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# DEVELOPMENT AND VALIDATION OF RP-HPLC METHOD FOR THE SIMULTANEOUS ESTIMATION OF DOXOFYLINE AND SERTRALINE IN PURE BULK AND PHARMACEUTICAL DOSAGE FORMS

K. Vikram*, R.V. Valli Kumari

Department of Pharmaceutical Analysis and Quality Assurance, Malla Reddy Institute of Pharmaceutical Sciences, Hyderabad, India

***Corresponding author e-mail:** katakamvikrampharma@gmail.com

**ABSTRACT**

A simple, Accurate, precise method was developed for the simultaneous estimation of the Doxofylline and Sertaline in Tablet dosage form. Retention time of Doxofylline and Sertaline were found to be 2.3min and 3.7min. %RSD of the Doxofylline and Sertaline were and found to be 0.98 and 1.14 respectively. %assay r was Obtained as 99.71% and 99.53% for Doxofylline and Sertaline respectively. LOD, LOQ values are obtained from regression equations of Doxofylline and Sertaline were 0.09ppm, 0.26ppm and 0.44ppm, 1.34ppm respectively. Regression equation of Doxofylline is of Sertaline $y = 14573x + 385.4$ And $y = 33848x + 4535$. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

**Keywords:** Doxofylline, Sertaline, RP-HPLC, Estimation, Tablets

## INTRODUCTION

Sertraline is an antidepressant in a group of drugs called selective serotonin reuptake inhibitors (SSRIs). Sertraline affects chemicals in the brain that may become unbalanced and cause depression, panic, anxiety, or obsessive-compulsive symptoms. Sertraline is used to treat depression, obsessive-compulsive disorder, panic disorder, anxiety disorders, post-traumatic stress disorder, and premenstrual dysphoric disorder. Doxofylline is a new generation long acting oral methyxanthine derivative. Its mainly used for maintenance therapy in patients suffering with Asthma and Chronic Obstructive Pulmonary Disease. Doxofylline is a theophylline derivative. Similarly, its mechanism of action is related to the inhibition of phosphodiesterase activities, resulting in bronchodilating effects.

Literature survey reveals that good analytical methods are not available for the drugs like Doxofylline and Sertraline and very few simultaneous estimation methods for these two drugs individually so many methods like UV-Visible, HPLC, HPTLC, Volta metric methods reported for the estimation sertraline and Doxofyline pharmaceutical dosage form.

Present work is aimed at to develop a new, simple, fast, rapid, accurate, efficient and reproducible RP-HPLC method for the analysis of sertraline and doxofyline and developed method will be validated for parameters like accuracy, linearity, precision, specificity, robustness, and system suitability according to ICH guidelines.

## MATERIALS AND METHODS:

**Instrumentation:** Chromatographic separation was performed on WATERS HPLC 2965 SYSTEM with Auto Injector and PDA Detector. Equipped with quaternary pump, Software used is Empower 2 solution software was employed for data collecting and processing. Chromatographic separation was achieved on BDS C-18 (150mm × 4.6 mm, i.d., 5µ.) column column. Over laid spectrum was recorded by using UV-3000$^+$ LABINDIA double beam UV-

Visible spectrophotometer (model no.UV-2371) with 1cm matched quartz cells. Weighing was done on Shimadzu electronic balance (AY-120). Global digital pH meter was used to adjust pH of the mobile phase. The mobile phase was degassed and sonicated by using PCI Mumbai 3.5 liter capacity Sonicator.

**Method development and optimization of chromatographic parameters:** The method is developed mainly based on $pk_a$ concept of drug and also different mobile phase compositions, flow rate, $\lambda_{max}$, different columns and column temperatures.

**Preparation of Buffer Solution: (0.01N $KH_2Po_4$)**
Accurately weighed 1.42gm of Potassium di hydrogen Ortho phosphate in a 1000ml of Volumetric flask add about 1000ml of milli-Q water and pH of this solution was adjusted to 3.0 with ortho phosphoric acid. The solution was mixed well and then filtered through 0.45µ filter paper.

