Available online at www.sciencedirect.com
ScienceDirect
Journal of Pharmaceutical and Biomedical Analysis 42 (2006) 213–217
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS
www.elsevier.com/locate/jpba

A sensitive LC–ESI-MS method for the determination of indapamide in human plasma: Method and clinical applications

Li Ding [a,*], Longhua Yang [a], Fang Liu [b], Wenzheng Ju [b], Ningning Xiong [b]

[a] *Department of Pharmaceutical Analysis, China Pharmaceutical University, 24 Tongjiaxiang, Nanjing 210009, China*
[b] *Organization for State Drug Clinical Trial, The Affiliated Hospital of Nanjing University of Traditional Chinese Medicine, Nanjing 210029, China*

Received 29 November 2005; received in revised form 7 March 2006; accepted 10 March 2006
Available online 16 June 2006

Abstract

A sensitive LC–ESI-MS method for the determination of indapamide in human plasma using glibenclamide as the internal standard (IS) was established. Following acidification with 1 M hydrochloric acid solution, plasma samples were extracted with ethyl acetate and separated on a C_{18} column with a mobile phase of 10 mM ammonium acetate–methanol (22:78, v/v). Indapamide was determined using electrospray 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* 364.3 for indapamide and *m/z* 492.4 for the IS. Calibration curves were linear over the ranges of 0.1–100 ng/ml for indapamide. The lower limit of quantification was 0.1 ng/ml. The intra- and inter-assay precisions were less than 9.5% and 10.6%, respectively. The mean plasma extraction recovery of indapamide was 90.5–93.9%. The method has been successfully applied to study the pharmacokinetics of indapamide in healthy male Chinese volunteers.

Keywords: Indapamide; LC–MS; Pharmacokinetics

1. Introduction

Indapamide (Fig. 1) is an oral antihypertensive diuretic agent indicated for the treatment of hypertensive and edema [1]. Indapamide inhibits carbonic anhydrase enzyme [2]. Recently, a new tablet formulation of indapamide was developed by Tianjin Pharmaceutical Group Xinzheng Co. Ltd. (Zhengzhou, China). As entrusted by Tianjin Pharmaceutical Group Xinzheng Co. Ltd., we carried out the study to determine the relative bioavailability of this new tablet formulation compared with Natrilix® (Servier Pharmaceutical Co. Ltd., France) and to evaluate the bioequivalence of the two products base on the plasma concentration data. Several articles [1,3,4] reported the pharmacokinetic parameters of indapamide in European volunteers, but there was no determination method described in these papers. Several HPLC methods [5–7] were developed for determination of indapamide in human whole blood, in which the lower limit of quantification (LLOQ) were 5–50 ng/ml. In fact, to evaluate the phamacokinetics of indapamide with its concentration in human whole blood, a method with an LLOQ of 5 ng/ml is enough. But, to evaluate the phamacokinetics of indapamide with its concentration in human plasma, a method with an LLOQ of 5 ng/ml is not sensitive enough, because the concentration of indapamide in human plasma is much lower than in human whole blood. Albu et al. [8] developed a liquid chromatography–electrospray tandem mass spectrometry method for determination of indapamide in serum, in which the LLOQ was 1 ng/ml. In this paper, we report an LC–ESI-MS method with an LLOQ as low as 0.1 ng/ml. This method was successfully applied to study pharmacokinetics of indapamide tablets in healthy male Chinese volunteers.

2. Experimental

2.1. Materials and reagents

Indapamide standard was from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Glibenclamide (IS, see Fig. 1) was a gift from Najing Xiandeng Pharmaceutic Science and Technology Development Co.

* Corresponding author. Tel.: +86 25 8327 1289; fax: +86 25 8327 1289.
E-mail address: dinglidl@hotmail.com (L. Ding).

0731-7085/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jpba.2006.03.039

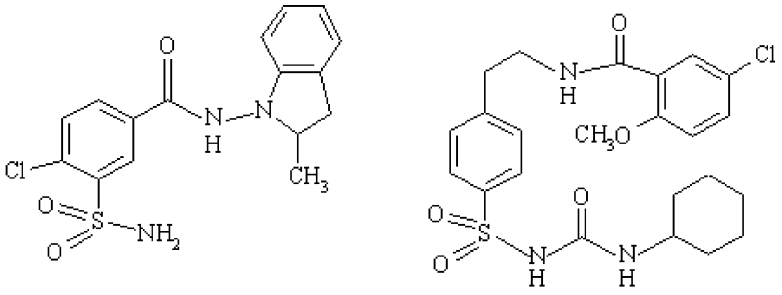

Fig. 1. Chemical strictures of indapamide (A) andglibenclamide (B).

