

Article

Influence of Chromatographic Conditions on LOD and LOQ of Fluoxetine and Sertraline Analyzed by TLC-Densitometric Method

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Abstract: This research introduces the analysis of fluoxetine and sertraline by means of the TLC-densitometric method. They provide information on LOD and LOQ under various chromatographic conditions. The study used adsorption (NPTLC) and partition (RPTLC) thin-layer chromatography in combination with a densitometric analysis. Four types of chromatographic plates precoated with: silica gel 60 F₂₅₄, silica gel 60, silanized silica gel 60 F₂₅₄ (RP-2), and a mixture of silica gel 60 and kieselguhr F₂₅₄, as well as three mobile phases: chloroform + methanol + ammonia (9:1:0.4, v/v/v), chloroform + methanol + glacial acetic acid (5:4:1, v/v/v), and acetone + toluene + ammonia (10:9:1, v/v/v), were used in NPTLC. RP-18F₂₅₄ and silanized silica gel 60 F₂₅₄ (RP-2) plates and four mobile phases: methanol + water (10:0 and 9:1, v/v), acetone + water (10:0 and 9:1, v/v), were used in RPTLC. The lowest LOD and LOQ values for fluoxetine were obtained using a silanized silica gel 60 F₂₅₄ (RP-2) with acetone + toluene + ammonia (10:9:1, v/v/v) in NPTLC, and with a silanized silica gel 60 F₂₅₄ (RP-2) in combination with methanol + water (10:0, v/v) in RPTLC. The lowest LOD and LOQ values of sertraline were obtained using a silica gel 60 with acetone + toluene + ammonia (10:9:1; v/v/v) in NPTLC. The smallest amount of sertraline was detected on the silanized silica gel 60 F₂₅₄ plate in combination with methanol + water (9:1, v/v) in RPTLC. The obtained results provide important information that can give a good basis and set the direction for further, more detailed research; the results can also benefit other researchers who analyze fluoxetine and sertraline with the TLC technique in model systems (testing standards) as well as in drug and biological samples.

Keywords: fluoxetine; sertraline; limit of detection (LOD); limit of quantification (LOQ); NPTLC; RPTLC



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1. Introduction

Depression is a multifactorial chronic disease; widespread worldwide; associated with serious health, social, and economic problems, and in some cases may lead to the suicidal thoughts of sick people. The most common symptoms are fatigue, malaise, insomnia, and mood changes. One of the factors that may lead to the development of depression when treatment is not started is anxiety [1]. Due to their different chemical structure or mechanisms of action, antidepressants can be classified in different ways. One of the most common classifications is their division into groups depending on the pharmacological action. There are four classes: tricyclic antidepressants (TLPD), selective norepinephrine reuptake inhibitors (SNRI), monoamine oxidase inhibitors (MAOIs), and selective serotonin reuptake inhibitors (SSRI), which include sertraline (Figure 1a) and fluoxetine (Figure 1b) [1,2]. Medicinal substances belonging to selective serotonin reuptake inhibitors (SSRIs) were developed and introduced in the 1980s [2,3].

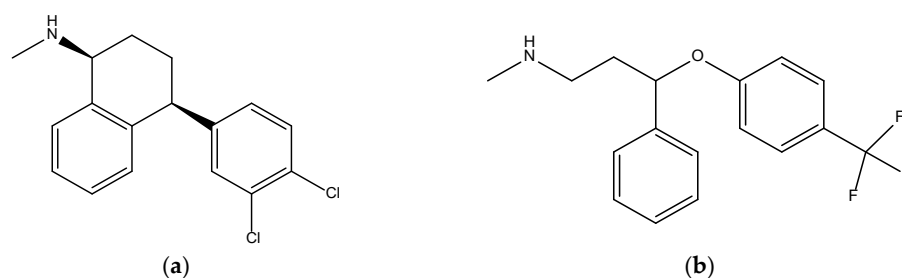


Figure 1. Chemical structures of sertraline (a) and fluoxetine (b).

A review of the literature shows that fluoxetine and sertraline in drugs or in biological material can be determined by various analytical methods such as spectrophotometry, spectrofluorimetry, voltamperometry, capillary electrophoresis, high-performance liquid chromatography (HPLC), and gas chromatography (GC) for pharmaceutical and clinical purposes [4–35]. However, chromatographic techniques are at the fore in the research.

Thin-layer chromatography (TLC) is a very effective tool for tracking the progress of a chemical reaction [36–38], whereas TLC in combination with densitometry or mass spectrometry is very often used in drug analysis. Several uses of TLC for the analysis of fluoxetine and sertraline in drugs are described in the scientific literature [39–46].

Compared to other chromatographic techniques such as gas chromatography (GC) and high-performance liquid chromatography (HPLC), TLC is the simplest and cheapest technique. The TLC technique in combination with densitometry is a good tool for the analysis of biologically active compounds, including drugs. Furthermore, TLC has high sensitivity, good selectivity and resolution, good reproducibility, and it is well suitable for qualitative and quantitative analyses. Occasionally, spectrophotometry has been used to analyze sertraline and fluoxetine [4]. However, compared to TLC, spectrophotometry has low selectivity and a lack of resolution. Despite the variety of analytical techniques available on the market for the identification of biologically active substances, TLC remains a key technique in the pharmaceutical industry. In addition, thin-layer chromatography studies are among the key identity studies in most pharmacopoeial monographs. Pharmacopoeial standards are typically used in the industry as a basis for meeting quality control requirements and current Good Manufacturing Practices (GMP). Many TLC tests to identify pharmaceuticals or contaminants are found in the US and European Pharmacopoeia monographs [47–50].

One of the most important stages of an analytical procedure is their validation. The determination of the limit of detection and the limit of quantification of a given analytical method is one of the most important elements of validation as it allows for the assessment of the sensitivity of the analytical method. The limit of detection (LOD) is the lowest concentration or the smallest amount of a substance that can be detected with sufficient probability using a given analytical technique. The limit of quantification (LOQ) is the lowest concentration or quantity of a substance that can be quantified using a specific analytical method with adequate accuracy and precision. Various methods of LOD determination are currently being used [51,52]. So far, there are existing no studies in the scientific literature on the sensitivity of TLC in terms of the effect of the mobile and stationary phases on the LOD and LOQ of fluoxetine and sertraline. In chromatography, following the ICH guidelines, LOD is most often calculated from the value of the standard deviation of the set of signals and the slope of the calibration curve. In this case, the LOD is calculated from the following formula:

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

The following formula is used to calculate the limit of quantification (LOQ):

$$\text{LOQ} = \frac{10 \sigma}{S} \quad (2)$$

where:

σ —standard deviation (standard deviation of the intercept σ_a and the residual standard deviation σ_{xy} of the calibration curve);

S—slope of the calibration curve [52].

Knowing the LOD, the LOQ value can usually be quickly calculated from the known LOD value, namely:

$$\text{LOD} = 3 \cdot \text{LOQ} \quad (3)$$

Correctly calculated values of the limit of detection should meet certain assumptions:

$$10 \cdot \text{LOD} > C \quad (4)$$

$$\text{LOD} < C \quad (5)$$

where C—concentration of the analyte in the standard samples [52].

The aim and assumption of this study was to determine the effect of chromatographic conditions on the limit of detection and the limit of quantification of the analyzed fluoxetine and sertraline using: adsorption thin-layer chromatography (normal-phase thin-layer chromatography, NP-TLC) in combination with densitometric analysis, and partition thin-layer chromatography (reversed-phase thin-layer chromatography, RP-TLC) in combination with densitometric analysis.

2. Materials and Methods

2.1. Chemicals and Reagents

Fluoxetine hydrochloride (United States Pharmacopeia (USP) Reference Standard) and sertraline hydrochloride (European Pharmacopoeia (EP) Reference Standard) were received from Sigma-Aldrich (St. Louis, MO, USA). Chloroform, methanol, ethanol (99.8%), ammonia (25%), glacial acetic acid (99.5%), acetone, and toluene were purchased from POCh (Gliwice, Poland). All solvents were of analytical purity.

2.2. Chromatographic Plates

The following chromatographic plates were used in the tests: silica gel 60 F₂₅₄ on glass plates (#1.05715); silica gel 60 on glass plates (#1.05721); mixture of silica gel 60 and kieselguhr F₂₅₄ on aluminum plates (#1.05567); silanized silica gel 60 F₂₅₄ (RP-2) on glass plates (#1.05747); silica gel RP-18F₂₅₄ on aluminum plates (#1.05559). All chromatography plates were for the TLC analysis and were purchased from Merck (Darmstadt, Germany).

