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Title: Confirmation of Aminoglycosides by HPLC-MS/MS		
Revision 02	Replaces: CLG-AMG1.01	Effective: June 1, 2005

Contents

A. INTRODUCTION

1. Theory

Aminoglycoside (AMG) residues are extracted from tissue using buffer containing trichloroacetic acid as a protein precipitant. The extract is neutralized and cleanup accomplished by passage through a weak cation exchange solid-phase extraction cartridge followed by elution with acidic methanol. The methanolic extract is evaporated and reconstituted in aqueous ion-pair reagent. It is analyzed by ion-pair reversed-phase liquid chromatography with detection by electrospray ion trap mass spectrometry. Confirmation is accomplished by comparison of retention time and full or partial scan MS/MS spectra with those of a fortified tissue standard and external standard respectively.

2. Applicability

This procedure is applicable to bovine, porcine, and avian (poultry) liver, kidney, and muscle. See I.6.b for specific capabilities.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

a. Centrifuges – IEC GP8R; 3000g fixed rotor for 50 mL centrifuge tubes; IEC-HN-S11 with swinging bucket rotor for 15 mL tubes; Eppendorf-5417C micro-centrifuge.

b. Blender or vertical cutter-mixer or cutting board and knives for mincing.

c. Homogenizer with 1 cm probe - Polytron.

d. Vortex mixer - Genie 2, Fisher.

e. Platform shaker - Cat. No. 6010, Eberbach.

f. pH meter - with ATC probe, Cat. No. 370, Orion.

g. Balance, accurate to 0.1 mg - Cat. No. MT5, Mettler.

h. SPE vacuum manifold - 24-port with polypropylene needles and stopcocks, Alltech.

i. SPE cartridges - BakerBond SPE Wide Pore CBX 500 mg, J. T. Baker.

j. Nitrogen evaporator - Turbovap LV, Zymark.

k. Centrifuge tubes - Polypropylene, 50 mL and 15 mL, for sample extraction and storage, Cat. No. 35-2070 and 35-2096 respectively, Falcon.

l. Centrifuge tubes -15 mL glass, Kimble #73790 for Zymark Turbovap

m. Autosampler vials - polypropylene, 750 µL crimp top, 12X32mm, Xpertek.

n. Volumetric flasks - polypropylene (preferred) or glass, 100 mL.

o. Sonicating waterbath - Aquasonic # 150T, VWR Scientific.

p. Microcentrifuge tubes - 0.6 mL with cap, Cat No. 02-681-240, Fisher.

2. Instrumentation

a. Ion trap mass spectrometer - Finnigan LCQ-deca XP-Plus equipped with electrospray (ESI) LC interface and Windows 2000 professional LCQ Xcalibur data system.

b. LC system - Quaternary pump equipped with degassing capability and autosampler. Thermo-Finnigan Surveyor HPLC and autosampler.

c. LC column - Xterra MS, 2.1 x 100 mm C_{18}, 3.5 µm particles, with guard cartridge containing same material, Waters.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents and solutions may be substituted.

1. Reagents

a. Methanol (MeOH) - LC grade, Mallinckrodt.

b. Water, LC grade - House distilled water passed through Waters MilliQ deionization system.

c. Heptafluorobutyric Acid (HFBA) - Lancaster Chemical.

d. Hydrochloric Acid (HCl), concentrated - J.T.Baker.

e. Acetic acid, glacial (HOAc) - ChromAR grade, Mallinckrodt.

f. Trichloroacetic Acid (TCA) - Cat. No. ACS T6399, Sigma.

g. Ethylenediaminetetraacetic acid, disodium salt (Na_2EDTA), 99+% - Cat. No. E5134, Sigma.

h. Potassium phosphate monobasic (KH_2PO_4) - HPLC grade, Cat. No. P286-1, Fisher.

i. Sodium Hydroxide (NaOH) - Cat. No. ACS S-0899, Sigma.

j. Formic acid - Cat. No. ACS F-4636, Sigma.

