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Estimating Limits of Detection and Quantification of Ibuprofen by TLC-Densitometry at Different Chromatographic Conditions

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Abstract: Ibuprofen is one of the best-known nonsteroidal anti-inflammatory and analgesic drugs. Following the previous work, the current study is focused on estimating the effect of different chromatographic conditions on the sensitivity of thin-layer chromatography in combination with UV densitometry, i.e., the detection and quantification of ibuprofen in a wide range of its concentrations including the lowest limits of detection (LOD) and quantification (LOQ). For this purpose, a reliable and easy-to-use calculation procedure for LOD and LOQ determination is presented in this work. In addition, the impact of type plates and mobile phase composition on the LOD and LOQ, respectively, of this active substance is accurately described. The results of detection and the quantification level of ibuprofen obtained under applied chromatographic conditions confirmed the utility of silica gel plates as well as silica gel bonded phases (i.e., reversed-phase (RP) plates) in the thin-layer chromatography (TLC)-densitometric analysis of ibuprofen at a low level, i.e., from several nanograms (below one microgram) to a few micrograms/spot. Among all chromatographic systems tested, the best are those consisting of silica gel 60F_{254} plates with concentrating zone (1.05583) and the mixture of *n*-hexane:ethyl acetate:glacial acetic acid in ratio 36:12:2 (*v*/*v*/*v*) as well as RP plates, i.e., RP-18F_{254} aluminum plates developed with methanol:water in volume composition 40:10 (*v*/*v*). These chromatographic systems allowed quantifying ibuprofen in the amount of 0.229 μg/spot and 0.228 μg/spot, which is less than 1 μg/spot. It can be stated that different chromatographic systems in combination with UV densitometric scanning at 224 nm proposed in this work can be successfully applied for the cost-effective and sensitive determination of ibuprofen as a widely used drug component as well as a residual in domestic wastewater. It was found that the modification of silica gel as well as the layer thickness of unmodified or modified silica gel 60 can influence the quality of chromatograms and the detection/quantification of ibuprofen in both normal phase (NP) and RP systems. Therefore, to obtain the best possible LOD and LOQ values of ibuprofen with precoated layers, suitable mobile phase and chromatographic plates are required.

Keywords: ibuprofen; limit of detection; limit of quantification; TLC-densitometry

1. Introduction

Ibuprofen (2-[4-(2-methylpropyl)phenyl]propanoic acid) shown in Figure 1 is a drug classified as a non-steroidal anti-inflammatory (NSAID) and analgesic agent. It is particularly indicated in treating some types of pain, inflammation, and symptoms associated with influenza [1–4]. Therefore, ibuprofen is a major constituent in many cold and flu medications in different dosage forms, e.g., tablets,

suspension, gel, suppositories, capsules, solution for injection, cream, and plasters. Because of its wide availability as over-the-counter (OTC) medication and relatively large application in human and veterinary medicine, the intake of this substance in the form of simple and combined pharmaceutical formulations for human and animal use increases, and, consequently, the amount of ibuprofen residuals in domestic wastewater grows. Therefore, an economical, fast, and sensitive method for the detection and quantification of ibuprofen is needed. A literature survey has revealed that ibuprofen in combination with other drugs or in human plasma can be determined by using different analytical methods such as spectrophotometry, high-performance liquid chromatography (HPLC), and gas chromatography (GC) for pharmaceutical and clinical purposes [5–14]. A high performance thin-layer chromatographic method (HPTLC) for the quantification of ibuprofen from human plasma at limit of detection (LOD) 50 ng has been developed by Save and coworkers [15]. In 2005, Krzek et al. developed a chromatographic-densitometric method for the identification and determination of both enantiomers of ibuprofen by means of reversed-phase thin-layer chromatographic plates and chiral mobile phase consisting of β-cyclodextrin [16]. The LOD was 1 μg/mg. Next, Starek and Krzek reported a TLC method for the quantitative estimation of ibuprofen and its impurities in pharmaceutical preparation in a normal phase system on silica gel plates at LOD and limit of quantification (LOQ) equal 0.24 and 0.72 μg/spot [17], respectively. In 2010, Pyka and Bochenska confirmed the utility of TLC with densitometry for the quantitative analysis of ibuprofen in selected pharmaceutical preparations. This work confirmed also the utility of a popular stationary phase, namely silica gel $60F_{254}$ plates in normal phase thin-layer chromatography (NP-TLC) and silica gel RP-$18F_{254}$ plates in reversed-phase thin-layer chromatography (RP-TLC) with LOD = 0.60 μg/spot for NP-TLC and LOD = 1.000 μg/spot for RP-TLC. The LOQ was 12.50 μg/spot in both cases [18].

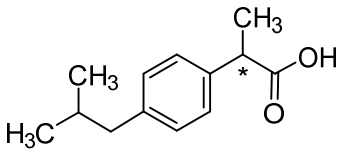


Figure 1. Chemical structure of ibuprofen.

These data confirmed the significant role of the two above parameters, i.e., LOD and LOQ, in describing the quality of the TLC method with UV-densitometry in the analysis of ibuprofen for clinical as well as pharmaceutical purposes. LOD and LOQ are terms used to describe the lowest amount of ibuprofen detectable with a precision and accuracy [19]. In general, there are different guidelines for the estimation of LOD and LOQ in an analytical method [19–21].

However, none of them is particularly dedicated to a TLC-densitometric method. Based on our previous study referring to the estimations of LODs and LOQs of some steroids, e.g., hydrocortisone acetate and spironolactone [22,23], by TLC-densitometry under different chromatographic conditions and using the calculation procedure recommended by the International Conference on Harmonisation (ICH) guideline as well as by the Konieczka and Namiesnik report [20,21], the current work is aimed at finding chromatographic conditions that can be useful to determine ibuprofen in a wide range of concentrations including the lowest one. In addition to this, the influence of the type of plates and mobile phase composition on the LOD and LOQ of ibuprofen is accurately described. Moreover, a reliable and easy-to-use calculation procedure of LOD and LOQ values of examined ibuprofen is presented here. The efficacy of NP and RP chromatographic systems consisting of different commercially available plates and proper mixtures as mobile phases are discussed in this work. The influence of all applied chromatographic systems on the determination of the LOD and LOQ of ibuprofen is presented.

2. Materials and Methods

2.1. Chemicals and Reagents

Ibuprofen was purchased from Sigma-Aldrich (St. Louis, MO, USA). Methanol, ethanol 96%, absolute ethanol (99.8%), 1,4-dioxane, acetonitrile, propan-1-ol, *n*-hexane, toluene, glacial acetic acid, ethyl acetate, and water were purchased from POCh (Gliwice, Poland). All solvents were of high-performance liquid chromatographic grade.

