



HAL
open science

Indirect determination of aflatoxin B1 in beer by a multicommuted optical sensor

Lucía Molina Molina García, M. Luisa Fernández de Córdoba, Antonio Ruiz Medina

► **To cite this version:**

Lucía Molina Molina García, M. Luisa Fernández de Córdoba, Antonio Ruiz Medina. Indirect determination of aflatoxin B1 in beer by a multicommuted optical sensor. *Food Additives and Contaminants*, 2012, pp.1. 10.1080/19440049.2011.643244 . hal-00780025

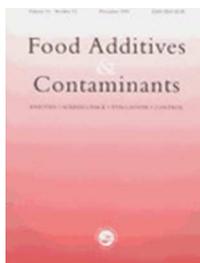
HAL Id: hal-00780025

<https://hal.science/hal-00780025>

Submitted on 23 Jan 2013

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Indirect determination of aflatoxin B1 in beer by a multicommuted optical sensor

Journal:	<i>Food Additives and Contaminants</i>
Manuscript ID:	TFAC-2011-397
Manuscript Type:	Original Research Paper
Date Submitted by the Author:	14-Sep-2011
Complete List of Authors:	Molina García, Lucía; University of Jaén, Physical and Analytical Chemistry Fernández de Córdoba, M. Luisa; University of Jaén, Physical and Analytical Chemistry Ruiz Medina, Antonio; University of Jaén, Physical and Analytical Chemistry
Methods/Techniques:	Screening - biosensor, Clean-up
Additives/Contaminants:	Mycotoxins – aflatoxins
Food Types:	Beer
Abstract:	This manuscript reports the determination of Aflatoxin B1 (AFB1), mycotoxin which is considered one of the most carcinogenic substances known. A multicommuted flow injection-solid phase spectroscopy (FI-SPS) system combined with photochemically-induced fluorescence (PIF) is developed, for the first time, for its determination with quantitative purposes. A strongly fluorescent degradation product is obtained on-line by irradiation with ultraviolet light. The determination is carried out by measuring the fluorescence intensity of the photoproduct at 353/424 ($\lambda_{ex}/\lambda_{em}$), once retained on C18 silica gel filling the flow-cell. A linear dynamic range of 0.09-12 $\mu\text{g L}^{-1}$, detection limit as sensitive as 29 ng L ⁻¹ and a relative standard deviation (RSD) of 1.4% were obtained. The method proposed has been satisfactorily applied to the determination of AFB1 in different types of beer (normal and non-alcoholic). Hydrophobic compounds are eliminated from beer samples and AFB1 is extracted with acetonitrile by solid-phase extraction on C18 sorbent. Recoveries of the target compound from spiked beers are between 94-106%. The results obtained in the analysis of real samples are in good agreement with those provided by a reference chromatographic method.

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

SCHOLARONE™
Manuscripts

For Peer Review Only

1
2
3 **Indirect determination of aflatoxin B₁ in beer by a multi-commuted**
4
5 **optical sensor**
6
7

8
9
10 Lucía Molina-García · M^a Luisa Fernández-de Córdoba · Antonio Ruiz-Medina*

11
12
13
14 Department of Physical and Analytical Chemistry

15
16 University of Jaén, Campus Las Lagunillas s/n, 23071 Jaén, Spain
17
18
19

20
21
22
23 *Corresponding author
24 Tel: (+34)953-212759
25 Fax: (+34)953-212940
26 e-mail: anruiz@ujaen.es
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Abstract

This paper reports the determination of aflatoxin B₁ (AFB₁), which is considered one of the most carcinogenic substances known. A multi-commuted flow injection-solid phase spectroscopy (FI-SPS) system combined with photochemically-induced fluorescence (PIF) was developed, for the first time, for its quantitative determination. A strongly fluorescent degradation product was obtained on-line by irradiation with ultraviolet light. The determination was carried out by measuring the fluorescence intensity of the photo-product at 353/424 ($\lambda_{\text{ex}}/\lambda_{\text{em}}$), once retained on C₁₈ silica gel filling the flow-cell. A linear dynamic range of 0.09-12 $\mu\text{g L}^{-1}$, detection limit as sensitive as 29 ng L^{-1} and a relative standard deviation (RSD) of 1.4% were obtained. The method proposed was satisfactorily applied to the determination of AFB₁ in different types of beer (normal and non-alcoholic). Hydrophobic compounds were eliminated from beer samples and AFB₁ was extracted with acetonitrile by solid-phase extraction on C₁₈ sorbent. Recoveries of the target compound from spiked beers were between 94-106%. The results obtained in the analysis of real samples are in good agreement with those provided by a reference chromatographic method.

Keywords: Aflatoxin B₁, sensor, multi-commuted, photochemical induced fluorescence, beer

Introduction

Aflatoxins are the main toxic secondary metabolites of the genus *Aspergillus flavus* and *Aspergillus parasiticus* (Asis et al. 2002; Ali et al. 2005). Under favourable conditions of temperature and humidity, these fungi grow in foods and produce aflatoxins. Mutagenic and carcinogenic activity, teratogenic properties and hepatotoxic action are the most dangerous damage caused by these compounds to human health (Krska et al. 2008). The occurrence of aflatoxins in food can be caused by both direct contamination via grain and grain products or “carry over” of these compounds and their metabolites into animal tissues, milk and meat after intake of contaminated foodstuffs. The most toxic aflatoxin is aflatoxin B₁ (AFB₁), classified as Group I human carcinogen by the International Agency for Research on Cancer (IARC) (Wang et al. 1998; IARC 2003), resulting in median lethal dose (LD₅₀) values ranging from 0.3 to 9.0 mg kg⁻¹ body weight. It is regulated by legislation in foods (EC 2006) for direct human consumption at 2 ng g⁻¹.

The analytical determination of AFB₁ is complicated by two main factors: (1) the complexity of the sample matrix in which it normally appears (corn, peanuts, cottonseed, nuts, almond, figs, fruits and spices) (Gourama and Bulleman 1995; Miller 2008) (AFB₁ can colonize and contaminate grain before harvest or during the storage); and (2) the low levels present in these samples. Analytical methodologies must be designed to address these requirements. Firstly, in order to avoid matrix interferences, a typical analysis of AFB₁ involves a liquid–liquid extraction (using solvents such as methanol, acetonitrile, and/or their combinations), followed by a clean-up step, e.g., using multifunctional, Florisil or immunoaffinity columns (Nawaz et al. 1995; Giray et al. 2007; Fu et al. 2008; O’Riordan and Wilkinson 2008). Secondly, with respect to its detection, a reliable and sensitive method must be selected for the screening and determination of this compound. Different methods have been established (Turner 2009), including capillary electrophoresis (CE) (Peña et al. 2002), thin-layer chromatography (TLC) (Stroka et al. 2000; Papp et al. 2002; Braicu et al. 2008), high-performance liquid chromatography (HPLC) (Abdulkadar et al. 2000; Gilbert and Vargas 2003; Brera et al. 2007), and enzyme-linked immunosorbent assay (ELISA) (Garden and Strachan 2001; Lee et al. 2004). For positive confirmation, LC-MS methods have also been reported (Sforza et al. 2005; Cavaliere et al. 2007). Although sensitive and accurate, most of the chromatographic methods require expensive

