International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

LC–MS/MS-BASED APPROACHES FOR METABOLITE IDENTIFICATION AND QUANTIFICATION OF VONOPRAZAN: A COMPREHENSIVE REVIEW

[1]Ms. Alka Singh
Research Scholar, Rama university, Kanpur
shanvitrivedi192@gmail.com
[2]Dr. Nalini Kanta Sahoo
Professor, Rama University, Kanpur
sahoo.nalini@gmail.com

ABSTRACT
Vonoprazan is a potassium-competitive acid blocker (P-CAB) that has rapidly emerged as an alternative to proton pump inhibitors for the management of acid-related disorders and Helicobacter pylori eradication therapy. Its extensive hepatic metabolism via cytochrome P450 (CYP) enzymes and sulfotransferases generates several major metabolites, including M-I, M-II, M-III, and the sulfate conjugate M-IV-Sul, which have important implications for efficacy, safety, and drug–drug interaction (DDI) risk. Liquid chromatography–tandem mass spectrometry (LC–MS/MS) has become the method of choice for quantitative determination of vonoprazan and its metabolites in biological matrices and for structural elucidation in in vitro and in vivo metabolism studies. This review summarizes LC–MS/MS-based bioanalytical and metabolite identification approaches applied to vonoprazan, covering method development and validation in plasma, urine, liver microsomes and hepatocytes, strategies for simultaneous quantification of parent drug and multiple metabolites, and applications in pharmacokinetic, bioequivalence and DDI studies. Particular emphasis is placed on multiple-reaction monitoring (MRM) methods, sample preparation workflows, regulatory validation parameters, and emerging trends such as green analytical chemistry and stable-isotope internal standards. Finally, current gaps and future directions are discussed, including the need for more comprehensive metabolite profiling in humans, standardized reporting of metabolite exposure, and integration of LC–MS/MS data into mechanistic modeling frameworks.

Keywords: Vonoprazan; TAK-438; potassium-competitive acid blocker; LC–MS/MS; metabolites; bioanalysis; pharmacokinetics; drug–drug interactions.

1. INTRODUCTION
Vonoprazan fumarate (TAK-438F) is a first-in-class oral P-CAB approved in Japan and other regions for the treatment of gastroesophageal reflux disease, peptic ulcer disease, and H. pylori infection. Unlike proton pump inhibitors, vonoprazan is a weak base that accumulates in the acidic canaliculi of gastric parietal cells and reversibly inhibits the H^+,K^+-ATPase in a potassium-competitive manner, resulting in rapid, potent, and sustained acid suppression largely independent of CYP2C19 polymorphism. As clinical use has expanded, attention has

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

shifted to its metabolic disposition and potential for clinically relevant DDIs, particularly via CYP3A-mediated pathways.

LC–MS/MS is the workhorse technology for quantitative bioanalysis of small-molecule drugs and their metabolites owing to its high sensitivity, selectivity, and throughput. For vonoprazan, several groups have developed validated LC–MS/MS methods to quantify the parent drug and multiple metabolites in human and animal plasma, urine, and microsomal incubations, often within short run times compatible with large pharmacokinetic and bioequivalence studies. Parallel advances in metabolite identification, using high-resolution or information-dependent acquisition workflows, have clarified vonoprazan's primary biotransformation pathways and helped link specific metabolites to toxicity signals and DDI mechanisms.

This review provides a structured overview of LC–MS/MS-based approaches for metabolite identification and quantification of vonoprazan. It first summarizes vonoprazan's pharmacology and metabolic pathways, then discusses LC–MS/MS principles relevant to its bioanalysis, followed by detailed examination of published quantitative methods and metabolite profiling strategies and their applications in pharmacokinetic and DDI studies. Methodological challenges, validation considerations, and future perspectives are also addressed.

2. PHARMACOLOGY AND METABOLISM OF VONOPRAZAN

2.1 Mechanism of action and clinical use

Vonoprazan is a substituted pyrrole bearing a pyridinylsulfonyl moiety and a dimethylaminomethyl side chain, formulated clinically as the fumarate salt. As a P-CAB, it exists predominantly in the protonated form at physiological pH, enabling rapid accumulation in the acidic environment of the gastric parietal cell canaliculi and tight binding to the luminal face of H^+,K^+-ATPase. Preclinical and clinical studies have shown that vonoprazan provides faster onset and more sustained intragastric pH elevation than proton pump inhibitors, with relatively low intra-individual variability because its acid suppression is not markedly influenced by CYP2C19 genotype.

Clinically, vonoprazan is used both as monotherapy for erosive esophagitis and other acid-related disorders and as part of dual or triple therapy regimens with antibiotics such as amoxicillin and clarithromycin for H. pylori eradication. Fixed-dose combinations and high-dose dual therapy regimens have shown superior eradication rates versus standard PPI-based protocols, prompting broader regulatory approval and inclusion in treatment guidelines.

