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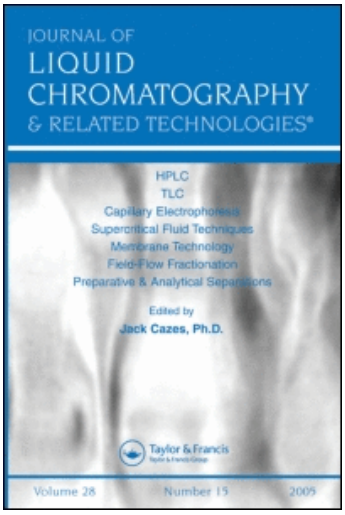

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Analytical Evaluation of Visualizing Reagents used to Detect Ibuprofen on Thin Layers

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Abstract: Six dyes as new visualizing reagents, namely, gentian violet, methylene violet, methylene blue, methyl green, malachite green, and Janus blue, have been used to detect ibuprofen on silica gel $60F_{254}$. Rhodamine B was used as the comparative visualizing reagent. The limit of detection (detectability), detection index, broadening index, modified contrast index, densitometric visualizing index, and linearity range were determined for ibuprofen following use of these visualizing reagents. It was stated that the earlier proposed densitometric visualizing index is an objective parameter describing the applied visualizing reagents. The best detection method for ibuprofen is the densitometric method without using a visualizing reagent. Among all studied new visualizing reagents, methylene violet is the best for detecting ibuprofen. The obtained visualizing effects and nondestructive properties of the applied visualizing reagents, in relation to investigated ibuprofen, indicate that progress in the range of analysis of ibuprofen on thin layer has taken place. This fact has definite analytical, pharmaceutical, and physicochemical significance.

Keywords: Broadening index, Densitometric visualizing index, Densitometry, Detectability, Detection index, Dyes, Ibuprofen, Modified contrast index, New visualizing reagents, NP-TLC, Pharmaceutical analysis

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INTRODUCTION

Currently, the most important field of application of thin layer chromatography is in pharmacy. The number of publications in the field of pharmacy has been steadily increasing.[1,2]

Many phenolic derivatives have definite pharmacological and biological properties. Ibuprofen is a non-steroidal anti-inflammatory drug. It is used for relief of symptoms of arthritis, primary dysmenorrhea, fever, and as an analgesic, especially where there is an inflammatory component. Ibuprofen may be useful in the treatment of severe orthostatic hypotension.[3]

In some studies, ibuprofen showed superior results when compared to a placebo in the prophylaxis of Alzheimer's disease, when given in low doses over a long time.[4] Ibuprofen has been also associated with a lower risk of Parkinson's disease, and may delay or prevent Parkinson's disease.[5]

Phenolic derivatives, including ibuprofen, can be investigated by thin-layer chromatography (TLC). Many visualizing reagents can be used to detect phenolic drugs on thin layers.[1,6] Phenolic compounds are characterized by electronic spectra in the UV range. Therefore, ibuprofen on a chromatographic plate can be detected in UV light.[7] Spots of ibuprofen were also located in an iodine chamber with the detection limit equal to 4.9 μg.[8] Generally, Rhodamine B as universally visualizing reagent, can be used to detect many organic compounds.[1]

We decided to examine a series of dyes as new visualizing reagents for their ability to detect ibuprofen on thin layer. This work also concerns the confirmation of the significance of the earlier proposed densitometric visualizing index[9] for the evaluation of the visualizing effects of ibuprofen.

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EXPERIMENTAL

Thin Layer Chromatography

TLC was performed on 10 cm × 20 cm aluminium plates precoated with 0.20 mm layers of silica gel $60F_{254}$ (E. Merck, #1.05554, lot: HX767953). The plates were prewashed with methanol and dried for 24 h at room temperature (20 ± 1°C). The plates were then activated at 120°C for 30 min. Standard solutions of ibuprofen (Sigma, USP, lot: 063K1117) containing 25.00, 20.00, 12.50, 15.00, 10.00, 7.50, 5.00, 2.50, 1.25, and 0.62 mg were prepared in 5 mL of 96% ethanol (POCh, Gliwice, Poland). The solutions of the studied compounds (5 μL) were spotted manually, using a microcapillary (Camag, Switzerland), onto the chromatographic plates. The mixture of chloroform and methanol

(50:1.4, v/v) as a mobile phase (50 mL) was placed in a classical chromatographic chamber (Camag, Switzerland) and, after saturation of the chamber with the mobile-phase vapor for 15 min, the plates were developed vertically at room temperature ($20 \pm 1°C$) to a distance of 7.5 cm. The plates were then dried for 20 h at room temperature ($20 \pm 1°C$) in a fume cupboard.

