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

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ABSTRACT

The present work describes a simple Reverse Phase HPLC method for the determination of Alprazolam and Sertraline Hydrochloride from tablet formulations. The determination was carried out on a Shimadzu, Intersil ODS-3V (250 x 4.6 mm, packed with 5 micron) column using a mobile phase of Acetonitrile: Acetate Buffer with 0.1ml Diethylamine (pH- 3) in the ratio of (70:30). The flow rate and runtime were 1 ml/min and 10 min, respectively. The eluent was monitored at 237 nm. The method was reproducible, with good resolution between Alprazolam and Sertraline Hydrochloride. The detector response was found to be linear in the concentration range of 4-32 µg/ml for Alprazolam and 200-1600 µg/ml for Sertraline Hydrochloride.

Key Words: Alprazolam, Sertraline Hydrochloride, Acetonitrile, Shimadzu HPLC and ammonium acetate buffer (pH - 3).

INTRODUCTION

Alprazolam is an Anxiolytic and acts by bind nonspecifically to benzodiazepine receptors BNZ1, which mediates sleep, and BNZ2, which affects affects muscle relaxation, anticonvulsant activity, motor coordination, and memory. As benzodiazepine receptors are thought to be coupled to gamma-aminobutyric acid-A ($GABA_A$) receptors, this enhances the effects of GABA by increasing GABA affinity for the GABA receptor. Binding of the inhibitory neurotransmitter GABA to the site opens the chloride channel, resulting in a hyperpolarized cell membrane that prevents further excitation of the cell. Chemically it is 8-Chloro-1-methyl-6-phenyl-4H-[1,2,4]triazolo[4,3-a] [1,4]-benzodiazepine. It is official in Indian Pharmacopoeia, British Pharmacopoeia and European Pharmacopoeia.

Sertraline is (1*S*,4*S*)-4-(3,4-Dichlorophenyl)-*N*-methyl- 1,2,3,4-tetrahydronaphthalen-1-aminehydrochloride. It is official in IP, USP and BP. Sertraline is primarily a serotonin reuptake inhibitor (SRI). Therapeutic doses of sertraline (50–200 mg/day) taken by patients for four weeks resulted in 80–90% inhibition of serotonin transporter (SERT) in striatum as measured by positron emission tomography.[1-3]

Literature survey reveals that several methods like Spectrophotometry, HPLC, HPTLC and LC-MS were reported for the Equimolar quantities of Isatin (0.01mol) and Para-substituted 4-phenylthiazole-2-amine (0.01mol) were added into 20ml of absolute ethanol containing a few drops of glacial acetic acid and irradiated at 200W in the microwave for 2-6 min. The solid was washed with distilled water and then dried. determination of Alprazolam and Sertraline Hydrochloride in simple and biological fluids. These above developed methods are too expensive and time consuming. An attempt has been made to develop a simple, economical, precise, accurate and reproducible HPLC method for estimation of Alprazolam and Sertraline Hydrochloride in bulk as well as pharmaceutical formulations.[4-8]

MATERIALS & METHODS

Instrumentation

SHIMADZU HPLC with class Vp version 6.12 software equipped with LC10AT & LC10 ATVp pumps & SPD10A UV Visible detector.A UV-Visible Spectrophotometer (Nicolet-e-100) with spectral bandwidth of 2 nm and wavelength accuracy of 0.5 nm, with automatic wavelength correction employing a pair of quartz cells. An electronic analytical balance (AFCOSET) was used for weighing the sample.

Optimized chromatographic conditions

Chromatograph: SHIMADZU HPLC with class Vp version 6.12 software equipped
Flow Rate: 1 ml/minute
Detector: SPD10A UV Visible detector
Detection Wavelength: 237 nm
Injection Volume: 20µl
Temperature: Ambient
Mobile Phase: Acetonitrile: 0.05M ammonium acetate buffer (pH - 3) (70:30).

Preparation of the mobile phase for the determination of Alprazolam and Sertraline Hydrochloride

2.31gm of Ammonium acetate was accurately weighed and dissolved in 200ml of distilled water by sonication and the volume was made upto 300ml with distilled

Water and was taken in a 1000ml bottle and the final volume was made upto 1000ml with Acetonitrile and 0.1ml diethylamine was added and filtered through 0.45µm (pore diameter) Whattman filter paper. The pH should adjust 3 with phosphoric acid. Out of the 1000ml, 500ml was taken for HPLC run and the rest was kept for dilution purpose for analysis of Alprazolam and Sertraline.