**Preparation of Mobile phase:** Mobile phase was prepared by mixing pH 3.0 buffer solution and acetonitrile in the ratio 60:40 v/v. prior to use the mobile phase was filtered through 0.45µ membrane filter after sonication for 8 mins.

**Diluent: (methanol)** sample firstly dissolved in methanol and made up with buffer

**Preparation of Standard Stock Solution of Doxofyline:** Accurately weighed 64 mg of doxofyline standard drug was transferred to 100 ml of volumetric flask. Then it was dissolved by adding a little amount of diluent. Mix it well, 20 min for sonicate the solution and volume was made up to 100 ml. This is 1000µg/ml. From this solution 10 ml was transferred to another 100 ml volumetric flask and volume was made up to 100 ml with diluent(100µg/ml).

**Preparation of Standard Stock Solution of Sertraline:** Accurately weighed 5 mg of sertraline standard drug was transferred to 25 ml of volumetric flask. Then it was dissolved by adding a little amount of diluent. Mix it well, sonicate the solution and volume was made up to 25 ml. This is 1000µg/ml. From this solution 10 ml was transferred to another 100 ml volumetric flask and volume was made up to 100 ml with diluent(100µg/ml).

**Preparation of Working Mixed Standard Solution:** Accurately Weighed and transferred 64mg of Doxofillyne and 5mg of Sertraline working Standards into 10ml and 25ml clean dry volumetric flasks, add 3/4$^{th}$ volume of diluent, sonicated for 5 minute and make up to the final volume with diluents. 1ml from the above two stock solutions was taken into a 10ml volumetric flask and made up to 10ml.

**Preparation of Sample Solution:** 5 tablets were weighed and calculate the average weight of each tablet then the weight equivalent to 5 tablets was transferred into a 100mL volumetric flask, 20mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered.

**System Suitability Studies:** The system suitability studies were done for parameters like theoretical plates, tailing factor, retention time, resolution. 10 µl of mixed drug solution containing 100µg/ml of sertraline and 100µg/ml of doxofyline was injected (n=6) in to the optimized HPLC system and the results obtained are given in the table no.1.

**Linearity studies:** Preparation of Working Standard Solution: A mixture of solutions is prepared using the above stock solutions and linearity was observed by injecting these prepared solutions. The results were obtained and a graph is plotted between peak area v/s concentration taking peak area on y-axis and concentration on x-axis.
Six Linear concentrations of Doxofylline (40-240ppm) and Sertaline (5-30ppm) are prepared and injected. Regression equation of the Doxofylline and Sertaline are found to be, y = 14573x + 385.4 y = 33848x + 4535. And regression co-efficient was 0.999.

**Precision studies:** For precision same concentration solution i.e, 400 mg of Doxofyline and 50 mg of Sertraline solution was injected 6 times and observed for any peculiar change in the areas and % RSD was calculated for each drug.
Precision is done by injecting six times the same sample solution of drug and checked for the change in the area and %RSD was calculated for each drug and the results are tabulated below individually for each drug
**Intraday precision (Repeatability):** Intraday Precision was performed and % RSD for Doxofylline and Sertaline were found to be 0.98% and 1.14% respectively.
**Inter day precision:** Inter day precision was performed with 24 hrs time lag and the %RSD Obtained for Doxofylline and Sertaline were 0.43% and 0.37%.

**Accuracy:** For Accuracy the standard drug is spiked and 50%, 100%, 150% solutions were prepared and

injected and averages of 3 readings are taken and recovery study is done.

**Specificity:** Specificity is the ability to assess unequivocally the analyte in the presence of components which may be expected to be present. Typically these might include impurities, degradants, matrix, etc.
The chromatogram was taken by injecting appropriate diluted standard solution, sample solution and also blank and it was found that there is no interference with the analyte peak.

**Limit of detection:** Limit of detection is the lowest concentration of the analyte that can be detected by injecting decreasing amount, not necessarily quantity by the method, under the stated experimental conditions. The limit of detection was calculated using the formula:

$$LOD = \frac{3.3 * S.D}{Slope}$$

The slope and standard deviation (SD) values were calculated using the linearity graph. The LOD values of Doxofylline and Sertraline were found to be 0.09 µg/ml and 0.44 µg/ml respectively.

