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ESTABLISHMENT OF A METHOD FOR MONITORING MEROPENEM CONCENTRATIONS IN PATIENTS WITH SEVERE ACUTE PANCREATITIS

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Abstract: Objective: To establish a population pharmacokinetic (PPK) model for meropenem in patients with severe acute pancreatitis (SAP), providing a valuable basis and method for developing individualized meropenem dosing regimens tailored to the pathophysiological state of SAP patients; Methods: Meropenem concentrations in plasma and abdominal drainage fluid were monitored using ultra-high performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) and homogeneous enzyme immunoassay. Nonlinear mixed-effects modeling was performed using Phoenix software; Results: (1) Meropenem concentration determination: A total of 20 SAP patients were enrolled, providing 99 qualified plasma samples and 42 qualified abdominal drainage fluid samples. Meropenem and the internal standard (metformin) were well separated in both plasma and drainage fluid samples, demonstrating good specificity. Meropenem showed excellent linearity within the ranges of 0.5 - 200 μg/mL in plasma and 0.1 - 10 μg/mL in drainage fluid. All methodological validation results fell within acceptable limits, with RSD ≤ 15%; Conclusion: This study established an LC-MS/MS method (referenced and quality-controlled by homogeneous enzyme immunoassay) for determining meropenem concentrations. The validated method is suitable for clinical therapeutic drug monitoring (TDM) of meropenem, particularly in critically ill ICU patients.
Keywords: Meropenem; Severe acute pancreatitis; Therapeutic drug monitoring; Monte Carlo simulation; Pharmacokinetics/Pharmacodynamics; Individualized therapy

1 INTRODUCTION

Meropenem exhibits time-dependent antibacterial activity. The pharmacokinetic/pharmacodynamic (PK/PD) index that best predicts its clinical efficacy is the duration of time that the free drug concentration remains above the minimum inhibitory concentration (fT > MIC) of the pathogen [1]. Studies suggest that for critically ill patients with severe bacterial infections, a target of 100% fT > MIC or 100% fT > 4x MIC may be necessary [2]. Therefore, understanding the population pharmacokinetics (PPK) of meropenem in pancreatitis is crucial. This study used body fluid samples (plasma and abdominal drainage fluid) from patients receiving meropenem, with meropenem concentration as the observation index. We established UPLC-MS/MS and homogeneous enzyme immunoassay (for rapid bedside monitoring) methods to detect meropenem concentrations, aiming to facilitate precise dosing (individualized therapy) for SAP patients.

2 MATERIALS AND METHODS

2.1 Reagents

Meropenem reference standard (purity >98%, Yuanye Bio-Technology, Shanghai, China); Metformin hydrochloride internal standard (IS) (purity >98%, Yuanye Bio-Technology); Acetonitrile, methanol (HPLC grade, Merck, Germany); Formic acid, ammonium formate (HPLC grade, Aladdin, Shanghai, China); Deionized water (prepared using a Millipore purification system).

2.2 Instruments

Waters ACQUITY UPLC H-Class System coupled with a Xevo TQ-S Micro Triple Quadrupole Mass Spectrometer (Waters Corp., Milford, MA, USA); ACQUITY UPLC® BEH Amide column (100 mm × 2.1 mm, 1.7 μm, Waters); Centrifuge (Eppendorf 5430R, Germany); Nitrogen evaporator (Organomation N-EVAP, USA); Vortex mixer (IKA

MS3 basic, Germany); Analytical balance (Mettler Toledo ME204, Switzerland); -80°C ultra-low temperature freezer (Thermo Scientific, USA).

2.3 Ethics

The study protocol for meropenem concentration determination in plasma and abdominal drainage fluid samples adhered to ethical guidelines. Sample collection obtained informed consent from patients or their families, complied with the Declaration of Helsinki, and was approved by the Ethics Committee of the Affiliated Hospital of Zunyi Medical University (Approval No.: KLLY - 2022 - 204).

2.4 Blank Plasma and Abdominal Drainage Fluid Sample Collection

2.4.1 Blank plasma

Plasma from 6 healthy adults was collected as blank plasma. Venous blood was drawn in the morning after fasting, promptly centrifuged, and stored at -80°C until use.

2.4.2 Blank drainage fluid

Abdominal drainage fluid (2 mL) was collected from 6 SAP patients with indwelling drainage catheters, no significant past medical history, and no prior meropenem use, using blank tubes. Samples were promptly centrifuged and stored at -80°C until use.

