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Title: Screening for Aminoglycosides by LC-MS-MS		
Revision: .03	Replaces: CLG-AMG4.02	Effective: 11/02/20

Contents

A. INTRODUCTION

1. Background / Summary of Procedure

Aminoglycosides (AMGs) residues are extracted from tissues using a buffer containing ammonium acetate / trichloroacetic acid as a protein precipitation. The extract is neutralized, cleaned using a weak-cation dispersive solid-phase extraction media , and analytes are captured with 10% formic acid in water. The final extract is analyzed using ion-pair reverse phase ultra high performance liquid chromatography (UHPLC) with detection by triple quadrupole mass spectrometry (MS/MS) using electrospray ionization in the positive mode (ESI^+).

2. Applicability

This method is suitable for the screening of the following aminoglycosides: amikacin, apramycin, dihydrostreptomycin, gentamycin, hygromycin B, kanamycin, neomycin B, spectinomycin (as spectinomycin hydrate) and streptomycin in bovine, porcine, poultry, ovine and caprine kidney and bovine, porcine, poultry, equine, ovine and caprine muscle at levels found in Section J.1. in Table 5.

Note: Refer to 21CFR for tolerance values set by FDA and 40CFR for tolerance values set by EPA.

Note: This method may be performed using standards/solutions that contain fewer analytes than the method is applicable for, if the excluded analytes will not be included in the reported results.

B. EQUIPMENT

Note: Equivalent equipment may be substituted.

1. Apparatus

a. Centrifuge – Thermo IEC , Centra GP-8

b. Cutting board and knives for mincing and removal of tendons and fat.

c. Vortex Mixer – Scientific Products, S8220

d. pH meter – with Ag/AgCl combination electrode Orion, Model 370

e. Top Loading Balance – Mettler, Model PB302

f. Analytical Balance – Mettler, Model X-205 Dualrange

g. Centrifuge tubes – polypropylene (PP), 50 mL, Falcon Part number 352070

h. Centrifuge tubes – polypropylene (PP), 15 mL, Falcon Part number 352096

i. Whatman Mini-Uniprep syringeless filter vials – VWR 0.2 µm, PVDF, Cat. No. 12000-524. Note: Avoid glass if the Mini-UniPrep filter vials are substituted with syringe filters and autosampler vials, and substitutes must be checked for possible retention of analytes.

j. Cryogenic tubes – Nalgene, 1.2 mL, Mfr. No. 5011 0012

k. Nalgene FEP bottle – Nalgene, 30 mL, Mfr. No. 1600 00901

l. Filters for mobile phases – VWR, Supor membrane disc filters, 47 mm i.d., 0.2 µm, Cat. No. 28147-978

m. Sorbent Selectra Bulk Sorbents – CUCCX1(carboxylic Acid) 40-63 µm, Part Number CUCCXOOK

n. Magnetic stirrer – Corning, Cat No PC-351

o. Repeating pipettes and tips – 25 µL and 2.5 mL-Eppendorf, 100 µL and 200 µL-Gilson, 1000 µL-VWR

p. Shaker – Eberbach, Cat. No. 6010

q. Glassware – Class A

r. Food Processor – Robot Coupe USA Inc., Robot Coupe model RSI6Y-1.

s. Freezer capable of -10^0 C – Fisher Scientific, Isotemp Freezer, Cat. No 13-986-149.

t. Freezer capable of -70^0 C – Fisher Scientific, Isotemp Freezer Ultra-Low Temperature, Cat. No 13-990-14.

u. PVDF filter disk – Xpertek, 0.2µm, Mfr. No. 9474051

v. Syringe filter – Becton Dickenson, 3mL, Mfr. No. 309657

w. Grade 313 fluted filter paper 15 cm, VWR, Cat. No 28333-043

x. Auto-titrator - SI Analytics, TitroLine 6000/7000.

y. Auto-titrator tray - SI Analytics, T W alpha plus.

z. Auto-titrator 50 mL beaker - Lab Synergy, Cat. No. TZ1783.

aa. Stirring rod – Lab Synergy, Cat. No. 285214232.

2. Instrumentation

a. Waters UPLC-MS/MS TQD system with MassLynx operating software.

b. UPLC Column – Waters UPLC BEH C18, 2.1 x 50 mm, 1.7µm with VanGuard Pre-column UHPLC BEH C18, 2.1 x 5.0 mm, 1.7 µm.

C. REAGENTS AND SOLUTIONS

Note: Equivalent reagents / solutions may be substituted.

1. Reagents

   a. Methanol (MeOH) – LC grade, Burdich and Jackson, Cat. No. H448-10.

   b. Acetonitrile (MeCN) – LC grade, CHROMASOLV, 99.9% Sigma-Aldrich, Cat. No. HP 412.

   c. Water (H_2O), LC grade – house deionized water passed through Barnstead E-pure 4 cartridge system.

   d. Heptafluorobutyric Acid (HFBA) – Sigma, Cat. No. 77249.

   e. Hydrochloric acid (HCl), concentrated – Mallinkrodt, Cat. No. 2612.

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

   g. Ethylenediaminetetraacetic acid, disodium salt, dihydrate ($Na_2EDTA•2H_2O$), 99+% – Sigma, Cat. No. E5134.

   h. Ammonium Acetate (NH_4OAc) – Mallinkrodt, Cat. No. 3272.

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

   j. Sodium Chloride (NaCl) – Sigma, Cat. No. S7653.

   k. Formic Acid (FA) – Fluka, Cat. No. 94318.

   l. 0.5N NaOH solution – VWR, Cat. No. BDH7221.

