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SPECTROSCOPIC METHOD DEVELOPMENT AND VALIDATION FOR ESTIMATION OF APIXABAN IN BULK AND PHARMACEUTICAL FORMULATION BY QbD APPROACH

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ABSTRACT
A simple, sensitive, rapid and reproducible UV method has been developed and validated for Calibration determination of Apixaban in bulk and in pharmaceutical formulation by applying Quality by Design. For development of UV method for Apixaban various trials are performed by using Stasticalsoftware by applying 3 level factorial design. Quantitative method development by optimization from trials by stastical software. The Optimized method Desirability **is** 1 for methnol:Buffer (30:70) in 8 pH at maximum Wavelength 277.8. Optimised Standard curve showed a regression coefficient is 0.999. The method was validated as per ICH guidelines. The precision and repeatability results showed % RSD less than 2%.

KEYWORDS: Quality by Design, Apixaban, UV, Stastical Software.

INTRODUCTION
Apixaban is an organic, heterocyclic compound with a phenylpiperidine skeleton (Fig. 1). It has the molecular formula, C25H25N5O4, weighing 459.5 g/mol.[27,28,29] Apixaban tablet is used for the prevention of serious blood clots. Apixaban belongs to the class of drugs called anti-coagulant drugs. This drug acts by inhibiting certain coagulation factors that are involved in clotting, which helps to prevent clot formation.[27,28,29] There were few methods are reported for analysis of Apixaban for Ultra-Voilet Spectroscopy(UV)[4,5], High Pressure Liquid Chromatography(HPLC)[4,7], High Pressure Thin Layer Chromatography(HPTLC).[6]

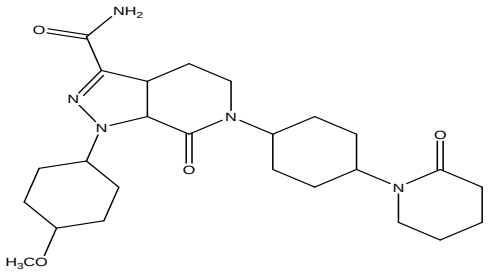


Fig. 1: Structure of Apixaban.

Quality by Design (QbD) is a concept first outlined by well-known quality expert Joseph M. Juran in various publications. [Quality by Design (QbD) is defined ICH guidelines Q8(R2) as "a systematic approach to development that begins with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management. The main two factors of QbD technique is by Developing a Design of experiments(DOE) and to establish a validated test method as per ICH guidelines.[8] Application of QbD concept to the UV-Visible method development leads to a more robust method. Design of Experiment is done by the Stastical software.

This research paper is based on a "3 Level Factorial design". 3 level factorial design is a miscellaneous, flexible to get trial for design of experiment.

MATERIAL AND METHOD
1. Drug and Chemicals
Apixaban API
Apixaban Formulation: Each Film Coated Tablet contains Apixaban 2.5mg, Pfizer Ltd.

Methanol, Disodium Hydrogen Phosphate, Potassium Dihydrogen Phosphate, Sodium chloride: all these chemicals are purchased from Shivaji Scientific.

2. Apparatus
Volumetric Flask, Beaker, Pipette, Funnel.

3. Instrument
Ultraviolet Spectrophotometer
Double beam UV-VIS spectrophotometer (UV-1800, Shimadzu, Japan) Software- UV probe (Version-2.35) Bandwidth-2 nm and wavelength accuracy of ± 0.3 nm

Cuvette pair of 10 mm matched quartz cells, The other instruments used during the process such as hot air oven, pH meter, digital weighing balance, filtration assembly with vacuum.

4. Preparation of Stalk Solution

Weighed accurately about 100mg of Apixaban drug, which is Dissolve in various combination of solvent that are Methanol:Water, Methanol:Phosphate Buffer, Acetonitrile:Water, Acetonitrile:Phosphate Buffer, Ethanol:Water, Ethanol:Phosphate Buffer, make up volume with it.

5. Preparation of Phosphate Buffer

Weighed accurately about 2.38gm Disodium Hydrogen Phosphate, 0.19gm Potassium Dihydrogen Phosphate, 8gm sodium chloride in 100ml volumetric flask and dissolve and make up volume in Distilled Water. Adjust ph 6-8 by adding 0.1N HCL and 0.1N NaOH.

