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# A MODIFIED LIQUID CHROMATOGRAPHIC METHOD DEVELPOMENT AND VALIDATION FOR SIMULTANEOUS ESTIMATION OF SILDENAFIL AND FLUOXETINE IN BULK AND TABLET DOSAGE FORM

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**Abstract**

A simple, specific, accurate, reproducible and economical reverse phase liquid chromatography method was developed and validated for the quantitative simultaneous estimation of Sildenafil and Fluoxetine in bulk and marketed formulations. Estimation of drugs in this combination was done with a C18 column Kromasil 100-5$C_{18}$ column [250mm x 4.6mm].using mobile phase of composition Acetonitrile and phosphate buffer (50:50 v/v, pH 5). The flow rate was 1 ml/min and the effluents were monitored at 212nm. The retention time of Sildenafil and Fluoxetine were 3.3 min and 6.2 min respectively. The linearity over a concentration range of 20-100 µg/ml for both Sildenafil and Fluoxetine.

The established method proved as reproducible one with a %RSD value of less than 2 and having the robustness and accuracy within the specified limits. Assay of marketed formulation was determined and find with 99.48% and 98.7% for Sildenafil and Fluoxetine respectively.

The method was validated according to the guidelines of International Conference on Harmonization (ICH) and was successfully employed in the estimation of commercial formulations. This liquid chromatographic method can be applied for the qualitative and quantitative determination of selected drugs by the modern chemist.

***Keywords:*** Sildenafil, Fluoxetine, RP-HPLC and Method validation.

**Introduction**

Sildenafil citrate and Fluoxetine is a combination of well-known Sildenafil citrate and Fluoxetine, a selective serotonin reuptake inhibitor (SSRI). Antidepressants of the SSRI class including Fluoxetine have long been used off-label to treat premature ejaculation. Delayed orgasm is a well-known effect of such drugs. Fluoxetine in combination with Sildenafil citrate has been shown to be more effective than Fluoxetine alone in delaying ejaculation and providing sexual satisfaction[1].

The stability of a drug dosage form refers to the ability of a particular form to maintain its physical, chemical, therapeutic, and toxicological specification presented in the monograph on identity, strength, quality, and purity.

The stability of a drug product should ordinarily be demonstrated by its manufacturer by methods appropriate for the purpose. Obviously, a stability testing problem is never simple. Stability testing is an important part of the process of drug product development.

The purpose of the stability testing is to provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors, such as temperature, humidity, and sun light, and enables recommendation of storage conditions, retest period and shelf life to be established[4]. The literature survey reveals that many analytical methods are reported for the determination of SC and FT individually and in combination. The literature survey revealed spectroscopic[5–12] and chromatographic methods[13–18].

It was found that number of studies involving method development for estimation of Sildenafil citrate and Fluoxetine alone are available including RP-HPLC, HPTLC, UPLC, UV Spectrophotometric and LC-MS[18-26].

But review of literature reveals that there is no any method reported till now for simultaneous estimation of Sildenafil citrate and Fluoxetine by stability indicating RP-HPLC in the fixed pharmaceutical dosage forms.

Extensive literature survey proved that very few methods were reported for the determination of Sildenafil and Fluoxetine by RP-HPLC.

So we attempted to develop an accurate, rapid, precise, stable, sensitive and economically viable liquid chromatographic method for the simultaneous determination of selected drugs in the present research.

## Materials and Methods

### Equipment used

The chromatographic separation was carry out on Agilent 1120 dense liquid chromatographic system integrated with a variable wavelength logic controller of UV detector and a Rheodyne injector instrument with 20µl fixed loop. A reverse phase C18[Kromasil 250mm × 4.6 mm]was used. Lab India $3000^{+}$double beam UV visible spectrophotometer and Axis AGN204-PO electronic balance was used for Spectrophotometric determinations and weighing purposes respectively.

