The Pharma Innovation

ISSN: 2277- 7695
CODEN Code: PIHNBQ
ZDB-Number: 2663038-2
IC Journal No: 7725

Received: 13-03-2013
Accepted: 11-04-2013

Vol. 2 No. 4 2013
Online Available at www.thepharmajournal.com

THE PHARMA INNOVATION - JOURNAL

Validated Spectrophotometric Methods for Simultaneous Estimation of Sildenafil Citrate and Dapoxetine Hcl in Tablet Dosage Form

Albin Pt[*1], Y Haribabu[1], Sosamma Cicy Eapen[1], Sheeja Velayudhan Kutty[1], Kumar P[1], Nithyamol P[2]

1. Department of Pharmaceutical Analysis, Grace College of Pharmacy, Kodunthirapully, Palakkad, India. [E-mail: aln.albin@gmail.com; Tele: +91 9744887787]
2. Department of Pharmaceutical Chemistry, Medical College, Trivandrum, India

Two simple UV-Spectrophotometric methods have been developed for simultaneous determination of sildenafil citrate and dapoxetineHClin pharmaceutical formulation. For both the methods stock solutions were prepared in methanol followed by the further required dilutions with methanol. Proposed Vierodt's method and double point standardization method, the λmax for the estimation of sildenafil citrate and dapoxetineHCl were selected at 292nm and 231nm respectively. In both methods, the linearity range lies between 2-20µg/mL for sildenafil citrate and 1.2-12µg/mL for dapoxetineHCl at their respective wavelengths. By Vierodt's method the percentage of sildenafil citrate and dapoxetineHCl was found to be 99.25%, dapoxetineHCl 99.08% respectively.It was estimated to be 98.1% for sildenafil Citrate and 99.16% for DdapoxetineHCl by double point standardization method. Both these methods were found to be accurate, precise, stable and robust as indicated by low values of % RSD. Thus the present study gives excellent method for the determination of both the drugs in combined tablet formulation.

*Keyword:*Sildenafil Citrate, DapoxetineHCl, UV Simultaneous Determination, Spectrophotometric Methods.

1. Introduction

Sildenafil citrate (SIL) was patented in1996 and launched in May 1998 as first oral drug approved by Food and Drug Administration (FDA) to treat erectile dysfunction (ED) in the United States. It is also effective for treatment of pulmonary arterial hypertension (PAH).

Sildenafil citrate is a white to off-white crystalline powder with a solubility of 3.5 mg/mL in water and a molecular weight of 666.7 Dalton. Molecular formula is $C_{22}H_{30}N_6O_4S$. .Chemically, designated as 1-[[3-(6, 7-dihydro-1-methyl-7-oxo-3-propyl-1*H*pyrazolo [4, 3-*d*] pyrimidin-5-yl)-4ethoxyphenyl] sulfonyl]-4-methylpiperazine citrate. Its structural formula is given below in the Fig.1.

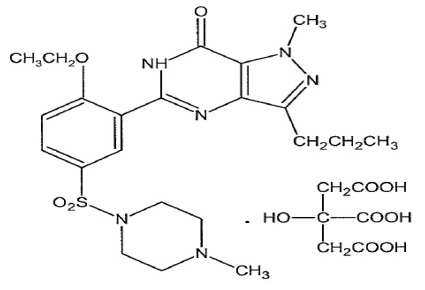

Fig 1.

The parasympathetic nerves are stimulated when man arouses sexually, leading to penile erection

as result of release nitric oxide (NO) which works by activation of the enzyme guanylatecyclase responsible for converting guanosine triphosphate (GTP) to 3'5' cyclic guanosine monophosphate (cGMP)[1].

The cGMP is a potent vasodilator vital erection of the penis. Sildenafil citrate selectively inhibits the enzyme PDE-5A (phosphodiesterase-5A) that hydrolyzes cGMP. Thus it increases level of cGMP by preventing it from breaking down. Consequently smooth muscle relaxation leads to vasodilation and increased inflow of blood into the spongy tissue of the penis causing an erection by fascinating the signaling actions of nitric oxide (NO) in penile smooth muscle. The most common side effects of Sildenafil citrate are headache, facial flushing, and upset stomach.Less commonly cyanopsia (bluish vision), blurred vision, or sensitivity to light may briefly occur[2].

