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Original Article

A stability-indicating method for Levetiracetam in tablets using advanced analytical quality-by-design approach

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ABSTRACT

Quality by design (QbD) framework focuses on identifying predetermined specifications where the constraints to the process and the material features of the products are critical. Implementing the QbD concept for the analytical method validation is the modern direction in the pharmaceutical industry This study's basic design involves applying analytical quality by design (AQbD) research phases in the authentication and application of the conventional approaches during the development of a dynamic principal composite design. The influential and predicted positive outcomes of developing the pragmatic AQbD method is an effective method to ensure that the method meets its designated requirements. The study aimed to develop a comprehensive stability-indicating approach of Levetiracetam using AQbD. This study has shown that the QbD approach allows different analytical parameters evaluation and measure. The proposed method for stability indication of Levetiracetam in tablets proved to be fast and straightforward, and the proposed methodology was valid for ruggedness, robustness, accuracy, specificity, and linearity.

Keywords: QbD, AQbD, Levetiracetam, Design of experiments

Introduction

The emergence of the Quality by Design (QbD) concept has enabled changes within pharmaceutical quality regulation over the experimental processes. QbD is an approach that focuses on quality risk management [1, 2]. Besides, recent discussions are based on quality by design, while the pharmaceutical industry tries to develop products and processes. The definition of QbD, according to the International Conference on Harmonization (ICH), entails that it is a systematic method of growth that emphasizes the proofs and product comprehensions and starts with predefined objectives using sound and science quality risk [3]. The process is concerned with the predetermination of specifications. It ensures adherence to the critical process constraints and material features of the Critical quality attributes (CQAs) of a drug product. QbD method ensures utilization of all aspects of a drug or pharmaceutical product ranging from its quality to active components properties. The current QbD concept ensures that the quality of pharmaceutical products is acceptable and helps understanding manufacturing variables and controlling drug dosage formulation [4]. Also, using the QbD frameworks in the implementation process of drug development procedures gives benefits felt by the target patient population, pharmaceutical industry, and regulatory [5]. The primary benefits of QbD include reducing costs at various stages, improving efficiency, and reducing instances of product differences. The period of introducing the product to the market is also reduced significantly. QbD enhances the product's quality process and systematic research [6, 7].

US food and drug administration (FDA), under the ICH, ICH Q9 (quality risk assessment), requires that pharmaceutical

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development (ICH Q8), and pharmaceutical quality systems (ICH Q10) be used to achieve the required quality of the product [8]. There are restraints and characteristics connected to the drugs, related processes, and the operating environment. Regarding the current trends, implementing the AQbD strategy is considered a primary task that provides exciting options for developing methods and specific chemometrics applications within the QbD methods [9].

The study seeks to validate and develop the HPLC method for Levetiracetam evaluation in tablets as a pharmaceutical dosage form. The research applies some AQbD steps in the validation of a vigorous developed central composite design.

Levetiracetam, as an antiepileptic drug, is (S)-2-(2-Oxopyrrolidin-1-yl) butanamide, $C_8H_{14}N_2O_2$ with a relative molecular mass of 170.2 gm/mole and a structural formula as indicated below. Levetiracetam is an off-white to white powder, with high solubility in water, methanol, and ethanol [10].

Materials and Methods

Chemicals and reagents

The active ingredient available in the pharmacy was purchased from a local pharmacy. Merck Specialists Limited provided the HPLC grade Acetonitrile and Methanol. Highly purified water was taken from a water purification system designed as an in-house system.

Instruments and chromatographic conditions

A Shimadzu HPLC system consists of an injector with 10 µL loop volume and LC-Solution helped in the data collection and data processing procedures. The chromatographic separation was performed using Symmetry C18 250 x 4.6 mm, 5µm columns, with a detection wavelength of 208 nm and a run time of 4.0 min. A variable wavelength detector which was programmable was used to detect the UV-Visible light. Furthermore, the Rheodyne injector helped investigate as the mobile phase degassing was conducted using the Loba ultrasonic bath sonicator. The mobile phase had a buffer pH of 5.5, 80: 20 rates, and 1 min/ml flow rate.

