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METHOD DEVELOPMENT AND VALIDATION FOR THE SIMULTANIOUS ESTIMATION OF SERTALINE AND ALPRAZOLAM USING RP HPLC IN BULK DOSAGE FORMS

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ABSTRACT

A reverse phase-liquid chromatographic method with UV detection at 225nm is described for simultaneous determination of Alprazolam and Sertaline. Chromatographic separation of the three drugs was achieved on an inertsil column, C18 (150x4.6 ID) 5µm column using a mobile phase consisting of a mixture of Ammonium Acetate buffer (pH 3.5): ACN: ACN in a ratio of 30:70. The liquid chromatographic method developed offers symmetric peak shape, good resolution, and reasonable retention time for both drugs. Linearity was found to be acceptable over the concentration ranges 1-3µg/ml for Alprazolam and 100-300µg/ml Sertaline. Accuracy was found to be acceptable over the concentration ranges 1-3µg/ml for Alprazolam and 100-300µg/ml Sertaline. The liquid chromatographic method was successfully applied to the quality control of formulated products containing Alprazolam and Sertaline.

Key Words: Alprazolam, Sertaline, reverse phase-liquid chromatographic method

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INTRODUCTION

Chromatography is the method of separation that finds applications in all branches of science. It was first invented by Russian Botanist Mikhail Twsett. This technique was used separate various plant pigments like chlorophylls and xanthophylls by passing solutions of these compounds through a glass column packed with finely divided calcium carbonate. The separated species appeared as colored Bands on the column hence the name of the process (Greek *chroma* meaning "color" and *graphein* meaning "writing"). Chromatography is defined as a non- destructive procedure for resolving multi-component mixture of trace, minor, or major constituents into its individual fractions. In chromatography, the sample is dissolved in the mobile phase which may be a gas, liquid, or a supercritical fluid. The principle involved in HPLC is that when a mixture containing different compounds is introduced into the mobile phase and allowed to flow over a stationary phase, the individual compounds travel at different speeds and get separated based on the relative affinities to the stationary phase and the mobile phase. The compounds are separated based on the polarity of the stationary phase and the mobile phase. High

Performance Liquid Chromatography is the most widely used of all the analytical separation techniques. The reasons for its popularity are its sensitivity, ready adaptability to quantitative determination, suitable for non- volatile and thermally fragile species, wide applicability to variety of substances such as amino acids, carbohydrates, nucleic acids, proteins, hydrocarbons, terpenoids, pesticides, steroids, metal-organic species and inorganic species. As high pressures (around 3000 psi) are used for the separation of the analytes down the column, it is often termed as High Pressure Liquid Chromatography.

Benzodiazepines bind nonspecifically to benzodiazepine receptors BNZ1, which mediates sleep, and BNZ2, which 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.

Mechanism of action sertraline is not fully known, but the drug appears to selectively inhibit the reuptake of serotonin at the presynaptic membrane. This results in an increased synaptic concentration of serotonin in the CNS, which leads to numerous functional changes associated with enhanced serotonergic neurotransmission. It is suggested that these modifications are responsible for the antidepressant action observed during long term administration of antidepressants. It has also been hypothesized that obsessive-compulsive disorder is caused by the dysregulation of serotonin, as it is treated by sertraline, and the drug corrects this imbalance (1-4).

Aim is to develop new RP HPLC method for the simultaneous estimation of Alprazolam and Sertaline in pharmaceutical dosage form.

MATERIALS AND METHODS

Determination of Working Wavelength (λmax) (5-7)

In simultaneous estimation of two drugs isobestic wavelength is used. Isobestic point is the wavelength where the molar absorptivity is the same for two substances that are interconvertible. So this wavelength is used in simultaneous estimation to estimate both drugs accurately.

Preparation of standard stock solution of Alprazolam

2.5mg of Alprazolam was weighed and transferred in to 100ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 4ml to 10ml with methanol.

