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Study of the feasibility of focused microwave-assisted Soxhlet extraction of *N*-methylcarbamates from soil

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Abstract

A study of the feasibility of a focused microwave-assisted Soxhlet device for the extraction of *N*-methylcarbamates from soil has been performed. The main factors contributing to the extraction efficiency—namely microwave power, irradiation time and number of cycles—were optimized by means of a two-level full factorial design. The extracts were analysed by HPLC–post-column fluorescence derivatization–detection at excitation wavelength 340 nm and emission wavelength 445 nm. The method has allowed the extraction of carbamate pesticides from contaminated soil with quantitative recoveries, similar to those provided by the US Environmental Protection Agency method 8318, without degradation of the target compounds during the extraction and using less organic solvent, as 75–80% of the extractant was recycled. © 2002 Elsevier Science B.V. All rights reserved.

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1. Introduction

N-Methylcarbamates represent an important category of pesticides since their introduction into the agrochemical market in the 1950s. They, together with organochlorine and organophosphorous compounds, act as inhibitors of acetylcholinesterase and the main hazards that they present relate to short-term toxicity. The stability of these compounds is low since they are readily degradable by chemical (hydrolysis), physical (light, temperature) and biochemical (microbial degradation) agents in the soil and, therefore, they do not usually raise problems of persistence. However, according to the capability of the soil retention, these compounds can remain in it for variable periods.

N-Methylcarbamates are hydrolysed by NaOH at elevated temperatures to yield methylamine, which subsequently reacts with *o*-phthalaldehyde (OPA) at high pH to produce a highly fluorescent isoindole. The use of these reactions for a two-step, post-column derivatization of *N*-methylcarbamates was first reported by Moye et al. [1]. This procedure has been usually applied for the determination of these compounds in water samples and adopted as an official method for drinking water by the US Environmental Protection Agency (EPA) [2].

The technique used for the extraction of *N*-methylcarbamate pesticides in solid samples plays a crucial role in the analytical process performed for

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determining these compounds due to their thermal lability. Well-established solid–liquid extraction techniques such as Soxhlet extraction cannot be used as the long heating periods in the Soxhlet flask cause degradation of these analytes. Alternative techniques such as supercritical fluid extraction [3–5] or pressurised solvent extraction [6] have been used for the removal of these compounds from fruits and foods principally. However, the recoveries obtained have been poor due to the fact that drastic conditions cannot be used in order to preserve the structure integrity of these pesticides. As far as we know, only supercritical fluid extraction has been used for the extraction of these compounds from soil [3] but the recoveries obtained from soils aged for 24 h, after 30 min of dynamic extraction were lower than 50%. In 1994, the EPA adopted a manual shaking method as reference [7] for the determination of *N*-methylcarbamates in soil, which remains nowadays in use.

Since 1975, when Abu Samra et al. [8] first demonstrated the use of microwave energy as a heat source in wet ashing procedures, several authors have applied microwave energy to accelerate a wide range of solid sample treatments such as digestion [9,10], extraction [11,12], distillation [13] and sample drying [14], among others. In 1998, Luque de Castro et al. [15] developed a new device (called focused microwave-assisted Soxhlet extractor [16]) with the aim of overcoming the main drawbacks of conventional Soxhlet extraction (long extraction time and high organic solvent consumption), but maintaining its advantages (sample fresh solvent contact during the whole extraction step, no filtration required after extraction, easy manipulation, well-known procedures and a large experience in the extraction field for more than a century). The use of this device has provided better results than conventional Soxhlet in shorter periods of time for the extraction of different pollutants such as polycyclic aromatic hydrocarbons (PAHs) [17], acid herbicides [18] and dioxins [19] and also as an alternative to traditional methods for lipid extraction from different foods [20,21].

Based on the shortening of the extraction time achieved using this device, and thus, in the reduction of the residence time of the analytes into the distillation flask, which is the cause of degradation of the *N*-methylcarbamates during Soxhlet extraction, this paper deals with the study of the feasibility of the focused microwave-assisted Soxhlet for the extraction of thermolabile compounds such as *N*-methylcarbamates from soil. The efficacy of the extraction was compared with that obtained by the reference EPA method 8318.