Preparation of the Stock Solutions

Exactly 2mg of Alprazolam and 100 mg of Sertraline were taken in a 50ml volumetric flask. Then 30ml of mobile phase was added to it and sonicated for 2 minutes. Then the final volume i.e., 50ml was made with mobile phase and filtered through 0.45 µ membrane to the get the stock solution (40 µg/ml Alprazolam and 2mg/ml Sertraline) as per formulation composition.

Preparation of the working standard solutions

In to a series of 10ml volumetric flasks containing aliquots of Alprazolam and Sertraline, standard stock solutions equivalent to 4-32 µg/ml Alprazolam and 200-1600 µg/ml Sertraline were taken and volume was made up to 10ml with mobile phase, sonicated and filtered through 0.45 µ membrane.

Procedure for Calibration curve

The column was equilibrated with the set chromatographic conditions for 30mins, then 20 µl each of the working standard solution in the determination of Alprazolam and Sertraline Hydrochloride were injected and average retention time and peak area ratio of drug were noted and a graph was plotted by taking concentration in mcg/ml on X-axis and peak area ratio on Y-axis, the results of which are shown in table-1 and figure-1 & 2.

Preparation of sample drug solution from formulations

Twenty tablets containing Alprazolam and Sertraline of marketed formulations [ANXIT PLUS] were taken and powered. The power equivalent to 0.4mg and 20mg of Alprazolam and Sertraline was dissolved in 10ml of mobile Phase to get a stock solution of 40µg/ml and 2mg/ml of Alprazolam and Sertraline Respectively and then sonicated for 30mins. This solution was filtered through a Whattman filter paper. From the filtrate 0.1ml and 0.2ml were taken and further diluted with mobile phase up to 10ml i.e, 0.1ml from stock (where 4µg/ml and 200µg/ml of Alprazolam and Sertraline was present respectively) which is within the range and was used for analysis.

Analysis of formulation

The amount of drug present in each pharmaceutical formulation was calculated by Using the standard calibration curves (concentration in µg/ml was taken on x-axis and peak area ratio on y-axis). The result is shown in Table-2. Chromatograms were Shown in Figure-4.

Validation

Validation of the developed method was done according to the USP 2006, Asian edition and ICH guideline.[09-15]

Method Validation Parameters

The precision of each method was ascertained separately from the peak area Ratios obtained by actual determination of six replicates of a fixed amount of Drug. The precision of the assay was also determined in terms of intra and interday variation in the peak areas of a set of drug solutions on three different days. The intra and inter day variation in the peak area ratio of the drug solution was calculated in terms of %RSD

and the results are presented in the Table-3, for Alprazolam and Sertraline.

Accuracy

To determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (80%, 100% and 120%) of bulk samples of drug to the pre-analyzed formulation solution of concentration 10µg/ml and 500µg/ml for Alprazolam and Sertraline respectively.Then the percentage recovery values were calculated. The results were shown in table-4, for Alprazolam and Sertraline.

System suitability parameters

System suitability parameters can be defined as tests to ensure that the method can generate results of acceptable accuracy and precision. The requirements for system suitability are usually developed after method development and validation has been completed. (or) The USP (2000) defines parameters that can be used to determine system suitability prior to analysis. The system suitability parameters like Theoretical plates (N), Tailing factor, LOD (µg/ml) and LOQ (µg/ml) were calculated and compared with the standard values to ascertain whether the proposed RP-HPLC method for the estimation of Alprazolam and Sertraline in pharmaceutical formulations was validated or not. The results were shown in table-5, for both Alprazolam and Sertraline.

Table No.1 Linearity table of Alprazolam and Sertraline

Concentration (µg/ml)		Area	
ALP	**SER**	**ALP**	**SER**
4	200	45.447	241.443
8	400	94.43	472.602
12	600	135.475	748.326
16	800	178.915	945.176
20	1000	227.087	1237.976
24	1200	274.682	1439.64
28	1400	320.129	1677.083
32	1600	356.93	1925.413

