

## A cost-effective and sensitive TLC-densitometric identification of meloxicam

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The influence of different chromatographic conditions and the process of spot visualization on determining the limit of detection as well as quantification (*LOD* and *LOQ*) of meloxicam by TLC-densitometric technique was estimated. Of all chromatographic conditions tested, the lowest limiting values, thus the best sensitivity, in the NP-TLC system was achieved on silica gel 60F<sub>254</sub> and neutral aluminum oxide plates developed with the mobile phase consisting of ethyl acetate/toluene/*n*-butylamine (2:2:1, *V/V/V*). In the case of the RP-TLC method, a mixture of methanol/water (8:2, *V/V*) enabled densitometric detection of meloxicam at the lowest concentration level on RP-8F<sub>254</sub> and RP-18F<sub>254</sub> plates. Additionally, the smallest *LOD* value of meloxicam ensured crystalline violet and gentian violet as visualization agents on silica gel 60F<sub>254</sub> and neutral aluminum oxide 150F<sub>254</sub> plates, resp. Comparison of the densitometrically obtained spectra of meloxicam drug and its standard after the use of appropriate visualization agents could be a good and cheap alternative tool for the identification of meloxicam as an active pharmaceutical ingredient.

*Keywords:* meloxicam, TLC-densitometry, identification, sensitivity, visualization agents

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Rheumatoid diseases belong to the most common diseases of the musculoskeletal system and connective tissues, especially in developed countries. They are one of the biggest issues of today's medicine due to the large number of people suffering from these diseases as well as their consequences. Non-steroidal anti-inflammatory drugs (NSAIDs), both traditional non-steroidal drugs and cyclooxygenase-2 inhibitors, are often used to treat patients with pain and inflammation. NSAIDs are very effective painkillers and one of the cornerstones of pain management in patients with arthritis (1). For example, meloxicam is a heterocyclic compound that belongs to the group of NSAIDs with a strong analgesic, anti-inflammatory and antipyretic effect. It is also a selective COX-2 blocking agent. During its use, the side-effects are less frequent and smaller compared to the drugs that block COX-1 and COX-2 non-selectively.

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The detection of various drugs by TLC was described in our earlier articles (2, 3). An extensive literature review reveals that till today the TLC detection of meloxicam on chromatographic plates is most often carried out under UV light without any visualization agent (4–11). To the best of our knowledge, there are no literature reports concerning the use of visualization agents to detect meloxicam on a thin-layer. Therefore, in this work, the utility of selected dyes as potential new visualization agents for the identification of meloxicam as a drug substance was also shown.

## EXPERIMENTAL

### *Chemicals and reagents*

The reference standard of meloxicam was procured from Sigma-Aldrich (USA). Organic solvents of analytical grade supplied by POCh (Poland) such as methanol, ethyl acetate, toluene, ethanol (96 %) and *N,N*-dimethylformamide, as well as *n*-butylamine (Acros Organics, Belgium) were applied as the components of mobile phases and as solvents for meloxicam.

### *Chromatographic plates*

Planar chromatography was performed on the following TLC plates purchased from Merck (Germany), TLC aluminum sheets (10 × 20 cm) precoated with: (i) neutral aluminum oxide 60F<sub>254</sub> (Al<sub>2</sub>O<sub>3</sub> 60F<sub>254</sub>), (ii) neutral aluminum oxide 150F<sub>254</sub> (Al<sub>2</sub>O<sub>3</sub> 150F<sub>254</sub>), (iii) silica gel 60 (SiO<sub>2</sub> 60), (iv) silica gel 60F<sub>254</sub> (SiO<sub>2</sub> 60F<sub>254</sub>), (v) mixture of silica gel 60 and Kieselguhr 60F<sub>254</sub> (SiO<sub>2</sub> 60/KG F<sub>254</sub>), (vi) silica gel RP-18F<sub>254</sub> (SiO<sub>2</sub> RP-18F<sub>254</sub>). Also, TLC glass plates precoated with silica gel RP-8 F<sub>254</sub> (SiO<sub>2</sub> RP-8F<sub>254</sub>) were used.

### *Instrumentation*

TLC Scanner 3 manufactured by Camag (Switzerland) was used in the reflectance/absorbance mode and controlled by WinCATS software (version 1.4.2) for spectrodensitometric and densitometric scanning, a twin-trough glass chamber (20 × 10 cm, Camag) was applied for the development of chromatographic plates and 5-mL microliters pipettes (Camag) were utilized for spotting the solutions of meloxicam.

