Polish J. of Environ. Stud. Vol. 20, No. 1 (2011), 231-235

Short Communication

Analysis of Zearalenone in Aqueous Environment Using GC-MS

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Received: 3 March 2010
Accepted: 16 August 2010

Abstract

In the article the method of the analysis of zearalenone in the aqueous environment using GC-MS chromatography with electron ionization (EI) is described. The separation of zearalenone from the aqueous matrix was performed by means of solid phase extraction with the use of Supelclean™ ENVI-18 tubes. Extraction efficiency was determined by the analysis of deionized and tap water samples of volume ranging from 100 to 500 cm^3, and zearalenone concentrations varying from 50 to 200 ng/dm^3. Chromatography analysis was preceded by the derivatization of samples via silylation reaction using a ternary mixture of BSTFA/TMCS/DTE. The developed method enabled us to determine the content of zearalenone in aqueous environment at a concentration level from 0.3 to 0.5 ng/dm^3, with the repeatability of the analysis 4-8%. The efficiency of the extraction exceeded 62% and depended on the investigated matrix and the volume of analyzed sample. In the final stage of the study the analysis of 500 cm^3 samples of natural water collected in the Upper Silesia Region was performed. The determined concentration of zearalenone was in the range from 0 to 3.2 ng/dm^3, depending on the character of the analyzed sample.

Keywords: zearalenone, gas chromatography, SPE, silylation, aqueous samples analysis

Introduction

Steroidal female estrogens and anthropogenic chemical compounds that are introduced to water environment, e.g. alkylphenols, bisphenol A, and chlorinated pesticides and herbicides (xenoestrogens), belong to the group of chemical microcontaminants that is the subject of the special interest of scientists according to high biological activity [1-4]. These studies covered methods of the analysis of those compounds in the aqueous environment as well as the determination of their concentration in wastewater and surface water [2-4]. However, previous investigations did not focus on the compounds that naturally occur in the environment (e.g. mycotoxins) that, except for toxic effects, also demonstrate estrogenic activity [5-6]. Thus, those compounds are also called mycoestrogens.

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Mycotoxins are secondary metabolites of fungi. The most popular toxin described in the literature is zearalenone ZON (the F-2 toxin, Fig. 1), which is produced by fungi of the type *Fusarium,* including *F. graminearum*, *F. culmorum*, *F. crookwellense*, *F. equiseti*, and *F. semitectum,* and mainly appears on crops, especially on corn and its products [7]. The increased hazard of feminization processes among animals is observed when the ZON concentration in the fodder reaches 0.06 mg/kg of body mass/day [5]. Analogically, there exists a danger that consumption of food and water containing ZON may affect humans. The permissible concentrations of mycotoxins in the European Union are established at the level of 100 μg/kg in crude crops other than corn, 200 μg/kg in the crude corn, 75 μg/kg in crops directly consumed by humans, 200 μg/kg in corn products (i.e. flour, seeds and corn oil), and 50 μg/kg in bakery products (except those consumed by infants and children in which the permissible level is 20 μg/kg; and http://eur-lex.europa.eu).

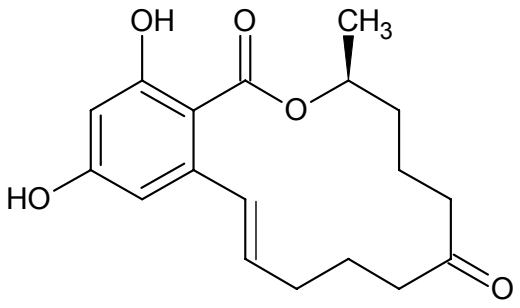

Fig. 1. The molecular structure of zearalenone ZON.

The presence of zearalenone is restrictively controlled in food and fodder samples [5]. However, information concerning its appearance in aqueous environments is limited. There are only several articles discussing the occurrence of ZON in surface water [6-9] and in influent and effluent of wastewater treatment plants [6, 7, 9, 10]. The concentration of mycotoxins in the aqueous environment varies from 0 to 60 ng/dm^3 [6-10]. In Poland, according to Gromadzka et al. [7], the content of ZON in investigated water samples did not exceed 43.7 ng/dm^3.

