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Determination of photoirradiated high polar benzoylureas in tomato by HPLC with luminol chemiluminescence detection

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ABSTRACT

This study reports the first analytical application of luminol chemiluminescence reaction for the sensitive detection of two benzoylurea insecticides (diflubenzuron and triflumuron). Off-line experiments demonstrated that previously irradiated traces of these benzoylurea insecticides largely enhanced the chemiluminescence emission yielded from the oxidation of luminol in methanol:water mixtures, by potassium permanganate in alkaline medium, the enhancement being proportional to the concentration of both pesticides. The two benzoylureas were determined in tomato samples by coupling liquid chromatography with post-column photoderivatization and detection based on this chemiluminescence reaction. Tomato samples were extracted using the QuEChERS method based on extraction with acetonitrile and dispersive solid-phase clean-up using primary and secondary amine (PSA). Interferences due to matrix effect were overcome by using matrix-matched standards. The optimised method was validated with respect to linearity, limits of detection and quantification, precision and accuracy. Under the optimised conditions, calibrations graphs were linear between 0.05 and 0.50 $\mu g\,mL^{-1}$ for diflubenzuron and between 0.10 and 1.00 $\mu g\,mL^{-1}$ for triflumuron. Method detection limits were 0.0025 and 0.0131 $\mu g\,mL^{-1}$ (equivalent to 0.0005 and 0.0026 $mg\,kg^{-1}$) and quantification limits were 0.05 and 0.10 $\mu g\,mL^{-1}$ (equivalent to 0.01 and 0.02 $mg\,kg^{-1}$) for diflubenzuron and triflumuron, respectively. In both cases, quantification limits were lower than the maximum residue levels (MRLs) established by the European legislation. The relative standard deviation of intra-day precision was below 10% and recoveries were between 79.7% and 94.2% for both pesticides.

1. Introduction

Substituted ureas are an important group of pesticides that are used as herbicides (phenylureas and sulfonylureas) and insecticides (benzoylureas). Benzoylureas are promising insecticides, widely used because their ability to act as powerful insect growth regulators which interfere with chitin synthesis in target pests and cause death. These insecticides show some attractive properties such as high selectivity, high biological activity, rapid degradation in both soil and water and acute low toxicity for animals, which make them suitable for inclusion in integrated pest management programs for crops [1]. Nevertheless, due to the high interest in the safety of products, the maximum residue levels (MRLs) established for these pesticides [2] are in the same order that those established for another ones considered with high toxicity.

Even though some papers reported the determination of benzoylureas by gas chromatography (GC) with different detectors [3–6], due to their thermostability, the technique of choice for analysing these pesticides has been high-performance liquid chromatography (HPLC) with UV [7–10], fluorescence [11–13] or mass spectrometry (MS) [14–19] detection. Thus, diflubenzuron, triflumuron, teflubenzuron, lufenuron and flufenoxuron were determined in grapes and wine [20] and diflumuron, flufenoxuron and hexaflumuron in citrus [21] by HPLC-UV; diflubenzuron, triflumuron, hexaflumuron, lufenuron and flufenxuron were determined in vegetables by HPLC with post-column photochemically induced fluorescence (PIF) derivatization and fluorescence detection [13] and finally some of this benzoylurea compounds were determined by HPLC–MS in fruits [16], apples [22] and vegetables [23] and by HPLC–MS/MS in processed fruit and vegetables [24] and citrus [19].

Nowadays, multiresidue methods by HPLC–MS or HPLC–MS/MS are becoming the most powerful techniques for the analysis of highly polar, less volatile and thermally labile compounds [25], but alternative low cost methods may be of high interest, mainly when a reduced number of pesticides must be determined.

Analytical interest in liquid-phase chemiluminescence (CL) has been continuously growing over the last 20 years, the best

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demonstration of this interest being the large number of recent manuscripts dealing with analytical applications for pesticides [26–30], drugs [31–33], antioxidants [34,35] and others [36–38] in a variety of industrial, clinical and environmental matrices.

CL is becoming an attractive technique to be used as detection system in LC due to its high sensitivity, wide linear range and simple instrumentation. Furthermore, CL detection is very sensitive because the absence of a light source reduces noise and eliminates Rayleigh and Raman scattering, allowing photon detectors to be operated at high gains to improve the signal-to-noise ratio (S/N). In addition, the elimination of the excitation source in CL can reduce stray light and background emissions and removes the possibility of light-source instability. Despite these advantages, CL has been used less than fluorescence and absorbance for pesticide residue analysis.

