

LC- MS/MSによる畜産食品中抗生物質のスクリーニング試験法

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Note

Screening Assay of Residual Antibiotics in Livestock Samples by LC-MS/MS

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A LC-MS/MS screening assay of multi-class antibiotics was developed for 19 residual antibiotics in livestock samples. Sample preparation employed the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach using 0.5% formic acid in acetonitrile-methanol (8 : 2), with salting-out using magnesium sulfate, trisodium citrate and sodium chloride. Recovery values from 5 different livestock samples ranged from 45.5 to 121.6%, and the RSDs were under 18% at two concentration levels. The limit of quantification values of 19 analytes were under 10 µg/kg in all livestock samples, and the procedure can detect almost all analytes under the MRL. Screening capability was confirmed by employing spiked samples. This new screening assay for residual antibiotics in livestock samples is expected to be useful for routine laboratory tests.

Key words: antibiotic; livestock sample; LC-MS/MS; QuEChERS approach; screening assay

Introduction

Antibiotics are widely used for the treatment and prevention of many kinds of infectious diseases in animals, and lead to increased productivity in farms. Tetracyclines and penicillins are often administered to animals because they have broad spectra and/or strong antibacterial activities, but residues may remain in the animal tissues. There is a lot of information about residual antibiotics detected at inspection institutes, because livestock products such as beef, pork and chicken are imported and widely sold in markets. Therefore it is necessary to develop a convenient screening assay which can analyze as many samples as possible at once.

Even though many reports have described analysis of antibiotics in livestock samples, there are few methods to analyze multi-class antibiotics including penicillins, tetracyclines and macrolides¹⁾⁻⁵⁾, because different classes of antibiotics have quite different chemical and physical properties. In addition, complicated clean-up has been necessary to analyze multi-class antibiotics by LC-MS/MS. Therefore the objective of this study is to develop an easy screening method for routine assay of antibiotics in livestock samples. We focused on the QuEChERS approach⁶⁾⁻⁹⁾ for LC-MS/MS analysis of residual antibiotics in livestock samples.

Materials and Methods

Chemicals and reagents

Ampicillin (ABPC, purity: 98.0%), benzylpenicillin potassium (PCG, 98.0%), cephalixin (CEX, 90.0%), chlorotetracycline hydrochloride (CTC, 98.0%), erythromycin (EM, 90.0%), kitasamycin (KT, 90.0%), oxytetracycline hydrochloride (OTC, 99.0%), phenoxymethylpenicillin (PCV, 95.0%), tetracycline hydrochloride (TC, 99.0%) and tylosin (TS, 93.0%) were purchased from Wako Pure Chemical Industries (Osaka, Japan). Cloxacillin sodium (MCIPC, 98.6%), dicloxacillin sodium (MDIPC, 98.2%), doxycycline hyclate (DC, 98.2%), nafcillin sodium (NFPC, 99.9%), oleandomycin (OM, 89.6%), oxacillin sodium (MPIPC, 99.0%) and spiramycin (SPM, 97.5%) were purchased from Hayashi Pure Chemical Industries (Osaka, Japan). Tilmicosin (TMS, 98.5%) was purchased from Eli Lilly Japan (Hyogo, Japan) and mirosamycin (MRM, 95.9%) was purchased from Kyoritsu Pharmaceutical Company (Tokyo, Japan).

ABPC, PCG, CEX, MCIPC, MDIPC, NFPC, MPIPC and PCV were accurately weighed in 10 mg portions, then diluted with distilled water to 10 mL and used as stock standard solutions (1,000 µg/mL). Other analytes were weighed in 5 mg portions, then diluted with methanol to 50 mL and used as stock standard solutions (100 µg/mL). Stock standard solutions were stored at -20°C for up to one month.

Working standard solution for calibration curves was prepared by mixing all analytes and diluting with 0.5% formic acid in acetonitrile-methanol (8 : 2) at the level

