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Photoinduced chemiluminescence determination of carbamate pesticides

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A liquid chromatography method with post-column photoinduced chemiluminescence (PICL) detection is proposed for the simultaneous determination of eight carbamate pesticides, namely aldicarb, butocarboxim, ethiofencarb, methomyl, methiocarb, thiodicarb, thiofanox and thiophanate-methyl. After chromatographic separation, quinine (sensitizer) was incorporated and the flow passed through an UV lamp (67 s of irradiation time) to obtain the photoproducts, which reacted qith acidic Ce(IV) and provided a CL emission. The PICL method showed great selectivity for carbamate pesticides containing sulphur in their chemical structure. A solid-phase extraction process increased sensitivity (LODs ranging from 0.06 to 0.27 ng mL^{-1}) and allowed the carbamate pesticides in surface and ground water samples to be determined, with recoveries in the range 87-110% (except for thiophanate-methyl, whose recoveries were between 60 and 75%). The intra- and inter-day precision was evaluated, with RSD ranging from 1.1 to 7.5% and from 2.6 to 12.3%, respectively. A discussion about the PICL mechanism is also included.

Introduction

Carbamate pesticides are extensively used in agriculture as insecticides, herbicides or fungicides. Their use is increasing due to their lower persistence in the environment than other pesticides, such as organophosphorus or organochlorine, and to their high pesticide efficiency and broad biological activities.[1] However, due to their toxicity there is an increasing demand for the development of sensitive and selective analytical methods for their determination. In fact, the maximum permitted concentration established by European Community is 0.1 μg L^{-1} for individual pesticides and 0.5 μg L^{-1} for total pesticides in drinking water[2] and 1-3 μg L^{-1} in surface water.[3]

Several detection techniques have been employed for carbamate analysis. Spectrophotometry determination is often based on diazotization reaction (several reagents have been proposed) to obtain azo-dye compounds.[4,5] Fluorescence has also been applied, often employing compounds such as cyclodextrins[6] to enhance the emission since most carbamates have poor fluorescence.

However, most of the published methods for determining carbamate pesticides in water samples are based on chromatographic methods, mainly liquid chromatography (LC), due to the thermal instability of several carbamates, which limits their direct determination by gas chromatography (GC). Therefore, prior derivatization is desirable when GC analysis is performed.[7]

In LC, different detection techniques have been used, such as ultraviolet[8,9] or fluorescence,[10,11] but mass spectrometry is the preferred technique[12-14] because it provides high sensitivity and selectivity with LODs of a few pg mL^{-1}. However, this technique is not available for all laboratories due to the high cost and complexity of the instrumentation. For this reason, it is necessary to develop alternative strategies. Chemiluminescence (CL) detection has also been employed in carbamate determination coupled to flow injection (FI) or multicommutation methods[15,16] generally for the determination of a single pesticide, or coupled to LC[17-19] for the simultaneous determination of several carbamate pesticides (no more than four pesticides). Post-column luminol reaction,[17] based on the enhancing effect of carbaryl, carbofuran and methiocarb on the oxidation of luminol with permanganate in a basic medium, has been proposed. Solid-phase extraction (SPE) was performed in order to obtain a preconcentration factor of 3000 for their determination in water samples. Tris(2,2′-bipyridyl)ruthenium(III),[18] photogenerated on-line, has also been proposed as a CL reactive for bendiocarb, carbaryl, promecarb and propoxur determination in water samples. The on-line photochemical conversion of the N-methylcarbamates into methylamine was necessary and SPE was used in order to obtain a preconcentration factor of 1000. Finally, peroxyoxalate[19] was used to determine carbaryl, carbofuran and propoxur in fruit juices. A pre-column hydrolysis of the pesticides, catalyzed by cetyltrimethyl ammonium bromide micelles, and derivatization of their hydrolytic metabolites with dansyl chloride was necessary. After separation of the dansylated phenols, the reaction with the bis(2,4,6-trichlorophenyl)oxalate-hydrogen peroxide system allowed light emission. Extraction and preconcentration of the pesticides was performed by a liquid-liquid extraction.

No CL method based on the reaction with strong oxidants has been previously described for carbamate pesticides, and it only has been

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occasionally employed for other pesticides, like organothiophosphorus[20], pyrethroids[21] or phenoxyacids.[22] The development of new LC-CL methods applied to other families of pesticides is very interesting due to the great advantages of these methods in terms of sensitivity, selectivity and simplicity. This paper presents a simple, sensitive and selective method for determining eight carbamate pesticides in surface and ground water samples. The method is based on the LC separation and post-column detection of pesticides by photoinduced chemiluminescence (PICL) sensitized by quinine. The UV irradiation is necessary to obtain photoproducts with adequate CL properties. Next, the photoproducts react with a strong oxidant (acidic Ce(IV)) to produce light emission. The off-line SPE allowed the sensitivity of the method to be increased.