2.2. Chromatographic Materials

The chromatographic plates used in this research were as follows: silica gel plates suitable for a RP-TLC system, such as RP-2F_{254} (glass plates, 1.05747), RP-8F_{254} (glass plates, 1.15424), and RP-18F_{254} (aluminum foil, 1.05559). For the purpose of chromatographic analysis in the NP-TLC system, different aluminum and glass plates precoated with silica gel 60F_{254} without concentration zone (1.05554, 1.05570, 1.05715) as well as specific, i.e., with a concentration zone (1.05583) and having enhanced brightness F_{254} indicator (Lux silica gel 60F_{254}, 1.05805), were used. All chromatographic plates of sizes 20 × 20 cm and 10 × 20 cm were purchased from E. Merck (Darmstadt, Germany). According to the manufacturer's specification, the applied RP modified silica layers in the form of RP-2F_{254}, RP-8F_{254}, and RP-18F_{254} plates coated with fluorescent indicator F_{254} show the same specific surface in the range of 480–540 m^2/g and a similar pore volume equal to 0.74–0.84 mL/g, but the layer thickness is 190–250 μm (RP-2F_{254}), 200–270 μm (RP-8F_{254}), and 160–200 μm (RP-18F_{254}). The second type of chromatographic plates tested, i.e., for the NP-TLC system, could be characterized similarly. All aluminum or glass silica gel plates for NP-TLC analysis, i.e., 1.05554 (aluminum foil), 1.05570 (aluminum foil), 1.05715 (glass), 1.05583 (glass), and 1.05805 (glass) indicate a comparable specific surface (480–540 m^2/g) and pore volume equal to 0.74–0.84 mL/g. However, they show a certain difference in layer thickness, which is 175–225 μm (1.05554), 165–235 μm (1.05570), 210–270 μm (1.05715), and 210–250 μm (1.05805), respectively. In the case of silica gel plates with a concentration zone (1.05583), the layer thickness is 160–220 μm and the layer thickness of the concentration zone is 90–140 μm.

2.3. Preparation of Standard Solution

Ibuprofen stock solution was prepared using absolute ethanol as solvent. A total of 25 mg of ibuprofen was weighted carefully and put in a 25 mL volumetric flask, then dissolved in absolute ethanol to the mark in order to obtain an ibuprofen solution containing 1 mg/mL. In the next step, this stock solution was further diluted with absolute ethanol to get a series of thirteen standard solutions in the wide range of concentrations, i.e., from 1 μg/μL to 0.04 μg/μL.

2.4. Equipment and Software

The TLC measurements were carried out using a TLC Scanner 3 (Muttenz, Switzerland) operated in the absorbance mode and a twin chamber of size 10 × 20 cm (Camag, Muttenz, Switzerland). The chromatograms were integrated by using WinCats software (version 1.4.2). Ibuprofen samples were applied onto chromatographic plates by means of precise Camag micropipettes (5 μL, Muttenz, Switzerland).

2.5. Development of Optimal Chromatographic Conditions

In order to obtain optimal chromatographic conditions suitable for the sensitive analysis of ibuprofen (i.e., mobile phase and plate type), several mixtures consisting of different components such as 1,4-dioxane:water, acetone:water, methanol:water, ethanol:water, propan-1-ol, and acetonitrile:water in various volume compositions for RP-TLC analysis as well as chloroform:acetone:toluene (32:13:5, *v*/*v*/*v*), *n*-hexane:ethyl acetate:glacial acetic acid in volume composition 36:12:2, chloroform:methanol:glacial acetic acid (27:22:1, *v*/*v*/*v*), chloroform:methanol (39:11, 49:1, 45:5, *v*/*v*), and toluene:ethyl acetate:glacial acetic acid in volume composition 27:21:2 (*v*/*v*/*v*) in the NP-TLC system were tested during preliminary

study. Besides, different types of sorbents in the form of commercially available chromatographic plates precoated with the popular sorbent, i.e., silica gel, as well as with other sorbents such as a mixture of silica gel and kieselguhr (diatomaceous earth), kieselguhr, Al_2O_3, or polyamide were tested. The use of the mixture consisting of chloroform and methanol in different volume compositions presented above showed that it is not good for obtaining appropriate chromatograms of examined ibuprofen. The application of chloroform:methanol in volume composition 39:11 resulted in the formation of spots with $R_f > 0.90$. Lower concentrations of methanol in this binary mobile phase (49:1 and 45:5, *v*/*v*) were associated with smaller R_f values of the spot, i.e., 0.30–0.51 and 0.62–0.68, respectively, depending on the kind of the used plates. However, the obtained spots were not compact. The addition of glacial acetic acid did not improve the quality of these chromatograms in the case of almost all initially tested chromatographic plates including those precoated with Al_2O_3 $60F_{254}$ and Al_2O_3 $150F_{254}$ (1.05550, 1.05551). The poor efficacy of the chromatographic analysis of ibuprofen, i.e., the lack of good-quality chromatographic spots (bands) of ibuprofen, was also stated for kieselguhr F_{254} plates (5568), a mixture of silica gel 60/kieselguhr F_{254} (1.05567), and polyamide $11F_{254}$ plates (5555). The process of the initial selection of an appropriate mobile phase for the RP-TLC analysis of ibuprofen by means of three types of RP-plates confirmed that 1,4-dioxane:water, acetone:water, and acetonitrile:water mixtures, in volume composition 40:10 each, were not well suited for the chromatographic analysis of ibuprofen. These mixtures led to the formation of large in height and in width spots that were not detectable and quantifiable with suitable precision.

Finally, of all the chromatographic systems tested, the best, thus, optimal for ibuprofen analysis, were those consisting of RP-$2F_{254}$, RP-$8F_{254}$, and RP-$18F_{254}$ plates and the mixture of methanol:water (40:10, *v*/*v*), ethanol:water (40:10), and propan-1-ol:water (40:10, *v*/*v*) for the RP-TLC system. Among the chromatographic conditions tested for the second, i.e., NP-TLC system, the best were the two mixtures of *n*-hexane:ethyl acetate:glacial acetic acid in the ratio 36:12:2 (*v*/*v*/*v*) and toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*) and the following plates for the adsorption TLC precoated with silica gel $60F_{254}$ with and without concentration zone: 1.05554, 1.05583, 1.05570, 1.05715, and 1.05805. These chromatographic conditions proved to be a good choice for the analysis of ibuprofen in both systems because they gave the optimal chromatograms of ibuprofen with compact spots and a suitable retardation factor (R_f value). 50 mL of the mobile phases were used for each development. The chromatographic chamber was previously saturated with mobile phase vapor for 20 min at 22 ± 2 °C before each run. The development distance was 7 cm. Solutions of 5 μL with a proper concentration within the range mention above, i.e., 1.00, 0.80, 0.60, 0.40, 0.20, 0.18, 0.16, 0.14, 0.12, 0.10, 0.08, 0.06, 0.04 μg/μL, were applied on the TLC plates. The position of application was at least 10 mm from the sides and 10 mm from the bottom of the plates (Figure 2). The distance between the spots was 1.5 cm. Each concentration was analyzed three times. After development, the plates were dried at room temperature and then densitometrically scanned.

