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Automated HILiC-MS/MS Method for Therapeutic Drug Monitoring of Aminoglycoside Antibiotics and Vancomycin

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Introduction

Aminoglycoside antibiotics are used for treatment of severe infections, especially in the case of Gram-negative bacilli infection. However, aminoglycosides have narrow therapeutic indexes due to their nephrotoxicity. Therefore, the benefit of therapeutic drug monitoring (TDM) for aminoglycoside has been well-established. Vancomycin, a glycopeptide antibiotic, often used with aminoglycosides because of their synergism, is also nephrotoxic and need to be monitored as well.

While LC-MS/MS is now considered as the gold standard method for TDM, many clinical laboratories still use immunoassays. Immunoassays suffer from cross-reactivity, difficult multiplexing and a higher cost-per-sample analysis. We present here a hydrophilic interaction liquid chromatography (HILiC) method with tandem mass spectrometry detection and automated sample preparation for the simultaneous analysis of 7 aminoglycosides and vancomycin.

Methods and Materials

Reagents

Analytical standards of Amikacin, Gentamicin (mixture of C1, C1a and C2/C2a), Kanamycin, Neomycin B, Paromomycin, Tobramycin and Vancomycin were purchased from Sigma-Aldrich. Hygromycin B was purchased from Wako Chemicals. Individual stock solutions at 100 mg/mL were prepared in water and further diluted in blank plasma to make calibration standards (7 levels) and QC (4 levels). The calibration range was from 0.1 to 50 µg/mL (0.1 to 100 µg/mL for Vancomycin).
As no analytical standard of Arbekacin is yet available on the market, a plasma calibrator set (5 levels + blank) from Microgenics Corporation was used. Additional dilution of the highest level were done to decrease the limit of quantification. Calibration range was then 0.1 to 30 µg/mL.
All other reagents were of analytical grade from Sigma-Aldrich. Solvents used were of LC-MS grade from Wako chemicals.

Sample Preparation

Automated sample preapration was performed using the CLAM-2000 device directly coupled to the LC/MS system (Figure 1).
Calibration standards, QC or samples were assayed the same way. Twenty microliters of water/Isopropanol (1/3) were deposited to activate the filter, and one-hundred microliters of precipitating reagent (trichloroacetic acid 100g/L in water) were added. Then 20 µL of internal standard (Hygromycin B at 50 µg/mL in water) and 20 µL of plasma. After vortexing for 1 minute, the precipitate was filtrated during 90 seconds and the extract transfered to the autosampler for injection. The sample preparation process is overlapped with the analysis to increase sample throughput.

Analytical Conditions

Analysis was performed using a Nexera X2 UHPLC system coupled with LCMS-8060 triple quad mass spectrometer. Parameters are described in Table 1 and 2.

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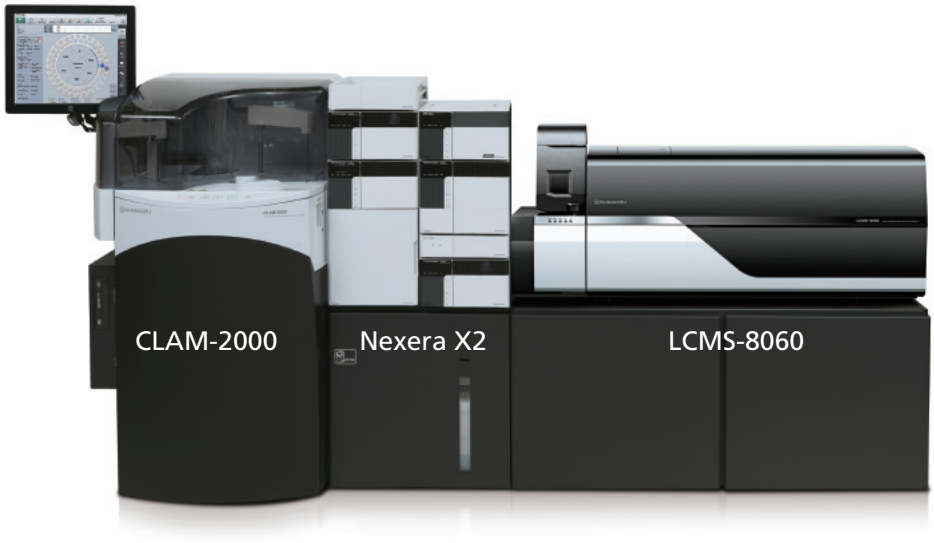

