

Development of a Bioanalytical LC/MS/MS Assay for the Quantitative Analysis of Amikacin, Neomycin and Gentamicin in Plasma and Tissue Samples

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Overview

- **Purpose** - Develop a bioanalytical HPLC/MS/MS method to determine concentrations of various aminoglycosides in plasma, urine and tissue samples.
- **Methods** – Methanol precipitation and reversed-phase HPLC/MS/MS using ion-pair reagent (API4000).
- **Results** – Range from 5 -10,000 ng/mL with accuracies and precision better than 20%.

Introduction

Aminoglycosides are antibiotics used mainly in the treatment of infections caused by aerobic gram-negative bacteria. The chemical structures of these compounds consist of two or more aminosugars joined by a glycosidic linkage to an aminocyclitol component. These compounds are very hydrophilic due to the large number of amino and hydroxyl groups in their structures. This presents a challenge when developing an HPLC/MS/MS method because the use of traditional reversed-phase HPLC will not retain these compounds on the column. Here we report on the development of an accurate, precise and specific HPLC/MS/MS assay for the determination of amikacin, neomycin and gentamicin from rat and mouse plasma as well as various tissues.

Method

Extraction

- For tissue samples, homogenize sample with equal volume of PBS (μL/mg).
- Precipitate sample with methanol containing 1% formic acid.
- Dilute supernatant to approximately 25% organic with aqueous mobile phase.

Mass Spectrometry

- Sciex API4000 operating in MRM mode
- Positive ion ESI MRM transitions:
 - Amikacin (586.4 → 425.3)
 - Neomycin (615.5 → 455.4)
 - Gentamicin (478.3 → 321.9)
 - Tobramycin IS (468.3 → 324.5)

