Comparison of the Limit of Detection of Paracetamol, Propyphenazone, and Caffeine Analyzed by TLC and HPTLC

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Article

Comparison of the Limit of Detection of Paracetamol, Propyphenazone, and Caffeine Analyzed by TLC and HPTLC

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Abstract: TLC and HPTLC in normal (NP) and reversed (RP) phase systems combined with densitometry were used to analyze caffeine, propyphenazone and paracetamol. Normal phase analyses were performed on five different stationary phases (chromatographic plates), testing three mobile phases. Reversed-phase analyses were performed on four different chromatographic plates using three mobile phases. Thus, it was checked whether analyses using TLC chromatographic plates could be as sensitive as those performed on HPTLC plates. For all analyzed biologically active substances, the limit of detection (LOD) was calculated, i.e. a parameter that describes the method sensitivity. It has been shown that using both TLC and HPTLC plates, it is possible to develop chromatographic conditions that enable the detection of caffeine, propyphenazone and paracetamol in amounts ranging from a dozen to several dozen μg/spot. In the reversed-phase (RP) system, lower LOD values for all tested compounds were obtained using TLC than HPTLC. However, using analyses in the normal phase system (NP), similar (of the same order) LOD values are obtained for caffeine, propyphenazone and paracetamol, both when using TLC and HPTLC plates. Therefore, for economic reasons, TLC plates should be recommended for analyses of caffeine, propyphenazone and paracetamol, which are several times cheaper than HPTLC plates.

Keywords: caffeine, propyphenazone, paracetamol, TLC-densitometry, limit of detection

1. Introduction

Each analytical method, including pharmaceutical analysis, requires validation. Validation of analytical procedures is carried out based on the guidelines of the International Conference on Harmonization (ICH) [1]. One of the parameters when performing method validation is the detection limit. The limit of detection (LOD) of the analyzed compound is usually defined as the lowest quantity or concentration of a component that can be reliably detected with a given analytical method [2]. There are several methods for determining the limit of detection (LOD), including the signal-to-noise method or by preparing the linear regression [3].

In the case of the analyzed compounds, namely caffeine, propyphenazone and paracetamol, many methods for their determination have been developed. All three compounds are popular ingredients of pharmaceutical preparations available on the market. They appear both as the sole active ingredient and in complex preparations. For example, there are known caffeine preparations combined with paracetamol or salicylic acid, as well as preparations that contain all three tested compounds, i.e. caffeine, propyphenazone and paracetamol [4]. Propyphenazone can be combined with ergotamine or allobarbital. However, paracetamol is the most often found in preparations as the only active ingredient, but it also can be combined with tramadol or diphenhydramine. Due to the multitude of pharmaceutical preparations containing the above-mentioned compounds (caffeine, propyphenazone and paracetamol), it seems necessary to propose new methods for their determination, for example, due to the need to control their quality or monitor the production process.

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The most common methods used in pharmaceutical analysis are chromatographic methods. It is, for example, micellar liquid chromatography for the analysis of caffeine in anti-inflammatory drugs containing caffeine [5]. The method was carried out on a C18-SN column and required daily tedious column washing to remove the mobile phase. In turn, caffeine in combination with ibuprofen was analyzed using gas chromatography at high temperatures [6]. A popular method in chemical, medical or pharmaceutical analysis is high-performance liquid chromatography, and therefore, many analyses of caffeine, paracetamol and propyphenazone are carried out using this method. The analyses concern single compounds or multi-component mixtures, also with substances other than those mentioned [7–16]. Most often, these are preparations available on the pharmaceutical market. Some of them were conducted in combination with HPLC and mass spectrometry [17–21]. Many analysts, however, tend to use a chromatographic method that is simpler, taking into account, for example, sample preparation. This method is thin-layer chromatography. One of the multi-component caffeine preparations (with codeine and paracetamol) was analyzed using two chromatographic methods - HPLC and TLC [22]. Both methods gave good results and proved to be useful for both quality control and routine analysis. Egyptian scientists also compared two chromatographic methods [23]. The assay concerned paracetamol, caffeine and propyphenazone in the presence of two paracetamol impurities - 4-aminophenol and 4-nitrophenol. RP-HPLC and TLC combined with densitometry were used. Both methods of simultaneous determination of the mentioned compounds have been validated. The comparison showed no significant differences. The literature base also includes studies concerning only thin-layer chromatography analysis, the most often combined with densitometric quantitative analysis of compounds such as caffeine, propyphenazone and paracetamol [24–28]. The most commonly used stationary phase are plates pre-coated with silica gel with an agent that allows visualization of chromatographic spots under UV light. There are also HPTLC (high-performance thin-layer chromatography) plates that are pre-coated with a thinner layer of a very fine-grained sorbent than in TLC [29]. It allows for greater sensitivity and resolution of the assay, which in practice should translate into a lower limit of detection value obtained during the determination. An interesting study is the analysis of a four-component preparation with anti-migraine activity (metoclopramide, ergotamine, caffeine, and paracetamol). The green high-performance thin-layer chromatography method was used for that purpose [30]. Validation parameters were checked in accordance with the International Conference of Harmonization guidelines. The analysis gave promising results. Propyphenazone and caffeine were also determined by thin-layer chromatography combined with densitometry in the presence of three other components, namely ergotamine tartrate and two impurities: phenazone and theophylline [31]. All compounds have been determined in human plasma and in one of the pharmaceutical preparations. The method was found economical and eco-friendly.