2.3. Standard Solutions

Standard solutions of fluoxetine and sertraline were prepared by dissolving 50 mg of the standard substance in 50 mL of ethanol (99.8%), resulting in a solution with a concentration of 5 mg/5 mL. A series of dilutions were then made from the resulting solution to obtain the following concentrations of fluoxetine and sertraline: 1.00, 0.90, 0.80, 0.70, 0.60, 0.50, 0.40, 0.30, 0.20, 0.10 mg/5mL.

Tables S1 and S2 (in Supplementary Materials) show the concentration values ($\mu\text{g}/\text{spot}$) of fluoxetine and sertraline, the results of which were included in the LOD and LOQ calculations after the TLC-densitometric analysis. Calibration curves were determined on the basis of the data contained in Tables S3–S37. After determining the parameters that characterize the calibration curve, the LOD and LOQ were then calculated (Tables S38 and S39).

2.4. Conditions for Analysis by Adsorption (NP-TLC) and Partition Thin-Layer Chromatography (RP-TLC)

The following plates were used for NP-TLC analysis: 1.05715, 1.05721, 1.05567, and 1.05747. Before starting the loading of the solutions, the chromatographic plates in the stationary phase were activated at 120 °C in an incubator for 30 min. After the specified time had elapsed, the plates were removed and set aside in order to cool them. Then,

the prepared solutions were applied to the designated start site with the use of 5 μ L microcapillaries.

The chromatographic plates were then developed in the following mobile phases:

- A—chloroform + methanol + ammonia, 9:1:0.4 (v/v/v);
- B—chloroform + methanol + glacial acetic acid, 5:4:1 (v/v/v);
- C—acetone + toluene + ammonia, 10:9:1 (v/v/v).

RPTLC analyses were performed using the 1.05559 and 1.05747 plates. The previously prepared standard solutions were applied to the designated starting places of the chromatographic plates using micropipettes with a volume of 5 μ L. The plates were developed in the following mobile phases:

- D—methanol + water, 10:0 (v/v);
- E—methanol + water, 9:1 (v/v);
- F—acetone + water, 10:0 (v/v);
- G—acetone + water, 9:1 (v/v).

After the chromatographic chambers were saturated with the eluent vapors, the chromatographic plates were placed in them and developed at room temperature until the mobile phase front had reached a height of 7.5 cm, which was measured from the starting line. The plates with developed chromatograms were removed from the chambers and placed in racks in a fume hood, where they remained for 24 h until the complete evaporation of the mobile phase.

2.5. Densitometric and Spectrodensitometric Analysis

Densitometric and spectrodensitometric analyses of fluoxetine and sertraline on individual chromatographic plates were performed using a TLC Scanner 3 densitometer with winCATS 1.4.2 software, manufactured by Camag, Muttenz, Switzerland. The measurement was performed in the absorption mode. The wavelength during the spectrodensitometric analysis was in the range of 200–700 nm. The scan speed was 100 nm/s, with a slit size of 6.0 \times 0.3 mm, Micro, and a resolution of 1 nm/step. The radiation sources were a deuterium and tungsten lamps.

Densitometric analysis was also performed using a TLC Scanner 3 densitometer with the winCATS 1.4.2 software by Camag, where the radiation source was a deuterium lamp. The optimal wavelengths during scanning were 200 nm and 202 nm for fluoxetine and sertraline, respectively. The analysis was performed in the absorption mode, with a scan speed of 20 mm/s, a slit size of 10.0 \times 6.0 mm, Micro, and a resolution of 100 μ m/step. The spectra of fluoxetine and sertraline are provided in the Supplementary Materials (Figures S1 and S2).

3. Results

The limit of detection (LOD) and the limit of quantification (LOQ) of fluoxetine and sertraline were calculated from the intercept standard deviation (σ_a) and the residual standard deviation (σ_{xy}) of the calibration curves, according to Formulas (1) and (2). The calibration curves were obtained from the arithmetic mean of the area of the chromatographic bands. Only the areas of the fluoxetine and sertraline chromatographic bands that met the conditions of Formulas (4) and (5) were taken into account in the calculations. Tables S3–S22 (Supplementary Materials) provide the full characterization of the chromatographic band area and calibration plots (i.e., regression equations) prepared to determine the LOD and LOQ results for fluoxetine when tested with the NP-TLC and RP-TLC techniques. Tables S23–S37 provide the full characterization of the chromatographic band area and calibration plots (i.e., regression equations) prepared to determine the LOD and LOQ results for sertraline with the NP-TLC and RP-TLC techniques. The LOD and LOQ values calculated from the intercept standard deviation (σ_a) and the residual standard deviation (σ_{xy}) of the calibration curve are presented in Tables S38–S41. From these values,

the mean LOD and LOQ values of fluoxetine and sertraline, analyzed under the particular chromatographic conditions, were calculated.

Figures 2 and 3 show a successive comparison of the mean values of the limits of detection and quantification of fluoxetine, obtained by adsorption thin-layer chromatography (NPTLC) with the use of three mobile phases and four chromatographic sorbents.

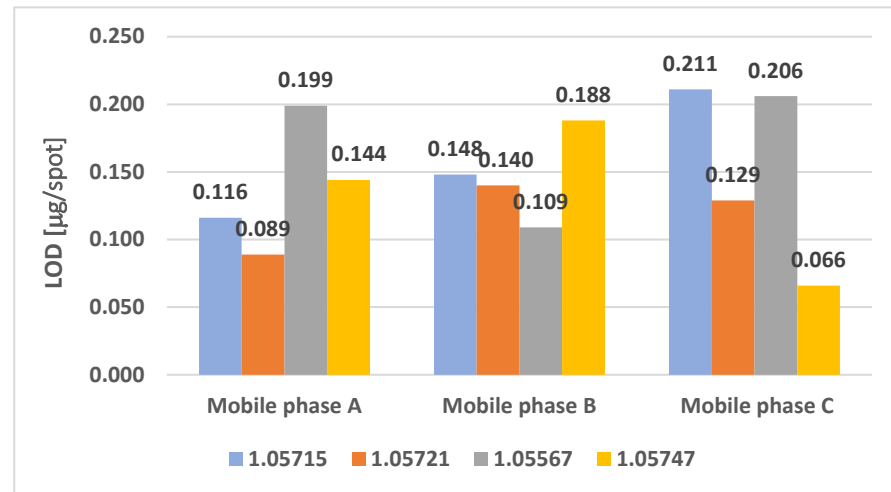


Figure 2. Mean values of the limit of detection of fluoxetine obtained by adsorption thin-layer chromatography, where the mobile phases were as follows: A—chloroform + methanol + ammonia, 9:1:0.4 (v/v/v); B—chloroform + methanol + glacial acetic acid, 5:4:1 (v/v/v); C—acetone + toluene + ammonia, 10:9:1 (v/v/v). The chromatographic plates were: 1.05715—silica gel 60 F₂₅₄ on glass plates; 1.05721—silica gel 60 on glass plates; 1.05567—mixture of silica gel 60 and kieselguhr F₂₅₄ on aluminum plates; 1.05747—silanized silica gel 60 F₂₅₄ (RP-2) on glass plates.

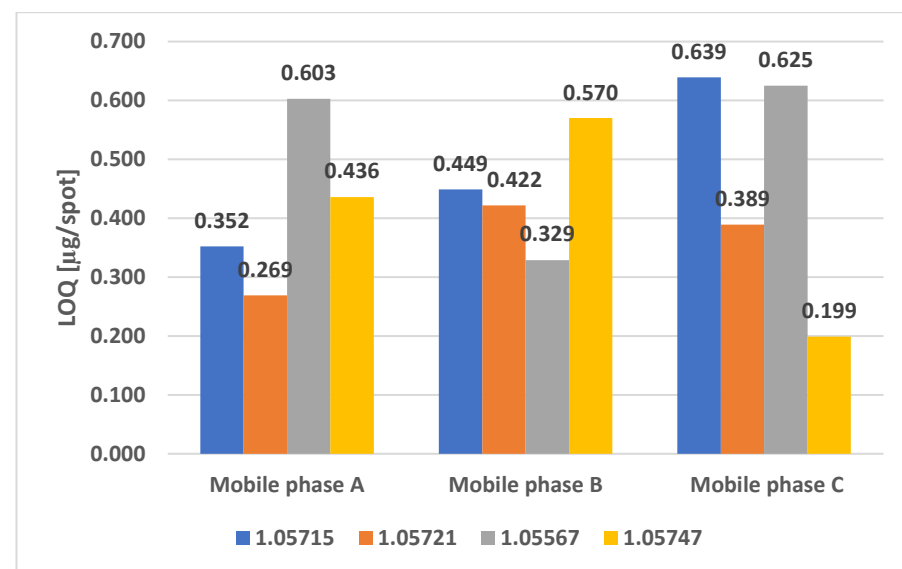


Figure 3. Mean values of the limit of quantification of fluoxetine obtained by NPTLC, where the mobile phases were as follows: A—chloroform + methanol + ammonia, 9:1:0.4 (v/v/v); B—chloroform + methanol + glacial acetic acid, 5:4:1 (v/v/v); C—acetone + toluene + ammonia, 10:9:1 (v/v/v). The chromatographic plates were: 1.05715—silica gel 60 F₂₅₄ on glass plates; 1.05721—silica gel 60 on glass plates; 1.05567—mixture of silica gel 60 and kieselguhr F₂₅₄ on aluminum plates; 1.05747—silanized silica gel 60 F₂₅₄ (RP-2) on glass plates.

