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Sensitive, Direct and Rapid Spectrophotometric Method for the Determination of Meloxicam through Ion-Associate Complex Formation

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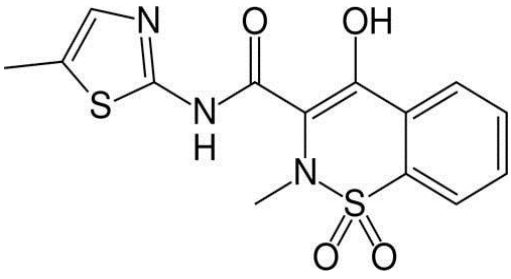
Abstract

A simple, accurate and sensitive spectrophotometric method has been developed and validated for the determination of meloxicam. The method is based on the formation of the ion-associate complexes between meloxicam and orange G (OG), methylene blue (MB) or copper chloride ($CuCl_2$) to give colored products maximally absorbed at 358, 652, 361 nm, with the three reagents respectively. Different factors affecting the reaction between the drug and the three reagents were carefully studied and optimized. Under the optimum conditions, linear relationships with good correlation coefficients (0.9956, 0.9934 and 0.9974) were found between absorbance and concentrations of the drug in the concentration range 1.0-22.1 μgml^{-1}. The assay limits of detection and quantitation were in the ranges 0.40-1.73, 0.66-1.87 and 0.8-1.33 μgml^{-1}, respectively. The method was validated, in terms of accuracy and precision and the results are satisfactory. The proposed method was successfully applied to the determination of the investigated drug in pure and pharmaceutical dosage forms (recovery is 99.63, 100.14 and 99.86 % for OG, MB and $CuCl_2$ respectively) without interference from the common excipients. The results obtained by the proposed method were comparable with those obtained by reference methods.

1. Introduction

Meloxicam, chemically is, 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, scheme (1).



Many techniques were utilized for the determination of meloxicam such as spectrophotometry [1- 7], flow injection [8,9] and first derivative spectroscopy [10,11]. These methods are not simple and required complicated instrument. The present study describes the development of a spectrophotometric method that can be used in laboratories without modern and expensive instrumentation such as that required for gas chromatography or HPLC. The proposed method involves the formation of ion-associate complexes of Meloxicam with methylene blue (MB) (OG) and $CuCl_2$as chromophoric reagents. The proposed procedure was applied successfully for the determination of Meloxicam in pure pharmaceutical preparations, with good accuracy and precision.

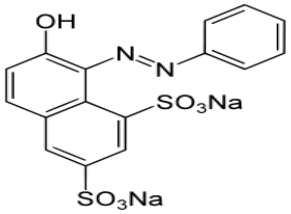
2. Experimental

2.1. Instrumentation

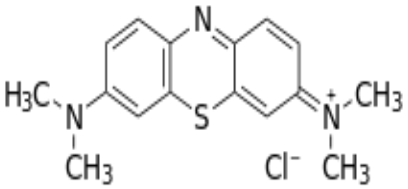
All absorption measurements were made by using a JASCO 530V (Tokyo. Japan; UV-Vis) spectrophotometer with a scanning speed of 400 nm/min and a band width of 0.2 nm and equipped with 10 mm matched quartz cells. An Orion research model 601 A/digital Ionalyzer pH meter was used to check the pH of the acetate buffer solution.

2.2. Reagents and Materials

All chemicals used were of analytical grade, and all solutions were freshly prepared in bidistilled water. OG, MB and $CuCL_2$ (scheme 1) (all from Aldrich, Milwaukee, WI) were used to prepare 1 x 10^{-3} M solutions by dissolving the calculated weight in 10 ml ethanol and diluting to the desired volume with the same solvent in a 100 ml volumetric flask.