Preparation of standard stock solution of Sertaline

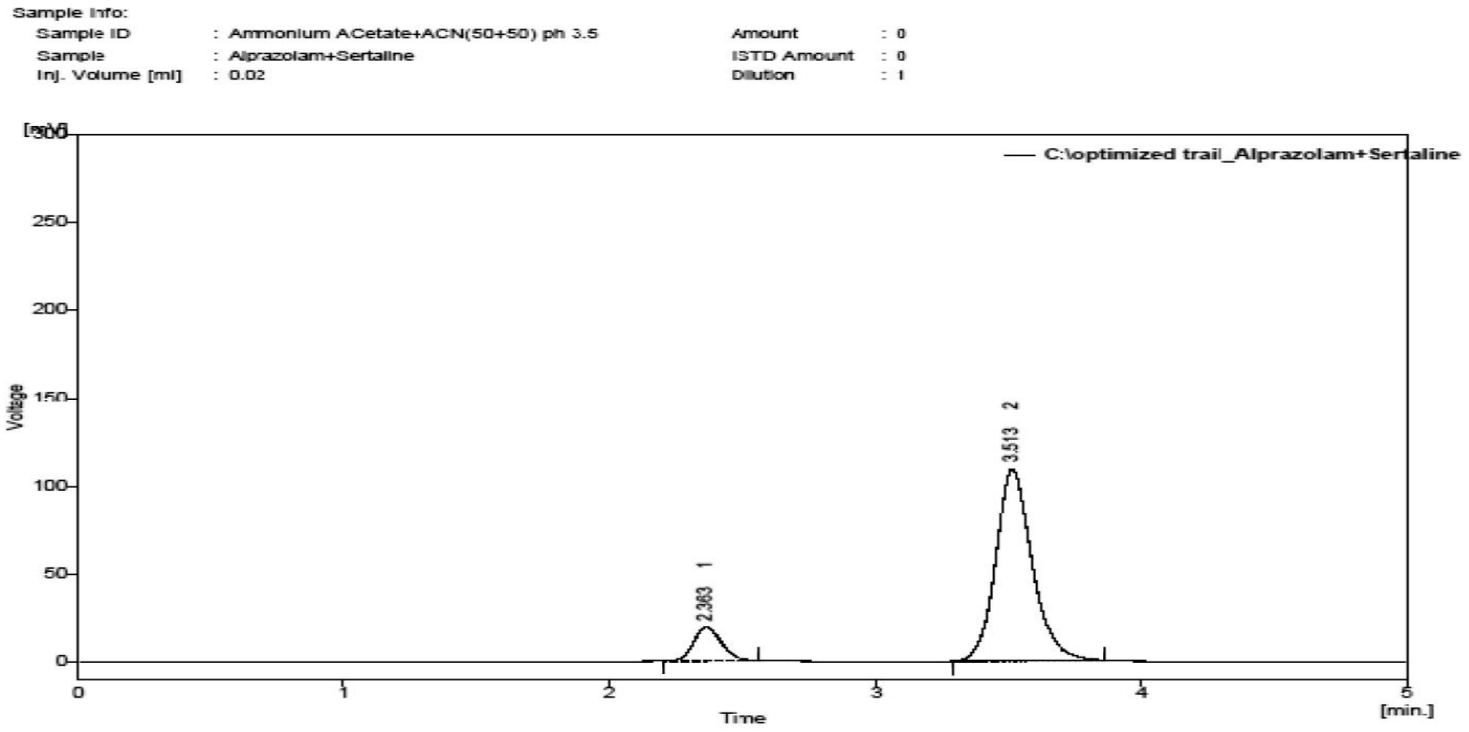
25mg of Sertaline was weighed in to 100ml volumetric flask and dissolved in Methanol and then dilute up to the mark with methanol and prepare 10 µg /ml of solution by diluting 0.4ml to 10ml with methanol.

Optimized chromatographic conditions

Optimized chromatographic conditions are shown in table-1 and fig-1.

