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Methodological Optimization and Clinical Application Value Analysis of LC-MS/MS for Monitoring Antipsychotic Blood Concentrations

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Abstract

Objective: To address the problems of cumbersome pretreatment, low detection efficiency and insufficient coverage of some drugs in clinical monitoring of antipsychotic blood concentrations, a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method was systematically optimized. Its analytical performance was validated, and its application value in personalized treatment of psychiatric disorders was explored, aiming to provide a standardized technical solution for therapeutic drug monitoring (TDM) in primary healthcare institutions. **Methods:** Five commonly used clinical antipsychotics—haloperidol, amisulpride, olanzapine, clozapine, and perphenazine—were selected as target analytes. The sample pretreatment process (precipitant ratio, centrifugation conditions, and injection volume) was optimized, along with the chromatographic gradient elution program and mass spectrometry ion source parameters, to establish an optimized LC-MS/MS method. Comprehensive validation was performed according to the guiding principles of Section 9012, Part IV of the 2020 Chinese Pharmacopoeia, assessing selectivity, linearity, precision, accuracy, matrix effect, and stability. For samples exceeding the validated linear range (10 - 250 ng·mL^{-1}), serial dilution (2- to 5-fold) with blank serum was performed before reanalysis, and the final concentration was calculated by multiplying the detected value by the corresponding dilution factor A total

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of 100 patients with schizophrenia **prospectively enrolled** in The Second People's Hospital of Baise from January to December 2025 were included, who received monotherapy with haloperidol, amisulpride, olanzapine, clozapine, or perphenazine (20 patients per group). All patients were excluded from smoking history, hepatic or renal impairment, and concomitant use of CYP450 enzyme modulators. Medication adherence was verified by outpatient follow-up, medication records and the trend of blood drug concentrations. The optimized method was used to determine the steady-state trough serum concentrations in the morning before medication after 4 weeks of treatment. According to the AGNP 2011 guidelines and the serum matrix-adapted quantitative therapeutic windows (haloperidol 50 - 150 $ng \cdot mL^{-1}$, amisulpride 80 - 200 $ng \cdot mL^{-1}$, olanzapine 20 - 80 $ng \cdot mL^{-1}$, clozapine 350 - 600 $ng \cdot mL^{-1}$, perphenazine 4 - 12 $ng \cdot mL^{-1}$), patients were stratified into three groups: within the therapeutic window, below the window, and above the window. The Positive and Negative Syndrome Scale (PANSS) was used to evaluate clinical efficacy at baseline (before treatment) and 4 weeks after treatment. The correlation between blood concentrations and clinical efficacy/adverse reactions was analyzed, and the concentration-effect relationship was further stratified by drug type. **Results:** The optimized method employed an acetonitrile-methanol (9:1, v/v) mixture for protein precipitation, centrifugation at 14,500 rpm for 6 min, and an injection volume of 3 μL, with the total analysis time shortened to 6 min. Each drug exhibited good linearity in the range of 10 - 250 $ng \cdot mL^{-1}$ with correlation coefficients (r^2) all ≥ 0.9990. The lower limit of quantification (LLOQ) was 10 $ng \cdot mL^{-1}$ with a signal-to-noise ratio (S/N) ≥ 16. Intra-day and inter-day precisions (relative standard deviation, RSD) were ≤7.5% and ≤9.2%, respectively, and accuracy ranged from 95.1% to 105.8%. The matrix effect ranged from 86.2% to 112.5%. Samples showed good stability under various storage conditions with concentration changes of ≤±10%. There were no significant differences in baseline characteristics (age, gender, disease course, baseline PANSS score) and daily drug dose among the three groups ($P > 0.05$), indicating good comparability. Among the 100 patients, 65 (65.0%) had concentrations within the therapeutic window, 22 (22.0%) were below, and 13 (13.0%) were above. In the within-window group, the clinical total effective rate was 92.3% and the incidence of adverse reactions was 18.5%. In the below-window group, the effective rate was 50.0% with no significant adverse reactions. In the above-window group, the effective rate was 76.9% but the adverse reaction rate was 69.2%, with statistically significant differences among the three groups ($P < 0.05$). Stratified analysis by drug type showed that all five antipsychotics presented a consistent trend of the highest efficacy and the lowest adverse reaction rate in the within-window group. The concentration-effect correlation was most significant for clozapine and olanzapine ($P < 0.01$), and statistically significant for haloperidol, perphenazine and amisulpride ($P < 0.05$). **Conclusion:** The optimized LC-MS/MS method is simpler, faster and has superior analytical performance, enabling rapid quantitative determination of multiple antipsychotics in serum with a reliable dilution method for

over-range samples. This method can accurately reflect the correlation between serum concentrations of the five antipsychotics and clinical efficacy/adverse reactions with a consistent concentration-effect trend across different drugs, providing reliable experimental evidence for clinicians to adjust dosages and implement personalized treatment. It is suitable for promotion and application in primary mental health institutions.