2. Solutions

   a. 1 N HCl:

      Dilute concentrated HCl 1:12 with LC water (e.g. add 7 mL acid to 77 mL water in a 100mL glass bottle for storage).

   b. 30% w/v NaOH:

      Add 30 g NaOH to a 100 mL graduated cylinder containing 90 mL of LC water. Mix with a magnetic stirbar then remove with retriever.

      Caution: This is an exothermic reaction; let the solution cool before adjusting to the 100 mL final volume. Store this solution in a plastic container.

   c. 1 N NaOH:

      Add 4 g NaOH to a 100 mL graduated cylinder containing 95 mL of LC water. Mix with a magnetic stirbar then remove with retriever.

      Caution: This is an exothermic reaction; let solution cool before adjusting to the 100 mL final volume. Store this solution in a plastic container.

d. Mobile Phase B (20 mM HFBA in MeCN) :

Measure 2.6 mL of HFBA and dilute to 1 L with LC grade MeCN. Filter through a 0.2 µm filter disc if necessary and transfer into UHPLC Reservoir B. Mobile phase B may be stored refrigerated or at room temperature if desired.

e. Mobile phase A (20mM HFBA in 95/5 water/MeCN):

Measure 2.47 mL of HFBA and 50 mL of the solution prepared in 2.d. (20 mM HFBA in MeCN) and dilute to 1 L with LC water. Filter through a 0.2 µm filter disc if necessary and transfer into UHPLC Reservoir A.

f. Extraction solvent mixture (10 mM NH_4OAc, 0.4 mM EDTA, 0.5% NaCl and 2% TCA in water):

Add 1.54 g of NH_4OAc to 2 L graduated cylinder. Dilute to 1.95 L with LC water and adjust the pH to 4.0 with 1 N HCl and/or 1 N NaOH using a calibrated pH meter to measure. Add 0.3 g $Na_2EDTA•2H_2O$, 10 g of NaCl, and 40 g TCA. Mix to ensure salts dissolve and adjust final volume to 2 L with LC water. Store in >2 L glass bottle

g. 10% FA in water:

Add 10 mL of formic acid (FA) to a 100 mL volumetric flask containing 80 mL LC water, then fill to mark with LC water.

D. STANDARD(S)

Note: Equivalent standards / solutions may be substituted. Purity and counterions are to be taken into account when calculating standard concentrations. In-house prepared standards shall be assigned an expiration date that is no later than the stability stated in the method.

1. Standard Information

Amikacin, Sigma (A-1774)	Kanamycin Sulfate, Sigma (K-1876)
Apramycin HCl, Sigma (A-2024)	Neomycin B Sulfate, Sigma (N-1876)
Dihydrostreptomycin Sulfate, USP (1203008)	Spectinomycin HCl, USP (1618003)
Gentamicin Sulfate, Sigma, (G-3632)	Tobramycin (int. std.), Sigma, (T-4014)
Streptomycin Sulfate, USP (1623003)	Hygromycin B, Sigma, (H-7772)

2. Preparation of Standard Solution(s)

a. Individual AMG stock solutions (2000 µg/mL in water):

For each stock solution, calculate the amount of material that contains 20 mg AMG base, accounting for purity and/or water and sulfate content. Weigh this amount to the nearest 0.1 mg. Transfer to a 30 mL Nalgene FEP bottle and add

by weight (1 g/mL density for water) the exact amount of water (≈10 mL) to yield 2000 µg/mL concentration of the pure drug. Mix well. This standard is stable for 3 months when stored at < -10°C.

b. Intermediate standard mixture of AMGs in water (50 µg/mL)

Pipet 250 µL each of amikacin, apramycin, hygromycin B, kanamycin, gentamicin, and spectinomycin into a 30 mL FEP bottle. Add 8.50 mL of water. Mix well. This standard is stable for 3 months when stored at < -10°C.

c. Mixed AMG calibration/spiking solution in water:

Following Table 1, combine the amounts of 2,000 µg/mL AMG stock solution for streptomycin, dihydrostreptomycin, neomycin and for Intermediate standard mixture of AMGs in water (50 µg/mL) to prepare the mixed working standards in a 30 mL FEP bottle for kidney, or muscle (use given volumes depending on matrix):

Table 1 AMG Calibration/ Spiking Solutions Preparation

AMG	Standard	Concentration (ug/mL)	Volume for Kidney, Fortification Standard, (mL)	Kidney Fortification Standard (µg/mL)	Volume for Muscle Fortification Standard, (mL)	Muscle Fortification Standard (µg/mL)
Neomycin	Stock	2000	0.72	144	0.12	24
Streptomycin	Stock	2000	0.2	40	0.05	10
Dihydrostreptomycin	Stock	2000	0.2	40	0.05	10
Hygromycin B	Mix AMG I.S.	50	0.4	2	0.4	2
Amikacin						
Kanamycin						
Apramycin						
Gentamicin						
Spectinomycin						
Water	N/A	N/A	8.48	N/A	9.38	N/A

Mix well. This standard is stable for 3 months when stored at < -10 °C.

The calibration/spiking solution is used for a spiking solution (recoveries and checks) and for preparation of calibration standards.

Note: the calibration/spiking solution should be portioned into polypropylene centrifuge tubes in quantities such that the volume in each tube is consumed on a sample set thus minimizing losses due to thawing and refreezing.

Table 2 provides AMG concentrations for each analyte when 100 µL of the appropriate fortification standard is spiked into 4 g of tissue.

Table 2 Analyte Concentrations as Fortified.

