CODEN [USA]: IAJPBB ISSN : 2349-7750

INDO AMERICAN JOURNAL OF
PHARMACEUTICAL SCIENCES

SJIF Impact Factor: 7.187
https://doi.org/10.5281/zenodo.7548297

Available online at: http://www.iajps.com *Research Article*

SIMULTANEOUS ESTIMATION OF SERTRALINE AND ALPRAZOLAM IN ITS BULK AND PHARMACEUTICAL DOSAGE FORM BY RP-HPLC METHOD

B.Sanghavi, P.Prapulla
Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar, Hyderabad, Telangana, India

Abstract:
A simple, accurate, rapid and precise isocratic reversed-phase high- performance liquid chromatographic method has been developed and validated for simultaneous determination of Sertraline and Alprazolam in tablets. The chromatographic separation was carried out on an cosmosil packed column 5c-18 ms II (250×4.6 i.d) with a mixture of acetonitrile: methanol: phosphate buffer pH 3 adjusted with orthophosphoric acid (20:50:30, v/v) as mobile phase; at a flow rate of1.0 ml/min. UV detection was performed at 239 nm. The retention times were 4.915 and 8.056 min. for Sertraline and Alprazolam respectively. Calibration plots were linear (r^2>0.998) over the concentration range 10-60 µg/ml for sertraline and 10-60 µg/ml Alprazolam. The method was validated for accuracy, precision, specificity, linearity, and sensitivity. The proposed method was successfully used for quantitative analysis of tablets. No interference from any component of pharmaceutical dosage form was observed. Validation studies revealed that method is specific, rapid, reliable, and reproducible. The high recovery and low relative standard deviation confirm the suitability of the method for routine determination of Sertraline and Alprazolam in bulk drug and tablet dosage form.

Keywords: *Sertraline, Alprazolam, RP-HPLC, Simultaneous estimation.*

Corresponding author:
B.Sanghavi,
M. Pharmacy,
Department of pharmaceutical Analysis,
Mother Teresa College of Pharmacy, Ghatkesar, NFC Nagar,
Hyderabad, Telangana,India
E-mail: priyankababy3466@gmail.com

QR code

Please cite this article in press B.Sanghavi et al, ***Simultaneous Estimation Of Sertraline And Alprazolam In Its Bulk And Pharmaceutical Dosage Form By RP-HPLC Method..,*** *Indo Am. J. P. Sci, 2022; 09(12).*

INTRODUCTION:

Sertraline is indicated for the management of major depressive disorder (MDD), post-traumatic stress disorder (PTSD), obsessive-compulsive disorder (OCD), panic disorder (PD), premenstrual dysphoric disorder (PMDD), and social anxiety disorder (SAD).[1] Common off-label uses for sertraline include the prevention of post stroke depression, generalized anxiety disorder (GAD), fibromyalgia, premature ejaculation, migraine prophylaxis, diabetic neuropathy, and neurocardiogenic syncope.[2] Sertraline selectively inhibits the reuptake of serotonin (5-HT) at the presynaptic neuronal membrane, thereby increasing serotonergic activity. This results in an increased synaptic concentration of serotonin in the CNS, which leads to numerous functional changes associated with enhanced serotonergic neurotransmission.[3] These changes are believed to be responsible for the antidepressant action and beneficial effects in obsessive-compulsive (and other anxiety related disorders). It has been hypothesized that obsessive-compulsive disorder, like depression, is also caused by the disregulation of serotonin.[4] IUPAC name of Sertraline is (1S,4S)-4-(3,4-dichlorophenyl)-N-methyl-1,2,3,4-tetrahydronaphthalen-1-amine. Molecular Formula is $C_{17}H_{17}NCl$. Molecular Weight is 306.23.

Alprazolam is indicated for the acute treatment of generalized anxiety disorder in adults.[5] Alprazolam is also indicated, either as a standard or extended-release formulation, for the treatment of panic disorder with or without agoraphobia in adults. 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. IUPAC name of Alprazolam is 3-O-(1-benzhydrylazetidin-3-yl) 5-O-propan-2-yl 2-amino-6-methyl-4-(3-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate. Molecular formula is $C_{33}H_{34}N_4O_6$. Molecular Weight is 582.6. Alprazolam is soluble in organic solvents such as ethanol, DMSO, and dimethyl formamide (DMF), which should be purged with an inert gas. The solubility of Alprazolam in ethanol is approximately 15 mg/ml and approximately 30 mg/ml in DMSO and DMF. Alprazolam is sparingly soluble in aqueous buffers.

