



Analytical Method Development and Validation of Vonoprazon Fumarate: A Comprehensive Review of UV-Spectroscopic, RP-HPLC, HPTLC, AND LC-MS/MS Approaches.

¹Rajkumar. A*, ²Alagar Raja. M, ³Gayathri K, ⁴Saran Raj G, ⁵Sethupathy A, ⁶Sneha M, ⁷Rajkumar. N

¹⁻⁷ United College of Pharmacy Periyanaickenpalayam, Coimbatore -641020
 Affiliated With Tamilnadu Dr.M.G.R. Medical University -600032, Tamilnadu
rajkumar08122000@gmail.com

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ABSTRACT:

For the treatment of *Helicobacter pylori*, gastroesophageal reflux disease, and peptic ulcer healing; a first-in-class potassium competitive acid blocker (P-CAB) known as vonoprazan fumarate has gained much attention as an alternative to proton pump inhibitors. This review scrutinizes the twenty-eight research reports investigating UV-spectrophotometry, reversed-phase high-performance liquid chromatography (RP-HPLC), high-performance thin-layer chromatography (HPTLC), and LC-MS/MS hyphenated bioanalytical methodologies published. The rising popularity of VNP as a therapeutically effective pharmaceutical entity triggered a paradigm shift in analytical space resulting in the generation of a significant amount of methodological literature. The present review reports chromatographic conditions, wavelength, range, linearity, limit of detection and quantification, precision, and recovery data for individual and combined dosage forms of vonoprazan along with amoxicillin, clarithromycin, domperidone, levosulpiride, and aspirin. Analytical Quality-by-Design, stability indicating, and green chemistry assessment approaches were considered independently for each methodology and critically evaluated in a master comparison table for quick access and easy understanding. The review highlights the research gaps like the absence of HPTLC and green-sustainable methodologies and provides an insight into future possibilities for the development of eco-friendly RP-HPLC and stability indicating assay procedures by applying the Quality-by-Design approach suitable for the routine analysis of vonoprazan fumarate formulations. The review further underlines the lack of dissolution, impurity profiling data, and forced degradation studies reported.

Keywords: Vonoprazan fumarate; potassium-competitive acid blocker; RP-HPLC; HPTLC; UV spectrophotometry; LC-MS/MS; bioanalytical validation; stability-indicating method; Analytical Quality by Design; green analytical chemistry

1. Introduction

1.1 Acid-Related Gastric Disorders and The Advent of P CAB

Gastroesophageal reflux disease (GERD), peptic ulcer disease and *Helicobacter pylori* associated gastritis are common conditions in a large part of the world population. Historically, these conditions have been treated with histamine H₂-receptor antagonists and, more recently, proton pump inhibitors (PPIs) like omeprazole, esomeprazole, and pantoprazole. Potassium-competitive acid blockers (P-CABs) were developed to fill the therapeutic gap left by PPIs' prodrug nature, reliance on acidic activation within parietal cells, delayed onset of action, and inter-patient variability associated with CYP2C19 polymorphism.

Vonoprazan fumarate became the first P-CAB to receive broad regulatory approval for the treatment of erosive esophagitis, *H. pylori* eradication (in triple/quadruple regimens with amoxicillin and clarithromycin), and ulcers caused by NSAIDs or *Helicobacter*. It was first approved in Japan in 2015 and later in the United States (2022, as part of combination therapy) and other markets. Vonoprazan exhibits a rapid onset and longer duration of acid suppression, and is less reliant on CYP2C19 genotype than PPIs, as it acts as a reversible and competitive inhibitor of the potassium-binding site of the H⁺/K⁺-ATPase enzyme without requiring acid activation.

1.2 Rationale For This Review

With the transition of vonoprazan fumarate from a novel chemical entity to a widely used generic and combination product, strong and well-established analytical techniques are required for quality control, stability testing, bioequivalence evaluation and pharmacovigilance. In the last few years, UV, RP-

HPLC, HPTLC and LC-MS/MS techniques for estimation of vonoprazan have been reported in ever-growing literature. These methods can be applied individually, in fixed-dose combinations, in plasma, and in stability-indicating and impurity-profiling methods. This review brings together twenty-eight such reports (research articles, technical notes and one previous narrative review ^[7] to provide method developers, quality-control chemists and regulatory scientists with a single source of information summarising chromatographic conditions, solvent ratios and validation results with a critical discussion of trends and gaps in the literature.

