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Analytical Method Development and Validation for the Simultaneous Estimation of Sildenafil and Dapoxetine by Using RP-HPLC Technique

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ABSTRACT

A new RP-HPLC method was developed for simultaneous estimation of sildenafil and Dapoxetine in pharmaceutical dosage form. The chromatographic conditions were successfully developed for the separation of Sildenafil and Dapoxetine by using Inertsil ODS C18 (4.6 X 250mm X 5µ) column, flow rate was 1.0ml/min, mobile phase ratio was Phosphate buffer (0.05M) pH 4.6: ACN (55:45%v/v) (pH was adjusted with orthophosphoric acid), detection wave length was 255nm. The retention time was found to be 2.984 and 3.525 for sildenafil and dapoxetine respectively. The analytical method was validated according to ICH guidelines. The linearity study for Sildenafil and Dapoxetine was found in concentration range of 1µg/ml to 5µg/ml and 100µg/ml to 500µg/ml and correlation coefficient (r2) was found to be 0.999 and % mean recovery was found to be 99.42% and 100.27%, %RSD for repeatability was 0.77 and 0.48, % RSD for intermediate precision was 0.18 and 0.39 respectively. The proposed method can be used for the routine analysis of sildenafile and Dapoxetine in API and pharmaceutical dosage form.

Keywords: Sildenafil, Dapoxetine and RP-HPLC techniques

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1. Introduction

Sildenafil (fig-1) chemically is 2 - Hydroxy - 1, 2, 3– propane tricarboxylate - 1-[[3-(6, 7-dihydro-1-methyl-7-oxo-3propyl1H pyrazolo [4, 3d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl] 4-methyl piperazine with molecular formula C22H30N6O4S. Sildenafil, sold as Viagra and other trade names, is a medication used to treat erectile dysfunction and pulmonary arterial hypertension (PAH). It acts by inhibiting cGMP-specific

phosphodiesterase type 5 (PDE5), anenzyme that promotes degradation of cGMP, which regulates blood flow in thepenis. The typical dosage of Sildenafil Citrate is 100 mg in men with erectile dysfunction.1,2.

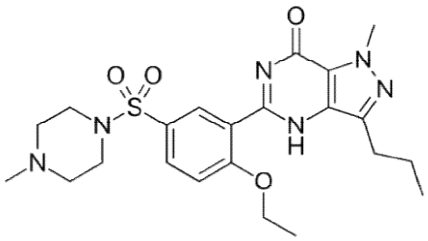

Fig 1: Structure of Sildenafil

Dapoxetine (fig-II) chemically is (S-(+)-N,N-Dimethyl-a-[2-(naphthalenyloxy) ethyl] benzene methanamine with molecular formula C21H23NO. Dapoxetine, marketed as Priligy and Westoxetin 30 mg.It is the first compound developed specially for the treatment of premature ejaculation (PE) in men,works by inhibiting the serotonin transporter, increasing serotonin's action at the post synaptic cleft, and as a consequence promoting ejaculatory delay. As a member of selective serotonin reuptake inhibitor (SSRI) family, Dapoxetine was initially created as an antidepressant. However, unlike other SSRIs, Dapoxetine is absorbed and eliminated rapidly in the body. Its fast acting property makes it suitable for the treatment of PE but not as an antidepressant.3,4.

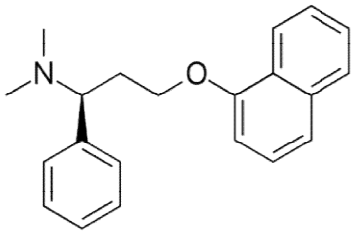

Fig 2: Structure of Dapoxetine

Literature survey revealed that very few methods were reported for the estimation of Sildenafil and Dapoxetine Hydrochloride by UV spectroscopy, HPTLC, UPLC-MS/UV and HPLC method 5 - 20. So an attempt has been made to develop an accurate precise and economically viable RP-HPLC method for the simultaneous estimation of combination of interest in the current research.

2. Materials and Methods

Chemicals and reagents:

HPLC grade acetonitrile and water purchased from E.Merck, Mumbai, India, and orthophosphoric acid and sodium hydroxide AR grade purchased from SD Fine Chem., Mumbai, India. The reference sample and branded formulation was supplied by Pharmatrain Analytical labs, Hyderabad, India.

