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Bithionol residue analysis in animal-derived food products by an effective and rugged extraction method coupled with liquid chromatography–tandem mass spectrometry

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ABSTRACT

Herein, we developed a simple analytical procedure for the quantitation of bithionol residues in animal-derived food products such as porcine muscle, eggs, milk, eel, flatfish, and shrimp using a modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) extraction method coupled with liquid chromatography–electrospray ionization tandem mass spectrometry (LC-ESI$^+$/MS-MS). Samples were extracted with 0.1% solution of formic acid in acetonitrile and the extract was purified using a C18 sorbent. Separation was performed on a Waters XBridge™ C18 reversed-phase analytical column using 0.1% solution of formic acid/acetonitrile as the mobile phase. Six-point matrix-matched calibration indicated good linearity, with the calculated coefficients of determination (R^2) being ≥ 0.9813. Intra- and inter-day recoveries (determined at spiking levels equivalent to 1 × and 2 × the limit of quantitation (0.25 μg/kg)) ranged between 80.0 and 94.0%, with the corresponding relative standard deviations (RSDs) being ≤ 8.2%. The developed experimental protocol was applied to different samples purchased from local markets in Seoul, which were tested negative for bithionol residues. In conclusion, the proposed method proved to be versatile and precise, being ideally suited for the routine detection of bithionol residues in animal-derived food products with various protein and fat contents.

1. Introduction

Anthelmintics are a class of veterinary drugs widely used for the prophylaxis and therapeutic treatment of parasitic infections in livestock, since animal health and meat quality maintenance requires the control of nematode (roundworm), cestode (tapeworm), and trematode (fluke) infections in food-producing animals [1]. Bithionol (Fig. 1) is a broad-spectrum anthelmintic drug mainly used to treat sheep and cattle [2], inhibiting oxidative phosphorylation and thus interfering with the mitochondrial ATP synthesis of parasites [3]. Formerly, bithionol was used as a bacteriostatic additive in cosmetics, being subsequently proven to cause photocontact sensitization and thus banned by the FDA [4]. However, this compound is still used to treat parasitic infections in some Asian and European countries and is an active ingredient of a formulation used to treat mouth and throat disorders in Argentina [5]. Additionally, bithionol inhibits caspases and reduces the detrimental effects of anthrax lethal toxin, diphtheria toxin, cholera toxin, *Pseudomonas aeruginosa* exotoxin A, *Botulinum* neurotoxin, ricin, and Zika virus in humans [5]. Although thyroid hormone–like activity caused by the presence of bithionol residues in food is unlikely to affect human thyroid homeostasis [3], consumers should be aware of the associated health and safety issues. Since bithionol is commonly used to prevent and/or treat fascioliasis in cattle [6], any inappropriate use or lack of adherence to the withdrawal period may lead to the presence of

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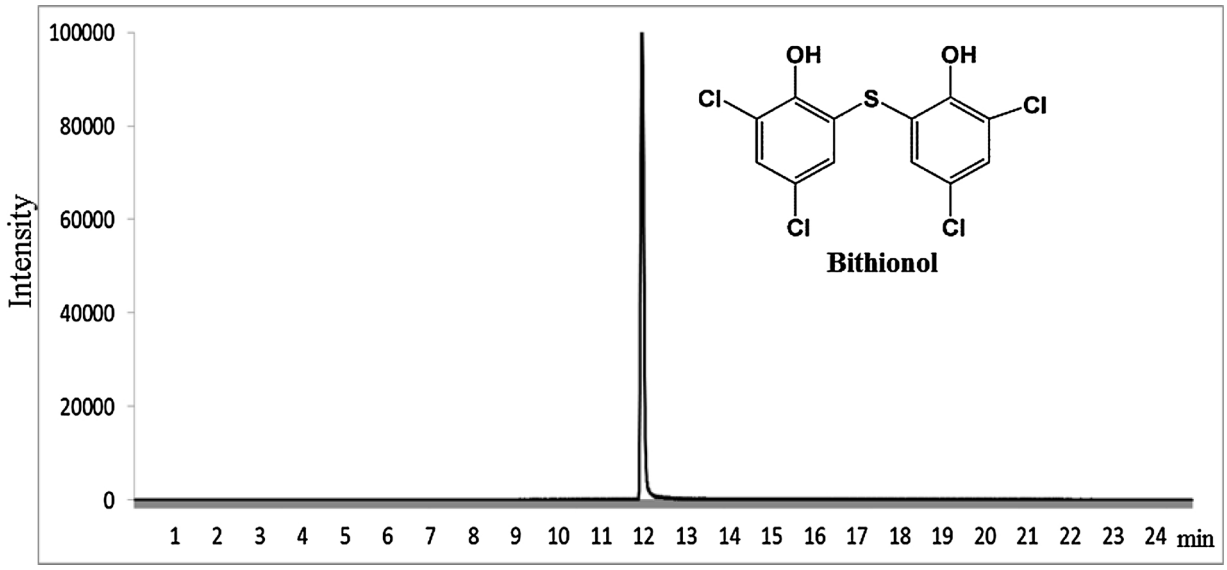

