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Development of an analytical protocol for detecting antibiotic residues in various foods

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Abstract

Medicines such as antibiotics and hormones have been widely used for treating diseases in animals. As more antibiotics are needed to prevent various diseases that occur, not only in domestic animals, but also in imported livestock, a quick and robust analytical method is required for detecting antibiotic residues. In this study, agricultural and fishery products, including beef ($n = 148$), pork ($n = 78$), chicken ($n = 88$), eel ($n = 70$), flatfish ($n = 17$), armorclad rockfish ($n = 18$), sea bream ($n = 18$), perch ($n = 18$), and oyster ($n = 4$) were obtained from local markets in different areas of Korea. A total of 13 antibiotics, including tetracycline, macrolide, penicillin, aminoglycoside, polyester, peptide, and chloramphenicol types, were analyzed by a microbial assay and high performance liquid chromatography (HPLC). In the microbial assay, 34 of the 459 screened samples had possible antibiotic residues. The antibiotic concentrations of the 34 samples were analyzed using HPLC with UV and fluorescence detection. The levels of oxytetracycline in pork and eel were 0.01 and 0.05 mg/kg, respectively. In eel and oyster, the concentrations of ampicillin were 0.4 and 0.32 mg/kg, respectively. In beef, the concentration of tylosin was 0.05 mg/kg. The levels of oxytetracycline and tylosin were below the Korean Food Code's recommended maximum residue limit (MRL). A maximum residue limit for ampicillin in fishery products, however, has not been established in Korea. These data indicate that an MRL for ampicillin should be set for sea foods and regulated by the Korean authorities.

Keywords: Analytical protocol; Antibiotic residues; HPLC; Microbial assay

1. Introduction

Antibiotics are one of the most important bioactive and chemotherapeutic groups of compounds made by microbiological synthesis (Joshi, 2002). Today, many antibiotics are widely used for preventing and treating several diseases, as well as for promoting growth in food-producing animals (Carson, Righter, & Wagner, 1994; Di Corcia & Nazzari, 2002). Currently, over 40,000 kinds of antibiotics have been discovered, and 80 of these are used in the agricultural and fishery industries (Kreuzig, Sherma, & Fried, 1996). This widespread use of antibiotic may cause residuals in foodstuffs, as well as the induction of allergic reactions in humans. In addition, resistance to pathogenic bacteria has been constantly weakening as a result of antibiotic use (Choma, Grenda, Malinowska, & Suprynowicz, 1999; Schenck & Callery, 1998).

In many countries, governmental authorities have established monitoring programmes to determine antibiotic levels in foods, as well as the highest allowable residue levels (Ramrrez et al., 2003). Foodstuffs containing levels of antibiotics that exceed the tolerance levels must be verified by highly selective and sufficiently sensitive chemical methods (Caren, Rosa, Merce, & Dolors-Prats, 2001). The official liquid chromatography (LC) method for determining antibiotic concentrations, as prescribed by the pharmacopoeias of Europe and the United States, was based on the work of Paesen, Roets, and Hoogmartens (1991). Using new stationary phase materials for reversed phase chromatography, a gradient LC method was developed by Govaerts,

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Chepkwony, Van Schepdael, Roets, and Hoogmartens (2000). Later, an isocratic LC method on XTerra™ RP_{18}, which separated more unidentified peaks from known related substances was developed by Chepkwony, Dehouck, Roets, and Hoogmartens (2000).

Although LC methods have been used extensively for individual antibiotics, a protocol to screen and determine antibiotic residues in foods has scarcely been reported. With HPLC, measuring the level of antibiotic residues in foods can be time- and labour-consuming work since the number of samples is so large. When determining antibiotic residues in foods, one or more microbial assays, in addition to HPLC methods, should be used. In this study, to save time and labour, we investigated an analytical method for measuring antibiotic residues in foods, using both a microbial assay and HPLC. The microbial assay was implemented to prescreen for possible antibiotic-containing foods.

2. Materials and methods

2.1. Chemicals

The antibiotics tylosin, erythromycin, spiramycin, ampicilin, chlorotetracycline, oxytetracycline, tetracycline, hygromycin B, spectinomycin, monensin, salinomycin, virginiamycin, bacitracin and chloramphenicol, as well as Amberlite XAD-2 resins and CG-50 resins, were bought from Sigma–Aldrich Corporation (Seoul, Korea). Muller Hinton agar, medium test agars (pH 8.0 and pH 6.0), and DST medium were bought from Difco Co. (Sparks, MD, USA). Potassium phosphate (KH_2PO_4), triethylamine, phosphoric acid, and sodium sulfate were purchased from Dae Jung (Seoul, Korea). HPLC grade solvents, such as acetonitrile, methanol, water, *n*-hexane and ethyl acetate, were purchased from J.T. Baker Co. (Phillipsburg, NJ, USA).

