



Aminoglycoside Antibiotics in Feed

Apramycin,
Gentamicin,
Neomycin

Aminoglycoside antibiotics are among those commonly used in animal feeds to manage intestinal microorganisms. The beneficial effects include improved growth and generally healthier animal populations. Use of antibiotics creates a demand for analytical procedures to verify concentrations in pre-mixes and feeds and in some instances for residue analysis in animal products.

This note describes a simple, robust analytical method for the family of Aminoglycoside antibiotics in feeds and animal products. The sample is homogenized with a generic extraction solution and the crude soluble portion is directly injected into an HPLC ion-exchange column. The column effluent is then mixed with an OPA/Thiofluor™ reagent which forms highly fluorescent derivatives with the primary amine moieties of the antibiotics.

Call if you need to analyze other Aminoglycosides.

EXTRACTION PROCEDURE

Take one part feed:10 parts Extraction solution (w/v) Catalog No. 1700-1118 and homogenize for five minutes. Centrifuge for 10 minutes. Three layers will form: the pellet, a supernatant emulsion and a soft layer of floating fat. Carefully lift the floating fatty layer with a spatula and discard. Transfer the emulsion to a sealable vial. Coagulate the emulsion by placing the vial in a boiling water bath for 15 minutes. Centrifuge for 10 minutes. The clear supernate is filtered (0.45um Nylon) and placed in an autosampler vial.

METHOD

Analytical Conditions

COLUMN: ALKION™ cation-exchange, K⁺ form,
4 x 150 mm, Catalog No. 9410917
ALKION™ Guard column, 3 x 20 mm,
Catalog No. 9493020

