EJPMR, 2026, 13(6), 856-863 **Review Article** **Impact Factor: 8.158**

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

ISSN (O): 2394-3211
ISSN (P): 3051-2573
Coden USA: EJPMAG

RP-HPLC METHOD DEVELOPMENT AND VALIDATION APPROACHES FOR QUANTITATIVE ESTIMATION OF VENETOCLAX: A COMPREHENSIVE REVIEW

Ms. Aditi Arun Mhatre[1*], Mr. Akash Darekar[2], Prof. M. T. Mohite[3], Dr. Hemant V. Kamble[5]

[1]Research Scholar Students, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata, Tal. Shirur, Dist, Pune 412211.
[2]Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata, Tal. Shirur, Dist, Pune 412211.
[3]Research Guide, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata, Tal. Shirur, Dist, Pune 412211.
[4]Principal, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata, Tal. Shirur, Dist, Pune 412211.

***Corresponding Author: Ms. Aditi Arun Mhatre**

Research Scholar Students, Loknete Shri Dadapatil Pharate College of Pharmacy, Mandavgan Pharata, Tal. Shirur, Dist, Pune 412211. **DOI:** https://doi.org/10.5281/zenodo.20642127

How to cite this Article: Ms. Aditi Arun Mhatre[1*], Mr. Akash Darekar[2], Prof. M. T. Mohite[3], Dr. Hemant V. Kamble[5] (2026). Rp-Hplc Method Development And Validation Approaches For Quantitative Estimation Of Venetoclax: A Comprehensive Review. European Journal of Pharmaceutical and Medical Research, 13(6), 856–863.

Article Received on 15/05/2026 **Article Revised on 05/06/2026** **Article Published on 10/06/2026**

ABSTRACT

Venetoclax is a selective B-cell lymphoma-2 (BCL-2) inhibitor widely used in the treatment of hematological malignancies such as chronic lymphocytic leukemia (CLL) and acute myeloid leukemia (AML). Due to its growing therapeutic importance, the development of accurate, precise, and reliable analytical methods for its quantitative estimation has become essential in pharmaceutical research and quality control. Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is one of the most preferred analytical techniques because of its high sensitivity, specificity, reproducibility, and cost-effectiveness in drug analysis. The present review focuses on various RP-HPLC method development strategies reported for Venetoclax, including selection of mobile phase, stationary phase, detection wavelength, and chromatographic conditions. The review also highlights validation parameters such as specificity, linearity, accuracy, precision, robustness, limit of detection, and limit of quantification according to ICH guidelines. In addition, stability-indicating methods and recent advancements in analytical approaches for Venetoclax estimation are discussed. This review aims to provide a comprehensive overview of RP-HPLC analytical methods for Venetoclax and emphasizes future perspectives toward the development of rapid, sensitive, eco-friendly, and regulatory-compliant analytical techniques in pharmaceutical analysis.

KEYWORDS: Venetoclax, RP-HPLC, Method Development, Method Validation, Quantitative Estimation, Pharmaceutical Analysis, Stability-Indicating Method, ICH Guidelines, Analytical Method Validation, Chromatographic Techniques.

INTRODUCTION

Cancer is one of the major causes of death worldwide and is characterized by uncontrolled proliferation of abnormal cells. Conventional chemotherapy often causes severe adverse effects because it affects both normal and cancerous cells. To minimize such limitations, targeted therapy has emerged as an effective treatment strategy that specifically acts on molecular targets involved in cancer progression and survival. Targeted anticancer agents improve therapeutic efficacy while reducing toxicity to healthy tissues, thereby enhancing patient safety and treatment outcomes.

Venetoclax is a potent and selective B-cell lymphoma-2 (BCL-2) inhibitor used in the treatment of hematological malignancies such as chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and non-Hodgkin lymphoma. BCL-2 is an anti-apoptotic protein that prevents programmed cell death and promotes survival of malignant cells. Overexpression of BCL-2 is commonly associated with resistance to chemotherapy and tumor progression. Venetoclax selectively binds to the BCL-2 protein and restores apoptosis in cancer cells, leading to their destruction. Due to its significant therapeutic efficacy and clinical importance, Venetoclax

has become an important targeted anticancer drug in modern oncology.

