Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 12 [3] February 2023 : 77-86

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Quality by Design (Qbd) Approach to Develop Stability Indicating RP-HPLC Method Development and Validation for Estradiol Valerate and Sildenafil

Sufiyan Ahmad[1]*, Patil Mohanesh[1], Vishal Lad[1], Bakhshi Abdul Rahman[2], Aditi Kulkarni[3]
[1]Gangamai College of Pharmacy, Nagaon, Dist. Dhule (M.S.), India.
[2]Royal College of Pharmaceutical Education and Research, Malegaon Dist: Nashik (M.S.), India.
[3]Smt. Kashibai Navale College of Pharmacy, Kondhwa, Affiliated to Savitribai Phule Pune University, Pune, Maharashtra, India.
Correspondence Email: sufimpharm@rediffmail.com

ABSTRACT

As per requisition of current regulatory requirements, simple, rapid and sensitive method by 3^3 factorial QbD approach was established and validated for Estradiol Valerate (EST) and Sildenafil (SDF) by RP-HPLC. A simple RP-HPLC method has been developed and validated with different parameters such as linearity, precision, repeatability, LOD, LOQ, accuracy as per International Conference for Harmonisation guidelines (Q_2R_1). Statistical data analysis was done for data obtained from different aliquots Runs on Agilent Tech. Gradient System with Auto injector, UV (DAD) & Gradient Detector. Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 100mm; 2.5µm), a 20µl injection loop and UV730D Absorbance detector at 282 nm wave length and running chemstation 10.1 software and drugs along with degradants were separated via Methanol: (0.1% OPA) Water (90:10) of pH 3.0 as mobile phase setting flow rate 0.9 ml/min at ambient temperature. The developed method was found linear over the concentration range of 2-10 µg/ml for EST and 25-125 µg/ml for SDF while detection and quantitation limit were found to be 0.05656 µg/ml and 0.17139 µg/ml for EST and 0.1272 µg/ml and 0.37810 µg/ml for SDF. There are no interfering peaks underperformed degradation conditions. Therefore, a sensitive, robust, accurate and stability indicating method was developed with high degree of practical utility.

Keywords: *Estradiol Valerate, Sildenafil Citrate, QbD, RP-HPLC, Stability Study, method development, validation*

Received 06.11.2022 Revised 22.01.2023 Accepted 24.01.2023

INTRODUCTION:

The concept of "Quality by Design" (QbD) was defined as an approach which covers a better scientific understanding of critical process and product qualities, designing controls and tests based on the scientific limits of understanding during the development phase and using the knowledge obtained during the life-cycle of the product to work on a constant improvement environment. QbD describes a pharmaceutical development approach referring to formulation design and development and manufacturing processes to maintain the prescribed product quality. Guidelines and mathematical models are used to ensure the establishment and use of the knowledge on the subject in an independent and integrated way [1-3]. Estradiol Valerate chemically is, (2*S*)-2-(6-methoxynaphthalen-2-yl) propanoic acid; (Fig. 1) act on the estrogen receptors to relieve vasomotor systems (such as hot flashes) and urogenital symptoms (such as vaginal dryness and dyspareunia). Estrogens cause an increase in hepatic synthesis of various proteins, which include sex hormone binding globulin (SHBG), and thyroid-binding globulin (TBG). Estrogen is known to suppress the formation of follicle-stimulating hormone (FSH) in the anterior pituitary gland [4]. Sildenafil chemically is 6-(difluoromethoxy)-2-[(3,4-dimethoxypyridin-2-yl) methylsulfinyl]-1*H*-benzimidazole (Fig. 1). Sildenafil is a potent and selective inhibitor of cGMP specific phosphodiesterase type 5 (PDE5) in the corpus cavern sum, where PDE5 is responsible for degradation of cGMP Sildenafil has a peripheral site of action on erections. Sildenafil has no direct relaxant effect on isolated human corpus cavern sum but potently enhances the relaxant effect of NO on this tissue. When

the NO/cGMP pathway is activated, as occurs with sexual stimulation, inhibition of PDE5 by sildenafil results in increased corpus cavern sum level of cGMP. Therefore, sexual stimulation is required in order for sildenafil to produce its intended beneficial pharmacological effects [5].

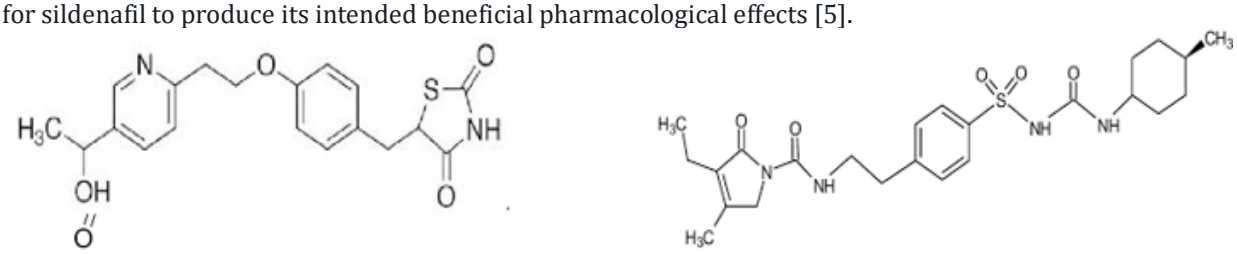


Fig. No. 1: Structure of estradiol valerate and sildenafil

Literature surveys revealed various RP-HPLC methods have been developed individually and combined with other drug [6]. The main objective of our work is to develop an improved RP-HPLC method suitable for the routine quality control of Sildenafil and Estradiol Valerate in a pharmaceutical industry and provide information on the sensitivity of chromatographic factors and their interaction effects on the separation characteristics. In our studies no method has been reported for simultaneous estimation of the Sildenafil and Estradiol Valerate drugs by using QbD based 3^3 factorial designing.

