AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Determination of Sildenafil Citrate In Pharmaceutical Dosage Forms by Reverse Phase High Performance Liquid Chromatography Method

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ABSTRACT

The main aim of the present study was to develop and validate a simple, rapid and sensitive assay method for sildenafil citrate in pharmaceutical dosage forms by reverse phase high performance liquid chromatography method. Determination Of Sildenafil Citrate was determined by reverse phase HPLC using Potassium dihydrogen phosphate Buffer (pH 7.0):ACN(45:55) and column Inertsil ODS-3V 5µ (150*4.6mm) as a stationary phase and the chromatogram of Sildenafil Citrate has shown in figure 10 and peak was observed at 228 nm which was selected as a wavelength for quantitative estimation. After development of the method, it was validated for system suitability, specificity, linearity, precision, accuracy, robustness and solution stability studies. The results system suitability was found to be within the limits. The limit were not more than RSD <2%. Precision RSD was found to be 0.43 for Sildenafil Citrate. The precision was found to be within the limits. The limit were not more than RSD <2%. Precision RSD was found to be 0.3 for Sildenafil Citrate. The accuracy was found that recovery value of pure drug from the reanalyzed solution of formulation were between 98.0 % to 102% which indicates that the method is accurate and also reveals that commonly used excipients. Theoretical plate for Sildenafil Citrate was found to be not less than 4500. Retention time was nearer to 5.0. In Robustness parameter in both conditions the R.S.D. was less than 2%. (-10 and +10) Hence this method was better for pharmaceutical formulations Analysis. In Solution Stability parameter the R.S.D. was less than 2%. Both Standard and Sample solution was stable up to 48 Hrs.

Keywords: Sildenafil Citrate, system suitability, specificity.

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Received 14 September 2017, Accepted 25 September 2017

Please cite this article as: Kumar M *et al*., Determination of Sildenafil Citrate In Pharmaceutical Dosage Forms by Reverse Phase High Performance Liquid Chromatography Method. American Journal of PharmTech Research 2017.

INTRODUCTION

Analytical chemistry may be defined as the science and art of determining the composition of material in terms of elements or compounds contained in it. Analytical chemistry is divided into two branches quantitative and qualitative. A qualitative method is the information about the identity of atomic or molecular species or functional groups in sample[1]. A quantitative method provides numerical information as to the relative amount of one or more of these components. Drug analysis reveals identification characterization & determination of the drugs in mixtures like dosage forms & biological fluids[2]. The number of drugs introduced in to the market has been increasing at very fast rate. These drugs may be either new entities in the market or partial structural modification of the existing drugs. The typical HPLC separation is based on the selective distribution of analyses between a liquid mobile phase and an immiscible stationary phase. The sample is first introduced by means of an injection port into the mobile phase stream that is delivered by a high-pressure pump. Next, the components of this sample mixture are separated on the column, a process monitored with a flow-through detector as the isolated components emerge from the column[3]. Each researcher has performed to develop a high performance Liquid Chromatography (HPLC) Method for the separation and analysis of new drug molecule either alone or in combination. Thus, the Analytical method development and validation is important in the Pharmaceutical industry[4-5]. To developed simple, rapid, specific and sensitive chromatographic method for determination of Sildenafil Citrate for routine quality control analysis. The developed method is validated according to ICH and USP Guidelines. The developed method used for assay and stability of drug combination. There is isocratic program throughout the chromatographic RP-HPLC method. Analysis of the developed method for their accuracy, precision and reproducibility.

MATERIALS AND METHOD

Materials:

Sildenafil Citrate[6-7] was gifted by Micro Laboratory (Bangalore, India), Potassium dihydrogen phosphate was gifted by Merck Chemicals(Bangalore, India), Sodium Hydroxide was gifted by SD Fine Chemicals(Bangalore, India), Pot. Phosphate monobasic, Acetonitrile, Methanol, Triethylamine, Ammonium Acetate, Ortho phosphoric Acid and R.O. Water was gifted by Merck Chemicals (Bangalore, India).

Assay Method Development for the Sildenafil Citrate by RP- HPLC

Initialization of The Instrument

First the column was placed on the instrument and switch on the instrument and washed with R.O. water for 30 min. Then run the mobile phase for 30 min for column saturation.

Chromatographic condition 1

Table 1

Parameters	Description
Column name	Spherisorb ODS 5µ (150*4.6mm)
Mobile phase	Buffer:ACN (60:40)
Flow rate	1.0ml/min
Detection	UV at 225nm
Temperature	25^0C
Injection volume	10µl
Run time	10 min

Filled the standard solution of Sildenafil Citrate in the syringe, set the HPLC as per Table 1 and saved the method and run the sequence for 10min.

Chromatographic Condition-2

In this chromatographic condition mobile phase was changed.

Table 2

Parameters	Description
Column name	Inertsil ODS-3 5µ (250*4.6mm)
Mobile phase	Buffer: ACN (60:40)
Flow rate	1.0ml/min
Detection	UV at 225nm
Temperature	25^0 C
Injection volume	10µl
Run time	10 min

Filled the standard solution of Sildenafil Citrate in the syringe, set the HPLC as per Table 2 and saved the method and run the sequence for 10min.

Chromatographic condition 3

Table 3

Parameters	Description
Column name	Bond pack C-18 10u (300*3.9mm)
Mobile phase	Buffer:ACN (50:50)
Flow rate	1.0ml/min
Detection	UV at 225nm
Temperature	25^0 C
Injection volume	10µl
Run time	10 min

Filled the standard solution of Sildenafil Citrate in the syringe set the HPLC as per Table 3 and run the sequence for 10 min.