Methods other than chromatography are also important, although used less frequently. For example, all compounds analyzed in our work (caffeine, paracetamol and propyphenazone) were determined simultaneously by two electrochemical methods [32]. Both methods were based on square-wave voltammetric detection and were statistically compared with each other. No significant differences have been found between them. Paracetamol, propyphenazone and caffeine were also determined using UV spectrophotometry [33,34]. An interesting approach was taken by researchers from Bulgaria then. The data obtained from the analysis were used to create chemometric models. The models were tested on an external data set at concentrations within the calibration range. Then, the obtained models were successfully used in the determination of active ingredients in pharmaceutical preparations containing paracetamol, caffeine and propyphenazone.

The aim of our work was to compare the LOD of caffeine, propyphenazone and paracetamol analyzed by thin-layer chromatography (TLC) and high-performance thin-layer chromatography (HPTLC) and to check whether it is possible to develop conditions using the TLC technique that would allow obtaining LOD lower or comparable to results obtained using the HPTLC technique.

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2. Materials and Methods

2.1. Compounds analyzed and their solutions

Three compounds with pharmacological activity were analyzed. These include caffeine (Fluka, USA), propyphenazone (Sigma-Aldrich, USA), and acetaminophen (Sigma-Aldrich, USA). Initial solutions of the analyzed substances with the following concentrations: 0.05, 0.1, 0.2, 0.3, 0.4 and 0.5 mg/mL were prepared in 99.8% ethyl alcohol (POCh, Poland).

2.2. Thin-layer chromatography

In order to determine the LOD, both adsorption (NP) and partition (RP) thin-layer chromatography were performed. Various chromatography plates were used as stationary phases, both for TLC and HPTLC. Seven different stationary phases were used for the analysis, namely TLC chromatographic plates, i.e. silica gel on aluminum foil (#1.05554), silica gel 60 RP-2 F_{254} silanized on glass (#1.05747) and silica gel RP-18 F_{254} (#1.05559) on aluminum foil, as well as HPTLC chromatographic plates: silica gel 60 F_{254} on aluminum foil (#1.05548), silica gel 60 F_{254} with a concentration phase on glass (#1.13728), silica gel 60 RP-2 F_{254s} on glass (#1.13726) and silica gel RP-18 F_{254} with concentration zone on glass (#1.15498). All chromatography plates were supplied by Merck, Darmstadt, Germany. Solutions of the tested substances were spotted on chromatographic plates in the amount of 5 µL. Various mobile phases prepared by mixing organic solvents and/or distilled water were also used. Table 1 shows the volumetric composition and symbols of the mobile phases used.

Table 1. Symbols and compositions of the mobile phases used.

Symbol	System	Composition	Volumetric ratio
A	normal phase system (NP)	acetone – chloroform - ammonia	10:40:0.5
B		n-hexane – acetone - ammonia	25:25:0.5
C		chloroform – toluene – ethylene acetate – methanol – 80% acetic acid	18:18:7.5:6:0.3
D	reversed phase system (RP)	methanol - water	25:25
E		methanol - water	30:20
F		methanol - water	40:10

Mobile phases A, B and C were used during studies using NP-TLC and NP-HPTLC, while phases D, E and F were used during studies using RP-TLC and RP-HPTLC analysis. All analyses were repeated five times.

2.3. Spectrodensitometric and densitometric analysis

For the purpose of the quantitative analysis of the tested compounds, densitometric analysis was performed, preceded by a spectrodensitometric analysis, during which λ_{max} was determined for individual compounds. Analyzes, spectrodensitometric and densitometric, were performed using a Camag TLC Scanner 3 densitometer (Switzerland) and winCATS software. Spectrodensitometric analysis was carried out in the wavelength range of 200-400 nm, and the radiation source was a deuterium lamp. Densitometric measurements were made in the absorption mode, with a resolution of 100 µm /step. The slit size was 10.00×0.40 mm and the scanning speed was 20 mm/s. The maximum wavelengths λ_{max} for individual compounds were, respectively: 276 nm for caffeine, 272 nm for propyphenazone and 248 for paracetamol.

