Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1083 (2005) 7–13
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Determination of ochratoxin A in beer marketed in Spain by liquid chromatography with fluorescence detection using lead hydroxyacetate as a clean-up agent☆

Ángel Medina[a], Misericordia Jiménez[a,*], José V. Gimeno-Adelantado[b], Francisco M. Valle-Algarra[b], Rufino Mateo[b]

[a] *Departamento de Microbiología y Ecología, Facultad de Biología, Universitat de Valencia, Dr. Moliner 50, E-46100 Burjassot, Valencia, Spain*
[b] *Departamento de Química Analítica, Facultad de Química, Universitat de Valencia, Dr. Moliner 50, E-46100 Burjassot, Valencia, Spain*

Received 30 March 2005; accepted 31 May 2005
Available online 21 June 2005

Abstract

A new sample treatment for liquid chromatographic analysis of ochratoxin A (OTA) in beer is proposed. Degassed beer is mixed with lead hydroxyacetate, which precipitates some bulk components but does not remove OTA. The precipitate is separated and the acidified liquid is extracted with chloroform. The solvent is evaporated and the residue is dissolved in mobile phase (acetonitrile–water, 40:60, v/v; acidified at pH 3.0 with phosphoric acid) and separated by liquid chromatography using fluorescence detection. The limit of detection was 0.005 ng/ml. The average recovery rate and the average RSD of recovery in the spiking level range 0.01–0.5 ng/ml were 95.5% and about 5%, respectively. The method is cheaper that other alternative ones using immunoaffinity columns or other solid-phase extraction cleanup. The separation was optimised with regard to composition and flow of the mobile phase and no interference from the matrix was found. The method was applied to 88 samples of beer (domestic and imported) marketed in Spain. OTA was detected in 82.9% of them. The range for positive samples was 0.007–0.204 ng of OTA/ml.

Keywords: Beer; Clean-up; Lead hydroxyacetate; Liquid chromatography; Ochratoxin A; Mycotoxins

1. Introduction

Ochratoxin A (OTA) is the most prevalent among ochratoxins [1,2]. It is a widely distributed mycotoxin that is produced by some species of the genus *Aspergillus*, such as *A. ochraceus*, *A. niger*, and *A. carbonarius*, *Penicillium verrucosum* or the genera *Petromyces* and *Neopetromyces* [3–6]. Its occurrence has been reported in cereals [7–9], coffee [10–13], grape must and wine [14–17], beer [18,19] and human blood serum after intake of contaminated food [20–23]. This mycotoxin has been shown to be nephrotoxic, hepatotoxic, teratogenic and immunotoxic to animals, and its carcinogenicity in rats and mice is well established [24,25]. OTA has been related to Balkan Endemic Nephropathy (an endemic fatal disease in south-eastern Europe) and the development of urinary tract tumours in humans [20,23,26]. The International Agency for Research on Cancer has classified this toxin as a possible carcinogen to humans (group 2B) [27]. On April 19 2002 the Joint FAO/WHO Expert Committee on Food Additives recognized that OTA is a human nephrotoxic and causes carcinogenic effects in kidney [28]. The half-life of OTA in human blood serum may reach about 35 days [29].

Although reported levels are usually low in each commodity, there is great concern over this metabolite at present because it can be taken from a variety of food sources and the concurrent intake of different contaminated food and drinks

☆ Presented at the fourth Scientific Meeting of the Spanish Society of Chromatography and Related Techniques, Madrid, 5–7 October 2004.

* Corresponding author. Tel.: +34 963543144; fax: +34 963543099.
E-mail address: misericordia.jimenez@uv.es (M. Jiménez).

0021-9673/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2005.05.089

might provide a total amount of OTA near the provisional tolerable levels. In 1998, the Scientific Committee on Food of the European Commission considered that it would be prudent to reduce the tolerable daily intake to less than 5 ng/kg body mass [30].

Reported occurrence of this mycotoxin in beer before year 1990 is very scarce or null because six screening studies carried out in European and American beers were negative [31–36]. However, the limits of detection (LOD) of the analytical methods used in these studies were in the 1–10 ng/ml range. In further years, the use of more accurate and sensitive analytical methods for this toxin, with LOD between 0.05 and 0.1 ng/ml, led to its detection in beer in different countries like Germany [37–39], Canada, UK, Switzerland, Belgium, Morocco, Japan or Spain [18,40–43].

Maximum allowable limits for OTA in beer and wine have been laid down in various countries such as the Netherlands (0.3 ng/ml), Finland (0.5 ng/ml) [44] or Italy (0.2 ng/ml) [19].

