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**Der Pharmacia Sinica, 2014, 5(6):36-46**

Der Pharmacia Sinica
**ISSN: 0976-8688**
**CODEN (USA): PSHIBD**

# Method development and validation of simultaneous determination of Sertraline Hydrochlorde and Alprazolam in pharmaceutical dosage form by RP-HPLC

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**ABSTRACT**

*The present work describes a new, simple, precise, and accurate RP-HPLC method for simultaneous estimation of Sertraline Hydrochloride and Alprazolam in pharmaceutical dosage form. Chromatographic separation of the drugs was achieved on a Symmetry C18 (4.6 x 250mm, 5µm, Make: Waters) using $KH_2PO_4$ buffer: acetonitrile (adjusted to pH 3.6 with ortho phosphoric acid) in the ratio of 40:60 v/v as mobile phase. Flow rate was 1.0 mL/min and the detection wavelength was 225 nm. The two drugs were satisfactorily resolved with retention time values 2.344 min and 3.286 min for Sertraline Hydrochloride and Alprazolam, respectively. Linearity was found to be in the range of 100 -500 µg /mL for Sertraline Hydrochloride and 1 – 5 µg /mL for Alprazolam with significantly high value of correlation coefficient, ($R^2$ = 0.999 for Sertraline Hydrochloride and 0.999 for Alprazolam). The accuracy of the method was assessed by evaluation of precision (intra-day and inter-day precision % RSD was less than 2% for both Sertraline Hydrochloride and Alprazolam), accuracy (99.84 % for Sertraline Hydrochloride and 100.51 % for Alprazolam). The LOD and LOQ were found to be 0.12 µg /mL and 0.42 µg /mL for Sertraline Hydrochloride and 0.015 µg /mL and 0.05 µg /mL for Alprazolam respectively. All the parameters were found to be within limits, which indicate that the above method was accurate, precise and validated as per ICH guidelines. Hence, it was concluded that the developed method is suitable for routine analysis of Sertraline HCL and Alprazolam due to its less analysis time.*

**Key words**: Sertraline Hydrochloride, Alprazolam, Estimation, RP-HPLC, Validation.

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## INTRODUCTION

**Sertraline hydrochloride**

Sertraline hydrochloride belongs to a class of antidepressant agents known as selective serotonin-reuptake inhibitors (SSRIS), chemically known as (1s-cis)-4-(3,4-dichlorophenyl)-1,2,3,4-tetrahydro- n-methyl-1-naphthalenamine hydrochloride with molecular formula $C_{17}H_{17}N C_{L2}$• HCL, molecular weight-342, melting point 243-245 °c, boiling point-416.3 °c at 760 mmHg, density 1.37 g/cm3, and pka values 8.5 in ethanol: water, 1:1 v/v, 9.48 in water, 8.6 in methanol: water, 40:60 v/v. Sertraline Hydrochloride is white crystalline solid powder, soluble in water (3.8 mg/ml), ethanol (10 mg/ml), 0.1n hcl (0.51 mg/ml), isopropyl alcohol (4.3 mg/ml), chloroform (110 mg/ml), and dimethyl sulfoxide (147 mg/ml). Its structural formula is shown in fig.1

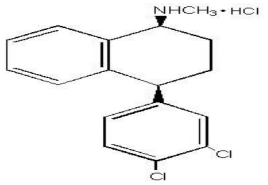


**Fig. 1: Structure of Sertraline Hydrochloride**

**Alprazolam**

Alprazolam is a drug which belongs to a group of medicines known as benzodiazepines. It works by binding to a specific site (benzodiazepines have their own receptor site) on the GABA-A receptors. Since GABA (gamma-amino butyric acid) is the brain's major inhibitory neurotransmitter. Alprazolam has a sedative effect on the nervous system. Alprazolam chemically known as 8-Chloro-1-methyl-6-phenyl-4H-s-triazolo [4,3-α] [1,4] benzodiazepine, with molecular formula $C_{17}H_{13}CLN_4$, molecular weight-308.76, melting point 228 -229.5 °c, boiling point-508.959 °C at 760 mmHg, density of 1.389 g/cm3, pka/ pkb value of 11.6. Alprazolam is a white crystalline powder and virtually insoluble in water (0.11 g/l) ; soluble in alcohol; sparingly soluble in acetone; freely soluble in chloroform; slightly soluble in ethyl acetate. Its structural formula is shown in fig.2.

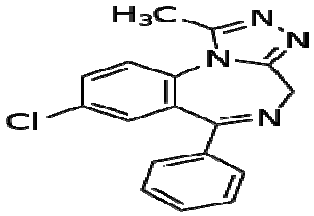


**Fig. 2: Structure of Alprazolam**

Some literatures revealed the high performance liquid chromatography (HPLC) method for Sertraline Hydrochloride and Alprazolam [5-9]. However, these methods are time consuming, so it is necessary to develop a cost-effective and less time consuming method for the estimation of Sertraline Hydrochloride and Alprazolam in API as well as pharmaceutical formulation. In the present study the authors report a rapid, sensitive, accurate and precise RP-HPLC method for the estimation of Sertraline Hydrochloride and Alprazolam in bulk drug and tablet dosage forms.

