Food Chemistry 115 (2009) 1120–1127

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

Analytical Methods

Determination of *Fusarium* mycotoxins beauvericin and enniatins (A, A1, B, B1) in eggs of laying hens using liquid chromatography–tandem mass spectrometry (LC–MS/MS)

Marika Jestoi [a,*], Mervi Rokka [a], Eila Järvenpää [b], Kimmo Peltonen [a]

[a] Finnish Food Safety Authority (Evira), Research Department, Chemistry and Toxicology Unit, Mustialankatu 3, FIN-00790, Helsinki, Finland
[b] University of Turku, Department of Biochemistry and Food Chemistry, Vatselankatu 2, FIN-20014, Turku, Finland

ARTICLE INFO

Article history:
Received 30 June 2008
Received in revised form 9 September 2008
Accepted 24 December 2008

Keywords:
Beauvericin
Eggs
Enniatins
Mycotoxin
Residues

ABSTRACT

An existing sample preparation technique used for the determination of ionophoric coccidiostats was modified to permit the analysis of *Fusarium* mycotoxins beauvericin and enniatins in egg samples. The validation results indicated that the sample preparation method developed is well applicable to the determination of the related compounds in eggs. The presence and contamination levels of beauvericin and enniatins A, A1, B and B1 were studied in Finnish egg samples in 2004–2005. The egg sample analyses (112 whole eggs and 367 egg yolk) revealed that the occurrence of beauvericin as well as enniatins B and B1 is very common in Finnish eggs. The contaminations were, however, in most cases in trace-levels (<limit of quantification). Enniatin A and A1 were not found in any of the whole egg samples, and furthermore enniatin B1 was present only in samples from 2004. The general contamination levels of beauvericin and enniatins in whole egg samples were similar in 2004 and 2005. The prevalence and concentration levels of mycotoxins were higher in the market samples (egg yolk) as compared to samples collected in the national residue monitoring programme (whole egg) samples suggesting that there may be bioaccumulation of these mycotoxin contaminants in egg yolk. This is the first study to report the presence of *Fusarium* mycotoxins beauvericin and enniatins in egg samples.

1. Introduction

Beauvericin (BEA) and enniatins (ENNs) are mycotoxins produced by different *Fusarium* species such as *F. avenaceum, F. poae* and *F. tricinctum* (Logrieco, Rizzo, Ferracane, & Ritieni, 2002; Thrane et al., 2004). These particular mycotoxins are cyclic hexadepsipeptides consisting of alternating hydroxy acid and N-methylamino acid residues. BEA has phenyl substituents on the N-methylamino acid residue whereas ENNs have various aliphatic substituents at the same positions. For comparison, the chemical structures of BEA and ENNs are shown in Fig. 1.

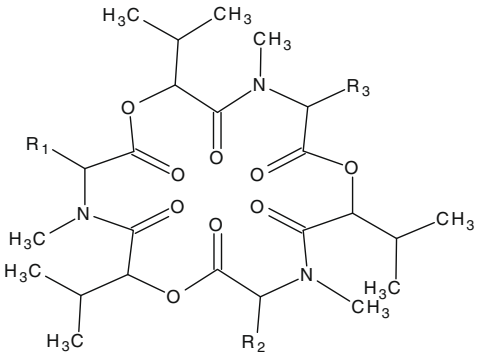
BEA and ENNs are common contaminants in grains originating from Scandinavia (e.g. Uhlig, Jestoi, & Parikka, 2007). The contamination level is usually low, especially in the case of BEA. Interestingly, ENNs may be detected even at mg/kg-levels, particularly in samples harvested in the late autumn. *Fusarium* species are worldwide pathogens in cereals and therefore it is most likely that BEA and ENNs are also present in grains harvested in other countries. In fact, the BEA contamination of cereals has been reported worldwide as reviewed by Jestoi (2008). However, to the best of our knowledge, there are no reports on the levels of ENNs in other countries than Finland, Norway and Italy.

The primary toxic action of BEA and ENNs is related to their ionophoric properties. They interfere with electrochemical gradient of membranes with the toxic response resulting from disturbances in the normal physiological level of cations in the cell (Hilgenfeld & Saenger, 1982; Kamyar, Rawnduzi, Studenik, Kouri, & Lemmens-Gruber, 2004; Ovchinnikov et al., 1974). In addition, BEA induces apoptosis by triggering an increase in the cytoplasmic calcium concentration (Dombrink-Kurtzman, 2003). BEA is also reported to intercalate with double stranded DNA and therefore it can interfere with DNA replication (Pocsfalvi et al., 1997). On the other hand, recently it was demonstrated that enniatins B, B1 and D inhibit adenosine triphosphate (ATP)-binding cassette transporter protein Pdr5p in *Saccharomyces cerevisae* at non-cytotoxic levels and suggested that they could be potent compounds used in cancer chemotherapy (Hiraga, Yamamoto, Fukuda, Hamanaka, & Oda, 2005). BEA and ENNs share similar chemical structures, and therefore the same toxicodynamic actions can be assumed to take place. At present, the toxic outcomes of BEA and ENNs are based on

Abbreviations: BEA, beauvericin; ENNs, enniatins.

* Corresponding author. Tel.: +358 20 77 24431; fax: +358 20 77 24359.
E-mail address: marika.jestoi@evira.fi (M. Jestoi).

0308-8146/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2008.12.105



	R1	R2	R3
BEA	Phenyl	Phenyl	Phenyl
A	*sec*-butyl	*sec*-butyl	*sec*-butyl
A1	*sec*-butyl	*sec*-butyl	*iso*-propyl
B	*iso*-propyl	*iso*-propyl	*iso*-propyl
B1	*iso*-propyl	*iso*-propyl	*sec*-butyl

Fig. 1. The structures of beauvericin and enniatins.

in vitro tests (Jestoi, 2008). This is clearly a shortcoming because BEA and ENNs may exert their toxic effects in different tissues and organs, although no distinct mycotoxicoses attributable to these particular mycotoxins have been reported. In the few *in vivo* studies that have been carried out so far, the toxicity of BEA and ENNs was reported to be low as reviewed by Jestoi (2008), though this was not confirmed by McKee et al. (1997).

