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Review Article

RP-HPLC method development and validation for simultaneous estimation of sertraline HCL and alprazolam in tablet dosage form

Paul Richards M*[1], A Sunil Kumar Reddy[2] and G V Chowdary[3]

[1]JNTUH, Telangana, Andhra Pradesh, India
[2]Aurobindo Pharma Ltd, Hyderabad Telangana, India
[3]Horizon college of Pharmacy, Hyderabad Telangana, India

ABSTRACT

To develop a simple and selective method for the simultaneous determination of sertraline and Alprazolam in tablet dosage forms. A simple, rapid, accurate, specific and sensitive reverse phase-HPLC method has been developed and validated for the simultaneous estimation of Sertraline HCl and alprazolam in pharmaceutical dosage form. The chromatographic separation was performed on Symmetry C18 (4.6 x 250mm, 5µm, Make: Waters) using a mobile phase of phosphate buffer: acetonitrile (40:60v/v), at a flow rate of 1.ml/min at ambient temperature with the detection wave length at 225nm. The retention times of Alprazolam and Sertraline HCl were 2.342 min and 3.282 min respectively. The linearity was performed in the concentration range of 5 -25 µ g/ml and 100-500 µg/ml with a correlation coefficient of 0.999 respectively. The percentage purity of Sertraline HCl and Alprazolam was found to be 99.8 and 100.4% w/v respectively. The proposed method has been validated for linearity, precision, accuracy and robustness were within the acceptance limit according to ICH guidelines and the developed method was successfully employed for routine quality control analysis in the combined pharmaceutical dosage forms.

Keywords: Alprazolam, ICH guidelines, RP-HPLC, Sertraline Hydrochloride

INTRODUCTION

Aquatic plants form one of the most productive Sertraline hydrochloride (STL) is chemically, (1S-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro-N- Methyl-1-napthalenamaine hydrochloride belongs to third-generation, structurally novel phenethyl bicyclic antidepressant (Goodman & Gilman's). It inhibits Antidepressant selective serotonin reuptake inhibitor, and it slightly soluble in water and isopropyl alcohol, and scarcely soluble in ethanol (Holliday MS). Alprazolam (ALP) prescribed principally to treat Anti-anxiety, is undergoing assessment for other neuropsychiatric disorders and medical conditions. The neurochemical substrates of alprazolam are unresolved. It has been postulated that alprazolam is soluble in methanol or ethanol but which has no considerable solubility in water at physiological pH (Madras BK, Xie Z et al., 2006).

There are several methods reported for the determination of sertraline in biological fluids (Raut BB et al.,2003- Cherkaoui S et al., 2001) However, for its determination in drug formulations only two methods have been reported (Makhija SN et al., 2002- Kaur Jet al., 2010).

Determination of alprazolam in biological fluids (Burnat P et al., 1998- Schwertner HA et al., 2005) and formulations, very few methods have been reported. The aim of present study is to develop a novel simultaneous method for the determination of sertraline and Alprazolam in their respective dosage forms using RP-HPLC.

EXPERIMENTAL

Materials and equipment

High Performance Liquid Chromatographic system equipped with a diode array detector and auto-sampler was used. C18 symmetry column (4.6 mm x 250 m) was used for separation. Chromatographic and integrated data were recorded using Empower 2 software. All the reagents were of analytical grade unless stated otherwise. Milli Q water, HPLC-grade acetonitrile (Rankem, Mumbai, India), methanol (Rankem, Mumbai, India) and ammonium acetate (AR grade, S.D. Fine Chem, Mumbai, India) were used. All solutions were filtered through 0.45 µm membrane filters procured from Pall Pharma Lab Filtration Pvt Ltd (Mumbai, India).

* Corresponding Author
Email: richie2626@gmail.com
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Preparation of standard solutions

A working standard of sertraline (25 mg) was taken into a 25 ml volumetric flask, dissolved in 15 ml of diluent, ultra-sonicated for about 10 min, filtered and the volume made up to the mark with the diluent (Standard Stock). From this solution, 0.5 ml of solution was transferred to 10 ml volumetric flask, 5 ml of diluent was added, sonicated to mix and the volume made up to mark with diluent. Further dilutions were made from reference standard to obtain final linear concentrations.

