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Simultaneous determination of concentration of sulfamethoxazole and trimethoprim in human plasma by dual-quaternary HPLC and its clinical application

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ABSTRACT

Sulfamethoxazole compound, a combination of sulfamethoxazole and trimethoprim, is commonly prescribed to organ transplant recipients for infections caused by *Nocardia*, *Burkholderia cepacia*, and *Pneumocystis jirovecii*. The intricate pharmacokinetics and pharmacodynamics of this compound pose challenges in achieving optimal therapeutic drug levels. Therefore, monitoring the plasma concentration of this compound drug is necessary. To address this need, we have developed a rapid and straightforward dual-quaternary two-dimensional high-performance liquid chromatography (2D-HPLC) method for the simultaneous quantification of sulfamethoxazole and trimethoprim. This technique involves a one-step protein precipitation procedure, with the initial stage utilizing a solid-phase extraction column followed by an analytical column for the subsequent stage. Detection is achieved using a dual-quaternary 2D-HPLC system. Calibration curves for both sulfamethoxazole and trimethoprim were constructed, and the method underwent thorough validation in accordance with the Chinese Pharmacopoeia (Ch.P) standards, demonstrating remarkable accuracy and precision. This technique has been effectively employed for routine therapeutic drug monitoring (TDM) in organ transplant patients and other individuals requiring medical intervention.

Introduction

In China, recent advancements in organ transplantation have led to a growing number of transplant recipients who require long-term immunosuppressive therapy. Post-transplant infections caused by opportunistic pathogens, such as *Pneumocystis jirovecii*, have become a common and potentially life-threatening issue, with their incidence on the rise.[1] Effective management and prevention of these infections can help alleviate financial burdens, mitigate organ dysfunction, and decrease mortality risks. The management of *Pneumocystis jirovecii* infections typically involves the administration of high-dose oral compound sulfamethoxazole, which can lead to fluctuations in blood drug levels and a heightened risk of adverse reactions. Therefore, monitoring the therapeutic drug levels of compound sulfamethoxazole can provide substantial advantages to patients.

Existing techniques for evaluating the plasma concentration of compound sulfamethoxazole in human plasma include liquid chromatography-tandem mass spectrometry (LC-MS/MS) and high-performance liquid chromatography (HPLC). Although LC-MS/MS offers high sensitivity, its high cost for both instrumentation and analysis limits its broader application. In contrast, HPLC requires intricate sample preparation, specific chromatographic column criteria for plasma sample analysis, and is susceptible to interference from various blood components, which can result in false peaks and other complications.

The aim of this study was to develop a simple and efficient method using a dual-quaternary two-dimensional liquid chromatography system for analyzing plasma levels of sulfamethoxazole and trimethoprim in organ transplant recipients. These antibiotics are often prescribed to treat severe infections caused by *Pneumocystis jirovecii* in individuals who have undergone organ transplantation. Due to the immunosuppressive medications required to prevent organ rejection, transplant recipients are particularly susceptible to infections. Monitoring sulfamethoxazole and trimethoprim plasma concentrations

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is crucial for ensuring effective treatment and minimizing toxicity. The dual-quaternary liquid chromatography system method developed in this study offers a reliable and accurate way for the simultaneous quantification of sulfamethoxazole and trimethoprim in organ transplant recipients. This method could be of significant value in managing infections and optimizing therapeutic outcomes for organ transplant recipients.

Materials and methods

Experimental chemicals and reagents

Sulfamethoxazole and trimethoprim were purchased from Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China. High-quality acetonitrile (ACN) and methanol (MeOH) for HPLC were purchased from Fisher Scientific, Fairlawn, NJ, USA. Ammonium acetate, Type I protease inhibitor, and IIB protease inhibitor were supplied by Suzhou Innovation General Chromatography Instruments Co., Ltd. For method validation, a drug-free biological matrix comprising normal human plasma, serum, and whole blood was obtained from the Second Affiliated Hospital of Guangxi Medical University.

