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Research Article

DEVELOPMENT OF NEW COLORIMETRIC METHOD AND VALIDATION FOR DETERMINATION OF MELOXICAM IN BULK AND MARKETED FORMULATION

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ABSTRACT

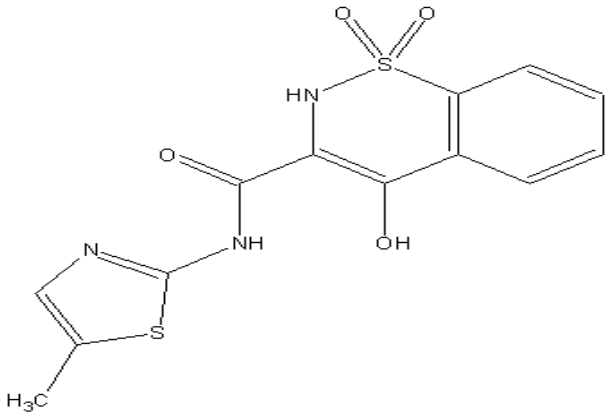
In the present work, simple, sensitive, specific, and validated colorimetric method has been developed for the quantitative estimation of Meloxicam in bulk and pharmaceutical dosage form. Meloxicam in presence of acidic medium reacts with MBTH reagent and $FeCl_3$ solution and gives yellow colour. The λ_{max} was found to be 465nm for assay. The linearity was found in concentration range of 10-60µg/ml. The correlation coefficient was found 0.9854. The regression equation was found as Y= 0.0128X + 0.0171. The method was validated for linearity, accuracy, precision and ruggedness. The LOD and LOQ for estimation of Meloxicam was found as 0.3769, 1.1456 respectively. Recovery of Meloxicam was found to be 95.33%. Proposed method was successfully applied for the quantitative estimation of Meloxicam in marketed formulations.

***Key Words**: Meloxiam, MBTH reagent, $FeCl_3$, 0.1N NaCl and distilled water.*

INTRODUCTION

A study of the interaction of light (or other electromagnetic radiation) with matter is an important and versatile tool for the chemist. Indeed, much of our knowledge of chemical substances comes from their specific absorption or emission of light. In this experiment, we are interested in analytical procedures based on the amount of light absorbed (or transmitted) as it passes through a sample.[1]

Meloxicam is a nonsteroidal anti-inflammatory drug with analgesic and fever reducer effects. It is a derivative of oxicam, closely related to piroxicam, and falls in the enolic acid group of NSAIDs. Meloxicam inhibits cyclooxygenase (COX), the enzyme responsible for converting arachidonic acid into prostaglandin H2—the first step in the synthesis of prostaglandins, which are mediators of inflammation. Meloxicam has been shown, especially at its low therapeutic dose, selectively to inhibit COX-2 over COX-1.



Its IUPAC name is 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide. And formula is C14H13N3O4S2. Molecular mass of meloxicam is 351.403 g/mole. It is freely soluble in dimethyl formamide and 0.1M NaOH. Up to now there is HPLC and spectrophotometric method developed on Meloxicam.[3-8]

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The USP has published specific guidelines for method validation for compound evaluation. USP defines eight steps for validation: Accuracy, Precision, Specificity, Limit of detection, and Limit of quantitation, Linearity and range, Ruggedness, Robustness [9]

MATERIALS AND METHODS

Meloxicam was determined spectrophotometrically in bulk and marketed formulation by using MBTH reagent in presence of ferric chloride as strong oxidizing agent.

Experimental:

a) Instrumentation: Experiment was performed on JASCO V-630 series UV spectrophotometer and simadzu 1700 with 1 cm path length matched glass cuvettes.
b) Preparation of standard stock solution of Meloxicam: Standard stock solution was prepared by accurately weighing 100 mg of meloxicam in 100 ml calibrated volumetric flask and 10 ml 0.1M NaOH solution was added and made up the volume with distilled water up to 100 ml.
c) Preparation of working standard solution of Meloxicam: Working standard was prepared by transfer of 10 ml standard stock solution into 100 ml calibrated volumetric flask and made up the volume with distilled water to get concentration of 100µg/mL.

