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ORIGINAL RESEARCH

Simultaneous Determination of Venetoclax and Posaconazole in Human Plasma by UPLC-MS/MS: Application to Therapeutic Drug Monitoring in Acute Myeloid Leukemia Patients

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Background: Due to the high risk of invasive fungal disease (IFD) in acute myeloid leukemia (AML) patients, the antifungal drug posaconazole is often co-administered with venetoclax. As posaconazole is a potent inhibitor of CYP3A4, the standard dosing regimen may lead to the elevated plasma concentrations of venetoclax due to potential drug-drug interactions (DDIs). Therefore, therapeutic drug monitoring (TDM) is necessary to optimize the dosage.

Methods: This study developed and validated a rapid and sensitive ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method for the simultaneous quantification of venetoclax and posaconazole in human plasma. Plasma samples were pretreated using acetonitrile precipitation. The chromatographic separation was achieved using an Acquity BEH C18 column with gradient elution. The mobile phase consisted of 0.1% formic acid in water and acetonitrile, with a flow rate of 0.4 mL/min.

Results: The method demonstrated good sensitivity and linearity within the concentration ranges of 10–15,000 ng/mL for venetoclax and 10–10,000 ng/mL for posaconazole, respectively. Additionally, the method showed acceptable selectivity, intra-day precision, inter-day precision, accuracy, matrix effects (95.2% to 102.0% for venetoclax and 98.4% to 102.5% for posaconazole), extraction recovery (93.2% to 95.4% for venetoclax and 87.8% to 95.8% for posaconazole), and stability under various conditions. The trough concentrations of venetoclax were 9326.88 ± 12,169.05 ng/mL in patients treated with venetoclax alone, and 31,623.55 ± 28,453.67 ng/mL in patients treated with venetoclax in combination with posaconazole.

Conclusion: A rapid and simple method was established and successfully applied to the simultaneous determination of the concentrations of venetoclax and posaconazole in AML patients, providing a basis for TDM and clinical pharmacokinetic analysis of these drugs in AML patients.

Keywords: invasive fungal disease, acute myeloid leukemia, therapeutic drug monitoring, UPLC-MS/MS

Introduction

Acute myeloid leukemia (AML) is the most common acute leukemia in adults.[1] AML is a biologically heterogeneous disease originating from myeloid hematopoietic stem cells, characterized by the presence of a large number of immature myeloid cells in the bone marrow and peripheral blood.[2,3] Genetic mutations play a crucial role in the pathogenesis, progression, and prognosis of AML. Currently identified mutations in genes encoding epigenetic regulators, such as DNMT3A, ASXL1, TET2, IDH1, and IDH2, significantly impact patient prognosis, treatment response, and overall survival rates.[4–6] Chemotherapy based on hypomethylating agents (HMAs) or low-dose cytarabine (LDAC) is not very effective in elderly AML patients.[7–9] Therefore, elderly AML patients lack effective and well-tolerated treatment options, especially those who are not eligible for intensive chemotherapy.

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B cell lymphoma 2 (BCL-2) protects cells from death by inhibiting the mitochondrial-mediated apoptosis pathway.[10] Additionally, BCL-2 regulates the cell cycle by maintaining cells in the quiescent state (G0 phase), which is crucial for the self-renewal and survival of both stem cells and cancer cells.[11] Overexpression of BCL-2 is associated with the development and progression of various tumors by promoting tumor cell survival and proliferation through apoptosis inhibition. In certain cancer stem cells, such as those in AML, high BCL-2 expression may lead to chemotherapy resistance by maintaining mitochondrial function and energy metabolism. Inhibiting BCL-2 in these leukemia stem cells can reduce oxidative phosphorylation, leading to decreased energy production and cell death.[12,13] Due to its critical role in tumor cell survival, BCL-2 has become a potential target for cancer therapy.

As the first approved selective small-molecule BCL-2 inhibitor, venetoclax has been approved for the treatment of adult AML.[14–16] However, with the widespread use of immunosuppressants, high-dose chemotherapy drugs, and hematopoietic stem cell transplantation (HSCT), the number of fungal infection cases has been increasing yearly. Patients with hematological malignancies have a higher incidence of invasive fungal diseases (IFD), particularly common among those with AML.[17]

Antifungal prophylaxis can reduce the incidence and mortality of IFD. Posaconazole, a second-generation triazole antifungal agent, is commonly used to prevent invasive aspergillosis and candidiasis.[18,19] A randomized controlled trial showed that in patients undergoing chemotherapy for AML, posaconazole was more effective than fluconazole or itraconazole in preventing invasive fungal infections and improving overall survival.[20] In addition, another Phase 3 randomized controlled trial demonstrated that posaconazole is as effective as voriconazole in treating invasive aspergillosis, with a significantly lower incidence of treatment-related adverse events in patients receiving posaconazole.[21] Therefore, in the early stages of treatment, AML patients often receive a combination of venetoclax and posaconazole due to the high risk of neutropenia and the occurrence of IFD.

