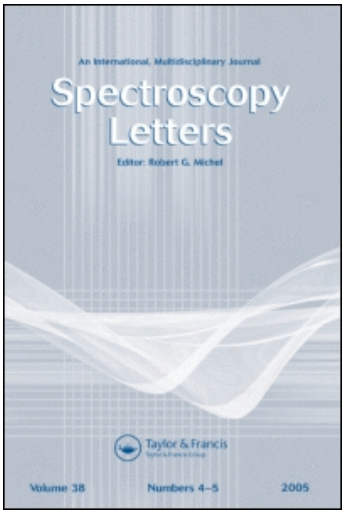

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Elham A. Taha[a]; Nahla N. Salama[a]; Laila S. Abdel Fattah[b]
[a] National Organization for Drug Control and Research, Cairo, Egypt [b] Department of Analytical Chemistry, Cairo University, Cairo, Egypt

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STABILITY-INDICATING METHODS FOR DETERMINATION OF MELOXICAM AND TENOXICAM IN THE PRESENCE OF THEIR DEGRADATION PRODUCTS

Elham A. Taha,[1,*] Nahla N. Salama,[1] and Laila S. Abdel Fattah[2]

[1]National Organization for Drug Control and Research, Cairo, Egypt
[2]Department of Analytical Chemistry, Faculty of Pharmacy, Cairo University, Cairo, Egypt

ABSTRACT

A spectrophotometric and a spectrofluorimetric methods are developed for the determination of two nonsteroidal anti-inflammatory drugs meloxicam **I** and tenoxicam **II** in the presence of their degradation products, namely 5-methyl-2-aminothiazole (**III**), benzothiazine carboxylic acid (**IV**), for meloxicam, pyridine-2-amine (**V**) and methyl 4-hydroxy-2-methyl-2H-thienol[2,3-e]1,2-thiazine-3-carboxylate-1,1-dioxide (**VI**) for tenoxicam, Fig. 1.

Both methods are based on the oxidative coupling reaction of these drugs with 3-methylbenzothiazolinone

*Corresponding author. E-mail: dr_elhamtaha@hotmail.com

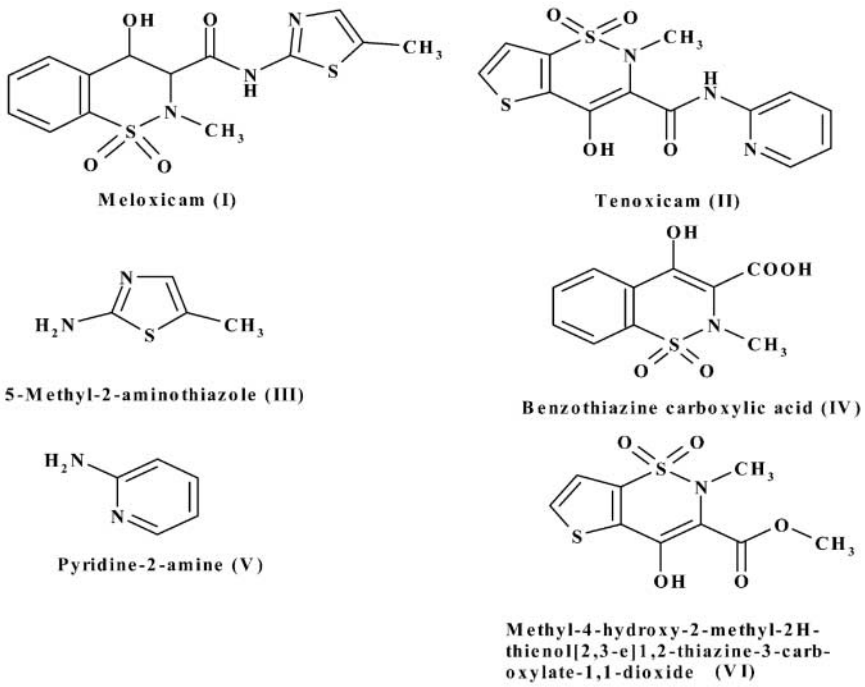


Figure 1. The structure of the studied drugs and their degradation products.

hydrazone (MBTH) hydrochloride in presence of ceric ammonium sulphate in an acid medium.