Instrumentation and LC conditions

A dual-quaternary two-dimensional liquid chromatography system (GI-3000XY, Suzhou Innovation General Chromatography Instruments Co.) was used to separate compound sulfamethoxazole. The GI-3000 liquid chromatograph is a fully intelligent quadrupole liquid chromatography system that features low-pressure gradient and online solid-phase extraction capabilities. The chromatograph conducts online solid-phase extraction initially through the first column, with individual target ingredients being temporarily stored using a computer-controlled, time-based six-port valve. Detailed settings for the runtime program are provided in Table 1.

Stock solutions, calibration standards, and quality control (QC) samples

Separate stock solutions were prepared by dissolving each powdered antimicrobial in its proper solvent: sulfamethoxazole and trimethoprim were dissolved in MeOH. Stock solution concentrations were prepared to 0.5 mg/mL. All prepared stock solutions were stored in 1 mL aliquots at − 20 ℃. Working solution (206.30 μg/mL for sulfamethoxazole and 20.63 μg/mL for trimethoprim) was then prepared by diluting the stock solutions in MeOH. Calibration standards and QC samples for sulfamethoxazole and trimethoprim were prepared by spiking the appropriate working solutions into blank plasma. The exact concentrations of the calibration standards and QC samples are listed in Table 2.

Sample processing

The sample was first centrifuged at 4000 rpm for 5 min, and 400 μL of the supernatant was collected. This supernatant was then mixed with 600 μL of Type I protease inhibitor to remove proteins. After vortexing for 1 min, the mixture was centrifuged at 10,000 rpm for 10 min, and approximately 800 μL of the supernatant was transferred to the sample injection bottle. Finally, 20 μL of the processed sample was injected into the 2D-HPLC system for quantitative analysis using an external standard.

Method validation

Validation procedures were carried out according to Ch.P guidelines for bioanalytical method validation,[2] covering parameters such as selectivity, lower limit of quantification (LLOQ), calibration curve, carry-over, accuracy, precision, dilution integrity, stability.

(1) *Selectivity*

The selectivity of the method was assessed through the analysis of sulfamethoxazole and trimethoprim in blank sample sources. A comparison was made between the chromatograms of the blank samples and the standards set for the lower limit of quantification (LLOQ). Chromatograms of the blank samples were compared with those of the standards at the LLOQ. The response from interfering substances should typically not exceed 20 % of the LLOQ for the compound being analyzed.

(2) *LLOQ, calibration curve and carry-over*

The LLOQ is considered as the lowest calibration standard. The analyte signal for the LLOQ sample should be at least 5 times higher than that of the blank sample. A linear regression model was used to generate the calibration curve, and the linear correlation coefficient was applied to assess the fit. The daily calibration curve for sulfamethoxazole ranged from 1.88 to 206.30 μg/mL, and for trimethoprim, it ranged from 0.19 to 20.63 μg/mL, with a total of six calibrators included in the calibration curve construction. The back-calculated concentrations of the calibration standards should fall within ± 15 % of the nominal value, except for the LLOQ, for which the deviation should be within ± 20 %. Carry-over was assessed by injecting blank samples after the upper limit of quantification (ULOQ) to ensure that it did not influence precision or accuracy. Carry-over should not exceed 20 % of the LLOQ and 5 % for the internal standard.

(3) *Within-run and between-run accuracy and precision*

Accuracy and precision were determined by analyzing the LLOQ and three QC levels at low, medium, and high concentrations over three

Table 1
GI-3000 liquid chromatograph system running time program.

