Journal of Food and Drug Analysis

Volume 9 | Issue 2 Article 6

Multiresidue determination of veterinary drugs in chicken and swine muscles by high performance liquid chromatography

Journal of Food and Drug Analysis, Vol. 9, No. 2, 2001, Pages 84-95 藥物食品分析 第九卷 第二期

Multiresidue Determination of Veterinary Drugs in Chicken and Swine Muscles by High Performance Liquid Chromatography

YA-MIN KAO*, MEI-HUA CHANG, CHIEU-CHEN CHENG AND SHIN-SHOU CHOU

National Laboratories of Foods and Drugs, Department of Health, Executive Yuan
161-2, Kuen-Yang Street, Nankang, Taipei, 115, Taiwan, R.O.C.

(Received: July 13, 2000; Accepted: September 21, 2000)

ABSTRACT

A high performance liquid chromatographic (HPLC) method equipped with a photodiode array detector was evaluated for the residual determination of 13 veterinary drugs, including clopidol, sulfadiazine, sulfathiazole, carbadox, sulfamerazine, ormethoprim, sulfamethazine, furazolidone, sulfamonomethoxine, sulfamethoxazole, ethopabate, sulfaquinoxaline, and sulfadimethoxine in chicken and swine muscles. Test samples were extracted with acetonitrile and filtered. The filtrate was partitioned with acetonitrile-saturated n-hexane for removing the interference. After evaporation to dryness, the residue was passed through a Sep-Pak C_{18} cartridge for sample cleanup prior to HPLC analysis. Veterinary drugs were determined by HPLC equipped with a photodiode array detector using a Luna 5 μ C_{18} (2) 25 cm × 4.6 mm i.d., 5 μm) analytical column and a gradient elution of acetonitrile and 0.05M sodium dihydrogen phosphate. The average recoveries of 13 veterinary drugs from chicken and swine muscles at the levels of 0.1, 0.2 and 0.4 ppm were in the range of 71.9 ～96.9% and 71.1~99.6%, respectively, with coefficients of variation less than 8%. The detection limits were 0.04 ppm for sulfathiazole and 0.02 ppm for other 12 drugs.

Twenty-five samples each of chicken and swine muscles collected from local markets in Taipei were investigated for veterinary drug residues. One chicken muscle sample was found to contain 1.23 ppm sulfaquinoxaline, the level of which exceeded the regulated tolerance.

Key words: veterinary drug, multiresidue, photodiode array detector, high performance liquid chromatography

INTRODUCTION

The purpose of using veterinary drugs is to prevent animals from getting disease, enhance feed efficiency, promote animal growth, and improve productivity. The Council of Agriculture of the Republic of China announced the "Rules of Use of Feeds Additives Announcement"[(1)] on October 1982, in which 64 feed additives were approved. The residue of veterinary drugs in food has received great attention in recent years because of concerns over the request in food safety by consumers. The improper use of veterinary drugs (such as over-dose or not obeying the off-drug regulation) could lead to drug residue in animal products. A detrimental effect on human health could occur when people consume those products containing veterinary drug residues. To solve the problems of veterinary drug residue in animal products, related authorities should take extra efforts in monitoring veterinary drug usage and enforcing inspection of commercial products.

To prevent the abuse of veterinary drugs, the ROC Department of Health announced the "Tolerances for Residues of Animal Drugs"[(2)] in April 1987, in which the tolerance levels of 19 veterinary drugs in animal products were declared. The analytical methods for most of these drugs are established and some are still being determined. However, the established methods are capable of analyzing only one or a few drugs at one time. To improve the inspection efficiency, it is necessary to establish a method, which allows multiple drugs to be analyzed simultaneously. In compliance with the "Tolerances for Residues of Animal Drugs", 13 veterinary drugs with similar chemical properties, including 8 sulfadrugs, clopidol, furazolidone, and carbadox (which tolerance levels are available), and ormethoprim and ethopabate (which tolerance levels are not available) were selected in this study. The purpose of this research was to develop an analytical method for multiresidue determination of veterinary drugs in chicken and swine muscles.

According to the ROC "Tolerances for Residues of Animal Drugs", the tolerance levels for sulfadrugs in both chicken and swine muscles are set at 0.1 ppm and for clopidol are 5 and 0.2 ppm in chicken and swine muscles, respectively. Residual furazolidone is not allowed in animal products. The tolerance level for carbadox in swine muscles is 0.03 ppm; however, the residual carbadox in animal products is not permitted in the United States[(3)]. There is no tolerance level for ormethoprim and ethopabate according to the "Tolerances for Residues of Animal Drugs". In such cases, the residual ormethoprim and ethopabate in animal products are not allowable. In the US, the tolerance levels for the above two drugs in chicken are set at 0.1 and 0.5 ppm, respectively.

A satisfactory analysis of veterinary drugs merely using simple extraction and fixed wavelength detection is difficult to achieve due to various veterinary drugs possessing differ-

* Author for correspondence. Tel: 02-26531258;
Fax: 02-26531256; E-mail:kaoamy@nlfd.gov.tw

Journal of Food and Drug Analysis, Vol. 9, No. 2, 2001

ent physical and chemical properties. Several approaches to veterinary drug analysis have been reported, including using a single solvent system to extract several veterinary drugs with similar chemical properties from animal products followed by a single[4-9] or several cleanup steps[10, 11]. The mobile phase elution for HPLC analysis can be isocratic[4, 5, 7, 9], gradient[6, 12], or using several mobile phase systems, and the detection can be a fixed wavelength[3] or multiple wavelengths[5-10, 12]. Because the photodiode array (PDA) detector possesses the characteristics of wavelength selection and spectrum identification, HPLC equipped with PDA was therefore used as a tool to analyze 13 residual veterinary drugs in chicken and swine muscles in this study.

MATERIALS AND METHODS

I. *Materials*

Test samples, including 25 chicken (breast) and 25 swine muscle (loin) samples, were purchased from local markets in April 1999.

II. *Reagents*

(I) *Standards*

Sulfathiazole (STZ), sulfamonomethoxine (SMMX), clopidol (CLP), and furazolidone (FZD) were supplied by the Bureau of Standards, Metrology and Inspection, Ministry of Economic Affairs, ROC. Ormethoprim (OMP) and ethopabate (ETB) were obtained from the National Institute for Animal Health, Council of Agriculture, ROC. Sulfadiazine (SDZ), sulfamerazine (SMR), sulfamethazine (SMT), sulfamethoxazole (SMXZ), sulfaquinoxaline (SQX), sulfadimethoxine (SDMX), and carbadox (CDX) were purchased from the Sigma Chemical Co. (St. Louis, Missouri, USA).

(II) *Solvents and Other Reagents*

LC grade acetonitrile was purchased from Lab-Scan Co. (Dublin, Irene) and methanol was obtained from BDH (Poole, England). GR grade sodium dihydrogen phosphate, disodium hydrogen phosphate, and phosphoric acid (reagent grade) were purchased from Merck (Darmstadt, Germany).

(III) *Solid Phase Extraction Cartridge*

The Sep-Pak C_{18} cartridge (500 mg) was obtained from Waters Co. (Milford, Massachusetts, USA).

