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ORIGINAL RESEARCH

Simultaneous Determination of Multiple Acid-Suppressing Drugs by UPLC-MS/MS Method and Application for Pharmacokinetics Study

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Background: Proton pump inhibitors (PPIs) and potassium competitive acid blockers (P-CABs) are widely used to treat acid-related diseases (ARDs). Precisely quantifying their plasma levels is crucial for clinical pharmacokinetic assessments and therapeutic drug monitoring.

Aim: This study aimed to establish a generic and efficient ultra-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) assay for the determination of five PPIs (esomeprazole, rabeprazole, ilaprazole, lansoprazole, and pantoprazole) and the P-CAB (vonoprazan) in human plasma.

Methods: The six analytes were extracted from human plasma via protein precipitation and a single dilution step. Detection was performed on a triple quadrupole tandem mass spectrometer with positive electrospray ionization. Chromatographic separation was achieved on the ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm) using gradient elution. The mobile elution was composed of 0.2% formic acid in acetonitrile (mobile phase A), 0.1% ammonium hydroxide and 10 mmol/L ammonium formate in deionized water (mobile phase B). The flow rate was 0.4 mL/min, the run time was 4.5 minutes, and the injection volume was 20 μL.

Results & Conclusions: The method exhibited excellent linearity across the ranges of 0.2–200 ng/mL for PPIs and 0.5–500 ng/mL for the P-CAB. Both intra- and inter-day precision and accuracy were within the acceptance criteria, with precision ranging from 1.1% to 14.6% and accuracy ranging from 0.0% to 14.7%. Extraction recoveries were consistent, ranging from 88.1% to 96.7%, with no significant matrix effects observed. The stability of the six analytes under diverse storage and processing conditions was also confirmed, with both precision and accuracy falling within the acceptable range of 15%. The UPLC-MS/MS assay provided an efficient and reliable approach for the simultaneous determination of six acid-suppressing medications in a single analytical run. It has been successfully applied to the pharmacokinetic studies of PPIs and P-CABs, offering a valuable tool for clinical research and therapeutic drug monitoring.

Keywords: acid-suppressing drugs, PPIs, P-CAB, UPLC-MS/MS

Introduction

Acid-related diseases (ARDs) are the major global healthcare concern, mainly including peptic ulcers, gastroesophageal reflux disease, erosive esophagitis, dyspepsia, etc.[1] Acid-suppressing drugs including proton pump inhibitors (PPIs) and potassium competitive acid blockers (P-CAB) are the most efficacious treatments for ARDs.[2]

Currently, PPIs including esomeprazole, rabeprazole, ilaprazole, lansoprazole, and pantoprazole, are widely used in clinical settings. Despite their widespread use, PPIs have certain limitations. As prodrugs, they require activation under acidic conditions to inhibit the activity of the proton pump. Consequently, they are optimally administered 30 to

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60 minutes prior to meals to maximize their acid-suppressing capabilities.[3] It typically takes 3 to 5 days of continuous administration to achieve the peak acid-suppressing effects. Moreover, PPIs generally have a short half-life, ranging from 1 to 3 hours, which leads to a lack of sustained acid suppression. As a result, patients with gastroesophageal reflux disease (GERD) may still experience nocturnal acid breakthroughs even after treatment with PPIs.[1] Furthermore, PPIs are mainly metabolized by the cytochrome P450 (CYP) 2C19 enzyme, and their efficacy can be significantly affected by the genetic polymorphism of CYP2C19.[4] Patients who are rapid metabolizers may experience a reduced acid-suppressing effect from PPIs. In contrast, P-CABs can directly and competitively bind to the potassium-binding site of the proton pump, bypassing the need for gastric acid activation. By accumulating at the target site, P-CABs inhibit both the resting and active states of the H+/K+-ATPase in gastric parietal cells, effectively controlling gastric acid secretion. The development of novel P-CABs is currently a prominent area of research in the field of acid-suppressing medications.

Drug concentration monitoring is an essential method for supporting clinical pharmacokinetic studies of novel drugs and optimizing clinical diagnosis and treatment. Currently, several methods based on LC-MS/MS have been reported for the determination of PPIs[5–10] and P-CAB.[11] However, existing methods predominantly focus on single-analyte assays, which fall short of meeting the evolving needs of clinical studies involving novel acid-suppressing drugs.[12] In such studies, multiple PPIs or P-CABs are often selected as control drugs.[13–17] A multi-analyte assay can circumvent the limitations of frequent switching inherent in single-analyte approaches, thereby improving research efficiency and reducing the costs associated with drug research and development (R&D).

In this study, we developed a versatile multiple-analyte assay for the rapid and concurrent quantification of esomeprazole, rabeprazole, ilaprazole, lansoprazole, pantoprazole, and vonoprazan in human plasma. The method has been effectively utilized in the clinical study of PPIs and P-CABs, thus supporting the R&D of novel acid-suppressing medications.

Experimental Procedures

Reagents and Chemicals

Vonoprazan was sourced from TLC Pharmaceutical Standards; D3-vonoprazan was provided by Chemstrong Scientific Co., Ltd (Shenzhen, China); Beijing Putian Genesis Biotechnology Co., Ltd (Beijing, China) supplied esomeprazole, rabeprazole, ilaprazole, lansoprazole, pantoprazole, and D3-omeprazole. For chromatographic solvents, Honeywell Burdick & Jackson (MN, USA) was the source of HPLC-grade acetonitrile, and Sigma-Aldrich Chemicals (MO, USA) supplied formic acid. Additionally, Beijing Chemical Reagent Company (Beijing, China) furnished Dimethyl Sulfoxide (DMSO), and Sinopharm Chemical Reagent Co., Ltd supplied both ammonium formate and ammonium hydroxide. Deionized water was prepared by Milli-Q from Millipore (Bedford, MA, USA).

Instruments & UPLC–MS/MS Conditions

Employing a Xevo TQ-S triple quadrupole mass spectrometer and an ACQUITY UPLC I-Class PLUS system from Waters Corporation (Milford, MA, USA), we conducted the analysis. The chromatographic separation was executed on an ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm), also from Waters.

The mobile phase was composed of 0.2% formic acid in acetonitrile (mobile phase A), and 0.1% ammonium hydroxide and 10 mmol/L ammonium formate in deionized water (mobile phase B). The gradient elution commenced at 20% of mobile phase A, enduring for the initial 0.5 minutes. It then transitioned linearly, reaching 30% of mobile phase A over the next minute, where it was sustained for an additional 0.8 minutes. Subsequently, the gradient sharply increased to 95% of mobile phase A within 0.2 minutes, and this condition was maintained for 1.0 minutes. The system promptly reverted to the initial 20% of mobile phase A, marking the start of a 1.0-minute column re-equilibration. Throughout, the flow rate was maintained at 0.4 mL/min, with each sample's analysis requiring 4.5 minutes. The column was kept at a temperature of 40°C, while the autosampler was cooled to 10°C.

