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Analytical Method Development and Validation for the Simultaneous Estimation of Sertraline hydrochloride and Alprazolam by RP-HPLC Method in Bulk and Pharmaceutical Dosage Form

Gobala Krishnan P*, Munisekhar V, Ranjit Singh B Rathore

Department of Pharmaceutical Chemistry, Vagdevi college of Pharmacy and Research centre, Nellore, A. P, India.

A B S T R A C T

The chromatographic conditions were successfully developed for the separation of Sertraline hydrochloride and Alprazolam by using C_{18} Column (150mm x 4.6mm) 5 µm, flow rate was 1 ml/min, mobile phase ratio was Methanol: Phosphate buffer P^H 2.8 (55:45 v/v), detection wavelength was 225 nm. The Spectroscopic method was done in solvent using methanol and the instrument lab India 3000+ with UV win software. The instrument used was WATERS HPLC Auto Sampler, Separation module 2695, UV detector, Empower-software version 2. The retention times for Sertraline hydrochloride and Alprazolam were found to be 2.344 min and 3.286 min. The analytical method was validated according to ICH guidelines (ICH, Q2 (R1)). The linearity study of Sertraline hydrochloride and Alprazolam was found in the concentration range of 100 ppm-500 ppm and 1ppm -5 ppm and correlation coefficient (r^2) was found to be 0.999 and 0.999 respectively, % recovery was found to be 98% and 102% respectively. %RSD for repeatability and precision was found to be <2. LOD and LOQ values were found to be within the limits for Sertraline hydrochloride and Alprazolam.

Keywords: Sertraline hydrochloride, Alprazolam, HPLC.

A R T I C L E I N F O

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***Corresponding Author**
Gobala Krishnan P
Department of Pharmaceutical Chemistry,
Vagdevi College of Pharmacy and Research Centre, Nellore, Andhra Pradesh, India.
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1. Introduction

Analytical methods

Methods are developed for new products when no official methods are available. Alternate methods for existing (non-pharmacopoeial) products are developed to reduce the cost and time for better precision and ruggedness [1]. Trial runs are conducted, method is optimized and validated. When alternate method proposed is intended to replace the existing procedure comparative laboratory data including merit/demerits are made available [2].

Description of the Various Analytical Methods

Titrimetric and gravimetric method of analysis is suitable when the sample is present in pure form or when no interference is observed in the mixture with other materials [3]. Ultraviolet and visible spectrometric method is suitable when no Interference is observed in the mixture [4]. HPLC and GC methods are more advantageous than the above due to their capability in separating organic mixtures and quantitative estimations. AAS is used mainly for quantitative estimation in ppm and ppb levels of elements [5]. Infra-red spectroscopy though mainly used for qualitative analysis can be used for quantitative estimation also. Out of all the above methods, thin layer chromatography plays a very important role in analysis due to its adaptability, flexibility, and cost and time. It can be used both for qualitative and quantitative determination. After separation spots can be scanned with the help of a scanner and quantitative measurement can be made [6].

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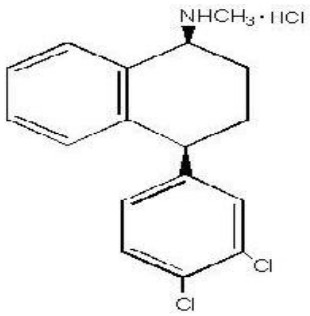

Figure 1: Sertraline hydrochloride

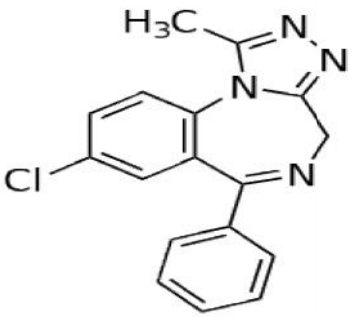

Figure 2: Alprazolam

Chromatography:

Chromatography is a technique used in analytical chemistry to separate and identify components of mixtures. The name comes from the Greek term for "color writing" because this method was originally used to separate colored samples. The advent of high-performance liquid chromatography (HPLC).in this system pressure is applied to the column, forcing the mobile phase through at much higher rate [7].

The pressure is applied using a pumping system. The action of the pump is critical, since it must not pulsate and mix up the sample being separated in the solvent, causing it to lose resolution [8]. Development of pumps has proceeded quite quickly over the last several years, and now it is possible to achieve good resolution under the conditions required for HPLC.

2. Materials and Methods

Apparatus

The instrument used for the study was Waters HPLC Auto Sampler, Separation module 2695, UV detector with Empower-software version-2.

