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Stability studies of carbamate pesticides and analysis by gas chromatography with flame ionization and nitrogen–phosphorus detection☆

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Abstract

As a result of thermal stability studies of carbamate pesticides, a method has been proposed for their direct determination by gas chromatography in the ranges 1–20 and 0.1–1 mg l^{-1}, using flame ionization and nitrogen–phosphorus detection, respectively. The method allows the determination of propham, propoxur, carbofuran, carbaryl, methiocarb, isopropoxyphenol and naphthol in powdered potato samples. The analytes were previously extracted with a light petroleum–dichloromethane (1:1, v/v) mixture and preconcentred by solid-phase extraction through a C_8 cartridge. The recoveries obtained from spiked potato samples ($n=4$ replicates) at two concentration levels, 10 and 0.5 mg of pesticide per kg of sample, were in the ranges 72–115 and 50–73%, with relative standard deviations of 2–7 and 5–8%, respectively. The detection limits were 50–210 and 41–53 μg kg^{-1} with flame ionization and nitrogen–phosphorus detection, respectively, and reaching the maximum residue levels, 0.05 mg kg^{-1} for methiocarb and propoxur, set by the Real Decreto 280/1994 (based on the European directive).

Keywords: Detection, GC; Food analysis; Stability studies; Carbamates; Pesticides

1. Introduction

The proven persistence and toxicity of organochlorine pesticides justify their replacement by other more easily degradable, polar, labile and less persistent pesticides, such as *N*-methylcarbamates. The carbamates are a family of compounds whose general structures ($R_1OCONR_2R_3$) are derived from carbamic acid by the introduction of different substituents. Their great success in agricultural applications has led to a continued increase in the use of these pesticides, but their acute toxicity is of great concern and makes necessary the determination of the carbamates at very low concentrations.

Analytical techniques more often used to determine carbamate pesticides are gas chromatography (GC) [1–10] and high-performance liquid chromatography (HPLC) [11–29].

Carbamates in general and their transformation products in particular are often quoted as being polar and thermally labile; these properties limit the use of GC. With regard to this, a clear discrepancy appears in the literature. Different authors propose methods

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based on GC with derivatization reactions [1–5], but it seems that the direct determination of carbamate pesticides is possible at very low concentrations [6–10]. Other authors do not recommend the use of this technique. Due to the thermal instability of these compounds, the use of HPLC with fluorometric detection [11–20] and UV detection [21–29] is increasing, although the detectors' sensitivities require more sample preparation and a higher concentration. As a rule, GC and, in particular, nitrogen–phosphorus detection (NPD) is preferred because resolution is higher and the detectors are more sensitive than in HPLC. In this paper we describe an exhaustive study of the thermal stability of several carbamate pesticides and metabolites (propham, *N*-phenylcarbamate herbicide; propoxur, carbaryl, methiocarb, *N*-methylcarbamate insecticides; isopropoxyphenol metabolite of propoxur, naphthol metabolite of carbaryl). The study results permitted development of a simple, rapid and sensitive method for their direct determination in powdered potato samples by GC with flame ionization detection (FID) and NPD at a 0.05 mg kg^{-1} concentration, the maximum residue level (MRL) allowed by the Real Decreto 280/1994 (based on the European directive), for propoxur and methiocarb pesticides [30].

2. Experimental

2.1. Reagents and standards

Isopropoxyphenol (IPF), naphthol (NAP) from Aldrich (Milwaukee, WI, USA) 97–99%; carbofuran (CBF), propham (PHM), methiocarb (MET), propoxur (PRO), carbaryl (CAR), from Chem Service (West Chester, PA, USA) 98.5–99%; aldicarb, aldicarb sulfoxide, methomyl from Riedel-de Haën (Seelze, Germany) 98–99%; phenanthrene from Sigma (St. Louis, MO, USA) 99%, and 4-nitrophenol from Fluka (Buchs, Switzerland) 99% as internal standard were used. HPLC-grade acetonitrile and methanol were purchased from Romil (Barcelona, Spain); light petroleum, dichloromethane, and ethyl acetate from Carlo Erba (Barcelona, Spain); 1,2-dichloroethane from Panreac (Barcelona, Spain), and *n*-hexane from Chromar HPLC (Paris, France). Water was purified with a Milli-Q system from Millipore (Milford, MA, USA).

