

Article

New TLC Method Combined with Densitometry for Determination of Sertraline and Fluoxetine in Pharmaceutical Preparations

Alina Pyka-Pająk 

Department of Analytical Chemistry, Faculty of Pharmaceutical Sciences in Sosnowiec, Medical University of Silesia in Katowice, Jagiellońska 4, 41-200 Sosnowiec, Poland; apyka@sum.edu.pl; Tel.: +48-32-364-15-30

Abstract: TLC combined with densitometry was used, and chromatographic conditions were developed to determine both fluoxetine and sertraline in pharmaceuticals. The mobile phase with the composition of acetone, chloroform, and ammonia (10:5:1, *v/v*) allowed separation of the most degradation products of sertraline and fluoxetine from all nineteen used in the study of mobile phases. Therefore, this mobile phase was selected as optimal for the analysis of sertraline and fluoxetine in pharmaceutical formulations. The R_F values of sertraline and fluoxetine differ from the degradation products of fluoxetine and sertraline. Therefore, the developed chromatographic conditions can be used for the simultaneous determination of fluoxetine and sertraline. Confirmation of the identity of the active substance can be made on the basis of the compliance of the R_F values and the spectrodensitograms of the substance from the pharmaceutical preparation and the standard of fluoxetine or sertraline, respectively. The developed method was simple, economical, specific, precise, accurate, sensitive, and robust, with a good range of linearity for the quantification of fluoxetine and sertraline in pharmaceutical preparations. TLC in combination with densitometry can be recommended for the analysis of fluoxetine and sertraline in the absence of HPLC in the laboratory or to confirm results obtained with other analytical techniques.



Citation: Pyka-Pająk, A. New TLC Method Combined with Densitometry for Determination of Sertraline and Fluoxetine in Pharmaceutical Preparations. *Processes* **2022**, *10*, 2083. <https://doi.org/10.3390/pr10102083>

Academic Editor: Yunfei Du

Received: 5 September 2022

Accepted: 11 October 2022

Published: 14 October 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2022 by the author. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

Keywords: TLC; densitometry; fluoxetine; sertraline; stability; pharmaceutical preparation

1. Introduction

Depression is an affective disorder. It manifests itself in emotional changes and changes in the patient's activity. The disease affects about 3–5% of the world's population, and more often it affects women, but the mechanism of its development has not been fully understood [1]. At the same time, the presence of a somatic disease such as Parkinson's disease, pain syndromes, malignant tumors, thyroid diseases, diabetes, heart attack, stroke, ischemic heart disease, AIDS, and Alzheimer's disease, may increase the likelihood of a depressive episode [2–9].

Various groups of drugs are used in the pharmacotherapy of depression, e.g., tricyclic antidepressants (TLPDs), selective serotonin reuptake inhibitors (SSRIs), selective norepinephrine reuptake inhibitors (SNRIs), receptor drugs, and MAO-A inhibitors. Fluoxetine and sertraline belong to the SSRIs, and in addition to the treatment of depression, they also find a number of other applications, including in the treatment of anxiety disorders, eating disorders, and alcoholism [1]. Fluoxetine and sertraline may also be administered as an adjunct to cancer pain management together with essential pain relievers [1–3]. Fluoxetine is a frequently prescribed antidepressant and is also used in bulimia nervosa, where it reduces bulimic and depressive symptoms [10]. Sertraline is indicated in the treatment of most depressive disorders and prevents them from coming back, as well as in the treatment of alcohol addiction [11].

The key aspect in the pharmacotherapy of depression is the selection of the type of active substance, as well as the dose of the drug for the existing disorders. Therefore, there

is a need to determine the concentrations of fluoxetine and sertraline in pharmaceutical preparations (ensuring the accuracy of dosing) or in biological material (effectiveness of therapy, dose matching to the patient's metabolism, and post-mortem examination excluding or confirming drug overdose). The development of analytical methods is particularly important, especially since the therapy is burdened with the risk of side effects. There are reports in scientific journals about the use of various analytical methods for the determination of antidepressants, including fluoxetine and sertraline. The tests were carried out on various types of samples, such as pharmaceutical preparations, whole blood, plasma, and others, using, among others, thin layer chromatography (TLC) or high performance thin layer chromatography (HPTLC) [12–22], high performance liquid chromatography (HPLC) [16,17,23–33], ultra-performance liquid chromatography (UPLC) [34–40], and gas chromatography—mass spectrometry (GC/MS) [41–47], in addition to spectrophotometry [48–50], spectrofluorimetry [51], capillary electrophoresis [52,53], differential impulse voltammetry [54,55], and ion mobility spectrometry [56]. Fluoxetine can metabolize to fluoride ions [57]. Fluoride ions may also be formed as a result of fluoxetine degradation [58,59]. The concentration of fluoride ions coming from fluoxetine was determined potentiometrically using a fluoride ion-specific electrode (Orion) and an Ag/AgCl reference electrode [57], by ion chromatography (IC) with an electrical conductivity detector [58], and by ^{19}F nuclear magnetic resonance spectroscopy (^{19}F NMR) [59].

The aim of this study was to propose a new validated thin layer chromatography method in combination with densitometry for the determination of sertraline and fluoxetine in the presence of their potential impurities. The developed chromatographic conditions were used for the qualitative and quantitative determination of sertraline and fluoxetine in pharmaceutical preparations.

2. Materials and Methods

2.1. Pharmaceutical Reference Standards, Drugs, and Chemicals

Fluoxetine hydrochloride and sertraline hydrochloride (European Pharmacopoeia Reference Standards; Sigma-Aldrich, St. Louis, MO, USA), were used as standards. Two drugs were tested: *Fluoxetin Polpharma* (POLPHARMA SA, Starogard Gdański, Poland) in capsules (20 mg of fluoxetine) and *Sertagen* (Mylan Hungary Kft., Komárom, Hungary) in tablets (50 mg of sertraline). All chemicals and reagents for the TLC method were analytical grade and were purchased from POCh (Gliwice, Poland).

2.2. Investigations of Selectivity of Method

Primary standard solutions of sertraline (S) and fluoxetine (F) at a concentration of 100 mg of drug in 100 mL of methanol were prepared. From these solutions were prepared:

- Solution I—acid hydrolysis—3 mL of sertraline (fluoxetine) primary solution was taken, 1 mL of methanol and 1 mL of 2 M NaOH were added;
- Solution II—alkaline hydrolysis—3 mL of sertraline (fluoxetine) primary solution was taken, 1 mL of methanol and 1 mL of 2M HCl were added;
- Solution III—3 mL of sertraline (fluoxetine) primary solution was taken, 1 mL of methanol and 1 mL of distilled water were added;
- Solution IV—oxidation—3 mL of the primary solution of sertraline (fluoxetine) were taken, 1 mL of methanol and 1 mL of 3% H_2O_2 were added;
- Solution V—3 mL of sertraline (fluoxetine) primary solution was taken, 1 mL of methanol and 1 mL of physiological saline (normal saline, 0.9% solution of sodium chloride) were added;
- Solution VI—3 mL of sertraline (fluoxetine) stock solution was taken and 2 mL of methanol was added.
- Solution VII—3 mL of sertraline (fluoxetine) stock solution was taken, 2 mL of methanol was added.
- Standard solutions—3 mL of sertraline (fluoxetine) stock solution was taken, 2 mL of methanol was added.

Solutions I–VI were heated at 70 °C for 2 hours on a TLC PLATE HEATER III heating plate (Camag). Solution VII was irradiated with a lamp (Cobrabid) for 2 hours with UV radiation with a wavelength of 254 nm.

For the chromatographic analysis, silica gel (TLC Silica gel 60 F₂₅₄) plates with dimensions of 10 × 20 cm (# 1.05570, E. Merck) were used. The test solutions were applied with the use of microcapillaries in the amount of 5 µL on the chromatographic plates which had been previously activated for 30 minutes in an incubator at the temperature of 120 °C. Analyses were performed using mobile phases listed in Table 1.

Table 1. Qualitative and quantitative composition (v/v) of the mobile phases used.

Mobile Phase Symbol	Composition of the Mobile Phase	Composition of the Solvents in Volume Ratio	Refs.
A	Toluene + ethyl acetate + ammonia	2: 10: 0.2	[18]
B	Chloroform + methanol	8.5: 1.5	-
C	Toluene + glacial acetic acid	4: 5	[19]
D	Toluene + ethyl acetate + methanol + glacial acetic acid	4.5: 1.5: 1: 0.5	[20]
E	Methanol + toluene	4: 2	[13]
G	Acetone + benzene + ammonia	50: 45: 5	[21]
G1	Acetone + toluene + ammonia	10: 9: 1	-
G2	Acetone + toluene + ammonia	9.5: 9.5: 1	-
G3	Acetone + toluene + ammonia	10.5: 8.5: 1	-
H	Methanol + toluene + ammonia	7: 3: 0.1	[12]
I	Toluene + ethyl acetate + ethanol + ammonia	8: 2: 0.5: 0.1	[15]
I1	Toluene + ethyl acetate + ethanol + glacial acetic acid	8: 2: 0.5: 0.4	-
J	Ethyl acetate + toluene + methanol + ammonia	4: 3: 1: 0.1	[22]
J1	Ethyl acetate + toluene + methanol + ammonia	4: 4: 1: 0.1	-
J2	Ethyl acetate + toluene + methanol + ammonia	5: 3: 1: 0.1	-
K	Carbon tetrachloride + methanol + acetone + ammonia	12: 3: 5: 0.1	[14]
L	Chloroform + methanol + glacial acetic acid	8.5: 1.5: 0.5	-
L1	Chloroform + methanol + glacial acetic acid	8.5: 1.5: 0.3	-
M	Acetone + chloroform + ammonia	10: 5: 1	-

Densitometric and spectrodensitometric analyses were performed using a CAMAG TLC3 densitometer. In the densitometric analysis, the wavelength range was 200–380 nm, at wavelength intervals of 30 nm at each step. A deuterium lamp and the slit dimensions 12.00 × 0.4 mm, Macro, were used. The scanning speed was 20 nm/s and the data resolution was 100 µm/step. In the spectrodensitometric analysis, the wavelength range was 200–400 nm. A deuterium lamp with the slit dimensions 12.00 × 0.4 mm, Macro, was used. Scanning speed was 20 nm/s and data resolution was 1 nm/step.

