Improvement of Chemical Analysis of Antibiotics. 21.[†] Simultaneous Determination of Three Polyether Antibiotics in Feeds Using High-Performance Liquid Chromatography with Fluorescence Detection

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A simultaneous determination of three polyether antibiotics (PEs), salinomycin, monensin, and lasalocid, in feeds has been established by high-performance liquid chromatography with a precolumn reaction system using 1-(bromoacetyl)pyrene as a fluorescence reagent. PEs were extracted from feeds with acetonitrile and cleaned up using prepacked silica gel cartridges to eliminate coexisting materials such as fatty acids. An addition of triethylamine into the reaction solution was essential to derivatize efficiently. The separation was performed on Develosil 5 C_{18} (5 μm, 250 × 4.6 mm) with methanol/water (97:3) as a mobile phase, and the separated PEs were detected at λ_x 360 nm and λ_m 420 nm. The internal standard, 18,19-dihydrosalinomycin, was effective for the simultaneous determination of PEs, and the recoveries of PEs from various feeds fortified at levels from 25 to 100 ppm were 95–100%.

INTRODUCTION

Three polyether antibiotics (PEs; Figure 1), salinomycin (SL), monensin (MN), and lasalocid (LA), are structurally characterized as sodium salts of a carboxylic acid ionophore and a number of cyclic ether moieties, which show characteristic biological properties. These compounds have been used for poultry as feed additives to prevent coccidiosis as well as growth promoters for cattle to increase feed conversion efficiency (Westley, 1982). SL, MN, and LA have been administered to poultry at concentrations of 50, 80, and 75 ppm, respectively, since 1978 in Japan. Although microbiological assays have been adopted as official analysis methods of these compounds in Japan (Abe and Kono, 1980; Kusama, 1986; Sugano, 1984), they are complicated, time-consuming and nonspecific. The development of a simple, rapid, and specific method has been desirable for the determination of PEs in feeds.

A typical chemical analysis for SL and MN is a colorimetry in which vanillin is used as a reagent to form a colored complex with these compounds. The reaction mechanism was first proposed by Duke in 1947. It has not been completely understood yet, but vanillin has been frequently used for feed analysis using colorimetry (Golab *et al.*, 1973; Kono and Yamamoto, 1979; Marten, 1982; Kozak, 1984; Suhara, 1986; Oyama, 1986; Illing and Mueller, 1986; Sokolic and Pokorny, 1991), spectrodensitometric methods (Koufidis, 1976; Owles, 1984; Kovacs-Hadady and Kupas, 1984), and flow injection analysis (Suzuki *et al.*, 1987). These methods are not suitable in feed analysis, because Suhara (1986) and Oyama (1986) reported that the blank values determined by colorimetry were 4 and 1.8–5.6 ppm for SL and MN in various feeds, respectively.

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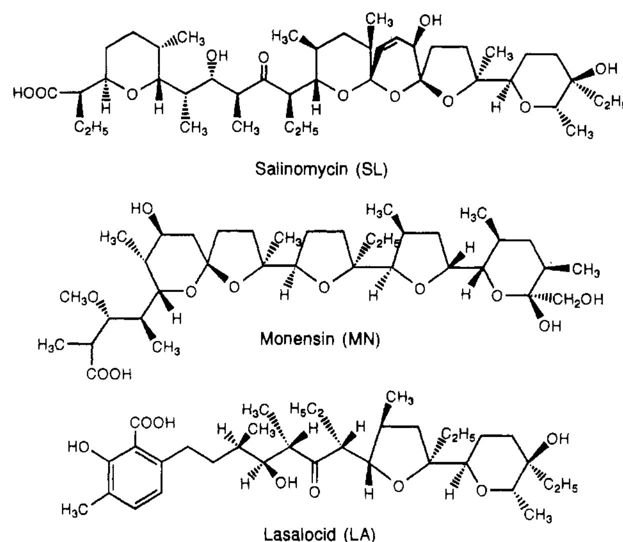

Figure 1. Anticoccidial polyether antibiotics.