DapoxetineHCl(DAP) is designated chemically as (S)-N, N-dimethyl-3-(naphthalen-1-yloxy)-1 phenylpropan-1-amine with an empirical formula of $C_{21}H_{23}NO$ and molecular weight of 305.413g. This drug is mainly useful in erectile dysfunction as selective serotonin reuptake inhibitor (SSRI)[3]. SSRI's are a class of compounds typically used as antidepressants in the treatment of depression, anxiety disorders, and some personality disorders. They can also sometimes be effective and used in treating premature ejaculation problems, impotence and some cases of insomnia. The drug's mechanism of action is thought to be related to inhibition of neuronal reuptake of serotonin and subsequent potentiation of serotonin activity and increase the ejaculation time[4]. Its structural formula is given below in the Fig.2.

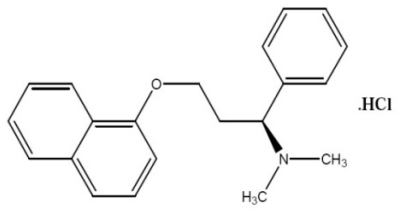

Fig 2.

2. Materials and Methods:

The spectrophotometric measurements were carried out using a Shimadzu double beam UV-Visible Spectrophotometer model 1800 with 1cm matched quartz cell.

2.1 Reagents:

Pure sample of sildenafil and dapoxetine were collected from Emcure pharmaceuticals, Pune. Methanol was used as solvents throughout the experiment. Pharmaceutical preparation was purchased from local industry.

2.2 Preparation of Standard Stock solution

Primary solutions (1000µg/ml) of sildenafil citrate and dapoxetineHCl (600µg/ml) were prepared by accurately weighing 10mg sildenafil citrate and 6mg dapoxetineHCl and diluted to 10ml respectively by methanol. Stock solutions of sildenafil(200µg/ml) and dapoxetine (120µg/ml) were prepared by diluting 2ml primary solutions to 10ml respectively by methanol. A series of standard drug solutions in concentration range of 2-20µg/ml of sildenafil and 1.2-12µg/ml of dapoxetine were prepared by diluting appropriate volumes of standard stock solutions.

Method I: Vierodt's method

This method of analysis was based on the absorption of SIL and DAP at the wavelength maximum of each drugs. Two wavelengths selected for the development of simultaneous equations were 292nm and 231nm which were λ_{max} of SIL and DAP respectively. The absorbances of SIL and DAP were measured at the selected wavelengths. The absorptivity values E (1%, 1cm) were determined for both drugs at the selected wavelengths. These values were mean of six estimations[5].

The concentration of both drugs in mixture can be calculated by using following equations-

$Cx = A1ay2 - A2ay1 / ax1ay2 - ax2ay1$ .....Eq (1)
$Cy = A1ax2 - A2ax1 / ay1ax2 - ay2ax1$ .....Eq.(2)

Where, A1 and A2 are absorbances of mixture at 292 and 231nm respectively
ax1 and ax2 are the absorptivities of SIL at 292 and 231nm respectively
ay1 and ay2 are the absorptivities of DAP at 292 and 231nm respectively
Cx and Cy are the concentrations of SIL and DAP respectively.

Method II: Double point standardization

In double point standardization, the concentration of one of the standard solutions is greater than that of the sample while the other standard solution has a lower concentration than the sample solution[6]. The concentration of the substance in the sample solution is given by

Ctest = [(A test – A std1) (C std1-C std2) +C std1 (A std1-A std2)] / (A std1-A std2).....Eq (3)

Where, C std is the concentration of standard solution
A test and Astd are the absorbance of the sample and standard solutions, respectively
std1 and std2 are the more concentrated standard and less concentrated standard solutions, respectively.

