

NEW ANALYTICAL METHODS AND THEIR VALIDATION FOR THE ESTIMATION OF CARVEDILOL IN BULK AND MARKETED FORMULATION

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ABSTRACT

Carvedilol is cardiovascular agent. Mainly used for the treatment of hypertension, heart failure, and cardiovascular diseases.

Purpose: Analytical methods are required to characterize drug substances and drug products composition during all phases of pharmaceutical development. Development of methods to achieve the final goal of ensuring the quality of drug substances and drug products must be implemented in conjunction with an understanding of the chemical behavior and physicochemical properties of the drug substance. This determination requires highly sophisticated instruments and methods like HPLC, HPTLC and Spectrophotometer. Hence there was a need for the development of newer, simple, sensitive, rapid, accurate and reproducible analytical methods for the routine estimation of metoprolol succinate in bulk and pharmaceutical dosage form.

Methods: The present work describes two new spectrophotometric methods for the determination of carvedilol in bulk and marketed formulations. Method A is based on oxidation of Carvedilol with ferric ammonium sulphate followed by complex formation of resulting ferrous ion (Fe^{2+}) with potassium ferricyanide to form bluish green coloured chromogen which showed λ_{max} at 582 nm. In Method B is based on the oxidation of 2, 4-Dinitrophenylhydrazine and coupling of the oxidized product with drugs to give brown coloured chromogen which showed λ_{max} at 500.8 nm.

Results: The linearity was found in concentration range of 5-30 $\mu\text{g/ml}$ for both Method A and Method B. The correlation coefficient was found 0.997 and 0.9997 for both Method A and Method B respectively. The methods were validated as per ICH guidelines. The LOD and LOQ for estimation of Carvedilol were found as 0.0840, 0.2545 for method A and 0.0667, 0.2021 for method B respectively.

Conclusion: Proposed methods were successfully applied for the quantitative estimation of Carvedilol in marketed formulations.

KEY WORDS Carvedilol, potassium ferricyanide, ferric ammonium sulphate, 2, 4-dinitro phenyl hydrazine, sodium hydroxide, potassium iodate, validation and colourimetry.

INTRODUCTION

Carvedilol is a beta-blocker. Beta-blockers affect the heart and circulation (blood flow through arteries and veins). Carvedilol is used to treat heart failure and hypertension. Carvedilol is a racemic mixture in which nonselective beta-adreno receptor blocking activity is present in the S(-) enantiomer and alpha-adrenergic blocking activity is present in both R(+) and S(-) enantiomers at equal potency. Carvedilol's beta-adrenergic receptor blocking ability decreases the heart rate, myocardial contractility, and myocardial oxygen demand. Carvedilol also decreases systemic vascular resistance via its alpha adrenergic receptor blocking properties. Carvedilol and its metabolite BM-910228 (a less potent beta blocker, but more potent antioxidant) have been shown to restore the inotropic responsiveness to Ca^{2+} in OH^- free radical-treated myocardium. Carvedilol and its metabolites also prevent OH^- radical-induced decrease in sarcoplasmic reticulum Ca^{2+} -ATPase activity. Therefore, carvedilol and its metabolites may be beneficial in chronic heart failure by preventing free radical damage. It has been determined by different techniques including UV spectrophotometric method^[1,7], analytical methods, RP-HPLC method^[3,5], Simple and facile methods^[4], Simple spectrophotometric estimation^[2,6].

In the present work, a simple, accurate and sensitive method for determining carvedilol in pure form and pharmaceutical formulations was introduced.

The literature survey on the analytical applications of potassium ferricyanide and 2, 4-DNP indicates that these compounds have not been earlier reported as reagents for the spectrophotometric determination of carvedilol in either biological fluids or pharmaceutical formulations. Hence the author has made an attempt to develop simple and rapid methods for the estimation of the sited drug in bulk and pharmaceutical formulations.

MATERIALS AND METHODS

Carvedilol was obtained in highly pure form (pharmaceutical grade) from the local pharmaceutical industry. Its pharmaceutical preparations obtained from different commercial sources. All other reagents were of analytical grade. Methanol, Sulphuric acid, distilled water was used for preparation of all solutions. Ultraviolet and visible spectrophotometry were carried out through Systronics PC based Double Beam Spectrophotometer 2202 and JascoV-630 spectrophotometer.

Standard drug solution:

Accurately weighed 100mg of carvedilol was dissolved in 100ml methanol to give a concentration of 1000 µg/ml. The final concentration was brought to 50 µg/ml for Methods A and B.