Preparation of reagent:

a) Preparation of 1% MBTH reagent: 1.0 gm. of MBTH was weighed and transferred into 100 ml volumetric flask and made up the volume with distilled water.
b) Preparation of 0.5% of Ferric chloride: 500 mg of Ferric chloride was transferred into 100 ml volumetric flask and dissolved in 5 ml 0.1N HCl made up the volume with distilled water.
c) Preparation of 0.1M NaOH: 400mg NaOH pallets was taken and dissolved in 100ml distilled water
d) Preparation of 0.1N HCl: 8.5 ml concentrated HCl dissolved in 1000 ml distilled water.

Preliminary investigation:

a) 1ml of Meloxicam working standard solution was transferred into 10 ml volumetric flask. Then 1 ml of 1% MBTH reagent and 1 ml of 0.5% Ferric chloride solution were added and kept aside for 10 minutes. Made up the volume with distilled water. Absorbance was recorded against reagent blank.

Parameter fixation:

Determination of absorbance maximum:

An absorption maximum (or) λ max is the wavelength at which maximum absorption takes place. It is important to know the absorption maximum of the substance under study, since it helps to avoid any interfering impurities.

Procedure: 1 ml of working standard solution of Meloxicam was transferred into 10 ml volumetric flask. Then 0.5 ml of 1% MBTH reagent and 0.3 ml of 0.5% ferric chloride reagent were added and kept aside for 10 minutes until completion of reaction. Made up the volume with distilled water. Absorbance was recorded against reagent blank. Shown in figure-1 these solutions were scanned in UV spectrophotometer between 400-800 nm.

Model: JASCO V-630
Band width: 1.5 nm
Response: Medium
Measurement: 800-400 nm
No. of cycle: 1
λmax: 465 nm
Absorbance: 0.24609

Stability of color:

Procedure: In 10 ml volumetric flask 1 ml of working standard of Meloxicam and 0.5 ml of 1% MBTH solution and 0.3 ml of 0.5% ferric chloride solution were added. Kept aside for 10 minutes until completion of reaction. Made up the volume with distilled water. Absorbance was recorded against reagent blank. And reading was taken of same solution for every 10 minutes intervals. The result is recorded in table no.1 and graph is given in table no. Reported in table no. 1 and figure no. 2.

Investigation:

a) Effect of concentration of reagent: Experiments was carried out to ascertain the optimum concentrations of reagents needed for rapid and quantitative formation of yellowish-green colored species by measuring the absorbance of series of solutions in which one parameter was varied and others fixed.

PROCEDURE: Eight 10ml volumetric flask were taken and 1 ml of working standard solution of meloxicam was added and different concentration of MBTH reagent and 1ml of 1% Ferric chloride solution were taken. Kept for

10 minutes. Made up the volume with distilled water. Absorbance was recorded against reagent blank. Reported in table no.2 and figure no. 3.

b) Effect of volume of reagent:
PROCEDURE: Eight 10ml volumetric flask were taken and 1 ml of working standard solution of meloxicam was added and different volume of 1.0% MBTH reagent and 1 ml of 1% Ferric chloride solution were taken. Kept aside for 10 minutes. Made up the volume with distilled water. Absorbance was recorded against reagent blank. Reported in table no. 3 and figure no. 4.

c) Effect of concentration of oxidizing agent (ferric chloride)
PROCEDURE: Eight 10ml volumetric flask were taken. 1 ml of working standard of meloxicam was added into it. Then 1ml 1% MBTH and different concentration of ferric chloride solution was taken. Kept aside for 10 minutes. Made up the volume with distilled water. Absorbance was recorded against reagent blank. Reported in table no. 4 and figure no. 5.

d) Effect of volume of oxidizing agent:
PROCEDURE: Eight 10ml volumetric flask were taken and 1 ml of working standard of meloxicam was added into it. 0.5ml 1% MBTH and different volume of 0.5% ferric chloride solution were added.Kept a side for 10 minutes. Made up the volume with distilled water. Absorbance was recorded against reagent blank. Reported in table no. 5 and figure no. 6.

Optical characters:

Determination of concentration range:
For spectrophotometric analysis determination of the concentration range which obeys the Beer- Lambert's law is necessary for accuracy and reproducibility.

