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# Determination of *N*-methylcarbamate pesticides in environmental samples by an automated solid-phase extraction and liquid chromatographic method based on post-column photolysis and chemiluminescence detection

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**Abstract**

An automated solid-phase extraction–high performance liquid chromatography method has been developed to determine trace concentration of *N*-methylcarbamate pesticides in water and fruits. The method is based on the post-column conversion of the pesticides into methylamine by irradiation with UV light. The resultant methylamine was subsequently detected by chemiluminescence using tris(2,2′-bipyridyl)ruthenium(III), which was on-line generated by photo-oxidation of the ruthenium(II) complex with peroxydisulfate. Factors affecting the rate of the reactions were optimized so that their contribution to the total band-broadening was negligible. This detection system was used to determine bendiocarb, carbaryl, promecarb and propoxur, which were separated on an ODS $C_{18}$ column. The mobile phase consisted of water and acetonitrile using a gradient elution. A linear relationship between peak area and concentration was obtained for all pesticides ($r^2 > 0.999$). Intra- and inter-day precision values of about 0.64–1.3% RSD ($n = 10$) and 2.2–2.8% RSD ($n = 15$), respectively, were obtained. *N*-Methylcarbamate pesticide residues at ultratrace levels could be determined in environmental samples when an automated solid-phase extraction device was coupled on-line with the HPLC system. Detection limits were within the range 3.9–36.7 ng $l^{-1}$ for water samples and 0.5–4.7 μg $kg^{-1}$ for fruits.


*Keywords:* *N*-Methylcarbamate pesticides; HPLC; Chemiluminescence detection; Photodecomposition; Tris(2,2′-bipyridyl)ruthenium(III) photogenerated; Automated solid-phase extraction; Water and fruit samples

## 1. Introduction

*N*-Methylcarbamates (NMCs) comprise a large class of pesticides widely used in agriculture and noted for their relatively short persistence in the environment. There are approximately 30 commercially available NMCs in the market, some of the most common ones being carbaryl, propoxur, promecarb and bendiocarb; residues may appear in fruits, vegetables and ground and surface waters and, therefore, pose a potential hazard for consumers.

Liquid chromatography (HPLC) is the favoured technique for the determination of NMC pesticides as a class since many of them lack the thermal stability necessary for gas chromatographic determination. For HPLC, no derivatization is required and the aqueous samples, analysed either directly or after preconcentration, are very compatible with reversed-phase LC [1–3].

Prior to about 1990, UV absorbance was the most commonly used detection method in HPLC determination of NMC pesticides [4]. However, UV is subject to interference from sample co-extractives and also lacks sensitivity for some compounds, two factors which limit its usefulness for analysing environmental samples [5].

Post-column hydrolysis and derivatization of NMCs coupled with fluorescence detection works for samples with complex matrices such as plants, soil, meat as well as water [6–9]. Post-column reactions were first reported by Moye et al. [10], where NMCs were hydrolyzed by NaOH at 90 °C to form methylamine (MA) followed by condensation with *o*-phtaldehyde (OPA) and 2-mercaptoethanol (MERC) to procedure a highly fluorescent

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isoindole. This technique forms the basis of the EPA Method 531 [11]. Subsequent modifications include a catalytic solid-phase reactor consisting of a column packed with an anion-exchanger resin maintained at 100–120 °C [12], an UV photolytic reactor [13] or a quaternary ammonium salt [14] in order to accelerate the hydrolyse of NMCs before labelling with OPA-MERC.

HPLC with mass spectrometry (LC–MS) or tandem mass spectrometry (LC–MS/MS) have been widely accepted as useful techniques for the identification and quantification of NMCs and other polar and thermally labile pesticides [2,15,16]. The reason is that the progress in LC–MS, in recent years, has remarkably improved the possibility of identification and/or confirmation of these compounds at very low concentration [17,18].

Chemiluminescence (CL) is quite a new detection technique for use in conjunction with HPLC due to its good selectivity, enhanced sensitivity and wide linear working ranges, which can be obtained with relatively simple and inexpensive instrumentation. Recently, CL detection using tris(2,2′-bipyridyl)ruthenium(III), $Ru(bipy)_3^{3+}$, has been used for the HPLC determination of numerous compounds [19,20]; e.g. amines, aminoacids, carboxylic acids and pharmaceuticals. Common to all these applications is the formation of $Ru(bipy)_3^{3+}$, which is then reduced by the analyte to emit light. The active reagent $Ru(bipy)_3^{3+}$ has been generated by chemical, photochemical and electrochemical oxidation and in situ electroluminescence. In previous works, it was found that the photochemically assisted oxidation of $Ru(bipy)_3^{2+}$ to $Ru(bipy)_3^{3+}$ in the presence of peroxydisulfate is very effective and reproducible and it has been successfully used in the CL determination of several analytes [21–24].