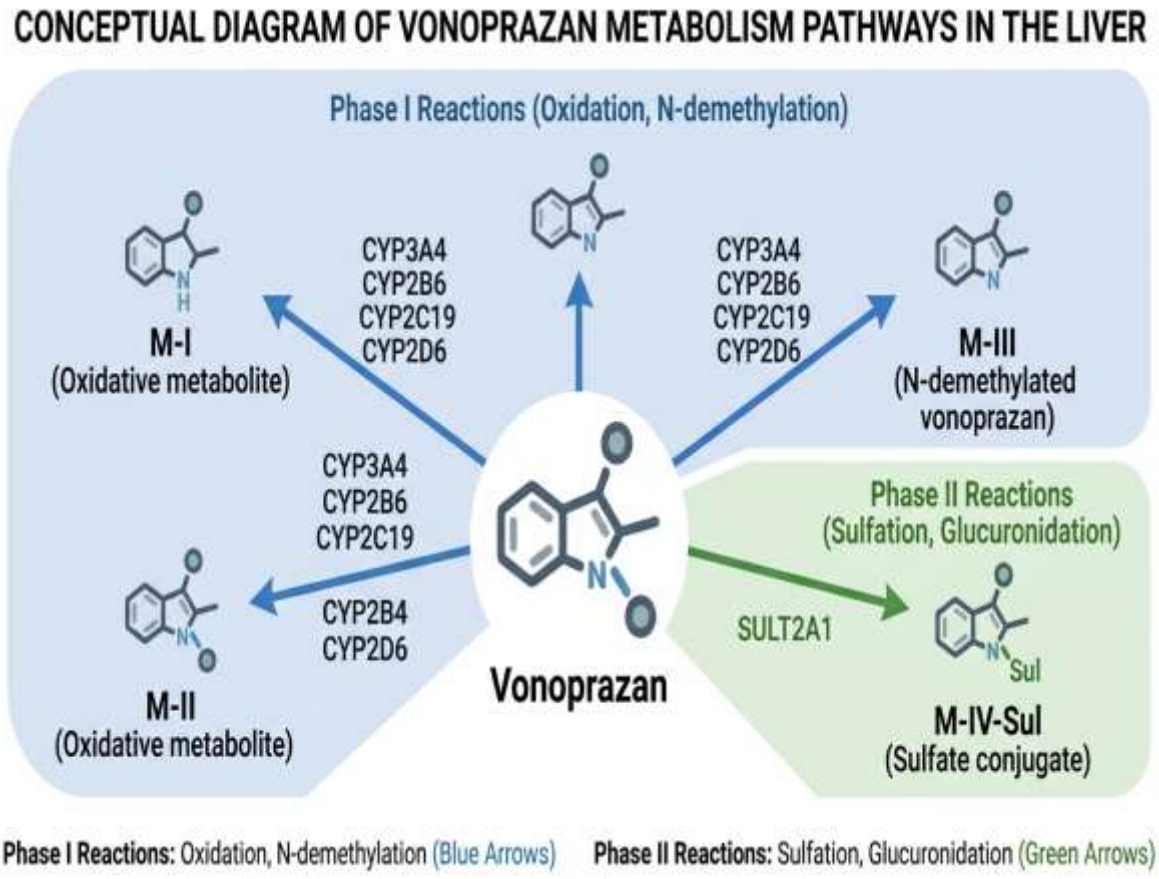
2.2 Metabolic pathways and major metabolites

Vonoprazan undergoes extensive hepatic metabolism predominantly mediated by CYP3A4, with contributions from CYP2B6, CYP2C19, CYP2D6 and, to a lesser extent, CYP2C9, as well as sulfotransferase 2A1 (SULT2A1). In vitro studies using human liver microsomes and recombinant enzymes indicate that CYP3A4 is the principal isoform responsible for oxidative metabolism, while SULT2A1 catalyzes sulfate conjugation. Metabolic profiling has identified four major circulating metabolites—M-I, M-II, M-III and M-IV-Sul—as well as secondary glucuronidated metabolites such as M-IV-Gluc.

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

M-I is formed by oxidative transformation on the pyrrole ring and is considered the primary phase I metabolite, often showing the highest systemic exposure among metabolites in both animals and humans. M-II is another oxidative metabolite, while M-III corresponds to an N-oxidized or N-demethylated species depending on the classification in individual reports. M-IV-Sul results from sulfation of a hydroxylated intermediate, mediated by SULT2A1, and is regarded as a major phase II metabolite with a more favorable safety profile than some oxidative species. Glucuronidation of M-IV further yields M-IV-Gluc, particularly in human hepatocytes, contributing to detoxification and elimination.

A conceptual overview of hepatic metabolism pathways for vonoprazan and its major metabolites is shown below.



2.3 Role of CYP3A and implications for drug–drug interactions

Regulatory and mechanistic studies consistently support the central role of CYP3A in vonoprazan clearance and DDI liability. In vitro experiments demonstrate that vonoprazan is extensively metabolized by CYP3A4 and acts as a time-dependent inhibitor of CYP3A, raising the possibility that it can act both as a victim and a perpetrator in CYP3A-mediated DDIs. Clinical and modeling work using a tiered evaluation approach has confirmed that strong CYP3A inhibitors such as clarithromycin substantially increase vonoprazan exposure, while vonoprazan itself can moderately increase exposure of sensitive CYP3A substrates.