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Table 1. Multiple reaction monitoring conditions

| Group | Analytes | Molecular weight | Precursor ion | Quantitative product ion | Collision energy (eV) | Qualitative product ion | Collision energy (eV) | Polarity |
|----------------|------------|------------------|---------------|--------------------------|-----------------------|-------------------------|-----------------------|----------|
| Tetracyclines | OTC | 460.4 | 461 | 426 | 19 | 443 | 12 | + |
| | TC | 444.4 | 445 | 410 | 18 | 427 | 11 | + |
| | DC | 444.4 | 445 | 428 | 16 | 154 | 32 | + |
| | CTC | 478.9 | 479 | 444 | 19 | 154 | 27 | + |
| Cephalosporins | CEX | 347.4 | 348 | 158 | 6 | 140 | 25 | + |
| Penicillins | ABPC | 349.4 | 350 | 106 | 21 | 79 | 38 | + |
| | PCG | 334.4 | 333 | 192 | 15 | 74 | 26 | - |
| | PCV | 350.4 | 349 | 93 | 46 | 114 | 21 | - |
| | MPIPC | 401.4 | 400 | 259 | 16 | 356 | 11 | - |
| | MCIPC | 435.9 | 434 | 293 | 15 | 390 | 9 | - |
| | NFPC | 414.5 | 413 | 272 | 16 | 243 | 27 | - |
| | MDIPC | 470.3 | 468 | 327 | 14 | 424 | 12 | - |
| | Macrolides | SPM | 843.1 | 844 | 174 | 34 | 101 | 46 |
| | TMS | 869.1 | 870 | 174 | 41 | 88 | 62 | + |
| | MRM | 727.9 | 729 | 158 | 26 | 116 | 33 | + |
| | OM | 687.9 | 689 | 158 | 26 | 544 | 13 | + |
| | EM | 733.9 | 735 | 158 | 29 | 577 | 19 | + |
| | TS | 916.1 | 917 | 174 | 37 | 156 | 41 | + |
| | KT | 771.9 | 773 | 109 | 34 | 174 | 29 | + |

of 1 µg/mL.

Working standard solution for spiking was prepared by mixing analytes with methanol at the level of 100 times fortification (PCG 0.4 and 1 µg/mL, NFPC 0.5 and 1 µg/mL and others 1 and 10 µg/mL). 500 µL of this solution was added to each livestock sample at 30 minutes before sample preparation.

Acetonitrile, distilled water and methanol (both HPLC grade), formic acid and ammonium formate (both LC/MS grade), EDTA-2Na (ethylenediaminetetraacetic acid disodium salt), magnesium sulfate and sodium chloride were purchased from Wako Pure Chemical Industries. Trisodium citrate dehydrate was purchased from Kanto Chemical Company (Tokyo, Japan).

Samples

The livestock samples (cattle muscle, swine muscle, chicken muscle, egg and milk) were purchased from local supermarkets in Tokyo and confirmed to be free from the targeted analytes. Each of them except milk was homogenized and stored at -20°C. Milk was stored at 4°C.

LC-MS/MS conditions

The analysis was carried out using a Prominence series (Shimadzu Corporation, Kyoto, Japan) HPLC system and L-column 2 ODS (2.1 mm i.d. × 150 mm, 5 µm, Chemicals Evaluation Research Institute, Tokyo, Japan) at 40°C. The gradient was applied with 0.1% formic acid in 10 mmol/L ammonium formate (pH 4.0, A) and acetonitrile (B). The initial condition (A : B=95 : 5) was maintained for 3 min, and then the acetonitrile concentration was increased to 90% over 7 min, and maintained for 5 min. Then, the mobile phases were re-equilibrated to the initial condition for 5 min. The flow rate was 0.2 mL/min.

A TSQ Quantum Access MAX (Thermo Scientific Ja-

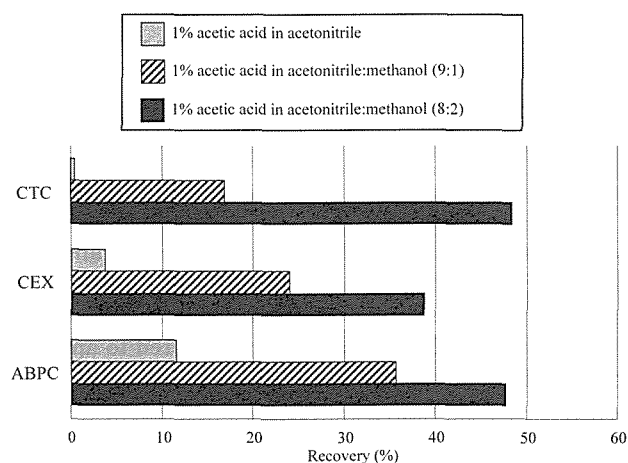


Fig. 1. Comparison of extraction solvents and recoveries of analytes spiked into cattle muscle at the level of 100 µg/kg

pan, Kanagawa, Japan) mass spectrometer, was used and operated in positive and negative electrospray ionization modes, with voltages of 3.0 kV and 2.5 kV, respectively. Vaporizer temperature was 465°C and capillary temperature was 220°C. Tuning was performed by direct infusion of 1 µg/mL of each standard solution, and the optimized conditions of multiple reaction monitoring are presented in Table 1.

