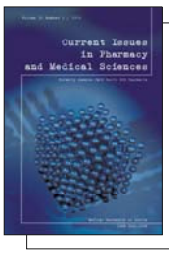

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Qualitative and quantitative analysis of diosgenin in pure substance by TLC-densitometry

MAŁGORZATA DOŁOWY

Department of Analytical Chemistry, Faculty of Pharmacy, Silesian University of Medicine, Poland

ABSTRACT

A simple and sensitive TLC-densitometric method for qualitative and quantitative analysis of diosgenin in pure sample was elaborated. Satisfactory results of the densitometric analysis of examined compound was obtained using chloroform-acetone in volume composition 40:10 (v/v) as a mobile phase and by means of the chromatographic plates precoated with silica gel $60F_{254}$ and silica gel 60. Visualization of the diosgenin spots was achieved by dipping the plates into 10% ethanol solution of phosphomolybdic acid and 10% ethanol solution of sulfuric acid respectively. The limit of detection (LOD) and the limit of quantification (LOQ) of diosgenin investigated under different chromatographic conditions was determined. The results obtained in this work indicate that, the elaborated method is suitable for densitometric analysis of diosgenin but only in pure sample.

Keywords: diosgenin, TLC-densitometry, thin-layer chromatography

INTRODUCTION

Diosgenin chemically known as (25R)-5-spirosten--3-ol is a naturally occurring steroidal sapogenin. It has a significant medicinal value. Diosgenin is a source of steroidal hormones mainly estrogens [11]. Besides estrogenic effect and influence for cholesterol metabolism, diosgenin and its derivatives indicate antitumor activities, because it inhibits reproduction of tumor cells by inducing apoptosis (e.g. in the case of human leukemia) [4].

Hence, it is necessity to develop a suitable analytical method for determination of diosgenin as a bioactive substance in pure sample and in form of different pharmaceutical formulations.

Some of the methods so far for the estimation of the ratio of diosgenin mostly in plant samples are based on spectrophotometry, immunoenzyme method (ELISA assay), liquid chromatography including: TLC, HPLC-UV and HPLC-ELSD [3,5,13]. As has been reported in literature among methods used in diosgenin analysis content, TLC-densitometry has emerged as an efficient tool for the simultaneous quantification of diosgenin in plant samples and herbal drugs [1,10,12,13].

In this work, a qualitative and quantitative TLC-densitometric analysis of diosgenin in pure substance was performed. Densitometric analysis was carried out under different chromatographic conditions such as: various chromatographic plates for TLC (aluminum and glass) precoated with silica gel 60 and silica gel $60F_{254}$: Art. 1.05553, Art. 1.05554, Art. 1.05715 (E. Merck), different volume composition of both mobile phases used: chloroform-acetone and *n*-hexane-ethyl acetate-acetic acid and using two visualizing agents (10% ethanol solution of sulfuric acid and 10% ethanol solution of phosphomolybdic acid). These chromatographic conditions were satisfactorily applied in our department in previous TLC analysis of another steroidal compounds e.g. bile acids [2,6-9]. Comparison of the obtained results especially LOD and LOQ values with those presented in literature reports allow to estimate the useful of the chromatographic systems proposed in this study for qualitative and quantitative analysis of diosgenin in pure sample.

Corresponding author
* Department of Analytical Chemistry, Faculty of Pharmacy,
Silesian University of Medicine,
4 Jagiellońska St., PL-41-200 Sosnowiec, Poland
e-mail: mdolowy@sum.edu.pl

MATERIALS AND METHODS

Chemicals

Diosgenin min. 99% was purchased from Sigma–Aldrich, (St. Louis, MO, USA). The following components of mobile phases: chloroform, acetone, *n*-hexane, ethyl acetate, acetic acid and methanol used for preparing of standard solution of diosgenin at a concentration 2 $mg \cdot mL^{-1}$ were from POCh (Gliwice, Poland). All chemicals were analytical grade. Sulfuric acid 95% and phosphomolybdic

acid used as the visualizing reagents were from POCh, (Gliwice, Poland). Ethanol (96%) with its chemical grade (POCh, Gliwice, Poland) was applied to prepare the solutions of above-mentioned visualizing agents.