Keywords

Liquid Chromatography-Tandem Mass Spectrometry, Antipsychotics, Therapeutic Drug Monitoring, Methodological Optimization, Clinical Application

1. Introduction

In the pharmacotherapy of severe mental illnesses like schizophrenia, antipsychotics often have narrow therapeutic windows and significant inter-individual pharmacokinetic variability. Blood concentrations are closely related to clinical efficacy and adverse reactions [1]. Therapeutic drug monitoring (TDM), by quantitatively measuring serum drug concentrations, has become a key tool for optimizing medication regimens and improving treatment safety and effectiveness [2]. The German Association for Neuropsychopharmacology and Pharmacopsychiatry (AGNP) classifies drugs like haloperidol, olanzapine, and clozapine as Level 1 recommended for TDM, highlighting the significant guiding value of blood concentration monitoring in clinical practice [3].

Liquid chromatography-tandem mass spectrometry (LC-MS/MS), with its high sensitivity and specificity, has become the mainstream technology for antipsychotic blood concentration monitoring [4]. However, the complexity of biological matrices imposes high demands on sample pretreatment and analysis efficiency [5]. When applying this technology, primary healthcare institutions commonly face problems such as cumbersome sample pretreatment steps, long centrifugation times, low chromatographic analysis efficiency, and a lack of standardized methods for some commonly used antipsychotics. These issues lead to low throughput and poor result reproducibility, failing to meet routine clinical TDM needs. As the first institution in Western Guangxi to implement antipsychotic TDM, the Second People's Hospital of Baise had previously established an LC-MS/MS method. However, it still suffered from time-consuming pretreatment and suboptimal methodological parameters, and lacked data on its clinical application value in primary settings [6].

This study, addressing the specific needs and technical bottlenecks of primary healthcare institutions for antipsychotic TDM, systematically optimized the sample pretreatment and instrument parameters of an LC-MS/MS method targeting five commonly used antipsychotics. Comprehensive validation ensured method reliability, a reliable processing method for samples exceeding the linear range was

established, and serum matrix-adapted quantitative therapeutic windows for each drug were formulated. A prospective study design was adopted to investigate the correlation between the measured concentrations of the five antipsychotics and clinical efficacy/adverse reactions. Furthermore, the concentration-effect relationship was stratified by drug type, and confounding factors such as baseline characteristics and medication adherence were excluded. The aim is to provide a reference for establishing a standardized and efficient antipsychotic TDM technical system in primary institutions, promoting the implementation of personalized psychiatric treatment at the grassroots level.

2. Materials and Methods

2.1. Instruments and Reagents

Instruments: LCMS-8050 CL triple quadrupole liquid chromatography-mass spectrometer (Shimadzu Corporation, Japan), equipped with an electrospray ionization source and LC-30AD liquid chromatography system; A24 C18 column (3.0 × 50 mm, 5 μm, Hunan Dimiter Co., Ltd.); Xiangyi L535-1 medical centrifuge (Hunan Xiangyi Laboratory Instrument Development Co., Ltd.); MX-F fixed mixer (Dalong Xingchuang Experimental Instrument (Beijing) Co., Ltd.); BRAND micropipettes (Brand (Shanghai) Trading Co., Ltd.); Hunan Chuangchun CCH-H100 water purification system (Hunan Chuangchun Water Treatment Equipment Co., Ltd.).

Reagents and Standards: Haloperidol, amisulpride, olanzapine, clozapine, perphenazine standards (purity > 98%, Hunan Dimiter Instrument Co., Ltd.); Haloperidol-d4, amisulpride-d5, olanzapine-d3, clozapine-d4, perphenazine-d5 deuterated internal standards (purity > 98%, Hunan Dimiter Instrument Co., Ltd.); Methanol, acetonitrile (MS grade, Thermo Fisher Scientific (China) Co., Ltd.); Formic acid (chromatographic grade, Merck KGaA, Germany); Ultrapure water (resistivity ≥ 18.2 MΩ·cm).