2.4. LOD calculation

The LOD values of caffeine, propyphenazone, and acetaminophen were calculated from the calibration curve data. LOD was calculated according to the formula:

$$LOD = \frac{3.3 * \sigma}{S} \quad (1)$$

where:

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σ – standard deviation; s – standard deviation of the intercept (s_a) or residual standard deviation (s_{xy})

For calculations were used only the results for which the standard solutions of caffeine, propyphenazone and paracetamol show the following conditions::

$$10 * LOD > C \quad (2)$$

$$LOD < C \quad (3)$$

where:

C – concentration of compound in solution; LOD – limit of detection.

2.5. Statistical calculation

Calculations were made using STATISTICA 13 software. Plots were prepared using EXCEL and STATISTICA 13 software.

3. Results and Discussion

The tables below present a summary of RF and LOD values for the tested compounds obtained by adsorption thin-layer chromatography (Table 2) and partition thin-layer chromatography (Table 3), respectively. The LODs presented in this paper are the average LOD values calculated based on the standard deviation of the intercept (s_a) and the residual standard deviation (s_{xy}).

Table 2. LOD values [μg/spot] of caffeine, propyphenazone and paracetamol obtained by use of adsorption thin-layer chromatography (NP-TLC and NP-HPTLC).

Mobile phase	Technique	Chromatographic plates	LOD of caffeine [μg/spot]	R_F	LOD of propyphenazone [μg/spot]	R_F	LOD of paracetamol [μg/spot]	R_F
A	NP-TLC	1.05554	0.059	0.12	0.099	0.73	0.106	0.13
		1.05747	0.023	0.76	0.091	0.83	0.032	0.66
	NP-HPTLC	1.05548	0.023	0.16	0.091	0.84	0.077	0.16
		1.13728	0.080	0.34	0.048	0.86	0.016	0.17
		1.13726	0.144	0.52	-	-	0.021	0.73
B	NP-TLC	1.05554	0.133	0.15	0.076	0.83	0.054	0.25
		1.05747	0.048	0.77	0.086	0.89	0.195	0.86
	NP-HPTLC	1.05548	0.010	0.38	0.046	0.75	0.030	0.57
		1.13728	0.090	0.39	0.175	0.79	0.025	0.40
		1.13726	0.193	0.89	0.247	0.94	0.090	0.77
C	NP-TLC	1.05554	0.054	0.47	0.029	0.60	0.016	0.38
		1.05747	0.067	0.76	0.045	0.86	0.025	0.72
	NP-HPTLC	1.05548	0.073	0.55	0.039	0.72	0.037	0.42
		1.13728	0.057	0.52	0.026	0.70	0.035	0.39
		1.13726	0.091	0.92	0.084	0.98	0.052	0.84

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Table 3. LOD values [µg/spot] of caffeine, propyphenazone and paracetamol obtained by use of partition thin-layer chromatography (RP-TLC i RP-HPTLC).

Mobile phase	Ttechnique	Chromatographic plates	LOD of caffeine [µg/spot]	R_F	LOD of propyphenazone [µg/spot]	R_F	LOD of paracetamol [µg/spot]	R_F
D	RP-TLC	1.05747	0.040	0.52	0.086	0.19	0.133	0.65
		1.05559	0.120	0.20	0.085	0.06	0.170	0.55
	RP-HPTLC	1.13726	0.104	0.48	0.046	0.15	0.084	0.57
		1.15498	-	-	-	-	-	-
E	RP-TLC	1.05747	0.074	0.60	0.030	0.39	0.203	0.85
		1.05559	0.072	0.32	0.089	0.13	0.104	0.65
	RP-HPTLC	1.13726	0.117	0.62	0.062	0.36	0.058	0.70
		1.15498	0.051	0.21	0.035	0.10	0.135	0.48
F	RP-TLC	1.05747	0.084	0.75	0.041	0.61	0.056	0.85
		1.05559	0.019	0.51	0.024	0.47	0.053	0.74
	RP-HPTLC	1.13726	0.055	0.71	0.041	0.70	0.088	0.79
		1.15498	0.086	0.62	0.030	0.60	0.199	0.91

Both adsorption (NP-TLC) and partition (RP-TLC) thin layer chromatography analyzes were performed, with plates 1.05554, 1.05548 and 1.13728 used for NP-TLC analysis, plates 1.05559 and 1.15498 used for RP-TLC analysis, and plates 1.13726 and 1.05747 were used for both types of analyses. In the case of mobile phase A (acetone - chloroform - ammonia) and chromatographic plates #1.13726, it was impossible to determine the detection limit for propyphenazone. The reason was the fact that under these chromatographic conditions, the analyzed compound migrated with the front of the mobile phase. It was not possible as well to determine detection limits for all compounds analyzed using mobile phase D (methanol-water, 25:25, v/v) and #1.15498 chromatographic plates because all compounds remained at the start. Based on Tables 2 and 3, plots were prepared to show the results of the limit of detection for individual tested compounds, namely for caffeine (Figure 1), propyphenazone (Figure 2) and paracetamol (Figure 3) for TLC and HPTLC plates, respectively. When analyzing the LOD values for the conducted analyses, the limit value of the detection limit was assumed to be 0.05 µg/spot, and the possibility of detecting caffeine, propyphenazone and paracetamol was determined based on this value.

