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TLC DETERMINATION OF MELOXICAM IN TABLETS AND AFTER ACIDIC AND ALKALINE HYDROLYSIS

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Abstract: A simple and rapid method for separation and determination of meloxicam and its degradation products by thin-layer chromatography with densitometric detection in pharmaceutical preparations was described. The method employed TLC F_{254} plates as the stationary phase. The solvent system consisted of ethyl acetate : toluene : butylamine (2:2:1, v/v/v). Densitometric analysis was carried out in absorbance mode at wavelength of 297 nm. The method was validated for linearity, precision and accuracy. The limits of detection and determination were 0.96 μg per spot and 2.90 μg per spot, respectively. The drug was degraded in acidic and basic environment, at different temperatures. The degradation products were well resolved from the active substance. The HPLC-MS/MS method for the identification of degradation products of meloxicam (i.e. 5-methylthiazol-2-ylamine and 5-(dioxide-l^6-sulfanylidene)-6-methylidenecyclohexa-1,3-diene) was investigated. Because the presented method allows the efficient separation of the drug from some of its degradation products, so it can be used as a stability-indicating analysis.

Keywords: meloxicam, thin-layer chromatography, validation, drug analysis

Meloxicam (chemical name: 4-hydroxy-2-methyl-N-(5-methyl-2-thiazolyl)-2H-1,2-benzothiazine-3-carboxamide-1,1-dioxide) is a selective cyclooxygenase (COX-2) inhibitor, which is successfully used to treat many rheumatic disorders including rheumatoid arthritis and osteoarthritis. The drug has a high gastric and renal tolerance and high therapeutic index, both experimentally and clinically comparable to conventional non-steroidal anti-inflammatory drugs (NSAIDs) (1).

Pharmacopoeia recommends potentiometric titration in anhydrous environment for the assay of meloxicam and liquid chromatography for the determination of related substances (2). There are many different methods reported for the determination of meloxicam in pharmaceuticals and biological samples. They include colorimetric (3), spectrophotometric and spectrofluorimetric (4–6), voltammetric (7, 8), flow-injection spectrophotometric (9, 10) and capillary electrophoresis (11, 12) methods. Meloxicam has been also been determined employing HPLC with UV detection (13–18) and LC-mass spectroscopy (19, 20). Taha et al. reported two methods for the determination of lornoxicam, tenoxicam and meloxicam in the presence of their alkaline degradation products (13). They used a TLC technique for the determination of meloxicam beside its major alkaline degradation products using: chloroform : n-hexane : glacial acetic acid as a mobile phase, and results were compared with those obtained by LC method. Bebawy described two methods for the determination of meloxicam and tetracaine hydrochloride in the presence of their degradation products: spectrophotometry at 338 nm and TLC densitometry at 365 nm (21). Some of oxicams have also been analyzed by videodensitometric TLC method with toluene : acetic acid : methanol as a mobile phase and identified at 254 nm (22). An HPTLC method for analysis of meloxicam from tablets in the presence of commonly used excipients was presented (23). The degradation of meloxicam solution was carried out using 1 mol/L HCl and 1 mol/L NaOH. Only in an alkaline degradation one product was found.

Thin layer chromatography is a method successfully used, both in the analysis of pharmaceuticals and biological material. TLC is a very useful technique because of the relatively low cost of analysis and need for minimum sample purification. TLC offers the advantages of automatic application,

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where the conditions can be more easily controlled. The main advantage of TLC is a reduction of analysis time and costs, by allowing the implementation of several indications simultaneously using a small quantity of solvents, unlike HPLC, for which substantial amounts of mobile phase and long analysis times are required for quantification of multiple samples.

The aim of this work, after reviewing the available literature, was to develop a fast, simple and sensitive TLC procedure for separation and identification of meloxicam in pharmaceutical formulations. Developed and validated method was also used in studies of the kinetics of decomposition of meloxicam in different environments (acid and alkaline conditions), at different temperatures. This paper also describes a precise, repeatable and stability-indicating method, that can be applied for the determination of meloxicam beside their possible degradation products and the purity and stability studies of drugs.

EXPERIMENTAL

Reagents and chemicals

Standard substance of meloxicam was purchased from Sigma-Aldrich (UK). Reagents: methanol, ethyl acetate, toluene, butylamine were purchased from POCH Gliwice (Poland). The following medicines were analyzed: Mobic (Boehringer Ingelheim, Allemagne, France) s.902471 tablets containing 7.5 mg of meloxicam, Mobic (Boehringer Ingelheim, Allemagne, France) s.103123 tablets containing 15 mg of meloxicam, Mobic (Boehringer Ingelheim, Allemagne, France) s.002007 tablets containing 7.5 mg of meloxicam, Meloksam (Polfa Sp. z o.o., Grodzisk Mazowiecki, Poland) s.0010605 tablets containing 7.5 mg of meloxicam.