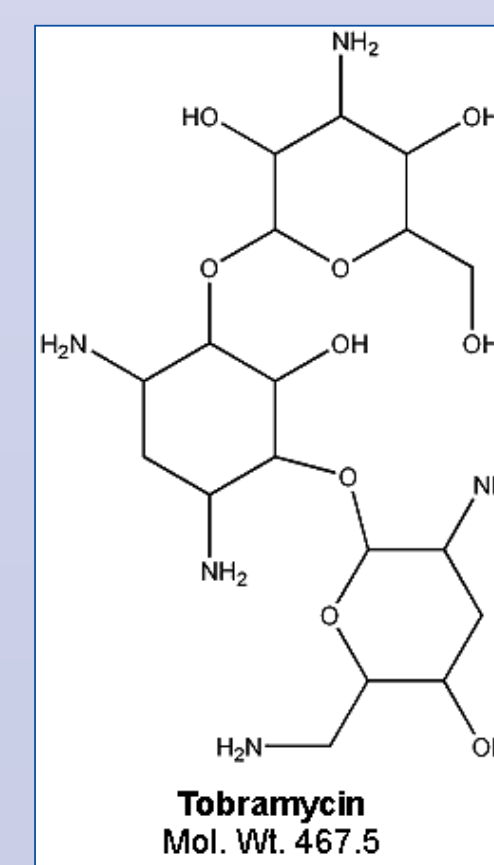
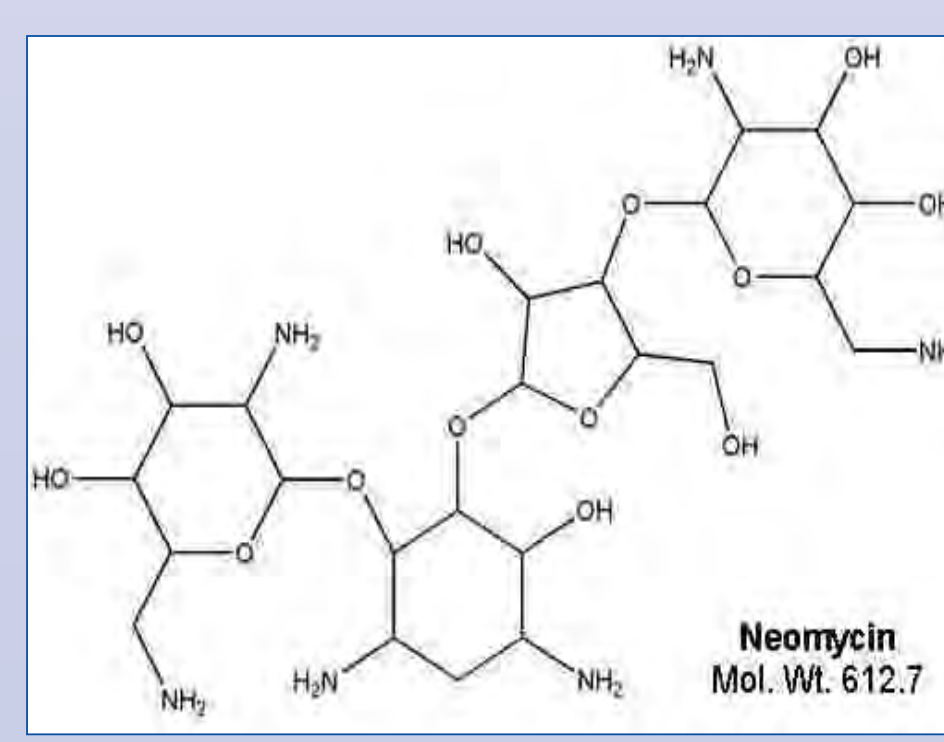
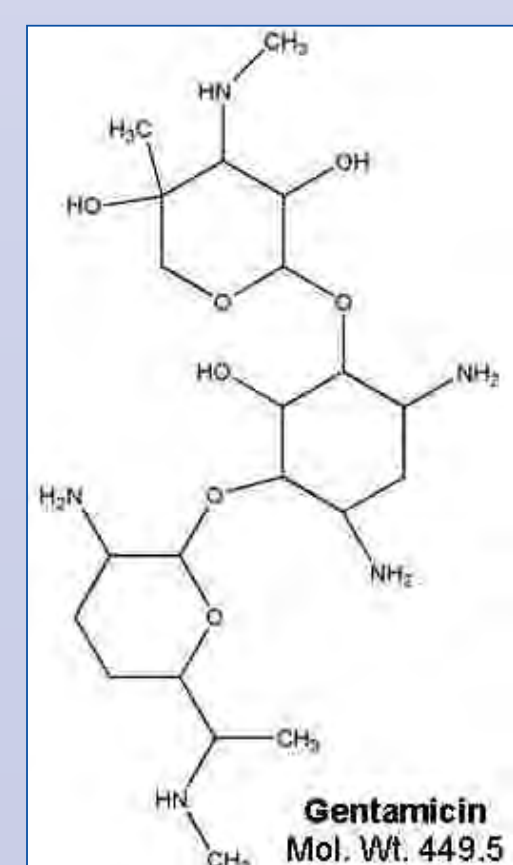
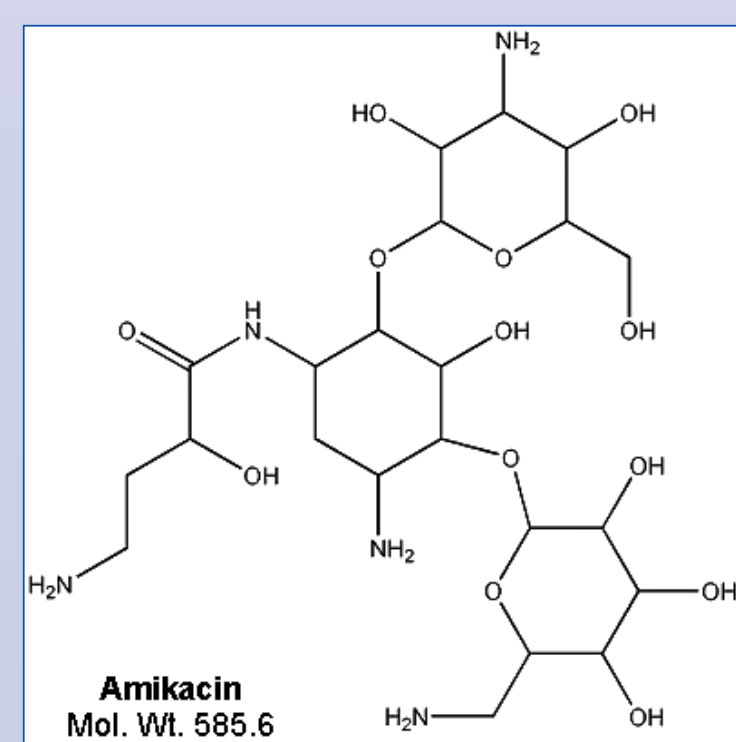
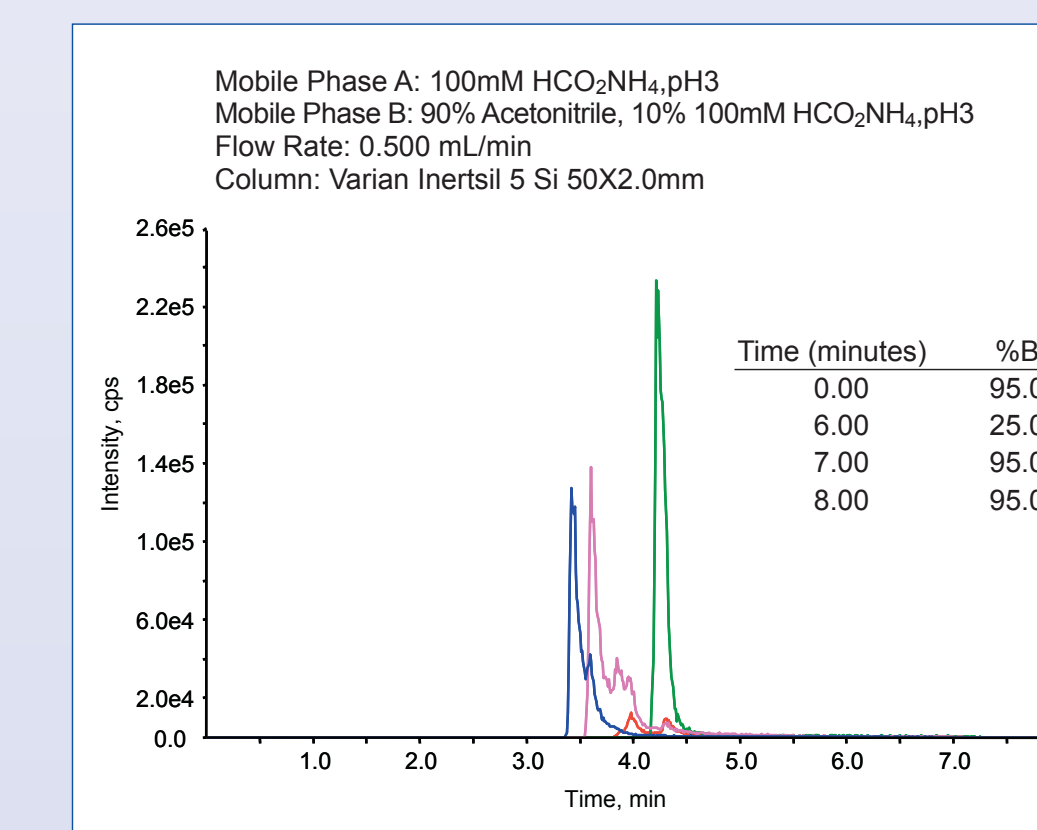


