Spectrophotometric Estimation of Meloxicam Using Charge Transfer Complex

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ABSTRACT:-

In this study we developed cheap and precise analytic method to measure Meloxicam concentration in bulk and pharmaceutical formula. The method based on the reaction between ferric ion and Meloxicam to yield ferrous ion. Then the former ion Fe$^{2+}$ reacted coupled with potassium ferric cyanide to forming colored compound has a maximum absorbance at 708 nm. The method was validated by calculating the precision and accuracy. The method follows Beer-Lamber law at a range of (0.1-11) µg/mL with correlation coefficient is 0.9978. Accuracy and precision of the proposed method were estimated and shown average of recoveries between (98.7-99.5 %) with precision represented by RSD% equal to 0.56. We also calculated the detection limit (LOD) and quantitative of limit (LOQ) were 0.0092 µg/mL 0.030 µg/mL respectively. The modified suggested method was used to measure Meloxicam in pharmaceutical preparation without additives interference and shows a good agreement with the standard method. Therefore, it can be used for routine works for quality control for estimation of the Meloxicam in bulk and pharmaceutical preparation as tablets.

KEYWORDS:- Meloxicam, UV Spectrophotometry, Charge transfer

INTRODUCTION:-

Meloxicam is an enolic acid group of nonsteroidal anti-inflammatory drugs NSAIDs, an oxicam as well as it is consider as analgesic and antipyretic drugs. Pharmacologically, Meloxicam inhibits prostaglandin synthetase enzyme, which inhibits prostaglandins production resulting to inhibit inflammation process$^{(1)}$. 
The chemical formula of Meloxicam is 4-hydroxy-2-methyl-N-5-methyl-2-thiazolyl-2H-1,2-benzothiazine-3-carboxamid-1,1-dioxide(2), $\text{C}_{14}\text{H}_{13}\text{N}_{3}\text{O}_{4}\text{S}_{2}$\(^{(3)}\) see Figure 1.

![Meloxicam Chemical Structure]

Figure 1 represents the chemical formula of Meloxicam

Actually, there are several analytical methods to detect Meloxicam in pure pharmaceutical dosage forms like spectrophotometric\(^{(4-11)}\), electrophoretic\(^{(12)}\), chromatographic\(^{(13)}\), polarographic\(^{(14-18)}\), calorimetric, FTIR\(^{(19)}\), HPLC\(^{(20-25)}\), LCMS\(^{(26-28)}\) and flow injection analysis\(^{(9)}\).

We designed this study to use spectrophotometric technique to measure Meloxicam in pure pharmaceutical formula. The principle of the method depends on the chemical reaction of the drug with ion $\text{Fe}^{+3}$ to form the corresponding ferrous ion $\text{Fe}^{+2}$, which consequently reacts with potassium hexacyanoferrate \([\text{K}_3\text{Fe(CN)}_6]\) to form a colored compound that is absorbed in 708 nm. Hence, our results show the modified analytic method we used is cheap, sensitive and precise comparing to the other analytical methods.

**MATERIALS AND METHODS:**

**Instruments:**

- All absorption measurement were carried out using UV-Visible Spectrophotometer SHIMADZU-1800, (Kyoto, Japan) with 1.0 cm quartz cells.
- pH meter, HANA 300
- Water Bath, ISO 9001
- Sensitive balance (Sartorius BL 210S)
REAGENT AND CHEMICAL: -

All chemicals used were of analytical reagent grade. Ferric chloride FeCl₃, potassium ferricyanate, hydrochloric acid HCl and ethanol. The drug was obtained in pure from the company of PIONER-Sulaymaniyah, Iraq. Meloxicam (15 mg) tablets were taken up from the local a market.

Standard stock solution: -

A standard solution of Meloxicam (100 µg/mL) was prepared by dissolving 0.0100 g of pure meloxicam in 95% Ethanol in 100 ml volumetric, and appropriate volume was taken was diluted to get 50 µg/mL.

Ferric Chloride FeCl₃: -

A 0.0147M of ferric chloride (FeCl₃) was prepared by dissolve 0.240 g in 1 mL concentrated HCl in 100 mL volumetric flask. Then the volume was finished to the mark by adding distilled water.

Potassium Ferricyanate K₃Fe(CN)₆: -

A 0.0045 M of K₃Fe(CN)₆, was prepared by dissolving 0.150g of K₃Fe(CN)₆ in distilled water in 100 ml volumetric flask. Then we finished the volume of the mixture with distilled water.