Apparatus

All designed experiments were carried out with a densitometer TLC Scanner 3 with Cats 4 software (Camag, Switzerland). Solutions were applied by sample applicator Linomat V (Camag, Switzerland). Silica gel aluminium TLC F_{254} plates, art. No. 1.05554 were obtained from E. Merck (Germany). Chromatograms were developed in a TLC chamber of 18×9×18 cm in size (Sigma-Aldrich). HPLC-MS-MS system was used: the triple quadrupole mass analyzer (MS API 2000; Applied Biosystems MDX SCIEX, Concorde, Ontario, Canada) connected with a HPLC system (with Xterra Waters column and DAD detector).

Solutions for analysis

Standard solution at a concentration of 0.1 mg/mL for meloxicam was prepared by dissolving amount of substance in methanol. Sample solutions: ten tablets of preparation were powdered and weighed, corresponding to approximately 10 mg of meloxicam, to an accuracy of 0.1 mg. Methanol was added and the mixture was shaken for 15 min, filtered, filled up to the volume of 100.0 mL and used for analysis.

Chromatographic conditions

The conditions for analysis of meloxicam were established by experimental selection of the appropriate stationary and mobile phases. Experiments were performed on the TLC silica gel aluminium plates 60 F_{254}. Samples were applied as 10 mm wide bands, with 10 mm distance between adjacent bands, under a continuous flow of air, by means of Camag Linomat V sample applicator fitted with a microlitre syringe. A constant application rate of 200 nL/s was used. The plates were developed to different distances in chamber previously saturated with mobile phase vapor for 15 min, at room temperature. Good separation and well developed peaks were obtained in a short time, approximately 20 min, by developing chromatograms over the distance of 10 cm, using the mixture of ethyl acetate : toluene : butylamine (2:2:1, v/v/v) as a mobile phase. After development, plates were dried in the air. Densitometric scanning was performed in the range from 200 to 400 nm with a Camag TLC Scanner 3 with the deuterium light source in absorbance/remission mode. The slit dimensions were 8×0.6 mm and the scanning speed was 20 mm/s. Evaluation was based on linear regression of peak areas. Compounds were identified by retention factor (R_F) values and UV spectra along with the peak area measurement.

Validation of the method

To confirm reliability of the results the method was validated (24).

Linearity: Standard solutions of meloxicam were prepared with six concentrations ranging from 50 to 2100 mg/mL. Each solution was spotted three times. Standard curves were constructed by defining the relationship between the peak area of the analyte and the concentration. The linearity plots were made, and the data were calculated using linear regression analysis. Parameters: standard error of estimate S_e, standard deviation of the slope S_a and standard deviation of the intercept S_b, were calculated.

Specificity: Specificity is defined as the ability to execute of unequivocal analysis in the presence of

components that can be expected. Typically they can include impurities and degradation products, matrix components or other potentially interfering substances. The specificity of the method was checked by blank sample detection, peak purity and experiments with standard compound. The bands for meloxicam in the sample were confirmed by comparing the R_F values and UV spectra of the bands from the sample with those from the standard drug. The peak purity of meloxicam was estimated by comparing the spectra at three different levels, i.e., peak start, peak apex, and peak end. The method was considered to be specific since there was no interfering peak at the R_F of meloxicam, and also the peak was well resolved from the peaks of all impurities.

Precision: The precision of an analytical procedure expresses the consistency of results between a series of measurements obtained from the same sample under the same conditions. The repeatability of sample application and measurement of peak area was determined by performing six replicate measurements of the same band. Intra-day precision was estimated from results obtained from sixfold replicate analysis of samples of three different concentrations on the same day. Inter-day precision was calculated from results for the same samples analyzed on five running days. The percent relative standard deviation and standard error of peak area were calculated. The results obtained are listed in Table 1.

Detection Limit and Quantification Limit: The detection limit (LOD) is defined as the lowest amount of an analyte which can be readily detected but not determined quantitatively. It is usually regarded as the amount for which the signal-to-noise (SNR) ratio is 3:1. The quantification limit (LOQ) is defined as the lowest amount of an analyte that can be determined quantitatively with acceptable precision and accuracy. It is usually regarded as the

Table 1. The results of intra-day and inter-day precision of the method.

Concentration added (μg/mL)	Intra-day precision			Inter-day precision		
	Mean area	SD	RSD (%)	Mean area	SD	RSD (%)
100	1154.22	19.29	1.67	1146.02	20.81	1.82
500	3158.13	45.73	1.44	3177.52	40.55	1.28
1500	8332.75	23.69	0.28	8278.70	54.26	0.66

SD = standard deviation; RSD = relative standard deviation (%).

Table 2. The results of meloxicam determination in pharmaceutical preparations with statistical analysis.