Fig. 1. Chemical structure of bithionol and an LC–MS/MS chromatogram of the bithionol standard (0.1 mg/L).

residues in milk. Indirectly, the parent drug can be excreted with feces and urine, entering surface water run-off and subsequently contaminating both aquatic organisms and other livestock. For instance, the above residues are likely to enter rivers inhabited by eel, shrimp, and flatfish, accumulating in these organisms [7]. Moreover, swine and chicken industrially raised along with cattle may also ingest drinking water containing drug residues. Animal proteins such as bovine blood are rich in protein/amino acids and available phosphorus, providing moderate amounts of other minerals and energy and therefore being commonly employed as beneficial poultry diet components, bringing about bithionol accumulation in products such as eggs [8]. All contaminants accumulated in food products may cause allergic reactions in sensitive individuals or result in the growth of antibiotic-resistant pathogenic bacterial strains [9]. In addition, the rapid population expansion observed in the past decades has significantly increased the global food consumption, especially that of porcine muscle, eggs, and milk, which are essential daily-life foods for both minors and adults, and that of aquatic products, which are rich in proteins and other nutrients. Consequently, bithionol residues accumulated in edible tissues pose a great threat to human health, necessitating the development of an accurate monitoring protocol.

Several studies dealing with anthelmintic drug analysis have employed liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC/MS/MS) [10–12], which is capable of providing a high degree of specificity for reliable identification and quantification of compounds of interest. In view of the satisfactory results previously obtained using this method, it was also used in this study. However, a reliable protocol should not only rely on a proven detection methodology but also feature a proper pretreatment, chosen according to matrix properties. Previous studies [6,13,14] utilized sample preparation by liquid–liquid extraction (LLE) to screen and quantify bithionol residues in milk. In 2003, the “quick, easy, cheap, effective, rugged, and safe” (QuEChERS) sample preparation method for multi-residue analysis was reported by Anastassiades et al. [15], rapidly gaining popularity due to its operation simplicity, low cost, and high efficiency, finding applications in the analysis of anthelmintic drug residues [16–18]. To the best of our knowledge, three studies [12,19,20] used the QuEChERS method for the determination of bithionol residues, with most of them [6,12,19,14] analyzing bithionol as a single analyte in the presence of other analytes in bovine milk or as part of a multiple determination of residual veterinary drugs (including bithionol) in animal muscle [13,20]. However, no analysis of bithionol in fishery products has been reported to date. Consequently, this study aimed to develop a sensitive and effective approach to the quantitation of bithionol residues in porcine muscle, milk, eggs, eel, flatfish, and shrimp using EN-QuEChERS extraction and purification followed by LC–MS/MS analysis.

2. Materials and methods

2.1. Chemicals and reagents

Bithionol (98.4%) and analytical-grade formic acid (98%) were purchased from Sigma-Aldrich Corporation (St. Louis, MO, USA). HPLC-grade methanol (99%) and acetonitrile (100%) were provided by J. T. Baker Chemicals (Phillipsburg, NJ, USA). GH polypro () membranes and syringe filters (0.45 μm) were provided by Pall (Michigan, USA). Ultrahigh purity water used for preparing the mobile phase was obtained using an Aqua MAX™ water purification system (Young Wha, Seoul, Republic of Korea). EN extraction kits and dispersive-solid phase extraction (d-SPE) tubes were supplied by Agilent Technologies (CA, USA).

2.2. Standard solutions

A standard stock solution of bithionol (1000 μg/mL) was prepared by precisely weighing 10 mg of bithionol powder using an AG 285 analytical balance (METTLER TOLEDO, Seoul, Republic of Korea) followed by its transfer to a 15-mL high-clarity polypropylene conical brown tube (Falcon, Corning Science Mexico S. A. de C.V., Tamaulipas, Mexico) and dissolution in 10 mL of acetonitrile. Intermediate and working standard solutions were prepared by further dilution with acetonitrile (mobile phase B), yielding diverse concentrations for calibration curve construction. All solutions were stored at −20 °C in the dark and analyzed within a week after preparation.

2.3. Sample preparation

Samples for bithionol detection and quantitation were collected from local markets in Seoul, Republic of Korea, with sample preparation performed using a modified EN-QuEChERS method [21]. Samples of chopped porcine muscle (5 g), chopped eel (5 g), chopped shrimp (5 g), homogenized whole egg (without shell, 5 mL), and homogenized milk (5 mL) were weighed, transferred to 50-mL centrifuge tubes, spiked with 0.5 mL of working standard solutions, left undisturbed for 10 min, and finally extracted with 20 mL of 0.1% solution of formic