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ORIGINAL ARTICLE / ÖZGÜN MAKALE

RPLC METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANEOUS DETERMINATION OF SELECTIVE SEROTONIN REUPTAKE INHIBITORS IN TABLET FORMULATIONS

SEÇİCİ SEROTONİN GERİ ALIM İNHİBİTÖRLERİNİN TABLET FORMÜLASYONLARINDA EŞ ZAMANLI TAYİNİ İÇİN RPLC YÖNTEM GELİŞTİRİLMESİ VE VALİDASYONU

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ABSTRACT

Objective: *Selective serotonin reuptake inhibitor (SSRIs) compounds are the most used compounds in the treatment of depression. The determination of chromatographic separation and quantitative determination of these compounds is very important in the clinical use of these compounds and the success of biopharmaceutical studies. For this reason, this study, it was aimed to optimize the chromatographic conditions for the quantitative determination of SSRIs in tablet formulation by Reversed phase liquid chromatography method.*

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Material and Method: *The optimum separation condition for the studied compounds was determined based on the relationship between the retention values of the compounds and the pH and the content of the mobile phase. Chromatographic determination was made on the X Terra C18 column (250 x 4.6 mm I.D., 5 µm), which is widely used for the determination of hybrid-based and basic compounds. The validation of the developed method was carried out based on the parameters of linearity, precision, and accuracy.*

Result and Discussion: *The developed and validated method was successfully applied for the determination of active ingredients in the tablet dosage form. The experimental results of the amount of studied SSRIs in selected commercial tablets are in good agreement with the label claims. The calculated percent recoveries show that the sample preparation techniques developed for the quantification of the compounds studied are not affected by interferences. The evaluation of the obtained results showed that the developed method is suitable for the routine use of studied compounds.*

Keywords: *Antidepressant, method optimization, quantitative analysis, RPLC*

ÖZ

Amaç: *Seçici serotonin geri alım inhibitörleri (SSRI) depresyon tedavisinde en çok kullanılan bileşiklerdir. Bu bileşiklerin kromatografik ayrımları ve kantitatif tayinlerinin belirlenmesi, bileşiklerin klinik kullanımlarında ve yapılan biyofarmasötik çalışmaların başarısında oldukça önemlidir. Bu nedenle bu çalışmada tablet formülasyonunda SSRI'ların kantitatif tayini için kromatografik koşulların Ters faz sıvı kromatografi yöntemi ile optimize edilmesi amaçlanmıştır.*

Gereç ve Yöntem: *Çalışılan bileşikler için optimum ayırma koşulu, bileşiklerin alıkonma değerleri ile pH ve mobil faz bileşimi arasındaki ilişkiye göre belirlenmiştir. Kromatografik ayrım, hibrit bazlı ve bazik bileşiklerin tayini için yaygın olarak kullanılan X Terra C18 kolonu (250 x 4.6 mm I.D., 5 µm) kullanılarak gerçekleştirilmiştir. Geliştirilen yöntemin validasyonu; doğrusallık, kesinlik ve doğruluk parametrelerine göre yapılmıştır.*

Sonuç ve Tartışma: *Tablet formülasyonlarındaki etken maddelerin tayini için geliştirilen yöntem başarıyla uygulanmış ve valide edilmiştir. Elde edilen deneysel SSRI miktarları, ticari formülasyonlarda belirtilen miktarlar ile uyumlu olarak bulunmuştur. Hesaplanan geri kazanım yüzdeleri, çalışılan bileşiklerin kantitatif tayini için geliştirilen numune hazırlama tekniklerinin dış etkenlerden etkilenmediğini göstermektedir. Elde edilen sonuçlar değerlendirildiğinde, geliştirilen yöntemin çalışılan bileşiklerin rutin analizlerine uygun olduğu görülmüştür.*

Anahtar Kelimeler: *Antidepresan, kantitatif analiz, metot optimizasyonu, RPLC*

INTRODUCTION

Selective serotonin reuptake inhibitors (SSRIs) are frequently used in the treatment of psychiatric disorders such as depression, obsessive-compulsive disorder, and anxiety [1,2]. SSRIs have advantages over tricyclic antidepressants such as less pronounced anticholinergic side effects and no severe cardiotoxicity [3]. These drugs inhibit the uptake of serotonin across the plasma membrane before it is stored in specific organelles [4].

Sertraline, citalopram, and fluvoxamine, which belong to the SSRI group, are basic compounds due to the basic functional group in their chemical structure (Figure 1). These compounds with primary, secondary, and tertiary amine groups were apolar compounds with poor water solubility according to the log P values of 5.15, 3.76, and 2.80, respectively [5].

In recent years, the identification of drug candidates has often faced the problem that many new molecules in drug discovery are less water-soluble and more lipophilic. This problem can be solved by using water-organic solvent binary mixtures. In addition to the discovery and design of a compound used as a drug in pharmaceutical chemistry, it is important to develop effective analytical methods for chemical analysis and quality control. There are studies in the literature for the determination of sertraline, citalopram, and fluvoxamine alone or together with their degradation products in various samples [6-8]. Among these methods, reverse phase liquid chromatography (RPLC) is mostly preferred due to its advantages such as accuracy, precision, and repeatability of the measurements taken [9-12]. The primary purpose of RPLC studies is to ensure that the studied compounds are separated from each

other as soon as possible or to make simultaneous determinations provided that certain validation conditions (ICH parameters) are met [13].