### *Preparation of sample solutions*

Working standard solutions of meloxicam were prepared in a mixture of methanol and *N,N*-dimethylformamide as a solvent (1:1, V/V) in the following concentrations: 1.60, 1.40, 1.20, 1.00, 0.80, 0.60, 0.40, 0.25, 0.20, 0.18, 0.16, 0.14, 0.12, 0.10, 0.08, 0.06, 0.04, and 0.02 mg mL<sup>-1</sup>. The spot volume was 5 µL.

### *Chromatographic conditions*

Different types of chromatographic plates as mentioned in the previous section were tested in TLC analysis of meloxicam in both, *i.e.*, normal-phase and reversed-phase system.

In the case of adsorption TLC, the plates were pre-washed with methanol and then activated at 120 °C for 30 min prior to chromatographic analysis. After sample application, the plates were developed in a Camag twin-trough chamber pre-saturated with mobile phase vapor for 30 min at the room temperature, up to 75 mm, using two mixtures: ethyl acetate/toluene/*n*-butylamine (2:2:1, V/V/V) (mobile phase I) and ethyl acetate/ethanol/toluene/25 % NH<sub>4</sub>OH (6:3:1:0.06, V/V/V/V) (mobile phase II). For the RP-TLC study, a mixture of methanol and water in a volume ratio of 5:5 (mobile phase III) and 8:2 (mobile phase IV) were used. After development, the plates were dried at room temperature (20 ± 2 °C) for 24 h. In the next step, the plates were directly scanned densitometrically or after the treatment with an appropriate visualization agent. All analyses were repeated six times. Averages were calculated from the results obtained.

### *Visualization agents*

Processes of spot visualization were carried out using several visualization agents procured from different suppliers. The solutions of these reagents were prepared as follows:

- (i) rhodamine B (POCh), (ii) Janus blue (Michrom, UK), (iii) methyl green (Fluka, Switzerland), (iv) brilliant green (POCh), (v) crystalline violet (Sigma-Aldrich), (vi) alkaline blue (Merck), (vii) gentian violet (Fluka), and (viii) methylene violet (Michrom) were used as 0.50 mg mL<sup>-1</sup> solutions in distilled water,
- fuchsine procured from Serva (Germany) was used as 0.150 mg mL<sup>-1</sup> solution in distilled water,
- brilliant cresyl blue supplied by Michrom was used as 0.50 mg mL<sup>-1</sup> solution in 2 % aqueous NaOH.

Taking into account the visualization manner, all obtained chromatograms have been divided into four groups. The first group was dipped in an individual visualization agent for 5 s and then left at room temperature until dry. The second group was immersed in an individual visualization agent for 5 s and then dried in a laboratory dryer at 110 °C for 1 hour. The third group was sprayed with a visualization agent and left at room temperature until dry. The fourth group was sprayed with a visualization agent and dried for one hour in a laboratory dryer at 110 °C.

### *Spectrodensitometry and densitometry of chromatograms*

Spectrodensitometry and densitometry were carried out with a Camag Scanner TLC 3 operated in absorbance mode, fitted with a WinCATS 1.4.2 software. The detector system was a deuterium lamp emitting a UV spectrum in the range 190–450 nm and a tungsten lamp emitting a spectrum in the range 370–800 nm. The starting point was at 200 nm and the end wavelength was 800 nm. The slit dimensions were set at 10.00 × 0.40 mm, the scanning speed of 20 nm s<sup>-1</sup> and the data resolution of 1 nm per step were used for the spectrodensitometric analysis. Densitometric scanning was conducted at the respective absorption maximum (Table I). The slit dimensions were set at 10.00 × 0.40 mm, the scanning speed of 20 mm s<sup>-1</sup> and the data resolution at 100 μm per step were suitable for densitometric analysis. This was done in triplicate and baseline correction was employed.

