chemistry proceedings

MDPI

Proceeding Paper

Development and Validation of RP-UHPLC Method for Determination of Sertraline in Bulk Drug and Dosage Form †

Vaishnavi Chaudhari [1],*, Sunila Patil [1], Sulbha Patil [2] and Sunil Pawar [1]

[1] Department of Quality Assurance, P.S.G.V.P.Mandal's College of Pharmacy, Dist-Nandurbar, Shahada 425409, Maharashtra, India; sunila_patil@rediffmail.com (S.P.); sppawar75@gmail.com (S.P.)
[2] Department of Pharmaceutics, P.S.G.V.P.Mandal's College of Pharmacy, Dist-Nandurbar, Shahada 425409, Maharashtra, India; sulbha.pharma@gmail.com
* Correspondence: vaishnavi4542@gmail.com; Tel.: +91-9604171788
† Presented at the 26th International Electronic Conference on Synthetic Organic Chemistry, 15–30 November 2022; Available online: https://ecsoc-26.sciforum.net/.

Abstract: Objective: The new, rapid, sensitive, simple, precise and accurate Reversed-Phase Ultra High Performance Liquid Chromatography (RP-UHPLC) method was developed and validated for determination of Sertraline in bulk drug and Pharmaceutical dosage form. Method: The UV Spectrum of Sertraline in water showed maximum wavelength at 273 nm. In RP-UHPLC method separation achieved by Agilent C18 (75 mm × 3.9 mm, 2 µm particle size) column using Acetonitrile: (0.1% OPA) Water (80:20 *v*/*v*) as mobile phase at flow rate 0.7 mL/min. Injection volume was 20 µL. RP-UHPLC detection carried out at 273 nm. Results: In RP-UHPLC method retention time was found to be 3.75 min. The Calibration curve was found to be linear ($r^2 = 0.999$) with concentration range of 10–50 µg/mL. The Accuracy (% recovery) for Sertraline was found to be 99–100%. The % RSD (intra-day and inter precision) values are not more than 2% hence the developed method is accurate and precise. The LOD and LOQ were found to be 0.2085 µg/mL and 0.6321 µg/mL respectively. Conclusion: The developed method was validated with respect to linearity, accuracy, precision, repeatability, robustness, LOD and LOQ as per ICH guidelines. The proposed method was used for routine analysis of Serraline in Bulk Drug and Solid Dosage form.

Keywords: Sertraline; antidepressant agent; method development; method validation; UV-Spectrophotometer; RP-UHPLC

Citation: Chaudhari, V.; Patil, S.; Patil, S.; Pawar, S. Development and Validation of RP-UHPLC Method for Determination of Sertraline in Bulk Drug and Dosage Form. *Chem. Proc.* **2022**, *4*, x. https://doi.org/10.3390/xxxxx

Academic Editor(s): Julio A. Seijas

Published: 15 November 2022

1. Introduction

Ultra-high performance liquid-chromatography (UHPLC) covers liquid chromatography separations implementing columns enclose particles smaller than the 2.5–5 µm sizes typically used in high-performance liquid chromatography (HPLC) [1]. UHPLC work on the same assumption as that of HPLC and of which governing principle is that, as column packing particle size decrease, efficiency and thus resolution increases. Separation using column contains smaller particles display enhance efficiency per unit time. High strength silica (HSS) is another type of column used in UHPLC. In UHPLC, high pore volume UHPLC particles do not acquire the mechanical stability necessary to hold up the high pressure innate of UHPLC separations. For that, there is established a novel silica particle and appropriate morphology required to give long and lifetime efficiency UHPLC column at high pressure likely 1000 bars [2].

Analytical methods development and validation are the continuous and inter-dependent task associated with the research and development, quality control and quality assurance department. Analytical method development is the process of selecting an accurate assay procedure to determine the composition of formulation [3].

Method validation is the process of demonstrating that an analytical method is suitable for its intended use, and involves conducting a variety of studies to evaluate method performance under defined conditions. The most compelling reasons to optimize and validate pharmaceutical productions and supporting processes are quality assurance and cost reduction [4].

