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A Liquid Chromatography-Tandem Mass Spectrometry Method for Simultaneously Determining Meropenem and Linezolid in Blood and Cerebrospinal Fluid

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Antibiotic therapy requires appropriate dosage of drugs for effective treatment. Too low antibiotic concentrations may lead to treatment failure and the development of resistant pathogens, whereas overdosing may cause neurological side effects or hemolytic diseases. Meropenem and linezolid are used only in the treatment of serious infections or when other antibiotics are no longer effective as well as for treating central nervous system infections. It is difficult or sometimes even impossible to predict the relation between dosing of antibiotics and its cerebrospinal fluid (CSF) concentration; thus, a method of determining antibiotics not only in the blood but also in the CSF is needed. Analytical method validation is an integral part of good laboratory practice and ensures high accuracy of the results. We performed complete validation process according to the Food and Drug Administration and European Medicine Agency, covering the aspects precision, specificity, accuracy, recovery, limit of detection, limit of quantification, stability, carry-over, and matrix effects. Our liquid chromatography-tandem mass spectrometry method for the simultaneous measurement of meropenem and linezolid in different matrix meets all the acceptance criteria. The method was successfully applied to determine meropenem and linezolid concentrations in serum and CSF samples obtained from children treated with these antibiotics.

Key Words: Antimicrobial agent, Blood, Cerebrospinal fluid, Linezolid, Liquid chromatography, Mass spectrometry, Meropenem, Validation study

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Widespread antimicrobial agent overuse leads to antibiotic resistance [1]. Low rates of new antibiotic approval, especially for pediatric patients, necessitate better drug management. Meropenem and linezolid are reserved for serious infections or cases where other antibiotics are ineffective. Additionally, meropenem and linezolid are frequently used in combination therapies for initially treating serious infections caused by multidrug resistant gram-positive and gram-negative pathogens [2]. They are also used to treat central-nervous system infections such as bacterial meningitis [3]. Meropenem and linezolid can cross the blood–brain barrier but are effective only when they reach a sufficient level. However, the antibiotic structure, disease-specific factors, inflammation stage, and renal clearance can influence penetration across the blood–brain barrier [4].

Predicting antibiotic levels in the cerebrospinal fluid (CSF) based on plasma concentrations is challenging. Thus, a method for monitoring both blood and CSF antibiotic concentrations is needed for individualizing antimicrobial therapies, especially in critically ill patients. Commercial methods are unavailable for measuring antibiotic concentrations; thus, we developed a liquid

chromatography-tandem mass spectrometry (LC-MS/MS) method to simultaneously determine meropenem and linezolid in serum/plasma and CSF. We completely validated the method according to the Food and Drug Administration (FDA) and European Medicines Agency (EMA) Guidelines [5, 6]. Method validation covered aspects such as the specificity, accuracy, recovery, precision, limit of detection (LOD), lower limit of quantification (LLOQ), stability, carryover, and matrix effects.

We prepared calibrators by adding pure meropenem and linezolid to artificial serum/plasma, artificial CSF, native serum, native plasma EDTA, and native CSF. We mixed 50 µL of each calibrator sample with 5 µL internal standard (IS) mixture and 100 µL precipitation agent (0.1% formic acid in acetonitrile). After centrifugation (5 mins/17,300 *g*, 4°C), we diluted each supernatant (1:1, v/v, 0.1% formic acid in water) and analyzed it via LC-MS/MS. We prepared QC samples at three different concentrations by spiking artificial serum/plasma or CSF with meropenem and linezolid. Details regarding calibrators, ISs, controls, and sample preparation are provided in Supplemental Data Table S1.

