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A NEW RP HPLC METHOD FOR THE ESTIMATION OF SILDENAFIL AND DAPOXETINE IN PHARMACEUTICAL DOSAGE FORM

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ABSTRACT

A simple and selective LC method is described for the determination of Sildenafil and dapoxetinein tablet dosage forms. Chromatographic separation was achieved on a c_{18} column using mobile phase consisting of a mixture of 30 volumes of ammonium acetate buffer,40 volumes of acetonitrile and 30 volumes of Methanol with detection of 239 nm. Linearity was observed in the range 50-175 µg/ml for Sildenafil (r^2 =0.998) and 50-175 µg /ml for dapoxetine(r^2 =0.999) for the amount of drugs estimated by the proposed methods was in good agreement with the label claim. The proposed methods were validated. The accuracy of the methods was assessed by recovery studies at three different levels. Recovery experiments indicated the absence of interference from commonly encountered pharmaceutical additives. The method was found to be precise as indicated by the repeatability analysis, showing %RSD less than 2. All statistical data proves validity of the methods and can be used for routine analysis of pharmaceutical dosage form.

Key Words: Sildenafil, dapoxetine, LC method

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INTRODUCTION

Pharmaceutical analysis simply means analysis of pharmaceuticals. Webster' dictionary defines a pharmaceutical is a medical drug. A more appropriate term for a pharmaceutical is active pharmaceutical ingredient (API) or active ingredient to distinguish it from a formulated product or drug product is prepared by formulating a drug substance with inert ingredient (excipient) to prepare a drug product that is suitable for administration to patients. Research and development (R&D) play a very comprehensive role in new drug development and follow up activities to ensure that a new drug product meets the established standards is stable and continue to approved by regulatory authorities,assuring that all batches of drug product are made to the specific standards utilization of approved ingredients and production method becomes the responsibility of pharmaceutical analysts in the quality control (QC) or quality assurance department . The methods are generally developed in an analytical R&D department and transferred to QC or other departments as needed. At times they are transferred to other divisions.Chromatography is a family of analytical chemistry techniques for the separation of mixtures. It involves passing the sample, a mixture that contains the analyte, in the "mobile phase", often in a stream of solvent, through the

"stationary phase." The stationary phase retards the passage of the components of the sample. When components pass through the system at different rates they become separated in time, like runners in a marathon. Ideally, each component has a characteristic time of passage through the system. This is called its "retention time." A physical separation method in which the components of a mixture are separated by differences in their distribution between two phases, one of which is stationary (stationary phase) while the other (mobile phase) moves through it in a definite direction. The substances must interact with the stationary phase to be retained and separated by it.A chromatograph takes a chemical mixture carried by liquid or gas and separates it into its component parts as a result of differential distributions of the solutes as they flow around or over a stationary liquid or solid phase. Various techniques for the separation of complex mixtures rely on the differential affinities of substances for a gas or liquid mobile medium and for a stationary adsorbing medium through which they pass; such as paper, gelatin, or magnesium silicate gel. Analytical chromatography is used to determine the identity and concentration of molecules in a mixture. Preparative chromatography is used to purify larger quantities of a molecular species [1-3].**Sildenafil** is a phosphodiesterase inhibitor used for the treatment of erectile dysfunction

Sildenafil is an oral therapy for erectile dysfunction. In the natural setting, i.e. with sexual stimulation, it restores impaired erectile function by increasing blood flow to the penis.The physiological mechanism responsible for the erection of the penis involves the release of nitric oxide (NO) in the corpus cavernosum during sexual stimulation. Nitric oxide then activates the enzyme guanylate cyclase, which results in increased levels of cyclic guanosine monophosphate (cGMP), producing smooth muscle relaxation in the corpus cavernosum and allowing inflow of blood [4]. Dapoxetine is a selective serotonin reuptake inhibitor used in the treatment of premature ejaculation.The drug's mechanism of action is thought to be related to inhibition of neuronal reuptake of serotonin and subsequent potentiation of serotonin activity. The central ejaculatory neural circuit comprises spinal and cerebral areas that form a highly interconnected network. The sympathetic, parasympathetic, and somatic spinal centers, under the influence of sensory genital and cerebral stimuli integrated and processed at the spinal cord level, act in synergy to command physiologic events occurring during ejaculation. Experimental evidence indicates that serotonin (5-HT), throughout brain descending pathways, exerts an inhibitory role on ejaculation. To date, three 5-HT receptor subtypes (5-HT(1A), 5-HT(1B), and 5-HT(2C)) have been postulated to mediate 5-HT's modulating activity on ejaculation [5].

Aim of the study is to develop new RP HPLC method for the simultaneous estimation of sildenafiland dapoxetine pharmaceutical dosage form.

MATERIALS AND METHODS

Preparation of samples for Assay

Preparation of mixed standard solution

Weigh accurately 10mg of Sildenafil and 10 mg of dapoxetinein 100 ml of volumetric flask and dissolve in 10ml of mobile phase and make up the volume with mobile phase.From above stock solution 10 µg/ml of Sildenafil and Dapoxetineis prepared by diluting 1ml to 10ml with mobile phase. This solution is used for recording chromatogram.

Tablet sample

10tablets (each tablet containsDapoxetine-30mg Sildenafil-50 mg) were weighed and taken into a mortar and crushed to fine powder and uniformly mixed. Tablet stock solutions of DAPOXETINE and SILDENAFIL (µg/ml) were prepared by dissolving weight equivalent to 10 mg of DAPOXETINE andSILDENAFIL and dissolved in sufficient mobile phase. After that filtered the solution using 0.45-micron syringe filter and Sonicated for 5 min and dilute to 10ml with mobile phase. Further dilutions are prepared in 5 replicates of 10µg/ml of DAPOXETINE and SILDENAFILwas made by adding 1 ml of stock solution to 10 ml of mobile phase.

