Talanta 82 (2010) 1286–1291

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Sensitive determination of pesticides residues in wine samples with the aid of single-drop microextraction and response surface methodology

Aimilia Garbi, Vasilios Sakkas*, Yiannis C. Fiamegos, Constantine D. Stalikas, Triantafyllos Albanis

Department of Chemistry, University of Ioannina, Ioannina 451 10, Greece

ARTICLE INFO

Article history:
Received 20 January 2010
Received in revised form 7 June 2010
Accepted 28 June 2010
Available online 6 July 2010

Keywords:
Single-drop microextraction
Response surface methodology
Pesticides
Wine samples

ABSTRACT

The multi-residue trace-level determination of six pesticides (diazinon, dimethoate, chlorpyrifos, vinclozolin, fenthion and quinalphos) in wine samples, after their single-drop microextraction (SDME) is presented herein. The extraction procedure was optimized using the multivariate optimization approach following a two-stage process. The first screening experimental design brought out the significant parameters and was followed by a central composite design (CCD) experiment, which revealed the simultaneous effect of the significant factors affecting the SDME process. High level of linearity for all target analytes was recorded with r^2 ranging between 0.9978 and 0.9999 while repeatability (intra-day) and reproducibility (inter-day) varied from 5.6% to 7.4% and 4.9% to 12.5%, respectively. Limits of detection (LODs) and limits of quantification (LOQs) were found to range in the low $\mu g\,L^{-1}$ level. In general, the developed methodology presented simplicity and enhanced sensitivity, rendering it appropriate for routine wine screening purposes.

1. Introduction

The widespread use of pesticides in agriculture has raised great concern about the health and safety of consumers. Monitoring pesticide residues in wine is mandatory for consumer protection, compliance with good agricultural practice and fair trade certification. The European Union (EU) has set maximum residue limits (MRLs) for pesticide residues in wine grapes (0.01–10 $mg\,kg^{-1}$ depending on the particular pesticide) [1]. The widespread use of pesticides in grape production resulted in the occurrence of pesticide residues in wines worldwide. There is, at present, a great deal of uncertainty surrounding the limits in wine that can be safely tolerated for these potentially toxic substances. According to EU, the MRLs for processed food products like wine, is the same with the raw material (e.g. the grape) while Environmental Protection Agency's (EPA) guidelines set MRLs for processed stuff only when concentration of residues is applied over the production process. Insecticide residues on grapes may pass to the must and therefore to wine, with consequent toxicological risk for the consumer. Although vinification involves many different steps that modify the concentration of pesticide residues in wine, it is generally accepted that this concentration decreases during wine making [2,3]. However, some exceptions have been reported so that some pesticides were present in wine at the same concentration as on the grapes [3]. As a consequence, sensitive and selective methods are required for the determination of pesticide residues.

The current trend towards multi-residue analytical methods has been successfully met by the use of liquid or gas chromatography hyphenated with mass spectrometry [4,5]. As a rule, the multi-residue chromatographic analysis requires a preconcentration step. Various methods have been reported using: liquid–liquid extraction (LLE) and gas chromatography (GC) with nitrogen–phosphorus (NPD) and electron capture detection (ECD) [6,7], gas chromatography–mass spectrometric detection (GC/MS) [8], solid-phase microextraction (SPME) GC/ECD method [9] and SPME-GC/MS [10]. A preconcentration step is required for liquid chromatographic analysis, as well. The liquid chromatography–mass spectrometry (LC/MS) multi-residue determination of pesticides in wines has been reported in combination with LLE [11], solid-phase extraction (SPE) [11,12], SPME [13] and hollow-fiber liquid-phase microextraction [14]. Recently, stir-bar sorptive extraction and membrane-assisted solvent extraction were successfully applied to the determination of oxazole fungicide residues in wines, using ultra-performance liquid chromatography with UV detection [15].

SPME has been accepted as a straightforward, rapid, easily automated and reliable technique for sample preconcentration [16]. However, single-drop microextraction (SDME) was introduced as the newer and less expensive variable of miniaturized liquid-phase extraction processes. The solvent drop, which can easily and reproducibly be formed into the sample is usually employed as a static method in both equilibrium and non-equilibrium modes, aiming at

* Corresponding author. Tel.: +30 26510 08303; fax: +30 26510 98795.
E-mail address: vsakkas@cc.uoi.gr (V. Sakkas).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.06.046

extracting volatile analytes or generated volatile derivatives [17]. SDME provides, analyte extraction, avoiding some inherent problems of SPME such as fiber degradation and thus, SDME has been used quite often for the determination of analytes of environmental [18–24] and biological interest [25–27].