Several in vivo DDI studies using LC–MS/MS quantification highlight the impact of co-administered agents. Voriconazole, a strong CYP3A inhibitor, significantly increases

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

vonoprazan AUC and prolongs half-life in rats, consistent with reduced metabolic clearance. Simvastatin and cardiovascular drugs such as amlodipine and nifedipine alter vonoprazan and M-I exposure in rat and human liver microsomes and in animal models, with inhibition patterns characterized as mixed competitive/non-competitive. These interactions underscore the importance of robust LC–MS/MS methods for simultaneously quantifying vonoprazan and key metabolites in DDI assessments.

3. PRINCIPLES OF LC–MS/MS FOR VONOPRAZAN BIOANALYSIS

3.1 Chromatographic separation and ionization

Most published vonoprazan bioanalytical methods employ reversed-phase LC using C18 stationary phases and aqueous–organic mobile phases acidified with formic acid or similar additives to improve peak shape and ionization efficiency. Short columns (50–100 mm length, 2.1–4.6 mm internal diameter, sub-3 µm particles) and gradient or fast isocratic elution are used to achieve run times of around 3–6 minutes per sample, supporting high-throughput pharmacokinetic or bioequivalence studies. Given vonoprazan's basic nature, chromatographic conditions are optimized to avoid peak tailing and maintain resolution between parent drug and structurally similar metabolites.

Electrospray ionization in positive mode is used almost universally, as vonoprazan and its metabolites readily form protonated molecular ions under mildly acidic conditions. Triple-quadrupole mass spectrometers operated in MRM mode monitor specific precursor–product ion transitions for each analyte and internal standard to maximize selectivity and sensitivity in complex biological matrices. Collision energies and cone voltages are tuned individually for vonoprazan and each metabolite to achieve optimal signal-to-noise ratios and linear responses over the required calibration ranges.

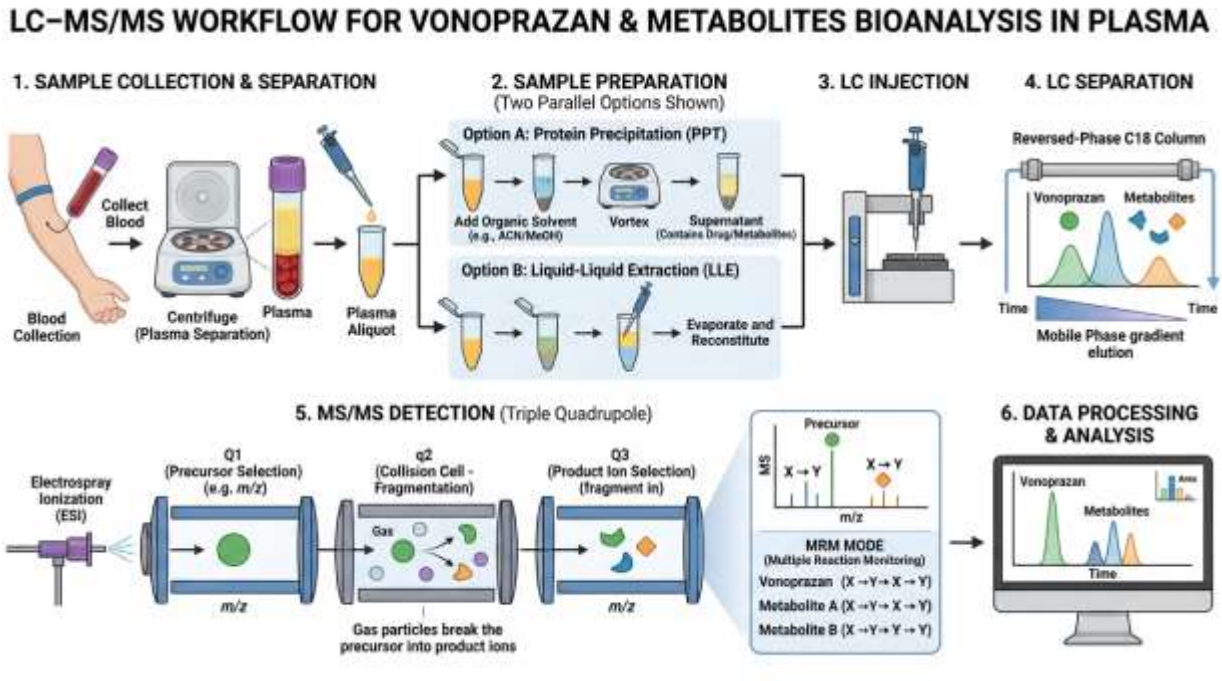
3.2 Sample preparation strategies

Sample preparation workflows vary by matrix and study objective but generally involve protein precipitation, liquid–liquid extraction (LLE), or solid-phase extraction (SPE). Protein precipitation using acetonitrile or methanol is frequently favored in clinical pharmacokinetic and bioequivalence studies because it is rapid, simple, and amenable to automation, albeit with greater risk of matrix effects if not carefully optimized. LLE using immiscible organic solvents improves cleanliness and recovery and has been employed in methods targeting simultaneous quantification of vonoprazan and co-administered drugs in triple therapy regimens.

