Spectrophotometric Method for Determination of Meloxicam in Pharmaceutical Formulations Using N-bromosuccinimide as an Oxidant

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Abstract

A simple, rapid and sensitive spectrophotometric method has been developed for the determination of meloxicam (MX) in pure form and in its pharmaceutical preparations. The proposed method involve the addition of a measured excess of N-bromosuccinimide (NBS) in acid medium followed by determination of unreacted NBS by reacting with indigo carmine (IC) and measuring the absorbance at 610 nm. The optimum reaction conditions and other analytical parameters have been evaluated. Linearity was observed from 0.2-50 µg/ml meloxicam. Statistical analysis of the results and comparison with results by the British Pharmacopoeia method are also reported.

Keywords: Meloxicam, Spectrophotometry, Indigo carmine, N-bromosuccinimide.

1. Introduction

Meloxicam, chemically named 4-hydroxy-2-methyl-N (5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide- 1,1-dioxide with chemical formula ($C_{14}H_{13}N_3O_4S$) and molecular weight 351.4 g/mol (Fig. I), is a non-steroidal anti-inflammatory drug (NSAID)[1,2] used to relieve the symptoms of arthritis. In contrast with other NSAIDs currently available, meloxicam appears to have greater inhibitory activity against the inducible isomer of cyclo-oxigenase, which is involved in the inflammatory response, than against the constitutive isomer, inhibition of which is associated with adverse gastrointestinal and renal events [3]. Spectrophotometric and fluorimetry [4-8], polarography [9, 10], turbidimetry [11], voltammetry [12], capillary zone electrophoresis [13] HPTLC [14], and high performance liquid chromatography [15, 16] methods have been reported for the determination of meloxicam in pharmaceuticals and plasma. Based on its high sensitivity, wide linear range, good reproducibility as well as its simplicity and affordability, flow-injection chemiluminescence has been extensively used to pharmaceuticals and plasma determinations [17, 18]. However the use of NBS has been extensively used as brominating and oxidizing agents for organic compounds. In this paper, a simple, rapid and sensitive spectrophotometric method for the determination of MX is presented. The method is based on the oxidation of MX by a known excess of NBS in acidic medium followed by a reaction of the excess oxidant with IC to bleach its blue color. The method utilized NBS-IC reagents (Fig. II) and their developed offer the advantage of simplicity, sensitivity, speed, accuracy, and precision without the need for costly equipment/chemicals. The proposed method has been successfully applied to the determination of the drug in pharmaceutical formulations.

2. Experimental

2.1. Apparatus

All spectral and absorbance measurements were performed on a (Cecil CE3021-England) UV-VIS spectrophotometer was used for all spectral and absorbance measurements with matched 1 cm quartz cells.

2.2. Reagents

All chemicals were of analytical reagents grade.

2.2.1. Stock solution of meloxicam.

A stock solution, 500 µg/ml, of the drug under investigation was prepared by dissolving 50 mg (provided by Awamedica Company for Drug Industries and Medical Applications Awa, Erbil, Iraq) in 100 ml of 0.02 M NaOH [19], and working standard solutions were prepared by suitable dilution of the stock solution with 0.02 M NaOH.

2.2.2. N-bromosuccinimide

A stock solution of 5.6 x 10^{-4} M NBS (Fluka) [1-Bromo-2,5-pyrrolidinedione], ($C_4H_4BrNO_2$, M.Wt. 177.98 g /mole) was freshly prepared by dissolving 10 mg of NBS in a minimum amount of warm water in a 100 ml measuring flask and then completed with distilled water to the mark.

2.2.3. Indigo carmine

A stock solution of 5 x 10^{-4} M indigo carmine (BDH) ($C_{16}H_{10}N_2O_2$, M.Wt. 262.27 g/mol) was prepared by dissolving accurately weighed 13.11 mg of dye (99% purity) in 100 ml distilled water in a calibrated flask with the same solvent.

2.2.4. Hydrochloric acid

A 0.5 M of Hydrochloric acid solution was prepared by diluting 4.2 ml of concentrated acid (Merck, Darmstadt, Germany, sp. gr. 1.18%, 37%) to 100 ml with distilled water.

2.2.5. Sodium hydroxide solution

A solution of 0.1 M was prepared by dissolving 1 g of sodium hydroxide (Fluka) in distilled water and completed to the marked with the same solvent in 250 ml volumetric flask. Working solution (0.02 M NaOH) was prepared by diluting 50 ml of stock solution to 250 ml with distilled water.