1
2
3 equipment and derivatisation after extraction in order to remove interfering substances.
4 Also, commercially available ELISA methods require enzymatic reactions, washing and
5 separation of bound and free label, while CE has remained a research topic rather than
6 finding application in routine analysis (Shepard 2009).
7
8

9
10 The intrinsic fluorescence of AFB₁, which is due to the presence of a chain of
11 conjugated bonds and heteroatoms in its molecule, could be used for its determination.
12 Nevertheless, the use of spectrofluorimetric analysis is difficult due to the complexity of
13 the matrix, which shows a great variety of natural fluorescent compounds whose spectra
14 often overlap the analyte signal. This situation demands tedious separation steps to
15 enable the AFB₁ determination. Different approaches can be used in order to both avoid
16 these inconveniences and increase the sensitivity of the spectrofluorimetric methods; for
17 example: chemical modification of the molecule with chlorine or bromine (Mably et al.
18 2005), the use of β -cyclodextrin as fluorescence enhancer (Hashemi et al. 2008) or
19 organised media based on surfactants (Shtykov 2002; Goryacheva et al. 2008), and the
20 possibility of working with photochemically induced fluorescence (PIF).
21
22
23
24
25
26
27

28 The current work focuses on the development of a novel method for the detection
29 of AFB₁ in beer. Its incidence in this product has been already described (Scott and
30 Lawrence 1997; Mably et al. 2005; Zollner and Mayer 2006; Romero González et al.
31 2009; Pietri et al. 2010). This presence is due to its transfer from contaminated grain
32 (barley and maize) during the brewing process. The use of adjuncts during the
33 processing, normally maize grit, explains this contamination; moreover, AFB₁ can be
34 also found in malted barley (Pietri et al. 2010). Sensors may be a good choice for the
35 analysis of AFB₁ due to their fast, simple, and low-cost detection capabilities.
36 Nevertheless, to date only electrochemical sensors (Ammida et al. 2004; Owino et al.
37 2008; Tan et al. 2009) can be found in literature for the determination of this aflatoxin
38 in alcoholic beverages. No optical sensors have been developed for this purpose.
39
40
41
42
43
44
45

46 In this paper we evaluate the potential of combining PIF with multi-commutation
47 to determine AFB₁ in the complex matrix of beer. The method is based on the on-line
48 generation of a fluorescent photoproduct from AFB₁ by UV-irradiation and its
49 monitoring when adsorbed onto C₁₈ silica gel. The measurement of the photoproduct
50 retained on this solid support, packed in the flow-cell placed in the detection area, was
51 adopted for improving both detection limit and selectivity. The enhancement in
52 selectivity makes it possible to analyse complex samples, such as beer, whose analysis
53 would not be possible in homogeneous solution due to the high number of interfering
54
55
56
57
58
59
60

1
2
3 species. This methodology, called flow injection-solid phase spectroscopy (FI-SPS) or
4 flow-through optosensor, combines advantages of FI with the analyte pre-concentration
5 on a small amount of a solid support (López Flores et al. 2005). The use of multi-
6 commutation, as alternative to conventional FIA, is introduced due to its favourable
7 intrinsic advantages such as low-cost equipment, high sample throughput, and
8 simplicity, as long as automation is complete (Reis et al. 1994; Catalá-Icardo et al.
9 2002). To the authors' knowledge, this is the first PIF determination of AFB₁ in beer to
10 be reported. This is also the first application of multi-commuted-flow methodology to
11 the determination of this aflatoxin. Spiked as well as real samples of beer were used to
12 validate the results.
13
14
15
16
17
18
19

20 21 **Materials and methods**

22 *Apparatus and Instrumentation*

23
24 A Varian Cary-Eclipse Luminescence spectrometer (Varian Inc., Mulgrave, Australia)
25 was used for recording spectra and making fluorescence measurements. It was
26 controlled by a microprocessor fitted with a Cary-Eclipse (Varian) software package for
27 data collection and treatment. The following instrumental parameters were used:
28 excitation and emission slit widths were set at 10 and 20 nm, respectively and
29 photomultiplier voltage was 630 V. The excitation and emission wavelengths
30 established were 353/424 nm for the fluorescent photoproduct.
31
32
33
34
35
36

37 The multi-commuted flow system is shown in Fig 1. It was built with a four
38 channel Gilson Minipuls-3 (Villiers Le Bell, France) peristaltic pump fitted with a rate
39 selector and pump tubing type Solvflex (Elkay Products, Shrewsbury, MA, USA), three
40 161T031 NResearch three-way solenoid valves (Neptune Research, MA, USA) and an
41 electronic interface based on ULN 2803 integrate circuits. The valves were operated at
42 an electric potential of 12 V and a direct current of 100 mA. PTFE tubing (0.8 mm i.d.)
43 and methacrylate connections were also used. The software for controlling the system
44 was developed in Visual Basic 6.0 by our research group. A 176.752-QS Hellma flow
45 cell (Müllheim, Baden, Germany) (inner volume, 25 µL; light path length, 1.5 mm)
46 filled with C₁₈ silica gel was used in the detection area. The solid support was loaded as
47 methanol slurry just up to a height which enabled the light beam to pass completely
48 through the solid phase and the outlet was locked with glass wool, to avoid the beads
49 movement and allow the continuous flow.
50
51
52
53
54
55
56
57
58
59
60

1
2
3 For the photochemical on-line AFB₁ conversion, a home-made photoreactor was
4 prepared by loosely coiling 400 cm of PTFE tubing around a low-pressure mercury
5 lamp (15 W, 254 nm). The UV lamp was wrapped in aluminium paper and it was
6 introduced into an aluminium box for maximum light reflection and heat dissipation.
7 The photoreactor was placed just between V₃ and the detection area (see Figure 1). All
8 the experiments were carried out at room temperature.
9

10
11
12 Other apparatus consisted of a vacuum system 12-port Visiprep SPE Vacuum
13 Manifold (Supelco, Bellefonte, PA), a Crison Model 2002 pH-meter with a
14 glass/saturated calomel combination electrode (Crison, Barcelona, Spain) and a Selecta
15 Ultrasons ultrasonic bath (Barcelona, Spain).
16
17
18

19 **Figure 1**

20 *Reagents and solutions*

21
22
23 AFB₁ from *Aspergillus flavus* was purchased from Sigma-Aldrich, St. Louis, MO, USA.
24 The stock standard solution (50 mg L⁻¹) was prepared by dissolving 5 mg of the analyte
25 in a 50% (v/v) methanol:water solution. It was kept away from light with aluminium
26 foil and stored at 4°C in a refrigerator, remaining stable for at least four weeks. Working
27 standard solutions were prepared daily by taking an aliquot of the stock solution and
28 diluting with ultrapure water obtained from a Milli-Q system (Millipore, Bedford, MA).
29 All reagents were analytical reagent grade. The carrier solution (25% (v/v)
30 methanol:water solution) was prepared by dissolving the required volume of methanol
31 in water. C₁₈ bonded phase silica gel beads (55-105 µm average particle size) (Waters,
32 Milford, MA, USA) was used as active solid support in the detection area. Cation and
33 anion exchangers (Sephadex SP C-25, Sephadex CM C-25, Sephadex QAE A-25 and
34 Sephadex DEAE A-25, all of them having 40-120 µm average particle size) (Sigma,
35 Alcobendas, Madrid, Spain) were also tested for the retention of AFB₁ photoproduct.
36
37
38
39
40
41
42
43
44
45

