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DEVELOPMENT AND VALIDATION OF CHROMATOGRAPHIC DETERMINATION OF CARVEDILOL PHOSPHATE IN BULK AND PHARMACEUTICAL DOSAGE FORM USING FLUORESCENCE DETECTOR

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ABSTRACT

An accurate, sensitive and precise RP-HPLC –Fluorescence method has been developed and validated for the estimation of Carvedilol Phosphate (CP) from bulk drug and Pharmaceutical Dosage form. The separation was achieved by a Brownlee analytical C18 column (250mm X 4.6mm, 5µm) in isocratic mode, with mobile phase comprises of Acetonitrile : Methanol : Buffer in proportion of 70:20:10v/v/v, buffer was 5mM Potassium Di-hydrogen Phosphate (pH 3.5 adjusted with Ortho Phosphoric Acid). The flow rate of mobile phase was 1.0ml/min and employing fluorescence detection with 280nm excitation and 340nm emission wavelengths. The retention time of Carvedilol Phosphate was 2.20 min.The calibration curve was found to be linear within the concentration range of 10ng/ml to 60ng/ml. The regression data for calibration curve shows good linear relationship with r^2 = 0.990. The method was validated in accordance with the requirements of ICH guidelines. Moreover, the proposed analytical method was applied to monitor the formulation commercially available.

INTRODUCTION: Carvedilol-Phosphate(CP) is a non cardioselective beta-adrenoceptor antagonist and arteriolar vasodilating properties, attributed mainly to its blocking activity at alpha1 receptor [1]. Carvedilol is official drug in British Pharmacopoeia [2].

Carvedilol Phosphate has much greater antioxidant activity than other commonly-used β–blockers. It has been prescribed as an antihypertensive agent and an angina agent and for treatment of congestive heart failure [3].

Chemically CP is (2RS)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy) ethyl] amino] propan -2-ol [**Figure 1**].

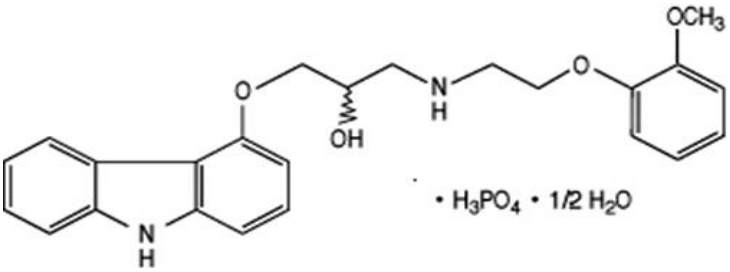

FIGURE 1: CARVEDILOL PHOSPHATE

Several analytical techniques are available for estimation of Carvedilol Phosphate in bulk dosage form by HPLC, HPTLC and UV Spectrophotometric method [4-14]. Keeping this objective in mind an attempt has been made to develop and validate the HPLC-fluorescence method for the analysis of Carvedilol Phosphate which would be highly sensitive, having good resolution and

reproducible. Various validation aspects of the analysis, accuracy, precision, recovery, and the limits of detection and quantification etc. have been measured as per ICH guidelines [15].

MATERIALS & METHODS:

1. **Material**: The HPLC system consisted of following components: Perkin-Elmer- Model Series 200 and software –Turbo chrome. Rhenodyne valve with 20µl fixed loop, quaternary gradient system pump, Chromatographic analysis was performed on Brownlee Analytical C18 column 250×4.6 mm, 5µm particle size. Analytically pure carvedilol Phosphate was procured as gift samples from Torrent research centre, Ahmedabad, Gujarat, India. All other chemicals and reagents used were analytical grade and purchased from Merck Chemicals, India. Tablets were procured from the local market.