Visualizing Reagents Investigated

New Visualizing Reagents

Gentian violet (Fluka, Switzerland), methylene violet (Michrom, England), methylene blue (POCh, Poland), methyl green (POCh, Poland), malachite green (POCh, Poland), and Janus blue (Michrom, England) were used as 50 mg/100 mL solutions in distilled water.

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Comparative Visualizing Reagent

Rhodamine B was used as the comparative visualizing reagent.[1] Rhodamine B (POCh, Poland) reagent was used as a 50 mg/100 mL solution in distilled water.

The dried plates were dipped in particular visualizing reagent solutions for 15 sec. Then, after dipping in the solution of visualizing reagent, they were dried for 24 h at room temperature ($20 \pm 1°C$).

Spectrodensitometric Analysis

A spectrum scan was recorded using a Camag Scanner TLC 3 operated in absorbance mode and controlled by WinCATS 1.4.2 software. The radiation sources were a deuterium lamp emitting a continuous UV spectrum between 190 and 450 nm and a tungsten lamp emitting a spectrum between 370 and 800 nm. The starting wavelength was 200 nm and the ending wavelength was 700 nm. The slit dimensions were 8.00×0.40 mm, Macro; the optimized optical system was resolution; the scanning speed was $20\,\text{nm}\,\text{s}^{-1}$; the data resolution was $1\,\text{nm step}^{-1}$; the measurement type was emission; and the measurement mode was absorption; the optical filter was second order.

Densitometric Analysis

Densitometric scanning was then performed at respective absorption maxima (Table 2). The radiation source was a deuterium lamp emitting

a continuous spectrum between 190 and 450 nm. The slit dimensions were 8.00×0.40 mm, Macro; the optimized optical system was light; the scanning speed was $20\,\text{mm}\,\text{s}^{-1}$; the data resolution was $100\,\mu\text{m}$ step^{-1}; the measurement type was emission; and the measurement mode was absorption; the optical filter was second order. Each track was scanned three times and baseline correction (lowest slope) was used.

Modified Broadening Index, Detection Index, Modified Contrast Index, and Densitometric Visualizing Index

Broadening Index

The broadening index was modified and was calculated as:[10,11]

$$I_{broad} = \frac{25}{p_2} \times 1000 \quad \left[\frac{\mu g}{AU}\right] \tag{1}$$

where 25 μg of the analyzed substance in 5 μL of solution was applied to the chromatographic plate, and p_2 is the spot area [AU] of 25 μg of analyzed substance.

Detection Index

The detection index is defined as:[11]

$$I_{det} = \frac{m_1}{p_1} \quad \left[\frac{\mu g}{AU}\right] \tag{2}$$

where m_1 is the smallest quantity of substance detected [μg] with the visualizing reagent (limit of detection), and p_1 is the spot area of the substance [AU] at the limit of detection of the substance.

Modified Contrast Index

The modified contrast index was calculated as:[12]

$$I_{Contr(\text{mod if})} = \frac{h}{\alpha} \quad \left[\frac{AU}{^\circ}\right] \tag{3}$$

where h is the height of denstitometric band [AU] of 25 μg of analyzed substance, and α is the angle [°] between the tangents at the inflection points to the curves of the densitometric band of substance.

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Densitometric Visualizing Index

The densitometric visualizing index (*DVI*) was calculated as:[9]

$$DVI = \frac{p_2}{m_1 \times \alpha} \times 10^{-4} \quad \left[\frac{AU}{\mu g \cdot {}^\circ}\right] \tag{4}$$

where m_1 is limit of detection of the analyzed substance [μg], p_2 is the spot area [AU] of 25 μg of analyzed substance after the plate has been dipped in reagent solution, and α is the angle [°] between the tangents at the inflection points to the curves of the densitometric band of 25 μg of analyzed substance.

The broadening index, detection index, modified contrast index, and densitometric visualizing index were calculated for ibuprofen by use of the Eqs. (1), (2), (3), and (4), respectively.

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RESULTS AND DISCUSSION

Six new visualizing reagents (dyes), namely gentian violet, methylene violet, methylene blue, methyl green, malachite green, and Janus blue were used to detect the ibuprofen. However, Rhodamine B was used as comparative visualizing reagent for detection of ibuprofen. The colors of the chromatographic spots for ibuprofen and background colors, without use of a visualizing reagent and after detection with visualizing reagents on silica gel $60F_{254}$, are presented in Table 1. It was stated that studied ibuprofen without use of a visualizing reagent is invisible on the chromatogram in visible light. Spots of ibuprofen after the detection with the use of specific visualizing reagents are visible on the chromatograms. All obtained chromatographic spots of ibuprofen after the application of particular visualizing reagents were durable and visible for over 6 weeks. The visual limits of detection of ibuprofen after their detection with the use of particular visualizing reagents on silica gel $60F_{254}$ are also presented in Table 1.

