Available online at www.sciencedirect.com
SCIENCE DIRECT
Journal of Chromatography B, 834 (2006) 204–207
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Short communication

Determination of mitiglinide in rat plasma by high-performance liquid chromatography with UV detection

Lushan Yu, Su Zeng *

Department of Pharmaceutical Analysis and Drug Metabolism, College of Pharmaceutical Sciences, Zhejiang University, Hangzhou, Zhejiang 310031, China

Received 7 February 2006; accepted 9 February 2006
Available online 6 March 2006

Abstract

A selective and sensitive high-performance liquid chromatography method has been developed and validated for determination of mitiglinide (MGN) in rat plasma using 2-(4-biphenylyl) propionic acid (BPA) as internal standard. Liquid–liquid extraction was used for sample preparation. Chromatographic separation was achieved on a C_{18} column using acetonitrile and 0.02 mol/l KH_2PO_4 buffer (pH 4.0) (45:55, v/v) as mobile phase delivered at 1.0 ml/min. The UV detector was set at 210 nm. The assay was linear over the range 0.1–20 μg/ml for MGN. The average extraction recoveries of MGN and BPA from rat plasma were 98.6 and 97.4%, respectively. The developed method has been applied to the pharmacokinetic study of MGN in rats.

Keywords: Mitiglinide; Pharmacokinetic

1. Introduction

Mitiglinide (MGN, Fig. 1A), (−)-2(*S*)-benzyl-4-(*cis*-perhydroisoindol-2-yl)butyric acid, is a new insulinotropic agent of the glinide class with rapid onset [1]. The calcium hydrate form is used in clinical practice. MGN is thought to stimulate insulin secretion by closing the ATP-sensitive K(+) (K (ATP)) channels in pancreatic beta-cells. Its early insulin release and short duration of action could be effective in improving postprandial hyperglycemia [2]. In vitro and in vivo studies have demonstrated that the insulinotropic effect of MGN is more potent than that of nateglinide [3]. To date, little information concerning the pharmacokinetic properties of MGN and analytical methods in biological samples is available. Therefore, the development of a simple and rapid method to determine MGN is necessary and valuable for further study.

In this paper, a high-performance liquid chromatography method was developed for determination of MGN in rat plasma. The technique was fully validated and successfully applied to the plasma pharmacokinetic study of MGN in rats after oral administration.

2. Experimental

2.1. Chemicals and regents

MGN calcium hydrate was kindly provided by Shangdong Chengchuang Medicine Co. Ltd. (Shangdong, China) with purity of 99.8%. 2-(4-Biphenylyl) propionic acid (BPA, Fig. 1B) was supplied by the Department of Pharmaceutical Chemistry (Zhejiang University, Hangzhou, China). Acetonitrile (HPLC grade) was purchased from Tedia (Tedia Company Inc., USA). All other chemicals and solvents were analytical reagents and obtained from commercial sources.

2.2. Standards

Standard stock solutions of MGN and BPA, each with a known concentration of 1 mg/ml, were accurately prepared in methanol. The stock solutions were diluted stepwise with methanol to form working standards of 5, 20 and 100 μg/ml for MGN. A 400-μg/ml solution of internal standard (BPA)

* Corresponding author. Tel.: +86 571 87217060; fax: +86 571 87217060.
E-mail address: zengsu@zju.edu.cn (Z. Su).

1570-0232/$ – see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2006.02.029

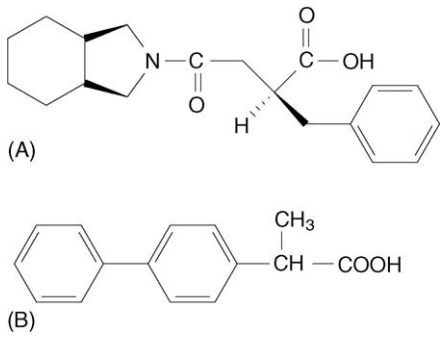

Fig. 1. Chemical structures of MGN (A) and BPA (B, internal standard).

was also prepared in methanol. Stock solutions were stored at 4 °C.

