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Mass spectrometry strategies for mycotoxins analysis in European beers

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ABSTRACT

In this work, an existent solid-phase extraction (SPE) procedure was used to study the occurrence of mycotoxins in different European beers. HPLC-QqQ-MS/MS and ultra high resolution mass analyser have been optimized for the analysis of 18 mycotoxins: the methods were validated according to the EU Commission Decision 2002/657/EC guidelines. In this sense, matrix-matched calibration was performed for each type of beer, obtaining an effective quantification. The recoveries ranged from 63 to 91% and repeatability and reproducibility expressed as relative standard deviation (RSD%) were lower than 17%. On one hand, HPLC-LTQ-Orbitrap® was used for unambiguous identification of target mycotoxins, as well as for screening non-target mycotoxins, such as enniatins, fusaproliferin and deoxynivalenol-3-glucoside. On the other hand, the quantification was carried out using HPLC-QqQ-MS/MS instrument. At the end, Ochratoxin A, fumonisins, HT-2 and T-2 toxins were detected and quantified in European beers. Moreover, pale lager beer showed higher mycotoxin incidence than the other studied beers.

1. Introduction

Beer is the oldest alcoholic beverages and the cereal-based product worldwide consumed. This invention has been argued to be responsible for humanity's ability to develop technology, concretely food technology. Nowadays, brewing and fermentation of starches, mainly derived from cereal grains, such as barley, wheat, maize and rice produce beer. The fermentation generates substances such as carbohydrates, mainly sugars or starches, which produce a benefit beverage (Bamforth, 2002).

It is important to keep in mind that cereal grains could be contaminated by intentioned addition or natural contaminants. For example, metals have been detected in cereal and beer (Donadini, Spalla, & Beone, 2008), and pesticides have been also identified in these matrix (Bolaños, Romero-González, Frenich, & Vidal, 2008; Navarro, Pérez, Navarro, Mena, & Vela, 2007). Focus on natural contaminants, mycotoxins have been wide reported in cereals, hop and beer (Běláková, Benešová, Mikulíková, & Svoboda, 2011; Romero-González, Vidal, Aguilera-Luiz, & Frenich, 2009).

Theses contaminants or its residues could reach the consumers, and a frequent consume of the contaminated product could suppose a risk for the health of the consumers. For this reason, maximum levels (MLs) for mycotoxins have been established (EC, 2006; EC, 2010) and moreover, several mycotoxins have been classified by IARC (IARC, 1993).

Beer is a complex matrix; it composition contains water, carbohydrates, protein substances, mineral salts and alcohol. The alcohol (ethanol) is a fermentation sub-product and it can strongly influence the extraction of mycotoxins. For this reason, extraction procedures for mycotoxins from beer have been carefully developed.

At the beginning of the decade, the developed mycotoxins methods in beer were focused on one mycotoxin as, for example, OTA (Araguás, González-Peñas, & López De Cerain, 2005; Aresta, Palmisano, Vatinno, & Zambonin, 2006; Bacaloni et al., 2005; Visconti, Pascale, & Centonze, 2000) or on the simultaneous detection of a group of mycotoxins as aflatoxins and OTA (Ventura et al., 2006), fumonisins (Shephard et al., 2005) or trichothecenes (HT-2 toxin (HT-2), T-2 toxin (T-2), deoxynivalenol (DON), nivalenol (NIV) and 3-acetyldeoxynivalenol (3-ADON)) (Suga, Mochizuki, Harayama, & Yamashita, 2005).

Nowadays, HPLC followed by tandem mass spectrometry have allowed the development of fast multi-residue methods in foods (Desmarchelier et al., 2010; Ediage, Di Mavungu, Monbaliu, Van Peteghem, & De Saeger, 2011; Rubert, Soler, & Mañes, 2011; Sulyok, Krska, & Schuhmacher, 2010). Focussing on beer, several methods have been also developed using HPLC-MS/MS (Romero-González et al., 2009; Rudrabhatla & Wood, 2007). In these studies, triple–quadrupole detector has demonstrated to be robust and useful instrument for routine analysis of mycotoxins in beer.

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However, the last trends in organic contaminant analysis have been to take advantage of ultra-high resolution mass spectrometry, which allows qualitative and quantitative analysis for target, non-target and unknown compounds to be analysed. For example, this technology has been recently applied to detect mycotoxins in beer (Rubert, Mañes, James, & Soler, 2011a; Zachariasova et al., 2010). In these cases, Orbitrap® technology also demonstrated to be useful for routine analysis, since effective quantification and unambiguous identification were obtained.

Thereby, the main aim of this work was to develop a rapid and sensitive method to identify and quantify 18 mycotoxins in beer. For that purpose, a previous SPE method was used to extract selected mycotoxins. HPLC-QqQ-MS/MS method was optimized to identify selected mycotoxins, as well as to obtain lowest levels of quantification (LOQs). On the other hand, hybrid linear ion trap-high resolution mass spectrometry was used to unambiguous identification of target compounds studying its fragmentation pathways and accurate mass. Owing to the capacity of Orbitrap® technology to acquire data essential for the identification of other "non-target" mycotoxins that maybe present in beer, several "emergent" mycotoxins as such enniatins (ENs): ENA, ENA1, ENB, ENB1, fusaproliferin (FUSA) and deoxynivalenol-3-glucoside (D3G) were tested.

At the end, the developed analytical methods were applied to monitor commercial beers from main manufacture countries in Europe.