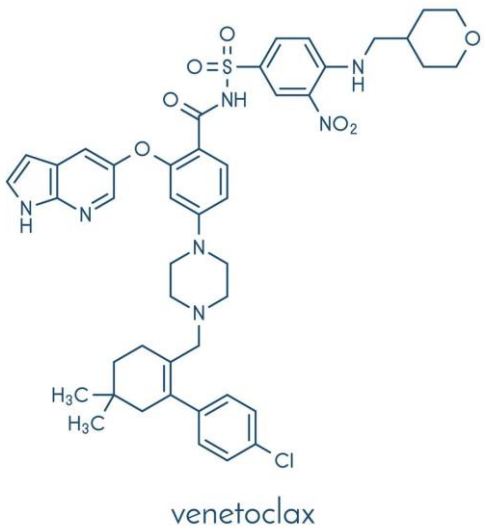
The growing use of Venetoclax in pharmaceutical formulations and biological studies necessitates the development of reliable analytical methods for its quantitative estimation. Accurate estimation of the drug is essential for quality control, dosage uniformity, dissolution testing, pharmacokinetic studies, bioequivalence studies, and stability analysis.[4] Analytical methods ensure the safety, efficacy, and regulatory compliance of pharmaceutical products throughout their lifecycle.

Among various analytical techniques, Reverse Phase High Performance Liquid Chromatography (RP-HPLC) is one of the most preferred methods for pharmaceutical analysis because of its high sensitivity, specificity, precision, reproducibility, and rapid separation capability. RP-HPLC effectively separates complex mixtures and provides accurate quantification of drug substances even at low concentrations. The technique is extensively used in routine quality control laboratories and research studies for assay determination and stability-indicating analysis. RP-HPLC method development involves optimization of chromatographic conditions such as mobile phase composition, stationary phase selection, flow rate, and detection wavelength to achieve satisfactory resolution and peak symmetry.[6] Furthermore, validation of RP-HPLC methods according to International Council for Harmonisation (ICH) guidelines ensures reliability, accuracy, and reproducibility of analytical results.

The present review focuses on RP-HPLC method development and validation approaches for the quantitative estimation of Venetoclax. The review also discusses various validation parameters, stability-indicating methods, analytical challenges, and recent advancements in chromatographic techniques used for Venetoclax analysis.

DRUG PROFILE OF VENETOCLAX

Venetoclax is a novel targeted anticancer agent belonging to the class of selective B-cell lymphoma-2 (BCL-2) inhibitors. Venetoclax acts by selectively inhibiting the anti-apoptotic BCL-2 protein, thereby restoring programmed cell death in malignant cells. Due to its high therapeutic efficacy and targeted mechanism, Venetoclax has gained considerable importance in modern cancer therapy.

Sr. No.	Properties	
1	Chemical Name	4-(4-{[2-(4-chlorophenyl)-4,4-dimethylcyclohex-1-en-1-yl]methyl}piperazin-1-yl)-N-{3-nitro-4-[(tetrahydro-2H-pyran-4-yl)methoxy]phenyl}sulfonyl-2-(1H-pyrrolo[2,3-b]pyridin-5-yloxy)benzamide
2	Molecular Formula	$C_{45}H_{50}ClN_7O_7S$
3	Molecular Weight	868.44 g/mol
4	Structure	Venetoclax contains aromatic rings, a piperazine moiety, sulfonamide linkage, and heterocyclic functional groups responsible for its selective BCL-2 inhibitory activity.  venetoclax
5	Category/Class	Anticancer agent Targeted therapy BCL-2 inhibitor Antineoplastic agent

6	Mechanism of Action	Venetoclax selectively binds to the BCL-2 protein, an anti-apoptotic protein overexpressed in many hematological malignancies. BCL-2 prevents apoptosis by inhibiting mitochondrial outer membrane permeabilization. Venetoclax displaces pro-apoptotic proteins from BCL-2, thereby activating the apoptotic pathway and inducing programmed cell death in cancer cells.[2,3]
7	Solubility Profile	Venetoclax is practically insoluble in water but shows solubility in organic solvents such as methanol, ethanol, dimethyl sulfoxide (DMSO), and acetonitrile. Its poor aqueous solubility presents challenges during formulation development and analytical method optimization. [4]
8	Pharmacokinetic Properties	Absorption Venetoclax is administered orally and exhibits good absorption when taken with food. Distribution The drug is highly protein bound (>99%) and shows extensive tissue distribution. Metabolism Venetoclax is primarily metabolized in the liver by cytochrome P450 enzyme CYP3A4. Elimination The drug is mainly eliminated through feces, with minimal renal excretion. Half-life The elimination half-life of Venetoclax is approximately 16–19 hours.
9	Marketed Formulations	Venetoclax is marketed under the brand name: Venclexta tablets Available strengths: 10 mg, 50 mg, and 100 mg tablets
10	Uses	It is commonly used in combination therapy for hematological malignancies such as chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), and small lymphocytic lymphoma (SLL).

