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High sensitive and selective HPTLC method assisted by digital image processing for simultaneous determination of catecholamines and related drugs

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A highly sensitive and selective thin layer chromatographic (TLC) method was developed for simultaneous determination of catecholamines and their related drugs using a new detection method and digital image processing of chromatographic plates. For the quantitative evaluation of the investigated compounds, the chromatographic separation was followed by spraying the plate with 0.02% solution of 2,2-diphenyl-1-picrylhydrazyl radical (DPPH$^{\bullet}$) in ethanol. The BioDit Thin Layer Chromatography (TLC) Scanner device and advanced specific software (ImageDecipher-TLC, Sorbfil TLC Videodensitometer and JustTLC) were used for the detection and quantification of chromatographic spots. For an accurate determination, the RGB colored images of the bright-white spots detected against a purple background were inverted and processed after their conversion into green scale. The results showed a strongly linear correlation between area ($R^2 > 0.99$) and volume ($R^2 > 0.99$) of spots and concentration of investigated compounds in all cases. The limit of detection (LOD) and the limit of quantification (LOQ) were below 49.3 ng/spot and 69.6 ng/spot respectively in all cases. The evaluation of the method was performed using different pharmaceutical samples spiked with the investigated amines and validated with respect to accuracy and precision.

1. Introduction

The analysis of biologically active compounds, most often responsible for the functioning of natural systems is particularly important in both the biomedical and pharmaceutical fields. Among the compounds with important biological activity, biogenic amines, especially psychoactive catecholamines (considered important neurotransmitters in the central nervous system) and their related compounds (synthetic drugs that mimic the amines behavior in the human body) have currently constituted a research topic of great interest both in neuroscience [1] and food industry [2]. In humans, the most abundant natural catecholamines (epinephrine, norepinephrine and dopamine) are secreted by the adrenal glands, from the aminoacids phenylalanine and tyrosine, in response to stress [3]. Usually the catecholamines cause general physiological changes preparing the body for physical activities [4] but they have been associated also with oxidative stress related-pressor responses [5] being important markers for the diagnosis of several diseases. In addition, they are widely used in the treatment of bronchial asthma, hypertension, heart failure associated with organic heart disease and cardiac surgery [6,7].

Therefore, a number of electroforetic [8,9], electrochemical [4,10] spectrofluorimetric [11] and spectroscopic methods [12] have been developed for the determination of biogenic amines in pharmaceutical preparations and biological samples. Amongst these techniques, some of them proved to be very sensitive for the determination of catecholamines but they require expensive and sophisticated instrumentation, time consuming and complex preparation of the samples in some cases and are not so popular in average analytical laboratories. During the last years, the most used method for analysis of catecholamines in biological fluids is HPLC in conjunction with various detection systems like mass spectrometry (MS) [13,14], electrochemical [15,16] and fluorescence detection [17,18]. The analysis of some amines in HPLC gives rise to considerable problems due to their hydrophilic nature, which can result in low retention necessitating the use of ion pair agents and/or very low concentration of organic modifier that can causes phase dewetting. Furthermore, the presence of ionized silanol groups on the stationary phase may give rise to peak tailing and overloading effects [19]. Because of the high polarity of amines, some limitations were encountered also by using thin layer chromatography (TLC). In this case they tend to streak during the chromatographic development limiting the resolution. Due to the presented limitations, usually these compounds were determined after their conversion to dansyl-derivatives which have better properties than the free amines [20,21]. Nevertheless, the separation and quantification of amines derivatives, using a single development technique, seems to be limited to the separation of five amines [20] and moreover, their separation was often adversely affected by ammonia derivatives [21]. Although some studies were developed

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concerning the separation and estimation of underivatized catecholamines by HPLC [16,22] the analysis employs expensive and sophisticated equipment, such as electrochemical or MS detectors [14,23,24].

In the last years, thin layer chromatography (TLC) assisted by digital image processing of chromatographic plates made this technique a convenient choice for many quantitative applications [25–29]. Currently, there are two common methods to acquire the TLC plate image. These methods use a flatbed scanner or employ a charge-coupled device (CCD) camera. TLC imaging using charge-coupled device (CCD) detectors has been shown to enable rapid and efficient analysis [30] because entire TLC plate can be imaged in a single exposure, yielding a rapid quantification of the chromatographic spots. For imaging system, in addition to digital scanning, image acquisition by digital camera has also been successfully used for TLC in case of fluorescent compounds [31]. In addition, due to the advantages of the uniform lighting of surface, short time of scanning and high optical resolution, different flatbed scanner systems have been employed in the TLC-quantitative evaluation of various classes of compounds [25,28,32] and in some cases the results were compared to those obtained with the use of slit-scanning densitometry [33]. Moreover, taking into account the advantages of chemometrics, Hemmateenejad et all. have successfully applied multivariate analysis to the images of TLC plates for simultaneous determination of compounds even in the presence of overlapped spots [34].

In view of the above considerations this study aimed the development and validation of a high sensitive and selective, simple, fast and convenient method for simultaneous determination of catecholamines and some related drugs using a new detection method and digital image processing of the chromatographic plates.