Photolysis of NMCs is strongly enhanced in the presence of acetone and has been coupled to flow injection manifolds for the CL determination of these pesticides using either $Ru(bipy)_3^{3+}$ [23–25] or the participation of a fluorescent derivate of the pesticide in the peroxyoxalate chemiluminescent system [26].

In this work, we attempted to establish a simple, sensitive and selective HPLC method for determining NMCs (bendiocarb, carbaryl, promecarb and propoxur) in real samples. The post-column CL detection used two photoreactors, in one of which $Ru(bipy)_3^{3+}$ is generated by on-line photo-oxidation of $Ru(bipy)_3^{2+}$ with peroxydisulfate while in the other the NMCs eluted from the column are first photodegraded to MA, which reacts with $Ru(bipy)_3^{3+}$ to give strong CL.

Finally, a fully automatic solid-phase extraction (SPE) module was connected on-line to the LC system in order to improve the trace enrichment of the water samples or the clean-up of fruit samples. This assembly provides a sufficiently low detection limit (ca. 3.9 ng $l^{-1}$ in waters and 0.5 μg $kg^{-1}$ in fruits) to permit virtually direct multiresidue analysis of NMC pesticides in environmental samples.

## 2. Experimental

### 2.1. Chemicals and solutions

Ultrapure water from a Milli-Q plus system (Millipore-Ibérica, Madrid, Spain) was used throughout. Methanol, acetonitrile and acetone were of chromatographic grade and provided by Romil (Loughborough, UK). Bendiocarb, carbaryl, promecarb and propoxur in purity higher than 98%, were purchased from Riedel-de Haën (Sigma–Aldrich Quimica, Madrid, Spain). Standard stock solutions were prepared in 30% acetonitrile–water containing each NMC at 150 μg $ml^{-1}$ and were stored in glass-stoppered bottles at 4 °C in a refrigerator. Standard working solutions of various concentrations were prepared daily by appropriate dilution of aliquots of the stock solution with 25% acetonitrile–water. A stock solution of $Ru(bipy)_3^{2+}$ ($2 \times 10^{-3}$ M) was prepared by dissolving tris(2,2′-bipyridyl)ruthenium(II) dichloride hexahydrate from Fluka (Steinheim, Switzerland) in ultrapure water. The solutions of $KIO_4$ ($1 \times 10^{-3}$ M) and $K_2S_2O_8$ ($1.5 \times 10^{-3}$ M) were prepared by dissolving the product from Sigma in ultrapure water. Phosphate buffers were prepared by dissolving $NaH_2PO_4$ in ultrapure water and adjusting to the pH desired with sodium hydroxide. All solutions were filtered through a 0.45 μm membrane filter (Millipore).

### 2.2. Apparatus and procedure

A schematic diagram of the detection system is shown in Fig. 1. The HPLC assembly consisted of a solvent delivery system, column and an injector. The column was a Beckman Ultrasphere (5 μm $C_{18}$, 45 mm × 4.6 mm). Solvents that constituted the mobile phase were water (A) and acetonitrile (B). The elution conditions applied were 0–11 min isocratic 22% B; 11–18 min isocratic 40% B and finally reconditionating the column for 5 min with 22% B. The flow rate of the eluent was 1.2 ml $min^{-1}$. A System Gold 125 NM solvent Module (Beckman Coulter, Fullerton, CA, USA) was used to deliver the eluent. The samples were injected either manually with a Rheodyne Model 7125 valve or automatically with the Aspec XLi (Gilson, Middleton, WI, USA). Chromatography was performed at room temperature and the injected volume was 50 μl. The CL detector was a Camspec CL-2 (Cambridge, UK) luminometer equipped with a three-port flow cell.

CL detection was conducted on a flow injection-CL system consisting of two Gilson Miniplus-3 peristaltic pumps to deliver the reagents. Two photoreactors, each consisting of PTFE tubing (0.5 mm I.D., length $L_1$ = 200 cm and $L_2$ = 175 cm) coiled around Spectronic (Westbury, NY, USA) rod-shape low pressure mercury lamps (50 mm × 5 mm diameter) were incorporated in the manifold. The lamps operated at 6 W and the main spectral line was at 254 nm. Both photoreactor-lamp assemblies were housed in fan-ventilated metal boxes covered with a mirror to increase the photon flux by reflection. The eluate from the HPLC column was mixed with the oxidizing agent and then irradiated with UV light as it passed through the photoreactor $L_1$ where the NMCs were photodegraded to MA. The oxidizing solution was 1 mM $KIO_4$ in 50 mM phosphate buffer pH 6.5 containing 2% acetone, and was delivered at flow rate of 1.4 ml $min^{-1}$. The effluent of the photolysis coil $L_1$ was directed to one of the flow cell ports. For the on-line generation of $Ru(bipy)_3^{3+}$, solutions containing 2 mM $Ru(bipy)_3^{2+}$ and 1.5 mM $K_2S_2O_8$ in 50 mM phosphate buffer of pH 5.7 were pumped at 1.1 ml $min^{-1}$