Figure 1: Overview of the Analytical System

Table 1 HILiC conditions

System	: Nexera X2
Column	: GL Sciences InertSustain Amide 3µm 50x2.1mm
Temperature	: 50°C
Mobile Phases	: A: Water + 250 mM ammonium formate + 1% formic acid B: Acetonitrile
Flow Rate	: 400 µL/min
Injection Volume (Quant screening)	: 0.5 µL
Gradient	: 75 % B (0.2min) to 55%B in 1.3 min. 55%B (1.5 min). 55%B to 75%B in 0.1 min
Total Run Time	: 4.75 min

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Table 2 MS/MS conditions

System : LCMS-8060
Ionization : Heated ESI
Probe Voltage : +5 kV (positive ionization)
Temperature : Interface: 300°C
Desolvation Line: 150°C
Heater Block: 500°C
Gas Flow : Nebulizing Gas: 2.5 L/min
Heating Gas: 10 L/Min
Drying Gas: 3 L/min
Dwell Time / Pause time : 23 ms / 1.5 ms
MRM :

Compound	MRM Quant	MRM Qual
Amikacin	586.3 > 163.3	586.3 > 425.2
Arbekacin	553.4 > 163.1	553.4 > 425.3
Gentamicin C1a	450.4 > 322.1	450.4 > 163.1
Gentamicin C1	478.4 > 322.2	478.4 > 157.1
Gentamicin C2	464.4 > 322.1	464.4 > 160.1
Hygromycin B	528.3 > 352.1	528.3 > 256.9
Kanamycin	485.4 > 163.1	485.4 > 324.0
Neomycin B	615.4 > 163.1	615.4 > 163.0
Paromomycin	616.4 > 293.1	616.4 > 163.1
Tobramycin	468.3 > 324.1	468.3 > 163.1
Vancomycin	725.4 > 1307.2	725.4 > 144.4

Results

Calibration

Calibration curves were calculated by internal standardization using a quadratic regression model with 1/x or $1/x^2$ weighting. Acceptance criteria was an accuracy comprised between 85 to 115%.
Some typical calibration curves are presented in Figure 2 and mass chromatograms at the LLOQ in Figure 3.

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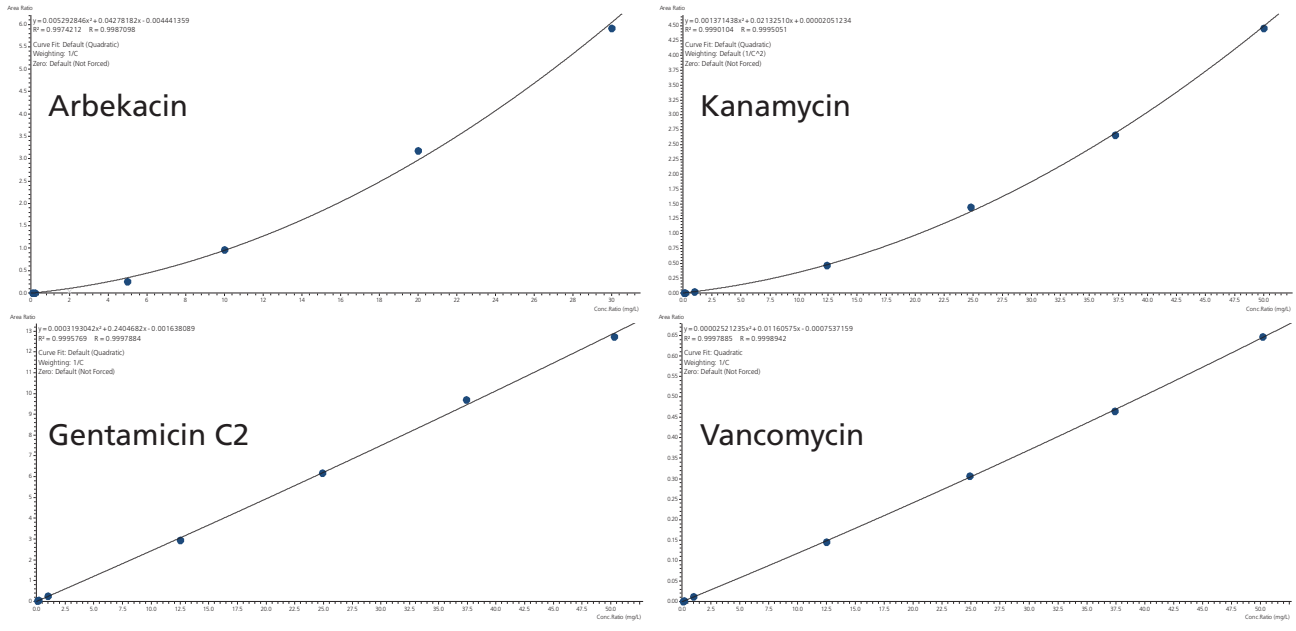

