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ANALYTICAL METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF ALPRAZOLAM AND SERTRALINE HYDROCHLORIDE BY HPLC AND UV

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ABSTRACT: The present study involves the development and validation of simple, accurate, and precise analytical methods for the simultaneous estimation of Alprazolam and Sertraline Hydrochloride in combined pharmaceutical dosage forms. Two methods were developed: UV spectrophotometry and reverse-phase high-performance liquid chromatography (RP-HPLC). The UV spectrophotometric method utilized the simultaneous equation method based on absorbance measurements at 260 nm and 273 nm, the respective λ_{max} values of Alprazolam and Sertraline HCl. Both drugs obeyed Beer's Law in the concentration range of 5–50 $\mu\text{g/mL}$, with high correlation coefficients, indicating good linearity. The method showed satisfactory precision and accuracy, making it suitable for routine analysis. The RP-HPLC method was performed using a C18 column and a mobile phase composed of methanol, acetonitrile, and phosphate buffer (pH 4.5) in the ratio 50:15:35 v/v, at a flow rate of 1 mL/min. Detection was carried out at 239 nm, and the retention times were found to be approximately 3.0 minutes for Alprazolam and 6.4 minutes for Sertraline HCl. The method was validated according to ICH guidelines, and parameters such as specificity, linearity, accuracy, precision, robustness, and sensitivity were within acceptable limits. Both methods were successfully applied to the estimation of the drugs in commercial tablet formulations. The developed methods are reliable and can be effectively employed in routine quality control and pharmaceutical analysis.

INTRODUCTION: Sertraline and alprazolam belongs to the class of medications called 'antidepressant' used in the treatment of panic disorder with or without agoraphobia (fear of leaving one's own home or being in open or crowded places) ¹. Panic disorder is characterized by a panic attack in which the patient experiences a sudden feeling of terror even if there is no real danger. Sertraline and alprazolam contains Alprazolam and Sertraline. Alprazolam is a benzodiazepine ².

It works by increasing the activity of GABA (a chemical messenger in the brain that acts as a natural nerve-calming agent). Thereby, Sertraline & alprazolam relaxes muscles, produces a calming effect, and helps to fall asleep. Sertraline is a selective serotonin reuptake inhibitor (SSRI) ³. It increases the release of the serotonin hormone in the body, which is responsible for improving mood, cognition, and memory. The present UV and HPLC methods are relatively simple, rapid, and highly sensitive in the determination of sertraline and alprazolam.

Only limited methods have been reported in the literature survey ⁴⁻⁷. The aim of the present work was to develop and validate a simple, fast, and reliable isocratic RP-HPLC and UV method for the determination of sertraline and alprazolam in

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pharmaceutical dosage forms. The important features and novelty of the proposed method included simple sample treatment with sonication of small amount of powder sample at ambient temperature, centrifugation, and dilution; short elution time with internal standard eluted prior to sertraline and alprazolam; good precision (R.S.D. less than 5%) and high recovery (greater than 95%). Confirmation of the applicability of the developed method was validated according to the International Conference on Harmonisation (ICH), to determination of sertraline and alprazolam in pharmaceutical dosage forms.

MATERIALS AND METHODS:

Materials: A market sample of BEREST was analyzed, which is labeled to contain Alprazolam 0.25 mg and Sertraline HCl 25 mg. The analysis was carried out using an ATCO Balance for accurate weighing of the sample and a SHIMADZU UV-1700 double beam digital UV-Visible spectrophotometer for spectroscopic measurements. Methanol (AR grade) was used as the solvent for the preparation of standard and sample solutions. The instruments used in the study included a Shimadzu UV-Visible SPD-20A detector, an LC-20AT isocratic system, a Rheodyne injector, and a C18 column. The reagents and chemicals employed for the analysis were

acetonitrile, HPLC grade water, buffer solution, and methanol, all of which were of analytical or HPLC grade to ensure accuracy and reliability of the results.

