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Fast low-pressure gas chromatography–mass spectrometry method for the determination of multiple pesticides in grapes, musts and wines

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ABSTRACT

A fast method using low-pressure gas chromatography coupled to mass spectrometry (LP-GC/MS) was implemented and optimized to yield a complete separation of 27 representative pesticides in grapes, musts and wines. Extraction was performed with acetonitrile, applying quick, easy, cheap, effective, rugged and safe (QuEChERS) methodology. Several LP-GC/MS conditions such as column temperature, injection conditions, flow rate, MS conditions and matrix effects were evaluated to achieve the fastest separation with the highest sensitivity in MS detection (selected ion monitoring mode). After optimization, all 27 pesticides were extracted, chromatographically separated and detected in less than 20 min. Acceptable recoveries for nearly all pesticides at three different spiking levels (from 0.04 to 2.5 μg/g) were achieved with good repeatability (from 3 to 21%). Limits of quantification (from 0.02 to 5 μg/g) were lower than the maximum limit of residues, when established for pesticides.

1. Introduction

Viticulture and wine production play a fundamental role in socio-economic terms around the world. According to the Organisation Internationale de la Vigne et du Vin (OIV) vineyard area in the world is around eight million ha and about 80% of the grape production is used in winemaking. World wine production has varied along the years reaching 300×10^8 l in 2004. Italy, France, and Spain produce about 50% of the world's wine. Other important wine producers are USA, Argentina, Australia, China, Germany, South Africa, Chile, and Portugal [1].

The use of pesticides to control pest and diseases are a common practice in vineyards to increase crop yield. However, these chemicals can reach plant tissues, leaving residues that can be detected in grapes and in processed products, such as wine [2,3]. This may become a significant route to human exposure to these toxic compounds.

In order to protect consumer's health, maximum residue levels (MRLs) in grapes have been established in different countries and internationally by Codex Alimentarius. Nevertheless, this is not applied to processed products, such as musts or wines [4]. European Union (EU) proposes harmonised MRLs instead of national MRLs. Thus far, EU have established MRLs ranging from 0.02 to 100 μg/g, for 65 pesticide residues in table and wine grapes, with 9 applied specifically for wine grapes and 9 for table grapes [5].

The high number of pesticides to be monitored in those matrices, along with the typically low concentrations of the MRLs, requires highly sensitive and selective methods. Consequently, sample preparation becomes a key step of the analytical procedure.

In recent years, extensive efforts have been made to the development of new sample preparation techniques that save time, labor and solvent consumption and, therefore, improve the analytical performance of the procedure. In this respect several procedures based on solid-phase extraction (SPE) [6–10], solid-phase microextraction (SPME) [11–13], pressurized hot water extraction–microporous membrane liquid–liquid extraction (PHWE–MMLLE) [14] and stir bar sorptive extraction (SBSE) [3], have been used in wine grapes and wine matrices.

Recently a quick, easy, cheap, effective, rugged and safe (QuEChERS) extraction procedure described by Anastassiades et al. [15] based on liquid–liquid partitioning with acetonitrile followed by a cleanup step with dispersive-SPE, was applied in multiresidue analysis of pesticides in fruits and vegetables. The QuEChERS method is a simple, rapid, and inexpensive procedure requiring little labor and few materials, space, and solvents. Two similar QuEChERS methods achieved the status of Official Method of AOAC International [16] and a draft from a technical commission of the European Committee for Standardization (CEN) [17]. This makes QuEChERS a "gold

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Table 1
List of pesticides MRLs in wine grapes, their chemical class and the equivalent concentration of the spiking level for the pesticides in experiments (http://mrldatabase.com/query.cfm, accessed August 2008).

Pesticide	Chemical class	LMR (μg/g)				Spiked level A (μg/g)
		Portugal	EU	Codex	USA	
Mevinphos	Organophosphate	–	0.2	–	0.5	1
Omethoate		–	0.2	–	–	1
Dimethoate		–	0.02	–	1	1
Diazinon		–	1	–	0.75	1
Vinclozolin	Dicarboxamide	5	–	–	–	5
Chlorpyrifos-methyl	Organophosphate	0.2	0.05	–	–	1
Metalaxyl	Phenylamide	1	0.5	1	2	2
Malathion	Organophosphate	0.5	2	5	8	5
Thiobencarb	Thiocarbamate	–	–	–	–	1
Fenthion	Organophosphate	–	–	–	–	1
Tetraconazole	Triazole	0.2	–	–	–	1
Chlorpyrifos	Organophosphate	0.5	0.3	–	–	1
Cyprodinil	Anilinopyrimidine	5	–	–	–	5
Chlorfenvinphos	Organophosphate	–	1	–	–	1
Captan	Phtalimide	–	0.1	–	25	5
Procymidone	Dicarboxamide	5	0.02	–	–	5
Folpet	Phtalimide	5	–	–	–	5
Methidation	Organophosphate	–	2	–	–	5
Endolsulfan	Cyclodiene	0.5	–	–	2	2
Ethion		–	2	–	–	2
Endolsulfan-beta		–	–	–	–	2
Endolsulfan sulfate		–	–	–	–	2
Iprodione	Dicarboxamide	10	–	–	–	5
Phosmet	Organophosphate	–	–	10	10	5
Phosalone		1	1	–	10	5
Azinphos-methyl		1	1	–	4	5
Deltamethrin	Pyrethroid	0.2	0.05	–	–	1

