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Enantioselective determination of cetirizine in human plasma by normal-phase liquid chromatography–atmospheric pressure chemical ionization–tandem mass spectrometry

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ABSTRACT

A highly sensitive and enantioselective method has been developed and validated for the determination of levocetirizine [(R)-cetirizine] in human plasma by normal-phase liquid chromatography coupled to tandem mass spectrometry with an atmospheric pressure chemical ionization (APCI) interface in the positive ion mode. Enantioselective separation was achieved on a CHIRALPAK AD-H column using an isocratic mobile phase consisting of a mixture of n-hexane, ethyl alcohol, diethylamine, and acetic acid (60:40:0.1:0.1, v/v/v/v). Levocetirizine-D_8 was used as an internal standard (IS). Levocetirizine and the IS were detected by multiple-reaction monitoring (MRM). Mass transitions of analyte and IS were m/z 389.2 → 201.1 and 397.2 → 201.1, respectively. Under optimized analytical conditions, a baseline separation of two enantiomers and IS was obtained in less than 11 min. Samples were prepared by a simple two-step extraction by protein precipitation using acetonitrile followed by liquid–liquid extraction with a *n*-hexane–dichloromethane mixture (50:50, v/v). The standard curve for levocetirizine was linear ($r^2 > 0.995$) in the concentration range 0.5–300 ng/mL. Recovery was between 97.0 and 102.2% at low, medium, and high concentration. The limit of quantification (LOQ) was 0.5 ng/mL. Other method validation parameters, such as precision, accuracy, and stability, were very satisfactory. Finally, the proposed method was successfully applied to the study of enantioselective oral pharmacokinetics of levocetirizine in healthy Korean volunteers.

1. Introduction

Cetirizine, (±)-[2-(4-[(4-chlorophenyl) phenylmethyl]-1-piperazinyl) ethoxy] acetic acid (Zyrtec®), is an orally active potent third-generation histamine H_1 receptor antagonist. This antihistamine has shown significant advantages over the first-generation agents, including less sedative effect, minor anticholinergic activity, and longer duration of the antihistaminic effect [1,2]. Chemically, levocetirizine, the (R)-enantiomer of racemic cetirizine and the component of Xyzal®, is the active enantiomer (eutomer) of cetirizine. It is well known that in most of the racemic drugs, one of the enantiomers is more active than the other [3], and cetirizine follows this rule, showing an approximately 30-fold higher affinity for human histamine H_1-receptors compared with dextrocetirizine, the (S)-enantiomer of racemic cetirizine [4]. In addition, the bioavailability of levocetirizine is higher than that of dextrocetirizine and the elimination half-life time ($t_{1/2}$) is prolonged due to minimal metabolism [5]. Therefore, the recommended dosage of levocetirizine for most adults and children who are at least 12 years old is only 5 mg per day, compared with 10 mg for cetirizine.

A number of analytical methods have been used to quantify cetirizine in pharmaceutical formulations and biological fluids (serum, plasma, urine, and tears) including high performance thin layer chromatography (HPTLC) [6], gas chromatography [7], HPLC with ultraviolet detection [8–24], HPLC with fluorescence detection (pre-column derivatization) [25], HPLC with on-line liquid scintillation counting [26], and capillary electrophoresis (CE) [14,27–29]. However, these methods have a number of disadvantages; for example, they require a large volume of sample (≥1.0 mL), and have long run time, low separation resolution, and poor sensitivity. More recently, liquid chromatography–mass spectrometry (LC–MS) [30–32] and tandem mass spectrometry (LC–MS/MS) [33–43] have been used to determine cetirizine in a biological matrix because of their high sensitivity and specificity.

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Due to the polarity of cetirizine, which makes it difficult to retain on reversed-phase columns using a mobile phase with a high percentage of organic solvent, hydrophilic interaction liquid chromatography–tandem mass spectrometry (HILIC–MS/MS) methods [34,38,40] were developed and applied to quantify cetirizine in human plasma. When operated under higher organic content in the mobile phase, HILIC columns showed good retention and peak shape of polar analytes. In addition, high organic mobile phases usually improved spraying conditions, which enhanced the LC–MS/(MS) sensitivity [44].

