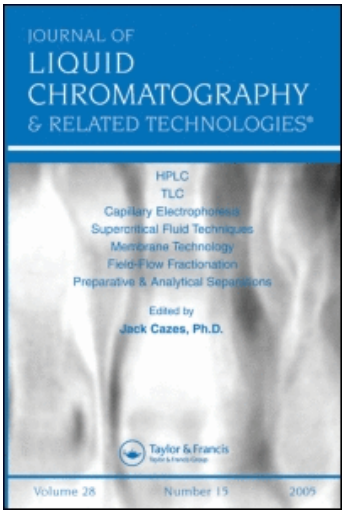

Journal of Liquid Chromatography & Related Technologies
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Analytical and Densitometric Evaluation of Visualizing Reagents of Selected Aliphatic Compounds on Thin Layer

A. Pyka[a]; W. Klimczok[a]
[a] Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, Sosnowiec, Poland

To cite this Article Pyka, A. and Klimczok, W.(2008) 'Analytical and Densitometric Evaluation of Visualizing Reagents of Selected Aliphatic Compounds on Thin Layer', Journal of Liquid Chromatography & Related Technologies, 31: 10, 1492 — 1510
To link to this Article: DOI: 10.1080/10826070802039556
URL: http://dx.doi.org/10.1080/10826070802039556

Journal of Liquid Chromatography & Related Technologies®, 31: 1492–1510, 2008

ISSN 1082-6076 print/1520-572X online
DOI: 10.1080/10826070802039556

Analytical and Densitometric Evaluation of Visualizing Reagents of Selected Aliphatic Compounds on Thin Layer

A. Pyka and W. Klimczok

Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, Sosnowiec, Poland

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 stearic acid, stearyl alcohol, and methyl stearate on silica gel 60. 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 stearic acid, stearyl alcohol, and methyl stearate 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 obtained results in this work indicate that all of the studied new visualizing reagents for detection of stearic acid, stearyl alcohol, and methyl stearate are better visualizing reagents in comparison with universally applied Rodamine B to detect these lipophilic compounds. The best visualizing reagents for quantitative determination of stearic acid are methylene blue and Janus blue. The best visualizing reagents for quantitative determination of stearyl alcohol are malachite green and Janus blue. The best visualizing reagents for quantitative determination of methyl stearate are methylene blue, Janus blue, and malachite green.

Keywords: NP-TLC, Stearic acid, Stearyl alcohol, Methyl stearate, New visualizing reagents, Dyes, Densitometry, Detectability, Broadening index, Detection index, Modified contrast index, Densitometric visualizing index

Correspondence: A. Pyka, Faculty of Pharmacy, Department of Analytical Chemistry, Medical University of Silesia, 4, Jagiellonska Str., PL-41-200 Sosnowiec, Poland. E-mail: apyka@slam.katowice.pl

INTRODUCTION

Higher fatty acids, higher fatty alcohols, and fatty acid methyl esters are used indirectly in a wide range of food, pharmaceutical, cosmetic, and industrial applications. Stearic acid is useful as an ingredient in marking candles, soaps, plastics, oil pastels, and cosmetics, and for softening rubber. Stearic acid also has medical and biological significance. Stearyl alcohol has a wide range of uses as an ingredient in lubricants, resins, perfumes, and cosmetics. It is also used as an emollient, emulsifier, and thickener in ointments of various sorts, and is widely used as a hair coating in shampoos and hair conditioners.

Higher fatty acids, higher fatty alcohols, and fatty acid methyl esters can be investigated by thin-layer chromatography. These compounds are characterized by very poor electronic spectra in the UV range.[1] Because of this, it is not possible to study these compounds, especially after their chromatographic separation by TLC, by a densitometric method. Therefore, respective visualizing reagents can be used to detect the above mentioned compounds. For example, iodine, Rhodamine B, sulfuric acid, 2′,7′-dichlorofluorescein-aluminum chloride-ferric chloride reagent,[2] berberin-reagent, Tillmanns-reagent, *tert*-butylhypochlorite,[3] uranyl acetate,[4] phosphomolybdic acid, bromothymol blue,[5] fuchsine dyes[6] have been used to detect free fatty acids; vanillin-sulfuric acid reagent and, Rhodamine B were used for detection of higher fatty alcohols.[5] Generally, Rhodamine B and Rhodamine 6G can be used to detect lipophilic substances.[1,3,7]

