Scientific Research Publishing

American Journal of Analytical Chemistry, 2026, 17(2), 9-22
https://www.scirp.org/journal/ajac
ISSN Online: 2156-8278
ISSN Print: 2156-8251

Simultaneous Quantification of Haloperidol and Other Antipsychotics in Human Serum by LC-MS/MS: Method Development and Clinical Application

Peng Huang[1*], Guosheng Su[2*], Jieling Lu[1#], Tiaoping Tao[3#], Liwen Huang[1], Liju Liao[1], Ruijian Lu[1], Yan Liao[1], Liefu Long[1]

[1]Clinical Laboratory, Baise Second People's Hospital, Baise, China
[2]Clinical Laboratory, Guangxi-ASEAN Technological Development Zone People's Hospital (The Tenth People's Hospital of Nanning), Nanning, China
[3]Baise Institute for Food and Drug Control, Baise, China
Email: [#]13117763266@163.com, [#]107842411@qq.com

How to cite this paper: Huang, P., Su, G.S., Lu, J.L., Tao, T.P., Huang, L.W., Liao, L.J., Lu, R.J., Liao, Y. and Long, L.F. (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

Received: January 5, 2026
Accepted: January 31, 2026
Published: February 3, 2026

Copyright © 2026 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Objective: To develop a high-throughput method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) for the simultaneous determination of serum concentrations of five commonly used antipsychotics (haloperidol, amisulpride, olanzapine, clozapine, and perphenazine) in order to address the issues of incomplete coverage and low efficiency in clinical monitoring during polypharmacy, thereby providing experimental evidence for personalized and precise medication in psychiatry. **Methods:** Haloperidol, amisulpride, olanzapine, clozapine, perphenazine and their corresponding deuterated internal standards (IS) were selected as analytes. Sample pretreatment procedures, chromatographic separation conditions, and mass spectrometric detection parameters were systematically optimized. Protein precipitation using acetonitrile as the precipitant was employed for serum sample preparation. Chromatographic separation was performed on an Agilent ZORBAX Eclipse Plus C18 column (2.1 × 100 mm, 1.8 μm) using a gradient elution program with mobile phases consisting of ultrapure water containing 0.1% formic acid and acetonitrile. The mass spectrometer operated in electrospray positive ionization mode with multiple reaction monitoring (MRM) for quantification. Comprehensive method validation, including specificity, linearity, lower limit of quantification (LLOQ), precision, accuracy, matrix effect, extraction recov-

*Co-first authors.
#Co-corresponding authors.

ery, and stability, was conducted according to the guidelines of "9012 Guidance for Validation of Quantitative Analytical Methods for Biological Samples" from the Chinese Pharmacopoeia (2020 Edition, Volume IV). The established method was applied to therapeutic drug monitoring (TDM) in 150 patients with psychiatric disorders admitted to Baise Second People's Hospital from January 2025 to December 2025. **Results:** An LC-MS/MS method capable of simultaneous analysis within 8 minutes was successfully established. All analytes showed good linearity in the range of 10 - 250 ng/mL with correlation coefficients (r^2) greater than 0.998. The LLOQ was 10 ng/mL, with signal-to-noise ratios (S/N) exceeding 15. For all concentration levels, intra-day and inter-day precision were ≤8.1% and ≤9.7%, respectively; accuracy ranged from 94.2% to 106.5%. The matrix effect values were between 85.6% and 113.2%, and extraction recoveries ranged from 86.3% to 102.4%. Samples demonstrated good stability under various storage conditions. Clinical application to 150 samples revealed that in 97 samples (64.7%), the concentration of at least one drug was within the recommended therapeutic reference range; 41 samples (27.3%) had concentrations below the therapeutic window, and 12 samples (8.0%) exceeded the potential toxicity threshold. The results provided direct evidence for dosage adjustment in 35 patients. **Conclusion:** The developed LC-MS/MS method is simple, highly sensitive, specific, rapid, and allows for the simultaneous determination of multiple antipsychotics. Its performance characteristics meet the requirements for clinical TDM. This method addresses the shortcomings of the previous detection system by extending coverage to key antipsychotics, improving testing efficiency, and providing a reliable tool for personalized treatment in psychiatric disorders, demonstrating good potential for clinical application and promotion.