Standard solutions: 1000 mg l^{-1} stock solutions were prepared by dissolving 25 mg of each carbamate pesticide in 25 ml methanol. Working standard solutions were prepared taking the corresponding aliquots from these solutions. 1000 mg l^{-1} internal standard solutions, phenanthrene in GC–FID, and 4-nitrophenol in GC–NPD were also prepared. Standard solutions were stable for at least 4 months at −22°C, and working solutions were stable for about 18 days.

Powdered potato sample was from Maggi (Barcelona, Spain) and contained 99% dehydrated potato, emulsifier (E-471), stabilizer (E-450a), antioxidant (E-300), preservative (E-223) and spice.

2.2. Material

Extraction tubes were from Pyrex: 8 ml, 10 cm×1 cm I.D. Silica (500 mg, 6 ml) from Waters (Milford, MA, USA), C_8 (500 mg, 3 ml) from Teknokroma (Barcelona), and C_{18} (500 mg, 6 ml) from Varian (Sunnyvale, CA, USA) cartridges were used. A hypodermic syringe (Hamilton, Reno, NV, USA) was 10 μl with a 70 mm needle. Another hypodermic syringe (Hamilton Teknokroma, Barcelona, Spain) was 10 μl with a 51 mm needle.

2.3. Apparatus

The chromatographic system consisted of the following components: a gas chromatograph, Hewlett-Packard (HP) 5890 I (from USA), equipped with two different detection systems (FID and NPD) and a normal injector for packed columns. The carrier gas was nitrogen. The operating conditions were: splitless injection through glass-liner; injection volume, 1 μl. Temperature program: injector temperature, 220°C; detector temperature, 250°C; gradient: initial temperature, 110°C for 5 min; 5°C min^{-1} to 140°C for 2 min; 5°C min^{-1} to 210°C for 2 min and 7°C min^{-1} to 240°C for 2 min. Semicapillary columns (from USA): HP-5 (30 m×0.53 mm, 2.65 μm) and OV-101 (10 m×0.53 mm, 2.65 μm) were used. Capillary column (from Hewlett-Packard, USA): HP-1 (25 m×0.25 mm, 0.25 μm) was used. For data acquisition ChemStation software HP-3365

was used (from USA). Also a gas chromatograph, Hewlett-Packard 5890 II, equipped with a split–splitless injector; injection volume, 1 μl; and Varian VA-5 column (30 m×0.25 mm, 0.25 μm) was used. A mass spectrometer with a quadrupole filter (USA) HP 5989A, Wiley library HP 59943B and integrator was also used. The operating conditions were: ionization source temperature, 220°C; quadrupole filter temperature, 100°C; ionization energy, 70 eV; temperature program, the same one used in GC–FID/NPD; scan mode (35–320 u), slected ion monitoring (SIM) mode (M^+ ions), *m/z*: 93, 109, 110, 115, 119, 131, 144, 149, 152, 153, 164, 168, 178, 179, 201, 221.

A Visidrep vacuum manifold system (Supelco, USA); a vacuum pump (Barna, USA); an ultrasonic bath (P-Selecta, Spain); a centrifuge (P-Selecta ''Angular 6'', Spain) and a Flash shaker (Griffin, UK) were also used for sample and solution preparation.

2.4. Procedures

2.4.1. Calibration for carbamate pesticides

Four standard work solutions of pesticide mixture were obtained in the concentration range 1–20 mg l^{-1} using 50 mg l^{-1} phenanthrene as internal standard (FID) and in the range of 0.1–1 mg l^{-1} using 5 mg l^{-1} 4-nitrophenol also as internal standard (NPD). A 1-μl volume was injected into the gas chromatograph.

2.4.2. Determination of carbamate pesticides in powdered potato samples

The sample preparation procedure was as follows: 0.5 g powdered potato sample was weighed into an 8-ml extraction tube, and spiked with work standard solutions of pesticide mixture in the concentration range 0.1–20 mg kg^{-1} of potato sample.

2.5. Liquid–liquid extraction (LLE)

A 1.75-ml volume of methanol was added to the spiked potato and shaken for 30 s using the ultrasonic bath. Then, 1.75 ml of purified water and 2.5 ml of light petroleum–dichloromethane (1:1) were added and shaken for 5 min in the ultrasonic bath. The mixture was centrifuged at 3000 rpm for 17 min. The organic phase was collected and evaporated to dryness with argon. The yellow–greenish extract was dissolved with 1 ml of methanol.

