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Journal of Chromatography B, 846 (2007) 105–111
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Development and evaluation of an efficient HPLC/MS/MS method for the simultaneous determination of pseudoephedrine and cetirizine in human plasma: Application to Phase-I pharmacokinetic study

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Received 1 April 2006; accepted 18 August 2006
Available online 12 September 2006

Abstract

A sensitive, simple and highly selective liquid chromatography/tandem mass spectrometry (LC/MS/MS) method was developed and evaluated to determine simultaneously the concentrations of pseudoephedrine and cetirizine in human plasma. The chief benefit of the present method is the minimal sample preparation, as the procedure is only one-step protein precipitation. Two drugs were separated on a C_8 column and analyzed by LC/MS/MS using positive electrospray ionisation (ESI). The method had a chromatographic run time of 12.0 min and a linear calibration curve over the concentration range of 1.0–800 ng/ml for pseudoephedrine and 1.0–400 ng/ml for cetirizine, respectively. The lower limit of quantification of the two drugs was 1.0 ng/ml, respectively. The intra- and inter-batch precisions were less than 9.7%. The method described herein has been first used to reveal the pharmacokinetic characters in healthy Chinese volunteers treated with oral administration of different dosages of cetirizine dihydrochloride and controlled-released pseudoephedrine hydrochloride compound tablet, and approached the influence of a standard meal on the extent and rate of absorption of the combination tablet.

Keywords: Pseudoephedrine; Cetirizine; HPLC–MS/MS; Human plasma; Pharmacokinetics

1. Introduction

Cetirizine dihydrochloride, is the carboxylated metabolite of hydroxyzine, and has high specific affinity for histamine H_1-receptors. Pseudoephedrine hydrochloride, which is a sympathomimetic drug acts directly on alpha-adrenergic receptors.

Extended-release of pseudoephedrine/cetirizine tablet formulations twice- or once-daily have been marketed in the Belgium since 1997. The twice-daily tablet consists of cetirizine dihydrochloride 5 mg in an immediate-release coating and pseudoephedrine hydrochloride 120 mg, of which 60 mg is in an immediate-release coating and 60 mg is in a barrier-protected core. To reveal the pharmacokinetic characters of two components at the combinatorial condition and direct the reasonable usage of the drug, an effective, accurate and rapid method to determine both cetirizine and pseudoephedrine in biologic fluids should be developed.

To date, no analytical methods have been reported for simultaneous determination of the two drugs in pharmaceutical preparations and in biological samples. Various analytical methods have been reported to determine them, respectively.

Many methods exit for pseudoephedrine quantification in human plasma including HPLC with ultraviolet (HPLC-UV) [1,2] and GC [3]. A liquid chromatography–tandem mass spectrometry [4,5] was reported to determine the plasma level of pseudoephedrine, but the extraction recovery was all less than 80%. For cetirizine, HPLC-UV [6] and GC [7], TLC [8] were reported. Recently, a hydrophilic interaction liquid chromatographic–tandem mass spectrometric method (HILIC-MS/MS) [9,10] and a column switching HPLC method [11] were reported to determine cetirizine in human plasma. The former achieved a lower limit of quantization (LLOQ) of 1 ng/ml, but the sample preparation was tedious, as solid phase extraction was used. The latter provided a LLOQ higher than 10 ng/ml, and the chromatographic run time for one sample was a little longer (about 23 min).

Considering the huge numbers of the sample, building a simple, sensitive, rapid and accurate method to be convenient

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doi:10.1016/j.jchromb.2006.08.026

to pharmacokinetic research was necessary. In this study, a LC–MS/MS method with selected-reaction monitoring (SRM) mode was first developed for simultaneous assay of the two drugs in human plasma samples. This assay method was simple, sensitive, stable and relatively rapid, without any tedious procedure approached the LLOQ as the literature in the following section.

2. Experimental

2.1. Reagents and materials

Pseudoephedrine hydrochloride (98% purity), cetrizine dihydrochloride (99% purity) and phenylalanine hydrochloride [internal standard (I.S.)](>98% purity) were supplied by Jiangsu Institute for Drug Control (Nanjing, China). Methanol was HPLC/Spectro grade and purchased from Merck Company (Darmstadt, Germany). Other chemicals were all of analytical grade and were used as received. Water was purified by redistillation before use.

