WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 5, 213-222. Review Article ISSN 2277– 7105

A MODIFIED UV-SPECTROPHOTOMETRIC METHOD DEVELOPMENT AND VALIDATION FOR THE QUANTITATIVE ESTIMATION OF SILDENAFIL AND FLUOXETINE IN PURE AND MARKETED FORMULATIONS

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Article Received on
23 Feb. 2020,
Revised on 15 March 2020,
Accepted on 05 April 2020
DOI: 10.20959/wjpr20205-17159

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ABSTRACT

A simple, accurate, precise, economical and reproducible UV Spectrophotometric method has been developed for the simultaneous estimation of Sildenafil and Fluoxetine in bulk and in combined tablet dosage form. The stock solutions were prepared in distilled water. This method involves the formation and solving of simultaneous equations at 228 nm and 216 nm, as absorbance maxima of Sildenafil and Fluoxetine, respectively. Beer's law obeyed the concentration range of 2-10mcg/mL for Sildenafil and Fluoxetine. The results of analysis were validated statistically and by recovery studies. The % RSD for the recovery study was less than 2. The proposed method can be effectively applied for the simultaneous estimation of these three drugs in bulk and in combined tablet dosage form.

KEYWORDS: Sildenafil and Fluoxetine, Method development and validation.

Preparation of Standard Stock Solution

25 mg each of standard Sildenafil Citrate and Fluoxetine Hydrochloride were weighed accurately and transferred in to two separate 25ml volumetric flasks, dissolved in 5ml of solvent and made up to the mark with methanol to obtain a final concentration of 1000 µg/ml of each Sildenafil Citrate and Fluoxetine Hydrochloride (standard stock solutions A1 and A2 respectively). From the above stock solution 'A1' and 'A2' 1 ml aliquots were pipetted in to

two separate volumetric flasks and dissolved in 5ml of solvent and made up to the mark with distilled water to obtain a final concentration of 100µg/ml. (Standard stock solutions 'B1' and 'B2' respectively).

Selection of Analytical Wavelengths

Appropriate dilution of the standard stock solutions 'A1' and 'A2' were scanned separately in the entire ultraviolet range. The λmax of each standard was selected in such a way that at each absorption maxima the difference in absorption of the two components should be as large as possible. The two wavelengths were 228nm and 216nm for Sildenafil Citrate and Fluoxetine Hydrochloride respectively. At 228nm Sildenafil Citrate has higher absorbance than Fluoxetine Hydrochloride and at 216 Fluoxetine Hydrochloride has higher absorbance than Sildenafil Citrate which were shown in figure.

Selection of Analytical Concentration Range and Construction of Calibration Graph

Sildenafil Citrate: Appropriate aliquots ranging from 0.2 ml to 1ml (1ml=100 µg/ml) was pipetted out in to a series of 10ml volumetric flasks. The volume was made up to the mark with distilled water to obtain a concentration range, ranging from 2-10µg/ml (2,4,6,8,10µg/ml). Absorbance of the above solutions was measured at 228 nm and a calibration curve of absorbance against concentration was plotted.

Fluoxetine Hydrochloride: Appropriate aliquots ranging from 0.2 ml to 1ml (1ml=100 µg/ml) was pipetted out in to a series of 10ml volumetric flasks. The volume was made up to the mark with distilled water to obtain a concentration range, ranging from 2-10µg/ml (2,4,6,8,10 µg/ml). Absorbance of the above solutions was measured at 216 nm and a calibration curve of absorbance against concentration was plotted.

Both drugs follow Beer-Lambert's law in the concentration range of 2-10µg/ml. Regression equation was established and the correlation coefficient was determined. The results were given in table and calibration curves of both the drugs were shown in figure…

B. Analysis of Tablet Formulation

Twenty tablets of Sildenafil Citrate and Fluoxetine Hydrochloride combination dosage forms were weighed and their average weight was determined. The tablets were crushed in to fine powder. From the tablet triturate a tablet mass equivalent to 10mg of Sildenafil Citrate or 5mg of Fluoxetine Hydrochloride was transferred in to a 10ml volumetric flask, dissolved in

a small quantity of methanol by sonication for 10min and finally the volume was made up to the mark with methanol. The resultant solution was filtered through a Whatmann filter paper no. 41 and used as sample stock solution 'A' (1000µg/ml Sildenafil Citrate and 500µg/ml Fluoxetine Hydrochloride).

From the above stock solution 1ml aliquot was transferred in to a 10 ml volumetric flask, dissolved in a small quantity of distilled water and the volume was made up to the mark with distilled water to obtain a final concentration of 100µg/ml Sildenafil Citrate and 50µg/ml Fluoxetine Hydrochloride. This solution was used as the sample stock solution 'B'.

