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Advanced method using microwaves and solid-phase microextraction coupled with gas chromatography–mass spectrometry for the determination of pyrethroid residues in strawberries

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Abstract

A microwave-assisted desorption method was developed and coupled with solid-phase microextraction and GC–MS for the analysis of pyrethroid residues in strawberries. In the first step, pyrethroid analytes were desorbed from the whole fruits in an aqueous acetonitrile solution at 50% under microwave assistance, so preventing these compounds to be captured with strong matrix effects by endogenous constituents. Then, the 100 μm poly(dimethylsiloxane)-coated fibre was exposed for 30 min in the obtained extracting solution. Calibration curves, realised from blank strawberries spiked at different concentrations with standards, showed a linear range between 1 μg/kg and 250 μg/kg with $r^2 > 0.992$ and variation coefficients below 15%. Limits of detection and quantitation were found lower than 14 μg/kg and 40 μg/kg, respectively. Observed analysis results by using this method and relative to field incurred strawberry samples were also compared to those obtained by two accredited trading laboratories using traditional methods.

Keywords: Food analysis; Extraction methods; Pesticides; Pyrethroids

1. Introduction

Pyrethroids are organic synthetic insecticides that are widely used for the protection of crops and food storage against insects and acarids, and in the control of the forestry [1]. Due to their broad spectrum of insecticide activity and low dose of application needed, their use has dramatically increased in recent years.

As a consequence, and notwithstanding their relatively low mammalian toxicity, analysis of pyrethroid residues in harvest products, foods and environmental matrices is of the most importance in agricultural and environmental sciences. In these conditions, elaborating simple, direct and adapted analytical method for routine analysis of pyrethroid residues in food at the lowest cost is a permanent challenge.

In fact, numerous methods for the determination of pyrethroid residues in agricultural commodities, based on the use of chromatographic techniques [2–9], mainly gas chromatography (GC), have already been proposed. In most cases, extensive sample pre-treatments are required, including pre-concentration and clean-up steps. For example, representative sub-samples of fresh fruit material are homogenised and extracted once or several times using either a single solvent or binary solvent mixtures [5–8].

Since the beginning of the 1990s, original methods using solid-phase microextraction (SPME) have been regularly developed and published in this field [10]. This multi-residue technique is simple and combines extraction and concentration of the analytes, directly from the environmental sample in one step. So, it is not time-consuming nor does it require large quantities of expensive and toxic solvents. In the field of pesticide residues in food, it has already been successfully applied to analysis of aqueous media [11–14], honeys [15], fruits and vegetables [16–18], and many other matrices.

In the case of strawberries, the determination of some pesticide residues with low solubility in water was shown to necessitate a preliminary step in order to facilitate the transfer of pesticide analytes from the fruit into the aqueous

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extracting solution [19]. The microwave-assisted extraction (MAE) technique was used for this purpose. On another hand, pyrethroids are known for their very low solubility in water and considered as nearly insoluble. So, addition of an organic co-solvent is necessary to extract this type of compounds from fruit samples into the aqueous solution. Moreover, it appeared of major importance not to degrade the fruit tissues to prevent eventual matrix effects between analytes and endogenous substances, like strawberry constituents liberated by blending, as observed in a previous work [17]. In the case of pyrethroid molecules, this type of interaction was such that it reduced to nearly zero most of the observed signals.

In this paper, an analytic method using focused microwave-assisted extraction (FMAE), coupled with SPME and gas chromatography–mass spectrometry (GC–MS), has been developed for the determination of residues corresponding to the four pyrethroid pesticides authorised for strawberry cultivation. The FMAE and SPME conditions have been optimised, including the use of a co-solvent to increase the transfer of the analytes into the SPME analysed solution. A validation experiment was also realised from field incurred strawberry samples that were simultaneously analysed by this method in the author laboratory and by traditional methods in two other accredited laboratories.

2. Experimental

2.1. Materials

Pyrethroids used in this work (acrinathrin, bifenthrin, λ-cyhalothrin and deltamethrin) were supplied by CIL (France) and were 98% to 99.9% pure. Some of their characteristics [20] are indicated in Table 1.