In feeding studies using chickens and turkeys, the interaction of various mycotoxins (BEA, moniliformin, deoxynivalenol, fumonisin B_1) did not reveal any synergistic or even additive effects (Leitgeb, Lew, Wetscherek, Böhm, & Quinz, 1999; Leitgeb et al., 2000). Bioaccumulation of lipophilic feed contaminants into egg yolks has been observed previously (Mortier et al., 2005; Rokka et al., 2005; van Eijkeren, Zeilmaker, Kan, Traag, & Hoogenboom, 2006). One possible explanation for this phenomenon is that laying hens transport substantial amounts of dietary and endogenous lipids to the forming egg yolks through the VLDL (very low density lipoprotein) particles synthesised in the liver (Griffin, Grant, & Perry, 1982). In contrast, the lipid metabolism of non-laying poultry resembles that of mammals, and VLDL particles transport fat to tissues (Elkin (1997) and references therein). Probably at least some of the lipophilic contaminants end up as constituents of VLDL particles during their formation in the liver. Due to their lipophilic nature, BEA and ENNs may bioaccumulate (Thakur & Smith, 1997), and therefore residues in animal tissues need to be monitored. In fact, we have recently reported the presence of BEA and ENNs -residues in poultry tissues (Jestoi, Rokka, & Peltonen, 2007).

The structures and particularly the mode of action of BEA and ENNs are closely related to the ionophoric coccidiostats, which have been reported to accumulate in eggs of laying hens (Kennedy, Hughes, & Blanchflower, 1998; Rokka et al., 2005). Due to the similarities in the mode of action as well as the fact that both coccidiostats and BEA/ENNs are detected in poultry tissues (Jestoi et al., 2007), we posed the question whether the lipophilic mycotoxins can also bioaccumulate in eggs.

To answer this question, the aims of our study were (i) to study the suitability of the existing sample preparation technique used for ionophoric coccidiostats for the analysis of BEA and ENNs in egg samples, (ii) to validate the instrumental method for the analysis of BEA and ENNs from eggs and (iii) to study the presence and contamination levels of BEA and ENNs in Finnish egg samples.

2. Materials and methods

2.1. Samples

Egg samples originated from the Finnish residue control programme and from the Finnish markets. Samples from the residue control programme in 2004 ($n = 62$) (3 organic, 11 barn and 48 cage egg samples) and in 2005 ($n = 50$) (3 organic, 15 barn and 32 cage egg samples) were collected from egg producers from various locations in Finland. One sample consisted of six whole eggs that had been pooled. In addition, 367 samples (138 organic, 112 barn and 117 cage egg samples) were purchased from grocery stores around Finland in 2005. From the samples originating from grocery stores, yolks and egg whites were separated, and yolks were analysed separately, as was reported in our earlier work on ionophoric coccidiostats (Rokka et al., 2005). Coccidiostats become accumulated particularly in the yolk of hens' eggs and therefore we hypothesised that BEA and ENNs would behave similarly.

2.2. Chemicals and reagents

Mycotoxin standards (BEA and ENN A + A1 + B + B1) were purchased from Sigma (St. Louis, MO, USA). The standard solutions of BEA (0.01–0.1 μg/ml) and ENNs (0.02–0.2 μg/ml) were prepared in acetonitrile. Acetonitrile, methanol, acetic acid and ammonium acetate were purchased from J.T. Baker (Deventer, Holland). The water used was Milli-Q water purified with a Millipore Milli-Q Plus System (Millipore, Espoo, Finland). Silica solid phase extraction columns (Bond elut, 500 mg, 3 ml) were purchased from Varian (Lake Forest, CA, USA). Argon (AGA, Finland) was used as the collision gas in the MS.

2.3. Sample preparation and LC–MS/MS-analysis

Sample preparation was performed as described by Rokka and Peltonen (2006). In brief, homogenised material (5 g) was dried using anhydrous sodium sulphate and extracted with acetonitrile (20 ml). During the extraction, the samples were shaken using a horizontal shaker (30 min) and centrifuged at 4000 rpm for 15 min. Crude extracts (5 ml) were purified with solid phase extraction methodology using silica cartridges. Before loading the sample onto the column, the column was conditioned with acetonitrile (2 ml). A sample was passed through the conditioned column and the eluate was collected into a test tube. The column was further washed with acetonitrile (2 ml), which was also collected. Eluates were pooled and concentrated under a stream of nitrogen, dissolved into the same HPLC mobile phase used to separate the coccidiostats (95% acetonitrile: 5% 2 mM ammonium acetate containing 2% acetic acid). The dissolved samples were injected into a liquid chromatography–tandem mass spectrometer (LC–MS/MS) (Waters Alliance 2695 liquid chromatograph; Waters Co., Milford, MA, USA and MicroMass Quattro Micro triple-quadrupole mass spectrometer; MicroMass Ltd, Manchester, UK).

