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UV first order and second order derivative spectrophotometric method using amplitude and AUC technique for determination of sildenafil in bulk and in pharmaceutical formulation

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Abstract
Four simple, rapid, accurate, precise, reliable, and economical UV-spectrophotometric methods have been proposed for the determination of Sildenafil in bulk and in pharmaceutical formulation. “Method A” is first order derivative UV spectrophotometry using amplitude, “method B” is first order derivative UV spectrophotometry using area under curve technique, “method C” is second order derivative UV spectrophotometry using amplitude, and “method D” is second order derivative UV spectrophotometry using area under curve technique. The developed methods have shown best results in terms of linearity, accuracy, precision, and LOD and LOQ for bulk drug and marketed formulation as well. In N, N-dimethyl formamide (DMF), Sildenafil showed maximum absorbance at 292 nm. For “method A” amplitude was recorded at 313 nm while for “method B” area under curve was integrated in the wavelength range of 302-326 nm. For “method C” amplitude was measured at 297 nm while for “method D” area under curve was selected in the wavelength range of 281-310 nm. For methods A, B, C and D, Sildenafil obeyed Lambert-Beer’s law in the range of 05–50 µg/mL, and the correlation coefficients were found to be > than 0.999.

Keywords: Sildenafil, UV spectroscopy, derivatives, area under curve

1. Introduction
Sildenafil citrate is designated chemically as 1-[[3-(6, 7-dihydro-7-oxo-3 propyl-1Hpyrazolo [4, 3-d] pyrimidin-5-yl)-4-ethoxyphenyl] sulfonyl]-4-methylpiperazine and it is popularly known as Viagra. It is a novel oral agent for the treatment of penile erectile dysfunction [1-2]. It is an active inhibitor of the type V-cyclic guanosine monophosphate (cGMP) specific phosphodiesterase on penile erectile activity, and causes cGMP to accumulate corpus cavernosum [3-6]. The structural formulae is $C_{22}H_{30}N_6O_4S$.
A detailed literature survey of Sildenafil revealed that several analytical methods are reported for the determination of Sildenafil by high-performance liquid-chromatography [7–8], UV-spectrophotometry [9-10]. To our knowledge no methods were found in literature for determination of Sildenafil in bulk and pharmaceutical formulation using derivative spectroscopic techniques with Water. Therefore, our attempt is to develop first order and second order derivative spectroscopy using amplitude and also area under curve (AUC) techniques.
The AUC method is applicable where there is no sharp peak or when broad spectra are obtained. It involves the calculation of integrated value of area with respect to the wavelength between the two selected wavelengths λ_1 and λ_2. Selection of wavelength range is on the basis of repeated observations so as to get the linearity between AUC and concentration [11]. Further, methods were validated as per ICH guidelines [12].

2. Experimental Work
2.1 Material and Methods
Sildenafil working standard was obtained from Glenmark Pharmaceuticals Ltd., Mumbai, India. The marketed formulation (Winagra) 100 mg was purchased from local market. N, N Dimethylformamide (DMF) (analytical grade) and R.O. water were used for the experiment.

2.2 Instrument
A double beam UV-VIS spectrophotometer (UV-1700, Shimadzu, Japan) connected to computer loaded with spectra manager software UV Probe 2.21 with 10 mm quartz cells was used. The spectra were obtained with the instrumental parameters as follows: wavelength range: 400– 200 nm; scan speed: medium; sampling interval: 1.0 nm; band width (Δλ): 1.0 nm; spectral slit width: 1 nm. An electronic balance (Model Shimadzu AUX 120) was used for weighing purpose.

2.3 Preparation of Stock Standard Solution and Selection of Wavelengths
The stock standard solution of Sildenafil was prepared by dissolving accurately weighed 25 mg in 50 mL of DMF. It was further diluted with water to obtain concentration of 10 µg/mL which was scanned in UV range 400–200 nm. Sildenafil showed a maximum absorbance at 313 nm. For "method A" amplitude in first order derivative spectrum was determined at (313) nm while for "method B" AUC in first order derivative spectrum was selected in between (302) and (326) nm. In "method C" amplitude of second order derivative spectrum was recorded at (297) nm while in "method D" AUC of second derivative spectrum was selected in between (281) and (310) nm.

