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Sensitive bioassay for the simultaneous determination of pseudoephedrine and cetirizine in human plasma by liquid-chromatography–ion trap spectrometry

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Abstract

A liquid chromatography–ion trap mass spectrometry coupled with electrospray ionization (HPLC–ESI–ion trap mass spectrometry) method for simultaneous determination of cetirizine and pseudoephedrine in human plasma is presented. Chromatographic separation was performed on a Hypurity C18 column (Thermo Hypersil-Keystone 2.1 mm × 150 mm, 5 μm, USA), The mobile phase was composed of 65% methanol and 35% water (contained 0.1% formic acid, 10 mM ammonium formate), which was run with a flow-rate of 0.2 ml/min at 40 °C. Quantitation was achieved by monitoring the product ions at *m/z* 166 → *m/z* 148 (pseudoephedrine), *m/z* 389.9 → *m/z* 201.1 (cetirizine), *m/z* 264 → *m/z* 246 (tramadol, IS). The calibration curve of pseudoephedrine and cetirizine was established with standard solutions. The limit of detection for pseudoephedrine and cetirizine each was 5 ng/ml. This simplified analytical method is sensitive, specific and accurate enough for simultaneous determination of pseudoephedrine and cetirizine in human plasma and is successfully applied to the pharmacokinetic study of pseudoephedrine and cetirizine.

Keywords: HPLC–ESI–ion trap mass spectrometry; Human plasma; Pseudoephedrine; Cetirizine; Pharmacokinetics

1. Introduction

Cetirizine (CET) is the carboxylated metabolite of hydroxyzine, and it has high specific affinity for histamine H_1 receptors [1]. Pseudoephedrine (PSE) is a sympathomimetic drug that acts directly on alpha-adrenergic receptors [2]. The combination of pseudoephedrine and cetirizine, with a long acting antihistaminic and slow release pseudoephedrine, a sympathomimetic decongestant widely used in the comprehensive management of allergic rhinitis [3].

Several gas chromatographic [4,5] and high performance liquid chromatographic (HPLC) [6–10] methods have been described for determination of pseudoephedrine in plasma. The limit of quantitation (LOQ) of HPLC assays ranges from 10 to 50 ng/ml. Sample preparation is performed by liquid extraction [6,10], solid-phase extraction [7] or column switching [8,9]. Recently, several HPLC [11] and liquid chromatography-mass spectrum (LC/MS) [1,12,13] methods have been reported to detect cetirizine in plasma. Although some of them were also sensitive and simple, there was no method to simultaneously determine pseudoephedrine and cetirizine in human plasma. To find a convenient, rapid, sensitive and selective method for the determination of pseudoephedrine and cetirizine in human plasma, we developed a method to simultaneously quantitate pseudoephedrine and cetirizine based on a liquid chromatography–ion trap mass spectrometry coupled with electrospray ionization (HPLC/ESI/ion trap mass spectrum).

2. Experimental

2.1. Drugs and chemicals

Pseudoephedrine (purity, 99.8%) and cetirizine (purity, 99.7%) were from HaoSheng Pharmaceutical Ltd. Company, JiangSu, China. Tramadol (internal standard, IS) (purity, 99.7%)

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was purchased from Sigma company (USA). Acetonitrile and methanol purchased from Dikma Comp (Guangzhou, China) were of HPLC grades, all other reagents were of analytical grades.

2.2. Biosamples

Blank human plasma samples were supplied from our local blood bank. Clinical blood samples were collected from 18 adult health volunteers.

2.3. Preparation of standard solutions

Stock standard solutions of pseudoephedrine, cetirizine, and IS were prepared in methanol at a concentration of 1.00, 1.00, and 0.9 mg/ml, respectively. These were further diluted in methanol to give appropriate working solutions used to prepare the calibration solutions. Standard curves were prepared in human plasma to yield final concentrations of 1000.0, 500.0, 200.0, 100.0, 50.0, 20.0, 10.0 and 5.0 ng/ml for pseudoephedrine and cetirizine. A 9 μg/ml tramadol work solution was prepared in methanol. All solutions were stored at 2–8 °C.

