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METHOD DEVOLOPMENT AND VALIDATION OF SILDENAFIL CITRATE IN TABLET DOSAGE FORM BY USING NEW RP-HPLC METHOD

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ABSTRACT

A simple and selective LC method is described for the determination of sildenafil citrate dosage forms. Chromatographic separation was achieved on a c18 column using mobile phase consisting of a mixture of Triethylamine: Acetonitrile (50:50 v/v), with detection of 290 nm. Linearity was observed in the range 2.5-7.5 µg /ml for sildenafil citrate (r^2 =0.994) 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: LC method, sildenafil citrate, Chromatographic separation

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INTRODUCTION

A drug includes all medicines intended for internal or external use for or in the diagnosis, treatment, mitigation or prevention of disease or disorder in human beings or animals, and manufactured exclusively in accordance with the formulae mentioned in authoritative books (1). Pharmaceutical analysis is a branch of chemistry involving a process of identification, determination, quantification, purification and separation of components in a mixture or determination of chemical structure of compounds. There are two main types of analysis – analysis is performed to establish composition of a substance. It is done to determine the presence of a compound or substance in a given sample or not. The various qualitative tests are detection of evolved gas, limit tests, color change reactions, determination of melting point and boiling point, mass spectroscopy, determination of nuclear half life etc. Quantitative analysis techniques are mainly used to determine the amount or concentration of analyte in a sample and expressed as a numerical value in appropriate units. These techniques are based on suitable chemical reaction and either measuring the amount of reagent added to complete the reaction or measuring the amount of reaction product obtained the characteristic movement of a substance through a defined medium under controlled conditions, electrical measurement or measurement of spectroscopic properties of the compound (2).

Quality investigation plays a very important role in quality specification establishment of chemical drugs.

The number of drugs introduced into the market every year .very often there is a time lag from the date of introduction of a drug into the market to the date of its inclusion in pharmacopoeias. Hence, standards and analytical procedures for these drugs may not be available in the pharmacopoeias. It becomes necessary, therefore to develop newer analytical methods for such drugs. Aim is to develop new RP HPLC method for the estimation of Sildenafil citrate in pharmaceutical dosage form.

MATERIALS AND METHODS

Preparation of standard stock solution of Sildenafil citrate

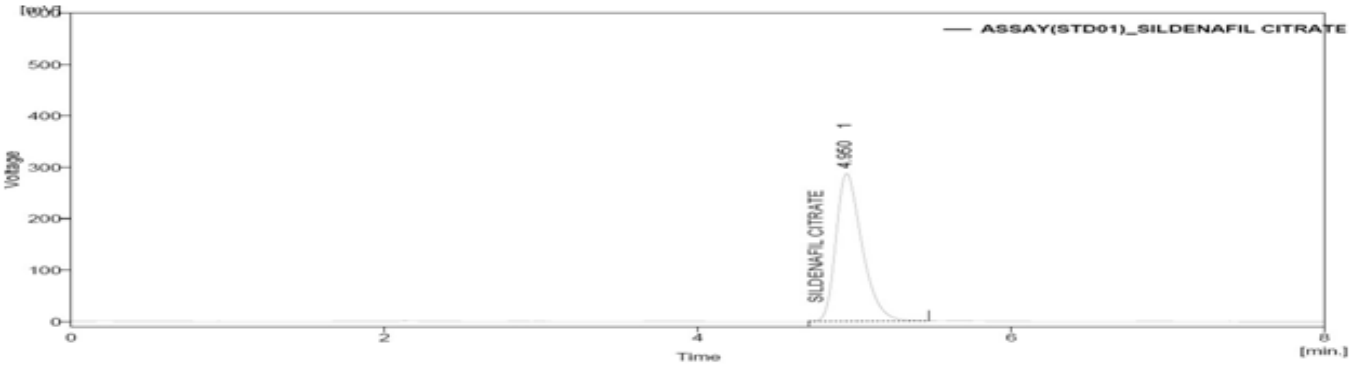
10mg of sildenafil citrate was weighed and transferred in to 10ml volumetric flask and dissolved in methanol and then make up to the mark with methanol and prepare 10 µg /ml of solution by diluting 1ml to 10ml with methanol (3-5).

Optimized chromatographic conditions

Optimized chromatographic conditions is given in table-1 and fig-1.