2.5 Patient Plasma and Abdominal Drainage Fluid Sample Collection

Sampling time points (0 h [pre-dose], 0.5 h, 1 h, 2 h, 4 h, 6 h, and 8 h post-dose) were set based on meropenem's PK parameters and half-life from literature and the drug label. A sampling time error of approximately 5 minutes was allowed. At least 3 plasma and 3 drainage fluid samples were collected per patient, covering absorption, distribution, and elimination phases. Samples were centrifuged at 12,000 rpm for 10 min, and the supernatant was stored at -80°C.

2.6 Preparation of Standard and Internal Standard Solutions

Meropenem standard stock solution (10 mg/mL) was prepared in water and stored at -20°C. Metformin IS stock solution (10 mg/mL) was prepared similarly. Working solutions were prepared by dilution.

2.7 Sample Preparation

2.7.1 Plasma and drainage fluid samples

To labeled centrifuge tubes, add 20 μL IS solution (500 μg/mL metformin) and 205 μL water. Add 25 μL sample. Mix thoroughly. Add 750 μL acetonitrile for protein precipitation. Vortex, centrifuge (12,000 rpm, 10 min). Filter supernatant through a 0.22 μm membrane into an injection vial. Injection volume: 5 μL.

2.7.2 Blank samples

Thaw blank plasma/drainage fluid. Add 225 μL water to tubes. Add 25 μL blank sample. Mix. Add 750 μL acetonitrile. Vortex, centrifuge. Filter. Combine filtered blank plasma and drainage fluid, mix. Injection volume: 5 μL.

2.8 Mass Spectrometric and Chromatographic Conditions

2.8.1 Chromatographic conditions

Column: ACQUITY UPLC® BEH Amide (100 mm × 2.1 mm, 1.7 μm); Mobile phase: Acetonitrile (A) - Water (B) (each containing 0.29 g ammonium formate and 0.5 mL formic acid per liter); Gradient: 0-9.5 min: 85%-65% A; 9.5-10 min: 65%-40% A; 10-10.5 min: 40%-85% A; 10.5-14 min: 85% A; Flow rate: 0.25 mL/min; Injection volume: 5 μL; Column temperature: 40°C; Autosampler temperature: 4°C.

2.8.2 Mass spectrometric conditions

ESI ion source; Positive ion mode; MRM mode; Temperature: 450°C; Capillary voltage: 3.5 kV; Gas flow: 750 L/hr; MRM transitions: m/z 384.6→141.2 (meropenem), m/z 130.2→60.1 (metformin IS).

2.9 Plasma Standard Curve Preparation

Serial dilutions of the meropenem stock solution (10 mg/mL) in blank plasma were prepared to obtain calibration standards at concentrations of 200, 100, 50, 10, 5, 2, 1, and 0.5 μg/mL. Each concentration was prepared in triplicate. Processed as per section 1.7.1.

2.10 Drainage Fluid Standard Curve Preparation

Serial dilutions were prepared in blank drainage fluid to obtain calibration standards at concentrations of 0.1, 0.2, 0.5, 1, 2, 5, and 10 μg/mL. Each concentration was prepared in triplicate. Processed as per section 1.7.1.

2.11 Quality Control (QC) Sample Preparation

2.11.1 Plasma QC samples

Low (4 μg/mL), medium (20 μg/mL), and high (125 μg/mL) concentration QC samples were prepared in blank plasma. Processed as per section 1.7.1. QC samples were prepared fresh for use.

2.11.2 Drainage fluid QC samples

Low (0.4 μg/mL), medium (2 μg/mL), and high (9 μg/mL) concentration QC samples were prepared in blank drainage fluid. Processed as per section 1.7.1. QC samples were prepared fresh for use.

2.12 Methodological Validation

2.12.1 Specificity

Analyzed blank plasma/drainage fluid, blank spiked with meropenem, and blank spiked with meropenem and IS. Checked for interference at the retention times of meropenem and IS.

2.12.2 Linearity and LOD

Calibration curves were constructed by plotting peak area ratio (meropenem/IS) against concentration using weighted ($1/x^2$) least squares regression. The limit of detection (LOD) was defined as the lowest concentration on the standard curve with signal-to-noise (S/N) ≥ 3.

2.12.3 Recovery

Recovery was determined by comparing the peak areas of extracted QC samples (low, medium, high) with the peak areas of post-extraction blank samples spiked with equivalent amounts of meropenem and IS (n=5 per level).

