



## Development and Validation of HPTLC Method for Estimation of Carvedilol Phosphate in Bulk and Pharmaceutical Dosage Form

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### ABSTRACT

A simple, accurate, and precise HPTLC method has been developed and validated for the estimation of Carvedilol Phosphate (CP) from bulk drug and tablet formulations. The method employed TLC aluminum plates precoated with silica gel 60 GF<sub>254</sub> as the stationary phase. The solvent system comprised n-hexane: ethyl acetate: methanol (3:5:2 v/v/v). This system was found to give good result for Carvedilol (R<sub>f</sub> value: 0.36cm). Spectrodensitometric scanning-integration was performed at a wavelength of 243nm. The calibration curve was found to be linear within the concentration range of 100ng/spot to 200ng/spot. 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. The method was successfully applied for determination of drug in tablets, wherein no interference from tablets excipients was observed, indicating the specificity of the developed method. Thus, the proposed method can be used successfully for routine analysis of Carvedilol Phosphate from bulk and formulation.

**Key Word:** Validation, HPTLC, Carvedilol Phosphate

### INTRODUCTION

Carvedilol-Phosphate, Figure.1, (2R,3S)-1-(9H-Carbazol-4-yloxy)-3-[[2-(2-methoxyphenoxy)ethyl] amino]propan-2-ol, is a non cardioselective beta-adrenoceptor antagonist and arteriolar vasodilating properties, attributed mainly to its blocking activity at alpha<sub>1</sub> receptor [1]. Carvedilol is official drug in British Pharmacopoeia [2]. It has been prescribed as an antihypertensive agent and an angina agent and for treatment of congestive heart failure [3].

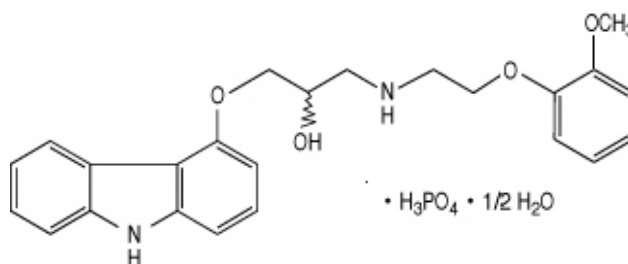


Fig. 1 Carvedilol phosphate

Several analytical techniques are available for estimation of Carvedilol in bulk dosage form by HPLC [4-15]. Keeping this objective in mind an attempt has been made to develop and validate the HPTLC method for the analysis of carvedilol 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 [16].

## EXPERIMENTAL SECTION

### *Chemicals and Reagents*

The working standard of Carvedilol was procured from Torrent Research Center, Ahmedabad, India. HPLC grade n-hexane, acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Deionized and ultra-pure water used in all experiments was obtained from Milli-Q System (Millipore). Glacial acetic acid of AR (Analytical Reagent) grade was procured from S. D. Fine Chemicals (India).

### *Equipment*

The HPTLC system employed in the method development and validation was Camag Linomat V applicator (Muttens, Switzerland), a Camag twin trough chamber of appropriate size, Camag TLC scanner III, Wincats (version 1.2.2) software as data integrator and a Hamilton syringe (Switzerland) of 100 µl capacity.

HPTLC was performed on 10 cm × 10 cm pre-coated silica gel GF<sub>254</sub> pre-coated HPTLC plates from E. Merck (Darmstadt, Germany).

## METHOD

### *Preparation of standard solution*

A stock solution of Carvedilol Phosphate (CP) (1mg/ml) was prepared in methanol. A standard solution was prepared by dilution of the stock solution with methanol to give solution in of 100 µg/ml. Further dilutions were made with methanol to give a solution in concentration range of 100-200ng/ml.

A methanolic solution of carvedilol phosphate (1 mg/ml) was prepared. This solution was further diluted with methanol to yield a solution containing 100 µg/ml. Different concentrations of CP in a concentration range of 100-200ng/ml were applied on plates as 3 mm bands, 3 mm apart and 1 cm from edge of the plate, by means of Camag Linomat V automatic sample applicator fitted with 100 µl Hamilton syringe. The mobile phase, n-hexane: ethyl acetate: methanol (3:5:2 %v/v) was poured into the Camag twin trough glass chamber and the mobile phase was left to equilibrate in the chamber for 20 min at room temperature. The plate was then put in the chamber containing the mobile phase and developed to a distance 75 mm. After development, the plate was removed from the chamber, dried in current of hot air, and scanned at 243 nm, using a deuterium lamp, by means of Camag TLC scanner III densitometer. Densitograms were obtained by HPTLC of CP at various concentrations. This method was followed for all quantitative analysis. The Wincats software (version 1.2.2) was used for data acquisition and processing of the plate. The scanning speed was 20 mm/s, the offset was 10%, and the sensitivity (SPAN) was optimized to 20 min. Peak height and peak area were integrated for the entire track. The calibration curve was established by plotting the obtained peak area on ordinate against corresponding concentration on abscissa. The reproducibility of this method was ascertained by repeating the experiment five times.