Materials and methods

Chemicals and reagents

Ultra-pure water, obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA) was used. Analytical standards (Pestanal) of aldicarb (ALD, 99.9%), butocarboxim (BUT, 99.2%), ethiofencarb (ETH, 99.0%), methiocarb (also called mercaptodimethur, MER, 99.8%), thiodicarb (TDC, 99.9%), thiofanox (TFN, 98.7%), thiophanate-methyl (TPM, 99.3%) were obtained from Fluka (Buchs, Switzerland). Methomyl (MET, 99.5%) was purchased from Chem Service (West Chester, PA, USA). Acetonitrile (ACN) and methanol gradient grade reagents for liquid chromatography were obtained from Merck (Darmstadt, Germany).

Individual stock standard solutions of 1000 mg L^{-1} of each pesticide were prepared by dissolving accurately weighed amounts of the pesticides in ACN and were stored in darkness at 4ºC. Working standard solutions were freshly prepared by dilution to the desired concentration with an aqueous solution containing 24% ACN. These solutions were filtered through nylon membrane filters (0.22 μm particle size) from Phenomenex (Torrance, CA, USA) before injection into the chromatographic system.

Mobile phases were filtered through a 0.20 μm nylon (for water) or polytetrafluoroethylene (PTFE) (for ACN) membrane filters from Phenomenex and degassed in an ultrasonic bath.

The pre-concentration of water samples was carried out with SPE using Bond Elut-Env (styrene-divinylbenzene), 200 mg / 3 mL cartridges from Agilent (CA, USA). Other SPE cartridges were: Strata-X (Phenomenex), Chromabond HR-X (Macherey-Nagel, Düren, Germany) and C18 (Phenomenex).

For the CL reaction the following reagents were used: ammonium cerium (IV) nitrate and sulphuric acid from Panreac (Barcelona, Spain) and quinine hydrochloride dihydrate from Sigma (Steinheim, Germany). All the reagents were analytically pure.

Instrumentation

Chromatographic analysis was carried out on an HPLC equipment from Jasco Analytica (Madrid, Spain), composed of a PU-2089 quaternary gradient pump, an AS-2055 autosampler with a 100 μL loop, a CO-2065 Plus oven, a MD-2018 photodiode array detector and a CL-2027 chemiluminescence detector. The system was controlled using the LC-NETII/AFC interface also supplied by Jasco. Acquisition and treatment of data was performed using the ChromNAV software (version 1.17.01).

HPLC separation was performed with a Kinetex C18 100 x 4.6 mm (2.6 μm particle size) core-shell column from Phenomenex, in conjunction with a security guard UHPLC C18 column from Jasco Analytica.

The reagent solutions for post column CL reaction were propelled by a Minipuls 2 peristaltic pump, provided with tygon pump tubes from Restec (Barcelona, Spain). The laboratory-made photoreactor consisted of PTFE tubing (0.5 mm i.d. x 400 cm) from Omnifit (Cambridge, UK) tightly coiled around a 15 W low-pressure mercury lamp (Sylvania) for germicidal use. Tree-way T-connectors (PEEK, 0.5 mm thru-hole from Phenomenex) were used for mixture.

Water samples preparation and SPE procedure

Surface and ground water samples from different origins, namely irrigation, river, dam, well and spring waters, were tested. They were collected in plastic flask and stored in the dark at 4ºC until analysis, performed before 48 h. In order to remove sand and other suspected solid matters, samples were filtered over a 0.45 μm membrane filter of cellulose acetate (Sartorius, Goettingen, Germany).

For the water samples determination spiking was done by adding the appropriate volume of standard to 500 mL of sample, in order to obtain five different concentrations (two replicates of each concentration were prepared), namely 0.4, 0.8, 1.2, 1.6 and 2 μg L^{-1} for ALD, BUT, MET, TDC and TFN or 1, 1.5, 2, 2.5 and 3μg L^{-1} for MER, TPM and ETH. Extraction and preconcentration were achieved by solid phase extraction (SPE) with Bond Elut-Env cartridges. These were pre-conditioned with 3 mL of methanol, 3 mL of ACN and 6 mL of water. Then, 500 mL of aqueous sample were passed through them at a flow rate of 8 mL min^{-1}. The cartridges were washed with 6 mL of water and dried with air for 10 min using the vacuum system. The retained pesticides were eluted by means of gravity with 1.2 mL of ACN and finally under vacuum. Then, 2 mL of water were passed through the cartridge to recover quantitatively the ACN. Both volumes were collected in a volumetric flask of 5 mL that was filled up with water (final ACN percentage of 24%). After filtration, 80 μL of this extract were injected in the HPLC system.

