI-MS–MS system. As the plasma concentration exceeded the upper limit of calibration curve, the plasma was reassayed after the appropriate dilution with control plasma.

2.6. Calibration curve, recovery and method validation

Calibration curves were daily prepared from human control plasmas spiked with olopatadine and M3 (1, 2, 4, 8, 20, 40, 100 and 200 ng/ml), and M1 and M2 (2, 4, 8, 20, 40 and 100 ng/ml). The linearity was determined by plotting the ratio of the analyte and I.S. areas versus analyte concentration. Calibration curves were constructed by weighted $(1/y^2)$ linear least-squares regression.

Recovery was calculated by comparison of the peak areas of analyte extracted from quality control plasmas to those of injected standards.

The method validation was carried out in accordance with a summary report of the conference on Analytical Methods Validation [11], that is, five replicates of the quality control plasmas containing LLOQ concentration were measured on one day, and a replicate of the quality control plasmas containing concentration near LLOQ was measured on four separate days.

2.7. Data analysis

The maximum plasma concentration (C_{max}) and time to reach the maximum concentration (t_{max}) were calculated directly from the plasma concentration data for each subject. The area under the plasma concentration–time curve from 0 to the last time point (AUC_{0-t}) was calculated by the linear trapezoidal rule.

3. Results and discussion

Direct injection of olopatadine, M1, M2, M3 and I.S. yielded protonated molecules of m/z 338, 324, 310, 354 and 352, respectively. Product ion mass spectra of the protonated molecule of olopatadine and its metabolites all showed an ion of m/z 165, which is probably formed by fission of the dibenzoxepin ring, as the most abundant ion (Fig. 2). This product ion was detected at m/z 179 in CAD of the protonated molecule of I.S.

Olapatadine, its metabolites and I.S. were isolated from plasma using solid-phase extraction. The recoveries were all more than 76.6%. The pre-conditioning of the cartridge with 1% BSA aqueous solution improved the poor recoveries of the metabolites. Olopatadine and its metabolites in human plasma were stable under storage at −20°C for at least two months. The mass chromatograms for olopatadine (m/z 338→165), M1 (m/z 324→165), M2 (m/z 310→165), M3 (m/z 354→165) and I.S. (m/z 352→179) in control plasma and plasma spiked with authentic standards (olopatadine and M3; 1