RESULT AND DISCUSSION

1. Design of Experiment

This design of experiment is done by the "Statistical Software". In that software the various factors are there, from that the 3 Level Factorial design selected for Apixaban. The various dependent (pH, Solvent) and independent (absorbance, wavelength) factor are selected for UV, which is directly or indirectly affect the result of UV.

2. Three Level Factorial Design

3 Level Factorial design is very Flexible, Miscellanious design.

Then software gives a design with 8 runs for every solvent. Then the optimization of accurate method and solvent from are selected by software and their validation is performed.

3. Solvent Solvent

1. Methanol:Water
2. Methanol:Phosphate Buffer
3. Acetonitrile:Water
4. Acetonitrile:Phosphate Buffer
5. Ethanol:Water
6. Ethanol:Phosphate Buffer

4. Ranges

4.1 Ranges of Dependent Factors are follow

i. Solvent: 20-40 (for Water and Buffer)
ii. pH: 6-8

4.2 Independent Factor

i. Absorbance
ii. Wavelength

5. Optimization

5.1 Result

Table 1: Apixaban trial in solvent ratio of 30:70 at pH 8.

Sr no.	Solvents	Observations	Results
1	Methanol:Water	Less Broad peak is observed.	Non-Satisfactory
2	Methanol:Phosphate Buffer	Broad peak is observed	Satisfactory
3	Acetonitrile:Water	Less Broad peak is observed.	Non-Satisfactory
4	Acetonitrile:Phosphate Buffer	peak was not observed	Non-Satisfactory
5	Ethanol:Water	Broad peak is observed.	Satisfactory
6	Ethanol:Phosphate Buffer	Less Broad peak is observed	Non-Satisfactory

5.2 Optimized trials suggested by software based on desirability value

This methodology is initially based on constructing a desirability function for each individual response. The scale of individual desirability function ranges between i= 0, for completely undesirable response and i =1, for fully desired response. Selection of trial was based on maximum desirability value. Therefore, first trial which was having desirability one (i=1) selected for method optimization.

Table 2: Optimized trials suggested by software based on desirability value.

Number	pH	Solvent	Absorbance	Maximum wavelength	Desirability	
1	8.00	30.00	0.356782	277.885	1.000	Selected
2	8.00	28.76	0.344694	277.947	1.000	
3	8.00	32.80	0.432591	277.745	1.000	
4	8.00	30.37	0.362954	277.866	1.000	
5	8.00	24.30	0.410376	278.169	0.971	

5.3.1 Optimized Spectroscopically conditions

The Optimized result of Apixaban is at 8 pH, Solvent combination of Methanol:Water(70:30) at Maximum Wavelength 277.885 nm gives 1 Desirability value.

6. Effect of independent variables

6.1 Effect of independent variables on absorbance (Y_1)

The equation for response surface quadratic model is as follows

$Y_1 = +0.55 -0.082 X_1 + 0.32X_2 -0.17 X_1X_2 -0.11 X_1^2 +0.43 X_2^2$

Where, X_1= A, X_2 = B

a graphical representation of amount of pH (A) and Solvent (B), An increase in pH resulted in decrease in Absorbance (Y_1), while increase in Solvent resulted in increase in Absorbance (Y_1). Combination of amount of pH and Solvent showed decrease in response.

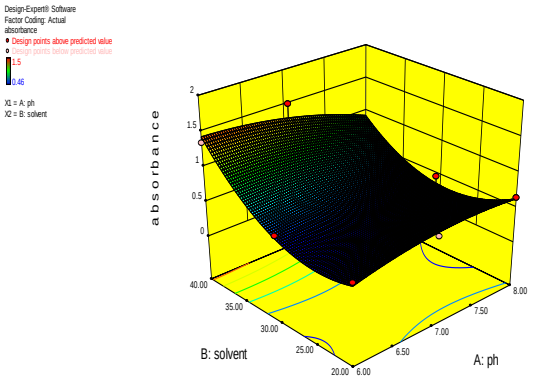


Fig. 2: Three-dimensional plot for Maximum Wavelength as a function of pH and Solvent.

Fit summary: Quadratic model was suggested by the software.

ANOVA: ANOVA of developed Full three level factorial model for retention time (Y_1).

Values of "Prob > F" (p- value) less than 0.1000 indicate model terms are significant.

