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Restricted access supramolecular solvents for sample treatment in enzyme-linked immuno-sorbent assay of mycotoxins in food

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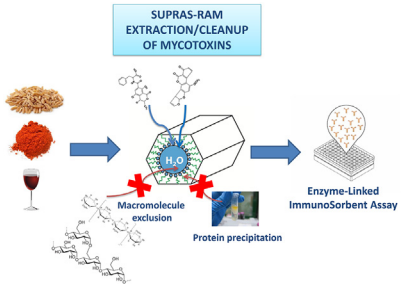
HIGHLIGHTS

- SUPRAS-RAM were proposed for the first time for general sample treatment prior to ELISA.
- The approach is a wide-scope, low-cost strategy for detection of mycotoxins in food.
- Mycotoxin extraction and sample cleanup were integrated in a single step.
- OTA and AFB1 were successfully analysed in wines, cereals and spices.

GRAPHICAL ABSTRACT



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ABSTRACT

A restricted access supramolecular solvent (SUPRAS-RAM) made up of tetradecanoic acid reverse micelles is proposed as a wide-scope and low-cost strategy for the treatment of agrifood samples prior to enzyme-linked immunosorbent assays (ELISA). The approach was assessed for the determination of ochratoxin A (OTA) in wines and spices and aflatoxin B1 (AFB1) in cereals, two ubiquitous mycotoxins that were selected as representative contaminants for this study. The samples were selected to cover a variety of matrices in terms diverse composition and high complexity. Macromolecules such as proteins and carbohydrates were not-co-extracted due to the restricted access properties of the SUPRAS that are provided by chemical and physical mechanisms. In this sense, analyte extraction and clean-up were carried out in a single step. Parameters determining the extraction efficiency were studied and optimized. Certified reference materials were used for method validation. Recoveries of OTA ranged between 83% and 96% in wines (with relative standard deviation, RSD, of about 10%) and between 81% and 93% in spices (RSD 7%). Recoveries for AFB1 in wheat ranged from 75% to 85% (RSD 8%). The detection limits were all below the maximum levels established for OTA and for AFB1 by EU directives. This method offers a green and low-cost alternative to the organic solvent-based extraction and/or immunoaffinity columns-based cleanup of complex samples prior to ELISA.

1. Introduction

Mycotoxins are toxic and ubiquitous metabolites produced by a great variety of filamentous fungi on agrarian-origin commodities, in both the field and the storage stage [1]. Around 400 mycotoxins have been described so far, although aflatoxins and ochratoxin A (OTA) have received the most attention due to their adverse effects (liver and kidney toxicity, teratogenicity and carcinogenicity) [2]. Both types of mycotoxins constitute a growing threat for animal and human health, and also cause high economic losses associated

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to crop production, so international organizations have established strict regulatory limits for their presence in food and feed [3–6]. In the last decade, around 25% of total notifications in The Rapid Alert System for Food and Feed (RASFF) were due to aflatoxins and OTA [7].

There is a growing need for rapid yes/no decisions for the control of mycotoxins in the food market and the compliance of regulations. Although methods based on liquid or gas chromatography coupled with mass spectrometry (LC-MS, GC-MS) have been reported as robust, sensitive and selective, they are also expensive, time consuming and require large volumes of hazardous solvents [reviewed in Refs. [8–10]]. These methods are mostly based on solvent extraction followed by clean up with solid-phase extraction based on multimode or immunoaffinity sorbents and are mainly used in routine analysis as confirmatory techniques. More rapid methods not requiring a chromatographic (or electrophoretic) separation and with the need of only minor or not sample preparation, have been developed based on matrix-assisted laser desorption ionization-MS [11,12] and ambient mass spectrometry, such as direct analysis in real time(DART)-MS (DART-MS) [13,14] and laser desorption ionization [15]. However, these methods still need to be carried out by experienced operators and are based on expensive techniques. As an alternative, there are available many immunoassay rapid test formats in the current market, namely enzyme linked immunosorbent assay (ELISA), lateral flow tests, flow through immunoassay, fluorescent polarisation immunoassay, and immunoaffinity columns coupled with fluorimetry [reviewed in Refs. [16–18]]. Other types of rapid formats based on piezoelectric sensors, enzyme inhibition assays or array biosensors have been reported in the literature too [reviewed in Ref. [18]].

Commercial ELISA kits for mycotoxins are simple, rapid, selective, sensitive, low-cost and portable and are one of the most common formats of kits in the market. Sample treatment in most of the commercial ELISA kits for both OTA and AFB1 (in spices, coffee, cereals, animal feed, etc.) is based on solvent extraction (mostly with 25–50 mL of methanol or acetonitrile), centrifugation for removal of solids and further dilution in buffer (total sample dilution factors typically range from 1:5 to 1:1000). Despite its claimed specificity, cross-reactivity and interferences from matrix components often cause overestimation or signal suppression in ELISA [19,20]. For this reason, many test kits are only semi-quantitative and they are mainly used to determine whether the levels of mycotoxins are above or below the established regulatory limits.