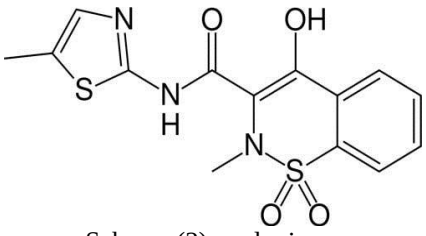
Orange G



Methylene blue

Scheme (1): structures of the reagents used

Meloxicam (99.8%) (Glaxo-Wellcome, London, UK) was kindly provided by the Egyptian International Pharmaceutical Industries Company (EIPICO), 10th of Ramadan city, Egypt and used as received. Chemically it is 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, scheme (2).



Scheme (2): meloxicam

The stock standard solution of 0.20 mg/ml was prepared by dissolving an accurately weighed amount (20 mg) of the drug in 100 ml bidistilled water in 100 ml calibrated flask. The working standard solutions were obtained by accurate dilution of the stock solution with bidistilled water.

Acetate buffer solutions of different pH values (3.6-5.6) were prepared as recommended earlier [12].

2.3. Pharmaceutical formulation

Melocam tablets (Amoun Pharmaceutical Co., El-Obour City, Cairo, Egypt) labeled to contain 7.5 mg of meloxicam/tablet, Neofloxin tablets (Alexandria Co. for pharmaceuticals, Alexandria, Egypt) labeled to contain 400 mg per tablet.

2.4. Preparation of pharmaceutical dosage form samples

A quantity of finely grounded tablet powder equivalent to 20 mg of meloxicam was accurately transferred into 100 ml calibrated flask; 60 ml of water was added and shaken for 10 minutes. The volume was then made to the mark with bidistilled water, mixed well and filtered using a Whatman No. 42 filter paper. The first 10 ml portion of the filtrate was discarded and the filtrate was diluted appropriately to get 20 µg/ml of meloxicam for assay by the recommended method.

2.5. General Procedure

In a 10 ml volumetric flask, an aliquot containing meloxicam at 2.0 - 20 µg/ml was added to 2.0 ml 10^{-3} M reagent solution (OG, MB or $CuCl_2$), followed by 3.0 ml acetate buffer solution at pH 3.60, 5.60, and 3.60, respectively. The

mixture was diluted with bidistilled water and the solution was allowed to stand for 5.0 min at room temperature (25 ± 2°C). The absorbance was then measured at λ_{max} 358, 652, and 361 nm, with OG, MB and $CuCl_2$ respectively, vs a reagent blank similarly prepared.

2.6. Procedure for studying interference

To an aliquot of 2.0 ml of solution containing 20 µg/ml of meloxicam, different amounts of various interfering compounds in an aliquot 2.0 ml were added individually and the recommended procedure was followed. Less than ±3.0 % change in absorbance is considered as non interference.

2.7. Stoichiometric Ratio

The stoichiometry of the ion - associate formed was studied using the mole ratio [13] and continuous variation [14] methods. By using the mole ratio method, the concentration of meloxicam was kept constant (0.1 ml of 10^{-3} M), whereas the concentration of the reagent was regularly varied (0.1-1.2 ml of 10^{-3} M). The absorbance of the prepared solutions was measured at the optimum λ_{max} for each ion-associate complex. The values were then plotted vs the mole ratio [reagent]/ [drug]. The intersections of the straight lines obtained showed the mole ratio of the most stable complexes.

In the continuous variation method, a series of solutions was prepared by mixing equimolar solutions of meloxicam and reagent in different proportions, while keeping the total molar concentration constant (2.0 ml of 10^{-3} M). A plot of the absorbance of the solutions measured at the recommended wavelength vs the mole fraction of the reagent shows a maximum at the mole ratio of the most stable complex.

3. Results and Dissection

Investigations were carried out to establish the most favorable conditions for the ion-associate complexation reaction of the reagents with meloxicam to obtain maximum color development for the determination. The influence of different variables on the reaction was tested as follows.