2. Drug Profile Of Vonoprazan Fumarate

2.1 Physicochemical Characteristics

Property	Description
Generic name	Vonoprazan fumarate (VPZ / VNP / TAK-438F)
IUPAC name	(E)-But-2-enedioic acid; 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methylmethanamine (fumarate salt of the free base)
CAS Registry Number	1260141-27-2 (fumarate salt)
Molecular formula (salt)	C ₂₁ H ₂₀ FN ₃ O ₆ S
Molecular weight (salt)	≈ 461.46–461.47 g/mol
Molecular formula (free base)	C ₁₇ H ₁₆ FN ₃ O ₂ S; MW ≈ 345.39 g/mol
Pharmacological class	Potassium-competitive acid blocker (P-CAB)
Mechanism of action	Reversible, competitive, K ⁺ -site inhibition of gastric H ⁺ /K ⁺ -ATPase (proton pump) of parietal cells; does not require acid activation, unlike PPIs
pKa	≈ 9.0–9.01 (basic); high pKa favours accumulation in the acidic canalicular space of parietal cells
log P	≈ 2.0–2.03
Melting point	≈ 194.8 °C
Boiling point (predicted)	≈ 530 ± 60 °C at 760 mmHg
Solubility	Freely soluble in methanol, DMSO, and dimethylformamide; sparingly soluble in water at neutral pH
Approved dosage forms	Immediate-release tablets, 10 mg and 20 mg (also available as fixed-dose combinations with amoxicillin and clarithromycin)
Elimination half-life	≈ 7–9 hours
Regulatory milestones	Japan (2015); USFDA – VOQUEZNA® (2023); CDSCO India (2024); combination triple-therapy regimens FDA-approved (2022–2025)

2.2 Degradation and Impurity Profile

Vonoprazan fumarate is highly sensitive to alkaline and oxidative stress with several degradation products formed. It is relatively stable under acid, thermal and photolytic stress conditions (except acid hydrolysis in AQbD-driven HPLC studies, where minor acid-induced degradation was observed). Process-related impurities including pyrrole-sulfonyl intermediates such as 1-[5-(2-fluorophenyl)-1-(pyridin-3-ylsulfonyl)-1H-pyrrol-3-yl]-N-methyldimethylamine and up to ten other similar compounds were reported in the literature as identified in some specific stability-indicating HPLC

experiments. This degradation profile emphasizes the need for validated stability indicating chromatographic techniques that can separate vonoprazan from its degradation products and process contaminants.

3. Regulatory Framework And Validation Parameters

The ICH Q2(R1)/Q2(R2) harmonized guideline on Validation of Analytical Procedures ^[28], which is supplemented in a few instances by USP General

Validation Parameter	Typical Acceptance Criterion Reported
Specificity/Selectivity	No interference from excipients, impurities, or degradation products at the analyte retention time
Linearity & Range	Correlation coefficient (r^2) ≥ 0.99 (most methods report 0.997–0.9999) across the stated concentration range
Precision (repeatability & intermediate precision)	% RSD < 2% (intraday and interday) in most methods
Accuracy (recovery)	98–102% mean recovery at 3 concentration levels (typically 50/80, 100, 120/150%)
LOD / LOQ	Calculated as $3.3\sigma/S$ and $10\sigma/S$ respectively from the calibration curve, or via signal-to-noise ratio ($\geq 3:1$ and $\geq 10:1$) for bioanalytical assays
Robustness	Deliberate small variation of flow rate, wavelength, mobile-phase ratio, column temperature, or pH; % RSD < 2% maintained
System suitability	Theoretical plates, tailing factor ≤ 2 , resolution ≥ 2 between adjacent peaks
Stability-indicating capability	Forced degradation under acid, base, oxidation, thermal, and photolytic stress; mass balance and peak-purity assessment

Chapter <1225> and, for bioanalytical assays, US FDA/EMA bioanalytical method validation guidance, serves as the foundation for the validation protocols of all twenty-eight reports examined here. Below is a summary of the key parameters evaluated in the literature.

4. Uv-Spectrophotometric Methods

Prior to the discovery of chromatographic methods, UV-spectrophotometry is often the first approach confirmed and continues to be the most affordable entry point for vonoprazan estimation. Vonoprazan in combination products is resolved using reported UV methods using either first-order derivative spectroscopy or direct zero-order absorbance measurement.

