## RESEARCH ARTICLE

# Development and estimation of carvedilol in oral dosage form using HPLC method

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### ABSTRACT

**Introduction:** Analytical chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. It deals with methods for determining the chemical composition of samples of matter. **Materials and Methods:** Chemicals and standards used as a material and methods to performing the task. Many trials were performed. **Results:** The present investigation reported in the thesis was aimed to develop a new method development and validation for the estimation of carvedilol by reversed-phase high-performance liquid chromatography (RP-HPLC) method. The literature reveals that there are no analytical methods reported for the estimation carvedilol by RP-HPLC method. Hence, it was felt that there is a need of new analytical method development for the estimation of carvedilol in pharmaceutical dosage form. **Conclusion:** A new method was established for estimation of Carvedilol by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Carvedilol using Phenomenex C18 column (4.6 × 150 mm) 5 μ, flow rate was 1.0 ml/min, mobile phase ratio was pH 3.4 Phosphate: meoH (65:35% v/v), and detection wavelength was 284 nm.

**Keywords:** Carvedilol, Chromatography, Reversed-phase high-performance liquid chromatography

## INTRODUCTION

Analytical chemistry is the study of the separation, identification, and quantification of the chemical components of natural and artificial materials. It deals with methods for determining the chemical composition of samples of matter. A Qualitative Method yields information about the identity of atomic or molecular species or the functional groups in the sample. A Quantitative Method, in contrast, provides numerical information as to the relative amount of one or more of these components.

Pharmaceutical analysis may be defined as a process or a sequence of processes to identify and/or quantify a substance or drug, the components of a pharmaceutical solution or mixture or the determination of the structures of chemical compounds used in the formulation of pharmaceutical product.

Analytical procedure refers to the way of performing the analysis. It should describe in detail the steps necessary to perform each analytical test. This may include but is not limited to: The sample, the reference standard and the reagents preparations, use of the apparatus, generation of the calibration curve, use of the formulae for the calculation, etc.

The Table 1 lists the names of instrumental methods that are based upon various analytical signals.

## CHROMATOGRAPHY

Chromatography is a technique, in which the components of a mixture are separated based on the

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rates at which they are carried through a stationary phase by a gaseous or liquid mobile phase. Chromatography involves two phases chosen such that the components of the sample have differing affinities in each phase and a sample (or sample extract) being dissolved in a mobile phase (which may be a gas, a liquid or a supercritical fluid). The mobile phase is then forced through the stationary phase. A component with high affinity toward the stationary phase will take longer to travel through it than a component with low affinity toward the stationary phase and high affinity toward the mobile phase. As a result of these differences in mobilities, sample components will become separated from each other as they travel through the stationary phase [Table 2].[1-10]

# HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

HPLC also known as high-pressure or high-price or high-speed liquid chromatography, HPLC is a form of column chromatography used frequently in analytical chemistry and biochemistry to identify, separate, and quantify compounds. It is a powerful tool in analysis. It is basically an improved form of column chromatography which has been optimized to provide rapid high resolution separations. Early LC used gravity fed open tubular columns with particles 100 s of microns in size; the human eye was used for a detector and separations often took hours or even days to develop. HPLC as compared with the classical technique is characterized by:

- Small diameter (2–5 mm), reusable stainless steel columns without repacking and regeneration
- Column packings with very small (3, 5, and 10 μm) particles and the continual development of new substances to be used as stationary phases
- Relatively high inlet pressures and controlled flow of the mobile phase
- Precise sample introduction without the need for large samples
- Special continuous flow detectors capable of handling small flow rates and detecting very small amounts
- Automated standardized instruments
- Rapid analysis
- Greater reproducibility due to close control of the parameters affecting the efficiency of separation
- Capable of handling macro molecule and viscous solutions
- Efficient analysis of labile natural products
- Reliable handling of inorganic or other ionic species.