Spectrophotometrically, the resulting stable coloured products showed absorption λ_{max} at 450 nm and 465 nm for meloxicam and tenoxicam respectively. The method was applicable over the concentration range of (2–20 μg mL^{-1}) with mean recoveries 99.90 ± 0.85 and 100.27 ± 1.27 respectively. The method is proved to be stability indicating method, as no reaction was observed with the degradation products.

Job plots of absorbance versus mole fraction of drug indicate a 1:1 ratio for both drugs. The variables have been optimized.

On the same principle a fluorescence quenching method for the determination of meloxicam and tenoxicam in the presence of their degradation products was developed. The λ_{ex} was 300 nm while the fluorescence intensity was measured at λ_{em} 360 nm.

This method is applicable over the concentration ranges (1–10 μg mL^{-1}) for both drugs with mean accuracies 99.88 ± 0.49 and 99.90 ± 1.02 respectively.

The proposed methods were successfully applied for the determination of the studied drugs in bulk powder, laboratory prepared mixtures containing different percentages of degradation products and pharmaceutical dosage forms.

The results of the analysis were found to agree statistically with those obtained with either the reported or the official methods. Furthermore the methods were validated and also assessed by applying the standard addition technique.

Key Words: Spectrophotometry; Spectrofluorimetry quenching; Stability-indicating method; Determination of meloxicam and tenoxicam; Drug formulations

INTRODUCTION

Meloxicam I and tenoxicam II are new nonsteroidal anti-inflammatory drugs. Few methods have been published for their determination. For meloxicam, spectrophotometric[1] and HPLC[2,3] methods were reported. Only two stability-indicating methods were mentioned for its determination.[4] For tenoxicam spectrophotometric,[5,6] infra-red[7] HPLC,[8] polarographic,[9,10] methods were reported, but no stability indicating assay have been published.

3-methylbenzothiazolone hydrazone (MBTH) hydrochloride has been used as chromogenic reagent for qualitative and quantitative determination of aromatic amines and amino compounds.[11] MBTH was also used for the determination of cholesterol in serum,[12] tricyclic antidepressants[13] and benzodiazines.[14]

The aim of this work is to establish simple, rapid, sensitive and reliable methods for routine quality control analysis and which can be used as stability-indicating assay.

EXPERIMENTAL

Materials and Standard Solutions

- Meloxicam I was kindly supplied by Adwia (Egyptian Co. for chemicals & pharmaceuticals). The purity of the sample was found to be 99.94 ± 0.76% according to the official method.[15]

- Tenoxicam was kindly obtained from Epico Co. (Egypt), its purity was labeled to be $99.74 \pm 0.86\%$ according to official method.[16]
- Anti-cox II capsules (Adwia-Egypt), labeled to contain 7.5 mg meloxicam/capsule.
- Anti-cox II tablets (Adwia-Egypt), labeled to contain 15 mg meloxicam/tablets.
- Anti-cox II ampoules (Adwia-Egypt) labeled to contain 15 mg meloxicam/ampoule.
- Epicotil tablets (Epico Co., Egypt), labeled to contain 20 mg tenoxicam/tablet.
- Epicotil vial (Epico Co., Egypt), labeled to contain 20 mg tenoxicam/vial.
- Degradation products of meloxicam and tenoxicam:

The degradation products were laboratory prepared according to the method mentioned for meloxicam.[4] As for tenoxicam the same procedure was followed, but the time needed for alkaline hydrolysis was two hours only, and the R_f values were 0.38 for tenoxicam, 0.88 for degradation product, pyridine-2-amine[V], and zero for the other degradation product named methyl-4-hydroxy-2-methyl-2H-thienol [2,3-e]1,2-thiazine-3-carboxylate 1,1-dioxide[VI].