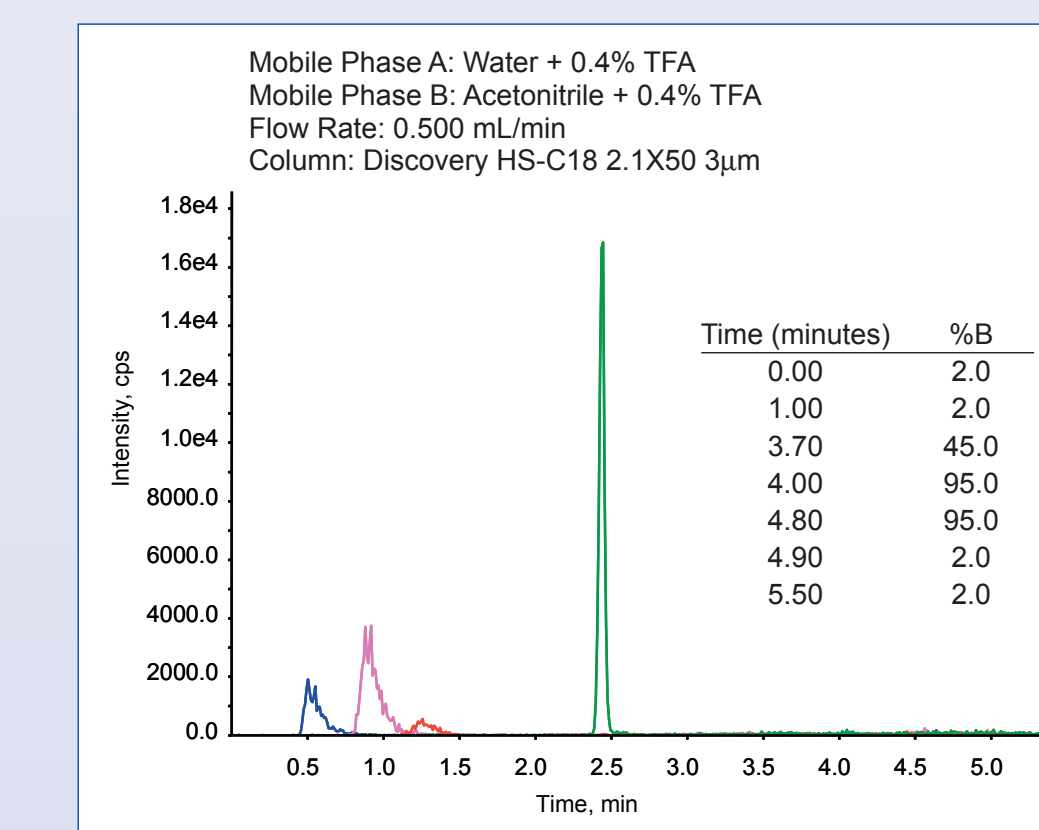
Table 1. Standard Curve and QC Results for the HPLC/MS/MS Analysis of Amikacin, Neomycin and Gentamicin from Rat Plasma.