## MATERIALS AND METHODS

**Instrument**

Chromatographic separation was performed on HPLC WATERS manufacturer, model 2695,empower -2 software, equipped with auto sampler and PAD 996 detector. UV/Vis spectrophotometer with Uv detector, LABINDIA manufacturer, UV 3000+ model, UV win software. Separation of the drugs was achieved on a Symmetry C18 (4.6 x 250mm, 5µm, Make: Waters).

**Reagents and chemicals**

Analytical pure drugs of Sertraline hydrochloride and Alprazolam were obtained from sura labs, Hyderabad, india. The combined tablet formulation ALPRAX FORTE, Manufacturer by TORRENT Pharmaceuticals Ltd With a labeled claim of Sertraline hydrochloride 50 mg and 0.5 mg Alprazolam respectively, were obtained from local drug store. Chemicals were procured from different brands like Acetonitrile HPLC grade from MOLYCHEM, potassium dihydrogen phosphate from FINER Chemicals ltd ,HLPC grade methanol from LICHOROSOLV ( MERK) ,and ortho phosphoric acid from MERCK.

**Method Development**

**Selection of wavelength: ($\lambda_{max}$)**

Initially method development work was started by taking UV-visible spectra from 400-200 nm of Sertraline HCL and Alprazolam, standard solution. By observing the spectra of standard solutions λmax 225 nm was selected as

common wavelength for simultaneous estimation of both the drugs as these are eluting in the same mobile phase at maximum absorbance. UV spectrum of Sertraline HCL and Alprazolam is shown in fig.3.

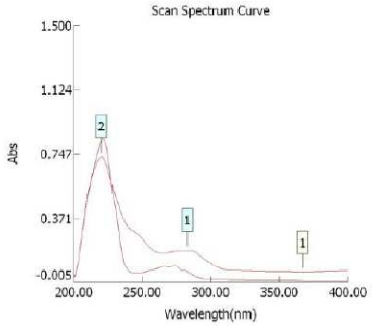


**Fig. 3: UV spectrum of Sertraline HCL and Alprazolam**

**Chromatographic conditions**

To optimize the chromatographic conditions, the effect of chromatographic variables such as mobile phase, pH, flow rate and solvent ratio were studied. Various solvent systems were tried for the development of a suitable HPLC method for determination of Sertraline Hydrochloride and Alprazolam in bulk drug and tablet dosage form.

**Mobile phase**

$KH_2PO_4$ buffer ( adjusted to pH 3.6 with ortho phosphoric acid ) and ACN in the ratio of 40 % : 60 % v/v was used for separation of these drugs after filtering through 0.45 µ membrane filter and sonicating each solvent for 10 min. The condition that gave best resolution and symmetry was selected. HPLC conditions are given in Table-1

**Table-1 HPLC conditions**

| PARAMETERS | CONDITIONS |
|---|---|
| Column (Stationary Phase) | Symmetry C18 (4.6 x 250mm, 5µm, Make: Waters) or equivalent |
| Mobile Phase | $KH_2PO_4$ buffer (pH 3.6) : ACN ( 40 % : 60 %) |
| Flow rate | 1.0 (ml/min) |
| Run time | 5 (min) |
| Column temperature | Ambient |
| Volume of injection loop | 20 20 (µl) |
| Detection wavelength | 225 nm |
| Buffer | 6.8 grams of potassium dihydrogen ortho phosphate in 1000ml HPLC water pH adjusted to 3.6 with orthophosporic acid. |

**Preparation of Phosphate buffer and mobile phase:**

**Preparation of Phosphate buffer:**

Accurately weighed 6.8 grams of $KH_2PO_4$ was taken in a 1000ml volumetric flask, dissolved and diluted to 1000ml with HPLC water and the volume was adjusted to pH 3.6 with Orthophosphoric acid.

**Preparation of mobile phase:**

Accurately measured 400 ml (40%) of above buffer and 600 ml of ACN HPLC (60%) were mixed and degassed in an ultrasonic water bath for 5 minutes and then filtered through 0.45 µ filter under vacuum filtration.

**Diluent Preparation:**

The Mobile phase was used as the diluent.

**Preparation of the Sertraline HCL & Alprazolam standard & sample solution:**

**Standard Solution Preparation:**

Accurately weigh and transfer 10 mg of Sertraline Hydrochloride and Alprazolam 10mg of working standard into a 10mL& 100ml clean dry volumetric flask add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)

Further pipette 3ml& 0.3ml of the above stock solutions into a 10ml volumetric flask and dilute up to the mark with diluent.