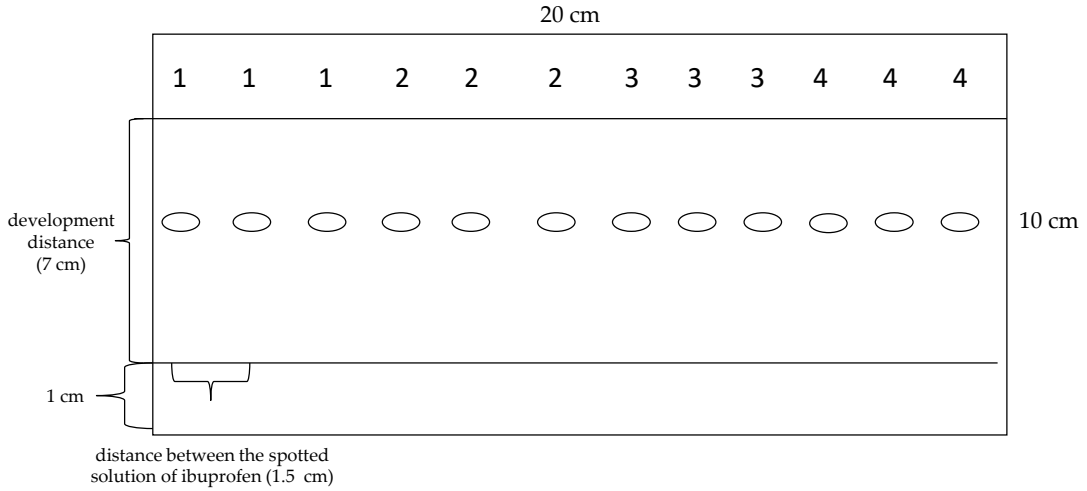


Figure 2. Scheme of application of ibuprofen solutions on chromatographic plate.

2.6. Densitometric Analysis

Densitometric scanning was performed in the absorbance mode with the wavelength of 224 nm by means of WinCats software (1.4.2). The slit was 12 mm long and 0.60 mm wide. The scanning speed was 20 mm/s. The data resolution was 1 nm/step. The representative chromatograms of ibuprofen obtained in the NP-TLC and RP-TLC systems are shown in Figure 3A–E.

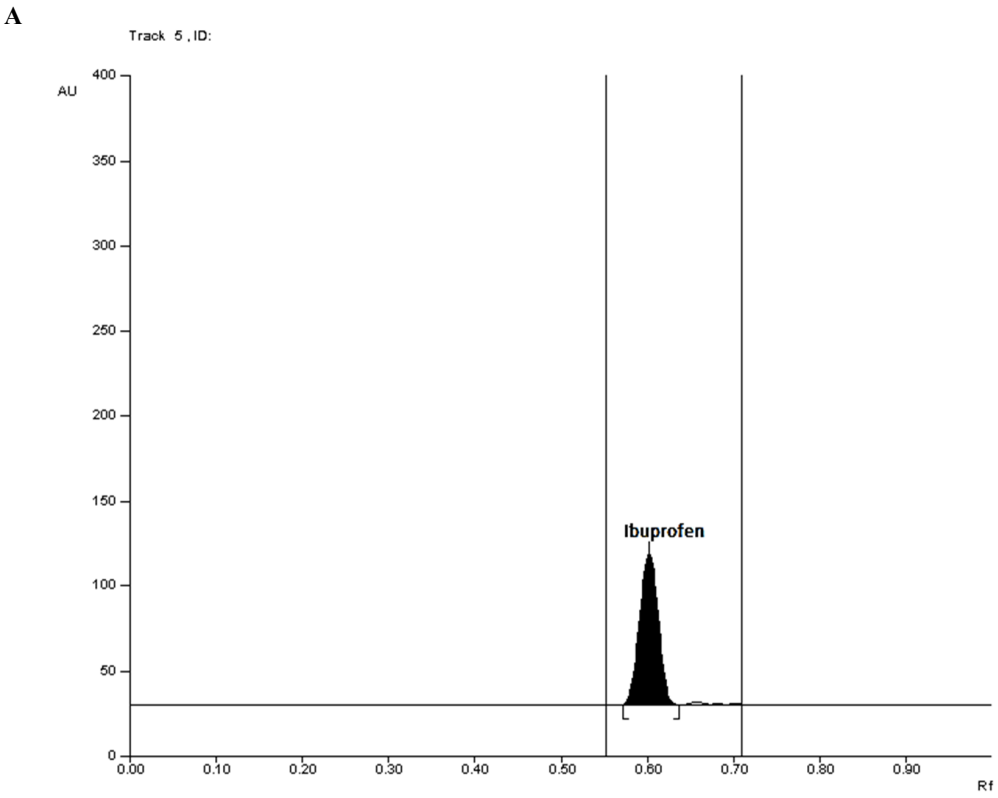
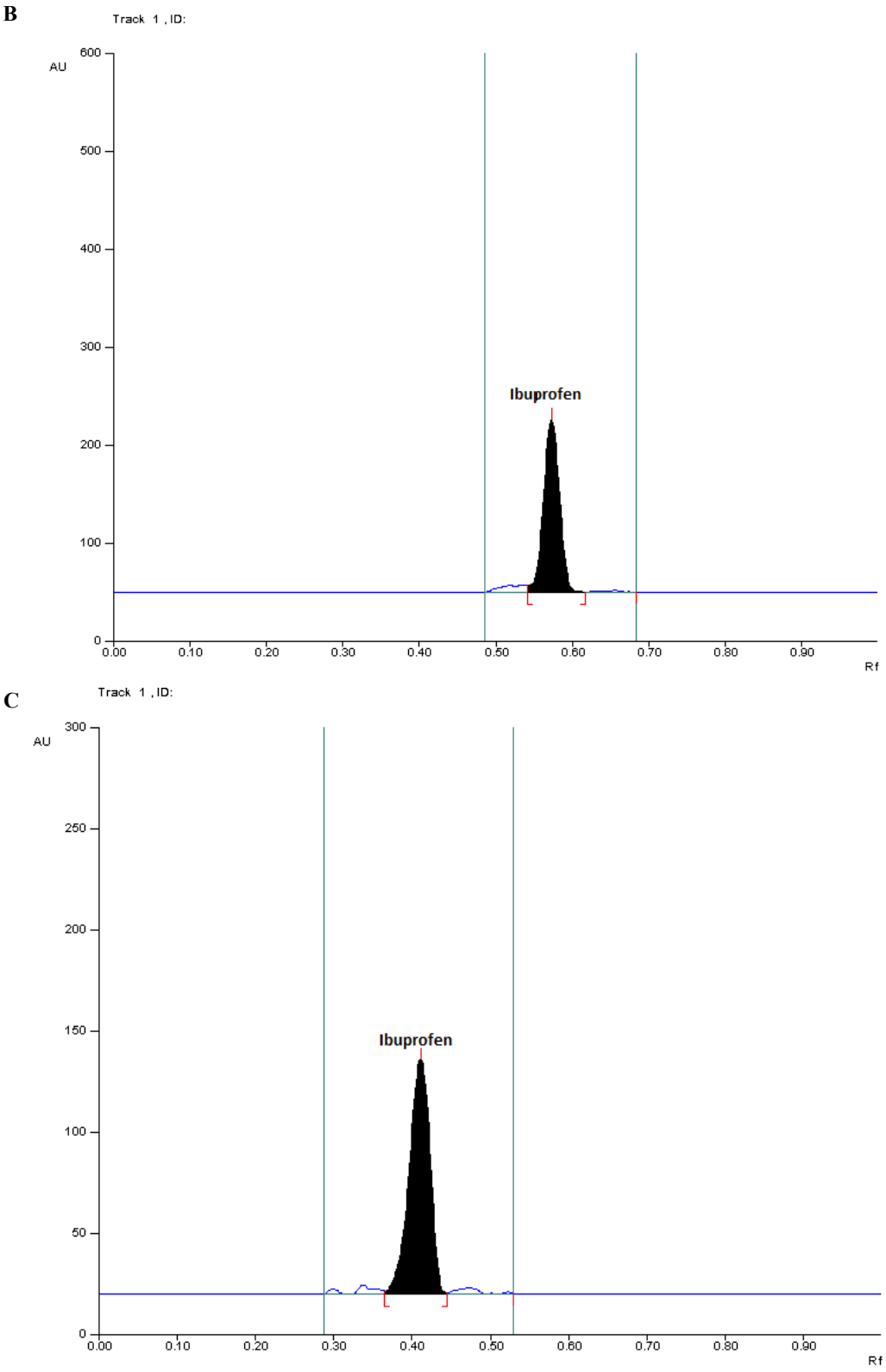