We analyzed meropenem and linezolid using 6460 Triple Quad LC-MS/MS instrument (Agilent Technologies, Santa Clara, CA, USA) connected to a 1260 Infinity II LC Instrument (Agilent Technologies) equipped with an Aquasil C_{18} column (100 × 3.0 mm, 5 µL; Thermo Fisher Scientific, Waltham, MA, USA). We conducted MS in the multiple-reaction-monitoring mode with positive electrospray ionization. After optimization, we used the following ion pairs: m/z 384.16→141.1 for meropenem, m/z 390.2→147.1 for $IS_{Meropenem.D-6}$, m/z 338.15→296.1 for linezolid, and 341.01→297.2 for $IS_{Linezolid.D-3}$. Chromatographic analysis was performed at 30°C using gradient elution with mobile phases A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile). The LC-MS/MS parameters are described in Supplemental Data Table S2. We analyzed the data using MassHunter Workstation Software Quantitative Analysis v.B.09.00 (Agilent Technologies) and Microsoft Office 365.

Representative LC-MS/MS chromatograms of meropenem and linezolid in plasma samples are presented in Fig. 1 showing respective retention times of 1.776 and 4.215 mins.

We established dynamic and linear ranges for the calibration curves using nine standard solutions for meropenem and linezolid (50, 25, 10, 5, 2.5, 1.0, 0.5, 0.25, and 0.1 µg/mL) matching the expected target range in patients treated with meropenem or linezolid. The standard meropenem and linezolid concentrations in the analyzed matrices (artificial serum/plasma and CSF, native serum, plasma EDTA, and CSF) were within ± 15% of the calculated nominal value. We observed good linear relationships between the response values and meropenem and linezolid concentrations (correlation coefficients ≥0.99) between 0.1 and 50 µg/mL (Supplemental Data Fig. S1). The meropenem and linezolid LOD in serum/plasma and CSF was 0.02 µg/mL, whereas the LOQ was 0.07 µg/mL. Comparing blank plasma and CSF samples with spiked samples demonstrated highly specific detection. No significant interference occurred at the retention times of meropenem, linezolid, and their ISs (meropenem-D6 and linezolid-D3). Analytes from the previous run did not remain in the LC-MS/MS system. The areas under the peaks for meropenem and linezolid in the blank samples did not exceed 20% of the corresponding areas for these analytes in the lowest calibrator-standard samples, which enabled injection of several samples without blank samples.

Accuracy and precision are essential metrics for assessing new analytical methods. Both the FDA and EMA recommend a relative standard deviation (RSD) of ≤15% as the acceptance criterion for accuracy and precision. The accuracy, presented as the percentage recovery at three different concentrations for

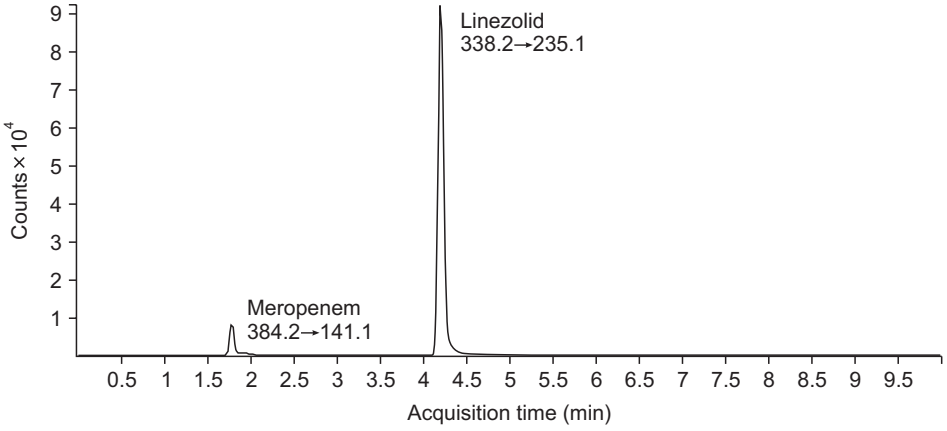

Fig. 1. Chromatogram of spiked plasma sample at 1 µg/mL for meropenem and linezolid.

both serum/plasma and CSF samples, was within acceptable limits (≤15%). The RSD did not exceed 10% for intra-day precision or 12% for inter-day precision (Table 1).