3.1. Effect of pH

The effect of pH on the ion – pair complex formation between miloxicam hydrochloride and the three reagents under investigation was studied in acetate buffer solutions within the pH range 2.60 – 11.62. A series of solutions containing 2.0 ml ($1.0x10^{-3}$ M) of reagent, 3.0 ml buffer solution of different pH values and 1.0 ml ($1.0x10^{-3}$ M) of the drug were prepared. Each solution was completed to 10.0 ml with bidistilled water and the content of each flask was mixed well. The absorption spectra were recorded using blank solutions prepared in the same way without drug at the same pH values. Illustrative spectra are shown in Fig. (1). Inspection of the data gathered from these figures shows that the optimum pH values giving maximum absorbance are 3.60 - 4.0 , 5.0 - 5.60 and 3.60 - 4.0 by using OG, MB and $CuCl_2$ respectively. Therefore, all subsequent studies were carried out at pH 3.60, 5.60 and 3.60, because the results were highly reproducible at these pH values. Moreover, the optimum volume of the acetate buffer solution was examined and found to be 3.0 ml in a total volume of 10 ml.

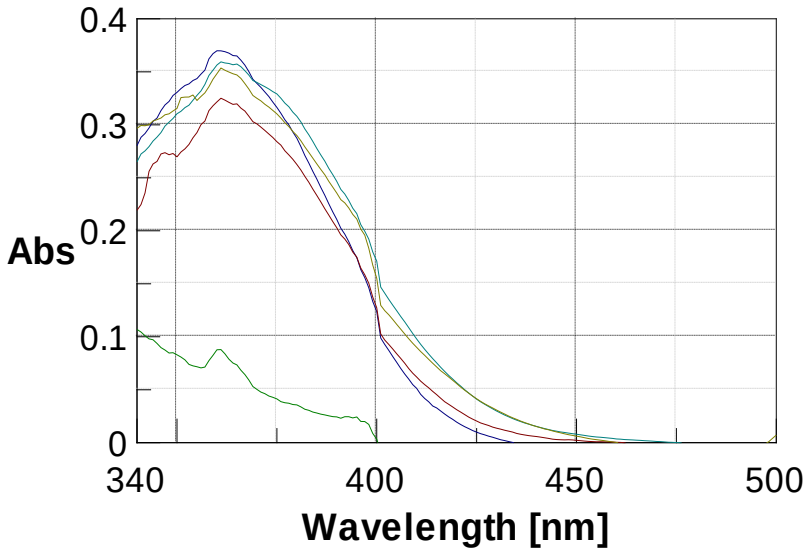


Fig 1: Effect of pH on absorption spectra of meloxicam - copper complex

3.2. Determination of λ_{max} of complex species

To determine the wavelength at which ion - pair complex species possesses maximum absorbance (λ_{max}), the following spectra must be recorded:

A- Spectrum of pure reagent; 1.0 ml (1×10^{-3} M) at the optimum pH value using the same buffer as a blank.

B- Spectrum of solution mixture of reagent (A) and drug (1.0 ml of $1x10^{-3}$ M) at the optimum pH value using the same buffer as a blank.

C- Spectrum of solution (B) against (A) as a blank.

The absorption spectra are shown in Fig. (2), from which the values of λ_{max} for each complex were determined and cited in Table (1). These optimal wavelengths are chosen for further investigation.

