Journal of
Food and Pharmaceutical Sciences

Original Article

A Simple LC-MS/MS Method for Determination of Gentamicin in Human Plasma and Uncertainty Evaluation

Dita Amalia Prihati*, Miftah Nurrokhimah, Nurul Fatimah, Sigit Sulistya Harjono, Nur Hasna Nafiisah, Hanifah Ratna Yuliani, and Tama Jahtra Palgunadi

Laboratory of Advanced Pharmaceutical Sciences, Faculty of Pharmacy, Universitas Gadjah Mada, Yogyakarta, Indonesia

*Corresponding author: Dita Amalia Prihati | Email: ditaamalia23@ugm.ac.id

Received: 2 October 2025; Revised: 4 March 2026; Accepted: 4 March 2026; Published: 25 April 2026

Abstract: Gentamicin is an antibiotic with a narrow therapeutic index that necessitates therapeutic drug monitoring (TDM) to enhance efficacy and reduce toxicity. The goal of this study was to a simple, sensitive, and reliable LC-MS/MS method for measuring gentamicin in human plasma with a key novelty being the comprehensive assessment and documentation of measurement uncertainty integrated into the method validation process. The method used a Sciex QQQ 4500 LC-MS/MS system equipped with a C18 column, 5 x 20 mm, 4 µm Fusion-RP 80 Å, and operated in multiple reaction monitoring (MRM) mode, employing amikacin as the internal standard. Method validation was conducted following ICH M10 (2022) guidelines, encompassing parameters of selectivity, linearity, accuracy, precision, stability, dilution integrity, and carry-over. The calibration curve showed good linearity (r^2 = 0.990–0.996) over the range of 100–5000 ng/mL, with a lower limit of quantification (LLOQ) of 100 ng/mL. Across all concentration levels, accuracy (within ±15%; ±20% at LLOQ) and precision (≤15% CV; ≤20% at LLOQ) met the acceptance criteria. Short term stability experiments indicated that the samples maintained enough rigidity for various handling and storage method. We used EURACHEM and JCGM GUM guidelines to figure out the measurement uncertainty. The standard error of the slope (SEm) was the biggest factor. The total uncertainty was found to be 0.27 ng/mL, which means that the expanded uncertainty is ±0.55 ng/mL with 95% confidence. The validated method is strong and dependable, which makes it good for use in clinical settings for gentamicin TDM.

Keywords: gentamicin; LC-MS/MS; measurement uncertainty; method validation; therapeutic drug monitoring

1. INTRODUCTION

Gentamicin is an aminoglycoside antibiotic that irreversibly binds to the 30S ribosomal subunits, consequently inhibiting bacterial protein production. Gentamicin is generally delivered intravenously or intramuscularly instead of orally due to inadequate absorption in the gastrointestinal tract. It is disseminated into the extracellular fluid and is excreted in its unaltered state via glomerular filtration [1]. It is well-known for its use to treat severe infections caused by Gram-negative bacteria. In pediatrics, gentamicin is useful for treating urinary tract infection [2].

Gentamicin is reported as an alternative treatment for gonorrhea when combined with azithromycin [3]. Although it has broad-spectrum efficacy, gentamicin is classified as a Narrow-Therapeutic-Index (NTI) drug. A slight change in treatment dose can lead to severe damage because of its toxicity. Gentamicin is reported to have serious adverse effects, such as nephrotoxicity (kidney damage) and ototoxicity (hearing or balance issues), due to repeated exposure to high concentrations. The nephrotoxicity potential study was conducted in rats, and the results show that the dose of 60 mg/kg/day of gentamicin for more than 7 days lead to kidney damage [4]. Its damages also include necrosis, apoptosis, and oedema. Thus, an appropriate dosing is needed to prevent its serious adverse effects. The main factors that affect gentamicin pharmacokinetics across various groups of patients are the volume of distribution (Vd) and

clearance (Cl). The increase in volume of distribution and clearance results in a lower peak plasma concentration (Cmax) for a given dose; therefore, both factors should be considered to determine the therapeutic dose [1]. The recommended initial dose for several groups of patients, including general adults, neonates, pediatric patients, elderly patients, obese patients, and critically ill patients, ranges from 4 - 7 mg/kg TBW (total body weight). However, the optimal initial dose for gentamicin treatment appears to be 7 mg/kg TBW. It is necessary to monitor the dosing to minimize the nephrotoxicity risk after the first administration. Therefore, therapeutic drug monitoring (TDM) has an important role during gentamicin treatment to maintain the therapeutic dose [5].