2.4. Sample preparation

Six hundred microlitres tramadol (internal standard) work solution was added to the sample of 200 μl plasma in Eppendorf tubes, the tubes were vortexed for 2 min, and centrifuged in 15,493 g/min for 10 min. Four hundred microlitres of the upper organic layer was transferred to autosampler vials. Ten microlitres was injected into the system.

2.5. Liquid chromatography–mass spectrometry

2.5.1. Equipment and chromatographic conditions

The LC–MS/MS system consisted of a Thermofinnigan Surveyor LC system (America) equipped with an antosampler. Compounds were screened for, identified, and quantified in plasma, using a Thermofinnigan LCQ Deca XP trap ion mass spectrometer, and the Thermofinnigan Xcalibur data system. Chromatographic separations were carried out by a 5 μm particle size Hypurity C18 column (150 × 2.1 mm i.d., ThermoHypersil-Keystone, America) whose temperature was maintained at 40 °C. Samples were eluted with a mobile phase (65% methanol and 35% water which contained 0.1% formic acid, 10 mM ammonium formate) delivered at a flow-rate of 0.2 ml/min. The entire flow was directed into the source without splitting. During use the mobile phase was degassed by an integrated Surveyor series degasser. In order to optimize the MS–MS parameters, infusion experiments were carried out with a 250 μl syringe connected to a pump with flow-rate of 3 μl/min.

2.5.2. Mass spectrometry conditions

The ionization technique used was electrospray ionization (ESI) in the positive-ion mode for both compounds. The spray needle was set at a potential of 5 kV. The heated capillary was set at 300 °C. Nitrogen was used as drying and nebulising gas. The shealf gas flow-rate of nitrogen was set at 25 (arbitrary unit), Aux gas flow-rate of nitrogen was set at 5 arb. The tube lens offset was set at 40 V and the electron multiplier voltage set at 950 V peak-to-peak. Helium was used in the trap as damping and collision gas. The instrument was set to acquire three microscans to detect cetirizine, pseudoephedrine and internal standard, respectively. And ion injection time into the trap was optimized by using the integrated automatic gain-control software.

2.5.3. MS conditions for identification

The detection of pseudoephedrine, cetirizine, and tramadol was performed by LC–MS–MS in full MS–MS scan mode (*m/z* 100–400). Full scan MS–MS spectra were produced by collision-induced dissociation (CID) of each molecular ion, using a normalized collision energy of 30% for pseudoephedrine, 32% for cetirizine, and 36% for tramadol, respectively. One analysis run, generating fragment ions of the molecular ion through CID, were carried out at *m/z* 148, *m/z* 201, and *m/z* 246, respectively, corresponding to the daughter ions of pseudoephedrine, cetirizine, and tramadol, respectively.

The reference MS–MS spectra of compounds of interest were previously collected individually using direct injection by using a normalized collision energy of 30% for pseudoephedrine and 32% for cetirizine.

2.5.4. MS conditions for quantitation

For quantitation, the selected reaction monitoring (SRM) precursor-product ion transitions *m/z* 166 → *m/z* 148 (pseudoephedrine), *m/z* 389.9 → *m/z* 201.1 (cetirizine), *m/z* 264 → *m/z* 246 (tramadol, IS), respectively.

2.5.5. Calculations

The calibration curves were calculated by weighted least-squares linear regression analysis ($1/x$) of the concentrations of the analyte versus the peak areas ratio of the target ion of pseudoephedrine (*m/z* 148) and cetirizine (*m/z* 201.1) to that of the IS (*m/z* 246). Concentrations of unknown samples were determined by applying the linear regression equation of the standard curve to the unknown sample's peak area ratio.