Apparatus

Densitometer: TLC Scanner 3 Camag (Muttenz, Switzerland) operated in absorbance equipped with winCATS 1.4.2 software. The source of the radiation was deuterium lamp. Chromatograhic chambers (Camag, Muttenz, Switzerland).

Chromatographic plates 20cm×20cm (E. Merck, Germany) for NP-TLC analysis: aluminum plates precoated with silica gel $60F_{254}$ (Art. 1.05554), aluminum plates precoated with silica gel 60 (Art. 1.05553) and glass plates precoated with a silica gel $60F_{254}$ (Art. 1.05715). Micropipettes 5μL Camag (Muttenz, Switzerland).

NP-TLC analysis

Firstly, in order to determine the suitable chromatographic conditions for TLC analysis of diosgenin 2 L of methanol solution of diosgenin at a concentration 2 mg·ml^{-1} were spotted on chromatographic plates 4cm×10cm (cut from 20cm×20cm): Art. 1.05715, Art. 1.05554 and Art 1.05715. The chromatographic plates were activated for 30 minutes at temperature 120°C prior chromatography. A mixture consisted of:

- chloroform-acetone in various volume compositions (Fig. 1);
- *n*-hexane-ethyl acetate-acetic acid in different volume compositions (Fig. 2) were applied per chromatography run.

Fifty mL of respective mobile phase was used in all cases. The chromatographic plates were developed to a distance of 8 cm at temperature 20±2°C in the chromatographic chambers saturated prior to analysis with the mobile phase vapors for 30 minutes. After developing, the plates were dried in a fume cupboard for 24 hours. The spots were visualized with the use of 10% ethanol solution of sulfuric acid. This visualizing agent was sensitive for TLC detection of different steroidal compounds e.g. bile acids [6-9]. Visualization of the spots was made by dipping the plates into ethanol solution of sulfuric acid for 15 seconds. Next, the plates were heated at temperature 120°C for 15 minutes. On the basis of obtained chromatograms, the R_F values of diosgenin were determined. Calculated R_F values were used to search the optimal mobile phase for diosgenin TLC analysis on all applied chromatographic plates.

Optimization of the NP-TLC densitometric method

The aim of this stage was to develop the next chromatographic conditions that would enable efficient qualitative and quantitative analysis of diosgenin such as: a proper visualizing agent and a suitable wavelength (λ_{max}) for densitometric scanning of the chromatograms after developing the plates using mobile phases: chloroform-acetone 40:10 (v/v) and *n*-hexane-ethyl acetate-acetic acid 24:25:1 (v/v/v). These mobile phases were previously described as the best, because allowed satisfactory identification of diosgenin on all applied chromatographic plates. Before TLC-densitometric analysis, the plates were activated at 120°C for 30 minutes. A quantity of 2 μL of methanol solution of diosgenin at a concentration 2 mg·mL^{-1} was spotted on chromatographic plates used in experiment. The mobile phase was run up to a distance 8 cm. After drying at room temperature for 24 h using a fume cupboard, the spots were visualized by dipping the plates into respective visualizing agent: 10% ethanol solution of sulfuric acid for 15 seconds or into 10% ethanol solution of phosphomolybdic acid respectively for 15 seconds. Then after heating at 120°C for 15 minutes, the chromatograms were densitometrically recorded under the following conditions:

- the slit dimensions were 8.00×0.40 mm Macro,
- the scanning speed was 20 mm·s^{-1},
- the data resolution was 100 m·step^{-1}.

Densitometric scanning was performed at multiwavelength in the range from 200 to 800 nm at interval 25 nm in order to select the appropriate wavelength for diosgenin quantitative analysis.

