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Continuous ultrasound-assisted extraction coupled to on line filtration–solid-phase extraction–column liquid chromatography–post column derivatisation–fluorescence detection for the determination of *N*-methylcarbamates in soil and food

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Abstract

A dynamic ultrasound-assisted method for the extraction of *N*-methylcarbamates (oxamyl, dioxacarb, metolcarb, carbofuran, carbaryl and isoprocarb) from soils and foods is proposed. The main factors affecting the extraction efficiency have been optimised by means of a central composite design. Pure water can be used as leaching agent. A flow injection manifold coupled to the extractor allows automation of the several steps involved in the analytical process. The method allows extraction of the carbamates from soil and food at 1 μg/g spiked level, with recoveries similar to those provided by the EPA 8318 method, without degradation of the target compounds during the extraction and with drastic shortening of the time required for this step (2 min vs. 4 h). Recoveries of the target analytes were 77–95% for spiked soil and 85–101% for spiked food. The detection and quantification limits were 12 and 40 ng/g, respectively, for all analytes, except carbaryl (detection and quantification limits 3 and 10 ng/ng, respectively). The relative standard deviations for repeatability and within-laboratory reproducibility were 3.1 and 7.5%, respectively.

Keywords: Derivatization, LC; Food analysis; Soil analysis; Continuous ultrasound-assisted extraction; *N*-methylcarbamates; Pesticides

1. Introduction

The technique used for the extraction of *N*-methylcarbamate pesticides from solid samples plays a crucial role in the subsequent analysis of these compounds due to their thermal lability. The tendency to thermal decomposition (to methylisocyanate and phenol) [1], makes most carbamate pesticides difficult to extract using conventional methods such as Soxhlet extraction. The long heating periods in the Soxhlet flask cause degradation of these analytes. Alternative techniques such as supercritical fluid extraction [2,3] or pressurised solvent extraction [4] have been used for the removal of these compounds from fruit and food, principally. However, the times required in both cases were long and the recoveries obtained poor, due to the fact that drastic conditions cannot be used in order to preserve

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the structure integrity of these pesticides. A good alternative are the methods reported by de Kok et al. [5–7] for analysing *N*-methylcarbamates from vegetables and fruit using liquid–solid extraction.

When supercritical fluid extraction was used for the extraction of these compounds from soil [3], the recoveries obtained for soils aged for 24 h, after 30 min of dynamic extraction, were lower than 50%. In 1994, the Environmental Protection Agency (EPA) adopted a manual shaking method as reference [8] for the extraction of *N*-methylcarbamates in soil, which remains in use today.

Analytical chemistry is one of the chemical areas where ultrasound radiation has not been frequently used. This auxiliary technique could be a powerful aid in the acceleration of various steps of the analytical process. Thus, this energy is of great help in the pretreatment of solid samples as it facilitates and accelerates operations such as the extraction of organic and inorganic compounds [9–12], slurry dispersion [13], homogeneisation [14] and various others such as nebulisation [15], washing [16] and derivatisation [17].

In many situations, ultrasound-assisted leaching is an expeditious, inexpensive and efficient alternative to conventional extraction techniques and, in some cases, even to supercritical fluid and microwave-assisted extraction, as demonstrated by application to both organic and inorganic analytes in a wide variety of samples [18].

At present, our research group has developed methods for the extraction of carbamates in soil and food with either focused microwaves for assisting Soxhlet extraction with organic solvents [19] or with a pressurised hot water extractor [20].

No extraction methods have so far been proposed for the extraction of carbamates from soil based on the use of ultrasounds as an auxiliary energy to accelerate the process. Moreover, there are only four cases in the literature in which this type of energy has been used in a similar dynamic system with this aim [21–24].

The research presented here is based on the use of a flow injection (FI) manifold coupled to an ultrasound-assisted extractor for full automation of all the steps of the analytical process. The inclusion of a minicolumn packed with C_{18} hydra in the flow injection system has allowed the concentration of the extracted carbamates before chromatographic separation derivatisation and detection, thus lowering the limits of detection.