Sertraline hydrochloride is a selective serotonin reuptake inhibitor (SSRI) for oral administration. Chemical name is (1S-cis)-4-(3, 4-dichlorophenyl)-1, 2, 3, 4-tetrahydro-Nmethyl-1-naphthalenamine hydrochloride. The empirical formula is $C_{17}H_{17}NCl_2$.HCl. Molecular weight of Sertraline is 306.229 g/mol. Sertraline is soluble in organic solvent like methanol, ethanol, DMSO. It is also soluble in water and acetonitrile. Sertraline is a popular anti-depressant medication commonly known for its selective serotonin reuptake inhibitor (SSRI) activity, and is similar to drugs such as Citalopram and Fluoxetine. Sertraline inhibits the reuptake of serotonine (5-HT) at the presynaptic neuronal membrane, thereby increasing serotonergic activity. This result in an increased synaptic concentration of serotonine in the CNS, which leads to numerous functional changes associated with enhanced serotonergic neurotransmission. Sertraline is effective for panic disorder, social anxiety disorder, generalized anxiety disorder, and obsessive compulsive disorder (OSD) [5]. The chemical structure of sertraline is shown in Figure 1.

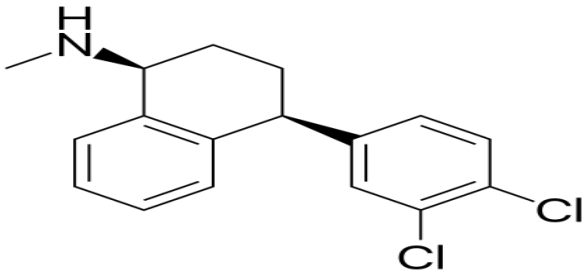

Figure 1. Structure of Sertraline.

The review of literature includes RP-HPLC [6–8], UV-Spectroscopy [9], HPTLC [10,11], UPLC [12], UPLC MS-MS [13], LC MS-MS [14], Stability indicating methods for determination of Sertraline either in individually or in combination with other drugs also in pharmaceutical dosage form. From the literature survey observed that UPLC method was developed for determination of Sertraline in biological fluid and not in mobile phase hence, the aim of the present work is to develop a simple, precise, and economical RP-UHPLC method for determination of Sertraline in bulk drug and dosage form. The RP-UHPLC method used the column having particle size 2 μm which having high resolution power than HPLC method. The developed method was validated for linearity, accuracy, precision, repeatability, robustness, LOD and LOQ.

2. Materials and Method

2.1. Materials

2.1.1. Chemicals and Reagents Used

Sertraline pure drug was a gift sample from Swapnroop drugs and Pharmaceuticals, Aurangabad. For UV-Spectroscopic determination water (HPLC grade) was used as solvent. HPLC grade Acetonitrile, methanol and 0.1% OPA was used for RP-UHPLC determination of Sertraline. Marketed formulation of Sertraline (SERTA-50 50 mg) was purchased from local pharmacist.

2.1.2. Instruments Used

A double beam UV-Spectrophotometer (Systronic-2201) was used for recording of spectrum and absorbance. The light source used is Deuterium lamp of Spectrophotometer,

a computer is attached which help in data processing. A Quartz cuvette with path length 1 cm was used. The HPLC analysis carried out on instrument (Agilent Technologies® Gradient System with Auto injector) with Reverse phase (Agilent) C18 column (75 mm × 3.9 mm, 2 μm particle size), SP930D pump, and 20 μL injection loop were used. Weighing balance (WENSAR™ High Resolution Balance), Sonicator (Ultrasonic electronic instrument) were used.

2.2. Method Development

2.2.1. Solubility Studies

This study was carried out to find an ideal solvent in which drugs are completely soluble. Various solvents were tried for checking solubility of Sertraline. From solubility studies it was concluded that of Sertraline is freely soluble in Acetonitrile, methanol and poorly soluble in water hence adjusted with 0.1% Orthophosphoric Acid, Buffer pH 3.