Figures 4 and 5 show a successive comparison of the limits of detection and quantification of fluoxetine, obtained by partition thin-layer chromatography (RPTLC) using four mobile phases and two chromatographic sorbents.

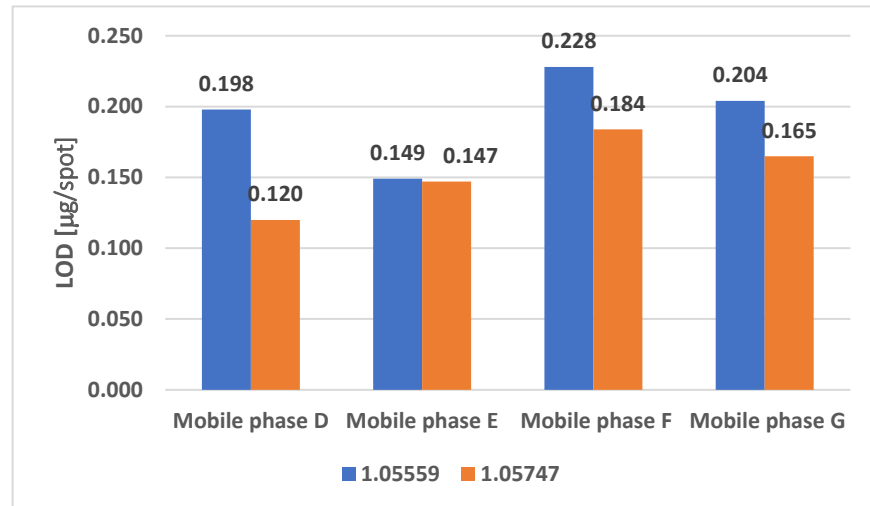


Figure 4. Mean values of the limit of detection for fluoxetine, obtained by partition thin-layer chromatography, where the mobile phases were as follows: D—methanol + water, 10:0 (*v/v*); E—methanol + water, 9:1 (*v/v*); F—acetone + water, 10:0 (*v/v*); G—acetone + water, 9:1 (*v/v*). The chromatographic plates were: 1.05747—silanized silica gel 60 F₂₅₄ (RP-2) on glass plates; 1.05559—silica gel RP-18F₂₅₄ on aluminum plates.

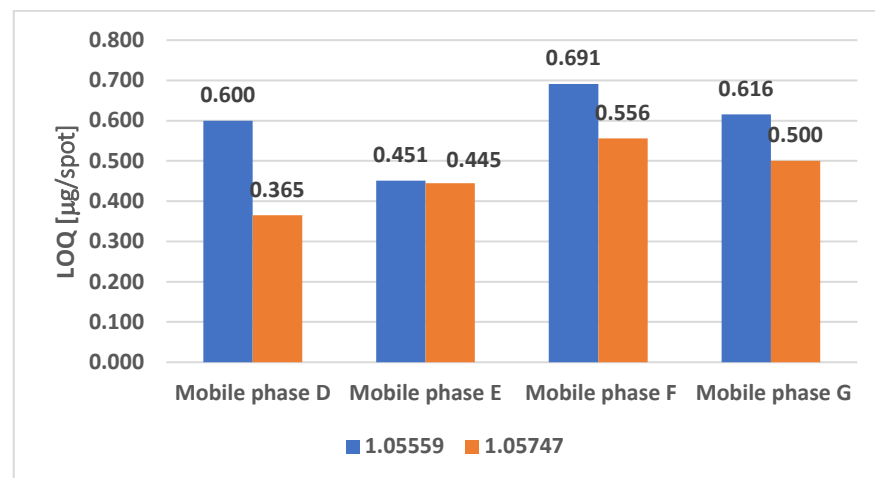


Figure 5. Mean values of the limit of quantification of fluoxetine, obtained by partition thin-layer chromatography, where the mobile phases were as follows: D—methanol + water, 10:0 (*v/v*); E—methanol + water, 9:1 (*v/v*); F—acetone + water, 10:0 (*v/v*); G—acetone + water, 9:1 (*v/v*). The chromatographic plates were: 1.05747—silanized silica gel 60 F₂₅₄ (RP-2) on glass plates; 1.05559—silica gel RP-18F₂₅₄ on aluminum plates.

Figures 6 and 7 show a successive comparison of the limits of detection and quantification of sertraline, obtained with adsorption thin-layer chromatography using two mobile phases and four chromatographic sorbents.

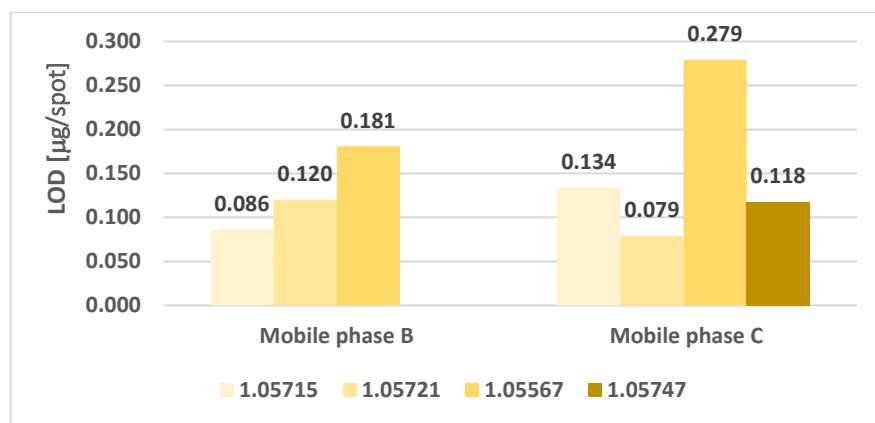


Figure 6. Mean values of the limit of detection of sertraline, obtained by adsorption thin-layer chromatography, where the mobile phases were as follows: B—chloroform + methanol + glacial acetic acid, 5:4:1 (*v/v/v*); C—acetone + toluene + ammonia, 10:9:1 (*v/v/v*). The chromatographic plates were: 1.05715—silica gel 60 F₂₅₄ on glass plates; 1.05721—silica gel 60 on glass plates; 1.05567—mixture of silica gel 60 and kieselguhr F₂₅₄ on aluminum plates; 1.05747—silanized silica gel 60 F₂₅₄ (RP-2) on glass plates.

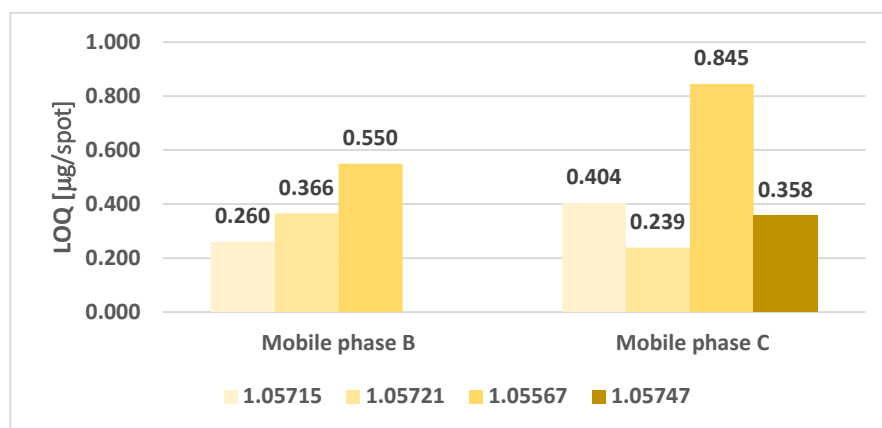


Figure 7. Mean values of the limit of quantification of sertraline, obtained by adsorption thin-layer chromatography, where the mobile phases were as follows: A—chloroform + methanol + ammonia, 9:1:0.4 (*v/v/v*); B—chloroform + methanol + glacial acetic acid, 5:4:1 (*v/v/v*); C—acetone + toluene + ammonia, 10:9:1 (*v/v/v*). The chromatographic plates were: 1.05715—silica gel 60 F₂₅₄ on glass plates; 1.05721—silica gel 60 on glass plates; 1.05567—mixture of silica gel 60 and kieselguhr F₂₅₄ on aluminum plates; 1.05747—silanized silica gel 60 F₂₅₄ (RP-2) on glass plates.