2.2. Instrumentation

A Thermo Finnigan TSQ Quantum Ultra tandem mass spectrometer equipped with electrospray ionization (ESI) source (San Jose, CA, USA), a Finnigan surveyor LC pump and an autosampler was used for LC–MS/MS analysis. Data acquisition was performed with Xcalibur 1.1 software (Thermo-Finnigan, San Jose, CA, USA). Peak integration and calibration were carried out using LC Quan software (Thermo-Finnigan).

2.3. Chromatographic condition

A Lichrospher C_8 column (5 μm, 250 mm × 4.6 mm i.d., Hanbang Co., Huaian, China) was used for all of the chromatographic separations. A two-solvent gradient elution was performed, with a flow rate of 1.0 ml/min. The solvent A was water with 0.13% formic acid, and solvent B was methanol. The mobile phase composition started at 45% solvent A and 55% solvent B, then was being increased linearly to 70% solvent B in 2 min, and then being held for 8 min. The column was equilibrated at 45% solvent A and 55% solvent B for 2 min before the second injection was initiated. The total period for one sample was about 12 min. The column temperature was maintained at 25 °C.

Mass spectrometric analysis was performed in the positive ion mode (ESI+) and set up in the selected reaction monitoring (SRM) mode. Nitrogen was used as the sheath gas (35 psi) and the auxiliary gas (5 psi). The capillary temperature was 350 °C, and the spray voltage was 4000 V. Collision induced dissociation (CID) studies were performed and argon was used as the collision gas with a collision cell gas pressure of 1.5 mTorr. The collision energy was 18 eV. On the basis of the full-scan MS and MS/MS spectra of the drug, the most abundant fragment ion was selected and the mass spectrometer was set to monitor the transition m/z 166.04 → 147.99 for pseudoephedrine, m/z 389.07 → 200.91 for cetirizine and 152.02 → 134.06 for internal standard. The scan time for each analyte was set to 0.5 s.

2.4. Preparation of stock and sample solution

The stock solutions of pseudoephedrine and cetirizine were prepared by dissolving the accurately weighed reference compound in water to give a final concentration of 50 μg/ml separately. The solutions were then serially diluted with water to obtain standard working solutions. Stock solutions of I.S. was prepared in methanol at the concentration of 100 μg/ml and diluted to 80 ng/ml with methanol. All the solutions were then stored at 4 °C and were brought to room temperature before use.

2.5. Sample preparation

0.8 ml I.S. solution (80 ng/ml) was added to 200 μl plasma in a 1.5 ml test tube. The samples were immediately vortexed for 3 min and centrifuged at 3000 rpm (revolutions per minute) for 10 min. 0.8 ml of the supernatant layer was transferred to another clean test tube, and centrifuged at 1,6000 rpm for another 10 min. Twenty microlitres of the clean supernatant was directly injected onto the LC/MS/MS for analysis. The chromatographic eluent was diverted to waste for 1.0 min after each sample injection in order to keep the ion source as clean as possible.

2.6. Calibration and quality control samples

Calibration standard solutions were prepared by spiking blank human plasma with standard solutions to give concentrations of 1.024/1.032, 2.048/2.046, 5.120/5.160, 10.24/10.32, 51.20/25.80, 102.4/51.60, 204.8/103.2, 409.6/206.4, and 819.2/412.8 ng/ml for pseudoephedrine and cetirizine. Quality control (QC) samples, which were used both in pre-study validation and during the pharmacokinetics study, were prepared separately to give concentrations of 2.048/2.048, 102.4/51.60, and 819.2/412.8 ng/mL of pseudoephedrine and cetirizine.

2.7. Method validation

The method was validated for selectivity, accuracy, precision, recovery, calibration curve range, and stability according to the US FDA [12]. The selectivity was investigated by preparing and analyzing six individual human blank plasma samples at the LLOQ.

Linearity was assessed by analyzing pseudoephedrine (1.024–819.2 ng/ml) and cetirizine (1.032–412.8 ng/ml) in human plasma. Calibration curves were analyzed by weighted linear regression (1/y, y: concentration of the analytes) of assayed peak areas ratios versus nominal drug concentrations.