Standard Stock Solutions

- For meloxicam **I** and tenoxicam **II**.
- Standard solution (1) $200\,\mu g\,mL^{-1}$ in methanol.
- Standard solution (2) $100\,\mu g\,mL^{-1}$ in methanol.
- For degradation products of meloxicam and tenoxicam:

Stock solutions ($100\,\mu g\,mL^{-1}$) in methanol were prepared. The solution can be used within one week and stored at 4°C.

Reagents

All reagents used were of analytical grade.

- Methanol (Lab-Scan).
- Sodium hydroxide 1 N aqueous solution.
- Hydrochloric acid 1 N aqueous solution.
- Sulphuric acid 1 N aqueous solution.

- 3-Methyl-2-benzothiazoline hydrazone (MBTH) hydrochloride monohydrate, (Sigma Co.), 0.2 % aqueous solution. It should be freshly prepared.
- Ceric ammonium sulphate, (Prolabo Co.), 1% in 1 N sulphuric acid in umber coloured container.
- Ethyl acetate-methanol-concentrated ammonia (85:10:5 v/v) as developing system.

Apparatus

- SHIMADZU 1601, UV/VIS Spectrophotometer.
- SHIMADZU RF-1501 Spectrofluorimeter.
- UV short wavelamp (254 nm).
- TLC plates (20×20) with 0.25 mm thickness silica gel GE 254, (E. Merck).

General Procedures

I.a Spectrophotometric Method, I.a.i Construction of Calibration Curves, I.a.ii Preparation of Working Standards

Transfer accurately aliquot portions equivalent to 20–200 μg for both meloxicam and tenoxicam from its stock solution (1) into a series of 10 mL volumetric flask, complete to volume with methanol. Transfer 1 mL of each of working solution in 10 mL flask, add 3 mL of MBTH for meloxicam solution and 4 mL for tenoxicam solution, allow to stand for 5 min., then add 1.5 mL of ceric ammonium sulphate for meloxicam and 2 mL for tenoxicam allow to stand 20 min and 10 min for both drugs respectively, complete to volume with methanol. Measure the yellow coloured produced at 450 nm and 465 nm for meloxicam and tenoxicam against a reagent blank prepared similarly without the drug. The concentration of the two anti-inflammatory drugs in the analyte solution can be determined by reference to corresponding calibration graphs or from a regression equation Tables 1, 3.

I.a.iii Assay of Laboratory Prepared Mixtures, I.a.iv Preparation of Working Solution

Laboratory prepared mixtures were prepared in different ratios from the stock solution (2) and stock solution of their degradation products as shown in Table 2.

Table 1. Comparison Between the Proposed Methods and Official Methods for the Determination of Meloxicam and Tenoxicam

	Meloxicam			Tenoxicam		
	Spectrophotometric Method Recovery %	Spectrofluorimetric Method Recovery %	Official Method[15] Recovery %	Spectrophotometric Method Recovery %	Spectrofluorimetric Method Recovery %	Official Method[16] Recovery %
	100.50	100.20	99.00	99.80	100.20	100.20
	99.60	99.80	101.00	101.00	99.80	99.50
	99.80	99.60	100.30	100.40	101.00	99.00
	100.80	99.00	99.50	99.55	99.80	101.00
	100.50	100.50	99.90	99.12	100.80	99.00
Mean* (at P = 0.05)	100.24	99.82	99.94	99.97	100.32	99.74
S.D.	0.51	0.58	0.76	0.74	0.56	0.86
V	0.26	0.34	0.58	0.55	0.31	0.74
S.E.	0.23	0.26	0.34	0.33	0.25	0.38
t test	1.20 (2.306)**	0.72 (2.306)**		0.76 (2.306)**	0.98 (2.306)**	
F test	2.23 (6.39)***	1.71 (6.39)***		1.35 (6.39)***	2.39 (6.39)***	

* Mean of five experiments.
** Theoritical t value.
*** Theoritical F value.