46 Methanol, acetonitrile, toluene and cyclohexane were obtained from Panreac
47 (Barcelona, Spain). Octadecyl (C₁₈) Bakerbond SPE cartridges of 6 mL with 500 mg
48 of packing material (J.T. Baker, Phillipsburg, NJ, USA) and 0.20 µm nylon filters
49 (Millipore Corporation, Bedford, MA) were also used for solid phase extraction (SPE)
50 procedure.
51
52
53
54
55

56 *Sample preparation*

57
58
59
60

1
2
3 Seventeen different Spanish beers were purchased from local markets. The sample was
4 kept away from the daylight, as far as possible, with the aid of aluminium foil, along all
5 the process.
6
7

8 Pre-cleaning of the sample. A previous hydrophobic compounds removal, with
9 toluene and cyclohexane, was carried out to improve the subsequent extraction. Initially,
10 beers were previously degassed by sonication and then aliquots of 1 mL were spiked
11 with known amounts of AFB₁. Next, they were treated under gentle stirring, in
12 consecutive 3 min steps, first with 1 mL of toluene (twice) and then with 1 mL of
13 cyclohexane (twice). At the end of each step, the mixture was centrifuged for 5 min at
14 3500 rpm, and the supernatant was discarded. Finally, cleaned beer was dried under a
15 gentle stream of nitrogen to get rid of residual solvent.
16
17
18
19
20

21 Extraction of AFB₁. A C₁₈ cartridge was conditioned by passing consecutively 10
22 mL of acetonitrile and 10 mL of water. Subsequently, cleaned beer was loaded onto the
23 column at a flow-rate of 2-3 mL min⁻¹ under vacuum. Then, the cartridge was washed
24 with 10 mL of water and vacuum dried for 1 min. Finally, AFB₁ retained in the
25 cartridge was eluted with 2×1 mL of acetonitrile and the eluate was concentrated to
26 dryness under a gentle stream of nitrogen. The residue was re-dissolved to a final
27 volume of 10 mL in a 5% (v/v) methanol:water solution and the pH value was adjusted
28 at 5.5 by adding an HNO₃ or NaOH solution.
29
30
31
32
33
34
35

36 *Procedure*

37 The schematic valve system diagram is shown in Fig. 1. All experiments were carried
38 out in triplicate, and the results are expressed as peak height mean values. In the initial
39 status, the UV lamp and the peristaltic pump were switched on to condition the flow
40 system (constant flow rate 2 mL min⁻¹) until a stable baseline was recorded. Being all
41 valves switched off, the carrier solution (25% (v/v) methanol:water solution) flowed
42 through the system, while the sample solution was recycled to its vessel. Then, in order
43 to insert the sample in the flow system, valves V₁ and V₂ were switched on for 75 s and
44 V₃ for 15 s. In this way the sample solution circulated through the system, whereas the
45 carrier solution was recycled to its recipient. For the first 15 s of this step, the sample
46 plug was directed towards the waste through V₃, so cleaning the tubing between V₁ and
47 V₃ with the new sample solution. Over the next 60 s, as V₃ was deactivated, the sample
48 plug was pumped towards the photo-reactor. After this, all valves were switched off
49
50
51
52
53
54
55
56
57
58
59
60

again for 35 s, the time required for the sample solution to stay into the photo-reactor. At this point, valve V₁ was switched on for 120 s, in order to recycle the carrier solution to its recipient and stop the flow, so allowing the photo-degradation process. Finally when valve V₁ was switched off and the photo-degradation product arrived to the flow-cell the analytical signal was measured at the corresponding wavelengths, 353/424 nm/nm. The lamp was always switched on during a whole experimental session in order to obtain the best precision.

Results and Discussion

Optimisation of AFB₁ extraction

The fluorescence photo-degradation product generated from AFB₁ is selectively adsorbed on the non-ionic exchanger C₁₈ silica gel in the working conditions. Nevertheless, owing to the fact that some mycotoxins found in cereals such as ochratoxin A, aflatoxins, fumonisins, and trichothecenes (deoxynivalenol, T-2, and HT-2 toxins) can survive the brewing process, they can also be detected in beer (Romero González et al. 2009). A previous clean-up step was necessary in order to ensure the elimination of others species, including these common mycotoxins present in beers, which could be retained on the solid-phase in the detection area and compete with the photo-degradation product for the active sites.

One of the most often used possibility as clean-up step and direct extraction of liquid samples, in mycotoxins analysis, is SPE. With the purpose of establishing a reliable SPE method for the extraction of AFB₁ from several types of beer, some considerations were taken into account according to the literature (Kralj Cigic and Prosen 2009). First, C₁₈ cartridges were selected for the extraction procedure due to the slightly better affinity for aflatoxins, compared with others mycotoxins. Second, to elute the target compound, acetonitrile was used because it has demonstrated a higher selectivity for aflatoxins (B₁, B₂, G₁, and G₂) and HT-2 and T-2 toxins, whilst fumonisins (B₁ and B₂) were not extracted when this solvent was applied. In addition these two latter mycotoxins were eliminated in the washing step carried out, before the elution, due to their high solubility in water. The washing and elution volumes were optimised taking into account both the elimination of the most polar interfering substances without eluting AFB₁ and the most complete extraction of this latter. The volumes selected were 10 mL of water and 2 mL of acetonitrile, respectively.

1
2
3 Due to a large decrease in the fluorescence of the AFB₁ and its photo-product
4 when they were diluted in acetonitrile, it was necessary to evaporate the extract and
5 solubilise it in methanol. The percentage of methanol necessary for the solubilisation of
6 the final residue, once the target compound extracted of the C₁₈ cartridge and
7 evaporated, was tested in the range 0-30% (v/v) methanol:water solution. For dilutions
8 with methanol in a percentage larger than 10% the fluorescence signal decreased.
9 Therefore, the minimum amount of methanol capable of dissolving AFB₁ which was
10 5% (v/v) methanol:water solution was selected as optimum.
11

12
13 A pre-cleaning step, prior to the SPE procedure, was also necessary in order to
14 remove hard and soft resins (Molina García et al. 2011). It was fulfilled in the way
15 described in *section 2.3*. If it is not carried out AFB₁ is poorly recovered, probably due
16 to the competition between the target compound and those high molecular weight
17 substances by the active sites of the C₁₈ cartridge.
18