Ltd. The reference preparation was Natrilix®, indapamide tablet which containing 2.5 mg of indapamide per tablet (Servier Pharmaceutical Co. Ltd., France). The test preparation was also indapamide tablet, which containing 2.5 mg of indapamide per tablet (Tianjin Pharmaceutical Groupe Xinzheng Co. Ltd., Zhengzhou, China). Methanol was of HPLC grade (Merck, Darmstadt, Germany). Hydrochloric acid, ammonium acetate and ethyl acetate were analytic-grade purity and purchased from Nanjing Chemical Regent Co. (Nanjing, China).

2.2. Instrument and conditions

HPLC analyses were performed using an Agilent 1100 LC–ESI-MS system (Agilent Technologies, Palo Alto, CA) with a column of Lichrospher ODS 5 μm, 250 mm × 4.6 mm i.d. (Jiangsu Hanbon Science & Technology Co. Ltd, China). The mobile phase was 10 mM ammonium acetate water solution–methanol (22:78, v/v) at a flow rate of 0.8 ml/min. The column temperature was maintained at 25 °C. 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, drying gas temperature of 350 °C, capillary voltage of 4 kV and the negative ion mode. The fragmentor voltage was 120 V. LC–ESI-MS was performed in selected-ion monitoring mode using target ions at *m/z* 364.3 for indapamide and *m/z* 492.4 for IS.

2.3. Preparation of standard solutions

Primary stock solutions of indapamide and IS were prepared at 1 mg/ml in methanol and stored at −20 °C. Standard solutions of indapamide were prepared at concentrations of 100 μg/ml, 10 μg/ml, 1 μg/ml, 100 ng/ml, 10 ng/ml and 1 ng/ml by serial diluting the primary stock solution with methanol in separate 10 ml volumetric flasks. A solution containing 1 μg/ml IS was also prepared by further diluting the primary stock solution of IS with methanol. All the solutions were stored at −20 °C.

2.4. Sample preparation

A 0.5-ml aliquot plasma sample was extracted by vortex-mixing for 3 min with 4 ml ethyl acetate after addition of 50 μl IS solution (1 μg/ml) and 0.1 ml of 1 M hydrochloric acid solution. Following centrifugation and separation, the organic phase was evaporated to dryness under a stream of nitrogen in a water bath of 35 °C. The residue was reconstituted in 100 μl of mobile phase, and a 40-μl aliquot was injected for analysis by HPLC–ESI-MS.

2.5. Calibration curves

Calibration standards of indapamide were prepared by spiking appropriate amounts of the standard solutions in 0.5 ml blank plasma obtained from healthy volunteers. Standard curves were prepared in the range of 0.1–100 ng/ml for indapamide at concentrations of 0.1, 0.3, 1, 3, 10, 20, 50 and 100 ng/ml. The calibration curve was prepared and assayed along with quality control (QC) samples and each batch of clinical plasma samples.

2.6. Preparation of quality control samples

QC samples were prepared in 0.5 ml blank plasma at concentrations of 0.1, 1, 10 and 100 ng/ml for indapamide and stored at −20 °C. QC samples were analyzed with processed test samples at intervals in each run. The results of the QC samples provided the basis of accepting or rejecting the run.

3. Clinical study design and pharmacokinetic analysis

Twenty healthy young male Chinese volunteers participated in the study. After an overnight fast, each volunteer received one tablet containing 2.5 mg indapamide. Blood was sampled pre-dose and at 0.25, 0.5, 0.75, 1, 2, 3, 5, 8, 12, 24, 48, 72 and 96 h post-dose for determination of plasma concentration of indapamide. Model-independent pharmacokinetic parameters were calculated for indapamide. The maximum plasma concentrations (C_{max}) and the time to those (t_{max}) were noted directly. The elimination rate constant (k_{el}) was calculated by linear regression of the terminal points of the semi-log plot of plasma concentration against time. Elimination half-life ($t_{1/2}$) was calculated from the formula $t_{1/2} = 0.693/k_{el}$. Area under the plasma concentration–time curve [$AUC_{(0-96)}$] to the last measurable plasma concentration (C_p) was calculated by the linear trapezoidal rule.