U.V. Method:

Fixation of Various Parameters (Sertraline HCl): The determination of absorption maximum (λ_{max}) for Sertraline HCl was performed by accurately weighing 30 mg of authentic Sertraline HCl and transferring it into a 50 ml volumetric flask, where it was dissolved in methanol and the volume was made up to the mark with the same solvent. From this stock solution, 5 ml was pipetted into another 100 ml volumetric flask and diluted to volume with methanol. The absorbance of the resulting solution was measured against a solvent blank in the UV region of 200–400 nm, and the λ_{max} was found to be 236 nm, as shown in the corresponding spectrum. For the Beer's Law plot, 100 mg of authentic Sertraline HCl was accurately weighed, dissolved in methanol in a 100 ml volumetric flask, and diluted to the mark. From this solution, aliquots of 0.5 ml to 5 ml were transferred into separate 100 ml volumetric flasks and diluted to volume with methanol. The absorbance of each solution was recorded at 273 nm against a reagent blank^{8,9}.

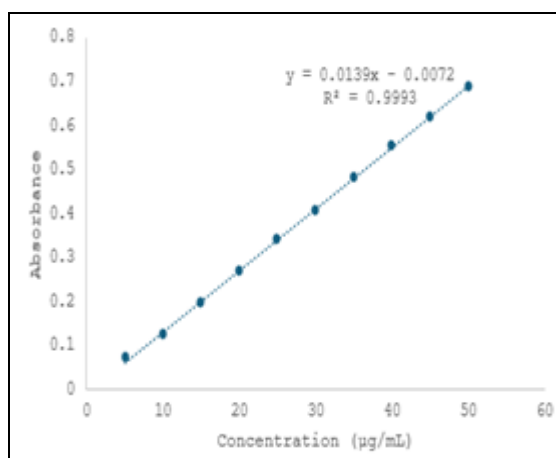


FIG. 1: SERTRALINE HCL PLOT

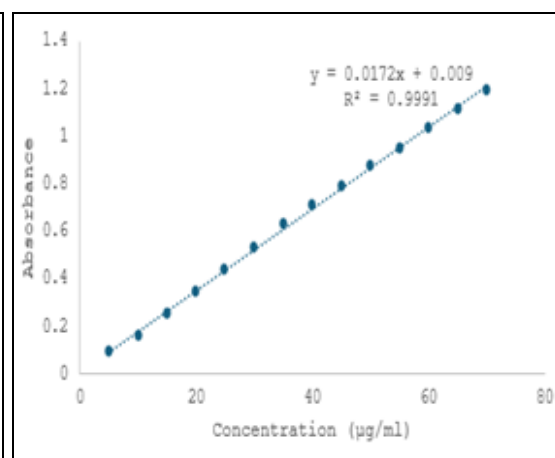


FIG. 2: DATA FOR DEVIATIONS FORM BEER'S LAW PLOT

Determination of Absorption Maximum (λ_{max})

Alprazolam: A 100 mg quantity of authentic alprazolam sample was accurately weighed and transferred into a 50 mL volumetric flask, dissolved in methanol, and the volume was made up to the mark with methanol. From this stock solution, 5

mL was pipetted into a separate 100 mL volumetric flask and diluted to volume with methanol. The absorbance of the resulting solution was measured against a solvent blank in the UV region between 200–400 nm, and the absorption maximum (λ_{max}) was found to be 260 nm. For Beer's Law plot

determination, 100 mg of alprazolam was accurately weighed and transferred into a 100 mL volumetric flask, dissolved in methanol, and the volume was made up to 100 mL with methanol to obtain a standard stock solution. From this, aliquots of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, and 5.0

mL were pipetted into separate 100 mL volumetric flasks and diluted to volume with methanol. The absorbance of each solution was measured at 245 nm against a reagent blank to establish the Beer's Law plot for alprazolam^{10, 11}.