19 20 21 22 23 24 25 26 *Preliminary test of sorption*

27 The native fluorescence of AFB₁ can be enhanced by photochemical derivatisation
28 procedures. This technique allows a significant increase in the fluorescence signal of
29 AFB₁ due to the reaction with hydroxyl radical produced from water by ultraviolet
30 radiation, which leads to new structures with stable and higher fluorescence (Joshua
31 1993). This is an interesting alternative to its chemical derivatisation in order to
32 simplify the system, reduce the consumption of reagents and enhance selectivity. With
33 the purpose of improving, even more, both sensitivity and selectivity of the
34 determination of AFB₁ after its photo-chemical conversion with UV irradiation, several
35 sorption tests of both the original compound and the fluorescent photo-product on
36 different solid supports were carried out. The retention assays were performed at
37 different pH values and with anion-exchangers (Sephadex QAE A-25, and Sephadex
38 DEAE-25), cation-exchangers (Sephadex SP C-25 and Chelex 100) and non-ionic
39 supports (C₁₈ silica gel). In all the cases a higher signal was obtained when a solid
40 support was used, being practically insignificant in the case of cationic exchangers.
41 However, the others (anionic and non- ionic exchangers) provided a significant
42 retention. Anion-exchangers allowed a very strong retention of the AFB₁ photoproduct,
43 but its complete elution was very difficult in a reasonable time. Finally, C₁₈ silica gel
44 was selected since it provided both a strong retention and a quick elution of the AFB₁
45 photo-product.
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 Fluorescence spectra of AFB₁ and its photo-degradation product, recorded in
4 homogeneous solution, showed maximum excitation/emission wavelengths at 359/446
5 nm and 359/435 nm, respectively (Fig. 2). This is in agreement with the theoretical blue
6 fluorescence under UV light (430-530 nm for emission) belonging to AFB₁ and AFB₂,
7 contrary to AFG₁ and AFG₂ which have green fluorescence (530-590 nm). The spectra
8 of the AFB₁ photo-degradation product retained on C₁₈ resin showed maxima
9 excitation/emission wavelengths at 353/424 nm (Fig. 2). As a result of the pre-
10 concentration process of the AFB₁ photo-degradation product on the active sensing
11 area, a 20-fold increase in the PIF signal was obtained when SPS methodology was
12 used.
13
14
15
16
17
18
19

20 Figure 2

21 AFB₂ aflatoxin (AFB₂) is an important interfering species in the fluorimetric
22 determination of AFB₁ due to the substantial overlapping of their spectra (Mohammad
23 et al. 2007). The tolerated AFB₂/AFB₁ (w/w) ratio in homogeneous solution is about
24 0.1, which makes impossible to determine AFB₁ in presence of AFB₂ without a
25 previous separation of this latter. As we could check, in the established working
26 conditions AFB₂ did not photo-degrade under UV irradiation, which agrees with the
27 results previously found by other authors in aflatoxin photo-degradation tests (Joshua
28 1993). On the other hand, although AFB₂ itself was retained on C₁₈ silica gel solid
29 support it did not interfere in the AFB₁ determination at the concentration levels usually
30 found in beer. This fact can be attributed to the increase of the AFB₁ fluorescence signal
31 provided by both its photo-degradation and the sorption of the photoproduct generated
32 on a solid support and to the improvement in selectivity originated by the measurement
33 of the analytical signal in solid phase.
34
35
36
37
38
39
40
41
42

43 Taking into account the possible retention of other organic compounds of the
44 matrix on C₁₈ silica gel, the emission and excitation slits and filters were established
45 providing the best sensitivity and selectivity, respectively. The selected excitation and
46 emission slits were 10 and 20 nm, respectively. Filter ranges were set at 250-395 nm for
47 excitation and 420-1100 nm for emission. These values also supplied the best ratio
48 between analytical signal and background noise. With the same criterion, the voltage of
49 the photomultiplier tube was set at 630 V.
50
51
52
53
54
55

56 *Chemical variables*
57
58
59
60

1
2
3 Photo-chemical conversion of AFB₁, as well as the fluorescence properties of the
4 obtained photo-product and its sorption on the solid support is governed by the pH
5 value. Therefore, this is one of the most important variables to be taken into account.
6
7 The influence of this variable was studied by inserting into the system different analyte
8 solutions adjusted, using HNO₃ or NaOH solutions, to pH values ranging from 1 to 11.
9
10 The maximum fluorescence signal was obtained when pH 5.5 was used (Fig. 3). Higher
11 or lower pH values caused a decrease in the intensity of fluorescence. Therefore, a pH
12 value of 5.5 was selected as optimum for the next experiments. Several buffer solutions
13 in a pH ranging 5.0-6.0 and concentration levels between 0.01-0.1 mol L⁻¹ were tested
14 (citric acid/sodium hydroxide; sodium acetate/acetic acid; tartaric acid/sodium
15 hydroxide). It was observed the same variation in all cases, fluorescence intensity
16 decreased by half when comparing to the absence of buffer solution. This performance
17 may be due to the interference of buffer ions in the photochemical conversion of AFB₁,
18 so sample solution without buffering was finally chosen.
19
20
21
22
23
24
25

26 **Figure 3**

27
28 The nature of the carrier solution will affect mainly to the sorption of the photo-
29 degradation product on the solid support. Taking into account the non-polar nature of
30 the solid support used in this case, aqueous solutions with different methanol
31 percentage, up to 45% (v/v), were tested in order to obtain the maximum retention of
32 the AFB₁ photoproduct. A solution of 20% (v/v) methanol provided the highest
33 analytical signal and a decrease in this latter was observed for higher and lower
34 methanol percentages. On the other hand, an incomplete elution of the photo-product
35 was achieved for methanol percentages lower than 25%. Therefore, as a compromise
36 between sensitivity and complete regeneration of the solid support, a solution of 25%
37 (v/v) methanol:water was selected as carrier solution.
38
39
40
41
42
43
44
45

46 *Flow system variables*

47
48 Irradiation time. The irradiation time is the residence time of the sample inside the
49 photo-reactor under UV radiation. It is another critical variable in PIF methodology. In
50 order to optimize this variable, 2 mL of 8 µg L⁻¹ AFB₁ solution were inserted in the
51 system (2 mL min⁻¹), the flow was stopped just when the whole plug of sample was
52 within the photo-reactor (400 cm) and this was irradiated for increasing periods of time.
53
54
55
56
57
58
59
60

1
2
3 recorded. The results showed that the kinetic of photo-degradation of AFB₁ in the
4 working conditions is slow (Figure 4). With an irradiation time of 120 s the maximum
5 fluorescence signal was obtained. Therefore, this value was selected as optimum one.
6
7

8 **Figure 4**

9
10 Flow rate and photo-reactor length. In general, the residence time of the sample in
11 the photochemical reactor and, consequently, the irradiation time can be controlled by
12 the flow-rate and/or the length of the tubing around the lamp. As the time required for
13 AFB₁ degradation is high, instead of using a long photo-reactor tubing or stopping the
14 peristaltic pump, the carrier solution was recycled for the required time (120 s) once the
15 sample plug was placed inside the photo-reactor, so stopping the circulation of the
16 sample solution through the system. Consequently, the length of the photo-reactor was
17 selected in order to be just the minimum one required to accommodate the whole
18 sample plug (2 mL), namely 400 cm.
19

20
21 2 mL min⁻¹ was the flow-rate established. This was the maximum flow-rate
22 allowed by the flow-system without overpressure problems and it also allowed the best
23 throughput of the method.
24

25
26 Sampling time. In flow-through sensors is possible to improve sensitivity by only
27 increasing the sample volume inserted in the system. This effect is a consequence of the
28 sorption on a constant amount of solid support of increasing amounts of analyte in the
29 detection area. This feature allows both the enhancement of sensitivity, by increasing
30 the sample volume, and the reduction of strong matrix effects by means of a previous
31 dilution of the sample before its insertion in the system.
32