Detection of the analytes was accomplished using positive mode electrospray ionization (ESI+), with the following source and gas settings: a temperature of 500 °C, a flow rate of 1000 liters per hour, and a capillary voltage of 3.5 kilovolts. The method employed multiple reaction monitoring (MRM) for the detection and quantification of analytes.

Table 1 MS Conditions

Analytes	MRM Transitions	Retention Time	Capillary Voltage	Cone Potential	Collision Energy	Dwell Time
		(Minute)	(kV)	(V)	(V)	(s)
Esomeprazole	346.3→198.1	1.90	3.5	50	12	0.038
Rabeprazole	360.2→242.1	1.57	3.5	40	10	0.038
Ilaprazole	367.2→184.0	2.34	3.5	30	10	0.038
Lansoprazole	370.2→252.0	2.79	3.5	30	12	0.038
Pantoprazole	384.2→200.2	2.23	3.5	30	20	0.038
D3- Omeprazole (IS)	349.2→198.1	1.88	3.5	60	15	0.038
Vonoprazan	346.1→315.1	1.63	3.5	30	15	0.038
D3-Vonoprazan (IS)	349.1→315.1	1.63	3.5	30	15	0.038

Abbreviation: MRM, Multiple reactions monitoring.

The compound-dependent parameters were meticulously fine-tuned for optimal performance and were detailed in Table 1. The product ion spectra were shown in Figure 1. Esomeprazole is characterized by a quantitative ion transition of m/z 346.3 to 198.1; Rabeprazole shows a transition of m/z 360.2 to 242.1; Ilprazole is identified by a transition of m/z 367.2 to 184.0; Lansoprazole is associated with a transition of m/z 370.2 to 252.0; Pantoprazole is marked by a transition

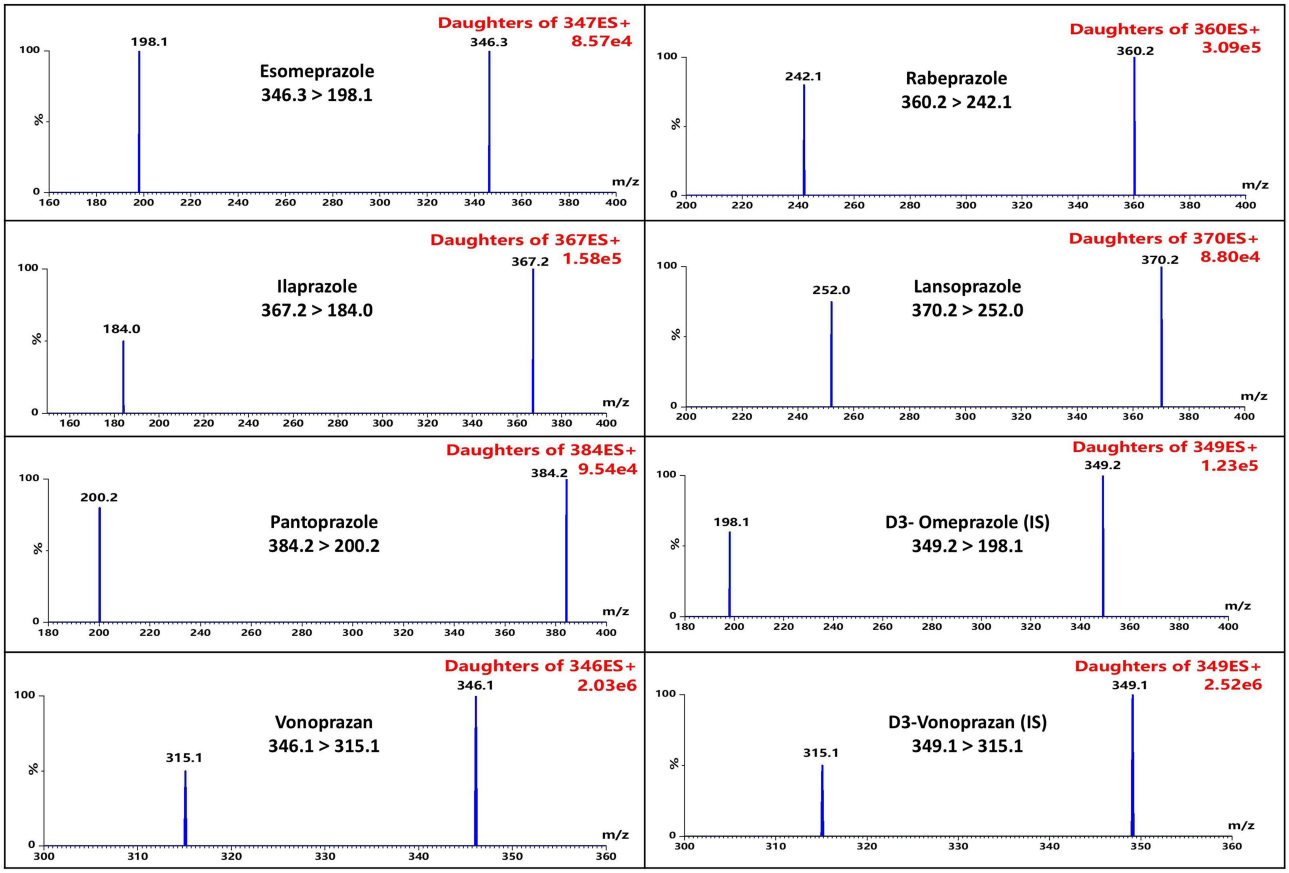

Figure 1 Product ion spectra. Esomeprazole; rabeprazole; ilprazole; lansoprazole; pantoprazole; D3- omeprazole (IS); vonoprazan; D3-vonoprazan (IS).

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of m/z 384.2 to 200.2; D3-Omeprazole (IS) exhibits a quantitative ion transition of m/z 349.2 to 198.1; Vonoprazan is indicated by a transition of m/z 346.1 to 315.1; and D3-Vonoprazan (IS) is represented by the same transition of m/z 346.1 to 315.1.

