Talanta 168 (2017) 263–268

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

# Rapid detection of five anesthetics in tilapias by *in vivo* solid phase microextraction coupling with gas chromatography-mass spectrometry

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ARTICLE INFO

*Keywords:*
Anesthetics
*In vivo*
Solid phase microextraction
On site sampling
Gas chromatography-mass spectrometry

ABSTRACT

The concentration of five rapidly metabolized anesthetics in living tilapias was determined in this study, by the presented method coupling *in vivo* solid phase microextraction (SPME) to gas chromatography-mass spectrometry (GC-MS), which was the first time that *in vivo* sampling method was adapted in detecting the anesthetic residue in the living aquatic product. The analytical performance of the developed method was evaluated in homogenized tilapia dorsal muscle, and the results demonstrated that the present method possessed low detection limits 1.7–9.4 ng $g^{-1}$), wide linear ranges (10 or 30–5000 ng $g^{-1}$), and satisfactory reproducibility (relative standard deviations no more than 8.1% and 10.8% for inter-fiber and intra-fiber assays, respectively). Standard curves were established in homogenized tilapia dorsal muscle for calibrating *in vivo* SPME in living tilapias. And the concentrations determined by *in vivo* SPME were close to those determined by the liquid extraction. By using the present method, one anesthetic residue was detected above the detection limit in tilapias from the local markets. Comparing to traditional methods, the present one exhibited superior time-efficiency and cost performance, as the extraction time was only ten minutes, which was short to successfully avoid the possible loss of analytes caused by elimination and sample storage. In addition, owing to the time-efficiency of the present method, the elimination of the anesthetics in tilapias was traced successfully in the laboratory.

## 1. Introduction

Aquaculture management once met challenges during processes such as handling, sorting, tagging, stripping and transportation, as stimulated fish stabbed and hurt each other in these processes, which eventually resulted in fatality rate increment, and profit declines [1]. Situation has been improved since anesthetics were used in aquaculture management, especially in the long-distance transport of living fish [2], which now have become indispensable to aquaculture.

Dozens of anesthetics or non-chemical anesthesia methods had been applied to aquatic products [3,4]. To living edible fish, anesthetics are required to be readily metabolized in fish with no side-effects, and safe to both fish and human [5]. Tricaine methane sulphonate [6,7], eugenol [8], isoeugenol [9], and benzocaine [10] are some of the most wildly used ones which were approved by the U.S. Food and Drug Administration (FDA) or other institutions. Anesthetics such as 2-phenoxyethanol [11], quinaldine, and procaine are also popular alternatives [4]. Albeit these anesthetics are considered safe, opposite opinion was proposed that care should be taken, as some anesthetics were reported to be anaphylactic and carcinogenic [12–14]. Therefore, it is necessary to build up standards to define the safe levels of residual anesthetics in fish.

To achieve this goal, developing a precise and convenient analytical method is crucial. Different from other food safety testing, it would be preferable if the analytical method aiming at anesthetics could be conducted on-site to snapshot the real-time concentrations, because certain portions of anesthetics might be metabolized during the transportation of fish to laboratory [15–17]. Several sample preparation methods aiming at determining anesthetics in fish samples were reported on the past years, such as solid-phase extraction (SPE) [6,8,15,18,19] and QuEChERs [7]. However, analysis had to be conducted until samples arrived laboratory. And the tedious and time-consuming steps such as solvent extraction and purification would also lead to the losses of analytes during sample preparation.

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http://dx.doi.org/10.1016/j.talanta.2017.03.045
Received 31 December 2016; Received in revised form 11 March 2017; Accepted 16 March 2017
Available online 18 March 2017