2.3.1 Methods A
The zero order absorption spectra of Sildenafil were derivatized in first order using software UV Probe 2.21 with delta lambda 8 and scaling factor 30. In "method A" the amplitudes were recorded at (313) nm. The calibration curves were constructed by plotting concentrations 05–50 µg/mL versus amplitude/AUC between selected wavelengths for "methods A.

2.3.2 Methods B
The zero order absorption spectra of Sildenafil were derivatized in first order using software UV Probe 2.21 with delta lambda 8 and scaling factor 30. In "method B" Area under curve between the two wavelengths (302) and (326) nm was selected. The calibration curves were constructed by plotting concentrations 05–50 µg/mL versus amplitude/AUC between selected wavelengths for "methods B".

2.3.3 Methods C
The zero order absorption spectra of Sildenafil was derivatized in second order using software UV Probe 2.21 with delta lambda 16 and scaling factor 50. In "method C" the amplitudes were recorded at (297) nm. The calibration curves were constructed by plotting concentrations 5–50 µg/mL versus amplitude/AUC for "method C".

2.3.4 Methods D
The zero order absorption spectra of Sildenafil was derivatized in second order using software UV Probe 2.21 with delta lambda 16 and scaling factor 50. In "method D" Area under curve was recorded in between the two wavelengths (281) and (310) nm. The calibration curves were constructed by plotting concentrations 5–50 µg/mL versus amplitude/AUC for "method D".

2.3.5 Preparation of Sample Solution
Ten Sildenafil tablets (label claim 100 mg) were weighed, transferred to a clean dry mortar, and grounded into a fine powder using a pestle. Tablet powder equivalent to 25 mg of Sildenafil was transferred to a 50 mL volumetric flask and 30 mL DMF was added. After ultrasonic vibration for 10 min, volume was made up to be marked with DMF and filtered through Whatman flter paper. From the filtrate, an appropriate volume was taken and diluted with water to get the final concentration of 30 µg/mL for "methods A, B, C and D." The responses measured and concentrations in the sample were determined from respective linearity equation.

3. Validation of Method
The proposed method was validated as per ICH guidelines

i) **Linearity:** The linearity of the "methods A, B, C and D" was evaluated by analysis of six standard solutions of Sildenafil of concentrations 05, 10, 20, 30, 40, and 50 µg/mL
ii) **Accuracy:** The accuracy of all methods was evaluated by measurement of recovery. To the reanalysed sample solutions (10 µg/mL in methods A, B, C, and D), known amounts of stock standard solutions were added at different levels, that is, 80%, 100%, and 120%. The solutions were reanalysed by the proposed methods. The experiments were repeated for three times at each level for each method.
iii) **Precision:** Precision of the methods was studied as intraday and inter-day variations. For "methods A, B, C, and D," precision was determined by analysing the 10, 20, and 30 µg/mL of Sildenafil solutions as intra-day and inter-day variations.
iv) **Sensitivity**: The sensitivity of measurements of Sildenafil by the use of proposed methods was estimated in terms of limit of detection (LOD) and limit of quantification (LOQ) which were calculated using formulae "LOQ = 10 × N/B" and "LOD = 3.3 × N/B," where "N" is standard deviation of the amplitude or peak areas of the Sildenafil (N = 3), taken as a measure of noise, and "B" is the slope of the corresponding calibration curve.
v) **Repeatability**: In "methods A, B, C, and D," repeatability was determined by analyzing 30 µg/mL concentration of Sildenafil solution for six times.
vi) **Ruggedness**: For "methods A, B, C, and D" ruggedness of the proposed method was determined by analyzing 20 µg/mL concentration of Sildenafil by two different analysts using similar operational and environmental conditions.

4. Results and Discussion
4.1 Method Validation
4.1.1 Linearity: From the linear regression data it is clear that for "methods A, B, C and D" calibration curves showed good linear relationship over the concentration range of 05–50 µg/mL for Sildenafil. The data of regression analysis is shown in Table 1 (a), (b), (c) and (d).

