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ORIGINAL RESEARCH PAPER

A novel HPLC method for selexipag in human plasma and application to a prototype pharmacokinetic study

Burhan Ceylan[1]*, Gizem Tırıs[2], S. Evrim Kepekci Tekkeli[3], Cem Önal[3] and Armağan Önal[4]

[1] Department of Pharmacognosy, Faculty of Pharmacy, Harran University, 63300 Sanlıurfa, Turkey

[2] Department of Analytical Chemistry, Faculty of Pharmacy, Bezmialem Vakif University, 34093 Fatih-Istanbul, Turkey

[3] Department of Analytical Chemistry, Faculty of Pharmacy, Istanbul Health and Technology University, Istanbul, Turkey

[4] Department of Analytical Chemistry, Faculty of Pharmacy, İstanbul University, 34116 Beyazit-Istanbul, Turkey

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ABSTRACT

A novel simple and cost effective HPLC technique was presented for the quantification of selexipag (SLP) in human plasma sample and the technique's applicability to a pharmacokinetic investigation. Chromatographic separation was achieved with C18 (5 µm × 4.6 mm × 150 mm) column, at 30 °C with isocratic elution, mobile phase composed of solution A (acetonitrile), and solution B (0.5% formic acid) (65:35 v/v) at flow rate 1.2 mL min^{-1}. The linearity range is 10–150 ng mL^{-1}. As sample preparation step human plasma was precipitated with acetonitrile and the detection was provided at 300 nm. The retention time is 8.20 ± 0.02 min. LOD is found to be 3.3 ng mL^{-1} for drug. The method was applied to the analysis of SLP in human plasma with good recovery as 97.83%. Validation of the studied methods was carried out according to EMA guideline. The new method applied on a prototype pharmacokinetic study by administration of 800 µg SLP to a healthy volunteer and parameters like AUC_{0-24}, $AUC_{0-\infty}$, C_{max}, t_{max}, and $t_{1/2}$ were assessed.

KEYWORDS

selexipag, HPLC-UV, pharmacokinetics, human plasma, pulmonary arterial hypertension

INTRODUCTION

Pulmonary arterial hypertension (PAH) is a disorder in which blood pressure is high in the blood vessels that carry blood from the heart to the pulmonary arteries. These arteries constrict in people with PAH, and the heart needs to work harder to pump blood through them to the lungs. This can make a person feel exhausted, dizzy, and short of breath, among other symptoms. Selexipag (SLP) is used for the long-term treatment of PAH in adult patients whose disease is not adequately controlled with other pulmonary arterial hypertension (PAH) drugs known as endothelin receptor antagonists or phosphodiesterase type 5 inhibitors. SLP dilates the pulmonary arteries by acting similarly to prostacyclin [1]. This makes it easier for the heart to pump blood into the pulmonary arteries. SLP lowers the pressure in the pulmonary arteries, relieves the symptoms of PAH and slows the progression of PAH disease [2, 3]. Recently an intravenous (IV) injection of SLP has been approved by FDA; Uptravi® for injection is supplied as a 10 mL single-dose vial containing 1,800 µg of

*Corresponding author. Department of Pharmacognosy, Faculty of Pharmacy, Harran University, 63050 Haliliye-Sanlıurfa, Turkey.
E-mail: burhanceylan@harran.edu.tr, b.ceylan022@gmail.com

SLP [4]. Molecular formula of SLP is 2-(4-((5,6-diphenylpyrazin-2-yl)(isopropyl)amino)butoxy)-N-(methylsulfonyl) acetamide. Chemical structure of SLP is shown in Fig. 1.

There are methods for the determination of SLP and its related metabolites in biological samples an at rat plasma. However there is a lack in the literature for the determination of SLP in human plasma by the high pressure liquid chromatography (HPLC) method. Existing method are not useful enough in the analysis of selexipag due to the expensive detection requirement, long sample preparation procedures, and low sensitivity. In addition, there are not enough methods that provide pharmacokinetic studies to be easily applicable for the determination of SLP in human plasma. Review of literature revealed that few methods were reported for the estimation of SLP including; spectrophotometric methods [5–7], gas chromatograhy [8] and chromatographic methods [9–12] in dosage form, in addition to, LC-MS/MS methods in plasma [13–17].

