Drug Design, Development and Therapy

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ORIGINAL RESEARCH

Therapeutic Drug Monitoring for Individualized Antidepressant Treatment

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Objective: This study aimed to establish a UPLC-MS/MS method for the simultaneous quantification of five antidepressants: venlafaxine (VEN) and its metabolite O-desmethylvenlafaxine (ODV), mirtazapine (MIR), sertraline (SER), escitalopram (ESC), and vortioxetine (VTX) in human plasma and saliva. By analyzing real-world therapeutic drug monitoring (TDM) data, this study aimed to identify key factors influencing drug concentrations thereby optimizing personalized treatment strategies for patients with depression and advancing precision medicine.

Methods: Following liquid-liquid extraction for plasma and protein precipitation for saliva, analyte concentrations were determined using a fully validated UPLC-MS/MS method. Validation included assessments of selectivity, linearity, accuracy, precision, extraction recovery, matrix effects, stability, and dilution integrity. The established method was applied to clinical samples, with further investigation into how clinical factors, including age, BMI, renal function (as measured by GFR), total protein (TP), albumin levels, and concomitant medications, influenced the concentration-to-dose ratio (CDR).

Results: The method demonstrated excellent linearity (5–500 ng/mL) with all validation parameters meeting acceptance criteria. The established method was successfully applied to analyze 566 plasma and 39 saliva samples. TDM revealed significant variations in target attainment rates among different antidepressants, along with varying degrees of dose-concentration correlations. Multivariate analysis demonstrated that the CDR of VEN + ODV was primarily influenced by age and GFR, while the CDR of MIR showed a significant association with BMI. The CDR of SER was affected by both BMI and TP levels. The CDR of ESC was modulated by age, concomitant medications, and renal function.

Conclusion: This study demonstrated that TDM-based individualized medication strategies can support the optimization of antidepressant efficacy. Saliva monitoring requires further validation. Clinicians should adopt dynamic, patient-specific monitoring to enhance precision medicine outcomes in depression management.

Keywords: UPLC-MS/MS, antidepressants, plasma, saliva, therapeutic drug monitoring

Introduction

Depression is a highly prevalent mental disorder worldwide, characterized by persistent and significant low mood, accompanied by symptoms such as psychomotor retardation, diminished volition, somatic disturbances, and suicidal behaviors, which severely impair patients' quality of life.[1,2] According to the World Health Organization (WHO), the global prevalence of depression is approximately 5%, with a steadily increasing trend and a significantly higher incidence among women than among men. This gender disparity highlights the complexity of depression pathogenesis and the importance of targeted interventions.[3,4] Pharmacotherapy remains the cornerstone of the clinical management of depression and plays a pivotal role in alleviating symptoms and restoring social functioning.

Current first-line antidepressant medications primarily modulate monoaminergic neurotransmitter systems, particularly serotonin (5-hydroxytryptamine, 5-HT), norepinephrine (NE), and dopamine (DA). Selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs) are recommended as first-line treatments owing to their favorable efficacy and safety profiles. For patients with treatment-resistant depression, noradrenergic and specific serotonergic antidepressants (NaSSAs) and multimodal antidepressants serve as second-line options, offering the

Received: 10 September 2025
Accepted: 18 December 2025
Published: 24 December 2025

synergistic modulation of multiple neurotransmitter systems to enhance therapeutic outcomes. Venlafaxine (VEN), mirtazapine (MIR), sertraline (SER), escitalopram (ESC), and vortioxetine (VTX) are widely endorsed in international guidelines as first-line agents because of their demonstrated efficacy and tolerability.[5,6]

Achieving an optimal balance between efficacy and safety is central to antidepressant therapy, with plasma drug concentrations serving as critical determinants of both therapeutic and adverse effects. Interindividual variability in drug concentrations arises from factors such as pharmacokinetic properties, patient-specific characteristics (eg, age and hepatic/renal function), and genetic polymorphisms in drug-metabolizing enzymes. Clinical challenges persist, including ambiguous efficacy markers, pronounced individual variability, and complex drug-drug interactions.[7,8] Therapeutic drug monitoring (TDM) has emerged as an indispensable tool in personalized antidepressant therapy. By systematically integrating dosage, plasma drug concentrations, and clinical response data, TDM enables clinicians to assess adherence, monitor concentration changes, adjust doses to avoid subtherapeutic or toxic levels, and tailor regimens based on individual pharmacokinetic profiles, thereby optimizing treatment safety and efficacy.[9–11]

The choice of biological matrix for TDM significantly affects the accuracy and clinical relevance of the measurements. Although plasma remains the gold standard for drug quantification, reflecting total drug concentrations with well-established correlations to pharmacological and toxic effects,[12] saliva has gained increasing attention as a noninvasive alternative. Salivary sampling offers advantages, such as ease of collection, improved patient compliance, and simplified storage and transport logistics, particularly in pediatric and geriatric populations.[13,14] Notably, salivary drug concentrations often correlate strongly with unbound plasma concentrations, as drugs in saliva predominantly exist in their free, pharmacologically active form.[14] Therefore, saliva is a promising matrix for drug monitoring, disease diagnosis, and therapeutic evaluation.

However, existing antidepressant TDM studies have notable limitations. On one hand, many focus on single-drug analysis, lacking synchronous comparison and comprehensive evaluation of multiple commonly used antidepressants. On the other hand, despite saliva's promising potential for noninvasive TDM, established and validated methods for simultaneous quantification of multiple antidepressants in saliva remain scarce. Therefore, developing a reliable, efficient, and sensitive method applicable to multiple drugs in dual matrices (plasma and saliva) is crucial for advancing TDM's clinical application and personalized antidepressant therapy.

To address these limitations and unmet clinical needs, an ultra-high-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method was developed for the simultaneous quantification of five commonly prescribed antidepressants, VEN and its metabolite O-desmethylvenlafaxine (ODV), MIR, SER, ESC, and VTX, in both human plasma and saliva. UPLC-MS/MS was selected for its superior separation efficiency, sensitivity, and specificity, which are critical for reliable high-throughput analysis in therapeutic drug monitoring, making it ideally suited for analyzing complex biological matrices like plasma and saliva. By analyzing real-world drug concentration data and identifying key influencing factors, this study aimed to provide a scientific foundation for optimizing personalized treatment strategies, ultimately advancing precision medicine in depression care while minimizing adverse event risks.

Materials, Subjects, and Methods

Study Subjects and Methodology

Study Subjects

This retrospective study analyzed plasma and salivary drug concentrations along with clinical data from patients diagnosed with depression who received antidepressant treatment (including VEN, MIR, SER, ESC, and VTX) at our institution between September 2023 and September 2024. The collected parameters included sex, age, body mass index (BMI), glomerular filtration rate (GFR), total protein (TP) level, albumin level, dosage regimen, and concomitant antidepressant use. This study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Hebei General Hospital Ethics Committee Review Board (Approval Nos. 2024382 and 2024383). Informed consent was obtained from all participants involved in the study.

The inclusion criteria were: (1) diagnosis of depression according to the International Classification of Diseases (ICD) or Diagnostic and Statistical Manual of Mental Disorders (DSM) criteria; (2) maintenance of a stable antidepressant regimen for 5–7 half-lives to achieve steady-state plasma concentrations; and (3) collection of trough drug concentrations immediately before the next scheduled dose administration. The exclusion criteria were as follows: (1) blood sampling before reaching steady-state drug concentrations; (2) incomplete clinical laboratory data; and (3) recent history of substance abuse.

Sample Collection and Preparation

After patients maintained a stable antidepressant regimen for 5–7 half-lives to ensure steady-state concentrations, fasting plasma and saliva samples were collected the morning before drug administration. After centrifugation, the supernatants were aliquoted and stored at −20°C until drug concentration analysis. Plasma samples were subjected to liquid-liquid extraction (LLE) using methyl tert-butyl ether (MTBE) as the extraction solvent. The saliva samples were processed using protein precipitation (PP) with methanol as the precipitating agent.

The choice of pretreatment methods was determined by matrix properties: plasma contains high levels of proteins and lipids with complex interferences, therefore LLE using MTBE was selected to efficiently eliminate impurities and minimize matrix effects; saliva is a relatively clean matrix with low protein content, accordingly PP with methanol was employed due to its simplicity, efficiency, and capability to maintain acceptable recovery.

Analytical Methods and Validation

Instruments and Reagents

The analytical system consisted of an LC-30A ultra-high-performance liquid chromatography (UPLC) system (Shimadzu, Japan) coupled with an AB Sciex 5500 triple quadrupole mass spectrometer (AB Sciex, USA). Data acquisition was performed using the Analyst 1.6.1 software (AB Sciex, USA). Additional equipment included an ST16R high-speed refrigerated centrifuge (Thermo Fisher, USA), AB204-S electronic balance (Mettler Toledo, Switzerland), nitrogen evaporator (Beijing Bafang Reagent, China), and vortex mixer (Thermo Fisher, USA). The reference standards for VEN, ODV, MIR, SER, ESC, VTX, and VEN-D_6 (IS) were obtained. HPLC-grade methanol, formic acid, and MTBE were used throughout this study.

UPLC-MS/MS Conditions

Chromatographic separation was performed on a Titank C18 column (2.1 mm × 50 mm, 3.0 μm) using a mobile phase consisting of 0.1% formic acid in water (A) and methanol (B). The gradient elution program was: 20% B (0–1.0 min), 20–90% B (1.0–2.0 min), 90% B (2.0–4.5 min), 90–20% B (4.5–4.6 min), and 20% B (4.6–6.5 min). The flow rate was maintained at 0.45 mL/min with an injection volume of 5 μL. Electrospray ionization (ESI) in the positive ion mode with multiple reaction monitoring (MRM) was used for detection. The detailed mass spectrometry parameters for each analyte are listed in Table 1.

Table 1 Parameter of Mass Spectrometry

Analyze	Precursor Ion (m/z)	Product Ion (m/z)	Collision Energy (eV)
VEN-D_6	284.2	64.0	22
VEN	278.2	58.0	21
ODV	264.0	58.0	18
MIR	266.2	195.1	28
SER	306.2	159.0	20
ESC	325.2	108.9	28
VTX	299.1	150.0	30

https://doi.org/10.2147/DDDT.S566716

Solution Preparation

Stock solutions of each analyte were prepared in methanol at concentrations of 5000, 2500, 1250, 600, 300, 150, and 50 ng/mL for calibration standards and 400, 200, and 10 ng/mL for quality control (QC) samples. The internal standard working solution (VEN-D_6) was prepared at a concentration of 300 ng/mL in methanol. All solutions were stored at −20° C until use.

