Available online at www.JGTPS.com

Research Article

ISSN:2230-7346

Journal of Global Trends in Pharmaceutical Sciences

Vol.3, Issue 3, pp-714-722, July–September 2012

DEVELOPMENT AND VALIDATION OF ANALYTICAL METHODS FOR THE ESTIMATION OF MEDICINAL COMPOUNDS HAVING HYDROXYL GROUP

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ABSTRACT

A Simple, Rapid, Reliable, Precise, Linear method was developed and validated for the estimation of medicinal compounds having Hydroxyl group. Hydroxyl group present in any drug molecule offer development of number of spectroscopic methods. Hydroxyl group renders sufficient polarity to the molecule. The work deals with development and validation of four UV-Visible spectrophotometric methods by NBS as a chromogenic reagent, four UV-Visible spectrophotometric methods by $FeCl_3$ as reducing reagent in bulk and pharmaceutical dosage form. RP-HPLC method was developed and validated for the estimation of carvedilol in bulk and pharmaceutical dosage forms. All the methods were found to be linear, precise, accurate, specific and all proved to be sensitive, convenient and effective for the determination of carvedilol, lumefantrine, alendronate sodium and simvastatin in bulk and pharmaceutical dosage forms.

Key Words: Hydroxyl group, NBS reagent, RP-HPLC, Carvedilol.

INTRODUCTION:

1. Materials Used

1.1 Preparation of Mobile phase

The mobile phase was prepared by filtered and degassed mixture of formic acid, acetonitrile and water in the ratio of 1:45:55.

1.2 Instrument Specification:

- HPLC : LC 2010 SHIMADZU AHT.
- Software : LC Solutions
- Detector : UV

Preparation of standard solution:

Accurately weighed 10.0 mg of carvedilol (bulk drug) was dissolved in 5.0 ml of mobile phase in 10 ml volumetric flask and sonicated for five minutes until it dissolves the material and the volume was made up with mobile phase. From this 1.0 ml of solution was taken and diluted to 10 ml (stock solution). Solution was filtered through 0.45 µm filter paper.

Preparation of sample solution:

Twenty carvedilol tablets were taken and finely powdered. The accurate quantity of powder equivalent to 10.0 mg of active ingredient was dissolved in 50.0 ml of mobile phase and sonicated for about 10.0 min with occasional shaking by maintaining sonicator bath temperature below 28 ^{0}C. The solution was cooled and the volume was made up with the mobile phase in 100 ml volumetric flask. From this 2.0 ml was taken and diluted to 10 ml and filtered through 0.45 µm filter paper.

Preparation of calibration curve:

Aliquots of carvedilol ranging from 1.7 – 2.1 ml were pipetted into as series of 10 ml volumetric flask and diluted to the mark with mobile phase. The linearity range of carvedilol was found to be 17.0 – 21.0 µg/ml. The calibration curve was constructed by plotting peak area against concentration. The column was equilibrated for at least 30 min, with the mobile phase flowing through the system with a flow rate of 0.5 ml/min. Detector was set at a wavelength of 280 nm. First, blank injection was given to show mobile phase does not account for any impurities. Six standard drug solutions were prepared as per the procedure mentioned in the preparation of calibration curve and injected in to the column in order to establish the linearity. The sample solution prepared as per the procedure mentioned above and was injected in order to know the percentage purity.

UV-Visible Spectrophotometric Methods the Estimation of carvedilol, lumefantrine, alendronate sodium and simvastatin by NBS in Bulk and Pharmaceutical Dosage Form[1-4]

Carvedilol, lumefantrine, alendronate sodium and simvastatin are having hydroxyl group at different positions. An attempt has been made to determine these drugs by reacting hydroxyl group with NBS. The reaction involves oxidation of hydroxyl group. Here NBS acts as an oxidizing agent which oxidizes the drugs. Residual NBS reacts with methyl orange, when fixed amount of methyl orange was added it reacts with decreasing amount of NBS, a constant increase in the dye concentration occurred. As a results absorbance increases linearly.

MATERIALS USED

NBS Solution (90 µg/ml): 9.0 mg of NBS was weighed, dissolved in distilled water in 100 ml volumetric flask and volume was made up to mark with distilled water. The solution was freshly prepared and protected from light during the use.

Hydrochloric acid (1.0M):

8.5 ml of concentrated hydrochloric acid was measured and transferred into a 100.0 ml volumetric flask and made up to the mark with distilled water.

