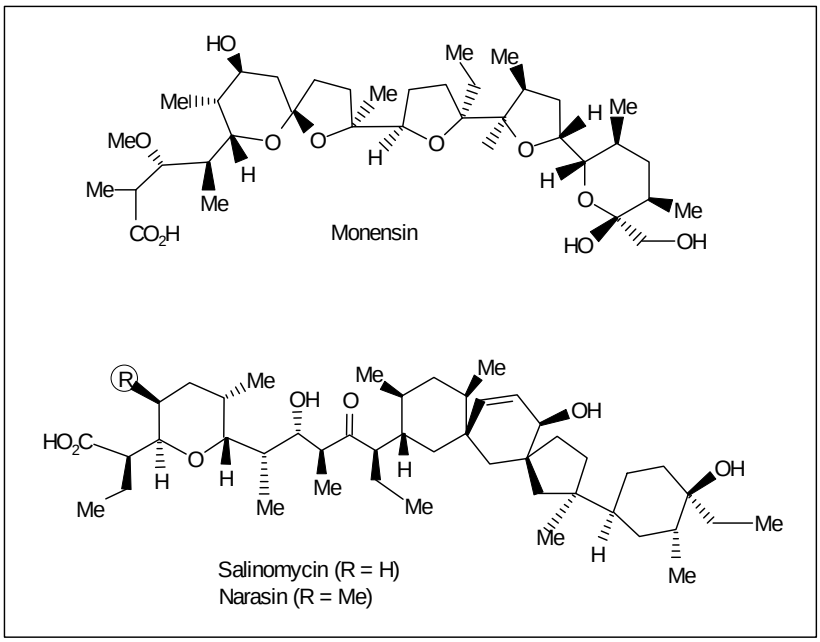
As of: April, 2008

POLYETHER ANTIBIOTICS

Monensin, narasin and salinomycin are substances belonging to the group of ionophore-antibiotics. As approved feed additives, these are used for the prevention of coccidiosis. Additionally, monensin and salinomycin carry a performance increasing effect.

For the detection of traces of most polyether antibiotics using HPLC, derivatization is indispensable. For this detection method, PICKERING offers the PINNACLE PCX post-column derivatization system. The derivatization reagents dimethylaminobenzaldehyde (DMAB, "Ehrlich's Reagent“) and vanillin are available from Pickering in a highly purified grade. The user, however, has to supply column, eluents and diluents.



Description of the Method

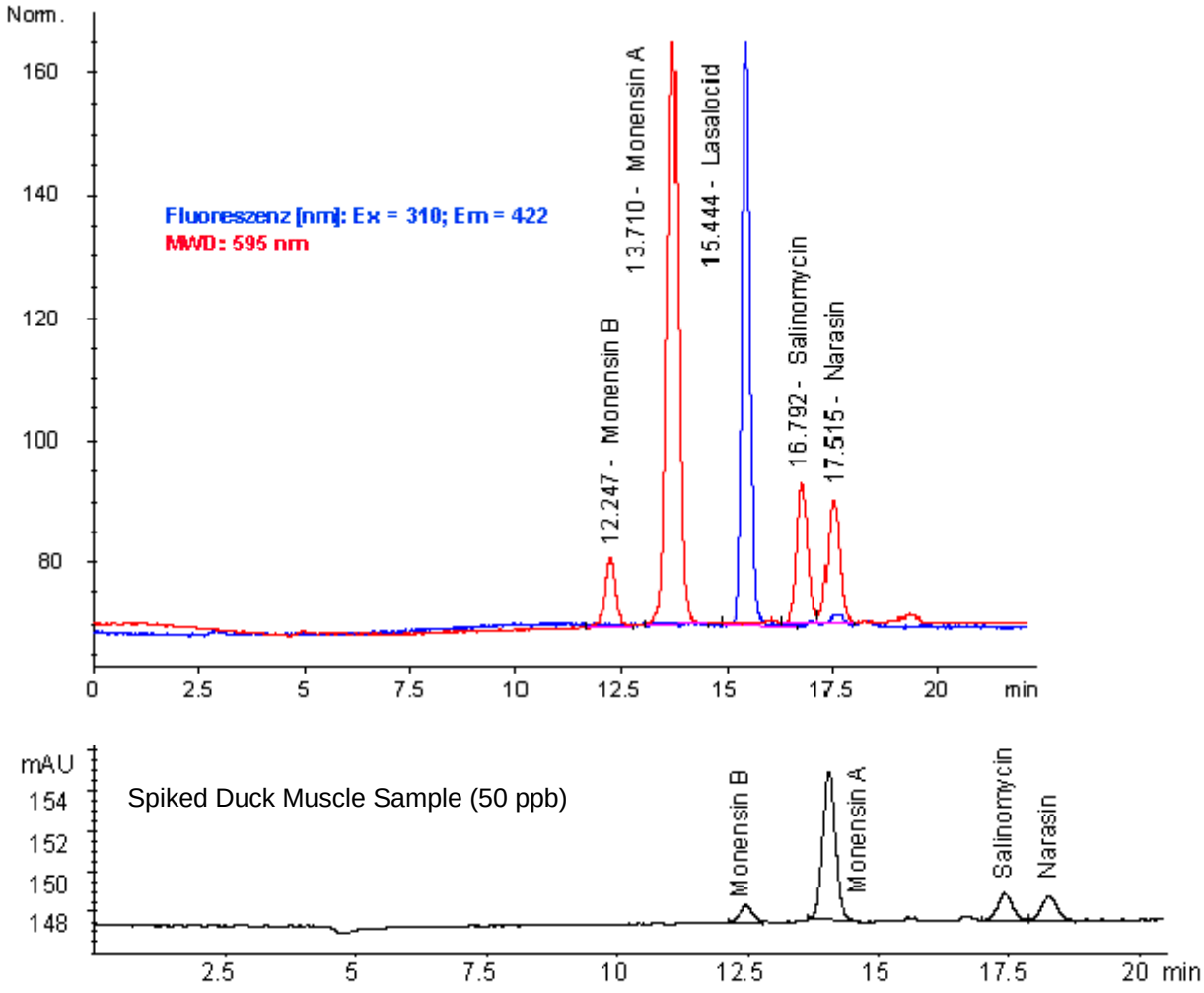
The derivatization of polyether antibiotics is performed in a one-step reaction using a methanol/sulphuric acid reagent solution. In this highly acidic environment, polyether antibiotics are hydrolysed and subsequently converted with DMAB or vanillin to colored derivatives at raised temperature. The sulphuric acid and the reagent solution should be added separately (two-step reaction), since the reagent solutions are stable for a longer period only, if they are separate (degradation occurs in a mixed solution after one day).