2.2.2. Chromatographic Conditions

The method was developed by using Agilent C18 (75 mm × 3.9 mm, 2 μm partical size) column with mobile phase Acetonitrile: (0.1% OPA) water (80:20). Flow rate was maintained 0.7 mL/min. The sample injection volume was 20μL and detected at 273 nm. The Optimized chromatographic conditions are shown in Table 2.

2.2.3. Preparation of 0.1% Orthophosphoric Acid Water

0.1% OPA acid prepared by transferring 0.1 mL of Orthophosphoric acid in 100 mL of volumetric flask and volume made up to the mark with water to obtained 0.1 % Orthophosphoric acid.

2.2.4. Preparation of Mobile Phase

Mobile phase was prepared by mixing the Acetonitrile and 0.1% OPA water in the ration of 80:20% *v/v* and the mixture degasified by vacuum filtration using 0.45 μ filter and sonication. Mobile phase used as diluent for the preparation of standard stock and working solutions.

2.2.5. Preparation of Standard Stock Solution

10 mg of Standard Sertraline was accurately weighed and transferred to 10 mL volumetric flask. Methanol was added up to the mark and sonicated for 15 min to dissolve to obtained 1000 μg/mL solution. It was filtered through 0.45 μ membrane filter.

2.2.6. Determination of Absorption Maxima

From Standard stock solution (1000 μg/mL) 0.2 mL was pipette out and transferred into 10 mL volumetric flask and volume made up to the mark with water to obtained 2 μg/mL solution. It was filtered through 0.45 μ membrane filter. This solution was scanned in the range of 200–400 nm for the analysis of absorption maxima of Sertraline.

2.2.7. Assay of Marketed Formulation

20 tablets of Sertraline were weighed and crushed into fine powder. A quantity of powder equivalent to10 mg of sertraline 13.66 mg transferred into 10 mL volumetric flask and sonicated to dissolve completely then volume made up to the mark with diluent to obtained 1000 μg/mL of solution. It was filtered through 0.45 μ membrane filter. From above solution 0.3 mL added to volumetric flask and made up to the mark with water to obtained 30 μg/mL solution for assay.

3. Results

3.1. UV-Spectrometric Determination (Absorption Maxima)

The Standard solution of Sertraline (2 µg/mL) was prepared from standard stock solution and scanned in the range of 200–400 nm and UV Spectrum was recorded. The absorption spectral analysis shows the maximum wavelength 273 nm.

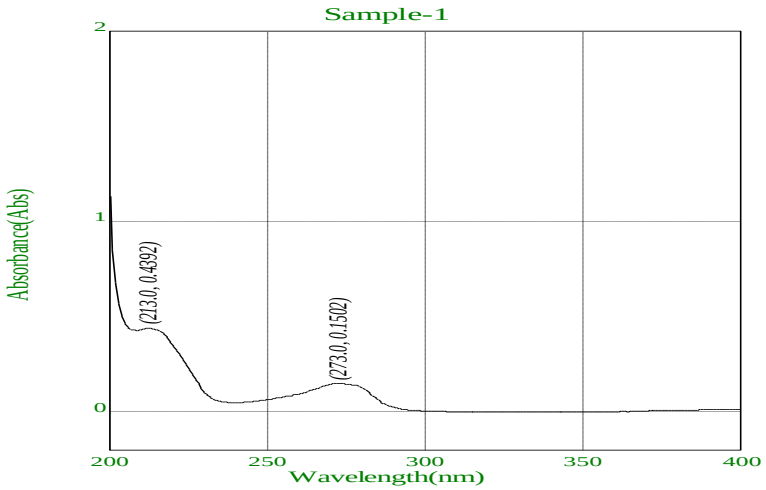

Figure 2. UV Spectrum of Sertraline at maximum wavelength 273 nm.

3.2. Method Optimization

Many trials have been performed by various mobile phases, flow rate, and stationary phase. After observing theoretical plates and stability factor, various chromatographic parameters were chosen. To optimize the HPLC method parameters, mobile phase ratios of different solvents were tried. Good separation and peak symmetry for Sertraline were developed with combination of Acetonitrile and 0.1% OPA water in the ratio of 80:20% *v*/*v*. System flow rate was confirmed as 0.7 mL/min. The peak was eluted at the retention time of 3.75 min at 273 nm wavelength.

