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Spectrophotometric and fluorimetric methods for the determination of meloxicam in dosage forms

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Abstract

Four simple and accurate methods are presented for the determination of meloxicam in dosage forms. These methods are based on: the direct measurements of the differential spectra at 339.9–384.7 nm (A), the ^{1}D-values at 322–368 nm and ^{2}D-values at 343.2–385.6 nm (B), the formation of an ion-association complex between the drug and safranin T with subsequent absorption measurement at 518 nm (C) and fluorescence measurement at 582 nm (D). All variables were studied to optimize the formation of the ion-association complex. Beer's law was valid over the concentration range 2–10 μg ml^{-1} (method A), 1–10 μg ml^{-1} (method B), 4.0–12 μg ml^{-1} (method C) and 0.4–1.2 μg ml^{-1} (method D). The detection limits were 0.11, 0.07, 0.10, 0.33 and 8.74×10^{-3} μg ml^{-1} for methods A, B, C and D, respectively. The proposed methods were successfully applied to the assay of meloxicam in tablets and suppositories. The procedures were rapid, simple and suitable for quality control applications.

Keywords: Meloxicam; Dosage forms; Spectrophotometry; Fluorimetry

1. Introduction

Meloxicam, chemically: 4-hydroxy-2-methyl-*N*-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide, is a newer NSAID in the group of enolic acids found to preferentially inhibit cyclo-oxygenase-2 (COX-2). Meloxicam is used in the treatment of rheumatoid arthritis, oesteoarthritis and other joint diseases [1].

The literature revealed that meloxicam has been determined by means of few analytical procedures, almost exclusively based on high performance liquid chromatography (HPLC) that permitted the assay of meloxicam in pharmaceutical preparations or in biological samples [2–5]. Only one paper has been published for the colorimetric determination of meloxicam in tablets [6]. Stability-indicating methods have been reported for determination of meloxicam in presence of its degradation product; by first-derivative spectrophotometry and by TLC on silica gel GE 254 with densitometry at 365 nm [7]. No fluorimetric

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methods have been published so far for the determination of meloxicam. As a result, it was considered very useful to develop selective spectrophotometric and fluorimetric methods for determination of meloxicam. These methods would serve as versatile tools suitable for the analysis of meloxicam and would be of interest for quality control.

In the present work, four simple, sensitive and accurate methods have been developed for the determination of meloxicam in pharmaceutical preparations. Method A depends on measuring the differential spectra of meloxicam. Method B is based on recording the first- and second-derivative spectra of meloxicam in 0.1 M ethanolic hydrochloric acid. Method C describes the spectrophotometric measurement of the ion-association complex, formed between meloxicam and safranin T, which is extractable into chloroform and absorbs maximally at 518 nm. Method D measures the fluorescence intensity of the ion-pair at 582 nm with excitation wavelength at 520 nm.

2. Experimental

2.1. Apparatus

The spectrophotometric determinations were performed using a Perkin-Elmer, Lambda EZ 201 (Version 1.0) UV/VIS spectrophotometer with matched 10 mm quartz cells, connected to a Panasonic Quiet KX-P 3626 printer. The spectral band width was 2 nm and the wavelength scanning speed was 200 nm min^{-1}.

The fluorimetric measurements were performed on a Perkin-Elmer Model 650-10S spectrofluorimeter equipped with 10 mm quartz cuvette, a 150-W Xenon lamp, excitation and emission grating monochromator and a Perkin-Elmer Model 56 recorder.

2.2. Materials and reagents

All experiments were performed with analytical reagent grade chemicals.

Authentic sample of meloxicam was kindly donated by Boehringer Ingelheim, Germany and was used as received. Mobic® tablets, containing 15 mg meloxicam, Mobic® suppositories, containing 15 mg meloxicam were obtained from the local market.

Safranin T (BDH) solution, 0.1% w/v, was prepared in distilled water.

Kolthoff's borax-phosphate buffer [8] was prepared by mixing different volumes of 0.05 M borax with 0.1 M potassium dihydrogen phosphate. The pH of the solutions was adjusted by adding more of the 0.05 M borax or the 0.1 M KH_2PO_4 solution as required.

2.3. Preparation of standard solution

A stock standard solution was prepared by dissolving meloxicam in ethanol to obtain 200 μg ml^{-1}. The solution was stable at 4 °C for at least 1 week.