Accuracy and precision were assessed by determining QC samples at three concentration levels (five samples each) on three different validation days. The precisions were determined as the RSD (%), and the accuracies were expressed as percentages of the nominal concentrations. The criteria used to assess the suitability of precision and accuracy was as follows: the RSD should not exceed 15% and the accuracy should be within 85–115%.

The absolute recoveries were evaluated for both pseudoephedrine and cetirizine by comparing peak areas of the precipitated samples with peak areas of the direct injection of

the pure authentic standard solutions dissolved in the methanol at three QC levels.

The stability of the analytes in stock solution was determined by placing the stock solutions in the refrigerator (4 °C) for a week. And the stability of the analytes in human plasma under different temperature and timing conditions was evaluated at three QC levels. The long-term stability was assessed after storage of the test samples at −20 °C for five days. The thaw-freeze stability was determined after five thaw-freeze cycles (−20 to 20 °C) on consecutive days. The extraction storage stability was assessed by placing QC samples being precipitated at −20 °C for five days and analyzed. The results were compared with those QC samples freshly prepared, and the percentage concentration derivation was calculated.

2.8. Pharmacokinetic study

The validated LC–MS/MS assay was applied to Phase-I pharmaceutics study in five female and five male healthy adult volunteers received oral administration. The study was approved by the Ethics Committee. All the volunteers who were selected after completing a thorough medical, biochemical and physical examination gave informed consent after they were explained the aims and risks of the study.

The pharmacokinetic experiment was containing two parts two-way crossover designs containing oral administration of single-dosage and different fasted and fed states.

Crossover 1, after an overnight fast (10 h), the volunteers took the assigned tablet orally with 200 ml of water. The volunteers were treated with an oral dose of one tablet, and with an oral dose of two tablets after 1 week washout period. Regular standardized low-fat meals were not provided until 4 h after dose administration; water intake was allowed after 2 h.

Crossover 2, the effect of a standard meal on the pharmacokinetics of one tablet was evaluated. After an overnight fast (10 h), for fed treatment, volunteers took one tablet after a standard breakfast whereas for the fasting treatment, volunteers took one tablet without breakfast and continued to fast for 4 h more. There was a 7-day drug-free period between the two treatments.

Following the drug administration, venous blood samples (5 ml) were collected into heparinized tubes according to the following schedule: immediately before administration and 0.17, 0.25, 0.33, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 8.0, 10.0, 13.0, 24.0, and 34.0 h after dosing. Blood samples were centrifuged at 1500 g for 10 min to obtain the plasma. The plasma samples were labeled and kept frozen at −30 ° until analysis.

Pharmacokinetic parameters were determined from the plasma concentration-time data. An analysis of log-transformed and non-transformed PK parameters was done using BAPP2 procedures.

3. Results and discussion

3.1. MS conditions selection

For the optimization of MS conditions, each of the analytes and I.S. was directly introduced into MS detector using

