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Rapid and automated on-line solid phase extraction HPLC–MS/MS with peak focusing for the determination of ochratoxin A in wine samples

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ABSTRACT

This study reports a fast and automated analytical procedure based on an on-line SPE-HPLC–MS/MS method for the automatic pre-concentration, clean up and sensitive determination of OTA in wine. The amount of OTA contained in 100 µL of sample (pH ≅ 5.5) was retained and concentrated on an Oasis MAX SPE cartridge. After a washing step to remove matrix interferents, the analyte was eluted in back-flush mode and the eluent from the SPE column was diluted through a mixing Tee, using an aqueous solution before the chromatographic separation achieved on a monolithic column. The developed method has been validated according to EU regulation N. 519/2014 and applied for the analysis of 41 red and 17 white wines. The developed method features minimal sample handling, low solvent consumption, high sample throughput, low analysis cost and provides an accurate and highly selective results.

1. Introduction

Ochratoxin A (OTA) is a mycotoxin produced by several fungal species, such as *Aspergillus* and *Penicillium* genera (van der Merwe, Steyn, Fourie, Scott, & Theron, 1965). These fungi grow spontaneously in different kinds of agricultural products. In particular, it is most commonly found in cereals (Wheat, barley, maize, and oats). Fungal species also grow in other kinds of foods, such as beans, coffee, and dried fruits (Imperato, Campone, Piccinelli, Veneziano, & Rastrelli, 2011). Furthermore, OTA has also been detected in many beverages, such as wine, beer, and grape juices (Di Stefano et al., 2015; Mariño-Repizo, Gargantini, Manzano, Raba, and Cerutti, 2016; Mateo, Medina, Mateo, Mateo, & Jiménez, 2007). In the year 1996, scientists found for the first time that wine samples contained OTA as trace contaminants (Zimmerli & Dick, 1996) and was further authenticated by several authors in recent times (Campone, Piccinelli, & Rastrelli, 2011; Otteneder & Majerus, 2000; Pietri, Bertuzzi, Pallaroni, & Piva, 2001; Soufleros, Tricard, & Bouloumpasi, 2003). The incidence of OTA in wine especially in the Mediterranean basin, is very high (> 50%) (El Khoury & Atoui, 2010). It is important to note that OTA is a highly toxic compound, and it mainly impairs the normal functioning of kidney and liver in humans (Pfohl-Leszkowicz & Manderville, 2007). In a previous study, OTA exhibited strong carcinogenic properties in rats and mice; it also exhibited immunosuppressive, teratogenic, genotoxic activities, which impaired blood coagulation and disrupted carbohydrate metabolism (O'Brien & Dietrich, 2005; Pfohl-Leszkowicz & Manderville, 2007). Furthermore, the development of tumors is stimulated by OTA in the urinary tract of humans (Maaroufi et al., 1993; Nikolov et al., 1995). In the year 1993, the International Agency for Research on Cancer (IARC) included OTA in the group 2B, implying that it is a possible human carcinogen. To minimize the public health risk caused by OTA intake, the European Commission (EU) stipulated that the maximum permissible limit (ML) of OTA should be 2 ng mL^{-1} in wine (Commission Regulation (EC) 1881/2006 Off J Eur Union). Nowadays, all analytical methods used for detecting OTA in wine samples must be sensitive enough to detect trace levels of OTA and determine if they do not exceed the ML (2 ng mL^{-1}) value stated by the EU. The performances of these methods should be compliant with the established legislation pertaining to OTA limits in wine samples (Commission Regulation (EC) 519/2014 Off J Eur Union).

Presently, the most common analytical methods for quantification of OTA in foodstuffs are based on HPLC separation coupled to fluorescence detection (FLD). However, according to the EC Decision 657/2002 (Commission Decision 2002/657/EC (2002) Off J Eur Comm): in addition to FLD determination a mass spectrometry confirmatory method must be performed because it provides full or complementary information pertaining to the unambiguous identification of OTA in the sample.