2.12.4 Precision

Intra-day precision (n=5 per level, analyzed three times within one day) and inter-day precision (n=5 per level, analyzed over three consecutive days) were expressed as RSD (%).

2.12.5 Matrix effect

The matrix effect was assessed by comparing the peak areas of analytes spiked into post-extraction blank matrix (from 6 different sources) with the peak areas of neat standard solutions at equivalent concentrations (n=6 per level). Calculated as (A/B * 100%).

2.12.6 Stability

Stability was evaluated under various conditions: short-term (room temperature for 8 h, autosampler (4°C) for 24 h), long-term (-20°C for 10 days), and freeze-thaw stability (3 cycles) using QC samples (n=5 per level). RSD should be ≤ 15%.

2.12.7 Data processing

Excel was used for calculating precision, stability, etc. Origin software was used for plotting standard curves and chromatograms.

3 RESULTS

2.1 Specificity Results

2.1.1 Plasma

Meropenem and metformin (IS) were well separated in plasma. No interference from endogenous plasma components or other co-administered drugs was observed. Chromatograms see Figures 1-3.

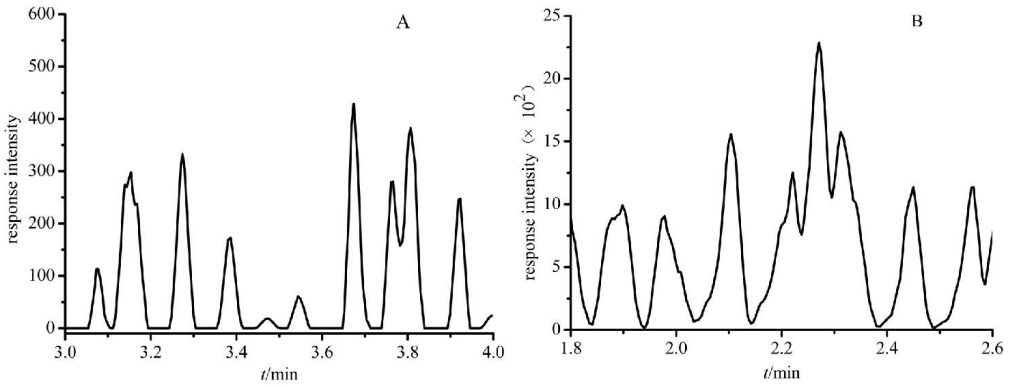

Figure 1 Blank Plasma Chromatogram: A. Meropenem Channel; B. Metformin Channel

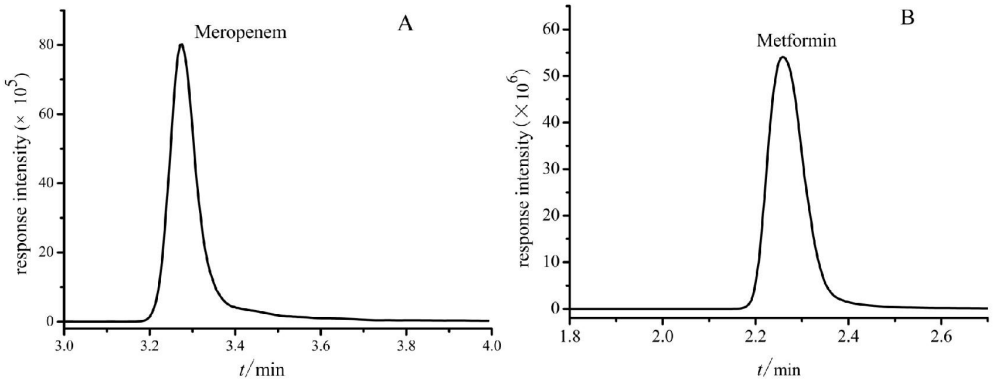

Figure 2 Blank Plasma Spiked with Meropenem Chromatogram

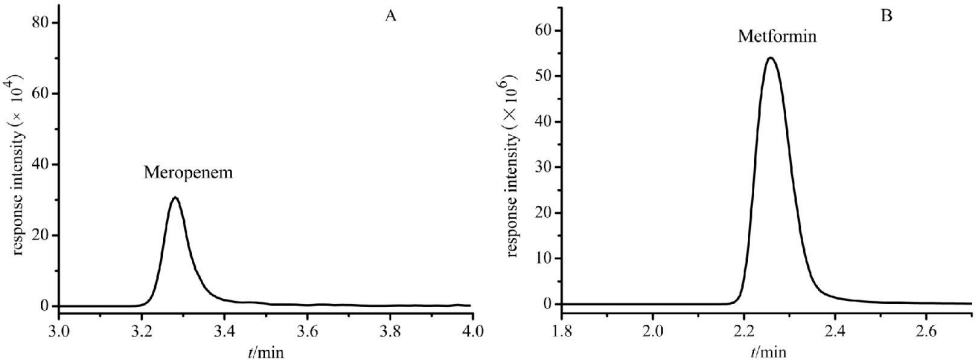

Figure 3 Blank Plasma Spiked with Meropenem and IS Chromatogram

2.1.2 Drainage fluid

Meropenem and IS were well separated in drainage fluid without interference, indicating good specificity. Chromatograms see Figures 4-6.

