Available online at www.derpharmachemica.com

ISSN 0975-413X
CODEN (USA): PCHHAX

Der Pharma Chemica, 2020, 12(4): 21-29
(http://www.derpharmachemica.com/archive.html)

Spectrometric Estimation of Gliclazide, Nortriptyline, Cilnidipine in Pure and Tablet Dosage Form Using MBTH

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ABSTRACT

The objective of the present work is to develop simple, precise and accurate colorimetric methods for the estimation of Gliclazide (Method A), Nortriptyline (Method B), Cilnidipine (Method C) using MBTH (3-Methyl-2-Benzothiaazide Hydrazine) reagent. All the three Methods were developed on Perkin Elmer LAMBDA 25 UV/VIS spectrophotometer with 1cm quartz cells. Quantification of Gliclazide, Nortriptyline, Cilnidipine were done by Oxidative coupling method using MBTH as reagent. Gliclazide (Method A), Nortriptyline (Method B) and Cilnidipine (Method C) undergoes oxidation with ferric chloride and couples with MBTH. The coupled oxidant shows maximum absorbance measured at 660 nm for Gliclazide (Method A), 650 nm for Nortriptyline (Method B), and 600 nm for Cilnidipine (Method C). The colorimetric method was extensively validated as per ICH guidelines and all the parameters were within the acceptance criteria with correlation of 0.999% RSD less than 2 for the method. The method was proved to be more accurate, simple, precise and rapid by statistical validation as well as recovery studies and can be used for routine laboratory analysis.

Keywords: Gliclazide, Visible spectrophotometry, Validation, Gliclazide, Nortriptyline, Cilnidipine, MBTH.

INTRODUCTION

Gliclazide1, chemically is 1-(3-azabicyclo (3.3.0) oct-3-yl)-3-(p-tolylsulfonyl) urea is an oral antihyperglycemic agent used for the treatment of non-insulin-dependent diabetes mellitus (NIDDM). Gliclazide binds to the β cell sulfonyl urea receptor (SUR1). Nortriptyline2, chemically is 3-(10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ylidene)-N-methyl-1-propanamine. It is the active metabolite of amitriptyline, is a tricyclic antidepressant (TCA). It is used in the treatment of major depression and is also used off-label for chronic pain and other conditions. Cilnidipine3, chemically is 1,4-dihydropyridine-3,5-dicarboxylic acid. It is the L-type calcium channel blocker [1-3].

The Chemical structures of Gliclazide, Nortriptyline and Cilnidipine are shown in Figure 1, 2 & 3 respectively. Literature survey of these drugs revealed that there are few methods for the determination of Gliclazide/Nortriptyline/Cilnidipine alone by HPLC and spectrophotometric methods [4-19]. The existing HPLC methods take more time for sample preparation & analysis, at the same time expensive. The reported UV methods may not be specific as there is no procedure for elimination of interference by the additives or active pharmaceutical ingredients. Gliclazide and Nortriptyline were chosen for the presence of active methylene groups. As such Cilnidipine would not react with MBTH to give colored product but the presence of nitro group could be helpful to reduce to amino group which helps for analysis by oxidative coupling with MBTH. There were no reported methods for the proposed drugs by visible spectrophotometry using ferric chloride and MBTH. The purpose of this work is to develop visible methods focused on economic point of view as well as stable and short analysis time by green solvents for routine analysis and to validate the methods according with ICH guidelines.

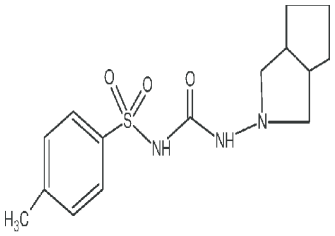

Figure 1: Chemical Structure of Gliclazide

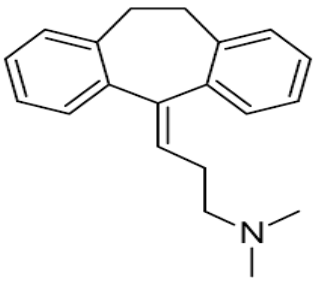

Figure 2: Chemical Structure of Nortriptyline

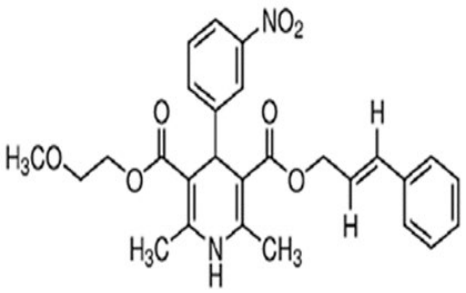

Figure 3: Chemical Structure of cilnidipine

MATERIALS AND METHODS

Equipment

Double-beam Perkin Elmer (LAMBDA 25) UV-Vis spectrophotometer was used for spectral measurements. Sarotius electronic balance was used for weighing the chemicals.