**Table 1:** Classification of instrumental methods based on various analytical signals

| Signal | Instrumental Methods |
| --- | --- |
| Emission of radiation | Emission spectroscopy (X-ray, UV, visible, electron, Auger); fluorescence, phosphorescence, and luminescence |
| Absorption of radiation | Spectrophotometry and photometry (X-ray, UV, visible, IR); photoacoustic spectroscopy; nuclear magnetic resonance; and electron spin resonance spectroscopy |
| Scattering of radiation | Turbidimetry; nephelometry; and Raman Spectroscopy |
| Refraction of radiation | Refractometry and interferometry |
| Diffraction of radiation | X-ray and electron diffraction methods |
| Rotation of radiation | Polarimetry; optical rotary dispersion; and circular dichroism |
| Electrical potential | Potentiometry and chronopotentiometry |
| Electrical charge | Coulometry |
| Electrical current | Polarography and amperometry |
| Electrical resistance | Conductometry |
| Mass-to-charge ratio | Mass spectrometry |
| Thermal properties | Thermal conductivity and enthalpy |
| Radioactivity | Activation and isotope dilution methods |

**Table 2:** Classification of column chromatographic methods

| General Classification | Specific Method | Stationary Phase | Type of Equilibrium |
| --- | --- | --- | --- |
| Liquid chromatography (LC) (mobile phase: liquid) | Liquid-liquid, or partition | Liquid adsorbed on a solid | Partition between immiscible liquids |
| | Liquid-bonded phase | Organic species bonded to a solid surface | Partition between liquid and bonded surface |
| | Liquid-solid, or adsorption | Solid | Adsorption |
| | Ion exchange | Ion-exchange resin | Ion exchange |
| | Size exclusion | Liquid in interstices of a polymeric solid | Partition/sieving |
| Gas chromatography (GC) (mobile phase: gas) | Gas-liquid | Liquid adsorbed on a solid | Partition between gas and liquid |
| | Gas-bonded phase | Organic species bonded to a solid surface | Partition between liquid and bonded surface |
| | Gas-solid | Solid | Adsorption |
| Supercritical-fluid chromatography (SFC) (mobile phase: supercritical fluid) | | Organic species bonded to a solid surface | Partition between supercritical fluid and bonded surface |

HPLC is probably the most universal type of analytical procedure. In addition, HPLC also ranks as one of the most sensitive analytical procedures and is unique in that it easily copes with multi-component mixtures. Its application areas include quality control, process control, forensic analysis, environmental monitoring, and clinical testing. It has achieved this position as a result of the constant evolution of the equipment used in LC to provide higher and higher efficiencies at faster and faster analysis times with a constant incorporation of new highly selective column packings.[11-15]

# DRUG PROFILE

## Carvedilol
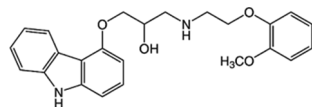


### Structure

- Molecular formula: $C_{24}H_{26}N_2O_4$
- Molecular mass: 406.474 g/mol
- IUPAC name: -[3-(9H-carbazol-4-yloxy)-2-hydroxypropyl][2-(2-methoxyphenoxy)ethyl] amine
- Description: White or almost white, slightly hygroscopic powder
- Solubility: Very soluble in water, freely soluble in methanol
- Drug Category: Anti-hypertensive Drug
- Class: Selective beta-blocker
- Pka: 8.77
- Half Life: 7–10 h
- Excretion: Primarily excreted in the bile and feces.

## Mechanism of action

Carvedilol inhibits exercise induce tachycardia through its inhibition of beta-adrenoceptors. Carvedilol's action on alpha-1 adrenergic receptors relaxes smooth muscle in vasculature, leading to reduced peripheral vascular resistance and an overall reduction in blood pressure. At higher doses, calcium channel blocking and antioxidant activity can also be seen. The antioxidant activity of carvedilol prevents oxidation of low density lipoprotein and its uptake into coronary circulation.

- Generic name: Carvedilol Tablets IP
- Brand Name: CardiVas 3.125 mg.