The separation of BEA and ENNs was achieved by applying the method developed by Jestoi, Rokka, Rizzo, Peltonen, and Aurasaari (2005). In the method, samples (10 μl) were injected into Symmetry C_{18} (3.5 μm), 2.1 × 150 mm analytical column (Waters Co., Milford, MA, USA) and an isocratic elution was applied. The eluent consisted of acetonitrile/methanol/ammonium acetate (10 mM, pH 7) in a ratio of 45:45:10 (v/v) with a flow rate of 0.2 ml/min. The instrument was operated in the positive ion electrospray mode using the following parameters: cone voltage 45 V, capillary voltage 3.80 kV, source temperature 150 °C, desolvation temperature 270 °C and collision gas energy 25 eV. SRM (selected reaction monitoring) technique was used for identification and quantification, in which protonated molecules $[M+H]^+$ of the analytes (BEA *m/z* 784.50, ENN A *m/z* 682.56, ENN A1 *m/z* 668.58, ENN B *m/z* 640.52, ENN B1 *m/z* 654.61) were fragmented in the collision cell to the product-ions (BEA *m/z* 244.20 and *m/z* 262.24, ENN A *m/z* 210.22 and *m/z* 228.26, ENN A1 *m/z* 210.22 and *m/z* 228.20, ENN B *m/z* 196.22 and *m/z* 214.18, ENN B1 *m/z* 196.20 and *m/z* 214.20). For the quantification, we used the product-ions *m/z* 244.20 for BEA, *m/z* 210.22 for ENN A, *m/z* 210.22 for ENN A1, *m/z* 196.22 for ENN B and *m/z* 196.20 for ENN B1.

2.4. Method validation

The following validation parameters were determined to describe the performance of the method in the case of whole egg as a matrix: selectivity, linearity, specificity, recovery, and repeatability, limit of detection (LOD) and limit of quantification (LOQ). Replicates (12) of spiked samples, at three concentration levels (Table 1.) and calibration curves with and without matrix were analysed. The calibration curves were prepared at five different concentration levels (BEA 0.2–20 μg/kg; ENN A 0.01–1.2 μg/kg; ENN A1 0.03–8.4 μg/kg; ENN B 0.03–8 μg/kg; ENN B1 0.09–22.4 μg/kg).

3. Results

3.1. Method validation

A chromatogram of a blank whole egg sample spiked with BEA and ENNs is shown in Fig. 2 and a chromatogram of an egg sample naturally contaminated with BEA and ENNs is shown in Fig. 3. The selectivity of the analytical method was tested by comparing (two-sided *t*-test) the slopes of the calibration curves obtained with and without matrix. Due to the presence of significant matrix effects ($p < 0.05$) (data not shown), the calibration curves for all compounds were prepared in whole egg matrix (a homogenised whole egg mixture). The matrix effect calculated as the signal suppression/enhancement-ratio (SSE%) as described in Sulyok, Berthiller, Krska, and Schuhmacher (2006) were 57%, 69%, 76%, 54% and 70% for BEA, ENN A, ENN A1, ENN B and ENN B1, respectively, further demonstrating the suppressive effect of the matrix components on the signal intensity. The acceptable linearity of each point of the matrix-assisted calibration curves was tested with the method of van Trijp and Roos (1991). A tolerance of 100 ± 10% was accepted for the separate calibration points for good linearity. On this basis, it can be stated that the measurement of BEA and ENNs in matrix is linear over the ranges studied (data not shown). The specificity of the method was tested by blank egg samples ($n = 20$) and no interference signals close to the retention times of BEA/ENNs were detected in any egg samples. The recovery and repeatability of the analysed mycotoxins in whole eggs are clearly acceptable for the monitoring purposes (Table 1). The determined LODs and LOQs were 0.50/1.0, 0.015/0.03, 0.21/0.42, 0.20/0.40 and 0.56/1.12 μg/kg for BEA, ENN A, ENN A1, ENN B and ENN B1, respectively.

3.2. Samples from the national residue monitoring programme (whole egg)

In 2004 BEA and/or ENNs were detected in 79% of all egg samples analysed. Eggs produced as organic products, or in barns or in cages were contaminated with mycotoxins in the following degrees: 100%, 82% and 77%, respectively. Only one kind of mycotoxin was detected in 25 samples whereas two or more mycotoxins were present in 24 samples. BEA and ENN B were found in more than 90% of the positive samples. The distribution of the contaminated samples in the 2004 sampling is presented in Fig. 4a. The contamination level was above the LOQs in 21%, 18% and 23% in organic, barn and cage produced eggs, respectively (Table 2a–c).

In the samples collected in the national residue control programme in 2005, 56% were contaminated with BEA and/or ENNs. The distribution of contaminated samples based on the farming method was 66%, 40% and 63% in organic, barn and cage produced eggs, respectively. One mycotoxin was detected in 27 samples and two or more compounds were present only in one egg sample. Only BEA and ENN B were found in the samples from the national residue control programme in 2005 (Fig. 4b.). The contamination level of the analysed mycotoxins exceeded the LOQs in 5% of the cage produced egg samples. In samples originating from organic or barn production, the contamination levels were at trace-level only (<LOQs) (Table 2a–c). ENN A and ENN A1 were never detected in the whole egg samples originating from the national residue monitoring programme.

3.3. Samples from the market (egg yolk)

In 2005, egg samples were also purchased from Finnish grocery stores. 367 samples were analysed and 366 samples were contaminated with BEA and/or ENNs. Only one organic egg sample did not contain any residues of BEA/ENNs. Only one type of mycotoxin was detected in 82 samples, whereas two or more compounds were present in 284 samples. Two organic and two cage produced egg

Table 1
The mean recoveries with the corresponding standard deviations of the mycotoxins analysed at three spiking level ($n = 12$).

