Focused Microwave Assistance for Extracting Some Pesticide Residues from Strawberries into Water before Their Determination by SPME/HPLC/DAD

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A novel and simple method for the determination of some pesticide residues in strawberries using both focused microwave-assisted extraction (FMAE) and solid-phase micro extraction (SPME), coupled with high-performance liquid chromatography (HPLC), has been developed. The pesticides were first extracted from strawberries with water and the assistance of focused microwaves at 30 W for 7 min. Then, an aliquot of the resulting aqueous extract was subjected to SPME with a 60-μm thick poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fiber for 45 min at room temperature, with the solution being stirred at 1000 rpm. The extracted pesticides on the SPME fiber were desorbed into the SPME/HPLC interface for quantitative analysis with a diode array detector (DAD). The whole sample pretreatment procedure before chromatographic analysis did not use any organic solvents or involve any blending or centrifugation steps. The five compounds (carbendazim, diethofencarb, azoxystrobine, napropamide, and bupirimate) were chosen because they cannot be analyzed easily by GC. The efficiency of this relatively fast procedure was comparable to that of previously reported methods, with detection limits at low μg/kg levels and linear responses in the range from 0.05 to 1 mg/kg of pesticide in strawberries, with RSDs between 3 and 7.3%, depending on the analyte. In all but one case results obtained by this method for field-incurred samples were comparable to those obtained with traditional methods.

Keywords: *Focused microwave-assisted extraction; solid-phase micro-extraction; pesticide residues; strawberries; high-performance liquid chromatography*

INTRODUCTION

Usually, pesticide residue analysis in food matrixes requires several procedures such as extraction, concentration, separation, and quantification. Chromatographic methods are the most commonly used techniques for the last two steps of this scheme, but traditional extraction procedures often involve the use of large volumes of organic solvents and are tedious and time-consuming (*1*−*3*).

Solid-phase micro extraction (SPME) is a recently developed sampling method (*4*). Owing to its convenience, solvent-free operation, and low cost, it is becoming more and more accepted by the scientific world. To date, SPME coupled with gas chromatography (GC) has been widely investigated for the analysis of volatile organic compounds (*5*), and it has been reported mainly for pesticide residue analysis (*6*, *7*). We also have reported pesticide residue analyses in wines (*8*, *9*) and strawberries (*10*) by SPME/GC.

However, some pesticides are nonvolatile or thermally unstable, and therefore, unsuitable for GC analysis, but they can be easily separated by high-performance liquid chromatography (HPLC). For this reason, an interface between SPME and HPLC has been recently developed (*11* ,*12*), but reports based on SPME/HPLC are currently still in their infancy.

Recently, we have reported the determination of four pesticide residues (methiocarb, napropamide, fenoxycarb, and bupirimate) in strawberries by SPME coupled with HPLC with diode array detection (DAD) (*13*), in which the strawberry samples were first blended in water and then centrifuged before SPME/HPLC analysis with a poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fiber.

On the other hand, microwave-assisted extraction (MAE) has been developed in the past few years as a new technique to facilitate the extraction of organic analytes from complex matrixes (*14*, *15*). It has been used recently for the extraction of many compounds including pesticides (*16*), polynuclear aromatic hydrocarbons (PAHs) (*17*), herbicides (*18*), and off-flavors (*19*). The rapid heat transfer provided by microwave heating can result in a much shorter extraction time for analytes in complex samples in comparison with more traditional Soxhlet or liquid extractions. Different apparatuses for this technique have been investigated. Zhu et al. (*19*) have shown that, by using closed vessels, MAE under pressure could be a substitute for conventional extraction techniques such as Soxhlet extraction.

Budzinski et al. (*20*) have demonstrated the efficiency of focused microwave-assisted extraction (FMAE) at atmospheric pressure for contaminants in environmental matrixes such as soils, sediments, and biological tissues. The advantages of using microwave focusing

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apparatus in FMAE mode include homogeneous and reproducible treatment of the sample owing to focusing of the microwaves on the medium, rapid heating to boiling temperature, and no thermal vessel inertia. In addition, the method is safe because the extraction is performed at atmospheric pressure. Nevertheless, to match the necessary separation and detection requirements, analytes in the solvent after extraction according to this procedure often need to be further concentrated and purified before chromatographic analysis.