Figures 8 and 9 show a successive comparison of the limits of detection and quantification of sertraline, obtained by partition thin-layer chromatography using four mobile phases and two chromatographic sorbents.

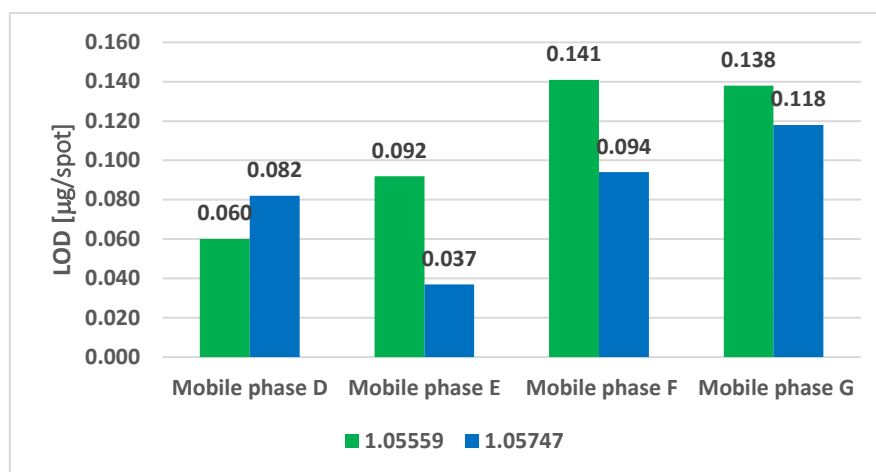


Figure 8. Mean values of the limit of detection of sertraline, obtained by the method of partition thin-layer chromatography, where the mobile phases were as follows: D—methanol + water, 10:0 (*v/v*); E—methanol + water, 9:1 (*v/v*); F—acetone + water, 10:0 (*v/v*); G—acetone + water, 9:1 (*v/v*). The chromatographic plates were: 1.05747—silanized silica gel 60 F₂₅₄ (RP-2) on glass plates; 1.05559—silica gel RP-18F₂₅₄ on aluminum plates.

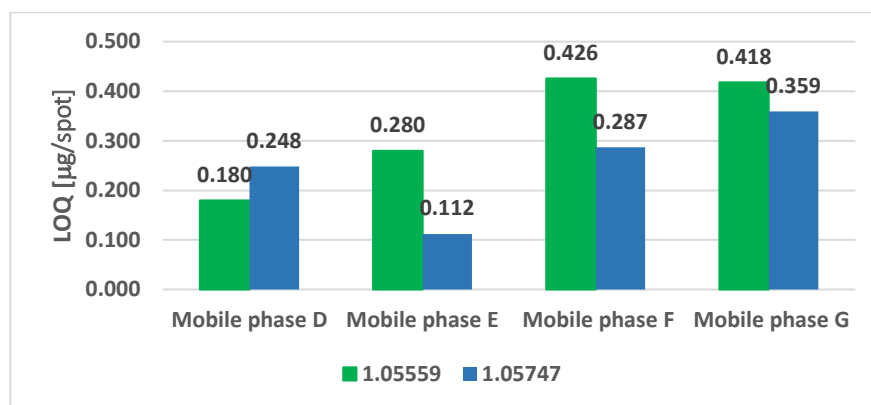


Figure 9. Mean values of the limit of quantification of sertraline, obtained by the method of partition thin-layer chromatography, where the mobile phases were as follows: D—methanol + water, 10:0 (*v/v*); E—methanol + water, 9:1 (*v/v*); F—acetone + water, 10:0 (*v/v*); G—acetone + water, 9:1 (*v/v*). The chromatographic plates were: 1.05747—silanized silica gel 60 F₂₅₄ (RP-2) on glass plates; 1.05559—silica gel RP-18F₂₅₄ on aluminum plates.

In addition, Figures 10 and 11 as well as Figures S3–S22 show exemplary densitograms of fluoxetine and sertraline (fluoxetine can be separated from sertraline under these chromatographic conditions).

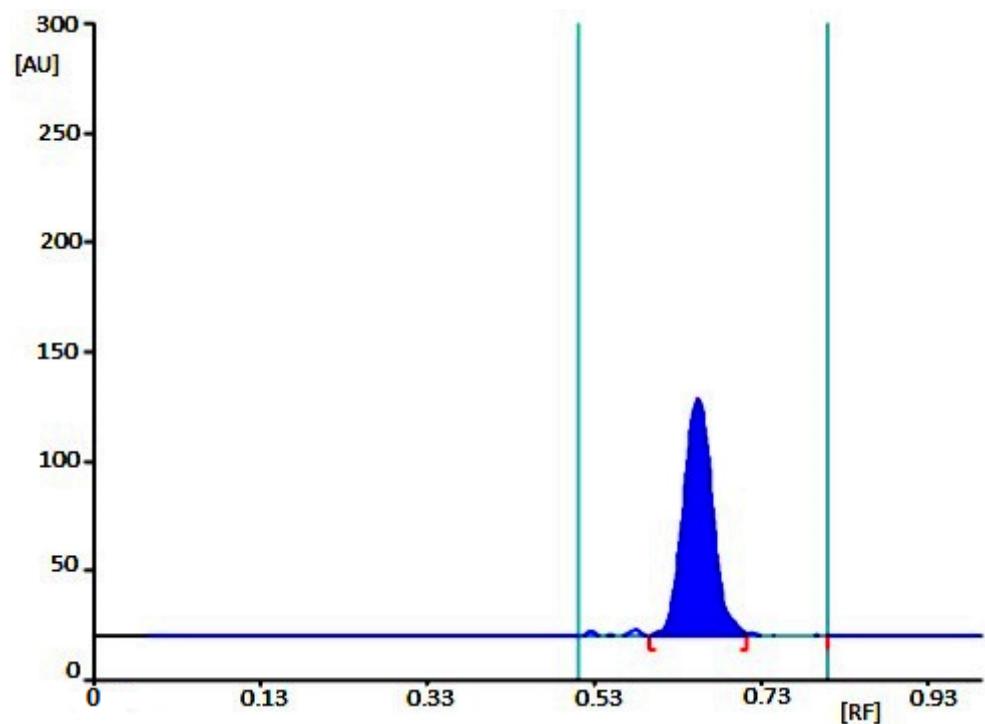


Figure 10. Chromatogram (densitogram) of sertraline ($R_F = 0.63$), analyzed on silica gel 60 (1.05721) using the acetone + toluene + ammonia, 10:9:1 ($v/v/v$) mobile phase.

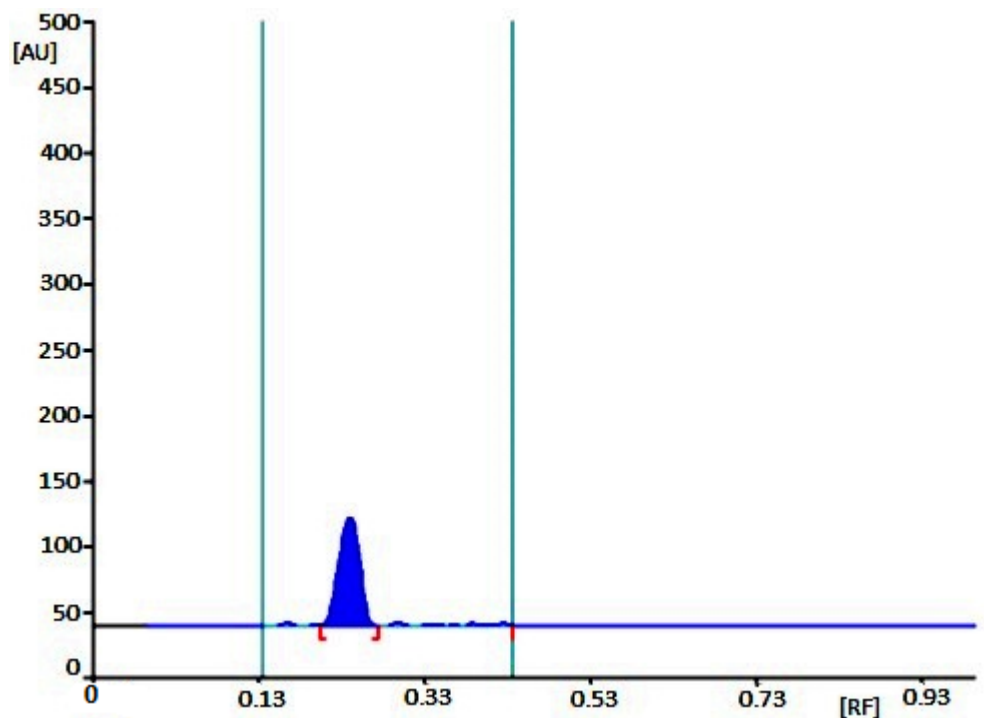


Figure 11. Chromatogram (densitogram) of fluoxetine ($R_F = 0.24$), analyzed on silica gel 60 (1.05721) using the acetone + toluene + ammonia, 10:9:1 ($v/v/v$) mobile phase.

