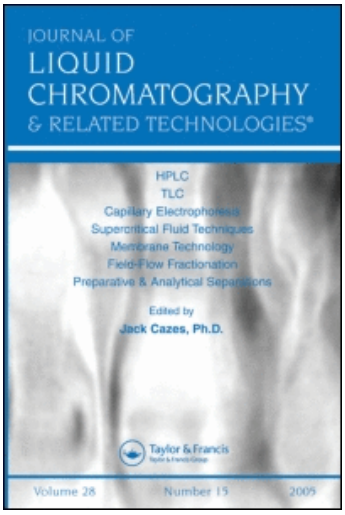

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Analytical Evaluation of Visualizing Reagents Used to Detect Tocopherol and Tocopherol Acetate on Thin Layer

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Analytical Evaluation of Visualizing Reagents Used to Detect Tocopherol and Tocopherol Acetate on Thin Layer

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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 (±)-α-tocopherol, and (+)-α-tocopherol acetate on silica gel 60. Rhodamine B and 2,2′-bipyridine–iron(III) chloride reagent were used as the comparative visualizing reagents. The limit of detection (detectability), detection index, broadening index, modified contrast index, densitometric visualizing index, and linearity range were determined for (±)-α-tocopherol, and (+)-α-tocopherol acetate following use of these visualizing reagents. It was stated, that earlier proposed densitometric visualizing index is an objective parameter describing the applied visualizing reagents. 2,2′-Bipyridine–iron(III) chloride reagent can be used only to detect (±)-α-tocopherol. Among all studied new visualizing reagents, methylene violet, and methyl green are the best to detect (±)-α-tocopherol. The best way of detection of (+)-α-tocopherol acetate is the densitometric method without using a visualizing reagent. Whereas, among all studied new visualizing reagents, gentian violet, methyl green, and Janus blue are the best for detection (+)-α-tocopherol acetate. These visualizing reagents have similar detection properties of (+)-α-tocopherol acetate in relation to rhodamine B.

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Keywords: (+)-α-Tocopherol acetate, (±)-α-Tocopherol, Broadening index, Densitometric visualizing index, Densitometry, Detectability, Detection index, Dyes, Modified contrast index, New visualizing reagents, NP-TLC

INTRODUCTION

Vitamins are organic compounds that have biochemical and physiological proprieties. Because of these qualities, they are the subject of numerous scientific investigations. Vitamins are classified according to their solubility in water and in fats. Fat-soluble vitamins are vitamins A, D, E, and K. Chromatography is useful in the identification and determination of vitamins in pharmaceutical preparations, the identification and determination of vitamins and related substances in natural materials and foodstuffs, and the chemical and biochemical determination of vitamins and their metabolites in fats and tissues. Vitamins that are soluble in fat (lipophilic or hydrophobic vitamins) are the subject of wide investigations because of their biological proprieties. HPLC, TLC, and GC are the principle techniques used for qualitative and quantitative investigations of fat soluble vitamins. Analysis of the fat soluble vitamins by liquid chromatography (TLC and HPLC) is the subject of many scientific publications.[1–4] Generally, TLC is useful for the investigation of a wide range of the fat soluble vitamins application, i.e., purification of samples, qualitative detection, quantitative determination, and the use of new visualizing agents, and also for separation of some optical isomers.

Vitamin E refers to a family of at least eight molecules having a biological antioxidant activity and a structure of a chromanol ring substituted by an aliphatic side chain (C-12) containing two methyl groups in the middle and two more at the terminal position. Vitamin E exists in eight different forms or isomers, four tocopherols and four tocotrienols. α-Tocopherol is recognized as the most effective biological antioxidant.[5] The ester of vitamin E is more stable to light and oxygen than tocopherol. The shelf life of the ester tocopherol is greater than that of the unesterified tocopherol. Tocopherol acetate is naturally converted by the body to vitamin E.[6]

In our earlier papers, we described brilliant green, gentian violet, methylene violet, methylene blue, methyl green, malachite green, and janus blue for the detection of salicylanilide,[7] estradiol,[8] dehydroepiandrosterone,[9] stearic acid, stearyl alcohol, and methyl stearate[10] in thin layer.