2.6. Clean-up by solid-phase extraction (SPE)

The methanolic extract obtained under LLE was passed through a C_8 microcolumn, which was previously conditioned (twice with 2 ml of methanol). Carbamates were eluted from the cartridge using 0.5 ml of acetonitrile at a flow-rate of 1 ml min^{-1} (Vacuum Manifold System pressure, 1 in.Hg= 338.638 Pa). Internal standard, phenanthrene and 4-nitrophenol, were added to the eluate at concentration levels of 50 and 5 mg l^{-1} respectively, for the corresponding analysis by GC–FID/NPD. A 1-μl volume was injected into the gas chromatograph.

3. Results and discussion

3.1. Preliminary

The degradation of carbamate pesticides, when they are injected directly without derivatization into the gas chromatograph, is not commonly studied. For this reason, a study of the temperature effect on the chromatographic column, using different compounds, was carried out on a potato sample for their direct determination by GC–FID/NPD.

Several carbamate pesticides PHM, PRO, CBF, CAR, MET and two metabolites, IPF from PRO and NAP from CAR were studied.

Two columns of different polarity and length were used, HP-5 and OV-101; the latter was older. Two temperature programs were selected.

It was shown that degradation increases when using old columns with respect to the new ones.

Other compounds tested; aldicarb, aldicarb sulfoxide and methomyl could not be detected under the same conditions, as stated in the literature [3]. These compounds differ structurally because they are polar aliphatic oximes.

3.2. Optimization of the chromatographic separation

3.2.1. Thermal stability studies

Different temperature programs were tested. The retention factor values (k), resolution (R_s) and separation factor (α) were taken into account in order to select two gradients, a strong one (I) and a weaker one (II) for thermal stability studies, which were as follows:

Gradient I: initial temperature, 110°C for 5 min; 70°C min^{-1} to 150°C for 0.5 min; 30°C min^{-1} to 175°C for 1 min and 4°C min^{-1} to 240°C for 2 min.

Gradient II: initial temperature, 110°C for 5 min; 5°C min^{-1} to 140°C for 2 min; 5°C min^{-1} to 210°C for 2 min and 7°C min^{-1} to 240°C for 2 min.

To select the optimal temperature gradient, a 50 mg l^{-1} standard mixture of pesticide solution was injected to observe the degradation effect on two columns of different characteristics: HP-5 (30 m) and OV-101 (10 m); the latter of less polarity and length. It was observed that a gradual temperature gradient II afforded a lesser degradation of the pesticides, with the consequent increase of the relative areas of the corresponding carbamates. The effect produced by both a gradual and a steep temperature gradient on the analytes studied, was increased in long columns, above all when steep temperature gradients are applied, involving sudden changes of temperature. However no variation was observed in the carbamate degradation using the OV-101 column and with the temperature gradients utilized. Using the HP-5 column, significant changes were obtained, with degradation increasing for the CBF and MET, 50 and 34%, respectively, when gradient I was applied. Through this study, gradient II was selected as optimum. The two columns continued to be used during the study because the OV-101 column minimizes the degradation, but it also gives less resolution than the HP-5 column. Fig. 1 shows the chromatogram obtained with the HP-5 column, using gradient II.

Once the temperature program was selected, other variables which can affect the degradation were performed to check the behavior of carbamate pesticides when they were injected using FID or NPD. The following parameters were studied: length of syringe needle, injector temperature, pesticide concentration, and volume of air in the end of the needle. The optimization of these experimental parameters was performed with four replicates.

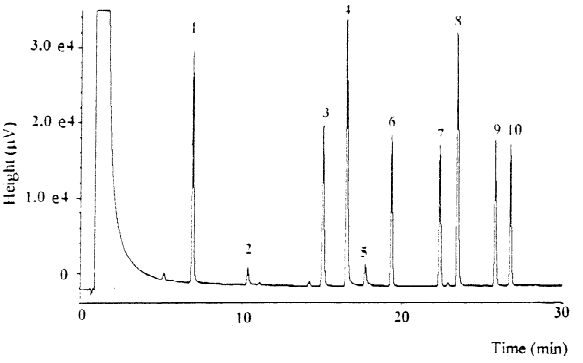

Fig. 1. Chromatogram obtained from a carbamate pesticide mixture using gradient II and a HP-5 (30 m×0.53 mm, 2.65 μm) column, [pesticide]=50 mg l^{-1}, injection volume, 1 μl. Temperature program: injector temperature, 220°C, detector temperature, 250°C, gradient: initial temperature, 110°C for 5 min, 5°C min^{-1} to 140°C for 2 min, 5°C min^{-1} to 210°C for 2 min and 7°C min^{-1} to 240°C for 2 min. Elution order: 1=IPF, 2=metabolite of CBF, 3=PHM, 4=NAP, 5=metabolite of MET, 6=PRO, 7=CBF, 8=phenanthrene, 9=CAR, 10=MET.