Reagents and Materials

The solvents used were Methanol, Ortho phosphoric acid, Acetonitrile, Potassium dihydrogen ortho phosphate and HPLC Water.

Selection of detection wavelength:

UV spectrum of 10 µg / ml Sertraline HCL and Alprazolam in diluents (mobile phase composition) was recorded by scanning in the range of 200 nm to 400 nm. From the UV spectrum wavelength selected as 225 [9]. At this wavelength both the drugs show good absorbance.

Selection of mobile phase

Initially the mobile phase tried was methanol: Ammonium acetate buffer and acetonitrile: phosphate buffer with various combinations of pH as well as varying proportions. Finally, the mobile phase was optimized to potassium dihydrogen phosphate with buffer (pH 3.5), acetonitrile in proportion 40: 60 v/v respectively [10].

Optimization Chromatographic trials for Simultaneous Estimation of Sertraline hydrochloride and Alprazolam by RP- HPLC.

Optimization Chromatographic conditions

Instrument used	: Waters HPLC with auto sampler and PAD or detector.
Temperature	: Ambient
Column	: Symmetry C18 (4.6 x 150mm, 5µm, Make: X Terra) or equivalent
Buffer	: 6.8 grams of potassium Dihydrogen ortho phosphate in 1000 ml water pH adjusted with Ortho phaosparic acid.
pH	: 2.8
Mobile phase	: 45% buffer 55% Methanol
Flow rate	: 1.0 ml per min
Wavelength	: 225 nm
Injection volume	: 20 µl
Run time	: 5 min.

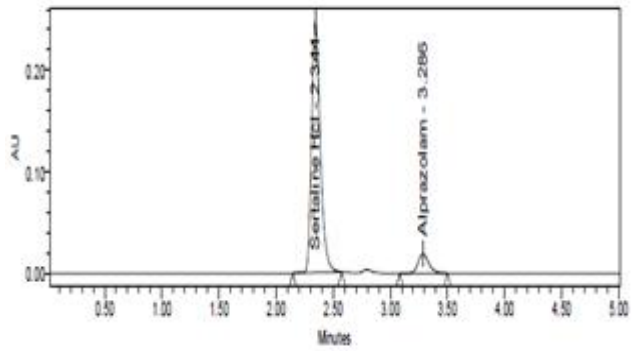

Figure 3: Optimization Chromatogram

Observation: The separation was good, peak shape was good, so we conclude that no trials required for separation.

Procedure

Preparation of phosphate buffer

Accurately weighed 6.8 grams of KH_2PO_4 was taken in a 1000ml volumetric flask, dissolved and diluted to 1000 ml with HPLC water and the volume was adjusted to pH 2.8 with Orthophosphoric acid [11].

Preparation of mobile phase

Accurately measured 450 ml (45%) of above buffer and 550 ml of Methanol HPLC (55%) were mixed and degassed in an ultrasonic water bath for 10 minutes and then filtered through 0.45 µ filter under vacuum filtration [12].

Sertraline hydrochloride and Alprazolam standard preparations

Accurately weigh and transfer 10 mg of Sertraline and Alprazolam 10 mg of working standard into a 10 mL & 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml& 0.3 ml of the above stock solutions into a 10 ml volumetric flask and dilute up to the mark with diluent.

Sample solutions preparation

Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Sertraline and Alprazolam (marketed formulation) sample into a 10 mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml of Sertaline and Alprazolam of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

3. Results and discussions

Method Validation Parameters

Specificity:

The system suitability for specificity was carried out to determine whether there is any interference of any impurities in retention time of analytical peak. The specificity was performed by Injecting blank.

Linearity

Preparation of stock solution:

Accurately weigh 10 tablets crush in mortor and pestle and transfer equivalent to 10 mg of Sertraline and Alprazolam (marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution)