Ibuprofen analyzed on silica gel $60F_{254}$ without use of a visualizing reagent and after detection with visualizing reagents were densitometrically and spectrodensimetrically evaluated. Spectrodensitogram characteristics of ibuprofen investigated on silica gel $60F_{254}$ are presented in Table 2. It was stated that the fundamental absorption band of ibuprofen without use of a visualizing reagent and after use following visualizing reagents, namely Rhodamine B, methylene violet, methylene blue, methyl green, malachite green, and Janus blue occurs at the wavelength equal to 200 nm. The fundamental absorption band of ibuprofen after detection

Table 1. Color of spot of ibuprofen, visual limit detection and background color after the detection using visualizing reagents on silica gel $60F_{254}$

Detection way	Spot color of ibuprofen	Background color	Visual limit detection of ibuprofen (μg/spot)
Without using visualizing reagent	Lack of colored spot in visible light	White	–
Rhodamine B	Light pink with dark pink border	Pink	2.50
Gentian violet	Light violet	Violet	0.62
Methylene violet	White-blue with dark blue border	Light	1.25
Methylene blue	Dark blue with white border	Blue	0.62
Methyl green	White-green with dark green border	Green-blue	0.62
Malachite green	White with dark green border	Green-blue	0.62
Janus blue	Light blue	Dark blue	0.62

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with the use of gentian violet occurs at 224 nm. It was found that the spectrodensitograms of ibuprofen on silica gel $60F_{254}$ plates and by use of particular visualizing reagents are different than the spectrodensitogram obtained on the plate without use of a visualizing reagent. The obtained spectrodensitograms of ibuprofen after the detection with the use of particular investigated visualizing reagents differ in the number and intensity of additional absorption bands. This fact has analytical and pharmaceutical significance in the identification of ibuprofen. The densitometric analysis of ibuprofen was performed at respective absorption maxima, which are given in Table 2.

The broadening indices for ibuprofen are presented in Table 3. A good visualizing reagent has a relatively large numerical value of modified broadening index for a particular substance detected (small spot area, which refers to 25 μg of a substance detected). The R_F value of ibuprofen investigated on silica gel $60F_{254}$ is equal to 0.61. The detection indices of ibuprofen investigated are also presented in Table 3. The detection index indicates the ratio of the minimal number of micrograms of ibuprofen to the area of the chromatographic spot, in AU. The area of the spot was assessed by the densitometric method.

The densitometric limits of detection of ibuprofen, without and with visualizing reagents tested, linearity range, densitometric visualizing index, and densitometric band characteristic of 25 μg investigated ibuprofen on silica gel 60 F_{254} are presented in Table 4. The densitometric

Table 2. Spectrodensitogram characteristics of ibuprofen on silica gel $60F_{254}$

Detection way of ibuprofen	Fundamental absorption band λ_{max} [nm][a]	Remaining absorption bands	
		λ [nm]	Intensity [AU]
Without using visualizing reagent	200	223	89.4
		265	15.9
		396	5.6
Rhodamine B	200	223	88.7
		266	7.3
		273	6.4
		325	2.7
		395	8.9
		444	8.2
		550	3.5
		599	21.4
Gentian violet	224	200	85.1
		266	54.5
		338	55.9
		406	60.0
		700	72.5
Methylene violet	200	224	85.8
		266	10.8
		396	5.9
		531	9.5
		571	10.8
		622	10.3
Methylene blue	200	223	85.5
		260	20.2
		320	22.4
		341	33.4
		449	35.9
		523	31.3
		619	2.4
		700	43.5
Methyl green	200	224	85.6
		265	7.1
		322	10.4
		349	4.6
		448	12.0
		473	22.0
		644	22.2
		224	93.7

(*Continued*)

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Table 2. Continued

Detection way of ibuprofen	Fundamental absorption band λ_{max} [nm][a]	Remaining absorption bands	
		λ [nm]	Intensity [AU]
Malachite green	200	265	32.5
		341	30.0
		403	20.8
		470	37.4
		681	36.7
		700	52.0
Janus blue	200	221	78.7
		249	41.3
		338	88.8
		420	90.6
		700	93.7

[a]Intensity of all absorption maximum is equal to 95 AU.