2.6. Method validation

2.6.1. Quality control

Quality controls were prepared from a pool of blank human plasma spiked with three different amounts of pseudoephedrine and cetirizine corresponding to the low, medium and high concentrations given in Table 1. Plasma aliquots were stored at −20 °C until assayed.

2.6.2. Precision and accuracy

Precision is measured using four concentrations that are LLOQ and three QC samples at 10, 50, 1000 ng/ml. Five determinations per concentration are included. Precision is reported as relative standard deviation (%R.S.D.) of the estimated concentrations and accuracy (bias) expressed as [100 − (mean calculated concentration/spiked concentration) × 100]. The within-run precision is described by the variation between

Table 1
Intra-day ($n = 5$) and inter-day ($n = 15$) precision, accuracy of the LC–MS–MS (SRM mode) assay for cetirizine (CET) and pseudoephedrine (PSE)

Analyte	Spiked low medium high (ng/ml)	Mean measured		R.S.D.[a] (%)		Bias[b] (%)	
		Intra-day	Inter-day	Intra-day	Inter-day	Intra-day	Inter-day
PSE	5	4.4	4.3	5.16	7.83	12	14
	10	11.2	11.2	10.58	12.39	12.5	12.2
	50	52.7	52.5	10.99	12.7	5.1	5.1
	1000	976.7	969.4	9.00	8.38	−2.3	−3.1
CET	5	4.5	4.1	6.12	10.65	10	18
	10	11.3	11.2	12.51	13.21	13.6	12.5
	50	49.9	52.13	8.12	8.48	−0.1	4.3
	1000	978.7	1004.7	9.23	8.98	−2.1	0.5

[a] Relative standard deviation.
[b] Expressed as [100 − (mean calculated concentration/spiked concentration) × 100].

these five mean test results obtained by the method during a single analytical run. The between-run precision is measured by the variation between fifteen mean test values obtained by the method during three analytical runs.

2.6.3. Recoveries

Absolute recoveries from human plasma were evaluated at low and high concentrations levels ($n = 5$). The samples were extracted without IS, according to the procedure described above. Ten microlitres of IS solution were added to the organic phase, and evaporated to dryness. The residue was dissolved in 200 μl of mobile phase prior to analysis. As controls ($n = 5$), pseudoephedrine and cetirizine solutions in mobile phase at the low and high levels to which 10 μl of IS solution were added were gently evaporated. The residues were then dissolved in 200 μl of mobile phase and analyzed. Recoveries were calculated by comparing peak areas of controls to those of spiked plasma samples.