Fischer Scientific, Merck and Aldrich (France) provided the organic solvents—methanol (MeOH), ethanol (EtOH) and acetonitrile (ACN), respectively. Stock solutions of each pyrethroid at a concentration of 1000 mg/L in ACN and a mix standard solution of the four compounds at 50 mg/L in ACN were prepared and stored at 4 °C. These solutions were used for spiking samples.

Strawberries were obtained from the Centre Interrégional de Recherche et d'Expérimentation de la Fraise (CIREF) from Bergerac (France). They were collected from both treated and untreated strawberry plants. All samples were frozen just after harvest and stored at −18 °C up to the analysis.

The SPME apparatus consists in a reusable manifold supplied by SupElco (France). The used micro-extraction fibres were coated with poly(dimethylsiloxane) (PDMS) of 100 μm of thickness or 65 μm poly(dimethylsiloxane)–divinylbenzene (PDMS–DVB).

The pyrethroid extraction from fruits was carried out with a focused microwave extractor Soxwave MAP 100 (Prolabo, France).

Chromatographic analyses were performed by means of a ThermoFinnigan GC–MS coupling system (polaris ion-trap MS and GC-Q2000) with an analytical capillary column DB-5MS (30 m length × 0.25 mm I.D., 0.25 μm thickness) and helium as carrier gas at a flow of 1.5 mL/min.

2.2. Method

The analytical method developed for the determination of pyrethroid residues is schemed on Fig. 1.

A 25 g sample of frozen strawberries was weighed into a 250 mL beaker and spiked with calculated aliquots of the mix standard solution, drop by drop by using a pipette. After being kept at room temperature overnight, the entire supplemented strawberries were introduced in a microwave heating tube. Two 15 mL aliquots of the extracting aqueous solution were used to rinse the beaker and added in the heating tube. This tube was then put in the microwave oven and the strawberries were irradiated for different duration at 30 W. Under microwave effect, the sample temperature increased up to about 65 °C. Vapour losses were prevented by the presence of a reflux system on the top of the extraction vessel. The washing solution was collected, cooled down to room temperature and decanted into a brown bottle. A volume of 9 mL of supernatant was taken and the SPME fibre was immersed for 30 min in the stirred solution at ambient temperature. After this time, the fibre was directly introduced in the GC–MS injector for thermal desorption. The temperature program used to separate all compounds was as follows. The initial oven temperature was held at 50 °C for 3 min, increased up to 240 °C with 20 °C/min, then up to 250 °C with 1 °C/min and finally raised up to 300 °C with 10 °C/min. This temperature was held for 2 min. The injector, transfer line and ion source temperatures were kept at 270 °C, 250 °C and 220 °C, respectively. For mass spectrometry analysis, electron impact (EI) mode at 70 eV was used. The mass range

Table 1
Main characteristics of the studied compounds

	Specific ions (*m*/*z*)	Pre-harvest delay (days)	Recommended dose (L/ha)	Solubility at 25 °C (μg/L)			
				Water	MeOH	EtOH	ACN
Bifenthrin	166–181	3	0.5	100	Slightly	–	–
Acrinathrin	93–181–289	3	0.8	<20	61×10^3	–	–
λ-Cyhalothrin	141–181–289	3	0.125	5	5×10^8	–	–
Deltamethrin	172–181–253	3	0.83	<0.2	8×10^6	15×10^6	7×10^7

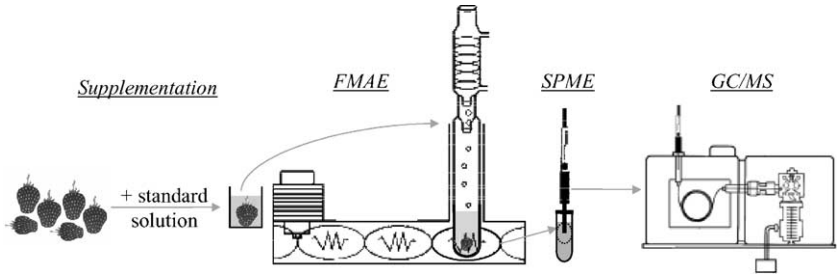

Fig. 1. Extraction and quantification method of pyrethroid residues in strawberries.

varied from 50 u to 650 u. For the calibration, the integration of the peak area was carried out by monitoring specific ions (SIM).