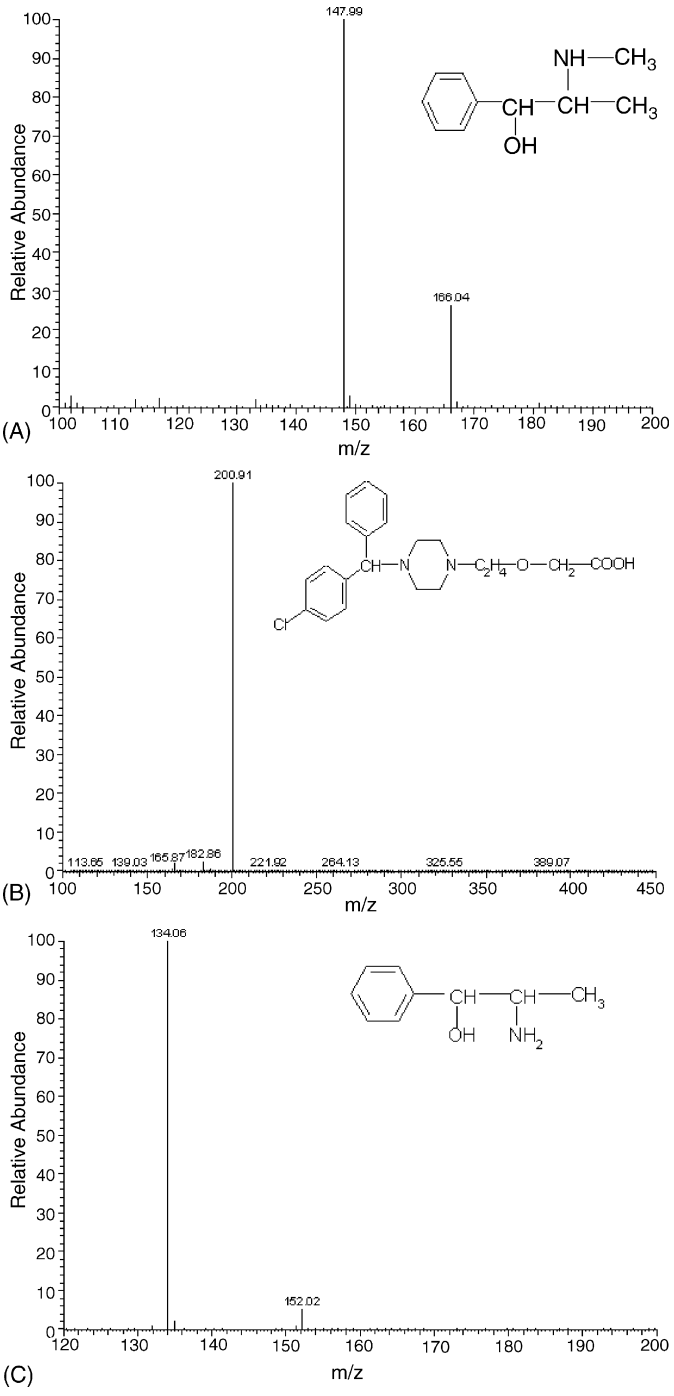

Fig. 1. Mass spectra and structures of the two analytes and I.S. (A) pseudoephedrine mother ion: *m/z* 166.04; daughter ion: *m/z* 134.06. (B) Cetirizine mother ion: *m/z* 389.07; daughter ion: *m/z* 200.91. (C) I.S. mother ion: *m/z* 152.02; daughter ion: *m/z* 134.06.

ESI ionization and parameters such as sheath gas, the auxiliary gas, collision induced dissociation and the collision energy were investigated to increase the detection sensitivity of pseudoephedrine, cetirizine and I.S.

Because three of the compounds were of weak base, dominantly protonated molecules $[M+H]^+$ in full scan spectra at *m/z* 166.04, *m/z* 389.07 and *m/z* 152.02 were observed. Fig. 1 displays the structures and the mass spectrum of $[M+H]^+$ ions from

three compounds. Pseudoephedrine gave an intense product ion at *m/z* 147.99, formed by losing [−OH]. Cetirizine showed an intense ion at *m/z* 200.91 corresponding to the loss of [CH (Ph)Cl] group from $[M+H]^+$ ion. Phenylalanine (I.S.) showed a major fragment ion at *m/z* 134.06 corresponding to a neutral loss of water. These major fragment ions at *m/z* 147.99, *m/z* 200.91 and *m/z* 134.06 were chosen in the SRM acquisition for pseudoephedrine, cetirizine and I.S., respectively.

3.2. Chromatographic conditions optimization

It is critical to optimize chromatographic conditions to obtain good selectivity, high sensitivity, quick speed and symmetrical peak shape. Various compositions of mobile phase were therefore, tried ae first by varying the percentages of organic solvent. It was found that under isocratic elution mode, an inverted peak always existed just before that of pseudoephedrine and that the peak made the baseline separation difficult, therefore, accurate measurement of pseudoephedrine area is impossible. To find the cause and avoid the overlaying of the two peaks, different volumes of methanol (as protein precipitant) or proper amounts of formic acid were added to black plasma to match its composition to the mobile phase,in a better manner but little improvement was observed. The disappearance of the inverted peak when water stead of blank plasma was used as analyte suggests that analytes coming from plasma contain certain kinds of compounds that reduce MS response. Various gradient conditions were then experimented and the best combination was selected to get baseline separation and symmetry peak shape.