As we know, venetoclax is extensively metabolized by CYP3A4 in the human body, while posaconazole is a potent inhibitor of CYP3A4.[22,23] Therefore, when these two drugs are used together, the plasma concentration of venetoclax would increase. Therapeutic drug monitoring (TDM) helps to optimize the dosage to achieve the desired therapeutic effect and minimize toxicity.

Existing studies have demonstrated significant pharmacokinetic interactions between venetoclax and posaconazole in patients with AML. De Gregori et al found that posaconazole, as a potent CYP3A4 inhibitor, significantly increased the plasma concentration of venetoclax.[24] However, this study focused solely on pharmacokinetic parameters and did not address the practical application of TDM. Guo et al explored the effect of posaconazole on venetoclax plasma concentration and efficacy, emphasizing the importance of BCL-2 expression levels. However, they similarly lacked a specific method for the simultaneous quantification of both drugs.[25]

Currently, studies have employed liquid chromatography tandem mass spectrometry (LC-MS/MS) to determine the concentration of venetoclax.[26,27] Meanwhile, for the quantitative detection of posaconazole, most methods focus on the simultaneous determination of several azole drugs.[28–30] However, to date, no method has been reported for the simultaneous detection of venetoclax and posaconazole. Existing methods require longer analysis times and still have certain limitations in terms of sensitivity and sample preparation steps (Table 1).

Therefore, a rapid, sensitive, and reproducible ultra performance liquid chromatography tandem mass spectrometry (UPLC-MS/MS) method was developed for the simultaneous quantification of venetoclax and posaconazole in human plasma in this study. The validated method was further applied to the analysis of plasma samples from AML patients

Table 1 Comparison of the Analytical Characteristics of the Present Assay with Previously Published Methods for Venetoclax Quantification

References	Analytical Method	Sample Preparation	Injection Volume (μL)	Run Time (Min)	Calibration Range (ng/mL)
Present study	UPLC-MS/MS	Acetonitrile precipitation	1.5	2	10-15,000
Simona De Gregori et al, 2023[24]	HPLC-MS/MS	Methanol precipitation	5	9	39.06-5000
Anuradha Reddy et al, 2025[26]	LC-MS/MS	Acetonitrile precipitation	5	6	5-500
Niels Westra et al, 2025[27]	LC-MS/MS	NA	0.5	1.5	50–5000

receiving treatment with venetoclax alone and in combination with posaconazole. This study is of significant importance for evaluating the clinical safety of venetoclax and posaconazole in humans.

Materials and Methods

Reagents and Chemicals

Venetoclax (purity ≥ 98%) and fluconazole (purity ≥ 98%, used as the internal standard, IS) were obtained from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Posaconazole (purity > 98%) was obtained from Nanjing XiZe Biotechnology Co., Ltd. (Jiangsu, China). HPLC-grade solvents, including methanol and acetonitrile, were supplied by Merck KGaA (Darmstadt, Germany). Ultra-pure water was generated by a Milli-Q water purification system manufactured by Millipore (Bedford, USA).

Chromatographic and Mass Spectrometric Conditions

The sample analysis was performed by UPLC-MS/MS consisting of a Waters Xevo TQ-S triple quadrupole tandem mass spectrometer (Milford, MA, USA) and a Waters Acquity UPLC I-Class system (Milford, MA, USA). The chromatographic analysis was achieved on an Acquity BEH C18 chromatography column (2.1 mm × 50 mm, 1.7 μm) at a flow rate of 0.40 mL/min with the column temperature set at 40 °C to separate the analytes in plasma. The mobile phase was composed of ultra-pure water containing 0.1% formic acid (solution A) and acetonitrile (solution B). The temperature of the autosampler was set at 10 °C, and the total run time of the method was 2.0 min. The gradient elution procedure for the separation of the analytes is detailed in Table 2. Figure 1 illustrates the chemical structures and mass spectrograms of venetoclax, posaconazole, and IS. The ion transitions monitored were *m/z* 868.80→636.40 for venetoclax, *m/z* 701.30→683.40 for posaconazole, and *m/z* 307.13→238.14 for IS, respectively (Table 3). Data were collected and analyzed using Masslynx 4.1 software (Milford, MA, USA).

Working Solutions, Calibrations and Quality Controls (QC)

Stock solutions of venetoclax and posaconazole were respectively prepared in methanol at a concentration of 1.00 mg/mL and stored at −80 °C. To obtain a series of working solutions, the stock solutions were serially diluted with methanol to concentrations of 100–150,000 ng/mL for venetoclax and 100–100,000 ng/mL for posaconazole. Following this, 10 μL venetoclax and 10 μL posaconazole were added to new EP tubes containing 80 μL blank plasma to prepare the 8-point calibration curves. The final concentrations of the calibration standards were 10, 20, 100, 500, 1000, 5000, 10,000, and 15,000 ng/mL for venetoclax, and 10, 20, 50, 100, 500, 1000, 5000, and 10,000 ng/mL for posaconazole in human plasma, respectively. The QC samples were prepared at four different concentration levels: high QC (HQC, 80% of the highest point of the calibration curve range), medium QC (MQC, middle of the calibration curve range), low QC (LQC, within 3 times the lower limit of quantification (LLOQ)), and LLOQ. The final concentrations of the LLOQ, LQC, MQC, and HQC samples were 10, 20, 6000, and 12,000 ng/mL for venetoclax, and 10, 20, 4000, and 8000 ng/mL for posaconazole in plasma, respectively. A stock solution of IS at a concentration of 1.00 mg/mL was prepared and subsequently diluted with methanol to a concentration of 1.00 μg/mL to be used as the IS working solution.