Overlain spectra of SIL and DAP were shown in Figure 3.

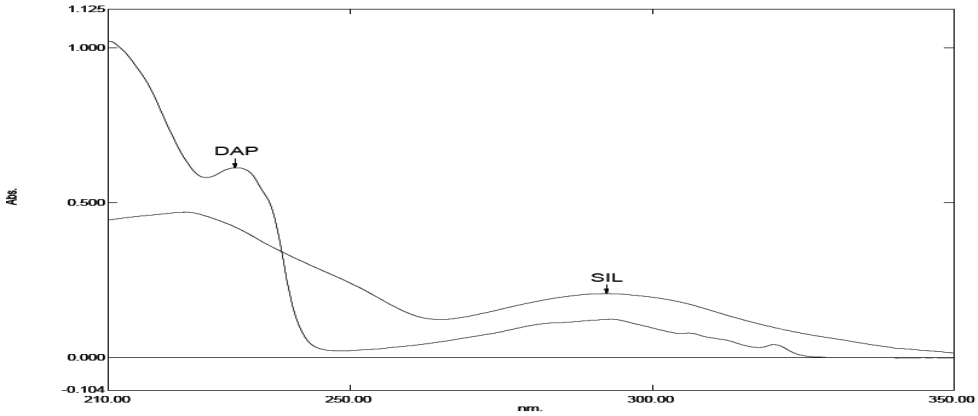

Fig 3: Overlain spectra of SIL and DAP

2.3 Analysis of Tablet Mixture

Twenty tablets were accurately weighed; average weight was determined and finely powdered. An accurately weighed quantity of tablet powder equivalent to 50mg of SIL and 30mg of DAP was transferred to 50ml volumetric flask and dissolved by sonication with sufficient quantity of methanol and then volume was made to the mark with methanol. The solution was then of filtered through Whatmann filter paper no. 41. The filtrate 2ml was taken in 10ml volumetric flask and volume made to the mark with methanol (Test stock solution). A series of different concentration range were prepared by diluting appropriate volumes of test stock solution in methanol. The above mixture was analyzed at 292 and 231nm wavelengths and values of the absorbance were substituted in respective equations (Eqn. 1, 2, and 3) to obtain the content of SIL and DAP respectively. The data of analysis is mentioned in Table 1.

2.4 Validation Parameters

Study of validation parameters like linearity, accuracy, precision, specificity, Limit of Detection (LOD, Limit of Quantization (LOQ) and robustness were carried out as per ICH guidelines (Table 2 to 5).

3. Results and Discussions:

Table 3: Results of Validation parameters

Parameters	Values	
	Sildenafil Citrate	**DapoxetineHCl**
Working λmax	292nm	231nm
Linearity (µg/mL)	2-20	1.2-12
Intercept*	0.0349	0.0109
Slope*	0.0961	0.0198
Correlation coefficient*	0.9957	0.9985
LOD* (µg/mL)	1.5	4
LOQ* (µg/mL)	0.6	1.5
Intra-day* (Precision) (%RSD)	0.1780	0.0045
Inter-day* (Precision) (%RSD)	0.0496	0.0031

*Average of six observations

The Beer- Lambert's concentration range is lies between 2-20µg/mL for sildenafil citrate at

292nm and 1.2-12µg/mL for dapoxetineHCl at 231nm with coefficient of correlation 0.9957 and 0.9985 respectively. The linearity graphs were shown in fig. 4 & 5. Drugs show good regression values at their respective wavelengths and the recovery study reveals that any small change in the drug concentration in the solution could be accurately determined by the proposed methods.

By Vierodt's method the percentage of sildenafil citrate and dapoxetineHCl was found to be 99.25%, dapoxetineHCl 99.08% respectively.It was estimated to be 98.1% for sildenafil Citrate and 99.16% for DdapoxetineHCl by double point standardization method with standard deviations <2.