HPLC procedure

A scheme of the HPLC system, with the diode array and PICL detectors (HPLC-DAD-PICL), is shown in Fig. 1. A volume of 80 μL of the water sample extract was separated in a C18 column at 32±0.1ºC using a mobile phase of ACN:H_2O flowing at 1 mL min^{-1} with the following gradient elution program: initially 24% ACN, then 8 min linear gradient to 30% ACN, followed by 0.6 min linear gradient to 50% ACN, and 1.9 min isocratic with 50% ACN; then an additional period of 1.3 min linear gradient to the initial conditions (24% ACN) and finally 2.2 min in the initial conditions was sufficient time before subsequent analysis runs. The UV spectra were

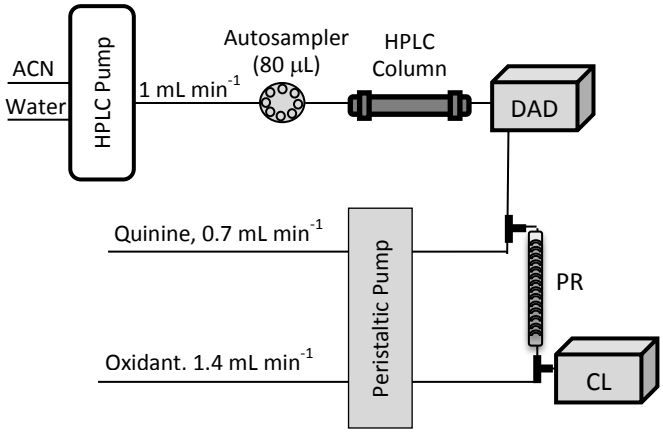


Fig. 1 Schematic diagram of the HPLC-DAD-CL system used in the determination of carbamate pesticides (PR, photoreactor (0.5 mm id x 4 m length); DAD, photodiode array detector; CL, chemiluminescence detector).

recorded between 190 and 350 nm in order to check the chromatographic system and to confirm the elution of the analytes from the chromatographic column. The column effluent from DAD was mixed, through a T-connector, with quinine 5 10^{-5} M at 0.7 mL min^{-1} and flowed through the photoreactor. Next, the products were mixed with the oxidant solution by means of a second T-connector placed immediately before the CL detector. The oxidant solution was 6 10^{-5} M Ce(IV) in 2.3 M H_2SO_4, at 1.4 mL min^{-1}. The CL emission was recorded as the background blank signal (baseline) and the quantification of carbamate pesticides was based on the peak area obtained due to the increase in the CL intensity when pesticides were detected.

Results and discussion

Optimization of the method

Chromatographic separation. In order to use the PICL for the simultaneous determination of several pesticides, a prior separation by HPLC was required. Sixteen pesticides were tested (ALD, BUT, carbaryl, carbendazim, carbofuran, chlorpropham, diethofencarb, ETH, MET, MER, phenmedipham, pirimicarb, pyraclostrobin, TDC, TFN, TPM), but only those containing sulphur in their chemical structure provided a CL signal. Therefore, these eight carbamates were selected for further studies (see molecular structures and other properties in Table 1).

Reverse-phase column (generally C18 column) and methanol-water mixtures at different pHs are the most employed when carbamates are determined by HPLC.[1] A core-shell C18 column was selected for the separation in the present work. However, mixtures of water and ACN were selected as mobile phase since it was found that methanol greatly inhibited the CL signal obtained with Ce(IV). Several gradient elution programs were tested using a flow rate of 1 mL min^{-1}, in order to obtain the complete separation of the eight carbamates in the minor time. Small percentages of ACN (below 30%) allowed the separation of seven of the carbamates, but MER was strongly retained and it was necessary to increase the ACN until 50% in order to elute it in a short time of analysis. Finally, the gradient program described in section 2.4 was selected. The temperature of the column was assayed in the range 20-55ºC. A temperature of 32ºC was selected in order to increase the reproducibility of the measures. At higher temperatures TFN and ETH overlapped. Throughout the optimization process of the PICL reaction, an injection volume of 40 µL was used.