The chiral separations of cetirizine in pharmaceuticals and biological samples (plasma, urine, and brain tissues) have been reported using capillary electrophoresis [27–29] and chiral HPLC systems [17,22–24,26,37,39]. Many different kinds of HPLC chiral stationary phases (CSPs) have been developed and used in a wide range of applications for pharmaceutical racemic mixtures [45,46]. In particular, chiral stationary phases based on the use of α_1-acid glycoprotein (AGP) [22,23,37,39] and polysaccharide types, Chiralcel OD-H [24,26] and OD-R [17], were used to separate the cetirizine enantiomers. Generally, polysaccharide-based CSPs are considered to be more effective under normal-phase conditions with alkanes/alcohols mobile phase, which may be more suitable for consistent and reliable quantification using atmospheric pressure chemical ionization–tandem mass spectrometry (APCI–MS/MS) [47].

To the best of our knowledge, no quantitative LC-APCI–MS/MS method for the stereoselective analysis of cetirizine enantiomers has been reported. In the present study, we have developed a rapid, sensitive, and enantioselective normal-phase liquid chromatography method using atmospheric pressure chemical ionization–tandem mass spectrometry (NP–LC-APCI–MS/MS) for the determination of cetirizine enantiomers in human plasma. This method could be readily adapted to clinical pharmacokinetic studies of levocetirizine.

2. Materials and methods

2.1. Chemical and reagents

Levocetirizine dihydrochloride and racemic cetirizine-D_8 dihydrochloride (Fig. 1) were purchased from TRC Inc. (Toronto Research Chemicals, North York, Canada). HPLC grade acetonitrile, *n*-hexane, dichloromethane, and ethyl alcohol were purchased from Burdick & Jackson (Muskegon, MI, USA). HPLC grade isopropanol was purchased from TEDIA (Fairfield, OH, USA) and HPLC grade acetic acid and high-purity grade diethylamine were from Sigma–Aldrich (St. Louis, MO, USA). Control plasma was obtained from BioChemed services Inc. (Winchester, VA, USA). All other reagents were of analytical grade except those for HPLC.

2.2. HPLC operation conditions

A Shimadzu (Shimadzu corporation, Tokyo, Japan) LC-20 series LC system equipped with degasser (DGU-20A_3), binary pump (LC-20AD), column oven (CTO-20A), and autosampler (SIL-20AC) in which rack changer was used to inject 10 μL aliquots of the processed samples onto a CHIRALPAK AD-H column (250 mm × 4.6 mm, 5 μm, Daicel Chemicals Industries Ltd., Tokyo, Japan) coupled with a guard column (CHIRALPAK AD, 10 mm × 4 mm, 5 μm, Daicel Chemicals Industries Ltd.), which was kept at 30 °C. The isocratic mobile phase, a mixture of *n*-hexane, ethyl alcohol, diethylamine, and acetic acid (60:40:0.1:0.1, v/v/v/v), was delivered to the column at a flow rate of 1 mL/min.

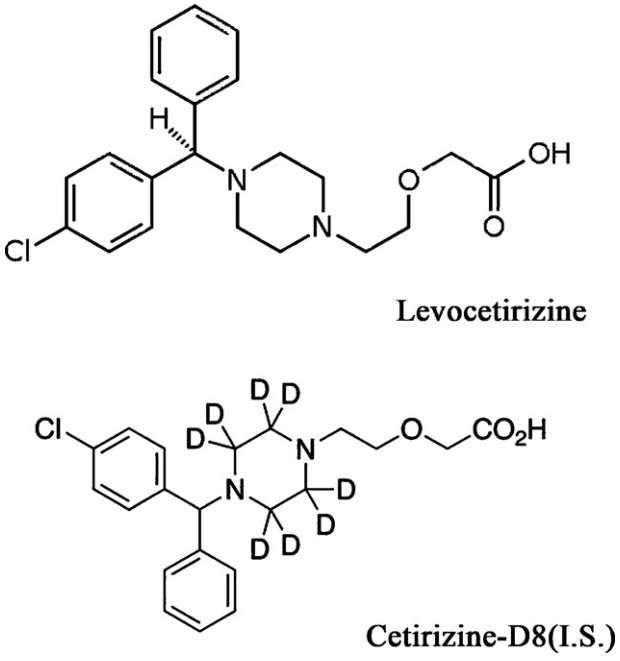

Fig. 1. Structural representations of levocetirizine and cetirizine-D_8 (IS).

