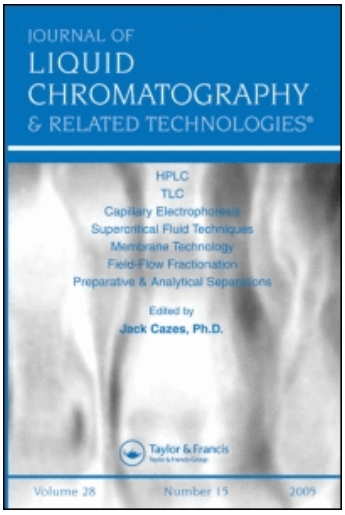

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LIQUID CHROMATOGRAPHY/PARTICLE BEAM/MASS SPECTROMETRY DETERMINATION OF CARBAMATES IN LETTUCE AND APPLE

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ABSTRACT

A method for extraction and determination of trace amounts of Carbaryl, Baygon, Methiocarb, and Methiocarb sulfoxide in lettuce and apple samples is described. The arrangement liquid chromatography-particle beam-mass spectrometry offers the possibility of confirmation and determination of these carbamates at concentrations lower than those admitted by the European Union. Recoveries of carbamates at the 0.16-4.9 mg/kg level were between 53 and 76% with standard deviation between 6 and 12% (n = 5). Detection limits for analytes single ion monitoring mode were 5, 30, 10, and 20 ng for Methiocarb sulfoxide, Baygon, Carbaryl, and Methiocarb, respectively.

INTRODUCTION

Pesticide use in agriculture and horticulture is widely accepted as necessary in the production and conservation of food sources. As a

consequence of pesticide use, the presence of residues in food is unavoidable. Indeed, pesticides are used widely to control infection by pests and diseases. Thus, carbamate insecticides are frequently employed to control insects that, for some reason, do not respond to organophosphorous compounds.[1] Residues of these pesticides are present in crops and in ground, so the separation and identification along with their main transformation products are important because of their proven or suspected toxicity. Unfortunately, the usual procedures for trace pesticides determination involving GC are limited, because carbamates in general and their transformation products in particular are often quoted as polar, non-volatile, and thermally labile compounds. Consequently, a wide variety of LC detection methods, such as diode array Uv-vis,[2] electrochemical,[3] and fluorescence[4] have been employed.

Although fluorescence and electrochemical methods provide higher sensitivity and selectivity, the number of compounds that can be detected is limited and, moreover, the use of other more universal detectors, as Uv-vis, present the disadvantage of their low confirmation power and, consequently, the large number of compounds to be identified and quantified push toward the use of LC/MS techniques. Different interfaces have been developed for coupling liquid chromatographs to mass spectrometers and, although thermospray (TS) is one of the most popular because of its high sensitivity, particle beam (PB) has proved to be the only LC-MS interface that provides classical electron ionization (EI) mass spectra for a wide variety of compounds of environmental and toxicological interest like carbamates.[5,6] Unfortunately, PB yields a poor sensitivity and non-linear response.[7] These problems can be partially overcome by the addition of mobile phase additives, such as ammonium acetate or ammonium oxalate.[8]

The aim of this work is to evaluate the capability of the LC-PB-MS combination to determine Carbaryl, Baygon, Methiocarb, and Methiocarb sulfoxide in apple and lettuce samples. All of them are N-methylcarbamates widely used in pest control. Carbaryl (1-naphtyl-N-methylcarbamate) has been employed against at least 150 major crop pests. It is specially useful against codling and tortrix moths in apples and other top fruits and several pests in vegetables. Baygon, also known as Propoxur (2-isopropoxyphenyl-N-methylcarbamate) has been used to control aphid and white flies on plants grown under glass; it is more persistent and toxic for mammals than Carbaryl. Methiocarb (3,5-dimethyl-4-methylthiophenyl-N-methylcarbamate) was introduced as an insecticide in 1965 and it has been used on vegetables for the control of fruit flies, codling moth and mites on apples. Finally, Methiocarb sulfoxide (3,5-dimethyl-4-methylsulfoxide-N-methylcarbamate) is the degradation product obtained from oxidation of Methiocarb.