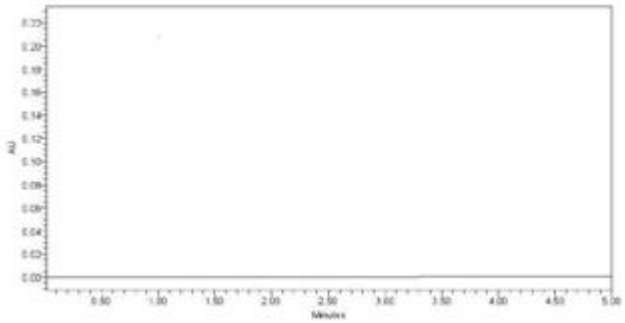

Figure 4: Chromatogram of Blank

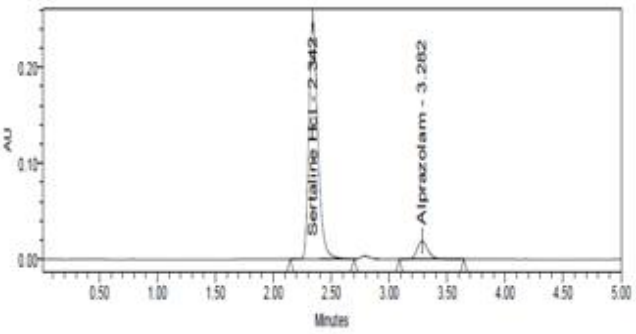

Figure 5: Chromatogram of Sample

Preparation of Level – I (100ppm of Sertraline &1ppm of Alprazolam): 1 ml and 0.1 ml of stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – II (200ppm of Sertraline &2ppm of Alprazolam): 2 ml and 0.2 ml of stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – III (300ppm of Sertraline &3 ppm of Alprazolam): 3 ml and 0.3 ml of stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent.

Preparation of Level – IV (400ppm of Sertraline &4ppm of Alprazolam):

4 ml and 0.4 ml of stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent

Preparation of Level – V (500ppm of Sertraline &5ppm of Alprazolam)

5 ml and 0.5 ml of stock solutions has taken in different 10 ml of volumetric flasks, dilute up to the mark with diluent

Procedure:

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.

Acceptance criteria: Correlation coefficient should be not less than 0.999.

Range:

Based on precision, linearity and accuracy data it can be concluded that the assay method is precise, linear and

accurate in the range of 100-500 ppm and 1-5 ppm for Sertraline hydrochloride and Alprazolam respectively

Accuracy

Preparation of Standard stock solution:

Accurately weigh and transfer 10 mg of Sertraline HCL and Alprazolam 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

Preparation Sample solutions:

For preparation of 50% solution (With respect to target Assay concentration): Accurately weigh and transfer 5 mg of Sertraline and 5.3 mg of Alprazolam working standard into a 10 mL and 100 ml of clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.(Stock Solution). Further pipette 3 ml of Sertaline & 0.3 ml of Alprazolam of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

For preparation of 100% solution (With respect to target Assay concentration):

Accurately weigh and transfer 10 mg of Sertraline and 10 mg of Alprazolam working standard into a 10mL and 100 ml 0f clean dry volumetric flask add about 7mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock Solution). Further pipette 3 ml of Sertraline & 0.3 ml of Alprazolam of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

For preparation of 150% solution (With respect to target Assay concentration): Accurately weigh and transfer 14.4mg of Sertraline and 14.5 mg of Alprazolam working standards into a 10 mL and 100ml of clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution).

Further pipette 3 ml of Sertraline & 0.3 ml of Alprazolam of the above stock solution into a 10 ml volumetric flask and dilute up to the mark with diluent.

Assay Procedure:

Inject the standard solution, Accuracy -50%, Accuracy -100% and Accuracy-150% solutions. Calculate the Amount found and Amount added for Sertraline &Alprazolam and calculate the individual recovery and mean recovery values.

Precision

Preparation of stock solution: Accurately weigh and transfer 25 mg of Sertraline and Alprazolam working standard into a 10 mL clean dry volumetric flask add about 7 mL of Diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent. (Stock solution). Further pipette 3 ml of Sertraline &Alprazolam of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

Procedure:

The standard solution was injected for five times and measured the area for all five injections in HPLC. The %RSD for the area of five replicate injections was found to be within the specified limits.

Acceptance Criteria: The % RSD for the area of five standard injections results should not be more than 2%**.**

Validation of the method

Linearity: The linearity range was found to lie from 100 µg/ml to 500 µg/ml of sertraline HCL, 5 µg/ml to 25 µg/ml of Alprazolam and chromatograms are shown below.