Chemicals

Gliclazide, Nortriptyline, Cilnidipine are obtained as a gift sample from Aurobindo pharma Ltd, Hyderabad. Triple distilled water, MBTH (3-Methyl-2-Benzothiaazide Hydrazine), sodium hydroxide, methanol and ferric chloride were used for the experimental work. All the chemicals used in the experimental procedure are of analytical reagent grade.

PREPARATION OF SOLUTIONS

Preparation of stock solution for estimation of Gliclazide (Method A)

About 25 mg of Gliclazide was weighed and transferred to a 25 ml volumetric flask, 0.1 N NaOH was added to dissolve it and diluted to volume with 0.1 N NaOH. The resulting solution has a concentration of 1 mg/ml.

Preparation of stock solution for estimation of Nortriptyline (Method B)

About 25 mg of Nortriptyline was weighed and transferred to a 25 ml volumetric flask, Ethanol was added to dissolve it and diluted to volume with Ethanol. The resulting solution has a concentration of 1 mg/ml.

Preparation of stock solution for estimation of Cilnidipine (Method C)

About 10 mg of Cilnidipine was weighed and transferred to a 10 ml volumetric flask, then 5 mg of zinc and 0.2 ml of 5N HCl was added for reduction. When the effervescence ceases the solution was filtered, final volume was made with methanol. The resulting solution has a concentration of 1 mg/ml of reduced Cilnidipine.

Preparation of 0.5% MBTH (3-Methyl-2-Benzothiaazide Hydrazine)

About 50 mg of MBTH (3-Methyl-2-Benzothiaazide Hydrazine) was dissolved in distilled water and made to 100 ml with the same solvent.

Preparation of 0.5% Ferric chloride (FeCl3)

About 50 mg of Ferric chloride (FeCl3) was dissolved in distilled water and made to 100 ml with the same solvent.

PROCEDURE FOR CALIBRATION PLOT

Method A

Into a series of 10 ml volumetric flasks, 0.4 ml-1.4 ml of working standard solution of Gliclazide was pipetted out and 0.6 ml of MBTH and 1 ml of $FeCl_3$ were added, then the final volume was made to 10 ml with distilled water. The absorbance of the greenish colored chromogen was measured at 660 nm against the reagent blank. The amount of Gliclazide present in the sample solution was computed from its calibration curve.

Method B

In a series of 10 ml volumetric flasks, 0.2 ml-1.0 ml of working standard solution of Nortriptyline was pipetted out and 0.8 ml of MBTH and 1 ml of $FeCl_3$ were added, then the final volume was made to 10 ml with distilled water. The absorbance of the bluish colored chromogen was measured at 650 nm against the reagent blank. The amount of Nortriptyline present in the sample solution was computed from its calibration curve.

Method C

In a series of 10 ml volumetric flasks, 0.4 ml-1.2 ml of working standard solution of Cilnidipine was pipetted out, then add 1 ml of MBTH and 1ml of Fecl3 were added, then the final volume was made to 10 ml with distilled water. The absorbance of the greenish colored chromogen was measured at 600 nm against the reagent blank. The amount of Cilnidipine present in the sample solution was computed from its calibration curve.

ASSAY PROCEDURE

Method A

Twenty tablets of commercial samples (Reclide 80mg TAB) of Gliclazide were accurately weighed and powdered. Tablet powder equivalent to 10 mg of Gliclazide was dissolved in 10 ml 0.1 N NaOH, filtered. The resulting solution has a concentration of 1 mg/ml then the procedure given for pure drug analysis was followed.

Method B

Twenty tablets of commercial samples (Nortri-Tab 25 mg) of Nortriptyline were accurately weighed and powdered. Tablet powder equivalent to 25 mg of Nortriptyline was weighed and transferred to a 25 ml volumetric flask, Ethanol was added to dissolve it and diluted to volume with Ethanol. The resulting solution has a concentration of 1 mg/ml.

