Sensitive determination of Fenamiphos in water samples by flow injection photoinduced chemiluminescence

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Abstract

In this work, a sensitive flow injection chemiluminescence (FI-CL) method for the determination of nematicide Fenamiphos in a rapid and simple way is proposed. Fenamiphos is firstly photodegraded in basic medium. These photofragments react with Ce(IV) providing the chemiluminescence signal. To the authors' knowledge, no chemiluminescence method has been described in the literature for the determination of the nematicide Fenamiphos. All physical and chemical parameters in the flow injection chemiluminescence system were optimized in order to obtain the best sensitivity, selectivity and sample throughput. Before the injection of the sample in the FI-CL system, a preconcentration step with solid phase extraction C18 cartridges was performed. By applying SPE to 250 mL of standard (final volume 10 mL), the linear dynamic range was between 3.4 and 60 μg L^{-1}, and the detection limit was 1 μg L^{-1}. When SPE was applied to 500 mL of standard (final volume 10 mL), the detection limit was 0.5 μg L^{-1}. These detection limits are below the emission limit value established by the Spanish Regulations of the Hydraulic Public Domain for pesticides (50 μg L^{-1}) and of the same order as the limit established for total pesticides (0.5 μg L^{-1}) at European Directive on the quality of water for human consumption. The sample throughput was 126 hour^{-1}. Intraday and interday coefficients of variation were below 10% in all cases. No interference was registered in presence of usual concentrations of anions, cations and other organophosphorus pesticides. The method was successfully applied to the analysis of environmental water samples, obtaining recoveries between 96 and 107.5%.

Keywords: Fenamiphos; chemiluminescence; flow injection analysis; solid phase extraction; water

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1. Introduction

Fenamiphos (ethyl 3-methyl-4-(methylthio)phenyl isopropylphosphoamidate, **Figure 1**) is an organophosphorus nematicide and insecticide that is mainly used to control soil and leaf nematodes such as *Meloidogyne spp., Tylenchulus semipenetrans, Aphelenchoides sp.* or *Ditylenchus dipsaci* on crops, agricultural plantations, nurseries and non-agricultural sites (i.e. turf and ornamentals). This systemic nematicide is active against free living ecto-endo parasites and root-knot nematodes.
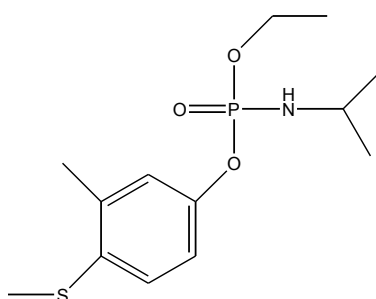
Figure 1. Molecular structure of Fenamiphos.

Fenamiphos has been extensively used in recent years in agriculture in the treatment of crops such as zucchini, peppers, watermelon, tomato, citrus fruit or bananas among others. In most treatments, the pesticide is applied as a spray on crops or on soil from where it can move to other parts of the environment such as surface water, drinking water and groundwater [1]. Due to the high stability, the water solubility and the toxicity of this nematicide, the development of simple and sensitive methods for its determination is needed for environmental protection and health-care. In this way, the European Union establishes the maximum allowable limit for total pesticide concentration in 0.5 μg L^{-1} both in the European Directive on the quality of water intended for human consumption [2] and in the European Directive on the protection of groundwater against pollution and degradation [3]. Moreover, the maximum concentration of pesticides established in the Regulations of public water domain [4] for the pesticide emission limit value is 50 μg L^{-1}. Therefore, monitoring the possible pesticide contamination in water samples is an important task for environmental protection.

The analytical methods proposed in recent years for the determination of the nematicide Fenamiphos in water samples are mainly based on separation techniques in which the separation and quantification of several pesticides of the organophosphorus family is proposed. Gas chromatography is the most widely used separation technique, and it is usually coupled with powerful detection systems like mass spectrometry [5-8], electron impact-mass spectrometry (EI-MS) and inductively coupled plasma mass spectrometry (ICP-MS) [9], or nitrogen phosphorus detection (NPD) [8, 10, 11] among others. This technique can achieve detection limits below the legislated value [2, 3] for most of the pesticides tested when it is combined with preconcentration and sample clean-up techniques, such as solid phase microextraction (SPME) [5-7, 9], multiwalled carbon nanotubes [10] or stir-bar sorptive extraction [12]. Besides gas chromatography, liquid chromatography has allowed the screening of 300 pesticides [13] using a MS/MS detection that achieves very low detection limits, near 0.1 μg/L, in the absence of sample preconcentration steps. In addition, other methods have been described for the separation and quantification of organophosphorus pesticides such as capillary electrophoresis coupled with off-line solid phase extraction [14] and capillary liquid chromatography combined or not with solid phase microextraction [15, 16]. These later methods provide detection limits for Fenamiphos between 1.0 and 5.8 μg L^{-1}.