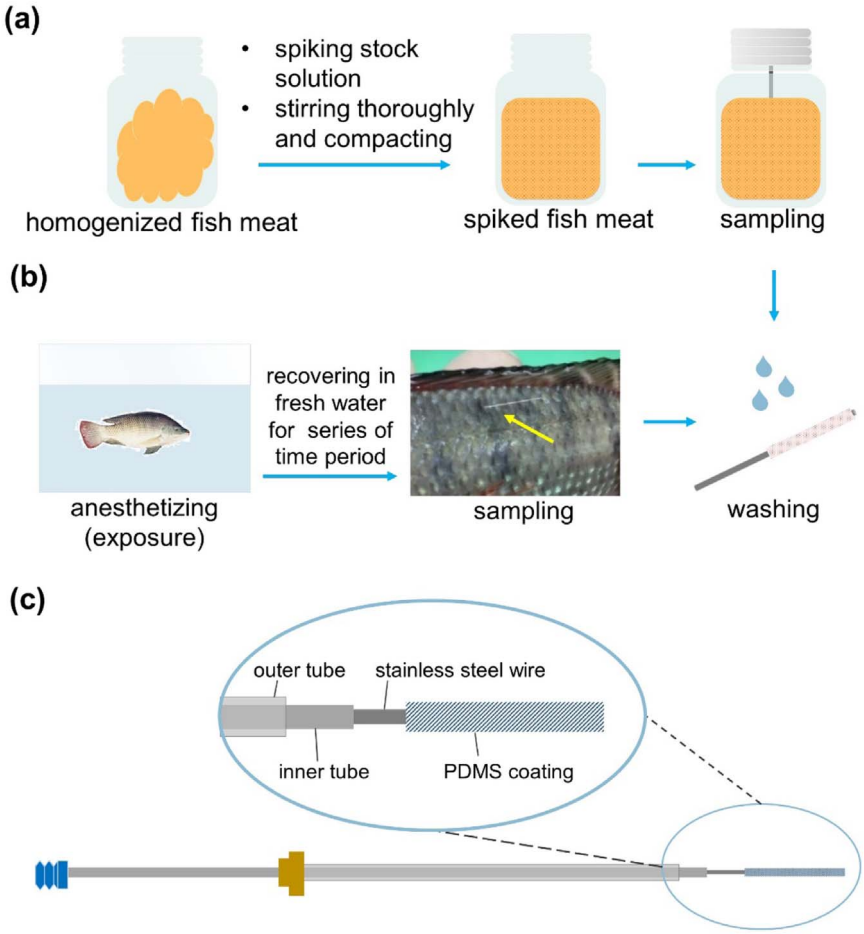


**Fig. 1.** The procedure of *in vitro* and *in vivo* experiment. (a) In vitro experiment was conducted in spiked homogenized fish dorsal meat; (b) It shows the procedure of elimination experiment (*in vivo* sampling), and the experiments on real samples purchased from local market were the same excluding the anesthetizing and recovering steps. (c) The schematic diagram of the commercial SPME fiber assembly fixed with a home-made PDMS fiber. After extraction, the PDMS fiber was inserted and fixed in the inner tube, which would be protected by the outer tube when inserting into the injection port, then pushed out and exposed.

As a promising method, solid phase microextraction (SPME) integrates extraction, enrichment and purification into one step, thereby greatly simplifies the procedure and shortens the time for sample preparation. Headspace (HS) SPME was used to examine 2-phenoxyethanol in fish meat. However, the extraction required two hours to achieve satisfactory detecting sensitivity, which greatly sacrificed the analytical efficiency [11,20]. Recently, direct immersion SPME has been adapted to detect analytes of interest in fish *in vivo* [21,22], in which way the elimination of target compounds was studied in the same individuals. Direct contact with the sample matrix could facilitate the mass transfer of analytes from the sample matrix to SPME fiber, so the extraction can be more efficient. Besides, *in vivo* SPME is feasible for on-site extraction and time-efficient monitoring [23,27], which is particularly valuable for the analysis of highly dynamic samples.

The object of this study was to develop an efficient and simple method to determine anesthetics residual in tilapias. We focused on five most used anesthetics, including tricaine methane sulphonate, eugenol, isoeugenol, benzocaine and 2-phenoxyethanol. Using the biocompatible custom PDMS (polydimethylsiloxane) fiber, equilibrium extraction was rapidly reached in 10 min. Quantification method was built *in vitro* in homogenized fish dorsal muscle. After that, anesthetics in living tilapias were determined with the developed method, and the elimination of the anesthetics in living tilapias was traced in real time.

## 2. Experimental

### 2.1. Chemicals and materials

Ethylene glycol monophenyl ether (also 2-phenoxyethanol, 2-PE, 99%), ethyl 4-aminobenzoate (also benzocaine, BENZ, 99%), isoeugenol (iso-EUG, 97.0%), and ethyl 3-aminobenzoate methanesulfonate (also tricaine, MS-222, 98%) were all purchased from Aladdin Reagent Company (Shanghai, China) and eugenol (EUG, 99.0%) was obtained from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). Polydimethylsiloxane (PDMS) tubing (i.d.=0.212 mm, o.d.=0.40 mm) was purchased from Helixmark (Carpinteria, CA, USA). Epoxy glue was purchased from Henkel Canada Corporation (Mississauga, ON, Canada). Stainless steel wires (SSWs, 127 μm in diameter, medical grade) were purchased from Small Parts Inc. (Miami Lakes, FL, USA). Standard stock solutions of five analytes (1000 μg $mL^{-1}$ for each analyte) were prepared in HPLC grade methanol and then diluted to prepare working solutions. All the solutions were store at −20 °C until use.

### 2.2. Fiber preparation

The preparation procedure of custom-made PDMS fibers was described in a previous study [21]. In brief, stainless steel wires were cut into sections about 3 cm, and ultrasonically cleaned in acetone and