2. Experimental

2.1. Reagents and standards

The *N*-methylcarbamate pesticides oxamyl, dioxacarb, metolcarb, carbofuran, carbaryl and isoprocarb were obtained from Sigma (St. Quentin, Fallavier, France) and Aldrich (Milwaukee, WI, USA). These compounds were used for preparing both the stock standard solutions and the internal standard solution (isoprocarb) at 100 μg/ml, by dissolving the required amount of each pesticide in the minimum volume of acetone (Panreac, Barcelona, Spain) necessary for total dissolution; then, dilution to the required volume was made with methanol (Panreac). Standard working solutions were prepared daily by appropriate dilution of aliquots of the stock solution in methanol.

A 0.03 mol/l NaOH solution was prepared using sodium hydroxide (Panreac) in deionised water of 18 MΩ cm resistivity obtained from a Milli-Q water purification system. The 0.625 m*M* stock solution *o*-phthaldialdehyde (OPA) was prepared by dissolving this compound (Sigma) in 1:4 methanol–boric acid buffer; then, the solution was made 6×10^{-4} *M* in thiolactic acid (Aldrich). The buffer was prepared by dissolving 12.5 mmol boric acid (Panreac) in 1 l 1:4 methanol–water. The derivatisation reagents were prepared daily and stored at 4 °C until use.

Water Milli-Q with HCl and NaOH to obtain different pHs was selected as leaching agent. All solvents were HPLC-grade.

2.2. Instruments and apparatus

Ultrasound irradiation was applied by means of a Branson 450 sonifier (20 kHz, 100 W) equipped with a cylindrical titanium alloy probe (2.54 cm diameter) which was immersed in a thermostated water bath (Ultraterm 6000383 P-Selecta, Barcelona, Spain) in which the sample cell was placed. An extraction chamber consisting of a stainless steel cylinder (10× 1.0×1.3 cm I.D.×O.D.) closed with screws at either

end, which permitted the circulation of the leaching solvent through it, was used.

Two Gilson Minipuls-3 low-pressure peristaltic pumps (Gilson, Worthington, OH, USA); five Rheodyne 5041 low-pressure injection valves—three adapted to work as selection valves—(Rheodyne, Cotati, CA, USA); a laboratory-made column (5.0 cm×4.0 mm I.D.×9.0 mm O.D.) packed with the sorbent C_{18}-Hydra (50 μm particle size from Panreac); a 0.45 μm nylon filter with a diameter of 29 mm (Scharlau, Barcelona, Spain) and PTFE tubing of 0.8 mm I.D. were used to build the FI manifold, which was connected to an HP 1100 liquid chromatograph (Agilent Technologies, Avondale, PA, USA) consisting of a G1311A high-pressure quaternary pump, a G1322A vacuum degasser, and a Rheodyne 7725 high-pressure manual injector valve (30 μl injection loop). An Ultrabase C_{18} (250 cm× 4.6 mm; 5 μm particle size, Scharlau, Barcelona, Spain) was the analytical column.

A low-pressure peristaltic pump (Gilson Minipuls-3) was used as propelling system of the reagents for post-column fluorescence derivatisation of the analytes at 0.5 ml/min (namely: a basic and OPA solutions). Alkaline hydrolysis of the compounds was developed in a mixing coil (86.6 cm×0.5 mm I.D.) located into a water bath (Tectron bio P-Selecta) at 85 °C. A mixing coil (65 cm×0.5 mm I.D.) was also used for derivatisation of the hydrolysed analytes by the fluorogenic reagent (OPA). The fluorescence detection of the target products was performed by a scanning wavelength molecular fluorescence detector (F-1050, Hitachi, Merck, Germany). The chromatograms were obtained and integrated using a D-2500, Hitachi device from Merck.