Although chromatographic methods are interesting due to their very low detection limits for Fenamiphos nematicide and other organophosphorus pesticides, it is important to note that the cost of the instrumental equipment is very high, and the time of analysis is usually very long, between 25 and 40 min per sample. Moreover, in some cases low recoveries have been obtained specifically in the analysis of Fenamiphos in real water samples [11, 12]. Therefore, simple, inexpensive and fast methods are required for routine analysis of this nematicide.

Chemiluminescence is the basis of a highly sensitive analytical technique that can be used for the determination of different compounds in a wide variety of matrices. This method also allows the determination of compounds that do not exhibit native chemiluminescence if they or their fragments obtained after photolysis, participate in the chemiluminescence reaction as precursors, catalysts, inhibitors, oxidants, etc [17, 18]. This detection technique combined with flow injection analysis provides simple and inexpensive methods, with a high level of automation and very short time of analysis. Due to the high sensitivity of the technique, low detection limits can be reached with a wide range of linearity. In recent years, there have been proposed in the

literature a wide variety of flow-injection chemiluminescence (FI-CL) methods that allow rapid, selective and sensitive determination of pesticides such as diuron [19], paraquat [20], imazalil [21], thiram [22], asulam [23], diquat [24], carbofuran and promecarb [25, 26], carbaryl [27] and pirimicarb [28] among others. However, to the authors' knowledge, none has been described the FI-CL method for the determination of Fenamiphos nematicide in the literature for any.

In this work, a selective and sensitive FI-CL method for the determination of nematicide Fenamiphos in a rapid and simple way is proposed. All physical and chemical parameters of the FI-CL system have been optimized. Fenamiphos is photodegraded in basic medium with an ultraviolet lamp. As it was established in the bibliography, in the direct photodegradation of Fenamiphos in water, the major photoproduct is fenamiphos sulphoxide [29]. After the photodegradation step, the fenamiphos photoproducts react with Ce(IV) in acid medium providing the direct chemiluminescence signal. In order to enhance the sensitivity of the method, a previous solid phase extraction (SPE) with C18 cartridges has been applied. The method has been satisfactorily applied to the analysis of Fenamiphos in environmental water samples.

2. Experimental

2.1. Reagents and solutions

All reagents were of analytical grade and all solutions were prepared in Milli-Q water (Millipore, Bedford, MA, USA).

The following chemical reagents were used: HCl, $HClO_4$, HCOOH, $Ce(SO_4)_2 \cdot 4H_2O$, KIO_4, NH_4Cl, KI and $CH_3COONa \cdot 3H_2O$ were purchased from Scharlau (Barcelona, Spain); $KMnO_4$, $K_3(Fe(CN)_6)$, $K_2S_2O_8$, $NaH_2PO_4 \cdot 2H_2O$, Na_2SO_3, NaCl, K_2SO_4, NaOH, $Na_2CO_3 \cdot 10H_2O$ and triton X-100 from Panreac (Barcelona, Spain); H_2SO_4, ethanol and acetonitrile from Merck (Darmstadt, Germany); sodium dodecyl sulphate (SDS) and hexadecyltrimethylamonium bromide (CTAB) from Fluka (Steinheim, Germany); and H_3PO_4, CH_3COOH, HNO_3, acetone and methanol were from J.T.Baker (Deventer, Holland).

Cations tested as potential inorganic interferents were prepared from chlorides (Ca^{2+}, Cr^{3+}, Pb^{2+}, Na^+, Mg^{2+}, Cd^{2+} and K^+ (Panreac) and NH_4^+, Co^{2+}, Ni^{2+}, Mn^{2+} and Fe^{3+} (Scharlau)), or from sulphates (Zn^{2+} and Cu^{2+} (Panreac)). Sodium anions such as nitrite and nitrate from Probus (Badalona, Spain) and chromate from Scharlau were also tested.

Potential organic interferents such as methidation, dichlorvos, glyphosate, glufosinate, dimethoate and methamidophos were purchased from Riedel de Haën (Seelze, Germany).

The following reagents were used as sensitizers: eosyn Y (Panreac), rhodamin B and 8-hydroxyquinoline (Merck), fluorescein (Scharlau), β-ciclodextrin (Fluka), riboflavin, hexadecylpiridinium and quinine hydroclhoride (Sigma, St. Louis, MO).

Fenamiphos (97.7%, Riedel de Haën, Seelze, Germany) stock standard solution of 100 mg L^{-1} was prepared by dissolving the pure compound in water. The solution was sonicated in an ultrasonic bath (J.P. Selecta, Barcelona, Spain) for 15 minutes and stored in the dark at 4ºC. This standard solution was stable for a month. Working Fenamiphos solutions were prepared by diluting the stock standard solution in water.