Drug	Combination of sulfamethoxazole and trimethoprim		
Valve switching	1→2	6→1	1→2
The operational principle of the instrument	Sample injection and separation of target components in the first dimension extraction column, as well as buffer storage	Reverse elution, transferring the target components from the extraction column to the second dimension analysis column	Separation and detection of target components in the second dimension chromatography
Time program configuration/min	0.0–1.0	1.0–2.0	2.0–14

Note: The chromatographic conditions employed LC1 (CAPCELLPAK MFPh-1S-5, 4.0 mm × 20 mm, 5 μm) with a mobile phase of methanol-water, and LC2 (SUNNIEST C18, 4.6 mm × 100 mm, 5 μm) with a mobile phase consisting of methanol and 0.05 mol/L ammonium acetate in water (68:32). The flow rate was set at 0.8 mL/min, and the column temperature maintained at 30 ˚C. UV detection was performed at 240 nm, and the elution process was carried out isocratically with an injection volume of 20 μL.

Table 2
Concentrations (μg/mL) of calibration standards and QC samples.

Drug	Calibration curve						QC			
	1	2	3	4	5	6	LLOQ	Low	Medium	High
Sulfamethoxazole	1.88	18.75	37.50	62.50	125.00	206.30	1.88	50.00	125	200.00
Trimethoprim	0.19	1.88	3.75	6.25	12.50	20.63	0.19	5.00	12.50	20.0

days. For within-run accuracy (n = 5) and between-run accuracy (n = 15), the mean concentration should be within ± 15 % of the nominal values for the QCs, except for the LLOQ, where it should be within ± 20 % of the nominal value. For within-run precision (n = 5) and between-run precision (n = 15), the coefficient of variation (CV) should not exceed 15 % for the QCs, except for the LLOQ, where it should not exceed 20 %.

(4) *Dilution integrity*

Dilution integrity was assessed by performing five replicate 4x and 8x dilutions above the ULOQ of a sample spiked with 225 μg/mL sulfamethoxazole and 22.50 μg/mL trimethoprim. Accuracy and precision are expected to be within ± 15 %.

(5) *Stability*

For sulfamethoxazole and trimethoprim, extensive stability studies were performed under different conditions using low, medium, and high QC samples (n = 3). To assess short-term stability, untreated QCs were stored at room temperature (RT) for 4 h and at 4 °C for 72 h. Post-extraction stability (autosampler stability) was evaluated by storing 5 samples of each QC level in the autosampler at 5 °C for 6 h. Freeze-thaw stability of plasma samples was evaluated after three freeze-thaw cycles (from − 20 °C to room temperature), with QC samples being frozen for at least 12 hours between cycles. Additionally, long-term storage stability was evaluated at − 20 °C for 4 weeks. For all experiments, stability was considered acceptable if the average concentration at each level is within ± 15 % of the nominal concentration. The stability of stock and working solutions was also tested at − 20 °C for 3 weeks.

Results and discussion

Method development

The GI-3000 liquid chromatograph is a fully intelligent quadrupole liquid chromatography system that features low-pressure gradient and online solid-phase extraction capabilities. The chromatograph conducts online solid-phase extraction initially through the first column, with individual target ingredients being temporarily stored using a computer-controlled, time-based six-port valve. An automated scheme for the concentration, collection, transfer, and liquid chromatography analysis of sulfamethoxazole was established based on preliminary experiments. The injection time ranges from 0.0 to 1.0 min, during which the target component is extracted and temporarily stored in the solid phase extraction column for one-dimensional extraction. Subsequently, around 1.0–2.0 min, the analytes of interest are transferred to a 2D analytical column. From 2.0 to 14.0 min, the target components are separated in the analytical column for two-dimensional chromatography.

Currently, the primary analytical techniques used to measure the plasma concentration of compound sulfamethoxazole are HPLC and LC-MS/MS.[3,4] HPLC requires complex sample preparation and precise mobile phase composition, leading to longer analysis times. In contrast, LC-MS/MS offers faster analysis and higher precision than HPLC but comes with higher instrumental costs. The use of solid-phase extraction as a preprocessing technique offers several advantages for transplant patients who often require multiple medications: 1. enhanced separation capability: 2D-LC employs two different separation mechanisms, which are beneficial for handling complex biological samples. This capability helps address complex matrix interferences, thereby improving the accuracy and sensitivity of the analysis; 2. improved sample purity: 2D-LC separates target compounds and interferents in two separation dimensions, which effectively enhances sample purity and reduces background interferences. This is particularly valuable for transplant patients, who have a higher likelihood to experience complex drug interactions and interferences.