Table-1 Optimized chromatographic conditions

Mobile phase	sodium di hydrogen phosphate buffer: Acetonitrile (30:70)
pH	3.5
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25ºC)
Sample temperature	Room temperature(20-25ºC)
Wavelength	225nm
Injection volume	20 µl
Run time	6 min
Retention time	About 2.363min for Alprazolam and 3.513min for Sertaline



Result Table (Uncal - C:\optimized trail_Alprazolam+Sertaline)

	Reter. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.363	136.129	19.057	11.69	14.8	0.11
2	3.513	1028.411	109.391	88.31	85.2	0.14
	Total	1164.539	128.449	100.00	100.0	

Column Performance Table (From 50% - C:\optimized trail_Alprazolam+Sertaline)

	Reten. Time	W05 [min]	Asymmetry [ ]	Capacity [ ]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [ ]
1	2.363	0.113	1.286	0.00	2409	24090	-
2	3.513	0.143	1.289	0.00	3329	33285	5.273

Fig-1 optimized chromatogram of Alprazolam and Sertaline

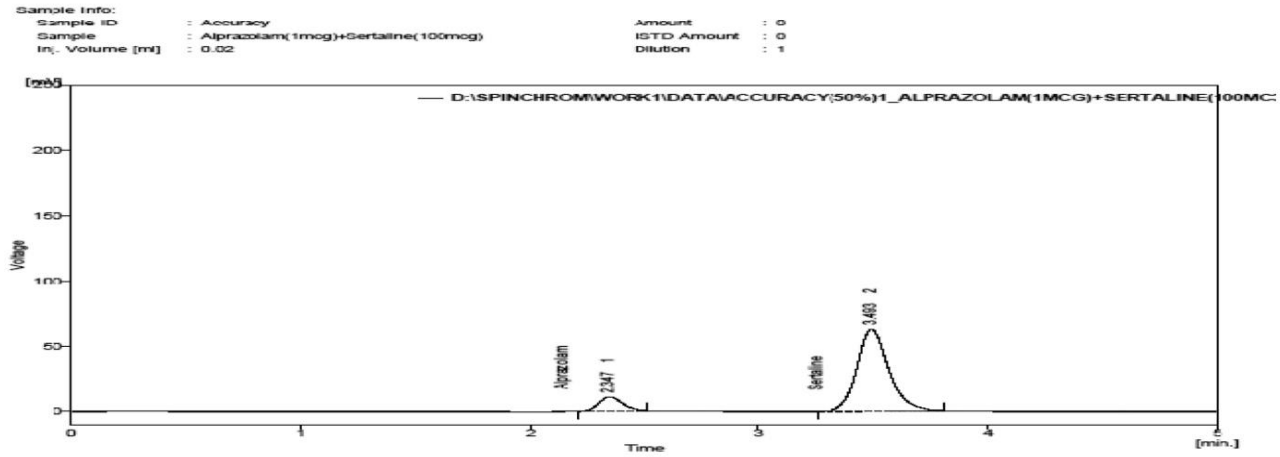
RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as blank. The resulting spectra show characteristic absorption maxima at 225nm for Alprazolam and nm for Sertaline 225nm for the combination. It is observed from the above data, diluents or excipient peaks are not interfering with the Alprazolam and Sertaline peaks.

The relationship between the concentration of Alprazolam and Sertaline and area of Alprazolam and Sertaline should be linear in the specified range and the correlation should not be less than 0.99. The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of Alprazolam and Sertaline is 0.998 and 0.999. The relationship between the concentration of Alprazolam and Sertaline and area of Alprazolam and Sertaline is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Accuracy of the method was determined by Recovery studies. To the formulation (pre analyzed sample), the reference standards of the drugs were added at the level of 50%, 100%, 150%. The recovery studies were carried out three times and the percentage recovery and percentage mean recovery were calculated for drug is shown in table. To check the accuracy of the method, recovery studies were carried out by addition of standard drug solution to pre-analyzed sample solution at three different levels 50%, 100% & 150% (Fig-2-4).

The percentage mean recovery of Alprazolam and Sertaline is 98.80% and 99.75% respectively.