2.2. Samples

The agricultural and fishery products (459 kinds), including beef ($n = 148$), pork ($n = 78$), chicken ($n = 88$), eel ($n = 70$), flatfish ($n = 17$), armorclad rockfish ($n = 18$), sea bream ($n = 18$), perch ($n = 18$), and oyster ($n = 4$) were obtained from various areas of Korea.

2.3. Microbial assay

2.3.1. General

We performed the microbiological screening tests for antibiotic residues in the foods according to the Korean Food Code. *Bacillus megaterium* ATCC 9885, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, and *Bacillus stearothermophilus* ATCC 10149 were inoculated in A–K #2 sporulating agar (BBL). Each species was allowed to sporulate for 7 days. The four test organisms were inoculated into the four different media as follows: 1 ml of the *B. megaterium* suspension (2×10^6 spores/ml) and 1 ml of TMP solution (15 μg/ml) were added to 100 ml of Mueller Hinton medium (Difco); 1 ml of the *B. subtilis* spore suspension of McFarland No. 1 standard was added to Antibiotic Medium No. 5 (Difco); 1 ml of the *B. cereus* spore suspension of McFarland No. 1 standard was added to Antibiotic Medium No. 8 (Difco), and 1 ml of the *B. stearothermophilus* spore suspension of McFarland No. 2 standard was added to Antibiotic Medium No. 2 (Difco). Next, 8 ml of each inoculated medium were put into a Petri dish (diameter = 90 mm). The antibiotics in the samples were extracted by both a buffer solution method and direct disc method. Paper discs containing absorbed sample buffer or sample extracts were placed on the surfaces of the inoculated media. The plates were then incubated for 16–18 h at the following temperatures: *B. megaterium* at 45 °C, *B. subtilis* at 37 °C, *B. cereus* at 30 °C, and *B. stearothermophilus* at 55 °C. A sample was presumed positive when the inhibition zone on the plate was greater than or equal to 2 mm.

2.3.2. Buffer solution assay

To extract the antibiotics from the samples, two kinds of solutions were used. One was a citric acid acetone solution. First, what we termed solution A was prepared by mixing 0.2 M citric acid solution with an equal volume of 0.5 M potassium hydroxide. Then the citric acid acetone extraction buffer was made by mixing solution A, acetone, and sterile distilled water in the ratios 35:35:30. The second solution was a 0.2 M phosphate buffer (pH 8.0). This buffer was prepared by adding both dibasic and monobasic potassium phosphate (K_2HPO_4) (21.75 and 8.5 g, respectively) to 1000 ml of distilled water.

The samples were weighed in two 10 g parts. Ten ml of the citric acid acetone buffer were added to the first 10 g portion, and 10 ml of the 0.2 M phosphate buffer were added to the second 10 g portion. Each sample, with an added buffer, was placed at 85 °C for 15 min, and then cooled. Next, the paper discs were dipped into the extracted buffer samples. Discs with absorbed citric acid acetone buffer were placed on the surfaces of the *B. subtilis*, *B. cereus*, and *B. stearothermophilus* plates. Discs with absorbed 0.2 M phosphate buffer were placed on the surface of *B. megaterium*.

2.3.3. Direct absorbing assay

This particular method did not use buffer solutions. Here, paper discs were inserted directly into incised tissue samples and left to absorb the sample fluids for 30–60 min. Then the discs were placed on the surfaces of the four different types of plates. All subsequent procedures followed the buffer solution method described above.

2.4. HPLC analytical procedures

2.4.1. General

The HPLC analyses of the various antibiotics were carried out according to Table 1. The employed analytical

methods were changed slightly from the Korean Food Code and published papers (Asukabe et al., 1994; Leal, Codony, Compano, Granados, & Prat, 2000).

2.4.2. Sample preparation

2.4.2.1. Ampicillin. Samples of 10 g were taken and homogenized for 1 min with 25 ml of methanol. The homogenized samples were then centrifuged at 3000 rpm for 20 min, and 2 ml of *n*-butanol and 20 ml of ethanol were added to the supernatants. Next, the samples were evaporated at 40 °C under reduced pressure and dissolved with 0.01 M monobasic potassium phosphate (K_2HPO_4) buffer solution (pH 6, 10 ml). This solution was mixed with ether (10 ml) and centrifuged at 3000 rpm for 10 min. Seven milliliters of supernatant were taken and filtered with a 0.45 μm membrane filter. Then, 50 μl of solution were injected into the HPLC.