	Spiking level 1 (μg/kg)	Recovery% (sp. level 1; $n = 12$)	Spiking level 2 (μg/kg)	Recovery% (sp. level 2; $n = 12$)	Spiking level 3 (μg/kg)	Recovery% (sp. level 3; $n = 12$)
BEA	2	67 ± 6.4	8.4	72 ± 3.5	16.7	52 ± 3.1
ENN A	0.12	51 ± 9.9	0.5	79 ± 9.2	1.0	61 ± 3.3
ENN A1	0.84	49 ± 7.5	3.5	68 ± 4.9	6.7	58 ± 3.3
ENN B	0.8	48 ± 7.0	3.2	73 ± 2.7	6.3	55 ± 3.0
ENN B1	2.24	50 ± 7.9	9.0	72 ± 4.2	18	56 ± 3.1

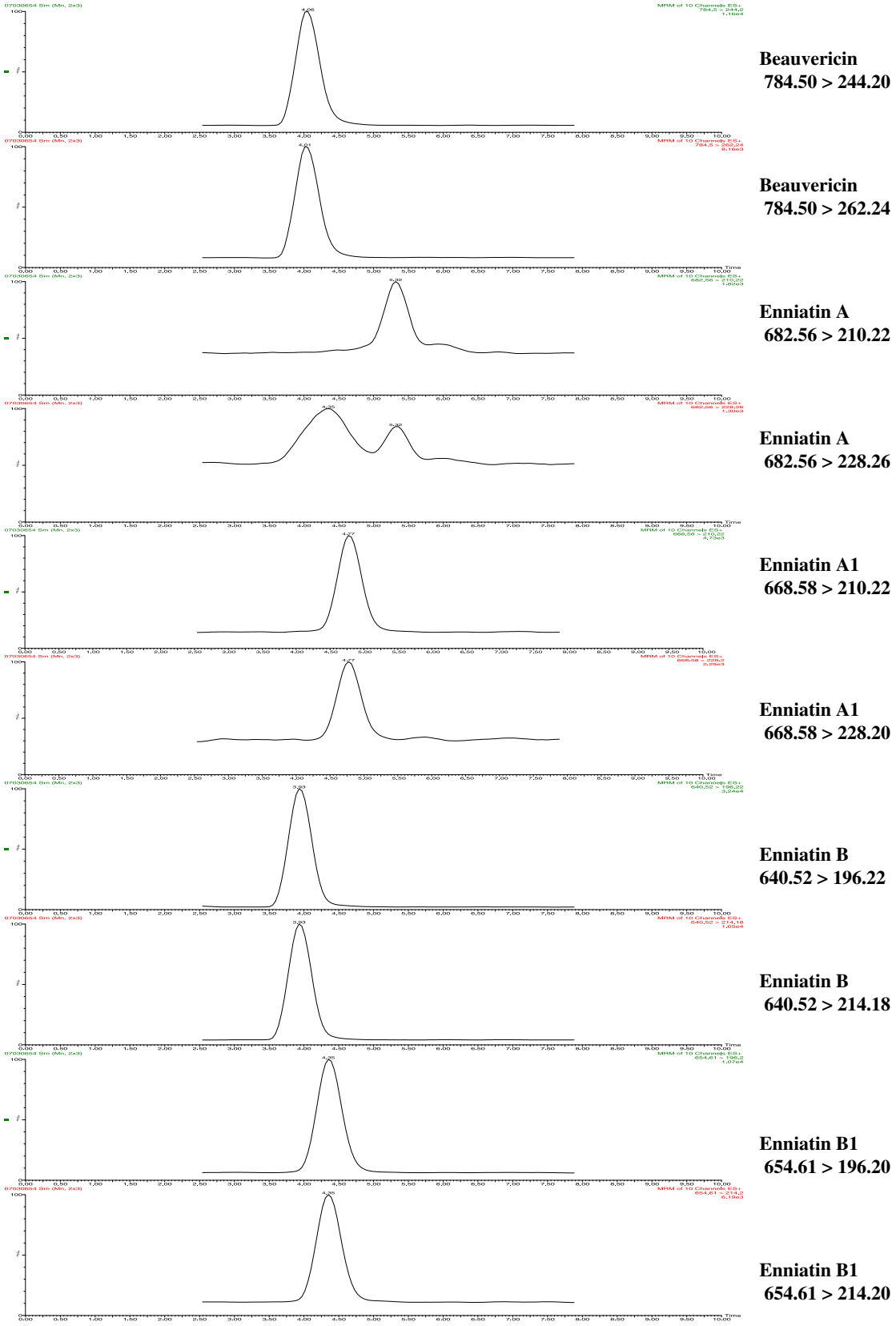


Fig. 2. The extracted ion chromatograms of beauvericin and enniatins of a spiked (BEA 2.0 μg/kg, ENN A 0.12 μg/kg, ENN A1 0.84 μg/kg, ENN B 0.8 μg/kg, ENN B1 2.24 μg/kg) blank whole egg sample. Two product ion tracings for each compound are presented (for further information see Section 2).

samples contained all of the analysed compounds. The distribution of contaminated samples between the different methods used in the production of the eggs bought from Finnish grocery stores in 2005 is presented in Fig. 4c. The percentages of the mycotoxins ex-