Since Goras and Lacourse (1984) reported a HPLC determination of SL in feeds using a postcolumn derivatization detection method with vanillin, Blanchflower *et al.* (1985) and Lapointe and Cohen (1988) reported successively the simultaneous HPLC determination of SL, MN, and narasin (4-methylsalinomycin) in feeds using this technique. However, LA is less sensitive to vanillin reagent than SL and MN (Marvin and Gerald, 1978), so it is difficult to simultaneously determine PEs including LA in feeds by the post-column derivatization system. Gamoh and Okada (1988; Okada and Gamoh, 1989) reported the high-performance liquid chromatographic methods with fluorescence detection of MN in feeds using derivatization with 9-anthryldiazomethane. Because LA has a unique cresotic acid moiety showing significant fluorescence in various organic solvents, a fluorometric method has been established for the analysis of LA and has been applied to the analysis in bulk, fermentation broth, premixes, feeds, and biological samples (Osadca

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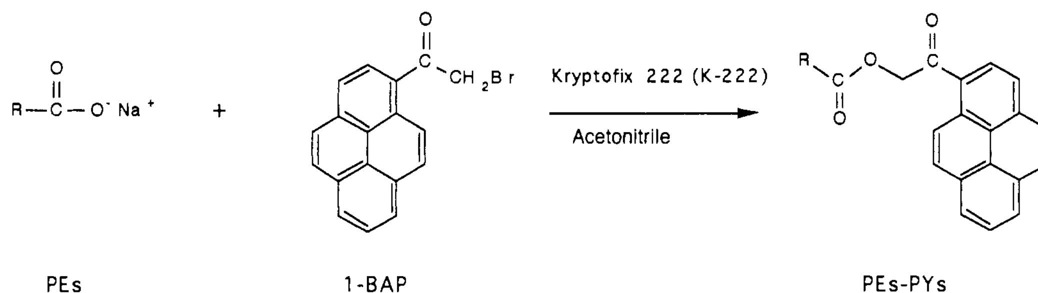


Figure 2. Derivatization of polyether antibiotics with 1-(bromoacetyl)pyrene.

and Araujo, 1974, 1975, 1978; Brooks *et al.*, 1975; Weiss *et al.*, 1983; Kaykaty and Weiss, 1983; Newkirk and Barnes, 1989; Frank and Barnes, 1989). However, no simultaneous HPLC determination method of SL, MN, and LA has been reported using fluorometric detection.

In our previous papers, we established techniques for the simultaneous determination of PEs using silica gel and RP-18 high-performance thin-layer chromatography (HPTLC) followed by fluorodensitometry (Asukabe *et al.*, 1987) and high-performance liquid chromatography (HPLC) with fluorescence detection (Asukabe *et al.*, 1993) using 1-(bromoacetyl)pyrene (1-BAP) as a fluorescence reagent and Kryptofix 222 (K-222) as a catalyst (Figure 2). Application of these methods to a simultaneous determination of PEs in feeds was planned, and we chose the HPLC method with fluorescence detection system because the HPLC system is more reliable and sensitive than the HPTLC system. When it was applied to feeds without any cleanup operation, many peaks of interfering substances appeared on the chromatograms. Additionally, it was anticipated it would be impossible to apply our previous derivatization system (Asukabe *et al.*, 1987, 1993) to analysis of feed samples.

To overcome the problem, the following experiments were carried out: (1) the elimination of interfering substances in the process of the derivatization and the establishment of the separation conditions between PEs and the interferences; (2) the establishment of a precolumn derivatization system by modification of the previous one (Asukabe *et al.*, 1987, 1993). Detailed investigation of the above points has led us to establish a simpler, more rapid, and more precise analytical method for PEs in feeds. This paper describes a useful technique for the simultaneous determination of PEs in feeds using a silica gel cartridge cleanup system followed by HPLC with fluorescence detection using 1-BAP as a fluorescence reagent.