2. Solutions

Note: Unless otherwise noted, solutions may be stored at room temperature.

a. 100 mM HFBA:

Dilute 6.5 mL HFBA to 500 mL with LC water. Stable for 6 months.

b. 5.0 mM HFBA:

Dilute 100 mM HFBA 1 + 19 with LC water. Store refrigerated. Stable for 6 months.

c. 10 mM KH_2PO_4 with 0.4 mM EDTA and 2% TCA:

Add 2.72 g KH_2PO_4 to 2 L graduated cylinder. Dilute to 2 L with LC water and adjust pH to 4.0 with 1 N HCl. Add 0.3 g Na_2EDTA dihydrate and 40 g TCA. Stable for 2 months.

d. 10% Acetic acid in Methanol (HOAc/MeOH):

Add 5 mL glacial acetic acid and 45 mL methanol to a 50 mL glass screw cap tube. Stopper and invert several times to mix. Stable for 1 week.

e. 30% w/v NaOH:

Add 30 g NaOH to a 100 mL mixing cylinder and dilute to volume with LC water. Store in a plastic container. Stable for 1 year.

f. 1 N NaOH:

Add 4 g NaOH to a 100 ml mixing cylinder, and dilute to volume with LC water. Store in a plastic container. Stable for 1 year.

g. 1 N HCl:

Dilute Concentrated HCl 1:12 with LC water. Stable for 1 year.

D. STANDARDS

1. Source

AMG standards. All AMGs in this procedure are available as the sulfate or chloride salt from Sigma. Some are also available as reference standards from U.S. Pharmacopeia. Note: Values in parentheses are Sigma catalog numbers.

Amikacin Sulfate (A-1774)	Kanamycin Acid Sulfate (K-1876)
Apramycin Sulfate (A-2024)	Neomycin Sulfate (N-1876)
Dihydrostreptomycin Sesquisulfate (D-7253)	Spectinomycin Dihydrochloride (S-9007)
Gentamycin sulfate (G-3632)	Streptomycin Sulfate (S-6501)
Hygromycin B (H-7772)	Tobramycin Sulfate (T-1783)

2. Preparation of Standard Solutions

Note: AMGs can adsorb to glass surfaces, causing losses, especially when dilute standards are prepared.

a. Individual AMG stock solutions (~100 µg/mL):

Using vendor's stated purity, or water and sulfate content, calculate the amount of material which contains 10 mg AMG base. Weigh out approximately this amount, accurately recording weight to nearest 0.1 mg. Transfer to a 100 mL volumetric flask and dilute to volume with water. Calculate exact concentration based on purity and actual weight. Transfer to a 50 mL polypropylene centrifuge tube. Store refrigerated. Stable for 1 year.

b. AMG working standards:

Prepare mixed standard solutions appropriate for use with liver, kidney, and muscle tissues by adding the amounts shown in Table 1 to 15 mL polypropylene centrifuge tubes (10 additions will be made to each tube). Dilute to 10 mL volume with water, cap tightly, and mix. Store refrigerated. Stable for one year.

Table 1. Fortification Volumes (mL) for working standards

	WS-L (Liver)	WS-K (Kidney)	WS-M (Muscle)
spectinomycin	1.0	0.4	0.25
hygromycin	1.0	1.0	0.4
streptomycin	0.4	0.4	0.4
dihydrostreptomycin	0.4	0.4	0.4
amikacin	1.0	1.0	0.4
kanamycin	4.0	2.0	0.4
aprimycin	0.4	0.1	0.1
tobramycin	1.0	0.1	0.1
gentamycin	0.4	0.1	0.1
neomycin	0.4	0.1	0.1
Total Volume Added	10.0	5.6	2.65

c. AMG External standards:

Add 250 µL of the appropriate mixed AMG working standard, 250 µL of water, and 1 µL of formic acid to a polypropylene centrifuge tube or autosampler vial, cap, and mix. Inject prior to each day's run of samples to determine instrument suitability. Prepare fresh each day of use.

3. Preparation of Controls (to be included as part of each sample batch):

   Note: Controls should be tissue matched to the samples to be confirmed. For example, if bovine kidney samples are to be analyzed, bovine kidney controls should be included in the sample set.

   a. Negative controls are tissues from animals known to be free of drugs. If these are not available, tissue from an unknown source may be used provided it is first tested and shown to be free of contaminants.

   b. Positive controls are negative tissues that have been fortified with AMGs before extraction. To prepare a fortified sample, add 200 µL of the appropriate mixed AMG working standard (D.2.b) to 2 g tissue.