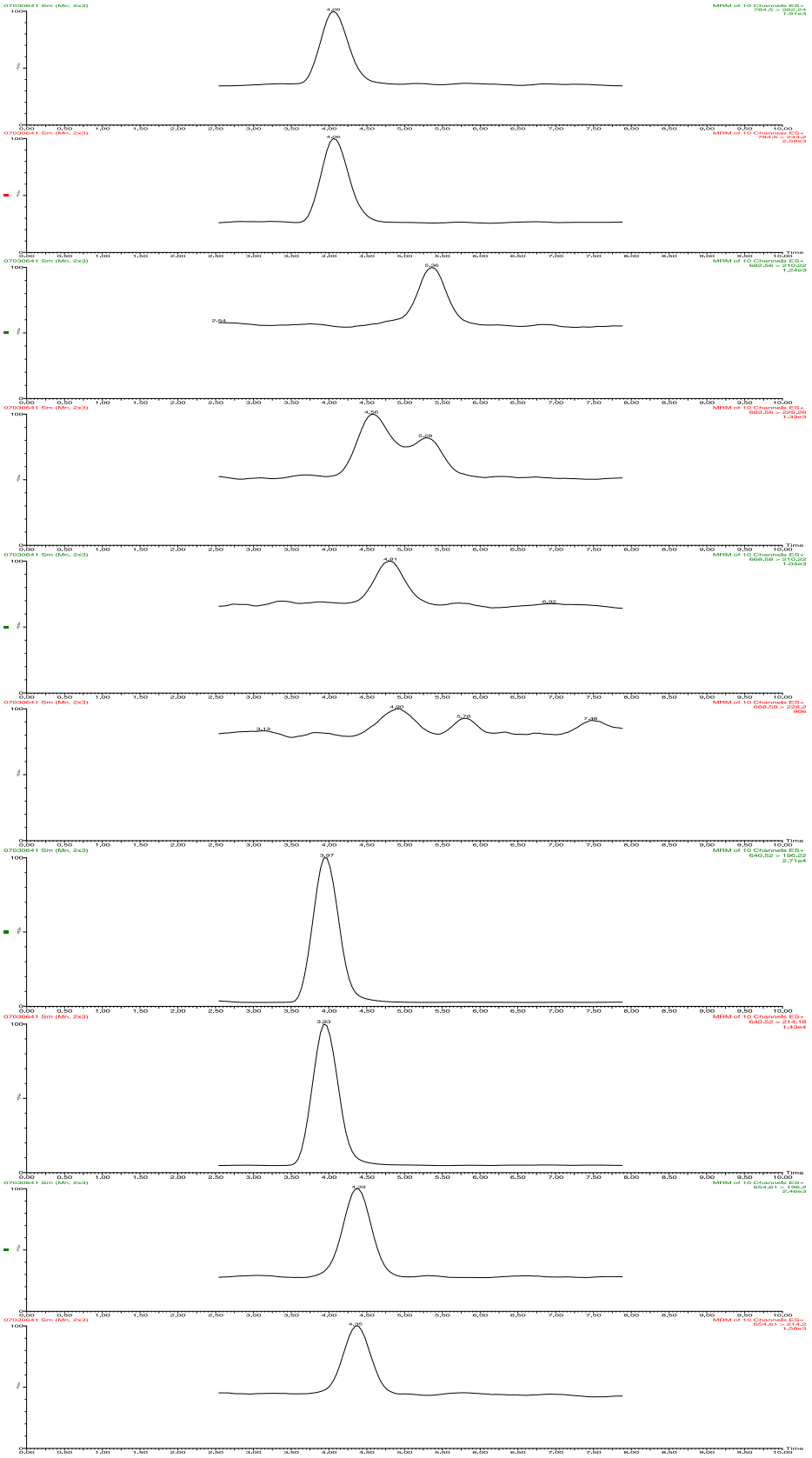


Fig. 3. The extracted ion chromatograms of beauvericin and enniatins of a naturally contaminated (BEA < 1.0 μg/kg, ENN A 0.03 μg/kg, ENN A1 < 0.42 μg/kg, ENN B 1.5 μg/kg, ENN B1 < 1.12 μg/kg) whole egg sample. Two product ion tracings for each compound are presented (for further information see Section 2).

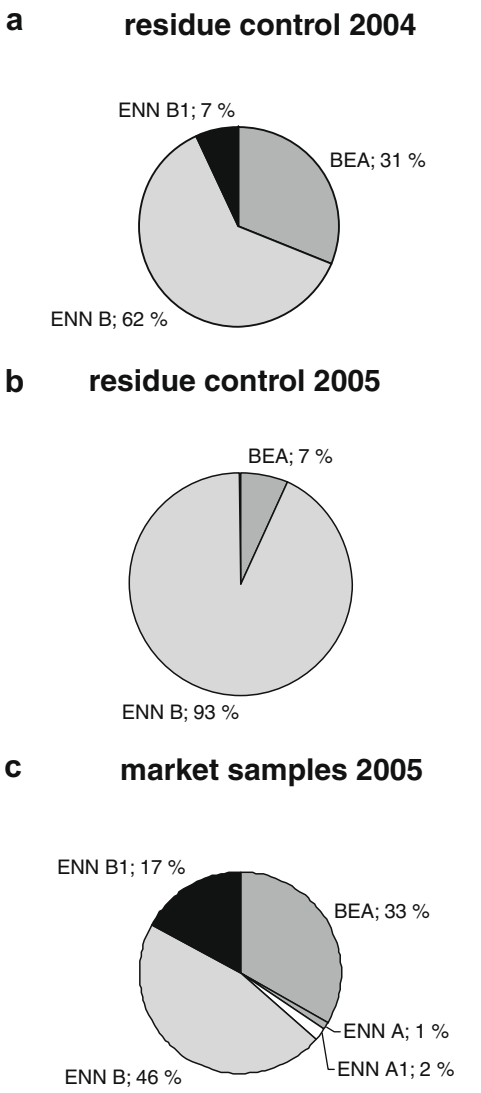


Fig. 4. (a) Distribution of the compounds detected in the positive samples of the national residue control programme of Finland in 2004. Enniatin A and A1 were not detected in any of the samples. (b) Distribution of the compounds detected in the positive samples of the national residue control programme of Finland in 2005. Enniatin A, A1 and B1 were not detected in any of the samples. (c) Distribution of the compounds detected in the positive samples (egg yolk) from local grocery markets of Finland in 2005.

Table 2
The concentration (μg/kg) ranges of beauvericin and enniatins in (a) organic, (b) barn and (c) cage eggs collected within the residue control programme of Finland in 2004 and 2005. (n.d. = not detected).

	BEA	ENN A	ENN A1	ENN B	ENN B1
(a) organic					
2004 (n = 3)	n.d. – <1	n.d	n.d.	n.d. – 0.7	n.d. – <1.12
2005 (n = 3)	n.d.	n.d.	n.d.	n.d. – <0.4	n.d.
(b) barn					
2004 (n = 11)	n.d. – <1	n.d.	n.d.	n.d. – 0.7	n.d.
2005 (n = 15)	n.d.	n.d.	n.d.	n.d. – <0.4	n.d.
(c) cage eggs					
2004 (n = 48)	n.d. – <1	n.d.	n.d.	n.d.– 1	n.d. <1.12
2005 (n = 32)	n.d. – <1	n.d.	n.d.	n.d. – 0.5	n.d.

ceeded the LOQs was 12%, 19% and 24% in organic, barn and cage produced egg samples, respectively (Table 3.).