Figure 3. *Cont.*



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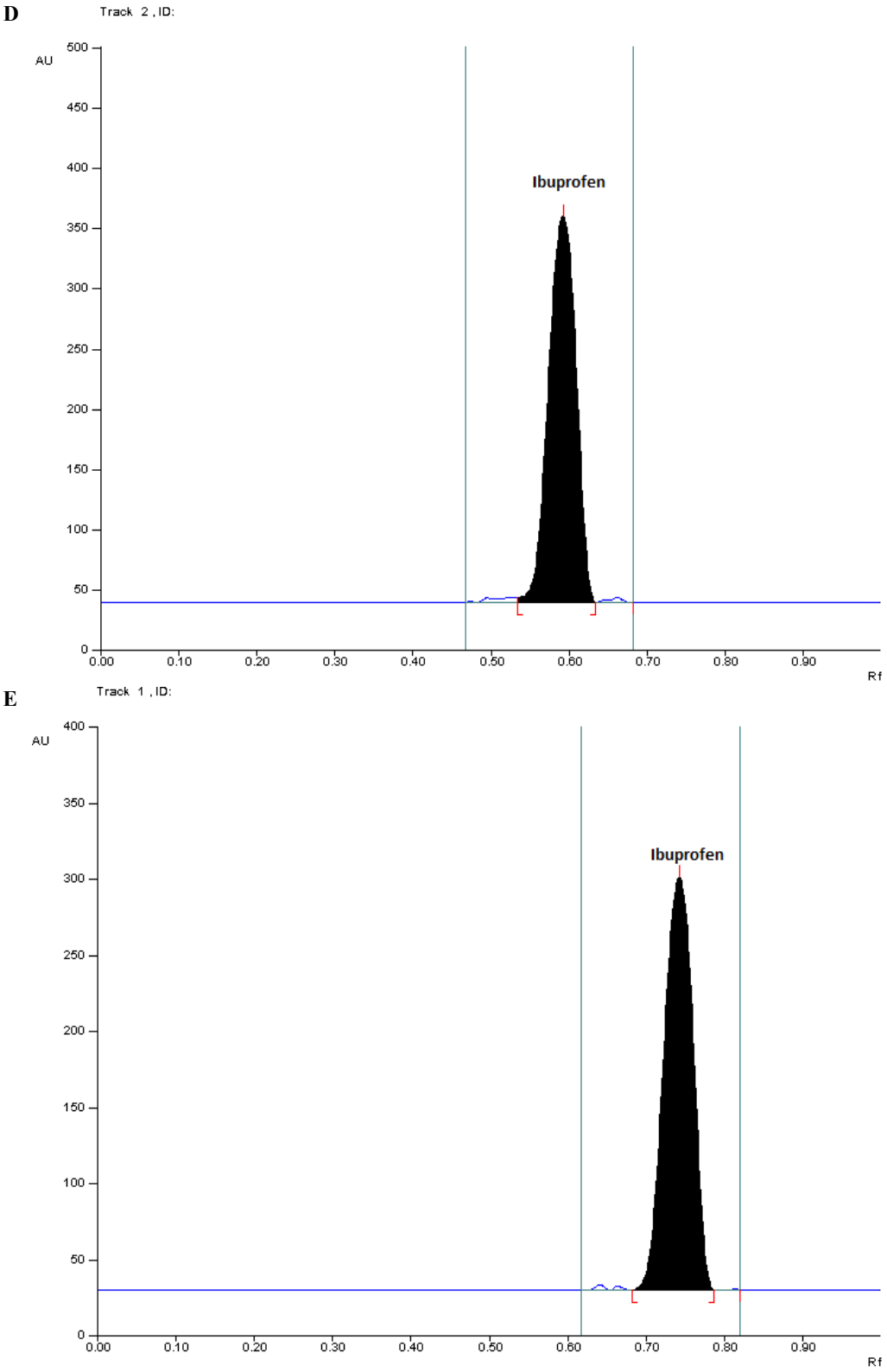

Figure 3. Exemplary chromatograms of ibuprofen registered on chromatographic plates 1.05554 developed with mobile phase toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*) (**A**) and using mixture *n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*) (**B**) as well as on RP-18F_{254} plates examined by means of methanol:water (40:10, *v*/*v*) (**C**), ethanol:water (40:10, *v*/*v*) (**D**), and propan-1-ol:water (40:10, *v*/*v*) (**E**).

Next, the measured spot area for each concentration of examined ibuprofen was used to prepare specific calibration curves. The calibration curves were developed by plotting the spot area versus the concentration of ibuprofen in the required range. The parameters of obtained linear regression equations were used in a further study to determine the limits of detection and quantification of ibuprofen as it is described in Section 2.7.

