Available online at www.sciencedirect.com
ScienceDirect
Journal of Chromatography B, 857 (2007) 219–223
JOURNAL OF CHROMATOGRAPHY B
www.elsevier.com/locate/chromb

Estimation of carvedilol in human plasma by using HPLC-fluorescence detector and its application to pharmacokinetic study

Rajeshwari Rathod, L. Poorna Chandra Prasad, Shubha Rani, Manish Nivsarkar, Harish Padh *

B.V. Patel Pharmaceutical Education and Research Development (PERD) Centre, Thaltej-Ghandhinagar Highway, Thaltej, Ahmedabad 380 054, India

Received 20 February 2006; accepted 13 July 2007
Available online 22 July 2007

Abstract

A simple, precise and sensitive high performance liquid chromatography procedure has been developed for determination of carvedilol in human plasma. The method was developed on Lichrosphere R CN column using a mobile phase of acetonitrile/20 mM ammonium acetate buffer with 0.1% triethylamine (pH adjusted to 4.5) (40/60, v/v). The peaks were detected by using fluorescence detector (excitation wavelength 282 nm and emission wavelength 340 nm). Carvedilol and domperidone (internal standard) were extracted by liquid–liquid extraction procedure using dichloromethane. This method was specific and had a linearity range of 1–128 ng/ml with intra- and inter-day precision (%C.V.) less than 15%. The accuracy ranges from 87.3 to 100.88% and the recovery of carvedilol was 69.90%. The stability studies showed that carvedilol in human plasma was stable during short-term period for sample preparation and analysis. This method was used to assay the carvedilol in human plasma samples obtained from subjects who had been given an oral tablet of 12.5 mg carvedilol.

Keywords: Carvedilol; HPLC-fluorescence detector; Liquid–liquid extraction; Pharmacokinetics

1. Introduction

Carvedilol (Fig. 1a) is a β-blocker with additional vasodilating, anti-proliferative and antioxidative properties [1]. Chemically it is 1-(9*H*-carbazol-4-yloxy)-3-[[2-(2-methoxy phenoxy) ethyl] amino]-2-propanol [2]. Several methods have been reported for estimation of carvedilol in human plasma using HPLC with fluorescence detector [3], electrochemical detector [4], LC–MS and capillary electrophoresis [5–7]. The limit of quantification (LOQ) has been reported as 1.30 ng/ml for HPLC-fluorescence detector and in LC–MS the report [5] shows LOQ up to 0.1 ng/ml. In some reported methods, solid phase extraction was used for extraction process [8]. However, the solid phase extraction and LC–MS methods are very costly. In this paper we report a simple, accurate and highly specific method for estimation of carvedilol in human plasma by using HPLC with fluorescence detector. This method has the advantage of using simple liquid–liquid extraction with good recovery and having LOQ of 1 ng/ml.

2. Experimental

2.1. Materials and chemicals

Carvedilol was a gift sample (Troikaa Pharmaceuticals Ltd., Ahmedabad, Gujarat, India). Domperidone (Fig. 1b) was used as an internal standard (Torrent Pharmaceuticals Ltd., Ahmedabad, Gujarat, India). All the solvents were of HPLC grade from Qualigen Fine Chemicals and Spectrochem, India. Ammonium acetate (S.D. Fine Chem. Ltd., Mumbai, Maharashtra, India) and acetic acid (S.D. Fine Chem. Ltd., Mumbai, Maharashtra, India) of analytical grade were used. Human plasma was obtained from Prathma blood bank, Ahmedabad, Gujarat, India.

* Corresponding author. Tel.: +91 79 27439375/27416409; fax: +91 79 27450449.
E-mail address: perd@perdcentre.com (H. Padh).
URL: http://www.perdcentre.com (H. Padh).

1570-0232/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.jchromb.2007.07.021

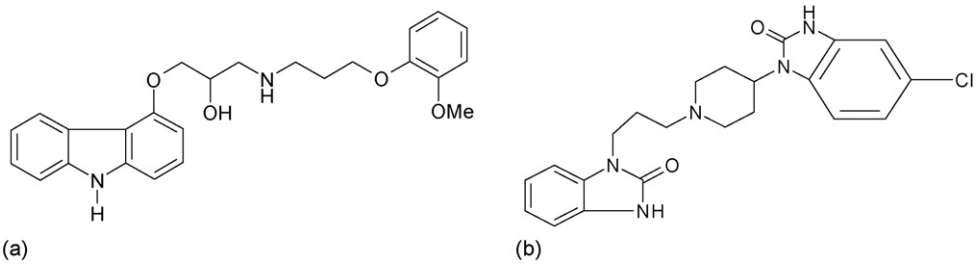

Fig. 1. Chemical structures of (a) carvedilol and (b) domperidone.