Chromatographic condition-4

Table 4

Parameters	Description
Column name	Symmetry C-18 5µ (250*4.6mm)
mobile phase	Buffer: ACN(55:45)
Flow rate	1.0ml/min
Detection	UV at 225nm
Temperature	25^0 C
Injection volume	10µl
Run time	10 min

Filled the standard solution of Sildenafil Citrate in the syringe set the HPLC as per Table 4

And run the sequence for 10min.

Chromatographic condition5

Table 5

Parameters	Description
Column name	Inertsil ODS-3 5µ (250*4.6mm)
mobile phase	Buffer: ACN(55:45)
Flow rate	1.0ml/min
Detection	UV at 228nm
Temperature	25^0 C
Injection volume	20µl
Run time	10 min

Filled the standard solution of Sildenafil Citrate in the syringe set the HPLC as per Table 5 and run the sequence for 10 min.

DEVELOPED METHOD

Preparation of Buffer

Dissolve 6.8 gm of Potassium dihydrogen phosphate in 1000ml water & adjust to pH 7.0 with sodium hydroxide solution.

Preparation of mobile phase

Here mobile phase was prepared as Buffer: Acetonitrile in the ratio of 45:55 v/v. Filter through 0.45u-membrane filter paper then sonicated for 2-3 min for degassing the air from mobile phase.

Preparation of Diluent

Here diluent was prepared as Buffer: Acetonitrile in the ratio of 60:40 v/v. Filters through 0.45u-membrane filter paper, then sonicated for 2-3 min for degassing the air from mobile phase.

Standard preparation of Sildenafil Citrate

Accurately weighed & transfer about 140 mg of Sildenafil Citrate working standard into 100.0 ml volumetric flask, add to it about 70 ml of diluent & sonicate to dissolve, dilute up to the mark with

diluent & mix well. Filter the solution through 0.45u-membrane filter. Discard first few ml of the filtrate.

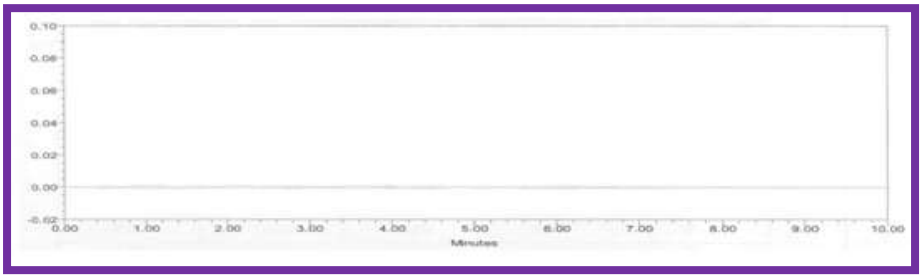
Chromatographic Condition for Developed Method.

Table 6

Parameters	Description
Column name	Inertsil ODS-3V 5µ (150*4.6mm)
mobile phase	Buffer: ACN(45:55)
Flow rate	1.0ml/min
Detection	UV at 228nm
Temperature	25^0 C
Injection volume	10µl
Run time	10 min

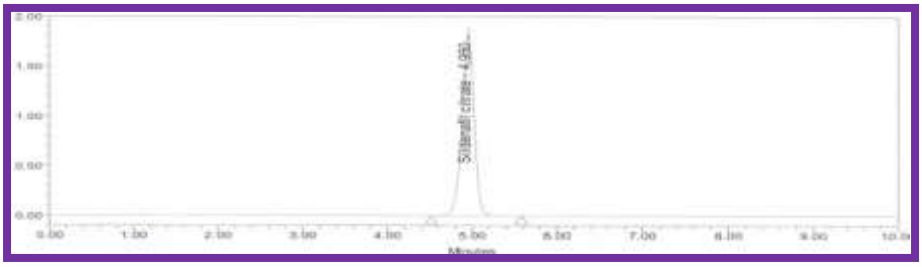
Filled the standard solution of Sildenafil Citrate in the syringe set the HPLC as per Table 6 and run the sequence for 10 min. The chromatogram obtained was shown below fig 1 and 2.

Figure 1 for Mobile Phase:-



Observation: No Peak was found in the mobile phase.

Figure 2 for Sample Solution:-



Observation: Sildenafil Citrate peak at 4.9 RT were obtained.

VALIDATION OF DEVELOPED METHOD[8-9]

The developed method was validated according to ICH and USP guidelines.

Analytical method validation of Sildenafil Citrate

Prepared the mobile phase diluent and arrange chromatographic conditions as per the developed method.

Validation Parameters

1. System Suitability
2. Accuracy
3. Precision
4. Linearity
5. Specificity
6. Robustness
7. Solution Stability

ASSAY OF SILDENAFIL CITRATE TABLET (100 mg) Chromatographic Condition For Assay Method.

Table 7

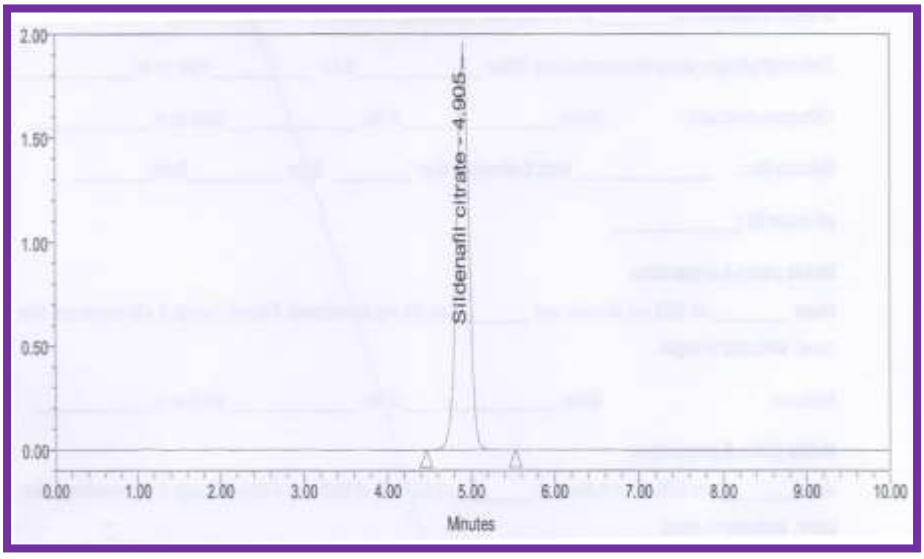
Parameters	Description
Column name	Inertsil ODS-3V 5µ (150*4.6mm)
mobile phase	Buffer:ACN(45:55)
Flow rate	1.0ml/min
Detection	UV at 228nm
Temperature	25^0 C
Injection volume	10µl
Run time	10 min

