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A NEW COLORIMETRIC DEVELOPMENT AND VALIDATION OF VISIBLE METHOD FOR ESTIMATION OF DICLOFENAC IN BULK FORMULATION

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ABSTRACT

A simple, sensitive, accurate, precise and economical visible Spectrophotometric method was developed and validated for the estimation of Diclofenac in Bulk form. The method is based on the reaction of Diclofenac with MBTH Reagent [3-Methyl-2-Benzothiazolinone Hydrazone] in the presence of ceric ammonium sulphate giving greenish blue colourchromogen which shows maximum absorbance at 580nm against reagent blank. The Chromogen obeyed Beer's law in the concentration range of 10-50 µg/ml for Diclofenac. The results of the analysis have been validated statistically and recovery studies.

Keywords: Diclofenac, MBTH Reagent [3-Methyl-2-Benzothiazolinone Hydrazone], ceric ammonium sulphate, Visible Spectrophotometric.

INTRODUCTION

Diclofenac is chemically 2-(2,6-dichloranilino) phenylacetic acid [1]. Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) taken to reduce inflammation and used as an analgesic reducing pain. Diclofenac is official in IP, USP and BP. Literature survey reveals Septrophotometric [2-4] methods for estimation of Diclofenac in pharmaceutical formulations. The present communication describes simple, sensitive, accurate, precise and economical visible spectrophotometric method using MBTH Reagent [3-Methyl-2-Benzothiazolinone Hydrazone] for the estimation of Diclofenac in bulk formulation.

Mechanism of action

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID). It is responsible for its anti-inflammatory, antipyretic and analgesic action by the inhibition of prostaglandin synthesis by inhibition of cyclooxygenase (COX). It also exhibit bacteriostatic activity by inhibiting bacterial DNA synthesis [2].

MATERIALS AND METHODS

Apparatus

A Shimadzu model T60 double beam UV/Vis Spectrophotometer with spectral width of 2nm wave length accuracy of 0.5 nm and a pair of 10mm matched quartz cells was used to measure absorbance of the resulting solutions. Shimadzu analytical balance, an ultra sonic cleaner were used in the study.

Reagents and Materials

Diclofenac drug was purchased from SD Fine Chemicals Pvt. Ltd. [Mumbai Maharashtra]. MBTH Reagent [3-Methyl-2-Benzothiazolinone Hydrazone] was purchased from lobachemie and Ceric ammonium sulphate was purchased from RL were used in the study.

Preparation of Reagent and Working standard stock solution

2% ceric ammonium sulphate

The solution was prepared by dissolving 2 gm of ceric ammonium sulphate in 100ml of water.

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Working Standard Stock Solution

Accurate weigh the 100mg of pure drug and transferred in 100 ml of volumetric flask later diluted with distilled water upto100ml gives 1000µg/ml.

Methodology

Different aliquots of working standard solution containing 10-50 µg/ml concentration of Diclofenac was transferred into series of volumetric flask. To it 1ml of MBTH reagent and 2 ml of 2% ceric ammonium sulphate was added and volume was made up to 10 ml with distilled water. The contents of the each flask was mixed well and allowed to stand at room temperature for 10 minutes. The absorbance of coloured species was measured at 580 nm against reagent blank. The amount of drug present in the sample solution was computed from the calibration curve.

Reaction Mechanism

The results obtained in this method were based on the reaction of MBTH with secondary amino group of Diclofenac and gives a greenish blue colour species which is measured at 580nm shown in the Figure 2.

Method Validation

Linearity

Five points calibration curve were obtained in a concentration range from 10-50 µg/ml for diclofenac. The response of the drug was found to be linear in the investigation concentration range and the linear regression equation was y = 0.010x+0.012 with correlation coefficient 0.997 results are tabulated in table No.1 & Figure 3.

Precision

Precision of the analytical method is ascertained by carrying out the analysis as per the procedure and as per normal weight taken for analysis. Repeat the analysis six times. Calculate the % assay, mean assay, % Deviation and % relative standard deviation and %RSD. The developed method was found to be precise as the %RSD values for the repeatability and intermediate precision studies were 0.61% and 0.68%, respectively shown results are tabulated in table No.2.

Accuracy

Accuracy of the method is ascertained by standard addition method at 3 levels. Standard quantity equivalent to 60%, 120% and 180% is to be added in sample. The result shown that best recoveries (97.4-99.3%) of the spiked drug were obtained at each added concentration, indicating that the method was accurate and results are tabulated in table No.3.

Robustness

Measure of the capacity of an analytical method to remain unaffected by small intentional variations in the operational parameters and provide an assurance of its reliability during the normal usage. It may be determined by various parameters like ph, flow rate, temperature etc. Robustness studies are performed during the method development stage. Results are tabulated in table No.4.

Sensitivity

Limit of Detection and Quantitation (LOD and LOQ)

From the linearity data calculate the limit of detection and quantitation using the following formula.

$$\textbf{LOD} = 3.3\sigma / S$$

σ = Standard deviation of response.

S = Slope of the calibration curve of the analyte.

$$\textbf{LOQ} = 10\sigma / S$$

σ = Standard deviation of response.

