Clinica Chimica Acta 577 (2025) 120448

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/cca

Research paper

Therapeutic drug monitoring of piperacillin: Agreement between quantitative nuclear magnetic resonance spectroscopy and liquid chromatography with tandem mass spectrometry

Alexander Dejaco [a], Michael Paal [b], Michael Gruber [a], Diana Drettwan [c], Christian Marquardt [c], Michael Helou [c], Tobias Wagner [d], Martin G. Kees [a], Bernhard M. Graf [a], Dominic Barthelmes [a,*]

[a] Department of Anesthesiology, University Hospital Regensburg, Franz-Josef-Strauß-Allee 11, Regensburg, 93053, Germany
[b] Institute of Laboratory Medicine, LMU University Hospital, LMU Munich, Marchionistr. 15, Munich, 91377, Germany
[c] Lifespin GmbH, Am Biopark 13, Regensburg, 93053, Germany
[d] Children's Hospital Ortenau, Ebertplatz 12, Offenburg, 77654, Germany

ARTICLE INFO

Keywords:
Piperacillin
Quantitative nuclear magnetic resonance spectrometry
Therapeutic drug monitoring
Liquid chromatography with tandem mass spectrometry

ABSTRACT

Background: In clinical practice, therapeutic drug monitoring (TDM) typically relies on specialized immunoassays or chromatography-based methods. These approaches require drug-specific setups and are limited to batch measurements, which can restrict their availability. Recent advances in quantitative nuclear magnetic resonance spectroscopy (qNMR) have made it suitable for rapid and reliable quantification of small molecules in human plasma. However, no qNMR-based method has yet been developed specifically for TDM.

Methods: A qNMR method for quantification of piperacillin (PIP) in human plasma was developed. Plasma samples were collected from 15 patients receiving PIP treatment in an intensive care unit. A total of 126 samples were subjected to analysis using ^{1}H-NMR spectroscopy and isotope dilution liquid chromatography–tandem mass spectrometry. Measurements were compared within the common quantifiable range of 10 to 100 mg/L. The degree of agreement between the methods was assessed using linear regression with post-hoc bootstrapping and Bland–Altman analysis.

Results: The concordance correlation coefficient between methods was 0.895, suggesting good alignment. Linear regression showed agreement with an intercept of –0.50 mg/L and a slope of 1.03. Bland–Altman analysis revealed a mean bias of –0.85 mg/L, with limits of agreement from –18.67 mg/L to +16.97 mg/L.

Conclusion: This study demonstrates the potential of qNMR as a viable alternative for therapeutic drug monitoring, with a promising level of agreement supporting further validation in larger patient cohorts.

1. Introduction

With a growing focus on individualized medicine, therapeutic drug monitoring (TDM) is increasingly used in clinical practice to guide dosing regimens for various drug classes, such as antiepileptics [1,2], antipsychotics [3,4], and antibiotics [5–8].

Several analytical approaches have been developed for TDM, including immunoassays and chromatographic techniques. Immunoassays are drug-specific and allow for the detection of very low concentrations, while chromatographic methods — such as liquid chromatography (LC) coupled with tandem mass spectrometry (MS/MS) — offer greater flexibility, enabling the quantification of different analytes or even multi-analyte detection [9,10]. In the context of TDM for betalactam antibiotics, isotope dilution LC-MS/MS [11] has emerged as the gold standard. However, individualized experimental preparations are required for each batch, limiting cost- and time-efficiency, especially for small sample runs.

Quantitative nuclear magnetic resonance spectroscopy (qNMR) has recently gained attention in clinical sciences and is already well-established in metabolomics [12–14]. It allows for fast, non-destructive quantification of small metabolites and exogenous organic compounds, with detection limits down to the low micromolar range [15]. To the best of our knowledge, qNMR has not yet been applied to TDM.

With increasing interest in TDM of antibiotics in intensive care settings — where patients often present with altered pharmacokinetics (PK) and pharmacodynamics (PD) [16–18] — we aimed to evaluate the applicability of qNMR for quantifying betalactam drug levels.