Calculation

The amount of Dapoxetineand Sildenafil present in the formulation by using the formula given below, and results shown in above table:

$$\%\ \text{Assay} = \frac{AT}{AS} \times \frac{WS}{DS} \times \frac{DT}{WT} \times \frac{P}{100} \times \frac{AW}{LC} \times 100$$

Validation

To verify that the analytical system is working properly and can give accurate and precise results were evaluated by 125µg/mLofBilastineand100µg/mLofMontelukast were injected six times and the chromatograms were recorded for the same [6-8].

RESULTS AND DISCUSSION

The amount of Sildenafil and Dapoxetinepresent in the taken dosage form was found to be 99.56 % and101.97 % respectively (Fig-1, Table-1).

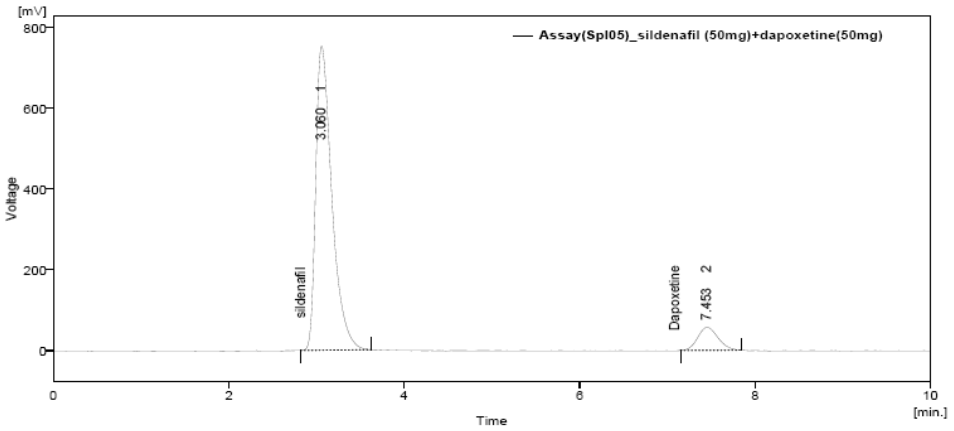

Fig-1 Chromatogram of Assay sample preparation

Table -1Assay Resultsof Sildenafil and Dapoxetine

SILDENAFIL			**DAPOXETINE**	
	Standard Area	**Sample Area**	**Standard Area**	**Sample Area**
Injection-1	9610.218	9618.037	929.107	933.278
Injection-2	9596.321	9610.218	898.860	902.356
Injection-3	9610.218	9545.801	909.64	928.769
Injection-4	9596.321	9394.586	898.86	934.043
Injection-5	9578.389	9612.063	891.613	918.958
Average Area	9598.293	9556.141	578.6002	923.4808
Assay(%purity)	99.5608344		101.972668	

The % RSD for the retention times and peak area of Sildenafil and Dapoxetinewere found to be less than 2%. The plate count and tailing factor results were found to be satisfactory and are found to be within the limit (Table-2 and 3).

Table-2 Results for system suitability of Sildenafil

Injection	**Retention time (min)**	**Peak area**	**Theoretical plates (TP)**	**Tailing factor (TF)**
1	3.073	9798.904	1353	2.083
2	3.073	9544.612	1400	1.919

3	3.073	9633.752	1400	1.892
4	3.070	9588.314	1397	1.892
5	3.073	9695.748	1353	2.083
Mean	3.060	9714.063	-	-
SD	3.0703	9662.566	-	-
%RSD	0.0052	92.301	-	-

Table-3 Results for system suitability of Dapoxetine

Injection	**Retention time (min)**	**Peak area**	**Theoretical plates**	**Tailing factor**
1	7.640	954.959	4909	1.250
2	7.497	970.903	4726	1.200
3	7.460	945.723	4933	1.206
4	7.443	964.109	4911	1.233
5	7.640	941.663	4909	1.254
Mean	7.453	935.153	-	-
SD	7.522	952.085	-	-
%RSD	0.093	13.731	-	-

The correlation coefficient for linear curve obtained between concentration vs. Area for standard preparations of Sildenafil is 0.998 and Dapoxetineis 0.999 and. The relationship between the concentration of Sildenafil and Dapoxetineand area of Sildenafil and Dapoxetineis linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits.

Table-4Linearity of Sildenafil

S.No.	**Conc.(µg/ml)**	**Area**
1	50	8118.069
2	75	8859.874
3	100	9690.218
4	150	10999.32
5	175	11897.59

Table-5Linearity of Dapoxetine

S.No.	**Conc.(µg/ml)**	**Area**
1	50	774.04
2	75	876.449

3	100	999.107
4	150	1199.86
5	175	1298.846

The percentage mean recovery of Sildenafil is 100.61 % and Dapoxetineis 100.17 % respectively. The LOD for this method was found to be 4.61µg/ml and 14.0 µg/ml for Dapoxetine. The LOQ for this method was found to be 0.21µg/ml for Sildenafil and 0.63µg/ml forDapoxetine.The observation between two analysts Assay values not greater than 2.0%, hence the method was rugged.

CONCLUSION

From the above experimental results and parameters, it was concluded that, this newly developed method for the simultaneous estimation Sildenafil and dapoxetinewas found to be simple, precise, accurate and high resolution and shorter retention time makes this method more acceptable and cost effective and it can be effectively applied for routine analysis in research institutions, quality control department in meant in industries, approved testing laboratories studies in near future.

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