Research Article

Quality by Design assisted the Ultraviolet Spectroscopic Method Development of Donepezil hydrochloride in Bulk and Dosage form

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ABSTRACT

Background and Objectives: A novel ultraviolet spectroscopic method was developed and validated for the drug Donepezil Hydrochloride using the latest Quality by Design software as per (ICH) guidelines. This modern scientific technology has been implemented to study the impact of independent factors affecting the resultant absorbance of the drug and the selection of the solvent.

Methods: A new method was developed by ultraviolet spectroscopy and the developed method was validated by using different validation parameters.

Results: Donepezil showed maximum absorbance at 250 nm in methanol. The concentration range for linearity was detected in the range of 60-100µg/ml. The accuracy was found to be in the range of 98-100%. The chemical behavior of the drug and drug product was indicated by conducting forced degradation studies. The LOD and LOQ were determined as 2.507 µg/ml and 7.599 µg/ml.

Conclusion: The results indicate that the QbD-assisted UV spectroscopic method for donepezil hydrochloride was simple, accurate, and precise. Therefore, it is suitable for routine analysis in quality control tests.

KEYWORDS: LOD, LOQ, ICH, Quality by design.

INTRODUCTION:

Dementia is a generalized word for various ailments such as the inability to recollect, think, and fulfill day-to-day activities. Such a condition worsens over some time. Dementia is generally noticed in elderly patients.

The following are the causes that gradually lead to dementia:

- age
- hypertension
- diabetes mellitus
- obesity
- smoking
- alcohol addiction
- being physically passive
- anxiety

At present, around 55 million people have been diagnosed with dementia, of which 33 million belong to low or average-income countries. Each year, new 10 million cases are recorded. Dementia is a result of certain diseases and head injuries. One of the common forms of dementia is Alzheimer's disease, where 60-70% of cases are reported[1]. Recently, dementia has been stated as the seventh primary cause of death. It leads to intellectual inability and reliance among older patients. Management for dementia cost economically 1.3 trillion US dollars in 2019, of which 50% is accountable to immediate family or care providers who give care of around 5 hours per day. Female patients undergo higher disability-adapted life and death due to dementia, yet 70% of care hours are provided to the people living with dementia.

Quality by Design (QbD) is a systematic approach to pharmaceutical development that focuses on predefined objectives, understanding the product and process, and implementing process control based on scientific principles and quality risk management[2].

QbD emphasizes the value of outlining quality in various steps of production from the very beginning, instead of examining quality at the end of production. By integrating quality considerations throughout the development process, including the identification and control of potential risks, QbD aims to create high-quality pharmaceutical products consistently.

This approach aligns with principles outlined in regulatory guidelines, such as those provided by the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH). Adhering to QbD principles can lead to more efficient development processes, reduced variability, and ultimately, improved patient outcomes.

Advantages:

Qbd offers several benefits, both from a business perspective and also in terms of scientific rigor[3].

- Through QbD, companies can minimize batch failure, reducing costly setbacks and ensuring consistent product quality.
- It helps in reducing deviations, which in turn saves resources that would otherwise be spent on investigations and corrective actions.

- Mitigating the risk of regulatory compliance issues for maintaining market presence and avoiding potential fines or product recalls.
- QbD is firmly grounded in scientific principles, emphasizing a methodical and risk-based outlook to product development.
- By integrating scientific knowledge and understanding into the development process, QbD enables informed decision-making at every stage.

Donepezil hydrochloride[4] is a hydrochloride salt that belongs to the class of acetylcholinesterase inhibitors and is effectively used in the treatment of dementia. It acts through the inhibition process of acetylcholinesterase which leads to obstruction of hydrolysis of the acetylcholine neurotransmitter. It improves the overall neurological function in Alzheimer's disease and also reduces the sedation in cancer patients due to the result of opioid treatment. It is a USFDA-approved drug for the therapy of mild to moderate dementia in Alzheimer's disease. It is currently being marketed under the brand name Aricept. The IUPAC name of donepezil hydrochloride is 2-[(1-benzylpiperidin-4-yl) methyl]-5,6-dimethoxy-2,3-dihydroinden-1-one; hydrochloride.

Donepezil hydrochloride **Figure 1** is a hydrochloride salt of piperidine derivative which possesses a neurocognitive enhancing activity. In this work, authors tried to develop a new QBD-assisted method development and validate all the parameters of Donepezil hydrochloride by UV spectrophotometry.