III. *Equipment*

1. ACE homogenizer: made by Nihonseiki Co. (Tokyo,

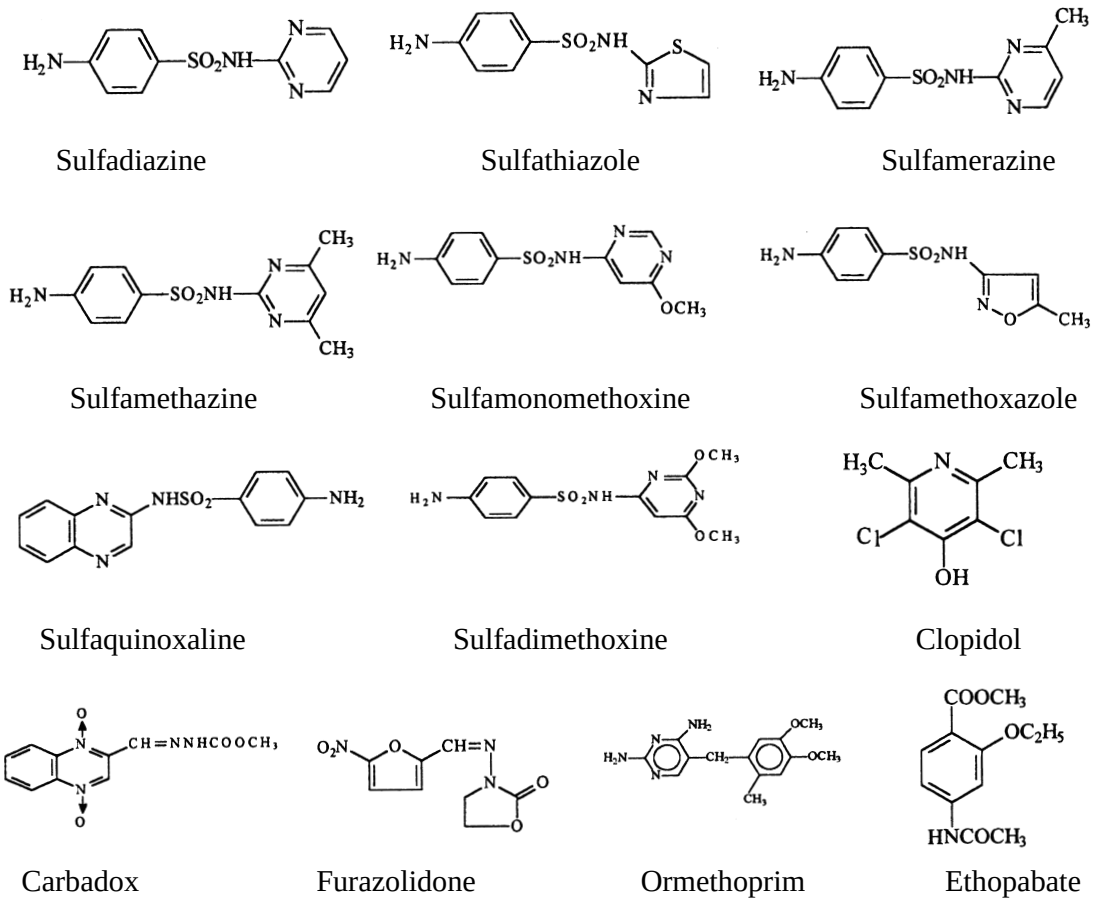

Figure 1. Chemical structures of 13 veterinary drugs.

Japan).

2. pH meter: made by Metrohm Co. (Herisan, Switzerland).

3. Rotary evaporator: made by Buchi Co. (Flawil, Switzerland).

4. High performance liquid chromatograph: Shimadzu LC-10AT equipped with a CBM-10A interface controller and an SPD-M6A photodiode array detector.

IV. *Preparation of 0.05M Sodium Dihydrogen Phosphate Solution*

A solution of 0.05M sodium dihydrogen phosphate was prepared and adjusted to pH 5.10 with 0.05M disodium hydrogen phosphate.

V. *Preparation of Standard Solution*

(I) *Standard Stock Solution*

CDX (50 mg), and FZD, OMP, ETB, SDZ, STZ, SMR, SMT, SMMX, SMXZ, SQX, and SDMX (100 mg of each) were separately weighed into a 100-mL volumetric flask and acetonitrile was added to the volume. CLP (100 mg) was accurately weighed and dissolved in 100 mL of acetonitrile/water (1/1, v/v) solution.

(II) *Standard Mix Solution*

The above stock solutions were diluted with acetonitrile/0.05 M sodium dihydrogen phosphate (3/7, v/v) to a series of concentrations ranging from 0.2 to 2.0 μg/mL.

VI. *Analytical Methods*

(I) *Extraction*

Test samples of chicken and swine muscles were homogenized. The homogenate (5 g) and 50 mL of acetonitrile were then added into a mixer and mixed for 3 min. After filtration, the residue was mixed with another 50 mL of acetonitrile. The mixing and filtration procedures were repeated. The combined filtrate was transferred into a separation funnel containing 30 mL of acetonitrile-saturated n-hexane and shaken for 5 min. The acetonitrile layer was collected into a concentration bottle and evaporated to dryness at 40˚C using a rotary evaporator.

(II) *Sample Cleanup Using a Sep-Pak C_{18} Cartridge*

The above dry residue was reconstituted with 20 mL of 0.05 M sodium dihydrogen phosphate and applied onto a Sep-Pak C_{18} cartridge, which was pre-conditioned with 10 mL of methanol and 10 mL of 0.05 M sodium dihydrogen phosphate. The concentration bottle was washed twice with 5 mL of sodium dihydrogen phosphate, which was then applied onto the same cartridge. The eluate was disregarded. The same concentration bottle was then washed twice with 5 mL of methanol and the resulting solution was passed through the same cartridge. The eluate was collected and evaporated to dryness at 40˚C using a rotary evaporator. The dry matter was reconstituted with 1 mL of acetonitrile/water (3/7, v/v) solution. After spiking 0.5 mL of acetonitrile-saturated n-hexane, the resulting solution was thoroughly mixed and then centrifuged at 3000 rpm for 5 min. The acetonitrile layer was collected and filtered through a membrane prior to HPLC analysis.

(III) *HPLC Analysis*

1. Analytical conditions

(1) Analytical column: Luna 5 μ C_{18} (2) column (25 cm × 4.6 mm i.d., 5μ), made by Phenomenex Co. (Torrance, CA, USA).

(2) Mobile phase: (A) acetonitrile, (B) 0.05 M sodium dihydrogen phosphate. A following gradient elution was performed:

Time (min)	Flow rate (mL/min)	%A	%B
0	1.00	10	90
5	1.00	15	85
20	1.00	15	85
35	1.00	30	70
45	1.00	10	90
60	1.00	10	90

(It took 65 min to make one run, 45 min for analysis and 20 min for equilibrium.)

(3) Detection wavelength: 200~400 nm.

(4) Wavelength resolution: 1 nm.

(5) Integration time: 0.64 sec.

(6) Injection volume: 20 μL.

2. Standard curves

Five concentrations (0.2, 0.5, 1.0, 1.5, and 2.0 of μg/mL) of mixed standard were prepared as described and 20 μL of each was injected. Standard curves were plotted according to the peak areas versus concentrations.

3. Quantification

Sample and standard solutions, 20 μL of each, were individually injected to the HPLC instrument. Peak identification was made by comparing the retention times and spectra of samples with those of standards. The following formula was used to calculate the amounts of veterinary drugs in test samples:

Amount of drug (ppm) = C x V/W

Where C is the drug concentration (μg/mL) calculated by standard curve, V is the volume of sample solution (mL), and W is the weight of sample (g).

VII. *Recovery Test*

Journal of Food and Drug Analysis, Vol. 9, No. 2, 2001

Recovery test was performed in triplicate by spiking standards at 3 levels (0.1, 0.2, and 0.4 ppm) into homogenate. The spiked samples and blank sample without standard were then analyzed by HPLC. Recovery was calculated by comparing the analyzed concentrations with spiked concentrations.

RESULTS AND DISCUSSION

I. *Study on the HPLC Conditions*

(I) *The Optimal Wavelength of Detection*

HPLC with photodiode array (PDA) detection has been used for multiresidue analysis of veterinary drug in many cases. In this study, a PDA detector was therefore used as a tool to optimize the wavelength for the detection of the 13 veterinary drugs. The UV spectra ranged at 200~400 nm for 13 veterinary drugs are shown in Figure 2 and 3. As can be seen, the maximum UV absorption of 8 sulfadrugs, CLP and ETB are at 250 nm; while the maximum UV absorption of other veterinary drugs are at 270 nm. Therefore, UV at 270 nm was used for the detection of most drugs in this study. The optimum detection wavelengths selected for 13 veterinary drugs analysis in this study are listed in Table 1. The optimum wavelengths for CDX and FZD detection were 308 and 368 nm, respectively. UV at 308 nm was selected for STZ analysis because the matrix interference at 308 nm was minimal. The absorbance intensity of OMP was decreased by increasing the wavelength detection. To minimize the matrix interference as well as maintain the absorbance intensity, the wavelength at 230 nm was selected for OMP detection.