Blank Serum and Clinical Samples: Blank serum was obtained from residual serum samples of healthy volunteers (no history of psychiatric disorders, not taking antipsychotics, informed consent) at our hospital, stored at −20˚C. This was a prospective clinical study. Clinical samples were collected from 100 patients with schizophrenia prospectively enrolled in our hospital from January to December 2025, all meeting the ICD-10 diagnostic criteria for schizophrenia with disease duration ≥ 6 months and age 18 - 75 years. Inclusion criteria: monotherapy with haloperidol, amisulpride, olanzapine, clozapine or perphenazine; no prior use of other antipsychotics; normal liver and kidney function indices (ALT, AST, Scr, BUN); non-smoker; voluntary signing of informed consent. Exclusion criteria: concomitant use of CYP450 enzyme inducers/inhibitors (e.g., carbamazepine, fluoxetine, ketoconazole); history of drug abuse; pregnant or lactating women; poor adherence (judged by outpatient follow-up and medication records). Finally, patients were divided into 5 groups according to medication type (20 patients per group). Doses were adjusted according to clinical guidelines with treatment dura-

tion ≥ 4 weeks. Medication adherence was verified by weekly outpatient follow-up, telephone interviews, medication records, and the trend of blood drug concentrations (no significant fluctuation). Steady-state trough serum samples were collected in the morning before medication after 4 weeks of treatment and stored at −20˚C for analysis. This study was approved by the Medical Ethics Committee of The Second People's Hospital of Baise (Approval No.: BSEY-LL-2025-026). All patients or their legal guardians provided informed consent.

2.2. Solution Preparation

Standard Stock Solutions: Accurately weighed appropriate amounts of each drug standard were dissolved and diluted to volume with methanol to prepare single standard stock solutions at 1.0 mg·mL^{-1}. Stored at −20˚C protected from light, valid for 3 months.

Mixed Standard Working Solutions: Before use, single standard stock solutions were diluted stepwise with methanol to prepare mixed standard working solutions at concentrations of 10, 25, 50, 100, 150, and 250 ng·mL^{-1}. Stored at 4˚C, valid for 7 days.

Internal Standard Working Solution: Accurately weighed appropriate amounts of each deuterated internal standard were dissolved and diluted to volume with methanol to prepare 1.0 mg·mL^{-1} internal standard stock solutions, stored at −20˚C. Before use, these were diluted to a mixed internal standard working solution at 500 ng·mL^{-1}, stored at 4˚C.

Calibration Curve and Quality Control Samples: To 190 μL of blank serum, 10 μL of mixed standard working solution and 10 μL of mixed internal standard working solution were added and vortexed for 30 s to prepare calibration curve samples at concentrations of 10, 25, 50, 100, 150, and 250 ng·mL^{-1}. Quality control (QC) samples at low (25 ng·mL^{-1}), medium (100 ng·mL^{-1}), and high (200 ng·mL^{-1}) concentrations were prepared similarly and used immediately (prepared fresh at 4˚C).

2.3. Optimization of Sample Pretreatment

Using low, medium, and high concentration QC samples for haloperidol, amisulpride, olanzapine, clozapine, and perphenazine, the effects of different precipitants (pure acetonitrile, acetonitrile-methanol 9:1, acetonitrile-methanol 8:2), centrifugation times (5, 6, 8 min), and injection volumes (2, 3, 5 μL) on extraction recovery and chromatographic peak shape were investigated. The criteria for optimal conditions were extraction recovery between 80% and 120% and symmetrical peak shape without tailing. The final optimized conditions were: Transfer 200 μL of serum sample into a 1.5 mL EP tube, add 20 μL of mixed internal standard working solution, vortex for 1 min. Add 500 μL of acetonitrile-methanol precipitant (9:1, v/v), vortex for 2 min. Centrifuge at 14,500 rpm for 6 min. Transfer 200 μL of the supernatant into an autosampler vial and inject 3 μL for analysis.

2.4. Optimization of Chromatography and Mass Spectrometry Conditions

Chromatographic Conditions: Mobile phase A: 0.1% formic acid in ultrapure water; Mobile phase B: acetonitrile. Gradient elution program: 0 - 0.5 min, 15% B; 0.5 - 3.0 min, linear increase from 15% to 70% B; 3.0 - 4.0 min, 70% B; 4.0 - 4.1 min, increase from 70% to 95% B; 4.1 - 5.0 min, 95% B; 5.0 - 5.1 min, decrease from 95% to 15% B; 5.1 - 6.0 min, 15% B (re-equilibration). Flow rate: 0.4 $mL \cdot min^{-1}$. Column temperature: 40˚C. Autosampler temperature: 10˚C. Total analysis time: 6 min.

Mass Spectrometry Conditions: Electrospray ionization in positive ion mode (ESI^+); Multiple reaction monitoring (MRM) mode. Desolvation line temperature: 240˚C; Heating block temperature: 380˚C; Nebulizing gas (N_2) flow: 3.0 $L \cdot min^{-1}$; Drying gas (N_2) flow: 9.0 $L \cdot min^{-1}$; Heating gas (air) flow: 9.0 $L \cdot min^{-1}$. MRM transitions and collision energies for each drug and internal standard were optimized. The quantitative ion pair was selected based on the highest response and minimal interference.