Table 3
The concentration (μg/kg) ranges of beauvericin and enniatins in market samples (egg yolk) purchased from local grocery markets of Finland in 2005.

	BEA	ENN A	ENN A1	ENN B	ENN B1
Organic (n = 138)	n.d. – 1.3	n.d. – 0.07	n.d. – <0.42	n.d.– 1.5	n.d. – <1.12
Barn (n = 112)	n.d. – < 1	n.d. – 1.3	n.d. – 7.5	n.d. – 3.8	n.d. – 7.0
Cage (n = 117)	n.d. – < 1	n.d. – <0.03	n.d. – <0.42	<0.4 – 1.8	n.d. – <1.12

4. Discussion

The validation results showed that the sample preparation method developed for the determination of ionophoric coccidiostats (Rokka & Peltonen, 2006) was well applicable for the determination of the related compounds – BEA and ENNs – in eggs. The repeatability of the method was acceptable for the purpose in the analysis of these particular mycotoxins in egg samples - although recoveries were low for some of the compounds. The repeatability as expressed as standard deviations was higher at the lowest spiking level, which is common in residue analyses. As far as the other validation parameters are concerned, the method performance was also acceptable. Matrix-effects (commonly suppression) have been described to dramatically affect the signal intensity obtained with electrospray-MS (e.g. Tang & Kebarle, 1993). This was also observed in our study, which was established with statistically significant differences between the slopes of calibration curves prepared with and without matrix. The best way to overcome this phenomenon is to use co-eluting stable-isotope-labelled internal standards for quantification. However, as these isotopes are not available for BEA or ENNs, we used matrix-assisted calibration curves to overcome the effect of suppression to the analytical data, which is also an accepted approach for the correction of matrix effects (Zrostlikova, Hajslova, Poutska, & Begany, 2002). The same sample preparation method used in this exercise has also been used successfully to determine BEA and ENNs in poultry meat and liver samples (Jestoi et al., 2007). The use of only one sample preparation method for mycotoxins and coccidiostats is very convenient. The simultaneous LC–MS/MS analyses can be performed utilising the same samples, resulting in savings in manpower, time and costs.

The analyses of the samples originated from the national residue control programme (egg samples with the yolk and white) showed that the presence of BEA, ENN B and ENN B1 is very common in Finnish eggs. However, the contamination levels were, in most cases, below the limit of quantification (Table 2a–c). ENN A and ENN A1 were not found in any of the samples, and furthermore ENN B1 was only present in samples from the year 2004. This is in accordance with the concentration levels generally found in Finnish grains (ENN B > ENN B1 > ENN A1 > ENN A) (Jestoi, 2008). If the years of 2004 and 2005 are compared in the sense of the general contamination level, then there is no major inter-year difference. With regard to the type of the mycotoxins, in 2004 the prevalence of ENN B contamination was lower and the prevalence of BEA contamination was higher in comparison to samples collected in 2005 (Fig. 4a and b).

The prevalence and concentration levels of mycotoxins were higher in the market samples (egg yolk) as compared to samples collected in the national residue monitoring programme (whole egg) samples pointing to the bioaccumulation of the mycotoxin contaminants in egg yolks. This is similar to the situation with the ionophoric coccidiostats reported previously (Mortier et al., 2005; Rokka et al., 2005). In addition, when the toxicokinetics of dioxins was examined in laying hens, it was observed that about 90% of the dioxins were deposited in egg yolk fat, whereas the abdominal fat is used only as storage, particularly at low exposure levels and at high exposure levels in depletion period (van Eijkeren

et al., 2006). The egg yolk accounts for about one third of the total weight of egg (excluding the shell) (Powrie & Nakai, 1985), and therefore the analysis of egg yolk only results in three fold higher levels of mycotoxins than if the egg *per se* is analysed. This is in good agreement with the contamination levels observed in the samples of grocery store samples (egg yolk) as compared to the samples from the national residue control programme (egg white + yolk) (Tables 2 and 3.). This partially explains why ENN A and ENN A1 were not detected in whole egg samples. In samples purchased in shops, the contamination levels exceeded the detection limit (LOD) of LC–MS/MS-instrument, and the contamination levels were more clearly observable. However, as BEA and ENNs are lipophilic compounds, we suggest that the bioaccumulation of mycotoxins to yolks via transportation by lipoproteins is the most obvious explanation for the results. The bioaccumulation to lipophilic media was seen also in our earlier study, in which the presence of BEA and ENNs in turkey tissues was detected (Jestoi et al., 2007). The fact that the bioaccumulation of food contaminants is highly dependent on their physico-chemical properties (e.g. polarity) highlights the necessity of substantial and expertised design to enable representative and expedient sampling for food safety purposes.

Other mycotoxins have also been observed to bioaccumulate in eggs, posing a threat for food safety. T-2 toxin and cyclopiazonic acid were reported to accumulate especially in egg whites (Chi, Robison, Mirocha, Behrens, & Shimoda, 1978; Dorner et al., 1994) whereas citrinin was found in both yolk and white (Abdelhamid & Dorra, 1990). Trucksess, Stoloff, Young, Wyatt, and Miller (1983) and Wolzak, Pearson, Coleman, Pestka, and Gray (1985) reported that aflatoxins and aflatoxicol reached their maximum concentration in eggs in 4–5 days after exposure to contaminated feed. After a four day withdrawal period, mycotoxins were not detected in egg samples. The concentration of deoxynivalenol in eggs rose rapidly during the first 7 days if laying hens were fed with mycotoxin contaminated feed (Sypecka, Kelly, & Brereton, 2004). After 7 days withdrawal time, the concentration of deoxynivalenol in eggs declined. In these studies yolks were not separated from albumen, thus possible accumulation can only be hypothesised. The egg yolk is formed from proteins and lipoproteins delivered from the liver 7–11 days before the laying of the egg as reviewed by Keshavarz (1998). Thus the components associated with lipoproteins in the liver can be found after a lag period in the eggs, and their clearance after withdrawal of the contaminated feed requires several days. In contrast, the proteins of egg white are mostly synthesised *in situ* in the oviduct during a very short time, about 1 day before laying. This time scale of egg development is relevant when considering into which part of the egg undesirable feed components will be deposited and how long it takes before they can be cleared from the eggs (Mortier et al., 2005).