Herein, we present the results obtained by coupling the two techniques (FMAE and SPME) for analyzing residues of five pesticides typically used for strawberry cultivation and analyzable by HPLC (carbendazim, diethofencarb, azoxystrobine, napropamide, and bupirimate). Analytes were transferred directly from the strawberry to the PDMS/DVB fiber via a pure water solution. So, blending the strawberries and centrifuging the resulting solution (*13*), which were two tedious and time-consuming steps of the previously described procedure, were avoided to simplify the approach. This combined technique, coupled with HPLC/DAD detection and quantification, was revealed to be an efficient tool for determining low level residues in artificially spiked samples as well as in field-incurred samples.

MATERIALS AND METHODS

Apparatus. A Spectra SYSTEM P1000 pump, SCM1000 vacuum membrane degasser, and UV6000LP detector were obtained from Thermo Separation Products (les Ulis, France). Ultra IBD 5-μm 250 × 4.6 mm column was bought from Restek (Evry, France). A Soxwave Map microwave oven adapted with one 250-mL long-neck quartz heating tube, was provided by Prolabo (Fontenay-sous-Bois, France). The interface between SPME and HPLC and 60-μm poly(dimethylsiloxane)/divinylbenzene (PDMS/DVB) fibers were from Supelco (St. Quentin Fallavier, France).

Reagents. Certified HPLC-grade water, methanol, and acetonitrile for UV detection were from Fisher Scientific International Company. Carbendazim (99.6%), diethofencarb (97%), and azoxystrobine (99.5%) were provided by CIL (Ste Foy la Grande, France), and napropamide (99.9%) and bupirimate (99.9%) were provided by Riedel de-Haën (Seelze, Germany).

Preparation of Solutions. About 5 mL of 1 mg/mL primary standard stock solutions were accurately prepared in acetonitrile for diethofencarb, azoxystrobine, napropamide, and bupirimate, respectively. Likewise, 5 mL of a 0.1 mg/mL standard stock solution of carbendazim in ethanol was made. The 5 solutions were stored at 4 °C. A mixture solution at the concentration of 0.05 mg/mL was freshly prepared every month by dilution of the above solutions in acetonitrile.

Strawberry Microwave-Assisted Extraction. A 25-g sample of frozen strawberries was weighed into a 150-mL beaker. Calculated aliquots of the pesticide standard mixture solution, containing 0.05 mg/mL of each of the five pesticides, were spiked onto the frozen strawberries, drop-by-drop using a pipet. To get a realistic model for naturally contaminated fruits, the spiked strawberries were extracted according to the following procedure: (1) after being kept at room-temperature overnight, the spiked strawberries were transferred from the beaker to the microwave heating tube. (2) Two aliquots of 15 mL of water each were used to rinse the beaker and transferred into the microwave heating tube. (3) The microwave heating tube was put into the microwave oven for 7 min at 30 W, and the temperature was increased up to about 65 °C. (4) After the microwave treatment, the mixture was cooled to room temperature, and the supernatant was decanted into a brown bottle and used for SPME/HPLC.

SPME Procedure. For the entire study, the 60-μm PDMS/DVB fiber and optimized working conditions were selected according to publications of the inventors (*4* and cited references therein) and other published results (*13*).

New fibers were conditioned with mobile phase until no interfering peaks appeared. Old fibers were conditioned with mobile phase for 20 min before the first use of the day. The conditioned fiber was allowed to air-dry for 1 min before each usage. A 4-mL aliquot of strawberry extract was transferred to a 5-mL Teflon-lined septum capped vial equipped with a glass-coated magnetic bar, and 50 mg of Na_2HPO_4 in powder was added which increased the pH of the solution to a value between 6 and 6.2. The SPME fiber was placed into the sample, which was stirred at 1000 rpm for 45 min. Once the extraction was completed, the fiber was withdrawn into the holder and removed from the vial. Then, the fiber was desorbed in the SPME/HPLC interface equipped with a six-port injection valve according to the procedure described by Chen and Pawliszyn (*11*) or Boyd-Boland and Pawliszyn (*21*), and immediately analyzed.