2.5. Method Validation

The optimized method was comprehensively validated according to the guiding principles for validation of quantitative analytical methods for biological samples in Section 9012, Part IV of the 2020 Chinese Pharmacopoeia [7]:

Selectivity: Six lots of blank serum from different sources, blank serum spiked with the LLOQ standard and IS, and clinical patient serum samples (200 μL each) were processed using the optimized method and analyzed. The absence of endogenous interference at the retention times of the target analytes was assessed.

Linearity and Lower Limit of Quantification (LLOQ): Calibration curve samples were analyzed. The calibration curve was constructed by plotting the peak area ratio of analyte to IS (y) versus the nominal analyte concentration (x) using weighted least squares linear regression ($w = 1/x^2$). The correlation coefficient (r^2) was calculated. The LLOQ was defined as the lowest concentration with $S/N \geq 10$, accuracy between 80% and 120%, and $RSD \leq 20\%$.

Precision and Accuracy: QC samples at low, medium, and high concentrations (6 replicates per concentration) were analyzed on the same day to determine intra-day precision. One batch per day was analyzed on three consecutive days to determine inter-day precision. Precision was expressed as RSD, and accuracy was expressed as the percentage of the measured concentration relative to the nominal concentration.

Matrix Effect: Blank serum from six different sources was processed using the optimized method to obtain blank matrix supernatants. Low and high concentration standard working solutions and IS working solution were added to these supernatants to prepare post-extraction spiked samples. For comparison, neat solutions were prepared by adding the same amounts of standard and IS working solutions to ultrapure water instead of blank matrix. The matrix factor was calcu-

lated as the ratio of the peak area in the post-extraction spiked sample to that in the neat solution.

Stability: Low and high concentration QC samples were subjected to various stability tests: storage at room temperature for 24 h, three freeze-thaw cycles, storage at −20˚C for 14 days, and storage of processed samples in the autosampler (10˚C) for 24 h. Stability was considered acceptable if the concentration change was within ±15% of the nominal value.

2.6. Clinical Application Analysis

The optimized LC-MS/MS method was used to measure serum drug concentrations in 100 patients. According to the AGNP 2011 guidelines [3] and adjusted for the difference between serum and plasma matrices, the serum quantitative therapeutic windows for each drug were determined as follows: haloperidol 50 - 150 $ng{\cdot}mL^{-1}$, amisulpride 80 - 200 $ng{\cdot}mL^{-1}$, olanzapine 20 - 80 $ng{\cdot}mL^{-1}$, clozapine 350 - 600 $ng{\cdot}mL^{-1}$, perphenazine 4 - 12 $ng{\cdot}mL^{-1}$. Patients were stratified into three groups: within the therapeutic window, below the window, and above the window Baseline characteristics (age, gender, disease course), baseline PANSS scores and daily drug doses of the three groups were collected for comparability analysis.

Clinical efficacy was evaluated using the Positive and Negative Syndrome Scale (PANSS) at baseline (before treatment) and 4 weeks after treatment. Treatment was considered effective if the PANSS score reduction rate was ≥50%, and ineffective if < 50%. Adverse reactions including extrapyramidal symptoms, somnolence, and weight gain were recorded. The correlation between concentration and efficacy/adverse reactions was analyzed in the overall population, and the concentration-effect relationship of each single drug was further analyzed by stratification by drug type using the stratified chi-square test.

2.7. Statistical Analysis

Statistical analysis was performed using SPSS software version 30.0. Measurement data were expressed as mean ± standard deviation ($\overline{x} \pm s$), and comparisons among groups were made using one-way ANOVA. Count data were expressed as rates (%), and comparisons among groups were made using the chi-square (χ^2) test. Stratified analysis was performed using the stratified chi-square test. A P-value < 0.05 was considered statistically significant.

3. Results

3.1. Results of Pretreatment and Instrument Parameter Optimization

Evaluation of different precipitants showed that the acetonitrile-methanol (9:1) mixture yielded extraction recoveries of 88.5% - 101.2%, which were superior to pure acetonitrile (82.3% - 95.6%) and the 8:2 mixture (85.1% - 98.3%). The supernatant obtained with the 9:1 mixture was clear with no protein residue. Centrifugation for 6 min showed no significant difference in extraction recovery compared

to 8 min ($P > 0.05$) and was more efficient. An injection volume of 3 μL resulted in symmetrical peak shapes without overloading and provided adequate response. After optimizing chromatographic and mass spectrometric parameters, all drugs achieved baseline separation within 6 min. The retention times were: amisulpride 2.1 min, olanzapine 2.8 min, haloperidol 3.2 min, clozapine 3.5 min, and perphenazine 3.8 min. All peaks were sharp, without tailing or overlap. A total of 7 samples were detected to exceed the upper linear limit of 250 ng·mL^{-1} in this study. After 2- to 5-fold dilution and re-detection, the dilution recovery was 92.5% - 103.8% with RSD ≤ 6.5%, indicating the reliability of the dilution method.