E. SAMPLE PREPARATION AND CLEANUP

1. Sample Handling and Preparation

   a. Freshly collected samples must be kept cold before and during shipping to laboratory. Once received at laboratory, samples must be frozen (< -10 °C) prior to mincing/grinding if they cannot be prepared on the day of receipt.

   b. If sample is frozen, allow to thaw, but keep as cold as possible. Dissect away fat and connective tissue from kidney or liver. Mince finely or grind tissue in blender or vertical cutter-mixer. Store frozen (< -10 °C) prior to analysis.

2. Extraction

   a. Weigh 2.00 ± 0.10 g ground or minced tissue in a 50 mL polypropylene centrifuge tube. Note: Prepare necessary controls (D.3) at this time.

   b. Add 10 mL phosphate buffer/TCA. Homogenize to an even suspension (30-60 seconds on a Polytron at moderate setting). Seal tube tightly, place on a platform shaker, and shake vigorously (fastest setting) for 10 minutes.

   c. Centrifuge at approximately 4000 rpm (3000 g) for 10 minutes.

   d. Decant supernatant solution into clean 50 mL tube. Add 10 mL phosphate buffer/TCA to pellets, vortex mix, and shake vigorously 10 min on a platform shaker. Repeat centrifugation and combine supernatants.

   e. Adjust pH of combined extract to 7.5–8.0 (This requires approximately 0.16 mL 30% NaOH). Use pH meter to check pH and adjust with 1 N HCI or 1 N NaOH, as needed.

   f. Centrifuge as above.

   g. If necessary, remove fat from top of extract with a clean spatula.

3. SPE cartridge Cleanup

   a. Attach SPE cartridge to vacuum manifold and condition using 5 mL MeOH followed by 5 mL water. Remove vacuum and decant extract onto SPE cartridge. Note: Cartridge reservoirs are not large enough to contain the entire extract. Either attach larger reservoirs or add extracts in batches.

   b. Apply sufficient vacuum to flow extract through cartridge at a rate of 1–3 mL/minute. After all extract has passed through, wash cartridge with 4–5 mL water using same elution rate.

   c. Fully dry the SPE cartridge by drawing air through it under a vacuum of approximately 10 in. of Hg for at least 5 minutes.

   d. Elute AMG residues into a 15 mL glass centrifuge tube with 3 mL 10% HOAc/MeOH at 1–3 mL/minute.
   Note: Losses due to adsorption on glass surfaces have not been observed with tissue extracts at levels tested. Substitution of a polypropylene receiving tube should eliminate any possibility of adsorptive losses, but may adversely affect evaporation times.

   e. Evaporate extract under a stream of nitrogen to ~0.1 mL at approximately 40 °C (This takes about 90 minutes). Remove tube from water bath and evaporate to dryness under a stream of nitrogen at room temperature

   f. Add 0.4 mL 5 mM HFBA, then cap tube. Vortex mix, sonicate 15 minutes, and vortex mix again.

   g. Centrifuge tube at approximately 2500 rpm (1200 g) for 10 minutes. Transfer the extract to a 0.6 mL polypropylene micro-centrifuge tube and centrifuge at approximately 16,000 rpm (23,000 g) for 10 minutes.

   h. Carefully transfer approximately half of supernatant to a polypropylene autosampler vial. *Important!* It is critical that the liquid transferred be free of particulates. Take care to avoid any solids at the top of the tube (usually muscle only). Transfer may require the use of a 250 µL hypodermic syringe.

   i. Freeze the remaining fraction of sample. Extracts are stable overnight at room temperature, several days if refrigerated, or at least 5 months when stored frozen.

F. ANALYTICAL PROCEDURE

1. Instrument Operating Parameters - LC System

   Note: Typical values listed below. Flows and elution gradient may be optimized, if necessary, for best separation and response.

   a. Install and degas mobile phases and install column and guard cartridge per manufacturers' instructions. Flush with 80:20 MeOH/water at a flow rate of 0.15 mL/minute.

b. Set initial composition to 5% MeOH, 75% water, 20% 100 mM HFBA at 0.15 mL/minute. Allow system to equilibrate at least 30 minutes.

c. Program pump for the following gradient

Time, min	% MeOH	% Water	% HFBA	Gradient
0	5	75	20	none
0.5	5	75	20	none
1.0	60	20	20	linear
12.0	70	10	20	linear
12.1	80	0	20	linear
15.0	80	0	20	none
15.1	5	75	20	linear
35.0	5	75	20	none

d. Set Flow rate to 150 µL/minute.

e. Set the run time to 35 minutes.

f. Set injection volume to 20 µL.

g. Use a needle wash step with MeOH or water.