MATERIALS

Equipment

Analyses were performed on a LC-PB-MS supplied by Hewlett Packard, equipped with a quaternary HP 1050 LC pump, an HP 59980B PB interface and a MS engine HP5989A coupled with an Apollo series 400 HP data system. Chromatographic separation of the carbamates was performed with a 250 x 4 mm id HP analytical column packed with 5 μm Spherisorb ODS (2).

Chemicals

Methiocarb and Baygon were obtained from Chem Service; Carbaryl from Riedel-de-Haën and Methiocarb sulfoxide from Greyhound. All compounds were at least 95% pure. HPLC-grade methanol, ammonium acetate, acetic acid, and dichloromethane were purchased from Carlo Erba. Purified water was obtained using a Milli-Q apparatus.

Standard solutions (300 mg/L) of all carbamates were prepared in methanol and diluted to appropriate concentrations in mixtures and samples prior to analysis. The standard solutions were stored in the dark at not more than 4°C.

METHODS

Sample Preparation

Amounts of 30 g of lettuce or 10 g of apple were spiked with each carbamate in the adequate proportion (5-8 μg of Methiocarb and Methiocarb sulfoxide and 70-100 μg of Carbaryl and Baygon in lettuce; and 20-50 μg of Carbaryl and Baygon in apple) to obtain those levels around the maximum admissible concentration dictated by the European Union (EU) and the Spanish regulations (0.20 mg/kg for Methiocarb and Methiocarb sulfoxide and 3.0 mg/kg for Carbaryl and Baygon in vegetables). The spiked samples were thoroughly blended with 100 mL of dichloromethane and the suspension was filtered and the residue washed with three volumes of c.a. 20 mL of dichloromethane. The resulting dichloromethane solution was evaporated to 1

mL under reduced pressure in a rotary evaporator. The residue was dissolved in methanol and the insoluble material was removed by filtration and washed with methanol. Finally, the resulting solution was evaporated in the rotary evaporator to a final volume of 1.0 mL prior to injection into the LC-system.

LC-PB-MS Determination

Twenty microlitres of sample were injected into the chromatographic system. Retention times were measured with the temperature maintained at 25°C and a flow rate of 0.7 mL/min of mobile phase (70% methanol and 30 % sodium acetate 0.05 M at pH adjusted to 5 by the addition of acetic acid). The He flow of the PB nebulizer was optimized by a standard procedure at 70-75 psi inlet pressure, and the desolvation chamber was kept at 65-70°C. The mass spectrometer was operated at 250°C source temperature and the quadrupole analyzer at 150°C. It was tuned with *m/z* 69, 219, and 502 ions of perfluorotributylamine (PFTBA) with maximum signal intensity tuning for *m/z* 219. Spectra were acquired in the mass range 80-300 u at a scan rate of 1.2 scan/s when TIC mode was used for identification purpose. For quantitation, SIM mode was used, and the ions monitored were *m/z* 107 for Methiocarb sulfoxide, 110 for Baygon, 144 for Carbaryl, and 168 for Methiocarb, and the Dwell (time that each ion is measured) was 120 milliseconds for each (1.3 scan/s).

RESULTS AND DISCUSSION

Optimization of Chromatographic Conditions

A mixture of water and methanol was chosen as mobile phase because the presence of methanol enhances the signal response with the particle beam interface, mainly when it is used at high concentrations. Ammonium acetate was added to the eluent to actuate as a buffer at pH 5, and also because, as demonstrated by Bellar,[7] the addition of this compound acts as a carrier and, in general, extends the PB-MS linear range and improves the sensitivity. Regarding the analytical column, those with 2.2 mm I.D. are preferred for LC-PB-MS analysis because of their relatively low flow rates. Thus, an ODS Hypersil 100 x 2.1 mm (5 μm) HP Analytical Column was tested, but a reasonable carbamate separation was achieved only when the ratio methanol: 0.05M ammonium acetate (pH 5) aqueous solution reached the proportion 55:45.