**Limit of quantification:** Limit of quantitation is the lowest concentration of the analyte in a sample that can be estimated quantitatively by injecting decreasing amount of drug with acceptable precision and accuracy under the stated experimental conditions of the method. The limit of quantitation was calculated using the formula:

$$LOQ = \frac{10 * S.D}{Slope}$$

The slope and standard deviation (SD) values were calculated using the linearity graph
LOQ values of Doxofylline and Sertaline were found to be 0.26 µg/ml and 1.34 µg/ml respectively.

**Robustness studies:** Robustness is generally done by deliberately changing the parameters like flow rate and column temperature in the optimised conditions. The results obtained are presented in the table no 6.

**Assay procedure:** 10µL of Standard solution and sample solution were injected automatically in to the chromatographic system and the peak areas responses for the analyte peaks were measured. The % content of was calculated. Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 99.71% and 99.53% for Doxofylline and Sertaline respectively.

## RESULTS AND DISCUSSION

To develop a new RP-HPLC method, several mobile phase compositions were tried. A satisfactory separation with good peak symmetry was obtained with C-18 (150mm X 4.6mm, i.d., 5µm,) column using mobile phase containing $KH_2PO_4$ Buffer ($P^H$=3): ACN: (60:40) (v/v) at a flow rate of 1 ml/min. Quantification was achieved at UV detection at 273 nm based on peak area. The retention time for Doxofyline and Sertraline were found to be 5.33 min and 9.32 min respectively. The optimized method was validated as per ICH guidelines.
System suitability parameters like retention time, resolution, tailing and plate count were shown uniformity and %RSD was less than 2 and the results are given in table 5.9 and from the obtained results we can say that the system is suitable for analysis.
A linearity range of Doxofylline (40-240ppm) and Sertaline (5-30ppm) with correlation coefficient 0.999 was observed for both the drugs. In linearity plot the graph with six different concentrations versus areas to construct the linear regression equation and to calculate the value of correlation co-efficient. Linear correlation of Doxofylline and Sertaline are found to be, y = 14573x + 385.4 y = 33848x + 4535. And regression co-efficient was 0.999. And calibration curve was shown in Fig.2 and 3.
The precision of the proposed method was carried in terms of the repeatability and the %RSD values were found to be Doxofylline and Sertaline were 0.43% and 0.37%. Which reveal that the proposed method is precise Precision studies were tabulated in table.no.3 and 4.
The study of robustness in the present method shows no significant changes either in the peak area or Rt. Rubustness data is tabulated in table no.6.
The method accuracy was evaluated by recovery studies. The percentage recovery of Doxofyline and sertraline was found to be 100.42% and 100.17 for 50% level; 100.59% and 99.41% for 100% level; 99.95% and 100.54% for 150% level and results was shown in table.no.5.
Method specificity was concluded and those figures are Doxofyline and Sertraline standard chromatogram and other one is formulation. There is no placebo and excipients peaks interference with standard and analytic peak so it proves method is selective

## CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Doxofylline

and Sertaline in Tablet dosage form. Retention time of Doxofylline and Sertaline were found to be 2.317min and 3.688min. %RSD of the Doxofylline and Sertaline were and found to be 0.98 and 1.14 respectively. %assay r was Obtained as 99.71% and 99.53% for Doxofylline and Sertaline respectively. LOD, LOQ values are obtained from regression equations of Doxofylline and Sertaline were 0.09ppm, 0.26ppm and 0.44ppm, 1.34ppm respectively. Regression equation of Doxofylline is of Sertaline y = 14573x + 385.4 And y = 33848x + 4535. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