4. Discussion

Our research aimed to find new or modified chromatographic conditions compared to those previously described in the literature in order to indicate the sensitive NPTLC and RPTLC analyses of fluoxetine and sertraline.

Four types of chromatographic sorbents were tested in adsorption thin-layer chromatography (NPTLC), all for classical TLC analysis. More specifically, the analyses were

performed on glass plates precoated with silica gel 60 without a fluorescent additive (#1.05721) and with the addition of fluorescent F₂₅₄ (#1.05715), dimethylsilyl-modified silica gel 60_{F254} (these plates are also referred to as silanized or RP-2F₂₅₄) (#1.05747), and on aluminum plates precoated with a mixture of silica gel 60 and kieselguhr F₂₅₄ (#1.05567). Three mobile phases that were used in the studies proved to be effective for fluoxetine analysis. On the other hand, when the chloroform + methanol + ammonia 9:1:0.4 (v/v/v) mobile phase was used, sertraline migrated from the front of the mobile phase on all the above-mentioned chromatographic plates. Therefore, the results of sertraline only apply to the other two mobile phases, i.e., chloroform + methanol + glacial acetic acid 5:4:1 (v/v/v) and acetone + toluene + ammonia 10:9:1 (v/v/v).

Two types of sorbents were used in partition thin-layer chromatography (RPTLC). More specifically, glass plates precoated with silanized silica gel 60 F₂₅₄ (#1.05747) and aluminum plates precoated with silica gel 60 F₂₅₄ were modified with the octadecyl groups (these plates are also referred to as RP-18F₂₅₄) (#1.05559). Four mobile phases were used in the research: methanol + water 10:0 (v/v), methanol + water 9:1 (v/v), acetone + water 10:0 (v/v), and acetone + water 9:1 (v/v).

4.1. Fluoxetine Analysis

The mean LOD and LOQ values were carefully analyzed to assess the effects of the type of chromatography plates used for NP-TLC and RP-TLC and the employed mobile phases on the detection and quantification of fluoxetine.

A comparison of the LOD and LOQ of fluoxetine tested with the NP-TLC technique (Figures 2 and 3) using three mixtures as mobile phases: chloroform + methanol + ammonia 9:1:0.4 (v/v/v), chloroform + methanol + glacial acetic acid 5:4:1 (v/v/v), and acetone + toluene + ammonia 10:9:1 (v/v/v), and four types of chromatography plates (1.05715, 1.05721, 1.05747, 1.05567) indicated that the limit of detection (LOD) of fluoxetine for the three mobile phases was generally comparable, i.e., it was in the range of 0.066–0.211 µg/spot. The lowest LOD and LOQ values of 0.066 and 0.199 µg/spot, respectively, were obtained on the 1.05747 plate using the C mobile phase composed of acetone + toluene + ammonia 10:9:1 (v/v/v). The second lowest results, 0.089 µg/spot for LOD and 0.269 µg/spot for LOQ, were obtained with the 1.05721 plate and mobile phase A composed of chloroform + methanol + ammonia 9:1:0.4 (v/v/v). The third lowest results, 0.129 µg/spot for LOD and 0.389 µg/spot for LOQ, were obtained with the 1.05721 plate and the C mobile phase composed of acetone + toluene + ammonia 10:9:1 (v/v/v). The use of these sorbents in combination with certain mobile phases enabled the detection and determination of the lowest fluoxetine concentration. Therefore, these chromatographic conditions are the most sensitive.

A comparison of the LOD and LOQ of fluoxetine tested by RP-TLC (Figures 4 and 5) using four mixtures as mobile phases: methanol + water 10:0 (v/v), methanol + water 9:1 (v/v), acetone + water 10:0 (v/v), acetone + water 9:1 (v/v), and two types of chromatography plates (1.05747, 1.05559) indicated that the limit of detection (LOD) of fluoxetine for the four mobile phases was generally comparable, i.e., it was in the range of 0.120–0.228 µg/spot. The lowest LOD and LOQ values, equal to 0.120 and 0.365 µg/spot, were obtained on the 1.05747 plate using mobile phase D with the following composition: methanol + water (10:0, v/v). The use of these chromatographic plates in conjunction with a given mobile phase makes it possible to detect and determine the lowest concentration of fluoxetine. The next lowest LOD and LOQ results were obtained on the 1.05559 (where LOD = 0.147 µg/spot, LOQ = 0.445 µg/spot) and 1.05747 (where LOD = 0.149 µg/spot, LOQ = 0.451 µg/spot) plates using mobile phase E composed of methanol + water (9: 1, v/v). The difference between the described results obtained with mobile phase E in combination with the mentioned plates is negligible. In all cases, lower LOD and LOQ values were achieved with carrier 1.05747, which can be attributed to better detectability and quantification of fluoxetine.

4.2. Sertraline Analysis

A comparison of the LOD and LOQ of sertraline tested with the NP-TLC technique (Figures 6 and 7) using two mixtures as mobile phases: chloroform + methanol + glacial acetic acid 5:4:1 (*v/v/v*), acetone + toluene + ammonia 10:9:1 (*v/v/v*), and four types of chromatography plates (1.05715, 1.05721, 1.05747, 1.05567) indicated that the limit of detection (LOD) of sertraline was generally comparable for the two mobile phases, i.e., in the range of 0.079–0.279 $\mu\text{g}/\text{spot}$. The lowest LOD and LOQ values of 0.079 and 0.239 $\mu\text{g}/\text{spot}$, respectively, were obtained on the 1.05721 plate using the C mobile phase, composed of acetone + toluene + ammonia 10:9:1 (*v/v/v*). Another slightly higher result was obtained on the 1.05715 plate (LOD = 0.086 $\mu\text{g}/\text{spot}$, LOQ = 0.260 $\mu\text{g}/\text{spot}$) in combination with mobile phase B, composed of chloroform + methanol + glacial acetic acid 5:4:1, (*v/v/v*). The third, slightly higher than the second result, was obtained on the 1.05747 plate (LOD = 0.118 $\mu\text{g}/\text{spot}$, LOQ = 0.358 $\mu\text{g}/\text{spot}$) in combination with the C mobile phase, composed of acetone + toluene + ammonia 10:9:1 (*v/v/v*). The use of the above-mentioned sorbents and mobile phases made it possible to detect and determine the lowest concentration of sertraline. On the 1.05747 plate, using mobile phase B, sertraline migrated to the front of the mobile phase. Therefore, this sorbent prevents the detection and determination of sertraline with mobile phase B.