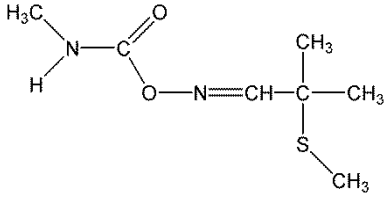
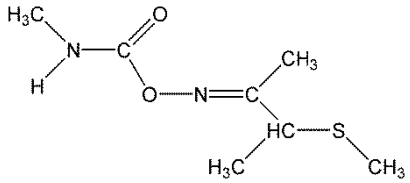
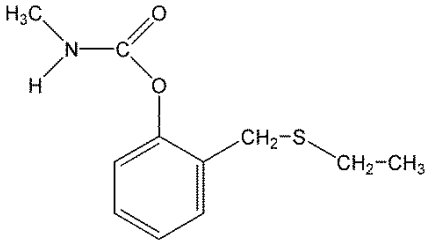
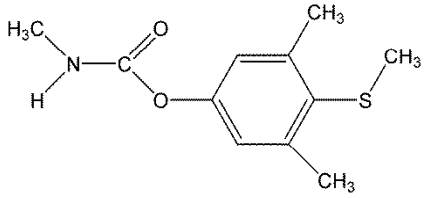
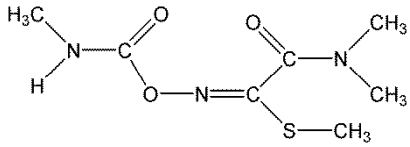
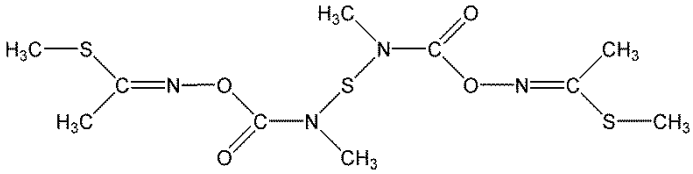
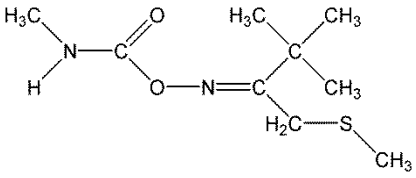
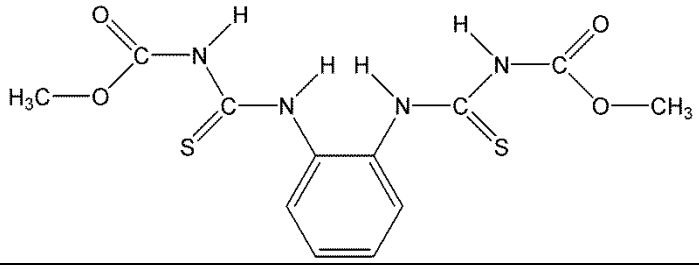
Post-column PICL reaction. The initial conditions for the PICL detection were based on our published results for methomyl,[15] modified according to a preliminary study about the effect of ACN in the system. They were as follows: 6 10^{-5} M Ce(IV) in 2 M H_2SO_4 flowing at 0.4 mL min^{-1} and quinine 10^{-4} M at 0.3 mL min^{-1}. In the absence of ACN, the best medium for the photoreaction is a basic one[15]; however, in the new conditions, basic and acid media showed an inhibition of the signal for all the assayed carbamate pesticides. Hence, water was employed as the photodegradation medium. The initial concentrations of carbamates in the standard solution were: 0.2 mg L^{-1} of MET, 0.5 mg L^{-1} of TDC and TFN, and 1 mg L^{-1} for the rest of carbamates. Through the optimization process, when a great increase in the signal was observed, the concentrations of carbamates were gradually reduced.

Changes in the chemical and physical parameters can affect the PICL reaction in different ways: enhancing the emission for some of the analytes and decreasing the emission for others. In general, the optimal conditions for MER, the less polar pesticide and therefore the strongest retained pesticide, differed from those of the rest of pesticides.

CL reactions are often very fast and high flow rates are usually required. However, the HPLC separation velocity is limited by the increase in the pressure of the system and by the decrease in the separation efficiency. Then, the global flow rate of PICL reagents (oxidant and quinine) was firstly studied. They were simultaneously varied in the range 0.7-2 mL min^{-1}. The CL signal increased with the flow, reaching a maximum at around 1.7 mL min^{-1} (1 mL min^{-1} for oxidant and 0.7 mL min^{-1} for quinine) for most of the analytes. Next, the oxidant was kept at 1 mL min^{-1}, and quinine flow rate was varied between 0.4 and 1 mL min^{-1}, but no improvements were observed and quinine flow rate was kept at 0.7 mL min^{-1}. This flow established the time of irradiation in the photoreactor which corresponded to 67 s. Next, the oxidant was varied in the range 0.6 – 1.6 mL min^{-1}, with the selected value being 1.4 mL min^{-1}.

