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Application News

Nexera™ lite High Performance Liquid Chromatograph/RF-20Axs

Analysis of Polyether Antibiotics in Animal Feeds Using Fluorescence Detection

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User Benefits

- The feed additive lasalocid sodium can be analyzed with good repeatability over a wide range of concentrations 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 No.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)]

However, the microbiological quantification method requires a long time to obtain results due to the complicated process of test organism storage and culture media preparation, along with 16 to 24 hours of cultivation. On the other hand, HPLC method can provide rapid analysis because it does not use microbes that require long term storage and/or cultivation.

In this article, we introduce a analysis of lasalocid sodium, for which the fluorescence detection method is specified.

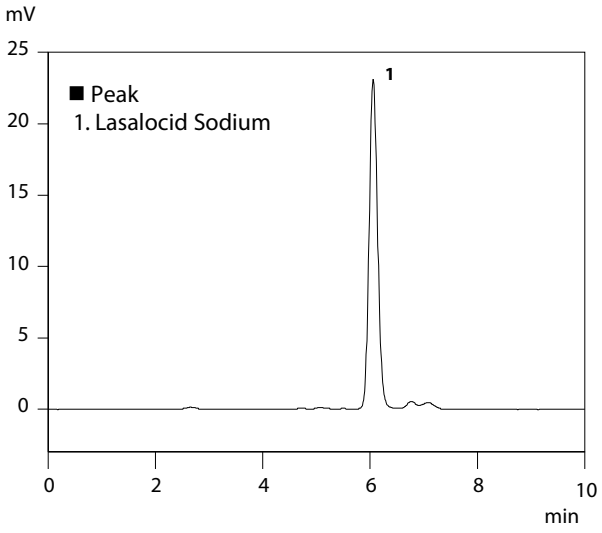

Fig. 2 Chromatogram of Lasalocid Sodium

■ Analysis of Standard Sample

Fig. 1 shows the structural formula of lasalocid sodium. Since lasalocid sodium has natural fluorescence, it can be easily measured using a fluorescence detector after chromatographic separation from other co-existing interferences. Furthermore, this system can provide fully automated analytical procedure from startup to shutdown.

Fig. 2 shows the chromatogram obtained by injecting 20 µL of 0.1 µg (potency)/mL lasalocid sodium standard solution, and Table 1 shows the analytical conditions. Rapid analysis requiring less than ten minutes for one analysis can be done.

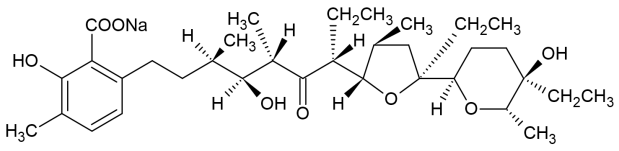

Fig. 1 Structural Formula of Lasalocid Sodium

Table 1 Analytical Conditions

System	: Nexera lite
Column	: Shim-pack Scepter™ C18-120 (250 mm × 4.6 mm I.D., 5 µm)*1
Mobile Phase	: Phosphate Buffer*2 / Methanol = 1 : 9 (v : v)
Flow Rate	: 1.0 mL/min
Column Temp.	: 40 °C
Injection Vol.	: 20 µL
Vial	: SHIMADZU LabTotal™ Vial for LC 1.5 mL, Glass*3
Detection	: RF-20Axs Ex: 310 nm, Em: 420 nm

*1 P/N: 227-31020-06
*2 Dissolve 2.72 g of KH_2PO_4 in 1000 mL of water and then adjust its pH within 2.9 to 3.1 by adding H_3PO_4 solution
*3 P/N: 227-34001-01

■ Linearity

The "Feed Analysis Standards" specifies to create a calibration curve based on the chromatogram obtained by injecting 20 µL of 1 to 15 µg (potency)/mL lasalocid sodium standard solution. Fig. 3 shows the calibration curve prepared in accordance with this documentation. Good linearity was obtained with a coefficient of determination (r^2) greater than 0.999.