The effect of the syringe needle length was developed using needles of 51 and 70 mm and pesticide standard mixtures of 20 and 50 mg l^{-1}. It was observed that using the needle of 70 mm the degradation was minimized, the relative areas of the chromatographic peaks of the metabolites were negligible at a 20 mg l^{-1} concentration. In a 50 mg l^{-1} solution, the degradation decreased from 22 to 15% for the metabolite of CBF and from 30 to 19% for the metabolite of MET (Table 1). Therefore, the 70-mm needle was used in all studies.

Three injector temperatures, 200, 220 and 240°C were tested. Whereas degradation increased at 240°C, no significant differences were observed in the values of the relative areas between 200 and 220°C. However, there was a slight increase at 220°C for the CBF and CAR compounds. This temperature was chosen as optimum value.

A carbamate pesticide concentration range of 1–75 mg l^{-1} was studied to ascertain which concentration level causes degradation subproducts to appear. No degradation was observed in the concentration range 1–10 mg l^{-1}. Above 20 mg l^{-1}, the metabolites of MET and CBF, absent in the working

Table 1
Effect of the syringe needle length on the degradation of the carbamate pesticides

Pesticide	[Pesticide]=20 μg ml^{-1}				[Pesticide]=50 μg ml^{-1}			
	Short needle		Long needle		Short needle		Long needle	
	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)
IPF	0.37	3	0.38	3	1.00	3	0.93	2
Metabolite of CBF	0.033	20	–	–	0.10	20	0.079	30
PHM	0.22	3	0.23	3	0.51	1	0.51	2
NAP	0.31	1	0.26	2	0.86	3	0.78	3
Metabolite of MTH	0.047	18	–	–	0.14	20	0.089	20
PRO	0.13	2	0.15	2	0.43	1	0.46	1
CBF	0.094	2	0.13	2	0.34	2	0.38	1
CAR	0.095	3	0.14	3	0.33	4	0.41	3
MET	0.093	3	0.13	3	0.32	5	0.37	4

Conditions: OV-101 (10 m×0.53 mm, 2.65 μm) column. Injection volume, 1 μl. Short needle, 51 mm. Long needle: 70 mm. A/A_{SI}, mean relative area. RSD, relative standard deviation (n=4).

standard mixture, began to be detected in both columns. PHM pesticide was stable in the whole range of concentration studied (Table 2).

In order to minimize the degradation, 1 μl of 50 mg l^{-1} standard mixture solution was drawn into the syringe, followed by 0.2 μl of air (so that there was no sample at the end of the needle or in the injection threshold). However, this effect increased the degradation from 7 to 12% for the metabolite of CBF and from 15 to 23% for the metabolite of MET; probably the air favors the hydrolysis of these compounds. With this experiment, it was also possible to find the dead time of the column.

It should be pointed out that PHM was completely stable under all the conditions studied.

3.3. Determination of carbamate pesticides in standard solutions. Analytical characteristics

The analytical characteristics of the method for the determination of carbamate pesticides in four standard work solutions, were achieved in the concentration ranges 1–20 mg l^{-1} using 50 mg l^{-1} phenanthrene as internal standard, the HP-5 and OV-101 columns and FID and 0.1–1 mg l^{-1} using 5 mg l^{-1} 4-nitrophenol as internal standard, the HP-5 column and NPD. The regression coefficients were in the range 0.991–0.999. The relative standard deviations (RSDs) (n=4) at the 10 mg l^{-1} concentration level were 5–9% with FID and 5–8% at the 0.5 mg l^{-1} concentration level with NPD. The sensitivity is