Table 2. Comparison Between the Proposed Methods and the Reported Methods for the Determination of Meloxicam and Tenoxicam in the Presence of Their Degradation Products

		Meloxicam			Tenoxicam		
Exp. No.	Degradation Products %	Spectrophotometric Method Recovery %	Spectrofluorimetric Method Recovery %	Reported Method[18] Recovery %	Spectrophotometric Method Recovery %	Spectrofluorimetric Method Recovery %	Reported Method[19]
1	10	99.00	100.20	103.50	99.20	100.19	109.77
2	20	99.20	99.10	105.20	101.00	99.59	115.84
3	40	101.10	100.30	115.06	99.48	99.89	129.52
4	60	99.30	99.80	123.00	99.70	99.95	154.21
5	80	101.00	100.50	130.50	100.00	99.86	178.68
6	90	100.00	101.00	139.08	101.00	100.30	190.50
Mean		99.93	101.15		100.06	99.96	
C.V.		0.93	0.65		0.77	0.25	

Table 3. Validation Report on Spectrophotometric and Spectrofluorimetric Methods for the Determination of Meloxicam and Tenoxicam

	Meloxicam		Tenoxicam	
Parameters	Spectrophotometric Method	Spectrofluorimetric Method	Spectrophotometric Method	Spectrofluorimetric Method
Beer's law ($\mu g\ mL^{-1}$)	2–20	1–10	2–20	1–10
Molar absorptivity ($L\text{-}mol^{-1}\text{-}cm^{-1}$)	2.03×10^3		1.78×10^3	
Regression equation				
Slope (b)	0.0574	49.570	0.0539	40.693
Intercept (a)	−0.0010	1.0189	−0.0001	2.8138
Correlation coeff.	0.9997	0.9999	0.9998	0.9997
RSD	0.51	0.58	0.74	0.56
LOD ($\mu g\ mL^{-1}$)	1.17	0.12	1.67	0.18
LOQ ($\mu g\ mL^{-1}$)	3.86	0.40	1.86	0.62

Transfer accurately aliquot portions of laboratory prepared mixtures containing different ratios of meloxicam and tenoxicam and their degradation products and proceed as directed in (1.a.ii) starting from "Transfer 1 ml of each of working solution . . . ". Calculate the concentration of meloxicam and tenoxicam from the regression equation, Table 3.

II.a Fluorimetric Method, II.a.i Construction of Calibration Curves

Follow the procedure mentioned as under construction of calibration curve for spectrophotometric using stock solution (2) and 15, 30 minutes as time of reaction for meloxicam and tenoxicam. Measure the difference in the relative fluorescence intensities at 360 nm emission wavelength with excitation at 300 nm The concentration of the two drugs can be determined by reference to corresponding calibration graphs or from regression equation, Table 3.

II.a.ii Assay of Laboratory Prepared Mixtures

The resulted solutions for assay of drugs colorimetrically were measured fluorimetrically at λ_{ex} 300 and λ_{em} 360.

Calculate the concentrations of both drugs from regression equation. Results obtained are shown in Table 3.

III.a Procedure for the Assay of Dosage Forms

Tablets

Weigh accurately twenty tablets and grind to fine powder. Transfer a weight of powdered tablets equivalent to 10 mg for meloxicam and tenoxicam into 100 mL volumetric flask, stir with magnetic stirrer for 30 min, filter and complete to volume with methanol. Complete as under I.a.i for spectrophotometric method and as under II.a.i for spectrofluorimetric method Table 4.

Capsules

Evacuate the contents of twenty capsules, mix thoroughly. Transfer an accurate weight of the mixed sample equivalent to 10 mg of meloxicam into

Table 4. Comparison Between the Proposed Methods* and the Reported Methods** for the Determination of Meloxicam and Tenoxicam in Pharmaceutical Preparations