Sample preparation

A 5 g aliquot of each sample was weighed and added into a 50 mL polypropylene centrifuge tube. 2.5 mL of 0.1 mol/L EDTA-2Na in distilled water and 15 mL of 0.5% formic acid in acetonitrile-methanol (8 : 2) were added. After homogenizing, 4 g of magnesium sulfate, 1.5 g of trisodium citrate dehydrate and 1 g of sodium

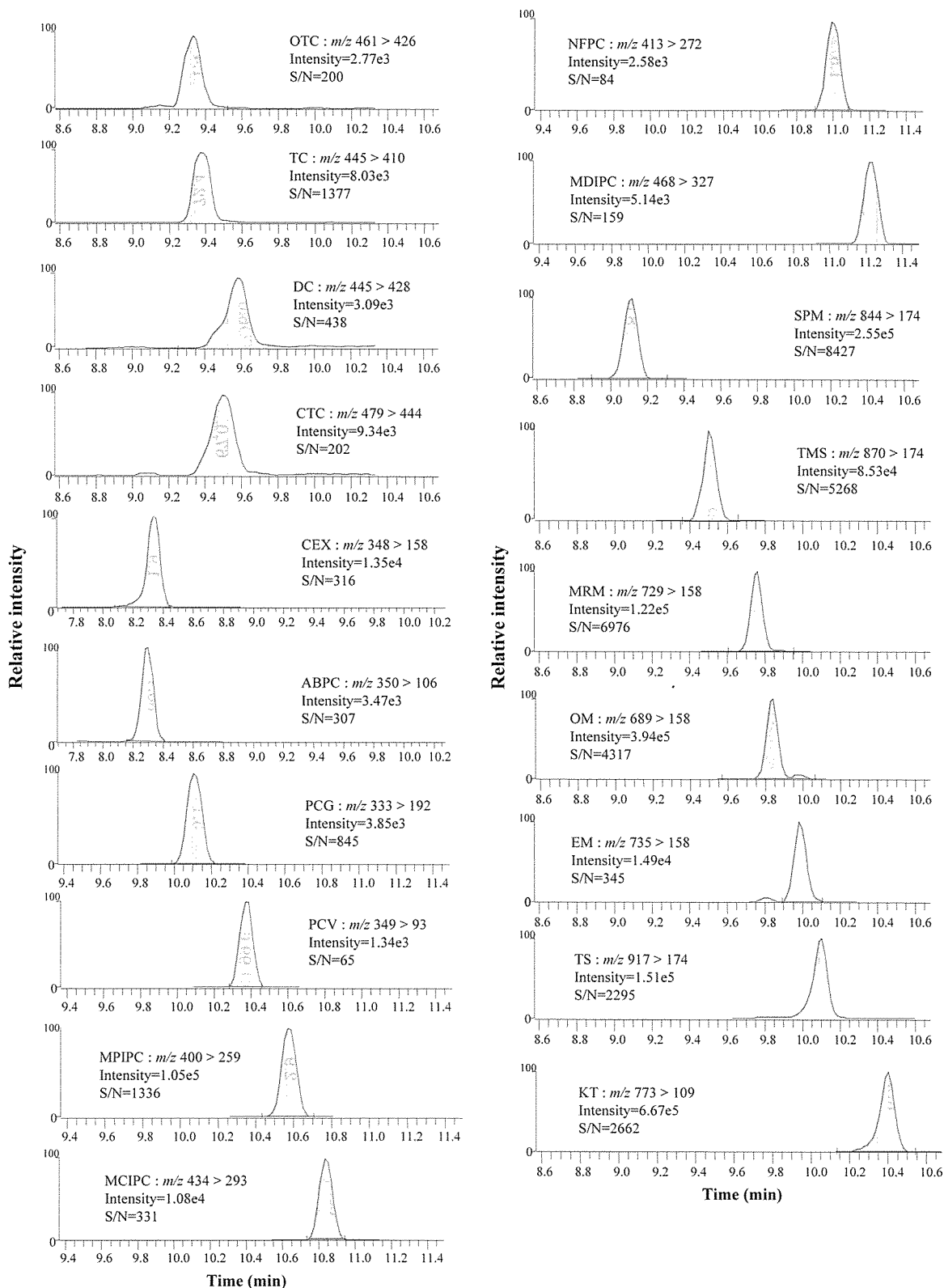


Fig. 2. MRL chromatograms of all analytes in cattle muscle for recovery test

Concentration levels: OTC (50 µg/kg), TC (50 µg/kg), DC (100 µg/kg), CTC (50 µg/kg), CEX (200 µg/kg), ABPC (30 µg/kg), PCG (50 µg/kg), PCV (10 µg/kg), MPIPC (300 µg/kg), MCIPC (40 µg/kg), NFPC (5 µg/kg), MDIPC (30 µg/kg), SPM (200 µg/kg), TMS (100 µg/kg), MRM (10 µg/kg), OM (50 µg/kg), EM (50 µg/kg), TS (50 µg/kg), KT (10 µg/kg).

Table 2. Recoveries and RSDs of antibiotics from 5 kinds of samples

| Analytes | Spiked level (µg/kg) | Cattle muscle | | Swine muscle | | Chicken muscle | | Egg | | Milk | |
|----------|-------------------------|---------------|---------|--------------|---------|----------------|---------|--------------|---------|--------------|---------|
| | | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) | Recovery (%) | RSD (%) |
| OTC | 10 | 61.5 | 5.6 | 63.1 | 2.2 | 69.0 | 3.0 | 66.3 | 5.5 | 107.3 | 4.4 |
| | 100 | 66.8 | 5.1 | 62.4 | 1.0 | 61.4 | 3.0 | 67.6 | 5.1 | 70.5 | 1.5 |
