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Worldwide interlaboratory study on the determination of ochratoxin A in different wine type samples

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Abstract

Interlaboratory studies are decisive tools to help the validation of a specific analytical methodology or to assess the reproducibility of the use of different methods to analyze a given compound or compounds in certain sample matrices. In this work, homogeneous samples of two white wines ("White Wine" and "White Liqueur Wine") and one red wine ("Red Fortified Wine") from Portugal with different production techniques and characteristics, namely in alcohol strength (10.5%, 16.0% and 19.0% ethanolic content, respectively), were analyzed for their contents in ochratoxin A (OTA), a mycotoxin generated from fungal contamination. White Liqueur Wine was naturally contaminated, whereas the other two wine type were spiked with ethanolic OTA solutions. The participation of 24 laboratories from 17 countries of five continents was ensured for this study. Although with no restrictions in terms of analytical methodology to employ, 75% of the laboratories resorted to immunoaffinity columns clean-up followed by high performance liquid chromatography with fluorescence detection (HPLC-FD), most of them in accordance with the European Standard EN 14133. For White Wine samples, the general mean OTA concentration was 1.96 μg/l (two outliers) with interlaboratorial standard deviation (s_L) of 0.53 μg/l; for White Liqueur Wine, mean of 1.59 μg/l (one outlier), with $s_L = 0.59$ μg/l; and for Red Fortified Wine, mean of 2.73 μg/l (no outliers), with $s_L = 0.96$ μg/l. Outliers were determined by Cochran and Grubbs tests. The Horrat index, recommended by the Association of Official Analytical Chemists (AOAC) for the quality assurance of the collaborative study was, on average, 1.7. This study proved that OTA determination in wines is reproducible, regardless of the methodology employed.

Keywords: Ochratoxin A; Wines; Interlaboratory study; *z*-Scores; Outliers

1. Introduction

Ochratoxin A (OTA) is a mycotoxin (fungal contamination-derived metabolites) produced by strains of *Aspergillus* and *Penicillium*, and recently detected in several food matrices, including alcoholic beverages. Since first reported in wines by Zimmerli and Dick in 1995 [1], the pressure for seeking food safety urged OIV to recommend a maximum limit of 2 μg/l for safe intake in wines [2]. The European Union recently adopted the value of 2.0 μg/kg as maximum residue level of OTA for wine (red, white and rosé) [3]. OTA has been classified as possessing nephrotoxic, carcinogenic, teratogenic, genotoxic and immunotoxic properties [4], amongst other hazardous effects. The main problem is that the routes of OTA intake for humans come from several food sources rather than from a specific acute one. Nevertheless, various data have been published in literature reporting OTA levels in wines ranging from a few nanograms to several micrograms per litre worldwide [5–18].

Different analytical methodologies have been proposed for OTA screening, mainly comprising liquid–liquid or immunoaffinity extraction steps, previous to liquid chromatographic analysis with fluorescence detection (HPLC-FD) [19,20]. Other detection methods used include mass spectrometry (MS/MS) [19,21] and photodiode array detection (PDA) [17]. Different clean-up procedures such as anion exchange [22] and molecular imprinted polymers (MIP) [23] were also

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recently reported. A reference method has been approved in 2003 by the European Committee for Standardization (CEN)—EN 14133 [24]. In general, immunoaffinity columns are recommended as clean-up procedure of OTA from wines and beers, prior to HPLC-FD analysis. The official method adopted by the Association of Official Analytical Chemists (AOAC) is also based on these techniques [25].

Interlaboratory studies aiming the validation of the reference method or focusing on proficiency objectives (ring-tests) have been performed for wine matrices, especially when alcoholic contents round up to 12% (v/v).

A proficiency test has been conducted on FTIR wine analysis with the participation of six laboratories [26]. The wine sample had an average 12% in alcohol strength. Reproducibility was evaluated for several analytical determinations such as density, ethanolic content, dry extract, total sugars, total acidity, pH, and total polyphenolic index. An interlaboratory study featuring six laboratories compared the determination of ethyl carbamate in alcoholic beverages (red, white and fortified wines, brandy and wine spirit) by HPLC–FLD and GC–MS [27].