2.7. Procedure for Determination of LOD and LOQ of Ibuprofen

There are various procedures for estimating both LOD and LOQ values as important parameters for the validation of analytical methods, which are recommended by regulatory authorities, such as the ICH, as well as in national pharmacopoeias [19–21]. So far, there is no calculation procedure designed especially for TLC-densitometric analysis. Of various methods, the most popular calculation for estimating the LOD and LOQ using an analytical method, including chromatography, is the calibration curve—Equations (1) and (2):

$$\mathrm{LOD} = \frac{\mathrm{f} \times \sigma}{\mathrm{S}} \quad (1)$$

$$\mathrm{LOQ} = \frac{\mathrm{f} \times \sigma}{\mathrm{S}} \quad (2)$$

where: f is a factor (f = 3.3 for LOD and f = 10 LOQ), S is the slope of the calibration plot; σ is the standard deviation of the intercept of the calibration or the residual standard deviation of the calibration curve [21]. In addition, based on our previous investigation, we tried to use and estimate the applicability of the modified procedure proposed by Konieczka and Namiesnik [21]. In accordance with this procedure, only every three concentrations of ibuprofen that meet the following requirements shall be used to prepare necessary calibration curves:

$$10 \times \mathrm{LOD} > \mathrm{C} \quad (3)$$

$$\mathrm{LOD} < \mathrm{C} \quad (4)$$

where: C is the quantity of ibuprofen

$$\mathrm{LOQ} = 3 \times \mathrm{LOD} \quad (5)$$

The amount of ibuprofen spotted on the chromatographic plates for NP-TLC and RP-TLC analysis in μg/spot performed under the proposed chromatographic conditions is given in Tables 1 and 2. Tables S1–S19 (Supplementary Materials) show the full characteristics of calibration plots (i.e., regression equations) prepared to determine both LOD and LOQ results of ibuprofen examined in the NP-TLC as well as in RP-TLC systems.

Table 1. Amount of ibuprofen used to prepare specific calibration curves (RP-TLC system).

Chromatography Conditions			Amount of Ibuprofen Spotted on Chromatographic Plates (μg/spot)		
Type of Chromatographic Plates	**Retardation Factor (R_f ± 0.02)**	**Mobile Phase Composition**			
1.05747	0.78	methanol:water (40:10, *v*/*v*)	1.00	2.00	3.00
	0.85	ethanol:water (40:10, *v*/*v*)	0.40	0.50	0.60
	0.86	propan-1-ol:water (40:10, *v*/*v*)	1.00	2.00	3.00
1.15424	0.56	methanol:water (40:10, *v*/*v*)	2.00	3.00	4.0
	0.69	ethanol:water (40:10, *v*/*v*)	0.30	0.40	0.50
	0.80	propan-1-ol:water (40:10, *v*/*v*)	1.00	2.00	3.00
1.05559	0.41	methanol:water (40:10, *v*/*v*)	0.20	0.30	0.40
	0.61	ethanol:water (40:10, *v*/*v*)	1.00	2.00	3.00
	0.75	propan-1-ol:water (40:10, *v*/*v*)	1.00	2.00	3.00

Table 2. Amount of ibuprofen used to prepare specific calibration curves (NP-TLC system).

Chromatography Conditions			Amount of Ibuprofen Spotted on Chromatographic Plates (μg/spot)		
Type of Chromatographic Plates	Retardation Factor (R_f ± 0.02)	Mobile Phase Composition			
1.05554	0.58	*n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*)	1.00	2.00	3.00
	0.61	toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*)	0.20	0.30	0.40
1.05583	0.79	*n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*)	0.20	0.30	0.40
	0.73	toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*)	1.00	2.00	3.00
1.05570	0.61	*n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*)	0.30	0.40	0.50
	0.68	toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*)	0.20	0.30	0.40
1.05715	0.57	*n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*)	1.00	2.00	3.00
	0.69	toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*)	0.20	0.30	0.40
1.05805	0.49	*n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*)	1.00	2.00	3.00
	0.60	toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*)	0.30	0.40	0.50

3. Results

Tables 3 and 4 show the LOD and LOQ values of investigated ibuprofen determined under different chromatographic conditions. Both parameters were calculated using the two procedures given in Equations (1) and (2) and according to the formulas in Equations (3)–(5). They are referred to as LOD^1/LOD^2 and LOQ^1 and LOQ^2.

Table 3. Limit of detection (LOD) of ibuprofen determined under different conditions in NP-TLC and RP-TLC systems.

Chromatographic System	Chromatographic Conditions		LOD^1 (μg/spot)	LOD^2 (μg/spot)
	Mobile Phase Composition	Type of Plate		
NP-TLC	*n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*)	1.05554	0.751	0.491
		1.05583	0.104	0.047
		1.05570	0.172	0.060
		1.05715	0.773	0.506
		1.05805	0.962	0.630
	toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*)	1.05554	0.156	0.071
		1.05583	0.686	0.449
		1.05570	0.128	0.058
		1.05715	0.310	0.044
		1.05805	0.146	0.051
RP-TLC	methanol:water (40:10, *v*/*v*)	RP-2F_{254}	0.499	0.327
		RP-8F_{254S}	1.218	0.554
		RP-18F_{254S}	0.103	0.047
	ethanol:water (40:10, *v*/*v*)	RP-2F_{254}	0.223	0.062
		RP-8F_{254S}	0.138	0.048
		RP-18F_{254S}	0.939	0.614
	propan-1-ol:water (40:10, *v*/*v*)	RP-2F_{254}	0.881	0.577
		RP-8F_{254S}	0.572	0.367
		RP-18F_{254S}	0.588	0.385

LOD^1 = value calculated by standard deviation of intercept of calibration curve; LOD^2 = value calculated by residual standard deviation of calibration curve.

Table 4. Limit of quantification (LOQ) of ibuprofen determined under different conditions in NP-TLC and RP-TLC systems.

Chromatographic System	Chromatographic Conditions		LOQ[1] (µg/spot)	LOQ[2] (µg/spot)
	Mobile Phase Composition	Type of Plate		
NP-TLC	*n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v/v/v*)	1.05554	2.275	1.489
		1.05583	0.314	0.143
		1.05570	0.522	0.181
		1.05715	2.344	1.534
		1.05805	2.915	1.908
	toluene:ethyl acetate:glacial acetic acid (27:21:2, *v/v/v*)	1.05554	0.474	0.216
		1.05583	2.080	1.361
		1.05570	0.387	0.176
		1.05715	0.938	0.133
		1.05805	0.443	0.152
RP-TLC	methanol:water (40:10, *v/v*)	RP-2F_{254}	1.513	0.991
		RP-8F_{254S}	3.691	1.679
		RP-18F_{254S}	0.313	0.143
	ethanol:water (40:10, *v/v*)	RP-2F_{254}	0.675	0.188
		RP-8F_{254S}	0.419	0.145
		RP-18F_{254S}	2.845	1.864
	propan-1-ol:water (40:10, *v/v*)	RP-2F_{254}	2.670	1.748
		RP-8F_{254S}	1.734	1.135
		RP-18F_{254S}	1.783	1.168

LOQ[1] = value calculated by standard deviation of intercept of calibration curve; LOQ[2] = value calculated by residual standard deviation of calibration curve.

In order to estimate the efficiency of all newly tested or modified, in relation to previously published documents, chromatographic conditions composed of different types of chromatographic plates (which are commercially available) for TLC sensitivity with UV densitometry in NP and RP systems, an average LOD value was calculated from LOD[1] and LOD[2] values in each case. The mean LOQ value was also determined in a similar manner. All average LOD and LOQ values of ibuprofen analyzed under different chromatographic conditions are shown in Figures 4–7.