The recoveries obtained for native serum and CSF samples spiked with different meropenem and linezolid quantities ranged from 85.9% to 107.2% with an RSD of <15%. Interfering substances in serum samples (such as lipids, hemoglobin, and bilirubin) did not impair recovery (Supplemental Data Tables S3 and S4).

In contrast to linezolid, meropenem showed better stability in blood samples stored at approximately 4°C than at 22–25°C indicating that blood samples should be analyzed immediately or stored as soon as possible at approximately 4°C (ice water) for up to 30 mins. Meropenem and linezolid were more stable in CSF than in blood, probably because of the lower protein content in the CSF; however, CSF samples should be analyzed within 60 mins after collection/drawing. Storing pooled plasma or serum samples at −80°C for 6 months did not cause significant meropenem or linezolid degradation regardless of the concentration. Meropenem and linezolid were also stable for 30 days in frozen pooled CSF samples. Samples prepared for LC-MS/MS analysis were stable for 24 hrs at autosampler temperature (4°C), reflecting the good stability indicated by the RSD of <15%.

We applied the validated LC-MS/MS method to measure meropenem and linezolid levels in serum samples from 100 children (65 boys and 35 girls, aged 6 days–18 yrs old) after obtaining informed consent from their parents. The method was validated in 2020; the samples were collected during 2021–2022 and analyzed freshly or frozen up to 2 days. We treated 50 children each with meropenem or linezolid and measured the CSF meropenem and linezolid concentrations of 10 children. The Bioethics Committee of Jagiellonian University, Krakow, Poland, approved this study (protocol number: 1072.6120.291.2019). We collected blood from patients 1 hr before and after each dose in steady state and collected CSF between doses. Supplemental Data Fig. S2 shows the CSF meropenem concentrations in two children treated with meropenem for *Klebsiella pneumoniae* infection.

Antibiotic penetration into the CSF shows high intra-variability. Meropenem CSF penetration was observed in 20% of children with normal or mildly infected meninges and in 39% of children with inflamed meninges [7]; for linezolid, these values were approximately 70% [8]. The plasma and CSF meropenem concentrations did not correlate well in two patients treated for neuroinfections because meropenem CSF penetration depends on the disease severity and degree of impairment of the blood–brain barrier.

Too low of an antibiotic concentration may lead to treatment failure and resistant pathogens, whereas overdosing may cause neurological side effects or hemolytic diseases. The administered dose of a drug might not be effective because pharmacokinetic (PK) parameters differ among patients. Measuring plasma and CSF antibiotic concentrations in critically ill patients

Table 1. Intra-day and inter-day precision

Compound	Matrix	Spiked concentration, μg/mL	Within-day (N=15)		Between-day (N=15)	
			Mean±SD, μg/mL	RSD, %	Mean±SD, μg/mL	RSD, %
Meropenem	Plasma	0.5	0.52±0.04	8.5	0.49±0.05	9.1
		3.75	3.85±0.34	8.7	3.67±0.22	5.9
		30	30.38±2.86	9.4	30.57±3.15	10.3
Meropenem	CSF	0.75	0.72±0.03	4.2	0.76±0.04	5.7
		3.75	3.71±0.20	5.3	3.69±0.25	6.8
		30	30.29±1.81	6.0	30.77±2.12	6.9
Linezolid	Serum	0.5	0.48±0.04	8.3	0.51±0.06	11.6
		3.75	3.64±0.27	7.5	3.74±0.26	7.1
		30	29.48±1.68	5.7	31.10±2.70	8.7
Linezolid	CSF	0.75	0.70±0.03	5.0	0.73±0.04	5.1
		3.75	3.69±0.22	6.0	3.81±0.23	6.1
		30	31.39±1.75	5.6	32.43±1.81	5.6

Abbreviations: RSD, relative standard deviation; CSF, cerebrospinal fluid.