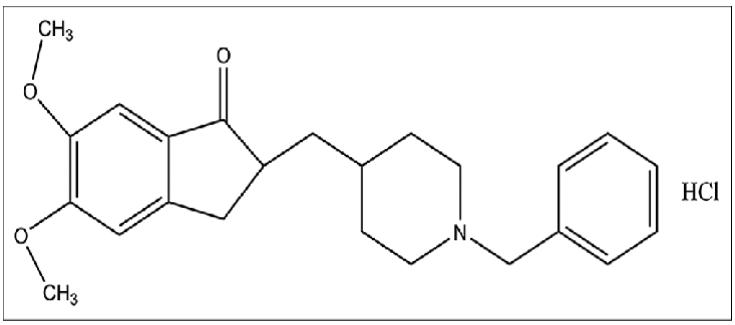

Figure 1: Structure of Donepezil hydrochloride

MATERIALS & METHODS:

Instruments:

The UV/Vis spectrophotometer employed in the validation process of donepezil was a UV3200 model from Lab India. Other instruments used were Weighing balance, Hot air oven, UV chamber, Rotary shaker, FTIR

Chemicals and reagents:

The API Donepezil hydrochloride was collected from Dr. Reddy's Laboratories.

Selection of Solvent with QBD software:

To select the appropriate solvent for dissolving donepezil hydrochloride, the QbD software was used. Through this software, numerous trials were conducted with different kinds of solvents like 0.1 N HCL, 0.1N NaOH, Acetonitrile, and methanol and tested for the

maximum absorbance of donepezil hydrochloride. The maximum absorbance (λ max) was found to be at 250 nm in methanol. Therefore, methanol was selected as the solvent for the donepezil hydrochloride validation process.

Impact of independent variables on λ max:

The QbD software- Design Expert 13 was applied to check the impact of independent variables on dependent variables. Sonication time, ratio of solvent, and scan speed were selected as independent variables. The impact of these variables on absorbance (dependent variable) was checked and tabulated in **TABLE 1**

	Factor 1	Factor 2	Factor 3
Run	A: Sonication(min)	B: Solvent (%)	C: Scan Speed
1	15	95	Fast
2	15	95	Fast
3	10	100	Fast
4	15	90	Slow
5	15	90	Medium
6	10	90	Fast
7	20	95	Slow
8	10	95	Slow
9	15	100	Medium
10	10	95	Medium
11	15	100	Slow
12	20	100	Fast
13	20	95	Medium
14	20	90	Fast
15	15	95	Fast
16	20	95	Slow

TABLE-1: Independent variables

Preparation of Standard Stock Solution:

A stock solution of donepezil hydrochloride [5] 1mg/ml solution was prepared.

Preparation of working standard solutions:

From the above stock solution, a series of dilutions of concentrations of 60,70,80,90,100 µg/ml were prepared as working standard solutions.

Preparation of test solution:

The average weight of 10 powdered tablets was taken and the weight equivalent to 1mg powder was measured and a stock solution of the drug product was prepared [6]. From this solution, serial dilutions were made.

Determination of absorbance maxima (λ max): A 10µg/ml was used to measure the absorbance of the donepezil hydrochloride solution. The solution was examined in the range of 200-400 nm. λ max of donepezil hydrochloride was noted at 250 nm.

Using the UV spectroscopy method, donepezil hydrochloride is further validated by the QbD process.

METHOD VALIDATION [7]:

The following parameters were validated for donepezil hydrochloride.

Linearity:

An array of solutions was prepared at concentrations of 60-100 µg/ml. These sample solutions were scanned for absorbance at 250 nm. Beer-Lambert's law compliance was checked by plotting a graph between concentration vs. absorbance. Linearity complied with Beer's law and the values were in optimal range.

Precision:

Intra-day and Inter-day studies were conducted on one particular concentration solution from a linearity range and the absorbance of that particular concentration was measured six times a day at different time intervals and also Inter-day absorbance was measured and the %RSD was calculated.