The validity and reliability of proposed methods are assessed by recovery studies. Sample recoveries for both the methods are in good agreement with their respective label claims, which suggests non-interference of formulation additives in estimation (Table 2).

Precision was determined by studying the intermediate precision and the result indicates the precision under the same operating conditions over a short interval time and inter-assay precision. The standard deviation and % relative standard deviation are calculated for both drugs (Table 1). Intermediate precision study expresses within laboratory variation in different days. In both intra and inter-day precision study for both the methods %RSD are not more than 1.0 indicating good intermediate precision (Table 3). The LOD values are 1.5 and 0.6µg/mL and LOQ values are 4 and 1.5µg/mL for sidenafil citrate and dapoxetineHCl respectively. Low LOD and LOQ indicates good sensitivity of proposed methods.

Table 1: Analysis data of tablet formulation

Method	Tablet Formulation	Labeled Amont (mg)	Estimated amount (mg)	% Estimated*	SD*	%RSD*
I	Sildenafil	50	49.625	99.25	0.023	0.234
	Dapoxetine	30	29.724	99.08	0.000	0.000
II	Sildenafil	50	49.05	98.10	0.004	0.041
	Dapoxetine	30	29.748	99.16	0.004	0.068

SD: Standard deviation RSD: Relative standard deviation,*Average of six observation

Table 2: Result of recovery studies

Recovery Level	% Recovery*		% RSD*	
	Sildenafil	Dapoxetine	Sildenafil	Dapoxetine
80%	99.20	100.14	0.0572	0.1157
100%	100.91	99.33		
120%	99.35	99.72		