3.3. Preparation of plasma samples

de Jager et al. [13] reported that protein precipitation for cetirizine can get a more satisified recovery than that of liquid–liquid extraction and that the latter was complex and time-consuming [1,14]. In this study, a one-step protein precipitation procedure was adopted to simplify the sample preparation and the treatment could provide LLOQ lower than 1.0 ng/ml for both of the analytes. The selected protein precipitant was methanol because of satisfactory efficiency in precipitating and less ion suppression compared with those observed with acetonitrile, ethanol and acetone.

3.4. Method validation

3.4.1. Selectivity

The LC/MS/MS method has high specificity because only a fragment ion derived from the $[M+H]^+$ ion of the analytes of interest was monitored. The selectivity toward endogenous plasma matrix components was assessed in six different batches of human plasma samples by analyzing blanks and spiked samples at LLOQ levels. Endogenous peaks at the retention time of the analytes were not observed for any of the plasma batches evaluated. This indicated no significant direct interference in the SRM channel for the analytes at the expected retention time. Fig. 2 shows the typical chromatograms of a blank plasma sample, a blank plasma sample spiked with pseudoephedrine, cetirizine at the LLOQ and I.S., and a plasma sample from a healthy volunteer 2 h after an oral administration. Typical retention times for pseudoephedrine, cetirizine and I.S. were 3.2, 7.7, and 3.2 min, respectively. The total run time was about 12 min.

3.4.2. Matrix effects

Matuszewski et al. [15] reported that matrix components, which co-elute with analytes, may adversely affect the reproducibility of analyte ionization in a mass spectrometer's electrospray source. For pseudoephedrine, cetirizine and I.S., the mean peak area from the six different samples sources had relative error of 2.3–5.6%, 3.6–4.8% and 3.2–5.1%, respectively compared with those from standard solutions. It was indicated that no endogenous substances significantly influenced the ionization of the analytes.

3.4.3. Linearity of calibration curves and lower limits of quantification

The typical calibration curves for cetirizine and pseudoephedrine were $Y = 139.23X - 3.3452$ (correlation coefficient, $r = 0.9996$) and $Y = 107.22\ X - 1.4467$ ($r = 0.9998$), respectively, *Y*: concentration in ng/ml, *X*: ratios of cetirizine/pseudoephedrine peak area to that of I.S.).

The limit of quantization was 1.0 ng/ml ($n = 6$) for pseudoephedrine and 1.0 ng/ml for cetirizine, and an acceptable accuracy of ±15% and a precision below 15% were obtained. This level was selected with respect to expected concentrations of the samples from the pharmacokinetic study.

3.4.4. Precision and accuracy

Intra- and inter precision was assessed from the results of QCs. The mean values and RSD for QCs at three concentration levels were calculated over three validation days by using a one-way analysis of variance (ANOVA). The intra- and inter-batch precision and accuracy data for cetirizine and pseudoephedrine are summarized in Table 1. Intra-batch precision ranged between 2.46–6.65% and 2.5–5.99% and the inter-batch precision were between 3.78–9.70% and 3.90–7.66% separately.

3.4.5. Recovery and stability

Table 2 shows the recovery (extraction efficiency) of pseudoephedrine and cetirizine from human plasma following

Table 1
Intra-, inter-batch precision and accuracy for analytes the in human plasma (in pre-study validation, $n = 3$ days, five replicates per day)

Concentration (ng/ml)		RSD (%)		Relative error (%)
Added	Found	Intra-batch	Inter-batch	
Pseudoephedrine				
2.048	2.000	6.65	9.70	2.34
102.4	107.4	4.67	5.39	4.88
819.2	811.8	2.46	3.78	0.90
Cetrizine				
2.046	2.031	5.99	7.66	0.73
51.60	50.53	3.60	5.48	2.07
412.8	395.2	2.46	3.90	4.62