AMG	Kidney (µg/g)	Muscle (µg/g)
Neomycin	3.6	0.6
Spectinomycin	0.05	0.05
Streptomycin	1	0.25
Dihydrostreptomycin	1	0.25
Gentamicin	0.05	0.05
Hygromycin B	0.05	0.05
Amikacin	0.05	0.05
Kanamycin	0.05	0.05
Apramycin	0.05	0.05

d. Tobramycin Internal standard(IS) in water (40 µg/mL)

Pipet 200 µL of 2000 µg/mL tobramycin stock solution from D.2.a into 9.8 mL of water in a 15 mL. polypropylene centrifuge tube. Mix well. This standard is stable for 3 months when stored at < -10 °C.

Note: Thorough mixing is critical for the preparation of these standards.

e. AMG External Standard in 9% FA Solution:

Add 50 µL of appropriate kidney or muscle calibration/spiking solutions plus 50 µL of the 40 µg/mL IS solution to the labeled bottom portions of Whatman Mini-Uniprep autosampler vials. Add 0.400 mL of 10% FA in water. Filter the reagent-only calibration standard by placing the upper filter caps on the bottom portion of the vials. Mix well by vortexing. Inject external or recovery standard prior to each day's run of samples to determine the instrument's suitability. These solutions can be stored at 2 - 8 °C and re-used for five days for routine monitoring.

Note: Solutions are stable for three months when stored at <-10 ºC, five days when stored at 2 - 8 ºC, and one day at ambient temperatures. The autosampler tray on the instrument keeps the solutions cold during analysis.

E. SAMPLE RECEIPT AND PREPARATION

Samples collected fresh must be kept cold before and during shipping to the laboratory. Once received at the laboratory, samples must be frozen (<10 °C) prior to grinding if

they cannot be prepared on the day of receipt. Once frozen, the sample should be allowed to thaw, while keeping it as cold as possible. Dissect away fat and connective tissue from kidney or liver. Grind tissue in blender or vertical cutter-mixer. Store samples frozen (<-10 °C).

Sample preparation may also be done by dry ice grinding as follows;

a. Chop 0.5 -1 lb of muscle tissue into small pieces and homogenize with an equal amount of dry ice in a large food processor. The resulting sample homogenate will be a frozen powder.

b. Transfer a portion of the homogenized sample into a loosely capped sample cup until the dry ice has sublimed. Excess sample from step E.a may be discarded.

c. For any retained sample, tighten the caps and store sample cups at ≤ -10 ºC.

F. ANALYTICAL PROCEDURE

1. Preparation of Controls and Samples

   a. Weigh 4.0 ± 0.1 g of homogenized samples into 50 mL polypropylene centrifuge tubes, allow sample to thaw, if necessary.

   b. Weigh three 4 g portions of blank tissue into 50 mL polypropylene centrifuge tubes, allow tissue to thaw and do the following described below:

      i. Prepare one each for the blank (negative control), the decision level recovery, and the recovery (positive control). Weigh one additional portion for an intra-laboratory check sample if necessary.

      ii. Prepare recoveries by fortifying with 100 µL of the appropriate fortification standard.

2. Extraction Procedure

   a. Add 20 mL of NH_4OAc/EDTA/NaCl/TCA buffer to each tube.

   b. To each tube add 200 µL of the 40 µg/mL tobramycin IS to yield 2 µg/g in the tissue.

   c. Shake for 10 minutes.

   d. Centrifuge at approximately 4000 rpm for 5 minutes. If floating material is observed, remove it with a spatula.

      Decant supernatant into another labeled 50 mL PP tube. If dry ice grinding was done for sample preparation, decant the supernatant through a fluted paper filter.

      Note: For non-dry ice ground tissue, the extract can as an optional step be filtered through a 0.20 µm PVDF syringe filter or equivalent or through fluted paper filters.

e. Using a calibrated pH meter or auto-titrator, adjust pH of the sample extracts to 7.50 ± 0.25 with a few drops of 30% NaOH followed by 1 N NaOH and/or 1 N HCl for fine adjustment.

Note: Using more dilute concentrations of NaOH and HCl, such as 0.5 N, is allowable for fine adjustment of the pH.

f. Centrifuge at approximately 4000 rpm for 3 minutes.

g. Decant each extract into a pre-labeled 50 mL polypropylene centrifuge tubes containing approximately 0.50 g of CUCCX1 Sorbent.

h. Cap tubes and vortex on a platform vortex for 3 minutes.

i. Centrifuge tubes at 4000+ rpm for 3 minutes.

j. Aspirate sample extract to waste.

k. Add 2 mL 10% Formic Acid to each tube containing sorbent, cap and vortex on a platform vortex for 3 minutes.

l. Centrifuge tubes at 4000 + rpm for 3 minutes.

m. For the samples and controls, place 500 µL of each final extract into bottom piece of Mini Uni-Prep PVDF syringeless filter vial. Then insert top filter vial and press together.

3. Instrumental Settings

Note: The instrument parameters may be optimized to ensure system suitability.

UHPLC-MS-MS Analysis

Instrument operating Parameters – UHPLC System

a. Mobile phases for AMG analysis:

Mobile Phase A – 95% water / 5% MeCN / 20 mM HFBA
Mobile Phase B – 100% MeCN / 20 mM HFBA

Flush column with 1:1 A/B at a flow rate of 0.5 mL/min for 3 minutes. Change the mobile phase initial conditions to 100% A. Allow column to equilibrate until the “delta” value on the pressure reading is < 20.

b. UHPLC gradient program: (Table 3)

Flow rate: 0.5 mL/min
Pressure Limits: 200 psi minimum; 12,000 psi maximum
Run time: 3.00 min