2.2.6. Solution of interferences

A stock standard solution of each interfering species (starch, glucose, sucrose, potassium chloride, sorbitol, magnesium stearate and sodium chloride) were prepared by dissolving 0.05 g of the compound in distilled water then the volume is completed to 50 ml in calibrated flask. Other solutions were prepared by serial dilutions of the stock solution.

2.3. Recommended procedure and calibration curve

Into a series of 10 ml volumetric flasks an increasing volume of meloxicam solution (50 µg /ml) were transferred to cover the range of the calibration curve (0.2 to 50) µg/ml. Then 1.4 ml of NBS (5.6 x 10^{-4} M) and 0.8 ml of HCl (0.5 M) were added. The solutions were lifted for 10 min. at room temperature (25˚C), finally 1.0 ml of IC (5.0 x 10^{-4} M) was added the diluted to the mark with distilled water. The absorbance was measured after 5 min at 610 nm versus the reagent blank, prepared in the same manner but containing no drug. A linear calibration curve was obtained with good value of determination coefficient (R^2) (Fig. III).

2.4. Procedure for pharmaceutical tablets

The contents of 10 finely ground tablets were weighed and mixed. An accurately weighed portion of the tablet powder equivalent to 50 mg of the drug under investigation was weighed, and dissolved in about 50 ml of 0.02 M NaOH. The solution was filtered (if necessary) and the clear solution was diluted to 100 ml with 0.02 M NaOH in a 100 ml calibrated flask.

3. Results and discussion

NBS has been extensively used as brominating and oxidizing agents for organic compounds [20]. NBS has been used as an oxidizing agent for several spectrophotometric and chemiluminescence reactions [21]. In the present work, it was found that NBS can oxidize MX in an acidic medium. In addition, it reacts immediately with IC in an acidic medium to bleach out its blue color. Therefore, after the oxidation of the drug under investigation by NBS, the excess NBS was reacted with the indigo carmine (scheme.1). The absorption spectrum of the IC has maximum absorption at 610 nm (Fig. IV). Therefore, the different parameters affecting the oxidation reaction, and hence the subsequent determination of these drugs were optimized.

3.1. Optimization of variables

The effect of various variables on the color development was tested to establish the optimum conditions for the determination of meloxicam in pharmaceutical preparation.

3.1.1. The chosen of dye and concentration

The preliminary experiment was performed to optimize the useful and optimum concentration of dye (indigo carmine, phenol red and crystal violate) that can be determined spectrophotometrically. The results indicated that indigo carmine was found to be a useful agent for the reaction. Then the optimum concentration selection of indigo carmine was studied. Fig.V shows that 1.0 ml of IC dye was the best volume; to give a stable color with highest intensity.

3.1.2. The effect of oxidant reagents

NBS was found to be a useful oxidizing agent, other oxidizing agents such as ($K_3Fe(CN)_6$,K_2CrO_4 and NBS) have also been tested, but none offered real advantages over NBS as in Fig. VI.

3.1.3. Effect of oxidant amount.

The effect of different volumes (0.2 to 2.2) ml of 5.6 x 10^{-4} M of NBS solution on the color of the dye was studied without meloxicam. Fig.VII shows that 1.4 ml of NBS solution was enough to obtain a maximum bleaching of the color of indigo carmine dye therefor it was recommended for the subsequent experiments.

3.1.4. Nature and amount of acid

The reactions were tested in HCl, H_2SO_4, HNO_3, and CH_3COOH solutions. The results indicate that hydrochloric acid was found to be the desirable medium for oxidation of MX by NBS; additionally it contributed in MX solubility and also increased NBS potential for IC oxidation. A 0.8 ml of 0.5 M HCl was found to be adequate for the oxidation of the drugs and was used for all subsequent experiments.

3.1.5. Effect of temperature

The effect of temperature on the IC color's intensity was studied. In practice a maximum absorbance was obtained when the color was developed at room temperature (25˚C), decrease in color intensity and stability was observed in low or high temperature, therefor room temperature is recommended for subsequent experiments.

3.1.6. Order of addition

To obtain optimum results the order of addition of components should be studied .The results shown in Table 1 indicated that order (II) addition of reagents was the optimum order due to the high intensity of the formed color and hence the same order of addition was followed throughout the investigation.

