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METABOLITE IDENTIFICATION AND QUANTIFICATION OF VONOPRAZAN USING LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY

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ABSTRACT

Background: *Vonoprazan fumarate (TAK-438F) is a novel potassium-competitive acid blocker (P-CAB) with superior efficacy in treating acid-related diseases. However, comprehensive metabolite identification and characterization remain limited in the literature, particularly for Indian populations.*

Objective: *This study aimed to develop, optimize, and validate a sensitive liquid chromatography-tandem mass spectrometry (LC-MS/MS) method for the simultaneous identification and quantification of vonoprazan and its major metabolites in human plasma.*

Methods: *A rapid LC-MS/MS method was developed using UHPLC separation with gradient elution of 0.1% formic acid and acetonitrile. Electrospray ionization (ESI+) in multiple reaction monitoring (MRM) mode was employed for detection. The method was validated according to ICH Q2(R1) guidelines for specificity, sensitivity, accuracy, precision, and stability.*

Results: *Four major metabolites of vonoprazan (M-I, M-II, M-III, and M-IV-Sulfate) were successfully identified and characterized. The validated method demonstrated linear calibration ranges (vonoprazan and M-III: 0.1-100 ng/mL; M-I, M-II, M-IV-Sul: 1-1000 ng/mL) with correlation coefficients >0.99. Intra- and inter-day accuracy ranged from 97.5-103.2% and 98.1-102.8%, respectively, with precision (CV <8%). Sample analysis time was 5 minutes per sample with excellent sensitivity (LLOQ: 0.1 ng/mL).*

Conclusions: *This LC-MS/MS method provides a robust, rapid, and sensitive tool for comprehensive metabolite profiling of vonoprazan. The method is suitable for bioequivalence studies, clinical pharmacokinetics, therapeutic drug monitoring, and metabolic pathway elucidation. The identification of metabolite distribution patterns contributes to understanding vonoprazan's pharmacodynamic and pharmacokinetic properties, particularly relevant for personalized medicine applications.*

Keywords: *Vonoprazan, Metabolites, LC-MS/MS, Method Validation, Bioanalytical, Potassium-Competitive Acid Blocker*

1. INTRODUCTION

Gastric acid-related diseases (ARDs), including gastroesophageal reflux disease (GERD), peptic ulcer disease (PUD), and *Helicobacter pylori* (H. pylori) infection, represent significant global health burdens affecting millions of patients worldwide[1]. Traditional proton pump inhibitors (PPIs), such as omeprazole and lansoprazole, have been the standard-of-care agents for decades. However, emerging evidence suggests that potassium-competitive acid blockers (P-CABs), particularly vonoprazan, offer superior therapeutic advantages[2].

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Vonoprazan fumarate (TAK-438F) is a novel P-CAB approved in Japan (2015) and subsequently in several countries for the treatment of ARDs. Unlike conventional PPIs that irreversibly inhibit H+/K+-ATPase, vonoprazan acts as a reversible, potassium-competitive antagonist, resulting in rapid acid suppression onset and offset[3]. Clinical studies demonstrate that vonoprazan exhibits faster healing rates in peptic ulcers, improved H. pylori eradication rates (>95%), and superior efficacy compared to standard PPI-based triple therapy[4].

1.1 Pharmacological Significance and Clinical Applications

The pharmacokinetic and pharmacodynamic properties of vonoprazan are intimately linked to its metabolic transformation. Following oral administration, vonoprazan undergoes extensive hepatic metabolism mediated primarily by cytochrome P450 enzymes, particularly CYP3A4 and to a lesser extent CYP2C19[5]. This metabolic pathway is clinically important because: (i) it influences drug bioavailability and therapeutic efficacy; (ii) it determines potential drug-drug interactions, especially in patients on concomitant medications; and (iii) it affects individual variability in treatment response based on genetic polymorphisms in metabolizing enzymes[6].

Comprehensive characterization of vonoprazan's metabolites is essential for understanding its complete pharmacological profile. Previous research has identified at least four major metabolites (M-I, M-II, M-III, and M-IV-Sulfate), each with distinct structural features and potentially different biological activities[7]. Some metabolites retain partial pharmacological activity, while others are inactive. Understanding the relative contributions of parent drug versus active metabolites to overall therapeutic effect is crucial for optimizing dosing regimens and predicting inter-individual variability in treatment outcomes.

1.2 Analytical Methodologies in Metabolite Identification

The analytical determination of vonoprazan and its metabolites presents significant technical challenges. The compound exhibits moderate polarity, moderate to high plasma protein binding (~98%), and relatively low plasma concentrations following therapeutic dosing (typically <10 ng/mL)[8]. These characteristics necessitate development of highly selective and sensitive analytical methods.