2. **Methods:**

a. **Preparation of standard stock solution and solutions for calibration curve:** Stock solutions of Carvedilol phosphate were prepared by dissolving 10 mg of Carvedilol Phosphate in 10 ml of volumetric flask with methanol. Aliquot of 0.1ml of the standard stock solution of carvedilol Phosphate were transferred using A-grade bulb pipette into 100 ml volumetric flask and from that appropriate aliquots were taken to give concentration range of 10-70ng/ml for calibration curve.

b. **Chromatographic conditions:** Chromatographic estimation was performed using an equilibrated Brownlee analytical C18 column (250mm×4.6mm i.d.), mobile phase consisting of Acetonitrile : Methanol : Buffer in proportion of 70:20:10v/v/v, buffer was 5mM Potassium Di-hydrogen Phosphate (pH 3.5 adjusted with Ortho Phosphoric Acid). Detection was done at excitation wavelength of 280 nm and emittation wavelength of 340 nm. The sample was injected using a 20µl fixed loop, flow rate 1ml/min and the total run time was 10 minutes.

c. **Validation:** The method was validated as per the ICH guideline.

i. **Regression analysis**- Regression of analytical method is expressed in terms of correlation co-efficient of the regression analysis. Accuracy**-** For determination of Accuracy, recovery study was carried out. That was performed by standard addition method at three different levels (50%, 100%, and 150%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed.

ii. **Precision**- The precision of analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogeneous samples. Intraday precision- Intraday variance for the Carvedilol phosphate was done at the interval of 3 hrs. Interday precision- Interday variance for the Carvedilol phosphate was done at the interval of one day.

iii. **Limit Of Detection (LOD)**- LOD was foundout based on the standard deviation of the response and the slope method. Limit Of Quantification (LOQ) - LOQ was foundout based on the standard deviation of the response and the slope method. Specificity- Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present.

d. **Determination of Carvedilol phosphate in Tablet Dosage Form:** Twenty tablets were weighed, finely powdered, and an accurately weighed sample of powdered tablets equivalent to 10 mg of Carvedilol phosphate was treated with mobile phase in a 10mL volumetric flask using ultra sonicator. This solution was filtered through 0.45 µm filter paper. Suitable aliquot of the filtered solution was added to a volumetric flask and make up to volume with mobile phase to get appropriate concentration in range.

RESULTS AND DISCUSSION: Several mobile phase compositions were tried to resolve the peak of CP. The mobile phase containing Acetonitrile : Methanol : Buffer in proportion of 70:20:10v/v/v, buffer was 5mM Potassium Di-hydrogen Phosphate (pH 3.5 adjusted with Ortho Phosphoric Acid) was found ideal to resolve the peak of CP.

Retention time of CP was 2.20 min [**Figure 2**]. Quantification was achieved by fluorescence detector with 280nm excitation and 340nm emission wavelengths.

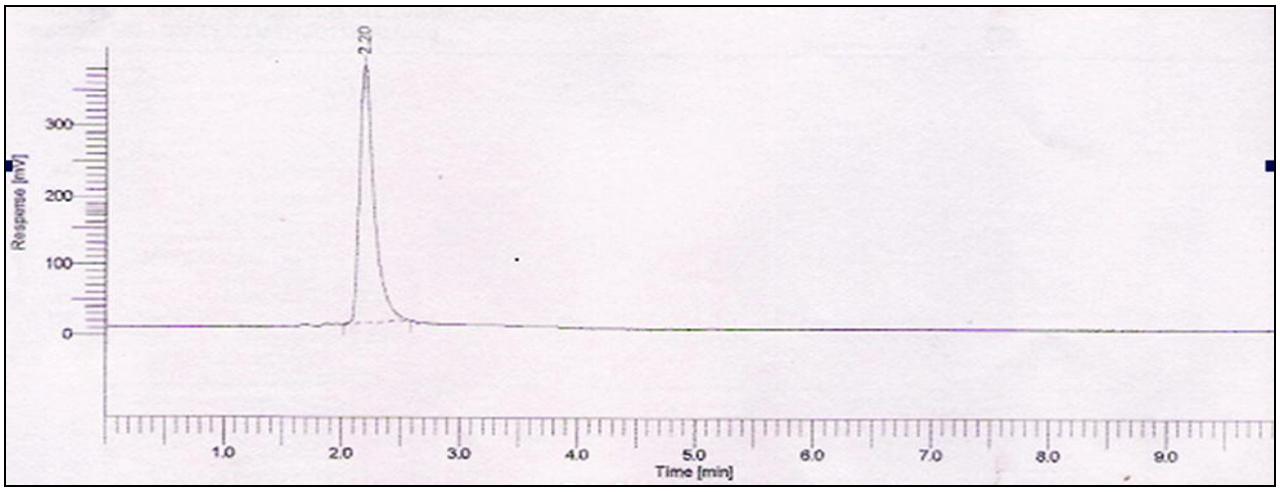

FIGURE 2: CHROMATOGRAM OF STANDARD CARVEDILOL PHOSPHATE SOLUTION (20 NG/ML)