In earlier our papers, we described many alkacymetric, and redoxymetric indicators as new visualizing reagents for the detection of selected fatty derivatives, including stearyl alcohol,[8,9] stearic acid,[9,10] and methyl stearate[11] on silica gel. On silica gel $60F_{254}$, 1 μg stearyl alcohol could be detected with thymol blue,[9] 0.5 μg stearic acid could be detected with thymol blue, bromophenol blue, aniline blue, and alkaline blue,[9,10] and 3 μg methyl stearate could be detected with erythrosine B.[11]

We decided to examine a series of dyes as new visualizing reagents for their ability to detect selected aliphatic compounds on thin layers. This work also concerns the conformation of the significance of the earlier proposed densitometric visualizing index[12] for the evaluation of visualizing effects of detected compounds.

EXPERIMENTAL

Thin Layer Chromatography

TLC was performed on 10 cm × 20 cm aluminium plates precoated with 0.20 mm layers of silica gel 60 (E. Merck, #1.05553). The plates were prewashed with methanol-chloroform (1:1, v/v) and dried for 24 h at room temperature

(18 ± 1°C). The plates were then activated at 120°C for 30 min. Standard solutions of:

- stearic acid (Sigma-Aldrich) containing 28.40, 22.72, 18.18, 14.54, 11.63, 9.31, 7.44, 5.96, 4.76, 3.81, 3.05, 2.44, 1.95, 1.56, 1.25, 1.00, 0.80, 0.64, 0.51, 0.40, 0.33, 0.20, 0.12, and 0.07 mg;
- stearyl alcohol (Sigma-Aldrich) containing 27.00, 21.60, 17.28, 13.82, 11.06, 8.85, 7.08, 5.66, 4.53, 3.62, 2.90, 2.32, 1.86, 1.48, 0.89, 0.53, 0.32, 0.19, and 0.11 mg;
- methyl ester of stearic acid (Sigma-Aldrich) containing 29.80, 23.84, 19.07, 15.26, 12.21, 9.76, 7.81, 6.25, 5.00, 4.00, 3.20, 2.56, 2.05, 1.64, 0.98, 0.59, and 0.35 mg were prepared in 5 mL chloroform.

The solutions of the studied compounds (5 μL) were spotted manually, using a microcapillary (Camag, Switzerland), onto the chromatographic plates. The plate with stearic acid was developed with a mixture of n-hexane-acetone (40:10, *v*/*v*) as mobile phase. The plate with stearyl alcohol was developed with a mixture of n-hexane-ethyl acetate-methanol (46:3.5:0.5, *v*/*v*/*v*) as mobile phase. The plate with the methyl ester of stearic acid was developed with a mixture of n-hexane-ethyl acetate (46:4, *v*/*v*) as the mobile phase. Mobile phase (50 mL) was placed in a classical chromatographic chamber (Camag, Switzerland) and, after saturation of the chamber with respective mobile-phase vapor for 30 min, the plates were developed vertically, at room temperature (18 ± 1°C), to a distance of 7.5 cm. The plates were then dried for 24 h at room temperature (18 ± 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 distillated water.

Comparative Visualizing Reagent

Rhodamine B was used as the comparative visualizing reagent.[1,3] Rhodamine B (POCh, Poland) reagent was used as 50 mg/100 mL solutions in distillated water.

The dried plates were dipped in particular visualizing reagent solutions for 5 sec. Then, after dipping in solution of visualizing reagents, they were dried for 24 h at room temperature (18 ± 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. Starting wavelength was 200 nm and ending wavelength was 800 nm. The slit dimensions were 12.00 × 0.90 mm, Macro; the optimized optical system was resolution; the scanning speed was 20 nm s^{-1}; the data resolution was 1 nm $step^{-1}$; the measurement type was remission; and the measurement mode was absorption; the optical filter was second order.

Densitometric Analysis

Densitometric scanning was then performed at respective absorption maximum (Table 1). The radiation source was a tungsten lamp emitting a continuous spectrum between 370 and 800 nm. The slit dimensions were 12.00 × 0.90 mm, Macro; the optimized optical system was light; the scanning speed was 20 mm s^{-1}; the data resolution was 100 μm $step^{-1}$; the measurement type was remission; 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[6,13]

The broadening index was modified and was calculated as

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

where m is weight of 0.1 μmol of the analyzed substance in 5 μL of solution was applied to the chromatographic plate, and p_2 is the spot area [AU] of 0.1 μmol of analyzed substance after the plate had been dipped into reagent solution.