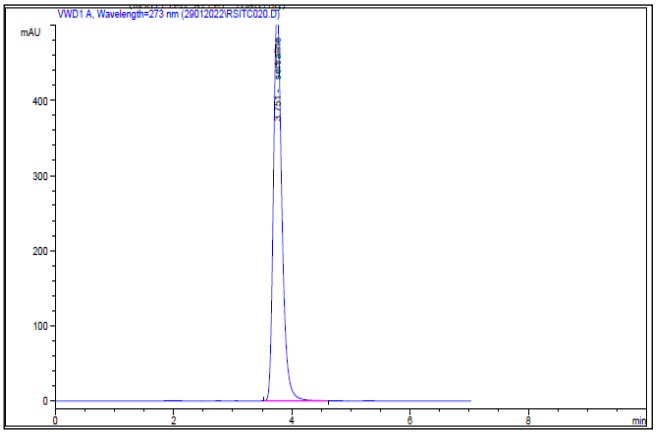

Figure 3. Optimized chromatogram for Sertraline.

Table 1. Optimized chromatographic parameters.

Parameters	Description
Stationary phase	Agilent C18 (75 mm × 3.9 mm, 2 µm particle size)
Mobile phase	Acetonitrile: (0.1%OPA) water 80:20 *v*/*v*
Flow rate	0.7 mL/min
Detection wavelength	273 nm

Injection volume	20 µL
Run time	10 min

OPA-Orthophosphoric Acid.

3.3. Method Validation

The method was validated for linearity, precision, accuracy, system suitability, robustness, ruggedness, LOD and LOQ according to ICH guidelines Q2.

3.3.1. Linearity and Range

Linearity is the ability of the method to elicit test results that is directly proportional to the concentration within a given range. It is generally reported as variance of slope or regression.

Accurately measured standard solution of sertraline (0.1, 0.2, 0.3, 0.4, and 0.5 mL) were transferred to series of 10 mL volumetric flasks and diluted to the mark with mobile phase to obtained the concentration range of 10–50 µg/mL. 20 µL of sample solution injected into the chromatographic system and chromatogram were recorded. Calibration curve was constructed by plotting Area versus Concentration of Sertraline and regression equation was calculated and shown in Table 3.

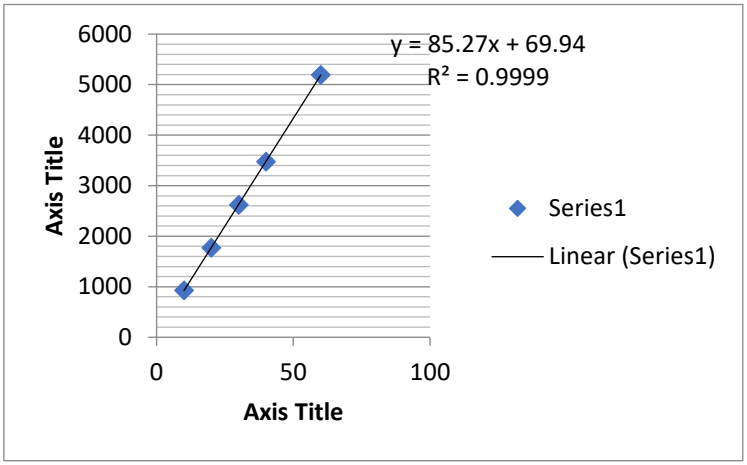

Figure 4. Calibration curve for Sertraline.

Table 2. Calibration curve results for Sertraline.

Concentration (µg/mL)	Area Sertraline
10	928.78
20	1773.78
30	2623.22
40	3477.225
50	5191.23

Table 3. Regression equation data for Sertraline.

Regression Equation Data Y = mx + c	
Slope (m)	85.27
Intercept (c)	69.94
Correlation Coefficient	0.999

3.3.2. Accuracy

The parameter accuracy is the extent to which the experimental results deviate from the expected results. The accuracy of method was determined by calculating recoveries of

Sertraline by Standard addition method. A known amount of drug (80, 100, and 120%) was added to pre analyzed sample solution. Good recovery of the spiked drug was obtained at each added concentration, indicating that the method was accurate. The results for accuracy studies for Sertraline are shown in Table 4. The %RSD was less than 2.

