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# Development of an SPME–GC–MS/MS procedure for the monitoring of 2-phenoxyethanol in anaesthetised fish

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**Abstract**

2-Phenoxyethanol (ethylene glycol monophenyl ether, $C_8H_{10}O_2$) is a promising anaesthetic agent used in fisheries and aquaculture. The aim of this study was to develop a fast and easy method to determine 2-phenoxyethanol residue levels in fish tissue and blood plasma, and, subsequently, to use the method to monitor the dynamics of 2-phenoxyethanol residues in fish treated with anaesthetic.

We developed a new procedure that employs solid phase microextraction (SPME) of the target analyte from the sample headspace followed by gas chromatography–mass spectrometry (GC–MS). Both sample handling, aimed at maximum transfer of 2-phenoxyethanol into the headspace, and SPME–GC–MS conditions were carefully optimised. Using a divinylbenzene/Carboxen/polydimethylsiloxane (PDMS/CAR/DVB) fiber for 60 min sampling at 30 °C and an ion trap detector operated in MS/MS mode, we obtained detection (LOD) and quantification (LOQ) limits of 0.03 and 0.1 mg $kg^{-1}$ of sample, respectively. The method was linear in a range of 0.1–250 mg $kg^{-1}$ and, depending on the sample matrix and spiking level, a repeatability (expressed as relative standard deviation, R.S.D.) of between 3% and 11% was obtained.


*Keywords:* Solid phase microextraction; 2-Phenoxyethanol; Fish samples; Matrix modification; Gas chromatography (GC); Ion trap mass spectrometry (ITMS)

## 1. Introduction

Anaesthetic agents are routinely used in aquaculture to allow the performance of disruptive procedures. They reduce the injuries and stress caused to fish in their handling; although, on certain occasions, anaesthesia itself may evoke a stress response or immunodepression [1,2,6,8]. Modern fish anaesthetics should meet a number of general requirements; in particular, high solubility of the substance, rapid effect, wide margin of safety, spontaneous recovery of fish and no residue. At the same time, the anaesthetics should be harmless to both fish and human beings, as well as to the environment.

Among the many anaesthetic agents used, 2-phenoxyethanol (ethylene glycol monophenyl ether, $C_8H_{10}O_2$) is considered to be highly suitable for aquacultural practices because of its easy synthesis, low price, bactericidal and fungicidal properties, and rapid action, together with the fast and uneventful recovery of the fish to which it is administered. Despite these advantages, 2-phenoxyethanol has not yet been approved for use in fish intended for human consumption. With no maximum residue limit (MRL) having yet been set, use of this promising anaesthetic agent remains illegal according to EEC Regulation 2377/90 [3].

To the best of our knowledge, existing papers are primarily concerned with the mode of action of anaesthetic agents used by fish biologists. Some comparative studies on the efficacy of anaesthetic chemicals have also been published, together with information about their effects on biochemical profile of blood [4–8]. However, because more data is required if

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2-phenoxyethanol is to be registered, our research encompassed the investigation of its acute toxicity, the histological examination of fish tissue and the determination of 2-phenoxyethanol residue levels in treated fish.

None of the aforementioned papers reported on analytical strategies applicable to the determination of anaesthetic residues in treated fish. To enable 2-phenoxyethanol analysis in experimental samples (fish tissue and blood plasma), we developed a new procedure that employs solid phase microextraction (SPME) [9,10] to sample the target analyte from the matrix headspace, followed by gas chromatography–mass spectrometry (GC–MS) to detect it. Subsequently, this method was used to monitor the dynamics of 2-phenoxyethanol residues in fish treated with anaesthetic.

## 2. Experimental

### 2.1. Chemicals and materials

A standard of 2-phenoxyethanol [CAS No. 56257-90-0] (p.a. standard for GC) was supplied by Sigma–Aldrich (Czech Republic). Stock solution I (40 g $L^{-1}$) of 2-phenoxyethanol was prepared in ethyl acetate (Merck, Czech Republic) and stored at +4 °C prior to use. Every day, fresh stock solution I was prepared. Working standards in ethyl acetate were prepared from stock solution I at concentrations in a range of 0.03–18 g $L^{-1}$.