Detection Index[6,13]

The detection index is defined as:

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

where m_1 is the smallest quantity of substance detected (μg) with the

Table 1. Spectrodensitogram characteristics of investigated aliphatic compounds on silica gel 60

	Detected compound								
	Stearic acid			Stearyl alcohol			Methyl stearate		
		Remaining absorption bands			Remaining absorption bands			Remaining absorption bands	
Visualizing reagent	λ_{max} (nm)[a]	λ (nm)	Intensity (AU)	λ_{max} (nm)[a]	λ (nm)	Intensity (AU)	λ_{max} (nm)[a]	λ (nm)	Intensity (AU)
Rhodamine B	585	243	52.3	586	243	37.6	587	243	34.1
		268	80.7		270	58.3		270	54.4
		288	65.8		317	68.5		318	79.7
		316	83.3		364	63.7		366	70.5
		362	63.2		407	48.3		412	50.4
		408	40.3		430	49.0		429	51.4
		430	38.9		485	33.8		481	14.9
		488	26.3		520	40.2		519	11.9
		521	40.7						
		556	46.2						
Gentian violet	602	214	24.6	610	218	25.2	605	216	21.3
		251	14.9		254	32.3		254	21.5
		306	33.0		310	46.0		310	38.2
		429	6.0		358	13.8		358	10.5
		548	59.2		373	13.7		375	11.8
					476	3.6		548	36.6
					547	27.8			

Methylene violet	616	208	60.3	616	286	77.7	618	226	25.5
		272	34.4		571	69.1		263	29.1
		289	37.8					290	40.6
								571	75.0
Methylene blue	687	251	37.3	689	218	23.8	686	211	19.8
		299	53.3		252	27.0		252	22.0
		333	34.3		305	38.7		275	15.0
		417	29.7		335	39.8		303	33.9
		623	44.7		405	33.0		334	28.7
		656	56.8		621	18.0		405	19.3
		795	38.3					504	16.1
								619	15.5
Methyl green	663	215	20.9	664	216	20.3	666	216	19.8
		261	12.2		265	20.1		264	18.2
		321	39.4		321	36.5		322	37.0
		441	33.0		445	30.7		446	29.8
		595	41.2		548	31.5		545	19.3
					592	43.7		595	28.4
Malachite green	640	215	16.9	644	217	33.4	643	217	30.0
		256	7.8		260	30.0		260	24.1
		321	30.4		303	28.6		302	24.2
		441	29.3		324	46.1		323	41.3
		579	21.1		447	44.3		447	43.1
					575	11.8		574	12.9

(*continued*)

Table 1. Continued

	Detected compound								
	Stearic acid			Stearyl alcohol			Methyl stearate		
		Remaining absorption bands			Remaining absorption bands			Remaining absorption bands	
Visualizing reagent	λ_{max} (nm)[a]	λ (nm)	Intensity (AU)	λ_{max} (nm)[a]	λ (nm)	Intensity (AU)	λ_{max} (nm)[a]	λ (nm)	Intensity (AU)
Janus blue	617	233	62.9	665	236	46.9	670	235	20.7
		261	55.0		263	45.1		262	20.2
		285	67.7		286	54.4		285	24.8
		397	20.8		308	46.3		311	28.7
		660	94.3		398	24.4		401	23.2
					618	81.3		440	13.5
								570	17.7
								619	55.2

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

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[14]

The modified contrast index was calculated as

$$I_{Contr(modif)} = \frac{h}{\alpha} \qquad \left[\frac{AU}{o}\right] \tag{3}$$

where *h* is the height of denstitometric band (AU) of 0.1 μmol of analyzed substance, and α is the angle (°) between the tangents at the inflection points to the curves of the densitometric band of substance.

Densitometric Visualizing Index[12]

The densitometric visualizing index (DVI) was calculated as

$$DVI = \frac{p_2}{m_1 \times \alpha} \times 10^{-4} \qquad \left[\frac{AU}{\mu g.^o}\right] \tag{4}$$

where m_1 is limit of detection of the analyzed substance (μg), p_2 is the spot area (AU) of 0.1 μmol 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 0.1 μmol of analyzed substance.