Keywords

Liquid Chromatography-Tandem Mass Spectrometry, Haloperidol, Amisulpride, Schizophrenia, Pharmacokinetics, Polypharmacy, Therapeutic Drug Monitoring, Method Validation

1. Introduction

With the intensification of social competition and the acceleration of the pace of life in modern society, the global incidence of mental disorders has shown a significant upward trend, becoming a major public health issue affecting public health and social development [1]. Schizophrenia, as a common and severe psychotic disorder, is characterized by complex etiology, high relapse rates, and high disability rates. Its pathophysiological mechanisms involve dysfunctions of multiple neurotransmitter systems in the brain, including dopamine, serotonin, and glutamate [2]. Pharmacotherapy is a core strategy for managing schizophrenia and other severe mental disorders. However, the clinical application of antipsychotic drugs faces several challenges: First, they have a narrow therapeutic win-

dow, meaning the blood concentration range for efficacy is very close to the concentration range that induces toxic side effects. Second, significant individual variability exists, where the same dosage produces markedly different blood concentrations and clinical effects among individuals, influenced by factors such as age, body weight, liver and kidney function, genetic polymorphisms, and drug interactions. Third, patient medication adherence is often poor, and long-term use of some drugs may induce severe adverse reactions such as extrapyramidal symptoms and metabolic syndrome [3] [4].

In this context, therapeutic drug monitoring (TDM), by quantitatively measuring the concentrations of drugs and their active metabolites in patient body fluids (primarily blood), provides objective and precise evidence for adjusting individualized dosing regimens. It has become a key tool for optimizing psychiatric pharmacotherapy and balancing efficacy and safety [5]. The consensus guidelines for TDM in neuropsychopharmacology issued by the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie (AGNP) systematically evaluate the evidence level and recommendation grade for TDM of various psychiatric drugs. Among them, drugs like haloperidol and amisulpride are classified as level 1 recommendations, indicating that TDM holds significant value for the clinical management of these drugs [4]. However, although traditional high-performance liquid chromatography (HPLC) methods are widely used, they have limitations such as insufficient sensitivity, poor selectivity, long analysis times, and difficulty in simultaneous multi-component determination [6], failing to meet the growing clinical demand for high-throughput, high-efficiency, and broad-coverage TDM.

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology combines the high separation efficiency of liquid chromatography with the high sensitivity and specificity of mass spectrometry, and has become the "gold standard" for quantitative analysis of trace substances in complex biological matrices [7] [8]. In recent years, the application of this technology in the field of psychiatric TDM has become increasingly widespread, with studies reporting methods for the simultaneous detection of several antidepressants or antipsychotics [9] [10]. However, a systematic literature review reveals that existing reported methods either fail to cover some key drugs such as haloperidol and amisulpride, or have cumbersome pretreatment steps, or excessively long chromatographic run times. To date, there is a lack of systematic reports on LC-MS/MS methods capable of simultaneously, rapidly, and accurately determining a panel of antipsychotics, including haloperidol, amisulpride, olanzapine, clozapine, and perphenazine, based on the spectrum of drugs commonly used in clinical practice in China [11] [12].

Baise Second People's Hospital, as a pioneer in performing antipsychotic TDM in the western Guangxi region, has successively implemented HPLC and LC-MS/MS technologies for TDM since 2017. However, the number of drugs routinely monitored is currently limited to 10, still unable to cover AGNP level 1 recommended drugs like haloperidol and amisulpride, which are also commonly used in our hospital's clinical practice. Furthermore, existing methods have not inte-

grated all commonly used drugs into a single analytical run, causing inconvenience for monitoring patients on polypharmacy. Therefore, developing a rapid and accurate LC-MS/MS method capable of simultaneously determining multiple key antipsychotics (especially those not yet covered by our hospital) in serum is of urgent practical significance for improving the TDM service system of our hospital and even the Baise region, and for enhancing the level of rational drug use in psychiatry.

Based on our hospital's clinical medication practice and association guidelines, this study aims to systematically develop and validate an LC-MS/MS method for the simultaneous determination of serum concentrations of haloperidol, amisulpride, olanzapine, clozapine, and perphenazine in human serum. The research encompasses method development, comprehensive method validation, and preliminary clinical application evaluation, with the goal of providing a reliable high-throughput TDM tool for clinical practice and promoting the implementation of personalized and precise treatment in psychiatry.

2. Materials and Methods

2.1. Instruments and Reagents

Instruments: LCMS-8050 CL triple quadrupole liquid chromatography-tandem mass spectrometer (Shimadzu Corporation, Japan) equipped with an electrospray ionization (ESI) source and an LC-30AD liquid chromatography system; Agilent ZORBAX Eclipse Plus C18 chromatographic column (2.1 × 100 mm, 1.8 μm); TG16-WS high-speed refrigerated centrifuge (Xiangyi Laboratory Instrument Development Co., Ltd., Hunan, China); XH-E vortex mixer (Kangjian Medical Supplies Co., Ltd., Jiangsu, China); micropipettes (Eppendorf, Germany); Milli-Q ultrapure water system (Millipore, USA).