**Sample Solution Preparation:**
Accurately weigh 10 tablets crush in mortar and pestle and transfer equivalent to 10 mg of Sertraline hydrochloride and Alprazolam(marketed formulation) sample into a 10mL clean dry volumetric flask add about 7mL of diluent and sonicate to dissolve it completely and make volume up to the mark with the same solvent.

(Stock solution)
Further pipette 3 ml of Sertraline and Alprazolam of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent.

**Assay:**
Inject 20 µl of the standard and sample solution into the chromatographic system and measure the areas for the Sertraline Hydrochloride and Alprazolam and calculate the % assay by using the formula. The standard and sample chromatograms were shown in fig. 4.

**Formula**

$$\text{Assay \%} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{\text{Avg. wt}}{\text{Label claim}} \times 100$$

Where:
AT = average area counts of sample preparation.
AS = average area counts of standard preparation.
WS = Weight of working standard taken in mg.
P = Percentage purity of working standard
LC = Label Claim of drug mg/ml.

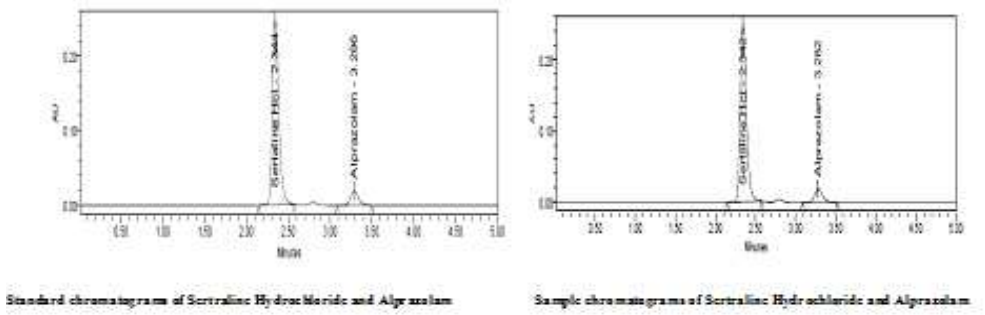


**Fig. 4: Standard and sample chromatograms of Sertraline Hydrochloride and Alprazolam**

**System Suitability:**
System suitability was daily performed during entire validation of this method. The results of system suitability were presented in Table 2.

**Table 2: System Suitability Parameters**

| S.No | Name | Retention time(min) | Area (µV) | Height (µV) | USP tailing | USP plate count | USP resolution |
|---|---|---|---|---|---|---|---|
| 1 | Sertraline HCL | 2.344 | 1311967.4 | 247438.6 | 1.3 | 4668.7 | 6.0 |
| 2 | Alprazolam | 3.286 | 124581.3 | 19195.3 | 1.3 | 6090.3 | |

**Method Validation**

**Accuracy:**

The accuracy of an analytical method expresses the closeness of agreement between the value, which is accepted reference value, and the value found. Accuracy studies were done by the standard addition method. Accuracy is expressed as % recovery of the standard spiked to previously analyzed test sample of tablet. The active ingredients were spiked in previously analyzed tablet powder sample at different concentration levels viz. 50%, 100%, and 150% each of the labeled claim and injected in developed chromatographic conditions in triplicate. The percentage recoveries were then calculated. The recovery data for accuracy studies were shown in Table 3. The accuracy chromatograms were shown in fig 5, 6, and 7.

