

Analysis of Polyether Antibiotics in Animal Feed

Polyether antibiotics sodium salinomycin and sodium monensin enhance the effectiveness of nutrients contained in animal feed, and the Ministry of Agriculture, Forestry, and Fisheries designates these antibiotics as feed additives. These components in poultry and cattle feed have been conventionally analyzed in microbiological quantification methods in accordance with the Animal Feed Analysis Standards. However this method requires two days for obtaining the results. Therefore, rapid quantification methods have been demanded.

Responding to this situation, the Animal Feed Analysis Standards were revised on April 10, 2002 to employ LC post-column derivatization method for the analysis of sodium salinomycin and sodium monensin. The Shimadzu LC-VP Polyether Antibiotic Analysis System is an application system conforming to the Animal Feed Analysis Standards that selectively detects sodium salinomycin and sodium monensin in animal feed. This article introduces the principles of this system and an example of application.

■ Detection Method

Sodium salinomycin and sodium monensin thermally react with vanillin (4-hydroxy-3-methoxybenzaldehyde) to form a colored complex in sulfuric acid-methanol. This reaction is commonly

known as Komarowsky reaction. This post-column derivatization system uses this reaction. This system also analyzes polyether antibiotics naracin and semduramicin.

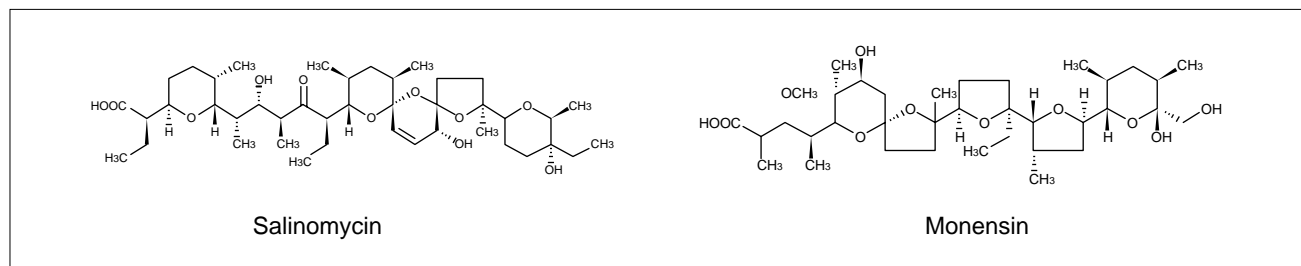


Fig.1 Structure of Salinomycin and Monensin

Fig. 2 shows a schematic of this system. Vanillin reagent is added continuously to polyether antibiotics separated by the reversed-phase column and, after thermal reaction at 95°C in the reaction bath, detected by the UV-visible absorption detector (520nm).

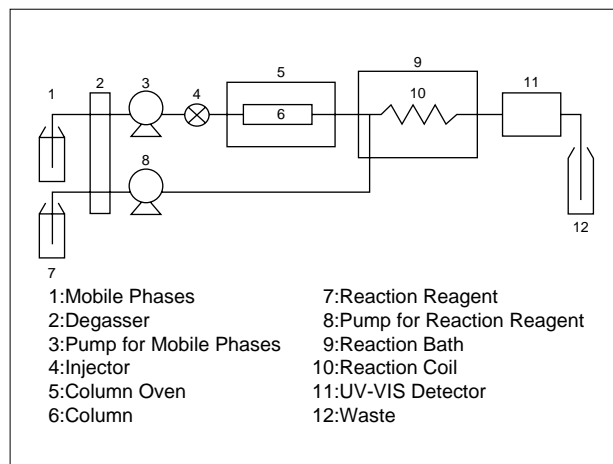


Fig.2 Flow Diagram

■ Separation of Standard Mixture

Fig. 3 shows a chromatogram of the standard mixture* of sodium salinomycin, sodium monensin and narasin. The sample concentrations are 10mg/L each (solution of methanol/water = 9/1) and 10 μ L were injected. Target substances were sharply separated under the analytical conditions shown at the right.

* Common standard products at Fertilizer and Feed Inspection Station (Japan).