MATERIAL AND METHODS

Reference standards of Estradiol Valerate was obtained as gift sample from Dr. Reddy's Laboratories, Hyderabad, India while Sildenafil was obtained as generous gift from Micro Labs Ltd., Bangalore, India. Pharmaceutical formulation was purchased from local market (Brand: Estroflow tablet labelled claim Estradiol Valerate 2mg and Sildenafil citrate 25 mg make Xeno Lifesciences). The HPLC grade solvents used were of E-Merck (India) Ltd., Mumbai. HPLC grade Acetonitrile, Methanol and Ortho Phosphoric Acid (Merck, Mumbai, India) were used in the analysis. HPLC grade water was prepared using Millipore purification system.

Instruments

The analysis of the drug was carried out on Agilent Tech. Gradient System with Auto injector, UV (DAD) & Gradient Detector. Equipped with Reverse Phase (Agilent) C_{18} column (4.6mm x 100mm; 2.5µm), a 20µl injection loop and UV730D Absorbance detector and running chemstation 10.1 software.

RP-HPLC Optimised Chromatographic Condition using QbD

Column C_{18} (100 mm× 4.6mm); particle size packing 5µm; detection wavelength 230 nm; flow rate 0.9 ml/min; temperature 26^0C ambient; sample size 20 µl; mobile phase methanol: water (OPA 0.1% PH 3) (90:10); run time 15 min. The retention time for Estradiol Valerate and Sildenafil were found at 2.183 min and 7.866 min respectively (Fig. 2). The RP-HPLC method developed for estimation of Estradiol Valerate and Sildenafil was validated as per ICH Q_2 (R_1) guidelines using various parameters (Table 1).

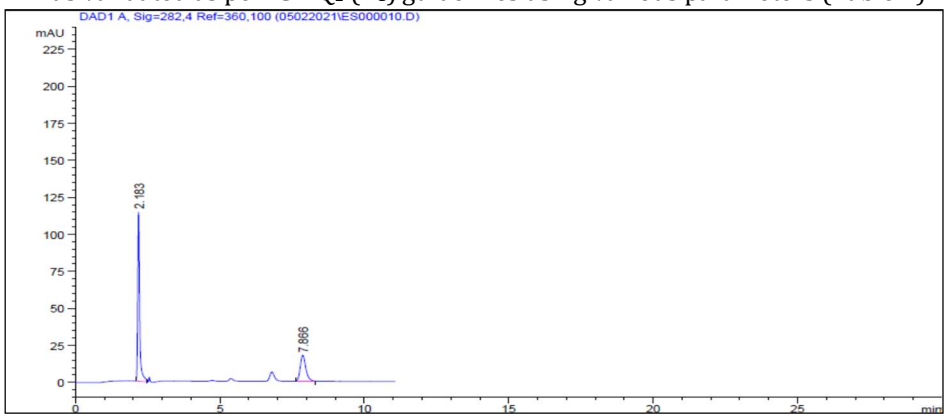


Fig. No. 2: Chromatogram of standard EST and SDF at 231 nm

Preparation of standard solution

All solutions were prepared on a weight basis and solution concentrations were also measured on weight basis to avoid the use of an internal standard Pharmaceutical formulation which is available in the market in the proportion of 1:12.5.

Stock preparations

Standard stock solution was prepared by dissolving 2 mg EST and 25 mg SDF in 10 ml clean dry volumetric flask and dilution was upto the mark with Methanol to obtain final concentration of EST (200 µg/ml) and SDF (2500 µg/ml). All the stock solutions were filtered through 0.45 µm membrane filter.

Detection of λ_{max} : The sample solution has been prepared and scanned in the UV region of 200-400 nm and the spectrum showed the maximum absorbance at 230 nm (Fig. 3).

QbD approach to analysis

The application of QbD in HPLC method development commences with establishing analytical objectives based on sound science to ensure consistent method performance characteristics are achieved [7-8]. The use of QbD for an analytical method commences with defining the target analytical profile in which the pre-defined objectives for method performance must be appropriately validated and documented [9-13].

Thus the objective of this work was to perform experimental design by using Design Expert Software leading to develop simple, rapid and sensitive method by QbD approach and validated as per ICH Guidelines (Q_2R_1) for Estradiol Valerate and Sildenafil citrate and its stability indicating method by RP-HPLC. Further statistical data analysis was done along with numerical and graphical optimization to develop Analytical Design Space (ADS) [14].

METHOD VALIDATION

Calibration Curve

A calibration curve was constructed succeeding replicate (n=6) analysis of five standards of 2,4,6,8 and 10 µg/ml of EST and 25, 50, 75, 100 and 125 µg/ml of SDF. The peak height ratio of drugs was calculated and plotted AUC versus concentration after which least squares linear regression analysis of data was undertaken to establish the equation for the best fit line and the correlation coefficient (R^2) to authorise linearity. Samples were injected and peaks were recorded at 230 nm and the graph plotted as concentration of drug verses peak area as shown in (Table 1).