Method C

Twenty tablets of commercial samples (Ciledge Tab 10 mg) of Cilnidipine were accurately weighed and powdered. Tablet powder equivalent to 10 mg of Cilnidipine was weighed and transferred to a 10 ml volumetric flask, then 5 mg of zinc and 0.2 ml of 5N HCl was added for reduction. When the effervescence ceases the solution was filtered, final volume was made with methanol. The resulting solution has a concentration of 1 mg/ml of reduced Cilnidipine.

RESULTS AND DISCUSSION

Method development

The method was optimized by selecting the proper solvent, reagent and oxidizing agent, order of addition, concentration of the reagent and the selection of the wavelength for quantification. The parameters were mentioned in Tables 1 to 2 and Figures 4, 5 & 6 represents absorption spectrum of Gliclazide, Nortriptyline and Cilnidipine, respectively.

Order of Addition

To find out whether the order of addition has any influence, the absorbance of a set of solutions prepared by mixing the reagents and the amounts of drug levels in different sequences, as given in the recommended procedure were measured, studied and presented in the Table 1 for all methods.

Table 1: Fixing order of addition for methods

S.No	Method	Order of Addition	Absorbance Mean ± Sd	ROA
1	Method A	GLI+$FeCl_3$+MBTH	0.033 ± 0.001	2
2		GLI+MBTH+$FeCl_3$	0.229 ± 0.001	
3		MBTH+$FeCl_3$+GLI	0.0095 ± 0.0001	

1	Method B	NOR+$FeCl_3$+MBTH	0.56 ± 0.01	1
2		MBTH+$FeCl_3$+NOR	0.256 ± 0.001	
3		NOR+MBTH+$FeCl_3$	0.35 ± 0.01	
1	Method C	CLN+$FeCl_3$+MBTH	0.56 ± 0.01	1
2		CLN+MBTH+$FeCl_3$	0.256 ± 0.001	
3		MBTH+$FeCl_3$+CLN	0.35 ± 0.01	

Reagent concentration

In order to obtain the optimum conditions for determination of drugs, the absorbencies were measured for a series of solutions by varying the concentration of one with respect to other against the corresponding reagent blank in each case. The optimum conditions were presented in Table 2.

Table 2: Fixing reagent concentration for methods

Method	Reagent	Vol of reagent used for test (ml)	Reagent vol. used in the procedure
Method A	MBTH	0.4-1.2	0.6 ± 0.01ml
	$FeCl_3$	0.4-1.2	1 ± 0.1ml
Method B	MBTH	0.2-1.0	0.8 ± 0.01ml
	$FeCl_3$	0.4-1.2	1 ± 0.1ml
Method C	MBTH	0.4-1.2	1 ± 0.1ml
	$FeCl_3$	0.4-1.4	1 ± 0.1ml

Effect of temperature

Effect of temperature on stability of reaction product was studied at 20°C-50°C. If the temperature increases above 40°C the stability of the product reduces and at low temperatures the reaction is very slow. So, these methods were carried out at room temperature.

Effect of time

The effect of time on the formation of the colored complex was studied for all the methods. The colored complex formation was complete within 5 min time interval at room temperature.

Stability of colored product

The influence of the time on the maximum color development and stability of the colored species were studied by measuring the absorbencies at gradual increase in time interval. The color of the product was stable for more than 2 h.