Unfortunately, the mycotoxin content of the feed used for laying hens was unknown. The average feed consumption by different avian species is from 100 to 150 grams per day. By combining this information with the fact that the BEA/ENNs contamination levels of Finnish raw grains (harvested in 2001–2002) ranging from trace-levels up to 25 ppm (Jestoi, 2008), one can estimate that the exposure of laying hens may have varied from negligible to 3.75 mg/day. The climatic conditions in which the mycotoxins are produced can have a significant effect on the levels and type of mycotoxins produced. Therefore the mycotoxin levels in the years from 2004 to 2005 may be quite different from the levels harvested in 2001–2002. For this reason it is not possible to determine the so-called transmission rate of BEA and ENNs from feed to chicken eggs. As far as the other mycotoxins are concerned, the determined transmission rates in poultry have usually been low, e.g. 0.31% for deoxynivalenol (Prelusky, Rotter, & Rotter, 1994) or 0.012–0.13% for T-2 toxin (Chi et al., 1978).

It is interesting that BEA was detected at the same level as ENNs in the Finnish egg samples, although ENNs are found at much higher levels in Finnish grains than BEA (Jestoi, 2008). This may be due to the higher transmission rate of BEA compared to ENNs or to the increased hydrophobicity of BEA due to the presence of the phenylmethyl side chain (Fig. 1.). The decreased polarity leads to a higher K_{ow}-value, which in turn leads to the increased adsorption and uptake into lipoproteins.

Despite the fact that trace amount of selected *Fusarium* mycotoxins can be detected in Finnish poultry tissues as well as in egg samples, the levels are most probably negligible if one is concerned about whether they can interfere with human or animal health (Jestoi et al., 2007). The observation that BEA and ENNs can accumulate in animal tissues and eggs is, however, interesting. Further investigations are needed to clarify the main metabolites of BEA/ENNs by using a metabolomic approach and also to determine the possible bioaccumulation of the key metabolites as well. This may also provide an alternative approach for food safety purposes.

Acknowledgements

The authors would like to thank Mrs. Merja Orpana for her technical assistance with the analysis.

References

Abdelhamid, A. M., & Dorra, T. M. (1990). Study of effects of feeding laying hens on separate mycotoxins (aflatoxins, patulin, or citrinin)-contaminated diets on the egg quality and tissue constituents. *Archives of Animal Nutrition, 40*, 305–316.

Chi, M. S., Robison, T. S., Mirocha, C. J., Behrens, J. C., & Shimoda, W. (1978). Transmission of radioactivity into eggs from laying hens (*Gallus domesticus*) administered tritium labeled T-2 toxin. *Poultry Science, 57*, 1234–1238.

Dombrink-Kurtzman, M. A. (2003). Fumonisin and beauvericin induce from seed of winter wheat in eastern Canada. *Canadian Journal of Plant Pathology, 8*, 282–288.

Dorner, J. W., Cole, R. J., Erlington, D. J., Suksupath, S., McDowell, G. H., & Bryden, W. L. (1994). Cyclopiazonic acid residues in milk and eggs. *Journal of Agricultural and Food Chemistry, 42*, 1516–1518.

Elkin, R. G. (1997). An overview of recent developments in avian lipoprotein metabolism. *Journal of Nutrition, 127*, 793S–794S.

Griffin, H., Grant, G., & Perry, M. (1982). Hydrolysis of plasma triacylglycerol-rich lipoproteins from immature and laying hens (*Gallus domesticus*) by lipoprotein lipase *in vitro*. *The Biochemical Journal, 206*, 647–654.

Hilgenfeld, R., & Saenger, W. (1982). Structural chemistry of natural and synthetic ionophores and their complexes with cations. In F. L. Boschke (Ed.). *Topics in current chemistry* (Vol. 101, pp. 1–82). Berlin, Germany: Springer.

Hiraga, K., Yamamoto, S., Fukuda, H., Hamanaka, N., & Oda, K. (2005). Enniatin has a new function as an inhibitor of Pdr5p, one of the ABC transporters in *Saccharomyces cerevisae*. *Biochemical and Biophysical Research Communications, 328*, 1119–1125.

Jestoi, M. (2008). Emerging *Fusarium*-mycotoxins fusaproliferin, beauvericin, enniatins and moniliformin – A review. *Critical Reviews in Food Science and Nutrition, 48*, 21–49.

Jestoi, M., Rokka, M., & Peltonen, K. (2007). Residues of emerging *Fusarium*–mycotoxins and coccidiostats in Finnish poultry tissues. *Molecular Nutrition and Food Research, 51*, 625–637.

Jestoi, M., Rokka, M., Rizzo, A., Peltonen, K., & Aurasaari, S. (2005). Determination of *Fusarium*-mycotoxins beauvericin and enniatins with liquid chromatography–tandem mass spectrometry (LC–MS/MS). *Journal of Liquid Chromatography and Related Technologies, 28*, 369–381.

Kamyar, M., Rawnduzi, P., Studenik, C. R., Kouri, K., & Lemmens-Gruber, R. (2004). Investigation of the electrophysiological properties of enniatins. *Archives of Biochemistry and Biophysics, 429*, 215–223.