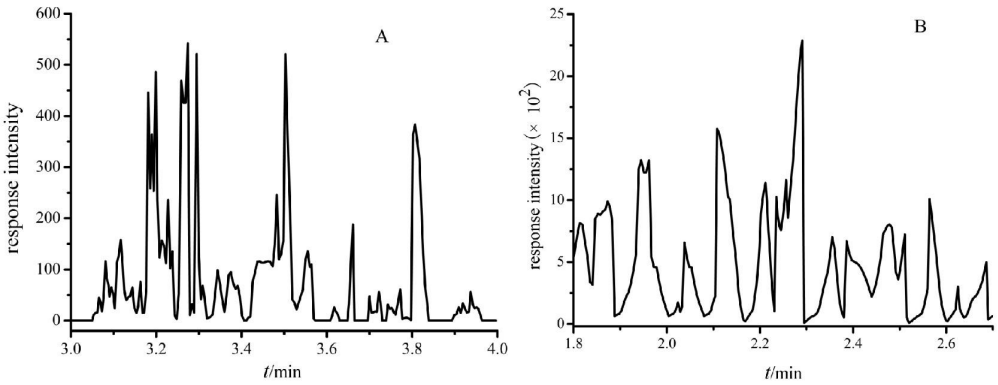

Figure 4 Blank Drainage Fluid Chromatogram

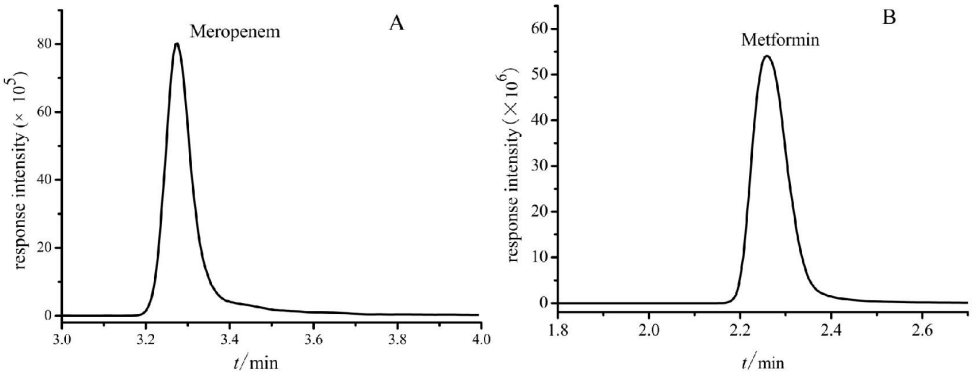

Figure 5 Blank Drainage Fluid Spiked with Meropenem Chromatogram

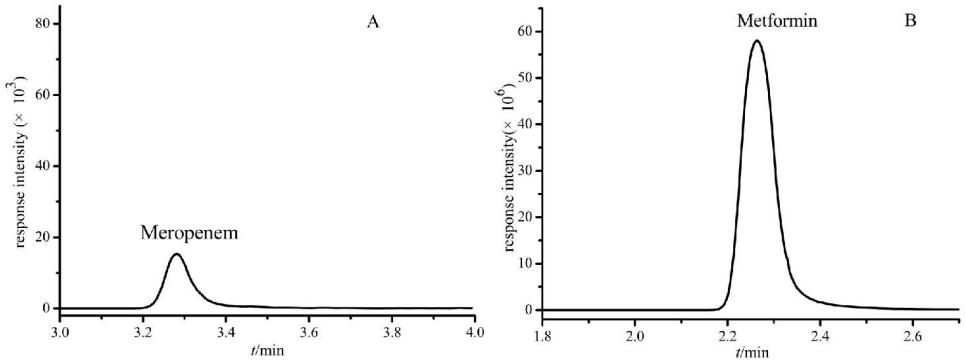

Figure 6 Blank Drainage Fluid Spiked with Meropenem and IS Chromatogram

2.2 Standard Curve and LOD Results

The standard curve equations were y = 0.0101x + 0.0047, r = 0.9998 (n=3) for plasma and y = 0.01x + 0.0023, r = 0.9999 (n=3) for drainage fluid. Linear ranges were 0.5-200 μg/mL (plasma) and 0.1-10 μg/mL (drainage fluid). LOD (S/N=3) in plasma: meropenem 1.1 ng/mL, metformin 0.024 ng/mL; in drainage fluid: meropenem 4.3 ng/mL, metformin 0.03 ng/mL. Standard curve shown in Figure 7.

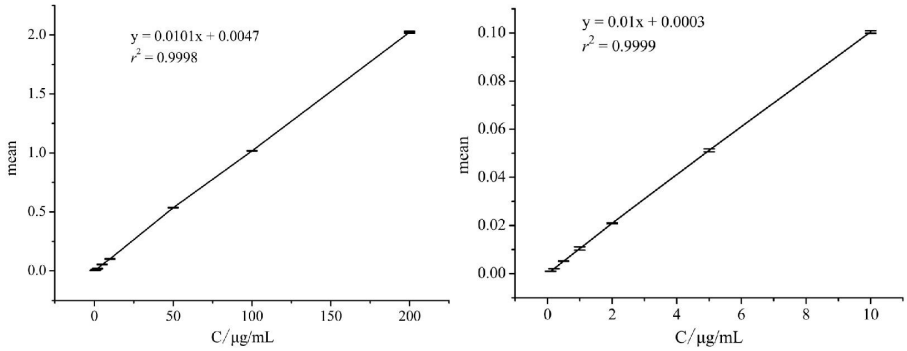

Figure 7 Standard Curves for Meropenem in Plasma and Drainage Fluid

2.3 Recovery Results

Recovery rates for meropenem ranged from 90% to 103% in both plasma and drainage fluid, with RSD < 15%, indicating good recovery.

2.4 Precision Results

Intra-day and inter-day RSD values were all ≤ 15%, indicating acceptable precision.

2.5 Matrix Effect Results

The matrix effect for QC samples ranged from 90% to 105% with RSD < 15%, indicating no significant matrix effect. Results shown in Tables 7 and 8.

2.6 Stability Results

Meropenem QC samples were stable under all tested conditions (short-term, long-term, freeze-thaw), with RSD values within 15%.

4 DISCUSSION AND CONCLUSION