Table 1 Analytical Conditions

Column	:Shim-pack FC-ODS (150mmL \times 4.6mmI.D.)
Mobile Phase	:Methanol/Water/Acetic Acid=940/60/1(v/v/v)
Flow Rate	:0.6 mL/min
Column Temp.	:40°C
Reaction Reagent	:Methanol/Sulfuric Acid/Vanillin=95/2/3(v/v/w)
Reaction Temp.	:95°C
Detection	:VIS at 520nm

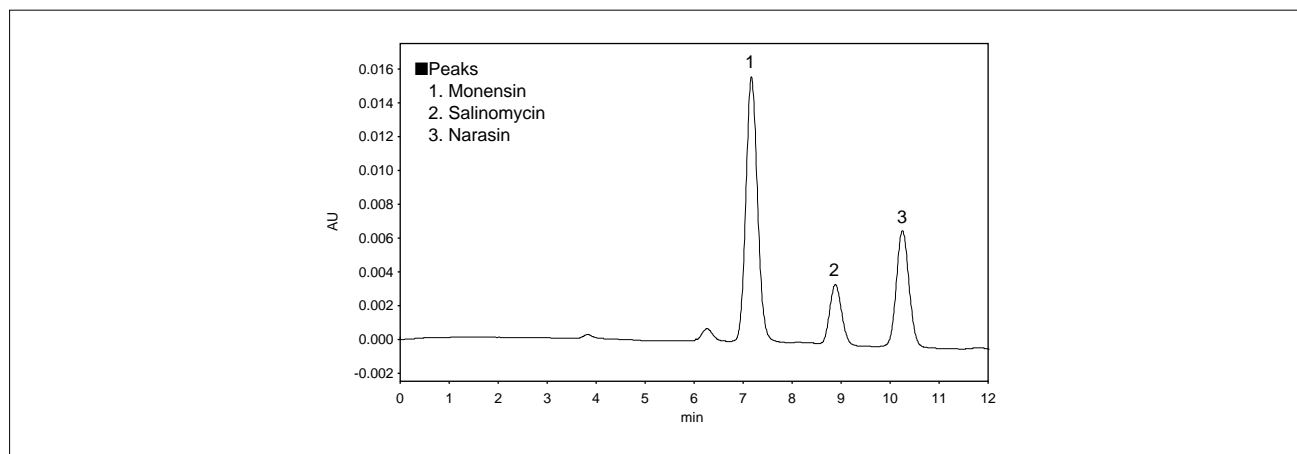


Fig.3 Chromatogram of Standard Mixture

■ Analysis of Feed Sample

Fig. 4 shows a chromatogram of animal feed sample with sodium monensin additive. The feed was extracted by agitation in a methanol/water=9/1 solution

and, after filtration, 10 μ L were injected. The same extraction method can be used for feed with sodium salinomycin and narasin.

Table 2 Pretreatment

Feed	10g
	← Methanol/Water=9/1(v/v) 100mL
Stir	20min
Filtration	
Inject	10 μ L

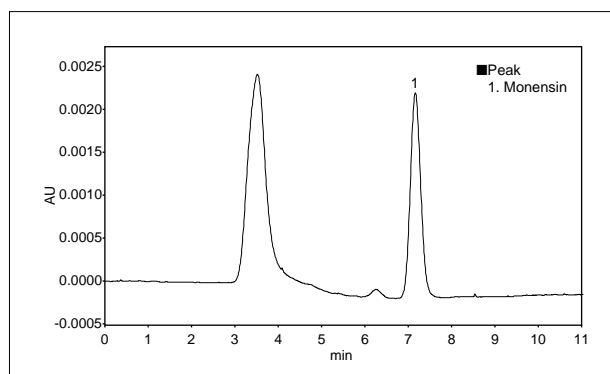


Fig.4 Chromatogram of Feed Sample

References

- 1) Toshiaki Hayakawa, Masahito Funatsu: Animal Feed Research Reports, Vol. 26, P 51-59 (2001)
- 2) Toshiaki Hayakawa, Daisaku Makino: Animal Feed Research Reports, Vol. 26, P 60-68 (2001)
- 3) Toshiaki Hayakawa: LCTalk, Vol. 46, p. 3 (2002)



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