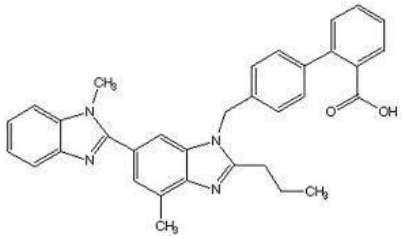

Figure 1: Structure of Sertraline

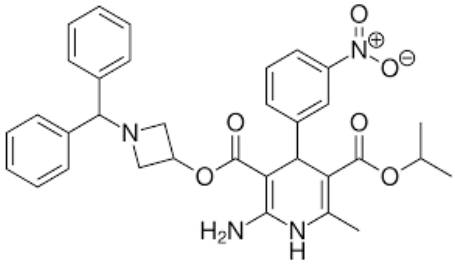

Figure 2: Structure of Alprazolam

Literature survey shows that a number of methods have been reported for estimation of Sertraline and Alprazolam individually or in combination with other drugs. [6-10] However, there is only few HPLC methods are reported for the simultaneous estimation of these drugs in combined dosage forms. I got better results than already published one. The aim of the present study was A New Rp-Hplc Method for Simultaneous Estimation of Sertraline and Alprazolam in Its Bulk and pharmaceutical Dosage Form and Its Force Degradation Studies as Per Ich.

MATERIALS AND METHODS:

Chemicals and Reagents: Sertraline and Alprazolam were Purchased from Sun Pharma India Limited. NaH_2PO_4 was analytical grade supplied by Finerchem limited, Orthophosphoric acid (Merck), and Water and Methanol for HPLC (Lichrosolv (Merck).

Equipment and Chromatographic Conditions: The chromatography was performed on a Waters 2695 HPLC system, equipped with an auto sampler, UV detector and Empower 2 software. Analysis was carried out at 239 nm with column cosmosil packed column 5c-18 ms II (250×4.6 i.d), dimensions at 25^0C temperature. The optimized mobile phase consists of acetonitrile: methanol: phosphate buffer pH 3 adjusted with orthophosphoric acid (20:50:30, v/v). Flow rate was maintained at 1 ml/min.

Preparation of solutions:

Standard solutions and calibration graphs for chromatographic measurement:

Stock standard solutions were prepared by dissolving separately 5 mg of Alprazolam and

Sertraline in 10 ml mobile phase (1000 µg/ml). The standard calibration solutions were prepared by appropriate dilution of the stock solution with methanol to reach a concentration range of 10-60µg/ml for Sertaline and 10-60 µg/ml for Alprazolam. 20 µl injections were made for each concentration and chromatographed under the optimized conditions described above. The peak area was plotted against the corresponding concentrations to obtain the calibration graphs.

Sample preparation: Twenty tablets' contents were accurately weighed; their mean weight was determined and they were mixed and finely powdered. A portion equivalent to about one tablet was accurately weighed and transferred into a 100 ml volumetric flask containing 50 ml mobile phase, sonicated for 15 min and diluted to 100 ml with mobile phase. The resulting solution was centrifuged at 100 rpm for 15 min. Supernatant was taken and after suitable dilution the sample solution was then filtered using 0.45 µ filter (Millipore, Milford, MA). The original stock solution was further diluted to get sample solution of drug concentration of 50 µg/ml Alprazolam and 25 µg/ml Sertraline. A 20 µl volume of sample solution was injected into HPLC, six times. The peak areas for the drugs were measured at 239 nm and amounts of Alprazolam and Sertraline were determined using the related linear regression equations.

RESULTS AND DISCUSSION:

During the optimization of HPLC method, columns (cosmosil packed column 5c-18 ms-II 250 mm × 4.6 i.d), two organic solvents (acetonitrile, methanol and phosphate buffer), two buffers (acetate and phosphate) at two different pH values (3 and 4) were tested. Initially methanol: water, acetonitrile: water, acetonitrile: phosphate buffer, methanol: phosphate buffer was tried in different ratios at pH 3 and 4. Sertalineeluted with the tried mobile phases, but Alprazolam was retained. Then, with acetonitrile: methanol: phosphate buffer all the two drugs eluted. The mobile phase conditions were optimized so the peak from the first-eluting compound did not interfere with those from the solvent, excipients. Other criteria, *viz.* time required for analysis, appropriate k range (1<k<10) for eluted peaks, assay sensitivity, solvent noise was also considered. Finally, a mobile phase consisting of a mixture of acetonitrile: methanol: phosphate buffer pH 3 adjusted with orthophosphoric acid in ratio20:50:30 (v/v), was selected as mobile phase to achieve maximum separation and sensitivity. Flow rates between 0.5 to 1.2 ml/min were studied. A flow rate of 1.0 ml/min gave an optimal signal to noise ratio with a reasonable separation time. Using a reversed phase C18 column, the retention times for Sertalineand Alprazolam were observed to be 4.915 and 8.056 min. respectively. Total time of analysis was less than 10 min. The chromatogram at 239 nm showed a complete resolution of all peaks. Representative chromatograms of standard solutions (a) standard solution of Alprazolam (50 µg/ml); (b) standard solution of Sertaline(25 µg/ml) and (c) a standard solution containing 50 µg/ml Alprazolam, 25 µg/ml Setraline.