Preparation	Declared concentration	Determined concentration		Statistical analysis (n = 8)	
Mobic s. 902471	7.5 mg per tab.	7.33 7.33 7.40 7.23	7.06 7.40 7.32 7.07	$x_m = 7.27$ S = 0.14 $\mu = x_m \pm 0.11$	$S_{x\,m} = 0.05$ RSD = 1.87
Mobic s. 103123	15 mg per tab.	14.67 14.80 15.05 14.84	15.01 14.98 15.06 14.77	$x_m = 14.91$ S = 0.15 $\mu = x_m \pm 0.12$	$S_{x\,m} = 0.05$ RSD = 0.97
Mobic s. 002007	7.5 mg per tab.	7.47 7.54 7.53 7.51	7.53 7.50 7.41 7.45	$x_m = 7.49$ S = 0.05 $\mu = x_m \pm 0.04$	$S_{x\,m} = 0.02$ RSD = 0.61
Meloksam s. 0010605	7.5 mg per tab.	7.46 7.54 7.45 7.42	7.46 7.57 7.50 7.41	$x_m = 7.48$ S = 0.06 $\mu = x_m \pm 0.05$	$S_{x\,m} = 0.02$ RSD = 0.75

s. = series; tab. = tablet; x_m = arithmetic mean; S = standard deviation; $S_{x\,m}$ = standard deviation for arithmetic mean; m = confidence interval at 95 % probability; RSD = relative standard deviation (%).

amount for which the SNR is 10:1. Detection limit and quantification limit were determined on the basis of the linear regression data, from the equations: LOD = 3.3 × σ/a and LOQ = 10 × σ/a, where σ is the standard deviation of the response and a is the slope of the calibration curve.

Accuracy: The accuracy of the presented method was calculated in term of percentage recovery by the standard addition technique. Recovery studies were run by adding 80, 100 and 120 % of the standard of meloxicam to the pre-analyzed samples and mixtures were analyzed by the proposed method. The experiment was repeated six times to check the recovery of the drug at different levels in the formulations.

TLC analysis of meloxicam in drugs

Solutions of meloxicam (10 μL of standard solutions, 10 μL of sample solutions for determining active substance and 100 μL of sample solutions for

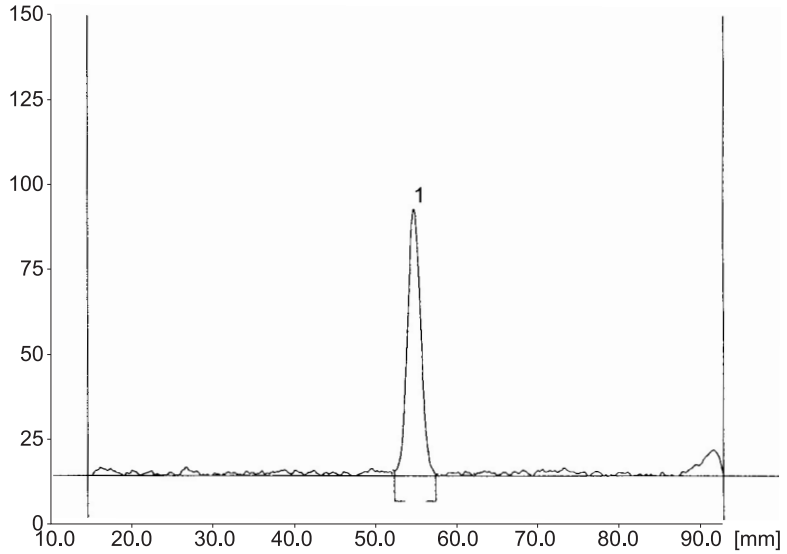

Figure 1. A typical TLC chromatogram, obtained for meloxicam, from analysis of a tablet formulation.

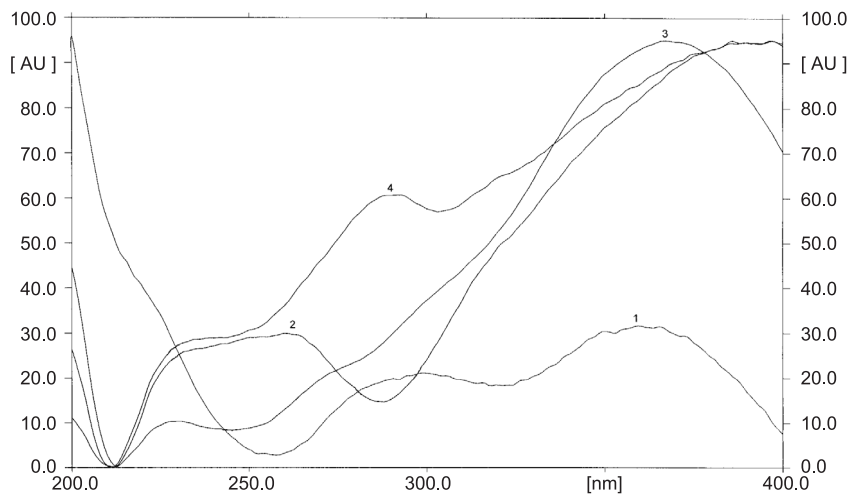

Figure 2. UV spectra for individual constituents, obtained directly from chromatograms by densitometer in absorption/remission mode (1 – meloxicam; 2 – IA; 3 – IB; 4 – IC).

