Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Pharmaceutical and Biomedical Analysis 40 (2006) 95–99
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

Determination of moxonidine in human plasma by liquid chromatography–electrospray ionisation–mass spectrometry

Luhua Zhao [a,*], Li Ding [b], Xin Wei [b]

[a] *Center for Instrumental Analysis, China Pharmaceutical University, 24th Tongjiaxiang Road, Nanjing 210009, PR China*
[b] *Department of Pharmaceutical analysis, China Pharmaceutical University, Nanjing 210009, PR China*

Received 31 March 2005; received in revised form 13 May 2005; accepted 2 June 2005
Available online 11 July 2005

Abstract

A sensitive and specific liquid chromatography–electrospray ionisation–mass spectrometry (LC–ESI–MS) method has been developed and validated for the identification and quantification of moxonidine in human plasma. After the addition of clonidine–HCl, the internal standard (IS) and sodium hydrogen carbonate, plasma samples were extracted using 5 mL ethyl acetate. The compounds were separated on a Lichrospher ODS (5 μm, 250 mm × 4.6 mm) column using an elution system of 10 mmol/L ammonium acetate buffer–methanol (20:80 v/v) as the mobile phase. Analytes were determined using electrospary ionization in a single quadrupole mass spectrometer. LC–ESI–MS was performed in the selected-ion monitoring (SIM) mode using target ions at *m*/*z*: 242.2 for moxonidine and *m*/*z*: 230.1 for the IS. The method has shown to be sensitive and specific by testing six different blank plasma batches. Linearity was established for the range of concentrations 0.01976–9.88 ng/mL with a coefficient of correlation (*r*) of 0.9999. The lower limit of quantification (LOQ) was identifiable and reproducible at 0.01976 ng/mL. The method has been successfully applied to study the pharmacokinetics of moxonidine in healthy male Chinese volunteers.

Keywords: Moxonidine; HPLC–ESI–MS; Human plasma; Pharmacokinetics

1. Introduction

Moxonidine–HCl (Fig. 1a) is a new centrally-acting antihypertension agent that reduces blood pressure by stimulating the central α_2-adrenoceptor. Determination of the pharmacokinetic profile of moxonidine is important for gaining a better understanding of its mechanism of action and for ensuring more efficient therapeutic application. Martin et al. [1,2] developed a gas chromatographic method with mass spectrometric detection for determination of moxonidine in human plasma. LOQ of moxonidine in plasma was 0.1 ng/mL. Qiang Zhang et al. [3] reported an RP-HPLC method for the quantification of moxonidine–HCl in material and preparations.

In this study, a sensitive liquid chromatography–electrospray ionisation–mass spectrometry (LC–ESI–MS) method has been novel developed for determination of moxonidine in human plasma. The assay is validated over the ranges of 0.01976–9.88 ng. The method has been successfully applied to study pharmacokinetics of moxonidine in healthy male Chinese volunteers.

2. Experimental

2.1. Chemicals and reagents

Moxonidine test patch, Moxonidine–HCl standard, and IS was supplied by Yabao Pharmaceutical Company (Shanxi Province, PR China); moxonidine reference patch was supplied by Tiantaishan Pharmaceutical Co. Ltd. (Chengdu Province, PR China). Methanol was chromatographic pure grade and purchased from Merck (Merck Company, Germany). Other chemicals were all of analytical grade. Deionized water was distilled before using.

* Corresponding author. Tel.: +86 2583 271185; fax: +86 2583 271399.
E-mail address: zhaoluhua@hotmail.com (L. Zhao).

0731-7085/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2005.06.002

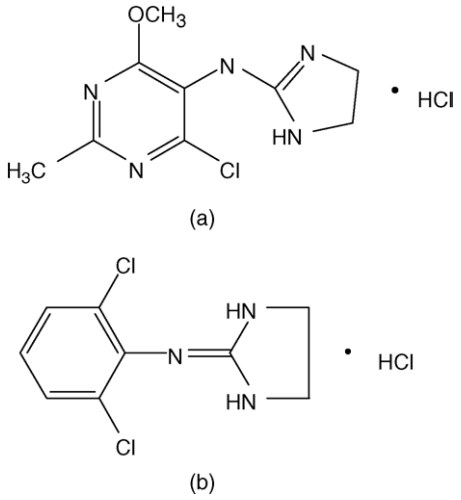

Fig. 1. Chemical structure of moxonidine hydrochloride (a) and clonidine hydrochloride (b).