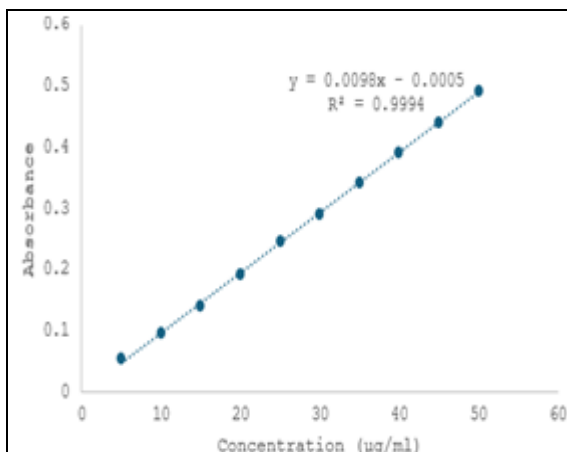


FIG. 3: DATA FOR BEER'S LAW PLOT FOR ALPRAZOLAM

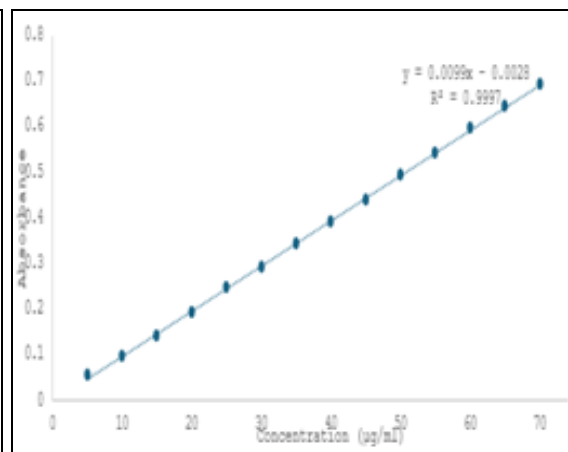


FIG. 4: DATA FOR DEVIATIONS FROM BEER'S LAW PLOT FOR ALPRAZOLAM

Preparation of Sertraline HCl Standard Solution: 100mg of authentic sertraline HCl sample is accurately weighed and transferred to 100ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made up to 100ml with methanol.

From this 1ml was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol. The absorbance and absorptivity values are shown in **Table 1A & 1B**¹².

Preparation of Sample Solution: Twenty tablets are weighed and average weight was calculated. The tablets are ground to a fine powder. A powder

equivalent to 25mg of sertraline and 0.25 mg of alprazolam was accurately weighed and transferred to 50ml volumetric flask and methanol was added and shaken until it dissolves and the volume was made up to 50ml with methanol.

This solution was filtered through Whatmann filter paper. From this 1ml was pipetted out in to separate 100ml volumetric flask and the volume was made up to 100ml with methanol. The absorbance of each solution was found out at 273nm (λ_{max} of sertraline HCl) and 260nm (λ_{max} of Alprazolam) against a reagent blank. The analysis values are given in **Table 2**¹³.

TABLE 1A: ABSORBANCE VALUES FOR STANDARD AND SAMPLE

Wavelength (nm)	Compound	Standard Absorbance	Notation	Sample Absorbance	Notation
273 nm (λ_1)	Alprazolam	0.2543	X ₁	0.6984	A ₁
	Sertraline HCl	0.1233	Y ₁		
260 nm (λ_2)	Alprazolam	0.1543	X ₂	0.5984	A ₂
	Sertraline HCl	0.1763	Y ₂		

TABLE 1B: ABSORPTIVITY VALUES FOR ALPRAZOLAM AND SERTRALINE HCL

Parameter	Alprazolam (a_x)	Sertraline HCl (a_y)
Absorptivity at 273 nm (λ_1) ($L \cdot mol^{-1} \cdot cm^{-1}$ or $L \cdot g^{-1} \cdot cm^{-1}$)	$a_{x1} = 212.6$	$a_{y1} = 1.233$
Absorptivity at 260 nm (λ_2)	$a_{x2} = 1232.1$	$a_{y2} = 1432.9$
Standard Deviation at 273 nm	$SD_{x1} = 0.3324$	$SD_{y1} = 0.2531$
Standard Deviation at 260 nm	$SD_{x2} = 0.4743$	$SD_{y2} = 0.1211$

* Absorptivity values are the mean of six determinations. S.D. is standard deviation. a_{x1} and a_{x2} are absorptivities of alprazolam at 260nm and 273nm respectively; a_{y1} and a_{y2} are absorptivities of sertraline hydrochloride at 260nm and 273nm respectively.