HPLC Conditions. The five pesticides were separated on an Ultra IBD 5-μm 250 × 4.6 mm column with acetonitrile/water/methanol (30:50:20, v/v) as mobile phase at a flow rate of 1 mL/min. For detection, the rise time was set at 1 s. Spectral scans were collected over the wavelength range from 200 to 360 nm in 5 nm intervals at a scan rate of 5 Hz.

Calibration Curve. A five-point calibration curve (0.05, 0.1, 0.3, 0.5, and 1 mg/kg) was made for spiked samples: the strawberry samples were prepared by adding 25, 50, 150, 250, and 500 μL of the mixed standard solution at 0.05 mg/mL, to 25 g of frozen strawberries, respectively. Spiked samples were extracted according to the previously described protocol and analyzed following the procedure described in the two previous sections.

General Remark. For each experimental condition, two strawberry samples were microwaved, and for each extracting solution, two SPME runs were performed (except for field-incurred samples for which 3 × 25 g of strawberries were microwaved and two SPME were performed from the resulting solution collected from the 3 samples). Therefore, every result given in this study is the average of four SPME runs in the case of spiked strawberries and of two SPME runs for field-incurred samples.

RESULTS AND DISCUSSION

Effect of Microwave Exposure Time on Extraction of Pesticides from Strawberries. To investigate the effect of focused microwave exposure time at constant wattage on the extraction of the pesticides in water, strawberry samples were treated under focused microwaves for 0, 3, 5, 7, 10, and 15 min. Samples had been previously spiked with the five compounds at 1 mg/kg and were immersed into water according to the described procedure. The lowest power setting (30 W) allowed by the device was used because other assays previously performed at higher power had caused a rapid decay of the sample and boiling of the solution. Then, the resulting aqueous solutions were subjected to the SPME procedure at room temperature and analyzed by HPLC/DAD as indicated. Obtained results for the five pesticides are represented in Figure 1.

As shown by these histograms, it can be seen that the signals for all 5 pesticides studied increased first and then decreased. The maximum response was reached in about 7 min. Before this duration and for these 5 compounds, it can be assumed that pesticide concentrations are increasing in the water phase because of their accelerated desorption out of the strawberries provoked by the combined effect of heat and microwaves. After 7 min of irradiation, analyte degradation probably occurred because of the increased temperature (up to 65 °C).

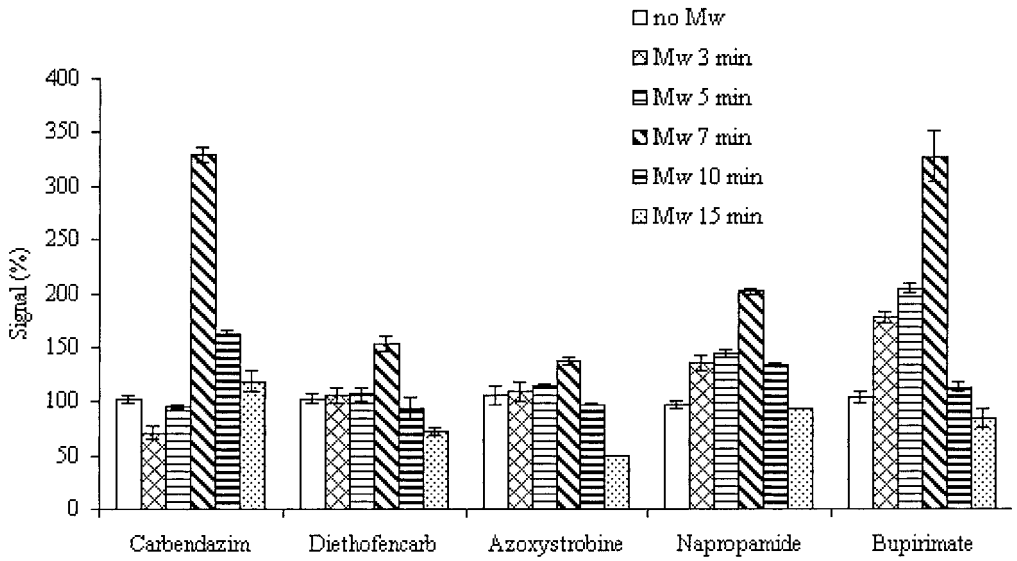

Figure 1. Effect of microwave irradiation time at 30 W on the signal obtained by SPME/HPLC/DAD from strawberries spiked at the concentration of 1 mg/kg (signals normalized to those obtained with no microwave).