Table I. Spectrodensitometric characteristics of meloxicam on different chromatographic plates after UV detection

TLC plate	Main absorption band $\lambda_{\max}$ (nm) <sup>a</sup>	Additional absorption bands	
		$\lambda$ (nm)	Intensity (AU)
Al <sub>2</sub> O <sub>3</sub> 60F <sub>254</sub>	369	211	36.0
		272	47.2
		300	44.8
Al <sub>2</sub> O <sub>3</sub> 150F <sub>254</sub>	367	211	35.9
		274	49.2
		207	82.2
SiO <sub>2</sub> 60	363	274	37.1
		292	35.3
		211	72.4
SiO <sub>2</sub> 60F <sub>254</sub>	361	277	71.6
		295	72.3
		208	71.6
SiO <sub>2</sub> 60/KG F <sub>254</sub>	368	266	15.2
		331	37.1
		274	32.9
SiO <sub>2</sub> RP-18F <sub>254</sub>	213	364	74.9
		267	16.9
SiO <sub>2</sub> RP-8F <sub>254</sub>	215	359	42.6

<sup>a</sup> Intensity of all absorption maxima is equal to 95 AU.

### Limit of detection (LOD) and limit of quantification (LOQ)

LOD and LOQ values were calculated according to ICH guidelines (12):

$$LOD = \frac{3.3 \times \sigma}{S} \quad LOQ = \frac{10 \times \sigma}{S}$$

where  $S$  is the slope of the calibration line and  $\sigma$  standard deviation of the response.  $\sigma$  was determined by using the standard deviation of the intercept ( $Y$ ) of the calibration plot ( $s_a$ ) and the residual standard deviation of the regression line ( $s_{xy}$ ). LOD and LOQ values were calculated from the results obtained by both criteria.

### Statistical analysis

All calculations were performed using Statistica program version 10.0 PL supplied by StatSoft (Kraków, Poland).

## RESULTS AND DISCUSSION

### Method development

The influence of mobile phase composition and the kind of the sorbent on the limit of detection and quantification of meloxicam was estimated. The most commonly used chroma-

tographic plates were selected as the stationary phases. The mobile phases were selected so that meloxicam travel properly either in NP-TLC or RP-TLC.

During methods optimization several mobile phases were tested; finally, the following were chosen: ethyl acetate/toluene/*n*-butylamine (2:2:1, V/V/V) (mobile phase I), ethyl acetate/ethanol/toluene/25 % NH<sub>4</sub>OH (6:3:1: 0.06, V/V/V/V) (mobile phase II), methanol/water (5:5, V/V) (mobile phase III) and methanol/water (8:2, V/V) (mobile phase IV).

### Detection of meloxicam without visualization agents

Meloxicam was detected firstly in UV without the use of visualization agents on different chromatographic plates. The resultant spectrodensitograms of meloxicam described in Table I indicate that the applied sorbents influenced the wavelength of the absorption peak ( $\lambda_{\max}$ ) and the additional absorption bands as well as their intensity [AU]. In NP-TLC analysis, the fundamental band of meloxicam shifts from 361 to 369 nm, and three additional absorption bands were observed. However, by using Al<sub>2</sub>O<sub>3</sub> 150F<sub>254</sub> plates, only two additional bands for meloxicam were noticed on its spectrodensitogram. In the case of RP-TLC plates, the fundamental bands of meloxicam occur at 213 nm and 215 nm, for SiO<sub>2</sub> RP-18F<sub>254</sub> and SiO<sub>2</sub> RP-8F<sub>254r</sub> respectively. The chromatographic plates influenced the meloxicam spectra markedly. This

Table II. Limit of detection (LOD) of meloxicam obtained by NP-TLC and RP-TLC methods after UV detection