### Reagents and chemicals

Pharmaceutical grade pure Sildenafil and Fluoxetine gift samples were procured from Mylan Laboratories, Hyderabad. Marketed tablet formulations (Malegra FXT) with of 100mg of Sildenafil and 60mg of Fluoxetine were procured from local market. (Mfd.by Aurochem Pharmaceuticals ltd). HPLC grade Acetonitrile and Water were commercially procured from Merck specialties private limited, Mumbai.

### Chromatographic conditions

Kromasil 100-5$C_{18}$ column [250mm x 4.6mm] was used for the chromatographic separation at a detection wave length of 212 nm. Mobile phase composition of Acetonitrile and Phosphate buffer pH 5 in a ratio of 50:50 v/v was selected for rines and same mixture was used in the preparation of solutions i.e (standard and sample). The adjusting of flow rate is 1ml/min and the injection volume was 20µl.

### Preparation of Mobile phase

Phosphate buffer pH 5 was prepared by dissolving 0.136 gm of Potassium dihydrogen phosphate and 2 ml of Triethyl amine in 80ml of HPLC grade water and adjusts the pH to 5.0 with orthophosphoric acid and volume was adjusted with water to produce100 ml, which is then filtered through 0.45µ membrane filter and sonicated for 20 minutes.

### Preparation of Standard solutions

25mg each of Sildenafil and Fluoxetine were accurately weighed and transferred into two 25ml volumetric flasks respectively and dissolved in mobile phase as mentioned above and the volume was made up with the same solvent to obtain primary stock solutions A (Sildenafil) B (Fluoxetine) to achieve standard of concentrations of 1000µg/ml of

each drug. From the primary stock solutions , 1 ml of each solution was pipette out and transferred to a 10ml volumetric flask and the volume was made up with the mobile phase to obtain final concentrations of 100 µg/ml of Sildenafil and Fluoxetine respectively and this solution is (working stock solution A).

**Preparation of Sample Solution**

Twenty tablets of Sildenafil and Fluoxetine were weighed and crushed. Tablet powder equivalent to 300mg of Sildenafil and 100mg of Fluoxetine was weighed accurately and transferred to a 25ml volumetric flask. The content was dissolved with 10ml of mobile phase and then sonicated for 15min. The volume was made up with the mobile phase and filtered with 0.45µmembrane filter and sonicated for 20min.0.1 ml of this solution was pipette out and transferred to a 10mlvolumetric flask and the volume was made up with the mobile phase to obtain a concentration of 120µg/ml of Sildenafil and 40µg/ml of Fluoxetine (working stock solution B).

**Optimization of RP-HPLC method**

The HPLC method was optimized with an aim to develop a simultaneous estimation procedure for the assay of Sildenafil and Fluoxetine. For the method optimization, different mobile phases were tried, but acceptable retention times, theoretical plates and good resolution were observed with Acetonitrile:Phosphate buffer pH5 (50:50 v/v) using Kromasil 100-5$C_{18}$ column [250mm x 4.6mm].

**Validation of the RP-HPLC method**

Validation of the optimized method was performed according to the ICH Q2 (B) guidelines.

**System suitability**

System suitability was carried out with five injections of solution of 100% concentration having 100 µg/ml of Sildenafil and Fluoxetine of each in to the chromatographic system. The theoretical plates values (N) obtained and calculated tailing factors (T) were showed in table 1.

**Linearity**

For the identification of linearity from the primary stock solution to a series of 10ml volumetric flasks and the volume was made up to the mark with mobile phase to have the concentration range of 20-100 µg/ml of both the drugs. Calibration curves of each drug were plotted with obtained peak areas vs concentration to determine the correlation

coefficients. The obtained linear graphs were shown in figure 3 and 4 their respective parameters were given in table 2.

**Limit of Detection (LOD) and Limit of Quantitation (LOQ)**

The LOD and LOQ were calculated from the slope(s) of the calibration plot and the standard deviation (SD) of the peak areas using the formulae LOD = 3.3 σ/s and LOQ = 10 σ/s. The results were given in table 2.