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evaluation of obtained densitometric bands of 25 μg ibuprofen was described by the area of the densitometric band [AU], the densitometric band height [AU], and the angle (α) between the tangents at the inflection points to the curves of the densitometric band, formulated in degrees [°]. The modified contrast index indicates the ratio of the height of the densitometric band [AU] of detected compound to the angle (α) between the tangents at the inflection points to the curves of the densitometric band formulated in degrees [°]. The best visualizing reagent has the highest value of the modified contrast index.

Table 3. Broadening index and detection index for ibuprofen detected on silica gel $60F_{254}$

Detection way	Broadening index (μg/AU)	Detection index (μg/AU)
Without using visualizing reagent	0.449	0.62/3325
Rhodamine B	0.656	1.25/2453
Gentian violet	1.092	5/7523
Methylene violet	0.466	1.25/4438
Methylene blue	0.816	2.5/4037
Methyl green	1.003	2.5/5893
Malachite green	1.459	2.5/1511
Janus blue	9.720	25/2572

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Table 4. Characteristic of densitometric band, modified contrast index, densitometric limit of detection, densitometric visualizing index, and linearity range of ibuprofen on silica gel $60F_{254}$

Detection way	Densitometric band characteristic of 25 μg ibuprofen			Modified contrast index $\left[\frac{AU}{°}\right]$	Limit of detection [g]	Densitometric visualizing index $\left[\frac{AU}{\mu g \cdot °}\right]$	Linearity range [μg spot^{-1}] (r, correlation coefficient)
	Area [AU]	Height [AU]	α [°]				
Without using visualizing reagent	55644	445	26	17.115	0.62	0.342	1.25 ÷ 10.00 (r = 0.9958)
Rhodamine B	38109	231	42	5.500	1.25	0.072	2.50 ÷ 25.00 (r = 0.9982)
Gentian violet	22883	136	117	1.162	5.0	0.004	10.00 ÷ 20.0 (r = 0.9977)
Methylene violet	53696	348	35	9.943	1.25	0.123	5.00 ÷ 25.00 (r = 0.99549)
Methylene blue	30616	203	53	3.830	2.50	0.023	5.00 ÷ 25.00 (r = 0.9936)
Methyl green	24914	162	78	2.077	2.50	0.013	5.0 ÷ 20.00 (r = 0.9936)
Malachite green	17132	124	89	1.393	2.50	0.008	5.00 ÷ 15.00 (r = 0.9896)
Janus blue	2572	36	113	0.318	25.00	0.00009	-

The densitometric visualizing index contains two very important characteristics of a densitometric band of a 25 μg studied substance, namely the area of the densitometric band [AU], the angle (α) between the tangents at the inflection points to the curves of the densitometric band, formulated in degrees [°]. The limit of detection of studied substance is the third most important element, which contains the densitometric visualizing index. The best way for substance detection has higher values of densitometric visualizing index.[9]

Obtained results in this work indicate that the best detection method for ibuprofen is the densitometric method without use a visualizing reagent. The densitometric limit of detection of ibuprofen is equal to 0.62 μg, and the linearity range is from 1.25 μg to 10.00 μg. However, among all studied new visualizing reagents, methylene violet is the best for detection ibuprofen. The densitometric limit of detection of ibuprofen after detection with methylene violet is equal to 1.25 μg, and the linearity range is from 5.00 μg to 25.00 μg.

It was stated that all applied ways of detection permit obtaining a linear dependence between the area of the densitometric band and the quantity of spotted ibuprofen; the Janus blue is the exception. The range of linearity varies for ibuprofen and depends on the particular applied visualizing reagents.

It was confirmed that the earlier proposed densitometric visualizing index is the objective parameter for evaluation of usefulness of definite visualizing reagents for the detection of ibuprofen. The visualizing reagents proposed in this work should serve as a supplement to those used previously for the detection of ibuprofen. The study also provides information about the analytical and pharmaceutical importance of the new proposed visualizing reagents. The visualizing reagents proposed in this work, i.e., dyes, are not universal visualizing reagents. In earlier work, the five dyes, namely gentian violet, methylene violet, methylene blue, malachite green, and Janus blue, were tried for nicotinamide detection. The above-mentioned visualizing reagents did not give coloured chromatographic spots with nicotinamide.[13] The applied new visualizing reagents are non-destructive reagents. This fact has definite analytical, pharmaceutical, and physicochemical significance. The obtained visualizing effects and non-destructive properties of applied visualizing reagents, in relation to investigated ibuprofen, indicate that rogress in range of analysis of ibuprofen on thin layer has taken place.

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