2.3. Chromatography

The HPLC system consists of a LC-10ATvp pump, a manual injector with a 20-μl fixed loop and a SPD-10Avp UV–vis detector (Shimadzu, Japan). Separation was performed on a Lichrospher C_{18} column (5 μm, 200 mm × 4.6 mm; Dikma Technologies, Dalian, China) preceded by an ODS guard column (10 μm, 10 mm × 5 mm) at ambient temperature. Analysis was isocratic at 1.0 ml/min flow rate with acetonitrile/0.02 mol/l KH_2PO_4 buffer (pH 4.0) (45:55, v/v) as mobile phase. Eluted peaks were detected at 210 nm. Chromatographic data were recorded and processed using a HS2000 Station Version 4.0 (Empire Science, Hangzhou, China).

2.4. Blanks and plasma standards for validation

Blank rat plasma was extracted and analyzed for the assessment of potential interference by endogenous substances. To prepare plasma standards for validation, blank plasma (0.1 ml) was spiked with known concentrations of MGN and internal standard. A standard curve consisted of samples containing 0.1, 0.2, 0.5, 1.0, 1.5, 2.0, 5.0, 10.0, 15.0 and 20.0 μg/ml of MGN. To evaluate the precision and accuracy of the assay, control standards were prepared at concentrations of 0.4, 2.5 and 12 μg/ml. To investigate the stability of the compound, three freeze–thaw cycles were adopted. To determine the recovery of the assay, control standards were prepared at concentrations of 0.5, 2.0 and 10.0 μg/ml for MGN, and 20.0 μg/ml for the internal standard (IS).

2.5. Sample preparation

In one 1.5-ml Ependorff tube, 100-μl rat plasma was mixed with 5 μl of internal standard stock solution, 100 μl of 0.05 mol/l KH_2PO_4 buffer solution (pH 2.0) and 1 ml chloroform. The mixture was vortexed for 2 min and centrifuged at 3000 × *g* for 10 min. The organic phase was transferred into a glass tube and evaporated to dryness at 45 °C under a stream of nitrogen. The residue was reconstituted with 50 μl of mobile phase and an aliquot of 20 μl was injected into the HPLC system.

2.6. Application of the assay

The method described above was applied to the pharmacokinetic studies of MGN in rats. Sprague-Dawley rats (180–200 g, equal numbers of male and female rats) were housed with free access to food and water, except for the final 12 h before experimentation. After a single oral administration of 2.5 mg/kg of MGN, 0.3 ml of blood samples via the caudal vein were collected at 5, 10, 15, 20, 30, 50, 70, 120, 180 and 300 min time-points. Plasma was separated by centrifugation and stored at −20 °C until analysis. Aliquots of 0.1 ml plasma samples were processed and analyzed for MGN concentrations.

3. Results and discussion

3.1. Selectivity and specificity

Typical chromatograms are shown in Fig. 2. Observed retention times are 14.1 and 18.8 min for MGN and BPA, respectively. Blank plasma was tested for endogenous interference. A representative chromatogram of the plasma blank is shown in Fig. 2A. No additional peaks of endogenous substances were observed. Fig. 2B shows the chromatograms of calibration standard containing 2.5 μg/ml of MGN in plasma. Typical chromatograms of plasma samples are shown in Fig. 2C, which were collected 15 min after oral administration of 2.5 mg/kg of MGN to a rat and the corresponding concentration of MGN was found to be 1.9 μg/ml.

3.2. Linearity and limit of quantitation

Linear calibration curves with correlation coefficients greater than 0.999 were obtained over the concentration range 0.1–20 μg/ml for MGN in rat plasma. The typical equation of the calibration curve is as follows: $y = 0.1471x + 0.0079$, $r = 0.9997$.The standard deviation (S.D.) for the slope and intercept were 0.0119 and 0.0013 ($n = 8$), respectively, showing that the calibration curve had good reproducibility. The limit of quantitation (LOQ), defined as the lowest concentration analyzed with an accuracy of ±15% and a coefficient of variation <15%, was 0.1 μg/ml for the determination of MGN in plasma ($n = 5$) Fig. 2D.