2.2. Apparatus

The flow injection chemiluminescence manifold is depicted in **Figure 2**. The assembly consists on a peristaltic pump (Gilson Minipuls, Wothington, OH, USA) equipped with polyvinyl chloride pumping tubes (Omnifit, Cambridge, UK). The whole flow system was designed by using PTFE tubing (0.8 mm i.d.). The insertion of the sample was carried out with a six-port injection valve Model V-450 (Upchurch Scientific, Oak Harbor, WA) equipped with a loop of 553 μL. The manifold includes a photoreactor consisting of a 400 cm length PTFE tubing (0.8mm i.d.) helically coiled around a 15W low pressure mercury lamp (Sylvania, Madrid, Spain). The detection cell is a flat-spiral glass tube (1 mm i.d., 3 cm diameter) backed by a mirror for maximum light collection. The photodetector package was a photomultiplier tube (P30CWAD5 type 9125B) supplied by Electron Tubes (Uxbridge, United Kingdom) that was located in a laboratory-made light-tight box. The output was fed to a computer equipped with a counter-timer, also supplied by Electron Tubes.

2.3. Flow injection procedure

In the final FI-CL assembly (**Figure 2**), the standard or sample (S) prepared in 0.05 M sodium hydroxide, flows at 3.1 mL min^{-1} along the photoreactor (Ph). In the photoreactor (Ph), Fenamiphos is degraded in basic media producing some photofragments that can later react with the oxidant and generate the chemiluminescence signal.

After crossing the photoreactor, the sample loop (L) in the injection valve (IV) is filled. On the other hand, the carrier stream (C) is Milli-Q water. It flows at 9.2 mL min^{-1} and collects the photodegraded standard or sample in the injection valve. Finally, the oxidant stream (O), 0.08 mM Ce(IV) in 1M sulphuric acid, flows at 3.1 mL min^{-1} and merges with the carrier stream just before the detection cell (D).
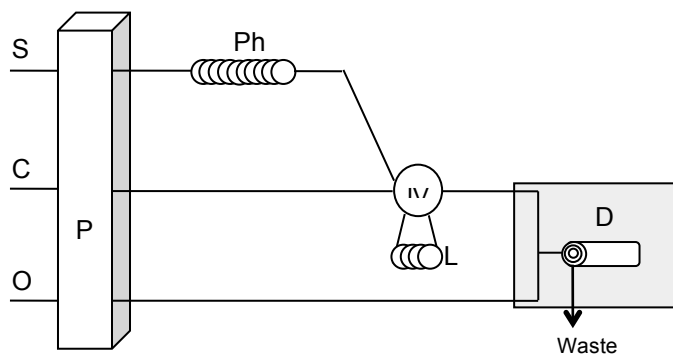
Figure 2. Flow injection chemiluminescence manifold. S: Sample stream: Fenamiphos prepared in 0.05M sodium hydroxide; C: Carrier stream: water; O: Oxidant stream: $8 \cdot 10^{-5}$ M Ce(IV) in 1 M H_2SO_4; P: Peristaltic pump; Ph: Photoreactor; L: 553 μL loop; IV: Injection valve; D: Chemiluminescence detector.

2.4. Standard preparation

The standard solutions of Fenamiphos in basic medium were prepared by mixing 0.5 mL of 1 M sodium hydroxide with variable amounts of Fenamiphos stock standard solution (100 mg L^{-1}). Standard solutions were diluted up to 10 mL with Milli-Q water.

2.5. Solid phase extraction

In general, liquid–liquid extraction (LLE) and SPE are the most commonly used sample pretreatment methods for the isolation and/or enrichment of pesticides [30]. In this work, solid phase extraction with C18 cartridges (Varian Bond Elut 200 mg, The Netherlands) has been applied to standards and water samples with the aim of preconcentrating the analyte and avoiding interferences.

To carry out the extraction of Fenamiphos in C18 cartridges, the cartridges were conditioned with 2 ml of ethanol, 2 mL of acetone, 2 mL of ethanol and 7.5 mL Mili-Q water. Then, variable volumes of standard solution (10–500 mL) or 250 mL of water sample were transferred through the cartridge, which was further washed with 25 mL of Mili-Q water and dried under vacuum for 5 minutes. The elution of the analyte was performed by adding 2 mL of acetone. Eluate was dried with N_2 at 40 ° C for 15 minutes. The dried residue was dissolved with Mili-Q water in an ultrasonic bath (5 minutes) and then 0.5 mL of 1M NaOH was added. Finally, it was diluted up to 10 mL with Mili-Q water prior to FI-CL analysis.

2.6. Sample preparation

The proposed method has been applied to the analysis of 7 water samples from different sources: 3 tap water samples (S1, S2 and S3), 2 bottled water samples (S4 and S5), 1 well water sample (S6) and 1 seawater sample (S7). The samples were collected in plastic bottles, filtered under vacuum with 0.45 μm polyamide membrane filters and stored in the refrigerator at 4 ° C until analysis.