Table 4. Accuracy (% recovery) results for Sertraline by RP-UHPLC method.

Drug	Level (%)	Amount Taken (µg/mL)	Amount Added (µg/mL)	* Area	* Amount. Recovered (µg/mL)	* %Recovery ± S.D.	%RSD
Sertaline	80%	10	8	1605.40	8.0	100.06 ± 0.98	0.97
	100%	10	10	1772.64	9.97	99.73 ±0.15	0.15
	120%	10	12	1941.34	11.94	99.97 ± 0.27	0.27

* Mean of two observations, SD-Standard deviation.

3.3.3. Precision

The precision of the method is degree of agreement among individual test results when the procedure is applied repeatedly to the multiple samplings. Precision of the method was studied as Inter-day precision and Intra-day precision. Intra-day precision was determined by analyzing 20, 30 and 40 µg/mL of Sertaline solution for RP-UHPLC method for two times in the same day. And Inter-day precision was determined by analyzing 20, 30 and 40 µg/mL of Sertaline solution for two days daily and results were recorded.

Intraday and Interday precision for Sertraline shows the high precision % amount in between 99% to 100%. Results are shown in Table 5.

Table 5. Intra-day and Inter-day precision results for Sertraline by RP-UHPLC method.

Drug	Concentration (µg/mL)	Intraday Precision			Interday Precision		
		* Peak Area ± SD	* %Amount Found	%RSD	Peak Area± SD	%Amount Found	%RSD
Sertaline	20	1778.58 ± 3.86	100.27	0.21	1774.80 ± 2.21	99.95	0.12
	30	2611.15 ± 11.40	99.33	0.44	2619.10 ± 4.41	99.63	0.17
	40	3461.64 ± 2.10	99.43	0.06	3465.77 ± 4.54	99.55	0.13

* Mean of two observations, SD-Standard deviation, %RSD-Relative standard deviation.

3.3.4. Repeatability (System Suitability)

The repeatability of method was assessed by two replicates analysis of Sertraline at concentration of 20 µg/mL prepared from Stock solution and the results are reported in Table 6. Different parameters The repeatability of method was assessed by two replicates analysis of Sertraline at concentration of such as number of theoretical plates, peak symmetery, and tailing factor were calculated from obtained data.

Table 6. Repeatability results for Sertraline (20 µg/mL).

Concentration of Sertraline (µg/mL)	Peak Area	Amount Found	% Amount Found	Retention Time	Theoretical Plate
20	1771.500	19.96	99.81	3.70	3581
20	1773.530	19.98	99.83	3.75	3663
	Mean	19.97	**99.82**	**3.72**	**3622**
	SD	1.44	0.014		
	%RSD	0.08	0.014		

SD-Standard deviation, %RSD-Relative standard deviation.

3.3.5. Robustness

Robustness is the measurement of capacity of analytical method to remain unaffected by small variations in method parameters.To evaluates the robustness of the proposed method, experimental conditions were deliberately altered and the response of the drug (10 μg/mL) was recorded. The mobile phase composition was changed in (±1 mL/min^{-1}) proportion and the flow rate was varied by (±1 mL/min^{-1}), and wavelength changes (±1 mL/min^{-1}) of optimized chromatographic condition.The results of minor variations in composition of mobile phase, wavelength, and flow rate are shown in Table 7. The reproducible results were obtained which proves that the method is robust.

Table 7. Robustness results for Sertraline (10 μg/mL) by RP-UHPLC method.