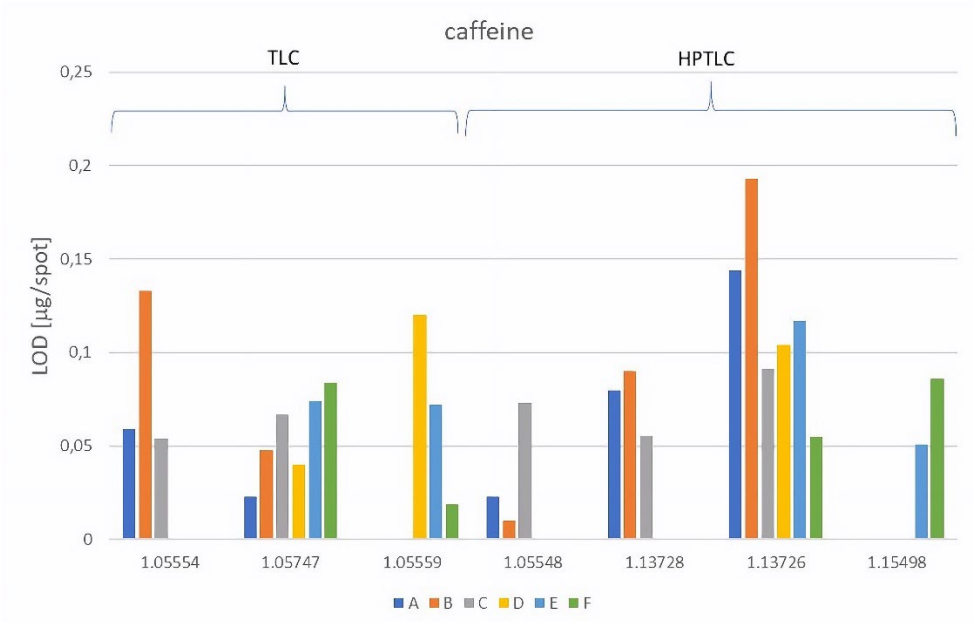

Figure 1. Comparison of the detection limits of caffeine analyzed under different chromatographic conditions.

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For caffeine, in the case of TLC plates, the condition that the LOD is less than 0.05 is met by four chromatographic conditions: chromatographic plates 1.05747 and mobile phases A, B and D, giving the following LOD values, respectively: 0.023, 0.048 and 0.040 µg/spot and chromatographic plates 1.05559 and mobile phase F with a LOD value of 0.019 µg/spot. In the case of HPTLC plates, only two chromatographic conditions met this condition, additionally only for one stationary phase - 1.05548 plates. The detection limit values are then 0.023 (mobile phase A) and 0.010 µg/spot (mobile phase B), respectively.

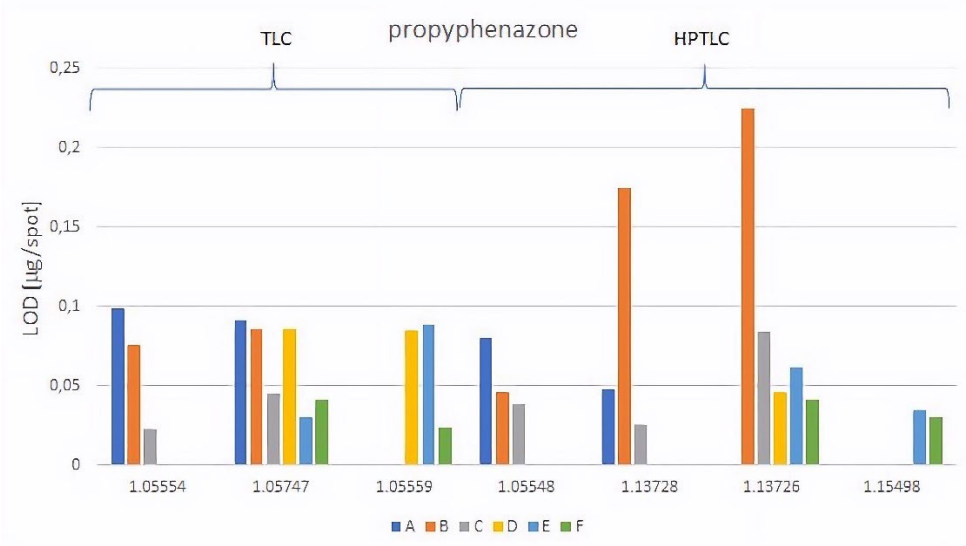

Figure 2. Comparison of the detection limits of propyphenazone analyzed under different chromatographic conditions.