Accuracy: Three percentage levels of concentration were selected i.e. 50%, 100%, and 150% for accuracy determination [8]. All the sample solutions were spiked at 70µg/ml concentration with drug product and the three replicates of concentrations of 35,70,105 ppm at each percentage were prepared and scanned in the UV spectrophotometer at 250 nm to measure the absorbance and calculated the % recovery.

$$\%\text{Recovery} = \frac{A^0mount\ found}{Amount\ taken} \times 100$$

Robustness:

This test was performed by evaluating values at two different wavelengths (250 ±1). The absorbance was recorded and %RSD was established.

Ruggedness:

The sample solutions were prepared and checked for various analytical parameters like different analysts and different laboratory conditions.

Forced Degradation Studies:

These are a type of stability testing studies that describe the degradation rate of the drug when it undergoes certain stressful conditions that might occur during storage and after administration to the body[9]. The following are the several types of forced degradation studies.

Acid Degradation:

A solution of 6 ml of 10 ppm drug solution and 10 ml of 0.1N HCl was taken in a 50 ml graded volumetric flask and kept in a rotary shaker for 6 hours. To neutralize the solution 0.1N NaOH is used and volume is made up with methanol.

Base Degradation:

A solution of 6 ml of 10 ppm drug solution and 10 ml of 0.1 N NaOH was taken into a 50 ml graded volumetric flask and kept in a rotary shaker for 6 hours. To neutralize the solution 0.1N HCl is used and the volume is made up with methanol.

Oxidative Degradation:

A 50 ml volumetric flask is taken and 6 ml of 10 ppm drug solution and 10 ml of 3% H_2O_2 is added and kept in a rotary shaker for 6-7 hours and made up to the mark with methanol.

Thermal Degradation:

In this test, a hot air oven is employed. In this study,5 mg of donepezil hydrochloride was taken in a petri dish and placed in a hot air oven for 6 hours. After cooling, the drug was taken into a 50 ml volumetric flask and the solution was made up of methanol.

UV Degradation: In this study, 5 mg of API was taken in a petri dish and placed in a UV chamber at a wavelength of 256 nm for around 6 hours. Later this Petri dish was removed and the drug was taken into a 50 ml marked volumetric flask and the volume was formulated with methanol.

Limit of detection and limit of quantification:

The parameters limit of detection and limit of quantification were determined using one of the three methods i.e., Residual SD of the regression line[10]. The following equations are as per ICH (International Conference on Harmonization) guidelines.

$$\mathbf{LOD=3.3\ x\ \sigma/S}$$

$$\mathbf{LOD=10\ x\ \sigma/S}$$

Where σ = the standard deviation of the response; S = the slope of the regression line.

RESULTS:

Linearity of Donepezil Hydrochloride:

The linearity of the drug was taken for the serial concentrations of 60,70,80,90,100 µg/ml and absorbance was noted at λmax 250 nm with the help of a UV3200 model -Lab India spectrophotometer. The absorbance was stated at 250 nm correlating to each concentration and was recorded **(Table 2)**. The calibration curve was marked with absorbance on the Y-axis and concentration on the X-axis **(Figure 2)**. The experiment was performed in triplicate and analyzed as ± SD of Donepezil hydrochloride.

S. No	CONCENTRATION(µg/ml)	ABSORBANCE
1	60	0.5531
2	70	0.6606
3	80	0.7567
4	90	0.8413
5	100	0.9354

Table 2: Linearity study for Donepezil Hydrochloride

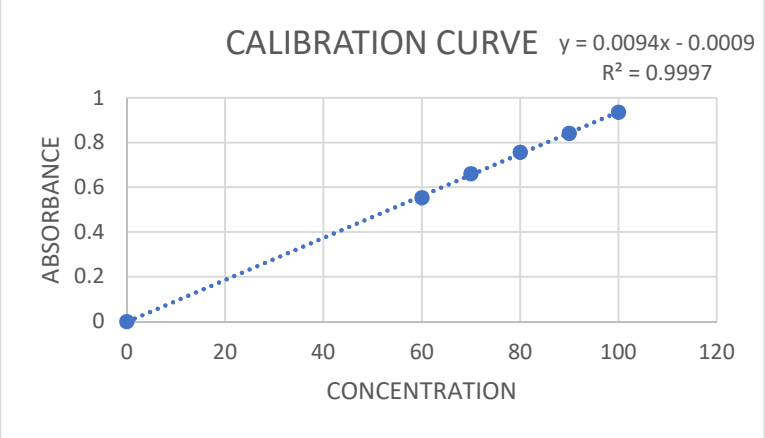

Figure 2: Calibration curve of Donepezil Hydrochloride

Precision:

For this parameter, the method was tested by using a tablet stock solution. Intraday precision was calculated by experimenting six times on the same day and on separate days for Interday precision studies. The obtained results are as below **(Table 3)**

S.no	Intra-day precision Absorbance	Inter-day precision Absorbance
1	0.9251	0.8316
2	0.9259	0.8317
3	0.9261	0.8320
4	0.9268	0.8304
5	0.9271	0.8314
6	0.9273	0.8322
Average	0.926383	0.83155
SD	0.000835	0.000632
%RSD	0.90164	0.075962

TABLE-3: Precision study for Donepezil Hydrochloride

Accuracy:

The accuracy of an analytical method indicates the similarity between the recorded value and reference value. The recovery studies were conducted by another calibrated method. The results recorded were found to be sufficiently good and are as follows. **(Table 4)**

Concentration	Drug Substance	Drug product	%recovery	Mean Recovery	% RSD
50%	35ppm	70ppm	99.8%	98.95	0.8859
50%	35ppm	70ppm	99.02%		
50%	35ppm	70ppm	98.05%		
100%	70ppm	70ppm	100.0%	100.93	0.9416
100%	70ppm	70ppm	100.9%		
100%	70ppm	70ppm	101.9%		
150%	105ppm	70ppm	99.4%	98.9	0.4557
150%	105ppm	70ppm	98.5%		
150%	105ppm	70ppm	98.9%		

TABLE-4: Accuracy study for Donepezil Hydrochloride

Robustness:

The Robustness of the method was calculated by modifying the wavelength ±1 nm from 249nm and 251nm (AML) and the results were satisfactory **(Table 5)**

S.No	Absorbance At 249nm	Absorbance At 251nm
1	0.8528	1.0216
2	0.8480	1.0226
3	0.8491	1.0260
4	0.8405	1.0223
5	0.8489	1.0242
6	0.8493	1.0239
Average	0.8481	1.02344
SD	0.004073	0.00159
%RSD	0.480231	0.15538

TABLE-5: Robustness study for Donepezil Hydrochlorid

Ruggedness:

Ruggedness was achieved by examining the of Donepezil hydrochloride sample with different instruments and analysts. The results were determined in terms of %RSD **(Table 6)**.

Instrument name: Shimadzu			**Instrument name: Lab India**	
Day 1:			**Day 2:**	
	analyst-1	**analyst-2**	**analyst-3**	**analyst-4**
Concentration	**Absorbance**	**Absorbance**	**Absorbance**	**Absorbance**
70PPM	0.8309	0.8201	0.709	0.671
70PPM	0.8307	0.8234	0.708	0.675
70PPM	0.8311	0.8241	0.703	0.660
70PPM	0.8316	0.8301	0.720	0.657

70PPM	0.8322	0.8160	0.701	0.653
70PPM	0.8311	0.8151	0.707	0.652
Average	**0.831267**	**0.821467**	**0.708**	**0.6613333**
SD	**0.000547**	**0.005612**	**0.006633**	**0.009564**
%RSD	**0.065744**	**0.683213**	**0.9369**	**1.446142**

TABLE-6: Ruggedness for Donepezil Hydrochloride

Limit of Detection and Limit of Quantification

The LOD & LOQ were evaluated by the equations LOD = 3.3 × σ/s and LOQ = 10 × σ/s, where σ is the standard deviation of the response areas of the drugs, taken as a measure of noise, and S is the slope of the corresponding calibration plot. The Limit of detection and Limit of quantification were recorded and are found within the limits **(Table 7).**

Parameter	**Type of method**	**Observation**
LOD	Residual SD of regression line	2.507µg/ml
LOQ	Residual SD of regression line	7.599µg/ml

TABLE-7: LOD & LOQ

Degradation studies:

Forced degradation studies were carried out for both drug substance and drug product and results were reported as follows **(Table 8, Figure 3)**

Nature of stress	**%Degradation of API**	**%Degradation of drug product**
Acid degradation	93.84	95.80
Base degradation	90.6	73.34
Oxidation degradation	83.73	94.22
Thermal degradation	93.38	92.54
Light degradation	88.12	93.09

