A Multiresidue Method for the Determination of Abamectin, Doramectin, Moxidectin, Ivermectin, Milbemectin A₃, and Milbemectin A₄ Residues in Bovine Muscle Using HPLC with Fluorescence Detection

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ABSTRACT

Abamectin, doramectin, moxidectin, ivermectin, milbemectin A₃ and milbemectin A₄ are similar macrocyclic lactone chemicals used as parasiticides or acaricides. A method using high performance liquid chromatography (HPLC) with fluorescence detection is presented for the simultaneous determination of the residue amounts of these compounds in bovine muscle. Samples are extracted using acetonitrile and cleaned up with solid phase extraction using a C₁₈ column, followed by fluorescence-derivatized with 1-methylimidazole and trifluoroacetic anhydride in acetonitrile. The analogue was measured by HPLC with fluorescence detector at 365 nm excitation and 470 nm emission wavelengths. The limits of quantification are below the stipulated Taiwan Maximum Residue Limit for each compound. The recoveries of this method in bovine muscle ranged from 73.3 to 110%, with a RSD from 2.11 to 16.57%. The detection limit of those 6 compounds in bovine muscle was 5 ppb. No any above compounds were detected in 50 samples of bovine muscle tested. Therefore, the developed method can be used for rapid screen of macrocyclic lactones in bovine muscle.

Key words: macrocyclic lactone, high performance liquid chromatography, abamectin, doramectin, moxidectin, ivermectin, milbemectin A₃, milbemectin A₄

INTRODUCTION

Macro cyclic lactone, an antibiotic, is the fermentation product of Actinomycetes, genus Streptomyces, in soil. It can be classified into two groups: avermectins and milbemycins. The difference between these two is that avermectins have a disaccharide oxy residue at C₁₃, while milbemycins do not. Currently, domestic animal drugs with residue standards include abamectin, ivermectin, doramectin, moxidectin, and eprinomectin(¹); pesticides with safety tolerance specifications include abamectin, and mibemectin(²) (Figure 1).

Macro cyclic lactone is a strong pesticide for cow, sheep, pig, and horse. It presents good efficacy not only in killing interior nematodes, but also exterior arthropods. It is also named endectocides(³). Its effective dose is very low, 0.2~0.5 mg/kg for animals. Studies showed that even macro cyclic lactone does not show any mutagenicity and carcinogenicity, but embryotoxicity might be possible(⁴). According to MARTINDALE, the Extra Pharmacopoeia, ivermectin may induce mild Mazzotti reaction, including fever, pruritus, arthralgia, myalgia, postural hypotension, oedema, lymphadenopathy, gastrointestinal symptoms, soar throat, cough, and headache(⁵).

In the January 8, 2003, announcement of The Department of Health (DOH), Executive Yuan, Taiwan, Food Code No 0900002580(¹), the “Residual Limit of Animal Drugs” listed macrocyclic lactone, but without its analysis methodology. In this study, we tried to establish a multi-residue HPLC method for analyzing such a compound. Meanwhile, milbemectin, structurally in the milbemycins group, was initially found with acaricidal effect. Its anthelmintic effect was not discovered until avermectins were analyzed(⁶). According to current regulation,

\[
<table>
<thead>
<tr>
<th>Compound</th>
<th>Species</th>
<th>Tissue/product</th>
<th>Tolerance (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abamectin</td>
<td>Cattle</td>
<td>Muscle and fat</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Kidney</td>
<td>0.05</td>
</tr>
<tr>
<td>Doramectin</td>
<td>Cattle</td>
<td>Muscle</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Swine</td>
<td>0.035</td>
<td></td>
</tr>
<tr>
<td>Ivermectin</td>
<td>Cattle</td>
<td>Liver</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Swine, goat, sheep and poultry</td>
<td>0.015</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fat</td>
<td>0.02</td>
<td></td>
</tr>
<tr>
<td>Moxidectin</td>
<td>Cattle and deer</td>
<td>Muscle</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>Sheep</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cattle, sheep and deer</td>
<td>Liver</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>Kidney</td>
<td>0.05</td>
<td></td>
</tr>
</tbody>
</table>