Validation experiments were carried out in accordance with EMA guideline [18], based on the chromatographic conditions and calibration. The determination for pharmacokinetic study was applied with the plasma specimen of a volunteer who is in good health with the agreement of the ethics committee after administration of 800 µg SLP.

The presented study provides simple and sensitive quantification of the SLP in human plasma sample and reveals the pharmacokinetic parameters as a prototype phase 1 clinical study.

MATERIAL AND METHODS

Used chemicals

SLP was obtained from Sigma Aldrich, St. (Louis, Missouri, USA). Formic acid, methanol and acetonitrile were obtained from Merck, Darmstadt, Germany. Human (Japan) ultrawater purification system was used to gain ultra pure water. Uptravi® tablets (Excella GmbH & Co. KG/Feucht/Germany) were purchased from local drug store.

Preparation of drug solution and plasma

The stock standard solution of SLP was dissolved in acetonitrile at 100.0 µg mL^{-1} concentration. It diluted with acetonitrile for working solutions. The stock standard and working solutions were kept at +4 °C and were remained stable throughout the research.

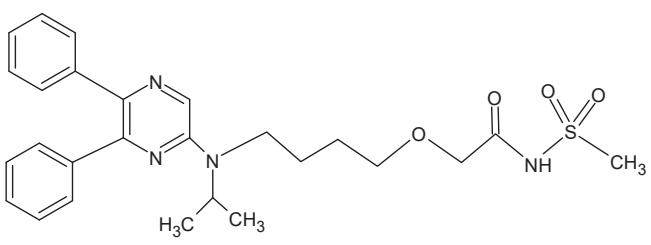

Fig. 1. Chemical structure of SLP

By spiking the different concentration of working solution to the blank plasma, the calibration graph was created. The calibration range was 10–150 ng mL^{-1}.

One healthy volunteer received a single oral dosage of SLP (800 µg). Blood was taken before medication and then again 1, 1.5, 2, 4, 6, 8, 10, and 24 h afterwards. Each blood sample was obtained in an EDTA tube and centrifuged for 15 min at 4,000 rpm. The plasma specimen was kept at −20 °C.

Instrument and chromatographic condition

HPLC experiments were done on A Shimadzu LC 20 (Japan). This chromatographic system is equipped with a LC-20AT pump, SIL-20A HT autosampler, RF-20A UV detector, and CTO 10 AC column oven. It temperature was 30 °C. Labsolutions® software and Inertsil® ODS-3 (5 µm, 150 mm × 4.6 mm) column (Shimadzu Corporations-Japan) was used for analysis.

In order to find most appropriate method different conditions were tried such as C8 and C18 column, different flow rate. The separation was C18 column with flow rate of 1.2 mL min^{-1}. The mixture of acetonitrile: 0.5% formic acid (65:35 v/v) were used as mobile phase. The specimen was injected into the column at 10.0 µL. A detection wavelength of 300.0 nm.

Precipitation procedure

Acetonitrile was added in the spiking and real human plasma in tube as ratio of plasma: acetonitrile 1:2. Then the tube was vortex-mixed for half a minute and the specimen was centrifuged at 4,100 rpm 15 min. 10 µL of the supernatant was given to HPLC.

Validation of the method

The following guidelines from the European Medical Agency (EMA) were used to verify the new method [18].

Linearity and limits of detection and quantification

The calibration graph was created with standard samples prepared ranging between 10 and 150 ng mL^{-1} of SLP. The peak area versus SLP concentration calibration curve was constructed. Limit of detection (LOD) is minimum concentration of a component in a specimen analyte that can be detectable but not quantifiable in at acceptable levels. Another parameter is limit of quantification (LOQ). It is the minimum concentration of analyte that can be quantified. The limit of detection (LOD) and limit of quantification (LOQ) were determined using the formula: LOD or LOQ = k × SDa/b, where k = 3 for LOD and 10 for LOQ, SDa is the standard deviation of the intercept, and b is the slope. The parameters for the analytical performance of the proposed method are summarized in Table 1.

Selectivity of the proposed method

Selectivity is defined as if the analysis can be carried out properly in the presence of components that may affect analysis or interfere with the substance. Throughout this study, these factors did not affect the result of the analysis.

