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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).

Table 1
MS/MS conditions

Parameter	Range tested
Precursor ions	94, 138
Daughter ions	77.1, 94.1
Isolation window	0.5, 1, 2
Excitation voltage	1, 5, 10

2.5. GC–ITMS conditions

Automated HS-SPME of 2-phenoxyethanol was performed using a CombiPal multipurpose sampler (CTC Analytics, USA) connected to a GC–ITMS system consisting of a Trace GC 2000 gas chromatograph (Thermo Quest, USA) equipped with a PTV injector (liner volume 95 μL), digital pressure flow control (DPFC) and an ion trap mass spectrometric detector POLARIS Q (Finnigan, USA). An HP-Innowax capillary column (30 m length × 0.25 mm i.d., coated with 0.25 μm film, Agilent, USA) was employed to separate the extracted compounds. The initial oven temperature of 45 °C was maintained for 1 min, before being increased to 225 °C at a rate of 3 °C min^{-1}, and subsequently to 275 °C at 10 °C min^{-1} (total GC run time was 30 min). The injector was operated in a splitless mode with a 1 min sampling time (splitless period). The injection port, the transfer line and the ion source temperatures were set at 250, 275 and 200 °C, respectively. The detector was operated in electron ionisation mode (70 eV) using either Segment Scan (MS spectra recorded in a range of *m/z* 35–520) or MS/MS mode. The tested range of optimised parameters is summarised in Table 1.

The data was processed using XCALIBUR software, version 1.2.2 (Finnigan, USA).

3. Results and discussion

SPME as an innovative, solvent free technique [9,10] has become widely used in the analysis of flavours and fragrances [11–15], pharmaceutics [16] and contaminants in environmental [17,18], food and biological matrices [19,20].

Our choice of this technique for the extraction of 2-phenoxyethanol from fish samples was based on the ability of SPME to concentrate analytes, as well as on the need for simple sample handling.

To develop the procedure, parameters influencing the SPME process, such as fiber selection, extraction time, extraction temperature and matrix modification were first optimised. In addition to the use of Segment Scan operation mode, MS/MS [21–23] mode was also applied to increase the sensitivity of MS determination. The optimisation steps are described in detail below.

3.1. Optimisation of SPME method

To obtain high sensitivity and good repeatability of determination, HS-SPME, as an equilibrium technique, requires careful optimisation. Consequently, the main factors influencing the whole analytical process were investigated.

Because of the complex character of the analysed samples, headspace sampling was the only SPME mode considered. To avoid any potential change to the sample matrix caused by elevated temperatures, the decision to keep the sorption temperature at 30 °C was made at the beginning of our study. This, together with the fact that 2-phenoxyethanol is a relatively polar compound ($\log K_{ow} = 1.16$) with relatively low vapour pressure (0.07 mmHg at 25 °C), makes the optimisation of headspace sampling difficult, but by no means impossible.

To identify the fiber most efficient for analyte extraction, three commercially available SPME fibers (PA, PDMS/CAR/DVB, CW/DVB), with different stationary phase selectivities, were evaluated. A fish tissue (unmodified) spiked at a level of 33 mg kg^{-1} served as the sample in these fiber testing experiments. All extractions were performed under the same conditions (60 min sorption at 30 °C). Unsatisfactory extraction efficiency results (based on detector response, i.e. signal to noise ratio comparison) were obtained using PA and CWX/DVB fibers. The mixed PDMS/CAR/DVB fiber produced the best results, and was therefore employed in our subsequent experiments.

Experiments focusing on the dynamics of 2-phenoxyethanol extraction were conducted with 5, 15, 30, 40 and 60 min extraction times at 30 °C. The experiments were carried out using samples of fish tissue spiked at a level of 1 mg kg^{-1}. The results obtained are shown in Fig. 1. The amount of extracted analyte increased continuously over the course of the extraction time range. To maintain laboratory sample throughput at an acceptable level, no further increase in extraction time was considered. The extraction time of 60 min was chosen for further analyses.