| TC | 10 | 70.1 | 3.8 | 65.8 | 5.1 | 78.3 | 5.7 | 73.5 | 4.8 | 113.3 | 1.5 |
| | 100 | 75.0 | 3.5 | 68.3 | 2.1 | 69.6 | 3.6 | 71.7 | 5.9 | 82.1 | 1.5 |
| DC | 10 | 84.3 | 9.7 | 76.3 | 3.1 | 84.4 | 6.1 | 78.2 | 7.4 | 121.6 | 4.9 |
| | 100 | 75.4 | 4.4 | 77.7 | 2.4 | 86.8 | 0.8 | 68.4 | 2.9 | 78.0 | 1.4 |
| CTC | 10 | 73.1 | 3.4 | 70.6 | 8.8 | 82.4 | 5.4 | 71.3 | 6.5 | 105.8 | 2.9 |
| | 100 | 80.6 | 3.7 | 69.8 | 2.1 | 83.2 | 1.2 | 67.7 | 3.2 | 77.6 | 0.5 |
| CEX | 10 | 56.1 | 9.3 | 51.4 | 9.1 | 64.9 | 8.4 | 45.5 | 5.5 | 81.3 | 2.7 |
| | 100 | 65.0 | 3.6 | 52.9 | 4.7 | 73.1 | 5.7 | 74.8 | 4.4 | 78.3 | 3.5 |
| ABPC | 10 | 67.3 | 5.3 | 60.0 | 8.1 | 71.0 | 5.3 | 50.2 | 8.6 | 83.1 | 3.2 |
| | 100 | 73.5 | 4.4 | 75.3 | 3.9 | 81.1 | 2.6 | 79.7 | 1.9 | 78.8 | 6.6 |
| PCG | 4 | 100.5 | 3.7 | 78.2 | 4.7 | 74.9 | 3.2 | 64.3 | 3.2 | 73.5 | 2.0 |
| | 10 | 86.0 | 14.8 | 73.5 | 9.1 | 98.3 | 4.1 | 88.6 | 6.3 | 108.0 | 4.0 |
| PCV | 10 | 77.3 | 10.6 | 86.2 | 5.8 | 108.8 | 4.3 | 90.1 | 17.9 | 115.2 | 3.5 |
| | 100 | 80.2 | 4.0 | 75.1 | 4.9 | 87.7 | 2.3 | 83.6 | 3.7 | 89.2 | 2.4 |
| MPIPc | 10 | 85.1 | 6.0 | 82.1 | 5.1 | 94.7 | 1.2 | 81.2 | 5.9 | 100.1 | 4.5 |
| | 100 | 81.2 | 3.1 | 77.2 | 2.1 | 82.2 | 1.8 | 78.2 | 2.2 | 85.8 | 3.1 |
| MCIPC | 10 | 88.8 | 8.7 | 94.7 | 4.2 | 101.5 | 4.8 | 82.9 | 6.6 | 106.5 | 3.8 |
| | 100 | 86.6 | 8.0 | 77.9 | 3.6 | 79.9 | 1.9 | 87.3 | 6.7 | 78.8 | 1.3 |