Amikacin $r^2 = 0.9849$
Neomycin $r^2 = 0.9868$
Gentamicin $r^2 = 0.9941$

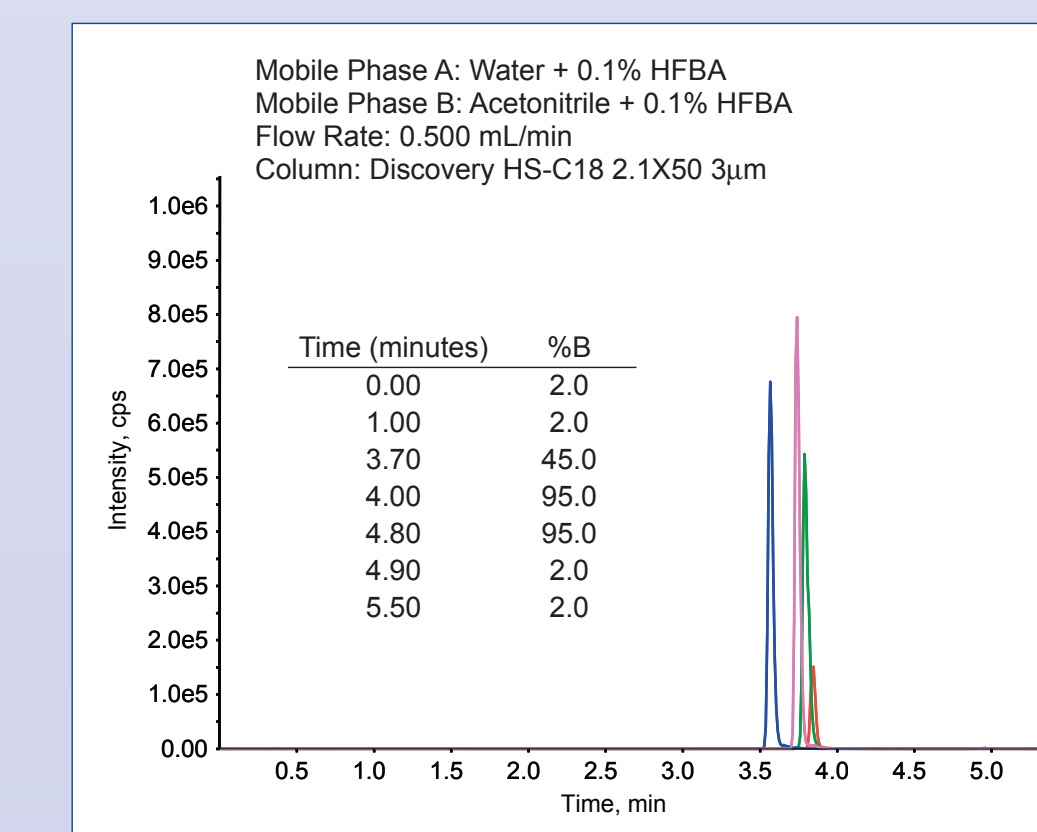
Sample Name	Amikacin		Neomycin		Gentamicin	
	Average Concentration (ng/mL)	Average Accuracy (%)	Average Concentration (ng/mL)	Average Accuracy (%)	Average Concentration (ng/mL)	Average Accuracy (%)
5	5.16	103	4.78	95.6	5.28	106
10	9.65	96.5	10.2	102	8.68	86.8
50	51.5	103	56.6	113	54.4	109
100	100	100	93.3	93.3	92.9	92.9
500	511	102	480	96.0	463	92.5
1000	984	98.4	969	96.9	1050	105
5000	5010	100	4960	99.1	5270	105
10000	10000	100	10200	102	9790	97.9
QC15	13.9	92.4	13.6	90.4	12.3	81.7
QC200	172	86.2	163	81.7	173	86.7
QC1500	1420	94.9	1310	87.1	1390	92.4
QC8000	8300	104	7220	90.3	7550	94.4