Several methods have been validated to analyze gentamicin for therapeutic drug monitoring. The LC-MS/MS method is the most reliable and sensitive for determining the concentration of gentamicin in various complex matrices with very low limit of quantification level [6 - 17]. The validated analytical methods for gentamicin and other various antibiotics in human plasma conducted in previous study were reported to be reliable and applicable for TDM [18 - 21]. Another LC-MS/MS method validated for gentamicin analysis in dried blood spots for therapeutic drug monitoring in neonates [22], [23]. In this study, the analytical method validation of gentamicin in human plasma is studied based on ICH M10 Bioanalytical Method Validation and Study Sample Analysis to achieve a sensitive, reliable, and reproducible method [24]. The uncertainty factors in the gentamicin analysis in human plasma were estimated based on EURACHEM and JCGM GUM [25], [26]. The uncertainty of the analysis was estimated from reference standard purity, sample preparation method, and method validation. The measurand and the uncertainty sources were identified, then calculated as combined and expanded uncertainty. In general, the coverage factor used in the uncertainty estimation is 2 for 95% confidence level [27], [28]. This study was conducted to widen the variety of analytical methods for gentamicin analysis with LC-MS/MS.

2. MATERIALS AND METHODS

2.1. Materials

The equipment utilized includes an analytical balance (OHAUS PX224E), a semi-micro balance (OHAUS Explorer EX225D), a Dynamica Velocity 18R refrigerated centrifuge, a Phenomenex column, an LCMS/MS Sciex QQQ 4500, a vortex mixer, a micropipette, nylon membrane filter with 0.45 μm pore, and glassware. The materials utilized include Gentamicin Sulphate standard (B0316ISB, BPFI), Amikacin as internal standard (LRAC 9136, Sigma Aldrich), gradient grade methanol (Sigma Aldrich), formic acid, ammonium formate, ultrapure water, and drug-free blood plasma from Indonesian Red-Cross.

2.2. Methods

The analysis was conducted using an LC-MS/MS instrument (Sciex QQQ 4500) integrated with a Luna C18 column (50 × 4.6 mm, 5 μm, 100 Å). There were two mobile phases: mobile phase A, which was 0.1% formic acid and 5 mM ammonium formate in water, and mobile phase B, which was 0.1% formic acid in methanol. The flow rate was 0.6 mL/minute, and the injection time was 5 μL. The gradient of the mobile phase started at 95% A (0 min), went to 75% A (2 min), and then went back to 95% A (4.5–7.5 min). The total time for the analysis was 7.5 minutes, and the retention time for gentamicin was 1.1 minutes and 1.4 minutes for amikacin. The injector system was cleaned with 75% methanol, and methanol and mobile phase A were used as solvents for dilution. The detection was done in MRM mode, with ion transitions of m/z 478.136 and 322.100 for gentamicin sulphate and m/z 586.136 and 163.00 for amikacin. The ion source parameters were set as follows: CUR 35.0; CAD 7; Ion Spray Voltage (IS) 5500 V; temperature (TEM) 500°C; GS1 50; and GS2 60. Figure 1 shows the structure and fragmentation of gentamicin as determined by the mass spectrometry method used.

2.2.1. Preparation of Standard Solutions

Gentamicin sulphate (B0316ISB, BPFI) was weighed (25.0 mg) and dissolved in distilled water in a 25 mL volumetric flask to yield the stock solution (Solution A, 1000 µg/mL). A 1.0 mL aliquot of Solution A was diluted to 10 mL with distilled water to obtain Solution B (100 µg/mL). Amikacin standard (LRAC 9136, Sigma) (25.0 mg) was dissolved in distilled water in a 25 mL volumetric flask to obtain Solution X (1000 µg/mL). A 1.0 mL aliquot of Solution X was diluted to 10 mL to yield Solution Y (100 µg/mL), which served as the internal standard (IS). Serial dilutions of gentamicin were prepared by pipetting appropriate volumes of Solution A or B into 10 mL volumetric flasks and diluting to volume with distilled water. Final concentrations ranged from 2 µg/mL to 100 µg/mL.

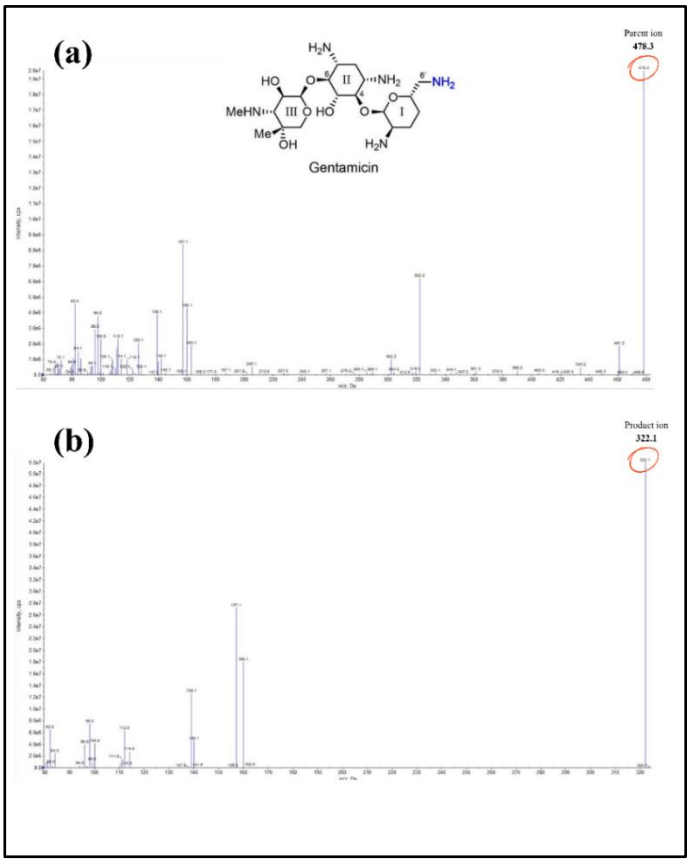

Figure 1. Structure and ion molecule of gentamicin (a); Product ion of gentamicin (b)