Table 2
Influence of carbamate pesticide concentration

Pesticide	[Pesticide] (μg ml^{-1})													
	1		5		10		20		30		50		75	
	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)	A/A_{SI}	RSD (%)
IPF	0.013	3	0.10	3	0.19	3	0.36	5	0.46	5	0.82	5	1.28	3
Metabolite of CBF	–	–	–	–	–	–	$8.6\cdot10^{-3}$	20	0.013	25	0.041	30	0.063	30
PHM	0.021	1	0.061	5	0.11	6	0.22	7	0.28	2	0.54	5	0.82	2
NAP	0.016	8	0.068	8	0.12	8	0.26	9	0.37	2	0.73	6	1.05	6
Metabolite of MET	–	–	–	–	–	–	$1.1\cdot10^{-2}$	30	0.011	20	0.067	30	0.077	30
PRO	0.013	5	0.044	4	0.078	6	0.15	8	0.23	3	0.42	5	0.43	3
CBF	0.021	4	0.043	4	0.075	5	0.12	9	0.16	5	0.29	6	0.50	6
CAR	$6.6\cdot10^{-3}$	8	0.031	8	0.054	5	0.14	10	0.22	10	0.29	8	0.52	5
MET	$5.4\cdot10^{-3}$	7	0.027	4	0.055	6	0.12	10	0.22	8	0.30	8	0.38	5

Conditions: OV-101 (10 m×0.53 mm, 2.65 μm) column. Injection volume, 1 μl. A/A_{SI}, mean relative area. RSD, relative standard deviation (n=4).

Table 3
Analytical characteristics of the method for the determination of carbamate pesticides in standard solutions by GC–FID/NPD

Pesticide	GC–FID						GC–NPD		
	OV-101 column			HP-5 column			HP-5 column		
	Sensitivity ($\mu V\,\mu g^{-1}\,l$)	LOD ($\mu g\,l^{-1}$)	LOQ ($\mu g\,l^{-1}$)	Sensitivity ($\mu V\,\mu g^{-1}\,l$)	LOD ($\mu g\,l^{-1}$)	LOQ ($\mu g\,l^{-1}$)	Sensitivity ($\mu V\,\mu g^{-1}\,l$)	LOD ($\mu g\,l^{-1}$)	LOQ ($\mu g\,l^{-1}$)
IPF	45	13	64	67	7	32	–	–	–
PHM	23	25	120	27	16	80	49	3	15
NAP	25	23	110	27	16	80	–	–	–
PRO	16	36	180	21	20	102	55	3	14
CBF	11	54	260	17	25	125	50	3	15
CAR	15	38	190	15	29	143	36	4	21
MET	10	57	290	12	48	240	25	6	30

LOD=Limit of detection, LOQ=limit of quantitation.

presented in Table 3. Limits of detection (LODs) and quantification (LOQs) using the OV-101 column, were higher because it is a shorter, older column.

3.4. Confirmation of carbamate pesticides by GC–MS

The analysis of a standard mixture of carbamate pesticides at the 100 mg l^{-1} concentration in scan and SIM modes confirmed all the compounds studied by comparison of their spectra with the library MS spectra, except CBF, CAR and MET, which were confirmed by their respective metabolites. The degradation was due to these compounds undergoing a McLafferty concerted fragmentation with a six-membered transition state, favored by the formation of very stable products. Fig. 2 shows the fragmentation for MET, as an example. A neutral molecule ''CH_3–N=C=O'' was lost in the fragmentation mechanism and the corresponding phenol tautomer was formed. MET, CBF and CAR were confirmed in the pesticide standard mixture.

3.5. Recovery of carbamate pesticides from powdered potato sample

Firstly, in order to check the absence of carbamate pesticides in the sample, the blank chromatogram was obtained by GC–FID/NPD. Fig. 3a shows the blank chromatogram obtained using the HP-5 col-