Precision

Intra-day (repeatability) precision was established following analysis of replicate samples (n=6) at three concentrations indicative of low, medium and high levels within the linear range *viz*., 4,6,8 µg/ml of EST and 50,75,100 µg/ml of SDF. Analysis was performed over a short period of time on the same day. Inter-day precision or reproducibility was assessed at low, medium and high concentration on three consecutive days and the percent relativestandard deviation (% RSD) was used to assess intra- and inter-day precision. An upper limit of 2% was used to confirm precision in our laboratory. Precision of an analytical method is usually expressed as standard deviation or relative standard deviation (Table 2-3) describes the Intraday, Interday and Repeatability of method.

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To pre-analysed tablet solution, a definite concentration of standard drug (80%, 100%, and 120%) was added and then its recovery was analyzed**.** Statistical validation of recovery studies shown in (Table 4).

Limits of detection (LOD) and quantitation (LOQ)

Several approach for the calculation of the LOD and LOQ of a method have been suggested in different guidelines and include visual evaluation, use of signal-to-noise (S/N) ratio, calculations based on the standard deviation of a response and the slope of the calibration curve [14].

By convention, the LOD is estimated as one third of the LOQ. A series of samples of 4,6,8,10 µg/ml of EST and 50,75,100,125 µg/ml of SDF were prepared and analysed using the optimized RP-HPLC method and the peak height ratio calculated. The LOQ was determined by establishing the lowest concentration of drugs that resulted in a % RSD value for precision of <2 %.

Specificity

The specificity of an analytical method is defined as the ability of a method to ensure that the peak(s) of interest elute as distinct responses in the presence of excipients, impurities or degradation compounds.

Robustness

To evaluate robustness few parameters were deliberately varied. The parameters include variation of flow rate, percentage of methanol as described in (Table 5).

Study of system suitability parameters

The system suitability is used to verify, whether the resolution and reproducibility of the chromatographic system are adequate for analysis to be done. The test was performed by collecting data from five replicate injections of standard solution as shown in (Table 6).

Forced degradation studies

Forced degradation study was performed to evaluate the stability of the developed method using the stress conditions like exposure of sample solution to acid, base, hydrogen peroxides (H_2O_2) and neutral. Investigation was done for the degradation products in different conditions and are shown in (Table 7).

Procedure for Estradiol Valerate and Sildenafil degradation

Acid hydrolysis The acid hydrolysis performed using 0.1N HCl at 70 ºC for 1st hr for both Estradiol Valerate and Sildenafil citrate indicated degradation. The major degradation products for Estradiol Valerate and Sildenafil citrate were observed at relative retention time (RRT) for 1st Hour.

Alkaline hydrolysis

The alkaline hydrolysis condition was performed using 0.1N NaOH at 70 ºC for 1st hr and 2nd hr both Sildenafil and Estradiol. The major degradation products for Sildenafil and Estradiol Valerate were observed at relative retention time (RRT) for 1st and 2nd Hours.

Oxidation

In the oxidation condition with 3% H_2O_2 for 1st hr and 2nd hr both Sildenafil and Estradiol Valerate show oxidative stress degradation peak in the chromatogram.

Neutral

There was no major degradation observed for both Sildenafil and Estradiol Valerate and hence they were not sensitive to light at 70 ºC for 1st hr and 2nd hr.

Application of analytical method

To determine the content of EST and SDF in marketed tablets (label claim 2mg of Estradiol Valerate and 25mg Sildenafil), 20 tablets powder weighed as 2.99 gm and average weight of powder was calculated in 0.149 gm. Tablets were triturated and powder equivalent to weighed in 149 mg. The drug was extracted from the tablet powder with 10 ml Methanol. To ensure complete extraction it was sonicated for 15 min. 0.1 ml of supernatant was then diluted up to 10 mL with mobile phase. The resulting solution was injected in HPLC and drug peak area was noted**.**

RESULTS AND DISCUSSION

Such analytical methods are, in fact, an indicator of a quality product and the robustness of that product for the duration on the lifecycle of that product. The main goal of any HPLC method is to separate and quantitate analyte(s) of interest from any impurity and/or excipients. Initially it is important to establish the critical quality attributes (CQA) of a system that may impact the quality of the analytical method. Development of Analytical RP-HPLC Method with Design Space and Control Strategy determination by optimization study all the computations for the current optimization study and statistical analysis were performed using Design Expert® software (Design Expert trial version). State-Ease Inc., Minneapolis, MN, USA).

Application of design of experiments for method optimization Design of experiments (DOE-1)

Thus, 3 randomized response surface designs with a full fraction design were used with 9 trial runs to study the impact of three factors on the three key response variables. In this design 3 factors were evaluated, each at 3 levels, and experimental trials were performed at all 3 possible combinations. The mobile phase composition (X1), Wavelength (X2) and flow rate (X3), were selected as independent variables and retention time (RT) and Resolution were selected as dependent variables. The resulting data were fitted into Design Expert 10 Software and analyzed statistically using analysis of variance (ANOVA) and F-Test. (Fig. 3) indicates the normal plot of residuals for retention time with other chromatographic parameters. The data were also subjected to 3-D response surface methodology to determine the influence of flow rate, Wavelength and mobile phase composition on dependent variables as shown in (Fig. 4). The probable trial runs using 3^3 full fraction designs are as shown in Table 4. Further ANOVA and F-test with variables are shown in (Table 8-12). More over degradation peaks of API were shown in (Fig. 5-8) from acidic, alkaline, peroxide and Heat.

