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Short communication

Monitoring some phenoxyl-type *N*-methylcarbamate pesticide residues in fruit juices using high-performance liquid chromatography with peroxyoxalate-chemiluminescence detection

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Abstract

A systematic investigation of optimal conditions for determining three of the most common phenoxyl-type *N*-methylcarbamate pesticides (carbaryl, carbofuran and propoxur) in fruit juices by HPLC with peroxyoxalate-chemiluminescence detection is described. The required pre-column hydrolysis of pesticides and derivatization of their hydrolytic metabolites with dansyl chloride was simultaneously carried out in a short time thanks to the micellar catalytic effect provided by cetyltrimethyl ammonium bromide micelles on the hydrolysis step. The liquid chromatographic separation of the dansylated phenols was performed on a reversed-phase C_{18} column with isocratic elution. The analytes were detected by using an integrated derivatization chemiluminescence detection unit based on the bis(2,4,6-trichlorophenyl)oxalate–hydrogen peroxide system. Fruit juice samples containing 4.0–1500 μg/l pesticides were analysed with a precision of ca. 6.5%. After contamination of the fruit juice samples, average recovery >93% at fortification levels of 10–100 μg/l was obtained.

Keywords: Fruit juices; Chemiluminescence detection; Carbaryl; Carbofuran; Propoxur; Pesticides

1. Introduction

N-Methylcarbamates (NMCs) comprise an important class of pesticides widely used in agriculture for crop protection [1,2] and, therefore, their residues may be encountered in fruits and vegetables, which poses a potential hazard for consumers. To watch over the safety of our food supply, international organisations regulate their maximum residue levels (MRLs) on crops, which are in the 0.02–7 mg/kg range depending on the particular pesticide/commodity combination [3]. Numerous analytical procedures have been developed for the determination of NMCs and metabolites in various matrices, including water, soil, fruits, vegetables and other crops. At present, HPLC with post-column derivatization and fluorescence detection, OPA method [4–9], is accepted as a standard protocol by many official organisations, including EPA and AOAC.

In the last few years we have developed a simple, flexible and inexpensive peroxyoxalate-chemiluminescence (PO-CL) detector for HPLC [10–14], which allows the efficient mixing of the mobile phase and PO-CL reactants with a zero dead-volume. In the present work, the first results obtained on the appli-

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cation of the CL detection in HPLC to the determination of three phenoxyl-type NMC pesticides (carbaryl, carbofuran and propoxur) in fruit juices are described. Pesticides were simultaneously hydrolyzed and derivatized with dansyl chloride (DNS-Cl) in a cetyltrimethyl ammonium bromide (CTAB) micellar medium, and the ensuing dansylated phenols separated by HPLC. PO-CL detection, based on the bis(2,4,6,-trichlorophenyl)oxalate (TCPO)–hydrogen peroxide system, was carried out by using the integrated derivatization-CL detection system [10]. Apple, pineapple, grapefruit and orange juices were spiked with the pesticides at several levels, and the fortified samples were analysed after a simple liquid–liquid extraction clean-up procedure.

2. Material and methods

2.1. Reagents

All chemicals and solvents were of analytical-reagent and chromatographic grade. Carbaryl, propoxur and carbofuran in purity higher than 98%, were purchased from Riedel-de Haën (Sigma–Aldrich Química, Madrid, Spain). Standard stock solutions were prepared in acetone (Merck, Darmstadt, Germany) contained each NMC at concentration of 1000 μg/ml 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 in the micellar derivatization buffer (pH 10.8), which was made from sodium carbonate (Merck) and CTAB (Sigma, Sigma–Aldrich Química) in such a way that the final concentrations were 0.15 and 0.11 *M* for carbonate and CTAB, respectively. DNS-Cl (Sigma, Sigma–Aldrich Química) solution was prepared at 2.6×10^{-3} *M* by dissolving 0.07 g in 100 ml acetone and stored cold.