OVERVIEW OF RP-HPLC TECHNIQUE

Reverse Phase High-Performance Liquid Chromatography (RP-HPLC) is one of the most widely used analytical techniques for the separation, identification, and quantitative estimation of pharmaceutical compounds. The technique utilizes a non-polar stationary phase and a polar mobile phase, allowing compounds to be separated based on their hydrophobic interactions. RP-HPLC offers high sensitivity, accuracy, precision, and reproducibility, making it an essential tool in pharmaceutical industries, research laboratories, and quality control departments.

RP-HPLC is extensively employed for assay determination, impurity profiling, dissolution testing, stability studies, pharmacokinetic analysis, and bioequivalence studies. The technique provides excellent peak resolution, shorter analysis time, and reliable quantitative results, even in complex sample matrices. Various chromatographic parameters, including mobile phase composition, pH, flow rate, and column temperature, can be optimized to achieve efficient separation and enhanced analytical performance.

Due to its versatility, robustness, and compatibility with different detectors, RP-HPLC has become a preferred method for routine pharmaceutical analysis and method development. It is particularly useful for the estimation of drugs in bulk materials, dosage forms, and biological samples, ensuring product quality, safety, and efficacy.

MATERIALS AND METHODS

MATERIALS

Table No.1: Active Pharmaceutical Drug.

Sr. No.	Name	Description
1.	Venetoclax	Yellowish amorphous powder. Venetoclax is selective BCL-2 inhibitor (anticancer drug) used in the treatment of hematological malignancies such as chronic lymphocytic leukemia (CLL), small lymphocytic lymphom (SLL), and acute myeloid leukemia (AML). It is a targeted anticancer agent, not an Anticoagulant.
2.	Venclexta 100mg Venetoclax Film-Coated Tablets	Each Tablet contains 100 mg Venetoclax, Manufactured and marketed by Abbvie Pharmaceutical Ltd..

Table No .2: List of Chemicals use in Research work.

Sr. No.	Name	Description / Use
1.	Venetoclax	Yellowish amorphous powder; BCL-2 inhibitor used as an anticancer drug. Used as the analyte (API) in the study.
2.	Acetonitrile (HPLC Grade)	Organic solvent used as a major component of the mobile phase due to its low viscosity and high elution strength.
3.	Methanol (HPLC Grade)	Organic solvent used in mobile phasepreparation and sample dilution. Helps in improving solubility of the drug
4.	Water (HPLC Grade)	Used for preparation of mobile phase and dilution of samples. Must be free from impurities to avoid interference.
5.	Orthophosphoric Acid (OPA)	Used to adjust the pH of the aqueous phase in the mobile phase. Helps in improving peak shape and resolution.
6.	Potassium Dihydrogen Phosphate (KH_2PO_4)	Used for preparation of phosphate buffer in the mobile phase to maintain pH stability.
7.	Sodium Hydroxide (NaOH)	Used for pH adjustment
8.	Hydrochloric Acid (HCl)	Used for pH adjustment

METHODS

1. Preliminary Analysis of Drug

a) Description

Color and texture of Venetoclax was compared with reported characters mentioned in drug bank.

b) Solubility

Venetoclax exhibits high solubility in organic solvents such as ethanol, dimethyl sulfoxide (DMSO), and dimethylformamide (DMF). This high solubility in organic media is attributed to its lipophilic chemical structure. These properties make organic solvents preferable for the preparation of standard and sample solutions in chromatographic analysis. Due to its poor aqueous solubility and stability, the use of mixed solvent systems (such as acetonitrile with buffer) is commonly employed in RP-HPLC method development to achieve optimal solubility, stability, and chromatographic performance.

c) UV Analysis

UV analysis was carried out by scanning the solution of Venetoclax at 200-400 nm. An accurately 10mg weighed quantity of Venetoclax mesylate was transferred into a clean and 100 ml dry volumetric flask and dissolved in a suitable solvent such as methanol (Stock Solution). The solution was sonicated, if necessary, to ensure complete dissolution, and the volume was made up to the mark with the same solvent to obtain a standard stock solution. From this stock solution, an appropriate aliquot was further diluted with the selected solvent to prepare a working standard solution (Sample Solution) of suitable concentration for UV analysis as Venetoclax 10µg/ml solution.

