# Determination of Amikacin in Human Serum Using Liquid Chromatography-Tandem Mass Spectrometry

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## INTRODUCTION

SLIT™Amikacin is a formulation of amikacin encapsulated inside nanoscale liposomal carriers being developed for administration via inhalation for the treatment of gram negative infections. Amikacin works by binding to the bacterial 30S ribosomal subunit, causing misreading of mRNA and leaving the bacterium unable to synthesize proteins vital to its growth.

Amikacin is an aminoglycoside used for treating severe, hospital-acquired infections with multidrug resistant Gram negative bacteria. The early phase studies have been conducted in cystic fibrosis patients with pseudomonas infections. This has required the development of a validated method for quantitation of amikacin in human serum.

No published article was found about the LC/MS/MS analysis of amikacin in human serum. In order to allow the chromatographic retention and sensitivity of the polar amikacin and the internal standard on a reverse-phase column, in combination with MS detection, the use of a volatile ion-pair agent in the mobile phase was necessary. We found that heptafluorobutyric acid (HFBA) showed a reasonable retention and less suppression than trifluoroacetic acid.

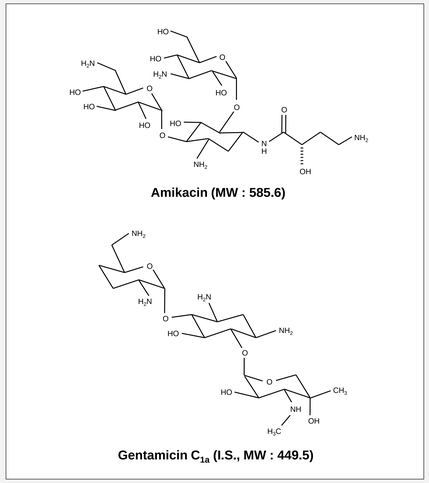


**Figure 1**. Structure of Amikacin and Gentamicin

## METHOD

The supernatants from clinical serum samples, following a protein precipitation procedure, were chromatographed on a Betasil phenyl column (5µ, 100x2.1mm) using heptafluorobutyric acid (HFBA) as ion-pairing agent to enhance sensitivity and eliminate the potential matrix effect through increased retention. During method development, butirosin and gentamicin were evaluated as internal standard candidates, with component $C_{1a}$ of gentamicin being selected as internal standard to eliminate interactions between amikacin and internal standard.

### Liquid Chromatography

Shimadzu 10ADvp HPLC system
Column : Betasil Phenyl (100 x 2.1 mm) 5µm, Thermo Electron Co.
Mobile Phase A :
MeOH/10 mM $NH_4OAc$ (pH 4.0)/Heptafluorobutyric acid (5/95/0.2)
Mobile Phase B : MeOH
Gradient Program : Flow rate (0.30 mL/min)

| Time (min) | 0.01 | 2.00 | 2.50 | 2.60 | 4.50 |
|---|---|---|---|---|---|
| % of B | 40.0 | 90.0 | 90.0 | 40.0 | stop |

### Mass Spectrometry

Applied Biosystem/MDS-Sciex API 3000
Polarity : positive
Scan type : Multiple Reaction Monitoring
Amikacin : 586.4 → 425.2
Gentamicin $C_{1a}$ (I.S.) : 450.2 → 322.2

### Extraction

Aliquot 0.100mL of human serum sample
↓
Add 0.100mL of IS solution (5µg/mL gentamicin) and 0.050mL of trichloroacetic acid (10%)
↓
Vortex and centrifuge
↓
Take 0.100mL of supernatant into 1.5mL tube and add 1.00mL of mobile phase A

## RESULTS

The method was fully validated over **a range of 0.15 to 40.0 µg/mL** with linear regression ($1/x^2$). The correlation coefficients for first three validation batches were 0.993 or better (Figure 2).

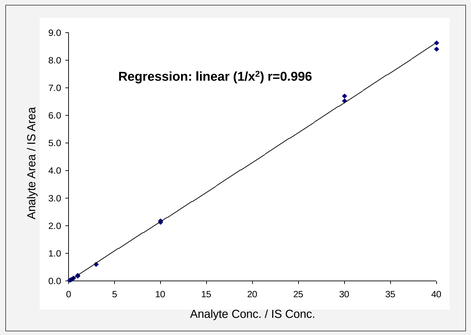


**Figure 2**. Calibration curve for 0.15 to 40.0 µg/mL Amikacin

**Figures 3 through 5** show typical chromatograms of extracted lower and upper limits of quatitation (LLOQ 0.15 µg/mL; ULOQ 40.0 µg/mL), and an extracted blank matrix injected after the ULOQ. At the LLOQ, the signal-to-noise ratio was greater than 20. No interference, or carryover was observed in the chromatogram of blank matrix.