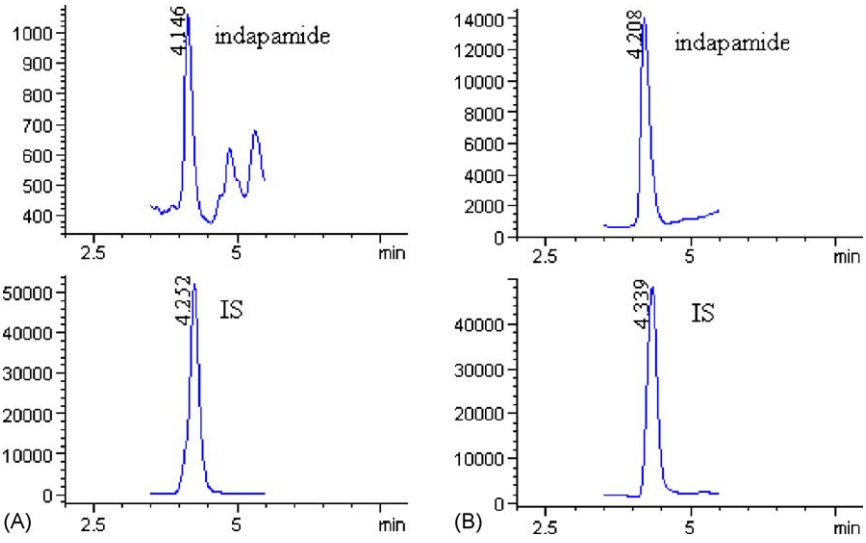

Fig. 2. Typical SIM chromatograms of LLOQ for indapamide in plasma (0.1 ng/ml) and IS (A), plasma obtained from a volunteer at 48 h after oral administration of 2.5 mg indapamide, the plasma concentration of indapamide was estimated to be 9.33 ng/ml (B).

4. Results and discussion

4.1. Conditions of chromatography

When selecting the mobile phase for LC–MS, attentions should be paid to the influence of mobile phase on the MS sensitivity. To achieve shorter run time and high ionization efficiency, a higher ratio of methanol was utilized in the mobile phase. Further experiment results showed that an ammonium acetate solution could not only improve peak shapes of indapamide and IS, but also increase the MS sensitivity to indapamide and IS. So, a 10 mM ammonium acetate solution was finally adopted in mobile phase. The ratio of aqueous solution in mobile phase could significantly affect the ionization efficiency and MS sensitivity. The experiment results showed that the MS sensitivity increased alone with the decreasing of the ratio of ammonium acetate solution in the mobile phase until the ratio decreased to 10% (v/v). But when the methanol ratio in the mobile phase exceeded 80%, the interference of indapamide from plasma would encounter. Finally, high sensitivity, good separation of indapamide and short run time were obtained by using an elution system of 10 mM ammonium acetate water solution–methanol (22:78, v/v) as the mobile phase. Representative selected-ion chromatograms are shown in Fig. 2 in which the retention times were 4.2 min for indapamide and 4.3 min for IS.

4.2. Conditions for ESI-MS

The ESI mass spectrum at a fragmentor voltage of 120 V showed that the negative ion $[M-H]^-$ of indapamide was at *m/z* 364.3. Fig. 3(A) shows a typical full-scan ESI-negative mass spectrum of indapamide at a 120 V fragmentor voltage. In order to determine the optimal fragmentor voltage, the intensities of selected ion of indapmide at *m/z* 364.3 was compared at fragmentor voltages of 90, 100, 120, 150 and 180 V. The result showed that the highest sensitivity was obtained using a fragmentor voltage of 120 V. Therefore, a fragmentor voltage of 120 V was used to carry out LC–ESI-MS in the assay. At this fragmentor voltage, the most intensive ion of IS was at *m/z* 492.4 (see Fig. 3(B)).