A 3.5×10^{-3} *M* TCPO (Fluka, Sigma–Aldrich Química) solution was made by dissolving 157 mg of chemical in 100 ml of ethyl acetate (Merck). The buffered oxidant solution was prepared by mixing 40 ml of concentrated hydrogen peroxide (Merck) and 1.0 ml of 0.15 *M* Tris (Merck) buffer, and making up to 100 ml with 2-propanol (Merck). 0.15 *M* Tris [tri(hydroxymethyl)methyl amine, Merck] buffer solution was prepared by dissolving 1.8 g of product in water and adding enough hydrochloric acid to adjust the pH to 9.5 in a final volume of 100 ml. These reagent solutions provide the maximum efficient CL production for the zero dead-volume PO-CL detector [10–14].

2.2. HPLC instrumentation

Experiments were conducted on a HPLC system consisted of a Nova-Pack C_{18} 250×4.6 mm (4 μm) column (Waters, Milford, MA, USA), a Waters 600 controller multisolvent pump and a Rheodyne 7161 injector. The mobile phase comprising a mixture of acetonitrile–water–methanol (55:37:8, v/v) was filtered through a 0.45-μm nylon membrane filter, degassed and delivered at a flow-rate of 1.0 ml/min. Chromatography was performed at ambient temperature and the injected volume was 20 μl. The reactant solutions (TCPO and oxidant/pH), both delivered at 0.5 ml/min, and the eluate from the column were mixed in the 1.0-cm quartz cell of the zero-dead-volume PO-CL detector reported elsewhere [10], and the resulting PO-CL signal was simultaneously monitored by the photomultiplier (PMT). Data acquisition and processing were performed using a Water Maxima 820 chromatographic workstation interfaced to a NEC PC-AT 33-MHz compatible computer.

2.3. Determination of NMCs in fruit juice extracts

Four samples of fruit juices were bought from a local market and analysed for incurred NMCs by the proposed method. No incurred residues were detected. For the preparation of fortified samples, volumes of 20.0 ml of fruit juice were spiked with NMC pesticides at 10, 50 and 100 μg/l levels, and after equilibration for 1 h at room temperature, 1 ml of 5 *M* sodium chloride was added prior to a liquid–liquid extraction step with 5.0 ml of a mixture of cyclohexane–ethylacetate–acetone (2:2:1, v/v) for 5 min. The organic layer was transferred to a graduate cylinder to determine the recovered volume, and a portion of 4.0 ml was evaporated to dryness under a nitrogen stream. For the hydrolysis of NMCs and dansylation of the corresponding phenols, 500 μl of the micellar derivatization buffer (0.15 *M* sodium

carbonate and 0.11 *M* CTAB adjusted to pH 10.8) and 1.0 ml of 2.6×10^{-3} *M* DNS-Cl were added to the extract, and the mixture was under reaction for 20 min at 65 °C. This solution was filtered through a 0.45-μm nylon membrane filter, and aliquots of 20 μl were injected into the HPLC system.

3. Results and discussion

3.1. Optimisation of the hydrolysis and dansylation conditions

Initial experiments were devoted to finding the most appropriate conditions for dansylation of the hydrolytic metabolites of the NMCs. The effect of the pH and the concentration of the derivatization buffer was studied over the range 10.5–12.0 and 0.01–0.5 *M*, respectively. A pH value of 11.5 was selected for further experiments on account that the maximum peak height achieved (the greatest reactivity between the hydrolysis product of NMCs and DNS-Cl was achieved when pesticides are completely dissociated), which was adjusted in the reaction medium by using 0.15 *M* carbonate buffer solution (pH 10.8): 500 μl of this solution yielded an apparent final pH of 11.5 in a 1:2 (v/v) water–acetone derivatization medium. The DNS-Cl concentration used for the derivatization was studied from 3.0×10^{-4} to 1.7×10^{-3} *M*. The signal increased with increasing concentration up to 8.5×10^{-4} *M* and then levelled off up to the maximum concentration tested. A concentration of 1.7×10^{-3} *M* (1.0 ml of 2.6×10^{-3} *M* DNS-Cl was added to the reaction mixture) was selected as optimal. Kinetic curves for the derivatization of the compounds studied were performed over the range 30–70 °C. The highest yields of the derivatives were attained at an incubation temperature of 65 °C in ca. 15 min.