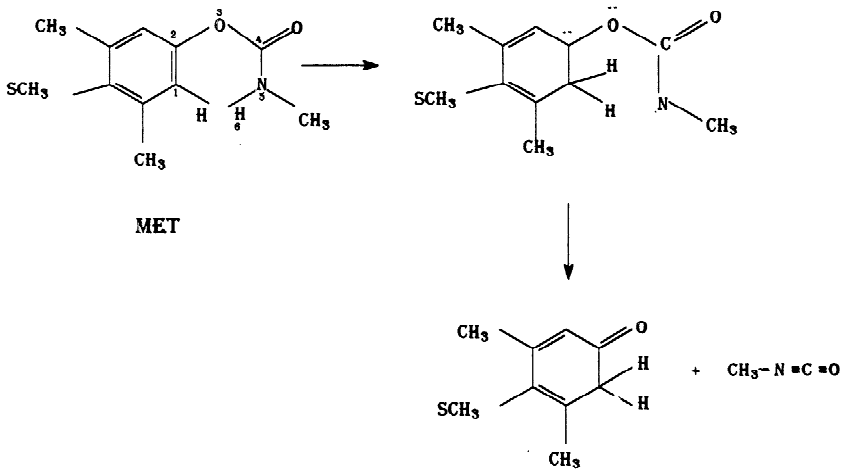

Fig. 2. Degradation mechanism of MET pesticide. McLafferty concerted fragmentation.

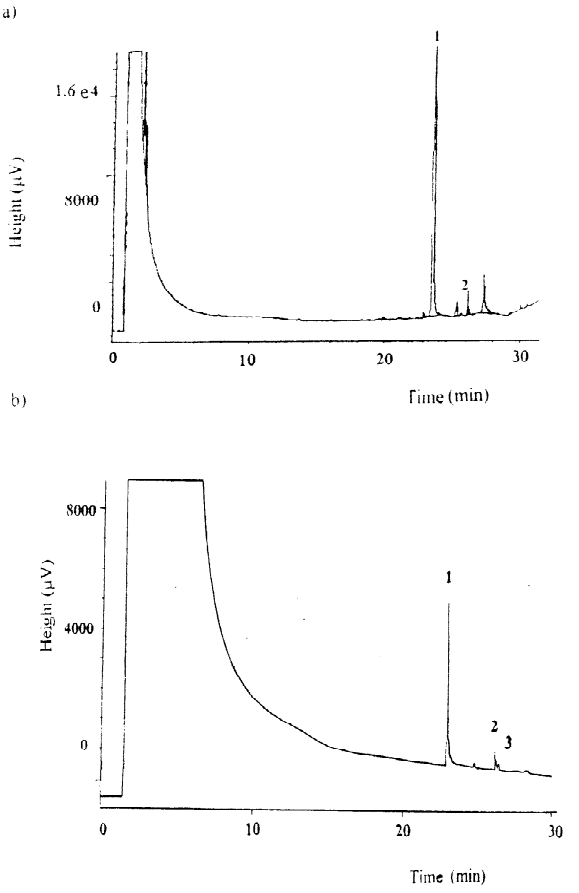

Fig. 3. Blank chromatogram obtained from a powdered potato sample. (a) HP-5 (30 m×0.53 mm, 2.65 μm) column, [phenanthrene]=50 mg kg^{-1}; elution order: 1=phenanthrene, 2=matrix peak. Injection volume, 1 μl. (b) HP-1 (25 m×0.53 mm, 2.65 μm) column, [phenanthrene]=50 mg kg^{-1}, [MET]=5 mg kg^{-1}; elution order: 1=phenanthrene, 2=matrix peak, 3=MET. Injection volume, 1 μl.

umn and GC–FID, in which can be seen the peak of the internal standard (retention time, 23.48 min) and other small peaks (peak 2), with relative retention time 1.13, which could belong to MET. To verify this, a HP-1 column whose polarity is different to the column mentioned above was used and the chromatogram of a spiked sample with only 5 mg kg^{-1} of MET was obtained, Fig. 3b. A new peak of the added MET pesticide appears (relative retention time 1.15) along with the peak of the matrix (relative retention time 1.14). Therefore, it could be confirmed that the powdered potato sample had none of the pesticides studied. The matrix peak was not obtained by GC–NPD, possibly because of a lack of nitrogen in its structure.

Secondly, recovery studies from a powdered potato sample spiked at the 10 mg kg^{-1} concentration level of IPF, PHM, NAP, PRO, CBF, CAR and MET were undertaken by GC–FID, using the HP-5 column. The optimization of experimental parameters involved in the sample preparation, LLE and SPE procedures, was performed according to the recoveries and relative areas obtained with their RSD values.

3.6. Sample preparation

0.25, 0.50 and 0.75 g of powdered potato sample were tested. A 0.5-g amount was chosen as the optimum sample amount, with recovery values of 79–108% and RSD, 2–8% (n=4). The recovery notably decreased for 0.25 g, possibly because the centrifugation gave a wide interphase between the aqueous and organic phases. For 0.75 g, there were limitations because of the size and diameter of the extraction tube.