We decided to examine a series of dyes as new visualizing reagents for their ability to detect (±)-α-tocopherol, and (+)-α-tocopherol acetate in thin layer. This work also concerns the conformation of the significance of the earlier proposed densitometric visualizing index[7] for the evaluation of visualizing effects of vitamin E.

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, lot: HX743154). The plates were prewashed with methanol-chloroform (1:1, v/v) 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 (±)-α-tocopherol (Fluka, lot: 41306116) and (+)-α-tocopherol acetate (Sigma, lot: 028H1164) containing 30.00, 25.00, 20.00, 16.00, 9.60, 5.76, 3.46, 2.08, 1.25, 0.75, 0.60, 0.45, 0.30 mg were prepared in 5 mL absolute ethanol (POCh, Gliwice, Poland). The solutions of the studied compounds (5 μL) were spotted manually, using a microcapillary (Camag, Switzerland), onto the chromatographic plates. Toluene 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 30 min, the plates were developed vertically, at room temperature (20 ± 1°C), to a distance of 7.5 cm. The plates were then dried for 24 h at room temperature (20 ± 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 and 2,2′-bipyridine–iron(III) chloride reagent were used as the comparative visualizing reagents.[11]

Rhodamine B (POCh, Poland) reagent was used as 50 mg/100 mL solutions in distillated water.

Ethanolic iron(III) chloride (0.5%) (POCh, Poland) solution and 0.5% ethanolic solution of 2,2′-bipyridine (POCh, Poland) were mixed in equal parts before use.

The dried plates were dipped in particular visualizing reagent solutions for 5 sec. Then, after dipping in a solution of visualizing reagents, they were dried for 24 h at room temperature (20 ± 1°C), with the

exception of 2,2′-bipyridine–iron(III) chloride reagent. The plates after dipping in the solution of 2,2′-bipyridine–iron(III) chloride reagent were dried in cold air.

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 start wavelength was 200 nm and 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 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 sources were a deuterium lamp emitting a continuous spectrum between 190 and 450 nm and a tungsten lamp emitting a spectrum between 370 and 800 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 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[12,13]

The broadening index was modified and was calculated as:

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

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.

Table 1. Color of spot of studied compounds, visual limit detection and background color after the detection using visualizing reagents on silica gel 60

Detection	Spot color of		Background color	Visual limit detection (μg/spot)	
	(±)-α-Tocopherol	(+)-α-Tocopherol acetate		(±)-α-Tocopherol	(+)-α-Tocopherol acetate
Without using visualizing reagent	Lack of colored spot in visible light	Lack of colored spot in visible light	White	–	–
2,2′-Bipyridine–iron(III) chloride	Raspberry red	Lack of colored spot in visible light	Light beige	2.08	–
Rhodamine B	Pink-violet	Dark pinka	Pink	0.75	3.46
Gentian violet	Dark violet with white border	Dark violet	Violet	2.08	3.46
Methylene violet	Azure with white border	Blue with white border	Light grey-blue	3.46	3.46
Methylene blue	Light blue	Light blue	Blue	2.08	3.46
Methyl green	Light green	Green (small contrast with background color)	Green	2.08	20.00
Malachite green	Celadon	Green-celadon	Green	3.46	9.60
Janus blue	Dark blue with white border	Dark blue	Blue	2.08	2.08

Detection Index[8,13]

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 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[10]

The modified contrast index was calculated as:

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

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

The Densitometric Visualizing Index[7]

The densitometric visualizing index (DVI) was calculated as:

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

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 β 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 by use of the Equations (1), (2), (3), and (4), respectively.