Stock Solutions, Calibration Standards, and Quality Controls (QC)

Stock solutions for esomeprazole, rabeprazole, ilaprazole, lansoprazole, pantoprazole, vonoprazan, and their corresponding internal standards were individually formulated to a concentration of 0.5 mg/mL in DMSO. Calibration standards in human plasma (heparin sodium anticoagulation) were established at levels of 0.2, 0.5, 2.0, 10, 20, 50, 100, and 200 ng/mL for the PPIs, and 0.5, 1.25, 5.0, 25, 50, 125, 250, and 500 ng/mL for the P-CAB. Quality control samples, including the Lower limit of quantitation (LLOQ), low-quality control (LQC), medium-quality control (MQC), high-quality control (HQC), and dilution-quality control (DQC) samples were crafted at specific concentrations: 0.2, 0.4, 16, 160, and 1600 ng/mL for PPIs, while 0.5, 1.0, 40, 400, and 4000 ng/mL for the P-CAB. The combined internal standard solution was adjusted to final concentrations of 20.0 ng/mL for D3-omeprazole and 50.0 ng/mL for D3-vonoprazan using acetonitrile. The above solutions and samples were preserved at −80°C until analysis.

Sample Preparation

The plasma samples were processed using a protein precipitation method. Initially, 200 μL of the combined internal standard solution—consisting of D3-omeprazole at 20.0 ng/mL and D3-vonoprazan at 50.0 ng/mL in acetonitrile—was added to a 1.5 mL centrifuge tube. Subsequently, 50 μL of plasma was introduced to the same tube. The mixture was homogenized using a vortex mixer for 60 seconds before being subjected to centrifugation at 13,300 rpm for 10 minutes. Then, 50 μL of the supernatant was diluted with 200 μL of acetonitrile-0.1% ammonium hydroxide water (2:8, v/v). This new mixture was briefly vortexed for 30 seconds to ensure thorough mixing. Finally, an aliquot of 20 μL of this prepared solution was loaded into the UPLC-MS/MS system for analytical evaluation.

Method Validation

The bioanalytical assay underwent comprehensive validation by the regulatory standards set by the United States Food and Drug Administration (FDA),[18] the European Medicines Agency (EMA),[19] and the Pharmacopoeia of the People's Republic of China,[20] encompassing a suite of evaluations including linearity, selectivity, precision and accuracy, recovery and matrix effects, stability, dilution integrity, and carryover.

Linearity

Linearity was confirmed by applying a regression analysis to the peak area ratios of six analytes to their corresponding internal standards for isotope labeling at eight different concentrations, comparing these ratios to the expected (theoretical) concentration (x). This was achieved using a least squares method with a weighting of $1/x^2$. Acceptance criteria for the calibration curves included correlation coefficients exceeding 0.98 and deviations from the nominal values not exceeding 15%, except LLOQ, which allowed for a 20% deviation.

Selectivity

Selectivity was assessed by analyzing six separate blank plasma samples and contrasting them with LLOQ samples, including 0.2 ng/mL for PPIs and 0.5 ng/mL for the P-CAB. The response of the blank samples, ie the peak area, not exceeding 20% of the LLOQ were considered satisfactory. We acquired blank plasma from six healthy volunteers, utilizing heparin sodium the anticoagulant, which was provided by Peking Union Medical College Hospital (Beijing, China). We obtained approval from the Ethics Committee of Peking Union Medical College Hospital, and ensured that all volunteers provided informed consent after receiving a comprehensive explanation of the study.

Precision and Accuracy

The accuracy and precision of the bioanalytical assay were ascertained through the analysis of six replicates for each level—LLOQ, LQC, MQC, and HQC—in plasma, considering both between-day and within-day variability across

a consecutive three-day period. The measure of precision was given by the relative standard deviation (RSD), and accuracy was indicated by the relative error (RE). For LQC, MQC, and HQC, the criteria were set for both RSD and RE to be within ±15%, while for LLOQ, a slightly broader range of ±20% was acceptable.

Matrix Effect and Extraction Recovery

The matrix effect on the response of analytes was assessed by contrasting the peak areas from six distinct blank plasma extracts with those from the solutions at LQC and HQC levels, aiming for the RSD below 15%. Extraction recovery was evaluated by comparing peak areas of QC samples at three levels—routinely extracted to those post-extraction from spiked blank plasma at equivalent concentrations, with the target range for recovery set between 85% and 115%, and an RSD threshold of 15% for all QCs.

Considering the complexity of clinical samples, the effect of sample-specific conditions such as hemolysis and hyperlipidemia was examined using LQC and HQC samples prepared in plasma with induced 2% hemolysis and elevated triglyceride levels of 300 mg/dL. Acceptable criteria for these samples were the RE and RSD both within the ±15%.

Stability

Stability assessments encompassed two QC levels (LQC and HQC), with six replicates each, subjected to defined storage and processing regimens. Freeze-thaw stability was determined following four cycles of alternating freezing at −80°C and thawing at room temperature before plasma pretreatment. Short-term stability was evaluated after a 24-hour period at room temperature, while long-term stability was examined after 112 days of storage at both −20°C and −80°C. Additionally, the autosampler stability was verified by maintaining the processed QC samples in an autosampler set to 10°C for 48 hours.

Dilution Integrity

For the evaluation of dilution integrity, DQC samples, prepared at concentrations of 1600 ng/mL for PPIs and 4000 ng/mL for P-CAB in plasma, were diluted by a factor of 10 with pooled plasma and analyzed. The assessment criteria required precision and accuracy to remain within 15%.

Carryover

The evaluation of carryover effects entailed the sequential analysis of a blank sample subsequent to the highest calibration standard. The criteria for acceptable carryover were defined as not exceeding 20% for the six analytes and 5% for the corresponding isotope-labeled IS.

Clinical Application

This generic multi-analyte assay could support clinical research on the aforementioned acid-suppressing drugs, including investigations into drug-drug interactions (DDIs) between PPIs and other medications such as clopidogrel, citalopram, methotrexate, and certain protease inhibitors like ritonavir and nelfinavir.[21] On the other hand, the PPIs and P-CAB included in this method are frequently utilized as positive control drugs in clinical studies for comparative analysis of novel acid-suppressing agents. For example, a pharmacokinetic study of tegoprazan by Yang et al in 2023 employed vonoprazan and esomeprazole as positive control drugs.[14] Furthermore, this method could inspire approaches for the quantitative detection of other emerging acid-suppressing drugs.