Table 3 – LC gradient

Time (min)	% Mobile Phase A	% Mobile Phase B	Gradient
0.00	100	0	none
0.50	80	20	linear
1.00	80	20	none
2.00	60	40	linear
2.05	10	90	linear
2.50	10	90	none
2.55	100	0	linear
3.00	100	0	none

c. Autosampler program:
   i. Run time: 3.0 min
   ii. Injection loop: 20 µL
   iii. Loop option: Partial loop needle overfill
   iv. Injection Volume: 15 µL
   v. Weak wash solvent: Mobile Phase A
   vi. Weak wash volume: 500 µL
   vii. Strong wash solvent: Mobile Phase B
   viii. Strong wash volume: 500 µL
   ix. Sample temperature: 7 ºC
   x. Column manager:
      (a) Column valve position: To match column location
      (b) Column manager temperature: 40 ºC
      (c) Use divert valve to divert eluant to waste 0.25 minutes prior to first peak and 0.25 minutes after last analyte peak.

d. Instrument Operating Parameters – Mass Spectrometer
   i. Mass spectrometer calibration and resolution were done according to the manufacturer's specification using the manufacturer's supplied calibration solution.

ii. Type: MS/MS

iii. Electrospray Source Parameters:

Capillary (kV): 3.0

Cone (V): Variable - analyte dependent

Extractor (V): 3.0

RF (V): 0.10

Source Temperature (°C): 150

Desolvation Temperature (°C): 450

Cone Gas Flow (L/hr): 20

Desolvation Gas Flow (L/hr): 900

Collision Gas Flow (mL/min): 0.20

iv. Analyzer Parameters:

LM1 Resolution 12.50

HM 1 Resolution: 12.50

MSMS Mode Entrance: -5

MSMS Mode Collision Energy: Variable – analyte dependent

MSMS Mode Exit: 1

LM 2 Resolution: 12.50

HM 2 Resolution: 12.50

v. MS Method Parameters:

Type: MRM

Ion Mode: ES+

Dwell (s): 0.005

Start time (min): 0.8

End time (min): 2.6

Table 4 – MRM Transitions

Start–End Time (min)	*Dwell Time (ms)*	*Compound*	*Precursor ion (m/z)*	*Product ions (m/z)*	*Cone (V)*	*Collision Energy (V)*
0.9-1.2	66	Spectinomycin Hydrate	351.24	333.33	40	20
1.1-1.3	66	Hygromycin B	528.20	177.05	44	30
1.2-1.4	44	Streptomycin	582.17	263.09	70	32
1.2-1.4	52	Dihydrostreptomycin	584.17	263.09	70	30
1.5-1.7	150	Amikacin	586.43	163.21	30	35
1.6-1.8	150	Kanamycin A	485.36	163.22	30	20
1.9-2.1	33	Apramycin	540.41	217.20	35	25
2.0-2.1	22	Tobramycin (IS)	468.36	163.19	25	25
2.0-2.2	33	Gentamicin c1a	450.39	160.16	35	25
2.0-2.2	33	Gentamicin c2+c2a	464.42	160.23	35	25
2.0-2.3	33	Gentamicin c1	478.42	157.25	40	30
2.1-2.3	22	Neomycin B	615.30	163.38	52	35

See Appendix for diagrams of proposed fragmentation patterns for each analyte.

Note: Screening of the presence of spectinomycin hydrate is considered to be screening of the parent spectinomycin. Screening for the presence of Gentamicin is determined when at least one of the complexes are present (i.e. either c1, c1a, or c2 + c2a).

e. UHPLC- MS/MS Analytical Procedure

i. Turn on UHPLC pump, set mobile phase to 100% A at a flow rate of 0.50 mL/min. Perform column equilibration for five minutes. Verify backpressure of column gives “delta” value < 20 in pressure fluctuations.

ii. Turn on MS and load appropriate MS Tune file (.ipr). Turn on API gas flow. Allow MS to achieve designated gas flow and desolvation temperature. Place MS valve position to LC.

iii. Inject 15 µL of external standard (appropriate for the tissue to be analyzed), followed by two injections of 10% FA in water (solvent blank). Verify the retention time, divert valve switching time, and detection of MS/MS ions using the TargetLynx sample processing program.

iv. Then inject recovery(ies), blank, followed by samples. One may want to put solvent blanks in between samples in case of high finding leads to carry-over.

v. As a test of retention time and instrument response stability, re-inject a calibration standard at the end of the injection sequence. Depending on instrument variability, additional injection of control standards may be interspersed mid-sample sequence.

vi. Column, Pump and ES interface care: At the end of set of analyses, the column should be flushed for 5-10 minutes with Acetonitrile. Then the instrument performs a shutdown procedure, turning off LC flow and MS desolvation temperature and gas flow. Inspect entrance cone for cleaning, following manufacturer's specification for cleaning the surfaces.

4. Sample Set
   a. Screening set
      i. External Standard (optional)
      ii. Decision Level recovery
      iii. Positive control (Recovery)
      iv. Intra-laboratory check sample (if necessary)
      v. Negative Control (Blank)
      vi. Up to 44 Samples
      vii. External standard or positive control

G. DECISION CRITERIA / CALCULATIONS

1. Screening
   a. The retention time for the recoveries and samples must match the retention time of the decision level recoverystandard within 5%.
   b. Blank must be less than 10% of the decision level recovery.
   c. The screening ion for a given analyte must be present. The required ion for each compound is listed in Table 4.
   d. The screening ion must have a signal-to-noise ratio ≥ 3. This may be verified by visual inspection.
   e. A sample is screened positive if the following criteria are met:
      i. The fortified recovery of the analyte must exceed 10% of the decision level recovery.
      ii. The sample response equals or exceeds the level of interest recovery (positive control) level.

H. SAFETY INFORMATION AND PRECAUTIONS

1. Personal Protective Equipment — Protective clothing, eyewear, and gloves, where applicable.

2. Hazards

Consult all Safety Data Sheets (SDS) associated with the method.

3. Disposal Procedures

Follow federal, state and local regulations.

