SHIMADZU
Excellence in Science

Application News

Nexera™ lite High Performance Liquid Chromatograph/CRB-40

Analysis of Polyether Antibiotics in Animal Feeds Using Post-Column Derivatization

E. Ando, H. Terada

User Benefits

- Three polyether antibiotics can be stably quantified over a wide concentration range in accordance with "Feed Analysis Standards".
- Rapid analysis can be performed with easy operation compared to the microbiological quantification method listed in "Feed Analysis Standards".
- Significant labor-saving and improved efficiency of analytical procedure can be achieved by automated entire analytical process from instrument startup to shutdown.

■ Introduction

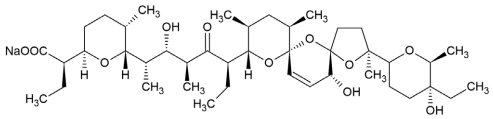
Polyether antibiotics are designated as feed additives in accordance with the Public Notice oNo.750[(1)] by the Japanese Ministry of Agriculture, Forestry and Fisheries (MAFF). For the analytical methods of these antibiotic components, microbiological quantification, HPLC, and LC/MS methods are specified in "Feed Analysis Standards," the Notice by the Director-General, Food Safety and Consumer Affairs Bureau, MAFF.[(2)] While the microbiological quantification method requires long pretreatment such as culturing test bacteria for 16 to 24 hours, the HPLC method can quickly obtain results with an average pretreatment time of 20 minutes.

This article introduces a analysis of salinomycin sodium (SL), monensin sodium (MN) and narasin (NR), specifically designated to apply post-column VIS photometric derivatization using HPLC.

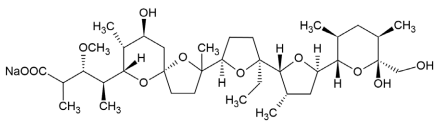
■ Principle of Detection Method

Fig. 1 shows the structural formulas of the three components targeted in this study. Each component is separated by reversed-phase chromatography, and thermally reacted (Komarowsky reaction) with vanillin (4-hydroxy-3-methoxybenzaldehyde) shown in Fig. 2 in sulfuric acid-methanol to produce a derivative with maximum absorption near 520 nm for detection.

Salinomycin (sodium salt)



Monensin (sodium salt)



Narasin

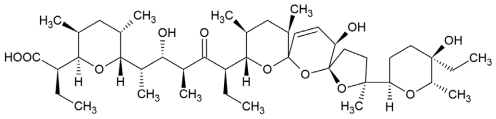


Fig. 1 Structural Formulas of Three Polyether Antibiotics

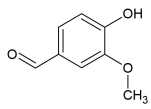


Fig. 2 Structural Formula of Vanillin

■ Analytical Instrument and Conditions

Fig. 3 shows the system flow path, and Table 1 shows the analytical conditions. The reaction reagent is delivered using an inert-type pump since it contains sulfuric acid. The chemical reaction box has an air circulation system to achieve uniform temperature control. This feature enables a stable derivatization reaction to occur in the reaction coil, thus ensuring highly repeatable analysis. Furthermore, this system can provide fully automated analytical procedure from startup to shutdown of the instrument.

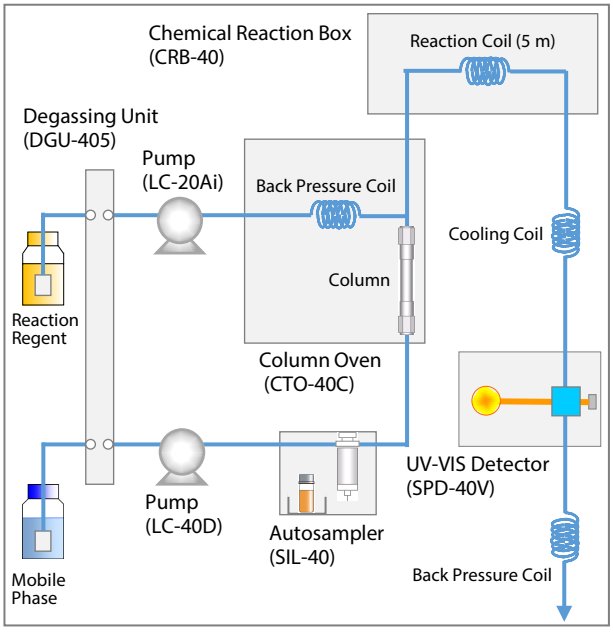


Fig. 3 Flow Diagram

Table 1 Analytical Conditions

<Separation>	
System	: Nexera lite
Column	: Shim-pack Scepter™ C18-120 (150 mm × 4.6 mm I.D., 5 µm)*1
Mobile Phase	: Water / Methanol / Acetic acid = 60 : 940 : 1 (v : v : v)
Flow Rate	: 0.6 mL/min
Column Temp.	: 40 ˚C
Vial	: SHIMADZU LabTotal™ for LC 1.5 mL, Glass*2
<Post-column reaction>	
Reaction Reagent	: Methanol / Sulfuric acid / Vanillin = 95 : 2 : 3 (v : v : w)
Flow Rate	: 0.6 mL/min
Reaction Temp.	: 95 ˚C
Reaction Coil	: 5 m × 0.5 mm I.D.
Detection	: SPD-40V (Inert-cell*3) at 520 nm (Lamp: W)

*1 P/N: 227- 31020-05 *2 P/N: 227-34001-01
*3 P/N: 228-64728-42

■ Calibration Curve Linearity and Area Repeatability

The minimum concentration of the standard solution for animal feed analysis listed in “Feed Analysis Standards” has been specified as 0.5 µg (potency)/mL. Figs. 4, 6, and 8 show the chromatograms obtained by injecting 20 µL of 0.25 µg (potency)/mL solution, one half of the specified concentration. Figs. 5, 7, and 9 show the calibration curves of the respective components created within the range including the concentrations listed in "Feed Analysis Standards". The red dots indicate the values converted from the standard reference values[3] for each component based on the pretreatment of the feeds listed in "Feed Analysis Standards". Good linearity was obtained for all components, with a coefficient of determination (r^2) greater than 0.999. Tables 2, 3, and 4 show the average and repeatability (%RSD) of the retention time and peak area of each component from six times consecutive analyses, respectively. Good repeatabilities were obtained even at low concentrations, indicating a reliable system performance.