2. Instrument Operating Parameters - Mass Spectrometer

Parameters listed below are typical. Parameters should be adjusted for optimum performance.

a. Mass Spectrometer. Calibrate the mass spectrometer and electrospray interface according to the manufacturer's specifications.

b. Set capillary temperature to 400 °C.

c. Operate in Positive mode.

d. Flow inject working standard WS-L (Sec. 2.b) to determine the precursor peak centroid and optimum collision energy for MS/MS for each of the AMG's. Inject standards on column to determine retention times. Set LCQ divert valve to divert LC effluent to waste until 1–2 minutes before elution of spectinomycin peak (about 6 minutes). Resume diversion after elution of neomycin (about 10 minutes).

e. Confirm appropriateness of divert valve settings by analyzing a fortified control tissue extract without using the divert valve.

Typical LC/MS system settings:

Capillary temp	400 ºC
Sheath gas flow	50
Aux/Sweep gas flow	5
Source voltage	4.5 kV
Capillary voltage	15 V
Tube lens offset	27 V
Microscans	1
Maximum inject time	100 msec
Segment 1	6.22 minute duration (0 - 6.22 minutes)
	Scan event 800 > (720 - 790), amp = 30%
Segment 2	1.86 minute duration (6.22 - 8.08 minutes)
	Scan event 333.2 > (90 - 300), amp = 34%
	Scan event 528.2 > (145 - 513), amp = 29%
	Scan event 582.4 > (160 - 578), amp = 33%
	Scan event 584.3 > (160 - 580), amp = 33%
	Scan event 586.3 > (160 - 580), amp = 32%
	Scan event 485.0 > (130 - 460), amp = 27%
Segment 3	3.54 minute duration (8.08 - 11.62 minutes)
	Scan event 540.3 > (145 - 530), amp = 32%
	Scan event 468.2 > (125 - 455), amp = 26%
	Scan event 478.2 > (130 - 465), amp = 28%
	Scan event 615.3 > (165 - 460), amp = 28%
Segment 4	23.38 minute duration (11.62 - 35 minutes)
	Scan event 800 > (720 - 790), amp = 30%
	For all scan acquisitions:
	Activation Q = 0.250
	Activation time = 30.0 msec
	Isolation width = 2.0 μ

3. Procedure for LC-MS/MS Analysis of Samples, Controls and Standards

   a. Turn on pump and set up mass spectrometer. Degas solvents. Equilibrate column in mobile phase at 0.15 mL/minute for at least 30 minutes.

   b. Inject 2 μL of working standard WS-L by flow injection three times and determine the centroids of the ten AG precursor ions (parent ions). For example, for streptomycin the first injection might yield ion 582.2 as the precursor, the second injection 582.4 and the third injection 582.4. Discard the highest (582.4) and the lowest (582.2) and retain 582.4 as the precursor to be used for the analyses.

c. Inject an external standard (appropriate for the tissue to be analyzed) twice, followed by a solvent blank of 5% HFBA solution. Inject an appropriate positive control to verify retention time, divert valve switching time, and spectral comparison to the external standard.

d. Inject sample extracts. If necessary to control carryover, precede each sample analysis with a blank buffer injection.

e. As a test of retention time and instrument response stability, reinject the positive control and one or more chromatographic standards at the end of the injection sequence. Depending on instrument variability and size of sample set, additional control or standard injections may be interspersed throughout the sequence.

f. Column, Pump, and ESI Interface Care.
At the end of set of analyses, flush the column for 10–20 minutes with 80:20 MeOH/water at 0.15 mL/min. Continue flushing the column for an additional 20 minutes with 50:50 MeOH/water. Follow manufacturer's specifications for cleaning ESI interface: Clean surface of the ion-guide with 50:50 MeOH/water and lab tissue. Spray orifice of the ion guide with 50:50 MeOH/water.

G. CONFIRMATION

1. Data Processing. Use the QUAL Browser to view total ion current, base ion chromatogram, and/or a reconstructed ion chromatogram for each AMG for each data file. Note retention time of any visible peaks in an AMG window. Generate averaged spectra across the retention time window for each AMG. This is usually from near the start to near the end of the peak visible in the chromatograms, though a smaller range may be used to avoid a spurious ion spike. Where no peak is visible, use the same settings as in a contemporaneous spiked or positive control extract.