Instruments and Columns:

Waters HPLC model 2695 equipped with UV Visible detector using data handling system-waters alliance empower software. Ultra Sonicator, model no: SE60US, Lab india. The column used in the development for determination is Inertsil ODS C18 (250mm X 4.6 mm, 5µ).

Selection of Chromatographic Method and Wave Length (λ max):

Selection of chromatographic method in general is done taking into consideration of several parameters like the nature of the drugs, molecular weight and solubility. Since both the drugs selected are polar in nature. RP-HPLC was selected for initial chromatographic condition because of its simplicity and suitability. The Chromatographic conditions are given in the table-I.

Table 1: Chromatographic conditions

Parameters	Conditions
Column	Inertsil ODS C 18 (250mm X 4.6 X 5µ)
Detection wavelength	255nm
Flow rate	1.0 ml/min
Injection volume	20µl
Mobile phase	Buffer pH 4.6:Acetonitrile (55:45%v/v) (diluent)
Column temperature	Ambient
Runtime	10min
Elution	Isocratic

Selection of Wave Length (λ max):

From the UV visible spectrophotometric results, Simultaneous estimation of two spectra shows maximum absorbance at 255nm (Fig-III).

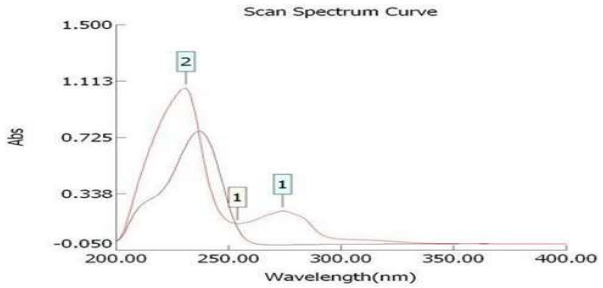

Fig 3: Overlay spectrum of Sildenafil and Dapoxetine

Preparation of mobile phase:

Accurately weighed the quantity of potassium dihydrogen phosphate dissolves in 1000ml of water and pH is adjusted to 4.6 ± 0.1 with ortho phosphoric acid. The solution was filtered through 0.45µ membrane filter and was degassed. A freshly prepared buffer solution: Acetonitrile in a ratio of (55:45% V/V) was filtered through 0.45µ membrane filter and sonicated.

Preparation of Stock solution:

Weighed accurately about 10 mg of Sildenafil and Dapoxetine and transferred into 10 ml volumetric flask, added 2.0 ml of diluent and the solution was sonicated to dissolve and dilute to the volume with mobile phase.

Standard preparation: Transfer10 ml of standard stock solution into 100 ml volumetric flask and dilute to volume with diluent.

Preparation of Sample solution:

Weighed twenty tablets of Sildenafil and Dapoxetine powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 50 mg of sildenafil and 30 mg

of Dapoxetine was transferred into 100 ml volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100 ml with mobile phase. Then the mixture was filtered through a 0.45µ membrane filter. From the above solution 10 ml aliquot was taken into a separate 100 ml volumetric flask and diluted up to the volume with the mobile phase and mixed well.

Optimization of HPLC method

The HPLC method was optimized with an aim to develop an accurate and precise method for the estimation of Sildenafil and Dapoxetine in pharmaceutical dosage forms. For the method optimization different mobile phases were tried but acceptable retention times, theoretical plates and good resolution observed with Buffer solution (pH = 4.6) : Acetonitrile in the ratio of 55:45 using Inertsil ODS C18, 250 X 4.6 mm, 5µ.

Validated RP-HPLC Method for Sildenafil and Dapoxetine

Validation of the optimized method was performed according to the ICH guide lines21-23.

Accuracy:

For accuracy determination, three different concentrations were prepared separately i.e.50%, 100% and 150% of analyte and the chromatograms were recorded for the same. The results obtained for recovery were found to be within the limits. The results were given in the table-II.

Precision:

The precision of an analytical procedure express the closeness of agreement between a series of measurement obtained from multiple sampling of the same homogenous sample under the prescribed conditions. Precision of an analytical procedure is usually expressed in terms of variance, standard deviation, coefficient of variation of a series of measurement.