Currently, this method has successfully facilitated several clinical studies, including a safety and pharmacokinetics study of ilaprazole in healthy Chinese individuals and a clinical study of vonoprazan with esomeprazole serving as a positive control. Taking the pharmacokinetic study of ilaprazole as an example, the research was conducted using a randomized, open-label trial design. A total of 16 volunteers received an intravenous infusion of 10 mg ilaprazole sodium for injection. Plasma samples were collected at various time points: before administration and at 15, 30, 45, and 50 minutes, and then at 1, 1.5, 2, 3, 4, 5, 8, 12, and 24 hours post-administration. In alignment with the ethical guidelines of the Declaration of Helsinki, our study obtained approval from the Ethics Committee of Peking Union Medical College Hospital, and ensured that all volunteers gave their consent after being fully informed about the study.

Data Analysis

Data acquisition and analytical processes were facilitated by the MassLynx software suite, specifically version 4.1 from Waters Corporation. For the calibration curve fitting and subsequent statistical evaluations, Microsoft Excel (Microsoft Office 2016, Microsoft Corp.) was utilized. The analysis incorporated a weighted least squares regression approach, applying weights based on the square of the independent variable ($1/x^2$).

Results & Discussion

Method Development

In the present study, we developed a specific and convenient method for the detection of multiple PPIs and the P-CAB in human plasma by UPLC-MS/MS. Compared with previously published methods, it has the following advantages: 1) Our approach extended beyond the method reported by Elkady EF. in 2018[9] and Yoneyama T. in 2016[11] by incorporating commonly utilized PPIs and a P-CAB, vonoprazan. Compared with the reported single-analyte assay,[5–8,10] this multi-analyte assay eliminated the need for frequent transitions between distinct analytical methods for different analytes, and met the current requirements of clinical studies of novel acid-suppressing drugs, in which more than one PPI or P-CAB would be selected as the control drug. Meanwhile, this advantage broadened the spectrum of acid-suppressing drugs that can be simultaneously determined, thereby enhancing the clinical applicability of our method; 2) We have significantly improved LLOQ for the four PPIs (esomeprazole, rabeprazole, lansoprazole, and pantoprazole) to 0.2 ng/mL, which was a 100-fold reduction compared to the 20 ng/mL of LLOQ reported by Elkady EF. in 2018.[10] The literature review and its details between reported and proposed methods were shown in Table 2.

Table 2 Summary of Reported and Proposed Methods

Literature number	Analytes	Internal standard	Matrix	Preparation	Mobile phase	Column	Run time	Linearity
Proposed method	Esomeprazole, rabeprazole, ilaprazole, lansoprazole, pantoprazole, vonoprazan	D3omeprazole, D3vonoprazan	Human plasma	Protein precipitation	0.2% formic acid in ACN and 0.1% ammonium hydroxide and 10 mmol/L ammonium formate in water	ACQUITY UPLC BEH C18 column (2.1 × 50 mm, 1.7 μm)	4.5 min	0.2 to 200 ng/mL for PPIs 0.5500 ng/mL for PCAB
5	Pantoprazole	Pantoprazole D3	Human plasma	Protein precipitation	10 mmol/L ammonium acetate (pH 7.10): ACN (30:70, v/v)	Zorbax SBC18 (4.6 mm × 75 mm, 3.5 μm)	2.5min	10 to 3000 ng/mL
6	Omeprazole and lansoprazole	Esomeprazole	Human plasma	Liquid-liquid extraction	0.25% formic acid in ACN and 0.25% formic acid in water	Thermo Betasil silica100 column (50× 3.0 mm, 5 mm particle size)	5min	1.5 to 100ng/mL for omeprazole 5 to 2000ng/mL for lansoprazole
7	Pantoprazole	Omeprazole	Human plasma	Protein precipitation	1% of 5 mol /L ammonium acetate in MeOH: water (60:40, v/v)	Lichrospher C18 column (5 μm, 2.1×9100 mm)	2.5min	5 to 5000 ng/mL
8	Esomeprazole and naproxen	Ibuprofen	Human plasma	Solidphase extraction	ACN: 25 mmol /L ammonium formate (70:30, v/v)	An XBridge C18 analytical column (50×3.0 mm, 3.5 mm)	4min	3.00 to 700.02 ng/mL for esomeprazole
9	Esomeprazole, lansoprazole, pantoprazole, rabeprazole	Escitalopram	Human plasma	Protein precipitation	10mM ammonium formate: ACN: MeOH (20:40:40% v/v)	C18 INERTSIL ODS3 (5 μm, 150×4.6 mm)	3.5min	20 to 5000 ng/mL for all analytes
10	Rabeprazole	Esomeprazole	Human plasma	Protein precipitation	10 mm ammonium acetate and 0.2% acetic acid in ACN: water (35:65, v/v)	Chiralpak IC column (4.6 mm × 150 mm, 5 μm).	8min	0.500 to 400 ng/mL for rabeprazole
11	Vonoprazan and its 4 metabolites	D4vonoprazan	Human plasma	Protein precipitation	ACN: 20 mmol/L ammonium formate (pH 3) (32:68, v/v)	An Acquity UPLC BEH C18 (2.1 mm I. D., 100 mm, particle size 1.7um)	5min	0.1 to 100 ng/mL for vonoprazan

During the method development, the mass spectrometric, the parameters of mass spectrometry and chromatography were meticulously optimized to enhance the accuracy and efficiency of the analysis, as well as sample preparation. Given the benzene of PPIs and the P-CAB, reversed-phase chromatography was chosen as the primary separation technique. Utilizing ESI in positive mode, which was advantageous due to the nitrogen atoms in all six target compounds, resulted in superior ionization efficiency and a consistent signal response. Following an evaluation of various chromatographic columns for their separation efficacy, the C18 column (2.1×50 mm, 1.7μm) was selected for its favorable retention characteristics and stable peak shapes. For mobile phase optimization, acetonitrile was implemented as the organic component, delivering effective elution and minimal background noise. The aqueous component was fortified with 0.1% ammonia and 10 mmol/L ammonium formate to augment the analytes' response. To ensure precise quantification of the analytes and mitigate matrix effects, stable isotope-labeled internal standards, D3-omeprazole for the PPIs and D3-vonoprazan for the P-CAB, were employed, respectively. In clinical laboratories, tasks characterized by high labor and extensive time requirements are typically not favored. However, in this study, we simplified the process by precipitating plasma samples with acetonitrile and subsequently diluting them fivefold, a procedure that required only a dozen minutes.

Method Validation

Linearity

Outstanding linearity across the entire analyte range was demonstrated for both PPIs (0.2–200 ng/mL) and the P-CAB (0.5–500 ng/mL) in plasma samples, with correlation coefficients (R^2) consistently achieving values between 0.987 and 0.994. Compliance with the calibration standard criterion as detailed in Linearity was confirmed for no less than 75% of the samples. The specific outcomes were shown in Table 3. Furthermore, the calibration standards for esomeprazole, rabeprazole, ilaprazole, lansoprazole, pantoprazole, and vonoprazan, with their back-calculated concentrations, were exhibited in Supplementary Table S1, all of which met the established acceptance criteria.

