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NEW RP-HPLC METHOD DEVELOPMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SERTRALINE AND ALPRAZOLAM IN PHARMACEUTICAL DOSAGE FORM

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Abstract:
***Objective**: A New method was established for simultaneous estimation of Sertraline and Alprazolam by RP-HPLC method.*
***Methods**: Chromatographic separations were carried using HPLC equipped with Auto Sampler and PDA Detector, Inertsil ODS (1.7 x 50 mm, 3µm) column with a mobile phase composition of Methanol: TEA Buffer (65:35 v/v) have been delivered at a flow rate of 1ml/min and the detection was carried out using waters HPLC auto sampler, separation module 2695 HPLC system with PDA detector at wavelength 230 nm.*
***Results**: The retention time for Sertraline and Alprazolam were 2.152 and 3.64 minute respectively. The correlation coefficient values in linearity were found to be 0.999 and concentration range 10-50 µg/ml for Sertraline and 20-100 µg/ml for Alprazolam respectively. For accuracy the total recovery was found to be 100.37 % and 100.34 % for Sertraline and Alprazolam. LOD and LOQ for Sertraline 0.9 and 1.2 µg/mL, LOD and LOQ for Alprazolam 2.7 and 3.6 µg/mL.*
***Conclusion**: The results of study showed that the proposed RP-HPLC method is a simple, accurate, precise, rugged, robust, fast and reproducible, which may be useful for the routine estimation of Sertraline and Alprazolam in pharmaceutical dosage form.*
***Keywords:** Sertraline, Alprazolam, RP-HPLC, Simultaneous estimation.*

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INTRODUCTION:

Sertraline is a selective serotonin reuptake inhibitor (SSRI) indicated to treat major depressive disorder, social anxiety disorder and many other psychiatric conditions. Sertraline is a popular antidepressant medication commonly known as a selective serotonin reuptake inhibitor (SSRI), and is similar to drugs such as Citalopram and Fluoxetine. Despite marked structural differences between compounds in this drug class, SSRIs exert similar pharmacological effects. Several weeks of therapy with sertraline may be required before beneficial effects are noticed. Sertraline displays enhanced safety or tolerability than other classes of antidepressants, which frequently cause high levels of drowsiness, dizziness, blurred vision, and other undesirable effects.[1-3] IUPAC name is (1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine.
Chemical formula $C_{17}H_{17}Cl_2N$. Molecular weight is 306.2.

Alprazolam is indicated for the acute treatment of generalized anxiety disorder in adults.18 Alprazolam is also indicated, either as a standard or extended-release formulation, for the treatment of panic disorder with or without agoraphobia in adults. Alprazolam may also be prescribed off-label for insomnia, premenstrual syndrome, and depression.[4] Neurotransmission relies on excitatory and inhibitory signalling. γ-aminobutyric acid (GABA) type-A receptors (GABAARs) are members of the pentameric ligand-gated ion channel (PLGIC) superfamily located synaptically and perisynaptically to mediate phasic inhibition and extrasynaptically to mediate tonic inhibition. GABAARs comprise a variety of subunits from a homologous family whose members are named based on sequence identity as one of α1-6, β1-3, γ1-3, δ, ε, θ, π, and ρ1-3. Each subunit possesses an extracellular (ECD), transmembrane (TMD), and intracellular (ICD) domain; inter-subunit interfaces are the primary points of neurotransmitter and modulator binding, described by coordination of the principal (+) and complementary (-) sites in each subunit. Binding of GABA to GABAARs induces pore opening, rapid flow of chloride ions, and synaptic hyperpolarization, which in turn manifests as an inhibitory signal.[5-6] IUPAC name of Alprazolam is 12-chloro-3-methyl-9-phenyl-2,4,5,8-tetraazatricyclo [8.4.0.0^ {2,6}] tetradeca-1(10),3,5,8,11,13-hexaene. Chemical formula $C_{17}H_{13}N_4Cl$. Molecular weight is 308.7.