2. Materials and methods

2.1. Chemicals and standard solutions

All chemicals used were of analytical-grade. Standards of catecholamines (dopamine hydrochloride (D), (−)-norepinephrine (NE), (−)-epinephrine (E)), and related drugs isoprenaline hydrochloride (IP) and α-methyl-3,4-dihydroxyphenylalanine (methyldopa (MD)) were purchased from Sigma-Aldrich (Steinheim, Germany). 2,2-diphenyl-1-picrylhydrazyl free radical (95%) was from Alfa Aesar (Karlsruhe, Germany). Analytical-grade methanol and formic acid (85%) were purchased from Chemical Company (Iaşi, Romania). Citrate buffer solution (pH=3.00) was from Sigma-Aldrich (Steinheim, Germany). The standard reference mixture of the catecholamines D, NE, E, IP and MD were prepared (0.5 mg/mL for the first four amines and 0.25 mg/mL for MD) as stock solution in methanol containing 0.1% hydrochloric acid 4 M. The working solution (used in method validation containing 0.05 mg/mL of D, NE, E, IP and 0.025 mg/mL of MD) were prepared by appropriate dilution of the stock solution with methanol.

2.2. TLC procedure

Chromatographic separations were performed on glass-backed LiChrospher® RP-18 WF$_{254S}$ HPTLC plates (20 cm × 10 cm, Merck, Darmstadt, Germany). The standards were applied as spots in increasing volumes (from 1 μL to 6 μL) using the Linomat 5 TLC applicator (Camag, Muttenz, Switzerland) with an application rate of 60 nL/s. The mobile phase, an optimized solvent system consisting of citrate buffer (pH=3.00): methanol: formic acid (96:4:5 v/v/v), was selected in order to obtain a good separation of all the investigated compounds. The plates were developed using the ascending technique, in a normal chromatographic chamber (Camag) saturated for 15 min with the vapors of mobile phase, to a developing distance of 7.0 cm, at room temperature (≈20°). After the chromatographic elution, the plates were dried at room temperature and prepared for the spots visualization procedure. Different common detection reagents including *o*-phtalaldehide, fluorescamine and ninhydrin were tested according to the procedures described in the literature [35] in all cases. Implementation of the DPPH$^\bullet$ reaction in the developed HPTLC method required optimization of several parameters (such as time/temperature needed to dry the chromatographic plates and DPPH$^\bullet$ solution concentration) in order to obtain accurate and reproducible results. Also, according to the best results, the plates were dried in the oven for 1 h at 65 °C, cooled at room temperature and sprayed with a freshly prepared 0.02% DPPH$^\bullet$ solution in ethanol. The investigated amines were detected in visible light as bright-white spots against a purple background.

2.3. Processing and evaluation of the chromatographic plates

A BioDit Thin Layer Chromatography (TLC) Scanner (the second-generation instrument for quantitative measurements in TLC, BioDit Technology, Co.), equipped with high qualified Micortek® 3-linear color CCD camera and coupled to a computer device, was used in visible mod for imaging the TLC plates with a resolution of 300 DPI. ImageDecipher-TLC software version 2.0 (BioDit Technology, Co.) was used for digital processing of captured images and quantitative evaluation of chromatographic spots on the TLC plates. To get more accurate concentration of the samples, the saved colorful RGB image of the plate can be opened for later analysis. Also, for the quantification of the investigated compounds based on the integrated peak area, the used software allows to invert the image and also to select a pure color (red, green or blue) or integrate all color (gray) for analysis. In addition, for the quantification of the investigated amines on the TLC plates, the previous converted images were processed also by using other two specialized software (Sorbfil TLC Videodensitometer (Sorbpolymer, Krasnodar, Russia) and JustTLC (Sweday, Sweden, www.sweday.com)) in order to calculate area and respectively volume of the chromatographic spots.

2.4. Method validation

For the calibration procedure, six different volumes of stock solution were used and the calibration function was constructed for each of the investigated amines by plotting the peak area and respectively peak volume values against the analyte concentration in all cases. The linearity was characterized by the linear range, the regression equation, and the coefficient of determination (R^2). The limit of detection (LOD) and the limit of quantification (LOQ) were calculated using the SMAC (Statistical Methods in Analytical Chemistry) software based on the confidence bands of the calibration function.

The precision of the method expressed as relative standard deviation (RSD) was determined on standard solutions at three concentration levels by analyzing six replicate spots for each concentration. The accuracy of the method expressed by percent recovery was investigated for standard solutions (125 ng/spot for D, NE, E, IP and 150 ng/spot for MD) by analyzing three replicate spots for each of the investigated compounds and also using the pharmaceutical samples spiked with known amounts of amines standard solution at two concentration levels (20 and 30 ng/spot for D, NE, E, IP and 200 ng/spot and 300 ng/spot for MD respectively). Statistica 8.0 software package was used for statistical data treatment.

2.5. *Preparation of method evaluation samples*

For the method evaluation, two different pharmaceutical samples were analyzed. The first one, the aqueous adrenaline injection (Terapia Ranbaxy, România, containing adrenaline (epinephrine) hydrochloride 1 mg/ml) for which no clean-up procedure was involved (only appropriate dilution) and the second one, pharmaceutical tablets (Dopegyt, Egis Pharmaceuticals PLC, Hungary, containing 250 mg methyldopa/tablet) which required an additional extraction step of methyldopa, before analysis. Therefore, two tablets were accurately weighted ($m_1=0.3450$ g and $m_2=0.3445$ g) and separately dissolved in 25 mL methanol containing 0.1% hydrochloric acid (4 M). Using an ultrasonic bath for 30 min, the powder tablet was completely disintegrated and the resulted solution was clarified by passing it through 0.45 μm syringe filter (Nylon, 25 mmθ, Teknokroma) in both cases. The resulted solutions were used for methyldopa analysis after appropriate dilutions with methanol. In addition to the method evaluation, other two mixed samples (containing 0.5 mg/mL concentration of norepinephrine, dopamine and isoprenaline respectively on a background level of epinephrine of 0.5 mg/mL) were prepared by spiking epinephrine injection with stock solution containing mixture of norepinephrine, dopamine and isoprenaline respectively and analyzed by the proposed method. In all cases, the investigated amines were identified by comparing their retention factors with those of standard solutions and the content in the samples was quantified based on linear calibration with known amounts of standards and using both, peak area and peak volume respectively in the chromatogram profiles of samples.