3. Results and discussion

Actually, two different extraction steps have been integrated in this method according which whole strawberries were immersed into an aqueous solution that was then analysed by using SPME fibres, as shown in Fig. 1. In the first one, pesticides were partially transferred into the solution from the fruits, and this was favoured by using microwaves and co-solvents. In the second one, residues were collected onto the fibre coating and then analysed by GC–MS. In this last step, the presence of an organic co-solvent in the aqueous solution, modified the partitioning of the pesticides between the solution and the polymeric coating, making all the parameters necessary to be optimised.

3.1. First step parameters

3.1.1. Use of microwaves

Microwave-assisted extraction has made significant strides for a few years in the field of pesticide residue analysis [21]. In order to evaluate the effect of this pre-processing technique for desorbing pyrethroids from samples, entire defrosted untreated strawberries were supplemented with a mix standard solution made from bifenthrin and acrinathrin, taken as examples, at the concentration of 200 μg/kg. They were then exposed to the lowest microwave power setting (30 W) allowed by the device for different times according to the described protocol. This microwave power was used as long as other assays previously performed at higher power had caused a rapid decay of the sample and the boiling of the solution. All samples were then analysed by using a standard SPME–GC procedure (100 μm PDMS fibre, 30 min exposure time) and results are indicated in Fig. 2.

In the first part of the curve, obtained signals increased up to 5 min of irradiation time, indicating that microwaves had a positive effect for transferring the analytes from the vegetal matrix to the liquid solution. Nevertheless, a longer exposure duration had a negative effect on the amount of analytes finally extracted by the fibre from the solution and the chromatographic signal abruptly decreased. This observation could be in relation with a potential degradation of pyrethroid molecules due to the temperature increasing of the solution, an inescapable consequence of the use of microwaves as already reported in literature for thermolabile compounds [22]. An exposure time of 5 min at the minimum power of 30 W was then chosen in the following of this study.

In order to verify this effect of microwaves for desorbing analytes from contaminated fruits, a comparison was made with results obtained from strawberries spiked according to the same procedure but blended and centrifuged before being SPME analysed. So, identical strawberry samples were spiked at the concentration of 200 μg/kg and divided into three lots. The first lot of samples were blended at 8000 rpm (Ultra-Turrax T25 blender) in distilled water and the supernatant was recovered after 20 min of centrifugation at 5200 $\times$ g. In the second lot, samples were blended in the same conditions but obtained mixtures were then exposed 5 min to microwave irradiation before being centrifuged as in the former case. Fruits of the last lot were immersed into the same volume of water and microwave irradiated for 5 min according to the proposed procedure. Finally, blended and not blended samples were analysed as described above in the previous experiment (100 μm PDMS fibre, 30 min of fibre exposure).

Results, shown in Fig. 3, clearly indicated that signals were notably increased, at least 100 times, for the four pesticides in the cases where MAE was used but without blending of the fruits. For deltamethrin, this change was of the main importance as no signals were observed when fruits were blended. This particular result was in agreement with pos-

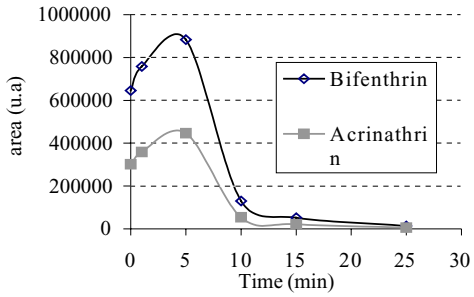

Fig. 2. Effect of the microwave irradiation time on the extraction efficiency.