2.3. Mass spectrometry operation conditions

Stereoselective quantitation was performed by MS/MS detection in positive ion mode for analytes and internal standard using a MDS SCIEX (Foster City, CA, USA) API 4000 triple quadrupole tandem mass spectrometer, equipped with a TurboIonSpray and APCI interface at 550 °C. Nitrogen was used as a nebulizer-, curtain-, auxiliary-, and collision-gas. The common parameters of mass spectrometer, including nebulizer-gas, curtain-gas, auxiliary-gas, and collision-gas, were optimized at 50, 20, 50, and 6 psig, respectively. The other operating parameters, declustering potential (DP), collision energy (CE), collision exit potential (CXP), and entrance potential (EP) were 46, 27, 16, and 10 V, respectively, for enantiomer analytes and 31, 27, 12, and 10 V for levocetirizine-D_8 (internal standard). Multiple-reaction monitoring of the precursor-product ion transitions were *m/z* 389.2 → 201.1 for levocetirizine and *m/z* 397.2 → 201.1 for internal standard. Quadrupole Q1 and Q3 were set on unit resolution. Analyst software (Version 1.4.2) was used to control the HPLC and mass spectrometer and to process the data.

2.4. Preparation of standard stock solutions and quality control (QC) samples

The stock solutions of levocetirizine (250 μg/mL) and the internal standard (500 μg/mL) were prepared in isopropanol and stored at 4 °C. A series of working standard solutions of levocetirizine in the concentration range of 5–3000 ng/mL was prepared by diluting the stock solution with isopropanol. Internal standard stock solution was diluted with isopropanol to a final concentration of 1000 ng/mL. Another set of working standard solutions of levocetirizine at 5, 50, 1000, and 3000 ng/mL was prepared from stock solution in isopropanol for preparation of samples. Standard plasma samples were prepared by spiking 30 μL of appropriate working standard solutions of the analyte to 270 μL of control pooled human plasma. Finally, standard plasma concentrations for the calibration curve were 0.5, 1, 5, 20, 100, and 300 ng/mL. Quality control samples (1, 20 and 300 ng/mL) and four different concentration levels (0.5, 5, 100, and 300 ng/mL) for determination

of precision and accuracy were prepared in the same manner. All samples were stored at −80 °C until analysis.

2.5. Sample preparation

All frozen human plasma samples were thawed at room temperature. Prior to the extraction of *in vivo* samples, 20 μL internal standard solution (1000 ng/mL) was added to 300 μL human plasma. Plasma samples were prepared by protein precipitation followed by liquid–liquid extraction (LLE). For protein precipitation, a 300-μL aliquot of acetonitrile was added to 320 μL of sample. After vortex mixing and subsequent centrifugation at 13,000 rpm for 5 min, the supernatant was transferred into a 5 mL glass tube. The procedure used for liquid-liquid extraction was as follows: 1.5 mL of hexane–dichloromethane mixtures (50:50, v/v) was added to the tube and shaken vigorously for 15 min. The samples were subsequently centrifuged at 13,000 rpm for 5 min and the supernatant was transferred into another new glass tube and evaporated in a stream of nitrogen at 40 °C (MG2200, EYELA, Tokyo, Japan). The residue was reconstituted in 200 μL of the mobile phase and a 10-μL aliquot was injected into the LC–MS/MS system.

2.6. Validation of the LC–MS/MS method

The method developed in this study was validated in terms of selectivity, linearity, limit of quantification (LOQ), precision, accuracy, recovery, and stability according to the guidelines of the Food and Drug Administration (FDA) for the validation of bioanalytical methods [48].

2.6.1. Matrix effect and selectivity

Qualitative matrix effects were assessed by post-column infusion experiments, which identified chromatographic zones most likely to experience ion suppressions [49]. The selectivity of the method was investigated by analyzing at least six different batches of blank human plasma samples. Double blank samples (processed without internal standard) and blank samples (processed with internal standard only) were prepared and tested for confirmation that endogenous components did not interfere with the LC peak region for analytes and the internal standard.

2.6.2. Calibration curve

Calibration curves were constructed by plotting the peak area ratio of levocetirizine to internal standard against the analyte concentration. The acceptance criterion for each back-calculated standard concentration was ±15% standard deviation (SD) from the nominal value except at the limit of quantification (LOQ), where it should not exceed ±20% of SD [48].

2.6.3. Precision and accuracy

The intra-day precision and accuracy was estimated by analyzing five replicates at four different concentration levels, 0.5, 5, 100, and 300 ng/mL. The inter-day precision was determined by analyzing the four different concentration levels samples on five different runs. Accuracy was expressed as the percentage of observed value to true value, and precision was expressed as the relative standard deviation (RSD). The criteria for acceptability of the data included accuracy within ±15% deviation from the nominal values and precision within ±15% RSD, except for the limit of quantification (LOQ) [48].

2.6.4. Recovery

The recovery of analyte was determined at three different concentrations (1, 20, and 300 ng/mL; $n = 3$) by comparing the area ratio with analyte and internal standard determined for the samples spiked with levocetirizine before extraction, with the area of samples spiked post-extraction with levocetirizine at the same concentration.

