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Development, Validation and Stability Study of UV Spectrophotometric Method for Determination of Carvedilol Phosphatein Bulk and Pharmaceutical Dosage Forms

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Abstract : A simple, specific and economic UV spectrophotometric method has been developed using as a solvent methanol:0.1N HCL (95:5) to determine the carvedilol phosphate content in bulk and pharmaceutical dosage formulations. The quantitative determination of the drug has been carried out at a predetermined $\lambda_{max}$of 242nm, it was proved linier in the range 2-12 µg/mL and exhibited good correlation coefficient (R^2=0.999) and excellent mean recovery (98-100.09%). The method was validated statically and by recovery studies for linearity, precision, repeatability and reproducibility as per ICH guideline. The obtained results proved that the method can be employed for the routine analysis of carvedilol phosphate in bulk as well as in the commercial formulations.
Key Words : Carvedilol Phosphate, UV Spectroscopy, Validation.

1. Introduction

Carvedilol phosphate is a non selective β-adrenergic blocking agent with α1-blocking activity. It is (2RS) 1-(9H-Carbazole-4-yloxy)-3-[(2-(2-methoxyphenoxy) ethyl) amino] propan-2-ol phosphate salt (1:1) hemihydrate. It is a racemic mixture with the following structure. Carvedilol phosphate is a white-to-almost white solid with a molecular weight of 513.5 (406.5carvedilol free base) and a molecular formula of $C_{24}H_{26}N_2O_4 \bullet H_3PO_4 \bullet ½\ H_2O$[1].

Literature survey reveals some UV spectrophotometric methods for estimation of carvedilol in pharmaceutical formulation. HPLC[9,10,11,12], HPLC-MS-MS[16], HPTLC[13] and GC-MS[21] determinations are available for the estimation of carvedilol in pharmaceutical dosage forms.In this study, efforts were made to develop a simple, easy and economic UV spectrophotometric method using diluents composed of methanol: 0.1N HCL (95:5) for the determination of carvedilol in raw material as well as in the marketed dosage formulations. The developed method was optimized and validated as per the guidelines of International Council on Hormonisation (ICH) and demonstrated excellent specificity, linearity, precision and accuracy for carvedilol.

2. Materials and Methods

2.1 Equipment

A Shimadzu UV–visible spectrophotometer (UV1800, Shimadzu Corporation, Kyoto, Japan)was used for all absorbance measurements with matched quartz cells.

2.2 Materials

All chemicals and reagents were of analytical grade. Carvedilol in the form of Carvedilol phosphate powder with certificate of analysis was provided by Hetero Drugs Limited, Hyderabad. Pharmaceutical grade excipients were obtained from Pharmaceutical Technology Lab. of Maharashtra.

2.3 Determination of wavelength of maximum absorption

A standard stock solution of carvedilol phosphate(100µg/mL) was prepared using diluents to further obtain 10µg/mL.An UV spectroscopic scanning (200-400 nm) was carried out with final diluted solution to determine $\lambda_{max}$for the detection of carvedilol phosphate using diluents as a blank.

2.4 Linearity and Range

For linearity study, six solutions at different concentrations (2, 4, 6, 8, 10 and 12 µg/mL) were prepared using six different aliquots of stock solution, and the obtained data were used for the linearity calibration plot. Limit of detection (LOD) and limit ofquantification (LOQ) for the assay were also calculated

2.5 Intra-day precision (repeatability) and inter-day precision study (intermediate precision)

Carvedilol phosphate sample stock solution of 10µg/Ml was prepared following the same dilution pattern of stock solution. Three different aliquots of stock solution were then diluted to 10 mL to obtain the concentrations of 4, 6 and 8 µg/mL. This procedure was repeated in the following days.

2.6 Stability study

Samples prepared for repeatability study were preserved for 24 h at room temperature and analyzed on the following day to test for short-term stability.

2.7 Accuracy/recovery study

This study was carried out using pre-formulated granules containing pure carvedilol phosphate and common excipients. Calculation was done from the label claim and the average weight of the final product. Previously used dilution pattern was followed for the granules to obtain three concentrations—80%, 100% and 120% of reference solution.

