International Journal of Advances in Pharmaceutical Analysis
IJAPA Vol. 3 Issue 3 (2013) 68-71
Journal Home Page http://www.ijapa.ssjournals.com

A SIMPLE REVERSED PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY METHOD DEVELOPMENT AND VALIDATION FOR DETERMINATION OF CARVEDILOL IN PHARMACEUTICAL DOSAGE FORMS

Md. Amran hossain, Dipu Rani Kundu, Tasnuva Sharmin, Mahfuza Maleque and **Sharmin Reza Chowdhury***

Department of Pharmacy, State University of Bangladesh

Abstract

A simple, sensitive and precise reverse phase high performance liquid chromatographic method has been developed for the estimation of Carvedilol in pharmaceutical preparations. Chromatographic determination was performed on a reversed phase C_{18} column (4.5 mm x 250 mm; 5 µm particle size) using a mixture of Phosphate buffer: Acetonitrile (65:35) as mobile phase at a flow rate of 1ml/min with UV detection at 240 nm. The method was validated for linearity, accuracy, repeatability, precision, reproducibility, and specificity as per International ICH guidelines. The method was also used in determination Carvedilol content in five commercial brands available in Bangladeshi market. The method was linear in the range between 5 – 35 µg/ml, exhibited good correlation coefficient (R^2 = 0.998) and good Accuracy study (98.08 %-99.91%). The method was found to specific for Carvedilol in presence of common excipients. Statistical analysis performed with proposed method proved it to be precise, accurate and reproducible. Hence it can be employed for routine analysis of Carvedilol both in bulk and commercial formulations.

Keywords: Carvedilol, HPLC method, Validation, Pharmaceutical formulations

1. Introduction

Carvedilol, or (±)-1-9H-(carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl]amino]-2-propanol, is an antihypertensive agent with β- and α_1-adrenergic receptor blocking activities.[1-3] Carvedilol has much greater antioxidant activity than other commonly-used β –blockers.[4-5] It has been prescribed as an antihypertensive agent and an angina agent[6-7] and for treatment of congestive heart failure.[8] High-performance liquid chromatography (HPLC) with fluorescence detector,[9-14] mass spectrometer[15-16] or electrochemical detection[17] has been used for the analysis of carvedilol and its enantiomers in biological samples. Determination of cavedilol by capillary electrophoresis has also been reported.[17,18] HPLC with UV detector[19-21] and differential pulse voltammetric determination[22] have been also used. There have been few published articles on the evaluation of carvedilol in pharmaceutical formulations. In this study, efforts were made for developing a simple, easy and cost effective HPLC method using UV detector and a readily available solvent. The method was optimized and validated as per the ICH guidelines.[23]

2. Material and Methods

2.1. ***Active drug and reagents:*** Carvedilol was kindly supplied by Incepta Pharmaceutical Ltd (Bangladesh) and was used as the reference standard. All chemicals and reagents were of analytical or pharmaceutical grade.

2.2. ***Instrumentation and chromatographic condition:*** An integrated high performance liquid chromatography system (Shimadzu) was used for this experiment. A C18 L1, pH resistant (4.5 mm x 250 nm: 5µm) column (Luna, Phenomenex) was used. The detector was set at 240 nm and the run time was 15 minutes at a flow rate of 1 ml/ minute at room temperature.

2.3. Pharmaceutical formulation: Commercial tablets (Effient) were procuredfrom the local market.

2.4. Method development: Buffer (Potassium Dihydro Phosphate was dissolved., pH was adjusted to 2 with the help of phosphoric acid) in different proportions were tried and finally buffer and acetonitrile (65: 35 v/v) was selected as an appropriate mobile phase which gave good resolution and acceptable system suitability parameters.

2.5 Linearity Study: Accurately weighed 25mg Carvedilol, was transferred into a 100ml volumetric flask. An amount of mobile phase (50ml) was added; it was then shaken for 5-10 minutes and diluted to the 100ml mark with same solvent to prepared C_s. For linearity study, five aliquots in the range of 0.2 ml to 1.4 ml of Cs were taken and diluted to 10 ml with mobile phase to obtain different concentrations within the range 5µg/ml to 35µg/ml and used for the linearity calibration plot.

2.6 Precision Study: Accurately weighed tablet powder, equivalent to 25mg Carvedilol, was transferred into a 100ml volumetric flask. An amount of mobile phase (50ml) was added; it was then shaken for 5-10 minutes and diluted to the 100ml mark with same solvent. It was then filtered and was further

Corresponding Author*: sharminreza10@yahoo.com

diluted 0.8ml, 1ml and 1.2ml to 10ml to obtain 20 µg/ml, 25 µg/ml, 30 µg/ml, respectively. Triplicate absorbance measurements of each were made and the mean, standard deviation and RSD were calculated.