Moreover, concentrations of the oxidation system were studied. The Ce(IV) concentration was varied from 2 10^{-5} to 1.4 10^{-4} M. Fig. 2 shows the behaviour of the eight pesticides with this parameter. For MET, TFN and MER the maximum signal was obtained for 4 10^{-5} M, whereas for the rest of the pesticides the maximum signal was at 6 10^{-5} M or higher (TPM). Thus, 6 10^{-5} M was the selected concentration for the oxidant. Sulphuric acid concentration in the oxidation system was studied in the range 0.5 – 3.0 M. The value selected for H_2SO_4 was 2.3 M because this was the optimum value for most of the pesticides although the optimum for MER was at 1.5 M, and for MET and TFN, the signals increased in the entire interval.

Table 1 Molecular structures, dissociation constant (pKa at 25ºC),[23] octanol-water partition coefficient (log P at pH 7 and 20ºC),[23] and retention times (t_R) of the carbamate pesticides in the PICL detection system.

Pesticide	Structure	pK_a	log P	t_R, min
Aldicarb (ALD)		-	1.15	4.4
Butocarboxim (BUT)		-	1.10	3.9
Ethiofencarb (ETH)		-	2.04	9.5
Methiocarb (mercaptodimethur) (MER)		-	3.18	12.5
Methomyl (MET)		-	0.09	2.1
Thiodicarb (TDC)		-	1.62	7.7
Thiofanox (TFN)		-	2.16	8.9
Thiophanate-methyl (TPM)		7.28	1.45	6.7

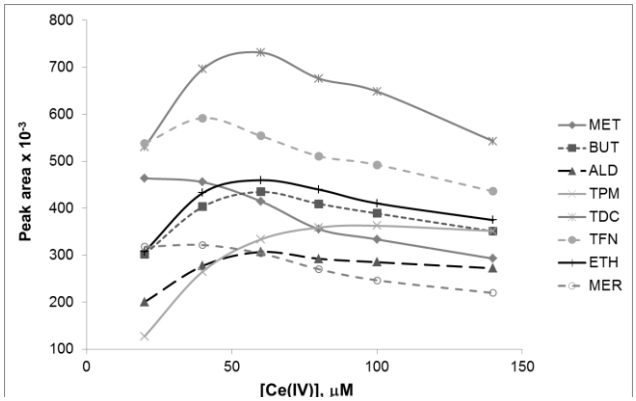


Fig. 2 Influence of the Ce(IV) concentration on the analytical CL signal for 0.2 μg L^{-1} of MER, 0.5 μg L^{-1} of TDC and TFN and 1.0 μg L^{-1} for the rest of pesticides.

The last chemical parameter involved was the sensitizer concentration. Quinine concentration was varied between 10^{-5} and 2 10^{-4} M, and 5 10^{-5} M was selected (for MER, the best results were obtained at 1.5 10^{-4} M concentrations).

Another strong oxidant that can provide a PICL signal with carbamate pesticides[15] is permanganate. We also optimized the HPLC-PICL system for this oxidant. The concentration of potassium permanganate, sulphuric acid as oxidation medium, and quinine as sensitizer were optimized, as well as the flow rate of all the reagents. However, no improvements in sensitivity compared to the Ce(IV) system were obtained and permanganate oxidation system was discarded.

With the system optimized for Ce(IV), the temperature in the flow cell of the luminometer was studied again in the range 26 (room temperature)-55ºC in order to study the effect of this parameter over the CL reaction. Only a slight increase was observed for some of the carbamates and room temperature was selected.

Sample volume and injection medium. Under optimal conditions, the effect of the injection volume was studied in the range 20-100 μL. We selected 80 μL as the optimum since higher values only provided slight increases in the analytical signal (between 2 and 22%) but amplified the peak width.

When a large injection volume is used, the strength of the solvent in which the analyte is dissolved has to be studied in order to avoid band distortions. When the percentage of ACN in the injected solution was increased, an important effect on the peak shape was observed. Thus, it was decided to keep the percentage of ACN in the injected solution at 24% (initial conditions for the chromatographic process).

PICL mechanism

CL determination employing strong oxidants of some carbamates without sulphur in their chemical structures have been reported[24-29] in FI and multicommutation systems, but this CL behaviour was only observed in one case after UV irradiation.[29] However, as mentioned above, in the assayed conditions only the carbamate pesticides containing sulphur in their chemical structure showed a PICL response. This suggests that sulphur has an important role in the PICL mechanism.

The PICL process involves two steps. Firstly the photoreaction with UV light of the pesticides and, secondly, the CL reaction with Ce(IV) in presence of quinine.