The most widely technique used for analysis of OTA is liquid chromatography with fluorescence detection (LC–FLD) [14,45]. Alternative detection methods, such as photodiode array detection (LC–PDA) [44,46] or mass spectrometry (LC–MS–MS) [29,47–50] have also been used. Other techniques for this analysis are TLC, GC–MS of the trimethylsilyl derivative [44], capillary electrophoresis with laser induced fluorescence [43] and enzyme immunoassay (EIA) [51].

Various extraction and cleanup protocols for OTA in beer have been reported. Some procedures use addition of $NaHCO_3$ and NaCl to degassed samples [41], followed by clean-up on immunoaffinity columns (IAC). Jorgensen [52] added degassed samples directly to IAC columns. A collaborative test where dilution with polyethyleneglycol 8000-$NaHCO_3$, IAC clean-up and analysis by LC–FLD were applied to beer and wine, led to acceptation of this method as official first action by the AOAC [45]. Various sample treatment procedures such as liquid-liquid extraction and clean-up using solid-phase extraction with regard to determination of OTA in beer and other beverages have been compared recently [53].

With regard to the LC separation, most authors used reversed-phase columns. Reported mobile phases used for separation are acetonitrile–water–acetic acid (99:99:2, v/v/v) [45], acetonitrile–water–acetic acid (50:49:1, v/v/v) [52], acetonitrile–0.2 M phosphate buffer, pH 8.5–water containing 3 mM cetyltrimethylammonium bromide (60:3:37, v/v/v) [18], 48% acetonitrile–52% 4 mM sodium acetate–acetic acid (19:1, v/v) [42], or methanol–9% aqueous acetic acid mixed in gradient mode [41]. Post-column addition of ammonia to acidic mobile phase has been shown to increase fluorescence yield, thus contributing to increase sensitivity [41,52,54]. The most critical step to get accurate and reproducible results is obtaining clean extracts free from matrix interferences.

The aim of this study was first to find a sensitive, accurate, reproducible and economic LC method for analysis of OTA in beer and then, its application to the knowledge of the occurrence of this mycotoxin in national and imported beers that are marketed in Spain. A clean-up procedure using lead hydroxyacetate as a precipitating agent for dyes and other components has been assayed successfully for the first time in the analysis of OTA in beer.

2. Materials and methods

2.1. Samples

A total of 88 beer samples were purchased in different Spanish retail markets. Thirty-one of them were Spanish beers. The 57 remaining beers had been manufactured in different countries as Germany (15), Denmark (8), the Netherlands (12), Belgium (13), Scotland (1), USA (4), Mexico (2) and Australia (1). One sample had non-defined origin but its label read ‘Made in EU’. The samples were stored in their original bottles or containers in fridge at 4–5 °C until analysis. Sample containers were opened the day before analysis to begin degasification and were still kept in fridge [53].

2.2. Standards and reagents

The ochratoxin A standard was purchased from Sigma (Sigma–Aldrich, Alcobendas, Spain). A stock solution of about 500 mg/l was prepared by solving 1 mg of OTA in 2 ml of toluene–acetic acid (99:1, v/v). A series of working standards was prepared by evaporation of known aliquots of the stock solution and dissolution in filtered LC mobile phase. They were used to calibrate the detector response. The concentration of the stock solution was determined by measuring absorbance at 333 nm of a diluted solution (20–30 mg/l) of OTA in toluene–acetic acid (99:1, v/v) [8].

Acetonitrile, chloroform, and methanol (all LC grade), acetic acid (A.R.) and phosphoric acid (85%, A.R.) were from J.T. Baker (Deventer, the Netherlands). Pure water was obtained from a Milli-Q apparatus (Millipore, Billerica, MA, USA) and was used when water was required. Lead hydroxyacetate solution (25% PbO) and sodium hydrogencarbonate were from Panreac (Panreac Química, Barcelona, Spain). Polyethylene glycol (PEG) 8000 was from Aldrich (Sigma–Aldrich). Glass microfiber filters (GF/C) were from Whatman (Maidstone, UK). OchraTest immunoaffinity columns were from Vicam Science Technology (Watertown, MA, USA).

2.3. Sample preparation

About 250 ml of cool beer (containers had been kept at 5 °C and opened the day before) was thoroughly degassed in ultrasonic bath for 40 min in 500-ml Erlenmeyer flask. Seventy milliliters of degassed beer was transferred to a 100-ml Erlenmeyer flask and 0.8 ml of 1 M aqueous solution of NaOH was added. After slight shaking, 1 ml of a 25% aqueous solution of lead hydroxyacetate was added. The mixture was vigorously shaken for 2 min and another 1 ml of lead

hydroxyacetate solution was added. The mixture was centrifuged at 4500 rpm for 10 min and the supernatant was collected.

