

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.

Method

Extraction Procedure

Take one part feed: 10 parts Extraction solution (30 g/L of Sulfosalicylic Acid) 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 (0.45 µm Nylon) and placed in an autosampler vial.

Analytical Conditions

Analytical Column: ALKION™ cation-exchange, 4 x 150 mm, Catalog No. 9410917

Guard Column: ALKION™ Guard column, 3 x 20 mm, Catalog No. 9493020

Column Temperature: 40 °C

Flow Rate: 0.8 mL/min

Mobile Phase: 1700-1101, Potassium buffer, K01
 1700-1102, Potassium titrant, K02
 1700-1103, Potassium ionic strength adjuster, K03

Post-Column Conditions

Post-Column System: Onyx PCX, Pinnacle PCX or Vector PCX

Reactor Volume: 0.15 mL

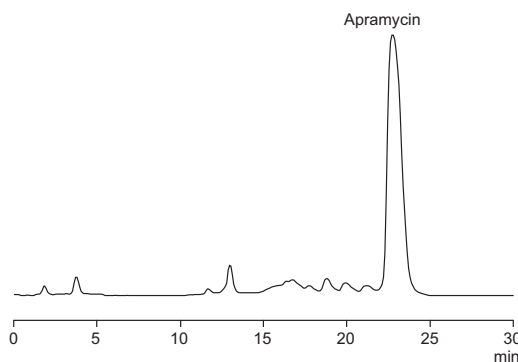
Reactor Temperature: 45 °C

Reagent: 300 mg of OPA, 2 g Thiofluor™, 3 mL of 30% Brij® 35 in 950 mL of OD104

Flow Rate: 0.3 mL/min

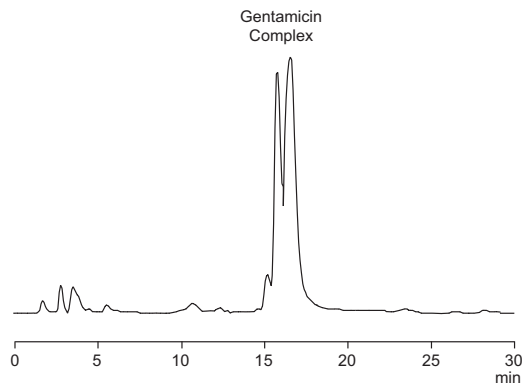
Detection: Fluorometer
 λ_{ex} : 330 nm, λ_{em} : 465 nm

Conditions for Apramycin Analysis			
Time	1700-1101 %	1700-1102 %	1700-1103 %
0	67	33	0
5	67	33	0
15	14.7	7.3	78
20	14.7	7.3	78
20.1	0	22	78
21	0	22	78
21.1	67	33	0
28	67	33	0



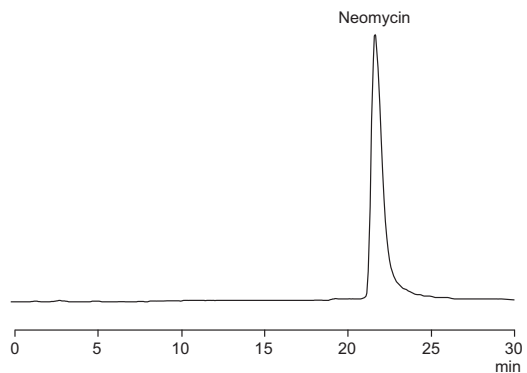
Conditions for Gentamicin Analysis

Time	1700-1101 %	1700-1102 %	1700-1103 %
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
38	43	31	26



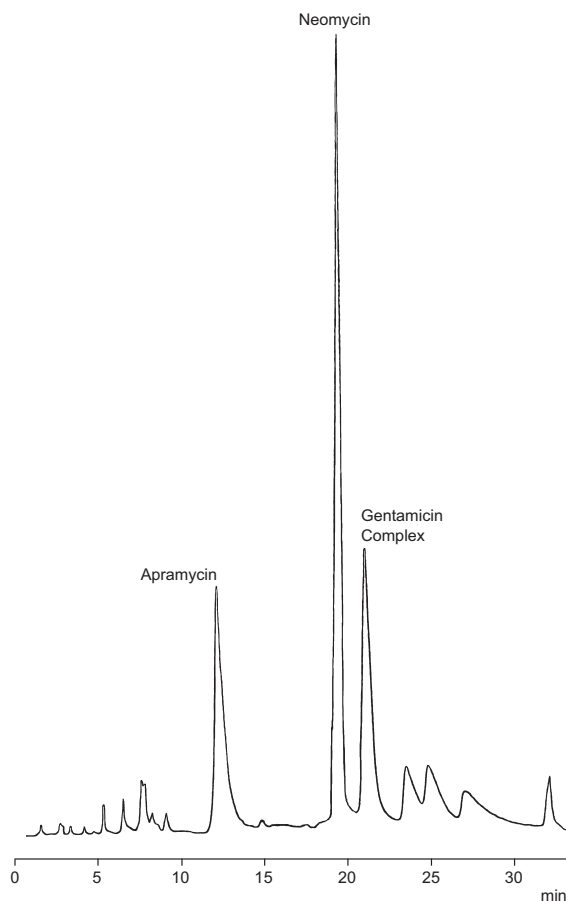
Conditions for Neomycin Analysis

Time	1700-1101 %	1700-1102 %	1700-1103 %
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
32	60	40	0



**Conditions for Separation of Apramycin
Gentamicin and Neomycin**

Time	1700-1101 %	1700-1102 %	1700-1103 %
0	60	40	0
15	13.2	8.8	78
15.1	12	10	78
30	12	10	78
30.1	0	22	78
31	0	22	78
31.1	60	40	0
37	60	40	0



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