MATERIALS AND METHODS

Chemicals. SL was supplied by Pfizer Pharmaceuticals (Tokyo), and MN and LA were purchased from Hexyst-Japan (Tokyo) and from Sigma (Tokyo), respectively. 18,19-Dihydrosalinomycin sodium (DSL) and 18,19-dihydro-20-ketosalinomycin sodium (DKSL) were prepared according to the procedure described in the previous paper (Asukabe *et al.*, 1987). 1-BAP was prepared according to the procedure of Kawahara *et al.* (1981). Mega Bond Elut silica gel cartridge (No. 601406, Lot 010692) was purchased from Varian (Harbor City, CA). All other chemicals were of analytical reagent grade. Four kinds of feed (standard experimental diets for livestock and poultry) were purchased from Nihon Haigou Shiryou (Yokohama).

Preparation of Standard Solution. Ten milligrams of SL, MN, and LA was accurately weighed into a 100-mL volumetric flask and diluted to volume in acetonitrile. A 5-mL volume of the resulting solution and a 5-mL volume of the internal standard solution were pipetted into a 20-mL round-bottom flask and evaporated to dryness using a rotary evaporator. Five milliliters of 1-BAP solution and 5 mL of K-222 solution were added into the round-bottom flask containing the PEs. The round-bottom flask was allowed to stand in an oil bath at 50 °C for 90 min. After the flask cooled to room temperature, the determination was carried out by HPLC.

Preparation of Internal Standard Solution. Ten milligrams of DSL was accurately weighed into a 100-mL volumetric flask and diluted to volume in acetonitrile.

Preparation of 1-BAP Solution. 1-BAP (132 mg) was weighed into a 25-mL volumetric flask and diluted to volume in acetonitrile.

Preparation of K-222 Solution. K-222 (32 mg) was weighed into a 25-mL volumetric flask and diluted to volume in acetonitrile.

Extraction and Cleanup Procedures. Five milliliters of internal standard solution was added to a sample (5 g). The sample was blended three times with 20, 30, and 30 mL of acetonitrile using a high-speed blender and centrifuged at 700*g*. After evaporation of the supernatant to dryness using a rotary evaporator, the residue was dissolved in 5 mL of chloroform. After filtration of the solution, the filtrate was applied on a silica gel cartridge activated with chloroform and the cartridge was washed twice with 10 mL of chloroform. PEs were eluted with two 10-mL portions of ethyl acetate and collected in a round-bottom flask. After evaporation of the solution to dryness using a rotary evaporator, 5 mL of 1-BAP solution, 5 mL of K-222 solution and 2 μL of triethylamine were added into the round-bottom flask. The round-bottom flask was allowed to stand in an oil bath at 50 °C for 90 min. After the flask cooled to room temperature, the reaction mixture was evaporated to dryness and the residue was dissolved in 5 mL of benzene. After filtration of the solution, the filtrate was applied again on a silica gel cartridge activated with benzene, and the cartridge was washed twice with 10 mL of benzene. Polyether pyrenacyl esters (PEs-PYs) were eluted with two 10-mL portions of benzene/acetone (7:3) solution. After evaporation to dryness using a rotary evaporator, the residue was dissolved in 10 mL of acetonitrile and the determination was carried out by HPLC.

Caution: Benzene and chloroform are carcinogenically active chemicals. The analyst should take appropriate precautions when handling these substances.

High-Performance Liquid Chromatography. A high-performance liquid chromatograph equipped with a constant-flow pump (Trirotar, JASCO, Tokyo) was used, with a spectrofluorometric detector (FP-210, JASCO) operated at λ_x 360 nm and λ_m 420 nm. The separation was performed on a Develosil 5 C_{18} (5 μm, 250 × 4.6 mm) (Nomura Chemical, Seto) with methanol/water (97:3) as the mobile phase at a flow rate of 1.0 mL/min at room temperature.

Gas Chromatography–Mass Spectrometry. A Model 9A gas chromatograph coupled to a GCMS-QP 1000 quadrupole mass spectrometer (Shimadzu, Kyoto, Japan) was used. The mass spectra from automatic repetitive scanning at 2.0-s intervals were recorded over the mass range *m/z* 100–400 and stored on a floppy disk. The GC conditions were as follows: column, 1-m glass packed 2% silicon OV-101 on Gaschrom Q column (Shimadzu); carrier gas, helium; flow rate 50 mL/min; column temperature, programmed from 130 to 250 °C at 5 °C/min; injection temperature, 250 °C. The MS conditions were as follows: ionization energy for the mass spectra, 20 eV; ion source temperature, 250 °C; separator temperature, 250 °C.