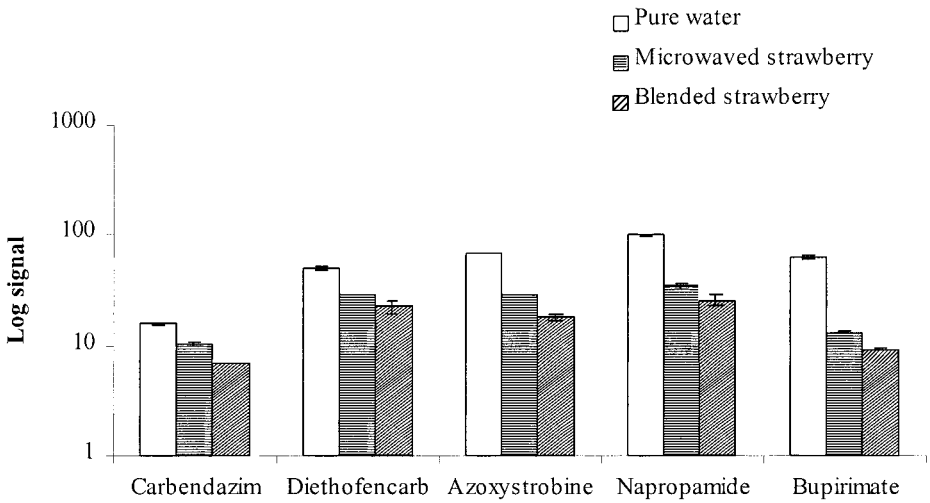

Figure 2. Comparison of signals obtained from pure water, microwaved strawberries, and blended strawberry solutions spiked at the concentration of 0.5 mg/kg.

Matrix Effect. Signals obtained from strawberries spiked at 0.5 mg/kg and analyzed by this method, and those from aqueous solutions containing the same amounts of the five pesticides in 30 mL of pure water, were compared in Figure 2. To give an idea of the relative efficiency of this method compared to the previously published method(*13*), the result obtained from a second sample spiked in the same conditions as the first one, but analyzed according to the previous procedure (spiked sample blended and the resulting suspension centrifuged before SPME/HPLC analysis), has been added for each analyte.

By comparison with the pure water solution, the strawberry-containing samples showed matrix diminishment effects for all five pesticides somewhat dependent on the nature of the pesticide. But in all cases, this effect seemed to be reduced with the FMAE method as compared to the blending method. In any case, the simultaneous partitioning of each compound between strawberry components and water, and water and the fiber in the different experimental conditions, made this effect hard to predict.

Calibration. Observed maximum absorption wavelengths for the five compounds are 207, 208, 203, 214, and 240 nm for carbendazim, diethofencarb, azoxystrobine, napropamide, and bupirimate, respectively. So, to simplify the data handling, the results were evaluated with detection at 205 and 240 nm for all five compounds although the DAD was set to monitor the whole range signal from 200 to 360 nm. Typical SPME/HPLC chromatograms of the pesticides from extraction of strawberries spiked at 0.3 mg/kg are presented in Figures 3 and 4. Most of the strawberry co-extractives were eluted before 4 min, and no interfering peaks were observed for the quantification of the five pesticides at either wavelength. In the case of carbendazim, a peak was always found in blank samples at the same retention time, but at such a low level ($s/n < 2$) that it was not significant compared to the various measured peaks relative to this compound, even at the LOD level.

A five-point calibration curve for strawberry samples was established for each pesticide. The results at both 205 and 240 nm detection are tabulated in Table 1, including the regression equation showing the relationship between the signal of the detector (y, peak area) and the concentration of the pesticide in strawberries (x, expressed as mg/kg), and the regression coefficients (r). Each curve was forced to go through the origin