TABLE 2: ANALYSIS DATA OF TABLET FORMULATIONS

Parameter	Sertraline HCl	Alprazolam
Label Claim	25 mg	0.25 mg
Amount Found	24.46 mg	0.234 mg
% Drug Content	99.8 %	99.95 %
Standard Deviation (SD)	0.2764	1.4320
% Relative Standard Deviation (RSD)	0.2439%	1.1543%

Recovery Studies: To check the accuracy of the developed method and to study the interference of formulation additives, analytical recovery experiments were carried out by standard addition

method at 80, 100 and 120% level. From the total amount of drug found the percentage recovery was calculated. The results are reported in **Table 3** and **4**¹⁴.

TABLE 3: RECOVERY STUDIES: SERTRALINE HCL

Range (%)	Amount Found (mg)	Recovery (%)	R.S.D (%)
80%	24.55	99.80	0.0995
100%	24.58	99.80	0.1892
120%	24.59	99.83	0.09

*Recovery is the mean of three estimations.

TABLE 4: RECOVERY STUDIES: ALPRAZOLAM

Range (%)	Amount Found (mg)	Recovery (%)	R.S.D (%)
80%	0.245	99.90	0.2652
100%	0.247	99.95	0.1321
120%	0.248	99.90	0.1875

*Recovery is the mean of three estimations.

HPLC Method Development: For the preparation of the standard solution of alprazolam, accurately 0.00025 g of alprazolam was weighed and transferred into a 100 mL volumetric flask, and the volume was made up to the mark with the mobile phase. From this solution, 5 mL was pipetted into a separate 50 mL volumetric flask and diluted to volume with the mobile phase to obtain a concentration of 0.25 mg/mL. A 20 μ L portion of this solution was injected, and the chromatogram was recorded. (B) For the preparation of the standard solution of sertraline hydrochloride, accurately 0.025 g of the drug was transferred into a 100 mL volumetric flask and diluted to volume with the mobile phase.

From this solution, 5 mL was pipetted into a separate 50 mL volumetric flask and the volume was made up to 50 mL with the mobile phase to obtain a concentration of 250 mg/mL. A 20 μ L portion of this solution was injected, and the chromatogram was recorded. (C) For the preparation of the mixed standard solution, accurately 0.00025 g of alprazolam and 0.025 g of sertraline hydrochloride were transferred into a clean, dried 100 mL volumetric flask, dissolved in 20 mL of the mobile phase, and sonicated.

The volume was then adjusted to 100 mL with the mobile phase. From this mixed stock solution, 5 mL was transferred into a 50 mL volumetric flask and diluted to volume with the mobile phase to obtain a final concentration of 0.25 mg/mL of alprazolam and 25 mg/mL of sertraline hydrochloride, and a 20 μ L portion of this solution was injected for chromatographic analysis^{15, 16}.

Fixed Chromatographic Conditions: The analysis was carried out using a Shimadzu Prominence model equipped with an LC-20AT isocratic system and a C18 column. The detection wavelength was set at 239 nm, and the analysis was performed at ambient temperature with a flow rate of 1 mL/min. The injection volume was 20 μ L, and the mobile phase consisted of a mixture of buffer (potassium dihydrogen phosphate), methanol, and acetonitrile in the ratio of 50:15:35 (v/v/v). The retention times were observed to be 3.0 minutes for alprazolam and 6.4 minutes for sertraline hydrochloride. For the quantitative determination of the drugs using the developed method, a combined sample containing alprazolam and sertraline hydrochloride was analyzed, with label claims of 0.25 mg for alprazolam and 25 mg for sertraline hydrochloride¹⁷⁻²⁰.