Searching for the optimal conditions for SDME analyses, it comes that usually, the traditional a one-factor-at-a time, approach is preferred. Nevertheless, this strategy fails to take into account interaction between or among variables. On the other hand, multivariate optimization strategies accommodate the need for simultaneous changing of variables levels in order to assess the interactions between the factors, during optimization. These interactions are unavoidable when headspace microextraction is employed, especially with complex samples, such as wine. Recently, Amvrazi et al. applied chemometrics for the SDME analyses of multiclass pesticide residues in fruits [28] while a multivariate approach for the optimization of a headspace SDME determination of 2,4,6-trichloroanisole and 2,4,6-tribromoanisole in wine samples was also reported [29].

In the present study, we describe a rapid and reliable multi-residue method for the determination of six insecticides widely used in vineyard. Although HS-SDME has been widely used for the analyses of a range of compounds in wine [30], to the best of our knowledge, this is the first time that a multi-residue method of wine analysis is developed combining the inherent advantages of direct SDME with the powerful tool of multivariate optimization process.

2. Experimental

2.1. Reagents and materials

Pesticides (diazinon, dimethoate, chlorpyrifos, vinclozolin, fenthion and quinalphos) were obtained from Riedel de Haën (Seelze, Germany). Stock standard solutions (from 460 to 3930 mg L^{-1}) were prepared in methanol and stored in a freezer, at −20 °C. The extraction solvents, *n*-hexane, toluene, chloroform and isooctane were acquired from Merck (Darmstadt, Germany). Pretilachlor (Riedel de Haën) was used as internal standard, at 10 μg L^{-1} (external calibration).

Sodium chloride from Merck was used to adjust the ionic strength of the aqueous samples. All reagents and solvents were of analytical purity. Wine samples used for the method development, optimization and validation, were analyzed in advance to ensure that they were free from pesticides contamination.

2.2. Single-drop microextraction (SDME)

Before each extraction, a 10-μl Hamilton syringe (Microliter Syringes) with a bevel needle tip was rinsed 10 times with acetone followed by 5 times with isooctane. No carry-over effect was observed with this cleaning procedure. The plunger was then placed at the 1-μl mark of barrel scale and 2 μl of the extraction solvent containing the internal standard was withdrawn into the syringe. The sample solution (wine sample, 4 ml) was conditioned and any air bubbles were removed by intensive stirring for 3 min, at 28 °C. Subsequently, the needle of the microsyringe was immersed into the sample and the microsyringe plunger was depressed to expose the microdrop, for a set period of time. The microsyringe was fixed with a stand and clamps so that the distance between the tip of the syringe and the stirring surface was set at 0.65 cm. Stirring rate and extraction time were selected at 180 rpm and 11.5 min, respectively. After extraction, the microdrop was withdrawn back into the syringe and injected into the GC–MS chromatographic system for further analysis. Before next extraction, the microsyringe was rinsed several times with acetone.

Table 1
Retention times and selected ions for the analysis of the target compounds.

Compound	Retention time (min)	Quantification ion (*m*/*z*)	Identification ions (*m*/*z*)
Dimethoate	9.52	125	87, 93, 229
Diazinon	10.51	137	179, 304
Chlorpyrifos	12.55	197	258, 286, 314
Vinclozolin	12.79	285	178, 198, 212
Fenthion	15.27	278	109, 125, 169
Quinalphos	18.16	146	118, 157, 298
Pretilachlor (IS)	21.66	162	176, 202, 238

2.3. GC–MS analysis

All analyses were carried out using a Shimadzu (Kyoto, Japan) GC-17A gas chromatograph, coupled with a QP 5000 mass spectrometer equipped with a fused-silica capillary column (J&W, Folsom, CA, USA) DB-5MS (30 m × 0.32 mm I.D., 0.25 mm), coated with 5% biphenyl and 95% dimethylsiloxane, used for chromatographic separation. Helium was used as the carrier gas, at a flow rate of 0.7 ml/min. The column oven temperature program was: initial temperature 150 °C, ramped at a 5 °C/min rate to 200 °C, followed by another ramp of 1 °C/min to 210 °C, held 2 min and finally ramped to 270 °C at a 20 °C/min rate and held for 3 min. The total run time was 28 min. For quantitative determination selective-ion monitoring (SIM) was used. The interface was kept at 280 °C and the ionization mode was the electron impact (70 eV). The analytes and IS were monitored according to the ions depicted in Table 1. Prior to quantification in the SIM mode, the full scan mode (*m*/*z* 40–400) was used for identification of all target compounds based on their mass spectra and GC retention times. Fig. 1a shows a typical chromatogram obtained using SDME combined to GC–MS in selected ion monitoring (SIM) mode, at the concentration level of 1 μg L^{-1}, for all the analytes tested.