The UV–Visible spectrophotometer was switched on and allowed to stabilize. A baseline correction was performed using the solvent as a blank over the wavelength range of 200–400 nm, employing matched quartz cuvettes of 1 cm path length. The prepared working solution was then scanned in the same wavelength range against the blank.

The absorption spectrum was recorded, and the wavelength corresponding to maximum absorbance was identified. The λmax of Venetoclax mesylate was observed at 248 nm. The spectrum was saved and documented for further analytical method development.

3. Preparation of stationary phase

The stationary phase used for the RP-HPLC method was a C18 reverse-phase column (250 mm × 4.6 mm, 5 µm particle size). Before analysis, the column was properly installed in the HPLC system and flushed with HPLC-grade methanol to remove any impurities and contaminants. The column was then equilibrated with the selected mobile phase consisting of Acetonitrile and Phosphate Buffer (60:40 v/v) at a flow rate of 1.0 mL/min until a stable baseline was achieved.

4. Preparation of mobile phase

Preparation of Phosphate Buffer (pH 4.0): Accurately weighed about 6.8 g of potassium dihydrogen phosphate (KH_2PO_4) was transferred into a 1000 mL volumetric flask. About 800 mL of HPLC grade water was added and the contents were stirred until completely dissolved and done sonication. The pH was adjusted to 4.0 using orthophosphoric acid. After adjusting the pH, the volume was made up to 1000 mL with HPLC grade water and the solution was mixed well. Mobile Phase: Mixed above buffer and acetonitrile in the ratio of 40:60 v/v. take 600 mL of acetonitrile and 400 mL of phosphate buffer (pH 4.0) in a clean reagent bottle and mix thoroughly.

5. Preparation of standard stock solutions of Venetoclax

An accurately weighed 10 mg of Venetoclax mesylate was transferred into a 100 mL volumetric flask. About 60–70 mL of methanol was added and the solution was sonicated for 5–10 minutes to ensure complete dissolution of the drug. After complete dissolution, the volume was made up to the mark with the same solvent and mixed well. This solution produced a standard stock solution containing 100 µg/mL of Venetoclax and was used for further dilution in the preparation of working

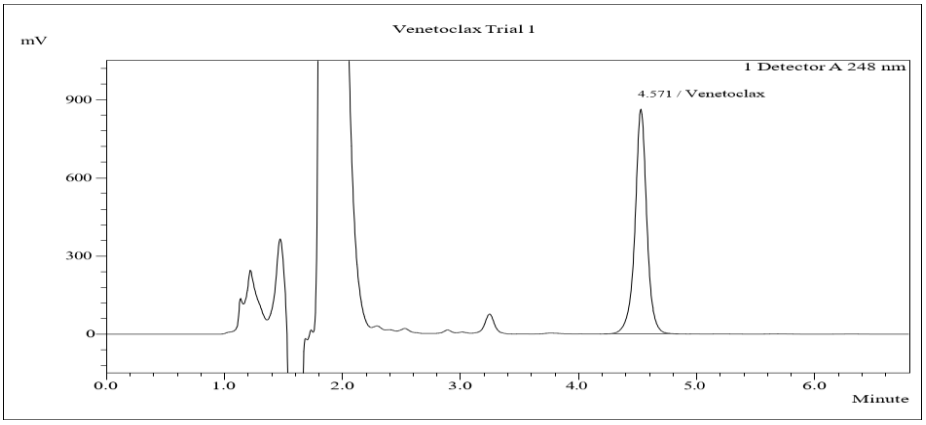
standard solutions and labelled as standard stock Venetoclax. From the resulting solution 0.1 ml was diluted to 10 ml with Methanol to obtain concentration of 10 µg/ml of Venetoclax.

6. Preparation of sample solution

20 tablets containing Venetoclax (100 mg) were weighed and the average weight was calculated. The tablets were carefully opened and the contents were mixed uniformly. An amount of powder equivalent to 10 mg of Venetoclax was accurately weighed and transferred into a 100 mL volumetric flask. About 60–70 mL of methanol was added and the solution was sonicated for 10–15 minutes to ensure complete extraction of the drug. The solution was then cooled and the volume was made up to the mark with the same diluent to obtain a stock solution of 100 µg/mL. The solution was filtered through Whatman filter paper or a 0.45 µm membrane filter to remove insoluble excipients. From this stock solution, 10 mL was pipetted into a 100 mL volumetric flask, and the volume was made up to the mark with the diluent to obtain a 10 µg/mL working sample solution for analysis.