2.4.2.2. Chlorotetracycline, oxytetracycline, tetracycline. Samples of 10 g were taken and homogenized for 1 min with 25 ml of methanol. The homogenized samples were then centrifuged at 3000 rpm for 10 min, and 20 ml of ethanol were added to the supernatants. The samples were evaporated at 40 °C under reduced pressure and dissolved with 0.02 M monobasic potassium phosphate buffer solution (pH 1, 30 ml). The solution was eluted onto an Amberlite XAD-2 column and extracted with 60% methanol (50 ml). Amberlite XAD-2 resins were prepared and conditioned by washing 3 times with methanol and water. The column (300 mm × 13 mm) was then packed with Amberlite XAD-2 (10 ml) and washed with 50 ml of water. The extracted solution was mixed with 25 ml of ethanol and concentrated using an evaporator at 40 °C. The residue was then dissolved with the HPLC mobile phase solution (5 ml). Finally, 50 μl of solution was injected into the HPLC.

2.4.2.3. Tyrosin, erythromycin, spiramycin. Samples of 10 g were taken and homogenized for 2 min with 70 ml of extracting solution (0.5% phosphoric acid:methanol = 8:2). The filtered solution was concentrated to 60 ml using an evaporator at 40 °C. The concentrated solution was eluted into a Sep-Pak C_{18} silica (500 mg) cartridge (Waters, Milford, MA, USA) and washed with water, followed by extraction with 50 ml of methanol. The extracted solution was evaporated at 40 °C under reduced pressure and dissolved with 1 ml of solution (water:acetonitrile = 75:25). Then, 50 μl of solution were injected into the HPLC.

2.4.2.4. Hygromycin B. Samples of 10 g were taken and homogenized for 1 min with 30 ml of 10% trichloroacetic acid. The homogenized samples were centrifuged at 3000 rpm for 5 min. The supernatants were eluted onto an Amberlite CG-50 column and extracted with 0.2 N HCl (50 ml). Amberlite CG-50 resins were prepared and conditioned by several washings with 10% KOH and water. A column (10 mm × 80 mm) was packed with Amberlite CG-50 (2 cm) and washed with 20 ml of water. A 0.5 ml amount of extracted solution was mixed with 0.5 ml of *O*-futalaldehyde. The mixed solution was reacted at 30 °C for 10 min and then kept at 50 °C for 5 min. Finally, 50 μl of solution were injected into the HPLC.

2.4.2.5. Monensin, salinomycin. Samples of 5 g were twice blended with 50 ml of acetonitrile, using a homogenizer, and then centrifuged. After evaporation of the supernatants, the residue was dissolved in 5 ml of chloroform. After filtration of the solution, the filtrate was applied into a Sep-Pak C_{18} silica (500 mg) cartridge (Waters, Milford, MA, USA) activated with chloroform, and then washed with 5 ml of chloroform. The samples were eluted with 15 ml of ethyl acetate and collected in a round-bottom flask. After evaporating the solution to dryness, using an evaporator, 5 ml of 1-BAP (1-bromoacetylpyrene), 5 ml of K-222 (kryptofix 222), and 2 μl of triethylamine were added to the flask. The flask was allowed to stand in a water bath at 50 °C for 90 min. After the flask had 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 to a silica gel cartridge activated with benzene, and then washed with 5 ml of benzene. The polyether pyrenacyl esters were eluted with 15 ml of solution (benzene:acetone = 7:3). After evaporation of the solution to dryness, the residue was dissolved in 10 ml of acetonitrile. Then, 50 μl of the solution were injected into the HPLC.

2.4.2.6. Virginiamycin, bacitracin. Samples of 10 g were taken and homogenized for 2 min with 50 ml of water and then heated for 5 min. The samples (pH 3.5) were centrifuged at 3000 rpm for 5 min with 60 ml of acetone. The supernatants were concentrated to 40 ml, using a rotary evaporator at 40 °C. The concentrated solution (pH 8.0) was moved into a separatory funnel and extracted with 80 ml of *n*-butanol, followed by 100 ml of 0.005 N HCl. The extracted solution was evaporated at 40 °C under reduced pressure and dissolved with 1 ml of water. Then, 50 μl of solution were injected into the HPLC.

