Development and validation of RP-HPLC method for estimation of Vonoprazan in bulk drug and formulation

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***Abstract*- A simple, precise, accurate, and robust reverse-phase high-performance liquid chromatography (RP-HPLC) method was developed and validated for the quantitative estimation of Vonoprazan in bulk drug and pharmaceutical dosage forms. Chromatographic separation was achieved on a C18 column using a suitable mobile phase consisting of [e.g., acetonitrile and phosphate buffer in a specific ratio, pH adjusted if applicable], at a flow rate of [e.g., 1.0 mL/min]. Detection was carried out at a wavelength of [e.g., 280 nm], and the retention time of Vonoprazan was found to be approximately [e.g., 3.5 minutes].**

The method was validated according to ICH Q2(R1) guidelines for parameters such as linearity, accuracy, precision, specificity, robustness, and system suitability. Linearity was observed in the concentration range of [e.g., 1–100 µg/mL], with a correlation coefficient (R^2) greater than 0.999. The percentage recovery ranged between [e.g., 98–102%], indicating the accuracy of the method. Intra-day and inter-day precision studies showed %RSD values less than 2%, confirming the reproducibility. The method was found to be specific for Vonoprazan with no interference from formulation excipients.

This validated method can be successfully applied for routine quality control analysis of Vonoprazan in bulk and pharmaceutical formulations.

***Index Terms*- HPLC, UV, Analytical Chemistry, Vanoprazon.**

I. INTRODUCTION

A systematic approach to pharmaceutical development begins with predefined objectives and emphasizes product and process understanding, along with process control based on sound science and quality risk management. In recent years, *Quality by Design (QbD)* has gained significant attention in the pharmaceutical industry. It focuses on designing quality into products rather than testing for it afterward.

Analytical techniques play a crucial role in ensuring the quality, safety, and efficacy of pharmaceutical compounds. They form a core component of QbD by providing the scientific basis for method control and risk management. The knowledge acquired during analytical method development helps in establishing control strategies and increases the likelihood of success throughout the product lifecycle. Here, validation serves to confirm method performance rather than expose potential weaknesses.

Pharmaceutical analysis is essential in quality control—monitoring raw materials, in-process materials, and finished products. It also aids in identifying and characterizing impurities, thereby ensuring consistent product quality and patient safety.

II. ANALYTICAL CHEMISTRY

Analytical chemistry is a measurement-based science focused on improving methods to determine the chemical composition of substances. It combines both theoretical and practical elements and is applied widely across laboratories in various fields. The discipline includes:

- **Qualitative analysis** – Identifying what substances are present.
- **Quantitative analysis** – Determining how much of each substance is present.

Analytical methods are continuously developed, refined, validated, and applied to solve specific problems.

III. HIGH PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC)

Chromatography involves the separation of mixture components based on their distribution between a stationary phase and a mobile phase. HPLC is a highly efficient and widely used chromatographic technique in pharmaceutical analysis due to its precision, sensitivity, and versatility.

IV. MODES OF CHROMATOGRAPHY

Chromatographic modes are classified based on the nature of interaction between solute and stationary phase. These interactions can involve hydrogen bonding, Van der Waals forces, electrostatic or hydrophobic forces, or molecular size. Common chromatographic modes include:

- **Normal Phase Chromatography**
- **Reverse Phase Chromatography**
- **Reverse Phase – Ion Pair Chromatography**
- **Ion Chromatography**
- **Ion-Exchange Chromatography**
- **Affinity Chromatography**
- **Size Exclusion Chromatography**

Each mode is suited for specific types of compounds and separation requirements.

Importance of Chromatography in Pharmaceutical Analysis

Chromatography has become increasingly vital in pharmaceutical analysis due to its ability to precisely separate, identify, and quantify structurally similar compounds. It is widely used for purity testing of final products and intermediates, including detection of degradation products and by-products. As a result, chromatographic techniques are gaining greater prominence in modern pharmacopoeias and regulatory standards.

High-Performance Liquid Chromatography (HPLC)

HPLC is a modern, advanced form of column chromatography where the mobile phase is pushed through the column under high pressure. This allows for much faster analysis and higher efficiency compared to classical techniques, thanks to the use of small particle-sized stationary phases.