Before the FI-CL analysis, the SPE procedure with C18 cartridges (sample initial volume 250 mL, final volume 10 mL) was applied to each water sample. Moreover, 250 mL of each water sample were also spiked at 3 or 4 levels of Fenamiphos concentration (10, 20, 30 and 40 μg L^{-1}) and analysed by the FI-CL method proposed.

3. Results and discussion

3.1. Optimization of chemical and physical parameters of the FI system

All physical and chemical parameters of the FI-CL system were optimized in order to reach the best sensitivity for Fenamiphos determination. The studied parameters will be described in the next sections.

Throughout the optimization process, a standard solution of Fenamiphos of 10 mg L^{-1} was used, and the standard/sample channel in the general FI-CL assembly (**Figure 2**) was divided into 2 sub-channels to introduce the photodegradation medium (NaOH 0.1M) separately.

3.1.1. Selection of the oxidation system

To select the oxidant system that will provide the maximum direct chemiluminescence signal in the reaction with the Fenamiphos photofragments, 5 strong oxidants in different oxidation media were tested. MnO_4^-, $Fe(CN)_6^{3-}$, $Ce(IV)$, IO_4^- and $S_2O_8^{2-}$ were tested in 1.8M H_2SO_4 as oxidation medium, and $Fe(CN)_6^{3-}$ was tested in 2.5M NaOH as oxidation medium. All oxidants were tested in a wide range of concentrations between $1\cdot10^{-5}$M and $2\cdot10^{-3}$ M.

$Fe(CN)_6^{3-}$, IO_4^- and $S_2O_8^{2-}$ in 1.8M H_2SO_4 did not provide any chemiluminescence signal when reacting with the Fenamiphos photofragments. So they were ruled out as oxidants.

Figure 3 shows the chemiluminescence signals obtained as a function of the concentration of oxidant for MnO_4^- in 1.8M H_2SO_4, $Fe(CN)_6^{3-}$ in 2.5M NaOH and Ce(IV) in 1.8M H_2SO_4. As shown, the oxidation system $Fe(CN)_6^{3-}$ in 2.5M NaOH provided a very weak chemiluminescence signal in all tested oxidant concentrations. MnO_4^- and Ce (IV) provided high chemiluminescence signals, being the chemiluminescence signal provided by Ce (IV) 2.4 times higher. Therefore, $8\cdot10^{-5}$M Ce(IV) in 1.8M H_2SO_4, was selected as oxidant.
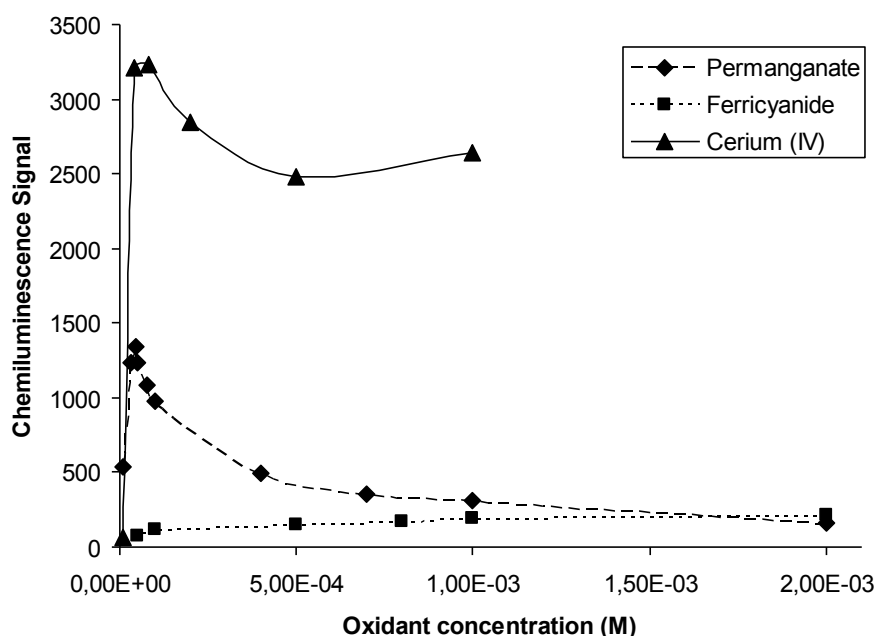
Figure 3. Chemiluminescence signal of a 10 mg L^{-1} standard of Fenamiphos versus oxidant concentration (M). Oxidation systems: ▲ Ce(IV) in 1.8 M H_2SO_4 , ◆ MnO_4^- in 1.8 M H_2SO_4, and ■ $Fe(CN)_6^{3-}$ in 2.5M NaOH.

3.1.2 Oxidation medium

With the optimum oxidant concentration for Ce(IV), $8 \cdot 10^{-5}$M, the oxidation medium was studied.