2.3. Procedure for soil contamination

Five types of soil were used. The sandy soil contained sand (65.9%), silt (18.8%), clay (13.2%) and organic matter (2.1%). The clayely soil had organic matter (1.0%), sand (20.6%), silt (37.4%) and clay (41%). The slimy soil contained organic matter (1.8%), sand (35.0%), silt (53.9) and clay (9.3%). The limy soil had little amounts of organic matter (0.7%) sand (35.1%), silt (45.0%) and clay (19.2%). The organic soil had large amounts of organic matter (18.0%) and also sand (19%), silt (37.3%), and clay (25.7%).

Then, 500 g of each soil was sieved to a size smaller than 1 mm and spiked with the *N*-methylcarbamates by adding to the soil 300 ml of methanol containing the necessary volume of stock standard solutions of the target analytes to obtain a final total concentration in the dry soil of 5 μg/g (1 μg/g of each carbamate). Then, the slurries were shaken for 72 h, and after evaporation of the solvent, the soils were completely dried under an N_2 stream. Finally, the soils were put into holders and maintained at environmental conditions for 3 months, then stored at 4 °C in the dark until use.

To evaluate the stability of the contaminated soil during the development of the method, soil extractions using the EPA procedure were performed once a week. The results obtained showed similar recoveries along all time with an average relative standard deviation <4%, thus demonstrating the stability of the soil during the overall research. Detectable levels of carbamates were not found in non-spiked soils using the EPA procedure (limit of detection=31 ng/g).

2.4. Food preparation

Apples were purchased at a local market. Spiked samples were used as no CRMs are available. Sampling was done according to the protocol established by legislation [25]. The samples were washed with water and cut into pieces. Approximately 5 g of each sample were accurately weighed and spiked with the carbamates by adding the stock standard solutions to obtain a final concentration in the food of 5 μg/g (1 μg/g of each carbamate). Then, the samples were refrigerated for 2 h at 4 °C in a sealed flask before extraction in order to simulate the normal contact between the food and the carbamates. A preliminary study showed an increase in the analytes retention when the storage time (under refrigeration conditions) increased from 0 to 2 h; times between 2 and 24 h provided similar results, so the former was used in all experiments.

Pear and cucumber samples were prepared and spiked in the same way as apple samples. None of the samples had detectable levels of the carbamates before spiking.

2.5. EPA method 8318

EPA method 8318 for the determination of *N*-methylcarbamates by high-performance liquid chromatography (HPLC) consists of three steps: extraction, solvent exchange and analysis [19].

The carbamates are extracted from soils with acetonitrile. The extract solvent is exchanged to methanol–ethylene glycol and then the extract is cleaned on a C_{18} cartridge. After separation the target analytes are hydrolysed and derivatised post-column for fluorometric quantitation.

2.6. Proposed method

The method was developed using the assembly shown in Fig. 1, which allows the coupling of leaching, filtration, preconcentration, individual chromatographic separation, post-column derivatisation and fluorescence detection.

2.6.1. Leaching step

Approximately 5 g of a food or soil sample was weighed and transferred to the extraction chamber together with glass beads (3 mm I.D.) to avoid compactness in the extraction cell, which was assembled to the FI manifold and immersed into the water bath at 40 °C. Then, it was filled—total volume 5 ml—with the leaching carrier (water at pH=10), impulsed by the peristaltic pump through the circuit in the closed position. The leaching carrier was then circulated through the solid sample for 2 min under ultrasound irradiation (duty cycle 0.8 s, output amplitude 70% of the nominal amplitude of the converter, applied power 100 W with the probe placed at 1 mm from the top surface of the extraction cell). During extraction, the direction of the leaching carrier (at 1.25 ml/min) was changed each 30 s, thus minimising both dilution of the extract and increased compactness of the sample in the extraction cell that could cause clogging of the system.