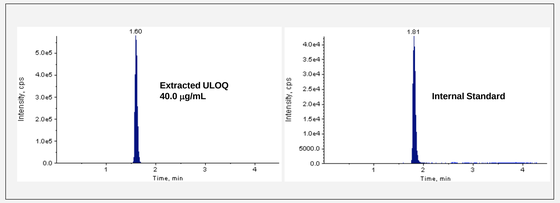


**Figure 3**. Chromatogram of an extracted ULOQ sample (40.0 µg/mL).

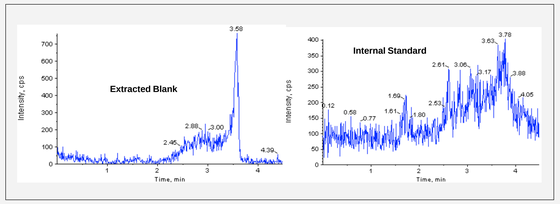


**Figure 4**. Chromatogram of an extracted blank sample injected after ULOQ.

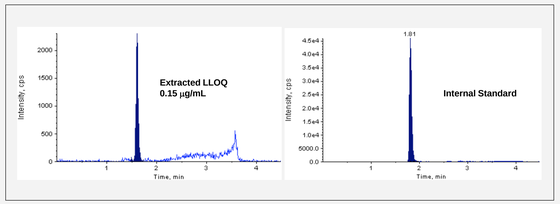


**Figure 5**. Chromatogram of an extracted LLOQ sample (0.15 µg/mL).

**Table 1 and 2** summarize results of inter-batch, intra-batch validation batches. All concentration levels demonstrate excellent precision and accuracy.

**Table 1**. Precision and Accuracy Results (inter-batch) for Amikacin Quality Control Samples.

| | 0.15 µg/mL (LLOQ) | 0.45 µg/mL (Low) | 15.0 µg/mL (Mid) | 25.0 µg/mL (High) |
|---|---|---|---|---|
| **n** | 18 | 18 | 18 | 18 |
| **Mean** | 0.148 | 0.417 | 15.6 | 27.0 |
| **Precision (cv %)** | 4.05 | 4.21 | 4.61 | 6.15 |
| **Accuracy (bias %)** | -1.63 | -7.25 | 3.98 | 8.19 |

**Table 2**. Precision and Accuracy Results (intra-batch) for Amikacin Quality Control Samples.

| | | 0.15 µg/mL (LLOQ) | 0.45 µg/mL (Low) | 15.0 µg/mL (Mid) | 25.0 µg/mL (High) |
|---|---|---|---|---|---|
| **Batch 1** | **n** | 6 | 6 | 6 | 6 |
| | **mean** | 0.147 | 0.422 | 16.2 | 28.6 |
| | **cv (%)** | 5.67 | 6.45 | 5.34 | 4.73 |
| | **bias (%)** | -2.27 | -6.32 | 7.89 | 14.37 |
| **Batch 2** | **n** | 6 | 6 | 6 | 6 |
| | **mean** | 0.149 | 0.414 | 15.6 | 26.6 |
| | **cv (%)** | 2.59 | 1.98 | 2.06 | 3.05 |
| | **bias (%)** | -0.86 | -8.03 | 3.91 | 6.32 |
| **Batch 3** | **n** | 6 | 6 | 6 | 6 |
| | **mean** | 0.147 | 0.417 | 15.0 | 26.0 |
| | **cv (%)** | 4.00 | 3.43 | 2.09 | 5.97 |
| | **bias (%)** | -1.77 | -7.40 | 0.14 | 3.87 |

Stability results for Amikacin are listed in **Table 3**. Selectivity was tested by determining accuracy and precision of mid-level QCs (0.600 µg/mL) in six different lots of blank matrix (**Table 4**). No interference or matrix effects were observed.

**Table 3**. Summary of Stability Results for Amikacin in Human Serum.

| | |
|---|---|
| Freeze-thaw stability | 3 cycles |
| Bench-top stability | 6 hours |
| Autosampler stability at room temperature | 6 days 9 hours |
| Refrigerated extract stability | 6 days 10 hours |
| Individual sample reinjection at room temp. | 8 hours |
| Primary solution stability at 4 °C * | 15 days |

* In the solution of MeOH/DI water (40/60).

**Table 4**. Matrix Effect for Amikacin in Human Serum.

| | Avg drug area | Avg IS area | Avg area ratio |
|---|---|---|---|
| **ME 1** | 20880.6 | 225018.1 | 0.0928 |
| **ME 2** | 22236.9 | 219719.6 | 0.1012 |
| **ME 3** | 23924.9 | 220034.6 | 0.1087 |
| **ME 4** | 24435.5 | 225815.0 | 0.1082 |
| **ME 5** | 23261.0 | 211193.3 | 0.1101 |
| **ME 6** | 20116.9 | 212723.4 | 0.0946 |
| **mean** | **22475.9** | **219084.0** | **0.1026** |
| **n** | **6** | **6** | **6** |
| **SD** | **1715.97** | **6075.31** | **0.0076** |
| **%CV** | **7.63** | **2.77** | **7.40** |

## CONCLUSION

A robust LC/MS/MS method for the determination of amikacin in human serum was developed and fully validated. This method showed acceptable accuracy, selectivity, and reproducibility, and was successfully applied to the analysis of clinical samples.