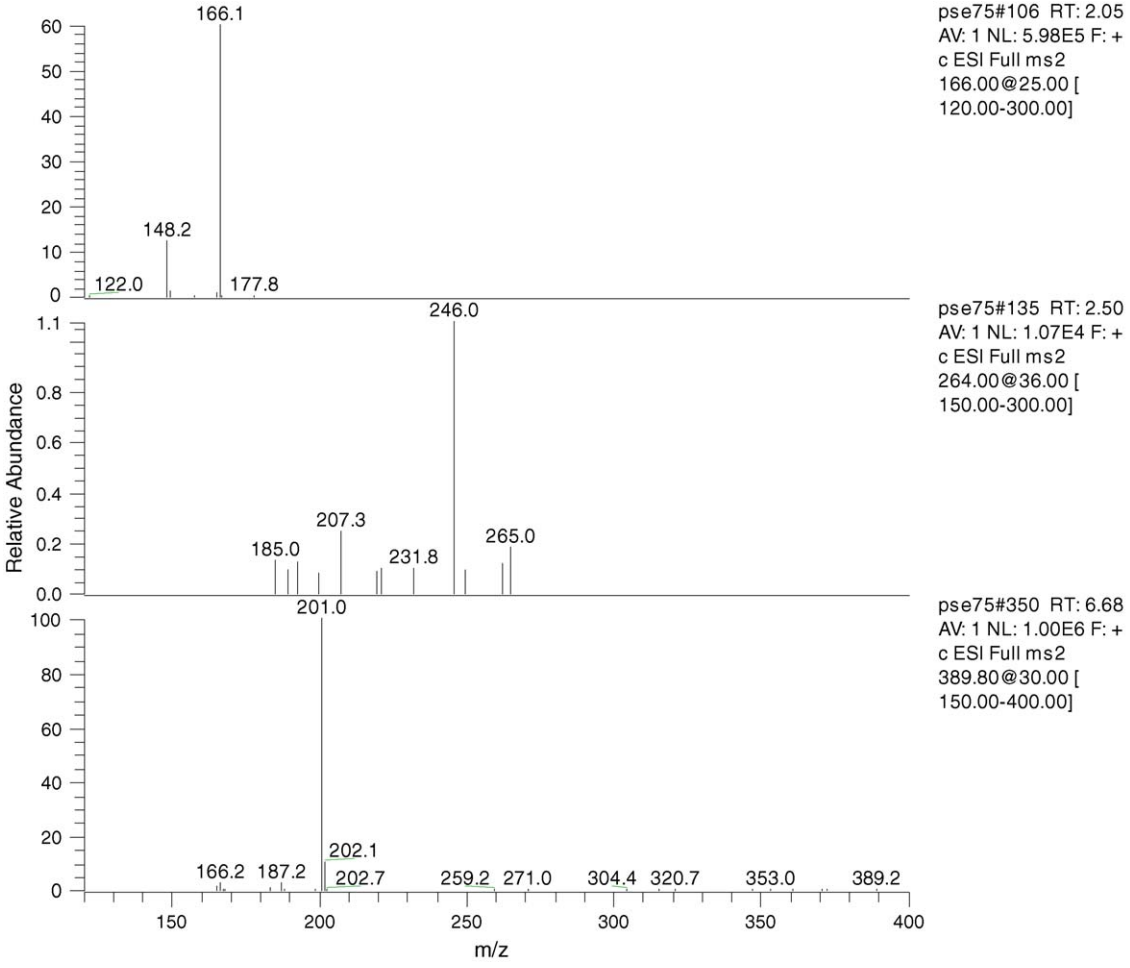


Fig. 1. LC–ESI–MS–MS spectra of pseudoephedrine,cetirizine and IS.

2.6.4. Ion suppression study

A 1 μg/ml cetirizine or pseudoephedrine was directed into the ESI source through syringe pump, while at the same time the blank plasma sample treated with acetonitrile was injected into the system through autosampler. The results showed the peak area of cetirizine or pseudoephedrine was little affected by the matrix. So the ion suppression effect is not obvious in this method.

2.6.5. Clinical application

We have employed this method to determine human plasma concentration of cetirizine and pseudoephedrine in healthy volunteers administered with these two compounds to test whether it was practicable. Thirty healthy subjects who gave written informed consent took part in this study. After the routine tests of blood, urine and stool, cardiogram, liver and kidney function examinations were made, any cardiac diseases, liver or kidney abnormalities and other diseases were exclusion criteria. This study was approved by the ethical Committee of Hunan Medical University (Changsha, China). After an overnight fast, the subjects were divided into three group, a group was given one pill of cetirizine and pseudoephedrine complex prescription capsule (a pill contained of 5 mg cetirizine and 120 mg pseudoephedrine); a group was given two pills; the third group was given three pills. Blood samples (5 ml) were taken before and 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 12, 24 and 36 h after drug administration. The plasma was separated by centrifugation at 1620 g/min for 10 min, and stored at −20 °C until analysis.

3. Results and discussion

3.1. LC–MS–MS analysis

We have employed ion trap MS for quantification of compounds in our methods, although it is not the first choice instrument, it can also meet the demand of quantitative analysis through method validation. And because of the condition limited, we had no choice to use the better instrument for analysis.