TRIALS AND DISCUSSION

Trial-1



igure No.1: Chromatogram of Venetoclax Trial 1.

Trial-2

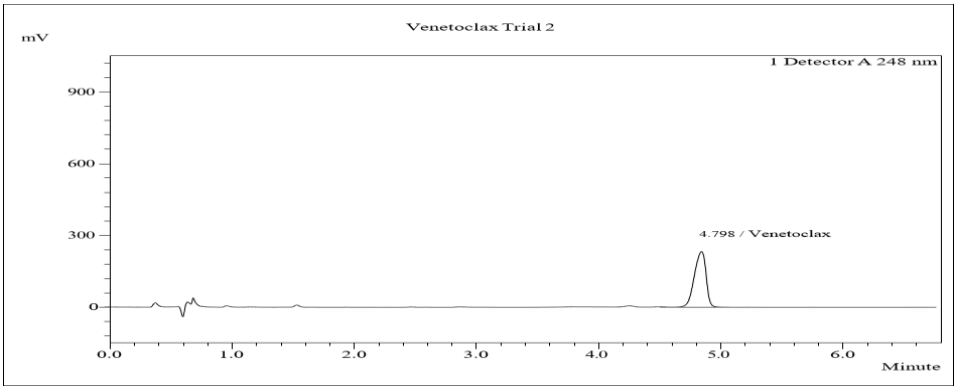


Figure No.2: Chromatogram of Trial 2.

Trial-3

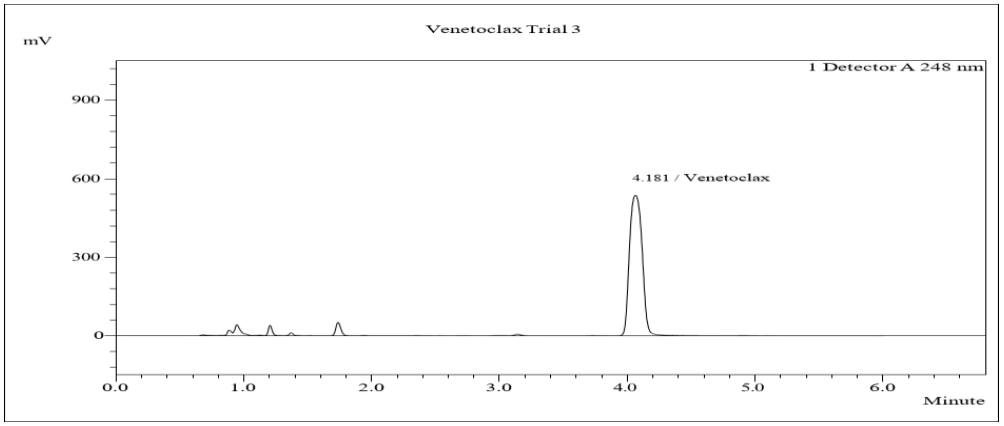


Figure No .3: Chromatogram of Trial 3.

Trial-4

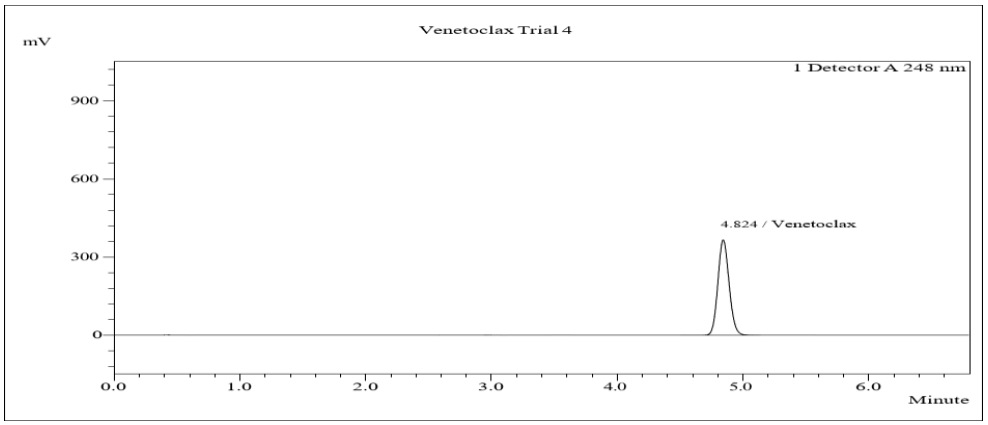


Figure No.4: Chromatogram of Trial 4.