| NFPC | 5 | 92.5 | 4.2 | 80.6 | 4.1 | 81.8 | 1.8 | 76.8 | 3.3 | 75.8 | 2.9 |
| | 10 | 88.2 | 3.4 | 86.6 | 8.1 | 86.9 | 4.7 | 84.7 | 7.9 | 101.4 | 5.6 |
| MDIPC | 10 | 104.1 | 2.4 | 90.8 | 5.8 | 98.3 | 1.4 | 95.2 | 6.9 | 112.8 | 6.2 |
| | 100 | 88.7 | 10.1 | 76.8 | 2.4 | 69.2 | 2.1 | 99.4 | 6.5 | 85.2 | 1.9 |
| SPM | 10 | 84.6 | 8.7 | 71.0 | 5.1 | 84.5 | 6.4 | 76.4 | 6.6 | 88.1 | 5.4 |
| | 100 | 99.4 | 2.9 | 77.1 | 2.6 | 85.5 | 2.6 | 87.0 | 3.9 | 99.5 | 1.2 |
| TMS | 10 | 105.5 | 14.6 | 94.4 | 6.2 | 98.5 | 5.9 | 82.6 | 5.5 | 115.8 | 13.4 |
| | 100 | 106.0 | 3.6 | 99.6 | 4.6 | 87.5 | 1.9 | 89.5 | 5.0 | 84.6 | 0.8 |
| MRM | 10 | 88.0 | 0.6 | 78.1 | 1.9 | 74.1 | 5.6 | 67.0 | 3.5 | 87.1 | 2.5 |
| | 100 | 100.0 | 3.5 | 74.0 | 3.1 | 77.7 | 2.5 | 65.2 | 7.8 | 72.4 | 1.4 |
| OM | 10 | 92.9 | 0.8 | 85.5 | 2.5 | 89.3 | 1.6 | 70.9 | 4.7 | 89.5 | 2.7 |
| | 100 | 104.1 | 2.4 | 82.7 | 3.5 | 80.1 | 1.9 | 80.4 | 7.6 | 80.1 | 0.9 |
| EM | 10 | 89.8 | 10.5 | 52.7 | 10.0 | 95.1 | 6.8 | 95.4 | 4.4 | 110.9 | 10.6 |
| | 100 | 100.4 | 5.9 | 65.3 | 2.0 | 98.6 | 5.8 | 95.6 | 5.3 | 76.4 | 4.8 |
| TS | 10 | 78.8 | 6.3 | 61.8 | 3.6 | 65.5 | 10.3 | 63.8 | 4.6 | 94.1 | 4.2 |
| | 100 | 77.9 | 7.5 | 66.7 | 2.5 | 73.5 | 3.5 | 67.5 | 4.3 | 82.8 | 0.9 |
| KT | 10 | 82.9 | 3.5 | 66.6 | 4.2 | 73.7 | 3.9 | 70.4 | 3.5 | 89.9 | 4.2 |
| | 100 | 102.6 | 7.1 | 65.5 | 1.6 | 73.3 | 2.0 | 69.7 | 1.3 | 81.0 | 1.0 |