3.2. Sample preparation and modification

Initially, sample preparation for SPME analysis consisted solely of the grinding of fish tissue. However, relatively poor repeatability of 2-phenoxyethanol determination (relative standard deviation, R.S.D. = 25%, $n = 5$, level of 50 mg kg^{-1}) was obtained; most likely as a result of the differing water content of the analysed samples. In addition, the method sensitivity was too low (LOQ 1 mg kg^{-1}). These results indicated that more atten-

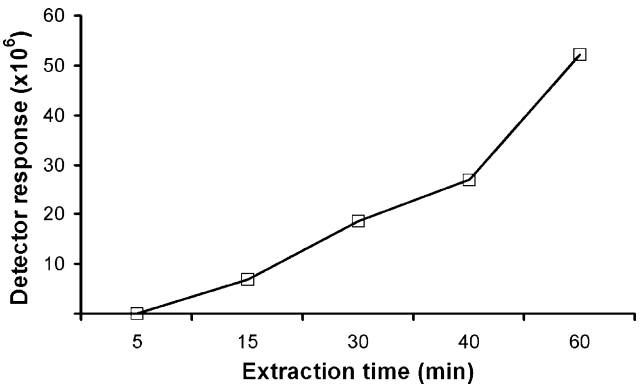

Fig. 1. Dynamics of 2-phenoxyethanol extraction.

Table 2
Comparison of relative responses of 2-phenoxyethanol in unmodified and modified samples

Sample	Without modification	Addition of water	Addition of sodium sulphate	Addition of sodium sulphate and water
Incurred residues	100%[a]	66% 10%	166%	100%[b]
Spiked sample	100%[a]	–	371%	

[a] Response of 2-phenoxyethanol in unmodified fish sample represents 100%.
[b] Response of 2-phenoxyethanol in fish sample ground with sodium sulphate and immersed in water represents 100%.

tion must be paid to the sample preparation step, and especially to unifying the water content of the samples. A well-known way of achieving this involves desiccating a sample by mixing it with a drying agent, such as sodium sulphate. Together with this method, the opposite approach, involving the addition of an excessive amount of water to the samples, was also investigated in our study.

To lower the LOQ and improve the repeatability of the method, several matrix modifications were tested: fish tissue immersed in ultrapure water; fish tissue ground with sodium sulphate; fish tissue ground with sodium sulphate and then immersed in ultrapure water. All modified samples were shaken vigorously for 20 min prior to SPME analysis.

For the experiments encompassing matrix modification, we used fish tissue samples spiked at a level of 40 mg kg^{-1}, as well as samples with incurred residues of 2-phenoxyethanol (fish after anaesthetic treatment, the level 100 mg kg^{-1}).

A comparison of the results obtained from these experiments is summarised in Table 2.

The addition of water always reduced the amount of 2-phenoxyethanol extracted. Conversely, grinding with sodium sulphate resulted in responses approximately 1.5 (166%)–3.5 (371%) times higher than the responses obtained for the unmodified samples. This result shows that homogenisation of a sample with sodium sulphate (i.e. simultaneous disintegration and drying of the sample) improves method sensitivity.

The difference between the increase in analyte response in the samples with incurred residues (166%) and the samples spiked with standard solution (371%) proves that dissimilar transitions to the headspace above the solid sample occurred. This can be a problem when the standard addition method (which involves spiking a matrix with a standard solution) is used for quantification.

Despite the fact that our earlier results indicated a possible reduction in the amount of 2-phenoxyethanol extracted following water addition, in an attempt to unify analyte transition, we also decided to test samples immersed in water after being ground with sodium sulphate. As can be seen from Table 2, the negative influence of water addition was not an issue in the case of these samples, for which 2-phenoxyethanol response was 10 times higher than for the samples only immersed in water.