Trial-5

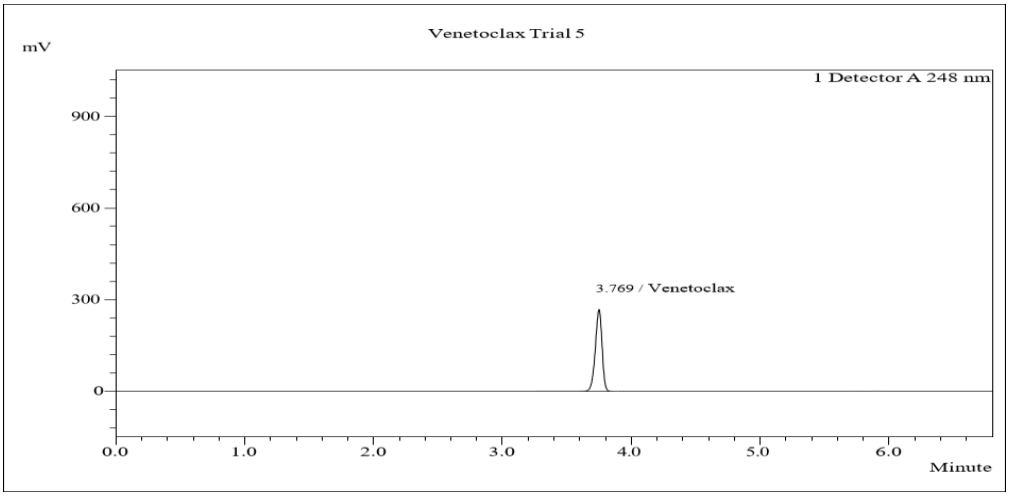


Figure No.5: Chromatogram of Venetoclax Trial-5.

Trial-6 (Optimized Trial)

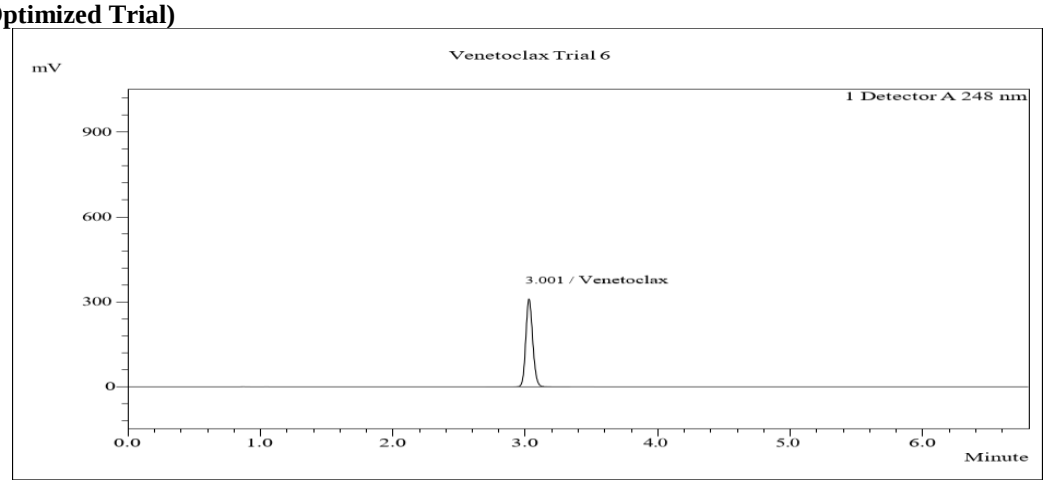


Figure No.6: Chromatogram of Venetoclax Trial 6 (Optimized Trial).

Several chromatographic trials were performed to optimize the RP-HPLC method for the analysis of Venetoclax. In Trial 1, using Acetonitrile (50:50), a retention time of 4.571 min was obtained; however, solvent peak splitting, poor baseline, and theoretical plates below 2000 resulted in rejection of the trial. Trial 2, with Methanol:Acetonitrile (25:25:50), showed peak tailing, high asymmetry, and increased retention time (4.798 min), making the chromatographic performance unacceptable.