determining impurities) were applied with an applicator to the plates of 7 × 11 cm in size (cut from 20 × 20 cm before use). Chromatograms were developed to a distance of 10 cm with ethyl acetate : toluene : butylamine (2:2:1, v/v/v) as the mobile phase. After development, plates were dried at room temperature. Densitometric measurements were acquired by scanning chromatograms at $\lambda = 297$ nm. The concentration of meloxicam in tested preparations was computed by comparing the peak areas for standard and sample solutions. Three measurements were made for each analysis. As a final result, the mean values were taken. Received results with the statistical estimation were assembled in Table 2. The typical chromatogram obtained from analysis of tablet formulations is shown in Figure 1. On the obtained chromatograms there were no other peaks, beside the peaks from the active substance.

Study of meloxicam stability in solutions

An influence of pH, temperature and incubation time on stability of meloxicam in solutions was considered. For this purpose, weighed amounts of tablets containing approximately 10 mg of meloxicam were dissolved in 5 mL of hydrochloric acid or sodium hydroxide solution. The solutions were incubated for particular length of time at temperatures of 60 and 120°C. Next, the samples were diluted with methanol (1:1, v/v) for further analysis. The resulting solutions were applied to TLC plates in triplicate and the chromatograms were run as described above. The measurements were made under conditions established for the method to determine the percentage constituent concentration, by the internal normalization method.

The process of meloxicam degradation was rated by means of some of kinetic and thermodynamic parameters (25). The order of reaction, the reaction rate constants k, half-life $t_{0,5}$ and the time $t_{0,1}$ at which the concentration of meloxicam is reduced by 10%, and activation energy E_a were calculated. Parameters were computed from the formulae $k = 2.303 \times (\log c_1 - \log c_2)/(t_2 - t_1)$, $t_{0.5} = 0.635/k$, $t_{0.1} = 0.1053/k$, and $E_a = -2.303 \times R \times (\log k_1 - \log k_2)/(1/T_1 - 1/T_2)$, where: R = 8.315 J/mol K, $k_2 > k_1$, $T_2 > T_1$.

Analysis of degradation products

To identify the meloxicam degradation products, beside densitometric analysis (R_F values and absorption spectra), HPLC-MS/MS analysis was carried out. The meloxicam solutions after hydrolysis in 0.5 mol/L HCl and NaOH, at 120°C were separated on a chromatotron plate (model 8924, Harrison Research, USA). Solution of hydrolyzed substance in methanol was applied to the plate (prepared with silica gel PF_{254} and water). Then, based on the TLC analysis, appropriate solvents of increasing polarity and different proportions were applied to the plate, and final mixture was obtained: ethyl acetate : toluene : butylamine (2:2:1, v/v/v). The separation obtained on the plate was observed under UV lamp. After separation of fractions, the products were identified by HPLC-MS/MS method. The triple quadrupole mass analyzer connected with a HPLC system was used. The analysis was carried out by using the mobile phase: acetonitrile : water (50:50, v/v) with addition of formic acid (10 mL/L), at fixed flow rate 600 mL/min, by using positive ionization and electrospray as an ion source.

RESULTS AND DISCUSSION

The aim of this paper was to establish a new, simple and accurate method for identification and quantitation of meloxicam in pharmaceutical preparations. The conditions for the analysis were established experimentally, based on eluotropic series. Finally, determination was performed on TLC 60 F_{254} plates with ethyl acetate – toluene – butylamine (2:2:1, v/v/v) as the mobile phase. On obtained chromatograms of the solutions recorded after extracting meloxicam with methanol the presence of main spot originated from meloxicam was observed at $R_F \approx 0.56$.

To investigate the appropriate wavelength for determination of meloxicam, the UV spectra in the range 200–400 nm were acquired from the chromatograms. The spot originating from the meloxicam ($R_F \approx 0.56$) shows the characteristic maxima at 297 and 365 nm (Figure 2). For monitoring a drug, the wavelength 297 nm was selected. It was observed that there was no interference from the mobile phase or baseline disturbance at 297 nm. Therefore, it was concluded that this wavelength was satisfactory for analysis of meloxicam with suitable sensitivity.