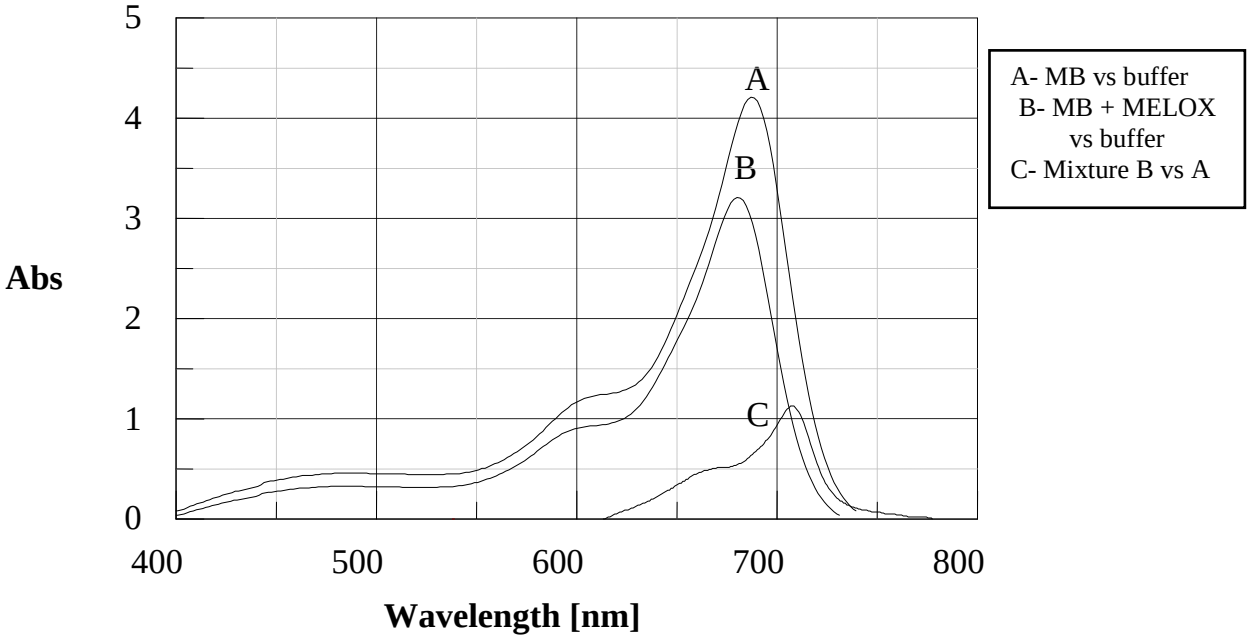


Fig 2: Difference curves of meloxicam-MB complex

3.3. Effect of time and temperature

The effect of time on complex formation was studied by measuring the absorbance of the complexes at optimum pH against a blank solution of the same pH at various time intervals. Also, the effect of temperature was studied for the same solution by incubating the sample and blank in water bath at different temperatures (25 - 50°C). The absorbance was measured after cooling to room temperature.

The experiments showed that complexes are formed within few minutes (5 minutes) after mixing drug with reagent in the buffered media and remain stable for about 6 hours. It was found also that, increasing the temperature up to 50°C has a slight effect on the absorbance, while boiling destroys the complex.

3.4. Effect of sequence of addition

The effect of sequence of addition on ion - pair complex formation was studied by measuring the absorbance of solutions prepared by different sequences of addition against a blank solution prepared in the same manner. Experiments showed that the sequence of reagent — buffer - drug is the best one. So, it seems that the buffer action must change the reagent to the anionic form [R] making it capable to interact with the drug in the cationic form [D+] to form the ion - pair association complex [R][D+].

3.5. Effect of reagent concentration

To study the effect of reagent concentration on the complex formation between meloxicam and different reagents under study, the concentration of meloxicam was kept constant (1.0 ml of 1x103 M) while that of the reagent was varied regularly (0.5, 1.0,1.5, 2.0, 2.5 and 3.0 ml of $1x10^{-3}$ M).The resulted spectra showed that 2.0 ml of each reagent is sufficient for complete complexation.

3.6. Effect of buffer volume

The effect of buffer volume on the reaction between meloxicam solution and the reagents OG, MB, Cucl2 was investigated by adding different buffer volumes of the selected pH (1.0, 2.0, ….. 4.0 ml) to fixed concentrations of drug and reagent (1.0 ml of 1x10'3 M drug solution + 1.0 ml of $1x10^{-3}$ M reagent solution) and the volume was completed to 10.0 ml with bidistilled water. The absorbance of each sample solution was measured against a blank solution of reagent at the same pH. The optimum volume of buffer was found to be 4.0 ml chosen from the highest absorbance value. This volume is used for further studies.

3.7. Stoichiometry of complexes

The molecular structure of the formed colored complex was determined by two spectrophotometric methods (molar ratio and continuous variation methods). The data obtained from these methods were used to calculate the stability constants of the colored products.