(II) *Mobile Phase Selection*

A mobile phase with gradient elution has been widely used for multiresidual analysis of veterinary drugs. In this study, a mobile phase consisted of acetonitrile and sodium dihydrogen phosphate was selected according to Horie *et al*.[5] and Ishii *et al*.[6]. In our preliminary study, we found that the mixing ratio of the above two phases could significantly affect the analysis of some veterinary drugs. The CLP peak was split into double peaks when a mobile phase containing more than 20% acetonitrile was used. When a mobile phase containing more than 40% acetonitrile was used, the OMP peak split into 2 peaks. A similar phenomenon was observed by Keuo *et al*.[13]. Therefore, to analyze CLP and OMP, a acetonitrile concentration higher than 20% and 40%, respectively, should be avoided. In addition, the resolution of the peaks of veterinary drugs was affected by the pH of sodium dihydrogen phosphate solution. The effect of pH, ranging from 3 to 6 adjusted by 1% phosphoric acid or 0.05M disodium hydrogen phosphate, on the retention times of 13 veterinary drugs is shown in Figure 4. The retention times of the

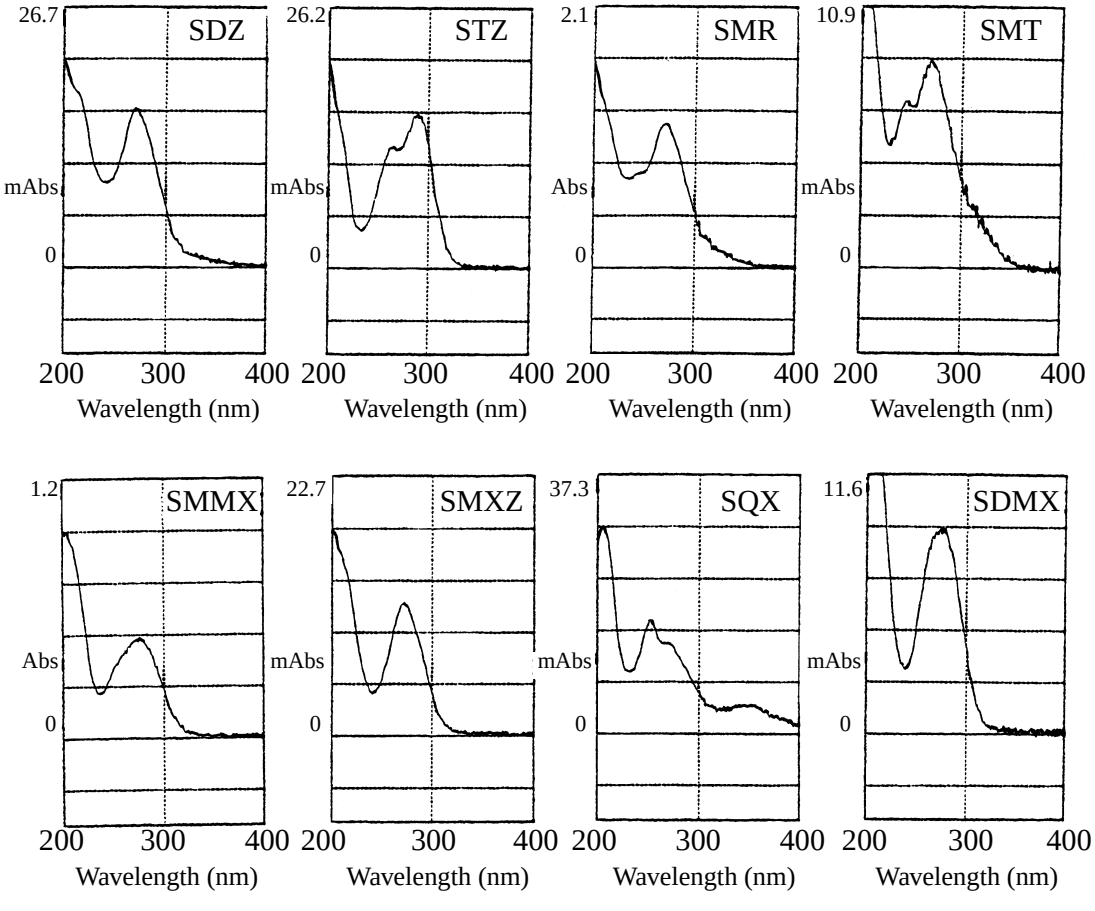

Figure 2. UV-absorption spectra of 8 sulfadrugs by photodiode array detector.

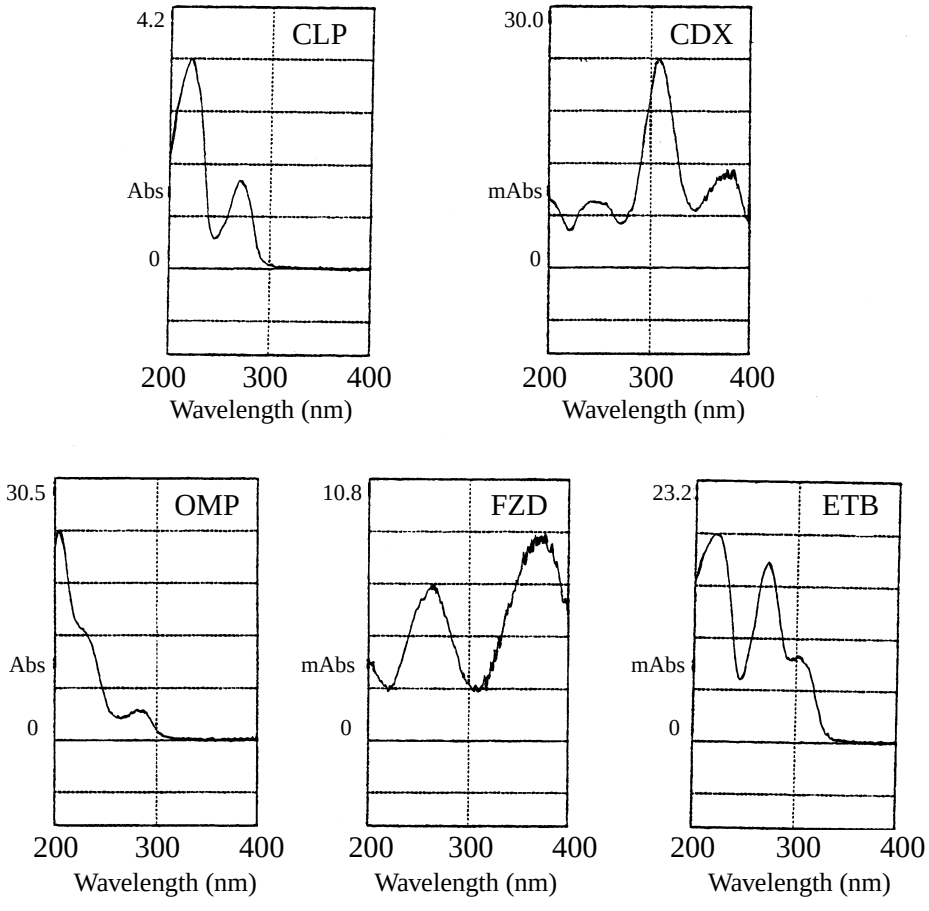

Figure 3. UV-absorption spectra of 5 veterinary drugs by photodiode array detector.

Table 1. Optimum detection wavelength of each veterinary drug analyzed by high performance liquid chromatography

Drug	λ_{max} (nm)
CLP[a]	270
SDZ	270
STZ	308
CDX	308
SMR	270
OMP	230
SMT	270
FZD	368
SMMX	270
SMXZ	270
ETB	270
SQX	270
SDMX	270

[a]CLP: clopidol; SDZ: sulfadiazine; STZ: sulfathiazole; CDX: carbadox; SMR: sulfamerazine; OMP: ormethoprim; SMT: sulfamethazine; FZD: furazolidone; SMMX: sulfamonomethoxine; SMXZ: sulfamethoxazole; ETB: ethopabate; SQX: sulfaquinoxaline; SDMX: sulfadimethoxine.