2.4. Extraction

Fifty-two milliliters of clear supernatant (equivalent to 50 ml of beer) was acidified to pH 2.8–3 with concentrated phosphoric acid. A precipitate was formed and the solution was filtered through glass microfiber filter paper Whatman GF/C. Then, the filtrate was extracted with 12 ml of chloroform in a separatory funnel. The organic phase was separated and the aqueous phase was re-extracted twice with 9 ml of chloroform. The organic extracts were reunified and evaporated in rotary evaporator at 40 °C. The residue was dissolved in 2 ml of methanol containing 5% (v/v) of acetic acid and transferred to a glass vial. The solvent was evaporated at 50 °C under N_2 stream and the residue was solved in 250 μl of mobile phase. The concentration ratio was 200:1.

2.5. Liquid chromatographic analysis

The LC system consisted of a Waters 600 pump, a Waters 717 automatic injector and a Waters 474 scanning fluorescence detector. Millennium 32 software, version 3.05.01 (Waters, Milford, MA, USA) was used to control the system and to process signals. Separation was performed on stainless steel LiChrospher 100 C_{18} reversed-phase column (250 mm × 4 mm, 5 μm particle size) connected to a guard column (4 mm × 4 mm, 5 μm particle size) filled with the same phase (Agilent Technologies, Waldbronn, Germany). The column was kept at 30 °C. Quantification of OTA was performed by measuring peak area at OTA retention time and comparing it with the calibration curve calculated from standard solutions. Different mobile phases containing either methanol and water or acetonitrile and water were assayed to optimize OTA detection and quantification. They were degassed by means of an on-line vacuum degassing device supplied by Waters. Because all tested mobile phases were acidic, the excitation and emission wavelengths were 330 and 460 nm, respectively. One hundred microliters of solution was injected into the liquid chromatograph.

2.6. Recovery evaluation

Recovery experiments were performed in triplicate according to Nakajima et al. [18] on an OTA-free Spanish pale beer sample, which was spiked with the mycotoxin to provide 0.01, 0.05, 0.1, and 0.5 ng of OTA/ml sample spiking levels. Appropriate volumes of OTA standard solution were added to 100-ml Erlenmeyer flasks. The solvent was evaporated under gentle stream of N_2. Seventy milliliters of a previously degassed OTA-free Spanish pale beer sample was added. The residue was dissolved in beer using ultrasonic bath for 1 min and homogenized by gentle mixing. Then, the previously indicated procedure for beer sample treatment was followed.

2.7. Alternative procedure: addition of PEG 8000-$NaHCO_3$ and IAC clean-up

The proposed method was compared with the AOAC official method [45], but using double sample amount [53]. Twenty milliliters of degassed beer sample was thoroughly diluted with 20 ml an aqueous solution containing 5% (w/v) of $NaHCO_3$ and 1% (w/v) of PEG 8000. The pH was set to 8.5 with 1 M NaOH solution. The solution was filtered through Whatman glass microfiber filter. Then, 20 ml of the filtrate (equivalent to 10 ml of sample) was passed through an OchraTest column at 1 drop/s flow-rate. The column was successively washed with 5 ml of an aqueous solution containing NaCl (2.5%, w/v) and $NaHCO_3$ (0.5%, w/v) and 5 ml of water to eliminate most impurities. OTA was eluted with 2 ml of methanol. After solvent evaporation under N_2 stream at 50 °C, the residue was dissolved in 0.250 ml of mobile phase. One hundred microliters was injected into the liquid chromatograph coupled to a fluorescence detector. Sample concentration ratio was 40:1. The mobile phase was acetonitrile–water (40:60, v/v) acidified to pH 3.0 with phosphoric acid (0.1 ml acid in 2 l of mobile phase).

3. Results and discussion

3.1. Optimisation of separation conditions

The chromatograms obtained by the method using precipitation of beer components with lead hydroxyacetate (Fig. 1) showed more peaks than those obtained by dilution with PEG-$NaHCO_3$ solution followed by IAC clean-up (Fig. 2). Therefore, to avoid co-elution of OTA with beer components and to optimise peak performance, several mixtures of methanol–water and acetonitrile–water were tested as mobile phases.

3.2. Methanol–water mixtures

The tested ratios of methanol–water mixtures were in the 40:60 to 70:30 (v/v) range (Table 1). Moreover, two pH modifiers (phosphoric acid and acetic acid) were used to separate OTA in its acidic form.