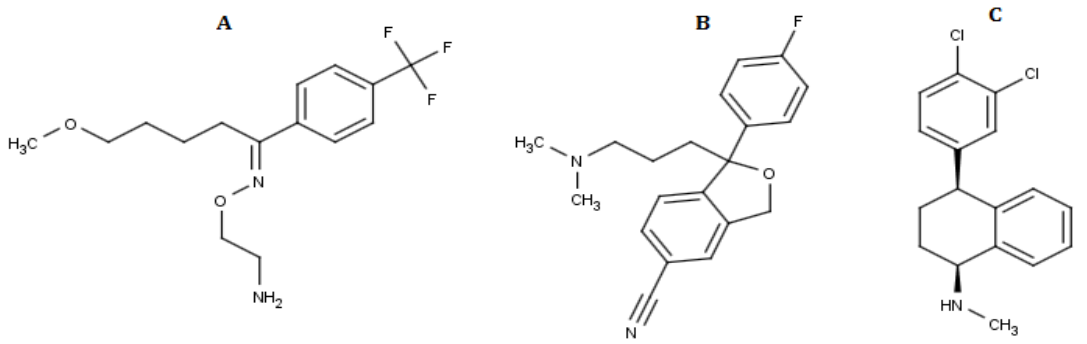

Figure 1. Chemical structure of studied compounds (A) fluvoxamine, (B) citalopram, and (C) sertraline [5]

In the HPLC method, the parameters known to affect the retention factor (*k*) values of the compounds are changed individually or randomly to determine the optimum separation condition in most studies. While this situation causes unnecessary time and material loss, in some cases, it is insufficient in determining the separation condition [14-18]. To determine the chromatographic working conditions, instead of this trial-and-error method, the chromatographic conditions (column temperature, mobile phase pH and organic solvent concentration, etc.) should be optimized in the developed method [15-19]. In this study, studies were found for the determination of the selected SSRIs sertraline, citalopram, and fluvoxamine by the RPLC method for the determination of the compounds alone or simultaneously [20-22]. In addition, there are few studies on the determination of optimum conditions of compounds with the experimental design method related to this group [23-25]. For the qualitative determination of the selected compounds in this study, the optimum chromatographic condition was determined by examining the change in *k* values depending on the mobile phase pH at the constant column temperature and the organic modifier concentration in the mobile phase. With this type of study, the simultaneous determination of compounds could be made in the shortest time possible. In addition, the method developed was validated according to International Conference on Harmonization (ICH) parameters and then quantitative determinations in drug formulations were performed.

MATERIAL AND METHOD

Apparatus

In this study, the qualitative and quantitative analyses of the compounds were made with a high-performance liquid chromatography device (Shimadzu Technologies, Japan). The system used consists of a UV detector (SPD-20A), pump (LC-20AD), column oven (CTO-20A), and degasser unit (DGU-20A3). Mobile phase pH measurements prepared for chromatographic determination were made using a Mettler Toledo pH/Ion analyzer (Schwerzenbach, Switzerland) and In Lab 413 Ag / AgCl pH electrode. Ultrapure water was supplied from the Direct-Q®3 UV (Millipore, Bedford, MA, USA) water purification system.

Chemicals

In this study, sertraline, citalopram, fluvoxamine, and uracil were obtained from Sigma-Aldrich (USA). Acetonitrile was used as an organic solvent in the preparation of the mobile phase, o-phosphoric acid, sodium hydroxide, ammonium bicarbonate, and ammonia were used as buffer components in the mobile phase, and potassium hydrogen phthalate was used as the primary standard reference in electrode calibration were supplied from Merck (Darmstadt, Germany). All chemicals used in the study are of analytical purity.

Chromatographic Study

In this study, the acetonitrile-water binary mixture containing 50% (v/v) acetonitrile was prepared as a mobile phase for the chromatographic determination of the compounds. o-phosphoric acid (85%, w/w) was added to the mobile phase medium at 25 mM and 1 M NaOH solution was added to reach the desired mobile pH. The pH values of the mobile phases were prepared between 6.0 and 10.5. o-phosphoric acid-sodium hydroxide and ammonia-ammonium bicarbonate were used as buffer compositions for the mobile phases. These solutions were used after degasification in an ultrasonic mixer. This work was carried out on an X Terra C18 column (250 x 4.6 mm I.D., 5 µm, Waters, USA), which is suitable for the analysis of hybrid-based and basic compounds. Separation was carried out at a column temperature of 30°C and a flow rate of 1.0 ml/min. The maximum absorbance wavelength of the studied compounds was determined as 210 nm with a UV detector.

Preparation of Standard and Calibration Solutions

Stock solutions of the analyzed compounds (100 µg/ml) were prepared by dissolving them in the mobile phase. For the calibration of the pH electrode, 0.05 mol/kg potassium acid phthalate solution was prepared in the working mobile phase binary mixture. The prepared solutions were protected from sunlight and stored at +4°C.

Calibration solutions of citalopram, fluvoxamine, and sertraline, whose linear working range was determined, were prepared in the optimum hydro-organic mixture at concentrations of 2-12 µg/ml, 4-32 µg/ml, and 1-10 µg/ml, respectively. The internal standard method was used for the calibration graph. The internal standard imipramine chosen for this was kept constant at 1 µg/ml throughout the entire study.