The European Standard EN 14133:2003 reports the results of an interlaboratory test according to AOAC guidelines for collaborative study procedures to validate characteristics of a method of analysis for OTA quantification in wines [24]. The study was conducted in 1999, with the participation of 16 laboratories, strictly applying the aforementioned standard. There is no information concerning the type of wines analysed, apart from the citation that the samples were white wine, red wine or beer, spiked at three concentration levels around 0.1–0.2 ng/ml, 0.9–1.1 ng/ml and 2.0–3.0 ng/ml, and one naturally contaminated sample (mean concentration of 0.283 ng/ml for white wine and 1.690 ng/ml for red wine).

The Bureau InterProfessionnel d'Etudes Analytiques (BIPEA) periodically organizes proficiency tests on several wine determinations, following ISO Guide 43:1997 [28]. It is probably the organization that covers a wider range of wine types [29]. For physico-chemical analysis, red wines, dry white wines, sparkling wines, dessert wines, rosé wines, aromatized wines have been included. Contaminants such as pesticides and mycotoxins are among the determinations. However, statistical reports are sent to the participating laboratories and therefore no statistical data is divulged.

Other matrices of study and reference materials were also reported in literature as a target for similar interlaboratory collaborations, such as sewage sludge [30], wastewater [31], water [32], saliva [33], ambient air [34], plants [35] or other food products [36–38]. A review on interlaboratory studies applied to analytical chemistry aiming their different purposes and evaluation methods was published by Hund et al. [39].

The importance of such interlaboratory studies, beyond the validation of an analytical method, is nowadays seen as an important contribution to the estimation of the global uncertainty associated to the results. Concerning the complex nature of many modern methods of analysis, proficiency testing schemes allowing laboratory specific standard operating procedures (SOPs), are more to the point than method-evaluating schemes like ISO 5725:1994 [40]. Several authors describe the calculation of the uncertainty with the inclusion of the interlaboratory variability [41–44].

In view of these foundations, in this work it is proposed to study three different wine matrices, which have undergone dissimilar vinification processes and therefore having different alcohol strength:

- Red Fortified Wine and White Liqueur Wine, in which fermentation is arrested before completion by alcohol distillate addition, allowing sugar and alcohol content to be higher (around 150 g/l total sugars and 18–19% alcohol strength (v/v)).
- White Wine, in which fermentation is complete, thus having lower sugar and alcoholic content (less than 0.3 g/l sugars and 9% alcohol strength). In the specific case of these samples, the acidity is slightly higher than in common table wines.

Being aware of the importance of interlaboratory studies to assess the fiability of OTA determination methods in alcoholic beverages, two Portuguese entities, LEPAE—Process Engineering, Environment and Energy Laboratory (a research group from the Faculty of Engineering of the University of Porto, FEUP) and ALABE, an Association of the Laboratories of Enology, organised such a study. ALABE acted as the guarantee of confidentiality of the process and LEPAE/FEUP performed all statistical treatment of data.

2. Experimental

2.1. Organization of the study

The main steps of this Interlaboratory Study are presented in Table 1.

2.2. Samples analyzed

The study was designed to comprise different types of wines and different levels of OTA contamination, both natural and spiked. As aforementioned, three different wines were chosen. In order for the participating laboratories to analyze all samples in triplicate, the necessary amount of each sample was sent, varying from 250 ml to 750 ml, according to the requirements of the methodologies employed by the different participants. Spiking was done with 9% and 18% ethanolic solutions of OTA.

Table 1
Steps of the ochratoxin A (OTA) interlaboratory study

Step	Action	Time frame
1	Last call for participants	May 2004
2	Setting of participating entities	May–June 2004
3	Samples preparation	June 2004
4	Samples delivery	June 2004
5	Experimental analysis	June–October 2004
6	Reception of the results	June–October 2004
7	Statistic analysis	November 2004
8	Final report/presentation of the results	December 2004