Methanol–water mixtures with 50:50 and 40:40 (v/v) ratios at pH 5.3 using acetic acid as acid modifier did not provide satisfactory results because OTA peaks were wide and showed long queues, which makes integration difficult and can give rise to overlapping. Reduction of pH to 3.5 increased OTA retention and band broadening. The methanol–water 70:30 (v/v) mixture provided a retention time of 7.9 min for OTA and acceptable peak shape but peaks from beer interfered avoiding the correct integration of the OTA peak. The

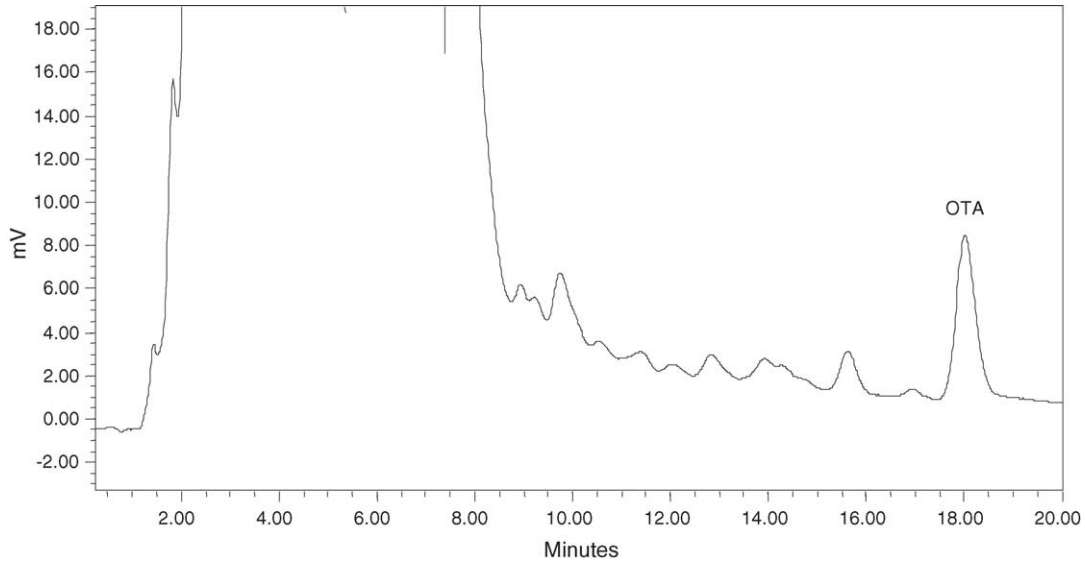

Fig. 1. Liquid chromatogram of a beer sample spiked with OTA after lead hydroxyacetate treatment and chloroform extraction. Spiking level: 0.5 ng/ml. Conditions: excitation wavelength (330 nm); emission wavelength (460 nm); mobile phase: acetonitrile–water (60:40, v/v) acidified at pH 3.0 with phosphoric acid as acid modifier at 1.4 ml min^{-1} flow-rate.

methanol–water 60:40 (v/v) mixture (pH 3.5) using phosphoric acid as modifier led to a quite broad OTA peak showing retention time >20 min. No assay with these kinds of mixtures provided satisfactory results with regard to reliable quantification of OTA.

3.3. Acetonitrile–water mixtures

The results observed with different mixtures of acetonitrile–water can be seen in Table 1. The first three mixtures were rejected because of interferences from matrix peaks. Acetic acid used as modifier proved worse than phosphoric acid with regard to OTA peak shape. The acetonitrile–water (35:65, v/v) mixture provided quite long retention time for OTA (near 30 min) and thus it was considered unsatisfactory. The best results in terms of lack of interfering peaks were obtained using the acetonitrile–water (40:60, v/v) pH 3.0 mixture but the OTA peak retention time was excessively long. However, it was lowered to 18 min when the flow was increased to 1.4 ml min^{-1} maintaining isocratic conditions. Then, this mixture was selected as mobile phase for further studies.