2.2. Chromatographic conditions

The HPLC system consisted of PU-980 intelligent HPLC pump (Jasco, Hachioji, Tokyo, Japan), Intelligent spectrofluorometer 821-FP (Jasco, Hachioji, Tokyo, Japan), Intelligent autosampler AS-950-10 (Jasco, Hachioji, Tokyo, Japan) and the data were analyzed by Borwin version 3.1 software. Chromatographic separation was achieved by using Lichrosphere R CN (250 mm × 4.0 mm, 5 μm) column maintained at room temperature. The mobile phase consisted of acetonitrile/20 mM ammonium acetate buffer with 0.1% (v/v) triethylamine (pH adjusted to 4.5) (40/60, v/v). Flow rate was maintained at 0.80 ml/min. The maximum response was obtained for carvedilol by fluorescence detector at an excitation wavelength 280 nm and emission wavelength 340 nm.

2.3. Preparation of standard solutions

Stock solutions of carvedilol and domperidone (100 μg/ml) were prepared in methanol. These solutions were protected from light by covering them with aluminium foil. Required aliquots of these solutions were diluted with methanol to obtain solutions of appropriate concentrations. For precision, accuracy, recovery and stability studies, aliquots of the above solution were added to blank plasma.

2.4. Calibration curve

Calibration curves were prepared by adding known amount of carvedilol (1, 2.00, 4.00, 8.00, 16.00, 32.00, 64.00 and 128.00 ng) to 1 ml of blank plasma. An aliquot of 100 μl of the internal standard solution (2 μg/ml) was added to each sample. The samples were extracted as described below. The standard curves were constructed by plotting the peak area ratio of carvedilol and domperidone (internal standard) on *Y*-axis and concentration of carvedilol on *X*-axis. Linearity was assessed by a weighted (1/*C*) least square regression analysis.

2.5. Preparation of quality control samples

The concentrations of carvedilol were 3.0, 20.00 and 100.00 ng/ml in human plasma to represent low, middle and high quality controls, respectively. Appropriate volumes from stock solution of carvedilol were added to normal human plasma to get low, middle and high quality control samples, respectively, and stored at −80 °C. The quality control samples were taken out from storage for analysis to determine intra- and inter-day precision and accuracy.

2.6. Extraction procedure

An aliquot of internal standard (100 μl of 2 μg/ml domperidone in methanol) was added to 500 μl of plasma sample and vortexed for 10 s. To this 100 μl of 0.50 M sodium bicarbonate was added followed by 5 ml of dichloromethane. The mixture was vortexed for 2 min and centrifuged at 2100 rpm for 10 min. After aspiration of plasma layer, the organic layer was transferred into conical tube and evaporated under nitrogen gas. The dry extract was reconstituted with 100 μl of mobile phase and 50 μl of this solution was injected in to HPLC with fluorescence detector.

2.7. Precision and accuracy

For the calculation of the intra-day precision and accuracy, five replicates of quality control samples (3.0, 20.0 and 100.0 ng/ml) were extracted as described above and the concentrations were calculated from the standard curve. For the calculations of inter-day precision and accuracy, five replicates of quality control samples (3.0, 20.0 and 100.0 ng/ml) were analyzed on three consecutive days along with the standard calibration curve.

2.8. Recovery

In order to calculate recovery of the extraction procedure, four replicates of quality control samples of carvedilol (3.0, 20.0 and 100.0 ng/ml) were extracted and analyzed. The peak area was compared with the same concentrations of unextracted standards of carvedilol reconstituted in mobile phase. The average value of area was taken into consideration to calculate the recovery. The recovery of internal standard was also calculated in similar way.

2.9. Stability

In bench top stability, three replicates of low and high controls of carvedilol (3.0 and 100.0 ng/ml) were analyzed at 0 and 6 h at room temperature and the deviation was calculated. In autosampler stability, three replicates of high and low quality control samples were analyzed at 0, 12 and 24 h by keeping

in autosampler at 5 °C and the deviation was calculated. In freeze–thaw stability, three replicates of low and high quality control samples of carvedilol were prepared, frozen at −80 °C and analyzed after 1, 2 and 3 freeze–thaw cycles. In dry extract stability, three replicates of high and low quality control samples were prepared. After evaporating the organic phase the tubes were stored at −80 °C and analyzed after 24 h by reconstituting with 100 μl of mobile phase and injected 50 μl in the HPLC system. The mean concentration of 24 h samples was compared with that of sample analyzed at 0 h long-term stability was done for 30 days by taking three replicates of high and low quality control samples. The mean concentration was taken into consideration which was compared with zero day sample concentration.