2. Confirmation of an analyte in any sample requires the following:

   a. Retention time of the analyte peak in one or more of the chromatograms listed in G.1. must match that found in the same chromatogram(s) from a contemporaneous (within same analysis set, analyzed on same day) positive control within 0.2 minutes.

   b. The AMG peak in the reconstructed ion chromatograms (RIC) is present at a S/N ratio of at least 3/1. This is estimated by visual inspection of the RIC. See below for ions used for each AMG's RICs.

   c. The spectra from the extract must visually match spectra from external standards in the same data set. The base ion must be the same. Qualifying ions should be present, readily distinguished from background and matrix ions, and have relative abundances comparable to those in the standard. There should be a general absence of nonspecific ions. Major specific ions for each AMG are listed below:

Table 2. Aminoglycoside Confirmation Criteria

Aminoglycoside	Precursor ion	Spectra Range	Base ion	Qualifying Ions[1]
spectinomycin	333.1	90 - 300[2]	189	140*, 158, 187, 227*, 289
hygromycin	528.3	145 - 513	352	177*, 303, 321*
streptomycin	582.2	160 - 578	263[3]	221, 246, 390, 407*, 540*, 565
dihydrostrepto.	584.2	160 - 580	263	221, 246, 392, 409*, 542*, 567
amikacin	586.2	160 - 580	425	264, 324*, 467, 485*
kanamycin	485.1	130 - 460	324	163*, 205, 366*
apramycin	540.1	145 - 530	378	217, 344*, 361*
gentamicin(C1)	478.1	130 - 465	322	157, 160*, 163, 205*[4], 461
neomycin	615.2	165 - 460	455	203, 293*, 323*
tobramycin	468.2	125 - 455	324	163, 205

[1] RICs comprised base ion and two qualifying ions. Recommended qualifying ions are marked with an *.
[2] Data acquired from 90 - 300. Matrix components generate potentially interfering ions between 300 and 333. Also, relative abundance of [MH-H2O]+ at m/z 315 is highly variable, limiting utility of this part of the spectral range.
[3] Usually. Can also be m/z 540 or, occasionally, m/z 407 or 565. Streptomycin is unique among the AMGs in this respect.
[4] The relative abundances of m/z 157,160, 163, and 205 are all very low. Although the presence of all four is desirable, only two of these four ions must be present for gentamicin presence to be confirmed.

d. The positive and negative (quality) control samples confirm and fail to confirm, respectively, for the presence of the appropriate AMG.

3. Criteria for Repeating an Analysis.

a. The conditions described in section G.2 are not met.

b. The instrument is suspected to be malfunctioning, as demonstrated by: clearly aberrant standard spectra; failure of a calibration check performed shortly after analysis of the sample set; instrumental parameters, especially vacuum readings, outside of normal operating range; or other conditions noted and documented by the analyst.

c. There is suspected carryover from a previous high concentration sample or standard. In this case, the sample should be reanalyzed after the cause of the carryover has been identified and measures taken to prevent its recurrence.

d. There is strong evidence of AMG presence, but multiple extraneous ions with relative abundance exceeding that of AMG's base ion prevent unambiguous confirmation. In this case, it may be appropriate to reanalyze the suspected positive sample together with a chromatographic standard, and negative and positive controls.

H. HAZARD ANALYSIS

1. Required Protective Equipment - Protective clothing, eyewear, and gloves, where applicable.

2. Hazards

Reagent	Hazard	Recommended Safe Procedures
AMG Standards	Ototoxic. Standards can cause kidney damage	Wear protective clothing and gloves when handling standards.
Methanol	Highly flammable. Explosive hazard. Vapors will explode if ignited. Irritating to skin and mucous membranes.	Keep container tightly closed and away from fire. Use under a fume hood. Avoid breathing vapors.
Concentrated Acids: HCl, Acetic, HFBA, TCA, Formic, and solutions made from same.	Corrosive substances. Danger of chemical burns. Potential for inhalation of corrosive fumes.	Prepare solutions in a fume hood. Wear protective equipment, avoid contact with skin.
NaOH and solutions made from same	Corrosive substances Danger of chemical burns.	Wear gloves when preparing solutions, and take care to avoid splashes or spills.