Preparation of Fatty Acid Pyrenacyl Esters (FAs-PYs). To each solution of 10–20 mg of sodium salt of fatty acid ($C_{14:0}$ myristic acid, $C_{16:0}$ palmitic acid, $C_{16:1cis9}$ palmitoleic acid, $C_{18:0}$

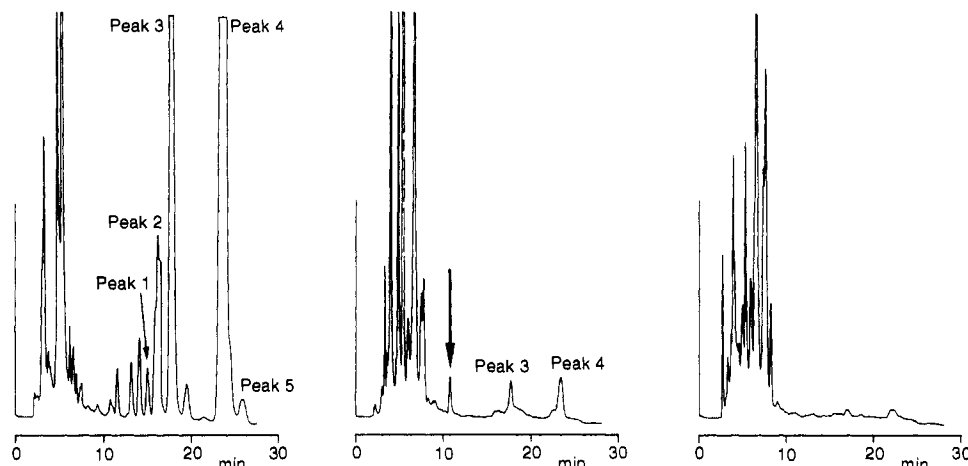


Figure 3. Effect of cleanup with silica gel on HPLC chromatograms of backgrounds: (left) without cleanup; (middle) after the first step cleanup; (right) after the second cleanup.

stearic acid, $C_{18:1cis6}$ petroselinic acid, $C_{18:1cis9}$ oleic acid, $C_{18:2cis9,12}$ linolenic acid, $C_{18:3cis9,12,15}$ linolenic acid) in 10 mL of acetonitrile were added 1-BAP (30 mg) and K-222 (15 mg). These solutions were stirred for 150 min and then concentrated under reduced pressure. The residues were chromatographed on a silica gel column (150 × 10 mm i.d.) with *n*-hexane/benzene (2:1) to give each FAs-PYs as a yellow oil. EI-MS: $C_{14:0}$-PY myristic acid pyrenacyl ester, *m/z* 470 ($M^{\cdot+}$); $C_{16:0}$-PY palmitic acid pyrenacyl ester, *m/z* 498 ($M^{\cdot+}$); $C_{16:1cis9}$-PY palmitoleic acid pyrenacyl ester, *m/z* 500 ($M^{\cdot+}$); $C_{18:0}$-PY stearic acid pyrenacyl ester, *m/z* 526 ($M^{\cdot+}$); $C_{18:1cis6}$-PY petroselinic acid pyrenacyl ester, *m/z* 524 ($M^{\cdot+}$); $C_{18:1cis9}$-PY oleic acid pyrenacyl ester, *m/z* 524 ($M^{\cdot+}$); $C_{18:2cis9,12}$-PY linoleic acid pyrenacyl ester, *m/z* 522 ($M^{\cdot+}$); $C_{18:3cis9,12,15}$-PY linolenic acid pyrenacyl ester, *m/z* 520 ($M^{\cdot+}$).