Reagents and Standards: Haloperidol (purity >98%, batch no. H1025), amisulpride (>98%, batch no. A1028), olanzapine (>98%, batch no. O1015), clozapine (>98%, batch no. C1032), perphenazine (>98%, batch no. P1011) and their corresponding deuterated internal standards: haloperidol-d4, amisulpride-d5, olanzapine-d3, clozapine-d4, perphenazine-d5 were all purchased from Hunan Demeter Instrument Co., Ltd. Methanol and acetonitrile were MS grade (Thermo Fisher Scientific, USA); formic acid was HPLC grade (Merck, Germany); all water used was ultrapure water (resistivity ≥18.2 MΩ·cm).

Blank Serum and Clinical Samples: Blank serum was obtained from leftover serum samples of healthy volunteers (with informed consent and confirmed not taking relevant psychiatric drugs) undergoing physical examination at our hospital, and stored at −80˚C for later use. Clinical validation samples were retrospectively analyzed steady-state trough concentration serum samples (collected in the morning before medication) from 150 patients with psychiatric disorders (diagnosis conforming to ICD-10 criteria) who were hospitalized or treated as outpatients at Baise Second People's Hospital and receiving relevant antipsychotic drug therapy from January 2025 to December 2025. This study was approved by the Medical Ethics Committee of Baise Second People's Hospital (Approval No.:

BSEY-LL-2025-018). Informed consent was obtained from all patients or their legal guardians.

2.2. Preparation of Solutions

Preparation of Standard Stock and Working Solutions: An appropriate amount of each drug standard was accurately weighed, dissolved, and diluted with methanol to prepare single standard stock solutions with a concentration of 1.0 mg/mL, stored at −20˚C protected from light. Mixed standard working solutions at appropriate concentrations were prepared by serial dilution with methanol before use. Internal standard stock and working solutions were prepared similarly.

Preparation of Calibration Curve and Quality Control (QC) Samples: 190 μL of blank serum was pipetted into a 1.5 mL EP tube, followed by the addition of 10 μL of mixed standard working solutions at different concentrations and 10 μL of mixed internal standard working solution (final IS concentration of 50 ng/mL for each), vortex-mixed for 30 seconds to prepare calibration curve samples at concentrations of 10, 25, 50, 100, 150, and 250 ng/mL. QC samples at low (25 ng/mL), medium (100 ng/mL), and high (200 ng/mL) concentration levels were prepared in the same manner.

2.3. Sample Pretreatment

200 μL of serum sample (calibration curve, QC, or clinical sample) was aliquoted into a 1.5 mL EP tube. 20 μL of mixed internal standard working solution (final concentration 50 ng/mL) was added, followed by 500 μL of ice-cold acetonitrile as protein precipitation reagent. The mixture was vortexed for 3 minutes for thorough mixing and then centrifuged at 4˚C, 14,500 rpm for 10 minutes. Approximately 200 μL of the supernatant was carefully transferred into an insert vial. 2 μL was injected for LC-MS/MS analysis.To assess potential solvent effects, the impact of injecting the undiluted acetonitrile supernatant (approximately 70% organic phase) on chromatographic peak shape was evaluated. Results showed symmetrical peaks without significant distortion, indicating negligible solvent effects under the initial gradient conditions (15% acetonitrile); therefore, an additional dilution step was omitted.

2.4. Chromatographic and Mass Spectrometric Conditions

Chromatographic Conditions: Mobile phase A was 0.1% formic acid in water, and mobile phase B was acetonitrile. The gradient elution program was set as follows: 0 - 1.0 min, maintain 15% B; 1.0 - 4.0 min, B linearly increased from 15% to 70%; 4.0 - 5.0 min, maintain 70% B; 5.0 - 5.1 min, B rapidly increased from 70% to 95%, and maintained until 6.5 min; 6.5 - 6.6 min, B rapidly decreased from 95% to 15%, followed by re-equilibration at 15% B until 8.0 min. The flow rate was set at 0.35 mL/min. Column oven temperature: 40˚C. Autosampler temperature: 10˚C. Total analysis run time: 8.0 minutes.

Mass Spectrometric Conditions: Electrospray ionization (ESI) source was used

with positive ion mode acquisition. Source parameters were set as: desolvation line (DL) temperature 250˚C; heat block temperature 400˚C; nebulizing gas (nitrogen) flow 3.0 L/min; drying gas (nitrogen) flow 10 L/min; heating gas (air) flow 10 L/min. The scan mode was multiple reaction monitoring (MRM). The optimized MS parameters for each analyte and corresponding IS are detailed in Table 1.