2.2.2. Preparation of Calibration Curve

A series of calibration curve solutions were produced by diluting gentamicin sulphate standard solution A or B to obtain final concentrations of 2 to 100 µg/mL.

2.2.3. Preparation of Spiked Plasma

Twelve blank plasma samples (100 µL each) were spiked with 100 µL of gentamicin standard solution with concentrations of 2–100 µg/mL. Then, 100 µL of internal standard (Solution Y) and 700 µL of methanol were added. The mixture was vortexed and centrifuged at 12,000 rpm at 4°C for 10 minutes. A 500 µL aliquot of supernatant was transferred to a vial, mixed with 500 µL of mobile phase A (0.1% formic acid + 5 mM ammonium formate in water), vortexed, filtered through a 0.45 µm nylon membrane filter, and injected into the LC-MS/MS (SCIEX) system. The final concentrations of spiked plasma was 0.1-5 µg/mL. To evaluate selectivity, 100 µL of blank plasma was treated similarly without the addition of a gentamicin and amikacin standard.

2.2.4. Quality Control (QC) Samples Preparation

QC samples were prepared at four concentration levels, 100 ng/mL (LLOQ), 300 ng/mL (Low), 2500 ng/mL (Medium), and 4500 ng/mL (High). Each was obtained by adding 100 µL of

blank plasma to the respective standard solutions. The extraction procedure was carried out in the same way as described for plasma samples.

2.2.5. Dilution Integrity

A high concentration stock plasma (100 μg/mL) was prepared by mixing 100 μL of Solution A with 900 μL blank plasma. This was subsequently diluted 2-, 5-, and 10-fold with blank plasma. A 100 μL aliquot was treated as per the extraction procedure above.

2.2.6. Stability Studies

Short-term stability was evaluated by storing QC samples at low and high concentrations at room temperature for 4 and 24 hours, respectively, and then processing and analyzing them according to the described procedure. Freeze-thaw stability was tested by performing three freeze-thaw cycles, namely storing QC samples at –80°C and thawing at room temperature, prior to processing and analysis. In addition, stability in the autosampler was determined by storing the processed QC samples in the autosampler for 24 hours and then reanalyzing them to assess their stability.

2.2.7. Method Validation and Analysis

Calibration standards were injected into the LC-MS/MS to construct a calibration curve based on the peak area ratio of gentamicin to the internal standard. QC samples were used to evaluate accuracy, precision, selectivity, dilution integrity, and stability parameters following ICH M10 (2022) guidelines.

3. RESULTS AND DISCUSSION

3.1. Results

Table 1 presents the analytical method validation results based on the ICH M10 (2022) guidelines.

Table 1. Validation results of gentamicin

Parameter			CV (%)	RE (%)	Mean Recovery %
			LLOQ		
	100 ng/mL		8.86	0.00	100.42
	200 ng/mL		5.35	0.02	102.40
	300 ng/mL		3.82	0.14	113.87
			Accuracy and Precision		
LLoQ	Intra-day		4.80	0.09	108.76
	Inter-day		2.59	0.11	111.46
LQC	Intra-day		0.65	-0.14	86.29
	Inter-day		12.08	-0.02	98.17
MQC	Intra-day		3.53	-0.11	88.65
	Inter-day		6.27	-0.05	95.05
HQC	Intra-day		2.00	-0.04	96.09
	Inter-day		5.10	-0.06	93.74
Dilution Factor	2 times		8.80	0.06	105.95
	5 times		2.82	0.11	110.76
	10 times		3.78	0.11	110.77
Stability Condition	T4h	LQC	0.16	0.15	114.51
		HQC	2.31	0.07	107.11
	T24h	LQC	9.12	0.07	107.39
		HQC	1.40	0.12	112.29
	Freeze and Thaw	LQC	13.68	-0.03	97.08
		HQC	4.99	0.00	100.42
	24h Autosampler	LQC	1.20	0.13	112.71
		HQC	9.53	0.05	105.20

3.1.1. Selectivity and specificity

There was no interfering peak observed at the same retention time as gentamicin in blank human plasma samples obtained from six different sources. The chromatogram of blank plasma, gentamicin, and amikacin as the internal standard is shown in Figure 2.