Preparation of standard curve:
Standard curve was prepared by using pure Meloxicam in the concentration range of 10-60µg/ml by this method and selecting absorbance maximum at 580 nm.

Reagent and chemicals:
1) Working standard stock solution (100µg/ml)
2) 1% MBTH reagent
3) 0.5% Ferric chloride solution

Procedure: Eight 10ml volumetric flask were taken and 1, 2, 3,4,5,6 ml of working standard of meloxicam was added into it. 0.5ml 1% MBTH and 0.3ml of 0.5% ferric chloride solution were added. Kept for 10 minutes. Made up the volume with distilled water. Absorbance was recorded against reagent blank at 465 nm. The result was recorded in table no. - 5.15 and graph was given in figure no.-5.16

The six such linearity was taken for regression co-efficient and eight such linearity was taken for standard deviation separately. Reported in table no. 6 and figure no. 7.

Analysis of marketed formulation:
Meloxicam is marketed as Muvera of 15mg tablet manufactured by SUN PHARMA were taken for analysis.

Reagent and chemicals:
1) Working standard stock solution (100µg/ml)
2) 1% MBTH reagent
3) 0.5% Ferric chloride solution

Preparation of sample solution:
Tablet powder equivalent to 100mg was weighed and transferred into 100ml volumetric flask. Made up the volume with distilled water along with 0.1M NaOH to get 1000µg/ml concentration. This solution was further diluted to get concentration of 100µg/ml.

Recovery experiments:
To keep an additional check on accuracy of developed assay method, analytical recovery experiments were performed. The different solutions of different concentration like 10, 20 and 30 µg/ml were prepared in case of both pure drug solution and formulation extract solution and these solutions were subjected to analysis by above developed method as mentioned in 5.2.6.2. The six such samples were prepared and average of that readings taken for calculation of % recovery. This is reported in following table no.7

Method validation:

1. **Linearity:**
   Linearity was determined over the range of 10-60 µg/ml. Different seven 10ml volumetric flask were taken and 1, 2, 3,4,5,6 ml of working standard of meloxicam were added into it. 0.5ml 1% MBTH and 0.3ml of 0.5% ferric chloride solution were added. Kept for 10 minutes.Made up the volume with distilled water. Absorbance was recorded against reagent blank.at 465 nm. The result was recorded in table no.6 And graph was given in figure no.7

2. **Recovery (%Accuracy):**
   The accuracy of the methods was determined by calculating % recovery of Meloxicam by standard addition method. Known volumes of standard solutions of Meloxicam were taken for recovery studies. It was mentioned in table no.8

3. **Method precision (% Repeatability)**
   The precision of the methods was checked by repeated measurement of the absorbance of standard solutions (n = 6) of 10 µg/mL without changing the parameters for the method. The repeatability was expressed in terms of relative standard deviation (RSD). Reported in table no. 9.

4. **Intermediate precision (Reproducibility)**
   The intraday and interday precision of the proposed methods were performed by analyzing the corresponding responses three times on the same day and on three different days over a period of one week for three different concentrations of standard solutions of Meloxicam (10, 15, 20 µg/ml). The results were reported in terms of relative standard deviation (RSD) in table no.10.

5. **Limit of detection and Limit of quantification**
   The limit of detection (LOD) and limit of quantification (LOQ) of the drug were derived by calculating the signal-to-noise (i.e. 3.3 for LOD and 10 for LOQ) ratio using following equations designated by International Conference on Harmonization (ICH) guideline: reported in table no. 11
   $LOD = 3.3 \times \sigma/S$ and $LOQ = 10 \times \sigma/S$

   Where, σ = the standard deviation of the response, S = slope of the calibration curve.