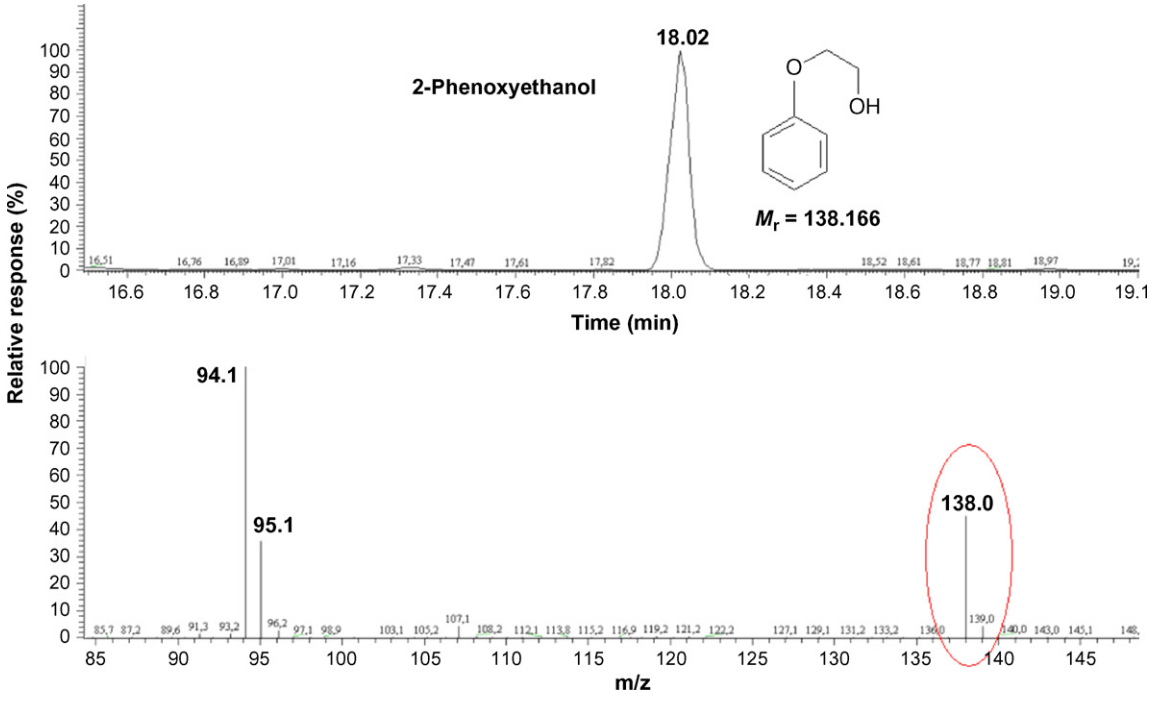

Fig. 2. Chromatogram and mass spectrum (Segment Scan) obtained by injection of standard of 2-phenoxyethanol in ethyl acetate (injection corresponds to 50 μg).