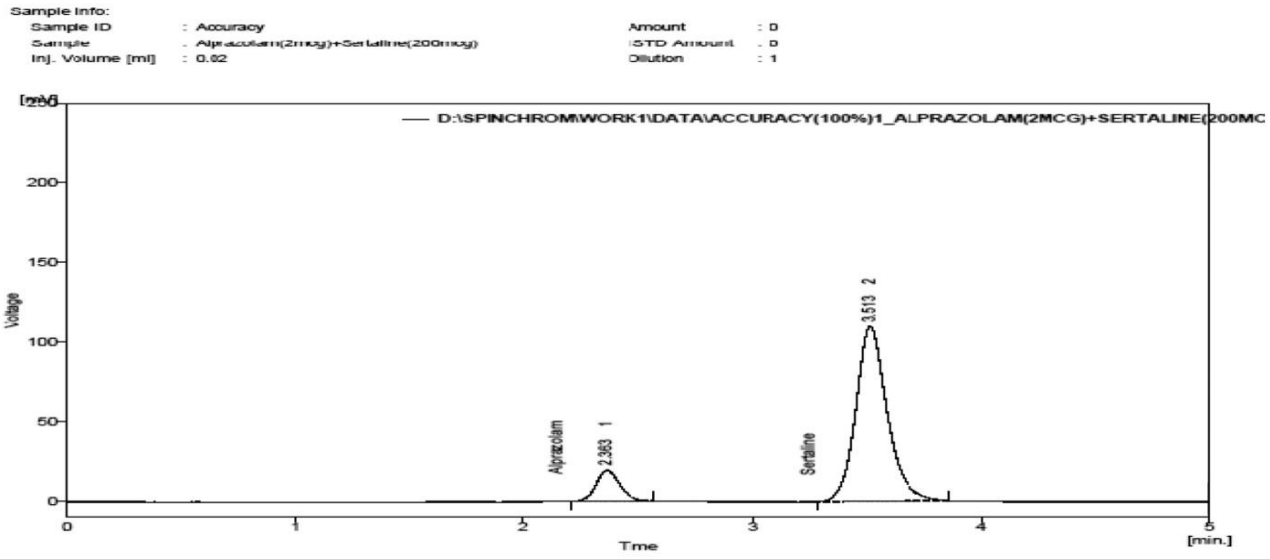
Result Table (Uncal - D:\SPINCHROM\WORK1\DATA\ACCURACY(50%)1_ALPRAZOLAM(1MCG)+SERTALINE(100MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.347	74.613	10.609	11.14	14.4	0.11
2	3.493	595.316	63.282	88.86	85.6	0.14
	Total	669.929	73.891	100.00	100.0	

Column Performance Table (From 50% - D:\SPINCHROM\WORK1\DATA\ACCURACY(50%)1_ALPRAZOLAM(1MCG)+SERTALINE(100MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.347	0.113	1.296	0.00	2375	23752	-
2	3.493	0.143	1.289	0.00	3291	32908	5.258