33
34 In a multi-commuted system the sample volume can be controlled by varying the
35 sampling time. With 8 µg L⁻¹ solution of AFB₁ and sampling times ranging from 10 s to
36 80 s, the fluorescence signal increased linearly until a value of 60 s. In addition, the
37 throughput of the system was also affected by this variable. Figure 5 shows the study of
38 the influence of sampling time. Sampling times lower than 60 s provided a quicker
39 elution of the photoproduct from the solid support but they also originated a significant
40 decrease in the analytical signal. Therefore, a 60 s sampling time was selected as a
41 compromise between fluorescence intensity and peak time. Higher sampling times
42 produced higher analytical signals than that obtained with 60 s. However, they also
43 originated wider flow peaks, so decreasing the sampling frequency.
44
45
46
47
48
49
50
51
52
53
54
55
56

57 **Figure 5**

Figures of merit

In the above established optimum working conditions, the calibration curve was established for the photoproduct of AFB₁ after injecting in triplicate, sample solutions containing increasing concentrations of the analyte. Analytical figures of merit are given in Table I. Quantification was carried out by using peak height as analytical signal. Data were fitted by standard least-squares treatment. The proposed methodology responds linearly in the AFB₁ concentration range 0.09-12 µg L⁻¹. The standard deviations of intercept and slope were also calculated (average of three determinations). Detection limit (LOD) and quantification limit (LOQ) were estimated as the concentration that produced a fluorescence signal equal to three and ten times the standard deviation of background fluorescence, respectively (MacDougall 1980). The LOD obtained with the proposed method allows the determination of this aflatoxin in beer at trace levels. This value, 0.029 µg L⁻¹, is similar or lower, in most of the cases, than others previously reported (Table II). On the other hand, LOQ attained by the method is much lower than the maximum level fixed by European Commission regulation for all cereals and products derived from cereals (2 µg kg⁻¹), so making the method suitable for routine analysis.

Tables I, II

Intra-day repeatability was established by comparing the response of 10 independent determinations of solutions containing 5 µg L⁻¹ of AFB₁. Inter-day repeatability was also performed for 10 consecutive days. RSDs obtained, in both cases, were low, and even though the measurements are made in solid phase.

Analytical applications

The proposed flow-through optosensor was successfully applied to the determination of AFB₁ in beer. The pre-treatment and procedure described in *Experimental* were used in each instance. The slope of the calibration curve obtained by spiking the final beer extracts with AFB₁ was different to that obtained by spiking the original samples, and both of them were different than that obtained by external calibration. This demonstrated the existence of a light negative matrix effect. This latter is due to the presence in the final extracts of interfering species and the incomplete recovery of AFB₁ after the pre-treatment. Consequently, the calibration curves were constructed with matrix-matched standards, that is, the analysis was carried out by spiking different

1
2
3 aliquots of a beer sample with increasing amounts of the analyte. The matrix effect was
4 evaluated by comparing the slopes of aqueous standards and standard addition
5 calibration curves for different beer samples ($m_{\text{standard}}/m_{\text{standard-addition}} \approx 0.9$). Of the
6 seventeen beers analysed, including normal ($n=12$) and non-alcoholic ($n=5$) beers, only
7 three samples contained traces of the target compound (0.86 , 0.28 and $0.17 \mu\text{g L}^{-1}$).
8 AFB₁ was not detected in the rest of beer samples investigated, including non-alcoholic
9 beers.
10

11
12
13
14 A recovery study, at three concentration levels, was also performed on both the
15 three samples containing AFB₁ and two of the samples no-containing this latter. The
16 results obtained summarized in Table III. In all cases good recoveries, ranging from 94
17 to 106% were achieved. The applicability of the proposed method to the analysis of
18 AFB₁ in beer was demonstrated by comparison with a reference method (Romero
19 González et al. 2009), based on ultrahigh-performance liquid chromatography-tandem
20 mass spectrometry. The statistical study of precision and accuracy of both the proposed
21 and the reference method was performed from F criterion and the t test, respectively
22 (Saunders and Fleming 1971). The results obtained in every case (Table III) show that
23 there is no significant statistical difference between the results obtained by both
24 methods, indicating the utility of the proposed method for routine analytical control.
25
26
27
28
29
30
31
32

33 **Table III**

34 35 36 37 38 **Conclusions**

39 An automatic spectroscopic method for the analysis of AFB₁ in beer has been
40 developed, for the first time. A photochemical induced fluorescence based flow-through
41 sensor, making use of the multi-commuted assembly, has been used by this effect. The
42 fluorescent photo-product generated on-line was selectively retained on an active solid
43 support, originating a transitory fluorescent signal. This fact added to the clean-up step
44 and SPE extraction carried out on the beer samples, makes the presented method to have
45 characteristics such as high sensitivity and selectivity, low reagent consumption, easy to
46 handle, rapidity and simplicity and low cost. Although the determination of AFB₁ in
47 complex matrices by the proposed method needs a previous pre-treatment, the time
48 consumed is compensated with the rapidity in the measurement of the analytical signal.
49
50
51
52
53
54
55

56 On the other hand, the detection limits of the proposed method are similar or even
57 quite lower than those obtained using reported chromatographic methods for AFB₁
58
59
60

1
2
3 determination in beer and other matrices. Therefore, it can be considered as an
4 interesting alternative to the chromatographic determination of this aflatoxin. In
5 addition, the results obtained in the analysis of several types of beer compare well
6 against those supplied by a reference liquid-chromatography method. All this shows the
7 feasibility of the proposed optosensor as screening method for determination of AFB₁ in
8 non-alcoholic and normal beer.
9
10
11
12

13 14 15 **Acknowledgements**

16
17 The authors acknowledge the financial support from “Consejería de Innovación, Ciencia
18 y Empresa” (Junta de Andalucía), Project P07-FQM-02673. L.M.G. also acknowledges
19 research fellowship from Spanish MEC.
20
21
22
23

24 25 **References**

- 26 Abdulkadar AHW, Al-Ali A, Al-Jedah J. 2000. Aflatoxin contamination in edible nuts
27 imported in Qatar. *Food Control* 11:157-160.
28
29 Ali N, Hasmin NH, Saad B, Safan K, Nakajima M, Yoshizawa T. 2005. Evaluation of a
30 method to determine the natural occurrence of aflatoxins in commercial traditional
31 herbal medicines from Malaysia and Indonesia. *Food and Chemical Toxicology*
32 43:1762-1772.
33
34 Ammida NHS, Micheli L, Palleschi G. 2004. Electrochemical immunosensor for
35 determination of aflatoxin B₁ in barley. *Analytica Chimica Acta* 520:159-164.
36
37 Asis R, Romina D, Paola DI, Mario AJ. 2002. Determination of aflatoxin B₁ in highly
38 contaminated peanut samples using HPLC and ELISA. *Food and Agricultural*
39 *Immunology* 14:201-208.
40
41 Braicu C, Puia C, Bodoki E, Socaciu C. 2008. Screening and quantification of
42 aflatoxins and ochratoxin A in different cereals cultivated in Romania using
43 thinlayer chromatography-densitometry. *Journal of Food Quality* 31:108-120.
44
45 Brera C, Debegnach F, Minardi V, Pannunzi E, De Santis B, Miraglia M. 2007.
46 Immunoaffinity column cleanup with liquid chromatography for determination of
47 aflatoxin B₁ in corn samples: interlaboratory study. *Journal of AOAC International*
48 90:765-772.
49
50 Catalá-Icardo M, García-Mateo JV, Martínez-Calatayud J. 2002. Multicommutation as a
51 powerful new analytical tool. *Trends in Analytical Chemistry* 21:366-378.
52
53
54
55
56
57
58
59
60