In order to give more accurate and reproducible results, some ELISA kits include a clean-up step for which immunoaffinity columns (IACs) or multimode solid-phase extraction are a common choice [21]. This more selective clean-up allows also further confirmation of ELISA positive samples by LC-MS or LC-fluorescence detection. IACs are however expensive, not recyclable and have a limited storage time. Considering that the most important features for test kits from an industrial point of view are cost-effectiveness and fit-for-purpose [16], an alternative sample treatment able to remove common sample interferences and to reduce costs in terms of solvents and reagents would be very desirable.

In this study, we propose supramolecular solvents with restricted access properties (SUPRAS-RAM) for the simultaneous extraction of mycotoxins and sample clean-up prior to ELISA determination of both liquid and solid food. For this purpose, SUPRASs made up of inverted hexagonal aggregates of tetradecanoic acid in mixtures of THF and water were selected [22]. These investigations aimed to develop generalized sample treatments for food matrices featuring a wide range of compositions (e.g. percentage of protein, fat, carbohydrates, water, etc.) or considered complicated (e.g. spices that have high content in pigments, essential oil compounds, carbohydrates and/or fats). The determination of ochratoxin A (OTA) in wines and spices and aflatoxin B1 (AFB1) in cereals were selected as representative contaminants/matrices. Extraction of OTA and AFB1 in the SUPRAS-RAM was based on both dispersion and hydrogen bonds analyte:extractant interactions. Macromolecules were excluded from extractions through both chemical and physical mechanisms. The developed method was simple, green and inexpensive and could constitute a valuable alternative for sample treatment prior to ELISA in the surveillance of mycotoxins in food.

2. Experimental

2.1. Chemicals

All chemicals were of analytical-reagent grade and employed as supplied. Tetradecanoic acid was obtained from Aldrich (Milwaukee, WI). Tetrahydrofuran (THF), HPLC-grade acetonitrile, methanol and acetic acid glacial were supplied by Panreac (Sevilla, Spain). Ultra-high-quality water was obtained from a Milli-Q water purification system (Millipore, Madrid, Spain). OchratoxinA (OTA) and Aflatoxin B1 (AFB1) were obtained from Sigma (St. Louis, MO, USA). The reference materials OWW-3 (OTA in white wine; 3.0 ± 0.5 μg L^{-1}) and ORW-5 (OTA in red wine; 5.0 ± 0.8 μg L^{-1}) were obtained from R-Biopharm (Glasgow, Scotland). Stock standard solutions of 10 mg L^{-1} of OTA and AFB1 were prepared in methanol and stored under dark conditions at −20 °C. Working solutions were prepared by dilution of the stock solutions with methanol.

2.2. Instrumentation

The Enzyme-Linked ImmunoSorbent Assays (ELISA): RIDASCREEN® Ochratoxin A 30/15 and RIDASCREEN® Aflatoxin B_1 30/15 and the RIDA® Ochratoxin A immunoaffinity column were obtained from R-Biopharm AG (Germany). A 1420 Multilaber counter Victor ^{3}V microplate reader (Perkin Elmer and Analytical Sciences, Wallac Oy, Turku, Finland) was used to perform photometry measurements. A Mixtasel Selecta and a High Speed Brushless (MPW-350R) centrifuges were employed for sample preparation. Centrifuge tubes (34 mm I.D., 80 mm height) with narrow necks (7 mm I.D, 60 mm height) were designed by authors and constructed by Pobel S.A. (Madrid, Spain) for treatment of liquid samples in order to make easier the measurement and collection of the SUPRAS after microextraction. A Spectra System SCM1000, (ThermoQuest, San Jose, CA, USA) consisting of a P2000 binary pump, a FL3000 fluorescence detector and an AS3000 autosampler was used for confirmatory purposes and optimization of the extraction parameters. The stationary-phase column utilized for the analysis was a 15 cm Kromasil C_{18} 5 μm column, with 4.6 mm I.D., from Análisis Vínico (Tomelloso, Spain). Excitation and emission wavelengths for OTA were 333 and 460 nm, respectively.

2.3. Supramolecular solvent extraction of mycotoxins in food prior to ELISA determination

A general workflow for mycotoxin determination in foodstuffs using SUPRAS-RAM extraction prior to ELISA is shown in Fig. 1.

2.3.1. Extraction of OTA in wine

Tetradecanoic acid (300 mg) was dissolved in THF (2 mL) and then, 8 mL of red or white wine (pH 3–3.5, adjusted with 1.0 M HCl) were added. Sweet wines were previously two-fold diluted with distilled water. The SUPRAS-RAM spontaneously formed in the wine and the mixture was stirred (5 min, 1500 rpm) to favour OTA