The coupling of luminol CL with HPLC provides high efficiency in separation and low limits of detection (LODs) inherent to CL, although its application in pesticide residue analysis has been limited [27].

The yield of strong CL emission by the oxidation of luminol in alkaline medium is one of the best known and most efficient CL reactions. Different oxidants can be used, such as hydrogen peroxide, molecular oxygen, hypochlorite or permanganate, mainly in the presence of some type of initiator or catalyst such as peroxidase, hexacyanoferrate(III), and compounds or metal ions (Co^{2+}, Cu^{2+}, Cr^{3+}, Ni^{2+}, etc.). The luminol–$KMnO_4$ CL system has been used for the selective detection of carbaryl [39] and carbofuran [40], both determinations being based on a FIA configuration. In both methods, luminol carrier was mixed with permanganate carrier before CL detector, and then, this oxidant solution was mixed with the analytes without any catalyst. The CL mechanism proposed for carbofuran consists in the oxidation of this compound with permanganate yielding an intermediate which could subsequently to oxidize luminol upto the excited state 3-aminophthalate anion; finally this excited anion decay to the ground state and produce CL [40].

On the other hand, it has been established that the irradiation of photoreactive analytes leads to the formation of species that can be detected by CL providing very sensitive procedures [41], but only some few papers dealing with photodegradation and CL have been published for pesticides determination [28–30,42–44].

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 [45–50]. 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 photoproducts need no stability for a long time [51].

The purpose of our study was to check the usefulness of the CL reaction of luminol for the determination of benzoylurea residues in vegetables. We found that after irradiation of both benzoylureas with UV light, the corresponding photoproducts produced a great enhancement on the CL emission from the luminol–$KMnO_4$ system in alkaline medium without any catalyst. This enhancement in the CL emission is proportional to the concentration of the selected compounds, which can be determined by measuring the increase in the CL intensity. Based on these findings, a new HPLC-CL method has been developed for the sensitive determination of diflubenzuron and triflumuron, which has been satisfactorily applied in tomato samples.

2. Experimental

2.1. Chemical and solvents

Analytical standards (pestanal quality) of diflubenzuron (99.5%) and triflumuron (99.9%) were obtained from Riedel-de Haën (Seelze, Germany).

Acetonitrile (ACN) and methanol (MeOH) of HPLC grade were obtained from Merck (Darmstadt, Germany); sodium acetate ($NaAc{\cdot}3H_2O$), magnesium sulphate anhydrous ($MgSO_4$) and acetic acid glacial (HAc) for pesticide residue analysis were obtained from Panreac (Barcelona, Spain). Luminol (5-amino-2,3-dihydro-1,4-phthalazine dione, $C_8H_7N_3O_2$), potassium permanganate ($KMnO_4$) and sodium hydroxide (NaOH) for analysis were obtained from Panreac (Barcelona, Spain). Primary and secondary amine (PSA)-bonded silica was supplied by Supelco (USA).

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 polytetrafluoroethylene (PTFE) (organic solvents) and degassed with helium prior and during use.

The luminol solution 0.1 mmol L^{-1}, prepared in NaOH 0.1 mol L^{-1} aqueous solution, and permanganate solution 0.001 mmol L^{-1} were filtered through a Millipore membrane of cellulose acetate (0.45 μm particle size) before pumping into the chromatographic system.

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 Model PHRED-8 (Aura Industries, USA) fitted with a knitted open tube reactor coil (7 m × 0.6 mm o.d. and 0.3 mm i.d.) of PTFE and an 8 W Mercury lamp. 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 CL detector was connected to the HPLC equipment through an interface (Waters busSAT/IN Module). The reagent solutions (luminol–NaOH and $KMnO_4$) were pumped with two systems Waters Model 510. The luminol solution was firstly mixed with the column effluent and $KMnO_4$ solution was after mixed with the resulting effluent inside the box containing the reaction cell and the CL detector. HPLC separations were performed with a Gemini C18 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 software (Chromatography Manager, Waters) was used for acquisition and treatment of data. A Model BV-401C blender (Fagor Guipuzcoa, Spain) was used for blending the vegetable samples.

2.3. Preparation of standards and spiked samples

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

Working standard solutions were prepared in MeOH:H_2O (50:50, v/v) as solvent. Calibration standard solutions of the analytes and extract of vegetable samples were prepared in MeOH:H_2O (50:50, v/v) and were filtered through Millipore membrane PTFE