3.1.7. The effect of time on oxidation

It was observed that if indigo carmine was added immediately to the solution containing MX and NBS in acidic medium the resulted solution is bleached rapidly and the absorbance is very low. This can be explained by the fact that the drug oxidation by NBS is a time developing reaction and thus the influence of the reaction time was studied. In this respect, solutions containing 1 ml of 30 µg/ ml MX, 1.4 ml of 5.6 x 10^{-4} M NBS and 0.8 ml of 0.5 M HCl have been let to react at darkness in different times before adding the indicator and measuring the absorbance at 610 nm. It was observed that the absorbance of these solutions increases with the time up to 10 minutes then it remains constant. So, for further measurements a reaction time of 10 minutes was selected, and the standing time for bleaching of the color of IC, 5 minutes was necessary for bleaching of the color of IC dye and it was stable for at least another 50 minutes.

3.2. Accuracy and precision

To check the accuracy and precision of the proposed method were evaluated by replicate analysis (n=5) of calibration standards at three concentration levels (5.0, 20.0 and 40.0 µg/ml). Precision and accuracy were based on the calculated relative standard deviation (RSD, %) and relative error (RE, %) of the experimentally determined concentration compared to the theoretical one, respectively. The results indicated that determination of MX by proposed method is satisfactory (average of five determinations of RSD %=0.095 and RE, %=1.57).

3.3. Analytical characteristics

Analytical characteristics such as regression equation, linear range, relative standard deviation, relative error, molar absorptivity and Sandell's sensitivity values of each method were determined under the optimized conditions as shown in Table 3. The limits of detection (LOD) and quantitation (LOQ) were calculated according to the International Union of Pure and Applied Chemistry (IUPAC) definition [22] using the formula:

$$LOD = 3S/b \text{ and } LOQ = 10S/b$$

Where S is the standard deviation of blank absorbance values and b is the slope of the calibration plot, are also presented in Table 2. The high values of molar absorptivity and low values of Sandell's sensitivity and LOD indicate the high sensitivity of the proposed methods.

3.4. Interference studies

In order to assess the possible analytical applications of the proposed analytical method described above to the assay of commercial meloxicam formulations, the effect of some common excipients (starch, glucose, sucrose, potassium chloride, sorbitol, magnesium stearate and sodium chloride) used in pharmaceutical preparations were investigated by carrying out the determination of MX in the presence of different excipients. Experimental results showed that there was no interference from excipients for the experimental method.

3.5. Application of the method

The proposed method was successfully applied to the determination of meloxicam in different brands of tablets and the results are summarized in Table 3. The results obtained were statistically [23] compared with those of the official BP method, HPLC method [24] by applying the student's t-test for accuracy and F-test for precision. The results indicated that there was no significant difference between the proposed method and the reference method with respect to accuracy and precision at 99% confidence level.

4. Conclusion

The proposed method has the advantage of simplicity and rapidity for the determination of meloxicam in pure and pharmaceutical preparations. The assay method involves less stringent control of experimental parameters such as the stability of the colored species, time of analysis and temperature independence. The reagents utilized in the proposed method are safe, inexpensive, have good shelf life, readily available and the procedure does not involve any sample preparation. These advantages encourage the application of the proposed method in routine quality control analysis of meloxicam in pharmaceutical formulations.

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Table 1: The effect of order of addition.

Reaction component	Order number	Absorbance
S+A+OX+DYE		0.753
S+OX+A+DYE		0.780
S+OX+DYE+A		0.668
S+A+DYE+OX	V	0.485

S = 30 µg/ ml Meloxicam, A = Hydrochloric acid, OX = N-bromosuccinamid, DYE = Indigo carmine.

Table 2: Quantitative parameters for the proposed method.

Parameter	The proposed method
Media	0.5 M of HCl
$_{max}$, nm	610
Beer's law range (µg /ml)	0.2-50
Detection limits (µg / ml)	0.126
Quantitation limit (µg/ ml)	0.420
Molar absorpitivity (L/mole.cm)	8.3633 x 10^4
Sandell's sensitivity (µg/cm^2)	0.0042
Regression equation (Y = a + bC) *	
Intercept (a)	0.059
Slope (b)	0.0238
Determination coefficient (R^2)	0.9977
Relative standard deviation, %	0.095
Relative error, %	1.57

* In Y = a + bC, Y is absorbance and C is concentration.

Table 3: Pharmaceutical applications for MX using the proposed method.