- 1
2
3 Cavaliere C, Foglia P, Guarino C, Nazzari M, Samperi R, Lagana A. 2007.
4 Determination of aflatoxins in olive oil by liquid chromatography-tandem mass
5 spectrometry. *Analytica Chimica Acta* 596:141-148.
6
7
8 European Commission Regulation (EC) No 1881/2006 of 19 December 2006. Official
9 Journal of the European Union L364/5.
10
11 Fu Z, Huang X, Min S. 2008. Rapid determination of aflatoxins in corn and peanuts.
12 *Journal of Chromatography A* 1209:271-274.
13
14 Garden SR, Strachan NJC. 2001. Novel colorimetric immunoassay for the detection of
15 aflatoxin B1. *Analytica Chimica Acta* 444:187-191.
16
17
18 Gilbert J, Vargas EA. 2003. Advances in sampling and analysis for aflatoxins. *Journal*
19 *of Toxicology* 22:381-422.
20
21
22 Giray B, Girgin G, Egin AB, Aydin S, Sahin G. 2007. Aflatoxin levels in wheat samples
23 consumed in some regions of Turkey. *Food Control* 18:23-29.
24
25
26 Goryacheva IY, Rusanova TY, Pankin KE. 2008. Fluorescent properties of aflatoxins in
27 organized media based on surfactants, cyclodextrins, and clixresorcinarenes. *Journal*
28 *of Analytical Chemistry* 63:751-755.
29
30
31 Gourama H, Bulleman LB. 1995. Relationship between aflatoxin production and mold
32 growth as measured by ergosterol and plate count. *Journal of Food Protection*
33 58:1395-1404.
34
35
36 Hajian R, Ensafi AA. 2009. Determination of aflatoxins B1 and B2 by adsorptive
37 cathodic stripping voltammetry in groundnut. *Food Chemistry* 115:1034-1037.
38
39
40 Hashemi J, Kram GA, Alizadeh N. 2008. Enhanced spectrofluorimetric determination
41 of aflatoxin B1 in wheat by second-order standard addition method. *Talanta*
42 75:1075-1081.
43
44
45 Herzallah Saqer M. 2009. Determination of aflatoxins in eggs, milk, meat and meat
46 products using HPLC fluorescent and UV detectors. *Food Chemistry* 114:1141-
47 1146.
48
49
50 International Agency for Research on Cancer (IARC). 1993. Monographs on the
51 evaluation of carcinogenic risks to humans. IARC, Lyon 6:245-395.
52
53
54 Joshua H. 1993. Determination of aflatoxins by reversed-phase high performance liquid
55 chromatography with post-column in-line photochemical derivatisation and
56 fluorescence detection. *Journal of Chromatography A* 654:247-254.
57
58
59
60

- 1
2
3 Kralj Cigic I, Prosen H. 2009. An overview of conventional and emerging analytical
4 methods for the determination of mycotoxins. *International Journal of Molecular*
5 *Sciences* 10:62-115.
6
7
8 Krska R, Schubert UP, Molinelli A, Sulyok M, MacDonald S, Crews C. 2008.
9 Mycotoxin analysis: an update. *Food Additives and Contaminants* 25:152-163.
10
11 Lee NA, Wang S, Allan RD, Kennedy IR. 2004. A rapid aflatoxin B1 ELISA:
12 development and validation with reduced matrix effects for peanuts, corn, pistachio,
13 and soybeans. *Journal of Agricultural and Food Chemistry* 52:2746-2755.
14
15
16 López-Flores J, Fernández-De Córdoba ML, Molina-Díaz A. 2005. Implementation of
17 flow-through solid phase spectroscopic transduction with photochemically induced
18 fluorescence: Determination of thiamine. *Analytica Chimica Acta* 535:161-168.
19
20
21 Mably M, Mankotia M, Cavlovic P, Tam J, Wong L, Pantazopoulos P, Calway P, Scott
22 PM. 2005. Survey of aflatoxins in beer sold in Canada. *Food Additives and*
23 *Contaminants* 22:1252-1257.
24
25
26 MacDougall D. 1980. Guidelines for data acquisition and data quality evaluation in
27 environmental chemistry. *Analytical Chemistry* 52:2242-2249.
28
29
30 Mansooreh M. 2009. Determination of aflatoxins in imported rice to Iran. *Food and*
31 *Chemical Toxicology* 47:2064-2066.
32
33
34 Miller JD. 2008. Mycotoxins in small grains and maize: old problems, new challenges.
35 *Food Additives and Contaminants* 25:219-230.
36
37
38 Mohammad A, Javad H, Gholamreza AK, Naader A. 2007. Enhanced synchronous
39 spectrofluorimetric determination of aflatoxin B1 in pistachio samples using
40 multivariate analysis. *Analytica Chimica Acta* 582:288-294.
41
42
43 Molina García L, Ruiz Medina A, Fernández-de Córdoba ML. 2011. A novel
44 multicommutated fluorimetric optosensor for determination of resveratrol in beer.
45 *Talanta* 83:850-856.
46
47
48 Nawaz S, Coker RD, Haswell SJ. 1995. HPTLC—a valuable chromatographic tool for
49 the analysis of aflatoxins. *Journal of Planar Chromatography* 8:4-9.
50
51
52 Papp E, Otta KH, Zaray G, Mincsovcics E. 2002. Liquid chromatographic determination
53 of aflatoxins. *Microchemical Journal* 73:39-46.
54
55
56 Peña R, Alcaraz MC, Arce L, Rios A, Valcarcel M. 2002. Screening of aflatoxins in
57 feed samples using a flow system coupled to capillary electrophoresis. *Journal of*
58 *Chromatogr A* 967:303-314.
59
60