2.4.2.7. Chloramphenicol. Samples of 10 g were taken and homogenized for 1 min with 6 ml of ethyl acetate and 2 ml of water. The homogenized samples were centrifuged at 4000 rpm for 5 min. A 4.2 ml amount of supernatant was evaporated at 40 °C under reduced pressure and dissolved with 1.4 ml of solution (hexane:chloroform = 1:1) and 0.7 ml of water. The solution was centrifuged and the supernatant was filtered with a 0.45 μm membrane filter. Then, 50 μl of solution were injected into the HPLC.

2.4.3. Standard curves of the antibiotics and determination of the limit of detection (LOD) and limit of quantification (LOQ)

A stock solution (0.1 mg/ml in methanol) of the antibiotics was diluted to concentrations of 0.5, 0.2, 0.1, and

Table 1
Analytical conditions for HPLC analysis of antibiotics

Antibiotics	Mobile phase	Detector	Flow rate (ml/min)
Ampicilin	0.01 M KH_2PO_4:acetonitrile:methanol (70:19:11)	UV detector; $\lambda = 225$ nm	1.0
Tetracycline	Water 760 ml, acetonitrile 240 ml, *N*,*N*-dimethylformamide 60 ml, ethanolamine 5 ml, $NaHPO_4$ 2.5 g	UV detector; $\lambda = 254$ nm	1.0
Tyrosin	Acetonitrile:methanol:0.002 M $(NH_4)_2PO_4$ (80:15:5)	UV detector; $\lambda = 280$ nm	1.0
Erythromycin	Acetonitrile:water:0.2 M CH_3COONH_4 (65:25:10)	UV detector; $\lambda = 215$ nm	1.0
Spiramycin	0.05 M phosphoric acid buffer:acetonitrile (44:56)	UV detector; $\lambda = 232$ nm	1.0
Hygromycin B	Methanol:water:acetonitrile (65:30:5)	Fluorescence detector; $\lambda_{ex} = 335$ nm; $\lambda_{em} = 440$ nm	0.8
Polether	Methanol:water (97:3)	Fluorescence detector; $\lambda_{ex} = 360$ nm; $\lambda_{em} = 420$ nm	1.0
Virginiamycin	0.01 M NaH_2PO_4:acetonitrile (65:35)	UV detector; $\lambda = 230$ nm	1.0
Bacitracin	0.05 M KH_2PO_4:MeOH:acetonitrile (2:1:1)	UV detector; $\lambda = 254$ nm	1.0
Chloramphenicol	0.005 M $(NH_4)_2PO_4$:acetonitrile (76:24)	UV detector; $\lambda = 278$ nm	1.0

0.05 μg/ml with the HPLC mobile phase to make the standard curves. All samples were prepared in triplicate. To determine the LOQ, 0.05 μg/ml of each antibiotic was injected 7 times and the standard deviation calculated. The LOD and LOQ were calculated, based on the following equations (Harris, 2001):

$$\text{LOD (limit of detection)} = 3 \times \text{SD/Slope (μg/ml)}$$

$$\text{LOQ (limit of quantification)} = 10 \times \text{SD/Slope (μg/ml)}$$

2.4.4. Recovery test

A 0.1 μg/ml amount of each antibiotic was added to food samples without antibiotics. The analyses were carried out according to the procedure described above. The recovery rate was calculated, based on the following equation (Harris, 2001):

$$\%\ \text{recovery} = \frac{(\text{Concentration of spiked sample} - \text{Concentration of unspiked sample})}{(\text{Concentration of added antibiotic})}$$

2.4.5. Analytical conditions of HPLC

The analytical conditions of HPLC for each antibiotic are shown in Table 1. These antibiotics were analyzed with a C_{18} (5 μm, 250 × 4.6 mm) reverse phase column.

3. Results

3.1. Results of the microbial assays

The results of the microbial assays, such as the buffer solution method and direct absorbing method, are shown in Table 2. The numbers of samples that potentially contained each antibiotic varied according to assay. In the buffer solution assay (BSA), only 46 (9.8%) of the 469 tested samples had antibiotics detected. However, in the direct absorbing assay (DAA), 274 samples (58.4%) were detected as being positive. We have considered two reasons as to why the percentage of positive sample was higher in DAA than in BSA. First, the contact surfaces of the disks used to dissolve the antibiotics of the foods into the buffer were much wider in DAA than in BSA. Another reason is that the antimicrobial materials in the food samples could interact directly with the disk in DAA. In the HPLC analyses, only five samples included antibiotics. When the microbial assay screening results were compared with the HPLC results, all the HPLC positive samples equal to or above LOQ were included in the positive results of the direct absorbing method. However, the buffer solution method only detected two samples as positive when compared with the HPLC results.