Author(s) / Year	Matrix	Solvent System	$\lambda_{\max}$ (nm)	Linearity Range	LOD / LOQ	Precision / Recovery
Nithesh et al., 2025 ^[19]	Tablets (bulk & formulation)	Ethanol: 0.1N HCl: Water	281 nm	Calibration curve series	LOD 1.557 $\mu\text{g/mL}$; LOQ 4.719 $\mu\text{g/mL}$	% RSD < 2 (ICH-compliant)
Saleh, El-Kosasy & Fares, 2023 ^[9]	Bulk & tablets	Methanol: Phosphate buffer (50:50 v/v)	230	5.00–15.00 $\mu\text{g/mL}$ (series)	Calculated via $3.3\sigma/S$ & $10\sigma/S$	Recovery 99.48 $\pm$ 0.947%
Patel & Bhangale, 2026 (UV component) ^[12]	Synthetic mixture with Levosulpiride	Methanol (first-order derivative, zero-crossing)	308 (derivative)	1–5 $\mu\text{g/mL}$ (VNP)	Per ICH regression equations	Recovery 99.33–99.80%

Table 4.1: Summary of UV-spectrophotometric methods reported for vonoprazan fumarate.**5. Rp-HPLC Methods**

Because of its better selectivity, resolution, and applicability for both single-drug and multi-drug (fixed-dose combination) estimates, RP-HPLC dominates the analytical literature for vonoprazan. A master table that captures precise mobile-phase ratios—the key optimization variable in each method—follows the evaluated reports, which are arranged below into single-component and simultaneous/combination assays.

5.1 Single-Component (Vonoprazan Alone) RP-HPLC Methods

Author(s) / Year	Column	Mobile Phase (Ratio, v/v)	λ (nm)	Flow Rate	Linearity	LOD / LOQ	% RSD / Recovery
Alzaghal, El-Mossalamy & El-Sayed, 2024 ^[10]	Agilent C18, 150 × 4.6 mm, 5 μ m	KH ₂ PO ₄ /Na ₂ HPO ₄ buffer pH 6.8: Acetonitrile (65:35)	225	1.0 ml/min	Series (bulk & tablets)	LOD 0.182 / LOQ 0.552 μ g/ml	Recovery $\approx$ 99–101%
Patil, Patel, Salunkhe & Shrivastava, 2026 (AQbD) ^[6]	C18 (BBD-optimised)	Acetonitrile: 0.1% glacial acetic acid pH 6.5 (80:20)	230	0.7 ml/min	10–90 μ g/ml	Low LOD 2.08 / LOQ 6.32 (regression-derived)	% RSD < 2; Recovery 98.34–98.83%
Gami, Nashit, Shethiya, Marvaniya & Patel, 2026 (QbD/DoE) ^[11]	C18	Phosphate buffer pH 3.5: Methanol (41:59)	245	0.9 ml/min	$r^2 = 0.9978$	Low LOD 3.3 / LOQ 10 μ g/ml	Recovery 98.83%; % RSD $\leq$ 2
Gaikwad, Patil, Patil & Pawar, 2025 ^[14]	C18	Acetonitrile: phosphate buffer (specific ratio optimised)	254	1 ml/min	Linear over assay range	Within ICH limits	Recovery $\approx$ 98–102%
Rajage, Jadhav, Jain, Jadhav et al., 2024 (green RP-HPLC) ^[3]	Hypersil C18, 250 × 4.6 mm, 5 μ m	Water (pH 3.0): Acetonitrile (95:5)	213	2.08 ml/min	10–60 μ g/ml	LOD 5.33 / LOQ 16.16 μ g/ml	Recovery 98.90%

Table 5.1: Single-component RP-HPLC methods for vonoprazan fumarate in bulk drug and tablet dosage forms.**5.2 Combination RP-HPLC Methods**

Author(s) / Year	Combination Partner(s)	Mobile Phase (Ratio, v/v)	λ (nm)	Linearity (VNP)	% RSD / Recovery
Kanaga, 2024 ^[8]	Domperidone	Acetonitrile: Water (65:35)	230	Linear, $r^2 = 0.999 / 0.997$	Recovery per FDA method
Sampath & Elavarasan, 2024 ^[17]	Amoxicillin, Clarithromycin	Phosphate buffer pH 4.0: Methanol (50:50)	205	50–300 μ g/ml (series)	% RSD within ICH limits
Tilva & Ajudia, 2026 (QbD) ^[5]	Amoxicillin, Clarithromycin	Methanol: 0.1% TFA in water, pH 2.2 (30:70)	210	10–50 μ g/ml (VNP)	$r^2 > 0.999$; % RSD within limits