Figure No.1 Standard graph for the estimation of Alprazolam

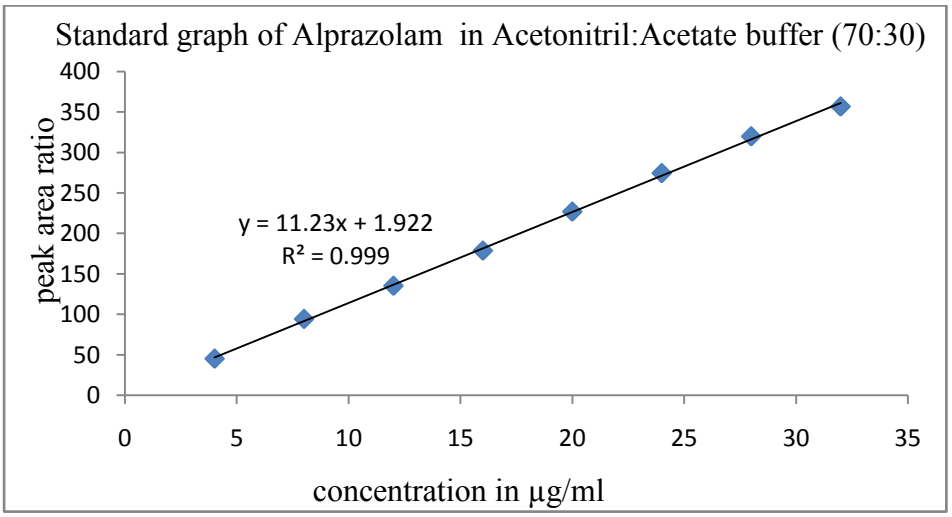


Figure No.2 Standard graph for the estimation of Sertraline

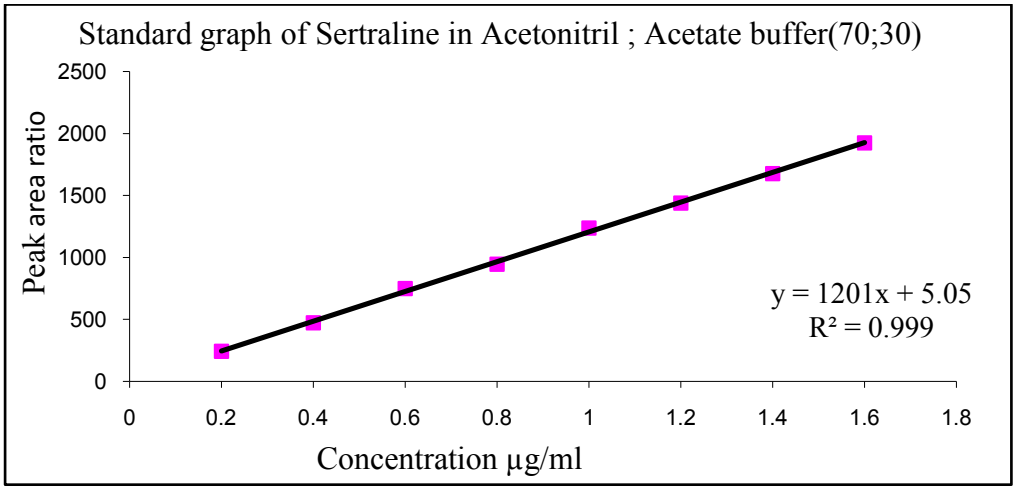
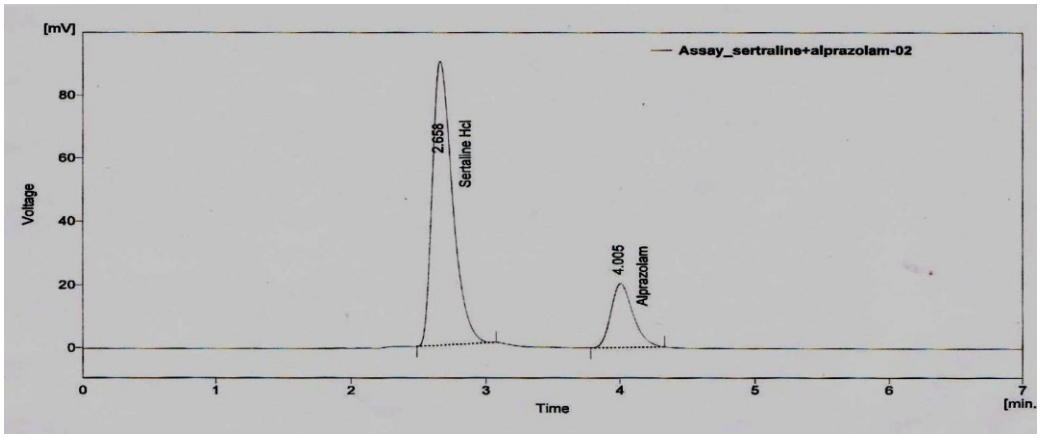


Figure No.3 Typical chromatogram of Alprazolam and Sertraline (Standard)



Name of the peaks	Retention time
Alprazolam	4.005
Sertraline	2.658

Table No.2 Amount of Alprazolam and Sertraline in their respective Formulations.