**Precision**

The method was observed for its reproducibility by calculating the %RSD of six replicate injections of the highest concentration of stock solutions on the same day and for intermediate precision. The obtained results were given in table 3.

**Accuracy**

Accuracy was performed by following standard addition method to know the recovery. A known amount of standard was added to pre-analyzed sample and the resultant solution was determined. The obtained recovery results were reported in table 4.

**Specificity**

Specificity of the method was established by examine the pure drug samples and the method was found to be specific and there was no interferences was found. The optimized chromatogram of the selected drugs of interest was shown in figure 2.

**Robustness**

Robustness of the method was examined by changing the different chromatographic conditions like wavelength and flow rate etc. and the % RSD were reported. The highest concentration of the linearity was injected and proved that the developed method was more robust. The obtained values were reported in table 5.

**Assay of Marketed Formulations**

20µl of sample solution of Sildenafil and Fluoxetine was injected into chromatographic system and the peak responses were determined. The percentage purity of the selected drugs were determined and the obtained respective chromatogram and the values were represented in figure 5 and table 6 respectively.

**Results and Discussion**

After a number of trials with mobile phases of different composition, Acetonitrile, Phosphate buffer pH 5 in the ratio 50:50v/v was selected as mobile phase because of better resolution and symmetric peaks. Sildenafil and Fluoxetine were found to show appreciable absorbance at 212nm when determined spectrophotometrically and hence it was selected as the detection wavelength. An optimized chromatogram showing the separation of Sildenafil and Fluoxetineat different $R_T$s was shown in figure 1.

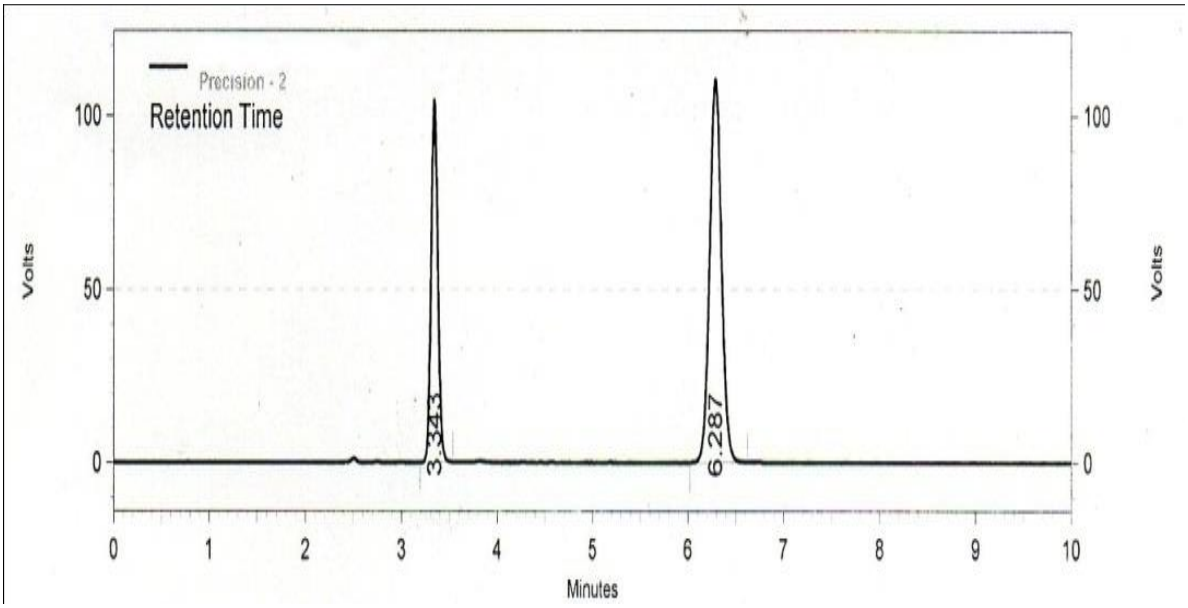


**Fig 1: Optimized Chromatogram of Sildenafil and Fluoxetine**

System suitability was carried out by injecting 5 replicate injections of 100% test concentration, number of theoretical plates, HETP and resolution were satisfactory. The chromatograms confirm the presence of Sildenafil and Fluoxetine at 3.3min and 6.2min respectively without any interference. The parameters were given in table 1.

