SHIMADZU APPLICATION NEWS

LAAN-A-LC-E057A

● HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

No.L338A

Determination of Polyether Antibiotics in Animal Feeds (Part 1) Analysis by Post-column VIS Photometric Derivatization Method

Five kinds of polyether antibiotics, including salinomycin sodium, are specified as feed additives based on the 750th notification of Japan's Ministry of Agriculture, Forestry and Fisheries, for addition to the feeds of domestic cattle and poultry to promote growth through the effectiveness of the nutritional content of these feeds. Analysis of these compounds in feed is conducted using microbiological quantitation methods and HPLC methods, as listed in the “Feed Analysis Standard”. HPLC allows more rapid analysis compared with microbiological quantitation methods, with post-column derivatization and VIS detection specified for four of the compounds and fluorometric detection specified for one of the compounds.
Here we introduce an example of analysis of salinomycin sodium (SL), monensin sodium (MN), narasin (NR) and senduramicin sodium (SD) using the post-column derivatization VIS method, as specified.

■ Principle of Detection Method

In this method, the four polyether antibiotic substances (Fig.1) are separated using reverse-phase chromatography, then reacted in hot sulfuric acid-methanol with vanillin (4-hydroxy-3-methoxybenzaldehyde) (Komarowsky coloration reaction), and VIS detection is conducted at 520 nm. The system flow diagram for this method is shown in Fig.2, and the analytical conditions are shown in Table 1. For the reaction coil, a 5 m coil was used for SL, MN and NR, and a 10 m coil was used for SD.