The linear regression analysis parameters for the calibration plot in the concentration range of 50–2100 μg/mL showed a good linear relationship (correlation coefficient, r = 0.9986). The regression equation obtained from the calibration plot: $y = 4.89 \cdot x + 675.03$ (where y – peak area, x – concentration in μg/mL), was used for quantitative determination of meloxicam in different samples. The values of the standard deviation of the slope (S_a), standard deviation of the intercept (S_b) and standard error of estimate (S_e) were 0.13, 142.02 and 238.39, respectively.

Developed method is specific for studied components occurring in pharmaceuticals with coexisting components. There are no peaks on recorded chromatograms for placebo solutions, where studied components occur. The peak purity for meloxicam was estimated by comparing the spectra of the standard with those acquired at the peak start, peak apex, and peak end positions of sample bands. Measured spots showed overlaying spectra. This indicates that the spot is free of any interference that might be present in the analysis. The R_F values for meloxicam were 0.56 (Fig. 1). No interference was observed in drug samples, indicating the high selectivity of the developed method.

The repeatability of sample application and measurement of peak area were expressed as % RSD, which was found to be 0.54 and 0.43, respectively. Table 1 shows intra- and inter-day variation of meloxicam concentrations. The RSD for those analyses was always less than 2%. These low values of the RSD for the repeatability and precision indicated good precision of the developed method. LOD and LOQ were found to be 0.96 and 2.90 μg per spot, respectively. The low LOD and LOQ values indicated high sensitivity of this method. When the developed method was applied for extraction and consequent evaluation of meloxicam from formulation after adding 80, 100 and 120% of additional

Table 3. Summary results of degradation of meloxicam under different stress conditions.

Temp. [°C]	Solution	Time	Concentration [%]			
			Meloxicam ($R_F \approx 0.56$)	IA ($R_F \approx 0.64$)	IB ($R_F \approx 0.76$)	IC ($R_F \approx 0.88$)
60	3 mol/L HCl	1 day	100.00	—	—	—
		42 days	100.00	—	—	—
		64 days	98.13	1.87	—	—
	1 mol/L HCL	1 day	100.00	—	—	—
		18 days	100.00	—	—	—
		42 days	64.56	25.22	10.22	—
		61 days	62.75	27.21	10.04	—
	1 mol/L NaOH	1 day	100.00	—	—	—
		7 days	100.00	—	—	—
		18 days	84.27	9.40	6.33	—
		42 days	54.48	27.11	18.41	—
		64 days	7.44	46.02	27.30	16.24
	3 mol/L NaOH	1 day	100.00	—	—	—
		7 days	97.03	2.97	—	—
		18 days	50.97	40.01	10.02	—
		42 days	12.32	56.61	31.07	—
		64 days	—	33.23	32.49	34.28
120	1 mol/L HCl	1 h	83.80	—	16.20	—
		2 h	81.49	—	18.51	—
		8 h	71.93	2.77	25.30	—
		24 h	66.75	6.44	26.81	—
	0.5 mol/L HCl	1 h	89.87	0.11	10.02	—
		2 h	85.10	1.90	13.00	—
		8 h	73.53	4.99	21.48	—
		24 h	70.28	7.31	22.41	—
	0.5 mol/L NaOH	1 h	100.00	—	—	—
		2 h	100.00	—	—	—
		8 h	57.09	23.25	19.66	—
		24 h	15.03	45.56	28.40	11.01
	1 mol/L NaOH	1 h	100.00	—	—	—
		2 h	87.53	5.22	7.25	—
		8 h	37.39	22.83	21.42	18.36
		24 h	—	—	—	100.00

(time "0" = 100% of meloxicam)