The liquid chromatography system used in this study differs from traditional HPLC and LC-MS setups by utilizing both extraction and analytical columns with distinct separation methods. Analytes are temporarily stored using computer-controlled valves, allowing for simultaneous one-dimensional and two-dimensional chromatographic separations. This approach reduces analysis time and improves methodological precision. To address potential contaminants in blood samples, the sample preparation process in this study is streamlined, yet effectively removes interfering substances from the plasma. This optimization ensures high methodological recovery and consistent reproducibility, particularly when analyzing large sample sets.

Method validation

(1) *Selectivity*

No interferences were observed in spiked (Fig. 1A), blank (Fig. 1B), or patient samples (Fig. 1C), within the retention time window of each compound.

(2) *LLOQ, calibration curve, and carry-over*

The LLOQ for sulfamethoxazole and trimethoprim were 1.88 μg/mL and 0.19 μg/mL, respectively. The signal-to-noise (S/N) ratios at the quantification limits for both sulfamethoxazole and trimethoprim were greater than 10. The average regression coefficient (r) of all standard curves in this study was exceeded 0.999. The obtained linear equations were Y (sulfamethoxazole) = 18.826X - 8.5968 (R^2 = 0.9999), and Y (trimethoprim) = 25.558X - 1.7849 (R^2 = 0.9997).

(3) *Accuracy and precision*

Within-run and between-run accuracy and precision for low, medium and high QC samples are presented in Table 3. The results were within the accepted criteria for all antimicrobials, which demonstrates that this assay possesses satisfactory accuracy and precision.

(4) *Dilution integrity*

The accuracy of the diluted samples was 103.67 %, with a precision of 4.65 %. These results illustrate that samples with concentrations exceeding the ULOQ can be accurately quantified after 4-fold or 8-fold dilution.