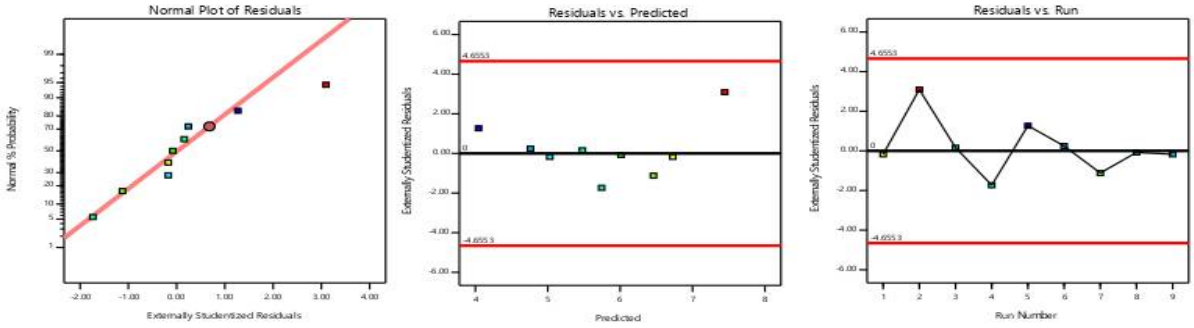


Fig. 3A: Normal plot of residuals for retention time and plot of predicted vs. actual data for retention time of cp.

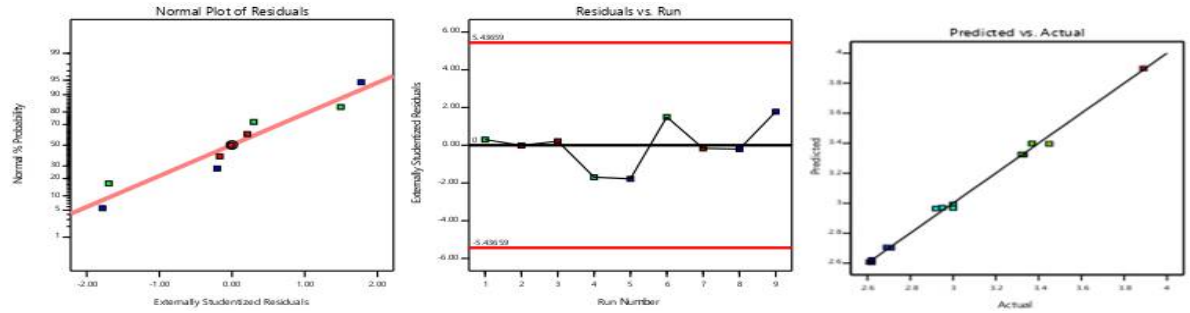


Fig. 3B: Normal plot of residuals for retention time and plot of predicted vs. actual data for retention time by the value of 2.61 to 3.89.