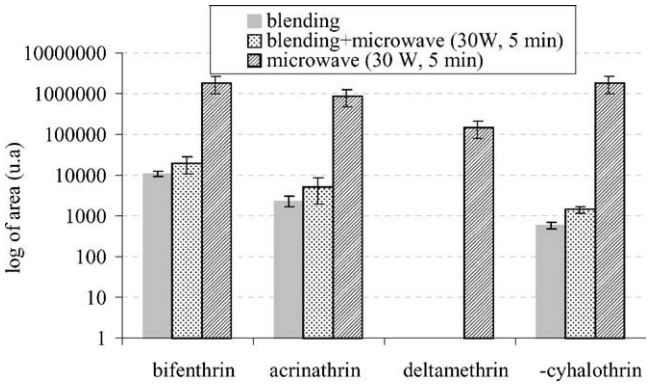

Fig. 3. Relative effects of blending, MAE treatment with blending and without blending on the extraction of the four pesticides from strawberry samples.

sible matrix effects that could interfere between strawberry endogenous substances and target compounds as already noticed by the authors in previous publications [17,19]. In fact, the fruit blending can liberate some active bio-molecules able to specifically trap the pyrethroid molecules and to bind them. In these conditions, the analytes are not available for the fibre and the signal is very low. On the contrary, molecules just desorbed from the entire fruits according to MAE conditions are transferred into the solution and collected onto the fibre.

3.1.2. Use of a co-solvent

Due to the integration of MAE in the proposed method, the choice of a co-solvent had to be done among liquids soluble in water and able to absorb and convert microwave energy into heat. This type of compounds are mainly characterised by a high dielectric constant like water. Methanol, ethanol and acetonitrile are belonging to this family and pyrethroid pesticides are much more soluble in these solvents than in water. For example, deltamethrin is 60 000 times more soluble in ACN than in water, and acrinathrin is 3000 times more soluble in ethanol than in water, whereas both compound solubility in water is less than 100 μg/L. In these conditions, aqueous solutions of these organic solvents at different percentage, respectively, were tested in order to increase the extraction efficiency of the method. So, untreated strawberries were spiked with the mix standard solution of the four pesticides at the concentration of 200 μg/kg and 25 g samples were immersed into 30 mL of aqueous solutions of the three solvents, respectively, at relative concentrations ranging from 10 to 100%, respectively, and finally microwave-assisted extracted before SPME analysis. Comparing histograms are presented in Fig. 4.

In all cases, addition of a co-solvent to the extraction solution enhanced the observed signal compared to this obtained in pure water. Among the three solvents, ACN revealed the most efficient and the 50% mixture, the most relevant for increasing the sensitivity at the maximum level. So, by using theses conditions, signals obtained for bifenthrin, acrinathrin, λ-cyalothrin and deltamethrin were 60 times, 80 times, 120 times and 200 times higher than this observed in pure water, respectively.

Moreover and in order to validate the positive overall effect of the coupling of the two processes, additional strawberry samples spiked at the same level, were microwave extracted in a ACN–water (50:50) solution and compared to others that were just blended and centrifuged without MAE treatment but in the same ACN–water mixture. Comparison

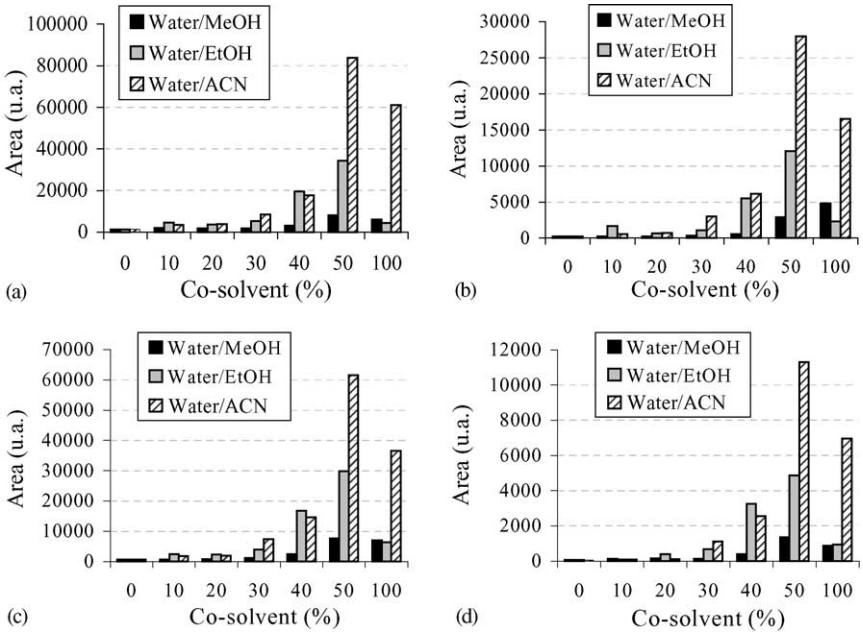

Fig. 4. Influence of a co-solvent for the MAE of bifenthrin (a), acrinathrin (b), λ-cyalothrin (c) and deltamethrin (d) from strawberry samples spiked at the concentration of 200 μg/kg.