Mobile phase <sup>a</sup>	Sorbent <sup>b</sup>	R <sub>F</sub> value <sup>c</sup>	LOD (µg per spot) calculated using <sup>d</sup>		Average value of LOD (µg per spot) <sup>e</sup>
			s <sub>a</sub>	s <sub>xy</sub>	
I	Al <sub>2</sub> O <sub>3</sub> 60F <sub>254</sub>	0.73 ± 0.05	0.098	0.034	0.066 ± 0.035
	Al <sub>2</sub> O <sub>3</sub> 150F <sub>254</sub>	0.75 ± 0.04	0.090	0.070	0.080 ± 0.011
	SiO <sub>2</sub> 60	0.59 ± 0.05	0.158	0.054	0.106 ± 0.057
	SiO <sub>2</sub> 60F <sub>254</sub>	0.58 ± 0.05	0.074	0.052	0.063 ± 0.012
	SiO <sub>2</sub> 60/KG F <sub>254</sub>	0.70 ± 0.05	0.266	0.074	0.170 ± 0.105
	Al <sub>2</sub> O <sub>3</sub> 60F <sub>254</sub>	0.07 ± 0.02	0.164	0.067	0.116 ± 0.053
II	Al <sub>2</sub> O <sub>3</sub> 150F <sub>254</sub>	0.28 ± 0.03	0.220	0.051	0.136 ± 0.093
	SiO <sub>2</sub> 60	0.80 ± 0.05	0.097	0.063	0.080 ± 0.019
	SiO <sub>2</sub> 60F <sub>254</sub>	0.75 ± 0.05	0.042	0.027	0.035 ± 0.008
III	SiO <sub>2</sub> 60/KG F <sub>254</sub>	0.73 ± 0.05	0.211	0.059	0.135 ± 0.083
	SiO <sub>2</sub> RP-18F <sub>254</sub>	0.48 ± 0.03	0.115	0.075	0.095 ± 0.022
	SiO <sub>2</sub> RP-8F <sub>254</sub>	0.38 ± 0.03	0.194	0.088	0.141 ± 0.058
IV	SiO <sub>2</sub> RP-18F <sub>254</sub>	0.90 ± 0.03	0.133	0.031	0.082 ± 0.056
	SiO <sub>2</sub> RP-8F <sub>254</sub>	0.80 ± 0.03	0.112	0.051	0.082 ± 0.033

R<sub>F</sub> – retention factor, LOD – limit of detection; <sup>a</sup> Mobile phase I: ethyl acetate/toluene/*n*-butylamine (2:2:1, V/V/V), II: ethyl acetate/ethanol/toluene/25 % ammonium hydroxide (6:3:1: 0.06, V/V/V/V), III: methanol/water (5:5, V/V), IV: methanol/water (8:2, V/V); <sup>b</sup> Sorbent Al<sub>2</sub>O<sub>3</sub> 60F<sub>254</sub> – neutral aluminum oxide 60F<sub>254</sub>, Al<sub>2</sub>O<sub>3</sub> 150F<sub>254</sub> – neutral aluminum oxide 150F<sub>254</sub>, SiO<sub>2</sub> 60 – silica gel 60, SiO<sub>2</sub> 60F<sub>254</sub> – silica gel 60F<sub>254r</sub>, SiO<sub>2</sub> 60/KG F<sub>254</sub> – mixture of silica gel 60 and Kieselgur F<sub>254r</sub>, SiO<sub>2</sub> RP-18F<sub>254</sub> – silica gel RP-18F<sub>254r</sub>, SiO<sub>2</sub> RP-8F<sub>254</sub> – silica gel RP-8F<sub>254r</sub>; <sup>c</sup> Mean ± SD, *n* = 6; <sup>d</sup> s<sub>a</sub> – standard deviation of the intercept (a) of calibration curve, s<sub>xy</sub> – residual standard deviation of a calibration curve.

might be explained by the physical and physicochemical properties of the chromatographic plates. This fact points to the need for standardization of chromatographic conditions during spectrodensitometric investigations of meloxicam.

LODs of meloxicam obtained by NP-TLC and RP-TLC on different chromatographic sorbents and by using four proposed mobile phases are presented in Table II, showing also the  $R_F$  values of meloxicam achieved under particular chromatographic conditions. As it is shown in Table II, NP-TLC analysis was performed using two mobile phases. For mobile phase I (ethyl acetate/toluene/*n*-butylamine (2:2:1, V/V/V), the lowest LOD (and consequently LOQ,  $LOQ = 3 \times LOD$ ) values were obtained on neutral aluminum oxide 60F<sub>254</sub> and silica gel 60F<sub>254</sub> plates. For mobile phase II (ethyl acetate/ethanol/toluene/25 % ammonia, 6:3:1:0.06; V/V/V/V), the lowest LOD (and LOQ) were achieved on silica gel 60F<sub>254</sub> plate. Comparing the results obtained for both mobile phases in the NP-TLC system leads to the highest LOD

Table III. Spectrodensitometric characteristics of meloxicam obtained on SiO<sub>2</sub> 60F<sub>254</sub> and neutral Al<sub>2</sub>O<sub>3</sub> 150F<sub>254</sub> plates after detection using visualization agents