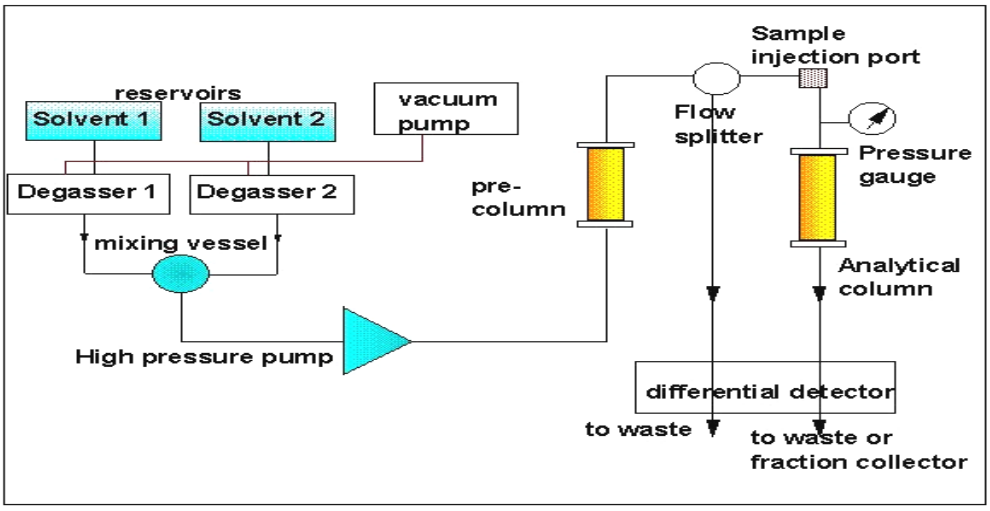


Fig.3. Instrumentation (Components) of HPLC System

An HPLC system typically includes:

- **Eluent reservoir**
- **High-pressure pump**
- **Sample injector**
- **Column with stationary phase**
- **Detector**
- **Data recorder**

Recent advancements in micro-particulate bonded phases have enhanced HPLC's ability to analyze complex mixtures with high resolution and speed.

Chromatographic Mechanisms

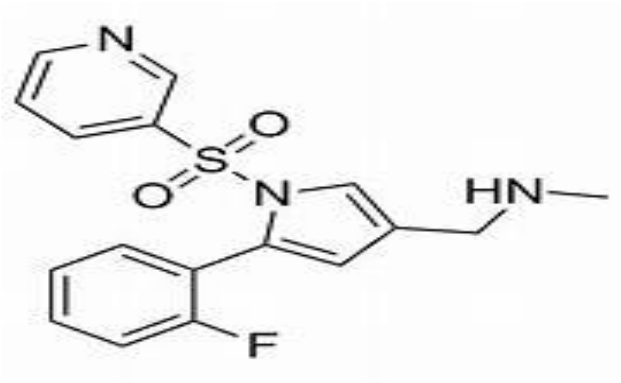
HPLC methods are classified into four main types based on separation mechanisms:

1. **Adsorption Chromatography** – Involves solute interaction with a solid stationary phase surface.
2. **Partition Chromatography** – Utilizes a liquid stationary phase coated on an inert solid; separation is based on solute partitioning.
    - *Normal phase*: Polar stationary phase, non-polar mobile phase

- *Reverse phase*: Non-polar stationary phase, polar mobile phase

3. **Ion-Exchange Chromatography** – Separates ions using a stationary phase with charged groups that attract oppositely charged solutes.
4. **Size-Exclusion Chromatography** – Separates molecules based on size using a stationary phase with controlled pore sizes; larger molecules elute first.

V. DRUG PROFILE



Vonoprazan

Table No.2: Profile of Vonoprazan

Molecular Formula	$C_{17}H_{16}FN_3O_2S$
Molecular weight	345.39 g/mol
Appearance	White, Crystalline powder
Solubility	soluble in water, freely soluble in Methanol,DMSO
Category	potassium-competitive acid blocker medication
IUPAC	(*E*)-but-2-enedioic acid;1-[5-(2-fluorophenyl)-1-pyridin-3-ylsulfonylpyrrol-3-yl]-*N*-methylmethanamine

Mechanism of Action:

Vonoprazan is a **potassium-competitive acid blocker (PCAB)** that inhibits the **H^+,K^+-ATPase** enzyme in gastric parietal cells. Unlike **proton pump inhibitors (PPIs)**, which form a covalent bond with the enzyme, vonoprazan **competitively blocks potassium binding**, rapidly and reversibly reducing gastric acid secretion.

Pharmacokinetics

- **Absorption:**
  - Steady state reached in 3–4 days.
  - Food slightly increases Cmax and AUC (~5–15%)—not clinically significant.

- Dose-proportional pharmacokinetics (10–40 mg/day).