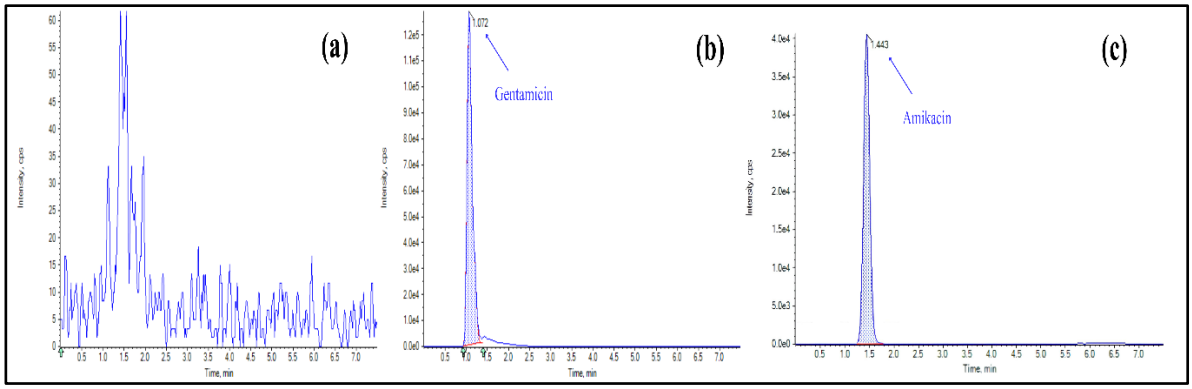

Figure 2. Extracted Ion Chromatogram (XIC) for blank plasma (a); Gentamicin standard at the concentration of 5000 ng/mL in the plasma (b); Amikacin internal standard at the concentration of 5000 ng/mL in the plasma (c). This chromatogram obtained under condition LC described in method section.

3.1.2. Lower Limit of Quantification (LLoQ)

The Lower Limit of Quantification (LLoQ) parameter was studied to quantify the lowest concentration of analyte in the sample for this method. To quantify the LLoQ values, the three lowest concentrations from the calibration curve range (100, 200, and 300 ng/mL) were injected, and their accuracy and precision were evaluated. The accuracy was assessed by the average % recovery, while precision was determined by the coefficient of variation (%CV). It was concluded that the average % recovery for gentamicin was 100.42% with a coefficient of variation of 8.86 % at the gentamicin concentration of 100 ng/mL. According to ICH M10, the acceptable %Recovery was 80 – 120% while the acceptable %CV was 20%. Therefore, the gentamicin concentration of 100 ng/mL was selected as the LLoQ of this method. The comparison of LLoQ values of this method to the other methods was shown in Table 2.

Table 2. The comparison of the developed method using LCMS-MS and other methods for the determination of gentamycin

Sample	LLoQ (ng/mL)	Methods	References
Bovine plasma	3.3	LC/MS/MS	[29]
Plasma	60 - 100	UHPLC-MS/MS	[30]
Cappilary plasma microsamples	500	LCMS/MS	[31]
Human plasma	630	HPLC-MS	[32]
Dried blood spots	25 - 100	UHPLC-MS/MS	[33]
Human plasma	100	LCMS-MS	This work

3.1.3. Calibration Curves

The calibration curve of gentamicin analysis was performed by using ten points of standard concentration in the range of 100 to 5000 ng/mL. The linearity was determined by the value of mean regression coefficients (r^2) between 0.990 and 0.996 with weighting $1/x^2$.

3.1.4. Accuracy and Precision

The accuracy and precision of this research were evaluated using the %Recovery (%RE) and %CV values for each of the three concentration levels investigated: LLoQ, LQC, MQC, and HQC. The results showed that the intra-day %Recovery was in the range of 86.29 – 108.76%. The inter-day %Recovery was in the range of 95.05 – 111.46%. Meanwhile, the intra-day %CV was in the range of 0.65 – 4.80%. The inter-day %CV was in the range of 2.59 – 12.08%. According to ICH M10, the %Recovery should be in the range of 85 – 115% except for LLoQ which was in the range of 80 – 120%. On the other hand, the acceptable %CV was less than 15% except for LLoQ which was less than 20%. Based on the result above, the accuracy and precision of LLoQ, LQC, MQC, and HQC had met the requirements of ICH M10.

3.1.5. Mean Recovery

The determination of gentamicin in the plasma sample method showed a good % recovery at all concentration levels. This method also produced a constant coefficient variation (CV) at all concentration levels, which indicated that this method was reliable for bioanalytical purposes. These results supported the validation of the bioanalytical method in accordance with the ICH M10 (2022) guidelines.

3.1.6. Carry-over

The carry-over effect was studied by injecting blank plasma immediately after the highest concentration of the calibration curve. The absence of a significant analyte peak in the blank chromatogram confirmed that there was no carry-over as shown in Figure 2(a).

3.1.7. Dilution Integrity

The plasma samples were diluted at ratios of 1:2, 1:5, and 1:10. It was found that the accuracy and precision requirements were in the acceptable range in accordance with ICH M10 (2022) guidelines.