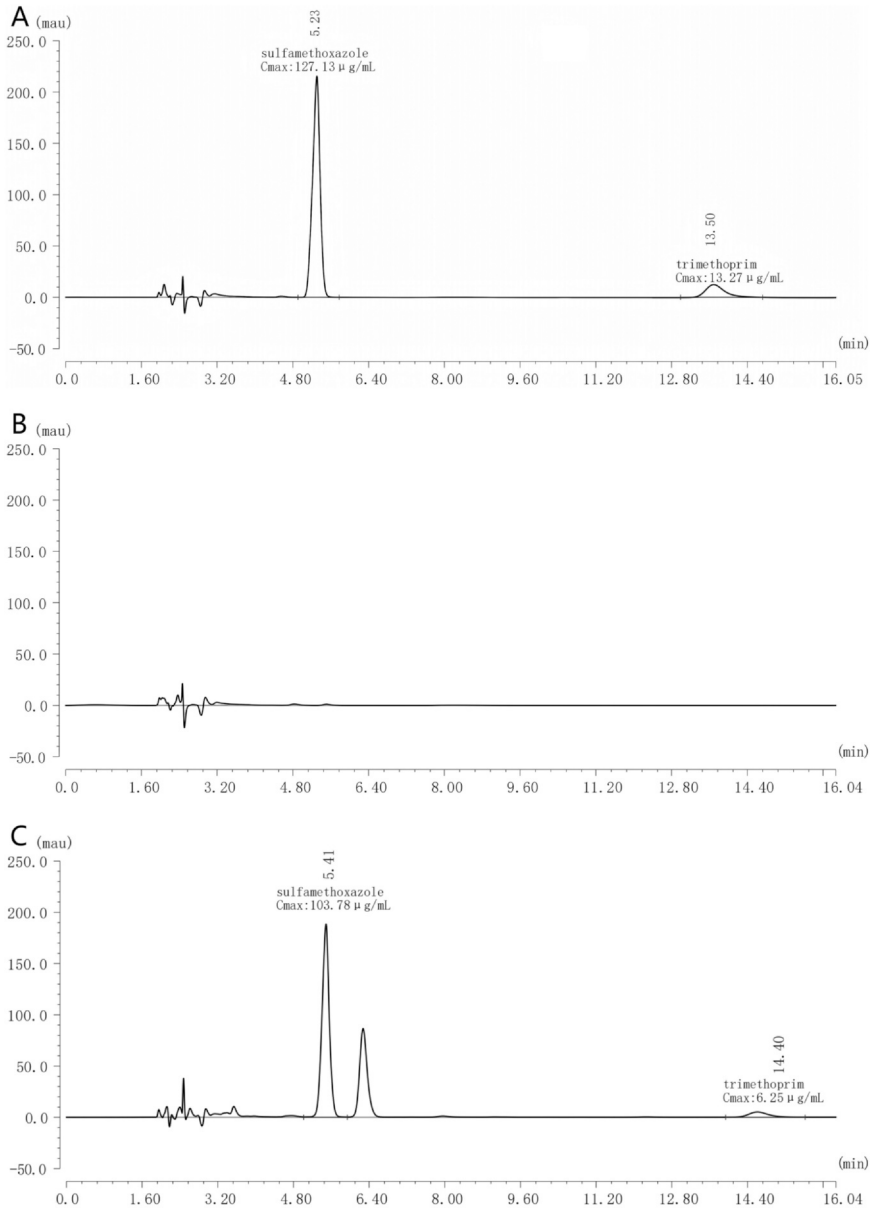

Fig. 1. Representative chromatograms of sulfamethoxazole and trimethoprim in human plasma. **Note:** (A) blank plasma sample spiked with analytes; (B) blank plasma sample; (C) clinical plasma sample.

Table 3
Summary of accuracy and precision.

Drug		Concentration (μg/mL)	Accuracy		Within-run precision		Between-run precision	
			Mean accuracy (%)	CV (%)	Mean Concentration (μg/mL)	CV (%)	Mean Concentration (μg/mL)	CV(%)
Sulfamethoxazole	Low	5.00	105.35	1.25	5.55	1.32	5.35	5.98
	Medium	125.00	101.64	1.74	136.65	1.74	132.31	3.33
	High	180.00	110.78	1.07	180.19	1.44	178.46	1.33
Trimethoprim	Low	0.50	107.17	1.65	0.48	4.03	0.52	8.10
	Medium	12.50	99.62	2.83	12.65	2.82	13.16	4.71
	High	18.00	105.80	3.86	7.79	2.17	18.20	4.34

Table 4
Stability for QCs. Values represent mean of accuracy (% CV), n = 3.

Drug	QC level	Room temperature for 24 h	4 ℃ for 24 h	-20 ℃ for 4 weeks	Freeze-thaw Stability
Sulfamethoxazole	Low	107.85(2.30)	101.15(0.11)	110.77(2.25)	103.04(0.46)
	Medium	108.16(1.84)	101.55(0.07)	109.88(8.01)	113.88(0.40)
	High	98.80(1.77)	101.44(0.42)	109.45(6.03)	95.10(0.64)
Trimethoprim	Low	101.50(0.57)	100.59(2.20)	102.82(8.41)	99.96(1.70)
	Medium	112.68(1.34)	101.58(0.09)	108.15(2.96)	111.09(0.95)
	High	98.64(3.00)	102.11(0.70)	111.40(4.56)	107.00(2.64)

(5) *Stability*

Table 4 summarizes the stability accuracy percentage of the chromatography under different storage conditions. Overall, all antibiotics in the QC samples were stable under the test conditions, with both accuracy and CV values falling within acceptable ranges.