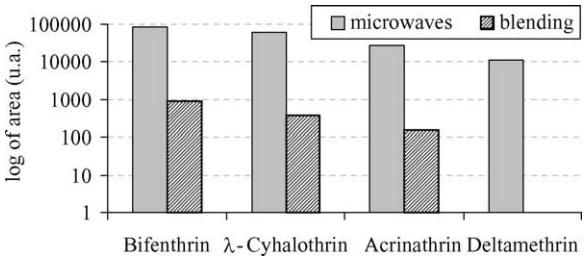

Fig. 5. Comparison of MAE and blending processes in aqueous solution of ACN at 50%.

of both conditions was made in Fig. 5 where it can be seen that the first type of signal is always at least 100 times more important compared to the second one.

3.2. SPME optimisation

3.2.1. Choice of the fibre

Two fibres were tested among those the most used for the determination of non-polar pesticide residues: the 100 μm PDMS and the 65 μm PDMS–DVB. Solutions obtained from 25 g strawberry samples spiked at the concentration of 100 μg/kg and microwave extracted in 30 mL of the ACN–water (50:50) mixture, were successively analysed with the two types of fibres. It appeared clearly (Fig. 6) that the 100 μm PDMS fibre was more efficient for the target analytes in the considered conditions.

3.2.2. Adsorption profiles

In order to optimise the exposure time during which the PDMS fibre had to be immersed into the extracting solution, adsorption profiles were realised by plotting observed signals according to times of immersion between 0 min and 80 min. The experimental results shown in Fig. 7 indicated that the thermodynamic equilibrium between the fibre and the liquid matrix was reached after 15 min for λ-cyhalothrin, acrinathrin and deltamethrin. For bifenthrin, 80 min were needed but the sensitivity of the fibre is higher than for the three other compounds and after 30 min, more than 70% of the maximum amount of bifenthrin was extracted. So, an extracting duration of 30 min was chosen as a compromise. It

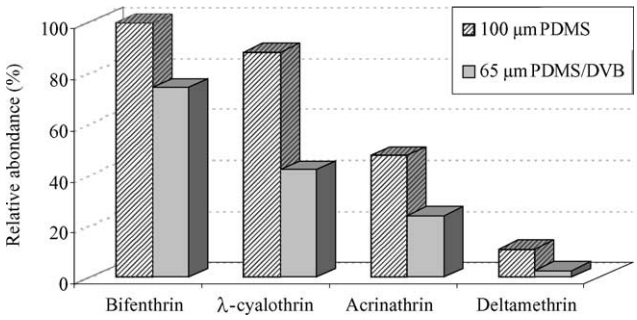

Fig. 6. Comparison between signals obtained by 100 μm PDMS and 65 μm PDMS–DVB fibres (exposure time of 30 min).

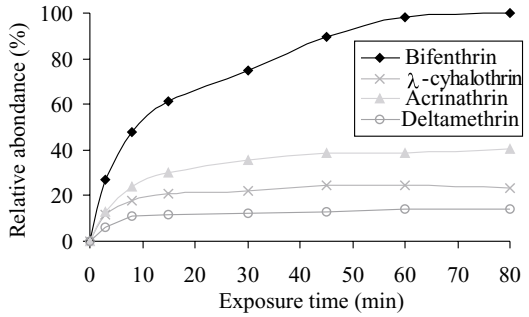

Fig. 7. Effect of the exposure time of the 100 μm PDMS fibre on the extraction efficiency of studied pyrethroids.