3.3. Precision and accuracy

Precision and accuracy of the assay were determined, using replicate analyses ($n = 6$) of quality control samples at three concentrations, by performing the complete analytical runs on the same day and also on three consecutive days. The data obtained for MGN were within the acceptable limits to meet the guidelines for bioanalytical methods. The results are summarized in Table 1.

3.4. Extraction recovery

Extraction recovery of MGN was determined by comparing peak areas obtained from extracted plasma samples with

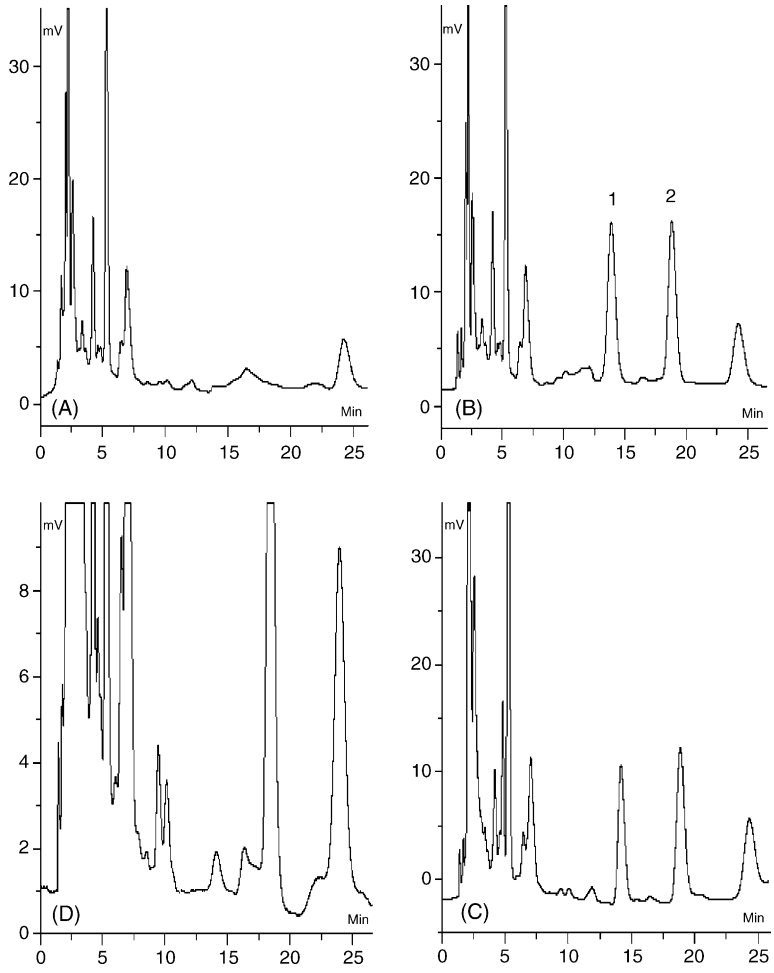

Fig. 2. HPLC trace of MGN and the internal standard (IS) using ultraviolet detection at 210 nm. (A) Blank plasma sample; (B) quality control standard (2.5 μg/ml); (C) plasma sample 15 min post administration of 2.5 mg/kg MGN (1.9 μg/ml); (D) plasma sample for LOQ (0.1 μg/ml). Peak 1 is MGN and peak 2 is IS.

those found by extracting blank matrices through the extraction procedure and spiking with a known amount of MGN. The results showed that the mean extraction recoveries of MGN were 98.9 ± 2.4, 98.3 ± 1.5 and 98.6 ± 0.6% at concentrations of 0.5, 2.0 and 10.0 μg/ml, respectively. The mean extraction recovery of the internal standard was 97.4 ± 0.4%.