2.4. Construction of calibration graphs

2.4.1. Method A

Accurate volumes of the standard solution, within the concentration range stated in Table 1, were transferred into two sets of 25 ml volumetric flasks, diluted to a constant volume with ethanol and then the first set was diluted to volume with ethanolic 0.1 M hydrochloric acid and the second set was diluted to volume with ethanolic 0.1 M sodium hydroxide. The differential spectra were scanned in the range 450–250 nm, using solution in 0.1 M sodium hydroxide as blank. The observed values of the ΔA amplitudes at 339.9–384.7 nm were plotted against the corresponding concentrations to obtain the calibration graphs.

2.4.2. Method B

Accurate volumes of the standard solution, within the concentration range stated in Table 1, were transferred into 25 ml volumetric flasks, diluted to a constant volume with ethanol and the volume was completed with 0.1 M ethanolic hydrochloric acid. The ^1D and ^2D spectra were scanned in the range 450–250 nm against a similarly prepared blank. The observed values of the ^1D amplitudes at 322–368 nm and the ^2D amplitudes at 343.2–385.6 nm were plotted against the

corresponding concentrations to obtain the calibration graphs.

2.4.3. Method C

Accurate volumes of the standard solution, within the concentration range stated in Table 1, were transferred into 60 ml separatory funnels. Five ml buffer solution pH 8.0 and 3 ml safranin T were added. The contents of each separator were mixed and then extracted with 10, 5 and 5 ml portions of chloroform. The chloroformic extracts were dried with anhydrous sodium sulphate, filtered through dry filter paper into 25 ml volumetric flasks and diluted to volume with chloroform. The absorbance of each solution was measured at 518 nm against a blank solution similarly prepared but omitting meloxicam. The absorbance values were plotted against the corresponding concentrations to obtain the calibration graphs.

2.4.4. Method D

One milliliter portion of each of the above solutions (prepared for method C), were transferred into 10 ml volumetric flasks and diluted to volume with chloroform. The fluorescence intensities were measured at 582 nm emission wavelength with excitation at 520 nm against chloroform as a blank. The fluorescence intensities were plotted against the corresponding concentrations to obtain the calibration graphs.

2.5. Analysis of pharmaceutical preparations

2.5.1. Assay of tablets

Twenty tablets were weighed and finely powdered. A portion of the mixed powder equivalent to 20 mg meloxicam was accurately weighed, transferred into 100 ml volumetric flask and dispersed in 50 ml ethanol. The flask was shaken for 5 min and the tablet extract was diluted to volume with ethanol and filtered. Aliquots of the filterate were subjected to the procedure previously described, for each method, under construction of calibration graphs.

2.5.2. Assay of suppositories

Five suppositories were weighed, melted by stirring on a water bath to homogenize and then cooled in an ice bath. A portion of the homogenized suppositories equivalent to 20 mg meloxicam was transferred into a beaker, melted on a water bath, dissolved in ethanol, cooled in an ice bath, filtered into 100 ml volumetric flask and diluted to volume with ethanol. Aliquots of the filterate were subjected to the procedure previ-

Table 1
Optical characteristics and statistical data of the regression equations for the determination of meloxicam

Parameter	ΔA	Derivative		Ion-pair	
		^{1}D	^{2}D	Spectrophotometry	Fluorimetry
Concentration range (μg ml^{-1})	2.0–10.0	1.0–10.0	1.0–10.0	4.0–12.0	0.4–1.2
λ (nm)	333.9–384.7	322–368	343.2–385.6	518	–
$\lambda_{excitation}$ (nm)	–	–	–	–	520
$\lambda_{emission}$ (nm)	–	–	–	–	582
Regression equation					
Intercept (a)	−0.014	0.016	-5×10^{-3}	−0.024	0.10
Slope (b)	0.478	1.696	1.23	0.074	31.4
Correlation coefficient, (r)	0.9999	0.9999	0.9999	0.9996	0.9996
Variance (S_o^2)	1.11	0.07	0.43	2.82	0.82
Detection limit (μg ml^{-1})	0.11	0.07	0.10	0.33	8.74×10^{-3}
S.D. of intercept (S_a)	1.7×10^{-2}	4.26×10^{-2}	4.06×10^{-2}	7.85×10^{-3}	0.091
S.D. of slope (S_b)	2.85×10^{-3}	1.0×10^{-2}	9.49×10^{-3}	1.18×10^{-3}	0.52
S.D. of residuals ($S_{y/x}$)	1.8×10^{-2}	3.16×10^{-2}	3.0×10^{-2}	7.18×10^{-3}	0.316
ε (l mol^{-1} cm^{-1})	–	–	–	2.5×10^4	–