In the case of propyphenazone, when using HPTLC plates, more (than in the case of TLC plates) chromatographic conditions give an LOD value of less than 0.05 µg/spot. These are 1.05548 plates and mobile phases B (LOD=0.046 µg/spot) and C (LOD=0.039 µg/spot), 1.13728 plates and mobile phases A (LOD=0.048 µg/spot) and C (LOD= 0.026 µg/spot), 1.13726 plates and mobile phases D (LOD=0.046 µg/spot) and F (LOD=0.041 µg/spot) and 1.15498 plates and mobile phases E (LOD=0.035 µg/spot) and F (LOD=0.030 µg/spot). However, in the case of HPTLC plates, there are also chromatographic conditions that give the detection limits values that are definitely unfavorable, being much higher than the others, namely 0.175 and 0.247 µg/spot for plates 1.13728 and 1.13726 and the mobile phase B, respectively. For propyphenazone analyzed using the method TLC, the best LOD values were obtained for 1.0554 plates and mobile phase C - then the LOD was 0.029 µg/spot; for 1.05747 plates - then the LOD was 0.045, 0.030 and 0.041 µg/spot for mobile phases C, E and F, respectively; for the mobile phase 1.05559 and mobile phase F (LOD=0.024 µg/spot).

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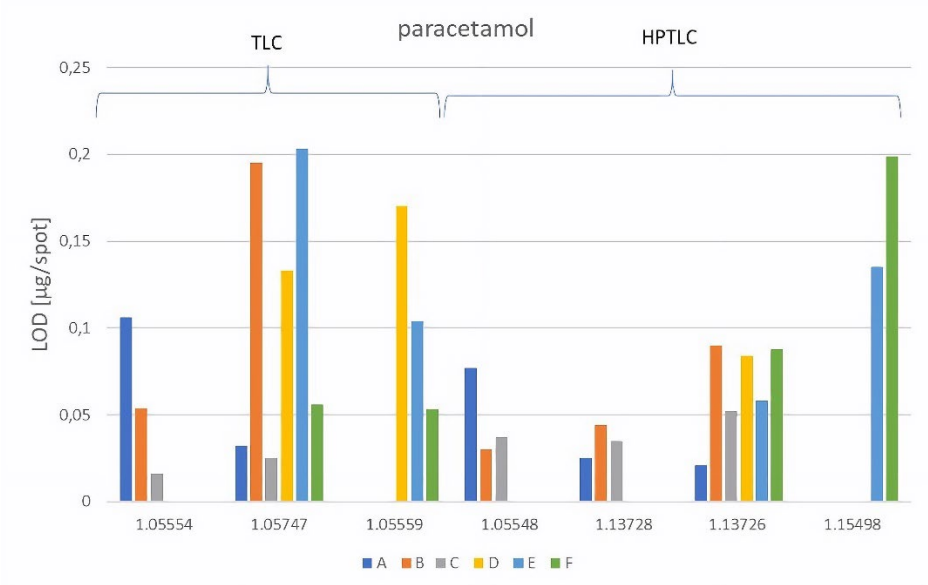

Figure 3. Comparison of the detection limits of paracetamol analyzed under different chromatographic conditions.

As in the case of propyphenazone, analysis on HPTLC plates gives more LOD of paracetamol results, which meet the condition that the LOD is less than 0.05 µg/spot. These are 1.05548 plates, mobile phases B and C, LOD 0.030 and 0.037 µg/spot, respectively; plates 1.13728, mobile phases A, B and C, LOD 0.016, 0.025 and 0.035 µg/spot, respectively; plates 1.13726 and mobile phase A, LOD=0.021 µg/spot. For TLC plates, there are only three results: plates 1.05554, mobile phase C, for which the detection limit is 0.016 µg/spot and plates 1.05747 and mobile phases A and C, for which the detection limits are 0.032 and 0.025 µg/spot, respectively. There are also significantly more cases of high LOD values for TLC plates.

The lowest LOD values for individual compounds obtained for caffeine, propyphenazone and paracetamol are 0.01, 0.024 and 0.016 µg/spot, respectively. For caffeine and propyphenazone, the analysis was carried out on TLC plates - 1.05548 (caffeine) and 1.05559 (propyphenazone). For paracetamol, a value of 0.016 µg/spot was obtained for plates TLC (1.13728) and HPTLC (1.05554). The list of the lowest and highest LOD values for caffeine, propyphenazone and paracetamol is shown in Table 4.

Table 4. The ranges of LOD for the tested compounds using all chromatographic conditions in µg/spot.