**Table 1: System Suitability Parameters**

| Parameters | Sildenafil | Fluoxetine |
|---|---|---|
| Retention time (min) | 3.3 | 6.29 |
| Theoretical plates (N) | 11456 | 10366 |
| Tailing factor (T) | 1.2 | 1.4 |
| Resolution ($R_{s)}$ | 2.99 | |

Concentration range of 20-100µg/ml for Sildenafil and Fluoxetine were found to be linear with correlation coefficients 0.998 and 0.999 for Sildenafil and Fluoxetine respectively. The respective calibrations curve was shown in Figure 3 and 4 respectively. The results were given in table 2.

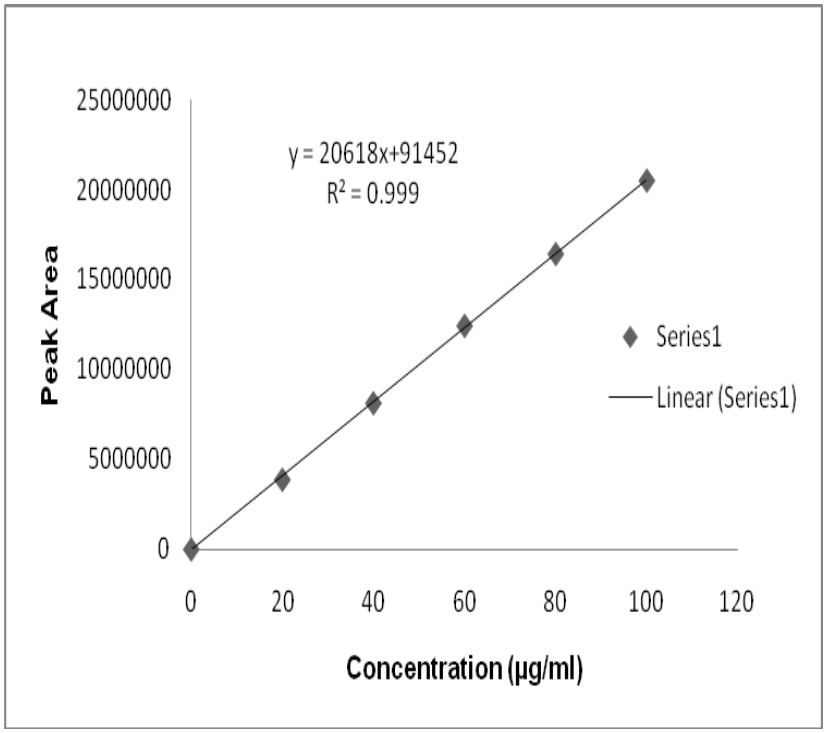


**Fig 2: Calibration Plot of Sildenafil.**

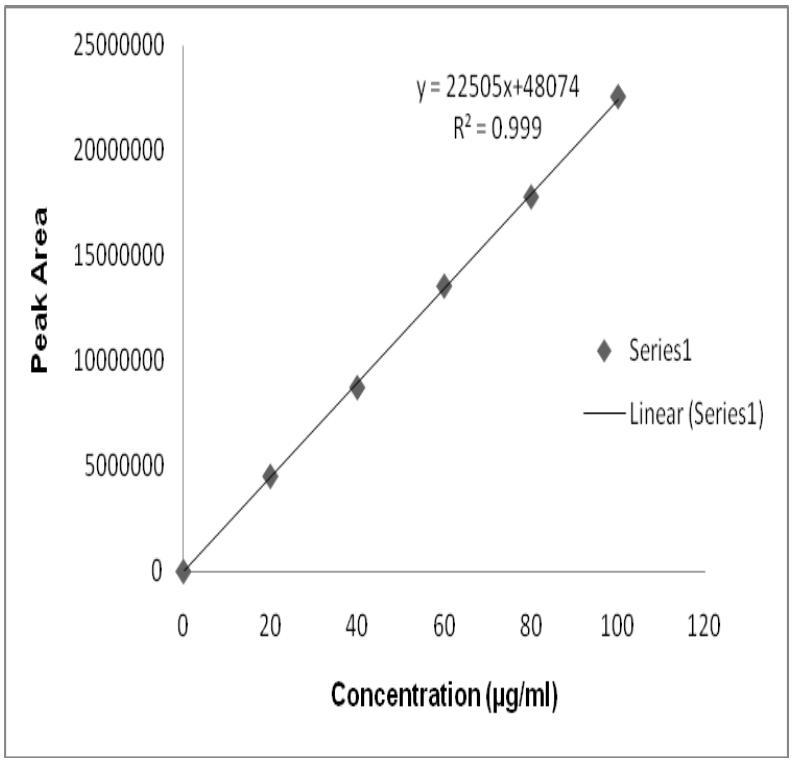


**Fig 3: Calibration Plot of Fluoxetine.**

The limits of detection for Sildenafil and Fluoxetine were found to be 0.65µg/ml and 0.54µg/ml respectively and the limits of quantization were 1.99µg/ml and 1.64µg/ml respectively. Values were represented in table 2.

**Table 2: Results for Linearity.**

| Parameter | Sildenafil | Fluoxetine |
|---|---|---|
| Linearity Range (µg/ml) | 20-100 | 20-100 |
| Regression Equation | y = 16324x+12346 | y = 86676x + 37329 |
| Slope (m) | 16324 | 86676 |
| Intercept (c) | 12346 | 37329 |
| Regression Coefficient ($r^2$) | 0.998 | 0.999 |
| Limit of Detection (µg/ml) | 0.65 | 0.54 |
| Limit of Quantitation (µg/ml) | 1.99 | 1.64 |

The proposed method was found to be precise and reproducible with %RSD of 0.42 and 0.19for Sildenafil and Fluoxetine respectively. %RSD was reported in table 3.

**Table 3: Results of Precision.**

| Drug | Intraday Precision (%RSD) | Interday Precision (%RSD) |
|---|---|---|
| Sildenafil | 0.42 | 0.34 |
| Fluoxetine | 0.19 | 0.11 |