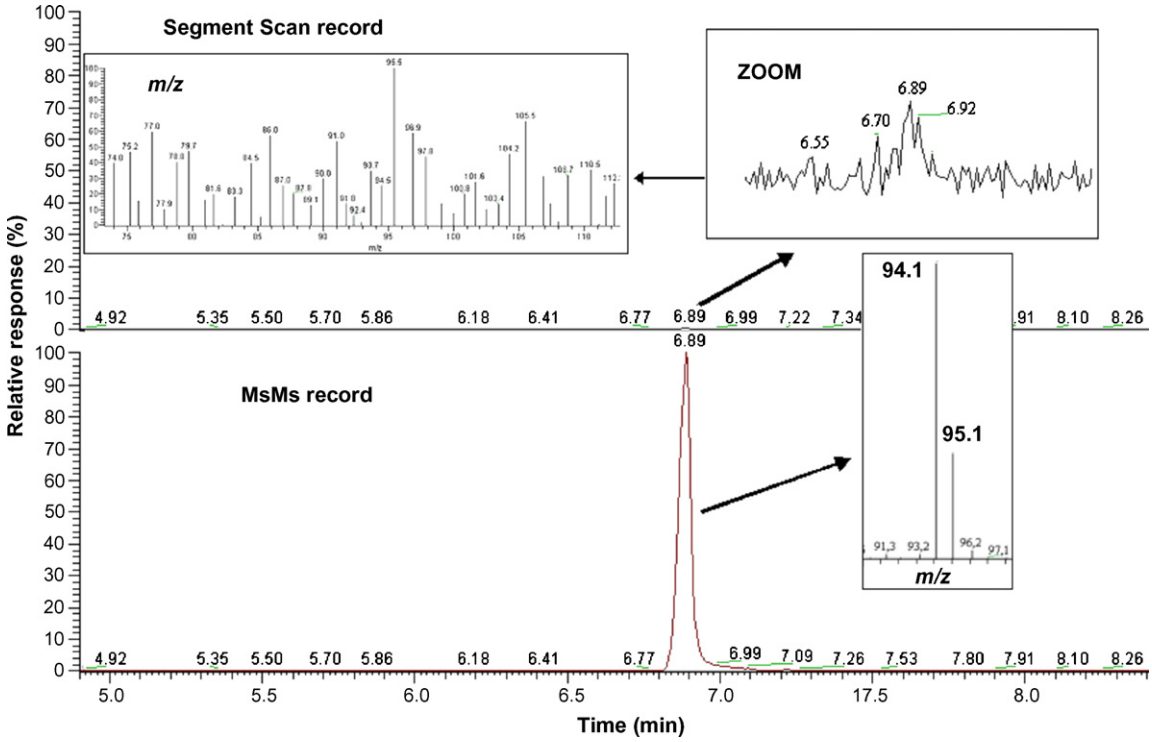

Fig. 3. Comparison of chromatograms obtained by Segment Scan and MS/MS detection modes. Sample of fish tissue spiked at a level of 0.36 mg kg^{-1}.

The blood plasma samples, having a completely different character, did not require any matrix modification, and were analysed immediately after being weighed into 10 mL glass vials.

3.3. Optimisation of MS detection

In addition to optimising the sample preparation strategy, the need to improve the sensitivity of the MS detection method was also addressed. The sensitivity of the ion trap detector was optimised by running it in MS/MS detection mode (rather than in the originally used Segment Scan single-MS mode) and by adjusting the relevant parameters (selection of precursor ion, excitation voltage, isolation window).

The spectra of 2-phenoxyethanol obtained in single-MS mode (EI, 70 eV) showed two major ions (*m*/*z* 94 and 138) in different ratios (see Fig. 2).

To achieve good selectivity of the MS/MS detection method, the *m*/*z* 138 ion (rather than the most abundant, but lower mass and, therefore, less selective *m*/*z* 94 ion) was selected for further optimisations. Evaluation of sensitivity was based on a comparison of the signal to noise ratios for these particular experiments. The best results were obtained with the following parameters: isolation window *m*/*z* 0.5; excitation voltage 1.0; excitation time 15 ms; medium excitation energy with the first product ion *m*/*z* 74 and the last product ion *m*/*z* 114.

The sensitivities of the Segment Scan and MS/MS modes are compared in Fig. 3. After fragmentation in MS/MS mode, the daughter ions *m*/*z* 94 and 95 were obtained (see zoom in MS/MS chromatogram). In comparison with Segment Scan mode (LOQ as high as 1 mg kg^{-1}), method sensitivity improved by one order of magnitude with the use of MS/MS detection mode (LOQ 0.1 mg kg^{-1}).

3.4. Method validation

Once the final SPME and MS detection conditions (see Sections 2.4 and 3.3) were optimised, validation of the developed HS-SPME–GC–MS/MS procedure was carried out in terms of linearity range, repeatability and sensitivity.

Repeatability was determined by performing five replicate analyses and expressed as a relative standard deviation. R.S.D. values of 11% and 3% were, respectively, achieved for fish tissue samples spiked at 40 mg kg^{-1} and samples containing incurred residues (100 mg kg^{-1}). The method was linear within a range of 0.1–150 mg kg^{-1}, with a regression coefficient of

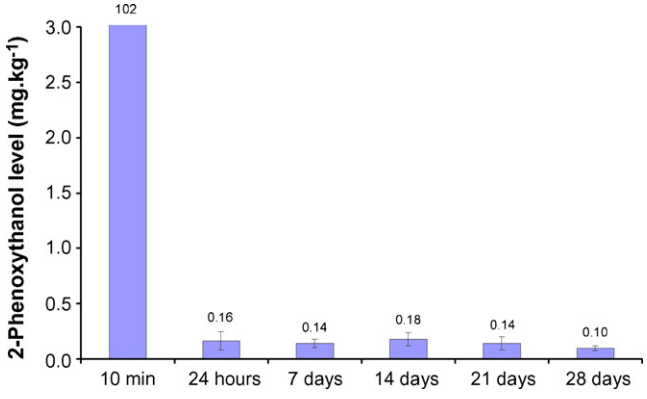

Fig. 4. Dynamics of 2-phenoxyethanol in fish tissue.