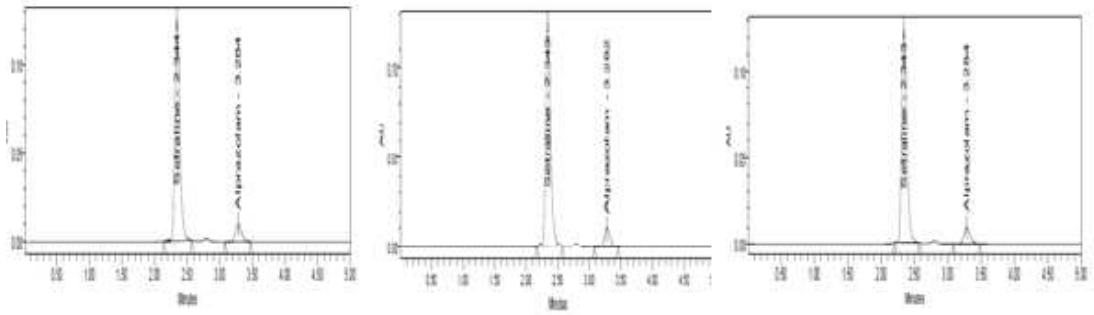

**Fig. 5: Accuracy chromatograms for 50% solution**

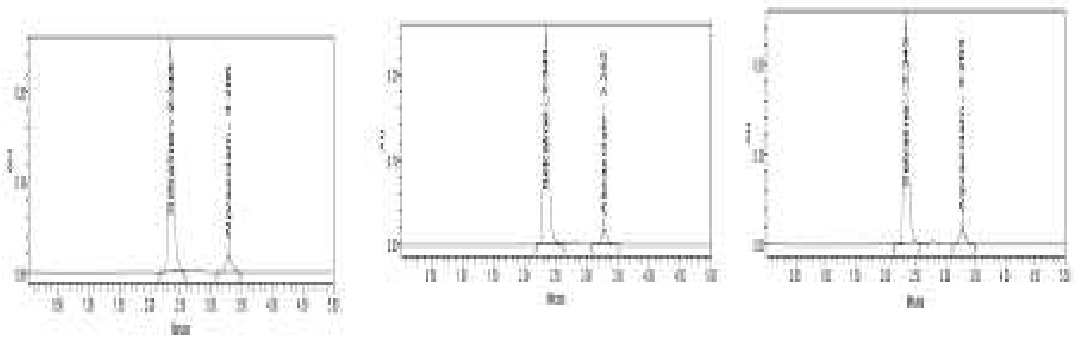

**Fig. 6: Accuracy chromatograms for 100% solution**

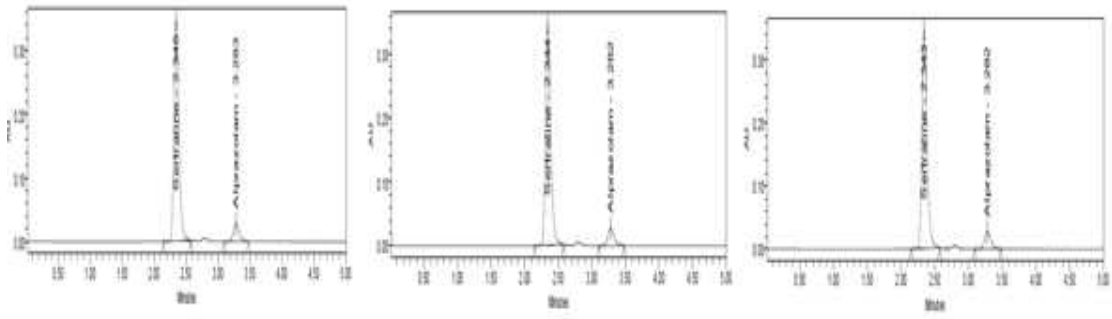

**Fig. 7: Accuracy chromatograms for 150% solution**

**Table 3: Accuracy (recovery) results of Sertraline HCL and Alprazolam**

| Drug | Accuracy level | Area | Amount Added (mg) | Amount Found (mg) | % Recovery | Mean Recovery |
|---|---|---|---|---|---|---|
| **Sertraline HCL** | 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% | |
| **Alprazolam** | 50% | 65800 | 5.3 | 5.34 | 100.8% | 10051 |
| | 100% | 124353 | 10 | 10.10 | 100.01% | |
| | 150% | 177940 | 14.2 | 14.45 | 99.68% | |

**Precision:**

The precision of an analytical method is a measure of the random error and is defined as the agreement between replicate measurements of the same sample. It is expressed as the percentage coefficient of variation (%CV) or relative standard deviation (RSD) of the replicate measurements. The standard solution was injected for five times and the area was measured for all five injections in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits. Results were reported in Table 4. Chromatograms were reported in fig 8.