Tablet MX sample	Label value (mg /tablet)	Obtained value (mg)	Recovery %[a], t[b] and F[c] value	Standard method (mg)	Recovery %[a]
Awamedica-Meloxicam Awa, Erbil- Iraq.	7.5	7.23	96.4 t=1.967 F=1.0	7.38	98.4
Boehringer Ingelheim-Mobic, Germany.	7.5	7.15	95.3 t=1.99 F=4.59	7.58	101.1
DEVA-Melocam, Istanbul-Turkey	15.0	14.87	99.13 t= 0.53 F= 0.002	14.92	99.5
BILIM-ZELOXIM ®FORT, Istanbul-Turkey	15.0	15.12	100.8 t= 1.994 F= 0.71	14.68	97.9

a Average of five determinations.

b Tabulated t-value for four degrees of freedom; and p=0.01 is 3.747.

c Tabulated F-value for four degrees of freedom; and p=0.01 is 15.97.

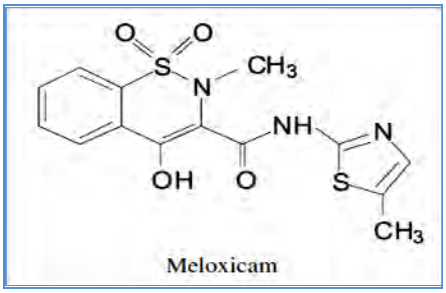

Fig. I: Chemical structure of MX.

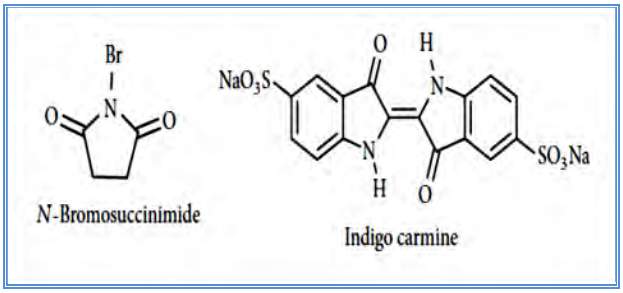

Fig. II: Chemical structure of the chemical reagents.

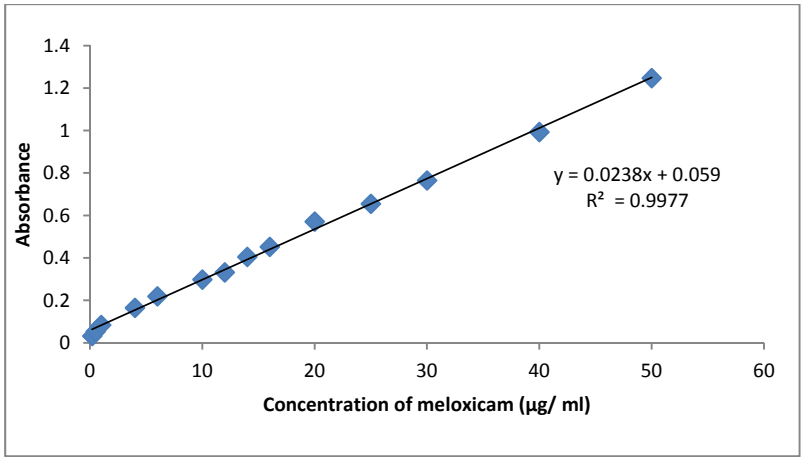

Fig. III: Calibration curve of meloxicam.

MX + NBS (excess) → MX (oxidized product) +NBS (unreacted)

NBS (unreacted) + IC →Oxidized product of IC + Unreacted IC (indicator bleaching)

↓

(Measured spectrophotometrically at λ_{max}=610 nm)

Scheme 1: Reactions of indirect determination of meloxicam by oxidation with N-bromosuccinamid.

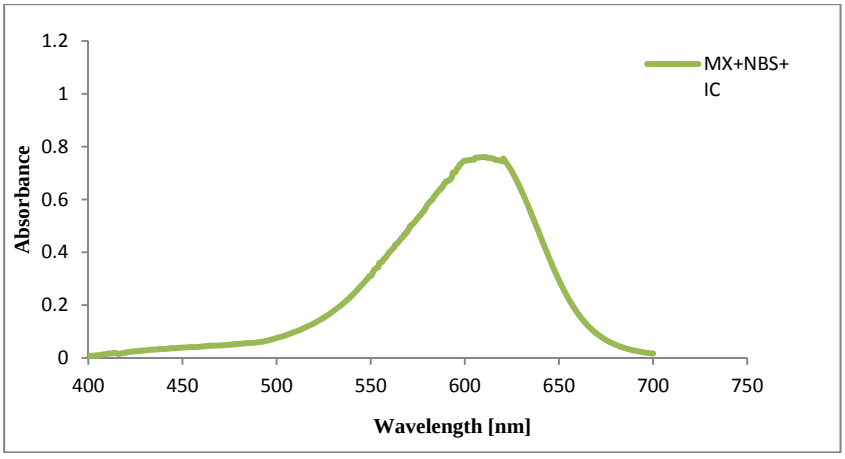

Fig. IV: Absorption spectra of solution containing 0.5 M HCl , 5 x 10^{-4} M (IC), 5.6 x 10^{-4} M (NBS) and 50 µg/ml (MX).