# MATERIALS AND METHODS

## Chemicals and standards used [Table 3]

**Table 3:** List of chemicals and standards used

| Chemicals | Manufacturer Name | Grade |
|---|---|---|
| Water | Merck | HPLC grade |
| Methanol | Merck | HPLC grade |
| Ortho phosphoric acid | Merck | G.R |
| KH2PO4 | Merck | G.R |
| K2HPO4 | Merck | G.R |
| 0. 22µ Nylon filter | Advanced lab | HPLC grade |
| 0.45µ filter paper | Millipore | HPLC grade |
| Carvedilol | In-House | In-House |

HPLC: High-performance liquid chromatography

## Instruments used [Take 4]

**Table 4:** List of instruments used

| | |
|---|---|
| UV-Visible Spectrophotometer | Shimadzu UV 1800 |
| UV-Visible Spec. software | UV Probe |
| HPLC software | Open lab software |
| HPLC | Agilent 1260 infinity binary pump |
| Ultra sonicator | Remi |
| pH meter | Systronics 335 |
| Electronic balance | Shimadzu |
| Syringe | Agilent manual syringe |
| HPLC Column | Zorbax C18 [250 × 4.6 mm] 5 µm |

HPLC: high-performance liquid chromatography

## Method development for the simultaneous estimation of uridine Triacetate using RP-HPLC

- Selection of mobile phase
- Selection of detection wavelength
- Selection of column
- Selection of solvent delivery system
- Selection of flow rate
- Selection of column temperature
- Selection of diluents
- Selection of test concentration and injection volume.

## Chromatographic trials for simultaneous estimation of Carvedilol by RP- HPLC [Figures 1-5]

### *Trial-1*
*Chromatographic conditions*
- Column: Phenomenex (150 × 4.6 mm) 5μ
- Mobile phase ratio: Methanol: 0.1% OPA (50:50%v/v) Detection wavelength: 285 nm
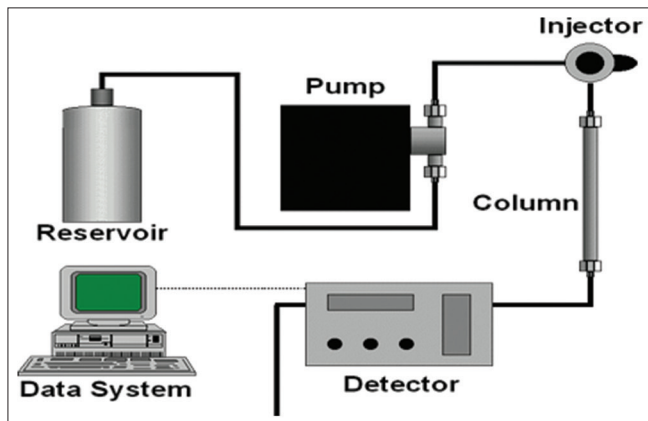- Flow rate: 1.0 ml/min
- Injection volume: 10 μl



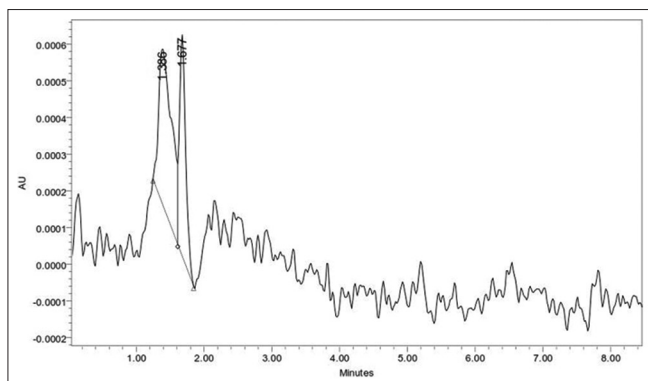**Figure 1:** Schematic diagram of high-performance liquid chromatography basic instrumentation



**Figure 2:** Chromatogram showing trial 1 injection
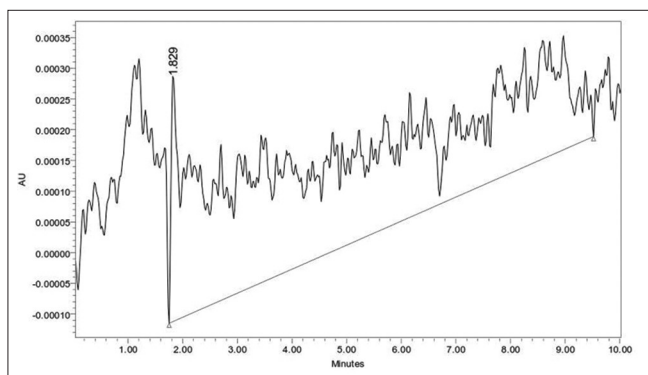


**Figure 3:** Chromatogram showing trial-2 injection

- Run time: 10 min
- Retention time: No peak.