2.3. Preparation of Drug Samples

In order to prepare the drug solutions, the contents of one capsule of Fluoxetine Polpharma and one tablet of Sertagen were transferred to plastic containers, and 15 mL of methanol and 3 metal balls were added. The whole solution was mixed on the Ika® Ultra Turrax® Tube Drive at the frequency of 8000 rpm for 30 minutes for extraction. The fluoxetine solution (F) was filtered through a filter into a 25 mL volumetric flask and made up to the mark with methanol. Thus, a concentration of 4 mg/5 mL (solution F1) was obtained. From this solution, dilutions were made to obtain a solution F2 with a concentration of 1 mg/5 mL and a solution F3 with a concentration of 0.5 mg/5 mL. The sertraline (S) solution was filtered into a 50 mL volumetric flask and made up to the mark with methanol, and solution S1 with a concentration of 5 mg/5 mL was obtained. From the S1 solution the following solutions were obtained: S2 with a concentration of 3 mg/5 mL, S3 with a concentration of 1.5 mg/5 mL, and S4 with a concentration of 0.6 mg/5 mL.

2.4. Conditions for the Analysis of Drug Samples by TLC Combined with Densitometry

Aluminum plates precoated with silica gel 60F₂₅₄ (# 1.05570 and #1.05554) from Merck (Germany) were used for the chromatographic analysis. The plates were heated in the incubator for 30 min at 120 °C. To test the robustness of the method, 1.05554 plates were used. The separations were performed using the mobile phase M: acetone + chloroform + ammonia in a volume composition of 10: 5: 1. The plates were developed in a Camag chromatography chamber with dimensions of 20 cm × 20 cm × 10 cm. The dried plates were scanned using a Camag TLC Scanner 3.

The wavelength of 200 nm was found to be optimal for performing densitometric measurements of both fluoxetine and sertraline.

2.5. Linearity and Range

The linearity of the TLC method was evaluated by analysis of standard solutions of: fluoxetine at concentrations: 0.50, 0.60, 0.70, 0.80, 0.90, 1.00, 2.00, 3.00, 4.00 and 5.00 mg/5 mL; and sertraline at concentrations: 0.60, 0.80, 1.00, 1.25, 1.50, 2.00, 2.50, and 3.00 mg/5 mL.

2.6. Accuracy of the Method

The accuracy of the TLC method combined with the densitometric analysis was determined based on the recovery measurements. For this purpose, three drug solutions containing 20 mg of fluoxetine and 50 mg of sertraline were prepared, and then internal standards were added to them in the amount of 50, 100, and 150% fluoxetine and sertraline. The solutions prepared in this way were applied to the activated plate and subjected to chromatographic and densitometric analysis. The densitometric measurement was repeated three times for each solution, and the experimental values were compared with the theoretical ones. On this basis, the mean recovery value and the coefficient of variation were calculated.

2.7. Precision of the Method

The precision of the method was determined based on the analysis of the surface area of the chromatographic bands of the tested samples. Test solutions of fluoxetine (Fluoxetin Polpharma 20 mg) F1, F2, F3, and sertraline (Sertagen 50 mg) S2, S3, S4, were used. Three determinations on the first day were made to determine the intra-day precision. The inter-day precision was determined by taking two consecutive measurements on two consecutive days and on days falling exactly one week and two weeks from the first day of the determinations.

2.8. Limit of Quantification and Detection Limit of the Method

The limit of quantification and detection limit of fluoxetine and sertraline were determined by analyzing standard solutions of fluoxetine at concentrations: 0.9, 0.8, and 0.7 mg/5 mL; and sertraline at concentrations: 0.36, 0.24, and 0.12 mg/5 mL.

2.9. Robustness

The robustness of the method was tested according to guidelines described in the papers by Nagy-Turák, and Ferenczi-Fodor et al. [60–62]. The robustness of the method was checked by spotting sample solutions on the plate and developing the plate after altering the conditions (Table 2). The conditions changed were the sorbent type, the temperature of plate activation, extraction time, saturation time of the chamber, the volume of acetone in mobile phase, and the volume of chloroform in mobile phase, and the wavelength in densitometric analysis. The method conditions and the selected factors with the values of their (+) and (−) levels are summarized in Table 3. A high level is represented by “+” and a low level by “−”. The main effects of seven factors were tested on two levels in eight experiments [60,61]. The levels of factors investigated and the experimental design matrix (2³) are shown in Table 3. The means of calculation of the effects (*E*) characterizing the particular individual factors and rank probabilities [63] were previously presented [61,62].

Table 2. The factors and their levels investigated in robustness tests.

Symbol	Factors	Method Condition	Levels	
			+	–
X ₁	Sorbent type (Merck, #)	Al sheet (1.05570)	Al sheet (1.05570)	Al sheet (1.05554)
X ₂	Temperature of plate activation [°C]	120	130	110
X ₃	Extraction time [min]	30	32	28
X ₄	Saturation time of the chamber [°C]	30	35	25
X ₅	Volume of acetone [mL]	10.0	10.1	9.9
X ₆	Volume of chloroform [mL]	5.0	5.1	4.9
X ₇	Wavelength in densitometric analysis at λ [nm]	200	205	200

catalog number.

Table 3. Experimental design matrix (2³) for robustness tests for biological active substances in pharmaceutical preparations containing fluoxetine and sertraline.

Experiment No		X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	Biological Active Substance ^a Content (y _i) [mg·tablet ^{−1}]	
									F	S
1		+	+	+	+	+	+	+	18.92	48.94
2		+	+	–	+	–	–	–	18.50	48.61
3		+	–	+	–	–	+	–	19.08	49.34
4		+	–	–	–	+	–	+	19.51	50.60
5		–	+	+	–	+	–	–	19.33	49.80
6		–	+	–	–	–	+	+	19.03	49.21
7		–	–	+	+	–	–	+	18.97	49.07
8		–	–	–	+	+	+	–	19.70	50.93
Size of effect	F	−0.255	−0.370	−0.110	−0.215	0.470	0.105	−0.045		
	S	−0.380	−0.845	−0.547	−0.348	1.010	0.082	−0.215		
The label claim [mg]									20	50
Average amount [mg]									19.1	49.6
Variance									0.141	0.673
Standard deviation (SD)									0.376	0.821
Coefficient of variation [CV, %]									2.0	1.7

^a F—fluoxetine, S—sertraline.

3. Results and Discussion

Fluoxetine and sertraline are SSRI antidepressants and are used for a wide range of indications. Due to their widespread use and the need for strict pharmacological control of depression, pharmaceutical preparations in which they occur should be analyzed in terms of the actual content of the active substance. For this purpose, among other methods, thin layer chromatography combined with densitometry, as examined in this work, can be used.

3.1. Validation of TLC-Densitometric Method

The applied method was fully validated and the validation results are presented in Figures 1–7, Figure S1–S44 and in Tables 3–7, and in the following subsections.

3.1.1. Selectivity of the Method

Optimum chromatographic conditions were used to separate fluoxetine and sertraline from their potential impurities. Both drugs were stressed by irradiation, heating, oxidation,

pH change, and addition of physiological saline. Nineteen mobile phases (Figures 1 and 2, and Figures S1–S42) were investigated to find the optimal one that would allow separation of the greatest amount of degradation products of both drugs.

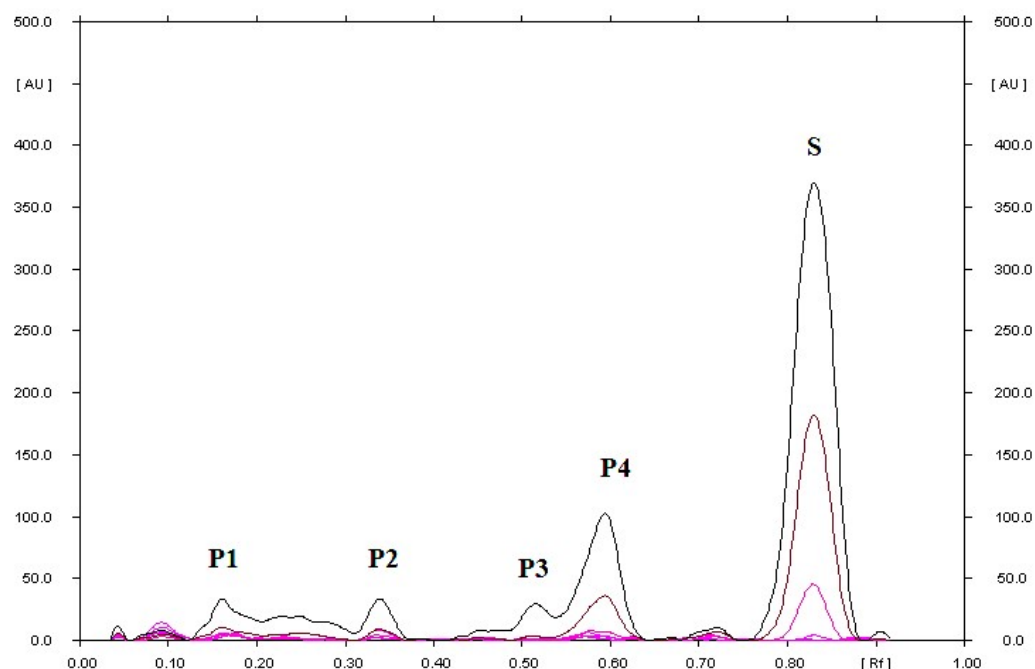


Figure 1. Densitogram of methanolic solution of sertraline, which after irradiation with UV radiation ($\lambda = 254$ nm) was separated on silica gel using a mobile phase M: acetone + chloroform + ammonia (10:5:1, $v/v/v$) (where: S—sertraline and P1, P2, P3, P4—degradation products of sertraline).

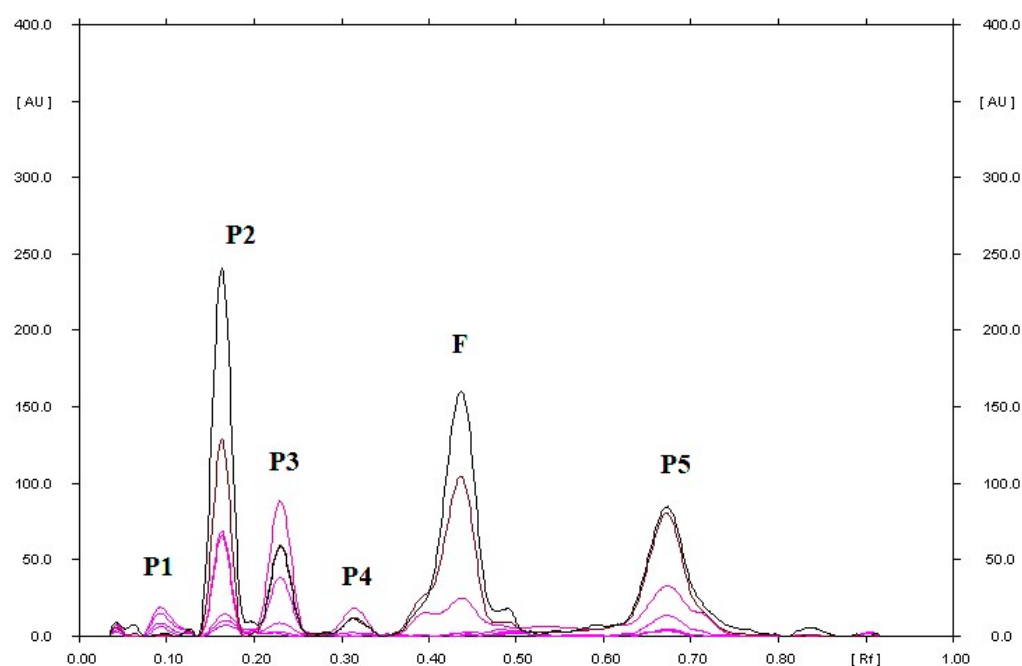


Figure 2. Densitogram of methanolic solution of fluoxetine, which after irradiation with UV radiation ($\lambda = 254$ nm) was separated on silica gel using a mobile phase M: acetone + chloroform + ammonia (10:5:1, $v/v/v$) (where: F—fluoxetine and P1, P2, P3, P4, P5—degradation products of fluoxetine).

