Electronic Supplementary Material (ESI) for Analytical Methods

Gas Chromatography – Mass Spectrometric Determination of Ivermectin following Trimethylsilyzation with Application to Residue Analysis in Biological Meat Tissue Samples

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Meat Sample Preparation

An edible horse meat sample (5.00 g) was spiked with 100 µg of IVM. The sample pretreatement followed the IVM analysis guideline of the Japanese Food sanitation law.[34] Namely, five grams of tissue is homogenized with 30 mL of 50:50 acetone-water which is contained five gram of sodium chloride, thereafter this sample transferred with this solution to a 100-mL centrifuge tube. The homogenizer cup is washed out with 20 mL of isooctane. The sample is shaken for 5 min and centrifuged for 5 min at 2500 rpm. For fat samples, 50 mL of isooctane are employed to homogenize the meat sample and the cup is rinsed with 10 mL of isooctane. After centrifugation, the isooctane layer is transferred to a clean 200-mL round-bottom flask. All isooctane extracts are combined together and evaporated (70ºC) with aspirate. The samples are redissolved in 20 mL of n-hexane and transferred to 100-mL centrifuge tube. Twenty milliliters of n-hexane saturated in acetonitrile are added to this centrifuge tube; Stir the tube and then centrifuged and repeated these extractions two times. The acetonitrile layer is transferred to a clean 100-mL centrifuge tube. The acetonitrile extracts are combined and further added 10 mL of hexane to this tube. After shaking and centrifuge, the solution (hexane) was evaporated with aspirate (40ºC) to be complete dryness. The dried sample was dissolved in 1.0 mL of carbon tetrachloride and then, the solution was filtered using SPE cartridge filter to remove protein residue. The eluate was finally made up to 2.00 mL with carbon tetrachloride. The derivatization reaction and GC-MS analysis were then conducted as described in text. Otherwise, in LC-MS as a cross check method, the condensed sample solution (carbon tetrachloride) was directly inject into LC-MS system

LC-MS method

Dionex Ultimate 3000 series liquid chromatograph (Dionex Japan, Tokyo, Japan) linked to a Bruker micrOTOF-QII series total of flight mass spectrometer (TOF-MS; Bruker Daltonics Japan, Yokohama, Japan) operating in electro-spray ionization (ESI) positive mode. Neat two µL of samples were introduced into this system using a Dionex same Ultimate 3000 series auto sample injector. An InertSustain®-C18 LC column (150 mm × 2.1 mm i.d., 3 µm particle size) (GL Science Co. Ltd., Tokyo, Japan) was employed. The carrier solvent was ultra-high purity grade water and methanol (10 : 90) isocratic flowing at a rate of 0.20 mL/min. The column temperature programmed run was constant temperature at 23 °C. A mass range of m/z 50 to 3,000 amu was scanned in each run. For the quantitative IVM analyses, the mass selective detector was operated

in the extraction of target ions (IVM: m/z 897.4974 ± 0.005 as sodium additive peak).