4.1.2 Accuracy: The solutions were reanalysed by proposed methods; results of recovery studies are reported in Table 2 (a), (b), (c) and (d). The % RSD values that were determined and found to be less than 2 indicate that the method is accurate

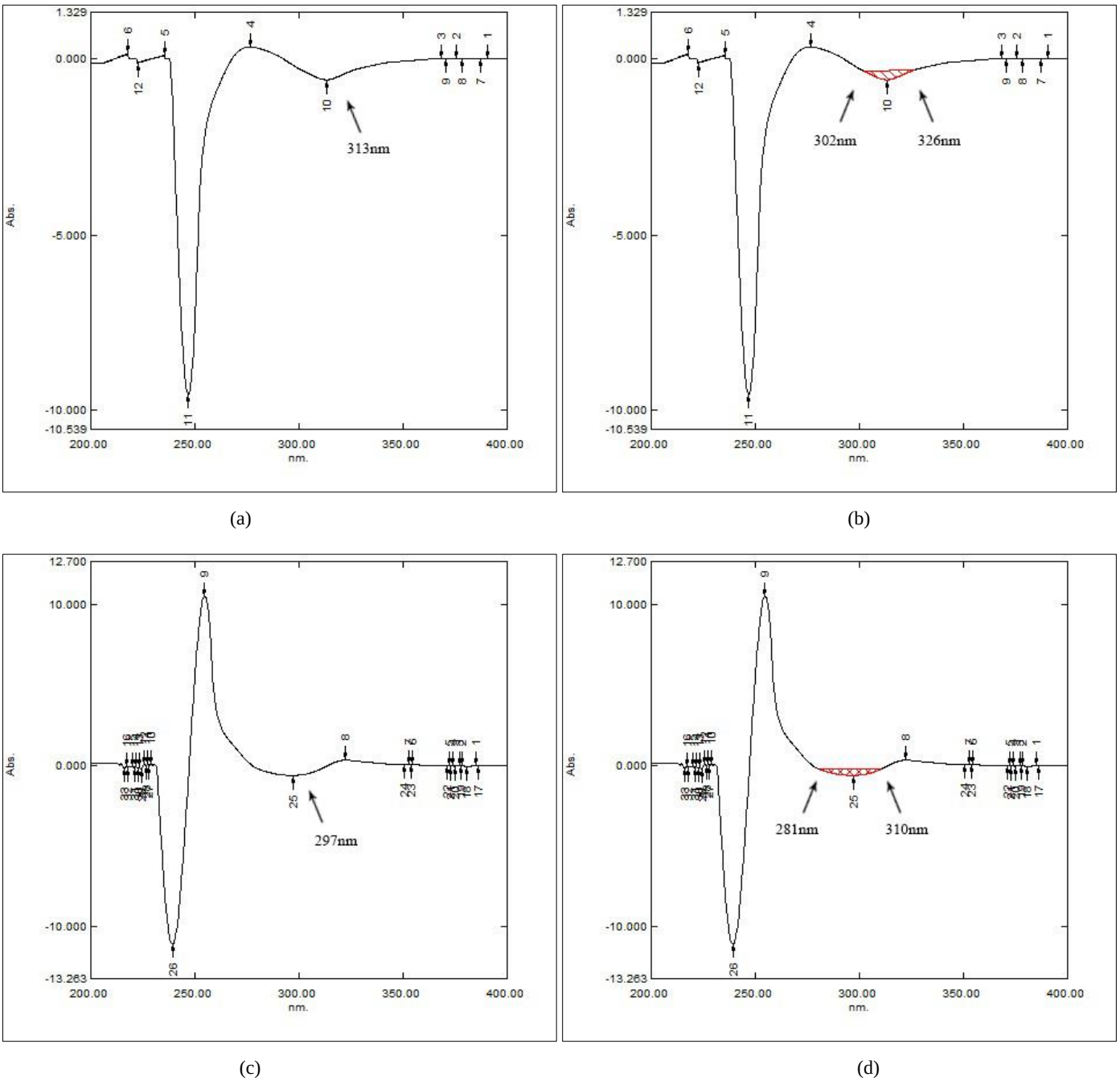
4.1.3 Precision: The precision of the developed methods was expressed in terms of % relative standard deviation % RSD. These results showed reproducibility of the assay. The % RSD values were found to be less than 2, so this indicates that the methods are precise for the determination of the Sildenafil

in pharmaceutical formulation. Results are shown in Table 3 (a), (b), (c) and (d).