2.6.2. Clean up-preconcentration step

After complete extraction, the selection valve was switched and the content of the closed circuit was led to the filtration-preconcentration manifold. The extract was firstly cleaned from particles by passage through a filter and the filtrate driven to a sorption column for analytes retention. The column was in the loop of an injection valve (IV_1), thus enabling

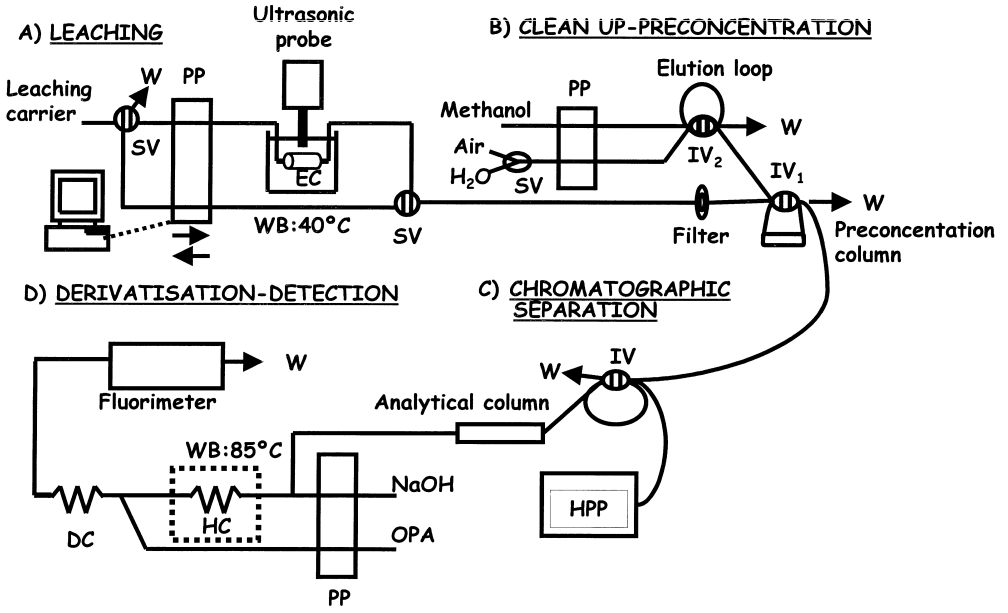

Fig. 1. Experimental setup of the fully automated approach. W, waste; SV, selection valve; PP, peristaltic pump; EC, extraction chamber; WB, water bath; IV, injection valve; HPP, high pressure pump; HC, hydrolysis coil; DC, derivatisation coil.

elution in the direction opposite to retention with a volume of methanol selected using valve IV_2.

2.6.3. Chromatographic separation step

The eluate from the sorption column was driven to the loop of the high-pressure injection valve of the chromatograph using as carrier an air stream at 1.3 ml/min. Between samples and during the chromatographic separation, the filter was changed and the sorbent in the column was conditioned with 5 ml methanol and 5 ml water at 1.5 ml/min. Separation of the *N*-methylcarbamates used a methanol–water gradient at a flow-rate of 0.8 ml/min. The gradient program was as follows: a 40:60 methanol–water mixture as initial mobile phase; two linear gradients to reach, first, a 50:50 methanol–water composition in 5 min and, then, a final 100% methanol composition in 18 min more. After separation, the derivatisation step started in the post-column flow manifold.

Different mixtures of methanol–water and different gradients were checked for separation of the analytes by the Ultrabase C_{18} column. The best separation was achieved using the gradient program given above. The influence of the flow-rate of the mobile phase was studied in the range 0.6–1.2 ml/min. Flow-rates higher than 0.8 ml/min caused overlapping of the analyte peaks, so 0.8 ml/min was selected as the value providing separation in a shorter time. A 30-μl injection volume was selected in order to obtain a quantifiable fluorimetric signal.