3. Results and discussion

3.1. *Optimization of the chromatographic conditions*

For the accurate separation of the underivatized proposed compounds, the TLC conditions were optimized using individual standard solutions and standard mix solution containing all of the investigated amines. Also, due to their polar nature, optimization of the chromatographic system was started (based on some few data collected from literature) using several stationary phases (such as silica gel 60, RP-18F$_{254}$, DiolF$_{254}$, RP-18WF$_{254}$, RP-2F$_{254}$) and different mixtures of solvents (such as acetate buffer: MeOH 96:4 (v/v); acetate buffer: acetonitrile 90:10 (v/v); phosphate buffer (pH=6.86): MeOH 1:1 (v/v); citrate buffer (pH=3): MeOH:formic acid 96:4:5 (v/v); amonium buffer: acetic acid 99:1 (v/v); i-butyric alcohol: acetic acid: acetonitrile: water 10:1:2:4 (v/v/v/v)) as mobile phases. In addition, the effect of temperature (over a range of −10 °C–60 °C) on the separation of the analytes was also tested. Thereby the RP-18WF$_{254S}$ chromatographic plates and a mobile phase consisting of citrate buffer (pH=3.00): methanol: formic acid (96:4:5 v/v/v) was selected as the best chromatographic system. Because the assessment of the effect of temperature showed an insignificant improvement in the separation process, all analyses were performed at ambient temperature (≈20 °C).

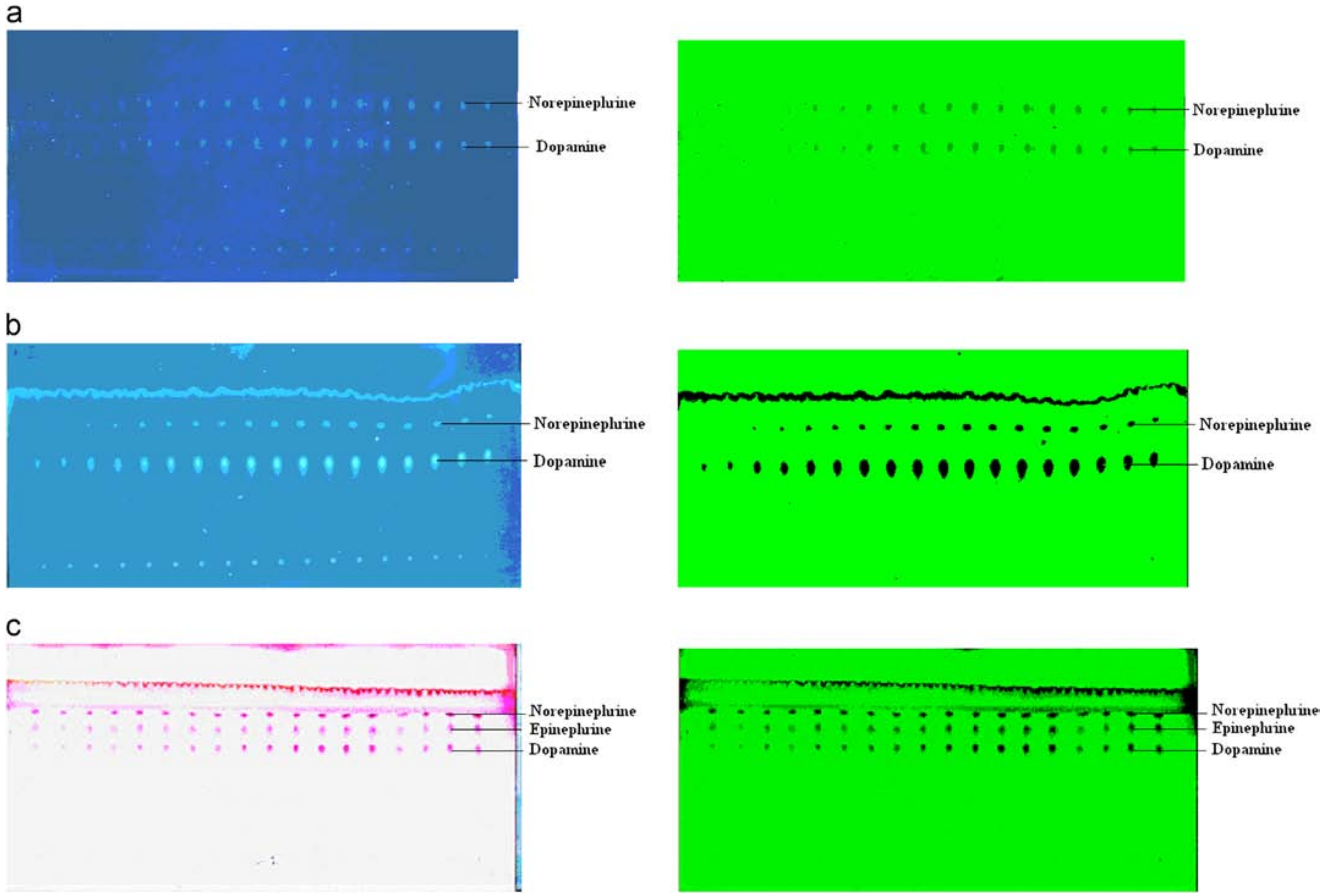

Fig. 1. Image of the chromatographic plate in visible mode and green scale respectively after the spot detection with (a) *o*-phtalaldehide; (b) fluorescamine; and (c) ninhydrin. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

The chosen TLC conditions have yielded to a good separation of the investigated compounds ($R_{F(\text{Norepinephrine})}=0.75$; $R_{F(\text{Epinephrine})}=0.63$; $R_{F(\text{Dopamine})}=0.58$; $R_{F(\text{Methyldopa})}=0.45$; and $R_{F(\text{Isopranaline})}=0.40$).