4.1.4 Sensitivity: The LOD and LOQ for Sildenafil in "method A" were found to be 0.60 µg and 1.82 µg while in "method B" 0.71 µg and 2.15 µg. Similarly in "method C" values for LOD and LOQ were as 0.58 µg and 1.71 µg and in "method D" 1.64 µg and 1.95 µg.

4.1.5 Repeatability: For "methods A, B, C and D" repeatability were determined by analyzing 30 µg/mL concentration of solution for six times with % RSD values < 2 for all the methods. Results are shown in Table 4 (a), (b), (c) and (d).

4.2 Analysis of Tablet Formulation: The amounts of Sildenafil estimated from tablet formulation using methods A, B, C, and D were found to be 101.79%, 99.58%, 100.95%, and 100.26%, respectively. The % amount estimated from tablet formulation indicates that there is no interference from excipients present in it.



(a) (b)

(c) (d)

Fig 1: Zero order spectrum of Sildenafil. (a) First order derivative spectrum. (b) First order derivative spectrum showing AUC between selected wavelengths. (c) Second order derivative spectrum. (d) Second order derivative spectrum showing AUC between selected wavelengths

Table 1(a): Optical characteristics of Sildenafil

Parameters	Method A
Beer Lambert's range (µg/mL)	5-50
λmax (nm)/wavelength range (nm)	313
Slope	0.0196
Intercept	0.0174
Correlation Coefficient	0.9998

Table 1(b): Optical characteristics of Sildenafil

Parameters	Method B
Beer Lambert's range (µg/mL)	5-50
λmax (nm)/wavelength range (nm)	302-326
Slope	0.117
Intercept	0.1093
Correlation Coefficient	0.9995

Table 1(c): Optical characteristics of Sildenafil

Parameters	Method C
Beer Lambert's range (µg/mL)	5-50
λmax (nm)/wavelength range (nm)	297
Slope	0.0188
Intercept	0.0156
Correlation Coefficient	09998

Table 1(d): Optical characteristics of Sildenafil

Parameters	Method D
Beer Lambert's range (µg/mL)	5-50
λmax (nm)/wavelength range (nm)	281-310
Slope	0.2426
Intercept	0.1594
Correlation Coefficient	09994

Table 2(a): Accuracy Studies

Drug	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount recovered [µg/mL]	% Recovered	% RSD
SLD	10	8	17.92	99.02	0.71
	10	10	20.03	100.30	0.25
	10	12	22.19	101.58	0.95

Table 2(b): Accuracy Studies

Drug	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount recovered [µg/mL]	% Recovered	% RSD n=3
SLD	10	8	17.98	99.75	0.67
	10	10	20.10	101.05	0.68
	10	12	21.97	99.81	0.25

Table 2(c): Accuracy Studies

Drug	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount recovered [µg/mL]	% Recovered	% RSD
SLD	10	8	17.94	99.33	0.59
	10	10	19.95	99.50	0.55
	10	12	22.02	100.20	0.27

Table 2(d): Accuracy Studies

Drug	Initial Amount [µg/mL]	Amount added [µg/mL]	Amount recovered [µg/mL]	% Recovered	% RSD [n]
SLD	10	08	17.93	99.13	0.38
	10	10	19.97	99.76	0.28
	10	12	22.02	100.23	0.54