Selectivity

In terms of selectivity, as illustrated in Figure 2, no significant interferences were detected in the retention times of the six analytes and corresponding ISs within the blank plasma samples. Figure 2A indicates a retention time of 1.9 min for esomeprazole, Figure 2B shows 1.57 min for rabeprazole, Figure 2C displays 2.34 min for ilaprazole, Figure 2D illustrates 2.79 min for lansoprazole, Figure 2E presents 2.23 min for pantoprazole, and its IS, D3-omeprazole, at 1.88 min. For vonoprazan and its IS, D3-vonoprazan, both elute at 1.63 min (Figure 2F). Notably, at these retention times, the blank plasma demonstrated no interference with the analytes.

Precision and Accuracy

Regarding precision and accuracy, the data presented in Table 4 reveal that both the RSD and RE for the LLOQ, LQC, MQC, and HQC levels were well within the acceptable criteria. This conformity to the acceptance criteria substantiates the method's reliability and reproducibility.

Matrix Effect and Extraction Recovery

Data about the matrix effect and extraction recovery for six analytes were presented in Tables 5 and 6. These findings indicated that ion suppression from human plasma was minimal. Supplementary Table S2 further illustrated that minor degrees of hemolysis and hyperlipidemia exert no significant influence on the quantification of analytes.

Table 3 Linearity for All Analytes

Analytes	Range	Regression Equation	R^2
Esomeprazole	0.20–200ng/mL	Y=0.0224X-0.0052	0.998
Rabeprazole	0.20–200ng/mL	Y=0.0372X-0.0052	0.996
Ilaprazole	0.20–200ng/mL	Y=0.0357X+0.0019	0.997
Lansoprazole	0.20–200ng/mL	Y=0.0264X+0.0180	0.996
Pantoprazole	0.20–200ng/mL	Y=0.0257X-0.0022	0.997
Vonoprazan	0.50–500ng/mL	Y=0.0068X+0.0119	0.997

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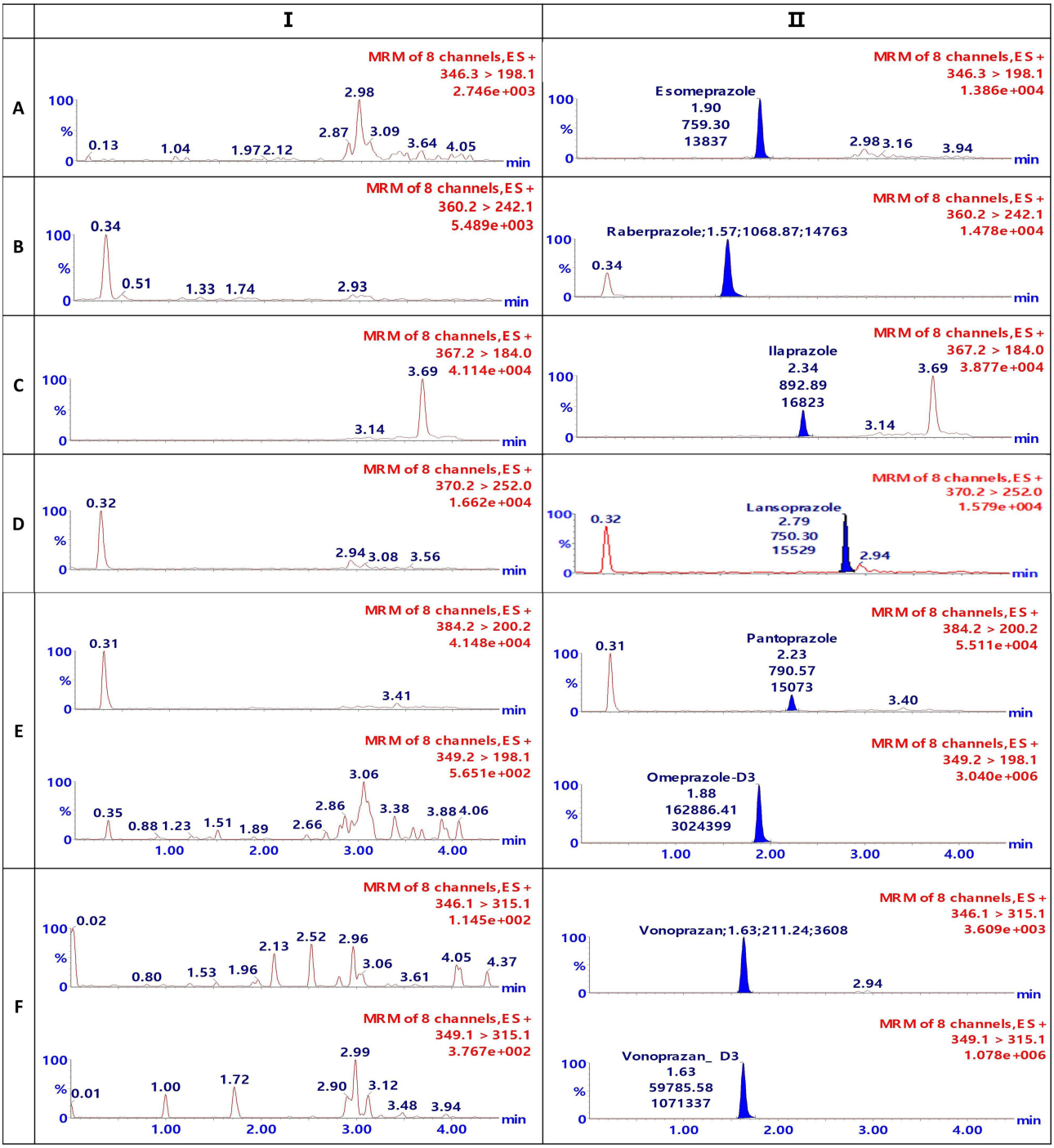

Figure 2 Typical multiple reactions monitoring chromatograms of analytes and ISs. (I) Blank plasma, (II) LLOQ sample; (**A**) Esomeprazole (0.2ng/mL), (**B**) Rabeprazole (0.2ng/mL), (**C**) Ilaprazole (0.2ng/mL), (**D**) Lansoprazole (0.2ng/mL), (**E**) Pantoprazole (0.2ng/mL) and D3- omeprazole (IS, 20ng/mL), (**F**) Vonoprazan (0.5ng/mL) and D3-vonoprazan (IS, 50ng/mL).