The catechol related compounds do not have native fluorescence and usually they are detected using different derivatization reagents [36,37]. In our case, different common detection reagents including o-phthalaldehyde, fluorescamine and ninhydrin were also tested according to the procedures described in the literature. In all cases some limitations regarding the number of the compounds detected and also sensitivity of the detection method were encountered. Also, by using *o*-phtalaldehide and fluorescamine reagent respectively, only norepinephrine and dopamine can be detected while the ninhydrin was able to detect three (norepinephrine, epinephrine and dopamine) of the five investigated amines (Fig. 1). In addition, in all cases a low intensity was observed and an increased interference from the background was encountered during the evaluation of the chromatographic spots, adding imprecision to the analysis.

Based on the structure of the investigated compounds it is expected that the free radical DPPH$^{\bullet}$ will act as a highly sensitive detection reagent after the chromatographic separation. By spraying the chromatographic plates with different concentrations of DPPH$^{\bullet}$ solution, all the investigated amines were detected in visible light as bright-white spots against a purple background. The background proved to be unstable and in some cases started to fade within approximately first minute after spraying. Also, implementation of the DPPH$^{\bullet}$ reaction in the developed TLC method required optimization of several parameters in order to obtain accurate and reproducible results. In this order, different concentrations of DPPH$^{\bullet}$ solutions (0.02%, 0.03% and 0.04% in methanol and ethanol) and different drying temperature (within the range of 40–70 °C) and drying periods (within the range of 30–60 min) were tested after development of chromatographic plates. According to the obtained results, the best conditions imply the drying of the chromatographic plates for 1 h at 65 °C after the developing process followed by cooling the plate at room temperature and spraying it with a freshly prepared 0.02% DPPH$^{\bullet}$ solution in ethanol. In these conditions, the background proved to be more uniform and stable starting to fade after approximately 10 min from spraying. After this period a poor peak shapes as well as decreased peak heights and areas and an increased interference from the background were observed. Due to these inconveniences the slit-scanning densitometry could not be applied for the quantitative determination of investigated compounds directly

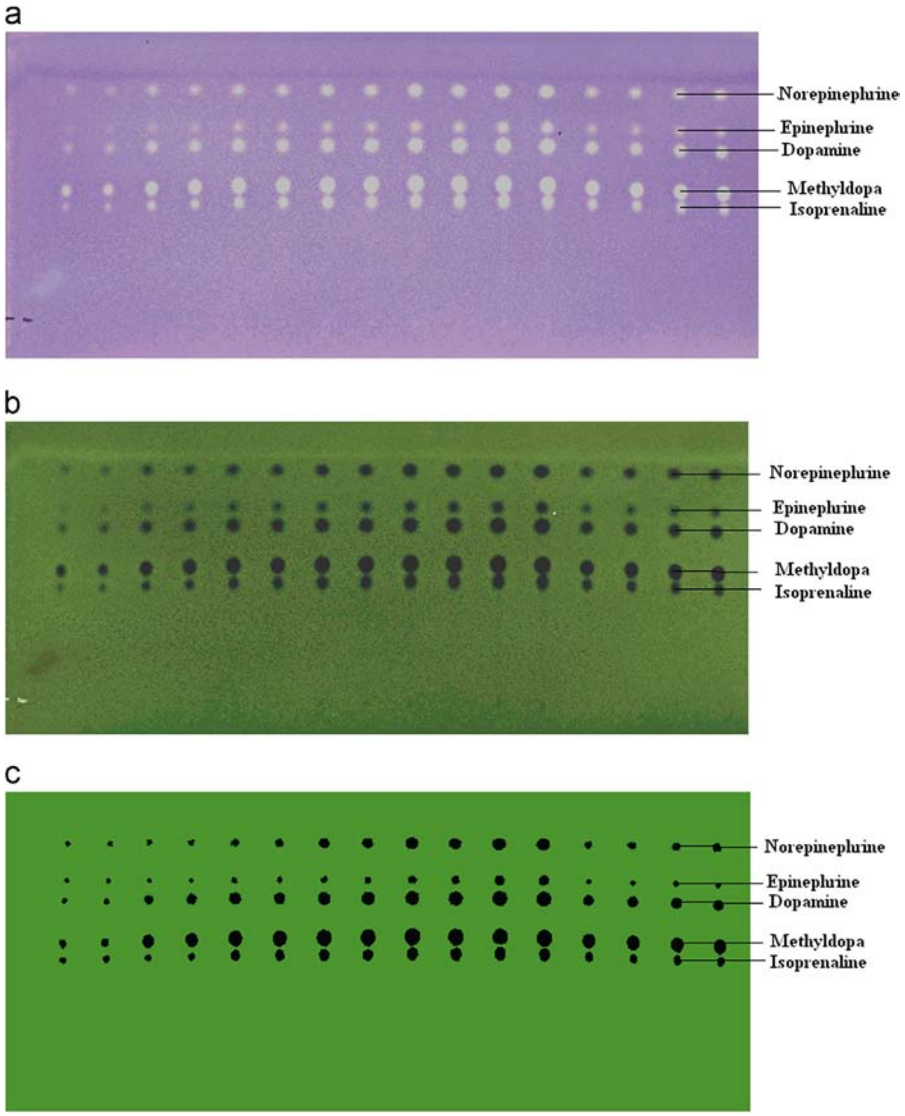

Fig. 2. Image of the chromatographic plate after the spot detection with DPPH$^{\bullet}$ radical in (a) visible mode; (b) inverted mode; and (c) green scale. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

from the TLC plate. This problem can be solved by using an image analysis system that can capture the image of the whole plate simultaneously before the fading of the background.