Methyl orange solution (50.0 µg/ml) 5.0 mg of the methyl orange was weighed dissolved in distilled water in 100 ml volumetric flask and volume was made up to mark with distilled water.

Preparation of standard stock solution:

Accurately weighed 10.0 mg of carvedilol, lumefantrine and simvastatin (bulk drugs) were dissolved in 40.0 ml of methanol and alendronate sodium was dissolved in 40 ml of double distilled water in 100 ml volumetric flask and sonicated for about 15 min to enhance the solubility and volume was made up to the mark with methanol and double distilled water respectively to obtain a final concentration of 100 µg/ml (Stock solution).

ANALYSIS OF TABLET DOSAGE FORM:

Ten Carvedilol (Carlac 25 mg), lumefantrine, alendronate sodium (Denfos 35 mg) and simvastatin tablets (Simvas 10 mg) were taken and finely powdered separately.

The accurate quantity of powder equivalent to 10 mg of active ingredient was dissolved in distilled water and then passed through the whatman filter paper, followed by adding double distilled water up to 100.0 ml to get the stock solution of 100 µg/ml.

From the 100.0 ml solution 0.4 ml, 0.6 ml, 1.2 ml and 4.0 ml solutions of Carvedilol, alendronate sodium and simvastatin respectively were pipetted into a 10.0 ml volumetric flask and then the resultant solutions were also analysed as per the procedure described under calibration graph.

Preparation of calibration curve:

Aliquots of Carvedilol, lumefantrine, alendronate sodium and simvastatin ranging from 0.1 – 0.5 ml, 0.2 – 1.0 ml, 0.6 – 1.8 and 2.0 – 6.0 ml respectively were pipetted into as series of 10 ml volumetric flask from above stock solution. To each flask, 1.5 ml of 1.0 M hydrochloric acid followed by 1.0 ml of NBS solutions were added and diluted to volume with distilled water.

The reaction was allowed to proceed at room temperature. The flasks were stoppered and allowed to stand for 20 min with occasional shaking. Finally, 1.0 ml of methyl orange solution was added to each flask, volumes diluted to the mark with distilled water. The absorption spectra of Carvedilol, lumefantrine, alendronate sodium and simvastatin were taken at 508 nm respectively (Fig.4-7).

The linearity range or Beer's range of Carvedilol, lumefantrine, alendronate sodium and simvastatin were found to be 1.0 – 5.0 µg/ml, 2.0 – 10 µg/ml, 6.0 – 18.0 µg/ml and 20 – 60 µg/ml respectively. The calibration curves were constructed by plotting absorbance against concentration (Fig. 8-11). The content of Carvedilol, lumefantrine, alendronate sodium and simvastatin were calculated from the calibration graph[5-6]

RESULTS AND DISCUSSION:

Table No.1: Optical characteristics for RP HPLC Method for carvedilol

PARAMETER	Carvedilol
Wavelength (nm)	280
Linearity range (µg/ml)	17 – 21
Correlation coefficient (R)	0.998
Regression equation (y)	y = 65823x + 3742
Slope, *b*	65823
Intercept, *c*	3742
Relative standard deviation %	0.00013
Limit of detection (µg/ml)	6.5×10^{-5}
Limit of quantification (µg/ml)	1.98×10^{-4}

Table No.2: System suitability parameters for Carvedilol

Parameter	Result
Retention time (min)	5.38
Tailing factor	1.331
Theoretical plates	3641.236
HETP	41.195

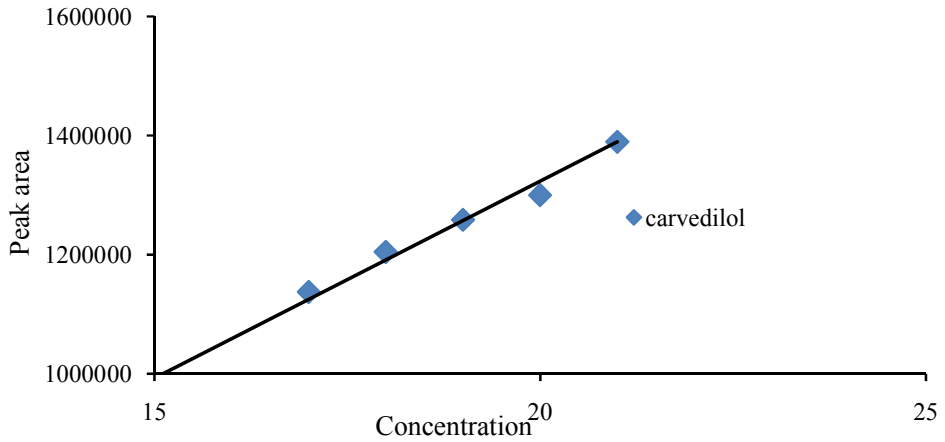
Table No.3: Validation Parameters of Carvedilol