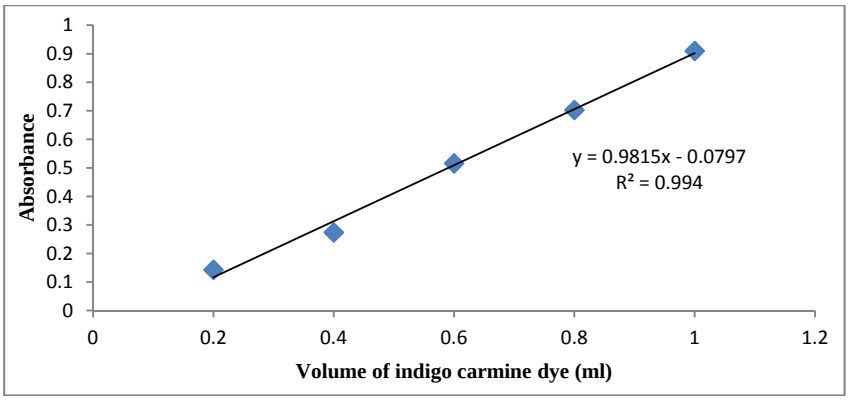

Fig. V: The effect of amount of IC on absorbance.

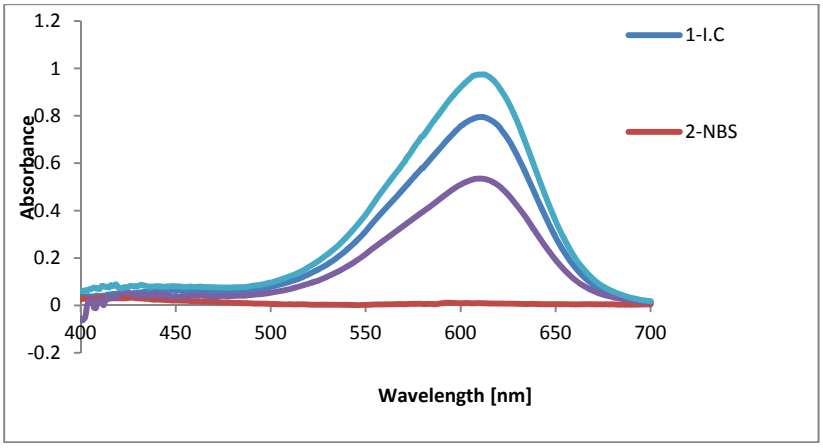

Fig. VI: The effect of oxidant on bleaching the dye when number 1- is IC, 2- is IC with NBS, 3- is IC with K_2CrO_4, and 4- with $K_3Fe(CN)_6$.

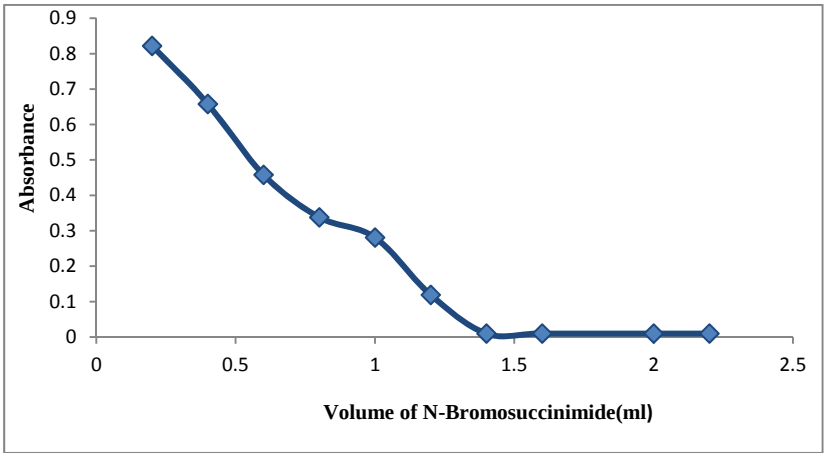

Fig.VII: The effect of NBS amount on bleaching the color of indigo carmine dye.