RESULTS AND DISCUSSION

Results for the analytical method Validation:-

SYSTEM SUITABILITY

Figure 3 for Standard



Observation: Sildenafil Citrate Standard peak at 4.9 RT was obtained.

Table: 8 Data for Area

Std Wt.(mg)	Area	RT	Mean Theoretical plate	Mean Assay
140 mg	20130785	4.9	5025	99.44%
	20110649	4.9	5022	
	20125045	4.9	5062	
	19948043	4.9	5059	
	19977882	4.9	5042	
Mean	20058481	4.9	5042	
SD	88137.51			
%RSD	0.439403			

Results:- In System Suitability Standard the S.D. for Sildenafil Citrate was 88137.51 and the R.S.D. was 0.43% & Mean RT, Theoretical plate and Assay were found to be 4.9, 5042 and 99.44%.

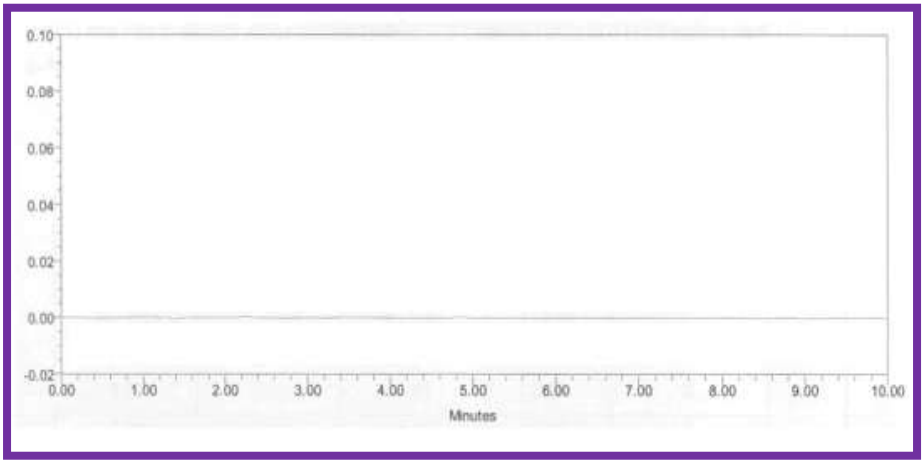
Table: 9 Data for Area

Sample Wt.(mg)	Area	RT	Mean Theoretical plate	Mean Assay
615 mg	20123353	4.9	5022	99.44
	20112701	4.9	5042	
	20126159	4.9	5025	
	20117191	4.9	5062	
	20153222	4.9	5059	
Mean	20126525	4.9	5042	
SD	15820.49			
%RSD	0.078605			

Results:- In System Suitability Sample the S.D. for Sildenafil Citrate was 15820.49 and the R.S.D. was 0.07% & Mean RT, Theoretical plate, Assay were found to be 4.9, 5042, 99.44%.

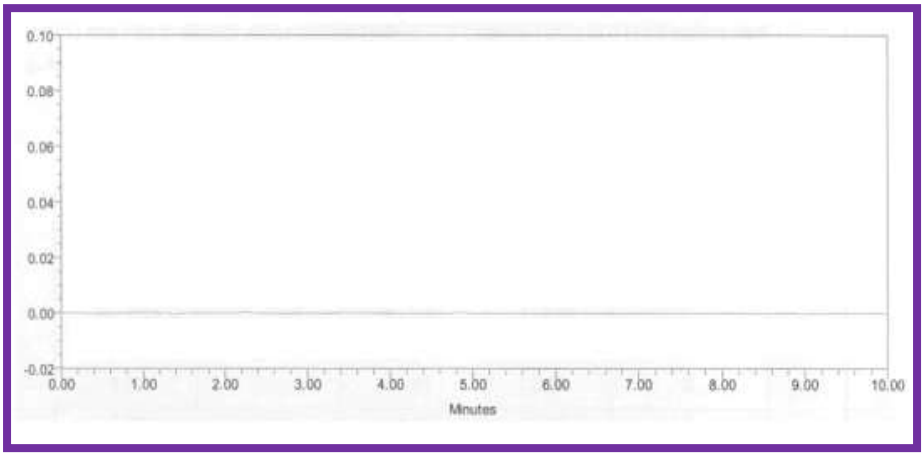
SPECIFICITY

Figure 4 for mobile phase



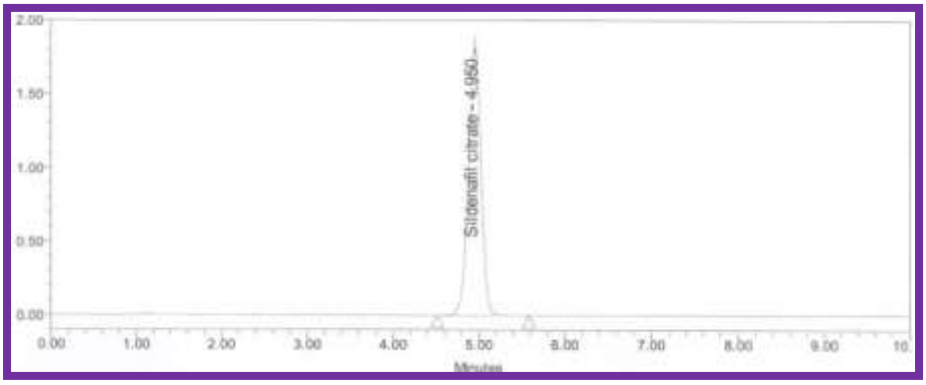
Observation: No Peak was found in the mobile phase.