	Caffeine	Propyphenazone	Paracetamol
Technique	Range of LOD [µg/spot]		
NP-TLC	0.023÷0.133	0.029÷0.091	0.016÷0.195
NP- HPTLC	0.010÷0.193	0.026÷0.247	0.016÷0.052
RP-TLC	0.019÷0.120	0.024÷0.089	0.053÷0.203
RP-HPTLC	0.051÷0.117	0.030÷0.062	0.084÷0.199

This comparison (Table 4) shows that in the reversed-phase system (RP), lower LOD values for all tested compounds can be obtained by TLC than by HPTLC. However, using analyses in the normal phase system (NP), similar (of the same order) LOD values are obtained for caffeine, propyphenazone and paracetamol when using both TLC and HPTLC plates. Therefore, for economic reasons, TLC plates should be recommended for the analysis of caffeine, propyphenazone and paracetamol because they are several times cheaper than HPTLC plates.

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Moreover, a cluster analysis was performed based on the obtained LOD values of caffeine, propyphenazone and paracetamol. It is illustrated in the next figure (Figure 4). Due to missing data, the LOD values for mobile phase A and 1.13726 plates (propyphenazone in this case migrates with the front of the mobile phase) and the LOD values for mobile phase D and 1.15498 plates (all analyzed compounds remain at the start during chromatographic analysis) were omitted.

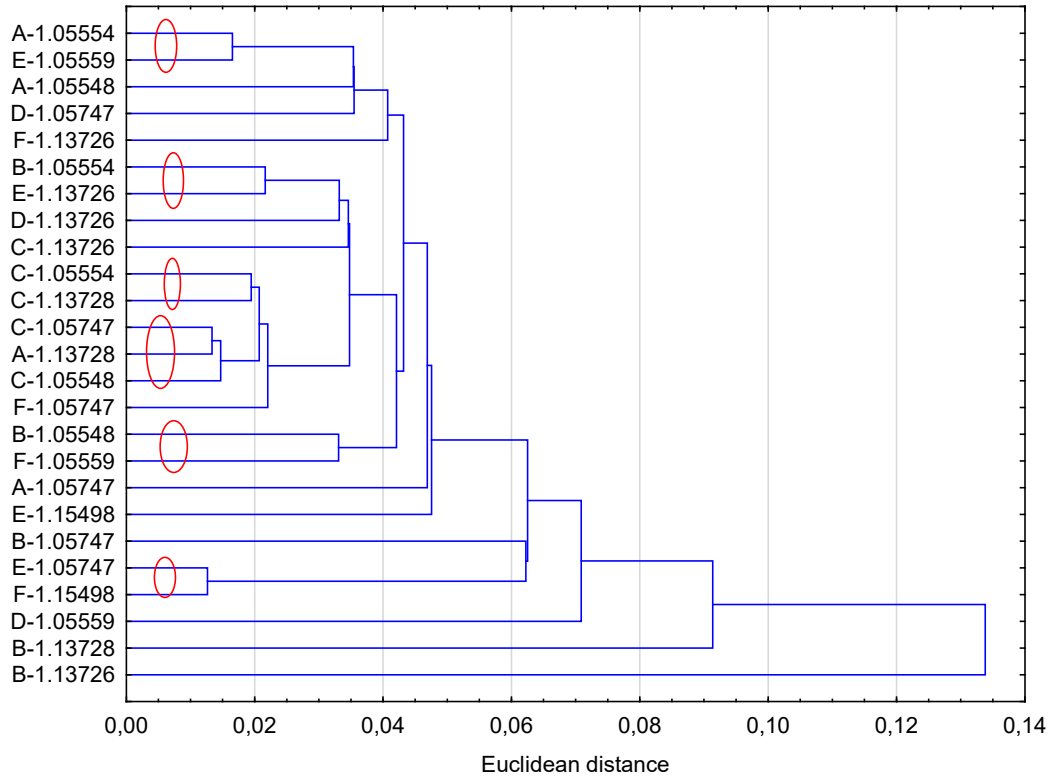

Figure 4. Cluster analysis of detection limit values for all tested compounds and all chromatographic conditions.

The analysis (Figure 4) indicates several clearly marked five-element clusters and one three-element cluster. These clusters include specific chromatography conditions for caffeine, propyphenazone, and acetaminophen. Subsequent clusters indicate similarity in LOD values for specific stationary and mobile phases. The similarities are as follows:

- mobile phase A and 1.05554 plates (TLC) and mobile phase E and 1.05559 plates (TLC)
- mobile phase B and 1.05554 plates (TLC) and mobile phase E and 1.13726 plates (HPTLC)
- mobile phase C and 1.05554 plates (TLC) and mobile phase C and 1.13728 plates (HPTLC)
- mobile phase C and 1.05747 plates (TLC), mobile phase A and 1.13728 plates (HPTLC) and mobile phase C and 1.05548 plates (HPTLC)
- mobile phase B and 1.05548 plates (HPTLC) and mobile phase F and 1.05559 plates (TLC)
- mobile phase E and 1.05747 plates (TLC) and mobile phase F and 1.15498 plates (HPTLC).

The above cluster analysis for all compounds shows that many chromatographic conditions give similar detection limits for the compounds tested. Often happens too that similar detection limits are obtained simultaneously on TLC and HPTLC plates, which is confirmed by the summary in Table 4. This is also confirmed by similarity analyses performed for each mobile phase separately. They are presented in Figure 5.

