Quantification of desloratadine in human plasma by LC-ESI-MS/MS and application to a pharmacokinetic study

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Abstract A simple, sensitive, and specific liquid chromatography tandem mass spectrometry (LC-MS/MS) method was developed for the quantification of desloratadine (DL) in human plasma using desloratadine-d5 (DLD5) as an internal standard (IS). Chromatographic separation was performed using an Xbridge C18 column (50 mm x 4.6 mm, 5 μm) with an isocratic mobile phase composed of 10 mM ammonium formate: methanol (20:80, v/v), at a flow rate of 0.7 mL/min. DL and DLD5 were detected with proton adducts at \(m/z\) 311.2-259.2 and 316.2-264.3 in multiple reaction monitoring (MRM) positive modes, respectively. Liquid–liquid extraction (LLE) method was used to extract the drug and the IS. The method was validated over a linear concentration range of 5.0–5000.0 pg/mL with a correlation coefficient of \(r^2\geq 0.9994\). This method demonstrated intra- and inter-day precision within 0.7–2.0% and 0.7–2.7%, and an accuracy within 101.4–102.4%, and 99.5–104.8%. DL was found to be stable throughout the freeze–thaw cycles, bench-top, and postoperative stability studies. This method was successfully applied in the analysis of plasma samples following oral administration of DL (5 mg) in 35 healthy Indian male human volunteers under fasting conditions.

1. Introduction

Desloratadine is an antihistamine drug used to treat the symptoms of allergies, such as sneezing, teary eyes, and runny nose. It is also used to treat skin hives and itching in people with chronic skin reactions. The molecular formula of desloratadine is \(C_{19}H_{19}ClN_2\) with a molecular weight of 310.8. The chemical name of the desloratadine is 8-chloro-6,11-dihydro-11-(4-piperidinylidene)-5H-benzo[5,6]cyclohepta[1,2-b]pyridine and has a structure as described below [1] (Fig. 1). DL is well absorbed and is extensively metabolized in the human body. The major metabolite of DL in human plasma and urine is the glucuronide conjugate of 3-hydroxydesloratadine. We found that the elimination of DL was gradual (mean \(t_{1/2}=19.5\) h).
and persisted in the plasma for 48–120 h postdose [2–4]. There were no statistically significant differences in the mean plasma concentrations in any of the main pharmacokinetic parameters of rupatadine, DL, and 3-hydroxydesloratadine when administered in combination with azithromycin or alone [5,6].

As of now, there have been several methods reported for quantification of DL in rat plasma with LC-MS [7], human plasma with LC-MS [8–12], LC-MS with nanospray ionization [13], human plasma with HPLC [14–16], dog plasma with HPLC [17], pharmaceutical formulation with HPLC [18,19], and pharmaceutical formulation with electrophoresis [20]. Wen et al. [8] reported having developed a method using LLE with a concentration range of 0.1–20 ng/mL. The sensitivity was improved by Xu et al. [9] with a concentration range of 0.05–10 ng/mL. Among all researchers, Yang et al. [12] achieved the best results with high sensitivity at a linearity range of 25–10000 pg/mL with SPE extraction.

The aim of the proposed method is to extract DL in human plasma by the LLE method with high sensitivity. Moreover, the analyte is to be compared with the deuterated internal standard (IS), which is most appropriate to develop in the bioanalytical method in terms of matrix effect and reproducibility. The developed method could be useful for application in clinical and pharmacokinetic studies.

2. Materials and methods

2.1. Chemicals and reagents

DL was obtained from Cadila Pharmaceuticals, India. DLD5 was procured from Clear Synth, India. Ammonium formate and sodium hydroxide (analytical grade) were purchased from Merck, Mumbai, India. Methanol, ethyl acetate, and dichloromethane (HPLC grade) were obtained from J.T. Baker, USA. Human plasma was procured from Navjeevan Blood Blank, Hyderabad. Milli Q water was taken from the in-house Milli-Q system.