Table 1. Multiple Reaction Monitoring (MRM) parameters for the five antipsychotics and their deuterated internal standards.

Analyte	Precursor Ion (m/z)	Product Ion (m/z)	CE (V)	Internal Standard	Precursor Ion (m/z)	Product Ion (m/z)	CE (V)
Haloperidol	376.2	**165.1***	30	Haloperidol-d4	380.2	165.1	30
		123.1	40				
Amisulpride	370.1	**242.1***	25	Amisulpride-d5	375.1	247.1	25
		112.1	40				
Olanzapine	313.2	**256.1***	25	Olanzapine-d3	316.2	256.1	25
		198.1	35				
Clozapine	327.1	**270.1***	25	Clozapine-d4	331.1	274.1	25
		192.1	40				
Perphenazine	404.2	**171.1***	30	Perphenazine-d5	409.2	171.1	30
		143.1	40				

***Note:** The product ion marked with an asterisk is the quantifier ion transition.*

2.5. Method Validation

Bbbnnn Method validation was performed systematically with reference to the "9012 Guidance for Validation of Quantitative Analytical Methods for Biological Samples" in the Chinese Pharmacopoeia (2020 Edition, Volume IV) and international standards [13].

1) Specificity: Six individual blank serum samples from different sources, blank serum spiked with analytes at LLOQ concentration and IS, and actual patient serum samples were analyzed to investigate the presence of interfering endogenous substances within the retention time windows of the target analytes and IS.

2) Linearity and Lower Limit of Quantification (LLOQ): Calibration curves were constructed by plotting the peak area ratio of each analyte to its corresponding IS versus the analyte concentration, using weighted ($1/x^2$) least squares linear regression analysis. The LLOQ was defined as the lowest concentration with a signal-to-noise ratio (S/N) ≥ 10, accuracy within 80% - 120%, and precision (RSD) ≤ 20%.

3) Precision and Accuracy: Intra-day precision was assessed by analyzing five replicates of QC samples at low, medium, and high concentration levels within a single batch. Inter-day precision was assessed by analyzing QC samples at the same three levels in three separate batches on different days. Precision was expressed as relative standard deviation (RSD). Accuracy was expressed as the percentage of the mean measured concentration relative to the nominal concentration.

4) Extraction Recovery and Matrix Effect: Matrix effect was evaluated using the post-column infusion technique. Extraction recovery was calculated by comparing the analyte peak area from pre-extraction spiked QC samples (low and high concentrations) after sample pretreatment to the analyte peak area from post-extraction spiked samples where the same amount of standard was added to the supernatant of processed blank matrix. Previous studies indicate that effective control of matrix effect and stable extraction recovery are key to ensuring the accuracy of biological sample analysis [14].

5) Stability: The stability of QC samples was investigated under various conditions: at room temperature for 24 hours; after three complete freeze-thaw cycles; after storage at −20˚C for 14 days. Considering that clinical samples are typically analyzed shortly after collection, the 14-day stability data are sufficient for routine testing cycles. For samples requiring long-term storage, storage at −80˚C is recommended, and their long-term stability will be addressed in future studies. and processed samples were placed in the autosampler (10˚C) for 24 hours. Comprehensive stability verification has been confirmed as a necessary prerequisite for applying a method to routine clinical testing [15].

2.6. Clinical Application

The validated method was integrated into the hospital's routine TDM workflow. Serum concentrations were determined in 150 samples from patients receiving relevant antipsychotic therapy. Measured drug concentrations were compared with the therapeutic reference ranges recommended in consensus guidelines [4] to assess patients' blood concentration levels. Combined with clinical efficacy records and adverse reaction reports, the results provided reference suggestions for clinicians regarding dosage adjustment. Studies have indicated that TDM based on LC-MS/MS technology also holds significant value in assessing medication adherence in psychiatric patients [16].

2.7. Data Analysis

Chromatographic data were acquired and processed using LabSolutions software (version 5.97, Shimadzu, Japan). Statistical analyses, including calibration curve fitting, precision, and accuracy, were performed using Microsoft Excel 2019 and SPSS 30.0 software.

3. Results

3.1. Method Development and Optimization

Through systematic optimization of mobile phase composition, gradient elution program, ion source parameters, and MRM transitions, an LC-MS/MS method capable of separating and detecting the five target drugs within 8 minutes was established. **Figure 1** shows representative MRM chromatograms under optimized conditions for a blank serum sample, a blank serum sample spiked with analytes at LLOQ and IS, and a typical patient serum sample. The results show sharp and sym-

metrical chromatographic peaks for all analytes and corresponding IS, with stable retention times. No significant interfering peaks from endogenous substances were observed at the elution positions of the target analytes in blank serum.