Reagents:

Method A:

- 0.6% ferric ammonium sulphate solution
- 0.3% Potassium ferricyanide solution
- Concentrated HCL

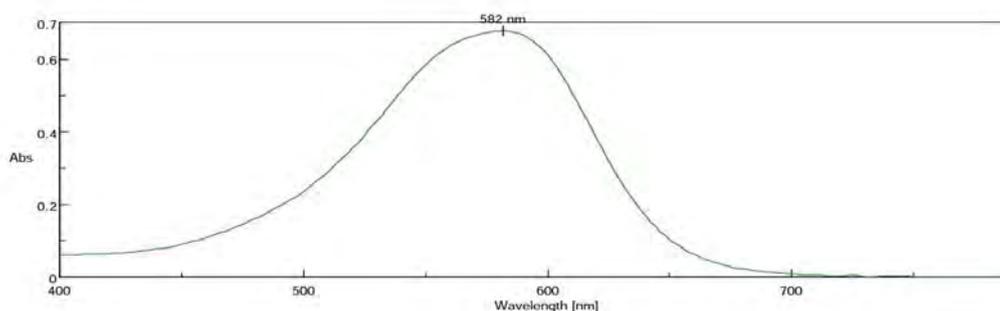
Method B:

- 0.06% 2, 4-DNP solution
- 4% potassium iodate solution
- 6N sodium hydroxide solution

Assay procedure for the determination of Carvedilol:

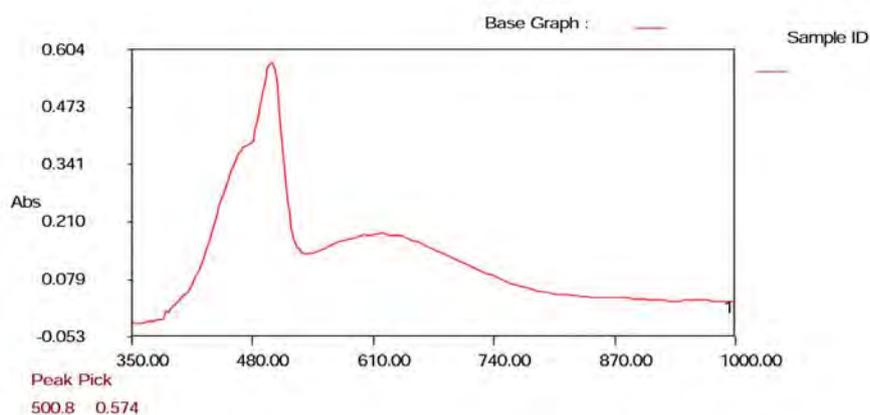
Method A: Seven 10ml volumetric flasks were taken. 0.5-3 ml (5-30µg/ml) of working standard of Carvedilol to each volumetric flask was added. Then 1 ml of 0.6% ferric ammonium sulphate and 1.5 ml of 0.3% potassium ferricyanide solution to each volumetric flask was added. The volumetric flask kept aside for 10 minute to complete the reaction. Then add 1ml of concentrated HCL and volume in flask was made up to the mark with methanol and the absorbance was recorded against reagent blank at 582 nm. (Fig: 1) against reagent blank which shown nil absorbance at corresponding wave length. The calibration curve was prepared to calculate the amount of the drug.

Fig: 1 Absorption spectrum of colored chromogen in method A



Method B: Seven 10ml volumetric flasks were taken. Then 0.5-3 ml (5-30µg/ml) working standard solution of Carvedilol was added. Then 1 ml of 4% potassium iodate and 1.5 ml of 0.06% of 2, 4-DNP solution were added. The volumetric flask kept aside for 10 minute to complete the reaction. Then add 1.5 ml of 6N NaOH to make solution alkaline and volume in flask is made up to the mark with methanol. Absorbance was recorded against reagent blank at 500.8 nm.

Fig: 2 Absorption spectrum of colored chromogen in method B



Assay of pharmaceutical formulations

Tablet powder equivalent to 100 mg was accurately weighed and dissolved in methanol and filtered. The filtrate was made up to 100 ml and appropriate aliquots of the drug solution were treated as described above and the results were tabulated.

RESULTS AND DISCUSSION

Method A is based on oxidation of Carvedilol with ferric ammonium sulphate followed by complex formation of resulting ferrous ion (Fe^{2+}) with potassium ferricyanide to form bluish green coloured chromogen which showed λ_{max} at 582 nm. In Method B is based on the oxidation of 2, 4-Dinitrophenylhydrazine and coupling of the oxidized product with drugs to give brown coloured chromogen which showed λ_{max} at 500.8 nm.