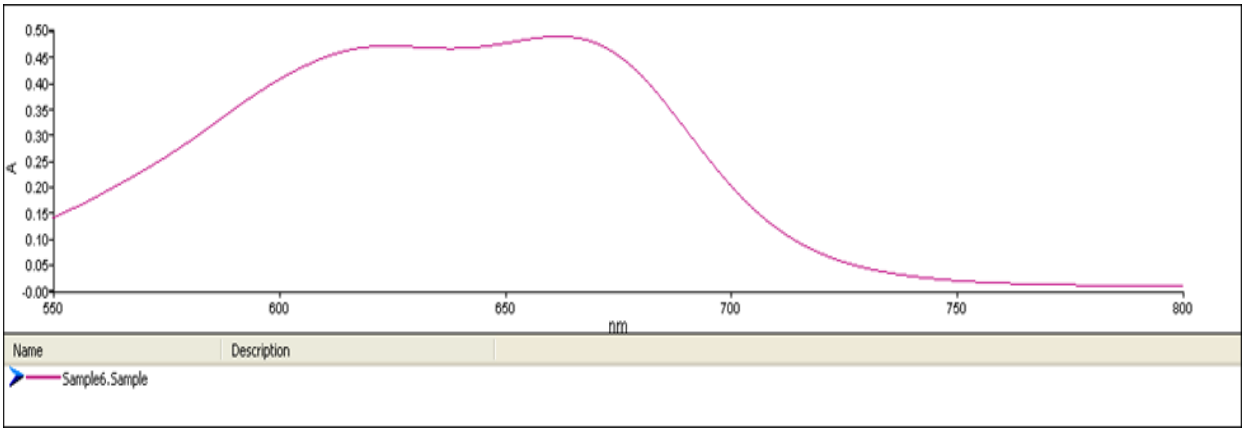

Figure 4: Absorption Spectrum of Gliclazide with MBTH

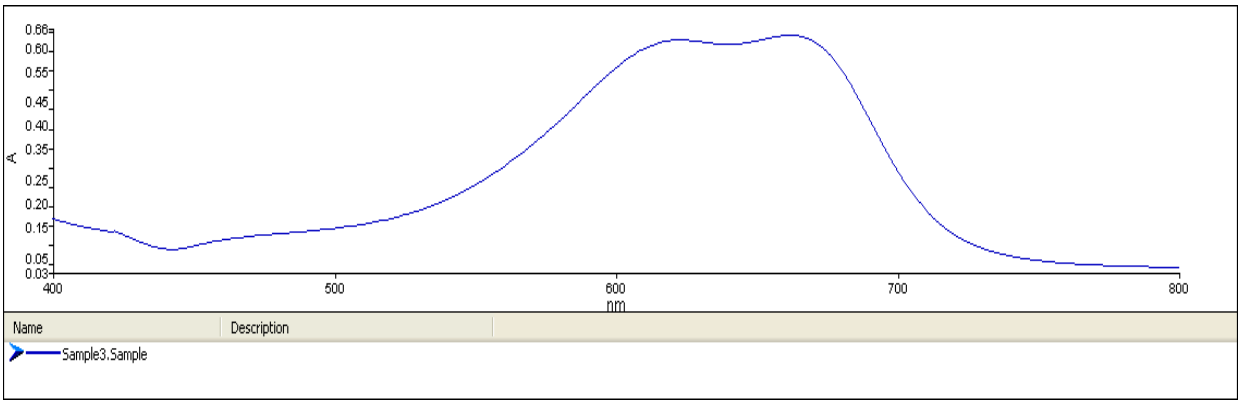

Figure 5: Absorption Spectrum of Nortriptyline with MBTH

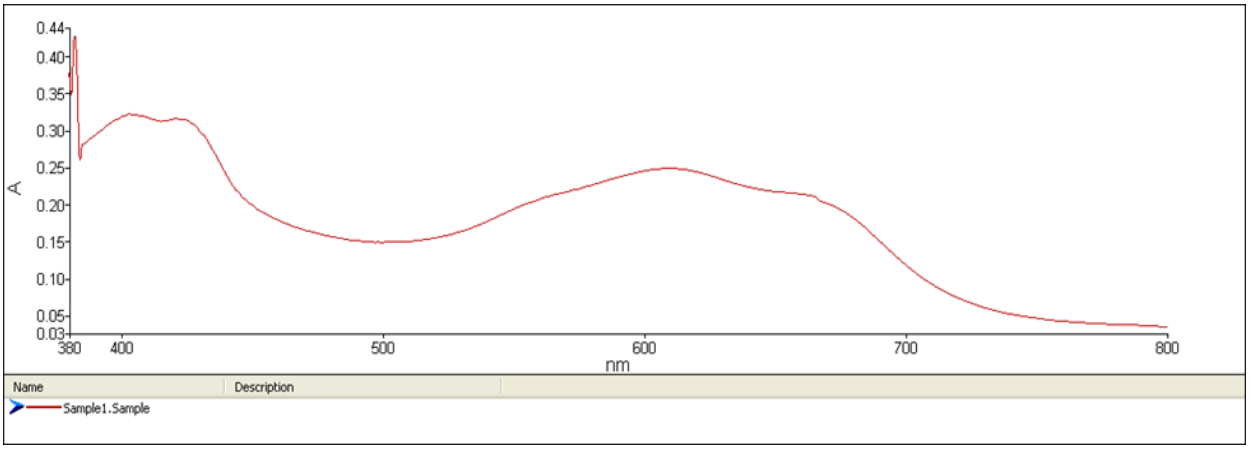

Figure 6: Absorption Spectrum of Cilnidipine with MBTH

Method validation

All the method was validated for accuracy, precision, linearity, LOD, LOQ, ruggedness and robustness and the results were found to be satisfactory. Regression parameters were presented in Table 3 for all methods.