Under our analytical conditions, pseudoephedrine is not fully chromatographically separated from the IS, with retention times of 2.26 and 2.42 min for pseudoephedrine and IS, respectively. However, due to the high selectivity of tandem MS, complete chromatographic separation is not necessary any more. By using an isocratic elution of compounds, total run-time was shorter than 7 min/sample. For application to pharmacokinetic studies, the single-step extraction procedure combined to a short chromatographic run-time could be considered as a major advantage.

Fig. 1 shows the full MS–MS spectra of cetirizine, pseudoephedrine, and IS. These show the abundant molecular cations ($[M+H]^+$) which were used as daughter ions for MS–MS analysis. We preferred the ESI source over the atmospheric pressure chemical ionization (APCI) source, since the latter resulted in bad ionization of the compounds of interest under our experimental conditions. Similarly, positive-ion mode was chosen in order to obtain the most intense signal of the molecular ion.

Quantitation of cetirizine and pseudoephedrine was performed in the SRM mode using the molecular ions of both cetirizine, pseudoephedrine, and IS as target ions. The most favorable transitions were selected for each drug, and product ions (*m/z* 148.1 for pseudoephedrine, *m/z* 201.1 for cetirizine, and *m/z* 246.0 for IS) were chosen by taking into account the fragment ions with the most intense signal. This approach resulted in a high sensitivity and specificity. Additionally, the high sensitivity of the method allowed us to use a small sample volume, which undoubtedly constitutes another important benefit for repeated measurements of drug concentrations in pharmacokinetics research. Fig. 2b shows a representative SRM-smoothed chromatogram from blank plasma spiked with cetirizine, pseudoephedrine, and IS.

3.2. Validation data

3.2.1. Specificity

Six different lots of blank plasma had been analyzed for specificity and there was no interference from endogenous compounds and from potentially co-administered drugs at the retention time of cetirizine and pseudoephedrin (Fig. 2a).

3.2.2. Precision and accuracy

Table 1 summarizes mean values, precision, and accuracy of intra- and inter-assay analyses. Precision and accuracy were within the ranges acceptable for bio-analytical purposes. Intra-day precision ranged from 8.38 to 12.51%, and accuracy (bias) was less than 15%. Inter-day precision did not exceed 15% over LLOQ and three quality control samples investigated. The accuracy of the technique was considered satisfactory, since between-day bias over the concentration range studied was found to be less than 15% except at LLOQ, where it was less than 20%.