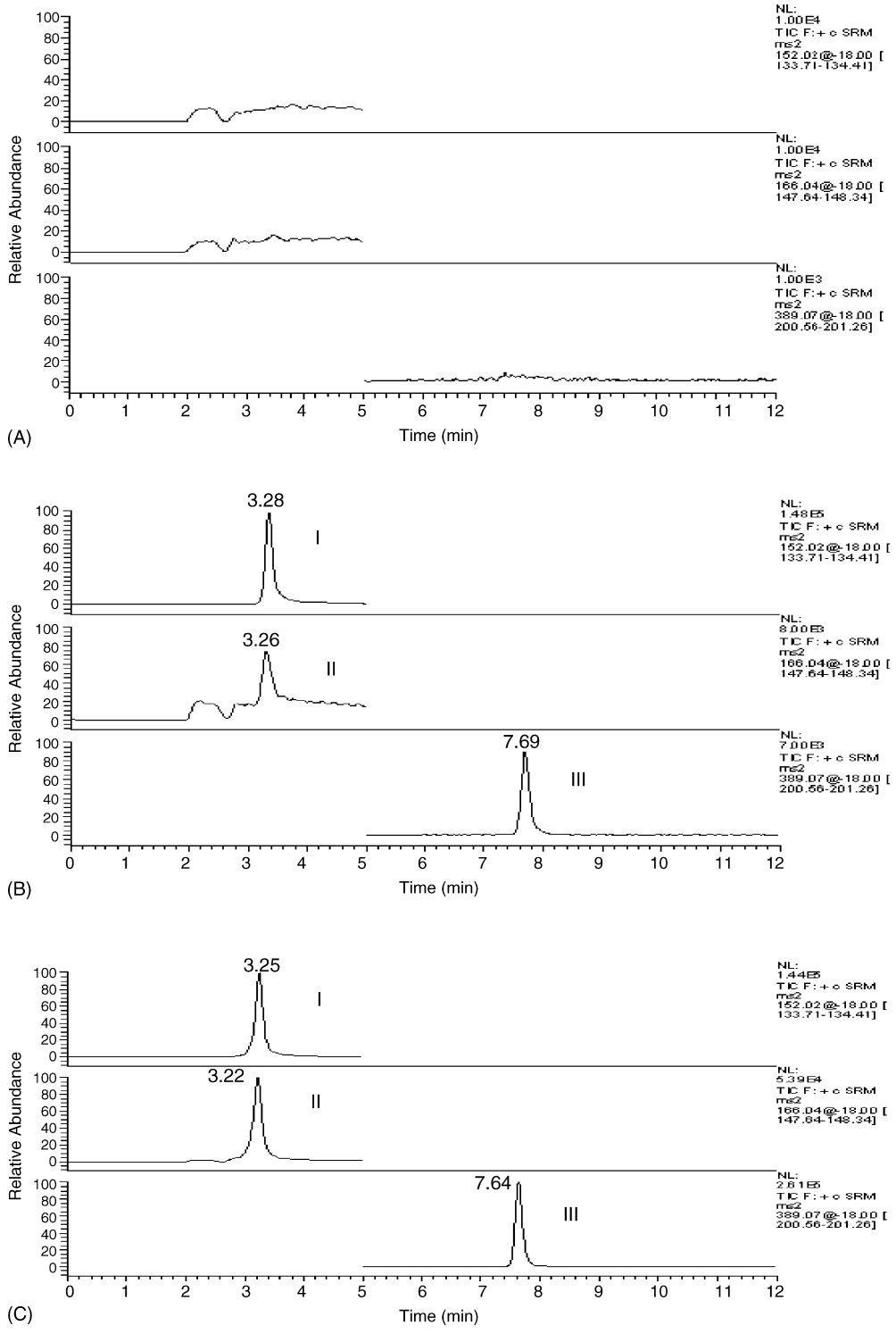

Fig. 2. Chromatograms of (A) blank plasma sample;(B) plasma sample spiked with pseudoephedrine (1 ng/ml), cetirizine (1 ng/ml) and I.S. (64 ng/ml); (C) volunteer plasma sample 0.5 h after oral dose of one tablet. I.S. (I), pseudoephedrine (II), and cetirizine (III) in human plasma samples.

methanol precipitation. The absolute recoveries were shown to be consistent, precise and reproducible.

The results of stability experiments showed that significant degradation occurred at −20 °C for 5 days and after five freeze-thaw cycles. The accuracy values at three QC levels of pseudoephedrine and cetirizine were within ±15%. The stock solutions in water were stable at 4 °C for a week. The methanol solution of I.S. (80 ng/ml) was proved stable at room temperature for more than 12 h and at 4 °C for a week. There was no significant degradation under the conditions described in this study and these results indicated that analytes were stable under routine laboratory conditions.