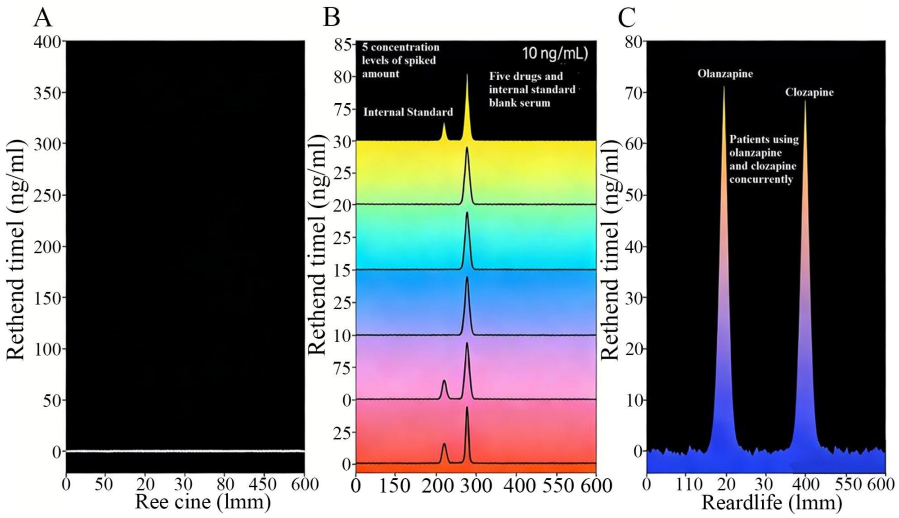

Figure 1. Representative MRM chromatograms. (A) Blank serum; (B) Blank serum spiked with LLOQ concentrations (10 ng/mL) of the five drugs and corresponding internal standards; (C) A typical patient serum sample from a patient concurrently taking olanzapine and clozapine).

3.2. Method Validation Results

1) Specificity: Six individual blank serum samples from different sources showed no significant interfering chromatographic peaks (response intensity less than 20% of the LLOQ response) within the retention time windows of the target analytes and corresponding IS, indicating good specificity of the method.

2) Linearity and LLOQ: All five target drugs showed good linearity in the concentration range of 10 - 250 ng/mL. Typical regression equations and correlation coefficients are detailed in **Table 2**. The correlation coefficients (r^2) for all analytes were greater than 0.998. The LLOQ was determined to be 10 ng/mL. At this concentration, the S/N for all drugs exceeded 15, accuracy ranged from 88.5% to 105.3%, and precision RSD was ≤12.8%, fully complying with the acceptance criteria for method validation. In similar studies, LLOQs for methods simultaneously detecting classical antipsychotics are mostly between 2 - 10 ng/mL [17]. The LLOQ set in this method aligns with clinical practical needs.

Table 2. Linear regression equations, correlation coefficients, and LLOQ for the five antipsychotics.

Analyte	Linear Range (ng/mL)	Regression Equation	Correlation Coefficient (r^2)	LLOQ (ng/mL)
Haloperidol	10 - 250	y = 0.0451x + 0.0023	0.9992	10
Amisulpride	10 - 250	y = 0.0387x + 0.0018	0.9987	10
Olanzapine	10 - 250	y = 0.0524x + 0.0031	0.9995	10
Clozapine	10 - 250	y = 0.0412x + 0.0045	0.9989	10
Perphenazine	10 - 250	y = 0.0368x + 0.0021	0.9991	10

3) Precision and Accuracy: As shown in Table 3, the intra-day precision RSD for QC samples at low, medium, and high concentration levels ranged from 2.5% to 8.1%, and inter-day precision RSD ranged from 3.8% to 9.7%; accuracy ranged from 94.2% to 106.5%. All results met the general acceptance criteria for bioanalytical method validation. Studies have reported intra-day and inter-day precision less than 10% for similar detection methods [18]. The precision indices of this method are consistent, indicating good reproducibility of the results.

Table 3. Method precision and accuracy validation results.