Figure 5 for Chromatogram for Placebo



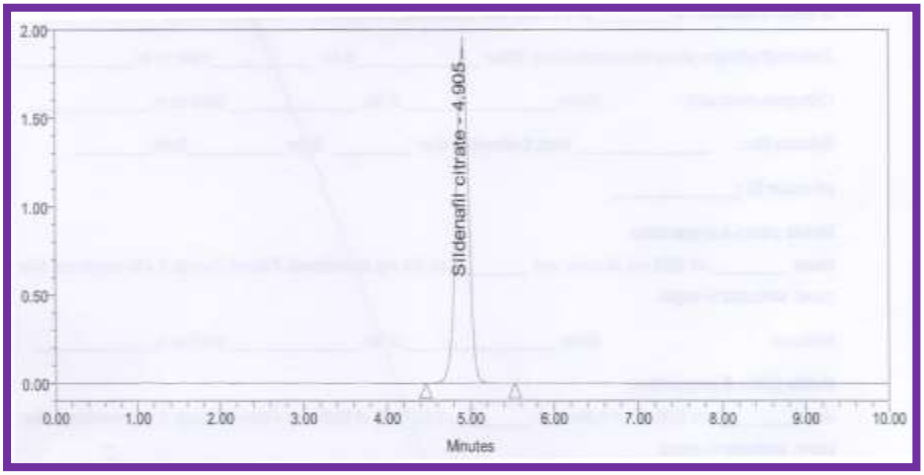
Observation: No Peak was found in the Placebo.

Figure 6 for Sample Solution



Observation: Sildenafil Citrate Sample peak at 4.9 RT were obtained.

Figure 7 for Standard Solution



Observation: Sildenafil Citrate Standard peak at 4.9 RT was obtained.

Table: 10 Data for Specificity

Sr. No.	Ingredient	RT (min.)
1	Mobile Phase	NA
2	Placebo	NA
3	Sildenafil Citrate	4.905
4	Sample	4.950

Results for Specificity: -

According to above Fig. 4 to 10 there was no interference in this method and good separation between all peaks, it means no impurity were interfered.

ACCURACY

Figure 8 for 80% Concentration Solution

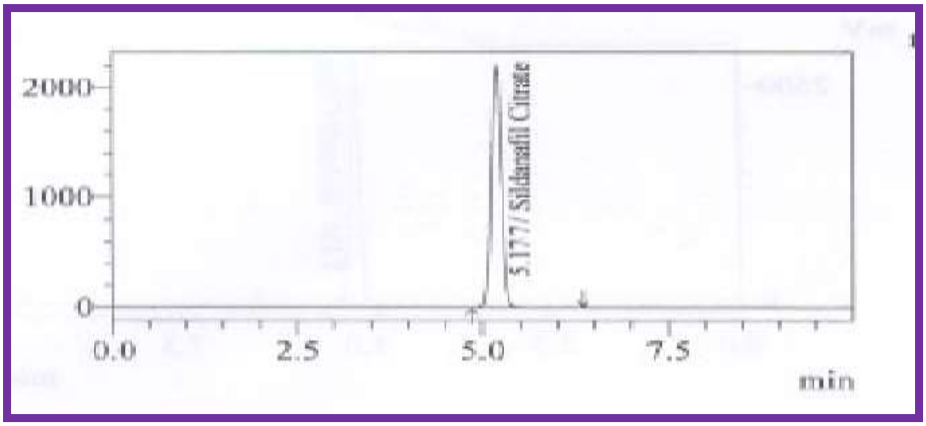


Table: 11 Data for Area

Standard wt. (mg)	Area	RT	Mean Theoretical Plate
140	16235689	5.1	4475
	16358255	5.1	4525
	16125478	5.1	4520
	16357056	5.1	4480
	16456982	5.1	4510
	16598764	5.1	4491
Mean	16355371	5.1	4500
SD	165378.76		
%RSD	1.0111588		

Results:- In Accuracy Standard the S.D. for Sildenafil Citrate was 165378.76 and the R.S.D. was 1.01% & Mean RT &Theoretical plate were found to be 5.1& 4500.

Figure 9 for 100% Concentration Solution

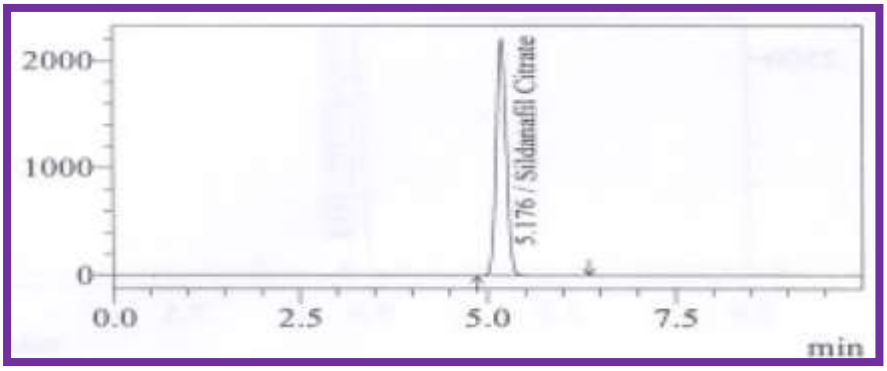


Table: 14 Data for Area

Standard wt. (mg)	Area	RT	Mean Theoretical Plate
140	20419564	5.1	4490
	20363214	5.1	4510
	20359874	5.1	4480
	20428946	5.1	4520
	20384546	5.1	4525
	20391245	5.1	4475
Mean	20385565	5.1	4500
SD	27725.47		
%RSD	0.136005		

Results: - In Accuracy Standard the S.D. for Sildenafil Citrate was 27725.47 and the R.S.D. was 0.13% & Mean RT &Theoretical plate were found to be 5.1& 4500.