Analysis of Tablet Solutions

For quantitative determination of citalopram, fluvoxamine and sertraline tablet analysis was performed. In this method, ten tablets were finely powdered and weighed in an equivalent amount to 1 tablet. Then, the powder in the amount of one tablet was put into the volumetric flask and by adding the mobile phase, its volume was made up to 100 ml. To dissolve the active ingredients of the drugs determined in the prepared sample solutions, the solutions were kept in an ultrasonic bath for 20 minutes. The insoluble part in the prepared solution was removed by filtration. Finally, the solution obtained was prepared at different dilution rates according to the concentration in the calibration range specified for each compound [16,26,27].

Recovery Experiment

A recovery study was conducted to determine the accuracy of the proposed method. Samples were analyzed by adding a known amount of pure standard and selected internal standard to the tablet sample containing a fixed amount of active ingredient. The percent recovery was calculated using the concentrations of the active ingredient in the sample and the added standard solution.

RESULT AND DISCUSSION

In general, the analysis of basic compounds is difficult due to peak asymmetry arising from secondary interactions between column residues of silanols and the ionized form of the compound. In addition, compounds with apolar properties cause higher retention times, peak broadening, and unnecessary mobile phase consumption because they interact more strongly with the apolar column. For this reason, suitable columns should be preferred considering the chemical properties of the compounds in RPLC analysis [28,29]. In this study, an X Terra column with a wide pH working range (pH 1-12) suitable for the analysis of basic compounds from the new generation columns was selected.

A mobile phase optimization study was performed to determine the optimum separation condition in the quantitative determination of sertraline, citalopram, and fluvoxamine used in the treatment of depression by the RPLC method. With knowing the pK_a values of the compounds, it is possible to determine the pH values at which they are in molecular or ionized form. For this, pH values above and below 1.5 units of pK_a value are determined as working pH ranges. For this, by keeping constant

chromatographic conditions and the acetonitrile concentration in the mobile phase, the effect of pH change on the *k* values of the compounds was investigated. The t_0 value was determined by using the standard solution of uracil, which was used as the non-retained species in the column. The *k* values at each pH value (6.0-10.0) studied were calculated by using the t_R and t_0 values of the studied compounds in the hydro-organic mixture containing 50% acetonitrile. When the *k* values of the compounds are plotted against the mobile phase pH values, the sigmoidal behavior belongs to the basic functional group (Figure 2).

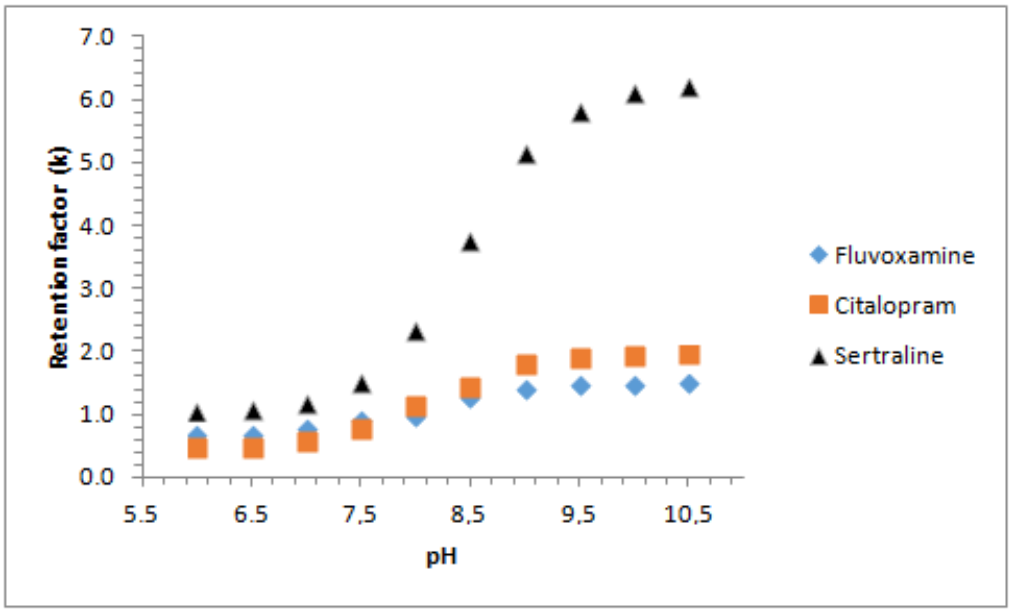

Figure 2. Sigmoidal behavior of studied compounds

In the optimization studies, it was aimed to determine the best chromatographic conditions in which the compounds were separated. The most basic method to find the optimum separation conditions for compounds containing ionizable functional groups in their structures is to examine the sequential change of chromatographic conditions. In particular, the effect of the pH of the mobile phase and the organic modifier concentration on the *k* value should be determined. Since compounds containing ionizable functional groups in their structure have different forms at different pH values, the affinity of these forms is different due to the second equilibrium requirement in the RPLC method. Therefore, the retention of these forms is also different. Furthermore, since the polarity of the mobile phase changes with the amount of organic modifier in the mobile phase, there are changes in the solvation of the compound. Because of the changes in the solvation of the compound, its affinity for the stationary phase changes, so the retention of the compound in the chromatographic column also changes [9,30,31].

