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Method Development and Analytical Method Validation of Carvedilol by High Performance Liquid Chromatography

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Abstract: *The goal of this study was to develop an easy, specific, accurate, precise and sensitive reversed-phase high performance liquid chromatographic (RP-HPLC) analytical method for the rapid quantitative determination of Carvedilol. Carvedilol is widely used in the early treatment of mild to severe congestive heart failure (CHF) and high blood pressure as it is a nonselective beta blocker/alpha-1 blocker. The chromatographic separation was carried out on Shimadzu 20-A series equipped with solvent delivery pump LC-20AT,autosampler SIL-20ACHT, PDA detector SPD-M20A and degassing unit. The HPLC column was Hibar® (250mm x 4.6mm, 5µm) C18 RP and perchlorate buffer (pH 2.0), Acetonitrile and Triethylamine in proportion of (45:55:0.3 V/V/V) as mobile phase at 1.0 mL/min flow rate while 240 nm wavelength used as UV detection. The ICH guideline Q2(R1) approach used for the Method Validation of this Analytical Procedures. The method found linear around the range of 1.88µg/mL to 11.25µg/mL with a (r2 = 0.9997) with adequate level of accuracy and precision. The method has shown acceptable level of robustness with repeatability, reproducibility and intermediate precision. The proposed method can be confidently employed with adequate level of assurance for the routine testing of carvedilol in pharmaceutical dosage form.*

Keywords – *Beta Blockers, Carvedilol, ICH, Method Validation, RP-HPLC*

I. Introduction

The development of absolute, as well as relative assay determination of carvedilol presents substantial challenges of analytical methods, and the results highly depends on method. Therefore for the purpose of accepting a consignment and lot release, an appropriate method should be selected and justified for determination of assay.

Complex and lengthy methods for the estimation of assay by titrimetric, Spectrophotometric and chromatographic techniques are available for carvedilol however limited data available for an easy and rapid assay determination method by reversed phase HPLC techniques. Therefore a method needs to be developed that is accurate, precise, robust and have a relatively short run time.

High Performance Liquid Chromatography (HPLC) is a form of column chromatography that pumps a sample mixture or analyte in a solvent (known as the mobile phase) at high pressure through a column with chromatographic packing material (stationary phase). HPLC has the ability to separate, and identify compounds that are present in any sample that can be dissolved in a liquid in trace concentrations as low as parts per billion. Because of this versatility, HPLC is used in a variety of industrial and scientific applications, such as pharmaceutical, environmental, forensics, and chemicals.

The objective of any analytical measurement is to obtain consistent, reliable and accurate data. Validated analytical methods play a major role in achieving this goal. The results from method validation can be used to judge the quality, reliability and consistency of analytical results, which is an integral part of any good analytical practice. Validation of analytical methods is also required by most regulations and quality standards that impact laboratories.

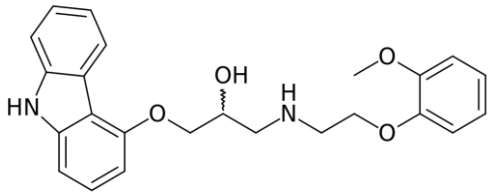
Method validation is the process used to confirm that the analytical procedure employed for a specific test is suitable for its intended use. Results from method validation can be used to judge the quality, reliability and consistency of analytical results; it is an integral part of any good analytical practice.

According to USP <1225> analytical methods should be validated through laboratory tests: "Validation of an analytical procedure is the process by which it is established, by laboratory studies, that the performance characteristics of the procedure meet the requirements for the intended analytical applications". The required laboratory tests for method validation include Specificity, accuracy, precision, linearity, range, limit of detection, limit of quantification, robustness and ruggedness.

Carvedilol is a nonselective beta blocker/alpha-1affect the heart and circulation (blood flow through arteries and veins). Carvedilol is used to treat heart failure and hypertension (high blood pressure). It is also used after a heart attack that has caused your heart not to pump as well. With respect to other beta blockers,

carvedilol has insignificant inverse agonist activity. This proposes that carvedilol has a diminished negative chronotropic and inotropic impact contrasted with other beta blockers, which may diminish its capability to intensify manifestations of heart failure.