Visualization agent	TLC plate					
	SiO <sub>2</sub> 60F <sub>254</sub>			Al <sub>2</sub> O <sub>3</sub> 150F <sub>254</sub>		
	Main absorption band $\lambda_{max}$ (nm) <sup>a</sup>	Additional absorption bands		Main absorption band $\lambda_{max}$ (nm) <sup>a</sup>	Additional absorption bands	
	$\lambda$ (nm)	Intensity (AU)		$\lambda$ (nm)	Intensity (AU)	
Janus blue	363	217	45.1	369	213	61.0
		271	51.4		273	62.2
		698	32.4		678	28.1
		278	76.4		249	79.7
Gentian violet	213	316	66.3	213	306	71.9
		359	71.3		360	64.3
		540	16.6		546	90.2
		635	23.0			
Brilliant green	346	200	66.0	378	210	73.7
		225	61.8		235	70.7
		283	66.3		290	78.1
		472	59.8		482	15.8
692	60.0					
Methyl green	357	209	69.5	363	210	56.6
		276	70.4		237	51.3
		292	71.1		276	57.7
		634	7.6		557	9.2
Crystalline violet	215	277	88.8	367	214	71.6
		359	71.8		254	74.8
		586	69.7		313	76.0
					556	65.2
			794	5.8		

<sup>a</sup> The intensity of all absorption maxima is equal to 95 AU.

(and *LOQ*) achieved using mobile phase I and SiO<sub>2</sub> 60/KG F<sub>254</sub> plates. Further, RP-TLC chromatographic analysis was also performed using two stationary phases and mobile phases consisting of methanol/water 5:5 (mobile phase III) and 8:2 (mobile phase IV). In this case, *LOD* (and *LOQ*) of meloxicam were very similar for both carriers, silica gel RP-18F<sub>254s</sub> and silica gel RP-8F<sub>254sr</sub> with mobile phase IV. In addition, values obtained using mobile phase IV were lower relative to the values obtained using mobile phase III.

#### *Detection of meloxicam with visualization agents*

Our earlier research (4) indicated that TLC coupled with densitometry on silica gel 60F<sub>254</sub> and neutral aluminum oxide 150F<sub>254</sub> plates using ethyl acetate/toluene/*n*-butylamine mixture (2:2:1, V/V/V) as the mobile phase was suitable for the successful separation of meloxicam from the potential impurities. For this reason, the same experimental conditions (stationary phases and mobile phase) were applied as the optimum in this work. Eight visualization agents (known as dyes), namely, brilliant cresyl blue, alkaline blue, methylene violet, Janus blue, brilliant green, methyl green, gentian violet and crystalline violet were used to detect meloxicam. Rhodamine B and fuchsin were used as visualization agents for comparison.

Meloxicam spots obtained on silica gel 60F<sub>254</sub> plates immediately after the use of an appropriate visualization agent were observed for all tested dyes except for fuchsin. After 60 min, the meloxicam spots were visible on the plates dried at room temperature when brilliant cresyl blue, methyl green, gentian violet, Janus blue, brilliant green and crystalline violet were applied. The spots obtained on the chromatographic plates dried in the laboratory dryer for 60 min were visible after the use of gentian violet, brilliant green, and crystalline violet. What is more, the meloxicam spots shown on the plates that were immersed in the visualization agents were more clearly visible than the spots on the plates sprayed with the same visualization agents. It was observed that the spots visible after immersion in Janus blue, methyl green, brilliant green, and gentian violet dried at room temperature and crystalline violet after drying in the laboratory dryer were the best for the analysis. The visual and densitometric evaluation of the chromatograms determined the choice of these visualization agents for further analysis of meloxicam (Table III). Figs. 1a-f show original photographs of chromatograms of meloxicam analyzed under applied chromatographic conditions and by using proposed visualization reagents.