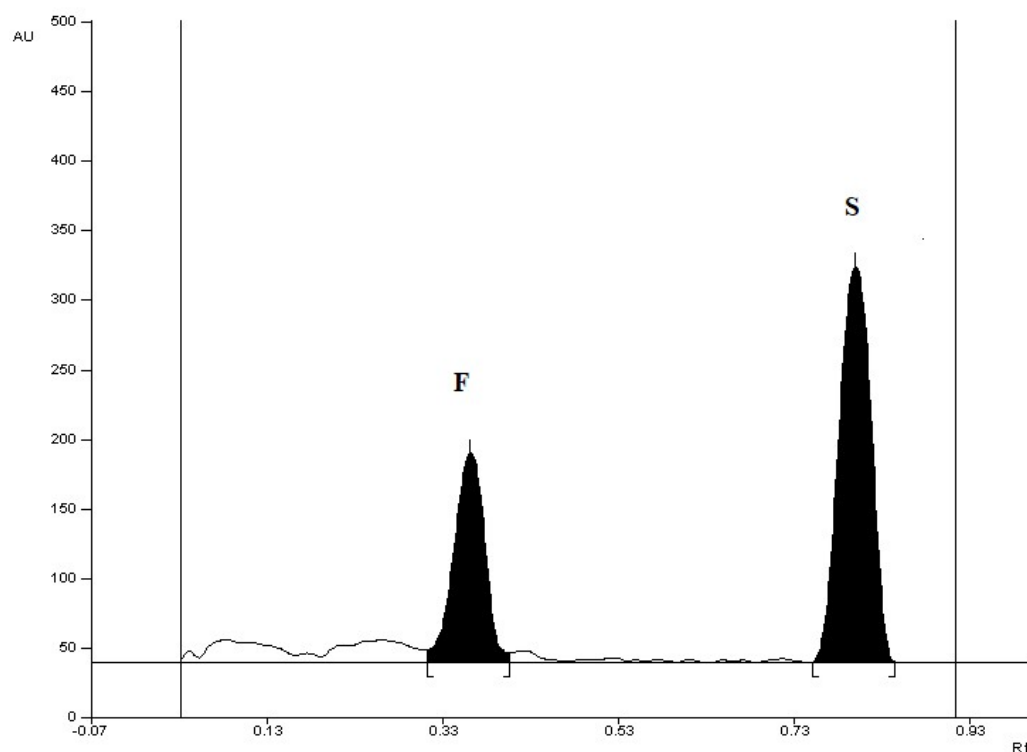


Figure 3. Densitogram of a mixture of drug standards: fluoxetine ($R_F = 0.42 \pm 0.05$) and sertraline ($R_F = 0.82 \pm 0.05$) (where F—fluoxetine and S—sertraline).

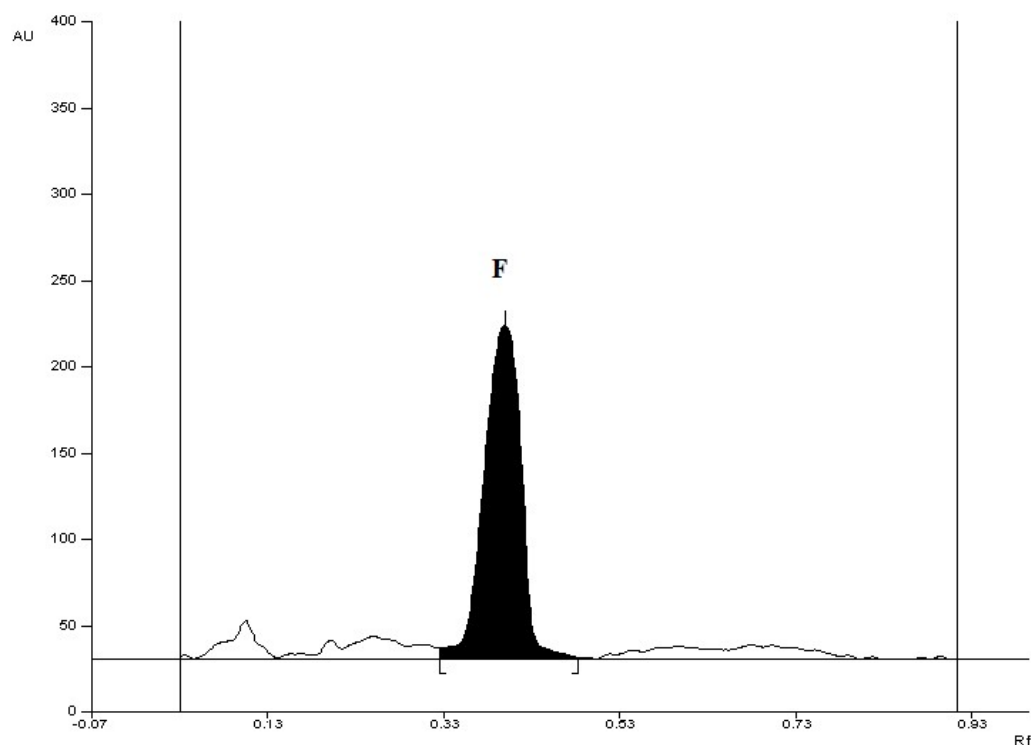


Figure 4. Densitogram of fluoxetine coming from the capsules ($R_F = 0.42 \pm 0.05$) (where F—fluoxetine and S—sertraline).

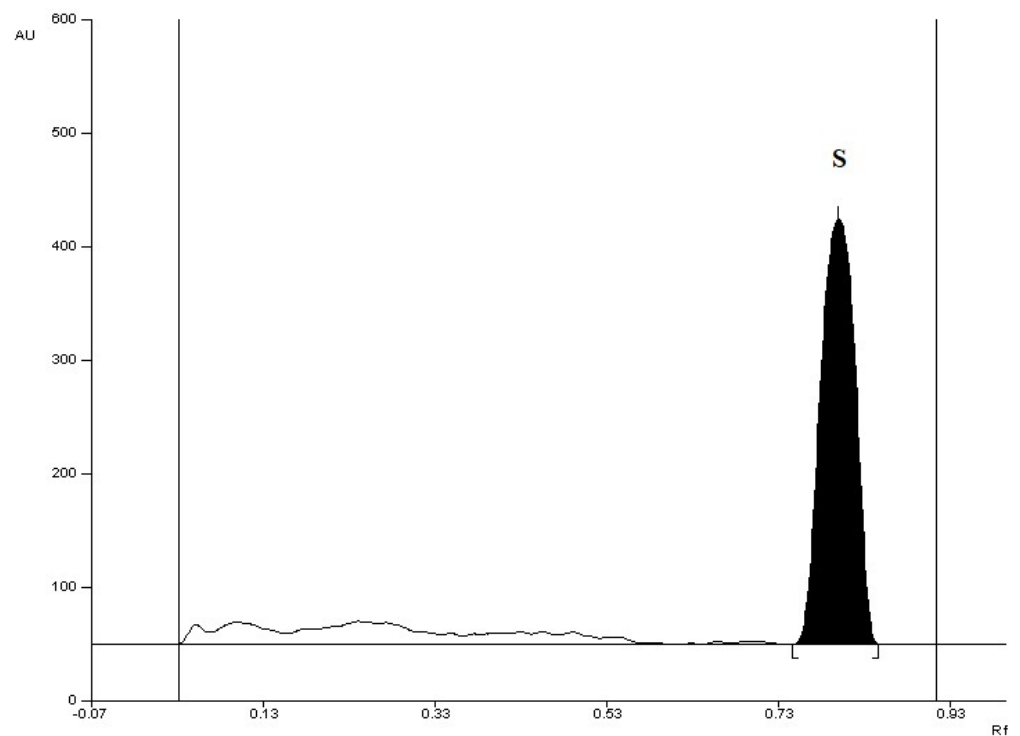


Figure 5. Densitogram of sertraline coming from the tablets ($R_F = 0.82 \pm 0.05$).

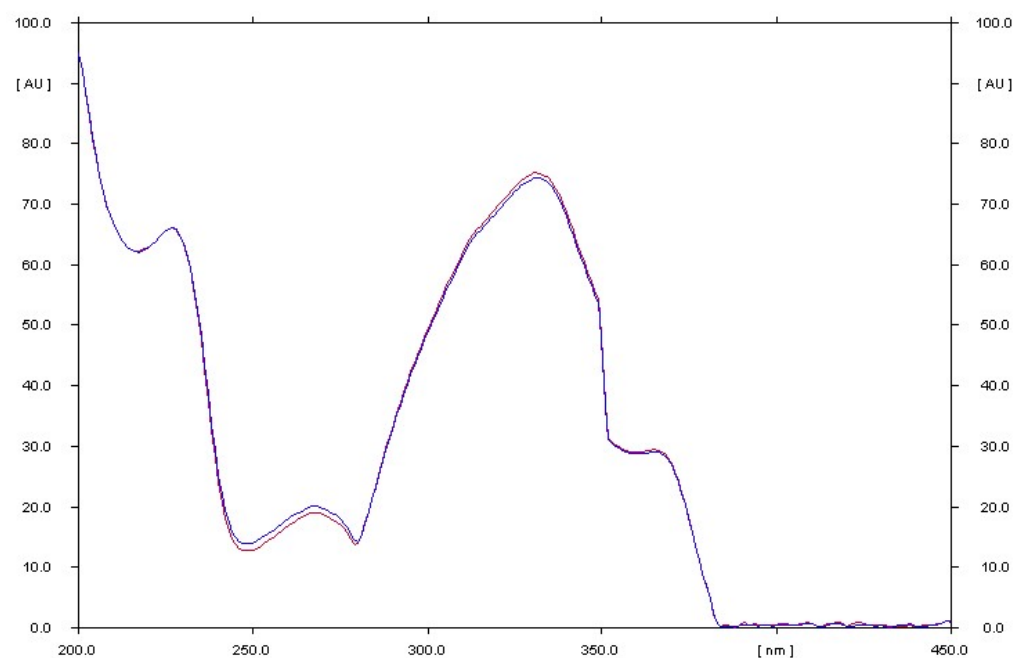


Figure 6. Comparison of the spectrodensitograms of the fluoxetine standard and fluoxetine from the pharmaceutical preparation.