3.7.1. The continuous variation method

In the present work, the modification of Job's [14] continuous variation method was used to investigate the stoichiometry of the complex formed between drug and reagent. A series of solutions were prepared by mixing equimolar solution of the reagent and drug in different preparations keeping the total molar concentration constant ($2.0x10^{-3}$ M) in the presence of *4* ml of the selected buffer. A plot of the absorbance of the solution at the maximum wavelength against the mole fraction of the drug gives the molar ratio of the most stable formed complex. Experimental results revealed that the complexes formed have 1:1 stoichiometric ratio.

3.7.2. The molar ratio method

In the molar ratio method described by Yoe and Jones [13], the concentration of the meloxicam was kept constant at (0.5 ml of $1x10^{-3}$ M) while that of the reagent was varied (0.2, .2.4 ml of $1x10^{-3}$ M), 3.0 ml of the selected buffer solution are added and the volume is completed to 10.0 ml with bidistilled water. The absorbance of the sample solution was measured against reagent blank at the maximum wavelength. The absorbance values were then plotted against the molar ratio [reagent/drug]. The inflection of the straight line obtained shows the molar ratio of 1:1 (drug : reagent) products. Results obtained from molar ratio and continuous variation methods are in agreement with each others.

3.8. Stability constants of the complexes

The stability constants of the formed complex were calculated using the data obtained from the mole ratio and continuous variation methods applying the equation of Yeo and Jones [ ] as modified by Issa *et al* [15].

$$K_n = \frac{(A/Amax)}{[1-(A/Amax)]^{n+1}\, C_R^n n^2} \qquad (1)$$

A : the absorbance at concentration C_R

A_{max}: the maximum absorbance value

n : the stoichiometric ratio of the complex

K_n : the stability constant

Log stability constants calculated from mole ratio and continuous variation methods are listed in Table (1). The values of log stability constants calculated by the two methods are in good agreement with each other. They indicate that the complexes are fairly stable

3.9. Validity to Beer's law

Under optimum conditions, mentioned above, different concentrations of meloxicam (ug/ml) were transferred into 10.0 ml measuring flask containing 1.0 ml ($1x10^{-3}$ M) of reagent and 3.0 ml of buffer solution of the optimum pH. The volume was completed to the mark by bidistilled water and the content of the flask was mixed well. The absorbance was measured at optimum A_{max}, then plotted against drug concentration [D] as shown in Fig.(3).

Limits of Beer's law, molar absorptivity (E; lmol'"cm"^{1}) and Sandell sensitivity [16] values were calculated and listed in Table (1). Regression analysis for the results was as carried out using least square method. In all cases, Beer's law plots were linear with very small intercepts and good correlation coefficients (0.999 and 0.999).

For more accurate analysis, Ringbom [17] optimum concentration range was determined by calculating the percent transmittance (%T) from the following equation:

$$\%T = 10^{-A} \times 100. \qquad (2)$$

where A is the absorbance of the complex.

By plotting logarithm of drug concentration; log[D] in ug/ml against %T as in Fig. (4), the linear portion of the sigmoid curve gave the accurate range of analysis. Results are listed in Table (1).

Table 1: Spectrophotometric cumulative data for meloxicam using OG, MB and $CuCl_2$ as chromophoric reagents

PARAMETER	**OG**	**MB**	**$CuCl_2$**
PH	3.6	5.6	3.6
λ_{max} (nm)	358	652	361
Beer's limits (µg/ml)	9.41	10.28	10.39
Ringbom range (µg/ml)	2.04 – 10.50	1.95 – 9.77	1.98 – 10.03
Molar absorptivity ($xl0^4$) (1 mol^{-1} cm^{-1})	4.36	5.04	4.70
Sandell sensitivity (µg cm^{-2})	8.06	7.39	6.29
Intercept (b)*	0.0132	0.0382	0.0277
Slope (m)*	0.1034	0.1440	0.1041
Correlation coefficient (r)	0.9956	0.9934	0.9974
RSD%	0.213	0.134	0.266
Stability constant**	4.35	5.21	4.84
RE%	0.364	0.405	0.153