In this case A, B, BC, A^2 and C^2 are significant model terms.

Table 3: Significance of *p* value on model terms of Absorbance.

Model terms	*p* value	Effect of factor	Remarks
A (X_1)	0.11118	-0.082	Insignificant
B (X_2)	**0.0002**	+0.32	**Significant**
AB (X_1X_2)	**0.0187**	-0.17	**Significant**
A^2 (X_1^2)	0.1400	-0.11	Insignificant
B^2 (X_2^2)	**0.0003**	+0.43	**Significant**
Overall model	**0.0004**	-	**Significant**

For response Y_1, factor pH and Solvent was having synergistic effect with *p* value 0.0004. Therefore, we can conclude that increase in pH was responsible for increment in absorbance and thus showed the direct relationship between them.

Combination of amount of Solvent was responsible for significant increase in absorbance with significant *p* value of 0.0002. Therefore, direct relationship between this combination and response is observed. Exponential terms (X^2_1) and (X^2_2) also showed direct relationship with response having significant *p* values 0.1400 and 0.0003 respectively. Factor pH and its exponential term differs from zero with a great margin therefore they were having more significant effect than other factors. Therefore, it is concluded that pH of buffer and its exponential term was responsible for significant change in response i.e. Absorbance.

6.2 Effect of independent variables on maximum wavelength (Y_2)

After applying experimental design, suggested Response Surface Linear Model was found to be significant with model F value of 23.79, *p* value less than 0.005 and R^2 value of 0.7563. There is only a 0.01% chance that a "Model F-Value" this large could occur due to noise. Values of % C.V. and adjusted R^2 were 3.78 and 0.7245 respectively. The model for response Y_2 (tailing factor) is as follows:

$Y_2 = +278.38 -0.50X_1 -0.50X_2$

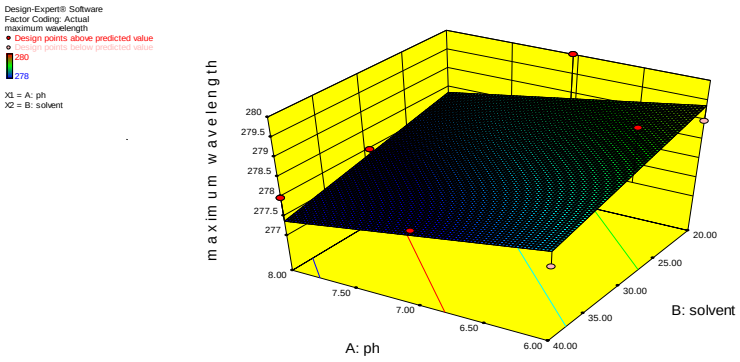


Fig. 3: Three-dimensional plot for Maximum Wavelength as a function of pH and Solvent.

Fit summary: Response Surface Linear Model was suggested by the software.
ANOVA: ANOVA of developed CCD model for tailing factor (Y_2).

Values of "Prob > F" (p- value) less than 0.1000 indicate model terms are significant.
In this case A, B are significant model terms.

Table 4: Significance of *p* value on model terms of Maximum wavelength.

Model terms	*p* value	Effect of factor	Remarks
A (X_1)	**0.0841**	-0.50	Significant
B (X_2)	**0.0841**	-0.50	Significant
Overall model	0.0635	-	**Significant**

For response Y_2, factor X_1 and X_3 was having synergistic effect with *p* value 0.0841 and 0.0841. Therefore, we can conclude that increment in amount of pH and Solvent was responsible for increase in Absorbance and thus showed the direct relationship between them. Terms X_2 were responsible for significant increase in Maximum Wavelength with *p* values 0.0841.

Validation

1. Linearity

The linearity was obeyed by Beer's law in the range of 5-30ug/ml and regression coefficient found to be 0.9987.

