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A Comprehensive Exploration of Method Validation and Development in Pharmaceutical Analysis: Focus on Vortioxetine Analysis

Devendra S. Mahale[1,*], Amit Kumar R. Dhankani[2], S.P. Pawar[3]

Abstract

The pharmaceutical industry relies on rigorous method validation to ensure the accuracy, precision, and reliability of analytical techniques employed in drug testing and quality control. This comprehensive exploration delves into the validation parameters and guidelines essential for method validation, emphasizing accuracy, precision, linearity, detection and quantitation limits, specificity, range, robustness, and ruggedness. Validation plays a pivotal role in guaranteeing high-quality products, adhering to good manufacturing practices (GMP), and optimizing manufacturing processes. The development of analytical methods for new drugs involves critical considerations, such as the absence of methods in pharmacopeia's, patent-related constraints, formulation ingredient influences, and the need to measure drug levels or combine drugs effectively. Method development is a crucial precursor to validation, requiring a strategic approach in line with International Conference on Harmonisation (ICH) guidelines and regulatory industry standards. Two analytical methods for vortioxetine are explored in detail: UV Spectrophotometric and High-Performance Liquid Chromatography (HPLC). The UV method involves determining the maximum wavelength, preparing standard stock solutions, dilutions, and analyzing tablet formulations. The HPLC method development encompasses steps such as choosing the procedure, setting up initial conditions, method development, and validation. Several studies exemplify different HPLC approaches for vortioxetine, showcasing their stability-indicating capabilities.

Keywords: Method validation, pharmaceutical analysis, UV spectrophotometry, HPLC, stability-indicating methods, drug development, regulatory guidelines, GMP, ICH, vortioxetine

***Author for Correspondence**
Devendra S. Mahale
E-mail: devendramahale2306@gmail.com

[1]Student, Department of Pharmaceutical Quality Assurance, Poojya Sane Guruji Vidya Prasarak Mandal's College of Pharmacy, Shahada, Maharashtra, India
[2]Assistant Professor, Department of Pharmaceutical Quality Assurance, Poojya Sane Guruji Vidya Prasarak Mandal's College of Pharmacy, Shahada, Maharashtra, India.
[3]Principal, Poojya Sane Guruji Vidya Prasarak Mandal's College of Pharmacy, Shahada, Maharashtra, India

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INTRODUCTION

This exploration delves into the critical realm of method validation and development in pharmaceutical analysis, shedding light on the intricate processes that ensure the accuracy, precision, and reliability of analytical techniques. From the inception of validation concepts in 1978 to the present, this discussion navigates through the parameters and guidelines vital for method validation, emphasizing their pivotal role in maintaining high-quality standards and optimizing manufacturing processes. The narrative extends its focus to the development of analytical methods for new drugs, necessitating strategic considerations and adherence to international guidelines. The study delves into two specific analytical methods for

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vortioxetine: (1) UV Spectrophotometric and (2) High-Performance Liquid Chromatography (HPLC). Each method is meticulously explored, detailing procedures, parameters, and validation aspects. Numerous studies showcasing different HPLC approaches for vortioxetine highlight the versatility and stability-indicating capabilities of these analytical techniques. In essence, this exploration underscores the significance of ongoing research and development in analytical methods to advance drug discovery, formulation, and quality assurance in the dynamic landscape of the pharmaceutical industry.

METHOD VALIDATION [1]

Validation was first introduced in the United States in 1978. Since then, it has expanded to cover different activities in fields like medical research and device manufacturing. It includes making sure computer systems, control procedures, labels, and tests for medical items work effectively. Usability, or how easy something is to use, is crucial, especially following rules for good manufacturing practices (GMP). Verification is about checking if something is true or valid. Multiple departments within a factory cooperate to observe this procedure. Verification is like gathering evidence to make sure a product or device meets its requirements. The validation parameters are as follows: (1) accuracy, (2) precision, (3) linearity, (4) limit of detection, (5) limit of quantitation, (6) specificity, (7) range, (8) robustness, and (9) ruggedness.