Table 1. System suitable parameters of the method

Capacity factor	Resolution	HETP (μm)	Tailing factor	Plate number
7.21	2.27	2.89	1.17	3,989

Accuracy

Different three concentrations named as quality control samples (QC) (10.0, 50.0 and 100.0 ng mL^{-1}) of standard solutions were spiked containing a fixed amount of SLP (50.0 ng mL^{-1}) human plasma. The prepared plasma sample was precipitated by adding acetonitrile. Then the experiment was done as explained in the part precipitation procedure. Recovery of the substance was calculated.

Precision of the method

Precision refers to the analysis can be done with same samples or solvents under the same condition in different hours or different days. Our precision studies were carried out with intraday and interday tests.

Robustness of the method

The robustness of the technique was assessed by varying the flow rate, composition, and column temperature. The mobile phase ratios were altered from 70:30 (v/v) (acetonitrile:% formic acid) to 60:40 where as 65:35 was the original ration. Flow rate and column temperature were changed 1.1 and 1.3 mL min^{-1} and 25, 35 °C, respectively.

Stability

The stability of working standard SLP solutions were tested at several storage conditions at QC levels and as three replicated. The trialed storage conditions are keeping at dark and at room temperature for 24 h, keeping in autosampler conditions for 24 h and keeping in refrigerated at 4 °C for 1 month. Recovery values for the trialed conditions are as follows; 94.2 ± 1.2%, 97.5 ± 1.5%, 95.4 ± 1.7% respectively. The highest RSD % for all these experiments was 3.35%. It is possible to mention under all tested conditions SLP were found to be stable.

RESULTS AND DISCUSSION

Chromatographic process

An isocratic elution system was used to achieve a good separation, as mentioned above. Figure 2 shows illustrative chromatograms of blank plasma (a), spiked plasma samples (b) and plasma samples from a volunteer who received Uptravi ® tablets containing 800 μg SLP at t_{max} (c). The plasma contents did not cause any interference. SLP has a retention time of 8.2 min.

Table 1 displays the system suitability parameters, which represent the separation process' quality.

Validation of the method

Linearity and sensitivity. The calibration graph was constructed with spiked plasma as 10–150 ng mL^{-1}. It was created by reading the area versus the concentration of the active ingredient. The experiments were carried out in triplicate.

The formula LOD = 3 × SDa/m and LOQ = 10 × SDa/m were used to compute the limit of detection (LOD) and limit of quantitation (LOQ). SDa was the standard deviation of the intercept, and m was the slope. The calibration graph's parameters were given in Table 2.

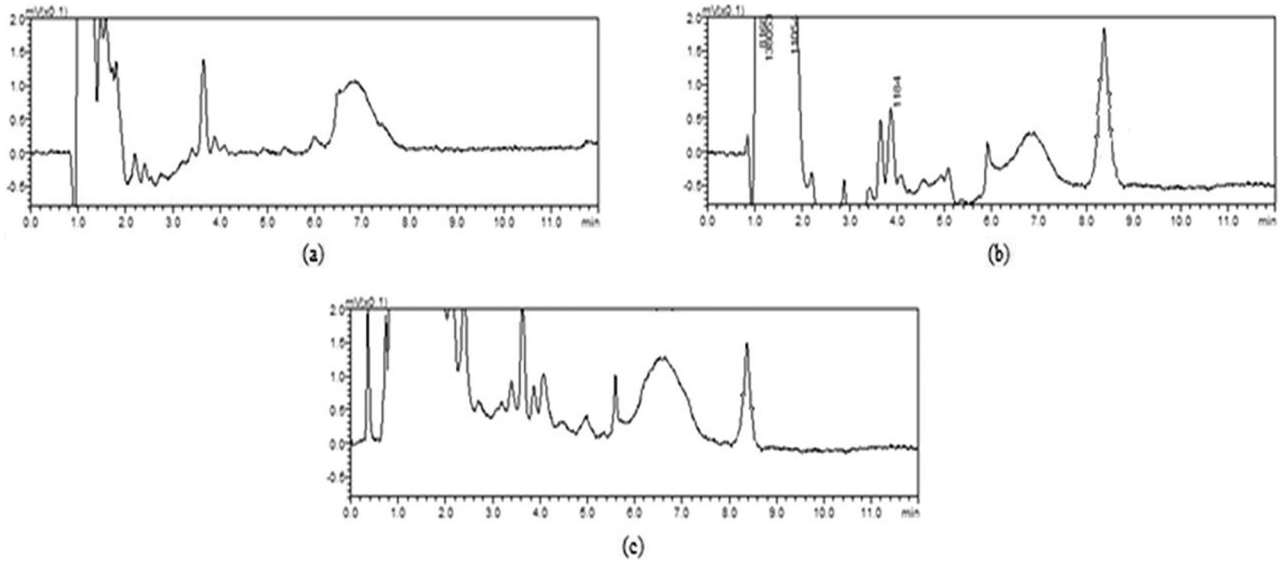

Fig. 2. Blank plasma (a), spiked plasma sample (50 ng mL^{-1}) (b), and plasma samples from a volunteer (C_{max}) (c)