RESULTS AND DISCUSSION

Six new visualizing reagents (known as dyes), namely gentian violet, methylene violet, methylene blue, methyl green, malachite green, and janus blue were used to detect the vitamin E, namely (±)-α-tocopherol, and (+)-α-tocopherol acetate. However, rhodamine B and 2,2′-bipyridine–iron(III) chloride were used as comparative visualizing reagents for detection of (±)-α-tocopherol, and (+)-α-tocopherol acetate. The colors of chromatographic spots for the investigated compounds and background colors, without use of a visualizing reagent and after

detection with visualizing reagents on silica gel 60, are presented in Table 1. It was stated, that studied (±)-α-tocopherol, and (+)-α-tocopherol acetate without use of a visualizing reagent are invisible on the chromatogram in visible light. Spots of (±)-α-tocopherol, and (+)-α-tocopherol acetate after the detection with the use of specific visualizing reagents are visible on the chromatograms; with the exception of 2,2′-dipirydyl–iron(III) chloride reagent, which did not give colored chromatographic spot with (+)-α-tocopherol acetate. However, the colored spots of (±)-α-tocopherol, and (+)-α-tocopherol acetate are similar with the use of a definite visualizing reagent. All obtained chromatographic spots of (±)-α-tocopherol, and (+)-α-tocopherol acetate after the application of particular visualizing reagents were durable and visible for over 6 weeks. The visual limit detections of (±)-α-tocopherol, and (+)-α-tocopherol acetate after their detection with the use of particular visualizing reagents on silica gel 60 are also presented in Table 1.

(±)-α-Tocopherol, and (+)-α-tocopherol acetate analyzed on silica gel 60 without use of a visualizing reagent and after detection with visualizing reagents were densitometrically and spectrodensimetrically evaluated. Spectrodensitogram characteristics of (±)-α-tocopherol, and (+)-α-tocopherol acetate investigated on silica gel 60 are presented in Table 2. It was stated that the fundamental absorption band of (±)-α-tocopherol without use of a visualizing reagent and after use following visualizing reagents, namely gentian violet, methylene violet, methyl green, malachite green, janus blue, and rhodamine B occurs at the wavelength equal to 270 nm. The fundamental absorption band of (±)-α-tocopherol after detection with the use of methylene blue occurs at 203 nm; however after detection with the use of 2,2′-dipirydyl–iron(III) chloride reagent occurs at 524 nm. However, the fundamental absorption band of (+)-α-tocopherol acetate without use of a visualizing reagent and after detection with all of the particular visualizing reagents, with the exception of 2,2′-dipirydyl–iron(III) chloride reagent, occurs at the wavelength equal to 203 nm. It was found that the spectrodensitograms of (±)-α-tocopherol, and (+)-α-tocopherol acetate on silica gel 60 plates and by use of particular visualizing reagents are different than the spectrodensitograms obtained on the plates without use of a visualizing reagent. 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. This fact has analytical significance in the identification of the investigated substances. The densitometric analyses were performed at respective absorption maxima, which are given in Table 2.

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

Table 2. Spectrodensitogram characteristics of (±)-α-tocopherol, and (+)-α-tocopherol acetate on silica gel 60

	Detected compound					
	(±)-α-Tocopherol			(+)-α-Tocopherol acetate		
		Remaining absorption bands			Remaining absorption bands	
Detection	Fundamental absorption band λ_{max} [nm][a]	λ [nm]	intensity [AU]	Fundamental absorption band λ_{max} [nm][a]	λ [nm]	intensity [AU]
Without using visualizing reagent	270	200	44.1	203	222	65.1
		227	25.4		284	31.6
		360	4.6			
2,2′-Bipyridine–iron(III) chloride	524	264	16.8			
		298	17.6	–	–	–
		399	36.3			
Rhodamine B	270	200	94.9	203	282	29.3
		224	59.4		319	13.0
		358	19.4		365	11.9
		398	14.8		398	12.4
		427	9.6		433	10.2
		560	86.9		587	16.1
Gentian violet		203	46.8	203	223	66.6
		224	35.7		281	24.7