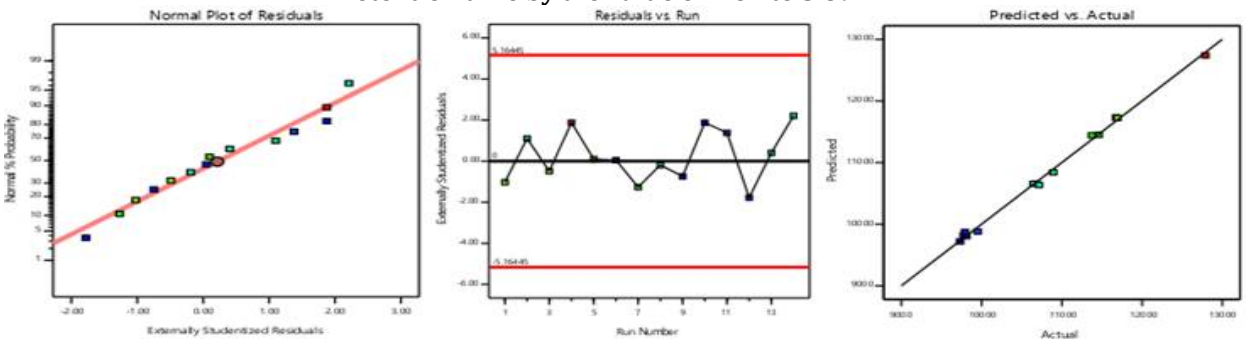
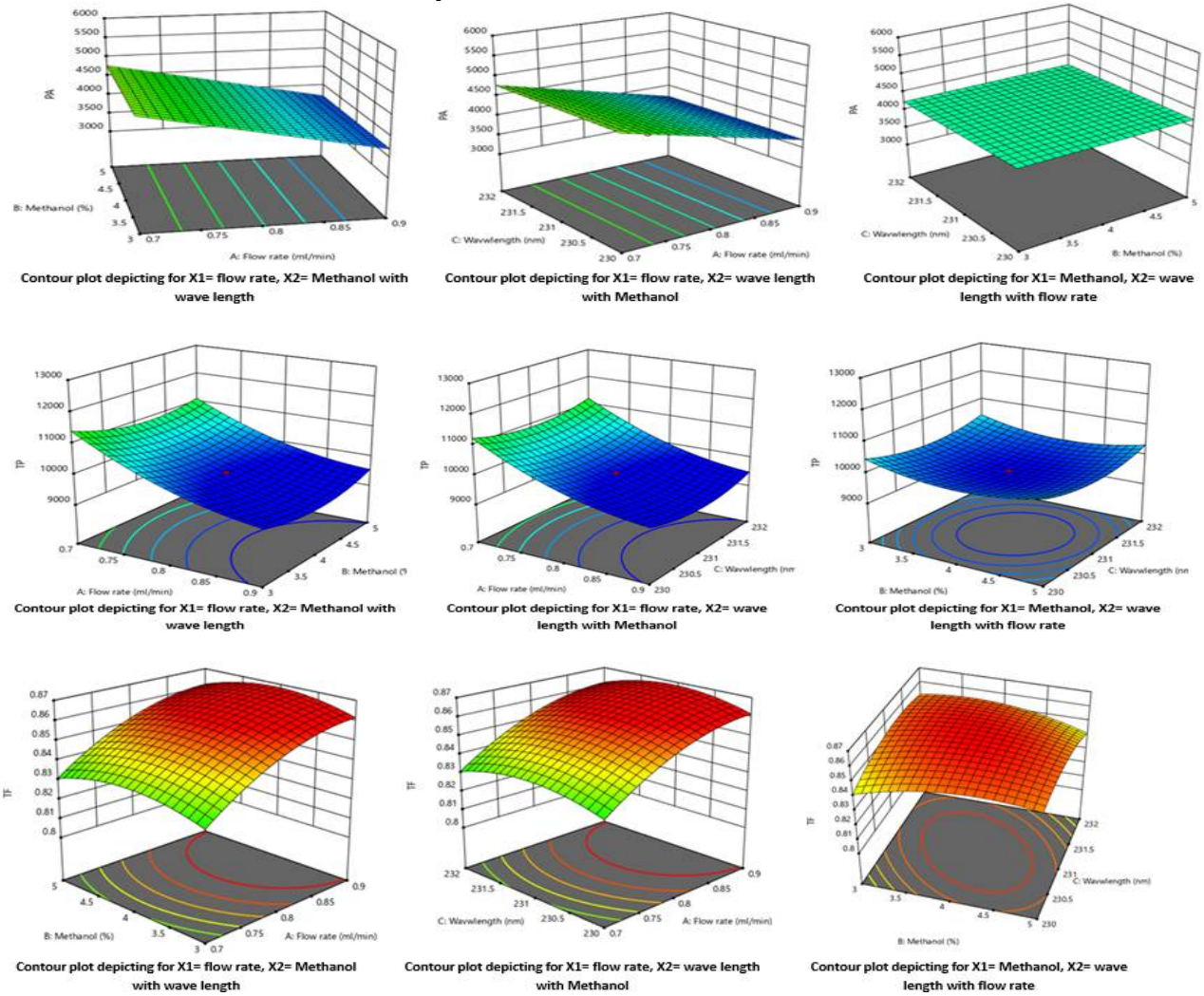


Fig. 3C: Normal plot of residuals for retention time and plot of predicted vs. actual data for retention time by the value of 9733 to 12789.



Contour plot depicting for X1= flow rate, X2= Methanol with wave length

Contour plot depicting for X1= flow rate, X2= wave length with Methanol

Contour plot depicting for X1= Methanol, X2= wave length with flow rate

Contour plot depicting for X1= flow rate, X2= Methanol with wave length

Contour plot depicting for X1= flow rate, X2= wave length with Methanol

Contour plot depicting for X1= Methanol, X2= wave length with flow rate

Contour plot depicting for X1= flow rate, X2= Methanol with wave length

Contour plot depicting for X1= flow rate, X2= wave length with Methanol

Contour plot depicting for X1= Methanol, X2= wave length with flow rate

Fig. No. 4: Contour plot for flow rate, mobile phase composition and wave length.

Table No. 1: Linearity Study

EST			SDF		
Conc. [µg/ml]	**Mean peak area ± SD [n=5]**	**%RSD**	**Conc. [µg/ml]**	**Mean peak area ± SD [n=5]**	**%RSD**
2	166.72±2.68	1.61	25	253.57±0.13	0.05
4	325.9±2.50	0.77	50	492.81±0.46	0.09
6	496.69±1.03	0.21	75	736.88±0.40	0.05
8	660.57±0.27	0.04	100	1002.97±0.35	0.03
10	827.98±0.62	0.08	125	1255.31±0.57	0.05

Table No. 2: Results of precision studies (intra-day and inter-day)

Drug	Conc. [µg/ml]	Intraday Amount Found [µg/ml]		Inter day Amount Found [µg/ml]	
		Mean ± S.D.	**% RSD [n= 3]**	**Mean ± S.D.**	**% RSD [n= 3]**
EST	4	322.99±0.60	0.34	324.43±1.95	0.69
	6	498.19±1.02	0.55	499.14±0.63	0.41
	8	657.58±0.75	0.27	652.00±0.53	0.38
SDF	50	498.87±0.23	0.29	497.09±1.38	0.28
	75	738.13±2.47	0.27	765.24±0.62	0.50
	100	1001.09±0.62	0.35	992.21±0.26	0.34

Table No. 3: Results of repeatability study

Drug	Conc. [µg/ml] [n=6]	Peak Area	Mean[µg/ml] ± SD	% RSD
EST	4	325.58	322.99±0.62	1.26
SDF	50	497.87	498.87±0.31	1.677

