Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Chromatography A, 1055 (2004) 247–249
JOURNAL OF CHROMATOGRAPHY A
www.elsevier.com/locate/chroma

Technical note

Development of an extraction method for the determination of zearalenone in corn using less organic solvents

Lea Pallaroni, Christoph von Holst*

European Commission, DG Joint Research Centre, Institute for Reference Materials and Measurements, Food Safety and Quality Unit, Retieseweg, B-2440 Geel, Belgium

Received 16 August 2004; received in revised form 27 August 2004; accepted 30 August 2004

Abstract

A method for the determination of zearalenone in corn has been developed applying pressurised liquid extraction (PLE) and using environmentally acceptable and less noxious organic solvents. The extracted samples were analysed with liquid chromatography coupled to mass spectrometry (LC–MS) equipped with an electrospray (ESI) ionisation interface. The optimised extraction mixture was isopropanol and an aqueous solution of triethylamine (1%) 50:50 (v/v), which allowed to halve the use of organic solvent compared to the method proposed by ISO. When applying the optimised method to five different naturally contaminated corn samples the obtained concentrations were slightly increased compared to the analysis using the previously used extraction solvent (acetonitrile–methanol). The relative standard deviation (RSD, $n = 3$) varied between 4 and 10% depending on the concentration level of the target analyte in the test material.

Keywords: Zearalenone; Pressurised liquid extraction; Environmentally friendly; Liquid chromatography–mass spectrometry

1. Introduction

Zearalenone (ZON) is a mycotoxin produced by several species of fungi belonging to the genus *Fusarium*, which is well known for colonising cereals [1]. ZON represents a health concern for animal husbandry (particularly for pigs) [1] and for humans due to its estrogenic property [2], thereby requiring the analysis of food samples for the presence of this compound. Various approaches for sample preparation including alternative extraction techniques such as microwave extraction and pressurised liquid extraction are applied as outlined in a previous paper [3]. The most commonly used solvent extraction mixture for ZON is acetonitrile–water in various ratios. For instance, the ISO [4] method requires the use of a mixture of acetonitrile–water 90:10 (v/v) whereas the method provided with the immunoaffinity column (Rhône-diagnostic) uses a mixture of acetonitrile–water 75:25 (v/v).

Evaluation of the safety data for organic solvents clearly shows the higher toxicity of acetonitrile compared to the non-aromatic alcohols used in this study [5]. Therefore a reduction in the use of acetonitrile moving towards less toxic and more environmentally- and user-friendly solvent is both, desirable and foreseeable.

The objective of the present study was to develop an alternative extraction method using less toxic solvents and showing an extraction efficiency for zearalenone that is comparable with the recovery rate obtained with the traditional extraction solvents containing acetonitrile. We applied the simplex method [6] to optimise the fraction of (1) isopropanol; (2) ethanol; (3) methanol; and (4) an aqueous solution of triethylamine at 1% (TAE 1%) of the solvent extraction mixture. In addition, the extraction temperature was included in the optimisation procedure. Extractions were performed on an automated extraction system (pressurised liquid extrac-

* Corresponding author. Tel.: +32 14 571221; fax: +32 14 571787.
E-mail address: christoph.von-holst@cec.eu.int (C. von Holst).

0021-9673/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2004.08.147

tion, PLE) and the extracts were analysed by using LC–MS [7].

2. Experimental

2.1. Chemical and reagents

All chemicals used in this study were described in detail in another publication [7].

2.2. Test material

The optimisation experiments were performed using corn fortified at a concentration of 400 ng/g. The selected extraction conditions were tested on naturally contaminated corn samples as described elsewhere [3]. In the present study we used as assigned value the results obtained with PLE utilising acetonitrile–methanol as extraction solvent [3].

2.3. Equipment

All extractions were performed on an ASE^{TM} 200 System (Dionex, Sunyvale, CA, USA) and analysed using an Agilent 1100 Series HPLC coupled to an ion-trap mass spectrometer equipped with an ESI Interface from Agilent. Details of the ASE and LC–MS parameters are given in [7].

2.4. Procedure

An amount of 5 g of sample was mixed thoroughly with 3 g of Hydromatrix to obtain a porous mixture to enable the extraction solvent to flow through the sample during the extraction. The mixture was poured into a 22 ml thimble, which was packed by adding a layer of Hydromatrix at the base and at the top (about 1 g), in order to fill the thimble completely according to the manufacturer's recommendations. ZAN was used as internal standard and added to the solution after the extraction [7].

Naturally contaminated corn samples were extracted in triplicate applying the selected extraction conditions using isopropanol–TEA 1% 50:50 (v/v) as extraction solvent and adjusting the temperature at 80 °C. Results from these experiments were compared with results obtained for the same samples extracted by PLE using methanol–acetonitrile 50:50 (v/v) [3]. The obtained LC chromatogram did not show any interference in the range of the interesting retention time and were very similar to those obtained when extracting the samples with methanol–acetonitrile [7].

2.5. Simplex

Multisimplex® (Grabitech Solutions AB, Sweden) was applied for the sequential simplex optimisation.