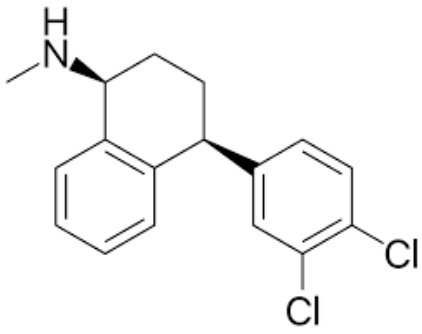

Figure 1: Structure of Sertraline

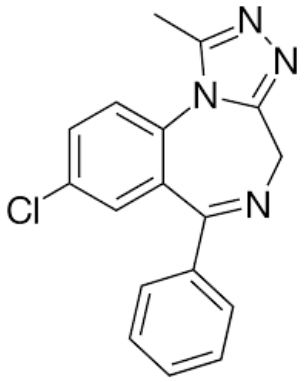

Figure 2: Structure of Alprazolam

The literature review reveals that these different dosage forms of Sertraline hydrochloride and Alprazolam are analyzed by a different method. Some literatures revealed the high-performance liquid chromatography (HPLC) method for Sertraline and Alprazolam.[7-11] However, these methods are time consuming, so it is necessary to develop a cost effective and less time-consuming method for the estimation of Sertraline Hydrochloride and Alprazolam in API as well as pharmaceutical formulation. The present study is to establish an apt stability indicating RP-HPLC method for evaluation of Sertraline and Alprazolam in pharmaceutical dosage form simultaneously and validate developed method as per frames and rules of ICHQ2 (R1).

MATERIALS AND METHODS:

Chemicals and Reagents: Sertraline and Alprazolam were obtained as a gift sample from Dr Reddys Laboratories India Pvt. Ltd, Hyderabad. Sodium

hydroxide, hydrochloric acid, Methanol for HPLC (Merck), Acetonitrile for HPLC (Merck) and Water for HPLC (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a HPLC equipped with Auto Sampler and PDA Detector and Empower 2 software. Analysis was carried out at 230 nm with column Inertsil ODS (1.7 x 50 mm, 3 µm), dimensions at 40^0C temperature. The optimized mobile phase consists of Methanol: TEA Buffer (65:35 v/v), Flow rate was maintained at 1.0 ml/min and run time for 6 min.

Preparation of solutions:

Preparation of buffer:

Take 6.0ml of Triethylamine in to 750ml of in a 1000ml volumetric flask and mix well. Make up the volume up to mark with water and adjust the pH to 4.0 by using Orthophosphoric acid, filter and sonicate.

Preparation of mobile phase:

Accurately measured 350 ml (35%) of TEA buffer and 650 ml of Methanol (65%) were mixed and degassed in digital ultrasonicater for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration**.**

The diluents:

The Mobile phase was used as the diluent.

Preparation of standard stock solution:

Accurately weigh and transfer 10 mg of Sertraline and Alprazolam working standard into a 10ml of clean dry volumetric flasks add about 7ml of Diluents and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

Further pipette out 0.3 ml of Sertraline and 0.6ml of Alprazolam from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

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Preparation of Sample stock solution:

Take average weight of one Tablet and crush in a mortar by using pestle and weight 10 mg equivalent weight of Sertraline and Alprazolam sample into a 10mL clean dry volumetric flask and add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. Filter the sample solution by using injection filter which contains 0.45µ pore size.

Further pipette out 0.3 ml of Sertraline and 0.6ml of from the above stock solutions into a 10ml volumetric flask and dilute up to the mark with Diluent.

Procedure: 10 µL of standard and sample solutions were injected into the LC-system and measure the peak areas for Sertraline and Alprazolam.

METHOD:

The developed chromatographic method was validated for system suitability, linearity accuracy, precision, ruggedness and robustness as per ICH guidelines.

System suitability parameters: To evaluate system suitability parameters such as retention time, tailing factor and USP theoretical plate count, the mobile phase was allowed to flow through the column at a flow rate of 1.0 ml/min for 6 minutes to equilibrate the column at ambient temperature. Chromatographic separation was achieved by injecting a volume of 10 µL of standard into Inertsil ODS (1.7 x 50 mm, 3 µm), the mobile phase of composition Methanol: TEA Buffer (65:35 v/v) was allowed to flow through the column at a flow rate of 1.0 ml per minute. Retention time, tailing factor and USP theoretical plate count of the developed method are shown in table 1.

Assay of pharmaceutical formulation: The proposed validated method was successfully applied to determine Sertraline and Alprazolam in their tablet dosage form. The result obtained for Sertraline and Alprazolam was comparable with the corresponding labeled amounts and they were shown in Table-2.

Validation of Analytical method:

Linearity and Range: Stock solution was prepared by dissolving the appropriate amount of Sertraline and Alprazolam in 10 ml of diluent and further diluted to the required concentrations with diluent. The solution was prepared at five concentration levels ranging from 10 µg/ml to 50 µg/ml of Sertraline and 20 µg/ml to 100 µg/ml of Alprazolam. Inject each level into the chromatographic system and measure the peak area. Plot a graph of peak area versus concentration (on X-axis concentration and on Y-axis Peak area) and calculate the correlation coefficient. The results are shown in table 3.

Accuracy studies: The accuracy was determined by help of recovery study. The recovery method carried out at three level 50%, 100%, 150%. Inject the standard solutions into chromatographic system. Calculate the Amount found and Amount added for v Sertraline and Alprazolam and calculate the

individual recovery and mean recovery values. The results are shown in table 4,5.