Fig-2 Chromatogram of 50%recovery



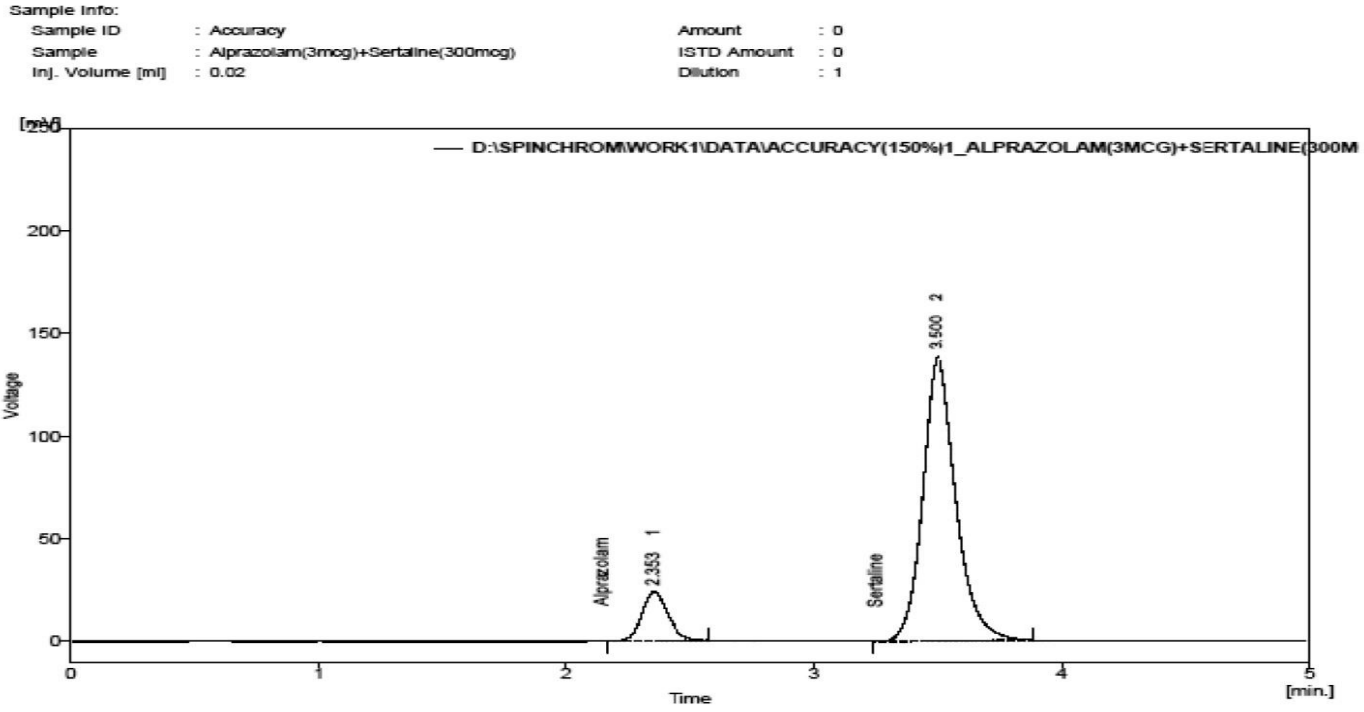
Result Table (Uncal - D:\SPINCHROM\WORK1\DATA\ACCURACY(100%)1_ALPRAZOLAM(2MCG)+SERTALINE(200MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.363	136.129	19.057	11.69	14.8	0.11
2	3.513	1028.411	109.391	88.31	85.2	0.14
	Total	1164.539	128.449	100.00	100.0	

Column Performance Table (From 50% - D:\SPINCHROM\WORK1\DATA\ACCURACY(100%)1_ALPRAZOLAM(2MCG)+SERTALINE(200MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.363	0.113	1.286	0.00	2409	24090	-
2	3.513	0.143	1.289	0.00	3329	33285	5.273

Fig-3 Chromatogram of 100% recovery



Result Table (Uncal - D:\SPINCHROM\WORK1\DATA\ACCURACY(150%)1_ALPRAZOLAM(3MCG)+SERTALINE(300MCG))

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.353	173.523	23.671	11.57	14.6	0.11
2	3.500	1325.818	138.662	88.43	85.4	0.14
	Total	1499.340	162.332	100.00	100.0	

Column Performance Table (From 50% - D:\SPINCHROM\WORK1\DATA\ACCURACY(150%)1_ALPRAZOLAM(3MCG)+SERTALINE(300MCG))

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	2.353	0.113	1.276	0.00	2389	23887	-
2	3.500	0.143	1.289	0.00	3303	33033	5.258

Fig-4 Chromatogram of 150% recovery

Test results for Alprazolam and Sertaline are showing that the % RSD of Assay results are within limits. The results were shown in table Table-2.

Table -2 Results for Method precision of Alprazolam and Sertaline

Alprazolam		
S.No.	Rt	Area
1	2.363	133.473
2	2.350	135.444
3	2.360	133.190
4	2.363	133.075
5	2.357	133.526
avg	2.358	133.742
stdev	0.0054	0.970
%RSD	**0.23**	**0.73**

Sertaline		
S.No.	Rt	Area
1	3.510	1015.834
2	3.500	1026.375
3	3.510	1023.904
4	3.513	1023.366
5	3.503	1021.136
avg	3.507	1022.123
stdev	0.0054	3.979
%RSD	**0.16**	**0.39**

From the observation it was found that the system suitability parameters were within limit at all variable conditions.

CONCLUSION

A simple and selective LC method is described for the determination of Alprazolam and Sertaline dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of Ammonium acetate buffer (pH 3.5) Acetonitrile (30:70v/v), with detection of 225nm. Linearity was observed in the range 1-3µg /ml for Alprazolam (r^2 =0.993) and 100-300µg/ml for Sertaline (r^2 =0.993) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

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