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

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 selected aliphatic compounds, namely stearic acid, stearyl alcohol, and methyl stearate. However, Rhodamine B was used as comparative visualizing reagent for detection of selected aliphatic compounds. Those compounds cannot be densitometricly detected without the use of a visualizing reagent. Spectrodensitogram characteristics of the investigated aliphatic compounds on silica gel 60 are presented in Table 1. It was stated that the studied compounds, after their detection with the use of investigated visualizing reagents, have the absorption maximum at similar wavelengths. The detection of stearic acid, stearyl alcohol, and methyl stearate with the use of Janus blue is an exception. Specifically, the absorption maximum for stearic acid, stearyl alcohol, and

methyl stearate is equal to 617 nm, 665 nm, and 670 nm, respectively. The obtained spectrodensitograms of studied compounds after the detection with the use of particular investigated visualizing reagents differ in the number and intensity of additional absorption bands. The densitometric analyses were performed at respective absorption maxima, which are given in Table 1. The colors of chromatographic spots for the investigated aliphatic compounds and background colors, without use of a visualizing reagent and after detection with visualizing reagents on silica gel 60, are presented in Table 2. It was stated that the studied aliphatic compounds without use of visualizing reagent are invisible on the chromatogram in visible light. Spots of stearic acid, stearyl alcohol, and methyl stearate after the detection with the use of specific visualizing reagents are visible on the chromatograms. However, the colored spots of stearic acid, stearyl alcohol, and methyl stearate are similar with the use of a definite visualizing reagent. All obtained chromatographic spots of investigated aliphatic compounds after the application of particular visualizing reagents were durable and visible for over 6 weeks.

The broadening index was calculated in this work. 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 0.1 μmol of a substance detected). The broadening indices for the investigated compounds are presented in Table 3. The R_F values of the stearic

Table 2. Color of spot of studied compounds and background color after the detection using visualizing reagents on silica gel 60

Detection way	Spot color of stearic acid	Stearyl alcohol	Methyl stearate	Background color
Without using visualizing reagent	Lack of colored spot in visible light	Lack of colored spot in visible light	Llack of colored spot in visible light	White
Rhodamine B	Dark pink	Dark pink	Dark pink	Pink
Gentian violet	Dark violet	Dark violet	Dark violet with light border	Violet
Methylene violet	Light blue	Light blue	Light blue	Light grey-blue
Methylene blue	Blue with light border	Light blue	Blue with white wide border	Blue
Methyl green	Dark green	Dark green	Dark green with light border	Green
Malachite green	Green with light border	Green with light border	Intensively green with light border	Green
Janus blue	Navy blue with white border	Navy blue	Navy blue with white border	Blue

Table 3. Broadening index and detection index for studied compounds detected on silica gel 60

	Stearic acid		Stearyl alcohol		Methyl stearate	
Detection way	Broadening index (μg/[AU)	Detection index (μg/[AU)	Broadening index (μg/[AU)	Detection index (μg/[AU)	Broadening index (μg/[AU)	Detection index (μg/[AU)
Without using visualizing reagent	—	—	—	—	—	—
Rhodamine B	4.32	1.25/900	7.49	1.48/234	17.41	5.00/320
Gentian violet	0.96	1.25/720	2.34	1.48/776	2.62	1.64/136
Methylene violet	5.68	0.80/380	4.87	1.48/766	9.95	0.98/323
Methylene blue	0.92	0.07/245	1.62	1.48/4307	2.15	0.98/750
Methyl green	1.03	0.64/391	3.13	1.48/807	3.77	1.64/279
Malachite green	0.89	1.00/2899	2.18	0.32/382	2.18	0.98/873
Janus blue	0.79	0.12/604	2.46	0.32/214	2.52	0.59/1155

acid, stearyl alcohol, and methyl stearate investigated on silica gel 60 are equal to about 0.38, 0.20, and 0.60, respectively. The detection indices of aliphatic compounds investigated are also presented in Table 3. The detection index indicates the ratio of the minimal number of micrograms of the detected compound to the area of the chromatographic spot, in AU. The area of the spot was assessed by the densitometric method.