Table 2 The Process of Linear Gradient Elution

Time (Min)	A (%)	B (%)
0.0	90	10
0.5	90	10
1.0	10	90
1.4	10	90
1.5	90	10
2.0	90	10

Note: 0.1% formic acid (solution A) and acetonitrile (solution B).

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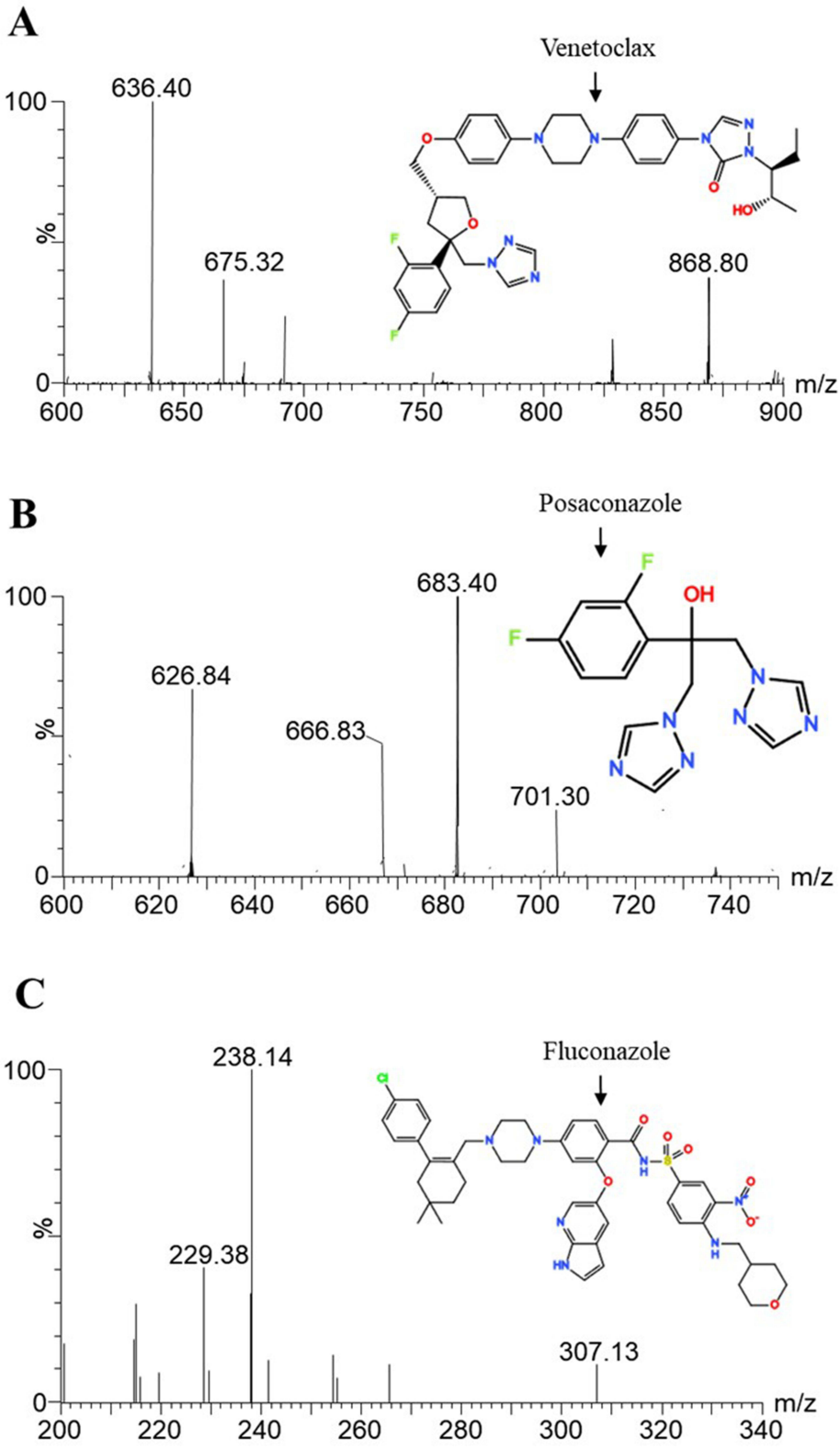

Figure 1 Mass spectrometry of venetoclax (**A**), posaconazole (**B**) and IS (**C**).

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Table 3 MRM Transitions and Compound-Specific MS Settings

Analytes	Precursor Ion (*m/z*)	Product Ion (*m/z*)	Cone (V)	Collision Energy (eV)	Capillary Voltage (kV)	Desolvation Temperature (°C)	Desolvation Gas Flow (L/h)
Venetoclax	868.80	636.40	6	16	2.0	600	1000
Posaconazole	701.30	683.40	80	30			
IS	307.13	238.14	50	22			

Abbreviations: MRM, Multiple reaction monitoring; MS, mass spectrometry.