- **Volume of Distribution:**
  - Single dose: ~1001 L
  - Steady state: ~782.7 L
- **Protein Binding:**
  - 85–88%, independent of concentration.
- **Metabolism:**
  - Mainly via **CYP3A4**; other CYPs and conjugation enzymes involved.
  - All metabolites are **inactive**.
  - **CYP2C19 polymorphisms** do not significantly affect metabolism.
- **Elimination:**
  - **Urine:** 67% (8% unchanged)
  - **Feces:** 31% (1.4% unchanged)
- **Clearance:**
  - Single dose: 97.3 L/h
  - Steady state: 81.3 L/h

Common Side Effects

- Anxiety
- Black, tarry stools
- Bladder pain
- Blood in urine
- Blurred vision
- Muscle/body pain
- Chest pain
- Cough
- Flu-like symptoms

VI. EXPERIMENTAL WORK

- **6.1. Chemicals used:**
- In method development and validation of preservatives following chemicals and reagents were used.

Table 3: List of chemicals

Ingredients	**Grade**	**Suppliers**
Vonoprazan	API	R.S.I.T.C Jalgaon.
Orthophopsphoric acid(OPA)	HPLC	Avantor Performance material India Ltd. Thane, Maharashtra
Formic acid	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager Estate ‘A’ Worli, Mumbai
methanol	HPLC	Merck Specialities Pvt. Ltd. Shiv Sager Estate ‘A’ Worli, Mumbai
Water	HPLC	Merck Specialities Pvt. Ltd.Shiv Sager Estate ‘A’ Worli, Mumbai

List of Marketed formulations:

Table No.4: List of brand names of combined formulations of Vonoprazan

Sr. No	Brand name	Formulation	Available strength	Address of manufacturer
1.	vocab	Tablet	Vonoprazan=20mg	Hetro Health Care

VII. RESULT AND DISCUSSION

Preliminary studies on Vonoprazan.

Melting point

The procured reference standard of Vonoprazan was found to melt in the range of 194.8 ºC respectively.

Solubility

The drug was found to be Vonoprazan slightly soluble in water, soluble in methanol DMSO, ethanol. Insoluble in ether.

UV Spectroscopy

UV absorption of 20mcg solution of Vonoprazan in MEOH was generated and absorbance was taken in the range of 200-400 nm. λ max of Vonoprazan was found to be 254 nm respectively.

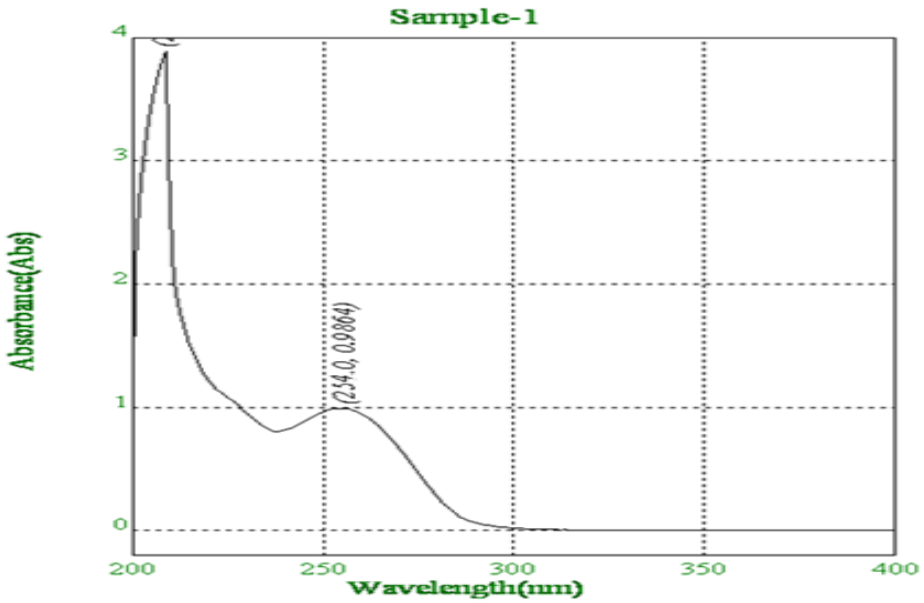


Fig No. 7: UV Spectrum of Vonoprazan

Studies on the chromatographic behaviour of Vonoprazan:

TABLE NO-10: Chromatographic behaviour of Vonoprazan mobile phase of various compositions.