The limits of detection of the aliphatic compounds investigated with visualizing reagents tested, linearity range, densitometric visualizing index, and densitometric band characteristic of 0.1 μmol investigated stearic acid, stearyl alcohol, and methyl stearate on silica gel 60 are presented in Tables 4, 5, and 6, respectively. The densitometric evaluation of obtained densitometric bands of 0.1 μmol aliphatic compound 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 (°). To depict the contrast index[13] the following test of aliphatic compound was carried out: 0.1 μmol of stearic acid, stearyl alcohol, and methyl stearate were dropped, in turn, on the starting line; next, their mobile phase was evolved and, afterwards, each spot was developed by means of another visualizing reagent. The contrast index represents two independent values, namely the angles (α) between the tangents at the inflection points to the curves of the densitometric band, formulated in degrees, and the densitometric band high (AU).[13] Earlier, we proposed the modified contrast index was for evaluation of visualizing reagents to detect estradiol.[14] 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.

In this work, we applied earlier proposed densitometric visualizing index[12] for the conformation of its significance to the evaluation of visualizing effects of studied aliphatic compounds detected with the use of investigated dyes. The densitometric visualizing index contains two most important characteristics of a densitometric band of a 0.1 μmol 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 of substance detection has higher values of densitometric visualizing index. Obtained results in this work indicate that all studied new visualizing reagents, namely gentian violet, methylene violet, methylene blue, methyl green, malachite green, and Janus blue, for detection stearic acid, stearyl alcohol, and methyl stearate, are better visualizing reagents in comparison with universally applied

Table 4. Characteristic of densitometric band, modified contrast index, limit of detection, densitometric visualizing index, and linearity range of stearic acid on silica gel 60

Detection way	Densitometric band characteristic of 0.1 μmol stearic acid			Modified contrast index (AU/o)	Limit of detection (μg)	Densitometric visualizing index (AU/μg·°)	Linearity range (μg spot^{-1}) (r, correlation coefficient)
	Area (AU)	Height (AU)	α (°)				
Rhodamine B	6567	82	31	2.645	1.25	0.017	1.56 ÷ 28.40(r = 0.9913)
Gentian violet	29550	403	11	36.636	1.25	0.215	1.95 ÷ 18.18 (r = 0.9989)
Methylene violet	5001	70	18	3.889	0.80	0.035	1.00 ÷ 18.18 (r = 0.9954)
Methylene blue	30918	368	14	26.286	0.07	3.155	0.41 ÷ 7.44 (r = 0.9975)
Methyl green	27452	386	11	35.091	0.64	0.390	0.80 ÷ 11.63 (r = 0.9953)
Malachite green	31748	456	12	38.000	1.00	0.265	4.76 ÷ 22.72 (r = 0.9953)
Janus blue	36175	462	11	42.000	0.12	2.741	1.56 ÷ 7.44 (r = 0.9928)

Table 5. Characteristic of densitometric band, modified contrast index, limit of detection, densitometric visualizing index, and linearity range of stearyl alcohol on silica gel 60

Detection way	Densitometric band characteristic of 0.1 μmol stearyl alcohol			Modified contrast index (AU/°)	Limit of detection (μg)	Densitometric visualizing index (AU/μg·°)	Linearity range (μg spot^{-1}) (r, correlation coefficient)
	Area (AU)	Height (AU)	α (°)				
Rhodamine B	3605	100	13	7.692	1.48	0.019	2.90 ÷ 27.00 (r = 0.9964)
Gentian violet	11549	305	7	43.571	1.48	0.111	2.90 ÷ 27.00 (r = 0.9970)
Methylene violet	5540	132	9	14.667	1.48	0.042	1.86 ÷ 11.06 (r = 0.9940)
Methylene blue	16647	372	7	53.143	1.48	0.161	7.08 ÷ 17.28 (r = 0.9776)
Methyl green	8638	242	7	34.571	1.48	0.083	2.32 ÷ 7.08 (r = 0.9945)
Malachite green	12378	333	7	47.571	0.32	0.553	0.89 ÷ 8.85 (r = 0.9959)
Janus blue	10963	269	13	20.692	0.32	0.264	0.53 ÷ 7.08 (r = 0.9995)

Table 6. Characteristic of densitometric band, modified contrast index, limit of detection, densitometric visualizing index, and linearity range of methyl stearate on silica gel 60