(*Continued*)

Table 2. Continued

	Detected compound					
	(±)-α-Tocopherol			(+)-α-Tocopherol acetate		
		Remaining absorption bands			Remaining absorption bands	
Detection	Fundamental absorption band λ_{max} [nm][a]	λ [nm]	intensity [AU]	Fundamental absorption band λ_{max} [nm][a]	λ [nm]	intensity [AU]
		375	16.0		308	6.5
	270	465	7.6	203	381	8.7
		547	9.2		457	7.9
		610	34.4		544	10.1
					601	28.1
Methylene violet	270	203	76.3	203	222	59.8
		222	49.8		277	29.7
		293	41.6		632	13.6
		369	14.6			
		582	3.3			
		646	38.3			
Methylene blue	203	265	94.9	203	244	21.7
		304	26.8		257	17.3

		337	29.5		292	43.3
		361	29.4		320	20.0
		622	11.4		366	30.4
		690	76.9		350	29.1
					418	43.3
					507	25.1
					661	67.4
Methyl green	270	203	64.7	203	223	66.6
		224	46.7		282	36.0
		334	21.4		341	14.7
		463	25.2		402	13.8
		691	30.1		461	18.8
Malachite green	270	200	49.0	203	223	73.1
		226	40.8		279	46.2
		345	30.1		336	31.1
		457	28.2		403	21.7
		665	35.3		459	33.4
					662	39.3
Janus blue	270	203	81.9	203	223	60.8
		363	14.1		283	34.3
		621	34.7	203	307	14.9
		670	45.7		397	8.8
					619	37.3
					660	38.6

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

Table 3. Broadening index and detection index for (±)-α-tocopherol, and (+)-α-tocopherol acetate detected on silica gel 60

Detection	(±)-α-Tocopherol		(+)-α-Tocopherol acetate	
	Broadening index (μg/AU)	Detection index (μg/AU)	Broadening index (μg/AU)	Detection index (μg/AU)
Without using visualizing reagent	0.724	0.45/3100	0.740	0.30/1100
2,2′-Bipyridine–iron(III) chloride	0.413	2.08/8915	–	–
Rhodamine B	0.814	0.30/1658	0.960	0.45/1511
Gentian violet	0.990	0.75/3359	0.849	0.75/1884
Methylene violet	0.618	0.30/3456	0.874	0.75/2514
Methylene blue	1.196	2.08/1670	1.170	0.75/2100
Methyl green	0.672	0.30/1564	0.827	0.75/3911
Malachite green	0.765	0.45/2860	0.848	0.75/2429
Janus blue	1.106	0.60/1300	0.830	0.75/3750

to 25 μg of a substance detected). The broadening indices for the investigated compounds are presented in Table 3. The R_F values of the (±)-α-tocopherol, and (+)-α-tocopherol acetate investigated on silica gel 60 are equal about to 0.41, and 0.44, respectively. The detection indices of (±)-α-tocopherol, and (+)-α-tocopherol acetate 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 densitometric limits of detection of the compounds investigated with and without visualizing reagents tested, linearity range, densitometric visualizing index, and densitometric band characteristic of 25 μg investigated (±)-α-tocopherol, and (+)-α-tocopherol acetate on silica gel 60 are presented in Tables 4, and 5, respectively. The densitometric evaluation of obtained densitometric bands of 25 μg compound was described by the area of the densitometric band [AU], the densitometric band height [AU], and the angle (β) 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 vitamin E was carried out: 25 μg of (±)-α-tocopherol, and (+)-α-tocopherol acetate were dropped in turn on the starting line, next their mobile phase was evolved,