The hydrolysis of phenoxyl-type NMCs was usually accomplished with sodium hydroxide at elevated temperatures. These experimental conditions preclude to fulfill the hydrolysis and dansylation of the pesticides in a single step. In view of the catalytic effect of quaternary ammonium salts with a long alkyl chain on the hydrolysis of NMCs reported earlier by Wu et al. [16], cationic surfactants such as CTAB, TTAB (tetradecyltrimethylammonium bromide), DTAB (dodecyltrimethylammonium bromide) and DPC (*N*-dodecylpyridinium chloride) were added to the reaction mixture at concentrations up to 6.0×10^{-2} *M*. Although all surfactants are useful for increasing the rate of the degradation of NMCs at concentrations higher than its critical micellar concentration, CTAB proved to be the most suitable because of its higher catalytic effect (see Fig. 1). Based on the results obtained, a 3.7×10^{-2} *M* CTAB concentration was chosen for subsequent experiments, at which the thermal decomposition (65 °C) of NMCs in alkaline medium (apparent pH 11.5) was achieved in no more than 5 min, and therefore the hydrolysis and dansylation reactions of phenoxyl-type NMCs can be concurrently carried out in 20 min.

3.2. Analysis of fruit juices

In view of the variety of approaches reported in

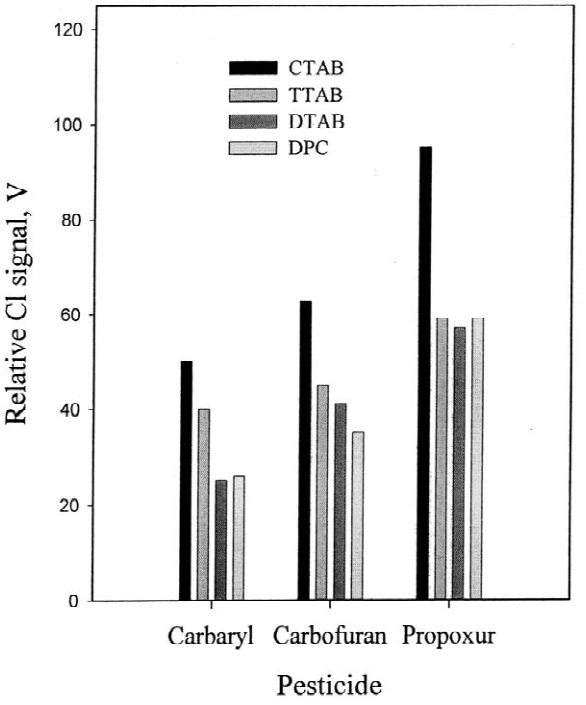

Fig. 1. Effect of type of quaternary ammonium salt on the hydrolysis of NMC assayed. Pesticide and surfactant concentrations: 150 μg/l and 3.7×10^{-2} *M*, respectively. All other conditions as in Section 2.

the literature for NMCs clean-up, several procedures were tested for applying the proposed method to the determination of these pesticides in fruit juice samples by using an orange juice free from residues of pesticides as a model fruit juice. Based on experimental results, a simple liquid–liquid extraction procedure earlier reported by us for clean-up of fungicides in fruit juice samples [15] was chosen. By using this protocol, NMCs were satisfactorily extracted into a mixture of cyclohexane–ethylacetate–acetone (2:2:1, v/v) in the presence of concentrated sodium chloride (see Section 2.3).

Table 1 gives the analytical figures of merit for the assayed pesticides obtained by processing 20 ml of orange juice spiked with variable concentrations of pesticides in the interval 4–1500 μg per ml of juice. The possible interaction between the pesticides and the sample matrix was also evaluated throughout 1 to 24 h. Similar figures of merit were obtained in all cases and, therefore, after spiking the samples were equilibrated for 1 h at room temperature prior to their analysis. As can be seen, the proposed method allows the determination of these compounds at low levels (μg/l) with good precision, viz. RSDs for peak height from 6.5 to 7.6%. These analytical figures of merit reveal that the proposed method would be suitable for the determination of NMCs in fruit juices.