2. Experimental

2.1. Instruments and apparatus

A conventional Soxhlet extractor was modified in order to facilitate accommodation of the sample cartridge compartment in the irradiation zone of a Microdigest 301 device of 200W maximum power (Prolabo, France). The latter was also modified: an orifice at the bottom of the irradiation zone enabled connection of the cartridge zone to the distillation flask through a glass siphon; an electrical isomantle (Prolabo) was used for heating the content of the distillation flask; a microprocessor programmer was used to control the microwave unit. Cellulose extraction thimbles (25×88 mm, Albet, Barcelona, Spain) were used to put the soil sample. Fig. 1 illustrates the operation of the overall extraction device.

A Soxhlet chamber (Afora, Seville, Spain) of 50

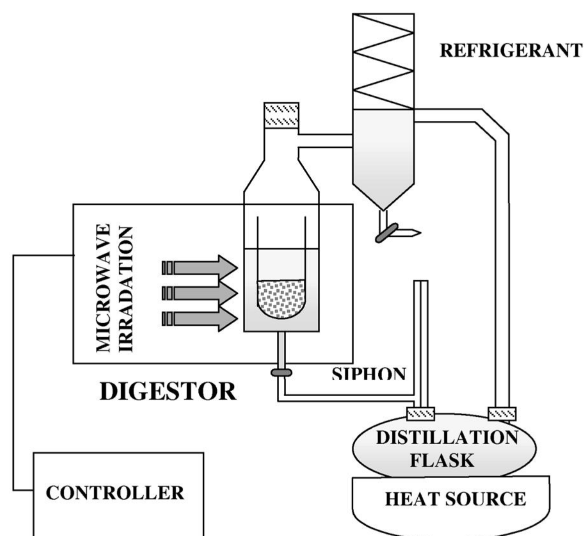

Fig. 1. Scheme of the prototype operation.

ml and the same electrical isomantle was used for the stability study.

The individual chromatographic separation step was performed by an HP1100 liquid chromatograph (Hewlett-Packard, Avondale, PA, USA) consisting of a G1311A high-pressure quaternary pump, a G1322A vacuum degasser, and a Rheodyne 7725 high-pressure manual injector valve (20 μl injection loop). An Ultrabase C_{18} (250×4.6 mm, 5 μm particle size, Scharlau) was the analytical column.

A low-pressure peristaltic pump (Gilson Minipuls-3, Worthington, OH, USA) was used as propelling system for both the alkaline and OPA solutions, at 0.5 ml/min each, which were used for post-column fluorescence derivatization of the analytes. Alkaline hydrolysis of the compounds was performed in a mixing coil (86.6 cm×0.5 mm I.D.) located in a water bath (Tectron bio P-Selecta, Barcelona, Spain) at 100 °C. A reaction coil (65 cm×0.5 mm I.D.) was also used for derivatization 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 (Model F-1050 Hitachi, Tokyo, Japan) at an excitation wavelength (λ_{ex}) of 340 nm and an emission wavelength (λ_{em}) of 445 nm. Chromatograms were obtained and integrated using a chromato-integrator (D-2500 Hitachi, Merck).

2.2. Reagents and chemicals

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 the stock standard solutions by dissolving the required amount of each pesticide in the minimum volume of HPLC-grade acetone (Panreac, Barcelona, Spain) necessary for total dissolution and diluted with HPLC-grade methanol (Panreac).

The NaOH solution (0.03 *M*) was prepared using sodium hydroxide (Panreac) in Milli-Q water. The stock OPA solution (0.625 m*M*) was prepared by dissolving *o*-phthalaldehyde (Sigma) in methanol–boric acid buffer (1:4) and adding an appropriate amount of thiolactic acid (Aldrich). The buffer was prepared by dissolving 12.5 mmol of boric acid (Panreac) in 1 l methanol–water (1:4). The derivatization reagents were prepared daily and stored at 4 °C until used.