In qualitative-quantitative analytical procedures, devoted to ZON determination in water, chromatographic techniques are used, among which liquid chromatography tandem mass spectrometry (LC-MS-MS) is the most popular [6, 8-10]. In this study the determination of zearalenone via gas chromatography and mass spectrometry (GC-MS) with electron ionization (EI) was proposed. The separation of zearalenone from water samples was made by means of solid phase extraction. Chromatographic analysis was preceded by the derivatization of ZON in the silylation reaction. During the study the efficiency of the zearalenone extraction was determined depending on the volume of water sample and the compound concentration. Two different matrices (i.e. deionized and tap water) were used. The developed method was applied to control the presence of zearalenone in water and wastewater samples collected in the Upper Silesia region.

The Methodology of the Study

Chemicals and Materials

Zearalenone (ZON), mirex (IS), *N,O*-bis(trimethylsilyl) trifluoroacetamide (BSTFA), trimethylchlorosilane (TMCS), and dithioerythritol (DTE) were supplied by Sigma-Aldrich (Poland). Organic solvents of HPLC quality, i.e. methanol and acetonitrile, were supplied by POCH S.A. (Poland).

The extraction was performed using SPE Supelclean™ ENVI-18 tubes of volume 6 cm^3 (1.0 g of the phase) by Supelco. The extraction was carried out in the SPE pressure chamber by Varian. Filters from cellulose acetate (0.45 μm) were supplied by Millipore (Poland).

The basic solution of zearalenone of concentration 1 mg/cm^3 and the working solution of concentration 10 ng/μl were prepared in methanol. The working solution was prepared the same day it was used.

Solid Phase Extraction SPE

SPE tubes were firstly washed with acetonitrile ACN (5 cm^3) and conditioned with water (5 cm^3). The extract was eluated with 4 cm^3 of ACN. The solvent from eluate was totally evaporated by a stream of nitrogen and the derivatization of the compound was performed.

The Derivatization Procedure

The silylation reaction of zearalenone was performed using a ternary mixture of BSTFA/TMCS/DTE in the ratio of 1000:10:2 (v/v/w). The derivation reaction was carried out for 5 min at 90ºC. The volume of the derivating mixture was equal to 50 μl. The sample of the zearalenone derivative was directly injected onto the chromatographic column from the derivating mixture. The derivatization reaction conditions were established according to Kinani et al. [11].

Chromatographic Analysis

Analysis was made by means of gas chromatograph with mass spectrometer (GC-MS) model Saturn 2100 T (ion trap) by Varian equipped with VF-5ms column of dimensions 30 m x 0.25 mm x 0.25 μm (film thickness). Other parameters of chromatographic analysis are shown in Table 1. Quantitative analysis was performed using internal standard (IS) method by the addition of mirex to the eluate in the amount of 250 ng/cm^3 before the evaporation of the solvent.

Table 1. GC-MS conditions for zearalenone analysis.

Instrument	Varian Saturn 2100T GC-MS (ion trap) with electron ionization at 70 eV	
Parameters for GC		
Carrier gas	Helium (purity>99.999%)	
Carrier gas flow rate	1.4 cm^3/min	
Injector temperature	300ºC	
Injected volume	3 μl	
Injection mode	splitless	
Oven program	Initial temperature of 140ºC, hold 0.5 min. 20ºC min^{-1} to 280ºC, hold 5.5 min	
Parameters for MS		
Compound	Retention time, min.	Selected ions in SIM, m/z
Mirex (IS)	9.25	272; 235; 187; 119
ZON	10.9	444; 430; 306; 150

Table 2. Limit of detection, precision and linearity of mass detector response.

Compound	Limit of detection LOD[a], ng/µl	Concentration		Correlation coefficient (R^2)[c]
		1 ng/3µl	3 ng/3µl	
		Relative standard deviation RSD[b], %		
ZON	0.1	10	0.3	0.982

[a]signal to noise ratio (S/N)>3,
[b]the precision was examined by replicate analysis (n=4),
[c]linear ranges of the calibration curve: 1 ng/3µl to 15 ng/3µl

Zearalenone and mirex were determined by selected ion monitoring (SIM). Four ions were selected for registration of zearalenone and mirex in order to increase identification sensitivity. In the applied chromatographic conditions, retention times of mirex and zearalenone were equal to 9.25 min and 10.9 min, respectively.