- 1
2
3 Pietri A, Bertuzzi T, Agosti B, Donadini G. 2010. Transfer of aflatoxin B₁ and
4 fumonisin B₁ from naturally contaminated raw materials to beer during an industrial
5 brewing process. *Food Additives and Contaminants* 27:1431-1439.
6
7
8 Polixeni V, Panagiota M. 2009. Aflatoxin B₁ and ochratoxin A in breakfast cereals from
9 Athens market: Occurrence and risk assessment. *Food Control* 20:455-461.
10
11 O'Riordan MJ, Wilkinson MG. 2008. A survey of the incidence and level of aflatoxin
12 contamination in a range of imported spice preparations on the Irish retail market.
13 *Food Chemistry* 107:1429-1435.
14
15
16 Owino JHO, Arotiba OA, Hendricks N, Songa EA, Jahed N, Waryo TT, Ngece RF,
17 Baker PGL, Iwuoha EI. 2008. Electrochemical immunosensor based on
18 polythionine/gold nanoparticles for the determination of aflatoxin B₁. *Sensors*
19 8:8262-8274.
20
21
22
23 Quinto M, Spadaccino G, Palermo C, Centonze D. 2009. Determination of aflatoxins in
24 cereal flours by solid-phase microextraction coupled with liquid chromatography
25 and post-column photochemical derivatisation-fluorescence detection. *Journal of*
26 *Chromatography A* 1216:8636-8641.
27
28
29
30 Reis BF, Gine MF, Zagatto EAG, Lima JLFC, Lapa RA. 1994. Multicommutation in
31 flow analysis. Part 1. *Analytica Chimica Acta* 293:129-138.
32
33
34 Romero González R, Martínez Vidal JL, Aguilera Luiz MM, Garrido Frenich A. 2009.
35 Application of conventional solid-phase extraction for multimycotoxin analysis in
36 beers by ultrahigh-performance liquid chromatography-tandem mass spectrometry.
37 *Journal of Agricultural and Food Chemistry* 57:9385-9392.
38
39
40 Saunders L, Fleming R. 1971. *Mathematics and Statistics*, 2nd ed.. Pharmaceutical
41 Press, London, pp. 192.
42
43 Scott PM, Lawrence GA. 1997. Determination of aflatoxins in beer. *Journal of AOAC*
44 *International* 80:1229-1234.
45
46
47 Sforza S, Dall'Asta C, Marchelli R. 2005. Recent advances in mycotoxin determination
48 in food and feed by hyphenated chromatographic techniques/mass spectrometry.
49 *Mass Spectrometry Reviews* 25:54-76.
50
51
52 Shepard GS. 2009. Aflatoxin analysis at the beginning of the 21st century. *Analytical*
53 *and Bioanalytical Chemistry* 395:1215-1224.
54
55
56 Shtykov SN. 2002. Chemical analysis in nanoreactor: main concepts and applications.
57 *Journal of Analytical Chemistry* 57:859-868.
58
59
60

- 1
2
3 Stroka J, Otterdijk RV, Anklam E. 2000. Immunoaffinity column clean-up prior to thin-
4 layer chromatography for the determination of aflatoxins in various food matrices.
5 *Journal of Chromatography A* 904:251-256.
6
7
8 Tan Y, Chu X, Shen GL, Yu RQ. 2009. A signal-amplified electrochemical
9 immunosensor for aflatoxin B1 determination in rice. *Analytical Biochemistry*
10 387:82-86.
11
12
13 Turner NW, Subrahmanyam S, Piletsky S. 2009. Analytical methods for determination
14 of mycotoxins: A review. *Analytica Chimica Acta* 632:168-180.
15
16
17 Ventura M, Guillén D, Anaya I, Broto-Puig F, Lliberia JL, Agut M, Comellas L, Rapad
18 C. 2006. Ultra-performance liquid chromatography/tandem mass spectrometry for
19 the simultaneous analysis of aflatoxins B1, G1, B2, G2 and ochratoxin A in beer.
20 *Rapid Communications in Mass Spectrometry. Mass Spectrometry* 20: 3199-3204.
21
22
23 Wang JS, Kensler TN, Groopman JD. 1998. Toxicants in food: fungal contaminants.
24 John Wiley and Sons, New York, pp. 29-57.
25
26
27 Wejdani SK, Baharuddin S, Chew B, Nor HH, Abdussalam SMA, Muhammad IS,
28 Salleh B. 2010. Determination of aflatoxins in animal feed by HPLC with
29 multifunctional column clean-up. *Food Chemistry* 118:882-886.
30
31
32 Zollner P, Mayer Helm B. 2006. Trace mycotoxin analysis in complex biological and
33 food matrices by liquid chromatography-atmospheric pressure ionisation mass
34 spectrometry. *Journal of Chromatography A* 1136:123-169.
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure captions

Figure 1. Multi-commuted flow-injection system. Carrier solution (25% (v/v) methanol:water solution); V_1 , V_2 and V_3 three-way solenoid valves; PH photo-reactor; Flow cell filled with C_{18} silica gel. For each solenoid valve, the solid and dotted lines refer to “Off” and “On” positions, respectively. Flow rate, 2 mL min^{-1} . The scheme at the lower part shows the valve time program (sampling time, 60 s).

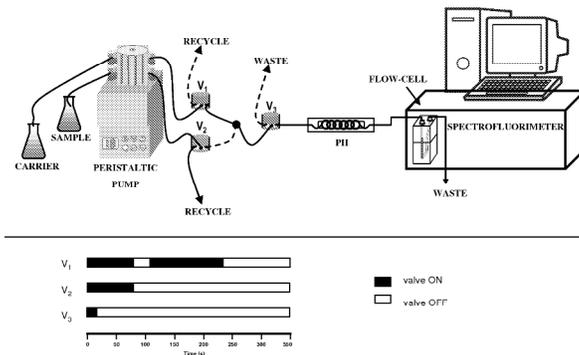
Figure 2. Excitation and emission fluorescence spectra of: (A) AFB_1 in homogeneous solution (0.2 mg L^{-1}); and (B) photoproduct of AFB_1 in homogeneous solution (continuous line) and retained on C_{18} silica gel (dashed line). 0.1 mg L^{-1} of AFB_1 (in solution); $12 \text{ } \mu\text{g L}^{-1}$ of AFB_1 (in solid phase); pH, 5.5; irradiation time, 120 s.

Figure 3. Influence of pH of the sample solution. $8 \text{ } \mu\text{g L}^{-1}$ AFB_1 ; carrier solution, 25 % MeOH (v/v); sampling time, 60 s; irradiation time, 120 s.

Figure 4. Influence of irradiation time in fluorescence signal of AFB_1 . $8 \text{ } \mu\text{g L}^{-1}$ of AFB_1 ; sampling time, 60 s; flow-rate, 2 mL min^{-1} ; photo-reactor length, 400 cm.

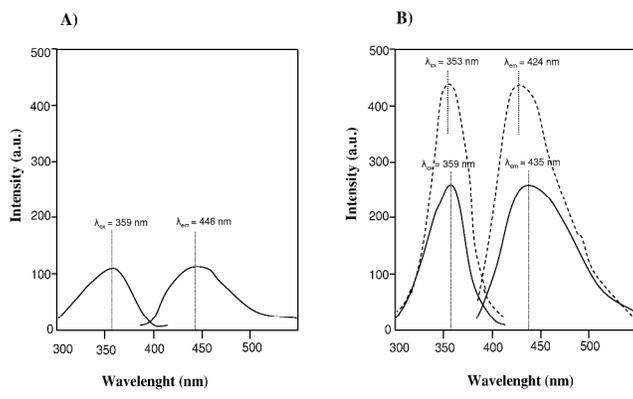
Figure 5. Influence of the sampling time in the fluorescence intensity (continuous line) and peak time (dashed lines). $8 \text{ } \mu\text{g L}^{-1}$ AFB_1 ; pH, 5.5; irradiation time, 120 s; flow-rate, 2 mL min^{-1} .