Accuracy of the method was verified by performing recovery studies by standard addition method. The percent recovery of the standard added to the pre-analysed sample was calculated and it was found to be 98.6% to 99.4% for Sildenafil and 98.4 to 99.3% for Fluoxetine. This indicates that the method was accurate. Values obtained were given in table 4.

**Table 4: Results for Accuracy.**

| Recovery level | Sildenafil | | | | Fluoxetine | | | |
|---|---|---|---|---|---|---|---|---|
| | Amount Added (µg/ml) | | Amount Found (µg/ml) | % Recovery | Amount Added (µg/ml) | | Amount Found (µg/ml) | % Recovery |
| | std | test | | | std | Test | | |
| 80% | 20 | 60 | 78.9 | 98.6 | 20 | 60 | 79.5 | 99.3 |
| 100% | 40 | 60 | 99.4 | 99.4 | 40 | 60 | 98.4 | 98.4 |
| 120% | 60 | 60 | 119.1 | 99.2 | 60 | 60 | 118.7 | 98.9 |
| **Mean recovery** | 98.6-99.4 | | | | 98.4-99.3 | | | |

The method was found to be robust after changing the conditions like detection wavelength (± 2nm) and flow rate (± 0.2 ml). %RSD was calculated for each variation and reported. Values obtained were given in table 5.

**Table 5: Results for Robustness.**

| Parameters | %RSD | |
|---|---|---|
| | Sildenafil | Fluoxetine |
| Detection wavelength at 210nm | 0.12 | 0.38 |
| Detection wavelength at 214nm | 0.36 | 0.66 |
| Flow rate 0.8ml/min | 0.34 | 0.52 |
| Flow rate 1.2ml/min | 0.31 | 0.35 |

The method was found to be specific for the combination of interest after verifying the chromatograms showing no interference of the excipients present. Hence, the method was well suitable for the estimation of the commercial formulations of the selected combination with a percentage purity of 99.48% for Sildenafil and 98.7% for Fluoxetine.

