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Generic sample treatment method for simultaneous determination of multiclass pesticides and mycotoxins in wines by liquid chromatography–mass spectrometry

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ABSTRACT

In this work, a generic sample treatment method for simultaneous determination of multiclass pesticides and mycotoxins in wines is presented. The proposed method is based on solid-phase extraction (SPE) using polymeric-type SPE cartridges. To evaluate the proposed sample treatment, a liquid chromatography electrospray time-of-flight mass spectrometry method was used for testing 60 selected representative multiclass pesticides and 9 mycotoxins. Two different polymeric sorbents were evaluated, with hydrophilic–lipophilic-balanced (HLB) polymer cartridges being selected (Oasis^{TM} HLB) as the most suitable for the present study. The identification and confirmation of the compounds was based on retention time and accurate mass measurements of the protonated molecules ($[M+H]^+$). Limits of detection were below 1 $\mu g\,L^{-1}$ for the 87% of the studied compounds. With the selected 4:1 preconcentration factor, 70% of the target compounds showed relatively low matrix effects, corresponding to signal suppressions lower than 30%. Recovery studies ($n=10$) were carried out at two concentration levels, 2.5 $\mu g\,L^{-1}$ and 25 $\mu g\,L^{-1}$, obtaining mean recovery rates between 70 and 120% for the 90% of studied analytes. The relative standard deviation (RSD%) values of the entire procedure were below 15% in most cases (97% of the studied analytes). The proposed method was successfully applied to 24 red wine samples produced in different regions of Spain. The concentration levels of the target compounds found in the studied samples were in compliance with the current regulations. Aflatoxin B_2 and metalaxyl were the most detected compounds (75% and 50% of the studied samples, respectively).

1. Introduction

Europe is the main producer of wine in the world, with an estimated production in 2010 of 66.5% of the wine produced worldwide, being Italy, France and Spain the largest producing countries [1]. Europe is also leading the consumption of wine in the world, partly fostered for the health benefits associated to a moderate consumption of wine [2]. On the other hand, the increasing public concern about the potential health risks posed by the presence of toxic residues in the human diet has focused all sights on food quality and safety. The two important classes of toxic organics that could be present in wine are mycotoxins and pesticides. Mycotoxins are secondary metabolites produced by several hundreds of fungi that grow in food under particular circumstances [3]. Within this group of compounds, only the content of ochratoxin A in wine samples is regulated in the EU, establishing a maximum permitted limit of 2 $\mu g\,kg^{-1}$ to those wines produced from 2005 harvest onwards [4]. In Spain, there is also an established generic maximum limit of 5 $\mu g\,kg^{-1}$ for aflatoxin B_1 and a limit of 10 $\mu g\,kg^{-1}$ for the sum of aflatoxins B_1, B_2, G_1 and G_2 in foodstuffs destined to human consumption [5].

Although there are extensive regulations for pesticide residues in fruits, vegetables, or drinking water, scarce attention is still devoted to derivate products – which may contain these commodities as an ingredient – such as wine. Despite the limited availability of specific regulation for pesticides in wine, several analytical methodologies have been reported, based on gas chromatography–mass spectrometry (GC–MS) and liquid chromatography–mass spectrometry (LC–MS). GC–MS(/MS) with quadrupole filters have been the most used [6–12], although time-of-flight mass spectrometers have been also used in the last years [13,14]. LC–MS(/MS) analyses of pesticides in wine have been usually performed by means of triple quadrupole analyzers operated in multiple reaction monitoring (MRM) mode [15–17], although the use of high resolution LC–MS instrumentation has been reported recently [17,18]. The development of analytical methods for testing

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mycotoxins in wine has been mainly focused in the determination of ochratoxin A (OTA). Liquid chromatography with fluorescence detection [20–22] and LC–MS/MS [23–25] have been the preferred techniques for the determination of OTA in wines. To date, only three multi-residue methods for the simultaneous determination of pesticides and mycotoxins in vegetable matrices have been described [26–28], although only one of them has been validated in wine [28].

The sample preparation for multi-residue pesticide testing in wines has been recently reviewed by Pang et al. [29]. The need of generic, universal extraction methods covering a wide range of targeted organic contaminants of different physicochemical properties is totally confronted with dedicated cleanup step stages biased to a specific class of contaminants. Several methodologies have been proposed for pesticide extraction in wines. Amongst them: (a) liquid–liquid extraction without further purification steps [9,28] or combined with a clean-up step generally based on sorbent-based strategies such as solid-phase extraction (SPE) [28,30,31], (b) solid-phase extraction using many different type of cartridges [6,32], (c) solid-phase microextraction [10], (d) stir bar sorptive extraction [33], (e) sorptive extraction with disposable silicon discs [19], (f) matrix solid-phase dispersion [34], (g) membrane assisted solvent extraction [33] and (h) hollow-fiber liquid-phase microextraction [15,25]. In contrast, only three methods have been described for simultaneous extraction of pesticides and mycotoxins from vegetable matrices [26–28].