3.2. Image analysis

From several systems commercially available, the BioDit Thin Layer Chromatography (TLC) Scanner coupled to a computer device and the ImageDecipher-TLC software were used for capture the image of the chromatographic plates, digital processing of captured images and quantitative evaluation of chromatographic spots on the TLC plates. The chromatographic plates were scanned under visible light at an optical resolution of 300 dpi in order to obtain the images of chromatographic plates (bmp file format). To get more accurate concentration of the samples, the saved colorful RGB image of the plate can be opened for later analysis. By using ImageDecipher-TLC, image of TLC plate was imported directly from the scanner (bmp file) and the image edition, optical density integrity, curves drawing, and peaks area calculation were done. Also, this software (ImageDecipher-TLC) allows the inverting of images and also selection of a pure color (red, green or blue) or integration of all color (gray) for analysis. In our case, for the chromatographic spots quantification was necessary to invert the RGB colored images (consisted of bright-white spots on a purple background) before their conversion into a pure color scale. Usually the images of the chromatographic plates are converted directly into a pure color scale, but in this case as the compounds appeared as bright-white spots on a purple background, the measured areas had negative values, therefore the images were firstly inverted and then converted into a pure color scale, thus obtaining positive values for the measured areas. According to the obtained results, the most uniform background and the best results were obtained by selecting the pure green color (Fig. 2) for the quantification analysis. The quantification of the investigated compounds was based on the integrated peak area in all cases.

In addition, other two specialized software (Sorbfil TLC Videodensitometer and JustTLC) were used for digital processing of images and quantification of amines on the TLC plates, thereby the images (in green scale, bmp files) were then converted in “jpg” format and processed by Sorbfil TLC Videodensitometer and JustTLC. Also, the previous converted images were processed by the two mentioned software in order to calculate area and volume of the chromatographic spots respectively. In case of spot volume evaluation, images were processed by JustTLC software—an advanced digital image analysis software packed with features for editing, quantifying and comparing spots by their automatically detection, only in gray scale. Unlike ImageDecipher-TLC and Sorbfil TLC Videodensitometer software that evaluate the chromatograms in two dimensions (2D analysis) by calculating the spots area, JustTLC performs the quantitative evaluation based on 3D analysis of the chromatographic spot, by measuring the spots area and color intensity, thus calculating the spots volume.

In all cases, the obtained results (based on the fact that both area and volume of the chromatographic spots are proportional with the amount of compound applied on the TLC plate) showed an accurate computation of peaks area and peaks volume of the chromatographic spots. Examples of computed 2D and 3D chromatograms in case of a selected plate area are shown in Fig. 3.