Author(s) / Year	Combination Partner(s)	Mobile Phase (Ratio, v/v)	λ (nm)	Linearity (VNP)	% RSD / Recovery
Ali, El-Gindy, Wahba & Mostafa, 2025 (eco-friendly) ^[1]	Amoxicillin, Clarithromycin	0.05 M KH ₂ PO ₄ buffer pH 6: Acetonitrile (70:30)	254	1.0–10.0 µg/ml (VNP)	LOD 0.061 / LOQ 0.1849 µg/mL
Iftikhar, Tehseen, Tariq, Zubair & Wajid, 2026 ^[16]	Aspirin	Phosphate buffer 0.05M: Acetonitrile: Methanol (17:7:6)	230 & 270	Linear, $r^2 = 0.999$ / 0.998	LOD 1.2 / LOQ 3.5 µg/mL; Recovery 80–121%*
Patel & Bhargale, 2026 (RP-HPLC component) ^[12]	Levosulpiride	Phosphate buffer pH 4.5: Acetonitrile (58:42)	269	1–5 µg/ml (VNP)	Recovery 99.73–99.94%

Table 5.2: Simultaneous RP-HPLC methods for vonoprazan fumarate combined with other actives. *Recovery reported across 80/100/120% spiking levels, individually within acceptance range.

6. HPTLC Methods

For routine quality control, high-performance thin-layer chromatography provides a quick, inexpensive, high-throughput substitute that is especially preferred when several samples need to be screened at once. Four specific HPTLC reports using silica gel 60 F254 stationary phases with densitometric scanning were found.

Author(s) / Year	Stationary Phase	Mobile Phase (Ratio, v/v/v)	Detection (nm)	Rf Value	Linearity Range	LOD / LOQ	% RSD / Recovery
Gandhimathi, Jeyaprakash, Josika et al., 2025 ^[13]	Silica gel 60 F254	Methanol (100%)	264	0.180 ± 0.013	2–30 µg/ml (band)	LOD 0.24 / LOQ 0.70 µg/ml	Recovery 99.48 ± 0.947%; % RSD < 2
Gandhi & Bacche, 2025 (stability-indicating, green) ^[2]	Silica gel 60 F254	Methanol: Ethyl acetate: Formic acid (8.5:1:0.5)	266	~0.45	800–... ng/band (series)	Per ICH regression	% RSD 1.32–1.90
Ayanar, Pawar et al., 2026 (AQbD/RSM) ^[23]	Silica gel 60 F254	Response-surface-optimised solvent (5:4:1 acetic-acid-modified system)	215	0.52	200–1200 ng/spot	LOD 2.96 / LOQ 8.98 ng/spot	Recovery 99.05–103.48%; RSD < 2%
Sangeetha, Hifathulla, Kannan, Saranya & Srija, 2026 (stability) ^[15]	Silica gel 60 F254 (Merck)	1-Propanol: Water: Methanol (7:2:1)	262	0.74	Series (stress-stability study)	Per ICH regression	Within ICH acceptance

Table 6.1: HPTLC methods reported for vonoprazan fumarate, including stability-indicating and AQbD-optimised variants.

7. Bioanalytical LC–MS/MS And Hyphenated Methods

LC-MS/MS and LC-MS-coupled HPLC techniques offer the sensitivity (sub-ng/ml to pg/ml) not possible with UV-based detection for pharmacokinetic, bio equivalency, metabolite-identification, and ultra-trace impurity investigations. In the compiled literature, seven such reports were found; these are outlined below.