Parameters	Modification	Concentration (μg/mL)	Area ± SD	%RSD
Flow rate change(0.7 mL/min)	0.6 mL/min	10	853.69 ± 2.02	0.24
	0.8 mL/min	10	695.25 ± 1.58	0.23
Wavelength 273 nm	272 nm	10	921.1 ± 1.32	0.14
	274 nm	10	796.60 ± 2.02	0.25
Mobile phase composition-ACN:(0.1%OPA) water(80:20 *v*/*v*)	(79:21 *v*/*v*)	10	920.35 ± 1.75	0.19
	(81:19 *v*/*v*)	10	918.9 ± 2.02	0.22

3.3.6. Li3.3.6.Lmit of Detection (LOD) and Limit of Quantificatication (LOQ)

Sensitivity of proposed method was estimated as LOD and LOQ. Limit of detection is the lowest concentration of analyte that can be detected. Limit of quantification is lowest concentration of analyte that can be quantified.

The LOD and LOQ of the method were determined by using the following equations:

$$LOD = 3.3\ \sigma/S$$

and

$$LOQ = 10\ \sigma/S$$

where,

σ = standard deviation of the response and
S = slope of the calibration curve

Table 8. LOD and LOQ results for Sertraline.

Drug	σ	Slope	LOD (μg/mL)	LOQ (μg/mL)
Sertraline	5.39	85.27	0.2085	0.6321

LOD-Limit of detection, LOQ-Limit of Quatitation.

3.4. Analysis of Sertraline in Tablet Formulation

Amount of drug present in SERTA-50® 50 mg tablet was calculated. The Results was found to be for Sertraline by RP-UHPLC method was 98.97% shown in Table 9.

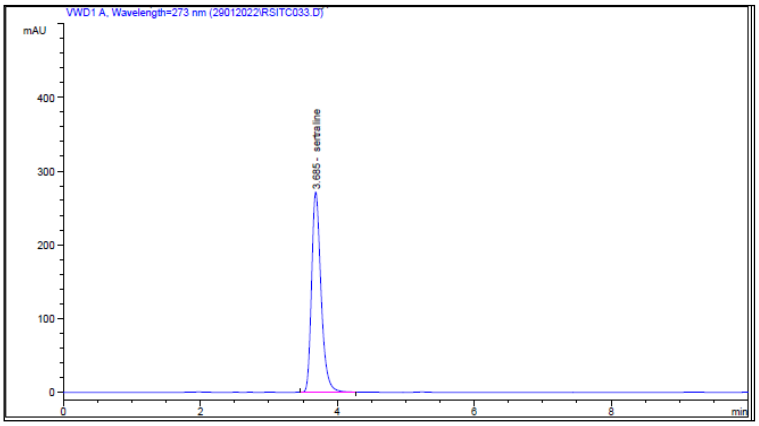

Figure 5. Chromatogram for marketed formulation of Sertraline (30 µg/mL).

Table 9. Analysis of marketed formulation.

Drug	Concentration (µg/mL)	Area	Amount Found	* %Label Claim
Sertraline	30	2599.470	29.66	98.97%
	30	2604.840	29.72	

4. Discussion

A precise and sensitive RP-UHPLC method using ACN: 0.1% OPA water (80:20 *v*/*v*) and the flow rate of 0.7 mL/min was developed and validated. The absorption maximum for Sertraline was found to be 273 nm. RP-UHPLC method was developed by using C18 (75 mm × 3.9 mm) Column having particle size 2 µm due to that method is rapid and have high resolution power than HPLC method. Linearity is the method ability to obtain test results, which are directly proportional to the concentration of analyte in sample. Sertraline showed a linear response curve for RP-UHPLC method. Correlation coefficient was found to be 0.9999 for Sertraline by RP-UHPLC method. To demostrate the accuracy of method, standard addition and recovery experiments were conducted. The % recovery was in the range of 99–100% by RP-UHPLC method for Sertraline. The method precision determines the closeness of agreement between series of measurement of the same sample. The % RSD for intraday and inter precision for both methods were obtained NMT 2%. The LOD and LOQ were found to be 0.2085 µg/mL and 0.6321 µg/mL respectively. The results obtained on validation parameters met the requirements. Validation of developed method was done as per ICH guidelines. Assay results found from the study show that the methods can be successfully applied for the estimation of Sertraline in tablet formulation. The proposed method was found to be simple, precise, accurate and validated with respect to Linearity, Accuracy, Precision, Robustness, LOD and LOQ which remained well within limit.