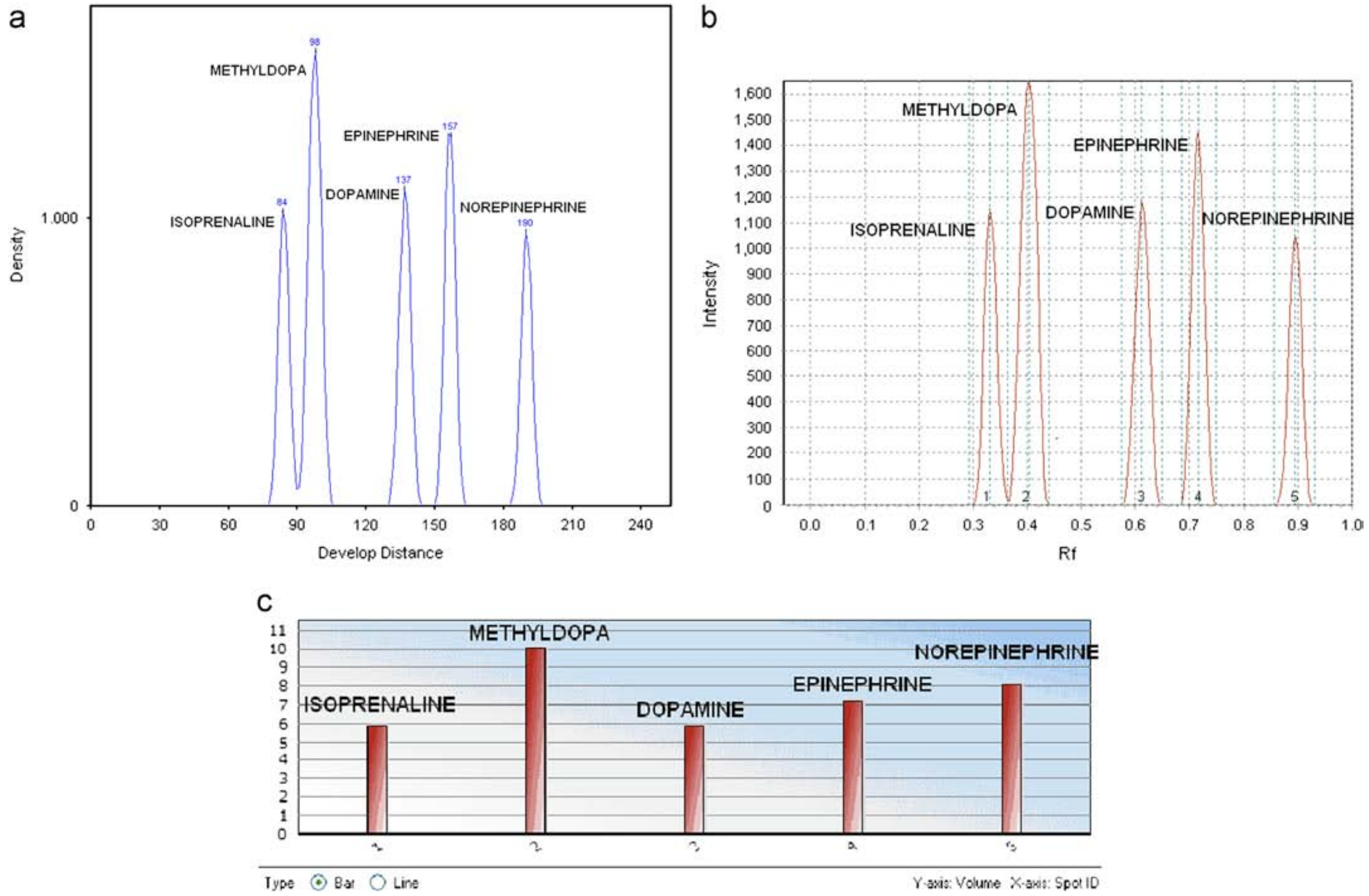

Fig. 3. The obtained chromatograms of the selected plate area using: (a) Image Decipher-TLC software (based on spot area—2D chromatogram); (b) Sorbfil TLC Videodensitometer software (based on spot area—2D chromatogram); and (c) JustTLC software (based on spot volume—3D chromatogram).

3.3. Method validation

For the calibration procedure 6 increasing volumes of the stock solution were applied on the chromatographic plate and the calibration function was constructed for each catecholamine and related compound. The chromatographic peak areas showed a linear relationship to the analyte concentration within the range of 50–200 ng/spot for norepinephrine, epinephrine, dopamine and isoprenaline and 50–300 ng/spot for methyldopa. The best linear regression parameter ($R^2 > 0.997$) was obtained when the ImageDecipher-TLC software was used for the quantification of the chromatographic spots (Table 1).

The precision of the method expressed as relative standard deviation was determined at three concentration levels by analyzing six replicate spots for each concentration. The RSD values of peak areas obtained with the ImageDecipher-TLC software were typically less than 5.42%, 3.04% and 3.84% at low, intermediate and higher concentrations respectively (Table 2).

The accuracy of the method expressed as percent recovery was investigated for standard solutions by analyzing three replicate spots for each of the compounds. In this case the results obtained with all three image analysis software were comparable, showing a percent recovery in range of 102.00%–111.92% for all the investigated compounds (Table 3).

The limits of detection and quantification, calculated using the SMAC software based on the confidence bands of the calibration function, were in range of 11.3–49.3 ng/spot and 21.8–69.6 ng/spot respectively (Table 4), thus sustaining the fact that the proposed free radical DPPH$^\bullet$ is indeed a highly sensitive detection reagent that can be successfully used for quantification of catecholamines and related compounds on the TLC chromatographic plates.

The specificity of the method was determined in relation to the interferences from other compounds in the tested pharmaceutical samples, thus observing that the compounds of interest have been determined using the method without any interference from the excipients used in the pharmaceutical preparations.

3.4. Evaluation of the method by pharmaceutical samples analysis

At present no certified reference materials are available for simultaneous evaluation of E, NE, D, IP and MP in pharmaceuticals. Thereby, the applicability of the method was tested on two kinds of pharmaceutical samples—the pharmaceutical tablets containing methyldopa as active compound and epinephrine injections (that were spiked with known quantities of norepinephrine, dopamine and isoprenaline) after a minimal sample preparation step. The evaluation of the method was performed at two concentration levels for the pharmaceutical samples containing mixture of E, NE, D and IP and also for the methyldopa containing samples (Table 5). The performance evaluation of the method showed a positive deviation from the designated values in the range 3.00%–6.00% for

Table 1
Method evaluation parameters (linearity range, regression equation and coefficient of determination (R^2)) for the investigated amines determination.

Compound	Linearity range (ng/spot)	Software	Regression equation	R^2
Norepinephrine	50–200	ImageDecipher-TLC	$y = 134599x + 4368$	0.9995
		Sorbfil TLC	$y = 119295x + 1699$	0.9947
		JustTLC	$y = 158.11x + 0.537$	0.9933
Epinephrine	50–200	ImageDecipher-TLC	$y = 229606x - 3899$	0.9984
		Sorbfil TLC	$y = 192975x - 7091$	0.9935
		JustTLC	$y = 177.99x - 4.890$	0.9973
Dopamine	50–200	ImageDecipher-TLC	$y = 264182x - 7937$	0.9986
		Sorbfil TLC	$y = 209008x - 7046$	0.9925
		JustTLC	$y = 210.15x - 6.287$	0.9960
Isoprenaline	50–200	ImageDecipher-TLC	$y = 169291x - 900$	0.9981
		Sorbfil TLC	$y = 144392x - 2857$	0.9946
		JustTLC	$y = 159.09x - 3.204$	0.9914
Methyldopa	50–300	ImageDecipher-TLC	$y = 91976x + 12013$	0.9983
		Sorbfil TLC	$y = 92178x + 11931$	0.9967
		JustTLC	$y = 103.80x + 9.178$	0.9951

Table 2
Method evaluation parameters (precision) for the investigated amines determination.