Figure 10 for 120% Concentration Solution

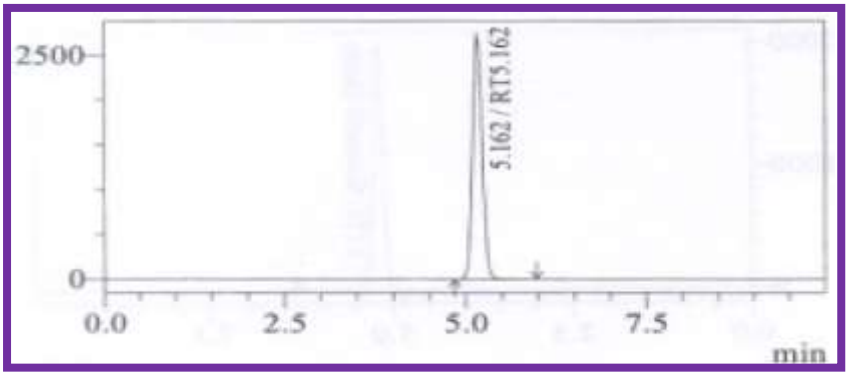


Table: 13 Data for Area

Standard .wt. (mg)	Area	RT	Mean Theoretical Plate
140	25123564	5.1	4550
	25110245	5.1	4450
	25101846	5.1	4475
	25124569	5.1	4525
	25110025	5.1	4480
	24949176	5.1	4520
Mean	25079172	5.1	4500
SD	73128.99		

%RSD	0.291593

Results: In Accuracy Standard the S.D. for Sildenafil Citrate was 73128.99 and the R.S.D. was 0.29 % & Mean RT &Theoretical plate were found to be 5.1& 4500.

Table: 14 Data for Recovery

Level		Wt. taken	Mg Spiked	Avg. Area	% Recovery	Mean Recovery	% RSD
80	1	112.2	79.9	16357656	98.9	98.9	0.3
	2	112.0	79.7	16291230	98.7		
	3	112.3	79.9	16417227	99.2		
100	1	140.1	99.7	20424255	99.0	98.9	0.1
	2	140.0	99.7	20373880	98.8		
	3	140.1	99.7	20380560	98.8		
120	1	168.4	119.9	25124067	101.3	101.2	0.2
	2	168.5	119.9	25110135	101.2		
	3	168.2	119.7	25025511	101.0		

Results: In Sildenafil Citrate Accuracy 80%, 100% & 120% was Recovered 98.9%, 98.9% & 101.2% and the R.S.D. were 0.3%, 0.1% and 0.2%.

PRECISION

Figure 11: Repeatability for 100% Concentration Solution

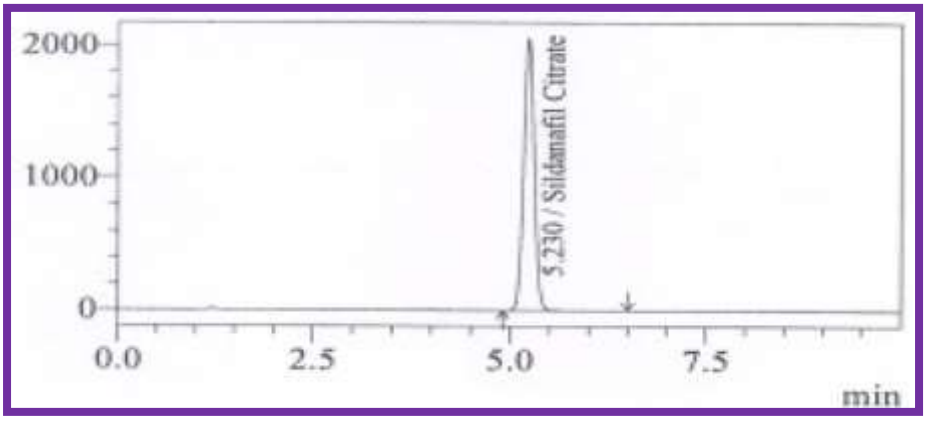


Table: 15 Data for Area

Std. Wt. (mg)	Area	RT	Mean Theoretical Plate
140	18524359	5.2	4575
	18613769	5.2	4425
	18608837	5.2	4550
	18602027	5.2	4450
	18600662	5.2	4500
Mean	18589931	5.2	4500
SD	37036.61		
%RSD	0.199229		

Results: - The S.D. for Sildenafil Citrate was 37036.61 and the R.S.D. was 0.19% and RT, Mean Theoretical plate were 5.2, 4500.

Table: 16 Data for Recovery

Sample	Wt. (mg)	Area	% Assay
615.3	Test1	18308091	98.2
		18330649	
615.4	Test2	18385912	98.6
		18433200	
615.1	Test3	18448119	99.0
		18476227	
Mean		18414821	98.6
SD		57310.333	0.40
%RSD		0.3112185	0.4

Results: The S.D. for Sildenafil Citrate was 57310.333 and the R.S.D. was 0.31% & Precision for 100% concentration solution was 98.6%.