2.6.4. Derivatisation-detection step

The effluent from the chromatograph was merged with a solution of NaOH and driven to a reactor placed into a water bath at 85 °C for the analytes hydrolysis (higher temperatures resulted in the formation of bubbles that disturbed the analytical signal); then, merged with an OPA stream and the mixture led to a reaction coil. Finally, fluorescence detection was performed at excitation and emission wavelengths of 340 and 445 nm, respectively.

To simplify the optimisation of the flow-rates, it was decided that the flow-rates of NaOH and OPA streams should be equal. Better signals were achieved as the flow-rate decreased, so the lowest value tested, 0.25 ml/min, was selected for further experiments (lower flow-rates are not precisely established by the impulsion system used).

3. Results and discussion

3.1. Optimisation of the preconcentration step

The variables affecting the preconcentration step and the ultrasound-assisted leaching itself are optimised. The ranges studied and the optimal values found for all variables are shown in Table 1. The optimal working conditions obtained provided the chromatogram in Fig. 2, where the I.S. peak appears after the analyte peaks. This unusual location for an I.S. was preferred because of the short retention time of the first eluted analyte (oxamyl). Maximum peak area was the criterion choose for optimisation.

The preconcentration step was studied using 5 ml of a standard solution containing 1 μg of each analyte to reproduce the volume of extract and concentration of the analytes obtained when quantitative extraction occured.

The flow-rate of the extractant was optimised by impulsing the standard solution through the minicolumn at flow-rates from 0.4 to 1.7 ml/min. The results showed an increase of the retention when the flow-rate decreased from 1.7 to 0.9 ml/min, thus demonstrating the influence of the retention kinetics. A flow-rate of 0.9 ml/min was selected for subsequent experiments.

The volume of eluent was optimised taking into account both the elution kinetics and the minimum elution volume—the latter in order to obtain the highest preconcentration factor. A volume of 0.25 ml circulated at 1.3 ml/min was selected as optimum. The eluent carrier was air, thus avoiding dilution of the analytes.

The breakthrough of the C_{18}-hydra minicolumn was determined with sample volumes in the range 5–30 ml containing 1 μg of each analyte. The signal remained constant up to 15 ml and decreased for higher volumes.

3.2. Optimisation of the continuous ultrasound-assisted leaching step

The variables optimised in the leaching step were

Table 1
Ranges assessed and optimal values for the variables in all steps

Step	Variable	Tested range	Optimum value
Leaching	Probe position (mm)	1–20 (3)[c]	1
	Radiation amplitude (%)	10–70 (3)	70[d], 30[e]
	Percentage of duty cycle (s)	0.2–0.8 (3)	0.8
	Extractant flow-rate (ml/min)	0.25–1.25 (3)	1.25
	Extractant pH	3–12 (5)	12
	Temperature (°C)	10–50 (5)	40
	Sonication time (min)	2–6 (3)	2
Preconcentration	Q[a] retention (ml/min)	0.6–1.2 (3)	0.9
	Q elution (ml/min)	0.8–1.8 (3)	1.3
	V eluent (ml)	0.25–0.5 (2)	0.25
Derivatisation-detection	Q[b] NaOH (ml/min)	0.25–0.5 (3)	0.25
	Q OPA (ml/min)	0.25–0.5 (3)	0.25
	NaOH concentration (M)	0.02–0.04 (3)	0.03
	OPA concentration (M)	0.4–0.8 (3)	0.625

[a] Volume.
[b] Flow-rate.
[c] Number of points tested.
[d] Soil.
[e] Fruit and vegetables.