Precision Studies: precision was calculated from Coefficient of variance for six replicate injections of the standard. The standard solution was injected for six times and measured the area for all six Injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 6.

Ruggedness: To evaluate the intermediate precision of the method, Precision was performed on different day. The standard solution was injected for six times and measured the area for all six injections in HPLC. The %RSD for the area of six replicate injections was found. The results are shown in table 7.

Robustness: As part of the Robustness, deliberate change in the Flow rate, Mobile Phase composition, Temperature Variation was made to evaluate the impact on the method. The flow rate was varied at 0.9 ml/min to 1.1ml/min. The resulte are shown in table 8,9.

LOD and LOQ: The sensitivity of RP-HPLC was determined from LOD and LOQ. Which were calculated from the calibration curve using the following equations as per ICH guidelines. The resulte are shown in table 10.

LOD = 3.3σ/S and

LOQ = 10 σ/S, where

σ= Standard deviation of y intercept of regression line,

S = Slope of the calibration curve

RESULTS AND DISCUSSION:

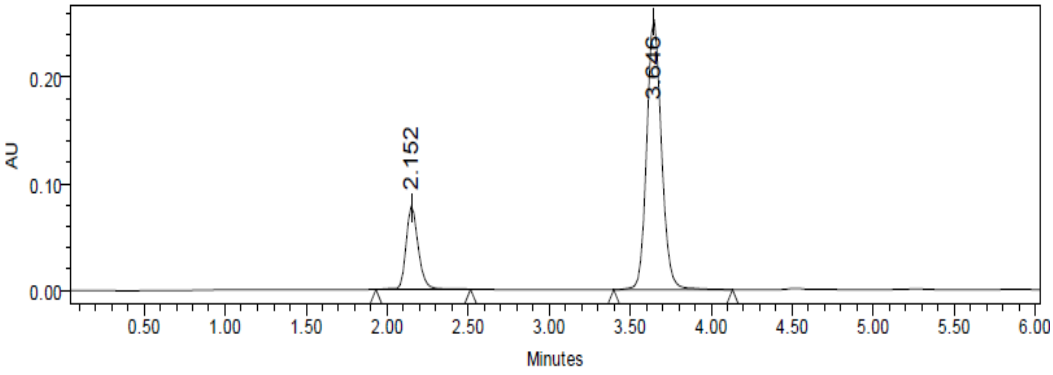

Figure 3: Standard chromatogram

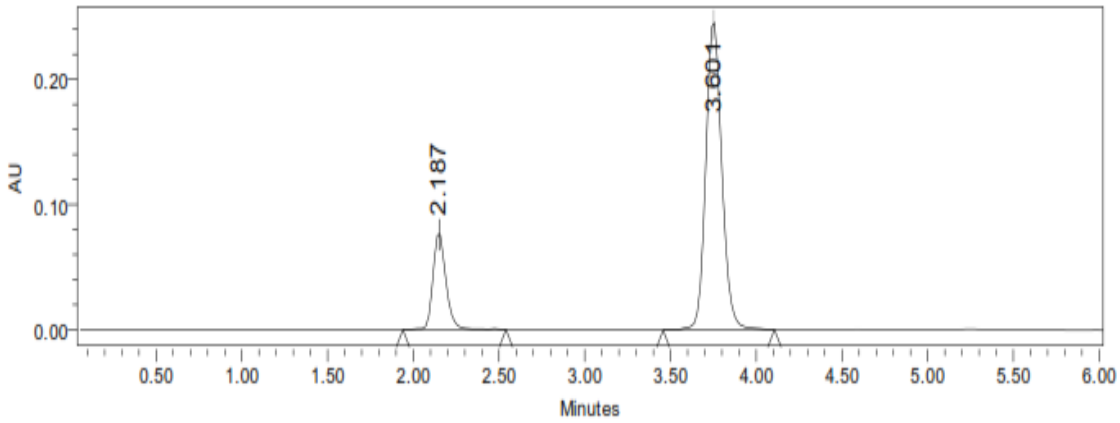

Figure 4: Sample chromatogram

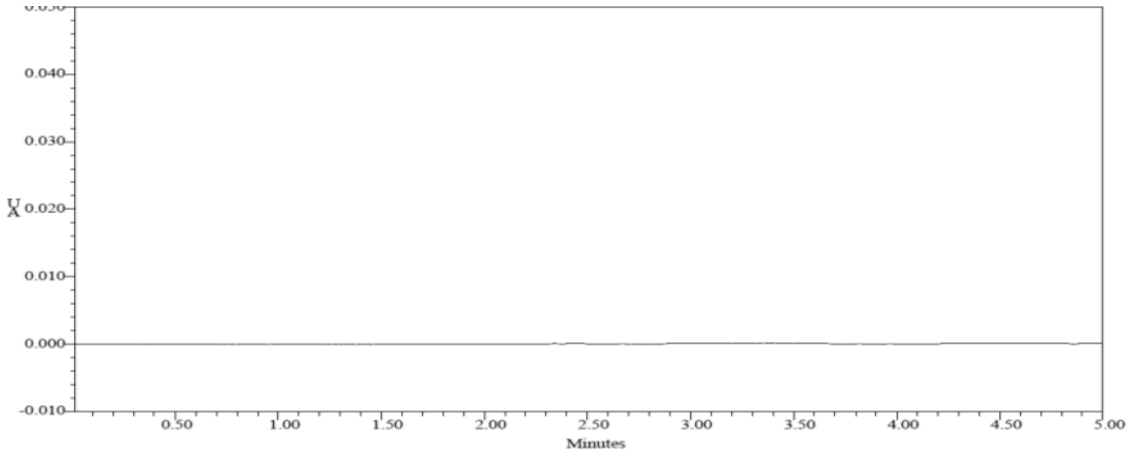

Figure 5: Blank chromatogram

Table 1: System suitability parameters

Parameters	Sertraline	Alprazolam
Retention time	2.152	3.64
USP Plate count	5969	8016
USP Tailing	1.64	1.49

Table 2: Assay results for Sertraline and Alprazolam

	Label Claim (mg)	% Assay
Sertraline	10	99
Alprazolam	20	99

Table 3: Linearity results for Sertraline and Alprazolam

Sertraline		Alprazolam	
Concentration(µg/ml)	**Area**	**Concentration(µg/ml)**	**Area**
10	185689	20	665985
20	349852	40	1298698
30	521541	60	1927852
40	685986	80	2548545
50	848265	100	3162468
Correlation coefficient	0.999	Correlation coefficient	0.999

Table 4: Showing accuracy results for Sertraline

(at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	263572	15	15.038	100.253%	100.37%
100%	518870.3	30	30.147	100.490%	
150%	772572.3	45	45.162	100.360%	