Table 1

Retention Times and Detection Limits Found for the Three Operation Modes of the Mass Spectrometer[a]

	tr (min)	LOD (ng) TIC	MC[b]	SIM
Methiocarb sulfoxide	3.20	9	13	5
Baygon	4.27	32	32	30
Carbaryl	4.80	24	8	10
Methiocarb	7.90	24	20	20

[a] LOD express as s/n=3/1.

[b] Corresponding to m/z 168 for Methiocarb; m/z 107 for Methiocarb sulfoxide; m/z 110.0 for Baygon and m/z 143.0-145.0 for Carbaryl.

Trying to carry out the analytes separation with mobile phases containing more proportion of methanol, two additional columns were tested: a Spherisorb ODS (2) 125 x 4 mm (5 μm) HP Analytical Column and a Spherisorb ODS (2) 250 x 4 mm (5 μm) HP Analytical Column, the last one being finally chosen because a reasonably separation of the four carbamates was achieved with it, using a mobile phase containing 70% methanol and 30% ammonium acetate 0.05 M (pH 5) aqueous solution . Flow rate selected was 0.7 mL/min, and 25°C column temperature was fixed. Under these conditions, all compounds are eluted in 8 min (Table 1).

Analytical Characteristics

Under these chromatographic conditions, the analytical characteristics were studied. The response of the PB-MS for each carbamate was studied for the three MS operating modes: total ion current (TIC), mass chromatogram (MC), and single ion monitoring (SIM). Mixtures of Methiocarb sulfoxide, Baygon, Carbaryl, and Methiocarb were injected into the chromatographic system in the concentration range 0.5-150 mg/L for Methiocarb sulfoxide and 1-300 mg/L for Baygon, Carbaryl, and Methiocarb in the TIC and MC acquisition mode, and between 0.2 to 50 mg/l for Methiocarb sulfoxide and 0.5 to 150 mg/L for the other three carbamates in the SIM mode. Mass chromatograms (MC) were calculated from the respective TIC chromatograms