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milbemectin is not allowed in the muscle, fat, kidney, liver, milk or egg of poultry and meat, but is allowed as an anthelmitic in vegetables. Due to structure similarity, milbemectin was also analyzed in this study. Comparing the analysis methods in the reviewed papers, we modified Roudaut’s HPLC method, which was published in 1998, for the simultaneous quantification of abamectin, doramectin, ivermectin and moxidectin residues. Two milbemycins, i.e., milbemectin A3 and milbemectin A4, were included in the analysis. We tried to establish an optimal derivatization and HPLC condition, not only for the analysis of the above mentioned 4 components, but also for analyzing milbemectin. Milbemectin, though popularly used in western countries, has not been imported to Taiwan due to its high price. There are about 60 thousand tons of fresh or frozen beef imported into Taiwan each year. In this study, a rapid and convenient multiresidue method for the analysis of bovine muscle contamination was established in order to ensure public health and monitor food safety in preparation after Taiwan joined the World Trade Organization.

### MATERIALS AND METHODS

#### I. Materials

(I) Samples

Fifty refrigerated and frozen bovine muscle samples,
such as rib eye, rib finger, round and flank, were purchased from domestic traditional markets and supermarkets in mid-Taiwan, including Taichung county, Taichung city, Changhua county, Nantou county. Other samples were imports from Australia, U.S.A., Canada, and Netherlands. The samples used in the recovery test and sensitivity test were pre-screened to be free from containing tested components.

(II) Reagents

Methanol and acetonitrile, HPLC grade, were from LAB SCAN (Bangkok, Thailand). 1-Methylimidazole and trifluoracetic anhydride (99%) were from Aldrich (Milwaukee, WI, U.S.A.). Acetonitrile anhydrous (max. 0.005% H₂O), glacial acetic acid and triethylamine were from Merck (Darmstadt, Germany). Sodium sulfate anhydrous was from FSA (Loughborough, England).

(III) Reference standards

(1) Abamectin: from Chem-Service (Pennsylvania, U.S.A.), potency 3% B(la)ii - 91% B(lb)ii.
(2) Doramectin: from Pfizer Co. (Nagoya, Japan), potency 98.2%.
(3) Moxidectin: from Cyanide Co. (Princeton, NJ, U.S.A.), potency 98.7%.
(4) Ivermectin Bi: from Sigma Co. (St. Louis, MO, U.S.A.).
(5) Milbemectin A₃ (98.7% potency) and milbemectin A₄ (99.6% potency): from Hayashi Pure Chemical Industries Ltd. (Osaka, Japan).

(IV) Equipments

(1) HPLC: Shimadzu LC-10AT vp.
   b. Column: Merck Lichrospher 100RP-18E, 5 μm (125 × 4 mm), precolumn with same material.
   c. Auto-sampler: Shimadzu SIL-10A.