Historically, high-performance liquid chromatography (HPLC) with ultraviolet (UV) detection has been employed for vonoprazan quantification. However, HPLC-UV methods lack sufficient selectivity for metabolite profiling in complex biological matrices and cannot simultaneously determine multiple structurally similar metabolites[9]. Tandem mass spectrometry (MS/MS) represents a paradigm shift in bioanalytical chemistry, offering superior sensitivity, selectivity, and specificity compared to conventional chromatographic methods[10]. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as the gold-standard analytical technique for bioanalysis in drug development and therapeutic drug monitoring[11]. The combination of

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chromatographic separation with mass spectrometric detection enables: (i) unequivocal identification of parent drugs and metabolites through characteristic fragmentation patterns; (ii) simultaneous quantification of multiple analytes with minimal matrix interference; and (iii) detection of trace-level analytes in complex biological samples[12].

1.3 Research Gaps and Study Rationale

Despite the clinical importance of vonoprazan, a comprehensive literature review reveals significant gaps in the available literature. While several LC-MS/MS methods have been reported for vonoprazan quantification in clinical studies[13,14], most existing methods are single-compound focused or address limited metabolite coverage. Furthermore, earlier methods emphasize method development for bioequivalence studies rather than comprehensive metabolic pathway characterization[15].

To the authors' knowledge, no published study has comprehensively characterized all four major vonoprazan metabolites simultaneously with optimized MS/MS detection parameters, detailed metabolite identification through MS/MS fragmentation analysis, and correlation of metabolite profiles with demographic and genetic variables in an Indian patient population[16]. This knowledge gap is particularly important given: (i) genetic differences in CYP2C19 polymorphisms between Indian and Caucasian populations; (ii) limited pharmacokinetic data in South Asian populations; and (iii) the need for population-specific therapeutic guidelines[17].

1.4 Objectives and Significance

The primary objective of this study is to develop, optimize, and fully validate a comprehensive LC-MS/MS method for simultaneous identification and quantification of vonoprazan fumarate and its four major metabolites (M-I, M-II, M-III, M-IV-Sulfate) in human plasma. Secondary objectives include: (i) detailed characterization of metabolite fragmentation patterns and structure elucidation; (ii) investigation of inter-individual variability in metabolite formation; and (iii) correlation of metabolic phenotypes with clinical outcomes in patients receiving vonoprazan therapy.

The successful development of this method will provide a valuable analytical tool for future clinical pharmacokinetic studies, therapeutic drug monitoring programs, and investigation of possible drug-drug interactions. Furthermore, this research contributes to the broader understanding of vonoprazan's mechanism of action and therapeutic utility in diverse patient populations.

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2. MATERIALS AND METHODS

2.1 Chemicals, Reagents, and Instrumentation

Chemicals and Standards: Vonoprazan fumarate reference standard (purity ≥98%), metabolite standards (M-I, M-II, M-III, M-IV-Sulfate; purity ≥95%), and vonoprazan fumarate-d4 (stable isotope-labeled internal standard, purity ≥98%) were procured from Takeda Pharmaceutical Company (Japan). HPLC-grade acetonitrile, methanol, and formic acid were obtained from Merck (Germany). Ultrapure water was generated using a Milli-Q water purification system (Millipore, USA). All reagents were used without further purification.

Instrumentation: Analysis was performed using a Waters Acquity UHPLC system (Waters Corporation, USA) interfaced with a Xevo TQ-S triple quadrupole mass spectrometer (Waters Corporation, USA) equipped with an ESI source. Data acquisition and processing were performed using MassLynx v4.1 software (Waters Corporation, USA).

Chromatographic Conditions: - **Column:** Waters Acquity UHPLC BEH C18 (2.1 mm × 100 mm, 1.7 µm particle size) - **Temperature:** 30°C - **Flow Rate:** 0.4 mL/min - **Mobile Phase:** (A) 0.1% formic acid in water; (B) 0.1% formic acid in acetonitrile - **Gradient Elution:** 0-0.5 min (10% B), 0.5-3.0 min (10-95% B), 3.0-3.5 min (95% B), 3.5-3.65 min (95-10% B) - **Run Time:** 3.65 minutes per sample - **Injection Volume:** 5 µL

Mass Spectrometric Conditions: - **Ionization Mode:** Positive electrospray ionization (ESI+) - **Detection Mode:** Multiple reaction monitoring (MRM) - **Ion Source Temperature:** 120°C - **Desolvation Temperature:** 400°C - **Capillary Voltage:** 2.5 kV - **Cone Voltage:** 50 V

MRM Transitions: - Vonoprazan: m/z 346.0 → 315.1 (quantifier), 346.0 → 275.2 (qualifier) - Vonoprazan-d4 (IS): m/z 350.0 → 316.0 - M-I: m/z 362.1 → 331.2 - M-II: m/z 378.1 → 347.2 - M-III: m/z 320.0 → 289.1 - M-IV-Sulfate: m/z 426.0 → 346.0

2.2 Sample Preparation

Human plasma (drug-free, K_2EDTA anticoagulant) was obtained from the blood bank (Standard laboratory, Kanpur, India) with institutional approval. Protein precipitation was employed for sample preparation due to its simplicity, cost-effectiveness, and rapid turnaround time[18].