Table 5: Showing accuracy results for Alprazolam

%Concentration (at specification Level)	Area	Amount Added (ppm)	Amount Found (ppm)	% Recovery	Mean Recovery
50%	972935.7	30	30.109	100.363%	100.34%
100%	1919319	60	60.100	100.166%	
150%	2877020	90	90.449	100.498%	

Table 6: Precision results for Sertraline and Alprazolam

Injection	Area for Sertraline	Area for Alprazolam
Injection-1	526854	1645879
Injection-2	523659	1648578
Injection-3	523856	1645985
Injection-4	523485	1648759
Injection-5	523485	1648572
Injection-6	523479	1648542
Average	524267.8	1647555
Standard Deviation	1453.805	1483.603
%RSD	0.277302	0.090049

Table 7: Intermediate precision results for Sertraline and Alprazolam:

Injection	Area for Sertraline	Area for Alprazolam
Injection-1	536598	1658254
Injection-2	536985	1659872
Injection-3	534587	1653589
Injection-4	536985	1658458
Injection-5	536985	1653652
Injection-6	538568	1652395
Average	536784.7	1656037
STD Deviation	1277.909	3175.804
%RSD	0.238067	0.191771

Table 8: Robustness results of Sertraline

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	526541	2.157	5859	1.62
Less Flow rate of 0.9 mL/min	589564	2.210	5635	1.61
More Flow rate of 1.1 mL/min	515246	2.184	5569	1.64
Less organic phase	502659	2.200	5154	1.63
More Organic phase	526485	2.172	5365	1.62

Table 9: Robustness results of Alprazolam

Parameter used for sample analysis	Peak Area	Retention Time	Theoretical plates	Tailing factor
Actual Flow rate of 1.0 mL/min	1645875	3.643	7965	1.48
Less Flow rate of 0.9 mL/min	1635985	4.498	7856	1.46
More Flow rate of 1.1 mL/min	1624587	3.505	7425	1.43
Less organic phase	1652834	4.504	7621	1.45
More organic phase	1625548	3.512	7582	1.42

Table 10: LOD, LOQ of Sertraline and Alprazolam

Drug	LOD	LOQ
Sertraline	0.9 µg/mL	2.7 µg/mL
Alprazolam	1.2 µg/mL	3.6 µg/Ml

CONCLUSION:

The proposed HPLC method was found to be simple, precise, accurate and sensitive for the simultaneous estimation of Sertraline and Alprazolam in pharmaceutical dosage forms. Hence, this method can easily and conveniently adopt for routine quality control analysis of Sertraline and Alprazolam in pure and its pharmaceutical dosage forms.

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