The selected concentrations for the intra-day precision study were again analyzed the following day and the mean, standard deviation and RSD were calculated.

2.7 Accuracy Study: This study was carried out using reformulated granules containing pure Carvedilol, and common excipients including maize starch, sodium starch glycol ate, avicel PH 101, magnesium stearate and purified talc. 50mg, granules was then transferred respectively into three 100ml volumetric flasks and diluted to the 100ml mark with mobile phase and then filtered. Same dilution pattern as reference solution was followed to obtain three concentrations, 80%, 100% and 120% of reference solution respectively. The solutions were analyzed then for the content of Carvedilol using the proposed method with a standard solution 15µg/ml of pure Carvedilol. All analysis was carried out in triplicate.

2.8 Specificity in the Presence of Excipients: This test was carried out using only excipients. Placebo granules devoid of the pure carvedilol were prepared, their absorbance reading at 240nm taken and compared with both that of the blank and that obtained for the recovery study.

Limit of detection (LOD) and Limit of quantification (LOQ): Limit of detection (LOD) and Limit of quantification (LOQ) for the assay were calculated using the following equations. [24]

$LOD = 3.3 \times S_0 / b$ and $LOQ = 10 \times S_0 / b$

Where S_0 and b are the standard deviation and the slope of the calibration line.

2.9. Assay of content of Carvedilol in selected marketed brands: This was carried out using the developed and validated method as follows-

Sample Preparation: 10 tablets is weighted and crushed and from that accurately weighed tablet powder, equivalent to 25mg Carvedilol, was transferred into a 100ml volumetric flask. An amount of mobile phase (50ml) was added; it was then shaken for 5-10 minutes and diluted to the 100ml mark with same solvent. It was then filtered and was further diluted 1ml to obtain 25 µg/ml. Triplicate absorbance measurements of each were made and the mean, standard deviation and RSD were calculated.

3.8.9 Reference Standard Preparation: 1ml of Cs was diluted to 10ml of mobile phase to obtain a 25 µg/ml of Carvedilol reference standard solution. The absorbance of the sample preparation and reference standard solution were taken using MeOH as blank. The content of carvedilol in the marketed brands was determined using the following equation-

Content of Carvedilol (%) per tablet = (As/Ast) × (Wst/100 × 1/10) × (100/Ws × 10/1) × W × P/100

Where,

As =area of generic sample solution,
Ast = area of reference Carvedilol standard solution,
Wst = weight of reference Carvedilol powder (mg)
Ws = weight of generic powder sample (mg)
W = average weight of tablet (mg)
P = potency of standard Carvedilol

Statistical analysis: *Where applicable, results were expressed as mean ± SD and analyzed statistically.*

3. Results and Discussion

The linearity parameter (Table 1 & Figure 1) and the corresponding regression data, indicated excellent linear relationship (R^2 = 0.998) over the working concentration range (5-35 µg/ml). Table 2 and Table 3 presents respectively the intra-and inter-day precision of the new method, confirming adequate sample stability and method reliability over a 24 h period. This is because for the three selected concentrations within the linearity range, the observed RSDs were all < 2.0 %. The result of Accuracy (Table 4) was within the range of ICH guideline. The % accuracy indicated non-interference from excipients of formulation. The results of analysis of 5 marketed brands were good and shown in Table 5. The limit of detection (LOD) and Limit of quantification (LOQ) were calculated as 35 µg/ml and 108 µg/ml respectively.

Table 1: Linear regression data for calibration curves

Parameter	Carvedilol
Linearity range (µg/ml)	5-35 µg/ml
Correlation coefficient	0.998
Slope	14625
Intercept	14778

Figure 1: Calibration curve for cavedilol

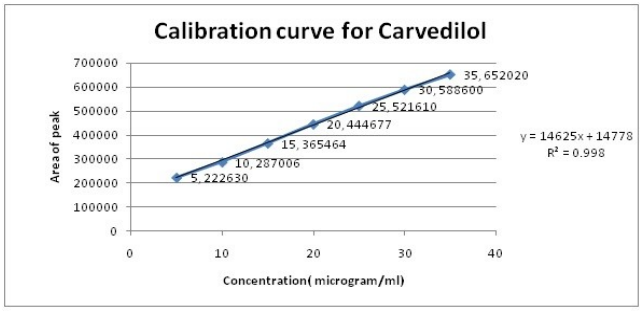

Table 2: Intra-day precision study of Carvedilo

Intra-day Precision study(n=3 replicates)						
Declared Conc. (µg/ml)	Calculated concentration (µg/ml)			Mean ± SD	RSD	Average Potency
	1	2	3			
20	19.8	19.9	19.7	19.8±0.1	0.50%	99.00%
25	25.1	24.8	24.9	24.9±0.15	0.61%	99.73%
30	29.8	29.7	29.9	29.8±0.1	0.33%	99.33%