Recommended procedure: -

In a series of 10 ml volumetric flasks, transfer increasing of volume of Meloxicam (50 µg/mL) to cover the range of calibration curve (0.1-11) µg/mL, then 1ml of (0.0045 M) potassium ferricyanate was added and shake well. A 1 ml (0.0147M) of FeCl₃ was added and left the reaction for 10 min, then 0.1 ml of concentrated HCl was added, leave the solution for 10 min. The solution make up to the mark with distilled water. The spectrum of the colored compound was recorder in the range of (400-800nm) which get a maximum absorption at 708 nm verses reagent blank prepare in the same way expect of absence drug.

Procedure for pharmaceutical preparation:-
Ten tablets of 15 mg of the drug was accurately weight and grounded well in pestle. Equivalent weight of 15 mg was taken and dissolved in 95% ethanol using 50 mL volumetric flask and shaken well for 10 min. The resulting solution was filtrated using A whatman filter paper no. 42) to get rid of all the impurities. The solution was complete to the mark using distilled.

RESULTS AND DISCUSSION:

We evaluated different parameters that could be impaired the stability, sensitivity and absorbance of the colored complex.

The spectrum of complex :-

The purposed method is based on the reduction of Fe$^{+3}$ to Fe$^{+2}$ by Meloxicam in presence of concentrated hydrochloric acid. Subsequently these ions reacted with potassium ferric cyanate to produce blue colored complex. The spectrum of complex was scanned against a reagent blank in the range(400-800 nm) and show that has a particular $\lambda_{max}$ at (708 nm) Figure2.

Figure2: Spectrum of complex (5 μg/mL meloxicam via reagent Blank.

Study the effects of Ferric Chloride FeCl$_3$ concentration:

We evaluated the effects FeCl$_3$ concentration on the intensity of the absorbance of the colored complex. We used different volume of a 0.0147 M Ferric Chloride (0.1-2.0) mL was added to the fixed volumes of the drug
(1) mL of 50 µg/mL and 1mL of A (0.0045) M of K₃Fe(CN)₆ M with 0.1 mL of concentrated HCl.

The complex formation reached at a maximum absorbance at 1mL of (0.0147 M) Ferric Chloride solution. This amount was used as suitable volume for the study because it gives high color intensity and less absorbance for blank and this was taken to subsequent experiment as in Figure 3.

![Figure 3: The correlation between Meloxicam absorbance and different volume of 0.0147 M Ferric Chloride](image)

Effect of potassium Ferricyanate K₃Fe(CN)₆ concentration:-

To find a suitable concentration of potassium ferricyanate a different volume (0.1-1.6 ml) of (0.0455 M) K₃Fe(CN)₆ with the fixed volumes of the dug (1.0 )mL of 50 µg/mL and 1mL of A (0.0045) M of FeCl₃ and 0.1 mL of concentrated HCl. A 1 ml of (0.0455M) K₃Fe(CN)₆ was founded to be optimum volume because it gives high color intensity of the colored compound because it gives high color intensity and less absorbance for blank and this was taken to subsequent experiment as in Figure 4.
Figure 4: effect of different volume of 0.0455 M \( \text{K}_3\text{Fe(CN)}_6 \).

Effect of type of Acids on the Absorbance of the Color Compound:

Different acids were individually taken study their effect on intensity of the colored complex as \( \text{H}_3\text{PO}_4 \), \( \text{H}_2\text{SO}_4 \), \( \text{HNO}_3 \), \( \text{HCl} \) and \( \text{CH}_3\text{COOH} \) acids. It was found that concentrated \( \text{HCl} \) acid gave the highest absorption with minimum absorbance of the reagent blank at the volume of (0.1 mL) and as shown in the Table 1.

Table 1 : Effect of acids

| Acids    | Absorbance |
|----------|------------|
| HCl      | 0.434      |
| \( \text{H}_2\text{SO}_4 \) | 0.418      |
| \( \text{HNO}_3 \)   | 0.422      |
| \( \text{CH}_3\text{COOH} \) | 0.417      |
| \( \text{H}_3\text{PO}_4 \)  | 0.346      |

Effect of Reaction Time:-

The intensity of the color complex was studied and found that the absorbance increased with increasing reaction time and reached maximum at 20 min. Therefore, 20 min was chosen for further study as in Figure 5.
Figure 5: Effect of Time

Effect of Temperature:

To study the effect of Temperature at the intensity of colored complex. The reaction was studied in different Temperature and it was found that the optimum temperature was at $C^o$ above Temperature the reaction was fast and the turbidity was appear, therefore a temperature 25 $C^o$ was adopted for subsequent study. The Figure 6 show optimum temperature to achieve the reaction.