The use of DMAB for derivatization is preferable to the use of vanillin, because the absolute sensitivity is increased by ca. 30 % with DMAB.

Chromatograms

Chromatograms of a Standard (200 ng abs.) and a Spiked Duck Muscle Sample

Figure above shows also the line of a fluorescence detector which was installed **before** the derivatization unit to determine the lasalocid.



HPLC Conditions and Derivatization Parameters

HPLC	
Operation Mode	Isocratic
Eluent	Methanol/ 5 % acetic acid in water (90/10)
Degassing	Helium- or vacuum-degassed
HPLC Column	RP C18
Column Oven	40 °C
Flow Rate	0.7 mL/min
Post-Column Derivatization	
Pinnacle PCX	Dual-pump
1. Reactor	0.15 mL; RT
2. Reactor	1.4 mL (DMAB or vanillin) Reactor
Reactor Temperature 2	90 °C
Reagent 1	Conc. sulphuric acid/methanol (4/96; v/v)
Reagent 2	DMAB or vanillin in methanol (60 g in 950 mL)
Reagent Flow	0.3 mL/min
Detection	
Detection Type	UV/VIS detection
UV/VIS	DMAB 450 nm; vanillin 520 nm
Flowcell	Analytic; pressure stable up to 7 bar

Literature

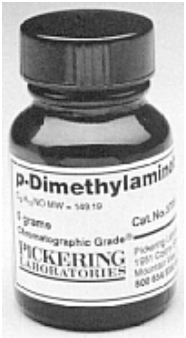
PICKERING LABORATORIES, application note 104.
J. T. Goras, W. R. Lacourse, *J. Assoc. Off. Anal. Chem*. **1984**, *67 (4)*, 701 – 706.
M. R. LaPointe, H. Cohen, *J. Assoc. Off. Anal. Chem.* **1988**, *71*, 480 – 484.
F. H. Johannsen, *Agribiological Research* **1991**, *44 (1),* 79 - 89.
J. M. Rodewald, J. W. Moran, A. L. Donoho, M. R. Coleman, *J. AOAC International* **1992**, *75 (2),* 272 – 279.
J. W. Moran, J. M. Turner, M. R. Coleman, *J. AOAC International* **1995** *78 (3)*, 668 - 673.

Order Information

Order Number	Description
1153-1082	PINNACLE PCX – dual-pump; 1.4 mL (DMAB or vanillin) reactor
2381750	Polyether RP C18 column
18ECG001	Guard cartridge holder with 3 guard cartridges

Chemicals and Columns

p-Dimethylaminobenzaldehyd (DMAB; Ehrlich's Reagent)



Low baseline noise for optimum sensitivity.

p-Dimethylaminobenzaldehyde reacts rapidly in an acidic environment with polyether antibiotics and sulfonamides to form colored complexes with maximum absorbance 450 nm.

Order Number	Description
3700-0400	p-Dimethylaminobenzaldehyde, *„Chromatographic Grade™“*, 5 g

Vanillin

When formulated as a reagent with sulphuric acid in methanol, this chemical permits post-column detection of polyether antibiotics such as monensin, narasin, and salinomycin at 520 nm. Additionally, Sulfonamides can be detected at 400 nm.

Order Number	Description
3700-2200	Vanillin, (4-Hydroxy-3-methoxybenzaldehyde), *„Chromatographic Grade™“*, 30 g; stored under CO_2