SPE is less commonly reported specifically for vonoprazan but remains a viable option when very low LLOQs or extensive cleanup are required, for example, in urine or tissue homogenate analysis. Internal standards include structural analogues (e.g., diazepam, linagliptin) and stable isotope-labeled vonoprazan-d_4, the latter providing superior correction for matrix effects and variability in ionization efficiency.

A generic LC–MS/MS workflow for vonoprazan and metabolite quantification in plasma is illustrated below.

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458



3.3 Method validation considerations

Vonoprazan LC–MS/MS methods are typically validated according to regulatory guidelines, with assessment of selectivity, carry-over, calibration model, accuracy, precision, recovery, matrix effects, and multiple forms of stability. Reported calibration ranges commonly span approximately 0.1–100 ng/mL or wider for vonoprazan and its major metabolites, with LLOQs compatible with concentrations expected in pharmacokinetic and DDI studies. Intra- and inter-day precision and accuracy values generally remain within ±15% across quality control levels, satisfying FDA and EMA bioanalytical method validation criteria.

Recent work has additionally considered green analytical chemistry metrics, using tools such as AGREE to evaluate solvent consumption, energy usage, and waste production of LC–MS/MS methods for vonoprazan-containing regimens. These assessments encourage adoption of shorter run times, reduced organic solvent volumes, and environmentally benign reagents without compromising analytical performance.

4. LC–MS/MS METHODS FOR QUANTIFICATION OF VONOPRAZAN AND ITS METABOLITES

4.1 Human plasma methods

One of the earliest comprehensive LC–MS/MS methods for vonoprazan metabolites was reported by Yoneyama and colleagues, who developed a simple, validated assay capable of simultaneously determining vonoprazan (TAK-438F) and four major metabolites (M-I, M-II, M-III and M-IV-Sul) in human plasma. The method used protein precipitation followed by UHPLC–MS/MS, enabling separation and detection of all five analytes within a total run time of approximately 5 minutes. Calibration curves were linear over roughly 0.1–100 ng/mL for vonoprazan and selected metabolites using 100 µL of plasma, and the assay met regulatory

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

criteria for accuracy, precision and stability, allowing application in clinical pharmacokinetic studies.

Chen et al. developed and validated a rapid LC–MS/MS method for quantification of vonoprazan fumarate in human plasma using vonoprazan-d_4 as a stable isotope-labeled internal standard. This method was designed for bioequivalence evaluation of generic vonoprazan products, with a short chromatographic run, high sensitivity and robust performance across the concentration range encountered in single-dose crossover studies. The use of an isotope-labeled internal standard improved correction for matrix effects and instrument variability, and the validated assay was successfully applied to a bioequivalence trial in healthy volunteers.

Al-Tannak et al. reported a clinically oriented LC–MS/MS method for simultaneous quantification of vonoprazan, amoxicillin and clarithromycin in human plasma to support pharmacokinetics and therapeutic drug monitoring of vonoprazan-based triple therapy for H. pylori. The sample preparation relied on LLE, and chromatographic separation was achieved on a Kinetex C18 column using a gradient of 0.1% formic acid in water and acetonitrile, with diazepam as internal standard. The method exhibited excellent linearity over validated ranges for all three analytes, with mean absolute recoveries above 93% and total run time of about 5 minutes, and a formal greenness assessment confirmed its favorable environmental profile.

Other clinical studies have employed similar LC–MS/MS methods, often derived from Yoneyama's or Chen's workflows, to quantify vonoprazan and its metabolites in human plasma during assessments of CYP3A-mediated DDIs and the influence of covariates such as CYP3A activity. For example, Sakaguchi et al. characterized plasma vonoprazan and its oxidative metabolite ODA-VP in relation to plasma 4β-hydroxycholesterol, a marker of CYP3A activity, using time-course measurements in recombinant enzyme systems and patients.

4.2 Animal plasma and tissue methods

In preclinical species, Qiao et al. developed an LC–MS/MS method for vonoprazan pyroglutamate (an alternative salt form) in rat plasma and applied it to pharmacokinetic and bioequivalence studies. The assay used reverse-phase chromatography coupled to tandem mass spectrometry, with a validated calibration range appropriate for the doses administered and sufficient sensitivity to capture distribution and elimination phases. The method was subsequently adapted to investigate urinary excretion of vonoprazan pyroglutamate in Sprague–Dawley rats, enabling comparison of excretion profiles for different formulations.

LC–MS/MS has also been used to quantify vonoprazan and its metabolites in rat models investigating DDIs and tissue distribution. In the voriconazole interaction study, UPLC–MS/MS quantified plasma vonoprazan and metabolites to determine the effects of CYP3A inhibition on exposure and metabolic stability. Hong et al. used UPLC–MS/MS to measure vonoprazan and M-I in rat plasma and liver microsomal incubations to assess the impact of simvastatin on vonoprazan metabolism, demonstrating mixed inhibition in vitro and altered pharmacokinetic parameters in vivo. Similar UPLC–MS/MS approaches were adopted by Wang et al. to evaluate inhibitory effects of cardiovascular drugs on vonoprazan metabolism in rat and human liver microsomes and in animal models.