2.4. Response surface methodology and experimental design

Response surface methodology (RSM) is a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes. The application of statistical experimental design techniques in the optimization of the analytical method can result in improved extraction efficiencies, reduced process variability mated to the requirement of less resources (time,

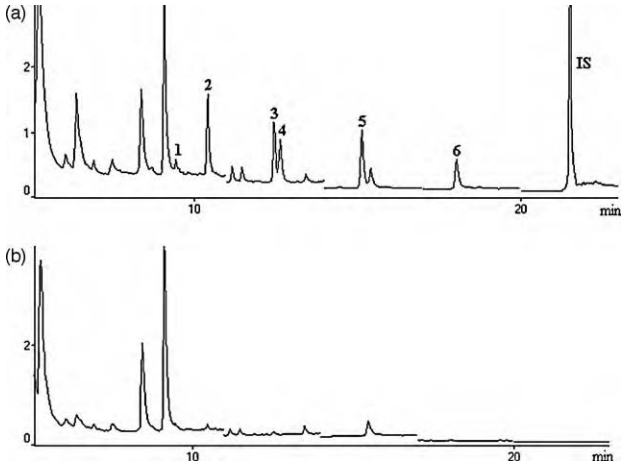

Fig. 1. Total ion chromatogram of (a) all the analytes at 1 μg L^{-1}: (1) dimethoate; (2) diazinon; (3) chlorpyrifos; (4) vinclozolin; (5) fenthion; (6) quinalphos; (7) pretilachlor (internal standard, IS), (b) real sample.

reagents, and experimental work) [31]. At first, two-level fractional factorial design with seven factors was used in order to exclude non-significant factors before investing time and money, in a more elaborate experiment. Seven factors i.e. sample volume, salt addition, pH, needle tip depth, organic drop volume, stirring rate and extraction time, were chosen, based on the literature and our experience. The selected response was the geometric mean of all the areas of the individual peaks of all analytes in order to obtain a unique set of optimum conditions for the extraction of all the target analytes.

The significant variables indicated by the Pareto chart (which was obtained after multiple linear regression and analysis of variance) were optimized using a central composite design (CCD) in order to investigate the simultaneous effect of the significant factors affecting the SDME process as well as to obtain a quadratic equation that correlates the variables with the response. The equation used to quantitatively describe the system and draw the response surface was built using STATISTICA 7.0 software (StatSoft, Inc., Tulsa, USA).

3. Results and discussion

3.1. Experimental variables

There are several parameters which influence the performance of SDME, as mentioned above. The experimental parameters investigated were: organic solvent, extraction temperature, sample volume, salt concentration, pH, needle tip depth, drop volume, stirring rate and extraction time.

The first step in the optimization procedure was to select an appropriate extraction solvent, which is essential for the development of a SDME method. In order to achieve a satisfactory extraction, several solvents with different polarity and water solubility were examined, according to their characteristics in extraction capacity, dissolution drop percentage and in their behavior in gas chromatographic analyses. Four organic solvents including *n*-hexane, toluene, chloroform and isooctane were tested for the extraction process. Isooctane was chosen as the extraction solvent because it is less water-soluble than the other solvents tested, presents adequately high boiling point and exhibits higher average peak area for the target analytes, while at the same time displays the lowest RSD values (data not shown).

Although higher extraction temperature is anticipated to produce better extraction efficiency, this parameter is not usually taken into consideration when liquid-phase microextraction is the case. This is because increased sample temperatures can also lead to elevated extraction solvent solubility. Additionally, the presence of ethanol in the samples in combination to temperatures above 35 °C led to droplet instability while intense bubble formation in the bulk solution caused its final dislodgment. Preliminary experiments, for a temperature range from 20 to 35 °C and an extraction time of 10 min, have shown that optimum extraction of the analytes and reproducible values were acquired, at 28 °C.