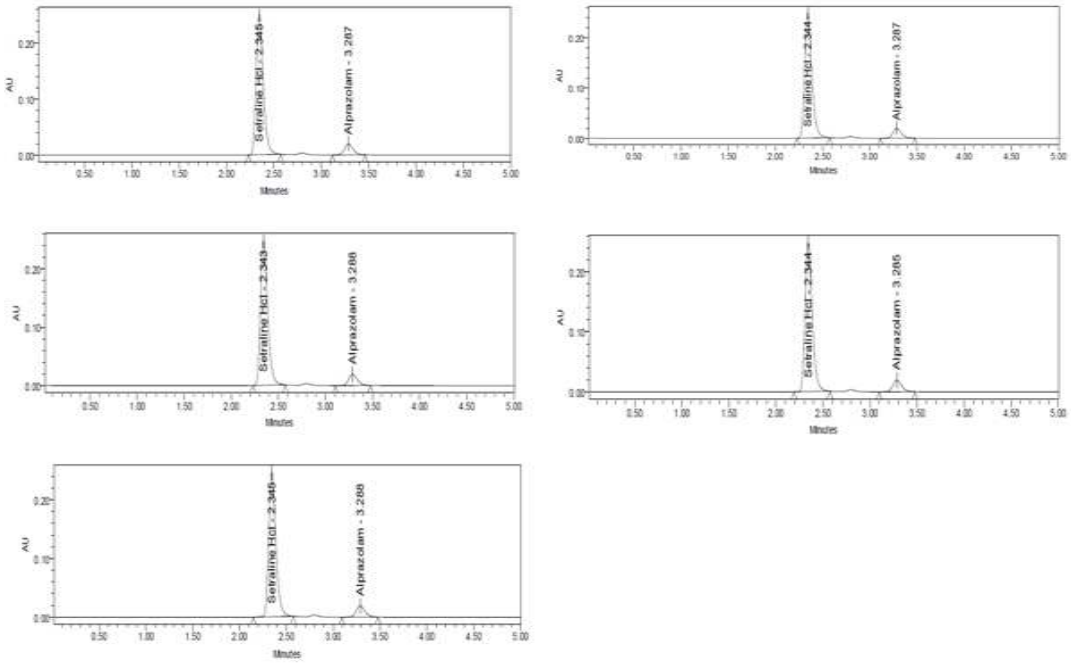


**Fig. 8: Precision chromatograms**

**Table 4: Precision results of Sertraline HCL and Alprazolam**

| Injection | Sertraline HCL | Alprazolam |
|---|---|---|
| | Area | Area |
| Injection-1 | 1302729 | 123149 |
| Injection-2 | 1302947 | 123766 |
| Injection-3 | 1303236 | 124271 |
| Injection-4 | 1303977 | 124691 |
| Injection-5 | 1309759 | 124956 |
| Average | 1304529.8 | 124162.7 |
| Standard Deviation | 2961.1 | 725.6 |
| %RSD | 0.2 | 0.6 |

**Intermediate Precision/Ruggedness:**

To evaluate the intermediate precision (also known as Ruggedness) of the method, Precision was performed on different day by using different make column of same dimensions. Results were reported in Table 5. Chromatograms were reported in fig 9.

**Table 5: Intermediate precision results of Sertraline HCL and Alprazolam**

| Injection | Sertraline HCL | Alprazolam |
|---|---|---|
| | Area | Area |
| Injection-1 | 1300148 | 122487 |
| Injection-2 | 1304520 | 122626 |
| Injection-3 | 1305937 | 122632 |
| Injection-4 | 1306476 | 122702 |
| Injection-5 | 130871 | 122962 |
| Average | 1305070.2 | 122681.8 |
| Standard Deviation | 3061.8 | 174.8 |
| %RSD | 0.2 | 0.1 |