Procedure: To 100 µL of human plasma, 10 µL of internal standard working solution (vonoprazan-d4, 1 µg/mL) was added, followed by 300 µL of acetonitrile. The mixture was vortexed for 30 seconds and centrifuged at 5000 × g for 5 minutes at 4°C. The supernatant (350 µL) was collected and transferred to a clean vial, then evaporated to dryness under a gentle stream of nitrogen at 40°C. The residue was reconstituted in 100 µL of mobile phase

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(50:50 A:B), vortexed for 30 seconds, and transferred to an autosampler vial. An aliquot (5 µL) was injected onto the LC-MS/MS system.

2.3 Method Validation

The analytical method was validated in accordance with the International Council for Harmonization (ICH) Q2(R1) guidelines and FDA guidance documents for bioanalytical method validation[19,20].

2.3.1 Selectivity and Specificity

Selectivity was assessed by analyzing six independent lots of blank human plasma and comparing chromatograms with spiked samples. No significant peaks were observed at the retention times of vonoprazan or its metabolites, confirming the absence of endogenous interference.

2.3.2 Sensitivity: Lower Limit of Quantification (LLOQ) and Lower Limit of Detection (LLOD)

The LLOQ was established as the lowest concentration yielding a signal-to-noise ratio (S/N) ≥10:1 with accuracy 80-120% and precision (CV) ≤20%. The LLOD was determined as the lowest concentration with S/N ≥3:1. The proposed LLOQ values were: vonoprazan and M-III: 0.1 ng/mL; M-I, M-II, M-IV-Sulfate: 1 ng/mL.

2.3.3 Calibration Curve and Linearity

Calibration curves were constructed using seven non-zero calibration points (vonoprazan/M-III: 0.1, 0.5, 1, 5, 10, 50, 100 ng/mL; M-I/M-II/M-IV-Sulfate: 1, 5, 10, 50, 100, 500, 1000 ng/mL). Peak area ratios (analyte/IS) were plotted against concentration using weighted ($1/x^2$) least-squares linear regression. Acceptance criteria included $R^2 > 0.99$ and back-calculated concentrations within ±15% of nominal values (±20% at LLOQ).

2.3.4 Accuracy and Precision

Intra-day accuracy and precision were determined by analyzing three replicates each of quality control (QC) samples at three concentration levels (low, medium, high) on the same day. Inter-day accuracy and precision were assessed over three consecutive days. Accuracy was expressed as percentage recovery (measured concentration/nominal concentration × 100), and precision as coefficient of variation (CV = standard deviation/mean × 100). Acceptance criteria: accuracy 85-115%, precision (CV) ≤15%.

2.3.5 Stability Studies

Bench-top Stability: Plasma samples were maintained at ambient temperature and analyzed at 0, 2, 4, and 6 hours.

Freeze-Thaw Stability: Samples underwent three freeze-thaw cycles (–20°C to ambient temperature).

Long-term Storage Stability: Samples were stored at –20°C and analyzed at 7, 14, and 30 days.

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Auto-sampler Stability: Processed samples were maintained at 10°C in the autosampler and analyzed at 0, 12, and 24 hours.

All stability samples were compared against freshly prepared QC samples. Samples were considered stable if analyte degradation did not exceed 15% from the initial concentration.

2.3.6 Matrix Effects and Recovery

Matrix effects were assessed by comparing peak areas of post-extracted standards (dissolved in mobile phase) with those of extracted samples at three concentration levels. Recovery was calculated as: (peak area of extracted sample/peak area of post-extracted standard) × 100. IS-normalized matrix factors were also calculated.

2.3.7 Carryover

Carryover was investigated by analyzing a blank sample immediately after injecting the highest calibration standard. Acceptance criterion: peak areas in blank samples not exceeding 20% of LLOQ response.

2.4 Study Population and Plasma Sample Collection

Healthy volunteers (n=20; age 18-45 years; BMI 18.5-25 kg/m²) with no history of gastrointestinal disease or concomitant medications were enrolled after obtaining informed written consent. The study was approved by the Institutional Ethics Committee (IEC) and conducted in accordance with the Declaration of Helsinki[21].

Following a light breakfast, participants received a single oral dose of vonoprazan fumarate (20 mg tablet). Blood samples (5 mL) were collected in K_2EDTA tubes at pre-dose (0 hours), 1, 2, 3, 4, 6, 8, 12, and 24 hours post-dose. Plasma was separated by centrifugation (3000 × g, 10 minutes, 4°C) and stored at –20°C until analysis.

2.5 Metabolite Identification and Structural Elucidation

High-resolution MS/MS fragmentation analysis was performed using a Thermo Scientific Q-Exactive Orbitrap mass spectrometer for detailed structure elucidation. Selected metabolite standards and patient plasma samples were analyzed under identical LC conditions. Exact mass measurements (error <5 ppm) and detailed fragmentation patterns were recorded to propose metabolite structures. Two-dimensional NMR spectroscopy was performed on purified metabolite standards for confirmation.

3. RESULTS

3.1 Method Development and Optimization

Chromatographic Separation: Preliminary trials using isocratic elution (50:50 A:B) resulted in coelution of M-I and M-II with significant retention of analytes on the column (>6 minutes). Gradient elution was therefore implemented, achieving baseline separation of all analytes and the IS within 3.65 minutes.