Table 3
Levels of 2-phenoxyethanol in tissue of rainbow trout

Purification time following anaesthetic treatment	Content in analysed sample (mg kg^{-1})												
	1	2	3	4	5	6	7	8	9	10	11	Average	R.S.D. (%)
10 min	120.18	100.16	88.95	116.65	98.69	95.73	105.34	116.01	94.24	97.01		101.88	10
24 h	0.33	0.25	0.24	0.17	0.19	0.10	0.11	0.10	0.10	0.13	0.14	0.16	36
7 days	0.19	0.15	0.18	0.12	0.20	0.12	0.11	0.10				0.14	27
14 days	0.26	0.27	0.17	0.12	0.14	0.14	0.11	0.15				0.18	39
21 days	0.18	0.17	0.23	0.14	<LOQ	0.10	0.16	0.14				0.14	41
28 days	<LOQ	0.12	<LOQ	0.13	<LOQ	<LOQ						0.10	24

Six to 11 samples were collected at each sampling time.

Table 4
Levels of 2-phenoxyethanol in fish blood samples

Sample	Code	Content in analysed sample (mg kg^{-1}) (% of initial content)			
		A	B	C	Average
Control sample (before anaesthetic treatment)	P1	–	–	–	–
Immediately after anaesthetic treatment	P2	254.6 (100)	252.1 (100)	137.1 (100)	214.6 (100)
15 min	P3	14.0 (5.5)	34.6 (13.7)	43.1 (31.5)	30.6 (14.3)
1 h	P4	12.8 (5.0)	5.6 (2.2)	14.7 (10.7)	11 (5.1)
4 h	P5	1.7 (0.7)	1.0 (0.4)	2.0 (1.5)	1,6 (0.7)
24 h	P6-a	1.4 (0.5)	0.8 (0.3)	0.9 (0.6)	1.0 (0.5)
24 h	P6-b	1.4 (0.6)	0.7 (0.3)	0.9 (0.6)	1.0 (0.5)

Samples from three fish were collected at each sampling time.

$r^2 = 0.9996$. An LOQ of 0.1 mg kg^{-1} was achieved in MS/MS detection mode.

An R.S.D. value of 10% was achieved for blood plasma samples containing incurred residues (level 250 mg kg^{-1}), and the method was linear within a range of 0.1–250 mg kg^{-1} ($r^2 = 0.9993$).

3.5. Dynamics of 2-phenoxyethanol in anaesthetised fish

The analytical procedure described above was used to analyse residues of 2-phenoxyethanol in fish tissue and blood plasma with the aim of monitoring its dynamics after anaesthetic treatment. As described in Section 2 (see Section 2.2), two groups