* Corresponding author.
E-mail address: dominic.barthelmes@ukr.de (D. Barthelmes).

https://doi.org/10.1016/j.cca.2025.120448
Received 22 May 2025; Received in revised form 29 May 2025; Accepted 22 June 2025
Available online 7 July 2025

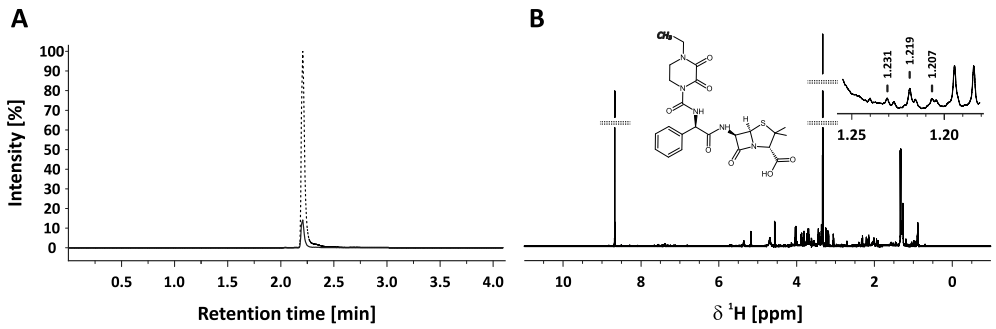

Fig. 1. (A) Exemplary liquid chromatography elution profile for piperacillin (PIP), with the quantifier (dashed line) and internal standard (d5-piperacillin, solid line). (B) Exemplary quantitative nuclear magnetic resonance spectroscopy profile, showing a ^{1}H-NMR spectrum with intense singlets at 8.673 ppm (pyrazine) and 3.329 ppm (deuterated methanol), and a highlighted triplet at 1.219 ppm originating from the indicated PIP methyl group used for quantification.

Specifically, we developed a qNMR-based method for measuring concentrations of piperacillin (PIP) in plasma samples from intensive care patients receiving piperacillin-tazobactam. Method performance was assessed by comparison with LC-MS/MS.

2. Methods

This work was approved by the Institutional Review Board (IRB) of the University of Regensburg (23-3311_3-101). Leftover material from routine blood gas analyses of patients who had received PIP within the previous 24 hours. Specimens were collected in the surgical intensive care unit at the University Hospital Regensburg between January and November 2024. Following collection, samples were centrifuged at 3000 g for 10 min, and plasma was stored at –80 °C until analysis. As only leftover material was used, the requirement for informed consent was waived by the IRB.

2.1. Liquid chromatography - tandem mass spectrometry

PIP was quantified within a range of 1.75 to 100 mg/L using a slightly modified isotope dilution LC-MS/MS method, based on [9]. Calibrators and quality controls were obtained from Recipe (Munich, Germany). For sample preparation, 100 µL of methanol containing the internal standard d5-piperacillin was added to 50 µL of plasma and briefly mixed. Following centrifugation, the supernatant was diluted 1:20 with HPLC-grade water, and 15 µL of the diluted sample was injected (Fig. 1A). Analysis was performed using a Waters Acquity UPLC system coupled to a Xevo TQD tandem quadrupole mass spectrometer (Waters, Milford, MA, USA). Intra- and inter-assay inaccuracy and imprecision (five replicates each) were ≤4.2% and ≤4.4%, respectively.

2.2. Nuclear magnetic resonance spectroscopy

PIP was quantified by qNMR within a concentration range of 10.0 to 350 mg/L using the triplet resonances at 1.219 ppm, analyzed with the proprietary Lifespin GmbH "Therapeutic Drug Profiler" software (Fig. 1B). To prepare samples, 500 µL of a precipitation reagent containing deuterated methanol and pyrazine (internal standard) was mixed with 250 µL of plasma. After incubation at –20 °C for 30 min, samples were centrifuged, and the supernatant was transferred to 5 mm Bruker NMR tubes. One-dimensional ^{1}H-NMR spectra were recorded at 38 °C on a 600 MHz Bruker Avance NEO spectrometer equipped with a 5 mm BBI probe. A NOESY-presaturation pulse sequence was used with 16 transients and 98,304 data points. The spectral width was 30 ppm, and the relaxation delay was 10 seconds, yielding a turnaround time of 7.2 min per sample. Intra- and inter-assay inaccuracy and imprecision (five replicates each) were ≤7.9% and ≤4.9%, respectively.