Its chemical structure and formula is as follows,
Chemical formula: $C_{24}H_{26}N_2O_4$
Molecular weight: 406.474
Chemical structure:



II. Materials and Method

2.1. Instrument

High performance liquid chromatograph (HPLC) Prominence series with, Auto Injector with built-in cooling system SIL-20ACHT, Column Oven CTO-20AC, solvent delivery pump LC-20AT, Degassing unit and PDA Detector SPD-M20A manufactured by Shimadzu Corporation, Japan, Ultrasonic Bath UCP10 manufactured by Jeiotech Korea, Magnetic Stirrer HS12-06P manufactured by MISUNG, Korea, pH meter S220 manufactured by Mettler Toledo Switzerland and Vacuum filtration pump manufactured by Millipore.

2.2. Reagents

The chemicals used during this research includes HPLC grade acetonitrile of Merck origin, sodium dihydrogen phosphate of Sigma Aldrich origin, sodium perchlorate of Fisher origin, orthophosphoric acid of Merck origin and triethylamin of Merck origin and purified water and Carvedilol was gift from a Pharmaceutical company.

2.3. Preparation of Phosphate Buffer

Dissolve 6.64 grams of sodium dihydrogen phosphate and 0.84 grams of sodium perchlorate in 1000 mL of purified water. Adjust pH 2.0 by dilute orthophosphoric acid.

2.4. Mobile Phase Preparation

Mix 900 mL Acetonitrile with 1100 mL of above buffer and 6mL Triethylamine. Filter through a filter of 0.45 micron or finer and degas for 15 minutes by an ultrasonic bath. This mobile phase will also be used as diluent.

2.5. Sample Preparation

Stock solution of Carvedilol (125µg/mL) was prepared in mobile phase and samples were prepared by serial dilution method. The standard solution of Carvedilol (7.5 µg/mL) was prepared by dissolving 25 mg of carvedilol into 100 mL volumetric flask. Further dilution was made by transferring 3 mL of this solution into 100 mL volumetric flask.

2.6. Chromatographic System

Shimadzu high pressure liquid chromatography (HPLC) prominence series model 20-A was used which were equipped with autosampler, built-in cooler, degassing unit and injection loop of 100µL with UV detector. The analysis was performed by using Isocratic reverse phase technique with Hibar® C18, 5µm (250mm X 4.6mm) HPLC column of Merck origin. The system also have Lab Solution software to control the chromatographic analysis and for the data acquisition and integration. The samples were analyzed by auto injection of 20µL solution at a flow rate of 1.0mL/minute with λmax of 240nm.

2.7. Validation Parameters

2.7.1. Suitability of HPLC system:

System suitability testing is an integral part of many analytical procedures. The tests are based on the concept that the equipment, electronics, analytical operations, and samples to be analyzed constitute an integral system that can be evaluated as such. The samples were run on system to check the system suitability before starting the validation.

2.7.2. Specificity

Specificity or selectivity of the method is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.The specificity of the method was established by analyzing the blank, diluents, mobile phase, standard and sample solutions.

2.7.3. Linearity and Range

The linearity of an analytical procedure is its ability (within a given range) to obtain test results which are directly proportional to the concentration (amount) of analyte in the sample and range of an analytical procedure is the interval between the upper and lower concentration (amounts) of analyte in the sample (including these concentrations) for which it has been demonstrated. Linearity and range of the method was established by analyzing the samples of six different concentrations prepared by serial dilution from standard stock solution.

2.7.4. Accuracy

The accuracy of an analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. The accuracy of the method was established by triplicate injection of 25%, 50%, 75%, 100%, 125% and 150% concentration samples prepared by separate pipetting for each sample from standard stock solution.

2.7.5. Precision

The precision of an analytical procedure expresses the closeness of agreement (degree of scatter) between a series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The precision of the method was demonstrated at two different levels i.e. repeatability and intermediate precision.

2.7.6. Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by small, but deliberate variations in method parameters and provides an indication of its reliability during normal usage.