TABLE 8: Forced Degradation Studies

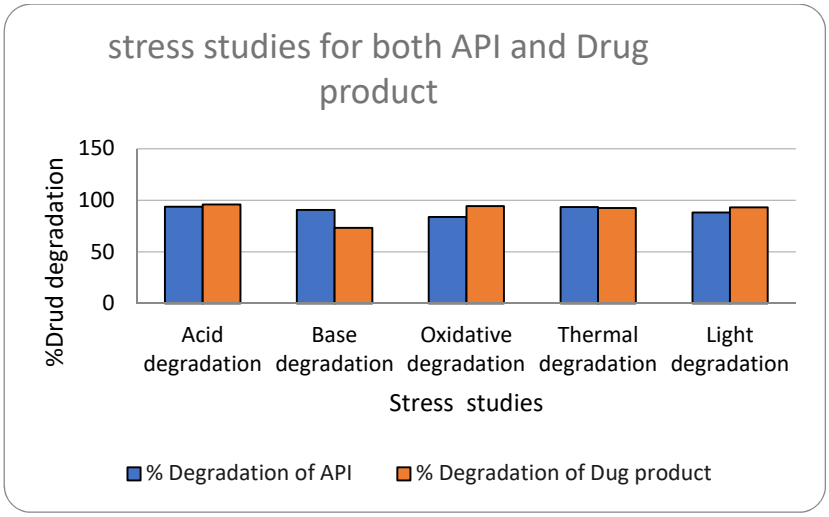

Figure 3: Graphical Representation of Forced Degradation Studies

Optimization formulation by the Custom model method by using Design Expert Software 13

The Method was optimized by taking three independent variables such as sonication time, the ratio of solvent, and scan speed, and their effect on the dependent variable which is the absorbance of the sample. The custom model method was used to optimize the method because this method only helps to determine both Qualitative and Quantitative factors simultaneously. In our method, we selected both quantitative factors (sonication time, ratio of solvent) and Qualitative factors (Scan speed) and their impact on absorbance (response). The results shown below indicate the sample showed good absorbance with sonication time between 15-20min, the ratio of solvent between 95-100%, and scan speed medium **(Table 9)**. In the below overlay plot, the optimized readings of the independent variables are shown **(Figure 4,5,6)** which are Scan speed –medium, Sonication time- 18 min, and Solvent –97 %. The designed model is desirable as the value we got 1 which is the maximum value (normal range is 0.6-1)

	factor 1	factor 2	factor 3	response
run	Sonication (min)	Solvent (%)	Scan Speed	Absorbance
1	15	95	Fast	1.213
2	15	95	Fast	1.211
3	10	100	Fast	1.542
4	15	90	Slow	1.411
5	15	90	Medium	1.409
6	10	90	Fast	1.642
7	20	95	Slow	1.153
8	10	95	Slow	1.577
9	15	100	Medium	0.914
10	10	95	Medium	1.573
11	15	100	Slow	0.91
12	20	100	Fast	0.852

13	20	95	Medium	1.157
14	20	90	Fast	1.333
15	15	95	Fast	1.243
16	20	95	Slow	1.151