Table IV summarizes the average values of *LOD* (and *LOQ*) of meloxicam and linearity range obtained on silica gel 60F<sub>254</sub> (SiO<sub>2</sub> 60F<sub>254</sub>) and neutral aluminum oxide 150F<sub>254</sub> (Al<sub>2</sub>O<sub>3</sub> 150F<sub>254</sub>) plates after detection using the best visualization agents. It can be observed that in the case of chromatographic plates precoated with neutral Al<sub>2</sub>O<sub>3</sub> 150F<sub>254</sub> the *LOD* values were the lowest without the use of a visualization agent (0.080 µg per spot). After the use of a proper visualization agent, the *LOD* value was for gentian violet 0.154 µg per spot and for Janus blue 0.192 µg per spot. Markedly higher *LOD* values were obtained using crystalline violet (0.394 µg per spot), brilliant green (0.697 µg per spot) as well as methyl green (0.706 µg per spot). The *LOD* of meloxicam on silica gel 60F<sub>254</sub> as the stationary phase was the lowest in the absence of a visualization agent and it was 0.063 µg per spot. Desain and Amin (6) reported a similar meloxicam *LOD* value on HPTLC plates (*LOD* 0.090 µg per spot). However, the best *LOD* of meloxicam on silica gel 60F<sub>254</sub> HPTLC plates of 0.023 µg per spot was obtained by Shaji and Varkey (9). Starek and Krzek (5) reported that *LOD* for meloxicam on silica gel 60F<sub>254</sub> is equal to 0.096 µg per spot. Moreover, it has been shown that by dipping in Janus blue, gentian violet, methyl green, and brilliant green and drying at room temperature, as well as dipping in crystalline violet and drying in the laboratory dryer, allow the identification of meloxicam due to the chromatographic spot color.

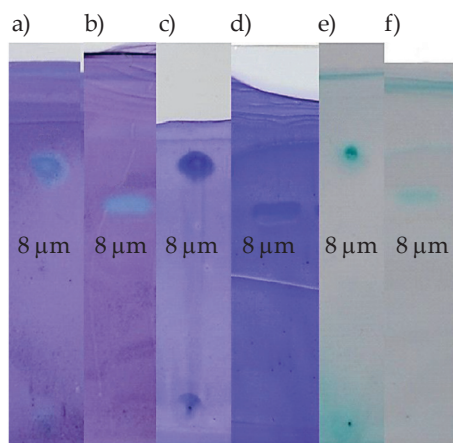


Fig. 1. Photographs of chromatograms of meloxicam analyzed on: a)  $\text{Al}_2\text{O}_3$  150F<sub>254</sub> and detected using Janus blue, b)  $\text{SiO}_2$  60F<sub>254</sub> and detected using Janus blue, c)  $\text{Al}_2\text{O}_3$  150F<sub>254</sub> and detected using crystalline violet, d)  $\text{SiO}_2$  60F<sub>254</sub> and detected using crystalline violet, e)  $\text{Al}_2\text{O}_3$  150F<sub>254</sub> and detected using brilliant green, f)  $\text{SiO}_2$  60F<sub>254</sub> and detected using brilliant green.

What is important, the lowest value of *LOD* for meloxicam was obtained on two applied sorbents without the use of any visualization agent. Of all visualization agents tested, the lowest detection limit of meloxicam was obtained using crystalline violet and gentian violet on silica gel 60F<sub>254</sub> and neutral aluminum oxide 150F<sub>254</sub> resp.

Table IV. Average values of the limit of detection (*LOD*) of meloxicam and linearity range obtained on  $\text{SiO}_2$  60F<sub>254</sub> and neutral  $\text{Al}_2\text{O}_3$  150F<sub>254</sub> plates after detection using the best visualization agents

Detection method	Sorbent	Average value of <i>LOD</i> ( $\mu\text{g}$ per spot) <sup>a</sup>	Linearity range ( $\mu\text{g}$ per spot)
No visualization agent	$\text{SiO}_2$ 60F <sub>254</sub>	$0.063 \pm 0.012$	0.2–5.0 ( $R = 0.989$ )
	$\text{Al}_2\text{O}_3$ 150F <sub>254</sub>	$0.080 \pm 0.011$	0.3–5.0 ( $R = 0.992$ )
Janus blue	$\text{SiO}_2$ 60F <sub>254</sub>	$0.272 \pm 0.058$	1.0–7.0 ( $R = 0.995$ )
	$\text{Al}_2\text{O}_3$ 150F <sub>254</sub>	$0.192 \pm 0.089$	0.7–6.0 ( $R = 0.992$ )
Gentian violet	$\text{SiO}_2$ 60F <sub>254</sub>	$0.436 \pm 0.074$	2.0–7.0 ( $R = 0.993$ )
	$\text{Al}_2\text{O}_3$ 150F <sub>254</sub>	$0.154 \pm 0.019$	0.5–5.0 ( $R = 0.991$ )
Brilliant green	$\text{SiO}_2$ 60F <sub>254</sub>	$0.149 \pm 0.097$	0.5–5.0 ( $R = 0.995$ )
	$\text{Al}_2\text{O}_3$ 150F <sub>254</sub>	$0.697 \pm 0.146$	3.0–7.0 ( $R = 0.996$ )
Methyl green	$\text{SiO}_2$ 60F <sub>254</sub>	$0.387 \pm 0.112$	2.0–8.0 ( $R = 0.993$ )
	$\text{Al}_2\text{O}_3$ 150F <sub>254</sub>	$0.706 \pm 0.135$	2.5–8.0 ( $R = 0.993$ )
Crystalline violet	$\text{SiO}_2$ 60F <sub>254</sub>	$0.105 \pm 0.089$	0.4–5.0 ( $R = 0.994$ )
	$\text{Al}_2\text{O}_3$ 150F <sub>254</sub>	$0.394 \pm 0.113$	2.0–7.0 ( $R = 0.990$ )