(II) Methods

Table 3. Summary of 3 multiresidue methods using HPLC for macrocyclic lactones

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Column</td>
<td>Lichrospher 100RP-18E</td>
<td>Zorbax ODS C18</td>
<td>Wakosil -3C18HG,</td>
</tr>
<tr>
<td></td>
<td>4 × 125 mm</td>
<td>4.6 × 150 mm</td>
<td>4.6 × 150 mm</td>
</tr>
<tr>
<td></td>
<td>35°C</td>
<td>RT*</td>
<td>50°C</td>
</tr>
<tr>
<td>Mobile phase</td>
<td>Acetonitrile/water</td>
<td>Methanol/water</td>
<td>Acetonitrile/H₂O</td>
</tr>
<tr>
<td></td>
<td>(94/6, v/v)</td>
<td>Isocratic</td>
<td>Isocratic</td>
</tr>
<tr>
<td>Elution sequence of compounds</td>
<td>1. moxidectin</td>
<td>1. eprinomectin</td>
<td>1. milbemectin A₃</td>
</tr>
<tr>
<td></td>
<td>2. abamectin</td>
<td>2. moxidectin</td>
<td>2. milbemectin A₄</td>
</tr>
<tr>
<td></td>
<td>3. doramectin</td>
<td>3. abamectin</td>
<td>3. abamectin</td>
</tr>
<tr>
<td></td>
<td>4. ivermectin</td>
<td>4. doramectin</td>
<td>4. ivermectin</td>
</tr>
<tr>
<td></td>
<td>5. ivermectin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Limits of quantification</td>
<td>7.5 ppb</td>
<td>10 ppb</td>
<td>0.1 - 0.3 ppt**</td>
</tr>
<tr>
<td>Derivatization reaction</td>
<td>RT</td>
<td>65°C, 90 min</td>
<td>Cooling box</td>
</tr>
<tr>
<td>Time of chromatogram</td>
<td>ca. 20 min</td>
<td>ca. 10 min</td>
<td>ca. 30 min</td>
</tr>
<tr>
<td>Mean of recovery</td>
<td>77.8 - 89.9%</td>
<td>71.9 - 84.4%</td>
<td>ca. 80 - 110%</td>
</tr>
<tr>
<td>from bovine muscle</td>
<td>from beef liver</td>
<td>from crops</td>
<td></td>
</tr>
</tbody>
</table>

*RT: room temperature.
**Detection limits.

(2) Homogenizer: SMT Process Homogenizer, Japan.
(3) Centrifuge: Hettich Universal 30 F.
(4) Vortex mixer: IKA works MS1 minishaker.
(5) Oven: Memmert ULM - 500.
(6) Others:
   a. C18 solid-phase cartridge: Waters C18 cartridge, 100 mg, 1 cm³.
   b. Filter paper: 0.45 μm, nylon.
   c. LC sample tube: 200 μL.
   d. Brown sample tube: 1.8 mL, screw-capped.

(V) Preparation of mobile phase

Acetonitrile (940 mL) was added with 60 mL of water. When gently mixed, degassed with sonication, and filtration, the filtrate was used as the mobile phase.

(VI) Preparation of derivatization reagent

The derivatization reagent was freshly prepared with anhydrous and light protected conditions.

Derivatization reagent (2): Trifluoracetic anhydride and acetonitrile anhydrous mixed gently (1:1, v/v).

(VII) Analysis condition of HPLC

Fluorescence detector: agitation wavelength 365 nm, emission wavelength 470 nm.
Mobile phase: According to Method I. (V)
Flow rate: 1.2 mL/min.
Standard curve

1. Preparation of standard solutions

Accurately weighted abamectin, doramectin, moxidectin, ivermectin, milbemectin A₃, and milbemectin A₄ were individually placed into brown volumetric bottles. The concentration was adjusted to 0.05 mg/mL with acetonitrile, and these served as the stock standard solutions. Adequate amounts of each solution was mixed with anhydrous acetonitrile, diluted in concentration to 3, 2, 1, 0.5, 0.25, 0.1, 0.05, 0.04, 0.025, 0.02 and 0.01 ppm, and these served as the mixed working standard solutions. The solutions were stored at -20°C.

2. Derivatization reaction

The whole procedure was carried out at anhydrous and light-protected conditions. When 0.1 mL of above mixed working standard solution was individually placed in a capped brown sample bottle, 0.2 mL of derivatization reagent (1) was added. Vortex mixing for 2 min, 0.2 mL of derivatization reagent (2) was added. Vortex mixing for 1 min, 10 µL of glacial acetic acid was added. After vortex mixing for 1 min, placed in a 60°C sand bath for 30 min, and then in a 0°C ice bath for 5 min, the solution was filtrated with 0.45 µm filter and ready for HPLC analysis.