TABLE-9: Effect of independent variables on absorbance

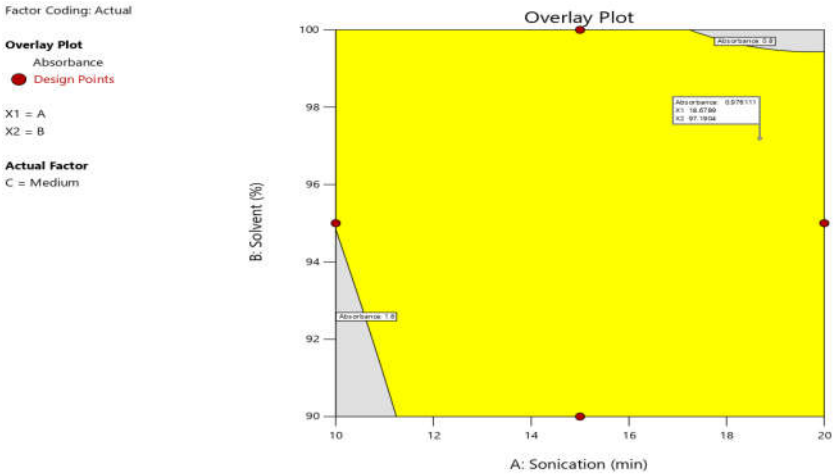

Figure 4: Overlay plot to determine sonication time and ratio of solvent

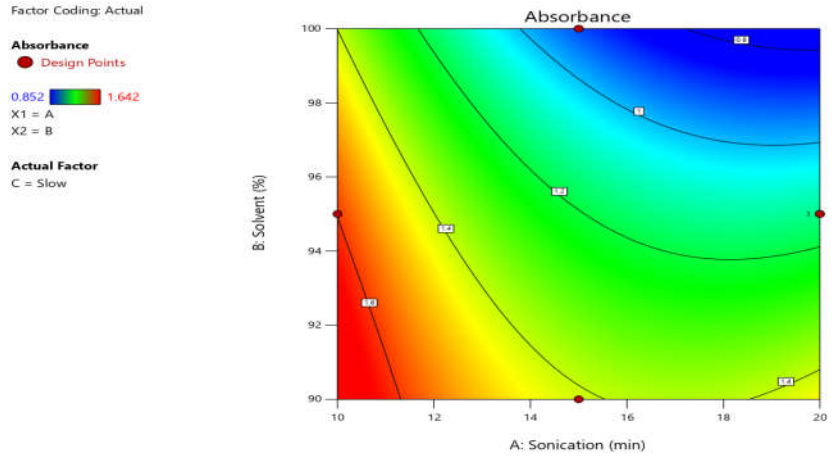

Figure 5: Effect of independent variables on dependent variables

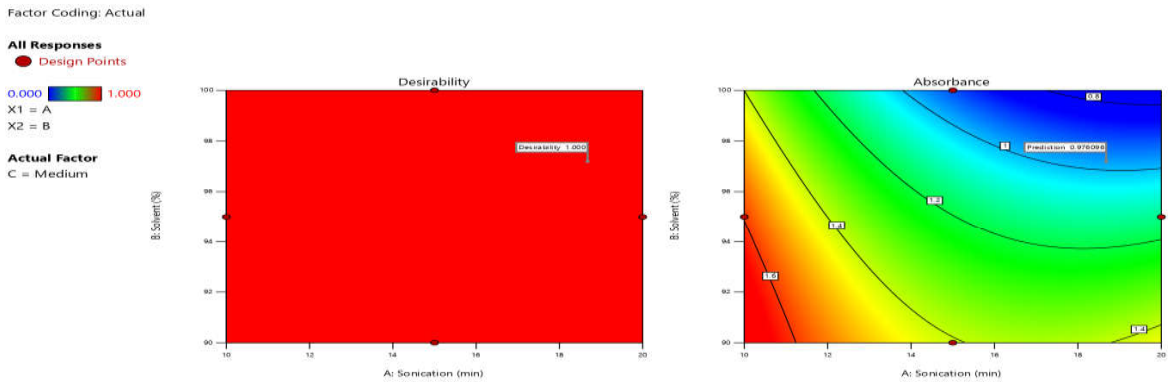

Figure 6: Plot to determine the desirability of the method

Anova results for the designed model:

The ANOVA results of the designed model are as follows **(Table 10)**. The < 0.0001 (which is lower than 0.0005) P value specifies the designed model is significant. Lack of fit F-value is not significant indicating that there is not much variability between the three repeated center points hence the designed model is significant. The F-value is 153.06 means the model is significant. The presence of square terms in ANOVA results indicates the model is a reduced quadratic model which indicates the 3-D surface plot is a curve **(Figure 7)**. In the below 3-D plot, as the sonication time increases initially the absorbance decreases and then increases and is represented in the form of a curve. Apart from this the FIT Statistics **(Table 11)** show the Adequate precision is 65.6567 which is >4 hence the model is significant. The difference between adjusted R^2 0.9892 and predicted R^2 0.8855 which is <0.2 indicates the model is significant.

source	**sum of squares**	**df**	**mean Square**	**f-value**	**P-value**	
model	0.9617	9	0.1019	153.06	<0.0001	Significant
A-Sonicatio n	0.4317	1	0.4317	648.72	<0.0001	
B-Solvent	0.3109	1	0.3109	467.16	<0.0001	
C-Scan speed	0.0012	2	0.0006	0.9304	0.4447	
AB	0.0363	1	0.0363	54.54	0.0003	
BC	0.0215	2	0.0108	16.18	0.0038	
A^2	0.1024	1	0.1024	153.89	<0.0001	
B^2	0.0072	1	0.0072	10.86	0.0165	
Residual	0.0040	6	0.0007			Not significant
Lack of fit	0.0033	3	0.0011	5.19	0.1046	
Pure error	0.0006	3	0.0002			
Cor Total	0.9207	15				