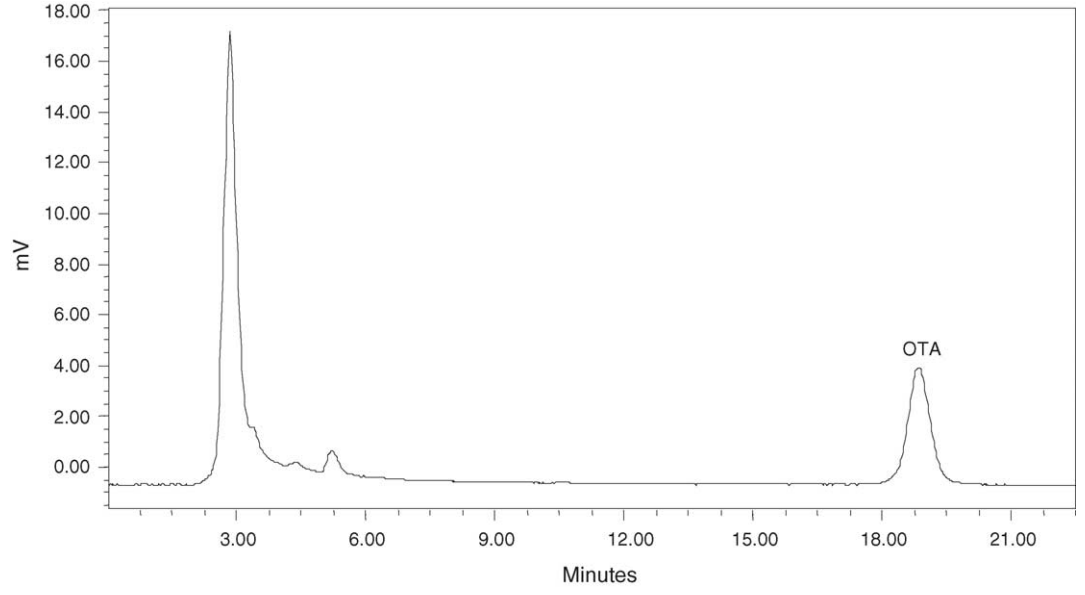

Fig. 2. Liquid chromatogram of a beer sample spiked with OTA without lead hydroxyacetate treatment. The sample was treated with PEG 8000/$NaHCO_3$ and cleaned up with immunoaffinity column. Spiking level: 0.5 ng/ml. Conditions: as in Fig. 1.

Table 1
Assayed mobile phases for OTA separation by LC

Mixture	pH	Flow (ml min^{-1})	Retention time (min)	OTA peak shape	Interference from matrix peaks
Methanol–water (40:60, v/v)	5.30	1	8.5	Broad with queue	Yes
Methanol–water (40:60, v/v)	3.50	1	10.6	Broad with queue	Yes
Methanol–water (50:50, v/v)	5.30[a]	1	7.7	Broad with queue	–
Methanol–water (50:50, v/v)	5.30	1	8.9	Broad with queue	–
Methanol–water (50:50, v/v)	3.50	1	7.7	Broad with queue	–
Methanol–water (60:40, v/v)	5.30	1	9.3	Broad with queue	–
Methanol–water (60:40, v/v)	3.50[a]	1	>20	Broad with queue	–
Methanol–water (60:40, v/v)	3.50	1	12.6	Acceptable	Yes
Methanol–water (70:30, v/v)	3.50	1	7.9	Acceptable	Yes
Acetonitrile–water (45:55, v/v)	4.50	1	10.4	Acceptable	Yes
Acetonitrile–water (45:55, v/v)	4.00[a]	1	6.2	Acceptable	Yes
Acetonitrile–water (45:55, v/v)	3.55	1.2	16.1	Acceptable	Yes
Acetonitrile–water (40:60, v/v)	3.55[a]	1.2	12.5	Acceptable	Yes
Acetonitrile–water (40:60, v/v)	3.00	1.2	21.4	Acceptable	No
Acetonitrile–water (40:60, v/v)	3.00	1.4	18	Acceptable	No
Acetonitrile–water (35:65, v/v)	3.55	1.2	∼30	Broad with queue	–

[a] Acetic acid was used as acid modifier.

3.4. Methanol washing

A washing step was required after the normal analysis time to speed late eluting peaks from some samples. It was done by changing the mobile phase to a methanol–water (80:20, v/v) mixture for 5 min. A methanol–water (70:30, v/v) mixture was used after analysis time by Aboul-Enein et al. [55] during 8 min to clean the chromatographic system and get more reproducible results. Sharp and symmetric peaks were obtained for OTA by these authors when this washing was applied.

Degassing time of beer samples by sonication was optimised. Forty minutes was selected after a trial of experiences instead of 1 h, a previously reported period.

Addition of lead hydroxyacetate as clarifying/precipitating agent helps to clean beer samples from bulk components, which results in flatter chromatographic baselines and lack of interfering neighbour peaks under appropriate LC conditions. In this way, the determination of OTA in beer samples is highly improved by this simple clean-up procedure before chloroform extraction.

3.5. Recovery of OTA

Once the optimised LC conditions were set, a calibration line for OTA was calculated using standard solutions. The OTA range was 0.05–10 ng of injected toxin (equivalent to 0.0025–0.5 ng/ml beer), and r^2 was 0.9998. The limit of detection of the method was 0.005 ng/ml beer (based on a signal/noise ratio of 3:1).

The results of recovery experiments of the proposed analytical procedure are shown in Table 2. Three replicates were carried out at each spiking level. The recovery range was 91.4–99.8% (average 95.5%) for spiking levels ranging 0.01–0.5 ng of OTA per milliliter of sample, respectively. The RSD range for recoveries was 4.0–6.7% at these spiking levels. On the basis of these data the method can be considered very good to be applied to OTA determination in beer. The alternative method using PEG-$NaHCO_3$ and IAC clean-up provided worse recoveries (61.0 and 63.7% at 0.1 and 0.5 ng/ml spiking levels, respectively according to a previous study [53]. Repeatability was also better working with the new procedure.