RESULTS AND DISCUSSION

As mentioned under Introduction, we set the goal to establish a simultaneous determination system using HPLC with fluorescence detection for analysis of PEs in feeds. For this purpose, it was most important to establish a cleanup procedure and to apply the previous simultaneous derivatization system (Asukabe *et al.*, 1987, 1993) without serious modification. Five grams each of four kinds of feeds was extracted with 50 mL of methanol, ethanol, ethyl acetate, acetone, acetonitrile, chloroform, benzene, and *n*-hexane. After evaporation of the supernatants to dryness, the previously reported derivatization system (Asukabe *et al.*, 1987, 1993) was applied without any cleanup and the reaction mixtures were injected into HPLC under the conditions described under Materials and Methods. About 10 peaks of interfering substances appeared on the chromatogram as indicated in Figure 3 (left). These peaks were commonly observed in any chromatograms of extracts from all kinds of the feeds. A more promising approach was to understand the structural characteristics of coexisting materials in feeds for establishing a cleanup system.

Characterization of Interfering Substances in Feeds. We used standard experimental feeds for broiler and chicken. Raw materials in feed are made of corn, rice bran, and soybean meal, and a large quantity of fatty acids (FAs) is contained in them. Tweeten and Wetzel (1979) reported the HPLC analysis of FAs as *p*-bromophenacyl esters from grain and feed extracts. An experiment using Soxhlet extraction showed 3–6% oil content in these feeds, suggesting that these peaks of interfering substances as shown in Figure 3 (left) correspond to pyrenacyl esters of fatty acids (FAs-PYs).

The identification of FAs was carried out using a GC–MS technique. After methylation of the extract from the feed with diazomethane, the determination of FAs was achieved by GC–MS under chemical ionization (CI) conditions. The CI mass spectra of the peaks exhibited protonated molecules, $(M + H)^+$, at *m/z* 243, 269, 271, 293, 295, 297, and 299, consistent with methyl esters of saturated FAs (*m/z* 243, $C_{14:0}$; *m/z* 271, $C_{16:0}$; *m/z* 299, $C_{18:0}$) and unsaturated FAs (*m/z* 269, $C_{16:1}$; *m/z* 293, $C_{18:3}$; *m/z* 295, $C_{18:2}$; *m/z* 297, $C_{18:1}$). The identification of FAs was also carried out by HPLC with fluorescence detection based on the results of the GC–MS. Retention times of derivatized FAs with 1-BAP were identical with those of the interfering substances in feeds. The results of the GC–MS and HPLC examinations indicated that they are pyrenacyl esters (PYs): peak 1, $C_{18:3}$ (linoleic acid); peak 2, a mixture of $C_{14:0}$ (myristic acid) and $C_{16:1cis9}$ (palmitoleic acid); peak 3, $C_{18:2cis9,12}$ (linoleic acid); peak 4 (main peak), a mixture of $C_{16:0}$ (palmitic acid) and $C_{18:1cis9}$ (oleic acid); peak 5; $C_{18:1cis6}$ (petroselinic acid). $C_{18:0}$ (stearic acid) was also observed at the retention time of 45 min.

Cleanup. It is known that alumina is effective in removing FAs in microbiological analysis of SL and MN (Chemical Society Analytical Methods Committee, 1977; Royal Society of Chemistry, 1981; Martinez and Shimoda, 1983; Kusama and Shimada, 1986; Takeba *et al.*, 1986), but LA is completely retained (Owles, 1984) because of its stronger adsorbing power. Therefore, we intended to use a silica gel cartridge for cleanup. Although FAs usually show tailing on silica gel TLC, only solvent systems containing chloroform and ethyl acetate gave relatively round spots of FAs compared with other solvent systems. Various combinations of chloroform/ethyl acetate were tested to obtain suitable solvent systems, and chloroform/ethyl acetate (8:2) gave excellent separation showing the following R_f values: SL, 0.04; MN, 0.05; LA, 0.07; and FAs, 0.17–0.54. On the basis of this experiment, PEs were applied to the silica gel cartridge, and their recoveries with the solvent systems are given in Table 1, indicating that when the cartridge retaining PEs is twice washed with 10 mL of chloroform, PEs are not eluted from the cartridge. However, 93–95% portions of FAs were eluted in this step. These experiments clearly show that PEs can be separated from FAs by the following way: the chloroform solution containing PEs and FAs is applied to a silica gel cartridge, FAs are washed out with two 10-mL portions of chloroform, and PEs are eluted with two 10-mL portions of ethyl acetate.