Chromatographic conditions

The mobile phase had 80% methanol, 20% acetonitrile mixed in the ratio of v/v, and then filtered through the membrane and finally degassed before use. There was an ambient temperature column, and the wavelength of the UV detected was 208 nm. Chromatographic analysis was conducted at a rate of 1 mL/min with the mobile phase's help mentioned above. This step was followed by adjusting the pH to 5.5 by using 0.1% of orthophosphoric acid. Furthermore, Chromosil C18 was used as the chromatographic column, measuring 250 mm × 4.6 mm, 5 µm. The volume injection rate 20 µL, while the runtime and retention time were 4 min and 2.8 min, respectively. The buffer solution of a 5.5 pH was prepared by dissolving 0.26 gm of potassium dihydrogen phosphate in 900 mL of water. It followed adjusting the pH to 5.5 by using 1M potassium hydroxide and complete the volume to1000 mL of water. The solvent was prepared with acetonitrile and buffer in the ratio of 70:30 (v/v).

Preparation of the standard solution

Prepare the standard solution by weighing 50 mg Levetiracetam working standard solution into a 100 mL volumetric flask. The powder was then dissolved in 80 mL of the solvent, then sonicated for 10-15 minutes. The volume was made up with the same solvent before 10 mL was pipetted in a 50 mL flask to mark the same solvent.

Test essay preparation analytical target profile (ATP)

Ten (10) tablets of Levetiracetam (KEPPRA, 500 mg) were accurately weighed, and their current average weight was considered before the tablets were crushed into powder form. The powder was then weighed into one tablet equivalent to 500 mg of the drug and then put into a 100 mL volumetric flask. 80 mL of the solvent sonicate was slowly added (25 to 30 min), accompanied by intermittent shaking to make sure the drug entirely dissolved before filling up the right volume. 2 mL of the solution was pipetted in a 100 mL volumetric flask and made up to the mark with the same solvent. The pipetted solution was filtered through a 0.45µm pore size membrane filter before it was injected.

Analytical target profile (ATP)

Identifying the ATP includes selecting method requirements, including the target analytes (product and impurities), analytical technique category, and product specifications. The target analytes selection in this session was Levetiracetam API (Active Product Ingredient), while the selected technique was the determination of Levetiracetam. This study had method requirements that included diluents, the mobile phase composition, and the column as per the HPLC.

Critical quality attributes (CQA) and initial risk assessment

Critical Quality Attributes include the method attributes and the method parameters. Every analytical technique differs from the other in terms of CQA. The CQA for the HPLC method is the column, the diluents, and the mobile phase composition. For the Initial Risk Assessment, the Ishikawa fishbone diagram was considered perfect for a task like this, and it is exemplified in **Figure 1**.

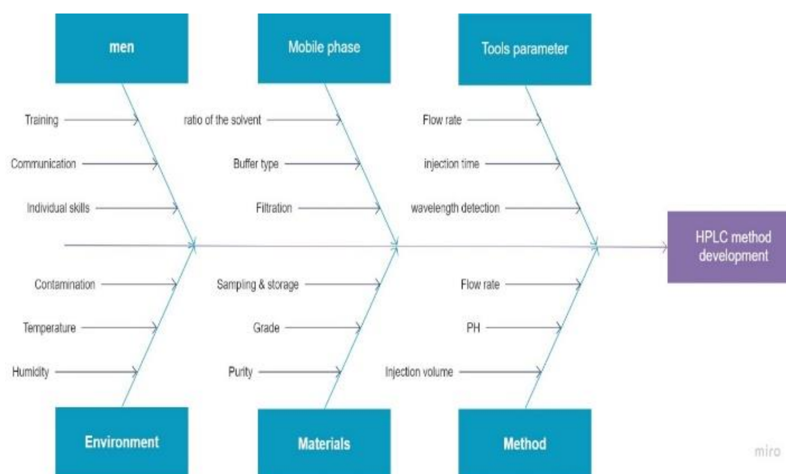

Figure 1. Ishikawa diagrams for risk identification

Results and Discussion

Design of experiments (DoE): (Method optimization and development)

As per the risk assessment, the DoE for this study confirmed and refined significant variables used in the methods. At this point, a more comprehensive and efficient design of the experiment was designed. It was designed as per the system requirements for the three significant components of the HPLC methods with Design Expert 12 software. Later, a database was built to help understand the procedure, optimization, and selection process. Additionally, the design helped to evaluate and implement change in method whenever it was required. The scouting of the parameters was as shown in **(Table 1)**.

Method operable design region (MODR) helps establish a suitable multidimensional space as per the DoE outcomes; the MODR can provide the perfect performance method. Moreover, more exercises can be performed to verify and establish the ATP performance and primarily defines the MODR.