* Linear regression equation: Y = mX + b
** Mean value of Molar ratio and continuous variation methods.
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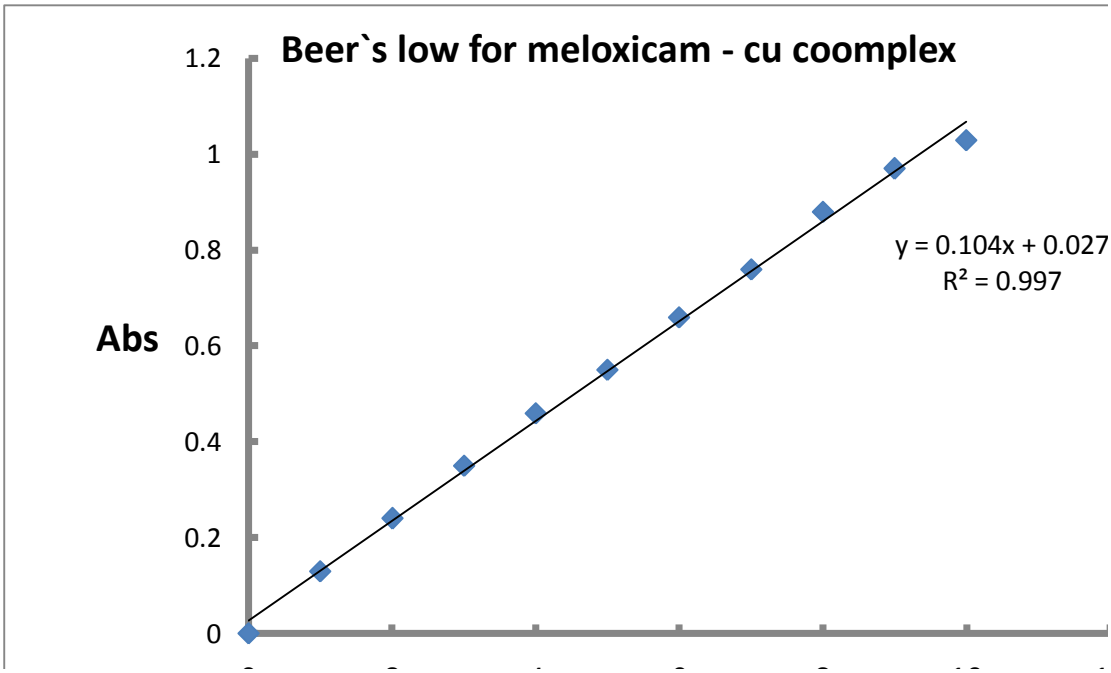

Fig 3: Beer´s low for meloxicam – cu complex.

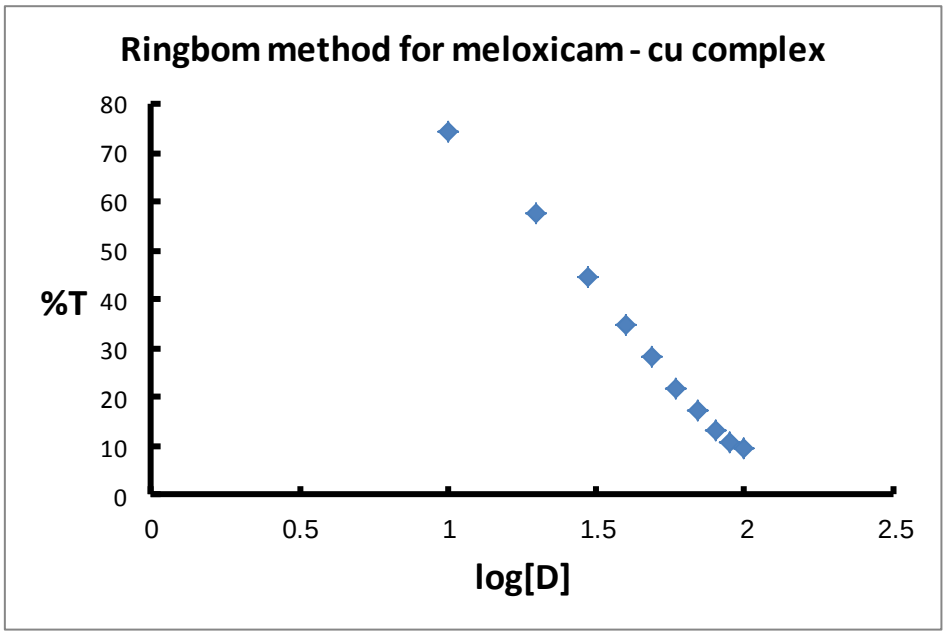

Fig 4: Ringbom method for meloxicam – cu complex.