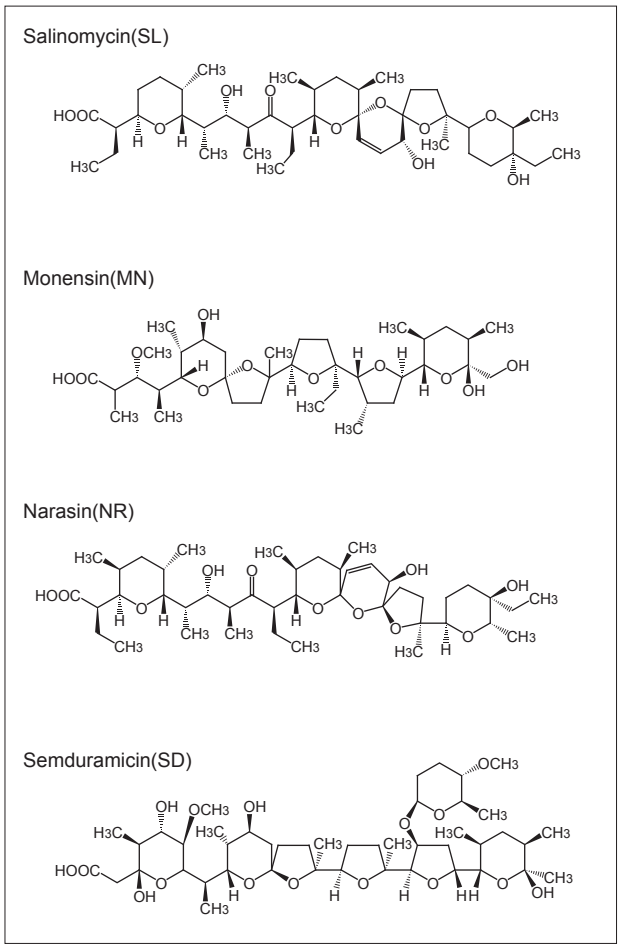


Fig.1 Structure of Four Polyether Antibiotics

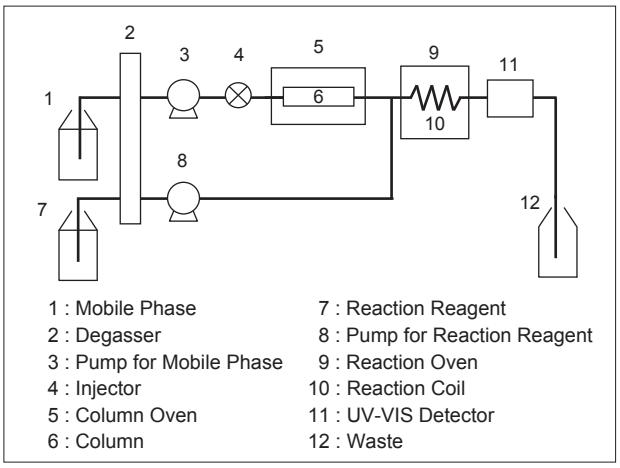


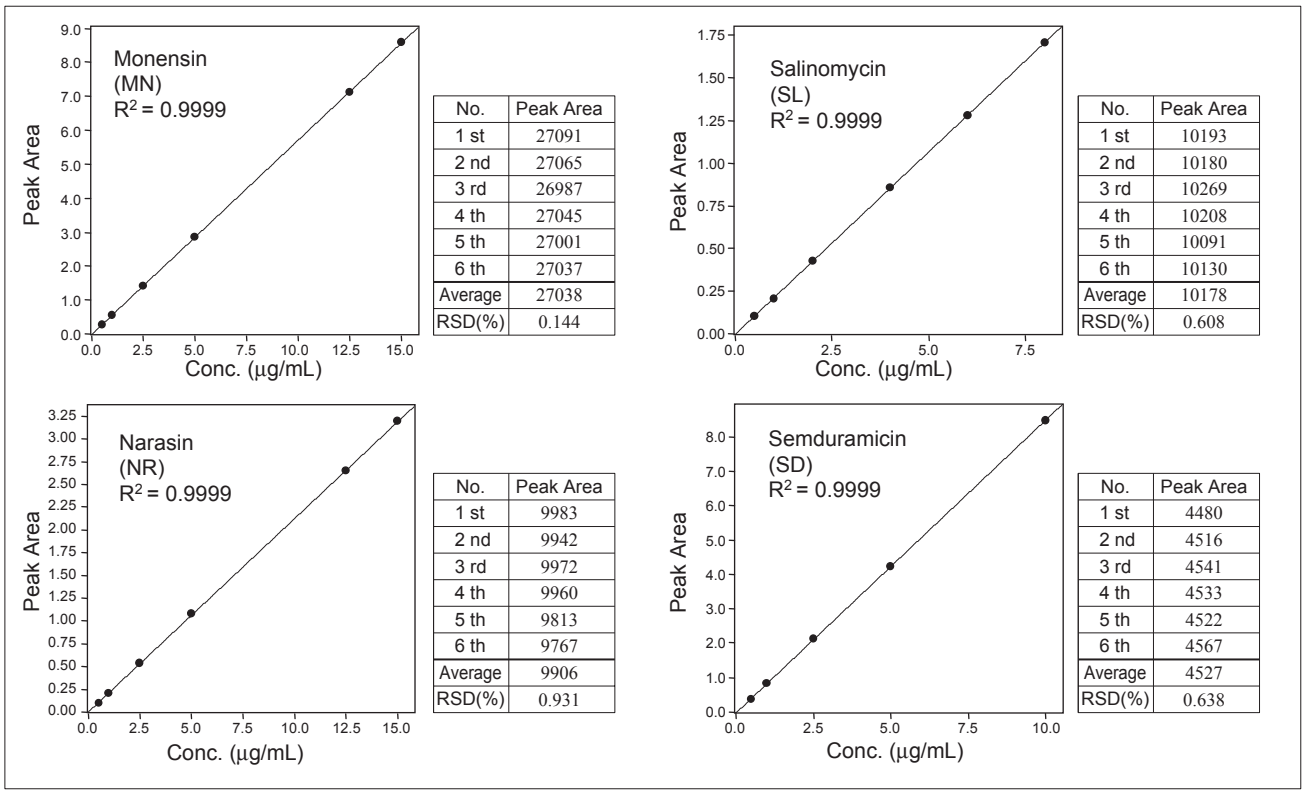
Fig.2 Flow Diagram

Table 1 Analytical Conditions

<Separaition Condition>	
Column	: Shim-pack VP-ODS (150 mmL. × 4.6 mmI.D.)
Mobile Phase	: Water / Methanol / Acetic acid =60 mL / 940 mL / 1 mL (v / v / v)
Flow Rate	: 0.6 mL/min
Column Temp.	: 40 ˚C
<Detection Condition>	
Reaction Reagent	: Methanol / Sulfuric acid / Vanillin =95 mL / 2 mL / 3 g (v / v/ w)
Flow Rate	: 0.6 mL/min
Reaction Temp.	: 95 ˚C
Reaction Coil	: 5 m.L (SL · MN · NR) or 10 m.L (SD) × 0.5 mmI.D.
Detection	: SPD-20AV at 520 nm (W Lamp)

■ Linearity and Repeatability

Fig.3 shows the calibration curve (horizontal axis: μg /mL (through titration)) for each compound generated within the concentration range specified in the “Feed Analysis Standard”, in addition to the peak area repeatability (n=6) for each standard solution (0.5 μg/mL (through titration)).