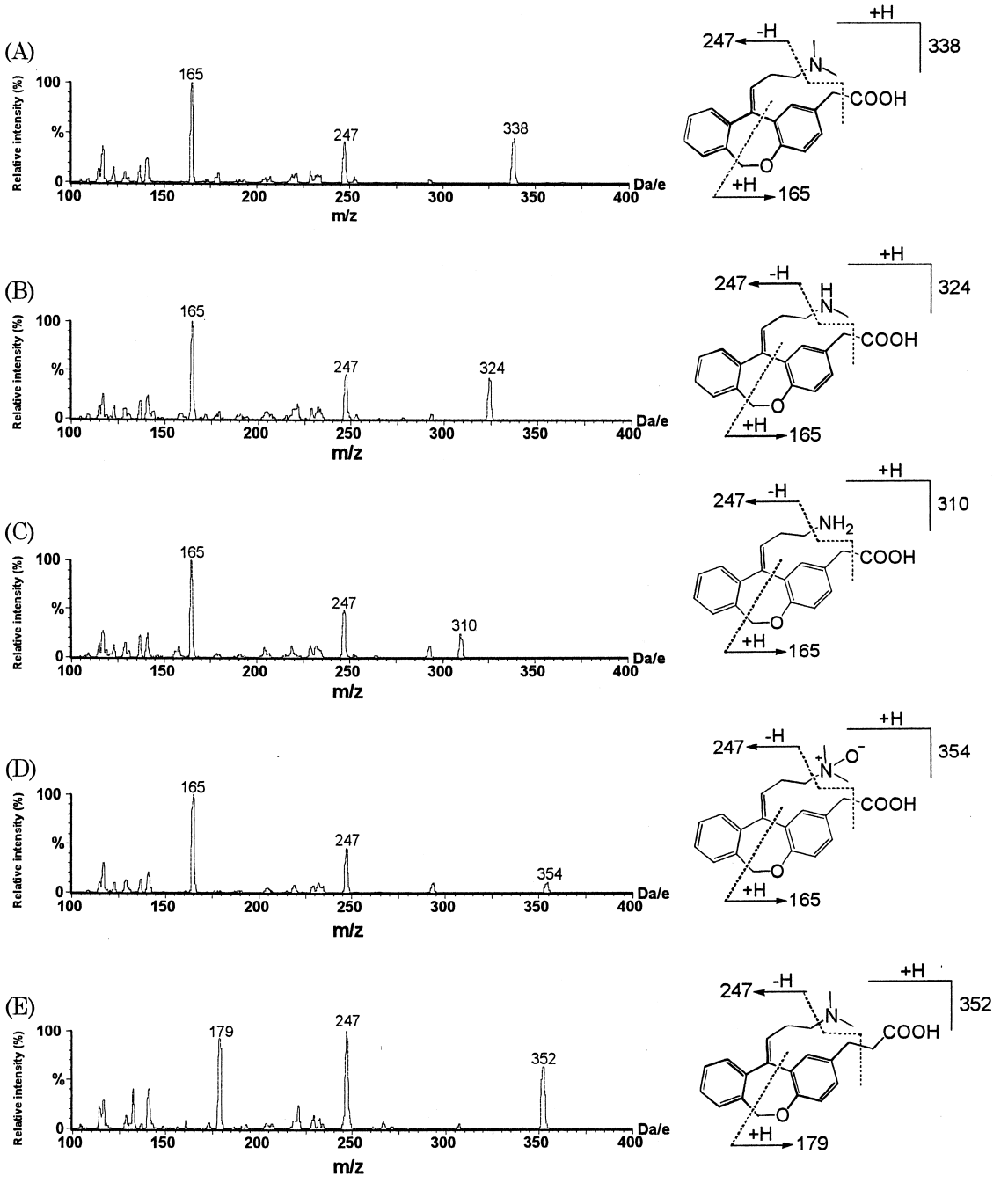

Fig. 2. Product ion mass spectra of olopatadine (A), M1 (B), M2 (C), M3 (D) and internal standard (E).

ng/ml, M1 and M2; 2 ng/ml) are shown in Fig. 3. No interference was observed in the determination of olopatadine, its metabolites and I.S. The detection limits of olopatadine and its metabolites injected into the LC–ESI-MS–MS were all 30 pg with a signal-to-noise ratio of 3:1.

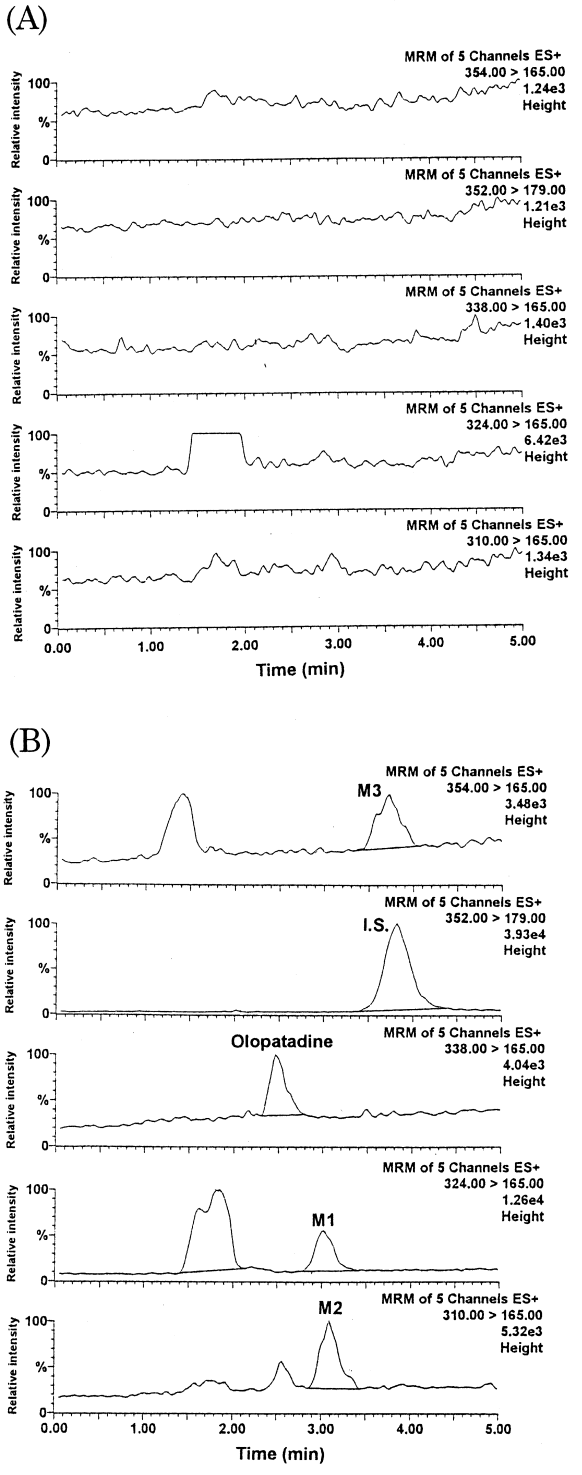

Fig. 3. Mass chromatograms of blank plasma (A) and plasma spiked with authentic compounds (olopatadine and M3; 1 ng/ml, M1 and M2; 2 ng/ml) (B).