### Observation

The trial shows no good peak separation, so more trials were required for obtaining peaks.

### *Trial-2*
*Chromatographic conditions*
- Column: Phenomenex (150 × 4.6 mm) 5μ
- Mobile phase ratio: Methanol: 0.1% OPA (70:30 %v/v)
- Detection wavelength: 285 nm
- Flow rate: 1.0 ml/min
- Injection volume: 10 μl
- Run time: 10 min
- Retention time: 1.217.

### Observation

In this trial, no proper peak separation was observed; still, more trials were required for good peaks.

### *Trial-3*
*Chromatographic conditions*
- Column: Phenomenex C18 (150 × 4.6 mm) 5μ
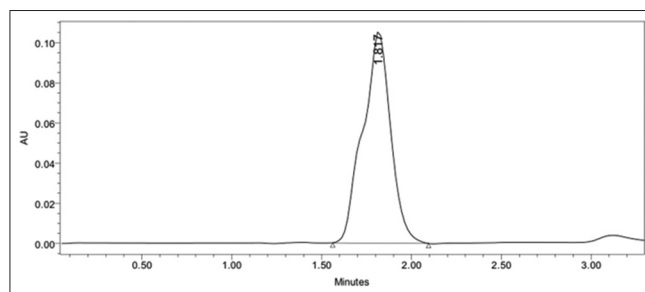- Mobile phase ratio: MeOH: pH 3.4 Phosphate buffer (50:50%v/v)



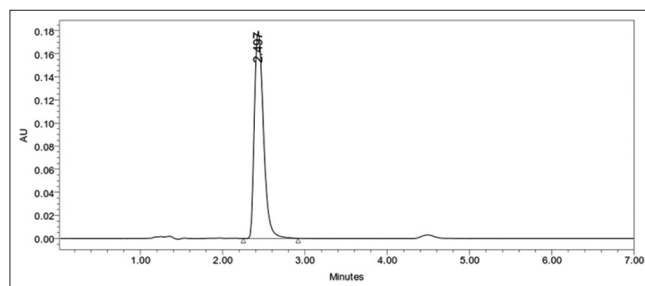**Figure 4:** Chromatogram showing trial-3 injection



**Figure 5:** Chromatogram showing trial-4 injection[16-19]

- Detection wavelength: 285 nm
- Flow rate: 1.0 ml/min
- Injection volume: 10 µl
- Run time: 10 min
- Retention time: 1.817 MIN.

## Observation

In this trial, Carvedilol was eluted, but there is no proper baseline and retention time; still, more trials were required for better peak.

### *Trial-4 (Optimized method)*
*Chromatographic conditions*
- Column: Phenomenex (150 × 4.6 mm) 5 µ
- Mobile phase ratio: MeOH: pH 3.4 Phosphate buffer (65:35%v/v)
- Detection wavelength: 285 nm
- Flow rate: 1.0 ml/min
- Injection volume: 10µl
- Run time: 7 min
- Retention time: 2.497 min.

## Observation

The separation was good, peak shape was good, so we conclude that there is no required for increase the retention times of peak, so it is taken as final method.

### *Assay calculation*

$$Assay\% = \frac{Sample\,area}{Standard\,\,area} \times \frac{Dilution\,sample}{Dilution\,of\,Standard}$$

$$\times \frac{P}{100} \times \frac{Avg\,wt}{Lc} \times 100$$

Avg. wt = average weight of tablets
P = Percentage purity of working standard
LC= Label Claim of Uridine Triacetate mg/ml.

## RESULTS AND DISCUSSION

The present investigation reported in the thesis was aimed to develop a new method development and validation for the estimation of carvedilol by RP-HPLC method. The literature reveals that there are no analytical methods reported for the estimation carvedilol by RP-HPLC method. Hence, it was felt that there is a need of new analytical method development for the estimation of carvedilol in pharmaceutical dosage form.

## Method development

The detection wavelength was selected by dissolving the drug in diluent to get a concentration of 10 µg/ml for individual and mixed standards. The resulting solution was scanned in U.V range from 200 to 400 nm. The spectrums are shown in Figure 6.

