

## ► ANALYSIS OF POLYETHER ANTIBIOTICS IN ANIMAL FEEDS BY HPLC WITH POST-COLUMN DERIVATIZATION

Polyether Antibiotics are commonly used for preventing coccidiosis and other infections in poultry and for improving feed efficiency for beef cattle and swine. The use of Polyether Antibiotics is strictly regulated, with only specific ionophores approved for use in feeds intended for different animals.

Analysis of Polyether Antibiotics by HPLC with post-column derivatization and UV/Vis detection has been proven to successfully identify and quantify Monensin, Narasin and Salinomycin in medicated feeds, supplements and premixes as well as to determine trace contamination levels in non-medicated feeds [1, 2].

Post-column derivatization of Polyether Antibiotics is done using highly acidic Vanillin or DMAB reagents. The Pinnacle PCX derivatization system (Pickering Laboratories, Inc.) has an inert flow path and automated system wash capabilities that make it uniquely suitable for handling corrosive reagents. The two-pump system is recommended to extend reagent stability, but the single-pump system for this application is also available.

Adding a Fluorescence detector to the instrumentation allows for using the same extraction procedure and HPLC conditions to also determine Lasalocid, which doesn't require post-column derivatization.

### METHOD

#### Sample Preparation

To 25 g of finely ground feed sample, add 100 mL of extraction solution (90% Methanol - 10% water). Shake for 1 hour at high speed using mechanical shaker. Let the solids settle and filter an aliquot of the extract for injection. Dilute with extraction solution if needed to fit the calibration curve. Use 2.5 g portion when testing premixes.

#### Analytical conditions

*Analytical Column:* Polyether Column, C<sub>18</sub>, 4.6 x 250 mm, Catalog No 2381750

*Temperature:* 40 °C

*Flow Rate:* 0.7 mL/min

*Mobile Phase:* 90% Methanol, 10% of 5% Acetic Acid solution in water, isocratic

*Injection volume:* 20 µL

#### Post-Column Conditions

*Post-column System:* Pinnacle PCX

*Reactor Volume:* 1.4 mL

*Reactor Temperature:* 90 °C

*Reagent 1:* Concentrated Sulfuric acid / Methanol (4:96 v/v)

*Reagent 2:* 60 g of Vanillin in 950 mL of Methanol

*Reagents Flow Rate:* 0.3 mL/min

*Detection:* UV/VIS 570 nm (for Lasalocid – FLD, Ex. 322 nm, Em. 370 nm)

#### Calibration

*Monensin A:* 0.1 ppm – 50 ppm, R<sub>2</sub> = 0.999

*Monensin B:* 0.0035 ppm – 0.7 ppm, R<sub>2</sub> = 0.999

*Lasalocid Acid:* 0.25 ppm – 50 ppm, R<sub>2</sub> = 0.999

TABLE 1. POLYETHER ANTIBIOTICS IN CERTIFIED MEDICATED FEEDS

ANTIBIOTIC	FEED TYPE	CERTIFIED AMOUNT	FOUND IN SAMPLE	RECOVERIES	RSD, N=4
Monensin	Beef feed	267 g/ton	275 g/ton	103%	0.7%
Lasalocid	Milk Replacer	72 g/ton	69 g/ton	96%	3.3%

TABLE 2. SPIKE RECOVERIES FOR MONENSIN

Antibiotic	NON-MEDICATED BIRD FEED		NON-MEDICATED RABBIT FEED	
	Monensin A	Monensin B	Monensin A	Monensin B
Spike Level	172 g/ton	8 g/ton	86 g/ton	4 g/ton
Recoveries	100%	100%	101%	102%
RSD, N=3	1.9%	2.1%	1.1%	0.6%
Spike Level	3.44 g/ton	0.16 g/ton	3.44 g/ton	0.16 g/ton
Recoveries	96%	95%	94%	88%
RSD, N=3	0.7%	3.1%	0.9%	1.6%