Figure 2 Typical Calibration Curves

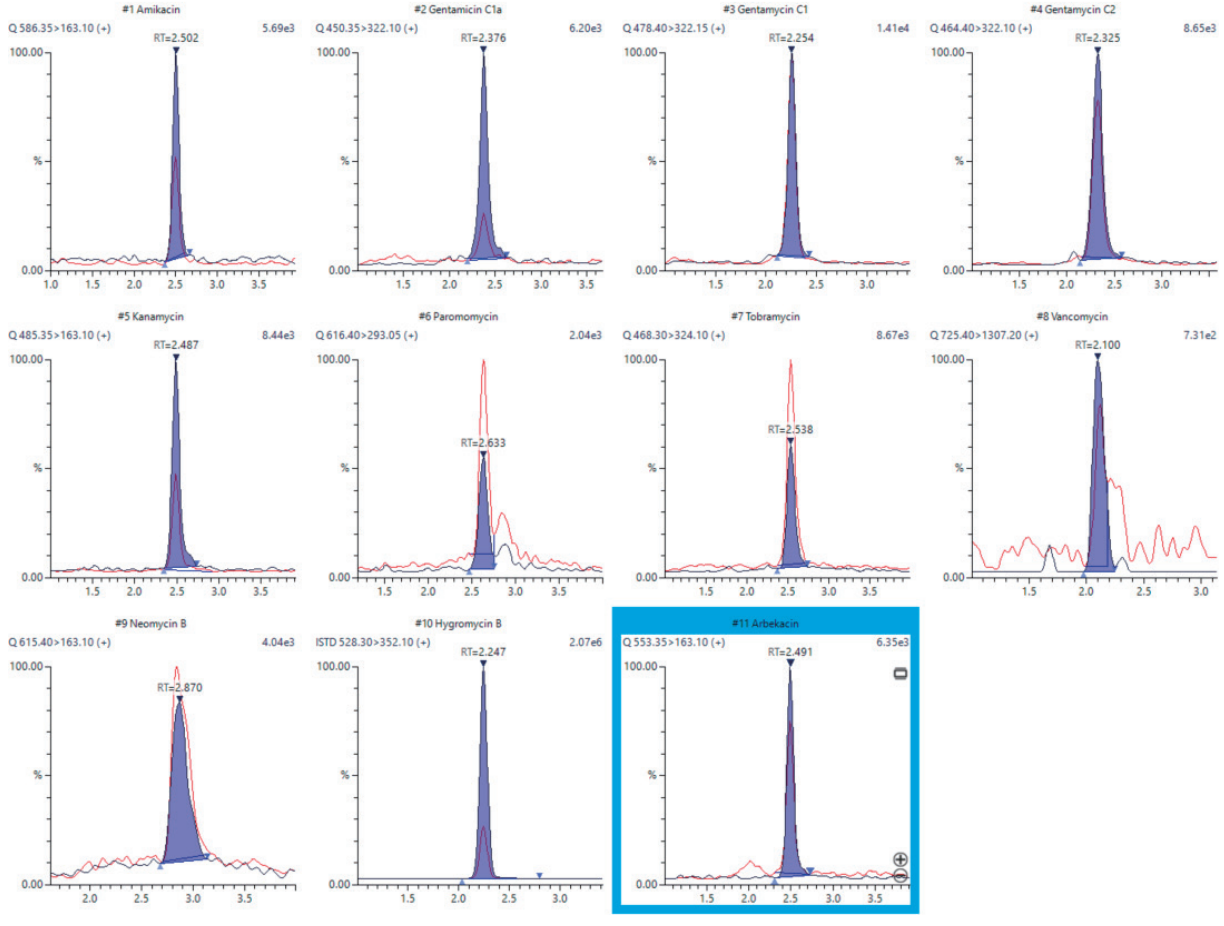

Figure 3 Mass Chromatograms at the Lower Limit of Quantification

Recovery

Total recovery (i.e. combining extraction and matrix effect) was evaluated by comparing peak areas in middle range level QC in plasma to an equivalent prepared in solution. Each type of sample was prepared in triplicate. Results are shown in Table 3. The mean recoveries were ranging from 89 to 105%, illustrating the good extraction rate and the low matrix effect.