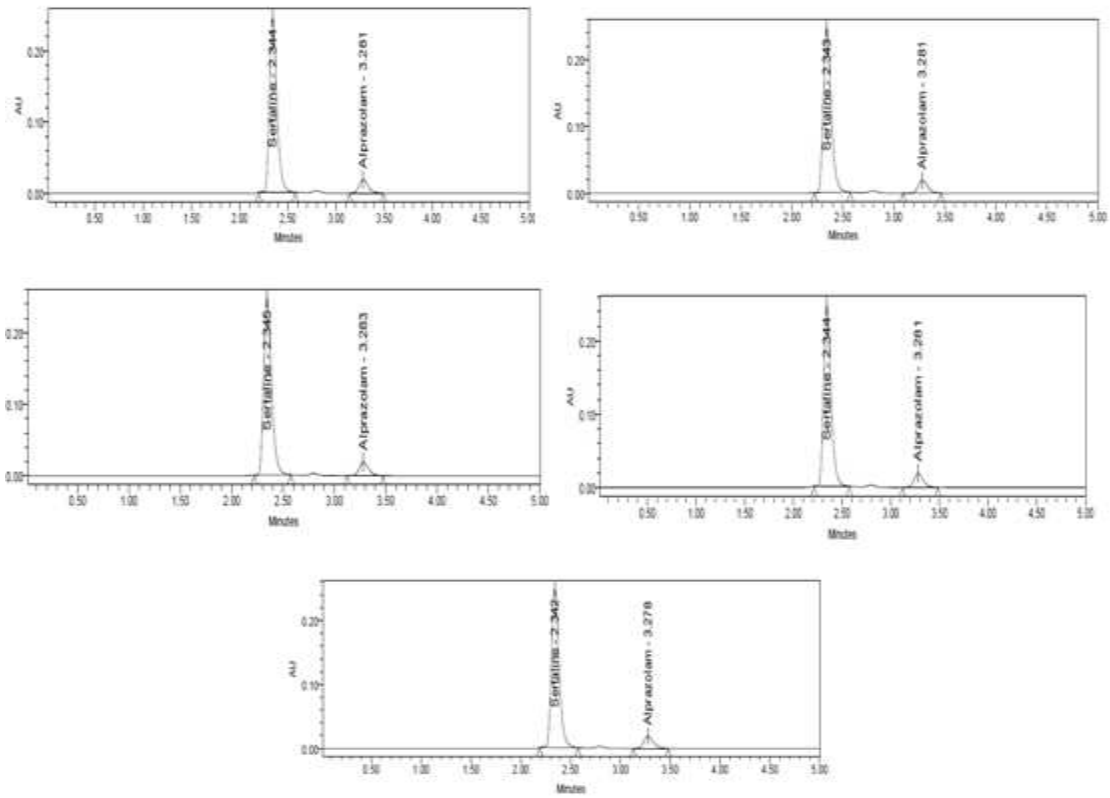


**Fig. 9: Intermediate Precision**

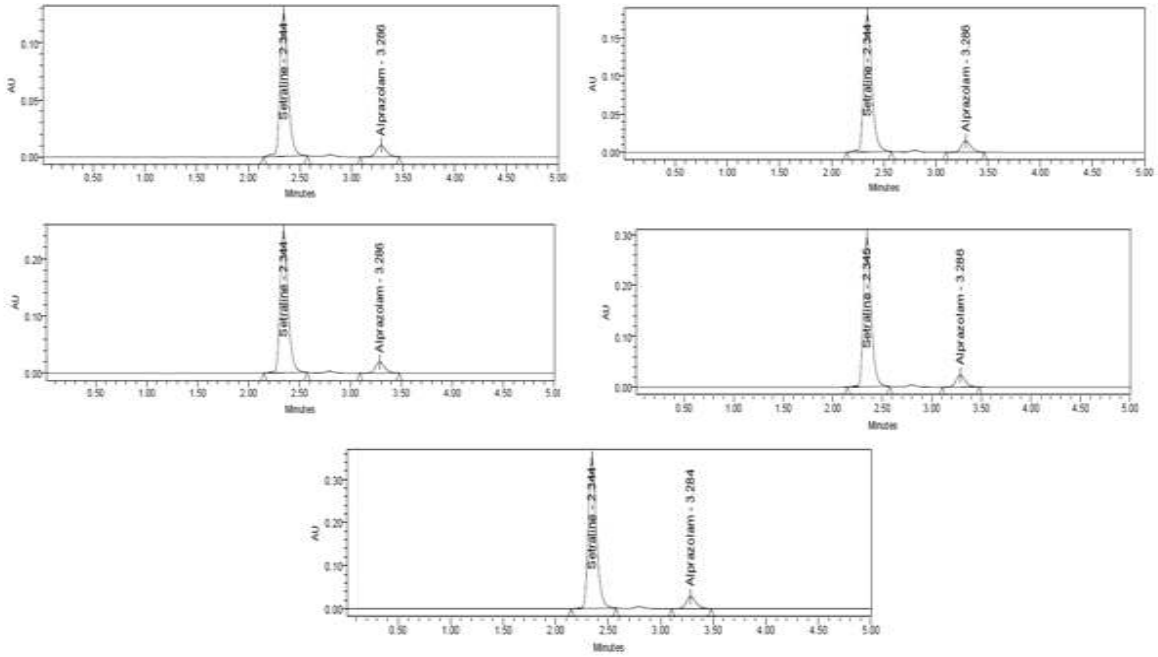


**Fig 10 Linearity chromatograms of Sertraline Hydrochloride and Alprazolam**

**Linearity:**

Aliquots of standard Sertraline hydrochloride and Alprazolam stock solution were taken in different 10 ml volumetric flasks and diluted up to the mark with the mobile phase such that the final concentrations of Sertraline

Hydrochloride and Alprazolam were in the range of 100-500 µg/ml. Each of these drug solutions (20 µL) was injected into the column, and the peak areas and retention times were recorded. Evaluation was performed with PDA detector at 225 nm and a Calibration graph was obtained by plotting peak area versus concentration of Sertraline Hydrochloride and Alprazolam (Fig 11 and 12). The linearity Chromatograms were presented in fig 9. Results were reported in table 6.

**Table 6: Linearity results of Sertraline HCL and Alprazolam**

| S. No | Linearity Level | Sertraline HCL | | Alprazolam | |
|---|---|---|---|---|---|
| | | Concentration | Area | Concentration | Area |
| 1 | I | 100ppm | 668934 | 1ppm | 66510 |
| 2 | II | 200ppm | 956781 | 2ppm | 94701 |
| 3 | III | 300ppm | 1313873 | 3ppm | 124802 |
| 4 | IV | 400ppm | 1563458 | 4ppm | 152731 |
| 5 | V | 500ppm | 1867084 | 5ppm | 179732 |
| | | Correlation Coefficient | 0.999 | Correlation Coefficient | 0.999 |