–, (not mentioned).

standard" method for pesticide residue analysis of fruits and vegetables, with applicability to other foods with proper validation. Despite the QuEChERS method has been applied in many different food solid and aqueous samples, such baby food [18] rice [19] and fruit juice [20] the method has received a limited attention in non-aqueous samples such as olive oil [21]. A far as we known, there is no report on the extraction of pesticides from wines (an hydroethanolic matrix) using this sample preparation procedure.

In the field of instrumental analysis the development of alternative methods, such as fast gas chromatography coupled to mass spectrometry (GC/MS) allowed simultaneously an increase in speed of analysis by the higher sample throughput, and lower detection limits of the analytes. The principles of fast gas chromatography have been recently reviewed in several papers [22,23]. Current fast GC/MS methodology employs short capillary columns and among the different approaches can be cited: (i) reduced column inner diameter using micro-bore capillary GC columns coupled with time-of-flight (TOF) MS or other high duty cycle detectors for analysis; (ii) fast temperature programming using resistive heating or conventional GC ovens; (iii) pressure-tunable (also called stop-flow) GC–GC; (iv) supersonic molecular beam (SMB) for MS at rather high carrier gas flow rates; (v) sub-ambient pressure in the analytical column in low-pressure (LP)-GC/MS. The last methodology does not require special techniques for injection or detection, which represents a significant advantage regarding the previous procedures. LP-GC/MS commonly uses a mega-bore analytical column (typically 10 m × 0.53 mm, from 0.25 to 1 μm), connected through a connector to a short, narrow restrictor column at the inlet, providing at the injector similar conditions to those of a conventional GC method, while sub-atmospheric pressure conditions occur throughout the analytical column (MS provide the vacuum source). LP-GC/MS is already a proven technique with a wide range of applications in the analysis of multi-pesticide residues in foods [22,24–26].

The aim of this work was to develop and validate a fast analytical method, not yet documented in literature, for the evaluation of multiple pesticide residues in grapes, musts and wines, using QuEChERS extraction and LP-GC/MS. Special attention was given on optimization of LP-GC/MS conditions, by the evaluation of column temperature, injection conditions, flow rate, MS conditions and matrix effects, to maximize sensitivity and selectivity in the detection and separation of 27 pesticide residues. An additional goal was to assess the applicability of the developed method in the analysis of pesticide residues in real samples at and below MRLs established by different countries (see Table 1).

2. Experimental

2.1. Reagents and solutions

Pesticide standards with 92.5% or higher purity grade were obtained from Dr. Ehrenstorfer (Augsburg, Germany). Purity-corrected individual pesticide stock solutions (1500–5000 μg/ml) were prepared in toluene or acetonitrile (MeCN). A composite stock standard solution of multiple pesticides (200 μg/ml azinphos-methyl, captan, phosalone, phosmet, malathion, methidation, procymidone, vinclozolin, iprodione, cyprodinil, folpet; 80 μg/ml endolsulfan, endolsulfan-beta, endolsulfan sulfate, ethion, metalaxyl; 40 μg/ml chlorfenvinphos, chlorpyrifos, chlorpyrifos-methyl, deltamethrin, diazinon; dimethoate, omethoate, fenthion, mevinphos, tetraconazole and thiobencarb) was prepared in MeCN (level A). The equivalent concentrations of the spiking level for the pesticides in experiments are shown in Table 1. Additional spiking solutions were appropriately diluted at 1/2 (level B), 1/5 (level C), 1/25 (level D) and 1/50 (level E), from level A with MeCN. Internal standards triphenylphosphate (TPP) and $[^2H_6]$malathion (malathion-d$_6$) solution (100 μg/ml in acetone) were obtained from Fluka (Neu-Ulm, Germany) and Dr. Ehrenstorfer, respec-