Compound	Concentration (ng/spot)	RSD (%)		
		I	II	III
Norepinephrine	50	5.42	3.49	5.71
	100	3.04	7.28	2.58
	150	3.84	7.23	8.31
Epinephrine	50	1.32	2.39	2.17
	100	0.66	0.64	3.35
	150	1.34	2.28	3.45
Dopamine	50	3.53	3.41	6.27
	100	1.82	1.68	3.21
	150	1.04	5.92	7.51
Isoprenaline	50	1.91	4.62	2.81
	100	2.58	2.67	1.36
	150	1.80	1.19	3.42
Methyldopa	50	2.39	2.24	3.78
	100	2.31	2.38	2.95
	150	0.98	2.09	3.24

(I)—ImageDecipher-TLC.
(II)—Sorbfil TLC.
(III)—JustTLC.

Table 3
Method evaluation parameters (accuracy) for the investigated amines determination.

Compound	Concentration (ng/spot)	Found concentration (ng/spot)			Recovery (%)		
		I	II	III	I	II	III
Norepinephrine	125	138.5	137.4	137.1	110.80	109.92	109.68
Epinephrine	125	134.1	133.3	129.7	107.28	106.64	103.76
Dopamine	125	139.9	135.9	133.1	111.92	108.72	106.48
Isoprenaline	125	127.5	128.5	131.6	102.00	102.80	105.28
Methyldopa	150	159.6	160.1	155.0	106.40	106.73	103.33

(I)—ImageDecipher-TLC.
(II)—Sorbfil TLC.
(III)—JustTLC.

Table 4
Method evaluation parameters (LOD and LOQ) for the investigated amines determination.

Compound	ImageDecipher-TLC (ng/spot)		Sorbfil TLC (ng/spot)		JustTLC (ng/spot)	
	LOD	LOQ	LOD	LOQ	LOD	LOQ
Norepinephrine	15.6	31.1	11.3	21.8	12.8	24.4
Epinephrine	29.0	41.3	46.2	57.6	34.0	41.6
Dopamine	41.4	54.2	44.1	56.3	49.3	69.6
Isoprenaline	17.7	29.5	29.6	40.1	32.6	45.6
Methyldopa	13.9	27.0	15.5	30.0	19.0	36.3

Table 5
Quantitative evaluation of the investigated amines in different pharmaceutical samples using the proposed TLC method.

Compound	Concentration of the pharmaceutical sample (μg/mL)	Estimated concentration in the pharmaceutical sample* (μg/mL)					
		ImageDecipher-TLC	Relative accuracy error (%)**	Sorbfil TLC	Relative accuracy error (%)**	JustTLC	Relative accuracy error (%)**
Norepinephrine	20	20.9 ± 0.6	4.5	20.7 ± 0.7	3.5	21.1 ± 0.7	5.5
	30	30.5 ± 0.1	1.7	31.8 ± 0.1	6.0	30.9 ± 0.6	3.0
Epinephrine	20	20.4 ± 0.3	2.0	19.4 ± 0.6	3.0	21.2 ± 0.3	6.0
	30	31.2 ± 0.6	4.0	30.9 ± 0.9	3.0	30.8 ± 0.2	2.7
Dopamine	20	20.6 ± 0.6	3.0	20.2 ± 0.6	1.0	20.8 ± 0.4	4.0
	30	29.4 ± 0.7	2.0	29.8 ± 1.1	0.7	29.2 ± 1.3	2.7
Isoprenaline	20	19.9 ± 0.3	0.5	20.9 ± 0.5	4.5	20.2 ± 0.1	1.0
	30	29.7 ± 0.5	1.0	29.4 ± 0.8	2.0	31.4 ± 1.1	4.7
Methyldopa	200	205.9 ± 3.2	2.9	212.3 ± 6.5	6.1	207.7 ± 1.5	3.8
	300	309.6 ± 4.1	3.2	300.5 ± 1.7	0.2	301.6 ± 1.6	0.5

* Data are mean of three replicate spots ± SD.

** Relative accuracy error (%) = [(experimental value − true value)/true value] × 100.

norepinephrine, 2.00%–6.00% for epinephrine and in range 0.17%–6.15% for methyldopa. In the other cases, generally a positive deviation (1.00%–4.00% for dopamine and 1.00%–4.50% for isoprenaline) from the designated values were observed for low analyte concentration and a negative deviation (2.67%–0.67% for dopamine and 2.00%–1.00% for isoprenaline) for a high level of analyte concentration. From these results it can be seen that the deviations obtained in the external quality control of the method were lower than the deviations derived from the method evaluation procedure (positive deviations in range 9.68%–10.8% for norepinephrine; 3.76%–7.28% for epinephrine; 6.48%–11.92% for dopamine; 2.00%–5.28% for isoprenaline and 3.33%–6.73% for methyldopa, in case of recovery experiments).

4. Conclusions

A simple, fast, highly sensitive and economical method was developed for simultaneous determination of catecholamines (norepinephrine, epinephrine and dopamine) and related drugs (isoprenaline and methyldopa) using a new detection method and TLC assisted by digital processing of chromatographic plates. The proposed detection reagent (free radical DPPH$^{\bullet}$ solution in ethanol) was found to be highly sensitive and appropriate for the quantitative evaluation of investigated compounds, after their chromatographic separation. The evaluation of the method indicated that it has a reasonable low standard deviation, a good precision and accuracy and no prederivatization step is needed. In addition, the performance evaluation of the method, based on external quality control on different spiked pharmaceutical samples, showed no significant deviations from the designated values in all cases. The high sensitivity and specificity of the developed method can extend its applicability for future simultaneous assessment of other related biologically important molecules as well.