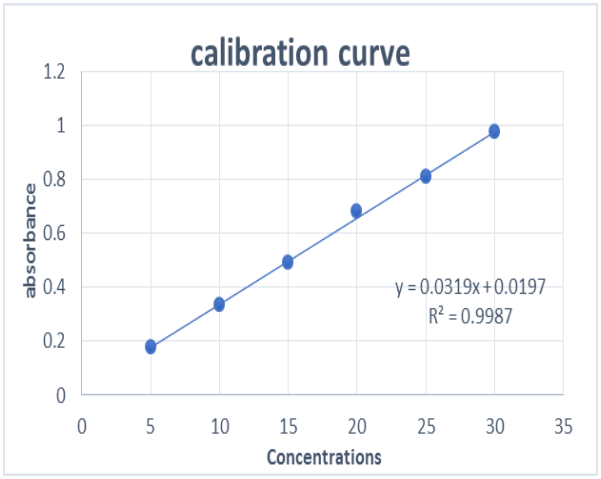


Fig. 4: Calibration of Apixaban.

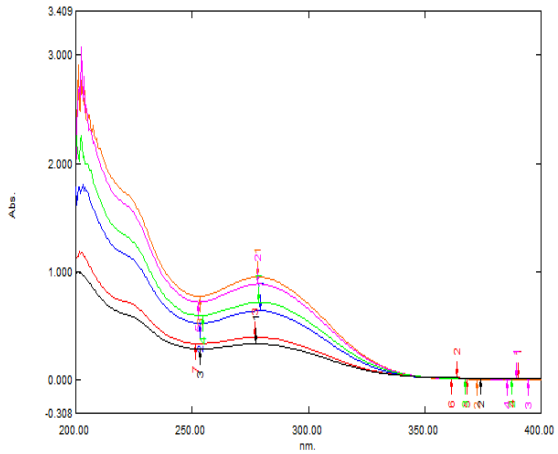


Fig. 5: Overlain of Apixaban.

2. Precision

The precision of an analytical procedure expresses the closeness of between the series of measurements obtained from multiple sampling of the same homogeneous sample under the prescribed conditions. The 6 concentrations of 20ug/ml at 277.8nm for interday and intraday precision. Results obtained are shown in table 5.

Table 5: Interday and Intraday precision of Apixaban at 277.8 nm.

	Precision Interday				Precision Intraday		
	0 min	1 hr	2 hr		1 day	2 days	3 days
SD	0.008758	0.012307	0.015336	SD	0.009042	0.011454	0.013277
RSD%	1.873338	1.921996	1.909867	RSD	1.586866	1.604238	1.549791

3. Robustness

Robustness is a measure of capacity of a method to remain unaffected by small, but deliberate variations in the method conditions, and is indications of the reliability of the method is robust, if it is unaffected by small changes in operating conditions. Prepare 6 concentrations of 20 ug/ml by water:methanol and water:methanol. result obtain is shown in table 6.

Table 6: Robustness of Apixaban at 277.8 nm.

	Absorbance of Apixaban at 277.8 nm	
	Buffer:Methanol	Water:Methanol
SD	0.012506	0.010113
RSD%	1.559351	1.44124

4. Ruggedeness

This is reproducibility of the results when the method is performed under actual use conditions. This includes different analyst, laboratories, column, instruments, sources of reagents, chemicals, solvents and so on.

Table 7: Ruggedeness of apixaban at 277.8 nm.

	Absorbance of Apixaban at 277.8 nm	
	277.8	250
SD	0.004041	0.004041
RSD%	1.599519	1.538624

5. Sensitivity

The limit of detection (LOD) and limit of quantification (LOQ) were calculated by using the equations LOD = 3 x σ / S and LOQ = 10 x σ /S, where σ is the standard deviation of intercept, S is the slope. The LOD and LOQ were found to be 2.838193 and 8.600586.

6. Accuracy

Accuracy of analytical procedure expresses the closeness of agreement between the value which is accepted either as a conventional true value or an accepted reference value and the value found. Prepare 6 concentrations of 32ug/ml, 40ug/ml and 48ug/ml from stock solution of bulk and pharmaceutical formulation. Results obtained are shown in Table 8.

Table 8: Accuracy for Apixaban at 277.8nm.

Actual conc.	Observed concentration	Recovery %	%RSD
16	15.89	99.34211	0.330544
20	20.09	100.4747	1.13778
24	23.98	99.9398	0.447184

CONCLUSIONS

A simple, sensitive, precise, accurate, rapid and reproducible UV method by applying QbD Method at 3 Level Factorial Design, has been developed and validated of Apixaban in bulk and in pharmaceutical formulation.

These all research describe the method is vary with change in solvent and its combination, pH, Wavelength and the identification of best method.

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