3.1.8. Short-Term Stability

Plasma samples were spiked with the standard of gentamycin and stored at –80 °C for 4 and 24 hours to study the short-term stability. Moreover, plasma spikes in 4- and 24-hour freeze-thaw cycles were also studied. In order to do the freeze-thaw cycles analysis, the standard solution was kept at room temperature for a maximum of 4 hours. Then, it was transferred to a freezer with a temperature of –80°C before further analysis. On the other hand, the 24-hour freeze-thaw cycles were conducted by storing the plasma for 24 hours at –80 °C. Then, the plasma-spiked samples were defrosted for further analysis. The obtained %recovery (97.08–114.51%) and %CV (0.16–13.68%) were within the acceptance limits defined by International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use M10, which require accuracy within ±15% (±20% at LLOQ) and precision not exceeding 15% CV (20% at LLOQ). Therefore, the results demonstrate that the method met the acceptable criteria for analytical performance. Thus, this method had good stability as long as it was kept at –80°C. Another point that should be considered is the condition of the deep freezer. The temperature of the deep freezer should be monitored daily, with the acceptable temperature variation up to ±5°C to minimize the probability of sample degradation due to unsuitable storage.

3.2. Uncertainty Evaluation

The uncertainty analysis of gentamicin quantification in plasma using LC-MS/MS is influenced by multiple parameters, which can be systematically illustrated using a fishbone diagram in the Figure 3. Sources of uncertainty arise from reference materials, particularly the concentration of the gentamicin standard and the internal standard (amikacin), which are associated with certificate of analysis values and weighing accuracy. Instrumentation enhances repeatability and the standard error of the slope (SEm), indicating signal stability, detector sensitivity, and injection reproducibility. Moreover, sample preparation raises uncertainty due to the dilution factor, as variations can occur in both human and automated dilution procedures. The analytical method influences measurement, with recovery indicating the effectiveness of analyte extraction from the plasma matrix, where fluctuations directly impact the final results. Operator-related issues, albeit generally minor, such as discrepancies in pipetting and dilution accuracy, might impact total measurement precision. The fishbone diagram offers a systematic framework for identifying and comprehending diverse sources of uncertainty, crucial for quantitatively evaluating their effect on overall measurement uncertainty [21]. Upon identifying items through the fishbone diagram, each source of uncertainty was quantified and incorporated into the calculation. The concentrations of gentamicin and the internal standard, along with instrument-related variables such as repeatability and the standard error of the mean (SEm), dilution factor, recovery, and operator-related variability, were articulated as relative standard uncertainties. The components were integrated using the root sum of squares (RSS) method, adhering to JCGM GUM and EURACHEM standards. This method enables the proportional assessment of each contributing factor and produces an adequate estimate of the overall combined measurement uncertainty.

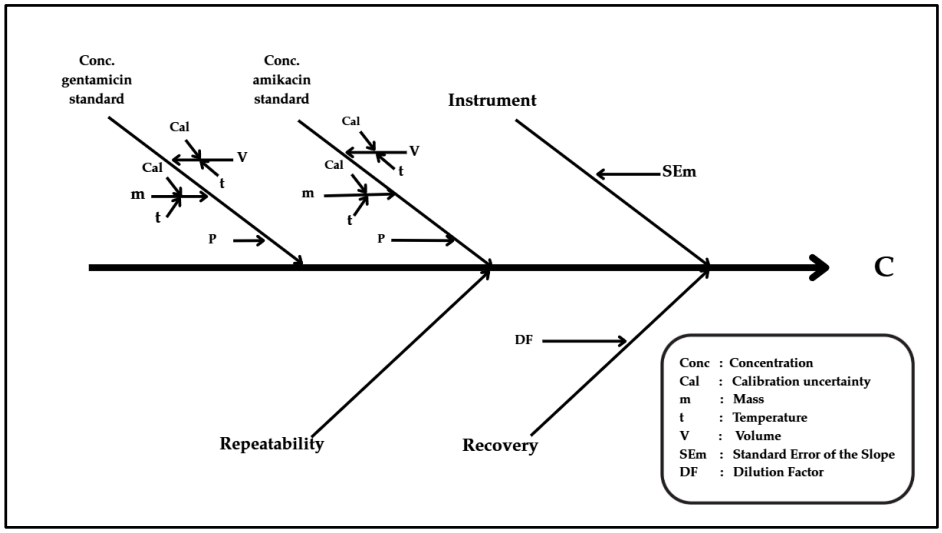

Figure 3. Fishbone diagram of uncertainty sources in gentamicin determination.