The Recovery of the Compound

The recovery of the compound was determined as the arithmetic average of four extractions (n=4) from water samples of volumes 100, 250, and 500 cm^3 and zearalenone concentrations equal to 50, 100, and 200 ng/dm^3. The samples were prepared from deionized and tap water. Tap water was analyzed according to the presence of zearalenone before the extraction. The repeatability of the analysis was expressed by relative standard deviation (RSD, %).

Environmental Samples

In the final stage of the study the determination of zearalenone content in natural water samples from Upper Silesia was performed. Water samples of volume of 500 cm^3 were introduced to the extraction procedure. Additionally, before the analysis samples were filtered using 0.45 µm filters from cellulose acetate. The natural water samples were collected in glass bottles and analyzed directly after delivery to the laboratory. The study was performed in autumn from September to November 2009. Based on the literature [7], rainfall that occurs in that period causes the washing of mycotoxins from soil, which results in an increase of their concentration in the aqueous environment. The increased concentration of mycotoxins in the environment observed in autumn is also caused by the transportation of those contaminants from crops (post-harvest time) to soil, and afterward to ground and surface water. Hence, this period was approved as the most suitable to collect environmental samples. The samples of water and wastewater used in the study were:

- three surface waters, i.e. Odra River – Kędzierzyn Koźle, Bytomka River – Zabrze, Gliwician Channel – Gliwice,
- lake and melioration ditch waters (Gliwice – Sośnica),
- tap water (Gliwician water supply system),
- influent and effluent of Wastewater Treatment Plant (Zabrze-Śródmieście).

In order to determine the contamination of the investigated waters with organic and inorganic substances, additional analyses of chosen physicochemical parameters were performed, i.e.:

- pH and conductivity using a multiparameter laboratory meter inoLab® 740 by WTW,
- absorbance at wavelength of 254 nm using UV VIS Cecil 1000 spectrometer by AGA Analytical,
- dissolved organic carbon (DOC) content using Multi N/C analyzer by Jena Analytic.

Results and Discussion

Detection Limit and Precision

The limit of detection (LOD) of the chromatographic analysis of zearalenone was equal to 0.1 ng/µl (Table 2). It was determined during the analysis of derivatized standard, assuming that the ratio of the signal intensity to noise is greater than 3. The precision of chromatographic analysis expressed as RSD was determined for two concentrations of the analyte introduced quadruply onto the chromatographic column. The precision of the GC-MS analysis did not exceed 10%, whereas the lowest value of the parameter was obtain for the higher concentration of the derivatized compound. The linear correlation coefficient (R^2) of the calibration curve determined for zearalenone derivative in the concentration range from 1 ng/3µl to15 ng/3µl exceeded 98%.

Recovery Study

The efficiency of the extraction (%) and repeatability of the method were determined by quadruple repetition of the whole procedure, i.e. SPE-derivatization-GC/MS. Deionized water and tap water were used as matrices for the preparation of solutions that were further put to the extraction. The volume of the prepared samples was equal to 500 cm^3. The concentration of zearalenone in the samples varied from 50 to 200 ng/dm^3. The detailed parameters of the quantitative analysis of the developed method are presented in Table 3.

The recovery of zearalenone was in the range from 62 to 80% in average in case of waters which were characterized by lower concentrations of the compound. An insignificantly higher recovery degree was observed for standards

Table 3. The efficiency of zearalenone extraction and accuracy of the SPE-derivatization-GC/MS method.

Matrix	Volume of the sample, cm^3	ZON concentration, ng/dm^3			Limit of quantification LOQ[b], ng/dm^3
		200	100	50	
		Recovery (RSD)[a], %			
Deionized water	500	69 (4)	71 (4)	77 (4)	0.5
Tap water		62 (7)	76 (8)	80 (8)	0.3

[a]the recovery and the precision were examined by replicate analysis (n=4),
[b]signal-to-noise ratio (S/N)>10

Table 4. Influence of the sample volume on the recovery of zearalenone.

Compound	Concentration, ng/dm^3	Sample volume, cm^3		
		500	250	100
		Recovery (RSD)[a], %		
ZON	200	69 (4)	68 (3)	64 (9)

[a]the recovery and the precision were examined by replicate analysis (n=4)

of ZON content equal to 50 ng/dm^3 and 100 ng/dm^3 based on tape water matrix, which can be explained by the presence of inorganic substances (desalination effect).

