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

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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

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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,

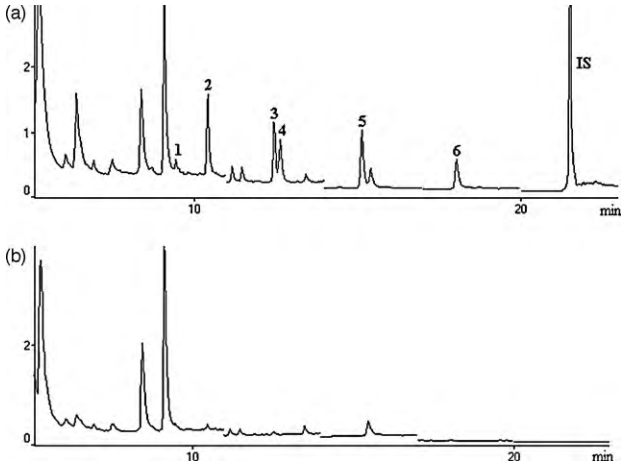

Fig. 1. Total ion chromatogram of (a) all the analytes at 1 μg L^{-1}: (1) dimethoate; (2) diazinon; (3) chlorpyrifos; (4) vinclozolin; (5) fenthion; (6) quinalphos; (7) pretilachlor (internal standard, IS), (b) real sample.