Finally, the proposed method was applied to the determination of the assayed pesticides in apple, pineapple and grapefruit juices (Fig. 2). Preliminary analyses of samples revealed that none of pesticides was present, and therefore they were spiked with NMC standard solutions to final concentrations of 10, 50 and 100 μg/l. These fortification levels are well below the MRLs for carbaryl, carbofuran and propoxur in these crops. As can be seen from Table 2, NMC pesticides were quantitatively recovered (recoveries ranged from 93 to 96%, with an average RSD of 6.5%). These results testify the good performance of the proposed HPLC-CL method in the determination of NMCs in this type of sample.

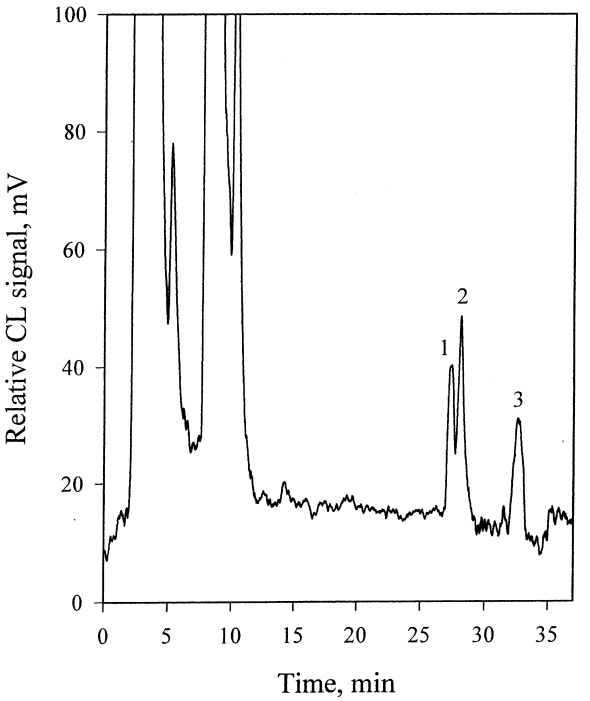

Fig. 2. Chromatograms of spiked apple juice sample. Pesticide concentration: 50 μg/l. Peak assignment: (1) carbofuran; (2) propoxur; (3) carbaryl. Other conditions as described in Section 2.

Table 2
Average recoveries (n=9) of NMC pesticides spiked at 10, 50 and 100 μg levels per ml of fruit juice

Pesticide	Apple	Pineapple	Grapefruit
Propoxur	94±6	93±6	93±6
Carbofuran	96±7	96±6	93±6
Carbaryl	93±6	93±7	94±5

Table 1
Characteristic parameters of the calibration graphs and analytical figures of merit for the determination of pesticides

Compound	Linear range (μg/l)	Regression equation[a]	r	LOD (μg/l)	RSD[b] (%)
Carbaryl	8.0–1500	$H=1.8\times10^{-4}+3.1\times10^{-4}\,C$	0.994	3	6.1
Carbofuran	7.0–1500	$H=1.3\times10^{-4}+3.8\times10^{-4}\,C$	0.993	3	7.6
Propoxur	4.0–1500	$H=1.6\times10^{-4}+5.8\times10^{-4}\,C$	0.996	2	6.3

[a] H, peak height (V); C, analyte concentration (μg/l).
[b] n=11 at a pesticide concentration of 50 μg/l.

4. Conclusions

In this paper, HPLC with PO-CL detection is shown to be an effective analytical technique for the rapid and reliable determination of carbaryl, carbofuran and propoxur in fruit juices. The results obtained warrant the following comments: (a) the method extends the scope of CL detection in HPLC to the determination of pesticides, for which PO-CL detection technique has not been used so far. (b) The proposed method can be a valuable tool (simple and inexpensive) for the determination of these pesticides in fruit juices regarding the well-established post-column OPA fluorescence method. (c) Although the PO-CL method involves the hydrolysis of NMCs and dansylation of their hydrolytic metabolites in a pre-column format, both reactions can be simultaneously performed in a short time thanks to the micellar catalytic effect provided by CTAB micelles on the hydrolysis step.

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