*LOD* – limit of detection, *R* – coefficient of correlation

<sup>a</sup> Mean  $\pm$  SD,  $n = 6$ .



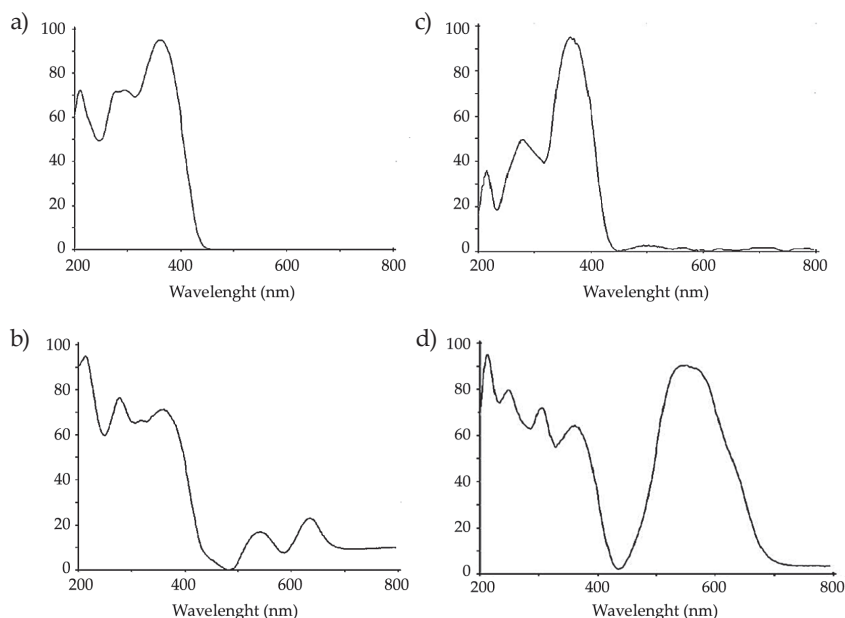


Fig. 2. A spectrum of meloxicam on: a) silica gel 60F<sub>254</sub> without the use of visualization agent, b) silica gel 60F<sub>254</sub> after detection with gentian violet, c) neutral aluminum oxide 150F<sub>254</sub> without the use of visualization agent, d) neutral aluminum oxide 150F<sub>254</sub> after detection with gentian violet.

The obtained spectrodensitograms of meloxicam indicate that applied visualization agents and the type of chromatographic plate can influence the wavelength of the obtained fundamental absorption band ( $I_{\max}$ ) and the additional absorption bands as well as their intensity values [AU]. The specific surface area of the stationary phase, its chemical composition/modification, the presence of a fluorescent additive, and other physicochemical characteristics affect the meloxicam spectra.

Generally, accurate and rapid identification of meloxicam as an API might rely on comparison with the spectrum of the reference standard. This might be assessed on silica gel 60F<sub>254</sub> and neutral aluminum oxide 150F<sub>254</sub> plates without the use of a visualization agent or after using gentian violet (Figs. 2a–d).