Among the many phases used during the experiment, the mobile phase M was found to be the best, consisting of acetone: chloroform: ammonia in a volume ratio of 10: 5: 1. This is the author's mobile phase. It was proposed on the basis of the knowledge of the elution strength of the individual components of this mobile phase. The obtained peaks in the chromatograms, showing drug degradation products, were clearly separated (Table 4) and clearly visible (Figures 1 and 2, Figures S1, S2, S5, S7–S10, S12). Using the proposed M mobile phase, it was possible to separate the largest amount of degradation products of

fluoxetine and sertraline. The remaining phases were not as favorable as the peaks were unresolved or poorly visible, tailing occurred, and the spots were blurred (Figures S13–S42). The R_F values of sertraline and fluoxetine differ from the degradation products of fluoxetine and sertraline (Table 4). Therefore, the developed chromatographic conditions enable the simultaneous determination of fluoxetine and sertraline.

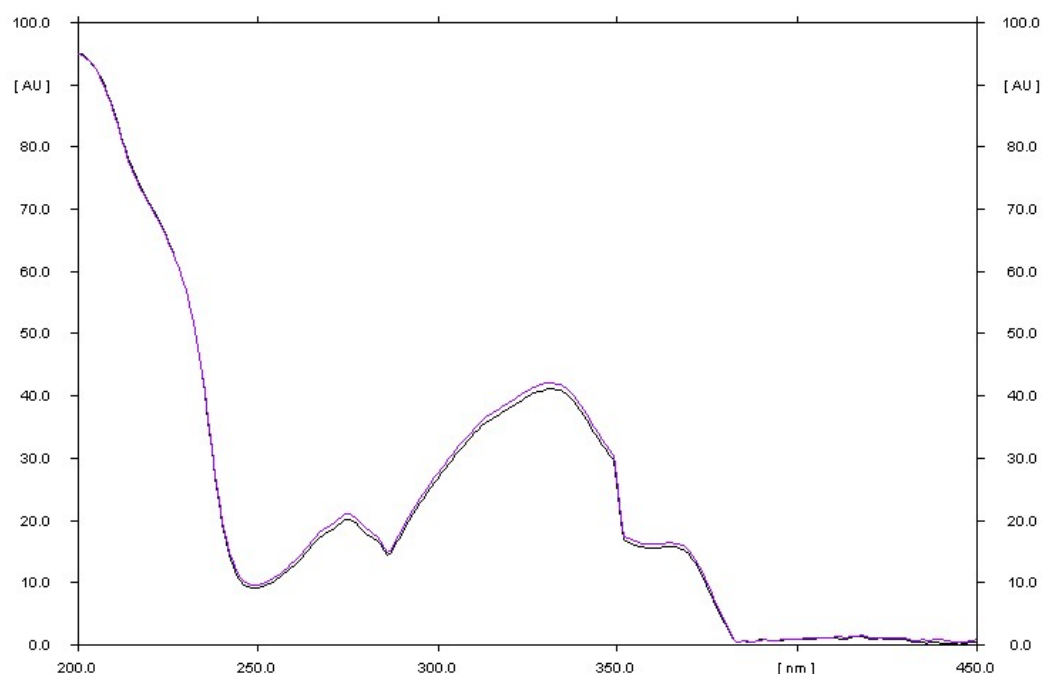


Figure 7. Comparison of the spectrodensitograms of the sertraline standard and sertraline from the pharmaceutical preparation.

Table 4. R_F values for sertraline (S), fluoxetine (F), and their degradation products (P) after analysis on silica gel using the acetone + chloroform + ammonia (10:5:1, v/v/v).

Stress Conditions	R_F	
	of Degradation Products of Sertraline (P) and R_F of Sertraline (S)	of Degradation Products of Fluoxetine (P) and R_F of Fluoxetine (F)
S or F with the addition of 2M NaOH heated at 70 °C for 2 h	0.04; 0.07 for P	0.04; 0.28 for P
	0.80 for S	0.39 for F
S or F with the addition of 2M HCl heated at 70 °C for 2 h	0.05; 0.19; 0.22; 0.68 for P	0.04; 0.63 for P
	0.79 for S	0.39 for F
S or F with the addition of H ₂ O heated at 70 °C for 2 h	0.78 for S	0.05; 0.17 for P
		0.38 for F
S or F with the addition of H ₂ O ₂ heated at 70 °C for 2 h	0.06; 0.91 for P	0.04; 0.12; 0.18; 0.82 for P
	0.78 for S	0.37 for F
S or F with the addition of physiological saline heated at 70 °C for 2 h	0.78 for S	0.40 for F
S or F in a methanolic solution, heated at 70 °C for 2 h	0.78 for S	0.04; 0.10; 0.90 for P
		0.38 for F
S or F in a methanolic solution irradiated with UV at $\lambda = 254$ nm for 2 h	0.16; 0.34; 0.52; 0.59 for P	0.10; 0.16; 0.23; 0.32; 0.69 for P
	0.83 for S	0.43 for F

Table 5. Method-validation data for the quantitative determination of fluoxetine by thin layer chromatography with densitometry.

Method Characteristic	Results
Retardation factor (R_F)	0.42 ± 0.05
Range [$\mu\text{g}/\text{spot}$]	0.5–5.0
Linearity [$\mu\text{g}/\text{spot}$]	$A = 1552.8 (\pm 31.7) + 1609.5 (\pm 13.2) \cdot x$ $n = 10; r = 0.9997; s = 63.8; F = 14802; p < 0.0001$
Limit of Detection (LOD) [$\mu\text{g}/\text{spot}$]	0.073
Limit of Quantification (LOQ) [$\mu\text{g}/\text{spot}$]	0.219
For capsules	
Accuracy	
for 50% fluoxetine added ($n = 6$)	$R = 97.2\%; CV = 0.88\%$
or 100% fluoxetine added ($n = 6$)	$R = 97.3\%; CV = 0.51\%$
for 150% fluoxetine added ($n = 6$)	$R = 99.2\%; CV = 1.57\%$
Precision (CV, [%])	
Intra-day	
for 0.5 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.68
for 1.0 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.69
for 4.0 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.17
Inter-day	
for 0.5 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.96
for 1.0 ($\mu\text{g}/\text{spot}$) ($n = 3$)	2.11
for 4.0 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.31
Robustness (CV, [%])	robust

Table 6. Method-validation data for the quantitative determination of sertraline by thin layer chromatography with densitometry.

Method Characteristic	Results
Retardation factor (R_F)	0.82 ± 0.05
Range [$\mu\text{g}/\text{spot}$]	0.5–3.0
Linearity [$\mu\text{g}/\text{spot}$]	$A = 2727.3 (\pm 77.6) + 3301.2 (\pm 43.8) \cdot x$ $n = 8; r = 0.9995; s = 98.6; F = 5676; p < 0.0001$
Limit of Detection (LOD) [$\mu\text{g}/\text{spot}$]	0.054
Limit of Quantification (LOQ) [$\mu\text{g}/\text{spot}$]	0.162
For tablets	
Accuracy	
for 50% sertraline added ($n = 6$)	$R = 99.9\%; CV = 1.19\%$
for 100% sertraline added ($n = 6$)	$R = 100.0\%; CV = 1.20\%$
for 150% sertraline added ($n = 6$)	$R = 99.7\%; CV = 0.91\%$
Precision (CV, [%])	
Intra-day	
for 0.6 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.12
for 1.5 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.26
for 3.0 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.20
Inter-day	
for 0.6 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.62
for 1.5 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.51
for 3.0 ($\mu\text{g}/\text{spot}$) ($n = 3$)	1.99
Robustness (CV, [%])	robust

Table 7. The results of the quantification determination of fluoxetine in the preparation Fluoxetine Polpharma and sertraline in the preparation Sertagen.

	Fluoxetin Polpharma (Fluoxetine, Capsules 20 mg)	Sertagen (Sertraline, Tablets 50 mg)
Number of analyzes	9	9
The drug content in a capsule/tablet declared by the manufacturer [mg]	20	50
Average drug content in capsule/ tablet [mg]	19.45	50.51
Minimum [mg]	19.03	49.54
Maximum [mg]	19.85	51.12
Variance (s^2)	0.098	0.324
Standard deviation (SD)	0.31	0.57
Coefficient of variation (CV) [%]	1.6	1.1
Confidence interval of the arithmetic mean with a confidence level of 95%	$\mu = 19.45 \pm 0.23$	$\mu = 50.51 \pm 0.43$
The content of the drug in relation to that declared by the manufacturer	97.2%	101.0%

Fluoxetine and sertraline changed the most after 2 hours of irradiation of methanolic solutions with UV radiation with a wavelength of 254 nm. Under these conditions, the most degradation products were formed that were separated from the drug using the M mobile phase (Figures 1 and 2). Five bands were observed for sertraline and six bands for fluoxetine. The percentage of peak areas of both drugs under these conditions was the lowest and was 75.21% and 30.33% for sertraline and fluoxetine, respectively.