	Spectrophotometric Method	Spectrofluorimetric Method	Reported Method
Preparations	Recovery % ± C.V.	Recovery % ± C.V.	Recovery % ± C.V.
Meloxicam			
Anticox II Capsules 7.5 mg	100.53 ± 0.78	99.88 ± 0.61	99.40 ± 0.71
Anticox II Tables 15 mg	100.38 ± 0.94	100.20 ± 0.59	99.82 ± 1.17
Anticox II Ampoules 15 mg	99.66 ± 0.68	100.62 ± 0.77	101.10 ± 0.38
Tenoxicam			
Epicotil tablets 20 mg	99.42 ± 0.54	100.35 ± 0.85	99.96 ± 0.83
Epicotil vial. 20 mg	101.11 ± 1.21	99.01 ± 0.94	97.40 ± 0.46

* Mean of five determinations.
** Mean of five determinations.

a 100 mL volumetric flask, stir with magnetic stirrer for 30 min, filter and complete to volume with methanol. Complete as directed in the two above methods, Table 4.

Vials

Mix the contents of six vials, transfer an accurately weighed amounts equivalent to 10 mg of tenoxicam into 100 mL volumetric flask, sonicate for 15 min, filter and complete to volume with methanol. Then complete as done in construction of calibration curves in I.a.i and II.a.i, Table 4.

Ampoules

Mix the contents of six ampoules, measure milliliters equivalent to 10 mg meloxicam in 100 mL volumetric flask, complete to volume with methanol, then complete as done in construction of calibration curves in I.a.i, II.a.i, Table 4.

RESULTS AND DISCUSSION

Spectrophotometric Method

The structure of meloxicam and tenoxicam are shown in Fig. 1.

The spectra of the reaction products for the two drugs with MBTH, show characteristic λ_{max} at 450 nm and 465 nm for meloxicam and tenoxicam Figs. 2, 3.

Also from these figures it is clear that, no reaction was observed between the alkaline hydrolytic products of the cited drugs and MBTH, blank reagent has no absorbance at the λ_{max} of the products. So this method was used for the stability studies of these drugs Figs. 2, 3.

The mechanism of the reaction is suggested to be an oxidative coupling reaction. Sch. 1 shows the proposed mechanism of reaction.

Under the reaction condition MBTH loses two electrons and one proton on oxidation forming the electrophilic intermediate which has been postulated to be the active coupling species, the intermediate reacts with

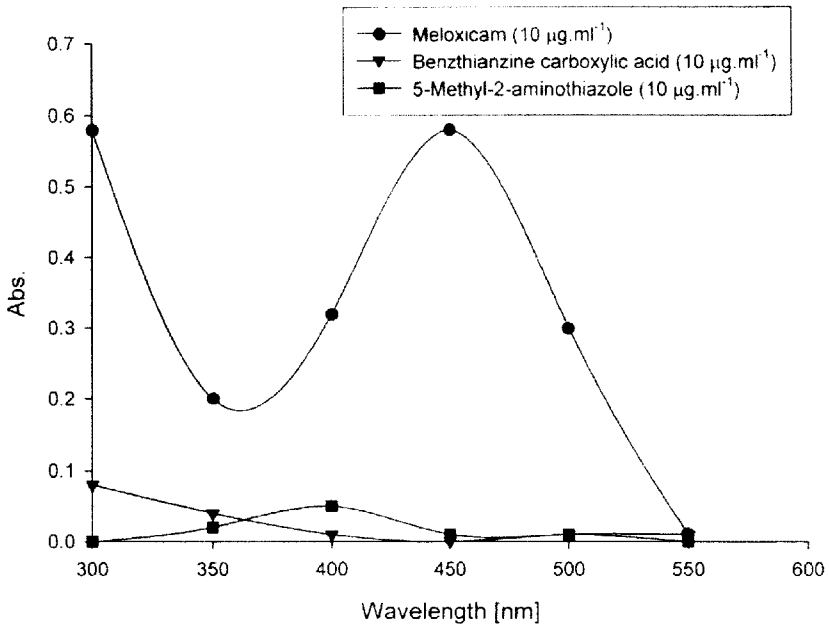


Figure 2. Absorption spectra of oxidative coupling products of meloxicam and its degradation products with MBTH reagent.