2.6.5. Stability experiments

The stability of levocetirizine in human plasma through three freeze-thaw cycles was assessed using two different stability control samples (1 and 300 ng/mL). The aliquots of samples, which were stored at −80 ± 5 °C, were thawed completely at room temperature, and then the samples were refrozen in the freezer. After three freeze–thaw cycles, these samples were analyzed along with freshly thawed QC samples to see if there was any variation due to thawing of the samples. Short-term stability of the analyte in human plasma on working bench over 6 h at ambient temperature (25 °C) was determined for two different stability control samples. Long-term stability of the analyte in human plasma was also assessed by analyzing two different stability control samples kept at −80 ± 5 °C for 112 days. The autosampler stability of levocetirizine was determined by injecting replicate preparations of processed samples at two concentrations (0.5 and 300 ng/mL) into the autosampler at 4 °C for up to 23 h after the initial injection. The all samples were processed using the procedure described in the sample preparation section. Samples were considered to be stable if assay values were within the acceptable limits of accuracy (±15% deviation) and precision (±15% RSD). All stability samples were estimated by analyzing three replicates.

2.7. Preliminary pharmacokinetic study

Ten healthy volunteers participated in this clinical study. A single dose of levocetirizine (2.5 mg) was administered to a test group and 5 mg racemic cetirizine was administered to a reference group. The study was performed by an open, randomized, two-way crossover design. The blood time points were 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, and 36 h post-dosing. The wash-out period of this study was a week. Plasma was harvested by centrifuging the blood using a Hanil Science centrifuge (Incheon, South Korea) at 3000 rpm for 3 min and was stored at −80 ± 5 °C until analysis. A 300-μL aliquot of thawed plasma was spiked with internal standard and processed as described in the sample preparation section. Along with study samples, QC samples at low, medium, and high concentration were assayed in duplicate and were distributed among unknown samples in the analytical run.

3. Results and discussion

3.1. Optimization of sample preparation

Tandem mass spectrometry using multiple-reaction monitoring (MRM) is a powerful analytical technique for selective and sensitive determination of drugs in biological fluids. Nevertheless, sample preparation is still an essential step in the bioanalytical process because of possible matrix effects. Residual endogenous components, formulation materials, and mobile phase additives are common sources of imprecision in quantitative analyses by affecting the ionization of the target analytes, causing ion suppression or ion enhancement [49]. To reduce or eliminate matrix effects, some attempts have focused on optimizing sample preparation methods by including protein precipitation (PPT) and liquid-liquid extraction (LLE), while others have investigated proper ionization sources and chromatographic parameters.

For the determination of cetirizine in human plasma, de Jager et al. reported that protein precipitation gave a better recovery than liquid–liquid extraction [42]. Initially, we also tested a PPT method for levocetirizine quantification and found a good linear response over the concentration range of 0.5–300 ng/mL. However