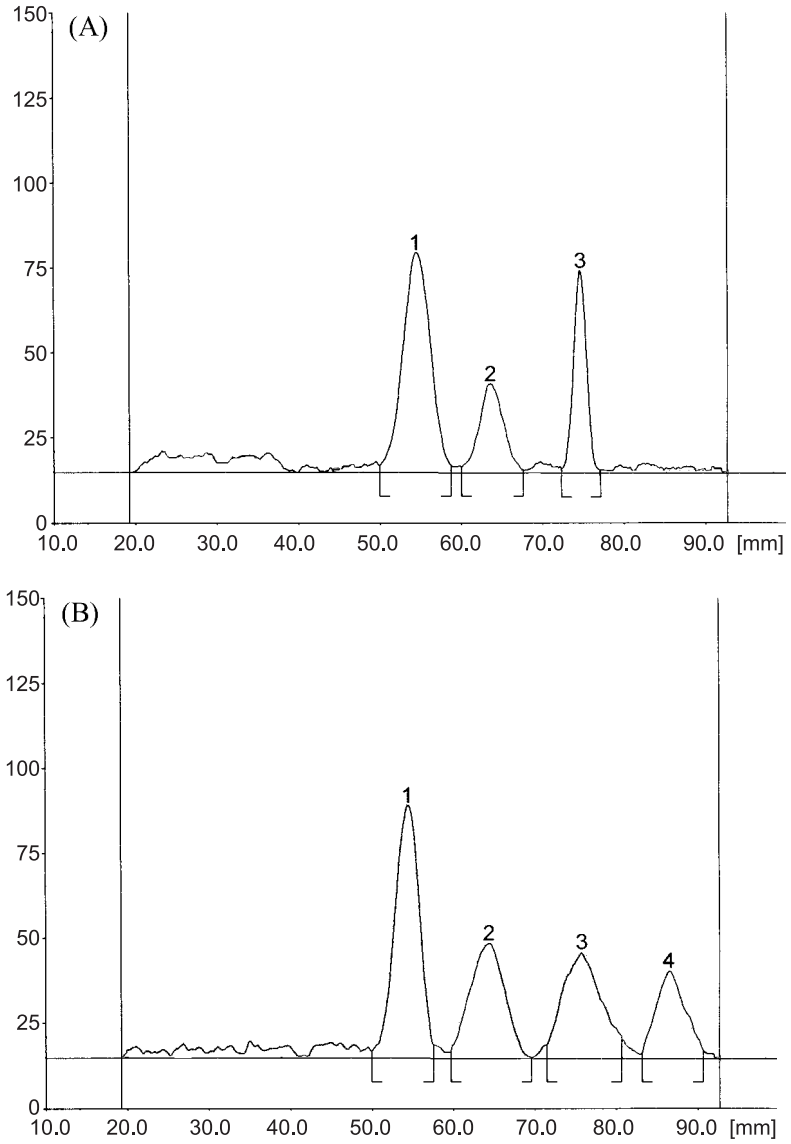

Figure 3. Examples of densitograms, obtained after meloxicam decomposition at 120°C in 1 mol/L HCl (A) and 1 mol/L NaOH (B) solution (1 – meloxicam; 2 – IA; 3 – IB; 4 – IC).

drug, the recovery values were in the range from 99.31 to 99.60%, The %RSD values were found in the range of 0.51–1.99%.

The presented method meets the fundamental requirements of good laboratory practices, both in the terms of qualitative analysis and assay of active ingredient. A single band at $R_F \approx 0.56$ was observed in chromatograms obtained for samples containing meloxicam, extracted from tablets. The results of determination of meloxicam in pharmaceutical preparations are repeatable and comparable with concentrations of active substance declared by the manufacturer. The obtained results with the statistical estimation were assembled in Table 2. The RSD values, varied from 0.61 to 1.87 %, were low, which confirms that the method is suitable for routine analysis of compound in drugs. The fact that no impurities under the study were found could indicate the high quality of the medicines available in the markets.

Such a conclusion led to further investigations focused on the behavior of meloxicam in solutions in different environments at different temperatures, and also on the suitability of the new method for this purpose. The results obtained in the tests show that

pH, temperature and incubation time affect the rate of meloxicam degradation.

The chromatograms obtained under stress conditions of the samples showed different peaks at various R_F values, except for the peak of meloxicam (Fig. 3). The concentration of meloxicam was also decreasing (in values of area) in the samples. Chromatograms obtained for the degraded samples showed well-separated spots of the pure drug as well as some additional peaks with good resolution. Resolution factor values (R_s) were in the range 1.28–2.67. It showed good separation of the obtained peaks. The R_F of meloxicam was not meaningly shifted in the presence of degradation peaks. This indicated the stability-indicating character of the developed method. The level of degradation at different stress conditions, and the number of degradation products with their R_F values are shown in Table 3.

On the chromatograms obtained under conditions of the acid degradation, two new products ($R_{F\ (IA)} \approx 0.64$ and $R_{F\ (IB)} \approx 0.76$) were observed, while three degradation products were observed in basic solutions ($R_{F\ (IA)} \approx 0.64$, $R_{F\ (IB)} \approx 0.76$ and $R_{F\ (IC)} \approx 0.88$) and their positions on chromatogram were comparable to that of a product formed in an acidic environment.

The degradation process was determined based on the decreasing concentration of meloxicam and increasing concentration of degradation products. The natural log (ln) of percentage drug remaining when plotted against time gave a straight line, which indicated that the degradation process of meloxicam followed the first-order kinetics at selected temperatures.

The number and concentrations of the degradation products of meloxicam changes with hydrochloric acid and sodium hydroxide concentrations, temperature and incubation time (Table 3). It was found that within the studied HCl and NaOH concentrations, degradation of meloxicam in basic solutions runs more quickly than in acidic solutions. The degradation constant (k) values, half-life ($t_{0.5}$) time and the time at which 10% of the original concentration of the drug is left ($t_{0.1}$) were obtained at each temperature for acidic and alkaline degradation processes. The activation energies of meloxicam in the acid and alkaline samples were calculated. The obtained kinetic and thermodynamic parameters are presented in Table 4.