Assay and quantitative determination of diosgenin

Preparation of calibration plot. Stock solution of examined diosgenin at a concentration 2 mg·mL^{-1} was serial diluted using methanol to the concentration: 1 mg·mL^{-1}, 0.66 mg·mL^{-1}, 0.4 mg·mL^{-1}, 0.2 mg·mL^{-1}, 0.1 mg·mL^{-1}, 0.04 mg·mL^{-1} and 0.02 mg·mL^{-1}. These diluted solutions of diosgenin and a stock solution at a concentration 2 mg·mL^{-1} were spotted on chromatographic plates 4cm x 10cm (Art. 1.05553, Art. 1.05554, Art. 1.05715) in quantity 1μL using micropipette equivalent to: 2, 1, 0.66, 0.4; 0.2, 0.1, 0.04 and 0.02 μg·spot^{-1}. Chromatograms were run up to distance 8 cm. Next, after drying the plates at room temperature for 24 h using a fume cupboard, the spots were visualized by dipping them into 10% ethanol solution of phosphomolybdic acid and 10% ethanol solution of sulfuric acid respectively for 15 seconds. Densitometric evaluation of the spots was performed at respective wavelength, suitable for each chromatographic system, after prior heating the plates at the temperature 120°C for 15 minutes. Each analysis was repeated three times.

On the basis of the densitometric measurements of the spot surface area obtained for respective amount of diosgenin a linearity of the relationship between the spot area ratio of diosgenin against corresponding amount of this substance (μg of diosgenin per spot) was determined. Im-

portant parameters of the obtained calibration curves type: Y=a·x+b, were determined. On the basis of each calibration plot parameters, the smallest amount of diosgenin determined under respective chromatographic conditions were estimated in term of limit of detection (LOD) and quantification limit (LOQ).

RESULTS

The aim of this paper was to use the TLC-densitometry for qualitative and quantitative analysis of diosgenin in pure substance. In order to search an appropriate mobile phase, several runs were exercised using the following solvent systems: chloroform-acetone and *n*-hexane-ethyl acetate-acetic acid mixed in different volume compositions. Graphical relationships between the R_F values of diosgenin obtained on all applied chromatographic plates (Art. 1.05554, Art. 1.05553, Art. 1.05715) and volume composition of both mobile phases used in experiment were presented in Fig. 1 and Fig. 2.

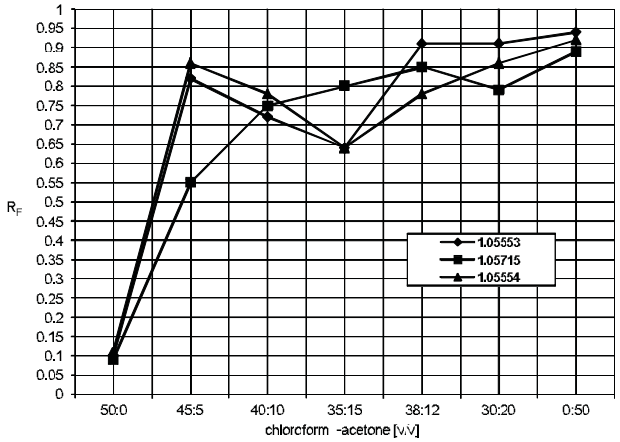

Fig.1. The relationship between the R_F values of diosgenin determined using chloroform-acetone in different volume compositions as a mobile phase on various chromatographic plates (Art. 1.05554, Art. 1.05553, Art. 1.05715) and volume composition of mobile phase

To compare the R_F results obtained using both mobile phases on all applied chromatographic plates, the similarity analysis was performed (Fig. 3 and Fig. 4).

Further step in this study referred to search the next optimal chromatographic conditions necessary for densitometric analysis of diosgenin. Hence, to find a sensitive visualizing agent for detection of examined diosgenin, the chromatographic plates were developed using both optimal mobile phases: *n*-hexane-ethyl acetate-acetic acid in volume composition: 24:25:1 (v/v/v) and chloroform-acetone 40:10 (v/v) respectively. Visualization of the diosgenin spots obtained under above described chromatographic conditions were achieved using 10% ethanol solution of phosphomolybdic acid and 10% ethanol solution of sulfuric acid. Chromatographic characteristic of the spots including the R_F values and the color of the spots produced by the studied visualizing agents with diosgenin on all chromatographic plates are listed in Tab. 1.