Analyte	Concentration (ng/mL)	Intra-day Precision (RSD, %)	Inter-day Precision (RSD, %)	Accuracy (%)
Haloperidol	25	7.2	8.9	98.5
	100	4.5	6.3	102.1
	200	3.8	5.1	99.8
Amisulpride	25	8.1	9.7	95.3
	100	5.8	7.4	101.7
	200	4.2	6.0	103.5
Olanzapine	25	6.3	8.2	101.2
	100	3.9	5.6	99.4
	200	2.5	4.1	98.7
Clozapine	25	7.5	8.5	94.2
	100	5.1	6.8	106.5
	200	3.6	5.3	104.8
Perphenazine	25	6.8	8.0	97.6
	100	4.7	6.2	102.9
	200	3.2	4.5	100.3

4) Matrix Effect and Extraction Recovery: Evaluation results showed that for the five drugs at low and high concentration levels, the IS-normalized matrix factor values ranged from 85.6% to 113.2%, with RSDs all less than 8.5%, indicating that the matrix effect was within a controlled range and reproducible. Using the one-step acetonitrile precipitation method for pretreatment, the average extraction recoveries for each drug at low and high concentrations ranged from 86.3% to 102.4%, with RSDs less than 7.8%, meeting methodological requirements. Studies using similar protein precipitation methods for serum sample processing have reported extraction recoveries of 86.2% - 104.8% [19]. The extraction efficiency of this method is comparable and the operation is simpler and faster.

5) Stability: Stability experiment data confirmed that for QC samples placed at room temperature for 24 hours, subjected to three freeze-thaw cycles, stored frozen at −20˚C for 14 days, and processed samples placed in the autosampler (10˚C) for 24 hours, the variation in each drug concentration was within ±12%, indicating

good stability of samples under these different storage and processing conditions.

3.3. Clinical Application Results

From January 2025 to December 2025, the method developed in this study was applied to TDM of 150 clinical samples. The basic demographic characteristics and medication profiles of the patients are shown in Table 4. The distribution of detection results is shown in Figure 2: in 97 samples, the blood concentration of at least one drug fell within the recommended therapeutic reference range; in 41 samples, drug concentrations were below the lower limit of the therapeutic window; and in 12 samples, drug concentrations exceeded the potential toxicity threshold.

Table 4. Basic characteristics of the clinical validation cohort patients (N = 150).

Characteristic	Value/Proportion	
Gender (Male/Female)	92/58	
Mean Age (years, range)	40.1 (13 - 77)	
Primary Diagnosis	Schizophrenia	132 (88.0%)
	Epileptic psychosis	7 (4.7%)
	Bipolar affective disorder	2 (1.3%)
	Organic mental disorders	2 (1.3%)
	Recurrent depressive disorder	1 (0.7%)
	Mental retardation	1 (0.7%)
	Mental retardation with psychotic disorders	1 (0.7%)
	Mental and behavioral disorders due to use of alcohol	3 (2.0%)
	Obsessive-compulsive disorder, Anxiety disorders	1 (0.7%)
Medication Pattern Involved in Testing	Monotherapy	65 (43.3%)
	Two-drug combination therapy	71 (47.3%)
	Three or more drug combination therapy	14 (9.3%)

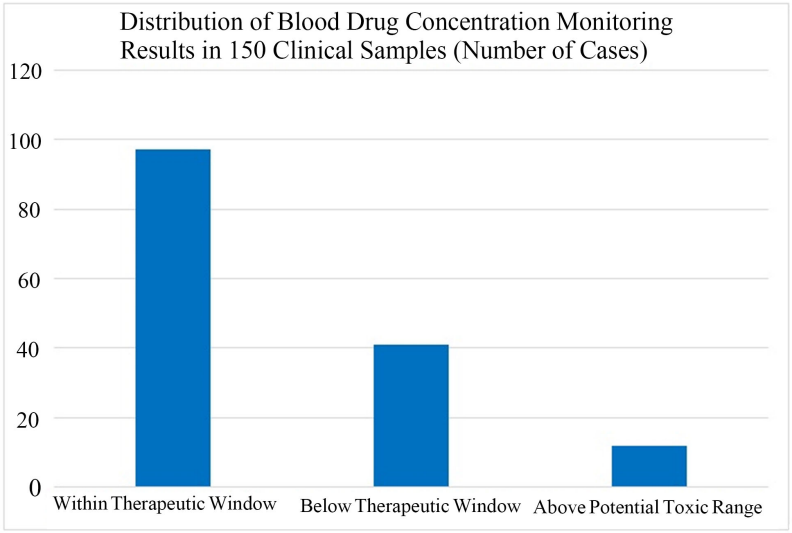

Figure 2. Distribution of blood concentration monitoring results from 150 clinical samples.

Based on TDM results combined with comprehensive clinical assessment, drug therapy regimens were adjusted for 35 patients. Subsequent follow-up showed that among these, 28 patients showed improved Clinical Global Impression (CGI) scores or reduced drug-related adverse reactions after regimen adjustment, preliminarily demonstrating the potential value of this method in guiding clinical individualized medication.