3.6. Survey of OTA in beer

The results of the analysis of 88 different beer samples are summarised in Table 3. OTA was detected in 73 samples (82.9%). The range for positive samples was 0.007–0.204 ng of OTA/ml. The occurrence level of this mycotoxin in Spanish beer was 83.8% (26 positives out of 31 samples) and the average concentration of OTA in Spanish beer was 0.0358 ng/ml. The incidence was quite similar to beers that had been brewed in the other countries (47 positive out of 57 samples, 82.45%) and the average concentration of OTA (0.0459 ng/ml) was rather similar.

Table 2
Recovery data of the LC method using lead hydroxyacetate for analysis of OTA in beer

OTA spiking level (ng/ml beer)	Mean recovery[a] (%)	Standard deviation of recovery	RSD (%)
0.50	99.8	4.0	4.0
0.10	96.1	6.0	6.2
0.05	94.7	5.4	5.7
0.01	91.4	6.1	6.7

[a] Number of replicates = 3.

Table 3
Occurrence of OTA in different beer samples marketed in Spain

Origin country	Analysed samples	Positive samples	Ochratoxin A (ng/ml sample)	
			Mean value	Overall range
Spain	31	26	0.0358	ND[a]–0.1468
Germany	15	10	0.0448	ND–0.2042
Denmark	8	6	0.0366	ND–0.096
The Netherlands	12	12	0.0254	0.0114–0.1320
Belgium	13	11	0.0595	ND–0.1204
Scotland	1	1	0.2011	–
Undefined[b]	1	1	0.0555	–
USA	4	3	0.0575	ND–0.1148
Mexico	2	2	0.0666	0.0322–0.101
Australia	1	1	0.0162	–

[a] ND: not detected at a LOD = 0.005 ng/ml (signal/noise ratio 3:1).
[b] "Made in EU" was labelled on the bottle.

This occurrence level is higher that those found by other authors who have carried out similar studies. In a screening study carried out on Italian and imported beers, Visconti et al. [19] found that 50% of beer samples contained detectable levels of OTA. The higher frequency of positive samples obtained in the present study can be due to the low LOD for the proposed method. However, Nakajima et al. [18], Burdaspal and Legarda [41] and Tangny et al. [43], found even higher occurrence levels (>90%), which can be due to the reported low detection limits of their methods (0.001, 0.004 and 0.003 ng/ml, respectively). The highest OTA level found by Nakajima et al. [18], in their analyses on 94 beer samples was 0.0662 ng/ml, which is quite lower than the highest level found by us and other European authors [19,41,43].

Therefore, the results shown in this article dealing with occurrence of OTA in beer marketed in Spain are comparable to those found in other screening studies made in different regions of the world like USA, Japan, Canada, and some European countries.

OTA is likely to enter the final beer from malt although other additional origins cannot be rejected. Some recent studies describe the occurrence of OTA in malt barley and malt that are used in breweries [56,57]. Other studies tried to find out the persistence of this toxin along the period of time necessary to convert the raw materials into beer. Baxter et al. [58] report that about 13–32% of the OTA present in malt barley reaches the final product. Thus, it may be taken into consideration the potential risk of this beverage regarding its incidence into the provisional tolerable daily intake (PTDI) of OTA. In spite of these considerations, the average level of OTA in beer manufactured in Spain is, according to our survey, 0.0358 ng/ml. One person weighing 60 kg and drinking 500 ml of beer daily would take 0.298 ng of OTA/kg body mass. This value is far below from the PTDI of 5 ng/kg body mass [30] established by the Scientific Committee on Food of the European Union. However, beer is not the only source of OTA in the typical Spanish diet, which also includes other products (vine, cereals products, bread, nuts, coffee, etc.) that can contain OTA.

4. Conclusions

A new method for determination of ochratoxin A in beer has been proposed. It uses reversed-phase LC separation and fluorescence detection. The main particularity is that many bulk components are precipitated by lead hydroxyacetate and, after separation of solid materials and acidification of the liquid, OTA is extracted with chloroform. Lead hydroxyacetate does not produce any removal of OTA. The selected mobile phase is acetonitrile–water 40:60 (v/v, pH 3.0) using phosphoric acid as acid modifier. No gradient is needed but a five minutes post-analysis run with methanol–water (80:20, v/v) as a mobile phase is helpful to clean following chromatograms from 'ghost' peaks. Chromatographic baselines are flat and there are no interfering peaks appearing in the vicinity of the OTA peak. The method is cheaper than others because solid-phase extraction cartridges or expensive IAC columns are not needed. However, the performance is good. The method is sensitive because LOD was 0.005 ng/ml, quite accurate as high recovery rates (average >95%) were obtained at low spiking levels (0.01–0.5 ng/l), and precise because RSD was 4.0–6.7% in this spiking range.