2.3. Procedure for soil contamination

It is well known that the efficacy of an extraction procedure depends on the type of matrix in which the analytes are retained and even more, on the aged of the sample. A procedure of soil contamination was carried out in order to reproduce as close as possible the real contamination conditions and the behaviour of the target compounds under natural conditions. A 500-g amount of clayey soil was sieved to a size smaller than 1 mm and spiked with the *N*-methylcarbamates by adding to the soil 300 ml of ether containing the necessary volume of stock standard solutions of the target analytes to obtain a final total concentration in the dry soil of 50 mg/kg (10 mg/kg of each carbamate). Then, the slurry was shaken for 72 h, and, after evaporation of the solvent, the soil was completely dried under an N_2 stream. Finally, the soil was put into a holder and subjected to environmental conditions for 3 months in order to simulate natural conditions and, after this time, the soil was homogenized and stored at 4 °C in the dark until used.

2.4. Soil homogeneity and stability studies

In order to evaluate the homogeneity of the soil, different amounts of soil were subjected to focused microwave-assisted Soxhlet extraction (FMASE) procedure based on the experience gathered in the preliminary experiments. Amounts ranging from 10 to 0.5 g were tested, each in triplicate. The results showed similar recoveries with an average relative standard deviation <3.5% for amounts between 10 and 1.0 g. For amounts <1.0 g the recoveries obtained were slightly different with an average relative standard deviation <10%. In order to avoid errors of lack of sample homogeneity an amount of 1.5 g was selected to carried out the subsequent experiments.

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 the time with an average relative standard deviation $<4\%$, thus demonstrating the stability of the soil during the overall research.

2.5. EPA method 8318

The EPA method 8318 for the determination of *N*-methylcarbamates by HPLC consists of three different steps: extraction, solvent exchange and analysis.

(a) Extraction: 20 g of dry sample was weighed and put into a 250-ml Erlenmeyer, 60 ml of hexane was added and the slurry was shaken for 1 h. Then, 50 ml of acetonitrile was added and the slurry was shaken for 3 h. After this time, the extract layers were decanted to a 250-ml separation funnel. The bottom acetonitrile layer was filtered into a 100-ml volume flask and the extraction was repeated with the same volumes of hexane and acetonitrile. The second extract layer was decanted on top of the first hexane layer. The funnel was shaken and the bottom layer was filtered into the same 100-ml volume flask and diluted to the mark.

(b) Solvent exchange: 15 ml of the extract was eluted through an acetonitrile pre-washed C_{18} cartridge and only the latter 13 ml was collected. A 10-ml volume of clean extract was mixed with 100 μl of ethylene glycol in a glass vial. The mixture was blown down in a heating block at 50 °C and N_2 was passed in order to remove the solvent. 1 ml of methanol was added to the residue.

(c) Sample analysis: the conditions used to carry out the chromatographic analysis were the same used in the proposed method. A fluorescence post-column derivatization reaction was performed to determine the analytes.

2.6. Proposed method

2.6.1. Extraction/leaching

A 100-ml volume of acetonitrile was poured into a distillation flask. A 1.5-g amount of dry soil sample was put into a cellulose extraction thimble, which was covered with cotton wool and inserted into the quartz extraction vessel placed in the microwave-extraction zone. The distillation flask was positioned on an electrical isomantle and connected to the sample vessel by a siphon and a distillation tube. After the extraction step (16 cycles, 20 s microwave irradiation at 100% of power each cycle), the extractant was collected in a reservoir by actuating a valve, which switched between the usual way of distilling in the Soxhlet extractor and the reservoir. A 75–80 ml volume of the solvent was thus recovered. The extract was completely dried under an N_2 stream and the residue was recomposed by adding 1 ml of HPLC-grade methanol.

2.6.2. Chromatographic separation

The HPLC separation of the *N*-methylcarbamates was performed using a methanol–water gradient at a flow-rate of 0.8 ml/min. The gradient program was as follows: a methanol–water (18:82) mixture was used as initial mobile phase. Two linear gradients were established in order to reach first, a 40:60 composition in 10 min and then, a final 70:30 composition in a further 20 min. Finally, 5 min was necessary for reestablishing the initial conditions. After separation, the derivatization step was started in the post-column flow manifold.