This study established an LC-MS/MS method (referenced against homogeneous enzyme immunoassay) for determining meropenem concentrations. Compared to established LC-MS methods [3-6], the retention time and total analysis time (~3-4 min) were similar, though some methods report shorter times (~1 min). Precision and stability were comparable, confirming the method's suitability for clinical TDM of meropenem, especially in critically ill ICU patients. Homogeneous enzyme immunoassay is also applicable but requires stringent temperature/humidity control. While certified and reimbursable in China, it's more commonly used for drugs like vancomycin, yielding accurate results. Whether its accuracy for meropenem matches LC-MS/MS requires further study.

For sample preparation, solid-phase extraction (SPE) and methanol/acetonitrile protein precipitation are common [7-9]. SPE can be tedious, have lower recovery, cause peak splitting/broadening, and be costly [10]. Protein precipitation with acetonitrile is simpler, cheaper, and widely used [11]. Acetonitrile is recommended due to its low ionization suppression [8]. Thus, acetonitrile protein precipitation was chosen, yielding recoveries within acceptable ranges.

Metformin was selected as the IS based on laboratory availability. It separated well from the analyte peak. Since enrolled patients were not taking metformin, it did not interfere. Methodological validation confirmed the method's sensitivity and effectiveness for meropenem TDM in plasma and drainage fluid. However, this method fails if patients are co-administered meropenem and metformin. Therefore, it's only suitable for TDM in patients not taking metformin. For patients on both drugs, suitable methods need exploration, as most existing studies don't specify metformin co-administration.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

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