2.10. Pharmacokinetic study

Total four subjects aged 26–28 years were included in the pharmacokinetic study. All subjects underwent a thorough physical and clinical examination. After an overnight fast, subjects received 12.5 mg carvedilol tablet (Cardivas, Sun Pharmaceuticals Ltd., India) with 240 ml of water. The blood samples (4 ml) were collected at pre-dose and 0.5, 0.75, 1, 1.25, 1.5, 2, 2.5, 3, 4, 5, 6, 8, 12, 24, 36, and 48 h post-dose. Up to 12 h post-dose, samples were collected by an indwelling venous cannula. From 24 to 48 h, the samples were collected by venipuncture into heparinised disposable syringes. Plasma was separated by centrifugation at 4000 rpm for 7 min at 4 °C, divided into two aliquots and stored in prelabeled polypropylene vials at −80 °C until analysis.

2.11. Data analysis

The maximum plasma concentration (C_{max}) and the time to reach maximum concentration (T_{max}) were directly determined from the plasma concentration versus time curves. The area under the curve from 0 to t (AUC_{0-t}) was calculated by the linear trapezoidal rule. The area under the curve from 0 h to infinity ($AUC_{0-\infty}$) was estimated by summing the area from 0 to t (AUC_{0-t}) and t to infinity ($AUC_{t-\infty}$), where $AUC_{t-\infty} = C_t/K_{el}$, with C_t defined as the last measured plasma concentration at time t, and K_{el} the slope of the terminal portion of the ln(plasma concentration) versus time curve. The elimination half-life ($t_{1/2}$) was calculated using the pharmacokinetic relationship $t_{1/2} = \ln(2)/K_{el}$.

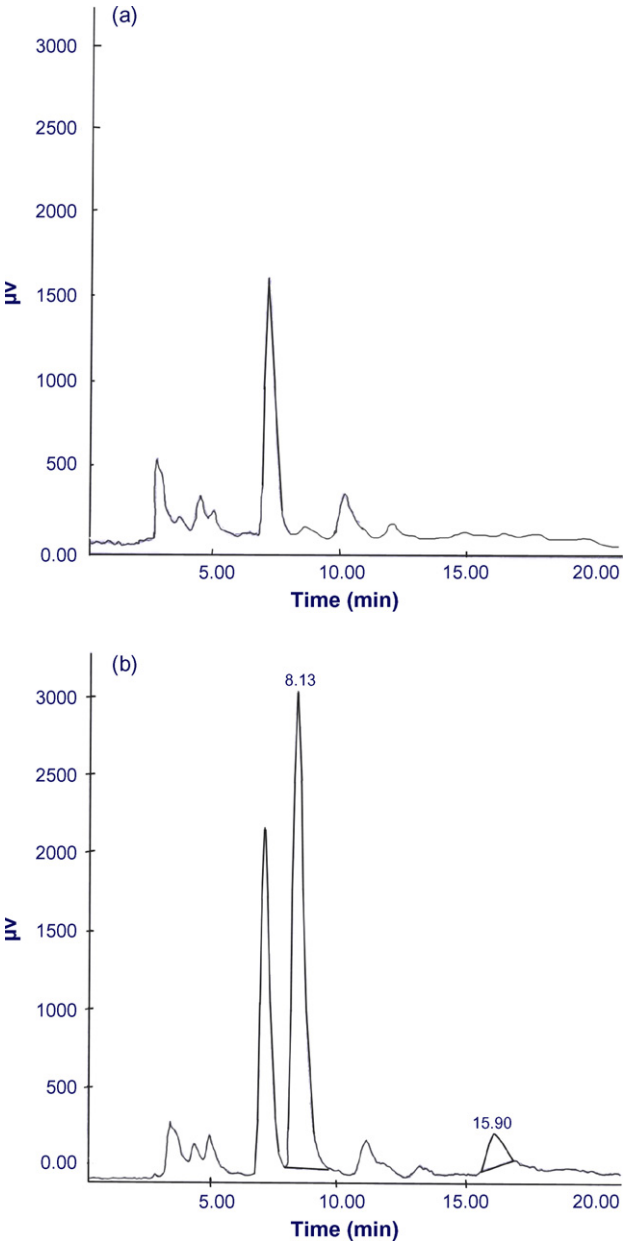

Fig. 2. Chromatograms of (a) blank plasma and (b) human plasma sample spiked with 1 ng/ml of drug (carvedilol). Approximate retention times of drug (carvedilol) = 15.9 min and I.S. (domperidone) = 8.13 min.