Table 1: Linearity Parameters for The Simultaneous Estimation of Sertraline and Alprazolam(*N*=6)

Parameters	Setraline	Alprazolam
μ_{max} (nm)	239	239
Beers law limit (µg/ml)	10-60	10 – 60
Correlation coefficient (r)	0.9994	0.9993
Regression equation (y=mx+c)	y= 230808.9x + 366397	y=249058 x + 2721008
Slope (m)	230808.9	249058
Intercept (c)	366397	2721008
LOD (µg/ml)	0.1379	0.0677
LOQ (µg/ml)	0.4180	0.2051
Standard Error	134339.4	162471.7

Validity of the analytical procedure as well as the resolution between different peaks of interest is ensured by the system suitability test. All critical parameters tested met the acceptance criteria on all days. As shown in the chromatogram, all three analytes are eluted by forming symmetrical single peaks well separated from the solvent front. Excellent linearity was obtained for all the two drugs in the range of 10-60 Sertralineand 10-60 Alprazolam. The correlation coefficients (r^2) were found to be greater than 0.999 (n=6) in all instances. The results of calibration studies are summarized in Table 1. The proposed method afforded high recoveries for Alprazolam and Sertraline tablet. Results obtained from recovery studies presented in Table 2, indicate that this assay procedure can be used for routine quality control analysis of this ternary mixture in tablet. Precision of the analytical method was found to be reliable based on % RSD (< 2%) corresponding to the peak areas and retention times. The % RSD values were less than 2, for intra-day and inter-day precision. Hence, the method was found to be precise for all the two drugs

Table -2: System Suitability Parameters for The Optimized Chromatogram by RP – HPLC

PARAMETERS	SETRALINE	ALPRAZOLAM
Tailing factor	1.10	1.18
Asymmetrical factor	1.10	1.18
Theoretical plates	24	11187
Capacity factor	6.697	14.377
Theoretical plate /unit Length	206.19	242.05
Resolution	Between AMLO and VALS 4.04	

The chromatograms were checked for the appearance of any extra peaks. It was observed that single peak for Sertraline (R_t±SD, 4.915±0.01) and Alprazolam (R_t±SD, 8.056±0.01) were obtained under optimized conditions, showing no interference from common tablet excipients and impurities. Also the peak areas were compared with the standard and % purity calculated was found to be within the limits. These results demonstrate the specificity of the method.

LOD and LOQ were found to be 0.1379 µg/ml and 0.4180 µg/ml for Alprazolam and 0.0677 µg/ml and 0.2051 µg/ml for Sertraline. In all deliberately varied conditions, the SD of retention times of Alprazolam and Sertalinewere found to be well within the acceptable limit. The tailing factor for all the two peaks was found to be < 1.5 (Table 3). The validated method was used in the analysis of marketed conventional tablet Amlopres with a label claim: 80 mg Alprazolam and 5 mg Sertralineper tablet. Representative chromatogram is shown in (fig. 4). The results for the drugs assay show a good agreement with the label claims.