1. *Accuracy:* The accuracy of an analytical method denotes the proximity between the measured value and the actual or anticipated value of the quantity being assessed. It is like checking how well our measurement matches what we know to be true. To test accuracy, we compare the values we get when we add something extra (spiking) to samples with the values from samples without the extra addition.
2. *Precision:* Precision in an analytical method means how consistent the method is when measuring many samples of the same thing in different situations. There are three types of precision: reproducibility, intermediate precision, and repeatability. They help us understand how dependable the method is in giving similar results under various conditions.
   i. *Repeatability:* Repeatability measures how consistent measurements are, when taken quickly and repeatedly in the same process.
   ii. *Intermediate precision:* This level of precision checks accuracy across different situations like different days, analysts, or products. It shows how the lab changes due to various factors.
   iii. *Reproducibility:* Reproducibility tells us how well results matchup between different labs.
3. *Linearity:* Linearity means that when you measure something in a sample, like how much of a substance is there, the test results change in a straight line as the amount of the substance changes.
4. *Limit of detection:* The analytical method's detection limit is the smallest quantity of a substance detectable within a sample, even if the measurement is not precise.
5. *Limit of quantitation:* The limit of quantitation in analytical methods is the smallest amount of substance that can be reliably measured in a sample.
6. *Specificity:* Specificity now refers to how clearly, we can tell if a test has the right properties.
7. *Range:* The analytical method's range refers to the variation between the highest and lowest concentrations of a substance in a sample where the method performs effectively.
8. *Robustness:* Robustness means that a method stays reliable even if small changes are made to the process.
9. *Ruggedness:* Ruggedness is about how consistent test results are when analysing the same samples under different conditions, like in different labs or with different analysts. It shows if results can be trusted across different situations.

Importance of Validation [1]

1. Quality guaranteed at a high level.
2. Time limits recognized and process improved.
3. Fewest product failures per batch.
4. Better productivity and manufacturing.

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5. Reduced costs for maintaining quality.
6. Less rejection of products.
7. More products produced successfully.
8. Fewer complaints about process problems.
9. Equipment starts quickly and reliably.
10. Employees become more aware of the process.

Guidelines for Validation [1]

1. Clarifications and language used in the ICH Q2A guide for validating analytical methods (March 1995).
2. Steps and procedures detailed in the ICH Q2B guide for validating analytical techniques (June 1997).
3. FDA's (Draft) Industry Guidance for Analytical Procedures and Method Validation.
4. Standards and guidelines established by the United States Pharmacopeia (USP) and European Pharmacopeia (EP) for pharmaceutical products.

METHOD DEVELOPMENT [1]

The development, production, and discovery of pharmaceuticals rely on creating and validating analytical methods. Before making a method, it is important to decide its requirements and choose the right tools. Picking the correct analysis strategy helps manage the editing sequence effectively. Analytical strategies should follow the methods and validation procedures in the ICH Guidelines Q2(R1) before being used in GMP and GLP environments.

Important Requirements for the Development of Analytical Methods for New Drugs [1]

- If a drug or mix of drugs is not mentioned in any pharmacopoeias.
- When there is a patent problem preventing the use of the right analytical methods.
- Analytical methods might not be available for drugs in development due to the influence of formulation ingredients.
- There might not be methods to measure drug levels in body fluids or to combine drugs with others [1].

UV Spectrophotometric Analytical Method on Vortioxetine in Pharmaceutical Preparations [2]

Materials and Methods

Instrument

The method was performed using a Shimadzu 1800 model UV spectrophotometer with 1 cm quartz sample cells. A Shimadzu Single pan electronic balance was used for weighing, and calibrated volumetric glassware was employed for the validation study.

Materials

Vortioxetine Hydrochloride standard was received as a gift from Apotex Research Limited in Bangalore. All reagents used were of analytical grade, and Brintellix (10 mg of Vortioxetine hydrochloride) tablets were bought locally. All chemicals and solvents, like methanol, were of analytical grade and used to prepare necessary solutions.

Methodology

- *Determining the maximum wavelength:* First, 50 mg of Vortioxetine Hydrochloride was precisely weighed and placed into a 50 ml volumetric flask. It was dissolved in 50 ml of Methanol and then the same solvent was added until reaching the mark, creating a solution with a concentration of 1000 µg/ml. Then, 5 ml of this solution was transferred to another 50 ml volumetric flask and diluted with Methanol to make a solution of 100 µg/ml. This solution was scanned from 200–400 nm against a blank, and a maximum wavelength of 230 nm was chosen for further analysis.

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Table 1. Summary of validation parameters [2].