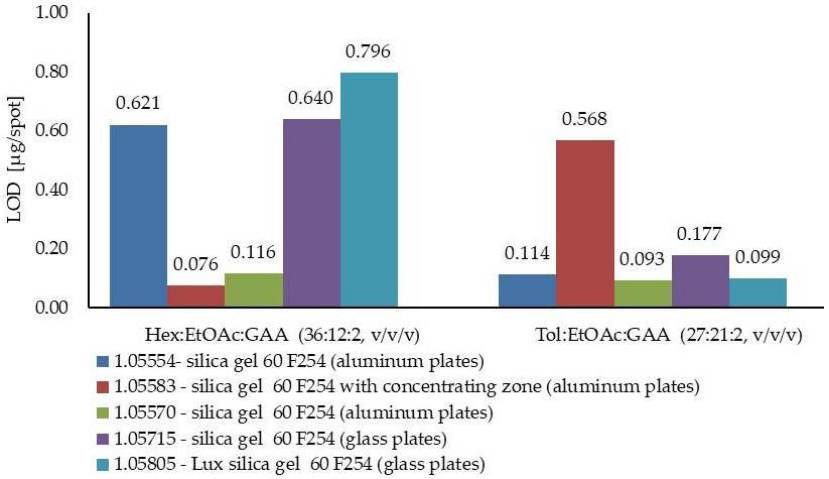

Figure 4. Comparison of average LOD values of ibuprofen determined by NP-TLC. (Hex = *n*-hexane, EtOAc = ethyl acetate, GAA = glacial acetic acid, Tol = toluene).

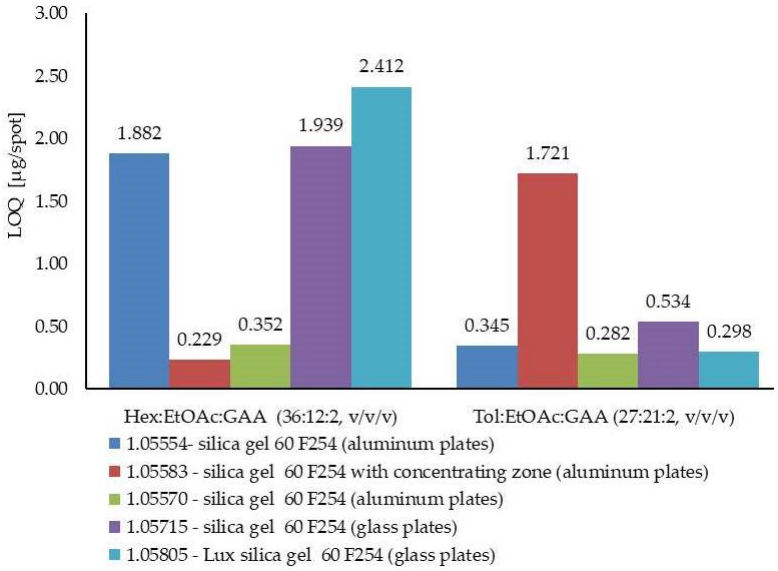

Figure 5. Comparison of average LOQ values of ibuprofen determined by NP-TLC. (Hex = *n*-hexane, EtOAc = ethyl acetate, GAA = glacial acetic acid, Tol = toluene).

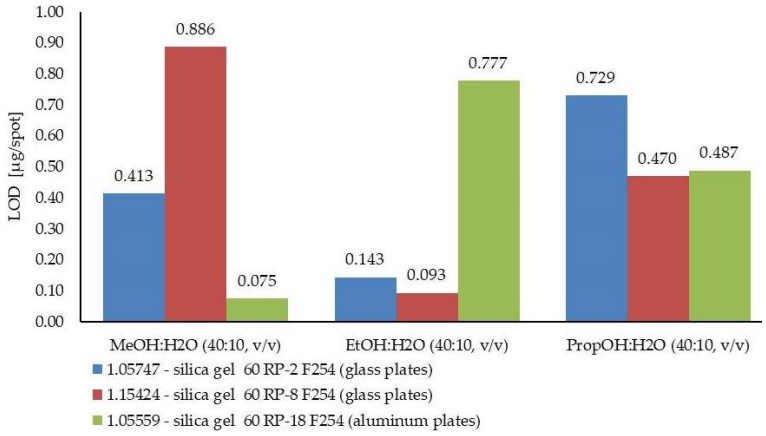

Figure 6. Comparison of average LOD values of ibuprofen determined by RP-TLC. (MeOH = methanol, EtOH = ethanol, PropOH = propan-1-ol).

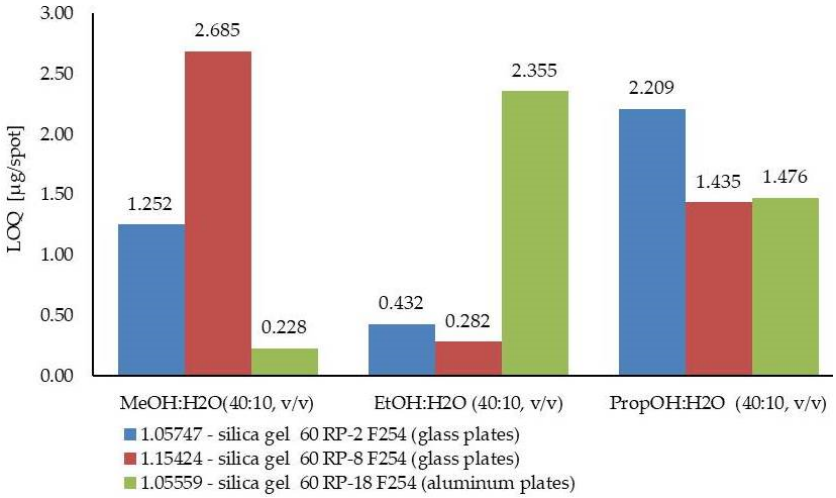

Figure 7. Comparison of average LOQ values of ibuprofen determined by RP-TLC. (MeOH = methanol, EtOH = ethanol, PropOH = propan-1-ol).

4. Discussion

Our preliminary study was aimed to find new or modified chromatographic conditions compared to those previously described in the literature including a chromatographic plate type and mobile phase composition suitable for the sensitive analysis of ibuprofen by TLC in two (NP and RP) chromatographic systems. The use of various chromatographic plates precoated with widely used chromatographic sorbents such as silica gel 60 and silica gel bound phases (RP plates) and other non-silica layers such as alumina, polyamide, a mixture of silica gel and diatomaceous earth (kieselguhr), and kieselguhr alone has proven that the most effective sorbent for analysis of ibuprofen is silica gel only. Therefore, this sorbent in the form of various commercially available plates for NP-TLC and RP-TLC with a UV indicator designated as s and in addition Lux TLC plates with increased brightness F_{254} indicators were tested in the current study to estimate the effect of chromatographic conditions on the sensitivity of the TLC analysis of ibuprofen, i.e., the determination of LOD and LOQ values. The accurate specification of five types of TLC plates used for the purpose of NP-TLC analysis indicates that they were in form of aluminum foil or glass plates of size 20 cm × 20 cm or 10 cm × 20 cm or coated with the same kind of sorbent, i.e., silica gel $60F_{254}$. However, they show some difference in the layer thickness of silica gel. Moreover, one of the chromatographic plates used under code 1.05583 had a special concentration zone. Therefore, during the conducted studies, we evaluated the influence of these properties on the quality of the obtained chromatograms and the quantitative analysis of ibuprofen.