4. Discussion

This study successfully developed and validated an LC-MS/MS method for the simultaneous determination of five commonly used antipsychotics—haloperidol, amisulpride, olanzapine, clozapine, and perphenazine—in human serum. Compared with similar methods reported in the literature, this method exhibits several significant advantages. First, the drug panel covered is more clinically practical and targeted. Closely aligned with our hospital's actual medication practices and referencing international authoritative guidelines, we have, for the first time, integrated haloperidol and amisulpride—two important drugs currently not routinely monitored in our hospital and region—into a single analytical run, effectively filling gaps in the TDM service. Second, the analytical method is highly efficient and rapid. By optimizing the chromatographic gradient conditions, the total analysis time was controlled within 8 minutes. Compared to some reported methods with run times exceeding 15 minutes, this significantly improves sample throughput, better meeting the timeliness requirements of clinical laboratories for high-volume sample screening. Third, the sample pretreatment procedure is simple. The one-step acetonitrile precipitation method is straightforward, fast, and reproducible, avoiding cumbersome steps like solid-phase extraction, reducing operational complexity and potential errors, and making it easier to implement and promote in routine clinical laboratory work.

Comprehensive method validation results confirm the reliability and robustness of this method. All validation indices met or exceeded the standards set by the Chinese Pharmacopoeia and international mainstream bioanalytical method validation guidelines. The LLOQ meets the needs for monitoring therapeutic concentrations of the relevant drugs. Good precision, accuracy, and stability provide solid assurance for the reliability and inter-laboratory comparability of the results. Controlled matrix effects and stable high extraction recoveries also attest to the robustness and reliability of the pretreatment protocol and chromatographic-mass spectrometric conditions.

Preliminary clinical application results demonstrate the practical value of this method. Among the 150 tests, 35.3% of patients had drug concentrations deviating from the ideal therapeutic range (27.3% below the therapeutic window, 8.0% above the potential toxicity threshold), once again highlighting the necessity of implementing routine TDM in psychiatry. The successful application of this method enables clinicians, for the first time, to obtain precise concentration data for drugs like haloperidol and amisulpride in local patients, providing the possi-

bility for evidence-based dose adjustments. The positive therapeutic outcomes observed in some patients following regimen adjustments based on monitoring results preliminarily corroborate the method's contribution to optimizing treatment outcomes, consistent with conclusions from other studies that TDM can improve the efficacy and safety of psychiatric treatment.

The method developed in this study not only improves the technical system for TDM in our hospital and expands the list of detectable drugs but also, due to its high-throughput nature, enhances the overall work efficiency of the laboratory. More importantly, this method provides a reliable and standardized technical model for promoting the application of psychiatric TDM in this region and even more broadly, contributing to the homogenization and improvement of the quality of regional mental health services.

5. Conclusion

This study successfully developed and validated a rapid, sensitive, and accurate LC-MS/MS method for the simultaneous determination of serum concentrations of haloperidol, amisulpride, olanzapine, clozapine, and perphenazine in human serum. The method underwent systematic and rigorous method validation with excellent performance indicators, fully meeting the requirements for quantitative analysis of clinical biological samples. This method effectively addresses the shortcomings of the previous detection system, such as insufficient coverage of some key drugs and inconvenience in monitoring polypharmacy, achieving efficient and simultaneous monitoring of clinically commonly used antipsychotics. Preliminary clinical application indicates that this method can provide key laboratory data support for implementing personalized and precise treatment in psychiatry. It holds significant practical importance for optimizing drug therapy strategies, improving efficacy, and reducing the risk of adverse reactions, demonstrating good potential for clinical application and promotion.

6. Study Limitations

This study also has several limitations. First, although the five included drugs are commonly used clinically, they do not cover all categories of antipsychotics; future work could further expand the detection panel. Second, while the sample size in the clinical validation phase was substantial, larger sample sizes and well-designed prospective intervention studies would more strongly demonstrate the impact of TDM on improving patients' clinical hard endpoints. Third, this is a single-center study; the transferability and reproducibility of this method across different laboratories and instrument platforms require validation involving more centers. Furthermore, future studies could consider incorporating the simultaneous monitoring of active metabolites to provide more comprehensive pharmacokinetic information.

Funding

This work was supported by the Second Batch of University-Level Scientific Re-

search Project of Youjiang Medical University for Nationalities in 2025 (Project No.: yy2025ky176).

Acknowledgements

We thank the Department of Science and Technology of Youjiang Medical University for Nationalities for the project approval and financial support. We sincerely thank all colleagues in the Department of Clinical Laboratory of Baise Second People's Hospital for their tremendous assistance in sample collection and testing. We also appreciate the valuable cooperation of clinicians from various departments in our hospital in integrating case information and interpreting clinical significance.