Table 10: ANOVA results for Absorbance

Fit Statistics:

standard deviation	mean	C.V%	R^2	adjusted R^2	predicted R^2	adequate precision
0.0258	1.27	2.03	0.9957	0.9892	0.8855	36.6567

Table 11: FIT Statistics

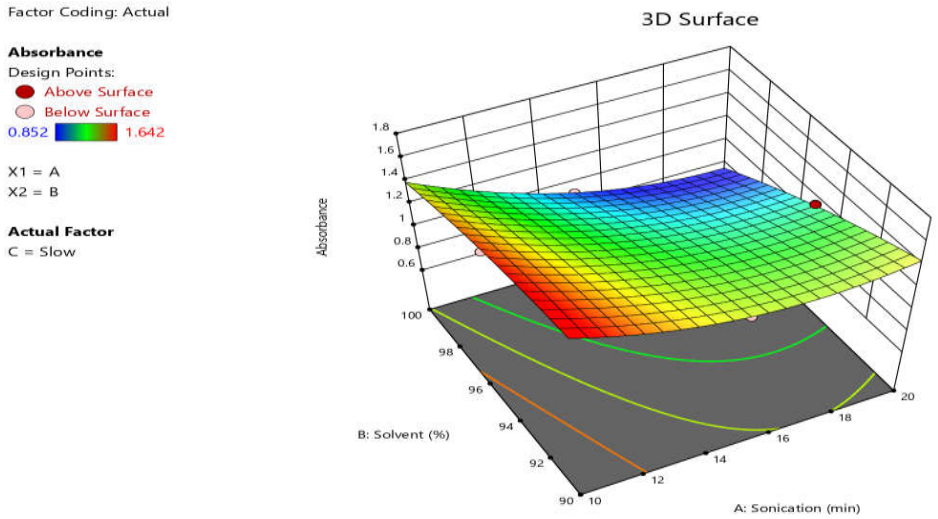

Figure 7: 3D surface plot for ANOVA results

DISCUSSION:

We developed and validated the QbD-assisted UV Spectrophotometric method for Donepezil Hydrochloride in both Bulk and Dosage formulation. Quality by design software method was used for the selection of solvent and also to study the effect of independent factors on the dependent factor which is absorbance has made this validation process more accurate and reliable. Based on the results the accurate percentage of solvent required for the method development was 97% of methanol with 18min sonication time required to solubilize the drug in the solvent. Based on these results we developed a method and validated the developed method where we got results within the limits. Apart from this we also carried out forced degradation studies in both bulk and dosage form. This advanced method was observed as robust and rugged in nature and was successfully used for the estimation of Donepezil Hydrochloride.

CONCLUSION:

We developed and validated the QbD-assisted UV Spectrophotometric method for Donepezil Hydrochloride in both Bulk and Dosage formulation. The developed method was successfully applied for the estimation of drug content in Donepezil Hydrochloride 10mg Tablets I.P. Percentage assay of Aricept®– ODT tablet was found to be 99.94. Quality by design software method was used for the selection of solvent and also to study the effect of independent factors on the dependent factor which is absorbance has made this validation process more accurate and reliable. After observing the validated parameters: accuracy, precision, LOD & LOQ were within limits. A lesser LOQ value specifies that the preferred method would be appropriate for analysing the samples containing even lower quantities of drugs. This advanced method was observed as robust and rugged in nature and was successfully used for the estimation of Donepezil Hydrochloride. Forced degradation studies of drugs and drug products were also studied. The results indicate that the QbD-assisted UV spectroscopic method for donepezil hydrochloride was simple, accurate, and precise. Therefore, it is suitable for routine analysis in quality control tests.

CONFLICT OF INTEREST: The author(s) declare(s) that they have no conflict of interest.

AUTHOR'S CONTRIBUTIONS:

The first author has contributed by doing a literature survey, performing the bench work, and writing the manuscript.

The corresponding author has contributed by editing the manuscript and providing guidance throughout the project.

Other authors have contributed by working on the QbD process and reviewing the manuscript.

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