Kennedy, D. G., Hughes, P. J., & Blanchflower, W. J. (1998). Ionophore residues in eggs in Northern Ireland: Incidence and cause. *Food Additives and Contaminants, 15*, 535–541.

Keshavarz, K. (1998). Investigation on the possibility of reducing protein, phosphorus, and calcium requirements of laying hens by manipulation of time of access to these nutrients. *Poultry Science, 77*, 1320–1332.

Leitgeb, M. D., Lew, H., Khidr, R., Böhm, J., Zollitsch, W., & Wagner, E. (2000). Influence of *Fusarium* toxins on growth and carcass characteristics of turkeys. *Die Bodenkultur, 51*, 71–178.

Leitgeb, M. D., Lew, H., Wetscherek, W., Böhm, J., & Quinz, A. (1999). Influence of fusariotoxins on growing and slaughtering performance of broilers. *Die Bodenkultur, 50*, 57–66.

Logrieco, A., Rizzo, A., Ferracane, R., & Ritieni, A. (2002). Occurrence of beauvericin and enniatins in wheat affected by *Fusarium avenaceum* head blight. *Applied and Environmental Microbiology, 68*, 82–85.

McKee, T. C., Bokesch, H. R., McCormick, J. L., Rashid, M. A., Spielvogel, D., Gustafson, K. R., et al. (1997). Isolation and characterization of new anti-HIV and cytotoxic leads from plants, marine and microbial origins. *Journal of Natural Products, 60*, 431–438.

Mortier, L., Huet, A.-C., Daeseleire, E., Huyghebaeert, G., Fodey, T., Elliott, C., et al. (2005). Deposition and depletion of five anticoccidials in eggs. *Journal of Agricultural and Food Chemistry, 53*, 7142–7149.

Ovchinnikov, Y. A., Ivanov, V. T., Evstratov, A. E., Mikhaleva, I. I., Bystrov, V. F., Portnova, S. L., et al. (1974). The enniatin ionophores, conformation and ion binding properties. *International Journal of Peptide and Protein Research, 6*, 465–498.

Pocsfalvi, G., Di Langa, G., Ferranti, P., Ritieni, A., Randazzo, G., & Malorni, A. (1997). Observation of non-covalent interactions between beauvericin and oligonucleotides using electrospray ionization mass spectrometry. *Rapid Communications in Mass Spectrometry, 11*, 265–272.

Powrie, W. D., & Nakai, S. (1985). In O. R. Fennema (Ed.), *Food chemistry* (2nd ed., pp. 829–855). New York: Marcel Dekker Inc.

Prelusky, D. B., Rotter, B. A., & Rotter, R. G. (1994). In J. D. Miller & H. L. Trenholm (Eds.), *Mycotoxins in grains compounds other than aflatoxin* (pp. 259–403). Minnesota, USA: Eagan Press, St.Paul.

Rokka, M., Eerola, S., Perttilä, U., Rossow, L., Venäläinen, E., Valkonen, E., et al. (2005). The residue levels of narasin in eggs of laying hens fed with unmedicated and medicated feed. *Molecular Nutrition and Food Research, 49*, 38–42.

Rokka, M., & Peltonen, K. (2006). Simultaneous determination of four coccidiostats in eggs and broiler meat: Validation of an LC–MS/MS method. *Food Additives and Contaminants, 23*, 470–478.

Sulyok, M., Berthiller, F., Krska, R., & Schuhmacher, R. (2006). Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Communications in Mass Spectrometry, 20*, 2649–2659.

Sypecka, Z., Kelly, M., & Brereton, P. (2004). Deoxynivalenol and zearalenone residues in eggs of laying hens fed with naturally contaminated diet: Effects on egg production and estimation of transmission rates from feed to eggs. *Journal of Agricultural and Food Chemistry, 52*, 5463–5471.

Tang, L., & Kebarle, P. (1993). Dependence of ion intensity in electrospray mass spectrometry on the concentration of the analytes in the electrosprayed solution. *Analytical Chemistry, 65*, 3654–3668.

Thakur, R. A., & Smith, J. S. (1997). Liquid chromatography/thermospray/mass spectrometry analysis of beauvericin. *Journal of Agricultural and Food Chemistry, 45*, 1234–1239.

Thrane, U., Adler, A., Clasen, P.-E., Galvano, F., Langseth, W., Lew, H., et al. (2004). Diversity in metabolite production by *Fusarium langsethiae*, *Fusarium poae* and *Fusarium sporotichioides*. *International Journal of Food Microbiology, 95*, 257–266.

Trucksess, M. W., Stoloff, L., Young, K., Wyatt, R. D., & Miller, B. L. (1983). Aflatoxicol and aflatoxins B_1 and M_1 in eggs and tissues of laying hens consuming aflatoxin-contaminated feed. *Poultry Science, 62*, 2176–2182.

Uhlig, S., Jestoi, M., & Parikka, P. (2007). *Fusarium avenaceum* – The North European situation. *International Journal of Food Microbiology, 119*, 17–24.

van Eijkeren, J. C. H., Zeilmaker, M. J., Kan, C. A., Traag, W. A., & Hoogenboom, L. A. P. (2006). A toxicokinetic model for the carry-over of dioxins and PCBs from feed and soil to eggs. *Food Additives and Contaminants, 23*, 509–517.

van Trijp, J. M. P., & Roos, A. H. (1991). Model for the calculation of calibration curves. RIKILT report 91.02.

Wolzak, A., Pearson, A. M., Coleman, T. H., Pestka, J. J., & Gray, J. I. (1985). Aflatoxin deposition and clearance in the eggs of laying hens. *Food and Chemical Toxicology, 23*, 1057–1061.

Zrostlikova, J., Hajslova, J., Poutska, J., & Begany, P. (2002). Alternative calibration approaches to compensate the effect of co-extracted matrix components in liquid chromatography–electrospray ionisation tandem mass spectrometry analysis of pesticide residues in plant materials. *Journal of Chromatography A, 973*, 13–26.