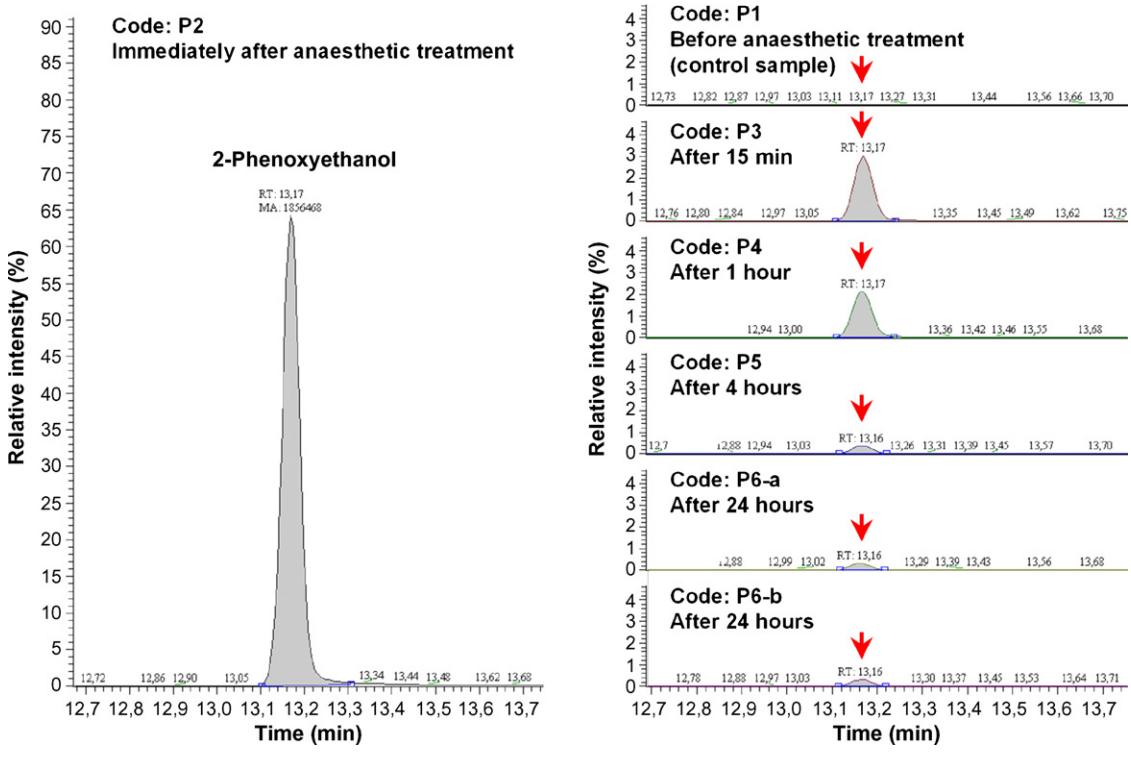

Fig. 5. Chromatograms of fish blood plasma samples taken from fish before and after anaesthetic treatment.

of samples, control fish and fish exposed to 2-phenoxyethanol, were studied.

3.5.1. Fish muscle tissue

At each sampling time, 6–11 samples were collected and analysed. The levels of 2-phenoxyethanol found in the analysed samples are summarised in Fig. 4 and Table 3.

No residues of 2-phenoxyethanol were detected in the control samples (fish not exposed to anaesthetic treatment). All fish samples collected 10 min after anaesthetic treatment showed high levels of 2-phenoxyethanol, with the mean being 102 mg kg^{-1}. Within the first 24 h after treatment, residue levels dropped remarkably to 0.16 mg kg^{-1}. This level remained more or less constant until the 28th day of purification, at which time a further slight decrease to 0.10 mg kg^{-1} was observed.

3.5.2. Blood plasma samples

At each sampling time, either one or two blood plasma samples were collected from three fish. The levels of 2-phenoxyethanol found in the analysed samples are summarised in Table 4 and Fig. 5.

During the first 15 min, a rapid decrease in 2-phenoxyethanol residue levels (down to 5–31% of the initial content) was observed. After 4 h, 2-phenoxyethanol content ranged from 1 to 2 mg kg^{-1} (0.4–1.5% of the original content) in all three analysed fish, and subsequently remained unchanged for the following 20 h. Twenty-four hours after anaesthetic treatment the mean residual amount of 2-phenoxyethanol in fish blood was 1 mg kg^{-1}, representing 0.5% of the initial amount detected immediately after anaesthetic treatment.