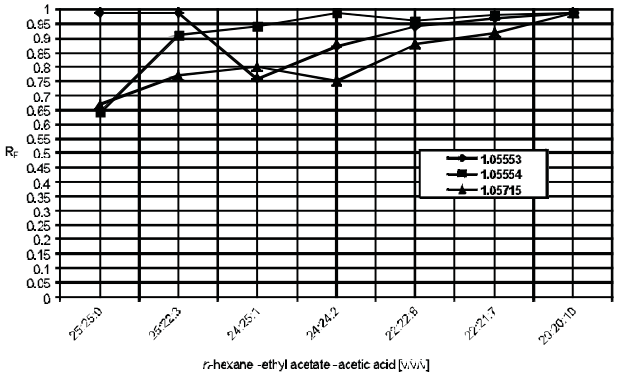

Fig. 2. The relationship between the R_F values of diosgenin determined using *n*-hexane-ethyl acetate-acetic acid in different volume compositions as a mobile phase on various chromatographic plates (Art. 1.05554, Art. 1.05553, Art. 1.05715) and volume composition of mobile phase

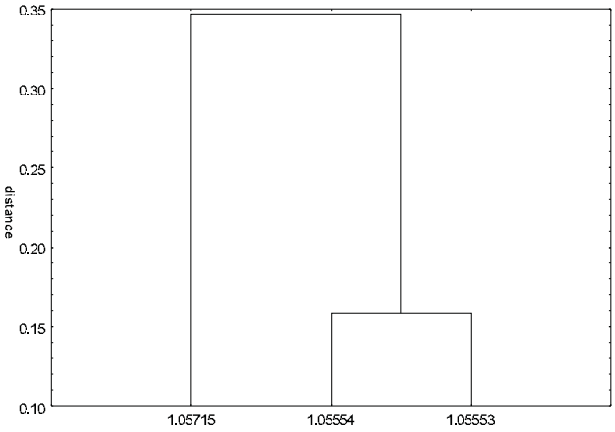

Fig. 3. Dendrogram of the similarity of the R_F values for diosgenin obtained using chloroform-acetone in different volume compositions as a mobile phase on various chromatographic plates (Art. 1.05554, Art. 1.05553, Art. 1.05715)

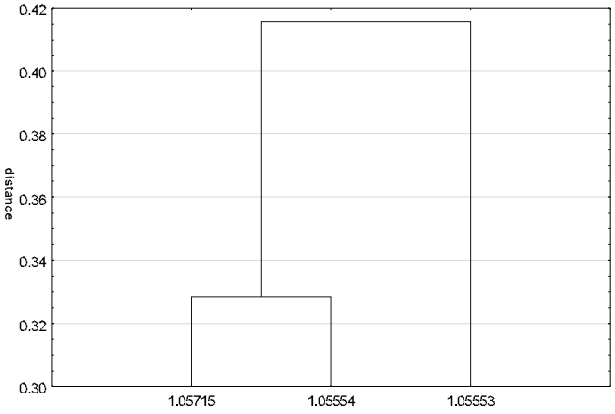

Fig. 4. Dendrogram of the similarity of the R_F values for diosgenin obtained using *n*-hexane-ethyl acetate-acetic acid in different volume compositions as a mobile phase on various chromatographic plates (Art. 1.05554, Art. 1.05553, Art. 1.05715)

Table 1. Chromatographic characteristic of the spots produced by the studied visualizing agents for diosgenin investigated on different chromatographic plates: Art. 1.05554, Art. 1.05553 and Art. 1.05715 using applied mobile phases

Mobile phase used	chloroform-acetone 40:10 (v/v)			*n*-hexane-ethyl acetate-acetic acid 24:25:1 (v/v/v)		
Substance	*Diosgenin*					
Chromatographic plates	1.05554	1.05553	1.05715	1.05554	1.05553	1.05715
R_F value	0.78	0.72	0.75	0.94	0.76	0.80
Visualizing agent	10% phosphomolybdic acid					
Spot color	dark blue	dark blue	dark blue	dark blue	dark blue	dark blue
Chromatogram backround	green	green	green	green	green	green
Visualizing agent	10% sulfuric acid					
Spot color	grey-green	grey-green	grey-green	grey-green	grey-green	grey-green
Chromatogram backround	light pink	light pink	light pink	light pink	light pink	light pink

Next, to search the optimum wavelength for TLC-densitometric analysis of diosgenin using phosphomolybdic acid and sulfuric acid, the multiwavelength spectrum of diosgenin was recorded in the range from 200 to 800 nm in both cases. The results of the maximum wavelengths for diosgenin spectra obtained under applied chromatographic conditions are shown in Tab. 2.