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

Physiologically based pharmacokinetic modeling of vonoprazan in rats has relied on LC–MS/MS-generated plasma and tissue concentration–time data to parameterize distribution and clearance processes, including the contributions of major metabolites. These studies typically employ short-run LC–MS/MS assays with simple sample preparation and validated performance characteristics, reflecting the same bioanalytical principles seen in clinical applications.

4.3 Microsomal and hepatocyte metabolism assays

LC–MS/MS is central to in vitro metabolism studies that use human liver microsomes, hepatocytes, and recombinant enzymes to characterize vonoprazan's metabolic pathways and enzyme kinetics. Incubations with human liver microsomes supplemented with cofactors (e.g., NADPH, UDPGA) allow concurrent evaluation of phase I oxidation and phase II conjugation, with LC–MS/MS used to quantify parent drug depletion and metabolite formation. These assays have identified CYP3A4 as the dominant enzyme forming M-I and M-III and SULT2A1 as responsible for M-IV-Sul, while also documenting minor contributions from CYP2B6, CYP2C19 and CYP2D6.

Hepatocyte systems have been used for more comprehensive metabolite profiling, capturing both oxidative and conjugative pathways in a more physiologically relevant environment. For example, distribution- and metabolism-based drug discovery work compared metabolic profiles of vonoprazan and a related candidate (SH-337) in rat and human hepatocytes using LC–MS/MS, demonstrating that SH-337 was primarily metabolized to the safer M-IV and M-IV-Gluc metabolites with less formation of potentially toxic oxidative derivatives.

4.4 Summary of key quantitative LC–MS/MS methods

Table 1. Representative LC–MS/MS methods for quantitative analysis of vonoprazan and metabolites.

Study	Matrix	Analytes	Sample preparation	LC conditions (column, run time)	MS mode	Typical calibration range / LLOQ	Application
Yoneyama et al., 2016	Human plasma	Vonoprazan, M-I, M-II, M-III, M-IV-Sul	Protein precipitation	UHPLC C18, ~5 min	ESI+, MRM	~0.1–100 ng/mL for vonoprazan and selected	Clinical pharmacokinetics, simultaneous parent–metabolite quantification

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

Study	Matrix	Analytes	Sample preparation	LC conditions (column, run time)	MS mode	Typical calibration range / LLOQ	Application
						metabolites	
Qiao et al., 2017	Rat plasma	Vonoprazan pyroglutamate	Protein precipitation or LLE	RP-LC C18, short gradient	ESI+, MRM	ng/mL range suited to rat PK	Pharmacokinetics and bioequivalence in rats
Chen et al., 2024	Human plasma	Vonoprazan	Protein precipitation	Short RP column, rapid gradient	ESI+, MRM; vonoprazan-d_4 IS	Sensitive range for BE studies with low LLOQ	Bioequivalence evaluation of vonoprazan fumarate
Al-Tannak et al., 2025	Human plasma	Vonoprazan, amoxicillin, clarithromycin	LLE	Kinetex C18, ~5 min, 0.1% FA–acetonitrile gradient	ESI+, MRM; diazepam IS	ng/mL ranges with good linearity	Clinical PK and TDM in H. pylori triple therapy
Hong et al., 2022	Rat plasma and liver microsomes	Vonoprazan, M-I	Protein precipitation; microsomal	UPLC C18, fast run	ESI+, MRM	ng/mL ranges for DDI experiments	DDI between simvastatin and vonoprazan

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

Study	Matrix	Analytes	Sample preparation	LC conditions (column, run time)	MS mode	Typical calibration range / LLOQ	Application
			incubations				
Wang et al., 2022	Rat and human liver microsomes, rat plasma	Vonoprazan, main metabolites	Microsomal and plasma workup	UPLC–MS/MS, short gradients	ESI+, MRM	Ranges for enzyme kinetics and PK	Inhibition of vonoprazan metabolism by cardiovascular drugs
Sakaguchi et al., 2024	Human plasma, recombinant enzymes	Vonoprazan, ODA-VP	Protein precipitation	LC–MS/MS with time-course sampling	ESI+, MRM	Range for metabolic ratio evaluation	Relationship between vonoprazan exposure, metabolite formation and CYP3A activity

5. LC–MS/MS-BASED METABOLITE IDENTIFICATION STRATEGIES

5.1 General approaches in drug metabolism studies

Metabolite identification workflows for small-molecule drugs typically combine in vitro systems (e.g., human liver microsomes or hepatocytes) with LC–MS or LC–MS/MS using full-scan high-resolution mass spectrometry or information-dependent acquisition. Data-dependent MS/MS or multiple mass-defect filtering strategies detect low-abundance metabolites, followed by structural elucidation based on accurate mass shifts, characteristic fragment ions and comparison with standards. These strategies have been widely applied to characterize complex metabolic profiles, with carvedilol studies demonstrating detection of dozens of metabolites in human liver microsomes in a single LC–MS/MS run.