2.6.3. Post-column fluorescence derivatization–detection

The effluent from the chromatograph was merged with a solution of NaOH and was driven to a reactor placed into a water bath at 100 °C in order to hydrolyse the *N*-methylcarbamate pesticides. After hydrolysis, the effluent was merged with an OPA stream and the mixture was led to a reaction coil as shown in Fig. 2. Finally, fluorescence detection was performed at excitation and emission wavelengths of 340 and 445 nm, respectively. The flow-rate of the post-column derivatization reagent streams, NaOH and OPA, was 0.25 ml/min each.

Quantitation of the analytes was carried out by running five calibration curves (one for each analyte) using standard solutions between 1 and 10 mg/l.

3. Results and discussion

3.1. Chromatographic conditions

The optimal working conditions summarized in Table 1 provide the chromatogram in Fig. 3. Overlapping of the peaks corresponding to metolcarb,

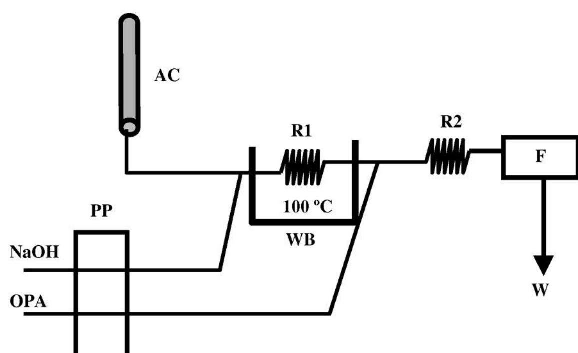

Fig. 2. Flow injection manifold for the post-column fluorescence derivatization–detection. PP, Peristaltic pump; AC, analytical column; R, reaction coil; WB, water bath; F, fluorimeter; W, waste.

carbofuran and carbaryl occurs at higher flow-rates. The 20-μl injection volume was selected in order to obtain a quantifiable fluorimetric signal.

3.2. Optimization of the post-column derivatization

A multifactorial design methodology for optimization, based on a half-fractioned factorial design 2^{4-1} type IV resolution allowing three degrees of freedom and involving eight randomized runs plus three centered points, was built for a screening study of the behaviour of the main factors affecting this step—namely, the flow-rates and the concentration of the NaOH and OPA solutions. Optimization of the length of the hydrolysis and derivatization reactors was not necessary as they are closed related with the flow-rate.

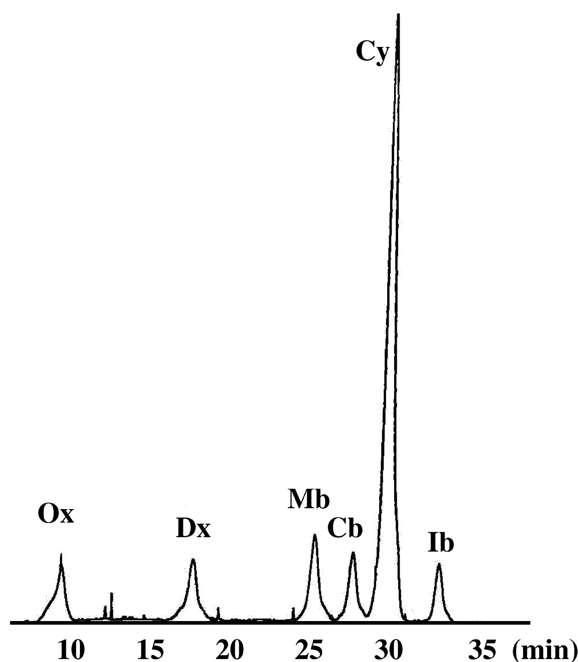

Fig. 3. Chromatogram for 10 mg/l calibration solution of *N*-methylcarbamate pesticides. Peaks: Ox=oxamyl, Dx=dioxacarb, Mb=metolcarb, Cb=carbofuran, Cy=carbaryl, Ib=isocarb.