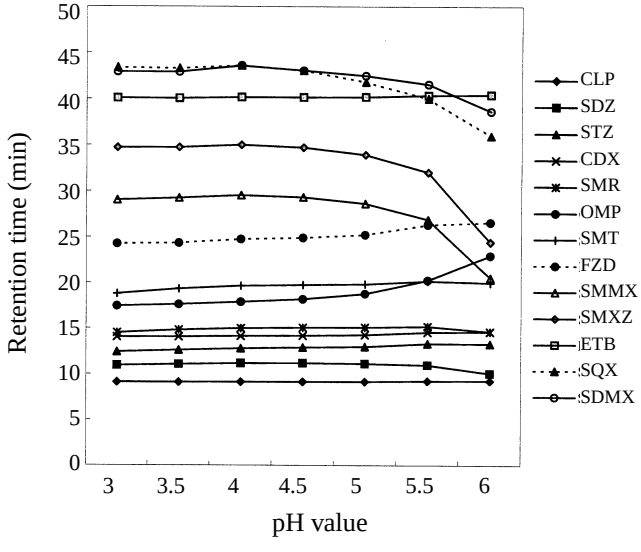

Figure 4. Effect of pH value in 0.05M sodium dihydrogen phosphate solution on the retention time of HPLC for each veterinay drug.

analyte were not affected by the mobile phase at pH lower than 4.5. However, under such conditions, the peak of SQX overlapped with the SDMX peak. Separation of these two peaks was achievable by increasing the pH of mobile phase higher than 5.0. This is due to a dissociation constant difference between SQX (pKa=5.5) and SDMX (pKa=5.9). Using a mobile phase with higher pH value was able to resolve the peaks of SQX and SDMX; however, at pH 5.5, an overlapping between ETB and SQX, FZD and SMMX, and OMP and SMT could occur. To optimize the resolution between SQX and SDMX, a mobile phase containing 0.05M sodium dihydrogen phosphate adjusted to pH 5.1 was thus used in

Journal of Food and Drug Analysis, Vol. 9, No. 2, 2001

this study. The HPLC chromatograms of 13 veterinary drugs by using a gradient elution of acetonitrile and 0.05M sodium dihydrogen phosphate (pH 5.1), a C_{18} analytical column, and a PDA detector, are shown in Figure 5. As can be seen, a satisfactory resolution for all analyte was obtained as using the HPLC conditions developed in this study.

II. *Sample Preparation*

(I) *Solvent Extraction*

Acetonitrile[10, 12], methanol[8], methanol/metaphosphoric acid solution[5], or acetonitrile/metaphosphoric acid solution are widely used as extraction solvents for multiresidue analysis of veterinary drugs in animal products. After extraction, a cleanup procedure using solid phase extraction (aluminum oxide[8] or C_{18}[5] cartridge) or liquid-liquid partition[8] (methylene chloride) is then carried out. Our preliminary study showed that methanol extract solution readily foamed, suddenly boiled, and took time to concentrate when evaporated under vacuum. Methanol/metaphosphoric acid and acetonitrile/metaphosphoric acid solutions are also not applicable because they are difficult to evaporate and can easily to block the cleanup cartridge. Acetonitrile was found to be the best solvent for sample extraction because it is easy to evaporate and the lipid/oil interference could readily be removed from the extract solution by introducing n-hexane.

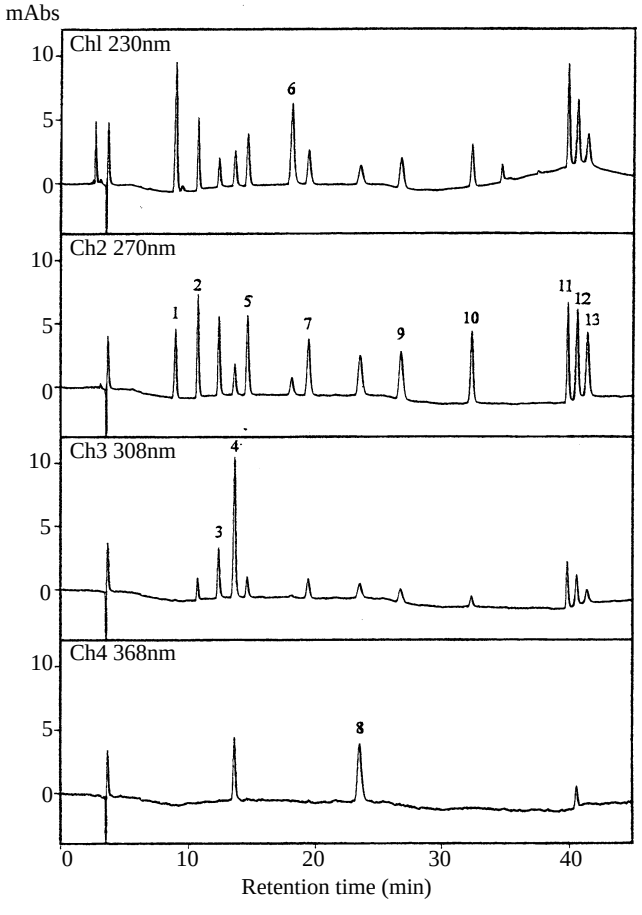

Figure 5. HPLC chromatograms of 13 veterinary drugs by photodiode array detector. HPLC conditions were described on text. Peaks: 1, clopidol; 2, sulfadiazine; 3, sulfathiazole; 4, carbadox; 5, sulfamerazine; 6, ormethoprim; 7, sulfamethazine; 8, furazolidone; 9, sulfamonomethoxine; 10, sulfamethoxazole; 11, ethopabate; 12, sulfaquinoxaline; 13, sulfadimethoxine.

(II) *Cleanup Conditions*

In the preliminary study, a de-fat acetonitrile extract without sample cleanup was analyzed by HPLC. Results showed that many co-extracts appeared on the chromatogram and interfered with the quantification of CLP, which was less retained on the column and co-eluted with other interference. To obtain a better result, a cleanup treatment is therefore required.

It has been reported by Hori[10] that sulfadrugs can be separated from other veterinary drugs using an aluminum oxide cartridge. Based on the cleanup treatment reported by Hori, in this study, veterinary drug standards were eluted from aluminum oxide cartridge with 85% methanol and methanol/acetic acid/water (30: 0.4: 70) followed by HPLC analysis. Results showed that separation of sulfadrugs from other spiked standards was not achievable. The recoveries of SMT, SQX, and SDMX were even less than 20%. A satisfactory result was not achieved after testing other cartridges and eluting conditions. Therefore, to search for a substitute cleanup method is necessary.