### **Validation**

The method was validated for specificity, accuracy, and precision by use of calibration standards of CP. Limit of detection and limit of quantitation were determined by the visual method by spotting different concentration of CP. The lowest concentration that could be detected for three replicate spots was regarded as the limit of detection. The lowest concentration for which the RSD [%] of five replicate spots less than 5 % was regarded as the limit of quantitation. LOD was calculated using formula  $LOD = 3.3 \times \sigma/S$ , where  $\sigma$  is the residual standard deviation of regression line and  $S$  is the slope of corresponding line. Accuracy was determined by measurement of the recovery of CP standard added at three different levels, each being analyzed as described for the assay. Intraday and interday precisions were determined by applying 140ng/spot standard CP solution. After the development and densitometric scanning of the plates the peak-area response was measured and precision was calculated as RSD (%). The method was validated as per ICH guidelines.

### **Application of the validated method**

To determine the content of the drug in a solid dosage form, 20 tablets of CP (10 mg) were accurately weighed, their average weight was calculated. Powder equivalent to 10 mg of the drug was dissolved in 10 ml methanol in a volumetric flask and sonicated for 20 min. (Solution A ~ 1000 µg/ml). Further appropriate dilutions were made with mobile phase to get 187.5 ng/ml. The concentration of CP was calculated from the standard graph, which was plotted using the area of known concentration (100-200ng/spot) of CP standard.

## RESULTS AND DISCUSSION

While choosing a solvent for HPTLC, the chemical nature and polarity of the drug molecule must take into account. Carvedilol Phosphate is practically insoluble in water and in the pH range of 3-9, freely soluble in methanol, soluble in strong base. Methanol: ethyl acetate (1:1) was used as the solvent system initially, but it was observed that the spot moved along with the solvent front and thus was not retained on the plate. When n-hexane was added, the R<sub>f</sub> decreased but tailing was observed. Finally, the solvent system comprising n-hexane: ethyl acetate: methanol (4:5:1 v/v/v) gave good separation and there was no interference around the drug R<sub>f</sub> value but it was less than 0.2cm. Thus, after many experiments, the system was developed which consisted of n-hexane: ethyl acetate: methanol (3:5:2 v/v/v). The optimized system gave a good separation and resolution of Carvedilol Phosphate.

The plate material employed was silica gel 60 GF<sub>254</sub> and scanning of CP on HPTLC plate at UV max at 243 nm. Hence, quantification was carried out at 243 nm using Camag TLC scanner III.

The optimized solvent system yielded a symmetrical peak for the drug with R<sub>f</sub> 0.36. A typical chromatogram of CP was shown in Figure 2.

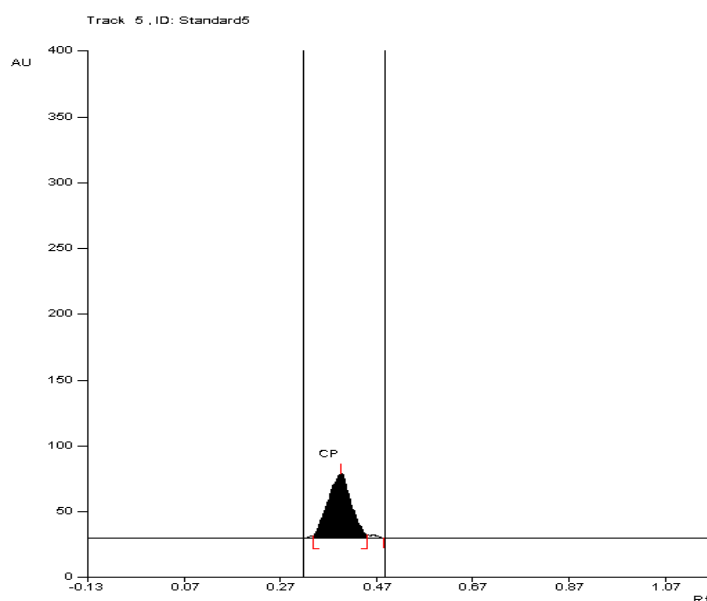


Figure.2 A Typical Chromatogram of Carvedilol phosphate

The developed method was then validated and successfully applied for quantification of CP from the formulation. To ensure accuracy of the method, recovery studies were performed by standard addition method at three different levels I, II and III (80%, 100%, and 120%), to the pre-analyzed samples and the subsequent solutions were re-analyzed. At each level, three determinations were performed and the results obtained are shown in Table 1.