2. Material and methods

2.1. Chemical and standards

Standard of aflatoxin B_1 (AFB_1), aflatoxin B_2 (AFB_2), aflatoxin G_1 (AFG_1), aflatoxin G_2 (AFG_2), Ochratoxin A (OTA), sterigmatocystin (STER), α-zearalenol (ZOL), zearalenone (ZEN), nivalenol (NIV), deoxynivalenol (DON), 3-acetyldeoxynivalenol (3-ADON), diacetoxyscirpenol (DAS), fumonisin B_1 (FB_1), fumonisin B_2 (FB_2) and beauvericin (BEA) were purchased from Sigma Aldrich (Madrid, Spain). T-2 and HT-2 toxin stock solutions (in acetonitrile) were obtained from Biopure referenzsubstanzen GmbH (Tulln, Austria). Fumonisin B_3 (FB_3) was supplied by the PROMEC unit (Programme on Mycotoxins and Experimental Carcinogenesis, Tygerberg, South Africa). The individual stock solutions were prepared as in previous work (Rubert et al., 2011a).

HPLC grade solvents, acetonitrile, methanol and water were supplied by ThermoFischer (Dublin, Ireland). Analytical grade reagent formic acid (purity > 98%), ammonium formate was obtained from Panreac Quimica S.A.U. (Barcelona, Spain). All solvents were passed through a 0.45 μm cellulose filter purchased from Scharlau (Barcelona, Spain). Oasis HLB SPE (200 mg/6 ml) cartridges were purchased from Waters (Milford, MA, USA) and Strata-X 33 μm Polymeric Reversed Phase cartridges (200 mg/6 ml) were purchased from Phenomenex (Phenomenex, Torrance, CA, USA). An extraction manifold from Waters connected to a Büchi Vac V-500 (Flawil, Switzerland) vacuum system was used for SPE experiments.

2.2. Samples and sampling

A total of 49 beer samples were purchased from different stores, supermarkets and specialized beer stores from Cork (Ireland) and València (Spain) and kept at −20 °C in a dark and dry place. Several imported beers from Belgium, Czech Republic, Germany, Italy and Poland were also included in the study. The samples with undetectable levels of mycotoxins were used for spiking and recovery studies.

2.3. Extraction procedure

Sample preparation was performed as described in a previous research (Rubert et al., 2011a). Beer was degassed by sonication for 25 min. The Oasis HLB cartridges were conditioned with 5 ml of acetonitrile/methanol (50:50, v/v) and 5 ml of water. Consequently, 10 ml of beer was loaded onto C_{18} cartridge. After that, SPE columns were washed with 5 ml of water. Then the cartridges were dried for 30 min. In the last step, the mycotoxins were eluted by adding 4 ml of acetonitrile/methanol (50:50, v/v). Then, the extract was transferred into a 15 ml conical tube and evaporated to dryness at 35 °C with a gentle stream of nitrogen using a multi-sample Turbovap LV Evaporator (Zymark, Hoptkinton, USA). The residue was reconstituted to a final volume of 1 ml with methanol/water (50:50, v/v), filtered through a 0.20 μm Millex-GN nylon filter (Millipore, Carrightwohill, Ireland) and collected into a vial.

2.4. Instrumental parameters

2.4.1. Liquid chromatography triple quadrupole mass spectrometry

The HPLC-QqQ-MS/MS system consists on a Finnigan Surveyor CTC (Autosampler ThermoFischer Scientifics), a Finnigan Surveyor LC quaternary Pump (Accelera, ThermoFischer Scientifics) and a Finnigan TSQ Quantum Discovery MAX triple quadrupole mass spectrometer (ThermoFischer Scienctific, Hemel Hempstead, UK). Chromatographic separation was performed with a reversed-phase analytical column (Gemini C_{18}, 150 mm, 2 mm i.d, 5 μm; Phenomenex). The mobile phase was a gradient of H_2O 5 mM ammonium formate and 0.1% formic acid (A) and methanol 5 mM ammonium formate (B) and the gradient conditions were as follows: 0–10 min, linear from 5 to 95% B; 10–15 min, isocratic 95% B. Ten minutes were used to equilibrate the column with initial conditions. The flow rate was 200 μl min^{-1}. The autosampler was set at 10 °C and column temperature was set at 35 °C, 10 μl was injection volume.

All mycotoxins were detected using heated electrospray (H-ESI) source. The first step was to infuse each mycotoxin standard solution (10 μg/ml) with a syringe pump (10 μl/min). Ion source parameters were optimized for each compound using the quantum tune application of Xcalibur 2.0.7 software. The source was operated in the positive heated ESI mode; spray voltage, 4500 V; vaporizer temperature, 300 °C; ion transfer capillary temperature, 350 °C; with both the sheath gas pressure set to 40, auxiliary gas pressure 55 arbitrary units and ion sweep gas was set to 0 arbitrary. Skimmer offset was set to −3 V and the collision gas pressure was 1.5 mTorr. Data processing was performed using the Xcalibur (Version 2.0.7) software (ThermoFischer Scientifics). Mass spectral data were acquired in SRM mode in a single time segment with 20 ms dwell time for each transition. Collision energy and tube lens offset voltages were optimized for each mycotoxin using the automated optimization procedure in syringe infusion mode provided by the manufacturer. Table 1 shows the two monitored transitions for each mycotoxin.

2.4.2. HPLC-LTQ-Orbitrap XL

The analytical method has been previously optimized and explained by Rubert et al. (2011a). The LC system was connected to a hybrid LTQ Orbitrap XL (Thermo Scientific), operating in positive ion mode. The column and the chromatographic separation used were the same as HPLC-QqQ-MS/MS method (Section 2.4.1).

The linear ion trap (LTQ) part of the hybrid MS system was equipped with heated electrospray interface (H-ESI). Full-scan accurate mass spectra (mass range from 90 to 900 Da) were obtained at high resolution (100,000 FWHM) and processed using Xcalibur v.2.0 software. The electrospray source conditions were: source voltage 4 kV, heated capillary temperature 275 °C, capillary