Table 2. Detection of the optimal wavelength (λ_{max}) for TLC-densitometric analysis of diosgenin examined under different chromatographic conditions

n-hexane-ethyl acetate-acetic acid 24:25:1 (v/v/v)			
Visualization of the spots	Chromatographic plates		
	1.05715	1.05554	1.05553
	Maximum wavelenght λ_{max} [nm]		
Without visualizing agent	200	200	–
10% phosphomolybdic acid	800	716	699
10% sulfuric acid	422	396	277
chloroform-acetone 40:10 (v/v)			
Visualization of the spots	Chromatographic plates		
	1.05715	1.05554	1.05553
	Maximum wavelenght λ_{max} [nm]		
Without visualizing agent	200	200	200
10% phosphomolybdic acid	800	756	745
10% sulfuric acid	396	598	200

Exemplary densitograms of diosgenin obtained under the optimal chromatographic conditions: using mobile phase chloroform-acetone 40:10 (v/v) and *n*-hexane-ethyl acetate-acetic acid 24:25:1 (v/v/v), which were visualized with the use of phosphomolybdic acid are presented in Fig. 5 and Fig. 6.

On the basis of the results of the spot surface area of diosgenin measured densitometrically, a linearity of the relation between spot surface area and diosgenin content was determined. In Tab. 3 the results of the linear regression analysis of the quantitative TLC-densitometric method of diosgenin in terms of: linearity, the limit of quantification (LOQ), the limit of detection (LOD) are performed.

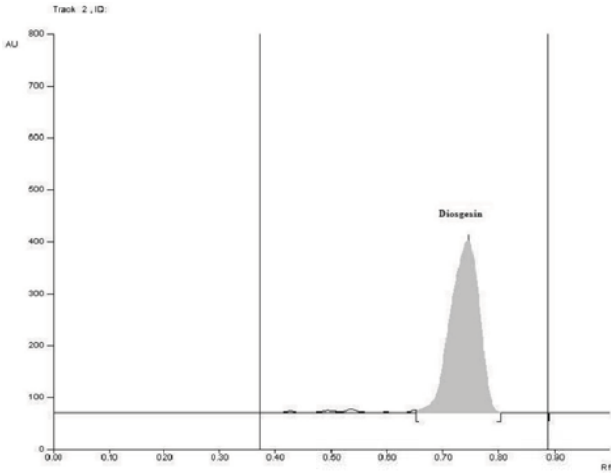

Fig. 5. TLC-densitogram of diosgenin examined on chromatographic plates Art. 1.05715 developed using chloroform-acetone 40:10 (v/v) and visualized by phosphomolybdic acid

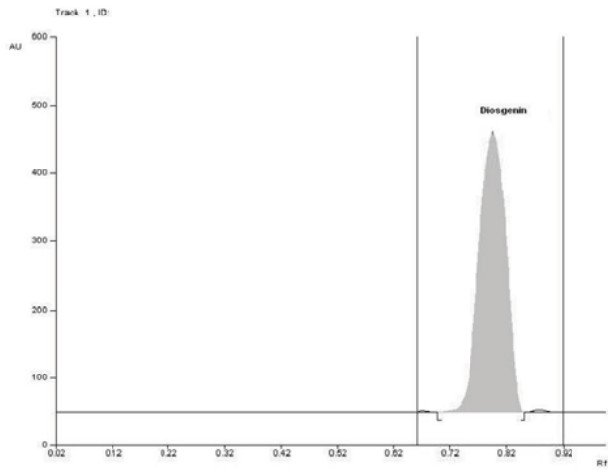

Fig. 6. TLC-densitogram of diosgenin examined on chromatographic plates Art. 1.05715 developed using *n*-hexane-ethyl acetate-acetic acid 24:25:1 (v/v/v) and visualized by phosphomolybdic acid