3. Disposal Procedures

Procedure Step	Hazard	Recommended Safe Procedures
Methanol	See section 2 above	Collect waste in a sealed container and store in a cool, well ventilated, flammable liquid storage area/cabinet for disposal in accordance with local, state, and Federal regulations.
Acids, acidic reagents.	See section 2 above	Collect waste in a sealed container and store in a cool, well ventilated, acid liquid storage area/cabinet for disposal in accordance with local, state, and Federal regulations.
NaOH and solutions made from same.	See section 2 above	Collect waste in a sealed container and store in a cool, well ventilated, base liquid storage area/cabinet for disposal in accordance with local, state, and Federal regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

   Refer to Section G.2 for Confirmation Criteria.

2. Readiness To Perform (FSIS Training Plan)

   a. Familiarization

      i. Phase I: Standards - Inject external standard solutions (D.2.c) in duplicate on at least three different days, and verify instrument response is adequate for confirmatory purposes.

      ii. Phase II: Fortified samples - Analyze one kidney, liver, and muscle blank fortified at minimum proficiency level (MPL, see I.6.b), on at least 3 different days (3 analyses per matrix, 9 total).

      Note: Phase I and Phase II may be performed concurrently.

      iii. Phase III: Check samples for analyst accreditation.

         (a) 6 check samples fortified at levels between 1–2 times MPL using analytes and concentrations unknown to the analyst. Any combination of tissues may be used, and set must include 1 blank.

         (b) Approval from the Supervisor and the Laboratory Quality Assurance Manager (QAM) is required to commence official analysis.

   b. Acceptability criteria.

      Refer to section I.1 above

3. Intralaboratory Check Samples

   a. System, minimum contents.

      i. Frequency: One per week per analyst when samples analyzed.

      ii. Records are to be maintained for review.

   b. Acceptability criteria.

      If unacceptable values are obtained, then:

      i. Stop all official analyses by that analyst.

      ii. Take corrective action.

4. Sample Acceptability and Stability
   a. Matrices: Bovine liver, muscle, and kidney.
   b. Sample receipt, minimum weight: approximately 50 grams.
   c. Condition upon receipt: chilled or frozen.
   d. Sample storage:
      i. Time: 30 days
      ii. Condition: frozen (≤ -10 °C)

5. Sample Set. Each sample set must include the following:
   a. Negative control sample (D.3.a)
   b. Positive control sample (D.3.b)
   c. Samples to be analyzed.

6. Sensitivity
   a. Lowest confirmable level: Varies with analyte and individual tissue background. Method has been demonstrated to function reliably at MPLs specified below.
   b. Minimum proficiency levels[1] (MPL), in ppm, by tissue.

	Liver	Kidney	Muscle
spectinomycin	1.0	0.4	0.25
hygromycin	1.0	1.0	0.4
streptomycin	0.4	0.4	0.4
dihydrostreptomycin	0.4	0.4	0.4
amikacin[2]	1.0[2]	1.0	0.4[2]
kanamycin	4.0	2.0	0.4
apramycin	0.4	0.1	0.1
tobramycin	1.0	0.1	0.1
gentamycin	0.4	0.1	0.1
neomycin	0.4	0.1	0.1

[1]Minimum analyte concentration analyst is expected to reliably confirm.

[2]Levels shown here are applicable only to bovine and avian tissues. Method will not reliably confirm amikacin in porcine liver or muscle tissues at these levels, and is only marginally successful in porcine kidney at a 1 ppm level.

J. WORKSHEET

See following page for example worksheet.

AMINOGLYCOSIDE CONFIRMATION FORM

Analyst (Confirmation) _____ Instrument used (LMS): _____

Date Started: _____ Method File Name: _____

Injection Volume (uL): _____

Date Completed: _____ Mixed Spiking Std. _____

Micropipettor (MCP): _____

Set Number: _____ Mobile Phase:

A - Methanol: _____

B - Water: _____

Reviewed by Init/Date _____ C - 100mM HFBA: _____

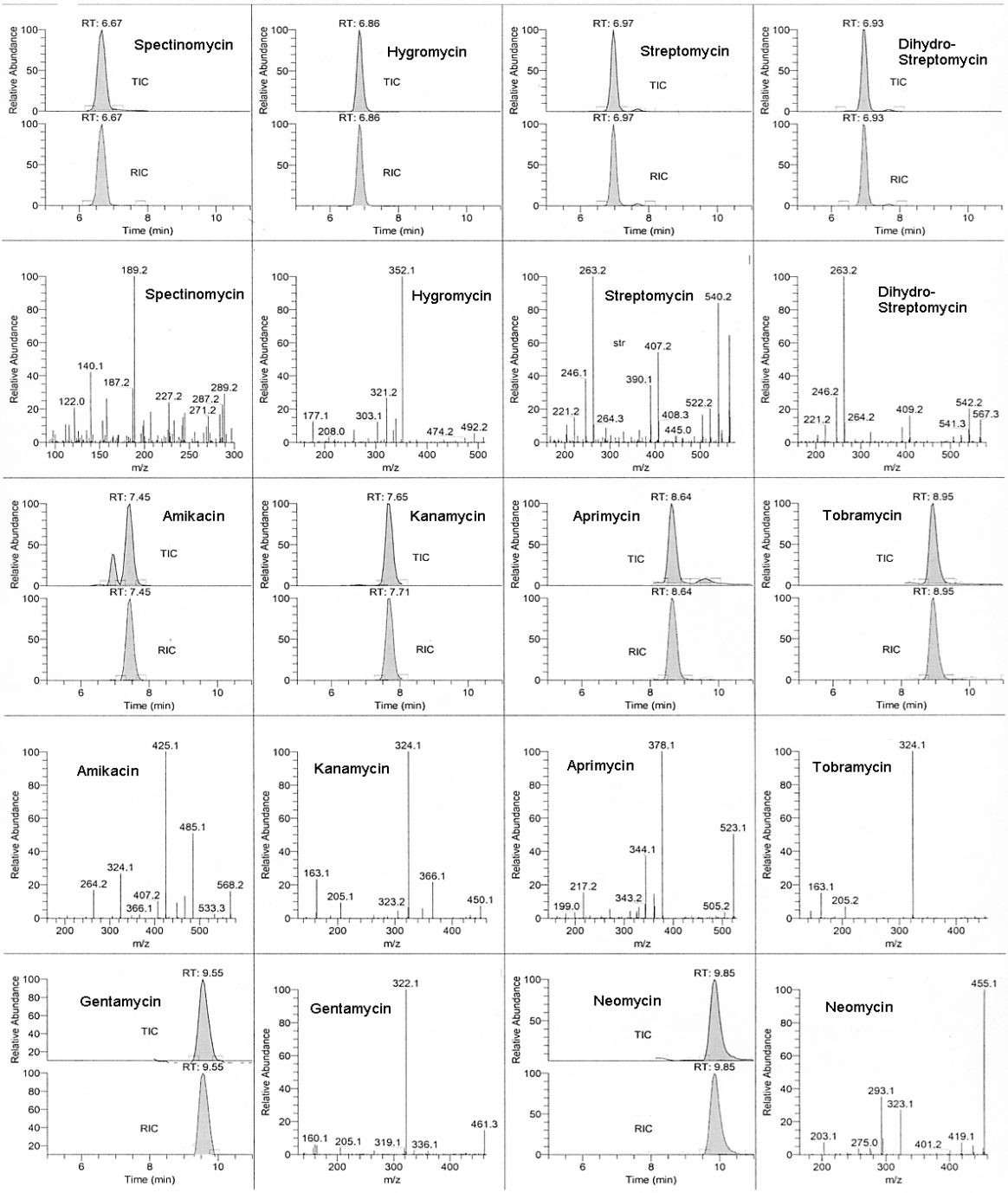
				ag-					ag-					ag-				
				Ret Time	Ret Time	RIC >3x	Qual ions		Ret Time	Ret Time	RIC >3x	Qual ions		Ret Time	Ret Time	RIC >3x	Qual ions	
			Tissue	Dilution	(nearest	within	noise	base ion	Visual	(nearest	within	noise	base ion	Visual	(nearest	within	noise	base ion	Visual
Sam.#	AT #	02,03,04	Factor	0.01 min.)	0.20 min?	level?	present?	match?	0.01 min.)	0.20 min?	level?	present?	match?	0.01 min.)	0.20 min?	level?	present?	match?