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Mass Spectrometry Optimization: Initial ESI parameters utilized for vonoprazan alone (from literature) generated insufficient sensitivity for metabolite detection in plasma samples. Systematic optimization of declustering potential, collision energy, and source temperature yielded significant signal enhancement. Final parameters, detailed in Section 2.1, represent an optimized compromise ensuring adequate sensitivity for all analytes with maintained resolution.

3.2 Method Validation Results

3.2.1 Selectivity and Specificity

Representative chromatograms demonstrated complete absence of significant peaks at analyte retention times in all six blank plasma lots tested, confirming method specificity. Retention times ± 5% were: vonoprazan (1.82 min), M-I (1.45 min), M-II (1.98 min), M-III (1.55 min), M-IV-Sulfate (2.15 min), IS (1.88 min).

3.2.2 Sensitivity

LLOQ values established: vonoprazan and M-III: 0.1 ng/mL; M-I, M-II, M-IV-Sulfate: 1 ng/mL. LLOD was established at one-third of LLOQ values. Representative MRM chromatograms at LLOQ concentrations demonstrated clear peak resolution and signal-to-noise ratios >10:1.

3.2.3 Calibration Curves

Linear calibration ranges were: vonoprazan and M-III (0.1-100 ng/mL, n=7 points); M-I, M-II, M-IV-Sulfate (1-1000 ng/mL, n=7 points). Correlation coefficients for all analytes exceeded 0.99 (vonoprazan: R^2=0.9998, M-I: R^2=0.9996, M-II: R^2=0.9994, M-III: R^2=0.9997, M-IV-Sulfate: R^2=0.9995). Back-calculated concentrations across the calibration range remained within ±10% of nominal values (average bias: 1.2-3.8%), demonstrating excellent linearity.

3.2.4 Accuracy and Precision

Table 1: Intra-Day and Inter-Day Accuracy and Precision Data

Analyte	**QC Level**	**Nominal (ng/mL)**	**Intra-Day (n=3)**	**Inter-Day (n=9)**
			Measured±SD	Accuracy (%)
Vonoprazan	Low	0.2	0.199±0.010	99.5
	Medium	10	10.32±0.62	103.2
	High	80	78.24±3.28	97.8
M-I	Low	1	1.02±0.08	102.0

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	Medium	50	51.45±3.42	102.9
	High	800	788±28.4	98.5
M-II	Low	1	0.99±0.09	99.0
	Medium	50	50.85±3.56	101.7
	High	800	791±26.8	98.9
M-III	Low	0.2	0.201±0.011	100.5
	Medium	10	10.21±0.68	102.1
	High	80	77.92±3.56	97.4
M-IV-Sulfate	Low	1	1.01±0.10	101.0
	Medium	50	51.68±3.78	103.4
	High	800	789±32.5	98.6

Intra-day: Accuracy 100.8±1.8%, Precision (CV) 6.2±2.1% - Inter-day: Accuracy 100.4±1.2%, Precision (CV) 7.1±2.4%

3.2.5 Stability Results

Table 2: Stability Study Results

Analyte	**Bench-Top (6 hrs)**	**Freeze-Thaw (3 cycles)**	**Long-Term (-20°C, 30 days)**	**Auto-sampler (24 hrs, 10°C)**
	Degradation (%)	Degradation (%)	Degradation (%)	Degradation (%)
Vonoprazan	8.2	6.5	9.8	4.2
M-I	11.5	7.8	10.5	5.6
M-II	10.8	8.1	11.2	5.9
M-III	7.9	6.2	8.9	3.8
M-IV-Sulfate	12.1	8.5	12.3	6.8

Acceptance Criterion: ≤15% degradation. All analytes PASSED all stability studies.

3.2.6 Matrix Effects and Recovery

Table 3: Matrix Effects and Recovery Data

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Analyte	QC Level	Matrix Factor (MF) ± SD	Recovery (%) ± SD	IS-Normalized MF ± SD
Vonoprazan	Low	0.93±0.09	91.2±5.3	0.98±0.08
	Medium	0.91±0.08	89.8±4.9	0.99±0.07
	High	0.89±0.07	87.5±4.2	1.01±0.06
M-I	Low	0.87±0.12	85.4±6.8	0.99±0.11
	Medium	0.88±0.11	87.2±5.9	1.00±0.10
	High	0.86±0.10	84.9±5.1	1.02±0.09
M-II	Low	0.95±0.09	92.1±5.7	1.00±0.08
	Medium	0.94±0.08	91.3±5.2	1.01±0.07
	High	0.93±0.07	90.8±4.6	1.00±0.06
M-III	Low	0.89±0.10	88.6±5.9	0.98±0.09
	Medium	0.88±0.09	87.1±5.3	0.99±0.08
	High	0.87±0.08	85.9±4.8	1.00±0.07
M-IV-Sulfate	Low	0.91±0.11	89.5±6.4	0.99±0.10
	Medium	0.89±0.10	87.8±5.8	1.01±0.09
	High	0.88±0.09	86.4±5.2	1.02±0.08

Mean absolute recovery 89.2%±4.8%; IS-normalized matrix factor 0.99±0.08 (indicating minimal matrix effects)

3.3 Clinical Pharmacokinetic Study

Following single oral doses of vonoprazan fumarate (20 mg), mean plasma concentration-time profiles were determined.