DISCUSSION OF THE RESULTS

The first stage in these investigations was to search the optimal chromatographic conditions enabling satisfactory identification of diosgenin such as: mobile phase composition, type of the chromatographic plates and the kind of visualizing agent. Fig. 1 shows that the R_F values change much when the content of acetone in mobile phase: chloroform-acetone is increased. To obtain a satisfactory R_F value of diosgenin ($0.30 \leq R_F < 80$), the content of acetone in mobile phase: chloroform-acetone should be equal to 10%. Less content of acetone in this mobile phase than 10% influences obtaining a very small R_F values (about 0.10). On the other hand, the amount of acetone of more than 10% gives very high R_F values (above 0.9), especially in the case of chromatographic plates Art. 1.05554. For this stationary phase, a strong influence of acetone content in mobile phase used for TLC study of diosgenin

Table 3. Calibration curves parameters, correlations, detection limit (LOD) and quantification limit (LOQ) of examined diosgenin studied on different chromatographic plates developed with the use of chloroform–acetone in volume composition 40:10 and visualized using 10% ethanol solution of phosphomolybdic acid and 10% ethanol solution of sulfuric acid

Substance	Chroma-tographic plates	Visualizing agent	Calibration curve parameters Y=a·x + b	Linearity range [μg·spot^{-1}]	$t_a = a/S_a$ $t_a \geq t_{\alpha,f}$ (2.571) statistically significant	$t_b = b/S_b$ $t_b \leq t_{\alpha,f}$ (2.571) statistically insignificant	$t_{calc} = \left\| \frac{r}{\sqrt{1-r2}} \cdot \sqrt{n-2} \right\|$ $t_{cal} \geq t_{\alpha,f}$ (2.571) linear relationship between Y and x	LOD [μg·spot^{-1}]	LOQ [μg·spot^{-1}]
Diosgenin	1.05554	10% ethanol solution of phosphomo-lybdic acid	a=5439.52±760.96 b=2212.33±683.49 r=0.954, p=0.0008 F=51.10, S=1291.73	0.04 – 2.00	7.148	3.237	6.364	0.784	2.375
	1.05553		a=7127.2±819.82 b=1454.16±688.82 r=0.962, p=0.0001 F=75.58, S=1467.81	0.02 – 2.00	8.694	2.111	7.048	0.680	2.059
	1.05715		a=4981.36±604.92 b=1475.18±543.33 r=0.965, p=0.0004 F=67.81, S=1026.84	0.04 – 2.00	8.235	2.715	7.068	0.680	2.061
Diosgenin	1.05554	10% ethanol solution of sulfuric acid	a=2124.54±237.84 b=420.80±213.62 r=0.970, p=0.0003 F=79.79, S=403.73	0.04 – 2.00	8.933	1.970	7.980	0.627	1.900
	1.05553		a=3177.07±257.34 b=407.83±231.14 r=0.984, p=0.0001 F=152.42, S=436.83	0.04 – 2.00	12.346	1.764	11.046	0.454	1.375
	1.05715		a=2381.99±261.16 b=591.44±234.55 r=0.971, p=0.0003 F=83.19, S=445.15	0.02 – 2.00	9.121	2.522	8.123	0.617	1.869

Y – area of spot, a and b – regression parameters, x – amount of analyzed anabolic [g], r – correlation coefficient, p – significance level, F – value of Fisher test, S – standard error of estimate, S_a – standard error of the slope, S_b – standard error of intercept, t_f – t-value in Student's test for =0.05 and f = n-2, where n=7

is observed (Fig. 1). On the basis of the R_F results obtained using chloroform-acetone as a mobile phase, it was suggested that of all combinations employed, a mixture of chloroform-acetone 40:10 (v/v) enables obtaining the optimal R_F values of diosgenin, which were located in the range 0.72-0.78 on all types of chromatographic plates used. For this reason, a solvent system: chloroform-acetone was recommended for further quantitative analysis of diosgenin as the optimal mobile phase.