2.2. Instrumentation and operating conditions

HPLC analyses were performed using an Agilent 1100 LC–MSD system (Agilent company, USA) with an Agilent Chem Station (Agilent company, USA) with a Lichrospher ODS C18 column (4.6 mm × 250 mm, 5 μm, Huaiyin Hanbang science Co. Ltd., PR China). Using an elution system of 10 mmol/L ammonium acetate buffer–methanol (20:80 v/v) as the mobile phase, and the column temperature was maintained at 25 °C. A constant mobile phase flow-rate of 1.0 mL/min was employed throughout the analyses. LC–ESI–MS was carried out using nitrogen to assist nebulization. A quadrupole mass spectrometer equipped with an electrospray ionization source was set with a drying gas (N_2) flow of 10 L/min, nebulizer pressure of 40 psi (1 psi = 6894.76 Pa), drying gas temperature of 350 °C and the positive ion mode The fragmentor voltage was 90 V. LC–ESI–MS was performed in SIM mode using target ions at *m*/*z*: 242.2 for moxonidine and *m*/*z*: 230.1 for the IS. The MS data acquisition started at 2 min after sample injection, and the stream selection valve was set to waste until data acquisition started.

2.3. Preparation of stock solutions

Primary stock solutions of moxonidine–HCl for preparation of standards and quality controls (QC) were prepared from separate weighings. The primary stock solutions were prepared in methanol at a concentration of 0.988 mg/mL. Working solutions of moxonidine–HCl were prepared daily in methanol by appropriate dilution at 0.988, 9.88, 98.8 and 197.6 ng/mL.

The IS stock solution was prepared by dissolving 5.56 mg of clonidine–HCl in 10 ml of methanol producing a concentration of 0.96 mg/mL. A 0.048 μg/mL internal standard working solution was prepared by diluting the stock standard solution of clonidine–HCl with the methanol.

All the solutions were stored at 4 °C and were brought to room temperature before use.

2.4. Calibration curves

Calibration curves were prepared by spiking different samples of 1 mL blank plasma each with one of the above mentioned working solutions to produce the calibration curve points equivalent to 0.01976, 0.0494, 0.0988, 0.1976, 0.494, 0.988, 1.976, 4.94 and 9.88 ng/mL of moxonidine. Each sample was also spiked with 40 μl IS working solution (0.048 μg/mL) and extract as the 2.6 procedure. In each run, a plasma blank sample (no IS) was also analyzed. Calibration curves were prepared by determining the best-fit of peak area ratios (peak area of analyte/peak area of IS) versus concentration, and fitted to the equation $f = bC + a$ by unweighted least-squares regression.

2.5. Preparation of quality control samples

Quality control samples were prepared at four different concentration levels. QC samples were prepared daily by spiking different samples of 1 mL blank plasma each with the corresponding standard solution to produce a final concentration equivalent to 0.0494, 0.1976, 0.988 and 4.94 ng/mL of moxonidine and 1.92 ng of internal standard.

2.6. Extraction procedure

QC, calibration curve, and clinical plasma samples were extracted employing a liquid–liquid extraction technique. To each tube containing 1 ml plasma, 40 μl of internal standard solution (0.048 μg/mL) was added, 200 μl of saturated sodium hydrogen carbonate was added for alkalinizing and 5 ml ethyl acetate were added and vortex for 3 min. Afterwards, samples were centrifuged for 5 min at 4000 × *g*. The organic layer was evaporated under a stream of nitrogen at 45 °C. The residue was reconstituted in 100 μl mobile phase. An aliquot of 50 μl was injected into the LC–MS system.

2.7. Assay validation

The method validation assays were carried out according to the currently accepted US Food and Drug Administration (FDA) bioanalytical method validation guidance [4]. The following parameters were considered.

The method's specificity was tested by screening six different batches of healthy human blank plasma. Each blank sample was tested for interference using the proposed extraction procedure and chromatographic/spectroscopic conditions and compared with those obtained with an aqueous solution of the analyte at a concentration near to the LLOQ.

The matrix effect on the ionization of analytes was evaluated by comparing the peak area of analytes resolved in blank sample (the final solution of blank plasma after extraction and reconstitution) with that resolved in mobile phase.

Four different concentration levels of moxonidine (0.0494, 0.1976, 0.988 and 4.94 ng/mL) were evaluated by analyzing five samples at each level. The blank plasma used in this study were five different batches of healthy human blank plasma. If the ratio <85 or >115%, an exogenous matrix effect was implied.