As already mentioned, different variables can affect the extraction in a SDME procedure, which in most cases, highly interact. Therefore, a multivariate approach is recommended for their optimization. However, some of them might not have a significant effect on the considered response and they can, thus, be left out of the optimization design. A screening study prior to optimization is helpful to assess the significant variables affecting the analytical response.

3.1.1. Screening step

For screening purposes, when a large number of variables are involved, the reduced factorial Plackett–Burman design is usually employed. The aim of such a design is to detect those variables presenting the greatest influence (significance) on the selected response, by calculation of the main effects. Eight combinations of all factors and three center points, in total 11 experimental runs were carried out (Table 2). For each variable, two levels were considered, which were chosen according to preliminary experiments. Analysis of variance (ANOVA) test was used for the evaluation of the data and statistically significant effects were determined, at a 5% significance level. The results were visualized using the bar charts, the well-known main effect Pareto chart (Fig. 2). Sampling volume is the most significant factor, displaying a negative effect. Ionic strength is the next most significant variable showing a negative sign followed by stirring rate, which exhibits a positive sign. The addition of a salt in a solution may have an impact on the extraction because it increases the ionic strength of the solution and affects the solubility of organic analyte, due to the salting-out effect. However, in our study the presence of salt was found to be a restrictive factor to the extraction of analytes. Apart from the salting-out effect, the presence of salt may have a secondary effect altering the physical properties of the Nerst diffusion film, thus, reducing the rate of diffusion of the target analytes into the drop. Taking into account that this variable had a negative effect on the extraction in addition to the visible drop instability, it was decided that no salt was used in the subsequent experiments.

Table 2
Factors, codes, low and high levels in 2^{7-4} Plackett–Burman design matrix.

Factors					Levels		
					Low (−1)		High (+1)
(X_1) Volume of sample (ml)					4		8
(X_2) Volume of drop (μl)					1		3
(X_3) Stirring rate (rpm/min)					120		180
(X_4) Extraction time (min)					5		10
(X_5) Ionic strength (NaCl concentration; w/v) (%)					0		10
(X_6) Depth (cm)					0.5		1.5
(X_7) pH value					3		9
Runs	X_1	X_2	X_3	X_4	X_5	X_6	X_7
1	−1	−1	−1	1	1	1	−1
2	1	−1	−1	−1	−1	1	1
3	−1	1	−1	−1	1	−1	1
4	1	1	−1	1	−1	−1	−1
5	−1	−1	1	1	−1	−1	1
6	1	−1	1	−1	1	−1	−1
7	−1	1	1	−1	−1	1	−1
8	1	1	1	1	1	1	1
9	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
11	0	0	0	0	0	0	0

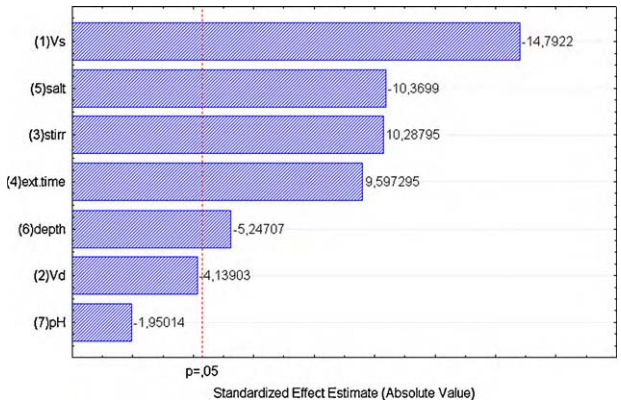

Fig. 2. Standardized main effect Pareto chart for the Plackett–Burman design of screening experiment. Vertical line in the chart defines 95% confidence level.

Table 3
Experimental conditions and responses obtained with the 2^3 central composite design.

Factors	Levels			Star point ($\alpha = 1.414$)	
	Low (−1)	Central (0)	High (+1)	$-\alpha$	$+\alpha$
(X_4) Extraction time (min)	8.00	9.50	11.00	7.38	11.62
(X_6) Depth (cm)	0.6	0.75	0.9	0.53	0.96

Run	X_4	X_6
1	−1	−1
2	1	−1
3	−1	1
4	1	1
5	0	−1.414
6	0	+1.414
7	−1.414	0
8	+1.414	0
9	0	0
10	0	0
11	0	0
12	0	0

Stirring speed was another significant factor affecting positively the extraction process. Increasing the stirring rate higher extraction efficiencies are attained, since the equilibrium between the aqueous and the organic phases is established faster. The agitation of sample improves the extraction and reduces the time required to reach thermodynamic equilibrium. The diffusion coefficient in the aqueous phase increases with increasing stirring rate, as fast agitation can decrease the thickness of the diffusion film in the aqueous phase [32]. This film theory was confirmed to be valid in the SDME method [33]. However, high stirring rates can result in instability of the microdrop leading to drop dislodgment. An additional issue with our matrix is the formation of air bubbles in the bulk of sample, which lead to quantification problems and reduced repeatability. Therefore, we opted for a 3-min sample conditioning step prior to extraction, with vigorous stirring to remove the air bubbles, before fixing the stirring speed at 180 rpm, which is the optimum setting.