Following the identification of factors through the fishbone diagram, each source of uncertainty was quantified and incorporated into the calculation. The concentrations of gentamicin and the internal standard, along with instrument-related data such as repeatability and the standard error of the slope (SEm), dilution factor, recovery, and operator-related variability, were articulated as relative standard uncertainties. The components were subsequently integrated utilizing the root sum of squares (RSS) method, in compliance with JCGM GUM and EURACHEM standards. This method facilitates the proportional evaluation of each contributing factor and yields a representative estimate of the total combined measurement uncertainty. Uncertainty estimation for gentamicin concentration measurement in plasma using LC-MS/MS presents in the Table 3.

Table 3. Uncertainty estimation for gentamicin determination in human plasma using LC-MS/MS

Parameter	Value	Unit	*u*	Unit	*u*C/C	(*u*C/C)^2
Conc. Gentamicin Standard	999.3991	ng/mL	0.0248	ng/mL	0.00002	0.0000
Conc. Amikacin Standard	994.8062	ng/mL	0.0249	ng/mL	0.00003	0.0000
SEm	0.000238		0.0001		0.2733	0.0747
Dilution Factor	100		0.4895		0.0049	0.00002
Recovery	99.47	%	0.8721	%	0.0088	0.0001
Repeatability	111.6133	ng/mL	1.8462	ng/mL	0.0165	0.0003
Total						**0.0751**
***u*C**						**0.27**

Table 3 presents the uncertainty estimation for gentamicin concentration measurement in plasma using LC-MS/MS. The calculation includes several important variables that contribute to the overall uncertainty, such as the concentrations of the gentamicin standard and internal standard (amikacin), the standard error of the slope (SEm), dilution factor, recovery, and method repeatability. Each parameter was converted into its standard uncertainty (u) and expressed as relative uncertainty (uC/C). The squared relative uncertainties were then summed to obtain the combined uncertainty (uC). Finally, the combined uncertainty was expanded using a coverage factor of k = 2 at a 95% confidence level, yielding a final expanded uncertainty of ±0.55 ng/mL.

The calculation showed that the largest contribution to uncertainty came from the standard error of the slope (SEm) with uC/C = 0.2733, yielding $(u\text{C/C})^2$ = 0.0747. Other components such as recovery (uC/C = 0.0088; $(u\text{C/C})^2$ = 0.0001 and dilution factor (uC/C = 0.0049; $(u\text{C/C})^2 \approx 0.00002$ contributed relatively little. The total $(u\text{C/C})^2$ was 0.0748, resulting in a combined uncertainty of 0.27 ng/mL. After applying a coverage factor of k=2, the expanded uncertainty was calculated as ±0.55 ng/mL at the 95% confidence level, confirming the reliability of the method for gentamicin determination in plasma matrix [21].

4. CONCLUSION

This study established and validated a robust and sensitive LC–MS/MS methodology for quantifying gentamicin in human plasma, adhering to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use M10 (2022). The primary innovation resides in the thorough assessment of measurement uncertainty, pinpointing the standard error of the slope (SEm) as the principal factor, with other elements exerting negligible influence. The method showed good selectivity, linearity, accuracy, precision, and stability, which means it can be used to monitor therapeutic drugs. However, potential limitations, including matrix effects, ion suppression, and individual variability, must be considered, and further validation in larger clinical populations is essential to enhance generalizability. The combined uncertainty (0.27 ng/mL) and the extended uncertainty (±0.55 ng/mL, 95% confidence level) indicate that the measurement is precise, and the uncertainty is well-defined.

Funding: This research was funded by Universitas Gadjah Mada in the Educational Laboratory Staff Competency Improvement Program
Acknowledgement: Appreciation is extended to the Advanced Pharmaceutical Sciences Laboratory, Faculty of Pharmacy Universitas Gadjah Mada for providing laboratory facilities for conducting this research
Conflicts of interest: The authors declare no conflict of interest.