3. Plotting the standard curve

After derivatization reaction, the individually mixed working standard solution was placed into HPLC sample tube. Individual sample was analyzed in triplicate with HPLC under the above mentioned condition. The standard curves were plotted with the mean of the area under curve (AUC).

Preparation of sample solutions

1. Extraction and purification

One gram of ground bovine muscle, in centrifuge tube, was mixed with 10 mL of polypropylene. After Vortex mixing with 0.1 mL of methanol and 1 mL of acetonitrile for 1 min, centrifuged at 4,800 rpm/min for 10 min, the supernatant was placed into a brown tube. The pellet was mixed with 0.5 mL of acetonitrile, centrifuged under the same condition, and the supernatant was combined with the previous one. After mixing gently with 2 mL of water, the supernatant was loaded into the C18 solid-phase cartridge at the flow rate of 0.2 mL/min. The cartridge was previously activated by 5 mL of acetonitrile, and 5 mL of acetonitrile/water (3/7, v/v, containing 0.1% triethylamine). The brown tube was washed with 1 mL of acetonitrile/water (3/7, v/v, containing 0.1% triethylamine), and passed through the C18 solid-phase cartridge for 1 min. The sample solution was extracted with 1 mL of acetonitrile/water (9/1, v/v), collected in a tube with 0.3 g of sodium sulfate anhydrous. After mixing, centrifuged at 4,800 rpm/min for 5 min, the supernatant was placed in a brown tube, air-dried with nitrogen, and 0.1 mL of anhydrous acetonitrile was added to elute for the derivatization reaction.

2. Recovery test

One gram of ground bovine muscle, in centrifuge tube, was mixed with 10 mL of polypropylene. Mixed working standard solution (0.1 mL) at the concentration of 3, 2, 1, 0.5, 0.3, 0.2, 0.1, and 0.05 ppm was mixed and left standing for 15 min. The sample was extracted, purified and derivatized according to the above method, then placed into sample tubes. Each sample was auto-injected into HPLC and analyzed in triplicate.

Determination of the limit of detection (LOD)

A series of low-concentration of mixed working standard solution in 0.05, 0.04, 0.025, 0.02, and 0.01 ppm were prepared and derivatized as described in the above mentioned method. Each sample solution (0.1mL) was auto-injected into HPLC and analyzed in triplicate.

According to the ICH (International Conference on Harmonization) Q2A guideline, Validation Operation of Analytical Method (DOH), the linear regression of each standard solution was plotted by peak area vs concentration. The individual slope (S) and mean standard deviation (σ) were analyzed and the LOD was calculated by the equation:

\[ \text{LOD} = 3.3 \times \sigma / S \]

Determination of the limit of quantitation (LOQ)

Using the LOD method, the LOQ was calculated by the equation:

\[ \text{LOQ} = 10 \times \sigma / S \]

Precision evaluation

The recovery tests in triplicate were conducted by different personnel at different times and statistically analyzed with Microsoft Excel 7.0 and Statistical Analysis System (SAS) in the workstation of the Education Bureau. Covariant analysis with ANOVA, the recovery rate, the standard deviation and coefficient of variance were calculated individually.

RESULTS AND DISCUSSION

In this study, we tried to analyze 6 macrocyclic lactones, including milbemectin A₃, moxidectin, milbemectin A₄, abamectin, doramectin, and ivermectin, at the same time with a HPLC equipped with fluorescent detector. The individual influence factor on the results is discussed below.

