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Chemometric study and optimization of extraction parameters in single-drop microextraction for the determination of multiclass pesticide residues in grapes and apples by gas chromatography mass spectrometry

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ABSTRACT

A simple and rapid single-drop microextraction method coupled with gas chromatography and mass spectrometry (SDME–GC/MS) for the determination of 20 pesticides with different physicochemical properties in grapes and apples was optimized by the use of a multivariate strategy. Emphasis on the optimization study was given to the role of ionic strength, sugar concentration and pH of the donor sample solution prepared from the fruit samples. Since all three variables were found to affect negatively SDME (a lower extraction efficiency was observed as the values of variables were increased for most of the pesticides studied), donor sample solution was optimized using a central composite design to evaluate the optimum pH value and the optimum dilution of the sample extract. With some exceptions (chlorpyrifos ethyl, α-endosulfan, β-endosulfan, pyriproxyfen, λ-cyhalothrin and bifenthrin), the optimum method included the dilution of the analytical sample by 12.5-fold with a buffered acetone/water solution at pH = 4 and exhibited good analytical characteristics for the majority of target analytes (pyrimethanil, pirimicarb, metribuzin, vinclozolin, fosthiazate, procymidone, fludioxonil, kresoxim methyl, endosulfan sulfate, fenhexamid, iprodione, phosalone, indoxacarb and azoxystrobin) by providing high enrichment factors (14–328), low limits of detection (0.0003–0.007 μg/g), and good precision (relative standard deviations below 15%).

1. Introduction

Direct single-drop microextraction (SDME) is one of the solvent-minimized analytical techniques for sample preparation where a microdrop of organic, water-immiscible solvent is suspended at the end of a microsyringe needle, which is then immersed in a stirred aqueous sample solution for a specified period of time [1–4] to simultaneously extract and pre-concentrate analytes. SDME offers significant advantages over liquid–liquid extraction (LLE) or solid-phase extraction (SPE) including near-total elimination of the use of toxic solvents, high enrichment factors of analytes due its comparatively small ratio of amount extracting solvent to sample, the use of analytical standards containing analyte of interest at much lower levels comparable to concentration in real samples, and integration of extraction and injection in a simple device. The importance of these advantages is more significant in pesticide residues analysis since the trend observed in current legislations to reduce the pesticide maximum residue levels (MRLs) allowed in a variety of matrices, is increasing the number of samples to be analyzed and the need for their accurate determination at very low levels.

SDME in pesticide residues analysis has been applied with success in both liquid (water [5–11], juices [12,13], wine [14]) and solid samples (vegetables [15,16], fish [17]) by providing low limits of detection and high selectivity as compared with classic robust sample preparation techniques [18,19]. All above mentioned reports on the determination of pesticide residues by SDME contain a specific section for optimization parameters, including almost invariably organic solvent, volume of extraction phase, extraction time, stirring rate, and ionic strength. However, although the study of the above mentioned parameters is enough for SDME application in water and relative simple aqueous samples (e.g. wine [14]), in more complex liquid and solid food samples extra parameters concerning mainly donor sample solution preparation usually require a further optimization. Organic solvent content, non-diluted suspended solids, and humic acids content in donor solution are some of these parameters that generally have been optimized by the initial extraction of pesticides with an organic solvent and then the dilution of an extract aliquot with water [15,17] and/or by the adjustment of an appropriate dilution of the analytical sample with pure water or a mixture of water and a miscible organic solvent [12,13,16]. Another factor that has not been studied exten-

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sively and may significantly affect SDME is the pH of the donor sample solution. In most SDME studies in pesticide analysis, the pH of the samples is not adjusted whereas a wide range of pH values between 2 and 10 has been reported for the analysis of organochlorine and organophosphorus pesticides by other liquid phase microextraction (LPME) techniques [13,20,21]. The limited number of reported studies on the optimization of these variables as well as the reported matrix effects on the SDME extraction efficiency [5,9,16] consist that a further study of these variables is required to evaluate the analytical performance of SDME procedure on pesticide residue analysis in solid food samples.

The aim of this study was to optimize and validate a simple and rapid SDME–gas chromatography with mass spectrometry detection (GC–MS) methodology in grapes and apples for the determination of 20 pesticides from different chemical families. As far as we know, no previous works dealing with the SDME extraction of pesticides from fruits have been reported. Variables that definitely affect SDME efficiency as drop type and volume, stirring rate and extraction time were optimized univariant since their role and significance on the extraction efficiency is well known. The different variables that may affect the analytical procedure through donor solution preparation and may be involved in matrix effects induced by the SDME procedure (salt concentration, sugar concentration, and pH) were studied initially using a fractional factorial design. In this first screening design, experiments were performed using solvent donor solutions. In a second step the variables that were found to affect the SDME were optimized using a central composite design and fruit matrix. Using the optimized conditions, precision, linearity, limits of detection (LOD) and quantification (LOQ), and accuracy of the method were further evaluated.