2.2. Instrumentation

The 1200 series HPLC system (Agilent Technologies, Germany) was used. Mass spectrometric detection was performed using an API 4000 triple quadrupole instrument (ABI-SCIEX, Toronto, Canada) and using multiple reaction monitoring (MRM). Data processing was performed using the Analyst 1.4.1 software package (SCIEX).

2.3. Detection

Detection was done by turbo ion spray (API) positive mode with unit resolution. For DL, mass transitions were obtained from 311.2 m/z (parent ion) to 259.2 m/z (product ion). Similarly, DLD5 mass transitions were obtained from (316.2 m/z) (parent ion) to 264.3 m/z (product ion).

2.4. Chromatographic conditions

Chromatographic separation was performed using an Xbridge C18 column (50 mm x 4.6 mm, 5 μm) at a temperature of 40 °C. The mobile phase was composed of 10 mM ammonium formate:methanol (20:80, v/v) at a flow rate of 0.7 mL/min. Deuterated IS DLD5 was used as the appropriate IS in terms of chromatography and extractability. DL and DLD5 were eluted at 0.9 ± 0.2 min, approximately, with a total run time of 3 min for each sample.

2.5. Preparation of standards and quality control samples

Standard stock solutions of DL (100.0 μg/mL) and DLD5 IS (100.0 μg/mL) were prepared in methanol. The IS spiking solutions (10.0 ng/mL) were prepared in reconstitution solutions (10 mM ammonium formate and methanol in the ratio of 20:80 (v/v) from IS stock solutions. Standard stock solutions and IS spiking solutions were stored in refrigerator conditions of 2–8 °C until analysis. Standard stock solutions of DL (100.0 μg/mL) were added to drug-free screened human plasma to obtain concentration levels of 5.0, 10.0, 200.0, 800.0, 1400.0, 2000.0, 3000.0, 4000.0, and 5000.0 μg/mL for analytical standards, and 5.0, 15.0, 2500.0, and 3500.0 μg/mL for quality control (QC) standards, and stored in the freezer at −30 °C until analysis. The aqueous standards were prepared in a reconstitution solution (10 mM ammonium formate and methanol in the ratio of 20:80 (v/v) and stored in the refrigerator at 2–8 °C until analysis.

2.6. Sample preparation

The LLE method was used to isolate DL and DLD5 from human plasma. For this purpose, 100 μL of DLD5 (10 ng/mL) and 400 μL of plasma sample were added to the labeled polypropylene tubes and vortexed briefly for about 5 min. Thereafter, 100 μL of 0.1 M NaOH solution and 3 mL of extraction solvent (in the ratio of ethyl acetate:dichloromethane 80:20(v/v)) were added and vortexed for about 10 min. Next, the samples were centrifuged at 4000 rpm for approximately 5 min at ambient temperature. From each, a supernatant sample was transferred into labeled polypropylene tubes and evaporated to a dryness of 40 °C briefly, and then reconstituted with a reconstitution solution (10 mM ammonium formate and methanol in the ratio of 20:80 (v/v), and the sample was transferred into auto sampler vials and injected into the LC-MS for study.

2.7. Selectivity and sensitivity

Selectivity was performed by analyzing human blank plasma samples from six different sources (donors) with an additional hemolyzed group and lipedimic group to test for interference.
at the retention times of analytes. The sensitivity was compared with the lower limit of quantification (LLOQ) of the analyte with its blank plasma sample. The peak area of blank samples should not be more than 20% of the mean peak area of the limit of quantification (LOQ) of DL and 5% of the mean peak area of DLD5.

2.8. Precision and accuracy

It was determined by replicate analysis of QC samples (n=6) at LLOQ, low QC (LQC), medium QC (MQC), high QC (HQC), and upper limit of quantification (ULOQ) levels. The % coefficient of variation (CV) should be less than 15%, and accuracy within 15%, except for LLOQ, where it should be within 20%.