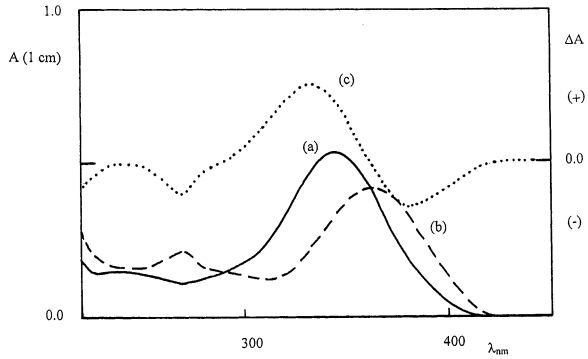

Fig. 1. Zero-order spectra for meloxicam 8.0 μg ml^{-1} in 0.1 M ethanolic HCl (a) in 0.1 M ethanolic sodium hydroxide (b) and their differential curves (c).

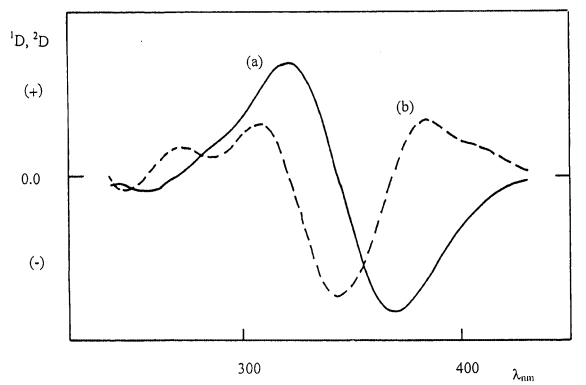

Fig. 2. First- and second-derivative spectra of meloxicam 4.0 μg ml^{-1} in 0.1 M ethanolic HCl (a) first-derivative and (b) second-derivative.

ously described, for each method, under construction of calibration graphs.

3. Results and discussion

3.1. Method A

Fig. 1 represents the zero-order spectra of meloxicam in acid and alkaline solutions (Fig. 1a and b) as well as their differential spectra (Fig. 1c). Being phenolic, meloxicam possesses a bathochromic shift with hypochromic effect when the ethanolic solution is made alkaline. Such a chemical shift may be used to cancel the effect of pH insensitive irrelevant absorption and therefore to improve the accuracy of the results obtained by the conventional spectrophotometric method [2]. The differential spectrum shows a maximum at 339.9 nm and a minimum at 384.7 nm (Fig. 1c). The peak-trough amplitudes at 339.9–384.7 nm were adopted for ΔA measurements.

3.2. Method B

Derivative spectrophotometry has been utilized successfully to overcome the problem of interference due to irrelevant spectral overlapping, which may be caused either by substances other than analytes or by excipient matrices commonly present in pharmaceutical formulations [9–11]. Besides, this technique offers a powerful enhancement of sensitivity and rapid determination without the requirement of extraction or separation. The ^{1}D- and ^{2}D-spectra of meloxicam in 0.1 M HCl are shown in Fig. 2. The ^{1}D curve displayed a maximum at 322 nm and a minimum at 368 nm (Fig. 2a), while the ^{2}D curve showed two maxima at 311.8 and 385.6 nm and a minimum at 343.2 nm (Fig. 2b). For quantitative analysis, the ^{1}D values at 322–368 nm and the ^{2}D values at 343.2–385.6 nm were chosen for the development of simple and specific procedure for analysis of meloxicam in dosage forms.

3.3. Method C

Due to its acidic properties, meloxicam reacts with safranin T, a basic dye, to give a highly colored ion-association complex which is easily extractable with chloroform and absorbs maximally at 518 nm (Fig. 3).

3.3.1. Optimization of conditions

The efficiency of extraction of the ion-pair with chloroform depends on the pH of solution. In order to establish the optimum pH range for the method, the drug was allowed to react with safranin T in pH range 6.5–9.0 and the complex formed was extracted with chloroform. Constant absorbance values were obtained over the pH range 7.8–8.4. At higher pH values, lower absorbance values were obtained with higher blank

readings. Hence a pH of 8.0 was chosen for subsequent studies. A volume of 5.0 ml of pH 8.0 Kolthoff's borax-phosphate buffer solution was required for maintaining the pH of aqueous phase at 8.0. The efficiency of extraction was also affected by the dye concentration. A 3 ml portion of safranin T solution, 0.1% w/v, was found to be optimal.