In Trial 3, Acetonitrile (50:50, pH 4.5) produced a retention time of 4.181 min with acceptable theoretical plates, but solvent peak splitting and higher retention

time led to rejection. Trial 4, employing Acetonitrile:Methanol (30:30:50, pH 4.5), generated a good peak shape and stable baseline; however, the retention time (4.824 min) remained higher than desired. Trial 5, using Acetonitrile (50:50, pH 4.0), improved chromatographic performance with a retention time of 3.769 min, good peak shape, and theoretical plates above 2000, but further reduction in retention time was required.

In Trial 6, the optimized chromatographic conditions consisting of Acetonitrile Buffer (60:40, pH 4.0) on a Phenomenex C18 column (250 mm × 4.6 mm, 5 µm) at a flow rate of 1.0 mL/min and detection wavelength of 248 nm produced a sharp, symmetrical peak with a retention time of 3.001 min. The chromatogram showed good peak height, acceptable theoretical plates, proper baseline, no tailing, and no interference from other components. Therefore, these conditions were selected as the optimized RP-HPLC method for the validation and quantitative analysis of Venetoclax.

DISCUSSION

The present study focused on the development of an RP-HPLC method for the quantitative estimation of Venetoclax. Several chromatographic trials were performed by varying the mobile phase composition, pH, and detection wavelength to obtain optimum chromatographic conditions. Initial trials using different combinations of acetonitrile, methanol, water, and buffer resulted in chromatograms with undesirable characteristics such as peak splitting, poor baseline stability, peak tailing, high asymmetry, and longer retention times. These observations indicated that further optimization of the chromatographic conditions was necessary.

The incorporation of phosphate buffer and adjustment of the mobile phase pH significantly improved peak shape and chromatographic performance. Progressive optimization of the organic solvent ratio resulted in better retention behavior and enhanced peak symmetry. Among all the trials, the mobile phase consisting of Acetonitrile Buffer (60:40 v/v) at pH 4.0 provided the most satisfactory chromatographic response.

Under the optimized conditions, Venetoclax exhibited a retention time of approximately 3.001 minutes with a sharp, symmetrical, and well-resolved peak. The chromatogram showed a stable baseline, acceptable theoretical plate count, and absence of peak tailing or interference from other components. The use of a Phenomenex C18 column and detection at 248 nm further contributed to improved chromatographic performance.

The results demonstrated that careful optimization of mobile phase composition and pH plays a crucial role in achieving efficient separation and reliable estimation of Venetoclax. The developed chromatographic conditions were found to provide consistent peak characteristics and suitable retention behavior, making the method appropriate for routine pharmaceutical analysis of Venetoclax.

CONCLUSION

A simple and effective RP-HPLC method was successfully developed for the estimation of Venetoclax. Various chromatographic conditions were evaluated by changing the mobile phase composition, pH, detection wavelength, and column parameters to obtain optimum separation and peak characteristics. Among the different trials performed, the optimized chromatographic condition consisting of Acetonitrile: Phosphate Buffer (60:40, v/v) at pH 4.0, using a Phenomenex C18 column with a flow rate of 1.0 mL/min and detection at 248 nm, produced a sharp, symmetrical, and well-resolved peak with a retention time of 3.001 minutes.

The optimized method showed good peak shape, satisfactory retention behavior, acceptable theoretical plates, and absence of interference from the mobile phase. Therefore, the developed RP-HPLC method was found to be suitable for the routine analysis and quantitative estimation of Venetoclax in pharmaceutical formulations.

ACKNOWLEDGEMENT

The author expresses sincere gratitude to the Principal, faculty members, and management of the institute for their valuable support and guidance during the preparation of this review article. Special thanks are extended to the guide and all researchers whose published work contributed to the successful completion of this review.

CONFLICT OF INTEREST

The author declares that there is no conflict of interest regarding the publication of this review article.

REFERENCES

1. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. 3rd ed. New Jersey: Wiley, 2010.
2. Meyer VR. Practical high-performance liquid chromatography. 5th ed. Chichester: Wiley, 2010.
3. Chatwal GR, Anand SK. Instrumental methods of chemical analysis. 5th ed. Mumbai: Himalaya Publishing House, 2017.
4. Sharma BK. Instrumental methods of chemical analysis. 24th ed. New Delhi: Goel Publishing House, 2014.
5. Skoog DA, Holler FJ, Crouch SR. Principles of instrumental analysis. 6th ed. Belmont: Thomson Brooks/Cole, 2007.
6. Kazakevich Y, LoBrutto R. HPLC for pharmaceutical scientists. New Jersey: Wiley-Interscience, 2007.
7. Dong MW. Modern HPLC for practicing scientists. New Jersey: Wiley-Interscience, 2006.