2.8 Specificity in the presence of excipients

The test for the specificity was carried out using only excipients. Spectra for placebo granules, blank, and sample were compared. Secondly the specificity was determined by subjecting the sample solution to accelerated degradation by heat (60 °C) for 48 h in order to verify that none of the degradation products interfered with the quantification of the drug.

2.9 Assay of content of Carvedilol in selected marketed brands

Market brands of carvedilol tablet from different manufacturers were randomly selected and analyzed using the newly developed and validated method. Sample solutions of each brand (10µg/mL) were also prepared and assayed for content of carvedilol against the standard. The content of carvedilolin the marketed brands was determined using standard calculations.

2.10 Stress degradation studies

i. Photolytic Degradation

Specific amount of drug carvedilol phosphate was weighed accurately & putted into the UVchamber for three days. After three days 10mg drug was weighed and madestock solution (100µg/mL) with diluents. Then an appropriate concentration(10µg/mL) wasprepared & absorbance was measured in UV spectrophotometer.

ii. Thermal Degradation

Drug was taken in a Petri dish which was previously cleaned & dried then was put it into the oven for 48 hrs then it was taken out & weighed 10mg drug was weighed and made stock solution (100µg/mL) with diluents. Then an appropriate concentration(10µg/mL) wasprepared & absorbance was measured in UV spectrophotometer.

iii. Acid Degradation

0.01N HCl was taken in a 10 ml volumetric flask then accurately weighed 10mg drug carvedilol phosphate was dissolved in it. Then the solution was refluxed for 4 hrs then from this solution anappropriate concentration(10µg/mL) wasprepared using diluents & absorbance was measured in UV spectrophotometer.

iv. Alkali Degradation

0.01N NaOH was taken in a 10 ml volumetric flask then accurately weighed 10mg drug carvedilol phosphate was dissolved in it. Then the solution was refluxed for 4 hrs then from this solution anappropriate concentration(10µg/mL) wasprepared using diluents & absorbance was measured in UV spectrophotometer.

v. Oxidation with H_2O_2

3% H_2O_2solutionwas taken in a 10 ml volumetric flask then accurately weighed 10mg drug carvedilol phosphate was dissolved in it. Then the solution was kept in dark for 4 hrs then from this solution anappropriate concentration(10µg/mL) wasprepared using diluents & absorbance was measured in UV spectrophotometer.

3. Results and discussion

3.1 Method development and optimization

Carvedilol Phosphate is almost insoluble in aqueous medium and freely soluble in organic solvents like methanol and 0.1N HCL. During the development phase, the use ofmethanol with 0.1N HCL as the diluent resulted in preferable outcome in UV analysis. The solvent composition was optimized to Methanol (95) and 0.1N HCL (5). The pre-determined wavelength of maximum absorption (λ_{max}) was 242 nm. (Fig. 2)

Table 1: Optical Parameters for Carvedilol Phosphate

Sr. No	Parameters	Data
1	λ max	242nm
2	Linearity	2-12µg/ml
3	Regression equation	Y= 0.182X + 0.068
4	Correlation coefficient	R^2 = 0.999
5	Slop	0.182
6	Intercept	0.0688
7	LOD	0.1490 µg/ml
8	LOQ	0.4517 µg/ml