Conflicts of Interest

All authors solemnly declare that there are no actual or potential conflicts of interest in the design, implementation, data analysis, manuscript writing, and publication of this study.

References

[1] GBD 2019 Mental Disorders Collaborators (2022) Global, Regional, and National Burden of 12 Mental Disorders in 204 Countries and Territories, 1990-2019: A Systematic Analysis for the Global Burden of Disease Study 2019. *Lancet Psychiatry*, **9**, 137-150.

[2] McCutcheon, R.A., Reis Marques, T. and Howes, O.D. (2020) Schizophrenia—An Overview. *JAMA Psychiatry*, **77**, 201-210. https://doi.org/10.1001/jamapsychiatry.2019.3360

[3] Schoretsanitis, G. and de Leon, J. (2021) Therapeutic Drug Monitoring of Antipsychotics: A Call for a Systematic Approach. *Journal of Clinical Psychopharmacology*, **41**, 623-628.

[4] Hiemke, C., Bergemann, N., Clement, H.W., *et al.* (2018) Consensus Guidelines for Therapeutic Drug Monitoring in Neuropsychopharmacology: Update 2017. *Pharmacopsychiatry*, **51**, 9-62.

[5] Gründer, G., Hahn, M. and Koller, G. (2020) TDM in Psychiatry and Neurology: A Comprehensive Review. *Therapeutic Drug Monitoring*, **42**, 1-10.

[6] Patteet, L., Maudens, K.E., Sabbe, B., *et al.* (2021) Advances in the Detection of Antipsychotics in Biological Matrices. *Clinica Chimica Acta*, **523**, 19-32.

[7] Vogeser, M. and Seger, C. (2021) Liquid Chromatography-Tandem Mass Spectrometry—Techniques and Applications for Clinical Diagnosis. *The Electronic Journal of IFCC*, **32**, 15-24.

[8] Seger, C. and Salzmann, L. (2020) After Another Decade: LC-MS/MS Became Routine in Clinical Diagnostics. *Clinical Biochemistry*, **82**, 2-11. https://doi.org/10.1016/j.clinbiochem.2020.03.004

[9] Wilhelm, A.J., den Burger, J.C.G., Chahbouni, A. and Swart, E.L. (2022) Simultaneous Quantification of Antipsychotic Drugs in Human Plasma by LC-MS/MS. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, **1188**, Article 123069.

[10] Koller, D., Zander, J., von Rheinbaben, F., *et al.* (2023) A Validated LC-MS/MS Method for Simultaneous Quantification of 15 Antipsychotics and 7 Metabolites in Human Serum and Its Application to TDM Data. *Journal of Pharmaceutical and Biomedical Analysis*, **222**, Article 115041.

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

[12] Fan, X.Y., Wang, S.W. and Fan, G.R. (2021) Simultaneous Determination of the Concentrations of Fluorouracil, Uracil and Dihydrouracil in Human Plasma by LC-MS/MS Method and Its Clinical Application. *Pharmaceutical Care and Research*, **21**, 12-18. https://doi.org/10.5428/pcar20210103

[13] Chinese Pharmacopoeia Commission (2020) Pharmacopoeia of the People's Republic of China: Volume IV. 2020 Edition. China Medical Science Press, 466-472.

[14] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER) and Center for Veterinary Medicine (CVM) (2018) Bioanalytical Method Validation Guidance for Industry.

[15] Zhang, G., Terry, A.V. and Bartlett, M.G. (2007) Sensitive LC-MS/MS Method for Simultaneous Determination of Antipsychotics in Rat Plasma. *Journal of Chromatography B*, **858**, 276-281.

[16] Patteet, L., Maudens, K.E., Sabbe, B., *et al.* (2014) High Throughput Identification and Quantification of Antipsychotics in Serum Using UHPLC-MS/MS. *Clinica Chimica Acta*, **429**, 51-58.

[17] Schoretsanitis, G., Paulzen, M., Unterecker, S., *et al.* (2018) TDM in Psychiatry and Neurology: A Comprehensive Review. *Therapeutic Drug Monitoring*, **40**, 389-413.

[18] Zander, J., Bruegel, M., Kleinhempel, A., *et al.* (2008) Method Development for Quantitative Determination of Antipsychotics by MEKC. *Journal of Chromatography B*, **863**, 1-9.

[19] Hiemke, C. (2008) Clinical Utility of Drug Measurement and Pharmacokinetics—Therapeutic Drug Monitoring in Psychiatry. *European Journal of Clinical Pharmacology*, **64**, 159-166. https://doi.org/10.1007/s00228-007-0430-1