Plasma Sample Preparation

Sample pretreatment was performed by mixing 100 μL plasma samples with 10 μL IS working solution in a 1.5 mL centrifuge tube. To precipitate the proteins, 300 μL acetonitrile was added, and the mixture was vortexed and centrifuged at 13,000 rpm for 10 min at 4 °C. Ultimately, 100 μL of the supernatant was transferred to an autosampler vial, and a volume of 1.5 μL was injected into the column for analysis.

Method Validation

The method was validated following the guidelines of the International Council for Harmonisation (ICH) Q2 and ICH M10 Bioanalytical Method Validation guidelines.[31] Key parameters such as selectivity, linearity, precision, accuracy, LLOQ, matrix effect, extraction recovery, and stability were assessed to ensure the reliability of this method.

Selectivity and Sensitivity

To determine the selectivity of the method, five different batches of blank human plasma were processed and tested according to the prescribed pretreatment method. It was ensured that no significant response attributable to interfering components was observed at the retention times of the analytes and IS in the blank plasma samples. The peak area of any interference peaks should not exceed 20% of the analyte response at the LLOQ and 5% of the IS response in the LLOQ sample for each matrix.

Calibration Curves and LLOQ

For the purpose of TDM, the range of calibration curves was selected based on the expected clinical concentrations. The calibration curves were prepared by mixing blank plasma with working standard solutions. A weighted least squares method with a weighting factor of $1/x^2$ was used, plotting the peak area ratio of the analyte to the IS (y) against the concentration of the analyte in the calibration samples (x) to assess the linearity. LLOQ was referred to the minimum concentration on the calibration curve, which could be accurately quantified. Precision and accuracy values of LLOQ were deemed acceptable if they fell within ±20%.

Precision and Accuracy

The intra-day and inter-day accuracy and precision were evaluated by analyzing QC samples at low, medium, and high concentrations, with five replicates for each concentration. Intra-day assessments were conducted on the same day, while inter-day assessments were performed on three different days. Accuracy was expressed as the relative error (RE%) between the measured concentration and the nominal concentration, and precision was expressed as the relative standard deviation (RSD%). The acceptance criteria required that the RE% for all QC levels, except for the LLOQ, should be within ± 15% of the nominal value. The precision was confirmed by ensuring that the RSD% for QC samples did not exceed 15%, and for LLOQ samples did not exceed 20%.

Extraction Recovery and Matrix Effect

The extraction recovery and matrix effect were assessed by comparing the peak areas of the analytes in different QC samples. Specifically, the extraction recovery was determined by calculating the ratio of the analytes' peak areas in plasma samples spiked before and after extraction, and the matrix effect was evaluated by comparing the peak areas of samples spiked post-extraction with those in neat solutions. The values of extraction recovery should fall within 85–100%, and the RE% of the matrix effect should be within ± 15%.

Stability

The stability of all the analytes at low, medium, and high concentration levels in plasma was assessed under various conditions. To evaluate the short-term stability, samples were kept at room temperature for 3 h, and freeze-thaw stability was determined by subjecting the samples to three cycles of freezing at −80°C and thawing at room temperature. Additionally, autosampler stability was evaluated by analyzing samples stored in an auto-sampler at 10 °C for 4 h. For long-term stability, QC samples were stored at −80 °C for three weeks. Accuracy across all stability conditions was required to be within ± 15%.

Application to Clinical Samples

Given the risk of DDI between posaconazole and venetoclax in AML patients, TDM is crucial. This study aimed to monitor the concentrations of both medications to ensure optimal dosing and minimize toxicity. This method was used to determine the concentrations of venetoclax and posaconazole in 63 plasma samples from 31 adult patients diagnosed with AML. Blood samples were collected and plasma concentrations were measured under steady-state conditions after the patients had been on regular medication for at least 3 days. Immediately after collection, the samples were centrifuged at 8870 × g (rotor radius: 12.4 cm) for 2 min to obtain the plasma. The plasma specimens were then stored at −80°C. Ethical approval was obtained from the Ethics Committee of the First Affiliated Hospital of Wenzhou Medical University (Zhejiang, China) (Acceptance Number: KY2024-R282), and the research was conducted in accordance with the guidelines of the Declaration of Helsinki. Besides, the informed consent was waived for participants in this study. Statistical analysis was performed using SPSS 23.0 software. Normality was assessed using the Shapiro–Wilk test, and the Wilcoxon signed-rank test was applied to compare the significant differences between samples.