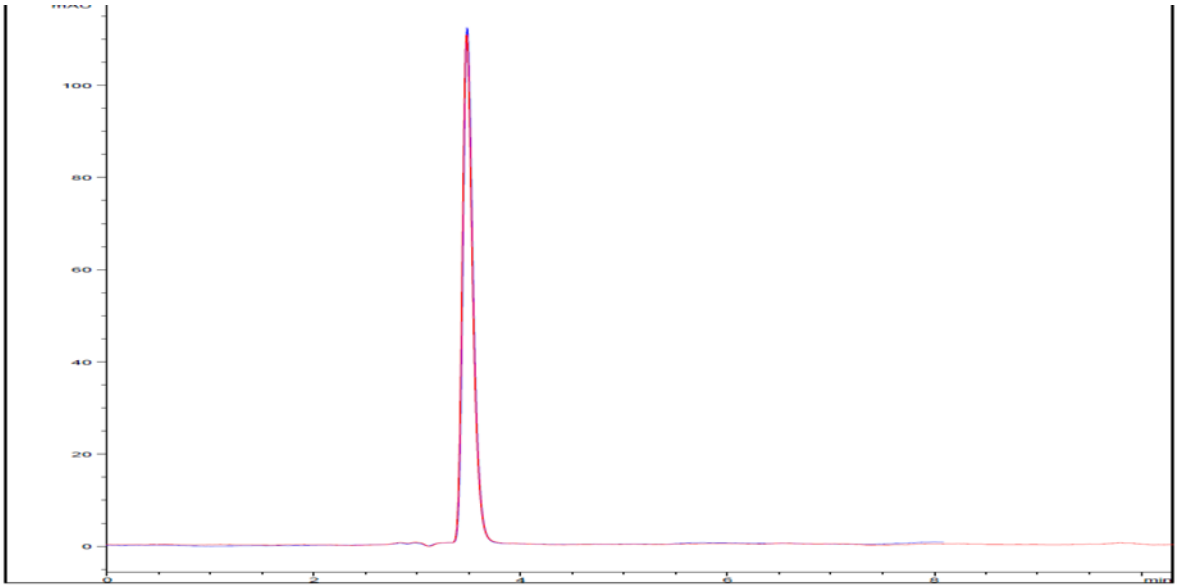
Sr No.	Mobile Phase	Retention time	Remark
1.	[90% MEOH +10% Water (0.1% Formic acid) Flow 0.7 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	4.011	Broad peak
2	[80% MEOH +20% Water (0.1% Formic acid) Flow 0.7 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	4.754	broad peak

3	[70 % MEOH + 30 % Water (0.1% Formic acid) Flow 0.7 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	3.83	Peak Fronting obtained
4	MEOH : 0. 1% formic acid 60:40 Flow 0.7 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	3.85	Larger RT
5	MEOH : 0. 1% formic acid 65:35 Flow 0.7 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	9.358	Sharpe peak were not obtained
6	MEOH : 0. 1% formic acid 65:35 Flow 1 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	6.478	Sharpe peak were not obtained
7	MEOH : 0. 1% formic acid 68:32 Flow 1 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	5.448	Sharpe peak were not obtained
8	MEOH : 0. 1% formic acid 58:32 Flow 1 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	2.753	Sharpe peak were not obtained
9	MEOH : 0. 1% formic acid 58:42 Flow 0.8 ml/min abs at 254 nm(column 250mm X 4.6, 5 µm)	3.445	Sharpe peak were obtained

Drug name	R.T	AREA	TH.PLATES	SYMM
Vonoprazan	3.423	777.56231	7823	0.70

Fig No.52: overlay for Marketed Formulation and STD

Analysis of marketed formulation were also %Label Claim was found to be 100-101% Satisfactory are concluded.



VIII. DISCUSSION

The developed RP-HPLC method provided an effective and reliable means for estimating Vonoprazan in both bulk and pharmaceutical formulations. The method demonstrated good separation with a sharp and symmetrical peak, indicating efficient chromatographic conditions. Linearity was established over a suitable concentration range with a high correlation

coefficient, confirming the method's suitability for quantitative analysis.

Precision and accuracy results were within acceptable limits, ensuring repeatability and reliability of the method. The method showed no interference from excipients, confirming its specificity. Robustness studies showed that minor deliberate changes in chromatographic conditions did not significantly affect the results, indicating method stability.

Overall, the validated method complies with ICH guidelines and is suitable for routine quality control of Vonoprazan in bulk and dosage forms.

IX. CONCLUSION

Simple, rapid, accurate and precise RP-HPLC as well as spectrophotometric methods have been developed and validated for the routine analysis of Vonoprazan in API and tablet dosage forms. Both methods are suitable for the simultaneous determination of Vonoprazan in multi-component formulations without interference of each other. The developed methods are recommended for routine and quality control analysis of the investigated drugs in two component pharmaceutical preparations. The amount found from the proposed methods was in good agreement with the label claim of the formulation. Also the value of standard deviation and coefficient of variation calculated were satisfactorily low, indicating the suitability of the proposed methods for the routine estimation of tablet dosage forms.

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