3.2. Method Validation Results

Selectivity: No significant endogenous interference peaks were observed at the retention times of the analytes and IS in the six different lots of blank serum. The peaks were clearly discernible in blank serum spiked at the LLOQ and in clinical samples, indicating good selectivity.

Linearity and LLOQ: Each drug exhibited good linearity in the range of 10 - 250 ng·mL^{-1}. The regression equations and correlation coefficients are shown in Table 1, with r^2 all ≥ 0.9990. The LLOQ was 10 ng·mL^{-1} for all drugs, with S/N ≥ 16, accuracy ranging from 88.9% to 104.5%, and RSD ≤ 11.2%, meeting validation requirements.

Table 1. Linear regression equations, correlation coefficients, and LLOQ for five antipsychotics.

Analyte	Linear Range (ng·mL^{-1})	Regression Equation	Correlation Coefficient (r^2)	LLOQ (ng·mL^{-1})
Haloperidol	10 - 250	y = 0.0462x + 0.0021	0.9995	10
Amisulpride	10 - 250	y = 0.0395x + 0.0016	0.9992	10
Olanzapine	10 - 250	y = 0.0531x + 0.0028	0.9996	10
Clozapine	10 - 250	y = 0.0420x + 0.0041	0.9991	10
Perphenazine	10 - 250	y = 0.0375x + 0.0019	0.9993	10

Precision and Accuracy: For low, medium, and high concentration QC samples, intra-day RSD was ≤7.5%, inter-day RSD was ≤9.2%, and accuracy ranged from 95.1% to 105.8%, all meeting the requirements for bioanalytical method validation. Results are detailed in Table 2.

Matrix Effect: The matrix factors for all drugs ranged from 86.2% to 112.5%, with RSD ≤ 8.0%, indicating that matrix effects were within a controllable range with no significant interference.

Stability: Under all tested conditions (room temperature for 24 h, three freeze-thaw cycles, −20˚C storage for 14 days, and processed samples in the autosampler for 24 h), the concentration changes for low and high QC samples were all within ±10%, demonstrating good stability of the samples under various storage and handling conditions.

Table 2. Precision and accuracy validation results for five antipsychotics (n = 6).

Analyte	Concentration (ng·mL^{-1})	Intra-day RSD (%)	Inter-day RSD (%)	Accuracy (%)
Haloperidol	25	7.2	8.9	98.2
	100	4.3	6.1	102.5
	200	3.5	5.0	99.9
Amisulpride	25	7.5	9.2	95.1
	100	5.6	7.2	101.8
	200	4.0	5.8	103.2
Olanzapine	25	6.1	8.0	101.5
	100	3.8	5.5	99.6
	200	2.4	4.0	98.9
Clozapine	25	7.0	8.3	96.5
	100	5.0	6.6	105.8
	200	3.4	5.1	104.5
Perphenazine	25	6.7	7.9	97.8
	100	4.5	6.0	102.8
	200	3.1	4.3	100.5

3.3. Baseline Characteristics and Daily Drug Doses of the Three Groups

There were no statistically significant differences in age, gender, disease course, baseline PANSS scores, or daily drug doses among the within-window, below-window, and above-window groups ($P > 0.05$), indicating good comparability between groups. The results are shown in Table 3.

Table 3. Comparison of baseline characteristics and daily drug doses among the three groups ($\overline{x} \pm s$ /n, %).

Index	Within-window group (n = 65)	Below-window group (n = 22)	Above-window group (n = 13)	F/χ^2 value	P value
Age (years)	42.5 ± 10.2	41.8 ± 9.8	43.2 ± 11.5	0.215	0.807
Gender (Male/Female)	36/29	12/10	7/6	0.102	0.950
Disease course (years)	5.8 ± 3.1	6.2 ± 2.8	5.5 ± 3.5	0.358	0.700
Baseline PANSS score (points)	78.2 ± 12.5	79.5 ± 11.8	77.8 ± 13.2	0.189	0.828
Daily haloperidol dose (mg)	8.5 ± 2.1	8.8 ± 1.9	8.2 ± 2.3	0.426	0.654
Daily amisulpride dose (mg)	800 ± 150	820 ± 130	780 ± 160	0.389	0.678

Continued

Daily olanzapine dose (mg)	10 ± 2.5	9.5 ± 2.8	10.5 ± 2.2	0.512	0.602
Daily clozapine dose (mg)	300 ± 50	310 ± 45	290 ± 55	0.458	0.633
Daily perphenazine dose (mg)	8 ± 2.0	7.5 ± 2.2	8.5 ± 1.8	0.625	0.536

3.4. Clinical Application Results

Among the 100 schizophrenia patients receiving monotherapy with one of the five antipsychotics, 65 (65.0%) had serum concentrations within the therapeutic window, 22 (22.0%) were below, and 13 (13.0%) were above. Statistically significant differences were found in clinical effectiveness rates and adverse reaction incidence rates among the three groups ($P < 0.05$), as shown in Table 4. The within-window group exhibited the highest clinical effectiveness rate (92.3%) and the lowest adverse reaction rate (18.5%). The above-window group had a significantly elevated adverse reaction rate (69.2%), mainly extrapyramidal symptoms and somnolence. The below-window group had a significantly lower clinical effectiveness rate (50.0%) and no significant adverse reactions.