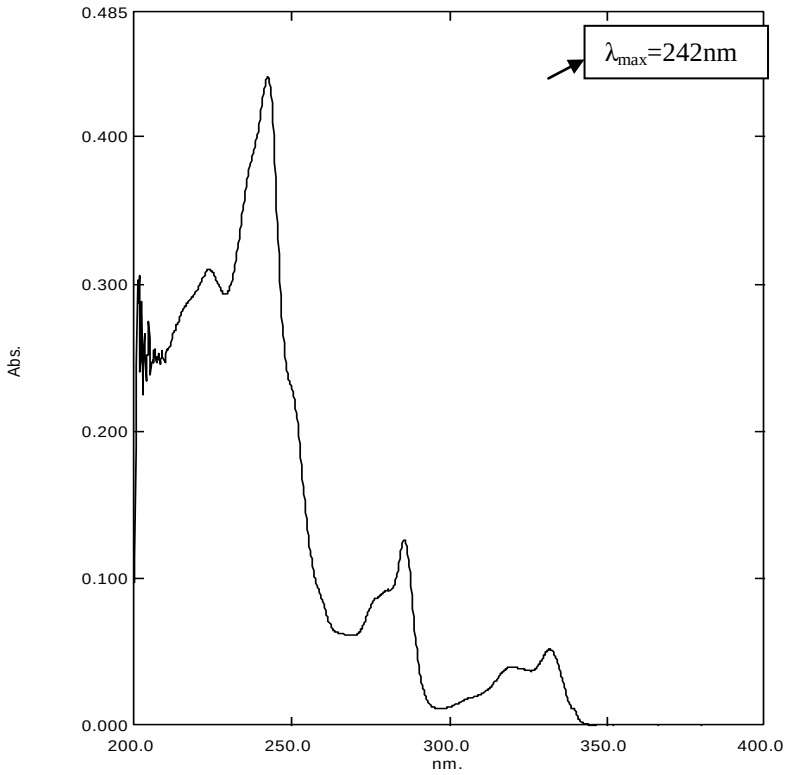

Figure 1- UV Spectrum of Carvedilol phosphate

3.2 Method validation

3.2.1 Linearity and range

The calibration curve obtained was evaluated by its correlation coefficient. The absorbance of the samples in the range of 2.0–12.0µg/mL was linear with a correlation coefficient (R^2) greater than 0.998 (Table 1). The LOD and LOQ were calculated as 0.858 mg/mL and 2.60 mg/mL respectively.

Table 1:Linearity data

Concentration µg/ml	**Absorbance**
2	0.439
4	0.805
6	1.155
8	1.522
10	1.897
12	2.268

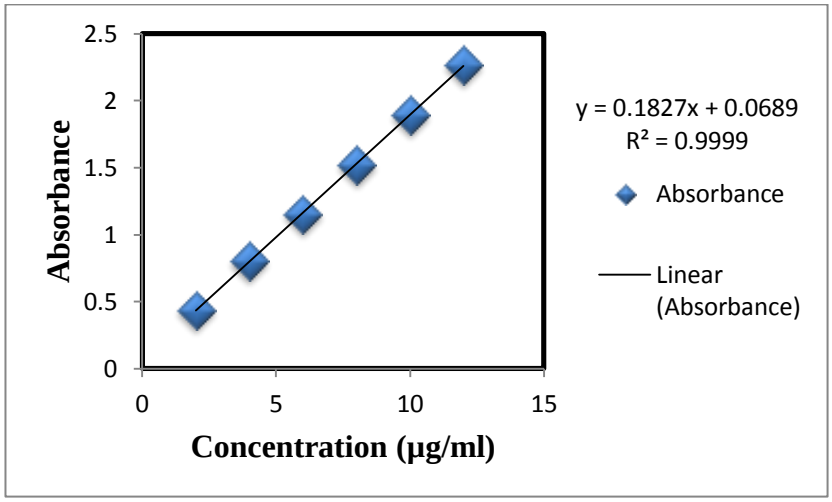

Figure 2- Calibration Curve

3.2.2 Intra-day and inter-day precision

The intra-day and inter-day precision study (Table 2) of the developed method confirmed adequate sample stability and method reliability where all the RSDs were below 2%.

Table 2:Intra-day and inter-day precision determined for three different concentrations of Carvedilol Phosphate (n=3).

Concentration (µg/ml)	Intra-day precision		Inter-day precision	
	Absorbance mean	%RSD	Absorbance mean	%RSD
4	0.797333	1.03016	0.857167	0.349342
8	1.508	0.565802	1.573333	0.191383
12	2.2585	0.283858	2.294167	0.193795

3.2.3 Stability

Stability study's results were within the acceptance range (Table 3) and indicated the samples stability over 24 h(short-term).