3.7. Liquid–liquid extraction

Four organic solvents were tested as extraction agents: *n*-hexane, light petroleum, dichloromethane and a light petroleum–dichloromethane mixture (1:1). Hexane and light petroleum solvents only extracted IPF, PHM, CAR and MET compounds, and dichloromethane was unsuitable for the analysis because of its higher density. The light petroleum–dichloromethane (1:1) binary mixture was chosen as optimum, with recovery values, 70–105% and RSD, 2–7% (n=4).

The aqueous phase, consisting of a methanol–water mixture, was optimized with different ratios, 1.0:2.5, 1.75:1.75 and 2.5:1.0 (v/v). The best recovery values obtained were 76–99% (1.75:1.75 ratio). Thereafter, the following phase ratio light petroleum–dichloromethane (V_o) and methanol–water (V_w) mixture were studied: 1:5, 1.5:4.5, 2:4, 2.5:3.5 and 3:3 (V_o/V_w). The best recoveries were obtained from the ratio 2.5:3.5 and were in the range of 76–99%, with RSD values of 2–5%.

Three agitation procedures, manual, shaker and

Table 4
Analytical characteristics of the method for the determination of carbamate pesticides in a powdered potato sample by GC–FID/NPD

Pesticide	GC–FID			GC–NPD		
	Sensitivity ($\mu V\ \mu g^{-1}\ l$)	LOD ($\mu g\ l^{-1}$)	LOQ ($\mu g\ l^{-1}$)	Sensitivity ($\mu V\ \mu g^{-1}\ l$)	LOD ($\mu g\ l^{-1}$)	LOQ ($\mu g\ l^{-1}$)
IPF	21	50	260	–	–	–
PHM	10	110	560	14	44	218
NAP	8	140	70	–	–	–
PRO	7	150	750	15	41	203
CBF	7	140	700	14	44	218
CAR	5	210	950	11	53	267
MET	6	180	900	12	50	254

LOD=Limit of detection, LOQ=limit of quantitation.

ultrasonic were tested, as well as agitation times of 2, 5 and 8 min. The best results were obtained for 5 min of ultrasonic agitation, with recovery values of 78–105% and RSD, 2–8%.

To optimize the centrifugation time, 12, 17 and 22 min were studied, and the best recovery values, 79–106%, were for 17 min.

Finally, 1 and 2 ml of methanol for the redissolution of the sample extract were tested and 1 ml of methanol was found to be sufficient for this step.

3.8. Solid phase extraction

An SPE procedure was carried out with silica, C_8 and C_{18} cartridges containing 500 mg adsorbent, previously conditioned with methanol. The C_8 cartridge gave better results than the C_{18} cartridge, with recovery values of 78–102%; IPF and NAP compounds were recovered in a small proportion with the silica cartridge; these polar compounds seem to be retained irreversibly.

The sample retained in the C_8 cartridge was eluted with two different flows, which corresponded to pressures of 1 and 4 in.Hg in the vacuum manifold system. The more suitable flow was 1 in.Hg, because a pressure of 4 in.Hg significantly decreased the retention.

Once the carbamate pesticides were retained in the C_8 cartridge, the drying time was optimized (1, 4 and 7 min). Good recoveries were obtained with 4 min, 78–102%.

Two volumes of acetonitrile, 0.5 and 1 ml, were used to elute the carbamate pesticides through the C_8 cartridge. The best recovery values were obtained

Table 5
Analytical characteristics of the method for the determination of carbamate pesticides in a powdered potato sample by GC–FID/NPD

Pesticide	GC–FID[a]				GC–NPD[b]			
	Reproducibility*		Repeatability**		Reproducibility*		Repeatability**	
	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)	RSD (%)	Recovery (%)
IPF	8	72	2	72	–	–	–	–
PHM	5	91	5	98	5	51	8	62
NAP	8	83	2	76	–	–	–	–
PRO	7	98	6	99	7	61	8	60
CBF	8	95	7	93	7	54	8	59
CAR	7	99	6	85	8	50	4	60
MET	6	115	2	115	8	73	7	72

*n=4, **n=6.
[a] [Pesticide]=10 mg l^{-1}.
[b] [Pesticide]=0.5 mg l^{-1}.

with 1 ml of acetonitrile, 79–105%, but 0.5 ml was considered as optimum elution volume with a recovery of 72–104%, since a preconcentration factor of 2 allowed the limits of detection established by the European directive to be reached.