6. **Statistical evaluation:**
   The precision of each proposed method was ascertained by analyzing the same concentration in freshly prepared sample solution of Chloramphenicol six times of each three sample combination of drug and extracted solutions of drug. The set absorbance values obtained were then used to calculate the drug content per tablet and this was used to obtain standard deviation (s), standard Error (S.E), and precision (P) value. Reported in table no. 12

RESULT AND DISCUSSION

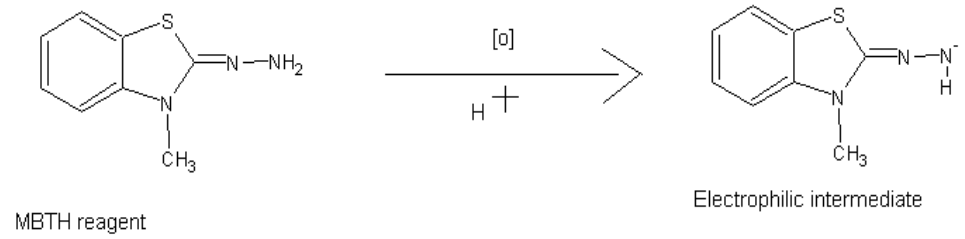
The objective of the proposed work was to develop new analytical method for the determination of Meloxicam and to validate the methods according to ICH guidelines and applying the same for its estimation in marketed formulations. Developed colorimetric method was found to be rapid, simple, precise, accurate and economic for routine estimation of Meloxicam in commercial dosage forms. Optical conditions, Optical characteristics and Statistical data of the Regression equation in colorimetric method:

The optical characteristics such as Beer's law limits, Molar absorptivity, Sandell's sensitivity, Limit of detection and Limit of quantitation etc., in each method were calculated and the results were presented in **Tables and figures** Meloxicam. Also the regression characteristics like slope (b), intercept (a), and correlation coefficient (R^2) using the method of least squares were calculated and were presented in **Tables** for Meloxicam. The results showed that the methods have reasonable precise.

Linearity: The linearity was found in the concentration range of 10-60 µg/ml respectively for meloxicam.
Precision: Values for both system precision and method precision in terms of % RSD were found to be < 2.0 % and were presented in **Table no 9, 10** for meloxicam.

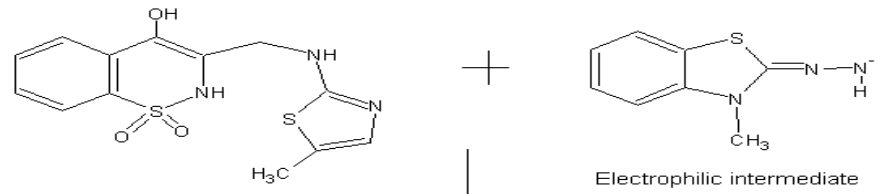
Accuracy: Accuracy of the method was confirmed by recovery studies and % recovery for two brands were determined and reported in **Table no 8** for Meloxicam.

MBTH reagent oxidized and produces electrophilic intermediate in presence of acidic medium. This reacts with Meloxicam and produces yellow color complex.



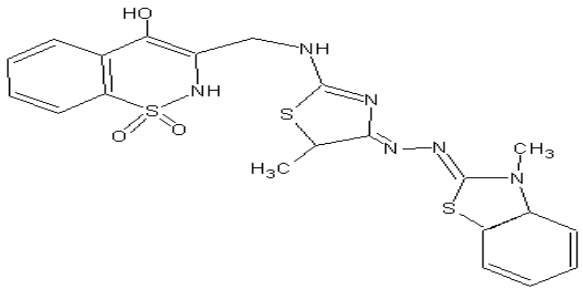
MBTH reagent

Electrophilic intermediate



Meloxicam

Electrophilic intermediate



Colored complex of Meloxicam and MBTH reagent

CONCLUSION

For routine analytical purpose, it is always necessary to establish methods capable of analysing huge number of samples in a short time period with due accuracy and precision. Meloxicam is official in only US Pharmacopoeia. A very few analytical methods appeared in the literature for the determination of Meloxicam includes LC, HPLC, HPTLC and UV-Visible spectrophotometric methods. In view of the above fact, some simple analytical method was planned to develop with sensitivity, accuracy, precision and economical. In the present investigation, colorimetric method for the quantitative estimation of Meloxicam in bulk drug and pharmaceutical formulations has been developed. The results are expressed in **Tables and figures** respectively for Visible Spectroscopy.