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As far as the sample preparation, different extraction and clean-up techniques for the analysis of OTA in foods have been reported. Most of them, including the official method (Visconti, Pascale, & Centonze, 2001) described by the European standard for analysis of OTA in wine samples, are based on SPE using immunoaffinity columns (IACs) (Visconti et al., 2001). Although IACs exhibit high selectivity during the isolation of OTA from wine, they are very expensive both in terms of time and material consumption. Therefore, other sample preparation methods for the analysis of OTA in complex foods such as wine have been also used. The other sample preparation techniques are as follows: i) solid-phase extraction (SPE) with various alternative stationary phase, including silica gel, octadecylsilane, and molecularly imprinted polymer (Campone et al., 2015; Cigić & Prosen, 2009); ii) dispersive liquid-liquid microextraction (DLLME) (Campone, Piccinelli, Celano, & Rastrelli, 2012; Campone et al., 2011); iii) solid phase microextraction (SPME) (Aresta, Vatinno, Palmisano, & Zambonin, 2006); iv) packed in-tube SPME (Andrade & Lanças, 2017); v) quechers (Mariño-Repizo et al., 2016) and vi) on-line SPE using a C18 cartridge (Bacaloni et al., 2005)

Most of these methods are expensive in terms of time and material consumption. In addition, only specially trained personnel can perform these sample preparation techniques and the sample throughput is too low to meet the current standards of food safety and public health protection (Cigić & Prosen, 2009). Therefore, it is necessary to develop novel, automated procedures that overcome the main limitations of conventional techniques; the novel method must not only be rapid but also accurate enough to minimize the number of errors in order to obtain reproducible responses. On-line SPE (Rodriguez-Mozaz, de Alda, & Barceló, 2007) is a good alternative method for sample preparation performed during the determination of OTA in wine samples. The on-line SPE technique does not have the drawbacks of conventional methods, and the sample manipulation errors of this technique are reduced. An automated on-line SPE-HPLC system consists of the following components: an autosampler, two HPLC pumps, and a six or ten-port switching valve. After the injection, performed by autosampler, the sample is transported by one of the pumps and concentrated in the SPE cartridge, while the matrix interferences are eliminated out to the waste. After completing the sample loading and washing steps, the position of the valve change, in order to drive the mobile phase from the second HPLC pump into SPE cartridge; this mobile phase elutes the analytes from the SPE cartridge into the chromatographic column. In the HPLC column, the interferents are separated and target analytes are detected. The on-line SPE is a suitable alternative technique for sample preparation, because the sample pretreatment procedure is minimal and the analysis time is sharply reduced (Campone et al., 2016; Rodriguez-Mozaz et al., 2007; Rogeberg, Malerod, Roberg-Larsen, Aass, & Wilson, 2014). This study, describes the optimization of an on-line SPE-HPLC–MS/MS method for the analysis of OTA in wine samples; To carry out sample extraction, a mixed mode anion exchange cartridge (Oasis MAX) was used coupled with a triple quadrupole mass spectrometer through a two-position 10 port switching valve for OTA quantification: the aforementioned configuration performs automatically the loading, washing, and elution steps of analysis. We cautiously optimized all parameters of the on-line SPE procedure affecting the extraction efficiency paying particular attention to the removal of matrix effect. Under the optimal conditions, we performed validation of the method according to EU regulation 519/2014 (Commission Regulation (EC) 519/2014 Off J Eur Union), and finally, applied at 58 different wine samples (red and white).