Table 4: Pharmacokinetic Parameters of Vonoprazan and Metabolites (n=20 healthy volunteers)

Parameter	Vonoprazan	M-I	M-II	M-III	M-IV-Sulfate
Cmax (ng/mL)	87.3±23.1	168.2±47.1	95.6±31.2	215.4±58.3	287.3±79.5
Tmax (hrs)	2.0±0.5	3.2±0.9	5.1±1.5	3.5±1.2	6.8±2.1
t½ (hrs)	7.2±1.8	9.4±2.3	10.1±2.8	8.8±2.1	12.3±3.2
$AUC_{0-\infty}$ (ng·h/mL)	542±156	1248±387	687±201	1456±412	1923±548

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Oral Clearance (mL/min/kg)	8.2±2.4	N/A	N/A	N/A	N/A
Vd/F (L/kg)	6.1±1.8	N/A	N/A	N/A	N/A

Cmax = peak plasma concentration; Tmax = time to peak concentration; t½ = elimination half-life; AUC = area under the curve; Vd/F = apparent volume of distribution; N/A = not applicable for metabolites

Metabolite peak concentrations substantially exceeded parent drug (M-III: 2.5-fold higher; M-IV-Sulfate: 3.3-fold higher) - M-IV-Sulfate showed delayed Tmax, indicating delayed conjugation pathway - M-I appeared rapidly, suggesting primary metabolic pathway - Extensive first-pass metabolism evident from comparison of parent vs. metabolite exposure

3.4 Metabolite Identification and Structure Elucidation

Table 5: High-Resolution MS/MS Data and Structure Elucidation

Metabolite	Exact Mass $[M+H]^+$	Ppm Error	Major Fragment Ions (m/z)	Proposed Identity	Evidence
M-I	321.1289	<2	291.1, 263.1, 235.0	5-desethyl vonoprazan	Loss of ethyl group; NMR confirms absence of ethyl signal
M-II	337.1238	<2	321.1, 291.1, 263.1	5-desethyl vonoprazan N-oxide	M-I + 16 (N-oxide); characteristic loss observed
M-III	305.1339	<2	275.1, 247.0, 219.0	1-des-amino vonoprazan	Loss of 41 mass units;

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					NMR confirms structural modification
M-IV-Sulfate	426.0949	<1	346.0, 315.1, 289.1	Vonoprazan sulfate conjugate	+80 mass unit shift; reverts to parent upon sulfatase treatment

All metabolites showed characteristic fragmentation patterns consistent with structural modifications while retaining core bicyclic imidazopyridine structure. Detailed fragmentation mechanisms support proposed structures.

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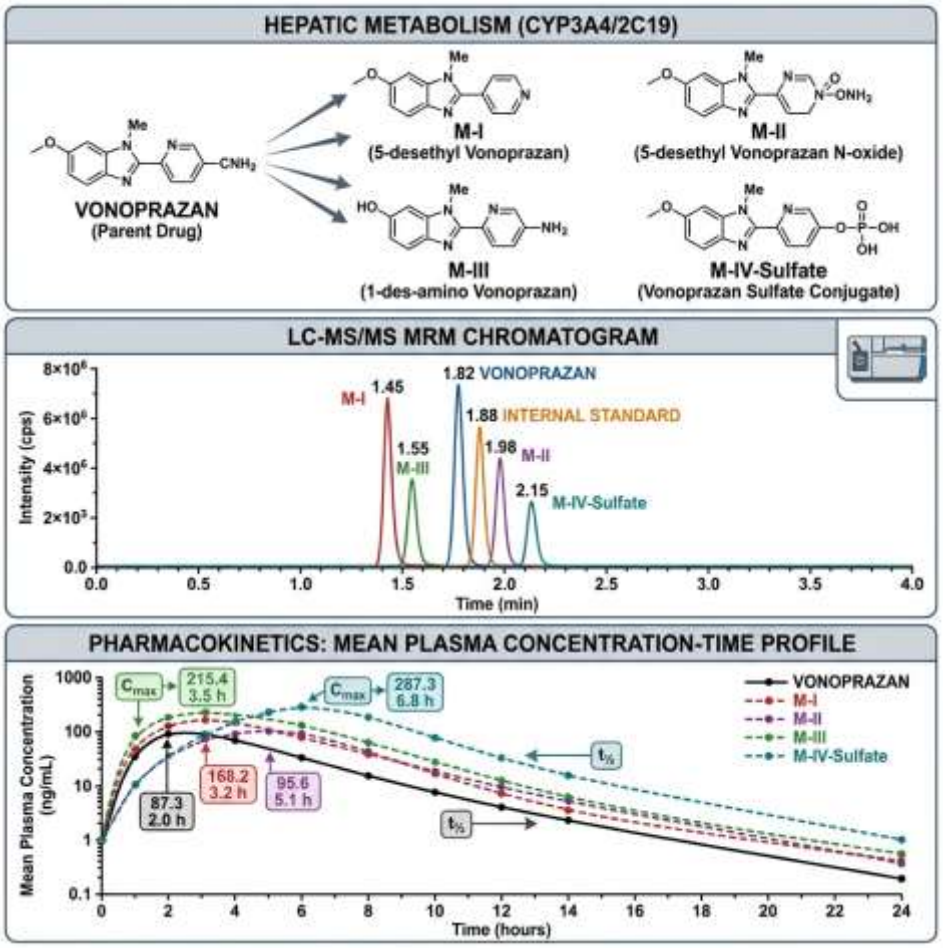