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TABLES:

Table no. 1: Stability study for meloxicam

Sr. no.	Volume of drug solution in 10ml vol. Flask(100µg/ml)	Time in minute	Absorbance
1	1 ml	0	0.2460
2	1 ml	10	0.2460
3	1 ml	20	0.2459
4	1 ml	30	0.2458
5	1 ml	40	0.2451
6	1 ml	50	0.2448
7	1 ml	60	0.2441
8	1 ml	70	0.2437
9	1 ml	80	0.2433
10	1 ml	90	0.2429
11	1 ml	100	0.2425
12	1 ml	110	0.2420
13	1 ml	120	0.2415

Table no. 2: Effect of concentration of reagent

Sr.no.	Concentration of MBTH (%)	Absorbance
1	0.5%	0.1625
2	0.75%	0.1957
3	1.0%	0.2443
4	1.25%	0.2439
5	1.50%	0.2397
6	1.75%	0.2407
7	2.0%	0.2401
8	2.25%	0.2379

Table no. 3: Effect of volume of reagent

Sr.no.	Volume of (1.0%) MBTH	Absorbance
1	0.1	0.1453
2	0.2	0.1987
3	0.3	0.2193
4	0.4	0.2237
5	0.5	0.2432
6	0.6	0.2387
7	0.7	0.2379
8	0.8	0.2307

Table no. 4: Effect of concentration of oxidizing agent

Sr.no.	Concentration of oxidizing agent (%)	Absorbance
1	0.1	0.1961
2	0.2	0.1993
3	0.3	0.2129
4	0.4	0.2397
5	0.5	0.2443
6	0.6	0.2395
7	0.7	0.2373
8	0.8	0.2295

Table no. 5: Effect of volume of oxidizing agent

Sr. no.	Volume of oxidizing agent (0.5%) in ml	Absorbance
1	0.1	0.1941
2	0.2	0.2153
3	0.3	0.2469
4	0.4	0.2449
5	0.5	0.2356
6	0.6	0.2293
7	0.7	0.2210
8	0.8	0.2109

Table no. 6: preparation of standard curve

Sr. no.	Volume of working standard of drug	Concentration of drug (µg/mL)	Absorbance
1	1.0	10	0.2461
2	2.0	20	0.3897
3	3.0	30	0.5421
4	4.0	40	0.6978
5	5.0	50	0.8457
6	6.0	60	0.9875

Table no. 7: Recovery study for meloxicam

Method	Sample	Labeled amount (mg)	Amount found (mg)	% Recovery
1	Muvera	15	14.3	95.33

Table no. 8: Accuracy of Meloxicam

Drug	Amount present in formulation (µg/ml)	Amount added (%)	% Recovery±sd*
MELOXICAM	10	50	96.27±0.79
		100	95.40±0.96
		150	97.89±0.41

* Average of three determination

Table no. 9: Method Precision (% Repeatability) of meloxicam

Concentration (µg/ml)	Absorbance
10	0.243
10	0.241
10	0.243
10	0.242
10	0.245
10	0.245
Mean	0.243
SD	0.001462
%CV	0.601

Table no. 10: Intermediate Precision (Reproducibility) of meloxicam

Concentration (µg/ml)	Mean absorbance ± %rsd
10	0.249±0.57
15	0.370±0.27
20	0.512±0.77

Table no. 11: LOD and LOQ of meloxicam

DRUG	LOD(µg/ml)	LOQ(µg/ml)
Meloxicam	0.3769	1.1456

Table no. 12: statistical data of meloxicam

PARAMETER	RESULT
λmax (nm)	465
Beer's law limits (µg/ml)	10-60
Sandell's sensitivity	0.0142
Molar absorptivity (1/mol.cm)	$2.46x10^4$
Regration equation (y=a+bc) a) Slope (b) b) Intercept (a)	 0.016 0.0509
Correlation coefficient	0.9854
%RSD	0.4128

FIGURES:

Figure 1: λ_{max} of Meloxicam with MBTH and ferric chloride

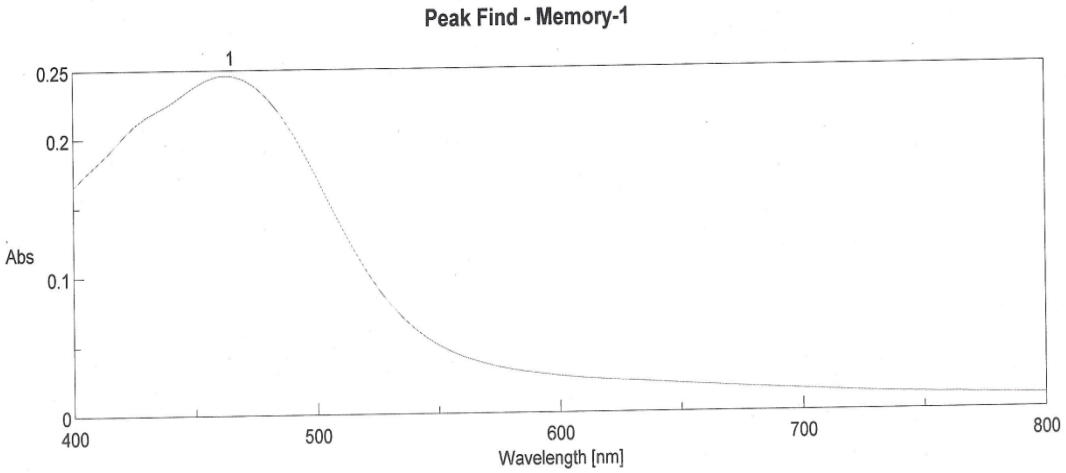
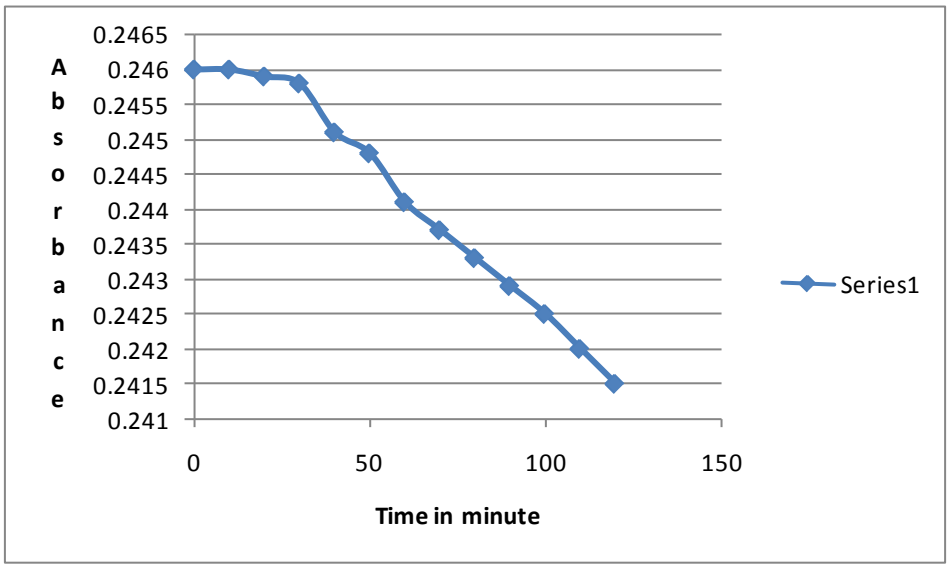
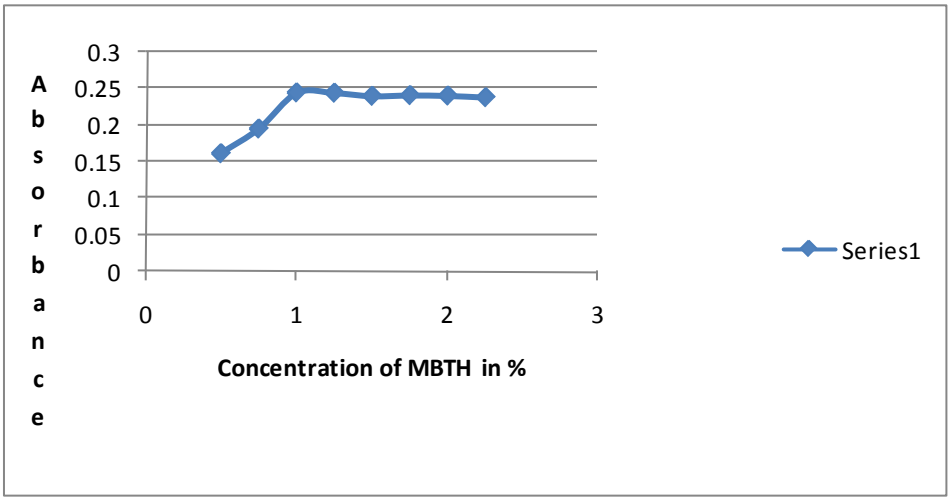