The calibration curve was linear over the range from 1 to 200 ng/ml for olopatadine ($y=0.0442x+0.0141$, $r=0.9992$) and M3 ($y=0.0449x+0.0007$, $r=0.9992$), and from 2 to 100 ng/ml for M1 ($y=0.0300x+0.0506$, $r=0.9992$) and M2 ($y=0.0204x+0.0309$, $r=0.9996$). The intra-day precision and accuracy for the measurement of quality control plasmas was less than 17.9% at the LLOQ concentration (olopatadine and M3; 1 ng/ml, M1 and M2; 2 ng/ml), and 14.0% at concentrations of 20 and 100 or 200 ng/ml with five replicates (Table 1). The inter-day precision and accuracy for the measurement of quality control plasmas was less than 14.5% at concentrations of 4, 20 and 100 or 200 ng/ml with a replicate on four separate days (Table 2).

This method was applied to the determination of olopatadine and its metabolites in plasma samples after a single 80 mg oral dose of olopatadine. The plasma concentration–time curves of olopatadine, M1 and M3 are shown in Fig. 4 and it can be seen that olopatadine, M1 and M3 reached a maximum concentration (C_{max}) at 1.0, 1.5 and 1.4 h, respectively. Following this, the plasma concentrations of olopatadine, M1, and M3 fell rapidly and could be detected until 48, 6 and 12 h, post-dosing, respectively. The concentration of M2 in all plasma samples was less than the LLOQ (2 ng/ml) in all subjects. The C_{max} values of M1 and M3 were considerably lower than that of olopatadine (Table 3). The ratio of the AUC_{0-6} of olopatadine to the AUC_{0-6} of M1 was about 100:1 and the ratio of the AUC_{0-12} of olopatadine to the AUC_{0-12} of M3 was about 100:7. The drug is predominantly in unchanged form in plasma after oral administration to humans, so the pharmacological contribution of its metabolites is likely to be very minor.

A species difference was found in metabolic profiles after a single oral administration of ^{14}C-olopatadine hydrochloride to rats and dogs [5]. M1, M2 and M3 in plasma collected 0.5 h after administration accounted for 7.7, 0.9 and 0.5% of the total plasma radioactivity in rats, respectively. While in dogs, the corresponding figures for M1, M2 and M3 were 2.1, 1.1 and 11.0%, respectively. The fact that M3 was the major metabolite in human plasma indicates that the metabolic pathway in humans is similar to that in dogs.

Table 1
Precision and accuracy of intra-day on LC–ESI-MS–MS for the determination of olopatadine and its metabolites in human plasma

Compound	Added (ng/ml)	Found (ng/ml)	Relative standard deviation (%)	Relative error (%)
Olopatadine	1	0.97±0.17[a]	17.9	2.8
	20	20.64±1.62	7.8	−3.2
	200	188.79±15.15	8.0	−5.6
M1	2	1.87±0.19	10.1	−6.4
	20	19.05±1.31	6.9	−4.8
	100	95.68±7.19	7.5	−4.3
M2	2	1.97±0.27	13.8	−1.5
	20	18.49±1.68	9.1	−7.6
	100	92.55±8.98	9.7	−7.5
M3	1	0.86±0.10	11.3	−14.0
	20	21.39±63	2.9	6.9
	200	195.60±13.73	7.0	−2.2

[a] Value represents the mean±SD (n=5).

Although an RIA method for the determination of olopatadine in plasma has been reported [7] and used to measure plasma concentrations of olopatadine in clinical trials [6], the antibody showed the cross-reactivity with the metabolites of olopatadine (27, 4 and <1% for M1, M2 and M3, respectively) [7]. This cross-reactivity could lead to overestimation of olopatadine in plasma. The values from RIA method [6] need to be validated using a more specific assay. On comparing plasma concentrations measured by the LC–ESI-MS–MS and RIA method, we obtained a good correlation coefficient of 0.9197 and a slope of 1.0629 (n=74, Fig. 5). The slope was almost unity. However, the values obtained by RIA differed apparently from those obtained by LC–ESI-MS–MS above 500 ng/ml. This difference would not be

Table 2
Precision and accuracy of inter-day on LC–ESI-MS–MS for the determination of olopatadine and its metabolites in human plasma

Compound	Added (ng/ml)	Found (ng/ml)	Relative standard deviation (%)	Relative error (%)
Olopatadine	4	4.35±0.63[a]	14.5	8.7
	20	19.01±1.29	6.8	−5.0
	200	194.05±21.62	11.1	−3.0
M1	4	3.92±0.44	11.3	−2.0
	20	20.02±1.08	5.4	0.1
	100	86.17±3.36	3.9	−13.8
M2	4	3.71±0.21	5.6	−7.3
	20	20.49±0.97	4.7	2.4
	100	86.67±4.42	5.1	−13.3
M3	4	4.06±0.53	13.1	1.4
	20	20.86±0.79	3.8	4.3
	200	188.05±9.06	4.8	−6.0

[a] Value represents the mean±SD (n=4).