Table 3: Inter-day precision study of Carvedilo

Inter-day Precision study(n=3 replicates)						
Declared Conc. (µg/ml)	Calculated concentration (µg/ml)			Mean ± SD	RSD	Average Potency
	1	2	3			
20	19.8	19.9	19.7	19.8±0.1	0.50%	99.00%
25	25.1	24.8	24.9	24.9±0.15	0.61%	99.73%
30	29.8	29.7	29.6	29.7±0.1	0.33%	99.00%

Table 4: Accuracy study of Carvedilol

Accuracy study (n=3)				
Dosage form	Labeled claim	Amount added (%)	Area of peak	% Recovered
Pre formulated granules	25 mg	80	294696±0.28	98.08%
		100	350485±0.16	99.02%
		120	421111±0.25	99.91%

Table 5: Assay of Carvedilol in marketed tablets

Assay of Carvedilol in marketed tablets(n=3)				
Formulation	Labeled claim	Amount found ±SD	Assay	RSD
Brand 1	25 mg	24.76±0.25	99.04	1.00
Brand 2	25 mg	24.56±0.89	98.24	3.60
Brand 3	25 mg	24.91±0.03	99.64	0.10
Brand 4	25 mg	24.38±0.78	97.52	3.10
Brand 5	25 mg	25.1±0.23	100.4	0.91

Table 5: Summary of validation parameters

Parameter	Carvedilol
Intraday precision	<2%
Retention time (min)	15min
Theoretical plates	2500
Tailing factor	1.2

4. Conclusion

The proposed method is simple and do not involve laborious time-consuming sample preparation. The results and the statistical parameters demonstrate that the proposed HPLC method is simple, rapid, selective, accurate, precise and highly sensitive. Therefore, it can be used for the determination of Carvedilol either in bulk or in their corresponding dosage forms. So this HPLC method can be used in the quality control department.

References

1. Ruffolo RR, Gellai M, Hieble JP, Willette RN, AJ Nichols. The pharmacology of carvedilol. *Eur J Clin Pharmacol* 1990; 38: S82-8.
2. Nichols AJ, Gellai M and Ruffolo Jr. RR. Studies on the mechanism of arterial vasodilation produced by the novel antihypertensive agent, carvedilol. *Fundam Clin Pharmacol* 1991; 5: 25-38.
3. Mey CD, Breithaupt K, Schloos J, Neugebauer G, Palm D and Belz GG. Dose-effect and pharmacokinetic-pharmacodynamic relationships of the beta 1-adrenergic receptor blocking properties of various doses of carvedilol in healthy humans. *Clin Pharmacol Ther* 1994; 55: 329-337.
4. Nakamura K, Kusano K, Nakamura Y, Kakishita M, Ohta K, Nagase S, Yamamoto M, Miyaji K, Saito H, Morita H, Emori T, Matsubara H,

Toyokuni S and Ohe T. Carvedilol. *Maejo Int J Sci Technol* 2010; 4(01); 8-19.