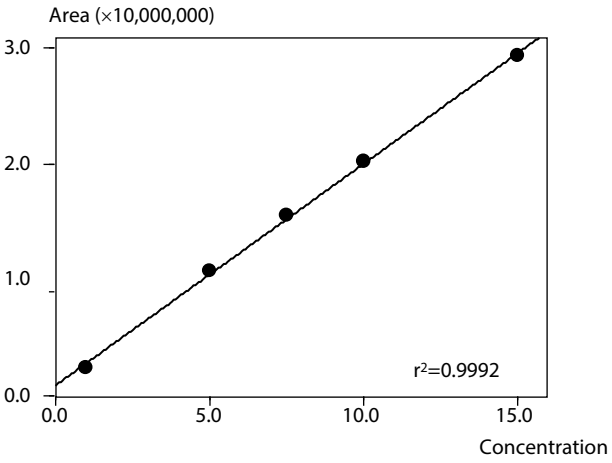

Fig. 3 Calibration Curve of Lasalocid Sodium Standard Solution
(Concentration: 1-15 µg (potency)/mL)

■ Repeatability

The minimum concentration of the standard solution for animal feed analysis specified in "Feed Analysis Standards" is 1 µg (potency)/mL. Six times consecutive analyses of 20 µL of 0.1 µg (potency)/mL solution, which is equivalent to one-tenth of the specified minimum concentration were carried out. Table 2 shows the retention times, peak areas, and their respective averages and repeatabilities (%RSD). Good repeatabilities were obtained even at low concentrations, indicating that reliable analysis can be performed over a wide range of concentrations.

Table 2 Repeatability of Retention Time and Peak Area of 0.1 µg (potency)/mL Standard Solution

No.	Retention time	Peak area
1st	6.059	252485
2nd	6.060	251855
3rd	6.059	251517
4th	6.060	252350
5th	6.059	252333
6th	6.059	250069
Average	6.059	251768
RSD(%)	0.009	0.361

■ Analysis of Animal Feeds

The maximum allowable amount of lasalocid sodium as a feed additive has been specified as 75 g (potency)/t for chickens (including broilers) by the "Ministerial Ordinance on the Specifications and Standards of Feeds and Feed Additives"[3] established by the MAFF. Therefore, we investigated the possibility of separation and quantitation in bird feeds using HPLC.

In accordance with the feed extraction procedure described in "Feed Analysis Standards" shown in Fig. 4, lasalocid sodium was added to 10.0 g of bird deed to make the specified concentration to check the resolution and spike recovery rate. following above mentioned procedure, the concentration of lasalocid sodium in the solution should be 7.5 µg (potency)/mL. Fig. 5 shows the chromatogram obtained when injecting 20 µL of this solution and Table 3 shows the spike recovery rate. We were able to successfully separate and quantify lasalocid sodium without any influence of co-existing interferences.

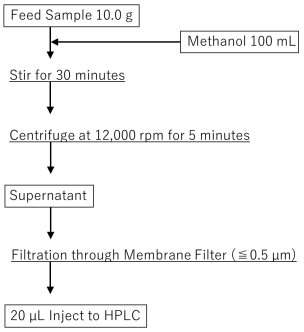

Fig. 4 Feed Extraction Procedure

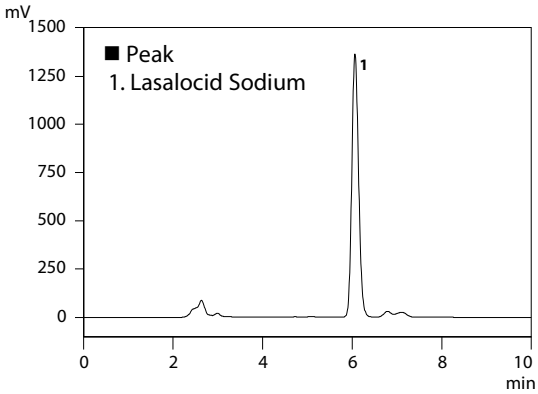

Fig. 5 Chromatogram of Extract of Bird Feeds with Lasalocid Sodium

Table 3 Spike Recovery Rate of Lasalocid Sodium

Concentration in feeds	Spike recovery rate
75 g (potency)/t	96.9%

■ Conclusion

This article introduced the analysis of lasalocid sodium, a polyether antibiotic, in animal feed using the Nexera lite equipped with a fluorescence detector. The HPLC method can provide rapid analysis requiring only less than 10 minutes per analysis with good repeatability over a wide range of concentrations. Furthermore, fully automated analytical procedures from startup to shutdown contributes to improved operational 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)

01-00128-EN First Edition: June 2022

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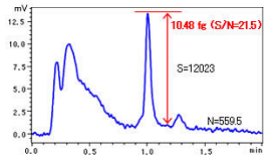

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