Table No. 4: Results of recovery studies

Drug	Initial amount [µg/ml]	Amount added [µg/ml]	Amt. recovered ± S.D. [µg/ml, n =3]	% Recovery	% RSD
EST	2	1.6	1.15±0.013	99.00±0.79	1.49
	2	2.0	1.96±0.015	97.84±0.73	1.45
	2	2.4	2.34±0.020	97.59±0.84	1.42
SDF	10	8	7.88±0.010	98.55±0.12	1.83
	10	10	10.13±0.07	101.27±0.77	1.67
	10	12	12.22±0.04	101.84±0.35	1.58

Table No. 5: Robustness evaluation of the HPLC method

Chromatographic conditions	EST			SDF		
	Tailing (T')	**Capacity Factor (K')**	**Theoretical Plate (N)**	**Tailing (T')**	**Capacity Factor (K')**	**Theoretical Plate (N)**
A: Mobile phase pH						
2.8	1.26	1.23	2683.9	1.28	0.99	7591.4
3.0	1.22	1.27	2683.5	1.23	1.09	7632.5
3.2	1.21	1.33	2625.5	1.25	1.15	7414.7
Mean ±SD	1.23±0.02	1.27±0.05	2687.63±36.80	1.25±0.02	1.07±0.02	7546.2±115.7
B: Flow rate (ml/min.)						
0.7 ml	1.23	0.98	2723.8	1.26	0.76	7587.3
0.9 ml	1.16	1.08	2818.9	1.29	1.10	7668.8
1.1 ml	1.15	1.09	2768.7	1.22	0.88	7423.5
Mean ±SD	1.18±0.04	1.05±0.06	2770.47±47.50	1.25±0.03	0.91±0.17	7593.2±75.82
C: Percentage methanol in mobile phase (v/v)						
80	1.09	1.22	2646.2	1.18	0.87	7623.8
90	1.06	1.13	2687.4	0.94	0.95	7667.3
100	1.19	1.18	2638.3	1.23	0.87	7433.2
Mean ±SD	1.11±0.06	1.17±0.04	2657.3±26.36	1.11±0.15	0.89±0.04	7574±124.51

Table No. 6: System suitability test

EST		SDF	
System suitability parameters	**Proposed method**	**System suitability parameters**	**Proposed method**
Retention time (Rt)	2.183	Retention time (Rt)	7.866
Capacity factor (K')	1.18	Capacity factor (K')	0.98
Theoretical plate (N)	2838.7	Theoretical plate (N)	7465.8
Tailing factor (T)	1.16	Tailing factor (T)	0.95

Table No.7: Forced degradation

Sample Exposure condition	Total Number of products with their Rt	EST		SDF	
		Degradation remained (4 µg/ml)	Recovery (%)	Degradation remained (50 µg/ml)	Recovery (%)
Acidic, 1N, 1 Hr	4 (2.191, 2.281, 2.444,7.590)	3.632	90.81	47.091	94.18
Basic, 1N, 1 Hr	6 (2.61, 2.80, 2.95, 3.38, 4.51, 7.20)	3.259	81.48	22.292	44.29
Per oxide, 30%, 1 Hr	4 (2.63, 2.83, 4.76, 7.03)	3.426	85.67	34.865	69.73
Heat, 50^{o}C, 1 Hr	3 (2.61,2.81,6.766)	3.642	91.05	37.005	74.01

Table No. 8: Probable trial runs using 3^3 full fraction designs

Std	Run	Factor 1	Factor 2	Factor 3	Response 1	Response 2	Response 3	Response 4
		A:Flow rate	B:Methanol	C:Wave length	RT	PA	TP	TF
		ml/min	%	Nm				
1	1	0.7	3	230	3.45	4850.37	11675	0.82
11	2	0.8	2.3	231	2.95	4019.71	10892	0.84
5	3	0.7	3	232	3.369	4521.28	11693	0.83
9	4	0.6	4	231	3.89	5516.24	12789	0.8
3	5	0.7	5	230	3.33	4896.5	11458	0.83
6	6	0.9	3	232	2.61	3555.04	9810	0.86
7	7	0.7	5	232	3.32	4665.06	11373	0.82
13	8	0.8	4	229.3	2.92	4373.36	10645	0.84
2	9	0.9	3	230	2.62	3755.37	9777	0.85
10	10	0.8	4	231	2.62	3707.75	9733	0.86
4	11	0.9	5	230	2.71	4018.88	9950	0.86
8	12	0.9	5	232	2.69	3785.56	9793	0.85
12	13	0.8	5.7	231	3	4326.7	10679	0.84
14	14	0.8	4	232.7	3	4484.22	10716	0.84

Table No. 9: ANOVA for Reduced Quadratic Model (Response 1: RT)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.95	7	0.2783	211.69	< 0.0001	significant
A-Flow rate	1.10	1	1.10	833.33	< 0.0001	
B-Methanol	0.0005	1	0.0005	0.4083	0.5464	
C-Wavelength	0.0000	1	0.0000	0.0124	0.9149	
AB	0.0144	1	0.0144	10.93	0.0163	
A^2	0.1479	1	0.1479	112.53	< 0.0001	
B^2	0.0882	1	0.0882	67.09	0.0002	
C^2	0.0810	1	0.0810	61.60	0.0002	
Residual	0.0079	6	0.0013			
Cor Total	1.96	13				