The best condition that satisfies the desired chromatographic conditions is determined as the optimum separation condition. In addition, if the *k* values of the compounds are in the range of $1 \leq k \leq 5$, the selectivity factor (α) is greater than 1.15, and the peak resolution (R_s) value is greater than 1.5, optimum chromatographic separation occurs when these favorable conditions are met. The selectivity factor is calculated by dividing the retention factor (k_2) of the second peak by the retention factor of the first peak (k_1).

When the experimental data obtained are examined, the *k* value of the compounds under pH 8 is below 1. At pH 8 and 8.5, compound pairs have α values below 1.15 and R_s below 1.5. At pH 9, *k* values for peak pairs are above 1, and α and R_s values for peak pairs are above 1.15 and 1.5, respectively (Figure 3).

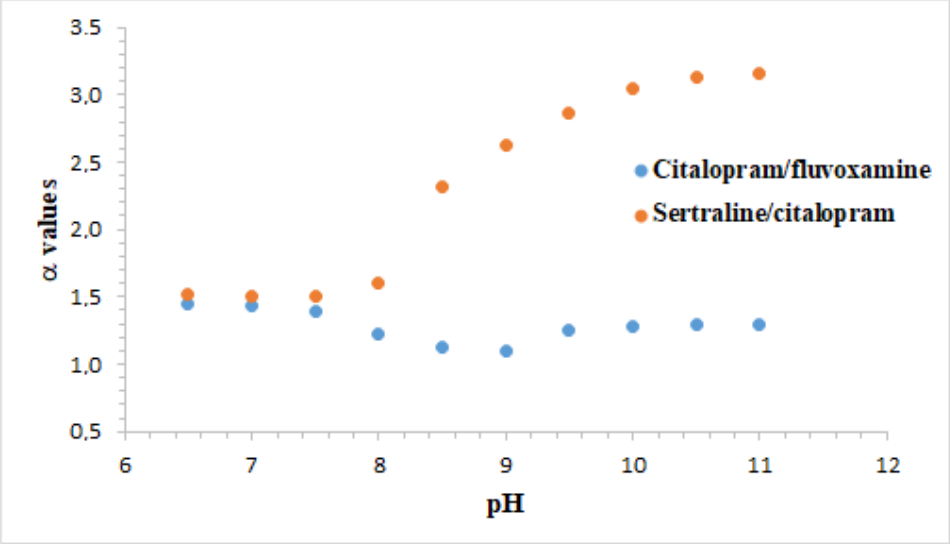

Figure 3. Variation in α values for compound pairs with mobile phase pH

For this, the pH value of the mobile phase was determined as pH 9.0 in the binary mixture containing 50% acetonitrile. The Purnell equation shows the relationship between the selectivity factor, peak resolution, and retention factor. For this reason, the R_s value between the two peaks must be calculated using this equation in the qualitative determination. The values calculated according to the Purnell equation under this mobile phase condition are given in Table 1.

Table 1. Calculated data of compounds at optimum separation condition

Compounds	k_2	α	k_2/k_2+1	$(\alpha-1)/\alpha$	$(\frac{1}{4})\sqrt{N}$	R_s
Fluvoxamine/citalopram	1.784	1.246	0.641	0.198	22.020	2.789
Citalopram/ imipramine (I.S)	3.713	2.082	0.788	0.520	24.477	10.022
Imipramine (I.S)/ sertraline	5.130	1.381	0.837	0.276	23.286	5.381

After the optimization of the liquid chromatographic method developed in the study, method validation was performed for the quantitative determination of the compounds. Analysis with the addition of an internal standard (IS) is common to eliminate systematic errors in analytical measurements [32,33]. The IS method is preferred to exclude systematic and random errors such as additives in drug formulations and volume errors during sample injection. When the internal standard is selected, it must be chromatographically separated from the compounds determined under optimal separation conditions. In this study, imipramine was selected as the IS. Under the selected optimal separation conditions, imipramine could be retained in this column because it was present in its molecular form. The chromatogram obtained under the optimal separation conditions is shown in Figure 4.

Once the optimal separation conditions were determined, the stability of the chromatographic system was determined according to the ICH guidelines [34]. For this purpose, chromatographic parameters were calculated by injecting the compounds into the HPLC system (Table 2).

The system suitability parameters results (Table 2) showed that the developed chromatographic method was suitable for the analysis and analytical method validation part.

A calibration curve was prepared to determine the linearity of the developed method. The regression parameters calculated when the peak area ratios of the compounds were plotted against the concentrations of the studied compounds are presented in Table 3.

A good correlation coefficient (0.999) from the graphs shows the appropriate linear concentration range for the three compounds. The precision of the developed method was determined by calculating the relative standard deviation (%RSD) of the compound concentrations calculated using the peak area ratios of five repetitive injections of the standard solution. For this purpose, intraday and interday studies were carried out. These studies were performed at two different concentrations for each compound and the results are shown in Table 4. It is seen that %RSD values calculated from Table 4 are less than 2%.