2.9. Matrix effect

The matrix effect caused due to the plasma matrix was used to evaluate the ion suppression/enhancement in a signal when comparing the absolute response of QC samples after pre-treatment (LLE) with the reconstitution samples extracted from the blank plasma sample spiked with analyte. Experiments in triplicates were performed at MQC levels with 6 different plasma lots with the acceptable precision (% CV) of ≤15%.

2.10. Recovery

The extraction recovery of analyte and IS from human plasma was determined by analyzing QC samples. Recovery at 3 concentrations (15.0, 2500.0, and 3500.0 pg/mL) was determined by comparing peak areas obtained from the plasma sample and the standard solution spiked with the blank plasma residue. A recovery of more than 50% was considered adequate to obtain required sensitivity.

2.11. Stability

LQC and HQC samples (n=6) were retrieved from deep freeze after 3 freeze-thaw cycles according to clinical protocol. Samples were stored at −30°C in 3 cycles of 24, 36, and 48 h. In addition, the long-term stability of DL in QC samples was also evaluated by analysis after 105 days of storage at −30°C. Auto-sampler stability was studied following a 53-h storage period in the auto sampler tray with control concentrations. Room temperature stability was studied over a 24.5-h period with control concentrations. Stability samples were processed and extracted along with the freshly spiked calibration curve standards. The precision and accuracy for the stability samples must be within ±15 and ±15%, respectively, of their nominal concentrations.

2.12. Analysis of Human samples

The bioanalytical method described above was used to determine DL concentrations in plasma following oral administration along with 240 mL of drinking water. The reference product, Clarinex tablets (Merck) 5 mg, USA, and test product, DL tablets (test tablet) 5 mg, were used. Study protocol was approved by IEC (Institutional Ethical committee) as per ICMR (Indian Council of Medical Research). Blood samples were collected at the predose 0 h 5 min prior to dosage followed by further samples at 0.333, 0.667, 1, 1.333, 1.667, 2, 2.5, 3, 3.5, 4, 4.5, 5, 6, 7, 8, 10, 12, 18, 24, 48, and 72 h. After dosage, 2 mL blood was collected each time in vacuutainers containing K2EDTA. A total of 44 (22 time points for test, and 22 time points for reference) time points were collected using centrifugation at 3200 rpm, 10°C, for 10 min, and kept stored at −30°C until sample analysis. Test and reference were administered separately to the same human volunteers under fasting conditions with proper washing periods as per protocol approved by the IEC.

2.13. Pharmacokinetics and statistical analysis

Pharmacokinetics parameters from the human plasma samples were calculated by a noncompartmental statistics model using WinNon-Lin5.0 software (Pharsight, USA). Blood samples were taken during a period of 3–5 times the terminal elimination half-life (t1/2), and it was considered as the area under the concentration time curve (AUC) ratio higher than 80%, as per FDA guidelines. Plasma DL concentration-time profiles were visually inspected, and Cmax and tmax values were determined. The AUC0–∞, was obtained by the trapezoidal method. AUC0–t was calculated up to the last measurable concentration, and extrapolations were obtained using the last measurable concentration and the terminal elimination rate constant (Kt). The terminal elimination rate constant, (Kt), was estimated from the slope of the terminal exponential phase of the plasma of DL concentration time curve (by means of the linear regression method). The terminal elimination half-life, t1/2, was then calculated as 0.693/Kt. Regarding AUC0–t and Cmax, bioequivalence was assessed by means of analysis of variance (ANOVA) and the standard 90% confidence intervals (CIs 90%) of the ratios’ test/reference (logarithmically transformed data) was calculated. The bioequivalence was considered when the ratio of averages of log transformed data was within 80–125% for AUC0–t, AUC0–∞, and Cmax [21,22].

3. Results and discussion

3.1. Method development

During development of the method, different options were evaluated to optimize mass spectrometry detection parameters, chromatography, and sample extraction.