3. Results and discussion

3.1. Impact of water in the extraction solvent

The first experiments focused on the use of pure water and mixtures of water with the selected alcohols. The trials showed that increasing the water percentage in the extraction mixture led to technical inconveniences and reduced efficiency, due to the presence of starch, which tended to cook forming thick porridge and clogging the thimble. Experiments with various ratios of the matrix and Hydromatrix were performed, in order to facilitate the solvent flow trough the matrix. Since the minimum sample portion was set at 5 g, the amount of Hydromatrix was limited by the size of the

Table 1
Extraction conditions and corresponding recovery obtained in the simplex optimization procedure

Trial number	TEA 1% (%)	Isopropanol (%)	Ethanol (%)	Methanol (%)	Temperature (°C)	Recovery (%)
1	15	15	15	55	70	93
2	15	35	35	15	90	94
3	35	15	35	15	70	98
4	35	15	15	35	90	87
5	35	35	15	15	70	95
6	15	35	35	15	60	91
7	20	30	30	20	68	98
8	25	20	20	35	72	99.
9	45	15	15	25	50	88
10	20	30	30	20	80	92
11	15	15	40	30	75	100
12	5	0	60	35	78	97
13	50	25	25	0	75	94
14	25	15	30	30	67	85
15	50	0	25	25	75	101
16	50	50	0	0	75	102
17	50	15	10	25	80	104
18	50	50	0	0	80	102

Target concentration of ZON in corn: 400 ng/kg.

Table 2
Comparison of ZON level of naturally contaminated corn samples determined using present extraction solvent mixture and a mixture of a previous study ($n = 3$)

Sample codes[a]	Isopropanol–TEA (1%), 50:50 (v/v)		Acetonitrile–methanol, 50:50 (v/v)	
	Average (ng/g)	R.S.D. (%)	Average (ng/g)	R.S.D. (%)
2	114	10	100	10
3	202	11	183	7
6	307	4	316	9
7	164	7	152	3
8	1320	10	1140	6

[a] Sample code same as those used in [3].

thimbles available with the ASE 200. Thus it was not possible to find a suitable ratio for using 100% water. In addition, using solvent mixtures with a water percentage higher than 50% led to technical problems, since the extracted volume slowly decreased until the instrument clogged when extracting a sequence of samples. The increase of the water percentage in the final extraction mixture was also limited by the fact that ZON is practically insoluble in water [8] leading to insufficient extraction efficiency when increasing the percentage of pure water (data not shown).

3.2. Stability study

To overcome the low extraction efficiency of water several trials were performed to find a suitable alkaline aqueous solution for substituting water, since the solubility of ZON is improved when increasing the pH value. On the other hand, ZON is not stable in alkaline conditions [2]. To find a compromise between increased solubility of the target analyte and sufficient stability, various solvents were evaluated revealing that a mixture of methanol–TEA 1% 50:50 (v/v) would fulfil these criteria. In addition, we showed that methanol can be substituted by other alcohols, maintaining the same stability of ZON (data not shown).

3.3. Simplex optimisation

The results of the experiments carried out consecutively according to the Simplex optimisation procedure are shown in Table 1. Interestingly, high values for the recovery rate were obtained irrespective of which parameter combination was used since the minimum recovery was 85% (trial 14).

The extraction conditions of trial 11 met the target of 100% recovery but the corresponding extraction solution contained only 15% of aqueous solution. In order to establish whether extraction solutions containing a higher percentage for water would gain comparable high values for the recovery of ZON, the percentage of TEA 1% was kept constant at 50% in trials 15–18. In these trials only the percentage of isopropanol, ethanol and methanol and the extraction temperature was varied by the simplex algorithm. Since the recovery of ZON in these trials was always above 100%, the optimisation was stopped after trial 18. This trial was selected as optimal parameter combination due to easier handling such as the facility for filtering the extract and due to the fact that the extraction solution contained only two different solvents. The extracts obtained with this trial were dark yellow, limpid and easy to be filtered, which was not the case for extracts of trial 15. Moreover isopropanol is far less toxic than acetonitrile and methanol. In addition, the selected solvent offers the most economical use of extraction solutions, since isoproponal is cheaper compared to the other solvents of these trials and also compared to the solvents of the ISO method.

Table 2 shows the comparison between the ZON concentrations in various naturally contaminated samples obtained with the presently proposed method and another PLE method using a 100% organic solvent mixture (acetonitrile–methanol 50:50 (v/v)) [3]. Since the results from the different methods are comparable, we concluded that the selected solvent mixture containing 50% aqueous solution was suitable for the determination of ZON in corn.

4. Conclusion

The present study showed that ZON could be extracted from corn with less toxic solvents applying pressurised liquid extraction and LC–MS detection. The results from the optimisation procedure indicated that quite different parameter combinations would yield high values for the extraction efficiency. In addition, the optimisation procedure allowed to establish parameter combinations that meet the objectives of this study.

References

[1] V. Betina, Bioactive Molecules, Mycotoxins, vol. 9, Elsevier, Amsterdam, The Netherlands, 1989.
[2] R. Krska, R. Josephs, J. Fresenius, Anal. Chem. 369 (2001) 469.
[3] L. Pallaroni, C. von Holst, Anal. Bioanal. Chem. 376 (2003) 908.
[4] Nederlands Normalisatie-Instituut ISO/TC 34/SC 10 N890, 2001-07-25.
[5] R.E. Lenga, K.L. Votoupal, The Sigma–Aldrich Library of Chemical Safety Data, Sigma–Aldrich Corporation, Milwaukee, WT, USA, 1993.
[6] E. Bergström, T. Öberg, MultiSimplex, 1.0 Version, Gullberna Park, Karlskrona, Sweden, 1997.
[7] L. Pallaroni, C. von Holst, J. Chromatogr. A 993 (2003) 39.
[8] S. Budavari, M.J. O'Neil, A. Smith, P.E. Heckelman (Eds.), The Merck Index, Merck & Co., Rahway, NJ, USA, 1989.