Table 4. Characteristic of densitometric band, modified contrast index, densitometric limit of detection, densitometric visualizing index, and linearity range of (±)-α-tocopherol on silica gel 60

Detection	Densitometric band characteristic of 25 µg (±)-α-tocopherol			Modified contrast index $\left[\frac{AU}{\circ}\right]$	Limit of detection [µg]	Densitometric visualizing index $\left[\frac{AU}{\mu g \cdot \circ}\right]$	Linearity range [µg spot^{-1}] (r, correlation coefficient)
	Area [AU]	Height [AU]	β [°]				
Without using visualizing reagent	34519	459	16	28.69	0.45	0.479	3.46 ÷ 20.00 (r = 0.9894)
2,2′-Bipyridine–iron(III) chloride	60474	698	9	77.56	2.08	0.323	5.76 ÷ 30.00 (r = 0.9927)
Rhodamine B	30712	483	12	40.25	0.30	0.853	0.75 ÷ 9.60 (r = 0.9914)
Gentian violet	25254	409	10	40.90	0.75	0.337	1.25 ÷ 9.60 (r = 0.9952)
Methylene violet	40426	636	8	79.50	0.30	1.684	0.75 ÷ 5.76 (r = 0.9949)
Methylene blue	20895	458	8	57.25	2.08	0.126	5.76 ÷ 25.00 (r = 0.9948)
Methyl green	37196	576	9	64.00	0.30	1.378	0.75 ÷ 5.76 (r = 0.9863)
Malachite green	32691	529	11	48.09	0.45	0.660	0.75 ÷ 20.00 (r = 0.9912)
Janus blue	22608	414	10	41.40	0.60	0.377	1.25 ÷ 9.60 (r = 0.9935)

Table 5. Characteristic of densitometric band, modified contrast index, densitometric limit of detection, densitometric visualizing index, and linearity range of (+)-α-tocopherol acetate on silica gel 60

Detection	Densitometric band characteristic of 25 μg (+)-α-tocopherol acetate			Modified contrast index $\left[\frac{AU}{°}\right]$	Limit of detection [μg]	Densitometric visualizing index $\left[\frac{AU}{\mu g \cdot °}\right]$	Linearity range [μg spot^{-1}] (r, correlation coefficient)
	Area [AU]	Height [AU]	β [°]				
Without using visualizing reagent	33796	591	11	53.73	0.30	1.024	0.75 ÷ 20.00 (r = 0.9978)
2,2′-Bipyridine–iron(III) chloride	–	–	–	–	–	–	–
Rhodamine B	26031	455	15	30.33	0.45	0.386	1.25 ÷ 16.00 (r = 0.9936)
Gentian violet	29441	545	10	54.50	0.75	0.392	2.08 ÷ 25.00 (r = 0.9908)
Methylene violet	28604	481	13	37.00	0.75	0.293	1.25 ÷ 25.00 (r = 0.9938)
Methylene blue	21369	412	13	31.69	0.75	0.219	3.46 ÷ 20.00 (r = 0.9927)
Methyl green	30226	564	11	51.27	0.75	0.366	1.25 ÷ 25.00 (r = 0.9943)
Malachite green	29491	507	12	42.25	0.75	0.328	1.25 ÷ 25.00 (r = 0.9945)
Janus blue	30125	565	11	51.36	0.75	0.365	1.25 ÷ 25.00 (r = 0.9938)