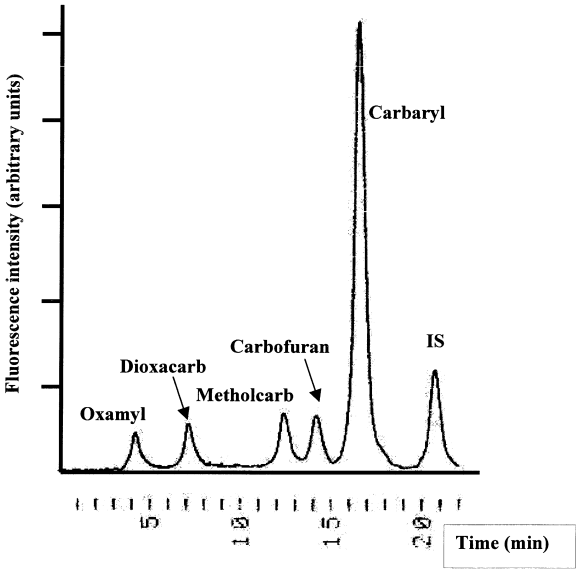

Fig. 2. Chromatogram of *N*-methylcarbamates from clayely soil (spiked with 1 μg/g each carbamate) under the optimal working conditions obtained from optimisation study. I.S.; internal standard.

the ultrasound radiation amplitude, the probe position (measured as the distance between the tip horn of the ultrasonic probe and the top surface of the extraction cell), the percentage of duty cycle of ultrasound exposure, the extractant flow-rate, the extractant pH, the extraction time and the temperature of the water bath in which the extraction cell is immersed. Water Milli-Q at different pHs was selected as leaching agent. The optimisation was performed with the clayey soil, unless stated otherwise.

The selection of a half-fraction 2^{7-2} type V resolution design allowing 13 degrees of freedom involved 32 randomised runs plus three centered points was built for a screening study of the behaviour of the main factors affecting the extraction process. The upper and lower values given to each factor were selected from the available data and experience gathered in the preliminary experiments.

The conclusions of this first screening study were that the probe position, the extractant flow-rate and the extraction time were factors not statistically influential at 95% confidence level in the ranges under study. However, the results showed better recoveries with the lower value tested for both the probe position and the extraction time—1 mm and 2

min, respectively—and with the upper value tested for the extractant flow-rate—1.25 ml/min. Thus, these values were selected for subsequent experiments. The other variables—namely radiation amplitude, duty cycle, temperature of the water bath and extractant pH—were factors statistically influential at 95% confidence level for the removal of *N*-methylcarbamates. Higher values for both temperature and extractant pH and lower values for both radiation amplitude and percentage of duty cycle were tested using a two-level full factor design involving 16 randomised runs plus three centered points. In this case, both the radiation amplitude and the percentage of duty cycle were factors not statistically influential in the ranges studied. However, the upper values tested for both, the percentage of duty cycle (0.8 s) and the radiation amplitude (70% of the nominal value of the converter) were selected for further experiments as they provided better recoveries. Analysing the design for temperature and extractant pH, which were the factors that showed a maximum of the response surface, a second-order polynomial equation was obtained. The optimal values were obtained by equalising to zero the first derivate of the polynomial. Optimum values of 40 °C and 10, for the bath temperature and extractant pH, respectively, were obtained and used for subsequent experiments.

Apple for optimisation of food extraction and the optimal values obtained for the clayely soil were assayed. The same values of all variables were selected, except for the radiation amplitude (30% of the nominal value of the converter, in this case). For radiation amplitude higher than 30%, the percentage extraction of the carbaryl was below 60%.

3.3. Validation of the method

3.3.1. Characterisation of the method

Five calibration curves were obtained using nine individual standard solutions of *N*-methylcarbamates, with correlation coefficient, r^2, better than 0.998. The linear concentration range of the calibration curve was 0.5–10 μg/ml for all analytes but carbaryl that was 0.03–5 μg/ml. The standards were injected in triplicate in all instances. The internal standard method was used for calibration and analysis of the samples.

To evaluate the precision of the proposed method, within-laboratory reproducibility and repeatability were estimated in a single experimental setup with duplicates [26], using the clayey soil and the optimal values of the variables. Two measurements of *N*-methylcarbamates signal per day were carried out on 7 days. The repeatability, expressed as RSD, was 3.1%; meanwhile, the RSD for within-laboratory reproducibility was 7.5%.