Drug	Brand	Formlulation	Company	Labeled Amount (mg)	Amount Obtained *(mg)	% Drug	% RSD
Alprazolam & Sertraline	Anxit Plus	Tablet	Micro labs	0.5 & 25	0.493±0.002454 & 24.943±0.038	98.75 & 99.77	0.4969 & 0.15234

*Each value is average of three determinations ±Standard deviation.

Figure No.4 A typical chromatogram of Alprazolam and Sertraline in formulation

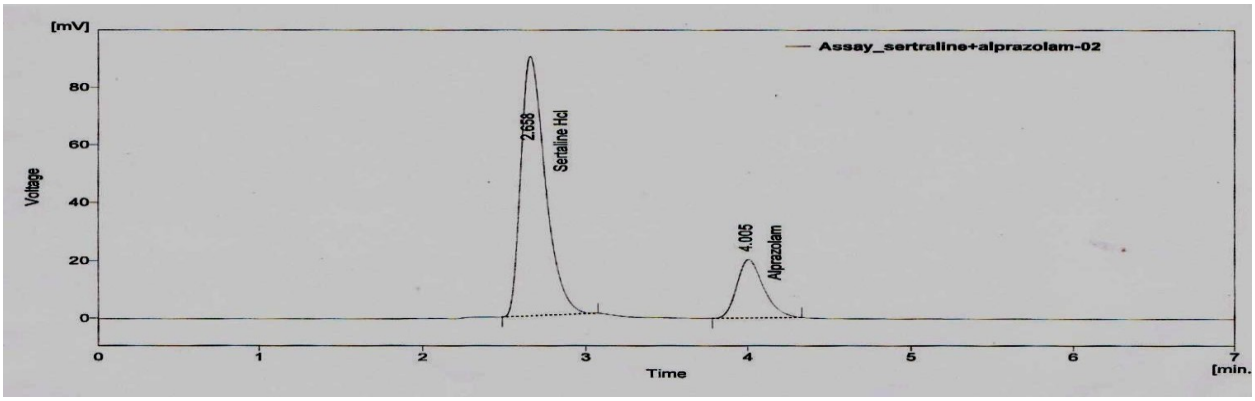


Table No. 3: Precision for Alprazolam and Sertraline

Drug	**Concentration (µg/ml)**	**Peak area ratio***	**%RSD**
Alprazolam	20	226.0023± 2.0104	0.8895
Sertraline	1000	1237.247± 1.7589	0.142

*Each value is average of six determinations ±SD

Table No. 4: Accuracy study of Alprazolam and Sertraline

Drug	Conc. Of Pure drug (µg/ml)	Conc. Of Formulation (µg/ml)	%Recovery*of pure drug	%RSD
Alprazolam	8	10	99.18±0.33005	0.3327
	10	10	98.54±0.68879	0.6989
	12	10	98.49±0.6264	0.6361
Sertraline	400	500	99.36±0.2112	0.2126
	500	500	99.59±0.22	0.2209
	600	500	99.67±0.1464	0.1468

*Each value is average of three determinations ±SD

Table No. 5: System suitability parameters

Sl.No	Parameters	Alprazolam & Sertraline
1	Theoretical plates (N)	2930, 2489
2	Tailing factor	1.516, 1.895
3	LOD	0.5907, 0.0048
4	LOQ	1.7902, 0.0146

RESULTS AND DISCUSSION

From the linearity table-1 it was found that these drugs obey linearity within the concentration range of 4-32µg/ml for Alprazolam and 200-1600µg/ml for Sertraline. From the results shown in precision table-3, it was found that %RSD is less than 2%; which indicates that the proposed methods have good reproducibility. From the results shown in accuracy table-4, it was found that the % recovery values of pure drug from the pre-analyzed solutions of formulations were in between 98.49-99.82 %, which indicates that the method was accurate and also reveals that the commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed method. The system suitability parameters table-5 also reveal that the values were within the specified limits for the proposed method.

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