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Determination of nine pyrethroid insecticides by high-performance liquid chromatography with post-column photoderivatization and detection based on acetonitrile chemiluminescence

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Abstract

An HPLC method was developed to determine pyrethroids, including fenpropathrin, β-cyfluthrin, λ-cyhalothrin, deltamethrin, fenvalerate, permethrin, acrinathrin, τ-fluvalinate, and bifenthrin, by coupling HPLC, post-column irradiation with UV light and chemiluminescence detection of the resulting photoproducts. It is based on the observation that photolyzed pyrethroids take part in a chemiluminescent reaction in presence of $K_3Fe(CN)_6$ and NaOH, whose signal increases with the percentage of acetonitrile in the reaction medium. As the yield of the photoderivatization process and the chemiluminescent signals depend on the percentage of acetonitrile, the chromatographic column (a Gemini C_{18}, Phenomenex 150 mm × 4.6 mm, 5 μm particle size) was chosen with the aim of using high percentages of this organic solvent in the mobile phase. Previous studies showed that the rate of the chemiluminescent reaction was very fast. Therefore, a modification was carried out in the detector in order to mix the analytes and reactives as near as possible to the measure cell. The optimised method was validated with respect to linearity, precision, limits of detection and quantification accuracy. Under the optimised conditions, linear working range extends three orders of magnitude with the relative standard deviation on intra-day precision below 10% and detection limits between 0.013 and 0.049 μg mL^{-1}, according to the compound. The proposed method has been successfully applied to the determination of pyrethroids in tomato with good results.

Keywords: Chemiluminescence detection; Photochemical derivatization; HPLC; Acetonitrile; Vegetables; Tomato

1. Introduction

In recent years, chemiluminescence (CL) is becoming an attractive technique to be used as detection in liquid chromatography (HPLC) due to its high sensitivity, wide linear range and simple instrumentation, these features being provided by the absence of an external light source, which allows to improve the signal-to-noise ratio (S/N). Because the emission intensity is a function of the concentration of the chemical species involved in the CL reaction, the technique is versatile for the determination of any species than can participate in the CL process [1].

HPLC is a useful technique for trace quantities of polar or termolabile compounds, in which selectivity and sensitivity of the more commonly used detectors may be, in some cases, improved by the use of suitable derivatization techniques. The main advantages of the post-column derivatization are that the analytes are separated in their original form, without the need for a complete derivatization reaction (assuming reproducibility) and the reaction products need no stability for a long time [2].

Photochemical reactions have been used to determine photoreactive analytes, in most of cases only using irradiation [3–6]. For analytical purposes, photochemical derivatization is extremely useful because of their selectivity and sensitivity and many of these reactions have been adapted as post-column detection systems in HPLC [7–13]. Photochemical reactions offers the advantage of simplicity over chemical post-column reactions in that post-column pumps and other devices are not required and, additionally, dilution is avoided.

CL-based techniques as a means of detection for HPLC have been developed since the 1980s. Although it is not as universal as other detection systems, i.e. mass spectrometry, CL detection is rapidly growing due to the above-mentioned features of CL reactions. Thus, a number of papers have been published dealing with

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the determination of pesticides [14,15], drugs [16–18], antioxidants [19,20] and others [21–23].

On the other hand, it has been established that irradiation of photoreactive analytes leads to species that can be detected by CL [24,25] and a number of publications are devoted to the coupling of photoderivatization and CL detection in flow injection analysis (FIA) [26–29] and HPLC [30,31].

Pyrethroid insecticides constitute the major alternative to the acutely toxic organophosphates and carbamates [32]. However, most of the synthetic pyrethroids show considerable field persistence due to their intrinsic molecular stability and to the type of formulation used for treatments [33]. In addition, some pyrethroid insecticides, such as esfenvalerate, fenvalerate, permethrin, and, in general, synthetic pyrethroids have been reported to have reproductive and endocrine disrupting effect [34]. Therefore, it may be interesting to develop analytical methods to determine these compounds in vegetables.

Pyrethroids are usually determined by GC with electron capture (ECD) or mass spectrometry (MS) detection [35–42]. However in GC methods, they usually appear at high retention times. The aim of this work was to develop a LC detection system based on the coupling of photochemical derivatization and CL detection to determine nine pyrethroids (fenpropathrin, β-cyfluthrin, λ-cyhalothrin, deltamethrin, fenvalerate, permethrin, acrinathrin, τ-fluvalinate, and bifenthrin) in tomato in a relatively short time interval. Analytical measurements are based on the direct CL generated in the reaction between the target analytes and hexacyanoferrate(III) in basic medium.