3.1.1. Mass spectrometry detection parameters optimization

Electrospray ionization (ESI) provided a maximum response over atmospheric pressure chemical ionization (APCI) mode, and was chosen for this method. The instrument was optimized to obtain sensitivity and signal stability during infusion of the analyte in the continuous flow of mobile phase to electrospray ion source operated at both polarities at a flow rate of 5 μL/min. DL gave a better response when in the
positive ion mode as compared to the negative ion mode. The predominant peaks in the primary ESI spectra of DL and DLD5 correspond to the (M+H)$^+$ ions at $m/z$ 311.2 and 316.2, respectively (Figs. 2(A) and 3(A)). Product ions of DL and DLD5 scanned in quadrupole 3 after a collision with nitrogen in quadrupole 2 had an $m/z$ of 259.2 and 264.3.

Figure 2  (A) Parent ion mass spectra of desloratadine and (B) product ion mass spectra of desloratadine.

Figure 3  (A) Parent ion mass spectra of desloratadine-D5 and (B) product ion mass spectra of desloratadine-D5.
respectively (Figs. 2(B) and 3(B)). Mass parameters were optimized as source temperature 500 °C, heater gas 45 psi (nitrogen), nebulizer gas 30 psi (nitrogen), curtain gas 20 psi (nitrogen), CAD gas 5 psi (nitrogen), ion spray (IS) voltage 5500 V, source flow rate 600 μL/min without split, entrance potential 10 V, declustering potential 70 V, collision energy 30 V, and collision cell exit potential 15 V for both analyte and IS.

3.1.2. Chromatography optimization

Initially, a mobile phase consisting of ammonium acetate and acetonitrile in varying combinations was tried, but a low response was observed. The mobile phase containing 5 mM ammonium formate, methanol (20:80 v/v), gave a better response, but poor peak shape was observed. A mobile phase of 0.1% formic acid in water in combination with methanol and acetonitrile with varying combinations was tried. Finally, a mobile phase containing 5 mM ammonium formate, acetonitrile (20:80 v/v), gave the best signal along with a marked improvement in the peak shape observed for DL and DLD5. Short-length columns, such as Symmetry Shield RP18 (30 mm × 2.1 mm × 3.5 μm), Inertsil ODS-2V (50 mm × 4.6 mm, 5 μm), Hypurity C18 (50 mm × 4.6 mm, 5 μm), and Hypurity Advance (50 × 4.0 mm, 5 μm), Xbridge C18 (50 mm × 4.6 mm, 5 μm), were tried during development of the method. Xbridge C18 (50 mm × 4.6 mm, 5 μm) column gave a relatively good peak shape with the best signal being obtained. It gave satisfactory peak shapes for both DL and DLD5. A flow rate of 0.7 mL/min without splitter was utilized and the run time was reduced to 2.5 min. Both drug and IS were eluted in a shorter time at 0.9 ± 0.2 min. For an LC-MS/MS analysis, utilization of stable isotope-labeled, or suitable analog drugs as an IS proved helpful when a significant matrix effect is possible. In our case, DLD5 was found to be best suited for the present purpose. The column oven temperature was kept at a constant temperature of about 40 °C. Injection volume of a 5 μL sample is adjusted for better ionization and chromatography.

3.1.3. Extraction optimization

Prior to loading the sample for LC injection, the coextracted proteins should be removed from the prepared solution. For this purpose, initially, we tested with different extraction procedures like protein precipitation (PPT), LLE, and solid phase extraction (SPE). We found an ion-suppression effect in the PPT method for the drug and IS. Further, we also tried this with SPE and LLE. Out of all these, we observed that LLE is suitable for extraction of the drug and IS. We tried with several organic solvents (ethyl acetate, chloroform, n-hexane, dichloromethane, and methyl tertiary butyl ether) individually as well as with various combinations of LLE to extract analyte from the plasma sample. In our case, an ethyl acetate:dichloromethane (80:20) combination served as a good extraction solvent. The auto sampler wash is optimized as 80% methanol. High recovery and selectivity was observed in the LLE method. These optimized detection parameters, chromatographic conditions, and extraction procedures resulted in reduced analysis time with accurate and precise detection of DL in human plasma.