TEMPERATURE: 40 °C

FLOW RATE: 0.8 mL/min

MOBILE PHASE: K01, Potassium buffer
K02, Potassium titrant

K03, Potassium ionic strength adjuster

Post-column Conditions

POST-COLUMN SYSTEM: Pinnacle PCX or Vector PCX

REACTOR VOLUME: 0.15 mL

TEMPERATURE: 45 °C

REAGENT: *o*-Phthalaldehyde/Thiofluor + Brij 35®

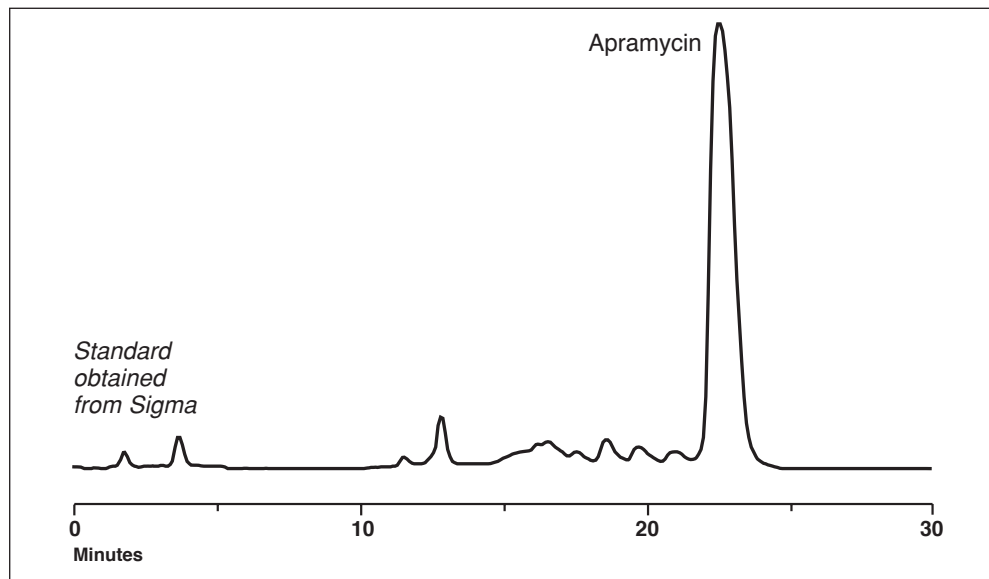
FLOW RATE: 0.3 mL/min

DETECTION: Fluorometer

λ_{ex} : 330 nm

λ_{em} : 465 nm

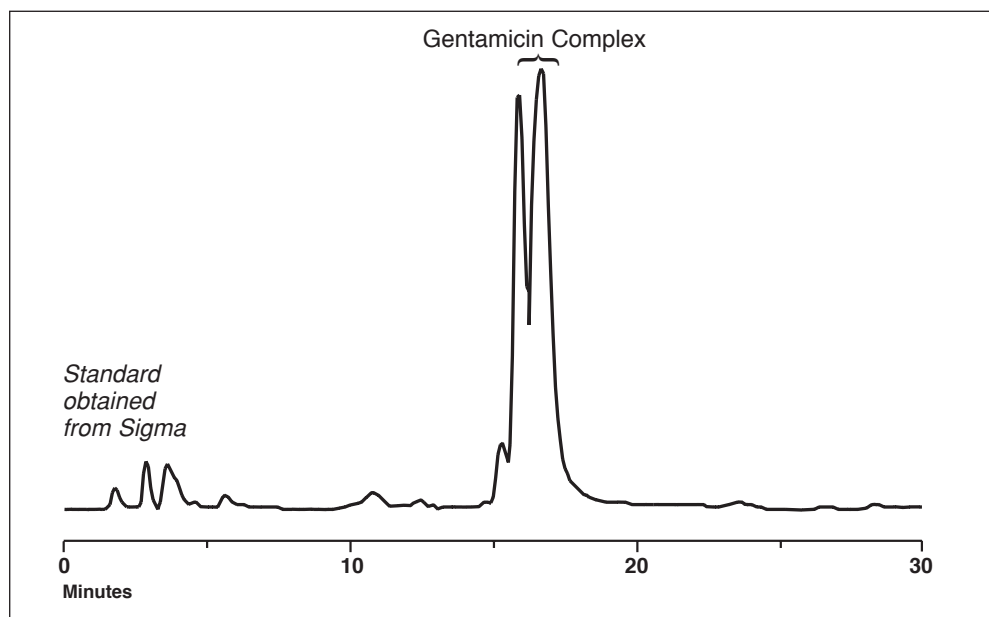
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Apramycin Analysis

Sample: 11.1 nmole Apramycin on column

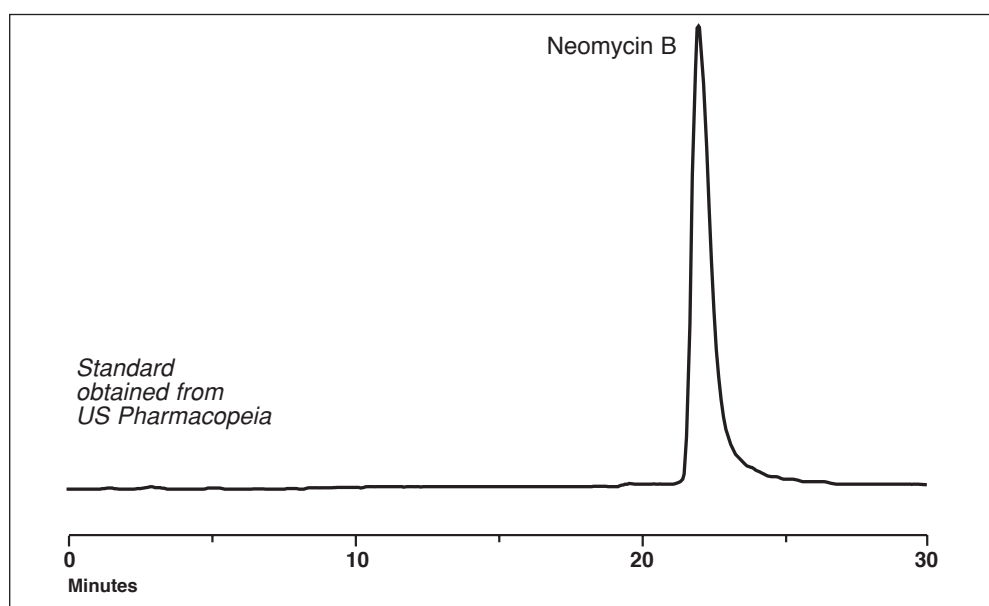
Time [min]	K01 [%]	K02 [%]	K03 [%]
0	67	33	0
5	67	33	0
15	14.7	7.3	78
20	0	7.3	78
20.1	0	22	78
21	0	22	78
21.1	67	33	0



Gentamicin Analysis

Sample: 5 x 10⁻⁶ g Gentamicin complex on column

Time [min]	K01 [%]	K02 [%]	K03 [%]
0	43	31	26
20	9	13	78
30	9	13	78
30.1	0	22	78
31	0	22	78
31.1	43	31	26



Neomycin Analysis

Sample: 2.75 nmole Neomycin on column

Time [min]	K01 [%]	K02 [%]	K03 [%]
0	60	40	0
15	13.2	8.8	78
25	11	11	78
25.1	0	22	78
26	0	22	78
26.1	60	40	0

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