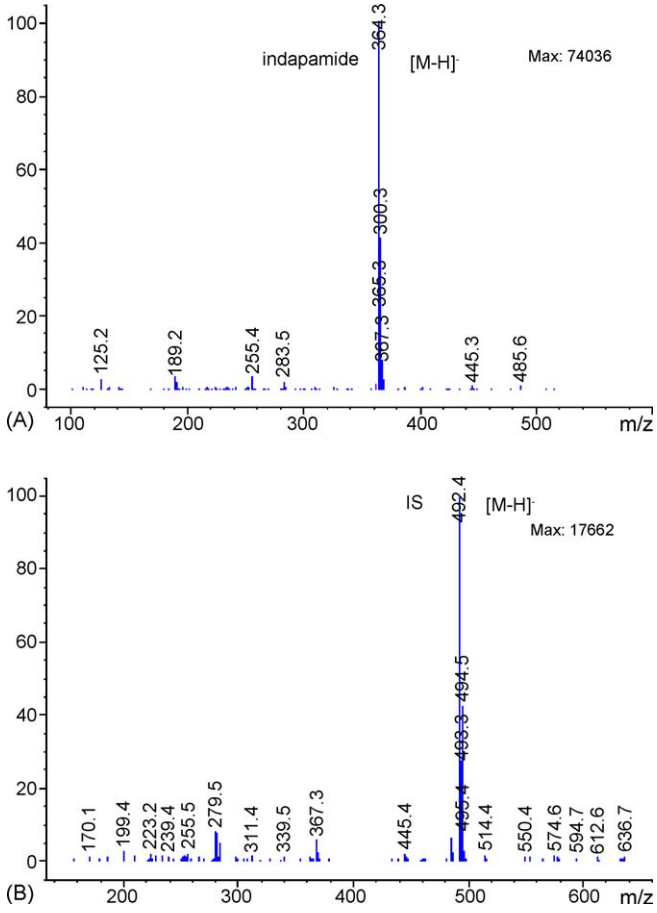

Fig. 3. Mass spectra of the negative ion of irdapamide (A) and IS (B) at 120 V fragmentor voltage.

Table 1
Precision and accuracy of the assay for determination of indapamide in plasma ($n = 3$ days, five replicates per day)

Added to plasma (ng/ml)	Mean measured concentration (ng/ml)	RE (%)	Intra-assay R.S.D.%	Inter-assay R.S.D.%
0.1	0.111	10.7	9.4	10.6
1.0	0.937	−6.3	3.1	6.4
10.0	9.52	−4.8	3.1	5.7
100	96.6	−3.4	2.1	3.3

Therefore, the negative ion $[M-H]^-$ (m/z 492.4) of IS was selected as the target ion of IS in the SIM.

4.3. Method validation

4.3.1. Calibration curve and sensitivity

The calibration curves were prepared in the range of 0.1–100 ng/ml for indapamide at concentrations of 0.1, 0.3, 1, 3, 10, 20, 50 and 100 ng/ml. The indapamide calibration curve was constructed by plotting the peak area ratio of indapamide to the IS versus the concentration of indapamide, using weighted least squares linear regression (weighting factor was $1/C^2$) [9–11]. The typical calibration curve for indapamide had a slope of 0.02464 ± 0.00018, an intercept of 0.00015 ± 0.00038 and $R = 0.9986$. Calibration curves were prepared with each batch of clinical samples. The LLOQ for indapamide in plasma was 0.1 ng/ml.

4.3.2. Assay precision and accuracy

Validation samples were prepared and analyzed on three consecutive days to evaluate the accuracy, intra-batch and inter-batch precisions of the analytical method. Accuracy is defined as the relative deviation in the calculated value (E) of a standard from that of its true value (T) expressed as a percentage (RE%). It was calculated using the formula: $RE\% = (E - T)/T \times 100$. The precision was calculated by using one-way ANOVA [12]. The intra- and inter-batch precision and accuracy are summarized in Table 1.

4.3.3. Extraction recovery

The ethyl acetate was chosen as the extraction solvent for its higher extraction efficiency to the two target compounds. The extraction recoveries of indapamide and IS can be promoted by acidizing the plasma samples with hydrochloric acid, so a 0.1 ml aliquot of 1 M hydrochloric acid was added to 0.5 ml plasma sample before extraction. Recovery was calculated by comparison of the peak areas of indapamide extracted from plasma samples with those of injected standards. The mean extraction recovery of indapamide from human plasma with ethyl acetate was $91.8 \pm 1.6\%$.

4.3.4. Stability

The stability of indapamide was studied under a variety of storage and handling conditions. The results showed that no significant degradation occurred at ambient temperature for 10 h and during the three freeze-thaw cycles for indapamide plasma samples. Indapamide in plasma at −20 °C was stable for 18 days at least. Indapamide and IS in extraction solution at ambient temperature were stable for 24 h at least. The stock solutions of indapamide and IS at −20 °C were stable for 2 month at least.

4.3.5. Assay selectivity

The selectivity of the assay was checked by analyzing blank plasma samples of twenty volunteers. Each blank plasma sample was tested using the proposed extraction procedure and HPLC–ESI-MS conditions to ensure no interference of indapamide and IS from plasma.

