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Determination of fenobucarb residues in animal and aquatic food products using liquid chromatography-tandem mass spectrometry coupled with a QuEChERS extraction method

Weijia Zheng[a,1], Jin-A Park[a,1], Dan Zhang[a], A.M. Abd El-Aty[a,b,**], Seong-Kwan Kim[a], Sang-Hyun Cho[a], Jeong-Min Choi[a], Jae-Han Shim[c], Byung-Joon Chang[d], Jin-Suk Kim[a], Ho-Chul Shin[a,*]

[a] Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Republic of Korea
[b] Department of Pharmacology, Faculty of Veterinary Medicine, Cairo University, 12211, Giza, Egypt
[c] Natural Products Chemistry Laboratory, College of Agriculture and Life Sciences, Chonnam National University, 300 Yongbong-dong, Buk-gu, Gwangju 500-757, Republic of Korea
[d] Department of Veterinary Anatomy, College of Veterinary Medicine, Konkuk University, Seoul 143-701, Republic of Korea

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ABSTRACT

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) was developed for quantification of fenobucarb residues in animal food products, such as porcine muscle, egg, and whole milk, and aquatic food products, such as eel, flatfish, and shrimp. Acetonitrile with the addition of 0.1% trifluoroacetic acid was employed as an extraction solvent and was compared with acetonitrile alone and 0.1% formic acid in acetonitrile. All extracted samples were purified using C18 sorbent. The best extraction efficiencies, expressed as recovery at two spiking levels equivalent to 1- and 2-times the limit of quantification (LOQ = 2 μg/kg) were achieved using 0.1% trifluoroacetic acid in acetonitrile and ranged from 61.38 to 102.21% in all matrices, with relative standard deviations (RSDs) < 13% (except for the low spiking of porcine muscle and the high spiking of whole milk, for which the RSDs were > 20%). Six-point matrix-matched calibration was used for quantification and the determination coefficients were good ($R^2 \geq 0.9865$). The method was verified by application to samples purchased from local markets and none of the samples tested positive. In conclusion, the developed method is simple and versatile and can be used for the routine detection of fenobucarb in different animal food products having varying protein and fat contents with satisfactory accuracy and precision.

1. Introduction

Pesticides play an essential role in pest and disease control for a diverse range of agricultural products. Although the use of these compounds brings enormous benefits, the spraying methods employed can lead to contamination and present a severe risk of bioaccumulation in food products [1,2]. The potential hazards this poses to human health, including dysfunction of the nervous and reproductive systems, have aroused a great deal of public concern worldwide [2]. Fenobucarb (Fig. 1) is a widely used carbamate insecticide derived from carbamic acid that inhibits cholinesterase enzymes (competitively) so as to affect nerve impulse transmission [3]. Carbamate pesticides are widely applied on grassland and farmland to protect crops from plagues. However, its careless use may give rise to the contamination of plants and by grazing on these contaminated crops, or by direct oral or injected administration, animals such as swine, cattle, and chickens may accumulate fenobucarb residues in their muscle tissues, milk, and eggs [4,5]. In recent years, rapid population expansion has led to a massive increase in food consumption throughout the world, especially aquatic food products as they are rich in proteins and other nutrient elements essential for both minors and adults. Drugs excreted in feces and urine as mixtures of unchanged parent compounds and their metabolites can enter aquatic environments through treated and untreated waste water. Consequently, these compounds may accumu-

* Corresponding author at: Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, Seoul, 143-701, Republic of Korea.
** Corresponding author at: Department of Veterinary Pharmacology and Toxicology, College of Veterinary Medicine, Konkuk University, Seoul, 143-701, Republic of Korea.
E-mail addresses: abdelaty44@hotmail.com, amabdelaty@konkuk.ac.kr (A.M. Abd El-Aty), hshin@konkuk.ac.kr (H.-C. Shin).
[1] The first two authors contributed equally to this article.

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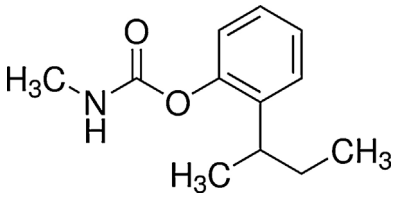

Fig. 1. Chemical structure of fenobucarb.

late in the edible tissues of aquatic species such eels, flatfish, and shrimps. Subsequent exposure presents a severe problem in terms of consumer safety and public health. Therefore, the accurate monitoring of pesticides is of extreme importance [2].

In the screening and detection of pesticide residues of animal origin, solid-liquid extraction (SLE) and solid-phase extraction (SPE) are widely applied for sample pretreatment. However, these techniques suffer from complicated handling, the formation of emulsions, and time consuming operation [6]. Owing to the complexity and diversity of biological matrices, as well as the low limit of quantification (< 10 μg/kg) stipulated by the Korean Ministry of Food and Drug Safety (MFDS) for drugs with no predefined maximum residue limits (MRL), these routine analytical procedures no longer satisfy the requirements of sample preparation [7]. Notably, the Japanese Ministry of Health, Labor, and Welfare (MHLW) is the only organizations, which prescribed the MRL of fenobucarb in porcine muscle and milk (20 μg/kg) and no MRL in egg, eel, flatfish, and shrimp has yet been established by other regulatory agencies [8–11].