Table No. 10: ANOVA for Reduced Linear model (Response 2: PA)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	3.294E+06	1	3.294E+06	57.63	< 0.0001	significant
A-Flow rate	3.294E+06	1	3.294E+06	57.63	< 0.0001	
Residual	6.858E+05	12	57151.71			
Cor Total	3.979E+06	13				

Table No. 11: ANOVA for Reduced Quadratic model (Response 3: TP)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	1.120E+07	7	1.600E+06	288.01	< 0.0001	Significant
A-Flow rate	6.682E+06	1	6.682E+06	1202.92	< 0.0001	
B-Methanol	40072.40	1	40072.40	7.21	0.0363	
C-Wavelength	358.64	1	358.64	0.0646	0.8079	
AB	60031.13	1	60031.13	10.81	0.0167	
A^2	7.371E+05	1	7.371E+05	132.68	< 0.0001	
B^2	7.252E+05	1	7.252E+05	130.54	< 0.0001	
C^2	5.848E+05	1	5.848E+05	105.27	< 0.0001	
Residual	33330.78	6	5555.13			
Cor Total	1.123E+07	13				

Table No. 12: ANOVA for Reduced Quadratic model (Response 4: TF)

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.0040	7	0.0006	646.40	< 0.0001	Significant
A-Flow rate	0.0020	1	0.0020	2334.61	< 0.0001	
B-Methanol	0.0000	1	0.0000	0.0000	1.0000	
C-Wavelength	0.0000	1	0.0000	0.0000	1.0000	
BC	0.0002	1	0.0002	228.17	< 0.0001	
A^2	0.0004	1	0.0004	427.66	< 0.0001	
B^2	0.0003	1	0.0003	298.35	< 0.0001	
C^2	0.0003	1	0.0003	298.35	< 0.0001	
Residual	5.259E-06	6	8.765E-07			
Cor Total	0.0040	13				