A cleanup method for analysis of 10 sulfadrugs using a C_{18} cartridge has been proposed by Horie *et al.*[5]. In this study, the acetonitrile extract was dissolved in 0.05M sodium dihydrogen phosphate solution, loaded onto a C_{18} cartridge, eluted with methanol, and analyzed by HPLC. It was found that not only the interference peaks were significantly

Table 2. Comparison of three kinds of C18 cartridges on the recovery of each veterinary drug

Drug	*Recovery (%)**		
	Sep-Pak C_{18} (360 mg)	Sep-Pak C_{18} (500 mg)**	Adsorbex RP-18 (400 mg)
CLP	54.2 ± 0.69^{b}***	87.7 ± 3.55^{a}	N.D.**** ^{c}
SDZ	45.2 ± 1.97^{b}	76.2 ± 3.96^{a}	N.D. ^{c}
STZ	71.0 ± 4.76^{b}	84.5 ± 6.23^{a}	N.D. ^{c}
CDX	88.9 ± 4.17^{a}	83.4 ± 2.71^{a}	12.6 ± 3.72^{c}
SMR	79.5 ± 6.04^{a}	82.8 ± 6.50^{a}	2.2 ± 3.15^{c}
OMP	89.8 ± 4.51^{a}	96.5 ± 0.58^{a}	14.1 ± 6.63^{c}
SMT	87.2 ± 5.80^{a}	84.6 ± 4.70^{a}	N.D. ^{c}
FZD	87.4 ± 4.51^{a}	94.5 ± 3.34^{a}	N.D. ^{c}
SMMX	89.8 ± 4.13^{a}	88.0 ± 4.25^{a}	N.D. ^{c}
SMXZ	85.1 ± 7.68^{a}	84.6 ± 3.68^{a}	N.D. ^{c}
ETB	92.9 ± 5.87^{a}	97.0 ± 1.85^{a}	61.6 ± 14.80^{b}
SQX	70.6 ± 6.86^{ab}	76.0 ± 1.92^{a}	46.6 ± 12.52^{bc}
SDMX	79.4 ± 8.26^{b}	82.3 ± 0.71^{a}	35.4 ± 13.44^{c}

* Mean ± S.D. (n=2), Chicken muscle was spiked with 0.2 ppm of each drug.

** Sorbent weight.

*** Means within a row followed by the same letter do not differ at the 5 % level by Duncan's multiple range test.

**** Not detected.

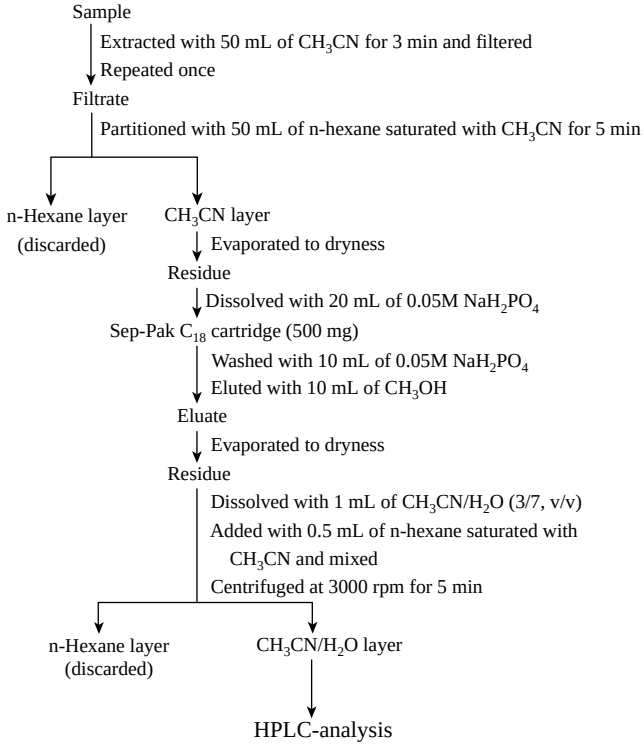

Figure 6. Analytical procedure for multiple residue of veterinary drugs in chicken and swine muscles.

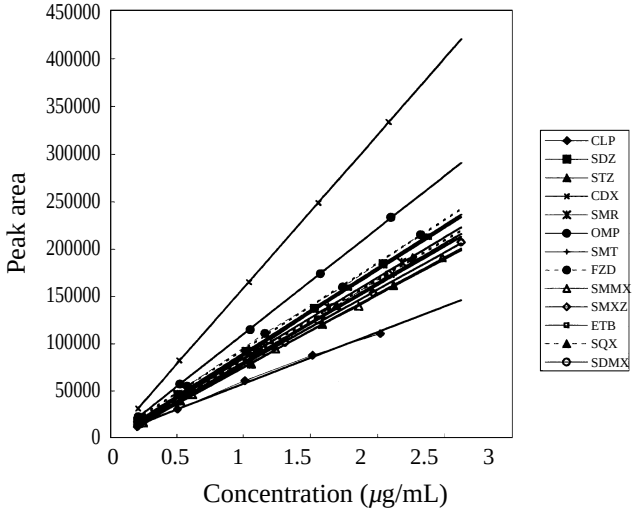

Figure 7. Standard curves of veterinary drugs with HPLC analyses.

reduced, but also the CLP peak clearly appeared on the chromatogram. Furthermore, 3 kinds of commercially available C_{18} cartridges were compared in the recovery of veterinary drugs after cleanup treatment. Results are shown in Table 2. A statistical study showed that the cartridge (Sep-Pak) packed with smaller sized (360 mg) packing material gave a lower recovery for polar compounds, such as CLP, SDZ, and STZ, as compared to the same brand 500 mg-packed cartridge. The Sep-Pak brand cartridges, both 360 and 500 mg-packing, showed higher recovery than the Adsorbex RP-18 (400 mg) cartridge. This result suggests that the ability to recover the veterinary drugs is not dependent on packing size but rather on the material size and shape, packing density, carbon content of packing material, and end capping treatment[5]. Based on the above study, the best cartridge for sample cleanup was found to be Sep-Pak C_{18} (500 mg).

To have more sulfadrugs retained in the cartridge, the

Table 3. Linear relationship of peak area versus concentration of each veterinary drug by high performance liquid chromatography

Drug	*Slope*	*Intercept*	r^2
CLP	68737	-1435.4	0.9999
SDZ	88531	-883.11	0.9998
STZ	46851	-1286.5	0.9999
CDX	158884	-5164.8	0.9998
SMR	85142	-2442.4	1.0000
OMP	121622	-1874.7	0.9999
SMT	84525	-4988.7	0.9998
FZD	83113	-7364.9	0.9948
SMMX	75568	-5115.9	0.9996
SMXZ	75757	-1645.3	0.9998
ETB	83763	-1125.5	0.9999
SQX	85551	-2153.7	0.9996
SDMX	80760	-4080.7	0.9999

Table 4. Recoveries of 13 veterinary drugs spiked into chicken muscle

Drug	Recovery (%)[a]		
	0.1 ppm[b]	0.2 ppm	0.4 ppm
CLP	85.9 (3.98)[c]	87.1 (4.67)	87.2 (2.62)
SDZ	71.9 (4.55)	75.1 (3.07)	74.2 (0.85)
STZ	86.5 (5.90)	84.4 (7.05)	84.3 (3.58)
CDX	77.2 (3.02)	82.4 (2.69)	87.0 (2.71)
SMR	90.7 (7.63)	88.1 (5.55)	90.1 (3.39)
OMP	90.9 (6.28)	96.1 (5.02)	92.5 (4.85)
SMT	90.4 (6.48)	84.1 (6.80)	87.6 (4.27)
FZD	87.8 (3.13)	88.4 (4.48)	90.6 (2.64)
SMMX	77.8 (7.59)	81.3 (1.99)	85.7 (1.12)
SMXZ	85.4 (3.59)	84.3 (4.03)	85.7 (2.00)
ETB	96.9 (4.90)	94.6 (3.51)	93.5 (2.03)
SQX	76.9 (6.62)	75.1 (4.51)	78.6 (1.97)
SDMX	82.7 (4.53)	81.2 (1.19)	82.1 (4.02)

[a] Average of triplicate.
[b] Spike level.
[c] Number in parentheses represents coefficient of variation (%).

Table 5. Recoveries of 13 veterinary drugs spiked into swine muscle

Drug	Recovery (%)[a]		
	0.1 ppm[b]	0.2 ppm	0.4 ppm
CLP	84.2 (5.36)[c]	86.9 (3.07)	85.4 (3.25)
SDZ	71.1 (5.89)	73.6 (1.16)	72.3 (4.18)
STZ	88.4 (4.15)	84.0 (2.42)	86.2 (3.17)
CDX	75.6 (5.91)	81.5 (3.11)	86.5 (2.74)
SMR	95.6 (0.39)	88.9 (2.18)	93.1 (2.38)
OMP	96.7 (2.16)	96.4 (0.92)	94.0 (1.36)
SMT	82.2 (6.05)	80.3 (2.22)	88.5 (2.27)
FZD	99.6 (5.30)	91.0 (4.94)	93.3 (1.31)
SMMX	77.8 (3.71)	83.6 (1.20)	90.5 (2.16)
SMXZ	82.4 (3.47)	84.4 (2.61)	88.5 (1.68)
ETB	91.4 (2.06)	95.2 (4.60)	96.6 (1.08)
SQX	71.7 (7.79)	73.1 (3.18)	79.5 (6.30)
SDMX	78.4 (4.67)	80.3 (4.93)	84.5 (4.83)

[a] Average of triplicate.
[b] Spike level.
[c] Number in parentheses represents coefficient of variation (%).