Firstly, the most suitable acid to carry out the oxidation reaction was selected. The following acids were tested at 1.8M concentration: H_2SO_4, HNO_3, $HCOOH$, HCl, CH_3COOH, $HClO_4$ and H_3PO_4. The best chemiluminescence signal was obtained by using H_2SO_4, but HNO_3 and CH_3COOH also gave a good chemiluminescence signal.

For these three acids, H_2SO_4, HNO_3 and CH_3COOH, its concentration was varied between 0.5 M and 2M. Among all assays, the maximum chemiluminescence signal was obtained by using 1.5M HNO_3, but the mixture Ce (IV)/HNO_3 was very unstable causing great variability in the signal. Therefore, 1M H_2SO_4 was selected as the optimum acid conditions for the oxidation reaction.

3.1.3. Photodegradation medium

Preliminary studies determined that the most suitable medium for Fenamiphos photodegradation was 0.1M NaOH.

With optimum oxidation system, $8 \cdot 10^{-5}$ M Ce (IV) in 1M H_2SO_4, the influence of the concentration of NaOH (between 0.01M and 1M) in the photodegradation step on the chemiluminescence signal was checked. Chemiluminescence signal increased when increasing the NaOH concentration, but by using 1M NaOH concentration, also the blank signal was enhanced. Finally 0.1M NaOH was selected as the optimal concentration for the photodegradation step.

3.1.4. Flow rate

In the FI system, the flow rate of the photodegradation and oxidation steps was evaluated separately.

In the photodegradation step, sample and photodegradation medium (0.1M NaOH) were circulating in a 1:1 ratio and merged just before the photoreactor. The total flow rate of the mixture was varied between 0.4 and 5.3 mL min^{-1}. **Figure 4** shows that in general, increasing the flow rate of the photodegradation step, the chemiluminescence signal

increased. However, above a rate of 3.1 mL min^{-1} a slight decrease in the chemiluminescence signal was observed. So 3.1 mL min^{-1} was established as optimal flow rate for the photodegradation step.

In the oxidation step, the oxidant and carrier flowed in proportion 1:3 in order to minimize analyte dilution. To optimize the flow rate, both velocities were varied simultaneously: the speed of the oxidant was varied between 0.22 and 3.3 mL min^{-1} and the speed of the carrier between 0.62 and 9.3 mL min^{-1}. The variation of the chemiluminescence signal versus the flow rate of the oxidant and the carrier are also shown in **Figure 4**. As can be seen in the figure, increasing the flow rates increases the chemiluminescence signal emitted. So the maximum flow rate tested was taken as the optimum speed for the oxidation step: 3.3 mL min^{-1} for the oxidant and 9.3 mL min^{-1} for the carrier. Higher flow rates were not tested because they gave high pressures on the flow system.
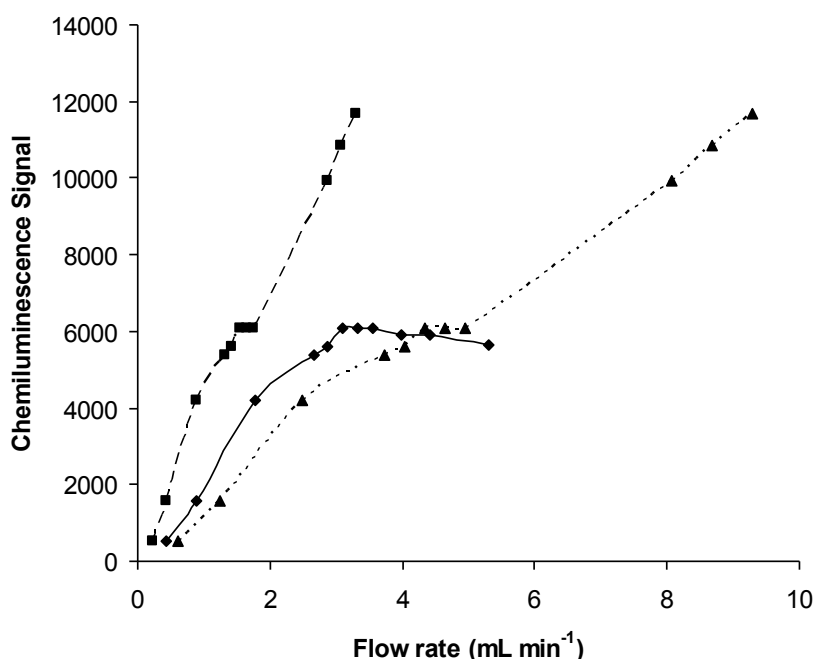
Figure 4. Chemiluminescence signal versus flow rate (mL min^{-1}) for a standard of 10 mg L^{-1} of Fenamiphos. ◆ Sample plus photodegradation medium total flow rate. ■ Oxidant flow rate. ▲ Carrier flow rate.

3.1.5. Sample volume

The volume of the sample was varied between 100 μL and 1005 μL. The chemiluminescence signal increased with the sample volume from 100 μL to 553 μL,

and above this sample volume it slightly decreased. As the maximum chemiluminescence signal was obtained with a loop of 553 μL, this was selected as the optimum sample volume.