Stability Assessments

The stability assessments encompassing short-term, reinject, autosampler, freeze-thaw, and long-term stability are outlined in Table 7. The findings indicated that both the measures of accuracy and precision adhered to the established acceptance criteria, signifying the robust stability of six analytes throughout standard preparative and preservation processes. In addition, Supplementary Table S3 showed that analytes in whole blood maintained stability at room temperature for up to 2 hours.

Table 4 Intra- and Inter-Run Accuracy and Precision of Quality Control Samples

Item	Intra-run (ng/mL)				Inter-run (ng/mL)			
Esomeprazole	**LLOQ**	**LQC**	**MQC**	**HQC**	**LLOQ**	**LQC**	**MQC**	**HQC**
Nominal Conc.	0.20	0.40	16.0	160	0.20	0.40	16.0	160
Precision (RSD%)	6.3	1.4	3.5	3.9	7.2	4.2	2.8	3.5
Accuracy (RE%)	3.3	1.2	1.1	1.0	5.6	0.7	0.6	1.6
Rabeprazole	**LLOQ**	**LQC**	**MQC**	**HQC**	**LLOQ**	**LQC**	**MQC**	**HQC**
Nominal Conc.	0.20	0.40	16.0	160	0.20	0.40	16.0	160
Precision (RSD%)	7.0	1.1	5.0	4.6	8.6	3.0	4.4	4.4
Accuracy (RE%)	13.3	2.9	2.6	0.0	9.7	3.1	3.5	0.6
Ilaprazole	**LLOQ**	**LQC**	**MQC**	**HQC**	**LLOQ**	**LQC**	**MQC**	**HQC**
Nominal Conc.	0.20	0.40	16.0	160	0.20	0.40	16.0	160
Precision (RSD%)	8.0	6.6	4.9	7.6	9.9	7.0	4.8	5.9
Accuracy (RE%)	1.7	3.3	0.2	7.0	5.0	0.4	0.4	3.9
Lansoprazole	**LLOQ**	**LQC**	**MQC**	**HQC**	**LLOQ**	**LQC**	**MQC**	**HQC**
Nominal Conc.	0.20	0.40	16.0	160	0.20	0.40	16.0	160
Precision (RSD%)	5.3	6.4	6.8	8.4	8.4	6.6	5.4	6.1
Accuracy (RE%)	1.7	2.5	2.0	2.2	5.8	0.8	1.5	0.1
Pantoprazole	**LLOQ**	**LQC**	**MQC**	**HQC**	**LLOQ**	**LQC**	**MQC**	**HQC**
Nominal Conc.	0.20	0.40	16.0	160	0.20	0.40	16.0	160
Precision (RSD%)	10.5	5.7	2.2	5.0	9.8	5.0	3.4	4.2
Accuracy (RE%)	5.0	1.7	1.2	3.4	5.6	3.3	1.2	2.8
Vonoprazan	**LLOQ**	**LQC**	**MQC**	**HQC**	**LLOQ**	**LQC**	**MQC**	**HQC**
Nominal Conc.	0.50	1.00	40.0	400	0.50	1.00	40.0	400
Precision (RSD%)	9.2	14.6	3.1	4.4	11.5	10.8	3.0	3.8
Accuracy (RE%)	14.7	5.2	0.0	2.9	10.1	5.7	0.3	4.2

Notes: n, the number of concentrations participating in standard curve fitting.
Abbreviations: Nominal Conc, Nominal Concentration; RSD%, Relative standard deviation; RE%, Relative Error. n, the number of concentrations participating in standard curve fitting.

Table 5 Extraction Recovery in Plasma

Item	Recovery		
Esomeprazole	LQC	MQC	HQC
Nominal Conc. ng/mL	0.40	16.0	160
Recovery %	95.5	90.9	93.5
RSD %	13.6	1.9	6.5
Rabeprazole	LQC	MQC	HQC
Nominal Conc. ng/mL	0.40	16.0	160
Recovery %	96.8	94.3	98.4
RSD %	10.6	5.9	7.9
Ilaprazole	LQC	MQC	HQC
Nominal Conc. ng/mL	0.40	16.0	160
Recovery %	91.9	92.7	92.5
RSD %	12.3	4.3	6.9

(*Continued*)

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Table 5 (Continued).

Item	Recovery		
Lansoprazole	LQC	MQC	HQC
Nominal Conc. ng/mL	0.40	16.0	160
Recovery %	95.4	95.9	96.8
RSD %	5.7	5.7	5.9
Pantoprazole	LQC	MQC	HQC
Nominal Conc. ng/mL	0.40	16.0	400
Recovery %	94.1	95.6	95.8
RSD %	8.9	4.9	6.6
Vonoprazan	LQC	MQC	HQC
Nominal Conc. ng/mL	1.00	40.0	400
Recovery %	88.5	91.0	94.5
RSD %	10.7	4.8	3.1

Abbreviations: LQC, Low concentration quality control; MQC, Middle concentration quality control; HQC, High concentration quality control; RSD%, Relative standard deviation.

Table 6 Matrix Effect in Plasma

Item	Matrix Effect	
Esomeprazole	LQC	HQC
Nominal Conc. ng/mL	0.40	160
Mean %	104.4	101.6
RSD %	3.7	0.6
Rabeprazole	LQC	HQC
Nominal Conc. ng/mL	0.40	160
Mean %	102.6	99.9
RSD %	3.7	1.2
Ilaprazole	LQC	HQC
Nominal Conc. ng/mL	0.40	160
Mean %	102.0	115.8
RSD %	10.8	7.4
Lansoprazole	LQC	HQC
Nominal Conc. ng/mL	0.40	160
Mean %	105.9	99.1
RSD %	3.9	4.2
Pantoprazole	LQC	HQC
Nominal Conc. ng/mL	0.40	160
Mean %	110.1	99.8
RSD %	3.1	2.1

(*Continued*)

Table 6 (Continued).

Item	Matrix Effect	
Vonoprazan	LQC	HQC
Nominal Conc. ng/mL	1.00	400
Mean %	97.6	101.9
RSD %	11.1	0.8

Abbreviations: LQC, Low concentration quality control; HQC, High concentration quality control; RSD%, Relative standard deviation.

Dilution Integrity

The RSD for the DQCs showed a range of 1.7% to 5.3%, while the RE showed a range of 2.0% to 5.3% across six analytes, after applying a 10-fold dilution. This indicated that plasma samples surpassing the ULOQ could be reliably analyzed post a 10-fold dilution with pooled plasma.