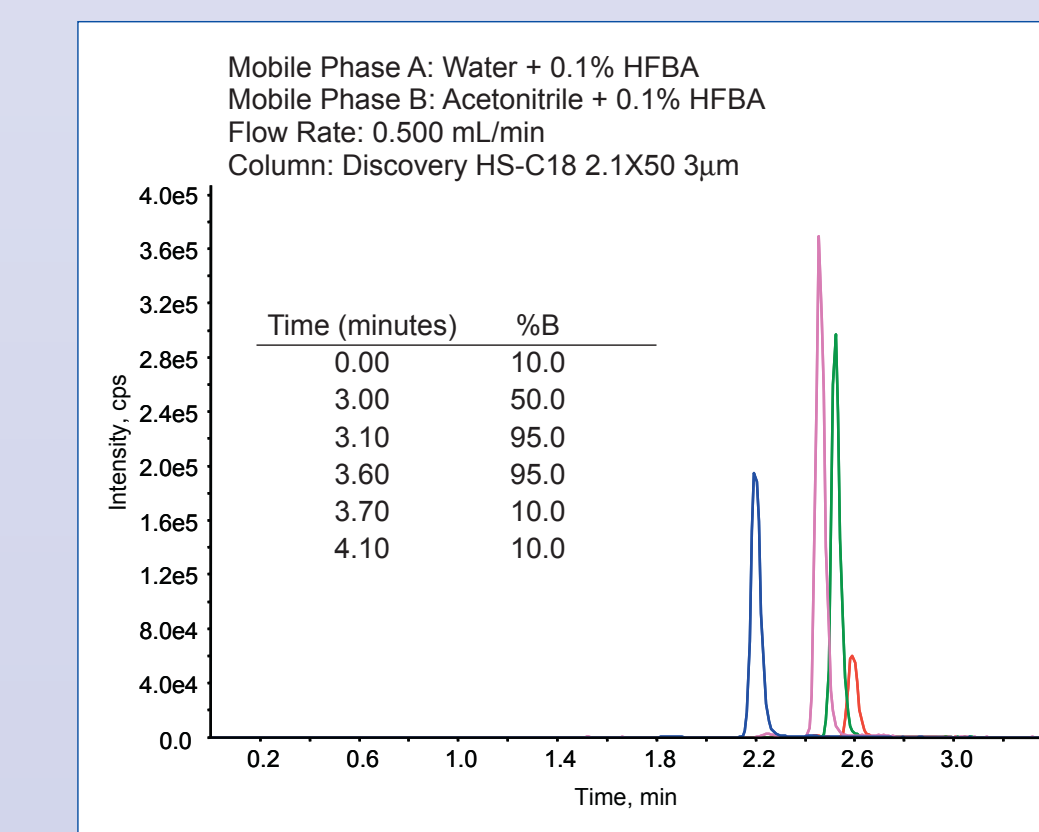
HILIC HPLC/MS/MS Chromatogram from the Analysis of a 1 μg/mL Solution of Aminoglycosides



HPLC/MS/MS Chromatogram from the Analysis of a 1 μg/mL Solution of Aminoglycosides using TFA as ion-pair reagent



HPLC/MS/MS Chromatogram from the Analysis of a 1 μg/mL Solution of Aminoglycosides using HFBA as ion-pair reagent



HPLC/MS/MS Chromatogram from the Analysis of a 5000 ng/mL Aminoglycoside sample from rat plasma using HFBA as ion-pair reagent

Conclusion

- For aminoglycosides, using HFBA as an ion-pair reagent shown to be the best method for HPLC/MS/MS analysis.
- Developed a bioanalytical HPLC/MS/MS method to quantify various aminoglycosides in rat and mouse plasma, rat and dog urine and various tissues.
- Method supports the analysis of aminoglycosides for PK/TK studies.