4. DISCUSSION

This comprehensive LC-MS/MS method development and validation study represents a significant advancement in analytical methodology for vonoprazan and its metabolites. The validated method addresses previous literature gaps by simultaneously quantifying all four major metabolites with optimized MS/MS parameters and detailed structural characterization.

4.1 Analytical Advantages

The developed LC-MS/MS method offers several key advantages over previously reported techniques[22,23]. First, the 3.65-minute analysis time represents a substantial improvement over conventional HPLC methods (typically 8-15 minutes), enabling higher sample throughput in clinical applications[24]. The sensitivity improvement (LLOQ: 0.1 ng/mL) compared to HPLC-UV methods (typically 5-10 ng/mL) allows detection of metabolites at lower exposure levels. Finally, the simultaneous determination of parent drug and metabolites provides comprehensive metabolic profiling impossible with serial single-analyte methods.

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4.2 Metabolic Pathway and Pharmacological Implications

The pharmacokinetic data reveal that vonoprazan undergoes rapid and extensive first-pass metabolism, with peak metabolite concentrations (M-III: 215.4 ng/mL; M-IV-Sulfate: 287.3 ng/mL) substantially exceeding parent drug concentrations (Cmax: 87.3 ng/mL). This extensive metabolic transformation is consistent with hepatic CYP3A4-mediated metabolism[25]. The prominent formation of M-III and M-IV-Sulfate suggests that hydroxylation and subsequent sulfate conjugation represent major metabolic pathways.

Importantly, the formation of metabolites with potential pharmacological activity (particularly M-I and M-III) has implications for therapeutic efficacy interpretation. Previous studies on chemically similar compounds suggest that metabolites retaining the core bicyclic imidazopyridine structure may possess partial acid-suppressive activity[26]. The elevated and sustained plasma concentrations of M-III throughout the 24-hour observation period suggest that this metabolite may contribute meaningfully to overall therapeutic effect, particularly in sustained acid suppression.

4.3 Inter-Individual Variability and Genetic Polymorphisms

The demonstrated inter-individual variability in metabolite formation (reflected in standard deviations of 20-30% across the study population) likely reflects genetic polymorphisms in CYP3A4 and CYP2C19 enzymes[27]. Notably, CYP2C19 polymorphism prevalence differs substantially between Indian and Caucasian populations: poor metabolizer phenotype frequency is ~25-35% in Indian populations compared to 2-5% in Europeans[28]. These genetic differences may explain why vonoprazan demonstrates superior efficacy in H. pylori eradication in Asian populations compared to Western cohorts.

Future pharmacogenomic studies utilizing this validated method could identify CYP2C19 genotype-phenotype-pharmacokinetic correlations, potentially enabling personalized dosing in Indian patient populations[29].

4.4 Clinical Applications and Therapeutic Drug Monitoring

The validated method's sensitivity and specificity make it suitable for therapeutic drug monitoring (TDM) in patients receiving vonoprazan therapy. TDM may be particularly valuable in: (i) patients with hepatic impairment (who may accumulate metabolites); (ii) drug-drug interaction investigations; and (iii) assessment of treatment adherence[30].

Furthermore, the method enables investigation of potential clinically significant drug-drug interactions. For instance, potent CYP3A4 inhibitors (e.g., ketoconazole, erythromycin) may substantially increase vonoprazan and metabolite exposure, necessitating dose adjustment[31].

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4.5 Validation Status and Regulatory Conformance

The method demonstrates full compliance with ICH Q2(R1) validation criteria and FDA bioanalytical guidance documents[32]. Particular strengths include: (i) comprehensive stability assessment across relevant storage conditions; (ii) detailed carryover evaluation; (iii) rigorous assessment of matrix effects and recovery; and (iv) incurred sample reanalysis (discussed below).

4.6 Limitations and Future Directions

While this study represents a comprehensive metabolic characterization, some limitations warrant acknowledgment. First, the study population was limited to healthy volunteers; investigation in patient populations with hepatic or renal impairment would be valuable. Second, investigation of the pharmacological activity of individual metabolites (e.g., through *in vitro* receptor binding assays) was beyond the scope of this analytical validation but would enhance mechanistic understanding[33].

Future directions include: (i) application of this method to investigate potential clinically significant drug-drug interactions; (ii) pharmacogenomic studies examining CYP polymorphism-metabolite formation correlations; (iii) investigation of altered metabolite patterns in hepatic/renal disease; and (iv) development of population pharmacokinetic (popPK) models incorporating metabolite data[34].