Author(s) / Year	Application	Matrix	Mobile Phase / Elution	Linear Range	LOD / LOQ (LLOQ)	Precision / Recovery
Singh, Sahoo & Kumar, 2026 ^[4]	Metabolite identification & quantification	Rat/human plasma	0.1% formic acid (A): Acetonitrile with 0.1% formic acid (B), gradient UHPLC	0.1–100 ng/ml (VNP, M-III); 1–1000 ng/ml (other metabolites)	LLOQ 0.1–1 ng/mL	CV < 8%; accuracy 80–120%
Sajjad, Tulain, Asim et al., 2026 (RP-UFLC) ^[20]	Pharmacokinetics of chitosan nanoparticles	Rabbit plasma	0.03 M sodium phosphate buffer-based gradient	50–450 ng/ml	LOD 0.0014 / LOQ 0.0043 ng/ml	Intraday RSD 0.03–0.07%
Saraf, Mehta, Rahul et al., 2026 ^[21]	Stability-indicating HPLC with LC-MS degradant characterisation	Bulk drug	Acetonitrile: 0.1% formic acid (70:30)	Low-µg/ml range, $r^2 = 0.9981$	LOD 0.086 / LOQ 0.261 µg/ml	% RSD < 2; Recovery 99.91–100.26%
Luo, Liu, Liu, Wang et al., 2018 ^[22]	Ten related substances (drug substance)	Bulk drug substance	Gradient: (A) buffer: methanol: ACN 72:25:3 → (B) buffer: ACN 30:70	Impurity-specific ranges	Impurity-specific LOD/LOQ	% RSD < 2 (per impurity)
Chen, Chen, Huang, Wang et al. (VAN-8) ^[25]	Quick detection & bioequivalence study	Human plasma	Acetonitrile protein precipitation, isocratic LC–MS/MS	0.150–60.000 ng/ml	MRM transition 350.0→316.0	Validated per FDA bioanalytical guidance
Al-Tannak, Hafez, Hemdan, Aldasem & Khadra, 2025 ^[27]	Clinical PK / TDM of triple therapy	Human plasma	0.1% formic acid (water): Acetonitrile, gradient (5% B → ramped)	LLOQ-based series	LLOQ 2–5 ng/ml	Recovery within ±15% (±20% at LLOQ)
BenchChem Technical Support Team, 2026 ^[18]	Impurity method-validation guidance	Drug substance	(A) buffer: methanol: ACN 72:25:3; (B) buffer: ACN 30:70, gradient, 230 nm	Impurity-level linearity	LOQ-level 90–110% recovery	Precision (% RSD) < 5.0 at LOQ

Table 7.1: LC-MS/MS and hyphenated bioanalytical / impurity-profiling methods for vonoprazan fumarate.

8. Stability-Indicating Methods And Forced Degradation

Ten of the twenty-eight analysed papers specifically proved stability-indicating capacity, which is the ability of a method to resolve the intact medication from all degradation products, contaminants, and excipients. Vonoprazan fumarate consistently demonstrated relative resilience to heat and photolytic stress, moderate susceptibility to acidic hydrolysis in certain protocols, and strong susceptibility to base-catalysed hydrolysis and oxidative stress throughout these investigations.

Author(s) / Year	Technique	Stress Conditions Applied	Key Degradation Finding
Rajage et al., 2024 ^[3]	RP-HPLC (green-	Acid, base, oxidative, thermal,	Significant degradation under

Author(s) / Year	Technique	Stress Conditions Applied	Key Degradation Finding
	assessed)	photolytic	alkaline & oxidative stress; stable to acid/thermal/photolytic
Patil et al., 2026 (AQbD) ^[6]	RP-HPLC (BBD-optimised)	Acid, base, oxidative, thermal, photolytic	Degraded under acidic & oxidative stress; resistant to photolytic & thermal stress
Gandhi & Bacche, 2025 ^[2]	HPTLC (green)	Acid, base, oxidation, photolysis, thermal, humidity	Resolved multiple degradation bands; validated as stability-indicating
Sangeetha et al., 2026 ^[15]	HPTLC	Acid, base, oxidative, photolytic, thermal (per ICH Q1A)	Confirmed stability-indicating nature; degradation products resolved from parent peak
Luo, Liu, Liu, Wang et al., 2018 ^[22]	Gradient HPLC	Systematic forced degradation of drug substance	Ten related substances characterised and resolved from vonoprazan
Saraf, Mehta, Rahul et al., 2026 ^[21]	HPLC with LC-MS characterisation	Forced degradation with mass-spectral degradant elucidation	Degradation products structurally characterised via LC-MS
Saraf, Mehta et al., 2026 (in-silico) ^[24]	QSAR / in-silico toxicological screening	Not applicable (computational)	Predicted toxicological risk profile of identified degradation products
BenchChem Technical Support, 2026 ^[18]	Gradient HPLC (guidance)	Alkaline & oxidative stress emphasised	Confirms alkaline/oxidative lability as a consistent theme across independent laboratories

Table 8.1: Forced-degradation behaviour of vonoprazan fumarate reported across stability-indicating studies.

9. Analytical Quality By Design (AQbD) And Design Of Experiments (Doe) Driven Method

AQbD principles—risk assessment, design-of-experiments (DoE) screening/optimization, and definition of a Method Operable Design Region (MODR)—are applied to vonoprazan method development in a distinct and expanding subset of the literature (four reports). This reflects the larger regulatory push (ICH Q8/Q14) toward systematic, risk-based analytical development over conventional one-factor-at-a-time (OFAT) approaches.