RSD: Relative standard deviation,*Average of six observations

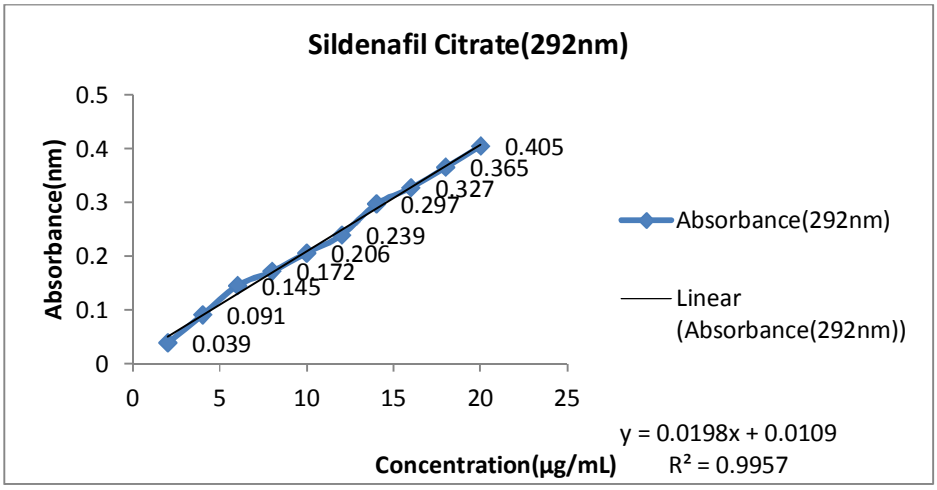

Fig. 4: Calibration curve of of Sildenafil Citrate

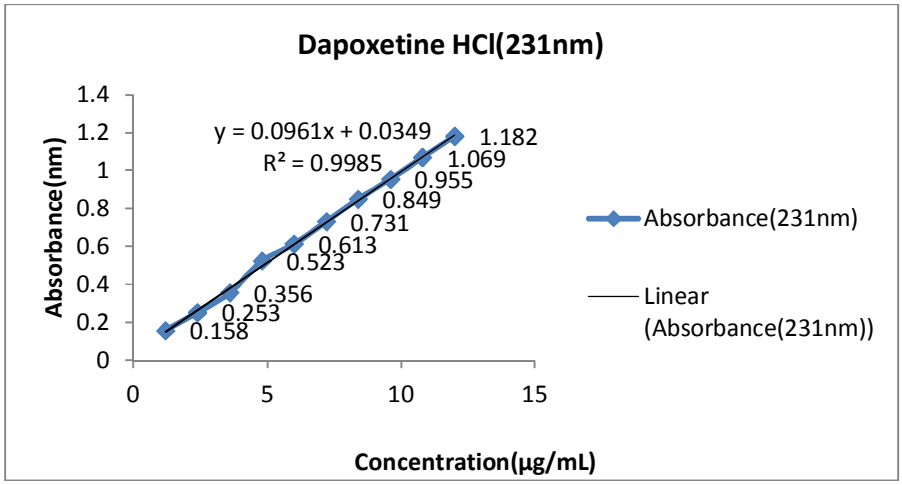

Fig. 5: Calibration curve of ofDapoxetineHCl

Table 4: Results of Specificity Parameters

Method	Specificity Parameters	Tablet Formulation	%Estimated*
I	Lactose	Sildenafil	98.56
		Dapoxetine	98.67
	Starch	Sildenafil	98.56
		Dapoxetine	98.67
II	Lactose	Sildenafil	98.11
		Dapoxetine	99.13
	Starch	Sildenafil	98.11
		Dapoxetine	99.13

*Average of six observations

Table 5: Results of robustness parameters

Method	Robustness Parameters	Tablet Formulation	%Estimated*
I	pH(2.051)	Sildenafil	98.95
		Dapoxetine	98.67
	Temperature 2-8^{0}C	Sildenafil	98.51
		Dapoxetine	98.67
II	pH(2.051)	Sildenafil	99.00
		Dapoxetine	99.28
	Temperature 2-8^{0}C	Sildenafil	98.05
		Dapoxetine	99.28

*Average of six observations

4. Conclusion

Sildenafil Citrate and DapoxetineHCl are available in combined pharmaceutical dosage form for the treatment of premature ejaculation. Here, two simple UV spectrophotometric methods were developed and validated for their simultaneous analysis. The standard deviation and RSD for the methods are low, indicating high degree of precision of the methods. The RSD was found to be less than 2%. The % recovery was between 98-102% indicating high degree of accuracy of the proposed methods. The developed methods are simple, rapid, precise, and accurate and can be employed for the routine estimation of Sildenafil Citrate and DapoxetineHCl.

5. References

1. Pfizer Viagra Product Monograph, 1-47,Submission Control No: 144432 (2009), http://www.pfizer.ca.
2. K.T. MahmoodAnd B. Khan*. A Validated Hplc Method for The Measurement Of Sildenafil Citrate in Different Formulations. Pakistan Journal of Science 192-196(Vol. 62 No. 3 September, 2010).
3. G.Abirami*, K.Anandakumar, R.Velmurugan. Development And Validation of UV-Spectroscopy Method For The Determination of Dapoxetine Hydrochloride in Pharmaceutical Formulation. G.Abirami*et al.* / Journal of Pharmacy Research 1949-1951 (2012,5(4)).
4. Abha D. Giri, Vidhya K. Bhusari, Sunil R. Dhaneshwar*.Validated Hplc Method for Simultaneous Quantitation of Tadalafiland Dapoxetine Hydrochloride in Bulk Drug And Formulation. International Journal of Pharmacy and Pharmaceutical Sciences, Issn- 0975-1491 Vol 4, 654-658 Issue 2, 2(2012).
5. Ramesh L. Sawant *, Manish A. Raskar, Raihan Ahmed and Sameer Pawar, Validated spectrophotometric methods for simultaneous estimation of telmisartan and indapamide in pharmaceutical dosage form, Journal of Chemical Metrology, ISSN 0975-413X, 633-638 (2012).
6. Jinendra M. Sonpetkar*, Deval V. Joshi, Nakul B. Patel and Mansi J. Wagdarikar. Uv-Spectrophotometric Method For Estimation of Secnidazole In Bulk And Tablet Dosage Form. Sonpetkar*et al.*, International Journal of Pharmaceutical Sciences and Research, Vol. 3(2): 536-538 (2012).