Table 2. The parameter of the calibration graph

Parameters	Method
Concentration range[a] (ng mL^{-1})	10.0–150.0
Regression equation[b]	
Intercept ± SD	58.09 ± 5.04
Slope ± SD	13,875 ± 10.11
Correlation coefficient (r^2)	0.9951
LOD (ng mL^{-1})	3.3
LOQ (ng mL^{-1})	10

[a] Average of six determinations.
[b] $y = xC + b$ where C is the concentration in ng mL^{-1} and y is the peak area.

Accuracy and precision

The specimens were determined at three different concentration levels to calculate precision and accuracy value. In plasma, QC samples were prepared (10, 50, and 100 ng mL^{-1}) as low, medium, and high concentrations (n = 3). Standard addition is one of the methods used to calculate recovery. One of the approaches for calculating recovery is standard addition. Three duplicate specimen at each QC concentration were tested the different experiments on the same day for intraday and on three different days for interday precision to demonstrate the precision of the established procedure. The results of these experiment are given in Table 3.

Robustness

Robustness studies were determined by doing minor changes to the flow, column temperature, and mobile phase ratio. The proportions of the mobile phase were adjusted from (65:35, v:v) to 70:30 and 60:40; the oven temperature was altered from 30 °C to 25 °C and 35 °C; and the flow rate was altered from 1.1 to 1.3 mL min^{-1}. The peak areas were unaffected by these modifications. The results were shown in Table 4. The recovery% values 104.45, 106.29 and 108.45 are better than the values in our previous study [19].

Pharmacokinetic analysis

The proposed method was utilized in human plasma for the pharmacokinetic study. SLP was given to a 42-year-old healthy volunteer in single oral dosages (800 µg). Before the analysis, 5 mL of venous blood samples were taken different hours. The plasma samples were studied as above explained. A chromatogram of the plasma specimen collected 1 h after the volunteer's oral intake of 800 mg SLP is shown in Fig. 3. The specimens were stored in the refrigerator at −20 °C. The pharmacokinetic parameters listed in Table 5 were calculated. The TOPFIT 2.0 pharmacokinetic and pharmacodynamic data analysis system [20] were used to quantify the area under the plasma concentration–time curves ($AUC_{0\text{-}24}$,

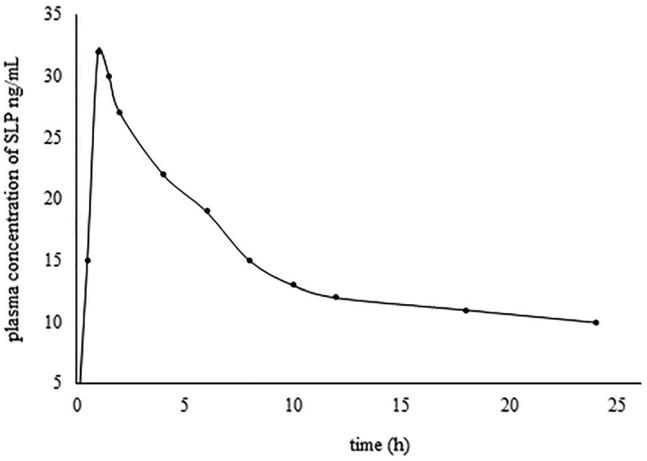

Fig. 3. Pharmacokinetic curve of SPL in human plasma after administration of 800 µg SLP

Table 3. The results of the accuracy and precision studies

Existent concentration (ng mL^{-1})	Added concentration (ng mL^{-1})	Found concentration (ng mL^{-1}) (Mean ± SD[1])	Recovery (%)	RSD of recovery	RSD of intraday variation	RSD of interday variation
	10.0	58.7 ± 0.42	97.83	3.26	3.67	5.91
50	50.0	90.53 ± 2.24	90.53	3.81	4.52	5.90
	100.0	146.40 ± 3.54	97.60	4.33	5.62	4.83
		Mean relative recovery = 95.32				

For each concentration n = 3.