Linearity and range

Linearity was assessed by performing single measurement at several analyte concentrations of Gliclazide, Nortriptyline, Cilnidipine and showed good correlation between concentration range of 40 µg/ml-140 µg/ml for Method A, 5 µg/ml-25 µg/ml for Method B and 50 µg/ml-250 µg/ml for Method C. The results were reported in Table 4 and shown in Figures 7, 8 & 9 respectively.

Precision

Precision of the method was determined by repeatability. Interday and intraday precision was studied by measuring the absorbances of the three sets of six similar concentrated solutions on the same day and on consecutive days. The % RSD was calculated and reported in Table 5 for all methods.

Robustness

Robustness was checked by narrow alteration of the optimized parameters and the % RSD were found to be satisfactory and reported in Table 6 for all methods.

Ruggedness

System to system/analyst to analyst variability study was conducted on different colorimeters and the results were satisfactory and reported in Table 7 for the three drugs.

Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ were determined by analyzing progressively lower concentrations of standard solution using optimized conditions and the results were presented in Table 3.

Accuracy

In order to determine the accuracy of the proposed methods, pure drug solution was spiked at three different concentration levels 80%, 100% and 120% (within the working range) were prepared and analyzed. The results of accuracy studies were presented in Table 8. The percentage relative error (RE%) indicates that the accuracy of the methods was good.

Table 3: Optical and regression parameters for all methods

PARAMETERS	Method A	Method B	Method C
λmax, nm	660	650	600
Beer's law range (µg/ml)	40-140	05-25	50-250
Molar extinction coefficient (L. mole-1 .cm-1)	$2.1x10^4$	3.8×10^3	$2.3x10^3$
Sandell' s sensitivity(µg/cm^2)/0.001 abs unit	$2.5x10^4$	2.5×10^{-3}	$1.42x10^4$
LOD, µg/ml	7.89	0.31	3.03
LOQ, µg/ml	26.07	0.845	9.36
Slope (m)	0.37	0.775	0.48
Intercept (b)	0.0257	0.049	0.024
Correlation coefficient (r)	0.9998	0.9999	0.9997

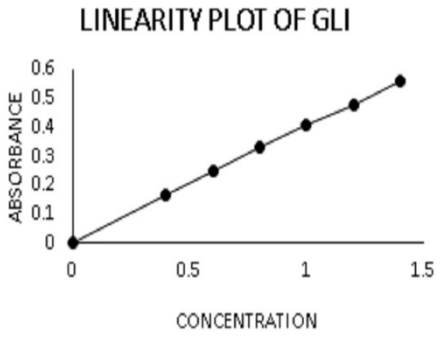

Figure 7: Linearity plot of Gliclazide

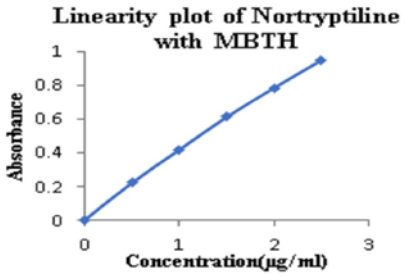

Figure 8: Linearity plot of Nortr

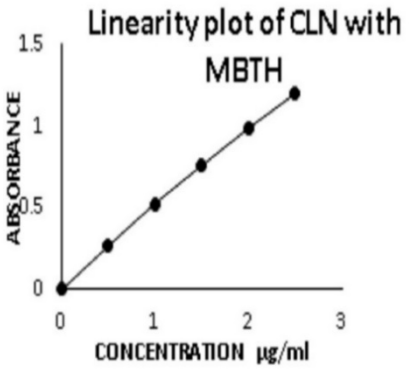

Figure 9: Linearity plot of Cilnidipine

Table 4: Linearity data for all methods

Method A		**Method B**		**Method C**	
Conc (µg/ml)	Abs	Conc (µg/ml)	Abs	Conc (µg/ml)	Abs
0.4	0.17	0.5	0.22	0.5	0.268
0.6	0.25	1	0.416	1.0	0.527
0.8	0.33	1.5	0.61	1.5	0.76
1.0	0.41	2	0.782	2	0.988
1.2	0.48	2.5	0.946	2.5	1.2
1.4	0.56	-	-	-	-

Table 5: Results showing Precision for all methods

Parameters	**Method A**		**Method B**		**Method C**	
	Intra*day	Inter*day	Intra*day	Inter*day	Intra*day	Inter*day
Conc (µg/ml)	120		20		150	
Mean abs	0.33	0.35	0.991	1.12	1.25	1.15
SD	0.009	0.008	0.707	0.71	0.45	0.45
%RSD	0.027	0.02	0.75	0.65	0.95	0.95