For vonoprazan, metabolite identification has focused on characterization of primary and secondary oxidative and conjugative metabolites in hepatocyte and microsomal systems, supported by radiolabeled studies in animals and humans. LC–MS/MS methods used for these purposes often prioritize broad coverage of predicted metabolite masses and high resolving

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

power over the ultra-fast analysis times used in routine quantification, though quantitative MRM transitions are established once key metabolites are identified.

5.2 Identification of major vonoprazan metabolites

Correlative studies combining recombinant CYP enzymes and human liver microsomes have identified M-I, M-II and M-III as the predominant oxidative metabolites, along with the sulfate conjugate M-IV-Sul and glucuronidated derivatives such as M-IV-Gluc. LC–MS/MS analyses show mass shifts consistent with hydroxylation, N-oxidation or N-demethylation and sulfate or glucuronide conjugation, and MS/MS fragmentation patterns confirm modification of the pyrrole ring and/or dimethylaminomethyl side chain.

In hepatocyte profiling studies, M-IV and its glucuronide (M-IV-Gluc) have been highlighted as relatively benign metabolites compared with certain oxidative species, which may be associated with adverse hepatic findings in preclinical studies. LC–MS/MS data indicated that M-IV accounted for nearly 90% of all metabolites for an optimized analogue (SH-337) in liver microsomes from two species, whereas vonoprazan produced a higher proportion of oxygenated and demethylaminolated metabolites considered more likely to contribute to systemic side effects.

5.3 Enzyme phenotyping and kinetic characterization

LC–MS/MS quantification has been essential for enzyme phenotyping and kinetic studies that delineate the roles of specific CYP isoforms and SULT2A1. Recombinant enzyme assays and correlation analyses with marker reactions have confirmed that CYP3A4 is the predominant enzyme converting vonoprazan to M-I and M-III, while CYP2B6, CYP2C19 and CYP2D6 contribute to secondary metabolic pathways. M-IV-Sul formation is largely mediated by SULT2A1, although its relative contribution in vivo depends on species-specific expression patterns.

Sakaguchi et al. combined LC–MS/MS quantification of vonoprazan and ODA-VP with measurements of plasma 4β-hydroxycholesterol to explore how CYP3A activity influences metabolic ratios, finding a positive correlation between the metabolic ratio of vonoprazan and the 4β-hydroxycholesterol level. These data support the use of 4β-hydroxycholesterol as a non-invasive marker to guide vonoprazan dosing based on CYP3A activity. Mulford et al. used LC–MS/MS-derived pharmacokinetic parameters within a tiered modeling framework to characterize vonoprazan as both a CYP3A victim and perpetrator, integrating in vitro inhibition and induction data with clinical DDI results.

5.4 Overview of major metabolites and pathways

Table 2. Major vonoprazan metabolites and metabolic pathways.

Metabolite	Structural change (conceptual)	Primary enzymes	Phase	Notes
M-I	Oxidation on pyrrole ring / side chain	CYP3A4 (major), CYP2B6,	Phase I	Primary oxidative metabolite; often highest exposure; key DDI marker

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

		CYP2C19, CYP2D6		
M-II	Additional oxidative transformation	CYP3A4 and minor CYPs	Phase I	Secondary oxidative metabolite; contributes to clearance
M-III	N-demethylated or N-oxidized vonoprazan	CYP3A4, CYP2B6, CYP2C19, CYP2D6	Phase I	Modification of dimethylaminomethyl moiety; low pharmacological activity
M-IV-Sul	Sulfate conjugate of hydroxylated metabolite	SULT2A1	Phase II	Major phase II metabolite; considered relatively safe; detoxification route
M-IV-Gluc	Glucuronide of M-IV	UGT isoforms (hepatocyte data)	Phase II	Major metabolite of SH-337; supports reduced accumulation of reactive species
ODA-VP	Oxidative desalkylated metabolite	CYP3A4 (predominant)	Phase I	Used as marker metabolite; metabolic ratio correlates with 4β-hydroxycholesterol

6. APPLICATIONS OF LC–MS/MS METHODS IN PHARMACOKINETIC, BIOEQUIVALENCE AND DDI STUDIES

6.1 Pharmacokinetics and bioequivalence

High-quality LC–MS/MS assays have enabled detailed characterization of vonoprazan pharmacokinetics in animals and humans. Rat studies using LC–MS/MS have measured plasma and tissue concentrations following intravenous and oral dosing, showing rapid absorption, moderate volume of distribution, and multi-exponential decline profiles suitable for physiologically based pharmacokinetic modeling. These data have been used to estimate tissue-to-plasma partition coefficients and simulate exposure under various dosing regimens.

In humans, validated LC–MS/MS methods have been applied to describe single- and multiple-dose pharmacokinetics in healthy volunteers and patients, including exposure under different dosing frequencies and in combination regimens. Bioequivalence studies such as the trial supported by Chen's method showed that generic vonoprazan fumarate formulations achieve systemic exposures within standard bioequivalence limits relative to reference products. Qiao's rat study on vonoprazan pyroglutamate similarly used LC–MS/MS to confirm bioequivalence between test and reference formulations.