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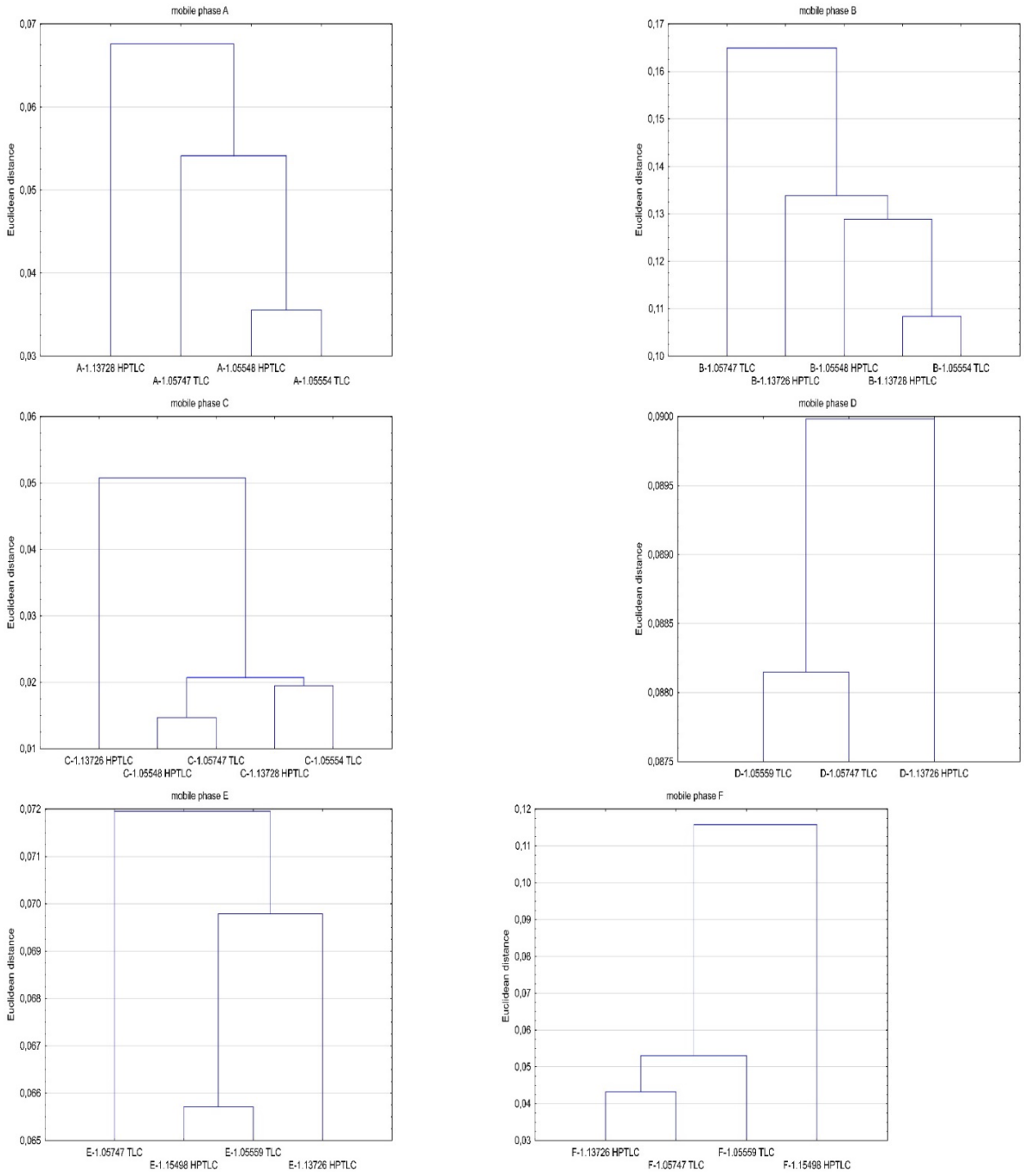

Figure 5. Similarity analyzes performed on the basis of data for each mobile phase separately.

Only in the case of the mobile phase D (methanol-water, 25:25, v/v) are similar results shown by analyses using the same type of plates, i.e. TLC (1.05559 and 1.05747). In the case of the remaining mobile phases, i.e. A, B, C, E, F, the highest LOD similarity was always obtained for TLC and HPTLC plates, which is another confirmation that cheaper TLC plates can be used and give values similar to those obtained on HPTLC plates.

Due to the fact that in the pharmaceutical market exist many multi-component preparations containing all the analyzed compounds, using the obtained LOD data, chromatographic conditions were searched for those which would give the best results in LOD values and would allow to determine all components during one analysis. For this purpose, only those values of the detection limit were considered that were less than 0.05 µg/spot, i.e. those that were discussed earlier in terms of the use of chromatographic plates for TLC and HPTLC. A table has been prepared, giving an overview of the chromatographic conditions and the compounds determined (Table 5). It was

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found that there is only one possibility for the analysis of all three compounds with such a limitation of the LOD value, namely using HPTLC plates 1.05548 and mobile phase B (n-hexane - acetone - ammonia, 25:25, 0.5, v/v/v).