A comparison of the LOD and LOQ of sertraline tested by RP-TLC (Figures 8 and 9) using four mixtures as mobile phases: methanol + water 10:0 (*v/v*), methanol + water 9:1 (*v/v*), acetone + water 10:0 (*v/v*), and acetone + water 9:1 (*v/v*), and two types of chromatographic plates (1.05747, 1.05559) indicated that the limit of detection (LOD) of sertraline is in the range of 0.037–0.141 $\mu\text{g}/\text{spot}$. Lower LOD and LOQ values were obtained for the methanol + water mobile phases (10:0 and 9:1, *v/v*). When these two mobile phases were used, the LOD of sertraline was in the range of 0.037–0.092 $\mu\text{g}/\text{spot}$. However, in the case of using the acetone + water mobile phases (10:0 and 9:1, *v/v*), the LOD of sertraline was in the range of 0.094–0.141 $\mu\text{g}/\text{spot}$. The lowest LOD and LOQ values, equal to 0.037 and 0.112 $\mu\text{g}/\text{spot}$, respectively, were obtained on the 1.05747 plate using the E mobile phase composed of methanol + water (9:1, *v/v*). The second lowest values for LOD (0.060 $\mu\text{g}/\text{spot}$) and LOQ (0.180 $\mu\text{g}/\text{spot}$) were obtained on the 1.05559 plate in combination with mobile phase D, which had the following composition: methanol + water (10:0, *v/v*). The third consecutive lowest LOD (0.082 $\mu\text{g}/\text{spot}$) and LOQ (0.248 $\mu\text{g}/\text{spot}$) results were obtained on the 1.05747 plate in combination with mobile phase D, with the following composition: methanol + water (10:0, *v/v*). The use of the mentioned sorbents together with the given mobile phases made it possible to detect and determine the lowest concentration of sertraline.

4.3. Comparison of the Detection of Fluoxetine and Sertraline as Well as Practical Utility of the Obtained Results

Under some of the chromatographic conditions used, it was possible to separate sertraline and fluoxetine. Figures 10 and 11 as well as Figures S3–S22 show densitograms (chromatograms) of sertraline and fluoxetine. Under these chromatographic conditions, sertraline and fluoxetine have different R_F values which allow for their separation. From a pharmaceutical point of view, chromatographic conditions are of particular importance as they allow for the separation of sertraline from fluoxetine and are characterized by high sensitivity (low LOD and LOQ) at the same time.

In general, when testing fluoxetine and sertraline, a very good chromatographic sorbent for both NP-TLC and RP-TLC is silanized silica gel 60 F₂₅₄ (RP-2F₂₅₄, #1.05747). Fluoxetine and sertraline can be analyzed on this chromatographic plate with very good sensitivity (low LOD and LOQ) using the following mobile phases: acetone + toluene + ammonia (10:9: 1, *v/v/v*), methanol + water (10:0, *v/v*), and methanol + water (9:1, *v/v*). These chromatographic conditions ensure the simultaneous determination of fluoxetine and sertraline because these chromatographic conditions ensure the separation of fluoxetine and sertraline, as shown by the R_F values listed in Tables S38 and S39 and the densitograms shown in Figures S13–S16, S19 and S20. Fluoxetine and sertraline can also be analyzed

with very good sensitivity (low LOD and LOQ) on chromatography plates precoated with silica gel 60 (#1.05721) and with the acetone + toluene + ammonia mobile phase (10:9:1, v/v/v). Again, it is possible to separate fluoxetine from sertraline (Figures 10 and 11). Under these chromatographic conditions, the R_F is 0.24 and 0.63 for fluoxetine and sertraline, respectively.

The obtained results are of practical use because, in accordance with pharmacopoeial recommendations, they allow the identity of fluoxetine and sertraline to be tested. The purpose of this test is to confirm that a given substance corresponds to the substance declared in a pharmaceutical preparation. The identity is most often tested by comparing the R_F value of the standard substance with the R_F value of the biologically active substance present in the drug. However, in complex mixtures, the retention parameters of a substance may be a criterion, but not the only one, for compound identification. Then, it is assumed that the substance is the same as the standard if the retention values (in the case of TLC—the R_F retardation factor) are the same for the three chromatographic systems with different stationary and mobile phases. On the other hand, the determination of the limit of detection allows for the discernment of the concentration in the given chromatographic conditions (chromatographic carrier, i.e., chromatographic plate and mobile phase) at which its presence can be detected on the chromatographic plate. Thus, the R_F values presented in Tables S38 and S39 for fluoxetine and sertraline, respectively, allow for the selection of three different chromatographic conditions to test the identity of fluoxetine and sertraline. The LODs for fluoxetine and sertraline in particular chromatographic conditions given in this work are an indication of the lowest possible concentrations of fluoxetine and sertraline that can be detected under given chromatographic conditions.

4.4. Comparison of the Obtained Values for the Limit of Detection (LOD) of Fluoxetine and Sertraline with the Literature Values

Table 1 shows the LOD results for fluoxetine and sertraline using the different methods for the stationary and mobile phases. The lowest LOD values obtained in this study are marked in red in this table.

Table 1. Summary of the lowest LOD values of fluoxetine and sertraline (marked in red) obtained during the study using the NPTLC and RPTLC techniques with the literature values.

Method	Stationary Phase	Mobile Phase	LOD of Fluoxetine (µg/Spot)	LOD of Sertraline (µg/Spot)	Ref.
NPTLC	Silanized silica gel 60 F ₂₅₄ (RP-2 F ₂₅₄ , 1.05747) on glass plate	acetone + toluene + ammonia (10:9:1, v/v/v)	0.066	0.118	in this work
NPTLC	silica gel 60 F ₂₅₄ (1.05721) on glass plate	acetone + toluene + ammonia (10:9:1, v/v/v)	0.129	0.079	in this work
RPTLC	Silanized silica gel 60 F ₂₅₄ (RP-2 F ₂₅₄ , 1.05747) on glass plate	methanol + water (10:0, v/v)	0.120	0.082	in this work
RPTLC	Silanized silica gel 60 F ₂₅₄ (RP-2 F ₂₅₄ , 1.05747) on glass plate	methanol + water (9:1, v/v)	0.147	0.037	in this work
NPTLC	silica gel 60 F ₂₅₄ on glass plate	acetone + benzene + 25% ammonia (50: 45: 5, v/v/v)	0.050	0.050	[39]
NPTLC	silica gel 60 GF ₂₅₄	benzene + acetone + ethanol + 25% aqueous ammonia (9:7:2:1, v/v/v/v)	0.05	-	[40]
HPTLC	silica gel 60 F ₂₅₄ on aluminum plate	methanol + toluene (40:20, v/v)	0.132	-	[41]
HPTLC	silica gel 60 F ₂₅₄ on aluminum plate	methanol + toluene (4:2, v/v)	0.300	-	[42]

Table 1. Cont.

Method	Stationary Phase	Mobile Phase	LOD of Fluoxetine (µg/Spot)	LOD of Sertraline (µg/Spot)	Ref.
RPTLC	RP-18 F _{254S} TLC on aluminum plate	methanol + 0.05 M phosphate buffer (pH = 5) + triethylamine (68:27:5, v/v/v)	0.100	-	[43]
HPTLC	silica gel 60 F ₂₅₄ on aluminum plate	toluene + ethyl acetate + ethanol + ammonia (8:2:0.5: 0.1, v/v/v/v)	-	0.670	[44]
HPTLC	silica gel 60 F ₂₅₄ on aluminum plate	toluene + ethyl acetate + ammonia (1:5:0.1, v/v/v)	-	0.015	[45]
HPTLC	silica gel 60 F ₂₅₄ on aluminum plate	n-propanol + hexane + toluene + triethylamine (7:2:1:0.1, v/v/v/v)	-	0.163	[46]

During the experiment, Gondová et al. [39] performed a simultaneous analysis of four antidepressants: fluoxetine, sertraline, fluvoxamine, and citalopram. They used the technique of adsorption thin-layer chromatography. The stationary phase employed glass plates precoated with silica gel 60 F₂₅₄, while the mobile phase consisted of acetone, benzene, and 25% ammonia (50:45:5, v/v/v). After developing the chromatograms, the plates were subjected to densitometric analysis. The results were determined on the basis of a calibration curve [39]. The literature LOD value for both fluoxetine and sertraline in this case is 0.050 µg/spot. Skibiński et al. [40] analyzed fluoxetine and paroxetine by NPTLC using silica gel 60 F₂₅₄ and benzene + acetone + ethanol + 25% aqueous ammonia (9:7:2:1, v/v/v/v). The limit of detection for fluoxetine was equal to 0.05 µg/spot. These values are lower than the lowest results obtained by this study during the test performed with adsorption thin-layer chromatography (Table 1). This means that the tested chromatographic conditions are characterized by worse detection and quantification properties as compared to the test conditions described in the publication by Gondová et al. [39] and Skibiński et al. [40]. This is because lower levels of fluoxetine and sertraline may be detected and quantified. However, the mobile phases proposed by Gondová et al. [39] and Skibiński et al. [40] cannot be recommended for use at present due to the fact that it contains benzene, which has carcinogenic properties.