References

[1] M. Crcek, J. Zdovc, and M. Kerec Kos, "A review of population pharmacokinetic models of gentamicin in paediatric patients," Oct. 01, 2019, *Blackwell Publishing Ltd*. doi: 10.1111/jcpt.12850.
[2] H. Kato, M. Hagihara, H. Matsuda, and T. Iwamoto, "Gentamicin Pharmacokinetics and Optimal Dosage in Infant Patients: A Case Report and Literature Review," *Int. J. Environ. Res. Public Health*, vol. 19, no. 22, Nov. 2022, doi: 10.3390/ijerph192215360.
[3] P. Burmeister, S. Su, A. J. Smith, and K. J. Wilby, "The Use of Gentamicin for Treatment of Urogenital and Extragenital Gonorrhea: A Systematic Review of Efficacy Data," Oct. 01, 2020, *SAGE Publications Inc.* doi: 10.1177/1060028020927057.
[4] P. M. Lintong *et al.*, "Gambaran Mikroskopik Ginjal Tikus Wistar (Rattus Norvegicus) Setelah Diinduksi Dengan Gentamisin."
[5] C. J. Hodiamont, A. K. van den Broek, S. L. de Vroom, J. M. Prins, R. A. A. Mathôt, and R. M. van Hest, "Clinical Pharmacokinetics of Gentamicin in Various Patient Populations and Consequences for Optimal Dosing for Gram-Negative Infections: An Updated Review," Aug. 01, 2022, *Adis*. doi: 10.1007/s40262-022-01143-0.
[6] H. Brozmanova, R. Urinovska, K. Safarcik, F. Vsiansky, I. Kacirova, and M. Grundmann, "Liquid chromatography-tandem mass spectrometry method for quantification of gentamicin and its individual congeners in serum and comparison results with two immunoanalytical methods (fluorescence polarization immunoassay and chemiluminiscent microparticle immunoassay)," *Clinica Chimica Acta*, vol. 521, pp. 191–198, Oct. 2021, doi: 10.1016/j.cca.2021.07.014.
[7] S. Altaweel, A. Van Schepdael, E. Adams, and A. Almomen, "The development of a bioanalytical method for the simultaneous analysis of gentamicin and tacrolimus in Rat whole blood using UHPLC–MS/MS," *Sci. Rep.*, vol. 15, no. 1, Dec. 2025, doi: 10.1038/s41598-025-92418-6.
[8] C. Zhang *et al.*, "Determination of four main components of gentamicin in animal tissues after solid-phase extraction by high-performance liquid chromatography/tandem mass spectrometry," *Rapid Communications in Mass Spectrometry*, vol. 32, no. 20, pp. 1766–1772, Oct. 2018, doi: 10.1002/rcm.8234.
[9] Z. Zhu, G. Liu, F. Wang, J. J. Sasanya, and A. Cannavan, "Development of a Liquid Chromatography Tandem Mass Spectrometric Method for Simultaneous Determination of 15 Aminoglycoside Residues in Porcine Tissues," *Food Anal. Methods*, vol. 9, no. 9, pp. 2587–2599, Sep. 2016, doi: 10.1007/s12161-016-0446-1.

[10] M. Rodriquez, D. S. Cretoso, M. A. Euterpio, P. Russo, C. Crescenzi, and R. P. Aquino, "Fast determination of underivatized gentamicin C components and impurities by LC-MS using a porous graphitic carbon stationary phase," *Anal. Bioanal. Chem.*, vol. 407, no. 25, pp. 7691–7701, Oct. 2015, doi: 10.1007/s00216-015-8933-6.

[11] X. Sun, Y. Yang, Q. Tian, D. Shang, J. Xing, and Y. Zhai, "Determination of gentamicin C components in fish tissues through SPE-Hypercarb-HPLC-MS/MS," *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, vol. 1093–1094, pp. 167–173, Sep. 2018, doi: 10.1016/j.jchromb.2018.07.011.

[12] U. Woiwode, A. Sievers-Engler, and M. Lämmerhofer, "Preparation of fluorescent labeled gentamicin as biological tracer and its characterization by liquid chromatography and high resolution mass spectrometry," *J. Pharm. Biomed. Anal.*, vol. 121, pp. 307–315, Mar. 2016, doi: 10.1016/j.jpba.2015.12.053.

[13] J. B. Arsand *et al.*, "Determination of aminoglycoside residues in milk and muscle based on a simple and fast extraction procedure followed by liquid chromatography coupled to tandem mass spectrometry and time of flight mass spectrometry," *Talanta*, vol. 154, pp. 38–45, Jul. 2016, doi: 10.1016/j.talanta.2016.03.045.

[14] F. Ibrahim *et al.*, "Toxicity Profile and Pharmacokinetic Study of Antibiotic Mixtures, Gentamicin and Vancomycin, in Rat Plasma by Ecofriendly Liquid Chromatography Coupled Tandem Mass Spectrometry," *Chem. Res. Toxicol.*, vol. 33, no. 10, pp. 2647–2658, Oct. 2020, doi: 10.1021/acs.chemrestox.0c00285.

[15] Y. Bijleveld *et al.*, "A simple quantitative method analysing amikacin, gentamicin, and vancomycin levels in human newborn plasma using ion-pair liquid chromatography/tandem mass spectrometry and its applicability to a clinical study," *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, vol. 951–952, no. 1, pp. 110–118, Mar. 2014, doi: 10.1016/j.jchromb.2014.01.035.

[16] A. P. Grando *et al.*, "Quantification of amikacin, gentamicin, and tobramycin in capillary plasma microsamples by LC-MS/MS," *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, vol. 1263, Sep. 2025, doi: 10.1016/j.jchromb.2025.124698.

[17] S. Lucha *et al.*, "An LC-MS/MS based candidate reference method for the quantification of total gentamicin in human serum and plasma using NMR characterized calibrator material," *Clinica Chimica Acta*, vol. 464, pp. 211–217, Jan. 2017, doi: 10.1016/j.cca.2016.11.029.

[18] S. Barco *et al.*, "A liquid chromatography-tandem mass spectrometry platform for the routine therapeutic drug monitoring of 14 antibiotics: Application to critically ill pediatric patients," *J. Pharm. Biomed. Anal.*, vol. 186, Jul. 2020, doi: 10.1016/j.jpba.2020.113273.