3.1.6. Temperature of the FI-CL system

In order to study the effect of temperature on the chemiluminescence signal generated in the oxidation reaction, a water bath (J.P. Selecta) was employed at temperatures between 20 °C and 80 ºC. To warm the different channels of the FI system, a reactor of 1005 μL was introduced into the bath water. The effect of temperature was studied by heating each channel separately (sample, photodegradation medium, oxidant and carrier) and by heating several channels at a time (sample + photodegradation medium, and sample + photodegradation medium + oxidant + carrier).

In all cases, there was a slight decrease on the chemiluminescence signal when the temperature increased. Therefore, working at room temperature was selected as the optimum option.

3.1.7. Effect of fluorescence compounds and organized media

In chemiluminescent reactions, fluorescent compounds and organized micellar media are commonly used to enhance the sensitivity of the reaction. Therefore, to assess the possibility of sensitizing the chemiluminescent reaction between the photofragments of Fenamiphos and Cerium (IV) in acid medium, the following common micellar media and fluorescence compounds were tested: 1.2%SDS, 0.6% Triton X-100, CTAB 0.14%, 20% Acetonitrile, 20% Ethanol, 1.2% β-cyclodextrin, 0.01 mM fluorescein in 0.1mM NaOH, 0.1 mM rhodamin B, 0.1 mM quinine, 0.1 mM Eosin Y, 0.1 mM riboflavin, 0.1 mM 8-hydroxyquinoline and 0.1 mM hexadecylpiridinium.

The sensitizer compounds were inserted into the FI system by using 2 different set-ups: in the first set-up, a mixture of photodegration medium and the sensitizer was prepared off-line and it was inserted into the FI system by means of the photodegradation medium channel (**Figure 2**). In the second set-up, the sensitizer was inserted by means of the carrier stream.

When inserting the sensitizer into the photodegradation medium channel, only quinine produced an increase in the chemiluminescence signal, but it was discarded because it precipitated in the photodegradation basic medium. When inserting the sensitizer in the carrier stream, only Eosyn-Y improved the chemiluminescence signal but it was

discarded because of the unstability of the blank and Fenamiphos signal. Therefore, the use of sensitizers was rejected.

3.2. Method validation

3.2.1. Analytical performance

Linear calibration curves were obtained by plotting the chemiluminescence signal versus Fenamiphos concentration (μg L^{-1}): $y = (a \pm s_a) + (b \pm s_b) \cdot C$, where **y** is the chemiluminescence signal and **C** is Fenamiphos concentration.

Some of the FIA-grams obtained after applying the SPE procedure to 250 mL of standards (final volume 10 mL) are shown in **Figure 5**. As can be seen, the chemiluminescence signal is stable and increases linearly with increasing concentration of Fenamiphos.
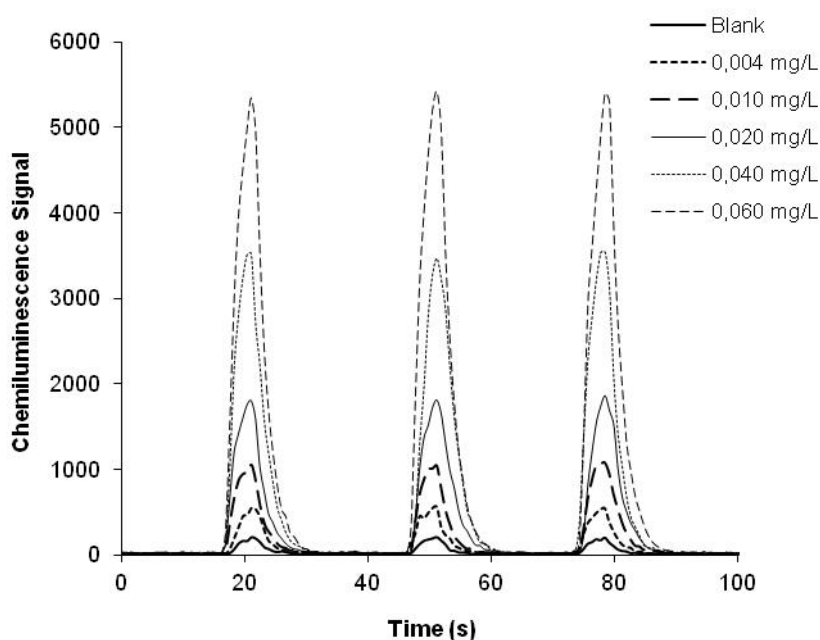
Figure 5. FIA-grams obtained after applying SPE to 250 mL Fenamiphos standards of 0, 4, 10, 20, 40 and 60 μg L^{-1}.