S.NO	Parameters	Carvedilol
1	Linearity (µg/ml)	17-21
2	Limit of detection (µg/ml)	6.5×10^{-5}
3	Limit of quantification (µg/ml)	1.98×10^{-4}
4	% Recovery	99.00
5	Intraday % RSD	25×10^{-3}
6	Interday % RSD	35×10^{-3}
7	Relative retention time (min)	5.386

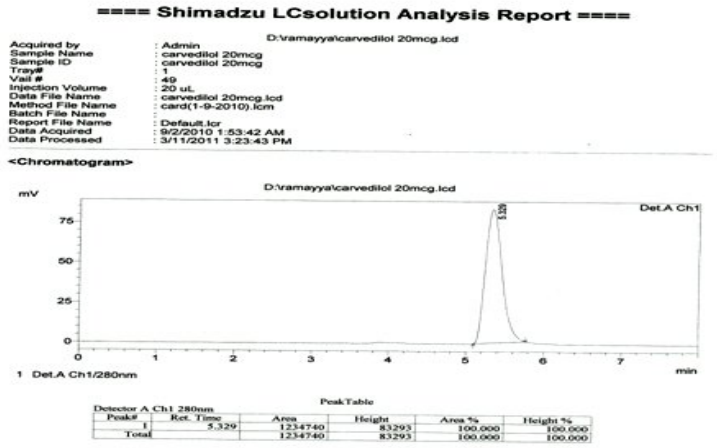
Table No.4: Optical characters of Carvedilol, Lumefantrine, Alendronate Sodium and Simvastatin for NBS method

S.NO	Parameter	Carvedilol	Lumefantrine	Alendronate sodium	Simvastatin
1	Limit of detection (µg/ml)	0.0200	0.060	0.10423	0.325
2	Limit of quantification (µg/ml)	0.0606	0.183	0.34744	1.083
3	% Recovery	98.06	97.9	99.36	98.66
4	Intraday % RSD	0.35	0.65	0.37	0.30
5	Interday % RSD	0.76	0.71	0.47	0.53
6	SEM	0.0004282	0.0005774	0.0006687	0.0005831

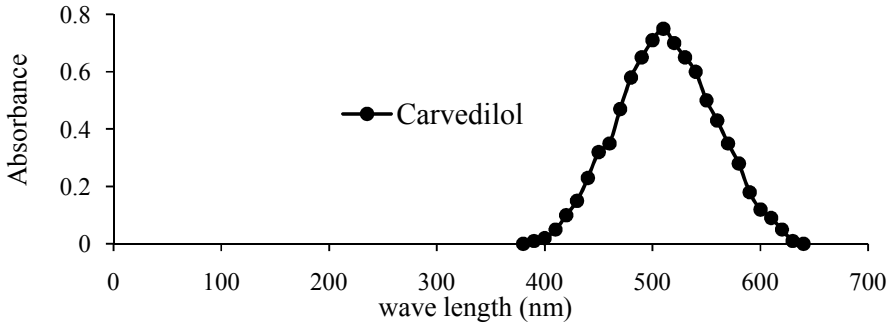
Graph 1: Calibration graph of carvedilol



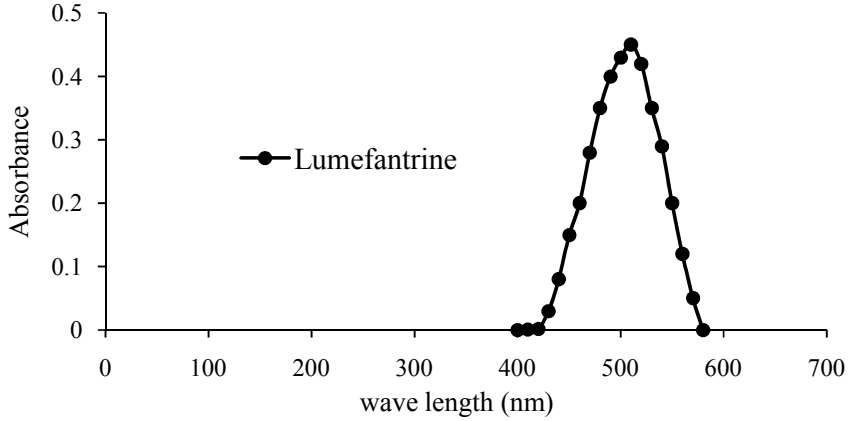
Graph 2: Standard graph of Carvedilol standard