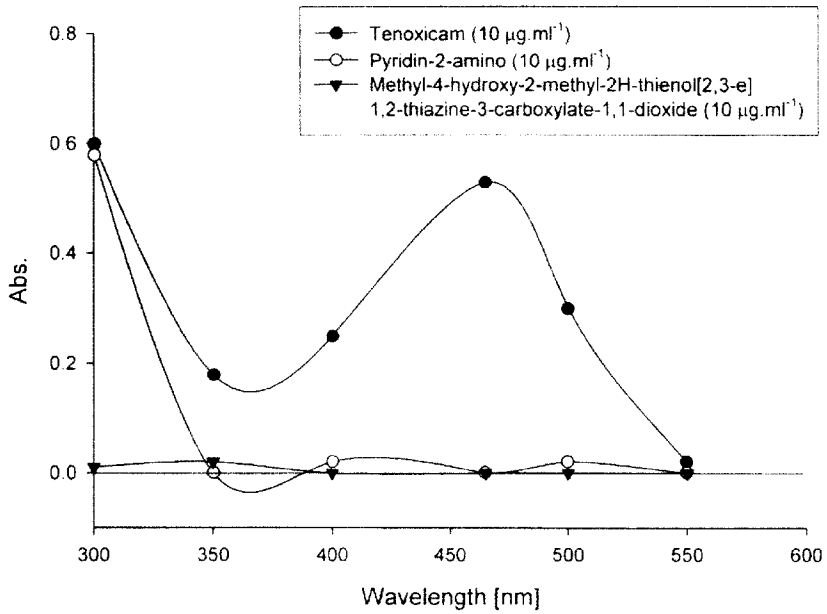


Figure 3. Absorption spectra of oxidative coupling products of tenoxicam and its degradation products with MBTH reagent.

meloxicam and tenoxicam by electrophilic attack on the most nucleophilic site on the aromatic ring.

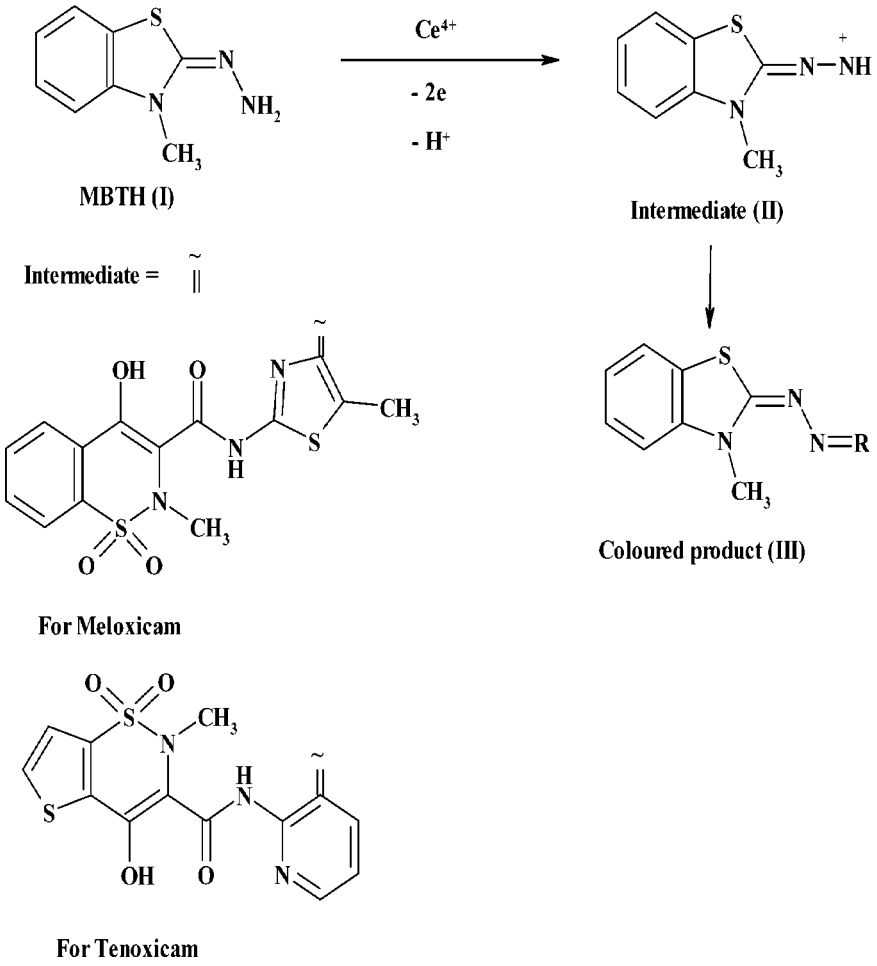
All factors affecting the production of the reaction products namely, reagent concentration, order of addition of reagents, solvent used and reaction time were thoroughly studied. The optimum conditions were incorporated into the general procedure. Beer's law was obeyed over concentration range 2–20 μg mL^{-1} for both drugs.