6.2 Drug–drug interaction assessments

Vonoprazan's extensive metabolism via CYP3A and its inhibitory effects on multiple CYP isoforms have stimulated a broad range of DDI investigations, almost all of which rely on LC–MS/MS quantification. Voriconazole co-administration in rats increased vonoprazan exposure and reduced clearance, with LC–MS/MS measurements defining changes in pharmacokinetic parameters. Simvastatin–vonoprazan interaction studies in rats employed UPLC–MS/MS to

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

determine plasma concentrations of vonoprazan and M-I, revealing that simvastatin inhibited vonoprazan metabolism in vitro but reduced vonoprazan exposure after multiple dosing in vivo, likely due to complex effects on hepatic enzymes and transport. Wang et al. used LC–MS/MS to evaluate the impact of eleven cardiovascular drugs on vonoprazan metabolism in rat and human liver microsomes and in vivo, identifying amlodipine and nifedipine as mixed-type inhibitors and highlighting amlodipine as a clinically relevant perpetrator.

Mechanistic modeling studies that integrate LC–MS/MS-derived pharmacokinetic data with in vitro inhibition parameters have been used to project DDI magnitude and guide clinical trial design. Mulford et al. implemented a tiered approach using LC–MS/MS data to understand vonoprazan's role as both CYP3A victim and perpetrator, informing label recommendations regarding co-administration with strong CYP3A inhibitors and sensitive substrates.

6.3 Impact on co-medications and pharmacodynamic endpoints

LC–MS/MS methods developed for vonoprazan have also been used to study its effects on the pharmacokinetics of co-administered drugs. Chen et al. investigated the impact of vonoprazan on venlafaxine in rats using UPLC–MS/MS to quantify venlafaxine and O-desmethylvenlafaxine, demonstrating that vonoprazan significantly inhibited venlafaxine metabolism and increased exposure. Similar analytical platforms have been used to evaluate potential interactions with antiplatelet agents such as prasugrel, where overlapping involvement of CYP3A4 in metabolism raises concerns about altered antiplatelet activity.

In clinical settings, LC–MS/MS data for vonoprazan and its metabolites have been linked to pharmacodynamic endpoints such as intragastric pH and H. pylori eradication rates, as well as safety markers including liver function tests and hematological parameters. These integrated analyses facilitate exposure–response modeling and optimization of dosing regimens when vonoprazan is used alone or in multidrug combinations.

7. METHODOLOGICAL CONSIDERATIONS AND EMERGING TRENDS

7.1 Matrix effects, selectivity and sensitivity

As with other LC–MS/MS bioanalytical methods, vonoprazan assays must carefully address matrix effects, especially when using simple protein precipitation in complex matrices. Post-column infusion experiments and matrix factor evaluations have been used to demonstrate that ion suppression or enhancement remains within acceptable limits for vonoprazan and its metabolites across multiple plasma lots. The use of stable isotope-labeled internal standards, such as vonoprazan-d_4, offers the most effective mitigation by closely tracking matrix-induced variability in ionization.

Selectivity is ensured by monitoring multiple MRM transitions per analyte, verifying absence of interfering peaks in blank matrices, and assessing carry-over with high-concentration samples followed by blank injections. Sensitivity requirements are driven by the lowest concentrations expected at late time points in pharmacokinetic profiles and by the need to quantify low-exposure metabolites; reported LLOQs for vonoprazan and key metabolites are generally in the low ng/mL or sub-ng/mL range.

International Journal of Advanced Research and Multidisciplinary Trends (IJARMT)
An International Open Access, Peer-Reviewed Refereed Journal
Impact Factor: 7.2 Website: https://ijarmt.com ISSN No.: 3048-9458

7.2 Green analytical chemistry and operational efficiency

Operational efficiency and sustainability have become important in LC–MS/MS method design. The vonoprazan–amoxicillin–clarithromycin method developed by Al-Tannak et al. was evaluated using the AGREE green analytical assessment tool, demonstrating favorable scores based on short run times, low solvent consumption, and minimal waste. Similar principles can be applied to future vonoprazan assays by optimizing gradient length, using smaller internal-diameter columns, and selecting less hazardous solvents when feasible.

Automation and high-throughput capabilities are increasingly important, particularly for large bioequivalence or population pharmacokinetic studies. Methods relying on straightforward protein precipitation or 96-well plate-based LLE workflows coupled with fast MRM acquisition make it feasible to analyze hundreds to thousands of samples while maintaining regulatory-compliant data quality.

7.3 Integration with modeling and systems pharmacology

Another emerging trend is integration of LC–MS/MS-derived concentration–time data and metabolite profiles into mechanistic models that capture vonoprazan's disposition and interactions. Physiologically based pharmacokinetic and pharmacodynamic models informed by rat and human data have been used to predict gastric pH control, tissue distribution, and DDI magnitude under various clinical scenarios. Incorporating explicit metabolite compartments (e.g., for M-I and M-IV-Sul) into such models could further clarify the contribution of individual pathways to efficacy and toxicity.