According to DeMarco and Hayes,[30] the irradiation of thiocarbamates with UV light causes the breakage of the carbonyl C-S bond resulting in two radicals, which combine with the hydrogen atoms present in the solvent to generate formamide and mercaptan. Formamide can continue the degradation process by UV light until dialkylamine is produced. On the other hand, two mercaptan radicals can lead to the formation of a disulphide. The bond S-S is quite susceptible to photolysis and can give back two separated mercaptan radicals by the effect of the UV light. Radical mercaptan, and therefore disulphide, could be obtained in the on-line irradiation process performed in the present paper. As it has been described,[31] both, mercaptan and disulphide, can be oxidized by Ce(IV) in the next step.

In the case of TPM, the photoreaction mechanism would probably be different, since a C=S double bond is present in the chemical structure. It is well know that photolysis is an important degradation rout of TPM being the carbendazim the main degradation product.[32] In the process the N-C bond adjacent to the C=S bond is broken and the resulting chain can produce the mercaptan or disulphide.

For ETH, as has been reported, the photocleavege of the carbon-sulphur band in aqueous solution gives 2-(methyl)phenyl-N-methylcarbamate as main product, and consequently a mercaptan is formed.[33] On the other hand, the irradiation of MER undergoes a photo oxidation to methiocarb sulfoxide. Further irradiation brings about loss of the sulfur moiety.[34]

In the second step, the photoproducts (PP) obtained are oxidised with Ce(IV) in acidic medium and in the presence of quinine. As has been reported,[35-37] the reduction of Ce(IV) produces excited Ce(III) which is deactivated by emitting a weak CL signal at 350 nm. Quinine, a good fluorescent substance with a emission maximum at about 450 nm,[38] has been often employed in order to increase the CL intensity via an energy-transfer excitation process. The excited quinine is the responsible for the strong CL observed. The schematic process is as follow:

$$\text{Carbamate} + h\nu \rightarrow \text{PP}$$

$$\text{Ce(IV)} + \text{PP} \rightarrow \text{Ce(III)*} + \text{oxidized PP}$$

$$\text{Ce(III)*} + \text{quinine} \rightarrow \text{Ce(III)} + \text{quinine*}$$

$$\text{quinine*} \rightarrow \text{quinine} + h\nu$$

Development of the SPE method

Although a wide variety of extraction methods has been proposed, SPE is still the most extended method used for clean-up and preconcentration of carbamate pesticides from water samples due to its large enrichment capacity[12] and simplicity. Four different cartridges were considered for SPE in the present study, namely

Table 2 Analytical figures of merit of the proposed method.

Pest	HPLC-PICL				HPLC-PICL with SPE (500 mL)		
	Dynamic range, ng mL^{-1}	LOD, ng mL^{-1}	LOQ, ng mL^{-1}	Calibration curve[a] (r^2)	Dynamic range, ng mL^{-1}	LOD, ng mL^{-1}	LOQ, ng mL^{-1}
MET	10-400	4	13	I=1.79C+37.4 (0.998)	0.10-3.0	0.06	0.20
BUT	20-250	7	23	I=0.75C+23.1 (0.994)	0.1-2.5	0.09	0.30
ALD	20-250	7	23	I=0.66C+25.2 (0.992)	0.1-2.5	0.09	0.30
PTM	60-350	20	67	I=0.36C+10.2 (0.992)	0.7-3.5	0.27	0.90
TDC	10-250	4	13	I=2.12C+27.7 (0.998)	0.1-2.5	0.06	0.20
TFN	10-350	4	13	I=1.65C+27.4 (0.999)	0.1-3.5	0.06	0.20
ETH	60-350	20	67	I=0.44C+11.3 (0.993)	0.7-3.5	0.23	0.77
MER	60-350	23	76	I=0.15C+9.66 (0.991)	0.7-3.5	0.26	0.87

a Where I is intensity in mV and C is concentration in ng/mL (n=7)

Strata-X, Chromabond HR-X, C18 and Bond Elut-Env. In all cases, the procedure was as described in *Water samples preparation and SPE procedure* section, but with 100 mL of standard solutions at initial concentrations five times higher than the ones employed with 500 mL (in order to obtain the same final concentrations). Recoveries for TPM with Strata-X and Chromabonds HR-X were less than 42% for the five concentrations assayed and, therefore, they were discarded. On the other hand, with C18 cartridges the recovery for MET was below 50%. Suitable recoveries for all the pesticides were only obtained with Bond Elut-Env cartridges and these were selected for further studies.