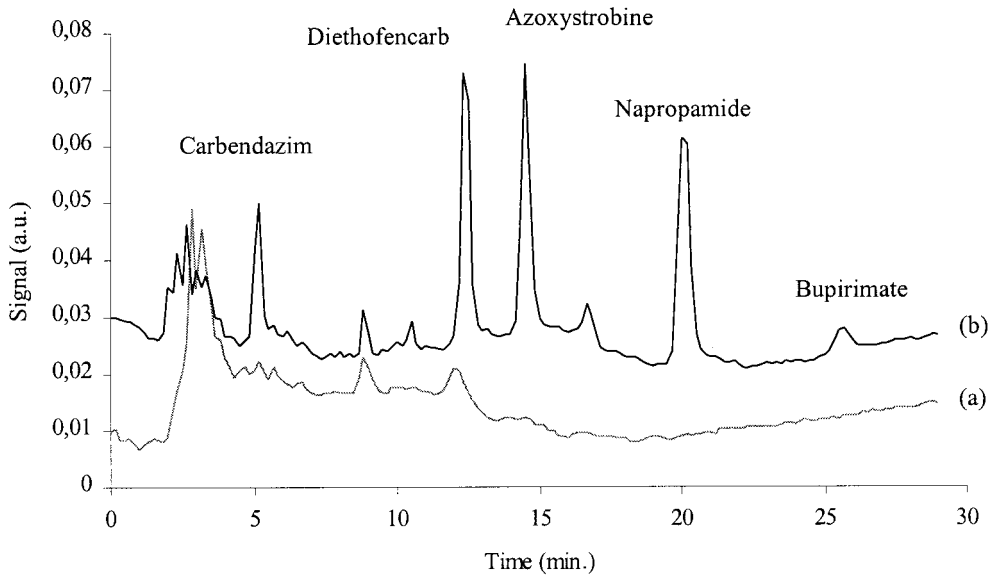

Figure 3. SPME/HPLC chromatograms from strawberry extract detected at 205 nm: (a) strawberry blank; (b) strawberry spiked at 0.3 mg/kg.

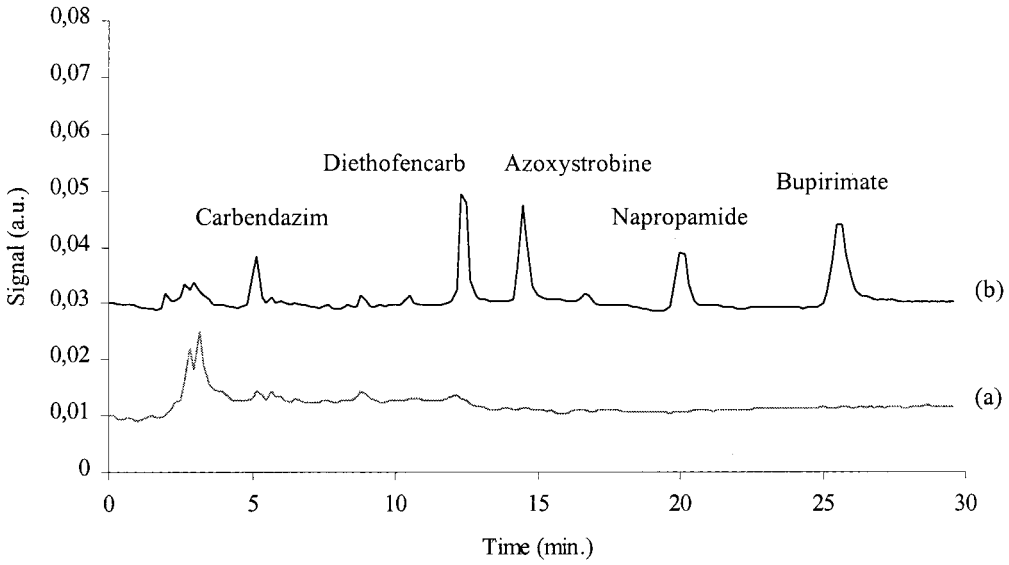

Figure 4. SPME/HPLC chromatograms from strawberry extract detected at 240 nm: (a) strawberry blank; (b) strawberry spiked at 0.3 mg/kg.

