



Aminoglycoside Antibiotics in Feed Apramycin, Gentamicin, Neomycin

Pickering Laboratories

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.

Extraction Procedure

Take one part feed: 10 parts Pickering Extraction solution (w/v) — catalog No. 1700-1118 and homogenize for 5 min. Centrifuge for 10 min. 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 seal able vial. Coagulate the emulsion by placing the vial in a boiling water bath for 15 min. Centrifuge for 10 minutes. The clear supernate is filtered (0.45 μm 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 × 20 mm,
Catalog No. 9493020

Temperature 40° C
Flow Rate 0.8 mL/min
Mobile Phase K01, Potassium buffer
K02, Potassium titrate
K03, Potassium ionic strength adjuster

Post-Column Conditions

Post-column system Pickering Laboratories 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

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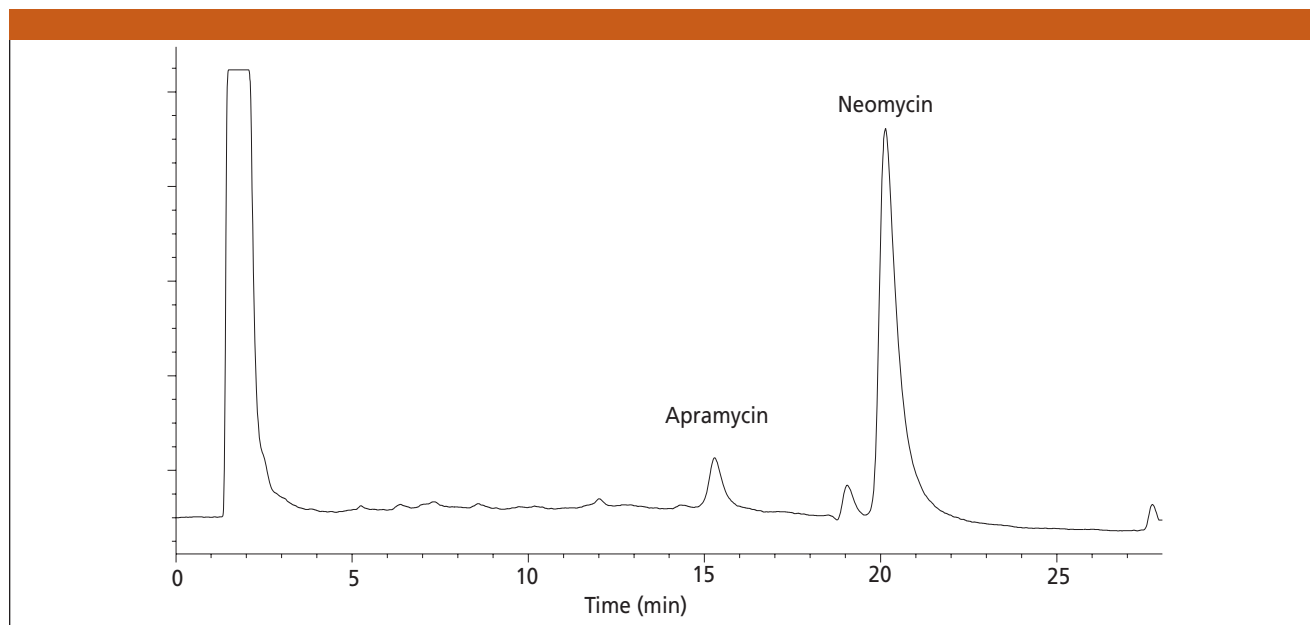


Figure 1: Corn meal sample spiked with apramycin and neomycin.

Improving the Analysis of Biodiesel Using Capillary Flow Technology

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Agilent Technologies

Improvements to the analysis of free and total glycerins using ASTM Method D6584 are made with a retention gap coupled to the analytical column with a new Capillary Flow Technology Union. A new heart-cutting 2-D GC technique using a Capillary Flow Technology Deans switch is also presented for the analysis of FAME content in biodiesel blends.