Next, the same assay was performed with 250 and 500 mL of standard solution with the Bond Elut-Env cartridges, in order to discard breakthrough volume and improve the preconcentration factor. The amount of pesticides in each standard solution was varied in order to obtain the same concentration in the final solution. Recoveries were between 82-110% for seven carbamates at any standard volume (100 mL to 500 mL). Only TPM presented lower recoveries, between 70-83%, but these data are inside the acceptable range for recoveries in water samples (70-110%, with a maximum RSD of 20%, IUPAC document[39]). The calibration curves obtained without and with SPE with 100, 250 and 500 mL of standard solutions gave statistically similar slopes by the t test at a 95% confidence level. 500 mL was selected as the sample volume for the SPE, which corresponds to a preconcentration factor of 100.

Validation of the method

Calibration graphs for determining samples were built by injecting, in duplicate, seven standard solutions within the linear dynamic range, and representing peak area versus standard concentration (ng mL^{-1}). Table 2 summarizes the linearity ranges and limits of detection (LOD) and quantification (LOQ) for the HPLC-PICL method with and without SPE (500 mL), as well as the calibration curve obtained.

LOD and LOQ were calculated on the basis of the equation $3s_b/b$ and $10s_b/b$ respectively, where s_b was the standard deviation of the blank (evaluated as the standard deviation of a very low concentration) and b the slope of calibration curve obtained with standard solutions. These values were experimentally confirmed. Fig. 3.a. shows a chromatogram obtained by spiking a river water sample with the eight pesticides at the LOD level, which was processed with the SPE and HPLC-PICL method. A peak at retention time around 1.4 min, which was not present in the standard solutions, was found when natural water samples were processed. This peak overlapped with the MET but it did not affect the recoveries obtained for this pesticide in the processed samples.

To evaluate the overall precision of the method, intra- and inter-day precision (as relative standard deviation, RSD) were assessed with standard solutions of the pesticides by applying the developed HPLC-PICL method with SPE at three concentration levels, namely 0.5, 1.0 and 2 ng mL^{-1} (1.0, 2.0 and 3.0 ng mL^{-1} for MER, TPM and ETH respectively). The procedure was repeated three times on the same day to evaluate repeatability (intra-day precision) and RSD values ranging from 1.1 to 7.5% were obtained. The same assay was performed for five days, randomly executed in a 22-day period, to determine inter-day precision (see ESI Table S1). RSD values in the range 2.6-12.3% were found. With these values, the precision of the method was adequate.

Furthermore, the trueness of the method was also evaluated with six water samples, namely spring, well, dam, irrigation and two river samples. Blank water samples were analysed with the proposed SPE and HPLC-PICL methods and it was observed that they did not contain the analytes or that they were below the LOD of the method. Moreover, the recoveries of known amounts of the tested compounds in the water samples at five concentration levels were evaluated. The obtained recoveries (see ESI Table S2) were close to 100% (ranging from 87 to 110), except for TPM whose recoveries ranged from 60 to 75%. This may be related to the weak acid character of this pesticide (pKa= 7.28), which may require a strict control of the sample pH.

All the slopes of the calibration curves obtained with the water samples were statistically similar to those obtained with 500 mL standard solutions, by the t test at a 95% confidence level, which