Results

UPLC-MS/MS Method Development

The optimization of chromatographic conditions can lead to a deep increase in the sensitivity and a reduction in costly consumption. The conditions of UPLC-MS/MS chromatography were optimized for the quantitative detection of venetoclax and posaconazole in human plasma in the present analysis. The processed plasma samples were chromatographically separated by gradient elution using a mixture of water containing 0.1% formic acid and acetonitrile. The chromatographic column chosen was a UPLC BEH C18 reversed-phase column (2.1 mm × 50 mm, 1.7 μm). Compared to other methods, the present analysis had shorter retention time (1.34 min for venetoclax, and 1.39 min for posaconazole, respectively) and lower LLOQ of 10 ng/mL for both drugs, compared to 20 or 25 ng/mL reported in the literature.[32,33] Moreover, the analysis time for each sample was only 2.0 min in the current research, which reduced the consumption of the mobile phase. It was also more practical and economical to use fluconazole as an IS for the present analysis, compared to the IS ([$^2H_7$]-venetoclax) used in other reports.[32,34]

Method Validation

Selectivity

Typical chromatograms of the blank plasma samples, blank plasma spiked with venetoclax and posaconazole at LLOQ and IS, and real human sample were shown in Figure 2. As illustrated, the method demonstrated good selectivity, as evidenced by the lack of endogenous interference at the retention times of venetoclax (1.34 min) and posaconazole (1.39 min).

Calibration Curves and LLOQ

Five batches of blank human plasma were used to confirm the selectivity, with the responses of interfering component being less than 20% of the LLOQ for the analytes and 5% for the IS. Calibration curves were established using weighted linear regression with $1/x^2$ weighting factors. The corresponding calibration curves for venetoclax and posaconazole were: y = 0.0023474*x - 0.00180204 (correlation coefficients, r^2 = 0.998) and y = 0.00457609*x + 0.0245412 (r^2 = 0.997), respectively, which showed good linearity across their respective concentration ranges. The LLOQ for venetoclax and posaconazole in plasma were both 10 ng/mL, with the linearity supported by r^2 exceeding 0.99.

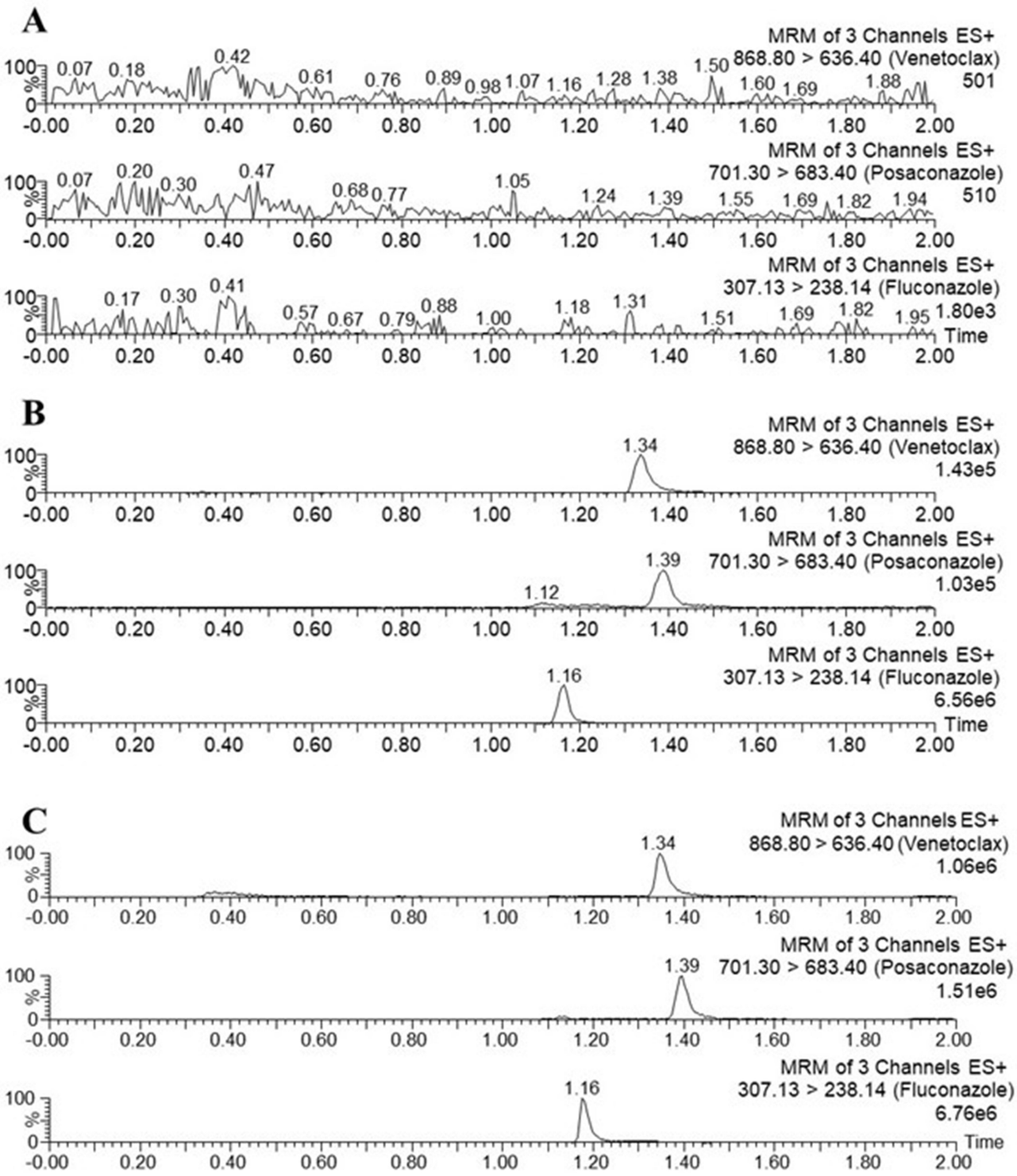

Figure 2 Representative MRM chromatograms of venetoclax, posaconazole, and IS in human plasma. (**A**) Blank plasma sample; (**B**) Plasma spiked with venetoclax and posaconazole at LLOQ and IS; (**C**) Plasma sample obtained from a patient at 7.5 h after oral administration of 100 mg venetoclax and 300 mg posaconazole. The unlabeled minor peaks in the figure represent minor fragments.