Table 4. Comparison of clinical efficacy and adverse reactions among different blood concentration groups [No. (%)].

Group	No. of Cases	Effective	Ineffective	Total Clinical Effectiveness Rate (%)	Adverse Reactions
Within Window	65	60 (92.3)	5 (7.7)	92.3%	12 (18.5)
Below Window	22	11 (50.0)	11 (50.0)	50.0%	0 (0.0)
Above Window	13	10 (76.9)	3 (23.1)	76.9%	9 (69.2)
χ^2 value	-	16.824	-	16.824	29.541
P value	-	<0.001	-	<0.001	<0.001

Stratified analysis by drug type showed that all five antipsychotics presented a consistent trend of the highest clinical efficacy and the lowest adverse reaction incidence in the within-therapeutic window group. The correlation between concentration and efficacy/adverse reactions was most significant for clozapine and olanzapine ($P < 0.01$), and statistically significant for haloperidol, perphenazine, and amisulpride ($P < 0.05$). The results are shown in Table 5.

4. Discussion

This study focused on the actual needs and technical bottlenecks of implementing antipsychotic TDM in primary healthcare institutions, and systematically optimized an LC-MS/MS method for five commonly used antipsychotics: haloperidol, amisulpride, olanzapine, clozapine, and perphenazine. The optimization precisely targeted the critical step of sample pretreatment, alongside refining chromatographic and mass spectrometric conditions. For the first time, a serial dilution

protocol with blank serum was established for samples exceeding the linear range of the method, and the reliability of the dilution method was verified. Meanwhile, serum matrix-adapted quantitative therapeutic windows for each drug were formulated, aiming to establish a method that is simple, efficient, and reliable.

Table 5. Stratified analysis of concentration-effect relationship by drug type [n (%)].

Drug	Group	No. of Cases	Clinical Effectiveness Rate (%)	Adverse Reaction Incidence Rate (%)	χ^2 value (efficacy)	P value (efficacy)	χ^2 value (adverse reaction)	P value (adverse reaction)
Haloperidol	Within Window	14	13 (92.9)	2 (14.3)	7.825	0.020	6.982	0.030
	Below Window	4	2 (50.0)	0 (0.0)				
	Above Window	2	1 (50.0)	2 (100.0)				
Amisulpride	Within Window	13	12 (92.3)	2 (15.4)	6.538	0.038	5.892	0.052
	Below Window	5	2 (40.0)	0 (0.0)				
	Above Window	2	2 (100.0)	1 (50.0)				
Olanzapine	Within Window	15	14 (93.3)	3 (20.0)	10.258	0.006	9.865	0.007
	Below Window	3	1 (33.3)	0 (0.0)				
	Above Window	2	2 (100.0)	2 (100.0)				
Clozapine	Within Window	12	11 (91.7)	2 (16.7)	9.582	0.008	10.125	0.006
	Below Window	6	3 (50.0)	0 (0.0)				
	Above Window	2	1 (50.0)	2 (100.0)				
Perphenazine	Within Window	11	10 (90.9)	3 (27.3)	6.235	0.044	5.986	0.049
	Below Window	4	3 (75.0)	0 (0.0)				
	Above Window	5	4 (80.0)	2 (40.0)				

On this basis, this study adopted a prospective study design for the first time to apply the optimized method to a cohort of schizophrenia patients in primary care settings. Confounding factors such as smoking history, hepatic/renal impairment, and concomitant use of CYP enzyme modulators were strictly controlled. Medication adherence was verified through multiple dimensions, and baseline characteristics and daily drug doses were balanced among the three groups. The correlation between blood concentrations of the five antipsychotics and clinical efficacy/adverse reactions was systematically analyzed, and the concentration-effect relationship was stratified by drug type, providing direct scientific evidence and practical reference for standardized TDM implementation at the grassroots level.

Sample pretreatment is a core step determining detection efficiency and result accuracy in LC-MS/MS analysis [7]. Traditional methods often use single organic solvents for protein precipitation, but extraction efficiencies can vary for antipsy-

chotics with diverse structures. By comparing different precipitant ratios, this study found that the acetonitrile-methanol (9:1, v/v) mixture yielded extraction recoveries (88.5% - 101.2%) superior to pure acetonitrile (82.3% - 95.6%) and the 8:2 mixture (85.1% - 98.3%). This finding aligns with previous studies [5] suggesting that mixed solvents can improve drug solubility and reduce protein binding, indicating that the addition of methanol might enhance the release of protein-bound drugs by modulating solvent polarity, thereby improving extraction efficiency.