The typical chromatogram for assay of marketed formulations was shown in figure.4 and Values obtained were given in table 6.

**Figure 4: A Typical Chromatogram for Assay of Marketed Formulation Containing 120µg/ml of Sildenafil And 40µg/ml of Fluoxetine**

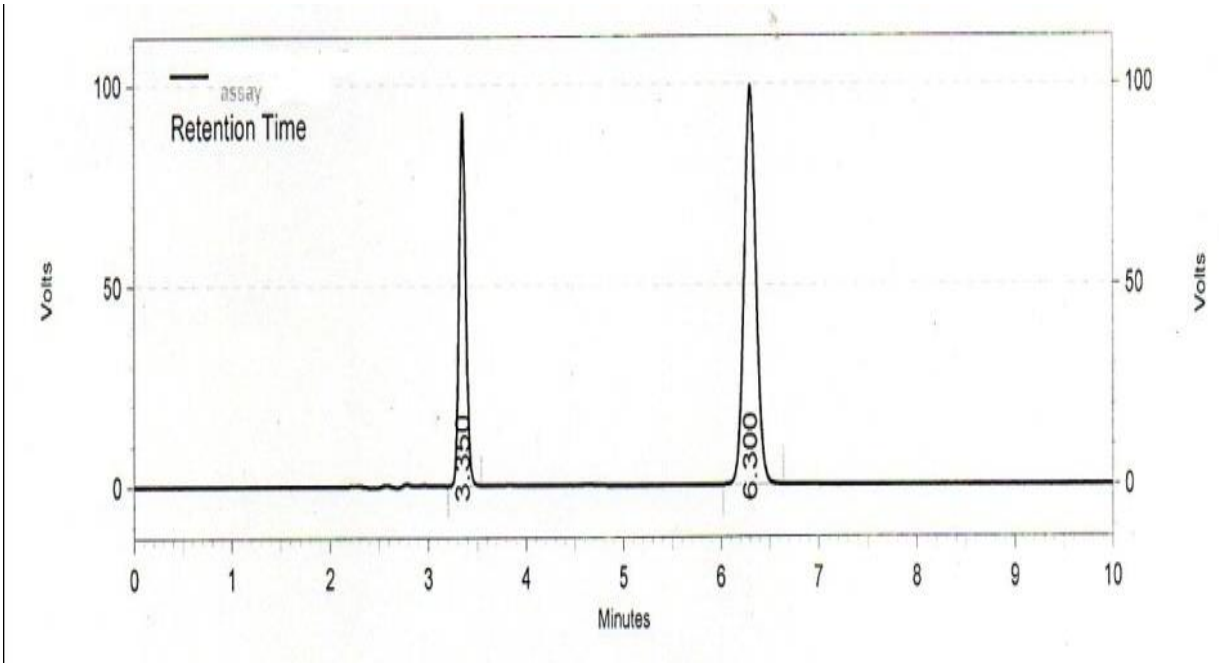


**Table 6: Results for Assay of Marketed Formulation Malegra Fxt (Sil-100mg, Flxt-60mg).**

| Drug | Label claim (mg/tab) | Amount recovered | % Amount found in drug |
|---|---|---|---|
| Sildenafil | 100 | 98.9 | 98.9 |
| Fluoxetine | 60 | 59.1 | 98.5 |

**Conclusion**

The RP-HPLC method developed and validated allows a simple and fast quantitative determination of Sildenafil and Fluoxetine from their formulations. All the validation parameters were found to be within the limits according to ICH guidelines. The proposed method was found to be specific for the drugs of interest irrespective of the excipients present and the method was found to be simple, accurate, precise, rugged and robust. So the established method can be employed in the routine analysis of the marketed formulations.

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