Journal of Food and Drug Analysis, Vol. 9, No. 2, 2001

cartridge needs to be treated with 0.05 M sodium dihydrogen phosphate to maintain a electron-neutral condition prior to sample application, because the sulfadrugs are amphoteric compounds with pKa in neutral or weak acid region. In addition, the eluate reconstituted with acetonitrile/water (3/7, v/v) needs to be extracted with n-hexane to remove oil/lipid and then centrifuged to obtain a clear solution prior to HPLC analysis. The operation procedure for the analysis of multiple residues of veterinary drugs is demonstrated in Figure 6.

III. *Standard Curve and Recovery Test*

The standard curves for the 13 veterinary drugs in the range of 0.2~2.0 μg/mL are shown in Figure 7. The slope, intercept, and linearity (r^2) of each curve are listed in Table 3. A satisfactory linearity with the r^2 greater than 0.99 was achieved.

A recovery test was performed in triplicate by spiking 0 (as blank), 0.1, 0.2, and 0.4 ppm of 13 veterinary drugs to 5 g ground samples. Test results are demonstrated in Table 4 and 5 and their HPLC chromatograms are shown in Figure 8. The average recoveries of 13 veterinary drugs in chicken and swine muscles were in the range of 71.9~96.9% and

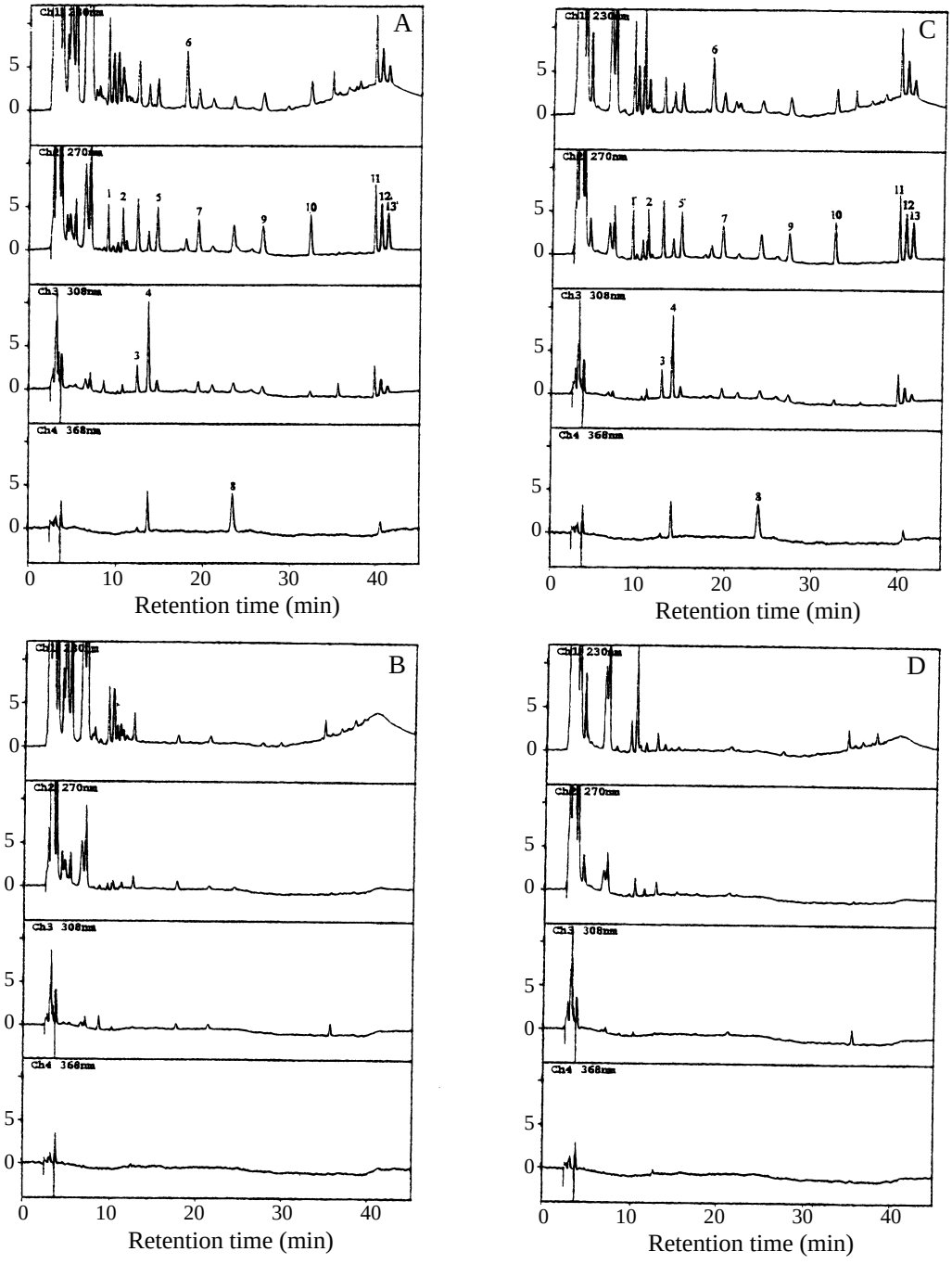

Figure 8. HPLC chromatograms of 13 veterinary drugs spiked into samples at the 0.2 ppm level (A,C) and blank sample (B,D). (A,B) Chicken muscle, (C,D) swine muscle. HPLC analytical conditions and peak numbers are the same as Figure 5.

71.1~99.6% with coefficient of variation ranging at 0.85~7.63% and 0.39~7.79%, respectively. Of the 13 veterinary drugs, SDZ and SQX showed the least recovery with average recoveries of 73.0 and 76.8%, respectively. This could be due to SDZ is a highly polar compound, which is less retained in C_{18} cartridge; while SQX shows less soluble in acetonitrile[13]. Except for SDZ and SQX, the average recoveries of other tested drugs were higher than 80%.

The detection limit for 13 veterinary drugs was also studied. Results showed that the detection limits of the tested drugs in both chicken and swine muscles were 0.02 ppm except for STZ, which detection limit was 0.04 ppm. Figure 9 shows the HPLC chromatograms for detection limit of 13 veterinary drugs.

IV. *Comparison of the Established Method in this Study and in Published Research*

Many methods for the multiple analysis of veterinary drugs have been developed on the basis of the drug varieties. In compliance with the "Tolerances for Residues of Animal Drugs" announced by the Department of Health, ROC, 13 veterinary drugs were selected in this study for developing a method of multiple residue analysis. A PDA detector, which possesses wavelength selection and spectrum identification characteristics, was used to detect the analyte eluted from HPLC column. Acetonitrile was used as an extraction solvent in this study in accordance with Murayama *et al.*[12] and Hori[10]. The extraction solution was extracted with n-hexane for removing fat/lipid and then analyzed by HPLC without passing through a cartridge for further cleanup according to analytical method reported by Murayama *et al.*[12]. This method resulted in many interference peaks showing in the front of HPLC chromatogram and peaks overlapping between FZD and SMMX, and SQX and SDMX. Therefore, it is not suitable for a quantification purpose. A cleanup