SPME fibers coated with: (i) divinylbenzene/Carboxen/polydimethylsiloxane (50/30 μm StableFlex, PDMS/CAR/DVB), (ii) polyacrylate (85 μm, PA) and (iii) carbowax/divinylbenzene (65 μm, CW/DVB) were supplied by Sigma–Aldrich (Czech Republic). Prior to use, all fibers were conditioned in accordance with the manufacturer's recommendations. Each day, before analysis of the samples began, short thermal "cleaning" of the fibers in a GC injector (30 min at 250 °C) was performed, together with a blank run, to verify that no extraneous compounds were desorbed from the fiber.

Ten millilitres headspace vials (Sigma–Aldrich, Czech Republic) were cleaned by sonication: 20 min in water with detergent, followed by 20 min in distilled water, and finally by 20 min in re-distilled acetone (Penta, Czech Republic). After heating at 220 °C for 4 h, the clean vials were covered with aluminium foil and stored. To verify that no interfering compounds were desorbed from the vial or chemicals, in each sample sequence a blank run from an empty vial was performed, together with analysis of a reagent blank sample.

Ultrapure water was obtained from a Milli-Q water purification system (Milipore, Germany).

An HS 250 basic device (IKA Laboratortechnik, Germany) was used to homogenize samples prior to SPME.

### 2.2. Fish samples

Anaesthetic treatment of fish was carried out by our project partner, the Institute of Fish Culture and Hydrobiology Vodňany.

Experimental fish were exposed to anaesthetic in a bath containing 0.30 mL of 2-phenoxyethanol per 1 L (dissolved in water at 10 °C). After exposure, the fish were transferred to a bath containing clean water. To investigate the dynamics of 2-phenoxyethanol residues in both fish tissue and blood plasma, the fish were sampled at various purification times following their treatment and subsequent transfer to clean water.

Fish tissue was collected from back musculature at different sampling times: 10 min; 24 h; 7, 14, 21 and 28 days. Anaesthetised fish samples were analysed together with control samples, i.e. fish not exposed to anaesthetic. Six to 11 fish were collected at each sampling time.

Blood plasma was obtained by centrifuging the blood (taken from a tail fin) in a cooled centrifuge (4 °C, 837 × *g*). The samples were collected from three fish (A–C) both before and after anaesthetic treatment with 2-phenoxyethanol. Two samples of blood were collected at each sampling time: immediately after exposure; 15 min; 1, 4 and 24 h.

All samples were maintained at −16 °C until analysis began.

### 2.3. Sample preparation

#### 2.3.1. Samples for method optimisation

Tissue samples from fish not exposed to 2-phenoxyethanol were used to develop and characterize the SPME method.

Spiked samples without matrix modification were prepared as follows: 5 μL of working standards was added to 2 g of ground fish tissue to obtain a final spiking level of 3–382 mg $kg^{-1}$.

Subsequently, several alternative matrix modifications were tested: (i) 2 g of either spiked tissue or tissue with incurred residue was transferred into a 10 mL headspace (HS) vial, to which 2 mL of ultrapure water was then added; (ii) 2 g of tissue with incurred residue was ground with 2 g of sodium sulphate; (iii) 2 g of tissue with incurred residue was ground with 2 g of sodium sulphate and then immersed in 3 mL of ultrapure water in a 10 mL HS vial.

All modified samples were shaken vigorously for 20 min prior to SPME analysis.

#### 2.3.2. Real samples of anaesthetised fish

*Fish muscle tissue*: 2 g of frozen sample was ground with 2 g of sodium sulphate and then immersed in 3 mL of ultrapure water in a 10 mL HS vial. The sample was vigorously shaken for 20 min prior to SPME analysis.

*Fish blood plasma*: 0.5 g of sample was weighed into a 10 mL HS vial and analysed.

### 2.4. Optimised SPME procedure

Samples prepared according to the procedure described in Section 2.3.2 were incubated for 5 min at 30 °C prior to automated SPME. The extraction was carried out using a divinylbenzene/Carboxen/polydimethylsiloxane (50/30 μm StableFlex, PDMS/CAR/DVB) fiber for 60 min at 30 °C. One minute desorption of the analyte took place in the injection port of the gas chromatograph, which was maintained at 250 °C. The fiber was kept inside the GC injector port until the end of the GC run (30 min).