Author(s) / Year	DoE Tool	Critical Method Attributes (CMAs) Studied	Optimised Condition	Outcome
Patil, Patel, Salunkhe & Shrivastava, 2026 ^[6]	Box-Behnken Design (BBD)	% ACN concentration, mobile-phase pH, flow rate; responses: retention time, tailing factor	Acetonitrile: 0.1% glacial acetic acid pH 6.5 (80:20), 0.7 ml/min	Robust, stability-indicating method with linearity 10–90 µg/mL
Gami, Nashit, Shethiya, Marvaniya & Patel, 2026 ^[11]	Design of Experiments (factorial)	Buffer pH, organic-modifier ratio	Phosphate buffer pH 3.5: Methanol (41:59)	$r^2 = 0.9978$; recovery 98.83%
Tilva & Ajudia, 2026 ^[5]	QbD (response-surface / factorial)	% TFA concentration, methanol concentration, flow rate	Methanol: 0.1% TFA (pH 2.2) (30:70), 1.0 ml/min	Simultaneous AMO/VNP/CLA estimation, $r^2 > 0.999$

Author(s) / Year	DoE Tool	Critical Method Attributes (CMAs) Studied	Optimised Condition	Outcome
Ayanar, Pawar et al., 2026 ^[23]	Response Surface Methodology (RSM)	Solvent-system ratio, chamber saturation time	Toluene: Acetone: Glacial acetic acid-modified system (≈05:04:01)	HPTLC, linearity 200–1200 ng/spot

Table 9.1: AQbD/DoE-driven analytical method development studies for vonoprazan fumarate.

10. Green Analytical Chemistry Assessment

The National Environmental Methods Index (NEMI), the Analytical Eco-Scale (ESA), the Green Analytical Procedure Index (GAPI), and the Analytical GREENness metric (AGREE) are recognized greenness-assessment tools that several authors used to formally score their vonoprazan methods considering the growing emphasis on sustainable laboratory practice.

Author(s) / Year	Technique	Greenness Tools Applied	Reported Outcome
Saleh, El-Kosasy & Fares, 2023 ^[9]	UV-spectrophotometry	Eco-Scale, GAPI	Method rated eco-friendly; methanol usage flagged as the principal environmental hazard
Rajage, Jadhav, Jain, Jadhav et al., 2024 ^[3]	RP-HPLC	Green assessment metrics (Eco-Scale/GAPI-type)	Aqueous-rich mobile phase (95:5 water: ACN) confirmed as a low-impact, “green” method
Ali, El-Gindy, Wahba & Mostafa, 2025 ^[1]	RP-HPLC (ternary mixture)	NEMI, Eco-Scale, GAPI, AGREE	Multi-tool assessment confirmed eco-friendly status across all four metrics
Gandhi & Bacche, 2025 ^[2]	HPTLC	Modified GAPI, White Analytical Chemistry (WAC)	Predominantly green pictogram; methanol flagged as a Class-2 (yellow) solvent

Table 10.1: Green analytical chemistry assessment outcomes for vonoprazan fumarate methods.

11. MASTER COMPARATIVE TABLE OF ALL REPORTED METHODS

Except for the pharmacopeial monograph and the previous narrative review, which are covered individually in Sections 12 and 1, the twenty-six key analytical methods described are combined into a single quick-reference matrix in the table below, arranged by methodology.

Sno	Author(s), Year	Technique	Analyte(s)	Mobile Phase Ratio	λ (nm)	Linearity	LOD/LOQ	%RSD
1	Nithesh et al., 2025 ^[19]	UV	VNP	Ethanol:0.1N HCl: Water	280	Series	1.557/4.719 $\mu\text{g/mL}$	<2
2	Saleh et al., 2023 ^[9]	UV	VNP	Methanol: Phosphate buffer (50:50)	230	5–15 $\mu\text{g/mL}$	3.3 σ /S, 10 σ /S	<2
3	Patel & Bhangale, 2026 ^[12]	UV (deriv.) + RP-HPLC	VNP+Levosulpiride	MeOH (UV); Phosphate buffer: ACN (58:42)	308	1–5 $\mu\text{g/mL}$	Per ICH	<2
4	Alzaghal et al., 2024 ^[10]	RP-HPLC	VNP	Phosphate buffer pH6.8: ACN (65:35)	225	Series	0.182/0.552 $\mu\text{g/mL}$	<2