Table 7 Stability of Analytes in Plasma Samples and Processed Samples

Analytes	Conditions	Nominal Conc.	Found Conc.	Precision (RSD %)	Accuracy (RE%)
	Plasma Samples	**(ng/mL)**	**(ng/mL)**	**(n = 3)**	**(n = 3)**
Esomeprazole	R.T. for 24 hours	0.40	0.39	4.4	2.5
		160	163.53	3.2	2.2
	80°C for 112 days	0.40	0.39	6.0	3.3
		160	167.56	1.9	4.7
	20°C for 112 days	0.40	0.38	9.3	5.8
		160	167.25	6.0	4.5
	Four Freeze-thaw circles	0.40	0.41	10.1	3.3
		160	165.17	3.3	3.2
	Autosampler; 10°C for 48 hours	0.40	0.36	8.3	10.8
		160	160.78	4.3	0.5
Rabeprazole	R.T. for 24 hours	0.40	0.41	10.2	1.7
		160	159.89	3.4	0.1
	80°C for 112 days	0.40	0.36	13.0	9.2
		160	161.43	2.2	0.9
	20°C for 112 days	0.40	0.37	4.1	6.7
		160	165.43	10.1	3.4
	Four Freeze-thaw circles	0.40	0.39	10.5	3.3
		160	166.32	3.9	3.9
	Autosampler; 10°C for 48 hours	0.40	0.39	2.7	3.8
		160	160.77	8.0	0.5
Ilaprazole	R.T. for 24 hours	0.40	0.45	1.3	11.7
		160	181.14	1.4	13.2
	80°C for 112 days	0.40	0.44	5.8	9.2
		160	172.19	0.4	7.6
	20°C for 112 days	0.40	0.44	6.0	10.0
		160	169.62	3.6	6.0
	Four Freeze-thaw circles	0.40	0.40	5.2	0.8
		160	173.23	3.8	8.3
	Autosampler; 10°C for 48 hours	0.40	0.45	5.7	12.1
		160	162.17	9.3	1.4

(*Continued*)

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Table 7 (Continued).

Analytes	Conditions	Nominal Conc.	Found Conc.	Precision (RSD %)	Accuracy (RE%)
	Plasma Samples	**(ng/mL)**	**(ng/mL)**	**(n = 3)**	**(n = 3)**
Lansoprazole	R.T. for 24 hours	0.40	0.36	4.8	10.0
		160	180.19	1.9	12.6
	80°C for 112 days	0.40	0.36	6.9	9.2
		160	176.62	1.1	10.4
	20°C for 112 days	0.40	0.40	5.2	0.8
		160	175.16	3.6	9.5
	Four Freeze-thaw circles	0.40	0.38	7.7	5.8
		160	164.92	3.6	3.1
	Autosampler; 10°C for 48 hours	0.40	0.39	3.0	2.9
		160	159.32	4.3	0.4
Pantoprazole	R.T. for 24 hours	0.40	0.39	1.5	3.3
		160	173.13	3.2	8.2
	80°C for 112 days	0.40	0.38	14.7	5.0
		160	172.07	3.3	7.5
	20°C for 112 days	0.40	0.38	5.4	4.2
		160	170.38	6.2	6.5
	Four Freeze-thaw circles	0.40	0.37	5.6	6.7
		160	170.90	3.1	6.8
	Autosampler; 10°C for 48 hours	0.40	0.39	7.1	2.1
		160	162.46	7.9	1.5
Vonoprazan	R.T. for 24 hours	1.00	0.90	7.5	9.7
		400	400.75	1.3	0.2
	80°C for 112 days	1.00	0.89	9.0	10.7
		400	397.25	1.7	0.7
	20°C for 112 days	1.00	0.96	14.0	4.0
		400	392.84	4.7	1.8
	Four Freeze-thaw circles	1.00	1.03	9.9	3.3
		400	407.60	3.5	1.9
	Autosampler;	1.00	0.85	7.3	14.7
	10°C for 48 hours	400	414.;52	2.9	3.6

Abbreviations: RSD%, Relative standard deviation; RE%, Relative Error; R.T., room temperature.

Carryover

Regarding carryover, the absence of peaks for both analytes and internal standards in the blank plasma after the ULOQ indicated a negligible carryover.

Clinical Application

The UPLC-MS/MS assay, once developed and validated, proved efficacious in its application to the pharmacokinetic analysis of ilaprazole. Up to now, the method analyzed a total of 224 plasma samples from 16 volunteers. A typical plasma concentration-time curve for ilaprazole was shown in Figure 3. The peak concentrations of ilaprazole in plasma (834 ng/mL) were observed within 0.75 to 1 hour after infusion, and the plasma clearance was approximately 3 L/h. The half-life of ilaprazole is about 3 to 4 hours, consistent with those reported in previous studies.[22,23] Furthermore, Figure 4 illustrated the UPLC-MS/MS chromatograms for both esomeprazole (Figure 4A) and vonoprazan (Figure 4B) in plasma, which confirmed that our method was also capable of quantitatively analyzing esomeprazole and vonoprazan in plasma samples.

The generic method enabled the simultaneous quantitative determination of all five PPIs and a P-CAB in human plasma. This capability would fulfill the essential need for monitoring drug levels in diverse patients who may be

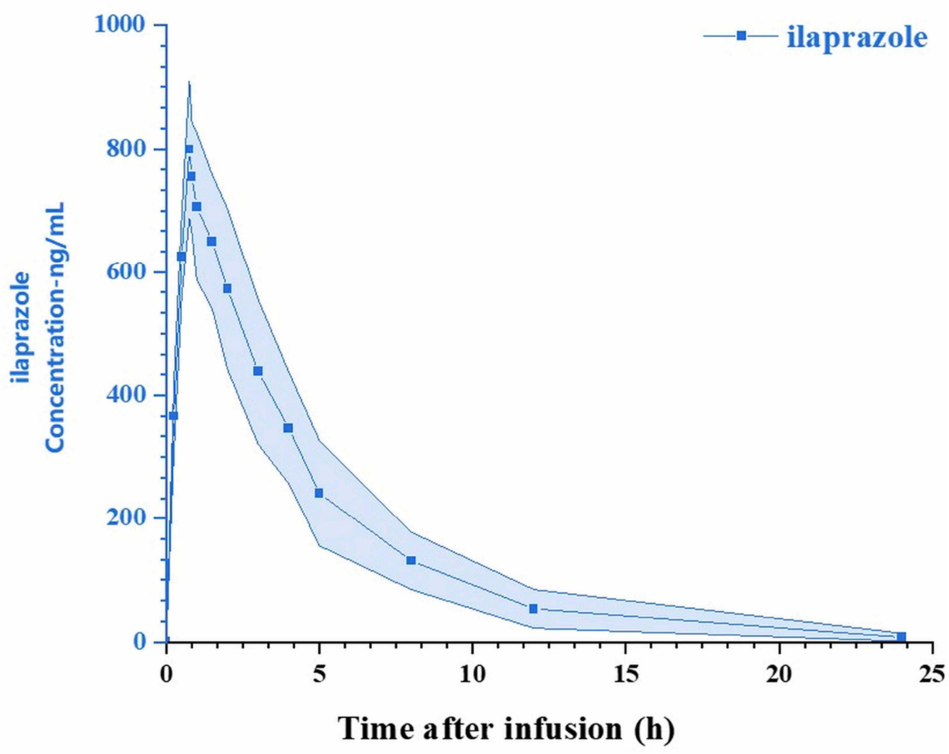

Figure 3 Plasma concentration over time profile for ilaprazole.