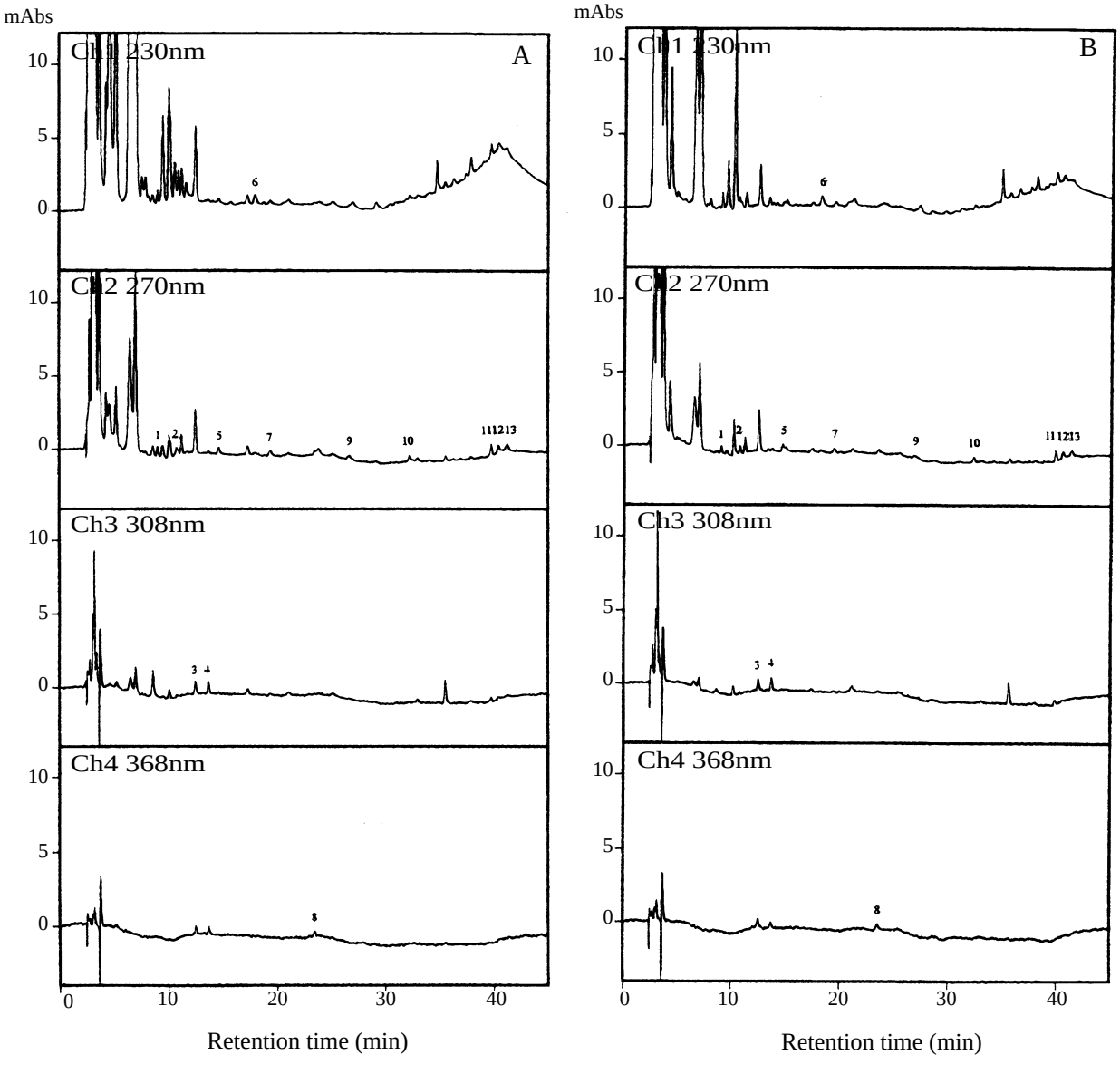

Figure 9. HPLC chromatograms for detection limit of 13 veterinary drugs. (A) Chicken muscle, (B) swine muscle. HPLC analytical conditions and peak numbers are the same as Figure 5.

Journal of Food and Drug Analysis, Vol. 9, No. 2, 2001

method using aluminum oxide and Sep-Pak C_{18} cartridges was introduced by Hori[10] to fractionate 11 veterinary drugs into 3 portions followed by HPLC analysis. This method is time-consuming and the fractionation of 13 studied veterinary drugs cannot be completed. Horie *et al*.[5] introduced an HPLC method using methanol/metaphosphoric acid as an extraction solvent and C_{18} cartridge for sample cleanup to analyze 10 sulfadrugs. This method is easy to operate but could result in blocking of the cleanup cartridge. The above method was modified by Ishii *et al*.[6] for HPLC analysis of 21 veterinary drugs by using a liquid-liquid partition with dichloromethane for sample cleanup. Using the modified method could give a cleaner chromatogram but less recovery caused by an emulsion during liquid-liquid partition. Using the HPLC condition developed by Ishii *et al*.[6] was not capable of giving a base-line separation between the peaks of FZD and SMMX, and SQX and SDMX. The method developed in this study used acetonitrile as an extraction solvent, n-hexane for removing fat/lipid as well as other co-extracts, and a Sep-Pak C_{18} cartridge for sample cleanup prior to HPLC analysis. This method is simple compared to the documented methods, capable of resolving FZD and SQX from SMMX and SDMX peaks, respectively, and can give more than 80% recovery of most test drugs (except for SDZ and SQX). The developed method in this study is recommended as an analytical method for multiresidual determination of 13 veterinary drugs, as it shows better precision and suitability as compared to methods in published reports.

V. *Investigation of Veterinary Drugs in Commercial Chicken and Swine Muscles*

Twenty-five samples each of chicken and swine muscles (50 samples in total) were collected from the retail markets in Taipei and analyzed using the developed method. No drug residue was detected in swine muscle samples, but SQX