I. QUALITY ASSURANCE PLAN

1. Performance Standard

a. For Screening:

i. For set acceptance, the nine analytes in the fortified recovery (positive control) must meet screening criteria.

ii. The blank (negative control) must be negative using the criteria in Section G.

2. Intralaboratory Check Samples

a. Acceptability criteria.

Refer to I. 1.

If unacceptable values are obtained, then:

i. Investigate following established procedures.

ii. Take corrective action as warranted.

J. APPENDIX

1. Screening levels

Table 5 - Screening level per species

AMG	Matrix	Bovine (µg/g)	Porcine (µg/g)	Poultry (µg/g)	Equine (µg/g)	Ovine (µg/g)	Caprine (µg/g)
Amikacin	Kidney	0.05	0.05	0.05	N/App	0.05	0.05
Amikacin	Muscle	0.05	0.05	0.05	0.05	0.05	0.05
Apramycin	Kidney	0.05	0.05	0.05	N/App	0.05	0.05
Apramycin	Muscle	0.05	0.05	0.05	0.05	0.05	0.05
Dihydrostreptomycin	Kidney	1	1	1	N/App	1	1
Dihydrostreptomycin	Muscle	0.25	0.25	0.25	0.25	0.25	0.25
Gentamicin	Kidney	0.05	0.05	0.05	N/App	0.05	0.05
Gentamicin	Muscle	0.05	0.05	0.05	0.05	0.05	0.05
Hygromycin B	Kidney	0.05	0.05	0.05	N/App	0.05	0.05

AMG	Matrix	Bovine (µg/g)	Porcine (µg/g)	Poultry (µg/g)	Equine (µg/g)	Ovine (µg/g)	Caprine (µg/g)
Hygromycin B	Muscle	0.05	0.05	0.05	0.05	0.05	0.05
Kanamycin	Kidney	0.05	0.05	0.05	N/App	0.05	0.05
Kanamycin	Muscle	0.05	0.05	0.05	0.05	0.05	0.05
Neomycin	Kidney	3.6	3.6	3.6	N/App	3.6	3.6
Neomycin	Muscle	0.6	0.6	0.6	0.6	0.6	0.6
Spectinomycin	Kidney	0.05	0.05	0.05	N/App	0.05	0.05
Spectinomycin	Muscle	0.05	0.05	0.05	0.05	0.05	0.05
Streptomycin	Kidney	1	1	1	N/App	1	1
Streptomycin	Muscle	0.25	0.25	0.25	0.25	0.25	0.25