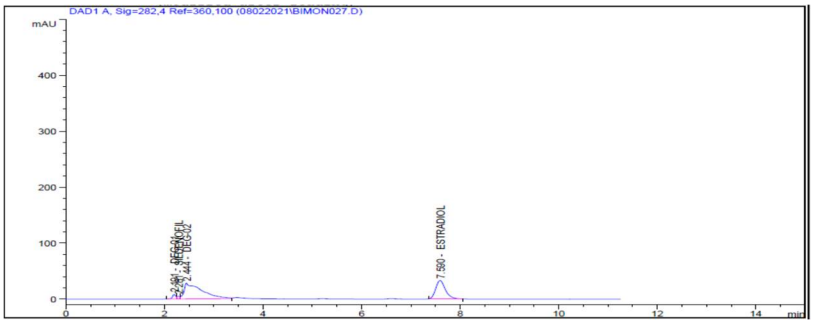


Fig. No. 5: Acidic degradation

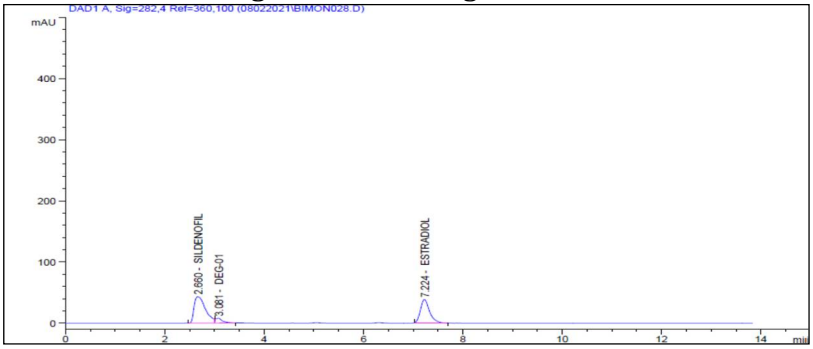


Fig. No. 6: Alkaline degradation

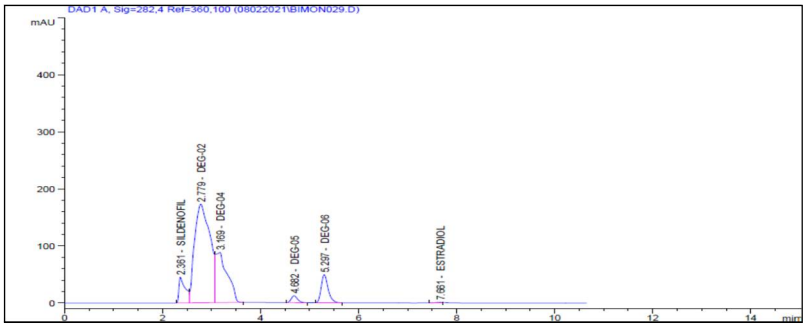


Fig. No. 7: Peroxide degradation

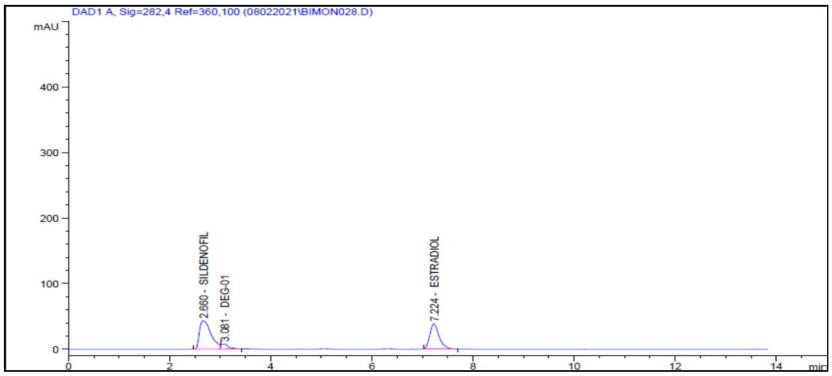


Fig. No. 8: Heat degradation

CONCLUSION

A simple, rapid, reliable, robust and optimized reversed phase high performance liquid chromatographic method for estimation of Estradiol Valerate and Sildenafil was successfully developed and validated as per International Conference on Harmonization guidelines. Percentage of mobile phase, flow rate and wave length wereoptimised by using QbD approach *i.e.* 3^3 factorialdesign. There are no interfering peaks in degradation conditions. Therefore, a sensitive, accurate and stability indicating method was developed with high degree of practical utility.

Acknowledgments

The authors are grateful thanks to the Management and Principal of Gangamai College of Pharmacy, Nagaon, Dhule, Maharashtra, for providing timely support for the research work.

Conflicts of interest

The authors declare no conflicts of interest.

REFERENCES

1. Raman, N. V. V. S. S., Mallu, U. R., & Bapatu, H. R. (2015). Analytical quality by design approach to test method development and validation in drug substance manufacturing. *Journal of chemistry*, *2015*.1-8.
2. Patel, M. N., & Kothari, C. S. (2016). Multivariate approaches for simultaneous determination of avanafil and dapoxetine by UV chemometrics and HPLC-QbD in binary mixtures and pharmaceutical product. *Journal of AOAC International*, *99*(3), 649-663.
3. Tol, T., Kadam, N., Raotole, N., Desai, A., & Samanta, G. (2016). A simultaneous determination of related substances by high performance liquid chromatography in a drug product using quality by design approach. *Journal of Chromatography A*, *1432*, 26-38.
4. Boyle, P. J., King, A. B., Olansky, L., Marchetti, A., Lau, H., Magar, R., & Martin, J. (2002). Effects of Estradiol and rosiglitazone on blood lipid levels and glycemic control in patients with type 2 diabetes mellitus: a retrospective review of randomly selected medical records. *Clinical therapeutics*, *24*(3), 378-396.
5. Inukai, K., Watanabe, M., Nakashima, Y., Takata, N., Isoyama, A., Sawa, T., ... & Katayama, S. (2005). Glimepiride enhances intrinsic peroxisome proliferator-activated receptor-γ activity in 3T3-L1 adipocytes. *Biochemical and biophysical research communications*, *328*(2), 484-490.
6. Yilmaz, B. (2010). Simultaneous determination of estradiol valerate and medroxyprogesterone

acetate in a tablet formulation by gas chromatography–mass spectrometry. *Analytical Sciences*, *26*(3), 391-393.
7. Junker, B., Zablackis, E., Verch, T., Schofield, T., & Douette, P. (2014). Quality-by-Design: as related to analytical concepts, control and qualification. In *Vaccine Analysis: Strategies, Principles, and Control* (pp. 479-520). Berlin, Heidelberg: Springer Berlin Heidelberg.
8. Guideline, I. H. T. (2005). Validation of analytical procedures: text and methodology. *Q2 (R1)*, *1*(20), 05.
9. Elder, D. P., & Borman, P. (2013). Improving analytical method reliability across the entire product lifecycle using QbD approaches. *Pharmaceutical Outsourcing*, *14*(4), 14-19.
10. Peraman, R., Bhadraya, K., & Padmanabha Reddy, Y. (2015). Analytical quality by design: a tool for regulatory flexibility and robust analytics. *International journal of analytical chemistry*, *2015*.
11. Ahmad, S., Ashwin, B., Rahman, B. A., Gorad, R. U., & Tare, H. (2022). Quality by design (QBD) approach to develop stability indicating RP-HPLC method development for naproxen and pantoprazole. *International Journal of Health Sciences*, *6*, 11450-11465.
12. Ahmad, S. U. F. I. Y. A. N., Rageeb, M., & Usman, M. (2017). Analytical method development and validation for the simultaneous estimation of emtricitabine and tenofovir by reversed phase high performance liquid chromatography in bulk and tablet dosage forms. *Asian J Pharm Clin Res*, *10*(11), 59-64.
13. Patel, M. M., & Patel, H. D. (2016). Development and validation of HPTLC method for simultaneous estimation of Terbinafine hydrochloride and Mometasone furoate in combined dosage form. *Journal of the Chilean Chemical Society*, *61*(2), 2958-2962.
14. Tol, T., Tawde, H., Gorad, S., Jagdale, A., Kulkarni, A., Kasbale, A., ... & Samanta, G. (2020). Optimization of a liquid chromatography method for the analysis of related substances in daclatasvir tablets using design of experiments integrated with the steepest ascent method and Monte Carlo simulation. *Journal of Pharmaceutical and Biomedical Analysis*, *178*, 112943.

CITATION OF THIS ARTICLE

Sufiyan Ahmad, Patil Mohanesh, Vishal Lad, Bakhshi Abdul Rahman, Aditi Kulkarni. Quality by Design (Qbd) Approach to Develop Stability Indicating RP-HPLC Method Development and Validation for Estradiol Valerate and Sildenafil. Bull. Env. Pharmacol. Life Sci., Vol 12[3] Feb 2023: 77-86