ELSEVIER

Journal of Chromatography B, 710 (1998) 243–246

JOURNAL OF CHROMATOGRAPHY B

Short communication

Simple high-performance liquid chromatographic method for the determination of metformin in human plasma

Kah Hay Yuen*, Kok Khiang Peh

School of Pharmaceutical Sciences, University of Science Malaysia, 11800 Penang, Malaysia

Received 14 November 1997; received in revised form 24 February 1998; accepted 24 February 1998

Abstract

A simple high-performance liquid chromatographic method using ultraviolet detection was developed for the determination of metformin in human plasma. The method entailed direct injection of the plasma sample after deproteination using perchloric acid. The mobile phase comprised 0.01 *M* potassium dihydrogen orthophosphate (pH 3.5) and acetonitrile (60:40, v/v). Analyses were run at a flow-rate of 1.0 ml/min with the detector operating at a detection wavelength of 234 nm. The method is specific and sensitive, with a quantification limit of approximately 60 ng/ml and a detection limit of 15 ng/ml at a signal-to-noise ratio of 3:1. The mean absolute recovery value was about 97%, while the within-day and between-day coefficient of variation and percent error values of the assay method were all less than 8%. The calibration curve was linear over a concentration range of 62.5–4000 ng/ml.

Keywords: Metformin

1. Introduction

Metformin–HCl is a biguanide type of oral antidiabetic agent that is used in the treatment of non-insulin-dependent diabetes mellitus and is highly recommended for obese patients [1]. Its structural formula is as shown in Fig. 1. Various analytical methods, including calorimetry [2], gas chromatography (GC) [3–7] and high-performance liquid chromatography (HPLC) [8–10], have been developed for the determination of metformin in biological samples. The calorimetry method [2] may not be sufficiently selective and sensitive for routine measurements of the drug in plasma, while the GC methods [3–7] require extensive sample preparation and derivatisation before analysis. HPLC methods using ultraviolet (UV) detection were reported by Charles et al. [8] and, more recently, by Huupponen et al. [9] and Caille et al. [10]. Charles et al. [8] reported a cation-exchange HPLC method with direct injection of the plasma samples after pretreatment with trichloroacetic acid. However, the pH of the mobile phase used was 2.4, which may be too acidic and could lead to rapid deterioration of the silica base stationery phase and, hence, shortening of the column's life. Huupponen et al. [9] and Caille et al.

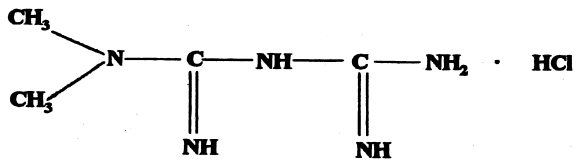

Fig. 1. Structural formula of metformin–HCl.

*Corresponding author.

0378-4347/98/$19.00
PII: S0378-4347(98)00117-0

[10], on the other hand, reported the use of a solid-phase extraction procedure in the sample preparation, which appeared to be tedious and time consuming. Furthermore, the method of Caille et al. [10] was not validated for accuracy, precision and sensitivity.

In this paper, we report a relatively simple, specific and sensitive HPLC method using UV detection for the determination of metformin in human plasma. We also demonstrated the applicability of this method in a bioavailability study.

2. Experimental

2.1. Materials

Perchloric acid (60%) was purchased from Merck (Darmstadt, Germany). Metformin–HCl was purchased from Tender Remedies (Bombay, India). Potassium dihydrogen orthophosphate, AR grade, was purchased from Ajax Chemicals (Sydney, Australia). All of the other solvents used were either of AR- or HPLC grade and were purchased from Mallinckrodt (Kentucky, USA).

2.2. Instrumentation

The HPLC system comprised a Jasco PU-980 pump (Jasco, Hachioji City, Tokyo, Japan), a Gilson 119 UV/Vis detector (Gilson Medical Electronics, Villiers-le-Bel, France) and an Hitachi D-2500 Chromato-integrator (Hitachi, Tokyo, Japan). The detector was operated using a sensitivity range of 0.005 AUFS, an output of 15 mV and a wavelength of 234 nm. A Supelcosil LC-CN column from Supelco (Bellefonte, PA, USA; 5 μm, 250×4.6 mm I.D.), fitted with a refillable guard column (Upchurch Scientific, Oak Harbour, WA, USA), packed with Spherisorb CN-5 μm (Keystone Scientific, Bellefonte, PA, USA), was used for the chromatographic separation. The mobile phase comprised 0.01 *M* potassium dihydrogen orthophosphate (adjusted to pH 3.5 with glacial acetic acid) and acetonitrile (60:40, v/v). Analyses were run at a flow-rate of 1.0 ml/min and the samples were quantified using peak height.

2.3. Sample preparation

A 250-μl aliquot of plasma sample was measured into an eppendorf microcentrifuge tube and deproteinized by adding 10 μl of 60% perchloric acid. The mixture was vortex-mixed for 1 min using a vortex mixer and then centrifuged at 12 800 *g* for 3 min. The supernatant was transferred to a new eppendorf microcentrifuge tube and 50 μl were injected onto the column.