Solid-phase extraction (SPE) is a convenient sample preparation technique, which permits in a single step a preconcentration step, a separation from the bulk matrix, and extract cleanup [35]. It can be easily automated and the extracts obtained are clean enough for further LC–MS analyses. SPE has been used combined with either GC–MS or LC–MS for pesticide testing using C_{18}, polymer type and mixed-mode anion exchange SPE cartridges [6,7,17,18,32]. In SPE, specific analyte–sorbent interactions usually yield cleaner extracts, enhanced preconcentration factors and minor matrix effects. This performance is achieved at the expense of limiting multi-analyte capability, since the applicability of the method is biased toward a specific class of analytes/species. Polymer-based SPE sorbents are suitable for multi-residue analysis involving compounds of a wide range of physicochemical properties, and have been satisfactorily tested for pesticide residue analysis in wine [6,7,17]. Thanks to the use of highly selective and sensitive mass spectrometry instrumentation, the tendency in food safety testing, moves toward the development of methods with minor cleanup stages, covering as many compounds as possible in a single sample preparation stage and LC–MS run [26,27,35]. In this article, a generic sample treatment approach is proposed for the simultaneous testing of multiclass pesticides and mycotoxins in wines. The developed method uses a single SPE step with polymeric cartridges. Several representative multiclass pesticides and relevant mycotoxins were included in the study (60 representative multi-class pesticides and 9 mycotoxins). Two different SPE sorbents were assayed and the recovery rates and matrix effects carefully evaluated using liquid chromatography time-of-flight mass spectrometry (LC–TOFMS). To our knowledge, this is the first generic method for large-scale testing of multiclass pesticides and mycotoxins in beverages using SPE. The proposed method was applied to the analysis of 24 market-purchased red wine samples produced in different regions of Spain.

2. Experimental

2.1. Chemicals and materials

Pesticide and mycotoxin analytical standards were purchased from Fluka, Pestanal® quality (Madrid, Spain) and Sigma–Aldrich (Madrid, Spain). Individual stock solutions of the studied compounds (ca. 500 μg mL^{-1} each) were prepared in methanol or acetonitrile and stored at −20 °C. HPLC-grade acetonitrile and methanol were obtained from Merck (Darmstadt, Germany). Formic acid was obtained from Fluka (Buchs, Switzerland). A Milli-Q-Plus ultra-pure water system from Millipore (Milford, MA, USA) was used throughout the study to obtain the HPLC-grade water used during the analyses. Oasis HLB™ SPE cartridges (200 mg, 6 mL) purchased from Waters (Milford, MA, USA) and Bond Elut™ Plexa SPE cartridges (200 mg, 6 mL) were obtained from Agilent Technologies (Madrid, Spain). A Supelco Visiprep™ (Bellefonte, PA, USA) SPE vacuum system was also used.

2.2. Sample treatment

The pesticides and mycotoxins were extracted using solid phase extraction with two polymer based SPE cartridges, namely Oasis HLB and Bond Elut Plexa. The cartridges were preconditioned with 4 mL of MeOH and 4 mL of ultrapure water at a flow rate of 2 mL min^{-1}. After the conditioning step, an aliquot of 4 mL of wine were passed through the cartridge at a flow rate of 1 mL min^{-1}. Then the cartridge was washed with 4 mL a mixture of MeOH/H_2O (5:95, v/v) and subsequently dried by vacuum during 1 min. The retained analytes were eluted with 2 mL × 4 mL of MeOH at 1 mL min^{-1}. This eluate was then evaporated until near dryness by a gentle nitrogen stream using a TurboVap LV from Zymark (Hopkinton, MA), with a water bath temperature of 37 °C and a N_2 pressure of 15 psi. The samples were then made up with 200 μL of MeOH and 800 μL of milli Q water (final preconcentration factor 4:1). Then this extract was filtered through a 0.45 μm PTFE filter (Millex FG, Millipore, Milford, MA, USA). For validation and quantification purposes, matrix-matched standards were prepared by spiking the filtered (final) extracts with appropriate volume of working standard solutions of the studied analytes.

2.3. LC–electrospray time-of-flight mass spectrometry

The separation of the species from the SPE extracts was carried out in a reversed phase C_{18} analytical column of 50 mm × 4.6 mm and 1.8 μm particle size (Zorbax Rapid Resolution Eclipse XDB-C18) by means of an Agilent HPLC system (Agilent 1290 Infinity, Agilent Technologies, Santa Clara, CA, USA), consisting of vacuum degasser, auto-sampler and a binary pump. 20 μL of extract was injected in each study. Mobile phases A and B were water with 0.1% formic acid and acetonitrile respectively. The chromatographic method held the initial mobile phase composition (10% B) constant for 2 min. Then the content of B was increased up to 50% at 5 min, followed by a linear gradient to 100% B at 15 min and held constant for 3 min at 100% B. The flow-rate used was 0.5 mL min^{-1}.

The HPLC system was connected to a time-of-flight mass spectrometer Agilent TOF 6220 (Agilent Technologies, Santa Clara, CA, USA) equipped with an electrospray interface operating in positive ion mode, using the following operation parameters: capillary voltage: 4000 V; nebulizer pressure: 40 psig; drying gas: 9 L min^{-1}; gas temperature: 325 °C; fragmentor voltage (in-source CID fragmentation): 190 V. LC–MS accurate mass spectra were recorded across the range 50–1000 *m/z*. Accurate mass measurements of each peak from the total ion chromatograms were obtained by means of an automated calibrant delivery system using a dual-nebulizer electrospray source that introduces the flow from the outlet of the chromatograph together with a low flow of a calibrating solution (calibrant solution A, Agilent Technologies), which contains the internal reference masses (purine ($C_5H_4N_4$ at *m/z* 121.050873 and HP-921 [hexakis-(1H,1H,3H-tetrafluoropentoxy)-phosphazene] ($C_{18}H_{18}O_6N_3P_3F_{24}$) at *m/z* 922.009798). Agilent MassHunter Data Acquisition software was used for method