Linearity was tested for the range of concentrations 0.01976–9.88 ng/mL. For the determination of linearity, standard calibration curves of at least seven points (non-zero standards, nine points in this research) were used. In addition, a blank plasma sample were also analyzed to confirm absence of interferences, these sample was not used to construct the calibration function. The acceptance criteria for correlation coefficient was 0.998 or more, otherwise the calibration curve should be rejected. Five replicate analyses were done.

The intra-day precision and accuracy of the assay was measured by analyzing five spiked samples of moxonidine at each QC level (0.0494, 0.1976, 0.988 and 4.94 ng/mL). The inter-day precision and accuracy was determined over 5 days by analyzing 15 QC samples. The acceptance criteria for precision and accuracy deviation values should be within 15% of the actual values.

The extraction yield (or absolute recovery) was determined by comparing the moxonidine/IS peak area ratios obtained following the outlined extraction procedure (the procedure was a little different from the outlined extraction procedure for QC, calibration curve and clinical plasma samples, that is IS was added to the organic layer after the extraction of moxonidine) and the result compared with those obtained from samples which contained the same amount of moxonidine in extracted plasma but not be extracted after addition of the drug. This procedure was repeated for the four different concentrations of moxonidine added, namely 0.0494, 0.1976, 0.988 and 4.94 ng/mL.

For sensitivity determination, the lowest standard concentration in the calibration curve was considered as the lower limit of quantification, and was to meet the following criteria: LLOQ response should be 10 times the response of the blank and the LLOQ response should be identifiable, discrete and reproducible with a precision corresponding to a maximum 20% R.S.D. The 0.01976 ng/mL concentration was investigated as the lower limit of quantification. Reproducibility and precision were also determined.

To evaluate stability on repeat analysis of samples, freezethaw stability was determined for three concentrations of moxonidine in plasma. QC plasma samples were tested after three freeze (−20 °C) and thaw (room temperature) cycles.

2.8. Clinical study design and pharmacokinetic analysis

Twenty healthy young male Chinese volunteers participated in this study. After an overnight fast, each volunteer received capsules containing 0.2 mg moxonidine–HCl, which was taken with 200 ml water. Standard meals were provided at 4 h post-dose. Blood was sampled pre-dose and at 0.167, 0.33, 0.5, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 16.0 h post-dose for determination of plasma concentration of moxonidine. Model-independent pharmacokinetic parameters were calculated for moxonidine. The maximum plasma concentrations (C_{max}) and the time to those (T_{max}) were noted directly.

3. Results and discussion

3.1. Selection of IS

It is necessary to use an IS to get high accuracy when a mass spectrometer is used as an HPLC detector. Clonidine–HCl was chosen in the end because of its similarity of structure (Fig. 1b), retention and ionization with the analyte and the less endogenous interferences at moxonidine $[M + H]^+$, m/z: 242.2.

3.2. Sample preparation

Liquid–liquid extraction [5–9] was necessary and important because this technique cannot only purify but also concentrate the sample. *n*-Hexane with or without saturated sodium hydrogen carbonate, ethyl acetate with or without saturated sodium hydrogen carbonate were all tested to do extraction and the ethyl acetate with 1 ml saturated sodium hydrogen carbonate was finally adapted because of its high extraction efficiency.

3.3. Conditions of chromatography

The selection of mobile phase components was a critical factor in achieving good chromatographic peak shape and resolution. Because ammonium acetate could improve the ionization efficiency of amines, a solvent system of ammonium acetate was selected as a buffer utilized in the mobile phase. Good separation of analytes and short run time were obtained by using an elution system of 10 mmol/L ammonium acetate buffer–methanol (20:80 v/v) as the mobile phase. Typical SIM mass chromatograms are shown in Fig. 2 in which the retention times were 3.0 min for moxonidine and 3.1 min for the IS.