System Precision:

System precision was determined by injecting six homogenous preparation solutions into HPLC System of a concentration 50µg/ml Sildenafil and30µg/ml Dapoxetine. The mean, standard deviation and % RSD for peak areas of Sildenafil and Dapoxetine from standard solutions were calculated. The % RSD of both the drugs was found to be below 1. Hence the method is said to be Precise. The results were given in the table-III.

Method Precision:

Method precision was determined by injecting six sample solutions of Single batch were analysed as per test method. The mean, standard deviation and % RSD for peak areas of Sildenafil and Dapoxetine from sample solutions were calculated. The % RSD Sildenafil and Dapoxetine was found to be below 1. Hence the method is said to be Precise. The results were given in the table-III.

Linearity:

Linearity for Sildenafil was determined in the range of 1.0µg/ml - 5.0µg/ml (Fig-IV) and Dapoxetine was determined in the range of 100.0µg/ml - 500.0µg/ml (Fig-V). A graph was plotted with concentration on X-axis and peak area on Y- axis and correlation coefficient was determined. The results were given in the table-IV.

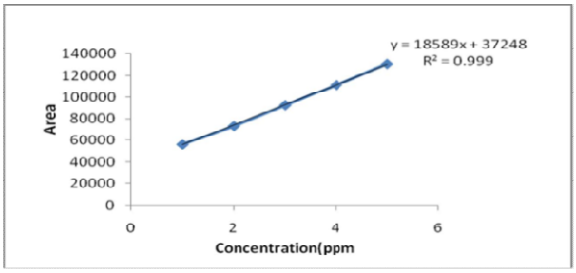

Fig 4: Calibration curve for Sildenafil

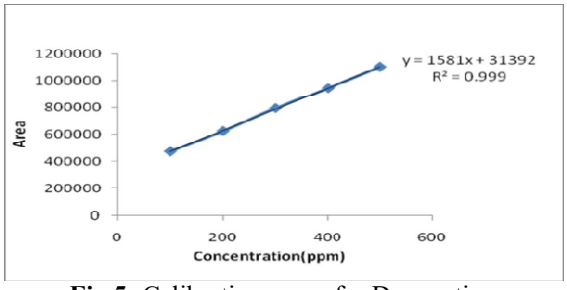

Fig 5: Calibration curve for Dapoxetine

Ruggedness (Intermediate Precision):

The Ruggedness of the method has been verified by analysing the six samples of the same batch for method precision as per test method by different analyst using different instrument, different days. The analyst's prepared six samples of the same batch for two different days. Calculated % RSD for two different days by analyst in six samples for ruggedness results with the method precision reported in the table-III.

Robustness:

To evaluate the robustness, the following small deliberate variations are made in the method and analysed the sample in triplicate, flow rate (±0.2 ml/min) and mobile phase (±5%). The system suitability was evaluated in each condition and compared the results with method precision results. The method is robust for change in flow rate and mobile phase. The% RSD reported in the table-III, IV.

Specificity:

Specificity shall be established by demonstrating that the procedure is unaffected by the presence of interference at the retention time of the Sildenafil and Dapoxetine with respect to mobile phase, diluents and degradants. Specificity studies include blank and sample solution (control sample), Sildenafil and Dapoxetine standard solution were injected into the HPLC system. There was no interference from the blank at the retention time of the peaks. Peak purity data reveals that Sildenafil and Dapoxetine were homogeneous and there was no interference at the retention time of Sildenafil and Dapoxetine peaks.

Limit of Detection (LOD) and Limit of Quantification (LOQ): The LOD and LOQ of the developed method were determined by analysing progressively low concentration of the standard solutions using the developed methods. The results are given in the table-III.

$$LOD = 3.3\ \sigma / S \text{ and } LOQ = 10\ \sigma / S$$

σ = standard deviation of the response

S = slope of the calibration curve of the analyte.

Analysis of Marketed Formulations:

Twenty tablets of sildenafil and dapoxetine are weighed powdered uniformly in a mortar. An accurately weighed portion powder equivalent to 50 mg of sildenafil and 30 mg of dapoxetine was transferred into 100 ml volumetric flask. The contents of the flask were sonicated for about 15 min for complete solubility of the drug and the volume was made up to 100 ml with mobile phase. Then the mixture was filtered. From the above solution 10 ml aliquot was taken into a separate 100 ml volumetric flask and diluted up to the volume with the mobile phase and mixed well. Initially inject 20 µL of blank solution, sample solution and standard solution, Disregard peaks due to blank and placebo if any.