N/App = Not applicable

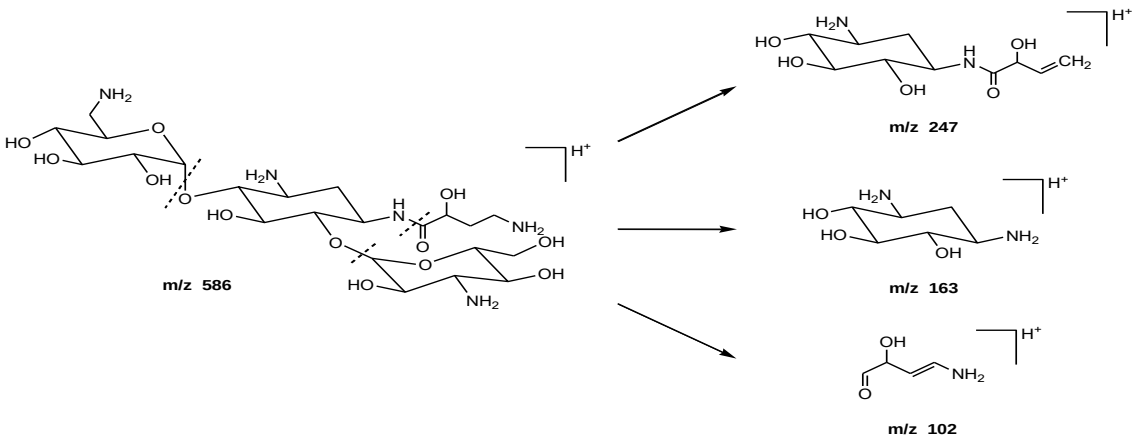
2. Proposed Fragmentation Pattern

Amikacin Formula: $C_{22}H_{43}N_5O_{13}$ MW: 585.60 g/mol

m/z 586.43 → *m/z* 247.37

m/z 586.43 → *m/z* 163.21

m/z 586.43 → *m/z* 101.98

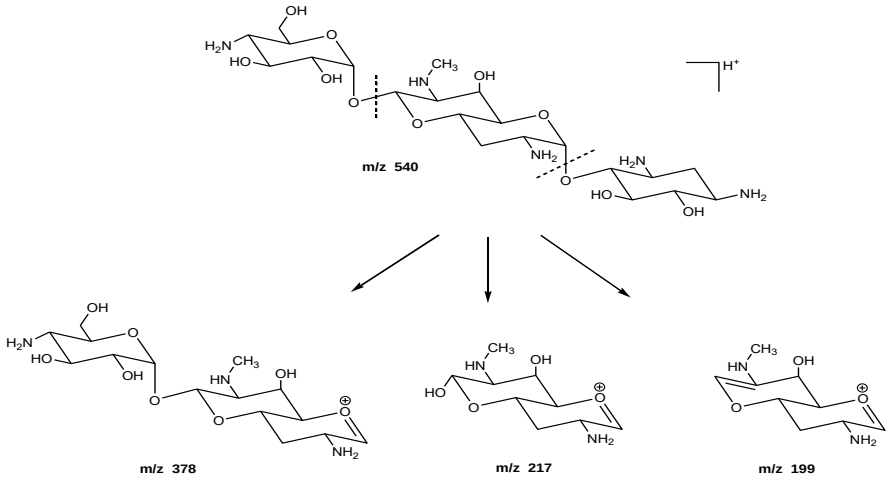


Apramycin Formula: $C_{21}H_{41}N_5O_{11}$ MW: 539.28 g/mol

m/z 540.41 → *m/z* 378.31

m/z 540.41 → *m/z* 217.20

m/z 540.41 → *m/z* 199.35

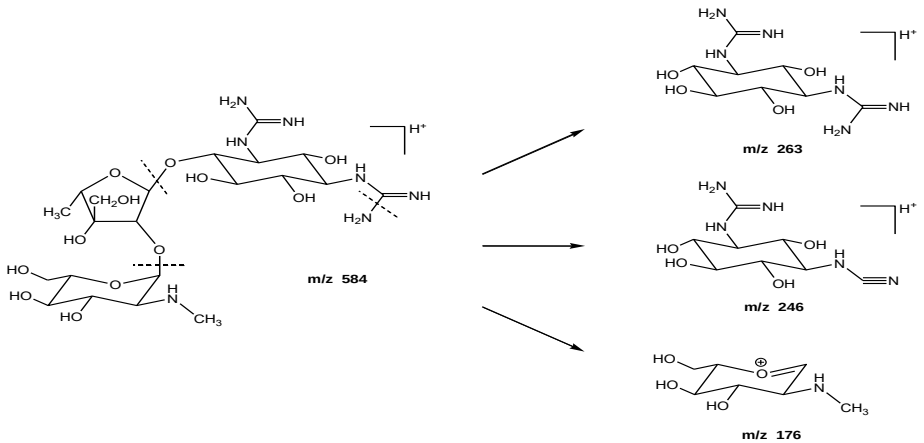


Dihydrostreptomycin Formula: $C_{21}H_{41}N_7O_{12}$ MW: 583.21 g/mol

m/z 548.17 → *m/z* 263.09

m/z 548.17 → *m/z* 246.05

m/z 548.17 → *m/z* 176.00

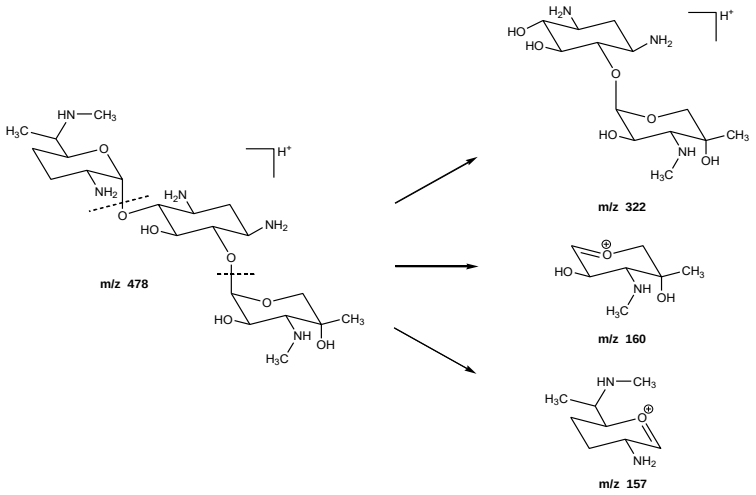


Gentamicin C_1 Formula: $C_{21}H_{43}N_5O_7$ MW: 477.32 g/mol

m/z 478.42 → *m/z* 322.42

m/z 478.42 → *m/z* 160.16

m/z 478.42 → *m/z* 157.25



Gentamicin C_{1a} Formula: $C_{19}H_{39}N_5O_7$ MW: 449.29 g/mol

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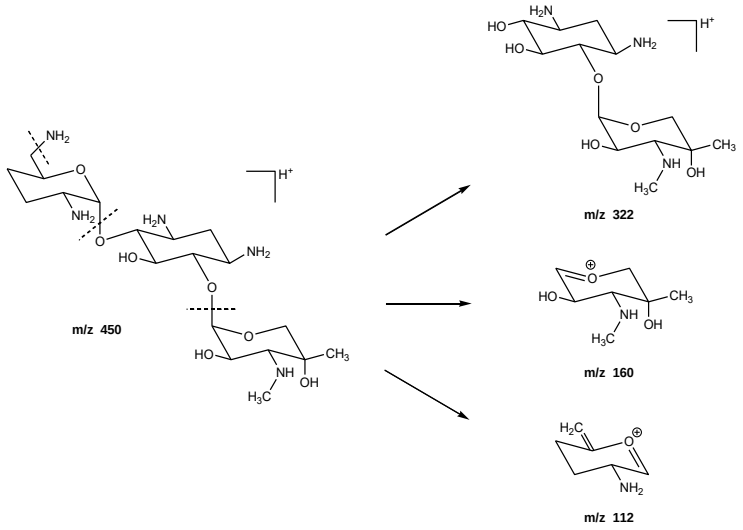
m/z 450.39 → *m/z* 160.16