2. Experimental

2.1. Chemical and solvents

Analytical standards (pestanal quality) of fenpropathrin (FP), *beta*-cyfluthrin (β-CF), *lambda*-cyhalothrin (λ-CH), deltamethrin (DL), fenvalerate (FV), permethrin (PM), acrinathrin (AC), *tau*-fluvalinate (τ-FL) and bifenthrin (BF) were obtained from Riedel-de Haën (Seelze, Germany).

Acetonitrile (ACN) of HPLC grade was obtained from Merck (Darmstadt, Germany); n-hexane, dichloromethane and anhydrous sodium sulphate for pesticide residue analysis were obtained from Panreac (Barcelona, Spain) and potassium hexacyanoferrate(III) ($K_3Fe(CN)_6$) and sodium hydroxide (NaOH) for analysis from Panreac (Barcelona, Spain).

Ultrapure water, obtained from a Milli-Q water purification system from Millipore (Bedford, MA, USA), was used.

Mobile phases were filtered through a 0.45 μm cellulose acetate (water) or PTFE (ACN) and degassed with helium prior and during use.

2.2. Instrumentation

The HPLC-CL system consisted of a Waters (Milford, MA, USA) HPLC equipment, composed of a Model 600E multisolvent delivery system and a Rheodyne 7725i manual injector valve with a 200 μL sample loop. The photochemical step was carried out on a photochemical reactor (Supelco, USA) fitted with a knitted open tube reactor coil (5 m × 1.6 mm O.D. and 0.8 mm I.D.) of PFA (perfluoroalkoxy) and a 8 W Xenon lamp. The CL detection was conducted on a CL detector from Jasco CL-2027 (Tokyo, Japan), which incorporated a modification consisting of placing the mixing chamber as near as possible to the detection cell. The HPLC-CL detection system was as depicted in Fig. 1.

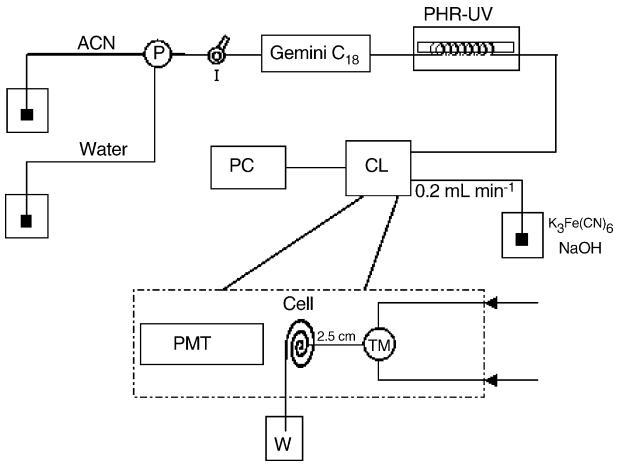

Fig. 1. Schematic diagram of the HPLC-CL system used in the determination of pyrethroids. P (HPLC pump); I (injector); PHR-UV (photochemical reactor UV); CL (chemiluminescence detector); PC (personal computer); TM (T-type mixing device); PMT (photomultiplier); W (waste).

The CL detector was connected to the HPLC equipment through an interface (Waters busSAT/IN Module). The reagent solution (containing $K_3Fe(CN)_6$ and NaOH) was pumped with a system Waters Model 510 and was mixed with the column effluent inside the box containing the reaction cell and the CL detector. HPLC separations were performed with a Gemini C_{18} 150 mm × 4.6 mm (5 μm particle size) column from Phenomenex (USA).

A digital venturis FP 575 pentium personal computer using a Millenium 32 (Chromatography Manager, Waters, Milford, MA, USA) software was used for acquisition and treatment of data.

A model VV2000 LIF rotary vacuum evaporator (Heidolpf) termostated by water circulation with a N-010 KN-18 vacuum pump (Telstar) was used to evaporate the extracts. A Model PT 2100 polytron (Kinematica, Luzern, Switzerland) and a Model BV-401C blender (Fagor Guipuzcoa, Spain) were used for blending the samples.

2.3. Preparation of standards and spiked samples

Individual analytical standard solutions of pesticides (400 mg L^{-1}) were prepared by exactly weighing and dissolving the corresponding compounds in n-hexane. Furthermore, the standard solutions were protected against light and stored at 4 °C in a refrigerator. In these conditions, they were stable for at least 3 months.

Working solutions were prepared daily by evaporating to dryness aliquots of the standard solutions in n-hexane under a gentle N_2 stream and redissolving in ACN. Calibration standard