Figure no. 2: Stability study for meloxicam



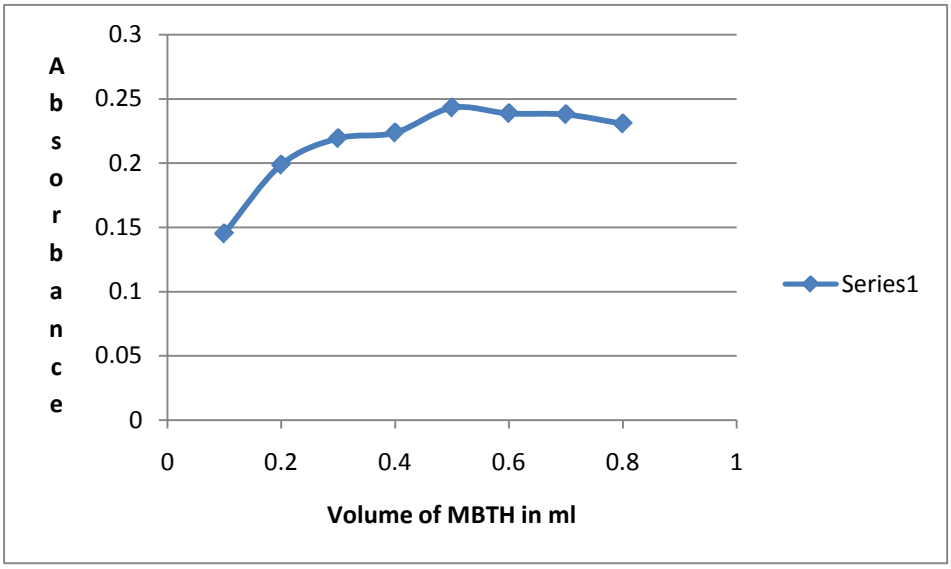
CONCLUSION: The stability study of developed color was performed and from graph it was proved that color was stable for 2 hours.

Figure no.3: Effect of concentration of reagent



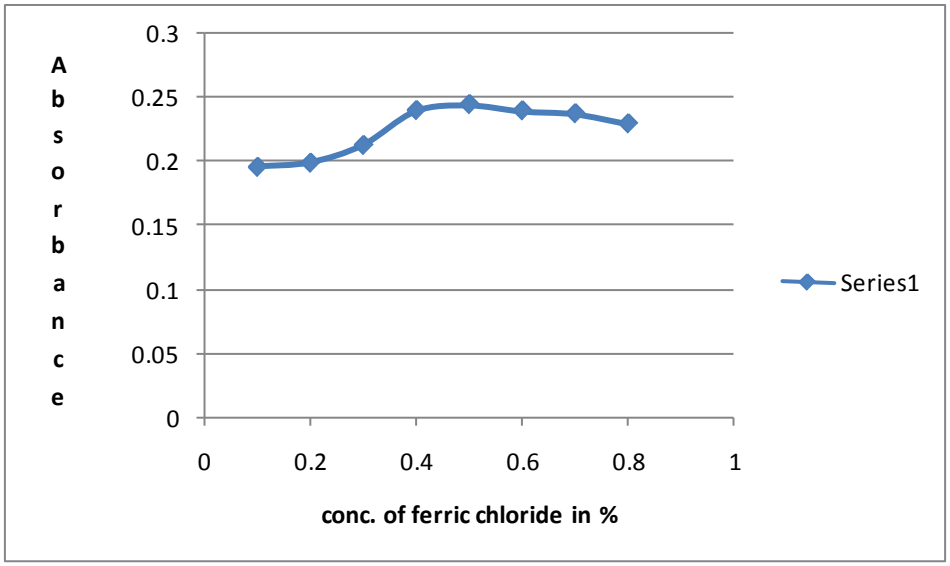
CONCLUSION: Highest absorbance was found in 1.0% conc. of MBTH solution.