Method Validation

The method was validated according to the Bioanalytical Method Validation Guidance (Chinese Pharmacopoeia 2020 Edition) using healthy human plasma and saliva samples.

Selectivity

Selectivity was evaluated by comparing chromatograms of blank plasma with those of the lower limit of quantification (LLOQ) samples to confirm the absence of interfering peaks at the retention times of the analytes and internal standard. The same procedure was applied to the saliva samples. The interfering component signal for each analyte should be less than 20% of the LLOQ response for the corresponding analyte and less than 5% of the IS response.

Linearity and LLOQ

Calibration curves were constructed using blank plasma spiked with standard working solutions at concentrations of 5, 15, 30, 60, 125, 250, and 500 ng/mL. The curves were fitted by weighted ($1/x^2$) least-squares linear regression of peak area ratios versus nominal concentrations over the range of 5–500 ng/mL. The same procedure was performed for the saliva samples. The LLOQ was determined with a signal-to-noise ratio of at least 10:1 and was required to demonstrate acceptable accuracy and precision.

Precision and Accuracy

Intra- and inter-day precision and accuracy were assessed by analyzing six replicates of QC samples at four concentration levels (LLOQ, low-quality control (LQC), medium-quality control (MQC), and high-quality control (HQC)) on three consecutive days. Precision was expressed as the relative standard deviation (RSD), while accuracy was calculated as the percentage deviation of the measured concentration from the nominal concentration. The pre-defined acceptance criteria were: precision (RSD) and accuracy (relative error, RE) within ±15% for all QC levels except the LLOQ, for which ±20% was acceptable.

Extraction Recovery and Matrix Effect

Extraction recovery was determined by comparing the peak areas of pre-extraction spiked samples (A) with those of post-extraction spiked samples (B) at three QC levels (n=6). Matrix effects were evaluated by comparing the peak areas of analytes in post-extraction spiked samples (B) with those of neat standard solutions (C) at equivalent concentrations. The same evaluations were performed for the saliva samples. The coefficient of variation (RSD) for both extraction recovery and matrix effect was required to be less than 15% to demonstrate consistency and reliability.

Stability

The stability of the analytes in plasma and saliva was evaluated against pre-defined criteria. Using LQC and HQC samples (n=6), the acceptance criteria were an RSD of ≤15% and a RE within ±15% compared to freshly prepared samples. The testing conditions were as follows: (1) Freeze-thaw stability: three complete cycles (frozen at −20°C for ≥12 h, then thawed at 20–25°C for 30 min before immediate refreezing); (2) Long-term stability: storage at −20°C for 30 consecutive days; (3) Post-preparation stability: processed samples kept at 20–25°C for 6 h; (4) Autosampler stability: reconstituted samples stored in the autosampler at 4°C for 24 h.

Dilution Integrity

To address potential sample concentrations exceeding the upper limit of quantification, the dilution integrity was evaluated by preparing samples at 2000 ng/mL and diluting them 5- and 10-fold with a blank matrix. Six replicates of

each dilution were processed and analyzed to verify accuracy and precision. The precision and accuracy of the measured concentration for diluted analytes should be within ±15%.

Clinical Application and Data Analysis

The developed method was clinically validated by analyzing 566 plasma and 39 saliva samples collected from patients receiving antidepressant therapy (VEN, MIR, SER, ESC, or VTX). The concentration-to-dose ratio (CDR) serves as the primary pharmacokinetic parameter to systematically evaluate the impact of clinical factors on antidepressant plasma concentrations. The patients were stratified into three age groups: pediatric (<18 years), adult (18–65 years), and elderly (≥65 years). BMI-based classification included underweight (<18.5 kg/m²), normal weight (18.5–24 kg/m²), and overweight/obese (≥24 kg/m²) categories.

Based on their metabolic characteristics, antidepressants were categorized as either CYP450 enzyme-inhibiting antidepressants (EIADs: fluoxetine, paroxetine, fluvoxamine, duloxetine, clomipramine, and VEN) or neutral antidepressants (NIADs: SER, MIR, ESC, VTX, and agomelatine) with minimal enzyme modulation effects. Renal function was assessed using GFR and classified as normal (>90 mL/min/1.73 m²), mild impairment (60–90 mL/min/1.73 m²), or moderate-severe impairment (<60 mL/min/1.73 m²). Total protein (TP) and albumin levels were dichotomized using cutoff values of 65 g/L and 40 g/L, respectively. All data underwent standardized processing and were analyzed using appropriate statistical methods to quantify the influence of clinical variables on CDR variations.

Statistical Analysis

All statistical analyses were performed using SPSS 21.0 (IBM Corp.) and GraphPad Prism 9.5.1 (GraphPad Software). The normality of continuous variables was assessed using the Shapiro–Wilk test. Normally distributed quantitative data are presented as mean ± standard deviation (SD), with between-group comparisons conducted using independent samples *t*-tests. Non-normally distributed data are expressed as medians with interquartile ranges (IQR, Q25%-Q75%). The Mann–Whitney *U*-test was used for two-group comparisons, whereas the Kruskal–Wallis test was used for multiple-group comparisons. Correlation analyses were performed using Spearman's rank correlation coefficient.

To comprehensively evaluate the influence of clinical factors on the CDR, an analytical strategy of univariate screening followed by multivariate verification was employed: univariate analysis was first conducted to screen potential factors, followed by the construction of multiple linear regression models incorporating age, BMI, GFR, TP, and albumin levels to further validate independent associations. It should be noted that, given the primary exploratory aim of this study to identify potential clinically relevant factors, no adjustment for multiple comparisons (eg, Bonferroni correction) was applied to the significance testing of individual predictors in the multivariate regression models, to mitigate the risk of Type II errors (false negatives) and preserve sensitivity to detect potentially important associations. Consequently, the relevant statistical findings should be interpreted as hypothesis-generating, and their importance should be evaluated in conjunction with effect sizes (eg, regression coefficient β) and clinical relevance. All statistical tests were two-tailed, with the significance level set at $\alpha = 0.05$. Statistical significance was defined as $P < 0.05$.

Results

Method Validation

Selectivity

The chromatograms of the analytes are shown in Figure 1. The retention times for VEN, ODV, MIR, SER, ESC, VTX, and VEN-D_6 in the plasma were 2.30, 2.08, 2.14, 2.49, 2.32, 2.52, and 2.30 min, respectively. For saliva, the retention times were 2.30, 2.11, 2.14, 2.49, 2.33, 2.52, and 2.30 min, respectively. No endogenous interference was observed in the blank plasma or saliva samples at the retention times of the analytes or IS.

Linearity and LLOQ

The calibration curves for VEN, ODV, MIR, SER, ESC, and VTX exhibited excellent linearity ($r > 0.99$) across a concentration range of 5–500 ng/mL in both plasma and saliva. The LLOQ was set to 5 ng/mL.

 https://doi.org/10.2147/DDDT.S566716

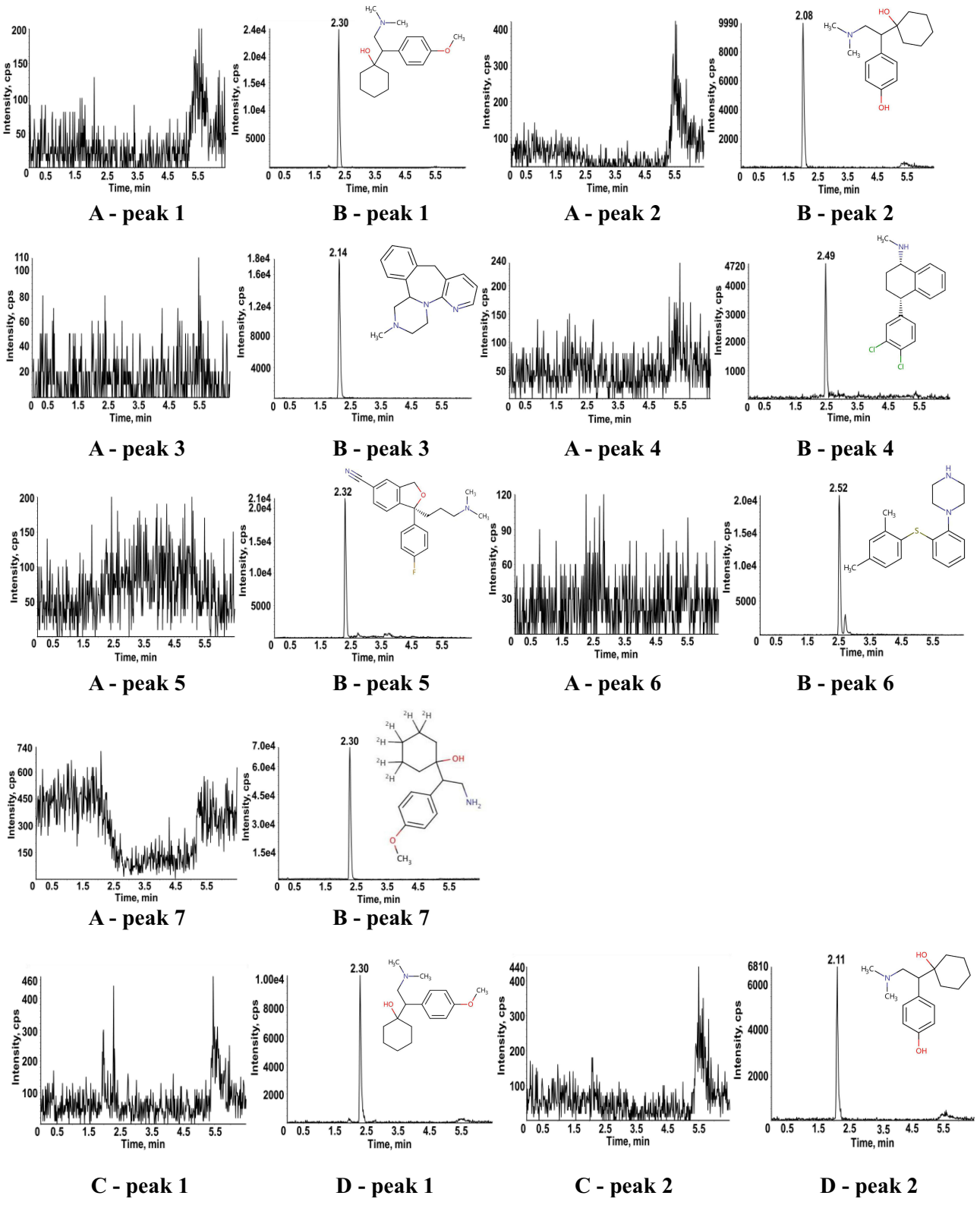

Figure 1 Continued.