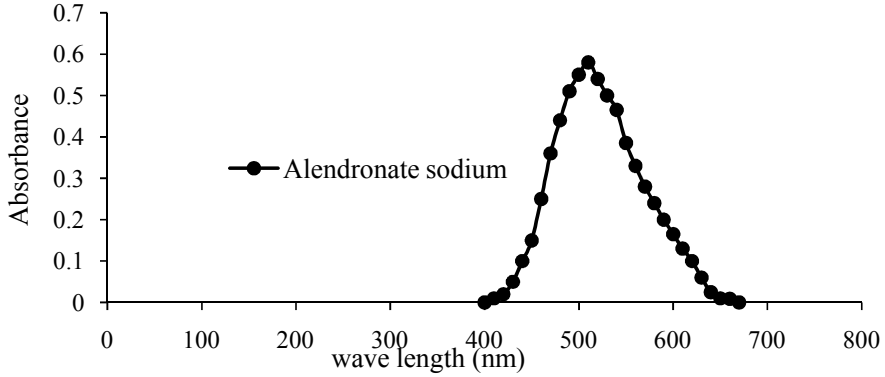
Graph 3: Absorption spectra of NBS with carvedilol against the reagent blank at 508 nm



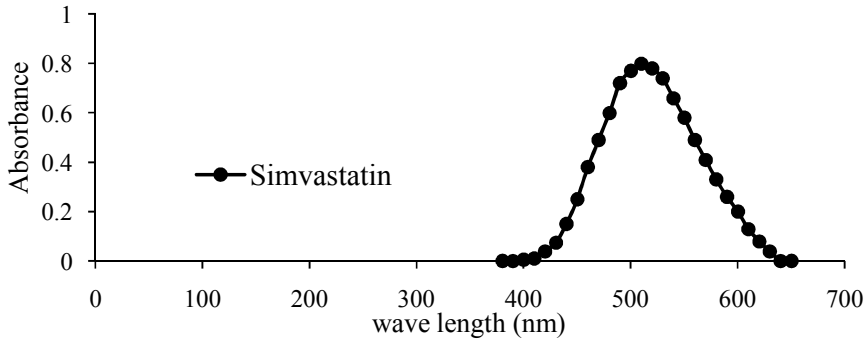
Graph 4: Absorption spectra of NBS with lumefantrine against the reagent blank at 508 nm



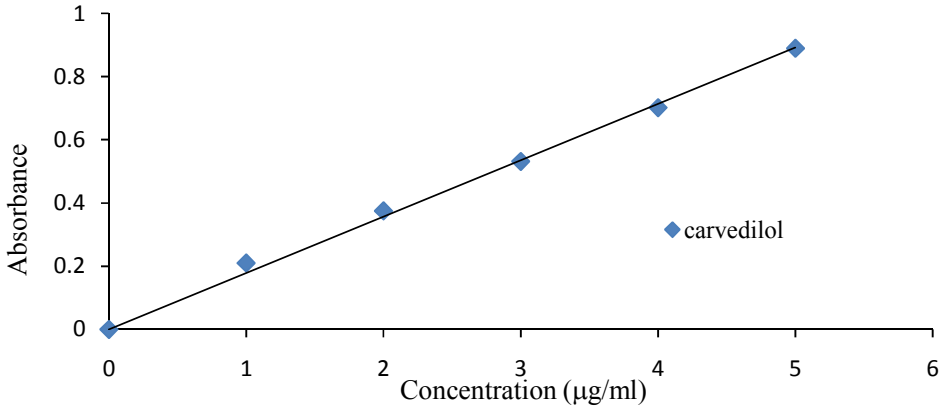
Graph 5: Absorption spectra of NBS with alendronate sodium against the reagent blank at 508 nm