Table 1
Linearity of carvedilol standards in human plasma

S. no.	Actual concentration (ng/ml)	Mean concentration found (ng/ml)	Precision (%C.V.)	% Accuracy
1	1.0	1.06	10.82	106.00
2	2.00	2.00	10.00	100.00
3	4.00	3.6	9.62	90.00
4	8.00	7.56	5.43	95.75
5	16.00	16.26	11.02	101.63
6	32.00	29.72	7.42	92.25
7	64.00	71.46	3.77	111.66
8	128.00	125.2	2.64	97.81

3. Results and discussion

The method was found to be relatively simple using liquid–liquid extraction. Dichloromethane was used as extraction solvent to get better recovery and good separation from plasma impurities. Before that we tried different solvents like methanol, acetonitrile, diethyl ether and ethyl acetate at different pH range. We got good result in dichloromethane in alkaline pH. Domperidone was chosen as internal standard because it showed similar chromatographic behavior to carvedilol with no interference by admixture in human plasma. Ammonium acetate 20 mM (pH 4.5) was used to get better resolution between carvedilol and plasma impurities. Triethylamine (0.1%) was used to get good peak shape. The chromatograms of blank plasma and LLOQ were shown in Fig. 2. The retention times of carvedilol and domperidone were observed about 15.9 and 8.13 min, respectively. Satisfactory peak resolution and reasonable retention of the drug and internal standard were obtained using the reverse phase cyano column.

3.1. Linearity and limit of quantification

The calibration curves were obtained by plotting the peak area ratio of carvedilol to internal standard against the respective concentration. Three calibration curves were used to show linearity over the range of 1–128 ng/ml (Table 1). The regression was found above 0.99 (Table 2). The limit of quantification was 1 ng/ml for carvedilol.

Table 2
Calibration curve data of carvedilol

	Slope	Intercept	Correlation coefficient
Mean ($n = 3$)	0.128	0.058	0.999
±S.D.	0.00288	0.00283	0.0049

3.2. Precision, accuracy and recovery

Intra- and inter-day precision and accuracy were determined by quality control samples at various concentrations as described in Section 2. The intra-day precision (%C.V.) was found to be less than 15% ($n = 5$) and accuracy ranged from 86.2 to 102.5% when determined at concentrations of 3.0, 20.0 and 100.0 ng/ml of carvedilol (Table 3). The inter-day precision (%C.V.) was found to be less than 15% and accuracy ranged from 87.3 to 100.88% (Table 3). The recovery for the carvedilol and internal standard were 69.90 and 71.38%, respectively.

Table 3
Precision and accuracy for quality control (QC) samples

QC samples	Concentration added (ng/ml)	Mean concentration observed (ng/ml)	Precision (%C.V.)	% Accuracy
Intra-day ($n = 5$)				
High	100.00	102.5	0.76	102.5
Middle	20.00	18.04	0.68	90.20
Low	3.0	2.58	3.49	86.20
Inter-day ($n = 3$)				
High	100.0	100.88	2.17	100.88
Middle	20.00	18.32	1.89	91.60
Low	3.0	2.62	5.34	87.3

Table 4
Stability for the quality control (QC) samples

QC samples	Mean concentration observed 0 h	Mean concentration observed last hour	% Deviation [%] [±]
Bench top stability ($n = 3$) (after 6 h)			
High	97.48	95.94	1.57
Low	2.58	2.46	9.3
Autosampler stability ($n = 3$) (24 h after extraction and reconstitution)			
High	103.78	100.5	2.78
Low	2.6	2.98	14.6
Freeze–thaw stability ($n = 3$) (three cycles)			
High	102.82	100.98	1.84
Low	2.42	2.56	5.7
Dry extract stability ($n = 3$) (24 h)			
High	101.48	103.66	1.75
Low	2.52	2.56	0
Long-term stability ($n = 3$) (30 days)			
High	102.72	100.8	1.86
Low	2.6	2.92	12.3