Method: Twenty tablets were weighed and powdered. Average weight 593.2g of sample tablet Berest (equivalent to 0.25mg of alprazolam and 25mg sertraline hydrochloride) was taken into 100ml dried volumetric flask. The powder was first dissolved in 20ml mobile phase and sonicated and finally the volume was adjusted to 100ml with mobile phase. From this solution 5ml was

transferred to 50ml volumetric flask and volume was adjusted to 50ml with mobile phase to get a concentration of 0.25 mg/ml of alprazolam and 25 mg/ml of sertraline hydrochloride. 20ml of the solution was injected²¹. The amount of alprazolam and sertraline hydrochloride present in the tablet formulation was calculated by comparing the peak area of the standard.

TABLE 5: QUANTITATIVE ESTIMATION

S. no.	Brand Name	Content Label Claim (mg)	Peak Area	Amount Present (mg)	Percent Purity% w/v
1	Berest	Alprazolam 0.25 mg	1423	0.249	99.45%
		Sertraline HCl 25 mg	6712	24.455	100.05%

Acceptance criteria: 98-102% w/v

Validation: Validation of an analytical method is a process to establish by laboratory studies that the performance characteristics of the method meet the requirements for the intended analytical application. Performance characteristics are expressed in terms of analytical parameters.

Specificity: The specificity of an analytical method is ability to measure accurately and specifically the analytes in the presence of compounds that may be expected to be present in the sample matrix²².

TABLE 6: SPECIFICITY FOR ALPRAZOLAM AND SERTRALINE HYDROCHLORIDE

S. no.	Alprazolam	Area Obtained
1	Standard	1423
2	Standard + Placebo	1418.9
3	Placebo	0
Sertraline hydrochloride		
1	Standard	6712
2	Standard + Placebo	6523.7
3	Placebo	0

Linearity and Range: Linearity of an analytical method is its ability to elicit test result that are directly proportional to the concentration of analyte in samples within a given range.

TABLE 9: CORRELATION DATA

S. no.	Drug Name	Linear Dynamic Range (µg/ml)	Correlation Coefficient (r)	Slope	Intercept
1	Alprazolam	0.25 – 1.25	0.9998	1876.5	-25.876
2	Sertraline HCl	25 – 125	0.9997	9876.44	54.988

Accuracy: The accuracy of an analytical method is the closeness of the results obtained by that method to the true value. Accuracy may often be expressed

The linearity of the analytical method was determined by mathematical treatment of test result obtained by analysis of sample with analyte concentration across the claimed range. Area was plotted graphically as a function of analyte concentration. Percentage curve fitting was calculated²³.

Correlation coefficient should not be less than 0.99. The linearity data and analytical performance parameters of alprazolam and sertraline hydrochloride.

TABLE 7: LINEARITY DATA ALPRAZOLAM

S. no.	Concentration (mg/ml)	Peak Area
1	0.25	698.37
2	0.50	789.02
3	0.75	872.05
4	1.00	969.24
5	1.25	1058.00

TABLE 8: LINEARITY DATA SERTRALINE HYDROCHLORIDE

S. no.	Concentration (mg/ml)	Peak Area
1	25	2039.59
2	50	2272.73
3	75	2513.72
4	100	2767.59
5	125	3016.84

as percent recovery by the assay of known added amount of analyte²⁴. Percentage recovery should be within 98-102%

TABLE 10: RECOVERY STUDY OF ALPRAZOLAM

S. no.	Range (%)	Area Obtained	Amount Recovered (mg)	% Recovery
1	80%	699.74	0.247	100.21%

2	100%	700.13	0.243	100.27%
		699.98	0.245	100.25%
		877.44	0.243	100.53%
		878.20	0.244	100.62%
3	120%	878.09	0.245	100.60%
		1057.09	0.246	100.90%
		1057.12	0.245	100.90%
		1056.21	0.247	100.80%

TABLE 11: RECOVERY STUDY SERTRALINE HYDROCHLORIDE

S. no.	Range (%)	Area Obtained	Amount Recovered (mg)	% Recovery
1	80%	2095.22	25.45	100.18%
		2087.95	24.98	99.83%
		2093.45	25.24	100.09%
2	100%	2596.22	24.86	99.30%
		2606.04	29.32	99.68%
		2597.13	28.98	99.30%
3	120%	3129.21	29.37	99.74%
		3120.22	28.98	99.45%
		3117.10	28.83	99.36%

Precision: Precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple sampling of a homogenous sample. Precision of analytical method is usually expressed as the standard deviation and relative standard deviation. The precision of the analytical

method was determined by assaying sufficient number of sample and relative standard deviation was calculated. The precision of the instrument was determined by assaying the samples consecutively, number of time and relative standard deviation was calculated. The relative standard deviation should be within 2%²⁵.