The relations of calculated kinetic and thermodynamic data were: $k_{NaOH} > k_{HCl}$ and $E_{a\ (NaOH)} < E_{a\ (HCl)}$. With increasing temperature, the time of incubation and pH of solutions, the reaction rate k attained maximum value at 120°C, after 1 day of heating in basic solutions. The values of $t_{0.1}$ and $t_{0.5}$ changed inversely. The obtained results confirm lower stability of meloxicam in alkaline than in acidic solutions.

Table 4. Kinetic and thermodynamic parameters describing degradation process of meloxicam.

Temperature	HCl or NaOH concentration / Kinetic parameters	
60°C	**1 mol/L HCl** $k = 3.08 \times 10^{-4}$ $t_{0.1} = 341.88$ $t_{0.5} = 2250.00$	**1 mol/L NaOH** $k = 1.72 \times 10^{-3}$ $t_{0.1} = 61.22$ $t_{0.5} = 402.91$
	3 mol/L HCl $k = 1.25 \times 10^{-5}$ $t_{0.1} = 8424.00$ $t_{0.5} = 55440.00$	**3 mol/L NaOH** $k = 2.13 \times 10^{-3}$ $t_{0.1} = 49.44$ $t_{0.5} = 325.35$
120°C	**0.5 mol/L HCl** $k = 1.07 \times 10^{-2}$ $t_{0.1} = 9.84$ $t_{0.5} = 64.77$	**0.5 mol/L NaOH** $k = 8.24 \times 10^{-2}$ $t_{0.1} = 1.28$ $t_{0.5} = 8.41$
	1 mol/L HCl $k = 9.89 \times 10^{-3}$ $t_{0.1} = 10.65$ $t_{0.5} = 70.07$	**1 mol/L NaOH** $k = 1.41 \times 10^{-1}$ $t_{0.1} = 0.75$ $t_{0.5} = 4.91$
$E_a = 6.29 \times 10^4$ (1 mol/L HCl) $E_a = 7.99 \times 10^4$ (1 mol/L NaOH)		

k = stability constants [h^{-1}]; $t_{0.1}$ = time, at which 10% of concentration is left [h];
$t_{0.5}$ = half-life time [h]; E_a = the energy of activation [J/mol K]

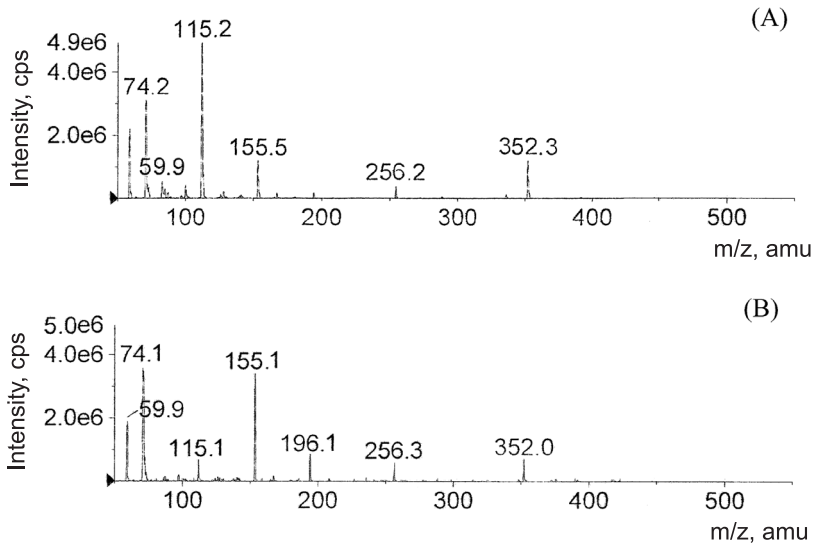

Figure 4. Mass spectra of tested samples in ESI mode (A) – acidic solution, (B) – alkaline solution).