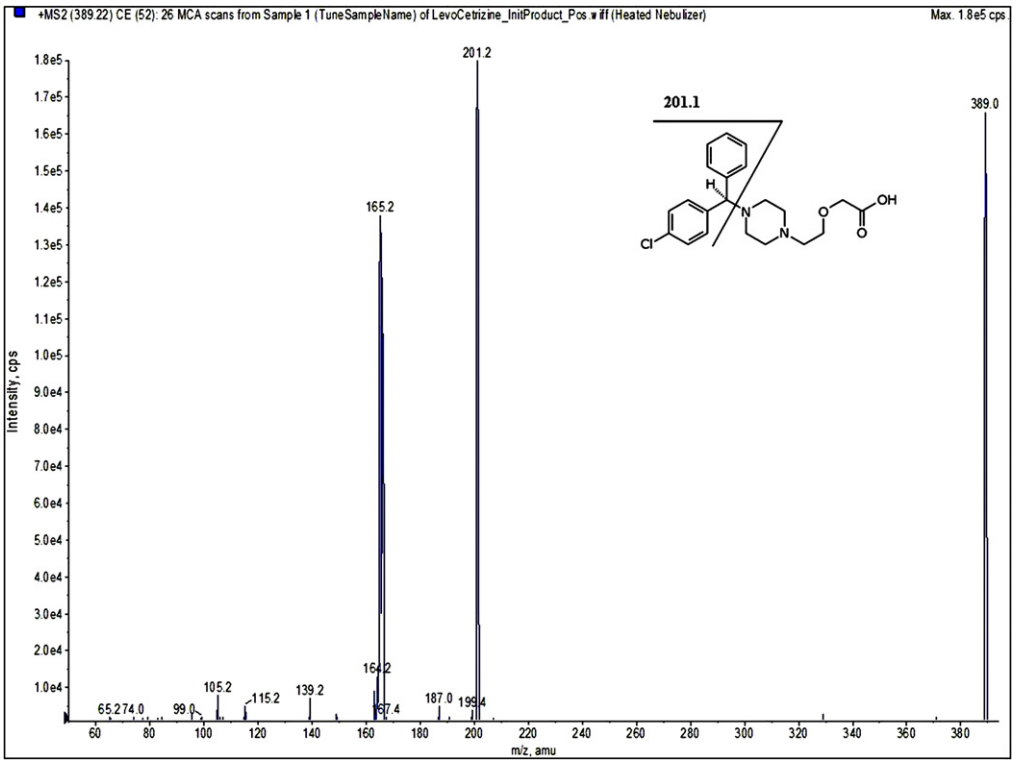

Fig. 2. Product ion spectra of $[M+H]^+$ ion of levocetirizine showing prominent fragment ions at *m/z* 201.1.

there was some interference in blank plasma and the final reconstituted solution was not clear. Moreover, the sensitivity was very poor. The dirty residues were deposited on the analytical column even though we used the guard column, which may negatively affect the method robustness and reduce the column durability. Generally, LLE methods provide clean extracts and reduce matrix effects compared with PPT. Hence, PPT with acetonitrile followed by LLE using a hexane/dichloromethane mixture (50:50, v/v) was chosen for clean extraction and enhanced concentration of levocetirizine. Under these conditions, levocetirizine was well resolved from the human plasma matrix with good selectivity, recovery, and sensitivity.

3.2. Optimization of HPLC conditions

As well as optimizing sample preparation, we have focused on manipulating chromatographic parameters to reduce the matrix effect and achieve fast separation of cetirizine enantiomers with good chromatographic resolution. Although we only measured levocetirizine, we needed to separate levocetirizine and dextrocetirizine in human plasma, because cetirizine racemic mixtures were administered to a reference group.

Many types of chiral stationary phases (CSPs) [50], including brush type (Pirkle columns), polysaccharide type, inclusion type (cyclodextrins, crown ethers, and polyacrylamides), ligand exchange type, and protein-based columns, have been used to separate chiral pharmaceutical drugs. These columns can be operated in the normal- or reversed-phase mode. Due to the broad applicability of α_1-acid glycoprotein (AGP) columns and polysaccharide-derived CSPs in which AGP and polysaccharide were immobilized and coated to porous and spherical support particles, these columns have been considered the first choice for stereoselective liquid chromatography. However, protein immobilized CSPs tend to be less stable and coated polysaccharide CSPs are somewhat limited in regard to the solvent [51,52] that can be used in the mobile phase.

In the present study, several protein immobilized silica columns, including Chiral AGP (Regis Technologies, 150 mm × 4.6 mm, 5 μm) and Ultron ES-OVM (Shinwa Chemical Industries Ltd., 150 mm × 4.6 mm, 5 μm), and several different polysaccharide CSP columns, including CHIRALPAK AD-H (Daicel Chemicals Industries Ltd., 250 mm × 4.6 mm, 5 μm), CHIRALPAK IA (Daicel Chemicals Industries Ltd., 150 mm × 4.6 mm, 5 μm), CHIRALPAK OD-H (Daicel Chemicals Industries Ltd., 150 mm × 4.6 mm, 5 μm), and Lux Amylose-2 (Phenomenex, 150 mm × 4.6 mm, 5 μm), were compared on the basis of peak separation and column lifetime in order to evaluate column performance (data not shown). The suitable mobile phase for protein-based CSPs is phosphate buffer solution with an organic solvent (reversed-phase mode) [22,23,39]. The most commonly used mobile phases with polysaccharide-based CSPs are alkane/alcohol mixtures (e.g., hexane/ethyl alcohol or heptane/isopropyl alcohol in normal-phase) [53,54].

Our results showed that each enantiomer was not well separated on Ultron ES-OVM, CHIRALPAK IA, CHIRALPAK OD-H, and Lux Amylose-2 columns and the retention time was long. Chiral AGP and CHIRALPAK AD-H columns showed the best performance, (i.e., a better resolution of enantiomers and shorter analysis time), however the very low durability of the Chiral AGP column has precluded its use in bioanalysis of cetirizine enantiomers. The retention times of cetirizine enantiomers on CHIRALPAK AD-H column were faster than that on Chiral AGP column. Thus, the CHIRALPAK AD-H column was selected as the chiral stationary phase for the determination of cetirizine enantiomers in human plasma.