Separation of sertraline from its degradation products using the mobile phase acetone + chloroform + ammonia (10:5:1, $v/v/v$) was also observed in methanolic drug solutions with the addition of 2M sodium hydroxide (Figure S1), 2M hydrochloric acid (Figure S2), and 3% hydrogen peroxide (Figure S5) which were heated at 70 °C for 2 hours. Under alkaline conditions, the percentage of the peak area of sertraline was 82.49%, and two degradation products were formed with a total peak area of 17.51%. Under acidic conditions, the percentage of the peak area of sertraline was 76.25%, and four degradation products were formed with a total peak area of 23.75%. However, after the addition of 3% hydrogen peroxide, the peak area percent of sertraline was 89.75%, and two degradation products were formed with a total peak area of 10.25%. Sertraline in methanolic solution (Figure S3) and in methanol solutions with the addition of distilled water (Figure S4) and physiological saline (Figure S6) did not undergo any chemical changes (Table 4). Under these conditions, there were no visible peaks on the densitogram to indicate the presence of degradation products. The obtained results can be compared to the HPTLC stability studies of sertraline carried out in previous years. Namely, Rao J. et al. [15] used HPTLC plates precoated with silica gel 60F₂₅₄ as the stationary phase, while the mobile phase consisted of toluene, ethyl acetate, ethanol, and ammonia in a volume ratio of 8: 2: 0.5: 0.1. In this study, to test the chemical stability of sertraline, TLC plates precoated with silica gel 60F₂₅₄ were also used, and the mobile phase used in the study by Rao J. et al. [15], which was marked with the letter I; however, this did not produce favorable results, because there was no separation of degradation products from sertraline (degradation substances remained immediately above the starting point). Rao J. et al. [15] showed that sertraline was degraded in an acidic environment (one degradation product was formed), in an alkaline environment (two degradation products were formed), after oxidation (two degradation products were formed), and after water hydrolysis (one degradation product was formed). The separation of sertraline from its degradation products using the M mobile phase in the presented work took place under similar stress conditions. However, in the acidic environment, four degradation products were formed; in the alkaline environment, two; after oxidation, two; and after adding water, no drug decomposition was observed. Comparing the results of both sertraline stability studies, it can be seen that the drug is not stable in acidic, alkaline, or oxidized environments. Moreover, the mobile phase consisting of acetone, chloroform,

and ammonia in a volume composition of 10: 5: 1 leads to a better separation of degradation products from sertraline than the mobile phase consisting of toluene, ethyl acetate, ethanol, and ammonia in a volume composition of 8:2:0.5:0.1. Hussain et al. [18] described the examination of the stability of sertraline by the HPTLC method. The stationary phase comprised TLC plates precoated with silica gel 60F₂₅₄, and the mobile phase consisted of toluene, ethyl acetate, and ammonia in a volume ratio of 2: 10: 0.2. This mobile phase was also used in this work as mobile phase A (Figure S13); however, it did not allow all degradation products to be separated from the drug. The M mobile phase allows for a better separation of the degradation products of sertraline. In a study by Hussain et al. [18], concentrated HCl, 10M NaOH, and H₂O₂ were added to methanolic solutions of sertraline and heated for 2 hours at 70 °C. The photochemical stability of the drug was also tested by irradiating the sample with UV radiation. In the acidic environment, three degradation products were formed, in the alkaline environment one degradation product was formed, and after oxidation and after exposure to UV radiation, one degradation product was also created. In the study from this work (Table 4), in the acidic environment and after UV irradiation, four and five degradation products were formed, respectively; in the alkaline environment and after oxidation, two degradation products were formed, which were separated from sertraline using the mobile phase M. Comparing the results from both studies, it can be observed that this drug is degraded under similar conditions and that the mobile phase consisting of acetone, chloroform, and ammonia in a volume ratio of 10: 5: 1 allows the separation of more degradation products from sertraline than the mobile phase consisting of toluene, ethyl acetate, and ammonia in a volume ratio of 2: 10: 0.2.

Separation of fluoxetine from its degradation products using the M mobile phase was observed not only in the solution irradiated with UV radiation, but also after adding to the methanolic solution of the drug, sodium hydroxide, hydrochloric acid, distilled water, and 3% hydrogen peroxide, and after heating for 2 hours at 70 °C (Figures S7–S10, S12, Table 4). Under alkaline conditions, the peak area percent for fluoxetine was 84.62%, and two degradation products were formed with a total peak area of 15.38%. Under acidic conditions, the peak area percent for fluoxetine was 76.87%, and two degradation products were formed with a total peak area of 23.13%. After the addition of distilled water, the peak area percent for fluoxetine was 82.18%, and two degradation products were formed with a total peak area of 17.82%. After the drug was oxidized by the addition of 3% hydrogen peroxide, the peak area percentage of fluoxetine was 68.6%, and four degradation products were formed with a total peak area of 31.4%. By heating a methanolic drug solution at 70 °C, the peak area percent of fluoxetine was 90.47%, and three degradation products were formed with a total peak area of 9.53%. Fluoxetine did not degrade in a physiological saline environment (Figure S11). No additional peaks were observed in the densitogram indicating the formation of degradation products. In a study of the stability of fluoxetine using the HPTLC method, as described in the publication of Shah et al. [13], the silica gel 60F₂₅₄ plates were used as the stationary phase, and the mobile phase methanol + toluene (4: 2, *v/v*), which was also used and denoted by the letter E in this study (Figure S32). Shah et al. [13] found that fluoxetine was degraded in acidic and alkaline environments and after oxidation to form one degradation product, while no changes were observed after heating and UV irradiation. In contrast, in this fluoxetine stability study (Table 4), the drug was degraded after the addition of 2M HCl, 2M NaOH, and 3% H₂O₂ and heating at 70 °C for 2 hours, and after irradiation with UV radiation at 254 nm for 2 hours. The mobile phase M, consisting of acetone, chloroform, and ammonia in the volume composition of 10: 5: 1, allowed the separation of two degradation products from fluoxetine in an acidic environment; also, two degradation products in the alkaline environment, four degradation products were observed after oxidation; and after irradiating the solution with UV radiation, five degradation products were visible on the densitogram. Comparing the results of both fluoxetine stability studies, it can be observed that the mobile phase M allows a better separation of degradation products from the drug than the mobile phase, which consists of methanol and toluene in a volume composition of 4: 2.

In this study, a mobile phase composed of toluene and glacial acetic acid in a volume ratio of 4: 5 was also used, which was marked with the symbol C (Figure S30). This mobile phase was previously used by Mennickent et al. [19] for the determination of fluoxetine in human serum by HPTLC. This method separated fluoxetine, imipramine, and norfluoxetine as related substances from fluoxetine. Comparing the results of both studies on the stability of fluoxetine, it can be observed that the mobile phase M allows better separation of degradation products from the drug than the mobile phase, which consists of toluene and glacial acetic acid in a volume composition of 4: 5.

Sertraline was found to be more stable than fluoxetine. The mobile phase M, consisting of acetone, chloroform, and ammonia with a volume ratio of 10: 5: 1, made it possible to separate the most degradation products from all nineteen used in the study of mobile phases. Therefore, this mobile phase was selected as optimal for the analysis of sertraline and fluoxetine in pharmaceutical formulations. In the course of the study, the values of the retardation coefficient R_F for the standards of both drugs were determined. The retardation coefficient for fluoxetine was $0.42(\pm 0.05)$ and for sertraline the value was $0.82(\pm 0.05)$ (Figure 3). The used chromatographic conditions allow the simultaneous determination of fluoxetine and sertraline, as the degradation products of both drugs are clearly separated. This is evidenced by other values of the coefficient R_F of fluoxetine and sertraline and their degradation products (Table 4).

The calculated values of the retardation coefficient R_F and the obtained spectrodensitograms allowed for the qualitative identification of fluoxetine and sertraline in pharmaceutical formulations (Figures 4–7). Moreover, the applied TLC method is highly selective and enables not only qualitative but also quantitative analysis of the investigated drugs in pharmaceutical preparations—fluoxetine in capsules and sertraline in tablets.

It was observed that excipients present in the formulation did not interfere with the fluoxetine (Figure 4) or with the sertraline peaks (Figure 5). The peak purities of fluoxetine from *Fluoxetin Polpharma* (POLPHARMA SA, Starogard Gdański, Poland) and sertraline from *Sertagen* (Mylan Hungary Kft., Komárom, Hungary) were also assessed by comparing the spectra obtained from fluoxetine and sertraline standards at the peak start (S), peak apex (M), and peak end (E) of the spot (Figures 6 and 7). It was found that $r(S,M) > 0.996$, and $r(M,E) > 0.990$ for all of the analyses performed by the TLC-densitometric technique. It should be stated that TLC combined with densitometry is highly selective for the determination of fluoxetine and sertraline in capsules and tablets, respectively.

3.1.2. Linearity and Range

The linearity of the method was established on the basis of the area measurements of the chromatographic bands. On the basis of the obtained results, calibration curves were prepared showing the dependence of the area of the spot [AU] on the amount of the drug [$\mu\text{g}/\text{spot}$] for fluoxetine and sertraline, respectively. The linear range of the assay for fluoxetine is 0.5–5.0 $\mu\text{g}/\text{spot}$ (Table 5, Figure S43A), whereas for sertraline analysis it is 0.6–3.0 $\mu\text{g}/\text{spot}$ (Table 6, Figure S44A). The differences between the real area of the band and the area calculated from the calibration curve equations for fluoxetine and sertraline are shown in Figures S43B and S44B, respectively. It can be observed that the residuals were distributed above and below the zero residual lines, thus confirming the linearity of proposed TLC methods for determination of fluoxetine and sertraline in pharmaceutical preparations.

3.1.3. Accuracy

The accuracy of the used method was established on the basis of the recovery value by calculating the mean value of recovery R [%] and the coefficient of variation CV [%]. The mean recovery values for fluoxetine ranged from 97.2% to 99.2%, and for sertraline it ranged from 99.7% to 100.0%. The coefficients of variation calculated were less than 2% and 1.5% for fluoxetine and sertraline, respectively. On the basis of the above values, it can

be concluded that the TLC method combined with densitometry is characterized by high measurement accuracy.

3.1.4. Precision

Based on the analysis of the area measurements of the chromatographic bands of fluoxetine and sertraline, the precision of the method was determined by calculating the coefficient of variation. The coefficient of variation for the intra-day precision of fluoxetine ranged from 1.17% to 1.69%, while for sertraline it ranged from 1.12% to 1.26%. The coefficient of variation calculated for the inter-day precision for fluoxetine ranged from 1.31% to 2.11%, and for sertraline from 1.51% to 1.99%. The obtained coefficients of variation for intra-day and inter-day precision do not exceed the value of 3%. On this basis, the applied analytical method can be described as highly precise.

3.1.5. Limit of the Quantification and Limit of the Detection of the Method

The limits of detection and quantification were 0.073 µg/spot and 0.219 µg/spot for fluoxetine, and 0.054 µg/spot and 0.162 µg/spot for sertraline. The proposed method is characterized by low LOD and LOQ for the determination of fluoxetine and sertraline, which confirm the sensitivity of the proposed method. The proposed TLC-densitometric method for the determination of fluoxetine and sertraline in pharmaceutical preparations is more sensitive than HPTLC method [13,15] and less sensitive than the HPLC [16,17,29–32], spectrophotometric [48–50], and spectrofluorimetric [51] methods.

3.1.6. Robustness

The main effects of seven factors were tested on two levels in eight experiments (Table 2). Table 3 shows the results obtained for fluoxetine and sertraline content (y_i) in commercial pharmaceutical preparations. These results show that no factor has a significant effect on the results. These results were also evaluated by half-normal probability plotting of rank probabilities (p_i) as a function of the absolute values of the main effects. The effects of factors, and the half-normal probability plot of effects for the determination of fluoxetine and sertraline in commercial pharmaceutical preparations, are presented in Figures 8 and 9, respectively. The points of all factors lie near the straight line, which indicates that their effect is negligible ($R^2 \geq 0.9552$). Therefore, the presented TLC-densitometric method can be regarded as robust. The standard deviation of fluoxetine and sertraline content (y_i) in commercial pharmaceutical preparations for seven parameters, which were changed in the conducted experiment in order to check the robustness of the applied method, is 2.0% and 1.7% for F and S, respectively. The value of % CV (<3) indicates the reliability of the proposed TLC-densitometric method during its normal use. However, the content of the ammonia in the mobile phase must be constant.