Clinical application

Sample for TDM

The patients administered the drug according to the recommended instructions, and the dose was adjusted according to the creatinine clearance. Patients were instructed to take compound sulfamethoxazole tablets for 48 h. Blood samples were then collected using Vacutainer Serum Plus Blood Collection Tubes 2–3 h after the fifth dose of the medication. These samples were required to be delivered to the laboratory within 2 h of collection for analysis.

The patients followed the prescribed dosage instructions, with dose adjustments made based on their creatinine clearance. Compound sulfamethoxazole tablets were administered for a duration of 48 h. Blood samples were collected 2–3 h after the fifth dose, using Vacutainer Serum Plus Blood Collection Tubes. All samples were delivered to the laboratory within 2 h of collection for analysis.

Clinical data

The dosage administered was 1.92 g three times a day. The initial blood concentration of sulfamethoxazole ranged from 2.83 to 281.30 μg/mL, while the initial blood concentration of trimethoprim ranged from 0.39 to 22.68 μg/mL. Samples exceeding the upper limit were measured according to method 2.4. Variability in plasma concentrations of sulfamethoxazole among patients is evident, as illustrated in Fig. 2. The figure highlights that only a small number of patients achieved the desired therapeutic drug levels within the specified range (sulfamethoxazole: 50–150 μg/mL, trimethoprim: 3–8 μg/mL. Hence, TDM plays a crucial role in ensuring treatment efficacy for these individuals.

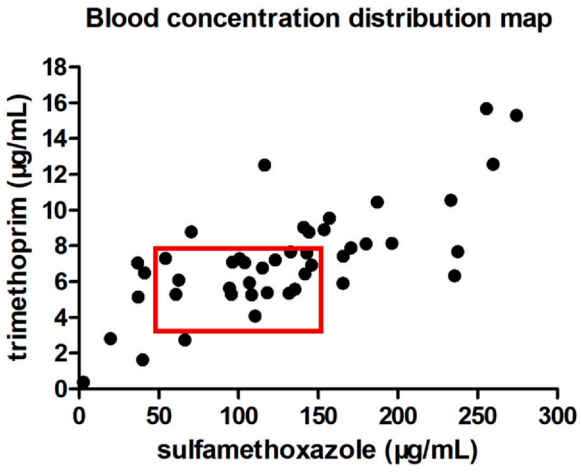

Fig. 2. Blood concentration distribution map.

Pneumocystis pneumonia (PCP) is a critical condition. Timely, adequate and appropriate anti-PCP treatment is key to good therapeutic outcomes. Although the oral absorption of compound sulfamethoxazole is minimally affected by food and other drugs, pharmacokinetic (PK) and pharmacodynamic (PD) variations still exist among different PCP patients. A standard therapeutic dosing regimen may lead to either excessive or suboptimal drug concentrations in some patients, which can affect treatment efficacy. Between November 2022 and March 2023, a total of 49 patients from the Organ Transplantation Department of our hospital were treated with compound sulfamethoxazole (containing 0.4 g sulfamethoxazole and 0.08 g trimethoprim) as part of their conventional regimen. The prescribed dosage was 1.92 g three times a day. The initial blood concentrations of sulfamethoxazole ranged from 2.83 to 281.30 μg/mL, while the initial blood concentrations of trimethoprim ranged from 0.39 to 22.68 μg/mL. Samples exceeding the upper limit of quantification were measured according to method 2.4. Significant variability in sulfamethoxazole plasma concentrations among patients was observed, as illustrated in Fig. 2. The results illustrate that only a small proportion of patients achieved the target therapeutic drug concentrations within the desired range (sulfamethoxazole: 50–150 μg/mL; trimethoprim: 3–8 μg/mL). Therefore, therapeutic drug monitoring (TDM) plays a crucial role in ensuring treatment efficacy for these individuals.