Evaluate the effect of order and addition:

The effect of the order of addition on the intensity of the colored compound was studied, it was found that order of add (Drug: $K_3Fe(CN)_6$: $FeCl_3$) gave the highest absorption value. Therefore, it was chosen for further study. The result obtained are shown in table 2.
Table 2: Effect of order of addition.

| Order of addition | Absorbance |
|------------------|------------|
| Drug: K₃Fe(CN)₆: FeCl₃ | 0.457 |
| Drug: FeCl₃: K₃Fe(CN)₆ | 0.427 |
| K₃Fe(CN)₆: FeCl₃: Drug | 0.396 |

Constricted of Calibration Curve:-

We obtained a linear calibration curve to determine Meloxicam that implies Beer’s law obeyed in concentration range 0.1–11 µg/mL. The linear regression equation obtained was \(y = 0.0974x + 0.0236\), the correlation coefficient 0.9978 and the molar absorptivity value was found to be \(3.42 \times 10^{4}\) L.mol\(^{-1}\).cm\(^{-1}\) which confirm the method was sensitive and accurate\(^{(31)}\) see figure 7. In addition we measured limit of detection LOD and limit of quantification LOQ based on the standard deviation blank. See table 3.

![Figure 7: Calibration Graph for the determination of Meloxicam in Complex.](image)

Table 3: Statistical parameters of proposed method

| Parameters | Value |
|------------|-------|
| Maximum absorption \(\lambda_{\text{max}}\) | 708 nm |
| Range of the linearity according to Beer Law | 0.1–11 µg/ML |
| Molar absorptivity | \(3.42 \times 10^{4}\) L.mol\(^{-1}\).cm\(^{-1}\) |
| Sandal Sensitivity | 0.010 Cm\(^2\) |
### Precision and Accuracy:

We statically evaluated the precision and accuracy of the proposed method to confirm the efficiency. To measure the precision of the method we determine the relative standard deviation RSD%, and to evaluate the accuracy, we measured percentage relative error RE% and percentage of recovery.

We used five replicates of Meloxicam drug at different concentrations (1.5, 6 and 10) µg/ml. The RSD% and RE% were 0.56% and 0.86% respectively. Interestingly, the experiment showed an excellent range between (98.7-99.5%, table 4) which refers that the modified method is definitely accurate and precise.

**Table 4: Presenting the accuracy and precision values of the modified method.**

| Concentration ppm | RE% | Recovery% | RSD% |
|-------------------|-----|-----------|------|
| Taken | Found |     |     |
| 1.5   | 1.510 | 1.3  | 98.7 | 0.72 |
| 6     | 6.016 | 0.39 | 99.5 | 0.25 |
| 10    | 9.972 | 0.9  | 98.8 | 0.73 |

**Interference:**

The effect of interference of the some excipients was examined to determine selectivity of the method by measuring the absorbance of the solution at (2mL) of 100 µg/mL of each excipients (Starch, Lactose, Sucrose, Mg Stearate and Glucose) and with 1ml of 50 µg/mL drug to the final volume.
of 10 ml. The study show there is no effect on the excipients on the intensity of the colored compound the results, as in Table 5.

Table 5: The effect of excipients (100 ppm) on the recovery. //RE: relative error.

| Excipients  | Found | RE % | Recovery % |
|-------------|-------|------|------------|
| Starch      | 5.04  | 0.8  | 99.2       |
| Lactose     | 4.98  | 0.4  | 99.6       |
| Sucrose     | 4.97  | 0.6  | 99.4       |
| Glucose     | 5.03  | 0.6  | 99.4       |
| Mg stearate | 4.98  | 0.4  | 99.6       |

Analytic Application:-

The suggested method was effectively applied to determine Meloxicam in its commercial formulation obtained. The amount of tablet containing 15 mg Meloxicam was determined for series of three replicates at three levels of Meloxicam concentration (1.5, 6 and 10) ppm as summarized in Table 6.