To investigate the suitability of the cleanup system for the analysis of PEs in feed, the following experiments were

Table 1. Recovery of SL, MN, LA, DSL, and DKSL from Mega Bond Elut Silica Gel

	recovery (%)				
	SL	MN	LA	DSL	DKSL
chloroform (10 mL × 2)	0	0	0	0	0
chloroform/ethyl acetate (10 mL × 2)					
3:7	97	100	99	66	90
2:8	99	100	99	78	95
1:9	100	100	99	83	97
ethyl acetate (10 mL × 2)	100	100	99	99	100

Table 2. Recovery of SL, MN, LA, DSL, and DKSL Pyrenacyl Esters (PYs) from Mega Bond Elut Silica Gel

	recovery (%)				
	SL-PY	MN-PY	LA-PY	DSL-PY	DKSL-PY
benzene (10 mL × 2)	0	0	0	0	0
benzene/acetone (10 mL × 2)					
19:1	31	12	98	7	90
9:1	95	79	99	88	99
8:2	99	98	98	99	98
7:3	100	100	100	101	101

carried out. Five grams of feed free from PEs was extracted with 50 mL of methanol. After evaporation of the supernatant to dryness, the residue was subjected to the cleanup and derivatization systems. The chromatogram in Figure 3 (middle) shows that the peaks of interfering substances are almost eliminated by the cleanup in comparison with the chromatogram in Figure 3 (left), but both peaks 3 and 4 still appeared together with one unidentified peaks (indicated by arrow). Since they could not be sufficiently eliminated in the washing stage and were coeluted with ethyl acetate, these results suggest that the second cleanup is required.

When chloroform was used as the solvent system of the silica gel TLC, R_f values of PEs and FAs ($C_{14:0}$, $C_{16:0}$, and $C_{18:0}$) were 0 and 0.02–0.2, respectively, whereas the R_f values of their pyrenacyl esters (PEs-PYs, FAs-PYs) were 0–0.05 and 0.68, respectively. The different chromatographic behavior was efficiently applied to the second-step cleanup. When benzene was used as the solvent system, the spots of PEs-PYs were at the origin, but the R_f values of FAs-PYs were 0.54–0.58. PEs-PYs were applied to the silica gel cartridge, and their recoveries with the solvent systems are given in Table 2, indicating that when the cartridge retaining PEs-PYs is twice washed with 10 mL of benzene, PEs-PYs are not eluted from the cartridge. However, 100% portions of FAs-PYs were eluted in this step. These experiments clearly showed that PEs-PYs can be separated from FAs-PYs in the following way: benzene solution containing PEs-PYs and FAs-PYs is applied to a silica gel cartridge, the FAs-PYs are washed two times with 10 mL of benzene, and PEs-PYs are eluted with two 10-mL portions of benzene/acetone (7:3). The obtained chromatogram in Figure 3 (right) shows that the peaks of interfering substances in feeds were completely eliminated by this cleanup system and that the two-step silica gel cleanup system is quite effective for the sample preparation of PEs in feeds.

Derivatization. In the previous paper we reported a simultaneous HPLC determination of SL, MN, LA, and narasin with fluorescence detection using DSL and DKSL (Figure 4) as internal standards (Asukabe *et al.*, 1993). The following derivatization conditions have been used: (1) molar excess of reagent (1-BAP), 50-fold molar excess of LA; (2) molar excess of catalyst (K-222), 10-fold molar excess of LA; (3) reaction temperature, 50 °C; (4) reaction

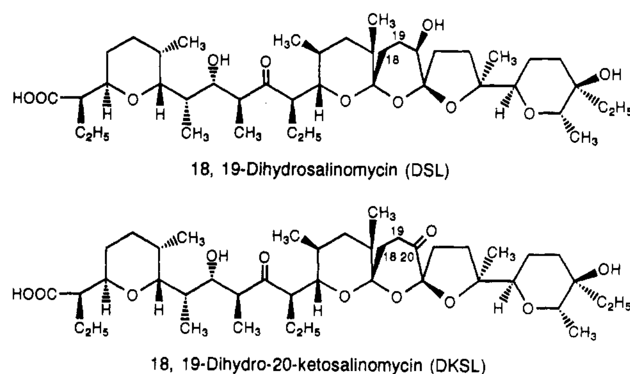


Figure 4. Internal standards for fluorometric determination.