3.2.3. Linearity and limit of quantitation

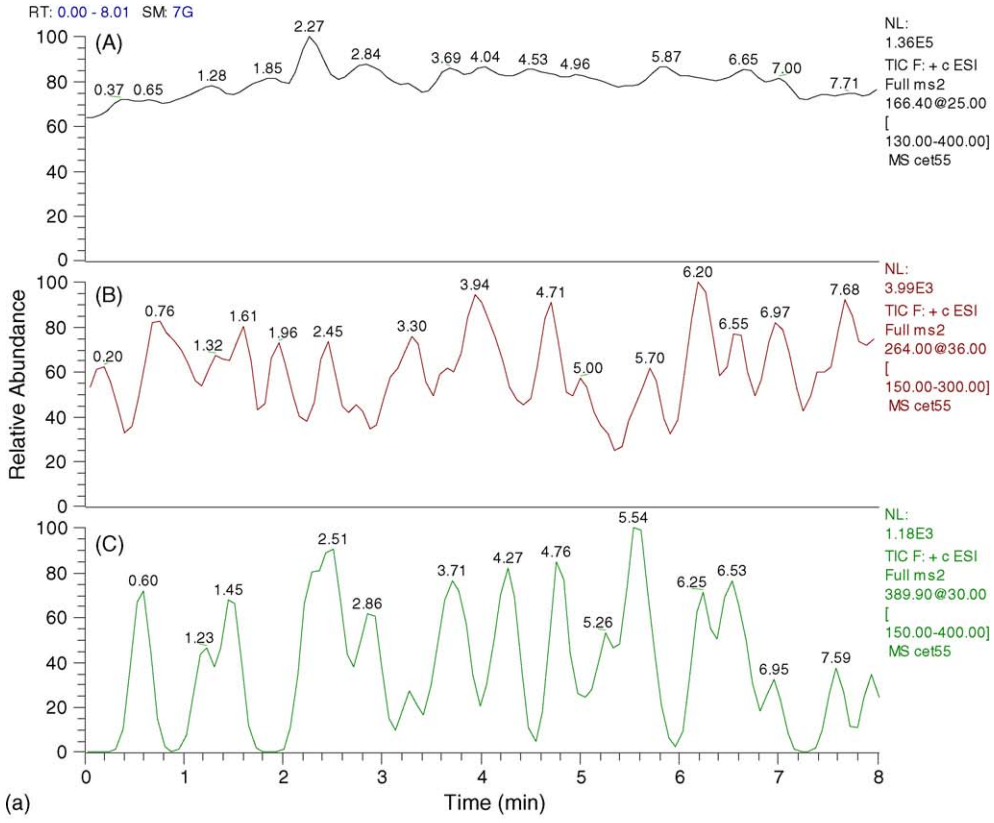
Calibration curves of pseudoephedrine and cetirizine were both found to be linear over the range of 10–1000 ng/ml with the lower limit of quantitation of 5 ng/ml. The coefficient of correlation were found to be better than 0.999 for calibration curves. The extrapolation outside the calibration curves was not permitted in our method.

3.2.4. Recoveries

The extraction efficiency of pseudoephedrine and cetirizine from human plasma was found to be more than 50% as described in Table 2, respectively. These results indicate that the single-step extraction procedure used in this assay is sufficient to ensure satisfactory extraction recovery.

Table 2
Absolute recovery of cetirizine (CET) and pseudoephedrine (PSE) ($n = 5$)

Analyte	Spiked low medium high (ng/ml)	Extraction recovery (100%)
PSE	10	89.5
	50	85.6
	1000	82.3
Cet	10	91.2
	500	86.5
	1000	85.6