n = 5

Table 3. MRL, LOD and LOQ values for each drug

| Analytes | Cattle muscle | | | Swine muscle | | | Chicken muscle | | | Egg | | | Milk | | |
|----------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|------------------------------------|
| | MRL ($\mu\text{g}/\text{kg}$) | LOD ($\mu\text{g}/\text{kg}$) | LOQ ($\mu\text{g}/\text{kg}$) | MRL ($\mu\text{g}/\text{kg}$) | LOD ($\mu\text{g}/\text{kg}$) | LOQ ($\mu\text{g}/\text{kg}$) | MRL ($\mu\text{g}/\text{kg}$) | LOD ($\mu\text{g}/\text{kg}$) | LOQ ($\mu\text{g}/\text{kg}$) | MRL ($\mu\text{g}/\text{kg}$) | LOD ($\mu\text{g}/\text{kg}$) | LOQ ($\mu\text{g}/\text{kg}$) | MRL ($\mu\text{g}/\text{kg}$) | LOD ($\mu\text{g}/\text{kg}$) | LOQ ($\mu\text{g}/\text{kg}$) |
| OTC | 200 ^{a)} | 2 | 4 | 200 ^{a)} | 1 | 2 | 200 ^{a)} | 2 | 4 | 400 ^{a)} | 1 | 4 | 100 ^{a)} | 1 | 4 |
| TC | 200 ^{a)} | 2 | 4 | 200 ^{a)} | 1 | 2 | 200 ^{a)} | 1 | 2 | 400 ^{a)} | 1 | 2 | 100 ^{a)} | 1 | 2 |
| DC | 100 | 2 | 4 | 50 | 2 | 4 | 50 | 1 | 2 | — ^{b)} | 1 | 4 | — ^{b)} | 2 | 4 |
| CTC | 200 ^{a)} | 2 | 4 | 200 ^{a)} | 1 | 4 | 200 ^{a)} | 1 | 2 | 400 ^{a)} | 1 | 2 | 100 ^{a)} | 1 | 2 |
| CEX | 200 | 2 | 4 | 10 | 2 | 4 | — ^{b)} | 2 | 4 | — ^{b)} | 4 | 10 | 100 | 2 | 4 |
| ABPC | 30 | 2 | 4 | 60 | 2 | 4 | 20 | 1 | 4 | 10 | 1 | 4 | 20 | 2 | 4 |
| PCG | 50 | 1 | 4 | 50 | 1 | 4 | 50 | 4 | 10 | 4 | 2 | 4 | 4 | 2 | 4 |
| PCV | — ^{b)} | 1 | 4 | 30 | 1 | 4 | — ^{b)} | 2 | 4 | — ^{b)} | 1 | 4 | — ^{b)} | 2 | 4 |
| MPIPC | 300 | 1 | 2 | 300 | 1 | 2 | 300 | 1 | 2 | — ^{b)} | 1 | 2 | 30 | 1 | 2 |
| MCIPC | 40 | 1 | 2 | 300 | 1 | 2 | 300 | 1 | 2 | — ^{b)} | 1 | 2 | 20 | 1 | 2 |
| NFPC | 5 | 1 | 2 | 5 | 1 | 2 | 5 | 1 | 2 | 5 | 1 | 2 | 5 | 1 | 2 |
| MDIPC | 30 | 1 | 2 | 300 | 1 | 2 | 300 | 1 | 2 | — ^{b)} | 1 | 2 | 10 | 1 | 2 |
| SPM | 200 | 0.1 | 0.5 | 200 | 0.5 | 2 | 200 | 0.1 | 0.5 | — ^{b)} | 0.1 | 0.5 | 200 | 0.1 | 0.5 |
| TMS | 100 | 1 | 4 | 100 | 1 | 4 | 70 | 0.1 | 0.5 | — ^{b)} | 0.5 | 2 | 50 | 0.1 | 0.5 |
| MRM | — ^{b)} | 0.1 | 0.5 | 50 | 0.1 | 0.5 | 40 | 0.1 | 0.5 | — ^{b)} | 0.1 | 0.5 | — ^{b)} | 0.1 | 0.5 |
| OM | 50 | 0.1 | 0.5 | 100 | 0.1 | 0.5 | 200 | 0.1 | 0.5 | — ^{b)} | 0.1 | 0.5 | 50 | 0.1 | 0.5 |
| EM | 50 | 0.5 | 2 | 50 | 0.5 | 2 | 50 | 1 | 4 | 90 | 1 | 4 | 40 | 0.5 | 2 |
| TS | 50 | 0.1 | 0.5 | 50 | 0.1 | 0.5 | 50 | 0.1 | 0.5 | 200 | 0.1 | 0.5 | 50 | 0.1 | 0.5 |
| KT | — ^{b)} | 0.1 | 0.5 | 200 | 0.1 | 0.5 | 200 | 0.1 | 0.5 | 200 | 0.5 | 2 | — ^{b)} | 0.1 | 0.5 |

$n = 3$

^{a)} MRLs for oxytetracycline, tetracycline and chlortetracycline are established for the sum of residues of these three drugs.

^{b)} MRL is not defined.

chloride were added to the tube, which was then vortexed immediately for 1 min. The sample was centrifuged at 3,000 rpm for 10 min, and the supernatant was poured into a 20 mL volumetric flask. After dilution with 0.5% formic acid in acetonitrile–methanol (8 : 2) to exactly 20 mL, a portion of the solution was transferred to a 1.5 mL microtube and centrifuged at 13,000 rpm for 5 min. 10 μ L of supernatant was injected into the LC-MS/MS.

Quantification

Calibration curves were obtained from matrix-matched calibration curves, *i.e.*, calibration curves were calculated from peak areas of each chromatogram obtained from blank samples spiked with working standard solution to the level of 1, 2.5, 5, 10, 20, 50 μ g/L. Five different concentrations among them were used.