8. Swartz ME, Krull IS. Analytical method development and validation. New York: Marcel Dekker, 1997.
9. Willard HH, Merritt LL, Dean JA, Settle FA. Instrumental methods of analysis. 7th ed. Belmont: Wadsworth Publishing; 1988.
10. United States Pharmacopeia. Chromatography general chapter <621>. Rockville: USP Convention, 2023.
11. Snyder LR, Kirkland JJ, Dolan JW. Introduction to modern liquid chromatography. 3rd ed. New Jersey: Wiley, 2010.
12. Roberts AW, Davids MS, Pagel JM, Kahl BS, Puvvada SD, Gerecitano JF, et al. Targeting BCL2 with Venetoclax in relapsed chronic lymphocytic leukemia. N Engl J Med, 2016; 374(4): 311-22.
13. International Council for Harmonisation. ICH guideline Q2(R1): Validation of analytical procedures: text and methodology [Internet]. Geneva: ICH, 2005 [cited 2026 May 22]. Available from: https: //database.ich.org
14. Blessy M, Patel RD, Prajapati PN, Agrawal YK. Development of forced degradation and stability indicating studies of drugs—A review. J Pharm Anal, 2014; 4(3): 159-65.
15. United States Pharmacopeia. Dissolution general chapter <711>. Rockville: USP Convention, 2023.
16. Salem AH, Agarwal SK, Dunbar M, Enschede SH, Humerickhouse RA, Wong SL. Clinical pharmacokinetics of Venetoclax, a selective BCL-2 inhibitor. Clin Pharmacokinet, 2018; 57(8): 1045-58.
17. Dong MW. Modern HPLC for practicing scientists. New Jersey: Wiley-Interscience, 2006.
18. Jones JA, Mato AR, Wierda WG, Davids MS. Clinical pharmacology and therapeutic use of Venetoclax in hematologic malignancies. Clin Lymphoma Myeloma Leuk, 2018; 18(11): 777–786.
19. Salem AH, Agarwal SK, Dunbar M, Kantarjian H. Pharmacokinetic variability and clinical implications of Venetoclax therapy. Cancer Chemother Pharmacol, 2019; 84(3): 593–602.
20. U.S. Food and Drug Administration. Venclexta (venetoclax) prescribing information, 2019.
21. DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, et al. Venetoclax combined with hypomethylating agents in AML: safety and efficacy analysis. Blood, 2020; 135(5): 341–349.
22. AbbVie Inc. Venclexta (venetoclax) prescribing information. North Chicago, IL, 2020.
23. D.Kelay, P.J.Haines, Instant notes Analytical chemistry, first ed., BIOS scientific, United Kingdom, 2002, pp. 1-18.
24. C. Marvin, MC Master, HPLC A practical use Guide, second ed., Willey-Inter sciences, A John Wiley & Sons, US, 2006, pp. 3-13.
25. L.R. Synder, J.J Kirkland, Introduction to modern Liquid chromatography, second ed., Willeys-Interscience, 1979.
26. ICH harmonized Tripartite Guidellines, validation of analytical procedure, and methodology, Q2 (R1), 2005; pp. 1-17.
27. Pungor, E. A Practical Guide to Instrumental Analysis. CRC Press: Boca Raton, FL, 1995.
28. ICH, Q2A validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, October 1994.
29. ICH, Q2B Validation of analytical procedure: Methodology International Conference on Harmonization, Geneva, March 1996.
30. Kar A. Pharmaceutical Drug Analysis. 2nd ed. New age international limited publishers, 2005. pp. 452-474.
31. Sethi P.D. Quantitative Analysis of drugs in Pharmaceutical, 1997
32. Formulations. 3rd ed. CBS publishers and distributors, New Delhi, pp. 17-19.
33. J. Bassett, R. C. Denney, G.H. Jeffery and J. Mendham. Vogel's Text Book of Quantitative Inorganic Analysis. 4th ed. Longman Group Ltd: England, 1978, pp. 1-9.