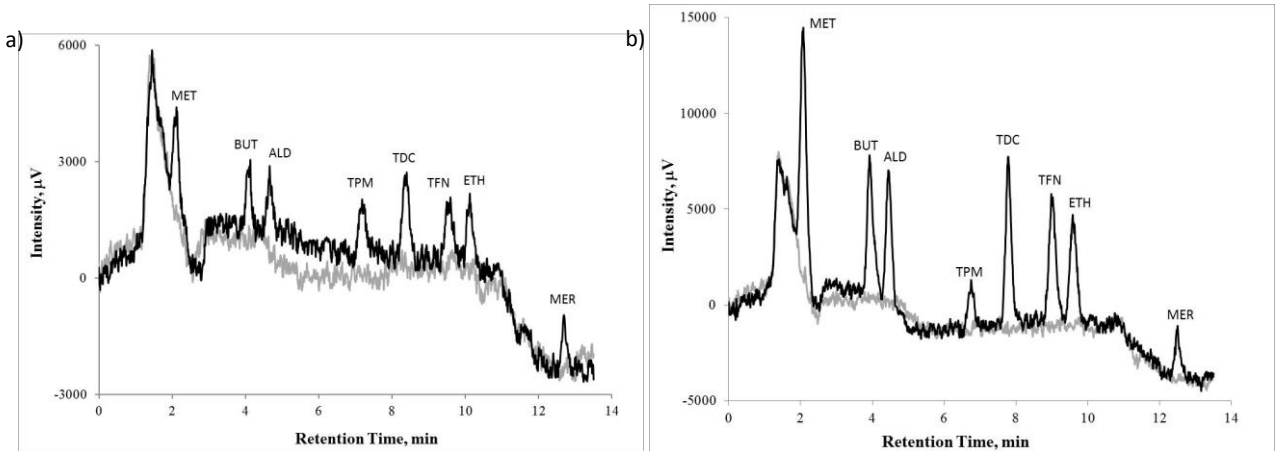


Fig. 3 HPLC-CL chromatograms obtained after SPE (500 mL) with a river water sample. Grey line is the blank and black line is the sample spiked with: a) the concentration corresponding to the LOD (0.06 μg L^{-1} of MET, TDC and TFN; 0.09 μg L^{-1} of BUT and ALD; 0.23 μg L^{-1} of ETH; 0.26 μg L^{-1} of MER; and, 0.27 μg L^{-1} of TPM); and the corresponding blank (grey line); b) 0.5 μg L^{-1} of MET, TDC and TFN; 1.0 μg L^{-1} of BUT and ALD; 1.8 μg L^{-1} of ETH, MER and TPM.

indicated that there is no matrix effect. Fig. 3.b. shows a chromatogram of a river water sample spiked with the eight pesticides in a concentration within the dynamic range.

Comparison with other CL methods

In Table 3 some of the characteristics of LC-CL methods described in the bibliography for carbamate pesticides determination are summarized. LODs obtained with luminol system[17] were much higher than those obtained by the present work. Moreover, this method required SPE preconcentration of 1.5 L of sample to achieve sensitivity levels below the maximum concentration allowed in water intended for human consumption.[2] Only one of the three pesticides considered in our paper, MER, was studied in that work. The LOD obtained with this luminol method without SPE was 6 times higher (140 ng mL^{-1}) than that obtained in our work (23 ng mL^{-1}).

In the other two references[18,19] included in Table 3, similar LODs to those obtained in our work were found without preconcentration, but none of the pesticides coincided with any included in the present work. In addition, we should point out the complexity of the derivatization and/or extraction methods, the limited number of pesticides analysed (4 and 3) in those studies and the longer time required for the chromatographic separation.

On the other hand, FI and multicommutation methods[15,16] have been described for the determination of a single carbamate pesticide. LODs obtained were, in general, similar to those provided by the present work, although in the optimization process they studied a single pesticide only, whereas in our HPLC-PICL method optimal conditions were established for eight pesticides.

Table 3 Comparison of the proposed HPLC-PICL method with other CL methods.

Pesticides	CL system	Pretreatment	LOD, ng mL^{-1}	Time,[a] min	LOD with SPE, ng mL^{-1} (preconc. factor)	Ref.
Carbaryl Carbofuran MER	Luminol/permanganate	SPE off-line	10-140	14	0.0064-0.0583 (3000)	[17]
Bendiocarb Carbaryl Promecarb Propoxur	tris(2,2´-bipyridyl)ruthenium(III) (generated on-line)	SPE off-line; photoreaction on-line (conversion to methylamine)	-	17	0.004-0.042 (1000)	[18]
Carbaryl Carbofuran Propoxur	Peroxyoxalate	Liquid-liquid extraction	-	35	2-3 (10-14)	[19]
MET,BUT, ALD,TPM, TDC,TFN, ETH,MER	Ce(IV)/H_2SO_4/quinine	SPE off-line and photoreaction on-line	4-23	13	0.06-0.27 (100)	This method

[a] Time for the chromatographic separation of the pesticides

Conclusions

The photoinduced chemiluminescence detection has been used as a selective technique for analysis of carbamate pesticides containing sulphur in their chemical structure. The UV irradiation in presence of quinine has been shown as an important tool to increase the number of compounds with adequate chemiluminescent properties. Practical application of the HPLC–Chemiluminescence (CL) technique for the determination of pesticides is still uncommon, probably because mobile phases are often incompatible with the CL emission. However, the developed method overcomes this problem and provides great precision and selectivity.

The method has been applied to a group of eight carbamate pesticides and their separation took less than 13 min. The intra- and inter-day precision is very good, with RSD ranging from 1.1 to 7.5% and from 2.6 to 12.3%, respectively. The method shows a great selectivity, since blank chromatograms present a near absence of interfering peaks. High sensitivity was obtained with LODs in the range 0.06-0.27 ng mL^{-1} when combined with SPE employing Bond Elut-Env cartridges. These values make the proposed method useful for determination of the pesticides in surface waters. The trueness of the method was evaluated satisfactorily by applying the method to six surface and ground water samples spiked at 5 concentration levels. Recoveries were around 100% except for thiophanate-methyl for which slight losses in the SPE procedure were found.

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