Table 1. Calibration Curve, Relative Standard Deviation (RSD), Limit of Detection (LOD), Limit of Quantification (LOQ), and French Maximum Residue Limit (MRL) Corresponding to the 5 Pesticides Analyzed

pesticide	λ	regression equation	r	RDS	LOD (mg/kg)	LOQ	MRL (mg/kg)
Carbendazim	205	$y = 1093x$	0.9994	5.8	0.022	0.074	0.100
Diethofencarb	205	$y = 3069x$	0.9977	7.3	0.018	0.060	0.500
Azoxystrobine	205	$y = 3149x$	0.9981	5.8	0.016	0.053	-[a]
Napropamide	205	$y = 4639x$	0.9964	3.0	0.013	0.067	0.100
Bupirimate	240	$y = 2331x$	0.9977	4.1	0.017	0.044	0.500

[a] Registration process for strawberry in progress.

because no significant blank sample signals existed at the retention time of the compounds under the experimental conditions. The regression coefficients were higher than 0.99, ranging from 0.05 to 1 mg/kg of these 5 pesticides in strawberries.

Limits of detection (LOD) and Quantification (LOQ). The detection and quantification limits with this procedure were defined as the concentrations of each of the pesticides in strawberries (expressed as mg/kg) that gave signals of 3 and 10 times the noise, respectively, within their retention time windows. Corresponding results are also presented in Table 1 and compared with the maximum residue limit (MRL) according to the French regulation.

Repeatability. With a single extracting solution from strawberries spiked at 0.5 mg/kg, five measurements were carried out under the same conditions. The relative standard deviations (RSDs) were 5.8, 7.3, 5.8, and 3.0% for carbendazim, diethofencarb, azoxystrobine, and napropamide, respectively, and 4.1% for bupirimate.

Table 2. Comparison of Concentrations Obtained by Using SPME and Traditional Methods from Field-Incurred Samples Coming from Experimental Assays and Commercial Production (n.a., not analyzed; LOD, limit of detection)

	experimental assay		commercial sample	
pesticide	SPME	traditional method	SPME	traditional method
Carbendazim	3.7	3.5		
	1.9	1.7		
	0.04	0.04		
	0.09	0.06		
Diethofencarb	1.8	2.07	0.66	0.73
	0.99	1.22	0.03	0.03
			0.03	0.27
Azoxystrobine	2.9	n.a.		
	0.38	n.a.		
Napropamide	< LOD	< LOD		
Bupirimate	< LOD	< LOD	0.09	0.09
			0.09	0.07
			0.19	0.21
			< LOD	0.03
			0.13	0.15

Field-Incurred Samples. To partially validate the results given by the proposed method, two types of field-incurred strawberry samples have been comparatively analyzed by the authors' laboratory (SPME method) and by a trading and certified laboratory approved by strawberry producers (traditional method). The first type concerned 6 samples of strawberries that had been cultivated in the frame of agronomic assays and treated with the five pesticides at experimental doses. The second type constituted 20 samples selected among commercial production and analyzed in blind fashion by both laboratories. Compared results are presented in Table 2.

Results obtained by using SPME were revealed to be in good agreement with those obtained by a traditional method. However, diethofencarb gave a lower SPME result in one case. Azoxystrobine, for which the registration process for strawberries is presently in progress, was not analyzed by the trading laboratory because of the lack of a described method. On the other hand, neither laboratory detected bupirimate nor napropamide that were used in the experimental assays. In commercial samples, residues of diethofencarb and bupirimate were found in the same proportion by both methods at concentration levels lower than the corresponding MRLs, except in one case where it was slightly above. Otherwise, bupirimate was analyzed by traditional method in a 5th sample and not detected by SPME, but at a concentration level very close to the corresponding LOD.

CONCLUSION

The method presented here was a successful combination of microwave-assisted extraction, SPME sampling technique, and HPLC analysis for some pesticide residues. Focused microwaves have shown to be an efficient tool for extracting pesticides from strawberries and reducing the strong matrix effects as observed in blended samples. Compared with the existing solvent extraction methods including blending and centrifuging steps, the sample preparation process described here, including microwave-assisted extraction and subsequent SPME sampling, appears to be a simple and time-saving procedure that could even be fully automated. The extracting step does not involve the use of organic solvents and therefore is environmentally friendly. In terms of sensitivity, this method, with detection limits at low μg/kg levels, can satisfy the requirements set by European and international regulations for the limits of maximum residues (MRL) which are usually at the mg/kg level for the majority of pesticides and μg/kg for some others.

Finally, this method provides an attractive approach for the determination of GC nonanalyzable pesticide residues in strawberries, especially for polar and thermally unstable compounds. Such an improvement should also be extendable to many other natural matrixes and even to other types of analytes that could be present at very low concentrations.

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