3.4. Conditions of ESI–MS

The ESI mass spectra of analytes at 50 V fragmentor voltage showed the protonated molecules of moxonidine ($[M + H]^+$) at m/z: 242.2. The intensities of its protonated molecules were compared at fragmentor voltages of 50, 70, 100, 120, 150, and 180 V in order to determine the optimal collision energy. The results showed that the highest sensitivities could be obtained by using a 90 V fragmentor voltage. Therefore, a 90 V fragmentor voltage was used to carry out LC–ESI–MS in the SIM mode. At this collision energy, the

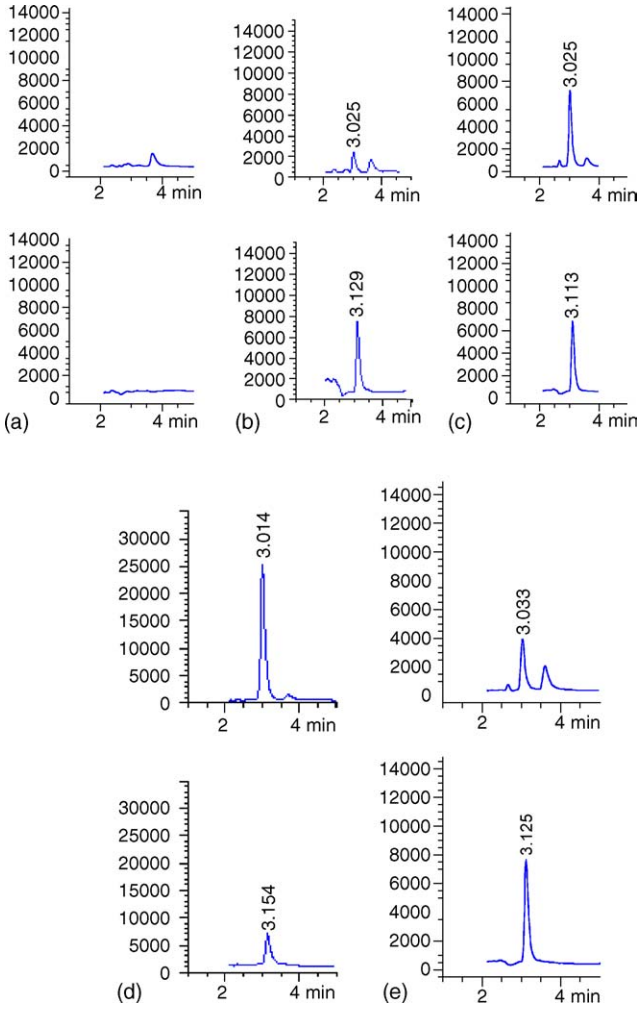

Fig. 2. Typical SIM mass chromatograms of: (a) blank plasma, (b) LLOQ (0.01976 ng/mL) moxonidine–HCl and the IS (1.92 ng/mL), (c) plasma spiked with moxonidine–HCl (1.976 ng/mL) and the IS (19.2 ng/mL), (d) plasma spiked with moxonidine–HCl (9.88 ng/mL) and the IS (19.2 ng/mL), and (e) plasma obtained from a volunteer after 0.167 h moxonidine–HCl administration.

most intensive ion in mass spectrum of IS was also its protonated molecule ($[M + H]^+$) m/z: 230.1. Therefore, protonated molecule m/z: 230.1 of IS was selected as the target ion of IS in the SIM.

3.5. Method validation

The calibration curves of moxonidine showed good linearity in the ranges of 0.01976–9.88 ng/mL. The linear regression calibration curve is $f = 0.5239C + 0.00336$ with a coefficient of correlation (r) of 0.99991. LLOQs for moxonidine in plasma, was proved to be 0.01976 ng/mL (Fig. 2, Table 1).

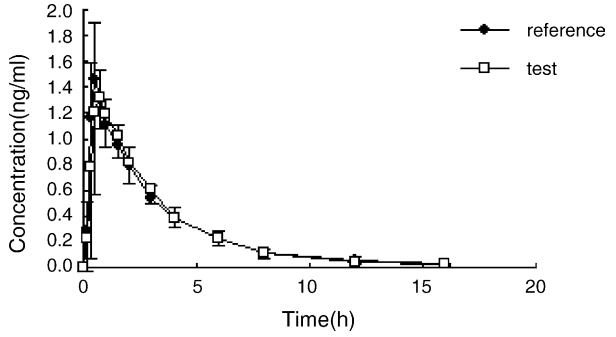

Fig. 3. Mean plasma concentration–time profile of moxonidine–HCl after an oral administration of 0.2 mg moxonidine–HCl to 20 healthy volunteers. Each point represents the mean + S.D. ($n = 20$).

Those data show that this assay is sensitive enough for pharmacokinetic study of moxonidine. Calibration curves were prepared with each batch of clinical samples.