A working standard of Alprazolam (10 mg) was taken into a 10 ml volumetric flask, dissolved in 5 ml of diluent, ultra-sonicated for about 10 min, filtered and the volume made up to the mark with the diluent (Standard Stock). From this solution, 0.5 ml of solution was transferred to 10 ml volumetric flask, 5 ml of diluent was added, sonicated to mix and the volume made up to mark with diluent. Further dilutions were made from reference standard to obtain final linear concentrations.

Preparation of Marketed formulation:

Twenty tablets of sertraline were accurately weighed, ground to powder and powder equivalent of 25 mg (1 tablet) of active ingredient was taken into a 50 ml volumetric flask, dissolved in 35 ml of 50% methanol in water (diluent), ultra sonicated for about 10 min, filtered and volume made up to the mark with the diluent. From this solution, 0.5 ml of solution was transferred to 10 ml volumetric flask, 5 ml of diluent was added, sonicated to mix and volume was made up to mark with diluent.

Twenty tablets of alprazolam were accurately weighed, powdered an equivalent of 200 mg of active ingredient was taken into a 100 ml volumetric flask, dissolved in 70 ml of diluent, ultra- sonicated for about 10 min, filtered and the volume made up to the mark with the diluent. From this solution, 0.1 ml of solution was transferred to a 10 ml volumetric flask, 5 ml of diluent was added, sonicated to mix and the volume made up to mark with diluent.

Preparation of 0.02 M ammonium acetate buffer (pH 4.0)

Ammonium acetate (1.07972 g) was transferred to a 1 L volumetric flask. Water (700 ml) was added and sonicated to dissolve and degas, filtered through 0.45µm filter paper and volume was made up to the mark with water. The pH of the resultant solution was adjusted to 4.0 with glacial acetic acid and sonicated for 2 min for proper mixing.

Preparation of 10 % methanol in acetonitrile

Methanol (5 ml) was taken in a 500 ml measuring cylinder, made up to volume with acetonitrile and sonicated for 2 min for proper mixing**.**

RESULTS & DISCUSSION

A simple and selective method was developed for the simultaneous determination of sertraline and Alprazolam in tablet dosage forms. Analysis was tried on a waters Kromasil C18 (4.6 mm x 250 mm i.d, 5 µm particle size) column, using 0.05M ammonium acetate buffer and methanol as well as methanol/acetonitr ile in varying proportions. The concentration of the organic modifier, buffer pH and column temperature were optimized to separate the two compounds with good resolution in less time. The optimized chromatographic conditions were shown in table 1. The column was maintained at different temperatures ranging from 25 to 50 ˚C. The UV overlaid spectra of both sertraline and alprazolam showed that both drugs absorb appreciably at 225 nm; hence, 225 nm was selected as the detection wavelength. The method showed good linearity for both sertraline and alprazolam. The retention time of sertraline hydrochloride was 4.4 min and that of alprazolam 6.3 min. Relative standard deviation (% RSD) of retention times (Rt) and peak areas were < 1 and means of tailing factor (> 2), resolution factor (> 2) and theoretical plates (> 2000) were well within the limits, hence the method passed system suitability tests. There was no interference of excipients with the analysis of the drugs. The standard and sample chromatograms were identical, which proves that the method is specific. The mean amount of drugs was 99.83 and 100.04 % for sertraline and alprazolam, respectively. When analysis was performed by a second analyst on a second system, RSD was < 1 %,, which proves the precision of the method. The method is robust and unaffected by small variations in test conditions. The method also satisfied stability requirements.

The proposed simultaneous RPHPLC method was validated according to ICH guidelines.

System suitability

Standard solutions of sertraline and alprazolam were injected six times and chromatograms were recorded. Relative standard deviation (% RSD) of retention time (Rt) and peak areas were calculated. The mean of tailing factor and theoretical plates were also calculated.

Linearity

From the working standard solutions various dilutions were prepared for both sertraline HCl and alprazolam. Solutions in the concentration range of 1-50 µg/ml were injected and chromatograms were recorded. The standard chromatograms for both sertraline and alprazolam were shown in figure 3 and 4. The calibration curve was plotted between concentrations against peak area and shown in figure 5 and 6 respectively. The regression coefficient was calculated from calibration data.