3. Results and Discussion

The goal of the study is to development of simple, rapid, sensitive, specific and accurate HPLC methods for the routine quantitative determination of samples. Inertsil C18 ODS Column (250 mm x 4.6 mm; 5µ) was used as stationary phase. The mobile phase composition of Buffer: Acetonitrile in the ratio of 55:45 and pH adjusted to 4.6±0.1 with 0.1% orthophosphoric acid were selected. A good linear relationship (r2 = 0.999 & r2 = 0.999) was observed in the range of 1.0 µg/ml - 5.0 µg/ml & 100.0 µg/ml – 500.0 µg/ml for sildenafil and Dapoxetine. Recovery values obtained by the proposed method are accurate.

The system precision was established by six replicate injections of the standard solutions containing analyte of interest. The value of relative standard deviation of Sildenafil and Dapoxetine was found to be 0.77 and 0.48 within the limit, indicating the injection repeatability of the method. The method precision was established by carrying out the analysis six times using the proposed method. The relative standard deviation of Sildenafil and Dapoxetine was found to be 0.18 and 0.39 within the limit, indicating the injection repeatability of the method. Six samples of the same batch were prepared on different days by the analysts. Calculated %RSD for two different days in six samples for ruggedness results with the method precision within the limits. The system suitability was evaluated in each condition and compared the results with method precision results. The method is robust for change in flow rate and mobile phase. The specificity studies blank and sample solution (control sample) Sildenafil and Dapoxetine standard solution were injected into the HPLC system. There was no interference from the blank at the retention time of the peaks. Peak purity data reveals that Sildenafil and Dapoxetine were homogeneous and there was no interference at the retention time of Sildenafil and Dapoxetine peaks. The method is specific for the estimation of Sildenafil and Dapoxetine tablets. (Fig VI).

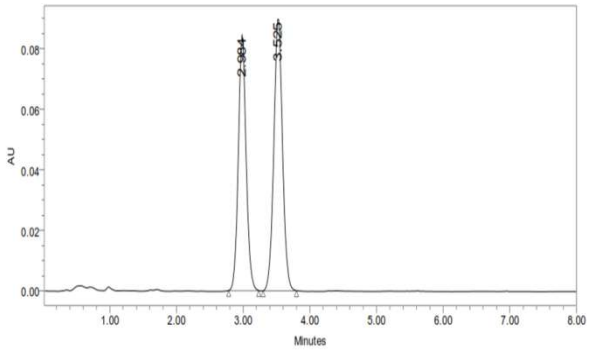

Fig 6: Sample Chromatogram for Sildenafil and Dapoxetine

Table 2: Results of Accuracy studies

Accuracy Level	Amount added		Amount recovered		% recovered	
	Sildenafil	Dapoxetine	Sildenafil	Dapoxetine	Sildenafil	Dapoxetine
50 %	5.0	5.0	5.01	5.08	100.2	101.6
100 %	10.0	10.0	9.94	9.99	99.40	99.90
150 %	15.0	15.0	14.8	14.9	98.67	99.33
Mean Recovery					99.42	100.27

Table 3: Linearity Results

Parameters	Sildenafil	Dapoxetine
Linearity range	1.0 – 5.0µg/ml	100.0 – 500.0 µg/ml
Intercept	37248	31392
Slope	18589	1581
R^2	0.999	0.999

Table 4: Summary of Validation Parameters

S.No	Parameters	Sildenafil	Dapoxetine
1	Method Precision (n=6) % R.S.D	0.77	0.48
2	Linearity Concentration range (µg/ml)	1.0 – 5.0	100.0 – 500.0
3	Precision (n=6) % R.S.D	0.65	0.38
4	Ruggedness % R.S.D	0.18	0.39
5	Specificity	No interference at the retention time of both drug peaks	
6	LOD	2.95	3.04
7	LOQ	9.87	10.0

4. Conclusion

The RP-HPLC method developed and validated allows a simple and fast quantitative simultaneous determination of Sildenafil and Dapoxtine from its formulation. All the validation parameters were found to be within the limits according to the ICH[21-23]. 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 and can be involved in the routine analysis of the marketed formulation. Therefore this method can be employed in quality control to estimate the amount of Sildenafil and Dapoxtine in pure and pharmaceutical dosage forms.

5. Acknowledgement

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