**Calibration curve**

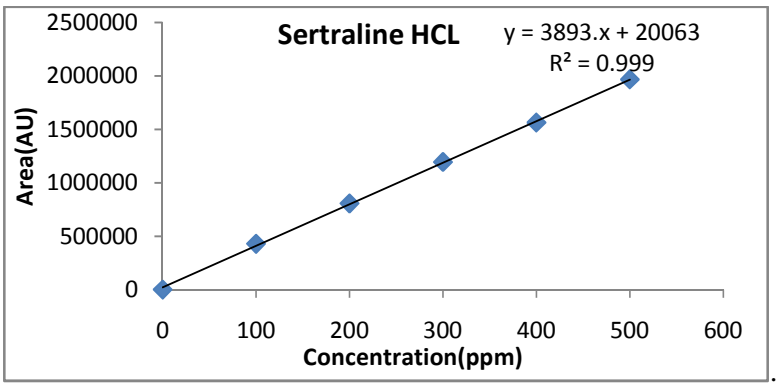


**Fig. 11: Calibration Curve for Sertraline HCl**

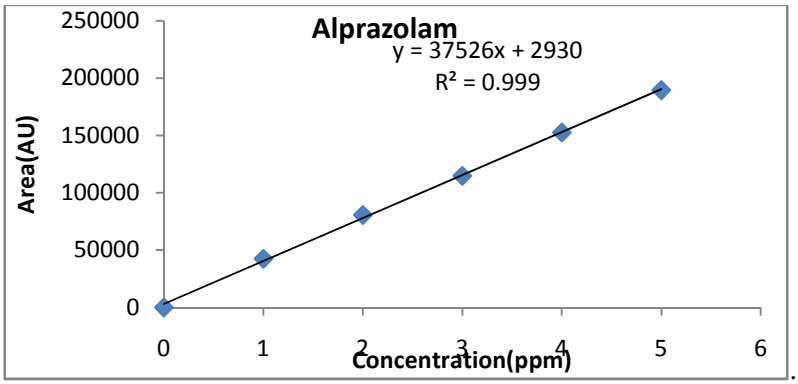


**Fig. 12: Calibration Curve for Alprazolam**

**Limit of Detection [LOD] and Limit of Quantification [LOQ]:**

The LOD and LOQ is the smallest concentration that can be detected but not necessarily quantified as an exact value. Results were reported in Table 7. Chromatograms were reported in fig 13, 14.

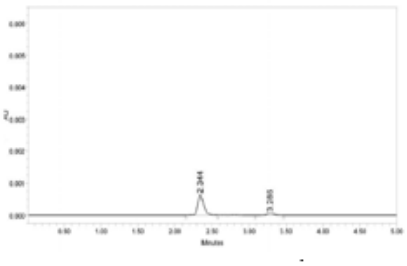

**Fig. 13: LOD Chromatogram**

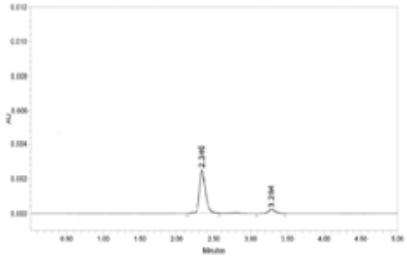

**Fig. 14: LOQ Chromatogram**

**Table 7: Results of LOD and LOQ**

| Drug name | Baseline noise(µV) | Signal obtained (µV) | S/N ratio |
|---|---|---|---|
| Sertraline HCL | 52 | 152 | 2.9 |
| | 52 | 522 | 10.03 |
| Alprazolam | 52 | 524 | 10.1 |
| | 52 | 156 | 3 |

**Robustness:**

Robustness of the method was determined by small deliberate changes in flow rate, mobile phase ratio and column oven temperature. The content of the drug was not adversely affected by these changes as evident from the low value of relative standard deviation indicating that the method was robust. The results of robustness were presented in table 8. The chromatograms for flow rate variation and mobile phase variation were shown in fig 15 and 16 respectively.