Table 4. Results of the robustness

Condition	Value	Recovery %	RSD %
Flow rate (mL min^{-1})	1.3	104.45	1.76
	1.1	106.22	1.31
Mobile phase composition	60:40	106.29	2.43
(methanol:aqueous phase)	70:30	107.61	3.21
Column temperature	25	108.45	2.53
	35	109.93	4.53

fn = 3 for all QC sample levels.

Table 5. The results of pharmacokinetic parameters

Parameter	Found value
T_{max}[a] (h)	1
C_{max}[b] (ng mL^{-1})	13.2
$t_{1/2}$[c] (h)	4.7
$AUC^{d}_{0\text{-}24}$ (ng h mL^{-1})	422.5
$AUC^{d}_{0\text{-}\infty}$ (ng h mL^{-1})	634.7

[a]Time to maximum concentration.
[b]Maximum concentration.
[c]Elimination half life.
[d]Area under the concentration-time curve.

$AUC_{0\text{-}\infty}$). Figure 3 illustrates the plasma concentration–time curve of SLP.

The previously developed methods [5–11] provide assays in bulk and pharmaceutical forms, they are not suitable for plasma assays and their sensitivity is not enough for bioanalytical studies. Other MS methods; LC-MS/MS [12, 13, 15–17] and UPLC-MS/MS [14] were sensitive but they are for rat plasma samples. The proposed method provides sensitive, cost reduced and simple assay of SLP in human plasma.

CONCLUSIONS

SLP is a relatively novel active substance for the treatment of pulmonary arterial hypertension. Existing methods for determination from plasma in the literature provide assays in rat plasma [7]. Human plasma analysis that provides quantitation in pharmacokinetic levels is a requirement for SLP. The developed and validated HPLC method provides fast, cost reduced and simple determination for SPL and has been successfully applied for the quantification of the SLP in human plasma. The rapid application of sample preparation is one of the advantages of the method. In this way, the method can be applied practically in routine analysis, bio-equivalence and bioavailability studies.

Conflict of interest: The author declares no conflict of interest.

Ethical approval: All procedure performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Bezmialem Vakıf University approved by the Clinical Trials Ethic Commitee (No: 2022/33).

Informed consent: Informed consent was obtained from all individual participants included in the study.