2.4. Assay validation

Standard calibration curves were constructed by spiking drug-free pooled plasma with a known amount of metformin in the concentration range of 62.5–4000 ng/ml. These plasma standards were also used to determine the within-day and between-day precision and accuracy ($n=6$) of the method. In addition, the absolute recovery ($n=6$) was estimated by comparison with directly injected aqueous drug solutions of corresponding concentrations.

3. Results and discussion

HPLC analysis of biological samples, such as plasma, requires sample preparation or clean-up prior to injecting into the HPLC system. Metformin is a biguanide compound that is highly polar in nature, making its extraction from biological samples using organic solvents difficult. Thus, methods using solid-phase extraction have been successfully employed and reported [9,10]. The method employed in our study involved the direct injection of the plasma sample after precipitation with perchloric acid. Since only 10 μl of the precipitating agent were used with 250 μl of plasma sample, there was minimal dilution with no significant loss in sensitivity of the assay method. The amount of perchloric acid used for deproteination was found to be critical. It was also observed that 10 μl of 60% perchloric acid was adequate for deproteinizing 250 μl of plasma sample. When the amount of perchloric acid used was increased to 30 μl, the metformin peak became skewed, non-symmetrical and broad.

An internal standard was used in the study of Charles et al. [8], but was deemed unnecessary in our

study as a linear curve was obtained between the peak height (signals) and the metformin concentration in the plasma over the range of concentrations (62.5–4000.0 ng/ml) measured.

Chromatograms obtained with blank plasma and plasma spiked with metformin are shown in Fig. 2A,B, while that of a volunteer 1 h after dosing with 850 mg of metformin is shown in Fig. 2C. It can be seen that the metformin peak, with a retention time of 5.9 min, was well resolved and free of interference from endogenous compounds in the plasma. In addition, the total run time for each injection/sample was only 8 min. The acetonitrile content in the mobile phase was found to be critical in separating

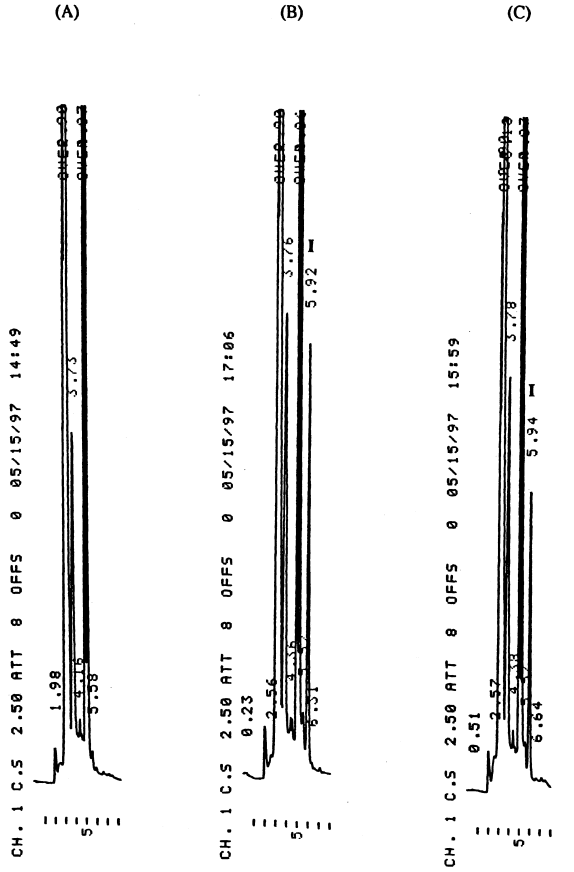

Fig. 2. Chromatograms of metformin in plasma. (A) Blank plasma. (B) Plasma spiked with 2000.0 ng/ml metformin. (C) Plasma from a volunteer containing 1517.8 ng/ml metformin 1 h after oral administration of 850 mg of metformin. (*Y*-axis, attenuation= 8; *X*-axis, chart speed=2.5 mm/min; I=metformin)

metformin from endogenous compounds, with the best resolution being achieved at a concentration of 40% (v/v). When the acetonitrile content was below 40% (v/v), the elution time of the metformin peak was correspondingly reduced and the peak was not well separated from an adjacent peak. The two peaks gradually overlapped as the acetonitrile content was further reduced to 20% (v/v). On the other hand, when the acetonitrile content was increased to above 40% (v/v), the elution time of metformin was correspondingly increased and the peak height was correspondingly decreased, resulting in a drop in the sensitivity of the assay method from 15 to 50 ng/ml. It is interesting to note that the retention time of metformin was increased on increasing the acetonitrile content, which is not in accord with reversed-phase chromatography. This could be due to the fact that the cyano (CN) column used for the chromatographic separation was more polar than the mobile phase used. Thus, the system behaved more like that of a normal phase chromatographic system.