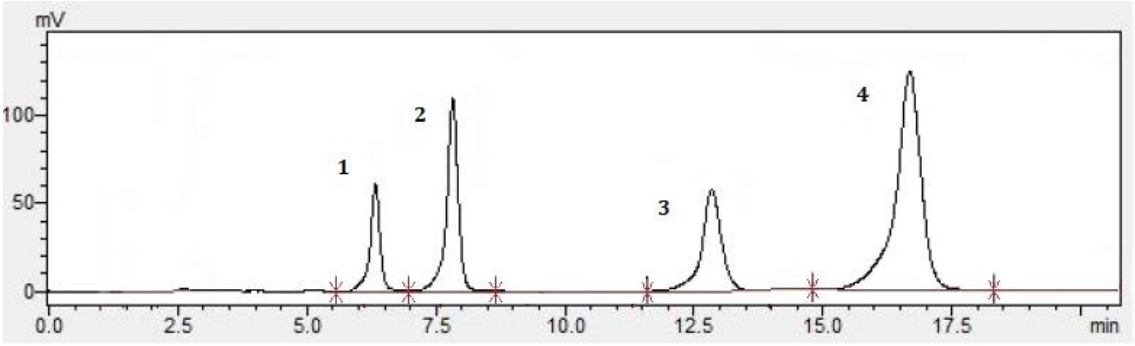

Figure 4. Chromatogram of standard mixture: 1) fluvoxamine, 2) citalopram, 3) imipramine (I.S), 4) sertraline

Table 2. System suitability parameters for studied compounds

Parameters	Fluvoxamine	Citalopram	Imipramine (I.S)	Sertraline	Recommended Value
Retention time (t_R)	6.299	7.678	12.695	16.513	
Retention Factor (k)	1.437	1.798	3.721	5.170	> 1
Tailing Factor (T_f)	1.250	1.176	1.389	1.682	≤ 2
Selectivity Factor (α)		1.575	1.248	2.212	> 1
Theoretical Plates (N)	6631.992	7950.845	9586.248	8640.986	>2000
Resolution (R_s)		2.875	9.971	9.971	>2
RSD% (for retention time)	0.216	0.590	0.141	0.885	≤1
RSD% (for peak area)	0.872	0.178	0.743	0.455	≤1

Table 3. The calibration data of studied compounds

Sample	Linearity Range (µg/ml)	Slope	Intercept	Correlation Coefficient (r)	Detection Limit (LOD) (µg/ml)	Quantitation Limit (LOQ) (µg/ml)
Fluvoxamine	4-32	0.265(0.003*)	-0.059(0.043*)	0.999	0.773	2.343
Citalopram	2-12	0.768(0.004)	-0.065(0.032)	0.999	0.147	0.446
Sertraline	1-10	2.916(0.011)	-0.085(0.065)	0.999	0.094	0.286

*Standard error

For the quantification of sertraline, citalopram, and fluvoxamine in tablet formulations, tablet solutions were prepared as described in the "Experimental" section, and the ratio of the peak area of the analyzed compounds to the peak area values of imipramine because of the analysis was evaluated in the corresponding calibration functions. Then, the amount of active compounds contained in the tablets was calculated (Table 5). Recovery studies were also performed to determine the accuracy of the method developed in the study. The calculated % recovery values are also shown in Table 5. Chromatograms showing the analysis of the tablet samples were given in Figure 5 for fluvoxamine, Figure 6 for citalopram, and Figure 7 for sertraline.

Table 4. Precision data of the developed method

Compounds	Theoretical concentration (µg/ml)	Intraday measured concentration, mean (µg/ml)	RSD (%)	Bias (%)	Interday measured concentration, mean (µg/ml)	RSD (%)	Bias (%)
Fluvoxamine	6	6.054	0.637	-0.894	6.083	0.787	-1.390
	16	16.036	0.689	-0.222	16.123	1.154	-0.768
Citalopram	4	4.019	0.752	-0.478	4.090	1.468	-2.247
	10	10.048	0.362	-0.482	9.946	1.493	0.540
Sertraline	2	2.016	0.645	-0.800	2.042	1.397	-2.075
	8	8.090	0.393	-1.126	8.213	0.447	-2.665

Table 5. Recovery results in drug formulation

Parameters	Fluvoxamine	Citalopram	Sertraline
Labeled claim (mg)	100	20	50
Amount found (mg)[a]	100.051±3.144[b]	20.341±0.327	49.919±0.938
RSD (%)	1.283	0.650	0.757
Bias (%)	-0.052	-1.706	0.162
Recovery (%)	100.051±1.137	101.705±2.006	99.838±0.755
RSD (%) of recovery	0.675	0.645	0.524