REFERENCES

1. Galiè, N.; Torbicki, A.; Barst, R.; Dartevelle, P.; Haworth, S.; Higenbottam, H. O.; Peacock, A.; Pietra, G.; Rubin, L. J.; Simonneau, G.; Priori, S. G.; Garcia, M. A. A.; Blanc, J-J.; Budaj, A.; Cowie, M.; Dean, V.; Deckers, J.; Burgos, E. F.; Lekakis, J.; Lindahl, B.; Mazzotta, G.; McGregor, K.; Morais, J.; Oto, Ali.; Smiseth, O. A.; Barbera, J. A.; Gibbs, S.; Hoeper, M.; Humbert, Marc.; Naeije, R.; Pepke-Zaba, J.; Force, T. Guidelines on diagnosis and treatment of pulmonary arterial hypertension. The task force on diagnosis and treatment of pulmonary arterial hypertension of the European society of cardiology. *Eur. Heart J.* **2004**, *25*, 2243–78.
2. Simonneau, G.; Torbicki, A.; Hoeper, M. M.; Delcroix, M.; Karlócai, K.; Galiè, N.; Degano, B.; Bonderman, D.; Kurzyna, M.; Efficace, M.; Giorgino, R.; Lang, I. M. Selexipag, an oral, selective prostacyclin receptor agonist for the treatment of pulmonary arterial hypertension. *Eur. Respir. J.* **2012**, *40*, 874–80.
3. Kaufmann, P.; Hurst, N.; Astruc, B.; Dingemanse, J. Absolute oral bioavailability of selexipag, a novel oral prostocyclin IP receptor agonist. *Eur. J. Clin. Pharmacol.* **2017**, *73*, 151–6.
4. UPTRAVI® (selexipag) receives FDA approval for intravenous use in adult patients with pulmonary arterial hypertension (PAH). News release, Janssen Pharmaceutical Companies. **2021**, Accessed July 30, 1–25. https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/214275s000lbl.pdf https://www.accessdata.fda.gov/drugsatfda_docs/nda/2015/207947Orig1s000SumR.pdf.
5. Gorumutchu, G. P.; Ratnakaram, V. N. Oxidative coupling: a tranquil approach for determination of selexipag by visible spectrophotometry. *Oriental J. Chem.* **2018**, *34*, 3112–7.
6. Kotwal, T. S.; Patwardhan, D. M.; Amrutkar, S. S.; Wagh, M. P. Development and validation of simple UV spectrophotometric method for the determination of selexipag in API and its bulk dosage form. *Indo Am. J. Pharm. Res.* **2017**, *7*, 8578–89.
7. Ratnakaram, V. N. Diazo coupling for the determination of selexipag by visible spectrophotometry. *Int. J. Green. Pharm.* **2018**, *12*, 822–8.
8. Li, L.; Wang, H.; Tian, H.; Li, J.; Gao, S.; Ha, J. Determination of 6 residual solvents in selexipag by headspace gas chromatoprahy. *J. China Pharma. Univ.* **2018**, *6*, 93–6.
9. Damireddy, S.; Pravalika, K.; Praveen, M.; Sathish, G.; Anusha, M. Method development and validation of selexipag in its bulk and dosage form by RP-HPLC. *Inter. J. Pharma. Biol. Sci.* **2017**, *7*, 84–92.
10. Santosh, S. S.; Devi, N. K. Stability indicating method development and validation of selexipag in bulk and pharmaceutical dosage form by using RP-HPLC. *Pharm. Res.* **2021**, *5*, 1–15.
11. Satheshkumar, S.; Muruganantham, V. Analytical development and validation of HPTLC method for determination of selexipag in bulk and formulation. *WJPPS* **2021**, *10*, 1661–70.
12. Shah, P.; Hadiyal, S.; Dhaduk, B. Stability indicating LC-MS/MS method and validation of selexipag impurities and identification of its force degradation products. *Results Chem.* **2023**, *6*, 101022.
13. Rao, K. P.; Babu, N. L.; Koganti, K.; Palakeeti, B.; Srinivas, K. S. V. Related substances method development and validation of an LC-MS/MS method for quantification of selexipag and its related impurities in rat plasma and application to pharmacokinetic studies. *SN Appl. Sci.* **2021**, *3*, 1–12.
14. Xie, S.; Shi, L.; Chen, J.; Xu, R. A.; Ye, X. Simultaneous quantification and pharmacokinetic investigation of selexipag and its main metabolite ACT-333679 in rat plasma by UPLC-MS/MS method. *J. Pharm. Biomed. Anal.* **2020**, *190*, 113496.

15. Babu, N. L. A. A.; Koganti, K.; Palakeeti, B.; Srinivas, K. S. V.; Rao, K. P. Development of an efficient stability-indicating LC-MS/MS method for the analysis of selexipag and characterization of its degradation products. *Biomed. Chromatograp.* **2021**, *35*, 1661–70.
16. Bhadru, B.; Rao, V. V.; Vidhyadhara, S. Development and validation of bioanalytical method for the quantitative estimation of selexipag in biological matrices using LC-MS/MS. *J. Pharm. Sci. & Res.* **2019**, *11*, 2722–7.
17. Satheshkumar, S.; Muruganantham, V. A rabit method for quantification of selexipag in human plasma using high performance liquid chromatography with electron spray ionzation tendem mass spectrometry. *Ann. R.S.C.B.* **2021**, *25*, 5689–707.
18. EMA (2011) European Medicines Agency (EMA)/Committee for Medicinal Products for Human Use (CHMP). Guideline on Bioanalytical Method Validation, EMA/CHMP, London, United Kingdom.
19. Ceylan, B.; Tekkeli, E. K.; Önal, C. Development of an HPLC method for the determination of mesalazine in human plasma by fluorimetric derivatization and application to a prototype pharmacokinetic study. *J. Fluoresc.* **2022**, *32*, 319–25.
20. Heinzel, G.; Woloszczak, R.; Thomann, S. *Version TOPFITT 2.0 Pharmacokinetic and Pharmacodynamic Data Analysis System for the PC*; VCH Publishers: Gustav- Fisher-Verlag: Stuttgart, Germany, **1993**.