Table 1
Intra-assay and inter-assay variability and accuracy of the quantitation of MGN ($n = 6$)

	Added C (μg/ml)		
	0.4	2.5	12
Intra-day run			
Mean found C (μg/ml)	0.42	2.50	12.14
S.D.	0.04	0.11	0.74
C.V. (%)	10.3	4.5	6.1
Accuracy (%)	104.1	100.1	101.2
Inter-day run			
Mean found C (μg/ml)	0.41	2.48	11.76
S.D.	0.04	0.16	0.61
C.V. (%)	8.9	6.3	5.2
Accuracy (%)	103.2	99.0	98.1

Different organic extraction solvents were evaluated in the experiment, including ethyl acetate, dichloromethane, chloroform and diethyl ether. Chloroform proved to be the most efficient in extracting MGN from rat plasma and had a small variation in extraction recoveries over the concentration range. In addition, pH values of the sample solutions were also evaluated for their extraction efficiency by adding 100 μl of KH_2PO_4 buffer solutions in different pH values (pH 1, 2, 3, 4, 5, 6 and 7). pH values of the buffer solutions were adjusted using KOH or H_3PO_4. Although the extraction efficiency were similar when adding 100 μl of pH 1 or 2 buffer solutions, the buffer solution (pH 2) was selected due to the possible instability of MGN and IS at low pH conditions.

3.5. Stability

The stability of MGN in rat plasma was investigated under different storage and process conditions. The analytes were found to be stable (coefficient of variation, C.V. <6%) in rat plasma after three freeze–thaw (−20 °C/room temperature) cycles. The analytes were also shown to be stable after 24 h storage in reconstitution solutions at room temperature (C.V. <5%). The good stability of MGN simplifies the precau-

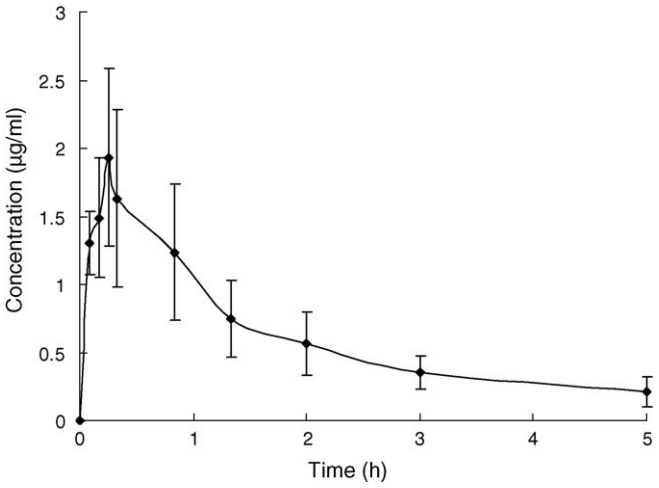

Fig. 3. Mean plasma concentration–time profile of MGN after oral administration of 2.5 mg/kg MGN in rats.

tions needed for laboratory manipulations during the analytical procedures.

3.6. Application of the analytical method in pharmacokinetic studies

The described method was applied to a pharmacokinetic study in rats. The mean plasma concentration–time curve after an oral dose of 2.5 mg/kg MGN is shown in Fig. 3. The C_{max} of MGN detected in the rats was 1.9 μg/ml, and the t_{max} was 15 min.

4. Conclusion

A sensitive and selective HPLC method was developed for the pharmacokinetic study of MGN in rats. The assay has been validated and the results have shown that the method is sensitive, accurate and reproducible.

Acknowledgements

This project was supported by Natural Science Foundation of China (30225047 and 39770868) and Zhejiang Provincial Natural Science Foundation of China (C397500 and RC97016).

References

[1] L.A. Sorbera, P.A. Leeson, R.M. Castańer, J. Castańer, Drugs Future 25 (2000) 1034.
[2] H. Ohnota, T. Kitamura, M. Kinukawa, S. Hamano, N. Shibata, H. Miyata, A. Ujiie, Jpn. J. Pharmacol. 71 (1996) 315.
[3] K. Ojima, Y. Kiyono, M. Kojima, Nippon Yakurigaku Zasshi 124 (2004) 245.