5. Kukin LM, Kalman J, Charney HR,. Levy KD, Buchholz-Varley C, Ocampo NO and Eng C. Prospective, randomized comparison of effect of long-term treatment with metoprolol or carvedilol on symptoms, exercise, ejection fraction, and oxidative stress in heart failure. *Circulation* 1999; 99: 2645-51.
6. Packer M, Fowler BM, Roecker BE, Coats JSA, Katus AH, Krum H, Mohacsi P,.Rouleau LJ, Tendera M, Staiger C, Holcslaw L T, Amann-Zalan I and DeMets LD. Effect of carvedilol on the morbidity of patients with severe chronic heart failure: results of the carvedilol prospective randomized cumulative survival (COPERNICUS) study. *Circulation* 2002; 106: 2194-99.
7. Ruffolo RR, Boyle DA, Brooks DP, Feuerstein GZ, Venuti RP, Lukas MA and Poste G. Carvedilol: a novel cardiovascular drug with multiple actions. *Cardiovasc. Drug Rev* 1992; 10: 127-57.
8. Poole PA, Wilson, Swedberg K, Cleland JG, Lenarda ADi, Hanrath P, Komajda M, Lubsen J, Lutiger B, Metra M, Remme WJ, Torp-Pedersen C, Scherhag A and Skene A. Rationale and design of the carvedilol or metoprolol European trial in patients with chronic heartfailure: COMET. *Eur J Heart Fail* 2002; 4: 321-29.
9. Hokama N, Hobara N, Kameya H, Ohshiro S and Sakanashi M. Rapid and simple microdeterminationof carvedilol in rat plasma by high-performance liquid chromatography. *J Chromatogr B* 1999; 732: 233-38.
10. Lamprecht G and Stoschitzky K. Determination of carvedilol in human plasma by highperformanceliquid chromatography applying on-line deproteination and column switching. *Chromatographia* 2004; 59: 551-54.
11. Lamprecht G, Gruber L, Stoschitzsky K and Lindner W. Enantioselective analysis of (R)- and(S)-carvedilol in human plasma by high-performance liquid chromatography. *Chromatographia* 2002; 56: S25-29.
12. Saito M, Kawana J, Ohno T, Kaneko M, Mihara K, Hanada K, Sugita R, Okada N, Oosata S, Nagayama M, Sumiyoshi T and Ogata H, Enantioselective and highly sensitive determinationof carvedilol in human plasma and whole blood after administration of the racemate using normalphasehigh-performance liquid chromatography. *J Chromatogr B* 2006; 843: 73-7.
13. Zarghi A, Foroutan SM, Shafaati A and. Khoddam A. Quantification of carvedilol in humanplasma by liquid chromatography using fluorescence detection: application in pharmacokineticstudies. *J Pharm Biomed Anal* 2007; 44: 250-53.
14. Clohs L and McErlane KM. Comparison between capillary electrophoresis and highperformanceliquid chromatography for the stereoselective analysis of carvedilol in serum. *J Pharm Biomed Anal* 2003; 31: 407-12.
15. Yang E, Wang S, Kratz J and Cyronak MJ. Stereoselective analysis of carvedilol in humanplasma using HPLC/MS/MS after chiral derivatization. *J Pharm Biomed Anal* 2004; 36: 609-15.
16. Carmo BN, Mendes GD, Oliveira Silva D.de, Marcondes Rezende V, Barrientos-Astigarraga RE and Nucci G. Quantification of carvedilol in human plasma by highperformanceliquid chromatography coupled to electrospray tandem mass spectrometry: application to bioequivalence study. *J Chromatogr B* 2005; 822: 253-62.
17. Machida M, Watanabe M, Takechi S, Kakinoki S and Nomura A. Measurement of carvedilolin plasma by high-performance liquid chromatography with electrochemical detection. . *Chromatogr B* 2003; 79: 187-91.
18. Oravcova J, Sojkova D and Lindner W. Comparison of the Hummel-Dreyer method in highperformanceliquid chromatography and capillary electrophoresis conditions for study of the interaction of (RS)-, (R) - and (S)-carvedilol with isolated plasma proteins. *J Chromatogr B* 1996; 682: 349-57.
19. Patel LJ, Suhagia BN, Shah PB and Shah RR. RP-HPLC and HPTLC methods for theestimation of carvedilol in bulk drug and pharmaceutical formulations. *Indian J Pharm Sci* 2006; *68*: 790-93.
20. Stojanovic J, Marinkovic V, Vladimirov S, Velickovic D and Sibinovic P. Determination ofcarvedilol and its impurities in pharmaceuticals. *Chromatographia* 2005; 62: 539-42.
21. Stojanovic J, Vladimirov S, Marinkovic V, Velickovic D and Sibinovic P. Monitoring of thephotochemical stability of carvedilol and its degradation products by the RP-HPLC method. *J Serb Chem Soc* 2007; 72: 37-44.
22. Radi A and Elmogy T. Differential pulse voltammetric determination of carvedilol in tabletsdosage form using glassy carbon electrode. *Il Farmaco* 2005; 60: 43-6.
23. ICH- Harmonised Tripartity Guideline, Validation of Analytical Procedures: Text and Methodology Q2 (R1). IFPMA: Geneva, (2005).
24. Moustafa AAM.Spectrophotometric methods for the determination of lansoprazole and pantoprazole sodium sesquihydrate. *J Pharm Biomed Anal* 2000; 22: 45 - 58.