The chromatographic method development for the estimation of carvedilol was optimized by several trials for various parameters as different column, flow rate, and mobile phase; finally, the following chromatographic method was selected for the separation and quantification of carvedilol in API and pharmaceutical dosage form by RP-HPLC method.

## Optimized chromatographic conditions for simultaneous estimations Carvedilol by RP-HPLC method

### *Chromatographic conditions*
- Column: Phenomenex (150×4.6mm) 5µ
- Mobile phase ratio: MeOH: pH 3.4 Phosphate buffer (65:35%v/v)
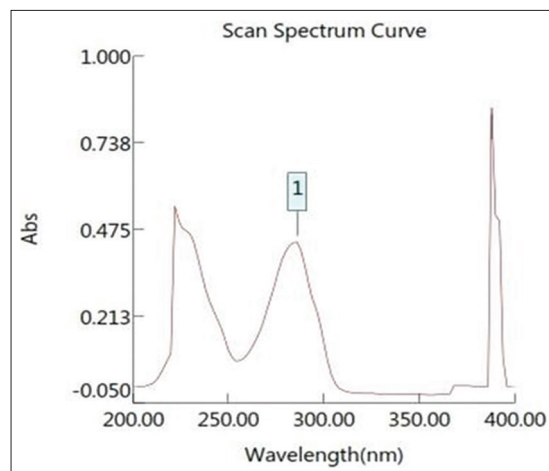- Detection wavelength: 285 nm
- Flow rate: 1.0 ml/min



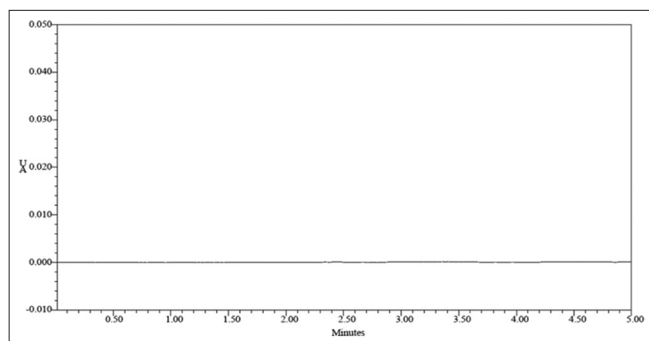**Figure 6:** Spectrum showing overlapping spectrum of Carvedilol

**Figure 7:** Chromatogram showing blank preparation (mobile phase)

**Table 5:** Linearity results for carvedilol

| Concentration (µg/ml) | Area |
| --- | --- |
| 20 | 264840 |
| 40 | 491415 |
| 60 | 677620 |
| 80 | 873311 |
| 100 | 1048958 |
| Correlation coefficient | 0.999 |

- Injection volume: 10µl
- Run time: 7 min
- Retention time: 2.497 min.

**Assay calculation for carvedilol**

The assay study was performed for the Carvedilol. Each three injections of sample and standard were injected into chromatographic system. The chromatograms are shown in Figures 1-7 and results are tabulated in Tables 3-5.

**SUMMARY AND CONCLUSION**

A new method was established for estimation of Carvedilol by RP-HPLC method. The chromatographic conditions were successfully developed for the separation of Carvedilol using Phenomenex C18 column (4.6 × 150 mm) 5 µ, flow rate was 1.0 ml/min, mobile phase ratio was pH 3.4 Phosphate: meoH (65:35% v/v), and detection wavelength was 284 nm. The instrument used was Agilent 1260 infinity binary pump HPLC, Open laboratory software. The retention times were found to be 2.425 min. The percentage purity of Carvedilol was found to be 99.94%. The system suitability parameters for Carvedilol such as theoretical plates

and tailing factor were found to be 4187, 1.5. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Carvedilol was found in concentration range of 20–100 µg and correlation coefficient ($r^2$) was found to be 0.999, % recovery was found to be 99.95%, % RSD for repeatability was 0.2, and % RSD for intermediate precision was 0.1. The precision study was precision, robustness and repeatabilty. LOD value was 3.06 and LOQ value was 10.14.

Hence, the suggested RP-HPLC method can be used for routine analysis of Carvedilol in API and pharmaceutical dosage form.

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