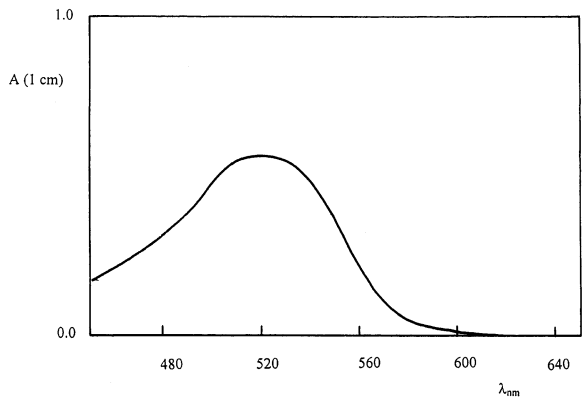

Fig. 3. Absorption spectrum of the ion-pair formed by reaction of 8.0 μg ml^{-1} meloxicam with safranin T.

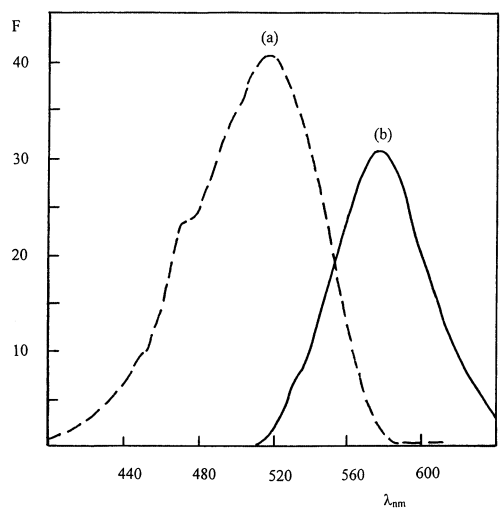

Fig. 4. Excitation (a) and emission (b) spectra of the ion-pair formed by reaction of meloxicam with safranin T, meloxicam final concentration 0.8 μg ml^{-1}.

3.3.2. Stoichiometric relationship

The reaction stoichiometry was determined by the method of continuous variation [12] and was found to be 1:1. This was anticipated by the presence of one acidic center, SO_2–N<, in meloxicam molecule.

3.4. Method D

Due to the fluorescent nature of the ion-pair, formed between meloxicam and safranin T, a fluorimetric method was adopted for the determination of meloxicam in dosage forms. The ion-pair exhibits maximum fluorescence intensity at 582 nm with excitation wavelength at 520 nm (Fig. 4).

3.4.1. Validation of the methods

Using the above mentioned spectrophotometric and fluorimetric procedures, linear regression equations were obtained over the concentration ranges stated in Table 1. The statistical parameters, regression equations, calculated from calibration graphs along with the S.D.s of the slope (S_b) and the intercept (S_a) on the ordinate and the S.D. of residuals ($S_{y/x}$) are given in Table 1. The good linearity of the calibration graphs is clearly evident from the values of the variances around the slopes (Table 1). The detection limits [13], varied from 8.74×10^{-3} to 0.33 μg ml^{-1}. In order to evaluate the precision of the proposed methods, solutions containing three different concentrations of meloxicam were prepared and analyzed in five replicates. The analytical results obtained from this investigation are summarized in Table 2. The mean standard deviation (R.S.D.%) and standard analytical error (SAE) are also shown in the same table and their low values indicate the precision and accuracy of the proposed methods.

The influence of commonly used tablet excipients (lactose, starch, magnesium stearate) and suppository base (suppocire) was investigated before the determination of the drug in dosage forms. No interference could be observed with the proposed methods.

Table 2
Evaluation of the precision of the proposed methods

Method	Added[a]	Found ± S.D.[b]	R.S.D.%[c]	SAE[d]	Confidence limit[e]
ΔA	4.0	4.012 ± 0.035	0.872	0.016	4.012 ± 0.044
	8.0	8.000 ± 0.055	0.687	0.025	8.000 ± 0.069
	10.0	9.991 ± 0.037	0.370	0.017	9.991 ± 0.047
^{1}D	2.0	1.989 ± 0.015	0.754	0.007	1.989 ± 0.019
	3.0	3.039 ± 0.040	1.316	0.018	3.039 ± 0.050
	5.0	4.919 ± 0.053	1.08	0.024	4.919 ± 0.066
^{2}D	2.0	1.984 ± 0.012	0.605	0.005	1.984 ± 0.014
	3.0	2.997 ± 0.011	0.370	0.005	2.997 ± 0.014
	5.0	5.016 ± 0.030	0.598	0.013	5.016 ± 0.036
Spectrophotometry	6.4	6.500 ± 0.017	0.262	0.008	6.500 ± 0.022
	9.6	9.622 ± 0.023	0.240	0.010	9.622 ± 0.028
	12.0	11.884 ± 0.054	0.454	0.024	11.884 ± 0.066
Fluorimetry	0.64	0.641 ± 0.007	1.09	0.003	0.641 ± 0.008
	0.96	0.966 ± 0.006	0.621	0.003	0.966 ± 0.008
	1.20	1.193 ± 0.006	0.503	0.003	1.193 ± 0.008