3.2. Quantitative Determination of Fluoxetine and Sertraline in Tested Pharmaceutical Preparations

The quantitative evaluation of drugs in the preparations was made on the basis of the chromatographic band measurements of the tested solutions of Fluoxetin Polpharma (fluoxetine 20 mg) and Sertagen (sertraline 50 mg). The equations of the calibration curves of fluoxetine (Table 5, Figure S43A) and sertraline (Table 6, Figure S44A) were used for the calculations. Table 7 shows the results of the quantification determination of fluoxetine in capsules and sertraline in tablets with the statistical description.

As a result of the measurements performed using the TLC method combined with densitometry, the real content of active substances was determined in relation to those declared by the manufacturer. The fluoxetine content in Fluoxetin Polpharma capsules was 97.2%. The content of sertraline in tablets Sertagen was 101.0%. According to the European Pharmacopoeia VIII, the deviation from the substance content in hard capsules and coated tablets is acceptable in the range of 85–115%. The American Pharmacopoeia 34 gives the range of acceptable deviation in the content of 90–110% for fluoxetine in capsules

and sertraline in tablets. The obtained results are within the standards specified in both Pharmacopoeias [64,65].

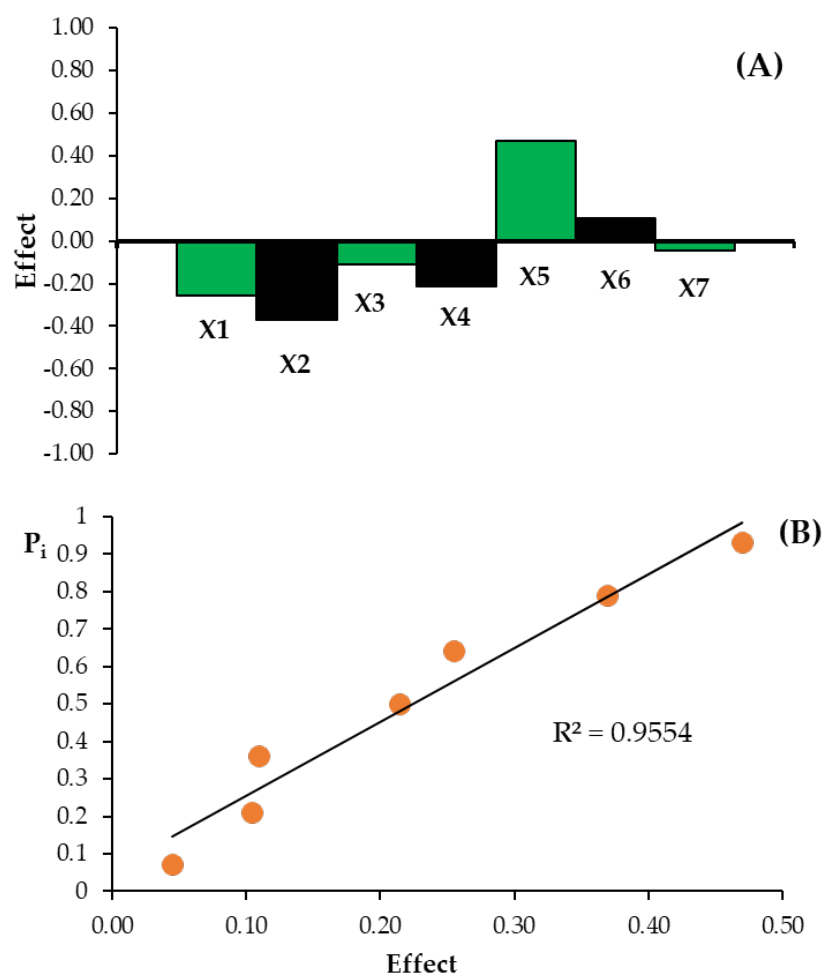


Figure 8. Robustness test: the effects of factors (A), and half-normal probability plot of effects (B) for determination of fluoxetine in commercial pharmaceutical capsules (Fluoxetin Polpharma).

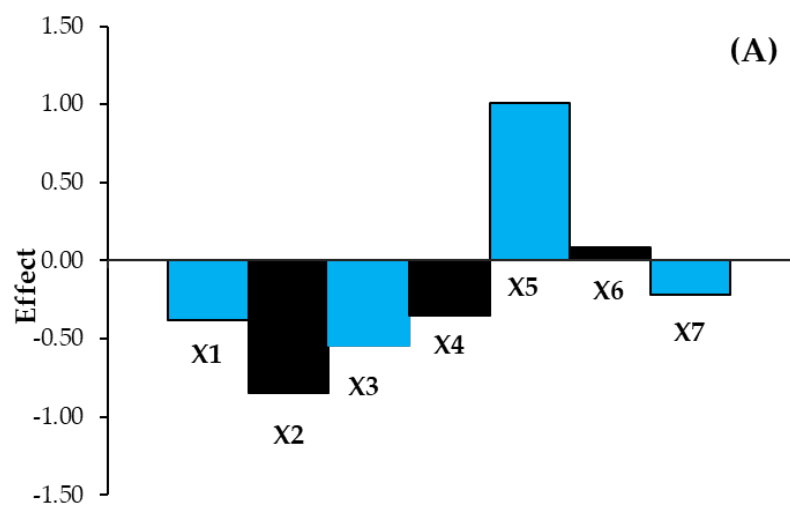


Figure 9. Cont.

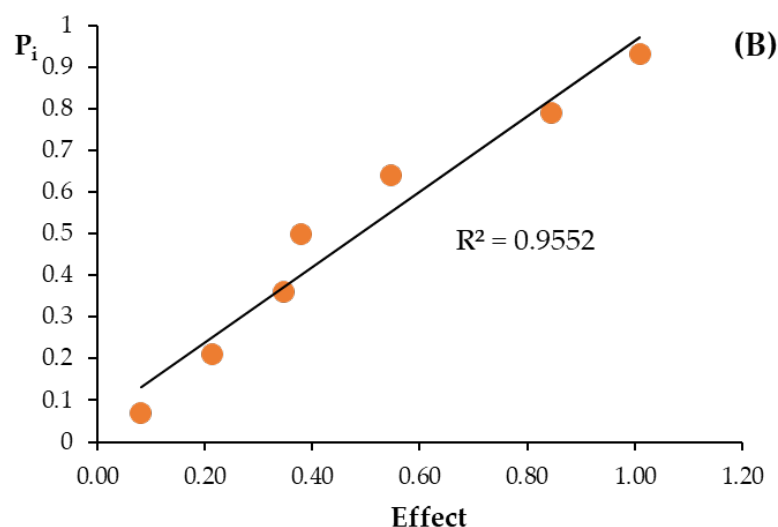


Figure 9. Robustness test: the effects of factors (A), and half-normal probability plot of effects (B) for determination of sertraline in commercial pharmaceutical tablets (Sertagen).

4. Conclusions

The applied TLC method combined with densitometry is selective, accurate, precise, and robust in the determination of fluoxetine and sertraline in pharmaceutical preparations. The identity of the active substance can be confirmed on the basis of the compliance of the R_F value and the spectrodensitograms of the substance coming from the pharmaceutical preparation and the standard of fluoxetine or sertraline. The linearity of the analytical method used for fluoxetine was achieved in the range from 0.5 to 5.0 $\mu\text{g}/\text{spot}$. For sertraline, linearity was found in the range from 0.6 to 3.0 $\mu\text{g}/\text{spot}$. The experimentally determined content of fluoxetine in hard capsules and sertraline in tablets meets the requirements for the content of the active substance of both the European Pharmacopoeia and the US Pharmacopoeia. It could be said that the developed TLC-densitometric method can be used for the routine simultaneous analysis of fluoxetine and sertraline in pharmaceutical formulations. This method is suitable for analyzing fluoxetine and sertraline in pharmaceutical preparations without any interferences from additives present in the pharmaceutical product. As elaborated in this work, the TLC-densitometric method for the determination of fluoxetine and sertraline in pharmaceutical preparations is more sensitive than HPTLC [13,15] method and less sensitive than the HPLC [16,17,29–32], spectrophotometric [48–50], and spectrofluorimetric [51] methods. The TLC-densitometric method can be used as a substitute method for the assay of fluoxetine and sertraline in pharmaceutical formulations, for example, in the case when HPLC is not available in the laboratory or to confirm results obtained with other analytical techniques (for example, spectrophotometric and spectrofluorimetric methods).

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/pr10102083/s1>. Figure S1: Densitogram of sertraline in alkaline solution, which after heating was separated on silica gel using the mobile phase M (where: S—sertraline and P1, P2—degradation products of sertraline). Figure S2: Densitogram of sertraline in acidic solution, which after heating was separated on silica gel using the mobile phase M (where: S—sertraline and P1, P2, P3, P4—degradation products of sertraline). Figure S3: Densitogram of methanolic solution of sertraline, which after heating was separated on silica gel using the mobile phase M (where: S—sertraline). Figure S4: Densitogram of methanolic solution of sertraline with the addition of H_2O , which after heating was separated on silica gel using the mobile phase M (where: S—sertraline). Figure S5: Densitogram of methanolic solution of sertraline with the addition of H_2O_2 , which after heating was separated on silica gel using the mobile phase M (where: S—sertraline and P1, P2—degradation products of sertraline). Figure S6: Densitogram of methanolic solution of sertraline with the addition of physiological saline, which after heating was separated on silica

[illegible]

J1. Figure S39: Densitogram of methanolic solution of fluoxetine, which after irradiation with UV light ($\lambda = 254$ nm) was separated on silica gel using a mobile phase J2. Figure S40: Densitogram of methanolic solution of fluoxetine, which after irradiation with UV light ($\lambda = 254$ nm) was separated on silica gel using a mobile phase K. Figure S41: Densitogram of methanolic solution of fluoxetine, which after irradiation with UV light ($\lambda = 254$ nm) was separated on silica gel using a mobile phase L. Figure S42: Densitogram of methanolic solution of fluoxetine, which after irradiation with UV light ($\lambda = 254$ nm) was separated on silica gel using a mobile phase L1. Figure S43: Calibration plot (A) and plot of residuals (B) for fluoxetine in the linear working range mobile phase M: acetone + chloroform + ammonia (10:5:1, v/v/v). Figure S44: Calibration plot (A) and plot of residuals (B) for sertraline in the linear working range mobile phase M: acetone + chloroform + ammonia (10:5:1, v/v/v).