In 2003, Anastassiades and his colleagues developed the concept of 'quick, easy, cheap, effective, rugged, and safe (QuEChERS) sample preparation, an approach that is now used in the treatment of samples for multi-residue pesticide analysis [12]. Due to its simplicity, automatability, time efficiency, and convenience, QuEChERS methodology has been rapidly and widely adopted for separating target analytes from lipids and proteins in pesticide residue analysis [13]. Several analytical methods have been applied for the determination of carbamate residues in foods, including biosensors [14], gas chromatography with electron capture detection (GC-ECD) [4], and gas chromatography-mass spectrometry (GC–MS) [15]. However, liquid chromatography coupled with tandem mass spectrometry (LC–MS/MS) has proved to be the most significant quantitative analytical technique in recent years, because it can be employed for the direct detection of target analytes and products susceptible to degradation in various matrices, and presents numerous advantages such as high selectivity, high sensitivity, reliable identification, and rapid cleanup procedures [16–18]. To the best of our knowledge, there are no published methods based on QuEChERS extraction followed by LC-electrospray ionization (ESI) MS/MS for analyzing fenobucarb residues, except for one 2013 report concerning residues in beef muscles [13]. Consequently, the aim of the study presented here was to develop an effective approach for the quantification of fenobucarb residues in porcine muscle, milk, egg, eel, flatfish, and shrimp using QuEChERS extraction and purification followed by LC–MS/MS sample analysis.

2. Materials and methods

2.1. Chemicals and regents

Fenobucarb (CAS Number: 3766-81-2), analytical-grade formic acid (98%), ammonium formate (97%), and trifluoroacetic acid (TFA, 99%) were provided by Sigma-Aldrich Corporation (St. Louis, MO, USA). HPLC-grade methanol (99%) and acetonitrile (100%) were supplied by J. T. Baker Chemicals (Phillipsburg, NJ, USA). GH polypro (GHP) membranes and syringe filters (0.45 μm) were purchased from Pall (Michigan, USA). Ultrahigh purity water was obtained from an aqua MAX™ water purification system (Young Wha, Seoul, Republic of Korea) for preparing the mobile phase.

2.2. Standard solutions

A 1000 μg/mL standard stock solution of fenobucarb was made by accurately weighing 10 mg of the solid using an AG 285 analytical balance (METTLER TOLEDO, Seoul, Republic of Korea) and transferring it to a 15-mL high-clarity polypropylene conical tube (Falcon, Corning Science Mexico S. A. de C.V., Tamaulipas, Mexico), then dissolving it in 10 mL methanol. The intermediate (100 μg/kg) and working solutions were prepared by further dilution in 0.2% formic acid + 10 mM ammonium formate in methanol (mobile phase B), yielding the various concentrations (2, 4, 6, 8, 10, and 12 μg/kg) that were used for constructing the calibration curves. All solutions were stored at −20 °C in the dark and analyzed within a week.

2.3. Sample preparation

Samples for detection and quantification were purchased from local markets in Seoul, Republic of Korea. The extraction and cleanup steps were carried out on the basis of EN QuEChERS methodology [19] with some improvements. Chopped porcine muscle (5 g), eel (5 g), shrimp (5 g), homogenized whole egg (without shell) (5 mL), and homogenized whole milk (5 mL) were transferred to 50-mL polypropylene conical tubes (Falcon, Corning Science Mexico S. A. de C.V., Tamaulipas, Mexico). After being spiked with working standard solution (0.5 mL) to a concentration of 20 μg/kg, the samples were left undisturbed for 10 min. The analyte was then extracted with 20 mL 0.1% TFA in acetonitrile, shaken vigorously for 5 min, and added to reagent kits (4 g magnesium sulfate, 1 g sodium chloride, 1 g sodium citrate tribasic dihydrate (SCTD), 0.5 g sodium citrate dibasic sesquihydrate (SCDS)), followed by vortex mixing (BenchMixer™ Multi-Tube Vortexer, Benchmark Scientific, NJ, USA) for 5 min. The tubes were then centrifuged at 2600*g* (Union 32 R Plus, Hanil Science Industrial Co., Ltd., Incheon, Republic of Korea) at 4 °C for 15 min. The supernatants were then transferred to 50-mL conical tubes and placed under gentle nitrogen gas flow at 50 °C (TurboVap®RV, Caliper Life Sciences, Hopkinton, USA) for evaporation until the volume reduced to 10 mL. The solutions obtained were transferred to 15-mL centrifuge tubes containing 150 mg C18 and 900 mg $MgSO_4$ (Agilent Bond Elut, Agilent Technologies, CA, USA) and then vortex mixed sufficiently prior to further centrifugation at 2600*g* and 4 °C for 15 min. The upper layer was transferred and concentrated to dryness under nitrogen gas as previously stated. The residue (approximately ~0.3 mL) was reconstituted in a 1:1 (*v*/*v*) mixture of mobile phases A and B to 1 mL, and the mixtures obtained were filtered through a 0.45-μm syringe filter prior to analysis.

2.4. LC–MS/MS analysis

LC was performed using an Agilent series 1100 HPLC system (Agilent Technologies, CA, USA) equipped with a G1311A Quart pump, a G1313A autosampler, a G1322A degasser, a G1316A column oven, and an API 2000TM LC–MS/MS detector (Applied Biosystems, NY, USA). Chromatographic separation was performed using a Waters XBridge™ C18 reversed-phase analytical column (2.1 × 100 mm; 3.5 μm particle size; Waters, Milford, CT, USA) maintained at 35 °C. A binary mobile phase system, consisting of (A) 0.2% formic acid with 10 mM ammonium formate in distilled water and (B) 0.2% formic acid with 10 mM ammonium formate in methanol (1:1, *v*/*v*) was employed in gradient pump mode with an injection volume of 10 μL. The linear mobile phase gradient at a flow rate of 0.3 mL/min was started at 5% B (0–1 min), increased to 95% B (1–2 min), maintained for 8 min (2–10 min), decreased to 5% B (10–11 min), and maintained to the end (11–15 min).

Triple quadrupole tandem mass spectrometric (MS/MS) analysis was employed under an ESI source in positive (ESI+) and negative