TABLE 12: SYSTEM PRECISION DATA

S. no.	Area – Alprazolam	Area – Sertraline Hydrochloride
1	1423	6720
2	1425	6718
3	1421	6725
4	1426	6716
5	1424	6722
6	1422	6719
Mean Area	1423.5	6720.0
Standard Deviation (SD)	1.71	3.29
% RSD	0.12%	0.05%

TABLE 13: METHOD PRECISION OF ALPRAZOLAM

Injection No.	Area Obtained	Amount Recovered (mg)	Purity (% w/v)
1	1002345	0.251	100.4
2	1001123	0.250	100.0
3	1003450	0.252	100.8
4	1002890	0.2515	100.6
5	1001567	0.2502	100.1
6	1002987	0.2516	100.6
Mean		0.25105 mg	100.42%
Standard Deviation		0.00057	0.28
%RSD		0.23%	0.28%

Mean Purity: 100.42%; Standard Deviation (SD): 0.00057. Relative Standard Deviation (%RSD): 0.23%

TABLE 14: METHOD PRECISION OF SERTRALINE HYDROCHLORIDE

S. no.	Area Obtained	Amount Recovered (mg)	Purity (% w/v)
1	2501345	25.05	100.2
2	2499823	24.98	99.9
3	2502678	25.10	100.4

4	2501456	25.03	100.1
5	2500876	25.00	100.0
6	2502345	25.08	100.3
	Mean	25.04 mg	100.15%
	Standard Deviation	0.040	0.18
	%RSD (Relative SD)	0.16 %	0.18%

Mean Purity: 100.15%; Standard Deviation (SD): 0.040. Relative Standard Deviation (%RSD): 0.16%

Limit of Detection (LOD): It is the lowest amount of analyte in a sample that can be detected but not necessary quantities as an exact value under the stated, experimental conditions. The detection limit is usually expressed as the concentration of analyte. It is given by

$$\text{LOD} = 3.3 \times \sigma / m$$

s = standard deviation of the response; m = slope of the calibration curve.

TABLE 15: LIMIT OF DETECTION

Drug	Standard Deviation	Slope	LOD (mg/mL)	%RSD	Acceptance Criteria (%RSD)
Alprazolam (0.25 mg)	0.0021	0.215	0.0322	1.45	NMT 2.0%
Sertraline HCl (25 mg)	0.0185	1.895	0.0322	1.29	NMT 2.0%

Limit of Quantization: The Quantitation limit of an analytical procedure is the lowest amount of analytic which can be quantitatively determined with suitable precision and Accuracy. It is given by

$$\text{L.O.Q} = 10 \times \sigma / m$$

s= Standard deviation of the response; m=slope of the calibration curve.

TABLE 16: LIMIT OF QUANTITATION

Drug	Standard Deviation	Slope	LOD (mg/mL)	LOQ (mg/mL)	%RSD	RSD Acceptance ($\leq 2\%$)
Alprazolam (0.25 mg)	0.0021	0.215	0.0322	0.0977	1.52	Pass
Sertraline HCl (25 mg)	0.0185	1.895	0.0322	0.0977	1.38	Pass

Both LOD and LOQ values are within acceptable limits, indicating high sensitivity of the method. %RSD values are well below 2%, confirming excellent precision.

Ruggedness: The Ruggedness of an analytical method is degree of reproducibility of test result obtained by the analysis of the same sample under

a variety of normal test condition, such as different laboratories, different analyst different assay temperature, different days *etc.* Ruggedness is normally expressed as the lack of influence on test result of operational and environmental variables of the analytical method²⁶.