Table 6: Determination of Meloxicam in pharmaceutical formulation.

| Company          | Concentration ppm | RE% | Recovery% | RSD% |
|------------------|-------------------|-----|-----------|------|
| Boehringer Ingelheim | Taken     | 1.5 | 2.5       | 97.5 | 1.7  |
|                   | Found           | 1.541| 2.5       | 97.5 | 1.7  |
|                   |                  | 5.838| 2.6       | 97.4 | 0.5  |
|                   |                  | 9.982| 0.9       | 99.1 | 0.72 |

Stoichiometric ratio for the formation colored complex:-

By application of Jobs method and mole ratio, It was found that drug form a colored complex product with stoichiometric (1:1) as shown in Figure (8 and 9).
CONCLUSION:-

A soluble colored complex has been formed successfully by chelation of potassium hexacyanoferrate (III) with iron (II) that was formed from reduction of iron (III) by Meloxicam. It is concluded that the suggested method is a precise, accurate and reproducible for determination of Meloxicam in pure and pharmaceutical formulation without the interference of any excipients. The suggested method was rapid, less time consuming than other spectrophotometric method and linked with a good accuracy and precision which make the method suitable for a routine assay of Meloxicam in quality control in laboratories and this simple doesn’t require any procedure of extraction, prior treatment of drug and expensive reagents.
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REFERENCES:-

1- Gurupadayya B. M., Trinath M.N. and Shilpa K., spectrophotometric determination of meloxicam by sodium nitroprusside and 1,10-phenanthroline reagent in bulk and its pharmaceutical formulation. Indian Journal of Chemical Technology, March 2013, p.111.

2- Ananth V., Venkatamahesh R., Ulaganathan C., Balaji M. and Sachin Kumar Singh.: Development and Validation Spectrophotometric Method for Quantitative Estimation of Meloxicam in bulk and Pharmaceutical Dosage forms. Der Pharma Chemical, 2011, 3(2): 352-357.

3- Emirhan. N., Filiz. S., Nursabah. E. B. and sedef. k. A, Validated HPLC Method for the Determination of Meloxicam in Pharmaceutical Preparations. Hacettepe University Journal of the Faculty of Pharmacy, July 2007, p.107.

4- Garcia, M.S., Pedreno, C.S., Albero, M.I., Marti, A., Spectrophotometric Methods for Determining Meloxicam in pharmaceuticals Using Batch and Flow-Injection Procedures, Eur. J. Sci. Pharm. (2000), 9, 311.

5- Bebewy, L. I., Stability-indicating Method for the Determination of Meloxicam and Tetracaine Hydrochloride in Presence of their Degradation Products, Spectrose. Lett., (1998), 31(4), 797.

6- Hassan, E.M., Spectrophotometric and Fluorimetric Methods for the Determination of Meloxicam in Dosage Forms, J. Pharm. Anal., (2002), 27, 771.

7- Joseph-Charles, J., Bertucat, M., Determination of Meloxicam in Tablets Formulations by Ultraviolet Spectrophotometry and High-performance Liquid Chromatography, Anal. Lett., (1999), 32(10), 2051.

8- Zawilla, N.H., Abdul-Azim Mohammed, M., El Kousy, N.M., El-Moghazy Aly, SM.: Determination of Meloxicam in Bulk and
Pharmaceutical Formulations, J. Pharm. Biomed. Anal., (2003), 32(6), 1135.

9- You W.W., Liu Y., Wang Z.B., Determination of Meloxicam by Ultraviolet spectrophotometry, Chinese J. Anal. Chem., (1999), 27(7), 841.

10- Taha E.A., Salama N.N. Fattah L.S.A., Stability-Indicating Methods for the Determination of Meloxicam and Tenoxicam in the presence of their Degradation products, Spectr. Lett. (2002), 35(4), 501.

11- Nemutlu E., Kir S., Validated Determination of Meloxicam in Tablets by Using UV Spectrophotometry, Hacettepe University Journal of the Faculty of Pharmacy, 2004, 24(1) 13-24.

12- Vignaduzzo, S.E., Casteiiano, P.M.M Kaufman, T.S., Method Development and Validation for the Simultaneous Determination of Meloxicam and Pridinol Mesylate Using RP-HPLC and its Application in Drug Formulations, J. Pharm. Anal. Corrected Proof (2007)

13- Altiookka, G., Atkosar, Z., Tuncel, M., Pulse Polarographic Determination of Meloxicam, Die pharmazie, (2000), 56(2), 184.

14- Radi, A., El-Ries, M.A., El-Anwar, F., El-Sherif, Z., Electrochemical Oxidation of Meloxicam and its Determination in Tablet Dosage Form, Anal. Lett., (2002), 34(5), 739.