4.3.6. System suitability test

Prior to running each batch of clinical plasma samples, the instrument performance (e.g., sensitivity, reproducibility of chromatographic retention and separation, plate number and tailing factor) was determined by analysis of the reference standard of indapamide, IS, blank plasma and plasma spiked with indapamide and IS.

4.4. Application

The method described above was successfully applied to the pharmacokinetic study in which plasma concentrations of indapamide in 20 healthy Chinese male volunteers were determined up to 96 h after administration of tablets containing 2.5 mg indapamide. The mean plasma concentration–time curve of indapamide is shown in Fig. 4. The mean pharmacokinetic parameter values are calculated and summarized in Table 2. The relative bioavailability of test preparation was $97.3 \pm 16.3\%$, base on the test-reference ratios of AUC. Result of variance analysis and two one-side t-test showed that there was no statistical significant difference between the two prepa-

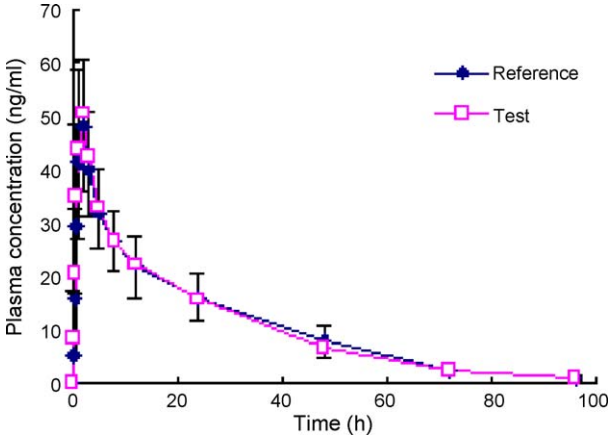

Fig. 4. Mean indapamide plasma concentration–time profile in 20 healthy volunteers after oral administration of 2.5 mg indapamide tablet.

Table 2
Mean pharmacokinetic parameters of indapamide for 20 volunteers after oral administration of 2.5 mg indapamide tablet

Parameters	Test tablet	Reference tablet
$t_{1/2}$ (h)	18.2 ± 4.3	18.4 ± 4.0
C_{max} (ng/ml)	55.8 ± 8.4	55.3 ± 10.5
t_{max} (h)	1.6 ± 0.6	1.7 ± 0.7
$AUC_{0–96}$ (h ng/ml)	1017 ± 249	1049 ± 204

rations in the AUC and C_{max}. In the case of t_{max} comparison between the two preparations was carried out by the Wiloxon-ranked sign test for the matched pairs, and the statistical result showed that there was no significant difference between the two preparations.

5. Conclusions

The method achieved good sensitivity and specificity for the determination of indapamide in human plasma. No significant interferences caused by endogenous compounds was observed. This simple and sensitive assay is suitable for pharmacokinetic studies of indapamide in human subjects.

References

[1] F.S. Caruso, R.R. Szabadi, R.A. Vukovich, Am. Heart 106 (1983) 212–220.
[2] M.M. Johnston, M.J. Rosenberg, A.K. Yeung, P.E. Grebow, J. Pharm. Sci. 69 (1980) 1158–1160.
[3] P.E. Grebow, J.A. Treitman, E.P. Barry, Eur. J. Clin. Pharmacol. 22 (1982) 295–309.
[4] P. Schiavi, R. Jochemsen, D. Guez, Fundam. Clin. Pharmacol. 14 (2000) 139–146.
[5] D. Zendelovska, T. Stafilov, M. Stefova, J. Chromatogr. B 788 (2003) 199–206.
[6] T.J. Hang, W. Zhao, J. Lin, J. Pharm. Biomed. Anal. 40 (2006) 202–205.
[7] X.L. Gao, J. Chen, N. Mei, W.X. Tao, W.M. Jiang, X.G. Jiang, Chromatographia 61 (2005) 581–585.
[8] F. Albu, C. Georgit, V. Davidb, A. Medvedovici, J. Chromatogr. B 816 (2005) 35–40.
[9] 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.
[10] B. Boulanger, P. Chiap, W. Dewe, J. Crommen, Ph. Hubert, J. Pharm. Biomed. Anal. 32 (2003) 753–765.
[11] C. Hartmann, J. Smeyers-Verbeke, D.L. Massart, R.D. McDowall, J. Pharm. Biomed. Anal. 17 (1998) 193–218.
[12] J. Xing, X.Y. Chen, D.F. Zhong, J. Pharm. Biomed. Anal. 39 (2005) 593–600.