The repeatability of the results in the developed method expressed as RSD was in the range 4 to 8%. The limit of quantification (LOQ) of zearalenone, assuming that the ratio of the signal intensity to the noise is greater than 10, was from 0.3 to 0.5 ng/dm^3, depending on the matrix. The lower value of LOQ in the case of tap water is caused by higher values of the recovery of zearalenone.

The influence of water sample volume on the standard recovery for comparative concentrations of zearalenone in deionized water equal to 200 ng/dm^3 was determined. Water samples of 100 to 500 cm^3 were prepared. The highest efficiency of the extraction of zearalenone was observed for the greatest volume of the sample, i.e. 500 cm^3, thus this volume was proposed as the optimal in the developed procedure (Table 4).

Application to Environmental Samples

Results of ZON content analysis and the physicochemical characteristics of investigated waters and wastewaters from Upper Silesia (September-November 2009) are shown in Table 5. Water samples of volume equal to 500 cm^3 were used during the procedure.

The highest concentration of zearalenone was observed in Wastewater Treatment Plant influent and it was equal to 3.2 ng/dm^3, whereas in the effluent the level of contaminant was below the limit of quantification of the developed

Table 5. The average concentration of zearalenone in different water and wastewater samples from Upper Silesia – physicochemical characteristics.

Compound	Tap water Gliwice	Odra River Kędzierzyn-Koźle	Lake Gliwice-Sośnica	Melioration ditch Gliwice-Sośnica	WWTP Zabrze		Bytomka River Zabrze
					influent	effluent	
Zearalenone	Concentration*, ng/dm^3						
	0	0.66	1.52	1.14	3.25	<LOQ	0
Physicochemical parameters							
pH	7.08	6.67	7.62	7.53	7.79	7.05	7.65
Absorbance at UV (254 nm), 1/cm	0.09	0.27	0.15	0.24	0.31	0.15	0.17
Dissolved organic carbon DOC, mg/dm^3	1.73	8.53	4.24	10.8	46.5	6.98	0.39
Coductivity, mS/cm	0.77	0.51	1.59	1.65	1.30	0.98	5.63

*average concentrations (n-number of analysis-3)

method. It indicates that zearalenone is effectively removed during activated sludge wastewater treatment. High concentrations of ZON equal to 1.52 ng/dm^3 and 1.14 ng/dm^3 in the lake water sample and in the water from melioration ditch localized near allotment gardens, respectively, were observed. Zearalenone was not present in water samples of Gliwician tap water and the Bytomka River. It was observed, however, that the river water was highly contaminated by inorganic substances (the conductivity value at the level of 5.63 mS/cm).

The high concentration of zearalenone was quite characteristic in water samples that contained high amounts of organic substances, i.e. wastewater plant influent, lake water and melioration ditch water. According to Gromadzka et al. [7], the concentration of zearalenone in water essentially depends on the season and sampling place. The authors determined the zearalenone content at the level of 43.7 ng/dm^3 in the Bogdanka River (Poznań environs) in autumn (October 2007), when heavy rainfalls caused the washing of the compound out from the soil. Additionally, the zearalenone concentration was higher in water samples collected near forested or agricultural areas. This dependence was also observed in the presented study.

Conclusions

The developed SPE-derivatization-GC/MS method is suitable for quantitative analysis of zearalenone in water samples at concentration levels from 0.3 ng/dm^3 to 0.5 ng/dm^3. The performed analyses of simulated water of various ZON concentrations from 50 ng/dm^3 to 200 ng/dm^3 characterized with satisfying repeatability in the range from 4 to 8%. The recovery of zearalenone varied from 62 to 80% in water of lower concentration of the compound. The optimal volume of the water sample introduced to the extraction was established at the level of 500 cm^3. The discussed method was successfully applied for the analysis of zearalenone in the environmental samples.

The content of zearalenone in investigated water and wastewater samples collected in Upper Silesia from September to October 2009 did not exceed 3.2 ng/dm^3. The concentration of zearalenone depended on the type of the analyzed sample. The highest concentration of the compound was determined in wastewater treatment plant influent, lake, and melioration ditch water samples.

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