Parameters	Results for AUC
Wavelength range	220–240 nm
Maximum wavelength	230 nm
Range of linearity	10–50 µg/ml
Slope of the plot	0.1424
Intercept of the plot	0
Correlation coefficient (R^2)	0.9986
Accuracy (mean recovery)	99.977
Precision (RSD)	0.1479
LOD and LOQ	2.276 and 6.8977

- *Area under the curve:* In this research, the area beneath the curve was determined by computing the integration spanning the wavelength spectrum from 220 to 240 nm.
- *Preparation of standard stock solution:* To prepare the standard stock solution, 50 mg of pure Vortioxetine hydrochloride was accurately weighed into a 50 ml volumetric flask. Then, 50 ml of methanol was added and dissolved to create a solution containing 1000 µg/ml Vortioxetine Hydrochloride. From this solution, 5 ml was transferred to another 50 ml volumetric flask, and 50 ml of methanol was added to create a solution containing 100 µg/ml of Vortioxetine hydrochloride.
- *Preparation of dilutions:* To examine the UV-spectrophotometric Area under Curve, we made a 100 µg/ml stock solution of Vortioxetine Hydrochloride using Methanol as the solvent. We then created dilutions by transferring 5, 10, 15, 20, and 25 ml of the 100 µg/ml solution into separate 50 ml volumetric flasks and topping them up with Methanol. This led to dilutions with concentrations of 10, 20, 30, 40, and 50 µg/ml. These dilutions were analyzed in a UV spectrophotometer for first-order derivative analysis within the 220–240 nm range with a sample size of N=5.
- *Tablet formulation analysis:* We analyzed a commercial tablet formulation (Brintellix) containing Vortioxetine Hydrochloride. After determining the total weight of the tablets, we weighed and crushed 10 tablets individually. We calculated the amount of powder needed for analysis and prepared a 100 µg/ml stock solution. We then measured the absorbance at 220–240 nm for practical analysis (Table 1).

HPLC METHOD DEVELOPMENT [3]

High Performance Liquid Chromatography (HPLC) is a widely used method for analysing drugs, with more than 85% of drugs tested using it. HPLC involves separating compounds using a setup with a stationary phase and a mobile phase of different polarities, often with a high-pressure pump. Choosing the stationary and mobile phases is critical for the process of separation. Factors like solution pH, column temperature, sample dilution, and detection wavelength are important in the design process. The aim is to minimize the number of experiments needed to develop an effective method while keeping it simple and utilizing tools like computer modelling [4–6]. When setting up an HPLC system, the typical steps include:

1. Choosing the HPLC procedure and setting up the initial system.
2. Selecting the starting conditions.
3. Developing the method.
4. Validating the method.

Steps for HPLC Method Development [3]

Step 1: Choosing the HPLC Method and Initial Setup

When starting with HPLC method development, it is important to be efficient and avoid unnecessary experiments to save time. Selecting the right HPLC system is crucial for effective sample analysis. For

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example, if a sample contains polar analytes, reverse phase HPLC works better than conventional HPLC [7–10].

Sample Collection and Preparation

Ideally, it is preferable for samples to be dissolved in the initial mobile phase. If there are solubility issues, adding formic acid, acetic acid, or salt can help. Sample preparation ensures compatibility with the HPLC method and proper dissolution.

Column Selection

Choosing the right column is crucial for accurate results. Different columns offer varying performance and selectivity. Manufacturers offer columns with different properties like pore volumes, sizes, and surface areas, which affect separation.

Step 2: Choosing the Initial Conditions

Ensure parameters are within appropriate limits, neither too low nor too high. This step establishes optimal conditions for parameter stability.

Step 3: Method Development

Identifying method weaknesses helps in model development. Consider variables, metrics, and model types during optimization. Mobile phase composition is the key, although stationary phase development is complex. Tests include HPLC parameters like organic content, pH, flow rate, and temperature, as well as sample preparation factors and calculation methods.

REVIEW OF LITERATURE

The proposed research work involved conducting an extensive literature survey by referring to various scientific research journals.

Wróblewski *et al.* devised a validated stability-testing method to determine vortioxetine in both bulk and pharmaceutical formulations. Their developed HPLC-DAD technique permits the acquisition of essential data for degradation kinetics assessment. They employed a Polar-RP column and a mobile phase consisting of acetonitrile (ACN), methanol (MeOH), acetate buffer pH 3.5, and the inclusion of diethylamine (DEA) under isocratic elution conditions. Ensuring the stability of vortioxetine is crucial for maintaining the effectiveness, safety, and quality of drug products [4].