The intra- and inter-day ($n = 5$) precision and accuracy are summarized in Table 2. Results in Table 1 demonstrate that the intra- and inter-assay precisions were measured to be below 5.4 and 9.3%, respectively. The intra- and inter-assay accuracy ranged from 88.5 to 104.6% and from 90.6 to 107.5%, respectively.

The extraction recovery determined for moxonidine was shown to be consistent, precise and reproducible. The mean recoveries of the four QC levels were 85.26, 87.38, 89.20 and 91.25%, respectively, whereas the precision (R.S.D.) were 8.02, 4.32, 3.98 and 2.88%, respectively.

3.6. Stability

The results of freeze–thaw stability indicated that the analyte is stable in plasma for three freeze–thaw cycles, when stored at −20 °C and thawed to room temperature. Long-term stability indicates that storage of moxonidine plasma samples at −20 °C is adequate when stored for 30 days and no stability related problems would be expected during routine analysis for the pharmacokinetic, bioavailability or bioequivalence studies.

3.7. Pharmacokinetic study

The method was applied to determine the plasma concentration of moxonidine following a single oral administration (0.2 mg) to 20 healthy volunteers. Mean plasma concentration–time profile of moxonidine was presented in Fig. 3. The main pharmacokinetic parameters of moxonidine in 20 volunteers were calculated. After oral

Table 1
Data for validating the LLOQ (0.01976 ng/mL)

Analyte	1	2	3	4	5	R.S.D. (%)
Measured concentration (ng/mL)	0.01317	0.025616	0.017732	0.023669	0.018229	19.10

Table 2
Precision of the assay for determination of moxonidine in plasma (mean ± S.D., $n = 5$)

Analyte	Added to plasma (μg/mL)							
	Intra-assay				Inter-assay			
	0.0494	0.1976	0.988	4.94	0.0494	0.1976	0.988	4.94
Measured concentration (%)	80.2	104.8	98.1	98.2	82.1	99.1	106.6	98.0
	88.0	110.3	97.0	99.3	96.3	111.6	103.4	100.9
	87.2	102.8	100.8	97.9	83.8	115.7	105.5	99.6
	93.6	97.2	101.2	100.7	88.9	101.1	94.2	100.8
	93.3	107.8	97.9	102.3	101.8	110.2	101.5	96.6
Mean	88.5	104.6	99.0	99.7	90.6	107.5	102.2	99.2
S.D.	0.00270	0.0099	0.0187	0.090	0.00413	0.0140	0.048	0.093
R.S.D. (%)	5.39	4.64	1.90	1.82	9.23	6.58	4.77	1.90

administration of 0.2 mg moxonidine, T_{max} and C_{max} values were found to be 0.7 ± 0.2 h and 1.62 ± 0.34 ng/mL, respectively. Plasma concentrations declined with $t_{1/2}$ of 2.79 ± 0.24 h.

4. Conclusion

The proposed method of analysis provided a sensitive and specific assay for moxonidine determination in human plasma. No significant interference caused by endogenous compounds was observed. Simple liquid–liquid extraction procedure and short run time can provide a short analysis time that is important for large sample batches. It was shown that this method is suitable for the analysis of moxonidine in plasma samples collected for pharmacokinetic, bioavailability or bioequivalence studies.

References

[1] R. Martin, J. Wilhelm, S. Manfred, J. Pharm. Biomed. Anal. (1992) 323–328.
[2] R.A. Theodor, H.J. Weimann, W. Weber, Eur. J. Drug Metab. Pharmacokinet. 17 (1992) 61–66.
[3] Q. Zhang, L.M. Ma, H. Zhang, J. Pharm. Anal. 17 (1997) 368–370.
[4] 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.
[5] F. Qiu, X. Chen, X. Li, D. Zhong, J. Chromatogr. B 802 (2004) 291–297.
[6] D. Zhong, B. Yang, X. Chen, K. Li, J. Xu, J. Chromatogr. B 796 (2003) 439–444.
[7] M. Zhou, G. Wei, Y. Liu, Y. Sun, S. Xiao, L. Lua, C. Liu, D. Zhong, J. Chromatogr. B 798 (2003) 43–48.
[8] L. Liu, M. Qi, P. Wang, H. Li, J. Pharm. Biomed. Anal. 34 (2004) 1013–1019.
[9] L. Ding, X. Hao, X. Huang, S. Zhang, Anal. Chim. Acta 492 (2003) 241–248.