Linearity

Table 17: Data for Linearity

S.no	Concentration %	Avg. Area Sildenafil Citrate
1	80	16049874
2	90	18105725
3	100	20121948
4	110	22141053
5	120	24048489
Correlation Coefficient		0.9998
Slope (m)		40201.39
Intercept(y)		60851.15

Results:-The correlation coefficient of Sildenafil Citrate was found to be = 1.

ROBUSTNESS

Data for Robustness

Change in Flow Rate Parameter

Table 18

-10 (0.9 ml)			+10 (1.1 ml)		
Std Wt.(mg)	**Area**	**RT**	**Std Wt.(mg)**	**Area**	**RT**
140 mg	20729847	5.4	140 mg	17106826	4.5
	20698661	5.4		17355468	4.5
	20714788	5.4		17067815	4.5
	20814637	5.4		17091249	4.5
	20756851	5.4		17105408	4.5
Mean	20742957	5.4	**Mean**	17145353	4.5
SD	45438.33		**SD**	118498.6	
%RSD	0.219054		**%RSD**	0.691141	

Observation: In above parameter in both conditions the R.S.D. was less than 2%.

Change in Column Temperature Parameter

Table 19

-10 (20^0C)			+10 (30^0C)		
Std Wt.(mg)	**Area**	**RT**	**Std Wt.(mg)**	**Area**	**RT**
140 mg	18950736	4.7	140 mg	18837701	4.9
	18939647	4.7		18769751	4.9
	18774019	4.7		18789206	4.9
	18876910	4.7		18831098	4.9
	18832938	4.7		18853922	4.9
Mean	18874850	4.7	**Mean**	18816336	4.9
SD	73968.04		**SD**	35331.59	
%RSD	0.391887		**%RSD**	0.187771	

Observation: In above parameter in both conditions the R.S.D. was less than 2%.

Change in Wavelength Parameter

Table 20

-10 (223 nm)			+10 (233 nm)		
Std Wt.(mg)	**Area**	**RT**	**Std Wt.(mg)**	**Area**	**RT**
140 mg	19493271	4.9	140 mg	17296784	4.9
	19479668	4.9		17320451	4.9
	19513508	4.9		17350885	4.9
	19520318	4.9		17314120	4.9
	19587546	4.9		17330178	4.9
Mean	19518862	4.9	**Mean**	17322484	
SD	41651.37		**SD**	20002.46	4.9
%RSD	0.21339		**%RSD**	0.115471	

Observation: In above parameter in both conditions the R.S.D. was less than 2%.

Change in Mobile Phase Parameter

Table 21

-10 (pH 6.8)			+10 (pH 7.2)		
Std Wt.(mg)	**Area**	**RT**	**Std Wt.(mg)**	**Area**	**RT**
140 mg	18869812	4.8	140 mg	19037662	4.9
	18921266	4.8		19150632	4.8
	18959486	4.8		19138337	4.9
	18966704	4.8		19130030	4.9
	18917142	4.8		19084371	4.8
Mean	18926882	4.8	**Mean**	19108206	4.85
SD	38833.63		**SD**	46713.74	
%RSD	0.205177		**%RSD**	0.24447	

Observation: In above parameter in both conditions the R.S.D. was less than 2%.

Solution Stability:-

Sample & Standard Stability after 48 Hrs.

Table 22

Std Wt.(mg)	Area	RT	Sample Wt.(mg)	Area	RT
140 mg	20121449	5.1	140 mg	20513587	5.1
	20205106	5.1		20545559	5.1
	20314515	5.1		20574251	5.1
	20532685	5.1		20684730	5.1
	21110264	5.1		21110264	5.1
Mean	20456804	5.1	**Mean**	20685678	5.1
SD	396493.7		**SD**	245936.4	
%RSD	1.938199		**%RSD**	1.188921	

Observation: In above parameter the R.S.D. was less than 2%.

ASSAY OF SILDENAFIL CITRATE Tablet (100 mg)

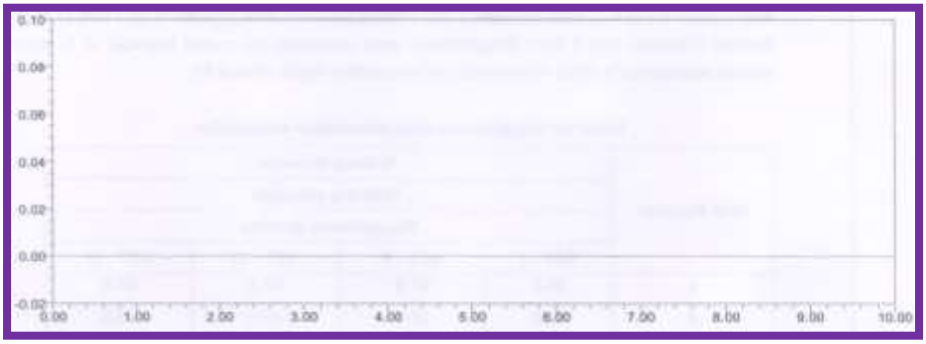
Table 23 Data for Area

Inj. No.	Area	RT	Theoretical Plate	Tailing Factor
1	17589792	4.886	6196	1.118
2	17584115	4.885	6193	1.118
3	17573361	4.886	6200	1.118
4	17571976	4.890	6204	1.119
5	17567825	4.894	6216	1.118
6	17563224	4.898	6245	1.117
Average	17572100	4.8906	6211.6	1.118
SD	7794.625	0.005459	20.45238	0.000707
%RSD	0.044358	0.111621	0.329261	0.063247