The method was applied to 88 different samples of beer that are marketed in Spain (both domestic and imported). OTA was detected in 82.9% of them. The occurrence of this mycotoxin in beer brewed in Spain was 83.8%, very similar to beer brewed in other countries.

Acknowledgements

The authors wish to acknowledge financial support from the 'Conselleria de Empresa, Universitat i Ciència (Generalitat Valenciana)' and from the Spanish Government 'Ministerio de Educación y Ciencia' (project ref. AGL-2004-07549-C05-02/ALI).

References

[1] D.B. Scott, Mycopathol. Mycol. Appl. 25 (1965) 213.

[2] K.J. Van Der Merwe, P.S. Steyn, L. Fourie, Nature (Lond.) 205 (1965) 1112.
[3] P. Krogh (Ed.), Mycotoxins in Food, Academic Press, London, 1987, p. 97.
[4] P.M. Scott, in: J.D. Miller, H.L. Trenholm (Eds.), Mycotoxins in Grain-Compounds other than Aflatoxin, Eagan Press, St. Paul, MN, 1997, p. 97.
[5] M.L. Abarca, M.R. Bragulat, G. Castellá, F.J. Cabañes, Appl. Environ. Microbiol. 60 (1994) 2650.
[6] J.C. Frisvad, R.A. Samson, Stud. Mycol. 45 (2000) 201.
[7] A.D. Damoglou, G.A. Downey, W. Shannon, J. Sci. Food Agric. 35 (1984) 395.
[8] G.M. Wood, S. Patel, A.C. Entwisle, A. Boenke, Food Addit. Contam. 13 (1996) 519.
[9] A. Rizzo, M. Eskola, M. Atroshi, Eur. J. Plant Pathol. 108 (2002) 631.
[10] M.E. Stack, P.B. Mislivec, T. Denizel, R. Gibson, E. Pohland, J. Food Prot. 46 (1983) 965.
[11] C. Micro, M. Grossi, M. Miraglia, C. Brera, Food Addit. Contam. 6 (1989) 333.
[12] I. Studer-Rohr, D.R. Dietrich, J. Schlatter, Ch. Schlatter, Food Chem. Toxicol. 33 (1995) 341.
[13] S. Patel, C.M. Hazel, A.G.M. Winterton, A.E. Gleadle, Food Addit. Contam. 14 (1997) 217.
[14] B. Zimmerli, R. Dick, Food Addit. Contam. 13 (1996) 655.
[15] P.A. Burdaspal, T.M. Legarda, Alimentaria 99 (1999) 107.
[16] H. Ottender, P. Majerus, Food Addit. Contam. 17 (2000) 793.
[17] C.A. Da Rocha, V. Palacios, M. Combina, M.E. Fraga, A. De Oliveira Rekson, C.E. Magnol, A.M. Dalcero, Food Addit. Contam. 19 (2002) 408.
[18] M. Nakajima, H. Tsubouchi, M. Miyabe, J. AOAC Int. 82 (1999) 897.
[19] A. Visconti, M. Pascale, G. Centonze, J. Chromatogr. A 888 (2000) 321.
[20] H. Bacha, K. Maaroufi, A. Achour, M. Hamammi, F. Ellouz, E.E. Creppy, in: E.E. Creppy, M. Castegnaro, G. Dirheimer (Eds.), Ochratoxines et Ochratoxicoses humaines en Tunisie, Human Ochratoxicoses and its Pathologies, John Libbey Eurotech, Montrouge, France, 1993, p. 111.
[21] E.E. Creppy, A.M. Betbeder, J.P. Godin, J.P. Fillastre, P. Simon, P. Lasseur C. Combe, M. Aparico, Landbauforschung Völkenrode, Sonderheft, vol. 157. Proceedings from 17 Mykotoxin-Workshop in der Bundesforschungsanstalt für Landwirtschaft Braunschweig, Völkenrode', 1995, p. 56.
[22] K. Maaroufi, A. Achour, A.M. Betbeder, M. Hammami, F. Ellouz, E.E. Creppy, H. Bacha, Arch. Toxicol. 69 (1995) 552.
[23] B. Radic, R. Fuchs, M. Peraica, A. Lucis, Toxicol. Lett. 91 (1997) 105.
[24] M. Castegnaro, U. Mohr, A. Pfohl-Leszkowicz, J. Esteve, J. Steinmann, T. Tillmann, J. Michelon, H. Bartsch, Int. J. Cancer 77 (1998) 70.
[25] A. Pfohl-Leszkowicz, E. Pinelli, H. Bartsch, U. Mohr, M. Castegnaro, Mol. Carcinog. 23 (1998) 76.
[26] I.G. Nikolov, D. Petkova-Bocharova, M. Castegnaro, A. Pfohl-Leskowicz, C. Gill, N. Day, I.N. Chernozemsky, J. Environ. Pathol. Toxicol. Oncol. 15 (1996) 201.