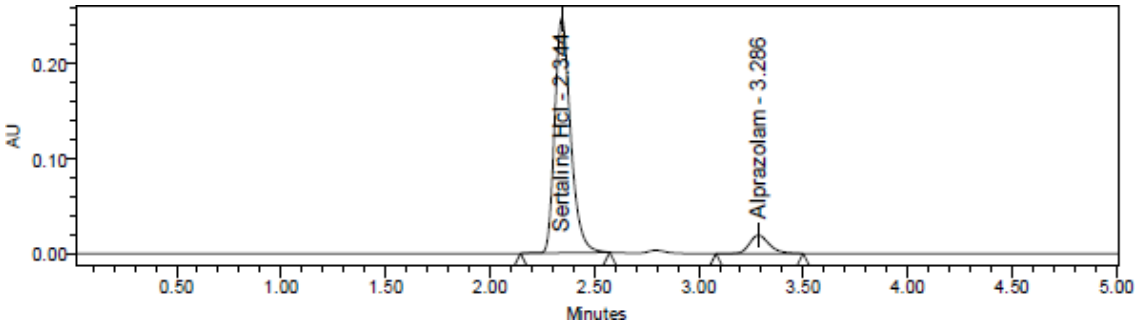

Figure 1: Chromatogram showing for Sertraline HCl and Alprazolam (Standard)

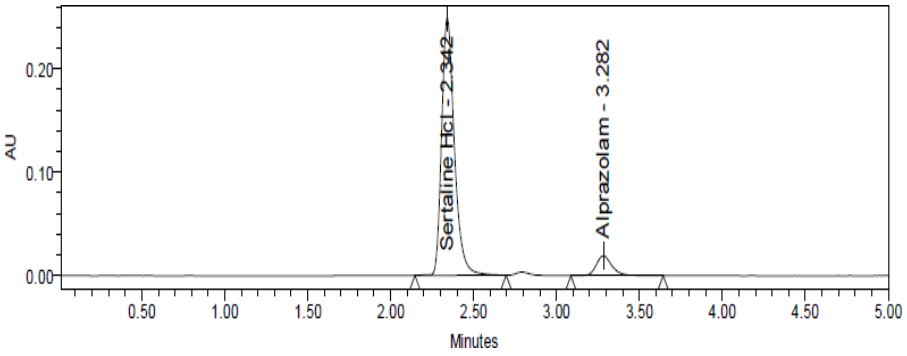

Figure 2: Chromatogram showing for Sertraline HCl and Alprazolam (sample)