To obtain complete separation and short retention times of enantiomers, several types of alkane/alcohol/base/acid mixtures in different proportions have been tried as a mobile phase. Initially, we used n-hexane/ethyl alcohol/diethylamine (80:20:0.1, v/v/v), in which the retention time of levocetirizine was over 20 min and there was some peak tailing. We then reduced the volume of *n*-hexane and added acetic acid to the mobile phase, which improved peak shape and separation and shortened retention time. Ultimately, the optimal mobile phase was *n*-hexane/ethyl

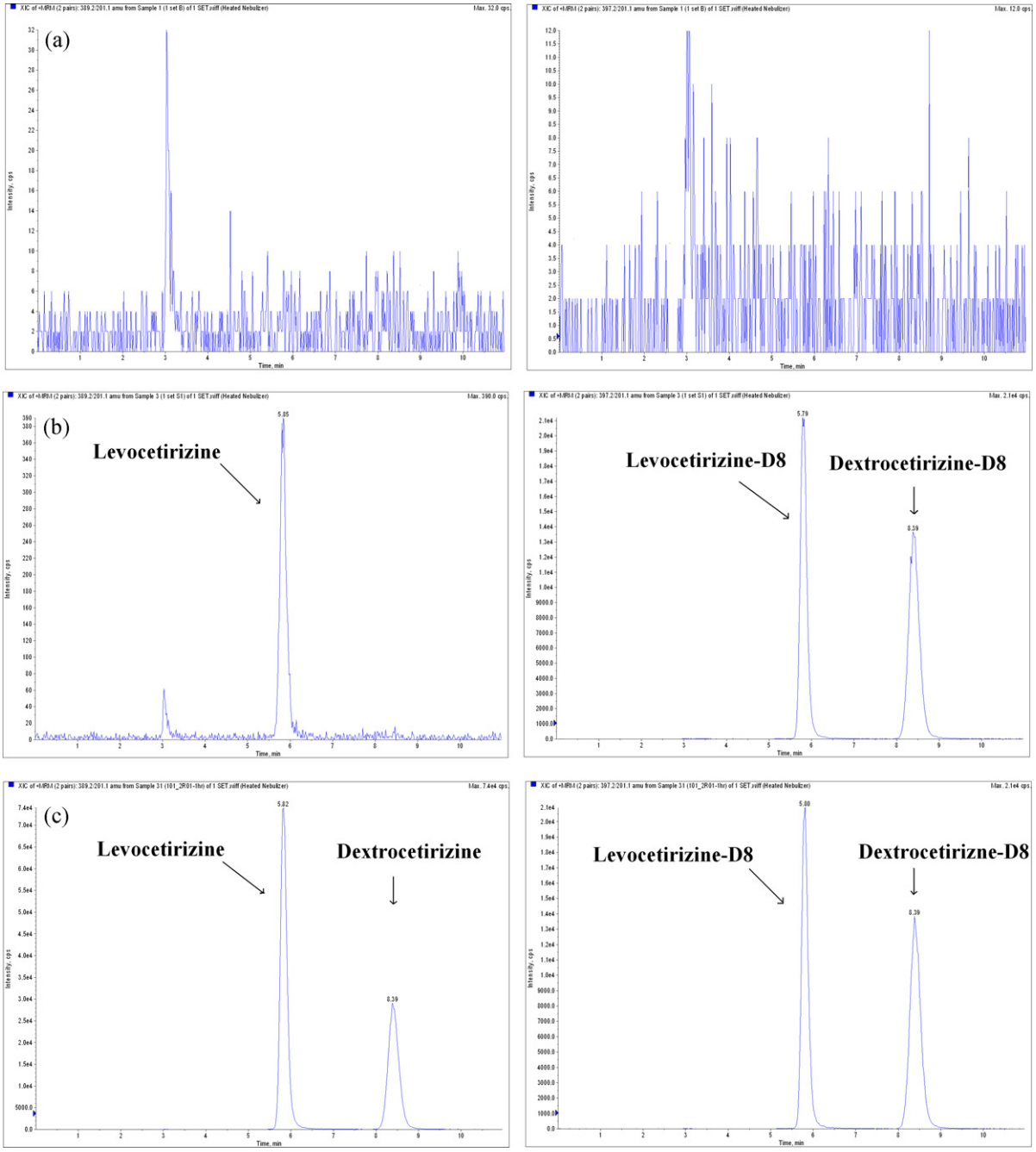

Fig. 3. Typical MRM chromatograms of cetirizine enantiomers (left panel) and racemic mixture of IS (right panel) in (a) double blank human plasma, (b) human plasma spiked with levocetirizine standard at LLOQ (0.5 ng/mL), and (c) human plasma sample obtained 1 h after oral administration of cetirizine racemic mixtures.

alcohol/diethylamine/acetic acid (60:40:0.1:0.1, v/v/v/v) in the isocratic elution mode at a flow rate of 1 mL/min.