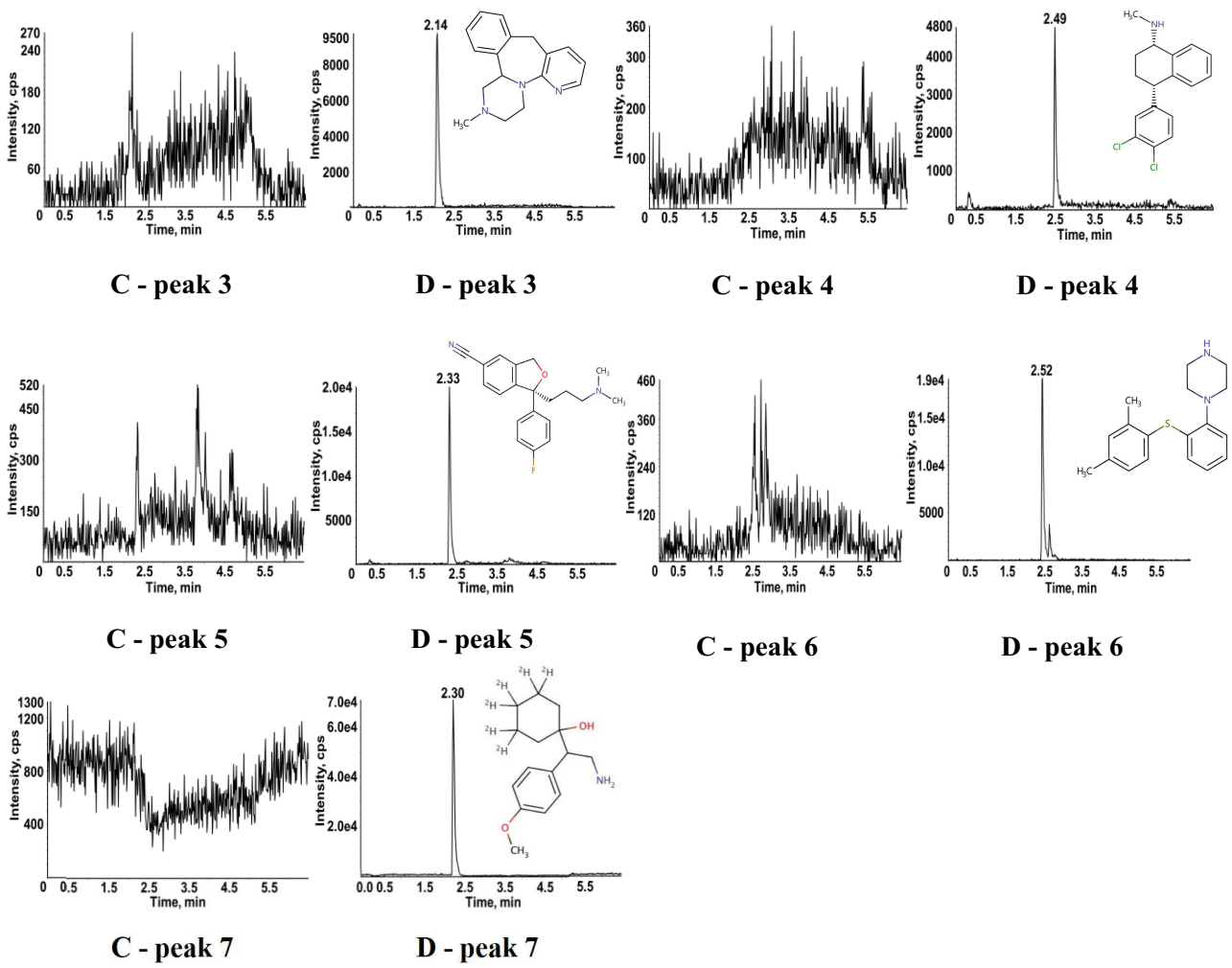

Figure 1 Extracted ion-current chromatograms of the analytes in different matrices. (**A**) Blank plasma sample. (**B**) Plasma sample spiked at the LLOQ. (**C**) Blank saliva sample. (**D**) Saliva sample spiked at the LLOQ. Peaks: (1) venlafaxine (VEN); (2) O-desmethylvenlafaxine (ODV); (3) mirtazapine (MIR); (4) sertraline (SER); (5) escitalopram (ESC); (6) vortioxetine (VTX); (7) venlafaxine-D_6 (IS).

Precision and Accuracy

The intra- and inter-day precisions for all analytes in the plasma and saliva were <12%, with accuracies ranging from −6.79% to 7.26%. These results confirm the reliability of the method for quantifying the six analytes at different concentrations (Tables 2 and 3).

Extraction Recovery and Matrix Effects

Extraction recovery in plasma was 85.91%-110.87%, with matrix effects of 84.18%-117.20%. In saliva, the recovery was 92.29%-101.08%, with matrix effects of 94.93%-107.78%. No significant matrix interference was observed (RSD <15%) (Supplementary Tables 1and 2).

Stability

All analytes remained stable under four different storage conditions (freeze-thaw cycles, long-term storage, short-term room temperature, and autosampler stability), with RSD <15% (Supplementary Tables 3 and 4).

Dilution Integrity

A simulated sample with a concentration 20-fold higher than the upper limit of quantification (2000 ng/mL) was diluted with a blank matrix to QC levels of 200 ng/mL and 400 ng/mL. The results demonstrated that dilution of high-concentration clinical samples with a blank matrix did not compromise the precision or accuracy of the assay (Supplementary Tables 5 and 6).

https://doi.org/10.2147/DDDT.S566716

Table 2 Precision and Accuracy in Human Plasma (n=6)

Analyte	Conc. (ng/mL)	Intra-Day (n=6)			Inter-Day (n=3)		
		Mean±SD (ng/mL)	RSD (%)	RE (%)	Mean±SD (ng/mL)	RSD (%)	RE (%)
VEN	5	4.81±0.17	3.51	−3.73	4.95±0.35	7.17	−1.08
	10	10.56±0.40	3.79	5.60	10.52±0.54	5.17	5.51
	200	202.0±9.59	4.75	1.00	208.7±14.05	6.73	4.36
	400	404.3±8.48	2.10	1.08	408.1±29.31	7.18	2.03
ODV	5	5.19±0.22	4.31	3.70	5.15±0.28	5.36	2.91
	10	10.69±0.44	4.14	6.87	10.73±0.50	4.69	7.26
	200	195.3±9.11	4.67	−2.33	208.3±15.01	7.21	4.14
	400	401.8±9.70	2.41	0.46	409.2±28.10	6.87	2.31
MIR	5	4.70±0.18	3.83	−6.03	4.77±0.39	8.15	−4.59
	10	10.49±0.44	4.16	4.92	10.13±0.69	6.81	1.34
	200	197.0±10.18	5.17	−1.50	202.8±12.56	6.19	1.42
	400	401.8±6.74	1.69	0.46	395.4±27.79	7.03	−1.41
SER	5	5.30±0.47	8.94	5.90	5.05±0.60	11.80	0.91
	10	10.13±0.35	3.47	1.30	10.35±0.60	5.77	3.52
	200	203.3±13.68	6.73	1.67	204.7±15.27	7.46	2.36
	400	405.5±10.58	2.61	1.38	406.3±35.22	8.67	1.58
ESC	5	5.01±0.12	2.42	0.23	4.85±0.34	6.95	−2.79
	10	10.15±0.26	2.61	1.45	10.31±0.46	4.45	3.08
	200	199.0±9.21	4.63	−0.50	205.7±11.65	5.67	2.83
	400	399.5±7.40	1.85	−0.13	401.8±23.40	5.82	0.46
VTX	5	4.69±0.33	7.02	−6.27	5.07±0.44	8.64	1.37
	10	9.93±0.42	4.21	−0.68	10.31±0.54	5.19	3.11
	200	194.2±14.20	7.32	−2.92	203.9±16.35	8.02	1.94
	400	388.8±14.19	3.65	−2.79	404.8±28.48	7.04	1.19

Abbreviations: Conc., Concentration; SD, Standard Deviation; RSD, Relative Standard Deviation; RE, Relative Error.

Table 3 Precision and Accuracy in Human Saliva (n=6)

Analyte	Conc. (ng/mL)	Intra-day (n=6)			Inter-day (n=3)		
		Mean±SD (ng/mL)	RSD (%)	RE (%)	Mean±SD (ng/mL)	RSD (%)	RE (%)
VEN	5	4.85±0.09	1.94	−3.93	4.75±0.18	3.76	−5.03
	10	10.11±0.31	3.05	1.05	10.40±0.54	5.23	4.03
	200	201.7±3.50	1.74	0.83	198.6±8.77	4.41	−0.70
	400	394.8±16.29	4.13	−1.29	417.0±22.36	5.36	4.25
ODV	5	5.15±0.20	3.94	3.00	4.98±0.40	8.05	−0.46
	10	9.99±0.39	3.95	−0.13	10.28±0.71	6.86	2.84
	200	204.0±7.56	3.71	2.00	197.1±9.35	4.75	−1.47
	400	409.8±14.69	3.58	2.46	396.6±17.52	4.42	−0.85
MIR	5	5.02±0.20	4.08	0.37	4.66±0.31	6.72	−6.79
	10	9.75±0.38	3.81	−2.50	10.15±0.51	5.04	1.17
	200	201.8±4.75	2.35	−0.92	196.2±8.31	4.24	−1.89
	400	418.2±12.27	2.93	4.54	395.4±22.45	5.68	−1.15
SER	5	4.84±0.38	7.83	−3.23	4.98±0.40	7.98	−0.34
	10	9.81±0.26	2.36	−1.92	10.36±0.61	5.89	3.62
	200	202.0±6.36	3.15	1.00	193.2±10.88	5.63	−3.42
	400	392.0±16.92	4.32	2.00	401.1±26.68	6.65	0.28

(*Continued*)

Table 3 (Continued).