and afterwards each spot was developed by means of another visualizing reagent. The contrast index represents two independent values, namely the angles (β) 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 salicylanilide,[7] estradiol,[8] and aliphatic compounds.[10] The modified contrast index indicates the ratio of the height of the densitometric band [AU] of detected compound to the angle (β) 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[7,10] for the conformation of its significance to the evaluation of visualizing effects of studied (±)-α-tocopherol, and (+)-α-tocopherol acetate detected with the use of investigated dyes. The densitometric visualizing index contains two most important characteristics of a densitometric band of a 25 μg studied substance, namely the area of the densitometric band [AU], the angle (β) 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 a 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 among all studied new visualizing reagents, methylene violet and methyl green are the best for detecting (±)-α-tocopherol. The densitometric bands of (±)-α-tocopherol after detection with methylene violet and methyl green are compacted (β are equal to 8°, and 9°, respectively) and linearity ranges are from 0.75 μg to 5.76 μg. Methylene violet and methyl green are better visualizing reagents in comparison with universally applied rhodamine and 2,2′-dipirydyl–iron(III) chloride reagent to detect (±)-α-tocopherol. The densitometric band of (±)-α-tocopherol after detection with rhodamine has β equal to 12°; however the densitometric band of (±)-α-tocopherol after detection with 2,2′-dipirydyl–iron(III) chloride reagent is compact (β is equal to 9°), but the limit of detection of (±)-α-tocopherol is then equal only to 2.08 μg. Whereas, the densitometric limit of detection of (±)-α-tocopherol after the use of methylene violet, methyl green, and rhodamine B is equal to 0.30 μg. Densitometric analysis of (±)-α-tocopherol without use of a visualizing reagent can also be recommended, because the limit of detection is equal to 0.45 μg, however the linearity range is from 3.46 μg to 20.00 μg. The densitograms of 25.00 μg (±)-α-tocopherol without use of a visualizing reagent and after detection with 2,2′-dipirydyl–iron(III) chloride reagent are presented in Figures 1a and 1b, respectively. The densitograms of 25.00 μg (±)-α-tocopherol after detection with rhodamine B and methylene violet are presented in Figures 2a and 2b, respectively.

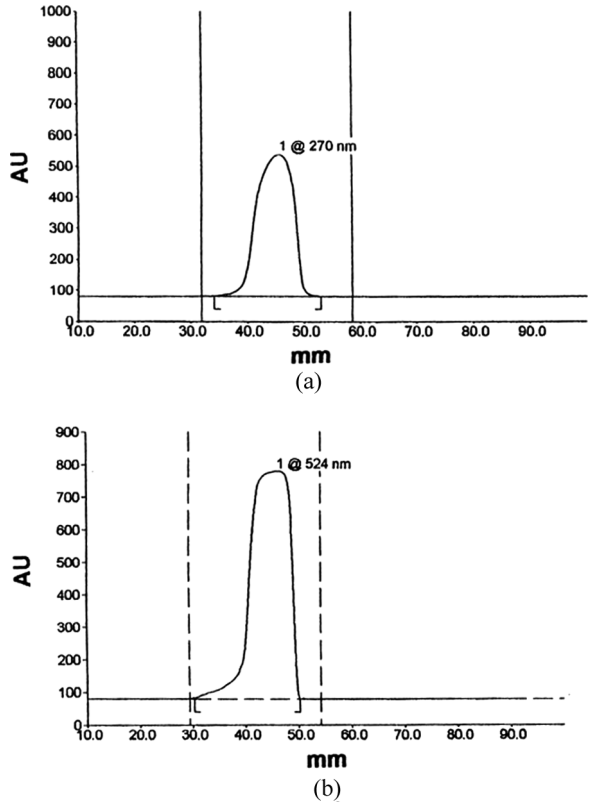
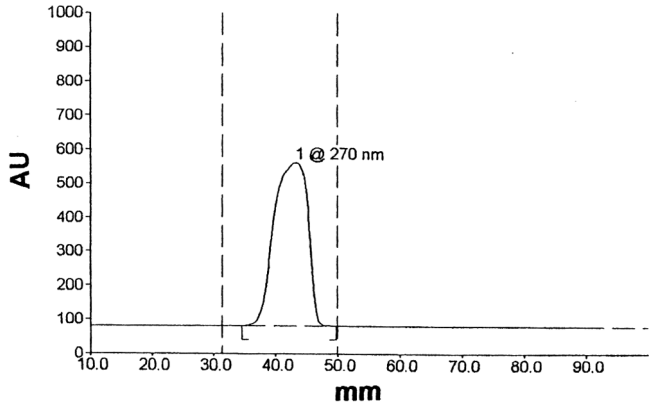