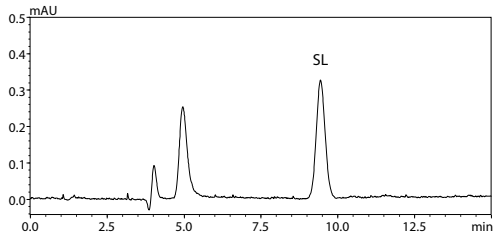


Fig. 4 Chromatogram of Salinomycin Sodium

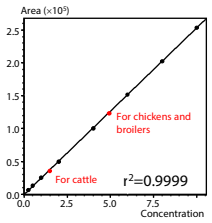


Fig. 5 Calibration Curve (Concentration: 0.25-10 µg (potency)/mL)

Table 2 Repeatability (0.25 µg (potency)/mL, n=6)

	Average	%RSD
Retention time	9.44	0.04
Area	6448	1.00

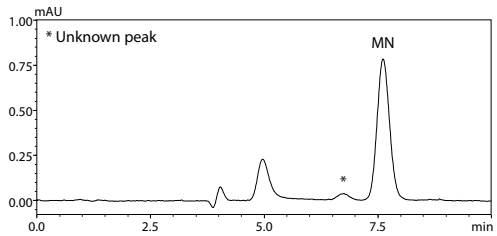


Fig. 6 Chromatogram of Monensin Sodium

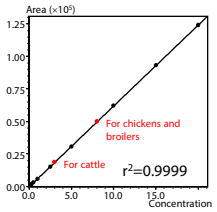


Fig. 7 Calibration Curve (Concentration: 0.25-20 µg (potency)/mL)

Table 3 Repeatability (0.25 µg (potency)/mL, n=6)

	Average	%RSD
Retention time	7.62	0.05
Area	14906	0.47

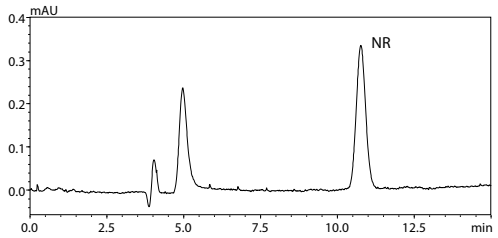


Fig. 8 Chromatogram of Narasin

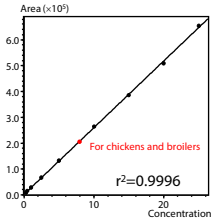


Fig. 9 Calibration Curve (Concentration: 0.25-25 µg (potency)/mL)

Table 4 Repeatability (0.25 µg (potency)/mL, n=6)

	Average	%RSD
Retention time	10.77	0.05
Area	7074	1.02

■ Analysis of Animal feeds

SL, MN, and NR were added to the extract of bird feeds prepared according to the procedure in "Feed Analysis Standards" at the respective concentrations of 0.5 µg (potency)/mL, the lowest concentration in the calibration range. Fig. 10 shows the chromatogram obtained by injecting 20 µL of this solution. Table 5 shows the results of the spike-and-recovery test, as well as the resolution (average of three analyses) among the unknown peak (*), MN, SL, and NR peaks. Good results were obtained for the resolution, greater than 1.5 for each peak, as well as the spike recovery rate.

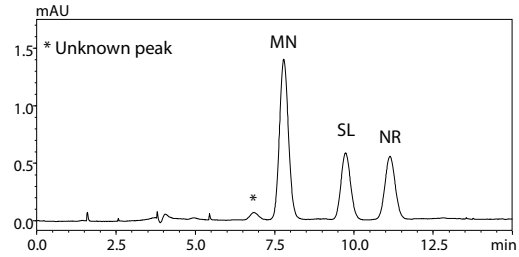


Fig. 10 Chromatogram of Bird Feed Extract with Three Polyether Antibiotics

Table 5 Resolution and Spike Recovery Rate

	Resolution	Spike recovery rate (%)
MN	1.93*1	103.3
SL	3.60	100.6
NR	2.45	104..6

*1 Resolution between MN and unknown peak

■ Conclusion

This article introduced the analysis of three polyether antibiotics using the post-column derivatization in accordance with "Feed Analysis Standards". Using the system configurated by Nexera lite, it is possible to quickly obtain reliable quantitative results over a wide concentration range. Furthermore, fully automated analytical procedures from startup to shutdown contributes to improved work efficiency.

<References>
(1) Establishing Feed Additives Based on the Provisions of the Act on Safety Assurance and Quality Improvement of Feed (Public Notice of the MAFF No. 750 of July 24, 1976)
(2) Feed Analysis Standards (Notice from the Director-General, Food Safety and Consumer Affairs Bureau, MAFF No. 19/14729 of April 1, 2008)
(3) Ministerial Ordinance on the Specifications and Standards of Feeds and Feed Additives (Ordinance No. 35 of July 24th, 1976 of the MAFF)

01-00127-EN First Edition: June 2022

Shimadzu Corporation
www.shimadzu.com/an/