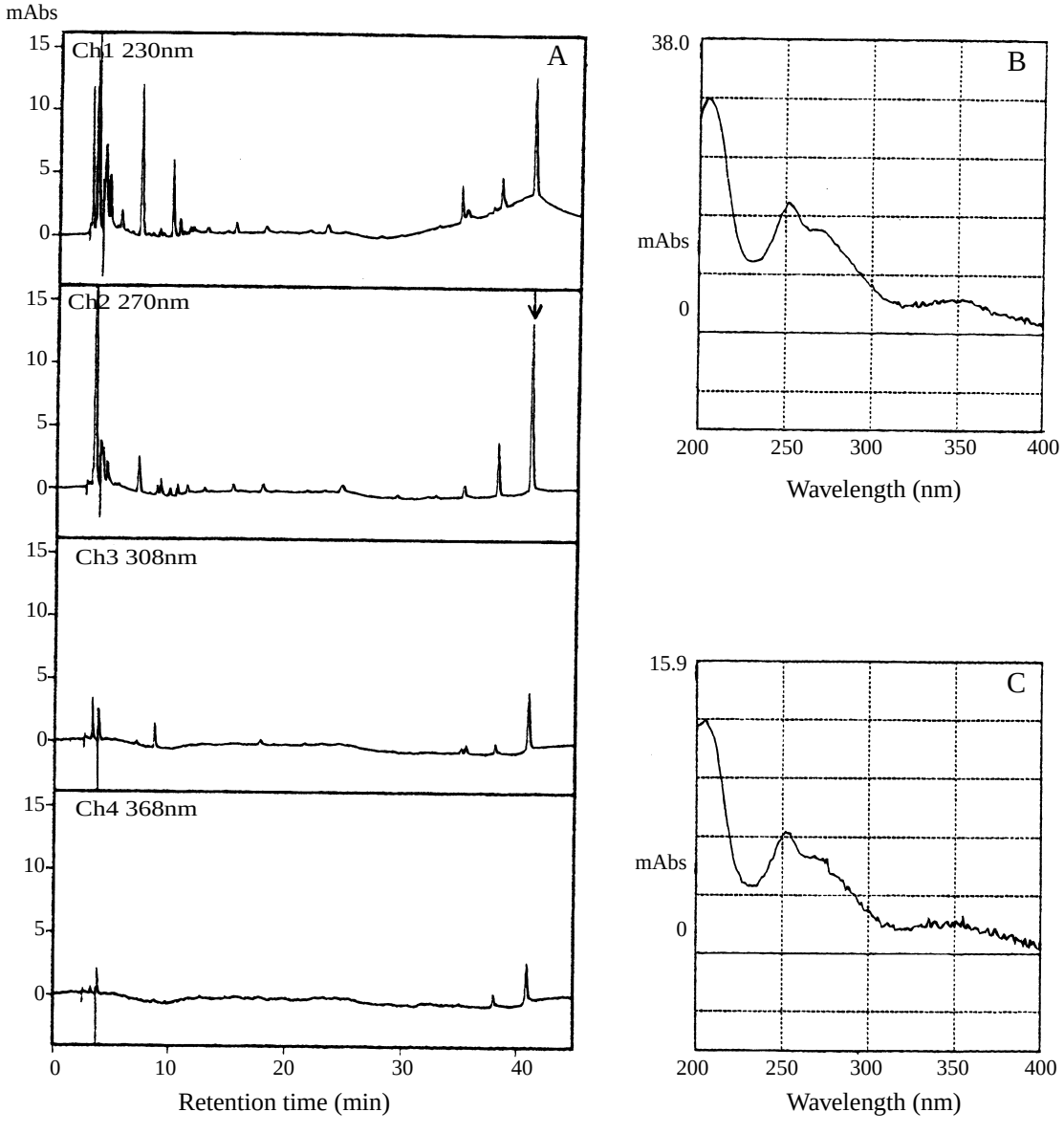

Figure 10. HPLC chromatograms for chicken muscle sample with sulfaquinoxaline residue of 1.23 ppm (A), and UV-absorption spectra of the peak from this sample (B) and sulfaquinoxaline standard (C). HPLC analytical conditions are the same as Figure 5.

(1.23 ppm) was found in 1 out of 25 chicken samples. The HPLC chromatogram of the detected sample is shown in Figure 10(A). SQX was further identified and confirmed by comparing the spectra of the sample and the standard (Figure 10 (B) and (C)). The similarity between these two peaks was 0.9986.

The regulated level for sulfadrugs in chicken and swine muscles is no more than 0.1 ppm according to the "Tolerances for Residues of Animal Drugs". One chicken sample with a SQX residue higher than the regulated level suggests that use of veterinary drugs, including usage, dosage, and off-drug regulation, is not strictly followed by some farmers. The results of this investigation could be a reference for authorities to further monitor the residue of veterinary drugs in commercial animal products and reinforce the administration of veterinary drug users.

CONCLUSIONS

A method using HPLC equipped with a PDA detector to simultaneously analyze 13 veterinary drugs (including 8 sulfadrugs) in chicken and swine muscles was developed in this study. This method is easy to operate and capable of removing most interference after cleanup with a Sep-Pak C_{18} cartridge. The average recoveries for most test drugs were greater than 80% except for SDZ and SQX. The detection limit was less than 0.04 ppm. PDA detector can be used to further confirm the compounds of interest by comparing the spectra of analyte with those of standards. The developed method is suggested to be a routine method for multiresidue analysis of veterinary drugs in animal products.

ACKNOWLEDGEMENTS

We would like to thank Dr. C. W. Chen for his translation work.

REFERENCES

1. Council of Agriculture, Executive Yuan. 1985. The Rules of Use of Feeds Additives Announcement. No. 65040. (in Chinese)
2. Department of Health, Executive Yuan. 1987. Tolerances for Residues of Animal Drugs. DOH Food No. 658449. April 11, Taipei. (in Chinese)
3. Federal Registration Office. 1997. 21 Code of Federal Regulations, Food and Drugs, part 556, The Office of the Federal Register National Archives and Records Administration, Washington, D. C., USA.
4. Haagsma ,N. and Van, D. W. C. 1985. Rapid determination of five sulphonamides in swine tissue by high performance liquid chromatography . J. Chromatogr. 333: 256-261.
5. Horie, M., Saito, K., Hoshino, Y., Nose, N., Hamada, N. and Nakazawa, H. 1990. Identification and quantitation of residual sulfa drugs in meat by high performance liquid chromatography with photodiode array detection. J. Food Hygi. Soc. Japan 31: 171-176.
6. Ishii, R., Horie, M., Hoshino, Y., Tokumaru, Y. and Nose, N. 1994. Simultaneous determination of residual synthetic antibacterials in fish and meat products by high performance liquid chromatography with photodiode array detector. J. Food Hygi. Soc. Japan 35: 173-179.
7. Long, A. R., Hsieh, L. C., Malbrough, M. S., Short, C. R. and Baker, S. A. 1990. Multiresidue method for the determination of sulfonamides in pork tissue. J. Agric. Food Chem. 38: 423-426.
8. Nagata, T. and Saeki, M. 1988. Simutaneous determination of 11 antibacteriales in chicken tissues by high performance liquid chromatography. J. Food Hygi. Soc. Japan 29: 13-20.
9. Thorpe, V. A. 1980. Sample preparation of carbadox, furazolidone, nitrofurazone and ethopabate in medicated feeds for high performance liquid chromatography. J. Assoc. Off. Anal. Chem. 63: 981-984.
10. Hori, Y. 1983. Systematic analysis of synthetic antibacterials in chicken muscles and eggs by high performance liquid chromatography . J. Food Hygi. Soc. Japan 24: 447-453.
11. Parks, O. W. 1985. Screening tests for sulfa drugs and/or dinitrobenzamide coccidiostats and their monoamino metabolites in chicken livers. J. Assoc. Off. Anal. Chem. 68: 20-23.
12. Murayama, M., Uchiyama, S. and Saito, Y. 1991. Rapid determination of residual synthetic antibacterials in fish and meat by high performamce liquid chromatography with stepwise gradient elution. J. Food Hygi. Soc. Japan 32: 155-160.
13. Kueo, M. Y., Lin, S. Y., Lin, K. M., Lin, M. C. and Liu. Y. F. Simultaneous determination of nineteen synthetic antibacterial drugs. Experimental Report of Taiwan Animal Health Research Institute. 33:103-116. (in Chinese)

Journal of Food and Drug Analysis, Vol. 9, No. 2, 2001

以高效液相層析法建立雞肉及豬肉中動物用藥之多重殘留檢驗方法

高雅敏*　張美華　鄭秋真　周薰修

行政院衛生署藥物食品檢驗局
台北市 115南港區昆陽街 161-2 號

（收稿： July 13, 2000 ；接受： September 21, 2000）

摘　要

本研究利用光二極體列陣檢測器之高效液相層析法建立雞肉及豬肉中13種動物用藥，包括氯煊啶（clopidol）、磺胺嘧啶（sulfadiazine）、磺胺噻唑（sulfathiazole）、卡巴得（carbadox）、磺胺甲基嘧啶（sulfamerazine）、歐美德普（ormethoprim）、磺胺二甲基嘧啶（sulfamethazine）、富來頓（furazolidone）、磺胺一甲氧嘧啶（sulfamonomethoxine）、磺胺噁唑（sulfamethoxazole）、衣索巴（ethopabate）、磺胺奎林（sulfaquinoxaline）及磺胺二甲氧嘧啶（sulfadimethoxine）等殘留量之檢驗方法。雞肉及豬肉檢體以乙腈萃取，經過濾、以乙腈飽和之正己烷去除雜質及濃縮後，以Sep-Pak C_{18}過濾層析匣淨化，最後利用高效液相層析儀分析定量。所使用之層析管柱為Luna 5 μ C_{18}（2）（25 cm × 4.6 mm i.d., 5 μm），並採用乙腈與磷酸二氫鈉溶液為梯度移動相溶液，以光二極體列陣檢測器偵測。檢體中添加13種動物用藥0.1、0.2及0.4 ppm時，其回收率於雞肉及豬肉中分別為71.9　96.9%及71.1　99.6%，變異係數介於0.85　7.63%及0.39　7.79%之間。本方法在此二檢體中之檢出限量除磺胺噻唑為0.04 ppm外，其餘均為0.02 ppm。將此方法應用於市售雞肉及豬肉之殘留量分析，結果於50件檢體中檢出1件雞肉殘留磺胺奎林1.23 ppm。

關鍵詞：動物用藥，多重殘留，光二極體列陣檢測器，高效液相層析