Table 1 summarizes the analytical figures of merit for the calibration curves obtained without applying the SPE procedure and applying the SPE procedure to 250 mL standards of Fenamiphos. As can be deduced from the slopes of the calibration curves (**Table 1**), the sensitivity of the reaction was enhanced about 22 times by applying the preconcentration procedure in C18 SPE cartridges.

Linear dynamic range without applying SPE procedure was between 16 and 2000 μg L⁻¹, and applying SPE procedure to 250 mL standards of Fenamifos, it was reduced to 3.4 – 60 μg L^{-1}. In both the calibration conditions (with or without SPE), the experimental detection limits are in accordance of the limits of detection calculated as 3 times the standard deviation of the blank signal divided by the slope ($3 \cdot s_{blank}/b$). Applying SPE procedure to 250 mL sample, the detection limit was 1 μg L^{-1}. This detection limit is below the emission limit value established by the Regulations of the Hydraulic Public Domain for pesticides (50 μg L^{-1}). Moreover, we have experimentally proved that a detection limit of 0.5 μg L^{-1} can be reached if 500 mL of Fenamiphos standard is preconcentrated with C18 cartridges. Thus, the method allows the detection of Fenamiphos at the limit established for total pesticides (0.5 mg L^{-1}) at European Directives about water quality [2, 3].

Sample throughput of the FI-CL system was 126 hour^{-1}.

Table 1. Analytical figures of merit for Fenamiphos determination. Conditions: without applying the SPE procedure and applying the SPE procedure to 250 mL standards of Fenamiphos.

Analytical figures of merit	Without SPE	With SPE
Calibration curve, r^2, n $y = (a \pm s_a) + (b \pm s_b) \cdot C$, n	$y = (330\pm60) + (3.84\pm0.08) \cdot C$, 0.9921, 20	$y = (180\pm30) + (83.90\pm1.10) \cdot C$, 0.9978, 16
Linear dynamic range (μg L^{-1})	56 - 2000	3.4 - 60
Experimental Detection Limit (μg L^{-1})	10	1.0
Calculated Detection Limit (μg L^{-1}) ($3 \cdot s_{blank}/b$, n=5)	16	1.0
Quantification Limit (μg L^{-1}) ($10 \cdot s_{blank}/b$, n=5)	56	3.4
Sample throughput (hour^{-1})	126	126
Repeatability (Fenamiphos concentration, %Variation coefficient, n)	250 μg L^{-1}, 6.9%, 3 500 μg L^{-1}, 3.5%, 3 1000 μg L^{-1}, 2.1%, 3 1250 μg L^{-1}, 3.5%, 3	10 μg L^{-1}, 6.5%, 3 20 μg L^{-1}, 3.7%, 3 40 μg L^{-1}, 0.5%, 3
Reproducibility (Fenamiphos concentration,	250 μg L^{-1}, 6.2%, 5 500 μg L^{-1}, 5.2%, 4	10 μg L^{-1}, 10%, 5 20 μg L^{-1}, 8.9%, 5

%Variation coefficient, n)	1000 µg L^{-1}, 2.1%, 3	40 µg L^{-1}, 7.4%, 5

The precision of the method was evaluated through repeatability (intra-day precision) and reproducibility (inter-day precision) studies at different concentration levels of Fenamiphos (**Table 1**). For repeatability studies, 3 replicates of the standards of Fenamiphos were prepared and measured the same day. For reproducibility studies, the standards of Fenamiphos were prepared and measured on different days (the number of replicates, n, are indicated on **Table 1**). Without SPE, coefficients of variation were below 6.9% for all concentration levels assayed. Applying the SPE procedure to 250 mL Fenamiphos standards, the coefficients of variation were always below 10%.

3.2.2. Interference study

Some ions and organic compounds can enhance or decrease the chemiluminescence signal causing errors in the determination of pesticide. So maximum allowable concentration must be established.

In order to study the selectivity of the proposed chemiluminescent method, the influence of the presence of cations (Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{3+}, Cu^{2+}, Fe^{3+}, Mg^{2+}, Mn^{2+}, NH_4^+, Ni^{2+}, Pb^{2+} and Zn^{2+}), anions (CH_3COO^-, Cl^-, CO_3^{2-}, CrO_4^{2-}, $H_2PO_4^-$, I^-, NO_2^-, NO_3^- and SO_4^{2-}) and other pesticides of the organophosphorus family (dichlorvos, dimethoate, glufosinate, glyphosate, metamidophos and methidation) on the chemiluminescence signal of Fenamiphos was assessed.

Standards of 100 mL containing 100 µg L^{-1} Fenamiphos in the presence of each one of the possible interferent were prepared and the SPE procedure was applied. After that, they were inserted on the FI-CL system. The tested concentrations for anions and cations were above their usual levels in water samples, and the tested concentration for pesticides of the organophosphorus family was the emission limit value established by the Regulations of the Hydraulic Public Domain for pesticides (50 µg L^{-1}).