The strategy of method control appears somehow different in the QbD process in comparison to the traditional approaches. However, establishing the control methods relies upon the CQA, DoE, and the MODR. This approach ensures a rigid connection between the purpose and the performance. Hence, the selected method against the attribute methods has higher chances of reliability and remaining operational over the material's lifetime. The robustness and the ruggedness of the evaluation method used in developing the method help verify and finalize. This study used a risk-based approach based on the principles of the QbD set out in the ICH Q8 and Q9 and was supplied for both ruggedness and robustness evaluation. ICH Q8 guidance process defines robustness as a process's ability to endure material variability and the process changes and the equipment exempting the adverse effects [3].

Table 1. Chromatographic factors variable for Central composite experimental design

Chromatographic conditions	Units	Low	High
Flow rate	mL min-1	0.9	1.1
Column temperature	µL	23	27
Methanol concentration	%	75	85

Table 2. Central composite design for method parameters

Std	Run	Factor 1 A: Flow rate mL min^{-1}	Factor 2 B: Column temp. °C	Factor 3 C: Mobile phase %	Response Retention time min.
12	1	1	28.36	80	2.32
18	2	1	25	80	2.22
5	3	0.9	23	85	4.95
11	4	1	21.63	80	5.54
19	5	1	25	80	2.46
1	6	0.9	23	75	5.58
6	7	1.1	23	85	3.74
3	8	0.9	27	75	3.51
15	9	1	25	80	2.59
13	10	1	25	71.59	3.12

4	11	1.1	27	75	2.12
16	12	1	25	80	2.62
2	13	1.1	23	75	4.17
14	14	1	25	88.40	2.054
10	15	1.168	25	80	2.22
9	16	0.831	25	80	4.95
8	17	1.1	27	85	1.91
17	18	1	25	80	2.41
7	19	0.9	27	85	2.95
20	20	1	25	80	2.55

The method responses analysis

As showen In **Table 2**. The analysis of variance (ANOVA) response method was performed for the retention time. The regression parameters ANOVA of the projected quadratic response surface model of the retention time was attained using DoE software and is presented in **Table 3**, **Figure 2** and **Figure 3**. F value of 82.09. The model also shows a low probability value. Thus, the model was significant for retention time. The prob. > F value of 0.0001 shows that the terms of the model terms were insignificant.

The 82.09 Model F-value infers that the model is substantial. The chance of the F-value to occur largely because the noise is minimal for about 0.01%.

Less than 0.0500 of the P-values show that the terms of the model are significant. Regarding this, A, B, C, A^2, B^2 becomes the significant model's terms. All the values that happen to be larger than 0.0001 show that the model terms are not substantial. When it results in many unimportant terms of the model (excluding the ones supporting the hierarchy), the decline of the model might help to advance the model.

The lack of fit F-value for about 2.40 infers the lack of fit is not significant in connection to the pure error. There is a greater chance of approximately 17.98% of the lack of fit F-value being that big because of the noise.

Table 3. Response: Retention time

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	27.43	9	3.05	82.09	< 0.0001	significant
A-flow rate	6.81	1	6.81	183.30	< 0.0001	
B-column temp.	13.08	1	13.08	352.26	< 0.0001	
C-mobile phase	0.9610	1	0.9610	25.88	0.0005	
AB	0.0045	1	0.0045	0.1215	0.7346	
AC	0.0378	1	0.0378	1.02	0.3367	
BC	0.0105	1	0.0105	0.2831	0.6063	
A^2	2.71	1	2.71	72.95	< 0.0001	
B^2	4.45	1	4.45	119.77	< 0.0001	
C^2	0.0939	1	0.0939	2.53	0.1429	
Residual	0.3713	10	0.0371			
Lack of Fit	0.2620	5	0.0524	2.40	0.1798	not significant
Pure Error	0.1093	5	0.0219			
Cor* Total	27.80	19				

*corrected sum of squares

Ruggedness

The analytical method's ruggedness is the degree of reproducibility of test samples under various conditions, including different laboratories, instruments, reagents, lots, assays, temperatures, days, or even various analytics. Consequently, studying robustness and ruggedness improves the performance of a method control strategy. The respective fitting system sustainability can be defined in the risk management and in ensuring that the present method gives the desired attributes [11]. Analysts get an opportunity when the risks are high and hard to control. They can look back at the described database in the CQA (Critical Quality Attribute) scouting parameters and then find the most appropriate approach strategy to ensure quality in the robustness and the ruggedness [12].