The experimental results obtained showed that better signals were obtained as the flow-rate of the

Table 1
Optimization of variables

Step	Variable	Tested range	Optimum value
Chromatographic separation	Injection volume (μl)	–	20
	Flow-rate (ml/min)	0.6–1.2	0.8
	Elution	–	See text
Post-column derivatization–detection	Q^{*}_{NaOH} (ml/min)	0.25–0.46	0.25
	Q_{OPA} (ml/min)	0.25–0.46	0.25
	NaOH concentration (*M*)	0.01–0.15	0.03
	OPA concentration (m*M*)	0.125–0.875	0.625
	λ_{ex} (nm)	–	340
	λ_{em} (nm)	–	445
FMASE	Power of microwaves (W)	100–200	200
	Irradiation time (s)	20–90	20
	Number of cycles	6–20	16

* Flow-rate.

NaOH and OPA solutions decreased, so the lowest value tested, 0.25 ml/min, was selected for further experiments (lower flow-rates are not precisely established by the propulsion system used). The second stage of the optimization procedure was to propose a new full factorial design where higher concentrations of the NaOH and OPA solutions were tested. In this case, the results showed that the highest fluorescence signals were obtained using intermediate values, 0.03 *M* and 0.625 m*M* for the NaOH and OPA solutions, respectively, which were selected for subsequent experiments.

The optimum temperature of the water bath where the hydrolysis of the analytes took place was 100 °C.

3.3. Selection of the extractant and degradation study

Four solvents (namely, water, acetonitrile, *n*-hexane and dichloromethane) were tested as extractants. The water cannot be used as leaching agent since the carbamates are rapidity hydrolysed into hot water, so an organic solvent must be used. The highest recoveries were obtained using acetonitrile.

In order to evaluate the grade of degradation of the analytes extracted in the distillation flask, 100 ml of acetonitrile with an amount of 10 μg of each analyte was put under reflux for different periods of time ranging from 1 to 8 h. After the preset time, the solvent was recycled in the same way as in the FMASE procedure and the residue was recomposed with 1 ml of methanol and injected into the liquid chromatograph. The results showed that for 4 h the analytes remain undegraded, but for longer times, the degradation of the analytes increased with increased reflux time.

3.4. Optimization of the focused microwave-assisted Soxhlet extraction

The variables optimized in the leaching step were the power of microwaves, the irradiation time and the number of cycles needed for total extraction of the target compounds. The overall optimization procedure was developed as follows: a first full factorial design was built for a screening study of the behaviour of the main factors and interactions within the domain imposed by the limits. The power of microwaves was tested between 50 and 100% of the power provided by the microwave device. The irradiation time was studied in the range 20–90 s and the number of cycles between 6 and 12. 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 the study are that both the power of microwaves and the number of cycles are the most influential factors on the extraction step, their increase having a positive effect on the analytes recovery; so higher values of both factors should be tested. In the case of the microwave power, the maximum value tested was the highest value provided by the device (200 W), which was selected as optimum. The irradiation time was not a significant factor for any of the compounds. However, the results showed better recoveries with shorter irradiation times so the minimum value tested, 20 s, was selected for further experiments. Finally, the results indicated an increase in the number of cycles in order to obtain better recoveries. Cycles ranging from 12 (the upper value tested previously) to 20 were performed. The analytes recovery increased when the number of cycles increased from 12 to 16 and remains constant up to 20 cycles. Thus, 16 cycles was selected as optimum value for complete removal of the target pesticides.

3.5. Study of the extraction kinetics

A 4-g amount of the contaminated soil was used to study the kinetics of the extraction process and the extract obtained in each cycle was removed from the distillation vessel and quantified by HPLC–post-column derivatization–fluorescence detection. The results obtained showed that the five pesticides are completely extracted after the 16th cycle. Differences between the extraction kinetics of the pesticides are shown in Fig. 4.