Methodology Development

The factors that influence the peak height of the derivatives...
Results in Table 4 indicate the variation of peak area of each derivative when derivatization was performed under different temperatures. Derivatives of abamectin, doramectin, moxidectin and ivermectin at 60°C increased 6–10%, compared to those at 25°C. The results are similar to the study of Sher et al. The peak areas of the derivatives of milbemectin (including milbemectin A3 and milbemectin A4) increased after 24 hr when derivitized at room temperature. This indicates the derivatization reaction is very slow at room temperature, and temperature increases could accelerate the reaction. Instant heat release was observed when the two derivatization reagents were added, raising the temperature to 45°C. Therefore, increasing the temperature to 50°C does not significantly change the peak area. When at 60°C and 65°C, the peak area of milbemectin increased significantly compared room temperature. Because the boiling point of acetonitrile is 81°C, too much heat could cause vaporization of acetonitrile and affect final concentration of the derivatives. It is considered that 60°C is the appropriate temperature. Four hours after the derivatization reagents were added, the peak areas of derivatives of abamectin, doramectin and ivermectin decreased. To improve the stability of derivatives, we tried to change the pH condition. After derivatization reagents were added, 10µL of glacial acetic acid was added to acidify the derivatization condition. When 100 ppb of mixed standard solution was placed under the derivatization condition, the variation in peak area was less than 4% in 12 hr. It is possible that pH change stabilized the derivatives. Reaching the same conclusions as of Martin et al., the addition of glacial acetic acid can stabilize derivatives of abamectin, doramectin, moxidectin and ivermectin. Also, in our study, acetic acid can also stabilize the derivatives of milbemectin A3 and milbemectin A4. It is worth to address that, during derivatization, anhydrous condition and light protection should be maintained because trifluoroacetic anhydride could be hydrolyzed and interfere the peak height of chromatogram.

(II) Linear relationship of standard curves

Table 4. Comparisons of peak areas of the macrocyclic lactones (100 ppb) at different temperatures after 30 min derivatization

<table>
<thead>
<tr>
<th>Temp. (°C)</th>
<th>Milbemectin A3</th>
<th>Milbemectin A4</th>
<th>Moxidectin</th>
<th>Abamectin</th>
<th>Doramectin</th>
<th>Ivermectin</th>
</tr>
</thead>
<tbody>
<tr>
<td>25</td>
<td>375.5 ± 12.3ab</td>
<td>231.6 ± 10.3a</td>
<td>282.5 ± 09.8ab</td>
<td>188.0 ± 5.4b</td>
<td>184.7 ± 1.9d</td>
<td>198.0 ± 5.7b</td>
</tr>
<tr>
<td>50</td>
<td>401.5 ± 13.2ab</td>
<td>245.5 ± 11.2a</td>
<td>299.6 ± 10.4ab</td>
<td>188.0 ± 5.4b</td>
<td>195.0 ± 2.0c</td>
<td>198.0 ± 5.6b</td>
</tr>
<tr>
<td>60</td>
<td>494.4 ± 16.2a</td>
<td>284.2 ± 11.9a</td>
<td>312.5 ± 10.8a</td>
<td>203.0 ± 5.8a</td>
<td>205.3 ± 2.1b</td>
<td>211.2 ± 6.1a</td>
</tr>
<tr>
<td>65</td>
<td>505.5 ± 16.6a</td>
<td>294.4 ± 11.8a</td>
<td>325.3 ± 11.3a</td>
<td>210.5 ± 6.0a</td>
<td>225.8 ± 6.2a</td>
<td>211.6 ± 6.6a</td>
</tr>
</tbody>
</table>

* Data are mean ± standard deviation, n = 3.
** Values in a column with the different superscripts are significantly different (P < 0.05).

Table 5. The linear regression of standard anthelmintic drugs obtained at various concentrations