Funding: This research was funded by Medical University of Silesia grant number PCN-1-040/K/2/F. The APC was funded by MDPI.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The author declares that there is no conflict of interests regarding the publication of this paper.

Sample Availability: Samples of the compounds are available from the author.

References

1. Katzung, B.; Trevor, A. *Basic and Clinical Pharmacology 15e*; McGraw-Hill Education/Medical: New York, NY, USA, 2021.
2. Kubanek, A.; Paul, P.; Przybylak, M.; Kanclerz, K.; Rojek, J.J.; Renke, M.; Bidzan, L.; Grabowski, J. Use of sertraline in hemodialysis patients. *Medicina* **2021**, *57*, 949. [\[CrossRef\]](#) [\[PubMed\]](#)
3. Miziak, B.; Błaszczyk, B.; Czuczwar, S.J. Some candidate drugs for pharmacotherapy of Alzheimer's disease. *Pharmaceuticals* **2021**, *14*, 458. [\[CrossRef\]](#)
4. Romero-Martínez, Á.; Murciano-Martí, S.; Moya-Albiol, L. Is sertraline a good pharmacological strategy to control anger? Results of a systematic review. *Behav. Sci.* **2019**, *9*, 57. [\[CrossRef\]](#)
5. Yue, J.K.; Burke, J.F.; Upadhyayula, P.S.; Winkler, E.A.; Deng, H.; Robinson, C.K.; Pirracchio, R.; Suen, C.G.; Sharma, S.; Ferguson, A.R.; et al. Selective serotonin reuptake inhibitors for treating neurocognitive and neuropsychiatric disorders following traumatic brain injury: An evaluation of current evidence. *Brain Sci.* **2017**, *7*, 93. [\[CrossRef\]](#) [\[PubMed\]](#)
6. Ramírez-Rodríguez, G.B.; Vega-Rivera, N.M.; Meneses-San Juan, D.; Ortiz-López, L.; Estrada-Camarena, E.M.; Flores-Ramos, M. Short daily exposure to environmental enrichment, fluoxetine, or their combination reverses deterioration of the coat and anhedonia behaviors with differential effects on hippocampal neurogenesis in chronically stressed mice. *Int. J. Mol. Sci.* **2021**, *22*, 10976. [\[CrossRef\]](#)
7. Zorzin, S.; Corsi, A.; Ciarpella, F.; Bottani, E.; Dolci, S.; Malpeli, G.; Pino, A.; Amenta, A.; Fumagalli, G.F.; Chiamulera, C.; et al. Environmental enrichment induces meningeal niche remodeling through TrkB-mediated signaling. *Int. J. Mol. Sci.* **2021**, *22*, 10657. [\[CrossRef\]](#) [\[PubMed\]](#)
8. Ahmed, H.; Khan, M.A.; Kahlert, U.D.; Niemelä, M.; Hänggi, D.; Chaudhry, S.R.; Muhammad, S. Role of adaptor protein myeloid differentiation 88 (MyD88) in post-subarachnoid hemorrhage inflammation: A systematic review. *Int. J. Mol. Sci.* **2021**, *22*, 4185. [\[CrossRef\]](#) [\[PubMed\]](#)
9. Dornquast, C.; Tomzik, J.; Reinhold, T.; Walle, M.; Mönter, N.; Berghöfer, A. To what extent are psychiatrists aware of the comorbid somatic illnesses of their patients with serious mental illnesses?—A cross-sectional secondary data analysis. *BMC Health Serv. Res.* **2017**, *17*, 162. [\[CrossRef\]](#)
10. Rajewski, A.; Komorowska-Pietrzykowska, R.; Rybakowski, F.P. Buspirone vs. fluoxetine in treatment of bulimia nervosa. *Eur. Neuropsychopharmacol.* **2005**, *15*, S601. [\[CrossRef\]](#)
11. McRae, A.L.; Brady, K.T. Review of sertraline and its clinical applications in psychiatric disorders. *Expert Opin. Pharmacother.* **2001**, *2*, 883–892. [\[CrossRef\]](#)
12. Tantawy, M.A.; Hassan, N.Y.; Elragehy, N.A.; Abdelkawy, M. Simultaneous determination of olanzapine and fluoxetine hydrochloride in capsules by spectrophotometry, TLC-spectrodensitometry and HPLC. *J. Adv. Res.* **2013**, *4*, 173–180. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Shah, C.R.; Suhagia, B.N.; Shah, N.J.; Patel, D.R.; Patel, N.M. Stability-indicating simultaneous HPTLC method for olanzapine and fluoxetine in combined tablet dosage form. *Indian J. Pharm. Sci.* **2008**, *70*, 251–255. [\[CrossRef\]](#) [\[PubMed\]](#)
14. Venkateswarlu, K.; Venisetty, R.K.; Yellu, N.R.; Keshetty, S.; Pai, M.G. Development of HPTLC-UV absorption densitometry method for the analysis of alprazolam and sertraline in combination and its application in the evaluation of marketed preparations. *J. Chromatogr. Sci.* **2007**, *45*, 537–539. [\[CrossRef\]](#) [\[PubMed\]](#)

15. Rao, J.; Kumar, M.; Sathiyarayanan, L.; Yadav, S.; Yadav, V. Application of a stability-indicating HPTLC method for quantitative analysis of sertraline hydrochloride in pharmaceutical dosage forms. *JPC-J. Planar Chromatogr.-Mod. TLC* **2011**, *24*, 140–144. [[CrossRef](#)]
16. Bhushan, R.; Agarwal, C. High-performance liquid chromatographic enantioseparation of (R,S)-fluoxetine using Marfey's reagent and (S)-N-(4-nitrophenoxycarbonyl) phenylalanine methoxyethyl ester as chiral derivatizing reagents along with direct thin-layer chromatographic resolution and isolation of enantiomers using L-tartaric acid as mobile phase additive. *Biomed. Chromatogr.* **2010**, *24*, 1152–1158. [[CrossRef](#)]
17. Patel, S.; Patel, N.J. Simultaneous RP-HPLC and HPTLC estimation of fluoxetine hydrochloride and olanzapine in tablet dosage forms. *Indian J. Pharm. Sci.* **2009**, *71*, 477–480. [[CrossRef](#)]
18. Hussain, A.; Rahman, A.; Hussain, S.; Mirza, M.A.; Iqbal, Z.; Harwansh, R.; Singh, L.R. HPTLC method for analysis of sertraline in pure bulk drug and lipidic nano delivery system: A stress degradation studies. *J. Liq. Chromogr. Relat. Technol.* **2013**, *36*, 700–716. [[CrossRef](#)]
19. Mennickent, S.; Fierro, R.; Vega, M.; De Diego, C.M.; Godoy, G. Quantitative determination of fluoxetine in human serum by high performance thin layer chromatography. *J. Sep. Sci.* **2010**, *33*, 2206–2210. [[CrossRef](#)]
20. Mennickent, S.; Fierro, R.; Vega, M.; De Diego, C.M.; Godoy, G.; Cifuentes, C.; Miranda, A. Quantification of sertraline in human serum by high-performance thin layer chromatography as a tool in pharmacotherapy adherence evaluation. *JPC-J. Planar Chromatogr.-Mod. TLC* **2013**, *26*, 358–362. [[CrossRef](#)]
21. Gondova, T.; Halamova, D.; Spacayova, K. Simultaneous analysis of new antidepressants by densitometric thin-layer chromatography. *J. Liq. Chromogr. Relat. Technol.* **2008**, *31*, 2429–2441. [[CrossRef](#)]
22. Thomas, A.B.; Naphade, A.; Karanjkele, S.S. Determination of alprazolam and fluoxetine HCl from spiked rat plasma using HPTLC with UV detection. *Int. J. Pharm. Pharm. Sci.* **2016**, *8*, 147–151.
23. Hamed, R.; Hadjmohammadi, M.R. Optimization of alcohol-assisted dispersive liquid-liquid microextraction by experimental design for the rapid determination of fluoxetine in biological samples. *J. Sep. Sci.* **2016**, *39*, 4784–4793. [[CrossRef](#)] [[PubMed](#)]
24. Zilfidou, E.; Kabir, A.; Furton, K.G.; Samanidou, V. An improved fabric phase sorptive extraction method for the determination of five selected antidepressant drug residues in human blood serum prior to high performance liquid chromatography with diode array detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2019**, *1125*, 121720. [[CrossRef](#)] [[PubMed](#)]
25. da Silva, A.C.C.; Raasch, J.R.; Vargas, T.G.; Peteffi, G.P.; Hahn, R.Z.; Antunes, M.V.; Perassolo, M.S.; Linden, R. Simultaneous determination of fluoxetine and norfluoxetine in dried blood spots using high-performance liquid chromatography-tandem mass spectrometry. *Clin. Biochem.* **2018**, *52*, 85–93. [[CrossRef](#)]
26. Weisskopf, E.; Panchaud, A.; Nguyen, K.A.; Grosjean, D.; Hascoët, J.M.; Csajka, C.; Eap, C.B.; Ansermot, N.; Collaborators of the SSRI-Breast Milk Study. Simultaneous determination of selective serotonin reuptake inhibitors and their main metabolites in human breast milk by liquid chromatography-electrospray mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2017**, *1057*, 101–109. [[CrossRef](#)]
27. López-Rabuñal, A.; Lendoiro, E.; Concheiro, M.; López-Rivadulla, M.; Cruz, A.; de-Castro-Ríos, A. LC-MS-MS method for the determination of antidepressants and benzodiazepines in Meconium. *J. Anal. Toxicol.* **2020**, *44*, 580–588. [[CrossRef](#)]
28. Rosetti, A.; Ferretti, R.; Zanitti, L.; Casulli, A.; Villani, C.; Cirilli, R. Single-run reversed-phase HPLC method for determining sertraline content, enantiomeric purity, and related substances in drug substance and finished product. *J. Pharm. Anal.* **2020**, *10*, 610–616. [[CrossRef](#)] [[PubMed](#)]
29. Batra, S.; Bhushan, R. Liquid chromatographic enantioseparation of (RS)-mexiletine and (RS)-fluoxetine using chiral derivatizing reagents synthesized with (S)-naproxen moiety. *Biomed. Chromatogr.* **2014**, *28*, 815–825. [[CrossRef](#)]
30. Pathak, A.; Rajput, S.J. Development of a stability-indicating high-performance liquid chromatographic method for the simultaneous determination of alprazolam and sertraline in combined dosage forms. *J. AOAC Int.* **2008**, *91*, 1344–1353. [[CrossRef](#)]
31. Chen, D.; Jiang, S.; Chen, Y.; Hu, Y. HPLC determination of sertraline in bulk drug, tablets and capsules using hydroxypropyl-beta-cyclodextrin as mobile phase additive. *J. Pharm. Biomed. Anal.* **2004**, *34*, 239–245. [[CrossRef](#)]
32. Rao, R.N.; Talluri, M.V.; Maurya, P.K. Separation of stereoisomers of sertraline and its related enantiomeric impurities on a dimethylated beta-cyclodextrin stationary phase by HPLC. *J. Pharm. Biomed. Anal.* **2009**, *50*, 281–286. [[CrossRef](#)]
33. Kertys, M.; Krivosova, M.; Ondrejka, I.; Hrtanek, I.; Tonhajzerova, I.; Mokry, J. Simultaneous determination of fluoxetine, venlafaxine, vortioxetine and their active metabolites in human plasma by LC-MS/MS using one-step sample preparation procedure. *J. Pharm. Biomed. Anal.* **2020**, *181*, 113098. [[CrossRef](#)]
34. Runfola, M.; Lima, D.L.D.; Fonseca, A.P.; Barbosa, Z. Optimization of a dispersive liquid-liquid microextraction method followed by UHPLC analysis for fluoxetine quantification in environmental water resources. *J. Sep. Sci.* **2018**, *41*, 4246–4252. [[CrossRef](#)]
35. Ma, W.; Gao, X.; Guo, H.; Chen, W. Determination of 13 antidepressants in blood by UPLC-MS/MS with supported liquid extraction pretreatment. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2021**, *1171*, 122608. [[CrossRef](#)] [[PubMed](#)]
36. Fernández, P.; Taboada, V.; Regenjo, M.; Morales, L.; Alvarez, I.; Carro, A.M.; Lorenzo, R.A. Optimization of ultrasound assisted dispersive liquid-liquid microextraction of six antidepressants in human plasma using experimental design. *J. Pharm. Biomed. Anal.* **2016**, *124*, 189–197. [[CrossRef](#)]
37. Cai, P.; Xiong, X.; Li, D.; Zhou, Y.; Xiong, C. Magnetic solid-phase extraction coupled with UHPLC-MS/MS for four antidepressants and one metabolite in clinical plasma and urine samples. *Bioanalysis* **2020**, *12*, 35–52. [[CrossRef](#)]