[19] J. Mula *et al.*, "Analytical validation of a novel UHPLC-MS/MS method for 19 antibiotics quantification in plasma: Implementation in a LC-MS/MS Kit," *Biomedicine and Pharmacotherapy*, vol. 163, Jul. 2023, doi: 10.1016/j.biopha.2023.114790.

[20] E. D. Utami *et al.*, "Development and validation of LC-MS/MS method for the determination of amikacin in human plasma and its application in adult hospitalized patients in Yogyakarta Indonesia," *J. Appl. Pharm. Sci.*, vol. 14, no. 3, pp. 108–118, Mar. 2024, doi: 10.7324/JAPS.2024.142046.

[21] M. A. A. van der Veer, M. van der Meer-Vos, T. R. de Haan, L. G. W. Franken, Y. A. Bijleveld, and R. A. A. Mathôt, "A quantitative method for the analysis of total and unbound concentrations of amoxicillin, ampicillin, ceftazidime, ceftriaxone, ertapenem, fosfomycin and penicillin G in human plasma with liquid chromatography–tandem mass spectrometry assay," *Biomedical Chromatography*, vol. 38, no. 10, Oct. 2024, doi: 10.1002/bmc.5956.

[22] A. L. Anibaletto dos Santos *et al.*, "Development and validation of an assay for the measurement of gentamicin concentrations in dried blood spots using UHPLC-MS/MS," *J. Pharm. Biomed. Anal.*, vol. 208, Jan. 2022, doi: 10.1016/j.jpba.2021.114448.

[23] Q. Liu, L. Liu, Y. Yuan, and F. Xie, "A Validated UHPLC–MS/MS Method to Quantify Eight Antibiotics in Quantitative Dried Blood Spots in Support of Pharmacokinetic Studies in Neonates," *Antibiotics*, vol. 12, no. 2, Feb. 2023, doi: 10.3390/antibiotics12020199.

[24] "INTERNATIONAL COUNCIL FOR HARMONISATION OF TECHNICAL REQUIREMENTS FOR PHARMACEUTICALS FOR HUMAN USE ICH HARMONISED GUIDELINE BIOANALYTICAL METHOD VALIDATION AND STUDY SAMPLE ANALYSIS M10."

[25] U. S. Ellison Secretary *et al.*, "EURACHEM/CITAC Guide Quantifying Uncertainty in Analytical Measurement Composition of the Working Group* EURACHEM members A Williams Chairman A Brzyski R Kaus E Amico di Meane M Rösslein A Fajgelj IAEA Vienna," 2009.

[26] "JCGM GUM-6:2020 Guide to the expression of uncertainty in measurement-Part 6: Developing and using measurement models Guide pour l'expression de l'incertitude de mesure-Partie 6: Élaboration et utilisation des modèles de mesure," 2020. [Online]. Available: www.bipm.org

[27] M. A. L. Traple, A. M. Saviano, F. L. Francisco, and F. R. Lourenço, "Measurement uncertainty in pharmaceutical analysis and its application," Feb. 2014. doi: 10.1016/j.jpha.2013.11.001.

[28] I. Farrance and R. Frenkel, "Uncertainty of Measurement: A Review of the Rules for Calculating Uncertainty Components through Functional Relationships," 2012.

[29] D. N. Heller, J. O. Peggins, C. B. Nochetto, M. L. Smith, O. A. Chiesa, and K. Moulton, "LC/MS/MS measurement of gentamicin in bovine plasma, urine, milk, and biopsy samples taken from kidneys of standing animals," *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, vol. 821, no. 1, pp. 22–30, Jul. 2005, doi: 10.1016/j.jchromb.2005.04.015.

[30] A. L. Anibaletto dos Santos *et al.*, "Sensitive determination of gentamicin in plasma using ion-exchange solid-phase extraction followed by UHPLC-MS/MS analysis," *Pract. Lab. Med.*, vol. 26, Aug. 2021, doi: 10.1016/j.plabm.2021.e00246.

[31] A. P. Grando *et al.*, "Quantification of amikacin, gentamicin, and tobramycin in capillary plasma microsamples by LC-MS/MS," *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.*, vol. 1263, Sep. 2025, doi: 10.1016/j.jchromb.2025.124698.

[32] L. Baietto *et al.*, "Development and validation of a simultaneous extraction procedure for HPLC-MS quantification of daptomycin, amikacin, gentamicin, and rifampicin in human plasma," *Anal. Bioanal. Chem.*, vol. 396, no. 2, pp. 791–798, 2010, doi: 10.1007/s00216-009-3263-1.

[33] A. L. Anibaletto dos Santos *et al.*, "Development and validation of an assay for the measurement of gentamicin concentrations in dried blood spots using UHPLC-MS/MS," *J. Pharm. Biomed. Anal.*, vol. 208, Jan. 2022, doi: 10.1016/j.jpba.2021.114448.ss

© 2026 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).