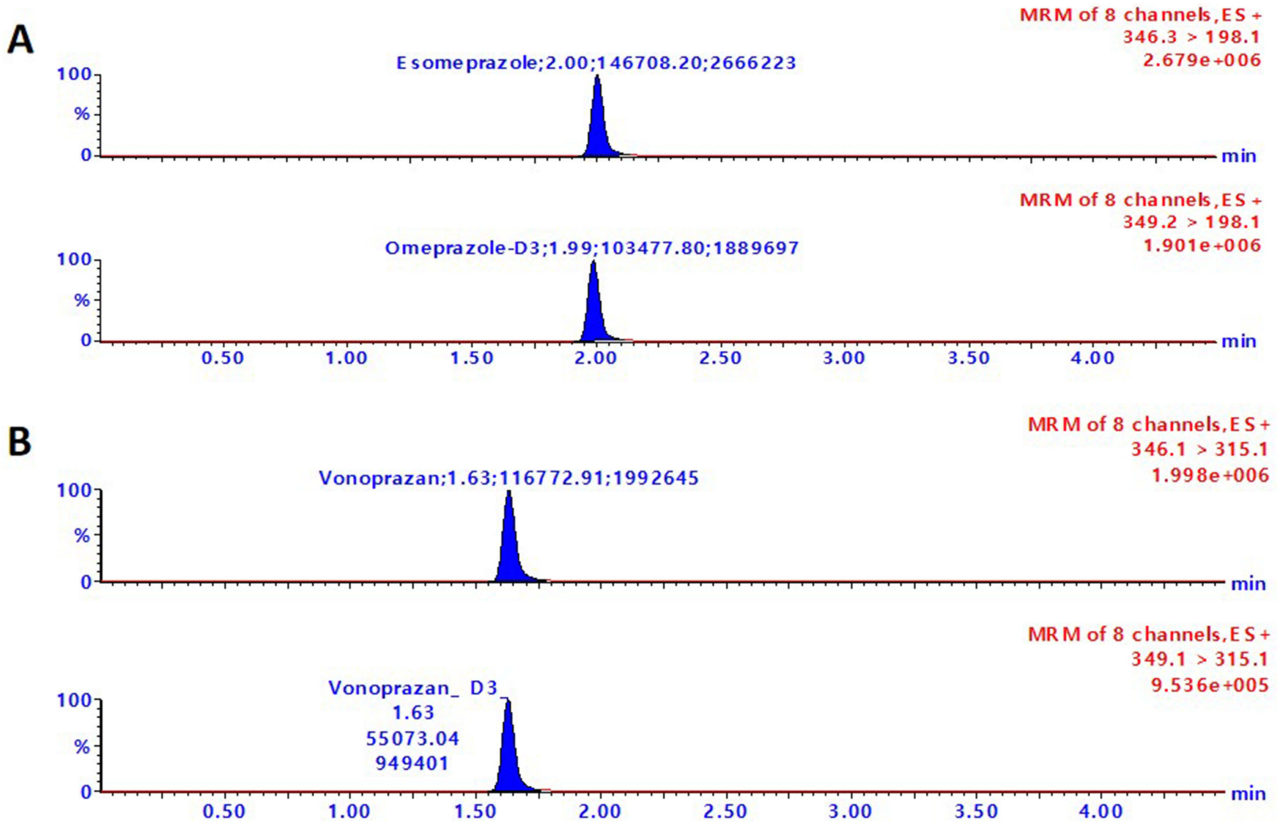

Figure 4 The UPLC/MS/MS chromatograms of the selected drugs in the plasma. (**A**) Esomeprazole, (**B**) Vonoprazan.

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concurrently utilizing PPIs or P-CAB. Since the analytes were the active pharmaceutical ingredients (APIs), this methodology would also be employed to support the formulation development of both PPIs and P-CAB. Consequently, this multi-analyte assay would offer a versatile and comprehensive approach to pharmaceutical analysis and hold promise for advancing drug monitoring and formulation strategies.

Conclusion

A comprehensive assay leveraging UPLC-MS/MS technology for the simultaneous measurement of five PPIs and a P-CAB in human plasma was developed and rigorously validated. Characterized by minimal sample preparation requirements, enhanced throughput, and expedited analysis, this assay has been effectively integrated into pharmacokinetic studies for both PPIs and P-CABs. Our method offers several advantages over previously reported methods, including the simultaneous analysis of multiple analytes, a lower LLOQ, improved time efficiency, and a broader range of clinical applicability. It serves as a robust analytical tool for clinical research and the precise monitoring of therapeutic drug levels. The method demonstrated excellent linearity across the ranges of 0.2–200 ng/mL for PPIs and 0.5–500 ng/mL for the P-CABs. Both intra- and inter-day precision and accuracy were within the acceptance criteria, with precision ranging from 1.1% to 14.6% and accuracy ranging from 0.0% to 14.7%. Extraction recoveries were found to range from 88.1% to 96.7%, with no significant matrix effects observed. The stability of the six analytes under various storage and processing conditions was also confirmed, with both precision and accuracy falling within the acceptable range of 15%. In conclusion, our UPLC-MS/MS assay provides a sensitive, accurate, and efficient approach for the quantification of PPIs and P-CABs in human plasma, contributing to the advancement of clinical pharmacokinetic studies and therapeutic drug monitoring.

Data Sharing Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Ethics Statement

The authors state that they have obtained appropriate institutional review board approval. This study was conducted in compliance with the local laws and regulations and accordance with the Declaration of Helsinki. All participants provided written informed consent prior to enrollment.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

All authors have reported that they have no known competing financial interests or personal relationships relevant to the contents of this paper to disclose.

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