The absolute recovery, within-day and between-day accuracy and precision values are presented in Table 1. The average absolute recovery value was about 97%, while the coefficient of variation (C.V.) and percent error values of both the within-day and between-day precision and accuracy were all less than 8%. The standard calibration curve ($n=6$) was found to be linear over the concentration range used, with a correlation coefficient of 0.99995. A detection limit of 15 ng/ml was obtained at a signal-to-noise ratio of 3:1, but could be further improved by using a larger volume sample loop. However, the quantification limit was set at 60 ng/ml, which was not more than 5% lower than the lowest concentration used to construct the standard curve. Our assay method was found to be more sensitive than that of Charles et al. [8], who reported a value of 100 ng/ml, even though a smaller injection volume was used in our study.

The present method was used to analyze plasma samples from twelve healthy adult male volunteers from a comparative bioavailability study of two different controlled release metformin–HCl tablet preparations, namely, Glucophage Retard and Diabetmin Retard, the latter being a generic preparation. Fig. 3 shows the individual plasma concentration–time profiles of the volunteers that were obtained using the two preparations. It can be seen from the

Table 1
Absolute recovery, within-day and between-day precision and accuracy (n=6)

Concentration (ng/ml)	Recovery		Within-day		Between-day	
	Mean (%)	C.V. (%)	Precision (C.V.%)	Accuracy (%)	Precision (C.V.%)	Accuracy (%)
62.5	97.4	7.1	5.3	101.9	6.6	108.6
125.0	92.0	6.2	3.6	99.3	7.5	91.4
250.0	98.6	4.8	1.6	103.6	6.3	102.3
500.0	96.4	1.4	2.8	97.9	4.6	102.4
1000.0	98.6	5.2	1.3	103.9	3.4	100.9
2000.0	100.4	4.0	0.7	104.4	1.8	101.6
4000.0	95.0	1.9	0.7	103.6	3.2	99.7

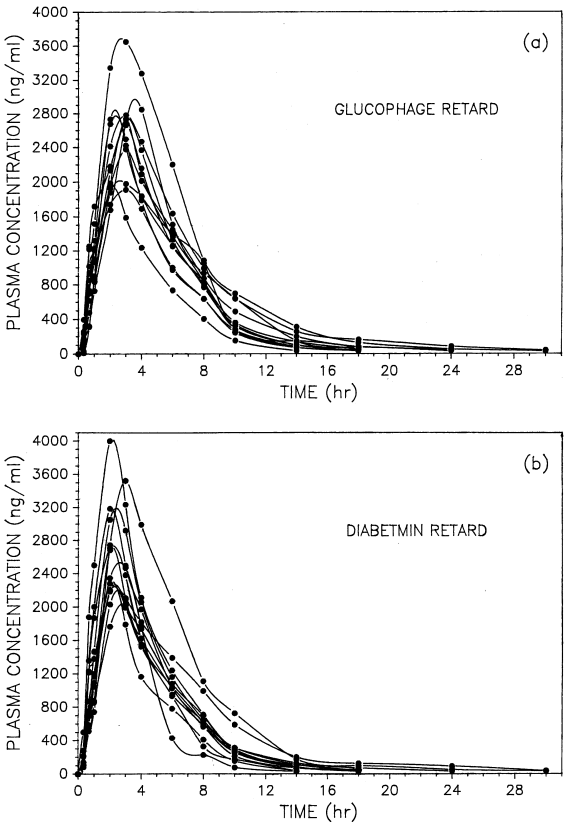

Fig. 3. Plasma metformin concentration versus time profiles for twelve volunteers following oral administration of 850 mg of Glucophage Retard (a) and Diabetmin Retard (b).

plasma profiles of both preparations that metformin could still be detected up to at least 18 h and, in all cases, the last detectable level was less than 10% of the peak plasma concentration.

In conclusion, the present HPLC method was simple, specific, sensitive and suitable for use in the determination of metformin in pharmacokinetic/bioavailability studies.

References

[1] R. Vigneri, I.D. Goldfine, Diabetes Care 10 (1987) 118.
[2] B.E.P. Murphy, Acta Endocrinol. Suppl. 147 (1970) 37.
[3] R. Beckmann, Diabetologia 5 (1969) 318.
[4] S.B. Martin, J.H. Karam, P.H. Forsham, J.B. Knight, Biomed. Mass. Spectrom. 1 (1974) 320.
[5] S.B. Martin, J.H. Karam, P.H. Forsham, Anal. Chem. 47 (1975) 545.
[6] J. Brohon, M. Noel, J. Chromatogr. 146 (1978) 148.
[7] M.S. Lannard, C. Casey, G.T. Tucker, H.F. Woods, Br. J. Clin. Pharmacol. 6 (1978) 183.
[8] B.G. Charles, N.W. Jacobsen, P.J. Ravenscroft, Clin. Chem. 27 (1981) 434.
[9] R. Huupponen, P. Ojala-Karlsson, J. Rouru, M. Koulu, J. Chromatogr. 583 (1992) 270.
[10] G. Caille, Y. Lacasse, M. Raymond, H. Landriault, M. Perrotta, G. Picirille, J. Thiffault, J. Spenard, Biopharm. Drug. Dispos. 14 (1993) 257.