Table 2
Recovery data for analytes in human plasma ($n = 5$)

Added concentration (ng/ml)	Recovery[a] (mean ± SD, %)	RSD[b] (%)
Pseudoephedrine		
2.048	96.24 ± 8.26	8.59
102.4	91.95 ± 4.11	4.48
819.2	95.81 ± 2.05	2.13
Cetrizine		
2.046	92.87 ± 7.11	7.66
51.60	93.78 ± 4.69	5.01
412.8	90.32 ± 1.91	2.11

[a] RSD = relative standard deviation.
[b] Mean relative error = (overall mean assayed concentration − added concentration)/added concentration ×100.

3.4.6. Application of the method to a pharmacokinetic study in healthy volunteers

The proposed method was applied to the determination of plasma concentrations and the pharmacokinetic parameters of the combination table were first reported in Chinese people.

The mean plasma concentration-time curve of oral administration is shown in Fig. 3A The comparison of corresponding pharmacokinetic parameters is listed in Table 3. The AUC, C_{max} are showing linear between one tablet and two tablets; T_{max} and $t_{1/2}$ were not statistically different for the two drugs. And the calculated pharmacokinetic parameters of pseudoephedrine and cetrizine are in agreement with literature separately [13,16].

Fig. 3B shows the profiles of the mean concentration of pseudoephedrine and cetrizine in fed and fasted states. The collected pharmacokinetic parameters are listed in Table 4. The results of our study demonstrated that the presence of standard meal had no effect on absorption of pseudoephedrine, which was the same as the former report [16,17]. The mean pharmacokinetic parameters of cetirizine had no significant statistical differences except for C_{max} and T_{max} which reduced 30% and delayed 2.4 h in the fed state. That means standard meal slightly reduce the rate but not the extent of cetirizine absorption. These results agree with previously published reports [18,19], and the present method is an excellent analytical option for quantifying pseudoephedrine and cetrizine in human plasma.

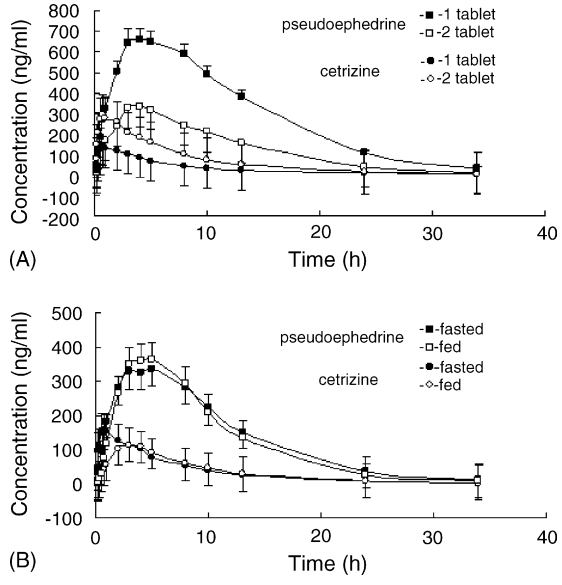

Fig. 3. Mean plasma concentration-time curve of pseudoephedrine and cetrizine at different conditions of administration. ($n = 10$).

Table 3
Comparison of mean pharmacokinetic parameters of single- and multiple- oral dosage administration ($n = 10$)

Parameter	One tablet		Two tablets	
	Mean	SD	Mean	SD
Pseudoephedrine				
AUC (h ng/ml)	4441.74	755.12	10404.74	1634.49
C_{max} (ng/ml)	350.36	57.33	701.83	57.30
T_{max} (h)	4.0	0.8	4.2	0.8
$t_{1/2}$ (h)	5.37	0.52	6.11	0.92
Cetrizine				
AUC (h ng/ml)	1099.77	255.49	2372.24	625.73
C_{max} (ng/ml)	167.06	47.17	323.20	77.16
T_{max} (h)	0.7	0.5	0.7	0.5
$t_{1/2}$ (h)	6.76	1.82	6.17	1.39

Table 4
Comparison of mean pharmacokinetic parameters under the fasted and fed conditions ($n = 10$)