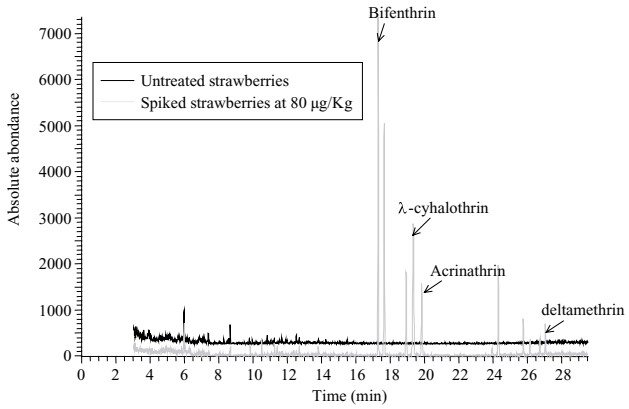

Fig. 8. SIM chromatograms (*m*/*z* 181) of unfortified and fortified samples.

avoided a too long adsorption time and gave good extraction efficiency for all the analytes.

3.3. Calibration curves, limits of detection (LODs) and quantitation (LOQs) and repeatability

For each compound, a calibration curve was built out from strawberry samples supplemented at the concentrations of 1, 2.5, 5, 10, 20, 40, 80, 140 and 250 μg/kg. All the curves were obliged to go through the origin because no signals were observed from blank samples analysed according to the global procedure (Fig. 8). In all cases, regression coefficients (r^2) revealed higher than 0.99. The characteristics of the calibration curves were gathered in Table 2.

The detection and quantification limits were defined as the concentrations corresponding to signal/noise values equal to 3 and 10, respectively.

Table 2
Quantification parameters of the proposed method

Pyrethroids	Calibration equation	r^2	R.S.D. (%)	LOD (μg/kg)	LOQ (μg/kg)
Bifenthrin	$y = 1060x$	0.9938	4	0.9	2.8
Acrinathrin	$y = 195x$	0.9962	12	5.1	15.4
λ-Cyhalothrin	$y = 508x$	0.9953	1.2	2.0	5.9
Deltamethrin	$y = 72.6x$	0.9974	14.2	13.8	41.3

Table 3
Pyrethroid residues found in field incurred strawberry samples by two trading laboratories and comparison with those found by the proposed method

Pyrethroids	Sample code	Laboratory 1 (μg/kg)	Laboratory 2 (μg/kg)	Proposed method (μg/kg)	MRL (μg/kg)
Bifenthrin	N21	<LOD	40	10	200
	N4	110	130	110	–
λ-Cyhalothrin	N1	49	70	20	200
	N31	14	40	30	–
	SVE11	21	<30	30	–
Acrinathrin	N21	n.d.	n.d.	30	200
Deltamethrin	N1	<LOD	100	<LOD	50

n.d.: not detected; MRL: maximum residue limit authorised by European legislation.

In order to determine the relative standard deviation (R.S.D.), five SPME replicates from a single extracting solution, obtained after irradiation of strawberries spiked at 50 μg/kg, were carried out under the same conditions. The calculated R.S.Ds. ranged between 1.2 and 14.2% depending on the studied pesticide.

3.4. Validation of the method from field incurred samples

As a validation of this approach, three series of field incurred strawberry samples produced by the professional association partner have been analysed. The two first series were sent to two certified private trading laboratories, respectively, and the third was blind analysed by the proposed method in the author's laboratory. Results given by the three laboratories are presented in Table 3.

Residue concentrations for bifenthrin, λ-cyhalothrin and acrinathrin were found in the same order of magnitude and far below the corresponding MRLs by the three operators. Deltamethrin was found at the level of 100 μg/kg by only one of the trading laboratory and not by the others. This could be due to an heterogeneity of the samples but clearly points out that the LOD corresponding to this compound in this method has still to be improved.

4. Conclusion

The coupling of two techniques, the first one using microwaves and the second using ACN as a co-solvent, allowed to elaborate an original method for the pre-treatment of pyrethroid residues in strawberries before their determination by using SPME. These type of molecules had been previously observed as able to be masked to the probe due to possible association with endogenous substrates liberated during blending of the fruits. For three of the four compounds authorised on strawberry by the French legislation, the proposed method revealed efficient for performing rapid routine analysis, without blending and centrifuging steps, at concentration levels corresponding to the MRLs and below. For deltamethrin, the LOD was found at a concentration level close to the MRL and this should allow an effective detection of overloaded samples.

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