## CONCLUSIONS

Based on the presented results, it can be concluded that the optimization of chromatographic conditions including the type of sorbent as well as modifying the mobile phase composition may significantly improve the *LOD* and *LOQ* values of meloxicam. The best sensitivity of developed TLC-densitometric method in the NP-TLC system was obtained on neutral aluminum oxide 60F<sub>254</sub> and silica gel 60F<sub>254</sub> plates by using the mixture of ethyl acetate/toluene/*n*-butylamine (2:2:1, V/V/V) as the mobile phase. However, in the case of RP-TLC, the best results for *LOD* and *LOQ* were achieved on silica gel RP-8F<sub>254</sub> and RP-18F<sub>254</sub> plates using a mixture of methanol/water (8:2, V/V) as the mobile phase.

Of all visualization agents tested, the lowest detection limit of meloxicam enabled crystalline violet and gentian violet as new visualization agents on silica gel 60F<sub>254</sub> and neutral aluminum oxide 150F<sub>254</sub>, resp. Thus, the developed TLC-densitometric method may be successfully applied for the detection of meloxicam at low LOD and LOQ range ( $\mu\text{g}$  per spot). What is more, the colored spots and spectrodensitograms of meloxicam obtained by using an individual visualization reagent might possibly be auxiliary tools for the identification of meloxicam.

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#### REFERENCES

1. A. Walsem, R. Nixon, P. Guyot, A. Karabis and A. R. Moore, Relative benefit-risk comparing diclofenac to other traditional non-steroidal anti-inflammatory drugs and cyclooxygenase-2 inhibitors in patients with osteoarthritis or rheumatoid arthritis, *Arthritis Res. Ther.* **19** (2015) 66–72; <https://doi.org/10.1186/s13075-015-0554-0>
2. A. Pyka, Detection progress of selected drugs in TLC, *Biomed. Res. Int.* **2014** (2014) Article ID 732078 (19 pages); <https://doi.org/10.1155/2014/732078>
3. M. Dołowy and A. Pyka-Pająk, Development of new procedures for the detection and separation of salicylic acid and acetylsalicylic acid using thin-layer chromatography with densitometry, *J. Planar Chromatogr. – Modern TLC* **30** (2017) 363–374; <https://doi.org/10.1556/1006.2017.30.5.5>
4. W. Parys, K. Bober, A. Pyka-Pająk and M. Dołowy, The application of TLC and densitometry for quantitative determination of meloxicam in tablets, *Curr. Pharm. Anal.* **15** (2019) 785–794; <https://doi.org/10.2174/1573412915666190212155740>
5. M. Starek and J. Krzek, TLC determination of meloxicam in tablets and after acidic and alkaline hydrolysis, *Acta Pol. Pharm. Drug Res.* **69** (2012) 225–235.
6. N. Desai and P. Amin, Stability indicating HPTLC determination of meloxicam, *Indian J. Pharm. Sci.* **70** (2008) 644–647; <https://doi.org/10.4103/0250-474X.45406>
7. M. Starek, Separation and determination of four oxicams in pharmaceutical formulations by thin-layer chromatographic-densitometric method, *J. Planar Chromatogr. – Modern TLC* **24** (2011) 367–372; <https://doi.org/10.1556/JPC.24.2011.5.1>
8. H. Hopkała and A. Pomykalski, TLC analysis of inhibitors of cyclooxygenase and videodensitometric determination of meloxicam and tiaprofenic acid, *J. Planar Chromatogr. – Modern TLC* **16** (2003) 107–111; <https://doi.org/10.1556/JPC.16.2003.2.4>
9. J. Shaji and D. Varkey, Development of a validated stability-indicating HPTLC method for determination of meloxicam in bulk and pharmaceutical formulations: Pertinence to ICH guidelines, *Int. J. Pharm. Pharm. Sci.* **4** (2012) 160–169.
10. B. Mamatha, G. Ushasree and V. U. Maheswara Rao, Analytical techniques for estimation of meloxicam in bulk and pharmaceutical dosage forms: A review, *Int. J. Pharm. Res. Anal.* **5** (2015) 74–77.
11. A. Noreen, S. Ahmed, Z. Anwar and I. Ahmad, Analytical techniques for determination of meloxicam in pharmaceutical formulations and biological samples, *Baqai J. Health Sci.* **19** (2016) 59–74.
12. International Conference of Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use, *ICH Harmonized Tripartite Guideline, Validation of Analytical Procedures: Text and Methodology Q2(R1)*, ICH, Geneva 2005; <http://www.ich.org>; last access date March 15, 2019.