To estimate the impact of the type of chromatographic plates for NP-TLC and RP-TLC for the detection and quantification of ibuprofen, the mean LOD and LOQ values were accurately analyzed.

The comparison of the LOD and LOQ of ibuprofen investigated by the NP-TLC system (Figures 4 and 5) using two mixtures as mobile phases: *n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*) and toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*) and five types of chromatographic plates precoated with silica gel $60F_{254}$, including concentration zone plates and plates with increased brightness F_{254} (1.05554, 1.05570, 1.05715, 1.05583, and 1.05805) indicated that the level of detection (LOD of ibuprofen) was generally comparable for both mobile phases, i.e., it is located in the range of 0.076–0.796 μg/spot for the first mobile phase and all plates used. For the second mobile phase consisting of toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*), it can be observed that the LOD value ranges from 0.093 to 0.568 μg/spot, so it is slightly shorter compared to the previous mobile phase. The lowest of all LOD values was obtained by a chromatographic system consisting of chromatographic plates 1.05583, i.e., precoated silica gel $60F_{254}$ and a mixture of *n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*) as mobile phase. In this case, the LOD of ibuprofen was 0.076 μg/spot and LOQ = 0.229 μg/spot. This chromatographic system for NP-TLC is therefore better than that which consisted of classical 1.05554 (aluminum plates) and 1.05715 (glass plates), for which the LOD is 0.621 and 0.640 μg/spot, respectively, and similarly LOQ = 1.882 and 1.939 μg/spot, respectively. The use of silica gel plates (1.05805) with increased brightness F_{254} did not improve the sensitivity of ibuprofen detection and quantification by NP-TLC with *n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*); the highest value of all obtained was observed; LOD = 0.796 and LOQ = 2.412 μg/spot. During another study, we tried to estimate the effect of the type of chromatographic plates on the levels of detection and quantification of ibuprofen using a second mobile phase consisting of toluene:ethyl acetate:glacial acetic acid (27:21:2, *v*/*v*/*v*). For this mobile phase, the results of the LOD and LOQ values were similar for most of the applied plates, i.e., 1.05554, 1.05570, 1.05715, 1.05805, except for those precoated with silica gel $60F_{254}$ with a concentration zone designated 1.05583. LOD = 0.568 μg/spot and LOQ = 1.721 μg/spot were for these plates. Of all the LOD and LOQ results, the lowest LOD = 0.093 μg/spot and LOQ = 0.282 μg/spot were observed for this mobile phase and 1.05570 silica gel plates. Very similar results can be observed for 1.05805, i.e., Lux plates of silica gel with increased brightness F_{254} (LOD = 0.099 and LOQ = 0.298 μg/spot). These results indicate that Lux silica gel plates with increased brightness F_{254} are a good alternative to conventional silica gel plates $60F_{254}$ and allowed to obtain low LOD and LOQ of ibuprofen using toluene:ethyl acetate:glacial acetic acid in a volume composition of 27:21:2. These chromatographic conditions have been reported to

improve the sensitivity of the TLC-densitometric method for the analysis of ibuprofen in relation to previously reported work [16–18]. Summarizing results obtained with the normal-phase system, it can be stated that besides the mobile phase composition, certain differences of the layer thickness of silica gel and the presence of a concentration zone influence the chromatographic analysis of ibuprofen, i.e., the quality of chromatograms (spot width and R_f value) as well as on the levels of detection and quantification. When silica gel plates with concentration zone were used (1.05583) and mobile phase consisted of *n*-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*), this allowed to obtain good quality chromatograms with very narrow and compact spots, which were detectable and quantifiable at the lower range in comparison with other classical silica gel plates, such as 1.05554, 1.05570 or 1.05715, used in this study.

As in the case of the described NP-TLC system, an effect of the type of chromatographic plates and the composition of the mobile phase on the LOD and LOQ of studied ibuprofen can be observed in the RP-TLC system as well.

In this part of the study, the following types of chromatographic plates based on silica gel 60 suited for RP and modified with aliphatic hydrocarbons, i.e., with partial octadecyl groups (C18, ODS) such as RP-18F_{254} (1.05559), with dimethylsilyl groups such as silica gel RP-2F_{254} plates 1.05747 of size 20 cm × 20 cm as well as glass plates RP-8F_{254} (1.15424) of size 10 cm × 20 cm were tested. Thus, the main difference in plates refers to the length of the hydrocarbon chain used to modify the silica gel layer. However, the manufacturer's specification of these modified silica gel plates (Section 2.2) indicates that there is also a certain difference in the layer thickness of these properly modified silica gel plates. The lowest thickness of the sorbent layer is observed in the case of RP-18F_{254}. Hence, the influence of the properties of the silica gel plates used in the RP system on the quality of chromatograms as well as the LOD and LOQ values of ibuprofen was discussed in further steps of this research.

The average LOD and LOQ of ibuprofen obtained using three types of RP-plates (RP-2F_{254}, RP-8F_{254}, and RP-18F_{254}) and different mobile phases consisting of methanol:water, ethanol:water and propan-1-ol:water in the ratio of 40:10 (*v*/*v*) are shown in Figures 6 and 7, respectively. In general, the LOD value of ibuprofen determined under the described chromatographic conditions ranged from 0.075 to 0.866 μg/spot. LOQ results were from 0.228 to 2.685 μg/spot. The lowest LOD (best sensitivity) was observed for the chromatographic system, consisting of RP-18F_{254} plates and a mixture of methanol:water 40:10 (*v*/*v*). Under these conditions, the LOD of ibuprofen was 0.075 μg/spot and LOQ = 0.228 μg/spot. A chromatographic system composed of RP-8F_{254} plates developed with ethanol:water (40:10, *v*/*v*) gave very similar results. For another type of plate, i.e., RP-2F_{254}, the best mobile phase was that containing ethanol:water (40:10, *v*/*v*); the LOD was 0.143 μg/spot, and the LOQ was 0.432 μg/spot. However, a poorer sensitivity of this method with RP-2F_{254} plates was observed for the mobile phase consisting of propan-1-ol: water, which allowed to obtain the highest LOD = 0.729 μg/spot and LOQ = 2.20 μg/spot for this type of plates. In the case of RP-8F_{254} plates and the proposed mobile phases, the most efficient mobile phase (allowing the lowest LOD and LOQ) was that consisting of ethanol:water (40:10, *v*/*v*). Under these conditions, the LOD and LOQ of ibuprofen were 0.093 and 0.282 μg/spot, respectively. The results of both estimated parameters obtained using propan-1-ol: water in the ratio of 40:10 (*v*/*v*) and RP-8F_{254} plates were much higher; LOD = 0.470 μg/spot and LOQ = 1.435 μg/spot. However, they are very similar to those obtained using the same mobile phases and RP-18F_{254} plates. It can be suggested that of all the proposed chromatographic conditions, the following should not be recommended for the detection and quantification of ibuprofen:

- RP-8F_{254} plates and methanol:water mixture (40:10, *v*/*v*): LOD = 0.886 μg/spot, and LOQ = 2.685 μg/spot;
- RP-18F_{254} plates and ethanol:water mixture (40:10, *v*/*v*): LOD = 0.777 μg/spot and LOQ = 2.355 μg/spot;
- RP-2F_{254} plates and propan-1-ol: water mixture (40:10, *v*/*v*): LOD = 0.729 μg/spot, LOQ = 2.209 μg/spot.