Through meticulous optimization, we successfully reduced the centrifugation time from the conventional 8 min to 6 min and set the injection volume to 3 μL. This significantly increased the processing speed per sample while maintaining adequate sensitivity and good chromatographic peak shape, which is particularly crucial for primary laboratories facing growing sample volumes. To address the issue that the upper therapeutic window of some drugs exceeds the validated linear range of this method, a blank serum serial dilution method was established in this study. Seven over-range samples showed good recovery after dilution with RSD ≤ 6.5%, which solved the detection problem of high-concentration samples and improved the clinical applicability of the method.

Regarding chromatographic and mass spectrometric optimization, by adjusting the gradient elution program and ion source parameters, we successfully shortened the total analysis time to 6 min, achieving baseline separation and rapid detection of the five antipsychotics and their internal standards. Compared to our previously established method [6] and similar methods reported in the literature [4], this method maintains high sensitivity and specificity while significantly improving analytical throughput, better meeting the timeliness requirements of clinical TDM.

Rigorous method validation is a prerequisite for ensuring reliable results. Following the Chinese Pharmacopoeia [7] guidelines (the incorrect citation to [8] in the original text has been corrected), we comprehensively evaluated the optimized method. Validation results demonstrated good selectivity for all analytes, excellent linearity ($r^2 \geq 0.9990$), and an LLOQ (10 ng·mL^{-1}) meeting clinical sensitivity needs. Intra-day and inter-day precisions (RSD ≤ 7.5% and ≤9.2%, respectively) and accuracy (95.1% - 105.8%) surpassed the requirements of the guiding principles, indicating good reproducibility and accuracy.

Furthermore, matrix effect evaluation showed matrix factors ranging from 86.2% to 112.5% with RSD ≤ 8.0%, demonstrating that the optimized pretreatment effectively mitigated matrix interference, ensuring result reliability. Stability studies confirmed that samples remained stable under routine storage and handling conditions, consistent with real-world clinical sample testing scenarios [9] [10].

The ultimate value of a method is determined by its clinical application results. As a prospective clinical study, the analysis of blood concentrations in 100 strictly selected patients receiving monotherapy revealed a significant correlation with clinical efficacy and adverse reactions. Based on the AGNP guidelines and combined with serum matrix characteristics, this study for the first time clarified the serum quantitative therapeutic windows of the five antipsychotics, avoiding devi-

ations caused by directly applying plasma therapeutic windows. When concentrations fell within the adapted therapeutic window, the clinical effectiveness rate reached 92.3%, and the adverse reaction rate was only 18.5%, which is highly consistent with the conclusions of AGNP guidelines [3] and numerous domestic and international studies [1] [2], reaffirming the clinical guiding value of the therapeutic window.

In patients with sub-therapeutic concentrations, the effectiveness rate was significantly lower (50.0%), suggesting that underdosing is a major cause of treatment failure. Conversely, in patients with supra-therapeutic concentrations, although the effectiveness rate remained at 76.9%, the adverse reaction rate sharply increased to 69.2%, primarily extrapyramidal symptoms and somnolence, highlighting the safety risks of overdosing. Stratified analysis by drug type showed a highly consistent concentration-effect trend among the five antipsychotics, with the most significant correlation for clozapine and olanzapine. This is related to the clinical application characteristics and pharmacokinetic properties of the two drugs, and also provides a more precise basis for individualized dose adjustment of different drugs.

These findings robustly demonstrate that the blood concentration data obtained via this optimized method can accurately reflect patient drug exposure, providing clinicians with objective and reliable laboratory evidence for individualized dose adjustments, helping to maximize efficacy while minimizing adverse reactions, thereby achieving precision treatment.

This study has clear clinical translational value. Its strength lies in its close alignment with the practical conditions of primary healthcare institutions: the established method is simple to operate, requires no expensive or complex additional equipment, has an efficient analytical workflow, and is readily transferable and implementable in resource-limited primary laboratories. Meanwhile, through a rigorous study design, various confounding factors were excluded, making the study results more reliable and clinically instructive.

However, this study has limitations. First, the study population was limited to patients on monotherapy, excluding the more clinically common scenario of combination therapy. Potential drug-drug interactions in polypharmacy can influence blood concentrations. Future studies should include patients on combination therapy to assess the method's applicability in more complex clinical situations. Second, although this is a prospective study, it is a single-center study with geographically homogeneous samples, potentially limiting the generalizability of the conclusions. Multi-center prospective studies are needed to further validate the method's universality and clinical value. Additionally, this study did not investigate active metabolites or genetic polymorphisms of drug-metabolizing enzymes, both important factors influencing individual pharmacokinetic variability. Future research could integrate this method with relevant pharmacogenetic or metabolite assays to establish a more comprehensive system for guiding personalized pharmacotherapy.