Of the different oxidizing reagents ceric ammonium sulphate was chosen due to production of cerrous, which can permit the assay of drug fluorimetrically.

Job plots of absorbance versus mole fraction of drug indicate a 1:1 ratio for both drug Fig. 6.

Fluorimetric Method

It was observed that the reagent blank itself has a characteristic fluorescence. Since the λ_{ex} and λ_{em} of Ce^{+3} were mentioned to be 300 nm,



Scheme 1. The suggested mechanism for the reaction of meloxicam & tenoxicam with 3-methyl benzothiazolinone hydrazone (MBTH).

360 nm respectively.[17] It is most probably that the obtained fluorescence in the reaction is due to Ce^{+3} resulted from reduction of Ce^{+4} by MBTH.

Upon addition of the cited drugs quenching of Ce^{+3} fluorescence was observed, this may be due to complexation reaction with coloured coupling products. Moreover addition of degradation products has no significant effect on fluorescence intensity, Figs. 4, 5. Thus, fluorescence quenching assay was developed.

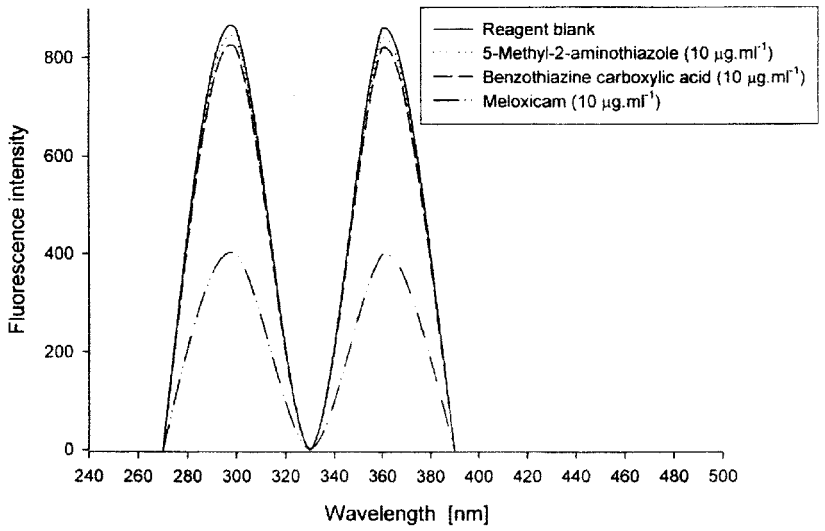


Figure 4. Uncorrected excitation and emission spectra of oxidative coupling products of meloxicam and its degradation products with MBTH reagent.