**Table.No.1:** System suitability studies of Doxofylline and Sertaline

| S.No | Parameter | Drug | Observed Value | Acceptance Criteria |
|---|---|---|---|---|
| 1. | Theoretical plates* | Sertraline | 4356 | NLT 2000 |
| | | Doxofyline | 3151 | |
| 2. | Tailing factor* | Sertraline | 1.09 | NMT 2 |
| | | Doxofyline | 1.26 | |
| 3. | Retention time (min)* | Sertraline | 2.346 | NLT 2 |
| | | Doxofyline | 4.714 | |
| 4 | %RSD* | Sertraline | 0.97 | NMT 2 |
| | | Doxofyline | 1.14 | |
| 5. | Resolution* | ------- | 3.20 | NLT 2 |

**Table: 2. Calibration data of Doxofylline and Sertaline method.**

| S.no | Linearity Level | Concentration Doxofylline(µg/ml) (µg/ml) | Area Response | Concentration Sertaline (µg/ml) | Area Response |
|---|---|---|---|---|---|
| 1 | I | 0 | 0 | 0 | 0 |
| 2 | II | 40 | 559927 | 5 | 177103 |
| 3 | III | 80 | 1196886 | 10 | 348146 |
| 4 | IV | 120 | 1765997 | 15 | 514507 |
| 5 | V | 160 | 2314611 | 20 | 681872 |
| 6 | VI | 200 | 2896222 | 25 | 833238 |
| 7 | VII | 240 | 3510567 | 30 | 1030897 |
| | Correlation coefficient | | 0.9997 | | 0.9994 |

**Table: 3. Repeatability results for Doxofylline and Sertaline.**

| Sr. No. | Doxofylline | Sertaline |
|---|---|---|
| 1 | 2144032 | 659367 |
| 2 | 2189195 | 663635 |
| 3 | 2161691 | 659826 |
| 4 | 2186600 | 677891 |
| 5 | 2173179 | 660594 |
| 6 | 2138796 | 671698 |
| Mean | 2165582 | 665502 |
| Std. Dev. | 21232.4 | 7606.7 |
| %RSD | 0.98 | 1.14 |

**Table .No:4. Inter day precision results for Doxofylline and Sertaline**

| Sr. No. | Doxofylline | Sertaline |
|---|---|---|
| 1 | 2193412 | 679404 |
| 2 | 2204983 | 674503 |
| 3 | 2197829 | 674622 |
| 4 | 2196594 | 680356 |
| 5 | 2192686 | 678873 |
| 6 | 2217749 | 676981 |
| Mean | 2200542 | 677457 |
| Std. Dev. | 9500.57 | 2497.6 |
| %RSD | 0.43 | 0.37 |

**Table: 5. Accuracy results of Doxofylline and Sertaline**

| Sample | Accuracy | Amount added (µg/ml) | Amount Recovered (µg/ml) | Recovery (%) | % RSD | SD |
|---|---|---|---|---|---|---|
| | 50% | 80 | 80.33 | 100.42 | 1.50 | 1.51 |
| | 100% | 160 | 160.94 | 100.59 | 0.75 | 0.75 |
| **Doxofyline** | 150% | 240 | 239.88 | 99.95 | 0.14 | 0.14 |
| | 50% | 10 | 10.01 | 100.17 | 1.42 | 1.43 |
| | 100% | 20 | 39.76 | 99.41 | 0.56 | 0.56 |
| **Sertraline** | 150% | 30 | 30.16 | 100.54 | 0.97 | 0.97 |

**Table 6. Robustness data of Doxofylline and Sertaline method**

| Condition | Variation | | | | | Retention time (min) | |
|---|---|---|---|---|---|---|---|
| | | DOXO | SERTA | DOXO | SER | DOXO | SER |
| Flow rate (ml/min) | Less flow 0.80 | 4304723 | 24398390 | 1.2 | 1.3 | 2.875 | 4.823 |
| | Actual flow 1 | 3819929 | 22066321 | 0.98 | 0.97 | 2.317 | 3.688 |
| | More flow 1.20 | 3414393 | 19104424 | 0.87 | 0.79 | 2.137 | 3.025 |
| Temperature (ºC) | Less temp 28 | 3940195 | 21760337 | 0.95 | 0.52 | 2.854 | 4.356 |
| | Actual temp 30 | 3819929 | 22066321 | 0.28 | 0.27 | 2.371 | 3.688 |
| | More temp 32 | 3921278 | 21410909 | 0.18 | 0.61 | 2.364 | 3.347 |