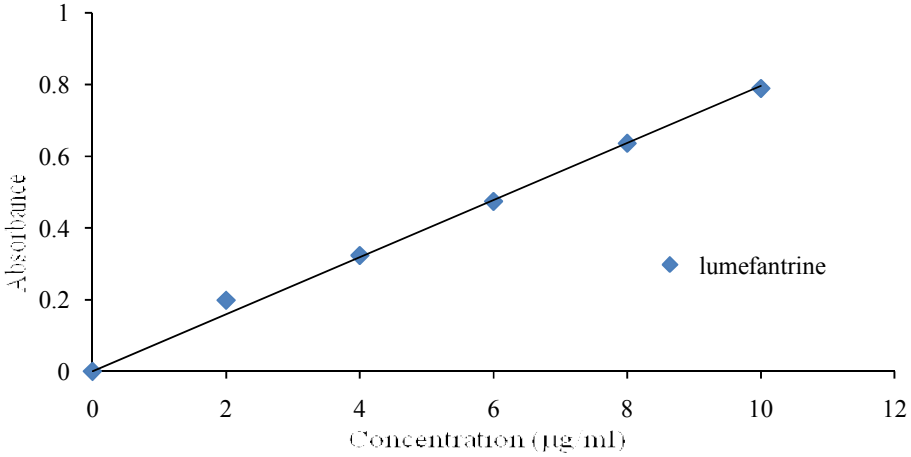
Graph 6: Absorption spectra of NBS with simvastatin against the reagent blank at 508 nm



Graph 7: Calibration graph of carvedilol by NBS



Graph 8: Calibration graph of lumefantrine by NBS



Validation parameters by NBS method for selected drugs

S.NO	Parameter	Carvedilol	Lumefantrine	Alendronate Sodium	Simvastatin
1	λ_{max} (nm)	508	508	508	508
2	Beers law limits (µg/ml)	1-5	2-10	6-18	20-60
3	Molar absorptivity (litre /mol/cm)	0.08536	0.05236	0.0143052	5.2111×10^{-3}
4	Correlation coefficient	0.997	0.996	0.999	0.998
5	Sandell's sensitivity (µg/ml 0.001 abs unit)	4.7619×10^{-3}	0.0101	0.02272	0.08032
6	Regression equation	Y=0.173x+0.0017	Y=0.077x+0.01	Y=0.047x+0.009	Y=0.012x+0.008
7	Slope, *b*	0.0173	0.077	0.047	0.012
8	Intercept, a	0.017	0.016	0.009	0.008
9	% Relative standard deviation	0.2	0.44	0.25	0.25

SUMMARY AND CONCLUSION:

In the current project work attempts were made to develop simple spectrophotometric and chromatographic methods for selected drugs. The hydroxyl group present in the selected drugs (carvedilol, lumefantrine, alendronate sodium and simvastatin) was exploited for development of spectrophotometric methods. The hydroxyl group was oxidised with many oxidising agents and best results were seen on oxidising the drugs with N- bromo succinamide and ferric chloride. Also, RP HPLC method was developed for quantitative estimation of carvedilol.

It explains the methodology of HPLC. It briefly explains about instrument specifications, selection of mobile phase, reagents and materials used, and their optimization followed by validation of developed method following ICH guidelines and also gives information regarding the spectrophotometric methods developed for Carvedilol, Lumefantrine, Alendronate sodium and Simvastatin using NBS reagent. The selected drugs possess hydroxyl groups at different positions. The hydroxyl group of the drug reacts with NBS reagent and get oxidized and residual NBS reacts with methyl orange and produces colored product which is determined spectrophotometrically. All the methods were found to be linear, precise, accurate, specific and all proved to be sensitive, convenient and effective for the determination of carvedilol, lumefantrine, alendronate sodium and simvastatin in bulk and pharmaceutical dosage forms.

REFERENCES:

1. Reynolds JEF. Martindale- The Extra Pharmacopoeia. 28th Edn. The Pharmaceutical Press. London. 198.
2. David Watson G. Pharmaceutical analysis. Harcourt Publishers Ltd. UK. 2000.
3. Ashutosh Kar. Pharmaceutical analysis.1st Edn. Vol. 1. CBS Publishers and Distributers. New Delhi. 2007.
4. John A. Adamoules. Chromatographic analysis of pharmaceuticals. Marcel Dekker. Inc. New York. 1990.
5. Raymond Scott PW. Gas Chromatography. Library 4 Science. UK. 2003.
6. Riley CM and Rosanske TW. Development and validation of analytical Methods. Elsevier. USA. 1996