4. Conclusions

An SPME–GC–MS/MS method for the quantitative determination of 2-phenoxyethanol in fish tissue and blood plasma samples was developed with the aim of achieving the lowest possible LOD. Several parameters influencing both the SPME procedure and the MS detection method were optimised. The best results were obtained using a PDMS/CAR/DVB fiber for 60 min extraction of 2-phenoxyethanol from the sample headspace at 30 °C. Matrix modification proved to be the crucial step in achieving good method repeatability and sensitivity. In particular, fish tissue should be both ground with sodium sulphate and immersed in water prior to SPME analysis. (This procedure does not apply to blood plasma samples, which can be analysed without matrix modification.) The optimised headspace SPME–GC–MS/MS procedure resulted in both a low detection limit (LOD 0.03 mg kg^{-1}) and a low quantification limit (LOQ 0.1 mg kg^{-1}) for 2-phenoxyethanol in both tested biological materials.

The applicability of our method was proven by analyses of fish samples collected at different times following anaesthetic treatment. Significant reductions in 2-phenoxyethanol residue levels in both fish muscle tissue and blood plasma were observed during the first 24 h after treatment. These promising results support the registration of 2-phenoxyethanol as an anaesthetic agent in fish intended for consumption.

Acknowledgments

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References

[1] H. Tsantilas, A.D. Galatos, F. Athanassopoulou, JHVMS 56 (2005) 130.
[2] P.L. Munday, S.K. Wilson, J. Fish Biol. 51 (1997) 931.
[3] Regulation (EEC) No. 2377/90, Council Regulation (EEC) No. 2377/90 of 26 June 1990 laying down a Community procedure for the establishment of maximum residue limits of veterinary medicinal products in foodstuffs of animal origin (OJ No. L 67 of 7.3.1997, p. 1).
[4] H. Tsantilas, A.D. Galatos, F. Athanassopoulou, N.N. Prassinos, K. Kousoulaki, Aquaculture 253 (2006) 64.
[5] C.C. Mylonas, G. Cardinaletti, I. Sigelaki, A. Polzonetti-Magni, Aquaculture 246 (2005) 467.
[6] J. Velisek, Z. Svobodova, V. Piackova, L. Groch, L. Nepejchalova, Vet. Med. Czech. 50 (2005) 269.
[7] J. Ortuno, M.A. Esteban, J. Meseguer, Vet. Immunol. Immunopathol. 89 (2002) 29.
[8] J. Velisek, Z. Svobodova, Acta Vet. Brno 73 (2004) 379.
[9] J. Pawliszyn, Theory and Practice, Wiley, New York, 1997.
[10] C.L. Artur, J. Pawliszyn, Anal. Chem. 62 (1990) 2145.
[11] V. Romeo, M. Ziino, D. Giuffrida, C. Condurso, A. Verzera, Food Chem. 101 (2007) 1272.
[12] T. Čajka, J. Hajšlová, J. Cochran, K. Holadová, E. Klimánková, J. Sep. Sci. 30 (2007) 534.
[13] T. Čajka, J. Hajšlová, LC GC Eur. 20 (2007) 25.
[14] M.C. Diaz-Maroto, M.S. Peréz-Coello, M.D. Cabezudo, Chromatographia 55 (2002) 723.
[15] R.A. Pérez, T. Navarro, C. de Lorenzo, Flav. Frag. J. 22 (2007) 265.
[16] X. Fu, Y. Liao, H. Liu, Anal. Bioanal. Chem. 381 (2005) 75.
[17] R. Stiles, I. Yang, R.L. Lippincott, E. Murphy, B. Buckley, J. Sep. Sci. 30 (2007) 1029.
[18] K. Holadová, G. Prokůpková, J. Hajšlová, J. Poustka, Anal. Chim. Acta 582 (2007) 24.
[19] F. Bianchi, M. Careri, M. Musci, A. Mangia, Food Chem. 100 (2007) 1049.
[20] S. Guillot, L. Peytavi, S. Bureaul, R. Boulangerc, J.-P. Lepoutred, J. Crouzeta, S. Schorr-Galindoa, Food Chem. 96 (2006) 147.
[21] J.J. Ramos, B. Gómara, M.A. Fernández, M.J. González, J. Chromatogr. A 1152 (2007) 124.
[22] B. Gómara, M.A. Fernández, M.J. González, L. Ramos, J. Sep. Sci. 29 (2005) 123.
[23] C. Zwiener, L. Kronberg, Fresenius J. Anal. Chem. 371 (2001) 591.