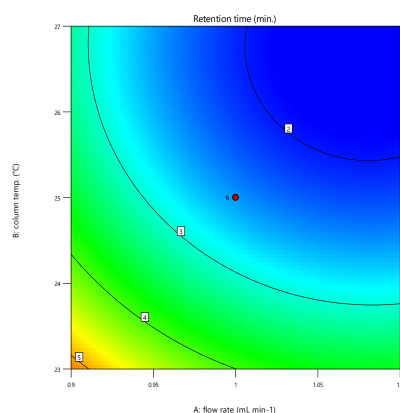

Figure 2. Contour plots as a function of mobile phase and temperature

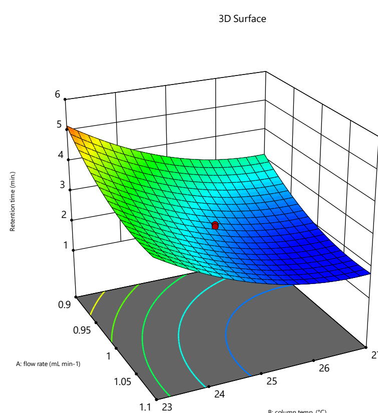

Figure 3. Response for retention time as a function of mobile phase and temperature.

These graphs show the combined and individual effect of the factors on the responses.

Validating analytical method

It is known that validation provides a more trusted assurance by documenting evidence for a specified method. The validation method followed the ICH guidelines. Validation parameters studied here, included linearity, specificity, precision, robustness, accuracy, system suitability, quantification limit, the limit of detection, and solution stability.

Specificity

Specificity was done by comparing the chromatograms, including blank, standard, and the sample prepared from the formulations. It was revealed that there is no evident interference because of the excipients found in the tablet formulations. Furthermore, it was revealed that the retention times for the standard and the sample had a good correlation.

Linearity

The HPLC system was injected with 20 µL for each concentration, and the response was read at precisely 208 nm. The corresponding chromatograms were tabulated **(Table 4)**.

Table 4. Levetiracetam Linearity

No. sample	Conc. (µg/ml)	Percent to the working concentration	Response	Average Response
1	25	25%	445.7 445.5 446.1	445.8
2	50	50%	866.7 867.4 866.6	866.8
3	75	75%	1312.6 1313.4 1312.7	1312.3
4	100	100%	1756.8 1756.9 1757.9	1756.6
5	127	125%	2207.8 2206.7 2205.6	2206.3
6	150	150%	2589.5 2587.1 2590.6	2589.2
7	200	200%	3424.7 3424.8 3424.6	3424.7
8	250	250%	4294.6 4292.6 2493.7	4293.7

Accuracy

A measured amount of the standard drug was mixed with the determined amount of the tablet's previously analyzed solution. Calculating the percent recovery followed and entailed comparing the area before and after adding the standard drug. According to the proposed method, analysis of the answers in triplicate at all the levels was the next step. The percentage recovery and the acceptable recovery limit were calculated through the proposed process, indicating that the proposed method was accurate.

Robustness

Robustness helps establish and demonstrate the reliability of the method's minority changes if the method conditions are changing [12]. Robustness was determined by slightly modifying the mobile phase flow rate, the buffer pH, the temperature, and the mobile phase composition. A value of 6ppm Levetiracetam concentration analysis was conducted under the changed experimental set conditions. There were no marked observable changes in the chromatograms demonstrating that it was developed a robust method.

System suitability

This process was conducted in all validation parameters through the injection of 6 replicates for the 12-ppm standard solution, and the acquired results lay within the acceptable limit.

Conclusion

This study aimed to develop a comprehensive stability-indicating method of using an advanced analytical Quality-by-

Design approach. Modern technologies dealing with the analysis of multiple compounds require more comprehensive plans than the old ways. This analytical QbD approach with the DoE algorithm and design could give room for measuring and evaluating various analytical parameters with their effects on the critical methodology properties. Applying such procedures affirms the significance of scientific knowledge over the general pharmaceutical analysis.

There are several reports variable to quantify Levetiracetam drug. However, there has been limited literature explaining the quantitation of Levetiracetam. The development method was validated successfully for the drug substance considering the ICH guidelines. The proposed way is better than the other reported methods considering the run-time facts, solvent consumption, selectivity, instrumental techniques (HPLC), drug product applicability, and the drug substance. The proposed method of assaying Levetiracetam of the tablets is simple and faster. The technique was valid for linearity, specificity, accuracy, robustness, and ruggedness.

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Conflict of interest: None

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Ethics statement: None

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