Results and Discussion

LC-MS/MS analysis of analytes

MRM transitions of each analyte were determined by using 0.1% formic acid in 10 mmol/L ammonium formate and acetonitrile as the mobile phase; this is used in our routine laboratory tests. Full scans showed that $[M+H]^+$ or $[M-H]^-$ ions had the highest abundance for each analyte. Using those ions as the precursor ion, product ion scan was performed. The most abundant product ion was selected as the quantitative ion, and the second most abundant as the qualitative ion.

For LC separation, we examined two columns, CAPCELL PAK C18 MG3 and L-column 2 ODS. Since DC and CTC were not eluted from the former column, the L-column 2 ODS was adopted. It was confirmed that all analytes could be detected.

Extraction process

Based on previous reports^{(6)–(9)}, an extraction process was developed using CTC, CEX and ABPC as indicators, because these analytes are amphoteric compounds and are expected to be hard to extract with acetonitrile only. All studies below were performed after spiking 50 μ L of 1 μ g/mL standard mixture into cattle muscle.

First, buffering effects were compared between sodium acetate buffer and sodium citrate buffer. With sodium acetate buffer, the muscle sample formed a thick mass when reagents were added, and mixing was difficult. Recoveries were also poor. Therefore citrate buffer was adopted.

Second, extraction solvents consisting of mixtures of acetonitrile and methanol with 1% acetic acid were compared. The result is shown in Fig. 1. Although recoveries improved as the ratio of methanol was increased, matrices derived from samples were increased and peak of matrices overlapped with analyte peaks. Therefore, we adopted acetonitrile–methanol (8 : 2) mixed solution.

Third, various acidic conditions were compared, *i.e.*, not adding or adding 0.1, 0.5 and 1% acetic acid or formic acid to the extraction solvent. Recoveries increased as the acidity was increased, but there was no difference

between 0.5% and 1% formic acid. Hence 0.5% formic acid was added to the extraction solvent because matrix levels were lower than with 1%.

Fourth, various amounts of water were compared, *i.e.*, addition of 0, 2.5, 5 and 10 mL of water. When no water was added, recoveries were much lower for example CEX recovery was under 30% *versus* over 50% when water was added. There were no significant differences from 2.5 mL to 10 mL, so 2.5 mL of water was adopted.

SPE clean-up was skipped, because multi-class antibiotics have wide-ranging properties and absorption might lead to decreased recoveries^{(8), (9)}. Instead, high-speed centrifugation was carried out to remove particulates.

Recovery tests

In this study, recovery tests were conducted at two concentration levels (10 μ g/kg for PCG and NFPC, and 10 and 100 μ g/kg for others). Typical MRL chromatograms of all analytes in cattle muscle are shown Fig. 2.

Quantification values were obtained from matrix-matched calibration curves. Although the ionization efficiencies of PCG, PCV, MPIP, MCIPC, NFPC, MDIPC, MRM, OM, EM, TS and KT were influenced slightly by matrices, others were greatly enhanced. Therefore matrix-matched calibration curves were used, and each calibration curve was exhibited good linearity ($r^2 > 0.999$). Results of recovery tests are shown in Table 2. Although recoveries of OTC, CEX and ABPC were slightly low and the precision, was insufficient, other analytes could be detected at the MRL. Therefore, this method is suitable for screening these targeted analytes.

Detection capacity as a screening method

The LOD and LOQ values were calculated at the S/N ratio of 3 and 10 for spiked samples. These results are shown in Table 3.

Application

This method was applied to samples purchased at

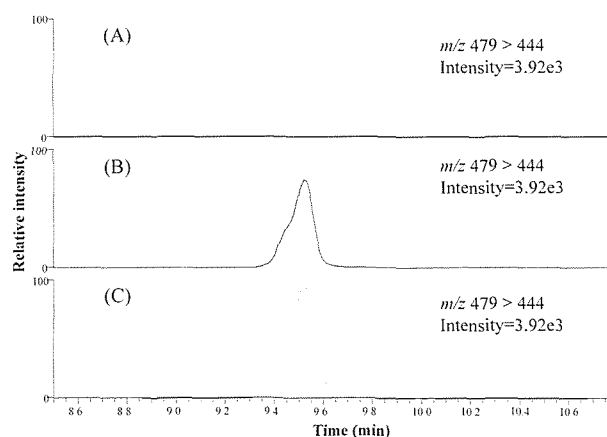


Fig. 3. Chromatograms showing the result of application (A) negative sample of swine muscle (B) positive sample of swine sample (C) 5 μ g/L standard solution of CTC.

markets in Tokyo. Ten samples each of muscles, egg and milk were analyzed, and no analytes were detected except in one sample of swine muscle, in which the MS/MS peak of CTC was detected and the quantification value was about 14 µg/kg. The chromatograms are shown in Fig. 3.