Detection way	Densitometric band characteristic of 0.1 μmol methyl stearate			Modified contrast index (AU/o)	Limit of detection (μg)	Densitometric visualizing index (AU/μg·°)	Linearity range (μg spot^{-1}) (r, correlation coefficient)
	Area (AU)	Height (AU)	α (°)				
Rhodamine B	1712	57	12	4.750	5.00	0.003	6.25 ÷ 29.80 (r = 0.9961)
Gentian violet	11364	381	6	63.500	1.64	0.115	2.56 ÷ 19.07 (r = 0.9972)
Methylene violet	2996	75	11	6.818	0.98	0.028	1.64 ÷ 12.21 (r = 0.9969)
Methylene blue	13829	401	6	66.833	0.98	0.235	1.64 ÷ 5.00 (r = 0.9959)
Methyl green	7903	253	7	36.143	1.64	0.069	2.05 ÷ 15.26 (r = 0.9984)
Malachite green	13651	383	8	47.875	0.98	0.174	1.64 ÷ 19.07 (r = 0.9973)
Janus blue	11837	238	11	21.636	0.59	0.182	2.05 ÷ 7.81 (r = 0.9958)

Rhodamine B to detect the lipophilic compounds. The best visualizing reagents for quantitative determination of stearic acid are methylene blue and Janus blue. The densitograms of 28.40 μg (0.1 μmol) stearic acid after detection with Rhodamine B and methylene blue are presented in Figures 1a and 1b, respectively. The best visualizing reagents for quantitative determination of stearyl alcohol are malachite green and Janus blue. The densitograms of 27.00 μg (0.1 μmol) stearyl alcohol after detection with Rhodamine B and malachite green are presented in Figures 2a and 2b, respectively. The best visualizing reagents for quantitative determination of methyl stearate are methylene blue, Janus blue, and malachite green. The densitograms of 29.80 μg (0.1 μmol) methyl stearate after detection

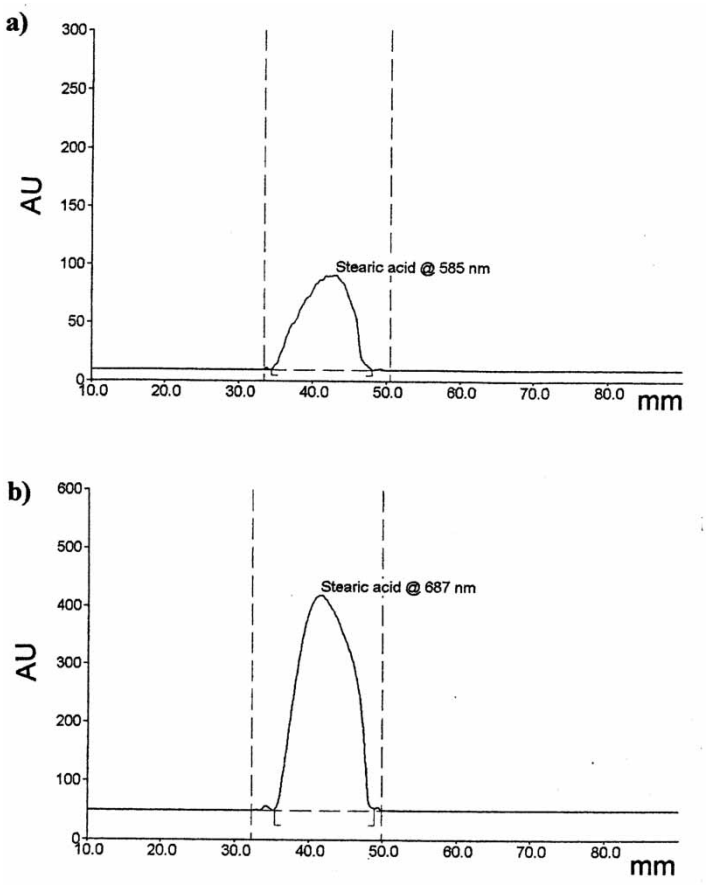

Figure 1. Densitograms of stearic acid after detection with (a) Rhodamine B; (b) methylene blue.