Table 3: Short term stability determined by the proposed method (n=3).

Concentration declared µg/mL	Concentration found µg/mL	RSD (%)	Average potency (%)
4	0.857	0.349342	98.36
8	1.573	0.191383	98.22
12	2.294	0.193795	98.00

3.2.4 Accuracy/Recovery

Results within the range of 98.00–100.97% ensure an accurate method (Table 4) as well as indicate non-interference with the excipients of formulation.

Table 4:Accuracy/Recovery for three different concentrations of carvedilol by the proposed method.

Dosage form	Label Claim	Amount added	Recovery (%)
Pre-formulated granules	12.5mg	80	99.01
		100	99.25
		120	99.69

3.2.5 Specificity in the presence of excipients

The specificity of the analytical method was proved by comparing the spectra of placebo and degradation productof sample solution with that of accuracy sample (Fig. 3).

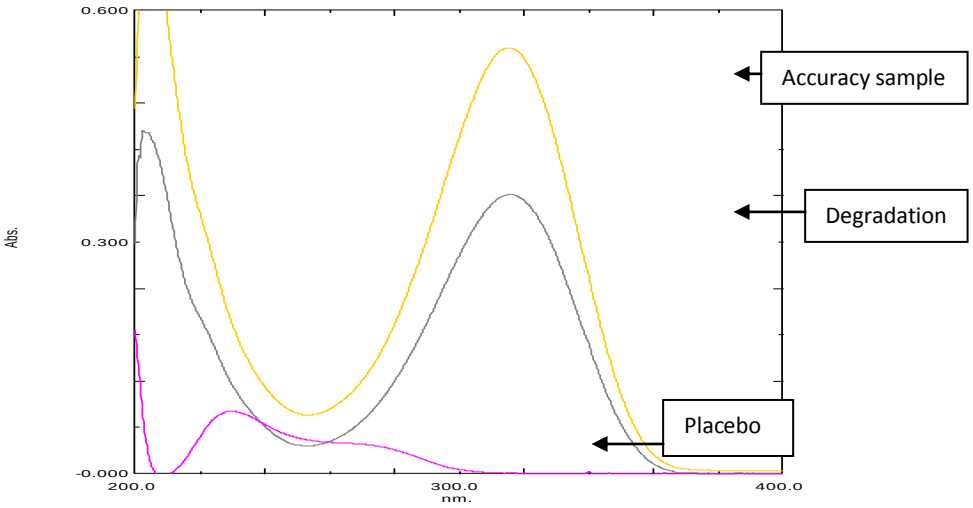

Figure 3: Specificity of the method determined by comparing the spectra of accuracy sample, placebo and degradation products

3.2.6 Stress degradation studies

The study conducted (Table5) shows that there is degradation of drug under the stress conditions like photolytic, alkali & oxidation.

Table 5: Summary of stress degradation results

Stress condition	Degradation%	Remark
Photolytic	42	Unstable
Thermal	11	Stable
0.01N HCl	19	Stable
0.01N NaOH	89	Unstable
H_2O_2	69	Unstable

3.2.7 Content of carvedilol phosphate in marketed brands

Carvedilol phosphate content of three marketed products determined by the proposed method (Table 6) was in good agreement with the label claims and was in the range of 98.45–100.50% with the RSD values of 0.107–0.140% respectively.

Table 6: Content of carvedilol phosphate in marketed products

Brand	Label claim (mg)	Amount found	Potency	RSD (%)
Brand A	12.5	12.4	98.93	0.125
Brand B	12.5	12.3	99.43	0.140
Brand C	12.5	12.4	99.00	0.107

4. Conclusion

The results and the statistical parameters demonstrate that the proposed UV spectrophotometric method is simple, rapid, specific, accurate and precise. Therefore, this method can be used for the determination of

carvedilol phosphate either in bulk or in the dosage formulations without interference with commonly used excipients and related substances.

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