3.2. Standard curves, recovery tests, LOD (limit of detection), and LOQ (limit of quantification) determination

In Table 3, the standard calibration equations, recovery rates, LOD and LOQ, are shown for the tested antibiotics. All samples were prepared in triplicate. The *R*-squares (R^2) of the standard curve for each antibiotic were between 0.9731 and 0.9996. The recovery rates were between 89.1% and 103%. The antibiotics with the lowest LOQ were hygromycin B and salinomycin (0.010 μg/ml).

3.3. Analysis of antibiotic residues in food samples

In the DAA microbial assay, 274 of the 459 samples were screened as having possible antibiotic residues.

Table 2
Number of detected samples, as determined by the two microbial assays and HPLC

Antibiotic types	Buffer solution assay	Direct absorbing assay	HPLC
Polyether	32	202	–
Chloramphenicol	2	1	–
Macrolide	1	3	1
Aminoglycoside	1	1	–
Penicillin	1	20	2
Novobiocin	0	1	–
Peptide	0	3	–
Tetracycline	0	1	2
Unknown	9	42	–
Total	46	274	5

Although BSA had also detected 46 samples with possible antibiotic residues, we chose to perform HPLC analysis on the 274 DAA samples. The antibiotic concentrations in the samples were analyzed using HPLC with UV and fluorescence detection. Varying amounts of antibiotics were detected in 13 samples. Among them, the antibiotic concentrations of seven samples were below the LOQ (limit of quantification). The antibiotics and their levels in foods are shown in Table 4. The antibiotics having concentrations higher than the LOQ were identified as oxytetracycline, tylosin and ampicillin. The levels of oxytetracycline in pork and eel were 0.01 and 0.05 mg/kg, respectively. In eel and oyster, the concentrations of ampicillin were 0.4 and 0.32 mg/kg, respectively. In beef, the concentration of tylosin was 0.05 mg/kg.

4. Discussion and conclusion

A total of 13 antibiotics, within the classes of tetracycline, macrolide, penicillin, aminoglycoside, polyester, peptide and chloramphenicol, were analyzed by microbial assay and high performance liquid chromatography (HPLC). For the microbial screening methods, the buffer solution assay had a lower number of false positive results than had the direct absorbing assay. The false positives that resulted with DAA were quite numerous, and among them the majority occurred with the *B. stearothermophilus* plates. However, BSA did not detect three of the five HPLC positive samples. Therefore, BSA had a greater possibility for false negatives than had DAA. In the HPLC analyses, the ampicillin levels of eel and oyster were 0.4 and 0.32 mg/kg, respectively, which were higher than the levels of all other antibiotics detected in this study. The levels of oxytetracycline (pork) and tylosin (beef) were lower than the recommended maximum residue limit (MRL) of the Korean Food Code. The MRL of ampicillin in fishery products, however, has not been established in Korea. Therefore, these data indicate that the MRL of ampicillin in sea foods should be set and regulated by the authorities.

As this study illustrates, an analytical protocol for the accurate and robust detection of residual antibiotics can be established. When the number of samples is plentiful, e.g. over 400, a microbial assay for antibiotic screening should be carried out prior to HPLC analysis to save time and labour. Although the numbers of positively screened samples in our two microbial assays were different, employ-

Table 3
Antibiotic calibration curve parameters, recovery rates, limits of detection (LOD), and limits of quantification (LOQ)

Antibiotics	Standard calibration equation ($y = ax + b$)			Recovery rates (%)	LOD (μg/ml)	LOQ (μg/ml)
	a	b	R^2			
Oxytetracycline	357,975	−1e + 06	0.9957	93.1	0.004	0.013
Tetracycline	513,971	38,359	0.9996	91.1	0.007	0.025
Chlortetracycline	85,134	1720.4	0.9973	90.6	0.005	0.018
Tylosin	96,934	−2e + 06	0.9981	95.5	0.007	0.020
Erythromycin	5286.1	−269,574	0.9731	89.1	0.006	0.015
Spiramycin	548,172	1e + 07	0.9869	92.5	0.007	0.023
Ampicillin	156,729	1e + 06	0.9919	97.8	0.004	0.016
Hygromycin B	3128.6	−225,022	0.9941	89.7	0.002	0.010
Monensin	355,964	5e + 06	0.9934	95.8	0.004	0.013
Salinomycin	4e + 06	2e + 07	0.9846	92.6	0.003	0.010
Virginiamycin	62,331	22,328	0.9903	92.8	0.005	0.028
Bacitracin	47,440	−2e + 06	0.9919	101.3	0.005	0.023
Chloramphenicol	750,689	−8e + 06	0.9893	102.6	0.004	0.020

