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Determination of organothiophosphorus pesticides in water by liquid chromatography and post-column chemiluminescence with cerium(IV)

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ABSTRACT

A new, fast, selective and sensitive method has been developed for the simultaneous determination of nine organothiophosphorus (OTP) pesticides, namely omethoate, dimethoate, disulfoton-sulfoxide, methidathion, phosmet, malathion, diazinon, pirimiphos-methyl and chlorpyrifos. The pesticides were separated on a Kinetex C18 column by gradient elution with acetonitrile:water. A post-column basic hydrolysis of the pesticides and later a chemiluminescence (CL) reaction with cerium (IV) in acid medium was carried out. Hexadecylpyridinium chloride highly enhanced the CL emission. Under optimized conditions, linearity, precision, limits of detection and quantification, and accuracy were determined. Both selectivity and sensitivity were compared with those obtained with UV detection. In combination with SPE, limits of detection in the range 15–80 ng/L and 5–30 ng/L were obtained when 250 mL and 1000 mL of solution were treated, respectively. When applied to 250 mL of sample the inter-day precision of the method was between 3.5% and 7.3% and the intra-day precision between 2.9% and 6.0%. The method was applied to determine OTP pesticides in spiked water samples from different origins: irrigation, river, sea, ground, spring, mineral and tap waters, being the percentage of recovery of added amounts near 100% form most of the pesticides.

1. Introduction

Organophosphorus pesticides (OPPs) are esters of phosphoric acid with diverse substituents. OPPs are extensively used for plant protection because of their insecticidal activity, ability to combat a large number of pest species, availability, and low cost. Many OPPs are highly toxic and pose a serious risk to humans and animals [1] because they are cholinesterase inhibiting substances, and are even used as chemical warfare agents [2]. The threat to human health arises from either direct contact or through residue in food and contamination of drinking water [3]. Moreover, use of OPPs is preferred over other pesticides (*e.g.* organochlorine compounds) because they exhibit less environmental persistence [4,5].

A number of methods have been proposed recently for the determination of OPPs [6,7]. Gas chromatography (often coupled with mass spectrometry (MS)) and high-performance liquid chromatography (HPLC) (often coupled with diode-array detection or MS) are by far the most employed analytical techniques.

Because of the low concentrations and the complexity of some sample matrices, determining OPPs in water often requires a sample preparation step, which involves extracting target compounds and cleaning them up, prior to chromatographic quantitative determination. Diverse extraction techniques, such as liquid–liquid extraction, solid-phase extraction, solid-phase microextraction, stir-bar sorptive extraction, or single drop microextraction, among others, have been proposed for preparing water samples containing OPPs [7].

Chemiluminescence (CL) can be used as a detection technique for analysis of a broad diversity of compounds in different fields [8]. In fact, CL has been gaining increasing acceptance and has been used to determine OPPs residues in recent years [9–15]. Nevertheless, practical application of the HPLC–CL technique for the determination of pesticides is still uncommon. The reason is probably that not enough CL reactions are available, or that the mobile phase of HPLC

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is often incompatible with the CL reactions, resulting in difficulties for determination. Consequently, it is essential for analysts to expand the practical application of current CL reactions and explore new ones with high sensitivity and good compatibility with HPLC. It would then be possible to take advantage of the post-column CL reaction: the analytes are separated into their original form and the reaction products of the reaction do not require a long period of stability [16].

The CL phenomenon can solve some of the problems concerning the monitoring of pesticide, such as lack of sensitivity or selectivity, and it is a more economical alternative to other powerful detection systems, such as MS.

To the authorsí knowledge, until now only two HPCL–CL methods employing strong oxidants have been described for the determination of pesticides: benzenediols and 1,2,4-benzenetriol [17], and pyrethroids [18].

The authors have previously published a flow injection analysis (FIA)–CL method for the determination of dimethoate in water samples [13]. The method developed was highly sensitive and, in general, good results were obtained. However, several compounds present in the environmental samples can often provide a similar response to the CL reaction and, therefore, they must first be removed. The aim of the present work was to develop an HPLC method, coupled with the post-column CL reaction, which would allow the simultaneous determination of several OTP pesticides with high sensitivity and selectivity. With this goal in mind, nine OPPs were selected, all of which were organothiophosphorus (OTP) compounds, in which the phosphorus component is also bonded to one or more sulfur atoms.