[a]Each value is the mean of 5 experiments, [b] confidence interval

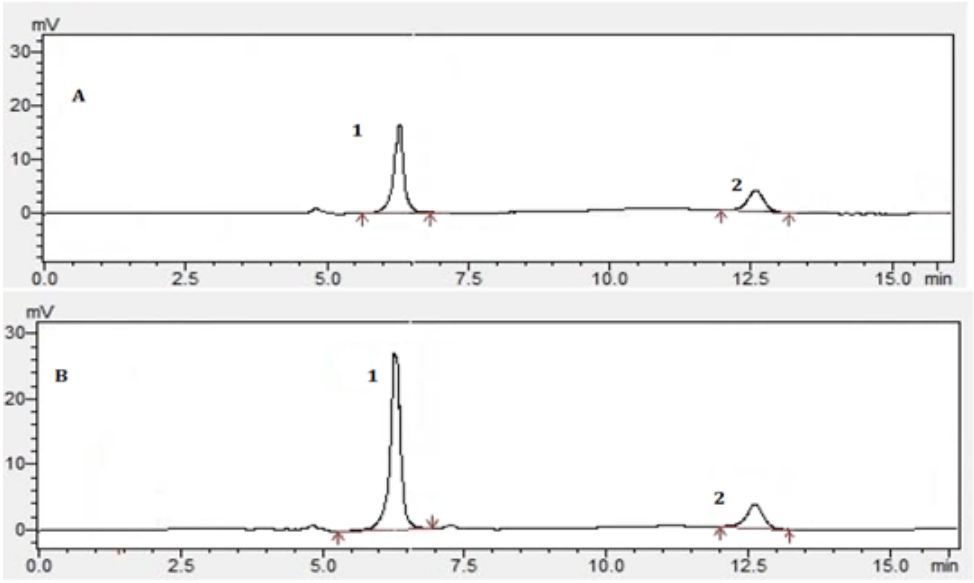

Figure 5. Chromatograms showing A) Faverin® tablet sample containing fluvoxamine (1) fluvoxamine (6 µg/ml) and (2) imipramine (I.S.) (1 µg/ml) B) tablet spiked with fluvoxamine (1) fluvoxamine (12 µg/ml) and (2) imipramine (I.S.) (1 µg/ml)

The experimental results of the amount of fluvoxamine, citalopram, and sertraline in selected commercial tablets are in good agreement with the label claims. The calculated percent recoveries show that the sample preparation techniques developed for the quantification of the compounds studied are not affected by interferences.

This is the first study of mobile phase optimization under specified chromatographic conditions. The retention time of the citalopram, fluvoxamine, and sertraline and the suitability of other chromatographic parameters were determined based on the combined effect of the percentage and pH of the organic solvent on the retention behavior of the compounds in RPLC. Moreover, the sufficient

reproducibility and good shapes of the peaks obtained throughout the liquid chromatographic study indicate the suitability of the selected column for this study. Method validation and quantitative determination of compounds in tablet formulations were performed under the determined optimal chromatographic conditions. The evaluation of the obtained results showed that the developed method is suitable for routine use.

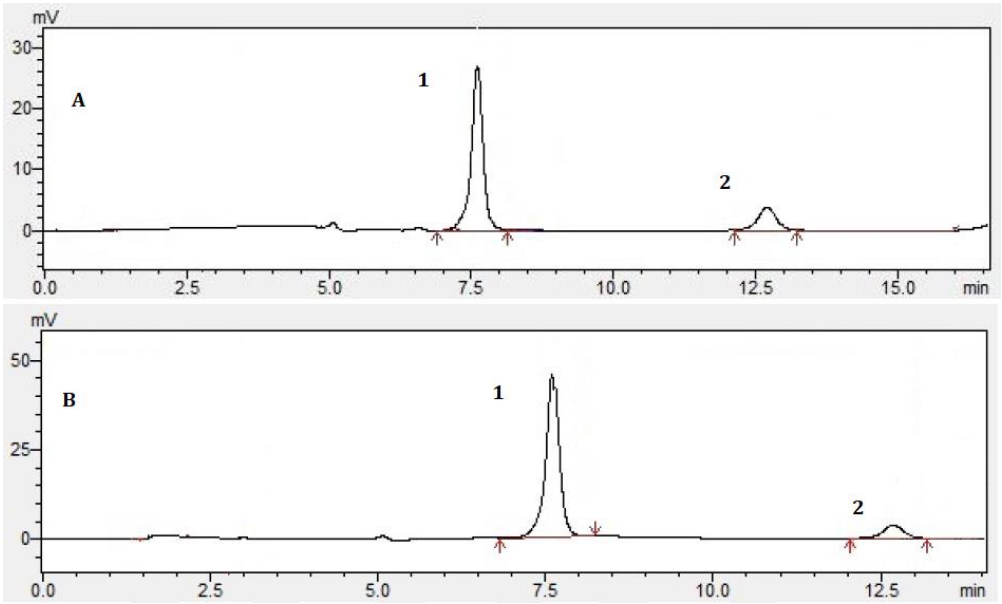

Figure 6. Chromatograms showing A) Citol® tablet sample containing citalopram (1) citalopram (4 µg/ml) and (2) imipramine (I.S.) (1 µg/ml) B) tablet spiked with citalopram (1) citalopram (8 µg/ml) and (2) imipramine (I.S.) (1 µg/ml)