0.8ml of the sample stock solution 'B' was transferred in to a 10 ml volumetric flask, dissolved in a small quantity of distilled water and the volume was made up to the mark with distilled water. The absorbance of the resultant solution was measured at the two absorption maxima 228nm and 216nm. This absorbance was noted as A_1 and A_2 respectively and amount of the drugs present was calculated using simultaneous equation method and the results were given in table. 9&16.

C. Method Validation

The following parameters were determined to validate the developed analytical method as per ICH guidelines (ICH Q2B, 1996).

1. Accuracy

Accuracy is the closeness of the test resultsobtained by the method to the true value. To study the accuracy, 20 tablets were weighed and powdered and analysis of the same was carried out. Recovery studies were carried out by addition of known amount of the Sildenafil Citrate and Fluoxetine Hydrochloride to the sample at three different concentration levels i.e. 80%, 100% and 120% (Standard addition method). The results were given in table and statistical data was given in table.

2. Precision

The precision of an analytical method is the degree of agreement among individual test results when the method is applied repeatedly to multiple samplings of homogenous samples. It provides an indication of random error results and was expressed as relative standard deviation (coefficient of variation).

Procedure for the Determination of Intra-day Precision

In intraday precision six replicate sample matrices separately containing 10 µg/ml of Sildenafil Citrate and Fluoxetine Hydrochloride were analyzed at different time intervals on the same day at 228 nm and 216 nm respectively. The variation of the results within the same day was analyzed and statistically validated.

Procedure for the Determination of Inter-day Precision

In inter-day precision six replicate sample matrices separately containing 10 µg/ml of Sildenafil Citrate and Fluoxetine Hydrochloride were analyzed on different days at 228 nm and 216 nm respectively. The variation of the results was analyzed and statistically validated, which were given in table.

3. Linearity and Range

The linearity of an analytical method is its ability to elicit test results that are directly proportional to the concentration of analyte in the sample with in a given range. Appropriate aliquots ranging from 0.2ml to 1ml were pipetted out separately from the 'standard stock solution B1 and B2' out in to a series of 10ml volumetric flasks. The volume was made up to the mark with distilled water to obtain a concentration range, ranging from 2-10 µg/ml (2,4,6,8,10 µg/ml). Absorbance of the above solutions was measured at 228 nm and 216 nm respectively.

A calibration curve of concentration vs. absorbance was established and shown in figure 11. Both drugs follow Beer's lamberts law in the concentration range of 2-10µg/ml. Regression equation was established and the correlation coefficient was determined. The optical and regression parameters were given in table.

4. Ruggedness

Ruggedness is the degree of reproducibility of results obtained by the analysis of the same sample under a variety of normal test conditions i.e. different analysts, laboratories, instruments, reagents, assay temperatures etc.

The solution of 10 µg/ml of Sildenafil Citrate and Fluoxetine Hydrochloride was prepared separately and analyzed with change in the analytical conditions like different instruments (Labindia 3200 and Elico SL 210) and different analysts (Analyst-1 and Analyst-2) and the results were given in table.

5. LOD and LOQ

The LOD and LOQ values were determined by the formulae LOD = 3.3 σ/S and LOQ = 10 σ/S (Where, σ is the standard deviation of y intercepts obtained from the replicate measurements (n=3) and S is mean of the slopes of the calibration curves) and were given in table. The results were given in table1-9

Table 1: Linearity Data at 228 nm for Sildenafil Citrate and 216 nm for Fluoxetine Hydrochloride.

S. No	Concentration	Absorbance	
		Sildenafil Citrate	Fluoxetine Hydrochloride
1	0	0	0
2	2	0.1458	0.1147
3	4	0.2865	0.2277
4	6	0.4098	0.3353
5	8	0.5548	0.4457
6	10	0.6756	0.5689

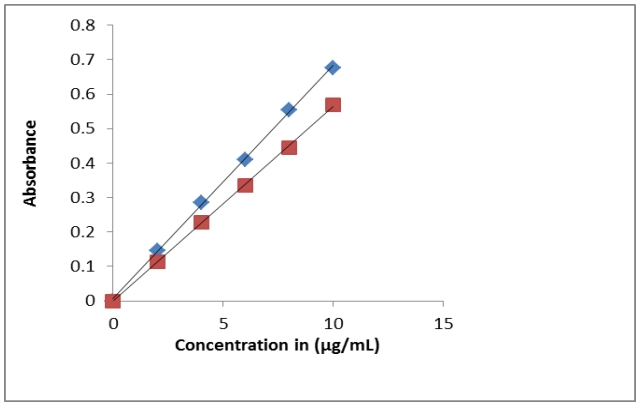

Fig-1: Calibration Curve of Sildenafil Citrate and Fluoxetine Hydrochloride.

Table 2: Optical and Regression Parameters of the Calibration Curve Obtained by UV Spectrophotometric Method.