No.	Peak Area
1 st	27091
2 nd	27065
3 rd	26987
4 th	27045
5 th	27001
6 th	27037
Average	27038
RSD(%)	0.144

No.	Peak Area
1 st	10193
2 nd	10180
3 rd	10269
4 th	10208
5 th	10091
6 th	10130
Average	10178
RSD(%)	0.608

No.	Peak Area
1 st	9983
2 nd	9942
3 rd	9972
4 th	9960
5 th	9813
6 th	9767
Average	9906
RSD(%)	0.931

No.	Peak Area
1 st	4480
2 nd	4516
3 rd	4541
4 th	4533
5 th	4522
6 th	4567
Average	4527
RSD(%)	0.638

Fig.3 Linearity and Repeatability of Peak Areas

■ Separation of Standard Solutions

Fig.4 shows the chromatographic results obtained following a 20 μL injection of the SL, MN and NR standard solution (1 μg/mL each (through titration)). The length of the reaction coil was 5 m. Fig.5 shows the results following a 20 μL injection of the SD standard solution (2.5 μg/mL (through titration)). In this case, a 10 m reaction coil was used.

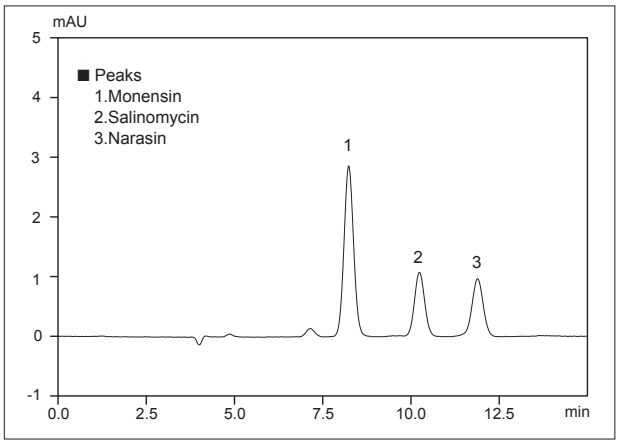


Fig.4 Chromatogram of Standard Mixture of Monensin, Salinomycin and Narasin (1 μg/mL each, 20 μL Inj.)

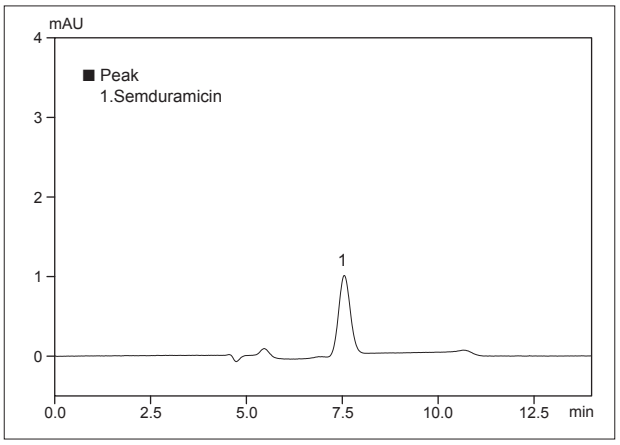


Fig.5 Chromatogram of Semduramicin (2.5 μg/mL, 20 μL Inj.)

[References] Feed Analysis Methods, Description (2004), Japan Scientific Feeds Association

NOTES:

*This Application News has been produced and edited using information that was available when the data was acquired for each article. This Application News is subject to revision without prior notice.

SHIMADZU

SHIMADZU CORPORATION. International Marketing Division

3. Kanda-Nishikicho 1-chome, Chiyoda-ku, Tokyo 101-8448, Japan Phone: 81(3)3219-5641 Fax. 81(3)3219-5710

Cable Add.:SHIMADZU TOKYO

Printed in Japan 3100-02702-10A-IK