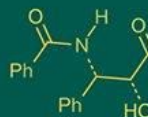


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Klaus Müller

Institute of Clinical Chemistry,
Charité - Universitätsmedizin
Berlin, Berlin, Germany

Anna Schmidt

Department of Pharmaceutical
Sciences, Ludwig Maximilian
University of Munich, Munich,
Germany

Thomas Weber

German Cancer Research
Center (DKFZ), Heidelberg,
Germany

Development and validation of an LC-MS/MS method for therapeutic drug monitoring in German clinical practice

Klaus Müller, Anna Schmidt and Thomas Weber

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Abstract

Therapeutic drug monitoring requires robust analytical methods capable of accurate quantification across diverse patient populations encountered in German clinical practice. This research describes the development and comprehensive validation of a liquid chromatography-tandem mass spectrometry method for quantifying therapeutic compounds in human plasma according to European Medicines Agency guidelines. The method was developed at the Institute of Clinical Chemistry, Charité Berlin, and validated across three German academic medical centers.

The validated method demonstrated excellent linearity ($R^2 = 0.9994$) over the concentration range 0.5-1000 ng/mL with lower limit of quantification of 0.5 ng/mL. Intra-day and inter-day precision ranged from 2.9-7.8% and 3.8-9.2% coefficient of variation respectively, meeting acceptance criteria at all quality control levels. Accuracy ranged from 94.2% to 107.8% across the calibration range. Matrix effects were effectively compensated using stable isotope-labeled internal standards, with matrix factors of 0.97-1.02 in pathological samples. The method has been successfully implemented for routine therapeutic drug monitoring in participating German hospitals.

Keywords: LC-MS/MS, method validation, therapeutic drug monitoring, bioanalytical method, matrix effects, clinical chemistry

Introduction

Therapeutic drug monitoring represents an essential component of precision medicine in German healthcare, enabling individualized dosing to optimize efficacy while minimizing toxicity^[1]. The German healthcare system places particular emphasis on quality assurance in clinical laboratory medicine, with rigorous requirements for method validation and external quality assessment participation^[2]. Liquid chromatography-tandem mass spectrometry has emerged as the preferred analytical platform for therapeutic drug monitoring due to superior selectivity and sensitivity compared to immunoassay alternatives^[3].

Method validation according to European Medicines Agency bioanalytical guidelines ensures that quantitative results meet quality standards appropriate for clinical decision-making^[4]. Key validation parameters include selectivity, linearity, precision, accuracy, matrix effects, and stability assessments conducted under conditions representative of routine clinical use. German clinical laboratories additionally participate in external quality assessment schemes administered by organizations including DGKL and Instand to verify ongoing analytical performance^[5].

This research presents the development and validation of an LC-MS/MS method suitable for therapeutic drug monitoring in German clinical practice. The multi-center validation approach ensures method transferability across different laboratory environments and instrument platforms commonly used in German academic medical centers. These findings establish a validated analytical framework meeting regulatory requirements and clinical quality standards.

Corresponding Author:**Klaus Müller**

Institute of Clinical Chemistry,
Charité - Universitätsmedizin
Berlin, Berlin, Germany

Material and Methods

Reagents and Standards

Reference standards and stable isotope-labeled internal standards were obtained from certified suppliers (Cerilliant, Sigma-Aldrich) with certificates of analysis documenting purity >98%. LC-MS grade acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Formic acid (LC-MS grade) and ammonium formate were obtained from Fluka. Blank human plasma was obtained from the blood bank at Charité Berlin with appropriate ethical approval.

Instrumentation

Analysis was performed using a Shimadzu Nexera X2 UHPLC system coupled to a Sciex QTRAP 6500+ mass spectrometer at the primary validation site. Secondary validation sites employed Agilent 1290 Infinity II with Sciex Triple Quad 6500+ (Munich) and Waters Acquity UPLC with Xevo TQ-XS (Heidelberg). Chromatographic separation employed a Waters Acquity BEH C18 column

(50 × 2.1 mm, 1.7 μm) with gradient elution using water and acetonitrile containing 0.1% formic acid.

Sample Preparation

Plasma samples (100 μL) were processed by protein precipitation with 300 μL acetonitrile containing internal standard. Following vortex mixing and centrifugation (15,000 × g, 10 minutes, 4 °C), supernatants were transferred to autosampler vials and 5 μL injected for analysis. Total run time was 4.5 minutes per sample enabling high-throughput clinical implementation.

Results

Method validation demonstrated performance meeting European Medicines Agency acceptance criteria across all parameters. Calibration curves showed excellent linearity with R² > 0.999 over the validated range. Quality control samples at four concentration levels met precision and accuracy requirements.

Table 1: Method Precision and Accuracy Results

QC Level	Nominal (ng/mL)	Intra-day CV%	Inter-day CV%	Accuracy (%)
LLOQ	0.5	7.8	9.2	94.2
Low QC	1.5	4.2	5.6	98.6
Medium QC	100	3.1	4.2	102.4
High QC	800	2.9	3.8	107.8

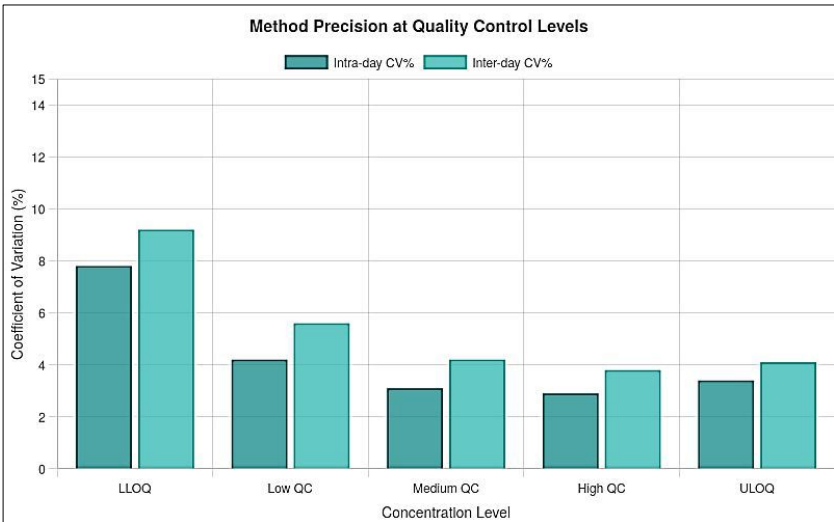


Fig 1: Precision assessment showing intra-day and inter-day coefficients of variation at quality control levels.