Table 3 Recovery

		Amikacin	Gentamicin C1a	Gentamycin C1	Gentamycin C2	Kanamycin
Plasma QC	Mean Area	10 113 056	3 527 104	43 678 905	8 757 317	9 596 851
	%RSD	9%	2%	0.3%	9%	7%
Solution QC	Mean Area	10 759 507	3 525 507	44 889 021	8 725 000	10 020 195
	%RSD	2%	4%	9%	4%	1%
%recovery		**94%**	**100%**	**97%**	**100%**	**96%**

		Paromomycin	Tobramycin	Vancomycin	Neomycin B	Hygromycin B
Plasma QC	Mean Area	2 588 488	20 326 679	3 188 732	2 505 853	6 872 652
	%RSD	12%	10%	0.4%	8%	2%
Solution QC	Mean Area	2 735 816	18 901 937	3 573 458	2 382 767	7 641 951
	%RSD	0%	1%	1%	9%	1%
%recovery		**95%**	**108%**	**89%**	**105%**	**90%**

Precision and Accuracy

Precision and accuracy were evaluated by measuring the concentration of QC samples at four levels across 3 independent runs (3 days). Each day, 5 replicates of each QC were prepared and analyzed. Acceptance criteria were a relative standard deviation <15% (20% at the LOQ) and accuracy between 80-120%. Results are presented in Table 4.

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Table 4 Precision and Accuracy

		Amikacin	Gentamicin C1a	Gentamycin C1	Gentamycin C2	Kanamycin
QC LOQ	Mean Calc. Conc. (µg/mL)	0.0984	0.0980	0.0968	0.0996	0.108
	%RSD	6.1%	10.4%	6.3%	9.5%	8.1%
	Theor. Conc. (µg/mL)	0.101	0.101	0.101	0.101	0.100
	Accuracy	97.4%	97.0%	95.8%	98.6%	108%
QC A (3x LOQ)	Mean Calc. Conc. (µg/mL)	0.283	0.303	0.269	0.308	0.273
	%RSD	2.5%	3.9%	5.4%	1.9%	2.1%
	Theor. Conc. (µg/mL)	0.302	0.302	0.302	0.302	0.300
	Accuracy	93.6%	100%	89.2%	102%	91.0%
QC B (mid-range)	Mean Calc. Conc. (µg/mL)	27.8	25.3	25.7	26.9	26.9
	%RSD	3.3%	3.8%	1.0%	2.8%	2.5%
	Theor. Conc. (µg/mL)	25.0	24.9	24.9	24.9	24.8
	Accuracy	111%	102%	103%	108%	108%
QC C (high-range)	Mean Calc. Conc. (µg/mL)	44.5	43.8	44.8	46.4	45.6
	%RSD	1.8%	3.2%	1.3%	3.0%	1.2%
	Theor. Conc. (µg/mL)	42.7	42.6	42.6	42.6	42.4
	Accuracy	104%	103%	105%	109%	107%

		Paromomycin	Tobramycin	Vancomycin	Neomycin B	Arbekacin
QC LOQ	Mean Calc. Conc. (µg/mL)	0.110	0.114	0.102	0.105	0.0998
	%RSD	13.8%	13.8%	6.3%	10.3%	11.1%
	Theor. Conc. (µg/mL)	0.100	0.100	0.100	0.100	0.099
	Accuracy	110%	114%	102%	105%	101%
QC A (3x LOQ)	Mean Calc. Conc. (µg/mL)	0.276	0.278	0.323	0.277	0.269
	%RSD	10.3%	3.2%	6.0%	7.1%	4.1%
	Theor. Conc. (µg/mL)	0.300	0.301	0.300	0.301	0.300
	Accuracy	92.1%	92.3%	108%	92.0%	89.5%
QC B (mid-range)	Mean Calc. Conc. (µg/mL)	27.8	26.8	51.8	27.4	16.0
	%RSD	3.8%	2.5%	4.7%	4.9%	3.1%
	Theor. Conc. (µg/mL)	24.8	24.9	49.6	24.9	15.0
	Accuracy	112%	108%	104%	110%	106%
QC C (high-range)	Mean Calc. Conc. (µg/mL)	43.4	45.5	90.4	40.3	26.5
	%RSD	1.0%	1.2%	2.1%	3.6%	1.0%
	Theor. Conc. (µg/mL)	42.4	42.6	84.9	84.9	25.2
	Accuracy	102%	107%	106%	47.5%	105%

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Conclusions

A fast and automated method was set-up to assay major aminoglycoside antibiotics and Vancomycin and improve therapeutic drug monitoring. The method performance were adequate to ensure routine accurate quantification. Automation of sample preparation also greatly enhance laboratory throughput and ease of use.

Acknowledgment

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