Fig. 2 shows the relation between R_F of diosgenin obtained on various chromatographic plates (Art. 1.05554, Art. 1.05553, Art. 1.05715) and the volume composition of mobile phase: *n*-hexane-ethyl acetate-acetic acid. On the basis of this relationship, it can be observed that similarly like in the case of Fig.1, the R_F values obtained on chromatographic plates Art. 1.05554 increase much (R_F>0.9) with the content of acetic acid in mobile phase: *n*-hexane-ethyl acetate-acetic acid. Of all applied volume compositions of this mobile phase, only mobile phase in volume composition 24:25:1 (v/v/v) allows obtaining the satisfactory R_F values on all applied chromatographic plates together. With the use of this volume composition of above-mentioned mobile phase, the R_F values of diosgenin are: 0.76 for Art. 1.05553, 0.80 in the case of Art. 1.05715 and 0.94 for Art. 1.05554. Another volume composition of a mixture: *n*-hexane-ethyl acetate-acetic acid gives the R_F values of diosgenin more than 0.94 especially for Art. 1.05553 and Art. 1.05554. The R_F values obtained on chromatographic plates Art. 1.05715 similarly like in the case of above described chromatographic conditions change slowly from 0.67 to 0.99 with increasing the content of acetic acid in mobile phase: *n*-hexane-ethyl acetate-acetic acid (Fig. 2). The results depicted in Fig. 2 indicate that the mobile phase: *n*-hexane-ethyl acetate-acetic acid in volume composition 24:25:1 (v/v/v) is the best of all used and can be applied in quantification of diosgenin by TLC-densitometric method.

Dendrogram of the similarity of the R_F values determined on all applied chromatographic plates developed using mobile phase: chloroform-acetone in various volume compositions (Fig. 3) indicates that the R_F values obtained on chromatographic plates Art. 1.05554 and Art. 1.05553 (the smallest Euclidean distance is observed) are very similar. Figure 4 shows that in the case of mobile phase: *n*-hexane-ethyl acetate-acetic acid, the biggest similarity is observed between R_F values obtained on chromatographic plates Art. 1.05715 and Art. 1.05554. It can be suggested that the similarity analysis can be useful in selection of the proper chromatographic conditions needed for diosgenin TLC analysis e.g. the kind of chromatographic plates. On the basis of the results presented in Tab. 1, it can be concluded that both visualizing agents: 10% ethanol solution of phosphomolybdic acid and 10% ethanol solution of sulfuric acid are suitable for TLC analysis of diosgenin, because they form with characteristic spots this compound. The color of the spots is blue in

the case of phosphomolybdic acid and grey-green in the case of sulfuric acid. Heating the chromatograms to 120°C for 15 minutes improve visualization of the diosgenin spots.

The data presented in Tab. 2 indicate that the maximum wavelength of diosgenin spectrum obtained in the presence of phosphomolybdic acid is located from 700 nm to 800 nm for all chromatographic plates used and both mobile phases applied. The maximum wavelength of diosgenin spectra obtained in the presence of sulfuric acid under above-mentioned chromatographic conditions is various for respective chromatographic plate and exists in the range from 200 nm to 598 nm. It could be assumed that phospomolybdic acid is much adequate visualizing agent for quantitative analysis of diosgenin than sulfuric acid because, in the same wavelength range, all chromatograms together (in one track) can undergo densitometric analysis. In the case of sulfuric acid used as a visualizing agent, each chromatogram (obtained under different chromatographic conditions) must be recorded at the individual wavelength. For this reason, densitometric measurements of diosgenin using sulfuric acid require more time to perform this analysis and they are time consuming. Moreover, on the basis of the data presented in Tab. 2, the following chromatographic conditions such as: chloroform-acetone 40:10 (v/v) as a mobile phase and 10% ethanol solution of phosphomolybdic acid as a suitable visualizing agent for quantitative TLC-densitometric analysis of diosgenin in pure sample was chosen. The quantitative TLC-densitometric analysis of diosgenin presented in this paper was validated in terms of: linearity, the limit of quantification (LOQ) and the limit of detection (LOD). The calibration plots were constructed by plotting spot areas against the corresponding amounts of diosgenin in $g \cdot spot^{-1}$. A linearity of the relations between spot areas and content of diosgenin was verified for the following amount ranges:

- 0.04-2.00 $\mu g \cdot spot^{-1}$ in the case of chromatographic plates Art. 1.05554 and both visualizing agents,
- 0.02-2.00 $\mu g \cdot spot^{-1}$ for chromatographic plates Art. 1.05553 and phosphomolybdic acid,
- 0.02-2.00 $\mu g \cdot spot^{-1}$ for chromatographic plates Art. 1.05715 and sulfuric acid,
- 0.04-2.00 $\mu g \cdot spot^{-1}$ for chromatographic plates Art. 1.05715 and phosphomolybdic acid,
- 0.04-2.00 $\mu g \cdot spot^{-1}$ for chromatographic plates Art. 1.05553 and sulfuric acid.