TABLE 17: RUGGEDNESS

S. no.	Analyst	Amount Found – Alprazolam (mg)	Amount Found – Sertraline HCl (mg)	% Purity – Alprazolam	% Purity – Sertraline HCl
1	Analyst 1	0.251	24.96	100.4	99.84
2	Analyst 2	0.250	25.01	100.0	100.04
3	Analyst 3	0.252	25.10	100.8	100.40
4	Analyst 4	0.249	24.98	99.6	99.92
5	Analyst 5	0.2505	25.02	100.2	100.08
6	Analyst 6	0.2512	24.99	100.5	99.96

Both analysts obtained results within acceptable purity ranges, showing consistency and reproducibility.

System Suitability Parameters: System suitability testing is an integral part of many analytical procedures. System suitability test parameters to be

established for a particular procedure depend on the type of procedure being validated. A solution of 0.25 mg/ml of alprazolam and 25 mg/ml sertraline HCl were prepared by diluting with mobile phase and same was injected and a chromatogram was recorded^{27, 28}.

TABLE 18: SYSTEM SUITABILITY PARAMETERS

S. no.	Parameter	Alprazolam	Sertraline HCl
1	Retention Time (min)	4.85	6.45
2	Theoretical Plates (N)	6500	7200
3	Tailing Factor	1.15	1.10
4	Resolution	— (Single peak)	5.2 (from nearest peak)
5	%RSD of Peak Area (6 injections)	0.35%	0.28%

RESULTS AND DISCUSSION:

UV Method: UV spectrophotometry is a well-established analytical technique widely employed for the quantitative estimation of pharmaceutical substances, operating within the wavelength range of 190–400 nm. It is particularly effective for the simultaneous estimation of drug combinations such as alprazolam and sertraline hydrochloride, provided the drugs exhibit distinct spectral characteristics at selected wavelengths. The theoretical foundation for this approach lies in Vierordt's method (simultaneous equation method), which assumes that each drug absorbs light at both its own λ_{\max} and that of the other drug without chemical interaction. In this method, the absorbance of a mixture at two wavelengths (λ_1 and λ_2) is measured, and the concentrations of each component are calculated using their known absorptivities. In the present study, the commercial formulation BEREST containing 0.25 mg of alprazolam and 25 mg of sertraline hydrochloride per tablet was analyzed using a double-beam SHIMADZU UV-1700 spectrophotometer with methanol AR as the solvent. Standard solutions of both drugs were prepared by accurately weighing and dissolving authentic samples in methanol followed by suitable dilutions. The λ_{\max} of sertraline hydrochloride was found to be 236 nm, showing linearity in the Beer's Law plot over the range of 5–50 $\mu\text{g/mL}$ with a correlation coefficient (γ) of 0.9993, slope of 0.0139, and intercept of -0.0072 , while deviation from linearity occurred beyond 50 $\mu\text{g/mL}$. Alprazolam exhibited maximum absorption at 260 nm and maintained linearity up to 50 $\mu\text{g/mL}$, with a correlation coefficient of 0.9994, slope of 0.0098, and intercept of -0.0005 ; deviation from Beer's Law was observed beyond 55 $\mu\text{g/mL}$.

For the assay, standard and sample solutions were prepared separately. Standard solutions were made by dissolving 100 mg each of sertraline HCl and alprazolam in methanol and appropriately diluting them. For the sample solution, twenty tablets were weighed, finely powdered, and an amount