2. Experimental

2.1. Chemicals and standards

Pesticide analytical standards of high purity (97.0–99.9%) were purchased from Riedel-de Haën (Seelze, Germany). Acetic acid (glacial, 99.5–100%) used for buffer solution preparation was obtained from J.T. Baker (Deventer, Holland). All other organic solvents used were of *Pestiscan* grade (pesticide residue analysis grade) and were obtained from Labscan (Dublin, Ireland). Water used for sample preparation by SDME was of HPLC grade and was purchased from Sigma–Aldrich (Steinheim, Germany). Hydrochloric acid (38%), sodium chloride, sodium hydroxide, saccharose, and sodium acetate were of suitable purity and were all obtained from Merck (Darmstadt, Germany).

Stock standard solutions of each pesticide were prepared in acetone at 1000 μg/ml and stored at −20 °C. A working standard solution at the same (20 μg/ml) or at different individual concentrations for each pesticide studied (2.5 μg/ml of pyrimethanil, 10 μg/ml of pirimicarb, chlorpyrifos ethyl, phosalone and pyriproxyfen, 20 μg/ml metribuzin, vinclozolin, procymidone, α-endosulfan, fludioxonil, kresoxim methyl, β-endosulfan, endosulfan sulfate and iprodione, 40 μg/ml fenhexamid and bifenthrin, 60 μg/ml indoxacarb, and 100 μg/ml fosthiazate, λ-cyhalothrin and azoxystrobin) was prepared in acetone and used in method validation studies. Other working standard solutions were obtained by appropriate dilutions with the appropriate organic solvent.

Internal standard (ethion) was prepared in the appropriate organic solvent used as acceptor phase in SDME at 1 μg/ml.

Buffered acetone/water, 10/90 (v/v), solution was prepared by mixing 0.1 M acetic acid and 0.1 M sodium acetate (both prepared in acetone/water, 10/90, v/v) in suitable proportions to get the required pH.

Matrix matched standards for SDME were used as donor solutions and were prepared by spiking fruit extracts free from pesticide residues (obtained by the optimum procedure described in Section 2.3) with appropriate amounts of a working standard solution.

2.2. Samples fortification

Apple and grape samples free from pesticide residues were used for method development and optimization studies. Appropriate amounts of a mix of pesticides working solution were spiked in suitable portions of homogenized fruit samples for recovery experiments and linearity studies. After agitation, the samples were allowed to equilibrate for 60 min prior to different extraction assays.

2.3. Proposed sample preparation

Two grams (2.0 g) of the homogenized fruit sample were weighted in a glass centrifugation tube and 25 ml of a mixture of acetone/water, 10/90 (v/v), buffered at pH 4 with sodium acetate and acetic acid were added. The solution was homogenized by an Ultra-Turrax (IKA, Werke, Staufen, Germany) at 13,000 rpm for 1 min and centrifuged at 4000 rpm for 10 min. Seven millilitres of the supernatant donor solution were placed into a 10 ml glass vial (6.5 cm high × 1.8 cm wide) equipped with a PTFE-coated magnetic stir bar (7 mm × 2 mm) and screw capped with a PTFE-faced silicone septum and suspended to the following SDME procedure.

2.4. SDME procedure

The SDME procedure was described as follows: one 10 μl microsyringe with a bevel needle tip (10F, SGE Australia) was used for introducing microdrop to the sample. Before each extraction, the microsyringe was washed at least 10 times with the drop extraction solvent (containing I.S.) in order to eliminate the bubbles in the barrel and the needle. The sample solution was agitated with a magnetic stirrer. A specified volume of organic solvent was drawn into the microsyringe before the extraction. The microsyringe fixed with a stand and clamps was then inserted through the septum of the sample vial and the plunger was pushed down to expose the microdrop in the stirred solution (the distance of the microdrop from the surface of the stirred donor solution was set at 1.5 cm). When the extraction was finished, the drop was retracted into the microsyringe and injected directly into the GC inlet for further analysis.

2.5. Gas chromatography

Analyses were performed on a GC–MS system from Thermo Electron Corporation consisting of a Trace GC Ultra gas chromatograph and a Polaris Q mass spectrometer system. Chromatographic separation was performed on a 30 m *L* × 250 μm ID HP-5MS capillary column coated with 5% diphenyl and 95% dimethylpolysiloxane (J&W Scientific Products) with a film thickness of 0.25 μm. The chromatographic temperature program was: 100 °C for 1 min, raised to 210 °C (5 °C/min) and held for 16 min; then raised to 285 °C (3 °C/min) and held for 20 min. The carrier gas (helium 99.999%) flow rate was in constant flow mode at 1.0 ml/min. Splitless injection of a 1 μl volume was carried out at 250 °C. The interface line and ion source temperatures were maintained at 300 and 200 °C, respectively. Analyses were performed in the full-scan mode whereas electron ionization mass spectra in the range of 45–450 Da were recorded at an electron energy of 70 eV.

Pesticide characteristic mass fragments used for their determination and the relative retention time used for the determination of each analyte are shown in Table 1.