3.9. Analytical characteristics of the method

The analytical characteristics of the carbamate pesticides in a powdered potato sample were studied. The sample was fortified for the analysis at four concentration levels in the ranges 1–20 mg kg^{-1} by GC–FID and 0.1–1 mg kg^{-1} by GC–NPD. The regression coefficients oscillated between 0.992 and 0.997 and between 0.995 and 0.999 by GC–FID and GC–NPD, respectively. Table 4 shows the sensitivity, detection limit and quantitation limit values.

Table 5 summarizes the recovery and RSD values, which belong to the reproducibility ($n=4$) and repeatability ($n=6$) studies by GC–FID and GC–NPD at 10 and 0.5 mg kg^{-1}, respectively.

Chromatograms obtained from a powdered potato sample using GC–FID/NPD and the HP-5 column are shown in Fig. 4.

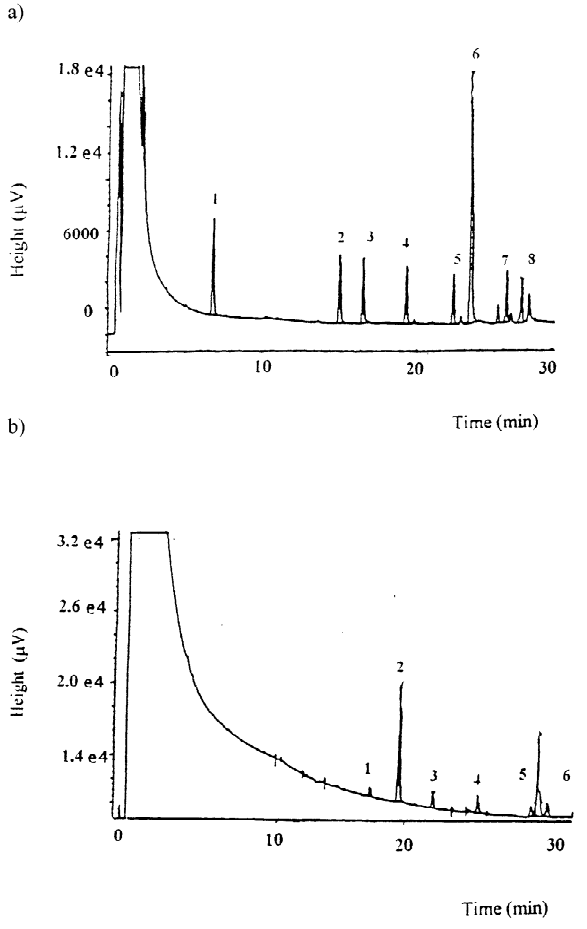

Fig. 4. Chromatogram obtained from a spiked powdered potato sample. HP-5 (30 m×0.53 mm, 2.65 μm) column. (a) GC–FID, [pesticide]=10 mg kg^{-1}, injection volume, 1 μl; elution order: 1=IPF, 2=PHM, 3=NAP, 4=PRO, 5=CBF, 6=phenanthrene, 7=CAR, 8=MET. (b) GC–NPD, [pesticide]=0.5 mg kg^{-1}, injection volume, 1 μl; elution order: 1=PHM, 2=4-nitrophenol, 3=PRO, 4=CBF, 5=CAR, 6=MET.

4. Conclusions

Degradation of carbamate pesticides was minimized using a temperature program with a gradual gradient, syringes with needles of 70 mm, longer than conventional ones (51 mm), and columns whose stationary phase had been less modified by use.

A wide concentration range of carbamate pesticides was studied (1–75 mg l^{-1}), which led to establishing 20 mg l^{-1} as the superior limit for their direct determination by GC, in the absence of their metabolites, using a temperature program with a gradual gradient. At higher concentrations, metabolites were detected and increased with the concentration of pesticide; this led to degradation in uncontrolled amounts. An exception was made for PHM, the most stable of all the studied compounds.

The proposed method for the determination of carbamate pesticides PHM, PRO, CBF, CAR and MET in a powdered potato sample by GC–FID/NPD is useful because of the low concentrations obtained with NPD, reaching the MRL established by the European directive, 0.05 mg kg^{-1} for PRO and MET.

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