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provides objective evidence for improving the treatment of bacterial infections, potentially lowering multidrug resistance and protecting patients from drug toxicity [1]. Exact antibiotic doses cannot be determined for individual patients without knowing the blood and/or CSF drug concentrations, estimating the minimal inhibitory concentration, or assessing a patient's condition [9, 10].

HPLC with ultraviolet detection [11, 12] or HPLC-MS can be used to measure antibiotic concentrations [13-15], although most methods are used to determine the plasma and serum concentration of one class of antibiotics, such as sulfonamides, β-lactams, and oxazolidinones. Only a few validated methods involving LC-MS/MS or ultra-performance liquid chromatography (UPLC-MS/MS) have been described for meropenem and linezolid determinations [16-19], but these methods determine meropenem and linezolid in either serum/plasma or CSF. To our knowledge, only Rehm, *et al*. [15] presented a method for quantifying meropenem in both plasma and CSF. Sun, *et al*. [20] demonstrated a UPLC-MS/MS method for simultaneously determining meropenem and linezolid in plasma and CSF samples. However, these methods differ in terms of sample preparation, equipment, and analytical performance in terms of specificity, selectivity, accuracy, linearity range, precision, LOD, and LOQ.

Our validated LC-MS/MS method for simultaneously measuring meropenem and linezolid in different matrices meets the acceptance criteria established by the FDA and EMA and is appropriate for determining their concentrations in serum/plasma and CSF samples. The main PK parameters can be calculated based on accurate meropenem and linezolid measurements, which helps clinicians prescribe the most appropriate treatment.

Our method is more suitable for pediatric patients unlike a previous method [20] because of the smaller sample volume required for analysis. Sample preparation was rapid and simple. This method can be used to conduct PK studies in children treated with meropenem or linezolid. In future, we intend to assess whether this method is useful for determining meropenem and linezolid dry blood spots. Most laboratories use ready-to-use kits for analyte determinations that are based on the manufacturer's validation data. As no commercial methods are available for determining antibiotic concentrations, we developed an LC-MS/MS method for simultaneous meropenem and linezolid determinations. A single LC-MS/MS method that can measure two or more antibiotics in different biological samples (serum/plasma and CSF) would be useful in hospitals, involving one analytical column, one calibration, the same reagents, and samples from patients receiving different antibiotics. Using one method involving only one complete validation process enables faster result reporting in routine practice.

SUPPLEMENTARY MATERIALS

Supplementary materials can be found via https://doi.org/10.3343/alm.2023.0250

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None.

AUTHOR CONTRIBUTIONS

Berska J: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Data curation, Writing—original draft, Writing—review & editing. Bugajska J: Methodology, Software, Validation, Investigation, Data curation, Writing—review & editing. Sztefko K: Conceptualization, Data curation, Writing—review & editing, Supervision. All authors reviewed and approved the final version of the manuscript.

CONFLICTS OF INTEREST

None declared.

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REFERENCES

1. Mian P, Flint RB, Tibboel D, van den Anker JN, Allegaert K, Koch BCP. Therapeutic drug monitoring in neonates: what makes them unique? Curr Pharm Des 2017;23:5790-800.
2. Wicha SG, Kees MG, Kuss J, Kloft C. Pharmacodynamic and response surface analysis of linezolid or vancomycin combined with meropenem against *Staphylococcus aureus*. Pharm Res 2015;32:2410-8.
3. Tan YC, Gill AK, Kim KS. Treatment strategies for central nervous system infections: an update. Expert Opin Pharmacother 2015;16:187-203.
4. Schneider F, Gessner A, El-Najjar N. Efficacy of vancomycin and meropenem in central nervous system infections in children and adults: current update. Antibiotics (Basel) 2022;11:173.
5. U.S Food and Drug Administration, Bioanalytical method validation guidance for industry. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/bioanalytical-method-validation-guidance-industry (Updated on May 2018).
6. European Medicines Agency. Guideline on bioanalytical method validation.https://www.ema.europa.eu/en/documents/scientific-guideline/