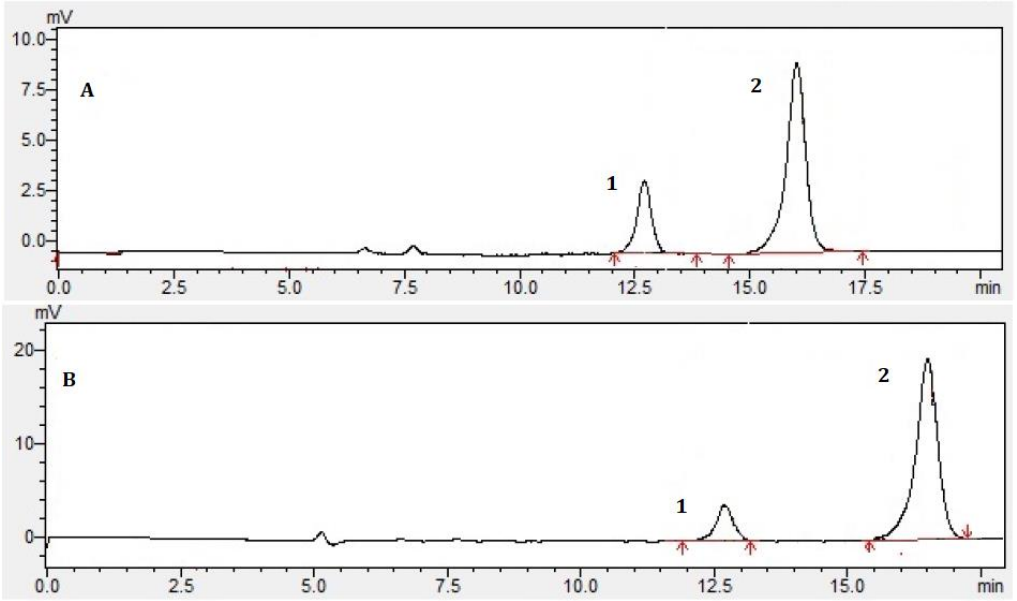

Figure 7. Chromatograms showing A) Lustral® tablet sample containing sertraline (1) imipramine (I.S.) (1 µg/ml) and (2) sertraline (2 µg/ml) B) tablet spiked with sertraline (1) imipramine (I.S.) (1 µg/ml) and (2) sertraline (4 µg/ml)

AUTHOR CONTRIBUTIONS

Concept: E.Ç.D., F.Ü., Y.D.D.; Control: E.Ç.D., Y.D.D.; Sources: E.Ç.D.; Data Collection and/or Processing: E.Ç.D., F.Ü., Z.Ü., Y.D.D.; Analysis and/or Interpretation: E.Ç.D., F.Ü., Y.D.D.; Literature Review: E.Ç.D., F.Ü., Z.Ü., Y.D.D.; Manuscript Writing: E.Ç.D.; Critical Review: E.Ç.D., Z.Ü., Y.D.D.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

REFERENCES

1. Jacobsen, N.W., Hansen, C.H., Nellemann, C., Styrishave, B., Halling-Sørensen, B. (2015). Effects of selective serotonin reuptake inhibitors on three sex steroids in two versions of the aromatase enzyme inhibition assay and in the H295R cell assay. Toxicology in Vitro, 29, 1729-1735. [CrossRef]
2. Bermel, R.A., Hashmonay, R., Meng, X., Randhawa, S., Von Rosenstiel, P., Sfikas, N., Kantor, D. (2015). Fingolimod first-dose effects in patients with relapsing multiple sclerosis concomitantly receiving selective serotonin-reuptake inhibitors. Multiple Sclerosis and Related Disorders, 4, 273-280. [CrossRef]
3. Aronson, J.K. (2016). Meyler's Side Effects of Drugs (16. edition). Netherlands, Elsevier.
4. Hoirisch-Clapauch, S., Nardi, A.E., Gris, J.C., Brenner, B. (2014). Are the antiplatelet and profibrinolytic properties of selective serotonin-reuptake inhibitors relevant to their brain effects? Thrombosis Research, 134(1), 11-16. [CrossRef]
5. Chemicalize program. http://www.chemicalize.org (accessed 29 October 2022).
6. Şentürk, Z., Saka, C., Teğin, İ. (2011). Analytical methods for determination of selective serotonin reuptake inhibitor antidepressants. Reviews in Analytical Chemistry, 30(2), 87-122. [CrossRef]
7. Murtada, K., de Andrés, F., Ríos, A., Zougagh, M. (2018). Determination of antidepressants in human urine extracted by magnetic multiwalled carbon nanotube poly (styrene-co-divinylbenzene) composites and separation by capillary electrophoresis. Electrophoresis, 39(14), 1808-1815. [CrossRef]
8. Annapurna, V., Jyothi, G., Nagalakshmi, V., Sailaja, B.B.V. (2010). Spectrophotometric methods for the assay of fluvoxamine using chromogenic reagents. E-Journal of Chemistry, 7(4), 1539-1545. [CrossRef]
9. Kazakevich, Y., Lobrutto, R. (2007). HPLC for Pharmaceutical Scientists (1st edition), Wiley-Interscience, Canada, p.1140.
10. Meyer, V.R. (2010). Practical High-Performance Liquid Chromatography (16th edition), John Wiley and Sons, United Kingdom, p.432.
11. Daldal, Y.D., Demiralay, E.Ç., Ozkan, S.A. (2016). Effect of organic solvent composition on dissociation constants of some reversible acetylcholinesterase inhibitors. Journal of the Brazilian Chemical Society, 27, 493-499. [CrossRef]
12. Daldal, Y.D., Demiralay, E.Ç. (2020). Chromatographic and UV-visible spectrophotometric pKa determination of some purine antimetabolites. Journal of Molecular Liquids, 317, 113930. [CrossRef]
13. Guideline, I.H.T. (2005). Validation of analytical procedures: Text and methodology. Q2 (R1), 1(20), 05.
14. Demiralay, E.Ç., Koç, D., Daldal, Y.D., Çakır, C. (2012). Determination of chromatographic and spectrophotometric dissociation constants of some beta lactam antibiotics. Journal of Pharmaceutical and Biomedical Analysis, 71, 139-143. [CrossRef]
15. Demiralay, E.Ç., Üstün, Z., Daldal, Y.D. (2014). Estimation of thermodynamic acidity constants of some penicillinase-resistant penicillins. Journal of Pharmaceutical and Biomedical Analysis, 91, 7-11. [CrossRef]
16. Demiralay, E.Ç. (2012). An experimental design approach to optimization of the liquid chromatographic separation conditions for the determination of metformin and glibenclamide in pharmaceutical formulation. Acta Chimica Slovenica, 59(2), 307-314.
17. Lundstedt, T., Seifert, E., Abramo, L., Thelin, B., Nyström, Å., Pettersen, J., Bergman, R. (1998). Experimental design and optimization. Chemometrics and intelligent laboratory systems, 42(1-2), 3-40. [CrossRef]
18. Bolton, S., Bor, S. (2003). Pharmaceutical Statistics: Practical and Clinical Applications, Revised and Expanded (4th edition), CRC press, Boca Raton, p.755.