[a] Final concentration in μg ml^{-1}.
[b] Mean ± S.D. for five determinations.
[c] R.S.D.%, relative standard deviation.
[d] SAE, standard analytical error.
[e] Confidence limits at $P = 0.05$ and four degrees of freedom.

Table 3
Assay results of meloxicam in pharmaceutical preparations using the proposed methods

	Method				
	ΔA	Derivative		Ion-pair	
		^{1}D	^{2}D	Spectrophotometry	Fluorimetry
Tablets					
Mean[a]	99.66	99.47	99.53	99.14	99.24
±S.D.	0.50	0.50	0.57	0.58	0.69
t-	1.10	0.60	0.72	0.25	
F-	1.90	1.90	1.46	1.41	
Suppositories					
Mean[a]	99.04	99.24	99.03	99.53	99.76
±S.D.	0.69	0.72	0.65	0.54	0.60
t-	1.76	1.24	1.84	0.64	
F-	1.32	1.44	1.17	1.23	

[a] Mean of five determinations.
Theoretical values of *t*- and *F*- at $P = 0.05$ are 2.31 and 6.39, respectively.

3.4.2. Analysis of commercial dosage forms

The applicability of the proposed methods was tested by the determination of meloxicam in commercial tablets and suppositories. The determinations were carried out on the same batch of samples. The results obtained, presented in Table 3, were compared statistically by the Student's *t*-test and Variance ratio *F*-test. The calculated *t*-

and F- values (Table 3) did not exceed the theoretical values, indicating no significant difference between the methods compared.

4. Conclusion

The proposed spectrophotometric and fluorimetric methods are suitable for the analysis of meloxicam in commercial dosage forms. The derivative spectrophotometric methods are simple, selective and rapid. Such features render it suitable for routine analysis in quality control laboratories. Although the fluorimetric method requires an extraction procedure, it possesses the advantage of high sensitivity, which may be an incentive to other workers to apply to the biological fluids.

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References

[1] E.F. Reynolds, Martindale The Extra Pharmacopoeia, 32nd ed., The Royal Pharmaceutical Society, London, 1999, p. 52.
[2] J. Joseph-Charles, M. Bertucat, Anal. Lett. 32 (10) (1999) 2051–2059.
[3] T. Velpandian, J. Jaiswal, R.K. Bhardwaj, S.K. Gupta, J. Chromatogr. B, Biomed. Appl. 738 (2) (2000) 431–436.
[4] J. Joseph-Charles, M. Bertucat, J. Liq. Chromatogr. Relat. Technol. 22 (13) (1999) 2009–2021.
[5] R.T. Sane, V. Surve, M. Francis, Indian Drugs 37 (5) (2000) 251–254.
[6] R.T. Sane, V. Surve, M. Francis, Indian Drugs 37 (8) (2000) 390–393.
[7] L.I. Bebawy, Spectrosc. Lett. 31 (4) (1998) 797–820.
[8] K. Diem (Ed.), Documenta Geigy-Scientific Tables, Sixth ed., 1969, p. 314.
[9] R.J. Forsyth, D.P. IP, J. Pharm. Biomed. Anal. 12 (1994) 1243–1248.
[10] B. Morelli, J. Pharm. Biomed. Anal. 13 (1995) 219–227.
[11] J.A. Murillo, J.M. Lemus, L.F. Garcia, J. Pharm. Biomed. Anal. 14 (1996) 257–266.
[12] D.T. Sawyer, W.R. Heineman, J.M. Beebe, Chemistry Experiments For Instrumental Methods, Wiley, New York, 1984, pp. 198–200.
[13] Nomenclature, symbols, units and their usage in spectrochemical analysis, II, Spectrochim. Acta, Part B, 33 (1978), 242–247.