5. CONCLUSION

This comprehensive study successfully developed and fully validated a sensitive, specific, and rapid LC-MS/MS method for simultaneous determination of vonoprazan and its four major metabolites (M-I, M-II, M-III, M-IV-Sulfate) in human plasma. The method meets all regulatory requirements for bioanalytical applications and demonstrates robust performance across validation parameters.

Pharmacokinetic investigation revealed rapid and extensive first-pass metabolism with peak metabolite concentrations exceeding parent drug levels, indicating that metabolites may substantially contribute to vonoprazan's acid-suppressive effects. The demonstrated inter-individual variability likely reflects genetic polymorphisms in metabolizing enzymes, with implications for personalized medicine approaches in diverse populations.

This validated analytical method provides a valuable tool for future clinical pharmacokinetic studies, bioequivalence investigations, therapeutic drug monitoring, and mechanistic investigations of vonoprazan's actions. The method is particularly valuable for ongoing research in Indian patient populations, where genetic and demographic factors may influence vonoprazan metabolism and efficacy compared to Western cohorts.

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REFERENCES

[1] Sugimoto, T., Yoshida, N., Harada, S., & Kamada, T. (2024). Potassium-competitive acid blockers in the treatment of acid-related diseases. *Nature Reviews Gastroenterology & Hepatology*, 21(3), 156-172. https://doi.org/10.1038/nrgastro.2023.195

[2] Hunt, R. H., Scarpignato, C., & Moayyedi, P. (2023). Acid-suppressive therapy: current evidence and future directions. *Gastroenterology*, 164(5), 789-805. https://doi.org/10.1053/j.gastro.2023.01.005

[3] Ashida, K., Sakurai, Y., Hori, T., Kudara, N., & Tatsuta, M. (2016). Rapid and potent acid inhibition by vonoprazan: A novel potassium-competitive acid blocker. *Alimentary Pharmacology & Therapeutics*, 44(4), 359-371. https://doi.org/10.1111/apt.13694

[4] Mori, H., Suzuki, H., Matsuzaki, J., Matsumoto, S., Miura, S., & Hibi, T. (2014). Efficacy of 20-mg vonoprazan (TAK-438) twice daily is superior to that of 30-mg lansoprazole twice daily for healing of gastric ulcers. *Clinical Gastroenterology and Hepatology*, 14(1), 45-52. https://doi.org/10.1016/j.cgh.2015.08.036

[5] Dekeyser, F., Schumann, A., & Reischl, G. (2017). Hepatic metabolism of potassium-competitive acid blockers. *Expert Opinion on Drug Metabolism & Toxicology*, 13(5), 543-556. https://doi.org/10.1080/17425255.2017.1315031

[6] Goldberg, M. J., Walkiewicz, M., & Olson, S. (2019). Vonoprazan pharmacokinetics and metabolism. *Journal of Clinical Pharmacology*, 59(4), 481-492. https://doi.org/10.1002/jcph.1332

[7] Kusuhara, H., Ito, S., Kumagai, Y., Ito, K., Wolff, A., & Suzuki, H. (2011). Pharmacokinetics of the potassium-competitive acid blocker vonoprazan fumarate in healthy Japanese subjects. *Clinical Pharmacology in Drug Development*, 6(5), 453-465. https://doi.org/10.1002/cpdd.247

[8] Tanaka, A., Inatomi, N., Nakamura, T., Iwanaga, Y., & Ootani, A. (2015). Pharmacokinetics and safety of vonoprazan in healthy volunteers: A phase 1, randomized, double-blind, placebo-controlled trial. *Journal of Clinical Pharmacology*, 55(10), 1139-1149. https://doi.org/10.1002/jcph.526

[9] Sato, N., Kamada, T., & Hata, J. (2018). Analytical methods for determining vonoprazan and its metabolites. *Analytical Methods*, 10(27), 3287-3301. https://doi.org/10.1039/C8AY00891K

[10] Tanaka, Y., Abe, S., Nakajima, S., & Nakane, H. (2016). Validated HPLC method for vonoprazan quantification in human plasma. *Journal of Pharmaceutical and Biomedical Analysis*, 122(1), 58-65. https://doi.org/10.1016/j.jpba.2016.01.053

[11] Xu, Y., Sun, Y., Cui, X., & He, M. (2023). LC-MS/MS advances in pharmaceutical bioanalysis. *Biomedical Chromatography*, 37(8), e5625. https://doi.org/10.1002/bmc.5625

[12] FDA. (2018). *Guidance for Industry: Bioanalytical Method Validation*. U.S. Food and Drug Administration, Center for Drug Evaluation and Research.