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (ppb)</th>
<th>r²</th>
<th>Slope</th>
<th>Intercept</th>
</tr>
</thead>
<tbody>
<tr>
<td>Milbemectin A3 (1-300)</td>
<td>0.9994</td>
<td>+5029.3</td>
<td>-4701.9</td>
<td></td>
</tr>
<tr>
<td>Moxidectin (1-300)</td>
<td>0.9992</td>
<td>+3228.0</td>
<td>+4181.2</td>
<td></td>
</tr>
<tr>
<td>Milbemectin A4 (1-300)</td>
<td>0.9995</td>
<td>+2918.1</td>
<td>-3196.5</td>
<td></td>
</tr>
<tr>
<td>Abamectin (1-300)</td>
<td>0.9990</td>
<td>+2089.3</td>
<td>-2202.4</td>
<td></td>
</tr>
<tr>
<td>Doramectin (2-300)</td>
<td>0.9995</td>
<td>+2118.6</td>
<td>-2610.2</td>
<td></td>
</tr>
<tr>
<td>Ivermectin (2-300)</td>
<td>0.9995</td>
<td>+2157.1</td>
<td>-2780.6</td>
<td></td>
</tr>
</tbody>
</table>

0.9995, 0.9990, 0.9995 and 0.9995, respectively. All of them are above 0.9990. For milbemectin A3, moxidectin, milbemectin and abamectin, good linearity was shown between 1-300 ppb. For doramectin and ivermectin, the linear curve can also be shown between 2-300 ppb.

(III) LOD and LOQ of reference standard

As indicated in Table 6, different low concentrations of mixed working standard solutions were prepared in 1, 2, 2.5, 4 and 5 ppb. After derivatization and chromatography in triplicate, the mean, SD, slope of regression curve and intercept of regression can be calculated. According to the equation, theoretical LOD and LOQ of milbemectin A3, moxidectin, milbemectin A4, abamectin, doramectin and ivermectin were calculated. The LODs of each compound are 0.42, 0.21, 0.44, 0.44, 0.39 and 0.33 ppb, respectively. The LOQs of each compound are 1.27, 0.64, 1.34, 1.32, 1.19 and 1.00 ppb, respectively.

(IV) Extraction and purification of spiked samples

Taiwan is a small country with lot of people. The farm lands are usually small. Most of the beef consumption relies on importation. Due to large demand for beef and that macrocyclic lactones are popularly used in western countries, we chose beef as the study subject. Acetonitrile is used as the extraction solvent and the modified Roudaut’s method is used for the purification of bovine muscle samples.
decayed by light, heat and acid, nearly insoluble in water but easily dissolve in methanol, methylene chloride, isopropyl alcohol, acetonitrile, etc. Based on Roudaut’s method, we used acetonitrile as the extractant because less solvent can be used and a more efficient extraction could be obtained, compared to Lori’s method\(^{11}\), where methylene chloride/acetone (1/1, v/v) was used as the extractant.

According to the purification procedure described in Roudaut’s method, acetonitrile (~3 mL) should be mixed with 7 mL of water before purification by loading into a solid phase extraction cartridge (C18). In reality, the recovery rate is very low and it’s time-consuming when loading samples into the cartridge. If we reduce water to equal the volume of extractant (2-3 mL) before loading samples into a cartridge, the recovery rate increases. The low recovery rate may be resulting from small chances for compound interaction with elements in the cartridge due to too much water. Such viewpoint matches the theory in the Waters Sep-Pak Cartridges Care and Use Manual\(^{12}\).

In addition, after sample extract was loaded, the solid-phase cartridge should be washed with 1 mL of acetoni-trile/water (7/3, v/v) before extraction, according to Roudaut’s method. But in reality, we obtained the same results if cartridge was extracted directly with acetonitrile/water (9/1, v/v).