The system suitability parameters are shown in **Table 1**.

TABLE 1: SYSTEM SUITABILITY PARAMETERS

System suitability Parameters	
Retention times (RT)	2.20
Theoretical plates (N)	1328.5
Tailing factor (AS)	1
Capacity factor	0.14

Linear regression data showed a good liner relationship over a concentration range of 10-60ng/ml for CP. The correlation coefficients (r^2) was 0.9906 [**Figure 3**].

The accuracy of the method was evaluated by carrying out recovery studies, were performed by standard addition method at three different levels I, II and III (50%, 100%, and 150%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed [**Table 2**].

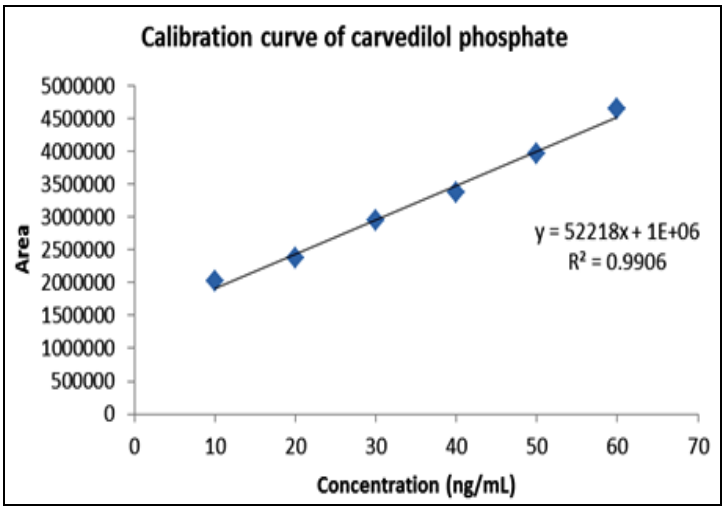

FIGURE 3: CALIBRATION CURVE OF CARVEDILOL PHOSPHATE

TABLE 2: RECOVERY STUDY DATA FOR CP

Level	Labeled amount (mg per tablet)	Amount obtained (mg per tablet)	Average assay recovery (%)	Assay % (RSD)
Level I	10	10.4	100.8	0.58
Level II	10	9.9	100.4	0.34
Level III	10	10.2	101.5	0.72

The limit of detection and limit of quantification were found to be 2.5ng/ml and 7.6ng/ml respectively. The intra-day and inter day precision was determined by analyzing standard solution of 40, 50 and 60 ng/mL and the results are reported in terms of relative standard deviation was 1.4, 1.2 and 1.0 respectively [**Table 3**].

TABLE 3: PRECISION DATA FOR CP

Concentration (ng/ml)	Area *(µV.s)	Standard Deviation	% Relative Standard Deviation
40 ng/ml	3148639	45298.03	1.438654
50 ng/ml	3546109	45198.12	1.274583
60 ng/ml	4677676	48925.89	1.045945

* Area: mean (n=3)

The assay result was repeated for three times which was found to be 99.2-101.3 % of labeled claim [**Table 4**].

TABLE 4: ASSAY RESULT

Formulation	Labeled claim	Amt. Recovered	% CP
TABLET	10mg per tablet	9.97 mg	99.7

CONCLUSIONS: A method of quantitative determination of carvedilol Phosphate using HPLC with fluorescence detector has been developed. The validation results have demonstrated that this method is accurate, precise, linear, specific and sensitive. The method can also be applied for drug content in pharmaceutical preparations.

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