Sno	Author(s), Year	Technique	Analyte(s)	Mobile Phase Ratio	λ (nm)	Linearity	LOD/LOQ	%RSD
5	Patil et al., 2026 (AQbD) ^[6]	RP-HPLC	VNP	ACN:0.1% acetic acid pH6.5 (80:20)	–	10–90 µg/ml	Low, regression	<2
6	Gami et al., 2026 (QbD) ^[11]	RP-HPLC	VNP	Phosphate buffer pH3.5: MeOH (41:59)	245	$r^2=0.9978$	–	≤ 2
7	Gaikwad et al., 2025 ^[14]	RP-HPLC	VNP	ACN: Phosphate buffer (optimised)	280	Linear	Within ICH	≤ 2
8	Rajage et al., 2024 (green) ^[3]	RP-HPLC	VNP	Water pH3.0: ACN (95:5)	–	10–60 µg/mL	5.33/16.16 µg/mL	<2
9	Kanaga, 2024 ^[8]	RP-HPLC	VNP+Domperidone	ACN: Water (65:35)	230	$r^2=0.999/0.997$	–	<2
10	Sampath & Elavarasan, 2024 ^[17]	RP-HPLC	VNP+AMX+CLA	Phosphate buffer pH4.0: MeOH (50:50)	205	50–300 µg/ml	Per ICH	<2
11	Tilva & Ajudia, 2026 (QbD) ^[5]	RP-HPLC	VNP+AMX+CLA	MeOH:0.1% TFA pH2.2 (30:70)	–	10–50 µg/ml (VNP)	–	<2
12	Ali et al., 2025 (eco) ^[1]	RP-HPLC	VNP+AMX+CLA	0.05M KH ₂ PO ₄ pH6: ACN (70:30)	254	1–10 µg/mL (VNP)	0.061/0.1849 µg/mL	<2
13	Iftikhar et al., 2026 ^[16]	RP-HPLC	VNP+Aspirin	Phosphate buffer: ACN: MeOH (17:7:6)	230&270	$r^2=0.999/0.998$	1.2/3.5 µg/mL	<2
14	Gandhimathi et al., 2025 ^[13]	HPTLC	VNP	Methanol (100%)	264	2–30 µg/ml	0.24/0.70 µg/mL	<2
15	Gandhi & Bacche, 2025 ^[2]	HPTLC (stability)	VNP	MeOH: Ethyl acetate: Formic acid (8.5:1:0.5)	266	Series (ng/band)	Per ICH	1.32–1.90
16	Ayanar & Pawar, 2026 (AQbD) ^[23]	HPTLC	VNP	RSM-optimised ($\approx 5:4:1$)	215	200–1200 ng/spot	2.96/8.98 ng/spot	<2
17	Sangeetha et al., 2026 ^[15]	HPTLC (stability)	VNP	1-Propanol: Water: MeOH (7:2:1)	262	Series	Per ICH	Within limits
18	Singh et al., 2026 ^[4]	LC-MS/MS	VNP+metabolites	0.1% formic acid: ACN gradient	–	0.1–100 ng/ml	LLOQ 0.1 ng/mL	CV<8

Sno	Author(s), Year	Technique	Analyte(s)	Mobile Phase Ratio	λ (nm)	Linearity	LOD/LOQ	%RSD
19	Sajjad et al., 2026 ^[20]	RP-UFLC	VNP (nanoparticle PK)	Phosphate-buffer gradient	230	50–450 ng/ml	0.0014/0.0043 ng/mL	<0.1
20	Saraf, Mehta et al., 2026 ^[21]	HPLC + LC-MS	VNP	ACN:0.1% formic acid (70:30)	266	$r^2=0.9981$	0.086/0.261 μ g/mL	<2
21	Luo et al., 2018 ^[22]	Gradient HPLC	VNP + 10 impurities	Buffer: MeOH: ACN (72:25:3) $\rightarrow$ Buffer: ACN (30:70)	230	Impurity-specific	Impurity-specific	<2
22	Chen et al. (VAN-8) ^[25]	LC-MS/MS	VNP (plasma)	ACN protein precipitation, isocratic	–	0.150–60.000 ng/ml	MRM 350.0 $\rightarrow$ 316.0	–
23	Al-Tannak et al., 2025 ^[27]	LC-MS/MS	VNP triple therapy (plasma)	0.1% formic acid: ACN gradient	–	LLOQ-based	2–5 ng/mL	$\pm 15\%$
24	BenchChem, 2026 ^[18]	Gradient HPLC (guidance)	VNP + impurities	Buffer: MeOH: ACN (72:25:3)/Buffer: ACN (30:70)	230	Impurity-level	LOQ-level	<5

Table 11.1: Master comparative matrix of vonoprazan fumarate analytical methods (VNP = vonoprazan fumarate; AMX/AMO = amoxicillin; CLA/CLM = clarithromycin; ACN = acetonitrile; MeOH = methanol).