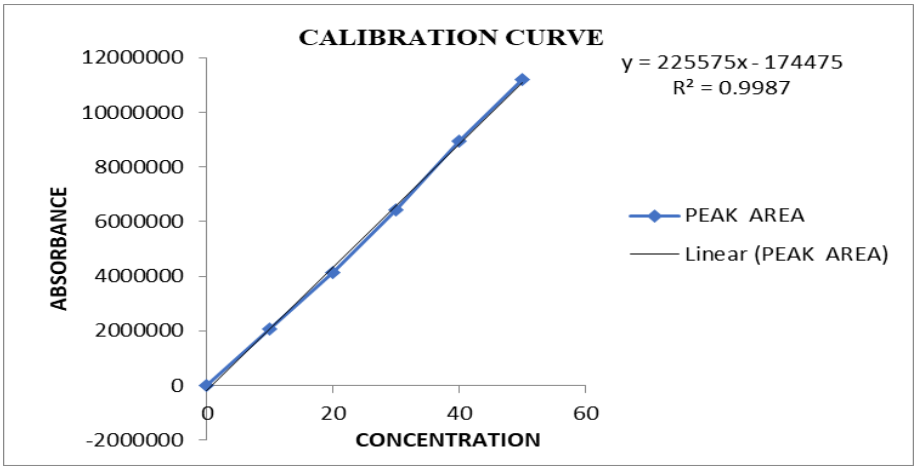

Fig.No.3. Calibration Curve of Sertaline

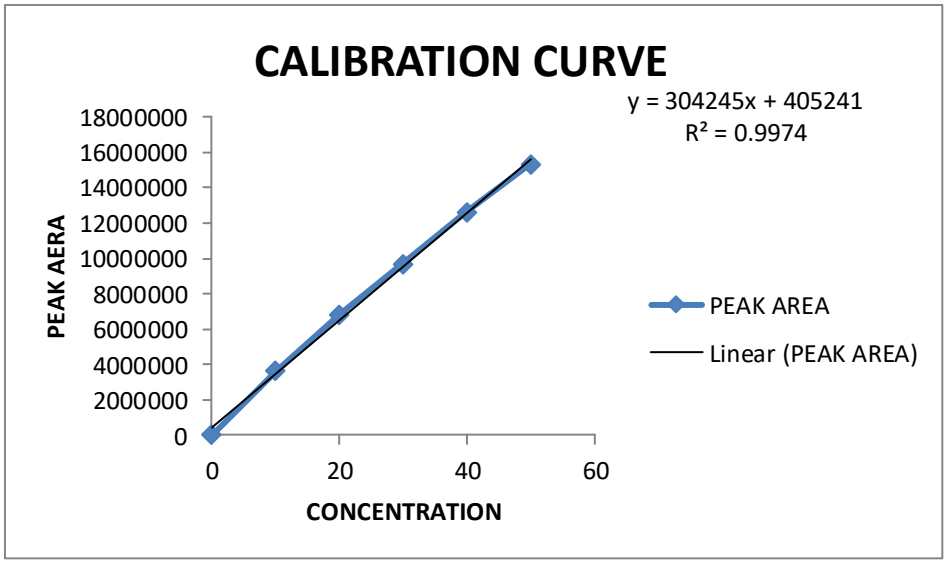

Fig.No.4. Calibration Curve of Alprazolam

CONCLUSION:

The developed HPLC method is simple, specific, accurate and precise for the simultaneous determination of Sertralineand Alprazolam from tablets. The developed method provides good resolution between Sertraline and Alprazolam and. It was successfully validated in terms of system suitability, linearity, range, precision, accuracy, specificity, LOD, LOQ and robustness in accordance with ICH guidelines. Thus, the described method is suitable for routine analysis and quality control of pharmaceutical preparations containing these drugs either as such or in combination.

REFERENCES:

1. FDA Approved Drug Products: Zoloft (sertraline hydrochloride) for oral use
2. Feng R, Wang P, Gao C, Yang J, Chen Z, Yang Y, Jiao J, Li M, Fu B, Li L, Zhang Z, Wang S: Effect of sertraline in the treatment and prevention of poststroke depression: A meta-analysis. Medicine (Baltimore). 2018 Dec;97(49):e13453. doi: 10.1097/MD.0000000000013453. (PubMed ID 30544429)
3. Rang, H. P. and Dale, M. M. (2012). Rang and Dale's Pharmacology (7th ed.). Edinburgh: Elsevier/Churchill Livingstone. (ISBN 978-0-7020-3471-8)
4. Pittenger C, Bloch MH, Williams K: Glutamate abnormalities in obsessive compulsive disorder: neurobiology, pathophysiology, and treatment. Pharmacol Ther. 2011 Dec;132(3):314-32. doi: 10.1016/j.pharmthera.2011.09.006. Epub 2011 Sep 22. (PubMed ID 21963369)
5. FDA Approved Drug Products: XANAX (alprazolam) tablets
6. Sanjay B, Manank Bhavsar, Indrajeet Singhvi, Anil Chandewar. Analytical method development and validation for the estimation of Alprazolam and Sertraline Hydrochloride by HPLC. Journal of Pharma Research. 11(1); 2014: 6-16.
7. Ramya S, Gowri Sankar D, Chaitanya KK, Gowri P. Simultaneous Determination of Alprazolam and Sertraline by RP-HPLC.Indian Journal of Pharmaceutical Sciences. 58(7); 2005: 352-360.
8. Lijuan He, Fang Feng, Jie Wu. Determination of Sertraline in Human plasma by HPLC-Electro sprays Ionisation mass spectrometry and method validation. J Chromatogr Sci. 2(3):2005: 43.
9. Venkateshwarlu K,Venisetty RK,Yellu NR, Keshetty S, Pai MG. Develpoment 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. 45(2); 2007:537-539.
10. Amir Farshchi, Golbarg Ghiasi, Gholamreza, Bahrami.High Performance Liquid Chromatography determination of Sertraline in Human plasma. Iranian Journal of Pharmaceutical Sciences. 5(3); 2009:171-178.