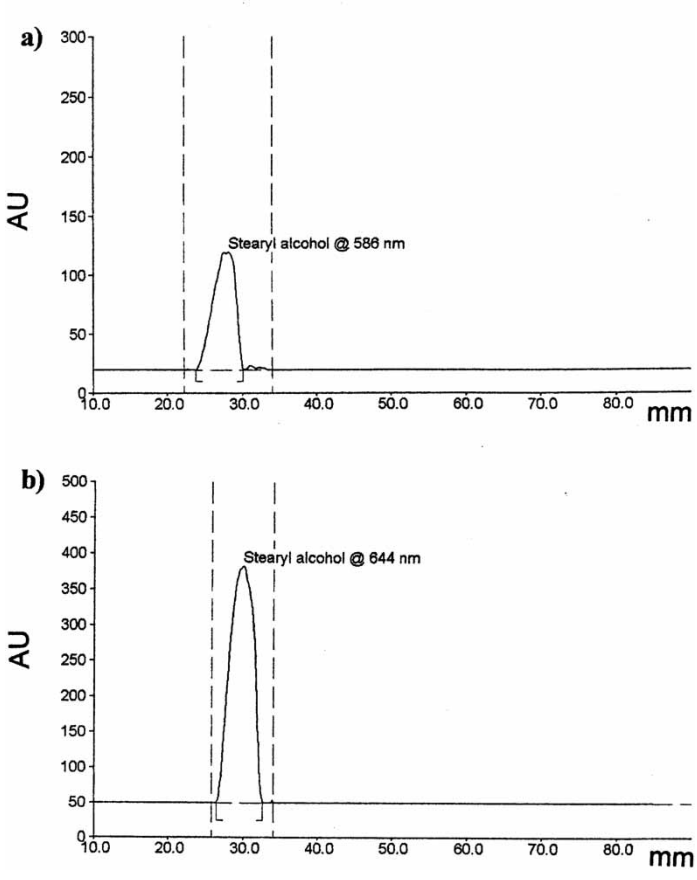

Figure 2. Densitograms of stearyl alcohol after detection with (a) Rhodamine B; (b) malachite green.

with Rhodamine B and methylene blue are presented in Figures 3 a and 3b, respectively.

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 to be used for nicotinamide detection. The above-mentioned visualizing reagents did not give coloured chromatographic spots with nicotinamide.[15] The applied new visualizing reagents are non-destructive reagents. This fact has definite analytical and physicochemical significance. The obtained visualizing effects and non-destructive properties of applied visualizing reagents, in relation to investigated aliphatic compounds, indicate that progress in range of analysis of stearic acid, stearyl alcohol, and methyl stearate on thin layer has taken place.

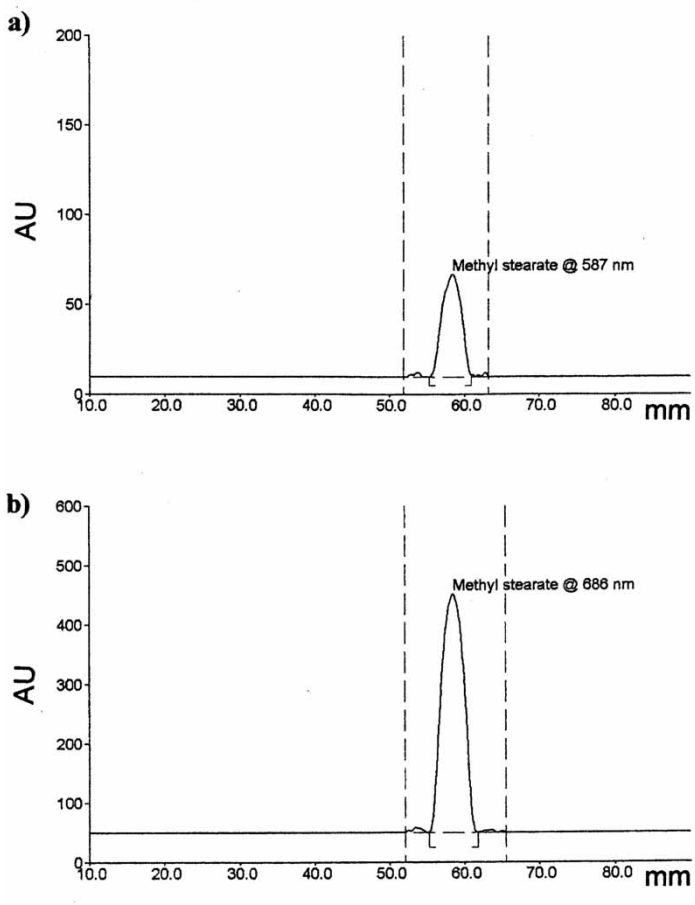

Figure 3. Densitograms of methyl stearate after detection with (a) Rhodamine B; (b) methylene blue.