Case report

A 46-year-old female patient, weighing 49 kg, was admitted to the transplant department of our hospital with a persistent cough and sputum production for 2 months following a kidney transplantation surgery 4 months prior. Upon admission, she was prescribed famciclovir (1.25 mg/kg/day), imipenem/cilastatin sodium (0.5 g q8h), and compound sulfamethoxazole (1.92 g tid). After 5 days of treatment, the patient developed nausea and vomiting. Blood drug concentration testing revealed high levels of sulfamethoxazole (281.30 μg/mL) and trimethoprim (22.68 μg/mL). These adverse reactions were attributed to the elevated blood concentration of compound sulfamethoxazole, prompting a dosage adjustment to 0.96 g tid. Following 4 days of the adjusted dosage, the patient's nausea and vomiting resolved. Subsequent blood concentration testing revealed a decrease in sulfamethoxazole (36.77 μg/mL) and trimethoprim (7.04 μg/mL) levels. Considering the possibility of a pulmonary infection, next-generation sequencing (NGS) of bronchoalveolar lavage fluid revealed the presence of *Staphylococcus epidermidis, Pseudomonas aeruginosa, human cytomegalovirus*, and *human herpesvirus 7*. *Pneumocystis jirovecii* infection was also suspected due to multiple potential pathogens. Considering the blood drug concentrations and the clinical picture, the dosage of compound sulfamethoxazole was adjusted to 1.44 g tid. After one week of treatment, the patient's cough and sputum production significantly improved, inflammatory markers decreased, and the pulmonary infection showed significant improvement. The patient was discharged thereafter.

The first six months following organ transplantation represent the period of the highest risk for PCP.[5,6] Compound sulfamethoxazole is the

preferred medication for PCP prophylaxis, with two internationally recognized low-dose regimens: once daily and three times weekly. The KDIGO guidelines recommend a once-daily dose ranging from 0.48 g to 0.96 g per dose. Both the once-daily and three-times-weekly regimens demonstrate comparable prophylactic efficacy. For the treatment of confirmed PCP, compound sulfamethoxazole is the first-line therapy, with a recommended dosage of 20 mg/kg/day, administered 2–4 times daily. Close monitoring for adverse drug reactions, such as bone marrow suppression, hepatitis, interstitial nephritis, pancreatitis, and skin rash, is necessary.[7]

In this case, the patient developed respiratory symptoms 4 months post-kidney transplantation and was empirically treated with compound sulfamethoxazole for infection. During treatment, the patient experienced nausea and vomiting. Blood drug concentration testing revealed significantly elevated levels of sulfamethoxazole and trimethoprim, exceeding the therapeutic range. According to the drug's guidelines, total sulfonamide concentrations exceeding 200 μg/mL can increase the risk of adverse reactions, such as loss of appetite, abdominal pain, nausea, vomiting, dizziness, headache, and, in severe cases, bone marrow suppression. The pharmacist suspected that the high blood concentrations were likely responsible for the adverse reactions and recommended a dosage adjustment. Following this adjustment, the patient's symptoms significantly improved. Subsequent blood concentration monitoring showed a reduction in sulfamethoxazole and trimethoprim levels, confirming the appropriateness of the adjusted dosage. Despite improvement in respiratory symptoms after dosage adjustment, further modifications were suggested based on microbiological findings and the continued presence of cough and sputum production. After the additional adjustment, the patient's respiratory symptoms improved significantly, leading to clinical improvement and eventual discharge.

TDM of sulfamethoxazole involves achieving specific target values concentrations for the treatment of different infections.[8] For *Pneumocystis jirovecii* and *Stenotrophomonas maltophilia* infections, recommended peak plasma concentrations (Cmax) for sulfamethoxazole range from 100 to 200 μg/mL, and for trimethoprim, from 5 and 8 μg/mL. In cases of *Burkholderia cepacia* complex and methicillin-resistant *Staphylococcus aureus* (MRSA) infections, monitoring trough concentrations (Cmin) is advised, with recommended levels of ≥ 38 μg/mL for sulfamethoxazole and ≥ 2 μg/mL for trimethoprim. For *Nocardia* species infections, the recommended trough concentrations are ≥ 78 μg/mL for sulfamethoxazole and ≥ 4 μg/mL for trimethoprim. Studies suggest that maintaining therapeutic concentrations between 100 and 150 μg/mL for sulfamethoxazole and 3–8 μg/mL for trimethoprim optimizes treatment outcomes.