Table 3(a): Precision Study

Drug	Concentration Drug [µg/mL]	Intra-day		Inter-day	
		Amount found [µg/mL]	%RSD [n = 3]	Amount found [µg/mL]	%RSD [n = 3]
SLD	10	10.12	0.70	10.08	1.69
	20	20.06	1.09	20.11	1.26
	30	29.97	1.32	29.78	1.35

n= Number of estimation

Table 3(b): Precision Study

Drug	Concentration Drug [µg/mL]	Intra-day		Inter-day	
		Amount found [µg/mL]	%RSD [n = 3]	Amount found [µg/mL]	%RSD [n = 3]
SLD	10	9.99	1.28	10.01	0.99
	20	20.03	1.48	20.00	1.21
	30	29.97	1.10	29.93	0.80

n= Number of estimation

Table 3(c): Precision Study

Drug	Concentration Drug [µg/mL]	Intra-day		Inter-day	
		Amount found [µg/mL]	%RSD [n = 3]	Amount found [µg/mL]	%RSD [n = 3]
SLD	10	10.03	1.08	9.98	1.29
	20	19.89	1.06	20.01	1.04
	30	29.80	0.71	29.79	1.02

n= Number of estimation

Table 3(d): Precision Study

Drug	Concentration Drug [µg/mL]	Intra-day		Inter-day	
		Amount found [µg/mL]	%RSD [n = 3]	Amount found [µg/mL]	%RSD [n = 3]
SLD	10	9.94	0.78	9.97	0.91
	20	19.82	0.36	19.84	0.40
	30	29.97	0.84	29.85	0.46

n= Number of estimation

Table 4(a): Repeatability studies

Drug	Concentration of SLD in µg/mL	Amount Found in µg/mL	% Amount Found
SLD	30	30.59	101.97
	30	29.97	99.93
	30	29.77	99.25
	30	30.23	100.78
	30	29.77	99.25
	30	29.97	99.93
	Mean ± SD	30.05± 0.31	100.18± 1.04
	% RSD	1.03	1.04

n= Number of estimation

Table 4(b): Repeatability studies

Drug	Concentration of SLD in µg/mL	Amount Found in µg/mL	% Amount Found
SLD	30	30.45	101.50
	30	30.50	101.67
	30	30.39	101.33
	30	29.79	99.30
	30	29.84	99.47
	30	30.56	101.87
	Mean ± SD	30.25± 0.34	100.86± 0.15
	% RSD	1.14	1.14

n= Number of estimation

Table 4(c): Repeatability studies

Drug	Concentration of SLD in µg/mL	Amount Found in µg/mL	% Amount Found
SLD	30	30.18	100.60
	30	30.12	100.42
	30	29.43	98.12
	30	29.59	98.65
	30	29.75	99.18
	30	29.84	99.89
	Mean ± SD	29.84± 0.29	99.47± 0.99
	% RSD	0.99	1.00

n= Number of estimation

Table 4(d): Repeatability studies

Drug	Concentration of SLD in µg/mL	Amount Found in µg/mL	% Amount Found
SLD	30	29.54	98.48
	30	29.78	99.28
	30	29.78	99.28
	30	30.06	100.21
	30	29.49	98.30
	30	29.88	99.60
	Mean ± SD	29.75 ± 0.21	99.19 ± 0.71
	% RSD	0.71	0.72

n= Number of estimation

Table 5(a): Ruggedness Studies

Analyst	Conc. of SLD	[%] Amount of SLD [n=6]	% RSD
I	20	99.94	0.98
II	20	100.57	0.81

n= Number of estimation

Table 5(b): Ruggedness Studies

Analyst	Conc. of SLD	[%] Amount of SLD [n=6]	% RSD [n]
I	20	100.97	1.52
II	20	99.05	0.67

n= Number of estimation

Table 5(c): Ruggedness Studies

Analyst	Conc. of SLD	[%] Amount of SLD [n=6]	% RSD
I	20	100.90	1.17
II	20	99.75	0.99

n= Number of estimation

Table 5(d): Ruggedness Studies

Analyst	Conc. of SLD	[%] Amount of SLD [n=6]	% RSD [n]
I	20	99.06	0.15
II	20	99.10	0.62

n= Number of estimation

5. Conclusion

All four methods were developed for the determination of Sildenafil based on different analytical techniques, UV spectrophotometric derivative, and AUC methods. The methods were validated and found to be simple, sensitive, accurate, and precise. Hence, the methods can be used successfully for routine analysis of pharmaceutical dosage form of Sildenafil. The proposed spectrophotometric methods will not be substituted to the existing known methods available for the analysis of Sildenafil. However, it can serve as an option where advanced instruments (e.g., HPLC) are not available for routine analysis.

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