3.10. Accuracy and precision

To determine the accuracy and precision of the proposed method; solutions of certain concentration (within the concentration range obtained from Beer's law and Ringbom methods) were prepared and analyzed in six replicates. The percentage relative standard deviation (% RSD) did not exceed 0.336 % indicating the high accuracy and reproducibility of the proposed method (Table 2). The percentage recovery and the range of error (%) at 95% confidence level indicate the reasonable accuracy and precision. The results are considered as very satisfactory for the examined concentration levels.

3.11. Interference

Before proceeding with the analysis of the investigated drug (meloxicaml) in its pharmaceutical dosage forms, interference liabilities were carried out to explore the effect of the presence of co-existing additives and excipients that might be added during formulation. Samples were prepared by mixing known amount (20 mg) of meloxicam with various amounts of the common excipients: sodium acetate, sodium bicarbonate, magnesium stearate, glucose, fructose, sucrose and lactose. The absorbance is measured in each case under optimum conditions. Less than ± 3.0 % in absorbance is considered non interference.

Studies on the effect of interfering species showed that such ingredients up to 10% do not interfere in the determination of miloxicam indicating the suitability for the analysis of this drug in its solid dosage forms without interference from the common additives.

3.12. Analytical applications

The validity of the proposed procedure was tested for determination of meloxicam in pharmaceutical preparations manufactured in local companies such as Melocam tablets (Amoun Pharmaceutical Co., El-Obour City, Cairo, Egypt) labeled to contain 7.5 mg of meloxicam/tablet, Neofloxin tablets (Alexandria Co. for pharmaceuticals, Alexandria, Egypt) labeled to contain 400 mg per tablet.

The concentrations of the studied drugs in dosage forms were calculated using the reagents under study from the appropriate calibration graphs. The assay results are presented in Table (3).

As can be seen from the data in the Tables, the results obtained agreed with the label claim and those of the reference method [11]. The performance of the proposed method was judged further by the Student's t-test for accuracy and F-test for precision. At 95% confidence level, the calculated t- and F-values did not exceed the tabulated values (t = 2.57 and F = 5.05) suggesting that the method is accurate and precise as the reference method.

The accuracy and precision of the proposed method was determined by analyzing 6 replicate samples, each containing meloxicam at 16, 14 and 14 µg/ml for OG, MB and $CuCl_2$, respectively in the final assay solution, (table 2). At these concentrations, the relative standard deviation (RSD) values are 0.234, 0.198 and 0.254 %, the detection limits

are 2.60 , 0.75 and 1.64 µg/ml and the quantification limits are 7.86, 2.56 and 5.62 µg/ml for OG, MB and $CuCl_2$, respectively.

The performance of the proposed method was assessed by calculating t- and F-values and comparing them with those obtained by standard method [6]. Mean values were obtained by Student's t-(for accuracy) and F-(for precision) tests at 95 % confidence limits and 10 degrees of freedom (table 3); the results showed that the calculated t- and F-values did not exceed the theoretical values.

When the results obtained with the proposed method were compared with those obtained earlier [7-9], they showed a better sensitivity and higher accuracy for the non-extractive method, which required less time and had a lower range for microdetermination. The proposed method is highly precise and is simpler and less time consuming than various HPLC methods [10-15]. Moreover, the proposed method could be used for routine determination of meloxicam in pure form or in pharmaceutical formulations.

The interference of excipients and additives usually present in pharmaceutical formulation was investigated. Preliminary experiments showed that all additives, excipients, and degradation products did not form ion-associate complexes with the reagents studied. These results indicate the high selectivity of the proposed method and its applicability for routine determinations of meloxicam in pure and dosage forms.