2.3. Data analysis and statistics

Concentration measurements from LC-MS/MS and qNMR were compared using linear regression and Bland–Altman analysis [19]. Bootstrapping with 1000 resampling steps was employed to estimate the confidence intervals of the regression parameters. Agreement was assessed by calculating bias, limits of agreement, and the concordance correlation coefficient. Data were visualized using Python 3.11.

3. Results

In total, 212 plasma samples were obtained from 15 patients. Among these, 126 samples had PIP concentrations within the common quantifiable range of 10 to 100 mg/L for both analytical methods and were included in the comparative analysis. The remaining samples outside this range (57 with concentrations below 10 mg/L and 29 above 100 mg/L) were excluded from analysis. The median PIP concentration quantified by LC-MS/MS was 40.0 mg/L (IQR 25.85 to 50.42), and by qNMR it was 37.51 mg/L (IQR 23.54 to 52.77). Linear regression displayed an intercept of −0.50 mg/L (95%-CI −3.33 to 2.41) and a slope of 1.03 (95%-CI 0.96 to 1.11; Fig. 2A). The concordance correlation coefficient was 0.895 (95%-CI 0.85 to 0.93). Mean bias was −0.85 mg/L with limits of agreement ranging from −18.67 mg/L to 16.97 mg/L (Fig. 2B).

4. Discussion

Current standard methodologies for TDM, such as isotope dilution LC-MS/MS, are generally reliable, highly sensitive, and participate in proficiency testing [20]. Commercial kits are even available for measuring multiple analytes in a single sample. However, in multi-analyte scenarios, LC-MS/MS requires tailored setups for each analyte, and the use of analyte-specific chromatography and stable-isotope labeled references limits its application for random-access testing [21]. qNMR offers a potential solution to these limitations. Unlike LC-MS/MS, qNMR does not require drug-specific reference substances or complex pre-analytical preparations, making it ideal for random-access testing. In this study, qNMR provided a run-time of just 7 min. Furthermore, qNMR has the advantage of enabling the simultaneous quantification of multiple compounds, as long as the corresponding resonances are adequately resolved in the NMR spectrum. However, compared to established TDM methods, qNMR's sensitivity remains inferior.

This study demonstrates that qNMR is suitable for quantifying the betalactam antibiotic piperacillin and guide dosing within the concentration range of 10 to 100 mg/L, which would have covered the majority of values (median 74.9 mg/L, IQR 60.6 to 91.0) in the TARGET trial—a large randomized clinical trial on TDM in ICU patients [22]. Quantifying drugs in critically ill patients presents particular challenges due to the complexities of the biological matrix, including interference

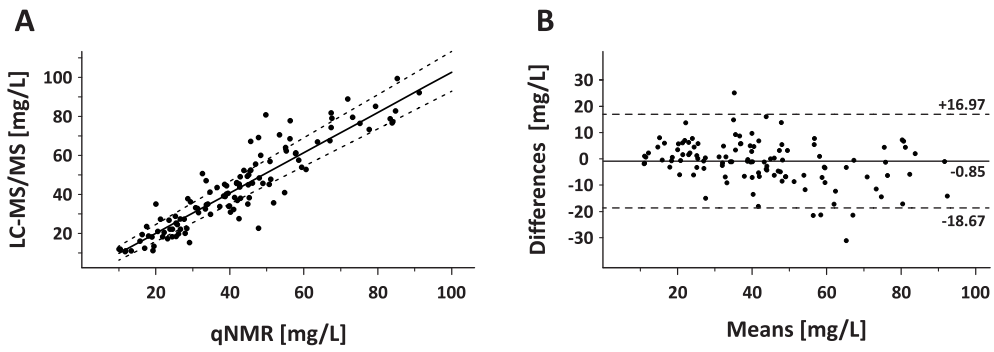

Fig. 2. Comparison of piperacillin (PIP) concentrations in intensive care patients measured by quantitative nuclear magnetic resonance (qNMR) and liquid chromatography-tandem mass spectrometry (LC-MS/MS): (A) linear regression analysis (solid line) with 95%-confidence intervals (dashed lines) computed by bootstrapping analysis; (B) Bland–Altman analysis showing mean PIP concentrations $(C_{qNMR} + C_{LC-MS/MS})/2$ against differences $(C_{LC-MS/MS} - C_{qNMR})$ between the two methods, and limits of agreement (dashed lines).