3.3. Optimization of MS/MS spectrometry conditions

Several methods have been reported for the quantitative analysis of cetirizine racemic mixture and cetirizine enantiomers in human plasma using only reversed-phase LC-ESI–MS/MS [33,35,36,41,42]. However, in the present study, the polysaccharide-based CHIRALPAK AD-H column showed the best separation efficiency with normal-phase condition. Although APCI is generally considered to be incompatible with alkane/alcohol solvent systems due to potential spark and explosion in the corona needle [52], we did not see any flames in the APCI ion source.

Compared with ESI, APCI has commonly been shown to be less susceptible to matrix ion suppression. In addition, APCI offers much wider linear range than ESI [55]. Thus, APCI can be a better choice for certain compounds based on detection sensitivity and accuracy, and precision of the analytical method.

Much higher ion intensities of levocetirizine and internal standard were observed in positive ion mode of APCI. The mass spectrometric parameters of desolvation temperature, curtain-gas, nebulizing-gas (GS1), declustering potential (DP), collision energy (CE), collision cell exit potential (CXP), and entrance potential (EP) were optimized to obtain the highest signal response for the precursor and product ions of levocetirizine and internal standard.

Under these conditions, protonated molecular ions, $[M+H]^+$, for cetirizine (both enantiomers) and internal standard (levocetirizine-D_8) were monitored at *m/z* 389.2 and 397.2, respectively, and the

product ion mass spectra showed the formation of characteristic product ions at *m/z* 201.1 and 165.1 (Fig. 2). The loss of protonated 2-piperazinyl-1-yl ethoxy acetic acid group from the protonated molecular ion of levocetirizine (*m/z* 389.2) yielded the stable product ion of *m/z* 201.1 due to stabilization by resonance [33].

3.4. Validation of the developed analytical method: matrix effect, selectivity, linearity, limit of quantification (LOQ), intra- and inter-day precision and accuracy, recovery, and stability

The post-column infusion method was used in this validation. There was almost no ion suppression at the region for retention time of analytes and IS. Fig. 3 shows typical MRM chromatograms for double blank human plasma (free of analyte and IS), human plasma spiked with levocetirizine standard at LOQ and internal standard, and plasma sample obtained 1 h after oral administration of cetirizine racemic mixtures to a reference group. No interfering peaks from endogenous components were observed at the retention times of analytes and IS. The retention times of levocetirizine, dextrocetirizine, and internal standard (levocetirizine-D_8) were 5.8, 8.4, and 5.8 min, respectively. The total chromatographic run was 11 min.

The standard calibration curve for levocetirizine was constructed using six calibration standard solutions (0.5–300 ng/mL). The linearity of the calibration curve was evaluated by determining the best fit of peak area ratios (peak area of analyte/peak area of IS) versus concentration using weighing factor ($1/X^2$). The coefficient of determination (r^2) was greater than 0.995. The % accuracy observed for the mean of back-calculated concentrations of calibration curves was within 94.2–104.4. The limit of quantification (LOQ) was determined as the concentration at which the signal-to-noise (S/N) ratio was greater than 10, with precision of <20% and accuracy between 80 and 120% for both intra- and inter-day assays. In this study, the LOQ for levocetirizine was 0.5 ng/mL, which was similar or slightly better than that of former reports using tandem mass spectrometry [33,35,36,38,40–42].

Precision and accuracy were assessed from replicated experiments ($n = 5$) at four different concentrations of the QC samples. Table 1 shows the results for intra- and inter-day precision and accuracy. The intra-day precision ranged from 0.9% to 3.9%, and the inter-day precision ranged from 1.0% to 4.6%. The intra-day accuracy was 97.6–111.7%, and the inter-day accuracy was 99.6–108.9%.

The results of the comparison of neat standards versus plasma-extracted standards of levocetirizine were estimated at 1, 20, and 300 ng/mL. The mean recovery at these three concentrations was 97.0 ± 3.3, 97.9 ± 1.2, and 102.2 ± 3.3%, respectively, and the overall average recovery was 99.0%. These results show that the two-step extraction (PPT and LLE) of this method results in satisfactory extraction recovery.