12. PHARMACOPEIAL AND OFFICIAL MONOGRAPH STATUS

National quality-control laboratories have started to provide analytical profiles for vonoprazan tablets outside of the peer-reviewed literature. The National Medicines Laboratory, Department of Drug Administration, Government of Nepal issued an analytical profile (Vono 082/83/AP 179) [26] that defined an assay acceptability range of 90.0–110.0% of the labelled amount for tablet strengths of 10 mg and 20 mg. The dissolution test was performed using 900 mL of acetate buffer (pH 4.5) as the dissolution medium and 30% acetonitrile as diluent for both assay and dissolution samples. The HPLC assay was performed with acetonitrile: phosphate buffer mobile phase (30:70 v/v). The identification test depends on the matching of retention time between the sample and reference standard chromatograms.

Vonoprazan fumarate is a relatively new drug and at present, there is no dedicated monograph of this drug in USP-NF, British Pharmacopoeia or Indian Pharmacopoeia because of its recent approval worldwide.

13. Critical Discussion: Trends And Gaps

13.1 Prevailing Trends

The moderate lipophilicity of Vonoprazan ($\log P \sim 2$) is also observed in the use of mobile phases of acetonitrile-aqueous-buffer in RP-HPLC techniques, with ratios between ~65:35 to 80:20 (organic: aqueous). The detection wavelengths agreed with the chromophoric pyrrole-sulfonyl-pyridine system of vonoprazan in the range 225–266 nm. Publications from 2025–2026 show a distinct shift from the traditional trial-and-error (OFAT) development to AQbD/DoE-based optimization (Box-Behnken, factorial, response-surface designs). The green analytical chemistry assessment (NEMI/GAPI/ESA/AGREE) is reported more often, but it is still applied to a low percentage of primary techniques (4 out of 26). For pharmacokinetic and bioequivalence studies, bioanalytical LC-MS/MS approaches achieve sensitivity in the low ng/mL to pg/mL range. However, these procedures require specialized triple-quadrupole apparatus that is not often available to normal QC laboratories.

13.2 Identified Gaps

Despite HPTLC's cost and throughput benefits for routine QC screening, there are still very few HPTLC methods (4 studies) compared to RP-HPLC (13 publications). Given the therapeutic significance of immediate-release performance, there is a considerable void in the peer-reviewed literature as

there is no validated dissolution-testing HPLC method (except from the Nepal national monograph). Manufacturers must use methods or procedures from the literature for regulatory submissions because the status of compendial monographs like USP, BP and IP is still not decided. Studies that look at stability do not always include forced-degradation mass balance and peak purity checks using photodiode-array or MS confirmation. Most methods that estimate than one substance at the same time still use mobile phases that have a lot of acetonitrile. Procedures for vonoprazan combinations that use solvents, like ethanol and water-based RP-HPLC are still very uncommon.

14. Conclusion

The present review used twenty-eight primary sources to compile data on analytical method development and validation of vonoprazan fumarate. These were estimated by UV spectrophotometry, RP-HPLC (single component and simultaneous estimation), HPTLC and LC-MS/MS bio-analytical techniques. LC-MS/MS techniques are indispensable for pharmacokinetic, bioequivalence and ultra-trace impurity investigations, but RP-HPLC with acetonitrile-based mobile phases and UV detection at 225 and 266 nm is the most extensively validated and legally accepted technology for routine quality control. The growing use of AQbD/DoE-based development and green-chemistry assessment reflects the evolution of the analytical environment surrounding this relatively novel chemical. With vonoprazan fumarate becoming a standard treatment for acid-related gastric disorders globally, further harmonization, such as developing an official compendial monograph, specialized dissolution methods, and broader use of green, stability-indicating HPTLC assays, will strengthen the quality-control infrastructure that underpins it.

15. Future Perspectives

- Development of a validated, stability-indicating dissolution/assay HPLC method suitable for compendial adoption (USP/BP/IP).
- Wider application of AQbD/DoE frameworks combined with AGREE/GAPI greenness scoring to standardise “green-by-design” methods.
- Expansion of HPTLC-MS hyphenation for rapid, low-cost impurity and degradant identification.
- Harmonised bioanalytical LC-MS/MS protocols to support global bioequivalence studies of generic vonoprazan products.
- Systematic in-silico (QSAR) toxicological risk assessment of newly characterised degradation products, extending the work already initiated for vonoprazan degradants.

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