The next most significant variables were extraction time and microdrop depth. Fig. 2 also, reveals that extraction time displayed a positive significant effect upon extraction, whereas, drop position in the solution shows a negative effect. Both variables along with their interaction were further studied during the optimization and the employment of CCD.

Drop volume was the variable that showed negative non-significant effect on the response, as Pareto chart reveals. Expectedly, the amount of the analytes extracted into an organic drop is linearly proportional to the drop size at equilibrium, as depicted by the following equation [34]:

$$N = K\, V_{\mathrm{org,eq}} C_{\mathrm{aq,in}} \quad (1)$$

where N is the number of moles of analytes extracted by the organic drop; K is the distribution coefficient of an analyte between the aqueous phase and the organic drop; $V_{\mathrm{org,eq}}$ is the volume of organic drop at equilibrium; and $C_{\mathrm{aq,in}}$ is the initial concentration of the analyte in aqueous solution. Nevertheless, the analytes get into the drop through the diffusion process, therefore, the larger the drop volume, the longer the time to reach the equilibrium. In order to obtain the highest sensitivity of the SDME method, experiments were performed increasing the isooctane drop volume from 0.5 to 3.0 μl. However, drop volumes higher than 2 μl were difficult to manipulate and were unstable in the needle tip. Thus, 2 μl of isooctane were chosen as the optimum drop volume. As well as being negatively affected by the drop volume, the response unexpectedly deteriorated by increasing the sample volume. Theoretically, larger sample volumes lead to increased response, nevertheless, in our case the hydrophilicity of the analytes in relation to the existence of ethanol in the sample matrix may be responsible for this odd behavior.

Finally, according to the Pareto chart (Fig.2), pH exhibited a negative (from low to high values) non-significant effect. The ionization form of the analytes changes with the modification of pH affecting their water solubility and extractability. In this study, the effect of pH was investigated by varying the pH from 3.0 to 9.0. For pH values higher than 6.0, the peak area was decreased displaying a negative effect. It is known that organophosphorous pesticides decompose presenting reduced extraction efficiency at alkaline pH. Therefore, the pH (∼3) of the original unadjusted sample was chosen for further experiments.

Relying on the obtained results and the discussions above, NaCl and pH should be eliminated from further studies (CCD) while the factors that should be considered in the following optimization step were extraction time and drop depth. A minimum sample volume of 4 ml was chosen based on the vial geometry (sufficient drop distance from the magnetic stirrer and sample surface), as well as for satisfactory preconcentration of the analytes into the organic drop. The other parameters should be set as follows: absence of salt; stirring speed, 180 rpm; drop volume, 2 μl at the original pH.

3.1.2. Optimization design

The second step was to optimize the values of the significant variables (extraction time and depth) in order to achieve the best response. Therefore, RSM was applied followed by a factorial experimental design to explore the region of interest of the factors identified by the preceding study. In order to evaluate the broader effects of the two significant factors (extraction time and depth) obtained from the screening test, the CCD was used. Several designs for modeling are based on the CCD, which is constructed by various superimposed designs and consists of a factorial design ($2k$) aggravated with ($2k$) star points, where k is the number of variables to be optimized, and with (n) central points [31]. The applied CCD was consisted of 12 experiments including 4 central points (Table 3).

The model coefficients were calculated by backward multiple regression and were validated by ANOVA.

The lack of fit was not significant ($p > 0.05$) and good values for r^2 (0.99718) and adjusted r^2 (0.99483) were observed. r^2 is the proportion of variance accounted for by the respective model, in the measurements of the dependent factor. The adjusted r^2 applies to the r^2 value an adjustment for the number of terms in the respective model. The large adjusted r^2-values indicate a good relationship between the experimental data and the fitted model.

Table 4
Analytical characteristics of the method (linear range, repeatability, reproducibility, LODs, LOQs).