from polypharmacy. While qNMR is still an emerging technique, ongoing advancements are continually improving its performance [23–27]. We anticipate that qNMR will become a viable method for therapeutic drug monitoring in the near future, with potential extension to other clinically relevant drugs — to name a few, meropenem, ceftazidime, fosfomycin, or valproate — possibly enabling simultaneous quantification of multiple therapeutics in complex matrices.

5. Conclusions

Our study indicates that qNMR is a promising tool for therapeutic drug monitoring of piperacillin in critically ill patients. The results demonstrate that qNMR is a viable alternative to LC-MS/MS for TDM, showing a strong level of concordance. These findings support the potential for further validation in larger patient cohorts to establish its broader clinical applicability.

CRediT authorship contribution statement

Alexander Dejaco: Writing – original draft, Validation, Software, Project administration, Investigation, Data curation, Writing – review & editing, Visualization, Supervision, Resources, Methodology, Formal analysis, Conceptualization. **Michael Paal:** Visualization, Supervision, Investigation, Conceptualization, Writing – review & editing, Validation, Methodology, Data curation. **Michael Gruber:** Writing – review & editing, Resources, Methodology, Supervision, Project administration. **Diana Drettwan:** Writing – review & editing, Visualization, Software, Project administration, Investigation, Data curation, Writing – original draft, Validation, Resources, Methodology, Formal analysis, Conceptualization. **Christian Marquardt:** Validation, Software, Methodology, Data curation, Supervision, Resources, Formal analysis, Conceptualization. **Michael Helou:** Visualization, Supervision, Methodology, Data curation, Validation, Software, Investigation, Conceptualization. **Tobias Wagner:** Writing – review & editing, Resources, Software, Methodology. **Martin G. Kees:** Writing – review & editing, Supervision, Methodology, Formal analysis, Validation, Resources, Investigation. **Bernhard M. Graf:** Writing – review & editing, Supervision, Project administration, Writing – original draft, Resources, Methodology. **Dominic Barthelmes:** Writing – review & editing, Visualization, Supervision, Resources, Writing – original draft, Validation, Software, Project administration, Investigation, Data curation, Methodology, Formal analysis, Conceptualization.

Funding

This study was supported by the Clinical Scientist program of the Faculty of Medicine of the University of Regensburg.

Declaration of competing interest

Authors Alexander Dejaco, Michael Paal, Michael Gruber, Tobias Wagner, Martin G. Kees, Bernhard M. Graf, and Dominic Barthelmes declare that they have no competing interests. Authors Diana Drettwan, Christian Marquardt, and Michael Helou declare that they are employees of Lifespin GmbH.

Acknowledgments

The authors would like to express their sincere gratitude to the staff of the Surgical Intensive Care Unit of the University Hospital Regensburg for their invaluable assistance in data collection for this study, and in particular Franz Eder for his unending support. We gratefully acknowledge Ramona Mirke and Uwe-Johann Kloos for their assistance in preparing and measuring all NMR samples.

Data availability

Some parts of the dataset are available from the corresponding author on reasonable request. Parts are confidential to comply with privacy regulations.