Table 2: Accuracy and precision of the method

PARAMETER	OG	MB	$CuCl_2$
Taken (µg/ml)	10	10	12
Found* (µg/ml)	9.94	10.03	11.92
Recovery%	99.63	100.13	99.89
RSD%	0.234	0.198	0.254
Relative error %	-0.40	0.30	-0.667
Confidence limit (µg/ml)**	16.08 ± 0.007	14.04 ± 0.002	15.98 ± 0.04
Detection limit (µg/ml)	2.60	0.75	1.64
Quantification limit (µg/ml)	7.86	2.56	5.62
t** value	1.94	1.75	0.89
F**	1.83	3.93	2.77

* Values obtained for six determinations.

** 95% confidence limits and five degrees of freedom (theoretical values of t and F values are 2.57 and 5.05, respectively)

3.13. Analytical Applications

The pharmaceutical formulations Zantac tablets and Ranitidine tablets (150 mg of ranitidine hydrochloride per tablet) and Zantac ampoules (50 mg of RAN per ampoule; 2.0 ml) were analyzed by the proposed method, and the accuracy of the method was confirmed by comparison of the results with those obtained earier. The standard additions method was used, in which variable amounts of the pure drug were added to the previously analyzed portion of the pharmaceutical formulations. Results (table 3) confirm that the proposed method is not subject to interference by the tablet fillers, excipients, and additives usually used in ranitidine hydrochloride formulations (Aluminum Lake, hydroxypropyl cellulose, hydroxypropyl methylcellulose, magnesium stearate, microcrystalline cellulose, triethyl citrate, sodium starch glycolate, titanium dioxide and flavoring). The proposed method is highly sensitive; therefore, it could be used easily for routine determination of RNH in its pure form and in its pharmaceutical formulations.

Table 3: Determination of meloxicam in dosage forms applying standard addition technique using OG, MB and $CuCl_2$ as chromophoric reagents

Dosage forms	Taken (µg/ml)	MB			OG			$CuCl_2$		
		Added µg/ml	Found* µg/ml	Recovery (%)	Added µg/m:	Found* µg/ml	Recovery (%)	Added µg/ml	Found* µg/ml	Recovery (%)
Milocam	10	0.0	9.98	99.80	0.0	10.02	100.2	0.0	9.89	98.90
		2.0	12.01	100.08	2.0	12.01	100.08	2.0	12.01	100.08
		3.0	12.97	99.77	3.0	12.94	99.54	3.0	13.02	100.15
		5.0	14.97	99.8	5.0	15.01	100.33	5.0	14.91	99.40
Neofloxin	10	0.0	10.02	100.2	0.0	10.02	100.20	0.0	9.89	98.90
		2.0	12.1	100.08	2.0	11.89	99.08	2.0	12.03	100.25
		3.0	12.96	99.69	3.0	12.93	99.46	3.0	12.94	99.54
		5.0	15.03	100.2	5.0	15.02	100.13	5.0	15.06	100.40
Mexicam	8	0,0	8.02	100.25	0.0	7.96	99.5	0,0	8.03	100.38
		2.0	10.02	100.2	2.0	10.03	100.3	2.0	9.95	99.50
		3.0	10.95	99.55	3.0	10.89	99.00	3.0	11.01	100.09
		4.0	11.96	99.67	4.0	12.03	100.25	4.0	11.93	99.42

Average of three determinations.

4. CONCLUSIONS

The proposed method is simple, less time consuming, and sensitive. It was advantageous over other reported visible spectrophotometric methods with respect to its higher sensitivity that permits the determination of 0.4, 2.4 and 1.73 µg/ml for OG, MB and $CUCl_2$ respectively, No interference from associated excipients, additives, and degradation products was observed. The proposed method can be used for routine analysis and quality control laboratories for the determination of meloxicam in raw materials and in pharmaceutical formulations, depending on the availability of the chemicals and the nature of other excipients present in the sample.

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