Compound	Linear range ($\mu g\,L^{-1}$)	r^2[a]	Repeatability[b] (RSD%)	Within-lab reproducibility[c] (RSD%)	LOD[d] ($\mu g\,L^{-1}$)	LOQ[e] ($\mu g\,L^{-1}$)	MRLs[f] ($\mu g\,kg^{-1}$)
Dimethoate	0.15–50	0.9999	6.3	11.4	0.0450	0.15	20
Diazinon	0.01–5	0.9999	5.6	12.5	0.0030	0.01	10
Chlorpyrifos	0.02–10	0.9999	5.7	4.9	0.0075	0.02	500
Vinclozolin	0.01–5	0.9994	6.5	6.4	0.0036	0.01	50
Fenthion	0.01–5	0.9999	7.4	5.0	0.0045	0.01	10
Quinalphos	0.15–50	0.9978	6.8	6.1	0.0450	0.15	50

[a] Squared correlation coefficient.
[b] Repeatability was investigated at concentration: 1 $\mu g\,L^{-1}$ of each analyte.
[c] Within-lab reproducibility was investigated at concentration: 1 $\mu g\,L^{-1}$ of each analyte.
[d] Limits of detection (LOD), for a S/N $\geq$ 3.
[e] LOQ, limits of quantification for a S/N $\geq$ 10.
[f] MRLs values are referred to fruits and not to derivate products.

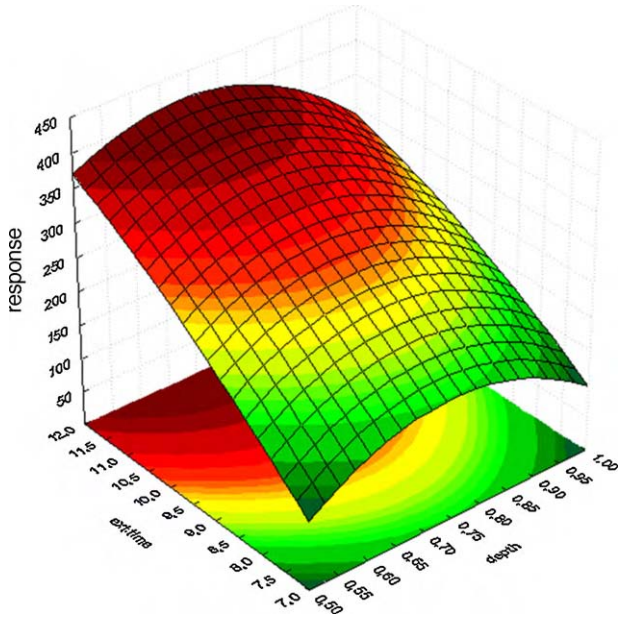

Fig. 3. Response surface for the 2^3 central composite design.

In the final step of the design, a response surface model was developed by considering only the significant interactions in the CCD. Data analysis permitted to obtain a semi-empirical expression of response R (the geometric mean of all the chromatographic areas of the individual peaks of all analytes) given below, in terms of coded values Eq. (2):

$$R = -1255 \times 10^3 + 2115 \times 10^3 X_6 - 1272 \times 10^3 X_6^2 + 123 \times 10^3 X_4 - 2.86 \times 10^3 X_4^2 - 30.55 \times 10^3 X_4 X_6 \quad (2)$$

The next step was to find the conditions of the factors (extraction time and depth) that maximize the response of the dependent factor. Fig. 3 shows the fitted response surface as a function of the interactive variables of drop depth and extraction time. As can be seen, extraction time shows a positive effect upon extraction, irrespectively of the range of the depth. Indeed, long extraction times could lead to high extraction efficiency, but prolonged extraction time is not practical. However, a certain extraction time was needed in order to extract adequate analyte quantities.

On the other hand, the distance between the needle tip and stirring bar exerts a negative effect on the response. Although, positioning the needle at a fixed height with stands and clamps, could reasonably improve the precision of the method, the drop was markedly unstable at a height of 0.5 cm from the stirring surface. Thus, by varying distance values from 0.5 to 1.5 cm we found that after 1.0 cm above the stirring bar, peak areas were decreased. This may be attributed to inadequate mass transference at the selected stirring rate.

For quadratic models, as in our case, the critical optimization condition (maximum) could be calculated through the first derivate of the mathematical function (Eq. (2)), which describes the response surface and equates it to zero. According to this, the optimum values obtained from the software were: drop distance from the stirring bar, 0.65 cm; extraction time, 11.5 min (Fig. 3).