Precision and Accuracy

The intra-day and inter-day precision and accuracy for venetoclax and posaconazole were evaluated using QC samples at various concentrations. The results, as shown in Table 4, demonstrated that both the RSD% and RE% for intra-day precision and accuracy fell inside the scope of ± 15%, and under ± 20% for LLOQ. For inter-day analysis, the RSD% and

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Table 4 Precision and Accuracy of Venetoclax and Posaconazole in Human Plasma (n = 5)

Analytes	Concentration (ng/mL)	Intra-Day		Inter-Day	
		Precision (RSD %)	Accuracy (%)	Precision (RSD %)	Accuracy (%)
Venetoclax	10	10.1	−1.8	8.3	−2.1
	20	2.0	−8.5	4.9	−3.4
	6000	2.7	−5.2	4.1	−0.7
	12,000	1.2	−6.4	5.4	0.2
Posaconazole	10	5.5	−9.5	5.6	−8.2
	20	4.0	−2.3	3.0	−1.7
	4000	2.1	2.0	2.6	3.3
	8000	1.2	−2.3	2.5	0.4

RE% were also within the acceptable limits, as outlined by ICH guidelines. Specifically, the intra-day and inter-day precision (RSD%) of venetoclax ranged from 1.2% to 10.1% and 4.1% to 8.3%, with accuracy values ranging from −8.5% to −1.8% and −3.4% to 0.2%, respectively. Additionally, the intra-day and inter-day precision (RSD%) of posaconazole ranged from 1.2% to 5.5% and 2.5% to 5.6%, with accuracy values ranging from −9.5% to 2.0% and −8.2% to 3.3%, respectively. The precision and accuracy values for each concentration level confirmed that all measurements met the required standards, ensuring the accuracy and reliability of the analytical method.

Matrix Effect and Recovery

The results of extraction recovery and matrix effect for venetoclax and posaconazole are summarized in Table 5. The data showed that the extraction recovery of venetoclax and posaconazole for plasma samples ranged from 93.2% to 95.4%, and 87.8% to 95.8%, respectively, indicating acceptable recovery rates. The matrix effect for venetoclax was between 95.2% and 102.0%, and for posaconazole between 98.4% and 102.5%, suggesting minimal interference from endogenous substances. The overall findings confirmed that the extraction method was effective, and the matrix effect did not significantly affect the signals of the analytes.

Stability

The stability was evaluated under various conditions, including short-term exposure at room temperature, storage in an autosampler at 10 °C for 4 h, multiple freeze-thaw cycles, and long-term storage. As detailed in Table 6, the stability of venetoclax and posaconazole was confirmed with RSD% and RE% values remaining within the acceptable range of ± 15%. Both venetoclax and posaconazole showed stable concentrations across all tested conditions, with deviations within the acceptable limits of ICH, demonstrating the stability of the analytical method was well.

Table 5 Extraction Recovery and Matrix Effect of Venetoclax and Posaconazole in Human Plasma (n = 5)

Analytes	Concentration (ng/mL)	Matrix Effect (%)		Recovery (%)	
		Mean ± SD (%)	RSD (%)	Mean ± SD (%)	RSD (%)
Venetoclax	20	102.0 ± 8.1	7.9	95.4 ± 7.6	7.9
	6000	95.2 ± 3.9	4.1	94.4 ± 2.7	2.9
	12,000	95.3 ± 2.1	2.2	93.2 ± 1.7	1.9
Posaconazole	20	100.5 ± 7.7	7.7	95.7 ± 8.6	9.0
	4000	98.4 ± 4.6	4.7	95.8 ± 2.9	3.0
	8000	102.5 ± 5.4	5.3	87.8 ± 4.4	5.0

Table 6 Stability of Venetoclax and Posaconazole Under Different Storage Conditions (n = 5)

Analytes	Concentration (ng/mL)	Room Temperature 3 h		Autosampler 10°C, 4 h		-80°C, 3 Weeks		Three Freeze-Thaw Cycles	
		RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)	RSD (%)	RE (%)
Venetoclax	20	5.0	11.0	4.6	−5.8	6.2	9.9	10.7	−4.9
	6000	2.4	1.6	5.1	−8.4	2.1	−10.0	4.0	−7.0
	12,000	1.8	−0.1	2.2	−7.8	2.6	−6.2	0.7	−11.1
Posaconazole	20	8.4	−9.4	6.1	−4.3	6.3	0.4	5.4	−8.7
	4000	3.8	−2.3	4.1	−1.2	2.9	−12.1	4.1	−10.7
	8000	2.1	−7.6	2.0	−4.1	4.9	−12.9	2.6	−1.0