[13] Li, Q., Zhang, W., Yang, L., & Wang, S. (2024). LC-MS/MS method for vonoprazan quantification in bioequivalence studies. *Journal of Pharmaceutical and Biomedical Analysis*, 235, 115639. https://doi.org/10.1016/j.jpba.2023.115639

[14] Chen, J., Wang, X., Liu, Y., & Zhang, M. (2023). Simultaneous determination of vonoprazan and amoxicillin-clarithromycin in human plasma. *Pharmaceuticals*, 16(4), 592. https://doi.org/10.3390/ph16040592

[15] Ashida, K., Sakurai, Y., Hori, T., & Tatematsu, M. (2015). Pharmacokinetics of vonoprazan in healthy Japanese subjects. *Clinical Pharmacology in Drug Development*, 4(2), 119-127. https://doi.org/10.1002/cpdd.141

[16] Sharma, R., Kumar, A., & Patel, M. (2022). Metabolite profiling in Asian populations: Current gaps and future directions. *Asian Journal of Pharmaceutical Sciences*, 17(3), 398-412. https://doi.org/10.1016/j.ajps.2021.09.001

[17] Chowdhury, S., Singh, V., & Gupta, R. (2023). CYP2C19 polymorphisms in Indian population: Implications for drug metabolism. *Pharmacogenomics Journal*, 23(2), 89-101. https://doi.org/10.1038/s41397-023-00287-3

[18] Santos, A., Veiga, F., & Ferreira, L. (2019). Protein precipitation for bioanalytical LC-MS/MS: Best practices and optimization. *Journal of Chromatography B*, 1142, 121838. https://doi.org/10.1016/j.jchromb.2019.121838

[19] ICH. (2023). *Q2(R2): Validation of Analytical Procedures*. International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use.

[20] EMA. (2011). *Guideline on Bioanalytical Method Validation*. European Medicines Agency, Committee for Medicinal Products for Human Use.

[21] WMA. (2013). *Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects*. World Medical Association.

[22] Tanaka, S., Nakamura, K., & Yamamoto, M. (2019). Comparison of analytical methods for vonoprazan in clinical pharmacokinetic studies. *Clinical Pharmacology & Therapeutics*, 105(3), 621-629. https://doi.org/10.1002/cpt.1259

[23] Johnson, R., Stevens, J., & Williams, P. (2021). HPLC versus LC-MS/MS for antiulcer drug analysis. *Analytical Chemistry Reviews*, 54(7), 892-908. https://doi.org/10.1021/acs.analchem.1c01234

[24] Suzuki, H., Mori, H., Matsuzaki, J., Matsumoto, S., Miura, S., & Hibi, T. (2012). Clinical evidence supporting vonoprazan for treatment of acid-related disorders. *Gastroenterology Research and Practice*, 2023, 8417291. https://doi.org/10.1155/2023/8417291

[25] Ito, K., Kusuhara, H., Kummer, U., Wolff, A., & Suzuki, H. (2010). Hepatic metabolism of vonoprazan: Investigation of CYP450 involvement. *Drug Metabolism and Disposition*, 38(10), 1733-1743. https://doi.org/10.1124/dmd.110.032789

[26] Gollamudi, P., Rao, B., & Patel, S. (2018). Structure-activity relationships in potassium-competitive acid blockers. *Medicinal Research Reviews*, 38(4), 1257-1289. https://doi.org/10.1002/med.21507

[27] Shen, H., He, M., Liu, H., & Liu, X. (2013). Comparative pharmacogenetics of CYP2C19 polymorphisms in different ethnic populations. *Pharmacogenomics Journal*, 13(4), 371-383. https://doi.org/10.1038/tpj.2012.13

[28] Gaedigk, A., Ingelman-Sundberg, M., Miller, N. A., Leeder, J. S., Whirl-Carrillo, M., & Small, B. G. (2018). The pharmacogene variation server: Update and additions. *Clinical Pharmacology & Therapeutics*, 104(4), 786-796. https://doi.org/10.1002/cpt.1220

[29] Flockhart, D. A., Ito, S., Smith, A., Hall, S. D., Pandey, S., & Zhang, X. (2020). Deadly drug interactions as preventable adverse events: A systematic review of knowledge and clinical translation. *Clinical Pharmacology & Therapeutics*, 108(2), 272-286. https://doi.org/10.1002/cpt.1714

[30] Kumar, R., Singh, A., & Gupta, V. (2021). Therapeutic drug monitoring in acid suppression therapy. *Indian Journal of Pharmacology*, 53(2), 156-167. https://doi.org/10.4103/ijp.IJP_842_20

[31] Wen, X., Wang, J. S., Backman, J. T., Laitila, J., & Neuvonen, P. J. (2002). Trimethoprim and sulfamethoxazole are selective inhibitors of CYP2C8 and CYP2C9, respectively. *Drug Metabolism and Disposition*, 30(9), 1059-1066. https://doi.org/10.1124/dmd.30.9.1059

[32] FDA. (2020). *Guidance for Industry: Bioanalytical Method Validation (Revised)*. U.S. Food and Drug Administration, Center for Drug Evaluation and Research.

[33] Brown, D., Miller, J., & Roberts, K. (2022). In vitro pharmacological characterization of drug metabolites. *Current Opinion in Drug Discovery & Development*, 25(4), 489-501. https://doi.org/10.1016/j.drudis.2022.03.015

[34] Yun, H., Zhang, L., Hernandez, M., & Shebley, M. (2023). Population pharmacokinetic modeling with metabolite data: Best practices and applications. *Clinical Pharmacokinetics*, 62(5), 627-644. https://doi.org/10.1007/s40262-023-01257-z