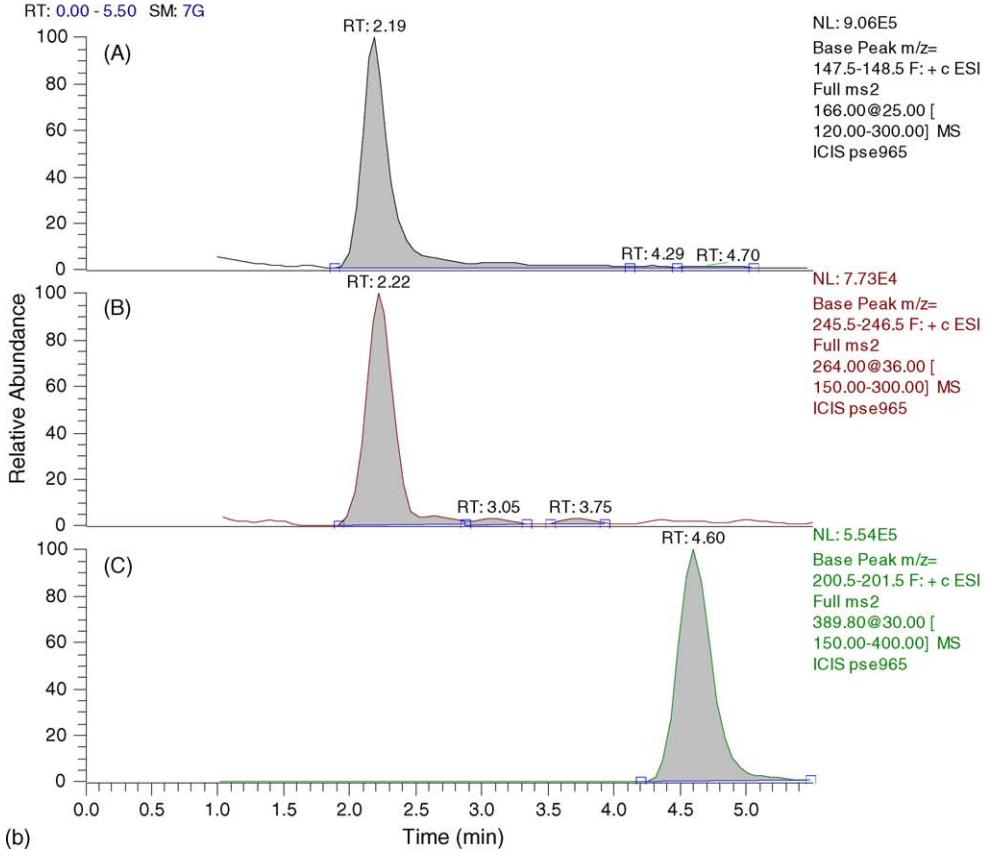


Fig. 2. LC–MS–MS scans: (a) blank plasma; (b) blank plasma with added pseudoephedrine, cetirizine and IS at the concentration of low QC. Key: (A) pseudoephedrine; (B) IS; (C) cetririzine.

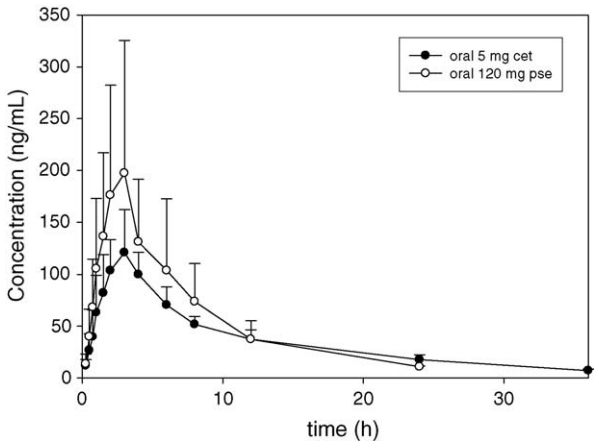


Fig. 3. Plasma concentration vs. time curves of cetirizine after oral administration of one pill of cetirizine and pseudoephedrine complex prescription capsule from 10 subjects.

3.2.5. Clinical application

Fig. 3 shows the time course of the pseudoephedrine and cetirizine plasma concentration after a single pill oral dose, respectively. The C_{max} of PSE and CET was 233 ± 111 and 138 ± 29 ng/ml at 3.1 ± 1.4 and 2.6 ± 0.9 h, respectively. $AUC_{0\rightarrow 24}$ and $AUC_{0\rightarrow 36}$ was 1459 ± 579.5 and 1285.9 ± 216.2 ng h/ml. $AUC_{0\rightarrow \infty}$ was 1462 ± 577.9 and 1290.7 ± 216.1 ng h/ml, respectively. The half-life calculated from the terminal phase was 5.2 ± 1.7 and 8.8 ± 0.9 h, respectively.

4. Conclusion

The bioanalytical methodology described in this study manuscript was specific, sensitive accurate and precise. The method employed HPLC coupled with electrospray ionization mass spectrometric detection (LC–ESI–MS). The method was involved in a simple sample preparation by protein precipitation using acetonitrile followed by isocratic chromatographic separation. The LC–ESI–MS method was capable of estimating 10 ng/ml of pseudoephedrine and cetirizine accurately in human plasma with high degree of reproducibility. The method was robust and it was successfully applied to pharmacokinetics study of pseudoephedrine and cetirizine in human plasma.

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