PCP patients are often in critical condition, with gastrointestinal dysfunction, and their blood drug concentrations are highly unstable following oral administration. Exceeding the target concentration range can adversely affect the treatment of PCP and may even cause adverse reactions in blood, liver, and kidney functions. Therefore, in addition to early diagnosis and treatment, TDM plays a crucial role in guiding clinical drug administration and improving patient outcomes.

Sulfamethoxazole targets dihydrofolate synthase, disrupting the initial step of folate synthesis, while trimethoprim acts on the subsequent phase by selectively inhibiting dihydrofolate reductase. The combined use of these agents significantly impedes folate metabolism, which can increase the incidence of adverse reactions. Previous studies have shown that elevated serum levels of both trimethoprim and sulfamethoxazole are associated with a higher rate of adverse reactions.[9,10] There is a correlation between hematological toxicity and the Cmax of trimethoprim, with toxicity increasing when the Cmax exceeds 8.0 μg/mL, and levels above 8.75 μg/mL may increase the risk of hyperkalemia.[11] Klinker et al. recommended monitoring sulfamethoxazole levels to reduce adverse effects, based on their finding that the occurrence of sulfamethoxazole-trimethoprim (SMX-TMP) side effects increased with higher SMX Cmax and prolonged treatment duration.[12] However, another study[14] showed that the correlation between increased adverse reactions and SMX Cmax was limited to several adverse reactions, such as anemia, neutropenia, and azotemia, while other adverse reactions were not related to SMX Cmax.[13] It is worth noting that both studies included patients with PCP in the context of HIV infection. In our study, the occurrence of adverse reactions, although significantly associated with SMX Cmax, was not related to the dose of SMX-TMP administration. This finding aligns with the study by Corsini et al., which also demonstrated that monitoring SMX-TMP serum concentrations could help mitigate adverse effects.[14] Additionally, recent case reports have suggested that compound sulfamethoxazole can induce antibiotic-associated encephalopathy and Drug Reaction with Eosinophilia and Systemic Symptoms (DRESS) syndrome.[15,16] Therefore, timely measurement of plasma drug concentrations is crucial to optimize the therapeutic efficacy of compound sulfamethoxazole and minimize adverse reactions.

Conclusion

A two-dimensional liquid chromatography (2D-LC) method was developed for the simultaneous quantification of sulfamethoxazole and trimethoprim in human plasma. This method underwent thorough validation and presents several advantages, including the requirement for a small sample volume, simplified sample processing, rapid analysis time, and broad applicability in routine analytical settings. Furthermore, this novel detection method has been effectively utilized for routine TDM of compound sulfamethoxazole in solid organ transplant patients.

Declarations

Not applicable.

Authors' contributions

L. Nong: Writing - original draft, Methodology, Funding acquisition, Formal analysis. Y. Tang: Writing - review & editing, Validation, Conceptualization. X. Qin: Writing - review & editing, Investigation. Z. Zhou: Data curation. Z. Lai and W. Wei: Resources, Supervision, Project administration. W. Tan: Writing - review & editing, Supervision.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of The Second Affiliated Hospital of Guangxi Medical University (2024 Scientific Research Ethics Review No. 23). All procedures involving human participants were conducted in accordance with the ethical standards set by the institutional and national research committees and with the 1964 Helsinki Declaration and its subsequent amendments or comparable ethical standards. Informed consent was obtained from the participant's legal guardian before treatment.

Consent for publication

Written informed consent for publication was obtained from all participants.

Availability of data and materials

Data will be made available on request.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Authors' other information

Not applicable.

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