The optical characteristics such as absorption maxima and Beer's law limits for these methods are presented in Table-1. The regression analysis using the method of least squares was made for the slope (a) and intercept (b) obtained from different concentrations are summarized in Table-1. The precision and accuracy were found by analyzing six replicate samples containing known amounts of the drug and the results are summarized in Table-1

TABLE – 1 Optical Characteristics, Precision and Accuracy of Proposed Methods

| Parameter | Results | |
|--|---------------------------|---------------------------|
| | Method A | Method B |
| λ_{max} (nm) | 582 nm | 500.8 nm |
| Beer's law limit | 5-30 $\mu\text{g/ml}$ | 5-30 $\mu\text{g/ml}$ |
| Regression Equation* (y) | $y=bx+a: 0.0185x -0.0045$ | $y=bx+a: 0.0233x +0.0045$ |
| Slope (b) | 0.0185 | 0.0233 |
| Intercept (a) | 0.0045 | 0.0045 |
| Correlation coefficient (R^2) | 0.997 | 0.9997 |
| Limit of Detection ($\mu\text{g/ml}$) | 0.0840 | 0.667 |
| Limit of quantitation ($\mu\text{g/ml}$) | 0.2545 | 0.2021 |
| Accuracy (% Recovery \pm SD) | 97.58 \pm 0.051 | 97.74 \pm 0.074 |
| Precision (Reproducibility) | | |
| Intraday (% Recovery \pm SD) | 0.179 \pm 0.0016 | 0.186 \pm 0.0024 |
| Interday (% Recovery \pm SD) | 0.176 \pm 0.0020 | 0.183 \pm 0.0015 |

$y = bx + a$, where y is the absorbance and x is the concentration of Metoprolol succinate in $\mu\text{g/ml}$.

RECOVERY EXPERIMENTS:

Weighed accurately tablet powder equivalent to 100mg and transferred into 100ml volumetric flask and dissolve in small volume of methanol. Then filter the solution and filtrate is made up the volume with methanol to get 1000µg/ml concentration. This solution was further diluted to get concentration of 100µg/ml. To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different concentrations like 5, 10 and 15 µg/ml were prepared in case of both pure drug solution and the formulation extract solution and these solutions were subjected to analysis by above developed method. The six such samples were prepared and average of that readings taken for calculation of % recovery. This is reported in following table no. 2.

TABLE - 2. Assay and Recovery of Carvedilol in Pharmaceutical Formulations

| Method | Sample | Labeled amount (mg) | Amount found (mg) | % Recovery |
|--------|------------|---------------------|-------------------|------------|
| A | CARCA-12.5 | 12.5 | 11.9 | 95.2 |
| B | CARCA-12.5 | 12.5 | 12.1 | 96.8 |

CONCLUSION

It could be concluded that the developed methods for metoprolol succinate assay is simple, sensitive, precise, accurate and can be satisfactorily applied to the analysis of metoprolol succinate in bulk and pharmaceutical formulations. The proposed methods are used for the routine analysis of the drug in the quality control.

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REFERENCES

- [1] Theivarasu C, Ghosh S, Indumathi T. UV spectrophotometric determination of carvedilol in pharmaceutical formulations. *Asian J Pharm Clinical Res* 2010;3(4):64-8.
- [2] Rani YN, Ravikumar BVV, Mohanty S. Development and validation of new analytical methods for the estimation of carvedilol in bulk and pharmaceutical dosage. *Asian J Pharm Clinical Res* 2013;6(2):138-40.
- [3] Rao BMV, Nagendrakumar AVD, Yedukondalu M, Raman BV. New validated RP-HPLC method for estimation of carvedilol in pharmaceutical formulation. *Int J Pharm Pharm Sci* 2012;4(2):353-8.
- [4] Sreevidya TV, Narayana B. Simple and facile methods for the determination of carvedilol in pharmaceuticals. *Ind J Chem Tech* 2009;16:74-8.
- [5] Edla S, Syamasundhar B. RP-HPLC method development and validation for the analysis of carvedilol in pharmaceutical dosage forms. *Int J Sci Innovations Discoveries* 2011;1(3):433-40.
- [6] Desai DC, Karkhanis VV. Simple spectrophotometric estimation of carvedilol phosphate in bulk and tablet dosage forms. *Int Res J Pharm* 2012;3(2):114-6.
- [7] Dattatraya SM, Dhake AS, Setty CM Development of UV spectrophotometric method for estimation of carvedilol in bulk and pharmaceutical formulations. *Asian J Res Chem* 2013;6(10):556-9.
- [8] <http://www.drugbank.ca/drugs/DB00264>
- [9] <http://en.wikipedia.org/wiki/Carvedilol>
- [10] <http://www.drugs.com/carvedilol.html>
- [11] <http://www.webmd.com/drugs/2/drug-5574/carvedilol-oral/details>
- [12] <http://www.drugbank.ca/drugs/DB01136>