Table-1 Optimized chromatographic conditions

Mobile phase	Triethylamine buffer : Acetonitrile
Ph	3.5
Column	INERTSIL column,C18(150x4.6 ID) 5µm
Flow rate	1.0 ml/min
Column temperature	Room temperature(20-25^{O}C)
Sample temperature	Room temperature(20-25^{O}C)
Wavelength	290 nm
Injection volume	20 µl
Run time	8min
Retention time	About 4.9 min for Sildenafil citrate



Result Table (Uncal - ASSAY(STD01)_SILDENAFIL CITRATE

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	4.950	3323.905	287.434	100.0	100.0	0.18
	Total	3323.905	287.434	100.0	100.0	

Column Performance Table (From 50% - ASSAY(STD01)_SILDENAFIL CITRATE

	Reten. Time	W05 [min]	Asymmetry [-]	Capacity [-]	Efficiency [th.pl]	Eff/l [t.p./m]	Resolution [-]
1	4.950	0.177	1.667	0.00	4349	86984	-

Fig-1 Chromatogram of Assay standard preparation

RESULTS AND DISCUSSION

The wavelength of maximum absorption (λ_{max}) of the drug, 10 µg/ml solution of the drugs in methanol were scanned using UV-Visible spectrophotometer within the wavelength region of 200–400 nm against methanol as

blank. The resulting spectra are shown in the fig. no. 8.1 and the absorption curve shows characteristic absorption maxima at 290 nm for sildenafil citrate.

The amount of SILDENAFIL CITRATE present in the taken dosage form was found to be 95.96% (Table-1).

Table-1 Assay Results

Sildenafil citrate		
	Standard Area	**Sample Area**
Injection-1	3323.905	3315.153
Injection-2	3320.771	2958.634
Injection-3	3293.678	3099.478
Injection-4	3274.549	3304.543
Injection-5	3193.689	3067.163
Average Area	3281.318	3148.994
Assay(%purity)	**95.96%**	

The % RSD for the retention times and peak area of sildenafil citrate were 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. The correlation coefficient for linear curve obtained between concentrations vs. Area for standard preparations of sildenafil citrate is 0.994. The relationship between the concentration of sildenafil citrate and area of sildenafil citrate is linear in the range examined since all points lie in a straight line and the correlation coefficient is well within limits (Table-2 and fig-2). The percentage mean recovery of sildenafil citrate is 98.40%.

Table-2 linearity of sildenafil citrate

S.No.	**Conc.(µg/ml)**	**Area**
1	2.5	1904.438
2	3.75	3061.665
3	5	3680.717
4	6.25	4770.500
5	7.5	5220.440

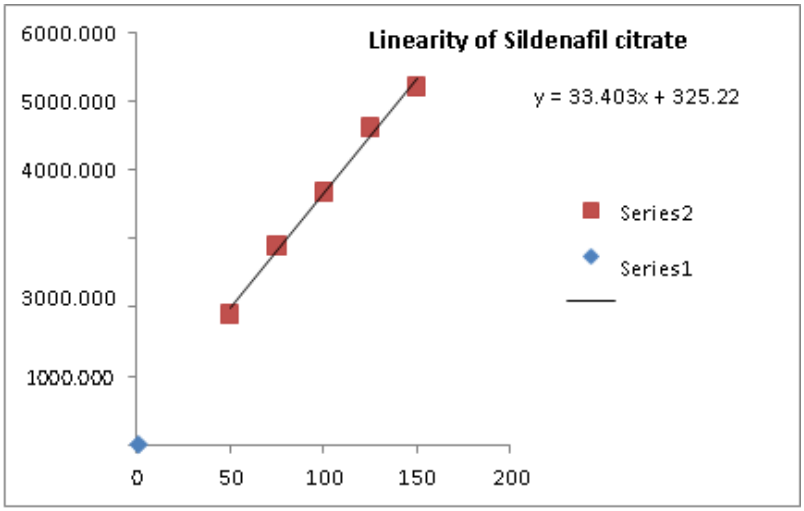


Fig-2 linearity graph of sildenafil citrate

Test results for sildenafil citrate are showing that the %RSD of Assay results are within limits. The results were shown in table-3

Table-3 Results for Method precision of sildenafil citrate

Sildenafil citrate		
S.No.	Rt	Area
1	4.987	3059.295
2	4.973	3091.558
3	4.950	3314.065
4	4.983	3293.678
5	4.987	3067.163
6	4.980	3315.153
avg	4.9767	3290.152
stdev	0.0141	23.203
%RSD	**0.28**	**0.70**

CONCLUSION

From the above experimental results and parameters it was concluded that, this newly developed method for the estimation of Sildenafil citrate was 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 industries, approved testing laboratories, bio-pharmaceutical and bio-equivalence studies and in clinical pharmacokinetic studies in near future

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