19. Bonazzi, D., Gotti, R., Andrisano, V., Cavrini, V. (1997). Analysis of ACE inhibitors in pharmaceutical dosage forms by derivative UV spectroscopy and liquid chromatography (HPLC). Journal of Pharmaceutical and Biomedical Analysis, 16(3), 431-438. [CrossRef]
20. Grover, P., Bhardwaj, M., Mukherjee, D. (2022). Identification and characterization of forced degradation products of sertraline hydrochloride using UPLC, ultra-high-performance liquid Chromatography-Quadrupole-Time of flight mass spectrometry (UHPLC-Q-TOF/MS/MS) and NMR. Journal of Pharmaceutical and Biomedical Analysis, 221, 115045. [CrossRef]
21. Ghane, M., Mohadesi, A., Ezoddin, M., Ali Karimi, M., Abdi, K. (2022). Dispersive liquid–liquid microextraction with back extraction based on in situ deep eutectic solvent decomposition and air-assisted for determination of some antidepressant drugs in biological samples prior to HPLC-UV. International Journal of Environmental Analytical Chemistry, 1-15. [CrossRef]
22. Kovatsi, L., Manousi, N., Papadoyannis, I. (2016). Direct UHPLC-DAD method to determine asenapine, paroxetine and fluvoxamine in human blood serum, urine and cerebrospinal fluid. Current Pharmaceutical Analysis, 12(4), 349-356.
23. Fernández, P., Taboada, V., Regenjo, M., Morales, L., Alvarez, I., Carro, A.M., Lorenzo, R. (2016). Optimization of ultrasound assisted dispersive liquid-liquid microextraction of six antidepressants in human plasma using experimental design. Journal of Pharmaceutical and Biomedical Analysis, 124, 189-197. [CrossRef]
24. Budău, M., Hancu, G., Muntean, D.L., Papp, L.A., Cârje, A.G., Garaj, V. (2020). Enantioseparation of citalopram enantiomers by capillary electrophoresis: Method development through experimental design and computational modeling. Chirality, 32(8), 1119-1128. [CrossRef]
25. Abdel-Raoof, A.M., Hasan, M.A., Madkour, A.W., Abdel-Fattah, A., Eissa, M.S. (2021). Simultaneous determination of citalopram and tadalafil by the second derivative synchronous fluorescence method in biological fluids; application of Box-Behnken optimization design. Luminescence, 36(1), 57-65. [CrossRef]
26. Yılmaz, H., Üstün, Z., Demiralay, E.Ç. (2016). RPLC determination of acid dissociation constants and quantitative estimation for sulfasalazine. Journal of the Iranian Chemical Society, 13, 103-110. [CrossRef]
27. Yılmaz, H., Demiralay, E.Ç. (2015). Investigation of chromatographic behavior of leflunomide and its identification in pharmaceutical dosage form by reversed phase liquid chromatography. Journal of Liquid Chromatography & Related Technologies**,** 38(1), 97-103. [CrossRef]
28. Miguel, L., Barbas, C. (2003). LC determination of impurities in azithromycin tablets. Journal of Pharmaceutical and Biomedical Analysis, 33(2), 211-217. [CrossRef]
29. Volná, T., Motyka, K., Hlaváč, J. (2017). RP-HPLC determination of dissociation constant using solely aqueous mobile phase. Journal of Pharmaceutical and Biomedical Analysis, 134, 143-148. [CrossRef]
30. Barbosa, J., Bergés, R., Sanz-Nebot, V., Toro, I. (1999). Chromatographic behavior of ionizable compounds in liquid chromatography. Part 2. Standardization of potentiometric sensors and effect of pH and ionic strength on the retention of analytes using acetonitrile-water mobile phases. Analytica Chimica Acta, 389(1-3), 43-52. [CrossRef]
31. Agrafiotou, P., Ràfols, C., Castells, C., Bosch, E., Rosés, M. (2011). Simultaneous effect of pH, temperature and mobile phase composition in the chromatographic retention of ionizable compounds. Journal of Chromatography A, 1218(30), 4995-5009. [CrossRef]
32. Vonaparti, A., Kazanis, M., Panderi, I. (2006). Development and validation of a liquid chromatographic/electrospray ionization mass spectrometric method for the determination of benazepril, benazeprilat and hydrochlorothiazide in human plasma. Journal of Mass Spectrometry, 41(5), 593-605. [CrossRef]
33. Paszun, S.K., Stanisz, B., Pawłowski, W. (2012). Rapid and simple stability indicating HPLC method for the determination of cilazapril in pure substance and pharmaceutical formulation in comparison with classic and derivative spectrophotometric methods. Acta Poloniae Pharmaceutica, 69(2), 193-201.
34. Swartz, M.E., Krull, I.S. (2012). Handbook of analytical validation (1st edition), CRC Press, Boca Raton, p.220.