TABLE: 1 ASSAY AND 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

TABLE: 2 PRECISION DATA FOR CP

	Intra day (140ng/spot)	Inter Day (140ng/spot)
	1699.54	1699.54
	1689.18	1658.9
	1703.6	1678.4
Mean	1696.39	1678.946667
Std Devi.	10.19647978	20.32551434
RSD	0.006010693	0.012106111
%RSD	0.601069317	1.210611078

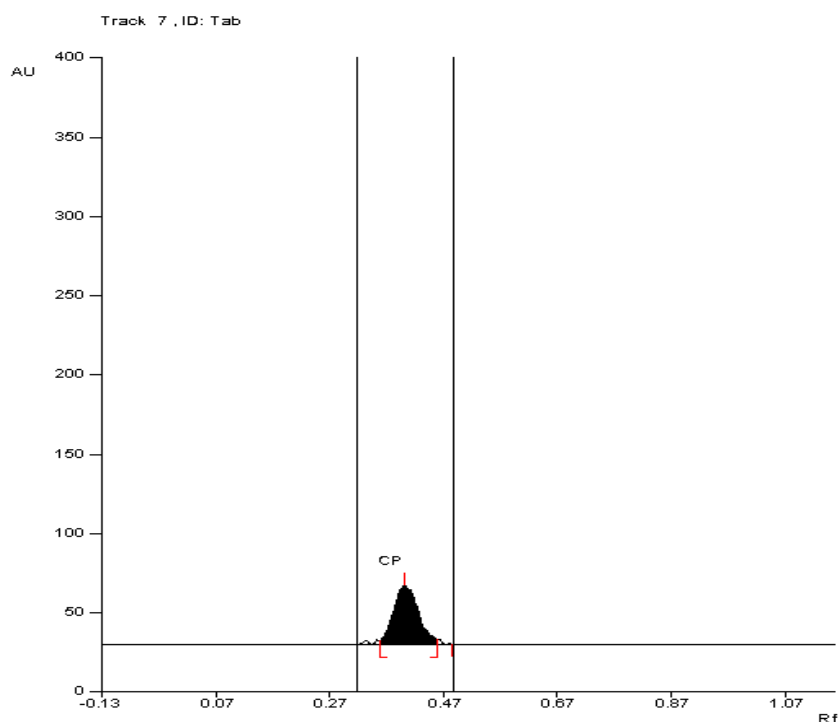
The results of recovery studies were within the specified limits of ICH guidelines. Lower values of %RSD reflect the accuracy of the method. Precision, expressed in terms of %RSD was determined in terms of intra-day and inter-day precisions, analyzing the drug at three different concentrations, determining each concentration thrice summarized in Table 2.

The sample solutions were analyzed using the method for three consecutive days, repeating the process twice a day at different periods. The results of regression analysis obtained are summarized in Table 3 which reflect high degree of precision. Two different analysts performed assay on marketed tablets of the drug, in similar operational and environmental conditions, using the developed method to determine its ruggedness.

**TABLE: 3 REGRESSION ANALYSIS DATA FOR CP**

Regression analysis data	
Linearity range (ng/spot)	100 to 200 ng/spot
Slope	15.09
Intercept	-375.3
Standard deviation of slope	15.04
Standard deviation of intercept	-368.9
Regression ( $R^2$ )	0.99
Correlation coefficient	1.2

The Chromatogram of the marketed formulation was shown in Figure 3 and that was identified by comparing the RF and also comparing its absorbance spectrum with that obtained with the standard drug



**Figure 3: Typical HPTLC Chromatogram of Carvedilol phosphate in Tablet dosage form**

A typical Purity comparison spectrum of the drug is shown in Figure 4.

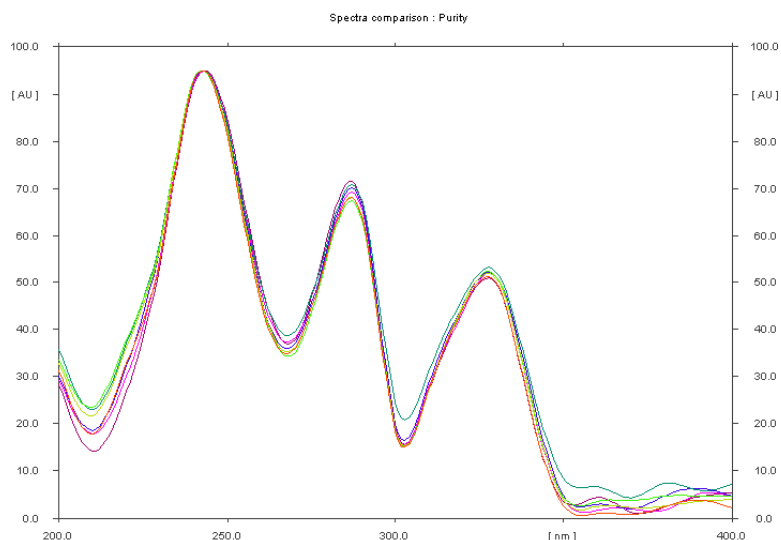


Figure 4: Purity Comparison Spectra of Bulk drug and Marketed formulation

### CONCLUSION

The developed and validated HPTLC method reported here is rapid, simple, accurate, sensitive, and specific. The method was also successfully used for quantitative estimation and analysis of Carvedilol Phosphate from formulation. Thus, the reported method is of considerable importance and has great industrial applicability for quality control and analysis of carvedilol phosphate from bulk drug and formulations.

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