The most optimal conditions for analyzing all compounds at once would be NP-HPTLC analysis. The detection limit values for caffeine, propyphenazone and paracetamol are 0.010, 0.046 and 0.030 µg/spot, respectively. The R_F values obtained under these chromatography conditions are 0.38, 0.75 and 0.57 for caffeine, propyphenazone and paracetamol, respectively. Differences in these values will allow for a good separation of compounds.

However, also good results that are comparable in quality to the above can be obtained on TLC plates:

a) precoated with silica gel 60 F_{254} (#1.05554) using mobile phase C (chloroform - toluene - ethyl acetate - methanol - 80% acetic acid, 18:18:7.5:6:0.3, v/v). The detection limit values for caffeine, propyphenazone and paracetamol are 0.054, 0.029 and 0.016 µg/spot, respectively. The R_F values obtained under these chromatography conditions are 0.47, 0.60 and 0.38 for caffeine, propyphenazone and paracetamol, respectively.

b) precoated with silica gel 60 F_{254}, modified with C18 groups (#1.05559) using a mobile phase F (methanol-water, 40:10, v/v). The detection limit values for caffeine, propyphenazone and paracetamol are 0.019, 0.024 and 0.053 µg/spot, respectively. The R_F values obtained under these chromatography conditions are 0.51, 0.47 and 0.74 for caffeine, propyphenazone and paracetamol, respectively.

Table 5. The overwiev of compounds investigated and chromatographic conditions for which LOD is less than 0.05 µg/spot.

Plate	Symbol of mobile phase					
	A	B	C	D	E	F
1.05554			propyphenazone paracetamol			
1.05747	caffeine paracetamol	caffeine	propyphenazone paracetamol	caffeine	propyphenazone	propyphenazone
1.05559						caffeine propyphenazone
1.05548		caffeine propyphenazone paracetamol	propyphenazone paracetamol			
1.13728	propyphenazone paracetamol	paracetamol	propyphenazone paracetamol			
1.13726	paracetamol			propyphenazone		propyphenazone
1.15					propyphenazone	propyphenazone

The overview presented in Table 5 shows that TLC plates may be particularly useful for the analysis of caffeine for which LOD is less than 0.05 in as many as four chromatographic conditions (plates 1.05747 and mobile phases A, B, and D, and also plates 1.05559 and mobile phase F). Only one chromatographic condition, using HPTLC plates, gave a LOD for caffeine of less than 0.05 (plates 1.05548 and mobile phase B, i.e. n-hexane – acetone – ammonia, 25:25:0.5, v/v/v). The data in Table 5 also indicate the conditions under which low LOD values can be obtained when testing caffeine, propyphenazone, paracetamol in single-component and two-component caffeine-paracetamol, caffeine-propyphenazone, propyphenazone-paracetamol samples.

5. Conclusions

Thin-layer chromatography combined with densitometry was used for the analysis of caffeine, propyphenazone and paracetamol. Analysis was performed in the normal and reversed phases using TLC and HPTLC plates. It has been shown that analyses performed using TLC chromatographic plates may be as sensitive as those performed on HPTLC plates. It turned out that using TLC as well

as HPTLC, it is possible to find chromatographic conditions that allow for the detection of caffeine, propyphenazone and paracetamol in amounts from several to several dozen µg/spot. In the reversed-phase (RP) analysis, the smaller LOD values for all results are obtained by using TLC rather than HPTLC plates. In the case of analysis in the normal phase system (NP), LOD values are similar (of the same order) for caffeine, propyphenazone and paracetamol for both TLC and HPTLC plates. For example, during the NP-HPTLC analysis using plates 1.05548 and mobile phase B (n-hexane - acetone - ammonia, 25:25:0.5, v/v/v), detection limit values for caffeine, propyphenazone and paracetamol are 0.010; 0.046 and 0.030 µg/spot, respectively. During NP-TLC analysis using 1.05554 plates and the mobile phase C (chloroform - toluene - ethyl acetate - methanol - 80% acetic acid, 18:18:7.5:6:0.3, v/v), the values of LOD for caffeine, propyphenazone and paracetamol are 0.054; 0.029 and 0.016 µg/spot, respectively. During RP-TLC analysis using TLC plates 1.05559 and mobile phase F (methanol-water, 40:10, v/v), the LOD values for caffeine, propyphenazone and paracetamol are 0.019; 0.024 and 0.053 µg/spot, respectively. Due to the economic purposes for caffeine, propyphenazone and paracetamol analyses, the TLC plates can be recommended because they are several times cheaper than HPTLC plates.

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