Analyte	Conc. (ng/mL)	Intra-day (n=6)			Inter-day (n=3)		
		Mean±SD (ng/mL)	RSD (%)	RE (%)	Mean±SD (ng/mL)	RSD (%)	RE (%)
ESC	5	4.94±0.12	2.36	−1.27	4.80±0.40	8.24	−4.04
	10	9.92±3.47	3.50	−0.82	10.22±0.61	5.97	2.25
	200	199.5±4.32	2.17	−0.25	197.5±8.50	4.30	−1.25
	400	406.8±15.00	3.69	1.71	398.1±21.88	5.50	−0.47
VTX	5	5.08±0.07	1.45	1.60	4.86±0.33	6.83	−2.69
	10	10.21±0.35	3.45	2.05	10.28±0.56	5.43	2.86
	200	203.8±3.76	1.85	1.92	195.6±9.64	4.93	−2.19
	400	401.8±18.13	4.51	0.46	391.0±18.92	4.84	−2.25

Abbreviations: Conc., Concentration; SD, Standard Deviation; RSD, Relative Standard Deviation; RE, Relative Error.

Clinical Application

The plasma and saliva assays developed were successfully applied to measure the antidepressant concentrations in clinical samples. From September 2023 to September 2024, 566 patients with depression were enrolled, including 48.2% males (n = 273) and 51.8% females (n = 293), with a median age of 71 years (range: 12–98) and a median BMI of 24.77 kg/m². The median estimated glomerular filtration rate (eGFR) was 93.1 mL/min/1.73 m², and 43% of patients exhibited varying degrees of renal impairment. The antidepressant treatment regimens included monotherapy (n = 441, 77.9%), dual therapy (n = 120, 21.2%), and triple therapy (n = 5, 0.9%).

Drug distribution analysis showed that among the 566 patients, 66 received VEN, 104 received MIR, 189 received SER, 206 received ESC, and 1 received VTX. Additionally, 39 saliva samples were analyzed, including 12 VEN + ODV, 3 MIR, 14 SER, and 10 ESC samples. Further analysis showed no significant difference between saliva and plasma concentrations of VEN + ODV and ESC, whereas SER exhibited a statistically significant difference ($P < 0.001$). While the observed patterns of saliva versus plasma concentrations (eg, the significant difference for SER) provide valuable preliminary insights, a definitive quantitative relationship between the two matrices could not be established in this study due to the limited saliva sample size and non-paired sampling design. Detailed demographic and concentration data are presented in Tables 4 and 5, respectively.

Table 4 Demographic Characteristics of the Patients in This Study

Characteristics	All Samples (n=566)
Sex, n (%)	
Males	273(48.2%)
Females	293(51.8%)
Age (years), Median (Min-Max)	71(12~98)
BMI (kg/m2), Median (IQR)	24.77(20.76~27.34)
GFR (mL/min·1.73m2), Median (IQR)	93.12(76.71~103.30)
GFR>90, n (%)	307(57%)
GER≤90, n (%)	232(43%)
TP (g/L), Median (IQR)	59.4(56.5~63.6)
Albumin (g/L), Median (IQR)	34.6(31.1~37.4)
Number of antidepressants, n (%)	
1	441(77.9%)
2	120(21.2%)
3	5(0.9%)

(*Continued*)

https://doi.org/10.2147/DDDT.S566716

Table 4 (Continued).

Characteristics	All Samples (n=566)
Plasma Drug Concentration, (ng/mL)	
VEN+ODV (n=66)	246.25(164.73~400.55)
MIR (n=104)	16.05(9.56~33.88)
SER (n=189)	23.60(14.00~35.10)
ESC (n=206)	41.35(24.00~68.20)
VTX (n=1)	15.8
Daily dose (mg/d)	
VEN+ODV	75(75~150)
MIR	7.5(7.5~15)
SER	50(25~50)
ESC	10(5~10)
VTX	10
Target rate (%)	
VEN+ODV	65.2
MIR	30.8
SER	85.2
ESC	72.3
VTX	/
Concentration-dose ratio (CDR, d/L/10^3)	
VEN+ODV	2.45(1.94~3.31)
MIR	1.45(0.95~2.09)
SER	0.47(0.31~0.69)
ESC	4.40(2.96~7.22)
VTX	1.58

Abbreviation: Median (IQR), Median (Interquartile Range).

Table 5 Determination of Antidepressant Concentrations in Biological Samples

Drug	Number in Plasma	Concentration in Plasma (ng/mL)	Number in Saliva	Concentration in Saliva (ng/mL)	*P* Value
VEN+ODV	66	246.3(164.7~400.6)	12	255.5(122.2~324.5)	0.489
MIR	104	16.1(9.6~33.9)	3	13.3(3.9~)	/
SER	189	23.6(14.0~35.1)	14	4.2(1.2~10.1)	<0.001*
ESC	206	41.4(24.0~68.2)	10	34.7(20.3~43.7)	0.306

Note: *P<0.05, the difference was statistically significant.
Abbreviation: /, No statistical comparisons were performed.

The plasma concentration distributions of the five antidepressants are shown in Figure 2. The target attainment rate, defined as the proportion of plasma concentrations within the therapeutic reference range, is a key indicator for evaluating treatment efficacy and safety. Among the 66 VEN-treated patients, the plasma concentrations of VEN + ODV showed a moderate positive correlation with dosage ($r = 0.606$, $P < 0.001$). Of these, 43 (65.2%) had concentrations within the therapeutic range (100–400 ng/mL), 16 (24.2%) exceeded the upper limit, and three surpassed the alert threshold of 800 ng/mL (Figure 2A).

Plasma concentrations correlated positively with dosage in the 104 MIR-treated patients ($r = 0.594$, $P < 0.001$). Although 98 patients (94.2%) received low-to-moderate doses (7.5, 15, or 30 mg), only 32 (30.8%) achieved concentrations within the reference range (30–80 ng/mL) (Figure 2B). Among 189 SER-treated patients, plasma concentrations demonstrated a moderate positive correlation with dosage ($r = 0.531$, $P < 0.001$), with 161 (85.2%) falling within the

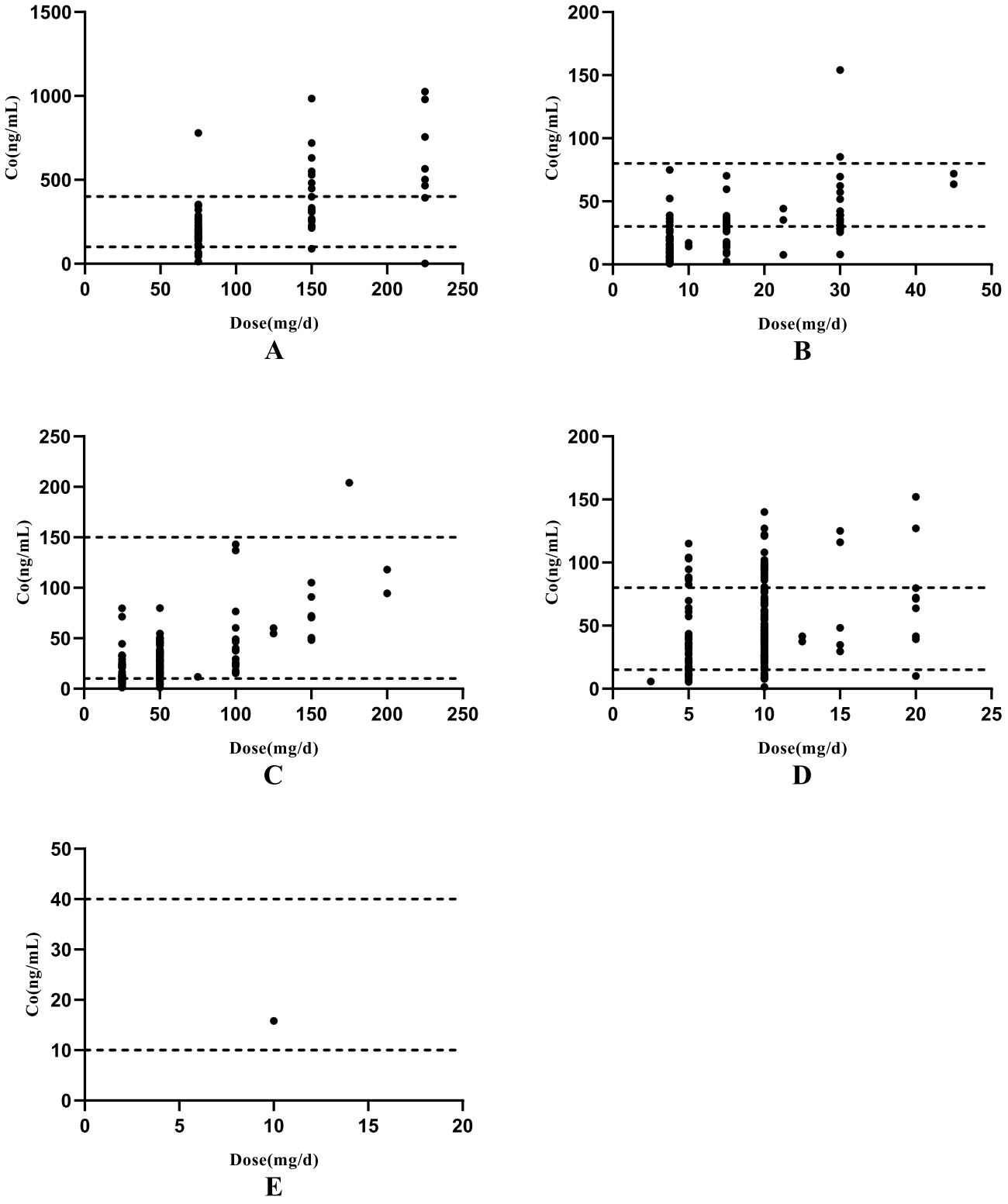

Figure 2 Scatter plot of plasma concentration distribution of 5 antidepressants. (**A**) VEN; (**B**) MIR; (**C**) SER; (**D**) ESC; (**E**) VTX.

therapeutic range (10–150 ng/mL) (Figure 2C). In the 206 ESC-treated patients, plasma concentrations showed a weak correlation with dosage ($r = 0.274$, $P < 0.001$). Most patients (187, 90.8%) received low doses (5 mg or 10 mg), of whom 149 (72.3%) had concentrations within the reference range (15–80 ng/mL), and 35 (17.0%) exceeded it (Figure 2D).

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Only one VTX sample was collected with a plasma concentration within the therapeutic range (10–40 ng/mL) (Figure 2E). Due to the insufficient sample size, dose-concentration correlation analysis was not performed for VTX.