3.6. Influence of fresh extractant presence

One of the main advantages of the focused microwave assisted Soxhlet extractor over commercial microwave assisted extractors is the possibility—characteristic of Soxhlet—of putting the sample into contact with fresh solvent in each cycle, thus

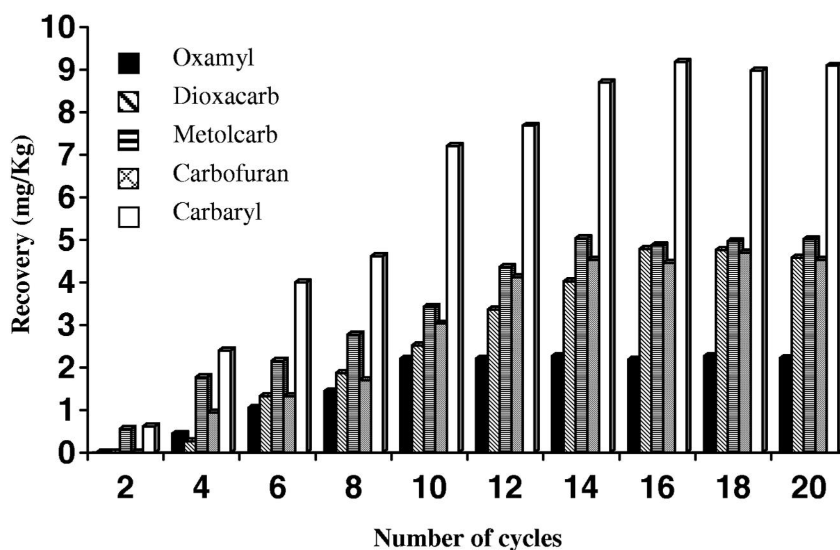

Fig. 4. Kinetics of extraction.

displacing partition of the analytes between the matrix and the extractant. In order to determine the influence of solvent re-cycling for the analyte removal, the proposed method was compared with the Soxwave method in which the sample was subjected to 320 s of continuous microwave irradiation, which was the total time at which the sample is under microwave irradiation in the FMASE method. The power used in the Soxwave method was also the same as in the FMASE method (200 W). The recoveries using the Soxwave method were lower than 10% of the recoveries provided by the proposed FMASE (expressed as percent of μg pesticides by Soxwave/μg of pesticides by FMASE), thus indicating either poor extraction efficiency of the Soxwave method or analytes degradation when subjected to continuous microwave irradiation. In order to discriminate between the two possible effects, the Soxwave method was applied to a pesticide standard solution. The results showed no degradation of the analytes under continuous microwave irradiation for 320 s at 200 W, thus demonstrating that the highest recoveries yielded using FMASE are due to re-cycling of the solvent.

3.7. Evaluation of the precision of the method

The precision of the proposed method (FMASE plus chromatographic–fluorescence detection steps) was studied by application of the whole analytical process to seven replicates of the same sample in different days, obtaining relative standard deviations ranging between 2.34 and 7.53%.

3.8. Comparison of the proposed method with the EPA method 8318

The FMASE method was compared with the EPA method 8318 in terms of efficiency. Table 2 shows the average recoveries obtained by both methods for each analyte and the value of f (FMASE recovery/EPA method recovery). As can be seen, similar recoveries were provided by both methods. Only for carbofuran, the recovery of the EPA method was

Table 2
Comparison of FMASE and EPA method

Analyte	Recovery (mg/kg)		f [b]
	FMASE method (n=7)	EPA method (n=5)	
Oxamyl	2.39±0.18[a]	2.42±0.28	0.99
Dioxacarb	4.71±0.11	2.43±0.16	1.94
Metolcarb	5.05±0.25	4.88±0.13	1.03
Carbofuran	4.50±0.22	5.14±0.27	0.88
Carbaryl	9.05±0.25	9.06±0.16	0.99

[a] Standard deviation.
[b] FMASE recovery/EPA method recovery.

slightly better; meanwhile in the case of dioxacarb the recovery provided by the FMASE was twofold higher than that provided by the EPA method.

4. Conclusions

The feasibility of FMASE as a prior step to the determination of *N*-methylcarbamates (namely, oxamyl, dioxacarb, metolcarb, carbofuran and carbaryl) in soil samples has been here demonstrated. FMASE is a good alternative for the extraction of these *N*-methylcarbamate pesticides without degradation, saving both time [2.5 h (9 min for each cycle, 16 cycles) vs. ≈6 h required for the EPA method] and organic solvent (75–80% of the extractant is recycled in FMASE), and avoiding manual and tedious operations. The recoveries were similar to those obtained by the EPA method and much better than those provided by the SFE previously reported [3] which ranged between 39.6 and 91.7%.

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