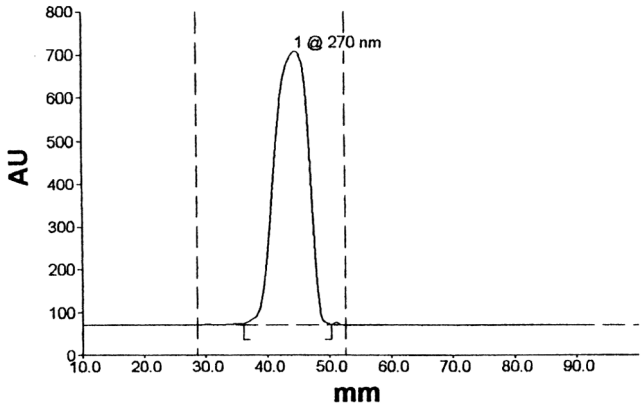
Figure 1. Densitograms of 25.00 μg (±)-α-tocopherol (a) without use of a visualizing reagent; (b) after detection with 2,2′-dipirydyl–iron(III) chloride reagent.

Obtained results in this work indicate, that the best detection way of (+)-α-tocopherol acetate is the densitometric method without using a visualizing reagent. Whereas, the densitometric limit of detection of (+)-α-tocopherol acetate is equal to 0.30 μg, and the linearity range is from 0.75 μg to 20.00 μg. However, among all studied new visualizing reagents, gentian violet, methyl green, and janus blue are the best for detection of (+)-α-tocopherol acetate. These visualizing reagents have similar detection properties of (+)-α-tocopherol acetate in relation to rhodamine B. The densitograms of 25.00 μg (+)-α-tocopherol acetate without use of a visualizing reagent and after detection with methyl green are presented in Figures 3a and b, respectively. The densitograms of 25.00 μg (+)-α-tocopherol acetate after detection with rhodamine B and gentian violet are presented in Figures 4a and b, respectively.

It was stated, that all applied ways of detection permit obtaining a linear dependence between the area of the densitometric band and the



(a)



(b)

Figure 2. Densitograms of 25.00 µg (±)-α-tocopherol after detection with (a) Rhodamine B; (b) methylene violet.

quantity of spotted (±)-α-tocopherol or (+)-α-tocopherol acetate. The range of linearity is different for particular applied visualizing reagents and depends on the detected compound.

It was confirmed that the earlier proposed densitometric visualizing index is the objective parameter for evaluation of the usefulness of definite visualizing reagents for the detection of (±)-α-tocopherol, and (+)-α-tocopherol acetate. The visualizing reagents proposed in this work should serve as supplements to those used previously for the detection of (±)-α-tocopherol, and (+)-α-tocopherol acetate. The study also provides information about the physicochemical, analytical, and pharmaceutical

