ELSEVIER

Journal of Chromatography A, 959 (2002) 327–330

JOURNAL OF CHROMATOGRAPHY A

www.elsevier.com/locate/chroma

Short communication

Optimization of solid-phase clean-up prior to liquid chromatographic analysis of ochratoxin A in roasted coffee

Liberty Sibanda[a,*], Sarah De Saeger[b], Carlos Van Peteghem[b]

[a]*TOXI-TEST, Harelbekestraat 72, B-9000 Ghent, Belgium*

[b]*Laboratory of Food Analysis, Faculty of Pharmaceutical Sciences, Ghent University, Harelbekestraat 72, B-9000 Ghent, Belgium*

Received 9 August 2001; received in revised form 11 February 2002; accepted 8 April 2002

Abstract

An extraction and clean-up method for ochratoxin A (OA) in roasted coffee has been developed and the HPLC method optimized. An interfering compound with a similar retention time as OA was adsorbed by the aminopropyl (NH_2) material at ≤5% $NaHCO_3$. Residual OA on the column was recovered by washing with the extraction solution followed with methanol. Fractions were mixed together for further clean-up with Ochratest™ immunoaffinity columns (IACs). Analysis by HPLC resulted in a well resolved OA peak and reduction in matrix interferences. Recoveries ranged from 72 to 84% and the detection limit was 1 ng/g.

Keywords: Food analysis; Coffee, roasted; Mycotoxins; Ochratoxin A

1. Introduction

Ochratoxin A (OA) is a naturally occurring toxicant produced mostly by *Aspergillus ochraceus* and *Penicillium verrucosum* [1]. This mycotoxin is reported to be highly toxic to a number of different animal species [2]. The occurrence of OA has been reported in various agricultural crops including cereals, coffee beans, pulses and cocoa beans [3].

The introduction of more sensitive High Performance Liquid Chromatography (HPLC) methods permitted the detection of trace levels of OA in roasted coffee [4]. However, the analysis of OA in coffee is still hampered by acidic substances extracted together with OA [5]. The HPLC method was recently improved by the introduction of the use of immunoaffinity columns (IACs) for the clean-up of coffee products [2]. In a 1996 study, a method was reported in which IACs were used directly after sample extraction without a clean-up step [5]. Due to extensive interferences by the coffee matrix it was necessary to increase the retention time of OA to nearly 14 min. Later in 1997 the use of a Sep Pak Silica column for solid-phase clean-up of the extract was reported and the resultant chromatograms showed a well resolved OA peak and a stable baseline [3]. However, this clean-up method employed extensive washing steps using chloroform, chloroform–methanol, toluene–acetic acid and acetonitrile. In this paper, we describe a new clean-up method employing aminopropyl (NH_2) as the solid-phase material. The method employs only three steps

*Corresponding author. Tel.: +32-9-264-8134; fax: +32-9-264-8199.

E-mail address: info@toxi-test.be (L. Sibanda).

0021-9673/02/$ – see front matter
PII: S0021-9673(02)00408-9

resulting in a sample extract which can be analyzed directly by an immunological method or further extracted by IAC for HPLC analysis.

2. Experimental

2.1. Sample extraction without aminopropyl clean-up

Green coffee samples were first analysed by HPLC and found to be OA negative [1]. Blank samples were then roasted and ground (particle size 1–2 mm) of which 20 g was extracted with 50 ml of methanol (analytical grade, BDH, Poole, UK)/3% aqueous $NaHCO_3$ (1:1, v/v) by shaking on an automatic shaker for 15 min [5]. The extract was filtered through a Schleicher and Schuell Faltenfilter (Dassel, Germany). An aliquot (4 ml) was diluted with PBS (pH 7.4) to 100 ml. The diluent was applied over a Ochratest™ IAC (Vicam, Watertown, MA, USA) and allowed to elute under gravity. The column was washed with 10 ml of HPLC water and blown to dryness with one syringe volume of air. OA was eluted with 4 ml of absolute methanol. The eluate was evaporated to dryness at 40 °C under a stream of nitrogen gas. The residue was redissolved in 150 μl of methanol for HPLC analysis.