The analysis of the chromatograms and the LOD and LOQ values of ibuprofen determined using the RP system indicates that the proposed mixture containing 80% of a proper organic solvent (i.e., methanol, ethanol, propan-1-ol) and 20% of water as a mobile phase is sufficient to obtain good quality chromatograms of ibuprofen and reliable detection of this compound. It allows to achieve compact spots and symmetric peaks of ibuprofen easy to interpret. In addition to this, it was found that an appropriate modification of silica gel 60 with different aliphatic hydrocarbons as well as layer thickness can improve the results of the LOD and LOQ of ibuprofen. This conclusion can be confirmed by the best LOD and LOQ values of ibuprofen obtained by using methanol-water (40:10, *v*/*v*) and RP-18F_{254} plates coated with the thinnest layer of modified silica gel 60.

A comparison of the LOD and LOQ results of ibuprofen obtained using NP- and RP-TLC systems and different mobile phases highlights the importance of 60F_{254} silica gel plates with concentration zones (1.05583) and a mixture of *n*-hexane:ethyl acetate:glacial acetic acid 36:12:2 (*v*/*v*/*v*) in the NP-TLC system to improve the sensitivity of the TLC-densitometric method for the estimation of ibuprofen. The levels of detection and determination of this active substance was comparable to RP-18F_{254} plates developed with methanol:water (40:10, *v*/*v*), for which LOD = 0.075 μg/spot and LOQ = 0.228 μg/spot.

5. Conclusions

In this work, an efficient and reliable procedure for the determination of LOD and LOQ of ibuprofen using TLC-densitometry was presented. The LOD and LOQ calculation procedure performed in accordance with the recommendations of Konieczka and Namiesnik made it possible to obtain reliable results of the two determined sensitivity parameters of the method for ibuprofen. The results of the detection and the quantification level of ibuprofen under the proposed chromatographic conditions confirmed the applicability of silica gel plates and silica gel-bound phases (RP plates) for sensitive analysis of ibuprofen (detection and quantification levels ranging from a few nanograms to a few micrograms depend on chromatographic systems used). Of all the tested chromatographic systems, the best are those consisting of 60F_{254} silica gel plates with a concentration zone (1.05583) and a mixture of *n*-hexane:ethyl acetate:glacial acetic acid in a ratio of 36:12:2 (*v*/*v*/*v*) and aluminum RP-18F_{254} plates developed with methanol:water in a volume composition of 40:10 (*v*/*v*). The two proposed chromatographic systems made it possible to quantify ibuprofen in the amount of 0.229 μg/spot and 0.228 μg/spot, respectively, i.e., in the amount significantly less than 1 μg/spot. The various chromatographic systems combined with the densitometric scanning at 224 nm described in this work can be successfully used for the cost-effective and sensitive determination of ibuprofen as a widely used drug substance and residue in domestic wastewater. It was found that the modification of silica gel as well as the layer thickness of unmodified or modified silica gel 60 can influence the quality of chromatograms and the detection/quantification of ibuprofen in both NP- and RP systems. Therefore, to obtain the best possible LOD and LOQ values of ibuprofen with precoated layers, suitable mobile phase and chromatographic plates are required.

Supplementary Materials: The following are available online at http://www.mdpi.com/2227-9717/8/8/919/s1, Table S1: Results obtained in RP-TLC system using methanol:water (40:10, *v*/*v*) as mobile phase and RP-2F254 plates. Table S2: Results obtained in RP-TLC system using methanol:water (40:10, *v*/*v*) as mobile phase and RP-8F254 plates. Table S3: Results obtained in RP-TLC system using methanol:water (40:10, *v*/*v*) as mobile phase and RP-18F254 plates. Table S4: Results obtained in RP-TLC system using ethanol:water (40:10, *v*/*v*) as mobile phase and RP-2F254 plates. Table S5: Results obtained in RP-TLC system using ethanol:water (40:10, v/v) as mobile phase and RP-8F254 plates. Table S6: Results obtained in RP-TLC system using ethanol:water (40:10, *v*/*v*) as mobile phase and RP-18F254 plates. Table S7: Results obtained in RP-TLC system using propan-1-ol:water (40:10, *v*/*v*) as mobile phase and RP-2F254 plates. Table S8: Results obtained in RP-TLC system using propan-1-ol:water (40:10, *v*/*v*) as mobile phase and RP-8F254 plates. Table S9: Results obtained in RP-TLC system using propan-1-ol:water (40:10, *v*/*v*) as mobile phase and RP-18F254 plates. Table S10: Results obtained in NP-TLC system using n-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*) as mobile phase and silica gel 60F254 plates (1.05554). Table S11: Results obtained in NP-TLC system using n-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*) as mobile phase and silica gel 60F254 plates with concentrating zone (1.05583). Table S12: Results obtained in NP-TLC system using n-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*) as mobile phase and silica gel 60F254 plates (1.05570). Table S13: Results obtained in NP-TLC system by using n-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v*/*v*/*v*) as mobile

phase and silica gel 60F254 plates (1.05715). Table S14: Results obtained in NP-TLC system using n-hexane:ethyl acetate:glacial acetic acid (36:12:2, *v/v/v*) as mobile phase and Lux silica gel 60F254 plates (1.05805). Table S15: Results obtained in NP-TLC system using toluene:ethyl acetate:glacial acetic acid (27:21:2, *v/v/v*) as mobile phase and silica gel 60F254 plates (1.05554). Table S16: Results obtained in NP-TLC system using toluene:ethyl acetate:glacial acetic acid (27:21:2, *v/v/v*) as mobile phase and silica gel 60F254 plates with concentrating zone (1.05583). Table S17: Results obtained in NP-TLC system using toluene:ethyl acetate:glacial acetic acid (27:21:2, *v/v/v*) as mobile phase and silica gel 60F254 plates (1.05570). Table S18: Results obtained in NP-TLC system using toluene:ethyl acetate:glacial acetic acid (27:21:2, *v/v/v*) as mobile phase and silica gel 60F254 plates (1.05715). Table S19: Results obtained in NP-TLC system using toluene:ethyl acetate:glacial acetic acid (27:21:2, *v/v/v*) as mobile phase and Lux silica gel 60F254 plates (1.05805).

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