Method Application

The method was used to measure the plasma concentrations of venetoclax and posaconazole in clinical patients. The study included a total of 31 adult inpatients taking venetoclax (100–400 mg qd) alone or in combination with posaconazole (venetoclax 100 mg qd; posaconazole 300 mg qd), and detailed information was shown in Table 7. All patients were diagnosed with AML, with a median age of 63 years (range: 26–80 years). The group was predominantly male, with 20 males (64.5%) and 11 females (35.5%). The average body weight of the patients was 62.6 kg (range: 36.5–84.0 kg). Notably, about 13% of patients had secondary AML, often associated with previous chemotherapy or radiation treatment. As shown in the results of Figure 3, the trough concentration of venetoclax was 9326.88 ± 12,169.05 ng/mL in patients treated with venetoclax alone, and 31,623.55 ± 28,453.67 ng/mL in patients treated with venetoclax in combination with posaconazole.

Table 7 Detailed Patient Information

	Overall (n = 31)
Age(years)	
Median(range)	63 (26–80)
Sex, n (%)	
Male	20 (64.5)
Female	11 (35.5)
Body weight (kg)	
No information	1
Mean (SD)	62.6 (12.7)
Median (range)	61.8 (36.5–84.0)
Hepatic function, n (%)	
Normal	14 (45.2)
Mild impairment	11 (35.5)
Moderate impairment	6 (19.3)
Severe impairment	0 (0)
Renal function, n (%)	
Normal	21 (67.7)
Mild impairment	6 (19.3)
Moderate impairment	3 (9.7)
Severe impairment	1 (3.2)
Neutropenia, n (%)	
No information	6
Normal	1 (3.2)
Grade 1	0
Grade 2	3 (9.7)
Grade 3	3 (9.7)
Grade 4	18 (58.1)

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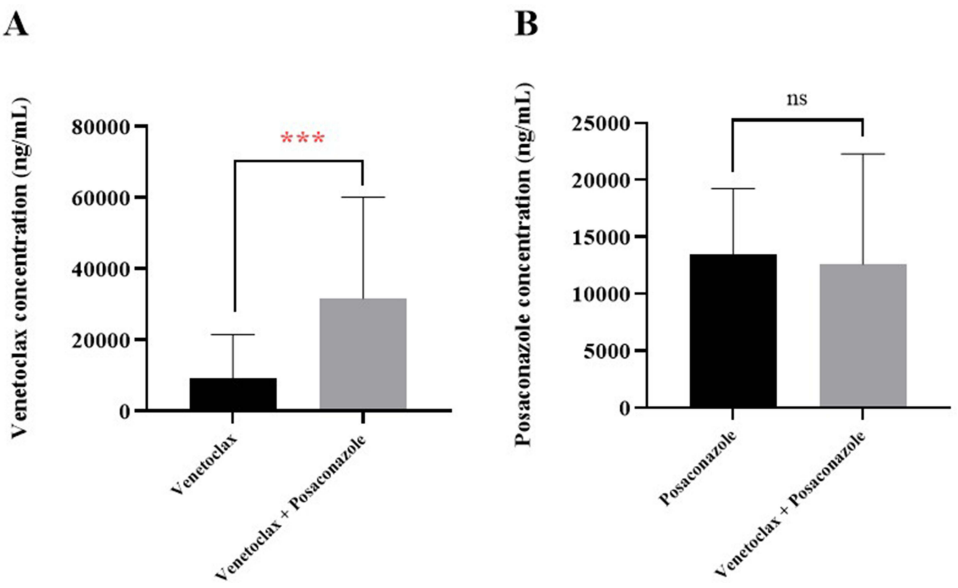

Figure 3 The trough concentrations of venetoclax (**A**) and posaconazole (**B**) were determined in 63 plasma samples. Results are presented as mean ± SD. ***$P < 0.001$, while ns indicates non-significance ($P > 0.05$), compared with venetoclax.

Discussion

A previous literature report indicated that posaconazole was a strong CYP3A4 inhibitor and contributed to increase the plasma concentration of venetoclax.[24] Therefore, when it was used in combination with moderate and strong CYP3A inhibitors, it was recommended to reduce the dose of venetoclax by at least 50% and 75%, respectively.[35] Agarwal et al studied the modifications of steady-state venetoclax plasma concentrations after introduction of oral posaconazole in patients with AML. They found a 7.1- and 8.8-fold increase in venetoclax dose-normalized C_{max} and AUC_{0-24} for the 100 mg and 50 mg daily dose, respectively, compared with the values observed when venetoclax was administered alone at the standard daily dose of 400 mg.[36] Posaconazole, a potent CYP3A inhibitor, significantly increased the trough concentration of venetoclax by approximately 3.11-fold when used in combination compared to venetoclax alone. The observed differences in the interaction effects between our study and that of Agarwal et al can be attributed to several key factors. Firstly, in pharmacokinetics and drug metabolism, trough concentrations and peak concentrations may be influenced differently. Secondly, the baseline characteristics of our study population, including age, hepatic and renal function, and concomitant medications, varied from those in Agarwal's study, potentially influencing drug exposure levels. Thirdly, blood sampling time points and the sensitivity of the detection methods also contributed to the discrepancies. Additionally, it was demonstrated that when posaconazole was co-administered with venetoclax, the majority of patients achieved adequate posaconazole exposure. Consequently, posaconazole was found to be safe, and the rates of invasive fungal infection were lower than those in previously reported cohorts who did not receive prophylaxis.[37,38]