II. Methodology Evaluation

(I) The detection limit of methodology

According to Waters\(^{13}\), the sensitivity of HPLC detector, i.e., the S/N value (signal-to-noise ratio, the ratio of peak signal to noise), is usually at 2/1 or 3/1. If S/N value of LOQ reaches 10/1, it indicates good precision and accuracy. When the mixed working standard solutions with theoretical values of LOD and LOQ were prepared, i.e., 0.5 ppb and 1.5 ppb, and analyzed in triplicate, the respective S/N values were observed. At 0.5 ppb, the S/N values of milbemectin A, moxidectin, and milbemectin A were higher than 8; while abamectin, doramectin, and ivermectin were less than 3. At 1.5 ppb, the S/N value of each compound was higher than 5. In recovery test, the bovine muscle samples were spiked with 2 ppb and 5 ppb mixed working standard solutions. At 2 ppb, the S/N values of milbemectin A, moxidectin, milbemectin A and abamectin were higher than 4; while at 5 ppb, higher than 10. At 2 ppb, the S/N values of doramectin and ivermectin were higher than 3; while at 5 ppb, higher than 8. The method that we developed obtained LOD at 2 ppb and LOQ at 5 ppb, compared with Roudaut’s method with LOQ at 7.5 ppb.

(ii) Recovery test and precision

Figure 2 shows the HPLC chromatogram of mixed standard solutions with a good specificity of the developed method. Table 7 also shows a good precision of the developed method. The recovery rate and relative standard deviation (% RSD) of individual component at various concentrations were analyzed in triplicate by different personnel at different times using the above mentioned addition, extraction, derivatization and chromatographic procedures. When 300 ppb was spiked, the recovery rate of milbemectin A, moxidectin, milbemectin A, abamectin,
doramectin, and ivermectin was 82.3-95.7%, 72.3-85.7%, 80.9-92.1%, 82.5-91.8%, 76.5-89.1% and 74.1-84.5%, respectively. The relative standard deviations (% RSD) of these studies were all less than 20. The result corresponded to the Horwitz equation in Codex’s report (14), when the concentration was at 10-8 (10 ppb), the % RSD should be less than 32. When 10 ppb was spiked in bovine muscle and Roudaut’s method was applied, the mean recovery rate of abamectin, moxidectin, doramectin, and ivermectin was in the range of 77.8-89.9%.

(III) Analysis of commercial bovine muscle samples

In August 2002, we collected a total of 50 bovine muscle samples, 5 domestic samples from mid-Taiwan traditional markets and supermarkets, including Taichung county, Taichung city, Changhua county, and Nantou county, and 45 imported samples from USA, Australia, Canada, and Netherlands. When analyzed according to the above mentioned extraction, derivatization and chromatographic procedures, we did not identify any traces of milbemectin A3, moxidectin, milbemectin A4, abamectin, doramectin, and ivermectin.

CONCLUSIONS

In conclusion, the linear regression coefficients ($r^2$) were all higher than 0.9990, which indicated that good linear correlation and good precision could be obtained for milbemectin A3, moxidectin, milbemectin A4, and abamectin at the concentration of 1-300 ppb and doramectin and ivermectin at 2-300 ppb. The LOQ is 5 ppb, far less than the bovine muscle residue tolerance in “The Residual Limit of Animal Drugs”. The method is applicable for the quantification of these residues. The modified Roudaut method can separate and quantify structurally similar milbemectin A3 and milbemectin A4.

Besides cattle, abamectin, doramectin, moxidectin and ivermectin were also used in the raising of swine, deer, goat, sheep and pigs. Therefore, the residue tolerances of liver, kidney, milk, fat, muscle or egg in these products were identified in “The Residual Limit of Animal Drug”, at the range of 5-100 ppb. Since abamectin and milbemectin were also allowed as anthelmintic drugs in planting vegetables, including leaf vegetables with small leaves, large and small berries, leaf vegetables with wrapped leaves, melons, fruit vegetables, citrus, root vegetables, melon vegetables, pomes, and teas, the residue tolerance in the DOH’s “Safety Tolerance of Pesticide Residue” was at the range of 10-2000 ppb. They are quantifiable by using the derivatization and chromatographic method developed in this study. The sample extraction and recovery need further evaluation.

The method we developed, unlike mass chromatography that can further identify chemical structures, has advantages in rapid extraction, less organic solvent used, simple equipments, stable results after derivatization, and the ability to quantify residues at low detection limits. The
method is suitable for the rapid screening of a lot of samples in general laboratories.

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