Analysis of Factors Influencing Plasma Drug Concentrations

Factors Affecting VEN and ODV (VEN+ODV)

Patient age and GFR were significant factors that influenced the CDR of VEN ($P < 0.05$) (Supplementary Table 7). Analysis of age groups showed that the exposure level of VEN + ODV in the <18-year age group was not significantly different from that in the other groups. However, elderly patients (≥65 years) exhibited a significantly higher CDR ($P = 0.0211$) with greater variability than those in the 18–65-year group (Figure 3A). Evaluation of BMI (Figure 3B), concomitant antidepressants (Figure 3C), TP levels (Figure 3E), and albumin levels (Figure 3F) revealed no significant impact on the CDR. In contrast, GFR analysis revealed that the $60 \leq GFR \leq 90$ mL/min/1.73 m² group had the highest CDR ratio (Figure 3D), indicating that renal function significantly affected the clearance of VEN and ODV. Compared with patients with normal renal function, those with mild renal impairment ($60 \leq GFR \leq 90$ mL/min/1.73 m²) showed the most pronounced CDR increase ($P = 0.0044$), with an approximate 40.5% elevation, whereas moderate-to-severe renal impairment ($GFR < 60$ mL/min/1.73 m²) resulted in only a 14.7% increase. Linear regression analysis incorporating age, BMI, GFR, TP, and albumin as covariates demonstrated no statistically significant associations ($P > 0.05$) (Supplementary Table 11).

Factors Affecting MIR

Univariate analysis identified BMI as the only significant factor influencing the CDR of the MIR ($P < 0.0001$) (Supplementary Table 8). Analysis of age groups showed that although age did not directly affect the CDR ($P > 0.05$), a trend of increasing CDR with age was observed, along with higher variability in elderly patients (Figure 4A). Intergroup comparisons revealed that both normal-weight ($P = 0.0002$) and overweight ($P = 0.003$) groups had significantly different CDR values than the underweight group (Figure 4B), suggesting that BMI was a potential independent risk factor for MIR CDR. Analyses of other clinical factors, including concomitant medications, renal function, and protein levels, showed no significant associations with MIR CDR (Figure 4C–F). Multivariate regression analysis adjusted for confounders confirmed that BMI ($P = 0.002$) and TP levels ($P = 0.010$) were independent predictors of CDR, whereas age ($P = 0.775$), GFR ($P = 0.584$), and albumin ($P = 0.234$) were not statistically significant (Supplementary Table 12). These results indicate that BMI and TP levels may be key modulators of MIR CDR.

Factors Affecting SER

BMI and TP levels significantly affected the CDR of SER ($P < 0.0001$; Supplementary Table 9). Although age did not reach statistical significance ($P > 0.05$), progressive CDR elevation was observed with advancing age (Figure 5A). Intergroup BMI comparisons revealed that both normal weight ($P = 0.0001$) and overweight ($P = 0.0012$) patients exhibited significantly lower CDR values than underweight individuals, with the overweight group showing the lowest CDR (Figure 5B). Concomitant antidepressants, GFR, and albumin levels showed no significant effect on SER exposure (Figure 5C, D and F). TP levels also exerted statistically significant effects ($P = 0.003$), with patients with normal TP showing a 1.7-fold higher CDR than those with low TP (Figure 5E). Multivariate linear regression identified BMI ($P = 0.017$), GFR ($P = 0.023$), and TP ($P = 0.005$) as independent CDR predictors after adjusting for covariates (Supplementary Table 13).

Factors Affecting ESC

Univariate analysis demonstrated that age, concomitant antidepressants, and renal function significantly influenced ESC CDR, whereas sex, BMI, TP, and albumin level showed no association (Supplementary Table 10). Comparative analysis between adults and elderly patients (excluding pediatric cases) revealed a statistically higher CDR in geriatric patients ($P = 0.0035$), with greater distribution variability (Figure 6A). Further analysis of BMI (Figure 6B) showed no significant association with ESC CDR. Concomitant antidepressant use markedly affected CDR ($P < 0.005$), although only the comparison between SER monotherapy and NIADs combination was significant ($P = 0.0078$; Figure 6C) due to the limited EIAD coadministration samples. Renal impairment significantly increased CDR ($P < 0.0001$), with mild, moderate, and severe renal impairment groups showing approximately 40% higher CDR than the normal renal function group (Figure 6D). Additional analyses of TP and albumin levels (Figure 6E and F) confirmed no statistically significant

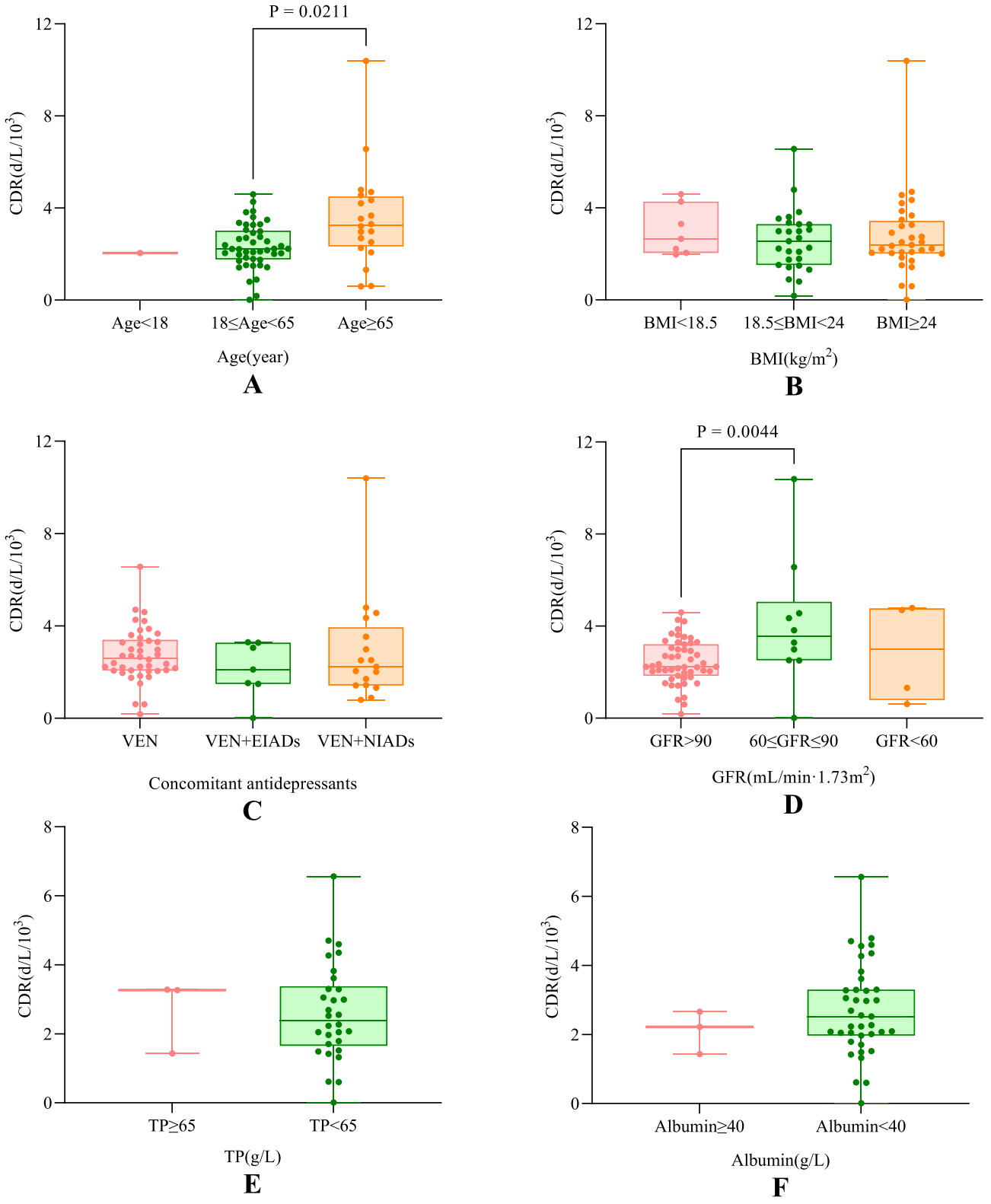

Figure 3 Analysis of factors influencing the CDR of VEN+ODV. (**A**) Age; (**B**) BMI; (**C**) Concomitant antidepressants; (**D**) GFR; (**E**) TP; (**F**) Albumin.

effects on ESC CDR. Multivariate regression confirmed GFR (P = 0.003) and TP (P = 0.002) as independent CDR determinants, whereas age (P = 0.263), BMI (P = 0.084), and albumin (P = 0.376) showed no significant effects (Supplementary Table 14).