The development and validation of vortioxetine hydrobromide in tablet form using the HPLC method by Ravisankar *et al*. were exceeded by the superior UPLC method during its establishment and validation for quantifying vortioxetine hydrobromide in tablet form. They employed an upgraded Aquity UPLC HSS system from Waters, coupled with an Aquity UPLC BEH C8 column (100×2.1 mm, 1.7 µ), operating at a flow rate of 0.4 ml/min and UV detection at 274 nm. The buffer consisted of acetonitrile, methanol, in proportions of 55, 35, and 10 v/v [5].

Gizem Tiris et al. (2020) described a new method for swiftly, accurately, and reliably determining vortioxetine (VRT) in bulk and pharmaceutical formulations. They introduced a reversed-phase high-performance liquid chromatography (RP-HPLC) method along with first derivative spectrophotometric determination. In their RP-HPLC method, they achieved optimal separation and vortioxetine quantification using a Waters Symmetry C18 column (100 × 4.6 mm, 3.5 µm) with a mobile phase consisting of methanol and 0.05 M potassium dihydrogen phosphate (pH: 3.0 ± 0.05) in a ratio of 30:70 (v/v) under isocratic conditions, operating at a flow rate of 1.3 mL min−1 [6].

Rathod K. G et al. (2019) conducted research on developing and validating an RP-HPLC method to determine vortioxetine in bulk and pharmaceutical formulations using chromatography. They utilized an ODS C18 column (4.6 x 250 mm, 5µm) with acetonitrile and methanol (in a 70:30 ratio) as the

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mobile phases, flowing at a rate of 1.0 mL/min. Detection was performed at 274 nm, and the retention time for vortioxetine was measured at 2.922 ±0.02 min [7].

Sakine Atila Karaca et al. (2020) devised a simple HPLC technique to quantify vortioxetine in pharmaceutical products using the Doe approach. They introduced an innovative HPLC method for quantifying vortioxetine, a novel antidepressant, in tablet form. Separation was conducted on a Zorbax Eclipse Plus C18 column (3.5 µm particles, 4.6 × 50 mm) at 35 °C, employing a flow rate of 1.0 mL/min, and chlorpromazine was chosen as the internal standard. They utilized the Box-Behnken design, a type of experiment design (DoE), to investigate the impact of mobile phase pH, acetonitrile content, and buffer concentration on the chromatographic separation of vortioxetine and chlorpromazine [8].

Marta de Diego et al. (2018) conducted an analysis to determine vortioxetine and its degradation product in bulk and tablet forms using LC-DAD and MS/MS techniques. They employed a C-18 column with a mobile phase comprising acetonitrile and water containing acetic acid and triethylamine, utilizing isocratic elution mode. Detection was performed at 228 nm, with a flow rate set at 1.0 mL/min. The assay displayed linearity within the concentration range of 25–125 µg/mL. Precision analysis indicated that the Relative Standard Deviation (RSD) remained below 1.8%, and recovery rates fell between 100.0 and 11.6%. The method exhibited satisfactory selectivity [9].

Pravallika, K.E. et al. (2017) developed and validated UV spectrophotometric techniques to assess vortioxetine hydrobromide in bulk and pharmaceutical formulations. Two methods were established using different solvents: methanol (method A) and a combination of 50% v/v water and methanol (method B). Vortioxetine hydrobromide was measured at 228 nm in method A and 224 nm in method B. Both methods exhibited high sensitivity and conformed to Beer-Lambert's law over concentration ranges of 2–12 µg/ml for method A and 3–15 µg/ml for method B, respectively, demonstrating a strong correlation coefficient (R^2 = 0.999). The linear regression equations obtained via the least squares regression method were Y = 0.061x + 0.01 for method A and Y = 0.061x + 0.03 for method B [1].

Lei Liu Na et al. (2016) documented the development and validation of a stability-indicating reversed-phase HPLC method for identifying potential impurities in vortioxetine. They performed the separation on a Shimadzu Inertsil ODS-3 C18 column (250 mm × 4.6 mm, 5 µm) located in Tokyo, Japan. The mobile phase A contained 0.05% trifluoroacetic acid in water, while phase B consisted of acetonitrile [10].