Table 24 Data for Recovery

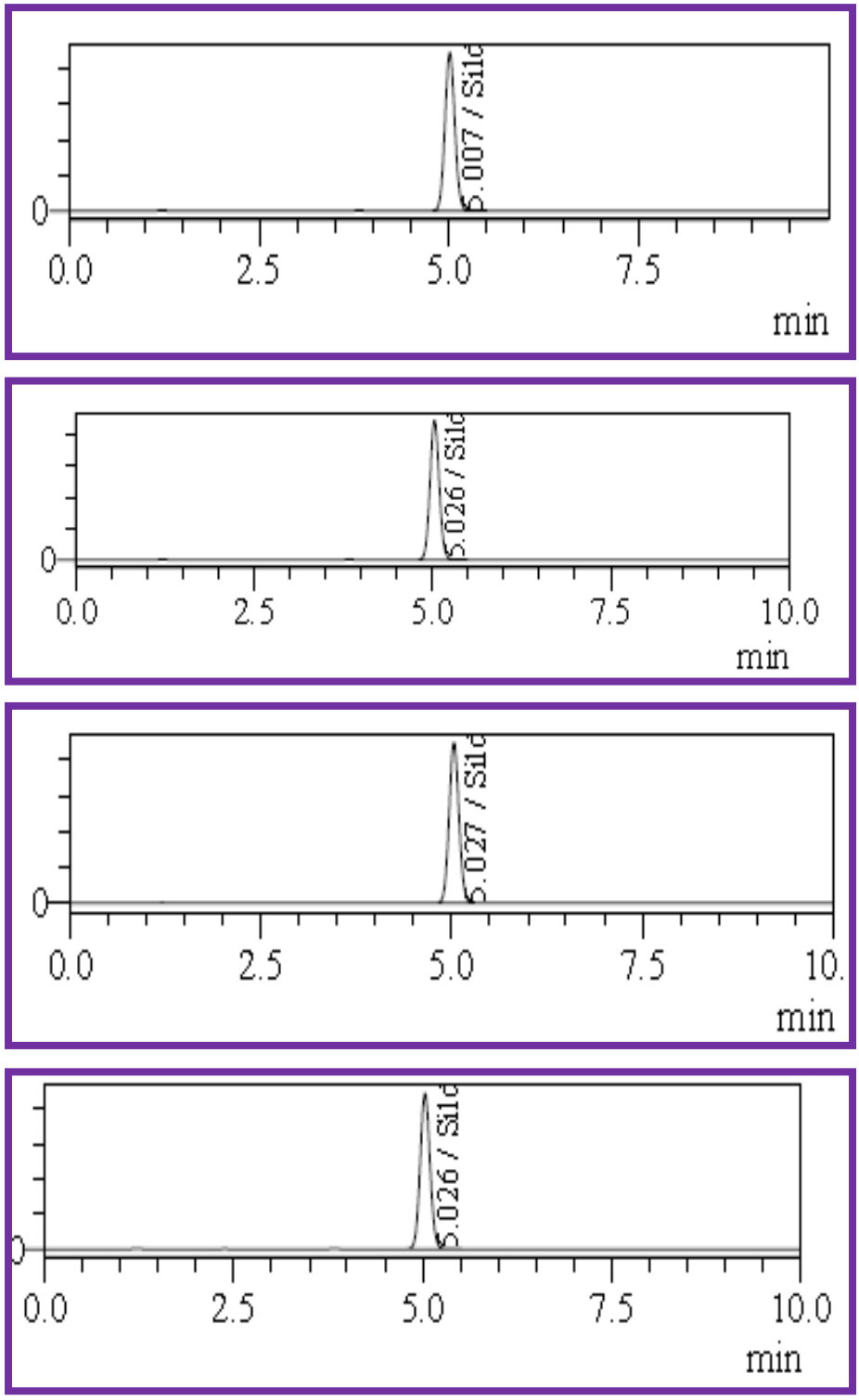
Inj. No.	Area	RT	Theoretical Plate	Tailing Factor
1	17460047	4.917	6301	1.117
2	17444308	4.915	6301	1.117
3	17534701	4.924	6310	1.119
4	17530550	4.926	6321	1.118
Average	17492402	4.9205	6308.25	1.11775
SD	46919.63	0.005323	9.5	0.000957
%RSD	0.268229	0.108178	0.150596	0.085657
% Assay	99.54645%			

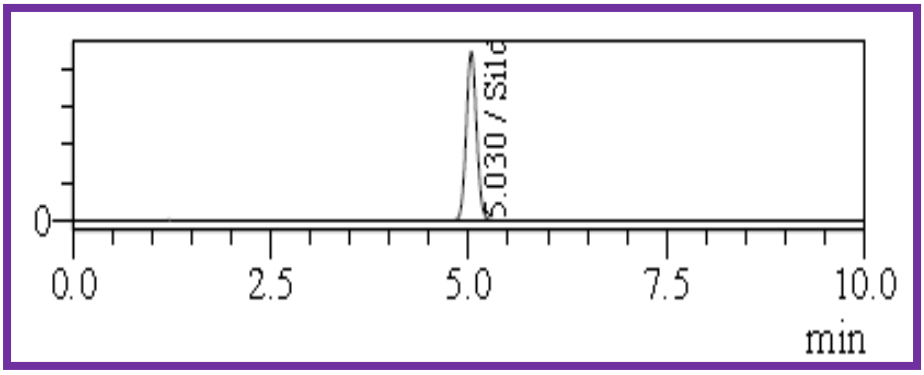
Figure 12 Blank For Assay



Observation: No Peak was found in the mobile phase.