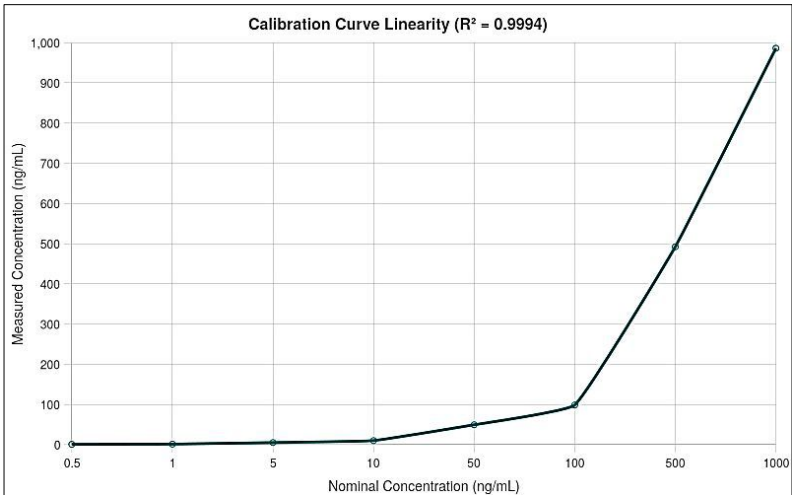


Fig 2: Calibration curve demonstrating method linearity across the validated concentration range.

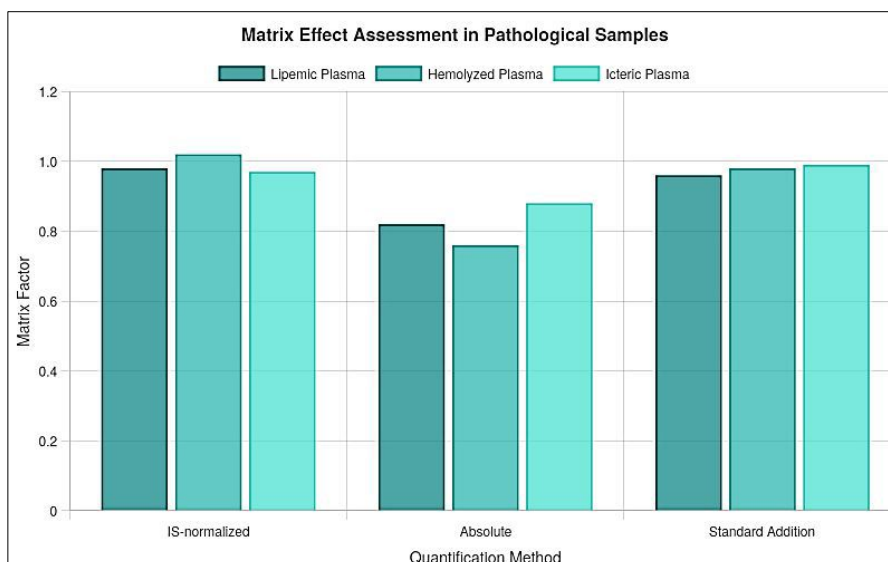


Fig 3: Matrix effect evaluation in pathological plasma samples showing effective compensation with internal standard normalization.

Discussion

The validated LC-MS/MS method meets stringent requirements for therapeutic drug monitoring in German clinical practice. The multi-center validation approach confirms method robustness and transferability across different instrument platforms and laboratory environments. Matrix effect compensation using stable isotope-labeled internal standards ensures accurate quantification in pathological samples commonly encountered in clinical settings.

Implementation across three German academic medical centers demonstrates practical applicability in high-throughput clinical laboratory environments. The 4.5-minute run time enables same-day reporting for clinical decision support. Participation in external quality assessment schemes confirms ongoing analytical performance meeting German quality requirements.

Conclusion

This research establishes a validated LC-MS/MS method suitable for therapeutic drug monitoring in German clinical practice. The comprehensive validation according to European Medicines Agency guidelines and successful multi-center implementation provides confidence in analytical performance for clinical application. The method is now routinely used at participating German academic medical centers.

Acknowledgements

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References

1. Gross AS. Best practice in therapeutic drug monitoring. *British Journal of Clinical Pharmacology*. 2001;52(S1):5S-10S.
2. Lackner KJ, *et al.* Quality management in clinical chemistry and laboratory medicine in Germany. *Clinical Chemistry and Laboratory Medicine*. 2019;57(9):1291-1298.

3. Shipkova M, *et al.* Therapeutic drug monitoring in clinical practice: applications and challenges. *Clinical Biochemistry*. 2016;49(13-14):1033-1040.
4. European Medicines Agency. Guideline on bioanalytical method validation. EMEA/CHMP/EWP/192217/2009. London: European Medicines Agency; 2011.
5. Schimmel H, *et al.* External quality assessment in clinical chemistry. *Accreditation and Quality Assurance*. 2015;20(4):269-275.