*Three sets of six determinations

Table 6: Results showing Robustness for all methods

Method	**λmax nm**	**% RSD**	**MBTH Vol**	**%RSD**	**$FeCl_3$ Vol**	**%RSD**
Method A	660 ± 2	0.06	0.6 ± 0.1 ml	0.042	1 ± 0.1 ml	0.024
Method B	650 ± 2	0.00785	0.8 ± 0.1 ml	0.0024	1 ± 0.1 ml	0.013
Method C	600 ± 2	0.9363	1 ± 0.1	0.9363	1 ± 0.1	0.263

Table 7: Results showing Ruggedness for the methods

Parameters	**Method A**	**Method B**	**Method C**
Analyst-1	0.33	0.785	0.748
Analyst-2	0.34	0.789	0.710
Mean abs	0.36	0.787	0.62383
SD	0.14	0.707	0.44906

%RSD	0.388	0.708	0.363

Table 7: Results showing Ruggedness for the methods

Parameters	Method A	Method B	Method C
Analyst-1	0.33	0.785	0.748
Analyst-2	0.34	0.789	0.710
Mean abs	0.36	0.787	0.62383
SD	0.14	0.707	0.44906
%RSD	0.388	0.708	0.363

Table 8: Results of accuracy studies

Method	Std added (mg)	Drug in formulation (mg)	%Recovery	SD	%RSD
Method A	80	100	99.99	0.14	0.388
	100	100	99.50	0.12	0.657
	120	100	99.95	0.14	0.818
Method B	12	15	99.41	0.47	0.436
	15	15	99.67	0.26	0.334
	18	15	99.83	0.317	0.636
Method C	80	100	99.32	0.406	0.363
	100	100	99.52	0.1906	0.96
	120	100	99.85	0.06	0.31

DISCUSSUION

The proposed visible spectrophotometric techniques are considered to be simple, less expensive and reliable by which it predominates the application of sophisticated techniques for regular analysis. Among the proposed methods, method B was more sensitive but range of linearity is medium. Methods A & C shows better linearity range between 40 µg/ml-250 µg/ml. Also, by the LOD and LOQ data from Table 3 it proves that method B is more sensitive due to very low values compared to other methods A & C. The sensitivity and selectivity of the colorimetric methods depend on the nature of the chemical reactions involved in color development and not on the sophistication of the equipment. The novelty of the method lies in the selection of appropriate chromogenic reagents for their assay by exploiting their characteristics, (physical and chemical properties) based on the oxidizable functional groups present in drug molecule. The choice of the chromogenic reagent for color development on a particular method is still a challenging problem. Selection of the chromogenic reagent is based on the presence of active methylene group in Gliclazide and Nortriptyline. Even though there is a heterocyclic amine in Cilinidipine, but it could not produce the oxidative coupled product with MBTH. But after the reduction of nitro group to amino group, Cilnidipine showed very good response with MBTH. This proves the specificity of the method. The careful consideration of factors such as the presence of other functional groups besides the chosen one that might be adversely affected by the reagents for the specificity study, the instability of desired colored product for stability of colored product, the rate of reaction and other related factors were all studied and the evidences presented in results section proved that the methods are specific, sensitive, linear. The validation studies proved the methods to be rugged, robust and accurate.

CONCLUSION

Even though there are some methods for the determination of Gliclazide, Nortriptyline, Cilnidipine by HPLC and spectrophotometric methods, the proposed colorimetric methods are simple, economic and sensitive with reasonable precision, accuracy and constitute better alternative to the existing ones for the routine determination in bulk and pharmaceutical formulations.

ACKNOWLEDGEMENTS

The authors are thankful to the management for providing the laboratory facilities to conduct the research work.

AUTHOR CONTRIBUTIONS

Concept – V.R; Design – V.R; Supervision – V.R; Resources – GIET School of Pharmacy, Materials – RN.C., S. R, S. S; Data Collection and/or Processing – RN.C., S. R, S. S.; Analysis and/or Interpretation – RN.C., S. R, S. S.; Literature Search – RN.C., S. R, S. S; Writing – V.R, RN.C., S. R, S. S.; Critical Reviews – V.R, RN.C., S. R, S. S.

CONFLICT OF INTEREST STATEMENT

The authors declared no conflict of interest.

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