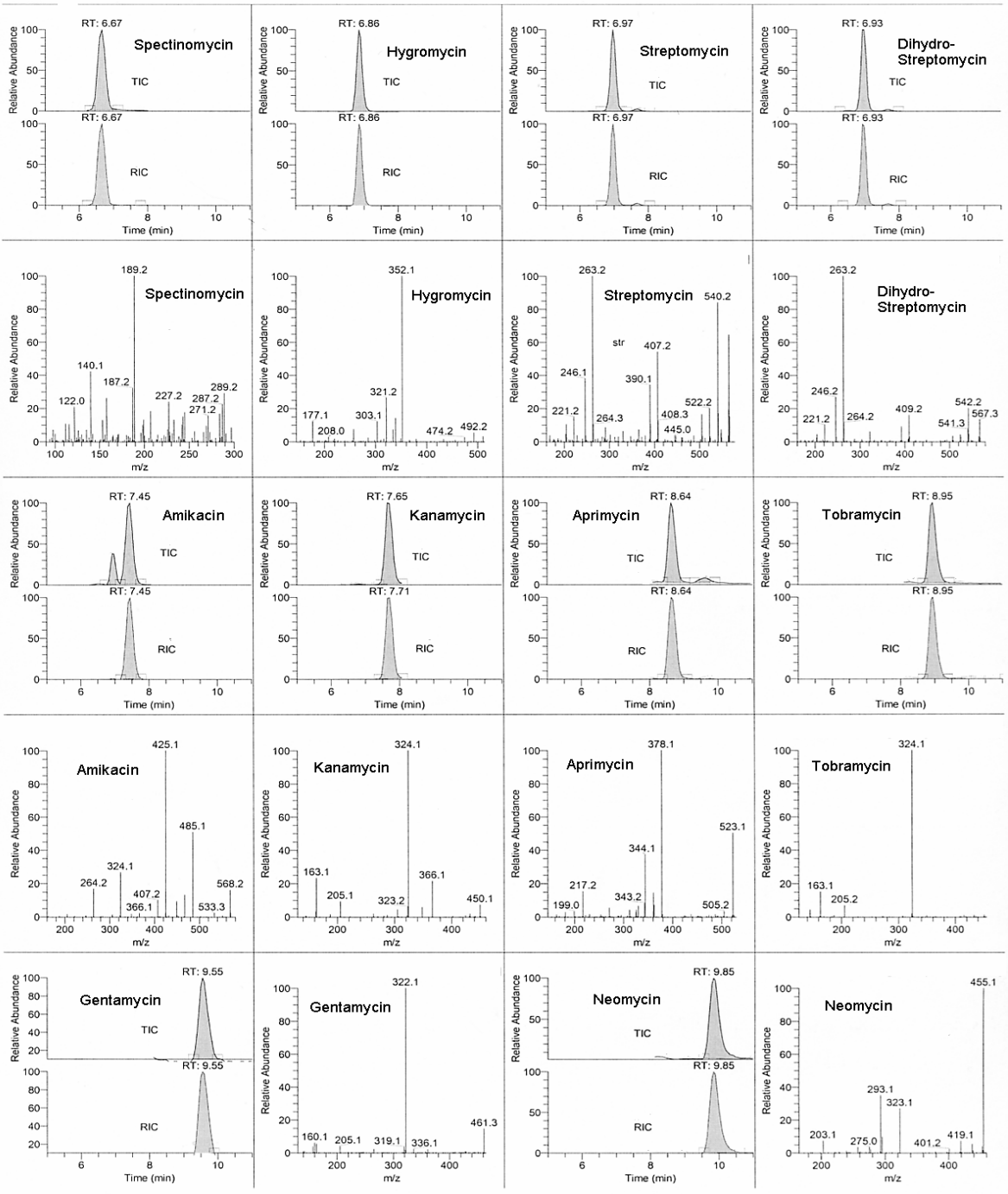
Remarks: Streptomycin base ion will vary.

Note: Place a check mark where applicable to indicate a positive response. A check mark in each of the four columns represents a positive confirmation.

K. APPENDIX

Figure 1. Retention times and spectra for Aminoglycosides




Spectinomycin
Hygromycin
Streptomycin
Dihydro-Streptomycin
Amikacin
Kanamycin
Aprimycin
Tobramycin
Gentamycin
Neomycin
TIC
RIC
Relative Abundance
Time (min)
m/z

Approvals

Approved by:	Date Approved
Tom Mallinson	05/09/2005
Terry Dutko	05/09/2005
Jess Rajan	05/10/2005
Charles Pixley	05/09/2005
Phyllis Sparling	05/20/2005