The detection and quantification limits can be obtained from the noise of the analytical signal as the concentration of analyte which gives a signal to noise is 3 and 10, respectively. The detection and quantification limits were 12 and 40 ng/g, respectively, for all analytes but for carbaryl (whose detection and quantification limits were 3 and 10 ng/ng, respectively). The detection and quantification limits were the same for soil and food samples.

3.3.2. Application

The proposed method was applied to five soil samples (spiked with 1 μg/g each carbamate) from different origin and the results were compared with those from the EPA method 8318. Table 2 shows the extraction efficiency for each analyte and the value of f (proposed method recovery/EPA method recovery). Similar recoveries were provided by both methods, the f value ranging between 0.97 and 1.16.

The proposed method was also applied to apple, pear and cucumber samples spiked with 1 μg/g of each carbamate (Table 3). The results show that the proposed method is a good choice for these matrices. The recoveries were higher than 94% for all analytes, except for carbaryl (85%).

4. Conclusions

The proposed method allows extraction of carbamate pesticides from soil and food with good recoveries, similar to those provided by both the EPA 8318 method and Ref. [19], in the case of soil, and Ref. [20] in the case of foods, without degradation of the target compounds during the extraction and with drastic shortening of the time required for this step (from ≈4 h of EPA method, 2.5 h of Ref. [19] and 50 min of Ref. [20], to 2 min).

Table 2
Comparison of the proposed and EPA methods

Sample (soil)	Analyte	Proposed method (μg/g)	EPA method 8318 (μg/g)	f [a]
Organic	Oxamyl	0.77[b]	0.78[c]	0.99
	Dioxacarb	0.86	0.80	1.08
	Metolcarb	0.87	0.87	1.00
	Carbofuran	0.87	0.86	1.16
	Carbaryl	0.91	0.92	0.99
Clayely	Oxamyl	0.80	0.79	1.01
	Dioxacarb	0.89	0.81	1.10
	Metolcarb	0.91	0.89	1.02
	Carbofuran	0.91	0.91	1.00
	Carbaryl	0.95	0.97	0.98
Slimy	Oxamyl	0.84	0.85	0.99
	Dioxacarb	0.92	0.85	1.08
	Metolcarb	0.93	0.88	1.06
	Carbofuran	0.92	0.93	0.98
	Carbaryl	0.96	0.95	1.01
Limy	Oxamyl	0.83	0.83	1.00
	Dioxacarb	0.92	0.87	1.06
	Metolcarb	0.93	0.90	1.03
	Carbofuran	0.93	0.96	0.97
	Carbaryl	0.94	0.96	0.98
Sandy	Oxamyl	0.78	0.77	1.01
	Dioxacarb	0.88	0.86	1.02
	Metolcarb	0.88	0.89	0.99
	Carbofuran	0.90	0.89	1.01
	Carbaryl	0.95	0.96	0.99

[a] Proposed method recovery/EPA method recovery.
[b] (0.03–0.05) standard deviation for the proposed method, $n=3$.
[c] (0.01–0.04) standard deviation for the EPA method, $n=3$.

The main advantages aspects of the proposed method are as follows:

The change of the extractant direction during the extraction avoids both overpressure problems caused by increased compactness of the sample in the extraction chamber and dilution of the extract. This is the first time that a method for the extraction of carbamates from soil and food based on the use of ultrasounds as an auxiliary energy to accelerate the leaching process has been proposed.

Table 3
Recoveries of *N*-methylcarbamates from different types of food (spiked with 1 μg/g of each carbamate)

Compound	Pear	Apple	Cucumber
Oxamyl	97±3[a]	98±3	97±1
Dioxacarb	101±2	95±2	96±3
Metolcarb	99±3	99±2	98±2
Carbofuran	100±3	97±2	99±2
Carbaryl	87±3	85±2	89±3

[a] Percentage of *N*-methylcarbamates±standard deviation for $n=3$.

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