Table 1
Intra- and inter-day precision and accuracy of levocetirizine determination in human plasma.

Nominal conc. (ng/mL)		Levocetirizine		
		Measured conc. (mean ± SD)	Precision (%, RSD)	Accuracy (%)
Intra-day variation ($n = 5$)				
	0.5	0.56 ± 0.02	3.9	111.7
	5	5.15 ± 0.14	2.7	103.0
	100	100.61 ± 1.81	1.8	100.6
	300	292.81 ± 2.73	0.9	97.6
Inter-day variation ($n = 5$)				
	0.5	0.55 ± 0.03	4.6	108.9
	5	5.06 ± 0.08	1.7	101.3
	100	100.65 ± 1.05	1.0	100.6
	300	298.96 ± 6.46	2.2	99.6

Table 2
Stability data of levocetirizine quality controls in human plasma.

Stability	Concentration (ng/mL)	Levocetirizine	
		Accuracy (%)	Precision (%, RSD)
Freeze and thaw	1	100.8	2.5
(three cycles)	300	104.3	1.5
Short-term (6 h	1	99.8	1.0
at 25 °C)	300	102.4	3.7
Autosampler	0.5	99.1	2.6
(23 h at 4 °C)	300	100.4	1.6
Long-term (112	1	102.9	6.2
days at −80 °C)	300	97.6	2.0

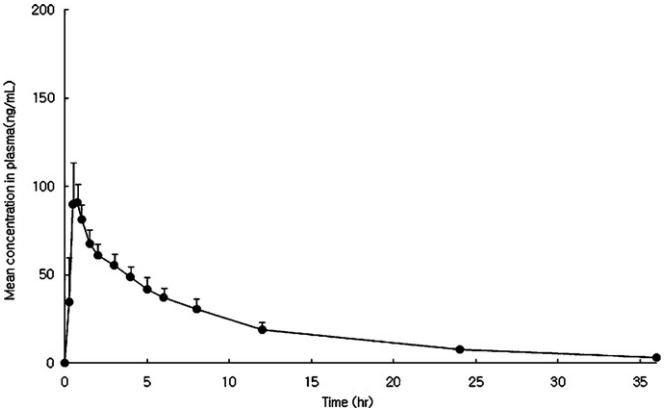

Fig. 4. Mean plasma concentration versus time profile of levocetirizine in human plasma following oral administration to a test group.

The results of freeze-thaw, short-term, long-term, and autosampler stability test for levocetirizine are shown in Table 2. Quantification of low QC and high QC samples slightly deviated (within about 5% except for long-term stability of low QC, 6.2%) from the nominal concentrations of levocetirizine in each stability tests. These results indicate that levocetirizine and internal standard are very stable after three freeze–thaw cycles (−80 °C to room temperature), over 6 h of storage at 25 °C, after 112 days of storage at −80 °C in plasma, and over 23 h at 4 °C in the autosampler tray as a processed sample solution.

3.5. Application of the method to a pharmacokinetic study

The proposed analytical method was successfully applied to a pharmacokinetic study in which plasma concentrations of levocetirizine in ten healthy Korean volunteers were determined up to 36 h after oral administration of a single dose of levocetirizine (2.5 mg) or racemic cetirizine (5 mg). Typical mean plasma concentration versus time profile for test drug is shown in Fig. 4. The observed maximum plasma concentration (C_{max}) was 97.47 ± 12.35 ng/mL and time to achieve maximum plasma concentration (t_{max}) was 0.63 ± 0.18 h. The elimination half-life ($t_{1/2}$) of levocetirizine was 8.92 ± 0.18 h, while the value of area under the curve from time 0 to ∞ ($AUC_{0-\infty}$) was 720.90 ± 111.12 ng · h/mL.

4. Conclusions

We have developed and validated a highly sensitive, specific, reproducible, and enantioselective NP-LC-APCI–MS/MS method to quantify levocetirizine using labeled IS from human plasma. Levo- and dextro-cetirizine and internal standard (levocetirizine-D_8) were baseline-separated with adequate specificity and selectivity. The novel method involved a simple two-step sample preparation of protein precipitation using acetonitrile followed by liquid–liquid

extraction with *n*-hexane–dichloromethane mixture, and did not require post-column reagent addition or a chemical derivatization step. From the results of all the validation parameters and the successful application of the method, we conclude that the proposed protocol will be very useful for pharmacokinetic studies.

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