guideline-bioanalytical-method-validation_en.pdf (Updated on July 2011).
7. Nau R, Sörgel F, Eiffert H. Penetration of drugs through the blood-cerebrospinal fluid/blood-brain barrier for treatment of central nervous system infections. Clin Microbiol Rev 2010;23:858-83.
8. Tsona A, Metallidis S, Foroglou N, Selviaridis P, Chrysanthidis T, Lazaraki G, et al. Linezolid penetration into cerebrospinal fluid and brain tissue. J Chemother 2010;22:17-9.
9. Germovsek E, Lutsar I, Kipper K, Karlsson MO, Planche T, Chazallon C, et al. Plasma and CSF pharmacokinetics of meropenem in neonates and young infants: results from the NeoMero studies. J Antimicrob Chemother 2018;73:1908-16.
10. Cojutti P, Maximova N, Crichiutti G, Isola M, Pea F. Pharmacokinetic/pharmacodynamic evaluation of linezolid in hospitalized paediatric patients: a step toward dose optimization by means of therapeutic drug monitoring and Monte Carlo simulation. J Antimicrob Chemother 2015;70:198-206.
11. Roth T, Fiedler S, Mihai S, Parsch H. Determination of meropenem levels in human serum by high-performance liquid chromatography with ultraviolet detection. Biomed Chromatogr 2017;31:e3880.
12. Milla P, Ferrari F, Muntoni E, Sartori M, Ronco C, Arpicco S. Validation of a simple and economic HPLC-UV method for the simultaneous determination of vancomycin, meropenem, piperacillin and tazobactam in plasma samples. J Chromatogr B Analyt Technol Biomed Life Sci 2020;1148:122151.
13. Decosterd LA, Mercier T, Ternon B, Cruchon S, Guignard N, Lahrichi S, et al. Validation and clinical application of a multiplex high performance liquid chromatography – tandem mass spectrometry assay for the monitoring of plasma concentrations of 12 antibiotics in patients with severe bacterial infections. J Chromatogr B Analyt Technol Biomed Life Sci 2020;1157:122160.
14. Phillips OA, Abdel-Hamid ME, Al-Hassawi NA. Determination of linezolid in human plasma by LC-MS-MS. Analyst 2001;126:609-14.
15. Rehm S and Rentsch KM. HILIC LC-MS/MS method for the quantification of cefepime, imipenem and meropenem. J Pharm Biomed Anal 2020;186:113289.
16. Zander J, Maier B, Suhr A, Zoller M, Frey L, Teupser D, et al. Quantification of piperacillin, tazobactam, cefepime, meropenem, ciprofloxacin and linezolid in serum using an isotope dilution UHPLC-MS/MS method with semi-automated sample preparation. Clin Chem Lab Med 2015;53:781-91.
17. Paal M, Zoller M, Schuster C, Vogeser M, Schütze G. Simultaneous quantification of cefepime, meropenem, ciprofloxacin, moxifloxacin, linezolid and piperacillin in human serum using an isotope-dilution HPLC–MS/MS method. J Pharm Biomed Anal 2018;152:102-10.
18. Ferrari D, Ripa M, Premaschi S, Banfi G, Castagna A, Locatelli M. LC-MS/MS method for simultaneous determination of linezolid, meropenem, piperacillin and teicoplanin in human plasma samples. J Pharm Biomed Anal 2019;169:11-8.
19. Barco S, Bandettini R, Maffia A, Tripodi G, Castagnola E, Cangemi G. Quantification of piperacillin, tazobactam, meropenem, ceftazidime, and linezolid in human plasma by liquid chromatography/tandem mass spectrometry. J Chemother 2015;27:343-7.
20. Sun H, Xing H, Tian X, Zhang X, Yang J, Wang P. UPLC-MS/MS method for simultaneous determination of 14 antimicrobials in human plasma and cerebrospinal fluid: application to therapeutic drug monitoring. J Anal Methods Chem 2022;2022:7048605.

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