m/z 450.39 → *m/z* 112.17

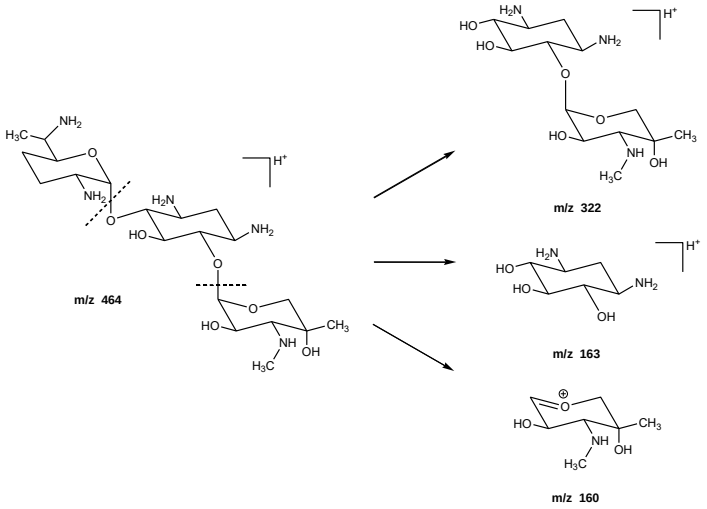


Gentamicin C_{1a} + C_{2a} Formula: $C_{20}H_{41}N_5O_7$ MW: 463.30 g/mol

m/z 464.42 → *m/z* 322.39

m/z 464.42 → *m/z* 163.14

m/z 464.42 → *m/z* 160.23

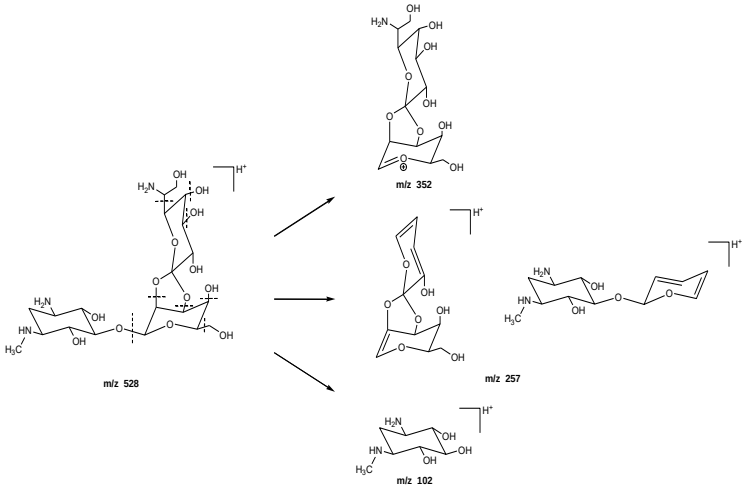


Hygromycin B Formula: $C_{20}H_{37}N_3O_{13}$ MW: 527.23 g/mol

m/z 528.20 → *m/z* 352.03

m/z 528.20 → *m/z* 257.00

m/z 528.20 → *m/z* 177.05

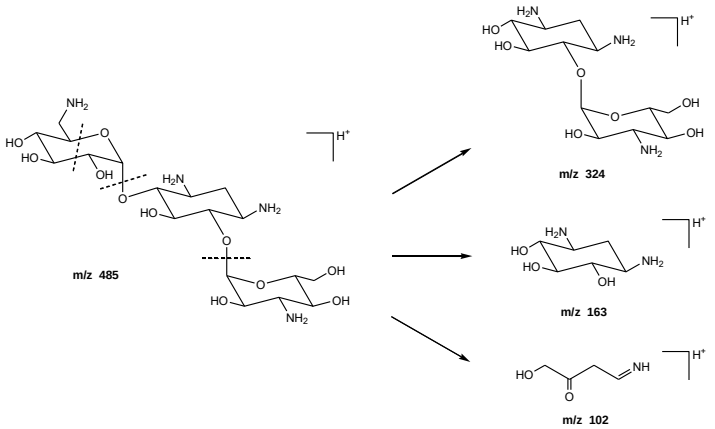


Kanamycin A Formula: $C_{18}H_3N_40_{11}$ MW: 484.24 g/mol

m/z 485.36 → *m/z* 324.33

m/z 485.36 → *m/z* 163.22

m/z 485.36 → *m/z* 102.14

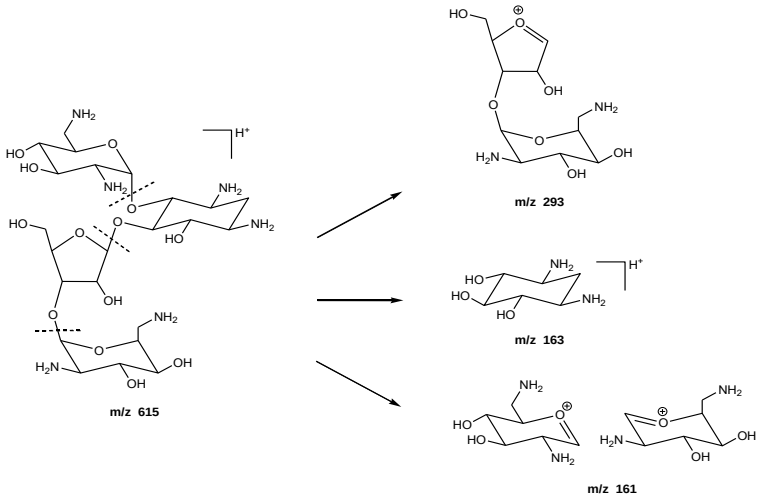


Neomycin B Formula: $C_{23}H_{46}N_60_{13}$ MW: 614.31 g/mol

m/z 615.30 → *m/z* 293.03

m/z 615.30 → *m/z* 163.38

m/z 615.30 → *m/z* 160.53

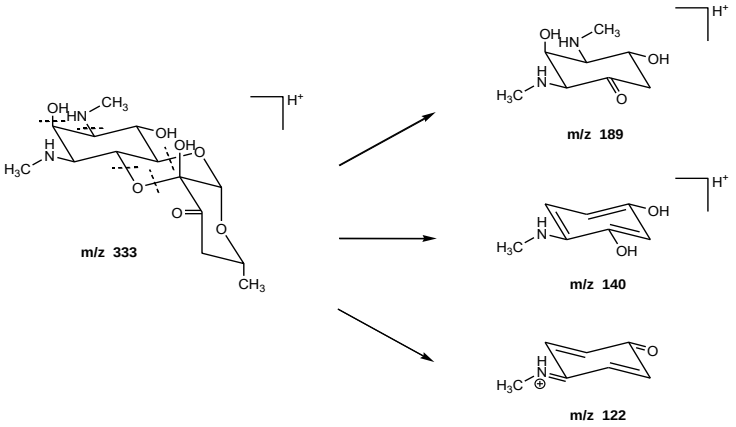