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



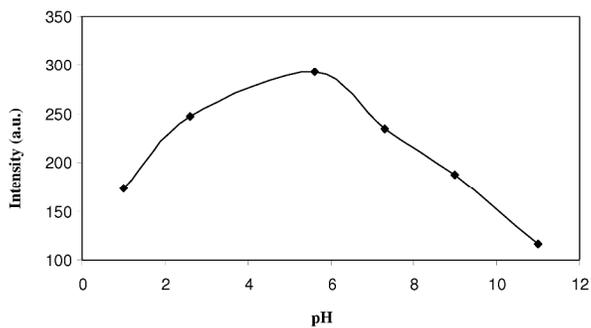
209x297mm (400 x 400 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



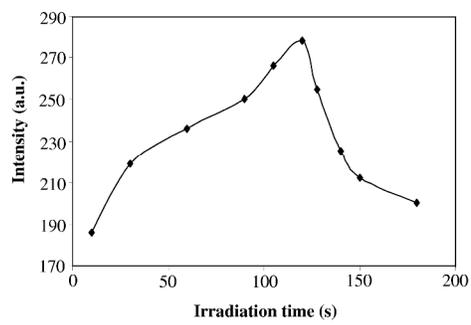
209x297mm (400 x 400 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



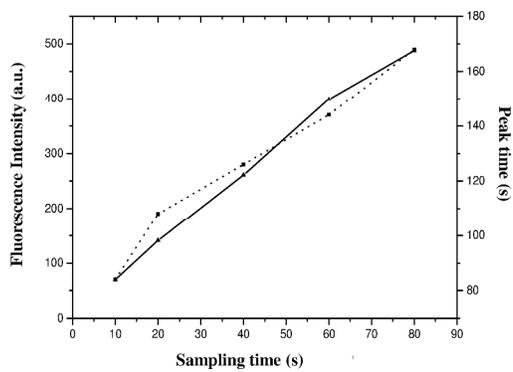
209x297mm (400 x 400 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



209x297mm (400 x 400 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



209x297mm (400 x 400 DPI)

Table I. Analytical parameters.

Parameter	Value
Linear dynamic range / $\mu\text{g L}^{-1}$	0.09 – 12
Calibration graph	
Intercept	6.68 ± 0.04
Slope / $\text{L } \mu\text{g}^{-1}$	34.8 ± 0.07
Correlation coefficient	0.9994
Detection limit / $\mu\text{g L}^{-1}$	0.029
Quantification limit / $\mu\text{g L}^{-1}$	0.09
Intraday RSD (%) ^a	1.4 ^b
Interday RSD (%) ^a	2.9 ^b
Sampling frequency / h^{-1}	10

^a n=10; ^b [AFB₁]= 5 $\mu\text{g L}^{-1}$

Table II. Comparison of the proposed method with others reported in literature.

Method	Linear range	LOD	Sample	Ref.
Liquid Chromatography (UHPLC) (mass spectrometry)	0.5-100 ($\mu\text{g L}^{-1}$)	0.04 ($\mu\text{g L}^{-1}$)	Beers	Romero González et al. 2009
Liquid Chromatography (UPLC) (mass spectrometry)	1-15 ($\mu\text{g L}^{-1}$)	0.1 ($\mu\text{g L}^{-1}$)	Beers	Ventura et al. 2006
Liquid Chromatography (fluorescent detection)	-	0.019 ($\mu\text{g L}^{-1}$)	Beers	Scott and Lawrence 1997
Liquid Chromatography (HPLC) (fluorescent detection)	5-35 ($\mu\text{g Kg}^{-1}$)	0.06 ($\mu\text{g Kg}^{-1}$)	Animal feeds	Wejdan et al. 2010
Liquid Chromatography (HPLC) (fluorescence detection)	0.05-24 ($\mu\text{g L}^{-1}$)	0.015 ($\mu\text{g L}^{-1}$)	Food samples	Herzallah Saqer 2009
Liquid Chromatography (HPLC) (fluorescence detection)	0.5-4 ($\mu\text{g Kg}^{-1}$)	0.02 ($\mu\text{g Kg}^{-1}$)	Breakfast Cereals	Polixeni and Panagiota 2009
Liquid Chromatography (UV detection)	20-200 ($\mu\text{g L}^{-1}$)	0.32 ($\mu\text{g Kg}^{-1}$)	Corn and Peanuts	Fu et al. 2008
Liquid Chromatography (HPLC-PD-FD)	0.1- 20 ($\mu\text{g Kg}^{-1}$)	0.035 ($\mu\text{g Kg}^{-1}$)	Cereal flours	Quinto et al. 2009
Liquid Chromatography (HPLC-PCD-FD)	-	0.1 ($\mu\text{g Kg}^{-1}$)	Rice	Mansooreh 2009
Spectrofluorimetry	0-18 ($\mu\text{g Kg}^{-1}$)	0.9 ($\mu\text{g Kg}^{-1}$)	Wheat	Hashemi et al. 2008
Spectrofluorimetry	0-15 ($\mu\text{g L}^{-1}$)	-	Pistachio	Mohammad et al. 2007
Spectrofluorimetry (one-and-two-photon-induced fluorescence)	-	46 ($\mu\text{g L}^{-1}$)	Wines and beers	Mably et al. 2005
Electrochemical immunosensor	0.1-10 ($\mu\text{g L}^{-1}$)	0.06 ($\mu\text{g L}^{-1}$)	Rice	Tan et al. 2009
Voltammetry	0.4-40 ($\mu\text{g L}^{-1}$)	0.15 ($\mu\text{g L}^{-1}$)	Groundnut	Hajian and Ensafi 2009

Table III. Determination of AFB₁ in beers.

Sample	Proposed method		Reference method	t_{calc}^b	F_{calc}^c
	Added ($\mu\text{g L}^{-1}$)	Found $\pm \sigma^a$ ($\mu\text{g L}^{-1}$)	Found $\pm \sigma^a$ ($\mu\text{g L}^{-1}$)		
Beer 1	-	0.86 \pm 0.05	0.90 \pm 0.02	1.28	6.25
(Normal)	1	1.90 \pm 0.09	1.95 \pm 0.05	0.87	3.24
	2	2.83 \pm 0.03	2.86 \pm 0.10	1.66	0.10
	4	4.84 \pm 0.04	4.96 \pm 0.04	2.12	0.21
Beer 2	-	0.28 \pm 0.03	0.29 \pm 0.08	0.20	0.14
(Normal)	2	2.33 \pm 0.05	2.25 \pm 0.06	1.77	0.70
	4	4.24 \pm 0.09	4.30 \pm 0.02	1.22	20.25
	6	6.30 \pm 0.06	6.36 \pm 0.08	1.04	0.56
Beer 3	-	0.17 \pm 0.07	0.15 \pm 0.06	0.37	1.36
(Normal)	6	6.22 \pm 0.05	6.19 \pm 0.02	0.96	6.25
	8	8.28 \pm 0.08	8.17 \pm 0.09	1.15	0.79
	10	9.95 \pm 0.02	10.13 \pm 0.03	2.16	1.96
Beer 4	-	<LOD	<LOD	-	-
(Normal)	1	0.94 \pm 0.07	1.06 \pm 0.04	2.56	3.06
	3	3.08 \pm 0.04	2.97 \pm 0.01	2.68	0.44
	5	5.11 \pm 0.08	5.20 \pm 0.06	1.56	1.77
Beer 5	-	<LOD	<LOD	-	-
(Non- alcoholic)	7	6.84 \pm 0.05	6.95 \pm 0.03	1.54	2.77
	9	9.19 \pm 0.02	8.88 \pm 0.01	2.72	0.44
	11	11.74 \pm 0.08	11.46 \pm 0.08	2.25	0.98

^aAverage of three replicates^bTheoretical value 2.772 (p=0.05)^cTheoretical value 39.00 (p=0.05)