Table 3. Derivatization of Polyether Antibiotics (PEs) in Feed Extract with 1-(Bromoacetyl)pyrene

molar excess			yield (%)				
reagent	catalyst	triethylamine	SL	MN	LA	DSL	DKSL
50-fold	10-fold		100	59	100	100	88
50-fold	10-fold	1 μL	100	89	99	100	87
50-fold	10-fold	2 μL	98	92	97	98	88
100-fold	10-fold		100	53	100	100	79
100-fold	10-fold	1 μL	99	87	99	100	91
100-fold	10-fold	2 μL	98	84	97	98	86
100-fold	20-fold		100	90	99	100	86
100-fold	20-fold	1 μL	98	99	98	99	89
100-fold	20-fold	2 μL	98	100	98	98	87
100-fold	20-fold	3 μL	98	100	98	98	87

time, 90 min. The derivatization conditions were applied to the analysis in feeds with modification of the following two points: (1) addition of triethylamine into the reaction solution; (2) increase of the amounts of reagent and catalyst.

Five grams of feed free from PEs was extracted with 50 mL of methanol, and the first-step cleanup system was achieved. After addition of 500 μg of PEs, DSL, and DKSL to the ethyl acetate fraction at the first-step cleanup, the reactivity of these compounds was examined under the following conditions: reaction temperature, 50 °C; reaction time, 90 min; followed by the second cleanup system. After the second-step cleanup, the residue from the desired fraction was dissolved in 10 mL of acetonitrile and the determination was achieved by HPLC. To obtain optimum conditions for the determination in feeds, several conditions were examined (Table 3).

The low reactivity of MN and DKSL was unchanged even if the molar excess of reagent was increased 100-fold (MN, 53%; DKSL, 79%). This was considered to be due to the fact that the carboxylate groups in the molecules were partially converted to free carboxylic acids through the first-step cleanup on the silica gel cartridge. Addition of triethylamine improved the reactivity of MN; that is, 1 μL of triethylamine corresponding to about 2-fold molar excess to be the total moles of PEs, DSL, and DKSL was directly added to the derivatizing solution. As expected, the reaction yield of MN increased remarkably from 59% to 89% (molar excess: 50-fold of reagent, 10-fold of catalyst) and from 53% to 87% (100-fold of reagent, 10-fold of catalyst). To obtain the higher reaction yield, 100-fold molar excess of the reagent, 20-fold molar excess of catalyst, and 1 μL of triethylamine were examined, so that the reaction yield of MN increased to 99%. Under these molar ratio conditions of reagent and catalyst, the amount of triethylamine was increased from 2 to 5 μL, but a marked change was not observed in reaction yield of DKSL, whereas those of SL, MN, LA, and DSL increased to approximately 100%.

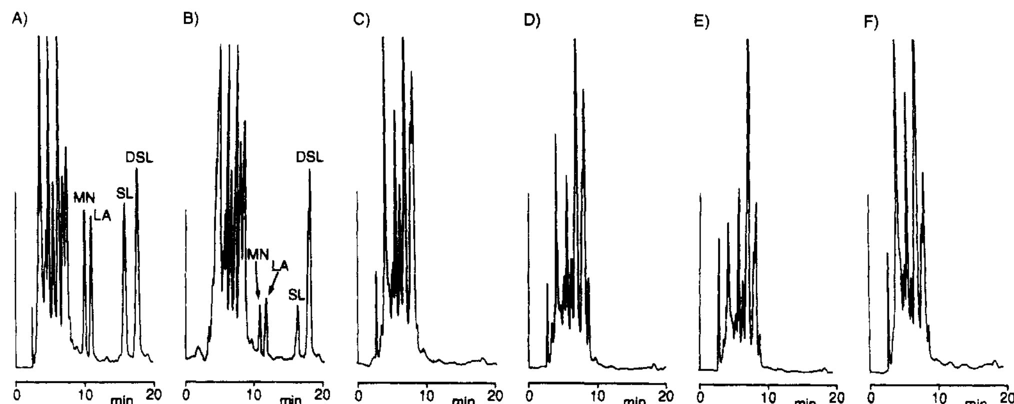
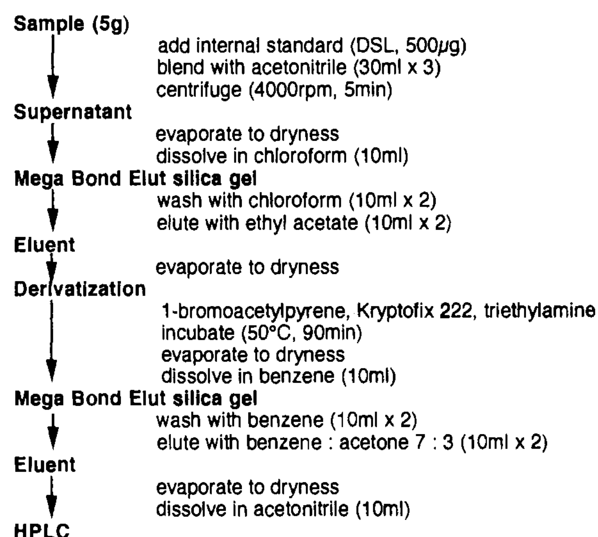