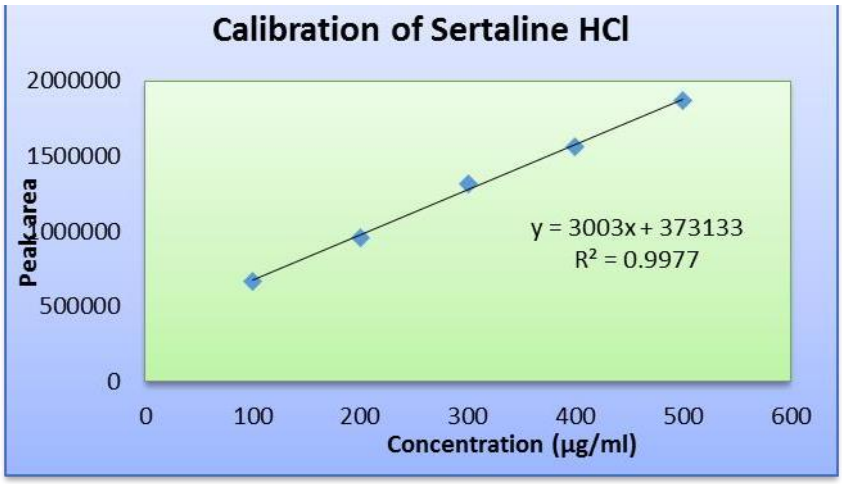

Figure 3: Chromatogram showing calibration curve for Sertraline HCl

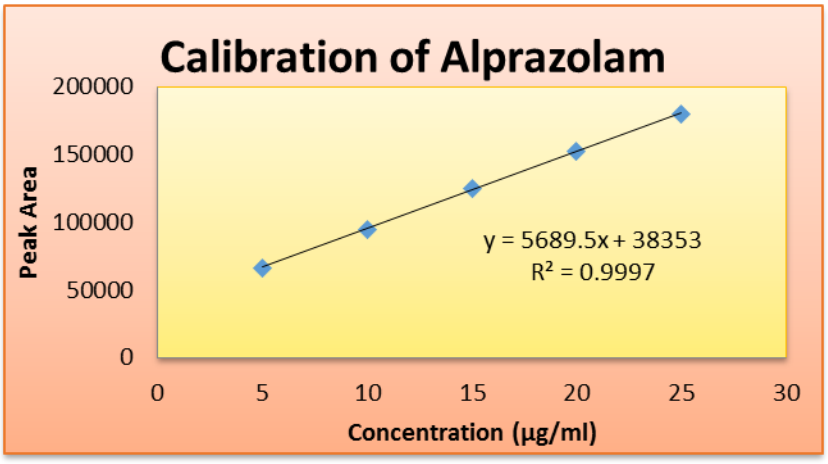

Figure 4: Chromatogram showing calibration curve for Alprazolam

Table 1: System suitability parameters for Sertraline HCl and Alprazolam

S. No	Name	Mobile phase	Flow Rate	Retention time (min)	Area (µV sec)	USP tailing	USP plate count
1	**Sertraline HCl**	Ammonium acetate (pH 4.0), 10 % methanol in acetonitrile (60:40)	1.0 ml/min	2.3	124505	1.2	4673.4
2	**Alprazolam**			3.2	1308495	1.3	6090.3

Table 2: Results of method precession for Sertraline HCl and Alprazolam

S.No.	Sertraline HCl		Alprazolam	
	Rt	Area	Rt	Area
1	2.345	1302726	3.287	123149
2	2.344	1302947	3.287	123766
3	2.343	1303236	3.288	124271
4	2.344	1303977	3.285	124691
5	2.345	1309759	3.284	124956
Mean		1304529.8		124162.7
Std. Dev.		2961.8		725.6
% RSD		**0.2**		**0.6**

Table 3: Accuracy (recovery) data for Sertraline HCl

%Concentration (at specification Level)	Area	Amount Added (mg)	Amount Found (mg)	% Recovery	Mean Recovery
50%	656659.5	5.0	5.036	100.7%	99.84%
100%	1304258	10.0	10.003	100.0%	
150%	1854608	14.4	14.224	98.780%	

Table 4: Accuracy (recovery) data for Alprazolam

%Concentration (at specification Level)	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%	

Table 5: Change in Flow Rate (ml/min) data for Sertraline HCl and Alprazolam

S. No	Flow Rate (ml/min)	Sertraline HCl		Alprazolam	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	0.8	5339.9	1.4	7063.3	1.3
2	1.0	4673.4	1.3	6090.3	1.2
3	1.2	5216.0	1.4	6998.0	1.3

Table 6: Change in Organic Composition in the Mobile Phase for Sertraline HCl and Alprazolam

S. No	Change in Organic Composition in the Mobile Phase	Sertraline HCl		Alprazolam	
		USP Plate Count	USP Tailing	USP Plate Count	USP Tailing
1	10% less	4508.4	1.3	6387.7	1.2
2	*Actual	4673.4	1.4	6090.3	1.2
3	10% more	4318.1	1.3	6232.5	1.2

Precision

Six different samples of both drugs were analyzed and % RSD of assay values was calculated. The %RSD was found to be within the limits and the values were shown in table 2.

Ruggedness (Intermediate precision)

The analysis was performed for both the drugs by a second analyst on Schimadzu HPLC system. The assay of six different samples was performed and % RSD of assay values was calculated. Different samples were performed and % RSD of assay values was calculated.

Accuracy

Accuracy of method was measured in terms of % recovery. Sample solutions were prepared at three different concentration levels, i.e., 50, 100 and 150 %. A predetermined amount of standard was added to these solutions and % recovery was determined by assaying the solutions. The results were presented in table 3 and 4.

Robustness

The samples of both drugs were subjected to different parameters by changing buffer, mobile phase, flow rate and column temperature. In the present study flow rate and mobile phase were slightly varied and standard solution was injected. Six replicates and system suitability tests were performed and the validation parameters indicated above were evaluated. The results were shown in table 5 and 6.

CONCLUSION

A simple and accurate reverse phase HPLC method has been developed for the determination of sertraline and alprazolam. The method was validated as per ICH guideline in terms of specificity, precision, accuracy, linearity, limit of detection, ruggedness, robustness and solutions and mobile phase stability. A single method can thus be used for the routine analysis of sertraline and alprazolam in dosage forms.

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