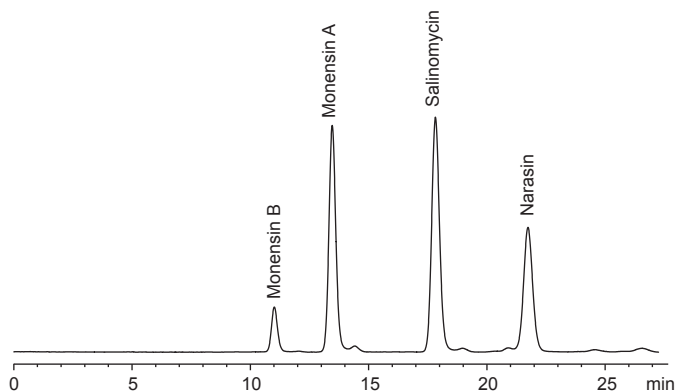


Fig. 1. Standard mixture of Monensin, Salinomycin and Narasin

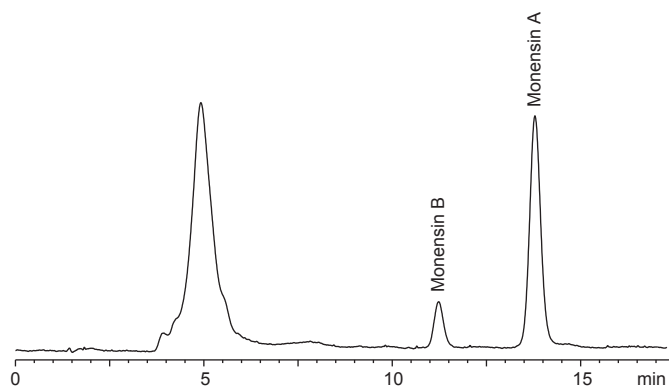


Fig. 3. Non-medicated bird feed sample spiked with Monensin A (3.44 ug/g) and Monensin B (0.16 ug/g)

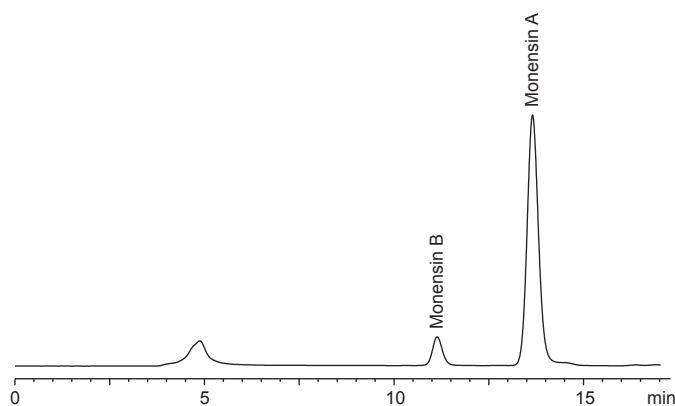


Fig. 2. Certified medicated beef feed sample containing 267 g/ton of Monensin

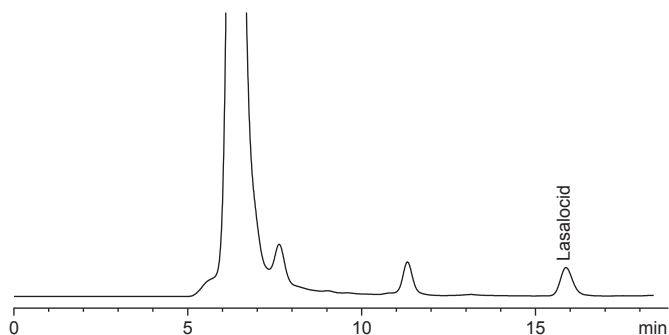


Fig. 4. Certified medicated milk replacer containing 72 g/ton of Lasalocid

### Conclusion

Analysis of Polyether Antibiotics by HPLC with Post-column derivatization is a robust and sensitive method that utilizes standard equipment and could easily be adopted by testing laboratories. It allows for testing of different ionophores at wide range of concentrations, including at trace levels. Using Pinnacle PCX post-column derivatization system, factory configured for the analysis, guarantees stable and reproducible results.

### REFERENCES

- 1) Campbell, H., Nayeri, G. (2006) J. AOAC Int. 89, 1229-1242, Determination of Monensin, Narasin, and Salinomycin in Mineral Premixes, Supplements, and Animal Feeds by Liquid Chromatography and Post-Column Derivatization: Collaborative Study
- 2) AOAC Official Method 997.04. Monensin in Premix and Animal Feeds