**Table.no.7: Results of analysis of commercial formulation**

| S.No | Drug | Label claim (mg) | Obtained (mg) | %Recovery |
|---|---|---|---|---|
| 1. | Doxofyline | 400 | 399.7 | 99.71 |
| 2. | Sertraline | 50 | 49.8 | 99.53 |

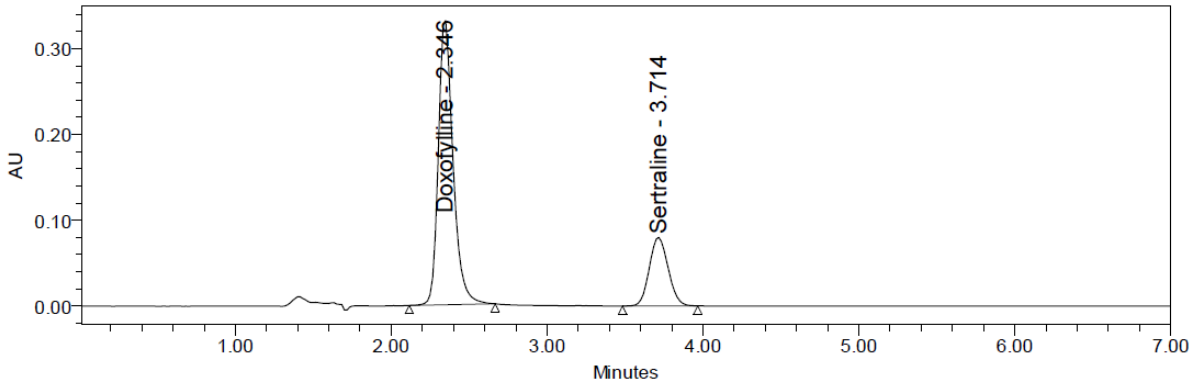


**Fig.no.1: Chromatogram of mixed standard solution**

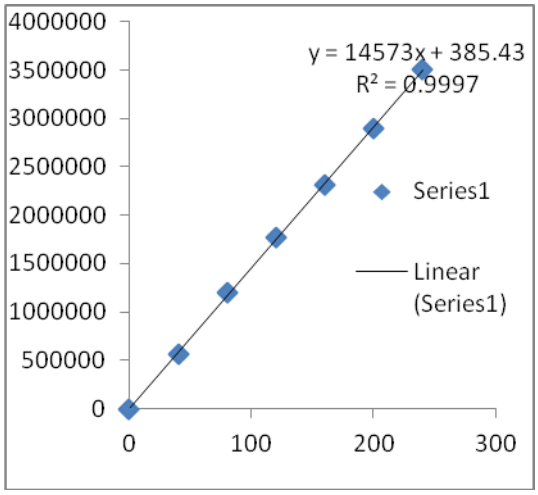


**Fig.2: Linearity curve for Doxofyline**

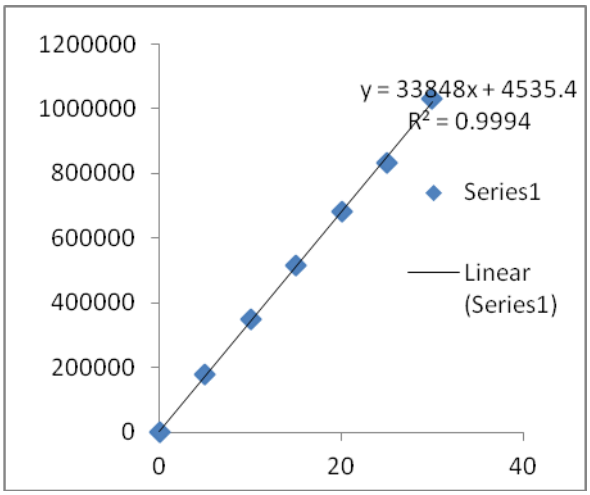


**Fig.3: Linearity curve for Sertraline**

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