Another method used, *inter alia*, to analyze the limit of detection was high-performance adsorption thin-layer chromatography (HPTLC). This method was used by Shah et al. [41] in a publication aimed at the simultaneous determination of fluoxetine and olanzapine. During the tests, the stationary phase made use of aluminum sheets precoated with silica gel 60 F₂₅₄ and the mobile phase methanol + toluene (40:20, v/v). The LOD value for fluoxetine was 0.132 µg/spot, and it was calculated using Formulas (1) and (2) [41]. Researchers using the adsorption HPTLC technique obtained a higher LOD result, equal to 0.132 µg/spot, as compared to the LOD values obtained by this study using the TLC adsorption technique (0.066 µg/spot). The same team of researchers, Shah et al. [42], again performed a study involving the simultaneous determination of fluoxetine and olanzapine using the same stationary and mobile phases (aluminum sheets precoated with silica gel 60 F₂₅₄, mobile phase—methanol + toluene (40:20, v/v)), but with a different concentration of the standard solutions. The LOD of fluoxetine obtained in this study was 0.300 µg/spot, which is higher than the previous one [42].

Gondová et al. [43] used RPTLC to quantify fluoxetine and citalopram. During the research, RP-18 F_{254S} TLC on an aluminum plate and methanol + 0.05 M phosphate buffer (pH 5) + triethylamine (68:27:5, v/v) were used. The limit of detection for fluoxetine was 0.100 µg/spot. This result is lower than the result of our partition TLC analysis (0.120 µg/spot).

Rao et al. [44] used the HPTLC adsorption technique to quantify sertraline hydrochloride. During the research, aluminum sheets for HPTLC precoated with silica gel 60 F₂₅₄ and the mobile phase toluene + ethyl acetate + ethanol + ammonia (8:2:0.5:0.1, v/v/v/v) were used. The limit of detection for sertraline was 0.670 µg/spot. This result is much higher than the result of our TLC adsorption analysis (0.079 µg/spot). This means that the chromatographic conditions used by Rao et al. [44] are characterized by lower detectability, i.e., lower sensitivity.

Hussain et al. [45], in a study that determined the content of sertraline in the lipid nanosystem, used aluminum plates for TLC adsorption, precoated with silica gel 60 F₂₅₄. The mobile phase consisted of the following solvents: toluene + ethyl acetate + ammonia (1:5:0.1, v/v/v). The LOD of sertraline was 0.015 µg/spot. This is the lowest value as compared to the results obtained by this study and the literature results that were previously mentioned. The chromatographic conditions used ensure that the lowest concentrations of sertraline can be detected.

Vahora et al. [46] analyzed brexpiprazole and sertraline HCl in bulk and synthetic mixtures. The stationary phase selected was a pre-coated silica gel aluminum plate 60 F₂₅₄, and n-propanol + hexane + toluene + triethylamine (7:2:1:0.1, v/v/v/v) was used as the developing mobile phase. The LOD of sertraline was 0.163 µg/spot. This result is much higher than the result of our TLC adsorption analysis (0.079 µg/spot).

In our previous work [53–57], we assessed the effect of stationary and mobile phases in NPTLC and RPTLC on the LOD and LOQ of hydrocortisone acetate, estradiol hemihydrate, nicotinamide, and ibuprofen. Dołowy [57] conducted similar research on spironolactone. Compared to other drugs, fluoxetine and sertraline as well as hydrocortisone acetate have a relatively narrow LOD range. Therefore, it should be assumed that the LOD of a compound depends on its chemical structure and the applied chromatographic analysis conditions, i.e., the type of chromatographic sorbent and the composition of the mobile phase.

5. Conclusions

The qualitative and quantitative composition of the mobile phase and the type of the stationary phase used affect the limits of detection and quantification of both fluoxetine and sertraline obtained by the technique of adsorption and partition thin-layer chromatography. It was also observed that generally lower LOD and LOQ values were obtained for sertraline as compared to fluoxetine. Therefore, the chemical structure of the detected and determined compound also affects the sensitivity of the analysis. The results of the detection and quantification of fluoxetine and sertraline under the proposed chromatographic conditions confirmed the usefulness of silica gel 60 plates and silanized silica gel 60 F₂₅₄ plates (RP-2 plates) for the sensitivity analysis of fluoxetine and sertraline. Using the adsorption thin-layer chromatography technique, the smallest amount of fluoxetine was detected and determined using silanized silica gel 60 F₂₅₄ (RP-2, #1.05747) in combination with a mobile phase composed of acetone + toluene + ammonia (10:9:1, v/v/v). The limit of detection (LOD) for fluoxetine was 0.066 µg/spot, and the limit of quantification (LOQ) for fluoxetine was 0.199 µg/spot. Using the partition thin-layer chromatography technique, the least amount of fluoxetine was detected and determined using silanized silica gel 60 F₂₅₄ (RP-2, #1.05747) in combination with the mobile phase methanol + water (10:0, v/v). The LOD of fluoxetine was 0.120 µg/spot and the LOQ of fluoxetine was 0.365 µg/spot. Using the adsorption thin-layer chromatography technique, the smallest amount of sertraline was detected and determined using silica gel 60 (#1.05721) in combination with a mobile phase composed of acetone + toluene + ammonia (10:9:1, v/v/v). The LOD of sertraline was 0.079 µg/spot, and the LOQ of sertraline was 0.239 µg/spot. Using the partition thin-layer chromatography technique, the smallest amount of sertraline was detected and determined using silanized silica gel 60 F₂₅₄ (RP-2, #1.05747) in combination with the mobile phase methanol + water (9:1, v/v). The LOD of sertraline was 0.037 µg/spot, and the LOQ of sertraline was 0.112 µg/spot. These chromatographic conditions allow both the lowest LOD and LOQ values to be obtained and the separation of fluoxetine

from sertraline. The obtained results are of practical use because, in accordance with pharmacopoeial recommendations, they allow for the identity of fluoxetine and sertraline to be tested. The purpose of this test is to confirm that a given substance corresponds to the substance declared in a pharmaceutical preparation. The LODs for fluoxetine and sertraline in particular chromatographic conditions given in this work are an indication of the lowest possible concentrations of fluoxetine and sertraline that can be detected under given chromatographic conditions. The obtained results provide important information that can give a good basis and set the direction for further, more detailed research as well as for other researchers who analyze fluoxetine and sertraline using the TLC technique in model systems (testing standards) and also in drug samples and biological samples.

Supplementary Materials: The following are available online on <https://www.mdpi.com/article/10.3390/pr10050971/s1>. Figure S1: Spectrum of fluoxetine. Figure S2: Spectrum of sertraline. Table S1: Concentrations of fluoxetine standard solutions used for the determination of LOD and LOQ. Table S2: Concentrations of sertraline standard solutions used for the determination of LOD and LOQ. Table S3: Results for fluoxetine obtained by NP-TLC technique using silica gel 60F254 on glass plates (#1.05715) and chloroform + methanol + ammonia (9:1:0.4, v/v/v) as the mobile phase. Table S4: Results for fluoxetine obtained by NP-TLC technique using silica gel 60 on glass plates (#1.05721) and chloroform + methanol + ammonia (9:1:0.4, v/v/v) as the mobile phase. Table S5: Results for fluoxetine obtained by NP-TLC technique using a mixture of silica gel 60 and kieselguhr F254 on aluminum plates (#1.05567) and chloroform + methanol + ammonia (9:1:0.4, v/v/v) as the mobile phase. Table S6: Results for fluoxetine obtained by NP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and chloroform + methanol + ammonia (9:10.4, v/v/v) as the mobile phase. Table S7: Results for fluoxetine obtained by NP-TLC technique using silica gel 60F254 on glass plates (#1.05715) and chloroform + methanol + glacial acetic acid (5:4:1, v/v/v) as the mobile phase. Table S8: Results for fluoxetine obtained by NP-TLC technique using silica gel 60 on glass plates (#1.05721) and chloroform + methanol + glacial acetic acid (5:4:1, v/v/v) as the mobile phase. Table S9: Results for fluoxetine obtained by NP-TLC technique using a mixture of silica gel 60 and kieselguhr F254 on aluminum plates (#1.05567) and chloroform + methanol + glacial acetic acid (5:4:1, v/v/v) as the mobile phase. Table S10: Results for fluoxetine obtained by NP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and chloroform + methanol + glacial acetic acid (5:4:1, v/v/v) as the mobile phase. Table S11: Results for fluoxetine obtained by NP-TLC technique using silica gel 60F254 on glass plates (#1.05715) and acetone + toluene + ammonia (10:9:1, v/v/v) as the mobile phase. Table S12: Results for fluoxetine obtained by NP-TLC technique using silica gel 60 on glass plates (#1.05721) and acetone + toluene + ammonia (10:9:1, v/v/v) as the mobile phase. Table S13: Results for fluoxetine obtained by NP-TLC technique using a mixture of silica gel 60 and kieselguhr F254 on aluminum plates (#1.05567) and acetone + toluene + ammonia (10:9:1, v/v/v) as the mobile phase. Table S14: Results for fluoxetine obtained by NP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and acetone + toluene + ammonia (10:9:1, v/v/v) as the mobile phase. Table S15: Results for fluoxetine obtained by RP-TLC technique using silica gel RP-18F254 on aluminum plates (#1.05559) and methanol+water (10:0, v/v/v) as the mobile phase. Table S16: Results for fluoxetine obtained by RP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and methanol+water (10:0, v/v/v) as the mobile phase. Table S17: Results for fluoxetine obtained by RP-TLC technique using silica gel RP-18F254 on aluminum plates (#1.05559) and methanol+water (9:1, v/v/v) as the mobile phase. Table S18: Results for fluoxetine obtained by RP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and methanol+water (9:1, v/v/v) as the mobile phase. Table S19: Results for fluoxetine obtained by RP-TLC technique using silica gel RP-18F254 on aluminum plates (#1.05559) and acetone+water (10:0, v/v/v) as the mobile phase. Table S20: Results for fluoxetine obtained by RP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and acetone+water (10:0, v/v/v) as the mobile phase. Table S21: Results for fluoxetine obtained by RP-TLC technique using silica gel RP-18F254 on aluminum plates (#1.05559) and acetone+water (9:1, v/v/v) as the mobile phase. Table S22: Results for fluoxetine obtained by RP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and acetone+water (9:1, v/v/v) as the mobile phase. Table S23: Results for sertraline obtained by NP-TLC technique using silica gel 60 on glass plates (#1.05721) and chloroform + methanol + glacial acetic acid (5:4:1, v/v/v) as the mobile phase. Table S24: Results for

