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

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INTRODUCTION

SLITT™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 quantification 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 heptfluorobutyric acid (HFBA) showed a reasonable retention and less suppression than trifluoroacetic acid.

METHOD

The supernatants from clinical serum samples, following a protein precipitation procedure, were chromatographed on a Betasil phenyl column (5µ, 100x2.1mm) using heptfluorobutyric acid (HFBA) as ion-pairing agent to enhance sensitivity and eliminate the potential matrix effect through increased retention. During method development, butisol and gentamicin were evaluated as internal standard candidates, with component C14 of gentamicin being selected as internal standard to eliminate interactions between amikacin and internal standard.

Liquid Chromatography


tables

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

<table>
<thead>
<tr>
<th>Drug Level (µg/mL)</th>
<th>Low</th>
<th>High</th>
<th>Mean</th>
<th>SD</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(ng/mL)</td>
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<td>(ng/mL)</td>
<td></td>
<td>(%, CV)</td>
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<tr>
<td>0.15</td>
<td>22475.9</td>
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<td>0.1026</td>
<td>0.128</td>
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- **Table 2.** Precision and Accuracy Results (intra-batch) for Amikacin Quality Control Samples.

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

<table>
<thead>
<tr>
<th>Stability Condition</th>
<th>% Mean Off Validation Level</th>
<th>% CV</th>
<th>% RSD</th>
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<td>Bench-top stability</td>
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<tr>
<td>Room temp. stability</td>
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<td>Primary Stability</td>
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- **Table 4.** Matrix Effect for Amikacin in Human Serum.

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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.