The LOD and LOQ values calculated on the basis of the calibration plots and standard error of estimation determined for all applied chromatographic conditions are given in Tab. 3. The data presented in Tab. 3 indicate that the lowest LOD and LOQ value of diosgenin could be determined for the chromatograms visualized with the use of sulfuric acid. In the case of the chromatograms visualized with phosphomolybdic acid the level of LOD values are similar to the LOD values obtained with the use of sulfuric acid but the LOQ values in this case are much higher.

Comparison of the obtained results especially of LOD and LOQ values with the data presented in literature reports [1,10,12-13] indicate that a proper choice of the chromatographic conditions such: mobile phase composition, kind of the chromatographic plates and the type of visualizing agent influence the effect of quantitative analysis of diosgenin. The mobile phase chloroform-acetone in volume composition 40:10 described in this paper as the best of all applied gives similar R_F values (about 0.76) like in the case of the solvent system: petroleum ether-isopropanol 12:1 (v/v) used for diosgenin analysis in rhizome of *Dioscorea deltoidea* [1]. However, in this case a lower LOQ value of diosgenin in comparison of chloroform-acetone mixture is observed. The results presented in the next paper concerning diosgenin study in plant sample show that similarly like in our paper *n*-hexane and chloroform as a mobile phase component are suitable for quantification of diosgenin in methanolic extracts from *Allium ursinum*. A mobile phase *n*-hexane-acetone in volume composition 4:1 (v/v) and sulfuric acid as a visualizing agent enabled successful TLC-densitometric analysis of diosgenin from *Allium ursinum* extract [10]. The limit of detection is similar to the LOD value presented in this paper, but LOQ value determined using this mobile phase is much lower [10]. Based on the data presented in another paper it can be concluded that the modified visualizing agent: anisaldehyde-sulfuric acid improves detection of diosgenin in different preparations (extract from plants and herbal drugs), but the correlation coefficient for this method is only 0.988 [12]. Based on the conducted comparison, it can be concluded that the method elaborated in this paper is suitable only for individual analysis of diosgenin in pure sample.

Further quantitative densitometric analysis of diosgenin e.g. in pharmaceutical formulations containing diosgenin is possible, but requires prior elimination of the influence of the excipients presented in diosgenin commercial products for identification and quantification of diosgenin as a bioactive substance in these pharmaceutical products like was described in scientific papers [1,10,12-13].

CONCLUSIONS

An TLC method described in this paper enables identification and quantification of diosgenin as a bioactive substance in pure samples. Optimization of the chromatographic conditions based on an selection of an appropriate mobile phase, type of chromatographic plates, visualizing agent and wavelength range for densitometric scanning, indicates that the satisfactory qualitative and quantitative analysis by means of all applied chroma-

tographic plates (E. Merck, Art. 1.05553, Art. 1.05554, Art. 1.05567) guarantees a mixture of chloroform-acetone in volume composition 40:10. Diosgenin can be identified chromatographically by retention factor (R_F) or absorption spectra but only after prior color reaction of the diosgenin spots with two visualizing agents: 10% ethanol solution of phosphomolybdic acid or 10% ethanol solution of sulfuric acid. Densitometric analysis of diosgenin chromatograms without visualizing agent is not effective. Big similarity in location of the maximum absorption of diosgenin spectra obtained on various chromatographic plates developed with chloroform-acetone (40:10) after visualization with phosphomolybdic acid indicate that diosgenin can be densitometrically examined using a phosphomolybdic acid at the similar wavelength range (from 700 to 800 nm) regardless of chromatographic plates used. The LOD and LOQ value of densitometric quantification of diosgenin determined under different chromatographic conditions show that the elaborated method is suitable for diosgenin analysis mainly in pure samples.

Further investigations could be referred to application of described TLC-densitometric method for diosgenin analysis as a component in pharmaceutical formulations.

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