References

[1] J. Martinho, A.Y. Simão, M. Barroso, E. Gallardo, T. Rosado, Determination of antiepileptics in biological samples—A review, Molecules 29 (19) (2024) 4679, http://dx.doi.org/10.3390/molecules29194679.
[2] M.D. Krasowski, G.A. McMillin, Advances in anti-epileptic drug testing, Clin. Chim. Acta 436 (2014) 224, http://dx.doi.org/10.1016/j.cca.2014.06.002.
[3] L. Patteet, K.E. Maudens, B. Sabbe, M. Morrens, M. de Doncker, H. Neels, High throughput identification and quantification of 16 antipsychotics and 8 major metabolites in serum using ultra-high performance liquid chromatography–tandem mass spectrometry, Clin. Chim. Acta 429 (2014) 51, http://dx.doi.org/10.1016/j.cca.2013.11.024.
[4] X.M. Hart, G. Gründer, N. Ansermot, A. Conca, E. Corruble, S. Crettol, et al., Optimisation of pharmacotherapy in psychiatry through therapeutic drug monitoring, molecular brain imaging and pharmacogenetic tests: Focus on antipsychotics, World. J. Biol. Psychiatry 25 (9) (2024) 451, http://dx.doi.org/10.1080/15622975.2024.2366235.
[5] S.G. Wicha, A.G. Märtson, E.I. Nielsen, B.C.P. Koch, L.E. Friberg, J.W. Alffenaar, et al., International society of anti-infective pharmacology (ISAP), the PK/PD study group of the European society of clinical microbiology, infectious diseases (EPASG), from therapeutic drug monitoring to model-informed precision dosing for antibiotics, Clin. Pharmacol. Ther. 109 (4) (2021) 928, http://dx.doi.org/10.1002/cpt.2202.
[6] A.J. Fratoni, D.P. Nicolau, J.L. Kuti, A guide to therapeutic drug monitoring of beta-lactam antibiotics, Pharmacotherapy 41 (2) (2021) 220, http://dx.doi.org/10.1002/phar.2505.
[7] S.K. Neef, A.-D. Hinderer, W. Arbash, M. Kinzig, F. Sörgel, C. Wunder, et al., A high performance liquid chromatography-tandem mass spectrometry assay for therapeutic drug monitoring of 10 drug compounds commonly used for antimicrobial therapy in plasma and serum of critically ill patients: Method optimization, validation, cross-validation and clinical application, Clin. Chim. Acta 559 (2024) 220, http://dx.doi.org/10.1016/j.cca.2024.119690.

[8] T. Liu, X. Chen, G. Zhang, J. Zhao, Q. Lu, F. Wang, et al., An LCMS/MS method for the simultaneous determination of ten antimicrobials and its application in critically ill patients, J. Pharm. Biomed. Anal. 252 (2025) 116489, http://dx.doi.org/10.1016/j.jpba.2024.116489.

[9] M. Paal, M. Zoller, C. Schuster, M. Vogeser, G. Schütze, 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. 152 (2018) 102.

[10] S. Barco, R. Bandettini, A. Maffia, G. Tripodi, E. Castagnola, G. Cangemi, Quantification of piperacillin, tazobactam, meropenem, ceftazidime, and linezolid in human plasma by liquid chromatography/tandem mass spectrometry, J. Chemother. 27 (6) (2015) 343, http://dx.doi.org/10.1179/1973947814Y.0000000209.

[11] J. Schäffler, K. Habler, M. Vogeser, A suggested protocol for value assignment using isotope-dilution-LC-ms/MS in reference measurement procedures – exemplary application for ciprofloxacin in serum, Clin. Chim. Acta 549 (2023) 117556, http://dx.doi.org/10.1016/j.cca.2023.117556.

[12] A.A. Crook, R. Powers, Quantitative NMR-based biomedical metabolomics: Current status and applications, Molecules 25 (21) (2020) 5128, http://dx.doi.org/10.3390/molecules25215128.

[13] D.S. Wishart, L.L. Cheng, V. Copié, A.S. Edison, H.R. Eghbalnia, J.C. Hoch, et al., NMR and metabolomics-a roadmap for the future, Metabolites 12 (8) (2022) 678, http://dx.doi.org/10.3390/metabo12080678.

[14] F.A. Castelli, G. Rosati, C. Moguet, C. Fuentes, J. Marrugo-Ramírez, T. Lefebvre, et al., Metabolomics for personalized medicine: the input of analytical chemistry from biomarker discovery to point-of-care tests, Anal. Bioanal. Chem. 414 (2) (2022) 759–789, http://dx.doi.org/10.1007/s00216-021-03586-z.

[15] A.-H. Emwas, R. Roy, R.T. McKay, L. Tenori, E. Saccenti, G.A.N. Gowda, et al., NMR spectroscopy for metabolomics research, Metabolites 9 (7) (2019) 123, http://dx.doi.org/10.3390/metabo9070123.