2. Experimental

2.1. Materials and reagents

Analytical standards (pestanal quality) of omethoate (OME, 98.5%), dimethoate (DIM, 99.6%), disulfoton-sulfoxide (DIS, 97.1%), methidathion (MET, 95.8%), phosmet (PHO, 99.9%), malathion (MAL, 97.2%), diazinon (DIA, 98.3%), pirimiphos-methyl (PIR, 99.5%) and chlorpyrifos (CLO, 99.9%) were obtained from Fluka (Buchs, Switzerland). Table 1 shows the structural formulas of the nine organothiophosphorus (OTP) pesticides. Acetonitrile (ACN) and methanol gradient grade reagents for liquid chromatography were obtained from Merck (Darmstadt, Germany).

Individual standard solutions of OTP (1000 mg/L) were prepared by exactly weighing and dissolving in ACN. Furthermore, the standard solutions were protected against light and stored at 4 °C.

Working standard solutions were prepared daily in an aqueous solution containing acetonitrile 40%, and were filtered through nylon membrane filters (0.22 μm particle size) from Phenomenex (Torrance, CA, USA), before injection into the chromatographic system.

Ultra pure water, obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA) was used. Mobile phases were filtered through a 0.20 μm nylon (for water) or polytetrafluoroethylene (PTFE) (for ACN) membrane filter from Phenomenex and degassed with an ultrasonic bath.

The solid phase pre-concentration (SPE) of water samples was carried out using Strata-X (polymeric reversed phase) 200 mg/6 mL cartridges from Phenomenex.

The ammonium cerium(IV) nitrate (Panreac, Barcelona, Spain), HCl (Scharlau, Barcelona, Spain), Hexadecylpyridinium chloride monohydrate (HPC) (Sigma, Steinheim, Germany) and NaOH (Panreac) were filtered through a Phenomenex filter membrane of nylon or PTFE (0.45 μm particle size) before pumping them into the chromatographic system.

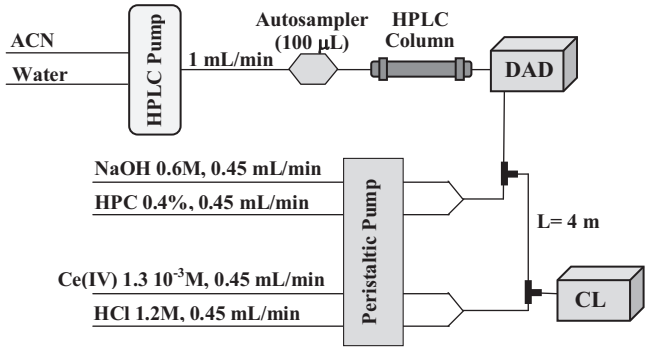

Fig. 1. Schematic diagram of the HPLC–DAD–CL system used in the determination of organothiophosphorus pesticides (DAD—photodiode array detector; CL—chemiluminescence detector; L, PTFE coil of 0.5 mm id × 4 m length).

2.2. 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 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 × 4.6 mm (2.6 μm particle size) column from Phenomenex, in conjunction with a security guard UHPLC C18 from Jasco Analítica.

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). Connections were carried out with PTFE coil of 0.5 mm i.d. from Omnifit (Cambridge, UK).

2.3. HPLC procedure

A scheme of the HPLC–DAD–CL system is shown in Fig. 1. A volume of 100 μL of the OTP solution was separated at room temperature (25 °C) with a gradient elution program at a flow rate of 1 mL/min. The mobile phase consisted of ACN and water, and the gradient elution program was: 30–44% ACN (0–0.5 min), 44% ACN (0.5–4.6 min), 44–76% ACN (4.6–8.2 min), 76–88% ACN (8.2–9.0 min), 88% ACN (9.0–9.4 min). This was followed by a 5 min equilibrium period with initial conditions prior to the injection of next sample. The UV spectra were recorded between 190 and 400 nm in order to check the chromatographic system and confirm the elution of the analytes from the chromatographic column, as well as to compare the results obtained with both detection systems. The column effluent from DAD was first mixed, through a T-piece, with a solution resulted of the confluence of 0.6 M NaOH and 0.4% HPC, both at 0.45 mL/min. After the mixture, a hydrolysis reactor of 4 m PTFE coil was inserted. Next, the hydrolysed solution was mixed with the oxidant solution by means of a second T-piece placed immediately before the CL detector. The oxidant solution resulted of the mixture of a 1.3 10^{-3} M Ce(IV) and a 1.2 M HCl solution, both at 0.45 mL/min. The CL emission was recorded as the background blank signal (baseline) and the quantification of OTP pesticides was based on the peak area obtained due to the increase in the CL intensity when pesticides were injected.

2.4. Water samples preparation and SPE procedure

Water samples from different origins, namely irrigation, river, sea, ground, spring, mineral and tap waters, were tested. They were collected in plastic flask and stored in the dark at 4 °C until analysis,