Last, in agreement with the results obtained from the screening and optimization study, the final optimal experimental conditions were: sample volume, 4 ml; NaCl, absence; stirring speed, 180 rpm/min; drop volume, 2 μl; pH, original unadjusted value; extraction time, 11.5 min and drop depth, 0.65 cm.

3.2. Analytical figures of merit

The optimum SDME conditions were used to verify the applicability of the proposed method for the quantitative determination of target analytes. The validation scheme followed was based on the SANCO/10684/2009 European Guidelines [35]. Quality parameters including the limit of detection (LOD), repeatability, within-lab reproducibility, linear range as well as specificity were investigated. The proposed analytical technique – SDME – by definition is an extraction technique, where the volume of the extracting phase is very small in relation to the volume of the sample, and extraction of analytes is not exhaustive. In such cases, at which the analytical method does not permit determination of recovery, the precision is determined from repeat analyses of calibration standards [35].

The calculated calibration curves gave a high level of linearity for all target analytes with coefficients of determination (r^2) ranging between 0.9978 and 0.9999 as shown in Table 4. Moreover residual plots (the difference between the best-fit calibration curve and the actual readings of the standards) were obtained for each analyte. The smaller these errors, the more closely the curve fits the calibration standards. Individual residuals were scattered randomly above and below zero with standard deviations ranging from 0.11% to 1.9% much lower than ±20% (±10% when the MRL is approached or exceeded) from the calibration curve, as suggested by SANCO/10684/2009 document.

The precision of the method consists of two terms: repeatability and within-lab reproducibility. The results were expressed as relative standard deviation (RSD). The calculated repeatability and within-lab reproducibility (Table 4) ranged from 5.6% to 7.4% and from 4.9% to 12.5%, respectively, complying with the requirements of SANCO document (≤20%).

The limits of detection (LODs) for all target analytes were determined according to the signal-to-noise-ratio (S/N) of three and the limits of quantification (LOQs) as ten times the above-mentioned

ratio. As shown in Table 4, the LOD and LOQ values were found to be in the low $\mu g\,L^{-1}$ level, ranging from 0.0030 to 0.0450 $\mu g\,L^{-1}$ and from 0.01 to 0.15 $\mu g\,L^{-1}$, respectively.

The specificity of the method was tested by the analysis of blank samples. The absence of any chromatographic peak in every matrix, at the same retention times as target pesticides, indicated that there were no matrix compounds that might give a false positive signal in these blank samples.

The enrichment factors can be defined as the ratio of the concentration of the target analytes in the microdrop (organic phase) to the concentration of the target analytes in the donor phase (wine sample). The enrichment factors were evaluated by three replicate extractions of wine samples containing 1 $\mu g\,L^{-1}$ of all target analytes. High enrichment factors of all analytes were obtained ranging from 46 for dimethoate to 192 for diazinon.

3.3. Analysis of real wine samples

To demonstrate the applicability of SDME method for routine analysis several samples were analyzed including wine samples kindly supplied from the Association of Robola of Cephalonia: (1) Robola of Cephalonia, (dry white wine), (2) San Gerrassimo, Robola of Cephalonia and (3) BIO, Robola of Cephalonia. Results have demonstrated that the selected wine samples were free of contamination from the target analytes (Fig. 1b).

4. Conclusions

The multi-residue determination of pesticides in wine products deserves special attention and development of accurate and sensitive analytical methodologies. The combination of SDME with a multivariate optimization approach and the GC/MS in the SIM mode was proved to be successful for the trace-level determination of diazinon, dimethoate, chlorpyrifos, vinclozolin, fenthion, and quinalphos in wine samples. The developed methodology presented adequate analytical features, while its enhanced sensitivity and simplicity, make it appropriate for routine wine screening purposes.