Table 3
Pharmacokinetic parameters of olopatadine and its metabolites in plasma after a single oral administration of olopatadine hydrochloride at a dose of 80 mg to healthy volunteers ($n=6$)

Compound		t_{max} (h)	C_{max} (ng/ml)	AUC_{0-6} (ng h/ml)	AUC_{0-12} (ng h/ml)	AUC ratio (%)
Olopatadine	Mean	1.0	1523	3816	4382	
	SD	0.5	314	528	637	
M1	Mean	1.5	12	41		1.1[a]
	SD	0.5	3	9		0.1
M3	Mean	1.4	85		289	6.6[b]
	SD	0.2	18		57	0.6

[a] AUC_{0-6}(M1)×100/AUC_{0-6}(olopatadine).
[b] AUC_{0-12}(M3)×100/AUC_{0-12}(olopatadine).

caused by the cross-reactivity with metabolites due to the fact that there are very low concentrations of metabolites in plasma. The maximum dilution ratio in the LC–ESI-MS–MS and RIA methods was 10-fold and 1000- to 10 000-fold, respectively. The dilution test up to 1000-fold indicated good precision and accuracy in RIA method (data not shown). However, the dilution test more than that fold was not examined. Therefore, it is expected that the difference of assay values in high concentration between LC–ESI-MS–MS and RIA methods might be due to the large dilution ratio in the RIA method.

4. Conclusion

In conclusion, we have developed a specific LC–ESI-MS–MS method to determine olopatadine and

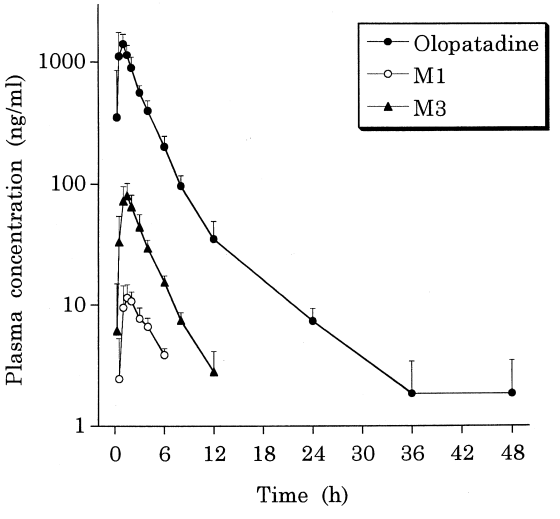

Fig. 4. Plasma concentration–time curves of olopatadine, M1 and M3 after a single oral administration of olopatadine hydrochloride at a dose of 80 mg to healthy volunteers. Each point represents the mean±SD ($n=6$).

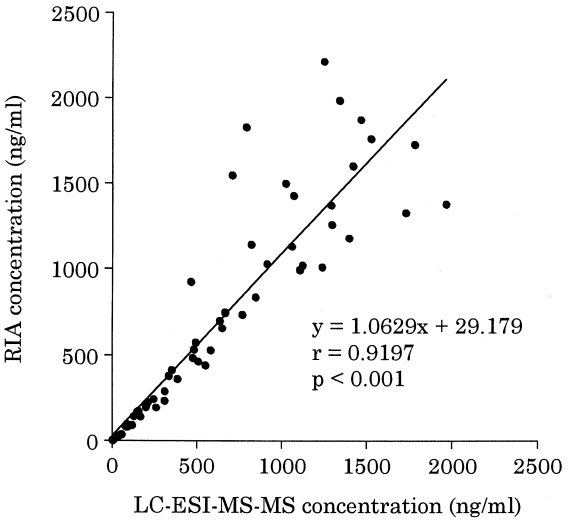

Fig. 5. The correlation of plasma concentrations of olopatadine determined by LC–ESI-MS–MS and RIA. The plasma samples were collected periodically after a single oral administration of olopatadine hydrochloride to six healthy volunteers at a dose of 80 mg. RIA data are from Ref. [6].

its metabolites in human plasma. The method is rapid, with a sample analysis times of 5 min from one injection to the next. Furthermore, we applied this method to clinical samples and showed that this method is useful for pharmacokinetic studies and monitoring olopatadine and its metabolites concentrations during clinical use.

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