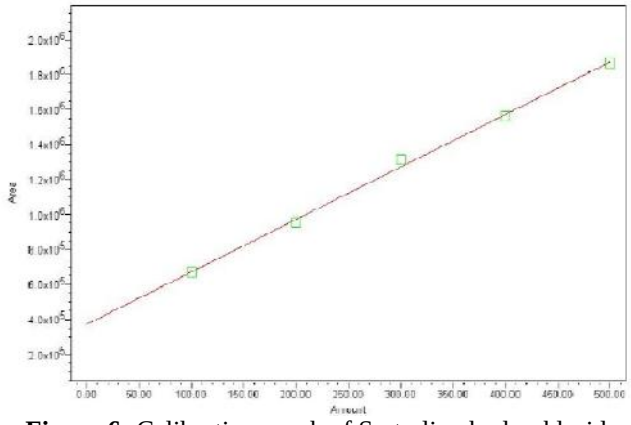

Figure 6: Calibration graph of Sertraline hydrochloride

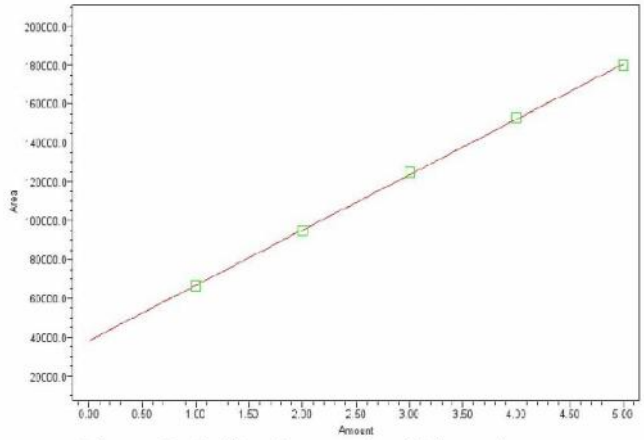

Figure 7: Calibration graph of Alprazolam

Table 1: Calibration data of Sertraline hydrochloride and Alprazolam

S.No.	Linearity Level	Concentration	Area
1	I	100ppm	668934
2	II	200ppm	956781
3	III	300ppm	1313873
4	IV	400ppm	1563458
5	V	500ppm	1867084
Correlation Coefficient			0.999

S. No	Linearity Level	Concentration	Area
1	I	1ppm	66510
2	II	2ppm	94701
3	III	3ppm	124802
4	IV	4ppm	152731
5	V	5ppm	179732
Correlation Coefficient			0.999

Recovery studies: Sample solutions at different concentrations (50%, 100%, and 150%) were prepared and the % recovery was calculated.

Table 2: Precision of Sertraline hydrochloride

Injection	Area
Injection-1	1302729
Injection-2	1302947
Injection-3	1303236
Injection-4	1303977
Injection-5	1309759
Average	1304529.8
Standard Deviation	2961.1
%RSD	0.2

Table 3: Precision of Alprazolam

Injection	Area
Injection-1	123149
Injection-2	123766
Injection-3	124271
Injection-4	124691
Injection-5	124956
Average	124162.7
Standard Deviation	725.6
%RSD	0.6

Table 4: LOD and LOQ

Drug name	Baseline noise(µV)	Signal obtained(µV)	S/N ratio
Sertraline HCL	52	152	2.9
Alprazolam	52	156	3

Drug name	Baseline noise(µV)	Signal obtained (µV)	S/N ratio
Sertraline HCL	52	522	10.03
Alprazolam	52	524	10.1

4. Conclusion

High performance liquid chromatography is at present one of the most sophisticated tool of the analysis. The estimation of Sertaline Hcl and Alprazolam was done by RP-HPLC. The Phosphate buffer was p^H 2.8 and the mobile phase was optimized with consists of Methanol: Phosphate buffer mixed in the ratio of 55:45 % v/ v. A C_{18} column C18 (4.6 x 150 mm, 5µm, Make: XTerra) or equivalent chemically bonded to porous silica particles was used as stationary phase. The detection was carried out using UV detector at 225 nm. The solutions were chromatographed at a constant flow rate of 1.0 ml/min. the linearity range of Sertaline HCL and Alprazolam were found to be from 100-500 µg/ml of Sertaline HCL and 1-5µg/ml of Alprazolam. Linear regression coefficient was not more than 0.999. The values of % RSD are less than 2% indicating accuracy and precision of the method. The percentage recovery varies from 98-102% of Sertaline HCL and Alprazolam. LOD and LOQ were found to be within limit. The results obtained on the validation parameters met ICH and USP requirements .it inferred the method found to be simple, accurate, precise and linear. The method was found to be having suitable application in routine laboratory analysis with high degree of accuracy and precision.

Table 5: Showing accuracy results for Sertraline hydrochloride

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	656659.5	5.0	45.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 6: Showing accuracy results for Alprazolam

%Concentration (at specification level)	Average area	Amount added (mg)	Amount found (mg)	% Recovery	Mean recovery
50%	65800	5.3	5.34	100.8%	100.51%
100%	124353	10	10.10	100.01%	
150%	177940	14.2	14.45	99.68%	

Robustness:

Table 7: System Suitability Results for Sertraline hydrochloride

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	5339.9	1.4
2	1	4673.4	1.3
3	1.2	5216.0	1.4

Table 8: System Suitability Results for Alprazolam

S. No	Flow rate (ml/min)	System suitability results	
		USP Plate Count	USP Tailing
1	0.8	7063.3	1.3
2	1	6090.3	1.2
3	1.2	6998.0	1.3

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