References

[1] Commission of the European Communities Regulation 396/2005, Council Directive 91/414/EEC (modification 148/2008 of 29th January, L58/1, 1.3.2008).
[2] P. Cabras, E. Conte, Food Addit. Contam. 18 (2001) 880–885.
[3] P. Cabras, A. Angioni, J. Agric. Food Chem. 48 (2000) 967–973.
[4] R. Flamini, A. Panighel, Mass Spectrom. Rev. 25 (2006) 741–774.
[5] L. Alder, K. Greulich, G. Kempe, B. Vieth, Mass Spectrom. Rev. 25 (2006) 838–865.
[6] E.J. Avramides, C. Lentza-Rizos, M. Mojasevic, Food Addit. Contam. 20 (2003) 699–706.
[7] Y. Saito, A. Yamamoto, S. Kodama, M. Ohto, T. Ohura, A. Matsunaga, J. Food Hyg. Soc. Jpn. 41 (2000) 321–325.
[8] M. Schellin, B. Hauser, P. Popp, J. Chromatogr. A 1040 (2004) 251–258.
[9] M. Correia, C. Delerue-Matos, A. Alves, J. Chromatogr. A 889 (2000) 59–67.
[10] C.G. Zambonin, M. Quinto, N. De Vietro, F. Palmisano, Food Chem. 86 (2004) 269–274.
[11] G.F. Pang, C.L. Fan, Y.M. Liu, Y.Z. Cao, J.J. Zhang, B.L. Fu, X.M. Li, Z.Y. Li, Y.P. Wu, Food Addit. Contam. 23 (2006) 777–810.
[12] M.J. Nozal, J.L. Bernal, J.J. Jimιnez, M.T. Martνn, J. Bernal, J. Chromatogr. A 1076 (2005) 90–96.
[13] J. Wu, C. Tragas, H. Lord, J. Pawliszyn, J. Chromatogr. A 976 (2002) 357–367.
[14] P. Plaza-Bolãnos, R. Romero-González, A. Garrido Frenich, J.L. Martínez Vidal, J. Chromatogr. A 1208 (2008) 16–24.
[15] P. Vinas, N. Aguinaga, N. Campillo, M. Hernandez-Cordoba, J. Chromatogr. A 1194 (2008) 178–183.
[16] M. Correia, C. Delerue-Matos, A. Alves, Anal. Bioanal. Chem. 369 (2001) 647–651.
[17] C.D. Stalikas, Y.C. Fiamegos, Trends Anal. Chem. 27 (2008) 533–542.
[18] M.C. Lopez-Blanco, S. Blanco-Cid, B. Cancho-Grande, J. Simal-Gandara, J. Chromatogr. A 984 (2003) 245–252.
[19] E.C. Zhao, W.l. Shan, S.R. Jiang, Y. Liu, Z.Q. Zhou, Microchem. J. 83 (2006) 105–110.
[20] M. Saraji, M. Bakhshi, J. Chromatogr. A 1098 (2005) 30–36.
[21] D.A. Lambropoulou, E. Psillakis, T.A. Albanis, N. Kalogerakis, Anal. Chim. Acta 516 (2004) 205–211.
[22] V. Colombini, C. Bancon-Montigny, L. Yang, P. Maxwell, R.E. Sturgeon, Z. Mester, Talanta 63 (2004) 555–560.
[23] E. Zhao, L. Han, S. Jiang, Q. Wang, Z. Zhou, J. Chromatogr. A 1114 (2006) 269–273.
[24] Y.C. Fiamegos, C.D. Stalikas, Anal. Chim. Acta 599 (2007) 76–83.
[25] Y.C. Fiamegos, C.D. Stalikas, Anal. Chim. Acta 609 (2008) 175–183.
[26] Y.C. Fiamegos, C.G. Nanos, C.D. Stalikas, J. Chromatogr. B 813 (2004) 89–94.
[27] E.M. Gioti, Y.C. Fiamegos, D.C. Skalkos, C.D. Stalikas, J. Chromatogr. A 1152 (2007) 150–155.
[28] E.G. Amvrazi, N.G. Tsiropoulos, J. Chromatogr. A 1216 (2009) 7630–7638.
[29] E. Martendal, D. Budziak, E. Carasek, J. Chromatogr. A 1148 (2007) 131–136.
[30] N. Garcia-Villar, J. Saurina, S. Hernandez-Cassou, Anal. Chim. Acta 575 (2006) 97–105.
[31] C. Stalikas, Y. Fiamegos, V. Sakkas, T. Albanis, J. Chromatogr. A 1216 (2009) 175–189.
[32] E.L. Cussler, Diffusion: Mass Transfer in Fluid Systems, Cambridge University Press, Cambridge, 1984.
[33] M.A. Jeannot, F.F. Cantwell, Anal. Chem. 69 (1997) 235–239.
[34] C.L. Arthur, J. Pawliszyn, Anal. Chem. 62 (2002) 2145–2148.
[35] SANCO/10684/2009 document, Method validation and quality control procedures for pesticide residues analysis in food and feed.