15- Altinoz, S., Nemutlu, E., Kir, S., Polarographic Behaviour of Meloxicam and its Determination in Tablet Preparation and Spiked Plasma, Il Farmaco, (2000), 57, 463.

16- Radi, A.E., Ghoneim, M., Beltagi, A., Cathodic Adsorptive Stripping Square-Wave Voltammetry of the Anti-Inflammatory Drug Meloxicam, Chem. Pharm. Bull., (2001), 49 (10), 1257.

17- Rao, R.N., Meena, S., Rao A.R., An Overview of the Recent Developments in Analytical Methodologies for Determination of COX-2 Inhibitors in Bulk Drugs, Pharmaceuticals and Biological Matrices, J. Pharm. Biomed. Anal., (2005), 39(3-4), 349.

18- Beltagi, A.M., Ghoneim, M.M., Radi, A., Electrochemical Reduction of MRLOXICAM AT Mercury Electrode and its Determination in Tablets, J. Pharm. Biomed. Anal., (2002), 27, 795.

19- Isaa AA, Marchidan D, Cojocaru V and Anuta V., Preparation and Evaluation of Meloxicam Solid Dispersion by Melting Method, J Farm., (2013), 61(6), 1216-1234.
20- Kulkarni V, Patil BS, Hariprassna RC, Borgaonkar PA, Hogada MG and Rabbani G., Formulation and Development of Fast Dissolving Meloxicam Tablet by solid Dispersion Technique for the Effective Treatment of dental Pain. International Journal of Current Pharmaceutical Research. (2010), 2(3), 82-85.

21- Ahmad S., Deepika S., Kapil W and Usman MR., Novel RP-HPLC Method Development and Validation of meloxicam suppository. Indian Journal of Pharmaceutical Education and Research. 2017, 51(4), 644-649.

22- Banerjee R., Chakraborty H and Sarkar M., Photophysical Studies of Oxicam Group of NSAID., Piroxicam, Meloxicam, and Tenoxicam. J Spectrochimical Acta. 2003, 59(6), 1213-1222.

23- Elbary AA, Foda N. and Elkhateeb M., Reversed –Phase Liquid Chromatographic Determination of Meloxicam in Human Plasma and its Pharmacoketic Application. J Analytical Letters 2001, 34(7)1175-1187.

24- Sahoo NK, sahu M., Rao PS, Rani NS, Devi J I and Ghosh G., Validation of Assay Indication Method Development of Meloxicam in Bulk and Some of its Tablet Dosage Forms by RP-HPLC. J Springerplus. 2014, 3(1),95. doi: 10.1186/2193-1801-3-95.

25- Bea JM., Kim MJ., Jang CG and Lee SK., Determination of Meloxicam in Human Plasma using an HPLC Method with UV Detection and its Application to a Pharmacokinetic Study. J of Chromatography. 2007, 859(1), 69-73.

26- JIHY, Lee HW, Kim YH, Jeong DW and LeeHS., Simultaneous Determination of Piroxica, Meloxicam and Tenoxicam in Human Plasma by Liquid Chromatography with Tandem Mass Spectroscopy. J of chromatography. 2005, 826:216-219.

27- Siddareddy K, Reddy MA, Surech B and Sreeramulu J., Development and Validation of Analytical Method for Simultaneous estimation of Bupivacaine and Meloxicam in Human Plasma using UPLC-MS/MS. J Pharmaceutical Methods. 2018. 9(1), 2-8.

28- Yuan Y., Chen X and Zhong D., Determination of Meloxicam in Human Plasma by Liquid Chromatography. 2007, 852, 650-654.

29- Al-Momani IF., Indirect Flow-Injection Spectrophotometric Determination of Meloxicam Tenoxicam and Piroxicam in Pharmaceutical Formulation. J Analytical Sciences. 2007, 22:1611-1614.
30- Abdulbari Mahdi Mahood and Sura L. Alkhafaji., Spectrophotometric Determination of Etodolac Using Charge Transfer complex., 2019 , 10(3): 1000-06.

31- Shaikh A, Singh G, Jain N and Gupta M., Development and Validation of New Simple , Sensitive and Validated UV-Spectrophotometric and RP-HPLC Method for the Simultaneous Estimation of Paracetamol and Etadolac in Marketed Formulation. Journal of Drug Delivery and Therapeutics 2017, 4:120-124.