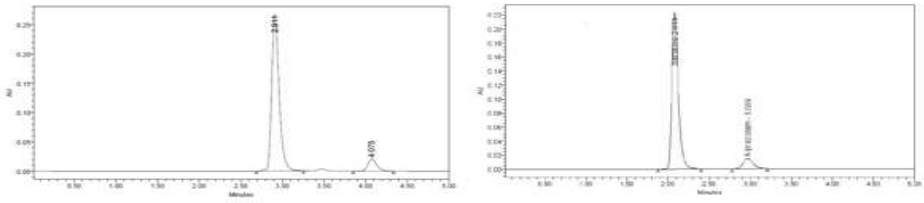

**Fig. 15: Flow rate variation chromatograms of Sertraline Hydrochloride and Alprazolam**

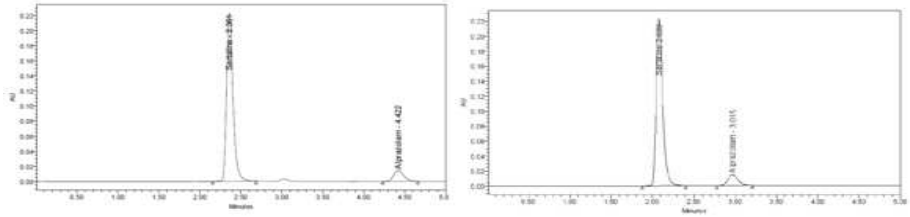

**Fig. 16: Mobile phase variation chromatograms of Sertraline Hydrochloride and Alprazolam**

**Table 8: Mobile phase and flow rate variation chromatograms of Sertraline Hydrochloride and Alprazolam**

| Drug | Flow Rate (ml/min) | System Suitability Results | | Change in Organic Composition in the Mobile Phase | System Suitability Results | |
|---|---|---|---|---|---|---|
| | | USP Plate Count | USP Plate Count | | USP Plate Count | USP Tailing |
| Sertraline HCL | 0.8 | 5339.9 | 1.4 | 10% less | 4508.4 | 1.3 |
| | 10 | 4673.4 | 1.3 | *Actual | 4673.4 | 1.4 |
| | 1.2 | 5216.0 | 1.4 | 10% more | 4318.1 | 1.3 |
| Alprazolam | 0.8 | 7063.3 | 1.3 | 10% less | 6387.7 | 1.2 |
| | 1.0 | 6090.3 | 1.2 | *Actual | 6090.3 | 1.2 |
| | 1.2 | 6998.0 | 1.3 | 10% more | 6232.5 | 1.2 |

## RESULTS AND DISCUSSION

To optimize the mobile phase, various proportions of buffers with acetonitrile were tested. Mobile phase composition was changed and the method development was started by symmetry C18 (4.6 x 250mm, 5µm, Make: Waters) or equivalent column and with a flow rate of 1.0 ml/min, and detection wavelength of 225 nm. Injection volume was 20 µL, and run time was for 5 min. The mobile phase consists of $KH_2PO_4$ buffer (pH 3.6): ACN (40 %: 60 %). The retention time of Sertraline Hydrochloride and Alprazolam was found to be 2.344 minutes and 3.286. The assay result of Sertraline Hydrochloride and Alprazolam was found to be 99.95 % and 100.24 %. Linearity was observed over the concentration range of 100 - 500 µg/ml for Sertraline hydrochloride and 1 – 5 µg /mL for Alprazolam with correlation coefficient ($R^2$=0.999). The numbers of theoretical plates obtained for Sertraline Hydrochloride and Alprazolam were 4668.7 and 6090.3 respectively which indicates the efficiency of the column. The LOD and LOQ were found to be 0.12 µg /mL and 0.42 µg /mL for Sertraline hydrochloride and 0.015 µg /mL and 0.05 µg /mL for alprazolam respectively which indicates the sensitivity of the method. The high percentage recovery indicates that the proposed method is highly accurate. Summary for RP-HPLC was shown in table No-9.

**Table No 9: Summary for RP-HPLC Method**

| S. No | Parameters | Acceptance Criteria | Results Obtained | |
|---|---|---|---|---|
| | | | Sertraline HCL | Alprazolam |
| 1 | System suitability (%RSD of tailing factor) | Theoretical Plates-NLT2000 | 4668.7 | 6090.3 |
| | | Tailing factor-NMT 2 | 1.3 | 1.3 |
| 2 | Retention time | | 2.344 | 3.286 |
| 3 | Precision | RSD NMT 2.0% | 0.2 | 0.6 |
| 4 | B).Intermediate Precision | RSD NMT 2.0% | 0.2 | 0.1 |
| 5 | Linearity | Correlation coefficient NLT 0.999 | 0.999 | 0.999 |
| 6 | Accuracy | %Recovery range 98-102 % | 99.84 % | 100.51% |
| 7 | LOD | S:N Ratio should be more than 3:1 | 2.9 | 3 |
| 8 | LOQ | S:N ratio should be more than 10:1 | 10.3 | 10.1 |

## CONCLUSION

A simple and rapid RP-HPLC method was developed for simultaneous estimation of Sertraline Hydrochloride and Alprazolam in API and pharmaceutical dosage forms.

Method was developed on Symmetry C18 (4.6 x 250mm, 5µm, Make: Waters) or equivalent column. The mobile phase was phosphate buffer (pH 3.6): acetonitrile 40:60 % ratio with a flow rate of 1.0 ml/min. The chromatograms were recorded at 225 nm wave length. The retention time of Sertraline Hydrochloride and Alprazolam was found to be 2.344 minutes and 3.286.

The developed method was validated in terms of accuracy, precision, linearity and robustness and results were validated according to ICH guidelines.

Therefore it was concluded that the proposed HPLC method was found to be simple, specific, precise, accurate, rapid and economical and can be used for the estimation of Sertraline Hydrochloride and Alprazolam in API as well as in pharmaceutical dosage forms.

**Acknowledgements**
The authors greatly acknowledge sura labs limited for providing the reference sample and sura labs, Dilsukhnagar, Hyderabad for providing facilities to carry out this work.

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