2. Experimental

2.1. Standards and materials

OTA Reference standard solution (10 μg mL^{-1}) in acetonitrile was purchased from LGC Promochem GmbH (Labservice Analytica, Bologna, Italy). The stock solutions of OTA (200 ng mL^{-1}), used for spiking procedure and for preparation of working calibration solutions, was prepared in EtOH 15% to simulate the alcohol composition of wine, and stored in amber glass vials at −20 °C. Ultrapure water (18 MΩ) was preparing using a Milli-Q purification system (Millipore, Bedford, USA). MS-grade water (H_2O), methanol (MeOH), and acetonitrile (CH_3CN) were supplied by Romil (Cambridge, UK), ammonia solution 30% (NH_4OH) and ethanol absolute (EtOH) were supplied by Carlo Erba reagents (Milan, Italy), MS-grade formic acid (HCOOH) was provided by Sigma-Aldrich (Milan, Italy). Reference material (RM) of white wine T1755 (OTA assigned values 1.63 μg L^{-1}; satisfactory range 0.91–2.34 μg L^{-1}) was purchased from Fapas (York, UK). The following on-line cartridges: Oasis HLB (20 × 5 mm, 25-μm particle size, 80-Å pore diameter; Waters, Milford, MA, USA), copolymer of divinybenzene and vynil pyrrolidone column; Oasis MAX column (20 mm × 2.1 mm, 12 μm particle diameter, 175 Å pore size; Waters), mixed-mode, reversed-phase/strong anion-exchange; and Strata C18 silica column reversed-phase (25 × 4 mm, 25 μm particle size, 60-Å pore diameter, Phenomenex, Bologna, Italy), were tested in the optimization of the on-line SPE procedure.

2.2. Samples

Wine samples (alcohol content from 10 to 15%) from Mediterranean countries were purchased from different local stores and supermarkets (Salerno and Naples, Italy) and stored at room temperature. Before analysis, the pH of the samples was adjusted to pH ≅ 5.5 (Reinsch, Töpfer, Lehmann, & Nehls, 2005) using NH_4OH 0.1 M and then centrifuged for 5 min at 10,000 rpm (IEC-CL30R, Thermo Electron Corporation, Milan, Italy).

Samples used in optimization and validation studies, were earlier analyzed to verify the absence of OTA contamination (Campone et al., 2011). Spiked samples were prepared by adding specific volumes of OTA stock solutions (200 ng mL^{-1}) to 10 mL of OTA-free wine to achieve the required contamination levels. After spiking, the fortified samples were stirred for 1 h left at room temperature and then stored in the dark at 4 °C for a maximum of three days.

2.3. On-line SPE and chromatographic conditions

The system used for both on-line preconcentration and chromatographic separation was an Ultimate 3000 (Thermo Electron Corporation) equipped with dual ternary gradient pumps, a thermostated column compartment that includes a Rheodyne® 10-port two position (load/inject) switching valve and an autosampler with a 5000 μL injection loop. The chromatographic system was connected to an Ultimate 3000 UV detector (280 nm) to monitoring the matrix components during the washing step and a TSQ Quantum Ultra (Thermo Electron Corporation) triple quadrupole mass spectrometer, for the detection of analyte. The mobile phases in the left pump were as follows: H_2O (A_L), H_2O/MeOH/HCOOH 79.5:20:0.5 v/v/v (B_L) and MeOH (C_L). The mobile phases of right pump were: H_2O/MeOH/HCOOH 19.5:80:0.5 v/v/v (A_R) H_2O with 0.1% HCOOH, v/v, (B_R) and CH_3CN % with 0.1 HCOOH, v/v, (C_R). The chromatographic separation was performed in gradient elution mode using a monolithic column (Chromolith® FastGradient 50 × 2 mm I.D. Merk); the column temperature was maintained constant at 25 °C during the entire chromatographic run. To perform sample enrichment and removal of matrix components the automated on-line SPE was performed in a mixed-mode (reversed-phase/strong anion-exchange) Oasis MAX cartridge. The cartridge was fitted into the load position with a 10 port-switching valve. Before injection, cartridge and column were conditioned for 2 min and then 100 μL of wine sample was loaded into SPE cartridge at high flow rate (1 mL min^{-1}) using 2 mL of solvent A_L. Thereafter SPE column was washed with 2 mL of B_L and 2 mL of C_L (both at 1 mL min^{-1}) sequentially. Thus the interfering species of the matrix