Liver and kidney function tests were performed to assess the safety and suitability of drug administration. Liver function was evaluated by measuring aspartate aminotransferase (AST) and bilirubin levels.[39] Liver function was classed: normal (bilirubin ≤ 1.0 mg/dL and AST ≤ 40 IU/L), mild (bilirubin ≤ 1.0 mg/dL and AST > 40 IU/L, or bilirubin > 1.0 mg/dL but ≤ 1.5 mg/dL), moderate (bilirubin > 1.5 mg/dL but ≤ 3.0 mg/dL), and severe (bilirubin > 3.0 mg/dL) impairment. Renal function was assessed using estimated glomerular filtration rate (eGFR).[40] Kidney function was classed: normal (≥ 90 mL/min), mild (≥60 but <90 mL/min), moderate (≥30 but <60 mL/min), and severe (≥15 but <30 mL/min). As shown in Table 7, a portion of patients (43%) exhibited mild to moderate liver dysfunction, with only one patient showing severe liver dysfunction. Additionally, approximately 36% of patients had mild renal impairment, while 19% had moderate renal impairment, requiring close monitoring of drug clearance and dose adjustments.

Neutropenia was the most frequently observed hematologic toxicity. According to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 by the National Cancer Institute, neutropenia is classified into four grades: Grade 1, Absolute Neutrophil Count (ANC) (≥ 1.5 but < lower limit of normal) × 10^9/L; Grade 2, ANC (≥ 1.0 but < 1.5) × 10^9/L; Grade 3, ANC (≥ 0.5 but < 1.0) × 10^9/L; Grade 4, ANC (< 0.5) × 10^9/L.[41] Adverse event monitoring revealed significant occurrences of neutropenia among patients treated with the combination of venetoclax and posaconazole.

The addition of posaconazole, a strong CYP3A4 inhibitor, led to increased venetoclax trough concentrations, which significantly aggravated the severity and frequency of neutropenia compared to venetoclax monotherapy. In the combination therapy group, 87.5% of patients developed Grade 4 neutropenia, compared to 50% in the venetoclax monotherapy group, which highlighted a strong correlation between increased drug exposure and severe neutropenia. To alleviate these adverse effects, it is crucial to adjust the dose of venetoclax and continuously monitor drug levels in patients.

To conclude, posaconazole inhibited venetoclax metabolism and significantly increased venetoclax trough concentrations, leading to increased systemic exposure and toxicity. A dose reduction of venetoclax and TDM are required when these two drugs are used in combination in the clinic. However, at present, there is uncertainty about how to translate the results of TDM into actual clinical decisions, so more research is needed to guide clinical practice. Future research should focus on evaluating the specific impact of TDM-guided dose adjustments on patient efficacy and toxicity to provide clearer guidance for clinical application. In addition, conducting multicenter studies will help assess the applicability of TDM in different healthcare settings.

Conclusion

The purpose of this study was to establish a sensitive and rapid UPLC-MS/MS method for the quantification of venetoclax and posaconazole in human plasma samples. The method was validated using human plasma and involved simple protein precipitation with acetonitrile, with a total run time of 2.0 min. This is the first reported UPLC-MS/MS method for the simultaneous determination of venetoclax and posaconazole concentrations in human plasma. The method was cost-effective, simple in sample preparation, and features a short run. These results indicated that when venetoclax and posaconazole were used together, posaconazole significantly increased the trough concentration of venetoclax. This necessitates the implementation of TDM to ensure that the concentrations of venetoclax remain within an acceptable range. In summary, this method was versatile, quick and easy to operate. Furthermore, the validated method was suitable for TDM in clinical settings.

Although this study presents an innovative method for the simultaneous quantification of venetoclax and posaconazole and demonstrates its application in AML patients, we must acknowledge some limitations inherent in our research. Firstly, our sample size is limited, which increases the possibility of chance findings and may affect the stability and reliability of the results. Secondly, the data in this study are derived from a single center, which may limit the generalizability of the findings. Furthermore, since venetoclax is metabolized by CYP3A4, this study did not take into account the inter-individual differences caused by genetic polymorphisms of CYP3A4. These differences could potentially influence the pharmacokinetics of the drug. In the future, with further refinement of this method, it could be used for clinical PK/PD analysis to evaluate the relationship between plasma drug concentrations, clinical efficacy, and the occurrence of adverse events. This will lead to the optimization of venetoclax dosing regimens for individual patients.

The efficacy of the drugs in AML patients can be more accurately assessed through this method, which allows for timely adjustment of the treatment regimen. This helps to increase the success rate of AML treatment, reduce the risk of treatment failure, and thereby ensure the effectiveness and safety of the combination therapy.

Disclosure

The authors report no conflicts of interest in this work.

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