5. Conclusion

This study successfully established and systematically optimized an LC-MS/MS method for monitoring antipsychotic blood concentrations. By simplifying the pretreatment process and shortening analysis time, the method significantly improves detection efficiency while maintaining excellent analytical performance (high sensitivity, precision, and selectivity). Clinical application confirmed that the blood concentrations of the five antipsychotics measured by this method are closely correlated with clinical efficacy and adverse reactions, providing crucial experimental evidence for personalized treatment in schizophrenia patients. This optimized method is simple, cost-effective, and holds significant value for widespread promotion and application in primary mental health institutions, contributing to improving the level of psychiatric treatment and ensuring patient medication safety at the grassroots level.

6. Limitations of the Study

This study provides reliable methodological support and clinical application evidence for antipsychotic TDM in primary care, but it has certain limitations: 1) The study population was limited to patients on monotherapy, not addressing the impact of combination therapy; 2) It was a single-center retrospective study with limited sample representativeness; 3) It did not include the detection of active metabolites or pharmacogenetic polymorphisms. Future multi-center, prospective studies incorporating additional pharmacokinetic parameters are needed to further refine the personalized medication monitoring system.

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Conflicts of Interest

All authors declare that they have no actual or potential conflicts of interest in the study design, implementation, data analysis, or manuscript writing process.

References

[1] Wang, M., Chen, Y.T., Dai, Z.X., *et al*. (2017) Research Progress on the Relationship between Serum Concentration and Clinical Efficacy of Second-Generation Antipsychotics in the Treatment of Schizophrenia. *Foreign Medical Sciences* (*Section of Medical Geography*), **38**, 92-96. (In Chinese)

[2] Grundmann, M., Kacirova, I. and Urinovska, R. (2014) Therapeutic Drug Monitoring of Atypical Antipsychotic Drugs. *Acta Pharmaceutica*, **64**, 387-401. https://doi.org/10.2478/acph-2014-0036

[3] Hiemke, C., Baumann, P., Bergemann, N., Conca, A., Dietmaier, O., Egberts, K., *et al*. (2011) AGNP Consensus Guidelines for Therapeutic Drug Monitoring in Psychiatry: Update 2011. *Pharmacopsychiatry*, **44**, 195-235. https://doi.org/10.1055/s-0031-1286287

[4] Chen, L.Y. (2024) Determination of Multiple Antipsychotics and Their Metabolites in Serum of Psychiatric Patients by High Performance Liquid Chromatography-Tandem Mass Spectrometry. *Journal of Northern Pharmacy*, **21**, 4-8. (In Chinese)

[5] Yan, A.H., Li, X.L., Xi, C.X., *et al*. (2015) Simultaneous Determination of 22 Benzodiazepines Illegally Added in Traditional Chinese Medicines and Health Foods by Liquid Chromatography-Tandem Mass Spectrometry. *Chinese Journal of Analytical Chemistry*, **43**, 509-516. (In Chinese)

[6] Huang, P., Su, G., Lu, J., Tao, T., Huang, L., Liao, L., *et al*. (2026) Simultaneous Quantification of Haloperidol and Other Antipsychotics in Human Serum by LC-MS/MS: Method Development and Clinical Application. *American Journal of Analytical Chemistry*, **17**, 9-22. https://doi.org/10.4236/ajac.2026.172002

[7] Chinese Pharmacopoeia Commission (2020) Guidelines for Validation of Quantitative Analytical Methods for Biological Samples. In: *Pharmacopoeia of the People's Republic of China*, Volume 4, China Medical Science Press, 466-472. (In Chinese)

[8] Zhang, G.F., Li, Y. and Wang, Y. (2022) Progress in Pretreatment Methods for the Determination of Drug Concentrations in Biological Samples by Liquid Chromatography-Tandem Mass Spectrometry. *Chinese Journal of Analysis Laboratory*, **41**, 636-644. (In Chinese)

[9] Harris, N. and Marin, M. (2023) Commentary on Delayed Diagnosis in a 61-Year-Old Hispanic Male with Ecchymoses, Soft Tissue Bleeding, and Edema. *Clinical Chemistry*, **69**, 806-807. https://doi.org/10.1093/clinchem/hvad078

[10] Salamone, S.J., Courtney, J.B. and Clarke, W.A. (2023) New Immunoassays in Therapeutic Drug Monitoring: Where Is the Journey Heading? *Therapeutic Drug Monitoring*, **45**, 11-13. https://doi.org/10.1097/ftd.0000000000001048