2.2. Sample clean-up with aminopropyl (NH_2)

A roasted coffee sample (20 g) was extracted with 50 ml of methanol/0, 1.5, 3, 4, 6, and 8% aqueous $NaHCO_3$ (1:1, v/v) by shaking for 15 min. The extract was filtered as described above and the filtrate (4 ml) was extracted over a NH_2 Sep Pak column (Baker, Utrecht, Holland) at a rate of 1 drop per s. The column should not be pre-conditioned as adsorptive efficiency is adversely impaired. The column was washed with 2 ml of methanol/3% aqueous $NaHCO_3$ (1:1, v/v) and lastly with 1 ml of absolute methanol. All fractions were collected into the same container. The eluate was then diluted to 100 ml with PBS (pH 7.4) and extracted over an IAC as described above (Section 2.1).

2.3. Sample clean-up with NH_2 and 5% aqueous $NaHCO_3$

The sample was extracted as described above (Section 2.2), but the extraction solution used was methanol/5% aqueous $NaHCO_3$ (1:1, v/v). The extract was cleaned-up and prepared for HPLC analysis using the method described above (Section 2.2). This method was also used to analyze nine commercial roasted coffee samples.

The HPLC method used was an adaptation of that described by Pittet et al. [5]. The sample (50 μl) was injected manually by means of a Rheodyne manual injector (Waters, Milford, MA, USA). The HPLC system consisted of a Waters™ 600 Controller and a Waters 610 Fluid Unit (Waters, Milford, MA, USA). The flow-rate was 1 ml per min over a Supelco Discovery™ C_{18} (25 cm×4.6 mm, 5 μm) reversed-phase column (Supelco, Bellafonte, USA) at ambient temperature. The mobile phase used was acetonitrile/water/acetic acid (99:99:2). OA detection was achieved by means of a Waters 474 scanning fluorescence detector (Waters, Milford, MA, USA) set at 333-nm excitation and 460-nm emission wavelengths.

2.4. Chemical confirmation of OA

OA was derivatized to its methyl ester derivative by mixing 100 μl of standard or sample with 50 μl of boron trifluoride (BF_3) (Fluka, Buchs, Switzerland) in 0.5 ml of methanol. The mixture was incubated at 60 °C for 30 min. The derivatized sample was evaporated to dryness, redissolved in 150 μl of methanol and analyzed by HPLC as described above for OA. The retention time of the OA methyl ester was ca. 22 min.

3. Results and discussion

The method described by Pittet et al. [5] for the analysis of OA in green coffee was used here for OA analysis in roasted coffee. It employed methanol/3% aqueous $NaHCO_3$ (1:1, v/v) as the extraction solution and immunoaffinity chromatography clean-up.

The HPLC conditions allowed retention of OA only up to 10 min. However, roasted coffee matrix interferences covered the chromatogram from ca. 1.5 min to over 15 min. There was a matrix peak with an identical retention time as that of OA in a blank roasted coffee sample after the IAC sample clean-up. This, therefore, masks the OA peak at ca. 10 min which appears as a shoulder on matrix peaks (Fig. 1). This, therefore, illustrates the inadequacy of using IACs in isolation as a clean-up method highlighting the need to add an SPE step prior to the IAC clean-up step.

Various solid phases [trimethylaminopropyl (SAX), *n*-propyl-ethylene-diamine (PSA), NH_2, octadecyl (18), Diol (2OH) and cyanopropyl (CN] were investigated for their ability to adsorb the matrix interferences and particularly the brown coffee color and the compound interfering with the OA peak. NH_2 was selected for its chromatographic elution of OA and adsorption of the interfering compound. Different concentrations of $NaHCO_3$ were investigated and there was an observed decrease in peak area of the interfering compound with increasing $NaHCO_3$ concentrations. From these results 5% aqueous $NaHCO_3$ was chosen as the optimum salt concentration in the extraction solution for the effective adsorption of the interfering compound on the NH_2 solid-phase material. The ability of the NH_2 solid-phase material to adsorb the matrix interferences is probably due to its weak anionic nature further enhanced by the ionic extraction solution.