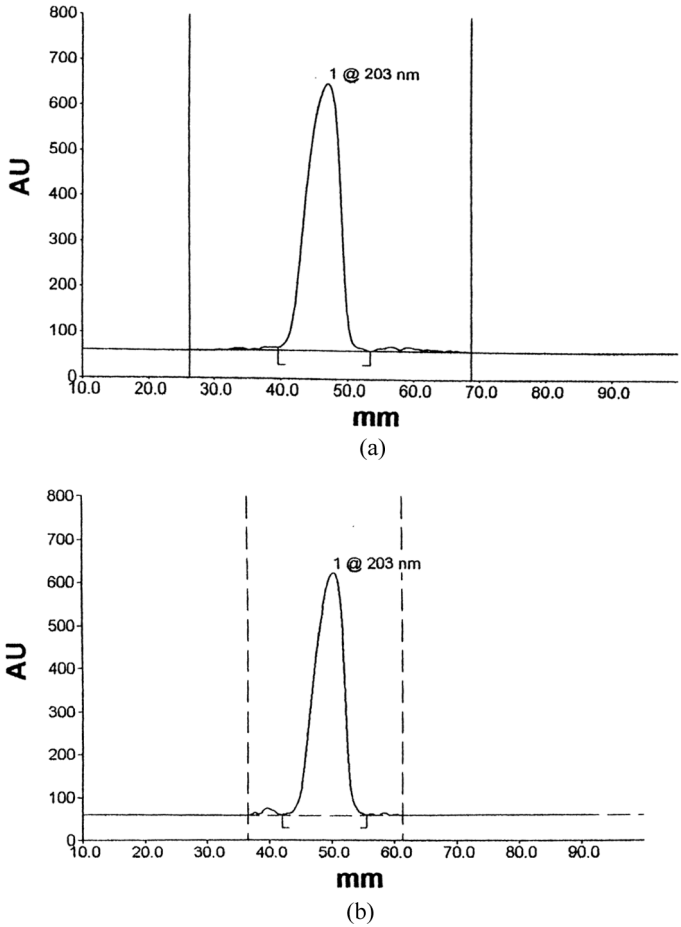
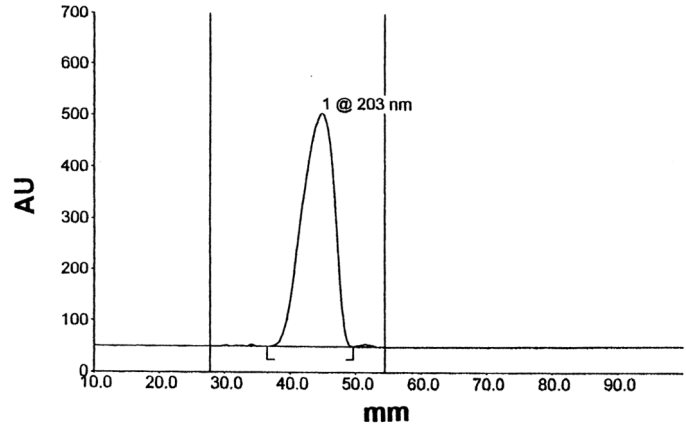
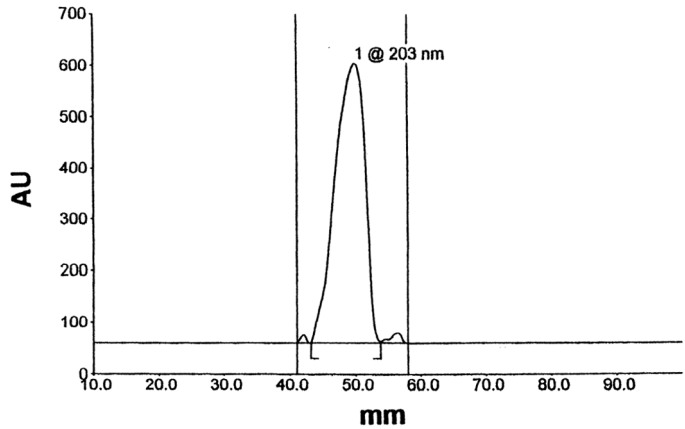


Figure 3. Densitograms of 25.00 μg (+)-α-tocopherol acetate (a) without use of a visualizing reagent; (b) after detection with methyl green.

importance of the new proposed visualizing reagents. The visualizing reagents proposed in this work, i.e., known 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.[14] 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



(a)



(b)

Figure 4. Densitograms of 25.00 μg (+)-α-tocopherol acetate after detection with (a) Rhodamine B; (b) gentian violet.

compounds, indicate that progress in the range of analysis of (±)-α-tocopherol, and (+)-α-tocopherol acetate on thin layer has taken place.

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