Table 5
Pharmacokinetic study results

Subject	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-t} (ng h/ml)	$AUC_{0-\infty}$ (ng h/ml)	K_{el} (h^{-1})	$t_{1/2}$ (h)
1	71.16	0.50	205.13	211.53	0.32	2.25
2	24.80	1.25	89.55	93.63	0.31	2.23
3	36.94	0.75	68.42	74.77	0.35	1.98
4	37.94	0.50	71.77	84.47	0.34	2.02
Mean	42.71	0.75	108.72	116.10	0.33	2.12
S.D.	19.89	0.35	64.94	64.08	0.02	0.14
%C.V.	46.56	47.14	59.73	55.19	6.61	6.59

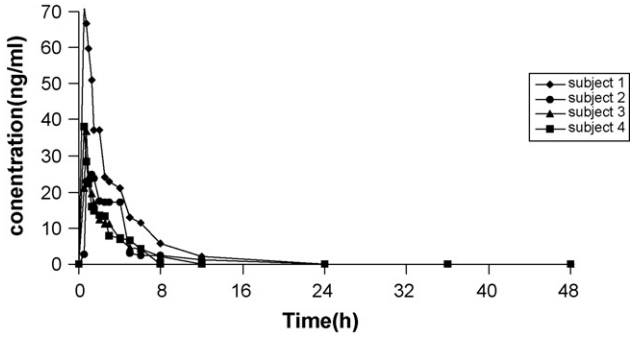

Fig. 3. Plasma concentration–time profiles of carvedilol after oral administration of 12.5 mg dose to four healthy subjects.

3.3. Stability

The percentage variation observed in bench top stability, autosampler stability, three freeze–thaw cycles and dry extract stability were within the limit of +15% (Table 4). The results of bench top stability suggested that carvedilol was stable at room temperature for 6 h. Autosampler stability showed that extracted samples were stable for 24 h at 5 °C. The results of freeze–thaw and dry extract stability showed that carvedilol was stable up to three freeze–thaw cycles and up to 24 h at −80 °C in the form of dry extract, respectively.

3.4. Pharmacokinetic study

Individual plasma concentration–time profiles of carvedilol following administration of single oral dose of 12.5 mg tablet to four healthy male subjects are shown in Fig. 3 and a summary of the pharmacokinetic parameters is presented in Table 5. The pharmacokinetic parameters (mean + S.D.) C_{max}, T_{max}, AUC_{0-t}, $AUC_{0-\infty}$ and $t_{1/2}$ were 42.71 + 19.88 ng/ml, 0.75 + 0.35 h, 108.72 + 64.94 ng h/ml, 116.10 + 64.08 ng h/ml and 2.12 + 0.14 h, respectively. High inter-subject variability has been observed in pharmacokinetic parameters C_{max} range (24.80–71.16 ng/ml), AUC_{0-t} range (71.77–205.13 ng h/ml) and $AUC_{0-\infty}$ range (74.77–211.53 ng h/ml). As carvedilol is metabolized by cytochrome P450 2D6 and cytochrome P450 2C9 [9], this high inter-subject variability may be due to genetic polymorphism in the cytochrome P450 2D6.

4. Conclusion

A simple, specific and sensitive method has been developed and validated for estimation of carvedilol in human plasma. The stability studies showed that carvedilol in human plasma were stable during short-term periods for sample preparation and analysis. This method has potential applicability in pharmacokinetic and bioequivalence studies of carvedilol.

Acknowledgements

The authors wish to thank Troikaa Pharmaceuticals Ltd., Ahmedabad, Gujarat, India and Torrent Pharmaceuticals Ltd., Ahmedabad, Gujarat, India for providing carvedilol and domperidone samples.

References

[1] K. Nolte, G. Backfisch, R. Neidlein, Arzneimittelforschung 49 (1999) 745.
[2] K. Parfitt (Ed.), MARTINDALE: The complete drug reference, Pharmaceutical Press, UK, 1999, p. 838.
[3] P. Ptacek, J. Macek, J. Klima, J. Chromatogr. B 789 (2003) 405.
[4] M. Machida, et al., J. Chromatogr. B 798 (2003) 187.
[5] do Carmo Borges, et al., J. Chromatogr. B 822 (1–2) (2005) 253.
[6] A. Portoles, A. Filipe, S. Almedo, A. Terleira, F. Vallee, F. Vargas, Arzneimittelforschung 55 (2005) 191.
[7] L. Clohs, K.M. Mc Erlane, J. Pharm. Biomed. Anal. 31 (2003) 407.
[8] F. Behn, Stephaneku, J. Chromatogr. Sci. 39 (2001) 121.
[9] H.H. Zhou, A.J. Wood, Clin. Pharmacol. Ther. 57 (1995) 518.