3.2. Method validation

A thorough and complete method of validation of metaxalone in human plasma was carried out following USFDA guidelines [23]. The method was validated for selectivity, sensitivity, matrix effect, linearity, precision and accuracy, recovery, and stability.

3.2.1. Selectivity and specificity

The analysis of DL and DLD5 using MRM function was highly selective with no interfering compounds (Fig. 4). Specificity was performed using 6 different lots of human plasma. Chromatograms obtained from plasma spiked with DL (5.0 pg/mL) and DLD5 (10.0 ng/mL) are shown in Fig. 5.

3.2.2. Matrix effect

Calibration curves were plotted as the peak area ratio (DL/ DLD5) vs. (DL) concentration. Calibration was found to be linear over the concentration range of 5.0–5000.0 pg/mL. The % CV was less than 3.2%, and the accuracy ranged from 97.6% to 102.2%. The determination coefficients (r²) were greater than 0.9994 for all curves (Table 1).

3.2.3. Linearity

Precision and accuracy for this method were controlled by calculating the intra- and inter-batch variations at four concentrations (5.0, 15.0, 2500.0, and 3500.0 pg/mL) of QC samples in 6 replicates. As shown in Table 2, the intrabatch % CV was less than 2.0%, and the accuracy ranged from 101.4% to 102.4%. Interbatch % CV was less than 2.7%, and the accuracy ranged from 99.5% to 104.8%. These results indicate

Figure 4 MRM chromatogram of blank human plasma.
the adequate reliability and reproducibility of this method within the analytical range.

3.2.5. Recovery
The recovery following the sample preparation using the LLE method was calculated by comparing the peak area ratios of DL in plasma samples with the peak area ratios of solvent samples, and was estimated at control levels of DL. The recovery of DL was determined at three different concentrations of 15.0, 2500.0, and 3500.0 pg/mL, and was found to be 89.6%, 90.2%, and 91.2%, respectively. The overall average recovery of DL and DL D5 was found to be 90.3% and 92.5%, respectively.

3.2.6. LOQ and limit of detection
The LOQ was determined at 5 pg/mL. The limit of detection (LOD) was determined at 50 fg/10 μL injection volume.

3.2.7. Stability
Quantification of the DL in plasma which was subjected to 3 freeze-thaw (−30 °C up to room temperature) cycles showed the stability of the analyte. The concentrations ranged from 99.3% to 98.5% of the theoretical values. No significant degradation of the DL was observed even after a 53-h storage period in the auto sampler tray, and the final concentration of DL was between 97.3% and 103.2% of the theoretical values. Room temperature stability at 24.5 h was between 98.0% and 99.4% of the theoretical values. In addition, the long-term stability of DL in QC samples after 105 days of storage at −30 °C was also evaluated. The concentrations ranged from 94.6% to 101.8% of the theoretical values. These results confirmed the stability of DL in human plasma for at least 105 days at −30 °C (Table 3).

3.3. Application to biological samples
The method validated above was used in the determination of DL in plasma samples for establishing the bioequivalence of a
single 5 mg dose (one 5 mg tablet) in 35 healthy volunteers. Typical plasma concentration vs. time profiles is shown in Fig. 6. All the plasma concentrations of DL were in the standard curve region and retained above the 5.0 pg/mL LOQ for the entire sampling period. Pharmacokinetic details are represented in Table 4. Test vs. reference $C_{\text{max}}$, AUC$_{0-\infty}$, AUC$_{0-\infty}$ were 101.1%, 99.3%, and 98.6%, respectively. On the basis of the pharmacokinetic data obtained from the test and reference values, it can be concluded that the two DL formulations (reference and test) analyzed are bioequivalent in terms of rate and extent of absorption.

4. Conclusions

The proposed method was five times higher in sensitivity than the method reported by Yang et al. [12] and the analyte was compared with deuterated IS. The method described here is quick (it requires less than 2.5 min of analysis time), is rugged, is a reproducible bioanalytical method, and was successfully applied to the bioequivalence and pharmacokinetic study of DL in 35 human volunteers after oral administration of 5 mg tablets under fasting conditions.
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