Parameter	Fasted		Fed	
	Mean	SD	Mean	SD
Pseudoephedrine				
AUC (h ng/ml)	4637.30	570.01	4384.13	488.69
C_{max} (ng/ml)	352.99	42.98	378.74	42.18
T_{max} (h)	4.2	0.8	4.2	0.8
$t_{1/2}$ (h)	5.44	0.58	4.94	0.61
Cetrizine				
AUC (h ng/ml)	1232.47	194.18	1153.21	207.26
C_{max} (ng/ml)	173.70	35.47	122.97	15.15
T_{max} (h)	0.6	0.2	3.0	1.3
$t_{1/2}$ (h)	5.88	0.79	5.84	0.73

4. Conclusions

A LC–MS/MS method to determine simultaneously the concentration of pseudoephedrine and cetrizine in human plasma was developed. The presented was then successfully applied for the evaluation of Phase-I pharmacokinetic study and the collected parameters were first reported in healthy Chinese people. The result proved that the method is sensitive, highly selective and stable.

References

[1] J. Macek, P. Ptáček, J. Klima, J. Chromatogr. B 766 (2002) 289.
[2] R. Herraez-Hernandez, P. Campins-Falco, Analyst 124 (1999) 239.
[3] M. Nieder, H. Jaeger, J. Chromatogr. 424 (1988) 73.
[4] J.G. Su, G.J. Wang, W. Wang, S. Zhao, Y. Gu, J.W. Zhang, M.W. Huang, F. Shao, H. Li, Q. Zhang, H.T. Xie, J. Pharm. Biomed. Anal. 39 (2005) 217.
[5] X.Y. Chen, Y. Zhang, D.F. Zhong, Biomed. Chromatogr. 18 (2004) 248.
[6] M.N. Muscará, G. De Nucci, Int. J. Clin. Pharmacol. Ther. 33 (1995) 27.

[7] E. Baltes, R. Coupez, L. Brouwers, J. Gobert, J. Chromatogr. 430 (1988) 149.
[8] K.K. Pandya, R.A. Bangaru, T.P. Gandhi, I.A. modi, R.I. modi, B.K. Chakrarthy, J. Pharm. Pharmacol. 48 (1996) 510.
[9] Q. Song, H. Junga, Y. Tang, A.C. Li, T. Addison, M. McCort-Tipton, B. Beato, W. Naidong, J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 814 (2005) 105.
[10] A.C. Li, H. Junga, W.Z. Shou, M.S. Bryant, X.Y. Jiang, W. Naidong, Rapid Commun. Mass Spectrom. 18 (2004) 2343.
[11] Chong-Kook Kim, Kyu Jeong Yeon, Eunmi Ban, Myung-Ja Hyun, Jin-Ki Kim, Mee-Kyung Kim, Su-Eon Jin, Jeong-Sook Park, J. Pharm. Biomed. Anal. 37 (2005) 603.
[12] Guidance for Industry, Bioanalytical Method Validation, US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), May 2001.
[13] A.D. de Jager, H.K.L. Hundt, K.J. Swart, A.F. Hundt, J. Els, J. Chromatogr. B 773 (2002) 113.
[14] H.Y. Yang, X.Y. Chen, H.Y. Xu, Y. Hou, D.F. Zhong, J. Shenyang Pharma. Univ. 18 (2001) 116.
[15] B.K. Matuszewski, M.L. Constanzer, C.M. Chavez-Eng, Anal. Chem. 70 (1998) 882.
[16] M.T. Wecker, D.A. Graves, L.P. Amsel, O.N. Hinsvark, K.S. Rotenberg, J. Pharm. Sci. 76 (1987) 29.
[17] S.T. Chao, D. Prather, D. Pinson, B. Pruitt, M. Knowles, V. Place, J. Pharm. Sci. 80 (1991) 432.
[18] Baltes E.L., Pharmacokinetic of Certizine, Allergy Forum, London, June 27, 1988. pp. 7–9.
[19] E. Baltes, L. Brouwers, J. Gobert, Pharmacokinetic of certizine in animal srecies and in man, in: Symposium Proceedings, Certizine: A New Era in Allergotherapy, Brussels, March 27–30, 1987, pp. 45–50.