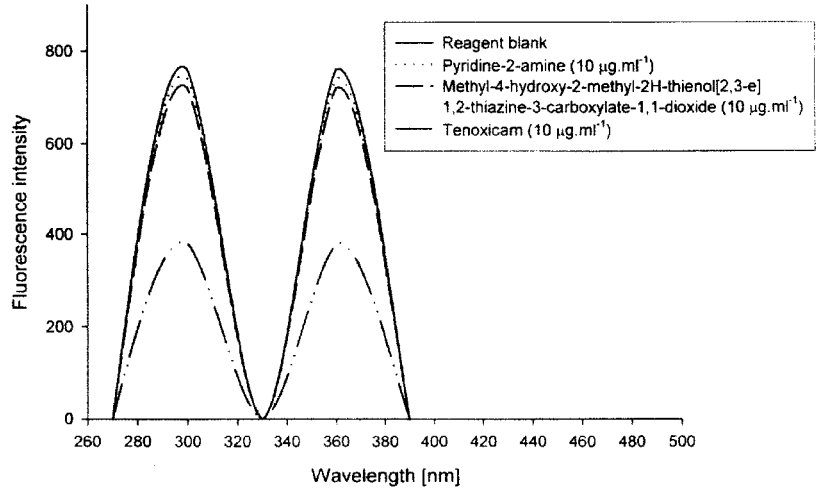


Figure 5. Uncorrected excitation and emission spectra of oxidative coupling products of tenoxicam and its degradation products with MBTH reagent.

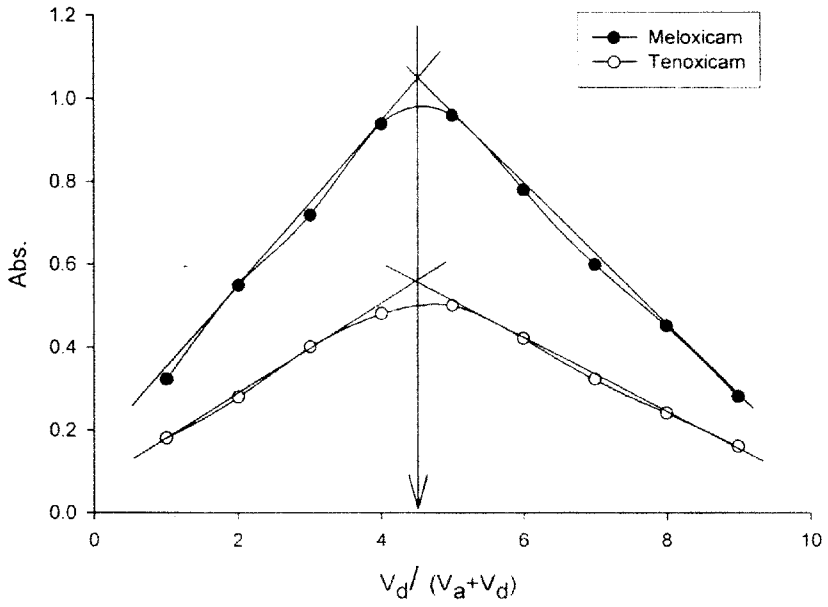


Figure 6. Determination of the stoichiometry of the reaction of meloxicam and tenoxicam with MBTH reagent by continueous variation method using [2.5×10^{-3} M] solution.

After studying of the reaction conditions, it was found that those conditions for spectrophotometric methods were optimum for spectrofluorimetric one. The decrease in fluorescence intensity was found to be quantitative in concentration range 1–10 $\mu g\,mL^{-1}$ for both drugs.

Table 1 shows a comparison between the proposed methods and official methods for the determination of meloxicam and tenoxicam from which the calculated t and F are less than that of the corresponding theoretical values indicating that there is no significant difference between the two methods with respect to both precision and accuracy.

Table 2 shows the results of determination of six synthetic mixtures of the intact drugs and their degradation products in different proportions.

These data indicated that the methods are accurate, sensitive, selective and stability indicating methods, as the presence of the degradation products did not interfere up to 90% for the two methods.

Validation of the proposed methods was assessed by applying the standard addition technique as well as in the presence of degradation products and the statistical analysis of the data are presented in Tables 3, 4.

High values of correlation coefficients and small values of intercepts validated the linearity of the calibration graphs and the obedience to Beer's law.

The RSD values and the slope and intercepts of the calibration graphs indicated the high reproducibility of the proposed methods.

Also, the proposed methods give accurate and reproducible results when applied for the determination of meloxicam and tenoxicam in pharmaceutical formulations. Moreover, these methods are simple and inexpensive that permits its applications in quality control laboratories and in biological fluids.

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