The chemiluminescence signal obtained in each assay was compared with the chemiluminescence signal of a 100 µg L^{-1} Fenamiphos standard (100 mL volume) to which the SPE procedure was applied. **Table 2** shows the maximum allowable concentrations for each one of the possible interferents assayed and the percentage of relative error of the chemiluminescence signal. It was considered that there was no

interference when the percentage of the relative error of the signal was below 10% because the previous precision studies gave coefficients of variation always below 10% (**Table 1**).

Table 2. Study of potential interferents: Maximum allowable concentrations and percentages of relative error.

Interferent	Maximum alloweable concentration (mg L^{-1})	%Relative error (%E$_r$)
CH_3COO^-	5	6.9
Cl^-	750	0.8
CO_3^{2-}	250	9.9
CrO_4^{2-}	1	0.8
$H_2PO_4^-$	50	-0.13
I^-	1	1.4
NO_2^-	10	-3.6
NO_3^-	50	2.4
SO_4^{2-}	250	-4.7
Ca^{2+}	250	3.7
Cd^{2+}	1	-3.9
Co^{3+}	0.1	-1.4
Cr^{3+}	1	-6.4
Cu^{2+}	2,5	1.7
Fe^{3+}	1	-1.3
Mg^{2+}	250	0.8
Mn^{2+}	0.1	4.4
NH_4^+	1	8.3
Ni^{2+}	0.02	-4.6
Pb^{2+}	0.01	-8.4
Zn^{2+}	1	-1.8
Dichlorvos	0.05	0.8
Dimethoate	0.05	-5.5
Glufosinate	0.05	3%
Glyphosate	0.05	-5.4
Metamidophos	0.01	-7.7
Methidation	0.05	5%

As can be seen in **Table 2**, neither anion nor cation interfered on the chemiluminescence signal of Fenamiphos at their usual concentrations in water samples. Among organophosphorus pesticides, only Methamidophos interfered at a concentration of 50 μg L^{-1}, but the interference disappeared at a concentration of Methamidophos of 10 μg L^{-1}, which is an unusual concentration in water samples. Therefore, it was concluded that the proposed method is selective for the determination of Fenamiphos in water samples.

3.3. Application to real water samples

The proposed method was applied to the analysis of 7 environmental water samples and none of them contained Fenamiphos at concentrations above the detection limit of this method.

Moreover, 250 mL of each sample were fortified at different concentration levels of Fenamiphos (10, 20, 30 and 40 μg L^{-1}), extracted with C18 cartridges, and analysed by the FI-Cl method proposed. **Table 3** shows the concentrations of Fenamiphos spiked and found, and the percentage recovery for each one of the fortified concentrations. Recoveries for each concentration were near 100% in all samples and at all levels of Fenamiphos concentration. The average recoveries for each sample (%REC), ranging from 96 to 107.5%, demonstrated that there was no matrix effect. With these results, the accuracy of the method in the determination of Fenamiphos was validated.

4. Conclusions

The proposed chemiluminescent method allows the determination of the nematicide Fenamiphos using a fast, simple and inexpensive flow injection system. This is an accurate, selective and sensitive method, by which Fenamiphos can be detected at the μg L^{-1} level if a previous SPE process with C18 cartridges is applied. Detection limit is below the emission limit value established by the Regulations of the Hydraulic Public Domain for pesticides (50 μg L^{-1}) [4] and of the same order as the limit established for total pesticides (0.5 μg L^{-1}) at European Directives on the quality of water [2, 3].

The method has been successfully applied to the determination of Fenamiphos in seven water samples from different sources, and no sample matrix effect appeared.

Table 3. Recoveries obtained for 7 environmental water samples fortified at different concentration levels of Fenamiphos. S1, S2 and S3: tap water samples; S4 and S5: bottled water samples; S6: well water sample; S7: marine water sample. %REC is the average percentage of recovery for each sample

Water Sample	Added concentration ($\mu g\ L^{-1}$)	Found Concentration ($\mu g\ L^{-1}$)	%Recovery	%REC ± s
S1	10	10.5	104.9	96 ± 6
	20	18.5	92.7	
	30	27.4	91.4	
	40	38.8	96.9	
S2	10	10.3	103.5	100 ± 4
	20	20.8	103.9	
	30	28.8	96.1	
	40	38.5	96.4	
S3	20	19.0	95.1	104 ± 8
	30	33.1	110.2	
	40	42.2	105.5	
S4	10	10.7	106.8	107.5 ± 1.8
	20	21.9	109.8	
	30	31.6	105.5	
	40	43.1	107.8	
S5	10	9.6	95.9	97 ± 3
	20	19.1	95.5	
	30	28.2	94.2	
	40	40.3	100.8	
S6	10	9.3	93.4	102 ± 8
	20	21.8	109.2	
	40	41.6	104	
S7	10	9.8	97.7	107 ± 9

	20	23.1	115.6	
	40	42.7	106.6	

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