Author Contributions: Conceptualization, V.C.; methodology, V.C.; software, V.C.; validation, V.C; formal analysis, S.P [2] and S.P [3].; investigation, S.P [2] and S.P [4].; resources, V.C.; data curation, V.C.; writing—original draft preparation, V.C.; writing—review and editing, V.C.; visualization, S.P [2] and S.P [3].; supervision, S.P [2] and S.P [3].; project administration, V.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Informed Consent Statement: Not applicable.

Acknowledgments: The authors would like to convey our obligation to Principal and Management of P.S.G.V.P.Mandal's College of Pharmacy, Shahada, Dis- Nandurbar (Maharashtra) India for providing the required facilities to carry out this research work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Walter, T.H.; Andrews, R.W. Recent innovation in UHPC columns and instrumentation. *Trends Anal. Chem.* **2014**, *63*, 14–20.
2. Rathod, R.H.; Chaudhari, S.R. Ultra-high performance liquid chromarography-MS/MS (UHPLC-MS/MS) in practice: analysis of drugs and pharmaceutical formulations. *Futur. J. Pharma. Sci*. **2019**, *5*, 6.
3. Chauhan, A.; Mittu, B.; Chauhan, P. Anlytical Method Development and Validation: A Concise Review. *J Anal. Bioanal. Tech.* **2015,** *6.*
4. Virani, P.; Raj, H.; Jain, V.; Jain, P. Updated Review: Validation and Method Validation Parameters. *PharmaTutor*. **2014**, *2*, 27–37.
5. Bairagi, S.H.; Ghosh, R.S. Development and Validation of RP-HPLC Method for Determination of Sertaline in Bulk drug and Dosage form. *World J. Pharm. Res*. **2020**, *9*, 1236–1246.
6. Bais, S.; Bhavsar, M.; Singhvi, I.; Chandewar, A. Analytical method development and validation for the estimation of Alprazolam and Sertraline Hydrochloride by HPLC. *Pharma. Res*. **2014**, *11*, 6–16.
7. Richards, P.M.; Reddy, S.K.; Chowdary, G.V. RP-HPLC method development and validation for simultaneous estimation of Sertraline HCL and alprazolam in tablet dosage form. *Int. J. Res. Pharma. Sci.* **2015**, *6*, 285–290.
8. Narkhede, S.P.; Singh. S.; Vidyasagar. G. A simple HPLC method for IN-VITRO dissolution study of sertraline hydrochloride in tablets. *Int. J. Lif. Sci. Pharm. Res*. **2016**, *6*, 22–29.
9. Ratnia, R.; Yadav, V.; Kumar, A. Method development and its validation for estimation of Sertraline Hydrochloride by using UV Spectroscopy. *Int. J. Pharm. Res. Health Sci.* **2015**, *3*, 616–620.
10. Venkateshwarlu, K.; Venisetty, R.K.; Yellu, N.R.; Keshetty, S. 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.
11. Gupta, K.R.; Tajne, M.R.; Wadodkar, S.G. A validated High Performance Thin Layer Chromatography method for Quantification of Sertraline in tablets. *J. Plane. Chromatogr*. **2008**, *23*, 134–136.
12. Bonchev, G.; Zleteva, S.; Marinov, P.; Yocheva, M.; Vazharov, I. A UHPLC Method for Sertraline determination. *J. IMAB*. **2017**, *23*, 1765–1768.
13. Yue, X.H.; Wang, Z.; Tian, D.D.; Zhang, J.W.; Zhu, K.; Ye, Q. Determination of Sertraline in human plasma by UPLC-MS/MS and its Application to a Pharmacokinetic Study. *J. Chromatogr. Sci*. **2016**, *54*, 195–199.
14. Zhang, M.; Gao, F.; Cui, X.; Zhang, Y.; Sun, Y.; Gu, J. Development and validation of an improved method for the Quantitation of Sertraline in Human plasma using LC-MS-MS and its application to Bioequivalence studies. *J. Chromatogr. Sci*. **2011**, *49*, 89–93.