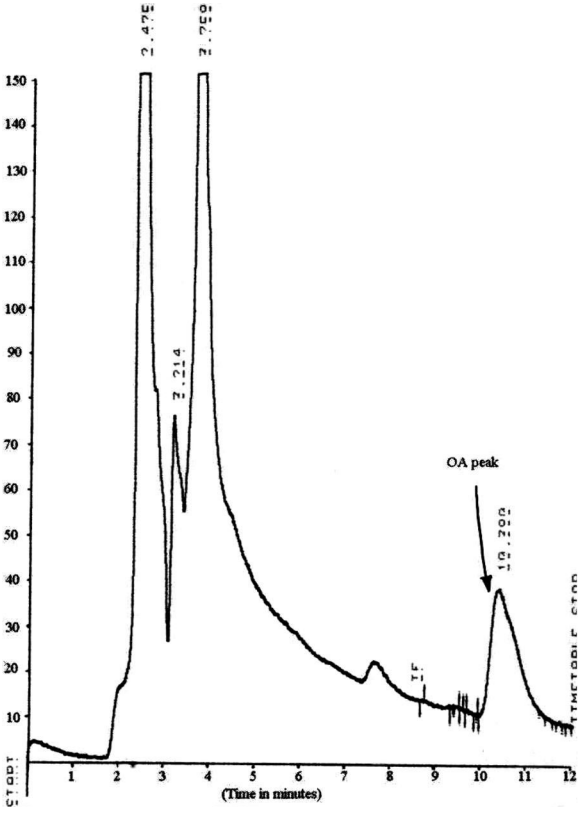

Fig. 1. Chromatographic analysis of an OA-spiked (10 ng/g) roasted coffee sample by HPLC after NH_2 solid-phase clean-up. The *y*-axis represents recorder responses to the fluorescence detector signal.

Eventually, after the application of methanol/5% aqueous $NaHCO_3$ (1:1, v/v) as an extraction solution, and cleaning up the sample on NH_2 solid-phase and IAC, an OA-spiked roasted coffee sample of 10 ng/g was analyzed by HPLC. The chromatogram showed extensive elimination of matrix interferences resulting in a well resolved OA peak (Fig. 1). Method recoveries ranged from 72 to 84% in spiked samples ($n=3$ replicated twice). Regression (r^2) of peak area on concentration for both standards and spiked samples were identical and these were 0.981 and 0.984, respectively. Analysis (see Section 2.1) of blank (false positive after analysis without NH_2 clean-up) sample extracts 24 h after extraction revealed that the interfering compound was unstable and the resultant chromatograms showed no peak at all. The recovery of OA from the NH_2 column was confirmed by derivatization to its methyl ester and the interfering compound peak disappeared after derivatization. The method (see Section 2.3) was successfully applied to nine commercial roasted coffee samples. The same samples were positive in a prior analysis (method in Section 2.1), however, after the NH_2 solid-phase clean-up method. These samples were known to be negative for OA because they were also analyzed before roasting [1]. Although this method requires NH_2 SPE plus IAC clean-up, it remains easily compatible with enzyme immunoassays. There is also no need to switch to different mobile phases when analyzing green and roasted coffee samples.

References

[1] L. Sibanda, S. De Saeger, T.G.M. Bauters, H.J. Nelis, C. Van Peteghem, J. Food Protect 64 (10) (2001) 1597.
[2] M. Nakajima, H. Terada, K. Hisada, H. Tsubouchi, K. Yamamoto, T. Uda, Y. Itoh, O. Kawamura, Y. Ueno, Food Agric. Immunol. 2 (1990) 189.
[3] S. Patel, C.M. Hazel, A.G.M. Winterton, A.E. Gleadle, Food Addit. Contam. 14 (1997) 217.
[4] H. Terada, H. Tsubouchi, K. Yamamoto, Y. Sakabe, J. Assoc. Anal. Chem. 69 (1986) 960.
[5] A. Pittet, D. Tornare, A. Hugget, R. Viani, J. Agric. Food Chem. 44 (1996) 3564.