Er-min Gu Chengke et al. (2015) The separation was conducted utilizing an Acuity UPLC BEH C18 column (2.1 mm × 50 mm, 1.7 µm), employing a gradient mobile phase containing 0.1% formic acid in water and acetonitrile [3].

CONCLUSION

Method validation is crucial in pharmaceutical analysis to ensure the accuracy, precision, and reliability of analytical methods used for drug testing and quality control. Guidelines from regulatory bodies and pharmacopeia's provide essential frameworks for validation protocols. The UV spectrophotometric method and HPLC methods developed for vortioxetine analysis demonstrate the versatility and robustness of analytical techniques in pharmaceutical research. Stability-indicating methods play a vital role in assessing drug stability and degradation kinetics, contributing to the quality and efficacy of pharmaceutical formulations. Further research and development in analytical methods are essential for advancing drug discovery, formulation, and quality assurance in the pharmaceutical industry.

REFERENCES

1. Pravallika KE, Ravi P, Abboodi AH, Razzaq AH, Sasivardhan O. Development and Validation of UV Spectrophotometric Methods for the Estimation of Vortioxetine Hydrobromide in Bulk and Pharmaceutical Dosage Forms. Res J Pharm Technol. 2017; 10(11): 3928–3932. https://doi.org/10.5958/0974–360X.2017.00713.2.

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2. Santosh Karajgi, Sushma Angadi, Shripad Potadar. Quantification of Vortioxetine in Pharmaceutical preparations by validated area under curve UV Spectrophotometric Analytical method. Res J Pharm Technol (RJPT). 2023; 16(12): 5986–5989.
3. Gu E, Huang C, Liang B, Yuan L, Lan T, Hu G, Zhou H. An UPLC–MS/MS Method for the Quantitation of Vortioxetine in Rat Plasma: Application to a Pharmacokinetic Study. J Chromatogr B. 2015; 997: 70–74. https://doi.org/10.1016/j.jchromb.2015.05.010.
4. Wróblewski K, Szultka-Młyńska M, Janiszewska D, Petruczynik A, Buszewski B. Development of the Validated Stability-Indicating Method for the Determination of Vortioxetine in Bulk and Pharmaceutical Formulation by HPLC-DAD, Stress Degradation Kinetics Studies and Detection of Degradation Products by LC-ESI-QTOF-MS. Molecules. 2022; 27(6): 1883. https://doi.org/10.3390/molecules27061883.
5. Ravisankar M, Alexandar S, Kumar M, Kumar RS, Venkateswarlu BS. Method Development and Validation of Vortioxetine Hydrobromide in Tablet Dosage Form. Indian Journal of Natural Sciences (IJONS). 2021; 12(67): 32769–32774.
6. Tiris G, Alver C, Erk N. A Novel Stability-Indicating Method for Determination of a New Antidepressant Effect of Vortioxetine in a Pharmaceutical Formulation by Using RP-HPLC. Future J Pharm Sci. 2020; 6(118): 1–7. https://doi.org/10.1186/s43094–020–00135-w.
7. Rathod KG, Bargaje GS, Rathod GR, Deshpande OV. Development and Validation of RP-HPLC Method for Estimation of Vortioxetine in Bulk and Pharmaceutical Dosage Form. Int J Trend Sci Res Dev. 2019; 3(6): 74–88.
8. Atila Karaca S, Rashidova N, Uğur A, Yeniceli Uğur D. Development of a Simple HPLC Method for the Quantitation of Vortioxetine in Pharmaceuticals Using DoE Approach. Chem Pap. 2020; 74(5): 1541–1549. https://doi.org/10.1007/s11696–019–01008–0.
9. De Diego M, Correa D, Mennickent S, Godoy R, Vergara C. Determination of Vortioxetine and Its Degradation Product in Bulk and Tablets, by LC-DAD and MS/MS Methods. Biomed Chromatogr. 2018; 32(11): e4340. https://doi.org/10.1002/bmc.4340.
10. Liu L, Cao N, Ma X, Xiong K, Sun L, Zou Q, Yao L. Stability-Indicating Reversed-Phase HPLC Method Development and Characterization of Impurities in Vortioxetine Utilizing LC–MS, IR and NMR. J Pharm Biomed Anal. 2016; 117: 325–332. https://doi.org/10.1016/j.jpba.2015.08.028.

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