sertraline obtained by NP-TLC technique using silica gel 60 on glass plates (#1.05721) and chloroform + methanol + glacial acetic acid (5:4:1, *v/v/v*) as the mobile phase. Table S25: Results for sertraline obtained by NP-TLC technique using a mixture of silica gel 60 and kieselguhr F254 on aluminum plates (#1.05567) and chloroform + methanol + glacial acetic acid (5:4:1, *v/v/v*) as the mobile phase. Table S26: Results for sertraline obtained by NP-TLC technique using silica gel 60F254 on glass plates (#1.05715) and acetone + toluene + ammonia (10:9:1, *v/v/v*) as the mobile phase. Table S27: Results for sertraline obtained by NP-TLC technique using silica gel 60 on glass plates (#1.05721) and acetone + toluene + ammonia (10:9:1, *v/v/v*) as the mobile phase. Table S28: Results for sertraline obtained by NP-TLC technique using a mixture of silica gel 60 and kieselguhr F254 on aluminum plates (#1.05567) and acetone + toluene + ammonia (10:9:1, *v/v/v*) as the mobile phase. Table S29: Results for sertraline obtained by NP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and acetone + toluene + ammonia (10:9:1, *v/v/v*) as the mobile phase. Table S30: Results for sertraline obtained by RP-TLC technique using silica gel RP-18F254 on aluminum plates (#1.05559) and methanol+water (10:0, *v/v/v*) as the mobile phase. Table S31: Results for sertraline obtained by RP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and methanol+water (10:0, *v/v/v*) as the mobile phase. Table S32: Results for sertraline obtained by RP-TLC technique using silica gel RP-18F254 on aluminum plates (#1.05559) and methanol+water (9:1, *v/v/v*) as the mobile phase. Table S33: Results for sertraline obtained by RP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and methanol+water (9:1, *v/v/v*) as the mobile phase. Table S34: Results for sertraline obtained by RP-TLC technique using silica gel RP-18F254 on aluminum plates (#1.05559) and acetone+water (10:0, *v/v/v*) as the mobile phase. Table S35: Results for sertraline obtained by RP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and acetone+water (10:0, *v/v/v*) as the mobile phase. Table S36: Results for sertraline obtained by RP-TLC technique using silica gel RP-18F254 on aluminum plates (#1.05559) and acetone + water (9:1, *v/v/v*) as the mobile phase. Table S37: Results for sertraline obtained by RP-TLC technique using silanized silica gel 60F254 (RP-2) on glass plates (#1.05747) and acetone+water (9:1, *v/v/v*) as the mobile phase. Table S38: Detection limit values and RF values for fluoxetine obtained in different chromatographic systems. Table S39: Detection limit values and RF values for sertraline obtained in different chromatographic systems. Table S40: Quantification limit values for fluoxetine obtained in different chromatographic systems. Table S41: Quantification limit values for sertraline obtained in different chromatographic systems. Figure S3: Densitogram of sertraline analyzed on silica gel 60 F₂₅₄ (1.05715) using the chloroform + methanol + glacial acetic acid- 5:4:1 (*v/v/v*) mobile phase. Figure S4: Densitogram of fluoxetine analyzed on silica gel 60 F₂₅₄ (1.05715) using the chloroform + methanol + glacial acetic acid- 5:4:1 (*v/v/v*) mobile phase. Figure S5: Densitogram of sertraline analyzed on silica gel 60 (1.05721) using the chloroform + methanol + glacial acetic acid- 5:4:1 (*v/v/v*) mobile phase. Figure S6: Densitogram of fluoxetine analyzed on silica gel 60 (1.05721) using the chloroform + methanol + glacial acetic acid- 5:4:1 (*v/v/v*) mobile phase. Figure S7: Densitogram of sertraline analyzed on a mixture of silica gel 60 and kieselguhr F₂₅₄ (1.05567) using the chloroform + methanol + glacial acetic acid- 5:4:1 (*v/v/v*) mobile phase. Figure S8: Densitogram of fluoxetine analyzed on a mixture of silica gel 60 and kieselguhr F₂₅₄ (1.05567) using the chloroform + methanol + glacial acetic acid- 5:4:1 (*v/v/v*) mobile phase. Figure S9: Densitogram of sertraline analyzed on silica gel 60 F₂₅₄ (1.05715) using the acetone + toluene + ammonia- 10:9:1 (*v/v/v*) mobile phase. Figure S10: Densitogram of fluoxetine analyzed on silica gel 60 F₂₅₄ (1.05715) using the acetone + toluene + ammonia- 10:9:1 (*v/v/v*) mobile phase. Figure S11: Densitogram of sertraline analyzed on a mixture of silica gel 60 and kieselguhr F₂₅₄ (1.05567) using the acetone + toluene + ammonia- 10:9:1 (*v/v/v*) mobile phase. Figure S12: Densitogram of sertraline analyzed on a mixture of silica gel 60 and kieselguhr F₂₅₄ (1.05567) using the acetone + toluene + ammonia- 10:9:1 (*v/v/v*) mobile phase. Figure S13: Densitogram of sertraline analyzed on silanized silica gel 60 (RP-2) (1.05747) using the acetone + toluene + ammonia- 10:9:1 (*v/v/v*) mobile phase. Figure S14: Densitogram of fluoxetine analyzed on silanized silica gel 60 (RP-2) (1.05747) using the acetone + toluene + ammonia- 10:9:1 (*v/v/v*) mobile phase. Figure S15: Densitogram of sertraline analyzed on silanized silica gel 60 (RP-2) (1.05747) using the methanol+water 10:0 (*v/v*) mobile phase. Figure S16: Densitogram of fluoxetine analyzed on silanized silica gel 60 (RP-2) (1.05747) using the methanol+water 10:0 (*v/v*) mobile phase. Figure S17: Densitogram of sertraline analyzed on silanized silica gel 60 (RP-2) (1.05747) using the acetone+water 10:0 (*v/v*) mobile phase. Figure S18: Densitogram of fluoxetine analyzed on silanized silica gel 60 (RP-2) (1.05747) using the acetone+water 10:0 (*v/v*) mobile phase. Figure S19: Densitogram of sertraline analyzed on silanized silica gel 60 (RP-2) (1.05747) using

the methanol+water 9:1 (*v/v*) mobile phase. Figure S20: Densitogram of fluoxetine analyzed on silanized silica gel 60 (RP-2) (1.05747) using the methanol+water 9:1 (*v/v*) mobile phase. Figure S21: Densitogram of sertraline analyzed on silica gel RP-18F₂₅₄ (1.05559) using the methanol + water 9:1 (*v/v*) mobile phase. Figure S22: Densitogram of fluoxetine analyzed on silica gel RP-18F₂₅₄ (1.05559) using the methanol + water 9:1 (*v/v*) mobile phase.

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