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References

[1] B. Assmann, Future Neurol. 1 (2006) 651–657.
[2] E. Dadáková, M. Křížek, T. Pelikánová, Food Chem. 116 (2009) 365–370.
[3] H.E. Himwich, W.A. Himwich, Biogenic Amines, Elsevier, Amsterdam, 1964.
[4] R.-S. Chen, W.-H. Huang, H. Tong, Z.-L. Wang, J.-K. Cheng, Anal. Chem. 75 (2003) 6341–6345.
[5] T. Aizawa, N. Ishizaka, S.-I. Usui, N. Ohashi, M. Ohno, R. Nagai, Hypertension 39 (2002) 149–154.
[6] J.J.B. Nevado, J.M.L. Gallego, P.B. Laguna, Anal. Chim. Acta 300 (1995) 293–297.
[7] S. Shioda, I. Homma, N. Kato, Transmitters and Modulators in Health and Disease, Springer, Tokyo, 2009.
[8] H. Sirén, M. Mielonen, M. Herlevi, J. Chromatogr. A 1032 (2004) 289–297.
[9] M. Moini, C.L. Schultz, H. Mahmood, Anal. Chem. 75 (2003) 6282–6287.
[10] C.R. Raj, T. Okajima, T. Ohsaka, J. Electroanal. Chem. 543 (2003) 127–132.
[11] X.L. Zhu, P.N. Shaw, D.A. Barrett, Anal. Chim. Acta 478 (2003) 259–269.
[12] M.R. Hormozi Nezhad, J. Tashkhourian, J. Khodaveisi, J. Iran. Chem. Soc. 7 (2010) 83–91.
[13] A. Törnkvist, P.J.R. Sjöberg, K.E. Markides, J. Bergquist, J. Chromatogr. B 801 (2004) 323–329.
[14] W.H.A. de Jong, E.G.E. de Vries, B.H.R. Wolffenbuttel, I.P. Kema, J. Chromatogr. B 878 (2010) 1506–1512.
[15] C.L. Guan, J. Ouyang, Q.L. Li, B.H. Liu, W.R.G. Baeyens, Talanta 50 (2000) 1197–1203.
[16] A. Kumar, J.P. Hart, D.V. McCalley, J. Chromatogr. A 1218 (2011) 3854–3861.
[17] M. Kamahori, M. Taki, Y. Watanabe, J. Miura, J. Chromatogr. B 567 (1991) 351–358.
[18] Å.M. Hansen, J. Kristiansen, J. Løgsted Nielsen, K. Byrialsen, J.M. Christensen, Talanta 50 (1999) 367–379.
[19] D.V. McCalley, J. Chromatogr. A 1217 (2010) 858–880.
[20] R.J. Shakila, T.S. Vasundhara, K.V. Kumudavally, Food Chem. 75 (2001) 255–259.
[21] N.P.J. Price, D.O. Gray, J. Chromatogr. A 635 (1993) 165–170.
[22] C. Sabbioni, M.A. Saracino, R. Mandrioli, S. Pinzauti, S. Furlanetto, G. Gerra, M.A. Raggi, J. Chromatogr. A 1032 (2004) 65–71.
[23] M. Segura, J. Ortuño, J.A. McLure, M. Pujadas, N. Pizarro, M. Farré, A. Llebaria, J. Joglar, J. Segura, R. de la Torre, J. Cromatogr. B 769 (2002) 313–321.
[24] S. Parrot, P.C. Neuzeret, L. Denoroy, J. Cromatogr. B 879 (2011) 3871–3878.
[25] F. Soponar, A.C. Moţ, C. Sârbu, J. Chromatogr. A 1188 (2008) 295–300.
[26] F. Soponar, A.C. Moţ, C. Sârbu, Chromatographia 69 (2009) 151–155.
[27] F. Soponar, A.C. Moţ, C. Sârbu, J. AOAC Int. 93 (2010) 804–810.
[28] D. Casoni, I.A. Tuhuţiu, C. Sârbu, J. Liq. Chromatogr. Relat. Technol. 34 (2011) 805–816.
[29] M. Olech, Ł. Komsta, R. Nowak, Ł. Cieśla, M. Waksmundzka-Hajnos, Food Chem. 132 (2012) 549–553.
[30] M. Lancaster, D.M. Goodall, E.T. Bergstrőm, S. McCrossen, P. Myers, J. Chromatogr. A 1090 (2005) 165–171.
[31] L.A. Fazakaş, R.D. Naşcu-Briciu, C. Sârbu, J. Liq. Chromatogr. Relat. Technol. 34 (2011) 2315–2325.
[32] K. Bansal, J. McCrady, A. Hansen, K. Bhalerao, Fuel 87 (2008) 3369–3372.
[33] S.C. Cobzac, D. Casoni, A.L. Fazakaş, C. Sârbu, J. Liq. Chromatogr. Relat. Technol. 35 (2012) 1–15.
[34] B. Hemmateenejad, N. Mobaraki, F. Shakerizadeh-Shirazi, R. Miri, Analyst 135 (2010) 1747–1758.
[35] H. Jork, W. Funk, W. Fisher, H. Wimmer, Thin-layer chromatography. Reagents and detection methods, vol. 1a: Physical and Chemical Detection Methods VHC, Weinheim, Germany, 1990.
[36] M. Yamaguchi, J. Ishida, M. Yoshimura, Analyst 123 (1998) 307–311.
[37] D. Casoni, C. Sârbu, J. Sep. Sci. 35 (2012) 915–921.