III. Results and Discussions

Validation is an integral part of analytical method development i.e. once the method has been devised, it is quite necessarily important to evaluate it under the conditions expected for actual samples before to use for a specific purpose. Though the validation stage is critical in method development, the importance of this step is often ignored. This study tries to explain the nomenclature of method validation and defines the validation procedure for analytical methods employed for the determination of carvedilol.

In this study we discussed the concept of method development and method validation which defined the numerous fundamentals and clarified its close association with suitability for purpose. It is based on the postulation that a bundle of necessities are satisfied and we explained how these necessities are chosen, what work will have to be carried out in the laboratory and the way by which evidence is provided. The inter-lab comparison method or carried out an in house validation are the different ways to validate a methodology. In this validation we covered all the parameters of validation and found our developed method as specific to our analyte, the method was also checked and verified for linearity over the whole range from 25% to 150% and found a remarkable coefficient of determination (R-Square) value of 0.9997 which is close to 1. The developed method also found accurate for the whole range i.e. from 25% to 150% method is accurate. The percent recovery found almost 99% for all the 18 samples of this range. During repeatability, the same analyst repeat the six samples and found the % RSD between replicates less than 0.35% and when run by two different analyst (as intermediate precision) more less % RSD achieved.

For checking method robustness, small but deliberate changes were introduce in mobile phase flow rate, its pH and even in wavelength as well. The method found entirely robust over all of these changes and remarkable % RSD achieved when compared results after changes with unchanged method results (repeatability results).

The stability of solution was also checked as per ICH Q2(R1) at room and refrigerator temperature i.e. (2 – 8 °C) for 24 and 48 hours. The sample solution found stable at both the temperature till 48 hours and give the assay content within specification (98.0 – 102)% criterion. However it is evident that the solution is not more stable at room temperature (20 – 25 °C) than at refrigerator temperature (2 – 8 °C). The solution should be considered as stable at room temperature for 24 hours' time period. Below is the results summary for the entire study conducted for method development and method validation.

Table -1 (Results Summary for Whole Study)

S. No.	Validation Parameters	Acceptance Criteria	Results	
01	System Suitability	Peak areas of six replicate should have the %RSD less than 2.0%.	0.0913%	
		Theoretical plates for column efficiency is not less than 1500	12206	
		The peak should have Tailing Factor less than 2.0	1.17	
02	Specificity	No any peak in diluents and mobile phase chromatograms on the equal retention time (RT) of sample and standard should be found.	Complies	
03	Linearity	The R-Square value should be ≥ 0.995	0.9997	
04	Accuracy	The % recovery should be 98 – 102 %	At 25% level	99.93
			At 50% level	99.28
			At 75% level	99.05
			At 100% level	98.98
			At 125% level	99.30
			At 150% level	99.22
05	**Precision** i. Repeatability	%RSD = ≤ 2%	0.34%	
	ii. Intermediate precision	%RSD between first& second analyst = ≤ 2%	0.32%	
06	Robustness	%RSD between repeatability assay & assay after applying small but deliberate changes should be ≤ 2%	At flow 0.9 mL/min	0.089
			At flow 1.1 mL/min	0.18
			At WL238 nm	0.25
			At WL242 nm	0.27
			At pH1.9	0.47
			At pH 2.1	0.64
07	Solution Stability	The results of sample solution should be between 98 – 102% stored at room and 2 – 8 °C temperature for 24 & 48 hours respectively.	At room temperature for 24 hours	0.04
			At 2 – 8 °C for 24 hours	0.48
			At room temperature for 48 hours	0.62
			At 2 – 8 °C for 48 hours	0.63

IV. Conclusion

The present work refers to the fact that an accurate, precise & robust assay determination method for carvedilol was developed and validated. The analytical method was not only successfully validated but also found simple, accurate and precise. The recovery percentages showed that the method found free from any interference of impurity present or developed during synthesis or storage. Further method can estimate the content of carvedilol in the range of 25 to 150% of label claim.

Therefore, the proposed method can be used confidently for routine analysis of carvedilol for routine quality control, stability studies and for further studies like standardization of working standards etc. The method is not affected by small variations between flow rate changes from 0.9 mL/min to 1.1 mL/min, wavelength changes from 238 nm to 242 nm and pH of mobile phases changes from 1.9 to 2.1.

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