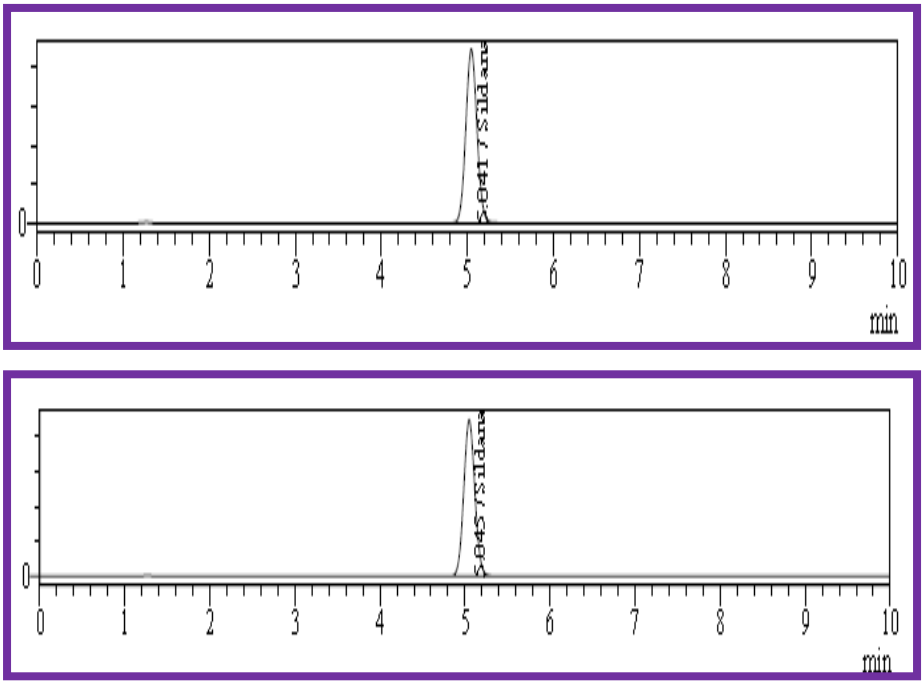
Figure 13 Standards for Assay





Observation: Standard peaks was separated within 10 min, Sildenafil Citrate peak at 5.0 RT was obtained.

Figure 14 Samples for Assay



Observation: Sample peaks was separated within 10 min, Sildenafil Citrate peak at 5.0 RT was obtained.

Results: -In the Standard of Sildenafil Citrate S.D. ,% R.S.D.,RT, Theoretical plate and Tailing Factor were 7794.62, 0.04, 4.89, 6211 and 1.1.

In the Sample of Sildenafil Citrate S.D. ,% R.S.D.,RT, Theoretical plate and Tailing Factor were 46919.63, 0.26, 4.9, 6308 and 1.1.

Assay of Sildenafil Citrate 100 mg Tablet was found to be 99.54%.

SUMMARY & CONCLUSION

On the basis of the experiments, we can conclude that the RP-HPLC method developed for the Determination of Sildenafil Citrate can be used for routine analysis.

Determination Of Sildenafil Citrate was determined by reverse phase HPLC using Potassium dihydrogen phosphate Buffer (pH 7.0):ACN(45:55) and column Inertsil ODS-3V 5µ (150×4.6mm) as a stationary phase and the chromatogram of Sildenafil Citrate has shown in fig. 2 and peak was observed at 228 nm which was selected as a wavelength for quantitative estimation. After development of the method, it was validated for system suitability, specificity, linearity, precision, accuracy, robustness and solution stability studies.

The system suitability was found to be within the limits. The limit were not more than RSD <2%. Precision RSD was found to be 0.43 for Sildenafil Citrate. This indicates that the method Suitable. The chromatograms for precision are shown in figure 3 and the data regarding the precision are shown in table 8 to 9.

The Specificity of Sildenafil Citrate Figure 4 to 7 there was no interference in this method and good separation between all peaks; it means no impurity was interfered.

The precision was found to be within the limits. The limit were not more than RSD <2%. Precision RSD was found to be 0.3 for Sildenafil Citrate. This indicates that the method is precise and accurate. The chromatograms for precision are shown in figure 11 and the data regarding the precision are shown in table 15 to 16.

From the linearity table, it was found that, the drug obeys beer's law and from the linearity studies the specified range for Sildenafil Citrate was found to be 80% to 120%.

From the results shown in the accuracy table, it was found that recovery value of pure drug from the reanalyzed solution of formulation were between 98.0 % to 102% which indicates that the method is accurate and also reveals that commonly used excipients and additives present in the pharmaceutical formulations were not interfering in the proposed methods. Theoretical plate for Sildenafil Citrate was found to be not less than 4500. Retention time was nearer to 5.0.

In Robustness parameter in both conditions the R.S.D. was less than 2%. (-10 and +10) Hence this method was better for pharmaceutical formulations Analysis.

In Solution Stability parameter the R.S.D. was less than 2%. Both Standard and Sample solution was stable up to 48 Hrs.

ACKNOWLEDGEMENTS:

Authors are thankful to Vinayaka Mission's College of Pharmacy, Salem, Tamilnadu and providing all the facilities for this research project.

REFERENCES:

1. Skoog, West and Holler, Fundamental of Analytical Chemistry**,** Saunders College,

Publishing, 7thEdn, 1992**,** pp.1

2. Corners' K. A., Textbook of Pharmaceutical Analysis, A Wiley Interscience Publication, 1st Edn 1967, pp.475-78
3. Beckett A.H. and Stanlake J.B., Practical Pharmaceutical Chemistry, Part 2, CBS Publishers and Distributors**,** 4th Edn, 2002**,** pp.157-174
4. Remington, The Science & Practice of a Pharmacy Vol. I, 20th Edn. pp 587-613
5. Snyder L.R., Kirkland J.J. and Glajch L.J., Practical HPLC Method Development, John
6. www.Drugbank.com
7. www.Wickipedia.com
8. Textbook of Pharmaceutical process validation 11nd edition Marcel Decker Inc. New York, Page no.10
9. C. Burgess, In; Valid Analytical methods and procedures Pub, Royals Society of Chemistry, 2002, Page no.20-37.