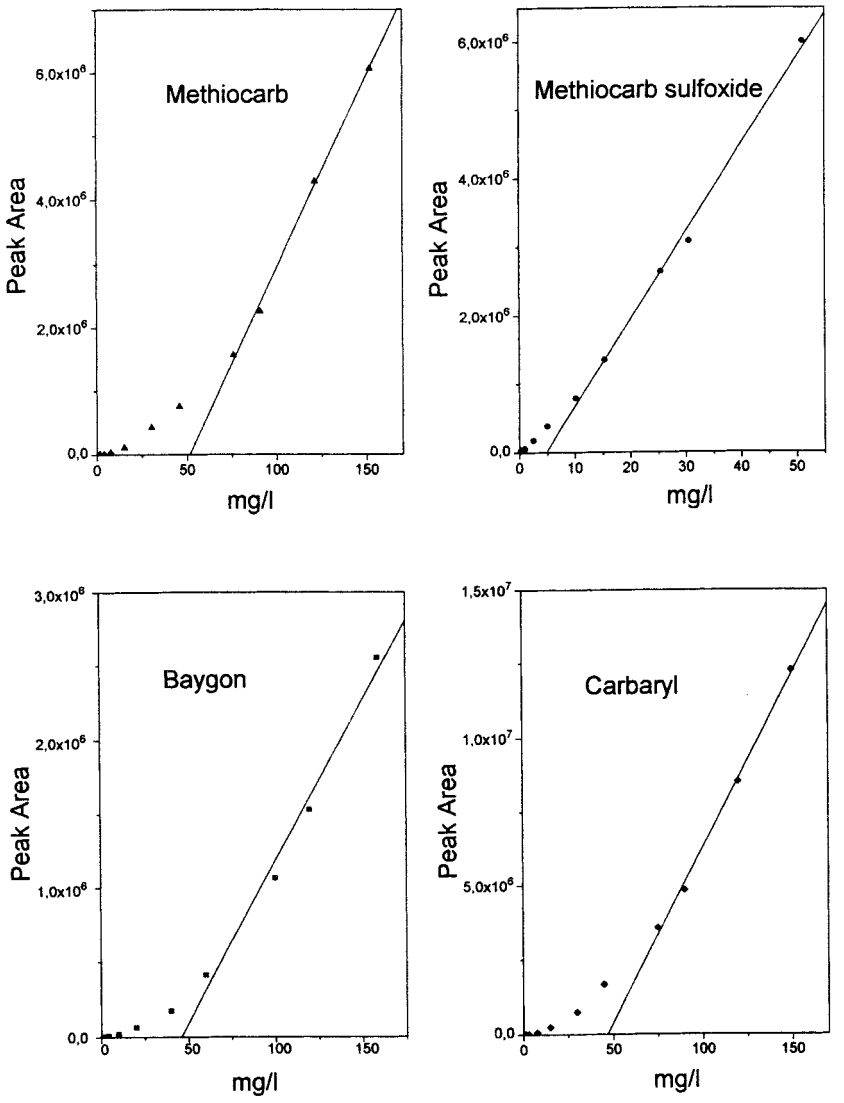

Figure 1. LC-PB-MS response graphs for Methiocarb sulfoxide, Baygon, Carbaryl and Methiocarb.

by selecting the *m/z* values of 168 for Methiocarb, 107 for Methiocarb sulfoxide, 110.0 for Baygon, and 143.0-145.0 for Carbaryl. The response of the PB-MS for the four carbamates studied was in all cases the one typical for this technique, with a linear range at higher concentrations and deviations of linearity at the lower levels.[8] The PB-MS response for each carbamate in the SIM mode is shown in Figure 1.

The detection limits for the analytes were calculated by injecting diluted solutions of the carbamates mixture in operative chromatographic conditions. The lowest amount was determined for a signal-to-noise ratio 3:1. Table 1 shows the detection limits achieved for the four carbamates and the three MS acquisition modes.

Determination of Methiocarb Sulfoxide, Baygon, Carbaryl, and Methiocarb in Lettuce

Determinations of Methiocarb sulfoxide, Baygon, Carbaryl, and Methiocarb in spiked lettuce samples were carried out by LC-PB-MS. Extraction and clean up of the carbamates were made using an adapted method previously reported by Engehart and Lilling.[9] Amounts of 30 g of lettuce were spiked with carbamates in concentrations around the maximum admissible levels dictated by the European regulations for vegetables (0.20 mg/kg for Methiocarb and Methiocarb sulfoxide and 3.0 mg/kg for Baygon and Carbaryl). As a previous step, in order to ensure that the lettuce samples were free of incurred residues before their use, all samples used were screened for carbamates residues using the proposed method. Afterwards, the capability of the LC-PB-MS was tested for both identification and quantitation of the carbamates Methiocarb sulfoxide, Baygon, Carbaryl, and Methiocarb. Figure 2 shows the TIC chromatogram corresponding to a sample of lettuce spiked with the four carbamates at levels under the maximum allowable by the European regulations. Identifications of the four carbamates was positive by comparison of their EI mass spectra obtained by LC-PB-MS with those contained in the Wiley 138.1 reference library. As an example, inside the figure, over the chromatogram, the EI mass spectrum obtained and the reference one are shown for Baygon, the less sensitive of the four carbamates.

For quantitation, SIM operating mode was preferred because of its better sensitivity and selectivity. Calibration prior to analysis was carried out in this mode for Methiocarb sulfoxide, Carbaryl, and Methiocarb in the concentration range 0-20.0 mg/L and between 0 and 30.0 mg/L for Baygon.