Table 4
The antibiotic residue levels in food samples analyzed by HPLC

No. of samples	Food Source	Part of sample	Concentration of antibiotic residues (mg/kg)	Regulation of Korea Food Code (mg/kg)
6	Pork	Muscle	Oxytetracycline; 0.01	Muscle 0.1
12	Eel	Tail	Ampicillin; 0.4	Meat 0.01
97	Eel	Tail	Oxytetracycline; 0.05	Fishes 0.1
105	Beef	Small intestine	Tylosin; 0.05	Meat 0.2
F-22	Oyster	Whole	Ampicillin; 0.32	Meat 0.01

ing an assay saved time compared with a whole examination by HPLC analysis alone. As a future study, we would like to develop a new microbial assay to decrease false positive detections by drawing on the advantages of the current two assays. For HPLC analysis, the present methodology of sample preparation would be modified to give a more accurate examination.

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References

Asukabe, H., Murata, H., Harada, K., Suzuki, M., Oka, H., & Ikai, Y. (1994). Improvement of chemical analysis of antibiotics: Simultaneous determination of three polyether antibiotics in feeds using high performance liquid chromatography with fluorescence detection. *Journal of Agricultural and Food Chemistry, 42*, 112–117.

Caren, L., Rosa, C., Merce, G., & Dolors-Prats, M. (2001). Determination of macrolide antibiotics by liquid chromatography. *Journal of Chromatography A, 910*, 285–290.

Carson, M. C., Righter, R. F., & Wagner, D. D. (1994). Residues of veterinary drugs and mycotoxins in animal products. In *Proceedings of forts electronic mail conference* (pp. 76). Wageningen: Wageningen Press.

Chepkwony, H. K., Dehouck, P., Roets, E., & Hoogmartens, J. (2000). Isocratic separation of erythromycin, related substances and degradation products by liquid chromatography on XTerra RP18. *Chromatographia, 53*, 159–165.

Choma, I., Grenda, D., Malinowska, I., & Suprynowicz, Z. (1999). Determination of flunequine and doxycycline in milk by a simple thin-layer chromatographic method. *Journal of Chromatography B, 734*, 7–14.

Di Corcia, A., & Nazzari, M. (2002). Liquid chromatographic–mass spectrometric methods for analyzing antibiotic and antibacterial agents in animal food products. *Journal of Chromatography A, 974*, 53–89.

Govaerts, C., Chepkwony, H. K., Van Schepdael, A., Roets, E., & Hoogmartens, J. (2000). Investigation of unknown related substances in commercial erythromycin samples with liquid chromatography/mass spectrometry. *Rapid Communication Mass Spectrometry, 14*, 878–884.

Harris, D. C. (2001). *Exploring chemical analysis* (pp. 91–98) (3rd ed.). New York: W.H. Freeman.

Joshi, S. (2002). HPLC separation of antibiotics present in formulated and unformulated samples. *Journal of Pharmaceutical and Biomedical Analysis, 28*, 795–809.

Korea Food and Drug Administration. (2005). *Food Code 2005* (pp. 424–423). Seoul: KFDA Press.

Kreuzig, F., Sherma, J., & Fried, B. (1996). *Antibiotics in hand book of TLC* (p. 445). New York: Marcel Decker.

Leal, C., Codony, R., Compano, R., Granados, M., & Prat, D. (2000). Determination of macrolide antibiotics by liquid chromatography. *Journal of Chromatography A, 910*, 285–290.

Paesen, J., Roets, E., & Hoogmartens, J. (1991). Liquid chromatography of erythromycin A and related substances on poly(styrene-divinylbenzene). *Chromatographia, 32*, 162–166.

Ramrrez, A., Gutierrez, R., Draz, G., Gonzalez, C., Perez, N., Vega, S., et al. (2003). High-performance thin-layer chromatography-bioautography for multiple antibiotic residues in cow's milk. *Journal of Chromatography B, 784*, 315–322.

Schenck, F. J., & Callery, P. S. (1998). Chromatographic methods of analysis of antibiotics in milk. *Journal of Chromatography A, 812*, 99–109.