Parameter	Sildenafil Citrate	Fluoxetine Hydrochloride
Linearity Range (µg/mL)	2-10	2-10
Λmax	228	216
Molar Extinction Coefficient (lit.mol^{-1} cm^{-1})	699.46	565.5
Sandell's Sensitivity (µgcm^{-2}/ 0.001 abs units)	0.0137	0.0174
Regression Equation (Y*)	Y=0.0675x+0.0077	Y=0.0564x+0.0003
Slope (m)	0.0675	0.0564
Intercept (c)	0.0077	0.0003
Regression Coefficient (r^2)	0.9992	0.9997
LOD (µg/mL)	0.12	0.11
LOQ (µg/mL)	0.38	0.34

*Y=mX+C where X is the concentration of drug in µg/mL and Y is the absorbance at the respective λmax.

Table 3: Assay of Sildenafil Citrate and Fluoxetine Hydrochloride in Tablet Formulation.

S. No	Amount Present in (mg/tab)		Amount Obtained in (mg/tab)		Label Claim %w/w	
	SIL	FLOX	SIL	FLOX	SIL	FLOX
1	100	60	98.2	59.1	98.2	98.5

Table 4: Determination of Accuracy for Sildenafil Citrate and Fluoxetine Hydrochloride.

Recovery Level	Amount of Standard Drug Added (µg/mL)		Amount of Test Added (µg/mL)		Total Amount Recovered (µg/mL)		% Recovery (w/w)	
	SIL	FLOX	SIL	FLOX	SIL	FLOX	SIL	FLOX
80%	8	4	2	1	9.99	4.90	100.5	98.5
	8	4	2	1	10.15	4.97	101.5	99.40
	8	4	2	1	10.03	4.91	100.3	98.20
100%	10	5	2	1	11.99	6.01	99.69	98.8
	10	5	2	1	12.01	6.02	100.08	100.33
	10	5	2	1	11.89	5.95	99.08	99.16
120%	12	6	2	1	13.97	7.01	99.78	100.14
	12	6	2	1	13.95	6.99	99.64	99.85
	12	6	2	1	14.12	7.08	100.85	101.11

Table 5: Statistical Validation Data for % Recovery Determinations.

Level of Recovery	Mean		Standard deviation		% RSD	
	SIL	FLOX	SIL	FLOX	SIL	FLOX
80 %	100.56	98.53	0.832	0.757	0.827	0.768
100 %	99.69	99.88	0.535	0.632	0.536	0.632
120%	100.09	100.36	0.661	0.659	0.661	0.657

Table 6: Precision Data of Sildenafil Citrate.

S. No	Concentration (µg/mL)	Absorbance	
		Intraday Precision	Interday Precision
1	10	0.6756	0.6721
2	10	0.6721	0.6705
3	10	0.6751	0.6695
4	10	0.6798	0.6698
5	10	0.6699	0.6685
6	10	0.6785	0.6697
Mean		0.6751	0.6700
SD		0.0037	0.0012
%RSD		0.558	0.180

Table 7: Precision Data of Fluoxetine Hydrochloride.

S. No	Concentration (µg/mL)	Absorbance	
		Intraday Precision	Interday Precision
1	10	0.5655	0.5576
2	10	0.5589	0.5549
3	10	0.5595	0.5587
4	10	0.5696	0.5698
5	10	0.5645	0.5543
6	10	0.5549	0.5605
Mean		0.5621	0.5593
SD		0.00534	0.00564
%RSD		0.949	1.009

Table 8: Ruggedness Data of Sildenafil Citrate.

S. No	Conditions	Conc. (µg/mL)	Absorbance	Mean	SD	%RSD
1	Analyst - 1	10	0.6721	0.6707	0.00131	0.195
2		10	0.6695			
3		10	0.6705			
4	Analyst-2	10	0.6697	0.6693	0.00075	0.113
5		10	0.6685			
6		10	0.6699			
7	Instrument-1	10	0.6721	0.6756	0.0038	0.574
8		10	0.6751			
9		10	0.6798			
10	Instrument-2	10	0.6721	0.6707	0.0013	0.195
11		10	0.6705			
12		10	0.6695			

Table 9: Ruggedness Data of Fluoxetine Hydrochloride.

S. No	Conditions	Conc. (µg/mL)	Absorbance	Mean	SD	%RSD
1	Analyst - 1	10	0.5756	0.5685	0.0061	1.08
2		10	0.5645			
3		10	0.5655			
4	Analyst-2	10	0.5598	0.5608	0.0011	0.210
5		10	0.5621			
6		10	0.5605			
7	Instrument-1	10	0.5798	0.5784	0.0047	0.819
8		10	0.5732			
9		10	0.5824			
10	Instrument-2	10	0.5698	0.5656	0.0051	0.917
11		10	0.5672			
12		10	0.5598			

CONCLUSION

UV-Spectrometric method developed and validated allows a simple and fast quantitative determination of Sildenafil and Fluoxetine from their formulations. All the validation parameters were found to be within the limits according to ICH guidelines. 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. So the established method can be employed in the routine analysis of the marketed formulations.

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