PICKERING LABORATORIES
CATALYST FOR SUCCESS

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 (0.45 um 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:
1700-1101, Potassium buffer, K01
1700-1102, Potassium titrant, K02
1700-1103, Potassium ionic strength adjuster, K03

Post-Column Conditions

Post-Column System: Pinnacle PCX

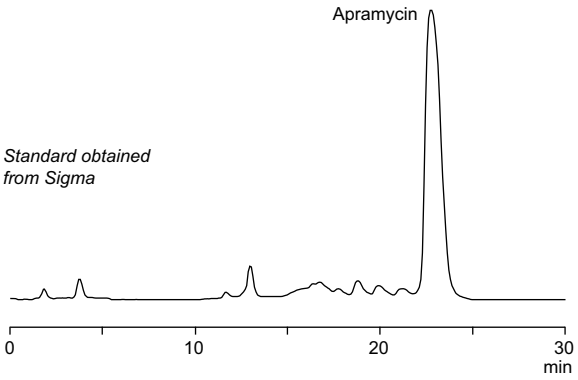
Reactor Volume: 0.15 mL

Temperature: 45 °C

Reagent: o-Phthalaldehyde/Thiofluor + Brij 35®

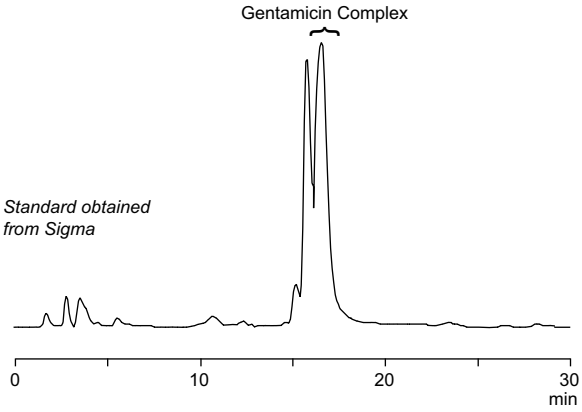
Flow Rate: 0.3 mL/min

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



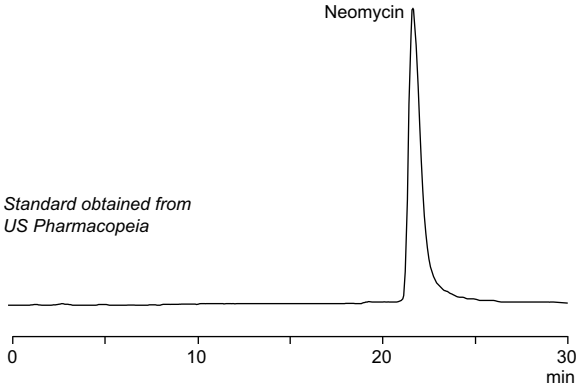
APRAMYCIN ANALYSIS			
TIME (Min)	1700-1101 %	1700-1102 %	1700-1103 %
0	67	33	0
5	67	33	0
15	14.7	7.3	78
20	14.7	7.3	78
20.1	0	22	78
21	0	22	78
21.1	67	33	0

SAMPLE: 11.1 nmole Apramycin on column



GENTAMICIN ANALYSIS			
TIME (Min)	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

SAMPLE: 5 x 10-6 g Gentamicin complex on column



NEOMYCIN ANALYSIS			
TIME (Min)	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

SAMPLE: 2.75 nmole Neomycin

PICKERING LABORATORIES
1280 Space Park Way / Mountain View, CA 94043
sales@pickeringlabs.com / support@pickeringlabs.com
800-654-3330 / 650-694-6700 / Fax: 650-968-0749