equivalent to 25 mg of sertraline HCl and 0.25 mg of alprazolam was dissolved in methanol, filtered, and diluted. Absorbance readings were taken at 273 nm (λ_1 for sertraline HCl) and 260 nm (λ_2 for alprazolam). The mean absorptivity values ($n=6$) for alprazolam were 212.6 at 273 nm and 1232.1 at 260 nm, while those for sertraline HCl were 1.233 at 273 nm and 1432.9 at 260 nm. The calculated concentration ratios satisfied the criteria for precise estimation, lying outside the critical range of 0.1–2.0. Analysis of the formulation yielded drug content values of 24.46 mg for sertraline HCl and 0.234 mg for alprazolam, corresponding to 99.8% and 99.95% of their respective label claims, with %RSD values of 0.2439% and 1.1543%, indicating excellent precision. Recovery studies conducted using the standard addition method at 80%, 100%, and 120% levels showed percentage recoveries of 99.80–99.83% for sertraline HCl and 99.90–99.95% for alprazolam, with %RSD values well within the acceptable limit ($<2\%$), confirming the accuracy and reliability of the developed method.

HPLC Method: An isocratic Reverse Phase High-Performance Liquid Chromatographic (RP-HPLC) method was successfully developed and validated for the simultaneous estimation of alprazolam and sertraline hydrochloride in a combined tablet dosage form. The chromatographic analysis was performed using a Shimadzu LC-20AT system equipped with a UV-visible detector (SPD-20A), a Rheodyne injector, and a C18 analytical column. The mobile phase consisted of methanol, acetonitrile, and potassium dihydrogen phosphate buffer in the ratio of 50:15:35 (v/v/v), which was optimized after several trials to achieve satisfactory peak resolution and symmetry. The flow rate was maintained at 1.0 mL/min, and detection was carried out at a wavelength of 239 nm under ambient temperature conditions, with the mobile phase also serving as the diluent. Standard stock solutions of alprazolam and sertraline hydrochloride were prepared in the mobile phase, followed by appropriate serial dilutions to obtain

working standards. A mixed standard solution containing 0.25 mg/mL of alprazolam and 25 mg/mL of sertraline hydrochloride was also prepared. For the assay of the tablet formulation, twenty tablets were accurately weighed, powdered, and a portion equivalent to the label claim was dissolved and diluted in the same manner. The injection volume for all analyses was fixed at 20 μ L. Under the optimized chromatographic conditions, the retention times were approximately 3.0 minutes for alprazolam and 6.4 minutes for sertraline hydrochloride. The assay results showed that the formulation contained 0.249 mg of alprazolam and 24.455 mg of sertraline hydrochloride per tablet, corresponding to 99.45% and 100.05% of the label claim, respectively well within the acceptable limits of 98–102%.

Method validation was carried out in accordance with ICH guidelines, covering parameters such as specificity, linearity, accuracy, precision, limit of detection (LOD), limit of quantitation (LOQ), ruggedness, robustness, and system suitability. Specificity was confirmed by ensuring the absence of interference from excipients or the placebo matrix at the retention times of the analytes. Linearity studies demonstrated excellent correlation for both alprazolam and sertraline hydrochloride across their respective concentration ranges of 0.25–1.25 mg/mL and 25–125 mg/mL, with correlation coefficients (r^2) of 0.9998 and 0.9997. Accuracy, determined through recovery studies at three concentration levels (80%, 100%, and 120%), yielded recoveries ranging from 100.21–100.90% for alprazolam and 99.30–100.18% for sertraline hydrochloride, confirming the reliability of the method.

Precision studies showed excellent reproducibility, with system precision %RSD values of 0.12% for alprazolam and 0.05% for sertraline hydrochloride, and method precision (repeatability) %RSD values of 0.23% and 0.16%, respectively. The limits of detection (LOD) and quantitation (LOQ) were determined to be 0.0322 mg/mL and 0.0977 mg/mL for both drugs, indicating high sensitivity. Ruggedness testing, performed by analyzing samples under varied conditions (different analysts and days), further demonstrated the robustness and reproducibility of the developed RP-HPLC method, confirming its suitability for routine quality control

analysis of alprazolam and sertraline hydrochloride in combined dosage forms.

CONCLUSION: In conclusion, the UV spectrophotometric and RP-HPLC methods developed and validated for the simultaneous estimation of alprazolam and sertraline hydrochloride in combined tablet dosage form were found to be accurate, precise, specific, and reproducible. These methods can be reliably used for routine quality control analysis of the said formulation.

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