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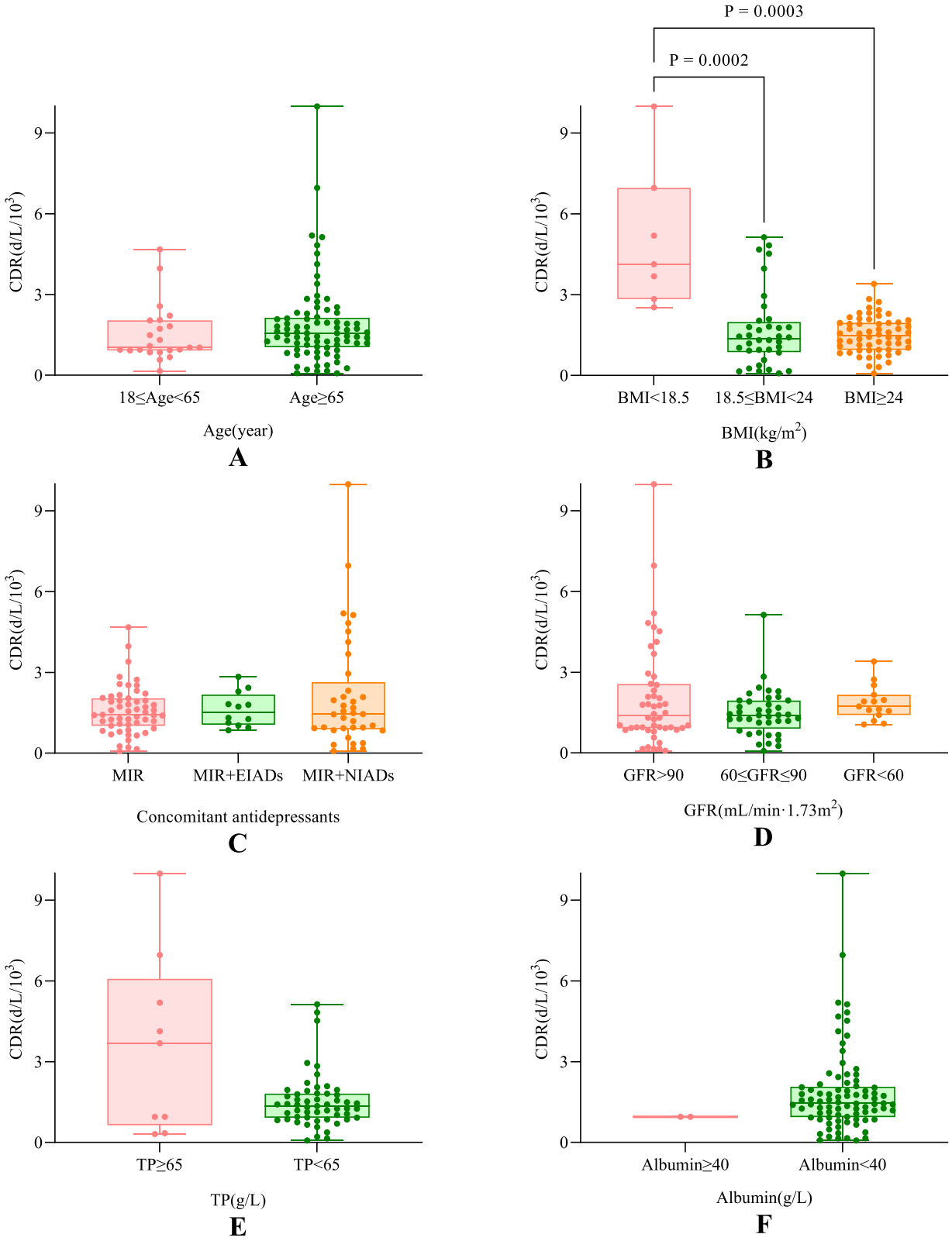

Figure 4 Analysis of factors influencing the CDR of MIR. (**A**) Age; (**B**) BMI; (**C**) Concomitant antidepressants; (**D**) GFR; (**E**) TP; (**F**) Albumin.

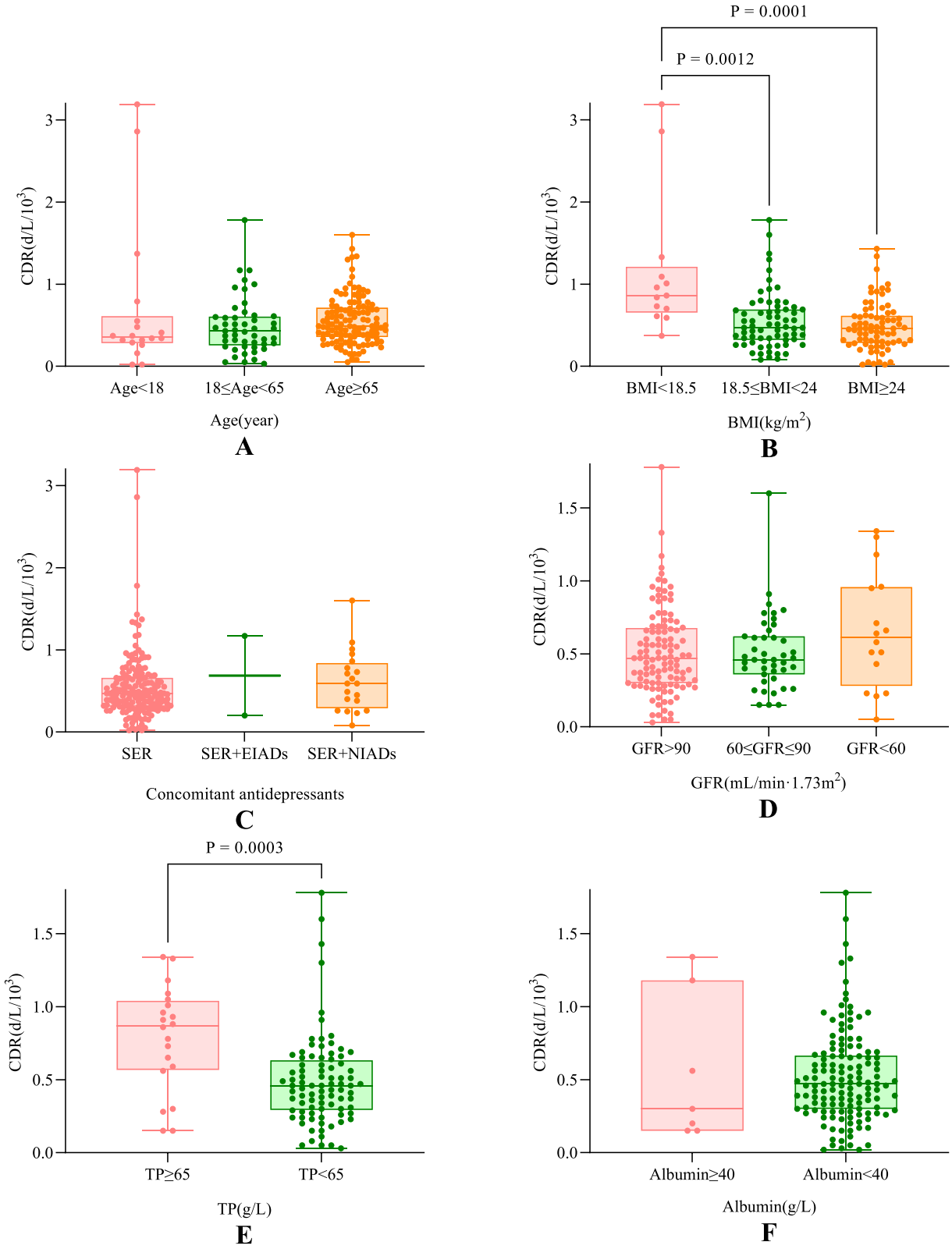

Figure 5 Analysis of factors influencing the CDR of SER. (**A**) Age; (**B**) BMI; (**C**) Concomitant antidepressants; (**D**) GFR; (**E**) TP; (**F**) Albumin.

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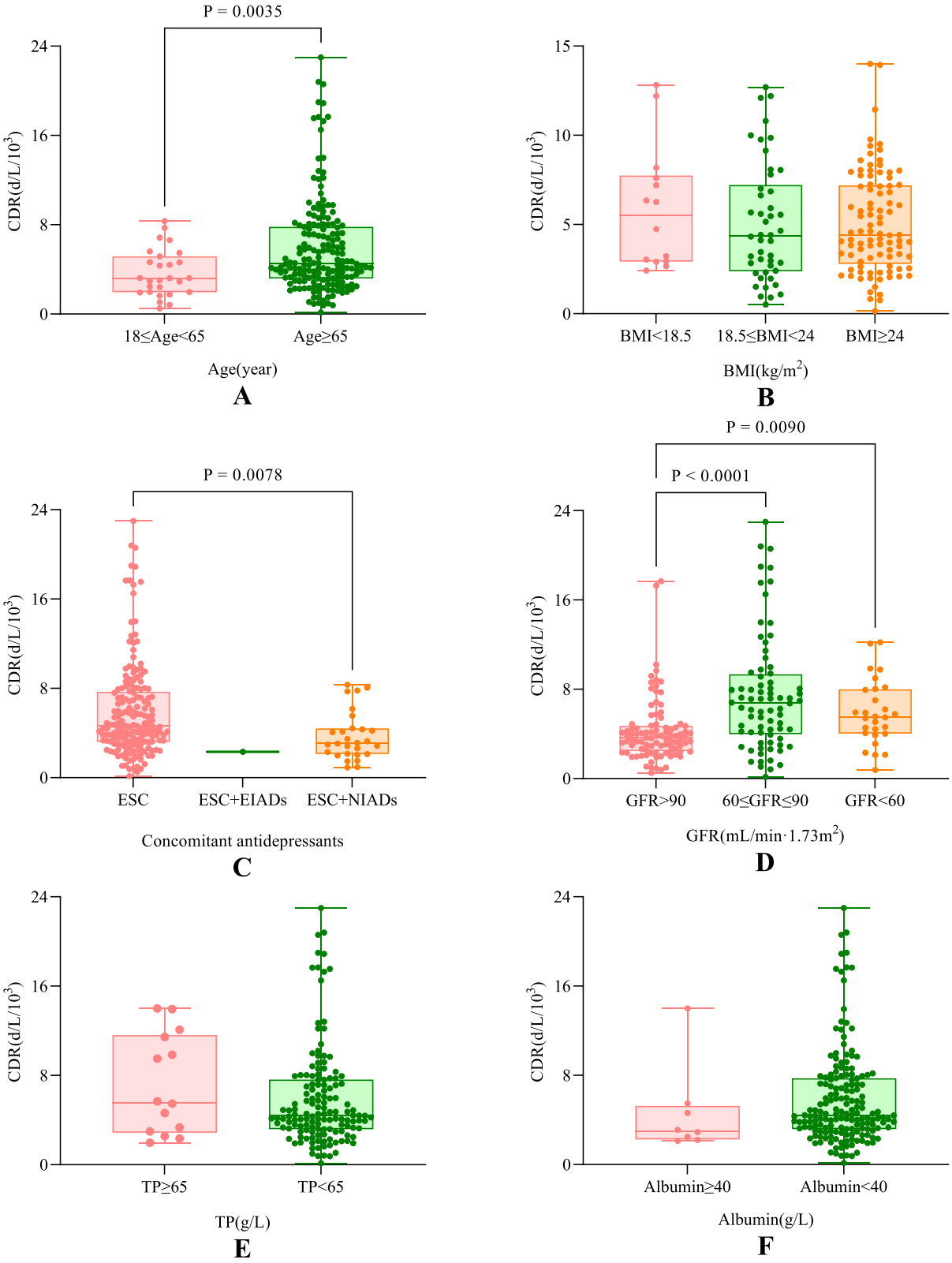

Figure 6 Analysis of factors influencing the CDR of ESC. (**A**) Age; (**B**) BMI; (**C**) Concomitant antidepressants; (**D**) GFR; (**E**) TP; (**F**) Albumin.