[16] M.H. Abdul-Aziz, J.W.C. Alffenaar, M. Bassetti, H. Bracht, G. Dimopoulos, D. Marriott, et al., Infection section of European society of intensive care medicine (ESICM), pharmacokinetic/pharmacodynamic and critically ill patient study groups of European society of clinical microbiology and infectious diseases (ESCMID), infectious diseases group of international association of therapeutic drug monitoring and clinical toxicology (IATDMCT), infections in the ICU and sepsis working group of international society of antimicrobial chemotherapy (ISAC), antimicrobial therapeutic drug monitoring in critically ill adult patients: A position paper, Intensive Care Med. 46 (6) (2020) 1127, http://dx.doi.org/10.1007/s00134-020-06050-1.

[17] N.A. Mohd Rozi, N.A. Mohd Tahir, S. Mohd Saffian, M. Makmor-Bakry, A. Mohamad Yusof, R. Mustafar, et al., Therapeutic drug monitoring-guided piperacillin dosing in critically ill patients undergoing continuous renal replacement therapy: A systematic review, J. Antimicrob. Chemother. 79 (12) (2024) 3078, http://dx.doi.org/10.1093/jac/dkae332.

[18] R. Tanaka, Pharmacokinetic variability and significance of therapeutic drug monitoring for broad-spectrum antimicrobials in critically ill patients, J. Pharm. Heal. Care Sci. 11 (1) (2025) 21, http://dx.doi.org/10.1186/s40780-025-00425-6.

[19] J.M. Bland, D.G. Altman, Statistical methods for assessing agreement between two methods of clinical measurement, Lancet 327 (8476) (1986) 307, http://dx.doi.org/10.1016/S0140-6736(86)90837-8.

[20] T. Tuzimski, A. Petruczynik, Review of chromatographic methods coupled with modern detection techniques applied in the therapeutic drugs monitoring (TDM), Molecules 25 (17) (2020) 4026, http://dx.doi.org/10.3390/molecules25174026.

[21] R.F. Greaves, LC-ms/MS random access automation- a game changer for the 24/7 clinical laboratory, Clin. Chem. Lab. Med. 62 (7) (2024) 1249, http://dx.doi.org/10.1515/cclm-2024-0501.

[22] S. Hagel, F. Bach, T. Brenner, H. Bracht, A. Brinkmann, T. Annecke, et al., TARGET trial investigators, effect of therapeutic drug monitoring-based dose optimization of piperacillin/tazobactam on sepsis-related organ dysfunction in patients with sepsis: A randomized controlled trial, Intensive Care Med. 48 (3) (2022) 311, http://dx.doi.org/10.1007/s00134-021-06609-6.

[23] J. McCullagh, F. Probert, New analytical methods focusing on polar metabolite analysis in mass spectrometry and NMR-based metabolomics, Curr. Opin. Chem. Biol. 80 (2024) 102466, http://dx.doi.org/10.1016/j.cbpa.2024.102466.

[24] V. Ribay, C. Praud, M.P.M. Letertre, J.N. Dumez, P. Giraudeau, Hyperpolarized NMR metabolomics, Curr. Opin. Chem. Biol. 74 (2023) 102307, http://dx.doi.org/10.1016/j.cbpa.2023.102307.

[25] X. Chen, G. Bertho, C. Caradeuc, N. Giraud, C. Lucas-Torres, Present and future of pure shift NMR in metabolomics, Magn. Reson. Chem. 61 (12) (2023) 654, http://dx.doi.org/10.1002/mrc.5356.

[26] A. Wong, A roadmap to high-resolution standard microcoil MAS nmr spectroscopy for metabolomics, NMR Biomed. 36 (4) (2023) e4683, http://dx.doi.org/10.1002/nbm.4683.

[27] J.R.J. Yong, A.L. Hansen, Ē. Kupče, T.D.W. Claridge, Increasing sensitivity and versatility in NMR supersequences with new HSQC-based modules, J. Magn. Reson. 329 (2021) 107027, http://dx.doi.org/10.1016/j.jmr.2021.107027.