8. CONCLUSION

LC–MS/MS-based bioanalysis and metabolite identification have played a central role in elucidating the pharmacokinetics, metabolism and DDI profile of vonoprazan across preclinical and clinical development. Validated quantitative methods now exist for human and animal plasma, urine and microsomal/hepatocyte systems, enabling simultaneous measurement of vonoprazan and key metabolites with high sensitivity, selectivity and throughput. These tools have supported bioequivalence evaluations, clarified the impact of co-administered drugs such as clarithromycin, voriconazole, simvastatin and cardiovascular agents, and informed regulatory labeling regarding CYP3A-mediated interactions.

Several areas merit further investigation. More comprehensive metabolite profiling in humans, including quantification of M-IV-Gluc and other secondary conjugates, would improve understanding of long-term safety and potential for metabolite-mediated toxicity. Standardized reporting of metabolite exposure (e.g., expressing metabolite AUCs as a fraction of total drug-related material) would facilitate cross-study comparisons and regulatory assessment, building on approaches used in radiolabeled studies. Broader adoption of green analytical principles and stable isotope-labeled internal standards may further enhance robustness and sustainability of LC–MS/MS assays in routine clinical laboratories.

Finally, integrating LC–MS/MS data on vonoprazan and its metabolites into systems pharmacology frameworks and real-world therapeutic drug monitoring may help personalize therapy, particularly in patients receiving multiple CYP3A substrates or inhibitors. Overall,

LC–MS/MS will remain indispensable for advancing knowledge of vonoprazan's disposition and optimizing its safe and effective clinical use.

REFERENCES

- Al-Tannak, N. F., Hafez, H. M., Hemdan, A., & co-authors. (2025). LC–MS/MS method development and validation for clinical pharmacokinetics and therapeutic drug monitoring of potassium-competitive acid blocker vonoprazan-based triple therapy for H. pylori in human plasma. Pharmaceuticals, 18(10), 1509. https://doi.org/10.3390/ph18101509
- Chen, X., Chen, T., Huang, Y., Wang, M., Wang, Y., Wu, P., Xia, X., Du, P., Wu, J., Shen, J., & Jia, Y. (2024). LC–MS/MS method for quick detection of vonoprazan fumarate in human plasma: Development, validation and its application to a bioequivalence study. Biomedical Chromatography, 38(6), e5860. https://doi.org/10.1002/bmc.5860
- Chen, F., et al. (2020). In vitro and in vivo rat model assessments of the effects of vonoprazan on the pharmacokinetics of venlafaxine. Drug Design, Development and Therapy, 14, 4815–4824. https://doi.org/10.2147/DDDT.S276704
- Hong, Y., Dai, D., Cai, J., Wang, S., Wang, Y., Zhao, F., Zhou, S., Zhou, Q., Geng, P., Zhou, Y., Xu, X., Shi, J., & Luo, Q. (2022). Effects of simvastatin on the metabolism of vonoprazan in rats both in vitro and in vivo. Drug Design, Development and Therapy, 16, 1779–1789. https://doi.org/10.2147/DDDT.S365610
- Jia, L., & Hahn, J. (2025). Clinical pharmacokinetics of potassium competitive acid blockers: A systematic review and meta-analysis. Frontiers in Pharmacology, 16, 1580969. https://doi.org/10.3389/fphar.2025.1580969
- Mulford, D. J., et al. (2023). Tiered approach to evaluate the CYP3A victim and perpetrator drug–drug interaction potential for vonoprazan. CPT: Pharmacometrics & Systems Pharmacology, 12(4), 532–544. https://doi.org/10.1002/psp4.12939
- Qiao, Y., Zhao, J., Yue, X., Zhang, Y., Zhang, R., Xu, Y, Tang, X., Liu, X., & Wang, Q. (2017). Study on pharmacokinetics and bioequivalence of vonoprazan pyroglutamate in rats by liquid chromatography with tandem mass spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1059, 56–65. https://doi.org/10.1016/j.jchromb.2017.05.013
- Sakaguchi, K., et al. (2024). Characterization of plasma vonoprazan and CYP3A activity using 4β-hydroxycholesterol as an endogenous biomarker. Drug Metabolism and Pharmacokinetics, advance online publication.
- Wang, Y., Shi, J., Dai, D., Cai, J., Wang, S., Hong, Y., Zhou, S., Zhao, F., Zhou, Q., Geng, P., Zhou, Y., Xu, X., & Luo, Q. (2022). Evaluation of commonly used cardiovascular drugs in inhibiting vonoprazan metabolism in vitro and in vivo. Frontiers in Pharmacology, 13, 909168. https://doi.org/10.3389/fphar.2022.909168

- Wang, M.-S., et al. (2022). Distribution- and metabolism-based drug discovery. Innovation, 3(3), 100312. https://doi.org/10.1016/j.xinn.2022.100312
- Yoneyama, T., Teshima, K., Jinno, F., Kondo, T., & Asahi, S. (2016). A validated simultaneous quantification method for vonoprazan (TAK-438F) and its 4 metabolites in human plasma by liquid chromatography–tandem mass spectrometry. Journal of Chromatography B: Analytical Technologies in the Biomedical and Life Sciences, 1015–1016, 42–49. https://doi.org/10.1016/j.jchromb.2016.01.051