Discussion

Selection of Pretreatment Methods

Sample preparation is a critical step in the development of quantitative analytical methods, directly influencing the sensitivity, accuracy, and reproducibility of the assay. During method development, this study systematically evaluated and optimized two pretreatment techniques, PP and LLE, to determine the most suitable approach for plasma and saliva samples. Owing to its simplicity and high efficiency, PP enables rapid sample processing and is an ideal choice for relatively clean matrices. Saliva, which is characterized by low protein content and minimal interference, is well-suited for PP. For saliva samples, methanol was selected as the precipitant for PP because it ensures high recovery rates while maintaining acceptable matrix effects, providing a reliable foundation for subsequent quantitative analysis.

LLE separates analytes based on their differential solubility in organic and aqueous phases, effectively removing interfering substances such as proteins and lipids from the plasma, thereby significantly reducing matrix effects, particularly for lipophilic compounds.[15,16] This study compared the performance of two extraction solvents: ethyl acetate and MTBE. The results demonstrated that ethyl acetate markedly reduced the response values and extraction recovery rates of VEN-D_6 and VEN, whereas MTBE exhibited superior extraction efficiency for all analytes, including VEN-D_6. MTBE not only efficiently extracts target analytes but also minimizes co-extracted interferences, protects the chromatographic column, and improves peak shape, making it the preferred extraction solvent. Future research could further optimize the extraction conditions (eg, solvent ratio and extraction time) and incorporate techniques such as solid-phase extraction (SPE) to enhance method sensitivity and throughput.

Therapeutic Target Attainment Rates of Antidepressants

The appropriate use of antidepressants is a key focus in contemporary psychiatric practice. Analysis of the target concentration attainment rate revealed significant differences between the five antidepressants. SER exhibited the highest attainment rate (85.2%), followed by ESC (72.3%) and VEN + ODV (65.2%), whereas MIR had the lowest rate (30.8%). Due to insufficient sample size, VTX was excluded from the final ranking, with only one case reported. The high attainment rates of SER and ESC may be attributed to the long-term use of fixed doses in most patients, which results in stable blood concentrations and effective symptom control. However, 24.2% of the VEN and 17% of the ESC samples exceeded the safety threshold, suggesting potential metabolic abnormalities (eg, altered enzyme activity, drug interactions, or hepatic/renal impairment). For these patients, continuous TDM is essential to assess intolerance or toxicity risks with timely dosage adjustments to ensure treatment safety.

The low attainment rate of MIR is closely associated with the dosing regimen. Although the recommended dose ranges from 15 to 45 mg/day, most patients (particularly the elderly) received only 7.5 mg/d. While low-dose MIR showed efficacy in improving sleep, mood, and appetite in older adults, its blood concentration often fell below the therapeutic reference range, potentially leading to suboptimal outcomes. Thus, for patients receiving low-dose MIR, clinicians should integrate clinical symptoms and concentration monitoring to evaluate potential undertreatment and consider dose optimization to enhance therapeutic efficacy.

Factors Influencing Plasma Drug Concentrations

Effect of Sex on Drug Concentrations

This study found no significant sex-based differences in the pharmacokinetics of VEN + ODV, MIR, SER, or ESC. This contrasts with some prior studies reporting higher CDRs of VEN and ODV in females, potentially because of their higher body fat percentage, which increases the volume of distribution of lipophilic drugs and elevates plasma concentrations.[17,18] Additionally, males generally exhibit higher renal clearance rates, accelerated drug elimination, and reduced plasma levels.[7,8] Unterecker et al[19] further demonstrated that dose-adjusted serum concentrations of antidepressants were significantly higher in females, attributing this not only to body fat and renal function differences, but also to sex-related variations in drug-metabolizing enzyme activity, with females showing lower enzymatic activity that may delay drug metabolism.[20,21] Clinically, sex alone does not warrant dose adjustment, but should be integrated with BMI/GFR for

personalized dosing - eg, females with low BMI may require cautious titration to avoid supratherapeutic exposure and improve tolerability.

Effect of Age and Renal Function on Drug Concentrations

Age exerted a stronger influence on antidepressant pharmacokinetics than sex, aligning with physiological drug metabolism patterns. It is important to distinguish between univariate and multivariate findings. Univariate analysis indicated an association between advancing age and elevated CDRs for VEN + ODV and ESC, with elderly patients (≥65 years) showing a 29.3% higher CDR than adults (18–65 years). However, age was not retained as a statistically independent predictor in the multivariate linear regression model for both VEN+ODV and ESC when modeled concurrently with other factors such as BMI and GFR. Age-related physiological changes, including increased body fat, reduced renal clearance, and reduced hepatic enzyme activity, likely contribute to this effect.[22–24] Specifically, CYP2D6 and CYP3A4 activity decreases with age, delaying the metabolism of VEN, ODV, and ESC, while a reduced GFR further impairs drug excretion, leading to dual accumulation risks.[25,26] These findings are consistent with the European College of Neuropsychopharmacology (ECNP) TDM guidelines, which recommend dose adjustment for elderly patients. Although the direct effect of age on MIR and SER CDRs was not statistically significant, subgroup analyses revealed an upward trend with advancing age.

Renal impairment affects drug metabolism primarily through reduced GFR and diminished tubular reabsorption/secretion, impairing the excretion of drugs and metabolites and elevating plasma concentrations.[27] This study confirmed that GFR decline is a key driver of increased CDRs. In VEN + ODV, renal dysfunction not only reduces the excretion of VEN but also significantly prolongs the clearance of its active metabolite ODV.[22] ODV has pharmacological activity comparable to that of VEN, but has a longer half-life, making it more prone to accumulation in renal impairment. Compared with patients with normal renal function, those with mild renal impairment ($60 \leq GFR \leq 90$ mL/min/1.73 m²) exhibited an approximately 40.5% increase in CDR, while patients with moderate-to-severe renal impairment (GFR < 60 mL/min/1.73 m²) showed a 14.7% elevation. This non-linear relationship, with the most pronounced CDR elevation observed in mild renal impairment, highlights the clinical necessity of adjusting VEN dosages based on GFR stratification to prevent potential toxic effects. This non-monotonic trend may be related to preemptive dose reductions in patients with moderate-to-severe renal impairment in clinical practice (to avoid overt toxicity) or compensatory metabolic adaptations (eg, enhanced hepatic metabolism of VEN/ODV to offset reduced renal excretion), although further studies are needed to verify these hypotheses.

Although ESC predominantly undergoes hepatic metabolism, approximately 30% of its clearance occurs through renal excretion. Consequently, renal impairment results in an approximately 40% increase in the CDR of ESC. These findings are consistent with those of previous studies demonstrating that ESC doses should be reduced by 50% in patients with moderate-to-severe renal impairment to maintain plasma concentrations within the therapeutic range.[28]

These findings highlight the pronounced effects of age and GFR on antidepressant concentrations, with potential "dual-factor synergy" exacerbating accumulation risks. We recommend lower initial doses for elderly and renally impaired patients, coupled with dynamic monitoring of hepatic/renal function and TDM-guided dose adjustments to maintain therapeutic levels. Based on these findings, we propose that for elderly patients (≥ 65 years), VEN and ESC be initiated at 50–75% of the standard adult dose. For those with renal impairment, a GFR-stratified approach is advised: reduce VEN by 25–30% for mild impairment ($60 \leq GFR \leq 90$ mL/min/1.73 m²) and by 50% for moderate-severe impairment (GFR < 60 mL/min/1.73 m²); similarly, reduce ESC by 50% for GFR < 30 mL/min/1.73 m². Future studies should explore age-renal function interactions and integrate pharmacogenomics to develop personalized dosing models.[29]

Effect of BMI on Plasma Drug Concentrations

BMI, as a measure of obesity, may influence plasma drug concentrations by altering the volume of distribution of antidepressants. This study found that BMI significantly affected the plasma concentrations of MIR and SER, whereas its effect on VEN + ODV and ESC was not statistically significant. The results demonstrated that compared with the low-body-weight group, the CDR was significantly lower in the normal-weight group ($18.5 \leq BMI < 24$ kg/m²) and the

overweight group (BMI ≥ 24 kg/m²), suggesting that BMI is an important variable influencing the pharmacokinetics of MIR and SER.

Both MIR and SER are highly lipophilic drugs, and their volume of distribution is positively correlated with the body fat percentage. In overweight patients (BMI ≥ 24 kg/m²), the substantial increase in adipose tissue leads to a greater accumulation of lipophilic drugs in fat, resulting in relatively lower initial plasma concentrations. Therefore, higher doses may be required to achieve effective therapeutic effects. Conversely, in low-body-weight patients (BMI < 18.5 kg/m²), the reduced adipose tissue results in a smaller volume of distribution, leading to significantly higher plasma concentrations and increased CDR at the same dose.

Existing research remains controversial regarding whether BMI definitively influences the plasma concentrations of antidepressants. For VEN + ODV, previous studies have reported findings inconsistent with this study; Schoretsanitis et al[17] and Warrings et al[30] demonstrated a negative correlation between BMI and the CDR of VEN and ODV. As for ESC, Hart et al[31] aligned with the present study and found no effect of BMI on ESC exposure. However, Jin et al[32] developed a pharmacokinetic model for ESC and observed that body weight significantly influenced its clearance and volume of distribution.

In clinical practice, the initial dosages of MIR and SER should be adjusted based on the patient's BMI. Specifically, for underweight patients (BMI < 18.5 kg/m²), we recommend initiating therapy at a dose 20–30% lower than standard to minimize the risk of central nervous system (CNS) toxicity (eg, dizziness, convulsions) and reduce adverse event rates. For overweight patients (BMI ≥ 24 kg/m²), a 10–15% dose increase may be required to elevate plasma concentrations into the therapeutic window, thereby improving the clinical response rate (eg, ≥50% reduction in Hamilton Depression Rating Scale [HAMD] scores) and remission rates. This tailored approach ensures that patients with low body weights are treated cautiously with reduced doses to avoid toxicity, while overweight patients receive adequate doses for optimal efficacy. Future studies should expand the sample sizes and incorporate population pharmacokinetic modeling to further explore the potential impact of BMI on antidepressant pharmacokinetics.