Figure no. 4: Effect of volume of reagent



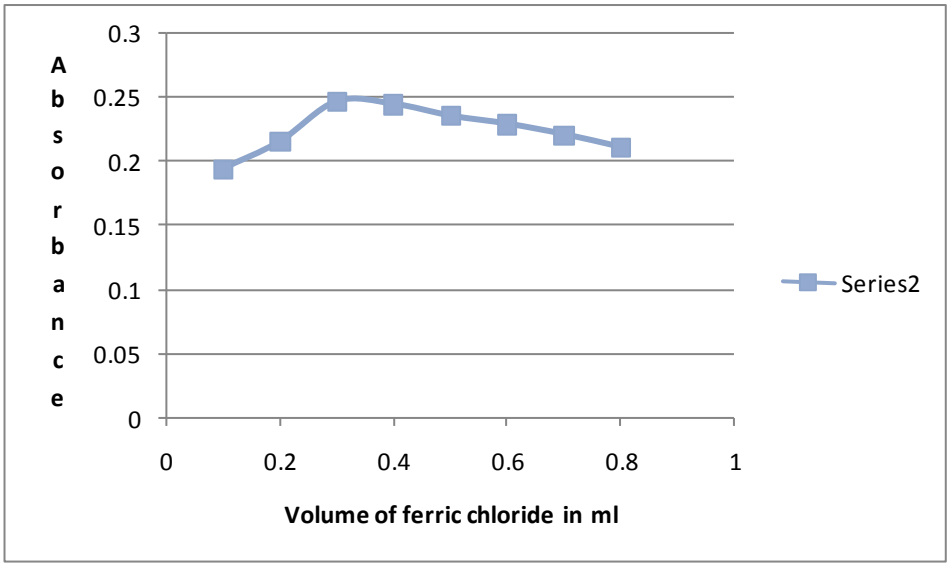
CONCLUSION: Highest absorbance found in 0.5 ml of (1%) MBTH solution.

Figure no. 5: Effect of concentration of oxidizing agent



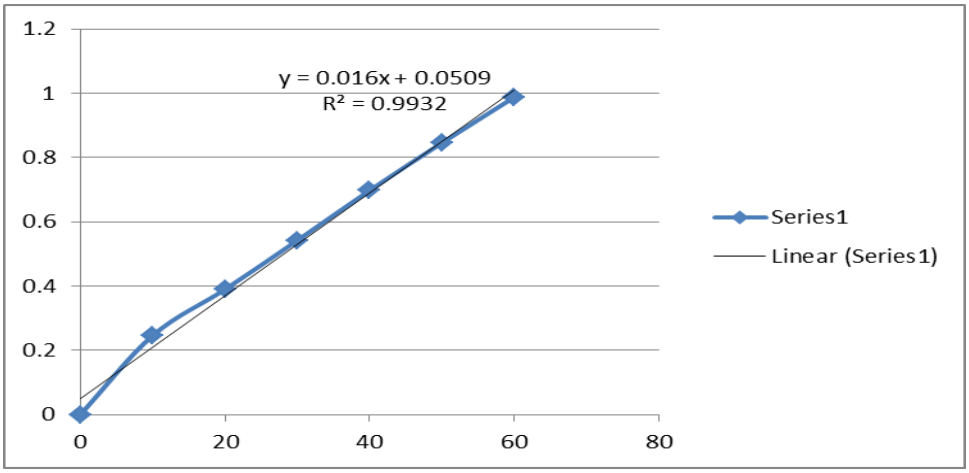
CONCLUSION: Highest absorbance found in 0.5% of ferric chloride solution.

Figure no.6: Effect of volume of oxidizing agent



CONCLUSION: Highest absorbance found in 0.3 ml of 0.05%of ferric chloride solution.

Figure no. 7: preparation of standard curve



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