[27] Some Naturally Occurring Substances: Food Items and Constituents, Heterocyclic Aromatic Amines and Mycotoxins, (IARC Monographs on Evaluation of Carcinogenic Risks to Humans, vol. 56) International Agency for Research on Cancer, Lyon, 1993, p. 489.
[28] T. Kuiper-Goodman, P.M. Scott, Biomed. Environ. Sci. 2 (1989) 179.
[29] I. Studer-Rohr, Dissertation No. 11071, Federal Institute of Technology, Zürich, Swiss, 1995.
[30] Health Scientific Committees. Outcome of discussions 14. Opinion of the Scientific Committee on Food on Ochratoxin A, Office for Official Publications of the European Communities, vol. 56, Luxembourg, 1998, p. 489.
[31] H. Frishbach, J.V. Rodricks, J. Assoc. Off. Anal. Chem. 56 (1973) 767.
[32] P. Majerus, R. Woller, Z. Lebensm, Unters. Forsch. A 178 (1984) 79.
[33] A. Vecchio, C. Finoli, M. Peccedi, G. Cerutti, Technol. Alimentari 8 (1985) 84.
[34] G. Cerutti, A. Vecchio, C. Finoli, A. Trezzi, Mon. schr. Brauwiss. 40 (1986) 455.
[35] Ministry of Agriculture, Fisheries and Food. Mycotoxins. Her Majesty's Stationary Office, London, No. 17, 1987.
[36] R. Tressl, E. Hommel, B. Helak, Mon. schr. Brauwiss. 42 (1989) 331.
[37] S. El-Dessouki, Dtsch. Lebensm. Rundsch. 88 (1992) 354.
[38] P. Majerus, I. Cutka, A. Dreyer, S. El-Dessouki, W. Eyrich, H. Reutsch, B. Schurer, H.U. Waiblinger, Dtsch. Lebensm. Rundsch. 89 (1993) 112.
[39] Y. Jiao, W. Blass, C. Ruehl, R. Weber, Dtsch. Lebensm. Rundsch. 90 (1994) 318.
[40] P.M. Scott, J. AOAC Int. 79 (1996) 875.
[41] P.A. Burdaspal, T.M. Legarda, Alimentaria 98 (1998) 115.
[42] A. Filali, L. Ouammi, A.M. Betbeder, I. Baudrimont, R. Soulaymani, A. Benayada, E.E. Creppy, Food Addit. Contam. 18 (2001) 565.
[43] E.K. Tangny, S. Ponchaut, M. Madoux, R. Rozenberg, Y. Larondelle, Food Addit. Contam. 19 (2002) 1169.
[44] Worldwide Regulations for Mycotoxins 1995 A compendium (FAO Food and Nutrition Paper, vol. 64) Food and Agriculture Organization of the United Nations, Rome, 1996, p. 6.
[45] A. Visconti, M. Pascale, G. Centonze, J. AOAC Int. 84 (2001) 1818.
[46] G.J. Soleas, J. Yan, D.M. Goldberg, J. Agric. Food Chem. 49 (2001) 2733.
[47] Scientific Committee on Food. Opinion on Ochratoxin A, CS/CNTM/MYC/14 final, Annex II to Document XXIV/2210/98, European Commission, Brussels, Belgium, 1998, p. 28.
[48] M. Becker, P. Degelmann, M. Herderich, P. Schreier, H.U. Humpf, J. Chromatogr. A 818 (1998) 260.
[49] P. Degelmann, M. Becker, M. Herderich, H.U. Humpf, Chromatographia 49 (1999) 543.
[50] G.S. Shephard, A. Fabiani, S. Stockenström, N. Mshicileli, V. Sewram, J. Agric. Food Chem. 51 (2003) 1102.
[51] I. Barna-Vetró, L. Solti, J. Teren, A. Gÿongÿosi, E. Szabo, A. Wölfling, J. Agric. Food Chem. 44 (1996) 4071.
[52] K. Jorgensen, Food Addit. Contam. 15 (1998) 550.
[53] J.M. Sáez, A. Medina, J.V. Gimeno-Adelantado, R. Mateo, M. Jiménez, J. Chromatogr. A 1029 (2004) 125.
[54] B. Zimmerli, R. Dick, J. Chromatogr. B 666 (1995) 85.
[55] H.Y. Aboul-Enein, O.B. Kutlut, G. Altiokka, M. Tunçel, Biomed. Chromatogr. 16 (2002) 470.
[56] A. Thellman, W. Weber, Dtsch. Lebensm. Rundsch. 93 (1997) 1.
[57] M. Gareis, Arch. Lebensm. Hyg. 50 (1999) 83.
[58] E.D. Baxter, I.R. Slaiding, B. Kelly, J. Am. Soc. Brew. Chem. 59 (2001) 98.