Effect of Concomitant Medications and Genetic Factors on Plasma Drug Concentrations

This study observed that concomitant antidepressant use may influence ESC plasma concentrations through CYP2C19 and CYP3A4 metabolic pathways. However, these results should be interpreted with caution. In the univariate analysis, statistical significance was achieved only for the comparison between ESC monotherapy and NIADs combination, and the sample sizes of the combination therapy groups were limited. Therefore, these findings are exploratory and hypothesis-generating, not definitive. Although the small sample size of combination therapy groups limited definitive conclusions, the observed pharmacokinetic interactions highlight important clinical considerations. For patients receiving ESC, co-prescription with potent CYP2C19/3A4 inhibitors (eg, fluvoxamine) warrants particular caution.[31,33] We recommend considering a 25–30% dose reduction in such cases to prevent supratherapeutic concentrations and associated adverse effects (eg, QTc prolongation, serotonin syndrome), thereby maintaining treatment safety without compromising efficacy. While no significant drug-drug interactions were observed for VEN+ODV, MIR, or SER, clinicians should remain cognizant of their metabolic pathways when prescribing complex medication regimens.

Genetic factors play a crucial role in the development of personalized antidepressant therapies. Most antidepressants are metabolized primarily by CYP450 enzymes, with polymorphisms in CYP2D6 and CYP2C19 significantly affecting the metabolic rate, clearance, and risk of drug accumulation.[34] Additionally, genetic variations in drug transporters (eg, ABCB1) and target genes (eg, SLC6A4, FKBP5, and HTR1A) can substantially influence antidepressant efficacy and safety.[35] Informed by the substantial interindividual variability in CDRs observed in this study-driven by non-genetic factors such as age, renal function, and BMI-we propose that future TDM protocols evolve to integrate pharmacogenetic data in a complementary manner. First, establish a genotype-informed starting dose through pre-emptive testing—for instance, applying a 30–50% dose reduction for CYP2D6/CYP2C19 poor metabolizers of ESC/VEN (genetically sensitive drugs) while using standard initial doses for MIR (genetically less responsive). Then, during TDM-guided dosing adjustments, the quantified clinical influences identified in this study must be fully incorporated.

In this synergistic framework, pharmacogenetics provides a personalized starting point, while TDM enables dynamic precision throughout treatment, collectively facilitating individualized dosing strategies and potentially leading to faster achievement of therapeutic efficacy with improved safety profiles.

Effect of TP and Albumin on Plasma Drug Concentrations

Most antidepressants exhibit high plasma protein binding, and TP and albumin levels, particularly albumin, serve as key indicators of drug-binding capacity, potentially influencing the free and bound fractions of antidepressants.[26] This study found that the plasma protein levels did not affect the CDR of VEN + ODV, MIR, or ESC. However, TP levels demonstrated a significant influence on SER concentrations, with a trend toward elevated SER exposure observed in patients with hypoalbuminemia (< 40 g/L).

The high plasma protein-binding rate of SER (up to 98%) makes it particularly sensitive to protein levels. For patients with hypoproteinemia (TP < 65 g/L), especially those with hypoalbuminemia (< 40 g/L), we recommend considering a 20–30% reduction in the initial SER dose. This pre-emptive adjustment aims to mitigate the risk of elevated free drug concentrations and associated central nervous system toxicity, while maintaining therapeutic efficacy. Therefore, TDM is recommended in patients with hypoproteinemia to dynamically assess free drug concentrations, guide dose adjustments, and optimize the treatment safety profile.

Factors Influencing Drug Concentrations in Saliva

From a pharmacokinetic perspective, plasma drug concentrations consist of both free and protein-bound fractions, with only the free fraction being capable of crossing the blood-brain barrier to exert antidepressant effects. Salivary drug concentrations primarily reflect the non-protein-bound form and exhibit a strong correlation with free drug concentrations in the plasma.[36] This characteristic makes saliva a potential non-invasive alternative matrix for TDM, offering improved convenience and patient compliance compared with plasma sampling.[37,38] However, establishing a reliable correlation requires specifically designed studies with paired samples and a sufficient sample size. In the present study, not only were plasma and saliva samples not collected as paired specimens from the same patients at concurrent timepoints, but the saliva sample size (n=39) was also limited. Thus, the current analysis of salivary drug concentrations should be framed as an exploratory validation rather than a full equivalence test between the two matrices. This dual methodological limitation (small sample size and non-paired design) makes it unfeasible to perform scientifically valid quantitative comparisons, such as calculating correlation coefficients or constructing Bland-Altman plots to confirm clinical substitutability. Therefore, while our data confirm the preliminary feasibility of detecting antidepressants in saliva, a definitive quantitative relationship between the two matrices could not be established herein, and its validation remains a goal for future research.

Antidepressants may reduce salivary secretion by activating the central α-2 adrenergic receptors, leading to xerostomia (dry mouth). Stimulation methods are sometimes required to obtain sufficient salivary volume.[39,40] However, stimulated saliva exhibits an altered composition, including increased sodium, chloride, and bicarbonate concentrations, which can modify salivary pH and subsequently affect drug dissociation. Therefore, naturally secreted saliva is preferable for drug concentration analyses.[41] In this study, naturally secreted saliva was used in 1.5 mL EP tubes to ensure sample reliability. Nevertheless, the choice of collection device may also influence salivary drug concentrations, warranting further optimization in future studies.[15]

Additionally, the plasma protein-binding rate is a critical factor affecting the correlation between salivary and plasma drug concentrations. Most antidepressants exhibit high protein binding, and special populations (eg, elderly patients, those with hepatic/renal impairment, or hypoalbuminemia) may demonstrate altered correlations owing to variable protein levels.[16,42,43] To minimize the impact of protein binding, future research should focus on establishing the relationship between salivary concentrations and free plasma drug levels, thereby optimizing the clinical applicability of saliva as a non-invasive biological matrix.

Clinical Translation and Practical Applications

The quantified influences of GFR, BMI, and age on antidepressant exposure identified in this study provide a foundation for more precise dosing. These relationships could be translated into structured dose-adjustment algorithms. For example, an algorithm could integrate patient-specific GFR, BMI, and age to calculate an individualized starting dose or suggest modifications for VEN, ESC, MIR, and SER. These drug-specific, factor-driven rules can be encoded into clinical decision-support systems (CDSS) and integrated with electronic health records (EHRs). This integration enables automated, real-time personalized initial dose recommendations at the point of prescribing, minimizing manual calculation errors and fostering standardized precision pharmacotherapy. Prospective validation of this CDSS-assisted, algorithm-guided dosing approach is a crucial next step to confirm improvements in therapeutic outcomes and safety.

Limitations

This study has several limitations. First, owing to the limited sample size, we only analyzed the plasma concentration characteristics of VEN + ODV, MIR, SER, and ESC. The VTX group included only one case, precluding any meaningful analysis of the influencing factors. Second, as a retrospective study, some patients lacked complete clinical evaluation indicators (eg, liver function tests), which may have affected the comprehensiveness of our findings. Third, as a single-center retrospective analysis, the enrolled population possesses a degree of homogeneity in clinical characteristics and diagnostic/therapeutic standards. Therefore, the external validity or generalizability of our findings may be limited and requires further validation through future prospective studies involving multi-center, geographically and ethnically diverse populations. Fourth, the regression analyses conducted in this study were exploratory in nature. As such, to avoid an increased risk of Type II errors (false negatives) that can arise from over-correction, no statistical adjustment for multiple comparisons (eg, Bonferroni correction) was applied. Therefore, statistically significant findings for individual factors (particularly those with p-values close to 0.05) should be interpreted as hypothesis-generating. Their clinical relevance should be evaluated in conjunction with effect sizes, confidence intervals, and prior biological plausibility, and warrants validation in future prospective studies. Additionally, constrained by experimental conditions, we were unable to establish correlations between individual genotypes and plasma antidepressant concentrations, limiting our ability to explore personalized medication mechanisms from a pharmacogenomic perspective. Regarding sample types, the insufficient number of saliva samples (n=39) and the absence of a paired design limited our ability to perform robust intergroup comparative analyses and define the current salivary data analysis as exploratory in nature. Most critically, the absence of paired plasma-saliva samples—collected simultaneously from the same individuals—represents a fundamental methodological limitation. Without such a paired design, it was methodologically unsound to perform quantitative correlation or agreement analyses to directly establish the relationship between concentrations in these two matrices. This is particularly relevant for drugs like SER, which demonstrated significant concentration differences between saliva and plasma, likely attributable to its exceptionally high protein-binding rate (98%).

Therefore, future studies must prioritize the collection of paired plasma-saliva samples and adopt a multi-center design with geographically and ethnically diverse populations to systematically validate both the plasma-saliva concentration correlation and the generalizability of the findings, thereby providing reliable evidence for the clinical translation of saliva-based TDM.

Conclusion

This study successfully established and validated a UPLC-MS/MS method for the simultaneous quantification of five antidepressants (VEN + ODV, MIR, SER, ESC, and VTX) in human plasma and saliva, demonstrating its applicability for clinical TDM. In contrast to conventional TDM assays that are often restricted to single-drug analysis or rely solely on plasma, this multiplexed, dual-matrix design enables a more comprehensive assessment of drug exposure. It particularly enhances practical applicability by complementing the gold-standard plasma matrix with a non-invasive saliva option, which may improve accessibility and compliance in vulnerable populations (such as the elderly or renal-impaired patients). Plasma sample analysis revealed that among the five antidepressants, SER exhibited the highest target attainment rate, whereas MIR showed the lowest. Based on the associations observed in this study, further exploratory investigation into key factors influencing plasma drug concentrations indicated the following: age and renal function as

significantly associated factors for VEN + ODV and ESC levels; BMI as a potential modifier for MIR and SER concentrations; and concomitant medications as potential contributors to variations in ESC metabolism. Clinicians should be vigilant regarding pathological conditions associated with significantly reduced plasma protein levels.

Saliva has emerged as a promising non-invasive alternative matrix for clinical applications, particularly in special populations, although the limited sample size precluded the establishment of reliable correlations with plasma concentrations. Collectively, these observed associations support the optimization of antidepressant treatment outcomes through TDM-based individualized dosing strategies. Future studies should expand the sample size to further validate the clinical utility of salivary drug monitoring and provide comprehensive scientific evidence for precision pharmacotherapy.

Data Sharing Statement

The authors confirm that the data supporting the findings of this study are available within the article.

Ethics Approval and Informed Consent

This study has been approved by the Ethics Committee of Hebei General Hospital (Approval Nos. 2024382; 2024383). Written informed consent was obtained from all the participants prior to the study.

Consent for Publication

All authors approved the final manuscript and the submission to this journal.

Acknowledgments

The authors gratefully acknowledge the contributions of data collectors and research supervisors.

Author Contributions

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

Funding

This work was supported by the Hebei Province Medical Applicable Technology Tracking Project(grant number GZ2023035).

Disclosure

The authors declare no competing interests.

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