Biodiesel is a motor or heating fuel produced from renewable vegetable oils derived from crops such as soybean, rapeseed and palm. Two chemical measurements in particular must be done in the production and usage of biodiesel; analysis of glycerins and analysis of blended biodiesel fuels. ASTM Method D6584 is used for the analysis of free and total glycerin in pure biodiesel (B100) (1). High levels of these compounds can cause engine deposits and fouling. The method is difficult to run because it requires derivatization and a challenging GC analysis using a cool-on-column inlet and high column temperature. Pure biodiesel (B100) is not commonly used as a fuel. Instead it is blended with petroleum diesel between 2 and 20 volume % (B2 to B20). Conventional one dimensional GC does not have the ability to resolve the FAMEs from the hydrocarbons in the blends.

Recently Agilent introduced the 7890A GC with Capillary Flow Technology. Capillary Flow Technology is a new way to make inert, reliable, leak-free column connections and control column flows within the GC oven. A family of Capillary Column Flow devices has been developed to enable everything from simple column unions to sophisticated two-dimensional GC. This application will show how Capillary Flow Technology can be used to improve the analysis of glycerins in B100 biodiesel and the measurement of FAMEs in biodiesel blends.

Experimental Conditions - ASTM D6584

Retention Gap: Deactivated fused silica, 1 m × 0.53 mm i.d. (part no.160-2535-30)

Column: DB-5HT, 15 m × 0.32mm i.d. × 0.1 μm (part no. 123-5711)

Column Union: Capillary Flow Technology Ultimate Union (part no. G3182-61580)

Column Flow: helium @ 3 mL/min

Inlet: Cool-On-Column, Initial temperature @ 50 °C, oven track mode

Detector: Flame Ionization @ 380 °C

Initial Oven Temperature: 50 °C for 1 min

Oven Ramp 1: 15 °C /min to 180 °C

Oven Ramp 2: 7 °C /min to 230 °C

Oven Ramp 3: 30 °C /min to 380 °C for 10 min

Results - ASTM D6584

To achieve the best possible chromatography with this method, a retention gap should be used to optimize peak shape. However, connecting the retention gap to the analytical column presents a challenge due to the high oven temperature (380 °C). The Agilent Capillary Flow Technology Ultimate Union is designed to provide a high temperature leak free connection between the retention gap and the analytical column. Figure 1 shows the improvement in peak shape when a retention gap is connected to the column with the Ultimate Union.

Samples and calibration standards were prepared according to the procedures specified in the ASTM D6584 method. A B100 sample made from rapeseed oil was run twice per day over a four day period. A typical chromatogram from this analysis is shown in Figure 2. The repeatability of this analysis is shown in Table I. Using the retention gap and the Agilent Capillary Flow Technology, the ASTM repeatability specification was exceeded over the four days of analyses.

Experimental Conditions - Biodiesel Blends

Primary Column: HP-5MS, 15 m × 0.25mm i.d. × 0.1 μm (part no. 19091S-331)

Primary Column Flow: He @ 1.5 mL/min.

Secondary Column: HP-Innowax, 30 m × 0.25mm i.d. × 0.5 μm (part no. 19091N-233)

Secondary Column Flow: He @ 3.5 mL/min

Deans Switch Restrictor: Deactivated fused silica, 0.77 m × 0.10 mm i.d.

Capillary Flow Technology Deans Switch

Inlet: Split/Splitless, Initial temperature @ 250 °C, Split Ratio 100:1

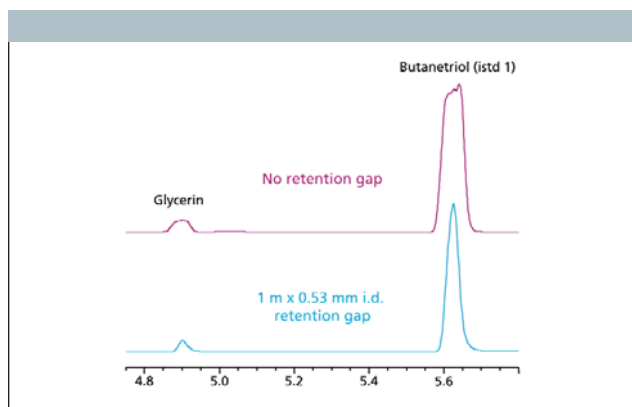


Figure 1: Peak shape can be improved for the analysis of free and total glycerins in B100 biodiesel using a retention gap before the analytical column. A Capillary Flow Technology Ultimate Union is used to join the retention gap and column so that the connection is reliable, inert and leak free even at the very high 380 °C oven temperature.