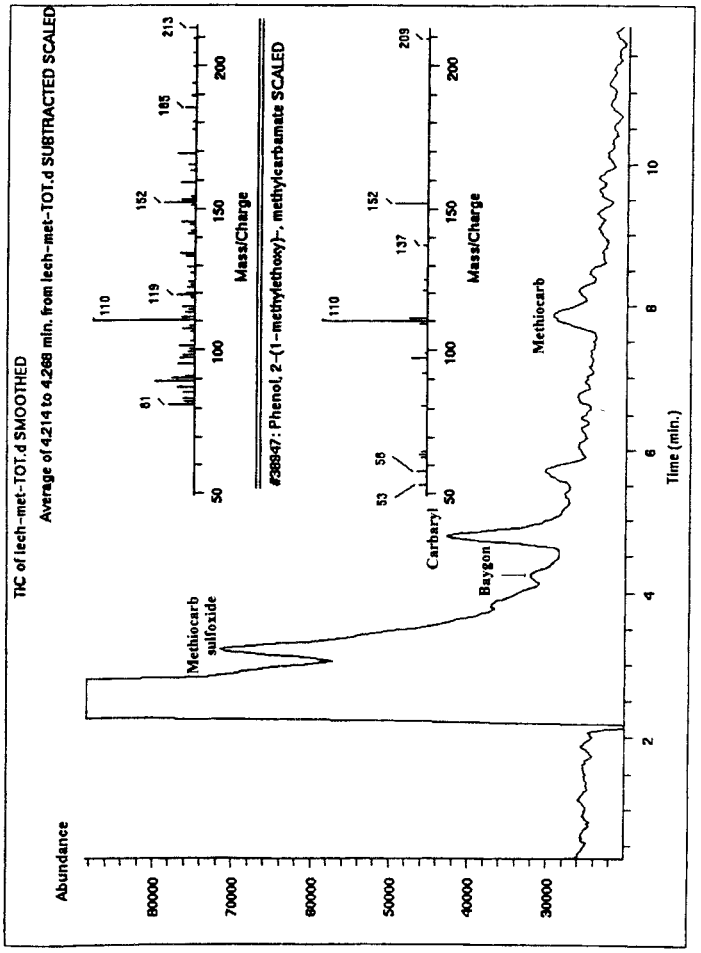

Figure 2. LC-PB-MS total ion chromatogram of lettuce extract spiked with 0.19 mg/kg of Methiocarb sulfoxide, 2.50 mg/kg of Baygon, 2.28 mg/kg of Carbaryl and 0.16 mg/kg of Methiocarb.

Table 2

Recovery Values for Spiked Lettuce

	Amount Added (mg/kg)	Recovery (%)[a]	SD
Metiocarb Sulfoxide	0.16 - 0.29	66	10
Baygon	2.19 - 3.80	53	6
Carbaryl	2.28 - 3.60	54	8
Methiocarb	0.19 - 0.33	76	15

[a] Mean of 5 additions.

To extend the applicability range, these calibrates were fitted to a second order polynomial, rather than to a straight line, to include in the calibration the typical deviation of linearity at lower levels, characteristic of PB-MS response. Over the concentration range tested, the correlation coefficients (R) were 0.9967, 0.9904, 0.9981, and 0.9951 for Methiocarb sulfoxide, Baygon, Carbaryl, and Methiocarb respectively. Recoveries achieved for five additions of the four carbamates to lettuce samples ant their standard deviation are shown in Table 2.

Determination of Baygon and Carbaryl in Apple Samples

One of the major problems observed in the LC-PB-MS analysis of the lettuce samples referred in the previous subsection was the broad peak appearing at 2.5 min (Figure 2). This peak corresponds to carbohydrates and other polar compounds coextracted with the carbamates. Since lettuce contains low amounts of carbohydrates, Methiocarb sulfoxide, which elutes at 3.20 min, could be identified and quantified. This was not possible when apple samples were tested, because of their high sugar content. Consequently, for this study only Baygon and Carbaryl were spiked into the apple samples.

As in the lettuce case the amount spiked was around the maximum allowable for these compounds (3.0 mg/Kg), and by following the same procedure, identification and quantitation ability of LC-PB-MS were tested satisfactorily. Recoveries obtained for this type of sample are shown in Table 3. It can be seen that values obtained for Baygon and Carbaryl (62 and 63% respectively) are slightly higher than those obtained in the lettuce study.

Table 3

Recovery Values for Spiked Baygon and Carbaryl in Apples

	Amount Added (mg/kg)	Recovery (%)[a]	SD
Baygon	2.07 - 4.85	62	10
Carbaryl	1.84 - 4.93	63	12

[a] Mean of 6 additions.

CONCLUSIONS

The LC-PB-MS method described is suitable to detect and quantify residues of the carbamates Baygon, Carbaryl, and Methiocarb and the degradation product Methiocarb sulfoxide in lettuce and apple samples. This method could be useful for other similar substances.

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