CONCLUSION

The visualizing reagents proposed in this work should serve as supplemental to those used previously for the detection of aliphatic compounds. The study also provides information about the physicochemical, analytical, and pharmaceutical importance of the new proposed visualizing reagents. The obtained results indicate that all of the studied new visualizing reagents, namely gentian violet, methylene violet, methylene blue, methyl green, malachite green, and Janus blue, for detection stearic acid, stearyl alcohol, and methyl stearate are better visualizing reagents in comparison with the universally applied Rhodamine B for detection of lipophilic compounds. It was confirmed that the earlier proposed densitometric visualizing index is the

objective parameter for evaluation of usefulness of definite visualizing reagents to the detection of investigated compounds.

ACKNOWLEDGMENT

This research was financed by the Ministry of Science and Higher Education by resources reserved for science in the years 2005-2008 as research project No. 3 T09A 155 29.

REFERENCES

1. Niestroj, A.; Stefaniak, M.; Sliwiok, J. Application of Rhodamine B in densitometric determination of long chain fatty acids in thin layer chromatography. *Chemistry and Biochemistry in the Agricultural Production, Environmental Protection, Human and Animal Health*; Gorecki, H., Dobrzanski, Z. and Kafarski, P., Eds.; Prague-Brussels: Czech-Pol-Trade, 2006; 1–4.
2. Fried, B. Lipids In *Handbook of Thin-Layer Chromatography: Third Edition, Revised and Expanded*; Marcel Dekker, Inc.: New York, 2003; 635–670.
3. Jork, H.; Funk, W.; Fischer, W.; Wimmer, H. *Dünnschicht-Chromatographie, Reagenzien und Nachweismethoden, Physicalische und Chemische Nachweismethoden: Grundlagen, Reagenzien I*; VCH: Weinheim: Germany, 1989.
4. Jork, H.; Funk, W.; Fischer, W.; Wimmer, H. *Thin-Layer Chromatography: Reagents and Detection Methods, Vol 1b, Physical and Chemical Detection Methods: Activation Reactions, Reagents Sequences, Reagents II*; VCH: Weinheim: Germany, 1994.
5. Gasparic, J.; Churacek, J. *Laboratory Handbook of Paper and Thin-Layer Chromatography*; John Wiley & Sons Inc.: New York, 1978.
6. Sliwiok, J. The Application of fuchsine dyes in the detection of higher fatty acids by thin-layer chromatography. Microchem. J. **1968**, *13* (1), 108–110.
7. Kishimoto, K.; Urade, R.; Ogawa, T.; Moriyama, T. Nondestructive quantification of neutral lipids by thin-layer chromatography and laser-fluorescent scanning: suitable methods for "Lipidome" analysis. Biochem. Biophys. Res. Comm. **2001**, *281*, 657–662.
8. Pyka, A.; Sliwiok, J. Application of thymol blue and bromothymol blue to the detection of the alcohols in TLC. J. Planar Chromatogr. Mod. TLC **1992**, *5*, 282–283.
9. Wardas, W.; Pyka, A. New visualizing agents for selected fatty derivatives in thin layer chromatography. J. Planar Chromatogr. Mod. TLC **1993**, *6*, 320–322.
10. Wardas, W.; Pyka, A. Visualizing agents for higher fatty acids in TLC. In *Dunnschicht-Chromatographie*; Kaiser, R.E., Gunther, W., Gunz, H. and Wulff, G., Eds.; Rudolf Stehle GmbH & Co.: Dusseldorf, KG, 1996, 196–201.
11. Wardas, W.; Pyka, A. Visualizing agents for esters of higher fatty acids in TLC. J. Planar Chromatogr. Mod. TLC **2001**, *14*, 8–15.
12. Pyka, A. The application of densitometry to evaluation of visualizing effects of salicylanilide using brilliant green. J. Liq. Chromatogr. & Rel. Technol. **2008**, *31*, in press.

13. Gregorowicz, Z.; Sliwiok, J. Indexes for estimation of developing reagents in thin-layer chromatography. Microchem. J. **1970**, *15* (1), 60–63.
14. Pyka, A.; Klimczok, W.; Gurak, D. Evaluation of visualizing reagents for estradiol on thin layer by densitometric method. J. Liq. Chromatogr. & Rel. Technol. **2008**, *31*, 555–566.
15. Klimczok, W.; Pyka, A.; Gurak, D. Application of densitometry and spectrodensitometry for the evaluation of the new visualizing reagents for selected drugsp61 The *XXXI* Symposium "Chromatographic Methods of Investigating the Organic Compounds", Katowice-Szczyrk, June 4th–6th, 2007, P-14, p. 16.

Received January 16, 2008
Accepted February 22, 2008
Manuscript 6301