Conclusion

We have developed a screening assay for residues of 19 antibiotics (4 tetracyclines, 9 β-lactams and 7 macrolides) in livestock samples using LC-MS/MS and QuEChERS. This method is suitable for monitoring these analytes in muscles, egg and milk, and should be applicable to routine laboratory testing for residual antibiotics in livestock samples.

References

- 1) Goto, T., Ito, Y., Yamada, S., Matsumoto, H., Oka, H. High-throughput analysis of tetracycline and penicillin antibiotics in animal tissues using electrospray tandem mass spectrometry with selected reaction monitoring transition. *J. Chromatogr. A*, **1100**, 193–199 (2005).
- 2) Granelli, K., Branzell, C. Rapid multi-residue screening of antibiotics in muscle and kidney by liquid chromatography-electrospray ionization-tandem mass spectrometry. *Anal. Chim. Acta*, **586**, 289–295 (2007).
- 3) Carretero, V., Blasco, C., Picó, Y. Multi-class determination of antimicrobials in meat by pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A*, **1209**, 162–173 (2008).
- 4) Marazuela, M. D., Bogialli, S. A review of novel strategies of sample preparation for the determination of antibacterial residues in foodstuffs using liquid chromatography-based analytical methods. *Anal. Chim. Acta*, **645**, 5–17 (2009).
- 5) Martos, P. A., Jayasundara, F., Dolbeer, J., Jin, W., Spilsbury, L., Mitchell, M., Varilla, C. Shurmer, B. Multiclass, multiresidue drug analysis, including aminoglycosides, in animal tissue using liquid chromatography coupled to tandem mass spectrometry. *J. Agric. Food. Chem.*, **58**, 5932–5944 (2010).
- 6) Aguilera-Luiz, M. M., Vidal, J. L., Romero-González, R., Frenich, A. G. Multi-residue determination of veterinary drugs in milk by ultra-high-pressure liquid chromatography-tandem mass spectrometry. *J. Chromatogr. A*, **1205**, 10–16 (2008).
- 7) Stubbings, G., Bigwood, T. The development and validation of a multiclass liquid chromatography-tandem mass spectrometry (LC-MS/MS) procedure for the determination of veterinary drug residues in animal tissue using a QuEChERS (QUick, Easy, CHEap, Effective, Rugged and Safe) approach. *Anal. Chim. Acta*, **637**, 68–78 (2009).
- 8) Martínez Vidal, J. L., Frenich, A. G., Aguilera-Luiz, M. M., Romero-González, R. Development of fast screening methods for the analysis of veterinary drug residues in milk by liquid chromatography-triple quadrupole mass spectrometry. *Anal. Bioanal. Chem.*, **397**, 2777–2790 (2010).
- 9) Frenich, A. G., Aguilera-Luiz, M. M., Martínez Vidal, J. L., Romero-González, R. Comparison of several extraction techniques for multiclass analysis of veterinary drugs in eggs using ultra-high pressure liquid chromatography-tandem mass spectrometry. *Anal. Chim. Acta*, **661**, 150–160 (2010).

LC-MS/MSによる畜産食品中抗生物質のスクリーニング
試験法 (ノート・英文)

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簡便で高精度な畜産食品中の抗生物質19剤のスクリーニング法を開発した。試料5.0 gに対し、0.5%ギ酸含有アセトニトリル-メタノール(8:2) 15 mLで抽出し、硫酸マグネシウム、クエン酸三ナトリウム、塩化ナトリウムで脱水、塩析した後、20 mLに定容、LC-MS/MSで測定した。5種類の畜産食品試料(牛筋肉、豚筋肉、鶏筋肉、鶏卵、牛乳)について、2濃度、 $n=5$ での添加回収実験を行った結果、回収率は45.5~121.6%、相対標準偏差は18%未満であった。本法の定量下限値は10 $\mu\text{g}/\text{kg}$ 以下であり、一部の薬剤を除き、残留基準値を検出することが可能であった。本法は、畜産食品中残留抗生物質の簡便な試験法であり、日常のスクリーニング検査に有用であると考えられる。

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