Figure 5. Typical chromatograms of feed extracts: (A) fortified broiler finisher (100 ppm); (B) fortified broiler finisher (25 ppm); (C) broiler finisher; (D) broiler starter; (E) chicken finisher; (F) chicken starter.

Scheme 1. Established Analytical Procedure for SL, MN, and LA in Feed Sample



Finally, the following derivatization conditions applicable to feeds were chosen: internal standard, DSL; molar excess of 1-BAP, 100-fold; molar excess of K-222, 20-fold; amount of triethylamine, 2 μL; reaction temperature, 50 °C; and reaction time, 90 min. To confirm the applicability of the cleanup and derivatization conditions, the experiment was performed as shown in Scheme 1. Good recoveries were obtained without interfering peaks, and the reactivity of DSL was almost same as those of SL, MN, and LA, showing that DSL gives a satisfactory result as an internal standard for the simultaneous determination of SL, MN, and LA in feeds.

Application to Feed Analysis. The extraction of PEs and DSL from feeds was investigated on the basis of the studies mentioned above. Five grams of sample (spiked at each level of 100 ppm) was extracted with 50 mL of the following solvents: methanol, acetone, ethyl acetate, acetonitrile, benzene, and *n*-hexane. Table 4 shows the recoveries of SL, MN, LA, and DSL from feeds, indicating that acetonitrile is most suitable for extraction solvent. PEs (each 125–500 μg) and DSL (500 μg) were spiked into 5 g of each feed, and the recoveries of PEs were investigated at levels of 25–100 ppm. As shown in Table 5, satisfactory recoveries were obtained even at a low concentration level of PEs. The typical high-performance liquid chromatograms of these feeds are shown in Figure 5. No interfering peaks appeared on the chromatograms.

Conclusion. A simple and rapid technique for the simultaneous analysis of PEs in feeds has been established with the following characteristics: (1) Acetonitrile is the most suitable solvent to extract PEs from feeds. (2) The internal standard, DSL, is effective for the simultaneous determination of PEs in feeds. (3) A two-step silica gel cartridge cleanup system makes possible the separation of PEs from FAs. (4) Addition of triethylamine to the reaction solution is essential to derivatize efficiently. (5) The recoveries of SL, MN, and LA from feeds spiked at levels of 25–100 ppm are 99%, 100%, and 100%, respectively. The analytical procedure is summarized in Scheme 1.

Table 4. Extraction of SL, MN, LA, and DSL from Feed Sample

solvent	recovery (%)			
	SL	MN	LA	DSL
methanol	95	98	86	95
acetone	90	96	84	91
ethyl acetate	94	99	91	91
acetonitrile	95	98	90	95
benzene	100	97	86	95
n-hexane	98	99	90	87

Table 5. Recovery of Polyether Antibiotics (PEs) from Feed Sample[a]

fortified (ppm)	recovery (%)		
	SL	MN	LA
25	99	98	96
50	99	100	95
75	98	100	95
100	100	104	94

[a] Results of three replicates.

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