Spectinomycin Formula: $C_{14}H_{24}N_2O_7$ MW: 332.16 g/mol

m/z 333.00 → *m/z* 189.21

m/z 333.00 → *m/z* 140.10

m/z 333.00 → *m/z* 122.15

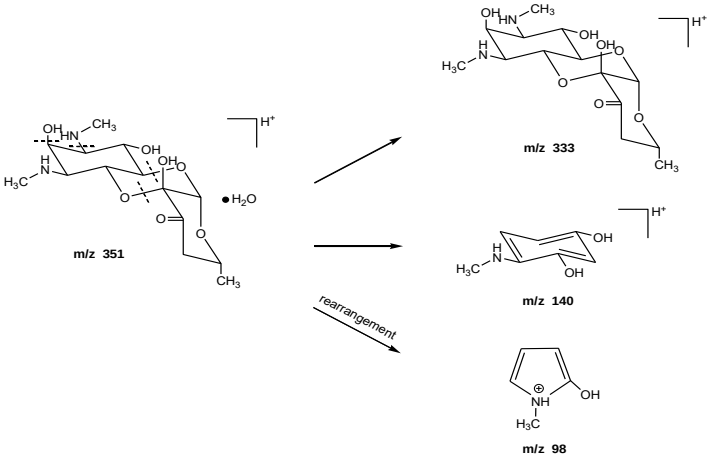


Spectinomycin Hydrate Formula: $C_{14}H_{26}N_2O_8$ MW: 350.17 g/mol

m/z 351.24 → *m/z* 333.33

m/z 351.24 → *m/z* 140.10

m/z 351.24 → *m/z* 98.00

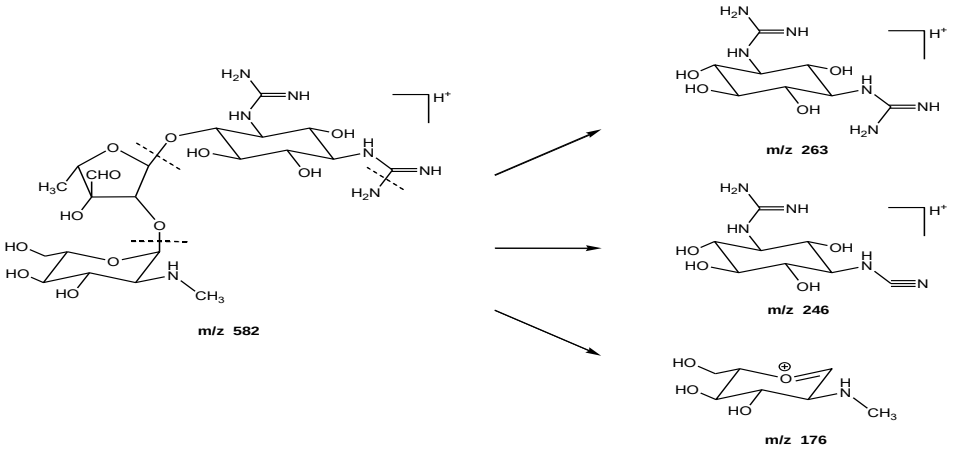


Streptomycin Formula: $C_{21}H_{39}N_7O_{12}$ MW: 581.27 g/mol

m/z 582.17 → *m/z* 263.09

m/z 582.17 → *m/z* 246.05

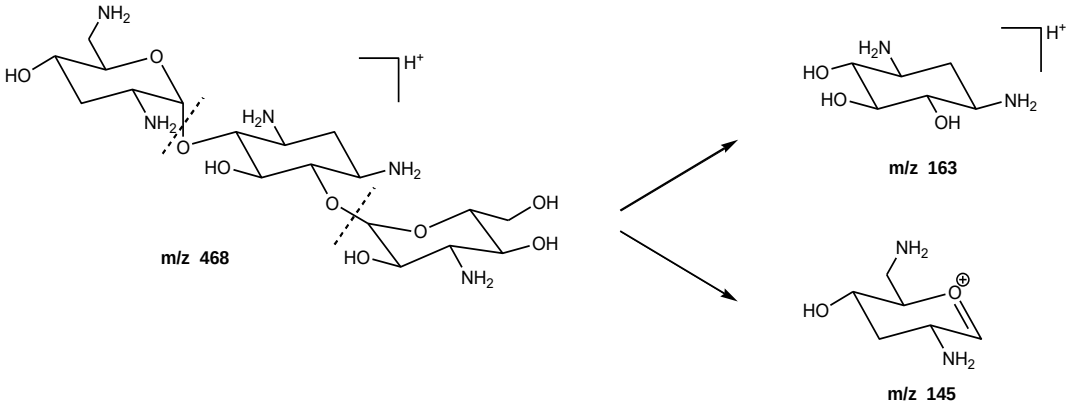
m/z 582.17 → *m/z* 176.00



Tobramycin Formula: $C_{18}H_{37}N_5O_9$ MW: 467.26 g/mol

m/z 468.36 → *m/z* 163.19

m/z 468.36 → *m/z* 145.10



K. APPROVALS AND AUTHORITIES

1. Approvals on file.

2. Issuing Authority: Director, Laboratory Quality Assurance Staff.