38. Snamina, M.; Wietecha-Postuszny, R.; Zawadzki, M. Postmortem analysis of human bone marrow aspirate—Quantitative determination of SSRI and SNRI drugs. *Talanta* **2019**, *204*, 607–612. [\[CrossRef\]](#)
39. Paíga, P.; Correia, M.; Fernandes, M.J.; Silva, A.; Carvalho, M.; Vieira, J.; Jorge, S.; Silva, J.G.; Freire, C.; Delerue-Matos, C. Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: Hourly variation. *Sci. Total Environ.* **2019**, *648*, 582–600. [\[CrossRef\]](#)
40. Li, D.; Xiong, X.; Zhuang, S.; Du, Z.; Xiong, C.; Jiang, H. Preparation of bovine serum albumin restricted access octadecyl/phenyl-mixed-functionalized magnetic silica nanoparticles for magnetic solid phase extraction of antidepressants in aquatic products followed by UHPLC-MS/MS. *Talanta* **2021**, *221*, 121458. [\[CrossRef\]](#)
41. Feng, Y.; Zheng, M.; Zhang, X.; Kang, K.; Kang Weijun Lian, K.; Yang, J. Analysis of four antidepressants in plasma and urine by gas chromatography-mass spectrometry combined with sensitive and selective derivatization. *J. Chromatogr. A* **2019**, *1600*, 33–40. [\[CrossRef\]](#)
42. Chen, X.; Zheng, S.; Le, J.; Qian, Z.; Zhang, R.; Hong, Z.; Chai, Y. Ultrasound-assisted low-density solvent dispersive liquid-liquid microextraction for the simultaneous determination of 12 new antidepressants and 2 antipsychotics in whole blood by gas chromatography-mass spectrometry. *J. Pharm. Biomed. Anal.* **2017**, *142*, 19–27. [\[CrossRef\]](#)
43. Koçoğlu, E.S.; Bakırdere, S.; Keyf, S. Sensitive Determination of sertraline in commercial drugs and its stability check in simulated gastric juice. *J. AOAC Int.* **2016**, *99*, 1527–1532. [\[CrossRef\]](#)
44. Luiz Oenning, A.; Birk, L.; Eller, S.; Franco de Oliveira, T.; Merib, J.; Carasek, E. A green and low-cost method employing switchable hydrophilicity solvent for the simultaneous determination of antidepressants in human urine by gas chromatography—Mass spectrometry detection. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2020**, *1143*, 122069. [\[CrossRef\]](#)
45. Boumba, V.A.; Rallis, G.; Petrikis, P.; Vougiouklakis, T.; Mavreas, V. Determination of clozapine, and five antidepressants in human plasma, serum and whole blood by gas chromatography-mass spectrometry: A simple tool for clinical and postmortem toxicological analysis. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2016**, *1038*, 43–48. [\[CrossRef\]](#)
46. Ntoupa, P.A.; Armaos, K.P.; Athanaselis, S.A.; Spiliopoulou, C.A.; Papoutsis, I.I. Study of the distribution of antidepressant drugs in vitreous humor using a validated GC/MS method. *Forensic Sci. Int.* **2020**, *317*, 110547. [\[CrossRef\]](#) [\[PubMed\]](#)
47. Gonçalves, R.; Ribeiro, C.; Cravo, S.; Cunha, S.C.; Pereira, J.A.; Fernandes, J.O.; Afonso, C.; Tiritan, M.E. Multi-residue method for enantioseparation of psychoactive substances and beta blockers by gas chromatography-mass spectrometry. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **2019**, *1125*, 121731. [\[CrossRef\]](#)
48. Ali, E.A.; Adawy, A.M.; El-Shahat, M.F.; Amin, A.S. Simple spectrophotometric methods for determination of fluoxetine and clomipramine hydrochlorides in dosage forms and in some post-mortem biological fluids samples. *Egypt J. Forensic Sci.* **2016**, *6*, 370–380. [\[CrossRef\]](#)
49. Nezhadali, A.; Motlagh, M.O.; Sadeghzadeh, S. Spectrophotometric determination of fluoxetine by molecularly imprinted polypyrrole and optimization by experimental design, artificial neural network and genetic algorithm. *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2018**, *190*, 181–187. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Walash, M.J.; Belal, F.F.; El-Enany, N.M.; Elmansi, H. Development and validation of stability indicating method for determination of sertraline following ICH guidelines and its determination in pharmaceuticals and biological fluids. *Chem. Cent. J.* **2011**, *5*, 61. [\[CrossRef\]](#) [\[PubMed\]](#)
51. Lotfi, A.; Manzoori, J.L. Determination of fluoxetine in pharmaceutical and biological samples based on the silver nanoparticle enhanced fluorescence of fluoxetine-terbium complex. *Luminescence* **2016**, *31*, 1349–1357. [\[CrossRef\]](#)
52. Wang, Z.R.; Hsieh, M.M. Ultrasound-assisted dispersive liquid-liquid microextraction coupled with field-amplified capillary electrophoresis for sensitive and quantitative determination of fluoxetine and norfluoxetine enantiomers in biological fluids. *Anal. Bioanal. Chem.* **2020**, *412*, 5113–5123. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Murtada, K.; de Andrés, F.; Ríos, A.; Zougagh, M. Determination of antidepressants in human urine extracted by magnetic multi-walled carbon nanotube poly(styrene-co-divinylbenzene) composites and separation by capillary electrophoresis. *Electrophoresis* **2018**, *39*, 1808–1815. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Alizadeh, T.; Azizi, S. Graphene/graphite paste electrode incorporated with molecularly imprinted polymer nanoparticles as a novel sensor for differential pulse voltammetry determination of fluoxetine. *Biosens. Bioelectron.* **2016**, *81*, 198–206. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Feroz, M.; Lopes, I.C.; ur Rehman, H.; Ata, S.; Vadgama, P. A novel molecular imprinted polymer layer electrode for enhanced sensitivity electrochemical determination of the antidepressant fluoxetine. *J. Electroanal. Chem.* **2020**, *878*, 114693. [\[CrossRef\]](#)
56. Aladaghlo, Z.; Fakhari, A.R.; Hasheminasab, K.S. Application of electromembrane extraction followed by corona discharge ion mobility spectrometry analysis as a fast and sensitive technique for determination of tricyclic antidepressants in urine samples. *Microchem. J.* **2016**, *129*, 41–48. [\[CrossRef\]](#)
57. Inkielewicz-Śtepiński, I. Impact of fluoxetine on liver damage in rats. *Pharmacol. Rep.* **2011**, *63*, 441–447. [\[CrossRef\]](#)
58. Silva, V.H.; Dos Santos Batista, A.P.; Silva Costa Teixeira, A.C.; Borrelly, S.I. Degradation and acute toxicity removal of the antidepressant Fluoxetine (Prozac®) in aqueous systems by electron beam irradiation. *Environ. Sci. Pollut. Res. Int.* **2016**, *23*, 11927–11936. [\[CrossRef\]](#)
59. Khan, M.F.; Murphy, C.D. Bacterial degradation of the anti-depressant drug fluoxetine produces trifluoroacetic acid and fluoride ion. *Appl. Microbiol. Biotechnol.* **2021**, *105*, 9359–9369. [\[CrossRef\]](#)

-
60. Ferenczi-Fodor, K.; Renger, B.; Végh, Z. The frustrated reviewer—Recurrent failures in manuscripts describing validation of quantitative TLC/HPTLC procedures for analysis of pharmaceuticals. *JPC-J. Planar Chromatogr.-Mod. TLC* **2010**, *23*, 173–179. [[CrossRef](#)]
 61. Nagy-Turák, A.; Végh, Z.; Ferenczi-Fodor, K. Validation of the quantitative planar chromatographic analysis of drug substances. III. Robustness testing in OPLC. *JPC-J. Planar Chromatogr.-Mod. TLC* **1995**, *8*, 188–193.
 62. Ferenczi-Fodor, K.; Nagy-Turák, A.; Végh, Z. Validation and monitoring of quantitative thin layer chromatographic purity tests for bulk drug substances. *JPC-J. Planar Chromatogr.-Mod. TLC* **1995**, *8*, 349–356.
 63. Hendix, C.D. What every technologist should know about experiment design. *Chem. Technol.* **1979**, *9*, 167–174.
 64. European Pharmacopoeia VIII. Council of Europe: Strasbourg, France, 2013; Volume 1, pp. 298, 780, 810.
 65. The United States Pharmacopeia 34. Twinbrook Parkway: Rockville, MD, USA, 2011; Volume 3, pp. 2877, 4219.