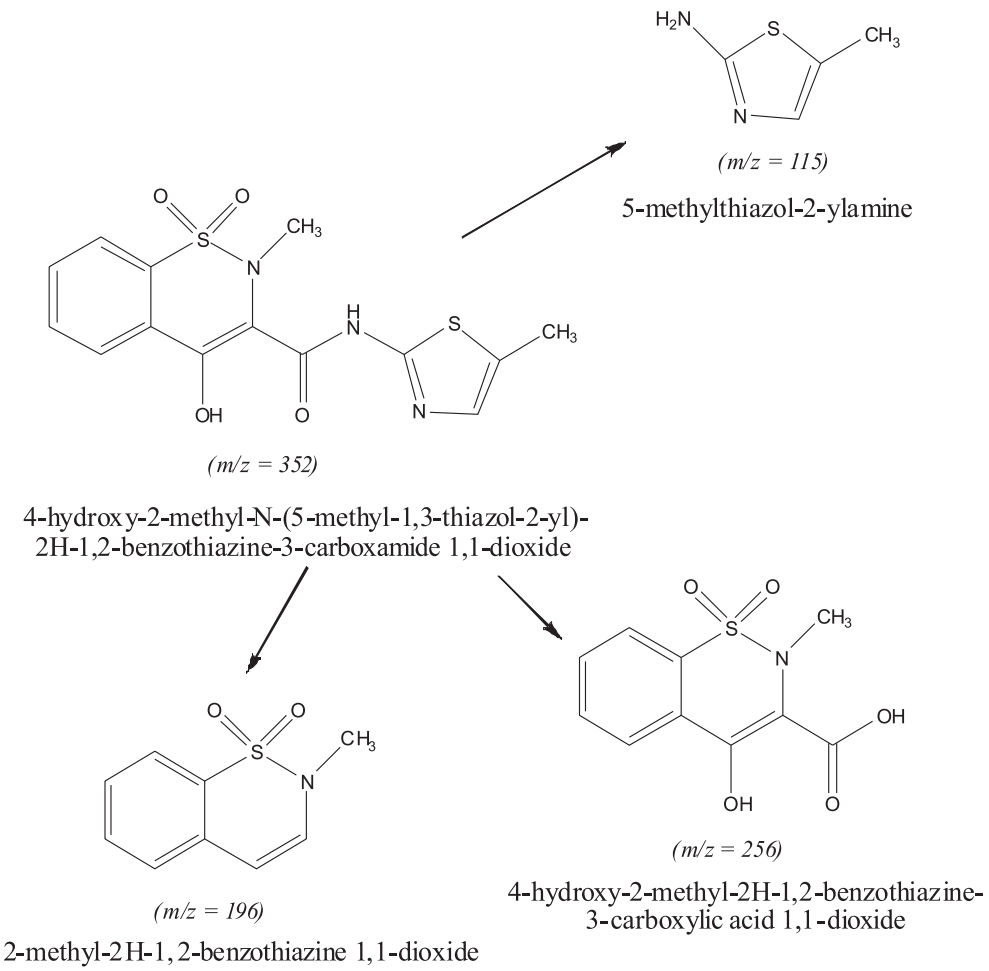

Figure 5. Scheme of the proposed products of meloxicam degradation in solutions under stress conditions.

Simultaneously, the number of additional peaks appearing on chromatograms was greater after alkaline degradation.

The degradation products were identified by using characteristic parameters, such as R_F values, UV spectra, and additionally, by HPLC-MS/MS analysis (Figs. 2–4). The results indicated the products' ion mass spectra obtained from collision-induced dissociation of the protonated molecular ions of meloxicam.

The HPLC-MS/MS method for the determination of degradation products of meloxicam was applied. The ionization process caused the protonation of the molecules of the analytes $[M-H]^+$. The protonated molecules at *m*/*z* 352 represented meloxicam. Under the experimental conditions, the ion mass spectra of degradation products of meloxicam produced an intense fragments at *m*/*z* 115, 196, 256 created by the division of an amide bond. The obtained MS spectra also indicate the presence of other ions, i.e., *m*/*z* 60, 74, 155. Analysis of the degradation process of meloxicam by TLC revealed the presence of three additional peaks, except for peak derived from meloxicam, i.e., IA, IB and IC. The proposed degradation pathway for meloxicam may lead to the creation of 5-methylthiazol-2-ylamine (*m*/*z* 115), 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylic acid 1,1-dioxide (*m*/*z* 256), 5-(dioxide-l^6-sulfanylidene)-6-methylidenecyclohexa-1,3-diene (*m*/*z* 196), and other products, less persistent in terms of the assay. The results enable the following meloxicam decomposition reactions to be identified as presented on scheme in Figure 5.

As the reported methods could effectively separate the drug from its degradated products, it can be employed as a stability indicating one. The system suitability tests performed verified the resolution, efficiency and repeatability of the chromatographic system.

CONCLUSIONS

A simple, rapid and specific analytical method for the determination of meloxicam in pharmaceutical preparations using the TLC technique with densitometric detection was proposed. The developed method is accurate, precise, reproducible, with a wide range of linearity and stability indicating. All these factors make this method suitable for quantification of meloxicam in drugs. The obtained results prove that the method can save time and money and it can be used with high accuracy and precision and may be useful for the routine analysis of meloxicam in pharmaceutical dosage forms without interference. This method is also highly sensitive and could effectively separate the drug from its degraded products. Solution of meloxicam is stable at room temperature, but demonstrated high susceptibility to degradation in alkaline and acidic environment. The products of decomposition are probably 5-methylthiazol-2-ylamine, 4-hydroxy-2-methyl-2H-1,2-benzothiazine-3-carboxylic acid 1,1-dioxide and 5-(dioxide-l^6-sulfanylidene)-6-methylidenecyclohexa-1,3-diene.

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