S = Slope of the calibration curve of the analyte.

The results are tabulated in Table No.5

RESULTS AND DISCUSSION

The analytical method was developed by studying different parameters. The method was validated for all validation parameters as per ICH guidelines. The lambda max of Diclofenac was found to be 580nm. Linearity was found with the concentration range 10-50 µg/ml and correlation coefficients found to be 0.997 indicate good linearity between concentration and slope area. Beer's law was obeyed by the fundamental spectrum. This method was found to be simple, sensitive, accurate, precise and economical for routine analysis for the estimation of Diclofenac in Bulk form. Recovery studies were found to be close 99% indicated the accuracy and precision of the above two proposed methods. Values of LOD and LOQ were found to be 3.96 and 12 respectively. The accuracy and robustness was calculated to be 99.3 and 100.4%.

Fig 1.Diclofenac structure

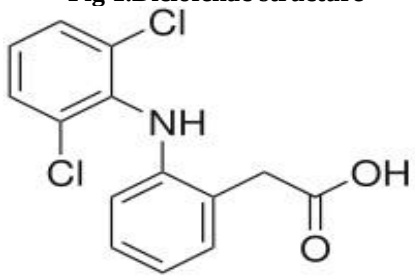

Fig 2. Reaction mechanism (λ max)

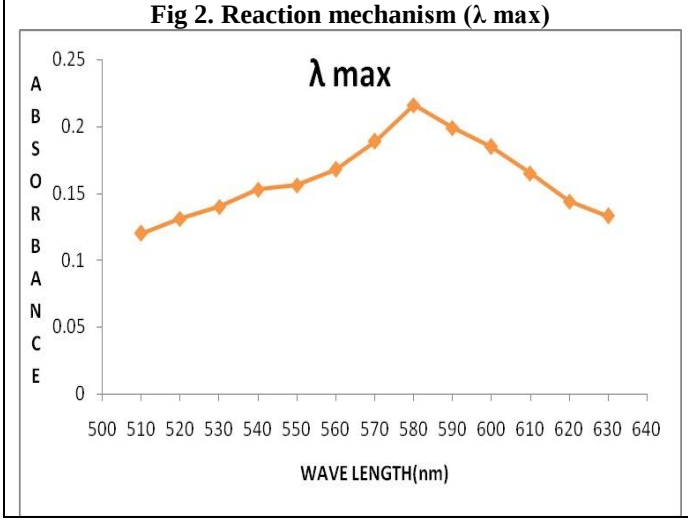

Fig 3. Linearity graph

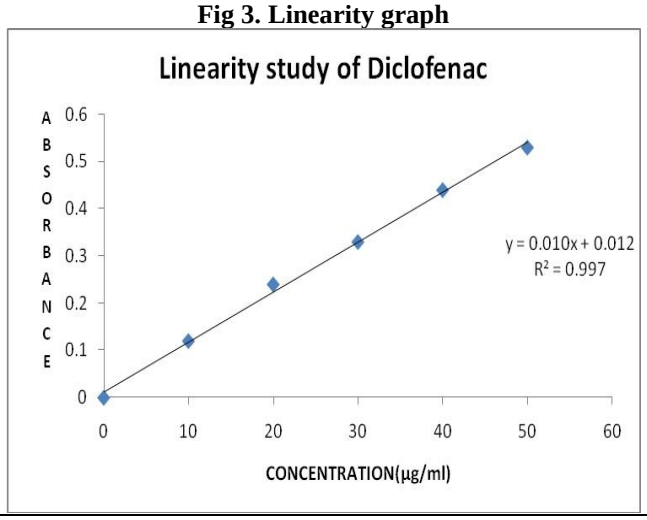

Table 1. Result for linearity

Concentration (µg/ml)	Absorbance
10	0.12
20	0.24
30	0.33
40	0.44
50	0.53
Correlation	0.997
Intercept	0.012
Slope	0.010

Table 2. Result for precision

Sample No.	% Assay	
	Intra day	Inter day
10	101.2	99.2
20	101.8	98.4
30	100.6	99.6
40	100	100.5
50	101.3	99.7
Mean	100.98	99.48
SD	0.62	0.685
% RSD	0.61	0.688

Table 3. Result for accuracy

% Recovery Level	% Recovery	Mean	SD	%RSD
60%	97.22	97.44	0.21	0.22
	97.74			
	97.37			
120%	98.61	98.6	0.224	0.227
	98.32			
	98.87			
180%	99.12	99.35	0.200	0.201
	99.34			
	99.61			

Table 4. Result for robustness

Parameter	Amount of Diclofenac (µg/ml)		%Recovery	SD	%RSD
	Taken	Found			
1.5ml MBTH, 1.5ml cerric ammonium nitrate	40	40.7	101.7	0.67	0.66
	50	50.2	100.4		

Table 5. Result for LOD and LOQ

LOD ((µg/ml)	3.96
LOQ ((µg/ml)	12

CONCLUSION

The proposed visible spectrophotometric method was found to be simple, sensitive, accurate, precise and economic for determination of Diclofenac in bulk formulation. Hence it can be conveniently adopted for routine quality analysis of drug in pharmaceutical dosage form.

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